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MexAB-OprM specific efflux pump inhibitors in *Pseudomonas aeruginosa*. Part 6: Exploration of aromatic substituents

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Abstract—A series of 4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine derivatives, derivatized at the 2-position with aromatic substituents, were synthesized by the Suzuki cross-coupling method and evaluated for their ability to potentiate the activity of the fluoroquinolone levofloxacin (LVFX) and the anti-pseudomonas β-lactam aztreonam (AZT) in *Pseudomonas aeruginosa*. By incorporating hydrophilic substituents onto the aryl nucleus, we found a morpholine analogue that possessed improved solubility, retained activity in vitro, and displayed potentiation activity in vivo in a rat model of *P. aeruginosa* pneumonia.

1. Introduction

In recent years, *Pseudomonas aeruginosa* has emerged as a problematic pathogen due both to innate and exposure-induced resistance to a variety of antibacterial agents, and the significance of the phenomenon of efflux has become apparent.[1–3] Seven drug efflux pumps of *P. aeruginosa* have been characterized thus far. The MexAB-OprM system contributes to drug resistance of wild-type strains and is commonly overexpressed in clinical isolates.[4] Therefore, we started research aimed at the identification of a molecule for combined use with existing antibacterial agents that can restore their efficacy through specific inhibition of drug efflux pumps, especially MexAB-OprM.[5–9] The clinical impact of such a drug combination, particularly in the treatment of refractory infectious diseases such as those caused by *P. aeruginosa*, would be significant.

In a previous paper, we reported that several vinyl stannanes, *B*-alkyl boranes, and boronic acids could be reacted with an aryl chloride by palladium(0)-catalyzed cross-coupling reactions, providing a powerful strategy for derivatization at the C-2 position in a series of pyridopyrimidines that inhibit MexAB-OprM.[9] Although the 2-carbon-linked variants, especially cyclic substituents, provided good potency, the high lipophilicity of many of these analogues limited their aqueous solubility and reduced their effectiveness in the presence of HSA. Therefore, we extended our studies to include aromatic substituents containing hydrophilic moieties.

Herein, we report the design, synthesis, and evaluation of the resulting derivatives.

2. Chemistry

We planned to generate the desired analogues from the appropriate boronic acids (**2a**–**c**, **2g**–**k**) or pinacol esters (**2d**–**f**) by typical Suzuki cross-coupling reactions[10] followed by hydrolysis of *t*-Bu ester moiety, as depicted in Scheme 1. In practice, this proved to be a powerful methodology, allowing for the incorporation of acidic,

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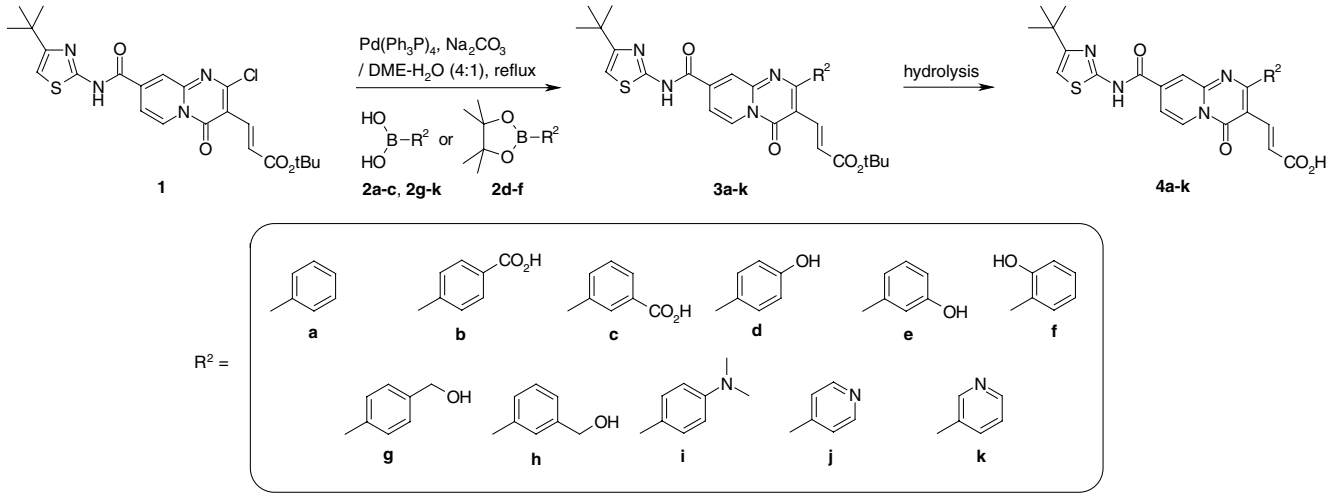


Scheme 1. General procedure for synthesis of 2-Ar analogues.

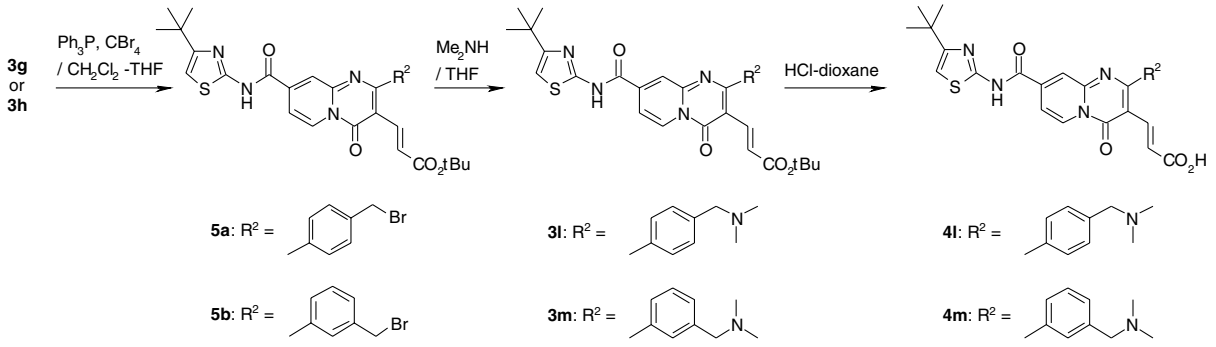
neutral, and weakly basic hydrophilic functional groups on phenyl and heteroaromatic substituents at the C-2 position.

The benzylic alcohols **3g** and **3h** proved useful intermediates in the preparation of analogues incorporating more basic benzylamines (Scheme 2). Reaction with Ph_3P and CBr_4 afforded the benzyl bromides (**5a**, **5b**). Treatment with amines, with subsequent ester hydrolysis, gave the desired products (**3l**, **3m**). This methodology was extensively applied to survey derivatives of **5a**, in particular (Scheme 3). The isonipecotic acid analogue (**4u**) was synthesized via the ethyl ester, with subsequent hydrolysis with lithium hydroxide. *N*-Cyclopropyl-*N*-methylamine (**6n**) and *N*-(2,2-difluoroethyl)-*N*-methylamine (**6o**), which were not commercially available, were prepared as shown in Scheme 4.

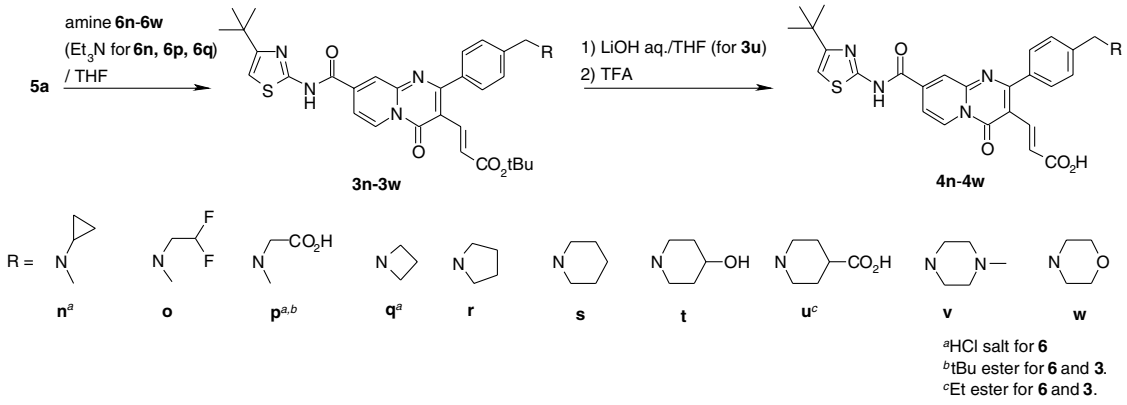
The functionalized piperazine analogues (**4x**, **4y**) were synthesized by N-alkylation of the parent piperazine (**10**), which itself was prepared via deprotection of the *N*-allyl intermediate (Scheme 5).

3. Results and discussion

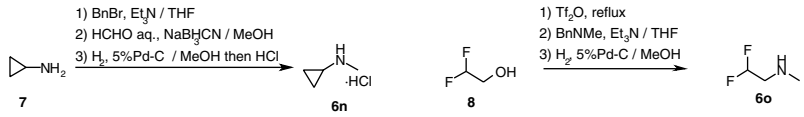
The compounds prepared in this study were tested for in vitro potentiation of the activity of levofloxacin



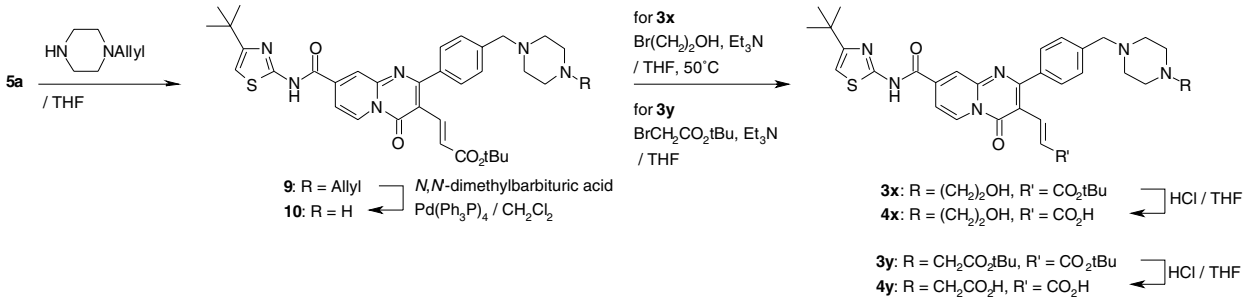
Scheme 2. Introduction of benzylamine moiety.



Scheme 3. Modification of benzylamine moiety.



Scheme 4. Preparation of secondary amines.



Scheme 5. Modification of piperazine moiety.

(LVFX) and aztreonam (AZT) against PAM 1723, a laboratory strain of *P. aeruginosa* in which the MexAB-OprM pump is overexpressed, and the MexCD-OprJ and MexEF-OprN pumps are disrupted.[11] The results, expressed as the minimum concentration of inhibitor required to decrease (potentiate) the minimum inhibitory concentration (MIC) of the combination agent 8-fold (MPC_8, μg/mL), together with aqueous solubilities (in pH 6.8 buffer solution, μg/mL) are presented in Table 1. Antimicrobial assays were conducted in the presence and absence of 0.125% human serum albumin (HSA) in order to evaluate the effects of protein binding.

The prototypical 2-Ph analogue (**4a**) showed encouraging potency, but the reduced activity in the presence of 0.125% HSA and the limited aqueous solubility suggested that the lipophilicity might be a liability for the series.[9] In order to overcome this, analogues bearing hydrophilic substituents were evaluated. Almost all compounds bearing aromatic substituents at C-2 were potent inhibitors, with 4-substituted analogues tending to be more active than 3-substituted (e.g., **4b** vs **4c**).

Although the 4-benzoic acid derivative (**4b**) displayed good activity which was not compromised in the presence of HSA, the solubility was still low. The introduction of hydroxyl groups was investigated in two ways. Phenol analogues (**4d**–**f**) were less active potentiators of AZT. On the other hand, the benzyl alcohol analogues (**4g**, **4h**) seemed to follow the same trend as that observed for the benzoic acids and also possessed poor solubility.

From the above observations, the introduction of acidic and neutral hydrophilic moieties had only incremental effects on solubility. Thus attachment of basic functional groups was next investigated. The conversion to *N*,*N*-dimethylaniline (**4i**) and the switch from phenyl to pyridyl substituents (**4j**, **4k**) resulted in failure (low solubility and low potency in the presence of HSA). However, a more basic *N*,*N*-dimethyl benzylamine analogue (**4l**) showed moderate activity and high solubility (654 μg/mL). An interesting feature of this series is that the migration of the *para*-*N*,*N*-dimethylaminomethyl group to the meta position led to a dramatic reduction in solubility (**4l** vs **4m**).

The water-soluble benzylamine analogue (**4l**), which showed moderate activity in vitro, had low potency in vivo in combination with AZT and was found to be acutely toxic in rats (data not shown). We therefore embarked upon a program to modify the amine substituents in the hopes of improving in vitro activity and reducing the acute toxicity (Table 2).

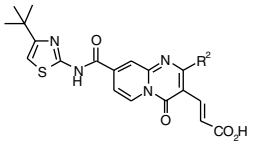
Attachment of sp^2-like carbon and electron-withdrawing substituents, based on the hypothesis that acute toxicity was the consequence of the strong basicity of the amine, was examined (**4n** and **4o**). Consistent with the results for other less basic analogues (**4i**–**4k**), activity improved but solubility was reduced. On the other hand, while conversion to the α-amino acid (**4p**) improved solubility, the potency was slightly reduced.

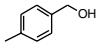
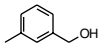
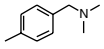
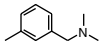
The 6-membered ring variant **4s** was slightly more potent than smaller congeners **4q** and **4r**. Attachment of a hydroxyl group at the piperidine 4-position (**4t**) did not increase solubility as much as expected, and the isonipecotic acid derivative (**4u**) was less active. The piperazine analogues (**4v**, **4x** and **4y**), which were synthesized for the sake of ready derivatization, were equipotent or slightly less active than the piperidine (**4s**).

Overall, the morpholine derivative **4w** showed the best combination of in vitro potency (with and without HSA) and solubility. In an acute toxicity assay, performed by intravenous administration in rats, the lethal dose was estimated to be above 100 mg/kg, consistent with the hope that reduction in basicity of the amine might prove beneficial.

Because of its safety characteristics, compound **4w** was chosen as a prototypical example with which to perform an exploratory in vivo experiment in combination with

Table 1. Incorporation of hydrophilic moiety on 2-Ar nuclei[a]



Compound	R^2	MPC_8 (LVFX) (μg/mL)		MPC_8 (AZT) (μg/mL)	Sol (pH 6.8) (μg/mL)
		Without HSA	With 0.125% HSA		
4a		4	16	0.5	2
4b		8	8	2	19
4c		16	32	8	—[c]
4d		8	>16[b]	4	—[c]
4e		8	>32	4	—[c]
4f		32	>32	8	—[c]
4g		4	8	1	21
4h		4	16	1	29
4i		4	16	2	—[c]
4j		2	8	1	6.8
4k		4	16	1	21
4l		16	>32	4	654
4m		16	>16[b]	2	2.7

[a] All compounds lacked intrinsic antibacterial activity.
[b] The apparent lack of activity is attributable to precipitation of the compound.
[c] Not tested.

AZT. We evaluated its potentiation activity in a pneumonia model against *P. aeruginosa* PAM 1020, a wild-type strain that exhibits basal levels of MexAB-OprM and does not express MexCD-OprJ or MexEF-OprN.[12] As shown in Table 3, when dosed at 10 mg/kg, **4w** in combination with 1000 mg/kg of AZT gave moderate survival at the end of the study, whereas no obvious effects were observed on treatment with AZT alone.

4. Conclusion

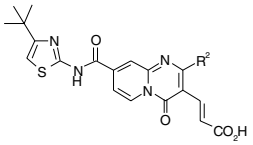
In summary, we have discovered a series of pyridopyrimidine derivatives, substituted at the 2-position with carbon-linked aryl groups, that display in vitro inhibition of the MexAB-OprM efflux pump in *P. aeruginosa*. The development of chemistry that introduces a variety of aromatic rings onto scaffold was accomplished by application of Suzuki cross-coupling methodology. Benzyl amine analogues that exhibited improved solubility stimulated the exploration of other substituents on benzylic nitrogen atom and culminated in the morpholine analogue, which showed potentiation activity in vivo and a reasonable safety profile in an acute toxicity assay. Additional research is required to further optimize the series, which will be reported in due course.

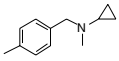
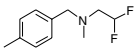
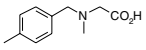
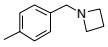
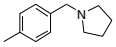
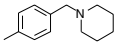
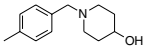
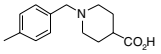
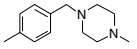
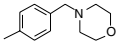
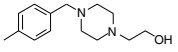
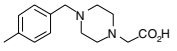
5. Experimental

5.1. General

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. *tert*-Butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (**1**), *tert*-butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-2-phenyl-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (**3a**) and (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]car-

Table 2. Modification of substituent of benzylic nitrogen atom[a]



Compound	R^2	MPC_8 (LVFX) (μg/mL)		MPC_8 (AZT) (μg/mL)	Sol (pH 6.8) (μg/mL)
		Without HSA	With 0.125% HSA		
4n		8	32	4	66
4o		4	16	2	14
4p		16	32	8	>830
4q		16	32	4	130
4r		16	32	4	—[c]
4s		8	32	4	—[c]
4t		8	16	4	74
4u		32	>32	16	—[c]
4v		8	>8[b]	4	12
4w		4	16	2	140
4x		8	16	4	—[c]
4y		16	32	8	—[c]

[a] All compounds lacked intrinsic antibacterial activity.
[b] The apparent lack of activity is attributable to precipitation of the compound.
[c] Not tested.

Table 3. Efficacy of AZT (1000 mg/kg) combined with ABS (10 mg/kg) by intravenous drip infusion in a model of *P. aeruginosa* pneumonia in rats

Conditions	Number of survival after infection			
	Day 0	Day 1	Day 3	Day 7
Non-treatment	8	2	0	0
AZT alone	8	8	1	1
AZT + **4w**	8	8	4	3

bonyl}-4-oxo-2-phenyl-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (**4a**) were prepared according to the literature procedures.[9] Melting points were taken on a Yanako MP-500D melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined on a JEOL JNM-EX400 spectrometer.

Chemical shifts are reported in parts per million relative to tetramethylsilane as internal standard. Significant ^{1}H NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), coupling constant(s) in hertz. Infrared (IR) spectra were obtained on a HORIBA FT-720 spectrometer. High-resolution mass spectra were obtained on a JEOL JMS-700 mass spectrometer under electron impact ionization conditions (EI), or fast

atom bombardment ionization conditions (FAB). Elementary analyses were conducted at Research Technology Center, Daiichi Pharmaceutical Co., Ltd. Column chromatography refers to flash column chromatography conducted on Merck silica gel 60, 230–400 mesh ASTM. Thin-layer chromatography (TLC) was performed with Merck silica gel 60 F_{254} TLC plates, and compound visualization was effected with a 5% solution of phosphomolybdic acid in ethanol, UV lamp, or Wako ninhydrin spray. Unless otherwise specified, reactions were carried out at ambient temperature. Combined organic extracts were dried over anhydrous Na_2SO_4. Reagents and solvents were removed from reaction mixture or combined organic extracts by concentration in vacuo using a rotary evaporator with bath at 35–45 °C.

5.2. Synthesis

5.2.1. 4-(3-[(1*E*)-3-*tert*-Butoxy-3-oxoprop-1-en-1-yl]-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-2-yl)benzoic acid (3b). To a solution of **1** (1.00 g, 2.05 mmol) in DME (20 mL)–water (5 mL) were added **2b** (339 mg, 2.05 mmol), Na_2CO_3 (434 mg, 4.09 mmol), and $Pd(Ph_3P)_4$ (118 mg, 0.102 mmol), and refluxed for 23 h under Ar. The mixture was concentrated, and the residue was diluted with EtOAc and washed with brine. The organic layer was dried and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3 \rightarrow CHCl_3$/EtOAc = 4:1 → 2:1 → $CHCl_3$/MeOH = 9:1) to afford **3b** (465 mg) as a yellow solid. ^1H NMR (DMSO-d_6) δ: 1.31 (9H, s), 1.42 (9H, s), 6.87 (1H, s), 7.24 (1H, d, J = 15.6 Hz), 7.40 (1H, d, J = 15.6 Hz), 7.71 (2H, d, J = 8.3 Hz), 7.94 (1H, dd, J = 7.6, 1.2 Hz), 8.14 (2H, d, J = 8.3 Hz), 8.47 (1H, s), 9.18 (1H, d, J = 7.6 Hz). HRMS (FAB) Calcd for $C_{30}H_{31}N_4O_6S$ ($[M+H]^+$): 575.1964. Found: 575.1936.

5.2.2. 3-(3-[(1*E*)-3-*tert*-Butoxy-3-oxoprop-1-en-1-yl]-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-2-yl)benzoic acid (3c). Following the procedures as described for **3b**, the title compound (896 mg) was prepared from **1** (1.00 g, 2.05 mmol) and **2c** (339 mg, 2.05 mmol) as a yellow solid. ^1H NMR (DMSO-d_6) δ: 1.30 (9H, s), 1.41 (9H, s), 6.80 (1H, s), 7.23 (1H, d, J = 15.5 Hz), 7.45 (1H, d, J = 15.5 Hz), 7.66 (1H, t, J = 7.6 Hz), 7.69–7.78 (1H, m), 7.90–7.98 (1H, m), 8.15 (1H, d, J = 7.1 Hz), 8.21 (s, 1H), 8.44 (1H, s), 9.15 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{30}H_{31}N_4O_6S$ ($[M+H]^+$): 575.1964. Found: 575.1936.

5.2.3. *tert*-Butyl (2*E*)-3-[8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(4-hydroxyphenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylate (3d). To a solution of **1** (100 mg, 0.205 mmol) in DME (4 mL)–water (1 mL) were added **2d** (45.0 mg, 0.205 mmol), Na_2CO_3 (43.4 mg, 0.409 mmol), and $Pd(Ph_3P)_4$ (11.8 mg, 10.2 μmol), and stirred at 80 °C for 12.5 h under N_2. The mixture was diluted with 10% citric acid aq and extracted with EtOAc (3×). The combined organic phases were dried and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3 \rightarrow CHCl_3$/MeOH = 98:2 → 95:5 and EtOAc/hexane = 1:9 → 1:4 → 1:2 → 1:1) to afford **3d** (35.3 mg) as an orange solid. ^1H NMR ($CDCl_3$) δ: 1.24 (9H, s), 1.37 (9H, s), 6.64 (1H, s), 7.02 (2H, d, J = 8.5 Hz), 7.40 (1H, d, J = 15.6 Hz), 7.62 (2H, d, J = 8.5 Hz), 7.68 (1H, d, J = 15.6 Hz), 7.70–7.74 (1H, m), 8.26 (1H, s), 9.18 (1H, d, J = 7.8 Hz). HRMS (FAB) Calcd for $C_{29}H_{31}N_4O_5S$ ($[M+H]^+$): 547.2015. Found: 547.1993.

5.2.4. *tert*-Butyl (2*E*)-3-[8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(3-hydroxyphenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylate (3e). Following the procedures as described for **3d**, the title compound (48.1 mg) was prepared from **1** (100 mg, 0.205 mmol) and **2e** (45.0 mg, 0.205 mmol) as an orange solid. ^1H NMR ($CDCl_3$) δ: 1.22 (9H, s), 1.24 (9H, s), 6.32 (1H, s), 6.51–6.65 (1H, m), 6.76–6.89 (1H, m), 6.94–7.07 (1H, m), 7.08–7.17 (1H, m), 7.43 (1H, d, J = 15.6 Hz), 7.58–7.68 (1H, m), 7.75 (1H, d, J = 15.6 Hz), 8.60 (1H, br s), 8.94–9.10 (1H, m). HRMS (FAB) Calcd for $C_{29}H_{31}N_4O_5S$ ($[M+H]^+$): 547.2015. Found: 547.2026.

5.2.5. *tert*-Butyl (2*E*)-3-[8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(2-hydroxyphenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylate (3f). Following the procedures as described for **3d**, the title compound (21.9 mg) was prepared from **1** (100 mg, 0.205 mmol) and **2f** (45.0 mg, 0.205 mmol) as a yellow solid. ^1H NMR ($CDCl_3$) δ: 1.35 (9H, s), 1.46 (9H, s), 2.75 (1H, dd, J = 14.8, 3.5 Hz), 2.87 (1H, dd, J = 14.8, 9.6 Hz), 6.14 (1H, dd, J = 9.8, 3.4 Hz), 6.64 (1H, s), 6.93–6.96 (1H, m), 7.07–7.12 (1H, m), 7.37–7.42 (1H, m), 7.58–7.63 (1H, m), 8.19 (1H, dd, J = 7.8, 1.7 Hz), 8.22–8.26 (1H, m), 9.06 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{29}H_{31}N_4O_5S$ ($[M+H]^+$): 547.2015. Found: 547.1992.

5.2.6. *tert*-Butyl (2*E*)-3-{8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-[4-(hydroxymethyl)phenyl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}acrylate (3g). Following the procedures as described for **3b**, the title compound (815 mg) was prepared from **1** (1.00 g, 2.05 mmol) and **2g** (311 mg, 2.05 mmol) as an orange solid. ^1H NMR ($CDCl_3$) δ: 1.35 (9H, s), 1.50 (9H, s), 4.78 (2H, s), 6.56 (1H, s), 7.38 (1H, d, J = 15.6 Hz), 7.45 (2H, d, J = 8.1 Hz), 7.55 (2H, d, J = 8.1 Hz), 7.60 (1H, d, J = 15.6 Hz), 7.67 (1H, dd, J = 7.3, 1.6 Hz), 8.23 (1H, d, J = 1.6 Hz), 9.13 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{30}H_{33}N_4O_5S$ ($[M+H]^+$): 561.2172. Found: 561.2212.

5.2.7. *tert*-Butyl (2*E*)-3-{8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-[3-(hydroxymethyl)phenyl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}acrylate (3h). Following the procedures as described for **3b**, the title compound (267 mg) was prepared from **1** (250 mg, 0.511 mmol) and **2h** (77.7 mg, 0.511 mmol) as an orange solid. ^1H NMR ($CDCl_3$) δ: 1.37 (9H, s), 1.53 (9H, s), 4.76 (2H, s), 6.44 (1H, s), 7.13–7.28 (3H, m), 7.32 (1H, d, J = 15.9 Hz), 7.47–4.67 (3H, m), 8.13 (1H, s), 8.90 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{30}H_{33}N_4O_5S$ ($[M+H]^+$): 561.2172. Found: 561.2194.

5.2.8. *tert*-Butyl (2*E*)-3-{8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-[4-(dimethylamino)phenyl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}acrylate (3i). Following the procedures as described for **3b**, the title compound (266 mg) was prepared from **1** (250 mg, 0.511 mmol) and **2i** (84.4 mg, 0.511 mmol) as a red solid. ^{1}H NMR ($CDCl_3$) δ: 1.32 (9H, s), 1.52 (9H, s), 3.01 (6H, s), 6.49 (1H, s), 6.66 (2H, d, J = 9.0 Hz), 7.35 (1H, d, J = 15.7 Hz), 7.47–7.54 (3H, m), 7.74 (1H, d, J = 15.7 Hz), 8.12 (1H, d, J = 1.2 Hz), 8.97 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{31}H_{36}N_5O_4S$ ($[M+H]^+$): 574.2488. Found: 574.2460.

5.2.9. *tert*-Butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-2-pyridin-4-yl-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (3j). Following the procedures as described for **3b**, the title compound (41.1 mg) was prepared from **1** (100 mg, 0.205 mmol) and **2j** (27.5 mg, 0.225 mmol) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.36 (9H, s), 1.51 (9H, s), 6.53 (1H, s), 7.43 (1H, d, J = 15.6 Hz), 7.47–7.51 (2H, m), 7.54 (1H, d, J = 15.6 Hz), 7.75 (1H, dd, J = 1.7, 7.4 Hz), 8.30–8.34 (1H, m), 8.75–8.81 (2H, m), 9.14 (1H, d, J = 7.4 Hz). HRMS (FAB) Calcd for $C_{28}H_{30}N_5O_4S$ ($[M+H]^+$): 532.2019. Found: 532.1996.

5.2.10. *tert*-Butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-2-pyridin-3-yl-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (3k). Following the procedures as described for **3b**, the title compound (63.4 mg) was prepared from **1** (100 mg, 0.205 mmol) and **2k** (27.5 mg, 0.225 mmol) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.35 (9H, s), 1.50 (9H, s), 6.51 (1H, s), 7.34–7.43 (1H, m), 7.39 (1H, d, J = 15.6 Hz), 7.51 (1H, d, J = 15.6 Hz), 7.66–7.75 (1H, m), 7.83–7.91 (1H, m), 8.30 (1H, s), 8.71–8.85 (2H, m), 9.08 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{28}H_{30}N_5O_4S$ ($[M+H]^+$): 532.2019. Found: 532.1989.

5.2.11. 4-{8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-3-[(*E*)-2-carboxyvinyl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-2-yl}benzoic acid (4b). To TFA (10 mL) was added **3b** (250 mg, 0.435 mmol) and stirred for 1 h. The mixture was concentrated, and the residue was diluted with Et_2O. The precipitated solid was collected by filtration and washed with Et_2O to afford **4b** (203 mg) as a yellow solid. Mp: 322–327 °C (dec). IR (ATR) cm^{-1}: 3180, 3095, 2964, 2871, 2800, 1678, 1604, 1568, 1547, 1508, 1448, 1396, 1367, 1311, 1292, 1242, 1205, 1153, 1103, 1061, 1018. ^{1}H NMR (DMSO-d_6) δ: 1.31 (9H, s), 6.89 (1H, s), 7.28 (1H, d, J = 15.5 Hz), 7.43 (1H, d, J = 15.5 Hz), 7.72 (2H, d, J = 7.9 Hz), 7.93 (1H, dd, J = 1.7, 7.6 Hz), 8.14 (2H, d, J = 7.9 Hz), 8.46–8.49 (1H, m), 9.20 (1H, d, J = 7.6 Hz), 12.92 (1H, br). Anal. Calcd for $C_{26}H_{22}N_4O_6S{\cdot}0.5H_2O$: C, 59.19; H, 4.39; N, 10.62; S, 6.08. Found: C, 58.95; H, 4.38; N, 10.51; S, 6.15.

5.2.12. 3-{8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-3-[(*E*)-2-carboxyvinyl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-2-yl}benzoic acid (4c). Following the procedures as described for **4b**, the title compound (24.4 mg) was prepared from **3c** (28.4 mg, 0.0494 mmol) as a yellow solid. Mp: 234–240 °C (dec). IR (ATR) cm^{-1}: 3062, 2962, 2870, 1678, 1583, 1547, 1500, 1437, 1400, 1288, 1254, 1190, 1140, 1097, 1059. ^{1}H NMR (DMSO-d_6) δ: 1.30 (9H, s), 6.88 (1H, s), 7.27 (1H, d, J = 15.6 Hz), 7.44 (1H, d, J = 15.6 Hz), 7.71 (1H, t, J = 7.8 Hz), 7.80–7.85 (1H, m), 7.88–7.93 (1H, m), 8.10–8.15 (1H, m), 8.20 (1H, s), 8.50 (1H, s), 9.18 (1H, d, J = 7.3 Hz). Anal. Calcd for $C_{26}H_{22}N_4O_6S{\cdot}0.5TFA{\cdot}1.5H_2O$: C, 53.82; H, 4.27; N, 9.30; S, 5.32; F, 4.73. Found: C, 53.82; H, 4.11; N, 9.03; S, 5.35; F, 5.23.

5.2.13. (2*E*)-3-[8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(4-hydroxyphenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylic acid (4d). Following the procedures as described for **4b**, the title compound (22.7 mg) was prepared from **3d** (35.3 mg, 0.06458 mmol) as a yellow solid. Mp: 241–246 °C (dec). IR (ATR) cm^{-1}: 3111, 2964, 2871, 1666, 1604, 1560, 1500, 1452, 1410, 1365, 1311, 1275, 1220, 1171, 1101, 1063. ^{1}H NMR (DMSO-d_6) δ: 1.30 (9H, s), 6.88 (1H, s), 6.94 (2H, d, J = 8.5 Hz), 7.25 (1H, d, J = 15.6 Hz), 7.49 (2H, d, J = 8.5 Hz), 7.57 (1H, d, J = 15.6 Hz), 7.82–7.86 (1H, m), 8.41-8.44 (1H, m), 9.12 (1H, d, J = 7.3 Hz), 10.04 (1H, s). Anal. Calcd for $C_{25}H_{22}N_4O_5S{\cdot}1.5H_2O$: C, 58.02; H, 4.87; N, 10.83; S, 6.20. Found: C, 58.25; H, 4.61; N, 10.47; S, 6.15.

5.2.14. (2*E*)-3-[8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(3-hydroxyphenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylic acid (4e). Following the procedures as described for **4b**, the title compound (29.0 mg) was prepared from **3e** (48.5 mg, 0.08874 mmol) as a yellow solid. Mp: 289–295 °C (dec). IR (ATR) cm^{-1}: 3099, 2964, 2870, 1658, 1610, 1560, 1510, 1477, 1412, 1367, 1311, 1282, 1242, 1203, 1103, 1066. ^{1}H NMR (DMSO-d_6) δ: 1.30 (9H, s), 6.86–7.00 (4H, m), 7.26 (1H, d, J = 15.6 Hz), 7.36 (1H, t, J = 7.8 Hz), 7.52 (1H, d, J = 15.6 Hz), 7.87–7.91 (1H, m), 8.41–8.44 (1H, m), 9.16 (1H, d, J = 7.3 Hz), 9.77 (1H, s). Anal. Calcd for $C_{25}H_{22}N_4O_5S{\cdot}H_2O$: C, 59.04; H, 4.76; N, 11.02; S, 6.31. Found: C, 58.83; H, 4.69; N, 11.01; S, 6.39.

5.2.15. (2*E*)-3-[8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(2-hydroxyphenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylic acid (4f). Following the procedures as described for **4b**, the title compound (17.4 mg) was prepared from **3f** (21.9 mg, 0.04003 mmol) as a yellow solid. Mp: 304–309 °C (dec.). IR (ATR) cm^{-1}: 2968, 1670, 1608, 1556, 1522, 1485, 1446, 1427, 1362, 1313, 1281, 1234, 1203, 1173, 1101, 1068, 1043. ^{1}H NMR (DMSO-d_6) δ: 1.31 (9H, s), 2.62 (1H, dd, J = 15.4, 3.2 Hz), 2.70 (1H, dd, J = 15.4, 10.0 Hz), 5.97 (1H, dd, J = 10.0, 3.2 Hz), 6.88 (1H, s), 6.96 (1H, d, J = 8.3 Hz), 7.14–7.20 (1H, m), 7.44–7.50 (1H, m), 7.76 (1H, dd, J = 7.6, 1.7 Hz), 8.17 (1H, dd, J = 7.6, 1.7 Hz), 8.47 (1H, s), 8.99 (1H, d, J = 7.3 Hz), 12.76 (1H, br s). Anal. Calcd for $C_{25}H_{22}N_4O_5S{\cdot}0.75H_2O$: C, 59.57; H, 4.70; N, 11.12; S, 6.36. Found: C, 59.58; H, 4.45; N, 11.11; S, 6.28.

5.2.16. (2*E*)-3-{8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-[4-(hydroxymethyl)phenyl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}acrylic acid (4g). Following the procedures as described for **4b**, the title compound (189 mg) was prepared from **3g** (250 mg, 0.446 mmol)

as a yellow solid. Mp: 282–295 °C (dec.). IR (ATR) cm^{-1}: 3103, 3053, 2960, 2871, 1670, 1639, 1603, 1560, 1549, 1500, 1442, 1402, 1363, 1311, 1267, 1201, 1147, 1101, 1057. ^1H NMR (DMSO-d_6) δ: 1.31 (9H, s), 4.62, 4.63 (total 2H, 2s), 5.36 (1H, t, J = 5.7 Hz), 6.86 (1H, s), 7.28 (1H, d, J = 15.6 Hz), 7.49–7.60 (5H, m), 7.88–7.93 (1H, m), 8.42–8.46 (1H, m), 9.16 (1H, d, J = 7.6 Hz). Anal. Calcd for $C_{26}H_{24}N_4O_5S{\cdot}2.25H_2O$: C, 57.29; H, 5.27; N, 10.28; S, 5.88. Found: C, 57.29; H, 4.80; N, 10.23; S, 6.02.

5.2.17. (2*E*)-3-{8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]-carbonyl}-2-[3-(hydroxymethyl)phenyl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}acrylic acid (4h). Following the procedures as described for **4b**, the title compound (27.0 mg) was prepared from **3h** (64.2 mg, 0.115 mmol) as a yellow solid. Mp: 246–252 °C (dec). IR (ATR) cm^{-1}: 2962, 1672, 1599, 1560, 1546, 1504, 1452, 1406, 1365, 1311, 1292, 1277, 1223, 1203, 1101, 1061. ^1H NMR (DMSO-d_6) δ: 1.29 (9H, s), 4.56–4.64 (2H, m), 5.33 (1H, t, J = 5.7 Hz), 6.81 (1H, s), 7.27 (1H, d, J = 15.6 Hz), 7.37–7.43 (1H, m), 7.45–7.53 (3H, m), 7.59 (1H, s), 7.92 (1H, d, J = 7.6 Hz), 8.42 (1H, s), 9.16 (1H, d, J = 7.6 Hz). Anal. Calcd for $C_{26}H_{24}N_4O_5S{\cdot}1.25H_2O$: C, 59.25; H, 5.07; N, 10.63; S, 6.08. Found: C, 59.18; H, 4.78; N, 10.52; S, 6.19.

5.2.18. (2*E*)-3-{8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]-carbonyl}-2-[4-(dimethylamino)phenyl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}acrylic acid (4i). To a solution of **3i** (266 mg, 0.463 mmol) in 4 N HCl solution in dioxane (5 mL) was added concentrated HCl (0.5 mL) and stirred for 1.5 h. The mixture was concentrated, and the residue was purified by silica gel column chromatography ($CHCl_3$ → $CHCl_3$/MeOH = 95:5 → 10:1). The combined fractions were concentrated and the residue was solidified with Et_2O. The precipitated solid was collected by filtration and washed with Et_2O to afford **4i** (153 mg) as an orange solid. Mp: 288–294 °C (dec). IR (ATR) cm^{-1}: 2964, 2860, 2798, 1672, 1599, 1558, 1508, 1441, 1406, 1362, 1311, 1292, 1255, 1228, 1196, 1151, 1124, 1101, 1063, 1005. ^1H NMR (DMSO-d_6) δ: 1.31 (9H, s), 3.04 (6H, s), 6.85–6.90 (3H, m), 7.27 (1H, d, J = 15.6 Hz), 7.56 (2H, d, J = 8.8 Hz), 7.65 (1H, d, J = 15.6 Hz), 7.80 (1H, dd, J = 1.8, 7.6 Hz), 8.40–8.43 (1H, m), 9.08 (1H, d, J = 7.6 Hz). Anal. Calcd for $C_{27}H_{27}N_5O_4S{\cdot}0.5H_2O$: C, 61.58; H, 5.36; N, 13.30; S, 6.09. Found: C, 61.58; H, 5.16; N, 13.33; S, 6.33.

5.2.19. (2*E*)-3-(8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]-carbonyl}-4-oxo-2-pyridin-4-yl-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (4j). Following the procedures as described for **4i**, the title compound (22.7 mg) was prepared from **3j** (41.1 mg, 77.3 μmol) as a yellow solid. Mp: 294–304 °C (dec). IR (ATR) cm^{-1}: 3435, 3373, 2968, 1691, 1637, 1603, 1556, 1495, 1462, 1414, 1365, 1336, 1288, 1230, 1192, 1144, 1107, 1061. ^1H NMR (DMSO-d_6) δ: 1.31 (9H, s), 6.89 (1H, s), 7.28 (1H, d, J = 15.6 Hz), 7.34 (1H, d, J = 15.6 Hz), 7.77 (2H, d, J = 5.6 Hz), 7.94–8.00 (1H, m), 8.48 (1H, s), 8.91 (2H, d, J = 5.6 Hz), 9.23 (1H, d, J = 7.3 Hz). Anal. Calcd for $C_{24}H_{21}N_5O_4S{\cdot}HCl{\cdot}3H_2O$: C, 50.93; H, 4.99; N, 12.37; S, 5.67. Found: C, 50.90; H, 4.85; N, 12.25; S, 5.84.

5.2.20. (2*E*)-3-(8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]-carbonyl}-4-oxo-2-pyridin-3-yl-4H-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (4k). Following the procedures as described for **4i**, the title compound (41.9 mg) was prepared from **3k** (63.4 mg, 119 μmol) as an orange solid. Mp: 266–271 °C (dec). IR (ATR) cm^{-1}: 3371, 3055, 2964, 2085, 1682, 1635, 1601, 1543, 1493, 1466, 1454, 1414, 1363, 1306, 1279, 1228, 1186, 1142, 1101, 1059. ^1H NMR (DMSO-d_6) δ: 1.31 (9H, s), 6.89 (1H, s), 7.29 (1H, d, J = 15.4 Hz), 7.41 (1H, d, J = 15.4 Hz), 7.76 (1H, dd, J = 5.1, 7.8 Hz), 7.95 (1H, dd, J = 2.0, 7.3 Hz), 8.18 (1H, d, J = 7.8 Hz), 8.49 (1H, s), 8.82–8.86 (1H, m), 8.87 (1H, d, J = 2.0 Hz), 9.21 (1H, d, J = 7.3 Hz). Anal. Calcd for $C_{24}H_{21}N_5O_4S{\cdot}0.9HCl{\cdot}2.4H_2O$: C, 52.26; H, 4.88; N, 12.70; S, 5.81; Cl, 5.78. Found: C, 52.38; H, 4.99; N, 12.49; S, 5.83; Cl, 5.66.

5.2.21. *tert*-Butyl (2*E*)-3-(2-[4-(bromomethyl)phenyl]-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (5a). To a solution of **3g** (615 mg, 1.10 mmol) in CH_2Cl_2 (6 mL)–THF (6 mL) were added CBr_4 (728 mg, 2.19 mmol) and Ph_3P (719 mg, 2.74 mmol) at 0 °C, and stirred for 30 min at 0 °C. The mixture was concentrated, and the residue was purified by silica gel column chromatography (EtOAc/hexane = 1:4) to afford **5a** (649 mg) as an orange solid. ^1H NMR ($CDCl_3$) δ: 1.33 (9H, s), 1.55 (9H, s), 4.46 (2H, s), 6.40 (1H, s), 7.389 (2H, d, J = 8.3 Hz), 7.392 (1H, d, J = 15.6 Hz), 7.46 (2H, d, J = 8.3 Hz), 7.52–7.58 (1H, m), 7.62 (1H, d, J = 15.6 Hz), 8.15 (1H, d, J = 1.0 Hz), 8.93–9.00 (1H, m).

5.2.22. *tert*-Butyl (2*E*)-3-(2-[3-(bromomethyl)phenyl]-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (5b). Following the procedures as described for **5a**, the title compound (261 mg) was prepared from **3h** (267 mg, 476 μmol) as an orange foam. ^1H NMR ($CDCl_3$) δ: 1.37 (9H, s), 1.53 (9H, s), 4.76 (2H, s), 6.44 (1H, s), 7.13–7.28 (3H, m), 7.32 (1H, d, J = 15.9 Hz), 7.47–4.67 (3H, m), 8.13 (1H, s), 8.90 (1H, d, J = 7.3 Hz).

5.2.23. *tert*-Butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-{4-[(dimethylamino)methyl]phenyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (3l). To a solution of **5a** (250 mg, 0.401 mmol) in THF (5 mL) was added Me_2NH (2.0 M THF solution, 401 μL, 0.802 mmol) at 0 °C and stirred for 64 h. The mixture was diluted with satd. $NaHCO_3$ aq and extracted with $CHCl_3$ (5×). The combined organic phases were dried and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 99:1 → 98:2) to afford **3l** (214 mg) as a yellow solid. ^1H NMR ($CDCl_3$) δ: 1.33 (9H, s), 1.50 (9H, s), 2.27 (6H, s), 3.50 (2H, s), 6.49 (1H, s), 7.36 (1H, d, J = 15.7 Hz), 7.41 (2H, d, J = 7.9 Hz), 7.51 (2H, d, J = 7.9 Hz), 7.58–7.63 (1H, m), 7.63 (1H, d, J = 15.7 Hz), 8.10 (1H, s), 9.05 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{32}H_{38}N_5O_4S$ ($[M+H]^+$): 588.2645. Found: 588.2628.

5.2.24. *tert*-Butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-{3-[(dimethylamino)methyl]phenyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (3m). Following the procedures as described for **3l**, the title compound (198 mg) was prepared from **5b** (261 mg, 418 μmol) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.32 (9H, s), 1.47 (9H, s), 2.29 (6H, s), 3.57 (2H, s), 6.54 (1H, s), 7.25–7.70 (5H, m), 7.31 (1H, d, J = 15.7 Hz), 7.54 (1H, d, J = 15.7 Hz), 8.11 (1H, s), 8.98–9.12 (1H, m). HRMS (FAB) Calcd for $C_{32}H_{38}N_5O_4S$ ($[M+H]^+$): 588.2645. Found: 588.2660.

5.2.25. (2*E*)-3-(8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-{4-[(dimethylamino)methyl]phenyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (4l). Following the procedures as described for **4i**, the title compound (190 mg) was prepared from **3l** (214 mg, 0.364 mmol) as a red solid. Mp: 216–223 °C (dec). IR (ATR) cm^{-1}: 2962, 2868, 2750, 2683, 2584, 1739, 1693, 1645, 1593, 1566, 1491, 1390, 1342, 1304, 1221, 1182, 1101, 1057. ^{1}H NMR (DMSO-d_6) δ: 1.30 (9H, s), 2.491, 2.494 (total 3H, 2s), 2.75, 2.76 (total 2H, 2s), 6.88 (1H, s), 7.27 (1H, d, J = 15.6 Hz), 7.44 (1H, d, J = 15.6 Hz), 7.68 (2H, d, J = 8.0 Hz), 7.73–7.79 (2H, m), 7.90–7.95 (1H, m), 8.40–8.43 (1H, m), 9.18 (1H, d, J = 7.6 Hz). Anal. Calcd for $C_{28}H_{29}N_5O_4S{\cdot}2.25HCl{\cdot}1.75H_2O$: C, 52.12; H, 5.43; N, 10.85; S, 4.97; Cl, 12.36. Found: C, 52.32; H, 5.50; N, 10.81; S, 4.99; Cl, 12.06.

5.2.26. (2*E*)-3-(8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-{3-[(dimethylamino)methyl]phenyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (4m). Following the procedures as described for **4i**, the title compound (185 mg) was prepared from **3m** (198 mg, 0.337 mmol) as an orange solid. Mp: 275–283 °C (dec). IR (ATR) cm^{-1}: 3464, 3128, 2964, 1730, 1674, 1649, 1579, 1500, 1475, 1346, 1311, 1250, 1205, 1167, 1149, 1097, 1061, 1014. ^{1}H NMR (DMSO-d_6) δ: 1.31 (9H, s), 2.74, 2.76 (total 6H, each s), 4.39, 4.40 (total 2H, each s), 6.89 (1H, s), 7.27 (1H, d, J = 15.6 Hz), 7.46 (1H, d, J = 15.6 Hz), 7.68–7.79 (3H, m), 7.81–7.86 (1H, m), 7.95 (1H, dd, J = 1.7, 7.3 Hz), 8.46 (1H, d, J = 1.7 Hz), 9.20 (1H, d, J = 7.3 Hz), 10.77–10.87 (1H, m). Anal. Calcd for $C_{28}H_{29}N_5O_4S{\cdot}2.4HCl{\cdot}2.5H_2O$: C, 50.63; H, 5.52; N, 10.54; S, 4.83; Cl, 12.81. Found: C, 50.69; H, 5.51; N, 10.50; S, 4.90; Cl, 12.43.

5.2.27. *tert*-Butyl (2*E*)-3-[8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(4-{[cyclopropyl(methyl)amino]methyl}phenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylate (3n). To a solution of *N*-cyclopropyl-*N*-methylamine hydrochloride **6n** (122 mg, 1.13 mmol) in THF (5 mL) was added Et_3N (166 μL, 1.19 mmol) and stirred for 10 min. To the mixture was added **5a** (200 mg, 0.321 mmol) and stirred for 17 h. The mixture was concentrated, and the residue was diluted with satd $NaHCO_3$ aq and extracted with $CHCl_3$. The organic layer was washed with brine. The organic layer was dried and concentrated. The residue was purified by PTLC ($CHCl_3$/MeOH = 10:1) to afford **3n** (176 mg, 90%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 0.38–0.56 (4H, m), 1.33 (9H, s), 1.53 (9H, s), 1.70–1.80 (1H, m), 2.25 (3H, s), 3.68 (2H, s), 6.45 (1H, s), 7.32 (2H, d, J = 7.8 Hz), 7.39 (1H, d, J = 15.7 Hz), 7.47 (2H, d, J = 7.8 Hz), 7.59 (1H, d, J = 7.3 Hz), 7.66 (1H, d, J = 15.7 Hz), 8.20 (1H, br s), 9.04 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{34}H_{40}N_5O_4S$ ($[M+H]^+$): 614.2801. Found: 614.2783.

5.2.28. *tert*-Butyl (2*E*)-3-[8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(4-{[(2,2-difluoroethyl)(methyl)amino]methyl}phenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylate (3o). Following the procedures as described for **3l**, the title compound (249 mg) was prepared from **5a** (250 mg, 0.401 mmol) and **6o** as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.32 (9H, s), 1.53 (9H, s), 2.36 (3H, s), 2.81 (2H, dt, J = 4.4, 14.9 Hz), 3.63 (2H, s), 5.88 (1H, tt, J = 4.4, 56.0 Hz), 6.43 (1H, s), 7.36 (2H, d, J = 7.9 Hz), 7.39 (1H, d, J = 15.6 Hz), 7.49 (1H, d, J = 7.9 Hz), 7.59 (1H, dd, J = 1.7, 7.3 Hz), 7.65 (1H, d, J = 15.6 Hz), 8.20 (1H, d, J = 1.2 Hz), 9.01 (1H, d, J = 7.3 Hz), 11.10 (1H, br). HRMS (FAB) Calcd for $C_{33}H_{38}F_2N_5O_4S$ ($[M+H]^+$): 638.2613. Found: 638.2597.

5.2.29. *tert*-Butyl (2*E*)-3-(2-(4-{[(2-*tert*-butoxy-2-oxoethyl)(methyl)amino]methyl}phenyl)-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (3p). Following the procedures as described for **3n**, the title compound (220 mg) was prepared from **5a** (200 mg, 0.321 mmol) and **6p** (175 mg, 0.963 mmol) as a yellow oil. ^{1}H NMR (CD_3OD) δ: 1.33 (9H, s), 1.44 (9H, s), 1.50 (9H, s), 2.39 (3H, s), 3.21 (2H, s), 3.77 (2H, s), 6.64 (1H, s), 7.13 (1H, d, J = 15.6 Hz), 7.46 (1H, d, J = 15.6 Hz), 7.51 (2H, d, J = 8.3 Hz), 7.56 (2H, d, J = 8.3 Hz), 7.75 (1H, d, J = 7.3 Hz), 8.14 (1H, s), 9.07 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{37}H_{46}N_5O_6S$ ($[M+H]^+$): 688.3169. Found: 688.3157.

5.2.30. *tert*-Butyl (2*E*)-3-(2-[4-(azetidin-1-ylmethyl)phenyl]-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (3q). Following the procedures as described for **3n**, the title compound (173 mg) was prepared from **5a** (250 mg, 0.401 mmol) and **6q** (75.0 mg, 0.802 mmol) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.34 (9H, s), 1.47 (9H, s), 2.01 (2H, quintet, J = 6.9 Hz), 3.32 (4H, t, J = 6.9 Hz), 3.80 (2H, s), 6.52 (1H, s), 7.34 (1H, d, J = 15.6 Hz), 7.42 (2H, d, J = 8.0 Hz), 7.51 (2H, d, J = 8.0 Hz), 7.59 (1H, d, J = 15.6 Hz), 7.61 (1H, d, J = 7.3 Hz), 7.95 (1H, d, J = 1.2 Hz), 9.05 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{33}H_{38}N_5O_4S$ ($[M+H]^+$): 600.2645. Found: 600.2672.

5.2.31. *tert*-Butyl (2*E*)-3-{8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-2- [4-(pyrrolidin-1-ylmethyl)phenyl]-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}acrylate (3r). Following the procedures as described for **3l**, the title compound (227 mg) was prepared from **5a** (250 mg, 0.401 mmol) and **6r** (66.9 μL, 0.802 mmol) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.35 (9H, s), 1.49 (9H, s), 1.66 (4H, br), 2.60 (4H, br), 3.78 (2H, s), 6.52 (1H, s), 7.35 (1H, d, J = 15.6 Hz), 7.49 (2H, d, J = 8.1 Hz), 7.54 (2H, d, J = 8.1 Hz), 7.57 (1H, dd, J = 1.7, 7.3 Hz), 7.62 (1H, d, J = 15.6 Hz), 7.89 (1H, s), 9.04 (1H, d,

J = 7.3 Hz). HRMS (FAB) Calcd for $C_{34}H_{40}N_5O_4S$ ($[M+H]^+$): 614.2801. Found: 614.2792.

5.2.32. *tert*-Butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-{4-[(piperidin-1-yl)methyl]phenyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (3s). Following the procedures as described for **3l**, the title compound (226 mg) was prepared from **5a** (250 mg, 0.401 mmol) and **6s** (79.4 μL, 0.802 mmol) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.33 (9H, s), 1.38–1.47 (2H, m), 1.49–1.58 (4H, m), 1.52 (9H, s), 2.42 (4H, br), 3.51 (2H, s), 6.45 (1H, s), 7.37 (1H, d, J = 15.6 Hz), 7.38 (2H, d, J = 8.1 Hz), 7.47 (2H, d, J = 8.1 Hz), 7.57 (1H, dd, J = 1.7, 7.3 Hz), 7.66 (1H, d, J = 15.6 Hz), 8.10 (1H, d, J = 1.7 Hz), 9.02 (1H, d, J = 7.1Hz). HRMS (FAB) Calcd for $C_{35}H_{42}N_5O_4S$ ($[M+H]^+$): 628.2958. Found: 628.2947.

5.2.33. *tert*-Butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-{4-[(4-hydroxypiperidin-1-yl)methyl]phenyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (3t). Following the procedures as described for **3l**, the title compound (184 mg) was prepared from **5a** (200 mg, 0.321 mmol) and **6t** (163 mg, 1.61 mmol) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 9.16 (1H, d, J = 7.59 Hz), 8.24 (1H, d, J = 1.47 Hz), 7.70–7.63 (2H, m), 7.56 (2H, d, J = 8.32 Hz), 7.45 (2H, d, J = 8.08 Hz), 7.40 (1H, d, J = 15.91 Hz), 6.57 (1H, s), 3.76 (1H, s), 3.57 (2H, s), 2.80 (2H, s), 2.21 (2H, s), 1.91 (2H, s), 1.64 (2H, q, J = 9.30 Hz), 1.49 (9H, s), 1.34 (9H, s). HRMS (FAB) Calcd for $C_{35}H_{42}N_5O_5S$ ($[M+H]^+$): 644.2907. Found: 644.2932.

5.2.34. Ethyl 1-[4-(3-[(1*E*)-3-*tert*-butoxy-3-oxoprop-1-en-1-yl]-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-2-yl)benzyl]piperidine-4-carboxylate (3u). Following the procedures as described for **3l**, the title compound (47.6 mg) was prepared from **5a** (50.0 mg, 0.0802 mmol) and **6u** (57.5 mg, 0.366 mmol) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 7.10 Hz), 1.34 (9H, s), 1.49 (9H, s), 1.77–2.07 (4H, m), 2.30 (1H, m), 2.89–2.91 (2H, m), 3.55 (2H, s), 4.14 (2H, q, J = 7.18 Hz), 6.57 (1H, s), 7.41 (1H, d, J = 15.66 Hz), 7.46 (2H, d, J = 8.08 Hz), 7.57 (2H, d, J = 8.08 Hz), 7.64–7.68 (2H, m), 8.24 (1H, s), 9.17 (1H, d, J = 7.34 Hz). HRMS (FAB) Calcd for $C_{38}H_{46}N_5O_6S$ ($[M+H]^+$): 700.3169. Found: 700.3159.

5.2.35. *tert*-Butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-{4-[(4-methylpiperazin-1-yl)methyl]phenyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (3v). Following the procedures as described for **3l**, the title compound (217 mg) was prepared from **5a** (250 mg, 0.401 mmol) and **6v** (88.3 μL, 0.802 mmol) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.33 (9H, s), 1.52 (9H, s), 2.30 (3H, s), 2.48 (8H, br), 3.51 (2H, s), 6.46 (1H, s), 7.38 (2H, d, J = 8.2 Hz), 7.39 (1H, d, J = 15.6 Hz), 7.48 (2H, d, J = 8.2 Hz), 7.59 (1H, dd, J = 1.7, 7.3 Hz), 7.65 (1H, d, J = 15.6 Hz), 8.16 (1H, d, J = 1.7 Hz), 9.04 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{35}H_{43}N_6O_4S$ ($[M+H]^+$): 643.3067. Found: 643.3073.

5.2.36. *tert*-Butyl (2*E*)-3-{8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-[4-(morpholin-4-ylmethyl)phenyl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}acrylate (3w). Following the procedures as described for **3l**, the title compound (176 mg) was prepared from **5a** (300 mg, 0.481 mmol) and **6w** (83.9 μL, 0.962 mmol) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.34 (9H, s), 1.50 (9H, s), 2.50 (4H, m), 3.56 (2H, s), 3.74 (4H, t, J = 4.5 Hz), 6.56 (1H, s), 7.41 (1H, d, J = 15.6 Hz), 7.47 (2H, d, J = 8.1 Hz), 7.57 (2H, d, J = 8.1 Hz), 7.65 (1H, d, J = 15.6 Hz), 7.68 (1H, dd, J = 1.7, 7.3 Hz), 8.24 (1H, s), 9.16 (1H, d, J = 7.6 Hz). HRMS (FAB) Calcd for $C_{34}H_{40}N_5O_5S$ ($[M+H]^+$): 630.2750. Found: 630.2778.

5.2.37. (2*E*)-3-[8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(4-{[cyclopropyl(methyl)amino]methyl}phenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylic acid (4n). Following the procedures as described for **4i**, the title compound (151 mg) was prepared from **3n** (176 mg, 0.287 mmol) as an orange solid. Mp: 227–232 °C (dec). IR (ATR) cm^{-1}: 2962, 2871, 2501, 1691, 1643, 1565, 1494, 1446, 1419. ^{1}H NMR (DMSO-d_6) δ: 0.70–0.85 (3H, m), 0.94–1.05 (1H, m) 1.31 (9H, s), 2.85 (3H, d, J = 3.9 Hz), 2.82–2.95 (1H, m), 3.62–3.75 (1H, m), 4.54 (2H, s), 6.90 (1H, s), 7.27 (1H, d, J = 15.6 Hz), 7.44 (1H, d, J = 15.6 Hz), 7.67 (2H, d, J = 8.2 Hz), 7.78 (2H, d, J = 8.2 Hz), 7.94 (1H, dd, J = 7.3, 1.5 Hz), 8.45 (1H, br s), 9.20 (1H, d, J = 7.3 Hz), 10.21 (1H, br s). Anal. Calcd for $C_{30}H_{31}N_5O_4S{\cdot}HCl{\cdot}1.9H_2O$: C, 54.20; H, 5.58; N, 10.53. Found: C, 54.70; H, 5.84; N, 10.00.

5.2.38. (2*E*)-3-[8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(4-{[(2,2-difluoroethyl)(methyl)amino]methyl}phenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylic acid (4o). Following the procedures as described for **4i**, the title compound (229 mg) was prepared from **3o** (249 mg, 0.390 mmol) as an orange solid. Mp: 253–256 °C (dec). IR (ATR) cm^{-1}: 3032, 2966, 2873, 1965, 1603, 1562, 1493, 1448, 1417, 1358, 1323, 1282, 1194, 1136, 1090, 1053. ^{1}H NMR (DMSO-d_6) δ: 1.31 (9H, s), 2.80 (2H, s), 4.48 (2H, br), 6.65 (1H, br t, J = 53.0 Hz), 6.89 (1H, s), 7.29 (1H, d, J = 15.6 Hz), 7.46 (1H, d, J = 15.6 Hz), 7.70 (2H, d, J = 8.3 Hz), 7.75–7.82 (2H, m), 7.94 (1H, dd, J = 1.7, 7.3 Hz), 8.42 (1H, s), 9.20 (1H, d, J = 7.6 Hz). Anal. Calcd for $C_{29}H_{29}F_2N_5O_4S{\cdot}1.6HCl{\cdot}1.4H_2O$: C, 52.36; H, 5.06; N, 10.53; Cl, 8.53; F, 5.71; S, 4.82. Found: C, 52.30; H, 4.80; N, 10.46; Cl, 8.37; F, 5.53; S, 4.89.

5.2.39. (2*E*)-3-[8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(4-{[(carboxymethyl)(methyl)amino]methyl}-phenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylic acid (4p). Following the procedures as described for **4i**, the title compound (188 mg) was prepared from **3p** (220 mg, 0.319 mmol) as an orange solid. Mp: 204–220 °C (dec). IR (ATR) cm^{-1}: 2962, 2873, 1724, 1685, 1565, 1496, 1446. ^{1}H NMR (DMSO-d_6) δ: 1.31 (9H, s), 2.85 (3H, s), 3.60–3.75 (2H, m), 4.17 (2H, s), 6.90 (1H, s), 7.31 (1H, d, J = 15.6 Hz), 7.47 (1H, d, J = 15.6 Hz), 7.72 (2H, d, J = 8.6 Hz), 7.75 (2H, d, J = 8.6 Hz), 7.95 (1H, d, J = 7.2 Hz), 8.42 (1H, s), 9.20 (1H, d, J = 7.2 Hz). Anal. Calcd for $C_{29}H_{29}N_5O_6S{\cdot}$

2.2HCl·1.9H_2O: C, 50.47; H, 5.11; N, 10.15. Found: C, 51.19; H, 5.31; N, 9.37.

5.2.40. (2*E*)-3-(2-[4-(Azetidin-1-ylmethyl)phenyl]-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (4q). Following the procedures as described for **4i**, the title compound (139 mg) was prepared from **3q** (173 mg, 0.289 mmol) as an orange solid. Mp: 271–274 °C (dec). IR (ATR) cm^{-1}: 2968, 2787, 2636, 2434, 1695, 1637, 1603, 1547, 1493, 1448, 1415, 1358, 1321, 1281, 1201, 1140, 1099, 1057. ^1H NMR (DMSO-d_6) δ: 1.31 (9H, s), 2.31–2.45 (2H, m), 4.00–4.19 (4H, m), 4.48 (2H, d, J = 6.1 Hz), 7.29 (1H, d, J = 15.5 Hz), 7.45 (1H, d, J = 15.5 Hz), 7.67 (2H, d, J = 8.3 Hz), 7.70 (2H, d, J = 8.3 Hz), 7.94 (1H, d, J = 8.3 Hz), 8.42 (1H, s), 9.20 (1H, d, J = 7.6 Hz), 10.7 (1H, br). Anal. Calcd for $C_{29}H_{29}N_5O_4S$·1.5HCl·1.7H_2O: C, 55.38; H, 5.43; N, 11.13; S, 5.10; Cl, 8.46. Found: C, 55.30; H, 5.24; N, 11.04; S, 5.17; Cl, 8.32.

5.2.41. (2*E*)-3-{8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-2-[4-(pyrrolidin-1-ylmethyl)phenyl]-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}acrylic acid (4i). Following the procedures as described for **4b**, the title compound (196 mg) was prepared from **3r** (227 mg, 0.370 mmol) as an orange solid. Mp: 219–226 °C (dec). IR (ATR) cm^{-1}: 2964, 2873, 2488, 1682, 1601, 1568, 1495, 1446, 1406, 1360, 1325, 1290, 1267, 1207, 1149, 1099, 1057. ^1H NMR (DMSO-d_6) δ: 1.30 (9H, s), 1.84–2.11 (4H, m), 3.06–3.17 (2H, m), 3.32–3.40 (2H, m), 4.46 (1H, d, J = 5.9 Hz), 6.88 (1H, s), 7.27 (1H, d, J = 15.6 Hz), 7.45 (1H, d, J = 15.6 Hz), 7.67, (2H, d, J = 8.0 Hz), 7.79 (2H, d, J = 8.0 Hz), 7.92 (1H, dd, J = 1.7, 7.6 Hz), 8.41 (1H, s), 9.18 (1H, d, J = 7.6 Hz), 10.8 (1H, br). Anal. Calcd for $C_{30}H_{31}N_5O_4S$·1.5HCl·2H_2O: C, 56.84; H, 5.55; N, 11.05; S, 5.06; Cl, 8.39. Found: C, 56.91; H, 5.60; N, 10.81; S, 5.03; Cl, 8.35.

5.2.42. (2*E*)-3-{8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-2-[4-(piperidin-1-ylmethyl)phenyl]-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}acrylic acid (4s). Following the procedures as described for **4i**, the title compound (207 mg) was prepared from **3s** (226 mg, 0.361 mmol) as an orange solid. Mp: 234–243 °C (dec). IR (ATR) cm^{-1}: 2966, 2871, 2650, 2557, 1699, 1635, 1604, 1547, 1493, 1446, 1415, 1354, 1323, 1282, 1194, 1149, 1092, 1055. ^1H NMR (DMSO-d_6) δ: 1.25–1.46 (2H, m), 1.30 (9H, s), 1.65–1.88 (6H, m), 2.85–2.98 (2H, m), 4.38 (2H, d, J = 4.9 Hz), 6.88 (1H, s), 7.26 (1H, dd, J = 1.2, 15.5 Hz), 7.42 (1H, dd, J = 1.2, 15.5 Hz), 7.67 (2H, d, J = 7.7 Hz), 7.78 (2H, d, J = 7.7 Hz), 7.92 (1H, d, J = 7.6 Hz), 8.39 (1H, s), 9.18 (1H, d, J = 7.3 Hz), 10.26 (1H, br). Anal. Calcd for $C_{31}H_{33}N_5O_4S$·1.5HCl·0.5H_2O: C, 58.60; H, 5.63; N, 11.02; S, 5.05; Cl. 8.37. Found: C, 58.48; H, 5.63; N, 10.84; S, 4.98; Cl, 8.54.

5.2.43. (2*E*)-3-(8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-{4-[(4-hydroxypiperidin-1-yl)methyl]phenyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (4t). Following the procedures as described for **4i**, the title compound (88.0 mg) was prepared from **3t** (184 mg, 0.286 mmol) as a yellow solid. Mp: 274 °C. IR (ATR) cm^{-1}: 1697, 1604, 1552, 1493, 1448, 1311, 1053. ^1H NMR (DMSO-d_6) δ: 1.31 (9H, s), 1.45–1.43 (2H, m), 1.75–1.73 (2H, m), 2.14–2.11 (2H, m), 2.73 (2H, br s), 3.49 (1H, br s), 3.58 (2H, s), 4.56 (1H, s), 6.85 (1H, s), 7.28 (1H, d, J = 15.62 Hz), 7.56–7.52 (5H, m), 7.92 (1H, dd, J = 7.45, 1.83 Hz), 8.42 (1H, s), 9.17 (1H, d, J = 7.57 Hz). Anal. Calcd for $C_{31}H_{33}N_5O_5S$·1.5HCl·0.25H_2O: C, 56.00; H, 5.61; N, 10.53. Found: C, 56.38; H, 5.24; N, 10.13.

5.2.44. 1-(4-{8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-3-[(*E*)-2-carboxyvinyl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-2-yl}benzyl)piperidine-4-carboxylic acid (4u). To a solution of **3u** (45.0 mg, 0.0643 mmol) in THF (10 mL) was added a solution of lithium hydroxide monohydrate (8.00 mg, 0.191 mmol) in H_2O (5 mL) and stirred for 16 h. To the mixture was added lithium hydroxide monohydrate (13.4 mg, 0.319 mmol) and stirred for 6 h. The mixture was acidified with 1 N HCl and concentrated. The residue was purified by PTLC ($CHCl_3$/MeOH/H_2O = 7:3:1, organic phase) to afford 1-[4-(3-[(1*E*)-3-*tert*-butoxy-3-oxoprop-1-en-1-yl]-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-2-yl)benzyl]piperidine-4-carboxylic acid (39.3 mg, 91%) as a yellow solid. Following the procedures as described for **4b**, the title compound (127 mg) was prepared from 1-[4-(3-[(1*E*)-3-*tert*-butoxy-3-oxoprop-1-en-1-yl]-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-2-yl)benzyl]piperidine-4-carboxylic acid (125 mg, 0.186 mmol) as a yellow solid. Mp: 121–127 °C. IR (ATR) cm^{-1}: 2966, 1668, 1566, 1502, 1448, 1414, 1290, 1182, 1134, 1061. ^1H NMR (CD_3OD) δ: 1.35 (9H, s), 1.86–1.99 (2H, m), 2.27–2.31 (2H, m), 2.65–2.69 (1H, m), 3.10–3.16 (2H, m), 3.60–3.65 (2H, m), 4.45 (2H, s), 6.76 (1H, br s), 7.35 (1H, br s), 7.50–7.58 (1H, m), 7.72 (2H, d, J = 8.2 Hz), 7.77 (2H, d, J = 8.2 Hz), 7.94 (1H, br s), 8.33 (1H, br s), 9.26 (1H, s, br s). Anal. Calcd for $C_{32}H_{33}N_5O_6S$·1.5TFA·1.5H_2O: C, 51.66; H, 4.64; N, 8.61. Found: C, 51.57; H, 4.59; N, 8.46.

5.2.45. (2*E*)-3-(8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-{4-[(4-methylpiperazin-1-yl)methyl]phenyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (4v). Following the procedures as described for **4i**, the title compound (204 mg) was prepared from **3v** (217 mg, 0.337 mmol) as an orange solid. Mp: 209–214 °C (dec). IR (ATR) cm^{-1}: 2964, 2436, 1685, 1637, 1603, 1560, 1495, 1446, 1414, 1360, 1321, 1279, 1178, 1151, 1099, 1059, 1016. ^1H NMR (DMSO-d_6) δ: 1.32 (9H, s), 2.51 (4H, m), 2.84 (3H, s), 3.65 (4H, m), 4.47 (2H, br), 6.90 (1H, s), 7.30 (1H, d, J = 15.6 Hz), 7.48 (1H, d, J = 15.6 Hz), 7.69 (2H, d, J = 7.7 Hz), 7.85 (2H, d, J = 7.7 Hz), 7.94 (1H, dd, J = 1.7, 7.6 Hz), 8.42 (1H, s), 9.20 (1H, d, J = 7.3 Hz). Anal. Calcd for $C_{31}H_{34}N_6O_4S$·2.3HCl·2.1H_2O: C, 52.56; H, 5.76; N, 11.86; S, 4.53; Cl, 11.51. Found: C, 52.53; H, 5.78; N, 11.76; S, 4.63; Cl, 11.58.

5.2.46. (2*E*)-3-{8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-[4-(morpholin-4-ylmethyl)phenyl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}acrylic acid (4w). Following the procedures as described for **4i**, the title compound (153 mg) was prepared from **3w** (176 mg, 0.280 mmol)

as an orange solid. Mp: 269–282 °C. IR (ATR) cm^{-1}: 3109, 2964, 2873, 2611, 2569, 2480, 1697, 1635, 1603, 1562, 1493, 1446, 1415, 1358, 1323, 1302, 1188, 1151, 1128, 1084, 1055. ^1H NMR (DMSO-d_6) δ: 1.31 (9H, s), 3.11–3.37 (4H, m), 3.71–3.82 (2H, m), 3.94–4.02 (2H, m), 4.48 (2H, d, J = 4.4 Hz), 6.90 (1H, s), 7.30 (1H, d, J = 15.6 Hz), 7.47 (1H, d, J = 15.6 Hz), 7.71 (2H, d, J = 8.2 Hz), 7.80 (2H, d, J = 8.2 Hz), 7.95 (1H, dd, J = 1.7, 7.5 Hz), 8.41 (1H, s), 9.20 (1H, d, J = 7.6 Hz), 10.82 (1H, br). Anal. Calcd for $C_{30}H_{31}N_5O_5S{\cdot}1.5HCl{\cdot}1.4H_2O$: C, 55.13; H, 5.44; N, 10.72; S, 4.91; Cl, 8.14. Found: C, 55.13; H, 5.15; N, 10.68; S, 5.00; Cl, 7.97.

5.2.47. *N*-Cyclopropyl-*N*-methylamine hydrochloride (6n). To a solution of cyclopropylamine (2.65 g, 46.4 mmol) and Et_3N (9.71 mL, 69.9 mmol) in THF (100 mL) was added dropwise benzyl bromide (6.62 mL, 55.7 mmol) and stirred for 17 h. The mixture was diluted with satd $NaHCO_3$ aq and extracted with EtOAc. The organic layer was washed with brine, dried, and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3 \rightarrow CHCl_3$/MeOH = 50:1) to afford *N*-benzyl-*N*-cyclopropylamine (3.27 g) as a colorless oil. To a solution of *N*-benzyl-*N*-cyclopropylamine (3.27 g, 22.2 mmol) in MeOH (60 mL) was added dropwise formalin (1.43 mL, 18.9 mmol) at 0 °C. To the mixture was added $NaBH_3CN$ (1.01 g, 15.2 mmol) and stirred for 20 min at 0 °C. After the mixture was stirred for 19 h, it was diluted with satd $NaHCO_3$ aq, and MeOH was removed by concentration. The residue was extracted with $CHCl_3$ and the combined organic layers were washed with brine, dried, and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3 \rightarrow CHCl_3$/MeOH = 98:1) to afford *N*-benzyl-*N*-cyclopropyl-*N*-methylamine (1.80 g) as a colorless oil. To a solution of *N*-benzyl-*N*-cyclopropyl-*N*-methylamine (1.80 g, 11.2 mmol) in MeOH (50 mL) was added 5% Pd-C (0.18 g, containing 50% water), and the mixture was stirred under atmospheric pressure of hydrogen for 16 h. The catalyst was filtered off and washed with MeOH. The combined filtrate and washings were concentrated. To the residue was added 4 N HCl–dioxane (20 mL) and concentrated to afford **6n** (1.18 g) as a pale yellow oil. ^1H NMR (CD_3OD) δ: 0.80–0.94 (4H, m), 2.70–2.80 (1H, br s), 2.77 (3H, s), 3.55–3.77 (1H, m).

5.2.48. *N*-(2,2-Difluoroethyl)-*N*-methylamine (6o). To a solution of *N*-methylbenzylamine (1.00 mL, 7.76 mmol) in THF (5 mL) was added 2,2-difluoroethyl trifluoromethanesulfonate[13] (830 mg, 3.88 mmol) and stirred for 21 h. To the mixture were added Et_3N (2.16 mL, 15.5 mmol) and a solution of 2,2-difluoroethyl trifluoromethanesulfonate (830 mg, 3.88 mmol) in THF (2 mL) and stirred for 16.5 h. The mixture was concentrated and the residue was purified by silica gel column chromatography ($CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH = 95:5 → 90:10) to afford *N*-benzyl-*N*-(2,2-difluoroethyl)-*N*-methylamine (677 mg) as a pale yellow oil. To a solution of *N*-benzyl-*N*-(2,2-difluoroethyl)-*N*-methylamine (677 mg, 3.67 mmol) in MeOH (6 mL) was added 5% Pd-C (200 mg, containing 50% water), and the mixture was stirred under atmospheric pressure of hydrogen for 14.5 h. The catalyst was filtered off and washed with MeOH to afford a solution of **6o** in MeOH (25 mL, ca. 0.146 mol/L). This compound was utilized for the next reaction without further purification.

5.2.49. *tert*-Butyl (2*E*)-3-(2-{4-[(4-allylpiperazin-1-yl)methyl]phenyl}-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (9). Following the procedures as described for **3l**, the title compound (34.0 mg) was prepared from **5a** (50.0 mg, 0.0802 mmol) and 1-allylpiperazine (46.7 μL, 0.321 mmol) as a yellow solid. ^1H NMR ($CDCl_3$) δ: 1.32 (9H, s), 1.52 (9H, s), 2.51 (8H, br), 3.01 (2H, d, J = 6.6 Hz), 3.51 (2H, s), 5.11–5.24 (2H, m), 5.87 (1H, ddt, 17.1, 10.2, 6.6 Hz), 6.44 (1H, s), 7.37 (2H, d, J = 7.7 Hz), 7.38 (1H, d, J = 15.6 Hz), 7.47 (2H, d, J = 7.7 Hz), 7.58 (1H, d, J = 6.8 Hz), 7.65 (1H, d, J = 15.6 Hz), 8.15 (1H, s), 9.02 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{37}H_{45}N_6O_4S$ ($[M+H]^+$): 669.3223. Found: 669.3244.

5.2.50. *tert*-Butyl (2*E*)-3-{8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-2-[4-(piperazin-1-ylmethyl)-phenyl]-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}acrylate (10). To a solution of **9** (185 mg, 0.276 mmol) in CH_2Cl_2 (4 mL) were added *N*,*N*′-dimethylbarbituric acid (129 mg, 0.829 mmol) and $Pd(PPh_3)_4$ (16.0 mg, 13.8 μmol), and stirred for 4 h under Ar. The mixture was concentrated, and the residue was diluted with EtOAc and washed with satd Na_2CO_3 aq (3×). The combined organic layers were dried and concentrated to afford **10** (210 mg) as inseparable mixture, which was utilized for the next reaction without further purification.

5.2.51. *tert*-Butyl (2i)-3-[8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(4-{[4-(2-hydroxyethyl)piperazin-1-yl]methyl}phenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylate (3x). To a solution of crude **10** (210 mg) in THF (5 mL) were added Et_3N (154 μL, 1.11 mmol) and 2-bromoethanol (39.2 μL, 0.553 mmol) and stirred for 14 h. After the mixture was further stirred for 9.5 h at 50 °C, Et_3N (154 μL, 1.11 mmol) and 2-bromoethanol (39.2 μL, 0.553 mmol) were added and stirred for 13 h at 50 °C. The mixture was concentrated, and the residue was diluted with satd $NaHCO_3$ aq and extracted with EtOAc (3×). The combined organic layers were dried and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 99:1 → 95:5) to afford **3x** (129 mg) as a yellow film. ^1H NMR ($CDCl_3$) δ: 1.33 (9H, s), 1.50 (9H, s), 2.42–2.62 (8H, m), 2.57 (2H, t, J = 5.4 Hz), 3.53 (2H, s), 3.65 (2H, t, J = 5.4 Hz), 6.51 (1H, s), 7.395 (1H, d, J = 15.6 Hz), 7.404 (2H, d, J = 8.1 Hz), 7.52 (2H, d, J = 8.1 Hz), 7.62–7.66 (1H, m), 7.65 (1H, d, J = 15.6 Hz), 8.21–8.24 (1H, m), 9.10 (1H, d, J = 7.6 Hz). HRMS (FAB) Calcd for $C_{36}H_{45}N_6O_5S$ ($[M+H]^+$): 673.3172. Found: 673.3185.

5.2.52. *tert*-Butyl (2*E*)-3-(2-(4-{[4-(2-*tert*-butoxy-2-oxoethyl)piperazin-1-yl]methyl}phenyl)- 8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (3y). To a solution of crude **10** (211 mg) in THF (5 mL) were added Et_3N (150 μL, 1.08 mmol) and bromoacetic acid *tert*-butyl ester (79.5 μL, 0.538 mmol) and stirred for 13 h. The mixture was concentrated, and the residue was diluted with satd $NaHCO_3$ aq and extracted with EtOAc. The organic layer was dried and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 10:1) to afford **3y** (183 mg) as a yellow oil. ^1H NMR ($CDCl_3$) δ: 1.33 (9H, s), 1.46 (9H, s), 1.50 (9H, s), 2.53 (4H, br), 2.60 (4H, br), 3.14 (2H, s), 3.52 (2H, s), 6.50 (1H, s), 7.391 (1H, d, J = 15.7 Hz), 7.394 (2H, d, J = 8.2 Hz), 7.50 (2H, d, J = 8.2 Hz), 7.64 (1H, dd, J = 1.7, 7.3 Hz), 7.65 (1H, d, J = 15.7 Hz), 8.22 (1H, d, J = 1.7 Hz), 9.09 (1H, d, J = 7.3 Hz). HRMS (FAB) Calcd for $C_{40}H_{51}N_6O_6S$ ($[M+H]^+$): 743.3591. Found: 743.3628.

5.2.53. (2*E*)-3-[8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(4-{[4-(2-hydroxyethyl)piperazin-1-yl]methyl}phenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylic acid (4x). Following the procedures as described for **4i**, the title compound (105 mg) was prepared from **3x** (129 mg, 0.191 mmol) as an orange solid. Mp: 209–216 °C. IR (ATR) cm^{-1}: 2966, 1691, 1637, 1603, 1568, 1495, 1446, 1414, 1360, 1327, 1277, 1207, 1176, 1151, 1097, 1059. ^1H NMR (DMSO-d_6) δ: 1.32 (9H, s), 3.02–4.06 (14H, m), 6.90 (1H, s), 7.30 (1H, d, J = 15.7 Hz), 1.49 (1H, d, J = 15.7 Hz), 7.69 (2H, d, J = 7.6 Hz), 7.80 (2H, br), 7.94 (1H, d, J = 7.1 Hz), 8.41 (1H, s), 9.20 (1H, d, J = 7.6 Hz). Anal. Calcd for $C_{32}H_{36}N_6O_5S{\cdot}2.5HCl{\cdot}2.5H_2O$: C, 51.05; H, 5.82; N, 11.16; S, 4.26; Cl; 11.77. Found: C, 51.04; H, 5.66; N, 10.99; S, 4.28; Cl; 11.63.

5.2.54. (2*E*)-3-[8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-(4-{[4-(carboxymethyl)piperazin-1-yl]methyl}phenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylic acid (4y). Following the procedures as described for **4i**, the title compound (164 mg) was prepared from **3y** (191 mg, 0.257 mmol) as an orange solid. Mp: 212–222 °C (dec). IR (ATR) cm^{-1}: 3014, 2978, 2936, 2536, 2441, 1718, 1685, 1651, 1583, 1558, 1498, 1444, 1429, 1373, 1344, 1277, 1230, 1196, 1169, 1099, 1061, 1030. ^1H NMR (DMSO-d_6) δ: 1.30 (9H, s), 3.32–3.80 (8H, m), 4.07 (2H, s), 4.47 (2H, s), 6.88 (1H, s), 7.28 (1H, d, J = 15.6 Hz), 7.46 (1H, d, J = 15.6 Hz), 7.69 (2H, d, J = 8.2 Hz), 7.82 (2H, d, J = 8.2 Hz), 7.93 (1H, dd, J = 2.0, 7.3 Hz), 8.38–8.40 (1H, m), 9.18 (1H, d, J = 7.3 Hz). Anal. Calcd for $C_{32}H_{34}N_6O_6S{\cdot}3.5HCl{\cdot}2.5H_2O$: C, 47.84; H, 5.33; N, 10.46; S, 3.99; Cl, 15.45. Found: C, 47.94; H, 5.17; N, 10.26; S, 3.94; Cl, 15.54.

5.3. In vitro potentiation activity

MIC assays against *P. aeruginosa* utilized Mueller–Hinton broth, following the broth microdilution methodology outlined by the National Committee for Clinical Laboratory Standards (NCCLS). Bacteria were inoculated at 1×10^6 CFU/mL and incubated at 37 °C for 18 h. MICs were determined by visual observation of growth.

5.4. In vivo efficacy

Pulmonary infection of SD rats by *P. aeruginosa* PAM1020 was instilled by intratracheal inoculation of bacteria enmeshed in agar beads. Two hours after bacterial challenge, rats were treated with AZT (1000 mg/kg/2 h) or AZT+ABS-EPI (ABS-EPI were dosed 10 mg/kg/2 h) by intravenous drip infusion.

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References and notes

1. Zhanel, G. G.; Hoban, D. J.; Schurek, K.; Karlowsky, J. A. *Int. J. Antimicrob. Agents* **2004**, *24*, 529.
2. Poole, K. *J. Antimicrob. Chemother.* **2005**, *56*, 20.
3. Kumar, A.; Schweizer, H. P. *Adv. Drug Delivery Rev.* **2005**, *57*, 1486.
4. Poole, K.; Krebes, K.; McNally, C.; Neshat, S. *J. Bacteriol.* **1993**, *175*, 7363.
5. Nakayama, K.; Ishida, Y.; Ohtsuka, M.; Kawato, H.; Yoshida, K.; Yokomizo, Y.; Hosono, S.; Ohta, T.; Hoshino, K.; Ishida, H.; Yoshida, K.; Renau, T. E.; Léger, R.; Zhang, J. Z.; Lee, V. J.; Watkins, W. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4201.
6. Nakayama, K.; Ishida, Y.; Ohtsuka, M.; Kawato, H.; Yoshida, K.; Yokomizo, Y.; Ohta, T.; Hoshino, K.; Otani, T.; Kurosaka, Y.; Yoshida, K.; Ishida, H.; Lee, V. J.; Renau, T. E.; Watkins, W. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4205.
7. Nakayama, K.; Kawato, H.; Watanabe, J.; Ohtsuka, M.; Yoshida, K.; Yokomizo, Y.; Sakamoto, A.; Kuru, N.; Ohta, T.; Hoshino, K.; Yoshida, K.; Ishida, H.; Cho, A.; Palme, M. H.; Zhang, J. Z.; Lee, V. J.; Watkins, W. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 475.
8. Nakayama, K.; Kuru, N.; Ohtsuka, M.; Yokomizo, Y.; Sakamoto, A.; Kawato, H.; Yoshida, K.; Ohta, T.; Hoshino, K.; Akimoto, K.; Itoh, J.; Ishida, H.; Cho, A.; Palme, M. H.; Zhang, J. Z.; Lee, V. J.; Watkins, W. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2493.
9. Yoshida, K.; Nakayama, K.; Kuru, N.; Koayashi, S.; Ohtsuka, M.; Takemura, M.; Hoshino, K.; Kanda, H.; Zhang, J. Z.; Lee, V. J.; Watkins, W. J. *Bioorg. Med. Chem.* **2006**, *14*, 1993.
10. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
11. Lomovskaya, O.; Warren, M. S.; Lee, A.; Galazzo, J.; Fronko, R.; Lee, M.; Blais, J.; Cho, D.; Chamberland, S.; Renau, T.; Leger, R.; Hecker, S.; Watkins, W.; Hoshino, K.; Ishida, H.; Lee, V. *Antimicrob. Agents Chemother.* **2001**, *45*, 105.
12. Lomovskaya, O.; Lee, A.; Hoshino, K.; Ishida, H.; Mistry, A.; Warren, M. S.; Boyer, E.; Chamberland, S.; Lee, V. J. *Antimicrob. Agents Chemother.* **1999**, *43*, 1340.
13. Reifenrath, W. G.; Roche, E. B.; Al-Turk, W. A.; Johnson, H. L. *J. Med. Chem.* **1980**, *23*, 985.