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MexAB-OprM specific efflux pump inhibitors in *Pseudomonas aeruginosa*. Part 5: Carbon-substituted analogues at the C-2 position

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Abstract—A series of 4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine derivatives, derivatized at the 2-position with carbon-linked substituents, were synthesized 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*. Palladium-catalyzed cross-coupling methods were applied for the incorporation of aliphatic and aromatic substituents.

1. Introduction

A particularly problematic pathogen in the clinical setting is *Pseudomonas aeruginosa*, an opportunistic Gram negative bacterium characterized by intrinsic resistance to a wide variety of antimicrobial agents.[1–3] This property has been attributed both to the impermeability of the outer membrane as well as to the activity of several efflux systems of the resistance-nodulation-division (RND) superfamily, five of which have been identified to date as: MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM, and MexJK-OprM.[1–5] The resistance might be overcome by the development of efflux pump inhibitors (EPIs) that could thereby enhance or restore the potency of anti-pseudomonas agents.

We recently reported the discovery of the first example of a MexAB-OprM specific efflux pump inhibitor and the early strategy for lead optimization through the use of a pharmacophore model.[6–9] This strategy furnished pyridopyrimidine-based inhibitors possessing nitrogen-linked C-2 substituents that play a critical role for potency (Fig. 1). The pharmacophore model also inspired us to incorporate some carbon-linked C-2 substituents to compare the potency and the opportunity for the improvement of physicochemical properties (Fig. 2).

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

2. Chemistry

We envisioned that an sp^2 carbon with π-electrons would mimic the nitrogen-linked leads, and thus investigated synthetic methodology to obtain vinyl or aryl substituents at C-2. The starting materials for the cross-coupling reactions were prepared as depicted in Scheme 1.

We explored the details of several palladium-mediated cross-coupling reactions (Stille,[10–12] Suzuki–Miyaura,[13,14] etc.) as shown in Scheme 2. The conditions depicted therein were found to be optimal for each type of reaction.

Keywords: Efflux pump inhibitor; MexAB-OprM efflux pump; *Pseudomonas aeruginosa*; Drug resistance.
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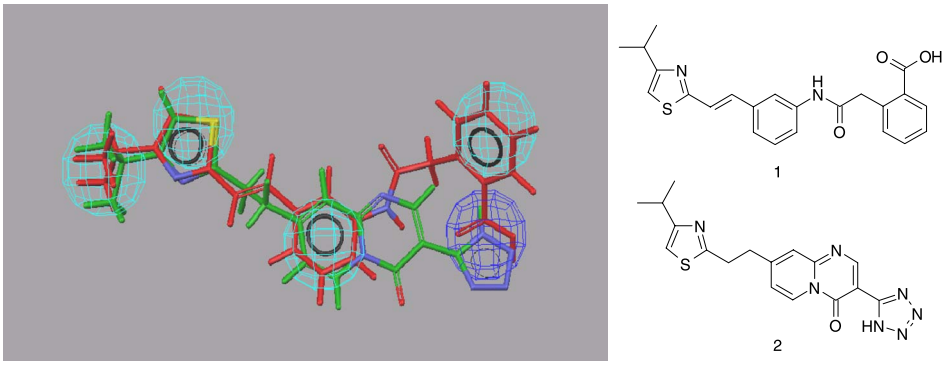


Figure 1. Structures of the first generation of MexAB-OprM specific efflux pump inhibitors and pharmacophore model generated by CATALYST™; compounds **1** (red) and **2** (green). Light blue spheres are hydrophobic sites, and the deep blue sphere represents an acidic ionizable site.

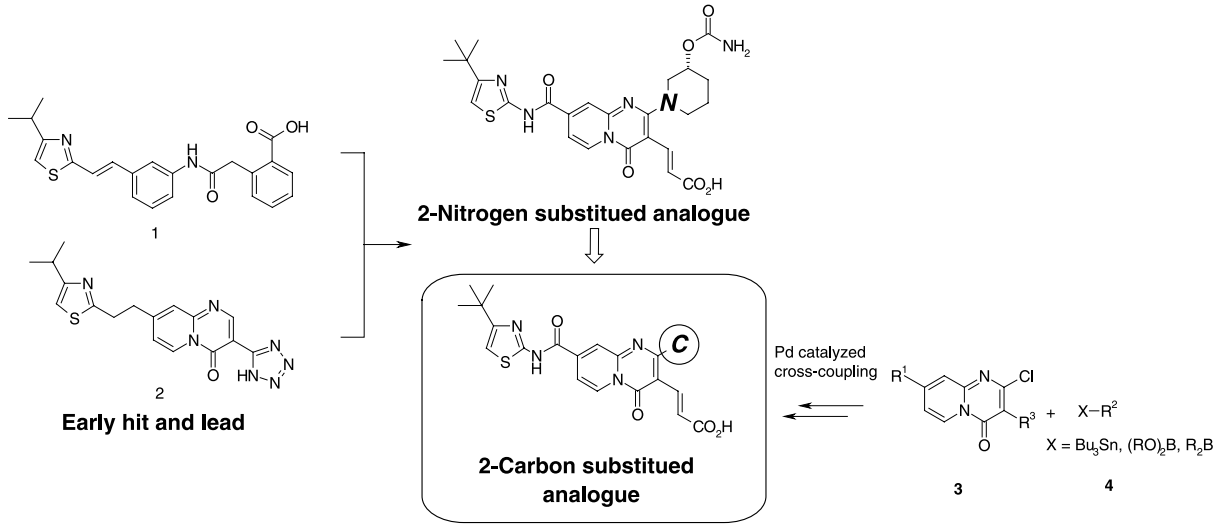
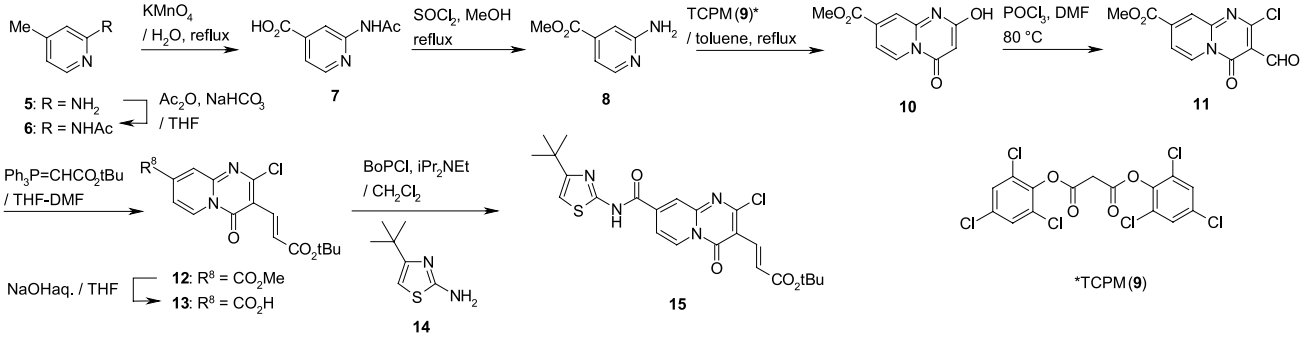
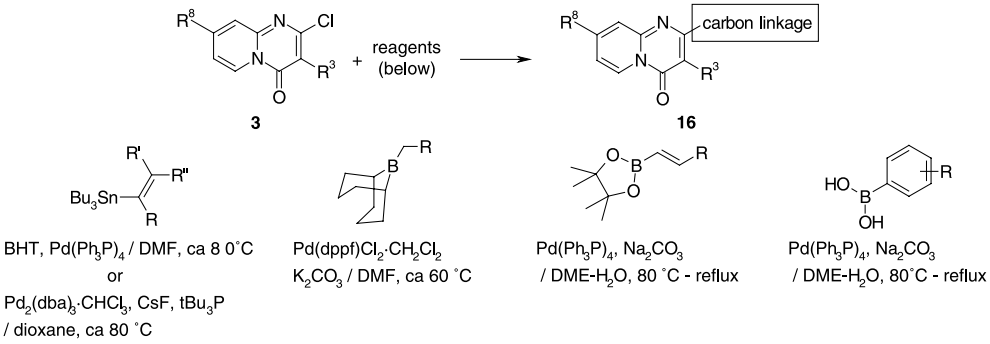


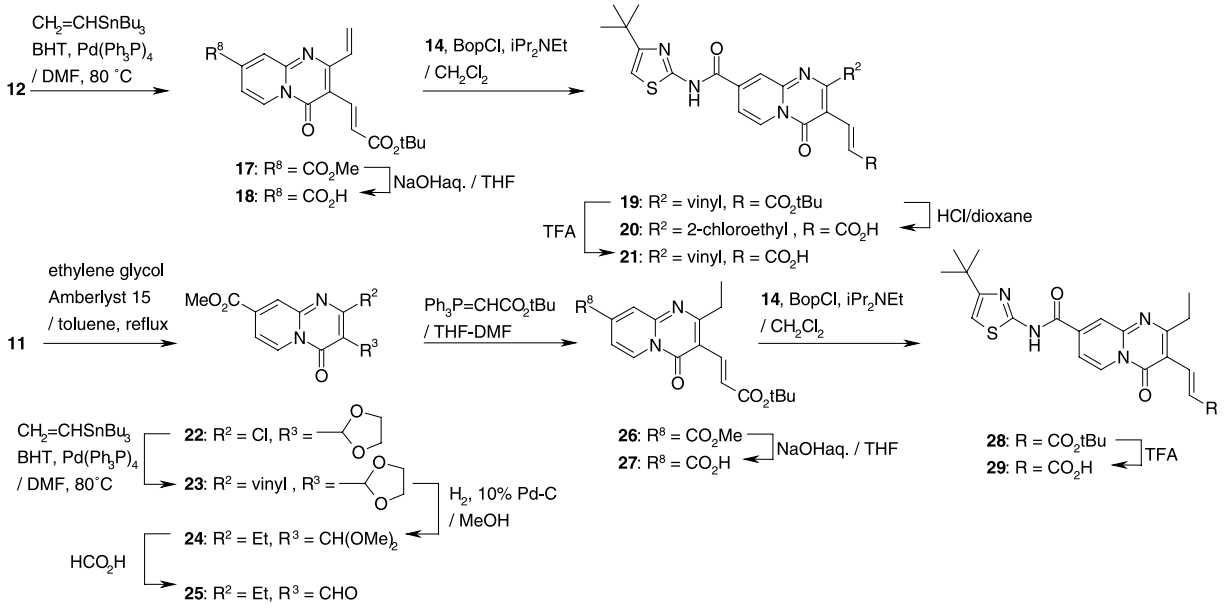
Figure 2. Novel MexAB-OprM specific efflux pump inhibitor and the design of 2-carbon-linked analogues.



Scheme 1. Preparation of ArCl for the cross-coupling reactions.



Scheme 2. Optimal reaction conditions for various reagents.

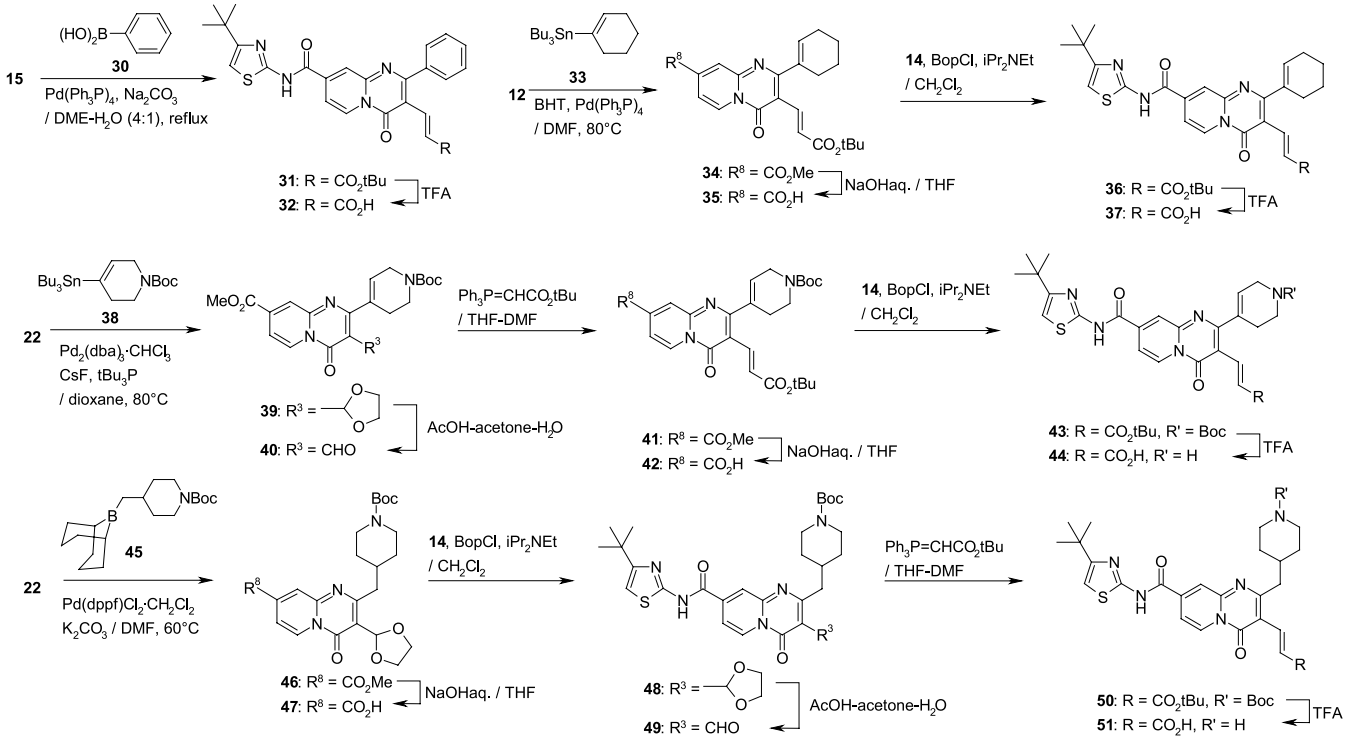


Scheme 3. Introduction of 2-carba substituent onto scaffold.

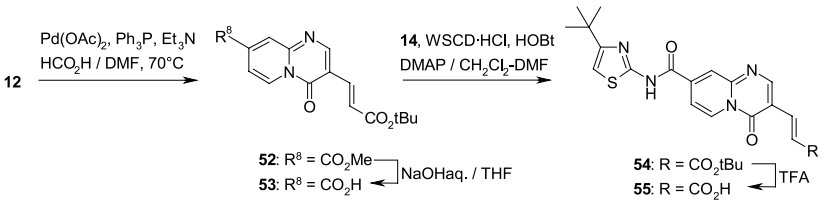
Thus, utilizing the Stille protocol, we initially generated the vinyl variant (**21**) (Scheme 3). In addition, ethyl (**29**) and 2-chloroethyl (**20**) variants were obtained via reduction and an unexpected chlorination, respectively; the final step for the deprotection of the *tert*-butyl ester using HCl afforded the latter via conjugate addition of chloride to the activated olefin.

In the case of nitrogen-linked analogues, variants containing a six-membered ring (piperidines) provided optimal potency.[8] Thus phenyl, cyclohexenyl, and 1,2,3,6-tetrahydropyridin-4-yl C-2 substituents were explored next. The syntheses of these derivatives are shown in Scheme 4. In addition to the optimized conditions for each reagent described above, the choice of substrate (**15** and **22**) was based on coupling efficiency.

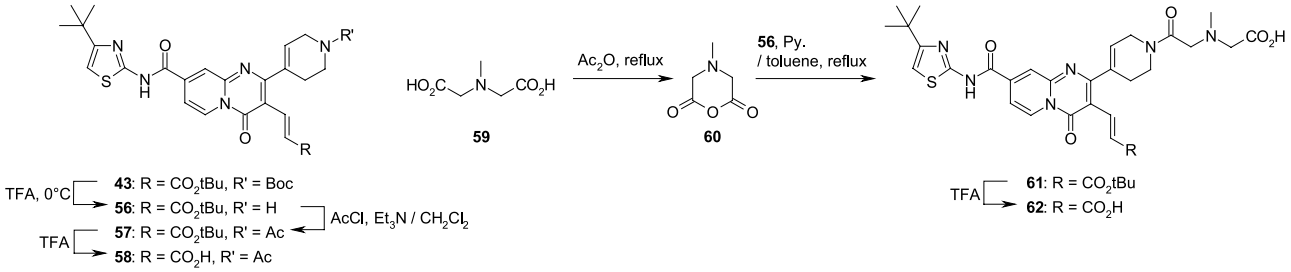
A Suzuki–Miyaura reaction, catalyzed by $Pd(Ph_3P)_4$ with Na_2CO_3 as base in DME–H_2O, smoothly introduced a phenyl group into the C-2 position of the pyridopyrimidine nucleus (for **32**). Cyclohexenyl variants (for **37**) were incorporated by Stille coupling with appropriate organostannanes, which were prepared according to the literature.[15,16] The substrate possessing a Boc-protected nitrogen (for **44**) required the more powerful coupling conditions reported by Littke and Fu[17] (i.e.,



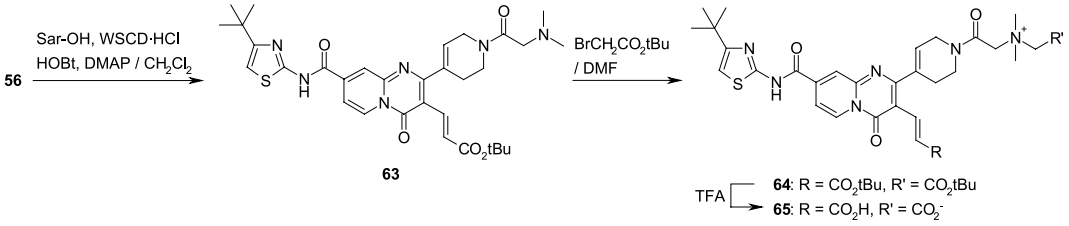
Scheme 4. Incorporation of six-membered ring at C-2 position.



Scheme 5. Synthesis of H-analogue.



Scheme 6. Functionalization on heterocycle.



Scheme 7. Water-soluble quaternary ammonium salt analogue.

$Pd_2(dba)_3{\cdot}CHCl_3$, CsF, and *t*-Bu_3P). Coupling reactions with *B*-alkyl boranes[18] (sp^3–sp^2 bond formation) were accomplished by a Suzuki–Miyaura reaction using $Pd(dppf)Cl_2$ as catalyst (for **51**). In addition, the unsubstituted (2-H) analogue **55**, which was synthesized from **12** (Scheme 5), served as useful comparator for analysis of the SAR of the novel 2-carba analogues.

In order to increase solubility, further functionalization of the tetrahydropyridine moiety by incorporation of an amino acid was also conducted. In addition, the *N*-Ac analogue **58** was synthesized as a comparator for SAR analysis (Scheme 6). Highly water-soluble derivative was synthesized by condensation of the tetrahydropyridine intermediate with sarcosine, followed by quaternization with bromoacetic acid ester and deprotection to afford ammonium analogue such as **65** (Scheme 7).

3. Results and discussion

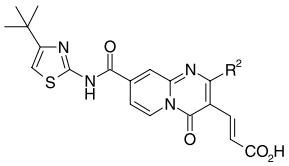
The series of 2-carbon-linked pyridopyrimidines prepared in this study was tested for in vitro potentiation of the activity of LVFX and AZT against PAM 1723,[19] a laboratory strain of *P. aeruginosa* in which the MexAB-OprM pump is over-expressed and the MexCD-OprJ and MexEF-OprN pumps are disrupted. The in vitro potencies, expressed as the minimum concentration of inhibitor required to decrease (potentiate) the MIC of the antibacterial agent 8-fold (MPC_8, μg/mL), and the 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.

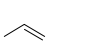
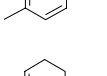
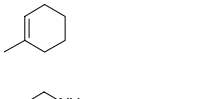
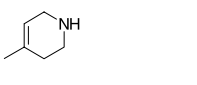
Introduction of the ethyl substituent increased the potency 4-fold by comparison with the corresponding 2-vinyl analogue or unsubstituted analogue (**29** vs **21** and **55**). The influence of the addition of HSA was somewhat greater than in the case of the 2-*N*-substituted compounds (e.g., the activity of the corresponding piperidine derivatives was reduced no more than 2-fold by the addition of HSA[9]). Introduction of the methylene-linked saturated piperidine (**51**) resulted in decreased activity. On the other hand, attachment of cyclic substituents directly at C-2 position, whether aryl or alkenyl, maintained activity (**32**, **37**, and **44**). The successful incorporation of a secondary amine (giving a zwitterionic analogue) with retention of good activity is particularly notable. Further functionalization of the nitrogen atom of the piperidine moiety successfully increased the solubility (**62**: 160 μg/mL and **65**: 1200 μg/mL at pH 6.8), although the activity was slightly compromised (**62** and **65** vs **58**).

4. Conclusions

In summary, we synthesized a series of 2-substituted pyridopyrimidine derivatives through the use of palladium-mediated cross-coupling reactions and explored

Table 1. Chemical structures, potentiation activity, and aqueous solubility of 2-carba analogues[a]



Compound	R^2	MPC_8 (LVFX) (μg/mL)		MPC_8 (AZT) (μg/mL)	Sol (pH 6.8) (μg/mL)
		Without serum	With 0.125% HSA		
55		8	32	2	2
20		4	16	0.5	1.8
21		8	32	2	2.6
29		2	8	0.5	—[b]
51		16	>32	4	37
32		4	16	0.5	2
37		1	4	1	—[b]
44		4	16	2	31
58		2	4	2	410
62		16	32	16	160
65		16	32	8	1200

[a] All compounds lacked intrinsic antibacterial activity.
[b] Not tested.

their potential as potentiators of the anti-pseudomonal activity of LVFX and AZT. Like the 2-nitrogen-linked analogues,[8,9] 2-carbon-linked variants, especially those with cyclic substituents, provided good potency. The high lipophilicity of many of these analogues limited their aqueous solubility and limited their effectiveness in the presence of HSA. Further work was stimulated by the observation that activity could be retained in more hydrophilic examples such as **44**, **62**, and **65**, and the results of these efforts 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. TCPM (**9**),[20] 1-tri-*n*-butylstannylcyclohexene (**33**),[15] 1-(*tert*-butoxycarbonyl)-4-tri-*n*-butylstannyl-1,2,3,6-tetrahydropyridine (**38**),[21] and 4-(9-borabicyclo[3.3.1]non-9-ylmethyl)-1-(*tert*-butoxycarbonyl)piperidine (**45**)[18] were prepared according to the literature procedures. Melting points were taken with 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. Methyl 2-hydroxy-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (10). To a suspension of **8** (2.77 g, 18.2 mmol) in toluene (50 mL) was added TCPM (**9**) (9.27 g, 20.2 mmol), and the mixture was refluxed for 1 h. The mixture was cooled and concentrated. The residue was diluted with Et_2O, and precipitated solid was collected by filtration, and washed with Et_2O to afford **9** (2.05 g, 51%) as a yellow solid. ^{1}H NMR (CD_3OD) δ: 4.04 (3H, s), 5.63 (1H, s), 7.64 (1H, d, J = 7.3 Hz), 8.05 (1H, s), 9.08 (1H, d, J = 7.3 Hz). HRMS (FAB) calcd for $C_{10}H_9N_2O_4$ ([M+H]$^+$): 221.0562. Found: 221.0557.

5.2.2. Methyl 2-chloro-3-formyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (11). To DMF (10 mL) was added phosphoryl chloride (1.27 mL, 13.6 mmol) at 0 °C. After the mixture was stirred at 0 °C for 40 min, a solution of **10** (1.00 g, 4.54 mmol) in DMF (10 mL) was added and stirred at 80 °C for 1 h. The mixture was cooled and concentrated in vacuo, and the residue was diluted with water and extracted with $CHCl_3$ (3×). The combined organic layers were washed with brine, dried, and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 9:1) to afford **11** (995 mg, 82%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 4.06 (3H, s), 7.86 (1H, dd, J = 1.7, 7.3 Hz), 8.34 (1H, dd, J = 0.7, 1.7 Hz), 9.25 (1H, dd, J = 0.7, 7.3 Hz), 10.45 (1H, s). HRMS (EI) Calcd for $C_{11}H_7{}^{35}ClN_2O_4$ (M$^+$): 266.0094. Found: 266.0084.

5.2.3. Methyl 3-[(1*E*)-3-*tert*-butoxy-3-oxoprop-1-en-1-yl]-2-chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (12). To a solution of **11** (995 mg, 3.73 mmol) in THF (40 mL)–DMF (10 mL) was added (*tert*-butoxycarbonylmethylene)triphenylphosphorane (2.11 g, 5.60 mmol). After stirred for 47 h, the mixture was concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1:2) to afford **12** (1.26 g, 93%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.55 (9H, s), 4.04 (3H, s), 7.40 (1H, d, J = 15.7 Hz), 7.74 (1H, dd, J = 1.8, 7.4 Hz), 7.92 (1H, d, J = 15.7 Hz), 8.27 (1H, dd, J = 0.7, 1.8 Hz), 9.13 (1H, dd, J = 0.7, 7.4 Hz). HRMS (EI) Calcd for $C_{17}H_{17}{}^{35}ClN_2O_5$ (M$^+$): 364.0826. Found: 364.0830.

5.2.4. 3-[(1*E*)-3-*tert*-Butoxy-3-oxoprop-1-en-1-yl]-2-chloro-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylic acid (13). To a solution of **12** (216 mg, 0.592 mmol) in THF (2 mL) was added 1 N NaOH aq (1.19 mL, 1.19 mmol) and stirred for 30 min. The mixture was acidified to pH 4 with 1 N HCl aq, diluted with water, and extracted with $CHCl_3$ (5×). The combined organic layers were dried and concentrated to afford **13** (194 mg, 93%) as an orange solid. ^{1}H NMR (CD_3OD) δ: 1.56 (9H, s), 7.34 (1H, d, J = 15.7 Hz), 7.84 (1H, dd, J = 1.7, 7.3 Hz), 7.89 (1H, d, J = 15.7 Hz), 8.23–8.28 (1H, m), 9.16 (1H, d, J = 7.3 Hz). HRMS (FAB) calcd for $C_{16}H_{16}{}^{35}ClN_2O_5S$ ([M+H]$^+$): 351.0748. Found: 351.0725.

5.2.5. *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 (15). To a solution of **14** (86.4 mg, 0.553 mmol) and **13** (194 mg, 0.553 mmol) in CH_2Cl_2 (5 mL) were added diisopropylethylamine (289 μL, 1.66 mmol) and *N*,*N*-bis-(2-oxo-3-oxazolidinyl)phosphinic acid chloride (211 mg, 0.829 mmol), and stirred for 12.5 h. The mixture was concentrated, and the residue was diluted with $CHCl_3$. The organic layer was washed successively with 10% citric acid aq, satd $NaHCO_3$ aq, and brine. The organic layer was dried and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$) to afford **15** (205 mg, 76%) as an orange solid. ^{1}H NMR ($CDCl_3$) δ: 1.35 (9H, s), 1.57 (9H, s), 6.51 (1H, s), 7.35 (1H, d, J = 15.7 Hz), 7.79–7.87 (1H, m), 7.83 (1H, d, J = 15.7 Hz), 8.22 (1H, d, J = 1.5 Hz), 9.02 (1H, d, J = 7.3 Hz), 11.0 (1H, br). HRMS (FAB) calcd for $C_{23}H_{26}{}^{35}ClN_4O_4S$ ([M+H]$^+$): 489.1363. Found: 489.1323.

5.2.6. Methyl 3-[(1*E*)-3-*tert*-butoxy-3-oxoprop-1-en-1-yl]-4-oxo-2-vinyl-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (17). To a solution of **12** (100 mg, 0.274 mmol) in DMF (2 mL) were added catalytic amount of 2,6-di-*tert*-butyl-*p*-cresol, tri-*n*-butylvinyltin (88.1 μL, 0.303 mmol) and $Pd(Ph_3P)_4$ (31.7 mg, 27.4 μmol), and stirred at 50 °C for 1 h and further at 80 °C for 3 h under Ar. The mixture was concentrated and the residue was purified by silica gel column chromatography (EtOAc/hexane = 1:5) to afford **17** (83.8 mg) as an inseparable mixture. This compound was utilized for the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ: 1.54 (9H, s), 4.02 (3H, s), 5.83 (1H, dd, J = 2.0, 10.8 Hz), 6.67 (1H, dd, J = 2.0, 16.6 Hz), 7.29 (1H, dd, J = 10.8, 16.6 Hz), 7.29 (1H, d, J = 15.4 Hz), 7.55 (1H, dd, J = 1.7, 7.3 Hz), 7.88 (1H, d, J = 15.4 Hz), 8.24 (1H, dd, J = 0.7, 1.7 Hz), 9.03 (1H, dd, J = 0.7, 7.3 Hz). HRMS (EI) Calcd for $C_{19}H_{20}N_2O_5$ (M$^+$): 356.1372. Found: 356.1368.

5.2.7. 3-[(1*E*)-3-*tert*-Butoxy-3-oxoprop-1-en-1-yl]-4-oxo-2-vinyl-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylic acid (18). Following the procedures as described for **13**, the title compound (220 mg) was prepared in 55% yield from **17** (418 mg, 1.17 mmol) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.54 (9H, s), 5.85 (1H, dd, J = 1.7, 10.5 Hz), 6.69 (1H, dd, J = 1.7, 16.6 Hz), 7.19–7.35 (2H, m), 7.54 (1H, dd, J = 1.7, 7.6 Hz), 7.9 (1H, d, J = 15.6 Hz), 8.31 (1H, m), 9.07 (1H, d, J = 7.6 Hz). HRMS (EI) Calcd for $C_{18}H_{18}N_2O_5$ (M$^+$): 342.1216. Found: 342.1194.

5.2.8. *tert*-Butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-2-vinyl-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-acrylate (19). Following the procedures as described for **15**, the title compound (274 mg) was prepared in 89% yield from **14** (100 mg, 0.640 mmol) and **18** (220 mg, 0.643 mmol) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ: 1.33 (9H, s), 1.55 (9H, s), 5.77–5.83 (1H, m), 6.59 (1H, s),

6.59–6.68 (1H, m), 7.15–7.29 (2H, m), 7.60 (1H, dd, $J = 1.8$, 7.3 Hz), 7.82 (1H, dd, $J = 3.9$, 15.7 Hz), 8.14 (1H, m), 9.03 (1H, dd, $J = 2.9$, 7.3 Hz), 10.33 (1H, br). HRMS (EI) Calcd for $C_{25}H_{28}N_4O_4S$ (M^+): 480.1831. Found: 480.1828.

5.2.9. (2*E*)-3-[8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]-carbonyl}-2-(2-chloroethyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylic acid (20). To 4 N HCl in 1,4-dioxane (4 mL) was added **19** (28.0 mg, 58.3 μmol) and stirred for 15 h. The mixture was concentrated, and the residue was diluted with Et_2O. Precipitated solid was collected by filtration and washed with Et_2O to afford **20** (16.7 mg, 62%) as a yellow solid. Mp: 241–250 °C (dec). IR (ATR) cm^{-1}: 3105, 2962, 2870, 1674, 1603, 1560, 1504, 1456, 1414, 1367, 1309, 1292, 1240, 1203, 1174, 1099, 1059. ^1H NMR (DMSO-d_6) δ: 1.31 (9H, s), 3.45 (2H, t, $J = 6.6$ Hz), 4.13 (2H, t, $J = 6.6$ Hz), 6.88 (1H, s), 7.28 (1H, d, $J = 15.5$ Hz), 7.75 (1H, d, $J = 15.5$ Hz), 7.88 (1H, d, $J = 7.3$ Hz), 8.44 (1H, s), 9.13 (1H, d, $J = 7.3$ Hz). Anal. Calcd for $C_{21}H_{12}N_4O_4ClS{\cdot}0.5H_2O$: C, 53.67; H, 4.72; N, 11.92; S, 6.82. Found: C, 53.40; H, 4.47; N, 11.65; S, 6.72.

5.2.10. (2*E*)-3-(8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-2-vinyl-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (21). To TFA (2 mL) was added **19** (100 mg, 0.208 mmol) and stirred for 30 min. 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 **21** (73.0 mg, 83%) as a yellow solid. Mp: 224–235 °C (dec). IR (ATR) cm^{-1}: 3111, 2962, 2908, 2870, 1678, 1599, 1564, 1504, 1446, 1367, 1311, 1292, 1240, 1201, 1176, 1101, 1059, 1032. ^1H NMR (DMSO-d_6) δ: 1.32 (9H, s), 5.90 (1H, dd, $J = 2.2$, 10.7 Hz), 6.62 (1H, dd, $J = 2.0$, 16.3 Hz), 6.88 (1H, s), 7.23 (1H, d, $J = 15.4$ Hz), 7.33 (1H, dd, $J = 10.7$, 16.3 Hz), 7.77–7.82 (1H, m), 7.86 (1H, d, $J = 15.4$ Hz), 8.43 (1H, s), 9.05 (1H, d, $J = 7.6$ Hz). Anal. Calcd for $C_{21}H_{20}N_4O_4S{\cdot}0.25H_2O$: C, 58.80; H, 4.82; N, 13.06; S, 7.48. Found: C, 58.78; H, 4.70; N, 12.98; S, 7.53.

5.2.11. Methyl 2-chloro-3-(1,3-dioxolan-2-yl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (22). To a suspension of **11** (1.16 g, 4.19 mmol) in toluene (20 mL) were added ethylene glycol (5 mL) and Amberlyst® 15 (120 mg), and refluxed for 2 h. The mixture was cooled and insoluble materials were filtered off and washed with $CHCl_3$. The combined filtrate and washings were concentrated. The residue was diluted with $CHCl_3$ and washed with water (3×). The combined organic layers were dried and concentrated to afford **22** (1.32 g, quantitative) as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ: 4.00–4.16 (2H, m), 4.02 (3H, s), 4.32–4.41 (2H, m), 6.43 (1H, s), 7.68 (1H, dd, $J = 2.0$, 7.3 Hz), 8.21–8.25 (1H, m), 9.05 (1H, d, $J = 7.3$ Hz). HRMS (FAB) calcd for $C_{13}H_{12}{}^{35}ClN_2O_5$ ($[M+H]^+$): 311.0435. Found: 311.0435.

5.2.12. Methyl 3-(1,3-dioxolan-2-yl)-4-oxo-2-vinyl-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (23). Following the procedures as described for **17**, the title compound (214 mg) was prepared in 44% yield from **22** (500 mg, 1.61 mmol) as a yellow solid. ^1H NMR ($CDCl_3$) δ: 4.01 (3H, s), 4.04–4.09 (2H, m), 4.24–4.30 (2H, m), 5.72 (1H, dd, $J = 10.5$, 2.2 Hz), 6.40 (1H, s), 6.67 (1H, dd, $J = 16.8$, 2.2 Hz), 7.29 (1H, dd, $J = 16.8$, 10.5 Hz), 7.53 (1H, dd, $J = 7.4$, 1.7 Hz), 8.24 (1H, dd, $J = 1.7$, 0.7 Hz), 8.98 (1H, dd, $J = 7.4$, 0.7 Hz). HRMS (EI) Calcd for $C_{15}H_{14}N_2O_5$ (M^+): 302.0903. Found: 302.0882.

5.2.13. Methyl 3-(dimethoxymethyl)-2-ethyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (24). To a solution of **23** (100 mg, 0.331 mmol) in MeOH (6 mL) was added 10% Pd–C (100 mg, containing 50% water), and the mixture was stirred under atmospheric pressure of hydrogen for 3 h. The catalyst was filtered off and washed with MeOH. The combined filtrate and washings were concentrated to afford **24** (98.9 mg, 98%) as a yellow oil, which was utilized for the next reaction without further purification. ^1H NMR ($CDCl_3$) δ: 1.30 (3H, t, $J = 7.5$ Hz), 3.05 (2H, q, $J = 7.5$ Hz), 3.49 (6H, s), 4.01 (3H, s), 5.88 (1H, s), 7.55 (1H, dd, $J = 7.4$, 2.0 Hz), 8.24 (1H, dd, $J = 2.0$, 0.7 Hz), 9.01 (1H, dd, $J = 7.4$, 0.7 Hz). HRMS (FAB) calcd for $C_{15}H_{19}N_2O_5$ ($[M+H]^+$): 307.1294. Found: 307.1277.

5.2.14. Methyl 2-ethyl-3-formyl-4-oxo-4*H*-pyrido[1,2-*a*]-pyrimidine-8-carboxylate (25). To formic acid (5 mL) was added **24** (231 mg) and stirred for 70 min. The mixture was concentrated to afford **25** (201 mg, 80% for two steps) as an orange oil, which was utilized for the next reaction without further purification. ^1H NMR ($CDCl_3$) δ: 0.92 (3H, t, $J = 7.3$ Hz), 3.22 (2H, q, $J = 7.3$ Hz), 4.05 (3H, s), 7.73 (1H, dd, $J = 1.7$, 7.3 Hz), 8.29–8.33 (1H, m), 9.15–9.20 (1H, m), 10.59 (1H, s). HRMS (EI) Calcd for $C_{13}H_{12}N_2O_4$ (M^+): 260.0797. Found: 260.0815.

5.2.15. Methyl 3-[(1*E*)-3-*tert*-butoxy-3-oxoprop-1-en-1-yl]-2-ethyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (26). Following the procedures as described for **12**, the title compound (111 mg) was prepared in 40% yield from **25** (201 mg, 0.772 mmol) as a yellow solid. ^1H NMR ($CDCl_3$) δ: 1.35 (3H, t, $J = 7.3$ Hz), 1.54 (9H, s), 3.01 (2H, q, $J = 7.3$ Hz), 4.02 (3H, s), 7.39 (1H, d, $J = 15.5$ Hz), 7.61 (1H, d, $J = 7.3$ Hz), 7.78 (1H, d, $J = 15.5$ Hz), 8.25 (1H, s), 9.10 (1H, d, $J = 7.3$ Hz). HRMS (EI) Calcd for $C_{19}H_{22}N_2O_5$ (M^+): 358.1529. Found: 358.1514.

5.2.16. 3-[(1*E*)-3-*tert*-Butoxy-3-oxoprop-1-en-1-yl]-2-ethyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylic acid (27). Following the procedures as described for **13**, the title compound (110 mg) was prepared from **26** (111 mg, 0.310 mmol) as a yellow solid. ^1H NMR (CD_3OD) δ: 1.34 (3H, t, $J = 7.3$ Hz), 1.56 (9H, s), 3.02 (2H, q, $J = 7.3$ Hz), 7.32 (1H, d, $J = 15.7$ Hz), 7.72 (1H, dd, $J = 1.2$, 7.3 Hz), 7.77 (1H, d, $J = 15.7$ Hz), 8.25 (1H, s), 9.11 (1H, d, $J = 7.3$ Hz). HRMS (EI) Calcd for $C_{18}H_{20}N_2O_5$ (M^+): 344.1372. Found: 344.1385.

5.2.17. *tert*-Butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-ethyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (28). Following the procedures as described for **15**, the title compound (122 mg) was prepared in 79% yield for two steps from **14** (48.6 mg, 0.311 mmol) and crude **27** (110 mg) as a yellow foam.

^{1}H NMR ($CDCl_3$) δ: 1.30–1.38 (3H, m), 1.32 (9H, s), 1.54 (9H, s), 2.93–3.02 (2H, m), 6.60 (1H, s), 7.35 (1H, dd, J = 2.2, 15.6 Hz), 7.66 (1H, dd, J = 1.7, 7.6 Hz), 7.75 (1H, d, J = 2.2, 15.6 Hz), 8.12–8.19 (1H, m), 9.13 (1H, d, J = 7.6 Hz), 10.09 (1H, br). HRMS (EI) Calcd for $C_{25}H_{30}N_4O_4S$ (M^+): 482.1988. Found: 482.1985.

5.2.18. (2*E*)-3-(8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-ethyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (29). Following the procedures as described for **21**, the title compound (88.8 mg) was prepared in 83% yield from **28** (122 mg, 0.252 mmol) as a yellow solid. Mp: 285–289 °C (dec). IR (ATR) cm^{-1}: 3107, 3059, 2962, 2871, 2166, 1678, 1597, 1566, 1500, 1452, 1412, 1365, 1311, 1294, 1240, 1217, 1201, 1176, 1099, 1063. ^{1}H NMR (DMSO-d_6) δ: 1.30 (3H, t, J = 7.3 Hz), 1.32 (9H, s), 2.97 (2H, q, J = 7.3 Hz), 6.88 (1H, s), 7.28 (1H, d, J = 15.4 Hz), 7.76 (1H, d, J = 15.4 Hz), 7.82–7.89 (1H, m), 8.40 (1H, s), 9.11 (1H, d, J = 7.3 Hz). Anal. Calcd for $C_{21}H_{22}N_4O_4S{\cdot}0.5H_2O$: C, 57.91; H, 5.32; N, 12.87; S, 7.36. Found: C, 58.00; H, 5.27; N, 12.84; S, 7.42.

5.2.19. *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 (31). To a solution of **15** (50.0 mg, 0.102 mmol) in DME (4 mL)–water (1 mL) were added phenylboronic acid (12.5 mg, 0.102 mmol), Na_2CO_3 (21.7 mg, 0.205 mmol), and $Pd(Ph_3P)_4$ (5.9 mg, 5.11 μmol), and refluxed for 3.5 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 (EtOAc/hexane = 1:3) to afford **31** (52.9 mg) as inseparable mixture, which was utilized for the next reaction without further purification. ^{1}H NMR ($CDCl_3$–CD_3OD) δ: 1.37 (9H, s), 1.48 (9H, s), 6.65 (1H, s), 7.32 (1H, d, J = 15.6 Hz), 7.54–7.67 (5H, m), 7.57 (1H, d, J = 15.6 Hz), 7.90–7.96 (1H, m), 8.37 (1H, m), 9.23 (1H, d, J = 7.6 Hz). HRMS (FAB) calcd for $C_{29}H_{31}N_4O_4S$: 531.2066. Found: 531.2044.

5.2.20. (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)acrylic acid (32). Following the procedures as described for **21**, the title compound (32.9 mg) was prepared in 68% for two steps from **32** (52.9 mg) as a yellow solid. Mp: 300–305 °C (dec). IR (ATR) cm^{-1}: 3102, 3051, 2962, 2871, 1687, 1666, 1601, 1549, 1506, 1454, 1414, 1365, 1311, 1292, 1271, 1223, 1196, 1099, 1057. ^{1}H NMR (DMSO-d_6) δ: 1.31 (9H, s), 6.89 (1H, br), 7.28 (1H, d, J = 15.6 Hz), 7.49 (1H, d, J = 15.6 Hz), 7.56–7.63 (5H, m), 7.88–7.93 (1H, m), 8.45 (1H, br), 9.18 (1H, d, J = 7.3 Hz). Anal. Calcd for $C_{25}H_{22}N_4O_4S{\cdot}H_2O$: C, 60.96; H, 4.91; N, 11.37; S, 6.51. Found: C, 60.85; H, 4.54; N, 11.37; S, 6.64.

5.2.21. Methyl 3-[(1*E*)-3-*tert*-butoxy-3-oxoprop-1-en-1-yl]-2-cyclohex-1-en-1-yl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (34). Following the procedures as described for **17**, the title compound (153 mg) was prepared from **12** (250 mg, 0.685 mmol) as inseparable mixture, which was utilized for the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ: 1.53 (9H, s), 1.70–1.89 (4H, m), 2.27–2.34 (2H, m), 2.41–2.48 (2H, m), 4.01 (3H, s), 5.94–5.99 (1H, m), 7.35 (1H, d, J = 15.7 Hz), 7.60 (1H, dd, J = 1.7, 7.6 Hz), 7.77 (1H, d, J = 15.7 Hz), 8.27–8.30 (1H, m), 9.10 (1H, d, J = 0.7, 7.6 Hz). HRMS (EI) Calcd for $C_{23}H_{26}N_2O_5$: 410.1842. Found: 410.1857.

5.2.22. 3-[(1*E*)-3-*tert*-Butoxy-3-oxoprop-1-en-1-yl]-2-cyclohex-1-en-1-yl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylic acid (35). Following the procedures as described for **13**, the title compound (67.4 mg) was prepared in 25% for two steps from **34** (153 mg) as a colorless solid, which was utilized for the next reaction without further purification. ^{1}H NMR (CD_3OD) δ: 1.54 (9H, s), 1.74–1.91 (4H, m), 2.28–2.35 (2H, m), 2.40–2.47 (2H, m), 5.91–5.95 (1H, m), 7.23 (1H, d, J = 15.7 Hz), 7.71 (1H, dd, J = 1.1, 7.4 Hz), 7.76 (1H, d, J = 15.7 Hz), 8.21–8.24 (1H, m), 9.09–9.14 (1H, m). HRMS (EI) Calcd for $C_{22}H_{24}N_2O_5$ (M^+) : 396.1685. Found: 396.1681.

5.2.23. *tert*-Butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-cyclohex-1-en-1-yl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (36). Following the procedures as described for **15**, the title compound (76.5 mg) was prepared in 84% yield from **14** (26.6 mg, 0.170 mmol) and **35** (67.4 mg, 0.170 mmol) as a yellow foam. ^{1}H NMR (DMSO-d_6) δ: 1.34 (9H, s), 1.54 (9H, s), 1.66–1.85 (4H, m), 2.21–2.32 (2H, m), 2.37–2.46 (2H, m), 5.91–5.97 (1H, m), 6.57 (1H, s), 7.30 (1H, d, J = 15.8 Hz), 7.61 (1H, dd, J = 2.0, 7.3 Hz), 7.76 (1H, d, J = 15.8 Hz), 8.12–8.16 (1H, m), 9.08 (1H, d, J = 7.3 Hz). HRMS (FAB) calcd for $C_{29}H_{35}N_4O_4S$ ($[M+H]^+$): 535.2379. Found: 535.2348.

5.2.24. (2*E*)-3-(8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-cyclohex-1-en-1-yl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (37). Following the procedures as described for **21**, the title compound (49.6 mg) was prepared in 73% yield from **36** (76.5 mg, 0.143 mmol) as a yellow solid. Mp: 225–233 °C (dec). IR (ATR) cm^{-1}: 3176, 3109, 2931, 2859, 2657, 1673, 1564, 1504, 1435, 1365, 1311, 1286, 1203, 1147, 1097, 1061. ^{1}H NMR (DMSO-d_6) δ: 1.31 (9H, s), 1.66–1.82 (4H, m), 2.21–2.28 (2H, m), 2.40–2.48 (2H, m), 5.89 (1H, br), 6.88 (1H, s), 7.24 (1H, d, J = 15.7 Hz), 7.73 (1H, d, J = 15.7 Hz), 7.81–7.86 (1H, m), 8.38 (1H, s), 9.09 (1H, d, J = 7.3 Hz). Anal. Calcd for $C_{25}H_{26}N_4O_4S{\cdot}0.4H_2O$: C, 61.81; H, 5.56; N, 11.53; S, 6.60. Found: C, 61.89; H, 5.35; N, 11.49; S, 6.60.

5.2.25. Methyl 2-[1-(*tert*-butoxycarbonyl)-1,2,3,6-tetrahydropyridin-4-yl]-3-(1,3-dioxolan-2-yl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (39). To a suspension of **38** (7.98 g, 16.9 mmol) and **22** (5.00 g, 16.1 mmol) in 1,4-dioxane (100 mL) were added CsF (5.38 g, 35.4 mmol), *t*-Bu_3P (212 mg, 0.966 mmol), and $Pd_2(dba)_3{\cdot}CHCl_3$ (250 mg, 0.241 mmol). The mixture was stirred at 80 °C for 14.5 h under Ar. To the mixture were added **38** (2.00 g, 4.23 mmol), CsF (1.35 g, 8.85 mmol), *t*-Bu_3P (212 mg, 0.966 mmol), and $Pd_2(dba)_3{\cdot}CHCl_3$ (250 mg, 0.241 mmol), and further stirred at 80 °C for 7 h under Ar. The mixture was cooled, and insoluble materials were filtered off through Celite, and washed with $CHCl_3$. The combined filtrate and washings were diluted with water and extracted with $CHCl_3$ (5×).

The combined organic layers were dried and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/EtOAc = 4:1 → 3:1 → 2:1) to afford **39** (5.51 g, 75%) as an orange foam. ^{1}H NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.59 (2H, br), 3.63–3.71 (2H, m), 3.98–4.14 (4H, m), 4.00 (3H, s), 4.31–4.37 (2H, m), 5.96 (1H, br), 6.16 (1H, s), 7.55 (1H, dd, J = 1.7, 7.6 Hz), 8.23 (1H, dd, J = 0.7, 1.7 Hz), 9.04 (1H, dd, J = 0.7, 7.6 Hz). HRMS (EI) Calcd for $C_{23}H_{27}N_3O_7$ (M^+): 457.1849. Found: 457.1867.

5.2.26. Methyl 2-[1-(*tert*-butoxycarbonyl)-1,2,3,6-tetrahydropyridin-4-yl]-3-formyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (40). To a solution of **39** (64.9 mg, 0.142 mmol) in acetone (2 mL)–H_2O (2 mL) was added AcOH (2 mL) at 0 °C, and the mixture was stirred for 1 h. The mixture was concentrated, and the residue was diluted with EtOAc, washed with satd $NaHCO_3$ aq and brine. The organic layer was dried and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1:2 → 1:1) to afford **40** (33.6 mg, 57%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.57 (2H, s), 3.67–3.77 (2H, m), 4.05 (3H, s), 4.14–4.19 (2H, m), 5.89–6.11 (1H, m), 7.75 (1H, dd, J = 7.3, 1.7 Hz), 8.33–8.36 (1H, m), 9.22 (1H, d, J = 7.3 Hz), 10.30 (1H, s). HRMS (EI) Calcd for $C_{21}H_{23}N_3O_6$ (M^+): 413.1587. Found: 413.1573.

5.2.27. Methyl 2-[1-(*tert*-butoxycarbonyl)-1,2,3,6-tetrahydropyridin-4-yl]-3-[(1*E*)-3-*tert*-butoxy-3-oxoprop-1-en-1-yl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (41). Following the procedures as described for **12**, the title compound (29.6 mg, 57.9 μmol) was prepared in 71% yield from **40** (33.6 mg, 81.2 μmol) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.51 (9H, s), 1.53 (9H, s), 2.62 (2H, s), 3.65–3.76 (2H, m), 4.02 (3H, s), 4.17 (2H, s), 5.96 (1H, br s), 7.36 (1H, d, J = 15.6 Hz), 7.62 (1H, dd, J = 7.3, 1.7 Hz), 7.73 (1H, d, J = 15.6 Hz), 8.27 (1H, dd, J = 1.7, 0.7 Hz), 9.11 (1H, dd, J = 7.3, 0.7 Hz). HRMS (EI) Calcd for $C_{27}H_{33}N_3O_7$ (M^+) : 511.2319. Found: 511.2327.

5.2.28. 2-[1-(*tert*-Butoxycarbonyl)-1,2,3,6-tetrahydropyridin-4-yl]-3-[(1*E*)-3-*tert*-butoxy-3-oxoprop-1-en-1-yl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylic acid (42). Following the procedures as described for **13**, the title compound (29.0 mg) was prepared in quantitative yield from **41** (29.6 mg) as a yellow solid, which was utilized for the next reaction without further purification. ^{1}H NMR (CD_3OD–$CDCl_3$) δ: 1.52 (9H, s), 1.54 (9H, s), 2.63 (2H, br), 4.18 (2H, br), 4.43 (2H, br), 5.98 (1H, br), 7.32 (1H, d, J = 15.8 Hz), 7.68–7.79 (1H, m), 7.23 (1H, d, J = 15.8 Hz), 8.29 (1H, s), 9.12 (1H, d, J = 7.3 Hz). HRMS (EI) Calcd for $C_{26}H_{32}N_3O_7$ (M^+): 498.2240. Found: 498.2215.

5.2.29. *tert*-Butyl 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)-3,6-dihydropyridine-1(2*H*)-carboxylate (43). Following the procedures as described for **15**, the title compound (86.2 mg) was prepared in 84% yield from **14** (38.9 mg, 0.249 mmol) and **42** (124 mg, 0.249 mmol) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.36 (9H, s), 1.52 (9H, s), 1.54 (9H, s), 2.57 (2H, br), 3.66 (2H, br), 4.14 (2H, br), 5.94 (1H, br), 6.56 (1H, s), 7.34 (1H, d, J = 15.7 Hz), 7.67 (1H, d, J = 7.3 Hz), 7.73 (1H, d, J = 15.7 Hz), 8.16 (1H, s), 9.08 (1H, d, J = 7.3 Hz), 10.59 (1H, br). HRMS (EI) Calcd for $C_{33}H_{41}N_5O_6S$ (M^+): 635.2778. Found: 635.2775.

5.2.30. (2*E*)-3-[8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]-carbonyl}-4-oxo-2-(1,2,3,6-tetrahydropyridin-4-yl)-4*H*-pyrido- [1,2-*a*]pyrimidin-3-yl]acrylic acid (44). Following the procedures as described for **21**, the title compound (73.7 mg) was prepared in 81% yield from **43** (86.2 mg, 0.136 mmol) as a yellow solid. Mp: 181–198 °C (dec). IR (ATR) cm^{-1}: 3062, 2968, 1684, 1604, 1552, 1508, 1446, 1415, 1360, 1313, 1284, 1186, 1138, 1099, 1059. ^{1}H NMR (CD_3OD) δ: 1.36 (9H, s), 2.86–2.93 (2H, m), 3.52–3.59 (2H, m), 3.94–3.99 (2H, m), 6.01–6.60 (1H, m), 6.74–6.77 (1H, m), 7.38–7.45 (1H, m), 7.78–7.85 (1H, m), 7.89–7.94 (1H, m), 8.26–8.30 (1H, m), 9.19–9.23 (1H, m). Anal. Calcd for $C_{24}H_{25}N_5O_4S{\cdot}1.5TFA{\cdot}H_2O$: C, 48.50; H, 4.30; N, 10.47; S 4.80. Found: C, 48.49; H, 4.16; N, 10.38; S, 4.86.

5.2.31. Methyl 2-{[1-(*tert*-butoxycarbonyl)piperidin-4-yl]-methyl}-3-(1,3-dioxolan-2-yl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (46). To a solution of **45** (95.2 mg, 0.4828 mmol) in THF (2 mL) was added 9-BBN (0.5 M solution in THF, 966 μL, 0.483 mmol) under Ar, and the mixture was refluxed for 1 h. To the mixture were added a solution of **22** (100 mg, 0.322 mmol) and Pd(dppf)$Cl_2{\cdot}CH_2Cl_2$ (13.1 mg, 0.0161 mmol) in DMF (3 mL) and K_2CO_3 (89.0 mg, 0.644 mmol) at room temperature, and the mixture was stirred at 60 °C for 4 h. The mixture was cooled and diluted with satd NH_4Cl aq and extracted with $CHCl_3$. The combined organic layers were dried and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1:2 → 1:1 → 3:2) to afford **46** (149 mg, 98%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.19–1.34 (2H, m), 1.46 (9H, s), 1.59–1.74 (2H, m), 2.08–2.21 (1H, m), 2.62–2.76 (2H, m), 2.85 (2H, d, J = 7.1 Hz), 3.99–4.15 (4H, m), 4.01 (3H, s), 4.22–4.29 (2H, m), 6.32 (1H, s), 7.53–7.57 (1H, m), 8.19–8.22 (1H, m), 9.00–9.04 (1H, m). HRMS (FAB) calcd for $C_{24}H_{32}N_3O_7$ ($[M+H]^+$): 474.2240. Found: 474.2237.

5.2.32. 2-{[1-(*tert*-Butoxycarbonyl)piperidin-4-yl]methyl}-3-(1,3-dioxolan-2-yl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylic acid (47). Following the procedures as described for **13**, the title compound (120 mg) was prepared in 83% yield from **46** (149 mg, 0.314 mmol) as a creamy solid. ^{1}H NMR (CD_3OD–$CDCl_3$) δ: 1.22–1.35 (2H, m), 1.46 (9H, s), 1.64–1.77 (2H, m), 2.10–2.23 (1H, m), 2.65–2.80 (2H, m), 2.88 (2H, d, J = 7.1 Hz), 4.01–4.12 (2H, m), 4.23–4.29 (2H, m), 4.52–4.63 (2H, m), 6.31 (1H, s), 7.55–7.58 (1H, m), 7.67–7.72 (1H, m), 9.02 (1H, d, J = 7.3 Hz). HRMS (FAB) calcd for $C_{23}H_{30}N_3O_7$ ($[M+H]^+$): 460.2084. Found: 460.2080.

5.2.33. *tert*-Butyl 4-{[8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)-amino]carbonyl}-3-(1,3-dioxolan-2-yl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-2-yl]methyl}piperidine-1-carboxylate (48). Following the procedures as described for **15**, the title compound (102 mg) was prepared in 65% yield from **14** (40.7 mg, 0.261 mmol) and **47** (120 mg, 0.261 mmol) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ: 1.20–1.35 (2H, m), 1.34 (9H, s), 1.47 (9H, s), 1.60–1.72 (2H, m), 2.07–2.10 (1H, m),

2.62–2.76 (2H, m), 2.78–2.88 (2H, m), 4.02–4.16 (4H, m), 4.24–4.31 (2H, m), 6.32 (1H, s), 6.60 (1H, s), 7.58 (1H, dd, $J = 1.7$, 7.4 Hz), 8.09 (1H, s), 8.97 (1H, d, $J = 7.4$ Hz). HRMS (FAB) calcd for $C_{30}H_{40}N_5O_6S$ ($[M+H]^+$): 598.2699. Found: 598.2687.

5.2.34. *tert*-Butyl 4-[(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)-amino]carbonyl}-3-formyl-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-2-yl)methyl]piperidine-1-carboxylate (49). Following the procedures as described for **40**, the title compound (93.4 mg) was prepared in 99% yield from **48** (102 mg, 0.170 mmol) as an orange foam. ^{1}H NMR ($CDCl_3$) δ: 1.28–1.37 (2H, m), 1.38 (9H, s), 1.49 (9H, s), 1.61–1.72 (2H, m), 1.60–1.75 (2H, m), 1.90–2.04 (1H, m), 2.59–2.76 (1H, m), 2.84–3.30 (2H, m), 4.00–4.14 (2H, m), 6.51 (1H, s), 7.94 (1H, d, $J = 7.3$ Hz), 8.39 (1H, s), 9.19 (1H, d, $J = 7.3$ Hz), 10.58 (1H, s). HRMS (FAB) calcd for $C_{28}H_{36}N_5O_5S$ ($[M+H]^+$): 554.2437. Found: 554.2453.

5.2.35. *tert*-Butyl 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)methyl]piperidine-1-carboxylate (50). Following the procedures as described for **12**, the title compound (96.1 mg) was prepared in 87% yield from **49** (93.4 mg, 0.169 mmol) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ: 1.31–1.37 (2H, m), 1.34 (9H, s), 1.47 (9H, s), 1.54 (9H, s), 1.62–1.71 (2H, m), 2.05 (1H, s), 2.62–2.76 (2H, m), 2.89 (2H, d, $J = 6.8$ Hz), 4.01–4.18 (2H, m), 6.58 (1H, s), 7.38 (1H, d, $J = 15.5$ Hz), 7.70 (1H, d, $J = 1.7$ Hz), 7.74 (1H, d, $J = 15.5$ Hz), 8.21 (1H, s), 9.12 (1H, d, $J = 7.3$ Hz), 10.45 (1H, br). HRMS (FAB) calcd for $C_{34}H_{46}N_5O_6S$ ($[M+H]^+$): 652.3169. Found: 652.3173.

5.2.36. (2*E*)-3-[8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-2-(piperidin-4-ylmethyl)-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylic acid (51). Following the procedures as described for **21**, the title compound (61.8 mg) was prepared in 65% yield from **50** (96.1 mg, 0.147 mmol) as a yellow solid. Mp: 154–161 °C. IR (ATR) cm^{-1}: 3045, 2966, 2866, 2754, 2517, 1670, 1604, 1549, 1502, 1458, 1408, 1360, 1300, 1198, 1130, 1061. ^{1}H NMR (CD_3OD) δ: 1.36 (9H, s), 1.54–1.66 (2H, m), 1.97–2.07 (2H, m), 2.23–2.36 (1H, m), 2.94–3.07 (4H, m), 3.35–3.44 (2H, m), 6.76 (1H, s), 7.43 (1H, d, $J = 15.4$ Hz), 7.85–7.92 (2H, m), 8.26 (1H, s), 9.18 (1H, d, $J = 7.3$ Hz). Anal. Calcd for $C_{25}H_{29}N_5O_4S{\cdot}TFA{\cdot}2H_2O$: C, 50.23; H, 5.31; N, 10.85; S, 4.97. Found: C, 50.20; H, 5.06; N, 10.58; S, 4.71.

5.2.37. Methyl 3-[(1*E*)-3-*tert*-butoxy-3-oxoprop-1-en-1-yl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylate (52). To a solution of **12** (1.16 g, 3.19 mmol) in DMF (30 mL) were added Ph_3P (42.0 mg, 0.160 mmol), $Pd(OAc)_2$ (14.3 mg, 0.0638 mmol), Et_3N (1.36 mL, 9.76 mmol), and formic acid (241 μL, 6.38 mmol), and stirred at 70 °C for 24 h. The mixture was concentrated, and the residue was diluted with $CHCl_3$. Insoluble materials were removed by filtration and washed with $CHCl_3$. The filtrate and washings were concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1:2) to afford **52** (149 mg, 14%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.54 (9H, s), 4.03 (3H, s), 7.16 (1H, d, $J = 15.7$ Hz), 7.57 (1H, d, $J = 15.7$ Hz), 7.68 (1H, dd, $J = 1.7$, 7.4 Hz), 8.30–8.31 (1H, m), 8.52 (1H, s), 9.18 (1H, dd, $J = 0.7$, 7.4 Hz). HRMS (EI) Calcd for $C_{17}H_{18}N_2O_5$ (M^+): 330.1216. Found: 330.1208.

5.2.38. *tert*-Butyl (2*E*)-3-(8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)-amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)-acrylate (54). To a solution of **52** (149 mg, 0.452 mmol) in THF (10 mL) was added 1 N NaOH aq (4.13 mL, 4.13 mmol) and stirred for 2.5 h. The mixture was acidified to pH 3 with 1 N HCl aq and concentrated. The residue was partitioned between water and $CHCl_3$, and the aqueous layer was extracted with $CHCl_3$ (5×). The combined organic layers were concentrated. To a solution of the residue in CH_2Cl_2 (40 mL)–DMF (40 mL) were added **14** (77.7 mg, 0.497 mmol), DMAP (110 mg, 0.904 mmol), HOBt (30.5 mg, 0.226 mmol), and WSCD·HCl (173 mg, 0.904 mmol). After stirring overnight, the mixture was concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1) to afford **54** (205 mg, quantitative) as a yellow powder. ^{1}H NMR ($CDCl_3$) δ: 1.35 (9H, s), 1.54 (9H, s), 6.61 (1H, s), 7.16 (1H, d, $J = 15.9$ Hz), 7.58 (1H, d, $J = 15.9$ Hz), 7.80 (1H, d, $J = 7.3$ Hz), 8.30 (1H, s), 8.52 (1H, s), 9.23 (1H, d, $J = 7.3$ Hz). HRMS (EI) Calcd for $C_{23}H_{26}N_4O_4S$ (M^+): 454.1675. Found: 454.1674.

5.2.39. (2*E*)-3-(8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]-carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (55). Following the procedures as described for **21**, the title compound (142 mg) was prepared in 79% yield from **54** (205 mg, 0.451 mmol) as a yellow foam. Mp: 302–305 °C. IR (ATR) cm^{-1}: 1689, 1670, 1608, 1576, 1481, 1323, 1286, 1234, 1051, 989, 737, 482. ^{1}H NMR (CD_3OD) δ: 1.31 (9H, s), 6.87 (1H, s), 7.08 (1H, d, $J = 15.6$ Hz), 7.64 (1H, d, $J = 15.6$ Hz), 7.90 (1H, dd, $J = 1.8$, 7.5 Hz), 8.45 (1H, s), 8.79 (1H, s), 9.16 (1H, d, $J = 7.5$ Hz). Anal. Calcd for $C_{19}H_{18}N_4O_4S{\cdot}0.75H_2O$: C, 55.40; H, 4.77; N, 13.60. Found: C, 55.42; H, 4.46; N, 13.35.

5.2.40. *tert*-Butyl (2*E*)-3-[8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-2-(1,2,3,6-tetrahydropyridin-4-yl)-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylate (56). To a solution of **43** (2.14 g, 3.37 mmol) in CH_2Cl_2 (40 mL) was added TFA (4 mL) at 0 °C, and the mixture was stirred for 15 h at 0 °C. Additionally, TFA (4 mL) was added to the mixture at 0 °C and stirred further for 4.5 h at 0 °C. The mixture was diluted with satd $NaHCO_3$ aq at 0 °C and extracted with $CHCl_3$ (3×). The combined organic layers were dried and concentrated to afford **56** (1.23 g, 68%) as a yellow solid. ^{1}H NMR (CD_3OD) δ: 1.36 (9H, s), 1.54 (9H, s), 2.82 (2H, br), 3.43–3.51 (2H, m), 3.85–3.92 (2H, m), 5.97–6.05 (1H, m), 6.72 (1H, s), 7.30 (1H, d, $J = 15.7$ Hz), 7.74 (1H, d, $J = 15.7$ Hz), 7.84–7.90 (1H, m), 8.20–8.25 (1H, m), 9.11–9.18 (1H, m). HRMS (FAB) calcd for $C_{28}H_{34}N_5O_4S$ ($[M+H]^+$): 536.2332. Found: 536.2310.

5.2.41. *tert*-Butyl (2*E*)-3-(2-(1-acetyl-1,2,3,6-tetrahydropyridin-4-yl)-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylate (57). To a solution of **56** (200 mg, 0.373 mmol) in CH_2Cl_2 (5 mL) were added Et_3N (78.1 μL, 0.560 mmol) and acetyl

chloride (29.2 μL, 0.411 mmol) at 0 °C, and stirred for 3.5 h. The mixture was concentrated, and the residue was diluted with EtOAc, and washed with 10% citric acid aq, satd $NaHCO_3$ aq and brine. The organic layer was dried and concentrated to afford **57** (172 mg, 80%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.36 (9H, s), 1.52 (9H, s), 2.29, 2.33 (total 3H, 2s), 2.65–2.73 (2H, m), 3.73–3.79, 3.97–4.03 (total 2H, 2m), 4.24–4.28, 4.35–4.39 (total 2H, 2m), 5.95, 6.04 (total 1H, 2s), 6.59 (1H, s), 7.35, 7.39 (total 1H, 2d, J = 11.5 Hz for each), 7.74, 7.70 (total 1H, 2d, J = 11.5 Hz for each), 7.70–7.74, 7.76–7.82 (total 1H, 2m), 8.36, 8.47 (total 1H, each s), 9.12, 9.14 (total 1H, 2d, J = 7.6 Hz for each). HRMS (FAB) calcd for $C_{30}H_{36}N_5O_5S$ ($[M+H]^+$): 578.2437. Found: 578.2419.

5.2.42. (2*E*)-3-(2-(1-Acetyl-1,2,3,6-tetrahydropyridin-4-yl)-8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)acrylic acid (58). Following the procedures as described for **21**, the title compound (121 mg) was prepared in 76% yield from **57** (172 mg, 0.297 mmol) as a yellow solid. Mp: 200–210 °C (dec). IR (ATR) cm^{-1}: 3049, 2960, 2873, 2789, 1682, 1597, 1493, 1446, 1414, 1365, 1288, 1238, 1203, 1144, 1099, 1061. ^{1}H NMR (DMSO-d_6) δ: 1.31 (9H, s), 2.08, 2.12 (total 3H, 2s), 2.49–2.69 (2H, m), 3.67–3.75 (2H, m), 4.14–4.25 (2H, m), 5.98 (1H, m), 6.89 (1H, s), 7.26 (1H, d, J = 15.6 Hz), 7.71 (1H, d, J = 15.6 Hz), 7.84–7.88 (1H, m), 8.40 (1H, s), 9.11 (1H, d, J = 7.6 Hz). Anal. Calcd for $C_{26}H_{27}N_5O_5S{\cdot}0.75H_2O$: C, 58.36; H, 5.37; N, 13.09; S, 5.99. Found: C, 58.28; H, 5.29; N, 12.83; S, 6.02.

5.2.43. [{2-[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)-3,6-dihydropyridin-1(2*H*)-yl]-2-oxoethyl}(methyl)amino]acetic acid (61). To **59** (100 mg, 0.680 mmol) was added acetic anhydride and refluxed for 4 h. The mixture was concentrated to afford crude **60**, which was utilized for the next reaction without further purification. To a solution of crude **60** in toluene (2 mL)–pyridine (0.5 mL) was added **56** and refluxed for 1 h. The mixture was concentrated and the residue was purified by silica gel column chromatography ($CHCl_3 \rightarrow CHCl_3$/MeOH = 10:1 → $CHCl_3$/MeOH/H_2O = 8:3:0.5) to afford **61** (233 mg) as a brown solid. This compound was utilized for the next reaction without further purification. HRMS (FAB) calcd for $C_{33}H_{41}N_6O_7S$ ($[M+H]^+$): 665.2757. Found: 665.2764.

5.2.44. (2*E*)-3-[8-{[(4-*tert*-Butyl-1,3-thiazol-2-yl)amino]-carbonyl}-2-(1-{[(carboxymethyl)(methyl)amino]acetyl}-1,2,3,6-tetrahydropyridin-4-yl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl]acrylic acid (62). Following the procedures as described for **21**, the title compound (152 mg) was prepared in 51% yield for two steps from **56** (233 mg) as a yellow solid. Mp: 222–236 °C (dec). IR (ATR) cm^{-1}: 2962, 2929, 2871, 1674, 1603, 1547, 1500, 1444, 1369, 1309, 1282, 1238, 1201, 1140, 1101, 1063. ^{1}H NMR (DMSO-d_6) δ: 1.27, 1.29 (total 9H, 2s), 2.30–2.41 (3H, m), 2.57–2.67 (2H, m), 3.03–4.46 (8H, m), 5.82–6.07 (1H, m), 6.48–6.67 (1H, m), 6.95–7.09 (1H, m), 7.38 (br, 1H), 7.63–7.80 (1H, m), 7.92–8.13 (1H, m), 8.78–8.91 (1H, m). Anal. Calcd for $C_{29}H_{32}N_6O_7S{\cdot}TFA{\cdot}4H_2O$: C, 46.85; H, 5.20; N, 10.57; S 4.03. Found: C, 46.89; H, 5.43; N, 10.84; S, 4.15.

5.2.45. *tert*-Butyl (2*E*)-3-{8-{[(4-*tert*-butyl-1,3-thiazol-2-yl)amino]carbonyl}-2-[1-(*N*,*N*-dimethylglycyl)-1,2,3,6-tetrahydropyridin-4-yl]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl}-acrylate (63). To a solution of **56** (200 mg, 0.373 mmol) and Sar-OH (42.4 mg, 0.411 mmol) in CH_2Cl_2 (2 mL)–DMF (2 mL) were added DMAP (91.2 mg, 0.747 mmol), HOBt (55.5 mg, 0.411 mmol), and WSCD·HCl (78.7 mg, 0.411 mmol) at 0 °C and stirred for 13 h. The mixture was concentrated, and the residue was diluted with $CHCl_3$, and washed with 10% citric acid aq, satd $NaHCO_3$ aq, and brine. The organic layer was dried and concentrated. The residue was purified by PTLC ($CHCl_3$/MeOH = 10:1) to afford **63** (89.5 mg, 39%) as a yellow solid. ^{1}H NMR (CD_3OD) δ: 1.35 (9H, s), 1.52, 1.53 (total 9H, 2s), 2.456, 2.465 (total 6H, 2s), 2.61–2.74 (2H, m), 3.49 (2H, s), 3.82–3.92 (2H, m), 4.30–4.38 (2H, m), 5.96–6.04 (1H, m), 6.73 (1H, s), 7.25 (1H, d, J = 15.6 Hz), 7.74 (1H, d, J = 15.6 Hz), 7.86 (1H, dd, J = 1.7, 7.3 Hz), 8.24 (1H, s), 9.14–9.18 (1H, m). HRMS (FAB) calcd for $C_{32}H_{41}N_6O_5S$ ($[M+H]^+$): 621.2859. Found: 621.2882.

5.2.46. [{2-[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)-3,6-dihydropyridin-1(2*H*)-yl]-2-oxoethyl}(dimethyl)ammonio]acetate (65). To a solution of **63** (89.5 mg, 0.144 mmol) in DMF (4 mL) was added *tert*-butyl bromoacetate (106 μL, 0.721 mmol) and stirred in the dark for 12.5 h. Additionally, *tert*-butyl bromoacetate (106 μL, 0.721 mmol) was added and stirred in the dark for 6.5 h. The mixture was concentrated to afford **64** (108 mg) as inseparable mixture, which was utilized for the next reaction without further purification. Following the procedures as described for **21**, the title compound (46.6 mg) was prepared in 45% yield for two steps from **64** (108 mg) as a yellow solid. Mp: 198–207 °C (dec). IR (ATR) cm^{-1}: 2962, 2868, 1635, 1547, 1502, 1441, 1387, 1311, 1273, 1236, 1200, 1130, 1101, 1061. ^{1}H NMR (CD_3OD) δ: 1.35 (9H, s), 2.62–2.79 (2H, m), 3.46 (3H, s), 3.47 (3H, s), 3.74–3.80, 3.88–3.94 (total 2H, 2m), 4.18–4.36 (4H, m), 4.86–4.98 (2H, m), 5.95–6.06 (1H, m), 6.74 (1H, s), 7.37 (1H, d, J = 15.7 Hz), 7.73 (1H, d, J = 15.7 Hz), 7.81–7.86 (1H, m), 8.22–8.26 (1H, m), 9.14–9.19 (1H, m). Anal. Calcd for $C_{29}H_{32}N_6O_7S{\cdot}0.5TFA{\cdot}2H_2O$: C, 52.02; H, 5.42; N, 11.74; S 4.48. Found: C, 51.78; H, 5.49; N, 11.58; S, 4.50.

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.

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References and notes

1. Poole, K.; Krebes, K.; McNally, C.; Neshat, S. *J. Bacteriol.* **1993**, *175*, 7363.
2. Poole, K.; Gotoh, N.; Tsujimoto, H.; Zhao, Q.; Wada, A.; Yamasaki, T.; Neshat, S.; Yamagishi, J.; Li, X. Z.; Nishino, T. *Mol. Microbiol.* **1996**, *21*, 713.
3. Köhler, T.; Michea-Hamzehpour, M.; Henze, U.; Gotoh, N.; Curty, L. K.; Pechere, J. C. *Mol. Microbiol.* **1997**, *23*, 345.
4. Mine, T.; Morita, Y.; Kataoka, A.; Mizushima, T.; Tsuchiya, T. *Antimicrob. Agents Chemother.* **1999**, *43*, 415.
5. Chuanchuen, R.; Narasaki, C. T.; Schweizer, H. P. *J. Bacteriol.* **2002**, *184*, 5036.
6. 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.
7. 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.
8. 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.
9. 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.
10. Stille, J. K. *Angew. Chem.* **1986**, *98*, 504.
11. Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508.
12. Mitchell, T. N. *Synthesis* **1992**, 803.
13. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
14. Suzuki, A. *Organomet. Chem.* **1999**, *576*, 147.
15. Gilbertson, S. R.; Challener, C. A.; Bos, M. E.; Wulff, W. D. *Tetrahedron Lett.* **1988**, *29*, 4795.
16. Laborde, E.; Kiely, J. S.; Culbertson, T. P.; Lesheski, L. E. *J. Med. Chem.* **1993**, *36*, 1964.
17. Littke, A. F.; Fu, G. C. *Angew. Chem., Int. Ed.* **1999**, *38*, 2411.
18. Vice, S.; Bata, T.; Bauer, A.; Evans, C. A.; Ford, J.; Josien, H.; McCombie, S.; Miller, M.; Nazareno, D.; Palani, A.; Tagat, J. *J. Org. Chem.* **2001**, *66*, 2487.
19. 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.
20. Varga, M.; Kapui, Z.; Bátori, S.; Nagy, L. T.; Vasvári-Debreczy, L.; Mikus, E.; Urbán-Szabó, K.; Arányi, P. *Eur. J. Med. Chem.* **2003**, *38*, 421.
21. Hertel, L. W.; Kohlmam, D. T.; Liang, S. X.; Wong, D. T.; Xu, Y-.C. PCT Int. Appl. WO 00/00198 A1, January 6, 2000.