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Bioorganic & Medicinal Chemistry Letters 14 (2004) 2493–2497
Bioorganic & Medicinal Chemistry Letters

MexAB-OprM specific efflux pump inhibitors in *Pseudomonas aeruginosa*. Part 4: Addressing the problem of poor stability due to photoisomerization of an acrylic acid moiety

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Received 16 February 2004; revised 1 March 2004; accepted 1 March 2004

Abstract—Exchange of the ethylene tether in a series of pyridopyrimidine-based MexAB-OprM specific efflux pump inhibitors to an amide bond stabilized the olefin of the acrylic acid moiety, preventing facile photoisomerization to the *Z*-isomer. Furthermore, the activity was drastically improved in the amide tether variants, providing extremely potent acrylic acid and vinyl tetrazole analogues.

The emergence of bacterial resistance continues to challenge effective chemotherapy, especially among the immune compromised. Measures to tackle the problem comprise the judicious use of antimicrobial agents together with discovery of new drugs, including those targeting the root cause of the resistance in question. *Peudomonas aeruginosa* is a problematic opportunistic pathogen that shows intrinsic resistance to a wide variety of antimicrobial agents (including quinolones, β-lactams, aminoglycosides, macrolides and tetracyclines). This is largely attributable to the expression of several efflux pumps, four of which have been identified to date: MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM.[1–4] We recently reported the discovery and optimization[5–7] of agents that are specific for the inhibition of MexAB-OprM. Our initial SAR studies included scaffolding methodology and pharmacophore-model-based design to improve both efficacy and physicochemical properties, furnishing pyridopyrimidine-based leads typified by **1**. This compound possessed high in vitro potency coupled with relatively low protein binding (for this series), and exhibited encouraging efficacy in vivo potency for the potentiation of the activity of Levofloxacin (LVFX). However, the series showed poor stability due to extremely facile photoisomerization of the olefin of the acrylic acid moiety, as shown Figure 1. Surprisingly, exposure of solutions of *E*-isomers such as **1** to ambient light for as little as 2 h was sufficient to generate predominantly the *Z*-isomer (**2**), which was found to be devoid of activity.

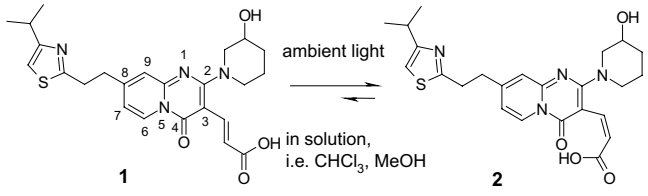

Figure 1. Structure of the pyridopyrimidine-based MexAB-OprM specific efflux pump inhibitor (**1**), and its isomerization to the *Z*-isomer (**2**).

Keywords: Efflux pump inhibitor; *Pseudomonas aeruginosa*; Photoisomerization.
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doi:10.1016/j.bmcl.2004.03.007

Herein, we report the results of structure–stability relationship studies to address the problem of photoisomerization by variation of the olefin, and through the incorporation of electron-withdrawing groups on the pyridopyrimidine scaffold so as to ameliorate the effect of the electron-rich substituent *ortho* to the acrylic acid moiety, together with the potency of the derivatives.

We envisioned that incorporation of small alkyl, alkoxy, or fluorine substituents might alter the electronic characteristics of the unsaturated olefin, leading to analogues with greater stability towards photoisomerization. Another strategy to circumvent the problem was based on the hypothesis that the isomerization was facilitated by the electron-donating 2-substituent in conjugation with the acrylic acid moiety. Incorporation of further substituents on the pyridopyrimidine scaffold might counterbalance this effect. Alternatively, exchanging the ethylene tether connecting the thiazole to the pyridopyrimidine nucleus to an amide surrogate would render the scaffold less electron rich. Conversely, an analogue with an ether tether (**29**) would donate electron density to the scaffold (Fig. 2).

Schemes 1–6 illustrate the routes by which these derivatives were prepared. The alkyl substituent on the

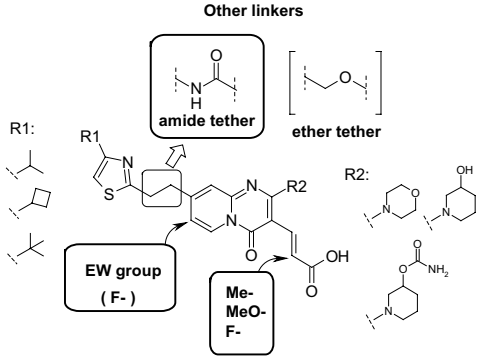
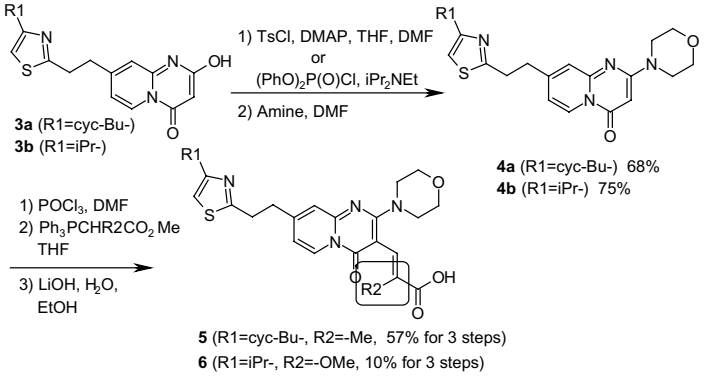


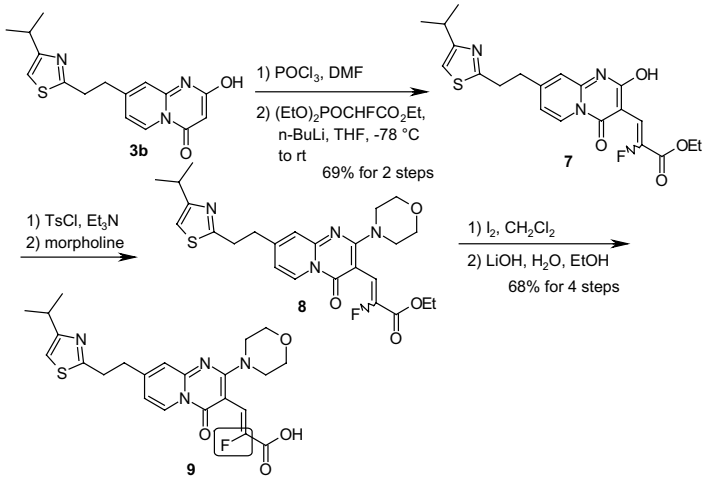
Figure 2. Outline of strategies to prevent photoisomerization of the olefin in the acrylic acid motif.

thiazole motif (R1) did not affect the stability (data not shown); its selection was driven by the availability of intermediates from earlier syntheses.[5]

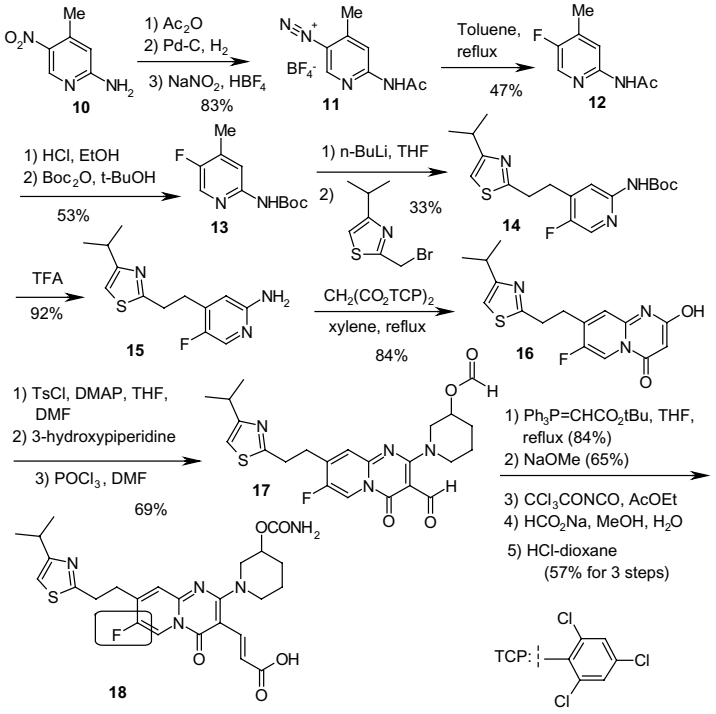
The routes for incorporation of methyl, methoxy, or fluorine substituents on the double bond of the acrylic acid are shown in Schemes 1 and 2. In general, Vilsmeier formylation of the parent pyrimidopyrimidine, followed by Wittig reactions provided the α-substituted acrylic



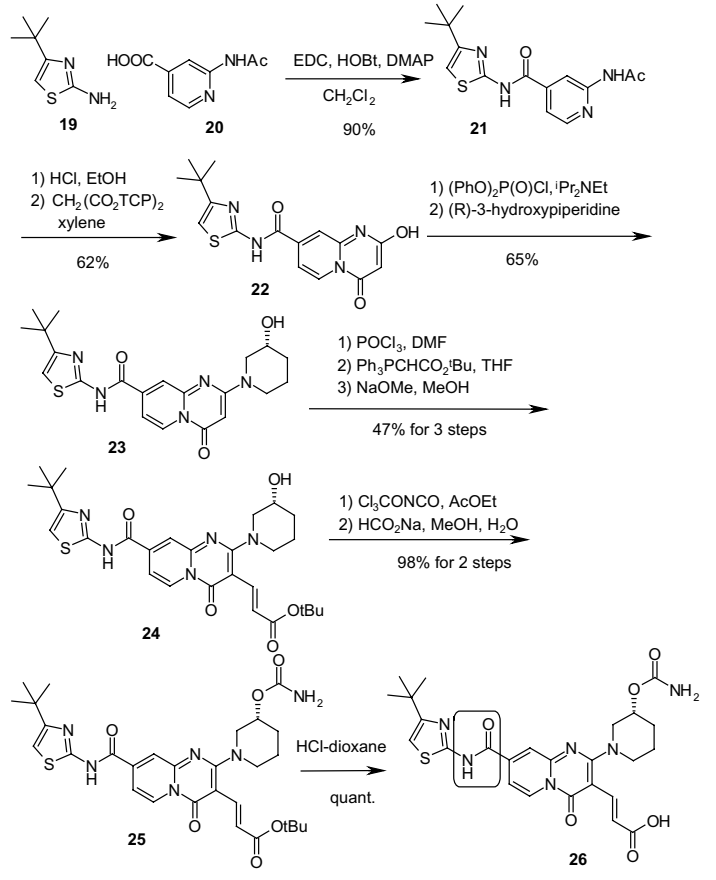
Scheme 1. Synthesis of methyl and methoxy α-substituted acrylic acid analogues.



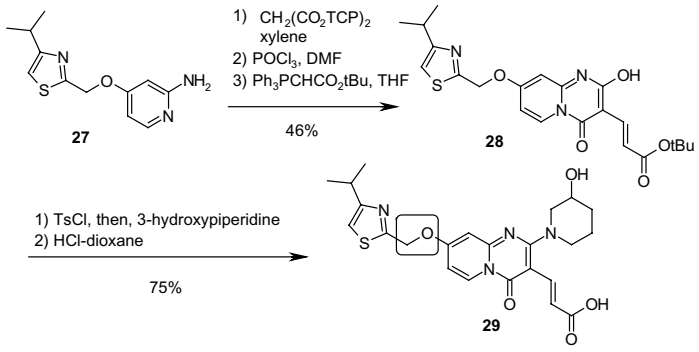
Scheme 2. Synthesis of α-fluorinated acrylic acid analogue.



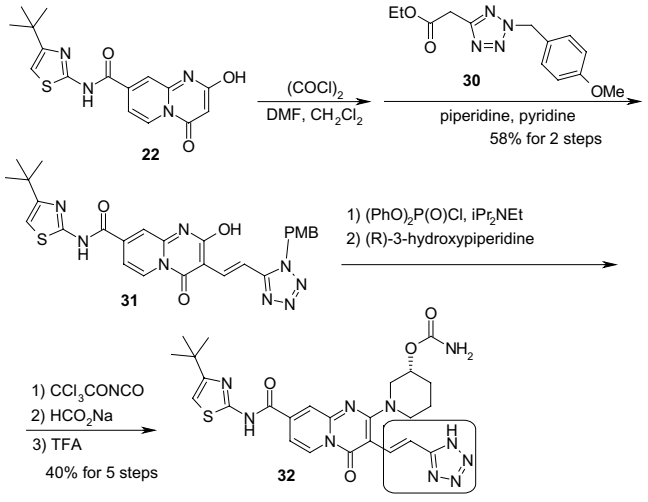
Scheme 3. Incorporation of fluorine onto the pyridopyrimidine scaffold.



Scheme 4. Synthesis of an amide tether variant.



Scheme 5. Synthesis of an ether tether variant.



Scheme 6. Synthesis of the vinyl tetrazole analogue.

acid derivatives. The reactivities of the Wittig reagents varied significantly, and we found that reactions proceeded most satisfactorily when matched with appropriate substrates: the 2-morpholino intermediate for Me- and MeO-containing analogues, and the 2-hydroxyl intermediate for Wittig–Horner reaction to generate a fluorine-containing analogue. In the latter case, a mixture of *E* and *Z*-isomers was generated. Treatment with iodine[8] furnished the *E*-isomer exclusively.

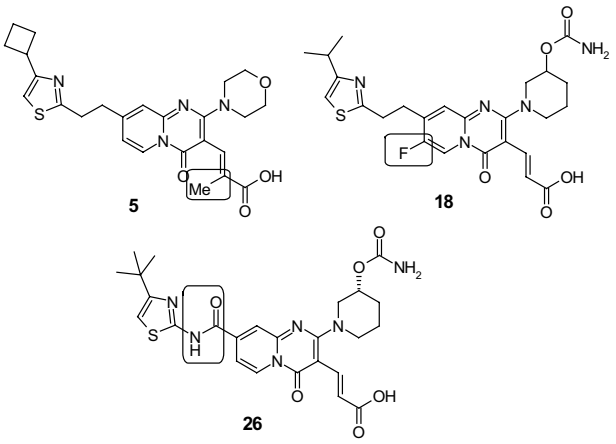
Incorporation of fluorine onto the pyridopyrimidine scaffold was achieved via the Shieman reaction[9] as the key step, starting with **10** (Scheme 3). The resulting product (**13**) was treated with excess base to generate a dianion, which coupled satisfactorily with the requisite 2-halomethylthiazole.[7] Subsequent steps were similar to those utilized in nonfluorinated variants.[7]

Scheme 4 outlines the synthesis of amide tether derivatives. Condensation of a 2-aminothiazole with a pyridine-4-carboxylic acid derivative using the conventional EDC protocol, removal of the acetyl protecting group, and cyclization with an active ester of malonic acid provided the 2-hydroxypyridopyrimidine **22**. For this analogue, diphenylphosphate was found to be a superior leaving group for the subsequent nucleophilic displacement by piperidine derivatives; by contrast, the more conventional tosylate gave starting material **22** predominantly. Vilsmeier formylation followed by Wittig reaction and deprotection led to the acrylic acid derivative. Finally, formation of the primary carbamate and deprotection gave **26**. A similar protocol was utilized for the ether tether analogue **29** (Scheme 5), but in this case tosylate was the preferred leaving group. The synthetic intermediate **27** was prepared via Curtius rearrangement.[5]

We next examined the photostability of the various analogues. The ratios of *E* and *Z*-isomers after exposure to light from a white fluorescent lamp are illustrated in Table 1.

The effect of a 6-fluoro substituent on the pyridopyrimidine framework was modest; the stability of analogue

Table 1. The ratio (*Z*/*E*) of the photoisomerization of the olefin with time[a]



Time (min)	**5**[b] (*Z*/*E*)	**18**[b] (*Z*/*E*)	**26** (*Z*/*E*)
0	4/96	3/97	0/100
60	6/94	24/76	1/99
120	7/93	31/69	1/99
240	10/90	35/65	1/99

[a] The ratio was calculated by peak area using LC–MS. Each sample was dissolved in H_2O–MeOH, 9:1 or MeOH (concentration; 25–50 μg/mL). Sample solutions were illuminated with a white fluorescent lamp (500 Lx). No peaks other than the *E* and *Z* isomers were detected during the study.

[b] The initial sample contained a slight amount of *Z*-isomer, presumably generated during the evaporation of the solvent after column chromatography.

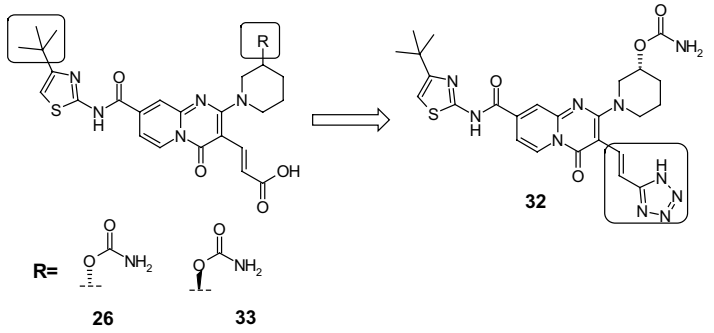


Figure 3. Advanced photostable analogues.

Table 2. In vitro potentiation activity of novel EPIs

	MPC_8 (AZT) μg/mL[a]		MPC_4 (LVFX) μg/mL[a]	MPC_8 (LVFX) μg/mL[a]
	Without HSA	With 0.125% HSA		
1	8	16	0.5	8
26	0.5	1	<0.25	1
32	<0.25	2	<0.25	0.5
33	1	4	0.5	2

[a] Values are for 100% growth inhibition.

18 (Table 1) was similar to that of the parent **1** (data not shown).

Compound **5**, bearing a methyl group on the acrylic acid moiety, was found to be more stable, though the isomerization was not prevented completely. Other modifications of the olefin gave no improvement (compounds **6** and **9**).

By contrast, introduction of the amide tether (**26**) gave a dramatic increase in stability. Conversely, the instability of the analogue bearing an ether tether (**29**) was such that a significant quantity of *Z*-isomer was already detected in solution after only a few minutes exposure to house light (data not shown).

The activities of the photostable efflux pump inhibitors were assessed using PAM1723,[10] an experimental strain of *P. aeruginosa* in which the MexAB-OprM pump is overexpressed and MexCD-OprJ and MexEF-OprN are genetically disrupted. Data for the potentiation of two MexAB-OprM substrates, LVFX and aztreonam (AZT), in the presence or absence of human serum albumin (HSA) is displayed in Table 2. To quantify the activity of the inhibitors, we defined the term MPC_n as the minimum concentration (μg/mL) of inhibitor required to reduce (potentiate) the activity of antibacterial drug *n*-fold.

The derivatives possessing the amide tether, in particular, showed excellent potency. We also extended the SAR at the 2-substituent[7] by the finding that the 3-(*R*)-carbamoyloxy-substituted piperazine (**26**) was more potent than the (*S*) isomer (**33**). Finally, we showed that the replacement of carboxylic acid by tetrazole (**32**, synthesized as shown in Scheme 6 via Knoevenagel reaction with tetrazole acetate **30**) gave a further enhancement in potency.[7] Like **26**, this compound was not susceptible to photoisomerization (Fig. 3).

In conclusion, replacement of the ethylene tether between the thiazole moiety and the pyridopyrimidine scaffold with an amide bond gave analogues whose stability with respect to photoisomerization was greatly enhanced. This change also unexpectedly led to significant improvement in potency. However, these compounds did not possess sufficient solubility for intravenous use. Efforts to address this issue will be reported in due course.

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