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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2627–2631

Studies on anti-*Helicobacter pylori* agents. Part 3: A novel, efficacious cephem derivative, FR193879

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Received 18 December 2003; accepted 17 February 2004

Abstract—The synthesis, therapeutic efficacy against *H. pylori*, and preliminary safety of the novel cephem derivative, FR193879 (**8a**) are described. Compound **8a** having a (4-carbamoylmethylthiazol-2-yl)thio moiety at the 3-position and a phenylacetamido at the 7-position was found to have good safety showing a nontoxic dose of >100 mg/kg in dogs in a 4-week repeat dose toxicity study and extremely potent therapeutic efficacy against *H. pylori*, showing 30 times superior activity compared to AMPC, and did not display cross-resistance with CAM or MNZ.

Since its discovery in the gastric mucosa of humans, the clinical importance of eradication of *Helicobacter pylori* (*H. pylori*) has increased significantly due to its relationship to many diseases.[1–3] While to date only a small number of multidrug therapy regimens are used for eradication of *H. pylori*, [4] these therapies are not entirely successful, and furthermore, problems remain such as drug resistance,[5,6] side effects,[7,8] and noncompliance.[9] Since there are currently no new anti-*H. pylori* compounds that show superior therapeutic eradication efficacy compared to AMPC and CAM, which are generally used for eradication, the need for alternative and novel structural types is evident and has stimulated the search for novel agents that have potent therapeutic efficacy and resolve the problems associated with current treatment regimens. In a previous paper, we reported the structure–activity relationships of certain cephem derivatives,[10] and from these studies, compound **1** was found to have extremely potent in vitro anti-*H. pylori* activity, superior therapeutic efficacy compared to AMPC and CAM, no cross resistance between CAM or MNZ and low potential for causing diarrhea due to instability to β-lactamase.[11] However, we found that the nontoxic dose for **1** in dogs in a 4-week repeat dose toxicity study was <32 mg/kg, which was considered to be too low in comparison to other oral cephem derivatives on the market, which are generally >100 mg/kg.[12,13] Since we postulated that the greater toxicity originated from decomposition of the 3-position side chain, we sought to obtain novel derivatives with comparable features to **1**, but with improved safety by 3-position modification. In this communication, we wish to report improvement of the toxicity profile as well as studies on therapeutic efficacy for a novel potent anti-*H. pylori* compound **8a** (Fig. 1).

In this research program, we designed novel compounds aiming for the following features: (1) good stability leading to a good recovery rate, (2) moderate lipophilicity

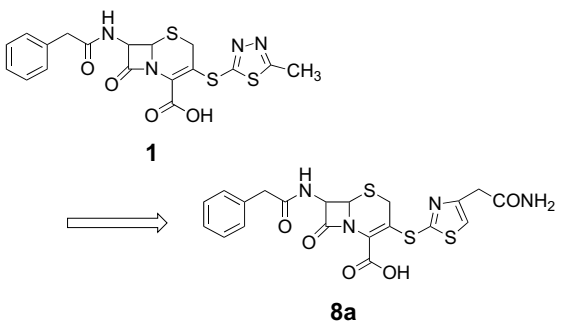

Figure 1.

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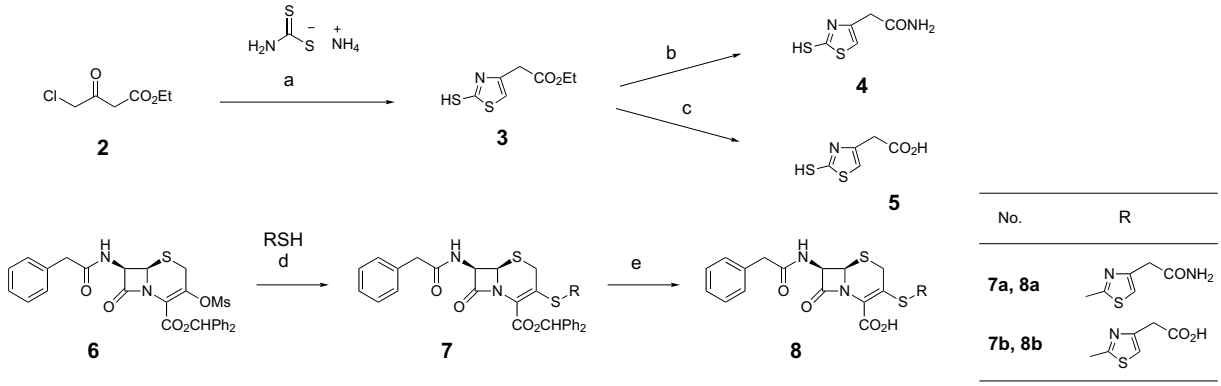
doi:10.1016/j.bmcl.2004.02.068



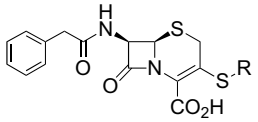
Scheme 1. Reagents: (a) water–EtOH; (b) NH_4OH, NH_4Cl, water; (c) 1 N–NaOH, dioxane; (d) RSH, *t*-BuOK, THF–DME; (e) TFA, anisole, CH_2Cl_2.

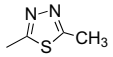
so that compound can enter into the phospholipid layer of the stomach, and (3) good water solubility under acidic conditions.

The derivatives described in this paper, 7-phenylacetamido derivatives having various heterocyclic thio moieties at the 3-position, were synthesized by the routes shown in Scheme 1. Coupling reactions of 3-mesylated compound **6** with heterocyclic mercaptans using potassium *t*-butoxide, followed by deprotection of the benzhydryl ester, afforded **8a**,**b**. Preparation of new heterocyclic mercaptans was performed according to the route shown in Scheme 1. Treatment of **2** with ammonium dithiocarbamate gave mercaptothiazole **3**,[14] which was treated with ammonium hydroxide and ammonium chloride to yield amide compound **4**. Saponification of **3** afforded carboxylic acid **5**. Compounds **1** and **9**–**12** were prepared by the same methods as described in our previous paper.[10] Selected data for compound **8a**: IR (KBr) 3440, 3284, 1776, 1664, 1539, 1357 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 3.52 (dd, 2H, $J = 13.9$, 17.8 Hz), 3.55 (s, 2H), 3.50 and 3.74 (ABq, 2H, $J = 17.6$ Hz), 5.20 (d, 1H, $J = 4.9$ Hz), 5.74 (dd, 1H, $J = 4.9$, 8.4 Hz), 6.99 (br s, 1H), 7.1–7.4 (m, 5H), 7.43 (br s, 1H), 7.53 (s, 1H), 9.21 (d, 1H, $J = 8.4$ Hz); FAB-MS m/z 491.0 (MH^+).

For biological evaluation, we attempted to determine the cause of the low nontoxic dose and estimated total recovery rate (urine and bile) of compound **1** after intravenous administration as a surrogate marker of stability, since we postulated that the toxicity displayed by **1** originated from decomposition of the 3-position side chain. As a result, we found that the recovery rate of **1** was only 33%. Therefore, the recovery rate after intravenous administration was investigated for various compounds bearing thiazole or thiadiazole rings at the 3-position and which had good anti-*H. pylori* activity (Table 1). Consequently, **11** having a thiazole ring at the 3-position had a 63% recovery rate, whilst the three thiadiazole compounds **1**, **9**, and **10** had low recovery rates (9–33%). This data indicated that a compound having a thiazole ring at the 3-position was more stable than compounds having a thiadiazole ring. Accordingly, we decided to search for novel compounds having a thiazole ring and prepared various thiazole derivatives in order to maintain stability, avoid toxicity, and maximize potency.

Table 1. Recovery rate after iv administration



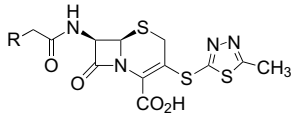
Compound	R	Recovery rate[a] (%)
1		33
9		9
10		21
11		63

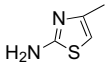
[a] Urine+bile, 20 mg/kg, iv, rat.

In addition to stability, we also screened for physicochemical properties with the objective of determining the main factors that influence treatment efficacy. Initially, we had taken notice of lipophilicity/hydrophilicity and evaluated high performance liquid chromatography (HPLC) retention times as an index of polarity, for compound **1** and especially **12**, which hardly showed therapeutic efficacy, in spite of superior in vitro activity to **1**. As a result, **12** was shown to be much more hydrophilic compared to **1** (Table 2). Therefore, we concluded that too high hydrophilicity was the reason **12** hardly displayed therapeutic efficacy.

Next, we speculated that the most important action route for these cephem compounds for therapeutic efficacy against *H. pylori* was direct action from the gastral cavity, since the oral bioavailability of compounds showing excellent therapeutic effect was not high. As a result, it was expected that the state of a compound in the stomach was an important factor for therapeutic effect. Accordingly, we concluded that a compound

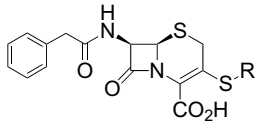
Table 2. Polarity of cephalosporin derivatives

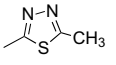
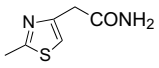
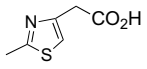


Compound	R	MIC (μg/mL)[a]	Therapeutic efficacy (ratio)[b]	LC-RT (min)[c]
		FP1757	Doses (mg/kg)	
			0.32	
			0.1	
1		0.00156	5/5	10.0
			2/5	
12		0.00078	1/8	1.0
			0/7	

[a] MIC (μg/mL), brucella agar +7% horse blood, 37 °C, 72 h, 10%-CO_2, stamp method.
[b] Mouse; PO, infection; *H. pylori* FP1757, therapy; 2/day × 4 days, termination; 2 weeks after final therapy.
[c] Compound **1** = 10.0, 0.1%TFA–H_2O/CH_3CN = 75/25, column: ODS-80TM, flow rate: 1 mL/min, detection: 254 nm.

Table 3. Therapeutic efficacy and toxicity of cephalosporin derivatives



Compound	R	MIC (μg/mL)[a]	Therapeutic efficacy (ratio)		Gastric mucosa concentration (μg/g)[d]	LC-RT (min)[e]	Solubility (mg/mL)	Recovery rate (%)[f]	Nontoxic dose (mg/kg)[g]
		FP1757	Mouse[b]	Guinea pig[c]	Hour (h)		pH		
		FP1836	Dose (mg/kg)						
			0.32	2.0	0.5		2.0		
			0.1	0.5	1.0		4.0		
			0.032	0.125	2.0				
					4.0				
1		0.00156	5/5	5/5	89	10.0	0.053	33	<32
		0.00313	2/5	2/5	83		1.05		
			1/5	0/5	48				
					23				
8a		0.00078	5/5	5/5	385	6.2	0.431	68	>100
		0.00313	4/5	3/5	163		2.21		
			1/5	0/5	68				
					38				
8b		0.00039	5/5	2/5	377	9.9	0.125	50	NT[h]
		0.00313	1/5	0/5	150		1.82		
			2/5	0/5	111				
					63				
	AMPC	0.0125	1/8	NT	33	NT	NT	NT	NT
		0.05	0/8		12				
			NT		<6				
					NT				

[a] MIC (μg/mL), brucella agar +7% horse blood, 37 °C, 72 h, 10%-CO_2, stamp method.
[b] PO, infection; *H. pylori* FP1757, therapy; 2/day × 4 days, termination; 2 weeks after final therapy.
[c] PO, infection; *H. pylori* FP1836, therapy; 3/day × 4 days, termination; 1 day after final therapy.
[d] Rat, 20 mg/kg (PO), gastric mucosa was scraped from the stomach and homogenated in phosphate buffer.
[e] Compound **1**=10.0, 0.1%TFA–H_2O/CH_3CN=75/25, column: ODS-80TM, flow rate: 1 mL/min, detection: 254 nm.
[f] Urine+bile, 20 mg/kg, iv, rat.
[g] Dog, orally administered for 4 weeks.
[h] NT = not tested.

Table 4. Activity against clarithromycin- and metronidazole-resistant strains and β-lactamase stability

Compound	MIC (μg/mL)			Stability toward β-lactamase					
	Helicobacter pylori			*B. fragilis* FP784[b]			TEM[b]		
	16021[a]	16043[a]	15069[a]	km (μg/mL)	V_{max}[c]	V_{max}/km[c]	km (μg/mL)	V_{max}[c]	V_{max}/km[c]
8a	0.0016	0.0016	0.0063	19.4	0.647	1.060	34.8	1.18	9.38
AMPC	0.025	0.05	0.2	27.3	0.0175	0.0521	22.0	2.94	11.1
CAM	50	50	25	NT[d]	NT	NT	NT	NT	NT
MNZ	50	50	25	NT	NT	NT	NT	NT	NT
CER	NT	NT	NT	31.7	1.0	1.0	276	1.0	1.0

[a] Clarithromycin- and metronidazole-resistant strains.
[b] *B. fragilis* FP784; cephalosporinase, TEM; penicillinase.
[c] Relative value (CER = 1.0), V_{max}/km; larger values mean more unstable.
[d] NT = not tested.

having better water solubility under acidic conditions may show a superior therapeutic effect compared to **1**, since the water solubility of **1** at pH 2.0 and 4.0 was low (Table 3). Therefore, we decided to search for a compound with moderate polarity and good water solubility under acidic conditions and prepared various cephem compounds containing suitable water-solublizing substituents on the thiazole ring at the 3-position. As a result, **8a** and **8b**, which were substituted by carbamoyl or carboxyl groups, respectively, showed similar polar character compared to **1** and much improved water solubility under acidic conditions. In particular, **8a** was 8-fold more soluble in a pH 2 medium.

Next, we estimated gastric mucosa concentration for these compounds since we speculated this might provide a rough standard for therapeutic effect (Table 3). As a result, compounds **8a** and **8b** showed far higher concentrations than **1**, especially after a short contact period. So we evaluated therapeutic efficacies in mice and guinea pigs. Consequently, **8a** showed marginally improved in therapeutic efficacies in terms of both eradication ratio in mice and clearance ratio in guinea pigs. On the other hand, **8b** showed decreased therapeutic efficacy in both models. We speculated that the reason for a lack of clearly improved therapeutic efficacy was as follows: gastric mucosa concentrations showed that the concentrations of **8a** and **8b** at 4 h after administration were not high, while those at a short contact period were much greater than **1**, thus these compounds may readily dissociate from the mucosal layer. However, **8a** is expected to show better therapeutic efficacy in humans, which have thicker gastric mucosa than model animals. Furthermore, the efficacy of **8a** in mice was at least 30 times superior compared to AMPC.

We next estimated recovery rate after intravenous administration as an estimate of stability as a surrogate index of toxicity. As a result, substituted compounds having a thiazole ring at the 3-position (**8a**,**b**) showed good stability rates (68%, 50%, Table 3). Concerning the toxicity of **8a**, the nontoxic dose in dogs in a 4 week repeat dose study was improved to >100 mg/kg, in comparison to the nontoxic dose of **1**, which was <32 mg/kg.

Additionally, we estimated the in vitro effect of **8a** against CAM- and MNZ-resistant strains and instability to β-lactamase (Table 4). Similar to **1**, **8a** showed excellent activity against these strains, which are highly resistant strains in the clinical environment, and there was no cross-resistance with CAM and MNZ. Moreover, low potential for causing diarrhea due to instability to β-lactamase was shown, since **8a** showed equal stability to CEZ, a typical unstable cephem drug, toward cephalosporinase, and toward penicillinase **8a** was 9-fold more unstable than CEM and almost similar to AMPC which is a penicillin derivative.

In summary, we have reported the discovery of FR193879 (**8a**), a novel, safe, and potent cephalosporin anti-*H. pylori* agent, that contains a (4-carbamoylmethylthiazol-2-yl)-thio moiety at the 3-position and a phenylacetamido group at the 7-position. Safety studies showed a nontoxic dose of >100 mg/kg in dogs in a 4 week repeated dose study as well as excellent anti-*H. pylori* therapeutic efficacy, far superior to AMPC, and with no cross-resistance to CAM or MNZ. These results suggest this compound is a suitable candidate for further development.

Acknowledgements

We are grateful to Dr. David Barrett, Medicinal Chemistry Research Laboratories, for his help in preparing this manuscript.

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