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SYNTHESIS AND ANTI-*HELICOBACTER PYLORI* ACTIVITY OF FR182024, A NEW CEPHEM DERIVATIVE

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Abstract

The synthesis and anti-*Helicobacter pylori* activity of a novel cephem derivative FR182024 (**1**) are described. FR182024 having a (5-methyl-1,3,4-thiadiazol-2-yl)-thio moiety at the 3-position and a phenylacetamido at the 7-position was found to have extremely potent *in vitro* anti-*H.pylori* activity, superior therapeutic efficacy to AMPC and CAM, and low potential for causing diarrhea.

Introduction

Since its discovery,[1] the clinical importance of eradication of *Helicobacter pylori* (*H.pylori*) has increased significantly[2] due to its relationship to diseases such as chronic gastritis, peptic ulcer and certain malignant peptic complications. Although multi-therapy regimens containing antibacterial agents, for example amoxicillin (AMPC) and clarithromycin (CAM), with an acid-lowering agent, antiprotozoal agent or bismuth salt, are used for eradication of *H.pylori*,[3] these therapies are not entirely successful. Furthermore, there remain problems such as drug resistance,[4] side effects[5] and non-compliance.[6] However, whilst new anti-*H.pylori* agents have been studied by many investigators,[7] there are few new anti-*H.pylori* compounds that show good therapeutic efficacy, although compounds with good *in vitro* activity[8] are known. As a result, the need for alternative and novel treatment is evident, and has stimulated the search for novel agents that have potent therapeutic efficacy against *H.pylori* and resolve the problems with current treatment. Although generally it has been believed that the anti-*H.pylori* activity of cephem derivatives is rather poor and weaker than that of AMPC, we found in our preliminary study that cephem derivatives display high potential for anti-*H.pylori* activity. Therefore, as part of a screening program of various compounds, we were drawn to cephem derivatives, since they are fundamentally stable under acidic conditions and are less susceptible to degradation in the stomach compared to AMPC and furthermore, much information is available on mechanism, bactericidal activity and toxicity. Furthermore, cephem compounds containing a non-oxime structure at the 7-position have low potential for causing diarrhea, a major side effect of AMPC, due to low

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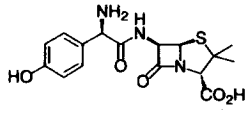
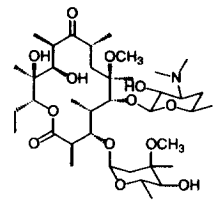
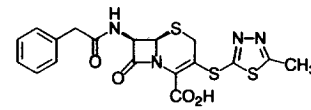
stability towards β-lactamase,[9] and thereby reduced potential for disruption of intestinal microbial flora, due to rapid deactivation by β-lactamase in intestine. Therefore, using this design concept we searched for a compound having a non-oxime structure at the 7-position for low stability towards β-lactamase, along with potent anti-*H.pylori* activity, and investigated the preparation of novel cephem analogs substituted at the 3- and/or 7- position of the cephem nucleus. In this communication, we wish to report the synthesis and biological evaluation of a potent anti-*H.pylori* compound FR182024 (**1**) with excellent *in vitro* activity and therapeutic efficacy and low potential for causing diarrhea.



Amoxicillin(AMPC)



Clarithromycin(CAM)

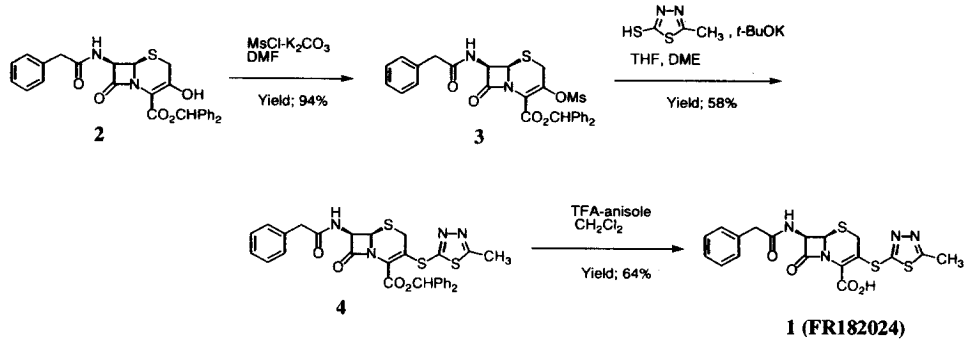


1 (FR182024)

Synthesis

The preparation of FR182024 (**1**) was performed according to the route shown in Scheme 1. 3-Mesylated compound **3** was obtained by mesylation (MsCl-K_2CO_3/DMF, -30°C) of the corresponding 3-hydroxy cephem derivative **2**[10] in good yield. Mesylation using *i*-Pr_2NEt as a base did not afford good results, since it was difficult to control the reaction even at low temperature. Subsequent coupling reaction with 2-mercapto-5-methyl-1,3,4-thiadiazole using *t*-BuOK as a base gave **4**. In this reaction, a mixture of tetrahydrofuran and dimethoxyethane as a solvent afforded good yields. Subsequent deprotection of the benzhydryl ester with TFA/anisole afforded FR182024 (**1**), which was obtained as white crystals from AcOEt by evaporation after the work-up step and recrystallized from water. Selected data for FR182024: mp >200°C (decomp.); IR (KBr) 1784, 1663, 1535 cm^{-1}; ^1H NMR (200MHz, DMSO-d_6) δ 2.73 (s, 3H), 3.52 and 3.62 (ABq, 2H, J = 14.0Hz), 3.55 and 3.85 (ABq, 2H, J = 17.6Hz), 5.22 (d, 1H, J = 5.0Hz), 5.78 (dd, 1H, J = 5.0, 8.3Hz), 7.15-7.35 (m, 5H), 9.24 (d, 1H, J = 8.3Hz); FAB-MS m/z 448.9 (M^+); Anal. Calcd. for $C_{18}H_{16}N_4O_4S_3$: C, 48.20; H, 3.60; N, 12.49. Found: C, 48.65; H, 3.68; N, 12.45.

Scheme 1



Biological Evaluation

The minimum inhibitory concentration values (MIC, μg/ml) against *H.pylori* and therapeutic efficacy in a mouse infection model of FR182024 in comparison to AMPC and CAM are shown in Table 1. The *in vitro* activity of FR182024 was superior to that of the reference compounds; 10-fold improved compared to AMPC and about 50-fold relative to CAM, against all sensitive strains tested. Furthermore, against *H.pylori* 16021, which is a serious clarithromycin-resistant strain in the clinical environment, FR182024 showed excellent activity and there was no cross resistance with CAM. Next, we examined the therapeutic efficacy in a mouse model. FR182024 showed superior therapeutic effect, reflecting the *in vitro* activity, compared to the reference compounds. FR182024 eradicated *H.pylori* in all mice at a dosage of 0.32mg/kg, at which AMPC was almost inactive and CAM was completely inactive. Furthermore, FR182024 showed an eradication effect even at a dosage of 0.1mg/kg in 5 out of 8 mice.

In the next phase, we evaluated the stability of FR182024 towards β-lactamase, and the relative values compared to AMPC and cephaloridine (CER), a typical drug that is unstable towards cephalosporinase, are shown in Table 2. FR182024 was more unstable than CER towards both β-lactamases, and towards penicillinase, FR182024 was only 2-fold more stable compared to AMPC.

These data showed that FR182024 is superior in terms of *in vitro* activity and therapeutic efficacy against *H.pylori* compared to the reference compounds, and has low potential for causing diarrhea due to low stability towards β-lactamase.

Table 1 *In vitro* Activity and *In vivo* Therapeutic Efficacy

Compound	MIC(μg/ml)[1]				Therapeutic Efficacy[3] (eradication ratio)			
	Helicobacter pylori				dose(mg/kg)			
	9005	13001	FP1757	16021[2]	0.1	0.32	1	3.2
FR182024	0.00625	0.00078	0.00156	0.0016	5/8	8/8	N.T.[4]	N.T.
AMPC	0.05	0.025	0.0125	0.025	0/8	1/8	3/8	7/8
CAM	0.2	0.05	0.1	50	N.T.	0/8	0/8	0/8

1): MIC(μg/ml) ; brucella agar + 7% horse blood, 37℃, 72h, 10%-CO_2, stamp method
2): clarithromycin-resistant strain
3): mouse, PO, infection: *H.pylori* FP1757, therapy: 2/dayx4days, termination: 2 weeks after final therapy
4): not tested

Table 2 Stability towards β-lactamase

Compound	*B. fragilis* FP784[1]			*TEM*[1]		
	Km(μg/ml)	Vmax[2]	Vmax/Km[2]	Km(μg/ml)	Vmax[2]	Vmax/Km[2]
FR182024	14.5	0.351	1.98	47.4	3.61	6.33
AMPC	27.3	0.0175	0.0521	22.0	2.94	11.1
CER	31.7	1.0	1.0	276	1.0	1.0

1): *B. fragilis* FP784; cephalosporinase, *TEM*; penicillinase
2): relative value (CER=1.0)

Summary

In this communication, we have reported the discovery of FR182024 (**1**), a novel, potent cephalosporin anti-*H.pylori* agent, that contains a (5-methyl-1,3,4-thiadiazol-2-yl)-thio moiety at the 3-position and a phenylacetamido group at the 7-position. Excellent anti-*H.pylori* therapeutic efficacy superior to AMPC and CAM as well as potenct *in vitro* activity, and low potential for causing diarrhea due to low stability towards β-lactamase make this compound a suitable candidate for further development. Future publications will report detailed therapeutic effects as well as structure activity relationships.

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