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The discovery and structure–activity relationships leading to CE-156811, a difluorophenyl cyclopropyl fluoroether: A novel potent antibacterial analog derived from hygromycin A

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ABSTRACT

SAR studies and optimization of various modified Hygromycin A fluoroalkyl ethers, which led to the discovery of the highly potent 4′-(2-cyclopropyl-2-fluoroethyl ether) antibacterial CE-156811 (**1**) derived from truncation of the ribose ring and difluorination of the phenyl found in Hygromycin A, are discussed.

Infections with bacterial pathogens that have developed resistance to a number of important classes of antibiotics continue to present highly challenging treatment problems for physicians.[1,2,8–10] As a consequence, discovery of novel antibacterial agents to combat these resistant strains remains of high interest due to high medical need.

The natural product Hygromycin A, discovered in 1953, has only weak activity against medically important Gram-positive organisms.[3,4] It demonstrates potent activity against the organism implicated in swine dysentery. From mode of action studies carried out a number of years ago, it was established that Hygromycin A interferes with bacterial growth by inhibition of protein synthesis involving inhibition of peptide bond formation.[5] Our study concerning Hygromycin A analogs as potential new leads within the Human Health anti-infective group at Pfizer was initiated as a consequence of an intensive examination of a broad selection of known natural products published in the literature. Earlier efforts reported a series of modified Hygromycin A analogs, resulting from investigations during a Pfizer Animal Health research program that was ultimately terminated. Hecker, Jaynes, and coworkers established that the Hygromycin A furanose ring could be effectively replaced by simple alkyl ether substituents, for example, 4′-allyl ether, and that the phenol hydroxyl could be replaced with fluorine. The 2,5-difluorophenyl analog in particular demonstrated improved activity against the animal pathogens of interest, as well as significant improvement against key Gram-positive human pathogens.[6,7]

CE-156811 (**1**) is a novel truncated Hygromycin A derivative possessing potent in vitro activities against important Gram-positive bacterial pathogens. It also demonstrated efficacy commensurate with linezolid in several in vivo infection models with subcutaneously (sc) dosing (Fig. 1).

The preparation of **5** started with condensation between phosphonate **3**[7] and commercially available aldehyde **2** under basic conditions (Scheme 1). Different ether substituents at the 4-aryl position of **5** were appended in a reasonably straightforward manner using nucleophilic aromatic substitution with a variety of alcohols on the trifluorophenyl intermediate **4**. This typically gave the desired *para* substituted analogs as the primary products in low-to-modest (15–45%) yields; minor amounts of the product of *ortho* substitution were also isolable. The selectivity can be inferred to arise from steric hindrance at the *ortho* site. As the isomers were separable by chromatography, the use of this method allowed us to prosecute the SAR in an expeditious manner.

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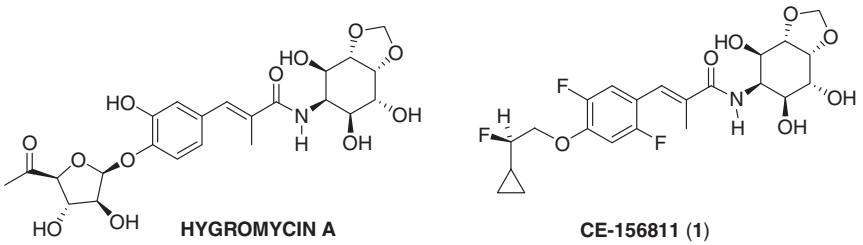
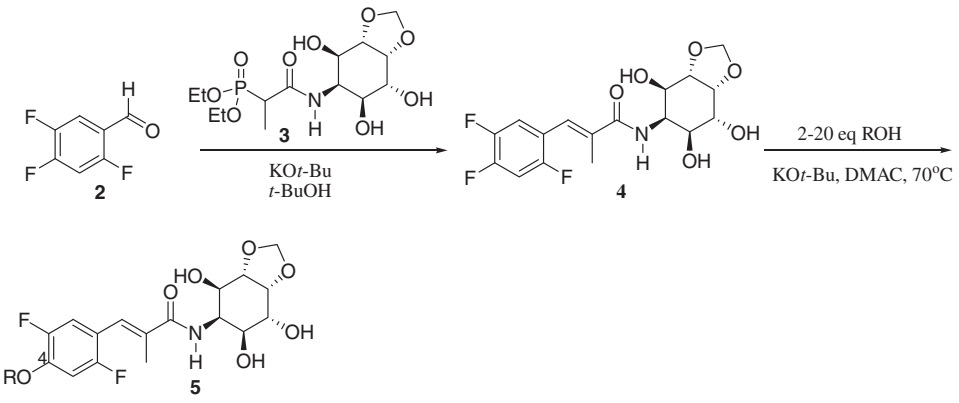


Figure 1. The structures of Hygromycin A and CE-156811 (**1**).



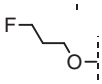
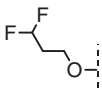
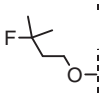
Scheme 1. Synthesis of 2,5-difluorophenyl 4-ethers.

Using this approach, the compounds in Table 1 were prepared. In the acyclic 4′-alkyl ether series, as exemplified in the generic structure **5**, going from ethyl ether **6** to hydroxyl ethyl ether **7**, potency dropped across species. However, when fluorine was introduced in place of the hydroxyl found in **7** to give **8**, the potency was regained; 3-fluoropropyl ether **9** provided the optimal antibacterial activity in this acyclic series. Based on this finding, we prepared additional 3-fluoropropyl analogs in an attempt to broaden the SAR. These efforts were generally unsuccessful, as a number of additional compounds demonstrated an overall weaker antibacterial activity spectrum (e.g., compounds **10** and **11**).

A more promising avenue became evident when we examined the addition of substituents to the C2-carbon of the 2-fluoroethyl ether moiety of **8**; it is to be noted that the parent 2-fluoroethyl ether itself has only modest antibacterial activity. However, placement of small alkyl moieties at this site proved to considerably boost the in vitro potency; for example, **12** demonstrated an 8- to 20-fold improvement in activity compared with **8**. In a similar manner, the importance of fluorination at the 2-ethyl ether position in potentiating activity was demonstrated; for example, **13** is 2-fold more potent than cyclobutylmethyl ether **14** (Table 2).

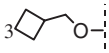
Due to these observations, we hypothesized that there was potentially a small binding pocket at the site of activity that accommodated these small alkyl substituents and the 2-fluoro heteroatom on the ethyl ether template. We therefore targeted a broader examination of related compounds to better understand

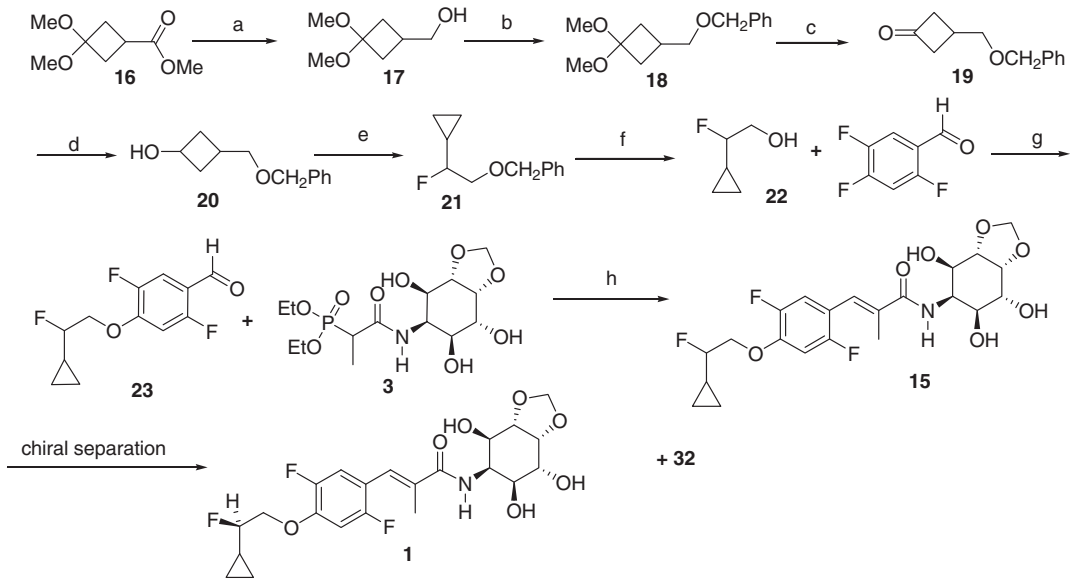
Table 1
In vitro antibacterial activities (MIC, μg/mL) of early analogs

Compound	R	*S. aureus* 1095[a]	*E. faecalis* 1085[b]	*E. faecium* 1022[c]	*S. pneumo* 1016[d]
6		100	25	12.5	NA
7		100	>100	>100	NA
8		50	25	25	6.2
9		1.56	1.56	0.78	2
10		12.5	12.5	3.13	1.56
11		100	100	50	25

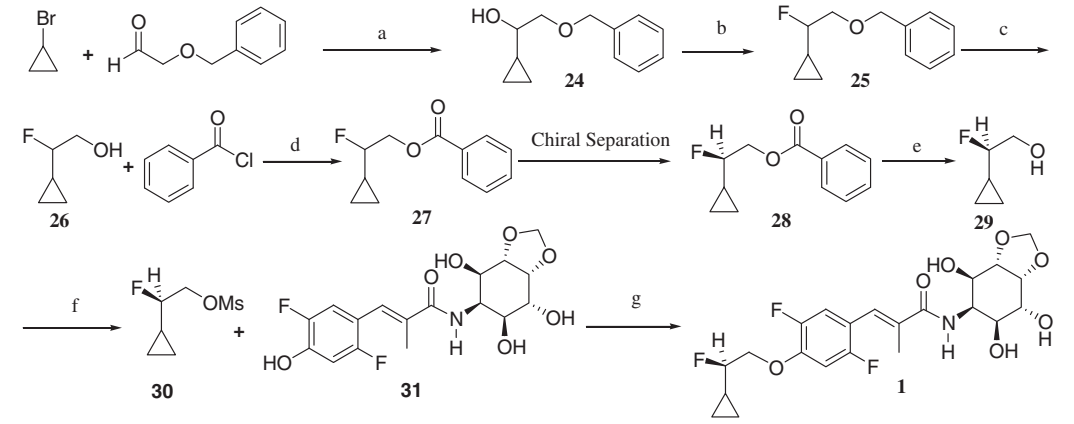
[a] *S. aureus, Staphylococcus aureus.*
[b] *E. faecalis, Enterococcus faecalis.*
[c] *E. faecium, Enterococcus faecium.*
[d] *S. pneumo, Streptococcus pneumoniae.*

Table 2
In vitro antibacterial activities (MIC, μg/mL) of follow-up analogs

Compound	R	*S. aureus* 1095	*E. faecalis* 1085	*E. faecium* 1022	*S. pneumo* 1016
12		6.25	⩽0.2	3.13	⩽0.2
13		6.2	6.2	6.2	3.1
14		12.5	12.5	6.2	6.2
15		1.56	1.6	1.6	0.4



Scheme 2. First generation synthesis of **1**. Reagents and conditions: (a) LAH, THF, −78 °C to rt (78%); (b) benzyl bromide 60% NaH, DMF; (c) 1 N HCl, MeOH/H_2O (1:3), rt; (d) LAH, THF, −78 °C to rt (44% for three-steps); (e) DAST, CH_2Cl_2, −78°C to rt (50%); (g) K_2CO_3, 65 °C, neat (22%); (h) KO*t*-Bu, *t*-BuOH, rt (36%).



Scheme 3. Second generation synthesis of **1**. Reagents and conditions: (a) *tert*-butyllithium in pentane THF, −78 °C to rt; (b) DAST, CH_2Cl_2 (68–73% for two-steps); (c) 10% Pd/C, H_2 , MeOH, rt; (d) TEA, CH_2Cl_2, rt (87% for two-steps); (e) K_2CO_3, MeOH, H_2O, rt; (f) MsCl, TEA, CH_2Cl_2, rt; (g) K_2CO_3, DMF, 75 °C, (33.6% yield for three-steps).

the SAR associated with other fluorinated cyclobutyl analogs, and search for improved activity.

One of these targeted cyclobutyl analogs had a fluorine introduced at the 3-position in the cyclobutyl methyl ether **14**. We envisioned this would be readily accessible in a straightforward manner from reaction of the corresponding alcohol **20** with DAST. In practice, however, this manipulation resulted in an unusual but precedented[13] rearrangement to give cyclopropyl fluoroethyl ether **21** (Scheme 2). This compound represented an attractive potential side chain for our examination. Preparation of **1** is shown in Scheme 2.

Cyclobutanol **20** was readily afforded by LAH reduction of the cyclobutanone **19**. This requisite ketone was obtained in a straightforward three-step transformation of the known cyclobutanone

Table 3
In vitro activity of **1** [MIC_{90} (μg/ml)]

Organism	Compound **1**	Linezolid
MSSA (28)[a]	1	2
MRSA (18)[b]	0.5	1
GISA (6)[c]	0.125–0.5	0.5–1
MSSE (23)[d]	0.25	1
PRSP (21)[e]	0.5	1
E. faecalis (27)	0.5	1
E. faecium (21)	1	2

[a] Methicillin-sensitive *S. aureus* (MSSA).
[b] Methicillin-resistant *S. aureus* (MRSA).
[c] Glycopeptide-intermediate *S. aureus (*GISA*)*.
[d] Methicillin-sensitive *Staphylococcus epidermidis* (MSSE).
[e] Penicillin-resistant *Streptococcus pneumoniae* (PRSP).

Table 4
In vivo PK data for **1**

PK species	Cl (mL/min/kg)	V_{dss} (L/kg)	$T_{1/2}$ (h)	*F*%[a]	Plasma protein binding (% unbound)[c]
Mouse	12.1	0.52	1.4	55	10.4
Rat	10.5	0.5	1.3	56	10.7
Monkey	8.6	0.59	1.0	ND[b]	17.2
Dog	5.4	0.71	1.9	ND	18.6

[a] *F*% oral bioavailability. Oral dose in rat = 10 mg/kg, iv dose = 5 mg/kg.
[b] ND = Not determined.
[c] CE-156811 concentration = 3 μg/ml.

ketal ester **16**.[11] Upon hydrogenation of **21** (Pd/C, H_2) to give the alcohol **22**, nucleophilic aromatic substitution of the alcohol **22** onto the 2,4,5-trifluorobenzaldehyde gave the 4-substituted ether benzaldehyde **23** after separation of isomers. This condensed with the phosphonate **3** under basic conditions to give **15** as a mixture of diastereomers. Compound **15** demonstrated highly potent antibacterial activity (Table 2). Upon chiral column HPLC purification, diastereomer mixture **15** was separated to give **1** and its corresponding diastereomer **32**. The absolute stereochemistry of the most active of these two (**1**) was inferred from an X-ray crystal structure.[14]

Because of the overall low yield and the fact that the chiral separation was done in the last step in this original route, a second generation synthesis was executed (Scheme 3). The approach began with *t*-BuLi metallation of bromocyclopropane and addition to 2-(benzyloxy)acetaldehyde to afford the secondary alcohol **24**. Compound **24** was treated with DAST in methylene chloride to give the desired fluorinated benzyl ether **25** in 68–73% yield over the previous two steps. After hydrogenation of benzyl ether **25**, 2-cyclopropyl-2-fluoroethanol **26** was then converted to benzoate **27** for ease of chiral separation, serving to both provide a UV chromophore and being less prone to loss during evaporative workup of column fractions than the more volatile alcohol **26**. After saponification of **28**, the unstable and low boiling alcohol was immediately converted to the mesylate **30**, which was then used to alkylate the difluorophenol **31** shown to provide **1**.

Compound **1** demonstrated potent in vitro activity across multiple drug-resistant organisms *S. aureus, S. pneumoniae, E. faecium, E. faecalis* and was comparable with linezolid (Table 3).

In in vivo studies across the four species examined (mouse, rat, monkey, and dog), **1** exhibited a good PK profile (Table 4). The human predicted $T_{½}$ based upon simple allometric scaling is approximately 2 h.

In vivo efficacy studies also showed that **1** had comparable activity to linezolid across several different acute models when dosed subcutaneously (sc) BID for one day and three days (Table 5). When compared to vancomycin, linezolid, and levofloxacin, **1** demonstrated activity comparable to that of linezolid.[12]

Table 5
In vivo efficacy of **1** (sc dosing) versus linezolid, (p.o. dosing) PD_{50} (mg/kg)

	1	Linezolid
Acute *S. aureus*[a]	4	4–7
Acute *E. faecium*[a]	8	4–8
Acute *E. faecalis*[a]	10	3–5
Lung *S. pneumoniae*[b]	23	10–13
Acute *S. pyogenes*[a]	3	3

[a] Dosed BID for one day.
[b] Dosed BID for three days.

Conclusions: We report the SAR in the 4′ position of the difluorophenyl ring of truncated Hygromycin A derivatives that led to the synthesis of **1**, a novel and highly potent inhibitor of Gram-positive bacteria with a very good PK profile in multiple animal species and excellent in vivo efficacy. Unfortunately, further development with this compound was precluded upon finding that **1** was insufficiently stable in water. When **1** was studied in water, it degraded to the extent of 4% per day at rt. After 5.5 days, compound **1** showed almost 25% conversion of the cyclopropyl fluoroethyl ether to the corresponding cyclopropyl hydroxyethyl ether.[12] Further lead optimization in the aminocyclitol has recently been reported.[15]

Acknowledgement

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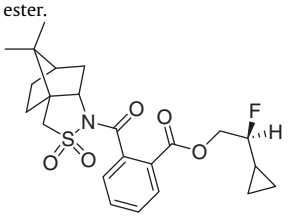
14. An X-ray crystal structure was obtained from the following structure, which was obtained from reacting the isomer of **29** with the corresponding substituted benzoate ester.



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