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Synthesis and antibacterial activity of alaremycin derivatives for the porphobilinogen synthase

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ABSTRACT

The preparation and the antibacterial activity of alaremycin derivatives such as their CF_3-derivatives and (*R*)- and (*S*)-4-oxo-5-acetylaminohexanoic acid for the porphobilinogen synthase (PBGS), were described. The IC_{50} values of the antibacterial activity of the prepared materials for the inhibitor of PBGS, were determined using PBGS assay.

The development of multi-drug-resistant pathogens has become a serious problem in the chemotherapy of bacterial infections diseases. One of the strategies to overcome this problem is to find a new drug with a molecular target. For this purpose, Wachi and co-workers have reported a new antibiotic, which is structurally related to 5-aminolevulinic acid, a precurser of heme biosynthesis, and named alaremycin, was isolated from the culture broth of *Streptomyces* sp. A012304.[1] The antibacterial activity of alaremycin was enhanced in the presence of 5-aminolevulinic acid (ALA). It is reported that alaremycin may affect the heme biosynthetic pathway.[1] Two ALA molecules would be condensed by porphobilinogen synthase (hemB protein; PBGS) to produce porphobilinogen reported by Frere et al.[2]

In the above reaction system, ALA first forms a Schiff base with the enzyme. Alaremycin might inhibit this reaction by competing with ALA. If so, the addition of an excess amount of ALA to the medium should relieve the lethal effect of alaremycin. Alaremycin contains amide group and methylidene group which are not contained in known PBGS inhibitors, so alaremycin might be expected as the novel backbone of PBGS inhibitor.[3–11] In 2010, the co-crystallization of *P. aeruginosa* phorphobiinogen synthase with alaremycin have been reported to understand the molecular basis of alaremycin's antibiotic activity at the atomic level. The crystal structure have revealed that the antibiotic efficiently blocked the active site of phorphobiinogen synthase. Further, in the above Letter, it is suggested that 4-oxo-5-acetylaminohexanoic acid derived from a reduced derivative of 4-oxo-5-acetylaminohexenoic acid (alaremycin) is important material to understand the alaremycin's antibiotic activity for *P. aeruginosa* phorphobiinogen synthase.[12]

In general, it is well known the introduction of fluorine atoms into the target hydrocarbon materials for the purpose of the improvement of biological activities.[13–16] As it is interesting in the introduction of fluorine atoms into the alaremycin and their derivatives to improve the activity against porphobilinogen synthase (PBGS), we have designed the introduction of fluorine atoms into the alaremycin and its derivatives.

In this Letter, we would like to describe the synthesis of CF_3–alaremycin derivatives, (*R*)- and (*S*)-4-oxo-5-acetylaminohexanoic acid and their fluorinated derivatives. Further, we describe the IC_{50} values of the antibacterial activity of the obtained derivatives for the inhibitor of porphobilinogen synthase (PBGS).

For the purpose of the development of the potent PBGS inhibitor for *Pseudomonas* sp. including the human pathogen *P. aeruginosa*, we have designed several kinds of alaremycin derivatives and their CF_3-derivatives as synthetic candidates as shown in Figure 1. Especially, we have challenged to search the improvement of antibacterial activity based on the effect of fluorine atoms, methyidene group and the carbon length.

At first, according to a total synthetic strategy devised by retrosynthetic analysis, alaremycin derivatives were successfully synthesized. In Scheme 1, we have examined the synthesis of 4-oxo-5-acetylaminopentanoic acid **1** which is the absence of methylidene group in alaremycin.

To transform from the methylidene group to methyl group, we have designed the synthetic route as shown in Scheme 2. To synthesize (*R*)- and (*S*)-4-oxo-5-acetylaminohexanoic acid **3**, the

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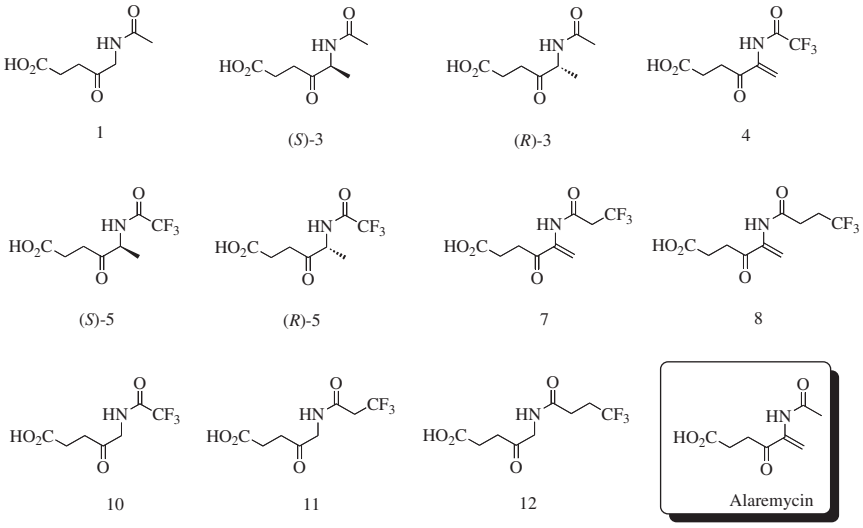
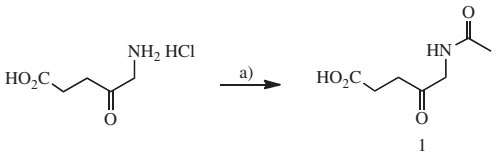
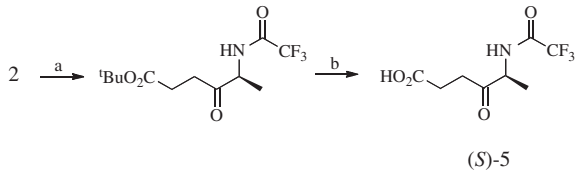


Figure 1. Structures of synthetic candidate.



Scheme 1. Reagents and conditions: (a) Ac_2O, Na_2CO_3, H_2O, 0 °C, 1 h then rt, 36 h, yield 28%.



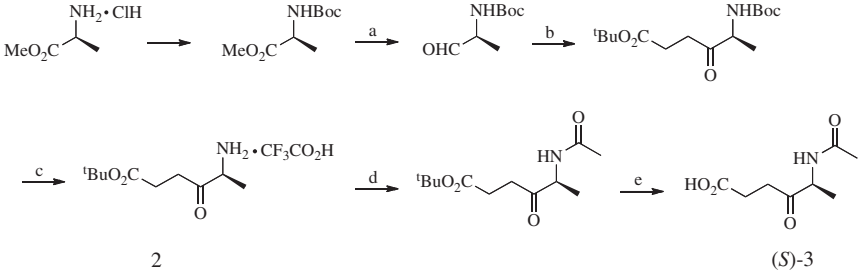
Scheme 4. Reagents and conditions: (a) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, rt; (b) H_2O, rt.

methylidene group of alaremycin was replaced by the chiral methyl group, and then (*R*)- and/or (*S*)-**3** was prepared from (*R*)- and/or (*S*)-alanine as a starting material. In the synthetic route to (*R*)- and/or (*S*)-**3**, the step b using Stetter reaction was the key step.

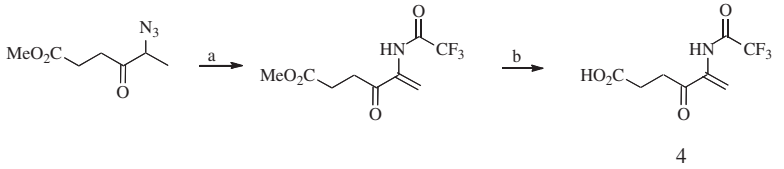
Further, as Kobayashi and co-workers have reported the synthetic route to alaremycin via azide intemediate,[17] we have designed the introduction of fluorine atoms into alaremycin to search the improvement of the activity. The CF_3–alaremycin (4-oxo-5-(trifluoroacetylamino)-5-hexenoic acid **4)** as the target material was prepared by the following Scheme 3.

To prepare the fluorinated derivatives of (*R*)- and (*S*)-4-4-oxo-5-acetylaminohexanoic acid **3**, we have examined the synthesis of (*R*)- and (*S*)-4-oxo-5-(trifluoroacetylamino)hexanoic acid **5** derived from (*R*)- and/or (*S*)-alanine as shown in Scheme 4.

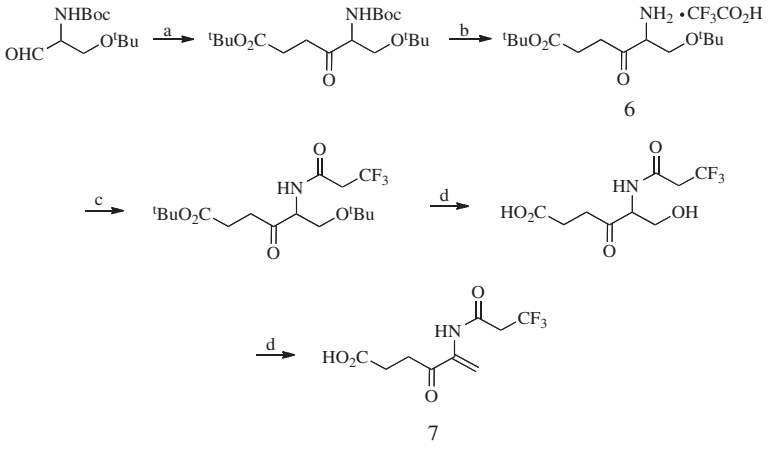
In the next step, to search the alaremycin derivatives with highly activity, we have designed the expansion of carbon length



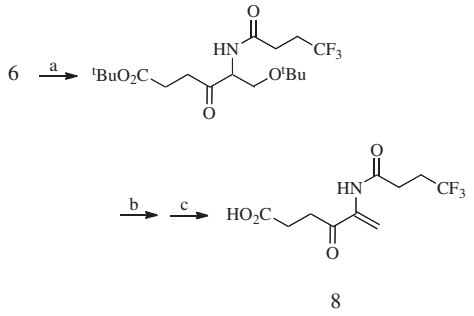
Scheme 2. Reagents and conditions: (a) DIBAL-H, toluene, −78 °C, 2 h, yield 53%; (b) 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide, *tert*-butyl acrylate, DBU, MS4A, THF, 50 °C, 10 h, yield 33%; (c) TFA, CH_2Cl_2, 0 °C, yield 43%; (d) $(CH_3CO)_2O$, Et_3N, CH_2Cl_2, rt, yield 86%; (e) H_2O, TFA, rt, yield 92%.



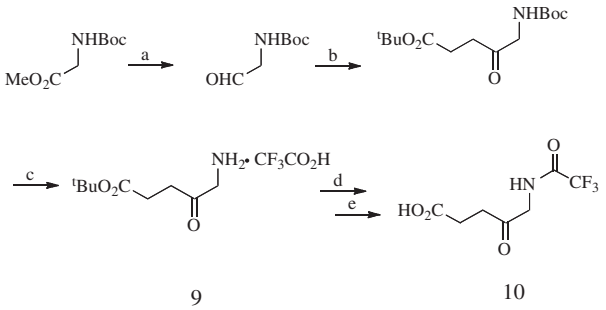
Scheme 3. Reagents and conditions: (a) $NaReO_4$, CF_3SO_3H, $(CF_3CO)_2O$, CCl_4, 50 °C, 12 h, yield 60%; (b) 1*N* LiOH, H_2O, THF, 0 °C, 1 h, yield 50%.



Scheme 5. Reagents and conditions: (a) 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide, methyl acrylate, DBU, MS4A, THF, 50 °C, 20 h, yield 41%; (b) 20% TFA in CH_2Cl_2, 0 °C, 2 h, yield 67%; (c) CF_3CH_2COCl, Et_3N, CH_2Cl_2, rt, overnight, yield 65%; (d) TFA, H_2O, rt, 3 h, yield 91%; (e) $(Boc)_2O$, DMAP, MeCN, rt, overnight then TMG, rt, 2 h, yield 43%.



Scheme 6. Reagents and conditions: (a) $CF_3CH_2CH_2COCl$, Et_3N, CH_2Cl_2, rt, overnight, yield 63%; (b) TFA, H_2O, 0 °C, 3 h, yield 88%; (c) $(Boc)_2O$, DMAP, MeCN, rt overnight then TMG, rt, 24 h, yield 52%.

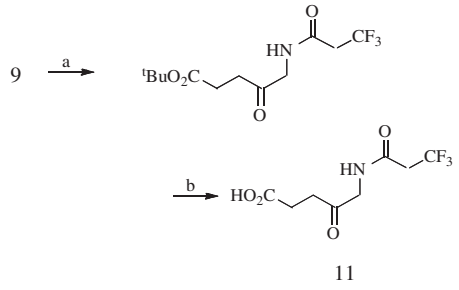


Scheme 7. Reagents and conditions: (a) DIBAL-H, toluene, −78 °C, 2 h, yield 82%; (b) 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide, *tert*-butyl acrylate, DBU, MS4A, THF, reflux, 12 h, yield 35%; (c) TFA in CH_2Cl_2; (d) $CF_3CH_2CO_2H$, Et_3N, CH_2Cl_2, DMF, $(COCl)_2$, yield 75%; (e) TFA, H_2O, rt, 3 h, yield 55%.

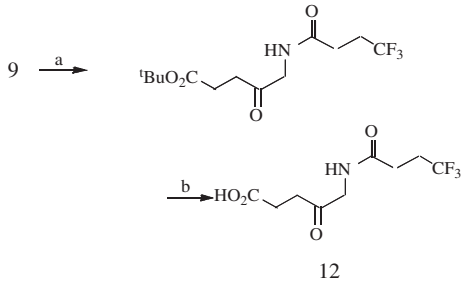
of the trifluoroacetylamido group in CF_3–alaremycin. At first, we have prepared 4-oxo-5-[(3,3,3-trifluoro-1-oxopropyl)amino]-5-hexenoic acid **7** and 4-oxo-5-[(4,4,4-trifluoro-1-oxobutyl)amino]-5-hexenoic acid **8** as shown in Schemes 5 and 6.

To prepare CF_3-derivative of compound **1** and the related derivatives, we have developed the synthetic routes for the introduction of fluorine atoms into the target materials (**10**–**12**) using the following Schemes 7–9.

The IC_{50} values of alaremycin and alaremycin derivatives were determined using PBGS assay.[18] The protocol is summarized in Figure 2.



Scheme 8. Reagents and conditions: (a) $CF_3CH_2CO_2H$, $(COCl)_2$, CH_2Cl_2, DMF, Et_3N, yield 74%; (b) TFA, H_2O, rt, 3 h, yield 88%.



Scheme 9. Reagents and conditions: (a) $CF_3CH_2CH_2CO_2H$, $(COCl)_2$, CH_2Cl_2, DMF, Et_3N, yield 44%; (b) TFA, H_2O, rt, 3 h, yield 71%.

Obviously, from the results of the IC_{50} values as shown in Figure 3 and Table 1, we have found that the PBGS inhibitory activity of (*R*)-**3** and (*S*)-**3** have been improved up to about two and/or four times more than that of alaremycin. Further, it is interesting the difference of antibacterial activity between (*R*)-**3** and (*S*)-**3**. However, the activity of compound **5** as fluorinated modification of compound **3** is not higher more than that of alaremycin as shown in Figure 5 and Table 1. Further, compound **1** which is the absence of methylidene group does not improve the activity, and its CF_3-derivative **10** is also no activity as shown in Figures 3 and 5 and Table 1. However, the IC_{50} values as shown in Figures 4 and 5 and Table 1 suggest the effect of the introduction of fluorine atoms for the improvement of antibacterial activity. Especially, we have found that CF_3–alaremycin derivatives (compounds **4**, **7**, **8**, **11** and **12**) inhibit the PBGS in *Pseudomanas fluorescens*, and that

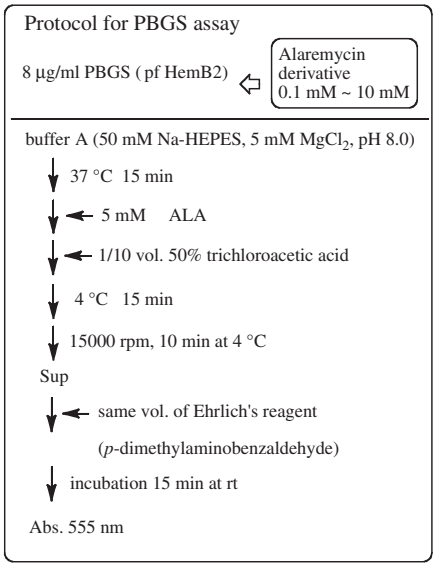


Figure 2. The IC_{50} values using PBGS assay.

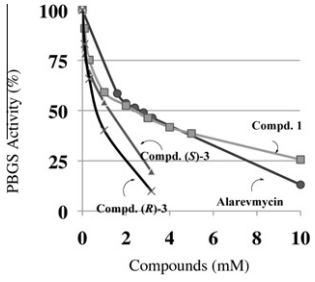


Figure 3. PBGS activity of compounds (*R*)-**3**, (*S*)-**3** and **1**.

Table 1
IC_{50} values of compounds

Compound no.	Activity IC_{50} (mM)	Compound no.	Activity IC_{50} (mM)
1	2.5	**7**	0.9
(*R*)-**3**	0.7	**8**	0.051
(*S*)-**3**	1.1	**10**	8.0
4	0.022	**11**	0.5
(*R*)-**5**	4.1	**12**	1.3
(*S*)-**5**	3.0	**Alaremycin**	2.6

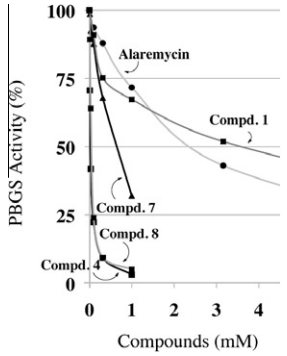


Figure 4. PBGS activity of compounds **4**, **7** and **8**.

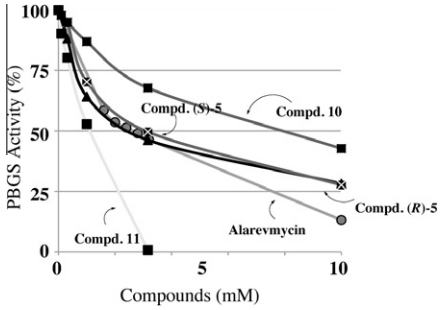


Figure 5. PBGS activity of compounds (*R*)-**5**, (*S*)-**5**, **10** and **11**.

the PBGS inhibitory activity of compounds **4** and **8** have been improved up to about 100 and/or 50 times more than that of alaremycin.

In conclusion, we have established the synthetic route for CF_3–alaremycin derivatives. Further, we have found the PBGS inhibitory activity of obtained CF_3–alaremycin derivatives have been increased up to about 50 and/or 100 times more than that of alaremycin. This is the first example for the preparation and the antibacterial activity for the porphobilinogen synthase (PBGS) of CF_3–alaremycin derivatives.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.106.

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