Bioorganic & Medicinal Chemistry 21 (2013) 5841–5850

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Metallo-β-lactamase inhibitory activity of 3-alkyloxy and 3-amino phthalic acid derivatives and their combination effect with carbapenem

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ARTICLE INFO

Article history:
Received 24 May 2013
Accepted 4 July 2013
Available online 18 July 2013

Keywords:
Metallo-β-lactamase
Pseudomonas aeruginosa

ABSTRACT

3-Alkyloxy and 3-amino phthalic acid derivatives were found to have metallo-β-lactamase inhibitory activity. Among them, 3-amino phthalic acid derivatives showed both potent activity against metallo-β-lactamase, IMP-1 inhibitory activity and a strong combination effect with biapenem (BIPM), carbapenem antibiotic. In particular, the 4′-hydroxy-piperidine derivative showed strong IMP-1 inhibitory activity and a combination effect with various antibiotics.

1. Introduction

Owing to their efficacy and safety, β-lactam antibiotics are widely used for the treatment of bacterial infection in the clinical field. It is well known, however, that mechanisms of resistance to β-lactam antibiotics exist. In this regard, inactivation by β-lactamase is the major mechanism of resistance against β-lactam antibiotics.

β-Lactamases are classified as four types A–D, based on their amino acid sequence homology by Amber classification.[1] Due to serine-catalysed hydrolysis of the β-lactam ring, class A, C, and D β-lactamases are called serine β-lactamases. On the other hand, class B β-lactamase is a metallo-β-lactamase (MBL) having one or two zinc ions in the active site.[2] Various types of metallo-β-lactamases, for instance, IMP, VIM, and SPM types etc., have been reported. In Japan, the IMP-1 metallo-β-lactamase has been reported so far.[3] MBLs can hydrolyze almost all β-lactam antibiotics, including penicillins, cephalosporins and carbapenems.

Carbapenems play an important role in the clinical field because they are effective against both Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*. Therefore, MBL-producing pathogens are a significant problem in the clinical field. In addition, multi-drug resistant *P. aeruginosa* (MDRP) strains are a significant problem because of their resistance against aminoglycosides, carbapenems and quinolones, which are normally effective against Gram-negative bacteria. Moreover, it is reported that MDRP strains produce MBLs at high frequency.[4]

As a result, we are interesting in developing a MBL inhibitor. In a previous study, we found that phthalic acids substituted with a bulky group at the 3-position had potent IMP-1 inhibitory activity.[5] In particular, compound **1**, which is substituted with a 4′-hydroxyphenyl group at the 3-position, was found to have the most potent IMP-1 inhibitory activity (IC_{50} = 1.55 μM) (Fig. 1). Furthermore, compound **1** showed a combination effect with biapenem (BIPM), a carbapenem antibiotic, against IMP-1 *P. aeruginosa* strains producing IMP-1. As a result, here we have continued to develop a more potent MBL inhibitor.

2. Results and discussion

2.1. Chemistry

Scheme 1 shows the synthesis of 3-alkyloxy phthalic acid derivatives from commercially available 3-hydroxyphthalic anhydride. Esterification of 3-hydroxyphthalic anhydride, followed by alkylation of the hydroxyl group, gave **4**. Hydrolysis of the diethylester **4** under alkaline conditions gave the phthalic acid **5** in good yield.

Scheme 2 shows the synthesis of 3-aminophthalic acid derivatives from commercially available 3-nitrophthalic acid. Two step esterification of the 3-nitrophthalic acid **6** gave the diethylester **7**. Hydrogenation of **7**, followed by alkylation of the 3-amino group of **8**, afforded **9**. Hydrolysis of the diethyl ester **9** under alkaline conditions afforded **10**.

3-Dimethylamino phthalic acid and its cyclic amine derivatives were synthesized from 3-fluorophthalic acid (Scheme 3). Esterification of the 3-fluorophthalic acid **11** afforded the diethyl

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http://dx.doi.org/10.1016/j.bmc.2013.07.006

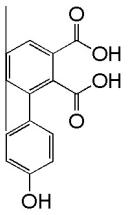

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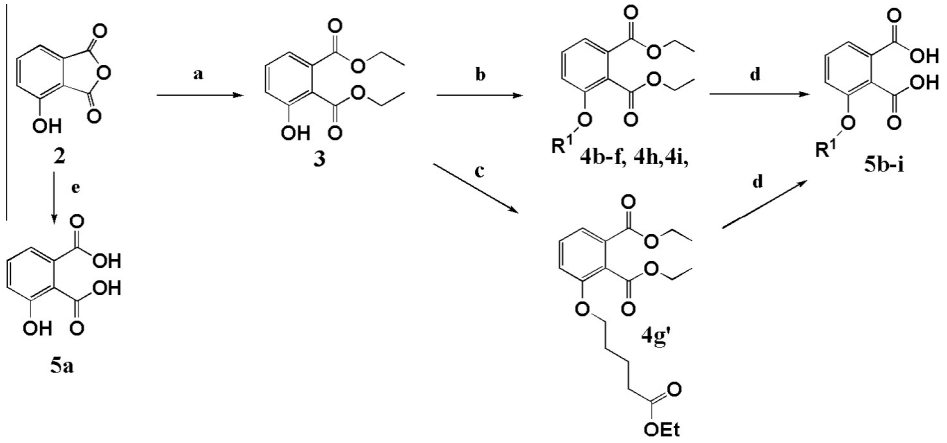
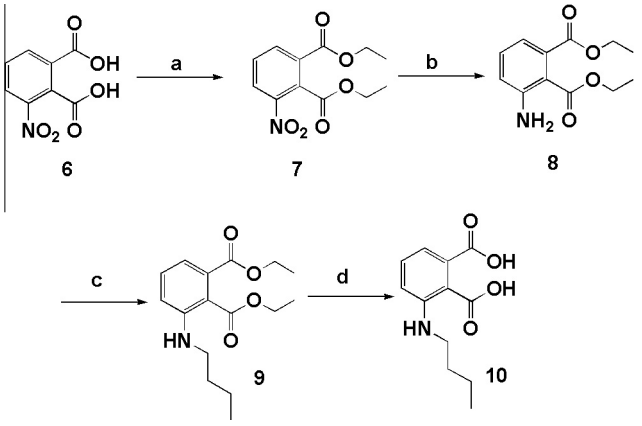
Figure 1. Lead compound of IMP-1 inhibitor



Scheme 1. Synthesis of 3-alkoxyphthalic acid derivatives. Reagents and conditions: (a) H_2SO_4, EtOH, reflux; (b) CH_3I, K_2CO_3, DMF, rt (**4b**); benzylbromide, K_2CO_3, DMF, rt (**4c**); *n*BuI, K_2CO_3, DMF, rt (**4d**); (3-bromopropyl)cyclohexane, K_2CO_3, DMF, rt (**4e**); 3-phenylpropylbromide, K_2CO_3, DMF, rt (**4f**); 2-bromoethanol, K_2CO_3, DMF, rt (**4h**); 3-bromo-1-propanol, K_2CO_3, DMF, rt (**4i**); (c) ethyl 5-bromovalerate, K_2CO_3, DMF, rt; (d) (1) NaOH, H_2O, 1,4-dioxane, 80 °C; (2) HCl, H_2O; (e) (1) NaOH, H_2O, rt; (2) HCl, H_2O.



Scheme 2. Synthesis of 3-aminophthalic acid derivatives. Reagents and conditions: (a) (1) *c*H_2SO_4, EtOH, reflux; (2) EtI, K_2CO_3, DMF; (b) H_2, 10% Pd–C, EtOH, rt; (c) *n*-butylaldehyde, acetic acid, triacetoxyborohydride, 1,2-dichloroethane, rt; (d) (1) NaOH, H_2O, 1,4-dioxane, 80 °C; (2) HCl, H_2O.

ester derivative **12**. Next, dimethyl amine or various cyclic amines were reacted with **12** to give diethyl esters of the 3-aminophthalic acid derivatives. Hydrolysis under alkaline conditions gave 3-aminophthalic acid derivatives.

2.2. Structure and activity relationship (biological activity)

The IMP-1 inhibitory activities of the 3-alkyloxy phthalic acid derivatives were shown in Table 1. Although the hydroxyl derivative **5a** showed no IMP-1 inhibitory activity, the methoxy derivative **5b** showed weak activity. The 3-benzyloxy phthalic acid **5c** showed about a 20-fold increase in activity as compared with **5b**. The derivatives **5d**, **5e** and **5f** with longer carbon chains showed potent IMP-1 inhibitory activity. In compounds **5g**, **5h** and **5i**, the hydrophilic hydroxyl or carboxyl group was introduced to the C2, C3 or C4 alkyl chain. These derivatives (**5g–i**) showed weak IMP-1 inhibitory activity (IC_{50} = 18.8–47.8 μM).

Next, we evaluated the combination effect with BIPM, a carbapenem antibiotic, against *P. aeruginosa* KG5002[6]/pMS363[7] (ΔMexAB) and PAO1/pMS363 strains that produce IMP-1. The 3-alkyloxy phthalic acids **5e** and **5f**, which were strong IMP-1 inhibitors, showed no combination effect with BIPM against *P. aeruginosa* PAO1/pMS363. Although the IMP-1 inhibitory activity of **5g**, **5h** and **5i** was weaker than that of **5e** or **5f**, the derivatives **5g–i** showed potent combination effect with BIPM against *P. aeruginosa* PAO1/pMS363. On the other hand, **5f** showed a strong combination effect with BIPM against *P. aeruginosa* KG5002/pMS363 (ΔMexAB). Interestingly, the hydroxyl derivatives **5h** and **5i** showed a combination effect against *P. aeruginosa* KG5002/pMS363 (ΔMexAB) that was equal to that against *P. aeruginosa* PAO1/pMS363. These results indicated that **5f** was affected by the efflux system of MexAB-OprM, whereas the hydroxyl derivatives **5h** and **5i** were not affected by this efflux system.

Table 2 shows the activities of the 3-aminophthalic acid derivatives. No substituted 3-aminiophthalic acid showed weak IMP-1 inhibitory activity, but the *n*-butyl (**10**) and *N*,*N*-dimethyl amino (**14**) derivatives showed improved IMP-1 inhibitory activity. Although the *N*,*N*-dimethyl amino derivative **14** had weak IMP-1 inhibitory activity, this compound showed a potent combination effect with BIPM against *P. aeruginosa* strains that produced IMP-1. On the other hand, cyclic amine compounds showed potent IMP-1 inhibitory activity and a combination effect with BIPM against *P. aeruginosa* strains producing IMP-1. In particular, the