Synthesis and antibacterial activity of racemic paecilocin A and its derivatives against methicillin-sensitive and -resistant Staphylococcus aureus

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Abstract: A total synthesis in four steps of racemic paecilocin A, a natural marine phthalide is reported. The synthetic pathway includes an iodine-magnesium exchange followed by a condensation on an aldehyde and represents a sufficiently flexible approach to allow the synthesis of twelve analogs. The synthesized compounds were investigated for their antibacterial activity against methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant strain (MRSA). Three analogs were found to have similar or better antibacterial activity than the natural compound on MRSA.

Introduction

The phthalides are bicyclic heterocycles resulting from the fusion of a γ-lactone and a benzene ring. This simple pattern was found in many natural compounds extracted from various biotopes like plants, fungi or marine organisms.[1] More than 200 natural phthalides are referenced in the literature,[2] most of them exhibit one or more biological activities such as antibacterial, antiviral, antifungal, antitumor. Among these, the paecilocin A (*S*)-**1a** was isolated in 2011 from the marine fungus *Paecilomyces variotii*[3] (and later in 2018 from the fungus *Byssochlamys spectabilis*)[4] as a single enantiomer. Evaluation of the biological properties of this natural polyketide showed (*S*)-**1a** exhibited an agonist activity toward peroxisome proliferator-activated receptor gamma PPAR-γ (just as its (*R*)-enantiomer), [5] an inhibitory effect on human carboxyesterase hCE2,[4] and a modest antibacterial activity (MIC > 40 µg/mL) against methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA 3090). [3]

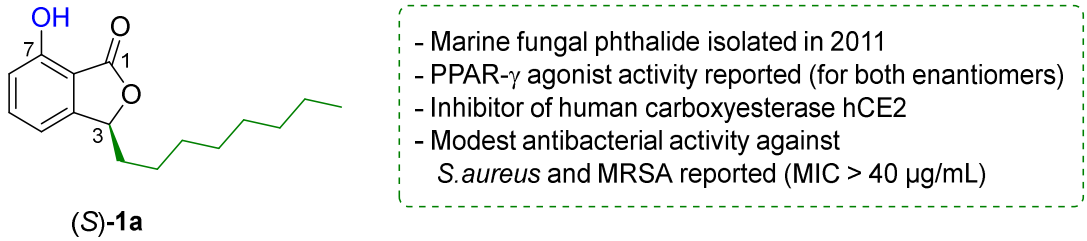


Figure 1. Structure and properties of paecilocin A

Two total synthesis of this natural compound were published in the literature to date. In 2012, Reddy and coworkers reported the synthesis of (*S*)-**1a** in eight steps which involved an enzymatic kinetic resolution and an Alder-Rickert reaction as key steps.[6] The same year, Jung reported the preparation of (±)-**1a** in one single step by condensing an excess of *n*-

octylmagnesium bromide on 4-hydroxyphthalic anhydride.[5] However, this straightforward method leads to a complex mixture of six compounds including **1a** which was obtained with a low yield of 12%.[7]

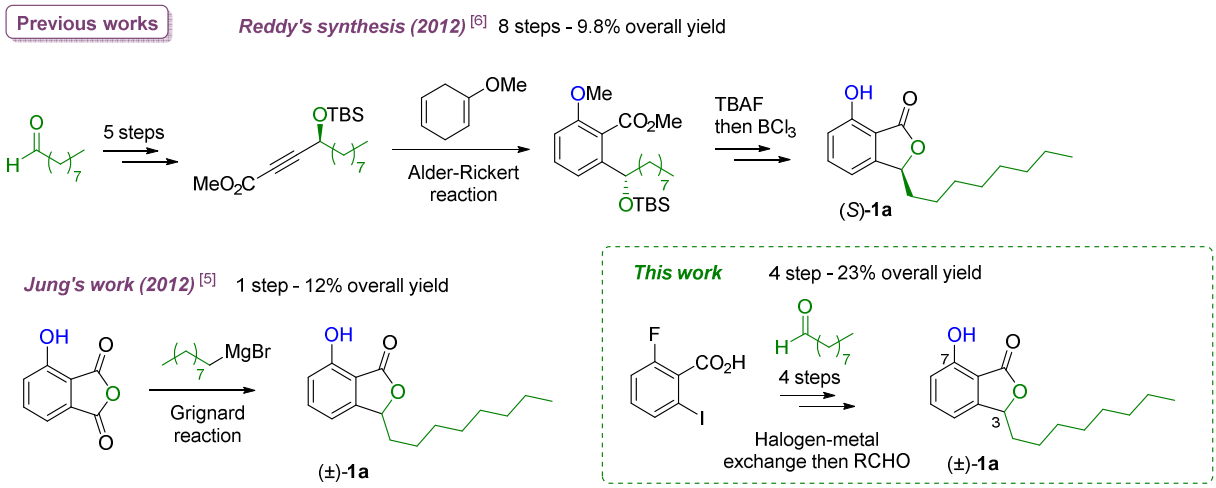


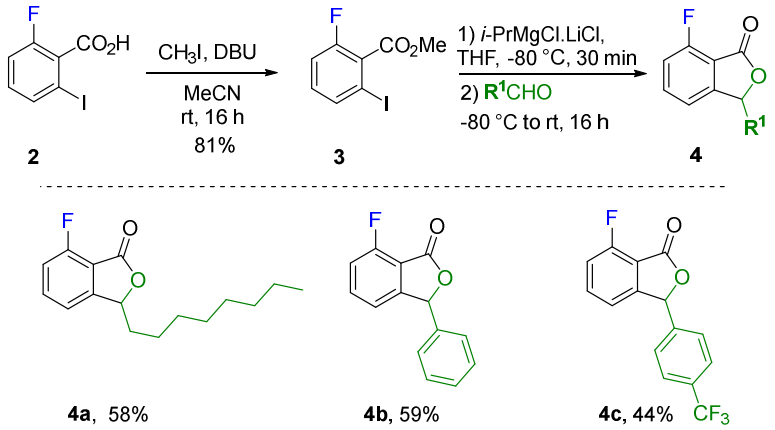
Figure 2. Approaches for the preparation of paecilocin A **1a**

While several references concerning the PPAR-γ activity of **1a** and its analogs were reported during this decade,[8] it is noteworthy that no further study concerning the antibacterial potentiate of paecilocin A have been reported, even though some natural phthalides which have structures very close to **1a** have shown good antibacterial properties[9] and have moreover been eventually subjected to structure-activity relationship studies.[10] Since the stereochemistry of position 3 did not appear to be determinant on the biological activity for such phthalides,[5, 11] we propose herein to carry out a rapid and efficient racemic synthesis of (±)-**1a**. This synthetic pathway would be taken advantage of for the preparation of analogues in both positions 3 and 7 of the phthalide core. The antibacterial activity of the latter would be evaluated against two strains of *S. aureus* including one sensitive (MSSA) and one resistant to methicillin (MRSA).

Results and Discussion

Various methodologies were developed for the preparation of the phthalides, all these approaches have been summarized in two recent reviews.[2a, 2b] Among these methodologies, the condensation of *ortho*-metalated benzoic esters or derivatives on an aldehyde is a strategy of choice allowing the introduction of various substituent on position 3 of the heterocycle. Such metalated benzoic esters and derivatives can be obtained for instance by ortho lithiation promoted by *sec*-BuLi-TMEDA[12] or by an halogen-metal exchange.[13] The iodine-magnesium exchange promoted by *i*PrMgCl.LiCl was reported to be an efficient strategy for the preparation of phthalides,[14] we therefore decided to use the latter as a key-step for the preparation of **1a** and its analogs.

The commercial 2-fluoro-6-iodobenzoic acid **2** was first esterified into the corresponding methyl ester **3** in the presence of iodomethane and DBU. The iodine-magnesium exchange on this substrate was cleanly performed by adding a freshly titrated homemade solution of *i*PrMgCl.LiCl in THF at −80 °C.[15] After 30 min, the organomagnesium intermediate was trapped by *n*-nonanal, benzaldehyde or 4-trifluoromethylbenzaldehyde to afford the 7-fluorophthalides **4a-c** with good yields (44-59%) (Scheme 1). These compounds were considered as analogs in C7 and/or C3 position of **1a**. At this stage, we envisaged performing the aromatic nucleophilic substitution of the fluorine in compounds **4** by methylate anion in order to afford the methoxylated derivatives, but this step led to low yields and is not reproducible.

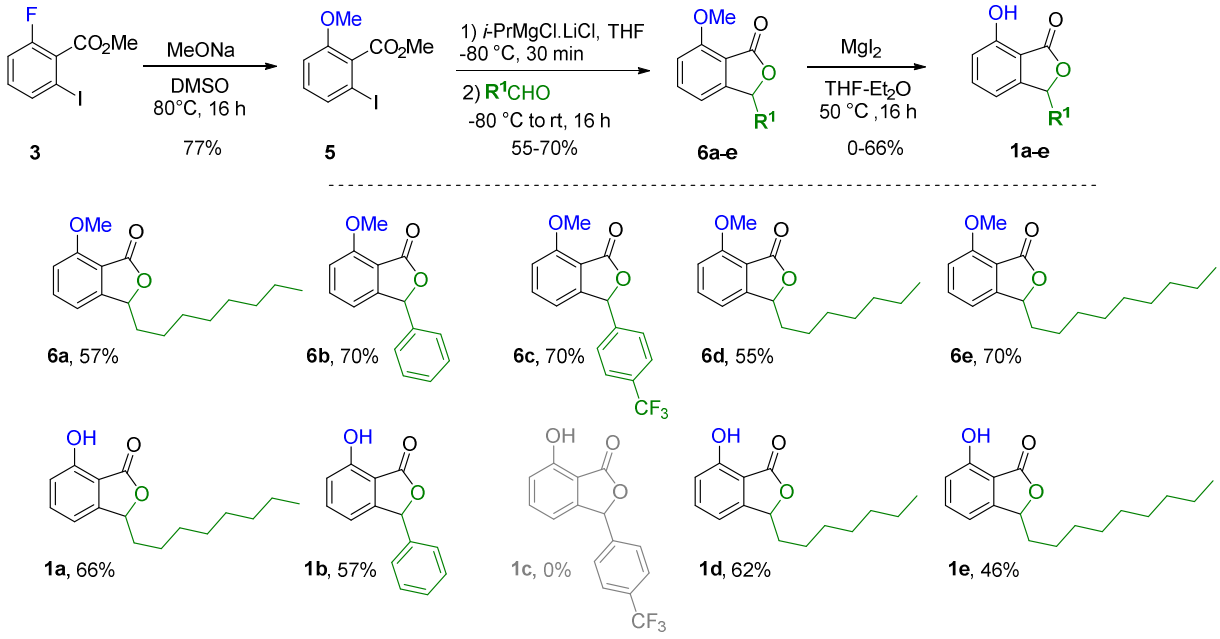


Scheme 1. Preparation of derivatives **4**

On the other hand, the ester **3** was cleanly transformed into **5** using sodium methylate in DMSO at 80 °C (Scheme 2). The latter was used as starting material for the preparation of the phthalides **6a-e** using *i*PrMgCl.LiCl then aliphatic or aromatic aldehydes, under similar conditions as those described previously. The use of octanal, nonanal and decanal led to good yields in **6a,d,e** (55-70%), as did benzaldehyde (**6b**, 70%). Only the 4-trifluoromethylbenzaldehyde led to a low yield of 13% in **6c**.

Finally, in order to achieve the total synthesis, the demethylation step was first attempted on **6a** in presence of BBr_3 or BCl_3 in CH_2Cl_2 (DCM). To our surprise, neither of these two reagents allowed to obtain **1a**, the starting material was entirely recovered or degraded when an excess of reagent was added.

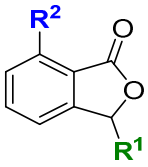
The demethylation step could be achieved by adding a freshly prepared solution of magnesium iodide in Et_2O then refluxing overnight in THF. In such conditions, the racemic paecilocin A **1a** was obtained with a good yield of 66%, as were analogs **1b, 1d** and **1e** (46-62%). However, we did not succeed in obtaining the compound **1c** under these conditions (Scheme 2).



Scheme 2. Synthesis of derivatives **6** and **1**

The antibacterial properties of phthalides **4, 6** and **1** were evaluated against one methicillin and one resistant strain of *Staphylococcus aureus* via broth microdilution method in accordance with the guidelines of the Clinical and Laboratory Standards Institute.[16]. The Minimum Inhibitory Concentrations (MIC) and the Minimum Bactericidal Concentrations (MBC) of these compounds are reported in $\mu g.mL^{-1}$ in Table 1.

Table 1: Minimum inhibitory concentrations (MIC) and Minimum Bactericidal concentrations of compounds **4, 6**, and **1** against MSSA ATCC 29213 and MRSA NCTC 12493

Compound			Minimum inhibitory concentration (MIC) (in µg.mL^{-1})[a]	
	R^2	R^1	Methicilin-sensitive *S. aureus* (MSSA) ATCC 29213	Methicilin-resistant *S. aureus* (MRSA) NCTC 12493
4a	F	*n*-octyl	>200	>200
4b	F	C_6H_5	>200	>200
4c	F	*p*-$CF_3C_6H_4$	>200	>200
6a	OMe	*n*-octyl	50 [b]	6.25 [b]
6b	OMe	C_6H_5	>200	>200
6c	OMe	*p*-$CF_3C_6H_4$	>200	>200
6d	OMe	*n*-heptyl	25 [b]	12.5 [b]
6e	OMe	*n*-nonyl	>200	>200
1a	OH	*n*-octyl	200 [b]	6.25 [b]
1b	OH	C_6H_5	n.d[c].	n.d.
1d	OH	*n*-heptyl	100 [b]	25 [b]
1e	OH	*n*-nonyl	>200	100 [b]
Daptomycin (lipopeptide)	-	-	0.25	0.25
Oxacillin (ß-lactamin)	-	-	0.25	4

[a] Assay experiments were performed in triplicates at three independent times. [b] MBC > 200 µg.mL^{-1}. [c] non-determined

The fluorinated bioisoster of paecilocin A **4a** did not showed any antibacterial activity below 200 µg.mL^{-1} on both strains. The pharmacomodulation of the alkyl chain by a benzene ring **4b** and a 4-trifluoromethylated benzene ring **4c** did not improve the antibacterial activity. Conversely, the "methylated" paecilocin A **6a** showed antibacterial activities of respectively 50 µg.mL^{-1} and 6.25 µg.mL^{-1}against MSSA and MRSA. ~~Similar~~ The antibacterial activity ~~profile~~ was ~~observed~~ improved when the C-3 alkyl chain was shortened (compound **6d**, MIC = 12.5-25 µg. mL^{-1}). Reversely, the elongation of the alkyl chain (**6e**) or the replacement by a benzene ring (**6b** and **6c**) were deleterious for the antibacterial activity (MIC > 200 µg.mL^{-1}). The racemic paecilocin A **1a** exhibit a weak activity against MSSA (MIC = 200 µg.mL^{-1}), this result was in accordance with the data reported for the natural product.[3] However, we were surprised to observe that **1a** was 32 times more active against MRSA (MIC = 6.25 µg.mL^{-1}) while the *n*-heptyl analog **1d** is only 4 times more active (MIC = 100 µg.mL^{-1} against MSSA and 25 µg.mL^{-1} against MRSA). Finally, the *n*-nonyl analog **1e** showed a weak activity only against the MRSA (MIC = 100 µg.mL^{-1}). It is noteworthy that **1a** and the analogs (**1d**, **6a** and **6d**) have a bacteriostatic effect against MRSA as the MBC was found to be superior to 200 µg.mL^{-1}.

In term of Structure-Activity Relationship (SAR), these assays showed the importance of the substituent on the C-7 position: while a hydrogen bond donor is apparently not required for the antibacterial activity, a good hydrogen-bond acceptor like an oxygen appears to be important.

Conclusion

In summary, we have developed a rapid synthesis for the preparation of racemic paecilocin A **1a** in four steps based on an iodine-magnesium exchange starting from 2-fluoro-6-iodobenzoic acid **2** (overall yield = 23%). This synthetic pathway allowed introducing simple chemical modifications on the both positions 3 and 7 of the phthalide and therefore the preparation of 11 analogs. The antibacterial activity of these compounds was evaluated against MSSA and MRSA strains. (±)-**1a** and three of these analogs (**6a**, **6d** and **1d**) were found to be 4 to 32 times more active on the resistant strain when compared to the sensitive strain. This study, which was to the best of our knowledge, the first SAR study on paecilocin A, revealed the importance of the oxygen atom on the C7 position as well as the lipophilic alkyl chain on the C3 position.

Acknowledgements

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Appendix A: Supplementary data

Supplementary data to this article can be found online at http://

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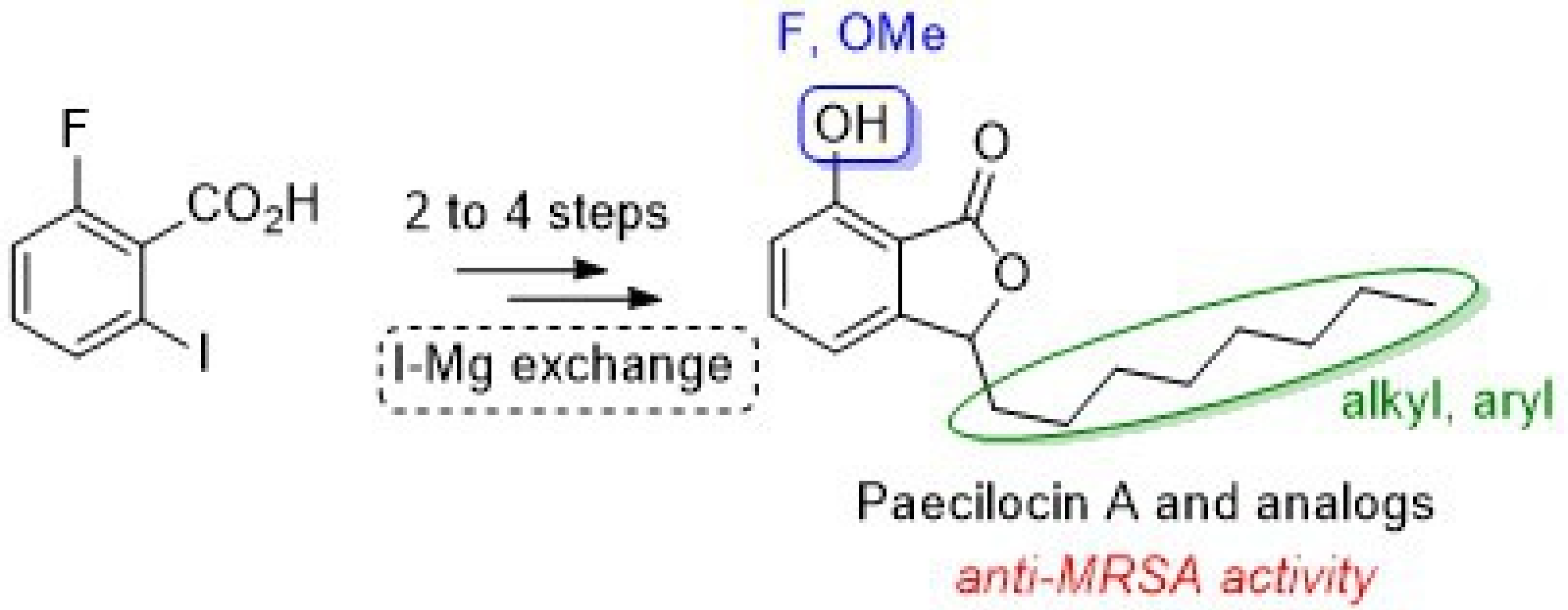

Paecilocin A and analogs

anti-MRSA activity