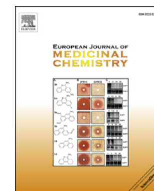
European Journal of Medicinal Chemistry 90 (2015) 351–359

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech



Short communication

Catechol-based substrates of chalcone synthase as a scaffold for novel inhibitors of PqsD

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ARTICLE INFO

Article history:
Received 2 September 2014
Received in revised form
24 November 2014
Accepted 26 November 2014
Available online 27 November 2014

Keywords:
Pseudomonas aeruginosa
PqsD
HHQ
SPR
Quorum sensing

ABSTRACT

A new strategy for treating *Pseudomonas aeruginosa* infections could be disrupting the *Pseudomonas* Quinolone Signal (PQS) quorum sensing (QS) system. The goal is to impair communication among the cells and, hence, reduce the expression of virulence factors and the formation of biofilms. PqsD is an essential enzyme for the synthesis of PQS and shares some features with chalcone synthase (CHS2), an enzyme expressed in *Medicago sativa*. Both proteins are quite similar concerning the size of the active site, the catalytic residues and the electrostatic surface potential at the entrance of the substrate tunnel. Hence, we evaluated selected substrates of the vegetable enzyme as potential inhibitors of the bacterial protein. This similarity-guided approach led to the identification of a new class of PqsD inhibitors having a catechol structure as an essential feature for activity, a saturated linker with two or more carbons and an ester moiety bearing bulky substituents. The developed compounds showed PqsD inhibition with IC_{50} values in the single-digit micromolar range. The binding mode of these compounds was investigated by Surface Plasmon Resonance (SPR) experiments revealing that their interaction with the protein is not influenced by the presence of the anthranilic acid bound to active site cysteine. Importantly, some compounds reduced the signal molecule production *in cellulo*.

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1. Introduction

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium, which is the etiological agent of chronic infections in immunocompromised patients [1] and in people affected by cystic fibrosis (CF) [2]. The treatment of the infections caused by this pathogen is very difficult due to its high intrinsic tolerance towards common antibiotics and the development of new resistant strains [3]. Consequently, novel therapeutic options are urgently needed for *P. aeruginosa*-related diseases. A potential approach could be targeting the quorum sensing (QS) which is a cell-to-cell communication system important for the regulation and coordination of the lifestyles of bacterial cells using diffusible small signal molecules [4].

P. aeruginosa employs three interconnected QS systems. The *las* and *rhl* systems use homoserine lactones as signal molecules (*N*-(3-oxo-dodecanoyl)-L-homoserine lactone and N-(butanoyl)-L-homoserine lactone, respectively) which are commonplace among Gram-negative bacteria [5]. The *pqs* system, on the other hand, is employed only by some *Pseudomonas* and *Burkholderia* species and operates via the autoinducers PQS (Pseudomonas Quinolone Signal; 2-heptyl-3-hydroxy-4(1*H*)-quinolone) and its precursor HHQ (2-heptyl-4(1*H*)-quinolone) [6]. PQS and HHQ interact with the transcriptional regulator PqsR (also called MvfR) controlling the production of virulence factors, such as pyocyanin, elastase and hydrogen cyanide [7], as well as the formation of biofilms [8]. Finally, activation of this receptor promotes expression of the enzymes encoded by the *pqsABCDE* operon is important for the synthesis of the HAQs themselves [9].

PqsD is encoded by this operon and is a key enzyme in the synthesis of the quionolone-based signal molecules catalyzing the conversion of anthraniloyl-CoA (ACoA) into the reactive 2-aminobenzoyl acetate (2-ABA) [10]. Subsequently, this

Abbreviations: QS, quorum sensing; SPR, surface plasmon resonance; CF, cystic fibrosis; PQS, pseudomonas quinolone signal; HHQ, 2-heptyl-4(1*H*)-quinolone; ACoA, anthraniloyl coenzymea; CoA, Coenzyme A; DMF, dimethylformamide; THF, tetrahydrofurane; BOP, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; TFA, trifluoroacetic acid; HRMS, high-resolution mass spectrum; IS, internal standard.

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http://dx.doi.org/10.1016/j.ejmech.2014.11.055
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intermediate reacts with PqsC which bears an octanoic acid residue, and, with the support of PqsB, gives the quinolone HHQ [10]. PqsD belongs to the β-ketoacyl-ACP synthase III (FabH)-type condensing enzyme family and possesses some functional and mechanistic properties of the polyketide synthase (PKS) family [11]. Another enzyme that belongs to the PKS family is the chalcone synthase (CHS2) expressed in *Medicago sativa* (alfalfa) [12]. Physiologically, this vegetable protein transforms *p*-coumaryl-CoA into the flavanone naringenin, the central intermediate for the biosynthesis of several classes of flavonoids [12,13]. However, CHS2 accepts other substrates *in vitro*, such as cinnamic acid [14], caffeic acid [14], ferulic acid [13].

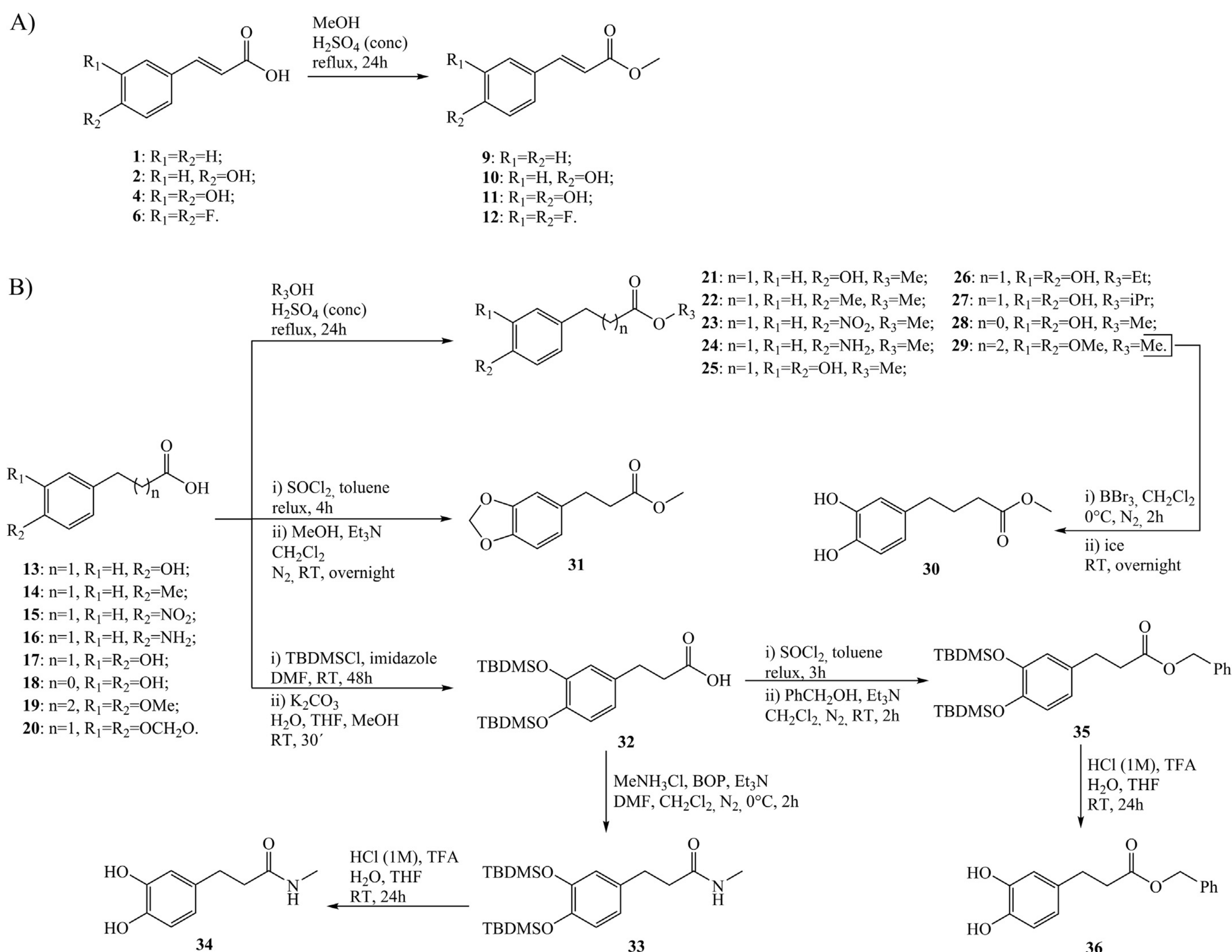
Comparing PqsD with CHS2, some matches were found. In fact, both are condensing enzymes with a similar volume of the active sites, 923 Å^3 for CHS2 and 890 Å^3 for PqsD [11], and use the same catalytic residues, such as cysteine, histidine, and asparagine [11–13]. Moreover, both the proteins accept malonyl-CoA as secondary substrate and the deepness of the binding pockets is comparable, 16 Å for CHS2 [12] and 15 Å for PqsD [11]. Finally, the entrances of the active sites of both enzymes are decorated with basic amino acid side chains [11,12].

Guided by the results of our previous work showing that the inhibition of PqsD is a promising approach for reducing the production of HHQ, PQS and biofilm [15], and the aim of this study was to identify and develop a new class of PqsD inhibitors using the molecular scaffold of described CHS2 substrates and understanding the binding mode of this series of compounds.

2. Results and discussion

2.1. Chemistry

The esters **9**–**12**, **21**–**29** of the respective carboxylic acids **1**, **2**, **4**, **6** and **13**–**19** were synthesized by a Fischer esterification with alcohol, as reactant and solvent, and drops of sulfuric acid 98% as catalyst (Scheme 1). **29** was demethylated by BBr_3 in dichloromethane obtaining **30**. **31** was synthesized by a two-step reaction starting with the conversion of **20** into the respective acyl chloride through thionyl chloride in toluene followed by subsequent methanolysis. The intermediate **32** was obtained by treating **17**, first, with *tert*-butyldimethylsilyl chloride (TBDMSCl) in DMF and, then, with potassium carbonate in THF/water/methanol for protecting the hydroxyl groups of the starting material. **32** was converted into the methyl amide **33** by BOP coupling and methylamine. Moreover, **32** was transformed into the benzyl ester **35** through acyl chloride intermediate formation and addition of benzyl alcohol.



Scheme 1. General synthesis of **9**–**12**, **21**–**28**, **30**–**31**, **34** and **36**.

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