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Research paper

Fusaric acid and analogues as Gram-negative bacterial quorum sensing inhibitors

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ABSTRACT

Taking advantage of microwave-assisted synthesis, efficient and expedite procedures for preparation of a library of fusaric acid and 39 analogues are reported. The fusaric acid analogues were tested in cell-based screening assays for inhibition of the *las* and *rhl* quorum sensing system in *Pseudomonas aeruginosa* and the *lux* quorum sensing system in *Vibrio fischeri*. Eight of the 40 compounds in the library including fusaric acid inhibited *lux* quorum sensing and one compound inhibited activity of the *las* quorum sensing system. To our delight, none of the compounds showed growth inhibitory effects in the tested concentration ranges.

1. Introduction

The mycotoxin fusaric acid (**FA**, Fig. 1) was originally isolated by Yabuta in 1934 from the fungus *Fusarium heterosporum* [1]. Later studies have revealed that the compound is produced by a large number of species belonging to the *Fusarium* genus and maybe even by all *Fusarium* species. In some cultures, more than 1 mg of **FA** per gram medium is produced. The agent was the first compound shown to have a pivotal role in plant diseases such as tomato, cucumber and banana wilt [2,3]. In addition to the involvement in plant diseases [2–4], **FA** has a number of pharmacological properties even though only in moderate to high doses [5,6] and possesses the ability to augment the effects of other mycotoxins. Despite a number of effects in ranging from alteration of membrane activity, decreased mitochondrial activity, inhibition of ATP synthesis and reduced production of polyphenol oxidase and peroxidases, the mechanism of **FA** is still not fully delineated [2]. **FA** is also reported to chelate divalent cations and inhibit of dopamine β-hydroxylase [7a-b]. Over the year, **FA** has undergone several preclinical and clinical trials for treatment of diseases such as mania, cancer and hypertension [7].

We hypothesized that **FA** can be considered a bioequivalent to *N*-hexanoyl-L-homoserine lactone, a signal molecule involved in quorum sensing (QS) in bacteria. In addition, **FA** like some QS inhibitors (QSIs) possesses a pyridine ring (Fig. 1c). QSIs are very encouraging for design of new antimicrobial drugs acting through a hitherto untapped bacterial pathway. No new molecular entities are currently in clinical trials for interfering with QS in patients. Notably however, azithromycin, one of the best-selling antibiotics, has been demonstrated to inhibit virulence and cooperation of *Pseudomonas aeruginosa* through QS mechanism [8a]. Recently, hamamelitannin analogues were suggested to increase the susceptibility of a methicillin-resistant *Streptococcus aureus* strain through QS inhibition [8b].

During QS bacteria use small signal molecules or autoinducers for cell-cell communication. QS enables bacteria to coordinate a collective response such as biofilm formation as a protection towards external factors. Therefore, compounds possessing QS inhibitory ability but void of bactericidal or bacteriostatic activity

Abbreviations: AHLs, *N*-Acyl-L-homoserine lactones; MW, Microwave; 4-NPO, 4-nitropyridine-*N*-oxide; Pma1, plasma membrane H^+-ATPase; QS, quorum sensing; QSI, quorum sensing inhibitor; TLC, thin layer chromatography; UPLC, ultra performance liquid chromatography.

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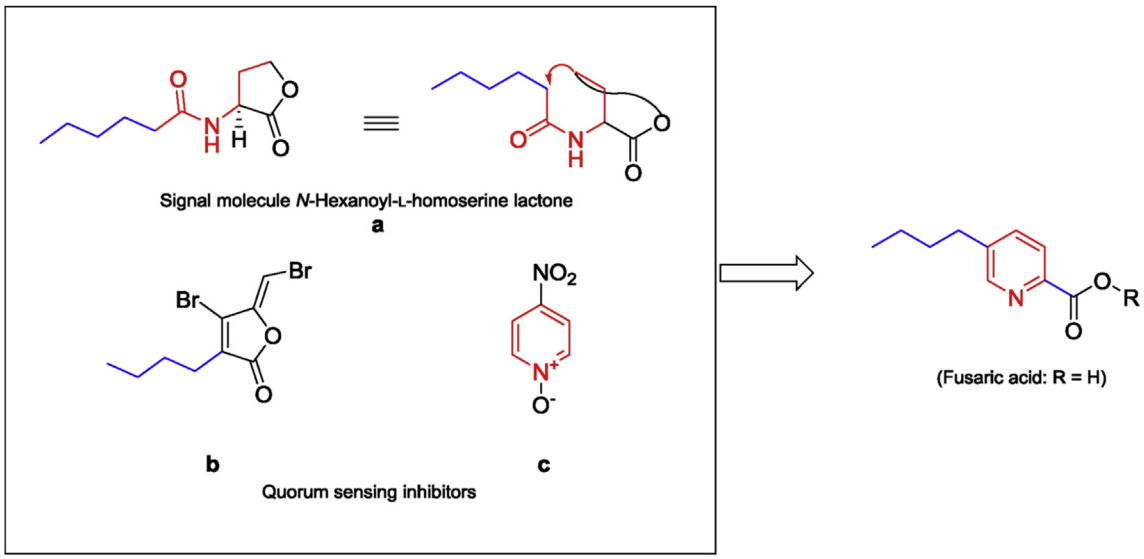

Fig. 1. Homologous structure of **FA** and analogues toward the QS signal molecule *N*-hexanoyl-L-homoserine lactone (**a**); QSI containing structural features of QS signal molecule 4-bromo-5-[1-bromo-meth-(*Z*)-ylidene]-3-butyl-5*H*-furan-2-one (**b**) and reported QSI containing pyridine 4-NPO (4-nitropyridine-*N*-oxide) (**c**).

inactivate bacteria defense systems, reduce the production of toxins and most importantly are less prone to selection for resistance than traditional cytotoxic remedies [9,10]. In Gram-negative bacteria, the QS signal compounds are *N*-Acyl-L-homoserine lactones (AHLs) [9–11]. Many QSIs based on structure of AHLs have been designed and tested [12]. As shown in Fig. 2 **FA** might be considered bioequivalent to the AHL.

FA comprises of a pyridine nucleus decorated with a carboxylic group at C-2 and an *n*-butyl moiety protruding from C-5. Overlay of the ester carbonyl of the two molecules, C-3 of homoserine lactone with C-2 of **FA**, and the methylene groups in the side chains of the two molecules gives a good match indicating that these groups might be bioequivalent (Fig. 2). In addition, molecules containing a pyridine ring (4-NPO, IC_{50} of 24 μg/ml or 171 μM [12c,13]) and these structural features are found to be QSIs (Fig. 1**b**, **c**). Bloemberg et al. [14] have also reported that **FA** prevents the production of the signaling molecule *N*-hexanoyl-L-homoserine lactone (C6-HSL) but no **FA** analogues were synthesized and evaluated to get structure-activity relationship and thereby maybe hint to understand the mechanism of action. Based on these arguments, **FA** was chosen as a suitable template for construction of a library of potential QSIs [15].

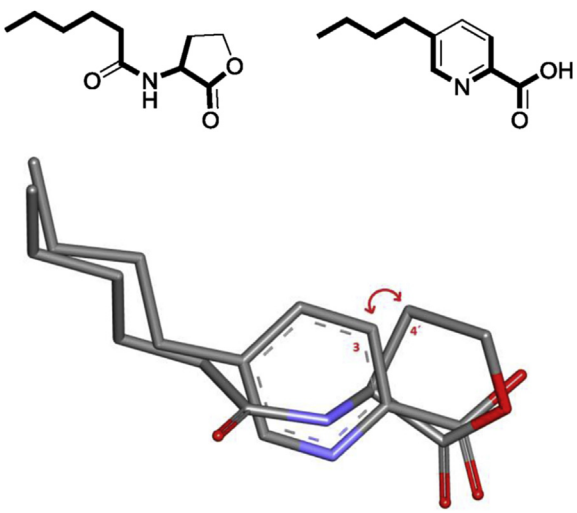

Fig. 2. Overlap between *N*-hexanoyl-L-homoserine lactone and **FA**.

2. Results and discussion

2.1. Strategy for design of library

The strategy behind the design of the library to get structure-activity relationship and thereby to identify pharmacophore descriptors is illustrated in Fig. 3. To further elaborate on these structural features the compounds shown in Table 1 were designed, prepared and screened as QSI. The importance of the substituent at C-5 was investigated by decorating with a broad plethora of substituents with different lengths, aromatic rings and functional groups.

2.2. Microwave assisted synthesis of fusaric acid and analogues

Only a few analogues of **FA** have been reported [16–19]. Common for all reported protocols, however, are that they only afford a limited spectrum of analogues. No biological studies have been performed on these compounds and none of the reported methods describe microwave-assisted synthesis. Taking advantage of microwave methodology, we have developed a protocol for fast preparation of **FA** and a diverse library of analogues (Scheme 1).

Treatment of 2,5-dibromopyridine (**1**) with $TMSCH_2Li$-LiDMAE in toluene at 0 °C for 30 min afforded the 2-lithiated intermediate selectively [20]. Addition of methyl or ethyl formate at −78 °C and stirring for 3 h followed by oxidation with iodine in methanol or ethanol introduced an ester group at C-2 in medium yield to give methyl 5-bromopicolinate (**2a**) or ethyl 5-bromopicolinate (**2b**), respectively.

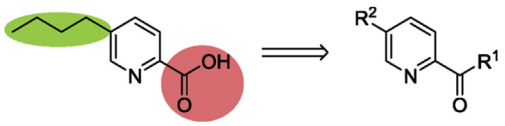

Fig. 3. FA and strategy for design of library.