

## Synthesis of (*R*)-Norbgugaine and its potential as quorum sensing inhibitor against *Pseudomonas aeruginosa*

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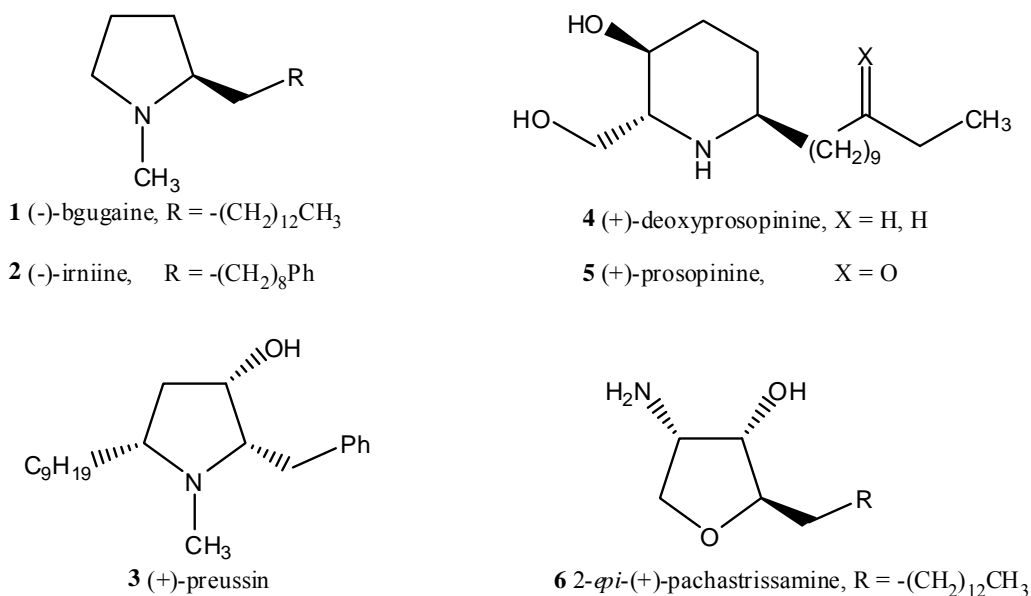
### Abstract:

(*R*)-Bgugaine is a natural pyrrolidine alkaloid from *Arisarum vulgare*, which shows antifungal and antibacterial activity. In this letter, we have accomplished the simple synthesis of norbgugaine (demethylated form of natural bgugaine) employing Wittig olefination and cat. hydrogenation as the key steps and its biological studies are reported for the first time. The synthesized norbgugaine was evaluated for inhibition of quorum sensing mediated virulence factors (motility, biofilm formation, pyocyanin pigmentation, rhamnolipid production and LasA protease) in *P. aeruginosa* wherein swarming motility is reduced by 95%, and biofilm formation by 83%.

**Keywords:** Norbgugaine, QS inhibitor, *Pseudomonas aeruginos*, Wittig reaction, pyrrolidine alkaloid

## Introduction:

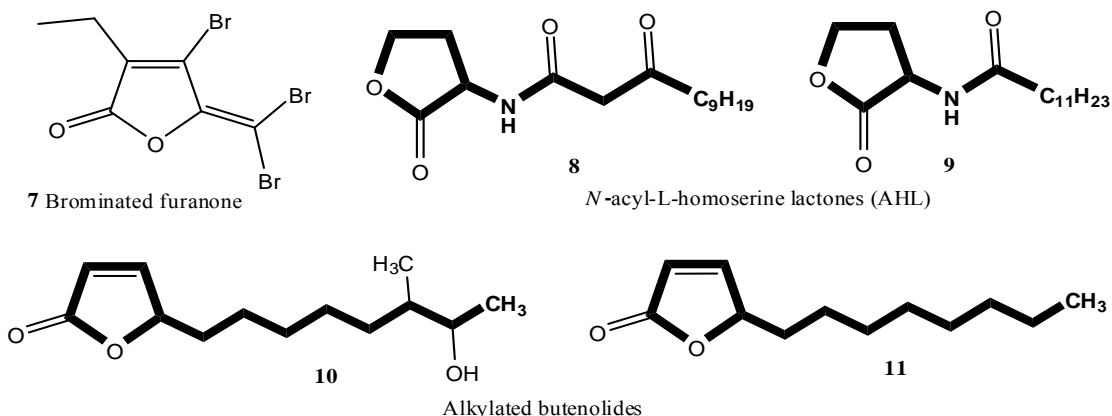
Small molecules created by nature possessing impressive biological activity define a particularly important area of new chemical space for drug discovery research. Natural products have been and continue to be a major source of new organic skeleton which has always inspired and challenged the synthetic chemists.<sup>1</sup> In fact, the structural designs of natural products are highly conserved in nature. And, it is well known fact that the natural products that share a common scaffold with different functionalized skeleton display different bioactivity profiles. Therefore, the scaffolds of natural products are considered as “privileged structures” for further modifications.<sup>2</sup>



**Figure 1:** Selected naturally occurring bioactive compounds with long chain hydrocarbon

The  $\alpha$ -substituted amines especially  $\alpha$ -substituted pyrrolidines, piperidines and related ring systems with long chain hydrocarbons are the common chemical entities that are found abundantly in nature as well as in many bioactive alkaloids and pharmaceutically relevant molecules.<sup>3</sup> For example, (-)-bgugaine (**1**) and (-)-irniine (**2**) (Figure 1) are alkaloids isolated from the tubers of *Arisarum vulgare*, a species that is found on the Mediterranean coasts of Morocco and Spain which show antibacterial activity against Gram-positive bacteria and antimycotic activity against some *Candida* and *Cryptococcus* strains.<sup>4</sup> (+)-Preussin (**3**), isolated from the fermentation broths of *Aspergillus ochraceus* ATCC 22947 and then from *Preussia* sp.

possesses a broad spectrum antifungal activity against both filamentous fungi and yeasts.<sup>5</sup> Moreover, other long chain containing compounds such as (+)-deoxyprosopinine (**4**), (+)-prosopinine (**5**), 2-*epi*-(+)-pachastrissamine (**6**) have been isolated and many of them showed important biological activities.<sup>6</sup> As a consequence, numerous synthetic methods have been developed for the construction of such ring systems.<sup>7</sup>

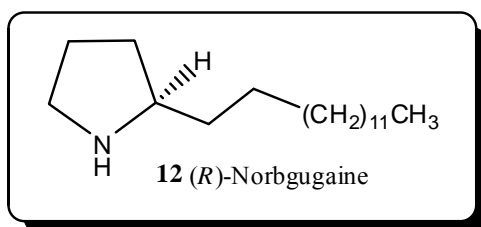


**Figure 2:** Structures of some natural furanones, and synthetic analogue in quorum sensing systems. Substructures of interest to present work are shown in bold.

Nowadays, the combination of natural products and combinatorial chemistry has proven new ground towards development of new classes of therapeutic agents. The treatment of infectious diseases using new agents has become an important research area worldwide. Bacteria can grow into surface-associated communities (biofilm) and this growth cause a significant complexity to the successful treatment of infectious diseases.<sup>8</sup> In particular, *Pseudomonas aeruginosa* is the Gram-negative bacterium responsible for biofilm growth has attracted considerable attentions, as this pathogen are able to form biofilms in lungs, kidney, urinary tract, causing inflammations and septic shock in patients.<sup>9</sup> Consequently, the research for the development of new therapeutic agent and convenient methods to attenuate bacterial biofilm growth has been greatly stimulated by medical needs.<sup>10</sup> A few natural products have been witnessed to inhibit bacterial QS or biofilm growth. The halogenated furanones e.g. brominated furanone **7** from the marine macroalga *Delisea pulchra* and their derivatives have been most intensively studied for their QS activities.<sup>11</sup> In fact, various QS-inhibiting marine natural products are also exhibiting antifouling

activity. The interference with bacterial quorum sensing (QS) has been proposed as one potential approach for controlling biofouling.<sup>12</sup> The natural *N*-acyl homoserine lactone (AHL) **8** family and their derivatives **9-11** can strongly modulate QS in Gram-negative bacteria (Figure 2),<sup>13</sup> and several of these AHLs also attenuate biofilm growth in *P. aeruginosa*.<sup>14</sup> Recently, Blackwell and co-workers observed that the AHL-derived biofilm inhibitors failed to remove the preformed biofilms.<sup>15</sup> Hence, the applicability of AHLs as biofilm or QS inhibitor involves few drawbacks in terms of their stability i.e. hydrolytic instability of the lactone group to furnish inactive hydrolysed AHL.<sup>15</sup> However, Horikawa and co-workers have demonstrated that the presence of the long acyl side chain with hydrophobic end is crucial for QS activity of *N*-acylated-L-homoserine lactone (AHL).<sup>16</sup> Despite the extent of detrimental effect of biofilms, examples of structural scaffolds that inhibit biofilm formation are rare in the literature.<sup>17</sup> Corresponding to these facts, the development of alternative hydrolytically stable molecular scaffolds for QS or biofilm inhibition represents an extremely important goal.

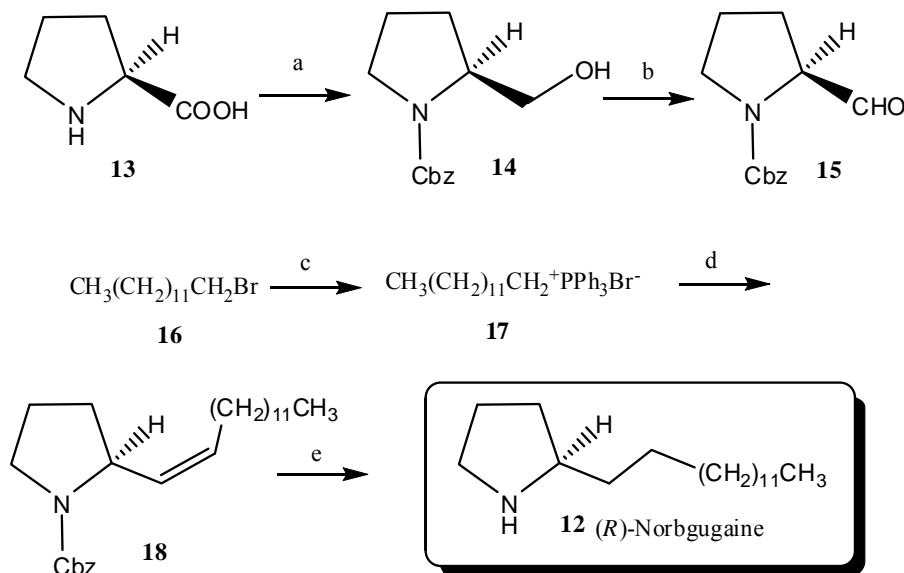
In the view of these observations and our research interest towards the syntheses of small bioactive molecules,<sup>18</sup> have encouraged us to undertake the synthesis of pyrrolidine derivative **12** and demonstrate its potential as QS inhibitor (Figure 3). We reasoned that combining structural attributes of these compounds (Figure 1-2) into simple molecular scaffold could give molecule with heightened QS properties. Herein, we have described the synthesis of norbgugaine, the *N*-demethylated form of natural pyrrolidine alkaloid and explored its biological properties associated with quorum sensing inhibition in *Pseudomonas aeruginosa*.



**Figure 3:** (*R*)-Norbgugaine as a potential QS inhibitor

Previously, we had reported the facile approach to the synthesis of (*R*)-bgugaine using Wittig olefination.<sup>19</sup> Herein, we have utilized the analogous approach for the synthesis of norbgugaine **12**, wherein Cbz is a choice of protecting group instead of Boc. This facilitates the deprotection as well as double bond reduction in single operation at the final stage of synthesis and thus

adding advantage in purification as well as conciseness in approach. Our synthetic strategy is depicted in Scheme 1.

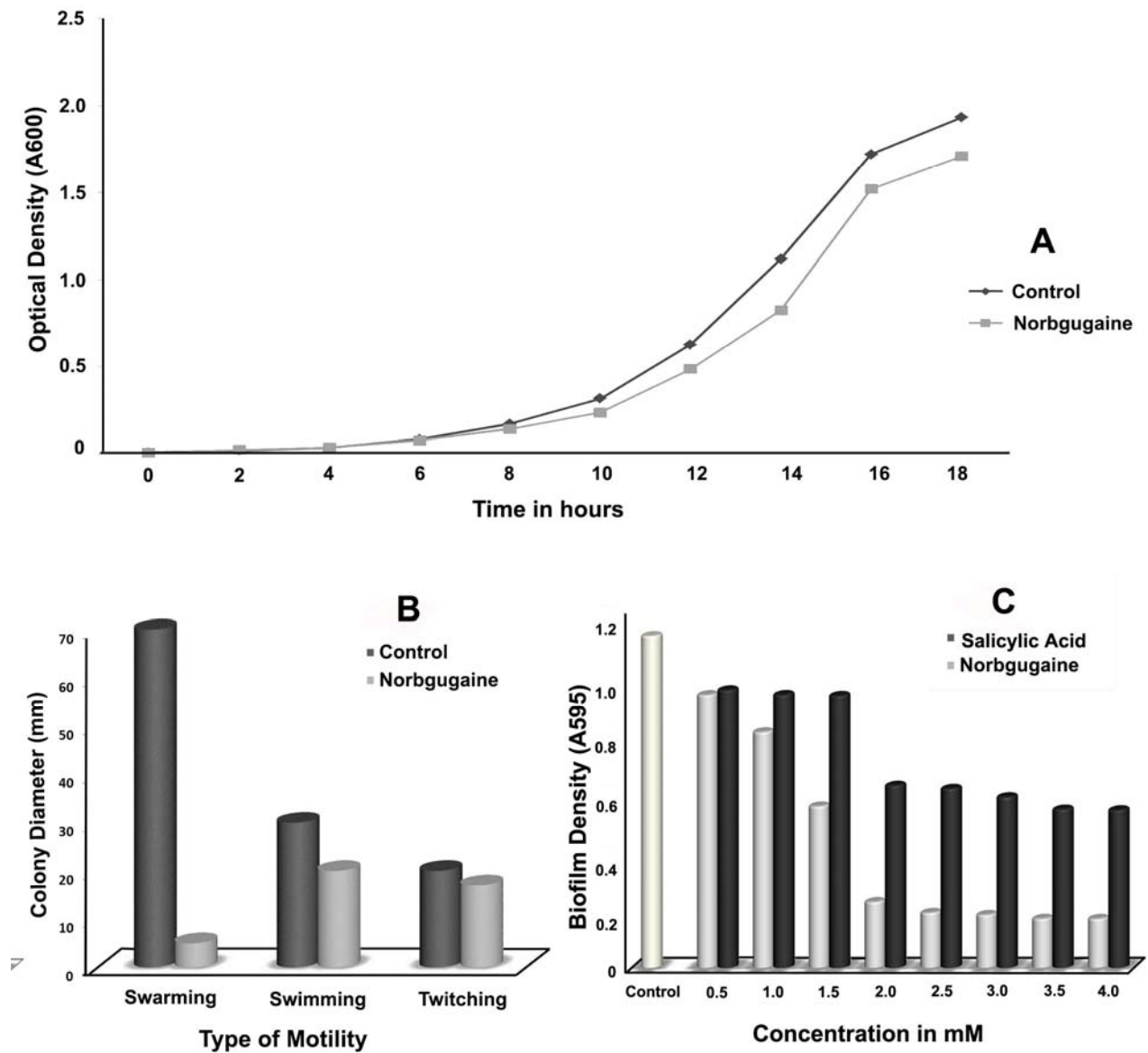


**Scheme 1.** Reagents and conditions: a) i)  $\text{LiAlH}_4$ , THF, reflux, 8 h (88%); ii)  $\text{K}_2\text{CO}_3$ , Benzyl chloroformate,  $\text{CH}_3\text{CN}$ ,  $-10-0^\circ\text{C}$ , 2 h (83%); (b) PCC,  $\text{CH}_2\text{Cl}_2$ , rt, 5 h (74 %); (c)  $\text{PPh}_3$ , toluene, reflux, 24 h; (d) *n*-BuLi,  $\text{Et}_2\text{O}$ , *N*-carbobenzyloxyprolinal **15** (two steps 50%); (e)  $\text{H}_2$ , Pd/C, EtOH, rt, 8 h (80%).

The synthesis commenced from commercially available L-proline **13** which was converted to *N*-carbobenzyloxy proline **15** using the literature procedure.<sup>20</sup> Treatment of tridecyl bromide **16** with  $\text{PPh}_3$  in refluxing toluene for 24 h gave the corresponding phosphonium salt **17** which was further treated with *n*-BuLi to generate *in situ* the desired phosphorane. Furthermore, the freshly prepared (*S*)-*N*-carbobenzyloxy prolinal was subjected to Wittig olefination in one-pot without isolating the intermediate phosphorane (Scheme 1) to give olefin **18**. The double bond geometry of olefin was assumed to be *cis* based on  $^1\text{H}$  NMR values [5.33-5.36 (m, 2H)]. In the end, the Cbz-deprotection and double bond reduction was achieved in one-pot by reaction of olefin **18** with  $\text{H}_2$ , Pd/C in ethanol, which resulted in a facile formation of the title compound **12**. This completed the first synthesis of norbgugaine in 6 steps using easily available L-proline as the starting material.

### **Biological activities/ Quorum Sensing Inhibition studies:**

Quorum sensing (QS) is cell to cell signaling mechanism that enables bacteria to collectively control gene expression and regulate diverse physiological processes.<sup>21</sup> QS is involved in expression of virulence genes in various bacteria, indicating the possible role of quorum sensing as a drug target. Molecules that target quorum sensing act by disarming pathogens by attenuating virulence factors rather than killing them.<sup>22</sup> This feature could greatly reduce the emergence of multi-antibiotic resistant bacteria often associated with traditional antimicrobial chemotherapy. In the present study, the synthesized norbgugaine **12** was evaluated for its potential inhibition of QS mediated virulence factors (motility, pyocyanin pigmentation, rhamnolipid production and LasA protease) in *P. aeruginosa* (ATCC 27853) at a variable concentration range from 0-0.5 mM.<sup>23</sup> The compound **12** showed a minimal effect on the growth rate of *P. aeruginosa* as indicated by growth curve experiment (Figure 4A). Whereas, it was found to exhibit significant effect on motility, particularly swarming as well as swimming motilities in *P. aeruginosa* (Figure 4B). Former was reduced by 95% ( $p < 0.0005$ ) and the latter by 33% ( $p < 0.0005$ ). However, no significant reduction was seen in twitching motility. The compound also showed significant reduction in rhamnolipid production by 42%, pyocyanin production by 37% and Las A production by 34% ( $p < 0.0005$ ) (Table 1).



**Figure 4:** A- Effect of norbgugaine **12** on growth of *P. aeruginosa*. B- Effect of norbgugaine **12** on motilities in *P. aeruginosa*. C- Effect of norbgugaine **12** and Salicylic acid on biofilm formation in *P. aeruginosa*.

Virulence parameters (% inhibition)							
Compound	Swarming motility	Swimming motility	Twitching motility	Pyocyanin pigmentation	Biofilm formation	Rhamnolipid production	LasA
Nor-bugaine	95	33	15	37	83	42	34
Salicylic acid	60	40	35	NA*	48	NA*	NA*

NA\* - not applicable

**Table 1:** Effect of norbugaine **12** on QS mediated virulence factors (Swarming, Pyocyanin pigmentation, Biofilm formation, Rhamnolipid production and LasA) in *P. aeruginosa* in comparison with salicylic acid.<sup>24</sup>

Furthermore, the effect of norbugaine **12** on biofilm formation in *P. aeruginosa* was assessed via static biofilm inhibition assay. *P. aeruginosa* strain demonstrated decreasing biofilm density with increasing concentration of norbugaine **12** from 0.0-4.0 mM (Figure 4C). A leveling off in decreasing biofilm densities with an average percentage of 83% was observed at a concentration of 1.8 mM and above. In comparison with salicylic acid,<sup>24</sup> a well known biofilm inhibitor, our synthesized compound **12** showed two times better biofilm inhibition i.e. norbugaine showed decrease in biofilm formation by 83% while salicylic acid reduced by 48% at 4 mM. Additionally, as *P. aeruginosa* growth was not inhibited by the pyrrolidine derivative **12** (Figure 4A), it is possible to conclude that the inhibition on virulence factor production is caused by an anti-QS mode of action.

In summary, a concise and simple synthesis of (*R*)-norbugaine has been achieved for the first time using L-proline through Wittig reaction as a crucial step. Furthermore, purification by column chromatography was required in only one step, which makes an efficient route to this alkaloid. The majority of infectious diseases are associated with the formation of biofilms. In this study, we found that norbugaine, inhibits various motilities, pyocyanin pigmentation, LasA protease rhamnolipid production, and biofilm formation in *Pseudomonas aeruginosa*. Swimming repression suggests that norbuganine affects the flagella related functions, which might lead to swarming inhibition. Rhamnolipids are known to modulate swarming motility in *P. aeruginosa*<sup>25</sup> and repression of rhamnolipid formation affects colony wetness which may be the reason for



almost the complete inhibition of swarming motility observed in the present case. Biofilm formation and swarming motility have been shown to be closely connected and regulated by a large set of overlapping genes. Moreover, swarming motility has been shown to be important for the early stages of biofilm formation. Therefore, it can be expected that the biofilm repression in the presence of norbgugaine may be mediated by a mechanism similar to that for swarming inhibition. Efforts are on in our laboratory to identify the exact mode of action of norbgugaine in *P. aeruginosa* and give the role QS plays in *P. aeruginosa* virulence. Thus, norbgugaine has immense potential to act as a next generation therapeutic agent.

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