Phytochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

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

Quorum sensing inhibitory potential and molecular docking studies of sesquiterpene lactones from *Vernonia blumeoides*

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ARTICLE INFO

Article history:
Received 4 September 2015
Received in revised form 17 February 2016
Accepted 19 February 2016
Available online xxxx

Keywords:
Vernonia blumeoides
Asteraceae
Quorum sensing inhibition
Molecular docking
Sesquiterpene lactones
Blumeoidolides

ABSTRACT

The increasing incidence of multidrug-resistant Gram-negative bacterial pathogens has focused research on the suppression of bacterial virulence *via* quorum sensing inhibition strategies, rather than the conventional antimicrobial approach. The anti-virulence potential of eudesmanolide sesquiterpene lactones previously isolated from *Vernonia blumeoides* was assessed by inhibition of quorum sensing and *in silico* molecular docking. Inhibition of quorum sensing-controlled violacein production in *Chromobacterium violaceum* was quantified using violacein inhibition assays. Qualitative modulation of quorum sensing activity and signal synthesis was investigated using agar diffusion double ring assays and *C. violaceum* and *Agrobacterium tumefaciens* biosensor systems. Inhibition of violacein production was concentration-dependent, with $\geqslant$90% inhibition being obtained with $\geqslant$2.4 mg ml^{-1} of crude extracts. Violacein inhibition was significant for the ethyl acetate extract with decreasing inhibition being observed with dichloromethane, hexane and methanol extracts. Violacein inhibition $\geqslant$80% was obtained with 0.071 mg ml^{-1} of blumeoidolide B in comparison with $\geqslant$3.6 mg ml^{-1} of blumeoidolide A. Agar diffusion double ring assays indicated that only the activity of the LuxI synthase homologue, CviI, was modulated by blumeoidolides A and B, and *V. blumeoides* crude extracts, suggesting that quorum sensing signal synthesis was down-regulated or competitively inhibited. Finally, molecular docking was conducted to explore the binding conformations of sesquiterpene lactones into the binding sites of quorum sensing regulator proteins, CviR and CviR′. The computed binding energy data suggested that the blumeoidolides have a tendency to inhibit both CviR and CviR′ with varying binding affinities. *Vernonia* eudesmanolide sesquiterpene lactones have the potential to be novel therapeutic agents, which might be important in reducing virulence and pathogenicity of drug-resistant bacteria *in vivo*.

1. Introduction

The increasing incidence of multi-drug resistant bacteria has prompted the search for potent, novel antibacterial drugs or complementary agents against resistant pathogens, with new targets or novel mechanisms, distinct from currently used antibacterial therapies. One such target mechanism which has garnered interest has been quorum sensing (QS), a cell density-dependent chemical signaling process, which is mediated by acyl homoserine lactones (AHL) in Gram-negative bacteria. QS regulates gene expression in bacteria for collective biological functions and significantly influences bioluminescence, plasmid transfer, bacterial virulence, the biosynthesis of secondary metabolites and antibiotics, and biofilm formation (Hirakawa and Tomita, 2013). Therefore, targeting QS mechanisms involving signal production, dissemination or reception could disrupt the QS circuits, curtail bacterial virulence and resistance (Hentzer and Givskov, 2003) and furthermore bacteria are unlikely to develop multi-drug resistance since no selection pressure is imposed (Koh et al., 2013).

Plants have been used for centuries in traditional medicine due to their diverse secondary metabolites such as alkaloids, flavonoids, saponin glycosides, anthraquinones and sesquiterpenoids among others. All plants grow in environments with high bacterial densities and have developed an evolutionary co-existence with QS bacteria. Plants have thus developed protective mechanisms against bacterial infections, e.g., being able to produce QS inhibitory compounds or QS mimic compounds, which reduce

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http://dx.doi.org/10.1016/j.phytochem.2016.02.012
0031-9422/

the pathogenic capability of bacteria (Koh et al., 2013; Nazzaro et al., 2013). Due to their diverse chemical repertoire, the anti-virulence properties of medicinal plants and their constituent phytochemicals are attracting attention since plants are able to interfere with bacterial communication processes thereby disrupting associated cellular mechanisms or functions (Koh et al., 2013; Nazzaro et al., 2013). Gram-negative bacteria primarily use the LuxR/I-type QS system. Plant compounds usually target these Gram-negative bacterial QS systems *via* three different ways, either by inhibiting the signaling molecules from being synthesized by the LuxI synthase, by inhibition of activity of AHL-producing enzymes, by degrading signaling molecules and/or by targeting the LuxR signal receptor (Koh et al., 2013; Nazzaro et al., 2013). Since QS is crucial to bacterial cellular functions and survival, disrupting the QS signal production or reception, facilitates control of bacterial virulence and resistance (Hentzer and Givskov, 2003).

Plants of the genus *Vernonia* (Asteraceae) represent about 500 species distributed in tropical regions of the world especially in Africa and South America (Bremer, 1994). *Vernonia* species, such as *Vernonia amygdalina*, is widely used in African traditional medicine due to its multiple therapeutic properties for various human and animal diseases (Yeap et al., 2010). Phytochemical studies on several *Vernonia* species have resulted in the isolation of flavonoids, triterpenoids and sesquiterpene lactones (SLs) with interesting biological activities (Toyang and Verpoorte, 2013). In the Asteraceae particularly, SLs which are typically localized in leaves and flowering heads, are one of the main contributors to the plant's defense mechanisms. Since plants are constantly under microbial attack, SLs are able to provide defense against fungi, bacteria, and viruses, by disruption of a microbe's cell membrane, due to their polar groups disrupting the phospholipid membrane. Sesquiterpene lactones function as phytoalexins in response to microbial attack, as anti-feedants to deter herbivores, as attractants of pest predators, as hormones, and as allelochemicals (Chadwick et al., 2013). Sesquiterpene lactones demonstrate a broad spectrum of biological activity including anti-tumor, anti-inflammatory anti-malarial, anti-viral, anti-bacterial, and anti-fungal activity. The QS and biofilm inhibitory potential of plant SLs has been reported by Cartagena et al. (2007) and Amaya et al. (2012), while that of drimane sesquiterpenoids has been reported by Paz et al. (2013) and Cárcamo et al. (2014).

The binding of the signal molecule to the sensor can be compared to that of a ligand-binding to an enzyme active site. According to Goh et al. (2005), it is important to analyze the binding of signal antagonists to the receptor protein in order to fully understand the inhibitory effect. In this study, we report the QS inhibitory potential of previously described eudesmanolide SLs (blumeoidolides) and crude extracts from *Vernonia blumeoides* Hook. f. (Asteraceae) (Aliyu et al., 2015) using *Chromobacterium violaceum* and *Agrobacterium tumefaciens* biosensor systems. In addition, *in silico* molecular docking of the SLs (blumeoidolides A, B, C, and D) with LuxR homologues, CviR and CviR′ was carried out to confirm and assess the molecular characteristics of the protein–ligand interactions.

2. Results and discussion

2.1. Chemical composition of extracts

Table 1 indicates the chemical composition of four solvent extracts of *V. blumeoides* as identified by GC–MS analysis and using the NIST library. Fatty acids/esters, terpenoids and steroids constituted the main classes of bioactive compounds. The major components in the hexane (VBL-Hex), dichloromethane (VBL-DCM), ethyl acetate (VBL-EA) and methanol (VBL-MeOH) extracts were 2-(octadeca-9*Z*,12*Z*-dienyloxy)ethanol (**5**) (12.5%), 14,15-epoxy-3,11-dihydroxy-(3β,5β,11α,15β)-bufa-20,22-dienolide (**6**) (33.5%), catechol (**7**) (19.5%) and 3,5-stigmastadien-7-one (**8**) (17.9%), respectively (Fig. 1). Of the main classes of bioactive compounds, terpenoids are ubiquitous components of plant extracts, but chemo-types vary in aggregate composition due to environmental influences or genetic evolution (Figueiredo et al., 2008). This probably determines the potency of biological or pharmacological action on microorganisms.

2.2. Biosensor antimicrobial susceptibility testing

The antimicrobial activity of crude extracts and eudesmanolide SLs from *V. blumeoides* against Gram-negative and Gram-positive indicator bacteria has already been reported (Aliyu et al., 2015). Crude extracts and blumeoidolide A (**1**) (2 and 4 mg ml^{-1}) were initially assessed for their antimicrobial effect against the biosensor and AHL over-producer strains (Table 2), where 2 mg ml^{-1} was observed to be a sub-inhibitory concentration.

2.3. Quantitative anti-quorum sensing activity-violacein inhibition

Inhibition of violacein pigment production by blumeoidolide A (**1**), blumeoidolide B (**2**) and four crude extracts (0.15–9.5 mg ml^{-1}), all obtained from our previous study (Aliyu et al., 2015), was measured spectrophotometrically and quantified (Fig. 2). Due to a lack of sufficient sample of blumeoidolides C (**3**) and D (**4**) also isolated in our previous study (Aliyu et al., 2015), the inhibition of violacein could not be determined for these samples. This range of concentrations was used to identify the lowest concentration at which QS was evident, as well as document any potential growth inhibitory effect. Given the limited yield of blumeoidolide B (**2**), QSI was investigated in a range of 0.003–0.19 mg ml^{-1}. Growth inhibition was observed at concentrations $\geqslant$5 mg ml^{-1} and these concentrations were not considered for QSI.

A concentration-dependent inhibition of violacein production by *C. violaceum* ATCC 12472 was observed with $\leqslant$4.75 mg ml^{-1} blumeoidolide A (**1**) and four crude extracts (Fig. 2), without inhibition of bacterial growth. The differences in the mean values among the treatment groups was greater than would be expected by chance, thus there was a statistically significant difference ($p < 0.001$). A similar concentration-dependent inhibition of violacein production has been reported with methanol extracts of dried *Capparis spinosa* fruit (Packiavathy et al., 2011), *Cuminum cyminum* extract (Packiavathy et al., 2012), and aqueous *Moringa oleifera* leaf and fruit extracts (Singh et al., 2009), without inhibiting bacterial growth. Inhibition of violacein production $\geqslant$90% violacein was observed at concentrations of 2.4 mg ml^{-1} of crude extracts. The four *V. blumeoides* extracts displayed varying levels of QSI potency, with 90% inhibitory activity in the following order: VBL-EA > VBL-DCM > VBL-Hex > VBL-MeOH (Fig. 2). An 88% inhibition in violacein production was obtained with 2 mg ml^{-1} of the *C. spinosa* methanol extract (Packiavathy et al., 2011), while 2 mg ml^{-1} of the methanol *C. cyminum* resulted in 90% inhibition (Packiavathy et al., 2012).

Violacein inhibition of $\geqslant$85% was obtained with blumeoidolide A (**1**) with $\geqslant$3.6 mg ml^{-1} and 22%, 81% and 97% with 0.048, 0.071 and 0.095 mg ml^{-1} of blumeoidolide B (**2**), respectively. The IC_{50} for blumeoidolides A (**1**) and B (**2**) were 1.55 and 0.055 mg ml^{-1}, respectively, while those of the crude extracts ranged from 0.45 (VBL-Hex) to 0.77 mg ml^{-1} (VBL-DCM). Blumeoidolide B (**2**) thus had a greater QSI effect than blumeoidolide A (**1**), since it inhibited violacein production at a much lower concentration. The difference in structure between blumeoidolide A (**1**) and B (**2**) is the position of the acetyl group from C-1 on the bicyclic ring to C-17 on the open side chain (Fig. 1). This is indicative that the acetyl group

Please cite this article in press as: Aliyu, A.B., et al. Quorum sensing inhibitory potential and molecular docking studies of sesquiterpene lactones from *Vernonia blumeoides*. Phytochemistry (2016), http://dx.doi.org/10.1016/j.phytochem.2016.02.012