Abstract PhD thesis Joshua Jacobtorweihen

Bromophenols from *Vertebrata lanosa* (L.) T.A.Christensen with antimicrobial activity against *Pseudomonas aeruginosa* – molecular biological and phytochemical investigations

The gram-negative bacterium *Pseudomonas aeruginosa* is responsible for a wide range of acute and chronic infections in both immunocompromised and cystic fibrosis patients. The ability to colonize the host and cause these infections can be attributed to the expression of a wide range of virulence traits by *P. aeruginosa*. Among these, specialized metal ion uptake systems enable the bacteria to evade certain mechanisms of the innate immune system associated with a so-called "nutritional immunity". Aim of the first part of this work was to utilize proliferation-based assays in order to identify potential inhibitors of the metal uptake systems of *P. aeruginosa*. Growth of *P. aeruginosa* ATCC 27853 in the metal ion-deficient Vogel-Bonner Minimal-Medium (VB-MM), which induces expression of relevant uptake systems, was assayed for 178 pure compounds and twelve fractions from two plant extracts. Ten of the 29 compounds showing growth inhibition in VB-MM did not exhibit antiproliferative effects on *P. aeruginosa* in the nutrient-rich lysogeny broth (LB). Methylrhodomelol, a bromophenol produced by the red alga *Vertebrata lanosa* (L.) T.A.Christensen, showed the strongest antiproliferative effect (MIC 50 µg/mL, VB-MM) of the ten screening hits while only exhibiting weak cytotoxicity (IC_{50} > 200 µM, MTT assay, Vero cells). The bacteriostatic effect was not antagonized by the physiologically important zinc(II) cation. Furthermore, methylrhodomelol neither reduced biofilm formation and extracellular proteolytic activity, nor swimming or swarming motility of *P. aeruginosa*.

Further experiments using drug affinity responsive target stability (DARTS) assays aimed at directly identifying potential target proteins interacting with methylrhodomelol in *P. aeruginosa*. DARTS assays suggested an interaction between the bifunctional protein FolD and the bromophenol, however this hypothesis could not be verified by additional assays (thermal shift assay, single protein DARTS and enzymatic activity). Therefore, a transcriptome analysis by RNA-seq was conducted to generate a hypothesis for the molecular target of methylrhodomelol in *P. aeruginosa*. Differential expression of 93 genes was observed in methylrhodomelol-treated bacteria, of which 28 were downregulated and 65 were upregulated. Network analysis of the differentially expressed genes demonstrated that genes associated with carbon utilization from various sources were upregulated, while downregulated genes were related to methionine biosynthesis (*metE*, *metH*), uptake of divalent metal ions (*cntI*, PA1925, PA2911, PA5534) and uptake of organic sulfur substrates (*tauB3*, *tauD*, *ssuCDF*). Validation of an increase or decrease in expression was achieved by real-time polymerase chain reaction for six selected genes (*metE*, *exaA*, *liuA*, *coxA*, *mmsB* and *hpd*). An inhibition of the *S*-adenosylmethionine synthetase MetK by methylrhodomelol could be a potential explanation for the observed changes in expression.

Apart from methylrhodomelol, the red alga *V. lanosa* also produces a variety of other bromophenolic secondary metabolites. Whereas compounds such as methylrhodomelol that are present in rather lipophilic fractions are well studied, little is known about the more polar components of extracts from *V. lanosa*. Within this study, a hydromethanolic extract of *V. lanosa* was fractionated yielding 24 pure compounds, eight of which are new natural products. Seven of these new compounds are polar bromophenols obtained from the aqueous fraction of the extract and were identified as 4-sulfo-7-dimethylsulfoniumlanosol (**7**), *N*-lanosylguanidine (**8**), 3,5-dibromo-L-tyrosine (**13**), 3-bromo-5-sulfo-L-dihydroxyphenylalanine (**14**), 3-bromo-6-lanosyl dihydroxyphenylalanine (**15**), 3-(6´-lanosyl lanosyl)-tyrosine (**16**) und 5-sulfovertebratol (**17**). This is the first report of naturally occuring bromophenols from algae containing a sulfonium or a guanidium moiety. In addition, the three mycosporine-like amino acids porphyra-334 (**18**), aplysiapalythine A (**19**) and palythine (**20**) were isolated from the aqueous fraction of the extract. Aside from the known bromophenol lanosol (**1**) and its derivatives (**2**, **3**, **5**, **6**, **9**, **10**), the four major glycerogalactolipids (**21**-**24**), including the new natural product (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoic acid-3´-[(6´´-*O*-*α*-galactopyranosyl-*β*-D-galactopyranosyl)]-1-glycerol ester (**24**) were isolated from the ethyl acetate phase of the extract.