Design and syntheses of potent antagonists of 3kPZS through medicinal chemistry optimization.

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ABSTRACT:

Pheromones are critical for sea lamprey spawning migration and reproduction and are potential targets for effective and environmentally benign control strategies. **Empirical evidence demonstrates that disrupting pheromone communication may provide a method for the sea lamprey lifecycle disruption and, consequently, a tactic for sea lamprey control.** We have previously shown that mature male sea lamprey release of 3-keto-petromyzonol sulfate (3kPZS) as a main component, of a more complex mixture of the mating pheromones, attract and retain ovulated females on nests. Unfortunately, 3kPZS (7α,12α, 24-trihydroxy-3-one-5α-cholan-24-sulfate or 3-keto-petromyzonol sulfate (**Fig. 1**) only moderately increases the trapping rate of female adult sea lampreys when used as a natural attractant. An alternative approach, taken herein, is to disrupt female attraction by disrupting with sexual pheromone antagonists to provide a more effective strategy for sea lamprey control. It has been previously found that individually or as the mixture, with even greater effectiveness, PZS (7α,12α,24-trihydroxy-5α-cholan-24-sulfate, Fig. 1) and 3sPZS (Petromyzonol 3,7,12,24-tetrasulfate, **Fig. 1**) significantly reduced, in field studies, the ability of the of female sea lamprey to find male sea lamprey nests. However, PZS and 3sPZS need to be applied 10~100 times the concentration of 3kPZS to be effective. PZS and 3sPZS disrupt mate searching behaviors in ovulated females and halve the number of females that reproduce on a spawning ground, demonstrating the potential utility of pheromone antagonists for integrated sea lamprey control. It was our goal to discover or design pheromone antagonists that are more potent than PZS or 3sPZS. To this end, we have prepared ~116 derivatives (multiple proposals), modulating substitution and ring conformations in the A, B, and C rings (**Fig. 1**) and the C24 position of both allocholic and cholic acid templates. Analogs have been tested in electro-olfactogram (EOG) recordings, examining the ability of sea lampreys to "smell" the compounds, and in behavioral response studies (2-choice maze), to elucidate the degree of sexual antagonism or agonism. In addition, we

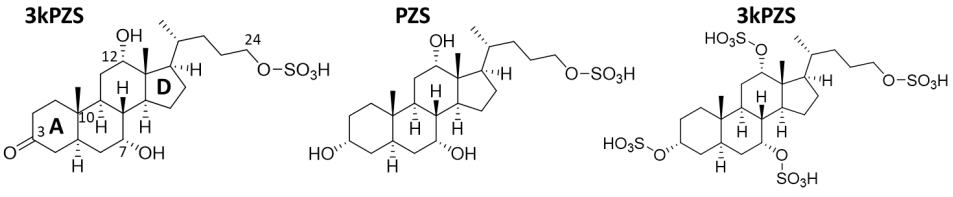

Fig 1. Structures of pheromone component (i) and its candidate antagonists (ii,iii). (i) **3kPZS**: 3-keto-petromyzonol sulfate sodium salt, showing canonical atom numbers for the steroid ring system and tail; (ii) **PZS**: Petromyzonol sulfate, sodium salt; (iii) **3sPZS**: Petromyzonol 3,7,12,24-tetrasulfate, tetrasodium salt.

previously identified three orphan receptors, putatively tied to the 3kPZS activity, and screened them against new analogs looking for correlation to either EOG activities or sexual behavioral response studies. No correlation of antagonism or agonism these receptors impacted EOG (smell), or sexual antagonism activities was observed. Multiple analogs were observed to independently modulate both EOG and / or sexual behavioral response. No analogs were identified as having both high EOG activity and high sexual antagonism. No structural correlation was identified connecting these two activities. Four analogs were identified as having significant sexual antagonism representing both the allocholic and cholic acid templates. More than 15 other analogs were observed to "neutralize the effects of 3kPZS. **These results provide the opportunity to further explore these 17 prepared analogs, individually, and in combination, to reduce the effects of 3kPZS, thereby disrupting the ability of females to find nests disrupting reproduction. It is currently not understood whether simply neutralizing the effect of 3kPZS or fully antagonizing its effect represents the superior strategy or if they are of similar impact. From our initial data, either strategy appears to disrupt the complex pheromone environment required for sea lamprey reproduction. Further studies need to be completed to address. If successful, we anticipate that these agents will provide an environmentally clean approach, relative to, for example, the use of lampricides, and fit into an integrated sea lamprey control program. Blocking reproduction, with sexual antagonists, represents an attractive approach to reduce the overall numbers of sea lamprey produced.**