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**Scale up isolation of aaptamine for in vivo evaluation indicates its neurobiological activity is linked to the delta opioid receptor**

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Scale up isolation of aaptamine for in vivo evaluation indicates its neurobiological activity is linked to the delta opioid receptor

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Introduction

Opioid receptors belong to the large superfAMILY of seven transmembrane-spanning (TM) G protein-coupled receptors (GPCRs). As a class, GPCRs are of fundamental physiological importance mediating the actions of the majority of known neurotransmitters and hormones. The Mu (µ), Delta (δ) and Kappa (κ) opioid receptors are particularly intriguing members of this receptor family as they are the targets involved in many neurobiological diseases such as addiction, pain, stress, anxiety, and depression. To date few marine natural products have been investigated for their neurobiological activities.1 One noteworthy example involves ziconotide (1) from the cone snail Conus magus.2 Compound 1 was the first marine natural product approved by the FDA and is used for the treatment of pain, marketed under the trade name Prialt® (2004).3 More recently Hamann reported that aaptamine (2) is the first marine natural product to show in vivo anti-depressant activity, however no mechanism of action was proposed.4,5 During a separate collaborative screening project we profiled 96 sponge-derived extracts and discovered that demethyl-aaptamine (3) and demethyl (oxy)-aaptamine (4) were selective DOR agonists using an LC-MS based library of an active methanolic extract coll. no. 92553 FM as shown in Fig. 1. We speculated that the in vivo activity for 2 could thus be linked to the DOR target and to test this hypothesis we conducted the following experiments below.

Experimental and Results

Our first step involved obtaining a source of aaptamine (2) for in vitro and in vivo evaluation. Compounds 3-4 were obtained from the sponge Auptos auptos (coll. no. 92553) but were devoid of 2. LC-MS analysis of sponge coll. no. 11308 (A. auptos) indicated m/z ions of 229 [M+H]+ consistent with that of 2 (not shown). We extracted coll. no. 11308 using a partition scheme shown in Fig. 2. The WB extract was enriched with 2 based on LC-MS data in Fig. 3a and used to scale up it’s isolation by HPLC shown in Fig. 3b. Chemical validation of pure 2 was confirmed by LC-MS and 1H NMR data in Fig. 4. This allowed us to screen 2 alone and confirm its DOR activity in vitro (2, EC50 = 5.1; 3, EC50 = 4.1; 4, EC50 = 2.3). In vivo evaluation indicated 2 was an antidepressant in wild type mice in the forced swim test (Fig. 5a, black bars) while having no effect on general locomotion (Fig. 5a, red bars). LC-MS analysis of sponge coll. no. 11308 (Add 180 mL CHCl3 (166.7 mg) and used to scale up its isolation by HPLC shown in Fig. 3b). We further found that the antidepressant activity was abolished in genetically modified mice where the DOR gene was knocked out (Fig. 5a, red bars, DOR KO). We also found 2 was an anxiolytic in the marble burying test (Fig. 5b). These results indicate the anti-depressant activity previously reported for 2 is mediated by it’s agonistic activity of the delta opioid receptor.


Conclusions

1) Scale up isolation of aaptamine (2) is best achieved through purification of water soluble extracts.
2) The mechanism of action for the in vivo anti-depressant-like and anxiolytic-like activity of 2 is mediated by it’s activity on the delta opioid receptor (DOR).
3) These data suggest that 2 can represent a novel chemical scaffold for the development of new DOR ligands in neurobiological research.

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