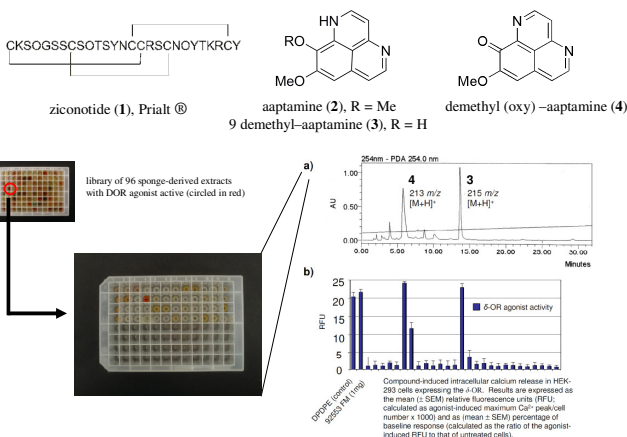


# Scale up isolation of aaptamine for *in vivo* evaluation indicates its neurobiological activity is linked to the delta opioid receptor

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## Introduction

Opioid receptors belong to the large superfamily of seven transmembrane-spanning (7TM) 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 ( $\mu$ ), Delta ( $\delta$ ) and Kappa (MOR, DOR, KOR) 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.<sup>1</sup> One noteworthy example involves ziconotide (**1**) from the cone snail *Conus magnus*.<sup>2</sup> 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).<sup>3</sup> More recently Hamman reported that aaptamine (**2**) is the first marine natural product to show *in vivo* anti-depressant activity, however no mechanism of action was proposed.<sup>1,4</sup> 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.



**Figure 1. a)** LC-MS library with annotations including  $m/z$  ions and **b)** Comparative DOR agonist activity of the methanol extract LC fractions of coll. no. 92553 FM.

## Experimental and Results

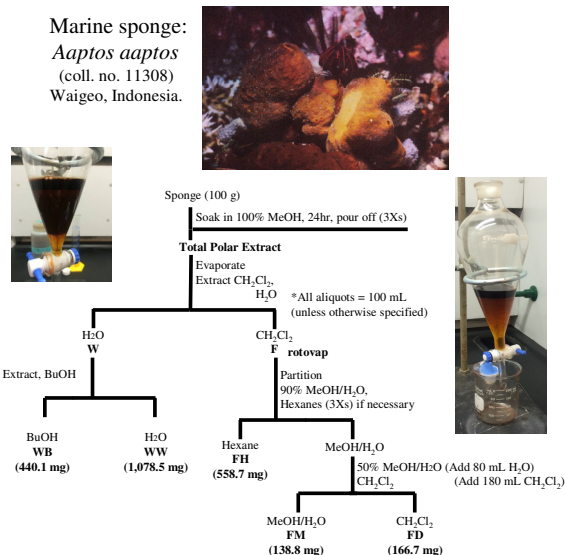
Our first step involved obtaining a source of aaptamine (**2**) for *in vitro* and *in vivo* evaluation. Compounds **3-4** were obtained from the sponge *Aaptos aaptos* (coll. no. 92553) but were devoid of **2**. LC-MS analysis of sponge coll. no. 11308 (*A. aaptos*) indicated  $m/z$  ions of 229 [M+H]<sup>+</sup> 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 its isolation by HPLC shown in Fig. 3b. Chemical validation of pure **2** was confirmed by LC-MS and <sup>1</sup>H NMR data in Fig. 4. This allowed us to screen **2** alongside **3-4** and confirm it's DOR activity *in vitro* (**2**, EC<sub>50</sub> = 5.1; **3**, EC<sub>50</sub> = 4.1; **4**, EC<sub>50</sub> = 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. 5b). 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. 5c). These results indicate the anti-depressant activity previously reported for **2** is modulated by its agonist activity of the delta opioid receptor.

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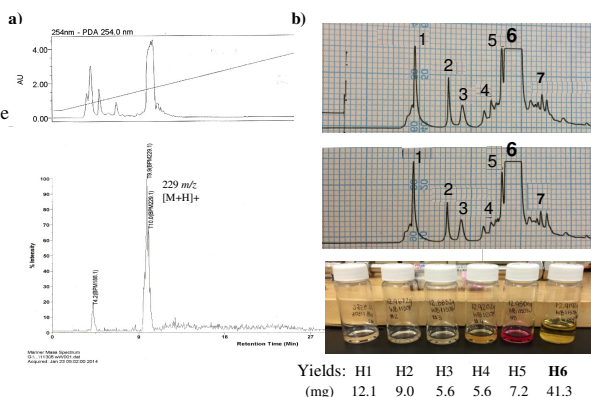
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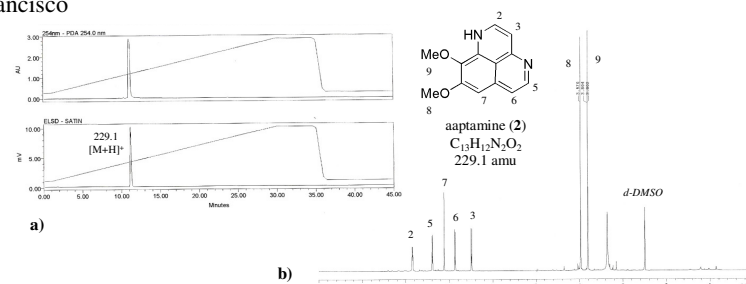
<sup>†</sup>These authors contributed equally to this work



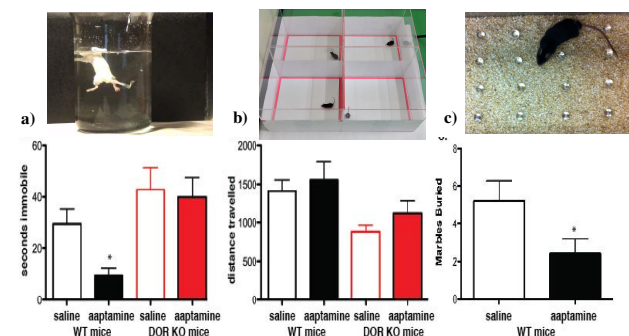
**Figure 2.** Extraction Scheme Flowchart of coll. no. 11308



**Figure 3. a)** Analytical traces LC (top) MS (bottom) of coll. no. 11308 WB and **b)** Preparative scale up HPLC traces of coll. no. 11308 WB fractions. Gradient: 10% → 100% CH<sub>3</sub>CN (45 min); [4.0 mg/100 μl] x 30 injections; λ<sub>max</sub> = 254 nm; sensitivity = 2.0 AU; flow 2.0 ml/min.



**Figure 4.** Chemical validation of aaptamine (**2**) using: **a)** LC-MS-ELSD analysis with annotations including  $m/z$  ions and **b)** <sup>1</sup>H NMR data of 11308 WB H6.



**Figure 5.** Mice were injected with saline or aaptamine (**2**, 40mg/kg, i.p.) and subjected to: **a)** a forced swim test (**a**), a locomotor test (**b**) or a marble burying test (**c**). Swim sessions lasted 10min (in ambient light of 580–680lx), and were conducted in individual clear plastic cylinders (35.5cm tall x 25cm in diameter) filled with water (26.5°C, ±0.7°C) to a depth of 20cm±1.5cm. A camera positioned on an angle to the cylinders recorded the sessions. Manual scoring was for total immobile time during the last 6min of the session. Immobility was defined as the absence of movement, except that necessary to keep afloat. Following the swim test, mice were placed in a locomotor chamber to assess general locomotion for 30 minutes. For the marble burying test, mice were placed in a cage with 5 cm of wood shavings and 15 evenly spaced marbles for 20 minutes. At the end of the session, the number of marbles buried and exposed were counted. Aaptamine (**2**) was effective in WT but not DOR knock out (KO) mice (\* p < 0.05 compared to saline).

## 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 its 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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