Screening of rare alkaloids as potential opioid analgesic

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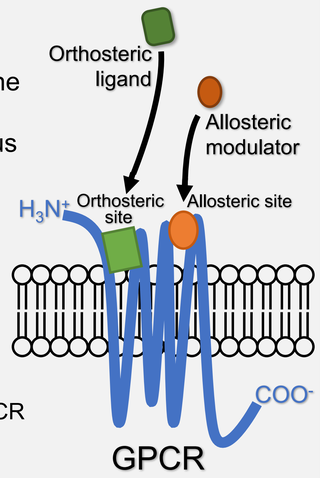
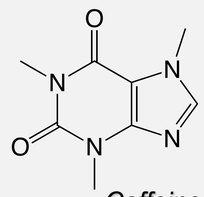
Introduction

G-protein-coupled receptors (GPCRs)

- Abundant and diverse human receptors
- Contain seven hydrophobic transmembrane segments with an extracellular amino terminus and intracellular carboxyl terminus
- Highly targeted in drug discovery for their role in nearly all physiological functions
- Extracellular stimuli result in internal signaling which alter cellular function:
  - Orthosteric ligands
    - Bind to natural ligand binding site
  - Allosteric modulators
    - Bind to allosteric sites which modulate GPCR activity in presence of orthosteric ligand





Caffeine, a common alkaloid

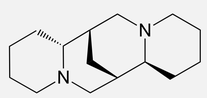
Alkaloids

- Have been used as empirical medicine as early as 2000 BCE
- Heterocyclic rings with nitrogen atoms of an amino-acid or pseudo-amino-acid derivative
- Underrepresented in today's pharmaceutical market despite their drug-like physiochemical properties

Receptor	Ligand
δ-opioid receptor (DOR)	D-Ala[2], D-Leu[5]-Enkephalin (DADLE)
μ-opioid receptor (MOR)	[D-Ala[2], *N*-MePhe[4], Gly-ol]-enkephalin (DAMGO)
κ-opioid receptor (KOR)	Salvinorin A (Sal A)
Nociceptin opioid peptide receptor (NOP)	Nociceptin

This project

- DOR, MOR, KOR, NOP are GPCRs involved in pain physiology
- Collaboration with Dr. Tony Durst (Department of Chemistry, University of Ottawa) made available 121 rare bioactive alkaloids that were screened at a single dose concentration.



Alkaloid 21, sparteine

Objectives

Secondary screening of positive bioactive alkaloids identified in the primary screening. Compounds 20, 21, 44, 45, 54, 85 and 121 are tested as potential allosteric modulators of DOR, MOR, KOR, or NOP using GloSensor™ assay to possibly lead to initiating development of innovative analgesic therapeutics.

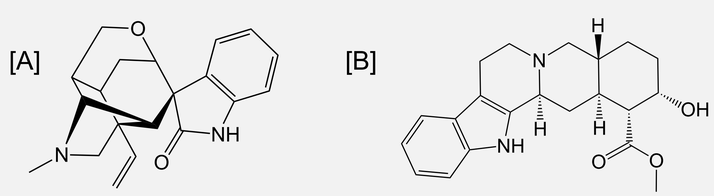


Figure 1. Scaffold structures of some alkaloids tested.
[A] Gelsemine chemotype. [B] Yohimbine chemotype.

Method

HEK-293 cells stably expressing opioid receptor and GloSensor™ luciferase used for screening

GloSensor™ assay: sensitive, real-time detection of cAMP in live cells following receptor activation

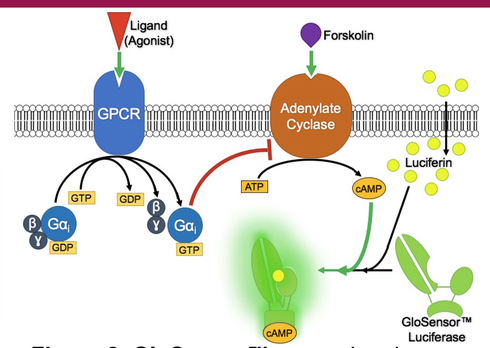


Figure 2. GloSensor™ assay. Luminescence from luciferase activity reports GCPR activity.

Protocol:

1. Addition of the luciferin substrate for 30 min
2. Alkaloids were added and incubated for 15 min
3. Agonist was added and incubated for 15 min
4. Forskolin was added and incubated for 15 min to elevate basal cAMP
5. Data read using a MicroBeta Luminescence Counter
6. Data analysis using Prism Software

Results

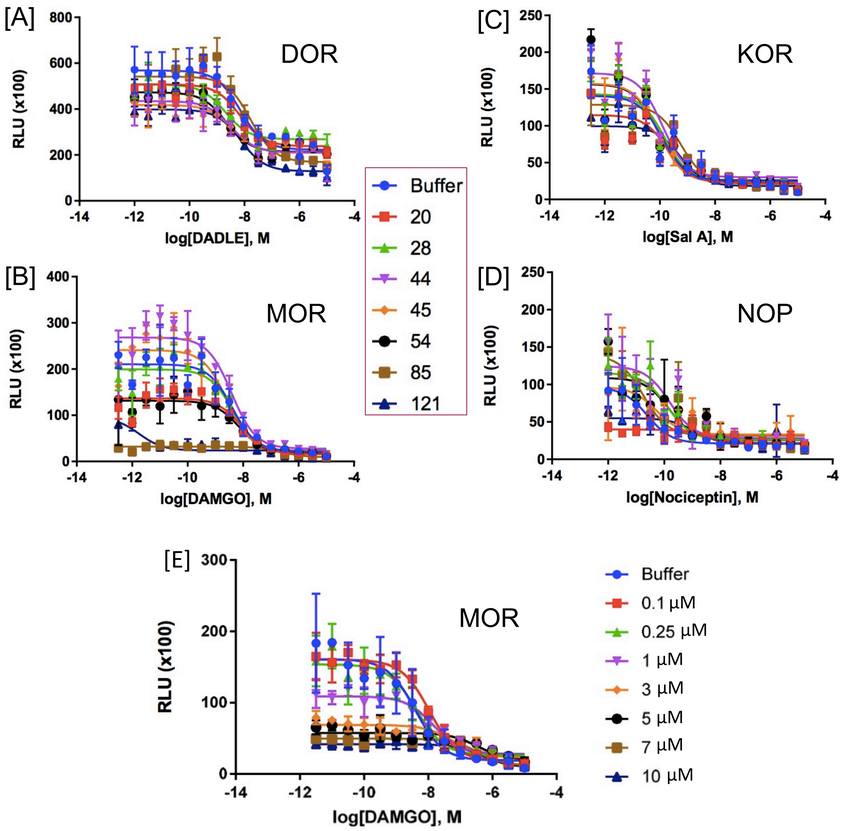


Figure 3. [A-D] Effect of various alkaloids at DOR, MOR, KOR, and NOP, respectively. All seven alkaloid compounds were tested at 10μM in triplicates in the presence of an endogenous ligand of each receptor. [E] Schild analysis of compound 85 on MOR. Because of its strong effect on MOR as shown in [B], various concentrations of compound 85 were tested for further study.

Discussion

Result	Implication
Figures 3[A,C] show all seven alkaloids have dose-curves very similar to buffer, the negative control, with only slight variation in dose-curve height and start plateau, respectively.	All seven alkaloids do not significantly modulate GPCR activity of DOR and KOR.
Figure 3[B] shows alkaloids 85 and 121 have very flat dose-curves compared to buffer and alkaloids 20 and 54 show a start plateau much lower than buffer. The remaining alkaloids show dose-curves very similar to buffer.	Alkaloids 85 and 121 have a strong activating effect on the MOR – even at low concentrations of DAMGO, they strongly activate the G-protein pathway, as seen by the low luminescence. Thus, they are positive allosteric modulators (PAMs) of MOR. Alkaloids 20 and 54 are PAMs to a lesser degree, and the remaining alkaloids do not significantly modulate MOR activity.
Figure 3[D] show alkaloids 20 and 121 have much flatter dose-curves than buffer. The remaining alkaloids show dose-curves very similar to buffer.	Alkaloids 20 and 121 are slight PAMs of NOP, and the remaining alkaloids do not significantly modulate NOP activity.
Figure 3[E] shows concentration of alkaloid 85 at 1μM and greater results in a start plateau lower than buffer.	Alkaloid 85 begins to show significant PAM effect on MOR activity at 1μM, and concentrations greater than that yield greater PAM effect on MOR activity.

Conclusion

Finding	Next steps
Alkaloids 85 and 121 are PAMs of MOR activity, with a minimum concentration of 1μM of 85 to see modulated effects.	Further Schild analysis of these alkaloids can be done to better characterize their allosteric modulation on their respective receptors.
Alkaloids 20 and 54 are slight PAMs of NOP activity.	
Schild analysis of 85 confirms it is a PAM of MOR and shows effect starting at 1μM.	Further characterization of the PAM effect of 85 to better understand how it affects activity.

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The Giguère Laboratory — Molecular Pharmacology & Drug Discovery

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