Control of the μ-Opioid Receptor Efficacy *In Vitro* and I*n Vivo* by Novel Conformationally Restricted Cyclopropane Carboxamide-derived *Cis/Trans* Isomers

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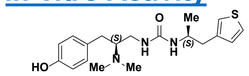
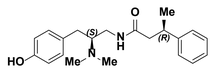
Introduction

- Opioid use disorder is widely recognized as a national epidemic and a health crisis in the US.[1,2]
- μOR agonists that are currently prescribed to manage pain, like morphine, codeine, fentanyl and oxycodone, can lead to tolerance and dependence resulting in respiratory depression ultimately causing hypoxia and death.[2]
- Towards better drugs to manage pain with reduced side effects, we have designed, synthesized and evaluated a novel series of rigid and conformationally constrained cyclopropane carboxamide derivatives with high affinity for the μOR to study how the ligand conformation affects activation (efficacy) of the receptor.
- The *cis* and *trans* configuration of our cyclopropane carboxamides have significantly different efficacy towards the μOR, acting as antagonists and agonists, respectively.
- In addition to *in vitro* and *in vivo* activity of our *cis* and *trans* isomers, protein-ligand docking studies of our ligands to the inactive form (pdb: **4DKL**)[3] and active form (pdb: **5C1M**)[4] of the μOR were performed using AutoDock4[5] to better understand the critical residue-ligand interactions underlying the observed difference in efficacy.

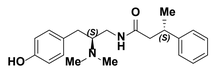
In Vitro Activity



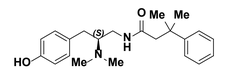
PZM21[6]
EC_{50} 13 nM (92%)



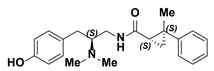
Compound A
EC_{50} 31 nM (71%)



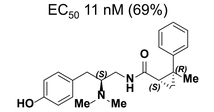
Compound B
EC_{50} 11 nM (69%)



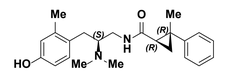
Compound C
EC_{50} 32 nM (37%)



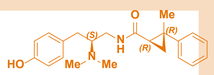
Compound D EC_{50} 7.3 nM (9%);
IC_{50} 220 nM (106%)



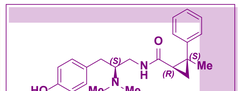
Compound E EC_{50} > 3000 nM;
IC_{50} > 3000 nM



Compound F
EC_{50} 0.33 nM (100%)



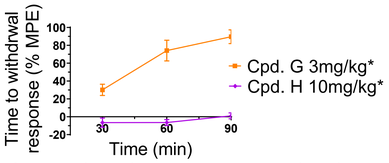
Compound G
EC_{50} 0.88 nM (90%)



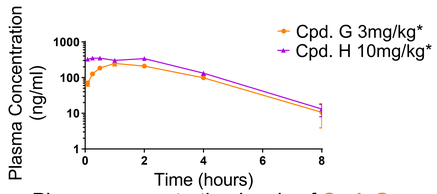
Compound H EC_{50} > 3000 nM;
IC_{50} > 120 nM (100%)

- μOR cAMP assays were performed using a stably transfected CHO cell line expressing human-μOR. Measurements were performed in triplicate.
- For the ***agonist*** assay the dose response are reported as mean values of the concentration that elicit 50% (EC_{50}) of the maximal response (%E_{max}) normalized to DAMGO.
- The ***antagonist*** assay was performed at the DAMGO EC_{90}, the dose response are reported as mean values of the concentration that elicit 50% (IC_{50}) of the maximal inhibition (%inhibition) normalized to naltrexone.

In Vivo Activity

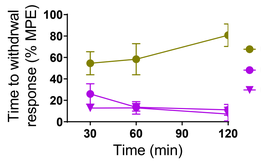


In a rat hot plate assay, an analgesic effect is observed for agonist **Cpd. G**, but not for antagonist **Cpd. H**.



Plasma concentration levels of **Cpd. G** correlate with analgesic effect. **Cpd. H** has roughly the same plasma stability.

**subcutaneous dose*

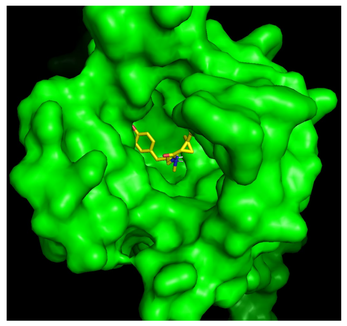


When co-administered with potent agonist **Cpd. F**, **Cpd. H** blocks analgesic effect in hot plate assay.

Color Legend:

Green: μOR protein (inactive 4DKL)	**Blue: μOR protein (active 5C1M)**
Yellow: Cpd. G pose in inactive μOR	**Orange: Cpd. G pose in active μOR**
Pink: Cpd. H pose in inactive μOR	**Purple: Cpd. H pose in active μOR**

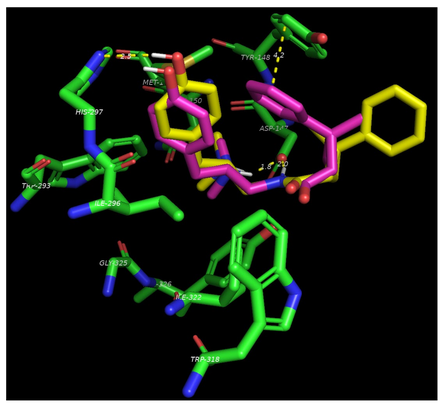
Binding cleft changes upon μOR activation



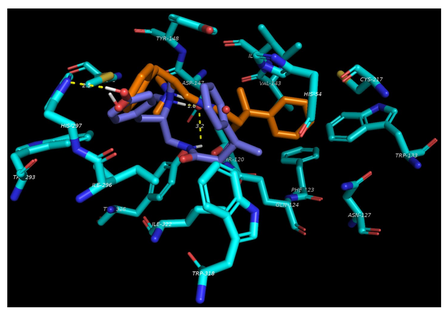
Molecular surface of the μOR with **Cpd. G** docked in the binding cleft.
Left: the inactive μOR **4DKL** has an open, solvent-accessible binding cleft.
Right: the active μOR **5C1M** has a closed cleft due to tertiary structure changes.

Docking to 4DKL (Inactive μOR)

- *Trans* analog **Cpd. G** and *cis* analog **Cpd. H** are docked to the inactive form of the μOR **4DKL**.
- Three hydrogen bonds are observed:
  - Phenolic OH to **HIS297** (2.5 Å)
  - NMe_2 to **ASP147** (1.8 Å)
  - Amide NH to **ASP147** (2.0 Å)
- A π-π interaction between the phenyl group on **Cpd. H** and **TYR148** is observed (4.2 Å)
- Docking Scores:
  - **Cpd. G: –9.13 kcal/mol**
  - **Cpd. H: –9.05 kcal/mol**

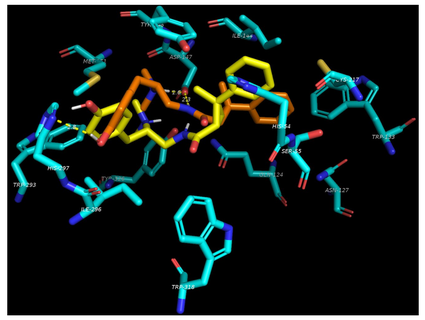


Docking to 5C1M (Active μOR)



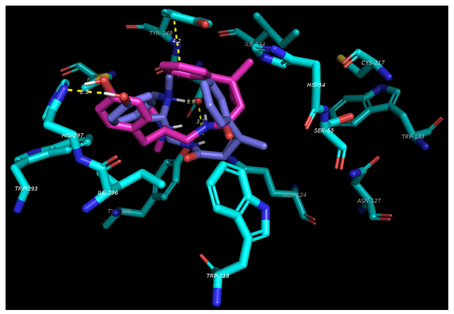
- *Trans* analog **Cpd. G** and *cis* analog **Cpd. H** are docked to the active form of the μOR **5C1M**.
- The H-bond network is maintained for **Cpd. G**. However, the H-bond network is disrupted for **Cpd. H**:
  - Phenol to **HIS297** maintained (2.5 Å)
  - NMe_2 to **ASP147** maintained (1.6 Å)
  - Amide NH to **ASP147** broken (3.2 Å)
- Docking Scores:
  - **Cpd. G: –11.2 kcal/mol**
  - **Cpd. H: –10.6 kcal/mol**

Conformational Response to μOR activation for Cpd. G



- Two poses of Cpd. G are shown: The **inactive μOR pose**, and the **active μOR pose**. The receptor displayed is the active form of the μOR **5C1M**.
- A steric clash is seen between the "lid" residue **HIS54** and the phenyl group of the **inactive pose**. This forces the phenyl group backwards in the **active pose**.
- Other conformational changes are seen throughout the ligand, but key H-bond interactions are maintained.

Conformational Response to μOR activation for Cpd. H



- Two poses of Cpd. H are shown: The **inactive μOR pose**, and the **active μOR pose**. The receptor displayed is the active form of the μOR **5C1M**.
- A steric clash is seen between the "lid" residue **HIS54** and the methyl group of the **inactive pose**. This forces the phenylcyclopropyl group downwards in the **active pose**.
- This conformational rearrangement breaks the hydrogen bond between the amide NH and **ASP147**.

Conclusions

- The use of stereochemically defined cyclopropane rings as conformational restraints is a useful strategy for controlling the efficacy of ligands for the μOR.
- *Trans* analog **Cpd. G** and *cis* analog **Cpd. H** bind equally well to the inactive form of the μOR (**4DKL**).
- *Cis* analog **Cpd. H** binds worse to the active form of the μOR (**5C1M**) by about 0.6 kcal/mol compared to *trans* analog **Cpd. G** and has one fewer hydrogen bond to the receptor.

References

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Acknowledgements

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