Design, Synthesis, Computational Studies, and Evaluation of Novel Salvinorin-Based Opioid Receptor Agonists and Antagonists

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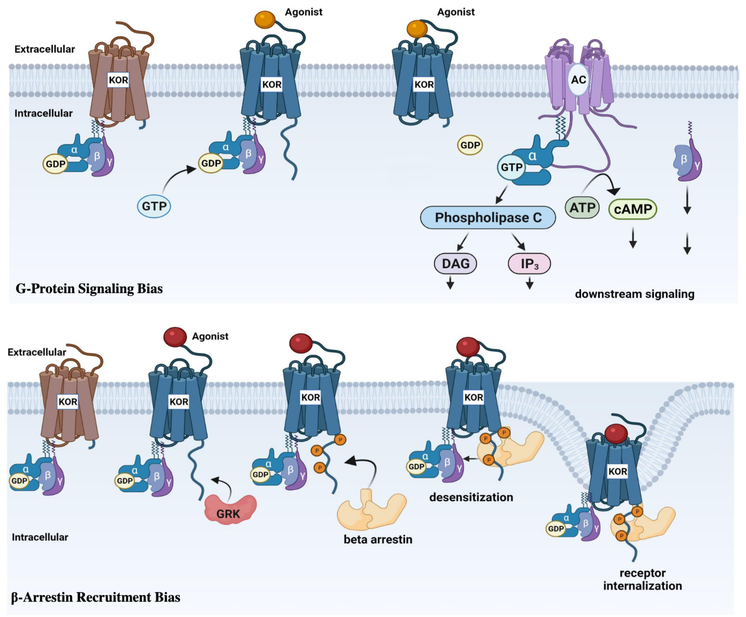
The Opioid Receptors and Salvinorin A

- Kappa opioid receptors (KORs) are one of the four ORs expressed throughout the nervous system.[1,2]
- KOR agonists have been employed clinically in the treatment of pain as they can elicit analgesia.[3,4]
- KOR antagonists have been used to stop common side effects of withdrawal which can lead to relapse in drug use, such as depressive and anxiety-related disorders.[5]
- Salvinorin A (SA) is isolated from *Salvia divinorum* and is a short-acting, naturally occurring, non-nitrogenous kappa-opioid-receptor (KOR) subtype-selective agonist.[6,7]



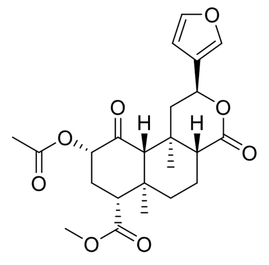


Figure 1: Signaling pathways in KOR agonism, *Salvia divinorum,* and structure of Salvinorin A

Design of Salvinorin–Based Compounds

- Several groups have undertaken a massive effort to synthesize a variety of analogs of salvinorin A.[6]
- This effort has produced some SAR hypotheses, but prediction of how each structural modification alters selectivity, affinity, and potency remains very difficult.[7]
- Overall, salvinorin-based opioid receptor ligands possess pharmacological liabilities:[7,10]
  - Side-effects such as hallucination and dysphoria, short-half life, etc.
- We aim to solve the problems for OR agonists and KOR antagonists with novel salvinorin-based compounds.[11]

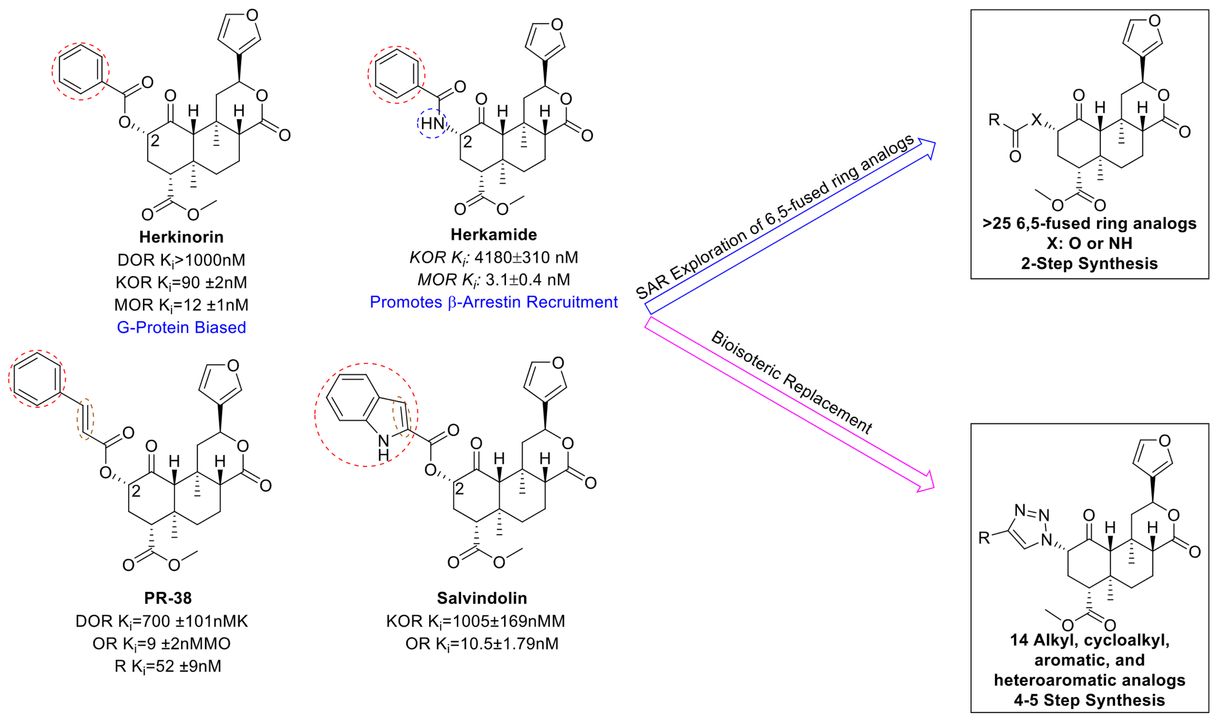
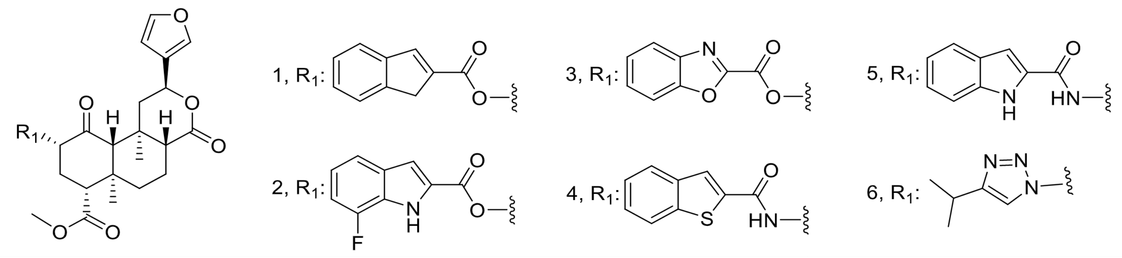


Figure 2: Salvinorin-based agonists

Top-Performing *In Vitro* Compounds



Cmpd	K_i ,nM (KOR)	EC_{50}, nM (KOR)	K_i, nM (MOR)	EC_{50}, nM (MOR)	K_i , nM (DOR)	EC_{50}, nM (DOR)
Nlxne	9.98 *(6.89–14.5)*	NT	4.39 *(3.14–6.11)*	NT	60.0 *(47.9–75.1)*	NT
1	49.7 *(23.5–105)*	853 *(456–1600)*	538 *(335–864)*	49.5 *(38.8–63.0)*	714 *(385–1330)*	2350 *(964–5710)*
2	53.8 *(36.0–80.6)*	36.5 *(13.6–98.2)*	58.4 *(26.2–130)*	132 *(46.3–378)*	ND[a]	ND[a]
3	>10,000	ND[a]	>10,000	ND	>10,000	ND[a]
4	>10,000	ND[a]	1.67±0.24	6.23±1.64	642±133	ND[a]
5	>10,000	ND[a]	54.5±11.9	38.4±9.57	>10,000	ND[a]
6	1089±505.8	NT	ND[a]	ND[a]	ND[a]	ND[a]

Table 1: Top performing ester, amide, and triazole salvinorin analogs[11]
[a]: Not tested further due to low displacement in screening assays
Italics 95% confidence intervals

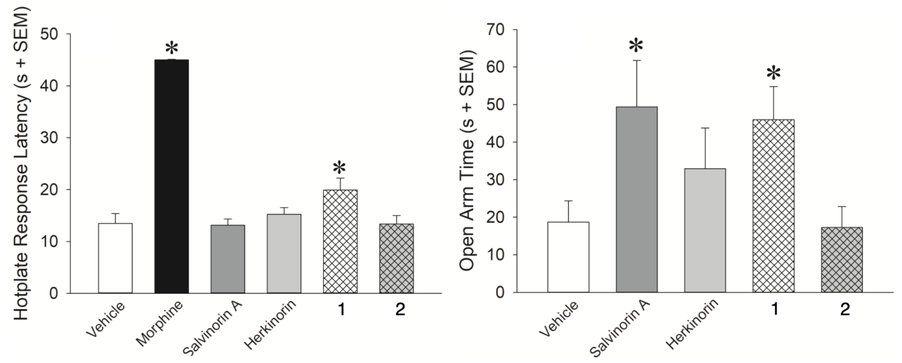


Figure 5: Effects of compounds **1** and **2** in the hot plate thermal anti-nociception assay and the elevated plus maze anxiety-like behavior assay. Morphine 5mg/kg, SalA 2.5mg/kg, **1** 2 mg/kg (HP) 5 mg/kg (EPM), **2** 2.5 mg/kg (HP) 5 mg/kg (EPM) @30 minutes.[11]

Computational and *In Vivo* Analysis

- Previous studies has suggested that ester-based compound, herkinorin, is a G-protein biased agonist due to interactions with the sodium allosteric pocket and similar to salvinorin A is thought to not interact with the conserved $D^{3.32}$ residue.[9,10]
- Alternative study has also identified $M151^{3.36}$ as a modulating residue for β-arrestin signaling.[12]
- Amide compounds exhibited hydrogen bonding within the linker region while esters do not **(Figure 4)**.
- Compound **4** exhibited an antinociceptive effect in hot-plate assay **(Figure 5)**.[11]

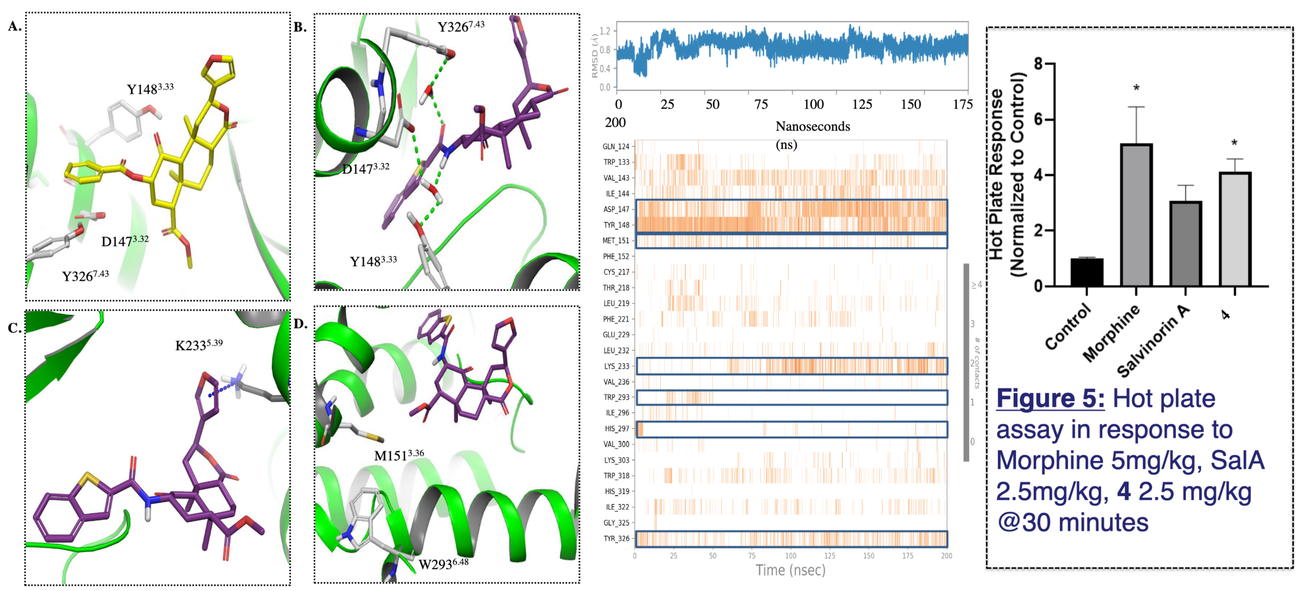
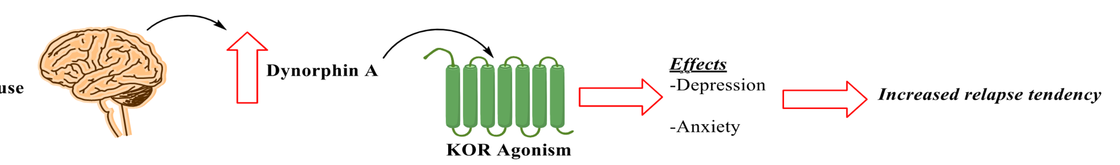


Figure 4: Computational Evaluation of Herkinorin and Compound **4**

In Vivo Evaluation of Salvinorin-Based KOR Antagonists



- The KOR is involved in response to stress and drug abuse[1,13] **(Figure 6)**.
- Only a handful of salvinorin-based antagonists have been discovered thus far[14] **(Figure 6)**.
- Current KOR antagonists possess unusually long PK profiles due to their long-acting effect,[1,7] so we took advantage of the short $t_{1/2}$ of salvinorin-based antagonist **7** to identify its pharmacological effect in withdrawal models **(Figure 7)**.

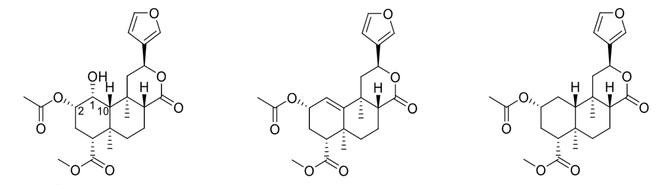


Figure 6: KOR function in drug-abuse and structures of salvinorin-based antagonists

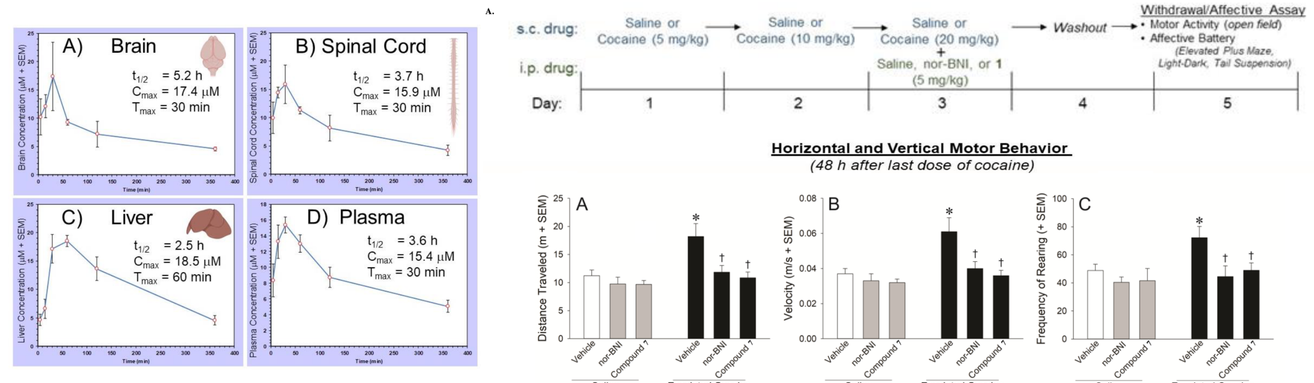


Figure 7: *In vivo* outcomes from pharmacokinetic and cocaine withdrawal symptomology in mice [15]

Computational Analysis of SA-Based Antagonists

- Previous study has suggested that SA does not interact with $D^{3.32}$ within the KOR.[8]
- Further studies have identified specific motifs important for function of class A GPCR's (PIF, KEY, DRY, NPxxY).[7]
- We examined compound **7** and salvinorin A binding to the KOR. **(Figure 8)** [15]

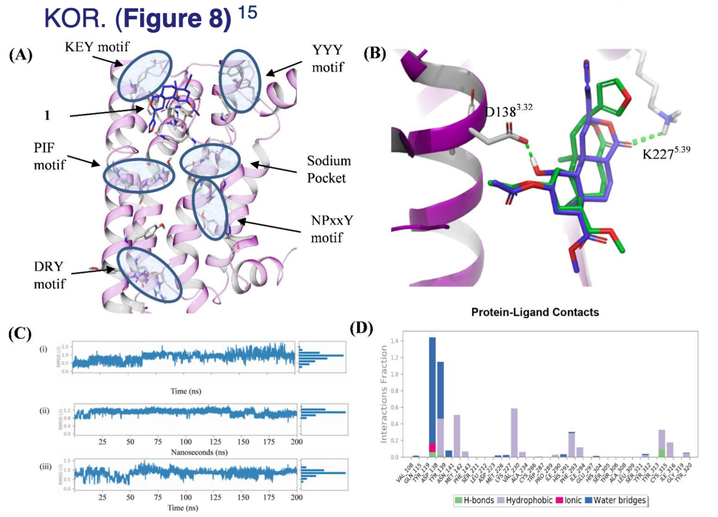


Figure 8: **(A)** Transducer regions during KOR agonism; **(B)** H-bonding interactions of **7** (in blue) and **8** (in green); **(C)** RMSD analysis of ligand C-α atoms; **(D)** Interaction histogram of **1** and KOR inactive state PDB (active): 6B73, PDB (inactive): 4DJH

Triazole-Linked SA-Based Compounds

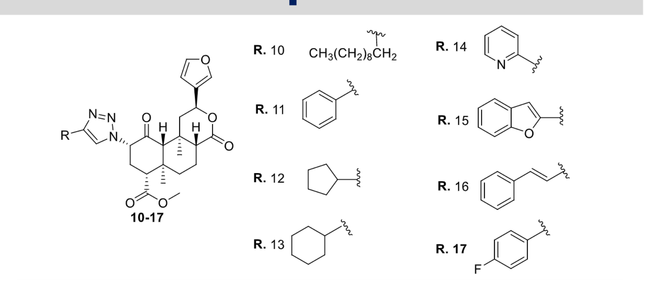


Figure 9: Preliminary results of triazole-linked SA-based compounds displaying delta (DOR) agonism

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