K32 Ethylenediamine Opioid Analogs, AH-7921, and U-47700 and Their Actions on Cloned Human OPRM1 Receptors

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Learning Overview: After attending this presentation, attendees will learn about the development of an *in vitro* human mu-opioid receptor assay (hOPRM1) and the testing of analogs related to emerging synthetic opioids AH-7921 and U-47700.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by allowing attendees to see how human receptor data compares to literature data that had only been performed in animals for some important emerging opioids of abuse.

The published pharmacology for the AH-7921 and U-47700 is more than 35 years old and was performed using non-human receptor systems.[1-5] While animal data and data using non-human receptors suggest similar action in humans, it is clearly more desirable to use human receptors to more accurately predict activity in humans. Considering the recent abuse of these opioids, this study sought to assess their pharmacology using a cloned human μ-opioid receptor (hOPRM1) in an *in vitro* stable human cell line.

Analogs within the AH-7921 and U-47700 series were synthesized and characterized by Nuclear Magnetic Resonance (NMR), Gas Chromatography/Mass Spectrometry (GC/MS), Infrared (IR), and Raman (1,064nm and 785nm). In the U-4770 series, the stereochemically pure R,R and S,S isomers were synthesized from single isomer intermediates.

An amino terminal HA-tagged hOPRM1 receptor was stably expressed using lentiviral transduction in human fibrosarcoma HT1080 under control of the EF1a promoter. Agonists acting at the hOPRM1 receptor activate G_{ai}, which in turn suppresses cAMP levels. Therefore, to characterize hOPRM1 pharmacology, analogue-mediated suppression of forskolin-induced cAMP accumulation was measured. In addition, to determine if changes in cAMP were mediated by hOPRM1 receptors, a treatment group containing 1mM naloxone in addition to the highest dose of the test analogue was assessed. The levels of cAMP were measured using a commercial kit, ELISA-based Catchpoint assay. Optimization of the assay involved dose ranging the amount of naloxone needed to show complete agonist reversibility, buffer optimization to maintain cell adherence and minimize cell loss during the assay, and optimizing the sequence of reagent introduction to obtain consistent results.

The optimized assay was used to run full-concentration response curves for morphine, AH-7921, and U-47700 (R,R stereochemistry). The EC_{50} values were 39nM, 16nM, and 10nM, respectively. These values are in agreement with the potencies reported in earlier literature. The (S,S) isomer of U-47700 had significantly less potency at this receptor. The literature suggests these analogs bind to both mu- and kappa-opioid receptors (OPRM1 and OPRK1) but with differing affinities based on their stereochemistry.[3] In a variety of series related to AH-7921 and U-47700, the 3,4-dichlorobenzoyl substitution consistently demonstrated the greatest potency relative to other substitutions on the benzoyl group.

In conclusion, an *in vitro* assay that can assess the human abuse potential of emerging opioids has been developed and the stereochemistry of the U-47700 stereoisomer more liable for abuse, (R,R), has been confirmed.

Reference(s):

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