K20 AH-7921 and U-47700 Series Analogs: Spectroscopic Characterization and mu-Opioid Receptor Pharmacology

John L. Krstenansky, PhD, Keck Graduate Institute, School of Pharmacy and Health Sciences, Claremont, CA 91711; Alexander C. Zambon, PhD, Keck Graduate Institute, Claremont, CA 91711; Thomas Hsu, PhD, Keck Graduate Institute, Claremont, CA 91711; Jayapal Reddy Mallareddy, Keck Graduate Institute, Claremont, CA 91711*

Learning Overview: After attending this presentation, attendees will know about the pharmacological differences between the structurally related emerging synthetic opioids, AH-7921 and U-47700.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by informing attendees of publicly deposited spectral information on two series of emerging synthetic opioids. This presentation will also highlight the pharmacological differences of the A-7921 analogs vs. the U-47700 analogs that may have bearing on the relative harm between the otherwise equipotent opioid series.

The published pharmacology for AH-7921 and U-47700 is more than 35 years old and was performed using non-human receptor systems.[1-5] This study synthesized and characterized 19 analogs of the AH-7821 and U-47700 series opioids. The spectra were deposited in publicly available databases for reference purposes and this study assessed their pharmacology using a cloned human μ-Opioid Receptor (hMOR) in an *in vitro* stable human cell line. This was to establish the potential for abuse of these analogs and to explore differences in ligand bias in these series.

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). In the U-47700 series, the stereochemically pure *R,R* and *S,S* isomers were synthesized from single isomer intermediates. These spectroscopic data have been deposited in publicly accessible repositories: Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) (GC/MS data & monographs) and "Ethylenediamine synthetic opioids," Mendeley Data, v1 http://dx.doi.org/10.17632/tjm8x4m93k.1.

Full concentration response curves, representing $G_{\alpha i}$ suppression of forskolin-stimulated adenylate cyclase, were obtained for morphine, AH-7921, and U-47700 (*R,R* stereochemistry). The EC_{50} values were 39nM, 27nM, and 9nM, 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 that these analogs bind to both mu- and kappa-opioid receptors (OPRM1 & OPRK1) but with differing affinities based on their stereochemistry.[3] In both AH-7921 and U-47700 series, the 3,4-dichlorobenzoyl substitution consistently demonstrated the greatest potency relative to other substitutions on the benzoyl group. Interestingly, U-47700, having the methyl group on the amide removed (Udes), retained good potency (EC_{50}=3nM), while other Udes analogs did not have a significant naloxone-reversible effect at 1μM.

In the AH-series, the rank order potency was as follows: 3,4-dichloro > 4-chloro > 4-trifluoromethyl > others not showing significant naloxone-reversible effect at 1μM. In the U-series, the rank order potency was as follows: 3,4-dichloro > 4-trifluoromethyl > 4-chloro; 4-methoxy; 4-bromo > others not showing significant naloxone-reversible effect at μM.

Considering that different opioid structural groups exhibit differing profiles of analgesia vs. adverse effects (respiratory depression, hyperalgesia and addictive potential, tolerance, etc.), this study explored the pharmacology of these analogs further. Receptor internalization was assessed for active analogs as a measure of β-arrestin recruitment. β-arrestin recruitment has been associated with adverse effects of opioids. Different opioid ligands display differing abilities to signal via G-proteins vs. β-arrestin recruitment. There is current debate whether biased ligands or ligands with balanced activities would produce the best analgesic/safety profile.[6] Regardless, this data show that AH-7921 leads to only modest levels of internalization similar to morphine, whereas U-47700 leads to high levels of receptor internalization, similar to the opioid peptide DAMGO.

In conclusion, these structurally similar compounds exhibit completely opposite signaling bias. These observations could indicate differences in the safety of these two emerging synthetic opioids that should be explored.

Reference(s):

1. Harper N.J., Veitch B.A., 1-(3,4-dichlorobenzamidomethyl)cyclohexyldimethylamine. *U.S. Patent* 3,975,443, Aug. 17, 1976.
2. Brittain, R.T., Kellett, D.N., Neat, M.L., & Stables, R. (1973). Proceedings: Anti-nociceptive effects in N-substituted cyclohexylmethyl-benzamides. *British Journal of Pharmacology*, 49(1), 158P–159P.
3. Szmuszkovicz J., VonVoigtlander P.F. (1982) Benzeneacetamide amines: Structurally novel non-mu opioids. *J. Med. Chem.* 25(10), 1125-1126.
4. Katselou, M., Papoutsis, I., Nikolaou, P., Spiliopoulou, C., and Athanaselis, S. (2015). AH-7921: The list of new psychoactive opioids is expanded. *Forensic Toxicology*, 33(2), 195–201.
5. Timothy P. Rohrig, Samuel A. Miller, Tyson R. Baird. (2018). U-47700: A Not So New Opioid, *Journal of Analytical Toxicology*, 2(1) e12–e14.
6. Johnson T.A., Milan-Lobo L., Che T., Ferwerda M., Lambu E, McIntosh N.L., Li F., He L., Lorig-Roach N., Crews P., Whistler J.L. (2017). Identification of the First Marine-Derived Opioid Receptor "Balanced" Agonist with a Signaling Profile That Resembles the Endorphins. *ACS Chem. Neurosci.* 8(3), 473-485.

*Presenting Author