Supplemental Information

Synthesis, Modeling and Pharmacological Evaluation of UMB 425, a Mixed μ Agonist/δ Antagonist Opioid Analgesic with Reduced Tolerance Liabilities

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KEYWORDS**:** Antinociception, conformationally sampled pharmacophore, delta antagonist, mu agonist, opioid receptor, tolerance

TABLE OF CONTENTS

Figure S1

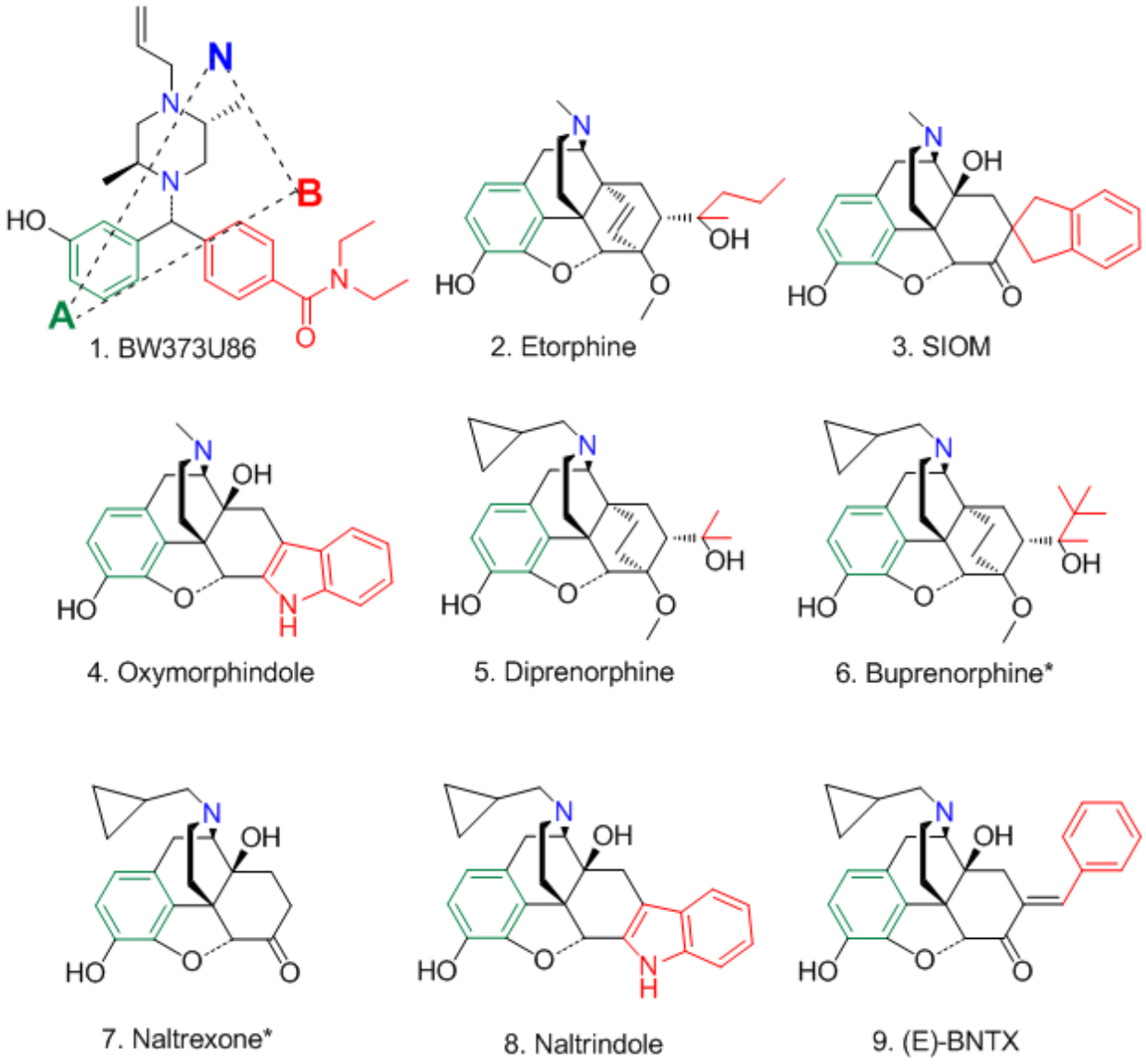

Figure S1. Compounds included in the δ receptor CSP training set. Pharmacophoric descriptors are designated in colors where green represents an aromatic ring (A), blue a basic nitrogen (N) and red a hydrophobic group (B).

SPECTROSCOPIC AND CHROMATOGRAPHIC METHODS

Chemicals and Materials. LC-MS grade acetonitrile, water, ammonium acetate and formic acid were purchased from Fisher Scientific (New Jersey, NJ).

High Resolution Mass Spectrometry (HRMS). Samples were prepared to an approximate concentration of 1 µM in water:acetonitrile (1:1) with 0.1% formic acid. Samples were analyzed by electrospray ionization in positive ion mode on a bench top quadrupole orbitrap mass spectrometer (Q Exactive; Thermo Fisher Scientific, Bremen, Germany). Samples were infused at a rate of 5.0 µL/min. Instrument calibration (< 1 ppm) and tuning parameters were optimized using the manufacturer's calibration mixture (consisting of caffeine, the tetrapeptide MRFA and Ultramark 1621). The ion source was set to 3.5 kV at a capillary temperature of 320 ^{0}C. All spectra were acquired over a time period of 1 min and averaged. Data were acquired and processed using Xcalibur, Version 2.2 (Thermo Fisher Scientific).

Liquid Chromatography for Sample Purity. Samples were prepared to an approximate concentration of 1 µM in water:acetonitrile (1:1). Samples were analyzed by high performance liquid chromatography (HPLC) equipped with a photo-diode array detector (Acquity UPLC H-Class, Waters, Milford, MA). The liquid chromatography separation was performed on a Develosil C18 column (2.0 x 150 mm, 5 µM) (Phenomenex, Torrance, CA) operated at 30 ^{0}C. Solvent A consisted of 0.1% formic acid and 10 mM ammonium acetate in water. Solvent B consisted of 0.1% formic acid in acetonitrile. The gradient program was 0.0-2.0 min, 1.0% B; 2.0-5.0 min, gradient to 50% B; 5.0-8.0 min, 50% B; 8.0-10.0 min, gradient to 95% B; 10.0-12.0 min, 95% B; 12.0-12.5 min, gradient to 1.0% B; 12.5-15.0 min, 1.0% B. The flow rate was to 0.5 mL/min during all separation steps and injection volume was 10 µL. Detection was performed using a photo-diode array (PDA) detector scanned over the following wavelength (210-400 nm) at a 4.8 nm resolution. Data collection and analysis was performed by Empower Pro 3 (Waters, Milford, MA).

Figure S2

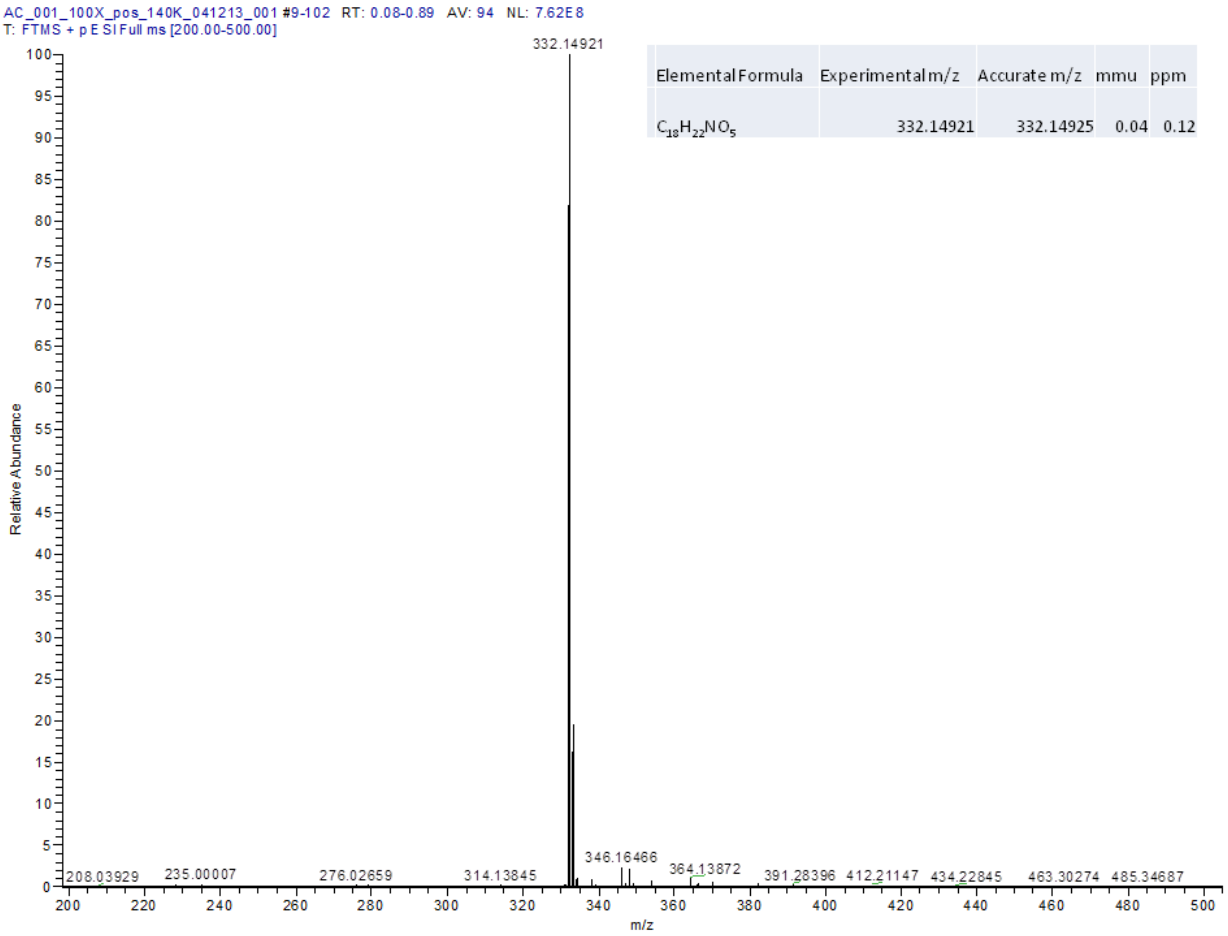

Figure S2. High resolution mass spectrum for UMB 425. The HRMS experiment yielded a measured experimental *m/z* value of 332.14921 for UMB 425 resulting in a mass accuracy of 0.04 milli mass units (mmu) which uniquely identified UMB 425 to have the elemental formula of $C_{18}H_{22}NO_5$.

Figure S3

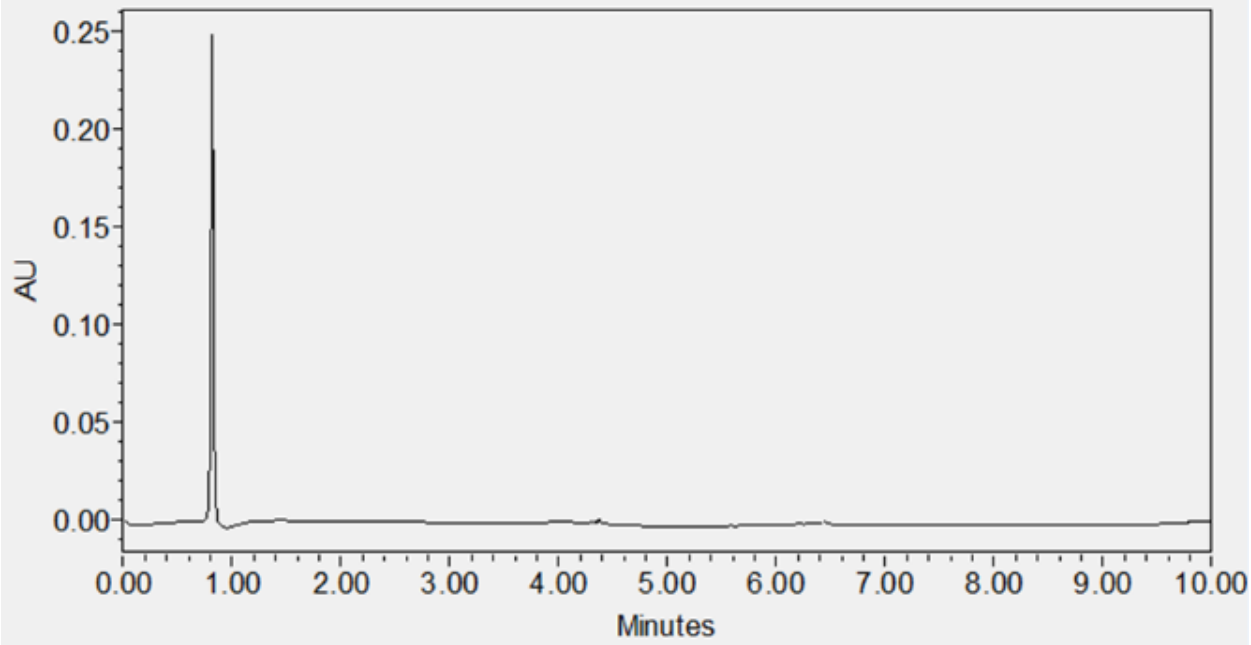

Figure S3. HPLC chromatogram for UMB 425. Sample purity for UMB 425 was assessed using HPLC coupled to a PDA detector. The HPLC separation yielded a liquid chromatogram with only one peak at retention time 0.8 min using a C18 reverse phase column. This separation effectively demonstrated the sample purity for UMB 425 was greater than 95%.