Ketamine Metabolites in the Treatment of Neuropathic Pain

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Objectives

- Investigation into Ketamine and its metabolites for their effect on serine racemase and subsequent intracellular and extracellular D-Serine concentrations.
- Computational analysis of the most potent molecules for active pharmacophores.
- Evaluation of active pharmacophores to further the understanding of agonist-peptide interactions
- Develop analogues based on our computational analysis and understanding of pharmacologically active substructures, to obtain optimized lead compounds capable of efficiently attenuating D-Serine concentrations

Cell-based Studies

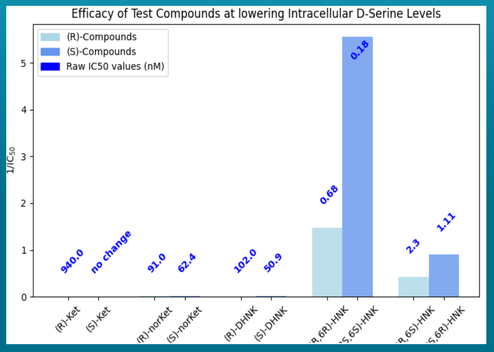
- PC-12 Neuronal Cell Line were incubated with each test compound in escalating concentrations 0μM-1μM
- After 36h of incubation with the test compounds, both intracellular and extracellular D-Serine concentrations were quantified
- Intracellular D-Serine concentration were measured using Capillary Electrophoresis-Laser Induced Fluorescence
- Extracellular D-Serine was quantified using Liquid Chromatography as a separation and Mass Spectrometry as a quantification method.

Computational Studies

- The test compounds were ranked based on their IC50 values associated with intracellular D-Serine reduction
- Based on their rankings as a bias, the test compounds were introduced into Comparative Molecular Field Analysis (CoMFA)
- Following CoMFA alignments, molecules that efficiently reduced intracellular D-serine levels were subjected to 3D-QSAR (quantitative structure-activity relationship)
- Molecules obtained from the CoMFA and 3D-QSAR were patented and await further studies on their D-Serine attenuation potential

Effect on Intracellular D-Serine

- All of Ketamine's metabolites reduced intracellular D-Serine concentrations by >30% at varying IC50 values
- Of the tested compounds, (2S,6S)-HNK and (2R,6R)-HNK were by far the most potent see graph below
- (R)-Ketamine also reduced intracellular D-Serine, but (S)-Ketamine did not.



Introduction

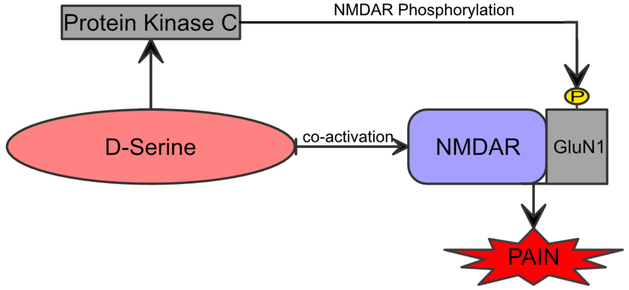
- The use of Ketamine in ER and perioperative settings has increased drastically over the past years.[1] Yet, the extensive psychodysleptic effects of Ketamine limit its use to these emergency situations. This is highlighted by studies[2] that limit ketamine to 1.0mg/kg due to the excessive hallucinogenic effects of (R,S)-Ketamine. We propose the use of a ketamine metabolite –Hydroxynorketamine (HNK)– as a safer and superior option in the treatment of neuropathic pain.
- HNK has been shown to be an excellent compound in the treatment of neuropathic pain in mouse models, as was demonstrated recently by Lefèvre and colleagues[3]. Additionally, it does not possess (R,S)-Ketamine's psychodesleptic side effects, and can thus be administered in higher doses.
- HNK's exact mode of action is not yet fully understood, but we speculate that it is related to the modulation of intracellular D-Serine levels.

Objectives

- Design a superior chemical D-Serine modulator based on the activity of Ketamine's metabolites using *in vitro* and *in silico* approaches

Why D-Serine

- D-Serine is a potent co-agonist for the NMDA receptor, a key receptor for hypersensitivity in chronic pain[3]
- Further, elevated intracellular D-Serine levels have been found to activate Protein Kinase C, which can post translationally phosphorylate the NMDA receptor's GluN1 subunit, thereby facilitating central sensitization[4]
- It follows that successful attenuation of D-Serine levels has important implications for the treatment of pain, aging and neurotoxicity.



Conclusion

- HNK is a superior metabolite in the attenuation of intracellular D-Serine levels.
- A resulting computational model was used to design multiple families of novel HNK analogs which are under investigation for use in the treatment of pain and inflammation.

Link to paper

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Computational Results

- COMFA (Fig 2) and QSAR modeling indicated that the C2 and C6 positions were key interaction points on the cyclohexanone ring of HNK
- The C2 position is defined by hydrophobic and hydrogen bonding capabilities
- The C6 position is only defined by hydrogen bonding
- Substituents at C2 and C6 without hydrogen bonding and/or hydrophobic interactions are associated with a loss of observed pharmacological activity.
- Based on our observations we designed analogues (Fig 3)

Fig 2: CoMFA alignment of HNK

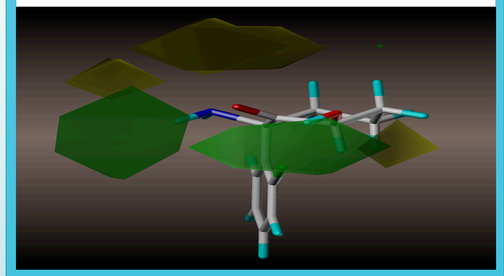
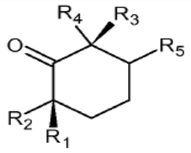
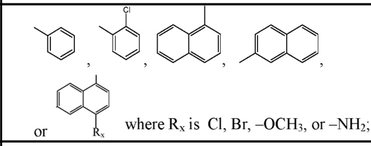
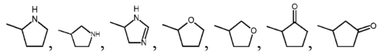


Fig 3: Families of New Drugs under Investigation



R_1	-OH
R_2	 or where R_x is Cl, Br, $-OCH_3$, or $-NH_2$;
R_3	$-OH$, $-OCH_3$, $-NH_2$, $-CN$, $-SO_2-NH_2$, 
R_4	-H, -OH, OCH_3
R_5	-OH, =O

Disclosures

Lucas B. Stolle has nothing to disclose. Joseph V. Pergolizzi is Consultant/Speaker, Owner, and Researcher for Spirify Pharma, US World Meds, BDSI, Salix, Enalare, Scilex, Pfizer, Lilly, Teva, Regeneron, Redhill, Grünenthal, and Neumentum. Irving W. Wainer is co-founder and CSO of Spirify Pharma.