2,5,6-Trisubstituted Pyridines as Novel, Highly Potent and Selective CB2 Agonists for the Prevention of Organ Damage

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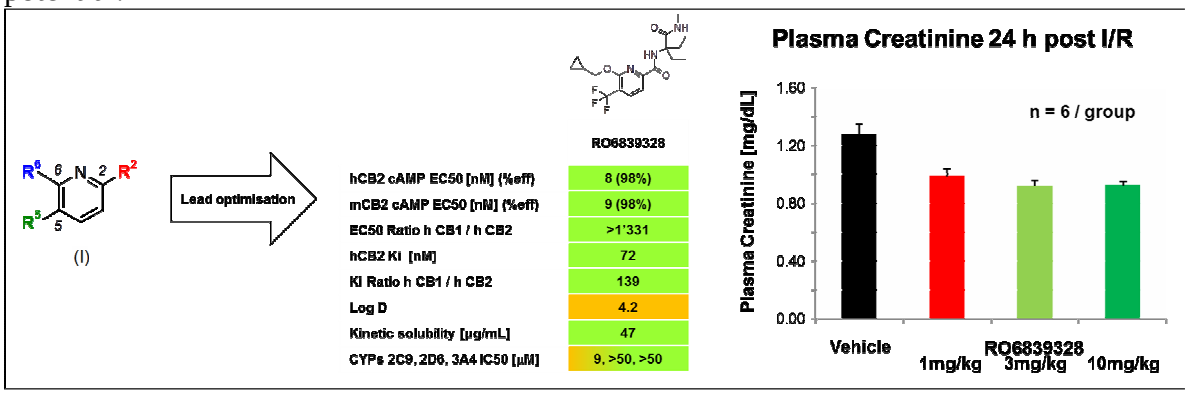
Throughout evolution the mammalian body has developed numerous protective mechanisms to prevent and limit tissue injury caused by various types of neuronal as well as non-neuronal insults. Lipid signaling through cannabinoid 2 (CB2) receptors is thought to be an important part of such a protective machinery and CB2 receptor stimulation triggers mostly protective activities.[1] Inflammation/tissue injury causes a rapid increase in local endocannabinoid levels which leads to a fast modulation of signaling pathways in immune and other cells and subsequently affects the function of these cells. It has been reported that CB2 agonists positively influence a large number of pathological conditions, spanning from cardiovascular, over gastrointestinal, liver, kidney, lung, neurodegenerative and psychiatric disorders to pain, cancer, bone, reproductive and skin pathologies. Taken together, we therefore hypothesize that selective CB2 agonists should be able to reduce kidney damage after a warm ischemia reperfusion injury.2,5,6-Trisubstituted pyridines (**I**) are highly potent CB2 agonists with CB2 cAMP EC_{50} values down to the picomolar range. By exploring the 3 exit vectors, a detailed structure activity relationship for CB2 binding and functional (cAMP and beta-arrestin) assays was elaborated. Subtle structural changes can turn agonists into inverse agonists. These effects can be rationalized by 3D homology models which are built on the crystal structures of bovine rhodopsin with *cis*- and *trans*-retinal. Furthermore, species differences between human and mouse receptor as well as the influence of structural changes on either of the three exit vectors on the selectivity against the CB1 receptor were investigated. Next to their *in vitro* potency on and selectivity for the target receptor, pyridines (I) were further optimized for their physicochemical properties including solubility, membrane permeation and lipophilicity as well as for their metabolic stability and cytochrome P450 inhibition potential.



Advanced compounds combining high *in vitro* potency with favourable early ADME properties have been profiled in *in vivo* pharmacokinetic studies and demonstrated to be efficacious in mouse ischemia reperfusion animal studies. RO6839328 exhibits a favourable *in vivo* mouse pharmacokinetic profile (clearance 37 mL/min/kg; bioavailability 41%; half-life 1.7 h). After administration by gavage to male mice (age ~10 weeks) 30 min before a warm ischemia/reperfusion (I/R), whereby kidney blood flow was interrupted for 25 min at

37°C, RO6839328 lowered plasma creatinine levels by 28% at 10 mg/kg and 23% at 1 mg/kg. This indicates that selective CB2 agonists might be beneficial for the treatment of acute kidney injury.

[1] Pacher P & Mechoulam R, Progress in Lipid Research 50:193, 2011.