Activity characterization of numerous stimulants towards human monoamine transporters and 5HT2A receptor

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Background: New psychoactive substances (NPS) have the last decades been used as legal alternatives to classical drugs. A large group of NPSs are synthetic stimulants, which mainly inhibit monoamine neurotransmitter reuptake from the synaptic cleft prolonging the synaptic activity. It has been shown that the relative inhibition of these transporters correlates to the drug-related effects in humans. Particularly, a high inhibition of dopamine transporter (DAT) and low inhibition of serotonin transporter (SERT) is associated with high self-administration potential. NPSs have a high enter-and-exit rate on the illicit drug market, and upon entry, limited pharmacological or toxicological data are available. Therefore, the study aimed to gain insight into the mechanism of action of different groups of synthetic stimulants.
Methods: The DAT, norepinephrine transporter (NET), and SERT inhibition and activity screening towards the 5HT2A receptor of 58 compounds were investigated using recombinant cell systems. The inhibition was measured using a fluorescent dye mix mimicking the neurotransmitter transport. Compounds were incubated in a 15-step dilution series in triplicates and fluorescence was measured on a TECAN Spark 10M.
The 5HT2A assay utilized AequoScreen® reporting system employing aequorin and coelenterazine luminescent interaction. The compounds were incubated in an 8-step dilution series in triplicates and luminescence was measured on a TECAN Spark 10M.
Results: In total 38 cathinones, 6 arylcyclohexylamines, 5 phenethylamines, 4 2C-B-like compounds, and 2 compounds labeled as "others" as well as cocaine (reference), amphetamine, and MDMA were included. The findings revealed potent inhibition of DAT, particularly by cathinones containing a pyrrolidine group with MDPiHP the most potent inhibition of DAT with an IC50 of 1.0 nM. Interestingly, this group also had the highest potency towards the NET, MDPiHP IC50 of 20 nM, shortly followed by smaller and more hydrophobic molecules like CMC-, MMC-, and amphetamine-like compounds. The SERT was inhibited the most by methamnetamine, with an IC50 of 80 nM, but none of the compounds targeted the transporter selectively.
Conclusion: These results show the diversity of targets and potencies of different classes of synthetic stimulants. Moreover, the high number of compounds with high potency toward the DAT raises concerns about their addictive potential.