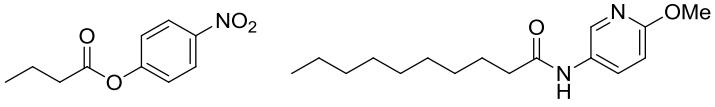
Abstract

The aim of the present thesis was the design, synthesis and kinetic characterization of new inhibitors of the serine hydrolases human monoacylglycerol lipase (hMAGL), human fatty acid amide hydrolase (hFAAH) as well as human and murine cholesterol esterase (hCEase and mCEase, respectively). To identify new lead structures, an *in silico*-screening of compounds, partly derived from the Zinc database,[1-2] was performed on hMAGL. Selected compounds of this screening were synthesized and investigated for their ability to inhibit the three serine hydrolases. For this purpose, a fluorometric FAAH assay and a colorimetric assay for MAGL were established. *N*-(6-methoxypyridin-3-yl)decanamide (D-MAP; **73**) D-MAP (**73**) was synthesized for the first time and characterized as a novel fluorogenic FAAH substrate. Compared to other literature-reported substrates, D-MAP (**73**) shows an increased water solubility and sensitivity towards FAAH: An enzyme concentration of 1 µg mL^{-1} was chosen for the kinetic measurements, which is below the FAAH concentrations of other reported fluorometric FAAH assays. In addition, *Z'* values of 0.76-0.86 were determined for D-MAP (**73**), which confirmed the excellent suitability of this substrate (**73**) for high throughput screening of compound libraries. The thiazole **135** was identified as a new inhibitor of hFAAH, inhibiting the enzyme with an IC_{50} of 52.4 µM. To establish the MAGL enzyme assay, the chromogenic substrate 4-nitrophenyl butyrate (4-NPB; **63**), previously described by Muccioli *et al.*[3], was kinetically characterized herein for the first time. Due to the superior substrate properties of 4-NPB, the experiments were performed with a MAGL concentration of 20 ng mL^{-1}, which is below the reported concentration of other colorimetric MAGL assays.



4-NPB (**63**)
K_m = 184 µM
V_{max} = 269 µmol min^{-1} mg^{-1}

D-MAP (**73**)
K_m = 8.43 µM
V_{max} = 9.08 nmol min-1 mg-1

Abb. 3: Molecular structures of the chromogenic MAGL substrate 4-NPB (**63**) and the fluorogenic FAAH substrate D-MAP (**73**).

Herein, ω-quinazolinonylalkyl aryl ureas were identified as a new class of reversible hMAGL inhibitors showing IC_{50} values in the range of 20-41 µM, with the most potent derivative **175** exhibiting a K_i value of 15.4 µM. The structurally related ω-phthalimidoalkyl aryl ureas **184** and **203** were identified and characterized as novel inhib-

itors of human and murine cholesterol esterase. Structural-activity relationship studies revealed that the urea moiety, an attached 3,5-bis(trifluoromethyl)phenyl group, and the aromatic character of the terminal phthalimide moiety of the alkyl chain were crucial for the inhibitory potency of these compounds. In contrast, the alkyl spacer length only slightly affected the inhibitory capacity of the compounds. The ω-phthalimidoalkylaryl ureas show K_i values in the low to medium micromolar range (K_i = 1.19-18.7 µM) and a complete, reversible and selective inhibition of the two CEases (in comparison to MAGL and FAAH) with both competitive and non-competitive behavior.

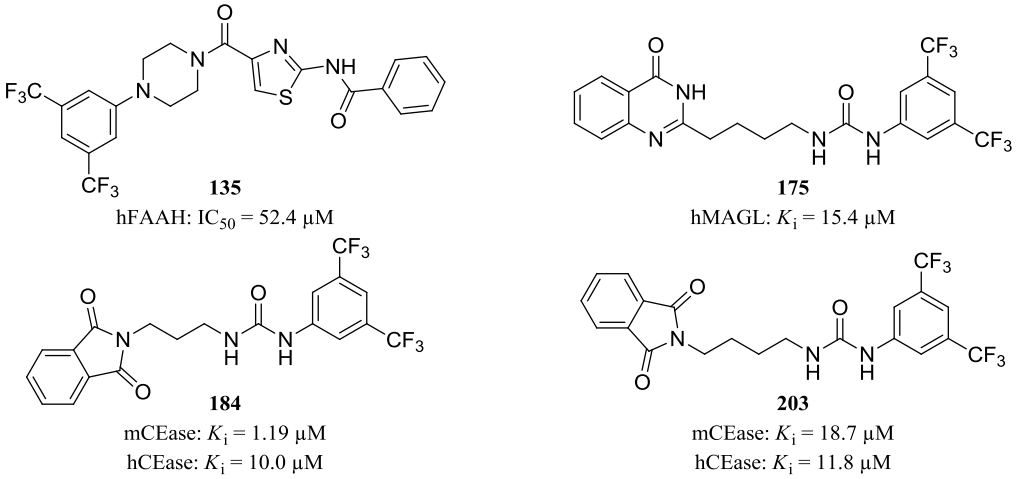


Abb. 4: Molecular structures of the thiazole inhibitor **135**, ω-quinazolinonylalkyl aryl urea inhibitor **175** and ω-phthalimidoalkyl aryl urea inhibitors **184** and **203**.