Potential drug metabolites as endocannabinoids and endovanilloids

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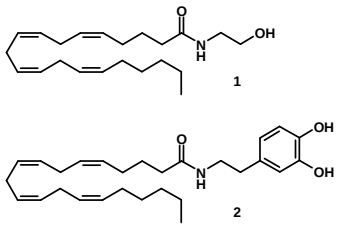
1. Introduction

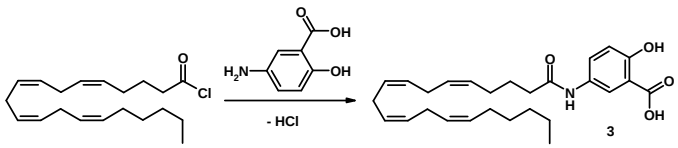
Δ^9-Tetrahydrocannabinol is the most famous plant-derived cannabinoid. Recently, N-arachidonoyl-ethanolamine (**1**, anandamide) and N-arachidonoyl-dopamine (**2**) were discovered as endogenous ligands of G-protein coupled receptors addressed by Δ^9-tetrahydrocannabinol [1], [2]. The arachidonic acid derivatives bind to cannabinoid receptors (CB_1 and CB_2), are degraded by fatty acid amide hydrolase (FAAH), are supposed to be transported inside cells by the anandamide transporter and interact with the vanilloid receptor 1 (TRPV1, a ligand gated Ca^{2+} channel). Due to their physiological activities these compounds are called endocannabinoids and endovanilloids.

We hypothesise that the activities of some known drug substances are partly effected by acyl metabolites. Here we present the synthesis of three potential drug metabolites generated by replacement of either the amine or the acid moiety in **1** or **2** along with the results of in vitro assays with CB_1, CB_2, and TRPV1 receptors and the putative anandamide transporter.

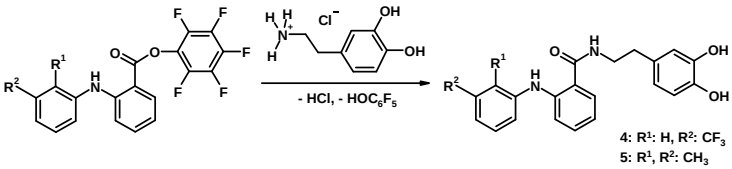


2. Synthesis and analysis

1. Arachidonic acid mesalazine amide (**3**)



2. Flufenamic (**4**) and mefenamic acid (**5**) dopamide



Analytical confirmation: ^{1}H-, ^{13}C-NMR, EI-MS, combustion analysis

3. Transporter assay

Assay procedure: The effect on the uptake of [^{14}C]-**1** by intact RBL-2H3 cells was studied by using 5.0 µM (20,000 cpm) of [^{14}C]-**1.** Cells were incubated with [^{14}C]-**1** for 5 min at 37 °C, in the presence or absence of **3** at a concentration of 25 µM. Residual [^{14}C]-**1** in the incubation media after extraction with $CHCl_3/CH_3OH$ (2:1), determined by scintillation counting of the lyophilized organic phase, was used as a measure of the [^{14}C]-**1** that was taken up by cells.

3 inhibited 80 % of [^{14}C]-**1** uptake at 25 µM. It is thus approximately equipotent with the established anandamide transporter inhibitor VDM-11 (IC_{50}: 10 µM) [3].

4. CB receptors assay

Assay procedure: For CB_1 and CB_2 receptor binding assays, the compounds were analyzed by using HEK-293 cells transfected stably with either the human CB_1 or CB_2 receptor and [^{3}H]CP-55,940 as the high-affinity ligand. WIN 55,212-2 was used to define specific binding in the assay. All experiments took place in the absence of a FAAH inhibitor.

Rates of displacement:

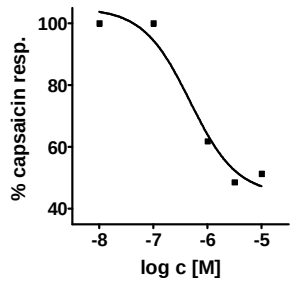
	1 µM -CB_1-	10 µM -CB_1-	1 µM -CB_2-	10 µM -CB_2-
3	32 %	64 %	12 %	29 %
4	31 %	100 %	13 %	73 %
5	21 %	67 %	3 %	34 %

5. TRPV1 assay

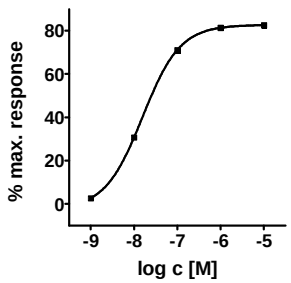
Assay procedure: HEK-293 cells transfected with human TRPV1 were used in the experiments. The effect of the compounds on Ca^{2+} influx was determined by using Fluo-4, a selective intracellular fluorescent probe for Ca^{2+}. Experiments were carried out by measuring cell fluorescence at 25 °C (λ_{EX}: 488 nm, λ_{EM}: 516 nm) before and after the addition of the test compounds at various concentrations. The efficacy of the agonists was determined by comparing to the maximal effect on Ca^{2+} influx observed with 4 µM ionomycin, whereas antagonistic baviour was evaluated by the reduction of Ca^{2+} influx provoked with 0.1 µM capsaicin (EC_{50} = 51 nM) [4].

Data points are means of n = 3 experiments:

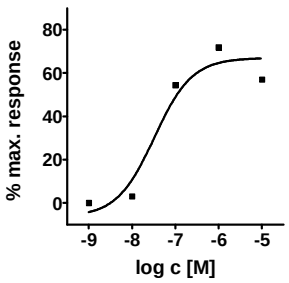
3: IC_{50} = 5 µM



4: EC_{50} = 16 nM



5: EC_{50} = 36 nM



6. Outlook

Anandamide transporter inhibitors are known to be effective in animal models of inflammatory bowel diseases [5]. So does **3** take part in the mechanism of action of mesalazine just as the anandamide transporter inhibitor N-arachidonoyl-phenolamine (AM404) does with paracetamol [6]? It was shown that the analgesic activity of paracetamol was abolished by CB_1 antagonists in rats [7]. Again, application of TRPV1 agonists like capsaicin or **4** and **5** elicits an effect called desensitisation that may be useful regarding analgesia [8]. TRPV1 antagonists should avoid neurotoxicity due to Ca^{2+} overload while retaining analgesic properties. Are **4** and **5** suitable templates for the development of high-affinity antagonists?

One gripping question remains: Do these potential drug metabolites really occur in vivo? We will try to answer this question especially in case of compound **3** by LC-MS analysis of suitable human tissue.

7. Literature

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8. Acknowledgment

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