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Design and synthesis of selective, dual fatty acid binding protein 4 and 5 inhibitors

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ABSTRACT

Dual inhibition of fatty acid binding proteins 4 and 5 (FABP4 and FABP5) is expected to provide beneficial effects on a number of metabolic parameters such as insulin sensitivity and blood glucose levels and should protect against atherosclerosis. Starting from a FABP4 selective focused screening hit, biostructure information was used to modulate the selectivity profile in the desired way and to design potent dual FABP4/5 inhibitors with good selectivity against FABP3. With very good pharmacokinetic properties and no major safety alerts, compound **12** was identified as a suitable tool compound for further in vivo investigations.

Fatty acid binding proteins 4 and 5 (FABP4 and FABP5) are members of a family of small, soluble proteins which contribute to the trafficking of fatty acids within the cytosolic compartments of cells. The proteins have no catalytic function but transport hydrophobic fatty acids within the aqueous environment of the cytosol to the various destinations enabling fatty acid oxidation, membrane homeostasis or nuclear signaling. In addition, they are likely involved in signaling processes which are so far poorly understood.[1–4]

FABP4 is highly expressed in adipose tissue, macrophages and endothelial cells. FABP5 is also expressed in macrophages and endothelial cells, as well as in skin and several other tissues.[3,5]

We became interested in FABP4 and FABP5 as targets for inhibition after the discovery that genetic deletion of FABP4 and FABP5 in mice improves insulin sensitivity, lowers glucose, and protects against atherosclerosis.[4] In a clamp study in *ob*/*ob* mice, a specific FABP4 inhibitor (BMS309403) showed a reduction of hepatic glucose production, increased glucose uptake in muscle and adipose tissue, and reduction in hepatic steatosis, but no change in body weight and energy consumption. Additionally, this compound showed a decrease in atherosclerotic plaque formation in ApoE KO mice.[6]

In humans, plasma levels of FABP4 are increased in patients with metabolic syndrome and atherosclerosis.[7] In addition, there is growing evidence for involvement of FABP4 in angiogenesis[8] and growth of certain tumors.[9] Increased levels of FABP5 have been found in human breast cancers and experimental results suggested that FABP5 is critical for mammary tumor development.[10]

The FABP family comprises nine isoforms, which differ in their sequence and tissue distribution, but have remarkably similar structures.[4] Our goal was to provide a dual FABP4/5 inhibitor selective against other FABP isoforms with good physicochemical and pharmacokinetic properties suitable as a tool for testing in models of metabolic diseases, angiogenesis or tumor growth. As selectivity filter, we chose activity against the structurally closely related FABP3 and the less similar FABP1.

A number of FABP inhibitors have been described in the literature. The pyrazole BMS309403 (**1**)[6,11] has been used extensively as a tool for FABP4 inhibition, both in vitro and in vivo.[12,13] Other structurally diverse compounds include indoles from Biovitrum (**2**),[14] thiophenes from the University of Minnesota (**3**),[15] as well as non-carboxylic acids from Biovitrum (**4**),[16] and Merck (**5**).[12] We tested a selection of published FABP inhibitors in our FABP binding assays measuring the displacement of a fluorescently labeled fatty acid.[17] However, we could not identify dual acting FABP4/5 inhibitors of reasonable selectivity against other FABP isoforms. Moreover, most compounds had no activity against FABP5 (Fig. 1).

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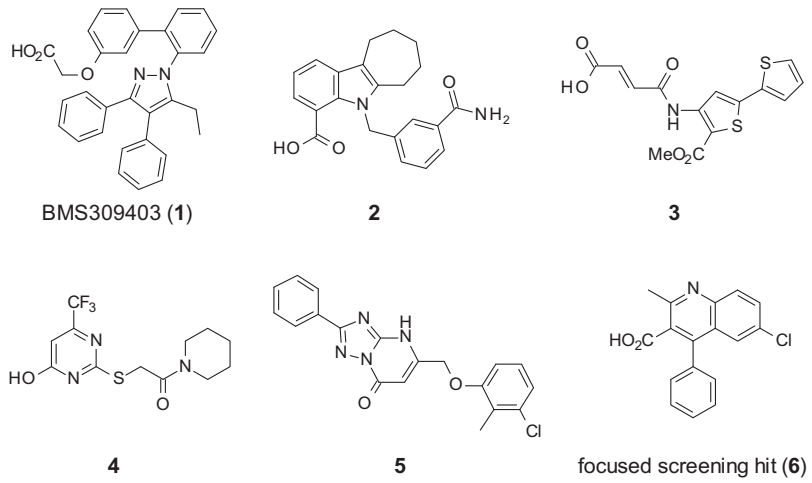

Figure 1. Structure of some published FABP4 inhibitors and focused screening hit quinoline **6**.

To identify novel chemical starting points for our program we conducted a focused screen against FABP4 and profiled the hits additionally against the FABP3 and 5 isoforms. The screening set consisted of about 1200 molecules from the Roche compound library which were selected based on 2D[18,19] and 3D similarity[20] to published FABP inhibitors. Although a number of structurally diverse candidates were found, most of the compounds were devoid of FABP5 activity. We decided to select hits for optimization based on their physicochemical and eADME properties and tried to design-in the desired dual FABP4/5 activity and selectivity against other FABP isoforms, especially FABP3. The quinoline carboxylic acid **6** appeared to be a good starting point (Table 1).

Despite the fact that compound **6** did not have the desired dual FABP4/5 selectivity profile, it turned out to be a very attractive starting point due to its favorable eADME properties such as high solubility, good permeability, low clearance in both human and mouse microsomes and low potential for drug–drug interactions. Considering the mostly lipophilic nature of the FABP4 binding pocket, compound **6** has a surprisingly low log*D*, which in combination with the low molecular weight offered good opportunities for further optimization. In addition, a crystal structure from the complex of **6** with FABP4 was obtained early in the program revealing its binding mode (Fig. 2). The ligand occupies a buried hydrophobic pocket with limited solvent access. The carboxylate group is engaged in a direct hydrogen bond with the guanidinium group of Arg127 as well as in two water-mediated hydrogen bonds with Tyr129. A large number of apolar π–π and dispersion interactions are made between the remainder of the inhibitor and the many lipophilic side chains pointing into the binding cavity.

One structure-based approach to modulate the selectivity profile of a small molecule is to exploit sequence differences in the ligand binding site. To identify these regions, we overlaid the crystal structure from Figure 2 with co-crystal structures of all three isoforms with the common ligand palmitic acid. From this analysis, several amino acids could be identified that are in close proximity to bound compound **6** and differ between FABP4, 5 and 3. As illustrated in Figure 3, four potential selectivity regions S1–S4 were identified, suggesting small differences in pocket size and shape between the three isoforms. Regions S1, S3 and S4 were most attractive as they could be readily reached with different substituents from the 4-phenyl quinoline scaffold of **6**.

We first focused our attention on region S4 because our analysis suggested a smaller binding pocket for FABP3 due to the presence of three Leu side chains which are bulkier compared to the set of Ile, Val, Cys residues in FABP4 and 5. Moreover, the quinoline 2-position was ideally suited to target this region. Increasing the size of the quinoline 2-substituent from methyl to isopropyl (compound **7**) to provoke a steric clash with FABP3 already changed the activity profile in the desired way. Not only did this modification lead to a drop of the FABP3 inhibition constant from 0.093 to 0.39 μM, it also afforded the first compound in this series with measurable FABP5 activity. The concomitant strong gain in binding

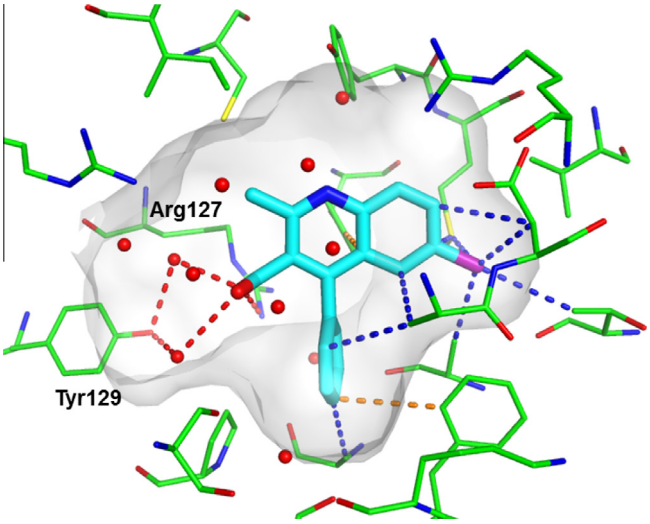

Figure 2. Crystal structure of human FABP4 with compound **6** (PDB code: 5edb). Protein residues are colored in green and the ligand in cyan. Water molecules are shown as red spheres. Favorable protein–ligand interactions[23] are shown as dashed lines (red: hydrogen bond, orange: π–π, blue: dispersion).

Table 1
Properties of screening hit **6**

hFABP4: K_i (μM)	0.105
hFABP5: K_i (μM)	>23.2
hFABP3: K_i (μM)	0.093
log*D* (pH 7.4)	−0.44
Solubility (μg/ml)[a]	>355
Permeability (10^{-6} cm/s)[b]	0.23
CYP450 (3A4/2D6/2C9): IC_{50} (μM)	>50/>50/>50
CL_{int} h/m (μl/min/mg of protein)[c]	<10/<10

[a] LYSA[21].
[b] PAMPA[22].
[c] From incubation with human and mouse liver microsomes.