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Substituted benzylamino-6-(trifluoromethyl)pyrimidin-4(1*H*)-ones: a novel class of selective human A-FABP inhibitors

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Abstract—The synthesis and biological evaluation of novel human A-FABP inhibitors based on the 6-(trifluoromethyl)pyrimidine-4(1*H*)-one scaffold is described. Two series of compounds, bearing either an amino or carbon substituent in the 2-position of the pyrimidine ring were investigated. Modification of substituents and chain length optimization led to novel compounds with low micromolar activity and good selectivity for human A-FABP.

Fatty acids are important fuels and cellular regulators that bind to cytoplasmic fatty acid-binding proteins (FABPs) in a noncovalent, reversible manner.[1] The expression of FABPs is fairly tissue specific and A-FABP (FABP4, adipocyte lipid-binding protein or aP2) is expressed in adipose tissue and in macrophages.[2] Dys-regulation of FA metabolism in adipose tissue is a prominent feature of insulin resistance and the transition from obesity to Type 2 diabetes. Hotamisligil et al. showed that A-FABP null mice remain insulin sensitive and appear protected against Type 2 diabetes, despite diet-induced obesity.[3] Inhibition of the human variant of A-FABP could be potentially therapeutically effective in the treatment of type 2 diabetes or effectively prevent this disorder.

Following a high throughput screen of our internal compound library on A-FABP followed by a selectivity filter with intestinal FABP (I-FABP), we identified the 4-hydroxypyrimidines **1** and **2** as relatively potent and selective inhibitors of human A-FABP (Fig. 1). These, and similar compounds have recently been claimed as A-FABP inhibitors in patent applications, although no binding data were given.[4] In addition, **1** and **2** lacked chemical stability in our hands. Ideally, an A-FABP inhibitor should not bind to other FABPs. In particular, binding to human heart and muscle isoform H-FABP should be avoided due to exercise intolerance seen in H-FABP null mice.[5] The aim of this investigation was to identify stable and novel compounds with selectivity over human H-FABP. The compounds were evaluated for A-FABP and H-FABP inhibitory activity using a fluorescence polarization assay.[6]

In order to better understand the binding mode, **1** was co-crystallized with recombinant human A-FABP (PDB code: 1tou).[7] The crystal structure in Figure 2 reveals that the hydroxyl group on the pyrimidine makes

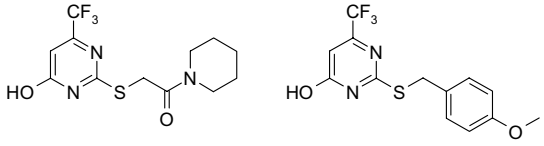

Figure 1. Thioether hit compounds **1** and **2**.

Keywords: A-FABP inhibitors; FABP4.
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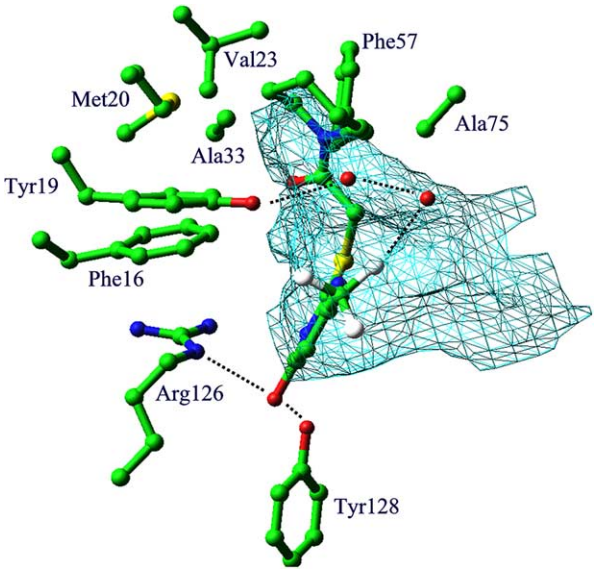


Figure 2. X-ray crystallographic representation of A-FABP in complex with **1** showing key hydrogen bonding interactions.

polar interactions with the side chains of Tyr128 and Arg126 similar to the binding of the carboxylate group seen with endogenous long chain fatty acids.[8] The piperidine moiety is nicely accommodated in a lipophilic pocket defined by Phe16, Met20, Val23, Ala33, Phe57, and Ala75. The CF3 group packs against a group of hydrophobic side chains and makes a polar contact with a conserved water molecule, judging from the distance of 2.9 Å (Fig. 3). These short contacts are not uncommon and have been previously observed with CF3 groups and polar amino acid residues.[9]

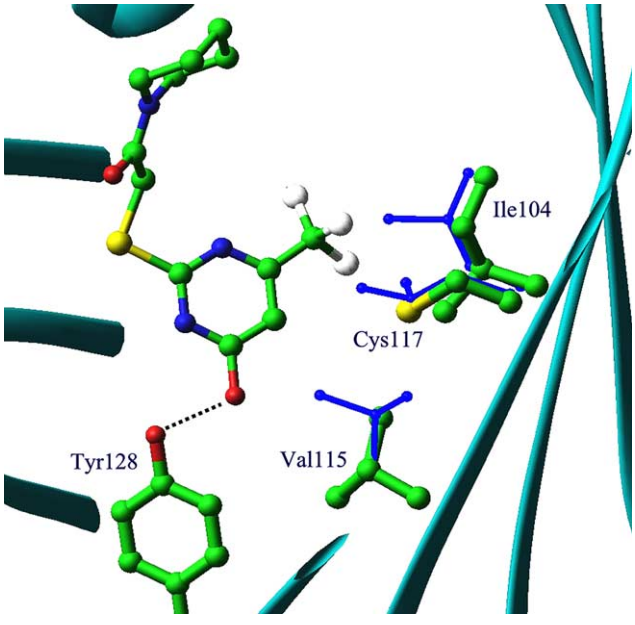
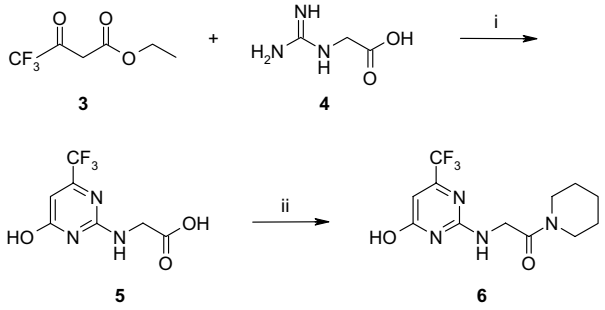


Figure 3. X-ray of the A-FABP–**1** complex showing neighboring residues of the CF3 group. In blue is shown three of the corresponding side chains (all leucines) from human H-FABP.

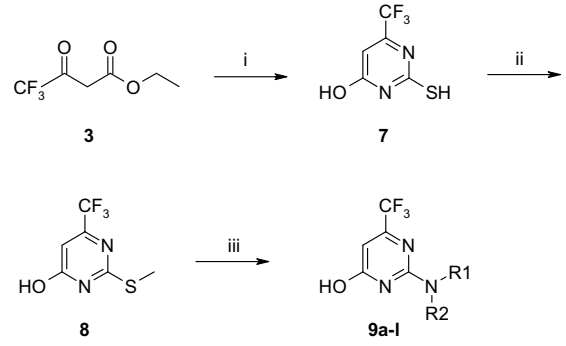


Scheme 1. Reagents and conditions: (i) NaOBu, *n*-butanol, 110 °C, 15 h; (ii) piperidine, *n*-butanol, 130 °C, 16 h.

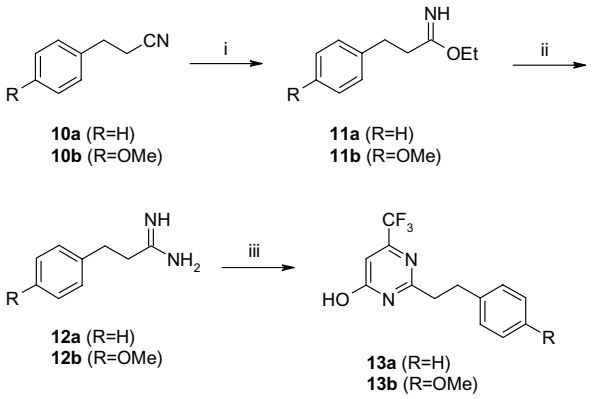
The observation that the thioether linker appeared to have no obvious contribution to binding, prompted us to prepare the amine analog of **1** (Scheme 1). Ethyl-4,4,4-trifluoroacetoacetate **3** and guanidoacetic acid **4** were heated under basic conditions to provide the carboxylic acid **5**. Subsequent heating with excess piperidine afforded the amine analog **6**, which showed an A-FABP inhibitory activity of 37 μM.

Despite the moderate A-FABP activity of compound **6** a synthetic program was initiated to more fully understand the structure–activity relationship (SAR) around this 6-trifluoromethylpyrimidine scaffold. Modest SAR studies were conducted by replacing the sulfur atom by nitrogen and carbon. We assumed that the amine and carbon analogs would give bioisosters that bind in the same way as the sulfur analogs. In addition, the CF3 group was exchanged with aliphatic and aryl substituents.

The synthetic strategy used to prepare the amino series is shown in Scheme 2. The thiol **7** was prepared according to a literature procedure by mixing ethyl-4,4,4-trifluoroacetoacetate **3**, thiourea, and sodium methoxide in refluxing ethanol.[10] Subsequent alkylation with methyl iodide under basic conditions furnished the methylthiopyrimidine **8**. The pyrimidines (**9a–l**) were prepared in parallel fashion by heating **8** and 3 equiv of the requisite primary or secondary amine at 130 °C overnight.



Scheme 2. Reagents and conditions: (i) thiourea, NaOMe, reflux, MeOH, 18 h; (ii) MeI, 25 °C, THF, 3 h; (iii) 3 equiv amine, 130 °C, neat, 18 h.

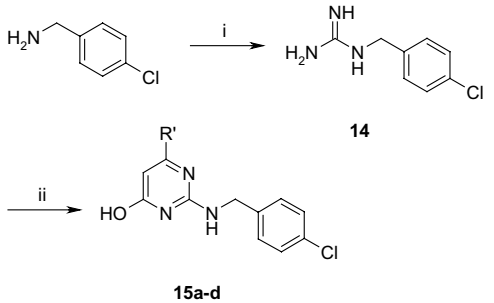


Scheme 3. Reagents and conditions: (i) HCl (g) satd in $EtOH/CH_2Cl_2$ 1:1, 0 °C, 20 h; (ii) 2 N NH_3, 0 °C, 18 h; (iii) ethyl-4,4,4-trifluoroacetoacetate, NaOMe, EtOH, 95 °C, 18 h.

Preparation of carbon analogs is described in Scheme 3. The nitriles (**10**) were converted to the amidates (**11**) with dry HCl in ethanol.[11] Subsequent reaction with 2 N NH_3 in methanol gave the amidines (**12**). Condensation of the amidines with ethyl-4,4,4-trifluoroacetoacetate in methanol under basic conditions provided the carbon analogs **13a** and **13b**.[12]

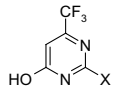
The preparation of analogs with different R′-groups in the 6-position of the pyrimidine ring is depicted in Scheme 4. The 4-chlorobenzylamine was heated with *S*-methylisothiourea sulfate to give the guanidine **14**. Subsequent condensation with the requisite acetate ester under basic conditions provided the pyrimidines **15a**–**d**.

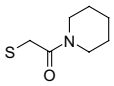
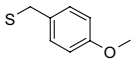
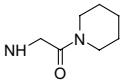
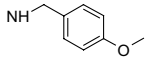
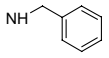
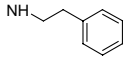
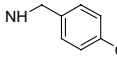
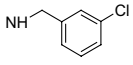
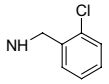
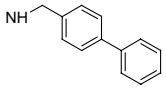
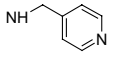
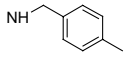
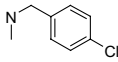
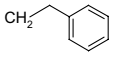
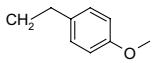
We started our exploration from derivative **2**, replacing the sulfur atom with nitrogen to give the compound **9a**. This modification decreased activity by approximately a factor of 7, as shown in Table 1. The identical carbon analog **13b** showed an additional 7-fold drop in activity compared to the amine **9a**. The length of the alkyl chain extending from the pyrimidine 2-position was modified to afford both chain-shortened and chain-elongated analogues of **9b**. Both modifications were detrimental to activity (**9c**, **9d** vs **9b**). Again, replacing the nitrogen with a carbon atom as in compound **13a** abolished inhibition.



Scheme 4. Reagents and conditions: (i) *S*-methylisothiourea sulfate, EtOH, 80 °C, 15 h; (ii) requisite ethylacetoacetate, NaOMe, EtOH, 80 °C, 16 h.

Table 1. Binding affinities for human A-FABP[a]

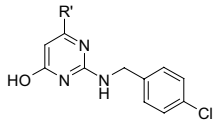


Compound	X	IC_{50} (μM)
1		1
2		0.6
6		37
9a		3.9
9b		33
9c		>100
9d		>100
9e		2.9
9f		33
9g		23
9h		>100
9i		>100
9j		33
9k		4.0
9l		24
13a		>100
13b		25

[a] In vitro binding data are reported as the mean of triplicate experiments performed on the same dilution.

The *para*-substituted benzyl groups give generally better potency than the *meta* and *ortho* analogs (**9e** vs **9f**, **9g**). This pattern is similar for methyl and methoxy substituents as well (not shown here). The electron poor 4-pyridyl analog **9i** was inactive. The most potent human A-FABP inhibitors possessed the *p*-chloro or *p*-methoxy moiety on the phenyl ring (**9e** and **9a**). Assuming a

Table 2. Replacement of the CF_3 group and the effect on A-FABP binding[a]



Compound	R′	IC_{50} (μM)
15a	Me	>100
15b	Et	47
15c	Phenyl	>100
15d	2-Fluorophenyl	>100

[a] In vitro binding data are reported as the mean of triplicate experiments performed on the same dilution.

binding mode for this series similar to compound **1**, the benzyl groups of **9e** and **9a** could fit well into a hydrophobic pocket that accommodates the piperidine of **1**. Modeling studies suggest that the phenyl group stacks on Phe16, and the smaller *para*-substituents fill a minor pocket defined by Tyr19, Met20, Val23, and Val25. Bulkier substituents such as *p*-phenyl become too large, and this explains the drop in activity for **9h**.

The CF3 group of the most potent amino analog **9e** was exchanged for a number of alkyl and phenyl groups. As shown in Table 2, all replacements gave rise to inactive compounds except for the ethyl group that retained some activity. It is obvious from the crystal structure that the larger substitutions such as phenyl will collide with Ile104. The size of the CF3 group is well adjusted to allow the positioning of the rest of the molecule as a fatty acid mimic. The impact of the CF3 group on binding as compared to equally sized but inactive methyl analog **15a** must have additional explanations. It seems to keep the network of water molecules intact, by the formation of at least one hydrogen bond. Finally, it may have an effect on the acidity of the pyrimidine hydroxyl group, which could be of importance for the polar interactions with Tyr128 and Arg126 (Fig. 2).

A selection of compounds was also evaluated for human H-FABP inhibitory activity (Table 3). An inherent selectivity for A-FABP against H-FABP is observed for all tested 6-trifluoromethylpyrimidine analogs, exemplified by the approximately 30-fold selectivity obtained with **2** and **9e**. There are three side chains on one face of the binding pocket that have close hydrophobic interactions with the ligands (Fig. 3). These side chains of Ile104, Val115, and Cys117 are not conserved in the related protein H-FABP, and are all replaced by leucines.[13] Alignment of the very similar A-FABP and H-FABP protein structures gave an rms distance of 0.8 Å over all alpha-carbons.[14] It is evident that the leucine side chains extend further into the binding pocket and obstruct the binding of this type of A-FABP inhibitors. Especially, the CF3 group clearly collides with Leu104 of H-FABP.

Table 3. Binding affinities for selected compounds for human A-FABP and human H-FABP[a]

Compound	A-FABP IC_{50} (μM)	H-FABP IC_{50} (μM)
2	0.6	17
9a	3.9	>100
9e	2.9	>100
9k	4.0	>100
9l	24	>100

[a] In vitro binding data are reported as the mean of triplicate experiments performed on the same dilution.

In summary, a novel structural class of human A-FABP inhibitors based upon a benzylamino-6-(trifluoromethyl)pyrimidine-4(1*H*)-ones is presented. These compounds were shown to have moderate to high inhibitory activity. The compounds display a good selectivity for A-FABP over H-FABP and represent potential leads for human A-FABP inhibition.

References and notes

1. Storch, J.; Thumser, A. E. A. *Biochim. Biophys. Acta* **2000**, *1486*, 28–44.
2. Hertzel, A. V.; Bernlohr, D. A. *TEM* **2000**, *11*, 175–180.
3. Hotamisligil, G. S.; Johnson, R. S.; Distel, R. J.; Ellis, R.; Papaioannou, V. E.; Spiegelman, B. M. *Science* **1996**, *274*, 1377–1379.
4. Robl, J. A.; Parker, R. A.; Biller, S. A.; Jamil, H.; Jacobson, B. L.; Kodukula, K. WO 200015229 and WO 200015230.
5. Binas, B.; Danneberg, H.; McWhir, J.; Mullins, L.; Clark, A. J. *FASEB J.* **1999**, *13*, 805–812.
6. For experimental details see: Van Dongen, M. J. P.; Uppenberg, J.; Svensson, S.; Lundbäck, T.; Åkerud, T.; Wikström, M.; Schultz, J. *J. Am. Chem. Soc.* **2002**, *124*, 11874–11880, The fluorescence polarization assay described herein was used with minor modifications.
7. Performed as described in: Lehmann, F.; Haile, S.; Axen, E.; Medina, C.; Uppenberg, J.; Svensson, S.; Lundbäck, T.; Rondahl, L.; Barf, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, in press. doi:10.1016/j.bmcl.2004.06.057.
8. (a) Xu, Z. H.; Bernlohr, D. A.; Banaszak, L. J. *J. Biol. Chem.* **1993**, *268*, 7874–7884; (b) Reese-Wagoner, A.; Thompson, J.; Banaszak, L. *Biochim. Biophys. Acta—Mol. Cell. Biol. Lipids* **1999**, *1441*, 106–116.
9. See for instance: Svensson, S.; Östberg, T.; Jacobsson, M.; Norström, C.; Stefansson, K.; Hallen, D.; Climent-Johansson, I.; Zachrisson, K.; Ogg, D.; Jendeberg, L. *EMBO* **2003**, *22*, 4625–4633.
10. Kaiser, C. *J. Org. Chem.* **1959**, *24*, 113–114.
11. Hirayama, F.; Koshio, H.; Katayama, N.; Kurihara, H.; Taniuchi, Y.; Sato, K.; Hisamichi, N.; Sakai-Moritani, Y.; Kawasaki, T.; Matsumoto, Y.; Yanagisawa, I. *Bioorg. Med. Chem.* **2002**, *10*, 1509–1523.
12. Elokdah, H. M.; Friedrichs, G. S.; Chai, S.-Y.; Harrison, B. L.; Primeau, J.; Chlenov, M.; Crandall, D. L. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1967–1971.
13. The H-FABP coordinates were taken from pdb entry 1HMS: Young, A. C.; Scapin, G.; Kromminga, A.; Patel, S. B.; Veerkamp, J. H.; Sacchettini, J. C. *Structure* **1994**, *2*, 523–534.
14. The alignment was made with the program O: Jones, T. A.; Zou, J. Y.; Cowan, S. W.; Kjeldgaard, M. *Acta Crystallogr. A* **1991**, *47*, 110–119.