Bioorganic & Medicinal Chemistry Letters 21 (2011) 2949–2952

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

New aromatic substituted pyrazoles as selective inhibitors of human adipocyte fatty acid-binding protein

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ARTICLE INFO

Article history:
Received 8 December 2010
Revised 11 March 2011
Accepted 16 March 2011
Available online 22 March 2011

Keywords:
a-FABP inhibitor
Aromatic substituted pyrazoles
Inflammatory response
Inflammation related diseases

ABSTRACT

a-FABP is indespensible in inflammation and may serve as a new potential drug target for inflammation related diseases. We have successfully designed and synthesized a series of aromatic substituted pyrazoles as new human a-FABP inhibitors. The compounds strongly bound to the hydrophobic binding pocket of a-FABP, while showed significantly lower binding affinities to the closely related homologue protein h-FABP. The most potent and selective compound **5g** bound to a-FABP with an apparent K_i value below 1.0 nM, while did not inhibit h-FABP at 50 μM and thus represents one of the most potent and selective a-FABP inhibitors to date. The strong binding capacity of these inhibitors was further validated by their effective blockade of inflammatory responses as determined by the production of pro-inflammatory cytokines upon LPS stimulation. Compound **5g** may serve as a lead compound for developing new effective therapeutic agent for prevention and treatment of atherosclerosis, type 2 diabetes and other inflammatory and metabolic related diseases.

Fatty acid-binding proteins (FABPs) are a class of low molecular weight cytoplasmic lipid chaperones that bind with hydrophobic lipids inside the cells.[1] Studies have suggested that FABPs coordinate intracellular lipid storage and trafficking, and cholesterol and phospholipids metabolism as well.[1] At least 9 FABPs have been identified which exhibit distinct expression profiles (FABP1–9, or liver (l-), intestinal (i-), heart (h-), adipocyte (a-), epidermal (e-), ileal (il-), brain (b-), myelin (m-) and testis (t-) FABPs).[1] The FABPs share highly similar three-dimensional structures, although their primary sequences show significant diversity (15–70% sequence identity).[2]

Adipocyte FABP (a-FABP), also known as ap2 or FABP4, is abundantly expressed in adipocytes and macrophages.[3] a-FABP deficient mice are significantly protected from the development of insulin resistance in both genetic and high-fat diet induced obese.[4–8] Molecular studies demonstrated that a-FABP physically associates with adipocyte hormone sensitive lipase (HSL), Jak2 and nuclear hormone receptor PPAR and thereby acts as a lipid sensor in adipocytes.[9–13] Furthermore, recent studies revealed that macrophage is a critical site of its action where a-FABP plays a key role in mediating inflammatory responses.[5,8,14] Additionally, a-FABP knockout mice are resistant to several other inflammation related disorders, including allergic airway inflammation[11] and experimental autoimmune encephalomyelitis (EAE)/multiple sclerosis (MS).[12] These evidence collectively support the notion that a-FABP is indespensible in inflammation and it may represent a new potential drug target for inflammation related diseases. Actually, it has been recently reported that inhibition of a-FABP by specific inhibitors suppressed inflammatory responses in macrophage cells.[9,10,14] More importantly, oral administration of the selective a-FABP inhibitor BMS309403, mitigated the onset of insulin resistance and atherosclerosis in mice, which are two main pathologies by chronic inflammation.[15] Several other small molecular a-FABP inhibitors have also been discovered with promising potential (Fig. 1).[16–22]

In this Letter, we report the design, synthesis and biological evaluation of a series of aromatic substituted pyrazoles (**5**) as new human a-FABP inhibitors.

The designed compounds were synthesized by condensation–cyclization of chalone derivatives **6** with phenylhydrazines **7** as the key step (Scheme 1). Briefly, condensation of chalone derivatives **6** with phenylhydrazine **7** under acidic conditions yielded tri-aromatic substituted 4,5-dihydro-1*H*-pyrazoles which were further converted to pyrazoles **8** by oxidization. Intermediates **9** were obtained by Suzuki coupling of pyrazoles **8** with boronic acid

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doi:10.1016/j.bmcl.2011.03.063

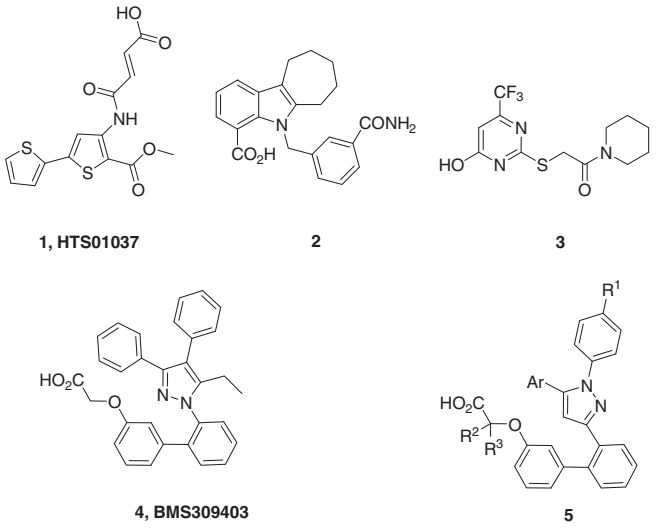
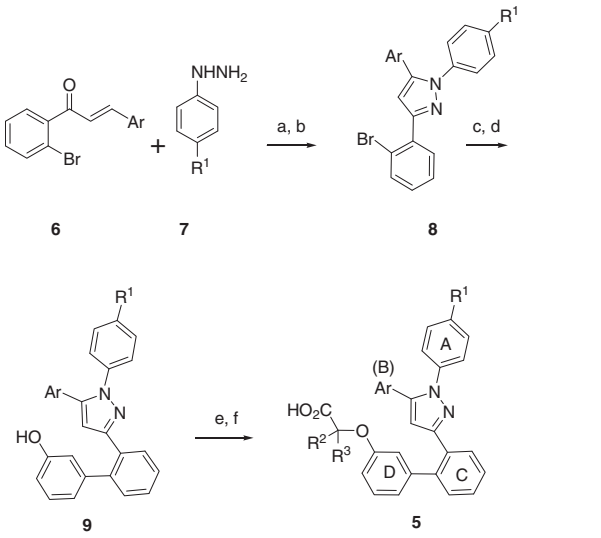


Figure 1. Chemical structures of some important reported a-FABP inhibitors and the designed aromatic substituted pyrazoles **5**.



Scheme 1. Synthesis of the designed aromatic substituted pyrazoles. Reagents and conditions: (a) CH_3CO_2H, C_2H_5OH, reflux, 80–85%; (b) MnO_2, toluene, separator, 75–80%; (c) 3-methoxyphenylboronic acid (**10**), $Pd(P(Ph)_3)_4$, dioxane, reflux, 70–95%; (d) BBr_3, CH_2Cl_2, 75–85%; (e) K_2CO_3, DMF, rt or 50–60 °C, 60–85%; (f) NaOH, H_2O, 85–95%.

10 and subsequent demethylation. O-Alkylation of compounds **9** with 2-bromoacetatic acid esters followed by hydrolysis of the corresponding esters provided the designed compounds **5a**–**5g**.

The binding affinity of the compounds with a-FABP was determined by an in vitro 1-anilinonapthalene 8-sulfonic acid (1,8-ANS) based fluorescence displacement assay.[23] BMS309403 or BMS309403 ethyl ester was respectively utilized as the positive or negative control to validate the screening assay. Under the screening conditions, BMS309403 displayed a K_i value of 15.0 nM binding to a-FABP, while its ethyl ester did not show obvious binding to the protein at 10 μM, which were consistent to the previous report (Table 1).[20] The results from the affinity binding assay showed that **5a** bound to a-FABP protein with an apparent K_i value of 32 nM which was twice less potent than BMS309403 (Table 1).

The predicted binding pose of compound **5a** with a-FABP had a similar orientation to that of BMS309403 (Fig. 2A and B).[25,26] The

Table 1
The binding affinities of the designed compounds against a-FABP and h-FABP[a]

Compds	R^1	R^2	R^3	Ar	Binding affinity (K_i, μM)	
					h-FABP	a-FABP
BMS309403					3.47 ± 0.18	0.015 ± 0.008
BMS309403 ester					NB[b]	NB[b]
5a	H	H	H	Ph	5.24 ± 3.70	0.032 ± 0.004
5b	Cl	H	H	Ph	17.00 ± 1.83	0.013 ± 0.008
5c	Cl	H	H	2-Furanyl	4.13 ± 1.03	0.007 ± 0.004
5d	Cl	H	H	2-Thienyl	4.24 ± 0.46	0.005 ± 0.002
5e	Cl	Me	H	2-Thienyl	2.27 ± 0.92	<0.001
5f	Cl	Et	H	2-Thienyl	3.72 ± 1.52	0.006 ± 0.002
5g	Cl	Me	Me	2-Thienyl	>50	<0.001

[a] The binding affinity with a-FABP or h-PABP was determined by an in vitro 1,8-ANS based fluorescence displacement assay.[23] The proteins were prepared and purified based on the published procedure.[24] The data were reported as the means of at least five independent experiments. The apparent K_i values were calculated using an equation reported by Sulsky.[20] The determined K_d values of 1,8-ANS for a-FABP and h-FABP were 0.25 and 0.60 μM, respectively.

[b] NB, no obvious binding at 10 μM.

carboxyl group formed direct hydrogen bonds with Arg126 and Tyr128 in a-FABP. It is also likely to form an extra interaction with Arg106 through a water bridge. The phenyl rings (A, B, C, D) achieved the hydrophobic interactions with Ile104, Val115, Met40 and Pro38. Additionally, the phenyl rings A and C could form π–π stacking interactions with aromatic residues of His93 and Phe57. Further analysis of the binding mode of compound **5a** with a-FABP indicated that an extra small hydrophobic space formed by the residues Met20, Val23 and Val25 in a-FABP was available. A tiny hydrophobic group might be introduced to the R^1-position of the compound **5a** to capture the interaction with this pocket. Therefore, the *p*-Cl substituted compound **5b** was designed and synthesized to display a binding affinity to a-FABP with a K_i value of 13 nM, which was almost equally potent to BMS309403 (Table 1).

Our modeling also indicated that the 5-phenyl group (phenyl ring B) could be replaced with a smaller aromatic group to achieve better binding mode with the less opened pocket surrounded by residues Ala33, Ala36, Pro38 and Ser55 (Supplementary data). Compounds **5c** and **5d**, which possess a 5- furanyl or 2-thienyl group respectively, indeed had a higher binding affinity than that of compound **5b**. Specifically, compound **5d** bound to a-FABP with a K_i value of 5.0 nM, which is three times more potent than compound **5b** or BMS309403 in a parallel comparison (Table 1).

Interestingly, compound **5e**, which was derived from the methylation of carboxyl α-carbon atom of **5d**, displayed the best binding affinity with a-FABP with the K_i value below 1 nM. This might be due to the fact that the methyl group would further strengthen the hydrophobic effects and van der Waals (VDW) interactions with Val115 and Cys117 (Fig. 2**C**). Furthermore, the introduction of a methyl group also caused a decrease in flexibility and degree of freedom for the carboxyl side chain to reduce the entropy effect of the conformation. The constraints in the orientation were conducive to the formation of salt bridge between the carboxyl group and the nitrogen atoms of Arg126, while oxygen atom on the side chains was fitted in right place to participate in the formation of hydrogen bonds with the receptor via a water bridge (Fig. 2**C**). The binding affinity to a-FABP was barely affected by the introduction of an extra methyl group at this position (compound **5g**) (Fig. 2**D** and Table 1). However, when a slightly bigger ethyl group

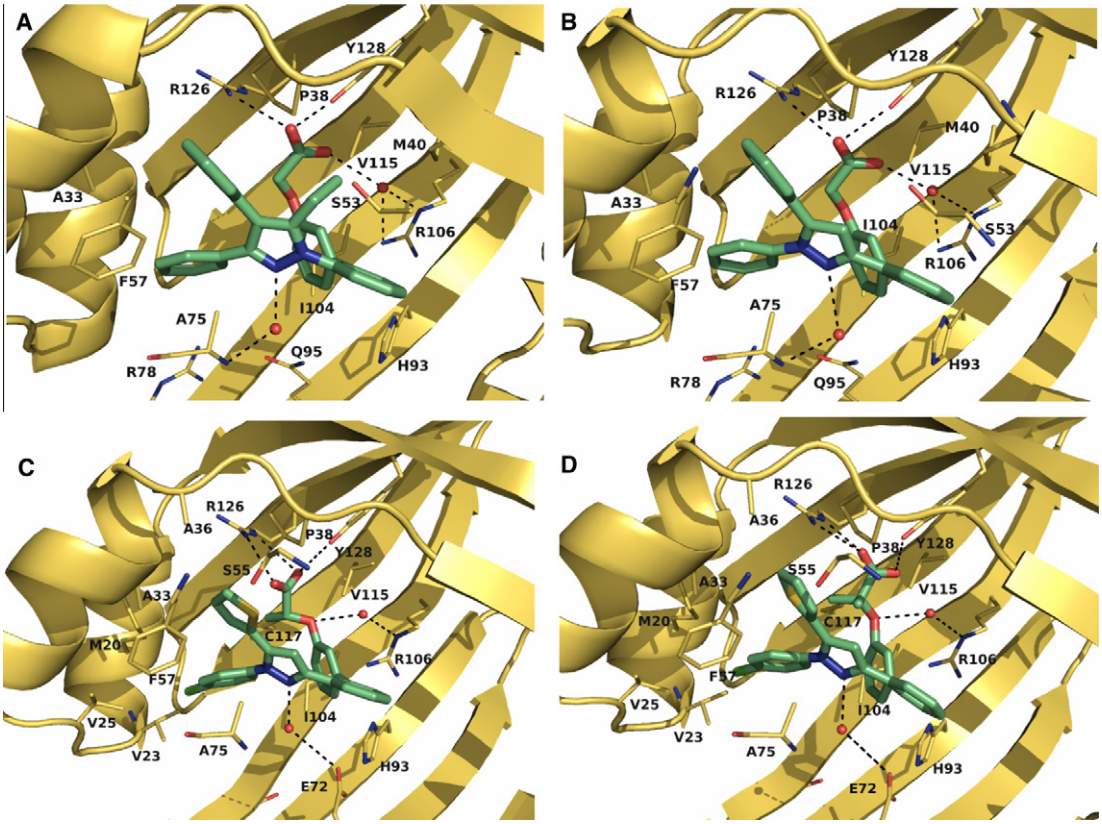


Figure 2. Co-crystal structure of a-FABP–BMS309403 complex (A) and the binding modes of the compounds **5a** (B), **5e** (C) and **5g** (D) with a-FABP. a-FABP is colored yellow. Key residues of the binding site are shown as solid lines and the hydrogen bonds are labeled as dash lines. The water molecules are represented with red balls. All the figures are prepared using Pymol 0.99 (http://www.pymol.org).

was introduced (**5f**), the binding affinity decreased by >6-fold. It is possible that the hydrophobic groove formed by Val115 and Cys117 is too small to accommodate an ethyl group in a proper orientation.

Nine FABPs have been identified to date and they share almost identical three-dimensional structures. In order to examine the specificity of these inhibitors, the binding affinities of these compounds against the human heart type FABP, that is, h-FABP (also

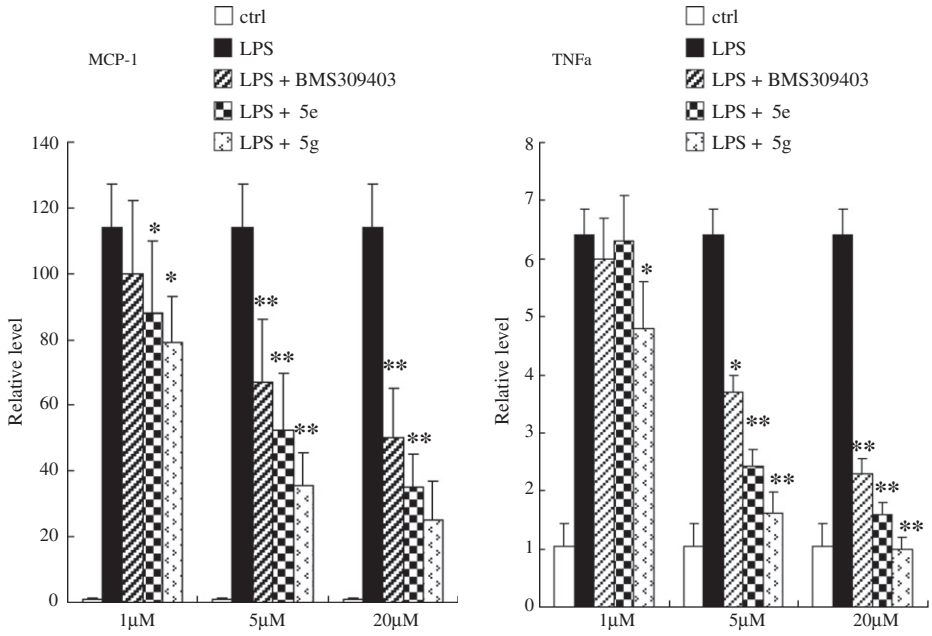


Figure 3. Compounds **5e** and **5g** potently inhibited the pro-inflammatory cytokine production in dose dependent manners. RAW264.7 cells were pretreated with BMS309403, **5e** and **5g** under indicated concentrations or DMSO for 1 h, followed by LPS stimulation. Cytokine production was determined by quantitative PCR. (A) MCP-1. (B) TNFα. $^{*}p$ <0.1, $^{**}p$ <0.05.

known as FABP3) were also evaluated by the fluorescence binding assay. Although h-FABP and a-FABP have a sequence identity of 64.4% and share a highly conserved functional domain, our data showed that almost all of the designed inhibitors and BMS309403 exhibited remarkable selectivity to a-FABP over h-FABP (Table 1). For instance, compound **5d** selectively bound to a-FABP with a K_i value of 5.0 nM, while its binding apparent K_i value against h-FABP was about 4.2 μM. This might be due to the relatively smaller hydrophobic groove in h-FABP as compared to a-FABP. The increase in the number of three-dimensional conformations compromised the binding affinity since it causes spatial collision with some larger residues (such as Tyr115, Tyr117 and Phe16), and hinders the formation of hydrogen bonds between key carboxyl pharmacophore with residues Phe16 and Arg126 on h-FABP as well (Supplementary data). Noticeably, when the carboxyl was substituted with dimethyl groups (compound **5g**), the biochemical activity against h-FABP was almost completely abolished due to the unavoidable spatial collision caused by the rigidity of the side chains.

Studies by us and others have demonstrated that targeted deletion or knockdown of a-FABP decreases inflammatory responses in macrophages.[5,8,14] The biological effects of some selected compounds were also evaluated in a murine macrophage cell line RAW264.7 to see whether they mitigate the activation of inflammatory responses. The results demonstrated that lipopolisacharide (LPS), an inflammatory stimulus, robustly enhanced the expression of two key pro-inflammatory cytokines MCP-1/CCL2 and TNFα (mRNA levels, Fig. 3). The effect was significantly attenuated by all of the three a-FABP inhibitors examined in dose dependent manners. It was noteworthy that the cellular cytokine suppressing activities of BMS309403, compound **5e** or **5g** were obviously less potent comparing with their strong a-FABP inhibitory effects. This may partially be due to the low cellular permeability of the compounds caused by the negatively charged carboxylic groups.

In conclusion, in the present study, a series of aromatic substituted pyrazoles were designed and synthesized as new human a-FABP inhibitors. The compounds strongly bound to the hydrophobic binding pocket of a-FABP, while showed significantly lower binding affinities to the closely related homologue protein h-FABP. Among them, the most potent and specific compound **5g** bound to a-FABP with an apparent K_i value below 1.0 nM while did not inhibit h-FABP at 50 μM and thus represents one of the most potent and selective a-FABP inhibitors to date. The strong and specific binding capacity of these inhibitors was further validated by their effective blockade of inflammatory responses as determined by the production of pro-inflammatory cytokines upon LPS stimulation. These inhibitors might serve as lead compounds for developing novel and effective therapeutic agents for prevention and treatment of atherosclerosis, type 2 diabetes and other inflammatory and metabolic related diseases.

Acknowledgments

We thank National Basic Research Program of China (Grant #2010CB529706, 2010CB9455000 and 2009CB940904), the 100-talent program of Chinese Academy of Sciences, and National Natural Science Foundation (Grant #30811120429, 3097063 and 90813033) for their financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.063.

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