Synthesis of BMS-309403-Related Compounds, Including [^{14}C]BMS-309403, a Radioligand for Adipocyte Fatty Acid Binding Protein

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Received September 14, 2011; accepted October 27, 2011; published online November 2, 2011

Adipocyte fatty acid binding protein (A-FABP; FABP4), which is predominantly expressed in macrophages and adipose tissue, regulates fatty acid storage and lipolysis, and is also an important mediator of inflammation. Here, we report a synthesis of ^{14}C-labeled 2-[2′-(5-ethyl-3,4-diphenyl-1*H*-pyrazol-1-yl)biphenyl-3-yloxy]acetic acid (BMS309403), a potent and selective small-molecular FABP4 inhibitor, as a chemical tool for investigating the roles of FABP4 in inflammatory and metabolic disorders. The structure–activity relationship of several BMS derivatives for inhibition of FABP4 is also reported.

Key words fatty acid binding protein 4; BMS-309403; radioligand; labelled synthesis; C-14; adipocyte fatty acid binding protein

Fatty acid binding proteins (FABPs) are small-molecular-weight hydrophobic proteins containing a large hydrophobic cavity, into which naturally occurring long-chain fatty acids and synthetic hydrophobic ligands can be accepted.[1] FABPs act as transporters of endogenous fatty acids from the cell surface to various sites of fatty acid storage and metabolism.[2] Nine isoforms of FABPs are currently known, and each FABP has a characteristic distribution pattern. Adipocyte FABP (also known as FABP4, A-FABP, ALBP and aP2), which is the only isoform predominantly expressed in macrophages and adipose tissue, regulates fatty acid storage and lipolysis in those tissues.[3] But, in addition to the roles of FABP4 in regulating lipid metabolism and insulin sensitivity,[4—6] recent pharmacological and biological findings have indicated a regulatory function of FABP4 in inflammation.[7—10] Because acute and/or chronic inflammation is involved in many diseases, we considered that visualization of FABP4 might be a powerful tool to investigate not only metabolic disorders, but also inflammatory disorders.

Since a labelled FABP4 ligand is expected to be a valuable tool for FABP studies, we have prepared ^{14}C-labelled 2-[2′-(5-ethyl-3,4-diphenyl-1*H*-pyrazol-1-yl)biphenyl-3-yloxy]-acetic acid (BMS-309403),[11] which is a potent and selective inhibitor of FABP4. Several derivatives of BMS-309403 were

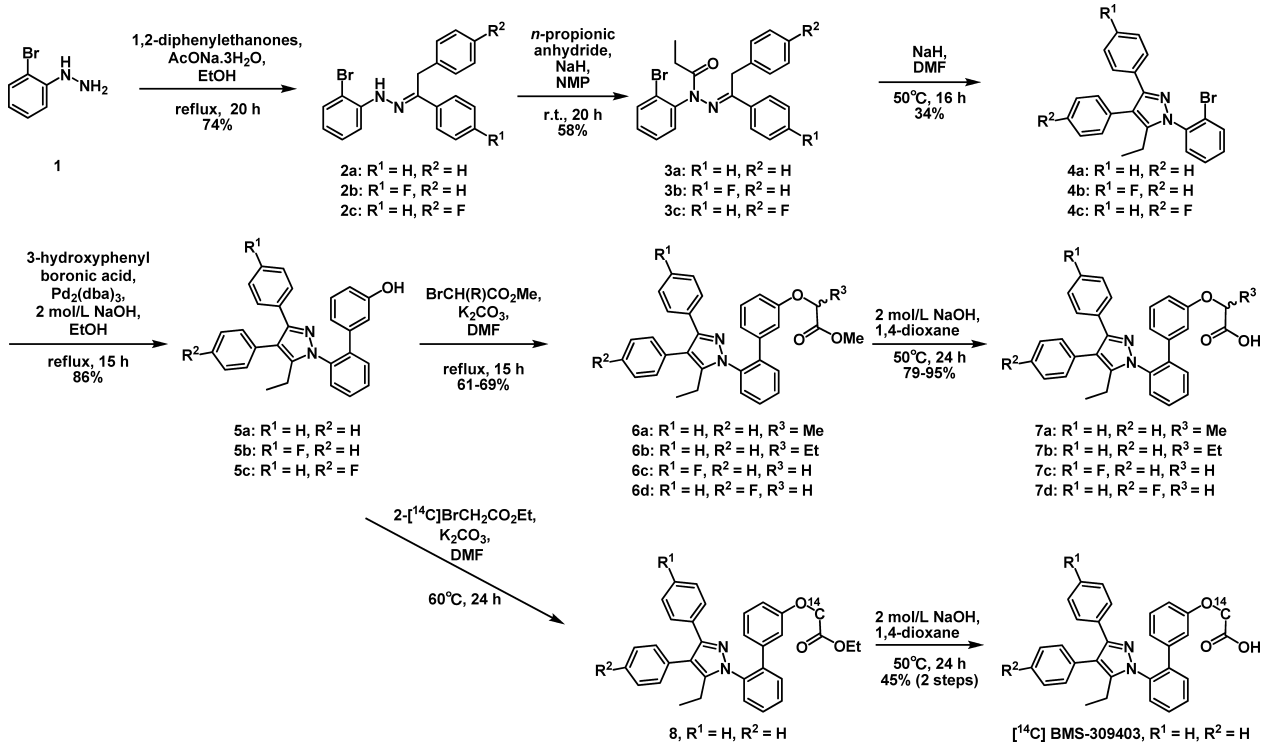

Chart 1. Synthesis of BMS-309403 Derivatives

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also prepared, and their structure–activity relationship for inhibition of FABP4 is also reported.

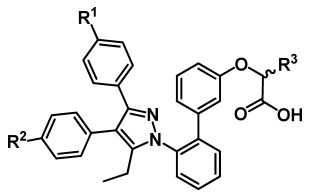
Synthesis of Side-Chain Alkyl Derivatives of BMS-309403 The binding pocket of FABP4 is reported to be extremely hydrophobic. Therefore, we first examined the effect of introducing an alkyl side chain at the α-position of the carboxyl group of BMS-309403.

2′-(5-Ethyl-3,4-diphenyl-1*H*-pyrazol-1-yl)biphenyl-3-ol (**5a**; R^1=H, R^2=H), used as a precursor for the present study, was prepared according to Sulsky *et al.*, from commercially available 2-bromophenyhydrazine HCl.[11] Compounds **5a** was treated with either methyl 2-bromopropionate or methyl 2-bromobutyrate in the presence of potassium carbonate as a base to afford ester derivatives **6a** and **b**. Alkaline hydrolysis of the ester moiety of **6a**, **b** afforded the desired acids **7a**, **b** (Chart 1).

Synthesis of Fluorinated Derivatives of BMS-309403 We also synthesized fluorinated derivatives of BMS-309403 (**7c**, **d**), in view of the potential applicability of ^{18}F-labeled derivatives for positron emission tomographic (PET) imaging of chronic inflammation. The synthetic routes are also depicted in Chart 1. 2-Bromophenylhydrazine (**1**) was treated with either 1-(4-fluorophenyl)-2-phenylethanone or 2-(4-fluorophenyl)-1-phenylethanone in the presence of sodium acetate in ethanol to afford the condensed derivatives **2b** and **c**. These compounds were treated with *n*-propionic anhydride, followed by cyclization and Suzuki coupling reaction to afford biphenyl-pyrazole derivatives **5b** and **c**. Compounds **5b** and **c** were treated with ethyl 2-bromoacetate in the presence of potassium carbonate as a base, and subsequent alkaline hydrolysis of the ester moiety afforded the desired acids **7c** and **d** (Chart 1).

Structure–Activity Relationship of BMS-309403 Derivatives The primary goal of this study is to prepare a FABP-4-selective radio isotope-labeled ligand as a candidate probe to image inflammation, especially inflammation-associated macrophages. Table 1 summarizes the FABP4-inhibitory activity of the prepared alkyl-substituted BMS-309403 derivatives, in addition to BMS-309403 itself and arachidonic acid, a naturally occurring unsaturated fatty acid, as a positive control. The assay-kit supplier reported that the IC_{50} value of arachidonic acid is 3 μM with this kit. We obtained a similar value, confirming the validity of the assay. We found that the introduction of a side-chain alkyl group at the α-position of the carboxyl group decreased the activity, and the decrease was greater as the alkyl chain length was increased.

Table 1. FABP4-Inhibitory Activity of BMS-309403 Derivatives



R^1	R^2	R^3		IC_{50} (μM)
H	H	H	(BMS-309403)	0.71
H	H	Me		2.35
H	H	Et		2.91
F	H	H		0.33
H	F	H		4.67
			Arachidonic acid	5.13

The inhibitory activities were determined with a Cayman FABP4 assay kit, according to the supplier's protocol. Arachidonic acid was used as a positive control.

The three-dimensional structure of the complex of BMS-309403 with FABP4 indicated that BMS-309403 formed a hairpin-like structure and its acetic acid moiety lay over the pyrazole moiety.[11] One of the methylene protons at the α-position of the carboxyl group of BMS-309403 was located near the side-chain benzyl group of 16Phe of FABP4 (within 3 Å), and the other was located near the phenyl group at the 4-position of the pyrazole ring of BMS-309403 (within 3 Å). We speculated that the introduction of an alkyl chain at the α-position of the carboxyl group changed the conformation of BMS-309403, interfering with one or more of these interactions. Therefore, alkylation of BMS-309403 does not appear to be a promising avenue, and so we considered radiolabeling of BMS-309403 itself.

First, we focused on the two benzene rings attached to the pyrazole backbone of BMS-309403. Although the detailed structure–activity relationship at the 3- and 4-position benzene rings of BMS-309403 has not been established, we were interested in the introduction of fluorine at the *para*-positions of both benzene rings, in view of the potential applicability of ^{18}F-labeled derivatives for PET imaging. Table 1 summarizes the FABP4-inhibitory activity of BMS-309403 and its fluorinated derivatives. It is noteworthy that the introduction of fluorine at the *para*-position of the 3-phenyl group of BMS-309403 resulted in retention or improvement of the FABP-4 inhibitory activity, while the introduction of fluorine at the *para*-position of the 4-phenyl group considerably decreased the FABP-4 inhibitory activity as compared to that of the parent BMS-309403. These data might indicate that the binding pockets hosting the 3-phenyl group and 4-phenyl group differ in volume and/or shape.

The X-ray crystal study of BMS-309403 complexed with FABP4 indicated that the 3-position phenyl group binds to the hydrophobic pocket formed by 16Phe, 19Tyr, 20Met, 25Val, 57Phe and 76Asp. The distance between the 4-position hydrogen atom of the 3-phenyl group of BMS-309403 and the nearest amino acid (25Val) was estimated to be more than 2.5 Å.[12] On the other hand, the 4-position phenyl group binds to the hydrophobic pocket formed by 33Ala, 34Gly, 36Ala, 38Pro, 55Ser, 57Phe and 126Arg. The distance between the 4-position hydrogen atom of the 4-phenyl group of BMS-309403 and the nearest amino acid (36Ala) was estimated to about 1.7 Å. These results indicated that there may not be enough space to accommodate a fluorine atom at the 4-position of the 4-phenyl group of BMS-309403. This might be the reason why the introduction of fluorine at the 4-position decreased the FABP4-inhibitory activity, even though fluorine is a small atom.

Based on these preliminary results, we decided that appropriate labeling positions would the α-position of the carboxyl group of BMS-309403 for ^{14}C, and the 4-position of the 3-phenyl group of BMS-309403 for ^{18}F.

Synthesis of [^{14}C]BMS-309403[13] [^{14}C]BMS-309403 was prepared analogously by the reaction of **5** with 2-[^{14}C]ethyl bromoacetate in the presence of potassium carbonate as a base. Alkaline hydrolysis, followed by column-chromatographic purification, afforded [^{14}C]BMS-309403 **9** in satisfac-

tory yield and high radiochemical purity of 94.8%.

Conclusion

We synthesized ^{14}C-labeled BMS-309403 from commercially available ^{14}C-labeled ethyl bromoacetate in excellent radiochemical purity and with sufficiently high specific activity. Several BMS-309403 related compounds were also prepared to enable us to select appropriate positions for labeling with ^{14}C and ^{18}F. As BMS309403 is a potent and selective small-molecular FABP4 inhibitor, and FABP4 is expressed mainly to macrophages and inflammatory response was reported to augment the expression of FABP4,[14—16] the labeled compound is expected to be useful as a chemical tool for investigating the roles of FABP4 in inflammatory and metabolic disorders. Studies along this line, including the synthesis of [^{18}F]BMS-309403, are in progress.

Experimental

General All reagents were of commercial grade unless otherwise stated. 2-[^{14}C]Ethyl bromoacetate (52 mCi/mmol) was purchased from Moravek Biochemicals and Radiochemicals, and was supplied as CH_2Cl_2 solution (0.5 mL). Radioactivity measurements were carried out using a Perkin Elmer liquid scintillation counter Tri-Carb 2800 TRs and Clera-sol Ias scintillant. Flash column-chromatography was performed using Merck silica gel 0.04—0.063 mm. Thin-layer chromatography (TLC) was run on Merck Kieselgel silica gel 60 F_{254} glass plates.

(*E*)-1-(2-Bromophenyl)-2-(1,2-diphenylethylidene)hydrazine, 2 To a stirred solution of 2-bromophenylhydrazine hydrochloride (**1**) (1.00 g, 4.47 mmol) in ethanol (20 mL) were added sodium acetate trihydrate (877 mg, 4.47 mmol) and benzyl phenyl ketone (608 mg, 4.47 mmol) under argon. The mixture was heated to reflux for 20 h and then cooled and evaporated. The reaction mixture was poured into saturated sodium hydrogencarbonate solution (10 mL), and extracted three times with ethyl acetate (50 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL), dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was purified by means of column chromatography (eluant; *n*-hexane : ethyl acetate=12/1 v/v) to afford **2** (1.21 g, 74%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.97—6.65 (m, 14H), 4.18 (s, 2H), 3.93 (s, 1H). MS (FAB) *m*/*z* 365, 367 $(M+H)^+$.

(*E*)-*N*-(2-Bromophenyl)-*N*′-(1,2-diphenylethylidene)propionohydrazide, 3 To a stirred mixture of sodium hydride (60% dispersion, 173 mg, 7.20 mmol) in *N*-methylpyrrolidone (NMP) (5 mL) was added a solution of **2** (1.21 g, 3.31 mmol) in NMP (5 mL) over 5 min under argon at room temperature. The reaction mixture was heated to 60°C for 3 h and then cooled to room temperature. To this solution was added propionic anhydride (0.57 mL, 4.62 mmol) at a rate appropriate to keep the reaction temperature below 35°C. The reaction mixture was stirred for 3 h, then quenched with aqueous HCl (0.5 mol/L, 100 mL) and extracted four times with ether (30 mL). The ether extracts were combined, washed with water (100 mL) and brine (100 mL), dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was purified by means of column chromatography (eluant; *n*-hexane : ethyl acetate=5/1 v/v) to afford **3** (805 mg, 58%) as an orange oil. MS (FAB) *m*/*z* 421, 423 $(M+H)^+$.

1-(2-Bromophenyl)-5-ethyl-3,4-diphenyl-1*H*-pyrazole, 4 To a solution of **3** (334 mg, 0.793 mmol) in dehydrated *N*,*N*-dimethylformamide (5 mL) at room temperature was added sodium hydride (60% dispersion, 79 mg, 3.30 mmol) in several portions over 1 min under argon. The reaction mixture was warmed to 38°C and then further heated to 50°C for 16 h. The deep red reaction mixture was cooled and quenched by the addition of water (10 mL). The mixture was acidified with aqueous HCl (1 mol/L, 20 mL) and then extracted four times with ether (30 mL). The organic extracts were washed with water (50 mL) and brine (50 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was purified by means of column chromatography (eluant; *n*-hexane : ethyl acetate = 5/1 v/v) to afford **4** (110 mg, 34%) as an orange oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.76—7.22 (m, 14H), 2.54 (m, 2H), 0.91 (t, J=7.8 Hz, 3H). MS (FAB) *m*/*z* 403, 405 $(M+H^+)$.

2′-(5-Ethyl-3,4-diphenyl-1*H*-pyrazol-1-yl)biphenyl-3-ol, 5 Aqueous NaOH (2 mol/L, 1.0 mL) was added to a mixture of bromophenyl pyrazole (**4**) (318 mg, 0.788 mmol), 3-hydroxyphenylboronic acid (130 mg, 0.946 mmol), and $Pd_2(dba)_3$ (22 mg, 0.023 mmol) in ethanol (2.0 mL). The reaction mixture was heated to reflux with stirring for 15 h under argon, then cooled to room temperature, quenched with water (50 mL) and extracted four times with ethyl acetate (30 mL). The organic extracts were washed with water (50 mL) and brine (50 mL), dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was purified by means of column chromatography (eluant; *n*-hexane : ethyl acetate=5/1 v/v) to give **5** (281 mg, 0.675 mmol, 86%) as a brown solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.64—6.55 (m, 18H), 2.16—2.05 (m, 2H), 0.60 (t, J = 7.6 Hz, 3H).

Methyl 2-((2′-(5-Ethyl-3,4-diphenyl-1*H*-pyrazol-1-yl)-[1,1′-biphenyl]-3-yl)oxy)propanate, 6a To a slurry of **5** (171 mg, 0.411 mmol) in anhydrous dehydrated *N*,*N*-dimethylformamide (4 mL) was added potassium carbonate (113 mg, 0.822 mmol), followed by methyl 2-bromopropionate (0.05 mL, 0.41 mmol). The reaction mixture was stirred at room temperature for 3 h. Additional methyl 2-bromopropionate (0.05 mL, 0.41 mmol) was added and stirring was continued for 21 h. The reaction mixture was cooled to room temperature, quenched with water (150 mL) and extracted four times with ethyl acetate (50 mL). The combined organic extracts were washed once with water (50 mL) and once with brine (50 mL), dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was purified by means of silica gel column chromatography (eluant; *n*-hexane : ethyl acetate=3/1, v/v) to give **6a** (126 mg, 61%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.67—6.66 (m, 18H), 4.58 (q, J=6.8 Hz, 1H), 3.61 (s, 3H), 2.08—2.05 (m, 2H), 1.44 (d, J=6.8 Hz, 3H), 0.60 (t, J=7.4 Hz, 3H).

2-((2′-(5-Ethyl-3,4-diphenyl-1*H*-pyrazol-1-yl)-[1,1′-biphenyl]-3-yl)oxy)propanoic Acid, 7a A solution of **6a** (121 mg, 0.241 mmol) in 1,4-dioxane (0.8 mL) was treated with sodium hydroxide solution (2 mol/L, 0.3 mL). The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was cooled to room temperature and acidified with aqueous citric acid solution (10%, 5 mL). The mixture was diluted with water (20 mL) and stirred vigorously for 30 min. The precipitate was collected by filtration, washed with water, and dissolved in ethyl acetate (100 mL). The solution was dried over anhydrous magnesium sulfate and concentrated

in vacuo. The resulting residue purified by silica gel column chromatography (eluant; *n*-hexane : ethyl acetate=5/1, v/v) to give **7a** (111 mg, 95%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.64—6.61 (m, 18H), 4.45 (m, 1H), 2.05 (m, 2H), 1.35 (m, 3H), 0.57 (t, J=6.8 Hz, 3H). High resolution (HR)-MS (FAB, MH^+) Calcd for $C_{32}H_{28}N_2O_3$ 489.2178, Found 489.2165.

2-((2′-(5-Ethyl-3,4-diphenyl-1*H*-pyrazol-1-yl)-[1,1′-biphenyl]-3-yl)oxy)butanoic Acid, 7b This compound (white solid, 79% yield) was prepared from **6b** by means of a procedure similar to that used for **7a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.64—6.64 (m, 18H), 4.32—4.15 (m, 1H), 2.05 (m, 2H), 1.74 (m, 2H), 0.83 (m, 3H), 0.57 (t, J=7.0 Hz, 3H). HR-MS (FAB, $(M+H)^+$) Calcd for $C_{32}H_{28}N_2O_3$ 503.2335, Found 503.2329.

2-((2′-(5-Ethyl-4-(3-fluorophenyl)-3-phenyl-1*H*-pyrazol-1-yl)-[1,1′-biphenyl]-3-yl)oxy)acetic Acid, 7c This compound (white amorphous solid) was prepared from **1** by means of a procedure similar to that used for **7a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.66—6.65 (m, 17H), 4.38 (s, 2H), 2.06—2.05 (m, 2H), 0.58 (t, J=7.4 Hz, 3H). HR-MS (FAB, $(M+H)^+$) Calcd for $C_{31}H_{26}FN_2O_3$ 493.1927, Found 473.1928.

2-((2′-(5-Ethyl-4-(4-fluorophenyl)-3-phenyl-1*H*-pyrazol-1-yl)-[1,1′-biphenyl]-3-yl)oxy)acetic Acid, 7d This compound (white amorphous solid) was prepared from **1** by means of a procedure similar to that used for **7a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.64—6.65 (m, 17H), 4.40 (s, 2H), 2.05 (m, 2H), 0.58 (t, J=7.5 Hz, 3H). HR-MS (FAB, $(M+H)^+$) Calcd for $C_{31}H_{26}FN_2O_3$ 493.1927, Found 493.1949.

2[^{14}C](2′-(5-Ethyl-3,4-diphenyl-1*H*-pyrazol-1-yl)biphenyl-3-yloxy)acetic Acid, [^{14}C]BMS 309403, 9 To a solution of **5** (12.0 mg, 30.0 μmol), potassium carbonate (4.00 mg, 30.0 μmol) in dehydrated *N*,*N*-dimethylformamide (5 mL) was added ethyl bromoacetate (3.06 μL, 20.0 μmol (containing 1.06 μL 2-[^{14}C]ethyl bromoacetate and 2.00 μL ethyl bromoacetate: total radioactivity was 6.852 MBq)) in a mixed solution of CH_2Cl_2 (0.5 mL) and dehydrated *N*,*N*-dimethylformamide (4.5 mL). The reaction mixture was stirred at 50°C for 27 h. The whole was quenched with water (100 mL) and extracted four times with ethyl acetate (30 mL×4). The combined organic extracts were dried over anhydrous magnesium sulfate, concentrated *in vacuo*. To the residue was added 1,4-dioxane (0.5 mL) and 2 mol/L sodium hydroxide solution (0.025 mL). The reaction mixture was stirred at 50°C for 22 h. The reaction mixture was cooled to room temperature and acidified with aqueous citric acid solution (10%, 5 mL), and extracted four times with ethyl acetate (30 mL×4). The combined organic extracts were dried over anhydrous magnesium sulfate, concentrated *in vacuo*. The residue was purified with column chromatography (eluant; *n*-hexane : ethyl acetate=5 : 1 to 3 : 1 v/v to ethyl acetate) to give the aimed [^{14}C]BMS-309403 as a blown oil. *Rf* values of this compound are the same as compared to cold BMS-309403. TLC: *Rf* 0.15 (*n*-hexane : ethyl acetate=1 : 1), *Rf* 0.35 (ethyl acetate3), *Rf* 0.60 (ethyl acetat : ethanol=1 : 1).

The average total radioactivity was calculated to 6.498 MBq, therefore the radiochemical purity of [^{14}C]BMS-309403 prepared was estimated to 94.8%.

FABP4-Inhibitory Assay The inhibitory activity of the present series of compounds were assayed with a FABP4 Inhibitor/Ligand Screening Assay Kit (Cayman Chemical Item Number 10010231). This assay makes use of a Detection Reagent that exhibits increased fluorescence at 500 nm when bound to FABP4. A ligand and/or inhibitor of FABP4 displaces the Detection Reagent, thereby reducing the fluorescence. The assay was performed according to the supplier's protocol.

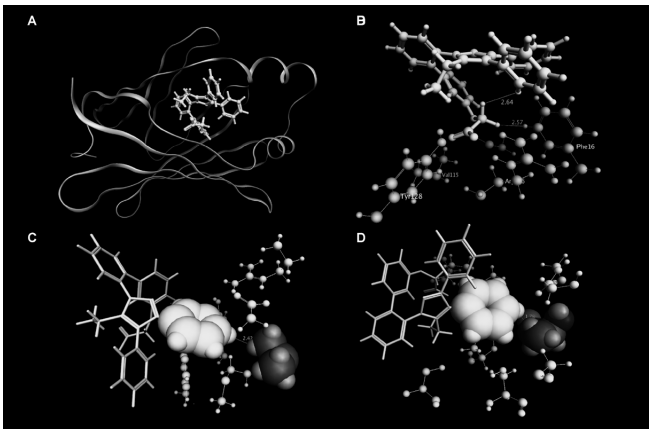


Fig. 1. X-Ray Crystallographic Structure of BMS-309403 Complexed with FABP4 (pdb : 2NNQ)

(A) The whole structure of BMS-309403 complexed with FABP4. FABP4 protein is depicted as ribbon model, and BMS-309403 as boll and stick model. (B) Expanded view of BMS-309403 (boll and stick model) showing the amino acid side chains within 4.5 A (boll and wire model) from the oxyacetic acid moiety of BMS-309403. (C) Expanded view of BMS-309403 (cylinder) and the amino acids hosting the 3-phenyl group of BMS-309403. The 3-phenyl group of BMS-309403 and its nearest amino acid, Val25, are illustrated as space-filling models. (D) Expanded view of BMS-309403 (cylinder) and the amino acids hosting the 4-phenyl group of BMS-309403. The 4-phenyl group of BMS-309403 and its nearest amino acid, Ala36, are illustrated as space-filling models.

Acknowledgement This work was supported in part by the Targeted Proteins Research Program of the Japan Science and Technology Corporation (JST), the Uehara Memorial Foundation and the Tokyo Biochemical Research Foundation (TBRF). We are grateful to Dr. Hiroki Kakuta for his help in FABP4 inhibitory activity assay.

References and Notes

1) Storch J., Thumser A. E., *Biochim. Biophys. Acta*, **1486**, 28—44 (2000).
2) Jenkins-Kruchten A. E., Bennaars-Eiden A., Ross J. R., Shen W. J., Kraemer F. B., Bernlohr D. A., *J. Biol. Chem.*, **278**, 47636—47643 (2003).
3) Chmurzyńska A., *J. Appl. Genet.*, **47**, 39—48 (2006).
4) Hotamisligil G. S., Johnson R. S., Distel R. J., Ellis R., Papaioannou V. E., Spiegelman B. M., *Science*, **274**, 1377—1379 (1996).
5) Maeda K., Cao H., Kono K., Gorgun C. Z., Furuhashi M., Uysal K. T., Cao Q., Atsumi G., Malone H., Krishnan B., Minokoshi Y., Kahn B. B., Parker R. A., Hotamisligil G. S., *Cell Metab.*, **1**, 107—119 (2005).
6) Uysal K. T., Scheja L., Wiesbrock S. M., Bonner-Weir S., Hotamisligil G. S., *Endocrinology*, **141**, 3388—3396 (2000).
7) Makowski L., Boord J. B., Maeda K., Babaev V. R., Uysal K. T., Morgan M. A., Parker R. A., Suttles J., Fazio S., Hotamisligil G. S., Linton M. F., *Nat. Med.*, **7**, 699—705 (2001).
8) Furuhashi M., Tuncman G., Görgün C. Z., Makowski L., Atsumi G., Vaillancourt E., Kono K., Babaev V. R., Fazio S., Linton M. F., Sulsky R., Robl J. A., Parker R. A., Hotamisligil G. S., *Nature* (London), **447**, 959—965 (2007).
9) Shum B. O., Mackay C. R., Gorgun C. Z., Frost M. J., Kumar R. K., Hotamisligil G. S., Rolph M. S., *J. Clin. Invest.*, **116**, 2183—2192 (2006).

10) Reynolds J. M., Liu Q., Brittingham K. C., Liu Y., Gruenthal M., Gorgun C. Z., Hotamisligil G. S., Stout R. D., Suttles J., *J. Immunol.*, **179**, 313—321 (2007).
11) Sulsky R., Magnin D. R., Huang Y., Simpkins L., Taunk P., Patel M., Zhu Y., Stouch T. R., Bassolino-Klimas D., Parker R., Harrity T., Stoffel R., Taylor D. S., Lavoie T. B., Kish K., Jacobson B. L., Sheriff S., Adam L. P., Ewing W. R., Robl J. A., *Bioorg. Med. Chem. Lett.*, **17**, 3511—3515 (2007).
12) Computer-graphic work and determination of hydrogen bonding distances were performed using MOE (Molecular Operating Environment) (Ryoka Systems Inc.).
13) All procedures to synthesize [^{14}C]BMS-309403 were performed in areas approved for radiation work at the Advanced Science Research Center, Okayama University.
14) Fu Y., Luo N., Lopes-Virella M. F., Garvey W. T., *Atherosclerosis*, **165**, 259—269 (2002).
15) Kazemi M. R., McDonald C. M., Shigenaga J. K., Grunfeld C., Feingold K. R., *Arterioscler. Thromb. Vasc. Biol.*, **25**, 1220—1224 (2005).
16) Pelton P. D., Zhou L., Demarest K. T., Burris T. P., *Biochem. Biophys. Res. Commun.*, **261**, 456—458 (1999).