Synthesis of Fatty Acid Binding Protein Inhibitors: A New Approach for Diabetes Treatment

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Adipocyte fatty acid binding protein (aFABP, aP2) is a 14.6 kDa cytosolic protein located in adipocytes and macrophages and assists in the intracellular transport of fatty acids. It is one of a class of fatty acid binding proteins (FABPs) that are found predominately in the liver, heart, intestine and connective tissues. Hotamisligal et al. have reported that aFABP-deficient mice, when placed on a high fat diet (40% of caloric intake as fat), were significantly protected from hyperinsulinemia and insulin resistance compared to the wild type. Additional genetic experiments have been reported in which aFABP null mice have been crossed with Ob/ob and in another instance apoE−/− mice. The aFABP-deficient ob/ob mice were more insulin sensitive when compared to ob/ob controls as demonstrated measuring by circulating glucose and insulin levels. Based on these genetic knock-out models, we pursued the development of inhibitors of aFABP for their therapeutic potential in the treatment of diabetes. Herein we disclose the synthesis of two azole inhibitors of P2 and the methods used to prepare them.

Introduction

With the spread of Western lifestyles, the prevalence of type 2 diabetes is rising. In the United States it is estimated that nearly 21 million children and adults (7.0% of the population) have diabetes. Of this number only 15 million are currently diagnosed. Alarming, it is also estimated that there are potentially 54 million people who are currently “pre-diabetic”. This is a situation where glucose is only partially controlled. If the situation is left unmanaged it is anticipated that these individuals will ultimately exhibit the disease. Taken together, there are 1.5 million new cases of diabetes being diagnosed in people aged 20 years or older (2005 data) within the United States each year. The expectation is that these numbers are likely to greatly increase in the near future.1

Diabetes is characterized by hyperglycemia and disturbances of carbohydrates, lipid and protein metabolism. As these metabolic disturbances become “chronic,” a wide variety of medical problems present themselves. Foremost among them these are heart disease and stroke accounting for 65% of deaths in people with diabetes. In addition, a high percentage of diabetics are afflicted with hypertension. Diabetic complications are not limited to cardiovascular concerns. Diabetes also has a detrimental impact on the retina, kidney and nervous systems. The disease becomes the basis for 24,000 new cases of blindness annually, nearly half of new cases of kidney failure, and a major percentage of lower limb amputations;2 the total cost of the disease is currently estimated at over 150 billion dollars in the United States annually.1

Current diabetes treatment strategies include: reducing insulin resistance using glitazones,3 supplementing insulin supplies with exogenous insulin,4 increasing insulin secretion with sulfonyl ureas,5 and reducing hepatic glucose production with biguanides.6 Recently, treatment strategies using DPP-IV inhibitors and GLP-1 agonists7 have also emerged. Of the aforementioned list, the mechanisms of action generally focus on treating type 2 diabetes predominately from the glucose/insulin axis. However, there is currently no chemical agent that addresses diabetes via altered energy homeostasis from the fatty acid vantage point. By altering the modulation of energy stores along the fatty acid axis an impact on circulating glucose levels would be expected. This strategy then provides a new basis for therapy.

Figure 1: Potent Inhibitors of aP2

A promising new diabetes target for investigation is the inhibition of aP2.8 aP2 is one of a family of homologous intracellular fatty acid binding proteins that is involved in the regulation of fatty acid trafficking. aP2 is found in adipocytes and mediates fatty acid fluxes in adipose tissue.9 Hotamisligal et al. have reported that aFABP deficient mice, when placed on a high fat diet (40% of caloric intake as fat), were significantly protected from hyperinsulinemia and insulin resistance compared to the wild type.10 Additional genetic experiments have been reported in which aFABP null mice have been crossed with ob/ob mice. The aFABP deficient ob/ob mice were more insulin sensitive when compared to ob/ob controls as demonstrated measuring by circulating glucose and insulin levels.11 Supported by these genetic knock-out models, aP2 inhibition presents itself as an perfect target for the treatment of diabetes.8 Moreover, workers at Harvard and Bristol-Myers Squibb recently reported successful intervention with an aP2 inhibitor against a wide variety of diabetes markers including fasting and parandial glucose levels in the ob/ob mouse.12

There are several reports of aP2 inhibitors in the literature;1 these papers typically reveal binding affinities to aP2 and very limited chemical synthetic procedures. Our goal was to
prepare two known potent inhibitors of aP2\(^{12}\) (Figure 1) and disclose needed experimental details to provide access in determining if these agents would serve as forerunners of a new therapeutic class of anti-diabetic agents. For this reason the preparation of the two compounds below was undertaken. They both have high binding affinity to aP2.

The first compound was prepared according to the methods in Scheme 1. The second compound was prepared as seen in Scheme 2.

**Chemistry:**

In Scheme 1, benzoin (3) was reacted with 2-bromo-benzoic acid under standard conditions to give benzoin 2-bromo-benzoate. The ester was reacted without purification and immediately treated with ammonium acetate in acetic acid to generate oxazole 4 (45% overall). Compound 4 smoothly underwent palladium catalyzed cross coupling with 3-methoxy phenyl boronic acid in 75% isolated yield. Deprotection of the methyl group was achieved with BBr\(_3\) to provide hydroxyl-biphenyl 6. For this conversion, maintaining the reaction temperature below 0 °C proved to be critical in the successful transformation of the ether to the alcohol. Warmer temperatures and prolonged reaction times generated multiple side products. Isolated yields of the alcohol were around 55%. The final two steps in generating the target inhibitor required coupling of the phenol with ethyl bromoacetate (DMF and K\(_2\)CO\(_3\)) followed by hydrolysis of the ester (KOH in alcohol 50%) to provide the target molecule (1). Isolation of the salt was accomplished with the agency of SP207 gel column chromatography.

**Scheme 1:**

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<th>Step</th>
<th>Reagents</th>
<th>Yields</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>(COCl)(_2), (\text{NH}_4)OAc, HOAc, (\Delta)</td>
<td>61%</td>
</tr>
<tr>
<td>b</td>
<td>methoxyphenyl boronic acid, Pd(PPh(_3))(_4), Na(_2)CO(_3)</td>
<td>85%</td>
</tr>
<tr>
<td>c</td>
<td>BBr(_3), -5 °C</td>
<td>82%</td>
</tr>
<tr>
<td>d</td>
<td>ethyl bromoacetate, K(_2)CO(_3)</td>
<td>70%</td>
</tr>
<tr>
<td>e</td>
<td>NaOH</td>
<td>50%</td>
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**Scheme 2:**

<table>
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<th>Step</th>
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</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>NH(_4)OAc, HOAc, 2-bromo-benzaldehyde</td>
<td>83%</td>
</tr>
<tr>
<td>b</td>
<td>ethyl iodide, K(_2)CO(_3)</td>
<td>82%</td>
</tr>
<tr>
<td>c</td>
<td>methoxyphenyl boronic acid, Pd(PPh(_3))(_4), Na(_2)CO(_3)</td>
<td>61%</td>
</tr>
<tr>
<td>d</td>
<td>BBr(_3), -5 °C</td>
<td>55%</td>
</tr>
<tr>
<td>e</td>
<td>ethyl bromoacetate, K(_2)CO(_3)</td>
<td>50%</td>
</tr>
<tr>
<td>f</td>
<td>NaOH</td>
<td>50%</td>
</tr>
</tbody>
</table>

**Materials and Methods**

Reagents were purchased from Aldrich Chemical (P.O. Box 2060 Milwaukee, WI 53201) and used without further purification. All column chromatography was carried out using ACROS silica gel 0.20 -0.50 mm, pore diameter – 4 nm. TLC was carried out on Merk Silica Gel Plates with a UV binder. Typical visualization was accomplished by UV, I\(_2\) or KMnO\(_4\) staining. \(^1\)H NMR was carried out on an Anasazi EFT-60 MHz Spectrometer referenced to TMS. HPLC was carried out using Waters 1525 HPLC. Column: Waters X-Terra Phenyl: pore 5um, length 4.6X150mm. UV detection at 220 and 245 nm. A linear gradient from 100% solvent A to 100% solvent B was run from time 0 min. to time 16 min. Solvent A: (water:methanol:acetic acid; 9/1/.025), Solvent B (water:methanol:acetic acid; 1/9/.025). SP207 gel was purchased from Sorbent Technologies (2377 John Glen Dr. Atlanta, GA 30341) and used after washing with solutions of NaOH, HCl, water, methanol, and acetone. The resin was stored over a solution of very dilute NaHCO\(_3\):methanol solution (95:5 v/v).

**Preparation of 2-(2-bromophenyl)-4,5-diphenyl-1,3-oxazole (2):**

A solution of 2-bromobenzoic acid (5.00 g, 24.77 mmol) and dichloromethane (50 mL) was treated with oxalyl phosphine under Suzuki conditions generating biphenyl 10. The yields of the reaction tended to vary but averaged around 70%. The methyl ether in 10 was deprotected with boron tribromide with reaction temperatures below CO to liberate phenol 11 (yields 35%-50%). The target molecule 2 was prepared by K\(_2\)CO\(_3\)/DMF and ethyl bromoacetate coupling followed by base hydrolysis. Overall conversions were around 35% for the last two procedures.
chloride (3.81 g, 30.00 mmol) and dimethyl formamide (5 drops). The reaction mixture was stirred until all gas evolution has stopped (0.5 h). The mixture was stripped on a rotovap and the remaining acid chloride dissolved with dichloromethane (100 mL). The slight yellow solution was mixed with benzoin (4.64 g, 22.00 mmol) and triethylamine (7.50, 75.00 mmol). The mixture was stirred overnight and diluted with water. The layers were separated and the dichloromethane fraction washed with Na₂CO₃ solution and then KHSO₄. The organics were dried over MgSO₄ and concentrated to give the ketoester as a colorless oil.

The crude ester (3.60 g, @ 9.4 mmol) from above was mixed with ammonium acetate (3.60 g, 47 mmol) and acetic acid (35 mL). The mixture was brought to reflux for 4 h and cooled to ambient temperature. The organics were diluted with ethyl acetate and water. The layers equilibrated and separated. The organic fraction was washed with water, NaHCO₃, brine, dried (MgSO₄) and concentrated to give yellow oil. The oil was recrystallized from a small volume of hot methanol to give the desired oxazole (2.20 g, 61%). TLC (1:3 ethyl acetate/hexane) Rf = 0.4; ¹H NMR (CDCl₃) δ 8.20 -6.90 (m, 14 H) ppm.

Preparation of 2-(2-(3-methoxyphenyl)-phenyl)-4,5-diphenyl-1,3-oxazole (4).

Nitrogen was bubbled through a solution of 3 (0.29 g, 0.78 mmol), 3-methoxy-phenyl boronic acid (0.15 g, 0.98 mmol) and aqueous Na₂CO₃ (0.7 mL, 2M) in toluene (3 mL) and ethanol (0.85 mL) for about 10 min. Tetrakis (triphenylphosphine) palladium (0) (50 mg) was added and the reaction was heated in an oil bath set at 77 °C for 14 h. The mixture was cooled and diluted with ethyl acetate (25 mL) and the organics were washed with water, NaHCO₃, dried and concentrated. The remainder was purified on silica gel chromatography with 1:5 ethyl acetate in hexanes as a mobile phase. The procedure provided 0.27 g (85%) of the title compound. TLC (1:3 ethyl acetate/hexane) Rf = 0.7; ¹H NMR (CDCl₃) δ 8.20 -6.90 (m, 18 H), 3.50 (s, 3H) ppm.

2'-((4,5-diphenyl-1,3-oxazol-2-yl)biphenyl-3-yl]oxy]acetate (6).

A 25 mL RB flask was charged with DMF (2.5 mL), K₂CO₃ (0.055 g, 0.40 mmol), ethyl bromoacetate (0.038 g, 0.02 mmol) and compound 5 (0.080 g; 0.20 mmol). The biphasic mixture was stirred overnight and diluted sequentially with ethyl acetate 15 mL and water 15 mL. The layers were separated and the organic fraction was washed with water, dried over Na₂SO₄ and concentrated. The remainder was purified by silica gel column chromatography with ethyl acetate and hexanes as a mobile phase. A gradient elution from hexane to 25% ethyl acetate in hexane provided the title compound. (0.80 g, 50%-85%). TLC (1:3 ethyl acetate/hexane) Rf = 0.5; ¹H NMR (CDCl₃) δ 7.90 -6.80 (m, 18 H), 4.60 (s, 2H), 4.20 (q, 2H, J=7.5 Hz), 1.60 (t, 3H, J=7.5 Hz) ppm.
reaction was heated in an oil bath set at 78°C for 14 h. The mixture was cooled and diluted with ethyl acetate (25 mL) and the organics were washed with water, NaHCO₃, dried and concentrated. The remainder was purified on silica gel column chromatography with 1:5 ethyl acetate in hexanes as a mobile phase. The procedure provided (0.23 g, 61%) of the title compound. TLC (1:3 ethyl acetate/hexane) Rf = 0.8; ¹H NMR (CDCl₃) δ 7.80-6.90 (m, 18 H), 3.60 (s, 2H), 4.60 (s, 2H), 3.20 (q, 2H, J=8.5 Hz), 0.60 (t, 3H, J=8.5 Hz) ppm.

1-ethyl-2-(3’-hydroxybiphenyl-2-yl)-4,5-diphenyl-1H-imidazole (11).

To a solution of 10 (0.25 g, 0.62 mmol) in anhydrous dichloromethane at -10°C was added boron trioxide in dichloromethane drop wise (2M, 0.65 mL). The reaction was stirred at -10°C for 90 min. and quenched with methanol (2 mL). The mixture was stirred for 1 h at RT. and the pH adjusted to 7 with dilute NaHCO₃ solution. The mixture was diluted with ethyl acetate and the organics separated from the aqueous fraction. The organics were washed with brine, dried over MgSO₄ and concentrated. The remainder was purified by silica gel column chromatography with dichloromethane and then 10% ethyl acetate/dichloromethane to give the title compound (0.13 g, 50%). Rf = 0.3; ¹H NMR (CDCl₃) δ 7.80-6.90 (m, 18 H), 3.30 (q, 2H, J=9 Hz), 1.70 (t, 3H, J=7 Hz), 0.60 (t, 3H, J=8.5 Hz) ppm.

ethy1 [[1-ethyl-2-4,5-diphenyl-1H-imidazole][biphenyl-3-yl]oxy]aceta te (12).

A 25 mL RB flask was charged with DMF (2.5 mL), K₂CO₃ (0.055 g, 0.04 mmol), ethyl bromoacetate (0.038 g, 0.02 mmol) and compound 11 (0.10 g; 0.22 mmol). The biphasic mixture was stirred overnight and was diluted sequentially with ethyl acetate 15 mL and water 15 mL. The layers were separated and the organic fraction was washed with water, dried over Na₂SO₄ and concentrated. The remainder was purified by silica gel column chromatography with ethyl acetate and hexanes as a mobile phase. A gradient elution from hexane to 25% ethyl acetate in hexane provided the title compound (0.80 g, 70%). Rf = 0.6; ¹H NMR (CDCl₃) δ 7.90-6.90 (m, 18 H), 4.60 (s, 2H), 4.20 (q, 2H, J=7 Hz) 3.30 (q, 2H, J=9 Hz), 1.70 (t, 3H, J=7 Hz), 0.60 (t, 3H, J=9 Hz) ppm.

[[1-ethyl-2-4,5-diphenyl-1H-imidazole][biphenyl-3-yl]oxy]acetic acid, sodium form (2) Inhibitor 2

A 25 mL RB flask was charged with ethanol (7 mL), NaOH (0.4 g, 10 mmol), compound 12 (0.10 g, 0.16 mmol) and 1 mL of water. The mixture was heated to reflux for 1 h and cooled to room temperature. The mixture was concentrated and purified on SP207 gel using a step gradient with 100 mL volumes starting with water; 10% methanol in water; 20% methanol in water; 30% methanol in water; 50% methanol in water; methanol in water; 90% methanol in water; methanol to give 0.09 g of final product. Rf = 0.05; HPLC: Retention Time: 15.0 min. ¹H NMR (CD₃OD) δ 7.90-6.90 (m, 18 H), 4.60 (s, 2H), 3.30 (q, 2H, J=9 Hz), 0.60 (t, 3H, J=9 Hz) ppm.

Results and Conclusion:

Herein we have described the preparation of two inhibitors of aP2. The methods disclosed are suitable to generate milligram to gram quantities of these inhibitors. All of the reactions are robust and give fair to excellent yields of products. A key component to the isolation of the final salts is the utilization of SP-207 resin. This resin provides a convenient system for preparative reverse phase isolation of organic salts. Herein the isolation of these fatty acid salts was demonstrated.

The synthesis of these potent inhibitors has been accomplished and it is intended that further utilization of these unique compounds will enhance our understanding of diabetes and new treatments for the disease. This rationale is consistent with the information reported by Hotamisligil utilizing the ob/ob mouse model. These animals suffer from severe obesity and very high glucose levels. In this animal model, the aP2 inhibitors not only reduced glucose levels but also demonstrated increased sensitivity to insulin. The Harvard results suggest that new diabetic treatments could be forthcoming by interference of the fatty acid trafficking pathway.

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References

1. Data taken from the American Diabetes Association Web Site 2006.


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