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Synthetic Studies of *cis*-4-Amino-L-proline Derivatives as Novel Lipid Lowering Agents☆

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Abstract—*cis*-4-Amino-L-proline derivatives **1** and **2** derived from quinazolinone and pyrazolo pyrimidone respectively were designed as novel lipid lowering agents. A preliminary in vivo screening indicates that **1b** and **2a** have moderate triglyceride lowering activity.

Coronary Heart Disease (CHD) is the major cause of mortality in the industrialized world.[1,2] Elevated low density lipoprotein (LDL) cholesterol has been identified as a key risk factor for CHD, and statin therapy for hypercholesterolemia has been shown to lower the risk for CHD by approximately one-third.[3] However, statin can hardly normalize the high density lipoprotein abnormality. It remains unclear whether more aggressive LDL-lowering therapy, or impact on related lipid risk factor such as low 'high density lipoprotein' (HDL) should be a target for further reduction in CHD risk. Currently, there has been an increased focus on the treatment of concomitant hypertriglyceridemia, and statins generally have a modest impact on lowering LDL level.

Earlier, we have reported[4] an α-alkoxy propionic acid derivative that has blood glucose and triglyceride lowering activity in experimental animal models. There are other reports[5] of few β-aryl α-hydroxy propionic acids and their analogues to be useful in the treatment of hyperglycemia and hyperlipidemia. Considering hyperlipidemia alone, there are only few statins and some fibrate class of molecules available in the market. So, there is a constant need of different class of potent compounds showing LDL lowering activity with HDL level elevation in the blood plasma. As an ongoing effort to study the specific triglyceride lowering effect, we searched for a new scaffold, *cis*-4-amino-L-proline (CAP), as a novel ligand. Our design is based on the derivatisation of CAP, which is amenable to three-way functionalisation in an orthogonal way. Thus, as depicted in the cartoon (Fig. 1) we envisioned a novel structure in which a heterocycle can be joined to the amino group via a linker and carboxyl group of CAP residue can be kept as such or manipulated to suitable hydrogen donor and/or acceptor. On the other hand the ring nitrogen of CAP can be hooked to lipophilic groups to strike a balance between lipophilicity and hydrophilicity. In this communication, we wish to report our preliminary findings along with the synthesis of potential lipid lowering compounds based on *cis*-4-amino-L-proline (CAP) template (Table 1).

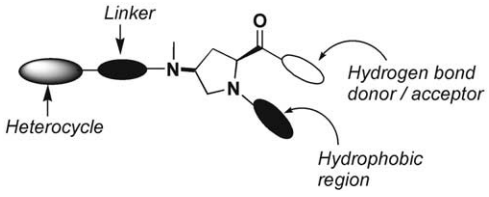

Figure 1. Design of 4-aminoproline derived template as novel lipid lowering agents.

We have identified two different heterocycles, quinazolinone **7** and pyrazolo pyrimidone **8**, which were joined

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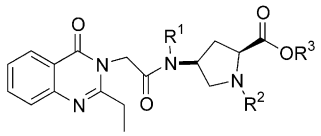
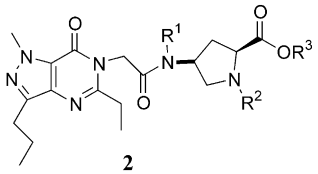
Table 1. List of compounds



1



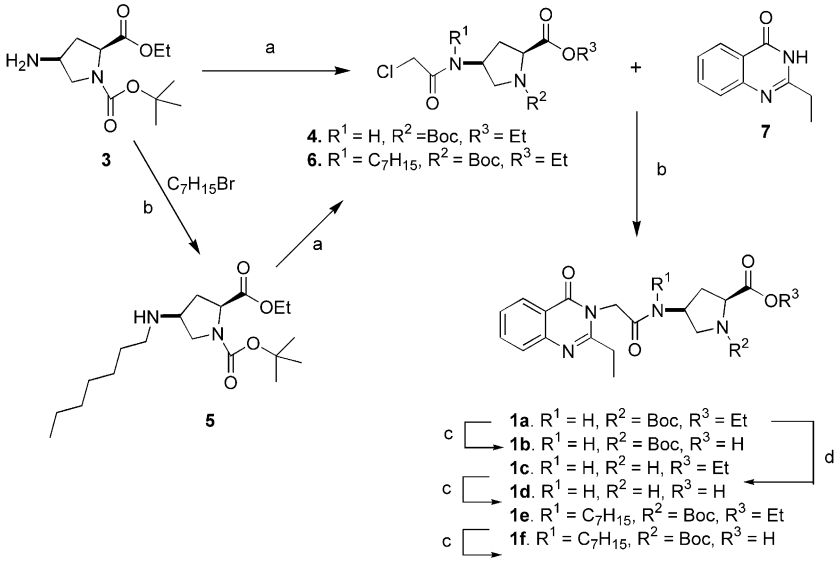
2

	R^1	R^2	R^3		R^1	R^2	R^3
1a	H	Boc	Et	**2a**	H	Boc	Et
1b	H	Boc	H	**2b**	H	Boc	H
1c	H	H	Et	**2c**	H	H	Et
1d	H	H	H	**2d**	C_7H_{15}	Boc	Et
1e	C_7H_{15}	Boc	Et	**2e**	C_7H_{15}	H	Et
1f	C_7H_{15}	Boc	H	**2f**	C_7H_{15}	Boc	H

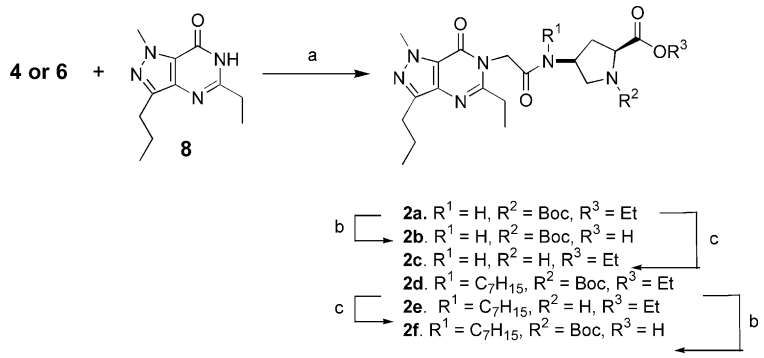
with the amino group of *cis*-4-amino-L-proline through an amide (carboxamidomethyl) linker. We have thus synthesized two series (**1** and **2**) of compounds, which are shown in Schemes 1 and 2, respectively. Thus (4*S*)-1-(*tert*-butoxycarbonyl)-4-amino-L-proline ethyl ester **3**[6] was treated with chloroacetyl chloride in the presence of triethyl amine to obtain the compound **4** in 89% yield. The alkylation of compound **3** with 1-bromoheptane using potassium carbonate followed by chloroacetyl chloride treatment of the product **5** gave compound **6** (51%, over two steps). Subsequently, 2-ethyl-3,4-dihydro-4-quinazolinone (**7**)[7] was allowed to condense with compound **4** using potassium carbonate to afford **1a** (96%), which was hydrolyzed (Na_2CO_3–MeOH) to yield **1b** (62%). Trifluoro acetic acid treatment of **1a** afforded **1c** (83%), which on subsequent hydrolysis gave **1d** (60%). On the other hand, **1e** was obtained in 98% yield when compound **6** was allowed to condense with quinazolinone **7** using potassium carbonate. Subsequent hydrolysis of **1e** (Na_2CO_3–MeOH) yielded **1f** (43%) (Scheme 1).

In order to synthesize the *cis*-4-amino-L-proline derivatives, based on pyrazolo pyrimidone (**8**),[8] the latter was condensed with compound **4** using potassium carbonate to afford **2a**[9] (91%) which upon hydrolysis (Na_2CO_3–MeOH) yielded **2b** (43%). The removal of Boc group of **2a** was conducted in dichloromethane using trifluoro acetic acid to provide **2c** in 73% yield. Similarly **2d** was obtained in 68% yield when compound **6** was allowed to condense with pyrazolo pyrimidone **8** using potassium carbonate. Subsequently **2d** was converted to **2e** on unmasking of Boc and to **2f** (79%) on hydrolysis using Na_2CO_3 in methanol (Scheme 2).

A preliminary in vivo screening[10] of **1a–f** and **2a–f** were performed for their triglyceride lowering activity. Results indicate that **1b** brought about 30% and **2a** brought about 40% reduction of plasma triglyceride when administered at 10 mg/kg/day dose for 6 days. The standard compound fenofibrate showed 36% triglyceride reduction in the same model when administered at 30 mg/kg/day dose for 6 days whereas there was no significant reduction at 10 mg/kg/day dose.



Scheme 1. Reagents and conditions: (a) CH_2Cl_2, Et_3N, $ClCH_2COCl$, rt, 48 h; (b) DMF, K_2CO_3, rt, 48 h; (c) MeOH–H_2O, Na_2CO_3, rt, 12–18 h; (d) CH_2Cl_2, TFA, rt, 12 h.



Scheme 2. Reagents and conditions: (a) DMF, K_2CO_3, rt, 48 h; (b) MeOH–H_2O, Na_2CO_3, rt, 12–18 h; (c) CH_2Cl_2, TFA, rt, 12 h.

Conclusion

We have designed and synthesized novel *cis*-4-amino-L-proline (CAP) based triglycerides lowering agents represented by general structures **1** and **2**. These compounds do not significantly activate PPAR (PPAR-α <0.5-fold activation with respect to 4.2-fold activity of WY14643;[12] and PPAR-γ <2.0-fold activation with respect to 11.5-fold activity of Rosiglitazone[13]) as indicated by in vitro transactivation studies. In view of preliminary indication obtained from our studies where two compounds (**1b** and **2a**) show moderate activity towards triglyceride lowering, it is hoped that little modification could lead to potent triglyceride lowering agents from this lead without significant PPAR activity. Further studies are in progress and will be published elsewhere.

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8. The detailed synthesis will be communicated soon.
9. All are isolated yields for our compounds, where compounds were fully characterized by ^{1}H NMR, Mass and IR spectra. A typical spectral data for **2a**: mp 130–134 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.99 (t, J=7.3 Hz, 3H), 1.25 (t, J=7.3 Hz, 3H), 1.34–1.56 (m, 12H), 1.60–1.98 (m, 6H), 2.30–2.45 (m, 1H), 2.73 (q, J=7.3 Hz, 2H), 2.84 (t, J=7.6 Hz, 2H), 3.45–3.62 (m, 1H), 4.05 (q, J=7.1 Hz, 2H), 4.21 (s, 3H), 4.72 (s, 2H). IR (KBr) cm^{-1}: 3319, 2977, 2875, 1744, 1695 (broad). Mass (CI) m/z: 519 (M^+ + 1, 100%).
10. An inbreed colony (at our own animal house) of Swiss Albino Mice (SAM) of 21–29 g body weight, moderately hypertriglycerimic, has been used for screening the compounds. Animals were treated orally with 10 mg/kg/day of **1a–f** and **2a–f** for 6 days. The control animals were treated with the vehicle (0.25% carboxymethyl-cellulose, 10 mL/kg) only. Animals were bled through retro orbital sinus on day-1 and day-6 of the experiment. Plasma samples were prepared and triglyceride levels were measured by using a commercial kit (Linco Research Lab., USA). For calculations of percentage reduction of tryglycerides, the standard method[11] was applied.
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