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Thiourea-Based Gemfibrozil Analogues as HDL-Elevating Agents

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Abstract—A series of gemfibrozil analogues with a thiourea moiety embedded in the side chain was prepared and evaluated as HDL-elevating agents. Derivatives **8b**, **9b**, **9c**, and **9d** were found to be approximately as effective as gemfibrozil (**1**) for HDL cholesterol elevation.

Cardiovascular disease remains the leading cause of death in the United States and many other industrialized countries.[1] Atherosclerosis, a progressive disease involving metabolic abnormalities in both lipoprotein and artery wall metabolism, underlies much of the total cardiovascular disease burden.[2] Major risk factors for atherosclerotic cardiovascular disease are well-known and include such dyslipidemias as elevated low-density lipoprotein (LDL) cholesterol, low levels of high-density lipoprotein (HDL) cholesterol, and high levels of triglycerides. The Scandinavian Simvastatin Survival Study[3] and the CARE trial[4] clearly established that lowering LDL cholesterol is associated with a reduction in total and cardiovascular mortality and nonfatal cardiac events.

Extensive epidemiological evidence supports the concept that HDL is a protective factor against atherosclerosis and that a low plasma HDL level is associated with a high risk of disease.[5] Several recent studies suggest the potential importance of pharmacological intervention to raise HDL levels. A proportional hazards model analysis of 12-year death rates of Framingham men indicated that a 10 mg/dL increase in HDL cholesterol was associated with a 19% decrease in coronary artery disease death and a 12% decrease in all-cause mortality.[6] The Helsinki heart study,[7] a primary prevention trial in dyslipidemic men, demonstrated a reduced incidence of cardiovascular events in response to treatment with gemfibrozil (**1**), a member of the fibrate class of drugs. The beneficial effect was associated with a drug-related increase in HDL levels. Additionally, the Veterans Administration HDL Intervention Trial (VA-HIT) demonstrated that gemfibrozil therapy in men with coronary heart disease and low HDL cholesterol levels raised HDL modestly and reduced coronary events by 22%, despite not lowering LDL cholesterol levels.[8] Triglyceride levels were also lowered in this study.

In contrast to the well-established pathways for LDL production and LDL receptor-mediated clearance, the molecular mechanisms involved in overall HDL metabolism have not been completely elucidated, and pharmacological approaches aimed at selectively modifying HDL levels in patients are not currently available. In response to this clear medical need, we embarked on a program to identify agents that would elevate HDL cholesterol.

A systematic analysis of our in-house chemical database resulted in the identification of thiourea **2** as a compound that was found to raise serum levels of HDL cholesterol and apolipoprotein A-I (Apo A-I) in rats fed a cholic acid and cholesterol-supplemented diet.[9]

Upon investigation of structures **1** and **2** one can envision certain similarities between the two. Two such similarities are the substitution pattern of the aromatic ring and the protonated oxygen atom at the terminus of both chains. We reasoned that combining the features common to both gemfibrozil and **2** into a new molecule

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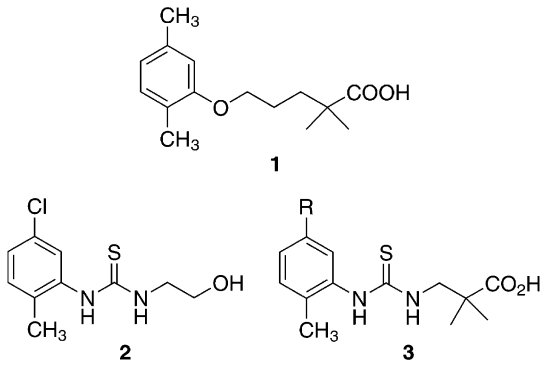
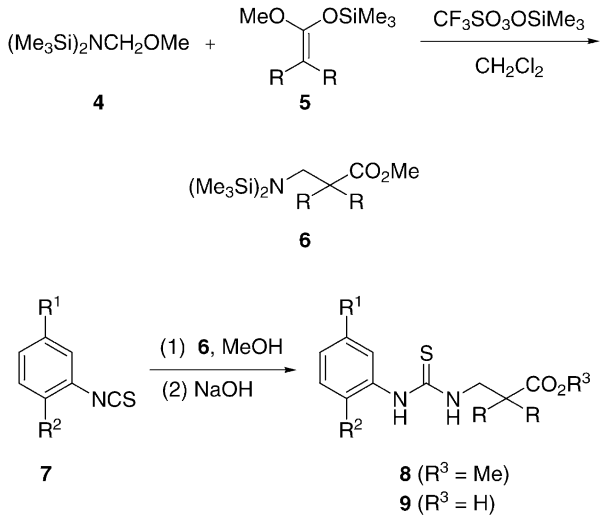


would produce a 'hybrid' structure **3**. Incorporation of the thiourea function into the chain portion of the molecule would also partially rigidify it thereby reducing the number of possible conformations the side chain can adopt. Since thioureas are known to raise HDL cholesterol[10,11] the inclusion of this function into **1** should be compatible.

This paper describes the synthesis of a series of analogues based on structure **3** and reports on the biological activity of these derivatives as HDL-elevating agents.



Scheme 1. General preparation of arylthiourea carboxylic acid derivatives.

Retrosynthetic analysis of generalized target structure **8** revealed that the skeletal framework of the molecule can be assembled convergently at the thiourea functionality. This can readily be accomplished by reacting an appropriately substituted aryl isothiocyanate **7** with an α-substituted-β-amino acid derivative. The flexibility of this synthesis would also allow the preparation of the corresponding urea derivatives by simply using an isocyanate in place of the isothiocyanate (Scheme 1).

The required 2,5-disubstituted phenyl isothiocyanates were commercially available. However, the β-amino acid reactants needed to be synthesized. These were readily prepared by amino methylation of ketene silyl acetals 5 with *N*,*N*-bis(trimethylsilyl)methoxymethylamine (**4**)[12] catalyzed with trimethylsilyltrifluoromethanesulfonate.[13] The resulting *N*,*N*-bis(trimethylsilyl)-β-amino acid esters **6** were allowed to react directly with the aryl isothiocyanate (or isocyanate) **7** in methanol at room temperature. Under these conditions, desilylation of **6** occurs slowly and the resulting free amine reacts with **7** to provide thiourea **8** (or urea) in good yields (Table 1). Hydrolysis of the ester with 1 N sodium hydroxide gives the desired acid **9**.

The primary amide **13** was prepared by an alternate route (Scheme 2). Reaction of the thiourea **10** with 3-chloropivaloyl chloride (**11**) under phase transfer conditions[14] produces the β-lactam **12** in 45% yield. Ring opening of the β-lactam heterocycle with lithium amide affords the desired amide 13 in 35% yield along with 24% of the corresponding acid **9b**.

In an effort to further conformationally restrict the chain in compound **9**, we designed a molecule where one of the methyl groups adjacent to the carboxylate is tethered to the closest thiourea nitrogen atom. The resulting cyclic analogue **19** would have the carboxylic acid group in a fixed location while still retaining the α-dialkylated motif.

The synthesis of **19** was accomplished in a convergent manner (Scheme 3). The requisite α-methyl nipecotate **17** was prepared from commercially available ethyl nipecotate (**14**) by protecting the nitrogen with a Boc group (98% yield), generating the enolate of ester **15** with LDA in THF at −78 °C, alkylation of the enolate with 1.1 equiv of methyl iodide (90% yield), then deprotection

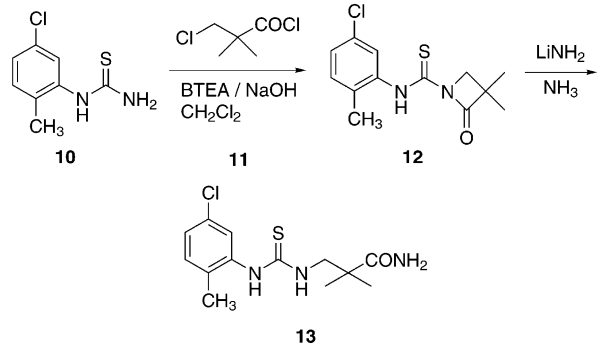
Table 1. Physical data for esters **8** and acids **9**

Compd	R^1	R^2	R	X	**8** Yield (%)	**8** mp (°C)[b]	**9** Yield (%)	**9** mp (°C)[b]
a	Me	Me	Me	S	66	80–83	75	174–176
b	Cl	Me	Me	S	82	95–98	85	164–167
c	Cl	Me	$-(CH_2)_3-$	S	62[a]	74–77	89	156–159
d	Cl	Me	$-(CH_2)_4-$	S	44	108–111	90	151–154
e	Cl	Me	$-(CH_2)_5-$	S	70	150–152	94	164–167
f	Cl	OMe	Me	S	95	81–84	86	138–141
g	Cl	Me	Me	O	51	138–140	79	190–191

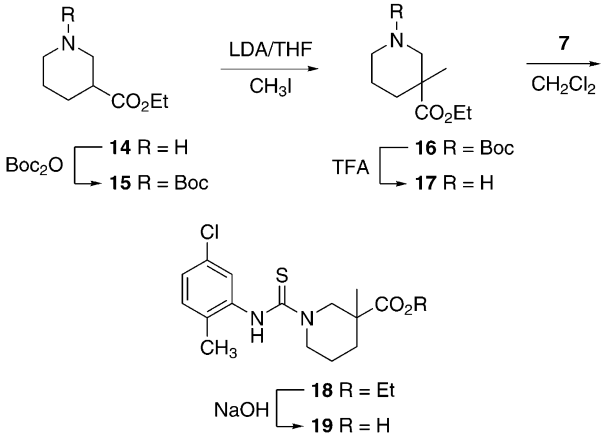
[a]Ethyl ester.
[b]Satisfactory elemental analysis obtained for all compounds.

of **16** with trifluoroacetic acid (85% yield). Reaction of **17** with **7** in methylene chloride furnishes **18** in 77% yield. Hydrolysis of the ester with 1 N sodium hydroxide in ethanol gives the desired product **19** in 51% yield.

Male Sprague–Dawley rats, weighing 200–225 g, were housed two per cage and fed Purina rodent chow (special mix 5001-S) which was supplemented with 0.25% cholic acid and 0.75% cholesterol, and water ad libitum



Scheme 2. Preparation of carboxamide derivative.



Scheme 3. Preparation of conformationally restrained derivative.

for 7 days. The compounds were then administered in the diet to groups of six rats for 8 days. At termination the rats were anesthetized with carbon dioxide and sacrificed by exsanguination from the corotid artery. The blood was centrifuged and the serum separated.

The lipoproteins were separated by classical ultracentrifugation techniques.[9,15] Serum was adjusted to a density of 1.060 g/mL with sodium chloride solution and 175 μL of adjusted sera were centrifuged at 42,000 rpm in a Beckman Ti42.2 rotor at 20 °C for 2.5 h. Lipid and apoprotein analyses were performed on fractions removed from the top of each tube and on the fraction remaining on the bottom. Cholesterol was quantitated by enzymatic colorimetric methods. Serum Apo A-I was determined by SDS polyacrylamide gel electrophoresis[16,17] or a rat competition ELISA.[18]

Within the series of analogues in Table 2, **9a**, which has the same aromatic substitution pattern as gemfibrozil, raised HDL only about half that of **1**, retained Apo A-I activity, but significantly raised triglycerides. Compound **9b** was nearly as potent as **1** with regard to HDL, raised Apo A-I almost 1.5 times that of **1**, and modestly lowered triglycerides. Ester **8b** exhibited roughly the same profile as the acid **9b**, which suggests that the ester is metabolized to the active analogue. Amide derivative **13** led to reduced HDL elevation levels while retaining Apo A-I levels, whereas cyclobutyl and cyclopentyl analogues **9c** and **9d** retained HDL levels while reducing Apo A-I levels. Conformationally restrained derivative **19**, where one methyl is tethered to the thiourea nitrogen, was essentially inactive. The importance of sulfur is illustrated by the lack of activity of the urea analogue **9g** in comparison to the corresponding thiourea **9b**. Of the most active compounds, **8b** and **9b** showed no elevation of total cholesterol levels.

The liver enzymes ALT, AST, and ALP were measured for all thiourea and urea analogues. Both AST and ALP remained at normal levels relative to untreated controls. ALT levels were elevated 60–127% for all compounds except **2**, **9a**, **9b**, and **9g** which remained normal. None

Table 2. In vivo biological results

Compd	Relative to gemfibrozil[a]		Total Cholesterol (% change)	Triglycerides (% change)	Dose (mg/kg/day)
	HDL	Apo A-I			
1	1	1	−23	−43[b]	46[b]
2	0.61[c]	1.06[c]	40	−56[c]	52[c]
8b	0.93	1.81	−12 (NS)	−12 (NS)	74
9a	0.54	0.93	73	69	78
9b	0.85[b]	1.44[b]	15(NS)	−19[b] (NS)	75[b]
9c	1.19	0.49	36	−39 (NS)	76
9d	1.09	0.55	41	−61	67
9e	0.73	0.32	21	−54	88
9f	0.62	0.27 (NS)	9 (NS)	−28 (NS)	77
9g	0.36	0.32 (NS)	74	−21 (NS)	73
13	0.57	1.15	−3 (NS)	−49	41
19	0.33	−0.16 (NS)	14 (NS)	15 (NS)	81

[a]Gemfibrozil = 1 (typical results for gemfibrozil: HDL cholesterol ↑113%; apo A-I ↑49%; triglycerides ↓43% relative to an untreated control group at about 46 mg/kg/day which is the maximally effective dose for this compound in this model).
[b]Mean value of three studies.
[c]Mean value of 26 studies. Values are significantly different ($P < 0.05$) relative to untreated controls unless otherwise indicated (NS).

of the gemfibrozil analogues had any statistically significant effect on body weight gain.

In conclusion, we have shown that a thiourea moiety can be incorporated into the gemfibrozil skeleton to produce derivatives which still exhibit varying degrees of HDL-elevating activity. Compounds **8b**, **9b**, **9c**, and **9d** were as potent as **1** while **8b** and **9b** raised Apo A-1 levels significantly higher than **1**.

References and Notes

1. Lacko, A. G. *Trends Cardiovascular Med.* **1994**, *4*, 84.
2. Gordon, T.; Castelli, W. P.; Hjortland, M. C.; Kannel, W. B.; Dawber, T. R. *Am. J. Med.* **1977**, *62*, 704.
3. Scandinavian Simvastatin Survival Study Group. *Lancet* **1994**, *344*, 1383.
4. Sacks, F. M.; Moye, L. A.; Davis, B. R.; Cole, T. G.; Roleau, J. L.; Nash, D.T; Pfeffer, M. A.; Braunwald, E. *Circulation* **1998**, *97*, 1446.
5. Gordon, D. J.; Rifkind, B. M. *N. Engl. J. Med.* **1989**, *321*, 1311.
6. Gordon, T.; Castelli, W. P.; Hjortland, M. C.; Kannel, W. B.; Dawber, T. R. *Am. J. Med.* **1977**, *62*, 704.
7. Frick, M. H.; Elo, O.; Kaapa, K.; Heinonen, O. P.; Heinsalmi, P.; Helo, P.; Huttunen, J. K.; Kaitaniemi, P.; Koskinen, P.; Manninen, V.; Maenpaa, H.; Malkonen, M.; Manttari, M.; Norola, S.; Pasternack, A.; Pikkarainen, J.; Romo, M.; Sjoblom, T.; Nikkila, E. A. *N. Engl. J. Med.* **1987**, *317*, 1237.
8. Rubins, H. B.; Robins, S. J.; Collins, D.; Fye, C. L.; Anderson, J. W.; Elam, M. B.; Faas, F. H.; Linares, E.; Schaefer, E. J.; Schectman, G.; Wilt, T. J.; Wittes, J. *N. Engl. J. Med.* **1999**, *341*, 410.
9. France, D. S.; Quinby, R. E.; Babiak, J.; Lapen, D. C.; Paterniti, J. R.; Weinstein, D. B. *Lab. Rob. Autom.* **1990**, *2*, 155.
10. Coppola, G. M.; Damon, R. E. Canadian Patent Application CA 2072704 1993. *Chem. Abstr.* **1993**, *119*, 95134.
11. Failli, A. A.; Shumsky, J. S. Internat. Patent WO 9911621. *Chem. Abstr.* **1999**, *130*, 209605.
12. Morimoto, T.; Takahashi, T.; Sekiya, M. *J. Chem. Soc., Chem. Commun.* **1984**, 794.
13. Okano, K.; Morimoto, T.; Sekiya, M. J. *Chem. Soc., Chem. Commun.* **1984**, 883.
14. Okawara, T.; Nakayama, K.; Furukawa, M. *Chem. Lett.* **1982**, 1791.
15. Bronzert, T. J.; Brewer, Jr., H. B. *Clin. Chem.* **1977**, *23*, 2089.
16. Hughes, T. E.; Sasak, W. V.; Ordovas, J. M.; Forte, T. M.; Lamon-Fava, S.; Schaefer, E. J. *J. Biol. Chem.* **1987**, *262*, 3762.
17. France, D. S.; Hughes, T. E.; Miserendino, R.; Spirito, J. A.; Babiak, J.; Eskesen, J. B.; Tapparelli, C.; Paterniti, Jr., J. R. *J. Lipid Res.* **1989**, *30*, 1997.
18. Burkey, B.; France, D. S.; Wang, H.; Ma, X.; Brand, B.; Abuhani, C.; Diffederfer, M.; Marsh, J.; Paterniti, Jr., J. R.; Fisher, E. *J. Lipid Res.* **1995**, *36*, 1463.