Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 809–812
Bioorganic & Medicinal Chemistry Letters

1-Hydroxyalkyl-3-phenylthioureas as novel HDL-elevating agents

Gary M. Coppola,[a,*] Robert E. Damon,[a] J. Bruce Eskesen,[a] Dennis S. France[a] and James R. Paterniti, Jr.[b]

[a]*Department of Diabetes and Metabolism, Novartis Institutes for Biomedical Research, 100 Technology Square, Cambridge, MA 02139, USA*
[b]*Amylin Pharmaceuticals, 9373 Towne Centre Drive, San Diego, CA 92121, USA*

Received 4 May 2004; accepted 29 October 2004
Available online 10 December 2004

Abstract—A series of 1-hydroxyalkyl-3-phenylthiourea analogs were prepared and evaluated as HDL-and Apo A–I-elevating agents. Derivatives **5h**, **7j**, **7n**, and **7o** were found to be as effective or superior to gemfibrozil (**1**).

Cardiovascular disease is the leading cause of mortality in the United States and many other industrialized countries and atherosclerosis accounts for the majority of these deaths.[1] Approximately 1% of the population have an inherited lipid disorder and are predisposed to premature onset of this disease. It is well known that major risk factors for atherosclerotic cardiovascular disease include such dyslipidemias as elevated low-density lipoprotein (LDL) cholesterol, low levels of high-density lipoprotein (HDL) cholesterol, and high levels of triglycerides.

Atherosclerosis is a complex disease where the progressive accumulation of cholesterol within the arterial wall eventually results in occlusion of the coronary or cerebral arteries ultimately leading to myocardial infarction or stroke. Extensive epidemiological studies have shown a strong inverse correlation between serum HDL cholesterol levels and coronary heart disease.[2–4] The Framingham heart study showed that a 10 mg/dL increase in HDL cholesterol was associated with a 19% decrease in coronary artery disease (CAD) death and a 12% decrease in all-cause mortality.[5] The Helsinki heart study,[6] a primary prevention trial in dyslipidemic men, demonstrated a reduced incidence of cardiovascular events in response to treatment with the PPARα agonist gemfibrozil (**1**). This study showed a 1% increase in HDL cholesterol was associated with a 2–3% decrease in CHD events independent of changes in LDL cholesterol levels.[7] Additionally, the Veterans Administration HDL Intervention Trial demonstrated that gemfibrozil therapy in men with coronary heart disease and low HDL cholesterol levels raised HDL cholesterol modestly and reduced coronary events by 22%, despite not lowering LDL cholesterol levels.[8]

One of the possible mechanisms for the protective nature of HDL against the development of atherosclerosis is its ability to extract cholesterol from cells,[9] thus counteracting the effects of LDL cholesterol and subsequently preventing the formation of foam cells, the genesis of atherosclerotic lesions. In vitro studies have supported this postulate.[10,11] The cholesterol-laden HDL particle is then transported to the liver where the cholesterol is recycled or removed by excretion in bile.[4,11]

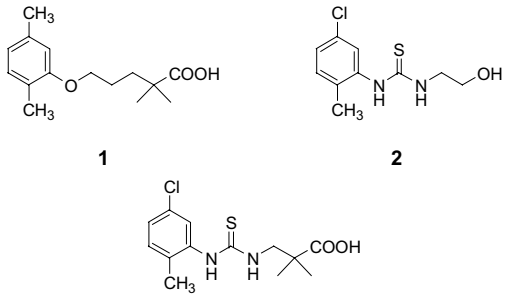
Further support that HDL has antiatherogenic properties was demonstrated in transgenic mice where over expression of the human apolipoprotein (Apo) A–I gene, the carrier protein for HDL cholesterol, resulted in elevated levels of HDL cholesterol and significant protection from the development of aortic fatty streak lesions.[12] This protection was also seen in spontaneously Atherosclerotic Apo E-deficient mice.[13,14]

Molecular mechanisms involved in overall HDL metabolism have not been completely elucidated. We therefore embarked on a program to identify agents that would elevate HDL cholesterol as well as Apo A–I plasma levels. Between 1967 and 1978 we (formerly Sandoz, Inc.) had a cholesterol-lowering program which used

* Corresponding author. Fax: +617 871 7043; e-mail: gary.oppola@pharma.novartis.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.10.094

rat as the in vivo model. At that time serum cholesterol was not fractionated and total cholesterol was measured. Since rats carry the majority of their cholesterol in the HDL fraction, we searched those compounds for the ones that raised cholesterol. We screened a subset of those compounds in rats fed with cholic acid and cholesterol-supplemented diet (a model of reduced HDL cholesterol and Apo A–I),[15] and identified thiourea **2** as a compound that raised serum levels of both HDL cholesterol and Apo A–I as well as reducing triglyceride levels.

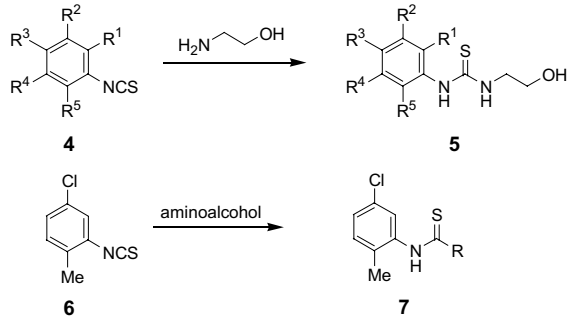


1 **2**

3

Reported in a previous publication, we combined the features of **1** and **2** to produce a gemfibrozil 'hybrid' **3** which raised HDL cholesterol and Apo A–I as effectively as **1**.[16] Since the thiourea group appears to be needed for biological activity[17,18] we undertook an investigation of the effects of aromatic substitution and the nature of the hydroxyalkyl chain of **2** on HDL elevation. The structure–activity relationship will be presented in this letter.

The aromatic substituted thioureas (**5**) were readily prepared by reaction of a phenyl isothiocyanate with ethanolamine in methylene chloride (Scheme 1). Conversely, chain variants **7** were synthesized by reaction of 5-chloro-2-methylphenyl isothiocyanate (**6**) with an appropriate amino alcohol. In many instances the products precipitated from the reaction mixture were isolated by simple filtration. All amino alcohols were commercially available with the exception of the chiral piperidine derivatives. Cbz-Pipecolic acid was resolved with L-Tyrosinehydrazide to give the (*R*)-enantiomer **8**.[19] Reduction of the acid with diborane followed by catalytic hydrogenolysis of the protecting group gave the

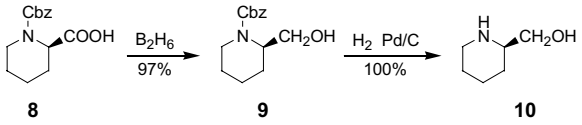


4 **5**

6 **7**

Scheme 1. General preparation of arylthiourea derivatives.

(*R*)-amino alcohol **10**. The opposite enantiomer (not shown) was prepared analogously by resolution with D-tyrosinehydrazide.



8 **9** **10**

Compounds were administered in diet (ad libitum) to groups of six male Sprague–Dawley rats for eight days as described in our previous publication.[16] The lipoproteins were separated by classical ultracentrifugation techniques and cholesterol was quantitated by enzymatic colorimetric methods. Serum Apo A–I was measured by SDS polyacrylamide gel electrophoresis[20] or a rat competition ELISA.[16] Within each study, gemfibrozil (**1**) was used as an internal positive control.

Initial modifications of structure **2** focused on the aromatic substituents (Table 1). Removal of the chlorine atom (**5a**) resulted in complete loss of activity whereas removal of the methyl group (**5b**) had no significant effect on HDL cholesterol or Apo A–I. Replacing the chlorine atom of **5b** with CF_3 (**5k**) also retained HDL activity, however, total cholesterol (TC) was raised slightly. Moving the chlorine around the ring produced varying results. The 6-chloro derivative **5d** did not raise HDL cholesterol and lowered Apo A–I whereas the corresponding 3- and 4-chloro analogues **5f** and **5e** showed moderately elevated HDL cholesterol levels. Replacing the 2-methyl group with either chlorine or methoxy (**5g** or **5h**) retained activity, however, TC levels in **5h** were raised 66%. Replacing the 5-chloro with methyl (**5j**) raised HDL cholesterol but not Apo A–I. All compounds in Table 1 exhibited reductions in body weight gain which were highly correlated to effects on food consumption ($R^2 = 0.89$, $p < 0.0001$; data not shown). It cannot be ruled out that a portion of the HDL effects in this series of compounds may be attributable to the diminished food consumption.

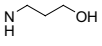
Another series of analogs with the same aromatic substitution pattern as **2** is shown in Table 2. Replacing the hydroxyl group with thiol (**7a**) results in loss of all activity. When the hydroxyl moiety is part of an acid functionality (**7c**) activity is also lost. Extension of the chain by one methylene (**7d**) retains HDL cholesterol elevating activity but not Apo A–I. A small series of C-methylated derivatives **7e–i** exhibited only modest increases in HDL cholesterol but none raise Apo A–I appreciably. The N-methyl analogue **7j**, however, retained HDL activity with no increase in TC. Conformationally restrained analogue **7k** loses all activity. The corresponding 6-membered ring analogue (not shown) had a profile identical to **7k**. Restraining the ring at the carbon adjacent to nitrogen (prolinol derivatives **7l** and **7m**) also resulted in complete loss of activity. Interestingly, the analogous piperidine derivatives **7n** and **7o** significantly increased HDL cholesterol by 134% and 156% over controls, respectively, however **7n** had no

Table 1. Lipid profiles of aromatic variants **5** in cholesterol-fed male rats

5[a]	R^1	R^2	R^3	R^4	R^5	Dose (mg/kg/day)	HDL (%)[b]	Apo A–I (%)[b]	Total chol. (%)[b]	TG (%)[b]	WG (%)[c]
a	H	H	H	H	Me	51	3 (NS)	−18	66	−51	−138
b	H	Cl	H	H	H	66	79	43	30 (NS)	−31	−70
c	H	Cl	Me	H	H	74	72	−5 (NS)	15 (NS)	−15 (NS)	−77
d	Cl	H	H	H	Me	40	23 (NS)	−34	8 (NS)	−48	−155
e	H	H	Cl	H	Me	42	53	18	17 (NS)	−8 (NS)	−229
f	H	H	H	Cl	Me	67	63	27	10 (NS)	−16 (NS)	−56
g	H	Cl	H	H	Cl	68	71	36	21	−13 (NS)	−66
h	H	Cl	H	H	OMe	80	106[e]	30[e]	66[e]	−27[e]	−38
i	H	Cl	H	Cl	H	60	50	48	9 (NS)	−35	−90
j	H	Me	H	H	Me	63	62	0 (NS)	32	−32	−117
k	H	CF_3	H	H	H	68	66[e]	36[e]	44[e]	−15[e]	−76[e]
2[f]	H	Cl	H	H	Me	55	75	48	39	−53	−104
1[d]						50	104	54	−23	−29	2

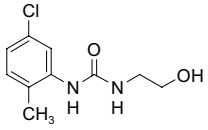
[a] Satisfactory elemental analysis obtained for all compounds.
[b] Relative to untreated controls. Values are significantly different ($p < 0.05$) relative to untreated controls unless otherwise indicated (NS).
[c] % Change in body weight gain relative to untreated controls.
[d] Mean value of 160 studies.
[e] Mean value of 3 studies.
[f] Mean value of 26 studies. TG = triglycerides. WG = weight gain.

Table 2. Lipid profile of chain variants **7** in cholesterol-fed male rats

7[a]	R	Dose (mg/kg/day)	HDL (%)[b]	Apo A–I (%)[b]	Total Chol. (%)[b]	TG (%)[b]	WG (%)[c]
a		36	25 (NS)	10 (NS)	8 (NS)	−3 (NS)	−20
b		36	30	33	20 (NS)	−47	−3 (NS)
c		38	8 (NS)	14 (NS)	24 (NS)	4 (NS)	10
d		74	82	24 (NS)	3 (NS)	−80	−63
e		75	13 (NS)	6 (NS)	85	−23	−4 (NS)
f		76	32	20	48	1 (NS)	10
g		80	58	23 (NS)	−13 (NS)	−15	−23
h		68	33	8 (NS)	30 (NS)	−75	−68
i		62	60	25 (NS)	−1 (NS)	−68	−69
j		75	100	40	29 (NS)	−47	−31
k		78	9 (NS)	4 (NS)	−5 (NS)	−24	2 (NS)
l		81	7 (NS)	25 (NS)	28	19	5 (NS)
m		83	19 (NS)	6 (NS)	43	30	29
n		74	134	15 (NS)	87	−36	−35
o		79	156	94	93	−67	−41
1[d]		50	104	54	−23	−29	2

[a] Satisfactory elemental analysis obtained for all compounds.
[b] Relative to untreated controls. Values are significantly different ($p < 0.05$) relative to untreated controls unless otherwise indicated (NS).
[c] % Change in body weight gain relative to untreated controls.
[d] Mean value of 160 studies. TG = triglycerides. WG = weight gain.

effect on Apo A–I whereas **7o** raised Apo A–I 94%, although both significantly raised TC, reduced triglycerides, and had modest effects on reduction of body weight gain. The sulfur atom of the thiourea in **2** is required for activity. The corresponding urea analogue of **2** (compound **11**) is totally inactive.



11

The liver enzymes ALT, AST, and ALP were measured for compounds **2**, **5h**, **7n**, and **7o**. The liver enzymes for **2** and **5h** remained at normal levels relative to untreated controls. Elevation of ALP (87%) was observed for **7n** whereas all three enzyme levels were elevated for **7o** (ALT, 142%; AST, 95%; ALP, 72%).

In conclusion, 1-hydroxyalkyl-3-phenylthioureas have been shown to be effective HDL and Apo A–I elevating agents as well as exhibiting TG-lowering properties. Several examples were superior to gemfibrozil. Aryl analogs **5** consistently showed reductions in body weight gain. Compound **7j** had a profile similar to **1** although it was superior at lowering TG. The most active compounds relative to controls were **7n** and **7o**. These compounds also raised TC levels, although much of that was in the HDL fraction.

References and notes

1. Lacko, A. G. *Trends Cardiovascular Med.* **1994**, *4*, 84.
2. Gordon, D. J.; Rifkind, B. M. *N. Engl. J. Med.* **1989**, *321*, 1311.
3. Gordon, D. J.; Probstfield, J. L.; Garrison, R. J.; Neaton, J. D.; Castelli, W. P.; Knoke, J. D.; Jacobs, D. R.; Bangdiwala, S.; Tyroler, H. A. *Circulation* **1989**, *79*, 8.
4. Barter, P. J.; Rye, K.-A. *Atherosclerosis* **1996**, *121*, 1.
5. Gordon, T.; Castelli, W. P.; Hjortland, M. C.; Kannel, W. B.; Dawber, T. R. *Am. J. Med.* **1977**, *62*, 704.
6. 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.
7. Manninen, V.; Elo, M. O.; Frick, M. H.; Haapa, K.; Heinonen, O. P.; Heinsalmi, P.; Helo, P.; Huttunen, J. K.; Kaitaniemi, P.; Koskinen, P.; Mäenpää, H.; Mälkönen, M.; Mänttäri, M.; Norola, S.; Pasternack, A.; Pikkarainen, J.; Romo, M.; Sjöblom, T.; Nikkilä, E. A. *J. Am. Med. Assoc.* **1988**, *260*, 641.
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. Picardo, M.; Massey, J. B.; Kuhn, D. E.; Gotto, A. M.; Gianturco, S. H.; Pownall, H. J. *Arteriosclerosis* **1986**, *6*, 434.
10. Miyazaki, A.; Rahim, A. T. M. A.; Ohta, T.; Morino, Y.; Horiuchi, S. *Biochim. Biophys. Acta* **1992**, *1126*, 73.
11. Fielding, C. J.; Fielding, P. E. *J. Lipid Res.* **1995**, *36*, 211.
12. Rubin, E. M.; Krauss, R. M.; Spangler, E. A.; Verstuyft, J. G.; Clift, S. M. *Nature* **1991**, *353*, 265.
13. Pászty, C.; Maeda, N.; Verstuyft, J.; Rubin, E. M. *J. Clin. Invest.* **1994**, *94*, 899.
14. Plump, A. S.; Scott, C. J.; Breslow, J. L. *Proc. Natl. Acad. Sci.* **1994**, *91*, 9607.
15. France, D. S.; Quinby, R. E.; Babiak, J.; Lapen, D. C.; Paterniti, J. R.; Weinstein, D. B. *Lab. Rob. Autom.* **1990**, *2*, 155.
16. Coppola, G. M.; Damon, R. E.; Eskesen, J. B.; France, D. S., Jr.; Paterniti, J. R., Jr. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2439.
17. Coppola, G. M.; Damon, R. E. Canadian Patent Application CA 2072704, 1993; *Chem. Abstr.*, 119, 95134.
18. Failli, A. A.; Shumsky, J. S. Internat. Patent WO 9911621, 1999; *Chem. Abstr.*, 130, 209605.
19. Baláspiri, L.; Penke, B.; Petres, J.; Kovács, K. *Monatsche Chem.* **1970**, *101*, 1177.
20. France, D. S.; Hughes, T. E.; Miserendino, R.; Spirito, J. A.; Babiak, J.; Eskesen, J. B.; Tapparelli, C.; Paterniti, J. R., Jr. *J. Lipid Res.* **1989**, *30*, 1997.