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Biological evaluation of 1-alkyl-3-phenylthioureas as orally active HDL-elevating agents

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Abstract—A series of 1-alkyl-3-phenylthiourea analogues were prepared and evaluated as HDL- and Apo A-I-elevating and triglyceride-lowering agents. Several derivatives were superior to gemfibrozil (**1**). The optimal analogue **8d** (HDL376) was shown to raise HDL cholesterol in the rat, hamster, dog, and monkey models.

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. 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. Extensive epidemiological studies have shown a strong inverse relationship between serum HDL cholesterol levels and coronary heart disease.[1–3] The Framingham Heart Study showed 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.[4] The Helsinki Heart Study[5,6] and the Veterans Administration HDL Intervention Trial[7] demonstrated a reduced incidence of cardiovascular events with increased HDL cholesterol levels in response to treatment with the PPARα agonist gemfibrozil (**1**). These two clinical trials raised HDL cholesterol by 9% and 6%, respectively.

One of the possible mechanisms for the protective nature of HDL against the development of atherosclerosis is its ability to mediate cholesterol efflux in the reverse cholesterol transport pathway. The HDL particle extracts cholesterol from cells,[8] thus counteracting the effects of LDL cholesterol and subsequently preventing the formation of foam cells, the genesis of atherosclerotic lesions.[9,10] The cholesterol-laden HDL particle is then transported to the liver where the cholesterol is recycled or removed by excretion in bile.[3,10]

Apolipoprotein A-I (Apo A-I), the major protein component of HDL, actively regulates the function of HDL. It has been demonstrated that transgenic mice which overexpress the human Apo A-I gene resulted in elevated levels of HDL cholesterol and significant protection from the development of aortic fatty streak lesions.[11] This protection was also seen in spontaneously atherosclerotic Apo E-deficient mice.[12,13]

We have previously disclosed a series of *N*-phenylthioureas as a new class of HDL-elevating agents.[14,15] The initial lead SDZ45-904 (**2**) was identified from our corporate compound archive. Initial modifications combined structural features from both gemfibrozil (**1**) and **2** to produce a 'hybrid' molecule **3**, which was shown to be as effective as **1** in raising HDL cholesterol and Apo A-I levels.[16] We then focused on probing the SAR around compound **2**. Varying the substitution pattern in the aromatic ring did not improve on the overall profile of **2** and generally elicited substantial reductions

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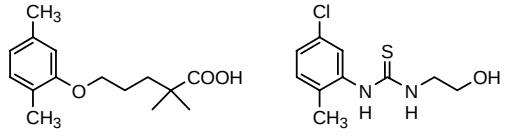
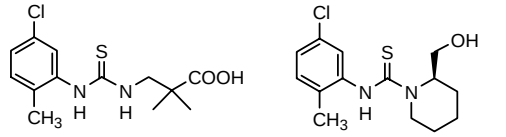
in body weight gain.[17,18] Chain modifications of **2** led to conformationally restricted analogue (*R*)-**4**, which was approximately 50% more potent than **1** in elevating HDL cholesterol and nearly twice that of **1** in raising Apo A-I levels. Total cholesterol was also raised significantly with moderate reduction in body weight gain. The (*S*)-enantiomer of **4** was equally efficacious at raising HDL cholesterol but had no effect on apo A-I.



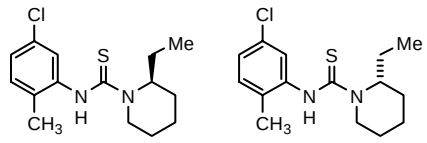
1 **2**



3 **4**

A common feature of **2** and its analogues is the primary alcohol, a potential metabolic liability. We were interested in exploring whether the alcohol could be replaced and maintain potency. We also incorporated fast protein liquid chromatography (FPLC) as another method for analyzing serum lipoproteins. This report will discuss the SAR around structural modifications to **2** as well as differences in results obtained with both ultracentrifugation and FPLC techniques.

The aromatic substituted thioureas (**6**) were readily prepared by reaction of a phenyl isothiocyanate with propylamine in methylene chloride (Scheme 1). Conversely, chain variants **8** were synthesized by reaction of 5-chloro-2-methylphenyl isothiocyanate (**7**) with an appropriate amine. All amines were commercially available with the exception of the chiral 2-ethylpiperidine derivatives. (*S*)-2-Ethylpiperidine was obtained by resolution of 2-ethylpiperidine with (*R*)-mandelic acid.[19] The corresponding (*R*)-enantiomer was obtained by resolution with (*S*)-mandelic acid. These were reacted with **7** to give **9** and **10**, respectively.

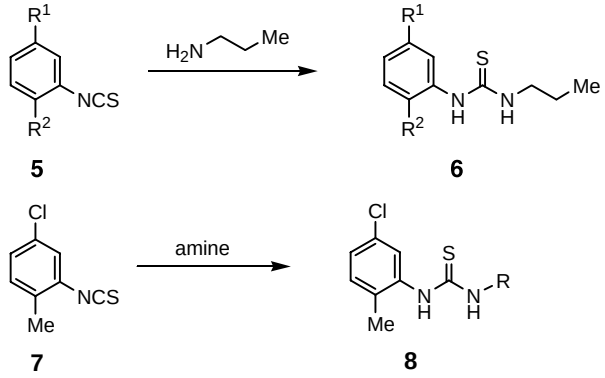


9 **10**

Compounds were administered in diet (ad libitum) to groups of six male Sprague–Dawley rats for 8 days as described in an earlier publication.[16] Serum lipoproteins were separated by classical ultracentrifugation (UC) 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] Concurrently, serum lipoproteins were also analyzed by FPLC,[21] which was modified for robotic automation.[22,23] Within each study, gemfibrozil (**1**) was used as an internal positive control.

Initial FPLC analysis of **2** provided conflicting results when compared with the UC method. HDL cholesterol values obtained by FPLC were significantly higher than the UC values. We then analyzed the serum samples using density gradient ultracentrifugation.[24] In contrast to classical UC where a static density of 1.06 g/ml is used, we used seven escalating densities. As seen in Figure 1, a density of 1.06 g/ml is sufficient to account for virtually all of the HDL cholesterol of **1** (fractions 20–44) but not for **2** when the same fraction numbers were collected. The HDL peak is shifted left (toward the β-VLDL region) indicating the presence of larger, more buoyant particles. However, all of the fractions of this peak contain Apo A-I and no Apo B, thus confirming the presence of HDL particles. Within the series of compounds listed in Tables 1 and 2 the HDL cholesterol values measured by classical UC at a density of 1.06 g/ml were underestimated in more than half of the cases.

The first structural change of **2** was the replacement of the hydroxyl group with methyl (**6a**, Table 1). Satisfyingly, it exhibited a profile similar to that of **2** with a smaller increase in total cholesterol (TC) and a smaller decrease in body weight gain (WG). We then made some minor changes to the aromatic substituents. Replacing chlorine with fluorine or bromine (**6b** and **6c**) retained the HDL effects, however, TC levels were higher than that of **6a**. Replacing methyl with ethyl (**6d**) resulted in a further increase of HDL cholesterol, although Apo A-I levels were statistically no different from controls. Replacement of methyl with methoxy (**6e–g**) resulted in a loss of activity relative to **6a**. The 2,5-dimethyl analogue **6h**, which has the same substitution pattern as gemfibrozil, had a similar profile as **6a**, although TC levels were elevated relative to **6a** and the WG effects were decreased. Within this series of compounds, it appears that the 5-chloro-2-methyl analogue **6a** has the



5 **6**

7 **8**

Scheme 1. General preparation of arylthiourea derivatives.

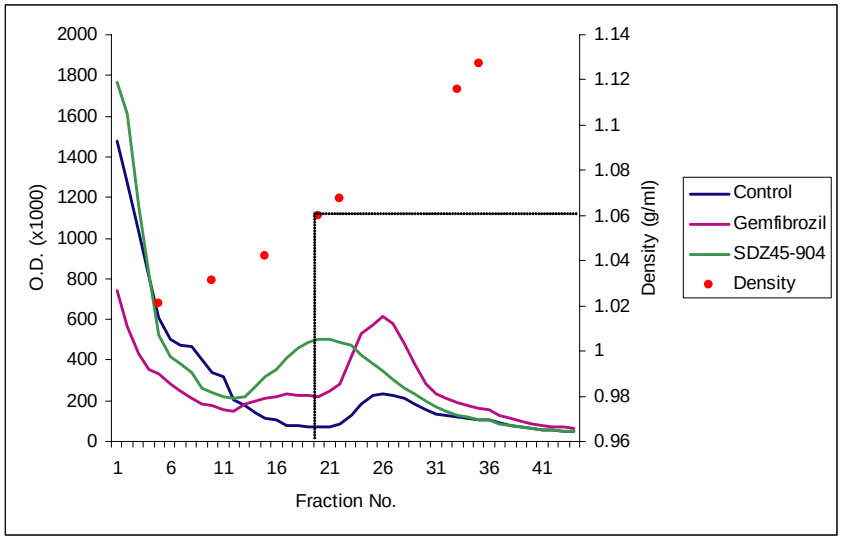


Figure 1. Rat serum density gradient cholesterol of SDZ45-904 (**2**) and gemfibrozil.

Table 1. Lipid profiles of aromatic variants **6** in cholesterol-fed male rats

6[a]	R^1	R^2	Dose (mg/kg/day)	HDL UC (%)[b]	HDL FPLC (%)[b]	Apo A-I (%)[b]	Total cholesterol (%)[b]	TG (%)[b]	WG (%)[c]
a	Cl	Me	55[f]	83[f]	292[f]	54[f]	6[f]	−54[f]	−78[f]
b	F	Me	57	152	312	18	54	−58	−77
c	Br	Me	63	142	306	46	47	−53	−29
d	Cl	Et	70	101	369	43 (NS)	28	−61	−33
e	Cl	OMe	78	99	97	58 (NS)	49	−43	−6
f	OMe	OMe	79	108	97	19 (NS)	60	−23 (NS)	14
g	Me	OMe	71	84	63	133	71	−54	−16
h	Me	Me	62	127	278	72	29	−59	−38
1[d]			50	104	139[g]	54	−23	−29	2
2[e]			55	75	241[h]	48	39	−53	−104

UC, ultracentrifugation; TG, triglycerides; WG, weight gain.
[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 26 studies.
[f] Mean value of 25 studies.
[g] Mean value of 97 studies.
[h] Mean value of 12 studies.

most desirable overall profile. This aromatic substitution pattern is consistent with earlier analogues reported by us[14–17] as well as others.[25]

We next focused our attention on optimizing the alkyl chain (Table 2). Either decreasing or increasing the chain length by one methylene unit (**8a** and **8b**) decreased the HDL and Apo A-I response relative to **6a** while raising TC. The *t*-butyl (**8c**) and closely related 1,1-dimethylpropyl analogue **8f** suffered dramatic loss of activity. The isobutyl analogue **8d** retained HDL activity, increased Apo A-I relative to **6a**, reduced TC level, and lowered adverse WG parameters to an acceptable level. Addition of another methyl group to the end of the chain (**8e**) resulted in some loss of HDL activity and an additional methyl next to the nitrogen (**8g**) further reduced HDL activity.

The carbon versions (**9** and **10**) of the most active conformationally restricted alcohol analogue (**4**) previously reported[17,26] were also the most active in this series, raising HDL cholesterol 404% and 614%, respectively; however, TC levels were also dramatically elevated and large reductions in WG were observed.

In general, nearly all of the thiourea analogues were effective at lowering TG levels. The liver enzymes ALT, AST, and ALP were measured for all compounds. Derivatives **6c**, **6e**, and **8b** elevated ALP 44–49% and **6h**, 75%. Elevation of ALT and AST was observed for **8a** (81%, 162%), **8b** (93%, 79%), and **8e** (111%, 49%). Compounds **9** and **10** elevated both AST (85% and 80%, respectively) and ALP (129% and 105%, respectively). Compounds **2**, **6a**, **8d**, **9**, and **10** were measured for PPARα and **6a**, **8d**, **9**, and **10** were measured for PPARδ activity. All of them were found to be inactive in both assays ($EC_{50} > 100$ μM).

Inspection of the data obtained for all compounds indicated that **8d** possessed the optimal overall profile.

Table 2. Lipid profiles of chain variants **8**, **9**, and **10** in cholesterol-fed male rats

8[a]	R	Dose (mg/kg/day)	HDL UC (%)[b]	HDL FPLC (%)[b]	Apo A-I (%)[b]	Total cholesterol (%)[b]	TG (%)[b]	WG (%)[c]
a	Et	73	110	215	27	32	−64	−107
b	*n*-Bu	55[e]	100[e]	203[e]	38[e]	39[e]	−44	−53[e]
c	*t*-Bu	77	30	22	60	−15 (NS)	−31	9 (NS)
d	CH_2CHMe_2	79[f]	104[f]	260[f]	77[f]	−11[f]	−54[f]	−12[f]
e	CH_2CMe_3	88	122	196	89	3 (NS)	−66	7
f	CMe_2Et	81	55	39	64	−10	−14 (NS)	15
g	$CHMeCMe_3$	82	93	109	34	15 (NS)	−35	−24
h	CH_2Ph	77	135	136	27	97	−21	41
9		62	124	404	39	126	−56	−88
10		63	129	614	30	167	−54	−105
1[d]		50	104	139[g]	54	−23	−29	2

UC, ultracentrifugation; TG, triglycerides; WG, weight gain.
[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 2 studies.
[f] Mean value of 51 studies.
[g] Mean value of 97 studies.

Table 3. Percent increase of HDL cholesterol of HDL376 (**8d**) and gemfibrozil in alternate animal models

Model	HDL376	Dose (mg/kg/day)	Gemfibrozil	Dose (mg/kg/day)
NF rat	75	80	25	50
CF hamster	15	64	NA	45
NF hamster	44	52	NA	45
NF dog	50	35	NA	250
CF rhesus monkey	75	12		
CF cynomolgus monkey	100	12		

NF, normal-fed; CF, chow-fed; NA, not active. Good dose responses were observed in each of the species mentioned in this table.

HDL cholesterol and Apo A-I levels were elevated nearly twice that of gemfibrozil, while TC was reduced by 11%. It was also twice as effective as gemfibrozil at reducing TG levels. Compound **8d** (designated HDL376) was further profiled against **1** in additional animal models (Table 3). In all models, HDL376 elevated HDL cholesterol, whereas **1** was not active in the CF hamster and NF hamster and dog. This suggests that the mechanism of action of this class of compounds, while currently unknown, may be broadly relevant to mammals and not specific to the rat.

In conclusion, 1-alkyl-3-phenylthioureas have been shown to be effective HDL and Apo A-I-elevating agents which also exhibit TG-lowering properties. Some analogues produced larger, more buoyant HDL particles. The optimal analogue **8d** was effective at raising HDL cholesterol in the CF and NF rat, CF and NF hamster, and NF dog and CF monkey models.

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