Design, Synthesis, and Biological Evaluation of Thio-Containing Compounds with Serum HDL-Cholesterol-Elevating Properties

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A novel series of substituted sulfanyldihydroimidazolones (**1**) that modulates high-density lipoprotein cholesterol (HDL-C) has been reported to have HDL-elevating properties in several animal models. Concerns about the chemical and metabolic stability of **1** directed us to explore the structure−activity relationship (SAR) of a related series of substituted thiohydantoins (**2**). Expansion of the scope of the thiohydantoin series led to exploration of compounds in related thio-containing ring systems **3**−**7** and the *N*-cyanoguanidine derivative **8**. Compounds were tested sequentially in three animal models to assess their HDL-C elevating efficacy and safety profiles. Further evaluation of selected compounds in a dose−response paradigm culminated in the identification of compound **2.39** as a candidate compound for advanced preclinical studies.

Introduction

Atherosclerosis, a major cause of death in the United States and other industrialized countries, is characterized by elevated levels of circulating plasma cholesterol and lipid accumulation in arterial lesion sites.[1] Therapeutic intervention for atherosclerotic coronary heart disease (CHD) has focused primarily on modulating cholesterol and low-density lipoproteins (LDL) rather than high-density lipoproteins (HDL) because highly effective pharmacologic agents exist for modifying risk due to hypercholesterolemia.[2−6] Unfortunately, reducing plasma cholesterol only benefits 30% of individuals and highlights the need for additional therapeutic approaches.[7] Strong epidemiological evidence links low HDL cholesterol to CHD and supports an independent inverse association of HDL levels and coronary heart disease event rates, for which risk decreases 2−3% for each 1 mg/dL incremental increase in HDL.[8−11] Mechanisms underlying this association include the role of HDL in promoting reverse cholesterol transport (RCT).[12−14] By this process, excess cholesterol removed from peripheral cells, including lipid-laden macrophages of the arterial wall, is returned via HDL to the liver for disposal by excretion into bile.

In vivo profiling of a series of 2-substituted sulfanyl-3,5-dihydroimidazol-4-one derivatives (**1**) disclosed their preferential activity for increasing HDL cholesterol relative to the other lipoprotein fractions as reported earlier (Chart 1).[15] Issues concerning the chemical and metabolic stability of this class of compounds led to the design and evaluation of a related series of substituted 2-thioxoimidazolidin-4-one (thiohydantoin) derivatives **2** and **3**. Subsequent expansion of the scope of the thiohydantoin chemical series encompassed the preparation of compounds in related series such as 3-thioxo-[1,2,4]oxadiazinan-5-one derivatives **4**, 2-thioxotetrahydropyrimidin-4-one derivatives **5**, and 2-thioxo[1,3]-diazepan-4-one derivatives **6**. A 6-thioxopiperazin-2-one derivative **7** and an *N*-cyanoguanidine derivative **8** were also prepared and tested. In this paper, the design and synthesis of these novel classes of compounds as well as their effects on altering the lipid profile following oral administration in various animal models are presented.

Chemistry

The 2-thiohydantoin derivatives (**2**) were prepared via the reaction of *N*-substituted amino acids **10**[16] with a variety of isothiocyanates, affording the thiourea derivatives **11** followed by cyclization in refluxing ethanol under basic conditions (Scheme 1). Mono- and di-α-substituted *N*-substituted amino acids **10** were prepared from α-halo α-substituted carboxylic acids **9** and the appropriate amines. Alternatively, *N*-substituted amino acids **10a** were prepared by the reductive amination of glyoxylic acid (**12**) with amines. Reaction of **10a** with isothiocyanates in chloroform or methylene chloride in the presence of a base such as triethylamine yielded substituted thiohydantoins **2** directly (Scheme 1).

2-Thioparabanic acid derivatives **3** were prepared by treatment of thioureas **14** with oxalyl chloride in methylene chloride to provide the iminothiazolidine-4,5-dione **15** and subsequent rearrangement to **3** in refluxing ethanol.[17] Additionally, it was later discovered that the reaction of thioureas **14** with ethyl chlorooxoacetate afforded the desired compounds **3** directly (Scheme 2).

3-Thioxo[1,2,4]oxadiazinan-5-one derivatives **4** were prepared by esterification of aminooxyacetic acid **16** to provide **17**, followed by conversion to the carbamate **18** (Scheme 3). Alkylation of **18** and subsequent deprotection furnished *N*-alkylaminooxyacetic acids **20**.[18] Alternatively, reductive amination of **17** followed by ester hydrolysis furnished **20**. Reaction of **20** with isothiocyanates as described above afforded thiourea **21**. Cyclization to provide the oxadiazinanone **4** was ac-

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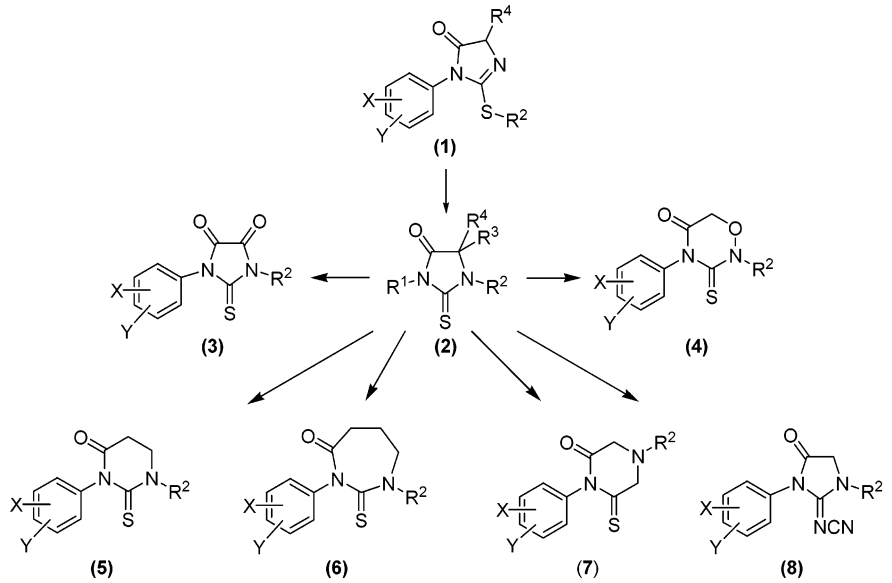
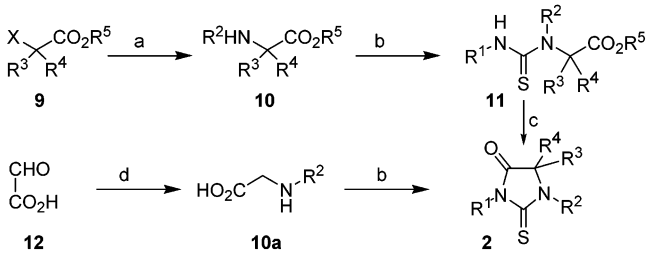
Chart 1. Exploration of Activities of Series Related to the 2-Ethylsulfanyl-3,5-Dihydroimidazol-4-one Lead (**1**)

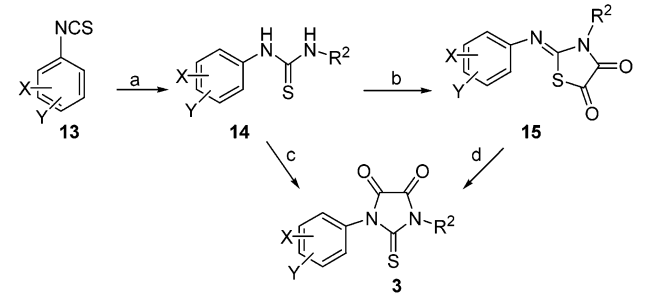


Scheme 1. Synthesis of Thiohydantoins **2**[a]



[a] (a) R^2-NH_2, ether, CH_2Cl_2, or H_2O; (b) R^1NCS, $CHCl_3$; (c) Et_3N, EtOH, reflux; (d) R^2-NH_2, H_2, 10% Pd−C, H_2O.

Scheme 2. Synthesis of 2-Thioparabanic Acids **3**[a]



[a] (a) R^2-NH_2, CH_2Cl_2; (b) $(COCl)_2$, CH_2Cl_2, reflux; (c) $ClCOCO_2Et$, CH_2Cl_2, reflux; (d) EtOH, reflux.

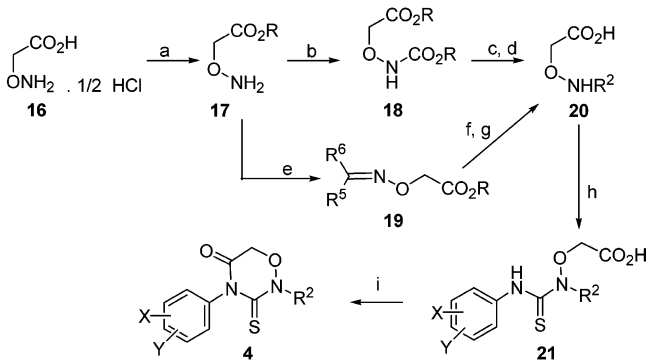
complished by treatment with phosphorus pentachloride in refluxing benzene.

Preparation of the thiouracil derivatives **5** and the ring-expanded diazepanone analogues **6** was affected by treatment of the appropriate halo acid **22** with amines to give the corresponding β- or γ-amino acids **23**. Reaction of **23** with an isothiocyanate afforded thiourea **24**. Cyclization of **24** was carried out either in refluxing acetone (containing 2% hydrochloric acid) or by the action of trifluoroacetic anhydride (Scheme 4).

Thiopiperazinone **7** was prepared by conversion of amino acid **10.02** to amide **26** via protection of the nitrogen[16] and subsequent coupling with 5-chloro-2-methylaniline (**29**) (Scheme 5). Amide **26** was converted to thioamide **27** using standard conditions.[19] Deprotection of **27** followed by treatment with ethyl bromoacetate furnished **7**.

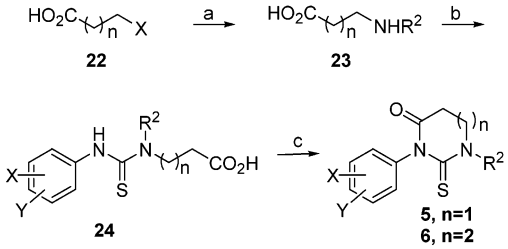
The 2-*N*-cyanoimidazolidin-4-one derivative **8** was prepared in two steps from **26** via deprotection with TFA and subsequent reaction with dimethyl *N*-cyanodithioiminocarbonate (Scheme 6).

Scheme 3. Synthesis of 3-thioxo[1,2,4]oxadiazinan-5-ones **4**[a]



[a] (a) ROH, HCl; (b) $ClCO_2R$, $NaHCO_3$, ROH; (c) R^2I, K_2CO_3, ROH; (d) HCl, reflux; (e) R^5COR,[6] EtOH, reflux; (f) $NaCNBH_3$, HCl, EtOH, (g) KOH, EtOH, H_2O, (h) ArNCS, Et_3N, $CHCl_3$, reflux; (i) PCl_5, C_6H_6, reflux.

Scheme 4. Synthesis of Thiouracils **5** and Diazepanones **6**[a]



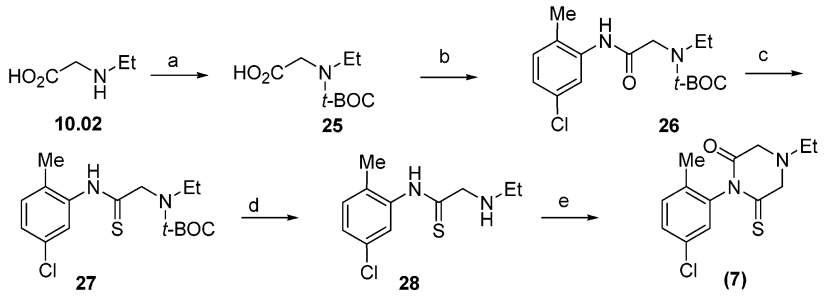
[a] (a) R^2NH_2, CH_2Cl_2; (b) ArNCS, Et_3N, $CHCl_3$; (c) acetone−HCl (2%), reflux, 18 h, or TFAA 0°C, 3 h.

The 2-*N*-cyanoimidazolidin-4-one derivative **8** was prepared in two steps from **26** via deprotection with TFA and subsequent reaction with dimethyl *N*-cyanodithioiminocarbonate (Scheme 6).

Results

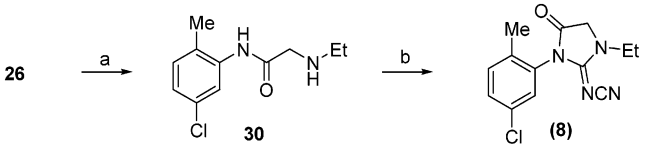
Target compounds were evaluated as HDL cholesterol (HDL-C) elevating agents in three animal models.

Scheme 5. Synthesis of 2-Thiopiperazine-4-one Derivative **7**[a]



[a] (a) $(t\text{-BOC})_2O$, KOH, H_2O; (b) 5-chloro-2-methylaniline (**29**), DCC, HOBT, CH_2Cl_2, (c) Lawesson's reagent, $PhCH_3$, reflux; (d) TFA, CH_2Cl_2, (e) $BrCH_2COOEt$, EtOH.

Scheme 6. Synthesis of 2-*N*-Cyanoimidazolidine-4-one Derivative **8**[a]



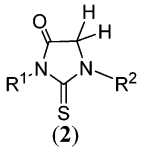
[a] (a) TFA, CH_2Cl_2; (b) dimethyl *N*-cyanodithioiminocarbonate, 175 °C.

Experimental animals on both a conventional dietary regiment and hypercholesterolemic diets were used to establish pharmacologic efficacy for raising HDL cholesterol. Initially, compounds were tested in a rat model of hyperlipidemia induced by feeding diet supplemented with 1% cholesterol and cholic acid.[20,21] Such diets attempt to elevate plasma cholesterol to levels approaching human dyslipidemias.[22] In this model, each test compound was admixed to the diet of a group of six animals. No correction in drug dose was made to account for small differences in food consumption of individual animals. The results shown reflect a drug effect at an average daily dose of 100 mg/kg. Compounds were ranked in this primary screen based on their potency for selective HDL elevation relative to the lower density lipoproteins, very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) fractions. Those compounds that demonstrated a significant increase in HDL-C of 50% or greater, without adversely influencing other measured parameters, were subsequently tested in a second animal model, the chow-fed rat. Treatments meeting the activity criteria for HDL elevation (50% or greater) in the animals maintained on chow diets and lacking significant safety issues were profiled further in the third model, the hypercholesterolemic hamster. Hamsters exemplify a small animal species possessing a lipoprotein metabolism consistent with that found in man and as such represents a significant hurdle for ensuing evaluation of active leads in higher species such as rabbits and primates.

Initial probing of the SAR requirements in the thiohydantoin series focused on modification of the N-1 (R^2) and N-3 (R^1) substituents. Maintaining R^2 as methyl or ethyl, various substituents at N-3 were evaluated (Table 1). In general, trends of HDL-C increases were observed with compounds where the R^2 group was an aryl ring directly attached to the nitrogen (**2.01** and **2.04**) (Table 1). A heteroaryl substituent (**2.03**) had diminished activity. With the exception of the benzhydryl analogue (**2.05**), the aryl−alkyl groups at N-3 yielded compounds devoid of significant HDL-C-enhancing activity. Saturation of the aromatic ring (**2.02**) led to loss of activity. The piperidinylethyl derivative (**2.12**) was also inactive.

The addition of aromatic substituents to **2.01** produced compounds that significantly ($p < 0.05$) increased HDL-C (**2.13**, **2.14**, **2.16**, and **2.19**). Introduction of a benzyl group at N-1 (**2.15** vs **2.16**) led to loss of activity. Refinement of SAR at the N-1 position confirmed the trend of activity in favor of a small substituent. Although some compounds produced increases in total plasma cholesterol (TPC), none of the increases were significant.

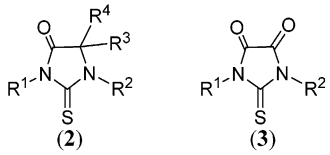
Table 1. Percent Changes in Serum Lipids Following Oral Administration of Compounds **2** in Hypercholesterolemic Rats[a] at 100 mg/kg/day for 8 Days; Variation of the R^1 and R^2 Groups



			% change[c]		
compd	R^1	R^2	TPC	HDL-C	TG
2.01	Ph	Me	25	66	10
2.02	cyclohexyl	Et	−7.4	5.4	28
2.03	3-pyridyl	Me	−11.4	29	150[b]
2.04	2-naphthyl	Et	−18.1	65	−33
2.05	$(Ph)_2CH$	Me	3.4	57[b]	92[b]
2.06	$PhCH_2CH_2$	Et	−14	30	6
2.07	$Ph(CH_2)_3CH_2$	Me	−4.3	−21	41
2.08	4-Me-$PhCH_2CH_2$	Me	−11	−26.6	27
2.09	4-Cl-$PhCH_2CH_2$	Me	−10.2	−15.5	24
2.10	1-(4-F-Ph)-CH_2CH_2	Et	−8.8	49	−17
2.11	2-Cl-$PhCH_2$	Me	14	23	11
2.12	piperidinylethyl	Me	−3.3	−40	33
2.13	5-indanyl	Me	−3	112[b]	−26
2.14	2-Me-Ph	Et	76.5	164[b]	38
2.15	2,6-di-Cl-Ph	$PhCH_2$	25.3	−2.2	−54
2.16	2,6-di-Cl-Ph	Et	53.4	142[b]	−37
2.17	2,6-di-Cl-Ph	Me	4.6	92	−43[b]
2.18	6-Cl-2-Me-Ph	Me	17	84	−56
2.19	6-Cl-2-Me-Ph	Et	−34	222[b]	−20
2.20	6-Cl-2-Me-Ph	*n*-Bu	30	53	−38
2.21	6-Cl-2-Me-Ph	Ph	2.2	39	16
2.22	5-Cl-2-Me-Ph	MeO	41	160[b]	−62[b]
2.23	2,6-di-Me-Ph	*i*-Pr	−25	146[b]	−43[b]
2.24	2,6-di-Me-Ph	allyl	−5	177[b]	−45[b]
2.25	2,6-di-Me-Ph	propargyl	−15	325[b]	0
2.26	4-Cl-2-Me-Ph	$CH_2CH_2CO_2Me$	14	27	−32

[a] Data are mean values for 6 rats per group. [b] Significantly different from control group at $p < 0.05$. [c] TPC, total plasma cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. Representative values for a control hypercholesterolemic rat: TPC, 167 mg/dL; HDL-C, 16.5 mg/dL; and TG, 107 mg/dL.

Table 2. % Changes in Serum Lipids Following Oral Administration of Compounds **2** and **3** in Hypercholesterolemic Rats[a] at 100 mg/kg/day for 8 Days; Effect of C-5 Substitution



(**2**) (**3**)

					% change[d]		
compd	R^1	R^2	R^3	R^4	TPC	HDL-C	TG
2.27	5-Cl-2-Me-Ph	Me	Me[c]	H	−8	220[b]	−58[b]
2.28	5-Cl-2-Me-Ph	Et	Ph[c]	H	60	187[b]	76[b]
2.29	4-F-Ph	Et	Ph[c]	H	31	67	35
2.30	2,6-di-Cl-Ph	Et	Ph[c]	H	18	39	25
2.31	5-Cl-2-Me-Ph	Et	OMe[c]	H	6	140[b]	−33
2.32	*i*-Bu	Et	Ph[c]	H	7	156[b]	92[b]
2.33	5-Cl-2-Me-Ph	Et	Me	Me	0	−9	15
2.34	2,6-di-Cl-Ph	Et	Me	Me	−16	−18	−19
2.35	2,6-di-Me-Ph	Et	Me	Me	13	−2	17
2.36	2-Cl,6-Me-Ph	Et	Me	Me	16	−5	−22
3.01	5-Cl,2-Me-Ph	Et	−	−	32	242[b]	−−22
3.02	5-Cl,2-Me-Ph	Me	−	−	44[b]	196[b]	−26
3.03	4-F-Ph	Et	−	−	44[b]	23	−48[b]
3.04	2,6-di-Me-Ph	Et	−	−	−21	186[b]	−48[b]

[a] Data are mean values for six rats per group. [b] Significantly different from control group at $p < 0.05$. [c] Racemic compound. [d] TPC, total plasma cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. Representative values for a control hypercholesterolemic rat: TPC, 167 mg/dL; HDL-C, 16.5 mg/dL; and TG, 107 mg/dL.

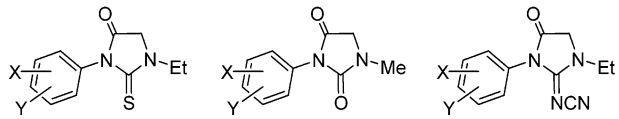
Exploration of C-5 on the thiohydantoin ring demonstrated that monosubstituted compounds showed enhanced activity (**2.27**−**2.32**, Table 2), while disubstituted compounds exhibited diminished activity (**2.33**−**2.36**, Table 2). The introduction of a carbonyl at C-5 (**3.01**−**3.04**, Table 2) was investigated and these compounds exhibited comparable activity to the unsubstituted counterparts.

Further investigation of the phenyl substituents at N-3 indicated that, with few exceptions (**2.48**, **2.50**, and **2.52**, Table 3), substituted phenyl derivatives induced favorable HDL-C increases that were significantly different from control. It is worth noting that, in addition to the HDL-C elevation, the 2,6-dialkyl phenyl derivatives **2.37**, **2.45**, and **2.46** showed a trend toward lowering of total plasma cholesterol (TPC). Of these, the effect of 6-isopropyl-2-ethyl analogue (**2.45**) on TPC lowering was significant (60%). The 4-dimethylamino derivative (**2.38**), the 4-fluoro derivative (**2.40**), and the 2-isopropyl derivative (**2.51**) effected significant increases in total plasma cholesterol (123%, 78%, and 71%, respectively).

We next embarked on exploration of ring-expanded scaffolds. As established by the SAR of the thiohydantoins, a small alkyl was maintained at R^2 and an aryl group was attached to N-3. Investigation of ring size demonstrated that while the activity profile of the six-membered ring analogues (**4** and **5**, Table 4) were similar to that of the thiohydantoins (e.g. **4.06** and **5.04**, Table 4, vs **2.19**, Table 1), the seven-membered ring analogues (**6.01** and **6.02**) did not significantly affect HDL-C levels.

The 2-thiopiperazin-4-one derivative (**7**, Table 4) and the *N*-cyanoguanidine [23,24] derivative (**8**, Table 3) exhibited no effect on serum lipoproteins. Replacement of

Table 3. Percent Changes in Serum Lipids Following Oral Administration of Compounds **2** in Hypercholesterolemic Rats[a] at 100 mg/kg/day for 8 Days; Optimization of the Aryl Substituents



(**2**) (**2.53**) (**8**)

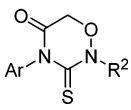
		% change[c]		
compd	X, Y	TPC	HDL-C	TG
2.37	2-Me, 6-Me	−28	181[b]	−23
2.38	4-$(Me)_2$N	123[b]	49[b]	−41
2.39	5-Cl, 2-Me	53	132[b]	−19
2.40	4-F	78[b]	59[b]	−18
2.41	5-Cl, 2-MeO	24	39[b]	51
2.42	4-CF_3O	−1	265[b]	−72[b]
2.43	5-F, 2-Me	35	112[b]	−36
2.44	2-CF_3	18	116[b]	35
2.45	2-Et, 6-*i*Pr	−62[b]	54[b]	5
2.46	6-Et, 2-Me	−19	112[b]	−10
2.47	2-Cl, 3-Cl	11	98[b]	−23
2.48	2-Me, 4-Me	25	83	−34[b]
2.49	2-Me, 5-Me	49	124[b]	−6
2.50	2-Me, 4-Me, 5-Me	39	112	−11
2.51	2-*i*Pr	71[b]	201[b]	10
2.52	2-MeS	13	60	−8
2.53	5-Cl, 2-Me	37	−8	−7
8	5-Cl, 2-Me	15	17	−25

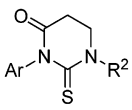
[a] Data are mean values for six rats per group. [b] Significantly different from control group at $p < 0.05$. [c] TPC, total plasma cholesterol, HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. Representative values for a control hypercholesterolemic rat: TPC, 167 mg/dL; HDL-C, 16.5 mg/dL; and TG, 107 mg/dL.

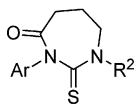
the sulfur atom of compounds **2** with oxygen abolished activity (**2.53**, Table 3).

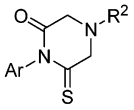
Of the compounds tested in the primary hypercholesterolemic rat model, 41 demonstrated a 50% or greater significant increase in HDL-C without adversely affecting other lipoproteins, total plasma cholesterol, or triglycerides. Differences exceeding 50% may fail to reach statistical significance as a result of animal variation; this was evident particularly in this hypercholesterolemic rat model, where animal variability reflects both treatment and dietary responsiveness. This subset of 41 compounds was advanced for further evaluation in the normal, chow-fed rat model (Table 5). This model offers an advantage for gathering preliminary information regarding safety parameters in normal animals. For this purpose, body weight gain, liver weights, lactic dehydrogenase (LDH), alkaline phosphatase (ALK PHOS), and the transaminases alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed along with total plasma cholesterol, triglycerides, and the lipoproteins. Since derivatives of thiourea are known inhibitors of thyroid function,[25] the inhibitory effects of the target compounds on thyroid (T_4) were assessed as an added safety parameter. Since the HDL cholesterol subfraction predominates in the lipoprotein profile of chow-fed rodents, changes in total cholesterol predictively parallel selective increases in HDL-C in this model. Thirty-three of the compounds tested in the chow-fed rat model induced 50% or greater HDL-C elevations (statistically significant at $p < 0.05$) and corresponding increases in total

Table 4. Percent Changes in Serum Lipids Following Oral Administration of Compounds **4**, **5**, or **6** in Hypercholesterolemic Rats[a] at 100 mg/kg/day for 8 Days; Exploration of Ring-Expanded Scaffolds


(**4**)


(**5**)


(**6**)


(**7**)

			% change[c]		
compd	Ar	R^2	TPC	HDL-C	TG
4.01	4-Cl,2-Me-Ph	Me	4	221[b]	−38
4.02	$(Ph)_2CH$	Me	22	60[b]	9
4.03	5-Cl, 2-Me-Ph	Me	5.7	236[b]	−10
4.04	4-indanyl	Me	−2	192[b]	−26
4.05	2,5-di-Me-Ph	Me	28	72[b]	26
4.06	2-Cl,6-Me-Ph	Me	−2.7	100[b]	−30
4.07	2-*i*Pr-Ph	Me	0	95[b]	−16
4.08	4-*t*Bu-Ph	Me	2	170[b]	−51[b]
4.09	4-PhO-Ph	Me	−25	102[b]	−43[b]
4.10	5-Cl,2-MeO-Ph	Me	44[b]	78[b]	7
5.01	2,6-di-Me-Ph	Et	11	184[b]	−12
5.02	2,4-di-Me-Ph	Et	27	−3	2
5.03	4-Cl,2-Me-Ph	Et	40[b]	126[b]	−16
5.04	2-Cl,6-Me-Ph	Et	23	199[b]	−16
5.05	5-Cl,2-Me-Ph	Et	53[b]	76[b]	44[b]
5.06	2-Et,6-*i*Pr-Ph	Et	2	34	−11
5.07	2-Et,6-Me-Ph	Et	52[b]	60	2
5.08	2-F-Ph	Et	21	72[b]	−26
5.09	2-*i*Pr-Ph	Et	44	108[b]	−31
5.10	2,6-di-Cl-Ph	Et	42[b]	16	−6
6.01	4-Cl,2-Me-Ph	Et	58.8[b]	15	−15
6.02	5-Cl,2-Me-Ph	Et	7	50	14
7	5-Cl,2-Me-Ph	Et	28	10	−27

[a] Data are mean ± SEM for six rats per group. [b] Significantly different from control group at $p < 0.05$. [c] TPC, total plasma cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. Representative values for a control hypercholesterolemic rat: TPC, 167 mg/dL; HDL-C, 16.5 mg/dL; and TG, 107 mg/dL.

plasma cholesterol. Of these, eight compounds (**2.22**, **2.27**, **3.01**, **3.04**, **2.42**, **2.43**, **4.01**, and **4.08**, Table 5) were excluded based on significant increases in other lipoprotein fractions, and 13 compounds (**2.14**, **2.16**, **2.17**, **2.18**, **2.24**, **2.25**, **2.37**, **2.44**, **2.46**, **4.03**, **4.04**, **5.08**, and **5.09**) were precluded from further analysis based on adverse events such as effects on body weight gain, liver weight, liver enzymes, or T4. The remaining 12 compounds met the activity criteria for profiling in the third model, the hypercholesterolemic hamster.

The hamster has proven to be a good model in which to study lipid-modulating agents, since it has significant levels of circulating LDL-C and responds to dietary lipid intake in a manner similar to man.[26] In hamsters and humans, LDL lipoproteins predominate as a major transporter of plasma cholesterol, in significant contrast to rodent species, where HDL lipoproteins perform this function. The goal for HDL-elevating agents in this model is to shift the HDL/LDL ratio in favor of an increase without increasing total plasma cholesterol levels. The redistribution of cholesterol from the non-HDL lipoproteins such as the VLDL-C lipoprotein fraction or a decrease in plasma triglycerides would be considered favorable secondary effects used for selection and further profiling. Of the 12 compounds tested in the hamster model, five compounds were effective in raising HDL-C significantly with concomitant lowering of LDL-C (Table 6). All but one compound (**2.31**) had no toxicological effects or adverse affects on other non-HDL-C lipid fractions. Further evaluation of these compounds was undertaken in dose−response studies in both the chow-fed rat at oral doses ranging from 1 to 100 mg/kg/day and the hypercholesterolemic hamster at oral doses ranging from 3 to 100 mg/kg/day. In addition to monitoring of lipid profile alterations, the effects of test compounds on body weight gain, liver enzymes, and T4 were monitored. Of the compounds profiled in these two models in a dose−response fashion, compound **2.39** emerged as the agent with the best overall safety and efficacy profile

Compound **2.39** caused a dose-dependent elevation in the HDL fraction at doses of 10 mg/kg/day and higher (Table 7) in chow fed rats. At a maximum dose of 100 mg/kg/day, HDL cholesterol was increased 85% relative

Table 5. Changes in Serum Lipids Following Oral Administration of Test Compounds in Normal Chow-Fed Rats[a] at 100 mg/kg/day for 4 Days

	% change[g]					
compd	TPC-C	HDL-C	LDL-C	VLDL-C	TG	AE
2.13	26	46	−40.3	26.3	−0.9	
2.14	52.1[b]	54.3[b]	18.7	0	−25	c
2.16	57[b]	60.2[b]	6.8	0	−35.8	e
2.17	83.9[b]	109.3[b]	39.6	−3.3	−33.3	e
2.18	71.2[b]	75.7[b]	0	89.4	−37.9	d
2.19	79.8[b]	62.9[b]	−35	41.4	−57.1	
2.22	134.4[b]	102.4[b]	87.5[b]	249.5[b]	−10.8	f
2.23	56.1[b]	71.2[b]	−5.8	−10.5	−42.9	
2.24	86[b]	120.4[b]	−18.8	−45.8	−29.9	c, e
2.25	98[b]	85.1[b]	27.9	−23	−48.5	e, f
2.27	276.5[b]	67.9[b]	800[b]	134.7	207.2[b]	c, e
2.31	102.3[b]	91.6[b]	11.9	83.6	−10.6	
2.32	−8.4	−3.1	−9.3	33.3	−1.6	
3.01	123.4[b]	87.6[b]	71.9[b]	67.3[b]		
3.04	138.2[b]	114.4[b]	73.2[b]	409.7[b]	−32.8	d, f
2.37	106.7[b]	93.6[b]	43.8	13	−12.8	d
2.39	72.8[b]	89.1[b]	9.6	−17.2	−23.1	
2.42	197.6[b]	71.4[b]	127.5	543.5[b]	2.8	d
2.43	95.2[b]	102.4[b]	14	131.6[b]	−6.3	d
2.44	67.2[b]	103.5[b]	−13.3	52.6	−6.3	d
2.45	30.9[b]	48.7[b]	−22.3	−50	−12.2	
2.46	52.8[b]	61.9[b]	−6.5	−29.2	−8.2	e
2.47	89.6[b]	85.3[b]	20.4	57.9	18.3	
2.48	55.5[b]	61.2[b]	8.3	15.6	−35.4	
2.49	26.4	31.9[b]	−8.3	31.3	−2.5	
2.51	111.4[b]	90.6[b]	24.6	73.7	−27.5	
4.01	51.4[b]	53.4[b]	20.8	114.7[b]	−4.3	
4.02	17.6	33.7[b]	43.8	−29.3	−44.1	
4.03	85.7[b]	83.5[b]	29.9	23.1	−26.2	d
4.04	78.5[b]	81.8[b]	42.3	68	−31.2	d
4.05	75.2[b]	87.2[b]	9.1	61.5	−31.6	
4.06	40.4	52.8	11.7	80.8	−61.5	
4.07	92.4[b]	98.2[b]	32.1	−8.3	−52.7[b]	f
4.08	62.0[b]	61.6[b]	2.6	176.5[b]	−24.6	
4.09	−13	0	−20.5	−9.1	−20.5	
4.10	67.7[b]	71.3[b]	8.6	25.0	−7.4	
5.01	70.4[b]	97.4[b]	0	−5.2	−11.4	
5.03	80.7[b]	75.5[b]	38.9	−15.0	−53.1[b]	
5.04	45.4	45.5	−1.1	−5.3	−35.7	
5.08	48.7[b]	55.3[b]	−4.0	20.1	−4.0	f
5.09	59.2[b]	67.8[b]	9.8	35.2	−12.4	e

[a] Data are mean values for six rats per group. [b] Significantly different from control group at $p < 0.05$. [c] Significant ($p < 0.05$) increase of one or more liver enzymes. [d] Significant ($p < 0.05$) decreases in body weight gain. [e] Significant ($p < 0.05$) increase in liver weight. [f] Significant ($p < 0.05$) decrease in T4. [g] TPC, total plasma cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; TG, triglycerides; AE, adverse event. Representative values for a control rat on normal chow diet: TPC, 62 mg/dL; HDL-C, 41 mg/dL; LDL-C, 6.3 mg/dL; VLDL-C, 3.7 mg/dL; and TG, 16 mg/dL.

Table 6. Changes in Serum Lipids Following Oral Administration of Test Compounds in Hypercholesterolemic Hamsters[a] at 100 mg/kg/day for 14 Days

	% change[d]				
compd	TPC	HDL-C	LDL-C	VLDL-C	TG
2.19	−32.2[b]	13.8	−39.1[b]	−74.4[b]	−77.1[b]
2.23	−3.1	1.7	17.2	8.9	−7.3
2.31[c]	3.8	57.4[b]	−19.0	−35.8	−36.4
2.39	−3.3	43.0[b]	−30.1	−33.4	−34.0
2.47	−8	0	−8.1	−5.9	−12.3
2.48	10.7	33.3[b]	−11.6	14.5	9.6
2.51	−5.9	19.5	−41.0[b]	−4.5	−10.1
4.05	−13.8	48.3[b]	−40.1[b]	−25.5	−23.8
4.06	−21.4[b]	−28.1[b]	−22.4[b]	5.2	38.6
4.10	−7.9	23.5	−20.2	−32.6	−26.5
5.01	−3.0	0.7	24.0	22.7	8.6
5.03	2.0	47.8[b]	−26.8	6.3	−30.7

[a] Data are mean values for six rats per group. [b] Significantly different from control group at $p < 0.05$. [c] Significant ($p < 0.05$) decrease in T4. [d] TPC, total plasma cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; TG, triglycerides. Representative values for a control hypercholesterolemic hamster: TPC, 448 mg/dL; HDL-C, 124 mg/dL; LDL-C, 203 mg/dL; VLDL-C, 121 mg/dL; and TG, 461 mg/dL.

to vehicle-treated controls ($p < 0.01$) in this model. At a lower concentration of 10 mg/kg/day, HDL was elevated 49% ($p < 0.05$). However, due to the steep rise in HDL cholesterol levels at the doses tested, a dose−response curve could not be generated through linear regression analysis. Increases in plasma total cholesterol range from 37% at 10 mg/kg/day to 90% at 100 mg/kg/day and were observed in parallel with HDL cholesterol elevations. Due to the close correlation between these two parameters in this model, the ratio of TC/HDL-C persists unchanged. Plasma triglycerides remain low with treatment and neither LDL nor VLDL cholesterol fractions were altered significantly at any dose. Safety measurements revealed few differences (Table 8). A small but statistically significant decrease in body weight gain was observed in animals given 100 mg/kg/day. No significant changes in liver enzymes were demonstrated. Changes in liver weights or relative liver weight, calculated as a percent of body weight, were unremarkable.

In a second dose−response study, serum apolipoprotein-A1 was measured by ELISA and shown to increase in parallel with the HDL cholesterol (Table 9) following treatment with **2.39**. At the two highest concentrations of 100 and 50 mg/kg, serum apo-A1 increased 52% and 41%, respectively. Trends that did not reach statistical significance were observed at lower doses. HDL cholesterol was raised approximately 2-fold at the 100 mg/kg dose and 68% at 50 mg/kg. Apo-A1 mRNA quantitation was performed by RNase protection analysis in both liver and intestinal samples from the same animals. However, no significant changes were observed in treated animal tissues relative to controls (data not shown).

Oral administration of compound **2.39** caused a dose-dependent elevation in HDL-C in male hypercholesterolemic hamsters at doses of 3−100 mg/kg/day (Table 10). HDL-C was raised 26% at 30 mg/kg/day and 54% at 100 mg/kg/day ($p < 0.01$), in the presence of diet-induced hypercholesterolemia. In addition, decreases in serum total cholesterol and LDL-C at doses of 10, 30, and 100 mg/kg/day partially reverse some of the diet-induced changes. Importantly, the TC/HDL-C ratio was reduced from 3.64 to 1.95 ($p < 0.01$), comparable to the ratio for chow-fed controls at the highest dose of 100 mg/kg/day. A trend toward decreasing T4 levels was observed with increasing doses of compound **2.39**. At the highest dose of 100 mg/kg/day, T4 levels, induced in response to the dietary cholesterol, were decreased to levels similar to hamsters maintained on chow. There were no significant changes in body weight gain, liver weights, or liver enzymes (Table 11) in any drug-treated group.

Table 7. Effects of Compound **2.39** on Serum Lipids and T4 Levels in Normal Chow-Fed Male Rats

	dose,[a]	serum lipids,[d] mg/dL					ratio	T4
groups	mg/kg/day	TG	TPC	HDL-C	LDL-C	VLDL-C	TPC/HDL-C	μg/dL
control		16 ± 1	62 ± 3	41 ± 1	6.3 ± 0.4	3.7 ± 0.9	1.51 ± 0.04	3.77 ± 0.23
1	1	18 ± 1	55 ± 2	38 ± 2	5.7 ± 0.4	4.3 ± 0.7	1.45 ± 0.05	3.65 ± 0.16
2	2	15 ± 1	56 ± 2	41 ± 2	5.7 ± 0.5	2.8 ± 0.2	1.37 ± 0.02	4.33 ± 0.30
3	5	18 ± 3	62 ± 3	45 ± 2	6.0 ± 0.5	3.9 ± 0.5	1.39 ± 0.02	4.23 ± 0.27
4	10	21 ± 2	85 ± 4[b]	61 ± 4[b]	8.1 ± 0.6	3.4 ± 0.3	1.39 ± 0.02	3.98 ± 0.20
5	20	19 ± 2	84 ± 3[b]	58 ± 1[b]	8.5 ± 0.4	5.3 ± 1.0	1.44 ± 0.02	4.05 ± 0.24
6	50	14 ± 1	101 ± 4[c]	61 ± 8[b]	7.1 ± 0.6	6.5 ± 1.5	2.03 ± 0.60	3.47 ± 0.13
7	100	12 ± 1	118 ± 5[c]	76 ± 2[c]	8.7 ± 0.4	5.7 ± 0.8	1.56 ± 0.03	3.40 ± 0.23

[a] Data are mean ± SEM for 6 rats per group. [b] Significantly different from control group at $p < 0.05$. [c] Significantly different from control group at $p < 0.01$. [d] TPC, total plasma cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; TG, triglycerides; T4, thyroxine.

Table 8. Effects of Compound **2.39** on Body Weight and Liver Parameters in Normal Chow-Fed Male Rats

	dose,[a]	body wt	liver	rel liver	liver enzymes,[d] U/L			
groups	mg/kg/day	gain, g	wt, g	wt, %	ALT	AST	ALKP	LDH
control		30.8 ± 2.2	8.0 ± 0.5	4.2 ± 0.1	56 ± 3	162 ± 11	306 ± 12	507 ± 47
1	1	26.3 ± 1.6	8.4 ± 0.5	4.3 ± 0.2	61 ± 3	216 ± 22	312 ± 18	724 ± 110
2	2	28.5 ± 1.3	7.3 ± 0.4	3.9 ± 0.1	59 ± 5	195 ± 22	373 ± 47	692 ± 68
3	5	30.7 ± 3.2	7.2 ± 0.5	3.8 ± 0.1	62 ± 5	216 ± 24	344 ± 38	743 ± 82
4	10	33.2 ± 2.2	8.3 ± 0.5	4.3 ± 0.2	58 ± 5	182 ± 21	327 ± 23	553 ± 87
5	20	30.3 ± 2.1	8.2 ± 0.5	4.3 ± 0.2	78 ± 5[c]	318 ± 37[c]	385 ± 25	1136 ± 151[c]
6	50	27.5 ± 0.8	7.3 ± 0.3	4.0 ± 0.1	62 ± 3	232 ± 19[b]	330 ± 21	815 ± 91[b]
7	100	22.5 ± 2.2[c]	7.8 ± 0.4	4.5 ± 0.1	67 ± 3	214 ± 26	382 ± 18	681 ± 112

[a] Data are mean ± SEM for six rats per group. [b] Significantly different from control group at $p < 0.05$. [c] Significantly different from control group at $p < 0.01$. [d] ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALKP, alkaline phosphatase; LDH, lactic dehydrogenase.

Table 9. Effects of Compound **2.39** on Serum HDL-C and Apolipoprotein-A1 Concentrations in Chow-fed Male Rats

dose,[a] mg/kg/day	HDL-C,[c] mg/dL	apo-A1,[d] mg/dL
0	51.9 ± 2.2	63.2 ± 2.4
5	52.4 ± 2.4	72.9 ± 3.6
10	73.1 ± 4.3[b]	79.2 ± 2.0
50	87.3 ± 3.3[b]	88.8 ± 6.9[b]
100	103.6 ± 4.2[b]	96.4 ± 5.3[b]

[a] Data are mean ± SEM for six rats per group. [b] Significantly different from control group at $p < 0.05$. [c] HDL-C, high-density lipoprotein cholesterol. [d] apo-A1, apolipoprotein A1.

Summary

Modulation of high-density lipoprotein cholesterol has been an area of intense research interest. The structure and metabolism of HDL are complex; however, studies in animals have defined several different mechanisms for increasing HDL cholesterol, the topic of several recent reviews.[27−29] In vitro assays have been developed to identify compounds that are effective in modulating one or more of these pathways. Often, the identified compound lacks in vivo activity or has adverse effects.[22] Since lipid modulators need to be taken on a chronic basis, maximum effectiveness and safety are prime concerns. We chose therefore to pursue a non-mechanism-biased approach for elevating HDL by using animal models for the initial evaluation of compounds. This approach presents several challenges. The initial quantity of test compound is increased, the throughput is significantly lower compared to the in vitro approach, and the SAR correlations are more challenging, but they are based on information obtained from several biological parameters, particularly bioavailability and safety aspects. These early indications can lead to a more focused direction and an actual overall time efficiency in candidate selection.

There is no single animal model that adequately predicts HDL-C elevation in man; however, several small animal models may provide a predictive approach toward the testing of novel lipid regulating drugs.[22] Our approach was to initially evaluate compounds in three sequential in vivo models. Subsequent complementary studies in nonhuman primates and experiments to elucidate the molecular mechanism of action were pursued prior to advancing selected drug candidates to preclinical safety assessment.[30,31]

The compounds disclosed here are potently efficacious in raising HDL cholesterol in both chow-fed and hypercholesterolemic rats as well as hamsters. Rodent species carry the majority of their cholesterol in the HDL cholesterol fraction but are susceptible to dietary cholesterol and elicit hypercholesterolemia when fed a diet containing cholesterol and cholic acid.[20,21] This animal model has been widely used in the testing of potentially valuable hypolipidemic compounds. The model's value for testing HDL-elevating drugs lies in its ability to shift the lipoprotein profile without increasing total cholesterol. Selective HDL elevation in the absence of significant diet-induced hypercholesterolemia was also achieved in chow-fed rats. Significantly fewer compounds exhibited efficacy for HDL elevation in rats on normal diets, analogous with the difficulty of raising plasma HDL in normolipidemic individuals with isolated low HDL cholesterol. The hamster model appears to more closely resemble human lipoprotein metabolism and as such was chosen as our third screen. Hamsters possess cholesteryl ester transfer protein (CETP) activity in plasma and share similarity with man in their regulation of LDL metabolism,[26] thus allowing for better predictability of the human HDL response.

The data from profiling 78 compounds yielded four candidate chemical entities (**2.39**, **2.48**, **4.05**, and **5.03**) with potent activity for mediating HDL elevation in all

Table 10. Effects of Compound **2.39** in Cholesterol-Fed Male Hamsters on Serum Lipids and T4 Levels after 14 Days of Treatment

		serum lipids,[f] mg/dL						
			cholesterol					
groups	dose, mg/kg/day	TG	TPC	HDL-C	LDL-C	VLDL-C	ratio TC/HDL-C	T4, μg/dL
cholesterol-fed control	0[b]	461 ± 107	448 ± 51	124 ± 4	203 ± 22	121 ± 32	3.64 ± 0.46	5.58 ± 0.23
1[a]	3[b]	431 ± 92	429 ± 34	136 ± 7	178 ± 14	115 ± 19	3.14 ± 0.12	5.80 ± 0.47
2[a]	10[b]	455 ± 35	370 ± 7[d]	133 ± 9	136 ± 5[e]	102 ± 8	2.85 ± 0.19[d]	5.73 ± 0.22
3[a]	30[b]	433 ± 46	372 ± 12[d]	156 ± 6[e]	125 ± 11[e]	91 ± 6	2.39 ± 0.06[e]	4.73 ± 0.20
4[a]	100[b]	342 ± 37	373 ± 12	191 ± 5[e]	102 ± 6[e]	81 ± 4	1.95 ± 0.03[e]	4.02 ± 0.23[e]
normal-fed control	0[c]	222 ± 20[d]	142 ± 13[e]	81 ± 2[e]	48 ± 11[e]	15 ± 2[e]	1.77 ± 0.12[e]	3.83 ± 0.71[e]

[a] All drug-treated groups were cholesterol-fed. [b] Data are mean ± SEM for six rats per group. [c] Data are mean ± SEM for four rats per group. [d] Significantly different from control group at $p < 0.05$. [e] Significantly different from control group at $p < 0.01$. [f] TPC, total plasma cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; TG, triglycerides; T4, Thyroxine.

Table 11. Effects of Compound **2.39** in Cholesterol-Fed Male Hamsters on Body Weight and Liver Parameters after 14 Days of Treatment

				liver enzymes,[e] U/L			
groups	dose, mg/kg/day	body wt gain, g	liver wt, g	ALT	AST	ALKP	LDH
cholesterol-fed control	0[b]	7.8 ± 3.4	8.97 ± 0.46	172 ± 12	267 ± 71	161 ± 13	1004 ± 243
1[a]	3[b]	10.2 ± 8.7	9.58 ± 0.64	182 ± 25	312 ± 35	154 ± 5	1234 ± 140
2[a]	10[b]	9.5 ± 8.3	9.08 ± 0.36	134 ± 7	345 ± 37	147 ± 9	1312 ± 129
3[a]	30[b]	8.2 ± 8.1	8.50 ± 0.28	112 ± 7	204 ± 18	136 ± 9	823 ± 67
4[a]	100[b]	9.0 ± 10.1	8.95 ± 0.69	639 ± 541	424 ± 227	136 ± 5	1387 ± 601
normal-fed control	0[c]	3.3 ± 6.1	7.98 ± 0.30	121 ± 17	272 ± 48	129 ± 17[d]	1154 ± 234

[a] All drug-treated groups were cholesterol-fed. [b] Data are mean ± SEM for six hamsters per group. [c] Data are mean ± SEM for four hamsters per group. [d] Significantly different from control group at $p < 0.05$. [e] ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALKP, alkaline phosphatase; LDH, lactic dehydrogenase.

three in vivo models. Several compounds modulate HDL very dramatically with elevations approaching 100% in selected models. Preliminary information gathered regarding general safety parameters (e.g. liver function enzymes, liver and body weight) was also quite favorable.

Earlier published work identified 2-ethylsulfanyl-imidazolone (**1**) as a potential biological lead exhibiting HDL-C elevating properties in several animal models.[15] Efforts to optimize the drug-like properties of **1** led us to explore the activity of the corresponding thiohydantoin series and other compounds with related ring systems. The presence of an embedded thiourea moiety was common to all active compounds. Prior work had established that replacement of the sulfur atom with oxygen abolished activity. Replacement of the sulfur with an *N*-cyano group, a well-established thiourea bioisostere,[23,24] or converting the thiourea into a thioamide also yielded inactive compounds. Also, activity of compounds diminished as the ring size became larger (seven-membered ring). Optimal activity was found in five- or six-membered systems with a small alkyl substituent at N-1 and an aryl substituent at N-3, preferably substituted in the ortho position. Of the various compounds tested, the thiohydantoin derivative **2.39** was found to have a superior efficacy and safety profile relative to other compounds tested in the same models at comparable doses.

Compound **2.39** induced dose-dependent increases in HDL-C in the chow-fed rat model and the hypercholesterolemic hamster model without adversely affecting non-HDL-C lipoproteins. In the rat, plasma apo-A1 increases in parallel with HDL cholesterol concentrations but without significant upregulation of liver or intestinal apo-A1 mRNA concentrations. Although the precise mechanism for the plasma apo-A1 increase is unknown, such phenotypic changes in the HDL fraction are expected to provide a cardioprotective benefit. Overexpression of apo-A1 has been shown to increase HDL and enhance the overall rate of reverse cholesterol transport in transgenic rabbits[32] and mice.[33,34] Apo-A1 influences HDL function as a coactivator for lecithin: cholesterol acyltransferase (LCAT)[35] and acceptor for ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux.[36] Its antiatherosclerotic potential has been well characterized in both animals and man.

Efforts toward identifying the molecular mechanism of **2.39** are ongoing. Results from initial studies in nonhuman primates, where **2.39** was identified as WAY-135433, revealed increases in apo-A1 levels and regulation of plasma post-heparin lipoprotein lipase (LPL) activity.[30] A role for lipoprotein lipase in modulating HDL levels has been clearly demonstrated in nonhuman primates[37] and man.[38] LPL-mediated lipolysis provides a means by which redundant phospholipid surface, from TG-rich lipoproteins, forms nascent HDL particles or is transferred to existing HDL pools.[39,40] This activity suggests a potential mechanism for the observed increases in apo-A1 and HDL, since these discoidal particles are excellent substrates for LCAT.

In conclusion, in an effort to discover novel compounds that selectively raise HDL-C, we optimized the structural properties, biological activity, and safety profiles of the earlier lead **1**. The work encompassed exploring SAR of substituted thiohydantoins and derivatives of related ring systems. Compound screening was carried out in three animal models leading to the discovery of the thiohydantoin derivative **2.39** as the optimal compound. The robust safety profile of **2.39** at high pharmacologic doses warranted further evaluation in nonhuman primates.[30] The positive results from testing in this model showed that our selection of small animal models for screening was successful in predicting lipid modulation in nonhuman primates and that agent **2.39** is a promising compound for raising HDL-C with a therapeutic potential as a cardioprotective agent for the prevention of atherosclerosis in man.

Experimental Section

Biological Materials and Methods. Animals and Diets: Hypercholesterolemic Rat. Male Sprague−Dawley rats weighing 140−170 g (Charles River, Kingston, NY) were fed Purina Rodent Chow Special Mix 5001-S supplemented with 0.25% cholic acid and 1.0% cholesterol and water ad libitum for 8 days.

At study initiation, animals were randomized into groups of six according to body weight and were housed three per cage. The test compound was admixed to the diet with the daily dose calculated on the basis of average daily food consumption. The actual average food consumption per cage was, however, monitored to verify that food intake amounts were consistent with the expected target dose. In addition, body weights taken on the first and last days of the study period were used to calculate average body weight gain and verify that different treatment groups have similar food intake and therefore receive roughly equivalent amounts of drug. At study termination, food is removed 2 h prior to blood collection from anesthetized rats.

Chow-Fed Rat. Male Sprague−Dawley CD rats (Charles River, Kingston, NY) weighing 60−80 g were maintained on Purina 5001 Chow with a reverse 12 h light/dark cycle (6:00 a.m. off/6:00 p.m. on). Water was provided ad libitum. The rats were fasted overnight and weighed for dose calculation on the first and third days of the study. Animals were administered a single daily dose of test compound suspended in 1.3% Tween 80, 0.25% CMC by oral gavage, for a period of 4 days. At the end of the study period, the animals were fasted overnight, weighed, and sacrificed for blood and tissue collection 24 h after the last treatment. Dose−response studies were performed as described above.

Hypercholesterolemic Hamster. Male Syrian Golden hamsters (Charles River, Kingston, NY) weighing 90−100 g were maintained on Purina 5001 Chow (Pet Specialties, Inc.), water ad libitum, and a 12 h light/dark cycle (6:00 a.m./6:00 p.m.). Hamsters were fed Purina 5001 Chow supplemented with 0.25% cholesterol (BioServe, Frenchtown, NJ) for 2 weeks prior to initiation of dosing and were maintained on this diet for the study duration. A group of four hamsters was maintained on normal chow for comparative purposes. Test compound was suspended in 2% Tween 80 in saline and administered orally by gavage for 14 days. Control animals received vehicle alone. Animals were fasted for 4 h and dosed the last time 2 h prior to sacrifice. Animals were weighed prior to collection of blood and liver. Multiple dose studies were performed in the same manner.

Serum Lipid Analysis. Serum was separated by centrifugation from blood collected on ice. Serum lipids (total cholesterol and triglycerides) were measured on the Hitachi 911 Clinical Analyzer using the Boehringer-Mannheim cholesterol and triglyceride (glycerol-blanked) reagents. Liver enzymes and T4 levels were measured on a Hitachi 911 Clinical Analyzer. Serum lipoproteins were analyzed by FPLC.[41] Cholesterol was quantitated in each of the lipoprotein fractions using the Boehringer-Mannheim cholesterol reagent.

Quantitation of Apolipoprotein A1. Plasma apolipoprotein A-1 was analyzed with an enzyme-linked immuno-

sorbant assay (ELISA). This assay utilized a rabbit anti-rat primary antibody,[42] an anti-rabbit IgG-alkaline phosphatase conjugate secondary antibody (Sigma, St. Louis, MO), and soluble alkaline phosphatase substrate (*p*-nitrophenyl phosphate) reagent (Sigma, St. Louis, MO).

Evaluation of Results. Cholesterol ratio, TC/HDL-C, body weight gain (calculated from the difference of the body weights at study initiation and termination), and relative liver weight (% of body weight) were calculated. All data were expressed as the mean ± SEM. The statistical significance of the data was assessed using the one-way analysis of variance and Dunnett's multiple comparison *t*-test in the Wyeth-Ayerst HT Basic Statistical Package or JMP (SAS Institute Inc., Cary, NC.). Differences in the means were considered significant at $p < 0.05$.

Chemistry. Melting points were determined on a Thomas-Hoover Mel-temp apparatus and are uncorrected. The NMR spectra were recorded on a Varian VXR300, or a Bruker AM-400 spectrometer in DMSO-d_6 as a solvent, unless otherwise stated. Mass spectra were obtained using Hewlett-Packard 5995A or a Finigan 8230 mass spectrometer. Elemental analyses were obtained using a Perkin-Elmer Model 240 analyzer. Flash chromatography was performed using EM Science 230-400 mess silica gel. Reported yields represent isolated products following purification by conventional methods including extraction, chromatography, and/or crystallization. Organic extracts were dried over anhydrous magnesium sulfate or sodium sulfate unless otherwise noted.

1-Methyl-3-phenyl-2-thioxoimidazolidin-4-one (2.01). A mixture of phenyl isothiocyanate (13.5 g, 0.1 mol), sarcosine ethyl ester hydrochloride (15.35 g, 0.1 mol), triethylamine (11.1 g, 0.11 mol), and chloroform (300 mL) was heated at reflux for 18 h. The mixture was cooled to room temperature, washed with 2 N hydrochloric acid and then with water, and evaporated to dryness. The residual solid was stirred in ethyl acetate (100 mL) for 1 h and then filtered. The solid was rinsed with ethyl acetate and air-dried to give 15.8 g (77%) of the title compound as a light peach solid: mp 163−165 °C; ^{1}H NMR δ 7.39−7.50 (m, 3H), 7.26−7.28 (m, 2H), 4.41 (s, 2H), 3.27 (s, 3H); MS (EI, $M^{\bullet+}$) *m*/*z* 206. Anal. for $C_{10}H_{10}N_2OS$: C, H, N.

The following compounds were prepared from sarcosine ethyl ester hydrochloride (50 mmol) and the appropriate isothiocyanate (55 mmol) in the manner described for compound **2.01**.

1-Methyl-3-(pyridin-3-yl)-2-thioxoimidazolidin-4-one (2.03): off-white solid (72%); mp 149−151 °C; ^{1}H NMR (DMSO-d_6) δ 8.60 (dd, J = 4.83 and 1.54 Hz, 1H), 8.51 (dd, J = 2.53 and 0.77 Hz, 1H), 7.75−7.78 (m, 1H), 7.53−7.56 (m, 1H), 4.44 (s, 2H), 3.29 (s, 3H); MS (EI, $M^{\bullet+}$) *m*/*z* 207. Anal. for $C_9H_9N_3OS$: C, H, N.

3-Benzhydryl-1-methyl-2-thioxoimidazolidin-4-one (2.05): off-white solid (68%); mp 139−141 °C; ^{1}H NMR δ 7.25−7.37 (m, 10H), 7.12 (s, 1H), 4.30 (s, 2H), 3.26 (s, 3H); MS (EI, $M^{\bullet+}$) *m*/*z* 296. Anal. for $C_{17}H_{16}N_2OS$: C, H, N.

1-Methyl-3-(4-phenylbutyl)-2-thioxoimidazolidin-4-one (2.07): yellow solid (79%); mp 76−77 °C; ^{1}H NMR (DMSO-d_6) δ 7.23−7.27 (m, 2H), 7.13−7.19 (m, 3H), 4.23 (s, 2H), 3.67 (t, 2H, J = 6.81 Hz), 3.18 (s, 3H), 2.57 (t, 2H, J = 7.25 Hz), 1.51−1.59 (m, 4H); MS (EI, $M^{\bullet+}$) *m*/*z* 262. Anal. for $C_{14}H_{18}N_2OS$: C, H, N.

1-Methyl-2-thioxo-3-[2-(*p*-tolyl)ethyl]imidazolidin-4-one (2.08): tan solid (90%); mp 126−128 °C; ^{1}H NMR (DMSO-d_6) δ 7.10 (s, 4H), 4.22 (s, 2H), 3.79−3.83 (m, 2H), 3.20 (s, 3H), 2.78−2.80 (m, 2H), 2.25 (s, 3H); MS (EI, $M^{\bullet+}$) *m*/*z* 248. Anal. for $C_{13}H_{16}N_2OS$: C, H, N.

3-[2-(4-Chlorophenyl)ethyl]-1-methyl-2-thioxoimidazolidin-4-one (2.09): light peach solid (84%); mp 116−118 °C; ^{1}H NMR δ 7.32−7.35 (m, 2H), 7.21−7.24 (m, 2H), 4.22 (s, 2H), 3.82−3.86 (m, 2H), 3.20 (s, 3H), 2.84−2.87 (m, 2H); MS (EI, $M^{\bullet+}$) *m*/*z* 268/270. Anal. for $C_{12}H_{13}ClN_2OS$: C, H, N.

3-(2-Chlorobenzyl)-1-methyl-2-thioxoimidazolidin-4-one (2.11): tan solid (77%); mp 144−146 °C; ^{1}H NMR δ 7.45−7.47 (m, 1H), 7.25−7.32 (m, 2H), 7.08−7.10 (m, 1H), 4.92 (s, 2H), 4.40 (s, 2H), 3.26 (s, 3H); MS (FAB, $[M + H]^+$) *m*/*z* 255/257. Anal. for $C_{11}H_{11}ClN_2OS$: C, H, N.

1-Methyl-3-(2-piperidin-1-ylethyl)-2-thioxoimidazolidin-4-one (2.12): off-white solid (68%); mp 153−155 °C; ^{1}H NMR δ 10.65−10.75 (br s, 1H), 4.25 (s, 2H), 4.04 (t, 2H, J = 6.26 Hz), 3.52 (br, d, 2H, J = 11.64 Hz), 3.26 (q, 2H, J = 5.93 Hz), 3.19 (s, 3H), 2.83−2.92 (m, 2H), 1.64−1.86 (m, 5H), 1.32−1.40 (m, 1H); MS (EI, $M^{\bullet+}$) *m*/*z* 241. Anal. for $C_{11}H_{19}N_3OS{\cdot}HCl$: C, H, N.

3-(Indan-5-yl)-1-methyl-2-thioxoimidazolidin-4-one (2.13): tan solid (73%); mp 158−160 °C; ^{1}H NMR δ 7.29(d, 1H, J = 7.91 Hz), 7.07 (s, 1H), 6.97 (dd, 1H, J = 7.80 and 1.87 Hz), 4.38 (s, 2H), 3.26 (s, 3H), 2.88 (t, 4H, J = 7.47 Hz), 2.04−2.08 (m, 2H); MS (EI, $M^{\bullet+}$) *m*/*z* 246. Anal. for $C_{13}H_{14}N_2OS$: C, H, N.

3-(2,6-Dichlorophenyl)-1-methyl-2-thioxoimidazolidin-4-one (2.17): light peach solid (88%); mp 207−209 °C; ^{1}H NMR δ 7.68 (d, 1H, J = 0.88 Hz), 7.66 (d, 1H, J = 0.44 Hz), 7.54−7.58 (m, 1H), 4.69 (s, 2H), 3.30 (s, 3H); MS (EI, $M^{\bullet+}$) *m*/*z* 274. Anal. for $C_{10}H_8Cl_2N_2OS$: C, H, N.

3-(2-Chloro-6-methylphenyl)-1-methyl-2-thioxoimidazolidin-4-one (2.18): orange solid (75%); mp 142−145 °C; ^{1}H NMR δ 7.33−7.47 (m, 3H), 4.60 (s, 2H), 3.30 (s, 3H), 2.14 (s, 3H); MS (EI, $M^{\bullet+}$) *m*/*z* 254. Anal. for $C_{11}H_{11}ClN_2OS$: C, H, N.

***N*-Ethylglycine (10.02).** Ethylamine (101 mL of 70% solution in water, 1.25 mol) was added slowly to the solution of glyoxylic acid monohydrate (92.05 g, 1 mol) in water (700 mL) while stirring. The reaction temperature was kept below 40 °C. The mixture was stirred at 35−40 °C for 1 h and then transferred to a pressure hydrogenation bottle containing 10% palladium on carbon (20 g) in water (200 mL). The mixture was hydrogenated at 50 psi for 18 h. The catalyst was removed by filtration. The filtrate was concentrated to half volume under reduced pressure (<50 °C). 2-Propanol (100 mL portions) was added and the mixture was repeatedly concentrated until a solid residue was obtained. The solid was collected, stirred in 2-propanol, and filtered. The solid was washed with ether and dried to give 57.5 g of *N*-ethylglycine (56%) as a white solid: mp 188−190 °C; ^{1}H NMR δ 3.10 (s, 2H), 2.82 (q, 2H, J = 7.25 Hz), 1.12 (t, 3H, J = 7.25 Hz); MS (FI, $M^{\bullet+}$) *m*/*z* 103. Anal. for $C_4H_9NO_2$: C, H, N.

3-(5-Chloro-2-methylphenyl)-1-ethyl-2-thioxoimidazolidin-4-one (2.39): A mixture of **10.02** (5.15 g, 0.05 mol), 5-chloro-2-methylphenyl isothiocyanate (9.18 g, 0.05 mol), triethylamine (15 mL), and chloroform (250 mL) was heated at reflux for 18 h. The solvent was evaporated. The residue was dissolved in ethyl acetate (400 mL) and water (300 mL). The organic phase was washed with 1 N HCl (2 × 300 mL) and then evaporated to dryness. The solid residue was crystallized from ethanol to give compound (12.5 g) of **2.39** as a pink solid (93%): mp 152−154 °C; ^{1}H NMR (DMSO-d_6; 400 MHz) δ 7.43 (dd, 1H, J = 8.35 and 2.20 Hz), 7.37−7.39 (m, 1H), 7.34 (d, 1H, J = 2.20 Hz), 4.53 (d, 1H, J = 19.8 Hz), 4.38 (d, 1H, J = 19.8 Hz), 3.76−3.82 (m, 2H), 2.07 (s, 3H), 1.21 (t, 3H, J = 7.14 Hz); MS (EI, $M^{\bullet+}$) *m*/*z* 268/270. Anal. for $C_{12}H_{13}ClN_2OS$: C, H, N.

The following compounds were prepared from **10.02** (50 mmol) and the appropriate isothiocyanate (50 mmol) in the manner described for compound **2.39**.

3-Cyclohexyl-1-ethyl-2-thioxoimidazolidin-4-one (2.02): tan solid (88%); mp 99−102 °C; ^{1}HNMR δ 4.35−4.43 (m, 1H), 4.18 (s, 2H), 3.69 (q, 2H, J = 7.25 Hz), 2.04−2.14 (m, 2H), 1.77 (d, 2H, J = 12.96 Hz), 1.55−1.62 (m, 3H), 1.07−1.28 (m, 3H), 1.23 (t, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) *m*/*z* 226. Anal. for $C_{11}H_{18}N_2OS$: C, H, N.

1-Ethyl-3-(naphthalen-2-yl)-2-thioxoimidazolidin-4-one (2.04): pink solid (69%); mp 156−159 °C; ^{1}H NMR δ 7.96−8.01 (m, 3H), 7.86 (d, 1H, J = 1.76 Hz), 7.55−7.62 (m, 2H), 7.41 (dd, 1H, J = 8.79 and 1.98 Hz), 4.48 (s, 2H), 3.83 (q, 2H, J = 7.25 Hz), 1.23 (t, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) *m*/*z* 270. Anal. for $C_{15}H_{14}N_2OS$: C, H, N.

1-Ethyl-3-phenethyl-2-thioxoimidazolidin-4-one (2.06): yellow solid (90%); mp 66−68 °C; ^{1}H NMR δ 7.28−

7.31 (m, 2H), 7.19−7.23 (m, 3H), 4.23 (s, 2H), 3.83−3.87 (m, 2H), 3.73 (q, 2H, J = 7.25 Hz), 2.83−2.87 (m, 2H), 1.14 (t, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) m/z 248. Anal. for $C_{13}H_{16}N_2OS$: C, H, N.

1-Ethyl-3-[1-(4-fluorophenyl)ethyl]-2-thioxoimidazolidin-4-one (2.10): light yellow solid (87%); mp 93−95 °C; ^{1}H NMR δ 7.39−7.43 (m, 2H), 7.11−7.17 (m, 2H), 5.89 (q, 1H, J = 7.25 Hz), 4.22 (d, 2H, J = 1.98 Hz), 3.68−3.78 (m, 2H), 1.73 (d, 3H, J = 7.25 Hz), 1.14 (t, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) m/z 266. Anal. for $C_{13}H_{15}FN_2OS$: C, H, N.

1-Ethyl-3-(2-tolyl)-2-thioxoimidazolidin-4-one (2.14): off-white solid (83%); mp 105−108 °C; ^{1}H NMR δ 7.26−7.35 (m, 3H), 7.15 (d, 1H, J = 7.47 Hz), 4.53 (d, 1H, J = 19.99 Hz), 4.43 (d, 1H, J = 19.99 Hz), 3.77−3.83 (m, 2H), 2.08 (s, 3H), 1.21 (t, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) m/z 234. Anal. for $C_{12}H_{14}N_2OS$: C, H, N.

3-(2,6-Dichlorophenyl)-1-ethyl-2-thioxoimidazolidin-4-one (2.16): tan solid (90%); mp 170−172 °C; ^{1}H NMR δ 7.67 (dd, 2H, J = 8.13 and 0.66 Hz), 7.54−7.58 (m, 1H), 4.70 (s, 2H), 3.80 (q, 2H, J = 7.18 Hz), 1.22 (t, 3H, J = 7.14 Hz); MS (EI, $M^{\bullet+}$) m/z 288/290/292. Anal. for $C_{11}H_{10}Cl_2N_2OS$: C, H, N.

3-(2-Chloro-6-methylphenyl)-1-ethyl-2-thioxoimidazolidin-4-one (2.19): tan solid (67%); mp 124−126 °C; ^{1}H NMR δ 7.33−7.47 (m, 3H), 4.62 (s 2H), 3.77−3.83 (m, 2H), 2.14 (s, 3H), 1.22 (t, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) m/z 268/270. Anal. for $C_{12}H_{13}ClN_2OS$: C, H, N.

3-(2,6-Dimethylphenyl)-1-ethyl-2-thioxoimidazolidin-4-one (2.37): tan solid (57%); mp 128−131 °C; ^{1}H NMR δ 7.22−7.26 (m, 1H), 7.15−7.17 (m, 2H), 4.57 (s, 2H), 3.80 (q, 2H, J = 7.25 Hz), 2.04 (s, 6H), 1.21 (t, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) m/z 248. Anal. for $C_{13}H_{16}N_2OS$: C, H, N.

3-(4-Dimethylaminophenyl)-1-ethyl-2-thioxoimidazolidin-4-one (2.38): light pink solid (80%); mp 159−161 °C; ^{1}H NMR δ 7.01−7.03 (m, 2H), 6.72−6.74 (m, 2H), 4.37 (s, 2H), 3.78 (q, 2H, J = 7.25 Hz), 2.92 (s, 6H), 1.19 (t, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) m/z 263. Anal. for $C_{13}H_{17}N_3OS$: C, H, N.

1-Ethyl-3-(4-fluorophenyl)-2-thioxoimidazolidin-4-one (2.40): pink solid (75%); mp 149−151 °C; ^{1}H NMR δ 7.28−7.36 (m, 4H), 4.40 (s, 2H), 3.80 (q, 2H, J = 7.18 Hz), 1.20 (t, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) m/z 238. Anal. for $C_{11}H_{11}FN_2OS$: C, H, N.

3-(5-Chloro-2-methoxyphenyl)-1-ethyl-2-thioxoimidazolidin-4-one (2.41): tan solid (7%); mp 171−174 °C; ^{1}H NMR δ 7.50 (dd, 1H, J = 9.01 and 2.64 Hz), 7.33 (d, 1H, J = 2.64 Hz), 7.19 (d, 1H, J = 9.01 Hz), 4.52 (d, 1H, J = 19.99 Hz), 4.39 (d, 1H, J = 20.21 Hz), 3.71−3.84 (m, 5H), 1.20 (t, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) m/z 284/286. Anal. for $C_{12}H_{13}ClN_2O_2S$: C, H, N.

1-Ethyl-2-thioxo-3-(4-trifluoromethoxyphenyl)imidazolidin-4-one (2.42): tan solid (46%); mp 116−119 °C; ^{1}H NMR δ 7.39−7.46 (m, 4H), 4.38 (s, 2H), 3.76 (q, 2H, J = 7.2 Hz), 1.17 (t, 3H, J = 7.2 Hz); MS (EI, $M^{\bullet+}$) m/z 304. Anal. for $C_{12}H_{11}F_3N_2O_2S$: C, H, N.

1-Ethyl-3-(5-fluoro-2-methylphenyl)-2-thioxoimidazolidin-4-one (2.43): pink solid (83%); mp 98−100 °C; ^{1}H NMR δ 7.36−7.40 (m, 1H), 7.23 (td, 1H, J = 8.50 and 2.79 Hz), 7.13 (dd, 1H, J = 9.35 and 2.75 Hz), 4.54 (d, 1H, J = 19.99 Hz), 4.40 (d, 1H, J = 19.99 Hz), 3.75−3.84 (m, 2H), 2.06 (s, 3H), 1.21 (t, 3H, J = 7.14 Hz); MS (EI, $M^{\bullet+}$) m/z 252. Anal. for $C_{12}H_{13}FN_2OS$: C, H, N.

1-Ethyl-2-thioxo-3-(2-trifluoromethylphenyl)imidazolidin-4-one (2.44): light pink solid (51%); mp 82−85 °C; ^{1}H NMR δ 7.81−7.88 (m, 2H), 7.69−7.73 (m, 1H), 7.50 (d, 1H, J = 7.91 Hz), 4.59 (d, 1H, J = 20.21 Hz), 4.44 (d, 1H, J = 20.21 Hz), 3.71−3.87 (m, 2H), 1.20 (t, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) m/z 288. Anal. for $C_{12}H_{11}F_3N_2OS$: C, H, N.

1-Ethyl-3-(2-ethyl-6-isopropylphenyl)-2-thioxoimidazolidin-4-one (2.45): white solid (52%); mp 77−79 °C; ^{1}H NMR δ 7.38 (t, 1H, J = 7.69 Hz), 7.28 (dd, 1H, J = 7.80 and 1.38 Hz), 7.18 (m, 1H), 4.61 (s, 2H), 3.72−3.87 (m, 2H), 2.60−2.66 (m, 1H), 2.28−2.38 (m, 2H), 1.22 (t, 3H, J = 7.25 Hz), 1.14 (d, 3H, J = 6.81 Hz), 1.08 (t, 3H, J = 7.47 Hz), 1.04 (d, 3H, J = 6.81 Hz); MS (EI, $M^{\bullet+}$) m/z 290. Anal. for $C_{16}H_{22}N_2OS$: C, H, N.

1-Ethyl-3-(2-ethyl-6-methylphenyl)-2-thioxoimidazolidin-4-one (2.46): light peach solid (66%); mp 82−84 °C; ^{1}H NMR δ 7.30 (t, 1H, J = 7.58 Hz), 7.18 (t, 2H, J = 7.80 Hz), 4.58 (s, 2H), 3.78−3.84 (m, 2H), 2.33−2.39 (m, 2H), 2.03 (s, 3H), 1.22 (t, 3H, J = 7.25 Hz), 1.08 (t, 3H, J = 7.58 Hz); MS (EI, $M^{\bullet+}$) m/z 262. Anal. for $C_{14}H_{18}N_2OS$: C, H, N.

3-(2,4-Dichlorophenyl)-1-ethyl-2-thioxoimidazolidin-4-one (2.47): light peach solid (74%); mp 144−146 °C; ^{1}H NMR δ 7.79 (dd, 1H, J = 8.02 and 1.65 Hz), 7.52 (t, 1H, J = 8.02 Hz), 7.46 (dd, 1H, J = 7.91 and 1.76 Hz), 4.61 (d, 1H, J = 20.21 Hz), 4.48 (d, 1H, J = 19.99 Hz), 3.77−3.83 (m, 2H), 1.22 (t, 3H, J = 7.25 Hz); MS (CI, $[M + H]^+$) m/z 289/291/293. Anal. for $C_{11}H_{10}Cl_2N_2OS$: C, H, N.

3-(2,4-Dimethylphenyl)-1-ethyl-2-thioxoimidazolidin-4-one (2.48): light peach solid (87%); mp 161−162 °C; ^{1}H NMR δ 7.14 (s, 1H), 7.08 (dd, 1H, J = 12.0 and 3.20 Hz), 7.01 (d, 1H, J = 13.2 Hz), 4.52 (d, 1H, J = 22.0 Hz), 4.42 (d, 1H, J = 21.2 Hz), 3.72−3.87 (m, 1H), 2.32 (s, 3H), 2.03 (s, 3H), 1.21 (t, 3H, J = 8.00 Hz); MS (EI, $M^{\bullet+}$) m/z 248. Anal. for $C_{13}H_{16}N_2OS$: C, H, N.

3-(2,5-Dimethylphenyl)-1-ethyl-2-thioxoimidazolidin-4-one (2.49): off-white solid (85%); mp 176−178 °C; ^{1}H NMR δ 7.21 (d, 1H, J = 7.91 Hz), 7.14−7.17 (m, 1H), 6.96 (m, 1H), 4.51 (d, 1H, J = 19.99 Hz), 4.42 (d, 1H, J = 19.99 Hz), 3.74−3.84 (m, 2H), 2.28 (s, 3H), 2.02 (s, 3H), 1.20 (t, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) m/z 248. Anal. for $C_{13}H_{16}N_2OS$: C, H, N.

1-Ethyl-2-thioxo-3-(2,4,5-trimethylphenyl)imidazolidin-4-one (2.50): off-white solid (95%); mp 162−164 °C; ^{1}H NMR δ 7.09 (s, 1H), 6.89 (s, 1H), 4.49 (d, 1H, J = 19.99 Hz), 4.40 (d, 1H, J = 19.99 Hz), 3.73−3.84 (m, 2H), 2.19 (d, 6H, J = 13.62 Hz), 1.99 (s, 3H), 1.20 (t, 3H, J = 7.14 Hz); MS (EI, $M^{\bullet+}$) m/z 262. Anal. for $C_{14}H_{18}N_2OS$: C, H, N.

1-Ethyl-3-(2-isopropylphenyl)-2-thioxoimidazolidin-4-one (2.51): white solid (86%); mp 125−127 °C; ^{1}H NMR δ 7.40−7.46 (m, 2H), 7.25−7.29 (m, 1H), 7.09−7.11 (m, 1H), 4.55 (d, 1H, J = 19.99 Hz), 4.43 (d, 1H, J = 19.99 Hz), 3.73−3.86 (m, 2H), 2.69−2.74 (m, 1H), 1.21 (t, 3H, J = 7.25 Hz), 1.14 (d, 3H, J = 6.81 Hz), 1.07 (d, 3H, J = 6.81 Hz); MS (EI, $M^{\bullet+}$) m/z 262. Anal. for $C_{14}H_{18}N_2OS$: C, H, N.

1-Ethyl-3-(2-methylsulfanylphenyl)-2-thioxoimidazolidin-4-one (2.52): tan solid (66%); mp 128−131 °C; ^{1}H NMR δ 7.43−7.48 (m, 2H), 7.26−7.30 (m, 1H), 7.19−7.21 (m, 1H), 4.54 (d, 1H, J = 20.21 Hz), 4.43 (d, 1H, J = 20.21 Hz), 3.76−3.82 (m, 2H), 2.39 (s, 3H), 1.21 (t, 3H, J = 7.14 Hz); MS (EI, $M^{\bullet+}$) m/z 266. Anal. for $C_{12}H_{14}N_2S_2O$: C, H, N.

1-Benzyl-3-(2,6-dichlorophenyl)-2-thioxoimidazolidin-4-one (2.15): light yellow solid (79%); mp 124−125 °C; ^{1}H NMR δ 7.71 (d, 1H, J = 0.88 Hz), 7.69 (s, 1H), 7.56−7.60 (m, 1H), 7.39−7.42 (m, 2H), 7.31−7.36 (m, 3H), 5.06 (s, 2H), 4.58 (s, 2H); MS (EI, $M^{\bullet+}$) m/z 350/352/354. Anal. for $C_{16}H_{12}Cl_2N_2OS$: C, H, N.

1-Butyl-3-(2-chloro-6-methylphenyl)-2-thioxoimidazolidin-4-one (2.20). 2-Chloro acetic acid (47.3 g) was added portionwise while stirring to *n*-butylamine (500.0 g) over a period of 30 min. The mixture was stirred at room temperature for 18 h. The excess *n*-butylamine was evaporated, leaving a viscous clear oil (120.0 g), which solidified upon standing. The crude product consisted of a 1:1 mixture of *N*-butylglycine and butylamine hydrochloride. The mixture of crude *N*-butylglycine (8.5 g, 0.035 mol), 2-chloro-6-methylphenyl isothiocyanate (6.5 g, 0.035 mol), triethylamine (14 mL), and methylene chloride (150 mL) was heated at reflux for 5 h. The solvent was evaporated and the residue was chromatographed on silica gel using methylene chloride as an eluant. Crystallization from ethanol afforded 3.85 g of the title compound (74%) as a light pink solid: mp 97−100 °C; MS (FAB, $[M + H]^+$) m/z 297/299. ^{1}H NMR δ 7.33−7.47 (m, 3H), 4.60 (s, 2H), 3.73−3.85 (m, 2H), 2.14 (s, 3H), 1.62−1.69 (m, 2H), 1.28−1.38 (m, 2H), 0.92 (t, 3H, J = 7.36 Hz). Anal. for $C_{14}H_{17}ClN_2OS$: C, H, N.

3-(2,6-Dimethylphenyl)-1-isopropyl-2-thioxoimidazolidin-4-one (2.23). Reaction of 2,6-dimethylphenyl isothiocyanate (50 mmol) with *N*-isopropylglycine[43] (50 mmol) in the manner described for compound **2.39** afforded the title compound as a white solid (94%): mp 135−137 °C; ^{1}H NMR δ

7.22−7.26 (m, 1H), 7.16 (dd, 2H, J = 7.25 and 0.44 Hz), 4.81−4.86 (m, 1H), 4.51 (s, 2H), 2.04 (s, 6H), 1.25 (d, 6H, J = 6.81 Hz); MS (FAB, $[M + H]^+$) m/z 263. Anal. for $C_{14}H_{18}N_2OS$: C, H, N.

1-Allyl-3-(2,6-dimethylphenyl)-2-thioxoimidazolidin-4-one (2.24). Reaction of 2,6-dimethylphenyl isothiocyanate (50 mmol) with *N*-allylglycine[44] (50 mmol) in the manner described for compound **2.39** afforded the title compound as an orange solid (64%): mp 43−46 °C; ^{1}H NMR δ 7.23−7.27 (m, 1H), 7.16 (d, 2H, J = 7.91 Hz), 5.84−5.94 (m, 1H), 5.23−5.29 (m, 2H), 4.51 (s, 2H), 4.43−4.44 (m, 2H), 2.05 (s, 6H); MS (EI, $M^{\bullet+}$) m/z 260. Anal. for $C_{14}H_{16}N_2OS$: C, H, N.

3-(2,6-Dimethylphenyl)-1-(prop-2-ynyl)-2-thioxoimidazolidin-4-one (2.25). Reaction of 2,6-dimethylphenyl isothiocyanate (50 mmol) with *N*-propargylglycine ethyl ester (50 mmol) in the manner described for compound **2.39** yielded a yellow solid (84%): mp 117−121 °C; ^{1}H NMR δ 7.24−7.28 (m, 1H), 7.16−7.18 (m, 2H), 4.68 (d, 2H, J = 2.42 Hz), 4.57 (s, 2H), 3.48 (t, 1H, J = 2.53 Hz), 2.04 (s, 6H); MS (EI, $M^{\bullet+}$) m/z 258. Anal. for C_{14} $H_{14}N_2S_2O$: C, H, N.

3-(2-Chloro-6-methylphenyl)-1-phenyl-2-thioxoimidazolidin-4-one (2.21). Reaction of *N*-phenylglycine ethyl ester (50 mmol) with 2-chloro-6-methylphenyl isothiocyanate (50 mmol) in the manner described for compound **2.39** furnished a thiourea derivative which upon refluxing in ethanol and triethylamine afforded the title compound as a white solid (56%): mp 154−156 °C; ^{1}H NMR δ 7.77−7.80 (m, 2H), 7.34−7.52 (m, 6H), 5.07−5.19 (m, 2H), 2.23 (s, 3H); MS (FAB, $[M + H]^+$) m/z 317/319. Anal. for $C_{16}H_{13}ClN_2OS$: C. H, N.

3-(5-Chloro-2-methylphenyl)-1-methoxy-2-thioxo-imidazolidin-4-one (2.22). The mixture of ethyl methoxyamino acetate (6.65 g, 50 mmol), 5-chloro-2-methylphenyl isothiocyanate (9.18 g, 50 mmol), and pyridine (4 mL) in ether (50 mL) was stirred at room temperature for 2 h. The ether was evaporated under vacuum. The residual solid was slurried in hexane and filter to give 13.8 g (88%) of *N*-[[(5-chloro-2-methylphenyl)amino]thioxomethyl]-*N*-methoxyglycine ethyl ester (**11.22**) as a white solid: mp 88−90 °C; ^{1}H NMR δ 10.09 (s, 1H), 7.29−7.24 (m, 2H), 7.17 (s, 1H), 4.85 (s, 2H), 4.13 (q, 2H, J = 7.03 Hz), 3.75 (s, 3H), 2.17 (s, 3H), 1.20 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) m/z 316/318. Anal. for $C_{13}H_{17}ClN_2OS$: C. H, N. This compound was suspended in 6 N hydrochloric acid solution (180 mL), and the mixture was heated at reflux for 1 h, cooled to room temperature, and diluted with water (100 mL). The solid was collected by filtration, washed with ether, and dried to give 9.5 g of the title compound (81%) as a white solid: mp 175−177 °C; ^{1}H NMR δ 7.45 (dd, 1H, J = 8.35 and 2.20 Hz), 7.38−7.40 (m, 2H), 4.80 (d, 1H, J = 18.01 Hz), 4.62 (d, 1H, J = 18.01 Hz), 3.90 (s, 3H), 2.09 (s, 3H); MS (FAB, $[M + H]^+$) m/z 271/273. Anal. for $C_{11}H_{11}ClN_2O_2S$: C, H, N.

3-[3-(4-Chloro-2-methylphenyl)-4-oxo-2-thioxoimidazolidin-1-yl]propionic Acid Methyl Ester (2.26). The mixture of 3-(ethoxycarbonylmethylamino)propionic acid methyl ester[43] (5.7 g, 30 mmol), 4-chloro-2-methylphenyl isothiocyanate (5.5 g, 30 mmol), triethylamine (5 mL), and methylene chloride (75 mL) was stirred at room temperature for 4 h. The solvent was evaporated and the residue was chromatographed on silica gel (20% ethyl acetate in hexane) to give 5.4 g (55%) of the title compound as a yellow solid: mp 112−114 °C; ^{1}H NMR δ 7.46 (dd, 1H, J = 2.31 and 0.55 Hz), 7.35−7.38 (m, 1H), 7.20 (d, 1H, J = 8.57 Hz), 4.53 (d, 1H, J = 19.77 Hz), 4.42 (d, 1H, J = 19.99 Hz), 3.97−4.01 (m, 2H), 3.63 (s, 3H), 2.78 (t, 2H, J = 7.25 Hz), 2.08 (s, 3H); MS (EI, $M^{\bullet+}$) m/z 326/328. Anal. for $C_{14}H_{15}ClN_2S_3O$: C, H, N.

2-Ethylamino-2-methylpropionic Acid (10.33). 2-Bromo-2-methylpropionic acid (33.4 g, 0.2 mol) was added portionwise to a 70% aqueous ethylamine solution (258 g, 0.4 mol). After stirring at room temperature for 18 h, the solvent was evaporated under reduced pressure. Chloroform (300 mL) was added and then evaporated. The residue was treated with ethanol and the formed solid was collected by filtration, washed with ether, and air-dried to give 27.9 g (71%) of the title compound as a white solid: mp >300 °C; ^{1}H NMR (D_2O) δ 2.87 (q, 2H, J = 7.25 Hz), 1.30 (s, 6H), 1.13 (t, 3H, J = 7.25 Hz); MS (FAB, $[M + H]^+$) m/z 132. Anal. for $C_6H_{13}NO_2$: C, H, N.

3-(5-Chloro-2-methylphenyl)-1-ethyl-5,5-dimethyl-2-thioxoimidazolidin-4-one (2.33). Reaction of **10.33** (50 mmol) with 5-chloro-2-methylphenyl isothiocyanate in the manner described for compound **2.39** furnished the title compound as a white solid (85%): mp 148−150 °C; ^{1}H NMR δ 7.45 (m, 2H), 7.38 (d, 1H, J = 8.13 Hz), 3.73−3.80 (m, 2H), 2.03 (s, 3H), 1.51 (d, 6H, J = 14.06 Hz), 1.28 (t, 3H, J = 7.14 Hz); MS (EI, $M^{\bullet+}$) m/z 296/298. Anal. for $C_{14}H_{17}ClN_2OS$: C, H, N.

3-(2,6-Dichlorophenyl)-1-ethyl-5,5-dimethyl-2-thioxoimidazolidin-4-one (2.34). Reaction of **10.33** (50 mmol) with 2,6-dichlorophenyl isothiocyanate in the manner described for compound **2.39** furnished the title compound as a white solid (79%): mp 151−153 °C; ^{1}H NMR δ 7.68 (d, 1H, J = 1.10 Hz), 7.66 (s, 1H), 7.54−7.58 (m, 1H), 3.79 (q, 2H, J = 7.10 Hz), 1.53 (s, 6H), 1.29 (t, 3H, J = 7.03 Hz); MS (FAB, $[M + H]^+$) m/z 317/319/321. Anal. for $C_{13}H_{14}Cl_2N_2OS$: C, H, N.

3-(2,6-Dimethylphenyl)-1-ethyl-5,5-dimethyl-2-thioxoimidazolidin-4-one (2.35). Reaction of **10.33** (50 mmol) with 2,6-dimethylphenyl isothiocyanate in the manner described for compound **2.39** furnished the title compound as an off-white solid (54%): mp 126−128 °C; ^{1}H NMR δ 7.24−7.28 (m, 1H), 7.17 (d, 2H, J = 7.25 Hz), 3.79 (q, 2H, J = 7.03 Hz), 2.02 (s, 6H), 1.51 (s, 6H), 1.29 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) m/z 276. Anal. for $C_{15}H_{20}N_2OS$: C, H, N.

3-(2-Chloro-6-methylphenyl)-1-ethyl-5,5-dimethyl-2-thioxoimidazolidin-4-one (2.36). Reaction of **10.33** (50 mmol) with 2-chloro-6-methylphenyl isothiocyanate in the manner described for compound **2.39** furnished the title compound as a white solid (61%): mp 135−137 °C; ^{1}H NMR δ 7.45−7.48 (m, 1H), 7.41 (t, 1H, J = 7.80 Hz), 7.35−7.37 (m, 1H), 3.79 (q, 2H, J = 7.10 Hz), 2.11 (s, 3H), 1.52 (d, 6H, J = 3.52 Hz), 1.29 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) m/z 296/298. Anal. for $C_{14}H_{17}ClN_2OS$: C, H, N.

3-(5-Chloro-2-methylphenyl)-1,5-dimethyl-2-thioxoimidazolidin-4-one (2.27): Reaction of 2-methylaminopropionic acid[44] (50 mmol) with 5-chloro-2-methylphenyl isothiocyanate in the manner described for compound **2.39** furnished the title compound as a white solid (64%): mp 106−108 °C; ^{1}H NMR δ 7.44 (dd, 1H, J = 8.35 and 2.20 Hz), 7.35−7.39 (m, 2H), 4.46 (q, 1H, J = 7.10 Hz), 3.26 (s, 3H), 2.03 (s, 3H), 1.47 (d, 3H, J = 7.25 Hz); MS (EI, $M^{\bullet+}$) m/z 268/270. Anal. for $C_{12}H_{13}ClN_2OS$: C, H, N.

3-(5-Chloro-2-methylphenyl)-1-ethyl-5-phenyl-2-thioxoimidazolidin-4-one (2.28). Reaction of ethylaminophenylacetic acid (50 mmol) with 5-chloro-2-methylphenyl isothiocyanate (50 mmol) in the manner described for compound **2.21** furnished the title compound as a white solid (68%): mp 148−150 °C; ^{1}H NMR (acetone-d_6) δ 7.36−7.55 (m, 8H), 5.56 (s, 1H), 4.25−4.30 (m, 1H), 3.28−3.33 (m, 1H), 2.16 (s, 3H), 1.19 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) m/z 344/346. Anal. for $C_{18}H_{17}ClN_2OS$: C, H, N.

1-Ethyl-3-(4-fluorophenyl)-5-phenyl-2-thioxoimidazolidin-4-one (2.29). Reaction of ethylaminophenylacetic acid (50 mmol) with 4-fluorophenyl isothiocyanate (50 mmol) in the manner described for compound **2.21** furnished the title compound as a white solid (74%): mp 215−216 °C; ^{1}H NMR δ 7.39−7.51 (m, 7H), 7.31−7.38 (m, 2H), 5.65 (s, 1H), 4.00−4.09 (m, 1H), 3.19−3.27 (m, 1H), 1.07 (t, 3H, J = 7.25 Hz); MS (+FAB, $[M + H]^+$) m/z 315. Anal. for $C_{17}H_{15}FN_2OS$: C, H, N.

3-(2,6-Dichlorophenyl)-1-ethyl-5-phenyl-2-thioxoimidazolidin-4-one (2.30). Reaction of ethylaminophenylacetic acid (50 mmol) with 2,6-dichlororophenyl isothiocyanate (50 mmol) in the manner described for compound **2.21** furnished the title compound as a white solid (59%): mp 192−194 °C; ^{1}H NMR δ 7.69−7.72 (m, 2H), 7.42−7.60 (m, 6H), 6.05 (s, 1H), 4.04−4.09 (m, 1H), 3.19−3.24 (m, 1H), 1.10 (t, 3H, J = 7.14 Hz); MS (EI, $M^{\bullet+}$) m/z 364/366/368. Anal. for $C_{17}H_{14}Cl_2N_2OS$: C, H, N.

1-Ethyl-3-isobutyl-5-phenyl-2-thioxoimidazolidin-4-one (2.32). Reaction of ethylaminophenylacetic acid (50 mmol)

with isobutylisothiocyanate (50 mmol) in the manner described for compound **2.21** furnished the title compound as a white solid (73%): mp 61−63 °C; ^{1}H NMR δ 7.40−7.48 (m, 3H), 7.22−7.25 (m, 2H), 5.54 (s, 1H), 3.98−4.07 (m, 1H), 3.50−3.62 (m, 2H), 3.08−3.17 (m, 1H), 2.13−2.20 (m, 1H), 1.02 (t, 3H, J = 7.25 Hz), 0.87 (d, 3H, J = 6.59 Hz), 0.81 (d, 3H, J = 6.81 Hz); MS (EI, M$^{\bullet+}$) m/z 276. Anal. for $C_{15}H_{20}N_2OS$: C, H, N.

3-(5-Chloro-2-methylphenyl)-1-ethyl-5-methoxy-2-thioxoimidazolidin-4-one (2.31). The mixture of **14.01** (11.43 g, 50 mmol), methyl 2-methoxy-2-hydroxy acetate (12 g, 100 mmol), and *p*-toluenesulfonic acid (1 g) in benzene (200 mL) was heated at reflux in a flask equipped with a water separator for 18 h. The solvent was evaporated and the residue was chromatographed on silica gel (5% ethyl acetate in hexane) to give the title compound (32%) as an off-white solid: mp 120−121 °C; ^{1}H NMR δ 7.46 (dd, 1H, J = 8.13 and 2.20 Hz), 7.37−7.41 (m, 2H), 5.55 (s, 1H), 3.88−3.95 (m, 1H), 3.58−3.65 (m, 1H), 3.34 (s, 3H), 2.06 (s, 3H), 1.25 (t, 3H, J = 7.25 Hz); MS (FAB, [M + H]$^+$) m/z 299/301. Anal. for $C_{13}H_{15}ClN_2O_2S$: H, N; C calcd: 52.26. Found: 51.84.

1-(5-Chloro-2-methylphenyl)-3-ethylthiourea (14.01). 5-Chloro2-methylphenyl isothiocyanate (18.3 g, 0.1 mol) was added dropwise to aqueous ethylamine (200 mL of 70% solution). The mixture was stirred for 18 h at room temperature. Excess ethylamine was removed under a stream of nitrogen. The residue was diluted with water. The solids were collected by filtration, washed with water, and dried to give 19.8 g of the title compound (87%). A sample crystallized from ethyl acetate afforded a white solid: mp 120−122 °C; ^{1}H NMR (DMSO-d_6; 300 MHz) δ 9.01 (br s, 1H), 7.67 (br s, 1H), 7.35 (br s, 1H), 7.24 (d, 1H, J = 8.12 Hz), 7.17 (dd, 1H, J = 8.14 and 1.8 Hz), 3.44 (br m, 2H), 2.13 (s, 3H), 1.08 (t, 3H, J = 7.25 Hz); MS (FAB, [M + H$^+$]) m/z 228/230. Anal. for $C_{10}H_{13}ClN_2S$: C, H, N.

1-(5-Chloro-2-methylphenyl)-3-ethyl-2-thioxo-4,5-imidazolidinedione (3.01). Ethyl chlorooxoacetate (12.3 g, 0.09 mol) was added dropwise to the solution **14.01** (13.7 g, 0.06 mol) in chloroform (300 mL). The mixture was heated at reflux for 3 h and then evaporated to dryness. The residue was dissolved in ethyl acetate (400 mL), washed with saturated sodium bicarbonate solution (300 mL) and then with water (300 mL), and evaporated to dryness. The residual solid was slurried in ether and filtered. The solid was washed with ether and air-dried to give 14.1 g of the title compound (83%) as a yellow solid: mp 173−174 °C; ^{1}H NMR δ 7.49−7.40 (m, 3H), 3.92 (m, 2H), 2.15 (s, 3H), and 1.23 (t, 3H, J = 7.25 Hz); MS (EI, M$^{\bullet+}$) m/z 282/284. Anal. for $C_{12}H_{11}ClN_2O_2S$: C, H, N.

1-(5-Chloro-2-methylphenyl)-3-methyl-2-thioxoimidazolidine-4,5-dione (3.02). Reaction of ethyl chlorooxoacetate (60 mmol) with 1-(5-chloro-2-methylphenyl)-3-methylthiourea (50 mmol) in the manner described for **3.01** afforded the title compound as a yellow solid (72%): mp 171−173 °C; ^{1}H NMR δ 7.49 (dd, 1H, J = 8.35 and 2.20 Hz), 7.41−7.43 (m, 2H), 3.31 (s, 3H), 2.15 (s, 3H); MS (EI, M$^{\bullet+}$) m/z 268/270. Anal. for $C_{11}H_9ClN_2O_2S$: C, H, N.

1-Ethyl-3-(4-fluorophenyl)-2-thioxoimidazolidine-4,5-dione (3.03). Reaction of ethyl chlorooxoacetate (60 mmol) with 1-ethyl-3-(4-fluorophenyl)thiourea (50 mmol) in the manner described for **3.01** afforded the title compound as a yellow solid (92%): mp 172−174 °C; ^{1}H NMR δ 7.35−7.42 (m, 4H), 3.92 (q, 2H, J = 7.18 Hz), 1.22 (t, 3H, J = 7.14 Hz); MS (FAB, [M + H]$^+$) m/z 253. Anal. for $C_{11}H_9FN_2O_2S$: C, H, N.

1-(2,6-Dimethylphenyl)-3-ethyl-2-thioxoimidazolidine-4,5-dione (3.04). Reaction of ethyl chlorooxoacetate (60 mmol) with 1-(2,6-dimethylphenyl)-3-ethylthiourea (50 mmol) in the manner described for **3.01** afforded the title compound as a yellow solid (71%): mp 103−105 °C; ^{1}H NMR δ 7.28−7.32 (m, 1H), 7.19 (d, 2H, J = 7.47 Hz), 3.94 (q, 2H, J = 7.18 Hz), 2.08 (s, 6H), 1.25 (t, 3H, J = 7.25 Hz); MS (FAB, [M + H]$^+$) m/z 263. Anal. for $C_{13}H_{14}N_2O_2S$: C, H, N.

Ethyl Aminooxyacetate Hydrochloride (17.01, R = Et). Aminooxyacetic acid hemihydrochloride (500 g, 5.487 mol) was suspended in ethanol (2000 mL). The mixture was saturated with hydrogen chloride and allowed to stand at room temperature for 24 h. The mixture was then concentrated to the precipitation point. The solid was collected by filtration, washed with ether, and dried to give 557 g of the title compound as a white solid (76%): mp 115−117 °C; ^{1}H NMR δ 11.08 (br s, 1H), 4.74 (s, 2H), 4.16 (q, 2H, J = 7.03 Hz), 1.21 (t, 3H, J = 7.03 Hz); MS (EI, M$^{\bullet+}$) m/z 119. Anal. for $C_4H_9NO_3$·HCl: C, H, N.

Ethyl *N*-(Ethoxycarbonyl)aminooxyacetate (18.01, R = Et). The mixture of **17.01** (542.3 g, 3.4 mol), sodium bicarbonate (300 g, 3.57 mol), and ethanol (1700 mL) was vigorously stirred with a mechanical stirrer. Ethyl chloroformate (395 g, 3.64 mol) was added dropwise. Additional sodium bicarbonate (300 g) was added in portions to keep the reaction mixture basic. The reaction mixture was stirred at room temperature for 3 h and then filtered to remove the solid. The solid was rinsed with fresh ethanol. The filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate (1500 mL), washed with water (2 × 500 mL), and evaporated to an oily residue, which solidified into a crystalline mass upon standing. The solid mass was crushed, stirred in ether (200 mL), filtered, and dried to give 645 g of the title compound (99%): mp 37−39 °C; ^{1}H NMR δ 10.45 (s, 1H), 4.36 (s, 2H), 4.12 (q, 2H, J = 7.25 Hz), 4.05 (q, 2H, J = 7.25 Hz), 1.19 (t, 3H, J = 7.25 Hz), 1.18 (t, 3H, J = 7.25 Hz); MS (FAB, [M + H]$^+$) m/z 192. Anal. for $C_7H_{13}NO_5$: C, H, N.

***N*-Methyl Aminooxyacetic Acid Hydrochloride (20.01). By Alkylation.** The mixture of **(18.01)** (306 g, 1.6 mol), methyl iodide (455 g, 3.2 mol), potassium carbonate (400 g, 2.9 mol), and ethanol (500 mL) was heated at reflux for 3 h, stirred at room temperature for 1 h, and then filtered. The filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate (1200 mL) and washed with water (3 × 700 mL), and the solvent was evaporated to give 290.5 g of ethyl *N*-methyl-*N*-ethoxycarbonylaminooxy acetate: ^{1}H NMR (DMSO-d_6; 400 MHz) δ 4.48 (s, 2H), 4.07−4.17 (m, 4H), 3.13 (s, 3H), 1.18−1.22 (m, 6H); MS (EI, M$^{\bullet+}$) m/z 205. Hydrochloric acid (350 mL) and water (150 mL) were added, and the mixture was heated at reflux for 45 min and then evaporated to dryness under vacuum. Treatment of the residue with acetonitrile (200 mL) and evaporation to dryness under vacuum afforded 176.4 g (78%) of **20.01** as a semisolid.

***N*-Methylaminooxyacetic Acid Hydrochloride (20.01). By Reductive Amination.** A solution of **17.01** (15.9 g, 0. 154 mol), paraformaldehyde (4.63 g, 0.154 mol), and ethanol (100 mL) was heated at reflux for 3 h, cooled to room temperature, and filtered to remove the solids. The filtrate was solvent evaporated to give 16 g (87%) of **19.01** (R = Et, R^5 = R^6 = H) as an oil: ^{1}H NMR δ 7.16 (d, 1H), 6.69 (d, 1H), 4.63 (s, 2H), 4.13 (q, 2H), and 1.20 (t, 3H, J = 7.25 Hz). To a stirred solution of **19.01** (11.9 g, 0.1 mol) in ethanol (75 mL) was added sodium cyanoborohydride (11.3 g, 0.18 mol). Ethanolic hydrogen chloride was then added to bring the pH to 1−2. The reaction mixture was stirred for 3 h and then evaporated to dryness. The residue was dissolved in ether (400 mL) and water (300 mL). The mixture was saturated with solid sodium bicarbonate. The organic phase was separated and washed with brine (200 mL), the and solvent was evaporated to give 7.0 g (58%) of ethyl *N*-methylaminooxyacetate as an oil. Ethanol (70 mL) was added followed by a solution of potassium hydroxide (2.8 g) in water (15 mL). The mixture was stirred at room temperature for 16 h. The solvent was evaporated under vacuum. The residue was treated with 2 N HCl (40 mL) and evaporated to dryness. The residue was stirred in ethyl acetate. The solvent was decanted. Drying of the residue under vacuum afforded 4.7 g (57%) of **20.01** as a semisolid: ^{1}H NMR δ 13−11 (br s, 2H), 4.71 (s, 2H), 2.8 (s, 3H); MS (EI, M$^{\bullet+}$) m/z 105. Anal. for $C_3H_7NO_3$·HCl: C, H, N.

[[[[(4-Chloro-2-methylphenyl)amino]thioxomethyl]-methylamino]oxy]acetic acid (21.01, R^2 = Me). A mixture of 4-chloro-2-methylphenyl isothiocyanate (12.8 g, 70 mmol), **20.01** (9.9 g, 70 mmol), triethylamine (7.5 g), and chloroform (100 mL) was heated at reflux for 1 h, stirred at room temperature for 1 h, and then evaporated to dryness. The residue was dissolved in ethyl acetate (200 mL) and extracted

with sodium bicarbonate saturated solution (2 × 200 mL). The aqueous extract was acidified with 6 N HCl and extracted with ethyl acetate (2 × 300 mL). The organic phase was washed with water (200 mL) and evaporated to dryness to give 13.9 g (69%) of the title compound as a white solid: mp 110−113 °C; ^{1}H NMR δ 13.30 (br s, 1H), 10.13 (s, 1H), 7.32−7.34 (m, 1H), 7.19−7.27 (m, 2H), 4.62 (s, 2H), 3.54 (s, 3H), and 2.17 (s, 3H); MS (EI, $M^{\bullet+}$) *m/z* 288/290. Anal. for $C_{11}H_{13}ClN_2O_3S$: C, H, N.

4-(4-Chloro-2-methylphenyl)-2-methyl-3-thioxo[1,2,4]-oxadiazinan-5-one (4.01). Phosphorus pentachloride (19.2 g, 92 mmol) was added in portions to the solution of **21.01** (26.5 g, 92 mmol) in benzene (250 mL) while stirring. The mixture was heated at reflux for 0.5 h and then evaporated to dryness. The residue was dissolved in ethyl acetate (500 mL), washed with saturated sodium bicarbonate solution (2 × 300 mL) and then with brine (300 mL), and evaporated to dryness. The residue was chromatographed on silica gel (40% methylene chloride in hexane). The title compound (12.8 g) was obtained as a light yellow solid: mp 141−143 °C; ^{1}H NMR δ 7.26−7.32 (m, 2H), 7.04 (d, 1H, J = 8.35 Hz), 4.84 (s, 2H), 3.74 (s, 3H), 2.14 (s, 3H); MS (EI, $M^{\bullet+}$) *m/z* 270/272. Anal. $C_{11}H_{11}ClN_2O_2S$: C, H, N.

In a similar manner as described for the preparation of **4.01**, reaction of compound **20.01** with the appropriate isothiocyanate furnished the thioureas **21**, which were cyclized with PCl_5 in refluxing benzene to produce compounds **4.02**−**4.10** (the reported percent yields for the two steps).

4-Benzhydryl-2-methyl-3-thioxo[1,2,4]oxadiazinan-5-one (4.02): white solid (33%); mp 152−154 °C; ^{1}H NMR δ 7.69 (s, 1H), 7.24−7.35 (m, 9H), 4.94 (s, 2H), 3.73 (s, 3H); MS (EI, $M^{\bullet+}$) *m/z* 312. Anal. for $C_{17}H_{16}N_2O_2S$: C, H, N.

4-(5-Chloro-2-methylphenyl)-2-methyl-3-thioxo[1,2,4]-oxadiazinan-5-one (4.03): white solid (35%); mp 100−102 °C; ^{1}H NMR ($CDCl_3$) δ 7.32 (dd, 1H, J = 8.24 and 2.09 Hz), 7.23−7.26 (m, 1H), 7.13 (d, 1H, J = 2.20 Hz), 4.84 (s, 2H), 3.74 (s, 3H), 2.13 (s, 3H); MS (EI, $M^{\bullet+}$) *m/z* 270/272. Anal. for $C_{11}H_{11}ClN_2O_2S$: C, H, N.

4-Indan-5-yl-2-methyl-3-thioxo[1,2,4]oxadiazinan-5-one (4.04): off-white solid (21%); mp 135−137 °C; ^{1}H NMR ($CDCl_3$) δ 7.26−7.32 (m, 1H), 7.04 (m, 1H), 6.96 (dd, 1H, J = 7.91 and 1.98 Hz), 4.84 (s, 2H), 3.75 (s, 3H), 2.96 (t, 4H, J = 7.47 Hz) 2.09−2.17 (m, 2H); MS (EI, $M^{\bullet+}$) *m/z* 262. Anal. for $C_{13}H_{14}N_2O_2S$: C. H, N.

4-(2,5-Dimethylphenyl)-2-methyl-3-thioxo[1,2,4]oxadiazinan-5-one (4.05): off-white solid (50%); mp 104−106 °C; ^{1}H NMR ($CDCl_3$) δ 7.17−7.26 (m, 2H), 6.92 (m, 1H), 4.84 (s, 2H), 3.75 (s, 3H), 2.35 (s, 3H), 2.12 (s, 3H); MS (EI, $M^{\bullet+}$) *m/z* 250. Anal. for $C_{12}H_{14}N_2O_2S$: C, H, N.

4-(2-Chloro-6-methylphenyl)-2-methyl-3-thioxo[1,2,4]-oxadiazinan-5-one (4.06): yellow solid (45%); mp 176−178 °C; ^{1}H NMR ($CDCl_3$) δ 7.35−7.37 (m, 1H), 7.26−7.31 (m, 1H), 7.21−7.23 (m, 1H), 4.89 (d, 1H, J = 15.38 Hz), 4.84 (d, 1H, J = 15.16 Hz), 3.76 (s, 3H), 2.21 (s, 3H); MS (ESI, $[M + H]^+$) *m/z* 271/273. Anal. for $C_{11}H_{11}ClN_2O_2S$: C, H, N.

4-(2-Isopropylphenyl)-2-methyl-3-thioxo[1,2,4]oxadiazinan-5-one (4.07): off-white sold (70%); mp 98−101 °C; ^{1}H NMR ($CDCl_3$) δ 7.35−7.42 (m, 2H), 7.21−7.25 (m, 1H), 7.06 (dd, 1H, J = 7.80 and 1.21 Hz), 5.12 (q, 2H, J = 14.43 Hz), 3.69 (s, 3H), 2.66−2.71 (m, 1H), 1.16 (d, 3H, J = 6.81 Hz), 1.04 (d, 3H, J = 6.81 Hz); MS (EI, $M^{\bullet+}$) *m/z* 264. Anal. for $C_{13}H_{16}N_2O_2S$: C, H, N.

4-(4-*tert*-Butylphenyl)-2-methyl-3-thioxo[1,2,4]oxadiazinan-5-one (4.08): light pink solid (66%); mp 168−170 °C; ^{1}H NMR ($CDCl_3$) δ 7.43−7.45 (m, 2H), 7.08−7.11 (m, 2H), 5.06 (s, 2H), 3.68 (s, 3H), 1.30 (s, 9H); MS (EI, $M^{\bullet+}$) *m/z* 276. Anal. for $C_{14}H_{18}N_2O_2S$: C, H, N.

2-Methyl-4-(4-phenoxyphenyl)-3-thioxo[1,2,4]oxadiazinan-5-one (4.09): light yellow solid (55%); mp 159−160 °C; ^{1}H NMR ($CDCl_3$) δ 7.41−7.45 (m, 2H), 7.17−7.21 (m, 3H), 7.07−7.10 (m, 2H), 6.99−7.03 (m, 2H), 5.07 (s, 2H), 3.68 (m, 3H); MS (EI, $M^{\bullet+}$) *m/z* 314. Anal. for $C_{16}H_{14}N_2O_2S$: C, H, N.

4-(5-Chloro-2-methoxyphenyl)-2-methyl-3-thioxo-[1,2,4]oxadiazinan-5-one (4.10): off-white solid (36%); mp 189−191 °C; ^{1}H NMR ($CDCl_3$) δ 7.44 (dd, 1H, J = 9.01 and 2.64 Hz), 7.31 (d, 1H, J = 2.64 Hz), 7.15 (d, 1H, J = 9.01 Hz), 5.06 (s, 2H), 3.73 (s, 3H), 3.67 (s, 3H); MS (EI, $M^{\bullet+}$) *m/z* 286/288. Anal. for $C_{11}H_{11}ClN_2O_3S$: C, H, N.

3-[3-(2,6-Dimethylphenyl)-1-ethyl-thioureido]propionic Acid (24.01). 3-Chloropropionic acid (31.5 g, 0.29 mol) was added portionwise while stirring to a 70% aqueous ethylamine solution (500 mL) while cooling in ice. The mixture was stirred at room temperature for 18 h and then evaporated to a viscous oily residue (45 g, 78%) of crude 3-(ethylamino)propionic acid (**23**, R^2 = Et, n = 1), which contained a 1 mol equiv of ethylamine hydrochloride. The mixture of crude 3-(ethylamino)-propionic acid (19.85 g, 0.1 mol), 2,6-dimethylphenyl isothiocyanate (16.3 g, 0.1 mol), triethylamine (20 g), and methylene chloride (200 mL) was heated at reflux for 3 h, cooled to room temperature, and extracted with 1 N NaOH solution (150 mL). The aqueous layer was separated and acidified with 2 N HCl. The formed solid was collected by filtration. Recrystallization from ethanol afforded 11 g (79%) of the title compound as a white solid: mp 115−118 °C; ^{1}H NMR δ 12.4 (br s, 1H), 8.17 (s, 1H), 7.05 (s, 3H), 3.85 (t, 2H, J = 8.13 Hz), 3.74 (q, 2H, J = 7.03 Hz), 2.68 (t, 2H, J = 8.13 Hz), 2.12 (s, 6H), 1.16 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) *m/z* 280. Anal. for $C_{14}H_{20}N_2O_2S$: C, H, N.

3-(2,6-Dimethylphenyl)-1-ethyl-2-thioxotetrahydropyrimidin-4-one (5.01). Concentrated hydrochloric acid (5 mL) was added to a solution of **24.01** (12 g, 42.8 mmol) in acetone (245 mL). The mixture was heated at reflux for 18 h and then evaporated to dryness. The residue was dissolved in methylene chloride (100 mL), washed with 1 N NaOH, and then evaporated to dryness. Crystallization from ethyl acetate afforded 5.3 g (47%) of the title compound as a white solid; mp 125−127 °C; ^{1}H NMR 7.08−7.12 (m, 1H), 7.03−7.05 (m, 2H), 4.00 (q, 2H, J = 7.03 Hz), 3.80−3.83 (m, 2H), 2.90−2.93 (m, 2H), 1.98 (s, 6H), 1.19 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) *m/z* 262. Anal. for $C_{14}H_{18}N_2OS$: C, H, N.

Reaction of 3-(ethylamino)propionic acid with the appropriate isothiocyanates afforded compounds **24**, which were cyclized in the same manner as described for the preparation of **5.01** to furnish compounds **5.02**−**5.10**. Compounds **6.01** and **6.02** were prepared similarly starting from 4-(ethylamino)-butyric acid (the reported percent yield is for the two steps).

3-(2,4-Dimethylphenyl)-1-ethyl-2-thioxotetrahydropyrimidin-4-one (5.02): white solid (31%); mp 153−156 °C; ^{1}H NMR δ 7.01 (s, 1H), 6.96−6.98 (m, 1H), 6.85 (d, 1H, J = 7.91 Hz), 3.92−3.99 (m, 2H), 3.72−3.86 (m, 2H), 2.83−2.94 (m, 2H), 2.27 (s, 3H), 1.95 (s, 3H), 1.18 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) *m/z* 262. Anal. for $C_{14}H_{18}N_2OS$: C, H, N.

3-(4-Chloro-2-methylphenyl)-1-ethyl-2-thioxotetrahydropyrimidin-4-one (5.03): white solid (51%); mp 133−136 °C; ^{1}H NMR δ 7.32−7.33 (m, 1H), 7.23−7.26 (m, 1H), 7.05 (d, 1H, J = 8.35 Hz), 3.96 (q, 2H, J = 7.03 Hz), 3.74−3.88 (m, 2H), 2.84−2.96 (m, 2H), 1.99 (s, 3H), 1.18 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) *m/z* 282. Anal. for $C_{13}H_{15}N_2ClOS$: C, H, N.

3-(2-Chloro-6-methylphenyl)-1-ethyl-2-thioxotetrahydropyrimidin-4-one (5.04): white solid (57%); mp 141−144 °C; ^{1}H NMR δ 7.31−7.34 (m, 1H), 7.20−7.27 (m, 2H), 3.94−4.03 (m, 2H), 3.75−3.88 (m, 2H), 2.96−3.04 (m, 1H), 2.85−2.92 (m, 1H), 2.07 (s, 3H), 1.19 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) *m/z* 282. Anal. for $C_{13}H_{15}N_2ClOS$: C, H, N.

3-(5-Chloro-2-methylphenyl)-1-ethyl-2-thioxotetrahydropyrimidin-4-one (5.05): white solid (38%); mp 142−145 °C; ^{1}H NMR δ 7.24−7.30 (m, 2H), 7.18 (s, 1H), 3.96 (q, 2H, J = 6.96 Hz), 3.73−3.89 (m, 2H), 2.84−2.92 (m, 2H), 1.98 (s, 3H), 1.16−1.20 (m, 3H); MS (EI, $M^{\bullet+}$) *m/z* 282. Anal. for $C_{13}H_{15}N_2ClOS$: C, H, N.

1-Ethyl-3-(2-ethyl-6-isopropylphenyl)-2-thioxotetrahydropyrimidin-4-one (5.06): white solid (17%); mp 129−132 °C; ^{1}H NMR δ 7.23−7.27 (m, 1H), 7.16 (dd, 1H, J = 7.91 and 1.54 Hz), 7.06−7.08 (m, 1H), 3.92−4.08 (m, 2H), 3.77−3.86 (m, 2H), 2.87−3.02 (m, 2H), 2.55−2.62 (m, 1H), 2.20−2.38 (m, 2H), 1.18 (t, 3H, J = 7.03), 1.14 (d, 3H, J = 8.57 Hz),1.07 (t, 3H, J = 7.58 Hz), 1.00 (d, 3H, J = 6.81 Hz); MS (EI, $M^{\bullet+}$) *m/z* 304. Anal. for $C_{17}H_{24}N_2OS$: C, H, N.

1-Ethyl-3-(2-ethyl-6-methylphenyl)-2-thioxotetrahydropyrimidin-4-one (5.07): white solid (30%); mp 74−77 °C; ^{1}H NMR δ 7.16 (t, 1H, J = 7.58 Hz), 7.07 (m, 2H), 3.95−4.04 (m, 2H), 3.81 (t, 2H, J = 6.92 Hz), 2.88−2.97 (m, 2H), 2.22−2.38 (m, 2H), 1.97 (s, 3H), 1.19 (t, 3H, J = 7.14 Hz), 1.07 (t, 3H, J = 7.58 Hz); MS (EI, $M^{\bullet+}$) m/z 276. Anal. for $C_{15}H_{20}N_2OS$: C, H, N.

1-Ethyl-3-(2-fluorophenyl)-2-thioxotetrahydropyrimidin-4-one (5.08): white solid (53%); mp 101−104 °C; ^{1}H NMR δ 7.34−7.41 (m, 1H), 7.18−7.26 (m, 3H), 3.92−4.01 (m, 2H), 3.83−3.90 (m, 1H), 3.72−3.78 (m, 1H), 2.85−2.99 (m, 2H), 1.19 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) m/z 252. Anal. for $C_{12}H_{13}FN_2OS$: C, H, N.

1-Ethyl-3-(2-isopropylphenyl)-2-thioxotetrahydropyrimidin-4-one (5.09): white solid (49%); mp 118−122 °C; ^{1}H NMR δ 7.24−7.34 (m, 2H), 7.13−7.17 (m, 1H), 6.95 (dd, 1H, J = 7.91 and 1.10 Hz), 3.93−4.02 (m, 2H), 3.82−3.89 (m, 1H), 3.72−3.79 (m, 1H), 2.84−2.99 (m, 2H), 2.59−2.66 (m, 1H), 1.18 (t, 3H, J = 7.03 Hz), 1.14 (d, 3H, J = 7.03 Hz), 1.02 (d, 3H, J = 6.81 Hz); MS (EI, $M^{\bullet+}$) m/z 276. Anal. for $C_{15}H_{20}N_2OS$: C, H, N.

3-(2,6-Dichlorophenyl)-1-ethyl-2-thioxotetrahydropyrimidin-4-one (5.10): white solid (72%); mp 114−117 °C; ^{1}H NMR δ 7.52−7.54 (m, 2H), 7.38−7.48 (m, 1H), 3.98 (q, 2H, J = 7.10 Hz), 3.82 (t, 2H, J = 7.03 Hz), 2.96 (t, 2H, J = 6.59 Hz), 1.19 (t, 3H, J = 7.03 Hz); MS (+FAB, $[M + H]^+$) m/z 303. Anal. for $C_{12}H_{12}N_2Cl_2OS$: C, H, N.

3-(4-Chloro-2-methylphenyl)-1-ethyl-2-thioxo[1,3]-diazepan-4-one (6.01): white solid (62%); mp 97−100 °C; ^{1}H NMR δ 7.33 (d, 1H, J = 2.20 Hz), 7.22−7.24 (m, 1H), 7.08 (d, 1H, J = 8.35 Hz), 3.92−4.02 (m, 3H), 3.78−3.85 (m, 1H), 2.51−2.64 (m, 2H), 2.17−2.33 (m, 2H), 2.08 (s, 3H), 1.25 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) m/z 296/298. Anal. for $C_{14}H_{17}ClN_2OS$: C, H, N.

3-(5-Chloro-2-methylphenyl)-1-ethyl-2-thioxo[1,3]-diazepan-4-one (6.02): white solid (45%); mp 142−144 °C; ^{1}H NMR δ 7.24−7.29 (m, 2H), 7.21 (d, 1H, J = 1.76 Hz), 3.92−4.03 (m, 3H), 3.77−3.83 (m, 1H), 2.51−2.64 (m, 2H), 2.18−2.31 (m, 2H), 2.06 (s, 3H), 1.26 (t, 3H, J = 7.03 Hz); MS (FAB, $[M + H]^+$) m/z 297. Anal. for $C_{14}H_{17}ClN_2OS$: C, H, N.

(*tert*-Butoxycarbonylethylamino)acetic Acid (25): *N*-Ethylglycine (10.3 g, 0.1 mol) was added to a solution of sodium hydroxide (4.4 g, 0.11 mol) in water (75 mL) and *t*-BuOH (75 mL). Di-*tert*-butyl dicarbonate (22.3 g, 0.1 mol) was added portionwise. The mixture was stirred at room temperature for 2 h, heated at reflux for 0.5 h, cooled to room temperature, and extracted with pentane (100 mL). The aqueous layer was acidified with hydrochloric acid and extracted with ether (3 × 100 mL). The ethereal extracts were combined and washed with water, and the solvent was evaporated to give 18.7 g (92%) of the title compound as an oil that solidified upon standing: mp 88−90 °C; ^{1}H NMR of a mixture of rotomers (45:55) δ 12.48 (br s, 1H), 3.82 and 3.79 (s, minor, s, major, 2H), 3.19 (m, 2H), 1.38 and 1.33 (s, minor, s, major, 9H), 0.99 (m, 3H); MS (ESI, $[M + H]^+$) m/z 204. Anal. for $C_9H_{17}NO_4$: C, N; H calcd: 8.43. Found: 8.00.

[(5-Chloro-2-methylphenylcarbamoyl)methyl]ethylcarbamic Acid *tert*-Butyl Ester (26). 5-Chloro-2-methylaniline (10.6 g, 0.075 mol) was added to a mixture of **25** (15.2 g, 0.075 mol) and HOBT (15.0 g, 0.113 mol) in dichloromethane (200 mL) while stirring and cooling in ice. The solution of DCC (17.0 g, 0.083 mol) in methylene chloride (40 mL) was then added. The mixture was stirred at 0 °C for 2 h and then at room temperature for 18 h. The mixture was filtered to remove the solids. The filtrate was evaporated and the residue was chromatographed on silica gel (10% ethyl acetate in hexane) to give 23.8 g (97%) of the title compound as an oil: ^{1}H NMR of a mixture of rotomers with overlapping peaks (1:1) δ 9.33 (br s, 1H), 7.62 and 7.56 (2 s, 1H), 7.25−7.23 (m, 1H), 7.14−7.13 (m, 1H), 3.98−4.10 (br m, 2H), 3.27−3.30 (br m, 2H), 2.20 (s, 3H), 1.42 and 1.36 (2 s, 9H), 1.07 (br m, 3H); MS (ES, $[M + H]^+$) m/z 327/329. Anal. for $C_{16}H_{23}ClN_2O_3$: C, H, N.

[(5-Chloro-2-methylphenylthiocarbamoyl)methyl]-ethylcarbamic Acid *tert*-Butyl Ester (27). The mixture **26** (9.7 g, 0.031 mol) and Lawesson's reagent (6.1 g, 0.015 mol) in toluene (70 mL) was heated at reflux for 2 h. The solvent was evaporated and the residue was chromatographed on silica gel (10% ethyl acetate in hexane) to give 9.2 g (87%) of the title compound as a solid: mp 117−119 °C; ^{1}H NMR δ 11.13 (br s, 1H), 7.22−7.34 (m, 3H), 4.28 (s, 2H), 3.32−3.34 (m, 2H), 2.14 (s, 3H), 1.40 (s, 9H), 1.06−1.10 (m, 3H); MS (EI, $M^{\bullet+}$) m/z 342/344. Anal. for $C_{16}H_{23}ClN_2O_2S$: C, H, N.

***N*-(5-Chloro-2-methylphenyl)-2-(ethylamino)thioacetamide (28).** Trifluoroacetic anhydride (18.5 mL) was added slowly to a solution of **27** (8.0 g, 0.023 mol) in methylene chloride (70 mL) while cooling in ice. The mixture was stirred at 0 °C for 1 h and then at room temperature for 18 h. The mixture was concentrated to half its volume and then diluted with ether (120 mL). The solid was collected by filtration, and dissolved in chloroform (200 mL) and saturated potassium carbonate solution (200 mL). The chloroform layer was washed with water and evaporated. Crystallization from ethyl acetate/hexane afforded 5.5 g (97%) of the title compound as a solid: mp 119−121 °C; ^{1}H NMR δ 7.77 (d, 1H, J = 2.2 Hz), 7.28 (d, 1H, J = 7.91 Hz), 7.19 (dd, 1H, J = 8.13 and 2.2 Hz), 3.65 (s, 2H), 2.64 (q, 2H, J = 7.03 Hz), 2.90−2.93 (m, 2H), 2.16 (s, 3H), 1.07 (t, 3H, J = 7.03 Hz); MS (ESI, $[M + H]^+$) m/z 243/245. Anal. for $C_{11}H_{15}ClN_2S$: C, H, N.

1-(5-Chloro-2-methylphenyl)-4-ethyl-6-thioxopiperazin-2-one (7). A solution of ethyl bromoacetate (3.7 g, 0.022 mol) in ethanol (20 mL) was added dropwise to a solution of **28** (4.85 g, 0.02 mol) and triethylamine (3 mL) in ethanol (150 mL). The mixture was stirred at room temperature for 2 h and then at reflux for 4 h. The solvent was evaporated and the residue was chromatographed on silica gel (40% ethyl acetate in hexane). Crystallization from ether furnished 3.4 g (60%) of the title compound as a solid: mp 120−122°C; ^{1}H NMR δ 7.24 (d, 1H, J = 8.13 Hz), 7.07 (dd, 1H, J = 8.13 and 2.1 Hz), 6.75 (d, 1H, J = 2.2 Hz), 4.48 (s, 2H), 3.67 (s, 2H), 3.45 (q, 2H, J = 7.03 Hz), 2.0 (s, 3H), 1.07 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) m/z 282/284. Anal. for $C_{13}H_{15}ClN_2OS$: C, H, N.

***N*-(5-Chloro-2-methylphenyl)-2-(ethylamino)acetamide (30).** Trifluoroacetic acid (100 mL) was added dropwise to a solution of **26** (40 g, 0.122 mol) in methylene chloride (300 mL) while cooling in ice. The mixture was stirred at 0 °C for 1 h and then at room temperature for 18 h. The mixture was concentrated to half its volume, diluted with ether (500 mL), and filtered. The solid was dissolved in ethyl acetate (500 mL) and K_2CO_3 solution (500 mL). The organic phase was evaporated to give 26.8 g (96%) of the title compound as a solid: mp 94−96 °C; ^{1}H NMR δ 8.00 (d, 1H, J = 2.19 Hz), 7.23 (d, 1H, J = 8.13 Hz), 7.07 (dd, 1H, J = 8.13 and 2.19 Hz), 3.32 (br s, 1H), 2.58 (q, 2H, J = 7.03 Hz), 2.20 (s, 3H), 1.04 (t, 3H, J = 7.14 Hz); MS (ESI, $M^{\bullet+}$) m/z 227/229. Anal. for $C_{11}H_{15}ClN_2O$: C, H, N.

3-(5-Chloro-2-methylphenyl)-1-ethyl-4-oxoimidazolidin-2-ylidenecyanamide (8). A mixture of **30** (6.78 g, 0.03 mol) and dimethyl *N*-cyanodithioiminocarbonate (9 g, 0.061 mol) was heated at 175 °C for 1 h. The mixture was cooled and purified by chromatography on silica gel (5−10% ethyl acetate in hexane) to give 1.3 g of the title compound as a solid: mp 210−212 °C; ^{1}H NMR δ 7.46−7.50 (m, 2H), 7.38 (d, 1H, J = 8.12 Hz), 4.47 (d, 1H, J = 18.90 Hz), 4.32 (d, 1H, J = 18.90 Hz), 3.61−3.73 (m, 2H), 2.12 (s, 3H), 1.23 (t, 3H, J = 7.03 Hz); MS (EI, $M^{\bullet+}$) m/z 276. Anal. for $C_{13}H_{13}ClN_4O$: C, H, N.

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References

(1) Rosamond, W. D.; Chambless, L. E.; Folsom, A. R.; Cooper, L. S.; Conwill, D. E.; Clegg, L.; Wang, C.-H.; Heiss, G. Trends in the incidence of myocardial infarction and in mortality due to coronary heart disease, 1987 to 1994. *N. Engl. J. Med.* **1998**, *339*, 861−867.

(2) Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: The Scandinavian simvastatin survival study (4S). *Lancet* **1994**, *344*, 1383−1389.
(3) Shepherd, J.; Cobbe, S. M.; Ford, I.; Isles, C. G.; Lorimer, A. R.; Macfarlane, P. W.; McKillop, J. H.; Packard, C. J. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia: West of Scotland coronary prevention study group. *N. Engl. J. Med.* **1995**, *333*, 1301−1307.
(4) Sacks, F. M.; Pfeffer, M. A.; Moye, L. A.; Rouleau, J. L.; Rutherford, J. D.; Cole, T. G.; Brown, L.; Warnica, J. W.; Arnold, J. M. O.; Wun, C.-C.; Davis, B. R.; Braunwald, E. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels: Cholesterol and recurrent events trial investigators. *N. Engl. J. Med.* **1996**, *335*, 1001−1009.
(5) Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels: The long-term intervention with pravastatin in ischaemic disease (LIPID) study group. *N. Engl. J. Med.* **1998**, *339*, 1349−1357.
(6) Downs, J. R.; Clearfield, M.; Weis, S.; Whitney, E.; Shapiro, D. R.; Beere, P. A.; Langendorfer, A.; Stein, E. A.; Kruyer, W.; Gotto, A. M., Jr. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: Results of AFCAPS/TexCAPS (Air Force/Texas coronary atherosclerosis prevention study). *J. Am. Med. Assoc.* **1998**, *279*, 1615−1622.
(7) Superko, H. R. Beyond LDL cholesterol reduction. *Circulation* **1996**, *94*, 2351−2354.
(8) Miller, G. J.; Miller, N. E.; Plasma high-density-lipoprotein concentration and development of ischaemic heart disease. *Lancet* **1975**, *1*, 16−19.
(9) 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. High-density lipoprotein cholesterol and cardiovascular disease: Four prospective American studies. *Circulation* **1989**, *79*, 8−15.
(10) Franceschini, G. Epidemiologic evidence for high-density lipoprotein cholesterol as a risk factor for coronary artery disease. *Am. J. Cardiol.* **2001**, Dec 20, 88(12A), 9N-13N.
(11) Assmann, G.; Schulte, H.; von Eckardstein, A.; Huang, Y. High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. *Atherosclerosis* **1996**, *124*, Suppl S11−20.
(12) Glomset, J. A. The plasma lecithin:cholesterol acyl-transferase reaction. *J. Lipid Res.* **1968**, *9*, 155−167.
(13) Tall, A. R. Plasma high-density lipoproteins. Metabolism and relationship to atherogenesis. *J. Clin. Invest.* **1990**, *86*(2), 379−384.
(14) Barter, P. J.; Rye, K. Molecular mechanisms of reverse cholesterol transport. *Curr. Opin. Lipidol.* **1996**, *7*, 82−87.
(15) Elokdah, H.; Sulkowski, T.; Cochran, D.; McKean, M.-L.; Quinet, E. Effects of 2-(substituted-sulfanyl)-3,5-dihydro-imidazole-4-one and 2-(substituted-sulfanyl)-1H-imidazole-4,5-dione derivatives on serum HDL-cholesterol. *Bioorg. Med. Chem. Lett.* **2000**, *10*(16), 1791−1794.
(16) Schutkowski, M.; Mrestani-Klaus, C.; Neubert, K. Synthesis of dipeptide 4-nitroanilides containing nonproteinogenic amino acids. *Int. J. Pept. Protein Res.* **1995**, *45*(3), 257−265.
(17) Ulrich, H.; Sayigh, A. A. R. The Reaction of Oxalyl Chloride with Substituted Ureas and Thioureas. *J. Org. Chem.* **1965**, *30*, 2781−2783.
(18) Kornowski, H.; Trichot, M.; Delage, B. 1,2,4-Oxadiazines. II. Preparation of N2- or N4-mono- or dimethyl derivatives. *Bull. Soc. Chim. Fr.* **1966**, (2), 683−686.
(19) Cava, M. P.; Levinson, M. I. Thionation Reactions of Lawesson's Reagents. *Tetrahedron* **1985**, *41*(22), 5061−5087.
(20) France, D. S.; Quinby, R. E.; Babiak, J. L.; Paterniti, D. C.; Weinstein, J. R.; D. B. *Lab. Rob. Autom.* **1990**, *2*, 155.
(21) Imaizumi, K.; Nagatomi, A.; Sato, M.; Tominaga, A.; Sugano, M. Cholesterol metabolism in ExHC (exogenous hypercholesterolemic) rats. *Biochim. Biophys. Acta* **1992**, *1123*(1), 101−109.
(22) Krause, B. R.; Princen, H. M. Lack of predictability of classical animal models for hypolipidemic activity: A good time for mice? *Atherosclerosis* **1998**, *140*(1), 15−24.
(23) Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Miles, P. D.; Parsons, M. E.; Prain, H. D.; White, G. R. Cyanoguanidine-Thiourea equivalence in the development of the histamine H2-receptor antagonist, cimetidine. *J. Med. Chem.* **1977**, *20*(7), 901−906.
(24) Manley, P. W.; Quast, U. Structure−activity studies of potassium channel opening in pinacidil-type cyanoguanidines, nitroethenediamines, thioureas, and ureas. *J. Med. Chem.* **1992**, *35*(12), 2327−2340.
(25) Gilman, A. G.; Murad, F. In *Pharmaceutical basis of therapeutics*; Goodman, L. S., Gilman, A., Eds.; Macmillan: New York, 1975; pp 1410−1415.
(26) Spady, D. K.; Dietschy, J. M. Interaction of Dietary Cholesterol and Triglycerides in the Regulation of Hepatic Low-Density Lipoprotein Transport in the Hamster. *J. Clin. Invest.* **1988**, *81*, 300−309.
(27) Kawashiri, M. A.; Maugeais, C.; Rader, D. J. High-density lipoprotein metabolism: Molecular targets for new therapies for atherosclerosis, review. *Curr. Atheroscler. Rep.* **2000**, *2*(5), 363−372.
(28) Rader, D. J.; Maugeais, C. Genes influencing HDL metabolism: New perspectives and implications for atherosclerosis prevention, Review, *Mol. Med. Today* **2000**, *6*(4), 170−175.
(29) Krause, B. R.; Auerbach, B. J. Reverse cholesterol transport and future pharmacological approaches to the treatment of atherosclerosis. *Curr. Opin. Investig. Drugs* **2001**, *2*(3), 375−381.
(30) McKean, M. L.; Quercia, S. M.; Ponticiello, R.; Clark, D. E.; Quinet, E. M.; Adelman, S. J. WAY-135433 increases plasma HDL cholesterol and post-heparin lipoprotein lipase activity in cynomolgus monkeys. *Suppl. Circulation* **1998**, *98*, I-663.
(31) Elokdah, H.; Sulkowski, T.; Chai, S.-Y.; McFarlane, G.; Butera, J.; McKean, Mar-Lee; Quinet, E. Design and synthesis of thioxoimidazolidine-diones and derivatives as high-density lipoprotein cholesterol (HDL-C) enhancers. *Abstracts of Papers*, 222nd National Meeting of the American Chemical Society, 2001; American Chemical Society: Washington, DC; ORGN-419.
(32) Duverger, N.; Knith, H.; Emmanuel, F.; Caillaud, J. M.; Viglietta, C.; Castro, G.; Tailleux, A.; Fievet, C.; Fruchart, J. C.; Houdebine, L. M.; Denefle, P. Inhibition of atherosclerosis development in cholesterol-fed human apolipoprotein A−I-transgenic rabbits. *Circulation* **1996**, *9*, 713−717.
(33) Rubin, E. M.; Krauss, R. M.; Spangler, E. A.; Veratuyft, J. G.; Clitt, S. M. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein A−I. *Nature* **1991**, *353*, 265−267.
(34) Plump, A. S.; Scott, C. J.; Breslow, J. L. Human apolipoprotein A−I gene expression increases high-density lipoprotein and suppresses atherosclerosis in the apolipoprotein H-deficient mouse. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9607−9611.
(35) Jonas, A.; von Eckardstein, A.; Kezdy, K. E.; Steinmetz, A.; Assmann, G. Structural and functional properties of reconstituted high-density lipoprotein discs prepared with six apolipoprotein A−I variants. *J. Lipid Res.* **1991**, *32*, 97−106.
(36) Lawn, R. M.; Wade, D. P.; Garvin, M. R.; Wang, X.; Schwartz, K.; Porter, J. G.; Seilhamer, J. J.; Vaughan, A. M.; Oram, J. F. The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. *J. Clin. Invest.* **1999**, *104*, R25−31.
(37) Goldberg, I. J.; Blaner, W. S.; Vanni, T. M.; Moukides, M.; Ramakrishnan, R. *J. Clin. Invest.* **1990**, *86*, 463−473.
(38) Reymer, P. W.; Gagne, E.; Groenemeyer, B. E.; Zhang, H.; Forsyth, I.; Jansen, H.; Seidell, J. C.; Kromhout, D.; Lie, K. E.; Kastelein, J.; Hayden, M. R. A lipoprotein lipase mutation (Asn291Scr) is associated with reduced HDL cholesterol levels in premature atherosclerosis. *Nat. Genet.* **1995**, *10*, 28−34.
(39) Redgrave, T. D.; Small, D. M. Quantitation of the transfer of surface phospholipid of chylomicrons to the high-density lipoprotein fraction during the catabolism of chylomicrons in the rat. *J. Clin. Invest.* **1979**, *64*, 162−171.
(40) Tall, A. R.; Blum, C. B.; Forester, G. P.; Nelson, C. A. Changes in the distribution and composition of plasma high-density lipoproteins after ingestion of fat. *J. Biol. Chem.* **1982**, *257*, 198−207.
(41) Wilson, T. A.; Nicolosi, R. J.; Lawton, C. W.; Babiak, J. Gender differences in response to a hypercholesterolemic diet in hamsters: Effects on plasma lipoprotein cholesterol concentrations and early aortic athersclerosis. *Atherosclerosis* **1999**, *146*, 83−91.
(42) Burkey, B. F.; France, D.; Wang, H.; Ma, X.; Brand, B.; Abuhani, C.; Diffenderfer, M. R.; Marsh, J. B.; Paterniti, J. R., Jr.; Fisher, E. A. Overexpression of human apolipoprotein A−I in transgenic rats and the hyperlipoproteinemia associated with experimental nephrosis. *J. Lipid. Res.* **1995**, *36*(7), 1463−1473.
(43) Loh, T.-P.; Wei, L.-L. Indium trichloride-catalyzed conjugate addition of amines to α,β-ethylenic compounds in water. *Synlett.* **1998**, *9*, 975−976.
(44) Spengler, J.; Burger, K. An efficient synthesis of *N*-methyl amino acids and some of their derivatives. *Synthesis* **1998**, *1*, 67−70.

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