Synthesis and Hypocholesterolemic Activity of 6,7-Dihydro-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-diones, Novel Inhibitors of AcylCoA:Cholesterol *O*-Acyltransferase

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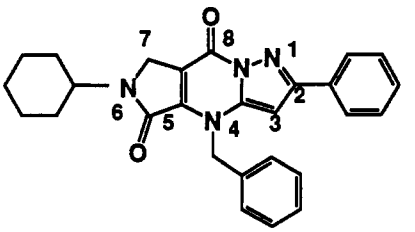
A novel series of 6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione inhibitors of the enzyme acyl-CoA:cholesterol *O*-acyltransferase is described. A number of these derivatives were found to be potent modulators of serum lipoprotein levels in cholesterol-fed rats. Further evaluation of one of the most effective analogues confirmed that it was significantly blocking the absorption of cholesterol from the gut.

The enzyme acylCoA:cholesterol *O*-acyltransferase (ACAT, EC 2.3.1.26) is believed to play a key role in two events which contribute to the atherosclerotic process: the absorption of dietary cholesterol[1] and the accumulation of cholesteryl esters within the cells of developing arterial lesions.[2] Inhibition of ACAT would thus be expected to retard the progression of atherosclerosis either by reducing serum cholesterol levels or by directly preventing the accumulation of cholesterol in arterial tissue.[3] The levels of ACAT activity in intestinal mucosal cells during cholesterol feeding[4] and in arterial cells undergoing atherogenesis[2] are known to be abnormally elevated, presenting prime targets for therapeutic inhibition. In fact, several ACAT inhibitors have been shown to be effective at attenuating hypercholesterolemia in animals during cholesterol feeding.[5] The relatively high levels of intestinal ACAT activity present in humans[6] provide further support for the hypothesis that ACAT inhibitors are potentially useful hypolipidemic agents.

During the course of broad screening for ACAT inhibitors of novel structure, we discovered the 6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione **4a**. The resulting analogue program revealed that a variety of related compounds possessed ACAT inhibitory activity. Structure–activity relationships were established by variation of the substituents at positions 2, 3, 4, and 6 of the tricyclic nucleus, and clear requirements for activity became readily apparent. The following report describes the synthesis and biological evaluation of this new class of compounds.



4a

Chemistry

Scheme I outlines the general synthesis of 4-substituted pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine diones **4**.

The requisite ethyl 4,5-dioxopyrrolidine-3-carboxylates **1** were prepared from a variety of primary amines as shown in Table I by using known methodology.[7] Similarly, Table II displays the established route[8] which was employed to obtain 1*H*-pyrazol-3-amines **2** from readily available esters. Condensation of **1** and **2** occurred smoothly in refluxing acetic acid[9] to give the highly insoluble 4-unsubstituted tricyclic compounds **3** (Table III). Reaction in ethanol was also successful, but was significantly slower. The products were all conveniently isolated analytically pure by simple filtration of the cooled reaction mixtures. Subsequent alkylation of the 4-nitrogen with alkyl halides could be effected with potassium carbonate in dimethylformamide (DMF) or dimethyl sulfoxide (DMSO).[10] The

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(5) (a) DeVries, V. G.; Bloom, J. D.; Dutia, M. D.; Katocs, A. S.; Largis, E. E. *J. Med. Chem.* **1989**, *32*, 2318. (b) Ishida, F.; Sato, A.; Iizuka, Y.; Kitani, K.; Sawasaki, Y.; Kamei, T. *Biochim. Biophys. Acta* **1989**, *1004*, 117. (c) Rucker, W.; Prop, G.; Huther, A. M. *Atherosclerosis* **1988**, *69*, 155. (d) Matsubara, K.; Matsuzawa, Y.; Jiao, S.; Kihara, S.; Takama, T.; Nakamura, T.; Tokunaga, K.; Kubo, M.; Tarui, S. *Atherosclerosis* **1988**, *72*, 199. (e) Natori, K.; Okazaki, Y.; Nakajima, T.; Hirohashi, T.; Aono, S. *Jpn. J. Pharmacol.* **1986**, *42*, 517. (f) DeVries, V. G.; Schaffer, S. A.; Largis, E. E.; Dutia, M. D.; Wang, C.; Bloom, J. D.; Katocs, A. S. *J. Med. Chem.* **1986**, *29*, 1131. (g) Heider, J. G. Agents Which Inhibit Cholesterol Esterification in the Intestine and Their Potential Value in the Treatment of Hypercholesterolemia. In *Pharmacological Control of Hyperlipidaemia*; J. R. Prous Science: Barcelona, Spain, 1986; p 423. (h) Heider, J. G.; Pickens, C. E.; Kelly, L. A. *J. Lipid Res.* **1983**, *24*, 1127.
(6) (a) Helgerud, P.; Saarem, K.; Norum, K. R. *J. Lip. Res.* **1981**, *22*, 271. (b) Norum, K. R.; Lilljeqvist, A.-C.; Helgerud, P.; Normann, E. R.; Mo, A.; Selbekk, B. *Eur. J. Clin. Invest.* **1979**, *9*, 55.
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(8) (a) Long, R. S. *J. Am. Chem. Soc.* **1947**, *69*, 990. (b) Tensmeyer, L. G.; Ainsworth, C. *J. Org. Chem.* **1966**, *31*, 1878. (c) Dymek, W.; Janik, B.; Ryznerski, Z. *Acta Pol. Pharm.* **1966**, *23*, 207.
(9) Related condensations of β-keto esters with 1*H*-pyrazol-3-amines have been reported: (a) Checchi, S.; Papini, P.; Ridi, M. *Gazz. Chim. Ital.* **1956**, *86*, 631. (b) Makisumi, Y. *Chem. Pharm. Bull.* **1962**, *10*, 612.
(10) For N-alkylations of related systems, see: (a) Senga, K.; Novinson, T.; Wilson, H. R. *J. Med. Chem.* **1981**, *24*, 610. (b) Auzzi, G.; Cecchi, L.; Costanzo, A.; Vettori, L.; Bruni, F. *Farm. Ed. Sci.* **1978**, *33*, 14. (c) Vettori, L.; Cecchi, L.; Costanzo, A.; Auzzi, G.; Bruni, F. *Farm. Ed. Sci.* **1981**, *36*, 441. The above references cite infrared data to support their structural assignments. The infrared data obtained for our analogues **4** are consistent with the proposed structures. Further confirmation of these assignments was obtained by an examination of NOE enhancements in the proton NMR spectrum of **4a**. Irradiation of the pyrazole proton (at C-3) resulted in approximately equivalent levels of signal enhancement of the benzyl protons and the ortho protons on the 2-phenyl substituent.

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Scheme I

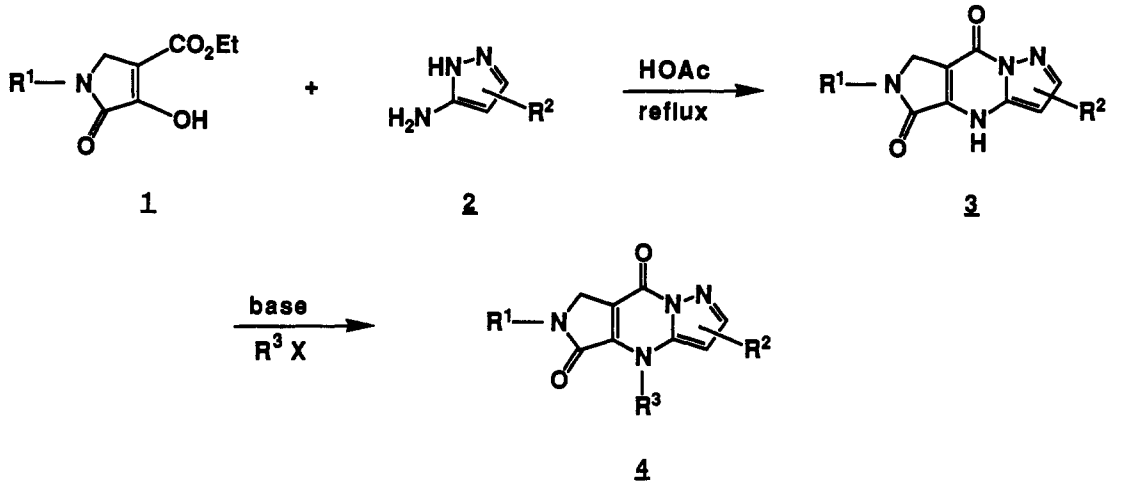
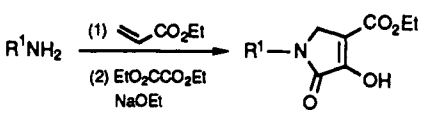
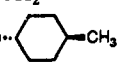


Table I. Synthesis of 4,5-Dioxopyrrolidine-3-carboxylic Acid, Ethyl Esters 1



compd	R^1	yield, %	mp, °C	formula[a]
1a	c-C_6H_{11}[b]	64	180–185	
1b	Ph[b]	66	153–155	
1c	CH_3[b]	49	154–156	
1d	$PhCH_2$[b]	70	134–135	
1e		61	305 dec	$C_{14}H_{21}NO_5$
1f	1,3-$(CH_3)_2$-n-C_4H_7	60	112–114	$C_{13}H_{21}NO_4$
1g	n-C_6H_{13}	63	127–128	$C_{13}H_{21}NO_4$
1h	c-C_6H_{15}	58	135–137	$C_{15}H_{23}NO_4$
1i	exo-2-norbornyl	43	134–135	$C_{14}H_{19}NO_4 \cdot 0.2H_2O$
1j	c-$C_{12}H_{23}$	71	155–158	$C_{19}H_{31}NO_4$
1k	n-$C_{12}H_{25}$	60	109–111	$C_{19}H_{33}NO_4$
1l	tert-octyl	48	109–111	$C_{15}H_{25}NO_4$
1m	oleyl	70	89–92	$C_{25}H_{43}NO_4$
1n	2,3-$(CH_3)_2$-c-C_6H_9	70	168–170	$C_{15}H_{23}NO_4$
1o	$PhCH(CH_3)$	59	121–122	$C_{15}H_{17}NO_4$
1p	p-MeO-$PhCH_2$	59	113–114	$C_{15}H_{17}NO_5$[c]

[a] Satisfactory C, H, and N analyses obtained except where indicated. [b] Previously prepared (ref 7). [c] N: calcd, 4.81; found, 5.36.

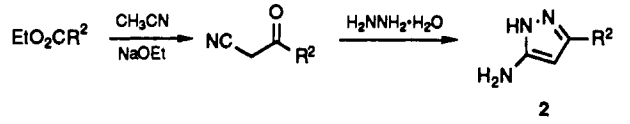
extreme insolubility of 3 resulted in rather slow reaction times, but this problem could be ameliorated by employing tetrabutylammonium fluoride as the base instead of potassium carbonate. This inevitably led to homogenous reaction mixtures and significantly accelerated reaction rates. Table IV presents the results of these alkylations and the particular alkylation method used.

Biology

Each of the new compounds was tested in vitro for ACAT inhibitory activity by using cultured Fu5AH cells. Measurements were performed initially at three different concentrations: 5, 10, and 15 μg/mL. The percents inhibition of ACAT observed at 10 μg/mL are recorded in Table IV. Sufficiently active compounds were further tested to establish IC_{50} values, which are also listed in Table IV.

Evaluation of hypocholesterolemic activity was performed in cholesterol-fed rats. Drug was administered for 7 days, and final serum levels of VLDL/LDL-cholesterol, HDL-cholesterol, and total cholesterol were determined.

Table II. Synthesis of 1H-Pyrazol-3-amines 2



compd	R^2	yield, %	mp °C	formula[a]
2a	Ph[b]	58	125–126.5	
2b	p-Cl-Ph[b]	28	173–174.5	
2c	p-EtO-Ph	32	155–157	$C_{11}H_{13}N_3O$
2d	p-$(CH_2{=}CHCH_2O)Ph$	35	158–159	$C_{12}H_{13}N_3O$
2e	c-C_6H_{11}	85[c]	oil	[d]

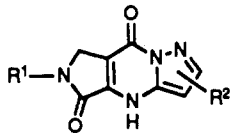
[a] Satisfactory C, H, and N analyses obtained. [b] Previously prepared (ref 8b,c). [c] Preformed lithioacetonitrile was used in the first step (ref 14; see preparation of 3h in Experimental Section). [d] Compound was not purified.

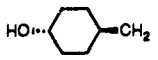
The results are presented in Table V as percentages of control values.

It is immediately evident from the in vitro data that substitution at the 4-nitrogen of the tricyclic nucleus is a requirement for activity. None of the 4-unsubstituted compounds 3 listed in Table III have significant ACAT inhibitory activity, except for the oleyl derivative 3n (59% at 10 μg/mL). Presumably this reflects the extremely poor solubility of this class of compounds which is overcome only by substitution at position 4 or by very large substituents elsewhere on the tricyclic nucleus. Furthermore, the 4-substituent needs to be fairly lipophilic. A simple methyl group (4b) is not sufficient, nor are short alkyl chains terminated with ester groups (4h,p). Significant levels of activity are observed with a variety of lipophilic 4-substituents both aromatic (e.g., 4a) and aliphatic (e.g., 4w), with the most potent analogues possessing a 2-oxo-2-phenylethyl group (4k and 4n). Further variation of this optimal substituent by including electron-withdrawing groups (4v and 4z) or an electron-donating group (4x) does not improve the activity. It is noteworthy that additional hydrocarbon bulk (4u, 4aa, and 4bb) on this 4-substituent significantly reduces activity. Furthermore, replacing the benzoyl ketone with an alcohol (4o) or a sulfone (4dd) is detrimental.

An optimal level of lipophilicity is clearly required at position 6 of the tricyclic nucleus. Alkyl groups in the 6–8-carbon range function well, whether they are acyclic (4ff and 4ii), cyclic (4k), or rigidly bicyclic (4ll). On the other hand, the methyl group (4g) is totally inadequate, while higher alkyl derivatives drop off steeply in activity with increasing molecular weight (4nn and 4hh). Surprisingly, although the benzyl group at position 6 (4j)

Table III. 4*H*-Pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-diones **3**



compd	R^1	R^{2a}	yield,[b] %	formula[c]
3a	c-C_6H_{11}	Ph	73	$C_{20}H_{20}N_4O_2$
3b	$PhCH_2$	Ph	65	$C_{21}H_{16}N_4O_2$
3c	CH_3	Ph	84	$C_{15}H_{12}N_4O_2$
3d	Ph	Ph	78	$C_{20}H_{14}N_4O_2$
3e	c-C_6H_{11}	*p*-ClPh	83	$C_{20}H_{19}ClN_4O_2$
3f	c-C_6H_{11}	*p*-EtOPh	81	$C_{22}H_{24}N_4O_3$
3g	c-C_6H_{11}	H	70	$C_{14}H_{16}N_4O_2$
3h	c-C_6H_{11}	c-C_6H_{11}	62	$C_{20}H_{26}N_4O_2$
3i	c-C_6H_{11}	Ph[a]	51[b]	$C_{20}H_{20}N_4O_2 \cdot 1H_2O$
3j		Ph	81	$C_{21}H_{22}N_4O_3 \cdot 0.25H_2O$
3k	c-C_6H_{11}	*p*-(CH_2=$CHCH_2O$)Ph	89	$C_{23}H_{24}N_4O_3$
3l	*n*-C_6H_{13}	Ph	73	$C_{20}H_{22}N_4O_2$
3m	*n*-$C_{12}H_{25}$	Ph	60	$C_{26}H_{34}N_4O_2$[d]
3n	oleyl	Ph	55	$C_{32}H_{44}N_4O_2$
3o	1,3-$(CH_3)_2$-*n*-C_4H_7	Ph	70	$C_{20}H_{22}N_4O_2$
3p	*tert*-octyl	Ph	55	$C_{22}H_{26}N_4O_2$
3q	2,3-$(CH_3)_2$-c-C_6H_9	Ph	40	$C_{22}H_{24}N_4O_2$
3r	*exo*-2-norbornyl	Ph	73	$C_{21}H_{20}N_4O_2$
3s	c-C_6H_{15}	Ph	72	$C_{22}H_{24}N_4O_2$
3t	c-$C_{12}H_{23}$	Ph	58	$C_{26}H_{32}N_4O_2$
3u	$PhCH(CH_3)$	Ph	69	$C_{22}H_{18}N_4O_2$
3v	*p*-$MeOPhCH_2$	Ph	60	$C_{22}H_{18}N_4O_3$

[a] All substituents at C-2, except for **3i** at C-3. [b] All compounds were obtained as microcrystalline precipitates by filtration of the cooled reaction mixture. Melting points were all >250 °C except for **3i** (mp 148 °C dec). [c] Satisfactory C, H, and N analyses obtained except where noted. [d] C: calcd, 71.86; found, 71.31.

provides excellent ACAT inhibitory activity, the corresponding phenyl derivative **4f** is almost totally inactive.

The substituent on the pyrazole ring is optimally placed at position 2 of the tricyclic nucleus (**4a** vs **4m**) and apparently needs to be aromatic, on the basis of the complete lack of activity of the 2-cyclohexyl derivative **4l**. Addition of electron-withdrawing or donating substituents to the aromatic ring do not significantly alter the activity (**4e** and **4n**).

Finally, it should be noted that inclusion of even mildly hydrophilic groups to any of the three major substituents results in dramatic reductions in activity. This is clearly illustrated with compounds **4q**, **4t**, and **4r**. This last observation is not surprising, considering the lipophilic nature of other known ACAT inhibitors.[2]

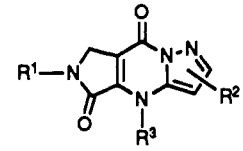
The mechanism by which this class of compounds is inhibiting ACAT is not known. There is no obvious structural similarity to other known ACAT inhibitors, except for a high degree of lipophilicity. In general, the ACAT inhibitors which have been reported to data are a structurally diverse lot, and it has been postulated that nonspecific mechanisms of inhibition, such as alteration of membrane fluidity, may be operating.[2] However, it should be noted that at least one claim of competitive inhibition of ACAT has appeared.[5h]

In addition to the most active in vitro ACAT inhibitors, several inactive compounds were chosen for in vivo testing in the cholesterol-fed rat to help determine if hypocholesterolemic activity might be resulting from mechanisms of action other than ACAT inhibition. With the exception of compound **4hh**, which is only weakly active, none of the nine in vitro inactives display hypolipidemic activity (Table V). Within the group of in vitro actives, the most striking requirement for activity is the 2-oxo-2-phenylethyl group at position 4. Even highly active compounds with the 4-benzyl group (**4a**, **4j**, and **4s**) or the 4-cyclohexylmethyl group (**4w**) have no hypocholesterolemic activity. On the other hand, the 4-(2-oxo-2-phenylethyl) analogues are consistently active with a reasonable correlation between in vitro and in vivo activity (**4k,n,ff–oo,qq**). Surprisingly, however, the 4-(2-oxo-2-phenylethyl) analogues which contained electron-withdrawing substituents (**4v** and **4z**) were not active in vivo. We define in vivo activity here as any combination of VLDL/LDL-cholesterol lowering or HDL-cholesterol raising, each of which would be expected to occur in cholesterol-fed, hypercholesterolemic rats if cholesterol absorption were blocked. Dose response follow-up of the most active compounds established **4n** as the most potent hypocholesterolemic analogue.

Because the hypocholesterolemic effect of **4n** is probably the result of its action on cholesterol absorption in the gut, we compared its effect with that of a known hypocholesterolemic agent that works by binding bile acids in the gut. Under the same experimental conditions used to obtain the data in Table V, colestipol hydrochloride (Colestid) granules administered at a daily dose of 1000 mg/kg resulted in serum VLDL/LDL, HDL, and total cholesterol levels of 48%, 129%, and 86% of control, respectively. Thus, ACAT inhibitor **4n** is slightly more effective than colestipol in this model at far smaller doses.

Additional in vivo experiments were performed to compare the effect of adding the ACAT inhibitor **4n** to the diet with the effect of removing cholesterol from the diet. Rats were fed the cholesterol-containing diet for one week, and then for an additional week were fed either the same diet with the ACAT inhibitor added at a dose of 20 mg/kg of body weight per day or were fed the synthetic diet without cholesterol. A third group was fed the cholesterol-containing diet both weeks. Blood samples were collected at the end of the first and second weeks to assay lipoprotein cholesterol levels. The results are presented in Table VI. When the animals were maintained on the cholesterol-containing diet for both weeks there was a decrease in HDL cholesterol, a slight increase in VLDL/LDL chole-

Table IV. Synthesis and in Vitro Biological Activity of 4-Substituted Pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-diones **4**



compd	R^1	R^{2a}	R^3	yield (method)[b]	mp, °C	form[c]	ACAT inhibition	
							%[d]	IC_{50}[e]
4a	c-C_6H_{11}	Ph	$PhCH_2$	84 (A)	276–278	$C_{27}H_{26}N_4O_2$	88	3.5
4b	c-C_6H_{11}	Ph	CH_3	83 (A)	276–279	$C_{21}H_{22}N_4O_2$	13	
4c	c-C_6H_{11}	Ph	CH_2=$CH(CH_2)_4$	93 (B)	145–146	$C_{26}H_{30}N_4O_2$	93	4.4
4d	c-C_6H_{11}	H	$PhCH_2$	76 (A)	284–286	$C_{21}H_{22}N_4O_2$	25	
4e	c-C_6H_{11}	*p*-EtOPh	$PhCH_2$	89 (A)	263–266	$C_{29}H_{30}N_4O_3$[f]	75	
4f	Ph	Ph	$PhCH_2$	80 (A)	271–272	$C_{27}H_{20}N_4O_2$[g]	18	
4g	CH_3	Ph	$PhCH_2$	47 (A)	283–288	$C_{22}H_{18}N_4O_2 \cdot 0.5H_2O$	8	
4h	c-C_6H_{11}	Ph	$CH_3O_2CCH_2$	85 (A)	257–259	$C_{23}H_{24}N_4O_4$	25	
4i	c-C_6H_{11}	Ph	$CH_3(CH_2)_5$	37 (A)	162–164	$C_{26}H_{32}N_4O_2$	48	
4j	$PhCH_2$	Ph	$PhCH_2$	58 (A)	232–234	$C_{28}H_{22}N_4O_2 \cdot 0.5H_2O$	90	4.7
4k	c-C_6H_{11}	Ph	$PhCOCH_2$	42 (A)	235–237	$C_{28}H_{26}N_4O_3$	92	1.7
4l	c-C_6H_{11}	c-C_6H_{11}	$PhCH_2$	63 (A)	296 dec	$C_{27}H_{32}N_4O_2 \cdot 0.2H_2O$	12	
4m	c-C_6H_{11}	Ph[h]	$PhCH_2$	54 (A)	295 dec	$C_{27}H_{26}N_4O_2$[i]	21	
4n	c-C_6H_{11}	*p*-ClPh	$PhCOCH_2$	40 (A)	237–239	$C_{28}H_{25}ClN_4O_3$	85	1.5
4o	c-C_6H_{11}	Ph	$PhCH(OH)CH_2$	30 (C)	263–266	$C_{28}H_{28}N_4O_3 \cdot 0.5H_2O$	66	
4p	c-C_6H_{11}	Ph	$EtO_2C(CH_2)_3$	84 (B)	195–196	$C_{26}H_{30}N_4O_4$[j]	28	
4q		Ph	$PhCH_2$	53 (A)	315 dec	$C_{28}H_{28}N_4O_3 \cdot 0.5H_2O$	23	
4r	c-C_6H_{11}	Ph	*p*-$(MeO_2C)PhCOCH_2$	80 (A)	215–217	$C_{30}H_{28}N_4O_5 \cdot 0.5H_2O$	1	
4s	c-C_6H_{11}	*p*-$(CH_2$=$CHCH_2O)Ph$	$PhCH_2$	93 (A)	226–227	$C_{30}H_{30}N_4O_3 \cdot 0.25H_2O$	65	5.7
4t	c-C_6H_{11}	*p*-HOPh	$PhCH_2$	68 (D)	307 dec	$C_{27}H_{26}N_4O_3$[k]	33	
4u	c-C_6H_{11}	Ph	$PhCOCH(CH_3)$	69 (E)	268–271	$C_{29}H_{28}N_4O_3 \cdot 0.5H_2O$	55	
4v	c-C_6H_{11}	Ph	m-$NO_2PhCOCH_2$	77 (E)	251–253	$C_{28}H_{25}N_5O_5$	87	1.4
4w	c-C_6H_{11}	Ph	(c-C_6H_{11})CH_2	57 (F)	254–256	$C_{27}H_{32}N_4O_2$[l]	97	3.9
4x	c-C_6H_{11}	Ph	*p*-$MeOPhCOCH_2$	76 (E)	250–252	$C_{29}H_{28}N_4O_4$[m]	52	
4y	c-C_6H_{11}	Ph	$PhSCH_2$	34 (E)	208–208.5	$C_{27}H_{26}N_4O_2S$	55	10.8
4z	c-C_6H_{11}	Ph	*p*-$FPhCOCH_2$	74 (E)	256–257	$C_{28}H_{25}FN_4O_3$	88	2.5
4aa	c-C_6H_{11}	Ph	2-(Napth)$COCH_2$	62 (E)	269–271	$C_{32}H_{28}N_4O_3$	19	
4bb	c-C_6H_{11}	Ph	*p*-$(CH_3(CH_2)_{15}O)$-$PhCOCH_2$	48 (E)	131–133	$C_{44}H_{58}N_4O_4 \cdot 0.5H_2O$	2	
4cc	c-C_6H_{11}	Ph	$PhCH$=$CHCH_2$	57 (E)	220–221.5	$C_{29}H_{28}N_4O_2$	75	7.2
4dd	c-C_6H_{11}	Ph	$PhSO_2CH_2$	65 (G)	267 dec	$C_{27}H_{26}N_4O_4S \cdot 0.5H_2O$	31	
4ee	c-C_6H_{11}	Ph	$PhCO(CH_2)_3$	29 (F)	256–258	$C_{30}H_{30}N_4O_3 \cdot 0.25H_2O$	78	6.8
4ff	*n*-C_6H_{13}	Ph	$PhCOCH_2$	80 (A)	219–220	$C_{28}H_{28}N_4O_3$	75	
4gg	*n*-$C_{12}H_{25}$	Ph	$PhCOCH_2$	70 (A)	196–197	$C_{34}H_{40}N_4O_3$	39	
4hh	oleyl	Ph	$PhCOCH_2$	88 (A)	189–191	$C_{40}H_{50}N_4O_3$	1	
4ii	1,3-$(CH_3)_2$-*n*C_4H_7	Ph	$PhCOCH_2$	74 (A)	204–206	$C_{28}H_{28}N_4O_3$	85	3.0
4jj	*tert*-octyl	Ph	$PhCOCH_2$	89 (A)	262–264	$C_{30}H_{32}N_4O_3$	80	4.4
4kk	2,3-$(CH_3)_2$-c-C_6H_9	Ph	$PhCOCH_2$	72 (A)	282–284	$C_{30}H_{30}N_4O_3$	79	3.3
4ll	*exo*-2-norbornyl	Ph	$PhCOCH_2$	87 (A)	276–278	$C_{29}H_{26}N_4O_3$	83	2.1
4mm	c-C_8H_{15}	Ph	$PhCOCH_2$	87 (A)	221–223	$C_{30}H_{30}N_4O_3$	68	
4nn	c-$C_{12}H_{23}$	Ph	$PhCOCH_2$	33 (A)	253–255	$C_{34}H_{38}N_4O_3$	39	
4oo	$PhCH_2$	Ph	$PhCOCH_2$	84 (A)	308 dec	$C_{29}H_{22}N_4O_3 \cdot 0.25H_2O$	70	2.6
4pp	*p*-$MeOPhCH_2$	Ph	$PhCOCH_2$	73 (A)	303 dec	$C_{30}H_{24}N_4O_4$	10	
4qq	$PhCH(CH_3)$	Ph	$PhCOCH_2$	85 (A)	236–237	$C_{30}H_{24}N_4O_2$	30	

[a] All substituents at C-2 unless otherwise noted. [b] Yield, % (reaction method: see Experimental Section). [c] All compounds exhibited satisfactory C, H, and N analyses except where indicated otherwise. [d] Percent inhibition of Fu5AH ACAT enzyme under the conditions described in the Biological Methods section at a concentration of 10 μg/mL. [e] IC_{50} (μg/mL) for the Fu5AH enzyme. [f] C: calcd, 71.76; found, 72.18. [g] Exact mass calcd: 432.1586; found: 432.1589. [h] Substituent at C-3. [i] C: calcd, 73.95; found, 73.50. [j] C: calcd, 67.51; found, 67.97. [k] C: calcd, 71.35; found, 69.77; N: calcd, 12.33; found, 12.78. [l] C: calcd, 72.95; found, 72.48. [m] C: calcd, 70.15; found, 69.51.

sterol but no change in total cholesterol between weeks one and two. Removing cholesterol from the diet at the end of week one resulted in a modest 14% increase in HDL cholesterol, a 73% decrease in VLDL/LDL cholesterol, and a 42% decrease in total cholesterol by the end of week two. In comparison, when **4n** was added to the cholesterol-containing diet during week two there was virtually no change in HDL cholesterol, a 57% decrease in VLDL/LDL cholesterol and a 42% decrease in total cholesterol by the end of week two. Therefore, the addition of an ACAT inhibitor to the hypercholesterolemic diet was about 78% as effective at lowering serum VLDL/LDL cholesterol as was completely removing cholesterol from the diet. Furthermore, the addition of the ACAT inhibitor to the diet caused exactly the same decrease in total serum cholesterol as did removing cholesterol from the diet.

We undertook further in vivo evaluation of **4n** to determine if cholesterol absorption was actually being inhibited. Cholesterol-fed rats were treated with **4n** and cholesterol absorption was determined by using the dual isotope ratio method as described in the methods section. As shown in Table VII, **4n** caused a 35% decrease in cholesterol absorption. The lipoprotein cholesterol values for these same rats are also shown in Table VII. After 16 days of treatment there was a highly significant increase in HDL cholesterol and a highly significant decrease in VLDL/LDL cholesterol. Thus, a 35% inhibition of cholesterol absorption led to a 62% decrease in the serum

Table V. Lipid Profiles of Cholesterol-Fed Rats Treated with 4

compd	dose,[a] mg/kg	serum cholesterol (% of control) VLDL/LDL-chol	HDL-chol	total chol
4a	50	110	97	104
4c	50	79	114*	95
4e	50	122*	93	110*
4h	50	109	112	109
4j	50	113	92	105
4k	13	84	120*	98
	25	75**	133**	97
	50	65**	128**	88**
4l	50	106	111	109
4n	13	37**	130**	81*
	25	32**	141**	84*
	50	40**	134**	85
4q	50	128	90	108
4r	50	116	99	107
4s	50	98	95	94
4v	50	126	83*	106
4w	50	144*	80**	111
4z	50	95	101	98
4ff	50	86	98	89
4gg	50	85	92	91
4hh	50	69*	86*	76*
4ii	50	57*	89	72*
4jj	50	96	122**	107
4kk	13	70*	105	86
	25	62*	109	83
	50	59**	122*	89
4ll	50	63*	90	75
4mm	50	67*	100	83
4nn	50	76	92	83*
4oo	50	101	100	100
4qq	50	90	92	91

[a] Compounds were administered for 7 days. *$P \leq 0.05$. **$P \leq 0.01$.

Table VI. Serum Lipoprotein Cholesterol in Cholesterol-Fed Rats Treated with the ACAT Inhibitor **4n**

group[a]	lipoprotein fraction	week one	week two	Δ
1	HDL	68.6 ± 6.6	52.2 ± 3.6*	-23.9%
	VLDL/LDL	77.8 ± 17.4	91.9 ± 5.6	+18.1%
	total	146.4 ± 17.4	144.1 ± 6.9	-1.6%
2	HDL	65.8 ± 6.3	75.2 ± 2.7	+14.3%
	VLDL/LDL	118.7 ± 16.7	32.6 ± 3.1**	-72.5%
	total	184.5 ± 17.3	107.8 ± 4.3**	-41.6%
3	HDL	52.9 ± 5.9	50.3 ± 3.5	-4.9%
	VLDL/LDL	129.5 ± 14.0	56.1 ± 4.6**	-56.7%
	total	182.4 ± 11.8	106.4 ± 6.3**	-41.7%

[a] Group 1: fed cholesterol diet both weeks. Group 2: fed cholesterol diet week one and cholesterol-free diet week two. Group 3: fed cholesterol diet week one and cholesterol diet containing ACAT inhibitor **4n** (20 mg/kg of body weight per day) week two. Tabular values are mg/dL, mean ± SEM. All groups contain 10 rats each. *,** Statistical comparisons for lipoprotein fractions between weeks: *$P \leq 0.05$, **$P \leq 0.001$.

concentration of VLDL/LDL cholesterol. The increase in HDL cholesterol is a reflection of the decrease in cholesterol absorption since cholesterol feeding causes a decrease in HDL cholesterol in this animal model.

The data in Tables VI and VII suggest that a 35% reduction in cholesterol absorption has nearly the same effect on serum lipoprotein cholesterol levels as does totally removing cholesterol from the diet. A possible explanation for this is that rats become hypercholesterolemic only after a threshold of cholesterol intake has been exceeded. It is known that rats are highly resistant to diet-induced hypercholesterolemia due to their ability to effectively counter a cholesterol challenge by increasing excretion of bile acids.[11] The 35% reduction in cholesterol absorption effected by **4n** may have been sufficient to allow this compensatory catabolic process to maintain normal serum cholesterol levels.

In summary, we have developed a novel series of ACAT inhibitors, some of which have demonstrated potent hypocholesterolemic activity. On the basis of the results of the cholesterol absorption study with compound **4n**, it is likely that the reductions in serum cholesterol are the result of interference in the uptake of exogenous cholesterol from the gut, presumably as a consequence of the inhibition of intestinal ACAT.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Mass spectra, infrared spectra, and combustion analyses were obtained by the Physical and Analytical Chemistry Department of The Upjohn Company. ^{1}H NMR spectra were recorded at 300 MHz with a Bruker AM-300 spectrometer. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. All other solvents were Burdick and Jackson or Fisher reagent grade.

Ethyl 1-(1,1,3,3-Tetramethylbutyl)-4,5-dioxopyrrolidine-3-carboxylate (11). *tert*-Octylamine (16 mL, 0.10 mol) and ethyl acrylate (10.8 mL, 0.100 mol) were stirred at room temperature in absolute ethanol (33 mL) for 24 h under nitrogen. Diethyl oxalate (13.6 mL, 0.100 mol) and sodium ethoxide (21 wt % in ethanol, 37 mL, 0.10 mol) were then added successively. The reaction mixture was refluxed on a steam bath for 1 h, and the resulting dark mixture was then cooled and concentrated in vacuo. The resulting orange solid was dissolved in boiling water (800 mL) and acidified with concentrated HCl (10 mL) to produce a brown gummy solid. After decanting, the solid was recrystallized from 3:2 ethanol/water (500 mL), affording 13.68 g (48%) of **11** as light orange crystals. An analytical sample was prepared by a second crystallization from 2:1 ethanol/water: ^{1}H NMR ($CDCl_3$) δ 8.6 (bs, 1 H), 4.39 (q, 2 H, J = 7 Hz), 4.11 (s, 2 H), 2.09 (s, 2 H), 1.57 (s, 6 H), 1.42 (t, 3 H, J = 7 Hz), 1.00 (s, 9 H); IR (mull, cm^{-1}) 1723, 1665; EI MS m/e (relative intensity) 283 (M^+, 3), 212 (100), 166 (80).

5-(4-(2-Propenyloxy)phenyl)-1*H*-pyrazol-3-amine (2d). A mixture of ethyl 4-(2-propenyloxy)benzoate (41.3 g, 200 mmol), sodium ethoxide (15.0 g, 220 mmol), and acetonitrile (13 mL, 240 mmol) in dry toluene (85 mL) was mechanically stirred at 108 °C under nitrogen for 24 h. The reaction was cooled and diluted with water (600 mL). After all of the solid had dissolved, the mixture was washed with ether (2 × 100 mL). The aqueous phase was then acidified to pH 5–6 with concentrated HCl (approx 15 mL). The resulting precipitate was collected by suction filtration, washed with water, and air-dried. The crude ketonitrile (22.5 g) was combined in 95% ethanol (150 mL) with hydrazine monohydrate (6.8 mL, 140 mmol) and stirred at reflux for 2 h. Upon cooling to room temperature and then to 0 °C, **2d** crystallized from the reaction mixture. It was collected by suction filtration, washed with cold ethanol, and dried in vacuo: yield 15.07 g (35% overall); ^{1}H NMR (DMSO-d_6) δ 7.55 (d, 2 H, J = 9 Hz), 6.95 (d, 2 H, J = 9 Hz), 6.04 (m, 1 H), 5.68 (bs, 1 H), 5.40 (dd, 1 H, J = 2, 16 Hz), 5.26 (dd, J = 2, 11 Hz), 4.57 (s, 2 H, J = 5 Hz); IR (mull, cm^{-1}) 3198, 1614, 1519, 1251; EI MS m/e (rel intensity) 215 (M^+, 71), 174 (100).

2,6-Dicyclohexyl-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione (3h). *n*-Butyllithium (1.48 M in hexane, 67.6 mL, 100 mmol) was added to THF (70 mL) at -78 °C under nitrogen. While stirring vigorously, dry acetonitrile (5.22 mL, 100 mmol) was added dropwise over 5 min. The mixture was stirred at -78 °C for 1 h before the addition of methyl cyclohexanecarboxylate (7.15 mL, 50.0 mmol) dropwise over 5–10 min. The resulting viscous mixture was stirred at -78 °C for 2 h, and then the reaction was warmed to room temperature over a period of 30 min and stirred another 1 h. Water (125 mL) was added, and the mixture was stirred until all solid dissolved. After the mixture was washed with ether (2 × 100 mL), the aqueous phase was acidified with concentrated HCl and extracted with ether (2 × 100 mL). The combined organic extracts were dried over magnesium sulfate and concentrated in vacuo to afford 1-cyclohexyl-2-cyanoethanone as a brown oil (6.76 g) of sufficient

(11) Wilson, J. D. *J. Lipid Res.* **1964**, *5*, 409.

Table VII. Cholesterol Absorption and Serum Lipoprotein Cholesterol Concentrations in Cholesterol-Fed Rats Treated with ACAT Inhibitor 4n[a]

group	cholesterol absorption, %[b]	Δ	lipoprotein cholesterol, mg/dL[c] HDL	Δ	VLDL + LDL	Δ	total	Δ
control	41.6 ± 2.7		37.0 ± 2.9		117.0 ± 13.7		154.0 ± 14.5	
4n[d]	27.0 ± 3.2**	-35%	76.4 ± 10.4**	+106%	41.8 ± 5.1**	-64%	118.2 ± 10.0*	-23%

[a] All tabular values are mean ± SEM. Groups contain 5 rats each. [b] Determined for day 7 of experiment. [c] Determined at day 16 of experiment. [d] Dosed at 20 mg/kg of body weight per day. *,** Different from control: *$P < 0.08$; **$P < 0.008$.

purity to carry directly into the next step.

Hydrazine monohydrate (0.41 mL, 8.5 mmol) was added to a solution of the crude ketonitrile from the previous step (1.00 g) in 95% ethanol (8.5 mL), and the reaction was refluxed for 2.5 h. After cooling, the solution was concentrated in vacuo, leaving crude 5-cyclohexylpyrazol-3-amine **2e** as a pale yellow viscous oil. This was dissolved in glacial acetic acid (8 mL), and to the solution was added dioxopyrrolidine **1a**[7] (1.65 g, 6.5 mmol). The mixture was heated to reflux and stirred for 4 h, during which time a copious crystalline precipitate appeared. After cooling, the mixture was diluted with 95% ethanol (30 mL) and stirred for 5 min. The mixture was subjected to suction filtration, and the collected solid was washed with 95% ethanol. Drying in vacuo left **3h** as an off-white microcrystalline solid (1.42 g, 62% yield from 5-cyclohexylpyrazol-3-amine, 27% overall from acetonitrile): ^{1}H NMR (DMSO-d_6) δ 6.05 (s, 1 H), 4.36 (bs, 2 H), 3.97 (m, 1 H), 2.71 (m, 1 H), 1.1–2.0 (m, 20 H); IR (mull, cm^{-1}) 1692, 1667, 1633, 1620, 1459; EI MS *m/e* (rel intensity) 355 (M + 1, 14), 354 (M$^+$, 56), 313 (22), 299 (100), 286 (77), 217 (34).

6-Cyclohexyl-6,7-dihydro-3-phenyl-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione (3i). 4-Phenylpyrazol-3-amine[12] (3.00 g, 18.8 mmol) and dioxopyrrolidine **1a** (4.77 g, 18.8 mmol) were combined in glacial acetic acid (19 mL) and stirred at reflux for 5 h. No precipitate formed during the reaction. Upon cooling, the reaction solidified to a light yellow mass. This was dissolved in boiling 95% ethanol (100 mL) before the addition of water (50 mL). The thick yellow precipitate was collected by suction filtration and recrystallized from 95% ethanol (200 mL), affording **3i** as a pale yellow solid (3.31 g, 51%): ^{1}H NMR ($CDCl_3$) δ 8.08 (s, 1 H), 7.44 (d, 2 H, *J* = 8 Hz), 7.36 (t, 2 H, *J* = 8 Hz), 7.23 (t, 1 H, *J* = 8 Hz), 4.37 (s, 2 H), 3.99 (m, 1 H), 1.1–1.9 (m, 10 H); IR (mull, cm^{-1}) 1692, 1638, 1613, 1455; EI MS *m/e* (rel intensity) 349 (M + 1, 23), 348 (M$^+$, 97), 266 (100).

6-Cyclohexyl-6,7-dihydro-2-(4-(2-propenyloxy)phenyl)-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione (3k). A mixture of pyrazole **2d** (14.1 g, 65.4 mmol) and ester **1a** (18.2 g, 71.9 mmol) in 95% ethanol (70 mL) was stirred at reflux for 24 h. The mixture was diluted with more ethanol (150 mL) and then suction filtered. The collected solid was washed with more ethanol and dried in vacuo, leaving **3k** as a white microcrystalline solid (23.6 g, 89%): ^{1}H NMR (DMSO-d_6) δ 7.91 (d, 2 H, *J* = 8 Hz), 7.04 (d, 2 H, *J* = 8 Hz), 6.58 (s, 1 H), 6.07 (m, 1 H), 5.42 (d, 1 H, *J* = 17 Hz), 5.28 (d, 1 H, *J* = 10 Hz), 4.63 (d, 2 H, *J* = 4 Hz), 4.38 (s, 2 H), 3.96 (m, 1 H), 1.1–1.9 (m, 10 H); IR (mull, cm^{-1}) 1694, 1668, 1633, 1620, 1612; EI MS *m/e* (rel intensity) 404 (M$^+$, 78), 363 (100), 281 (24).

2-Phenyl-6-(1,1,3,3-tetramethylbutyl)-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione (3p). A mixture of ester **1l** (11.68 g, 41.2 mmol) and 5-phenylpyrazol-3-amine[8] (6.6 g, 41.2 mmol) in glacial acetic acid (41 mL) was heated to reflux and stirred for 1.5 h. The mixture was cooled, diluted with 95% ethanol (300 mL), and suction filtered. The collected solid was washed with more ethanol and dried in vacuo, affording **3p** as a cream microcrystalline solid (8.53 g, 55%): ^{1}H NMR (DMSO-d_6) δ 8.02 (dd, 2 H, *J* = 2,8 Hz), 7.4–7.6 (m, 3 H), 6.68 (s, 1 H), 4.57 (s, 2 H), 2.08 (s, 2 H), 1.55 (s, 6 H), 0.93 (s, 9 H); IR (mull, cm^{-1}) 1681, 1642, 1609, 1449; EI MS *m/e* (rel intensity) 378 (M$^+$, 13), 266 (100).

Method A. 6-Cyclohexyl-6,7-dihydro-4-(phenylmethyl)-2-(4-(2-propenyloxy)phenyl)-4*H*-pyrazolo[1,5-*a*]pyrrolo-[3,4-*d*]pyrimidine-5,8-dione (4s). A mixture of **3k** (10.0 g, 24.7 mmol), benzyl bromide (5.9 mL, 50 mmol), and potassium carbonate (3.76 g, 27 mmol) in DMF (100 mL) was stirred at room temperature for 24 h. The turbid yellow mixture was poured into vigorously stirring water (300 mL) and stirred for 5 min. The resulting solid was collected by suction filtration and air dried. Recrystallization from 6:1 ethanol/chloroform (1100 mL) afforded **4s** as pale yellow fine needles (11.37 g, 93%): ^{1}H NMR ($CDCl_3$) δ 7.89 (d, 2 H, *J* = 9 Hz), 7.33 (m, 5 H), 6.95 (d, 2 H, *J* = 9 Hz), 6.35 (s, 1 H), 6.05 (m, 1 H), 5.90 (s, 2 H), 5.43 (d, 1 H, *J* = 17 Hz), 5.30 (d, 1 H, *J* = 10 Hz), 4.57 (d, 2 H, *J* = 5 Hz), 4.38 (s, 2 H), 4.16 (m, 1 H), 1.1–2.0 (m, 10 H); IR (mull, cm^{-1}) 1685, 1621, 1594; EI MS *m/e* (rel intensity) 494 (M$^+$, 80), 453 (38), 91 (100).

Method A. 6,7-Dihydro-4-(2-oxo-2-phenylethyl)-2-phenyl-6-(1,1,3,3-tetramethylbutyl)-4*H*-pyrazolo[1,5-*a*]-pyrrolo[3,4-*d*]pyrimidine-5,8-dione (4jj). A mixture of **3p** (8.0 g, 21 mmol), 2-chloroacetophenone (6.5 g, 42 mmol), and potassium carbonate (3.8 g, 28 mmol) in DMF (65 mL) was stirred at 30–35 °C for 24 h. The mixture was diluted with water (300 mL) and suction filtered. The collected solid was recrystallized from ethanol (3 L), affording **4jj** as a flocculent white solid (9.22 g, 89%): ^{1}H NMR (DMSO-d_6) δ 8.14 (d, 2 H, *J* = 7 Hz), 7.9–8.0 (m, 2 H), 7.8–7.9 (m, 1 H), 7.6–7.7 (m, 2 H), 7.4–7.6 (m, 3 H), 7.27 (s, 1 H), 6.3 (bs, 2 H), 4.62 (bs, 2 H), 1.95 (bs, 2 H), 1.50 (s, 6 H), 0.88 (s, 9 H); IR (mull, cm^{-1}) 1708, 1677, 1635, 1595, 1579; EI MS *m/e* (rel intensity) 496 (M$^+$, 35), 425 (100).

Method B. 6-Cyclohexyl-5,6,7,8-tetrahydro-5,8-dioxo-2-phenyl-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-4-butanoic acid, Ethyl Ester (4p). A mixture of **3a** (1.00 g, 2.87 mmol), ethyl 4-bromobutyrate (0.82 mL, 5.7 mmol), and potassium carbonate (0.41 g, 3.0 mmol) in DMSO (11 mL) was stirred at 50 °C for 17 h. Water (35 mL) was added, and the mixture was suction filtered. The collected solid was washed with water and air dried. Recrystallization from 95% ethanol (100 mL) afforded **4p** (1.11 g, 84%) as very fine white needles: ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 2 H, *J* = 8 Hz), 7.45 (m, 3 H), 6.70 (s, 1 H), 4.74 (t, 2 H, *J* = 7 Hz), 4.37 (s, 2 H), 4.16 (q, 2 H, *J* = 7 Hz), 4.15 (m, 1 H), 2.49 (t, 2 H, *J* = 7 Hz), 2.23 (m, 2 H), 1.2–2.0 (m, 10 H); IR (mull, cm^{-1}) 1728, 1698, 1679, 1615, 1590, 1576; EI MS *m/e* (rel intensity) 462 (M$^+$, 67), 115 (100), 87 (50).

Method C. 6-Cyclohexyl-6,7-dihydro-4-(2-hydroxy-2-phenylethyl)-2-phenyl-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]-pyrimidine-5,8-dione (4o). A mixture of **3a** (0.50 g, 1.4 mmol), triethylamine (0.30 mL, 2.2 mmol), and styrene oxide (0.33 mL, 2.9 mmol)in absolute ethanol (6 mL) was stirred at reflux for 16 h. The dark brown reaction was cooled and diluted with 95% ethanol (30 mL). After stirring for 5 min, the mixture was suction filtered, and the collected solid was washed with more ethanol and air dried. Recrystallization from 2.5:1 ethanol/chloroform (70 mL) provided **4o** (205 mg, 30%) as a light tan solid: ^{1}H NMR ($CDCl_3$) δ 7.98 (d, 2 H, *J* = 8 Hz), 7.56 (d, 2 H, *J* = 7 Hz), 7.3–7.5 (m, 6 H), 6.41 (s, 1 H), 5.27 (m, 1 H), 4.98 (dd, 1 H, *J* = 9, 13 Hz), 4.62 (dd, 1 H, *J* = 3, 13 Hz), 4.34 (s, 2 H), 4.14 (m, 1 H), 3.81 (d, 1 H, *J* = 6 Hz), 1.1–2.0 (m, 10 H); IR (mull, cm^{-1}) 3408, 1685, 1676, 1621, 1591, 1577; EI MS *m/e* (rel intensity) 468 (M$^+$, 13), 362 (100).

Method D. 6-Cyclohexyl-6,7-dihydro-4-(phenylmethyl)-2-(4-hydroxyphenyl)-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione (4t). To a suspension of 10% Pd/C (0.40 g) in 95% ethanol (50 mL) was added **4s** (2.00 g, 4.05 mmol), followed by 70% aqueous perchloric acid (0.4 mL, 4 mmol). The mixture was stirred at reflux for 29 h. After cooling, chloroform (70 mL) was added, and the mixture was filtered through Celite. The filtrate was concentrated by boiling to a volume of about 75 mL. Cooling to 0 °C for several days provided **4t** (1.25 g, 68%) as off-white prisms: ^{1}H NMR (DMSO-d_6) δ 7.77 (d, 2 H, *J* = 8 Hz), 7.35 (m, 5 H), 6.85 (d, 2 H, *J* = 8 Hz), 6.83 (s, 1 H), 5.87 (s, 2 H), 4.38 (s, 2 H), 3.96 (m, 1 H), 1.1–1.9 (m, 10 H); IR (mull, cm^{-1}) 3491, 3375, 3175, 1693, 1678, 1614, 1596, 1577, 1535; EI MS *m/e*

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(rel intensity) 454 (M^+, 44), 363 (10), 91 (100).

Method E. 6-Cyclohexyl-4-(2-(4-fluorophenyl)-2-oxoethyl)-6,7-dihydro-2-phenyl-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione (4z). A solution of tetrabutylammonium fluoride (1.0 M in THF, 1.3 mL, 1.3 mmol) was concentrated in vacuo to a white solid. DMF (4.0 mL) and **3a** (300 mg, 0.861 mmol) were added, forming a colorless solution. 4'-Fluoro-2-chloroacetophenone (223 mg, 1.29 mmol) was added and the reaction was stirred at room temperature for 19 h. The mixture was poured into water (40 mL) with stirring, and the solid precipitate was collected by suction filtration. After washing with water and air-drying, the solid was recrystallized from 4:1 ethanol/chloroform (125 mL), affording **4z** (309 mg, 74%) as pale yellow fine prisms: ^{1}H NMR ($CDCl_3$) δ 8.08 (m, 2 H), 7.95 (dd, 2 H, J = 2, 8 Hz), 7.4 (m, 3 H), 7.24 (t, 2 H, J = 8 Hz), 6.28 (s, 1 H), 6.05 (bs, 2 H), 4.37 (s, 2 H), 4.03 (m, 1 H), 1.1–1.9 (m, 10 H); IR (mull, cm^{-1}) 1704, 1675, 1625, 1597, 1592, 1580; EI MS m/e (rel intensity) 484 (M , 100), 361 (73), 123 (92).

Method F. 6-Cyclohexyl-4-(cyclohexylmethyl)-6,7-dihydro-2-phenyl-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione (4w). A solution of tetrabutylammonium fluoride (1.0 M in THF, 1.3 mL, 1.3 mmol) was concentrated in vacuo to a white solid. DMF (4.0 mL), **3a** (300 mg, 0.861 mmol), and (bromomethyl)cyclohexane (0.24 mL, 1.7 mmol) were added, and the solution was stirred at 60 °C for 8 h and 50 °C for 48 h. The reaction was poured into water (50 mL) and suction filtered. The collected solid was washed with water and air dried. Recrystallization from 10:1 ethanol/chloroform (60 mL) afforded **4w** (218 mg, 57%) as fine cream needles: ^{1}H NMR ($CDCl_3$) δ 8.05 (dd, 2 H, J = 2, 8 Hz), 7.45 (m, 3 H), 6.48 (s, 1 H), 4.52 (bd, 2 H), 4.36 (s, 2 H), 4.13 (m, 1 H), 1.1–2.1 (m, 21 H); IR (mull, cm^{-1}) 1699, 1680, 1615, 1588, 1575; EI MS m/e (rel intensity) 444 (M^+, 49), 349 (100), 319 (42).

Method G. 6-Cyclohexyl-6,7-dihydro-2-phenyl-4-((phenylsulfonyl)methyl)-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione (4dd). To a solution of sulfide **4y** (0.54 g, 1.2 mmol) in methylene chloride (55 mL) at room temp was added *m*-chloroperoxybenzoic acid (80%, 1.24 g, 5.75 mmol). After 1.5 h, the solution was poured into saturated aqueous sodium bicarbonate (100 mL) containing sodium thiosulfate (2.5 g). The mixture was shaken until gas evolution ceased, the organic layer was separated, and the aqueous phase was extracted once more with methylene chloride (30 mL). The extracts were dried over magnesium sulfate and concentrated in vacuo. The resulting crusty foam was crystallized from 5:1 ethanol/chloroform (250 mL), affording **4dd** (376 mg, 65%) as pale yellow fine needles: ^{1}H NMR ($CDCl_3$) δ 7.95 (d, 2 H, J = 8 Hz), 7.88 (d, 2 H, J = 8 Hz), 7.65 (t, 1 H, J = 8 Hz), 7.53 (t, 2 H, J = 8 Hz), 7.43 (m, 3 H), 6.59 (s, 1 H), 4.23 (s, 2 H), 3.99 (m, 1 H), 1.1–2.0 (m, 10 H); IR (mull, cm^{-1}) 1705, 1686, 1626, 1598, 1580; EI MS m/e (rel intensity) 502 (M^+, 6), 361 (100).

Biological Methods. In Vitro ACAT Assay. Initial testing of compounds for possible ACAT inhibitory activity was conducted in vitro in cultured Fu5AH cells by using methodology previously described.[13]

Cholesterol-Fed Rat Assay. Male Sprague-Dawley rats weighing 100–110 g were evenly distributed by weight into groups of 10. The animals were housed in stainless steel cages with five rats per cage. Food and water were provided ad libitum. All experiments started two days after grouping the animals and continued for seven days. The animals were fed either a standard rat chow (Rodent diet no. 5002, Purina Mills, Inc., St. Louis, MO) or a synthetic diet containing cholesterol (1% cholesterol, 20% casein, 57.85% sucrose, 10% lard, 6% cellulose fiber, 4% mineral mix, 1% vitamin mix, and 0.15% choline chloride, w/w). Test compounds were mixed into the diets in amounts that were calculated to provide the desired daily doses on the basis of the average daily food consumption and the average body weights of the animals.

Lipoprotein Isolation and Analysis. Rats were anesthetized with Cyclopal sodium (1 mL ip at a concentration of 2.5% in saline) and bled from the right jugular vein. Serum samples were obtained after low-speed centrifugation. β- and α- lipoproteins (VLDL + LDL and HDL, respectively) were isolated from individual serum samples by precipitation using PEG-8000 in 0.2 M glycine buffer, pH 9. Serum (300 μL) was mixed with 300 μL of Solution A (20 g PEG-8000 + 100 mL glycine buffer), and after 10 min at room temperature the samples were centrifuged for 45 min at 2000g at 4 °C. The α-lipoprotein supernatant was decanted, and the β-lipoprotein pellet was dissolved in 300 μL of Solution B (10 mL Triton X-100 + 1 L Milli Q water). Cholesterol concentrations in the α- and β-lipoprotein fractions were measured by using a Demand autoanalyzer (Cooper Biomedical, Diagnostics Division, Freehold, NJ) and Demand enzymatic reagents. The data were statistically analyzed by using a one-way classification design.[15] The mean response for each test compound was compared with the mean observed in the control animals by the LSD method.[16]

Cholesterol Absorption. The effect of **4n** on cholesterol absorption was determined in rats by using the dual-isotope plasma ratio method of Zilversmit.[17,18] Groups of rats were fed the cholesterol-containing diet with or without **4n** added to the diet at a rate to provide a daily dose of 20 mg/kg. After one week [^{3}H]cholesterol was administered iv and [^{14}C]cholesterol was administered orally by gavage. Blood samples were collected on days 4, 7, and 9 after the administration of radiolabeled cholesterol. The percentage of oral cholesterol absorbed was determined by using the isotope ratio method as described.[18] A mean value was calculated for each rat by using the individual values for the 3 days that blood samples were collected. Differences between the control and treated groups were statistically analyzed by using a one-way classification design.

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