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Synthesis, X-ray Crystal Structure, and Biological Activity of FR186054, a Novel, Potent, Orally Active Inhibitor of Acyl-CoA:Cholesterol *O*-Acyltransferase (ACAT) Bearing a Pyrazole Ring

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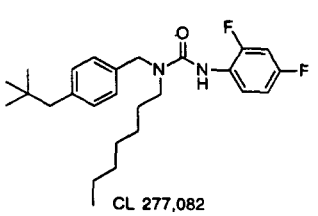
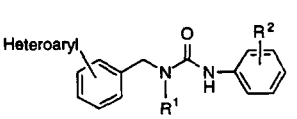
Abstract: The synthesis, single crystal X-ray structural analysis, and biological activity of FR186054 (**2c**), a new, potent, orally efficacious inhibitor of acyl-CoA:cholesterol *O*-acyltransferase (ACAT), containing a pyrazole ring are described. This compound displayed excellent *in vivo* efficacy, irrespective of dosing method, indicating superior characteristics compared to other ACAT inhibitors.

Introduction

Hypercholesterolemia is one of the major risk factors for the development of coronary heart disease (CHD).[1] Considerable efforts have been directed toward the development of potential therapies for the treatment of atherosclerotic disease.[2] Acyl-CoA:cholesterol *O*-acyltransferase (ACAT, EC 2.3.1.26)[3] is an intracellular enzyme responsible for catalyzing the esterification of free cholesterol with fatty acyl-CoA to produce cholesteryl esters. This enzyme plays an important role in the absorption of dietary and biliary cholesterol, the secretion of hepatic very low density lipoprotein (VLDL), and the accumulation of cholesteryl esters in arterial lesions. Inhibition of ACAT would be expected to reduce the absorption of cholesterol, lower plasma lipid levels, and prevent progression and promote regression of atherosclerotic lesions. Therefore, ACAT inhibitors offer potential as new treatments for hypercholesterolemia and atherosclerosis.[4]



CL 277,082



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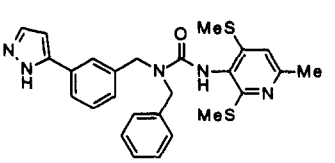

FR186054 (2c)

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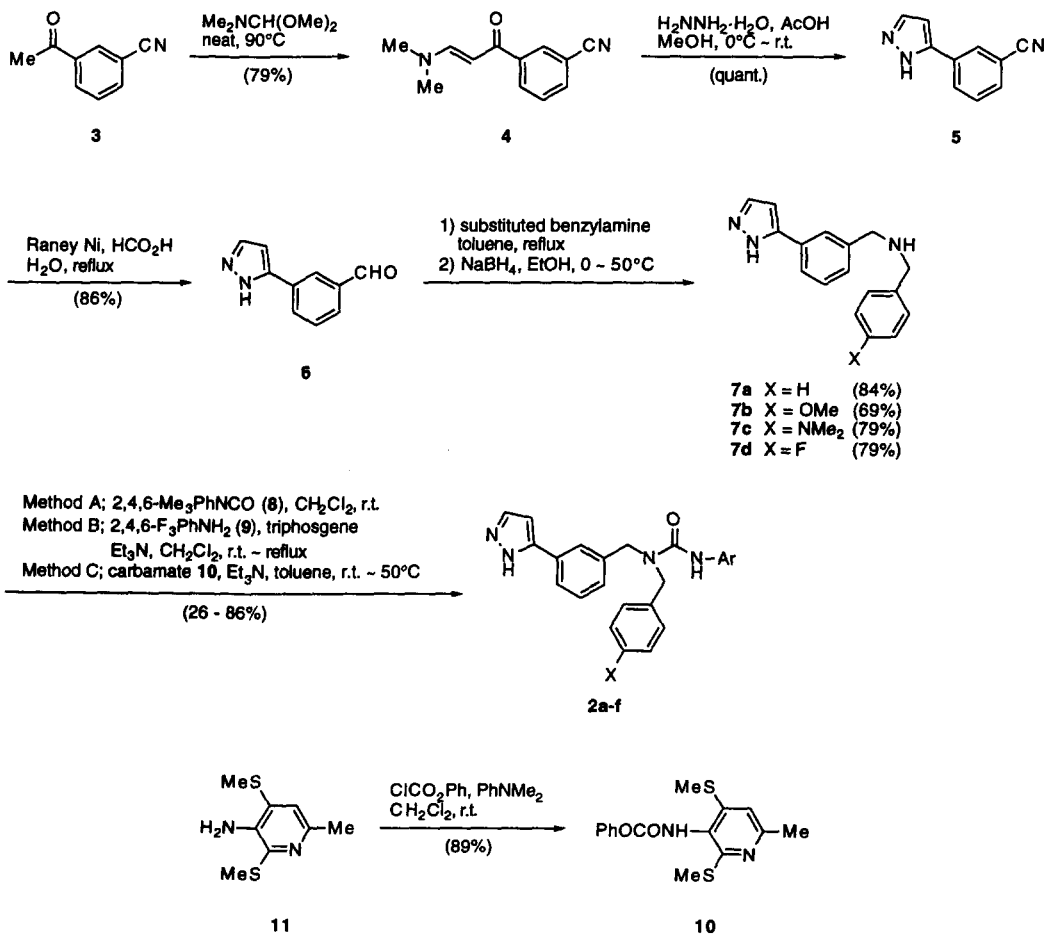
In recent years, a number classes of compounds have been shown to inhibit ACAT-catalyzed cholesterol esterification.[5] For example, in the *N*-alkyl-*N*-benzyl-*N'*-phenylurea series,[6] CL 277,082[6a] has been the subject of various animal and human studies;[7] although it produced no clinically detectable benefits.[7c] As part of our program directed at the development of potent ACAT inhibitors that are particularly effective in *in vivo* models of hypercholesterolemia, we have investigated a series of *N*-alkyl-*N*-heteroaryl-substituted benzyl-*N'*-arylureas represented by **1** as a series related to CL 277,082 designed to have improved physicochemical characteristics, and hopefully, thereby, superior bioavailability. As a result of these investigations, we have discovered FR186054 (**2c**), a new ACAT inhibitor, bearing a pyrazole ring that exhibited potent *in vitro* ACAT inhibitory activity and excellent hypocholesterolemic effects in cholesterol-fed rats. In this communication, we wish to disclose the synthesis, single crystal X-ray structural analysis, and biological activity of this new hypocholesterolemic agent.

Scheme 1



Synthesis

The synthetic route to the *N*-alkyl-*N*-pyrazolylbenzyl-*N'*-arylureas described in this paper is summarized in Scheme 1. Commercially available 3-acetylbenzonitrile (**3**) was converted to enamine (**4**) as a single isomer by treatment with *N*,*N*-dimethylformamide dimethylacetal at 90℃ (79%). Construction of the pyrazole ring was accomplished by reaction with hydrazine monohydrate - acetic acid in methanol at room temperature to afford 3-pyrazol-3-ylbenzonitrile (**5**) quantitatively. Smooth reduction to the corresponding aldehyde **6** was accomplished by exposure to Raney nickel in aqueous formic acid (86%). Reductive amination of the benzaldehyde **6** with various substituted benzylamines provided the desired secondary amines **7a-d** in excellent yield (69-84%). Elaboration to the final hypocholesterolemic agents **2a-f** was performed by one of three methods: (1) treatment with 2,4,6-trimethylphenylisocyanate (**8**) (Method A), (2) reaction with 2,4,6-trifluoroaniline (**9**) in the presence of triphosgene (Method B), (3) treatment with phenyl *N*-arylcarbamate **10**, which was readily obtained by the reaction of aminopyridine derivative **11**[8] with phenyl chloroformate (Method C). It is particularly noteworthy that no protecting group strategies were required in this synthesis, resulting in a concise, practical route.

Biological Activity

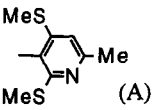
Biological data for the prepared urea compounds in comparison to CL 277,082 are shown in Table 1. *In vitro* inhibition of intestinal ACAT was evaluated by incubation with [1-^{14}C]oleoyl-CoA and the mucosal microsomes from the small intestine of cholesterol-fed rabbits[9] and *in vivo* hypocholesterolemic activity was assessed in cholesterol-fed rats by oral administration of the test compounds as a dietary admixture in a cholesterol-enriched diet[10] or by gavage in PEG400 as a vehicle.[11] The *in vitro* activity is expressed as the nanomolar concentration of a compound required to inhibit 50% of the enzyme activity (IC_{50}), the *in vivo* cholesterol-lowering activity is presented in terms of percent reduction at the dose or ED_{50}, the effective dose to reduce plasma total cholesterol level by 50%.

Although **2a** and **2b** showed more potent ACAT inhibitory activity *in vitro* than CL 277,082, **2c** bearing the substituted pyridine ring introduced into systemically available ACAT inhibitors by Pfizer[8] was 3-fold less potent. However, of these three compounds, the hypocholesterolemic effect *in vivo* of **2c** was clearly superior when dosed as a dietary admixture, being 100-fold more potent compared with the reference compound, presumably as a result of improved pharmacokinetics.

It has been shown previously that the bioavailability of ACAT inhibitors can be markedly influenced by modes of drug dosing.[12] Therefore, we next evaluated the hypocholesterolemic effects of **2b** and **2c** in a different administration model, *i.e.* by gavage in PEG400 as a vehicle.[11] Using this model, **2b** which showed excellent activity when dosing as a dietary admixture, was only modestly potent. However, it was gratifying that **2c** was still efficacious even when dosed by gavage in PEG400. Although the precise reasons why **2c** exhibited such high potency are still unclear at this point, it is possibly related to improved bioavailability of this inhibitor.

Introduction of a substituent on to the 4-position of *N*-benzyl group of **2c** resulted in reduced *in vitro* activity (**2d**, **2f**) irrespective of their electronic and/or steric effect, however their cholesterol lowering activity *in vivo* was retained, and especially **2d** exhibited about the same level as the parent compound (**2c**). As a consequence these modifications could not produce any significant improvement in the biological activity. On the basis of the results discussed above, *N*-benzyl-*N*-[3-(pyrazol-3-yl)benzyl]-*N'*-[2,4-bis(methylthio)-6-methylpyridin-3-yl]urea (**2c**, FR186054) was identified as a potent, orally efficacious ACAT inhibitor, independent of the administration mode, and selected for further development as a new treatment for hypercholesterolemia and atherosclerosis.

Table 1. Biological Activity

compound	Ar	X	yield[a] (method)	ACAT inhibitory activity[b] IC_{50} (nM)	hypocholesterolemic activity[c] ED_{50} (mg/kg) administration mode diet[d]	gavage[e]
2a	2,4,6-Me_3Ph	H	86 (A)	21	> 1 (36)	ND
2b	2,4,6-F_3Ph	H	26 (B)	14	0.096	8.6
2c	 (A)	H	78 (C)	99	0.046	0.44
2d	(A)	OMe	68 (C)	200	< 0.1 (81)**[f]	ND
2e	(A)	NMe_2	76 (C)	72	< 0.1 (61)*[f]	ND
2f	(A)	F	61 (C)	250	< 0.1 (66)**[f]	ND
CL 277,082				33	5.0	ND

[a] Yield (%) of final step. [b] IC_{50} (nM) for the enzyme obtained from rabbit intestinal microsomes. [c] ED_{50} values are the effective dose to reduce plasma total cholesterol level by 50% of the control value. Values in parentheses denotes percent reduction in total cholesterol at the dose indicated. [d] Compound was administered as a dietary admixture.[10] [e] Compound was administered by gavage in PEG400 as a vehicle.[11] [f] Significantly different from control using unpaired, two-tailed Student's *t*-test, $*p < 0.05$, $**p < 0.01$. ND denotes not determined.

Single Crystal X-Ray Structural Analysis of FR186054 (2c)

Despite a number of conformational analyses around the arylurea or anilide moiety of ACAT inhibitors derived from the results of SAR[13] and molecular modeling studies,[14] to our knowledge, X-ray crystal structure analysis has not been reported yet. Since we fortunately succeeded in efforts to obtain suitable large crystals of FR186054 from CH_2Cl_2-MeOH-hexane, we performed a single crystal X-ray structure determination.[15] Figure 1 shows the ORTEP plot of the obtained structure.

In general, one of the structural requirements for optimal ACAT inhibitory activity is 2,6-substitution on the phenyl ring in both main classes of ACAT inhibitors, the phenylureas and the anilides. On the basis of the results of SAR[13] and molecular modeling studies,[14] it is considered that the spatial arrangement of the carbonyl function, *i.e.* the carbonyl moiety oriented in a perpendicular direction to the aromatic ring, is important for good interaction at the enzyme site. In this X-ray structural analysis of FR186054, it was revealed that the orientation of the urea carbonyl relative to the substituted pyridine ring (C2-N1-C5-C6) is 105.8°. This is consistent with the earlier studies. In addition, it is very interesting that the pyrazolylbenzyl substituent, the largest substituent on the urea nitrogen, is oriented transoid to the urea carbonyl group, irrespective of the small environmental difference between the two benzyl groups on the same urea nitrogen. Furthermore, the two methylthio groups on the pyridine ring are oriented away from the urea moiety, presumably to limit steric interaction, and also they are in the plane of the pyridine ring (torsion angles: N9-C10-S14-C15: -8.6°; C7-C6-S11-C12: 1.4°). ^{1}H NMR spectra of FR186054 obtained in DMSO-d_6 shows equilibrium of the position of hydrogen on the pyrazole ring,[16] whereas in the crystal state, it is fixed on the inner nitrogen atom (N24). This may in part be attributed to the existence of an intermolecular hydrogen bond between the pyrazole nitrogen (N24) and the urea carbonyl oxygen (O3) of another molecule through this hydrogen (intermolecular N24-O3 distance is 2.92 Å). Additionally, the pyrazole ring - phenyl ring dihedral angle (C20-C19-C23-C27) is 18.3°.

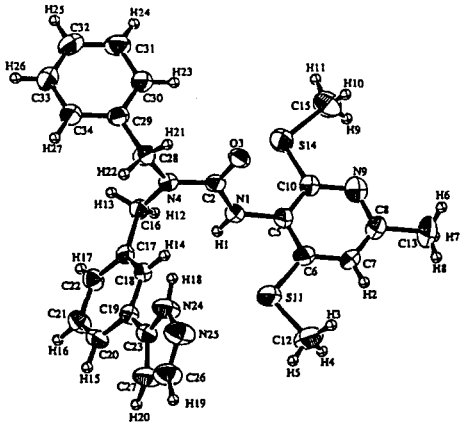


Figure 1. ORTEP drawing of FR186054 (**2c**) with crystallographic numbering scheme.

Summary

In this communication, we have described the discovery of FR186054, a new, orally potent ACAT inhibitor irrespective of dosing mode, that contains a pyrazole ring. Furthermore, the interesting crystal structure of this compound, which is the first report of X-ray structural analysis of an ACAT inhibitor, was disclosed. The full details of the structure-activity relationships, chemical synthesis, and pharmacological studies of this novel series of compounds will be the subject of future publications from these laboratories.[17]

References and Notes

1. (a) Martin, M. J.; Hulley, S. B.; Browner, W. S.; Kuller, L. H.; Wentworth, D. *Lancet* **1986**, *2*, 933. (b) Badimon, J. J.; Fuster, V.; Chesebro, J. H.; Badimon, L. *Circulation* **1993**, *87* (supple II) 3.
2. McCarthy, P. A. *Med. Res. Rev.* **1993**, *13*, 139.
3. For the role of ACAT, see: (a) Spector, A. A.; Mathur, S. N.; Kaduce, T. L. *Prog. Lipid Res.* **1979**, *18*, 31. (b) Suckling, K. E.; Stange, E. F. *J. Lipid Res.* **1985**, *26*, 647.
4. Sliskovic, D. R.; White, A. D. *Trends Pharmacol. Sci.* **1991**, *12*, 194.
5. For recent reviews of ACAT inhibitors, see: (a) Picard, J. A. *Curr. Opin. Ther. Patents* **1993**, *3*, 151. (b) Matsuda, K. *Med. Res. Rev.* **1994**, *14*, 271. (c) Sliskovic, D. R.; Trivedi, B. K. *Curr. Med. Chem.* **1994**, *1*, 204.
6. (a) DeVries, V. G.; Schaffer, S. A.; Largis, E. E.; Dutia, M. D.; Wang, C.-H.; Bloom, J. D.; Katocs, A. S., Jr. *J. Med. Chem.* **1986**, *29*, 1131. (b) DeVries, V. G.; Bloom, J. D.; Dutia, M. D.; Katocs, A. S., Jr.; Largis, E. E. *J. Med. Chem.* **1989**, *32*, 2318.
7. (a) Largis, E. E.; Wang, C. H.; DeVries, V. G.; Schaffer, S. A. *J. Lipid Res.* **1989**, *30*, 681. (b) Burrier, R. E.; Deren, S.; McGregor, D. G.; Hoos, L. M.; Smith, A. A.; Davis, H. R., Jr. *Biochem. Pharmacol.* **1994**, *47*, 1545. (c) Harris, W. S.; Dujovne, C. A.; von Bergmann, K.; Neal, J.; Akester, J.; Windsor, S. L.; Greene, D.; Look, Z. *Clin. Pharmacol. Ther.* **1990**, *48*, 189.
8. McCarthy, P. A.; Hamanaka, E. S.; Marzetta, C. A.; Bamberger, M. J.; Gaynor, B. J.; Chang, G.; Kelly, S. E.; Inskeep, P. B.; Mayne, J. T.; Beyer, T. A.; Walker, F. J.; Goldberg, D. I.; Savoy, Y. E.; Davis, K. M.; Diaz, C. L.; Freeman, A. M.; Johnson, D. A.; LaCour, T. G.; Long, C. A.; Maloney, M. E.; Martingano, R. J.; Pettini, J. L.; Sand, T. M.; Wint, L. T. *J. Med. Chem.* **1994**, *37*, 1252.

9. Sakuma, Y.; Hagihara, H.; Ohne, K.; Nagayoshi, A.; Mutoh, S.; Ito, Y.; Notsu, Y.; Okuhara, M. *Jpn. J. Pharmacol.* **1996**, *70*, 35.
10. The test compounds were administered to male rats (n = 4) by admixing with a diet supplemented with cholesterol (1%) and cholic acid (0.5%). After 7 days of feeding, plasma total cholesterol (TC) was measured and the percent change vs control was determined and expressed as the percent reduction at the dose or ED_{50}, the effective dose to reduce plasma total cholesterol level by 50%.
11. In this much more clinically relevant model, hypercholesterolemia was first established in male rats (n = 4) by feeding a diet supplemented with cholesterol (1%) and cholic acid (0.5%) for 2 days, and was then followed by oral administration of the test compound dissolved in PEG400 by gavage once a day in the evening for 3 days, continuing the cholesterol-enriched diet. Efficacy was determined on the day following 3 day administration of the compounds and presented in terms of ED_{50}.
12. (a) Trivedi, B. K.; Purchase, T. S.; Holmes, A.; Augelli-Szafran, C. E.; Essenburg, A. D.; Hamelehle, K. L.; Stanfield, R. L.; Bousley, R. F.; Krause, B. R. *J. Med. Chem.* **1994**, *37*, 1652. (b) Inskeep, P. B.; Davis, K. M.; Reed, A. E. *J. Pharm. Sci.* **1995**, *84*, 131.
13. (a) Trivedi, B. K.; Holmes, A.; Stoeber, T. L.; Blankley, C. J.; Roark, W. H.; Picard, J. A.; Shaw, M. K.; Essenburg, A. D.; Stanfield, R. L.; Krause, B. R. *J. Med. Chem.* **1993**, *36*, 3300. (b) Augelli-Szafran, C. E.; Blankley, C. J.; Roth, B. D.; Trivedi, B. K.; Bousley, R. F.; Essenburg, A. D.; Hamelehle, K. L.; Krause, B. R.; Stanfield, R. L. *J. Med. Chem.* **1993**, *36*, 2943. (c) Augelli-Szafran, C. E.; Roth, B. D.; Essenburg, A.; Hamelehle, K. L.; Krause, B. R.; Stanfield, R. L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1095 and references cited therein.
14. (a) Sekiya, T.; Inoue, S.; Shirasaka, T.; Miyajima, C.; Okushima, H.; Suzuki, K.; Kawai, M.; Mitsuka, M.; Umezu, K. *Chem. Pharm. Bull.* **1994**, *42*, 586. (b) Kumazawa, T.; Yanase, M.; Shirakura, S.; Ohishi, E.; Yamada, K.; Matsumiya, S.; Kono, M. *Chem. Pharm. Bull.* **1996**, *44*, 222.
15. Crystal data for FR186054 (**2c**): Crystal dimensions 0.20 × 0.10 × 0.10 mm, $C_{26}H_{27}N_5OS_2$, M = 489.65, triclinic, a = 11.558(1) Å, b = 12.057(1) Å, c = 9.5234(9) Å, α = 96.922(9)°, β = 99.110(8)°, γ = 104.772(8)°, V = 1248.9(2) Å^3, space group $P\bar{1}$(#2), Z = 2, Dc = 1.302 g cm^{-3}, μ(Cu-Kα) = 21.56 cm^{-1}, F(000) = 516.00. Data were obtained at 25.0℃ on a Rigaku AFC5R diffractometer using graphite monochromated Cu-Kα radiation. A total of 4480 refractions (4246 unique) were collected using the ω-2θ scan technique within a 2θ range of 130.2°. The structure was solved by direct methods and refined by a full-matrix least-squares method using 3471 reflections [$I > 3.00\sigma(I)$]. The final refinement converged to R = 0.048 and R_w = 0.042.
16. Selected spectroscopic data for FR186054 (**2c**): mp 209-210℃; ^{1}H NMR (200MHz, DMSO-d_6) δ 2.42 (6H, s), 2.46 (3H, s), 4.49 (4H, s), 6.67 (1H, br s), 6.90 (1H, s), 7.18-7.90 (10H, m), 8.29 (1H, s), 12.88, 13.30 (total 1H, each br s); IR (KBr) 3390, 3246, 2920, 1651, 1562, 1489, 1228 cm^{-1}; APCI-MS m/z 490 (MH^+). Anal. Calcd for $C_{26}H_{27}N_5OS_2$: C, 63.78; H, 5.56; N, 14.30. Found: C, 63.94; H, 5.65; N, 14.20.
17. In the SAR studies on compounds of type **1**, a pyrazol-3-yl group on the N-benzyl group of trisubstituted urea **1** was identified as a heteroaryl providing a good profile of activity. In this communication we have disclosed the results of the final optimization of R^1 and R^2. The full paper will describe this optimization in greater detail.