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# Inhibitors of acyl-CoA:cholesterol *O*-acyltransferase (ACAT). Part 1: Identification and structure–activity relationships of a novel series of substituted *N*-alkyl-*N*-biphenylylmethyl-*N'*-arylureas

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**Abstract**

A series of *N*-alkyl-*N*-biphenylylmethyl-*N'*-arylurea and related derivatives represented by **1** have been prepared and evaluated for their ability to inhibit acyl-CoA:cholesterol *O*-acyltransferase in vitro and to lower plasma cholesterol levels in cholesterol-fed rats in vivo. Linking of two phenyl groups via oxygen and introduction of fluorine at appropriate positions on the biphenyl moiety improved in vitro and in vivo activity. From this series of analogs, compound **40** (FR179254), which had potent in vitro potency (rabbit intestinal microsomes $IC_{50}$ = 25 nM), showed excellent plasma cholesterol-lowering activity when administered via the diet ($ED_{50}$ = 0.045 mg/kg). However, the hypocholesterolemic effect of this compound was moderate when dosed by oral gavage in PEG400 as a vehicle ($ED_{50}$ = 5.3 mg/kg). Modification of the *N'*-aryl moiety led to the identification of compound **50** (FR182980) which was efficacious in both dosing models ($ED_{50}$ = 0.034 mg/kg and 0.11 mg/kg, respectively). 

## 1. Introduction

Hypercholesterolemia is now well known as a major risk factor for the development of atherosclerosis [1], and the therapeutic reduction of serum cholesterol levels has been proven to be an effective treatment for 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. In the intestine, dietary and biliary cholesterol is esterified by ACAT prior to incorporation into chylomicrons and secretion into the lymphatic system. In addition, ACAT plays an important role for the hepatic production of very low density lipoprotein (VLDL), the precursor of the atherogenic low density lipoprotein (LDL). Atherosclerotic lesions in the arterial walls are considered to be promoted by the accumulation of cholesteryl esters in macrophages (foam cell formation) in which ACAT activity is enhanced. Inhibition of ACAT decreases the absorption of dietary cholesterol, reduces hepatic VLDL secretion, and consequently lowers plasma total cholesterol levels [4]. In addition to affecting plasma cholesterol levels, ACAT inhibition in arterial macrophages would also be expected to prevent accumulation of cholesteryl esters in the artery and result in a direct effect on the regression of arterial lesions [5]. Therefore, ACAT inhibitors offer significant potential as new treatments for hypercholesterolemia and atherosclerosis [6].

In recent years a large number of ACAT inhibitors have been reported [7]. Most of them are classified into two major groups, one being fatty acid anilide derivatives designed to mimic acyl-CoA, one of the substrates of ACAT, where the long-chain aliphatic acyl moiety was thought to be essential, and the other being urea derivatives. These types are represented by CI-976 [4a,8]

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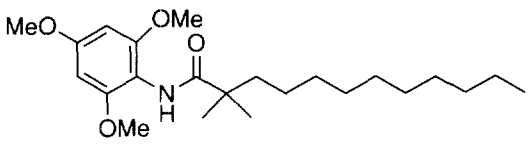
and CL 277,082 [4b,9] respectively (Fig. 1). However, despite numerous studies on ACAT inhibitors, clinical trials have revealed only poor efficacy, and as a result, up to the present time no drug candidates have shown clinical success [10]. Therefore, our interest was directed at identifying potent ACAT inhibitors, and in particular compounds with excellent in vivo activity. Trisubstituted urea derivatives seemed to be more tolerable to various chemical modifications than fatty acid anilide derivatives, since conversion of three substituents on urea nitrogens would be possible independently. Thus we opted to investigate the design and synthesis of novel trisubstituted urea derivatives having various bisphenyl moieties represented by **1** (Fig. 1). These derivatives were evaluated for their ability to inhibit intestinal microsomal ACAT in vitro and to reduce serum total cholesterol levels in cholesterol-fed rats in vivo. In this paper, we wish to disclose the synthesis and structure-activity relationships of this novel series of ACAT inhibitors.

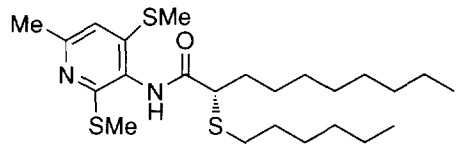
## 2. Chemistry

The basic synthetic route to the novel trisubstituted urea compounds **9–50** prepared in this work is summarized in Scheme 1 and Tables 1 and 2. Benzaldehyde derivatives **3b** and **3e–v** were prepared from the appropriate precursors **2a–h** by Methods A–K. The details are as follows: **3b** [11] was obtained by oxidation of **2a** with $MnO_2$ (Method A). **3e** [12] was prepared by the reaction of 4-fluorobenzaldehyde (**2b**) with thiophenol in the presence of $K_2CO_3$ (Method B). The preparation of **3f** was accomplished by activation of 4-formylbenzoic acid (**2c**) as the mixed anhydride with $ClCO_2{}^iBu$ followed by treatment with aniline (Method C). **3g** was prepared from ethyl 4-aminobenzoate (**2d**) by sequential treatment with benzenesulfonyl chloride, $LiAlH_4$, and $MnO_2$ (Method D). **3h** was prepared from 4-(chlorosulfonyl)benzoic acid (**2e**) by reaction with aniline, followed by amidation, and $LiAlH_4$ reduction (Method E). **3i** was obtained by the acylation of benzenesulfonamide with 4-formylbenzoic acid (**2c**) (Method F). **3j** was prepared from 4-sulfamoylbenzoic acid (**2f**) by formation of the Weinreb amide, $LiAlH_4$ reduction to the aldehyde, and acylation with benzoic acid (Method G). Substituted biphenylcarboxaldehydes **3k–n** [13–16] were obtained by the palladium-catalyzed cross-coupling reaction of 4-bromobenzaldehyde (**2g**) with the appropriate substituted phenylboric acid under modified Suzuki cross-coupling conditions [17a,b] (Method H). **3o** [18] was prepared from 4-carboxyphenylboric acid (**2h**) [19] by amidation, modified Suzuki cross-coupling reaction [17c] with 4-bromo-*N*,*N*-dimethylaniline, and $LiAlH_4$ reduction (Method I). **3p** was prepared from 4-carboxyphenylboric acid (**2h**) by amidation, modified Suzuki cross-coupling reaction [17c] with *N*-(4-bromophenyl)methanesulfonamide (obtained by mesylation of 4-bromoaniline) and $LiAlH_4$ reduction (Method J).

Fatty Acid Anilide Derivatives

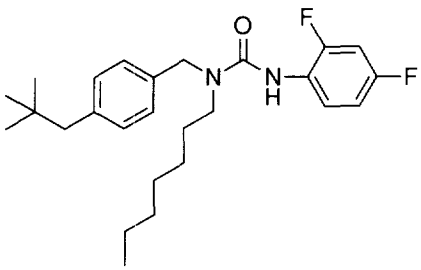



CI-976

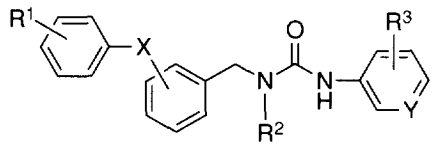



CP-113,818

Urea Derivatives

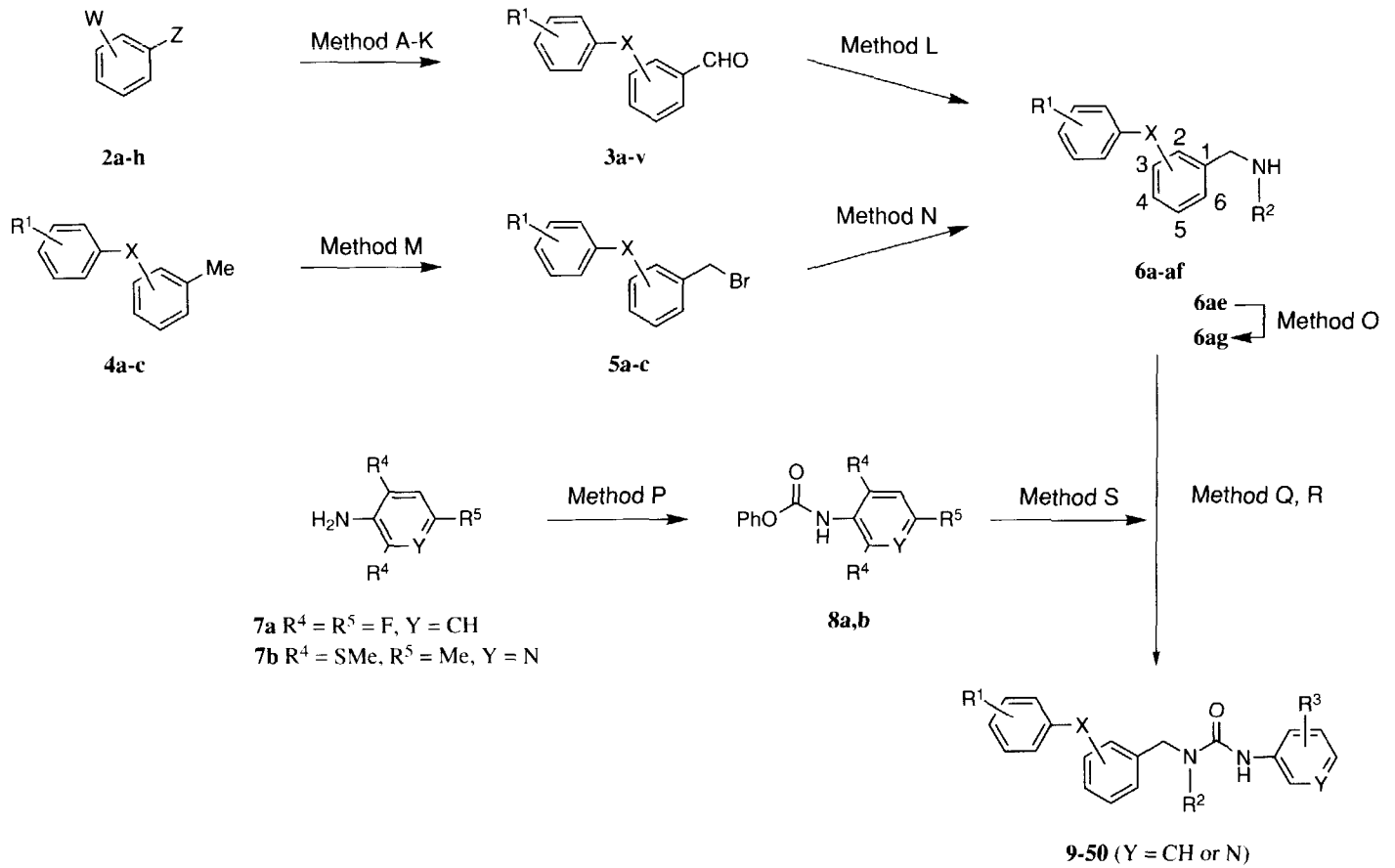



CL 277,082




**1**

Fig. 1. ACAT inhibitors.




Scheme 1. Preparation of target molecules. Reagents: Method A; **2a**, $MnO_2$, $CHCl_3$; Method B; **2b**, PhSH, $K_2CO_3$, DMF; Method C; (1) **2c**, $ClCO_2{}^tBu$, $Et_3N$, $CH_2Cl_2$; (2) aniline; Method D; (1) **2d**, $C_6H_5SO_2Cl$, pyridine; (2) $LiAlH_4$, THF; (3) $MnO_2$, $CHCl_3$; Method E; (1) **2e**, aniline, pyridine; (2) $SOCl_2$, DMF (cat.), $ClCH_2CH_2Cl$; (3) Me(MeO)NH·HCl, $Et_3N$, $CH_2Cl_2$; (4) $LiAlH_4$, THF; Method F; **2c**, $C_6H_5SO_2NH_2$, WSC·HCl, DMAP, $CH_2Cl_2$; Method G; (1) **2f**, Me(MeO)NH·HCl, WSC, $CH_2Cl_2$; (2) $LiAlH_4$, THF; (3) benzoic acid, WSC·HCl, DMAP, $CH_2Cl_2$; Method H; **2g**, substituted phenylboric acid, $Pd(PPh_3)_4$, $K_2CO_3$, toluene; Method I; (1) **2h**, Me(MeO)NH·HCl, HOBt, WSC, $CH_2Cl_2$; (2) 4-$Me_2NC_6H_4Br$, $Pd(PPh_3)_4$, 2M-$Na_2CO_3$, DME; (3) $LiAlH_4$, THF; Method J; (1) **2h**, Me(MeO)NH·HCl, HOBt, WSC, $CH_2Cl_2$; (2) 4-$MeSO_2NHC_6H_4Br$ (prepared from 4-$NH_2C_6H_4Br$ by treatment with *MsCl in pyridine*), *$Pd(PPh_3)_4$, 2M-$Na_2CO_3$, DME; (3) $LiAlH_4$, THF*; Method K; **2b**, substituted phenol, $K_2CO_3$, DMF; Method L; (1) alkylamine ($R^2NH_2$); (2) $NaBH_4$, EtOH; Method M; NBS, $Bz_2O_2$, $CCl_4$; Method N; alkylamine ($R^2NH_2$); Method O; $H_2NNH_2·H_2O$, KOH, $HOCH_2CH_2OH$; Method P; $ClCO_2Ph$, $PhNMe_2$, $CH_2Cl_2$; Method Q; arylisocyanate, $CH_2Cl_2$; Method R; substituted aniline, triphosgene, $Et_3N$, $CH_2Cl_2$; Method S; phenyl *N*-arylcarbamate (**8**), $Et_3N$, toluene

Table 1
Structures of precursors (**2**) of benzaldehyde derivatives (**3**)

| No. | W | Z |
|---|---|---|
| **2a** | 2-Ph | $CH_2OH$ |
| **2b** | 4-F | CHO |
| **2c** | 4-$CO_2H$ | CHO |
| **2d** | 4-$NH_2$ | $CO_2Et$ |
| **2e** | 4-$SO_2Cl$ | $CO_2H$ |
| **2f** | 4-$SO_2NH_2$ | $CO_2H$ |
| **2g** | 4-Br | CHO |
| **2h** [19] | 4-$B(OH)_2$ | $CO_2H$ |

4-Phenoxybenzaldehydes with various substituents **3q–v** were prepared by the reaction of 4-fluorobenzaldehyde (**2b**) with the appropriate substituted phenols in the presence of $K_2CO_3$ according to the reported method [20] with slight modifications (Method K). The compounds **3a**, **3c**, and **3d** were commercially available. Benzylbromide derivatives **5a-c** were obtained by bromination of the corresponding commercially available toluene derivatives **4a–c** with NBS in the presence of a catalytic amount of benzoyl peroxide (Method M) [21].

Reductive amination of benzaldehydes **3a–v** with various alkylamines (Method L) or monoalkylation of various amines with benzylbromides **5a–c** (Method N) provided the key intermediate secondary amines **6a–af**. Preparation of amine **6ag** (X = $CH_2$) was accomplished by Wolff–Kishner reduction of **6ae** with hydrazine-KOH in ethylene glycol (Method O).

The desired trisubstituted ureas **9–50** were prepared from amines **6a–ag** by one of three standard methods: (1) treatment with the appropriate arylisocyanates (Method Q), (2) reaction with substituted anilines using triphosgene activation (Method R), and (3) reaction with phenyl *N*-arylcarbamates **8** (Method S), which were readily obtained by the reaction of 2,4,6-trifluoroaniline (**7a**) or aminopyridine derivative **7b** [22] with phenyl chloroformate (Method P). Free rotation about the urea moiety of the compounds prepared seemed to be restricted, since no rotational isomers were detected in the $^1H$ NMR spectra of any of the final compounds **9–50**.

Table 2
Structures and synthetic methods of aldehydes (**3**), toluenes (**4**), bromides (**5**), and amines (**6**)

| No. | X (position) | $R^1$ | $R^2$ | Method |
|---|---|---|---|---|
| **3a**,[a] **6a–b** | bond (4) | H | *n*-$C_7H_{15}$ (**6a**), c-$C_7H_{13}$ (**6b**) | L |
| **3b**, [11] **6c** | bond (2) | H | c-$C_7H_{13}$ | A, L |
| **3c**,[a] **6d–f** | O (4) | H | c-$C_7H_{13}$ (**6d**), Bn (**6e**) 2-furfuryl (**6f**) | L |
| **3d**,[a] **6g** | O (3) | H | c-$C_7H_{13}$ | L |
| **3e**, [12] **6h** | S (4) | H | c-$C_7H_{13}$ | B, L |
| **3f**, **6i** | NHCO (4) | H | c-$C_7H_{13}$ | C, L |
| **3g**, **6j** | $SO_2NH$ (4) | H | c-$C_7H_{13}$ | D, L |
| **3h**, **6k** | $NHSO_2$ (4) | H | c-$C_7H_{13}$ | E, L |
| **3i**, **6l** | $SO_2NHCO$ (4) | H | c-$C_7H_{13}$ | F, L |
| **3j**, **6m** | $CONHSO_2$ (4) | H | c-$C_7H_{13}$ | G, L |
| **3k**, [13] **6n** | bond (4) | 4-F | c-$C_7H_{13}$ | H, L |
| **3l**, [14] **6o** | bond (4) | 4-Cl | c-$C_7H_{13}$ | H, L |
| **3m**, [15] **6p** | bond (4) | 4-Br | c-$C_7H_{13}$ | H, L |
| **3n**, [16] **6q** | bond (4) | 4-Me | c-$C_7H_{13}$ | H, L |
| **3o**, [18] **6r** | bond (4) | 4-$Me_2N$ | c-$C_7H_{13}$ | I, L |
| **3p**, **6s** | bond (4) | 4-$MeSO_2NH$ | c-$C_7H_{13}$ | J, L |
| **3q**, **6t–x** | O (4) | 4-F | c-$C_7H_{13}$ (**6t**), c-$C_6H_{11}$ (**6u**) c-$C_5H_9$ (**6v**), *n*-$C_5H_{11}$ (**6w**) Bn (**6x**) | K, L |
| **3r**, [20] **6y** | O (4) | 4-Cl | c-$C_7H_{13}$ | K, L |
| **3s**, [20] **6z** | O (4) | 4-Br | c-$C_7H_{13}$ | K, L |
| **3t**, **6aa** | O (4) | 3-F | c-$C_7H_{13}$ | K, L |
| **3u**, **6ab** | O (4) | 4-$CF_3$ | c-$C_7H_{13}$ | K, L |
| **3v**, **6ac** | O (4) | 3,4-$OCH_2O$- | c-$C_7H_{13}$ | K, L |
| **4a**,[a] **5a**, [21] **6ad** | bond (3) | H | c-$C_7H_{13}$ | M, N |
| **4b**,[a] **5b**, **6ae** | C=O (4) | H | c-$C_7H_{13}$ | M, N |
| **4c**,[a] **5c**, **6af** | bond (4) | 2-CN | c-$C_7H_{13}$ | M, N |
| **6ag** | $CH_2$ (4) | H | c-$C_7H_{13}$ | O |

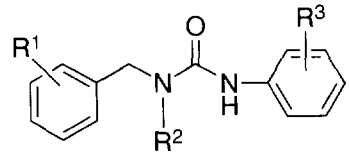
[a]Commercially available.

## 3. Results and discussion

The compounds prepared were evaluated for their ability to inhibit intestinal ACAT in vitro by incubation with [1-$^{14}$C]oleoyl-CoA and the mucosal microsomes from the small intestine of cholesterol-fed rabbits according to the method of Heider et al. [23] with minor modifications [24]. In vivo hypocholesterolemic activity was assessed in rats by admixing the test compounds 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 versus control was determined [24]. 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%.

To investigate the potency of this type of compound we first synthesized and evaluated the in vitro activity of **9** and **10** (Table 3), structurally similar analogs to CL 277,082. As we had expected, these compounds showed very potent in vitro ACAT inhibitory activity. With regards to their cholesterol-lowering effects in vivo, **9** was effective at a dose of 0.32 mg/kg, but **10** was inactive. Compound **11**, with a cycloheptyl group on the urea nitrogen, a cyclic analog of **9**, was also potent in vitro, however, its in vivo activity was only modest. In contrast however, conversion of the 2,4,6-trifluorophenyl group of **11** to 2,4,6-trimethylphenyl improved the in vivo activity dramatically (**13**, $ED_{50}$ = 0.29 mg/kg). Although it has been reported previously [25] that certain disubstituted ureas with a 2,6-diisopropylphenyl moiety have a good profile as potent ACAT inhibitors, unexpectedly the in vivo effect of the corresponding analog (**12**) in our series, which showed potent in vitro activity ($IC_{50}$ = 34 nM), was not attractive. As a result of these preliminary investigations, the best compound with potent hypocholesterolemic activity was **13**, containing a cycloheptyl group, therefore we examined the structure–activity relationships of compounds of the

Table 3
Biological activities of *N*-alkyl-*N*-substituted benzyl-*N'*-arylureas




| No. | $R^1$ | $R^2$ | $R^3$ | Formula[a] | mp (°C) | Yield[b] (method) | ACAT inhibitory activity[c] $IC_{50}$ (nM) | Hypocholesterolemic activity[d] $ED_{50}$ (mg/kg) |
|---|---|---|---|---|---|---|---|---|
| **9** | 4-Ph | *n*-heptyl | 2,4,6-$F_3$ | $C_{27}H_{29}F_3N_2O$ | oil | 54 (S) | 19 | > 0.32 (33) |
| **10** | 4-Ph | *n*-heptyl | 2,4,6-$Me_3$ | $C_{30}H_{38}N_2O$ | 131–132 | 88 (Q) | 21 | > 0.32 (−8) |
| **11** | 4-Ph | cycloheptyl | 2,4,6-$F_3$ | $C_{27}H_{27}F_3N_2O$ | 141–142 | 83 (R) | 21 | < 10 (70) |
| **12** | 4-Ph | cycloheptyl | 2,6-$^i Pr_2$ | $C_{33}H_{42}N_2O$ | 139–140 | 92 (Q) | 34 | > 10 (5) |
| **13** | 4-Ph | cycloheptyl | 2,4,6-$Me_3$ | $C_{30}H_{36}N_2O$ | 137–138 | 81 (Q) | 24 | 0.29 |
| **14** | 3-Ph | cycloheptyl | 2,4,6-$F_3$ | $C_{27}H_{27}F_3N_2O$ | amorphous solid | 73 (R) | 15 | 0.20 |
| **15** | 3-Ph | cycloheptyl | 2,4,6-$Me_3$ | $C_{30}H_{36}N_2O$ | 172–174 | 79 (Q) | 21 | 0.17 |
| **16** | 2-Ph | cycloheptyl | 2,4,6-$F_3$ | $C_{27}H_{27}F_3N_2O$ | 116–119 | 94 (R) | 110 | ND |
| **17** | 2-Ph | cycloheptyl | 2,4,6-$Me_3$ | $C_{30}H_{36}N_2O$ | 129–130 | 98 (Q) | 200 | ND |
| **18** | 4-PhO | cycloheptyl | 2,4,6-$F_3$ | $C_{27}H_{27}F_3N_2O_2$ | amorphous solid | 99 (R) | 11 | > 1 (47) |
| **19** | 4-PhO | cycloheptyl | 2,6-$^i Pr_2$ | $C_{33}H_{42}N_2O_2$ | 118–120 | 75 (Q) | 29 | > 1 (−10) |
| **20** | 4-PhO | cycloheptyl | 2,4,6-$Me_3$ | $C_{30}H_{36}N_2O_2$ | 128–129 | 90 (Q) | 16 | < 0.30 (59) |
| **21** | 4-PhO | benzyl | 2,4,6-$Me_3$ | $C_{30}H_{30}N_2O_2$ | 89–91 | 97 (Q) | 15 | < 1 (56) |
| **22** | 4-PhO | 2-furfuryl | 2,4,6-$Me_3$ | $C_{28}H_{28}N_2O_3$ | 119–120 | 98 (Q) | 31 | > 1 (−18) |
| **23** | 3-PhO | cycloheptyl | 2,4,6-$F_3$ | $C_{27}H_{27}F_3N_2O_2$ | amorphous solid | 76 (R) | 17 | < 1 (53) |
| **24** | 3-PhO | cycloheptyl | 2,4,6-$Me_3$ | $C_{30}H_{36}N_2O_2$ | 140–141 | 87 (Q) | 13 | 0.19 |

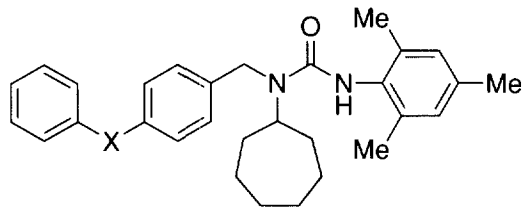
[a]Satisfactory elemental analyses were obtained for C, H, N unless otherwise indicated.
[b]Yield (%) of final step.
[c]$IC_{50}$ (nM) for the enzyme obtained from rabbit intestinal microsomes.
[d]$ED_{50}$ values are the effective dose to reduce plasma total cholesterol level by 50% of the control value. Compound was administered as a dietary admixture. Values in parentheses denotes percent reduction in total cholesterol at the dose indicated.
ND denotes not determined.

type **1** keeping the cycloheptyl group constant as substituent $R^2$.

The position of the second phenyl substituent markedly influenced biological activity (Table 3). Compounds substituted at position 2 were significantly less potent than 3- or 4-substitutions which retained high in vitro activity (**16** and **17** versus **9–15**). In terms of the in vivo activity, the choice of the aryl urea moiety has a large effect; in the case of compounds with $R^1$ at position 4, 2,4,6-trimethylphenylureas are superior to 2,4,6-trifluorophenylureas and/or 2,6-diisopropylphenylureas (**13** versus **11** and/or **12**), however in the case of inhibitors with $R^1$ at position 3, 2,4,6-trifluorophenylureas are equipotent to 2,4,6-trimethylphenylureas (**14** versus **15**). Similar trends in the structure–activity relationships were observed for phenoxy-substituted compounds (**20** versus **18** and/or **19**, **23** versus **24**). A simple benzyl group was also acceptable as substituent $R^2$ (**21**). Despite having high activity, the 2,4,6-trifluorophenylurea series were of unfavorable physical character (**9**, **14**, **18**, and **23**). As a consequence, the 2,4,6-trimethylphenylureas **13** and **20** were selected as lead compounds for further modification due to in vitro potency, in vivo efficacy, synthetic accessibility (substitution at position 4), and physicochemical characteristics (crystalline, not oils or amorphous).

Having identified an oxygen atom as a particularly acceptable spacer, we focused our attention on varying the linker unit in order to further examine the tolerance of hydrophilicity, size, and length, since altered molecular properties such as lipophilicity and shape can be expected to significantly influence biological activity (Table 4). The in vitro activity decreased in the order: O (**20**), $CH_2$ (**27**), S (**25**), bond (**13**) > NHCO (**28**) ≫ C=O (**26**), $SO_2NH$ (**29**) ≫ $SO_2NHCO$ (**31**), $NHSO_2$ (**30**), $CONHSO_2$ (**32**). In general, non-polar, small spacer groups retained the activity, whilst polar or large spacers decreased the activity. It can be concluded that appropriate size, lipophilicity, and lack of restriction to free rotation about the two phenyl rings were necessary for optimal inhibition of ACAT in vitro. The sulfide compound **25** possessed very low in vivo hypocholesterolemic activity, irrespective of its potent in vitro inhibitory activity. Although the reason is unclear at this time, it is possibly related to the metabolic instability of the compound or to its poor bioavailability. It is also interesting that there are large differences in activity between the isomers of the sulfonamide linker (**29** versus **30**). On the basis of these results, a simple bond (biphenyl unit) and oxygen (phenoxyphenyl unit) proved to be the optimal choices for linking the two phenyl rings.

Table 4
Effect of varying X-linker on biological activities



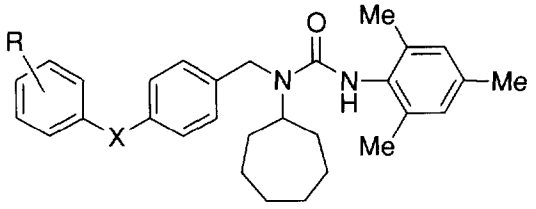

| No. | X | Formula[a] | mp (°C) | Yield[b] (method) | ACAT inhibitory activity[c] $IC_{50}$ (nM) | Hypocholesterolemic activity[d] $ED_{50}$ (mg/kg) |
|---|---|---|---|---|---|---|
| **13** | bond | $C_{30}H_{36}N_2O$ | 137–138 | 81 (Q) | 24 | 0.29 |
| **20** | O | $C_{30}H_{36}N_2O_2$ | 128–129 | 90 (Q) | 16 | < 0.30 (59) |
| **25** | S | $C_{30}H_{36}N_2OS$ | 103–106 | 94 (Q) | 19 | > 1 (24) |
| **26** | C=O | $C_{31}H_{36}N_2O_2$ | amorphous solid | 95 (Q) | 200 | ND |
| **27** | $CH_2$ | $C_{31}H_{38}N_2O$ | 138–139 | 62 (Q) | 17 | < 1 (72) |
| **28** | NHCO | $C_{31}H_{37}N_3O_2$ | 206–209 | 56 (Q) | 39 | < 1 (65) |
| **29** | $SO_2NH$ | $C_{30}H_{37}N_3O_3S$ | 208–210 | 50 (Q) | 220 | > 1 (−17) |
| **30** | $NHSO_2$ | $C_{30}H_{37}N_3O_3S$ | 214–215 | 77 (Q) | > 1000 | > 1 (14) |
| **31** | $SO_2NHCO$ | $C_{31}H_{37}N_3O_4S$[e] | amorphous solid | 25[f] (Q) | 920 | > 1 (−10) |
| **32** | $CONHSO_2$ | $C_{31}H_{37}N_3O_4S \cdot 1.6H_2O$ | 205–207 | 49 (Q) | > 1000 | > 1 (−44) |

[a–d] See corresponding footnotes of Table 3.
[e]Satisfactory analytical data could not be obtained for this compound. Anal. C: calcd, 67.98; found, 69.55.
[f]Two steps from aldehyde **3i**.
ND denotes not determined.

Effects of introduction of substituents on to the terminal phenyl ring of the biphenyl (**13**) and the diphenyl ether (**20**) were evaluated (Table 5). For the biphenyl series the electronic nature of the substituent did not correlate with in vitro activity, i.e. electron-withdrawing groups such as halogen (**33–35**) and methylsulfonylamino (**38**), and electron-donating groups such as methyl (**36**) and dimethylamino (**37**) showed variable activity. It seems that the in vitro activity depends mostly on the steric bulk of the substituent, thus fluorine was the most favorable, i.e. compound **33**. Groups larger than chlorine or methyl greatly reduced the activity (**13** and **33** versus **34–38**). In the case of the diphenyl ether analogs, potency was increased as a whole by the effect of the oxygen linker, therefore compounds with bulky substituents such as chlorine (**41**), bromine (**42**), and trifluoromethyl (**44**) maintained the potent activities of the parent compound (**20**) both in vitro and in vivo. It was of special significance that in this case also, a fluorine at the para position dramatically improved in vivo activity and gave the compound with the best profile (**40**, FR179254, $IC_{50}$ = 25 nM, $ED_{50}$ = 0.045 mg/kg). Substitution at the para position was better than in the meta position (**40** versus **43**) for in vivo activity. In general, the metabolic hydroxylation of monosubstituted benzene occurs at the para position and gives phenolic compound, thus introduction of a substituent to the para position may result in an increase in the metabolic stability (**33** versus **13**, **40** versus **20** and **43**). Interestingly, **45** which possesses a benzodioxole bicyclic system still had moderate activity. These results discussed above suggest that the binding site to enzyme for our compounds is not so deep as to accept a large substituent at the para position but is wide enough to allow rotation about the ether bond and the bicyclic core such as compound **45**. Whilst we are unsure about the nature of inhibition of ACAT by these urea derivatives (i.e. competitive versus non-competitive), and the literature contains examples of both types [4b,8b,23,26], CL 277,082, a trisubstituted urea structurally related to our compounds, has been established to be a non-competitive inhibitor with respect to acyl-CoA [4b]. Among the derivatives having a substituent on the terminal phenyl ring, fluorinated compounds (**33** and **40**) were selected for further studies.

At this stage optimization of the alkyl substituent on the urea nitrogen was examined since only *n*-heptyl and cycloheptyl had hitherto been evaluated in detail

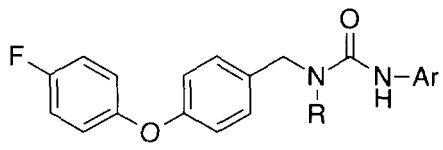
Table 5
Effect of varying outer phenyl substituent on biological activities

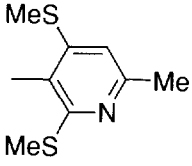



| No. | R | X | Formula[a] | mp (°C) | Yield[b] (method) | ACAT inhibitory activity[c] $IC_{50}$ (nM) | Hypocholesterolemic activity[d] $ED_{50}$ (mg/kg) |
|---|---|---|---|---|---|---|---|
| **13** | H | bond | $C_{30}H_{36}N_2O$ | 137–138 | 81 (Q) | 24 | 0.29 |
| **33** | 4-F | bond | $C_{30}H_{35}FN_2O$ | 179–180 | 91 (Q) | 16 | 0.13 |
| **34** | 4-Cl | bond | $C_{30}H_{35}ClN_2O$ | 187–188 | 79 (Q) | 240 | ND |
| **35** | 4-Br | bond | $C_{30}H_{35}BrN_2O$ | 185–188 | 96 (Q) | 210 | ND |
| **36** | 4-Me | bond | $C_{31}H_{38}N_2O$ | 163–165 | 98 (Q) | 93 | < 1 (69) |
| **37** | 4-$Me_2N$ | bond | $C_{32}H_{41}N_3O$ | 165–168 | 85 (Q) | 550 | ND |
| **38** | 4-$MeSO_2NH$ | bond | $C_{31}H_{39}N_3O_3S$ | 125–127 | 97 (Q) | 400 | > 1 (–3) |
| **39** | 2-CN | bond | $C_{31}H_{35}N_3O$ | 165–167 | 92 (Q) | 210 | > 1 (15) |
| **20** | H | O | $C_{30}H_{36}N_2O_2$ | 128–129 | 90 (Q) | 16 | < 0.30 (59) |
| **40** | 4-F | O | $C_{30}H_{35}FN_2O_2$ | 125–126 | 80 (Q) | 25 | 0.045 |
| **41** | 4-Cl | O | $C_{30}H_{35}ClN_2O_2$ | 153–154 | 67 (Q) | 25 | 0.17 |
| **42** | 4-Br | O | $C_{30}H_{35}BrN_2O_2$ | 147–149 | 99 (Q) | 26 | 0.052 |
| **43** | 3-F | O | $C_{30}H_{35}FN_2O_2$ | 127–128 | 47 (Q) | 23 | > 0.32 (37) |
| **44** | 4-$CF_3$ | O | $C_{31}H_{35}F_3N_2O_2$ | 146–147 | 74 (Q) | 48 | 0.25 |
| **45** | 3,4-$OCH_2O$- | O | $C_{31}H_{36}N_2O_4$ | 125–126 | 77 (Q) | 45 | < 1 (72) |

[a] [d]See corresponding footnotes of Table 3.
ND denotes not determined.

Table 6
*Optimization of N-alkyl group and modification of N'-aryl moiety*



| No. | R | Ar | Formula[a] | mp (°C) | Yield[b] (method) | ACAT inhibitory activity[c] $IC_{50}$ (nM) | Hypocholesterolemic activity[d] $ED_{50}$ (mg/kg) |
|---|---|---|---|---|---|---|---|
| **40** | cycloheptyl | 2,4,6-$Me_3$Ph | $C_{30}H_{35}FN_2O_2$ | 125–126 | 80 (Q) | 25 | 0.045 |
| **46** | cyclohexyl | 2,4,6-$Me_3$Ph | $C_{29}H_{33}FN_2O_2$ | 129–130 | 98 (Q) | 22 | 0.12 |
| **47** | cyclopentyl | 2,4,6-$Me_3$Ph | $C_{28}H_{31}FN_2O_2$ | 110–111 | 92 (Q) | 12 | > 1 (47) |
| **48** | *n*-pentyl | 2,4,6-$Me_3$Ph | $C_{28}H_{33}FN_2O_2$ | 104–105 | 94 (Q) | 13 | > 1 (11) |
| **49** | benzyl | 2,4,6-$Me_3$Ph | $C_{30}H_{29}FN_2O_2$ | 121–122 | 90 (Q) | 8.8 | < 1 (58) |
| **50** | cycloheptyl |  | $C_{29}H_{34}FN_3O_2S_2$ | 140–141 | 90 (S) | 30 | 0.034 |

[a] [d]See corresponding footnotes of Table 3.

(Table 6). Although replacement of the cycloheptyl group of **40** by smaller ring cycloalkyl groups (i.e. **46** and **47**) retained in vitro activity, in vivo hypocholesterolemic activity was reduced. Cycloalkyl groups were shown to be preferable to straight chain alkyl groups for in vivo activity (**47** versus **48**, **10** versus **13**, see Table 3). The best in vitro activity was obtained in the case of a benzyl group (**49**, $IC_{50}$ = 8.8 nM), however this compound lowered plasma total cholesterol only moderately in vivo. As a consequence a compound superior to **40** was not identified by varying the *N*-alkyl group.

It has been reported that the pharmacokinetics, especially bioavailability, of ACAT inhibitors can be markedly influenced by modes of drug dosing. For instance, PD 129337, identified at Parke-Davis, was effective when dosed in a lipid-rich diet or with an oil vehicle, but inactive when administered by gavage in an aqueous vehicle [25c]. In other cases, the bioavailability increases dramatically in the fed state as compared with the fasted state, for example TEI-6620 [27] and CP-105,191 [22,28]. Therefore, we further evaluated the hypocholesterolemic effect of the selected compounds (**33** and **40**) in a different drug dosing model, i.e. administration of the test compound by gavage in polyethylene glycol (PEG400) as a vehicle (Table 7). In this much more clinically relevant model, hypercholesterolemia was pre-established by feeding a cholesterol-enriched diet, and was then followed by oral administration of the compounds once a day for 3 days, continuing the cholesterol-enriched diet. As a result, a large difference in efficacy between the two models used was observed, presumably due to bioavailability. Although these compounds showed potent cholesterol lowering effects when dosed as a dietary admixture, they were only moderately potent in the case of a different dosing model. We thus

Table 7
Hypocholesterolemic activities of selected compounds and effect of administration mode

| No. | ACAT inhibitory activity[a] $IC_{50}$ (nM) | Hypocholesterolemic activity[b] $ED_{50}$ (mg/kg) administration mode: Diet[c] | Gavage[d] |
|---|---|---|---|
| **33** | 16 | 0.13 | 9.2 |
| **40** | 25 | 0.045 | 5.3 |
| **50** | 30 | 0.034 | 0.11 |
| CL 277,082 | 33 | 5.0 | ND |

[a]See footnote c of Table 3.
[b]$ED_{50}$ values are the effective dose to reduce plasma total cholesterol level by 50% of the control value.
[c]Compound was administered as a dietary admixture.
[d]Compound was administered by gavage in PEG400 as a vehicle.
ND denotes not determined.

decided to focus on modification of the 2,4,6-trimethylphenyl ring for further optimization of in vivo potency and improvement of bioavailability. Recently researchers at Pfizer have identified CP-113,818 [22,29] as an amide type ACAT inhibitor which is characterized by an asymmetric carbon and a novel trisubstituted pyridine ring (Fig. 1). Although the bioavailability of this inhibitor was also reported to be influenced by food, it was demonstrated to be systemically available. For the purpose of further improving the pharmacokinetics, introduction of a 2,4-bis(methylthio)-6-methylpyridine ring to our best compound **40** gave an excellent result (Tables 6 and 7). Compound **50** retained potent activity both in vitro and in vivo ($IC_{50}$ = 30 nM, $ED_{50}$ = 0.034 mg/kg dosing as a dietary admixture) which was much more potent with respect to hypocholesterolemic activity in vivo than CL 277,082 (see Table 7), and also displayed improved bioavailability, and consequently showed very potent hypocholesterolemic activity even when dosed by gavage in PEG400 ($ED_{50}$ = 0.11 mg/kg). Researchers at Parke-Davis have reported [30] a 2-methylthionicotinamide ACAT inhibitor, the 2-methylthiopyridine ring of which is structurally similar to 2,4-bis(methylthio)-6-methylpyridine, that exhibited improved in vivo potency comparable to CI-976. They referred to the possibility of protonation at the pyridine nitrogen atom leading to the observed improvement of solubility and absorption properties. Incorporation of this substituted pyridine part apparently makes the molecule more polar compared to **40**, however, it is unclear whether or not this is related to improved bioavailability. These results discussed above suggest that **50** (FR182980) is a potent, orally efficacious ACAT inhibitor, independent of the administration method, and has good potential for development as a new treatment of hypercholesterolemia and atherosclerosis.

## 4. Conclusion

In summary, we have prepared a novel series of biphenylylmethylurea derivatives and evaluated them as ACAT inhibitors. The SAR study in this series of compounds revealed the following main features. (1) Attachment of the terminal phenyl group to the 3- or 4-position of the inner phenyl ring was necessary for potent activity. (2) Optimized spacers linking two phenyl rings were found to be bond (none) and oxygen. (3) Introduction of fluorine at position 4 of the terminal phenyl ring resulted in significant reduction in plasma total cholesterol in vivo. (4) Various alkyl groups were acceptable as substituents for the urea nitrogen, but cycloheptyl was optimal for both in vitro and in vivo activity. (5) Combination with a 2,4-bis(methylthio)-6-methylpyridine ring greatly improved bioavailability. On the basis of the main results described above, *N*-cycloheptyl-*N*-[4-(4-fluorophenoxy)benzyl]-*N'*-[2,4-bis(methylthio)-6-methylpyridin-3-yl]urea (**50**, FR182980) was identified as a potent, orally efficacious ACAT inhibitor irrespective of dosing method and was selected for further development. The details of pharmacological and toxicological studies on this compound will be the subject of further communications from these laboratories.

## 5. Experimental

### *5.1 Chemistry*

#### *5.1.1 General procedures*

Melting points were measured on a Büchi 535 apparatus in open capillaries and are uncorrected. IR spectra were recorded on a Horiba Spectradesk FT-210 spectrometer as KBr disks, neat, or films as indicated. NMR spectra were measured on a Bruker AC200P ($^1$H, 200 MHz). Chemical shifts are given in parts per million, and tetramethylsilane was used as the internal standard for spectra obtained in DMSO-$d_6$ and $CDCl_3$. All *J* values are given in Hz. Mass spectra were measured on a Hitachi Model M-1000H mass spectrometer using APCI for ionization. Elemental analyses were carried out on a Perkin Elmer 2400 CHN Elemental Analyzer. Reagents and solvents were used as obtained from commercial suppliers without further purification. Column chromatography was performed using silica-gel, and reaction progress was determined by TLC analysis on silica-gel coated glass plates. Visualization was with UV light (254 nm) or iodine.

#### *5.1.2 Method A*

*5.1.2.1 2-Biphenylcarboxaldehyde (**3b**).* To a solution of 2-hydroxymethylbiphenyl **2a** (9.21 g, 50 mmol) in $CHCl_3$ (90 mL) was added activated $MnO_2$ (21.74 g, 250 mmol) and the mixture was refluxed for 5 h. The mixture was filtered and the filtrate was evaporated under reduced pressure to give crude **3b** (9.11 g, 100%) as an oil: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.38–7.67 (7H, m), 7.77 (1H, ddd, $J$ = 7.5, 7.5, 1.5 Hz), 7.93 (1H, dd, $J$ = 7.6, 1.5 Hz), 9.89 (1H, s); IR (neat) 3061, 3030, 2846, 2764, 1695, 1597, 1473, 1456 cm$^{-1}$; MS *m/z* 183 ($MH^+$).

#### *5.1.3 Method B*

*5.1.3.1 4-(Phenylthio)benzaldehyde (**3e**).* To a solution of 4-fluorobenzaldehyde **2b** (1.00 g, 8.06 mmol) and thiophenol (0.83 ml, 8.06 mmol) in DMF (10 ml) was added powdered $K_2CO_3$ (1.34 g, 9.67 mmol), and the mixture was stirred at 120°C for 4.5 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc and washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography (hexane–EtOAc 10:1

elution) to give **3e** (1.72 g, 100%) as a solid: $^1$H NMR ($CDCl_3$) $\delta$ 7.24 (2H, d, $J$ = 8.5 Hz), 7.37–7.60 (5H, m), 7.72 (2H, d, $J$ = 8.5 Hz), 9.91 (1H, s); IR (KBr) 3049, 2827, 2737, 1693, 1587, 1556, 1475 cm$^{-1}$; MS $m/z$ 215 ($MH^+$).

*5.1.4 Method C*

*5.1.4.1 4-Formyl-N-phenylbenzamide (**3f**).* To a suspension of 4-formylbenzoic acid **2c** (3.00 g, 20 mmol) and $Et_3N$ (3.07 ml, 22 mmol) in $CH_2Cl_2$ (50 ml) was added dropwise isobutyl chloroformate (2.85 ml, 22 mmol) at 5°C and the mixture was stirred at the same temperature for 40 min. To the reaction mixture was added aniline (2.0 ml, 22 mmol) and the mixture was stirred at room temperature for 16 h. Water was added to the reaction mixture, and the separated organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the resulting solid was triturated with hexane–EtOAc to give **3f** (2.24 g, 50%) as a powder: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.13 (1H, t, $J$ = 7.5 Hz), 7.38 (2H, dd, $J$ = 7.5, 7.5 Hz), 7.79 (2H, d, $J$ = 7.5 Hz), 8.06 (2H, d, $J$ = 8.4 Hz), 8.14 (2H, d, $J$ = 8.4 Hz), 10.12 (1H, s), 10.46 (1H, s); IR (KBr) 3338, 3053, 2821, 2725, 1705, 1651, 1599, 1533 cm$^{-1}$; MS $m/z$ 226 ($MH^+$).

*5.1.5 Method D*

*5.1.5.1 N-(4-Formylphenyl)benzenesulfonamide (**3g**).* To a solution of ethyl 4-aminobenzoate **2c** (8.26 g, 50 mmol) in pyridine (25 ml) was added dropwise $PhSO_2Cl$ (8.83 g, 50 mmol) at 5°C, and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into a mixture of EtOAc and 3N hydrochloric acid, and the resulting precipitate was collected by filtration, washed with EtOAc and isopropyl ether (iPE), and dried over phosphorus pentaoxide to give ethyl 4-(benzenesulfonylamino)benzoate (10.72 g, 70%) as a powder: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.27 (3H, t, $J$ = 7.1 Hz), 4.24 (2H, q, $J$ = 7.1 Hz), 7.22 (2H, d, $J$ = 8.8 Hz), 7.50–7.70 (3H, m), 7.75–7.90 (4H, m), 10.86 (1H, s); IR (KBr) 3228, 3068, 2987, 2939, 2881, 1693, 1608, 1512, 1475, 1410 cm$^{-1}$; MS $m/z$ 306 ($MH^+$).

To a suspension of $LiAlH_4$ (1.81 g, 47.6 mmol) in THF (500 ml) was added dropwise a solution of the above benzoate (14.53 g, 47.6 mmol) in THF (100 ml) at 5°C, and the mixture was stirred at room temperature for 1 h then stirred at 45°C for 3 h. After cooling to room temperature, the reaction mixture was poured into an ice cooled mixture of EtOAc and 1N hydrochloric acid. The separated organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography (hexane–EtOAc 1:2 elution) to give *N*-(4-hydroxymethylphenyl)benzenesulfonamide (5.75 g, 46%) as an oil: $^1$H NMR (DMSO-$d_6$) $\delta$ 4.36 (2H, d, $J$ = 5.8 Hz), 5.07 (1H, t, $J$ = 5.8 Hz), 7.02 (2H, d, $J$ = 8.6 Hz), 7.15 (2H, d, $J$ = 8.6 Hz), 7.50–7.65 (3H, m), 7.70–7.80 (2H, m), 10.21 (1H, s); IR (neat) 3516, 3265, 3062, 2935, 2875, 1705, 1614, 1514, 1452, 1333 cm$^{-1}$; MS $m/z$ 246 ($MH^+ - H_2O$).

To a solution of the above sulfonamide (5.75 g, 21.8 mmol) in $CHCl_3$ (60 ml) was added activated $MnO_2$ (18.98 g, 218 mmol) and the mixture was refluxed for 3 h. The mixture was filtered and the filtrate evaporated under reduced pressure to give crude **3g** (4.74 g, 83%) as a solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.29 (2H, d, $J$ = 8.6 Hz), 7.50–7.72 (3H, m), 7.72–7.92 (4H, m), 9.81 (1H, s), 11.01 (1H, s); IR (KBr) 3238, 3059, 2937, 2848, 2765, 1690, 1601, 1581, 1510 cm$^{-1}$; MS $m/z$ 262 ($MH^+$).

*5.1.6 Method E*

*5.1.6.1 4-Formyl-N-phenylbenzenesulfonamide (**3h**).* To a solution of aniline (8.20 g, 88 mmol) in pyridine (100 ml) was added dropwise 4-(chlorosulfonyl)benzoic acid **2e** (17.65 g, 80 mmol) at 5°C, and the mixture was stirred at 90°C for 6 h. The reaction mixture was poured into a mixture of EtOAc and 3N hydrochloric acid, and the resulting precipitate was collected by filtration, washed with EtOAc and iPE, and dried over phosphorus pentaoxide to give 4-(phenylsulfamoyl)benzoic acid (6.87 g, 31%) as a powder: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.00–7.18 (3H, m), 7.20–7.35 (2H, m), 7.85 (2H, d, $J$ = 8.4 Hz), 8.07 (2H, d, $J$ = 8.4 Hz), 10.45 (1H, s); IR (KBr) 3267, 2841, 2675, 2559, 1682, 1601, 1578 cm$^{-1}$; MS (neg.) $m/z$ 276 ($M-H^+$).

To a suspension of the above benzoic acid (13.43 g, 48.4 mmol) in 1,2-dichloroethane (130 ml) were added $SOCl_2$ (7.07 ml, 96.8 mmol) and DMF (2 drops), and the mixture was stirred at 100°C for 2 h. The resulting solution was evaporated and the residue was dissolved in $CH_2Cl_2$ (150 ml). To this solution was added *N,O*-dimethylhydroxylamine hydrochloride (5.19 g, 53.2 mmol), followed by dropwise addition of $Et_3N$ (13.5 ml, 96.8 mmol) at 5°C. The mixture was stirred at room temperature for 4 h. Water was added thereto, and the separated organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography (EtOAc–iPE 1:2 elution) to give *N*-methoxy-*N*-methyl-4-(phenylsulfamoyl)benzamide (11.31 g, 73%) as a solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 3.24 (3H, s), 3.48 (3H, s), 7.00–7.15 (3H, m), 7.15–7.32 (2H, m), 7.70 (2H, d, $J$ = 8.3 Hz), 7.80 (2H, d, $J$ = 8.3 Hz), 10.38 (1H, s); IR (KBr) 3149, 2976, 2904, 1624, 1601, 1568, 1497, 1429 cm$^{-1}$; MS $m/z$ 321 ($MH^+$).

To a suspension of $LiAlH_4$ (2.01 g, 53.0 mmol) in THF (150 ml), was added dropwise a solution of the benzamide (11.30 g, 35.3 mmol) in THF (150 ml) at 5°C and the mixture was stirred at room temperature for 2 h. To the mixture were added anhydrous NaF (8.90 g, 212 mmol) and water (2.87 ml), and the mixture was stirred at room temperature for 30 min. The insoluble materials were removed by filtration and washed with

THF. The filtrate was evaporated and the residue was purified by silica-gel column chromatography (hexane–EtOAc 2:1 elution) to give **3h** (4.45 g, 48%) as a solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.00–7.15 (3H, m), 7.15–7.33 (2H, m), 7.93 (2H, d, $J$ = 8.1 Hz), 8.05 (2H, d, $J$ = 8.1 Hz), 10.04 (1H, s), 10.48 (1H, br s); IR (KBr) 3261, 3053, 2860, 1697, 1597, 1491, 1400 cm$^{-1}$; MS $m/z$ 262 ($MH^+$).

*5.1.7 Method F*

*5.1.7.1 N-(4-Formylbenzoyl)benzenesulfonamide (**3i**).* To a suspension of 4-formylbenzoic acid **2c** (600 mg, 4.0 mmol) and benzenesulfonamide (691 mg, 4.4 mmol) in $CH_2Cl_2$ (30 ml) were added 4-dimethylaminopyridine (537 mg, 4.4 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC·HCl) (843 mg, 4.4 mmol) at room temperature and the mixture was stirred for 2 h. The reaction mixture was washed with water, 1N hydrochloric acid, and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography ($CH_2Cl_2$–MeOH 5:1 elution) to give **3i** (939 mg, 81%) as a syrup: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.50–7.75 (4H, m), 7.90–8.10 (6H, m), 10.07 (1H, s); IR (neat) 3261, 3066, 2860, 1701, 1589, 1549, 1446, 1344, 1246 cm$^{-1}$; MS $m/z$ 290 ($MH^+$).

*5.1.8 Method G*

*5.1.8.1 N-Benzoyl-4-formylbenzenesulfonamide (**3j**).* To a suspension of 4-sulfamoylbenzoic acid **2f** (4.02 g, 20 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (2.12 g, 22 mmol) in $CH_2Cl_2$ (120 ml) was added dropwise a solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (WSC) (5.15 ml, 22 mmol) in $CH_2Cl_2$ (30 ml) at room temperature and the mixture was stirred for 6 h. To the reaction mixture was added water, and the separated organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the resulting solid was triturated with EtOAc–iPE to give *N*-methoxy-*N*-methyl-(4-sulfamoyl)benzamide (3.55 g, 73%) as a solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 3.28 (3H, s), 3.54 (3H, s), 7.49 (2H, br s), 7.74 (2H, d, $J$ = 8.4 Hz), 7.88 (2H, d, $J$ = 8.4 Hz); IR (KBr) 3292, 3201, 3111, 2980, 2943, 1605, 1562, 1504, 1348, 1163 cm$^{-1}$; MS $m/z$ 245 ($MH^+$).

To a suspension of $LiAlH_4$ (750 mg, 19.8 mmol) in THF (100 ml) was added dropwise a solution of the above benzamide (3.22 g, 13.2 mmol) in THF (50 ml) at room temperature and the mixture was stirred at 50°C for 7 h. After cooling to room temperature, the reaction mixture was poured into 1N hydrochloric acid under ice cooling, and extracted with THF. The organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography (EtOAc–iPE 1:1 elution) to give 4-formylbenzenesulfonamide (1.27 g, 52%) as an amorphous solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.61 (2H, br s), 8.02 (2H, d, $J$ = 8.4 Hz), 8.10 (2H, d, $J$ = 8.4 Hz), 10.10 (1H, s); IR (KBr) 3361, 3248, 1703, 1576, 1340, 1321, 1155 cm$^{-1}$.

To a suspension of 4-formylbenzenesulfonamide (1.26 g, 6.8 mmol) and benzoic acid (830 mg, 6.8 mmol) in $CH_2Cl_2$ (30 ml) were added 4-dimethylaminopyridine (831 mg, 6.8 mmol) and WSC·HCl (1.30 g, 6.8 mmol) at room temperature and the mixture was stirred for 6 h. The reaction mixture was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography (EtOAc–MeOH 10:1 elution) to give **3j** (420 mg, 21%) as an amorphous solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.25–7.45 (3H, m), 7.85–7.95 (2H, m), 7.92 (2H, d, $J$ = 8.2 Hz), 7.99 (2H, d, $J$ = 8.2 Hz), 10.03 (1H, s); IR (KBr) 3381, 3057, 2883, 1697, 1599, 1560, 1329 cm$^{-1}$; MS $m/z$ 290 ($MH^+$).

*5.1.9 General procedure for Method H*

*5.1.9.1 4-(4-Fluorophenyl)benzaldehyde (**3k**).* To a suspension of 4-bromobenzaldehyde **2g** (1.85 g, 10 mmol) and 4-fluorophenylboric acid (1.40 g, 10 mmol) in toluene (50 ml) were added powdered $K_2CO_3$ (2.07 g, 15 mmol) and $Pd(Ph_3P)_4$ (578 mg, 0.5 mmol), and the mixture refluxed for 24 h. The reaction mixture was poured into a mixture of EtOAc and ice water. The separated organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography (hexane–EtOAc 2:1 elution) to give **3k** (1.67 g, 84%) as a solid: $^1$H NMR ($CDCl_3$) $\delta$ 7.10–7.25 (2H, m), 7.55–7.70 (2H, m), 7.71 (2H, d, $J$ = 8.2 Hz), 7.95 (2H, d, $J$ = 8.2 Hz), 10.06 (1H, s); IR (KBr) 3053, 2848, 2754, 1703, 1601, 1566, 1520, 1495 cm$^{-1}$; MS $m/z$ 201 ($MH^+$).

*5.1.10 Method I*

*5.1.10.1 4-(4-Dimethylaminophenyl)benzaldehyde (**3o**).* To a suspension of 4-carboxyphenylboric acid **2h** (9.00 g, 54 mmol), *N,O*-dimethylhydroxylamine hydrochloride (5.29 g, 54 mmol), and 1-hydroxybenzotriazole (HOBt) (7.32 g, 54 mmol) in $CH_2Cl_2$ (300 ml) was added dropwise a solution of WSC (6.59 g, 54 mmol) in $CH_2Cl_2$ (40 ml) at room temperature and the mixture was stirred for 72 h. The reaction mixture was poured into water. The separated organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography (EtOAc–MeOH 10:1 elution) to give impure 4-(*N*-methoxy-*N*-methylcarbamoyl)phenylboric acid (6.32 g, 56%) as a solid: $^1$H NMR (DMSO-$d_6$) $\delta$ *inter alia* 3.25 (3H, s), 3.53 (3H, s), 7.51 (2H, d, $J$ = 8.0 Hz), 7.83 (2H, d, $J$ = 8.0 Hz), 8.18 (2H, br); IR (KBr) 3381, 1610, 1554, 1508, 1404 cm$^{-1}$; MS $m/z$ 210 ($MH^+$).

To a solution of the above impure phenylboric acid (6.27 g, 30 mmol) and 4-bromo-*N,N*-dimethylaniline (3.96 g, 20 mmol) in 1,2-dimethoxyethane (100 ml) were added 2M $Na_2CO_3$ solution (30 ml) and $Pd(Ph_3P)_4$

(1.16 g, 1.0 mmol), and the mixture refluxed for 16 h. The reaction mixture was poured into a mixture of EtOAc and ice water. The separated organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography (EtOAc–iPE 1:4 elution) to give *N*-methoxy-*N*-methyl-4-(4-dimethylaminophenyl)benzamide (2.51 g, 44%) as a solid: $^1$H NMR ($CDCl_3$) $\delta$ 3.01 (6H, s), 3.38 (3H, s), 3.60 (3H, s), 6.80 (2H, d, $J = 8.9$ Hz), 7.54 (2H, d, $J = 8.9$ Hz), 7.59 (2H, d, $J = 8.6$ Hz), 7.74 (2H, d, $J = 8.6$ Hz); IR (KBr) 3253, 2899, 2814, 1603, 1541, 1506, 1471, 1381 cm$^{-1}$; MS *m/z* 285 ($MH^+$).

To a suspension of $LiAlH_4$ (334 mg, 8.8 mmol) in THF (100 ml) was added dropwise a solution of the above benzamide (2.50 g, 8.8 mmol) in THF (70 ml) at 5°C and the mixture was stirred at room temperature for 2.5 h. To the mixture were added anhydrous NaF (1.48 g, 35.2 mmol) and water (0.48 ml), and the mixture was stirred at room temperature for 30 min. The insoluble materials were removed by filtration and washed with THF. The filtrate was evaporated and the residue was purified by silica-gel column chromatography (hexane–EtOAc 2:1 elution) to give **3o** (652 mg, 33%) as a solid: $^1$H NMR ($CDCl_3$) $\delta$ 3.03 (6H, s), 6.81 (2H, d, $J = 9.0$ Hz), 7.58 (2H, d, $J = 9.0$ Hz), 7.72 (2H, d, $J = 8.4$ Hz), 7.90 (2H, d, $J = 8.4$ Hz), 10.01 (1H, s); IR (KBr) 2895, 2812, 2725, 1697, 1680, 1593, 1539, 1502 cm$^{-1}$; MS *m/z* 226 ($MH^+$).

*5.1.11 Method J*

*5.1.11.1 N-(4'-Formylbiphenyl-4-yl)methanesulfonamide (**3p**).* To a solution of 4-bromoaniline (6.88 g, 40 mmol) in pyridine (20 ml) was added dropwise methanesulfonyl chloride (3.1 ml, 40 mmol) at 5°C and the mixture was stirred at the same temperature for 1.5 h, then stirred at room temperature for 1.5 h. The reaction mixture was poured into a mixture of EtOAc and 3N hydrochloric acid and insoluble materials were filtered off. The separated organic layer of the filtrate was washed with water and brine, dried ($MgSO_4$), evaporated, and the resulting solid was triturated with iPE to give *N*-(4-bromophenyl)methanesulfonamide (8.30 g, 95%) as a solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 3.00 (3H, s), 7.16 (2H, d, $J = 8.7$ Hz), 7.52 (2H, d, $J = 8.7$ Hz), 9.92 (1H, br); IR (KBr) 3292, 1489, 1448, 1385, 1329, 1147 cm$^{-1}$.

To a solution of the above *N*-(4-bromophenyl)methanesulfonamide (4.36 g, 20 mmol) and impure 4-(*N*-methoxy-*N*-methylcarbamoyl)phenylboric acid (6.27 g, 30 mmol, see Method I for preparation) in 1,2-dimethoxyethane (100 ml) were added 2M $Na_2CO_3$ solution (30 ml) and $Pd(Ph_3P)_4$ (1.16 g, 1.0 mmol), and the mixture refluxed for 16 h. The reaction mixture was poured into a mixture of EtOAc and ice water. The separated organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography (EtOAc–iPE 1:1 elution) to give *N*-methoxy-*N*-methyl-4-[4-(methylsulfonylamino)phenyl]benzamide (1.54 g, 23%) as an amorphous solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 3.04 (3H, s), 3.28 (3H, s), 3.58 (3H, s), 7.32 (2H, d, $J = 8.6$ Hz), 7.60–7.80 (6H, m), 9.91 (1H, s); IR (KBr) 3209, 2937, 1622, 1608, 1525, 1468, 1340, 1221, 1157 cm$^{-1}$; MS *m/z* 335 ($MH^+$).

To a suspension of $LiAlH_4$ (175 mg, 4.61 mmol) in THF (40 ml) was added dropwise a solution of the above benzamide (1.54 g, 4.61 mmol) in THF (30 ml) at 5°C and the mixture was stirred at room temperature for 3 h. The reaction mixture was poured into an ice cooled mixture of EtOAc and 1N hydrochloric acid. The separated organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography (EtOAc–iPE 1:1 elution) to give **3p** (975 mg, 77%) as a solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 3.06 (3H, s), 7.33 (2H, d, $J = 8.5$ Hz), 7.78 (2H, d, $J = 8.5$ Hz), 7.89 (2H, d, $J = 8.2$ Hz), 7.98 (2H, d, $J = 8.2$ Hz), 9.98 (1H, br s), 10.04 (1H, s); IR (KBr) 3288, 2995, 1697, 1601, 1525, 1491, 1471, 1396 cm$^{-1}$; MS *m/z* 276 ($MH^+$).

*5.1.12 General procedure for Method K*

*5.1.12.1 4-(4-Fluorophenoxy)benzaldehyde (**3q**).* To a solution of 4-fluorobenzaldehyde **2b** (83.0 g, 669 mmol) and 4-fluorophenol (75.0 g, 669 mmol) in DMF (1.3 l) was added powdered $K_2CO_3$ (92.5 g, 669 mmol), and the mixture was stirred at 150°C for 8 h. The reaction mixture was cooled and poured into ice water (3 l). The resulting precipitate was collected by filtration, washed with EtOH and iPE, and dried over phosphorus pentaoxide to give **3q** (129.1 g, 89%) as a solid: $^1$H NMR ($CDCl_3$) $\delta$ 6.98–7.20 (6H, m), 7.85 (2H, d, $J = 8.7$ Hz), 9.92 (1H, s); IR (KBr) 3070, 2837, 2744, 1697, 1606, 1585, 1506, 1309 cm$^{-1}$; MS *m/z* 217 ($MH^+$).

*5.1.13 Typical procedure for Method L*

*5.1.13.1 N-Cycloheptyl-4-(4-fluorophenoxy)benzylamine (**6t**).* A mixture of 4-(4-fluorophenoxy)benzaldehyde **3q** (15.1 g, 70 mmol) and cycloheptylamine (10.7 ml, 84 mmol) was heated at 120°C for 5 h. The mixture was cooled to room temperature and dissolved in EtOH (200 ml). To the solution was added carefully $NaBH_4$ (2.65 g, 70 mmol), and the mixture was stirred at ambient temperature for 1.5 h. The mixture was poured into water and extracted with $CH_2Cl_2$. The organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography ($CH_2Cl_2$–MeOH 20:1 elution) to give **6t** (21.9 g, 100%) as an oil: $^1$H NMR ($CDCl_3$) $\delta$ 1.30–2.00 (12H, m), 2.62–2.82 (1H, m), 3.74 (2H, s), 6.85–7.13 (6H, m), 7.28 (2H, d, $J = 8.4$ Hz); IR (neat) 2926, 2854, 1498, 1458, 1250, 1213 cm$^{-1}$; MS *m/z* 314 ($MH^+$).

Compound **6n** was also prepared as described for **6t** from the appropriate starting material.

*5.1.13.2* N-*Cycloheptyl-4-(4-fluorophenyl)benzylamine* (***6n***). $^1$H NMR ($CDCl_3$) $\delta$ 1.30–1.98 (12H, m), 2.60–2.80 (1H, m), 3.81 (2H, s), 7.02–7.19 (2H, m), 7.38 (2H, d, $J = 8.3$ Hz), 7.42–7.62 (4H, m); IR (neat) 2926, 2854, 1498, 1458, 1232, 1159, 820 cm$^{-1}$; MS *m/z* 298 ($MH^+$).

*5.1.14 General procedure for Method M*

*5.1.14.1 3-Bromomethylbiphenyl* (***5a***). To a mixture of 3-methylbiphenyl **4a** (5.0 g, 29.7 mmol) and *N*-bromosuccinimide (5.29 g, 29.7 mmol) in $CCl_4$ (150 ml) was added benzoyl peroxide (144 mg, 0.59 mmol) and the mixture was refluxed for 6 h. After cooling to room temperature, insoluble materials were filtered off. The filtrate was evaporated under reduced pressure and the residue was purified by silica-gel column chromatography (hexane–EtOAc 20:1 elution) to give impure **5a** (6.59 g, 90%) as an oil: $^1$H NMR ($CDCl_3$) $\delta$ 4.56 (2H, s), 7.35–7.70 (9H, m); IR (neat) 3032, 1599, 1574, 1477, 1454 cm$^{-1}$; MS *m/z* 167 ($MH^+$–HBr).

*5.1.15 General procedure for Method N*

*5.1.15.1* N-*(Biphenyl-3-ylmethyl)cycloheptylamine* (***6ad***). A mixture of 3-bromomethylbiphenyl **5a** (6.58 g, 26.6 mmol) and cycloheptylamine (6.8 ml, 53.3 mmol) was stirred at 120°C for 3.5 h. After cooling to room temperature, a mixture of $CH_2Cl_2$ and water was added thereto. The separated organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography ($CH_2Cl_2$–MeOH 10:1 elution) to give **6ad** (4.49 g, 60%) as an oil: $^1$H NMR ($CDCl_3$) $\delta$ 1.30–2.00 (12H, m), 2.65–2.83 (1H, m), 3.85 (2H, s), 7.25–7.68 (9H, m); IR (neat) 3059, 3031, 2920, 2852, 1458 cm$^{-1}$; MS *m/z* 280 ($MH^+$).

*5.1.16 Method O*

*5.1.16.1* N-*(4-Benzylbenzyl)cycloheptylamine* (***6ag***). To a suspension of 4-(cycloheptylaminomethyl)benzophenone **6ae** (1.87 g, 6.1 mmol) in ethylene glycol (10 ml) were added KOH (511 mg, 9.1 mmol) and $H_2NNH_2 \cdot H_2O$ (1.9 ml, 39 mmol), and the mixture was stirred at 150°C for 5 h then at 200°C for 4 h. After cooling to room temperature, the reaction mixture was poured into a mixture of $CH_2Cl_2$ and water. The separated organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography ($CH_2Cl_2$–MeOH 20:1 elution) to give **6ag** (1.29 g, 72%) as an oil: $^1$H NMR ($CDCl_3$) $\delta$ 1.30–2.00 (12H, m), 2.58–2.78 (1H, m), 3.74 (2H, s), 3.96 (2H, s), 7.10–7.40 (9H, m); IR (neat) 3026, 2926, 2852, 1508, 1456, 1099 cm$^{-1}$; MS *m/z* 294 ($MH^+$).

*5.1.17 General procedure for Method P*

*5.1.17.1 Phenyl* N-*(2,4,6-trifluorophenyl)carbamate* (***8a***). To a solution of 2,4,6-trifluoroaniline (883 mg, 6.0 mmol) and *N,N*-dimethylaniline (0.91 ml, 7.2 mmol) in $CH_2Cl_2$ (18 ml) was added phenyl chloroformate (0.83 ml, 6.6 mmol) at room temperature, and the mixture was stirred for 4 h. The reaction mixture was washed with 1N hydrochloric acid (3×), water, saturated sodium hydrogen carbonate, water, and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The resulting solid was triturated with hexane to give **8a** (1.46 g, 91%) as a solid: mp 126–127°C; $^1$H NMR ($CDCl_3$) $\delta$ 6.26 (1H, br s), 6.70–6.86 (2H, m), 7.10–7.30 (3H, m), 7.30–7.46 (2H, m); IR (KBr) 3253, 1749, 1722, 1539, 1240, 1200 cm$^{-1}$; MS *m/z* 268 ($MH^+$). Anal. ($C_{13}H_8F_3NO_2$) C, H, N.

Compound **8b** was also prepared as described for **8a** from the appropriate starting material.

*5.1.17.2 Phenyl* N-*[2,4-bis(methylthio)-6-methylpyridin-3-yl]carbamate* (***8b***). MP 141–146°C (dec.); $^1$H NMR ($CDCl_3$) $\delta$ 2.46 (3H, s), 2.53 (3H, s), 2.59 (3H, s), 5.86 and 6.25 (total 1H, each br), 6.69 (1H, s), 7.08–7.45 (5H, m); IR (KBr) 3230, 3197, 3145, 2926, 1730, 1560, 1431, 1346, 1198 cm$^{-1}$; MS *m/z* 321 ($MH^+$). Anal. ($C_{15}H_{16}N_2O_2S_2$) C, H, N.

*5.1.18 General procedure for Method Q*

*5.1.18.1* N-*Cycloheptyl*-N-*[4-(4-fluorophenoxy)benzyl]*-N′-*(2,4,6-trimethylphenyl)urea* (***40***). To a solution of *N*-cycloheptyl-4-(4-fluorophenoxy)benzylamine **6t** (22.5 g, 71.8 mmol) in $CH_2Cl_2$ (300 ml) was added 2,4,6-trimethylphenylisocyanate (11.6 g, 71.8 mmol), and the mixture was stirred at room temperature for 2 h. After evaporation, the residue was purified by silica-gel column chromatography (hexane–EtOAc 4:1 elution) to give **40** (27.3 g, 80%) as a solid: mp 125–126°C; $^1$H NMR ($CDCl_3$) $\delta$ 1.40–2.10 (12H, m), 2.00 (6H, s), 2.22 (3H, s), 4.30–4.50 (1H, m), 4.47 (2H, s), 5.49 (1H, s), 6.81 (2H, s), 6.90–7.12 (6H, m), 7.36 (2H, d, $J = 8.6$ Hz); IR (KBr) 3406, 2922, 2856, 1659, 1498, 1250, 1209 cm$^{-1}$; MS *m/z* 475 ($MH^+$). Anal. ($C_{30}H_{35}FN_2O_2$) C, H, N.

Compound **33** was also prepared as described for **40** from the appropriate starting material.

*5.1.18.2* N-*Cycloheptyl*-N-*[4-(4-fluorophenyl)benzyl]*-N′-*(2,4,6-trimethylphenyl)urea* (***33***). MP 179–180°C; $^1$H NMR ($CDCl_3$) $\delta$ 1.40-2.10 (12H, m), 2.01 (6H, s), 2.21 (3H, s), 4.34–4.54 (1H, m), 4.55 (2H, s), 5.47 (1H, s), 6.80 (2H, s), 7.05–7.20 (2H, m), 7.40–7.62 (6H, m); IR (KBr) 3402, 3298, 2924, 2856, 1657, 1495, 1232, 1159 cm$^{-1}$; MS *m/z* 459 ($MH^+$). Anal. ($C_{30}H_{35}FN_2O$) C, H, N.

*5.1.19 General procedure for Method R*

*5.1.19.1* N-*Biphenyl-4-ylmethyl*-N-*cycloheptyl*-N′-*(2,4,6-trifluorophenyl)urea* (***11***). To a solution of 2,4,6-trifluoroaniline (441 mg, 3.0 mmol) and triphosgene (297 mg, 1.0 mmol) in $CH_2Cl_2$ (10 ml) was added $Et_3N$

(0.42 ml, 3.0 mmol) at 5°C, and the mixture was refluxed for 2 h. The mixture was cooled to room temperature and a solution of *N*-(biphenyl-4-ylmethyl)cycloheptylamine **6b** (559 mg, 2.0 mmol) in $CH_2Cl_2$ (3 ml) was added thereto. After stirring at room temperature for 1.5 h, the reaction mixture was poured into water. The separated organic layer was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography (hexane–EtOAc 4:1 elution) to give **11** (752 mg, 83%) as a solid: mp 141–142°C; $^1$H NMR ($CDCl_3$) $\delta$ 1.40–2.10 (12H, m), 4.30–4.50 (1H, m), 4.59 (2H, s), 5.58 (1H, s), 6.57–6.73 (2H, m), 7.30–7.50 (5H, m), 7.55–7.70 (4H, m); IR (KBr) 3286, 2927, 2858, 1635, 1518 cm$^{-1}$; MS *m/z* 453 ($MH^+$). Anal. ($C_{27}H_{27}F_3N_2O$) C, H, N.

*5.1.20 Typical procedure for Method S*

*5.1.20.1 N-Cycloheptyl-N-[4-(4-fluorophenoxy)benzyl]-N′-[2,4-bis(methylthio)-6-methylpyridin-3-yl]urea (50).* To a solution of *N*-cycloheptyl-4-(4-fluorophenoxy)-

Elemental Analyses Table

| Compounds | Formula | Analysis (%) Calcd (Found) C | H | N |
|---|---|---|---|---|
| **8a** | $C_{13}H_8F_3NO_2$ | 58.43 (58.72) | 3.02 (2.72) | 5.24 (5.22) |
| **8b** | $C_{15}H_{16}N_2O_2S_2$ | 56.23 (56.34) | 5.03 (5.08) | 8.74 (8.58) |
| **9** | $C_{27}H_{29}F_3N_2O$ | 71.35 (71.46) | 6.43 (6.44) | 6.16 (6.11) |
| **10** | $C_{30}H_{38}N_2O$ | 81.40 (81.24) | 8.65 (8.85) | 6.16 (6.31) |
| **11** | $C_{27}H_{27}F_3N_2O$ | 71.66 (71.77) | 6.01 (6.12) | 6.19 (6.10) |
| **12** | $C_{33}H_{42}N_2O$ | 82.11 (81.84) | 8.77 (8.83) | 5.80 (5.75) |
| **13** | $C_{30}H_{36}N_2O$ | 81.78 (81.58) | 8.23 (8.22) | 6.36 (6.32) |
| **14** | $C_{27}H_{27}F_3N_2O$ | 71.66 (71.39) | 6.01 (6.08) | 6.19 (6.28) |
| **15** | $C_{30}H_{36}N_2O$ | 81.78 (81.76) | 8.23 (8.49) | 6.36 (6.33) |
| **16** | $C_{27}H_{27}F_3N_2O$ | 71.66 (71.54) | 6.01 (6.06) | 6.19 (6.26) |
| **17** | $C_{30}H_{36}N_2O$ | 81.78 (81.68) | 8.23 (8.35) | 6.36 (6.32) |
| **18** | $C_{27}H_{27}F_3N_2O_2$ | 69.22 (69.40) | 5.81 (5.79) | 5.98 (5.92) |
| **19** | $C_{33}H_{42}N_2O_2$ | 79.48 (79.16) | 8.49 (8.46) | 5.62 (5.73) |
| **20** | $C_{30}H_{36}N_2O_2$ | 78.91 (79.05) | 7.95 (8.10) | 6.13 (6.18) |
| **21** | $C_{30}H_{30}N_2O_2$ | 79.97 (79.79) | 6.71 (6.77) | 6.22 (6.18) |
| **22** | $C_{28}H_{28}N_2O_3$ | 76.34 (76.57) | 6.41 (6.61) | 6.36 (6.34) |
| **23** | $C_{27}H_{27}F_3N_2O_2$ | 69.22 (69.39) | 5.81 (5.95) | 5.98 (5.96) |
| **24** | $C_{30}H_{36}N_2O_2$ | 78.91 (79.12) | 7.95 (7.96) | 6.13 (6.16) |
| **25** | $C_{30}H_{36}N_2OS$ | 76.23 (76.28) | 7.68 (7.93) | 5.93 (6.30) |
| **26** | $C_{31}H_{36}N_2O_2$ | 79.45 (79.24) | 7.74 (7.95) | 5.98 (5.92) |
| **27** | $C_{31}H_{38}N_2O$ | 81.90 (82.03) | 8.42 (8.52) | 6.16 (6.20) |
| **28** | $C_{31}H_{37}N_3O_2$ | 76.99 (76.93) | 7.71 (7.80) | 8.69 (8.54) |
| **29** | $C_{30}H_{37}N_3O_3S$ | 69.33 (69.47) | 7.18 (7.33) | 8.09 (8.03) |
| **30** | $C_{30}H_{37}N_3O_3S$ | 69.33 (69.57) | 7.18 (7.39) | 8.09 (8.05) |
| **31** | $C_{31}H_{37}N_3O_4S$ | 67.98 (69.55) | 6.81 (6.99) | 7.67 (7.88) |
| **32** | $C_{31}H_{37}N_3O_4S \cdot 1.6H_2O$ | 64.58 (64.53) | 7.03 (6.76) | 7.29 (6.94) |
| **33** | $C_{30}H_{35}FN_2O$ | 78.57 (78.94) | 7.69 (7.89) | 6.11 (6.04) |
| **34** | $C_{30}H_{35}ClN_2O$ | 75.85 (76.16) | 7.43 (7.57) | 5.90 (5.87) |
| **35** | $C_{30}H_{35}BrN_2O_2$ | 69.36 (69.75) | 6.79 (6.99) | 5.39 (5.37) |
| **36** | $C_{31}H_{38}N_2O$ | 81.90 (81.95) | 8.42 (8.70) | 6.16 (6.06) |
| **37** | $C_{32}H_{41}N_3O$ | 79.46 (79.11) | 8.54 (8.70) | 8.69 (8.61) |
| **38** | $C_{31}H_{39}N_3O_3S$ | 69.76 (69.45) | 7.36 (7.54) | 7.87 (7.56) |
| **39** | $C_{31}H_{35}N_3O$ | 79.96 (79.69) | 7.58 (7.77) | 9.02 (8.87) |
| **40** | $C_{30}H_{35}FN_2O_2$ | 75.92 (76.24) | 7.43 (7.51) | 5.90 (5.87) |
| **41** | $C_{30}H_{35}ClN_2O_2$ | 73.38 (73.29) | 7.18 (7.33) | 5.70 (5.61) |
| **42** | $C_{30}H_{35}BrN_2O_2$ | 67.29 (67.63) | 6.59 (6.71) | 5.23 (5.19) |
| **43** | $C_{30}H_{35}FN_2O_2$ | 75.92 (75.91) | 7.43 (7.54) | 5.90 (5.90) |
| **44** | $C_{31}H_{35}F_3N_2O_2$ | 70.97 (70.74) | 6.72 (6.64) | 5.34 (5.24) |
| **45** | $C_{31}H_{36}N_2O_4$ | 74.37 (74.09) | 7.25 (7.36) | 5.60 (5.49) |
| **46** | $C_{29}H_{33}FN_2O_2$ | 75.62 (75.72) | 7.22 (7.40) | 6.08 (6.06) |
| **47** | $C_{28}H_{31}FN_2O_2$ | 75.31 (75.02) | 7.00 (7.11) | 6.27 (6.23) |
| **48** | $C_{28}H_{33}FN_2O_2$ | 74.97 (75.03) | 7.41 (7.52) | 6.24 (6.20) |
| **49** | $C_{30}H_{29}FN_2O_2$ | 76.90 (76.51) | 6.24 (6.18) | 5.98 (5.91) |
| **50** | $C_{29}H_{34}FN_3O_2S_2$ | 64.54 (64.36) | 6.35 (6.50) | 7.79 (7.62) |

benzylamine **6t** (2.51 g, 8.0 mmol) in toluene (100 ml) were added phenyl *N*-[2,4-bis(methylthio)-6-methylpyridin-3-yl]carbamate **8b** (2.56 g, 8.0 mmol) followed by $Et_3N$ (3.4 ml, 24.0 mmol), and the mixture was refluxed for 4 h. After cooling to room temperature, the reaction mixture was washed with water and brine, dried ($MgSO_4$), evaporated, and the residue was purified by silica-gel column chromatography (hexane–EtOAc 2:1 elution) to give **50** (3.89 g, 90%) as a solid: mp 140–141°C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.30–1.90 (12H, m), 2.39 (6H, s), 2.45 (3H, s), 3.95–4.15 (1H, m), 4.45 (2H, s), 6.86 (1H, s), 6.94 (2H, d, $J$ = 8.4 Hz), 7.02 (2H, dd, $J$ = 9.0, 4.6 Hz), 7.22 (2H, dd, $J$ = 9.0, 9.0 Hz), 7.36 (2H, d, $J$ = 8.4 Hz), 7.83 (1H, br s); IR (KBr) 3379, 2924, 2856, 1651, 1568, 1529, 1497, 1207 cm$^{-1}$; MS $m/z$ 540 ($MH^+$). Anal. ($C_{29}H_{34}FN_3O_2S_2$) C, H, N.

### 5.2 *Biological methods*

#### 5.2.1 *Assay of in vitro ACAT inhibitory activity*

Rabbit intestinal microsomes for assay of in vitro ACAT inhibitory activity were prepared as follows. Male, Japanese white rabbits (2.0–2.5 kg body weight, 9-week-old; Kitayama Labes, Kyoto, Japan) were fed pelleted rabbit chow supplemented with 2% cholesterol (Oriental Yeast, Tokyo, Japan) *ad libitum* for 8 weeks. After 24 h of fasting, each animal was sacrificed and samples of the small intestine were excised immediately. The intestinal microsome fraction was prepared by the method of Heider et al. [23]

ACAT inhibitory activity was determined according to the method described by Heider et al. [23] with minor modifications [24]. Fatty acid free bovine serum albumin (1.7 nmol BSA; Sigma, St Louis, MO, USA) and equimolar [1-$^{14}$C]oleoyl-CoA (2.0 Mbq/$\mu$mol; New England Nuclear, Boston, MA, USA) in 0.154 M potassium phosphate buffer pH 7.4 (85 $\mu$l) and test compound in 10% DMSO (15 $\mu$l) were mixed and preincubated for 5 min at 37°C. To the mixture was added microsomes (20 $\mu$g of protein) in the buffer (50 $\mu$l), and incubated for 4 min at 37°C. The reaction was stopped by the addition of chloroform–methanol (2:1, v/v, 500 $\mu$l). After vigorous shaking, the reaction mixture was centrifuged at 1,500 × $g$ for 5 min. A 250-$\mu$l aliquot of the chloroform phase was extracted and transferred with chloroform containing cholesteryl oleate as a cold carrier to a silica-gel 60 plate (Merck, Darmstadt, Germany) for TLC using hexane–ether–acetic acid (73:25:2, v/v) as a solvent system. The band of cholesteryl oleate was visualized with iodine vapor. The fraction containing cholesteryl [1-$^{14}$C]oleate was cut out, and the radioactivity was measured by liquid scintillation counting as the ACAT activity. For all test compounds, the $IC_{50}$, which is the concentration that inhibited 50% of the enzyme activity, was determined.

#### 5.2.2 *Hypocholesterolemic effect*

*5.2.2.1 Administration of test compound as a dietary admixture.* Male Sprague–Dawley rats (180–220 g body weight, 6-week-old; CLEA Japan Inc., Japan) were fed the chow diet supplemented with 1% cholesterol and 0.5% cholic acid (high cholesterol diet) *ad libitum*. Normal control group rats were fed the standard chow. Test compounds were admixed with the high cholesterol diet. The duration of feeding was 7 days. Blood samples were taken for measurement of plasma lipid parameters at 9 a.m. through the tail vein in the nonfasted state. Plasma total cholesterol was measured by enzymatic methods using commercial kits (Wako Pure Chemical Industries, Osaka, Japan).

*5.2.2.2 Administration of test compound by gavage in PEG400.* Male Sprague–Dawley rats (180–220 g body weight, 6-week-old; CLEA Japan Inc., Japan) were fed the high cholesterol diet for 6 days. Normal control group rats were fed the high cholesterol diet for 3 days, then fed the standard chow for 3 days. Test compound suspended in PEG400 (in most cases they were dissolved) was orally administered by gavage once a day in the evening on the 3rd, 4th, and 5th day at a dose of 0.1–10 mg/kg. The rats in the normal control group and the high cholesterol control group were given the vehicle alone. The animals were anaesthetized with ether and exsanguinated via cardiac puncture at 9 a.m. on the day following 3 day administration of the compound. Plasma total cholesterol was measured as in the previous section.

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