Inhibitors of Acyl-CoA:Cholesterol *O*-Acyltransferase. 3. Discovery of a Novel Series of *N*-Alkyl-*N*-[(fluorophenoxy)benzyl]-*N*′-arylureas with Weak Toxicological Effects on Adrenal Glands

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A series of *N*-alkyl-*N*-[(fluorophenoxy)benzyl]-*N*′-arylureas were prepared and evaluated for their ability to inhibit intestinal acyl-CoA:cholesterol *O*-acyltransferase and to inhibit accumulation of cholesteryl esters in macrophages in vitro. In vivo hypocholesterolemic activity was assessed in cholesterol-fed rats by oral administration as a dietary admixture and/or by gavage in a PEG400 vehicle. Modification of the alkyl substituent on the *N*′-aryl moiety and on the urea nitrogen significantly influenced macrophage assay in vitro. Toxicological study revealed a distinct relationship between macrophage assay and the toxicity observed in adrenal glands of rabbits treated with representatives of this series of compounds. Investigations utilizing the macrophage assay as an indicator for adrenal toxicity led to the identification of compounds **1g** (FR190809) and **1k** (FR186485, or FR195249 as its hydrochloride salt) as potent, nonadrenotoxic, orally efficacious ACAT inhibitors irrespective of the administration method.

Introduction

Recent epidemiological studies have shown hypercholesterolemia to be associated with increased risk for the development of atherosclerosis.[1] Since therapeutic reduction of serum cholesterol levels has been proven to be an effective treatment of atherosclerotic disease,[2] agents to control plasma lipid levels have been sought as potential therapy.[3] Acyl-CoA:cholesterol *O*-acyltransferase (ACAT, EC 2.3.1.26)[4] 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. Furthermore, ACAT is implicated in the storage of cholesteryl esters to be used as substrate for steroidogenesis as cytosolic droplets in steroid hormone-producing tissues, such as the adrenal gland. Inhibition of ACAT would be expected to reduce the absorption of cholesterol, lower serum lipid levels,[5] and prevent progression and promote regression of atherosclerotic lesions.[6] Therefore, ACAT inhibitors are very attractive targets for development of new treatments for hypercholesterolemia and atherosclerosis.[7]

Despite numerous studies on potent ACAT inhibitors,[8] in clinical trials poor efficacy has been found, and up to the present time none have shown clinical success.[9] In addition, toxicological effects induced by various classes of ACAT inhibitors have consistently been observed in the adrenal glands of certain species[10−17] and are considered to be dose-limiting effects and thus make the development of ACAT inhibitors more difficult. With respect to the mechanisms of this toxicity, although some reports have been published,[11b−e,13b,15,16] it still remains unclear whether this adrenotoxicity is related to ACAT inhibition[13b,15] or not.[11c,14d−f] In our previously reported series,[17] FR182980 showed adrenal toxicity in rabbits and dogs. In contrast, drug-related histopathologic alterations to the adrenal glands of rabbits and/or dogs were not observed with the related compounds FR179254, FR186054, and FR180734 (Chart 1). The IC_{50} values of these compounds for the ACAT enzyme obtained from rabbit intestinal microsomes were 30, 25, 99, and 14 nM, respectively, and the toxicity of FR182980 was deemed to be unrelated to ACAT inhibitory activities (Table 2). However, in the macrophage assay, these compounds showed IC_{50}'s of 48, 80, 350, and 7400 nM (Table 2). Though the precise reason is unclear at this time, the compound with potent macrophage ACAT inhibitory activity induced adrenal toxicity, and the possibility was therefore suggested that macrophage ACAT inhibition and adrenotoxicity are related. With regards to reported ACAT inhibitors, though it has mostly been concluded that ACAT inhibition and adrenotoxicity are not mechanistically related,[11c,14d−f] when examining the published data in detail, the same tendency as our observations can be seen; thus, compounds possessing quite potent activity in the macrophage assay are found to be toxic.[14b−d] Therefore, our interests were directed at identifying ACAT inhibitors with high in vivo potency and no adrenotoxicity by chemical modification of FR182980 utilizing the macrophage assay as an indicator for

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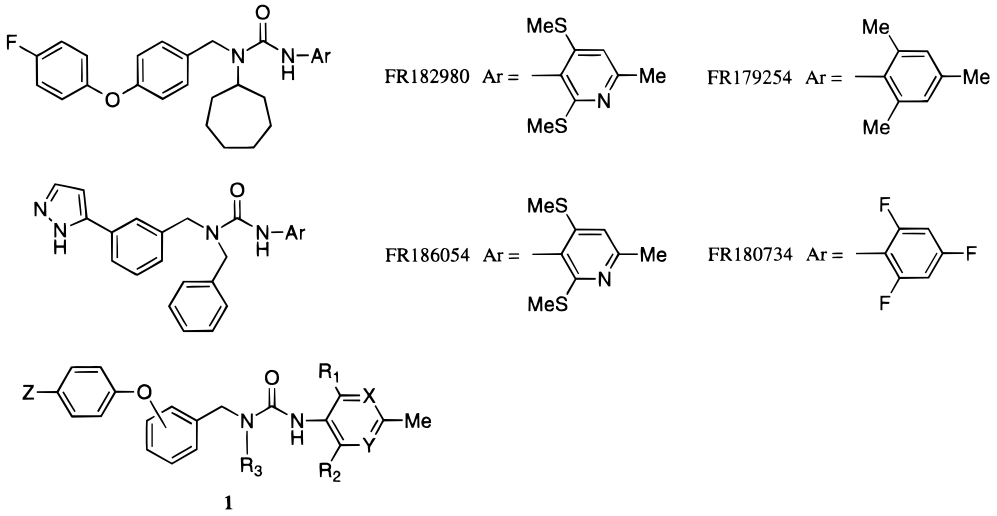
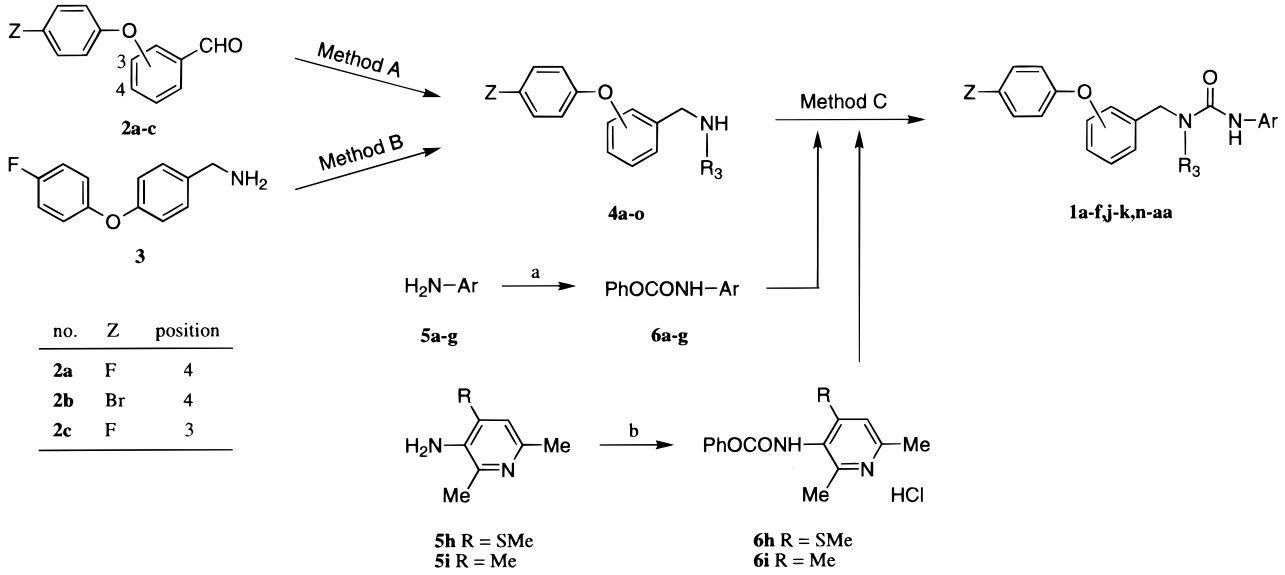
Chart 1. ACAT Inhibitors



Scheme 1. Preparation of Target Molecules[a]



no.	Z	position
2a	F	4
2b	Br	4
2c	F	3

[a] Method A reagents: (1) R_3NH_2; (2) $NaBH_4$, EtOH. Method B reagents: (1) ketone; (2) $NaBH_4$, EtOH. Method C reagents: phenyl *N*-arylcarbamate (**6**), Et_3N. Reagents: (a) $ClCO_2Ph$, $PhNMe_2$, CH_2Cl_2; (b) $ClCO_2Ph$, $ClCH_2CH_2Cl$.

adrenal toxicity. Thus we designed, prepared, and evaluated the novel series of *N*-alkyl-*N*-[(fluorophenoxy)benzyl]-*N′*-arylureas represented by **1** as a related series to FR182980 (Chart 1). Since replacement of the *N′*-aryl moiety reduced the toxicological effect exemplified by the conversion of FR182980 to FR179254, these modifications might be expected to strongly influence physicochemical characteristics and biological profile as well as adrenotoxicity. In this paper, we wish to disclose the synthesis, structure−activity relationships, and toxicological evaluation of this novel series of ACAT inhibitors.

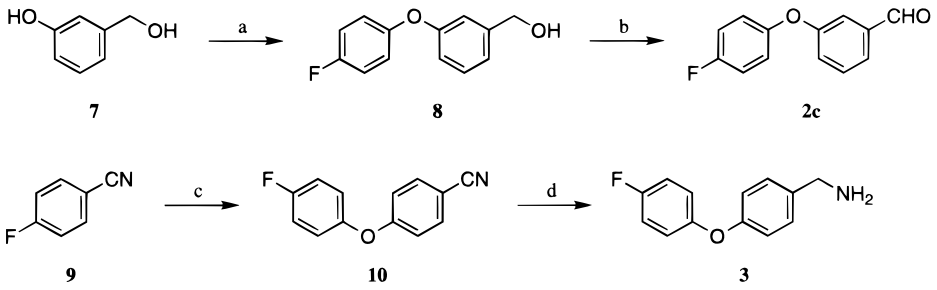
Chemistry

The basic synthetic route to the novel trisubstituted urea compounds **1a**−**f**,**j**−**k**,**n**−**aa** prepared in this work is summarized in Scheme 1 and Table 1. Reductive amination of benzaldehydes **2a**,**b**,[17a]**c** or benzylamine **3** with various alkylamines or carbonyl compounds (methods A, B) provided the secondary amines **4a**−**o** (Table 1). Transformation to the final urea compounds **1** was carried out by treatment with phenyl *N*-arylcarbamates **6a**,[17a]**b**−**i** in various solvents (toluene, DMF, and CH_2Cl_2) (method C). Carbamates **6a**−**g** were readily obtained from the corresponding arylamines **5a**,[18]**b**−**g** utilizing *N*,*N*-dimethylaniline as a base. However, in the case of **5h**,**i**,[19] the main product was a urea compound resulting from self-dimerization under the reaction conditions. Therefore, carbamates **6h**,**i** were prepared as hydrochloride salts under nonbasic conditions. In the free form, these carbamates **6h**,**i** gradually convert to the dimerized urea compounds.

The synthetic method for benzaldehydes **2a**,**b** was described previously.[17a] Meta-substituted benzaldehyde **2c** was prepared by the condensation of 3-hydroxybenzyl alcohol (**7**) with 1-chloro-4-fluorobenzene under the reported conditions,[20] followed by MnO_2 oxidation (Scheme 2). Benzylamine **3** was obtained from reaction of 4-fluorobenzonitrile (**9**) with 4-fluorophenol, followed by $LiAlH_4$ reduction (Scheme 2).

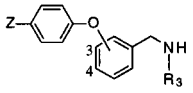
The synthetic routes to highly substituted aminopyridines **5b**−**f**,**h** or pyrimidine **5g**, the precurser of

Scheme 2[a]



[a] Reagents: (a) 4-F-C_6H_4Cl, K_2CO_3, CuCl, 8-quinolinol (cat.), DMI; (b) MnO_2, $CHCl_3$; (c) 4-F-C_6H_4OH, K_2CO_3, DMF; (d) $LiAlH_4$, THF.

Table 1. Structures and Synthetic Methods of Secondary Amines **4**



no.	Z	R_3	position	method (yield, %)
4a[17a]	F	c-C_7H_{13}	4	A (100)
4b[17a]	F	c-C_6H_{11}	4	A (100)
4c	F	tetrahydropyran-4-yl	4	B (86)
4d	F	CH_2CH_2OEt	4	A (95)
4e[17a]	F	Bn	4	A (72)
4f	F	CH_2CH_2Ph	4	A (98)
4g	F	$CH_2(CH_2)_2Ph$	4	A (83)
4h	F	4-MeO-Bn	4	A (89)
4i	F	(*S*)-CH(Me)Ph	4	A (100)
4j	F	(*R*)-CH(Me)Ph	4	A (95)
4k	F	$C(Me)_2Ph$	4	A (97)
4l	F	2,6-Me_2-Bn	4	A (98)
4m[17a]	Br	c-C_7H_{13}	4	A (95)
4n	Br	Bn	4	A (87)
4o	F	c-C_7H_{13}	3	A (91)

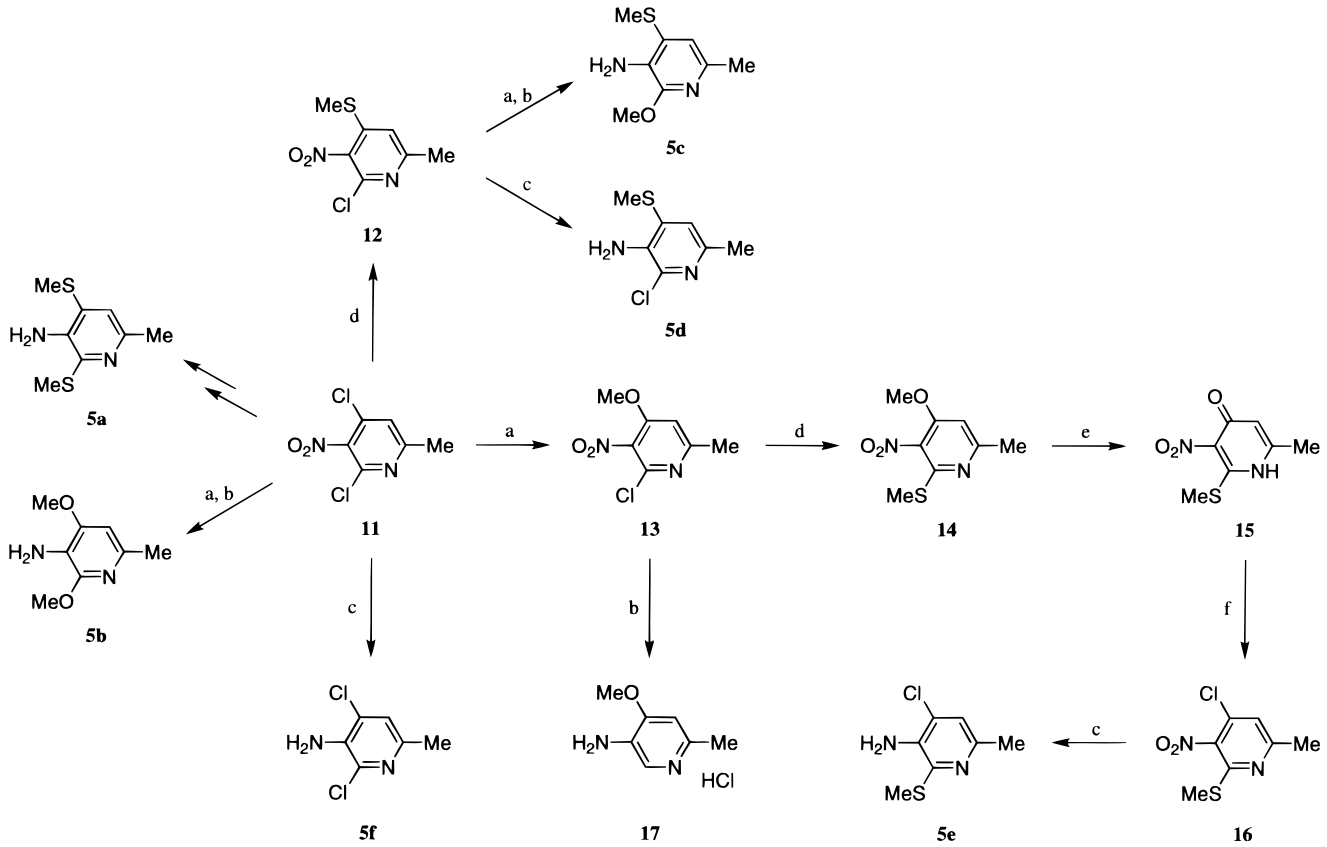
carbamates **6b**−**h** which were the reagents for urea formation, are shown in Schemes 3−5. **5b**−**f** were synthesized from **11**[18] as a key intermediate (Scheme 3). Reaction of this intermediate with an excess amount of NaOMe followed by reduction of the nitro function provided **5b**. Of the two chlorine atoms on the pyridine ring of **11**, the 4-chloro was more reactive; this is the same as the reactivity of 2,4-dichloropyridine without any other substituent such as a nitro group. Therefore, the reaction of **11** with 1 equiv of NaSMe provided 4-(methylthio)pyridine **12** which was converted to **5c** by substitution of the 2-position chlorine atom with NaOMe, followed by hydrogenation, or to **5d** by reduction of the nitro function. The regiospecific synthesis of aminopyridine **5e**, which has the same substituents as **5d** but in the opposite positions, was accomplished as follows. Highly substituted nitropyridine **14** was prepared by a similar procedure to the synthesis of **5c** and was then hydrolyzed to **15**. Subsequent chlorination with $POCl_3$ and reduction provided **5e**. **5f** was readily obtained from **11** by reduction of the nitro function. The reactivity of the two chlorine atoms of nitropyridine **11** was confirmed by structure determination of **12** and **13**. Hydrogenation of **13** provided **17**, which showed two singlet peaks at 7.29 and 7.75 ppm in the ^1H NMR spectrum. The structure of **12** was determined indirectly by desulfurization of ureas **1c**,**e**, which provided **1l**,**m**, respectively (Scheme 6). **1l** showed two coupled doublet signals at 6.65 and 8.24 ppm (J = 7.9 Hz), and **1m** showed a doublet signal derived from ortho coupling on the pyridine ring at 8.44 ppm (J = 8.3 Hz). These data suggested that the methylthio group on the pyridine ring of **12** was at the 4-position. Aminopyrimidine **5g** was prepared from **18**[21] by treatment with NaSMe, followed by reduction of the nitro group (Scheme 4). **5h** was synthesized from 4*H*-pyran-4-one **20**, which was converted to 1*H*-pyridin-4-one **22** in two steps, followed by nitration, chlorination, substitution with NaSMe, and hydrogenation (Scheme 5).

Ureas **1g**,**i** bearing sulfone substituents on the pyridine ring were obtained by *m*-CPBA oxidation of FR182980 and **1e**, respectively (Scheme 6). **1h** was prepared by selective monosubstitution of the methylsulfonyl group at the 4-position of the pyridine ring of **1g** as a leaving group, the structure of which was determined by desulfurization to provide **1ab**, which showed a doublet signal derived from ortho coupling on the pyridine ring at 8.81 ppm (J = 8.8 Hz) in the ^1H NMR spectrum. In this case also, the 4-position of the pyridine ring was more reactive.

Results and Discussion

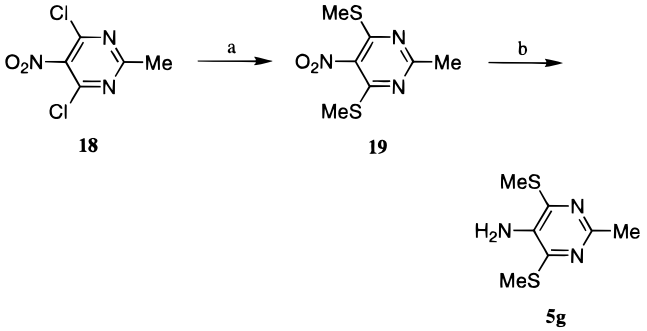
The compounds prepared were evaluated for their ability to inhibit rabbit intestinal ACAT and to inhibit acetylated LDL-induced accumulation of cholesteryl esters in mouse peritoneal macrophages in vitro.[17] In vivo hypocholesterolemic activity was assessed in cholesterol-fed rats by oral administration of the test compounds as a dietary admixture in a cholesterol-enriched diet.[17] Certain of the compounds possessing potent in vivo effects were also evaluated in a different administration model, i.e., dosing by gavage in poly(ethylene glycol) (PEG400) as a vehicle,[17] since it has been shown that the bioavailability of ACAT inhibitors can be markedly influenced by dosing mode.[10,18,22] 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%. Furthermore, selected compounds were examined for adrenal toxicity in rabbits (n = 2) at a single dose of 5 mg/kg iv which is the

Scheme 3[a]



[a] Reagents: (a) NaOMe, MeOH; (b) H_2, 10% Pd/C; (c) H_2, Raney-Ni; (d) NaSMe, MeOH; (e) concd HCl, EtOH; (f) $POCl_3$.

Scheme 4[a]

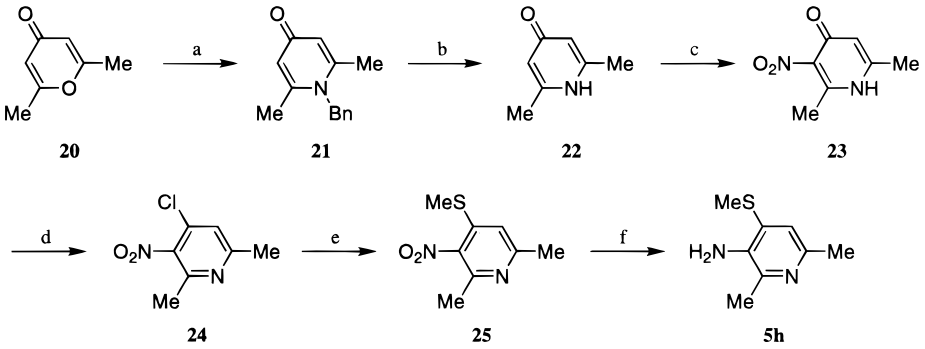


[a] Reagents: (a) NaSMe, DMF; (b) H_2, Raney-Ni, dioxane, MeOH.

dose our previously reported FR145237 induced significant adrenal toxicity.[16] PEG400 was used as a vehicle, and drug-induced necrosis of the adrenal glands was assessed at 24 h after dosing and is expressed as observed (+) or not observed (−), compared to the controls.

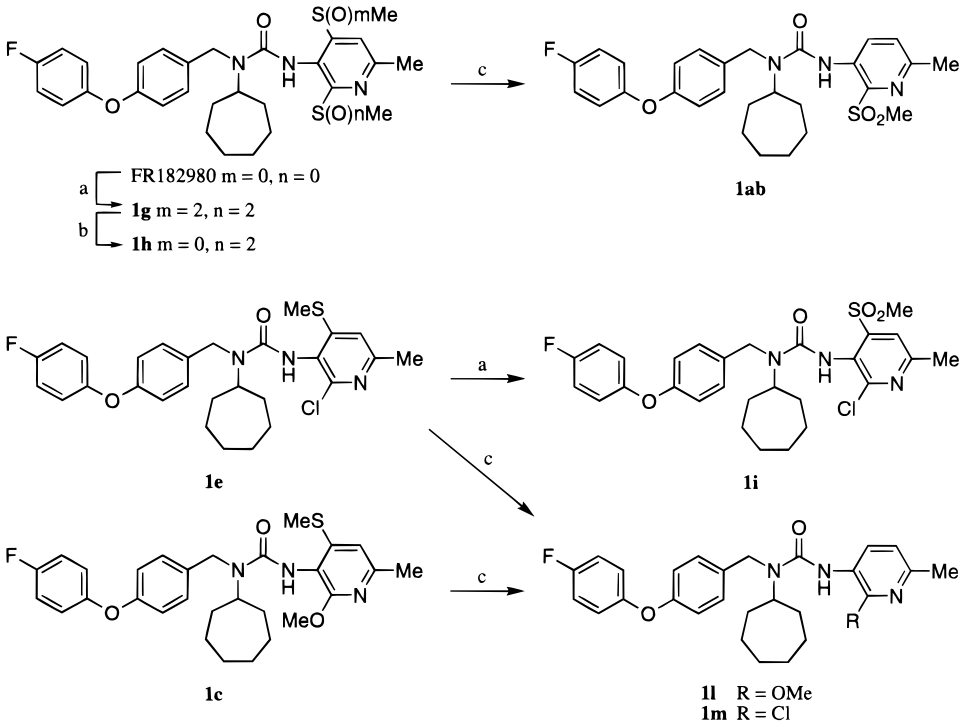
Biological data for urea compounds with various *N′*-aryl moieties are shown in Table 2. Although these modifications did not have a large effect on in vitro inhibitory activity of rabbit intestinal ACAT, the inhibitory activity of acetylated LDL-induced accumulation of cholesteryl esters in mouse peritoneal macrophages was markedly influenced. Conversion of the pyridine to a pyrimidine did not affect activity in the macrophage assay (**1a**). However, replacement of the bis(methylthio) group in FR182980 by a bis(methyloxy) group reduced the inhibitory activity against macrophage ACAT (**1b**). On the other hand, conversion of only R_2 into a methoxy group (**1c**), leaving the R_1 methylthio

Scheme 5[a]



[a] Reagents: (a) $BnNH_2$; (b) HCO_2H, Pd-black, MeOH; (c) HNO_3, H_2SO_4; (d) $POCl_3$; (e) NaSMe, MeOH; (f) H_2, 10% Pd/C, EtOH.

Scheme 6[a]



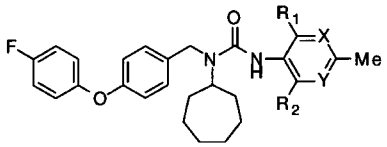
[a] Reagents: (a) *m*-CPBA, CH_2Cl_2; (b) NaSMe, MeOH; (c) Raney-Ni, EtOH.

group intact, significantly increased potency ($IC_{50} = 13$ nM). Furthermore, replacement of the methylthio group of R_1 by chlorine reduced the activity (**1f**), but a similar modification at R_2 retained high activity (**1e**). Oxidation of sulfide in **1e** to sulfone resulted in reduced activity (**1i**). Although **1g**, which is a bis(methylsulfonyl) compound derived from FR182980, also showed relatively weak activity, the monomethylsulfonyl compound **1h** had increased activity. Replacement of the methylthio group at R_2 by a simple methyl group still retained high activity (**1j**); however, conversion of the remaining methylthio group in **1j** to a methyl group showed relatively reduced activity (**1k**). On the basis of these results, it was considered that modification of R_1 significantly influenced macrophage ACAT activity, as compared to R_2. As a consequence of these modifications, we obtained many FR182980 derivatives possessing various macrophage activity as tools to investigate the relationship between macrophage assay and adrenotoxicity.

With respect to in vivo hypocholesterolemic activity, most compounds showed potent activity ($ED_{50} \leq 0.1$ mg/kg) dosing as a dietary admixture, with the exception of **1d**,**h**,**l**,**m**. Furthermore, in a different administration model, i.e., by gavage in PEG400 as a vehicle, several compounds exhibited potent cholesterol-lowering effects (**1a**,**e**,**g**,**k**). The selected five compounds, i.e., **1b** and the four compounds described above, were next evaluated for adrenal toxicity in rabbits ($n = 2$) at a single dose of 5 mg/kg iv as a solution in PEG400. Necrosis of the zona fasciculata cortical cells in the adrenal cortex was observed in the animals treated with compounds **1a**,**e**, which exhibit potent inhibitory activity of foam cell formation. However, drug-induced necrosis was not observed in the adrenal glands taken from rabbits treated with compounds **1b**,**g**,**k**, possessing relatively weak activity in the macrophage assay. Moreover, the hydrochloride salt of compound **1k** was assesed for adrenotoxicity in dog ($n = 1$), which has been reported to be the most sensitive species to the adrenal effects of ACAT inhibitors,[23] at a single dose of 10 mg/kg po as a suspension in PEG400. Though FR182980 induced severe toxicological effects under these conditions,[17c] drug-related histopathologic alterations to the adrenal gland of dog were not observed for the hydrochloride salt of compound **1k**, irrespective of its comparable plasma drug level (the maximum plasma concentrations (C_{max}) for this compound and FR182980 were 144 and 180 ng/mL, respectively). Among these compounds **1g**,**k** showed an excellent biological profile comparable to that of the previously reported FR186054, lacked adrenal toxicity, and were selected for further development. As a consequence of these toxicological evaluations, a distinct relation between macrophage assay and adrenotoxicity was observed in this series, though the reason and significance are still unclear at this point. In addition, it was deemed that the borderline in the macrophage assay for induction of adrenotoxicity was about 100 nM in this series. It should be noted that a correlation between toxicity and ClogP values (Table 2) or partial charge on the nitrogen atom of the aryl moiety (data not shown) was not observed.

Effects of the alkyl substituent (R_3) on the urea nitrogen were next examined (Table 3), since modification at this position was postulated to markedly influence the conformation of the molecule and as a result its biological activity. As described in the modification of the *N*′-aryl moiety, these modifications did not have a large effect on in vitro inhibitory activity of rabbit intestinal ACAT. Compounds **1n**,**q**,**r** showed very potent foam cell formation inhibitory activity ($IC_{50} \leq 10$ nM). In vivo hypocholesterolemic activity of compounds **1n**,**o**,**q**−**s**,**v** were very potent when dosed as a dietary admixture; however, **1r**,**v** only showed moderate effects

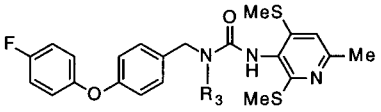
Table 2. Effect of Modification of *N'*-Aryl Moiety on Biological Activities

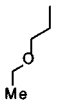
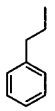
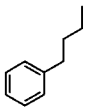
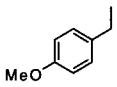
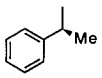
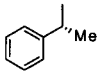
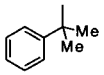


no.	R_1	R_2	X	Y	formula[a]	mp (°C)	yield[b]	ACAT inhibitory activity[c] IC_{50} (nM)	foam cell formation inhibitory activity[d] IC_{50} (nM)	hypocholesterolemic activity[e] ED_{50} (mg/kg) administration mode: diet[f]	hypocholesterolemic activity[e] ED_{50} (mg/kg) administration mode: gavage[g]	ClogP[h]	adrenal toxicity[i]
1a	MeS	MeS	N	N	$C_{28}H_{33}FN_4O_2S_2$	amorphous solid	63	56	20	0.069	0.45	9.23	(+)
1b	MeO	MeO	CH	N	$C_{29}H_{34}FN_3O_4$	92-94	74	33	350	0.032	4.5	8.79	(-)[j]
1c	MeS	MeO	CH	N	$C_{29}H_{34}FN_3O_3S$	amorphous solid	50	29	13	<0.032 (62)**	1.4	9.41	NT
1d	Cl	Cl	CH	N	$C_{29}H_{34}FN_3O_3S$	amorphous solid	72	14	ND	>0.1 (38)	ND	8.07	NT
1e	MeS	Cl	CH	N	$C_{28}H_{31}ClFN_3O_2S$	161-162	65	46	47	0.022	0.17	8.83	(+)
1f	Cl	MeS	CH	N	$C_{28}H_{31}ClFN_3O_2S$	132-133	45	12	350	<0.1 (77)**	>1 (48)	8.88	NT
1g	$MeSO_2$	$MeSO_2$	CH	N	$C_{29}H_{34}FN_3O_6S_2$	129-130	71	45	215	0.068	0.63	5.61	(-)
1h	MeS	$MeSO_2$	CH	N	$C_{29}H_{34}FN_3O_4S_2$	184-185	47	78	15	>0.1 (inactive)	ND	7.76	NT
1i	$MeSO_2$	Cl	CH	N	$C_{28}H_{31}ClFN_3O_4S$	amorphous solid	92	30	350	>0.1 (46)	>1 (34)	6.86	NT
1j	MeS	Me	CH	N	$C_{29}H_{34}FN_3O_2S$	amorphous solid	81	90	35	ND	<1 (72)*	8.56	NT
1k	Me	Me	CH	N	$C_{29}H_{34}FN_3O_2$	amorphous solid	73	62	240	0.044	0.77	7.49	(-)
1l	H	MeO	CH	N	$C_{28}H_{32}FN_3O_3$	132-133	64	39	ND	>0.1 (inactive)	ND	8.40	NT
1m	H	Cl	CH	N	$C_{27}H_{29}ClFN_3O_2$	128-129	35	19	ND	>0.1 (25)	ND	7.88	NT
FR182980	MeS	MeS	CH	N				30	48	0.034	0.11	9.63	(+)[k]
FR179254	Me	Me	CH	CH				25	80	0.045	5.3	8.01	(-)
FR186054								99	350	0.046	0.44	6.34	(-)[k]
FR180734								14	7400	0.096	8.6	4.30	(-)

[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] IC_{50} (nM) for acetylated LDL-induced accumulation of cholesteryl esters in mouse peritoneal macrophages. [e] ED_{50} values are the effective dose to reduce plasma total cholesterol level by 50% of the control value. Values in parentheses denote percent reduction in total cholesterol at the dose indicated. [f] Compound was administered as a dietary admixture. [g] Compound was administered by gavage in PEG400 as a vehicle. [h] ClogP values calculated using MacLogP v2.0.3 (BioByte Corp.). [i] Adrenal toxicity was assessed in rabbits at a single dose of 5 mg/kg iv, and drug-induced necrosis is expressed as observed (+) or not observed (−). [j] At a single dose of 10 mg/kg iv. [k] Data for dog at a single dose of 10 mg/kg po. *Significantly different from control using unpaired, two-tailed Student's *t*-test: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. ND and NT denote not determined and not tested, respectively.

Table 3. Effect of Varying *N*-Alkyl Group on Biological Activities

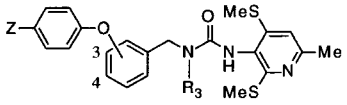


no.	R_3	formula[a]	mp (°C)	yield[b]	ACAT inhibitory activity[c] IC_{50} (nM)	foam cell formation inhibitory activity[d] IC_{50} (nM)	hypocholesterolemic activity[e] ED_{50} (mg/kg) administration mode: diet[f]	hypocholesterolemic activity[e] ED_{50} (mg/kg) administration mode: gavage[g]
1n		$C_{28}H_{32}FN_3O_2S_2$	160-161	87	23[h]	<10 (61)[i]	<0.01 (63)**	ND
1o		$C_{27}H_{30}FN_3O_3S_2$	143-145	41	46	ND	<0.1 (58)	ND
1p		$C_{26}H_{30}FN_3O_3S_2$	157-158	89	24	ND	>0.1 (26)	ND
1q		$C_{29}H_{28}FN_3O_2S_2$	116-118	82	9.1[h]	<10 (77)[i]	<0.01 (58)**	ND
1r		$C_{30}H_{30}FN_3O_2S_2$	amorphous solid	94	16	6.1	0.016	2.7
1s		$C_{31}H_{32}FN_3O_2S_2$	amorphous solid	91	28	40	0.025	ND
1t		$C_{30}H_{30}FN_3O_3S_2$	130-131	79	29	ND	>0.1 (24)	ND
1u		$C_{30}H_{30}FN_3O_2S_2$	amorphous solid	89	19	260	>0.1 (inactive)	ND
1v		$C_{30}H_{30}FN_3O_2S_2$	amorphous solid	71	19	110	0.095	4.6
1w		$C_{31}H_{32}FN_3O_2S_2$	144-145	66	24	52	ND	>1 (inactive)
1x		$C_{31}H_{32}FN_3O_2S_2$	92-93	80	26	21	ND	ND
FR182980					30	48	0.034	0.11

[a–g,]*See corresponding footnotes of Table 2. [h] Different lot of enzyme was used. IC_{50} is an estimated value based on the relative IC_{50} of FR182980 for this batch of enzyme. For example, **1n** showed 0.78 × IC_{50} of FR182980. [i] Values in parentheses denote percent inhibition at the concentration indicated. ND denotes not determined.

when dosed by gavage in a PEG400 vehicle. Compound **1t** was also weak in vivo in comparison to the unsubstituted **1q**; this may possibly be due to metabolism or poor bioavailability. Interestingly, although **1u**,**v**, a pair of enantiomers, exhibited about the same in vitro activity in both assays, the in vivo activity of **1v** (*R*-isomer) was higher. On the basis of the results discussed previously, **1u**,**v** were suggested to be nontoxic;

Table 4. Effect of Varying Substituent on Terminal Phenyl and Position of Phenoxy Moiety on Biological Activities



no.	Z	position	R_3	formula[a]	mp (°C)	yield[b]	ACAT inhibitory activity[c] IC_{50} (nM)	foam cell formation inhibitory activity[d] IC_{50} (nM)	hypocholesterolemic activity[e] ED_{50} (mg/kg) administration mode: diet[f]	hypocholesterolemic activity[e] ED_{50} (mg/kg) administration mode: gavage[g]
1q	F	4	benzyl	$C_{29}H_{28}FN_3O_2S_2$	116-118	82	9.1[h]	<10 (77)[i]	<0.01 (58)**	ND
1y	Br	4	cycloheptyl	$C_{29}H_{34}BrN_3O_2S_2$	161-162	86	28[h]	<10 (75)[i]	0.020	ND
1z	Br	4	benzyl	$C_{29}H_{28}BrN_3O_2S_2$	amorphous solid	87	10[h]	14	0.011	<0.1 (54)
1aa	F	3	cycloheptyl	$C_{29}H_{34}FN_3O_2S_2$	amorphous solid	86	73	2.9	<0.1 (79)**	ND
FR182980	F	4	cycloheptyl				30	48	0.034	0.11

[a–g,*] See corresponding footnotes of Table 2. [h,i] See corresponding footnotes of Table 3. ND denotes not determined.

however, further evaluation of toxicity was not performed because of their insufficient in vivo activity. Of particular note, the introduction of a methyl group onto the *N*-benzyl of **1q** reduced in vitro activity in the macrophage assay (**1u**,**v**); however, the activity was restored by introduction of a dimethyl group (**1w**,**x**).

In our previous report, a bromine atom had been identified as an alternative substituent for fluorine on the terminal phenoxy moiety.[17a] In addition, a benzyl group as an alkyl substituent on the urea nitrogen has been shown to give a good biological profile, both in this series (**1q**) and our previous series represented by FR186054.[17b,c] Therefore, we opted to examine the combination of bromine atom and a cycloheptyl or benzyl group (Table 4). These modifications gave compounds **1y**,**z** possessing excellent biological activity both in vitro and in vivo. In particular, **1z** was still efficacious when dosed by gavage. However, as predicted from its potent inhibitory activity of foam cell formation, this compound induced significant adrenotoxicity in treated rabbits at a single dose of 5 mg/kg iv.

For the purpose of investigation of the position of the 4-fluorophenoxy moiety, *N*-3-(4-fluorophenoxy)benzyl compound (**1aa**) was synthesized and evaluated (Table 4). This compound showed potent hypocholesterolemic effect; however, its macrophage ACAT inhibitory activity was extremely potent (IC_{50} = 2.9 nM), and it was rejected for development based on its predicted adrenotoxicity.

On the basis of the results discussed above, compounds **1g** and **1k** (or its crystalline hydrochloride salt, since **1k** was an amorphous solid) were identified as potent, nontoxic, orally efficacious ACAT inhibitors comparable to our previously reported FR186054,[17b,c] independent of the dosing mode, and selected for further development as a new treatment for hypercholesterolemia and atherosclerosis.

In this series, a distinct relationship between macrophage assay and adrenotoxicity was observed. Although the precise reason remains unclear at this time, it has been reported that ACAT inhibition results in the intracellular buildup of free cholesterol, some of which is then transferred to a membrane pool and induces a loss of cell viability leading to the cell toxicity.[15] Since adrenal gland is a steroid hormone-producing tissue and is replete with cholesteryl esters, it is considered that the effect of ACAT inhibition is direct. Furthermore, the possibility of the existence of isozymes of ACAT, or multiple cholesterol esterification enzymes, has been suggested recently.[24–28] It was also reported that ACAT-deficient (*Acact*$^{-/-}$) mice exhibited a marked reduction in cholesteryl ester levels in the adrenal glands and peritoneal macrophages. However, the livers of these mice still contained significant amounts of cholesteryl esters as well as esterification activity, and sterol absorption in the intestine was unaffected,[26] which was consistent with the results of tissue expression studies in wild-type mice that the expressions of mouse ACAT mRNA and protein were at a high level in steroidogenic tissues, sebaceous glands, peritoneal macrophages, and atherosclerotic lesions, but not in the liver or the intestine.[27] In this connection, recently an ACAT homologue, highly expressed in liver and intestine, has been cloned and designated ACAT II or ACAT-related gene product (ARGP1).[28] If adrenotoxicity is derived from ACAT inhibition, it must be related to inhibitory activity of adrenal ACAT by these inhibitors. Unfortunately, we do not yet have data for the adrenal ACAT assay. However, based on the reports described above, there is a possibility that ACAT in adrenal glands and ACAT in peritoneal macrophages are the same isoform, or derived from the same ACAT gene, and are different from that of the liver- or intestine-derived ACAT; it was thus considered that a relationship between macrophage assay and adrenotoxicity would indirectly be shown. If the adrenal toxicity observed is actually mechanism-based, drug-design directed at the development of inhibitors that cannot reach undesirable tissues (adrenal gland) or isozyme-specific inhibitors is a suitable objective for future research. Data from the adrenal ACAT assay, as well as more detailed molecular biological aspects of the ACAT enzyme, must be investigated further.

Conclusion

In summary, we have prepared a novel series of *N*-alkyl-*N*-[(fluorophenoxy)benzyl]-*N′*-arylureas as a related series to FR182980 and evaluated them as ACAT inhibitors. The SAR study in this series of compounds revealed the following features: (1) Modification of the substituent at the 4-position on the *N′*-pyridine ring does not influence in vitro inhibitory activity against intestinal ACAT; however, it did show a large effect on the macrophage assay. (2) Varing the alkyl substituent on the urea nitrogen also influences macrophage assay rather than the intestinal ACAT assay. (3) Changing the position of the 4-fluorophenoxy moiety to the 3-position on the inner phenyl ring retains activity. (4) Toxicological study revealed a distinct relationship between macrophage assay and adrenotoxicity in this series.

Utilizing the macrophage assay as an indicator for adrenotoxicity, *N*-cycloheptyl-*N*-[4-(4-fluorophenoxy)-benzyl]-*N′*-[2,4-bis(methylsulfonyl)-6-methylpyridin-3-yl]urea (**1g**, FR190809) and *N*-cycloheptyl-*N*-[4-(4-fluorophenoxy)benzyl]-*N′*-(2,4,6-trimethylpyridin-3-yl)urea (**1k**, FR186485, or FR195249 as its hydrochloride salt) were identified as potent, nonadrenotoxic, orally efficacious ACAT inhibitors irrespective of the administration method and were selected for further development. The details of pharmacological studies on these compounds will be the subject of further communications from these laboratories.

Experimental Section

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) spectrometer. 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 hertz. 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. The term "evaporated" or "evaporation" refers to removal of solvent on a rotary evaporator at reduced pressure.

3-(4-Fluorophenoxy)benzaldehyde (2c). To a solution of 3-hydroxybenzyl alcohol (**7**) (51.0 g, 411 mmol) and 1-chloro-4-fluorobenzene (65.7 mL, 617 mmol) in 1,3-dimethyl-2-imidazolidinone (DMI) (200 mL) were added powdered K_2CO_3 (34.1 g, 247 mmol), CuCl (814 mg, 8.2 mmol), and 8-quinolinol (1.19 g, 8.2 mmol). The mixture was stirred at 150 °C for 3 days. The mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated, and the residue was purified by silica gel column chromatography (hexane−EtOAc, 3:1 to 2:1 elution) to give 3-(4-fluorophenoxy)benzyl alcohol (**8**) (49.7 g, 55%) as an oil: ^{1}H NMR ($CDCl_3$) δ 4.66 (2H, s), 6.83−7.15 (7H, m), 7.31 (1H, dd, J = 8.0, 8.0 Hz); IR (neat) 3352, 3074, 2875, 1610, 1585, 1502, 1448 cm^{-1}.

To a solution of **8** (49.6 g, 227 mmol) in $CHCl_3$ (500 mL) was added activated MnO_2 (98.8 g, 1.14 mol), and the mixture was refluxed for 3.5 h. The mixture was filtered, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (hexane−EtOAc, 4:1 elution) to give **2c** (44.2 g, 90%) as an oil: ^{1}H NMR ($CDCl_3$) δ 6.93−7.17 (4H, m), 7.18−7.32 (1H, m), 7.35−7.64 (3H, m), 9.96 (1H, s); IR (neat) 3074, 2837, 2731, 1701, 1585, 1502, 1481, 1450 cm^{-1}; MS *m*/*z* 231 (MH^+ + MeOH − H_2O).

4-(4-Fluorophenoxy)benzylamine (3). To a solution of 4-fluorobenzonitrile (**9**) (50.0 g, 413 mmol) and 4-fluorophenol (50.9 g, 454 mmol) in DMF (400 mL) was added powdered K_2CO_3 (62.8 g, 454 mmol), and the mixture was stirred at 150 °C for 6 h. The reaction mixture was cooled and poured into ice water (2.5 L). The resulting precipitate was collected by filtration, washed with water, and dried to give 4-(4-fluorophenoxy)benzonitrile (**10**) (87.6 g, 99%) as a solid: ^{1}H NMR (DMSO-d_6) δ 7.08 (2H, d, J = 9.0 Hz), 7.13−7.40 (4H, m), 7.84 (2H, d, J = 9.0 Hz); IR (KBr) 3188, 3076, 2220, 1649, 1608, 1483 cm^{-1}; MS *m*/*z* 214 (MH^+).

To a suspension of $LiAlH_4$ (5.69 g, 150 mmol) in THF (300 mL) was added dropwise a solution of **10** (21.3 g, 100 mmol) in THF (200 mL) at 5 °C, and the mixture was stirred at room temperature for 4 h. To the mixture were added anhydrous NaF (16.8 g, 400 mmol) and water (5.41 mL) at 5 °C, and the mixture was stirred at room temperature for 45 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 (CH_2Cl_2−MeOH, 10:1 elution) to give **3** (21.4 g, 99%) as a solid: ^{1}H NMR (DMSO-d_6) δ 3.69 (2H, s), 6.86−7.13 (4H, m), 7.13−7.40 (4H, m); IR (KBr) 3352, 3269, 3043, 1645, 1606, 1495, 1217 cm^{-1}; MS *m*/*z* 201 (MH^+ − NH_3).

3-Amino-2,4-dimethoxy-6-methylpyridine (5b). To a solution of 2,4-dichloro-6-methyl-3-nitropyridine (**11**) (30.3 g, 147 mmol) in MeCN (100 mL) was added NaOMe (28% MeOH solution) (85 mL, 440 mmol) at 5 °C, and the mixture was stirred at 80 °C for 6 h. The mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated, and the residue was purified by silica gel column chromatography (hexane−EtOAc, 4:1 elution) to give 2,4-dimethoxy-6-methyl-3-nitropyridine (28.2 g, 97%) as a solid: ^{1}H NMR (DMSO-d_6) δ 2.44 (3H, s), 3.92 (3H, s), 3.94 (3H, s), 6.97 (1H, s); IR (KBr) 3035, 3005, 2960, 2868, 1601, 1581, 1531, 1460, 1375 cm^{-1}; MS *m*/*z* 199 (MH^+).

To a solution of the above nitropyridine (28.1 g, 142 mmol) in 1,4-dioxane (200 mL) and MeOH (100 mL) was added 10% palladium on carbon (14 g), and the mixture was hydrogenated for 4.5 h under atmospheric pressure. The catalyst was filtered off, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (hexane−EtOAc, 2:1 elution) to give **5b** (23.4 g, 98%) as an oil: ^{1}H NMR (DMSO-d_6) δ 2.26 (3H, s), 3.79 (3H, s), 3.82 (3H, s), 3.96 (2H, br s), 6.52 (1H, s); IR (neat) 3458, 3373, 2945, 2856, 1605, 1446, 1345 cm^{-1}; MS *m*/*z* 169 (MH^+).

3-Amino-2-methoxy-6-methyl-4-(methylthio)pyridine (5c). To a solution of **11** (20.7 g, 100 mmol) in MeOH (250 mL) was added dropwise a solution of NaSMe (7.01 g, 100 mmol) in MeOH (150 mL) at room temperature, and the mixture was stirred for 5 h. The mixture was evaporated, and the residue was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated, and the residue was purified by silica gel column chromatography (hexane−EtOAc, 3:1 to 1:1 elution) to give 2-chloro-6-methyl-4-(methylthio)-3-nitropyridine (**12**) (16.6 g, 76%) as a solid: ^{1}H NMR (DMSO-d_6) δ 2.53 (3H, s), 2.65 (3H, s), 7.53 (1H, s); IR (KBr) 3091, 2993, 2926, 1574, 1531, 1435, 1346, 1234 cm^{-1}; MS *m*/*z* 219, 221 (MH^+).

To a solution of **12** (13.3 g, 60.6 mmol) in MeOH (150 mL) was added NaOMe (28% MeOH solution) (23.4 mL, 121 mmol), and the mixture was refluxed for 7 h. After cooling, the resulting solid was collected by filtration and washed with MeOH−iPE to give 2-methoxy-6-methyl-4-(methylthio)-3-nitropyridine (10.3 g, 79%) as a solid: ^{1}H NMR (DMSO-d_6) δ 2.46 (3H, s), 2.57 (3H, s), 3.94 (3H, s), 7.07 (1H, s); IR (KBr)

3024, 2997, 2951, 2924, 2856, 1587, 1541, 1495, 1452 cm^{-1}; MS *m/z* 215 (MH^+).

To a suspension of the above nitropyridine (12.1 g, 56.5 mmol) in 1,4-dioxane (100 mL) and MeOH (100 mL) was added 10% palladium on carbon (9 g), and the mixture was hydrogenated for 3 h under atmospheric pressure. The catalyst was filtered off, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (hexane−EtOAc, 4:1 elution) to give **5c** (9.07 g, 87%) as an oil: ^1H NMR (DMSO-d_6) δ 2.26 (3H, s), 2.43 (3H, s), 3.84 (3H, s), 4.39 (2H, br s), 6.64 (1H, s); IR (neat) 3444, 3350, 2983, 2947, 2922, 1585, 1558, 1462, 1387 cm^{-1}; MS *m/z* 185 (MH^+).

3-Amino-2-chloro-6-methyl-4-(methylthio)pyridine (5d). To a suspension of **12** (16.0 g, 73.2 mmol) in 1,4-dioxane (200 mL) and MeOH (50 mL) was added Raney nickel (30 g), and the mixture was hydrogenated for 3 h under atmospheric pressure. Raney nickel was filtered off, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (hexane−EtOAc, 6:1 to 4:1 elution) to give **5d** (12.9 g, 93%) as an oil: ^1H NMR (DMSO-d_6) δ 2.29 (3H, s), 2.51 (3H, s), 4.93 (2H, br s), 6.98 (1H, s); IR (neat) 3429, 3350, 2922, 1606, 1570, 1529, 1468, 1444 cm^{-1}; MS *m/z* 189, 191 (MH^+).

3-Amino-4-chloro-6-methyl-2-(methylthio)pyridine (5e). To a solution of **11** (41.4 g, 200 mmol) in MeOH (400 mL) was added NaOMe (28% MeOH solution) (38.6 mL, 200 mmol), and the mixture was stirred at 60 °C for 1 h. The mixture was evaporated, and the residue was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated, and the residue was purified by silica gel column chromatography (hexane−EtOAc, 4:1 elution) to give 2-chloro-4-methoxy-6-methyl-3-nitropyridine (**13**) (30.4 g, 75%) as a solid: ^1H NMR (DMSO-d_6) δ 2.51 (3H, s), 4.01 (3H, s), 7.42 (1H, s); IR (KBr) 3088, 2987, 2953, 2883, 1601, 1552, 1524, 1471 cm^{-1}; MS *m/z* 203, 205 (MH^+).

To a solution of **13** (30.4 g, 150 mmol) in MeOH (300 mL) was added dropwise a solution of NaSMe (12.6 g, 180 mmol) in MeOH (200 mL) at room temperature, and the mixture was stirred at 50 °C for 4 h. The mixture was evaporated, and the residue was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated, and the residue was purified by silica gel column chromatography (hexane−EtOAc, 4:1 elution) to give 4-methoxy-6-methyl-2-(methylthio)-3-nitropyridine (**14**) (30.2 g, 94%) as a solid: ^1H NMR (DMSO-d_6) δ 2.51 (3H, s), 2.53 (3H, s), 3.95 (3H, s), 7.11 (1H, s); IR (KBr) 3066, 2997, 2956, 2933, 2858, 1585, 1549, 1514, 1466, 1352 cm^{-1}; MS *m/z* 215 (MH^+).

To a suspension of **14** (30.2 g, 141 mmol) in EtOH (300 mL) was added concentrated HCl (58.6 mL), and the mixture was refluxed for 10 h. After cooling to 5 °C, the resulting precipitate was collected, washed with EtOH and iPE, and dried to give 6-methyl-2-(methylthio)-3-nitro-1*H*-pyridin-4-one (**15**) (19.8 g 70%) as a solid: ^1H NMR (DMSO-d_6) δ 2.39 (3H, s), 2.50 (3H, s), 6.62 (1H, s); IR (KBr) 2989, 2920, 1551, 1518, 1309, 1246, 1225 cm^{-1}.

A suspension of **15** (30.7 g, 153 mmol) in $POCl_3$ (85.6 mL, 918 mmol) was stirred at 100 °C for 10 h. The mixture was poured into water, neutralized with 5 N NaOH solution, and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated, and the residue was purified by silica gel column chromatography (hexane−EtOAc, 5:1 elution) to give 4-chloro-6-methyl-2-(methylthio)-3-nitropyridine (**16**) (11.9 g, 36%) as a solid: ^1H NMR (DMSO-d_6) δ 2.55 (3H, s), 2.59 (3H, s), 7.55 (1H, s); IR (KBr) 3103, 3053, 2933, 1560, 1518, 1402, 1344 cm^{-1}; MS *m/z* 219, 221 (MH^+).

To a suspension of **16** (11.9 g, 54.3 mmol) in EtOH (200 mL) was added Raney nickel (20 g), and the mixture was hydrogenated for 4 h under atmospheric pressure. Raney nickel was filtered off, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (hexane−EtOAc, 6:1 elution) to give **5e** (9.83 g, 96%) as a solid: ^1H NMR (DMSO-d_6) δ 2.31 (3H, s), 2.51 (3H, s), 4.96 (2H, br s), 6.96 (1H, s); IR (KBr) 3417, 3300, 3207, 2922, 1618, 1558, 1443, 1367 cm^{-1}; MS *m/z* 189, 191 (MH^+).

3-Amino-2,4-dichloro-6-methylpyridine (5f). To a solution of **11** (4.14 g, 20 mmol) in 1,4-dioxane (50 mL) and MeOH (50 mL) was added Raney nickel (2 g), and the mixture was hydrogenated for 4 h under atmospheric pressure. Raney nickel was filtered off, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (hexane−EtOAc, 4:1 elution) to give **5f** (3.53 g, 100%) as an oil: ^1H NMR (DMSO-d_6) δ 2.28 (3H, s), 5.52 (2H, br s), 7.23 (1H, s); IR (neat) 3479, 3385, 3188, 2924, 1616, 1576, 1543, 1471, 1367, 1309 cm^{-1}; MS *m/z* 177, 179, 181 (MH^+).

5-Amino-4,6-bis(methylthio)-2-methylpyrimidine (5g). To a solution of 4,6-dichloro-2-methyl-5-nitropyrimidine (**18**) (7.95 g, 38.2 mmol) in DMF (150 mL) was added NaSMe (5.89 g, 84.0 mmol), and the mixture was stirred at room temperature for 6 h and then stirred at 150 °C for 10 h. After cooling, the mixture was poured into ice water and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated, and the residue was purified by silica gel column chromatography (hexane−EtOAc, 4:1 elution) to give 4,6-bis(methylthio)-2-methyl-5-nitropyrimidine (**19**) (5.19 g, 59%) as a solid: ^1H NMR (DMSO-d_6) δ 2.54 (6H, s), 2.66 (3H, s); IR (KBr) 3007, 2926, 1518, 1425, 1329, 1201 cm^{-1}; MS *m/z* 232 (MH^+).

To a suspension of **19** (5.17 g, 22.4 mmol) in 1,4-dioxane (120 mL) and MeOH (50 mL) was added Raney nickel (10 g), and the mixture was hydrogenated for 2.5 h under atmospheric pressure. Raney nickel was filtered off, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (hexane−EtOAc, 4:1 elution) to give **5g** (4.11 g, 91%) as a solid: ^1H NMR (DMSO-d_6) δ 2.45 (3H, s), 2.52 (6H, s), 4.57 (2H, br s); IR (KBr) 3363, 3284, 3197, 2922, 1625, 1531, 1417, 1363 cm^{-1}; MS *m/z* 202 (MH^+).

3-Amino-2,6-dimethyl-4-(methylthio)pyridine (5h). A mixture of 2,6-dimethyl-4*H*-pyran-4-one (**20**) (100 g, 806 mmol) and benzylamine (88 mL, 806 mmol) was heated at 180 °C for 10 h. After cooling, the reaction mixture was purified by silica gel column chromatography (EtOAc−MeOH, 10:1 to 5:1 elution) to give 1-benzyl-2,6-dimethyl-1*H*-pyridin-4-one (**21**) (135.2 g, 79%) as a solid: ^1H NMR (DMSO-d_6) δ 2.19 (6H, s), 5.22 (2H, s), 6.04 (2H, s), 6.92−7.02 (2H, m), 7.23−7.45 (3H, m); IR (KBr) 3377, 3167, 1641, 1550, 1477, 1450, 1371, 1346, 1207 cm^{-1}; MS *m/z* 214 (MH^+).

To a suspension of **21** (135 g, 633 mmol) in MeOH (800 mL) were added palladium-black (40 g) and HCO_2H (72.2 mL), and the mixture was refluxed for 10 h. The catalyst was removed by filtration, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH, 10:1 to 5:1 elution) to give 2,6-dimethyl-1*H*-pyridin-4-one (**22**) (68.0 g, 87%) as a solid: ^1H NMR (DMSO-d_6) δ 2.15 (6H, s), 5.84 (2H, s); IR (KBr) 3365, 2902, 2777, 1651, 1628, 1504, 1437, 1394, 1367, 1200 cm^{-1}; MS *m/z* 124 (MH^+).

To ice-cooled fuming H_2SO_4 (30% SO_3) (98 mL) was added fuming HNO_3 (d = 1.52) (90 mL), and the mixture stirred at 5 °C for 40 min. To the mixture was added **22** (66.0 g, 536 mmol) carefully at 5 °C, followed by stirring at room temperature for 3 days. The mixture was poured into ice water (600 mL) and adjusted to pH 3 with 5 N NaOH solution. The resulting precipitate was collected by filtration, washed with ice water, and dried to give 2,6-dimethyl-3-nitro-1*H*-pyridin-4-one (**23**) (67.2 g, 75%) as a solid: ^1H NMR (DMSO-d_6) δ 2.22 (3H, s), 2.28 (3H, s), 6.18 (1H, s), 11.83 (1H, br); IR (KBr) 3271, 3068, 2949, 1647, 1620, 1572, 1508, 1383, 1346 cm^{-1}; MS *m/z* 169 (MH^+).

To $POCl_3$ (260 mL, 2.8 mol) was added **23** (67.2 g, 400 mmol), and the mixture stirred at 100 °C for 10 h. The mixture was poured into water (1.2 L), adjusted to pH 8 with 5 N NaOH solution, and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated, and the residue was purified by silica gel column chromatography (hexane−EtOAc, 1:1 elution) to give 4-chloro-2,6-dimethyl-3-nitropyridine (**24**) (48.6 g, 65%) as a solid: ^1H NMR (DMSO-d_6) δ 2.49 (3H, s), 2.52 (3H, s), 7.67 (1H, s); MS *m/z* 187, 189 (MH^+).

To a solution of **24** (48.3 g, 259 mmol) in MeOH (400 mL) was added dropwise a solution of NaSMe (21.8 g, 311 mmol) in MeOH (200 mL) at room temperature, and the mixture was refluxed for 5 h. The mixture was evaporated, and the residue was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated, and the residue was purified by silica gel column chromatography (hexane−EtOAc, 1:1 elution) to give 2,6-dimethyl-4-(methylthio)-3-nitropyridine (**25**) (36.0 g, 70%) as a solid: ^{1}H NMR (DMSO-d_6) δ 2.48 (3H, s), 2.50 (3H, s), 2.57 (3H, s), 7.32 (1H, s); MS *m*/*z* 199 (MH^+).

To a suspension of **25** (35.8 g, 181 mmol) in EtOH (400 mL) was added 10% palladium on carbon (35 g), and the mixture was hydrogenated for 3 h under atmospheric pressure. The catalyst was filtered off, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH, 20:1 elution) to give **5h** (26.2 g, 86%) as a solid: ^{1}H NMR (DMSO-d_6) δ 2.26 (3H, s), 2.27 (3H, s), 2.43 (3H, s), 4.51 (2H, br s), 6.81 (1H, s); IR (KBr) 3429, 3307, 3194, 2927, 1633, 1568, 1552, 1468, 1431, 1389, 1292 cm^{-1}; MS *m*/*z* 169 (MH^+).

3-Amino-4-methoxy-6-methylpyridine Hydrochloride (17). To a solution of **13** (608 mg, 3.0 mmol) in EtOH (40 mL) was added 10% palladium on carbon (1.0 g), and the mixture was hydrogenated for 2 h under atmospheric pressure. The catalyst was filtered off, and the filtrate was evaporated. The residual solid was collected by filtration and washed with iPE to give **17** (419 mg, 80%) as a solid: ^{1}H NMR (DMSO-d_6) δ 2.51 (3H, s), 4.03 (3H, s), 5.78 (2H, br s), 7.29 (1H, s), 7.75 (1H, s), 14.70 (1H, br); IR (KBr) 3390, 3304, 2661, 1624, 1603, 1500, 1452, 1348, 1319 cm^{-1}; MS *m*/*z* 139 (MH^+ − HCl).

Synthesis of Phenyl *N*-Arylcarbamates 6b−i, Reagents for Urea Formation. (1) Phenyl *N*-[2-Methoxy-6-methyl-4-(methylthio)pyridin-3-yl]carbamate (6c). To a solution of **5c** (9.06 g, 49.2 mmol) and *N*,*N*-dimethylaniline (7.48 mL, 59.0 mmol) in CH_2Cl_2 (120 mL) was added phenyl chloroformate (6.79 mL, 54.1 mmol) at 5 °C, and the mixture was stirred at room temperature for 3 h. The reaction mixture was washed with 1 N HCl, water, saturated $NaHCO_3$ solution, water, and brine, dried ($MgSO_4$), and evaporated. The resulting solid was triturated with iPE to give **6c** (7.83 g, 52%) as a solid: ^{1}H NMR (DMSO-d_6) δ 2.39 (3H, s), 2.45 (3H, s), 3.86 (3H, s), 6.81 (1H, s), 6.85−7.50 (5H, m), 8.76 and 9.17 (total 1H, each br s); IR (KBr) 3217, 1740, 1649, 1541, 1518 cm^{-1}; MS *m*/*z* 305 (MH^+).

The following compounds **6b**,**d**−**g** were also prepared as described for **6c** from the appropriate starting material.

Phenyl *N*-(2,4-dimethoxy-6-methylpyridin-3-yl)carbamate (6b): yield 22.0 g (55%); ^{1}H NMR (DMSO-d_6) δ 2.38 (3H, s), 3.85 (6H, s), 6.72 (1H, s), 7.05−7.30 (3H, m), 7.35−7.47 (2H, m), 8.83 (1H, br); IR (KBr) 3251, 3147, 2983, 2947, 1713, 1593, 1497, 1454 cm^{-1}; MS *m*/*z* 289 (MH^+).

Phenyl *N*-[2-chloro-6-methyl-4-(methylthio)pyridin-3-yl]carbamate (6d): yield 47.2 g (68%); ^{1}H NMR (DMSO-d_6) δ 2.45 (3H, s), 2.51 (3H, s), 6.98−7.50 (6H, m), 9.36 and 9.76 (total 1H, each br s); IR (KBr) 3145, 2926, 1751, 1579, 1516, 1489, 1342, 1201 cm^{-1}; MS *m*/*z* 309, 311 (MH^+).

Phenyl *N*-[4-chloro-6-methyl-2-(methylthio)pyridin-3-yl]carbamate (6e): yield 11.9 g (62%); ^{1}H NMR (DMSO-d_6) δ 2.48 (3H, s), 2.51 (3H, s), 6.98−7.53 (6H, m), 9.37 and 9.77 (total 1H, each br s); IR (KBr) 3207, 3026, 2926, 1724, 1597, 1554, 1524, 1489 cm^{-1}; MS *m*/*z* 309, 311 (MH^+).

Phenyl *N*-(2,4-dichloro-6-methylpyridin-3-yl)carbamate (6f): yield 1.96 g (33%); ^{1}H NMR (DMSO-d_6) δ 2.27 (3H, s), 7.10−7.55 (5H, m), 7.65 (1H, s), 10.10 (1H, br s); IR (KBr) 3282, 3184, 3012, 1718, 1637, 1608, 1524, 1491 cm^{-1}; MS *m*/*z* 297, 299, 301 (MH^+).

Phenyl *N*-[4,6-bis(methylthio)-2-methylpyrimidin-5-yl]carbamate (6g): yield 5.74 g (88%); ^{1}H NMR (DMSO-d_6) δ 2.49 (6H, s), 2.59 (3H, s), 6.98−7.52 (5H, m), 9.27 and 9.68 (total 1H, each br s); IR (KBr) 3217, 3005, 2924, 1711, 1595, 1485, 1408, 1360, 1300 cm^{-1}; MS *m*/*z* 322 (MH^+).

(2) Phenyl *N*-(2,4,6-Trimethylpyridin-3-yl)carbamate Hydrochloride (6i). To a solution of phenyl chloroformate (25 mL, 199 mmol) in 1,2-dichloroethane (250 mL) was added dropwise a solution of **5i** (22.6 g, 166 mmol) in 1,2-dichloroethane (120 mL) at 100 °C, and the mixture was refluxed for 1 h. After cooling to room temperature, to the reaction mixture was added dropwise a mixture of EtOAc (2 L) and THF (1 L). The resulting precipitate was collected, washed with EtOAc and iPE, and dried to give crude **6i** (48.8 g, 70%) as a highly hygroscopic solid: ^{1}H NMR (DMSO-d_6) δ 2.49 (3H, s), 2.69 (6H, s), 7.15−7.75 (6H, m), 9.63 and 10.20 (total 1H, each br s); IR (KBr) 3413, 1741, 1645, 1541, 1483, 1234, 1201 cm^{-1}; MS *m*/*z* 257 (MH^+ − HCl).

Compound **6h** was also prepared as described for **6i** from **5h**.

Phenyl *N*-[2,6-dimethyl-4-(methylthio)pyridin-3-yl]-carbamate hydrochloride (6h): yield 9.76 g (38%); ^{1}H NMR (DMSO-d_6) δ 2.61 (3H, s), 2.66 (3H, s), 2.69 (3H, s), 6.68−7.55 (5H, m), 7.74 (1H, s), 9.65 and 10.14 (total 1H, each br s); IR (KBr) 3130, 2752, 1743, 1630, 1608, 1481, 1225, 1198 cm^{-1}; MS *m*/*z* 289 (MH^+ − HCl).

Method A. Reductive amination of benzaldehyde derivatives **2a**−**c** with various amine compounds was performed as previously described.[17]

Method B. *N*-[4-(4-Fluorophenoxy)benzyl]tetrahydropyran-4-ylamine (4c). A mixture of **3** (4.35 g, 20 mmol) and tetrahydropyran-4-one (2.22 mL, 24 mmol) was heated at 120 °C for 4 h. The mixture was then cooled to room temperature and dissolved in EtOH (80 mL). To the solution was added carefully $NaBH_4$ (757 mg, 20 mmol), and the mixture was stirred at ambient temperature for 2 h. The mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated, and the residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH, 20:1 elution) to give **4c** (5.15 g, 86%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.34−1.60 (2H, m), 1.77−1.95 (2H, m), 2.65−2.85 (1H, m), 3.30−3.50 (2H, m), 3.79 (2H, s), 3.88−4.08 (2H, m), 6.83−7.13 (6H, m), 7.28 (2H, d, J = 8.6 Hz); IR (neat) 2930, 2845, 1498, 1464, 1250, 1211 cm^{-1}; MS *m*/*z* 302 (MH^+).

Typical Procedure for Method C. *N*-Cycloheptyl-*N*-[4-(4-fluorophenoxy)benzyl]-*N*′-(2,4,6-trimethylpyridin-3-yl)urea (1k, FR186485). To a solution of *N*-cycloheptyl-4-(4-fluorophenoxy)benzylamine (13.9 g, 44.3 mmol) in DMF (150 mL) was added crude carbamate **6i** (13.0 g, 44.3 mmol) followed by Et_3N (18.5 mL, 133 mmol), and the mixture was stirred at 100 °C for 3 h. After cooling to room temperature, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated, and the residue was purified by silica gel column chromatography (EtOAc elution) to give **1k** (15.4 g, 73%) as a solid: ^{1}H NMR ($CDCl_3$) δ 1.38−2.10 (12H, m), 2.03 (3H, s), 2.19 (3H, s), 2.42 (3H, s), 4.30−4.50 (1H, m), 4.48 (2H, s), 5.51 (1H, br s), 6.81 (1H, s), 6.87−7.13 (6H, m), 7.36 (2H, d, J = 8.6 Hz); IR (KBr) 3300, 2926, 2856, 1632, 1498, 1250, 1213 cm^{-1}; MS *m*/*z* 476 (MH^+). Anal. ($C_{29}H_{34}FN_3O_2$) C, H, N.

***N*-Cycloheptyl-*N*-[4-(4-fluorophenoxy)benzyl]-*N*′-(2,4,6-trimethylpyridin-3-yl)urea Hydrochloride (FR195249, hydrochloride salt of FR186485).** To a solution of **1k** (23.9 g, 50.2 mmol) in EtOAc (100 mL) was added dropwise a solution of 4 N HCl in EtOAc (37.6 mL) at 5 °C, and the mixture stirred for 20 min. To the mixture was then added iPE (100 mL), followed by warming to room temperature, and then the mixture stirred for 3 h. The resulting precipitate was collected, washed with iPE, and recrystallized from EtOH−hexane to give FR195249 (16.7 g, 65%) as a solid: mp 174−176 °C; ^{1}H NMR (DMSO-d_6) δ 1.30−1.88 (12H, m), 2.32 (3H, s), 2.52 (3H, s), 2.65 (3H, s), 4.03−4.23 (1H, m), 4.53 (2H, s), 6.90−7.12 (4H, m), 7.14−7.38 (4H, m), 7.61 (1H, s), 8.30 (1H, br s); IR (KBr) 3369, 2925, 2858, 2619, 1645, 1497, 1248, 1211 cm^{-1}; MS *m*/*z* 476 (MH^+ − HCl). Anal. Calcd for $C_{29}H_{34}FN_3O_2$·HCl: C, 68.02; H, 6.89; N, 8.21. Found: C, 68.15; H, 7.03; N, 8.23.

***N*-Cycloheptyl-*N*-[4-(4-fluorophenoxy)benzyl]-*N*′-[2,4-bis(methylsulfonyl)-6-methylpyridin-3-yl]urea (1g, FR190809).** To a solution of *N*-cycloheptyl-*N*-[4-(4-fluorophenoxy)benzyl]-*N*′-[2,4-bis(methylthio)-6-methylpyridin-3-yl]-urea (FR182980) (26.1 g, 48.4 mmol) in CH_2Cl_2 (400 mL) was added dropwise a solution of 80% *m*-CPBA (41.7 g, 193 mmol)

in CH_2Cl_2 (900 mL) at room temperature, and the mixture was stirred for 3 days. Insoluble materials were removed by filtration, and the filtrate was washed with saturated $NaHCO_3$ solution, water, and brine, dried ($MgSO_4$), and evaporated. The residue was purified by silica gel column chromatography (hexane−EtOAc, 1:1 elution) to give **1g** (20.8 g, 71%) as a solid: mp 129−130 °C; ^{1}H NMR ($CDCl_3$) δ 1.40−2.13 (12H, m), 2.66 (3H, s), 3.19 (3H, s), 3.30 (3H, s), 4.05−4.30 (1H, m), 4.55 (2H, s), 6.90−7.10 (6H, m), 7.34 (2H, d, J = 8.6 Hz), 7.85 (1H, s); IR (KBr) 3361, 2927, 2860, 1664, 1500, 1325, 1248, 1211, 1159, 1128 cm^{-1}; MS m/z 604 (MH^+). Anal. ($C_{29}H_{34}FN_3O_6S_2$) C, H, N.

***N*-Cycloheptyl-*N*-[4-(4-fluorophenoxy)benzyl]-*N′*-[6-methyl-2-(methylsulfonyl)-4-(methylthio)pyridin-3-yl]urea (1h).** To a solution of **1g** (3.04 g, 5.0 mmol) in MeOH (100 mL) was added NaSMe (315 mg, 4.5 mmol) at room temperature, and the mixture was stirred at 50 °C for 1 h. After cooling, the resulting precipitate was collected, washed with MeOH and iPE, and dried to give **1h** (1.35 g 47%) as a solid: mp 184−185 °C; ^{1}H NMR ($CDCl_3$) δ 1.38−2.08 (12H, m), 2.44 (3H, s), 2.54 (3H, s), 3.23 (3H, s), 4.10−4.32 (1H, m), 4.55 (2H, s), 6.74−7.10 (7H, m), 7.36 (2H, d, J = 8.6 Hz); IR (KBr) 3377, 3072, 2926, 2858, 1657, 1572, 1498, 1309, 1248, 1209, 1142 cm^{-1}; MS m/z 572 (MH^+). Anal. ($C_{29}H_{34}FN_3O_4S_2$) C, H, N.

***N*-Cycloheptyl-*N*-[4-(4-fluorophenoxy)benzyl]-*N′*-[6-methyl-2-(methylsulfonyl)pyridin-3-yl]urea (1ab).** To a suspension of **1h** (172 mg, 0.3 mmol) in EtOH (20 mL) was added Raney nickel (1.0 g), and the mixture was refluxed for 15 min. Raney nickel was filtered off, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (hexane−EtOAc, 4:1 elution) to give **1ab** (127 mg, 81%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.38−2.00 (12H, m), 2.50 (3H, s), 3.21 (3H, s), 4.05−4.25 (1H, m), 4.52 (2H, s), 6.87−7.32 (9H, m), 8.81 (1H, d, J = 8.8 Hz), 9.12 (1H, br s); MS m/z 526 (MH^+).

***N*-Cycloheptyl-*N*-[4-(4-fluorophenoxy)benzyl]-*N′*-[2-chloro-6-methyl-4-(methylsulfonyl)pyridin-3-yl]urea (1i).** To a solution of *N*-cycloheptyl-*N*-[4-(4-fluorophenoxy)benzyl]-*N′*-[2-chloro-6-methyl-4-(methylthio)pyridin-3-yl]urea (**1e**) (1.0 g, 1.90 mmol) in CH_2Cl_2 (40 mL) was added 80% *m*-CPBA (1.23 g, 5.69 mmol) at room temperature. After stirring for 2 h, the reaction mixture was washed with saturated $NaHCO_3$ solution, water, and brine, dried ($MgSO_4$), and evaporated. The residue was purified by silica gel column chromatography (hexane−EtOAc, 3:2 elution) to give **1i** (976 mg, 92%) as a solid: ^{1}H NMR ($CDCl_3$) δ 1.40−2.10 (12H, m), 2.59 (3H, s), 3.09 (3H, s), 4.10−4.35 (1H, m), 4.54 (2H, s), 6.65 (1H, br), 6.90−7.10 (6H, m), 7.31 (2H, d, J = 8.6 Hz), 7.58 (1H, s); IR (KBr) 3367, 2927, 2858, 1664, 1498, 1317, 1213, 1149 cm^{-1}; MS m/z 560, 562 (MH^+). Anal. ($C_{28}H_{31}ClFN_3O_4S$) C, H, N.

***N*-Cycloheptyl-*N*-[4-(4-fluorophenoxy)benzyl]-*N′*-(2-chloro-6-methylpyridin-3-yl)urea (1m).** To a suspension of **1e** (730 mg, 1.38 mmol) in EtOH (30 mL) was added Raney nickel (5.0 g), and the mixture was refluxed for 3 h. Raney nickel was filtered off, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (hexane−EtOAc, 4:1 to 2:1 elution) to give **1m** (230 mg, 35%) as a solid: mp 128−129 °C; ^{1}H NMR ($CDCl_3$) δ 1.38−2.05 (12H, m), 2.43 (3H, s), 4.25−4.45 (1H, m), 4.50 (2H, s), 6.76 (1H, br s), 6.88−7.12 (7H, m), 7.30 (2H, d, J = 8.6 Hz), 8.44 (1H, d, J = 8.3 Hz); IR (KBr) 3386, 2927, 2858, 1676, 1512, 1497, 1460, 1296 cm^{-1}; MS m/z 482, 484 (MH^+). Anal. ($C_{27}H_{29}ClFN_3O_2$) C, H, N.

Compound **1l** was also prepared as described for **1m** from **1c**.

***N*-Cycloheptyl-*N*-[4-(4-fluorophenoxy)benzyl]-*N′*-(2-methoxy-6-methylpyridin-3-yl)urea (1l):** yield 215 mg (64%); mp 132−133 °C; ^{1}H NMR ($CDCl_3$) δ 1.40−2.05 (12H, m), 2.34 (3H, s), 3.74 (3H, s), 4.23−4.43 (1H, m), 4.45 (2H, s), 6.65 (1H, d, J = 7.9 Hz), 6.77 (1H, br s), 6.90−7.10 (6H, m), 7.32 (2H, d, J = 8.6 Hz), 8.24 (1H, d, J = 7.9 Hz); IR (KBr) 3394, 2927, 2858, 1666, 1591, 1520, 1497, 1454, 1379 cm^{-1}; MS m/z 478 (MH^+). Anal. ($C_{28}H_{32}FN_3O_3$) C, H, N.

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