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Studies on Cerebral Protective Agents. V.[1a]
Novel 4-(3-Nitrophenyl)pyridine and 4-(3-Nitrophenyl)pyrimidine Derivatives with Anti-anoxic Activity

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Novel 4-(3-nitrophenyl)pyridine and 4-(3-nitrophenyl)pyrimidine derivatives, possessing three-atom linkages between basic nitrogen and at the C-5 (or C-3) position of the pyrimidine (or pyridine) ring, were synthesized and tested for anti-anoxic (AA) activity in mice. Among them, 6-methyl-4-(3-nitrophenyl)-2-phenyl-5-(pyrrolidinomethylcarbonylamino)pyrimidine (10f) had the most potent AA activity (3.2 mg/kg, i.p.). Three-dimensional molecular electrostatic potentials (3D-MEP) around the nitrogeneous basic moiety of 6-methyl-5-(4-methylpiperazin-1-ylcarbonyl)-4-(3-nitrophenyl)-2-phenylpyrimidine (FK 360) and 10f were compared. The negative zone of 10f is broader and deeper and positioned somewhat differently to that of FK 360, although there is a co-occupied spacial area in part.

Keywords cerebral protective agent; anti-anoxia; structure–activity relationship; 4-(3-nitrophenyl)pyridine; 4-(3-nitrophenyl)pyrimidine; FK 360

Introduction

In the course of searching for new cerebral protective agents which have anti-anoxic (AA) and anti-lipid peroxidation (ALP) activities, we found that the 4-(3-, or 4-nitrophenyl)-2-phenylpyrimidine system (part A) and a nitrogenous basic moiety in the C-5 position of the pyrimidine ring (part B) appeared to be a prerequisite for the expression of ALP and AA activity, respectively (Fig. 1).[1c,d]

Three representative types of compounds are shown in Fig. 2.

Regarding the structure–activity relationships (SARs) on AA activity these compounds revealed that four-atom linkages between the pyrimidine ring and a basic nitrogen are a prerequisite and that five-atom linkages result in a loss of AA activity.[1b,c] The necessity of these SARs for the expression of AA activity was also confirmed in the case of the modification of the pyrimidine nucleus (*e.g.* pyridine, pyridazine).[1a] In this report we describe the synthesis and the SARs of new pyrimidine and pyridine derivatives which have three-atom linkages between the pyrimidine (or pyridine) ring and a basic nitrogen since, in regard to the SARs, this distance still remained ambiguous.[1c] A comparison of a three dimensional molecular electrostatic potential (3D-MEP) mapping between FK 360 and compound **10f** is also described.

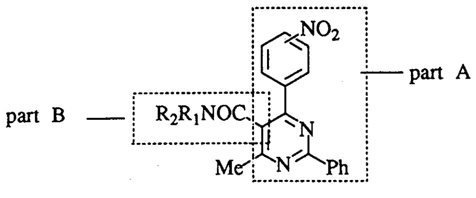

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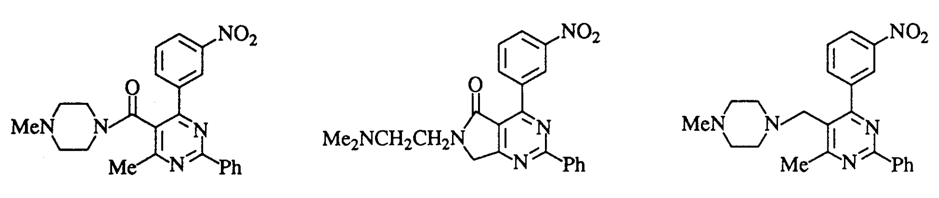
Fig. 1

Chemistry

Compounds **10a**—**g** and **11a**—**i** were prepared in a convergent manner as shown in Chart 1. The conversion of the carboxylic acid (**2**)[1a] to the urethane (**3**) was carried out through a Curtius rearrangement using diphenylphosphoryl azide (DPPA) in the presence of triethylamine (Et_3N) in *tert*-butanol (*tert*-BuOH). Deprotection of **3** with concentrated HCl aq. provided the 5-aminopyridine derivative (**6**). Acylation of **4**[1d] and **6** with appropriate acid bromides gave **5** and **7**—**9**, followed by amination with amines afforded **10a**—**g** and **11a**—**i**, respectively. Alkylation of **3** with *N*-(2-chloroethyl)morpholine gave **12**, followed by deprotection with concentrated HCl aq. afforded **13**.

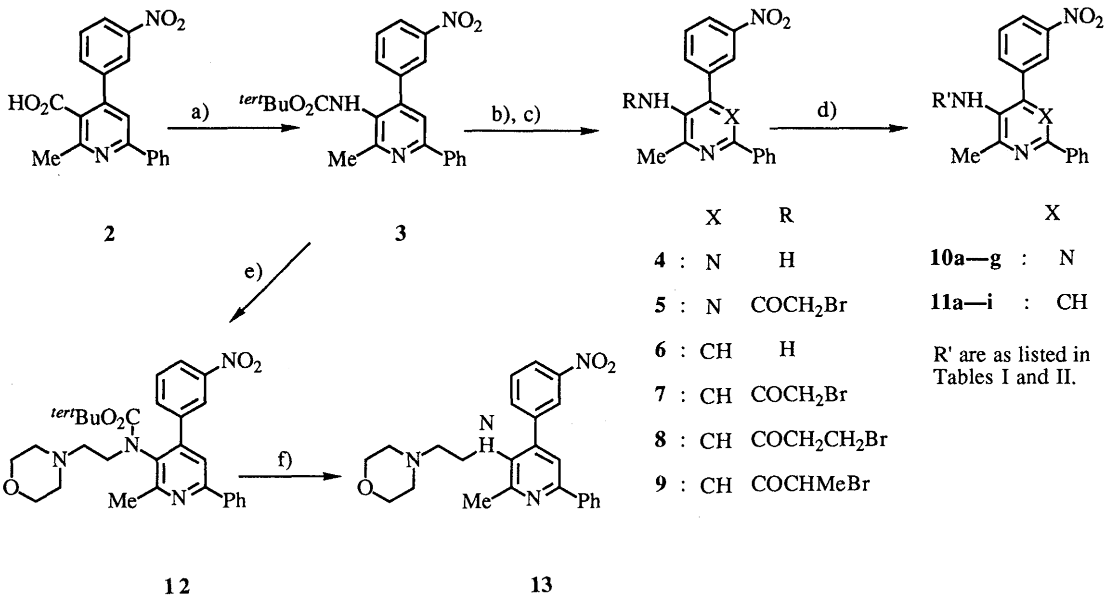
Compounds **20**, **24**, **28** and **33** were synthesized *via* the routes shown in Charts 2—5.

As shown in Chart 2, condensation of the 3-butenoate derivative (**14**)[2] with ethyl 3-aminocrotonate gave 1,4-dihydropyridine-2,5-dicarboxylate (**15**). On the basis of 200 MHz ^{1}H-NMR measurements in DMSO-d_6, **15** was found solely in the 1,4-dihydro form under these conditions. Thus, the proton signal of C_4-H (δ 4.80) of **15** was coupled with the proton C_5-H (δ 5.95) at a value of 6.0 Hz. Further, the signal of C_5-H was also long-range coupled with the proton of N_1-H (δ 8.51) at 1.8 Hz, which disappeared on the addition of D_2O.



FK 360 FR 75469 FR 72707

Fig. 2



	X	R
4 :	N	H
5 :	N	$COCH_2Br$
6 :	CH	H
7 :	CH	$COCH_2Br$
8 :	CH	$COCH_2CH_2Br$
9 :	CH	COCHMeBr

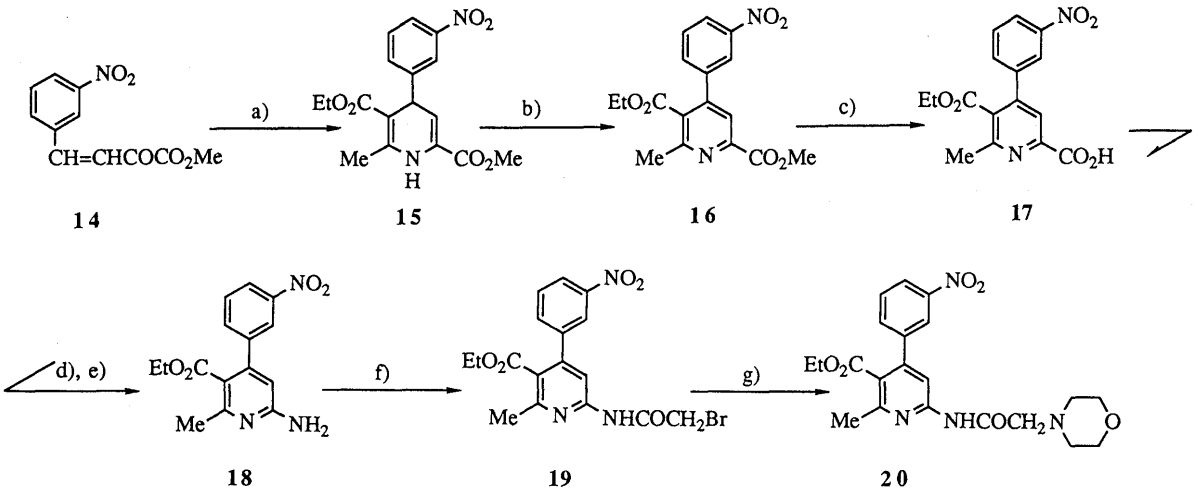
	X
10a—g :	N
11a—i :	CH

R' are as listed in Tables I and II.

a) DPPA, Et_3N / *tert*-BuOH ; b) conc. HCl aq. / EtOH ; c) RBr / pyridine—toluene ; d) amines / CH_2Cl_2

e) NaH, *N*-(2-chloroethyl)morpholine / DMF ; f) conc. HCl aq. / EtOH

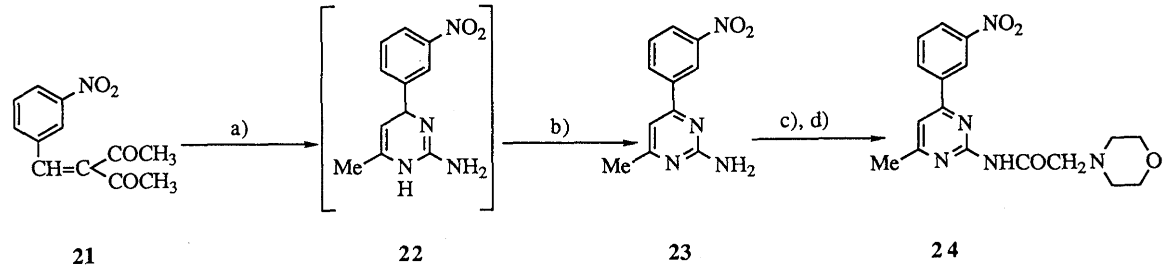
Chart 1



a) ethyl 3-aminocrotonate / *tert*-BuOH ; b) MnO_2 / $CHCl_3$; c) NaOH aq. / EtOH ; d) DPPA, Et_3N / *tert*-BuOH ;

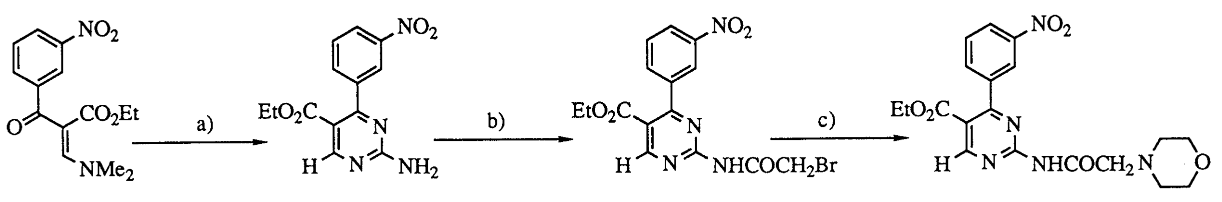
e) conc. HCl ; f) $BrCH_2COBr$ / pyridine—toluene; g) morpholine / CH_2Cl_2

Chart 2



a) guanidine / EtOH ; b) MnO_2 / $CHCl_3$; c) $BrCH_2COBr$ / pyridine—toluene ; d) morpholine / CH_2Cl_2

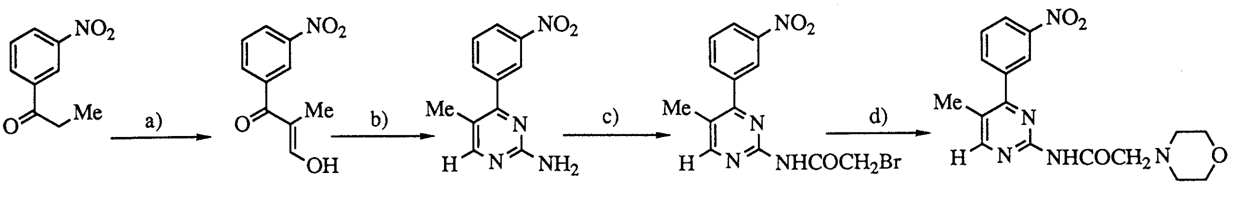
Chart 3



25 26 27 28

a) guanidine•HCl, Et_3N / EtOH ; b) $BrCH_2COBr$ / pyridine—toluene ; c) morpholine / CH_2Cl_2

Chart 4

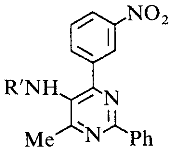


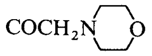
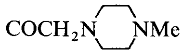
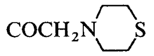
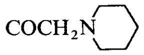
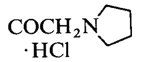
29 30 31 32 33

a) ethyl formate, DBU; b) guanidine • carbonate, AcOH / DMSO ; c) $BrCH_2COBr$ / pyridine—toluene ; d) morpholine / CH_2Cl_2

Chart 5

Table I. Physical Properties and AA Activity of 2-Methyl-4-(3-nitrophenyl)-2-phenylpyrimidine Derivatives (**10a**—**g**)



Compound No.	R′	Anti-anoxia[a] (% of control) (mg/kg, i.p.) 3.2	10	32	Yield (%)	mp (°C) (Recryst. solv.)	Formula	Analysis (%) Calcd (Found) C	H	N
10a	$COCH_2NMe_2$			96	72.4	142—144 (EtOH–Et_2O)	$C_{21}H_{21}N_5O_3$	64.44 (64.44	5.41 5.19	17.89 17.91)
10b			112	124[b]	67.6	145—147 (EtOH–Et_2O)	$C_{23}H_{23}N_5O_4$	63.73 (64.01	5.35 5.30	16.16 15.98)
10c				103	70.4	159—161 (EtOH–Et_2O)	$C_{24}H_{26}N_6O_3$	64.56 (15.03	5.87 5.85	18.82 18.84)
10d			105	120[c]	68.3	147—148 (EtOH–Et_2O)	$C_{23}H_{23}N_5O_3S$	61.46 (61.75	5.16 4.95	15.58 15.73)
10e			107	130[c]	79.2	145—147 (EtOH–Et_2O)	$C_{24}H_{25}N_5O_3$	66.81 (67.00	5.84 6.02	16.23 16.04)
10f	 ·HCl	115[b]	126[c]	172[c]	56.1	181 (dec.) (EtOH)	$C_{23}H_{23}N_5O_3$ ·HCl·0.5H_2O	59.67 (59.40	5.44 5.43	15.13 15.19)
10g	$COCH_2NH$(1-adamantyl) ·HCl		110[d]	117[c]	42.3	257—259 (EtOH)	$C_{29}H_{31}N_5O_3$ ·HCl	65.22 (65.25	6.03 5.82	13.11 13.08)

a) Each value represents the mean of 5 to 10 animals compared with control group. *b*) $p<0.05$. *c*) $p<0.001$. *d*) $p<0.01$. Values without marks are not statistically significant.

Oxidation of **15** with activated manganese (IV) oxide (MnO_2), followed by alkaline hydrolysis afforded the 2-pyridinecarboxylic acid derivative (**17**). Compound **17** was converted to **18** by the same procedures employed in the preparation of **6**.

The reaction of 3-(3-nitrophenylmethylene)acetylacetone (**21**) with guanidine, followed by oxidation with activated MnO_2 afforded **23** through a possible intermediate (**22**) (Chart 3). Similar deacetylated reaction has been reported by Weis *et al.*[3)]

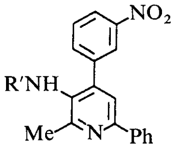
The reaction of ethyl 2-(3-nitrobenzoyl)-3-dimethylaminopropenoate (**25**)[1c)] with guanidine gave **26** (Chart 4).

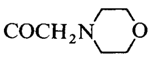
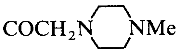
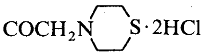
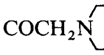
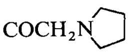
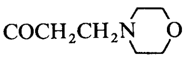
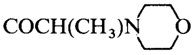
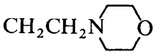
Formylation of 1-(3-nitrophenyl)propan-1-one (**29**)[4)] with ethyl formate in the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) gave **30**, followed by reaction with guanidine afforded **31** (Chart 5). The above mentioned amine derivatives (**18**, **23**, **26** and **31**) were converted to **20**, **24**, **28** and **33**, respectively, by the same procedures employed in the preparation of **10a**—**g** and **11a**—**i**.

Pharmacological Results and Discussion

The compounds listed in Tables I, II and IV were tested for AA activity in mice as described previously.[1d)] The

TABLE II. Physical Properties and AA Activity of 2-Methyl-4-(3-nitrophenyl)-6-phenylpyridine Derivatives (**11a**—**i** and **13**)



Compound No.	R′	Anti-anoxia[a] (% of control) (mg/kg, i.p.) 3.2	10	32	Yield (%)	mp (°C) (Recryst. solv.)	Formula	Analysis (%) Calcd (Found) C	H	N
11a	COCH$_2$NMe$_2$			118	40.7	148—149 (EtOH)	$C_{22}H_{22}N_4O_3$	67.68 (67.87	5.68 5.52	14.35 14.47)
11b	 COCH$_2$N O		111[b]	134[c]	81.4	167—168 (EtOH)	$C_{24}H_{24}N_4O_4$	66.65 (66.66	5.59 5.44	12.96 13.06)
11c	 COCH$_2$N NMe			114	15.5	103—106 (EtOH)	$C_{25}H_{27}N_5O_3$ $\cdot 0.3H_2O$	66.59 (66.50	6.16 6.22	15.53 15.27)
11d	 COCH$_2$N S·2HCl			106	71.4	212—215 (dec.) (EtOH)	$C_{24}H_{24}N_4O_3S$ $\cdot 2HCl$	50.08 (50.22	5.60 5.49	9.73 9.90)
11e	 COCH$_2$N			108	62.0	126—127 (Et$_2$O)	$C_{25}H_{26}N_4O_3$	69.75 (69.80	6.09 5.69	13.01 13.03)
11f	 COCH$_2$N		110[b]	132[c]	52.5	120—121 (Et$_2$O)	$C_{24}H_{24}N_4O_3$	69.21 (69.54	5.81 5.57	13.45 13.46)
11g	COCH$_2$NH(1-adamantyl)		109	119[c]	76.0	190—191 (EtOH-Et$_2$O)	$C_{30}H_{32}N_4O_3$	72.56 (72.99	6.49 6.33	11.28 11.32)
11h	 COCH$_2$CH$_2$N O			112	62.6	142—144 (Et$_2$O)	$C_{25}H_{26}N_4O_4$	67.25 (66.81	5.87 5.65	12.55 12.48)
11i	 COCH(CH$_3$)N O			107	61.2	177—178 (Et$_2$O)	$C_{25}H_{26}N_4O_4$	67.25 (66.75	5.87 5.64	12.55 12.47)
13	 CH$_2$CH$_2$N O		102	120[b]	66.1	164—165 (Et$_2$O)	$C_{24}H_{26}N_4O_3$	68.88 (68.52	6.26 6.26	13.39 13.22)

a) See footnote *a*) in Table I. *b*, *c*) See footnote *b*, *c*) in Table I.

results of the pyrimidine derivatives are shown in Table I.

Compounds **10b** and **10d**—**g** possessed significant AA activity at 32 mg/kg, i.p. Among them, **10f** had the most potent activity (3.2 mg/kg, i.p.) in this series. Compound **10c**, which possesses two basic nitrogens in the substituent resulted in a loss of activity. Activity of compound **10a**, unexpectedly, was not significant. Incongruous failures for the expression of AA activity such as this occurred in several of the previous pyrimidine series and may reflect negative pharmacokinetic issues resulting from metabolic degradation or low penetration into the brain. However, the substituent at the C-5 position, which has three-atom linkages between the nitrogenous basic moiety and pyrimidine nucleus (*e.g.* $R_1R_2NCH_2CONH$–), seemed also to be a prerequisite for the expression of AA activity. Moreover, these compounds, which have a reversal amide linkage to the pyrimidine ring, had a tendency to increase or to tolerate well the expression of AA activity compared with that of the four-atom linkaged normal amide ($R_1R_2NCH_2CH_2HNOC$–) derivatives. In order to confirm the necessity of these SARs for the expression of AA activity, we further synthesized the pyridine derivatives (**11a**—**i**) and similar results were obtained in the pyridine series as shown in Table II.

Compounds **11b**, **11f** and **11g** possessed significant AA activity at 32 mg/kg, i.p. Compound **11h**, which is elongated one methylene unit compared to **11b**, unexpectedly, resulted in a loss of activity. Introduction of a methyl group neighboring the nitrogenous basic moiety (**11i**) also resulted in a loss of activity. The 4-methylpiperazine derivative (**11c**), which has two basic nitrogens in the substituent, was not significant. Conversion of amide (–NHCO–) to aminomethyl (–$NHCH_2$–) (**13**) was tolerated. As well as the above mentioned pyrimidine derivatives, these pyridine derivatives which have reversed amide linkage to the pyridine ring had a tendency to increase or to tolerate well the expression of AA activity compared with that of the four-atom linkaged normal amide ($R_1R_2NCH_2CH_2HNOC$–) derivatives.[1a]

In order to reason these results we sought to compare 3D-MEP around the nitrogeneous basic moiety of FK 360 and **10f**.[1b,5] The 3D-MEP program was based on the modified neglect of diatomic overlap (MNDO) approximation and the definition of molecular electrostatic potential (MEP) calculations were carried out by the method of Politzer.[6]

The MEP result is represented in Fig. 3, and suggests that the negative zone is located around the basic nitrogen. It should be noted that the carbonyl oxygen of **10f** oriented in the same direction of the lone pair of the pyrrolidine nitrogen, and the negative zone of **10f** is broader and deeper and positioned somewhat different to that of FK 360, although there is a co-occupied spacial area in part. It is tempting to speculate that the three-atom linkaged reversed amide derivatives bear increased negative electrostatic potentials and/or preferred comformations of the molecule and this result in an increase of AA activity, although the mechanisms of action on AA activity are complex[7] and are far from being fully elucidated.

Subsequently, we further evaluated the compounds which were effective on the AA assay; on AA activity by oral-administration and/or actue toxicity in mice and/or ALP activity and/or arachidonate-induced cerebral edema in rats.

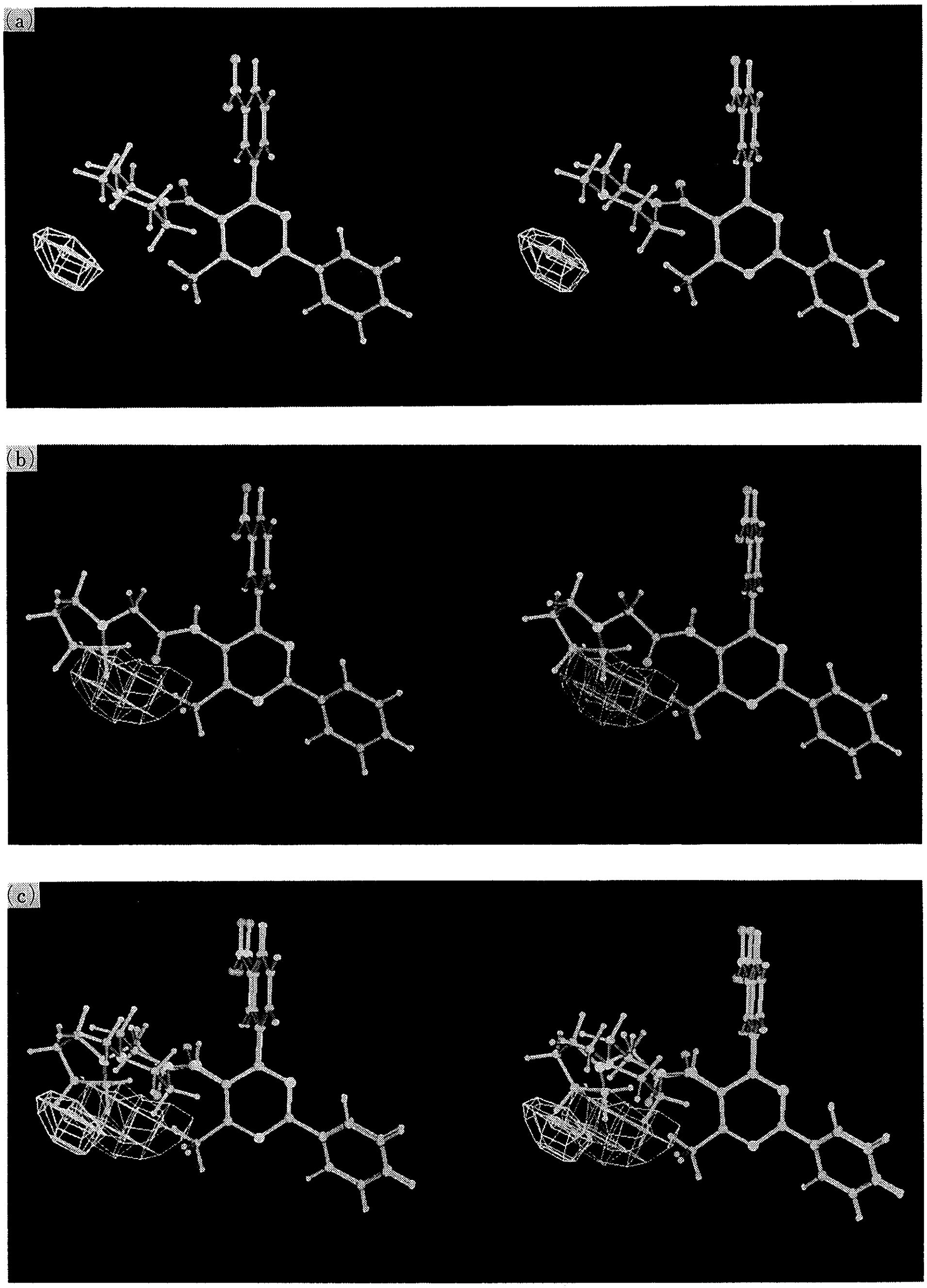


Fig. 3. (a) Stereo View of MEP Study around the Nitrogeneous Basic Moiety of FK 360, (b) Stereo View of MEP Study around the Nitrogeneous Basic Moiety of **10f**, (c) Superimposition of (a) and (b)

a: The contour denotes potential surface of −4 kcal/mol. b: The contour denotes potential surface of −10 kcal/mol.

Compounds **10b**, **10d**, **10e**, **10g** and **11g** were not effective on AA activity by oral-administration. Compounds **11f** and **13** were effective on AA activity by oral-administration. However, **11f** was more toxic ($LD_{50} < 320$ mg/kg, i.p.) than FK 360 ($LD_{50} > 560$ mg/kg, i.p.); **13** had low acute toxicity ($LD_{50} > 560$ mg/kg, i.p.), although observed sedation at 560 mg/kg, i.p. (details of these results are not presented here). The results of **10f** and **11b** are listed in Table III.

Compound **10f** was the most potent AA agent in our synthesized compounds, however, disappointingly, was not so effective on arachidonate-induced cerebral edema assay.

On the other hand, compound **11b** had a comparable potency on the AA assay to that of FK 360, however, this proved not to be superior on ALP activity and arachidonate-induced cerebral edema in rats to those of FK 360.

Introduction of a morpholinoacetylamino group in the C-2 (or C-6) position of the pyridine (or pyrimidine) nucleus, due to the fact that **11b** had potent AA activity and low acute toxicity, however, resulted in abolishment of AA activity (Table IV).

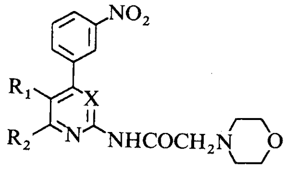
In conclusion, i) three-atom linkages between basic nitrogen and the pyrimidine (or pyridine) nucleus also seems to be a prerequisite for the expression of AA activity. ii) These pharmacological results and 3D-MEP study may be used, along with a model of the geometrical features of the recognition site for the expression of AA activity, to provide

Table III. Pharmacological Data of 4-(3-Nitrophenyl)-2-phenylpyrimidine Derivatives (**10f**, **11b**, FK 360)

Compound No.	Anti-anoxia (% of control) (mg/kg)			Upper: i.p. Down: *p.o.*	Lipid peroxidation IC_{50} (M)	Arachidonate-induced[a] cerebral edema ED_{50} (mg/kg)		Acute toxicity[b] LD_{50} (mg/kg, i.p.)
	3.2	10	32	100		i.p.	*p.o.*	
10f	115[c]	126[d]	172[e]	NT	9.8×10^{-6}	>100	NT	>210
	114[d]	119[d]	128[d]	155[c]				
11b		111[c]	134[d]	140[d]	6.0×10^{-5}	>100	NT	>560
			109[c]	118[e]				
Cf. FK 360		104	126[e]	168[e]	6.7×10^{-6}	18	32	>560
			114	125[e]				

a) The experiments were conducted using each group of 5 animals. The dose required to produce 50% of maximum inhibition produced by the test drug, was determined from log-probit plots of the individual. *b*) Male ICR mice weighing 25—35 g were used in groups of 5—10 animals for each test drug. The LD_{50} value was calculated from the lethality within 7 d after an intraperitoneal administration of a test compound. *c*) $p<0.05$. *d*) $p<0.001$. *e*) $p<0.01$. NT, not tested.

Table IV. Physical Properties and AA Activity of 4-(3-Nitrophenyl)-pyridine(or pyrimidine) Derivatives (**20**, **24**, **28**, **33**)



Compound No.	R_1	R_2	X	Anti-anoxia[a] (% of control) (mg/kg, i.p.)		Yield (%)	mp (°C) (Recryst. solv.)	Formula	Analysis (%) Calcd (Found)		
				10	32				C	H	N
20	CO_2Et	Me	CH		116	82.8	163—165	$C_{21}H_{24}N_4O_6$	58.87	5.65	13.08
							($EtOH-Et_2O$)		(59.30	5.69	13.01)
24	H	Me	N		117	28.4	206—208	$C_{17}H_{19}N_5O_4$	57.14	5.36	19.60
							($EtOH-Et_2O$)		(57.22	5.20	19.45)
28	CO_2Et	H	N		114[b]	95.3	108—110	$C_{19}H_{21}N_5O_6$	54.94	5.10	16.86
							($EtOH-Et_2O$)		(55.04	4.98	16.80)
33	Me	H	N		111	83.9	192—194	$C_{17}H_{19}N_5O_4$	55.73	5.50	19.12
							($EtOH-Et_2O$)	$\cdot 0.5H_2O$	(55.91	5.22	18.86)

a) See footnote *a*) in Table I. *b*) See footnote *b*) in Table I.

information for the design of AA agents.

Experimental

Melting points were determined using a Thomas–Hoover capillary melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on a Varian EM-390 NMR (90 MHz) or a Hitachi R90-H NMR (90 MHz) or a Bruker AC-200P (200 MHz) using tetramethylsilane (TMS) as an internal standard. IR spectra were recorded on a Hitachi 260-10 or Shimadzu IR-420 spectrophotometer. Mass spectral measurements (MS) were made on a Hitachi M-80 or a JEOL-D300 mass spectrometer.

3-*tert*-Butyloxycarbonylamino-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine (3) A mixture of **2** (10.7 g, Et_3N (3.3 g) and DPPA (8.98 g) in *tert*-BuOH (100 ml) was refluxed for 14 h. After evaporating the solvent, the residue was dissolved in a mixture of $CHCl_3$ (100 ml) and saturated $NaHCO_3$ aq. (100 ml) with stirring. The organic layer was successively washed with H_2O and brine, and dried over $MgSO_4$. The filtrate was evaporated *in vacuo* and the residue was purified by column chromatography on silica gel (SiO_2) (250 g) with $CHCl_3$ as eluent. The fractions containing **3** were combined and evaporated *in vacuo*. The residue was recrystallized from Et_2O to afford **3** (9.64 g, 74.3%), mp 153 °C (dec.). IR (Nujol): 3250, 1680, 1520, 1340 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (9H, s), 2.66 (3H, s), 5.97 (1H, s), 7.30—8.45 (9H, m), 7.54 (1H, s). MS *m/z*: 405 (M^+). *Anal.* Calcd for $C_{23}H_{23}N_3O_4$: C, 68.13; H, 5.71; N, 10.36. Found: C, 68.08; H, 5.76; N, 10.22.

3-Amino-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine (6) A mixture of **3** (9.6 g) and concentrated HCl aq. (5.0 ml) in EtOH (96 ml) was refluxed for 1.5 h. After evaporating the solvent, the residue was dissolved in a mixture of $CHCl_3$ (100 ml) and H_2O (100 ml). The mixture was adjusted to pH 10.0 with 4 N NaOH aq., and the organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was recrystallized from Et_2O to afford **6** (5.17 g, 71.5%), mp 155—157 °C. IR (Nujol): 3460, 3370, 3350, 1620, 1520, 1350 cm^{-1}. MS *m/z*: 305 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 2.58 (3H, s), 3.74 (2H, br s), 7.20—8.50 (9H, m), 7.33 (1H, s). *Anal.* Calcd for $C_{18}H_{15}N_3O_2 \cdot 0.2H_2O$: C, 69.98; H, 5.02; N, 13.60. Found: C, 70.22; H, 5.07; N, 13.40.

3-(Bromoacetylamino)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine (7) To a mixture of **6** (1.0 g) and pyridine (0.3 g) in toluene (10 ml) was added dropwise a solution of bromoacetyl bromide (0.7 g) in toluene (2 ml) under ice cooling. After stirring for 7 h at room temperature, the reaction mixture was poured into a mixture of AcOEt (50 ml) and ice-water (20 ml). The organic layer was successively washed with saturated $NaHCO_3$ aq. and brine, and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was pulverized from Et_2O to afford **7** (1.15 g, 82.1%), mp 198—200 °C. IR (Nujol): 3240, 1650, 1530, 1350 cm^{-1}. MS *m/z*: 425 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 2.53 (3H, s), 3.91 (2H, s), 7.40—8.46 (9H, m), 7.93 (1H, s). *Anal.* Calcd for $C_{20}H_{16}BrN_3O_3$: C, 56.35; H, 3.78; N, 9.85. Found: C, 56.44; H, 3.70; N, 9.93. The following compounds were obtained in a manner similar to **7**.

5-Bromoacetylamino-6-methyl-4-(3-nitrophenyl)-2-phenylpyrimidine (**5**): Yield 68.7%, mp 206—209 °C (dec.). IR (Nujol): 3220, 1660, 1520, 1345 cm^{-1}. MS *m/z*: 426 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 2.57 (3H, s), 4.02 (2H, s), 7.45—8.80 (10H, m). *Anal.* Calcd for $C_{19}H_{15}BrN_4O_3$: C, 53.41; H, 3.53; N, 13.11. Found: C, 53.28; H, 3.58; N, 13.01.

3-(3-Bromopropionylamino)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine (**8**): Yield 50.0%, mp 198—200 °C. IR (Nujol): 3230, 1650, 1530, 1350 cm^{-1}. MS *m/z*: 439 (M$^+$). ^{1}H-NMR (DMSO-d_6) δ: 2.54 (3H, s), 2.56—3.10 (2H, m), 7.35—8.50 (9H, m), 7.88 (1H, s), 9.81 (1H, s).

3-(2-Bromopropionylamino)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine (**9**): Yield 68.8%, mp 206—208 °C. IR (Nujol): 3200, 1645, 1525, 1345 cm^{-1}. MS *m/z*: 439 (M$^+$). ^{1}H-NMR (CDCl$_3$) δ: 1.76 (3H, d, J=7 Hz), 2.61 (3H, s), 4.40 (1H, q, J=7 Hz), 7.30—8.40 (9H, m), 7.74 (1H, s). *Anal.* Calcd for $C_{21}H_{18}BrN_3O_3$: C, 57.29; H, 4.12; N, 9.54. Found: C, 57.29; H, 4.02; N, 9.38.

Ethyl 2-Bromoacetylamino-6-methyl-4-(3-nitrophenyl)-5-pyridinecarboxylate (**19**): Yield 70.0%, mp 199 °C (dec.). IR (Nujol): 3300, 1680, 1570, 1550, 1520, 1340 cm^{-1}. MS *m/z*: 421 (M$^+$). ^{1}H-NMR (CDCl$_3$) δ: 1.07 (3H, t, J=7 Hz), 2.58 (3H, s), 4.04 (2H, s), 4.15 (2H, q, J=7 Hz), 7.50—7.93 (2H, m), 8.08 (1H, s), 8.10—8.50 (2H, m), 8.82 (1H, br s).

Ethyl 2-Bromoacetylamino-4-(3-nitrophenyl)-5-pyrimidinecarboxylate (**27**): Yield 31.7%, mp 142—144 °C. IR (Nujol): 1710, 1690, 1355 cm^{-1}. MS *m/z*: 408 (M$^+$). ^{1}H-NMR (CDCl$_3$) δ: 1.20 (3H, t, J=7 Hz), 4.23 (2H, q, J=7 Hz), 4.46 (2H, s), 7.53—8.01 (3H, m), 7.26—7.39 (2H, m), 9.23 (1H, s), 9.40 (1H, s).

2-Bromoacetylamino-5-methyl-4-(3-nitrophenyl)pyrimidine (**32**): Yield 42.8%, mp 167 °C (dec.). IR (Nujol): 1680, 1355 cm^{-1}. MS *m/z*: 350 (M$^+$). ^{1}H-NMR (CDCl$_3$) δ: 2.42 (3H, s), 4.40 (2H, s), 7.60—8.55 (4H, m), 8.60 (1H, s), 8.90 (1H, s). *Anal.* Calcd for $C_{13}H_{11}BrN_4O_3$: C, 44.46; H, 3.15; N, 15.95. Found: C, 44.58; H, 3.26; N, 15.96. Compounds **8**, **19** and **27** were not further purified or analyzed before use in the next step.

Typical Example for the Preparation of (10a—g, 11a—i, 20, 24, 28, 33) 2-Methyl-3-(morphorinoacetylamino)-4-(3-nitrophenyl)-6-phenylpyridine (**11b**): A mixture of **7** (0.50 g) and morpholine (0.25 g) in CH$_2$Cl$_2$ (5 ml) was stirred for 8 h at room temperature. The reaction mixture was poured into a mixture of CH$_2$Cl$_2$ (5 ml) and H$_2$O (10 ml). The organic layer was successively washed with saturated NaHCO$_3$ aq. and brine, dried over MgSO$_4$ and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (SiO$_2$) (25 g) with CHCl$_3$–MeOH (30 : 1) as eluent. The fractions containing **11b** were combined and evaporated *in vacuo*. The residue was recrystallized from EtOH to afford **11b** (415 mg, 81.4%). Compounds **11a**, **11c—i**, **10a—g**, **20**, **24**, **28** and **33** were prepared by the same procedures employed in the preparation of **11b** and these physical data are listed in Tables I, II, IV and V.

3-(*N-tert*-Butyloxycarbonyl-*N*-2-morphorinoethylamino)-2-methyl-4-(3-nitrophenyl)-6-phenylpyridine (12) To a solution of **3** (0.50 g) in *N*,*N*-dimethylformamide (DMF) (5 ml) was added NaH (0.07 g, 60% in mineral oil) and the whole was stirred for 30 min at room temperature. To this mixture *N*-(2-chloroethyl)morpholine hydrochloride (0.28 g) was added and the whole was stirred for 4.5 h at 55 °C. The reaction mixture was poured into a mixture of AcOEt (50 ml) and H$_2$O (50 ml). The organic layer was washed with brine, dried over MgSO$_4$ and evaporated *in vacuo*. The residue was purified by column chromatography on SiO$_2$ (15 g) with

TABLE V. Spectral Data of 4-(3-Nitrophenyl)pyridine and 4-(3-Nitrophenyl)pyrimidine Derivatives (**10a—g**, **11a—i**, **13**, **20**, **24**, **28**, **33**)

Compd. No.	MS *m/z*, M$^+$	IR (Nujol) cm^{-1}	Solvent[a]	^{1}H-NMR (ppm)[b]
10a	391	3290, 1690, 1530, 1350	A	2.32 (6H, s), 2.62 (3H, s), 3.05 (2H, s), 7.35—8.73 (9H, m), 8.80—9.30 (1H, br s)
10b	433	3330, 1700, 1525, 1350	A	2.33—2.80 (4H, m), 2.60 (3H, s), 3.10 (2H, s), 3.50—3.90 (4H, m), 7.35—8.62 (9H, m), 8.90 (1H, s)
10c	446	3330, 1690, 1520, 1350	A	2.20—2.84 (8H, m), 2.27 (3H, s), 2.60 (3H, s), 3.10 (2H, s), 7.33—8.70 (9H, m), 8.98 (1H, s)
10d	449	3200, 1675, 1520, 1340	A	2.47—3.05 (8H, m), 2.58 (3H, s), 3.10 (2H, s), 7.35—8.72 (9H, m), 8.86 (1H, s)
10e	431	3320, 1680, 1520, 1350	A	1.28—1.78 (6H, m), 2.25—2.72 (4H, m), 2.50 (3H, s), 3.02 (2H, s), 7.35—8.70 (9H, m), 8.90 (1H, s)
10f	417	3200, 1680, 1655, 1525	B	1.67—2.13 (4H, m), 2.63 (3H, s), 3.00—3.40 (4H, m), 4.27 (2H, s), 7.40—8.63 (9H, m), 10.50 (1H, s), 11.18 (1H, s)
10g	497	1710, 1525, 1350	B	1.30—2.40 (15H, m), 2.66 (3H, s), 3.97 (2H, br), 7.40—8.80 (9H, m), 8.90—9.40 (2H, br)
11a	390	3270, 1670, 1520, 1350	A	2.23 (6H, s), 2.63 (3H, s), 2.92 (2H, s), 7.25—8.35 (9H, m), 7.50 (1H, s), 8.75 (1H, s)
11b	432	3330, 1690, 1520, 1355	A	2.30—2.60 (4H, m), 2.62 (3H, s), 2.97 (2H, s), 3.45—3.72 (4H, m), 7.25—8.30 (9H, m), 7.50 (1H, s), 8.66 (1H, s)
11c	445	1660, 1520, 1350	A	2.10—2.75 (8H, m), 2.26 (3H, s), 2.63 (3H, s), 2.98 (2H, s), 7.20—8.35 (9H, m), 7.54 (1H, s), 8.76 (1H, s)
11d	448	3350, 1700, 1630, 1375	B	2.63 (3H, s), 2.65—3.65 (8H, m), 4.15 (2H, s), 7.35—8.45 (9H, m), 7.96 (1H, s), 11.05 (1H, s)
11e	430	3340, 1680, 1520, 1345	A	1.25—1.90 (6H, m), 2.20—2.65 (4H, m), 2.64 (3H, s), 2.92 (2H, s), 7.35—8.45 (9H, m), 7.55 (1H, s), 8.95 (1H, s)
11f	416	3250, 1665, 1520, 1350	A	1.56—1.98 (4H, m), 2.36—2.76 (4H, m), 2.65 (3H, s), 3.16 (2H, s), 7.35—8.44 (10H, m), 8.77 (1H, s)
11g	496	3250, 1660, 1525, 1350	A	1.20—2.20 (15H, m), 2.63 (3H, s), 3.24 (2H, s), 7.25—8.40 (9H, m), 7.56 (1H, s), 9.32 (1H, s)
11h	446	3240, 1630, 1520, 1345	A	2.20—2.85 (8H, m), 2.64 (3H, s), 3.40—3.80 (4H, m), 7.35—8.43 (9H, m), 7.58 (1H, s), 10.35 (1H, s)
11i	446	3325, 1690, 1525, 1340	A	1.13 (3H, d, J=7 Hz), 2.34—2.66 (4H, m), 2.63 (3H, s), 3.00 (1H, q, J=7 Hz), 3.50—3.85 (4H, m), 7.40—8.53 (9H, m), 7.57 (1H, s), 8.79 (1H, s)
13	418	3350, 1520, 1350	A	2.15—2.55 (6H, m), 2.45—2.80 (2H, m), 2.68 (3H, m), 3.46—3.80 (4H, m), 7.20—8.50 (9H, m), 7.37 (1H, s)
20	428	3310, 1710, 1580, 1345	A	1.06 (3H, t, J=7 Hz), 2.50—2.83 (4H, m), 2.62 (3H, s), 3.21 (2H, s), 3.70—4.10 (4H, m), 4.16 (2H, q, J=7 Hz), 7.45—7.90 (2H, m), 8.10—8.47 (2H, m), 8.18 (1H, s), 9.63 (1H, br)
24	357	3260, 1720, 1525, 1340	A	2.63 (3H, s), 2.55—2.80 (4H, m), 3.27 (2H, s), 3.70—4.00 (4H, m), 7.38 (1H, s), 7.53—7.80 (1H, m), 8.20—8.60 (2H, m), 8.75—8.93 (1H, m), 9.65 (1H, br s)
28	415	1710, 1690, 1350	A	1.26 (3H, t, J=7 Hz), 2.63—2.83 (4H, m), 3.33 (2H, s), 3.79—3.96 (4H, m), 4.36 (2H, q, J=7 Hz), 7.59—8.13 (3H, m), 8.33—8.60 (2H, m), 9.30 (1H, s), 10.03 (1H, s)
33	357	1690, 1350	B	2.40 (3H, s), 2.50—2.70 (4H, m), 3.34 (3H, s), 3.58—3.78 (4H, m), 7.72—8.72 (5H, m), 10.20 (1H, s)

a) A, CDCl$_3$; B, DMDO-d_6. *b*) Listed as chemical shifts (number of protons, multiplicity, constant).

$CHCl_3$ as eluent. The fractions containing **12** were combined and evaporated *in vacuo* to afford **12** (0.60 g, 93.8%). IR (film): 1670, 1530, 1350 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.51 (9H, s), 2.00—2.80 (6H, m), 2.66 (3H, s), 3.35—3.80 (6H, m), 7.25—8.40 (10H, m). This compound was not further purified or analyzed before use in the next step.

2-Methyl-3-(2-morpholinoethylamino)-4-(3-nitrophenyl)-6-phenylpyridine (13) A mixture of **12** (0.60 g) and concentrated HCl aq. (0.3 ml) in EtOH (6 ml) was refluxed for 2 h. After evaporating the solvent, the residue was dissolved in a mixture of $CHCl_3$ (10 ml) and H_2O (10 ml). The mixture was adjusted to pH 10.0 with 4 N NaOH aq. and the organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was recrystallized from Et_2O to afford **13** (0.32 g, 66.1%). Physical data are listed in Tables II and V.

Ethyl 1,4-Dihydro-2-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-5-pyridinecarboxylate (15) A mixture of methyl 4-(3-nitrophenyl)-2-oxo-3-butenoate (**14**) (4.25 g), ethyl 3-aminocrotonate (2.57 g) in *tert*-BuOH (9 ml) was stirred for 1 h at 80 °C. After being cooled to room temperature, the reaction mixture was poured into Et_2O with stirring. The resulting precipitate was collected by filtration, washed with Et_2O and recrystallized from EtOH to afford **15** (4.62 g, 73.8%), mp 124—125 °C. IR (Nujol): 3370, 1710, 1690, 1590, 1520, 1340 cm^{-1}. MS *m/z*: 346 (M$^+$). ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.02 (3H, t, J=7.1 Hz), 2.38 (3H, s), 3.74 (3H, s), 3.92 (2H, q, J=7.1 Hz), 4.80 (1H, d, J=6.0 Hz), 5.95 (1H, dd, J=6.0, 1.8 Hz), 7.56—7.70 (2H, m), 8.00—8.10 (2H, m), 8.51 (1H, s). *Anal.* Calcd for $C_{17}H_{18}BrN_2O_6$: C, 58.95; H, 5.23; N, 8.08. Found: C, 58.55; H, 5.15; N, 8.08.

Ethyl 2-Methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-5-pyridinecarboxylate (16) To a solution of **15** (4.57 g) in $CHCl_3$ (40 ml) was added activated MnO_2 (18.3 g), and the whole was refluxed for 1 h with vigorous stirring. After being cooled to room temperature, the MnO_2 was filtered off. The filtrate was evaporated *in vacuo*, and the residue was recrystallized from EtOH–Et_2O to afford **16** (3.94 g, 86.7%), mp 127—129 °C. IR (Nujol): 1740, 1725, 1575, 1520, 1350 cm^{-1}. MS *m/z*: 344 (M$^+$). ^{1}H-NMR ($CDCl_3$) δ: 1.13 (3H, t, J=7 Hz), 2.76 (3H, s), 4.05 (3H, s), 4.24 (2H, q, J=7 Hz), 7.55—7.90 (2H, m), 8.06 (1H, s), 8.25—8.50 (2H, m). *Anal.* Calcd for $C_{17}H_{16}N_2O_6$: C, 59.30; H, 4.68; N, 8.13. Found: C, 59.64; H, 4.58; N, 8.14.

5-Ethoxycarbonyl-6-methyl-4-(3-nitrophenyl)-2-pyridinecarboxylic Acid (17) A mixture of **16** (3.85 g) and NaOH (0.49 g) in a mixture of H_2O (20 ml) and EtOH (40 ml) was heated at 40 °C for 1 h with stirring. After being cooled to room temperature, the solvent was concentrated to half volume, added H_2O (30 ml), adjusted to pH 2.0 with 10% HCl aq. and extracted with AcOEt (40 ml × 2). The organic layer was washed with brine, dried over $MgSO_4$, and the solvent was evaporated *in vacuo*. The residue was recrystallized from EtOH–Et_2O to afford **17** (3.47 g, 93.8%), mp 126—128 °C. IR (Nujol): 1730, 1700, 1580, 1530, 1345 cm^{-1}. MS *m/z*: 330 (M$^+$). ^{1}H-NMR ($CDCl_3$) δ: 1.15 (3H, t, J=7 Hz), 2.75 (3H, s), 4.26 (2H, q, J=7 Hz), 7.60—7.95 (2H, m), 8.16 (1H, s), 8.23—8.65 (2H, m). *Anal.* Calcd for $C_{16}H_{14}N_2O_6$: C, 58.19; H, 4.27; N, 8.48. Found: C, 58.60; H, 4.27; N, 8.46.

Ethyl 6-Amino-2-methyl-4-(3-nitrophenyl)-3-pyridinecarboxylate (18) A mixture of **17** (3.40 g), Et_3N (1.15 g) and DPPA (3.12 g) in *tert*-BuOH (34 ml) was refluxed for 4.5 h. After evaporating the solvent, the residue was dissolved in a mixture of $CHCl_3$ (50 ml) and saturated $NaHCO_3$ aq. (50 ml) with stirring. The organic layer was successively washed with H_2O and brine, and dried over $MgSO_4$. The filtrate was evaporated *in vacuo* and to the residue was added EtOH (40 ml) and concentrated HCl aq. (2.2 ml), and the whole was refluxed for 2.5 h. After evaporating the solvent, the residue was dissolved in a mixture of $CHCl_3$ (50 ml) and H_2O (50 ml) and the mixture was adjusted to pH 10 with 4 N NaOH aq. The organic layer was washed with brine, dried over $MgSO_4$, and the solvent was evaporated *in vacuo*. The residue was recrystallized from EtOH–Et_2O to afford **18** (2.15 g, 69.4%), mp 143—145 °C. IR (Nujol): 3450, 3300, 3170, 1705, 1640, 1595, 1520, 1350 cm^{-1}. MS *m/z*: 301 (M$^+$). ^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, J=7 Hz), 2.54 (3H, s), 4.06 (2H, q, J=7 Hz), 4.01 (2H, br s), 6.31 (1H, s), 7.40—7.80 (2H, m), 8.06—8.47 (2H, m). *Anal.* Calcd for $C_{15}H_{15}N_3O_4$: C, 59.79; H, 5.02; N, 13.94. Found: C, 59.59; H, 4.80; N, 13.91.

2-Amino-6-methyl-4-(3-nitrophenyl)pyrimidine (23) To a solution of Na (0.1 g) in absolute EtOH (10 ml), guanidine hydrochloride (0.41 g) was added at room temperature. After being stirred for 20 min, NaCl was filtered off and a solution of **21**[1d] (1.0 g) in EtOH (10 ml) was added dropwise and then the whole was refluxed for 2 h. After being cooled to room temperature, the solvent was evaporated *in vacuo*. The residue was diluted with $CHCl_3$ (20 ml), washed with brine, and dried over $MgSO_4$. After filtration, to the filtrate was added activated MnO_2 (5.0 g) and it was refluxed for 1 h with vigorous stirring. After cooling, the MnO_2 was filtered off and the filtrate was evaporated *in vacuo*. The residue was pulverized from Et_2O to afford **23** (0.27 g, 27.4%), mp 199—200 °C. IR (Nujol): 3490, 3280, 3130, 1620, 1570, 1540, 1515, 1345 cm^{-1}. MS *m/z*: 230 (M$^+$). ^{1}H-NMR (DMSO-d_6) δ: 2.36 (3H, s), 6.73 (2H, s), 7.20 (1H, s), 7.65—7.93 (1H, m), 8.17—8.60 (2H, m), 8.80—9.00 (1H, m). *Anal.* Calcd for $C_{11}H_{10}N_4O_2$: C, 57.39; H, 4.38; N, 24.34. Found: C, 57.79; H, 4.27; N, 24.25.

Ethyl 2-Amino-4-(3-nitrophenyl)-5-pyrimidinecarboxylate (26) To a mixture of **25**[1c] (0.5 g) and guanidine hydrochloride (0.5 g) in EtOH (5 ml) was added Et_3N (0.75 ml), and the solution was refluxed for 5 h. After being cooled to room temperature, the solvent was evaporated *in vacuo*. The residue was diluted with AcOEt (50 ml), successively washed with saturated $NaHCO_3$ aq., H_2O and brine, and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was pulverized from Et_2O to afford **26** (0.25 g, 51.0%), mp 153—154 °C. IR (Nujol): 3425, 3300, 1705, 1650, 1350 cm^{-1}. MS *m/z*: 288 (M$^+$). ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7 Hz), 4.26 (2H, q, J=7 Hz), 6.00 (2H, s), 7.53—8.00 (2H, m), 8.26—8.50 (2H, m), 9.01 (1H, s). *Anal.* Calcd for $C_{13}H_{12}N_4O_4$: C, 54.16; H, 4.19; N, 19.43. Found: C, 54.25; H, 4.09; N, 19.13.

2-Hydroxymethylene-1-(3-nitrophenyl)propan-1-one (30) A mixture of **29** (60.0 g), ethyl formate (120 ml) and DBU (200 ml) was heated at 70 °C for 24 h. After being cooled to room temperature, the reaction mixture was poured into a mixture of ice-water (800 ml) and AcOEt (300 ml). The aqueous layer was washed with AcOEt (300 ml) and adjusted to pH 2.0 with concentrated HCl aq. The precipitated material was collected by filtration, washed with H_2O and dried *in vacuo* to afford **30** (33.8 g, 49.5%), mp 130—131 °C. IR (Nujol): 1650, 1530, 1220, 720 cm^{-1}. MS *m/z*: 207 (M$^+$). ^{1}H-NMR (DMSO-d_6) δ: 1.77 (3H, s), 7.41 (1H, s), 7.74 (1H, dd, J=8, 8 Hz), 7.93 (1H, dd, J=8, 2 Hz), 8.20 (1H, dd, J=2, 2 Hz), 8.33 (1H, dd, J=8, 2 Hz). *Anal.* Calcd for $C_{10}H_9NO_4$: C, 57.97; H, 4.35; N, 6.79. Found: C, 58.36; H, 4.36; N, 6.82.

2-Amino-4-(3-nitrophenyl)-5-methylpyrimidine (31) To a mixture of **30** (3.0 g) and guanidine carbonate (3.30 g) in dimethyl sulfoxide (DMSO, 30 ml) was added acetic acid (2.6 ml), and the solution was stirred for 4 h at 80 °C. After being cooled to room temperature, the solution was poured into a mixture of AcOEt (50 ml) and saturated $NaHCO_3$ aq. (50 ml). The organic layer was successively washed with water and brine, dried over $MgSO_4$. The solvent was evaporated *in vacuo*, and the residue was pulverized from Et_2O to afford **31** (1.67 g, 49.3%), mp 172—175 °C. IR (Nujol): 3440, 3280, 1630, 1345 cm^{-1}. MS *m/z*: 230 (M$^+$). ^{1}H-NMR ($CDCl_3$) δ: 2.26 (3H, s), 5.26 (2H, s), 7.69—8.56 (5H, m). *Anal.* Calcd for $C_{11}H_{10}N_4O_2$: C, 57.38; H, 4.37; N, 24.33: Found: C, 57.64: H, 4.26; N, 24.31.

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References and Notes

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