Studies on Aromatase Inhibitors IV.[1] Synthesis and Biological Evaluation of *N,N*-Disubstituted-5-aminopyrimidine Derivatives

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In order to study the potency of the 5-aminopyrimidine skeleton as an aromatase inhibitor, we synthesized various *N,N*-disubstituted-5-aminopyrimidine derivatives and evaluated their aromatase-inhibitory activity (*in vitro*) and their inhibitory activity on pregnant mare serum gonadotropin (PMSG)-induced estrogen synthesis (*in vivo*). Compounds with the fluoro-substituted benzyl group showed potent aromatase inhibition. Among them, 5-[(4-cyanophenyl)(3,5-difluorobenzyl)amino]pyrimidine (5w, YM553) was a highly potent compound with an IC_{50} value of 0.038 nM for aromatase from human placenta. Its inhibitory effect was approximately four times greater than that of YM511. In addition, YM553 was a weak inhibitor of other enzymes involved in steroid hormone synthesis. These results indicate that YM553, as well as YM511 (a 4-amino-4*H*-1,2,4-triazole derivative), is a promising agent for the treatment of estrogen-dependent diseases.

Key words aromatase; estrogen; 5-aminopyrimidine; steroid; aromatase inhibition

Breast carcinoma is one of the most common malignancies in women and its incidence is tending to increase throughout the world.[2] About 30—50% of breast cancers are considered to be estrogen-dependent.[2,3] Therefore, the inhibition of estrogen biosynthesis using an aromatase inhibitor is expected to be an effective therapy for breast cancer.[4,5] It is important to develop inhibitors with high selectivity for aromatase, because other steroidogenic enzymes, such as 11-β-hydroxylase, 21-hydroxylase and 18-hydroxylase, are also cytochrome P-450 enzymes like aromatase.[6] In recent years, several classes of aromatase inhibitors have been reported, and they can be classified into two groups based on their structure. One is substrate-based steroidal inhibitors,[7-11] and the other is non-steroidal inhibitors with an aza-hetero ring containing an sp^2 nitrogen atom that binds to the heme iron atom of aromatase.[12-20] Although many non-steroidal compounds exhibit potent aromatase inhibition, they often lack selectivity due to their mode of action.[21-23]

Previously, we reported[1a] that 4*H*-1,2,4-triazol-4-ylamino derivatives were potent non-steroidal aromatase inhibitors, and YM511, having the (4-bromobenzyl)(4-cyanophenyl)amino group as part of the side chain, showed the most potent and selective inhibition for aromatase among the compounds examined. In our continuing efforts[1c] to find more potent non-steroidal aromatase inhibitors, we have evaluated a number of other aza-heterocyclic compounds with the (4-bromobenzyl)(4-cyanophenyl)amino moiety in the side chain. Among them, the 5-aminopyrimidine derivative **1** inhibited aromatase to the extent of 85.4% at a concentration of 1 nM in the *in vitro* rat microsome assay and was a weak inhibitor of aldosterone synthesis. This suggests that 5-aminopyrimidine is available as a new azole moiety instead of the 4*H*-1,2,4-triazole-4-ylamino group. Therefore, we synthesized a series of *N,N*-disubstituted-5-aminopyrimidine derivatives by modifying the side chain of **1** with the aim of obtaining more potent and selective aromatase inhibitors (Chart 1). Among these derivatives, the difluoro-substituted compound, 5-[(4-cyanophenyl)(3,5-difluorobenzyl)amino]pyrimidine (**5w**, YM553) was a highly potent aromatase inhibitor and was also a weak inhibitor of other enzymes involved in steroid hormone synthesis. In addition, its inhibitory efficacy against human placental aromatase (IC_{50} = 0.038 nM) was four times more potent than that of YM511. We report here the synthesis and structure–activity relationships (SARs) of the *N,N*-disubstituted aminopyrimidine derivatives.

Chemistry

The synthetic procedures for the *N,N*-disubstituted aminopyrimidine derivatives (**4b—e** and **5a—y**) are shown in Chart 2. These derivatives were prepared by two-step reactions from 5-aminopyrimidine **2**.[24] The *para*-substituted phenylaminopyrimidine derivatives **3a—e** were obtained by the *ipso*-substitution reaction of the cor-

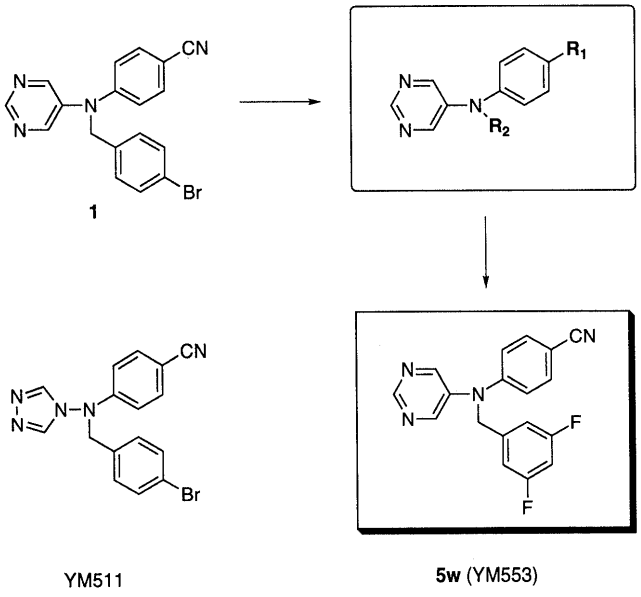

Chart 1

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responding fluorophenyl derivatives with 5-aminopyrimidine **2** in the presence of potassium *tert*-butoxide in dimethyl sulfoxide (DMSO). Compounds **3b—e** were reacted with *para*-bromobenzyl bromide using sodium hydride as a base to afford the *para*-bromobenzylaminopyrimidine derivatives **4b—e**. Other *N*,*N*-disubstituted amino derivatives **5a—y** were also synthesized by the reaction of **3a** with substituted phenyl or benzyl halides in a similar manner. In the case of **5c**, the intermediate 5-fluorobenzofurazane **7** was prepared by reduction of the commercially available *N*-oxide **6**.

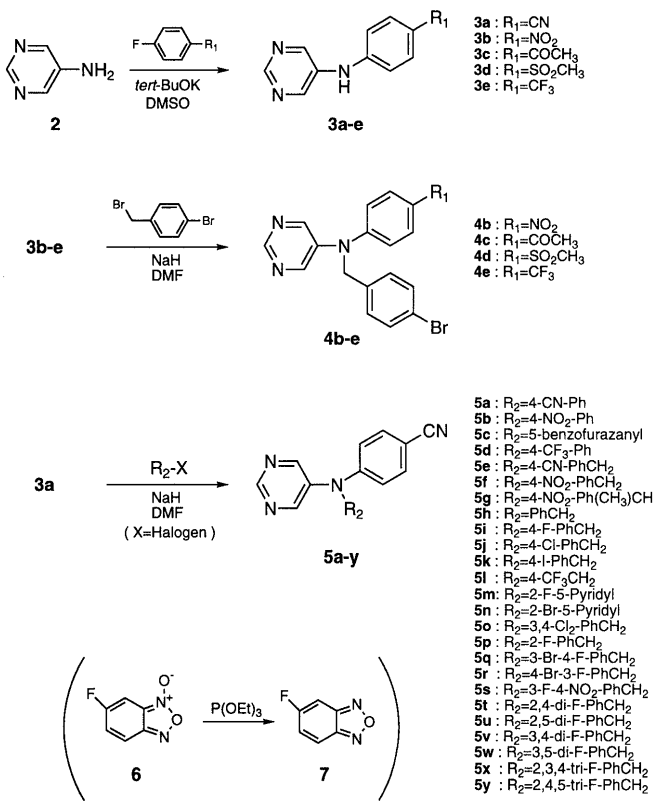


Chart 2

Results and Discussion

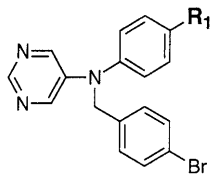
Inhibitory activities of the series of 5-aminopyrimidine derivatives on aromatase (*in vitro*) and pregnant mare serum gonadotropin (PMSG)-induced estrogen synthesis (*in vivo*) were evaluated. In the *in vitro* rat ovarian or human placental microsome assay, aromatase-inhibitory activity of the compounds at concentrations of 1 and 10 nM (rat ovary) or 0.3 and 1 nM (human placenta) was expressed as percent inhibition of the aromatization of androstenedione. In the rat *in vivo* assay, the estrogen synthesis-inhibitory activity of the compounds at the dosages of 0.03 and 0.3 mg/kg *p.o.* was expressed as percent inhibition of PMSG-induced estrogen synthesis.

The pharmacological data for the synthesized compounds are summarized in Tables 1—4.

First of all, the R_1 substituent on the phenyl ring was evaluated (Table 1). We introduced various kinds of electron-withdrawing substituents, such as a nitro (**4b**), acetyl (**4c**), sulfonyl (**4d**) or trifluoromethyl (**4e**) group, on the R_1 moiety because a strong electron-withdrawing group on the R_1 moiety was indispensable for high aromatase-inhibitory activity in the 4-amino-4*H*-1,2,4-triazole series.[1a] These derivatives, except **4e**, all exhibited potent inhibitory activities *in vitro*, and **4d** with the bulky methylsulfonyl substituent compared with the cyano substituent **1** also showed high activity (68.1% at 1 nM), suggesting that the steric factor at the R_1 moiety is not very important for aromatase inhibition. As regards estrogen synthesis (*in vivo*), however, only the nitro derivative **4b** was inhibitory, though its inhibitory efficacy was weaker than that of the cyano derivative **1**.

In the next step, the R_1 substituent was fixed as the cyano group and the R_2 substituent was examined (Table 2). The *para*-substituted phenyl derivatives tended to show decreased activity compared to the corresponding benzyl derivatives (**5b** *vs.* **5f**, **5d** *vs.* **5l**), especially in the case of the trifluoromethyl group (**5d**); its *in vivo* activity was over ten times less potent than that of the benzyl analog (**5l**). The bicyclic benzofurazanyl derivative **5c**, which is a bioisostere of the nitrophenyl group, showed decreased

Table 1. Physical and Biological Data for 5-[(4-Bromobenzyl)amino]pyrimidine Derivatives



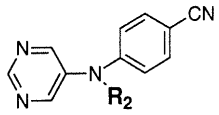
Compd.	R_1	Yield (%)	mp (°C)	Recryst. solvent	% inhibition of aromatase[a] (*in vitro*, nM) 1	10	% inhibition of PMSG-induced estrogen synthesis[b] (*in vivo*, mg/kg *p.o.*) 0.03	0.3
1[c]	4-CN				84.5	96.4	65.7	92.4
4b	4-NO_2	68	162—164	AcOEt	83.7	96.9	10.8	82.8
4c	4-CH_3CO	43	81—82	EtOH–iso-Pr_2O	77.6	95.8	−27.9	−33.6
4d	4-CH_3SO_2	68	157—158	EtOH–iso-Pr_2O	68.1	80.9	9.2	−14.4
4e[d]	4-CF_3	42	105—108	iso-Pr_2O	9.9	39.1	—	—

a) % inhibition of aromatization of androstenedione in the *in vitro* rat ovarian microsome assay. Values were determined in a single experiment. Each assay was performed in triplicate. *b*) % inhibition of estrogen synthesis in the *in vivo* rat PMSG-induced estrogen synthesis assay. Each compound was tested in groups of five rats and data represent mean values of peak inhibition. *c*) See ref. 1*c*. *d*) Monooxalate. —: Not tested.

activity. Introduction of a methyl group at the benzylic position of **5f** resulted in retention of the activity *in vitro*, but no inhibitory effect was seen *in vivo* (**5f** *vs.* **5g**), probably due to poor pharmacokinetic properties. These observations indicated that a benzyl group having some steric flexibility is a more suitable substituent on the R_2 moiety for high aromatase inhibition both *in vitro* and *in vivo*. Therefore, we focused on this benzyl substituent and synthesized various substituted benzyl derivatives.

The unsubstituted benzyl compound **5h** exhibited a relatively strong inhibition both *in vitro* and *in vivo*. Introduction of a halogen substituent at the *para* position of the phenyl ring of the benzyl group (**1** and **5i**—**k**) increased the inhibitory activity *in vitro*. Although both **5i** and **5k** showed nearly equal potency *in vitro*, only the fluoro derivative **5i** strongly inhibited estrogen synthesis, while the iodo derivative **5k** had no effect at a dose of 0.03 mg/kg upon oral administration. Introduction of

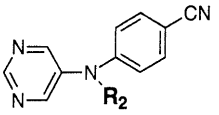
Table 2. Physical and Biological Data for 5-[(4-Cyanophenyl)amino]pyrimidine Derivatives



Compd.	R_2	Yield (%)	mp (°C)	Recryst. solvent	% inhibition of aromatase[a] (*in vitro*, nM) 1	10	% inhibition of PMSG-induced estrogen synthesis[b] (*in vivo*, mg/kg *p.o.*) 0.03	0.3
1[c]	4-Br–$PhCH_2$				84.5	96.4	65.7	92.4
5a	4-CN–Ph	37	206—207	AcOEt–Et_2O	34.7	80.9	59.3	90.9
5b	4-NO_2–Ph	80	197—198	AcOEt–Et_2O	16.4	67.7	48.7	81.0
5c	5-Benzofurazanyl	44	190—191	AcOEt	6.6	54.3	19.5	72.4
5d	4-CF_3–Ph	32	141—142	iso-Pr_2O	22.6	72.1	−3.2	42.0
5e	4-CN–$PhCH_2$	64	117—118	EtOH	38.5	83.8	59.6	83.8
5f	4-NO_2–$PhCH_2$	54	151—152	AcOEt–*n*-hexane	74.8	95.6	77.7	94.7
5g	4-NO_2–$Ph(CH_3)CH$	19	206—208	AcOEt	59.5	87.3	−45.4	−94.4
5h	4-$PhCH_2$	85	98—99	EtOH–iso-Pr_2O	62.4	86.7	60.7	85.0
5i	4-F–$PhCH_2$	72	92—93	EtOH–iso-Pr_2O	70.1	93.1	73.1	93.9
5j	4-Cl–$PhCH_2$	75	123—124	EtOH–iso-Pr_2O	76.9	95.2	52.9	91.6
5k	4-I–$PhCH_2$	80	176—177	EtOH–iso-Pr_2O	71.4	94.7	−7.4	83.2
5l	4-CF_3–$PhCH_2$	54	78—79	iso-Pr_2O	70.4	91.5	60.6	92.3
5m	2-F-5-Pyridylmethyl	66	130—131	EtOH–iso-Pr_2O	24.4	78.1	64.6	88.8
5n	2-Br-5-Pyridylmethyl	11	133—134	EtOH–iso-Pr_2O	59.1	92.2	73.4	88.6
5o	3,4-di-Cl–$PhCH_2$	68	128—129	AcOEt	84.5	96.1	24.6	89.8

a) % inhibition of aromatization of androstenedione in the *in vitro* rat ovarian microsome assay. Values were determined in a single experiment. Each assay was performed in triplicate. *b*) % inhibition of estrogen synthesis in the *in vivo* rat PMSG-induced estrogen synthesis assay. Each compound was tested in groups of five rats and data represent mean values of peak inhibition. *c*) See ref. 1*c*.

Table 3. Physical and Biological Data for 5-[(4-Cyanophenyl)amino]pyrimidine Derivatives



Compd.	R_2	Yield (%)	mp (°C)	Recryst. solvent	% inhibition of aromatase[a] (*in vitro*, nM) 1	10	% inhibition of PMSG-induced estrogen synthesis[b] (*in vivo*, mg/kg *p.o.*) 0.03	0.3
5i	4-F–$PhCH_2$	72	92—93	EtOH–iso-Pr_2O	70.1	93.1	73.1	93.9
5p[c]	2-F–$PhCH_2$	26	113—115	AcOEt	64.8	92.7	4.1	60.7
5q	3-Br-4-F–$PhCH_2$	65	138—139	AcOEt–Et_2O	85.8	97.0	52.3	94.0
5r	4-Br-3-F–$PhCH_2$	57	164—165	AcOEt–Et_2O	76.7	91.4	36.2	93.4
5s	3-F-4-NO_2–$PhCH_2$	15	165—167	AcOEt–*n*-hexane	68.6	90.0	27.6	80.2
5t	2,4-di-F–$PhCH_2$	72	114—115	EtOH–iso-Pr_2O	60.5	92.4	69.7	86.7
5u	2,5-di-F–$PhCH_2$	69	92—93	AcOEt–*n*-hexane	64.7	92.7	23.1	56.7
5v	3,4-di-F–$PhCH_2$	73	120—121	AcOEt–*n*-hexane	82.2	95.8	84.8	96.1
5w	3,5-di-F–$PhCH_2$	83	108—109	EtOH	79.9	96.1	85.9	96.8
5x	2,3,4-tri-F–$PhCH_2$	82	156—157	AcOEt–*n*-hexane	71.3	93.9	76.7	95.4
5y	2,4,5-tri-F–$PhCH_2$	70	147—148	AcOEt–iso-Pr_2O	80.1	95.3	58.0	87.6

a) % inhibition of aromatization of androstenedione in the *in vitro* rat ovarian or human placental microsome assay. Values were determined in a single experiment. Each assay was performed in triplicate. *b*) % inhibition of estrogen synthesis in the *in vivo* rat PMSG-induced estrogen synthesis assay. Each compound was tested in groups of five rats and data represent mean values of peak inhibition. *c*) Hemioxalate.

the dichloro substituent (**5o**) increased the aromatase inhibition *in vitro* over that of the mono-substituted compound **5j**, but the *in vivo* inhibition was reduced. On the other hand, replacement of the benzene ring with a pyridine ring (**5i** *vs.* **5m**, **1** *vs.* **5n**) resulted in retention of the inhibitory effect *in vivo* in spite of the decreased *in vitro* activity. These results suggested that introduction of a strong lipophilic substituent, such as an iodo, trifluoromethyl or dichloro atom, on the phenyl ring is unfavorable for improving the *in vivo* activity.

Among these substituents on the benzyl derivatives, we selected the fluoro atom due to its good efficacy for estrogen-synthesis inhibition and we further synthesized a number of fluoro-substituted analogs to examine the effect of a fluoro substituent on aromatase-inhibitory activity (Table 3). All of these compounds (**5i**—**y**) showed potent aromatase inhibition *in vitro*, while there were marked differences in *in vivo* potency. Compound **5p** with the ortho substituent had a remarkably reduced inhibitory activity compared with the *para* fluoro compound **5i**. The addition of another substituent such as bromo (**5q**, **5r**) or nitro (**5s**) to the mono fluoro-substituted phenyl ring did not improve the activity, though the introduction of one or two more fluorine atoms resulted in good activity (**5t**—**y**, except **5u**). In this series, the *in vitro* and *in vivo* activities were poorly correlated. It seems that the variation in the *in vivo* potency of these derivatives is caused by their pharmacokinetic characteristics (*e.g.*, absorption, duration of action or bioavailability).

Among the di- and trifluoro-substituted compounds, we selected three compounds, **5v**—**x**, which showed superior activity to compound **5i** and we further examined their inhibitory activities on human placental aromatase (Table 4). Surprisingly, there were great differences between the activities of the difluoro derivatives **5v** and **5w**. The 3,5-difluoro compound **5w** was more potent than the 3,4-difluoro compound **5v**, although their inhibitory activities for rat ovarian aromatase were almost equipotent, suggesting that there may be a species difference between the aromatases of human placenta and rat ovary.

Based on the above results, we identified the 3,5-difluorobenzyl derivative **5w** (YM553) as a highly potent

Table 4. Inhibitory Activities of Compounds **5v**—**x** towards Human Placental Aromatase

Compd.	% inhibition of human placental aromatase[a] (*in vitro*, nM)	
	0.1	0.3
5v	−8.1	39.9
5w	75.4	88.9
5x	68.8	85.6

a) % inhibition of aromatization of androstenedione in the *in vitro* human placental microsome assay. Values were determined in a single experiment. Each assay was performed in triplicate.

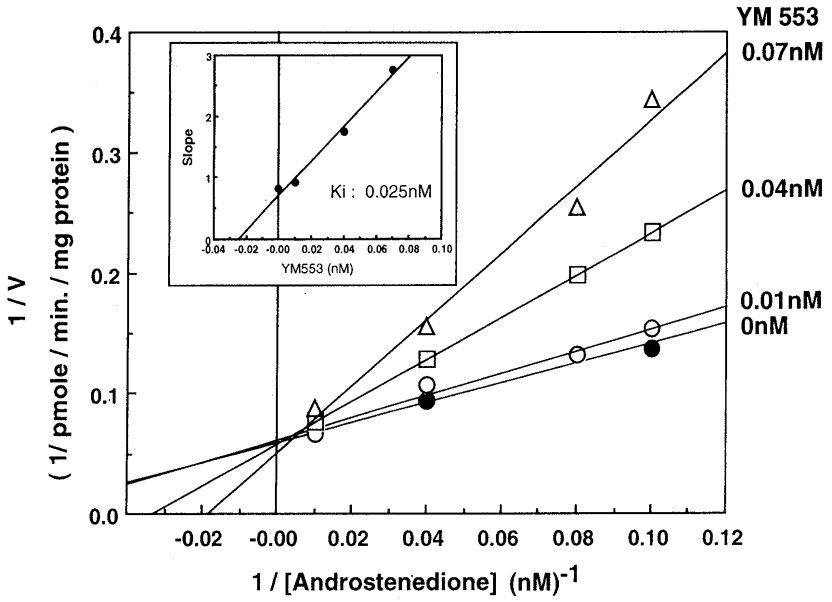


Fig. 1.

Table 5. Comparison of the Inhibitory Activities of Compound **5w** (YM553) and YM511 on the Synthesis of Various Steroid Hormones

Compd.	Inhibition of aromatase IC_{50} (nM)[a,c]		Inhibition of synthesis IC_{50} (μM)[b-d]		
			Aldosterone	Cortisol	Testosterone
	Rat ovarian microsomes	Human placental microsomes	Rat adrenal cells	Rabbit adrenal cells	Rat testicular cells
5w (YM553)	0.22	0.038	6.45	1.3	65% (3 μM)
YM511	0.40	0.13	2.19	3.9	52.9

a) Concentration required to inhibit aromatase activity by 50%. *b*) Concentration required to inhibit steroid synthesis by 50%. *c*) The number of experiments was 3 to 5. *d*) See ref. 30.

aromatase inhibitor both *in vitro* and *in vivo*. We conducted a kinetic analysis of its human placental aromatase inhibition (Fig. 1) and we examined its effects on other enzymes involved in steroid hormone synthesis in order to compare them with those of YM511 (Table 5). The IC_{50} values of YM553 for rat ovarian and human placental aromatases were 0.22 and 0.038 nM, respectively. Lineweaver–Burk plots showed that YM553 competitively inhibited human placental aromatase with a K_i of 0.025 nM, indicating that its effect is about four times more potent than that of YM511 ($K_i = 0.11$ nM).[1a] YM553 inhibited aldosterone synthesis with an IC_{50} value of 6.45 μM, and the aldosterone/aromatase inhibitory activity ratios of YM553 and YM511 in rats were 29000 and 5500, respectively, suggesting that YM553 is a more selective inhibitor than YM511 against aldosterone synthesis. Although the inhibitory activities of YM553 on cortisol and testosterone synthesis were stronger than those of YM511, these inhibitory potencies are sufficiently weak in comparison with its aromatase-inhibitory activity. Moreover, YM553 had no effect on aldosterone and cortisol synthesis *in vivo* even at doses of 100 and 30 mg/kg *p.o.*, respectively (data not shown). These results demonstrated that YM553 is a highly potent and selective aromatase inhibitor.

Conclusion

We prepared a series of *N*,*N*-disubstituted-5-aminopyrimidine derivatives and evaluated their aromatase-inhibitory activity. SAR studies of this series led us to identify the compound **5w** (YM553), which has a (4-cyanophenyl)(3,5-difluorobenzyl) moiety as part of its side chain, as a highly potent aromatase inhibitor both *in vitro* and *in vivo*. YM553 inhibited human placental aromatase approximately four times more potently than YM511, and also showed high specificity for aromatase. These results indicated that YM553, as well as YM511 (a 4*H*-1,2,4-triazol-4-yl type compound), is a promising agent for the treatment of estrogen-dependent diseases, and it is now under development.

Experimental

Melting points were determined on a Yanaco MP-500D micro melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL EX-90, a JEOL FX-100, a JNM-EX 400 and a JNM-GX 500 spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a Hitachi M-80 (EI) or a JEOL JMS DX-300 (FAB) mass spectrometer. Elemental analysis was performed with a Yanaco MT-5 analyzer. Column chromatography was performed on silica gel (Wakogel C-200 or Merck Kieselgel 60, 70—230 mesh). The synthetic procedures for compounds **1** and **3a** are described in our previous report.[1c]

5-(4-Nitrophenyl)aminopyrimidine (3b) 5-Aminopyrimidine (**2**,[24] 2.38 g, 25 mmol) was added portionwise to a suspension of potassium *tert*-butoxide (3.36 g, 30 mmol) in DMSO (20 ml) at 10—15 °C with stirring. The mixture was stirred for 1 h at room temperature, and then 4-fluoronitrobenzene (1.41 g, 10 mmol) in DMSO (5 ml) was added dropwise. The mixture was stirred for 30 min at 50 °C, then poured into water and neutralized with 1 N HCl. The resultant precipitate was collected by filtration and purified by silica gel column chromatography. Elution with $CHCl_3$–MeOH (50 : 1) gave a crystalline product, which was washed with ether to give **3b** (1.40 g, 65%). mp 224—225 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.20 (2H, d, J=9 Hz), 8.14 (2H, d, J=9 Hz), 8.76 (2H, s), 8.80 (1H, s), 9.54 (1H, br s). EI-MS m/z: 216 (M^+).

4′-(5-Pyrimidinyl)aminoacetophenone (3c), 5-(4-Methanesulfonylphenyl)aminopyrimidine (3d) and 5-(4-Trifluoromethyl)aminopyrimidine (3e) Compounds **3c**—**e** were prepared from 5-aminopyrimidine with 4-fluoroacetophenone, 4-fluorophenylmethylsulfone[25] and 4-fluorobenzotrifluoride, respectively, in a similar manner to that described for compound **3b**.

Compound **3c**: Yield 47%. mp 206—209 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.49 (3H, s), 7.17 (2H, d, J=9 Hz), 7.88 (2H, d, J=9 Hz), 8.70 (2H, s), 8.79 (1H, s), 9.08 (1H, br s). EI-MS m/z: 213 (M^+).

Compound **3d**: Yield 18%. mp 202—204 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.14 (3H, s), 7.25 (2H, d, J=9 Hz), 7.77 (2H, d, J=9 Hz), 8.71 (2H, s), 8.82 (1H, s), 9.16 (1H, br s). EI-MS m/z: 249 (M^+).

Compound **3e**: Yield 35%. mp 161—163 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.25 (2H, d, J=8 Hz), 7.59 (2H, d, J=8 Hz), 8.68 (2H, s), 8.77 (1H, s), 9.02 (1H, br s). EI-MS m/z: 239 (M^+).

5-[(4-Bromobenzyl)(4-nitrophenyl)amino]pyrimidine (4b) Compound **3b** (0.22 g, 1.0 mmol) was added to a suspension of sodium hydride (60% in mineral oil, 40 mg, 1.0 mmol) in dimethylformamide (DMF) (5 ml) with ice-cooling. The mixture was stirred for 30 min at 40—50 °C, and cooled to room temperature. 4-Bromobenzyl bromide (0.25 g, 1.0 mmol) was added and the reaction mixture was stirred for 1 h at 60 °C, then concentrated under reduced pressure. Water was added to the resultant residue and the whole was extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was subjected to silica gel column chromatography. The $CHCl_3$ eluate gave a crystalline product, which was recrystallized from AcOEt to give **4b** (0.26 g, 68%). Compounds **4c**—**e** and **5a**—**y** in Tables 1, 2 and 3 were similarly synthesized. Physical and spectral data for compounds **4b**—**e** and **5a**—**y** are summarized in Tables 1—3 and 6.

5-Fluorobenzofurazane (7) A mixture of 5-fluorobenzofuroxan (**6**, 1.54 g, 10 mmol) and triethyl phosphite (1.99 g, 12 mmol) in benzene (15 ml) was refluxed for 1 h. The reaction mixture was concentrated and the resultant residue was subjected to silica gel column chromatography. Elution with *n*-hexane–AcOEt (150 : 1) gave **7** (0.62 g, 45%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 7.28 (1H, m), 7.42 (1H, m), 7.91 (1H, dd, J=9, 1 Hz). EI-MS m/z: 138 (M^+).

Aromatase-Inhibitory Activity [1β,2β-^{3}H]Androstendione (0.1 μmol) (44.2 Ci/mmol, Du Pont New England Nuclear, Boston, MA, U.S.A.) was incubated with rat (Wistar strain, about 3 weeks old) ovarian microsomes (160 μg/ml, specific activity 0.021 pmol/min/mg of protein) in potassium phosphate buffer[26] (pH 7.4). The incubation medium also contained various concentrations of test compounds dissolved in DMF (final concentration 0.5%) in the presence of an NADPH-regenerating system[27] or 5 mM NADPH.[28] The reaction mixture was treated with $CHCl_3$ and activated charcoal to remove residual steroids. The radioactivity in an aliquot of the supernatant was determined with a Packard liquid scintillation spectrometer (model 2500TR). The inhibitory activity of test compounds was obtained as the percentage inhibition of the aromatization with respect to the solvent control. The IC_{50} was obtained from a line drawn by the least-squares method.

Inhibitory Activity of PMSG-Induced Estrogen Synthesis (*in Vivo*) The *in vivo* inhibition of aromatase activity by the test compounds was evaluated according to the literature methods.[26,29] Briefly, female rats (Wistar strain, about 3 weeks old, n=5) were injected subcutaneously with 100 IU/rat of PMSG. After 72 h, rats were administered 20% polyethylene glycol or various doses of the test compound orally. At 3 h after administration, the rats were killed, their ovaries were removed, and the estrogen content of the ovaries was measured by radioimmunoassay. The inhibitory activity of the test compound was expressed as the percentage inhibition with respect to the control.

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Table 6. Physical and Spectral Data for Compounds **4b—e** and **5a—y**

Compd.	Formula	Analysis (%) Calcd (Found) C	H	N	X	Y	^{1}H-NMR ($CDCl_3$) δ[a]	MS *m/z*
4b	$C_{17}H_{13}BrN_4O_2$	53.01 (52.90	3.40 3.35	14.54 14.48	20.74 (X=Br) 20.74)		5.04 (2H, s), 6.94 (2H, d, *J*=9), 7.16 (2H, d, *J*=9), 7.52 (2H, d, *J*=9), 8.14 (2H, d, *J*=9), 8.72 (2H, br s), 9.09 (1H, s)	385 (M^+)
4c	$C_{19}H_{16}BrN_3O$	59.70 (59.65	4.22 4.25	10.99 10.76)			2.54 (3H, s), 5.00 (2H, s), 7.03 (2H, d, *J*=9), 7.14 (2H, d, *J*=9), 7.47 (2H, d, *J*=9), 7.90 (2H, d, *J*=9), 8.59 (2H, s), 8.92 (1H, s)	383 $(M+H)^+$
4d	$C_{18}H_{16}BrN_3O_2S$	51.68 (51.45	3.86 3.73	10.05 9.99	19.10 (X=Br, Y=S) 18.90	7.67 7.68)	3.03 (3H, s), 5.01 (2H, s), 7.02 (2H, d, *J*=9), 7.15 (2H, d, *J*=9), 7.48 (2H, d, *J*=9), 7.80 (2H, d, *J*=9), 8.64 (2H, s), 9.00 (1H, s)	418 (M^+)
4e	$C_{20}H_{15}BrF_3N_3O_4$	48.21 (47.90	3.03 3.08	8.43 8.21	16.04 (X=Br, Y=F) 15.96	11.44 11.21)	5.14 (2H, s), 7.17 (2H, d, *J*=9), 7.30 (2H, d, *J*=9), 7.50 (2H, d, *J*=9), 7.59 (2H, d, *J*=9), 8.73 (2H, s), 8.88 (1H, s)[b]	407 $(M-H)^+$
5a	$C_{18}H_{11}N_5$	72.72 (72.19	3.78 3.88	23.56 23.34)			7.16 (4H, d, *J*=9), 7.64 (4H, d, *J*=9), 8.58 (2H, br s), 9.05 (1H, s)	297 (M^+)
5b	$C_{18}H_{11}N_5O_4$	56.98 (56.82	3.29 3.35	20.76 20.71)			7.33 (4H, d, *J*=9), 8.23 (4H, d, *J*=9), 8.78 (2H, br s), 9.12 (1H, s)[b]	337 (M^+)
5c	$C_{17}H_{10}N_6O$	64.96 (65.02	3.21 3.27	26.74 26.63)			7.20 (2H, d, *J*=9), 7.21—7.32 (2H, m), 7.68 (2H, d, *J*=9), 7.86 (1H, dd, *J*=9, 1), 8.61 (2H, s), 9.09 (1H, s)	314 (M^+)
5d	$C_{18}H_{11}F_3N_4$	63.53 (63.45	3.26 3.27	16.46 16.49	16.75 (X=F) 16.64)		7.07 (2H, d, *J*=9), 7.21 (2H, d, *J*=9), 7.59 (2H, d, *J*=9), 7.62 (2H, d, *J*=9), 8.59 (2H, s), 9.00 (1H, s)	340 (M^+)
5e	$C_{19}H_{13}N_5$	73.30 (73.37	4.21 4.15	22.49 22.53)			5.10 (2H, s), 6.95 (2H, d, *J*=9), 7.40 (2H, d, *J*=8), 7.55 (2H, d, *J*=9), 7.67 (2H, d, *J*=8), 8.64 (2H, s), 9.01 (1H, s)	311 (M^+)
5f	$C_{18}H_{13}N_5O_2$	65.25 (64.90	3.95 3.99	21.14 20.93)			5.15 (2H, s), 6.97 (2H, d, *J*=9), 7.47 (2H, d, *J*=9), 7.56 (2H, d, *J*=9), 8.23 (2H, d, *J*=9), 8.56 (2H, s), 9.03 (1H, s)	331 (M^+)
5g	$C_{19}H_{15}N_5O_2$	66.08 (66.03	4.38 4.42	20.28 26.32)			1.62 (3H, d, *J*=6), 5.49 (1H, d, *J*=6), 6.79 (2H, d, *J*=9), 7.50 (2H, d, *J*=9), 8.42 (2H, s), 9.18 (1H, s)	345 (M^+)
5h	$C_{18}H_{14}N_4$	75.51 (75.43	4.93 5.00	19.57 19.63)			5.05 (2H, s), 7.03 (2H, d, *J*=9), 7.19—7.40 (5H, m), 7.55 (2H, d, *J*=9), 8.65 (2H, s), 8.97 (1H, s)	286 (M^+)
5i	$C_{18}H_{13}F_4N$	71.04 (71.12	4.31 4.35	18.41 18.25	6.24 (X=F) 6.24)		5.01 (2H, s), 6.86—7.19 (6H, m), 7.54 (2H, d, *J*=9), 8.64 (2H, s), 8.99 (1H, s)	304 (M^+)
5j	$C_{18}H_{13}ClN_4$	67.40 (67.38	4.08 4.09	17.47 17.44	11.05 (X=Cl) 11.06)		5.01 (2H, s), 6.97 (2H, d, *J*=9), 7.27 (2H, d, *J*=9), 7.34 (2H, d, *J*=9), 7.53 (2H, d, *J*=9), 8.63 (2H, s), 8.89 (1H, s)	320 (M^+)
5k	$C_{18}H_{13}IN_4$	52.45 (52.32	3.18 3.08	13.59 13.55	30.78 (X=I) 31.06)		4.98 (2H, s), 6.96 (2H, d, *J*=9), 7.01 (2H, d, *J*=9), 7.53 (2H, d, *J*=9), 7.68 (2H, d, *J*=9), 8.64 (2H, s), 9.00 (1H, s)	421 (M^+)
5l	$C_{19}H_{13}F_3N_4$	64.41 (64.46	3.70 3.75	15.81 15.91	16.09 (X=F) 16.05)		5.10 (2H, s), 7.12 (2H, d, *J*=9), 7.30—7.70 (6H, m), 8.65 (2H, s), 9.01 (1H, s)	354 (M^+)
5m	$C_{17}H_{12}FN_5$	66.88 (66.84	3.96 3.92	22.94 23.01	6.22 (X=F) 6.10)		5.06 (2H, s), 6.89—7.07 (1H, m), 6.99 (2H, d, *J*=9), 7.56 (2H, d, *J*=9), 7.76 (1H, dd, *J*=9, 3), 8.17 (1H, d, *J*=9), 8.63 (2H, s), 9.00 (1H, s)	305 (M^+)
5n	$C_{17}H_{12}BrN_5$	55.76 (55.12	3.30 3.24	19.12 19.08	21.82 (X=Br) 21.80)		5.02 (2H, s), 6.95 (2H, d, *J*=9), 7.47 (2H, d, *J*=9), 7.56 (2H, d, *J*=9), 8.36 (1H, s), 8.36 (2H, s), 9.03 (1H, s)	365 (M^+)
5o	$C_{18}H_1Cl_2N_4$	60.86 (60.73	3.26 3.27	16.46 16.49	16.75 (X=Cl) 16.64)		4.99 (2H, s), 6.95 (2H, d, *J*=9), 7.10 (2H, dd, *J*=9, 2), 7.37 (1H, d, *J*=2), 8.63 (2H, s), 9.01 (1H, s)	354 (M^+)
5p	$C_{19}H_{14}N_4FO_2$	65.33 (65.26	4.04 4.09	16.04 16.05	5.44 (X=F) 5.25)		5.18 (2H, s), 7.03 (2H, d, *J*=9), 7.00—7.50 (4H, m), 7.66 (2H, d, *J*=9), 8.81 (2H, s), 8.98 (1H, s)	305 $(M+H)^+$
5q	$C_{18}H_{12}BrFN_4$	56.42 (56.29	3.16 3.19	14.62 14.56	20.85 (X=Br, Y=F) 20.95	4.96 4.79)	4.99 (2H, s), 6.96 (2H, d, *J*=9), 7.08—7.19 (2H, m), 7.41—7.52 (1H, m), 7.55 (2H, d, *J*=9), 8.62 (2H, s), 9.00 (1H, s)	383 (M^+)
5r	$C_{18}H_{12}BrFN_4$	56.42 (56.27	3.16 3.25	14.62 14.56	20.85 (X=Br, Y=F) 20.66	4.96 4.92)	4.99 (2H, s), 6.91—7.10 (4H, m), 7.45—7.63 (1H, m), 7.55 (2H, d, *J*=9), 8.63 (2H, s), 9.01 (1H, s)	383 (M^+)
5s	$C_{18}H_{12}F_2N_5O_2$	61.89 (61.71	3.46 3.49	20.05 19.95	5.44 (X=F) 5.28)		5.11 (2H, s), 6.96 (2H, d, *J*=9), 7.22—7.26 (2H, m), 7.58 (2H, d, *J*=9), 8.10 (1H, m), 8.67 (2H, s), 9.06 (1H, s)	349 (M^+)
5t	$C_{18}H_{12}F_2N_4$	67.08 (67.06	3.75 3.79	17.38 17.37	11.79 (X=F) 11.11)		5.02 (2H, s), 6.80—6.90 (2H, m), 6.97 (2H, d, *J*=9), 7.10—7.20 (1H, m), 7.54 (2H, d, *J*=9), 8.63 (2H, s), 9.01 (1H, s)	322 (M^+)
5u	$C_{18}H_{12}F_2N_4$	67.08 (67.13	3.75 3.70	17.38 17.32	11.79 (X=F) 11.59)		5.04 (2H, s), 6.91—7.01 (4H, m), 7.15—7.10 (1H, m), 7.57 (2H, d, *J*=9), 8.65 (2H, s), 9.02 (1H, s)	322 (M^+)
5v	$C_{18}H_{12}F_2N_4$	67.08 (66.96	3.75 3.73	17.38 17.31	11.79 (X=F) 11.76)		5.00 (2H, s), 6.99—7.01 (3H, m), 7.06—7.10 (1H, m), 7.13—7.19 (1H, m), 7.56 (2H, d, *J*=9), 8.64 (2H, s), 9.00 (1H, s)	322 (M^+)
5w	$C_{18}H_{12}F_2N_4$	67.08 (67.00	3.75 3.69	17.38 17.33	11.79 (X=F) 11.90)		5.05 (2H, s), 7.03 (2H, d, *J*=9), 7.19—7.40 (5H, m), 7.55 (2H, d, *J*=9), 8.65 (2H, s), 8.97 (1H, s)	322 (M^+)
5x	$C_{18}H_{11}F_3N_4$	63.53 (63.57	3.26 3.23	16.46 16.55	16.75 (X=F) 16.47)		5.05 (2H, s), 6.94—6.99 (4H, m), 7.56 (2H, d, *J*=9), 8.63 (2H, s), 9.02 (1H, s)	340 (M^+)
5y	$C_{18}H_{11}F_3N_4$	63.53 (63.40	3.26 3.22	16.46 16.42	16.75 (X=F) 16.63)		5.00 (2H, s), 6.80—7.30 (2H, m), 7.00 (2H, d, *J*=9), 7.56 (2H, d, *J*=9), 8.62 (2H, s), 9.03 (1H, s)	340 (M^+)

a) *J*=Hz. *b*) Measured in DMSO-d_6.

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