Synthesis and Aromatase-Inhibitory Activity of Imidazolyl-1,3,5-triazine Derivatives

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Triamino-substituted 1,3,5-triazine derivatives were synthesized and tested for inhibitory activities against the aromatase of human placental microsomes and the cytochrome P450 side chain cleavage of cholesterol ($P450_{SCC}$) of pig adrenal mitochondria. The compounds having imidazolyl and tertiary amino groups as substituents in the 1,3,5-triazine ring showed significant aromatase-inhibitory activity. Among them, compounds 17, 23, 26, 27 and 28 were more active than the reference compound, CGS 16949A. The inhibitory activities of these compounds against $P450_{SCC}$ were much weaker than their aromatase-inhibitory activities. These compounds may be regarded as selective aromatase inhibitors.

Key words 1,3,5-triazine; aromatase-inhibitory activity; CGS 16949A; $P450_{SCC}$

Estrogen receptor-positive breast cancer in either premenopausal women or postmenopausal women is likely to be promoted by estrogen.[1] In postmenopausal women, the major pathway of estrogen biosynthesis is the peripheral conversion of androstenedione secreted by the adrenal cortex to estrone, for example, in the adipose tissue. This conversion is catalyzed by aromatase, which is a cytochrome P450-dependent monooxygenase.[2] Thus, aromatase inhibitors might be applicable as antitumor agents for estrogen-dependent breast cancer of postmenopausal women. With the aim of pharmacologically interfering with steroidogenesis, Griffiths *et al.*[3] proposed the use of aminoglutethimide (AG) combined with dexamethasone to suppress adrenal function. AG is a potent inhibitor of the conversion of cholesterol to pregnenolone,[4] and it simultaneously acts as a nonsteroidal inhibitor of aromatase.[5]

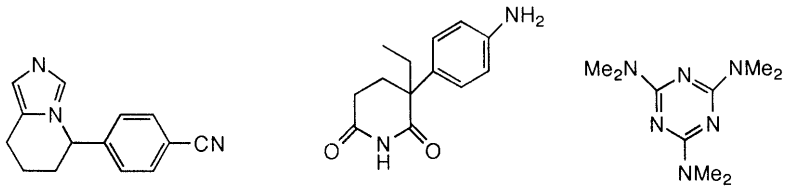
However, AG primarily acts as an inhibitor of cytochrome P450-catalyzed side chain cleavage of cholesterol ($P450_{SCC}$), depleting corticosteroid production.[6] The efforts of many groups to find selective aromatase inhibitors resulted in the discovery of CGS 16949A as a potent nonsteroidal aromatase inhibitor.[7]

On the other hand, hexamethylmelamine (HMM), a 1,3,5-triazine derivative, which has been used as an antitumor agent to treat breast cancer[8] and ovarian cancer,[9] does not show aromatase-inhibitory activity. Recently it has been suggested that an imidazole moiety is essential for aromatase-inhibitory activity, as in CGS 16949A or clotrimazole.[10] Therefore, imidazolyl-1,3,5-triazine, which combines the two moieties, may be worth testing.[11] The aim of the present study was to seek novel aromatase inhibitors which are more potent and selective than CGS 16949A, by synthesizing a series of imidazolyl-1,3,5-triazine derivatives, and examining their inhibitory activities towards aromatase and $P450_{SCC}$.

Chemistry The desired 1,3,5-triazine derivatives I—IV were prepared by nucleophilic substitution reaction of azoles or aliphatic amines as shown in Chart 1.

Monochlorotriazine **1** and dichlorotriazine **2** were synthesized according to the method of Thurston *et al.*[12] However, the method was found to be inconvenient in that it yielded a mixture of **1** and **2**. For the selective synthesis of either compound **1** or **2**, care should be taken that the initial reaction temperature is kept at -15 to $-5\,^{\circ}C$ for several hours using ethyleneglycol dimethyl ether as a reaction solvent for the synthesis of compound **2**, and at 0 to $10\,^{\circ}C$ using acetone as a reaction solvent for compound **1**. Furthermore, to avoid amine exchange reaction, the aliphatic amine should be employed in the first nucleophilic substitution reaction step. For example, when compound **3** was treated with morpholine for the synthesis of **14**, the product obtained was not the desired compound **14**, but **29**. It seems that the morpholino group was changed to an imidazolyl group, which acted as a leaving group to afford **29**.

Then, compound **2** was treated with amine and azole in the presence of base to afford **3** or **4** and compounds III, respectively. Because of the low activity of the monochlorotriazine for nucleophilic substitution reaction, the reactions of **1**, **3** and **4** with azole were carried out at higher temperature than that used for the synthesis of **3**



CGS 16949A AG HMM

Fig. 1

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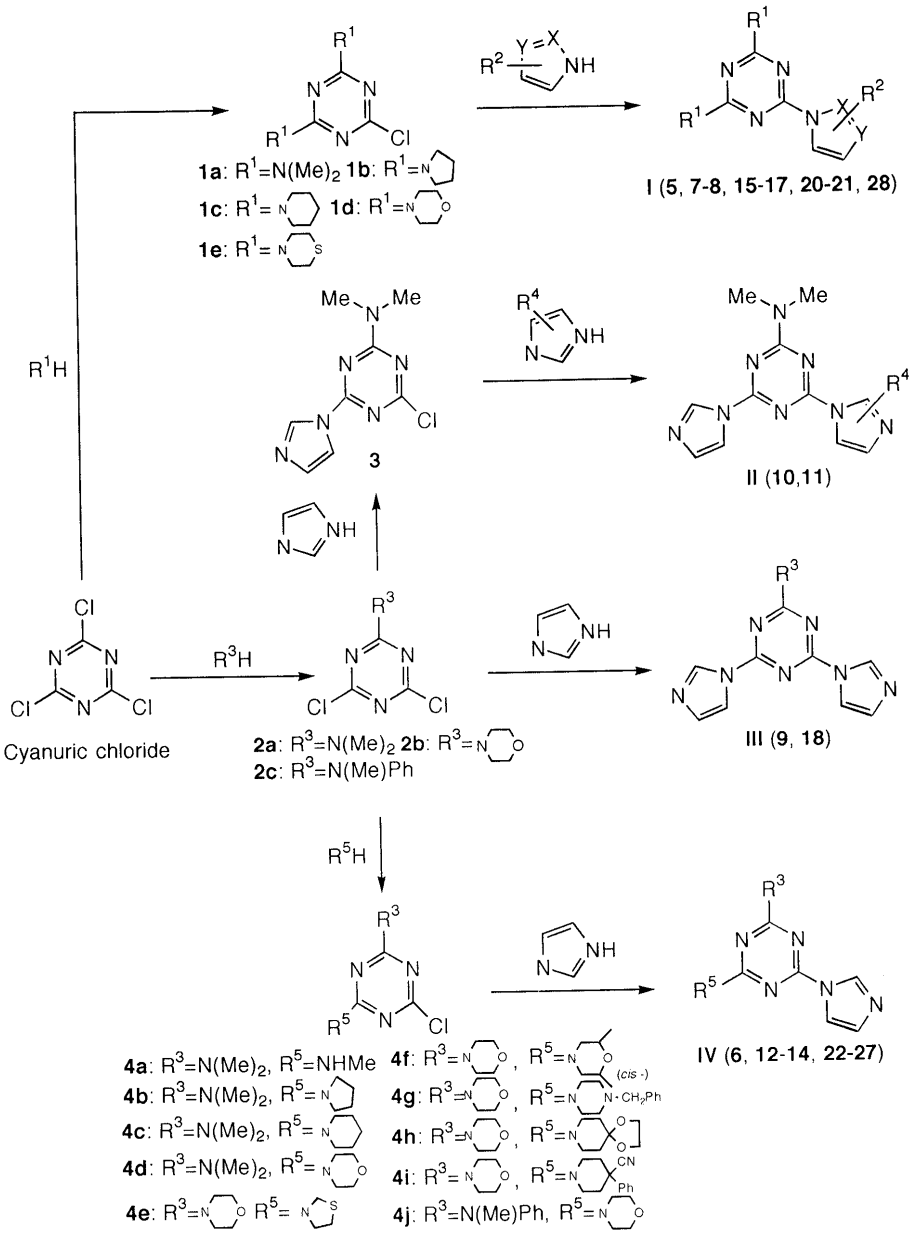


Chart 1

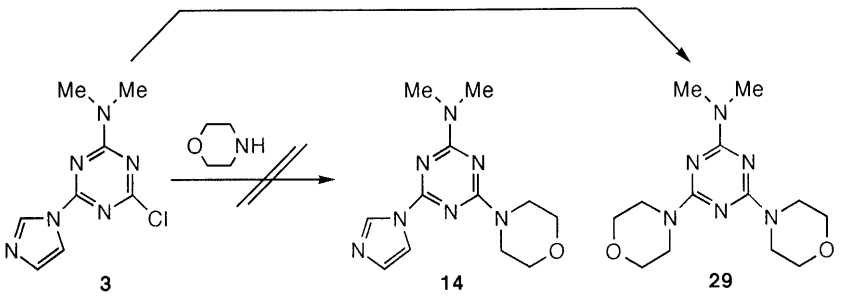


Chart 2

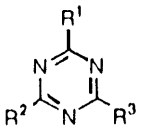
from dichlorotriazine **2**, to afford I, II and IV, respectively.

Because substituted imidazoles, triazoles and pyrazoles are tautomeric, it is difficult to determine the structure of the product. To avoid this problem, 2-isopropylimidazole or 4,5-dimethylimidazole was employed as a starting material. In the case of the reaction of 1,2,3-triazole, a single product was obtained. The NMR spectra indicated that this compound is not 2-substituted, but is the 1-substituted triazole derivative, because two protons in the triazole ring show two signals.[13]

Pharmacological Results and Discussion

The compounds listed in Table 1 were evaluated for

Table 1. Biological Properties of 1,3,5-Triazines



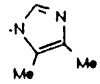
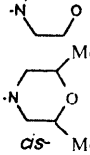
Compd.	R^1	R^2	R^3	Inhibition of aromatase IC_{50} (M) or inhibitory % (M)	Inhibition of $P450_{scc}$ IC_{50} (M) or inhibitory % (M)
5	$-NMe_2$	$-NMe_2$		4.1×10^{-6}	0% (1×10^{-4})
6	–NH–Me	$-NMe_2$		2.7% (1×10^{-6})	ND
7	$-NMe_2$	$-NMe_2$		0% (1×10^{-4})	ND
8	$-NMe_2$	$-NMe_2$		0% (1×10^{-4})	ND
9	$-NMe_2$			5.5×10^{-7}	8×10^{-5}
10	$-NMe_2$			4.3×10^{-6}	30% (1×10^{-4})
11	$-NMe_2$			2.0×10^{-7}	3% (1×10^{-4})
12	$-NMe_2$			1.3×10^{-6}	14% (1×10^{-4})
13	$-NMe_2$			7.7×10^{-7}	26% (1×10^{-4})
14	$-NMe_2$			1.0×10^{-8}	1% (1×10^{-4})
15				3.1×10^{-7}	40% (1×10^{-4})
16				3.1×10^{-7}	30% (1×10^{-4})
17				5.3×10^{-9}	1% (1×10^{-4})
18				2.1×10^{-8}	0% (1×10^{-4})
19				0% (1×10^{-6})	ND
20				3.8% (1×10^{-6})	ND
21				0% (1×10^{-4})	ND
22				3.1×10^{-8}	45% (1×10^{-4})
23				6.6×10^{-9}	7.2×10^{-5}
24				1.7×10^{-8}	36.8% (2×10^{-5})
25				2.4×10^{-8}	28% (5×10^{-5})
26				9.0×10^{-9}	2.2×10^{-5}
27				6.7×10^{-9}	3.1×10^{-5}

Table 1. (continued)

Compd.	R^1	R^2	R^3	Inhibition of aromatase IC_{50} (M) or inhibitory % (M)	Inhibition of $P450_{scc}$ IC_{50} (M) or inhibitory % (M)
28				5.1×10^{-9}	0% (1×10^{-6})
HMM	$-NMe_2$	$-NMe_2$	$-NMe_2$	5.4% (1×10^{-4})	ND
AG				3.8×10^{-5}	6.6×10^{-5}
	CGS-16949A			1.7×10^{-8}	6×10^{-5}

ND: not determined.

inhibitory activity against aromatase from human placental microsomes and for inhibitory activity against $P450_{SCC}$ from pig adrenal mitochondria. Almost all the compounds, which have one or two imidazolyl group as substituents on the 1,3,5-triazine moiety, showed potent aromatase-inhibitory activity. A remarkable decrease in potency was observed upon replacing the imidazolyl group of **17** with other functional groups, such as a morpholino group (**19**), pyrazolyl group (**20**), or triazolyl group (**21**). Moreover, replacing the dimethylamino group of **5** with a methylamino group resulted in a remarkable decrease in the aromatase-inhibitory activity (**6**). These results suggest that the imidazolyl group and the tertiary amino group are necessary for potent aromatase-inhibitory activity. However, the substitution of the 2-position on the imidazole ring with an isopropyl group as in compound **7** or the substitution of the 4- and 5-position on the imidazole ring with a methyl group as in compound **8** caused a reduction of the activity. This result suggests that a bulky isopropyl group or methyl group, which prevents the access of the imidazole ring to the active site of the enzyme, is unfavorable for the inhibitory activity. Compounds **17**, **18** and **22**—**27** all have a morpholino and an imidazolyl group, and showed high aromatase-inhibitory activity. Compound **28**, in which the morpholino group was replaced with a thiomorpholino group also showed high aromatase-inhibitory activity. Compounds **17**, **23** and **26**—**28** showed two- or three-fold higher activity than that of the reference compound, CGS 16949A. In order to understand whether these compounds have potent antitumor activity against estrogen-dependent breast cancer, we examined the effect of these compounds in suppressing uterine weight in rats. The results will be reported in a separate paper.

Experimental

Melting points were determined on a Yamato melting point apparatus Model Mp-21, and are uncorrected. Mass spectra (MS) were recorded on a JEOL GX102 spectrometer. ^{1}H-NMR spectra were taken at 270 MHz with a JEOL spectrometer with tetramethylsilane as an internal standard. Elemental analysis was performed using a Yanagimoto MT-2 analyzer. Column chromatography was carried out on silica gel (Wakogel C-200). CGS 16949A,[14] HMM[15] and 19[16] were prepared according to the literature.

General Procedure for Preparation of Monochloro-1,3,5-triazines 1 from Cyanuric Chloride Synthesis of 2-Chloro-4,6-bis(dimethylamino)-1,3,5-triazine (**1a**): An aqueous solution of dimethylamine (50% solution, 19.0 ml, 211 mmol) was slowly added dropwise to a solution of cyanuric chloride (9.22 g, 50.0 mmol) in Me_2CO (100 ml) at 0 to 10 °C. The reaction mixture was stirred at 0 °C for 2 h and then at room temperature for 18 h. After removal of the solvent, the residue was extracted with CH_2Cl_2. The extract was washed with brine, dried over $MgSO_4$ and concentrated. The residue was column-chromatographed on silica gel with hexane–ethyl acetate (AcOEt) (8 : 2) to give **1a** (9.9 g) in 98% yield. Colorless crystals, mp 89—91 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.13 (12H, s). MS *m/z*: 201 (M^+).

Compound **1b**—**e** were similarly prepared from cyanuric chloride.

2-Chloro-4,6-dipyrrolidino-1,3,5-triazine (**1b**): 25% yield. Colorless crystals, mp 112—115 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.9—2.0 (8H, m), 3.5—3.7 (8H, m). MS *m/z*: 253 (M^+).

2-Chloro-4,6-dipiperidino-1,3,5-triazine (**1c**): 75% yield. Colorless crystals, mp 114—117 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.5—1.7 (12H, m), 3.73 (8H, t, J=5 Hz). MS *m/z*: 281 (M^+).

2-Chloro-4,6-dimorpholino-1,3,5-triazine (**1d**): 91% yield. Colorless crystals, mp 173—174 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.6—3.8 (16H, m). MS *m/z*: 285 (M^+).

2-Chloro-4,6-dithiomorpholino-1,3,5-triazine (**1e**): 12% yield. Colorless crystals, mp 192—194 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.6—2.7 (8H, m), 4.07 (8H, br s). MS *m/z*: 317 (M^+).

These were used for the next reaction without further purification.

General Procedure for Preparation of Dichloro-1,3,5-triazines 2 Synthesis of 2,4-Dichloro-6-dimethylamino-1,3,5-triazine (**2a**): An aqueous solution of dimethylamine (50% solution, 10.8 ml, 120 mmol) was slowly added dropwise to a solution of cyanuric chloride (11.1 g, 60.2 mmol) in ethyleneglycol dimethyl ether (130 ml) at −15 to −5 °C. The reaction mixture was stirred at −15 °C for 2 h and then at room temperature for 20 h. After removal of the solvent, the residue was extracted with CH_2Cl_2. The extract was washed with brine, dried over $MgSO_4$ and concentrated to give **2a** (11.0 g) in 95% yield. Colorless crystals, mp 122—124 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.13 (6H, s). MS *m/z*: 192 (M^+).

Compounds **2b** and **2c** were similarly prepared from cyanuric chloride.

2,4-Dichloro-6-morpholino-1,3,5-triazine (**2b**): 63% yield. Colorless crystals, mp 162—164 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.6—3.8 (8H, m). MS *m/z*: 234 (M^+).

2,4-Dichloro-6-(*N*-methyl-*N*-phenylamino)-1,3,5-triazine (**2c**): 61% yield. Colorless crystals, mp 131—133 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.55 (3H, s), 7.2—7.5 (5H, m). MS *m/z*: 254 (M^+).

These were used for the next reaction without further purification.

2-Chloro-4-dimethylamino-6-(1-imidazolyl)-1,3,5-triazine (**3**): Imidazole (0.681 g, 10.0 mmol) and K_2CO_3 (1.38 g, 10.0 mmol) were added to a solution of **2a** (1.93 g, 10.0 mmol) in *N*,*N*-dimethylformamide (DMF) (8 ml) at room temperature. The reaction mixture was stirred at room temperature for 17 h, then evaporated *in vacuo*, and the residue was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was column-chromatographed on silica gel with AcOEt–methanol (MeOH) (95 : 5) to give **3** (0.87 g) in 39% yield. Colorless crystals, mp 87—90 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.27 (6H, s), 7.13 (1H, s), 7.78 (1H, s), 8.54 (1H, m). MS *m/z*: 224 (M^+).

This was used for the next reaction without further purification.

General Procedure for Preparation of Monochloro-1,3,5-triazines 4 Synthesis of 2-Chloro-4-dimethylamino-6-methylamino-1,3,5-triazine (**4a**): An aqueous solution of methylamine (40% solution, 0.16 ml, 2.0 mmol) was slowly added dropwise to a solution of **2a** (0.39 g, 2.0 mmol) in DMF (6 ml) at −5 to 0 °C in the presence of K_2CO_3 (0.56 g, 4.1 mmol) as a hydrogen chloride scavenger. The resulting reaction mixture was stirred at −5 °C for 2 h and then at room temperature for 20 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$ and concentrated. The residue was column-chromatographed on silica gel with AcOEt (7 : 3)

to give the crude product **4a** (0.18 g) in 47% yield. Colorless crystals, mp 206—208 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.98 (3H, d, J=5 Hz), 3.16 (6H, s), 6.27 (1H, br s). MS m/z: 187 (M^+).

Compounds **4b—j** were similarly prepared from dichloro-1,3,5-triazine **2**.

2-Chloro-4-dimethylamino-6-pyrrolidino-1,3,5-triazine (**4b**): 45% yield. Colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.9—2.0 (4H, m), 3.13 (6H, s), 3.5—3.6 (4H, m). MS m/z: 227 (M^+).

2-Chloro-4-dimethylamino-6-piperidino-1,3,5-triazine (**4c**): 81% yield. Colorless crystals, mp 68—72 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.5—1.8 (6H, m), 3.13 (6H, s), 3.74 (4H, t, J=5 Hz). MS m/z: 241 (M^+).

2-Chloro-4-dimethylamino-6-morpholino-1,3,5-triazine (**4d**): 55% yield. Colorless crystals, mp 96—98 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.13 (6H, s), 3.6—3.8 (8H, m). MS m/z: 243 (M^+).

2-Chloro-4-morpholino-6-thiazolidino-1,3,5-triazine (**4e**): 97% yield. Colorless crystals, mp 137—139 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.07 (2H, t, J=6 Hz), 3.6—4.0 (10H, m), 4.66 (1H, s), 4.71 (1H, s). MS m/z: 287 (M^+).

2-Chloro-4-(*cis*-2,6-dimethylmorpholino)-6-morpholino-1,3,5-triazine (**4f**): 73% yield. Colorless crystals, mp 185—187 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (6H, d, J=6.0 Hz), 2.57 (2H, t, J=12 Hz), 3.5—3.9 (10H, m), 4.4—4.6 (2H, m). MS m/z: 313 (M^+).

2-(4-Benzylpiperazino)-4-chloro-6-morpholino-1,3,5-triazine (**4g**): 88% yield. Colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 2.47 (4H, br s), 3.56 (2H,

Table 2. Properties of the Compounds in Table 1

Compd.	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	MS m/z [M^+]	^{1}H-NMR ($CDCl_3$) δ
5	80	129—131	$C_{10}H_{15}N_7$	51.49 (51.63)	6.48 (6.54)	42.03 (42.11)	233	3.17 (12H, s), 7.08 (1H, d, J=1 Hz), 7.80 (1H, d, J=1 Hz), 8.53 (1H, s)
6	91	164—166	$C_9H_{13}N_7$	49.30 (49.32)	5.98 (6.08)	44.72 (44.60)	219	3.01 (3H, d, J=5 Hz), 3.20 (6H, s), 5.17 (1H, br s), 7.08 (1H, s), 7.75 (1H, s), 8.51 (1H, s)
7	50	130—131	$C_{13}H_{21}N_7$	56.71 (56.53)	7.69 (7.67)	35.61 (35.53)	275	1.36 (6H, d, J=7 Hz), 3.16 (12H, s), 4.18 (1H, septet, J=7 Hz), 6.91 (1H, d, J=2 Hz), 7.80 (1H, d, J=2 Hz)
8	42	135—137	$C_{12}H_{19}N_7$	55.15 (55.01)	7.33 (7.33)	37.52 (37.40)	261	2.18 (3H, s), 2.50 (3H, s), 3.16 (12H, s), 8.43 (1H, s)
9	65	172—174	$C_{11}H_{12}N_8$	51.56 (51.69)	4.72 (4.77)	43.72 (43.70)	256	3.31 (6H, s), 7.16 (2H, s), 7.83 (2H, s), 8.59 (2H, s)
10	60	130—131	$C_{14}H_{18}N_8$	56.36 (56.12)	6.08 (6.05)	37.56 (37.43)	298	1.43 (6H, d, J=7 Hz), 3.30 (3H, s), 3.32 (3H, s), 4.09 (1H, septet, J=7 Hz), 6.99 (1H, s), 7.17 (1H, s), 7.82 (1H, s), 7.87 (1H, s), 8.57 (1H, s)
11	88	192—194	$C_{13}H_{16}N_8$	54.92 (54.90)	5.67 (5.57)	39.41 (39.54)	284	2.21 (3H, s), 2.54 (3H, s), 3.27 (3H, s), 3.29 (3H, s), 7.15 (1H, s), 7.80 (1H, s), 8.50 (1H, s), 8.56 (1H, s)
12	63	101—103	$C_{12}H_{17}N_7$	55.58 (55.48)	6.61 (6.60)	37.81 (37.65)	259	1.9—2.0 (4H, m), 3.17 (6H, s), 3.5—3.7 (4H, m), 7.07 (1H, s), 7.80 (1H, s), 8.53 (1H, s)
13	65	98—99	$C_{13}H_{19}N_7$	57.12 (56.96)	7.01 (7.07)	35.87 (35.64)	273	1.5—1.8 (6H, m), 3.17 (6H, s), 3.80 (4H, t, J=5 Hz), 7.07 (1H, s), 7.79 (1H, s), 8.52 (1H, s)
14	92	156—158	$C_{12}H_{17}N_7O$	52.35 (52.31)	6.22 (6.17)	35.61 (35.41)	275	3.15 (3H, s), 3.20 (3H, s), 3.74 (4H, t, J=4 Hz), 3.85 (4H, t, J=4 Hz), 7.08 (1H, s), 7.77 (1H, s), 8.51 (1H, s)
15	87	162—163	$C_{14}H_{19}N_7$	58.93 (58.72)	6.71 (6.63)	34.36 (34.25)	285	1.9—2.0 (8H, m), 3.5—3.7 (8H, m), 7.07 (1H, s), 7.80 (1H, s), 8.53 (1H, s)
16	96	154—156	$C_{16}H_{23}N_7$	61.32 (61.38)	7.40 (7.29)	31.28 (31.32)	313	1.3—1.7 (12H, m), 3.71 (8H, br s), 7.00 (1H, s), 7.71 (1H, s), 8.44 (1H, s)
17	88	248—249	$C_{14}H_{19}N_7O_2$	52.99 (52.97)	6.03 (6.00)	30.90 (30.64)	317	3.74 (8H, t, J=5 Hz), 3.85 (8H, br s), 7.09 (1H, s), 7.75 (1H, s), 8.49 (1H, s)
18	33	268—270	$C_{13}H_{14}N_8O$	52.34 (52.14)	4.73 (4.81)	37.56 (37.46)	298	3.82 (4H, t, J=4 Hz), 3.99 (4H, t, J=4 Hz), 7.17 (2H, s), 7.82 (2H, s), 8.58 (2H, s)
20	71	220—221	$C_{14}H_{19}N_7O_2$	52.99 (52.75)	6.03 (6.10)	30.90 (30.87)	317	3.75 (8H, t, J=5 Hz), 3.8—3.9 (8H, m), 6.42 (1H, dd, J=1, 2 Hz), 7.79 (1H, d, J=1 Hz), 8.51 (1H, d, J=2 Hz)
21	43	239—240	$C_{13}H_{18}N_8O_2$	49.05 (49.22)	5.70 (5.66)	35.20 (35.06)	318	3.76 (8H, t, J=5 Hz), 3.8—3.9 (8H, m), 7.78 (1H, d, J=1 Hz), 8.48 (1H, d, J=1 Hz)
22	73	165—167	$C_{16}H_{23}N_7O_2$	55.64 (55.49)	6.71 (6.71)	28.39 (28.36)	345	1.26 (6H, d, J=6 Hz), 2.5—2.7 (2H, m), 3.5—3.9 (10H, m), 4.5—4.7 (2H, m), 7.09 (1H, t, J=1 Hz), 7.76 (1H, t, J=1 Hz), 8.49 (1H, t, J=1 Hz)
23	89	185—187	$C_{13}H_{17}N_7OS$	48.89 (49.12)	5.37 (5.41)	30.70 (30.47)	319	3.10 (2H, t, J=7 Hz), 3.74 (4H, t, J=4 Hz), 3.85 (4H, br s), 3.9—4.0 (2H, m), 4.74 (2H, br s), 7.09 (1H, s), 7.77 (1H, s), 8.50 (1H, s)
24	99	207—209	$C_{21}H_{26}N_8O$	62.05 (62.08)	6.45 (6.54)	27.57 (27.35)	406	2.49 (4H, br s), 3.55 (2H, br s), 3.73 (4H, t, J=5 Hz), 3.83 (8H, br s), 7.07 (1H, dd, J=1, 2 Hz), 7.25—7.34 (5H, m), 7.74 (1H, d, J=2 Hz), 8.48 (1H, d, J=1 Hz)
25	99	174—176	$C_{17}H_{23}N_7O_3$	54.68 (54.63)	6.21 (6.29)	26.26 (26.08)	373	1.74 (4H, t, J=6 Hz), 3.75 (4H, t, J=4 Hz), 3.84 (4H, br s), 3.94 (4H, br s), 4.01 (4H, s), 7.08 (1H, s), 7.76 (1H, s), 8.50 (1H, s)
26	64	160—161	$C_{22}H_{24}N_8O$	63.44 (63.29)	5.81 (5.87)	26.90 (26.75)	416	2.02 (2H, dt, J=4, 13 Hz), 2.2—2.3 (2H, m), 3.3—3.4 (2H, m), 3.76 (4H, t, J=4 Hz), 3.86 (4H, br s), 4.9—5.1 (2H, m), 7.10 (1H, t, J=1 Hz), 7.32—7.52 (5H, m), 7.77 (1H, t, J=1 Hz), 8.51 (1H, t, J=1 Hz)
27	53	119—120	$C_{17}H_{19}N_7O$	60.52 (60.62)	5.68 (5.70)	29.06 (29.02)	337	3.54 (3H, s), 3.7—3.8 (8H, m), 7.04 (1H, s), 7.2—7.4 (5H, m), 7.65 (1H, br s), 8.39 (1H, br s)
28	83	239—241	$C_{14}H_{19}N_7S_2$	48.12 (48.18)	5.48 (5.50)	28.06 (28.00)	349	2.66 (8H, t, J=5 Hz), 4.14 (8H, br s), 7.09 (1H, d, J=1 Hz), 7.75 (1H, d, J=1 Hz), 8.49 (1H, s)

br s), 3.7—3.9 (12H, m), 7.3—7.4 (5H, m). MS *m/z*: 374 (M^+).

2-Chloro-4-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-6-morpholino-1,3,5-triazine (**4h**): 89% yield. Colorless crystals, mp 169—171 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.72 (4H, t, J=6 Hz), 3.7—4.0 (12H, m), 3.99 (4H, s). MS *m/z*: 341 (M^+).

2-Chloro-4-(4-cyano-4-phenylpiperidino)-6-morpholino-1,3,5-triazine (**4i**): 99% yield. Colorless crystals, mp 203—205 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.99 (2H, d, J=13 Hz), 2.19 (2H, d, J=13 Hz), 3.32 (2H, t, J=13 Hz), 3.6—3.9 (8H, m), 4.8—5.1 (2H, m) 7.3—7.5 (5H, m). MS *m/z*: 384 (M^+).

2-Chloro-4-(*N*-methyl-*N*-phenylamino)-6-morpholino-1,3,5-triazine (**4j**): 84% yield. Colorless crystals, mp 97—100 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.49 (3H, s), 3.6—3.9 (8H, m), 7.2—7.5 (5H, m). MS *m/z*: 305 (M^+).

These were used for the next reaction without further purification.

General Procedure for Preparation of Synthesis of Triamino-substituted 1,3,5-Triazines I, II, III and IV Synthesis of 2-(1-Imidazolyl)-4,6-dimorpholino-1,3,5-triazine (**17**): Imidazole (0.694 g, 10.2 mmol) and NaOH (0.41 g, 10.3 mmol) was added to a solution of **1d** (1.44 g, 5.04 mmol) in DMF (10 ml) at room temperature. The reaction mixture was stirred at 110 °C for 30 min, then extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography with CH_2Cl_2–MeOH (95:5) to afford **17** (1.36 g, 85%) as colorless crystals.

Compounds **5**—**16**, **18** and **20**—**28** were obtained by a similar procedure to that described for **17**.

2-Dimethylamino-4,6-dimorpholino-1,3,5-triazine (29) Morpholine (0.18 g, 2.1 mmol) and NaOH (0.084 g, 2.1 mmol) were added to a solution of **3** (0.45 g, 2.0 mmol) in DMF (10 ml) at room temperature. The reaction mixture was stirred at 110 °C for 30 min, then extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography with CH_2Cl_2-MeOH (95:5) to afford **29** (0.18 g, 31%) as colorless crystals, mp 188—190 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.09 (6H, s), 3.7—3.9 (16H, m). MS *m/z*: 294 (M^+).

The properties of the prepared compounds **5**—**18** and **20**—**28** are shown in Table 2.

Aromatase-Inhibitory Activity The inhibitory activity was measured by the method of Thompson and Siiteri.[2a] Briefly, the assay measures the loss of tritium to the aqueous phase of the incubation medium during aromatization of androstenedione ^{3}H-labeled at positions 1 and 2. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and further dilution was done with 3% DMSO (v/v) in 0.05 M phosphate buffer (pH 7.4). The reaction mixture contained [1β, 2β-^{3}H]-4-androstene-3,17-dione (500 pmol), human placental microsomes (20 μg protein), and various concentrations of test compound (10^{-4}—10^{-9}) in phosphate buffer in a final volume of 450 μl. The preincubation was carried out at 37 °C for 3 min. The reaction was started by the addition of NADPH (125 nmol/5 μl) and stopped after 15 min of incubation by the addition of 25% trichloroacetic acid (50 μl). A suspension of activated charcoal (50 μl, containing 5% activated charcoal and 0.5% dextolane) was added to the reaction mixture. The resulting mixture was vortexed for 30 min and centrifuged at 600 × *g* for 5 min. After centrifugation, 400 μl of aqueous phase was dissolved in Aquazol-2 (5 ml) and the radioactivity of the resulting solution was measured with a liquid scintillation spectrometer (Aloka LSC-3100). Thus, the aromatase-inhibitory activities of the test compounds were radiometrically determined. The IC_{50} was determined by using an inhibition curve constructed by plotting the reciprocal percent conversion *versus* the concentration of the test compound.

$P450_{SCC}$-Inhibitory Activity The inhibitory activity was measured by the method of Shikita and Hall.[17] Briefly, the assay radiometrically measures the rate of transformation to the organic phase of the incubation medium during the biosynthetic transformation of cholesterol to pregnenolone. Test compounds were dissolved in DMSO and further dilution was done with 2% DMSO (v/v) in 0.05 M phosphate buffer (pH 7.4). The reaction mixture contained [4-^{14}C]cholesterol (5 nmol/250 Bq/10 μl ethanol), sonicated pig adrenal mitochondria (320 μg protein) and various concentrations (10^{-4}—10^{-7} M) of test compound in phosphate buffer in a final volume of 900 μl. The preincubation was carried out at 37 °C for 3 min. The reaction was started by the addition of NADPH (250 nmol/100 μl) and stopped after 15 min by the addition of CH_2Cl_2 (3 ml). Following extraction and centrifugation, the organic layer was removed and evaporated to dryness. The residue was taken up in CH_2Cl_2, and chromatographed on a silica gel (13181 Silicagel Kodak) thin layer plate with AcOEt–hexane (3:7, v/v). The radioactive spots were identified by comparison with authentic standards and quantified by the use of a liquid scintillation spectrometer (Aloka LSC-3100). Thus, the $P450_{SCC}$-inhibitory activities of the test compounds were radiometrically determined. The IC_{50} was determined by using an inhibition curve constructed by plotting the reciprocal percent conversion *versus* the concentration of the test compound.

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