Studies on Aromatase Inhibitors. I. Synthesis and Biological Evaluation of 4-Amino-4*H*-1,2,4-triazole Derivatives

Minoru OKADA,*[,a] Toru YODEN,[a] Eiji KAWAMINAMI,[a] Yoshiaki SHIMADA,[a] Masafumi KUDOH,[a] Yasuo ISOMURA,[b] Hisataka SHIKAMA,[a] and Takashi FUJIKURA[a]

Endocrinology/Metabolic Disease Laboratories[a] and Infectious Disease/Immunology Research Laboratories,[b] Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba 305, Japan.
Received March 26, 1996; accepted May 28, 1996

Various 4-*N*-substituted amino-4*H*-1,2,4-triazole derivatives were synthesized and evaluated for aromatase-inhibitory activity (*in vitro*) and for pregnant mare serum gonadotropin (PMSG)-induced estrogen synthesis-inhibitory activity (*in vivo*). The 4-(4-cyanophenyl) amino derivative and 4-(4-nitrophenyl)amino derivative, each possessing a strong electron-withdrawing group on the phenyl moiety, showed potent aromatase-inhibitory activity. Structure–activity relationship studies indicated that 4-[(4-bromobenzyl)(4-cyanophenyl)amino]-4*H*-1,2,4-triazole (5k, YM511) is a highly potent aromatase inhibitor with IC_{50} values of 0.4 and 0.12 nM in *in vitro* experiments using rat ovary and human placenta, respectively, and an *in vivo* ED_{50} of 0.002 mg/kg in rats on oral administration. YM511 was also a weak inhibitor of other steroid hormone synthesis enzymes. These data suggest that YM511 is a highly selective aromatase inhibitor and may be a useful agent for the treatment of estrogen-dependent diseases such as breast cancer.

Key words aromatase; estrogen; 4-amino-4*H*-1,2,4-triazole; YM511

Aromatase is a cytochrome P-450 enzyme which is responsible for the conversion of androgen to estrogen in the final step of the steroid biosynthesis cascade. Inhibition of this enzyme is therefore of practical importance in the treatment of estrogen-dependent diseases, for example breast cancer, cancer of the uterine body, endometriosis and uterine myoma.[1-5] Like aromatase, several other steroidogenic enzymes are also cytochrome P-450 enzymes.[5] Therefore, lack of selectivity of P-450 could lead to inhibition of the biosynthesis of other important steroid hormones such as aldosterone, cortisol and testosterone, causing undesirable side effects.

Several non-steroidal aromatase inhibitors are now under clinical trial or in clinical use, including CGS16949A[6] (imidazole type), D1033[7] and CGS20267[8] (1*H*-1,2,4-triazol-1-yl type). Recently, CGS16949A was launched in Japan for the treatment of advanced breast cancer in postmenopausal women. However, this agent was shown to cause a significant decrease in serum aldosterone level,[9] demonstrating that it shows insufficient selectivity for aromatase. Thus, in an attempt to develop more useful aromatase inhibitors, we have focused our attention on not only potency, but also enzyme selectivity.

It is well known that compounds having an aza-hetero ring, such as imidazole,[10] triazole[11,12] and pyridine,[13] show inhibitory activity against aromatase. The sp^2 nitrogen atom in an azole or azine ring binds to the heme iron atom of aromatase.[4] Inhibitory potency and selectivity for aromatase depend on the number and position of sp^2 nitrogen atoms in the hetero ring.[13] During the course of our studies on azole-type compounds as aromatase inhibitors, we found that 4-[bis(4-cyanophenyl)-amino]-4*H*-1,2,4-triazole (**5c**) has good selectivity for aromatase inhibition. Although its aromatase-inhibitory

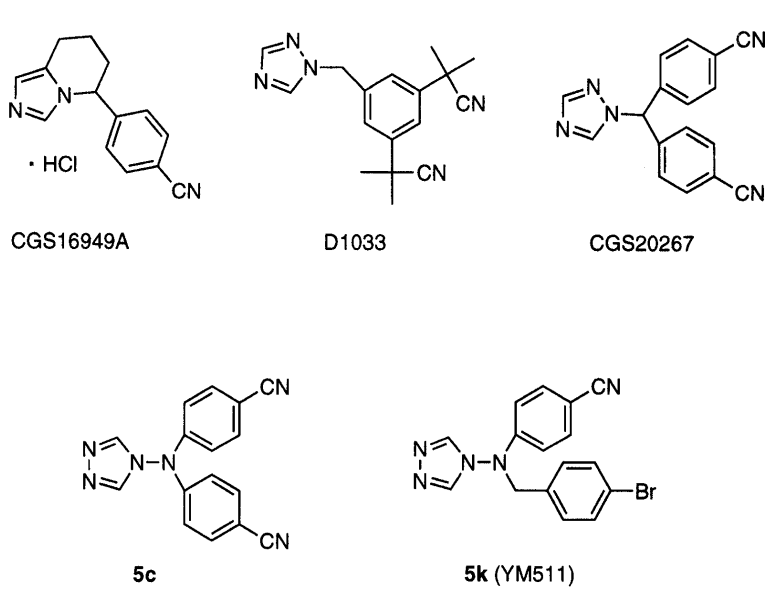


Fig. 1

* To whom correspondence should be addressed.

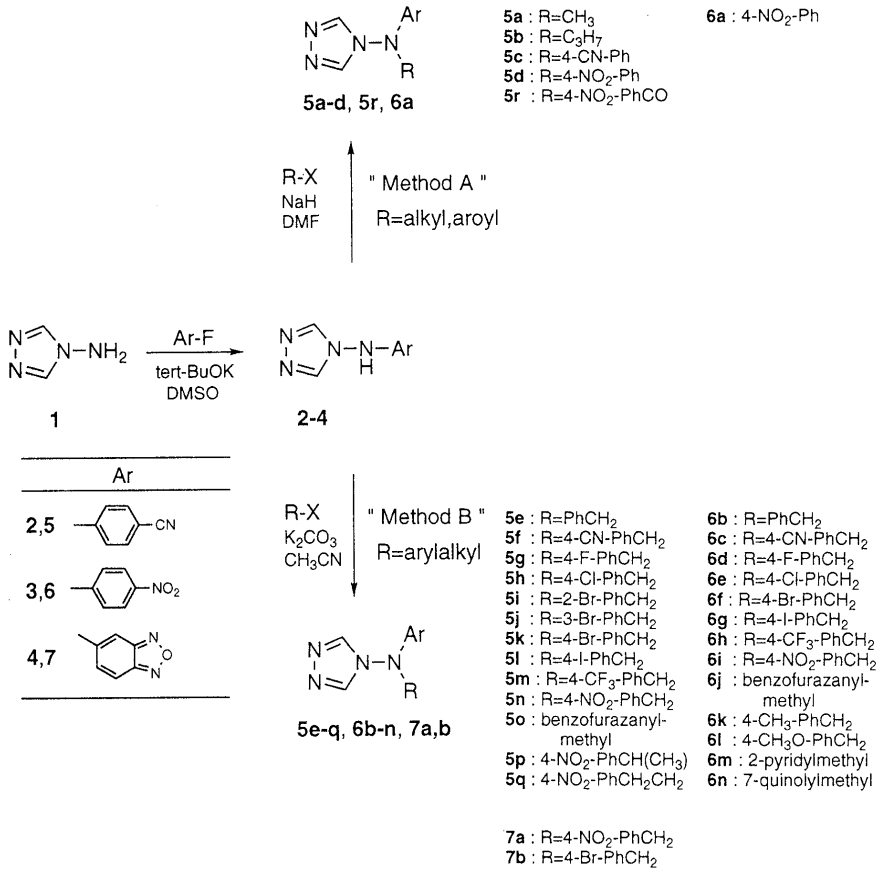


Chart 1

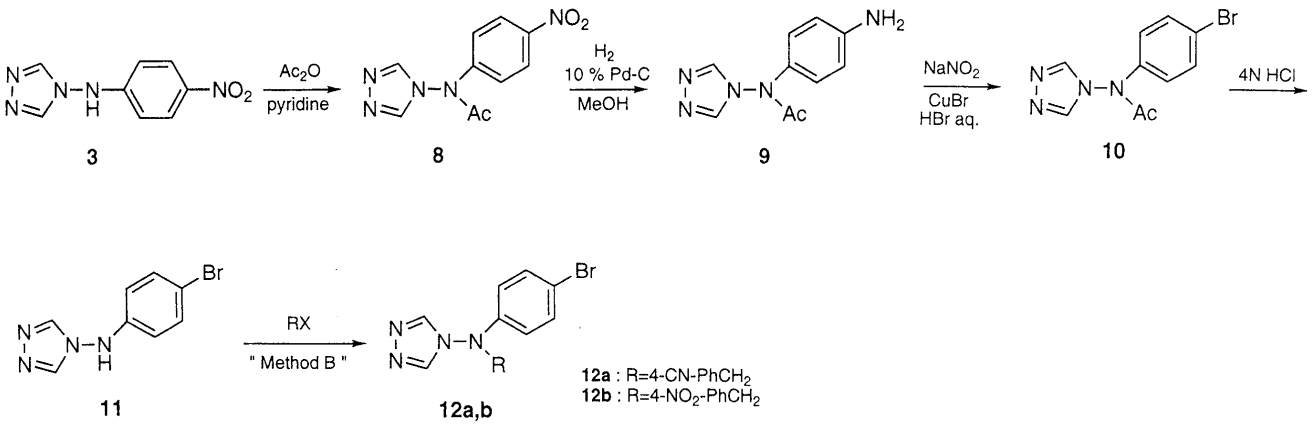


Chart 2

potency was only moderate, its aldosterone synthesis-inhibitory activity was very weak. We therefore selected this compound as the lead compound and synthesized a series of 4-*N*-substituted 4*H*-1,2,4-triazol-4-ylamino derivatives to examine the structure–activity relationships (SARs). Among these compounds, 4-[(4-bromobenzyl)(4-cyanophenyl)amino]-4*H*-1,2,4-triazole (**5k**) (YM511) was a highly potent and selective aromatase inhibitor.

Chemistry

Most of the *N*,*N*-disubstituted derivatives were synthesized by two-step reactions from 4-amino-4*H*-1,2,4-triazole (**1**). General synthetic procedures for the *para*-cyanophenylamino (**5**), *para*-nitrophenylamino (**6**) and 5-benzofurazanylamino derivatives (**7**) are shown in Chart 1, and the synthesis of *para*-bromophenylamino derivatives (**12**) is shown in Chart 2. Excess 4-aminotriazole (**1**) was reacted with fluoroaryl derivatives in the presence of potassium *tert*-butoxide in dimethyl sulfoxide (DMSO) to afford the 4-*N*-mono-substituted amino-4*H*-1,2,4-triazole derivatives (**2**—**4**). *N*,*N*-Diphenyl derivatives (**5c**, **d**, **6a**) were obtained by reaction of **2** and **3** with the fluoroaryl derivatives using sodium hydride as a base (method A). Compounds **5a**, **b** with a short alkyl substituent and compound **5r** with a benzoyl substituent were also synthesized in a similar manner.

Preparation of compounds **5e**—**q**, **6b**—**n**, **7a**, **b** and **12a**, **b** having an arylalkyl group on the amino moiety was performed by the reaction of **2**, **3**, **4** and **11** with arylalkyl halide derivatives in the presence of potassium carbonate

in acetonitrile (method B). The *para*-bromophenylamino derivative (**11**) was synthesized from the nitro derivative (**3**) as shown in Chart 2. The mono-substituted amino group of **3** was protected with an acetyl group, and the protected compound was hydrogenated with 10% palladium on carbon under atmospheric pressure to afford the *para*-aminophenyl derivative (**9**). This amino group was replaced with a bromo group by means of the Sandmeyer reaction to give **10** in moderate yield. Compound **10** was treated with 4 N hydrochloric acid to give the amine derivative (**11**).

Results and Discussion

Inhibitory activities of the series of triazole derivatives on aromatase (*in vitro*), aldosterone synthesis (*in vitro*) and pregnant mare serum gonadotropin (PMSG)-induced estrogen synthesis (*in vivo*) were evaluated. In the *in vitro* rat ovarian microsome assay, aromatase-inhibitory activity of the compounds at concentrations of 1 and 10 nM 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 *p.o.* was expressed as percent inhibition of PMSG-induced estrogen synthesis.

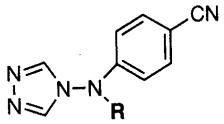
The pharmacological data for these derivatives are summarized in Tables 1—4.

In a series of *para*-cyanophenylamino derivatives (**5**) (Table 1), the unsubstituted compound **2** and compounds **5a** and **5b** with a short alkyl substituent showed no aromatase inhibitory activity *in vitro*. Compounds possessing a phenyl moiety (**5c—r**), except **5r**, exhibited the inhibitory activities *in vitro*, and substituted benzyl derivatives (**5e—q**), except **5e** and **5p**, were more potent inhibitors than phenyl derivatives (**5c, d**). These results indicated that a sufficiently bulky substituent such as a benzene ring is necessary as the R moiety for a potent aromatase inhibitor. Moreover, as shown in Table 4, the inhibitory activities of compounds **5c, d** (phenyl type) and **5f, k** (benzyl type) on aldosterone synthesis were very weak, being comparable to that of CGS16949A. This shows that these compounds have good selectivity for aromatase inhibition.

Introduction of a cyano (**5f**), halogen (**5g, h, k, l**), trifluoromethyl (**5m**) or nitro group (**5n**) at the *para* position of the phenyl ring of the benzyl derivatives increased the aromatase-inhibitory activity. Among them, the *para* bromo-substituted derivative (**5k**) showed the most potent activity both *in vitro* and *in vivo*, while the inhibitory activity of compounds with an *ortho* (**5i**) or a *meta* (**5j**) bromo substituent was weak, especially *in vivo*.

Introduction of a methyl or carbonyl group at the benzylic position of compound **5n** resulted in decreased

Table 1. Physical and Biological Data for 4-[(4-Cyanophenyl)amino]-4*H*-1,2,4-triazole Derivatives



Compd.	R	Method[a]	Yield (%)	mp (°C)	Recryst. solvent	% inhibition of aromatase[b] (*in vitro*, nM) 1	10	% inhibition of PMSG-induced estrogen synthesis[c] (*in vivo*, mg/kg *p.o.*) 0.03	0.3
2	H	[d]	69[e]	206—208	Acetone	NE	NE	NT	NT
5a	CH_3	A	34	184—186	AcOEt–Et_2O	NE	NE	30.0	67.2
5b	C_3H_7	A	59	108—109	AcOEt–Et_2O	NE	NE	19.3	56.7
5c	4-CN–Ph	A	60	218—220	AcOEt	NE	13.9	49.8	81.8
5d	4-NO_2–Ph	A	71	203	EtOH–iso-Pr_2O	NE	24.5	57.2	89.8
5e	$PhCH_2$	B	48	193—194	AcOEt	NE	10.5	21.5	66.5
5f	4-CN–$PhCH_2$	B	48	214—215	EtOH–iso-Pr_2O	14.7	50.1	27.2	87.6
5g	4-F–$PhCH_2$	B	40	193—194	AcOEt	11.9	20.1	38.1	81.6
5h	4-Cl–$PhCH_2$	B	45	172—173	AcOEt	60.2	57.4	71.6	92.2
5i	2-Br–$PhCH_2$	B	46	234—235	MeOH	22.4	43.4	NE	14.2
5j	3-Br–$PhCH_2$	B	45	195—196	MeOH	25.5	51.1	38.8	86.2
5k	4-Br–$PhCH_2$	B	68	201—202	EtOH	68.2	92.0	90.0	97.8
5l	4-I–$PhCH_2$	B	40	242—243	MeOH	28.9	64.9	55.6	89.1
5m	4-CF_3–$PhCH_2$	B	52	157—158	AcOEt	55.3	54.7	68.8	92.2
5n	4-NO_2–$PhCH_2$	B	32	213—215	EtOH	22.1	68.3	76.2	92.3
5o	5-Benzofurazanylmethyl[f]	B	39	200—205	AcOEt	13.9	59.7	58.1	96.9
5p	4-NO_2–$PhCH(CH_3)$	B	33	191—194	EtOH	NE	22.7	NE	20.4
5q	4-NO_2–$PhCH_2CH_2$	B	24	213—218	MeOH	54.7	71.8	57.3	86.8
5r	4-NO_2–PhCO	A	64	235—237	AcOEt–acetone	NE	NE	NT	NT
CGS16949A						37.0	85.2	81.3	92.3

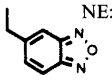
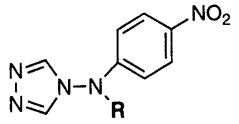
a) A: NaH, DMF B: K_2CO_3, CH_3CN. *b*) % inhibition of aromatization of androstenedione in the *in vitro* rat ovarian microsome assay. Values were determined in a single experiment. Each assay was performed in triplicate. *c*) % inhibition of estrogen synthesis in the *in vivo* rat PMSG-induced estrogen synthesis assay. Each compound was tested in groups of five rats and data represent mean values of peak inhibition. *d*) See experimental section. *e*) Yield is based on 4-fluorobenzonitrile. *f*) 5-Benzofurazanylmethyl:  NE: no effect. NT: not tested.

Table 2. Physical and Biological Data for 4-[(4-Nitrophenylamino]-4*H*-1,2,4-triazole Derivatives



Compd.	R	Method[a]	Yield (%)	mp (°C)	Recryst. solvent	% inhibition of aromatase[b] (*in vitro*, nM) 1	10	% inhibition of PMSG-induced estrogen synthesis[c] (*in vivo*, mg/kg *p.o.*) 0.03	0.3
6a	4-NO_2–Ph	A	46	217—218	MeOH	13.4	38.9	61.7	78.2
6b	$PhCH_2$	B	51	203—204	AcOEt	NE	21.1	NE	16.0
6c	4-CN–$PhCH_2$	B	41	211—212	EtOH	15.6	47.0	62.3	87.2
6d	4-F–$PhCH_2$	B	64	178—179	EtOH–iso-Pr_2O	16.1	33.5	75.0	92.3
6e	4-Cl–$PhCH_2$	B	76	207—208	EtOH	29.4	72.4	77.4	77.9
6f	4-Br–$PhCH_2$	B	45	237	EtOH–acetone	68.0	92.3	91.9	96.9
6g	4-I–$PhCH_2$	B	31	225—226	MeOH	66.1	82.7	17.7	92.0
6h	4-CF_3–$PhCH_2$	B	56	156—157	AcOEt	21.2	63.6	86.3	93.4
6i	4-NO_2–$PhCH_2$	B	41	211—212	EtOH	37.0	85.2	91.3	97.1
6j	5-Benzofurazanylmethyl[d]	B	25	217—218	AcOEt	31.4	75.0	70.4	92.6
6k	4-CH_3–$PhCH_2$	B	24	213	EtOH–iso-Pr_2O	16.9	56.1	63.5	63.8
6l	4-CH_3O–$PhCH_2$	B	49	178	EtOH–iso-Pr_2O	18.3	40.4	13.6	46.4
6m	2-Pyridylmethyl	B	61	137—138	MeOH	53.1	73.4	NE	NE
6n	7-Quinolylmethyl	B	61	198	MeOH–acetone	52.5	76.2	NE	NE

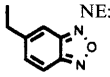
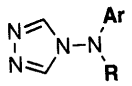
a) A: NaH, DMF B: K_2CO_3, CH_3CN. *b*) % inhibition of aromatization of androstenedione in the *in vitro* rat ovarian microsome assay. Values were determined in a single experiment. Each assay was performed in triplicate. *c*) % inhibition of estrogen synthesis in the *in vivo* rat PMSG-induced estrogen synthesis assay. Each compound was tested in groups of five rats and data represent mean values of peak inhibition. *d*) 5-Benzofurazanylmethyl:  NE: no effect.

Table 3. Physical and Biological Data for 4-(*N*,*N*-Disubstituted amino)-4*H*-1,2,4-triazole Derivatives



Compd.	Ar	R	Method[a]	Yield (%)	mp (°C)	Recryst. solvent	% inhibition of aromatase[b] (*in vitro*, nM) 1	10	% inhibition of PMSG-induced estrogen synthesis[c] (*in vivo*, mg/kg *p.o.*) 0.03	0.3
7a	5-Benzofurazanyl[d]	4-NO_2–$PhCH_2$	B	38	218—220	MeOH	36.2	46.4	18.5	76.5
7b	5-Benzofurazanyl	4-Br–$PhCH_2$	B	50	177—178	EtOH	NE	63.7	37.0	80.8
12a	4-Br–Ph	4-CN–$PhCH_2$	B	79	215—216	MeOH	NE	6.5	NE	49.1
12b	2-Br–Ph	4-NO_2–$PhCH_2$	B	37	205	EtOH	NE	28.2	NE	69.2

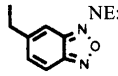
a) B: K_2CO_3, CH_3CN. *b*) % inhibition of aromatization of androstenedione in the *in vitro* rat ovarian microsome assay. Values were determined in a single experiment. Each assay was performed in triplicate. *c*) % inhibition of estrogen synthesis in the *in vivo* rat PMSG-induced estrogen synthesis assay. Each compound was tested in groups of five rats and data represent mean values of peak inhibition. *d*) 5-Benzofurazanylmethyl:  NE: no effect.

Table 4. Inhibitory Activities of Compound **5** and CGS16949A against Aldosterone Synthesis

Compound	% inhibition of aldosterone synthesis[a] (*in vitro*, μM) 1	3
5c	NE	NE
5d	NE	NE
5f	NE	NE
5k	35	52
CGS16949A	99 (IC_{50} = 0.04 μM)	NT

a) % Inhibition of aldosterone synthesis in the *in vitro* rat adrenal cell assay. Values were determined in a single experiment. Each assay was performed in triplicate. NE: no effect. NT: not tested.

activity (**5n** *vs.* **5p**, **5r**), but elongating the benzyl moiety to a phenethyl moiety (**5q**) resulted in retention of potency both *in vitro* and *in vivo*. These results suggested that steric and electronic effects in the benzylic position are the main determinant factors of the inhibitory activities. Compound **5o** having the 5-benzofurazanyl group, which is well known as a bioisostere of the nitrophenyl moiety, exhibited comparable potency to that of the parent compound **5n**.

The inhibitory activities of various *para*-nitrophenylamino derivatives (**6**) are listed in Table 2. Introduction of an electron-withdrawing substituent on the benzene ring of benzyl substituents showed potent activity, as did a series of *para*-cyanophenylamino derivatives (**5**). In particular, compounds **6f** and **6i** showed the most potent

inhibitory activity *in vivo*. In contrast, compound **6l** possessing a methoxy substituent, which has an electron-donating effect, showed remarkably decreased potency. These results suggested that the electron-withdrawing effect on the benzene ring of the benzyl moiety is the strongest determinant of the potency of aromatase-inhibitory activity.

Replacement of the benzene ring with a hetero aryl ring, such as a pyridyl group (**6m**) resulted in no loss of potency of aromatase inhibition *in vitro*, but no inhibitory activity was seen *in vivo* even at a dosage of 0.3 mg/kg. Compound **6n** having a quinolyl hetero ring also exhibited no inhibitory activity *in vivo*, although it had distinct *in vitro* activity. These observations suggested that introduction of an aza-hetero ring as the R moiety is disadvantageous from the viewpoint of pharmacokinetic properties *in vivo*.

From the results of these two series (Tables 1, 2), it was clear that phenylamino derivatives possessing a strong electron-withdrawing group (*e.g.*, cyano or nitro substituent) on the *para* position of the phenyl ring exhibited potent aromatase-inhibitory activity. To study the degree of influence of the substituent on the potency, we investigated other electron-withdrawing substituents, such as halogen and benzofurazanyl (Table 3). 5-Benzofurazanyl ring analogs (**7a**, **7b**) showed moderate activity *in vitro* and *in vivo*. On the other hand, the *para*-bromophenyl compounds **12a** and **12b** were about 10 times less potent than the corresponding nitrophenylamino derivatives (**6c**, **6i**), respectively. These observations demonstrated that a very strong electron-withdrawing group, that is a cyano or nitro group, is indispensable on the phenylamino moiety for high potency.

From the SAR studies in this series, we selected compound **5k** (YM511) as a highly potent, novel aromatase inhibitor and further evaluated its effect on various steroid hormone synthesis activities *in vitro* and *in vivo* in comparison with those of other aromatase inhibitors.

The results of these evaluations are summarized in Table 5. YM511 inhibited aromatase in human placental and rat ovarian microsomes with IC_{50} values of 0.12 and 0.4 nM, respectively. Thus, YM511 is about 3 times more potent than CGS16949A and CGS20267. A kinetic analysis of human placental aromatase inhibition by YM511 is shown in Fig 2. Lineweaver–Burk plots indicated that YM511 was a competitive, reversible inhibitor with a K_i of 0.11 nM.

YM511 inhibited aldosterone synthesis, cortisol synthesis and testosterone synthesis with IC_{50} values of 2.19, 3.9 and 52.9 μM, respectively, indicating that its inhibitory activity against synthesis of other steroids is very weak in comparison with its aromatase-inhibitory activity. In particular, with regard to its inhibitory activity on aldosterone synthesis, the aldosterone/aromatase inhibitory activity ratios of CGS16949A, CGS20267 and YM511 were 27, 1500 and 5500, respectively, showing that YM511 has more than 200 times and 3 times greater selectivity for aromatase inhibition than CGS16949A and CGS20267, respectively.

Further, YM511 showed good enzyme selectivity *in vivo*. YM511 inhibited estrogen synthesis with an ED_{50} of 0.002 mg/kg *p.o.*, while its inhibitory activities for aldosterone and cortisol synthesis were extremely weak, being undetectable even at a dose of 100 mg/kg. In contrast, CGS16949A and CGS20267 significantly inhibited the synthesis of aldosterone with ED_{50} values of 0.26 and 32.6 mg/kg, respectively. These results suggested that YM511 is the most potent and selective aromatase inhibitor among the three compounds.

In conclusion, we have identified a novel series of 4*H*-1,2,4-triazol-4-ylamino derivatives which are potent aromatase inhibitors. SAR studies of this series have led to the conclusion that compound **5k** (YM511) is optimal

Table 5. Comparison of the Inhibitory Activities of Compound **5k** (YM511) and Other Aromatase Inhibitors on the Synthesis of Various Steroid Hormones

In vitro

Compound	Inhibition of aromatase IC_{50} (nM)[a,c]		Inhibition of synthesis IC_{50} (μM)[b,c,d]		
			Aldosterone	Cortisol	Testosterone
	Rat ovarian microsomes	Human placental microsomes	Rat adrenal cells	Rabbit adrenal cells	Rat testicular cells
5k (YM511)	0.4	0.12	2.19	3.9	52.9
CGS16949A	1.5	0.37	0.04	2.4	1.8
CGS20267	1.8	0.39	2.61	1.6	18.4

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 reference 20.

In vivo

Compound	Inhibition of synthesis ED_{50} (mg/kg)[a,b]		
	PMSG-induced estrogen synthesis in rats	ACTH-induced aldosterone synthesis in rats	ACTH-induced cortisol synthesis in guinea pigs
5k (YM511)	0.002	NE (100 mg/kg)	NE (100 mg/kg)
CGS16949A	0.006	0.26	NT
CGS20267	0.003	32.6	NT

a) Dose required to decrease the concentration of estrogen in ovary or the serum concentration of aldosterone or cortisol by 50%. In each experiment 5—65 animals were used. *b*) See reference 20. NE: no effect. NT: not tested.

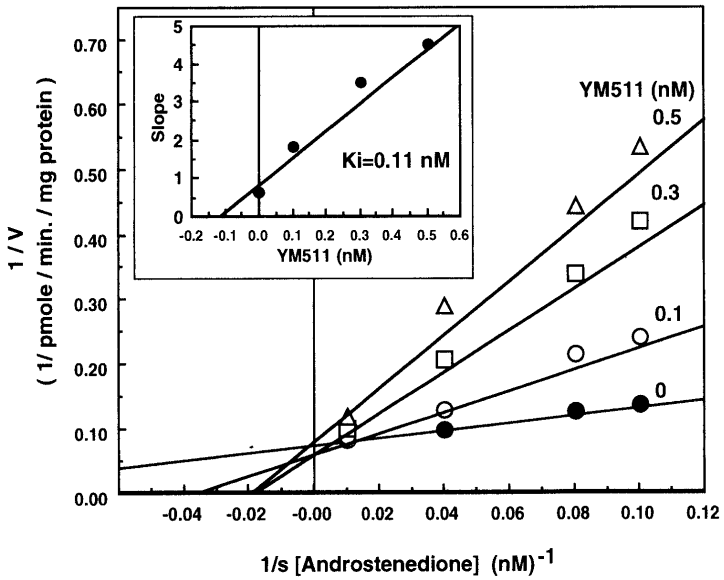


Fig. 2. Kinetic Analysis of the Mechanism of Inhibition of Androstenedione Aromatization in Human Placental Microsomes by YM511 (Lineweaver–Burk Plots)

The results are mean values of three experiments.

in this series. YM511 showed potent and highly selective aromatase inhibition, and is currently under clinical study.

Experimental

Melting points were determined on a Yanaco MP-500D micro 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).

4-[(4-Cyanophenyl)amino]-4*H*-1,2,4-triazole (2) 4-Amino-4*H*-1,2,4-triazole (**1**) (1.68 g, 0.02 mol) was added portionwise to a suspension of potassium *tert*-butoxide (22.4 g, 0.02 mol) in DMSO (10 ml) at 10—15 °C with stirring. The mixture was stirred for 30 min at room temperature, and then 4-fluorobenzonitrile (1.21 g, 0.01 mol) in DMSO (3 ml) was added dropwise below 30 °C. The mixture was stirred for 30 min at room temperature, 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 (100 : 1) gave a crystalline product, which was recrystallized from acetone to give **2** (1.33 g, 72%). mp 206—208 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.57 (2H, d, J=9 Hz), 7.69 (2H, d, J=9 Hz), 8.83 (2H, s). FAB-MS m/z: 186 $(M+H)^+$.

4-[(4-Nitrophenyl)amino]-4*H*-1,2,4-triazole (3) and 5-[(4*H*-1,2,4-Triazole-4-yl)amino]benzofurazan (4) Compounds **3** and **4** were prepared from **1** with 4-fluoronitrobenzene and 5-fluorobenzofurazane, respectively, in a similar manner to that described for compound **2**.

Compound **3**: Yield 61%. mp 176—178 °C (recrystallized from EtOH). ^{1}H-NMR (DMSO-d_6) δ: 6.61 (2H, d, J=9 Hz), 8.17 (2H, d, J=9 Hz), 8.88 (2H, s), 10.52 (1H, s), EI-MS m/z: 205 (M^+).

Compound **4**: Yield 78%. mp 250—253 °C (recrystallized from EtOH). ^{1}H-NMR (DMSO-d_6) δ: 6.09 (1H, s), 7.29 (1H, dd, J=10, 2 Hz), 8.05 (1H, d, J=10 Hz), 8.90 (2H, s). EI-MS m/z: 202 (M^+).

4-[Bis(4-cyanophenyl)amino]-4*H*-1,2,4-triazole (5c). Method A Compound **2** (0.3 g, 1.6 mmol) was added to a suspension of sodium hydride (60% in mineral oil, 65 mg, 1.6 mmol) in *N*,*N*-dimethylformamide (DMF) (5 ml) with ice-cooling. The mixture was stirred for 30 min at 40—50 °C, and cooled to room temperature. 4-Fluorobenzonitrile (0.2 g, 1.62 mmol) was added and the reaction mixture was stirred for 2 h at 80 °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 $CHCl_3$–MeOH (100 : 1) eluate gave a crystalline product, which was recrystallized from AcOEt to give **5c** (0.28 g, 60%). Using this procedure, compounds **5a, b, d, r** and **6a** in Tables 1 and 2 were synthesized.

4-[Benzyl(4-cyanophenyl)amino]-4*H*-1,2,4-triazole (5e). Method B A mixture of compound **2** (0.5 g, 2.7 mmol), benzyl bromide (0.46 g, 2.7 mmol) and K_2CO_3 (0.75 g, 5.4 mmol) in CH_3CN (20 ml) was stirred for 2 h at room temperature. The reaction mixture was diluted with water and extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography. Elution with $CHCl_3$–MeOH (100 : 1) gave a crystalline product, which was recrystallized from AcOEt to give **5e** (0.36 g, 48%). Using this procedure, compounds **5f—q, 6b—n, 7a, b** and **12a, b** in Tables 1, 2 and 3 were synthesized.

4-[Acetyl(4-nitrophenyl)amino]-4*H*-1,2,4-triazole (8) Acetic anhydride (47.2 ml, 0.5 mol) was added dropwise to a solution of compound **3** (10.26 g, 0.05 mol) in pyridine (200 ml), and the mixture was stirred for 30 min at room temperature. Removal of the solvent under reduced pressure afforded a residue, which was taken up in water and extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and evaporated *in vacuo* to give **8** (10.61 g, 86%) as a foam. ^{1}H-NMR ($CDCl_3$) δ: 2.13 (3H, s), 7.49 (2H, d, J=9 Hz), 8.28 (2H, d, J=9 Hz), 8.52 (3H, s). EI-MS m/z: 274 (M^+).

4-[Acetyl(4-aminophenyl)amino]-4*H*-1,2,4-triazole (9) Palladium on carbon (10%, 0.05 g) was added to a solution of compound **8** (0.38 g, 1.5 mmol) in MeOH (15 ml) and the mixture was stirred under H_2 gas at atmospheric pressure and room temperature. After the theoretical amount of hydrogen had been consumed, the mixture was filtered and the filtrate was concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 50 : 1) to give **9** (0.3 g, quant.) as a foam. ^{1}H-NMR (DMSO-d_6) δ: 1.97 (3H, s), 5.53 (2H, br), 6.58 (2H, d, J=9 Hz), 7.35 (2H, d, J=9 Hz), 8.88 (2H, s). EI-MS m/z: 217 (M^+).

4-[Acetyl(4-bromophenyl)amino]-4*H*-1,2,4-triazole (10) A solution of sodium nitrite (0.1 g, 1.47 mmol) in water (1 ml) was added dropwise to a solution of compound **9** (0.32 g, 1.47 mmol) in 47% hydrobromic acid (3 ml) at 0—5 °C, and the mixture was stirred for 20 min at 0—5 °C. The reaction mixture was poured into a solution of cuprous bromide (0.53 g, 3.7 mmol) in 47% hydrobromic acid (1 ml) with ice-cooling, and stirred for 20 h at room temperature. The mixture was neutralized with a solution of sodium hydrogen carbonate and extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. The crystalline residue was washed with ether to give **10** (0.29 g, 71%). ^{1}H-NMR (DMSO-d_6) δ: 2.00 (3H, s), 7.45—7.95 (4H, m), 9.06 (2H, s). EI-MS m/z: 281 (M^+).

4-[(4-Bromophenyl)amino]-4*H*-1,2,4-triazole (11) A solution of com-

Table 6. Physical and Spectral Data for Compounds **5a—r**

Compd.	Formula	Analysis (%) Calcd (Found) C	H	N	X	^{1}H-NMR (DMSO-d_6) δ	MS m/z
5a	$C_{10}H_9N_5$	60.29 (60.24	4.55 4.66	35.15 35.12)		3.56 (3H, s), 6.60 (2H, d, J=9 Hz), 7.60 (2H, d, J=9 Hz), 8.41 (2H, s)[a]	199 (M^+)
5b	$C_{12}H_{13}N_5$	63.42 (63.41	5.77 5.82	30.82 30.77)		1.03 (3H, t, J=7 Hz), 1.45—1.76 (2H, m), 3.67 (2H, dd, J=7, 7 Hz), 6.54 (2H, d, J=9 Hz), 7.56 (2H, d, J=9 Hz), 8.33 (2H, s)[a]	227 (M^+)
5c	$C_{16}H_{10}N_6$	67.13 (66.92	3.52 3.62	29.35 29.23)		7.04 (4H, d, J=9 Hz), 7.69 (4H, d, J=9 Hz), 8.44 (2H, s)[a]	286 (M^+)
5d	$C_{15}H_{10}N_6O_2$	58.82 (58.79	3.29 3.46	27.44 27.37)		6.98—7.16 (4H, m), 7.72 (2H, d, J=9 Hz), 8.26 (2H, d, J=9 Hz), 8.46 (2H, s)[a]	307 $(M+H)^+$
5e	$C_{16}H_{13}N_5$	69.80 (69.66	4.76 4.84	25.44 25.43)		5.07 (2H, s), 6.76 (2H, d, J=9 Hz), 7.32 (5H, s), 7.76 (2H, d, J=8 Hz), 8.80 (2H, s)	275 (M^+)
5f	$C_{17}H_{12}N_6$	67.99 (67.91	4.03 3.96	27.98 27.97)		4.98 (2H, s), 6.64 (2H, d, J=9 Hz), 7.26—7.74 (6H, m), 8.20 (2H, s)[a]	300 (M^+)
5g	$C_{16}H_{12}FN_5$	65.52 (65.53	4.12 4.16	23.88 23.93	6.48 (X=F) 6.43)	5.05 (2H, s), 6.77 (2H, d, J=9 Hz), 7.04–7.44 (4H, m), 7.76 (2H, d, J=9 Hz), 8.78 (2H, s)	293 (M^+)
5h	$C_{16}H_{12}ClN_5$	62.04 (61.97	3.90 4.10	22.61 22.59	11.45 (X=Cl) 11.26)	5.07 (2H, s), 6.75 (2H, d, J=9 Hz), 7.37 (4H, s), 7.76 (2H, d, J=9 Hz), 8.80 (2H, s)	309 (M^+)
5i	$C_{16}H_{12}BrN_5$	54.26 (54.10	3.41 3.32	19.77 19.85	22.56 (X=Br) 22.72)	5.14 (2H, s), 6.75 (2H, d, J=9 Hz), 7.27—7.36 (3H, m), 7.65 (1H, d, J=7 Hz), 7.78 (2H, d, J=9 Hz), 8.80 (2H, s)	354 (M^+)
5j	$C_{16}H_{12}BrN_5$	54.26 (54.16	3.41 3.29	19.77 19.89	22.56 (X=Br) 22.59)	5.09 (2H, s), 6.75 (2H, d, J=9 Hz), 7.27—7.34 (2H, m), 7.50 (1H, d, J=7 Hz), 7.77 (2H, d, J=9 Hz), 8.86 (2H, s)	354 (M^+)
5k	$C_{16}H_{12}BrN_5$	54.26 (53.96	3.41 4.48	19.77 19.72	22.56 (X=Br) 22.65)	5.06 (2H, s), 6.75 (2H, d, J=9 Hz), 7.27 (2H, d, J=9 Hz), 7.53 (2H, d, J=9 Hz), 7.75 (2H, d, J=9 Hz), 8.81 (2H, s)	354 (M^+)
5l	$C_{16}H_{12}IN_5$	47.97 (47.76	3.01 3.05	17.46 17.46	31.63 (X=I) 31.51)	5.03 (2H, s), 6.74 (2H, d, J=9 Hz), 7.13 (2H, d, J=8 Hz), 7.68 (2H, d, J=8 Hz), 7.76 (2H, d, J=9 Hz), 8.88 (2H, s)	401 (M^+)
5m	$C_{17}H_{12}F_3N_5$	59.48 (59.40	3.52 3.59	20.40 20.41	16.60 (X=F) 16.48)	5.20 (2H, s), 6.75 (2H, d, J=9 Hz), 7.58 (2H, d, J=8 Hz), 7.71 (2H, d, J=9 Hz), 8.20 (2H, d, J=9 Hz), 8.88 (2H, s)	343 (M^+)
5n	$C_{16}H_{12}N_6O_2$	60.00 (59.75	3.78 3.71	26.24 26.28)		5.27 (2H, s), 6.74 (2H, d, J=9 Hz), 7.65 (2H, d, J=9 Hz), 7.77 (2H, d, J=9 Hz), 8.20 (2H, d, J=9 Hz), 8.90 (2H, s)	320 (M^+)
5o	$C_{16}H_{11}N_7O$	60.56 (60.56	3.49 4.41	30.90 31.05)		5.27 (2H, s), 6.75 (2H, d, J=9 Hz), 7.61 (1H, d, J=9 Hz), 7.78 (2H, d, J=9 Hz), 8.02 (1H, s), 8.08 (1H, d, J=9 Hz), 8.99 (2H, s)	317 (M^+)
5p	$C_{17}H_{14}N_6O_2$	61.07 (60.92	4.22 4.27	25.14 25.11)		1.48 (3H, d, J=9 Hz), 5.88 (1H, q, J=7 Hz), 6.66 (2H, d, J=9 Hz), 7.68 (2H, d, J=9 Hz), 7.74 (2H, d, J=9 Hz), 8.20 (2H, d, J=9 Hz), 8.88 (2H, s)	334 (M^+)
5q	$C_{17}H_{14}N_6O_2$	61.07 (61.01	4.22 4.26	25.14 25.14)		2.99 (2H, t, J=7 Hz), 4.18 (2H, t, J=7 Hz), 6.26 (2H, d, J=9 Hz), 7.61 (2H, d, J=9 Hz), 7.72 (2H, d, J=9 Hz), 8.17 (2H, d, J=9 Hz), 8.88 (2H, s)	334 (M^+)
5r	$C_{17}H_{10}N_6O$	64.96 (64.81	3.21 3.35	26.74 26.72)		7.61 (2H, d, J=9 Hz), 7.77—7.99 (6H, m), 9.13 (2H, s)	314 (M^+)

a) Measured in $CDCl_3$.

pound **10** (0.22 g, 0.78 mmol) in 4 N hydrochloric acid (5 ml) was heated for 40 min at 90 °C, then concentrated under reduced pressure. The residue was taken up in water, neutralized with a solution of saturated sodium hydrogen carbonate, and extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was subjected to silica gel column chromatography, and elution with $CHCl_3$–MeOH (50 : 1) gave a crystalline product **11** (0.18 g, 96%). ^{1}H-NMR (DMSO-d_6) δ: 6.45 (2H, d, J=9 Hz), 7.41 (2H, d, J=9 Hz), 8.77 (2H, s), 9.62 (1H, s). EI-MS m/z: 239 (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 ovarian microsomes (160 μg/ml, specific activity 0.021 pmol/min/mg of protein) in potassium phosphate buffer[14] (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[15] or 5 mM NADPH.[16] The reaction mixture was treated with $CHCl_3$ and 2.5% 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 in the solvent control. The IC_{50} was obtained from a line drawn by the least-squares method. Using this method, aromatase inhibitory activity of human placental microsomes was measured ([1β,2β-^{3}H]androstendione 1.04 nmol, human placental microsomes 7.5 μg/ml, specific activity 0.351 pmol/min/mg of protein).

Inhibition of Aldosterone Synthesis Aldosterone synthesis activity was measured according to the method described by De Coster *et al.*[17] Rat adrenal cells suspended (3×10^5 cells/ml) in 199 medium containing 0.2% bovine serum albumin were preincubated at 37 °C for 30 min with various concentrations of test compounds dissolved in DMSO, then incubated for 2 h with 1 ng/ml ACTH to stimulate aldosterone synthesis. The amount of aldosterone released from the cells in the presence or absence of the test compound was measured by radioimmunoassay. The inhibitory activity of test compounds was obtained as the percentage inhibition 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 by literature methods.[18,19] Briefly, female rats (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. ED_{50} was expressed as the dose required to decrease the concentration of estrogen in the ovaries by 50%.

Table 7. Physical and Spectral Data for Compounds **6a—n**

Compd.	Formula	Analysis (%) Calcd (Found) C	H	N	X	^{1}H-NMR (DMSO-d_6) δ	MS m/z
6a	$C_{14}H_{10}N_6O_4$	51.54 (51.59)	3.09 (3.14)	25.76 (25.80)		7.24 (4H, d, J=9 Hz), 8.30 (4H, d, J=9 Hz), 9.28 (2H, s)	326 (M^+)
6b	$C_{15}H_{13}N_5O_2$	61.01 (60.68)	4.44 (4.49)	23.72 (23.67)		5.13 (2H, s), 6.79 (2H, d, J=9 Hz), 7.33 (5H, s), 8.20 (2H, d, J=9 Hz), 8.83 (2H, s)	295 (M^+)
6c	$C_{16}H_{12}N_6O_2$	60.00 (59.94)	3.78 (3.98)	26.24 (26.21)		5.27 (2H, s), 6.76 (2H, d, J=9 Hz), 7.57 (2H, d, J=9 Hz), 7.84 (2H, d, J=9 Hz), 8.20 (2H, d, J=9 Hz), 8.91 (2H, s)	320 (M^+)
6d	$C_{15}H_{12}FN_5O_2$	57.51 (57.44)	3.86 (3.98)	22.35 (22.27)	6.06 (X=F) (5.85)	5.12 (2H, s), 6.81 (2H, d, J=9 Hz), 7.05—7.46 (4H, m), 8.20 (2H, d, J=9 Hz), 8.81 (2H, s)	313 (M^+)
6e	$C_{15}H_{12}ClN_5O_2$	54.64 (54.59)	3.67 (3.85)	21.24 (21.13)	10.75 (X=Cl) (10.72)	5.14 (2H, s), 6.79 (2H, d, J=9 Hz), 7.36 (2H, d, J=9 Hz), 7.40 (2H, d, J=9 Hz), 8.20 (2H, d, J=9 Hz), 8.84 (2H, s)	329 (M^+)
6f	$C_{15}H_{12}BrN_5O_2$	48.15 (48.07	3.23 (3.27	18.72 (18.74	21.35 (X=Br) (20.64)	5.12 (2H, s), 6.79 (2H, d, J=9 Hz), 7.29 (2H, d, J=9 Hz), 7.54 (2H, d, J=9 Hz), 8.19 (2H, d, J=9 Hz), 8.84 (2H, s)	374 (M^+)
6g	$C_{15}H_{12}IN_5O_2$	42.77 (42.68	2.87 (3.01	16.63 (16.46	30.13 (X=I) (30.26)	5.10 (2H, s), 6.78 (2H, d, J=9 Hz), 7.14 (1H, d, J=9 Hz), 7.70 (2H, d, J=9 Hz), 8.19 (2H, d, J=9 Hz), 8.84 (2H, s)	422 $(M+H)^+$
6h	$C_{16}H_{12}F_3N_5O_2$	52.90 (52.88	3.33 (3.36	19.28 (19.38	15.69 (X=F) (15.60)	5.27 (2H, s), 6.78 (2H, d, J=7 Hz), 7.59 (2H, d, J=8 Hz), 7.72 (2H, d, J=9 Hz), 8.21 (2H, d, J=9 Hz), 8.84 (2H, s)	363 (M^+)
6i	$C_{15}H_{12}N_6O_4$	52.94 (52.94	3.55 (3.62	24.70 (25.02)		5.33 (2H, s), 6.77 (2H, d, J=9 Hz), 7.66 (2H, d, J=9 Hz), 8.20 (4H, d, J=8.93 (2H, s)	340 (M^+)
6j	$C_{15}H_{11}N_7O_3$	53.41 (53.27	3.29 (3.38	29.07 (29.08)		5.33 (2H, s), 6.78 (2H, d, J=7 Hz), 7.61 (1H, d, J=9 Hz), 8.04 (1H, s), 8.09 (1H, d, J=9 Hz), 8.21 (2H, d, J=7 Hz), 9.03 (2H, s)	337 (M^+)
6k	$C_{16}H_{15}N_5O_2$	62.13 (61.87	4.89 (5.00	22.64 (22.43)		2.34 (3H, s), 4.90 (2H, s), 6.68 (2H, d, J=7 Hz), 7.08 (2H, d, J=8 Hz), 7.16 (2H, d, J=8 Hz), 8.10 (2H, s), 8.19 (2H, d, J=7 Hz)[a]	309 (M^+)
6l	$C_{16}H_{15}IN_5O_3$	59.07 (59.05	4.65 (4.61	21.53 (21.50)		3.73 (3H, s), 5.04 (2H, s), 6.76—6.92 (4H, m), 7.22 (2H, d, J=9 Hz), 8.19 (2H, d, J=9 Hz), 8.75 (2H, s)	325 (M^+)
6m	$C_{14}H_{12}N_6O_2$	56.75 (56.67	4.08 (4.23	28.36 (28.36)		5.23 (2H, s), 6.72 (2H, d, J=9 Hz), 7.40 (2H, d, J=6 Hz), 8.19 (2H, d, J=9 Hz), 8.55 (2H, d, J=6 Hz), 8.97 (2H, s)	297 $(M+H)^+$
6n	$C_{18}H_{14}N_6O_2$	62.42 (62.42	4.07 (4.22	24.26 (24.30)		5.52 (2H, s), 6.70 (2H, d, J=8 Hz), 7.61 (1H, t, J=6 Hz), 7.67 (1H, d, J=7 Hz), 7.76 (1H, t, J=6 Hz), 7.98—8.03 (2H, m), 8.42 (1H, d, J=7 Hz), 9.08 (2H, s)	347 $(M+H)^+$

a) Measured in $CDCl_3$.

Table 8. Physical and Spectral Data for Compounds **7a, b** and **12a, b**

Compd.	Formula	Analysis (%) Calcd (Found) C	H	N	X	^{1}H-NMR (DMSO-d_6) δ	MS m/z
7a	$C_{15}H_{11}N_7O_3$	53.41 (53.13)	3.29 (3.28)	29.07 (29.10)		5.29 (2H, s), 7.04 (1H, dd, J=10, 2 Hz), 7.15 (1H, d, J=2 Hz), 7.69 (2H, d, J=9 Hz), 8.05 (1H, d, J=10 Hz), 8.21 (1H, d, J=10 Hz), 8.92 (2H, s)	337 (M^+)
7b	$C_{15}H_{11}BrN_6O$	48.54 (48.36	2.99 (3.03	22.64 (22.71	21.53 (X=Br) (21.67)	5.07 (2H, s), 7.02 (1H, dd, J=10, 2 Hz), 7.18 (1H, d, J=2 Hz), 7.31 (2H, d, J=8 Hz), 7.54 (2H, d, J=8 Hz), 8.03 (1H, d, J=10 Hz), 8.83 (2H, s)	370 (M^+)
12a	$C_{16}H_{12}BrN_5$	54.26 (54.17	3.41 (3.55	19.77 (19.70	22.56 (X=Br) (22.43)	5.07 (2H, s), 6.66 (2H, d, J=9 Hz), 7.45—7.90 (6H, m), 8.84 (2H, s)	354 (M^+)
12b	$C_{15}H_{12}BrN_5O_2$	48.15 (48.08	3.23 (3.39	18.72 (18.66	21.35 (X=Br) (21.19)	5.13 (2H, s), 6.68 (2H, d, J=9 Hz), 7.51 (2H, d, J=9 Hz), 7.65 (2H, d, J=9 Hz), 8.19 (2H, d, J=9 Hz), 8.88 (2H, s)	374 (M^+)

Acknowledgment We are grateful to the staff of the Division of Molecular Chemistry Research Laboratories for measurement of ^{1}H-NMR, MS and elemental analyses.

References

1) Fishman J., *Cancer Res.*, **42** (Suppl.), 3277—3280 (1982).
2) Ibrahim N. K., Buzdar A. U., *Am. J. Clin. Oncol.*, **18**, 407—417 (1995).
3) Bossche H. V., *J. Steroid. Biochem. Molec. Biol.*, **43**, 1003—1021 (1992).
4) Cole P. A., Robinson C. H., *J. Med. Chem.*, **33**, 2939—2942 (1990).
5) Nelson D. R., Kamataki T., Waxman D. J., Guengerich F. P., Estabrook R. W., Feyereisen R., Gonzalez F. J., Coon M. J., Gunsalus I. C., Gotoh O., Okuda K., Nebert D. W., *DNA Cell Biol.*, **12**, 1—51 (1993).
6) Raats B. J., Falkson G., Falkson H. C., *J. Clin. Oncol.*, **10**, 111—116 (1992).
7) Plourde P. V., Dyroff M., Dukes M., *Breast Cancer Res. Treat.*, **30**, 103—111 (1994).
8) Iveson T. J., Smith I. E., Ahern J., Smithers D. A., Trunet P. F., Dowsett M., *Cancer Res.*, **53**, 266—270 (1993).
9) Dowsett M., Stein R. C., Mehta A., Coombes R. C., *Clin. Endocr.*, **32**, 623—634 (1990).
10) Browne L. J., Gude C., Rodriguez H., Steel R. E., *J. Med. Chem.*, **34**, 725—736 (1991).
11) Bhatnager A. S., Häusler A., Schieweck K., Lang M., Bowman R., *J. Steroid Biochem. Molec. Biol.*, **37**, 1021—1027 (1990).
12) Bossch H. V., Willemsens G., Roels I., Bellens D., Moereels H.,

Coene M. C., Jeune L. L., Lauwers W., Paul A. J., *Biochem. Pharm.*, **40**, 1707—1718 (1990).
13) Jones C. D., Winter M. A., Hirsch K. S., Stamm N., Taylor H. M., Holden H. E., Davenport J. D., Krumkalns E. V., Suhr R. G., *J. Med. Chem.*, **33**, 416—429 (1990).
14) Steel R. E., Mellor L. B., Sawyer W. K., Wasvary J. M., Browne L. J., *Steroids*, **50**, 147—161 (1987).
15) Bullion K. A., Osawa Y., Braun D. G., *Endocr. Res.*, **16**, 255—267 (1990).
16) Hirsch K. S., Jones C. D., Lindstrom T. D., Stamm N. B., Sutton G. P., Talor H. M., Weaver D. E., *Steroids*, **50**, 201—217 (1987).
17) De Coster R., Wouters W., Beerens D., Haelterman C., Doolege R., Goemine N., Krekels M., *J. Vet. Pharmac. Ther.*, **11**, 345—353 (1988).
18) Steel R. E., Mellor L. B., Sawyer W. K., Wasvary J. M., Browne L. J., *Steroids.*, **50**, 147—161 (1987).
19) Wouters W., De Coster R., Krekels M., Van Dun J., Beerens D., Haelterman C., Raeymaekers A., Freyne E., Van Gelder J., Venet M., Janssen P. A., *J. Steroid Biochem.*, **32**, 781—788 (1989).
20) Kudoh M., Susaki Y., Ideyama Y., Nanya T., Okada M., Shikama H., Fujikura T., *J. Steroid Biochem. Molec. Biol.*, **54**, 265—271 (1995).