Synthesis and Aromatase Inhibitory Activity of Novel 1-(4-Aminophenyl)-3-azabicyclo[3.1.0]hexane- and -[3.1.1]heptane-2,4-diones

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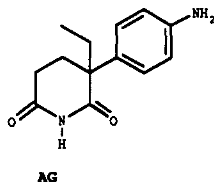
The synthesis of 3-(cyclohexylmethyl)-1-(4-aminophenyl)-3-azabicyclo[3.1.0]hexane-2,4-dione (**1h**), with its optical enantiomers, and a series of novel achiral 1-(4-aminophenyl)-3-azabicyclo[3.1.1]haptane-2,4-diones (**2a–i,k**) is described. These compounds were tested in vitro for inhibition of human placental aromatase, a cytochrome-P_{450}-dependent enzyme responsible for the conversion of androgens to estrogens. All of them displayed enzyme-inhibiting activity, and 3-cyclohexyl derivative **2g** and 3-cyclohexylmethyl derivative **1h** both proved more potent (>140-fold) than the clinically effective agent aminoglutethimide [3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione, AG]. As with AG and its derivatives, the 1*R*-(+)-enantiomer of **1h** was responsible for the enzyme inhibitory activity. These novel compounds are of interest as potential drugs for endocrine therapy of hormone-dependent tumors, e.g. breast cancer.

Introduction

Aromatase is a cytochrome-P_{450}-dependent enzyme which catalyzes the conversion of androgens to estrogens.[1,2] Its inhibition, leading to the reduction of circulating estrogens, is of practical importance in the treatment of hormone-dependent tumors, which comprise 30–40% of all cases of breast cancer.[3,4] These endocrine effects have already been demonstrated experimentally and clinically with some compounds of different structures.[5] One of the best known drugs, aminoglutethimide [3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione, AG] was introduced

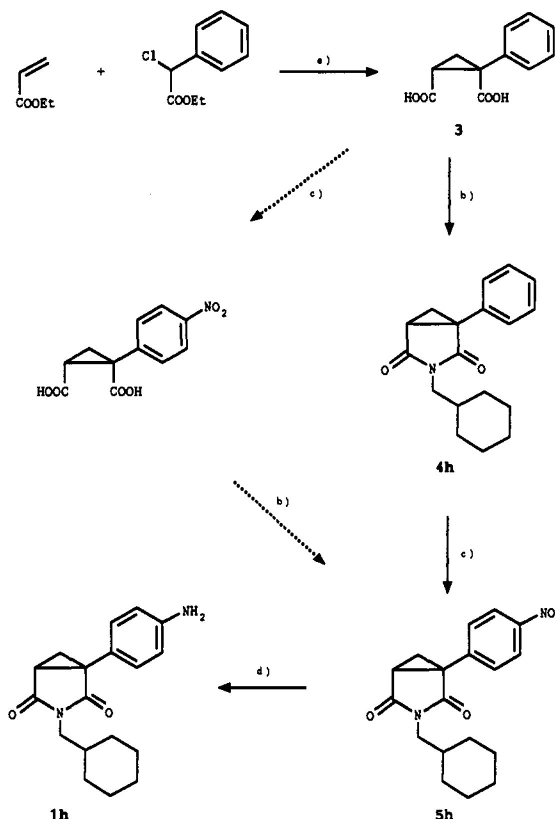


on the market in 1981. Meanwhile, two further selective and even more potent compounds, 4-hydroxyandrostene-3,17-dione[6] and 4-(5,6,7,8-tetrahydroimidazo[1,5-*a*]-pyridin-5-yl)benzonitrile[7,8] are in phase III clinical trials. Despite the availability of these substances, it seemed worthwhile to synthesize further analogues of AG with a view to obtaining compounds possessing greater selectivity and higher affinity for aromatase. Our criterion of selectivity is the absence of any inhibitory action on the biosynthesis of progesterone, corticosterone, and aldosterone. Compounds fulfilling this criterion would not require supplementation therapy with exogenous steroids.[9,10]

Till now, efforts have been made to improve the selectivity of AG by extensive modification of its chemical structure. These variations have included pyridyl analogues,[11] enlargement of the ethyl side chain, or alkyl substitution of the imide function,[12,13] and have led to 3-(4-aminophenyl)-3-cyclohexyl-[14] and 3-ethyl-3-(4-pyridyl)piperidine-2,6-diones[15] as new model structures. Finally, the glutarimide ring was modified. While the corresponding pyrrolidinedione analogues were as active as AG in vitro,[16] a new group of sterically more rigid cyclopropanedicarboximides (type 1) with the lead compound **1d** (R = *n*-pentyl) met our criterion for new aromatase inhibitors.[17]

In the present paper we describe the synthesis and biological activity of **1h**, a further cyclopropanedicarboximide of type **1**, the enantiomers of **1h** and **1a**, and a series of new achiral cyclobutanedicarboximides of type **2**.

Scheme I[a]



[a] (a) (1) NaH, toluene, (2) NaOH, H_2O; (b) $C_6H_{11}CH_2NH_2$, xylene, reflux; (c) HNO_3, H_2SO_4; (d) H_2, Pd–C, ethyl acetate.

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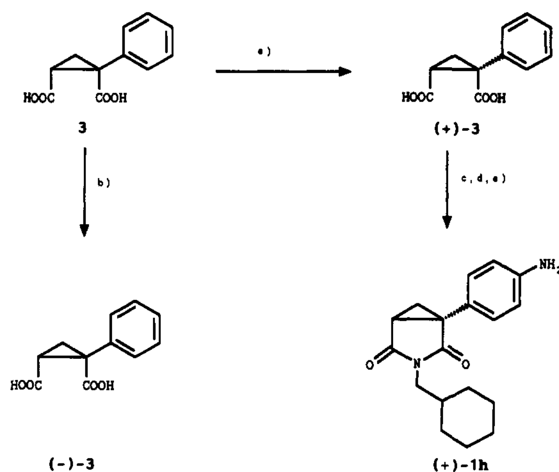
Scheme II[a]



[a] (a) (+)-Phenethylamine; (b) brucine *or* (-)-phenethylamine; (c) $C_6H_{11}CH_2NH_2$, xylene, reflux; (d) HNO_3, H_2SO_4; (e) H_2, Pd-C.

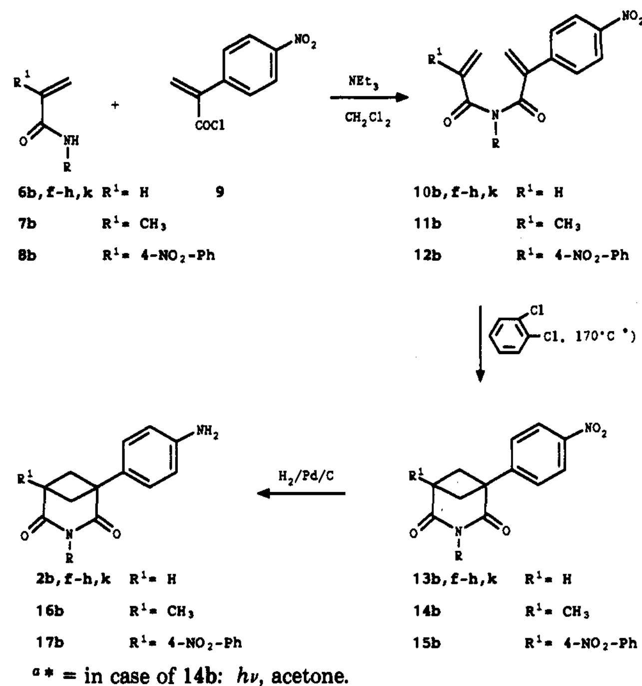
Chemistry

Synthesis of Type 1 Imides. In continuation of our work about 1-(4-aminophenyl)-3-azabicyclo[3.1.0]hexane-2,4-diones we have prepared the 3-(cyclohexylmethyl) derivative **1h** by analogy with the previously published synthesis (Scheme I).[17] By starting from easily accessible substances, the *cis*-dicarboxylic acid **3** was obtained in few steps and in a good overall yield according to McCoy's procedure.[18,19] Although the introduction of the amino function by nitration could be carried out before or after the formation of imide **4h**, the latter sequence gave better results, yielding the 4-nitro derivative **5h** directly by crystallization. The good biological activity and selectivity of the resultant compound, which will be discussed later, encouraged us to prepare the pure enantiomers. Attempts to crystallize salts of aniline derivative **1h** with optically active acids, however, all failed. The first successful separation of the enantiomers was achieved, analytically and preparatively, by chromatography on a cellulose triacetate column. Only then were we able to obtain the pure enantiomers in sufficient quantities to test for biological activity. Similar separation of the N-unsubstituted imide **1a** was already published.[20]

In order to obtain optically pure substances of type 1 in greater quantities, we examined new synthetic approaches with the help of examples described in the literature. To this end, the idea of separating the racemates at the earlier dicarboxylic acid **3** step seemed very attractive.[21] The (+)-dicarboxylic acid **3** ultimately crystallized in pure form as the (+)-phenethylammonium salt (Scheme II) and gave (+)-**1h** in the same way as for the racemic material. The absolute configuration 1*R* of (+)-**3** and (+)-**1h** could be assigned indirectly, since the absolute configuration of (-)-**3** is known to be 1*S*.[21]

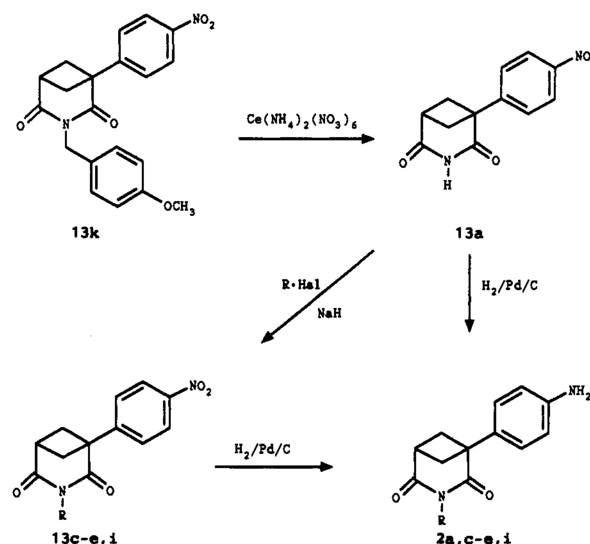
Synthesis of Type 2 Imides. As will be discussed later, (+)-**1h** was identified as an interesting substance, but one that was only synthesizable via separation of racemates. We therefore began to look for qualitatively and quantitatively equivalent achiral compounds. One elegant solution to this problem was to replace the three-membered

Scheme III[a]



[a] * = in case of **14b**: $h\nu$, acetone.

Scheme IV



a: R= H; c: R= $CH_2CH(CH_3)_2$; d: R= n-C_5H_{11}; e: R= n-C_7H_{15}; i: R= benzyl;

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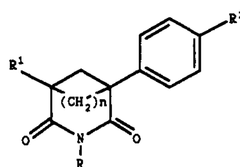
Table I. Physical Properties of Preparations **1a,h**, **3**, **4h**, and **5h** (R^1 = H)



Type 1: n=0

Type 2: n=1

compd	R	R^2	$[\alpha]^{20}_D$, deg/solvent	mp, °C	recryst solvent	% yield	formula[a]
1*R*,2*S*-(+)-3		H	+185.6/MeOH	164	EtOAc	84	$C_{11}H_{10}O_4$
1*S*,2*R*-(−)-3		H	−185.0/MeOH	161–165	EtOAc	67	$C_{11}H_{10}O_4$
4h	$CH_2C_6H_{11}$	H		64–65	MeOH	81	$C_{18}H_{21}NO_2$
1*R*,5*S*-(+)-**4h**	$CH_2C_6H_{11}$	H	+82.1/MeOH	69–70	MeOH	72	$C_{18}H_{21}NO_2$
1*S*,5*R*-(−)-**4h**	$CH_2C_6H_{11}$	H	−84.4/MeOH	67–70	MeOH	81	$C_{18}H_{21}NO_2$
5h	$CH_2C_6H_{11}$	NO_2		125–127	MeOH	60	$C_{18}H_{20}N_2O_4$
1*R*,5*S*-(+)-**5h**	$CH_2C_6H_{11}$	NO_2	+91.5/$CHCl_3$	105	MeOH	45	$C_{18}H_{20}N_2O_4$
1*S*,5*R*-(−)-**5h**	$CH_2C_6H_{11}$	NO_2	−92.7/$CHCl_3$	103–105	MeOH	46	$C_{18}H_{20}N_2O_4$
1*R*,5*S*-(+)-**1a**	H	NH_2	+64.0/MeOH	198–201	EtOH	68	$C_{11}H_{10}N_2O_2$
1*S*,5*R*-(−)-**1a**	H	NH_2	−66.0/MeOH	203–205	EtOH	81	$C_{11}H_{10}N_2O_2$
1h	$CH_2C_6H_{11}$	NH_2		130–133	EtOAc	84	$C_{18}H_{22}N_2O_2$
1*R*,5*S*-(+)-**1h**	$CH_2C_6H_{11}$	NH_2	+88.6/MeOH	157–159	EtOAc	77	$C_{18}H_{22}N_2O_2$
1*S*,5*R*-(−)-**1h**	$CH_2C_6H_{11}$	NH_2	−88.1/MeOH	159–160	EtOAc	79	$C_{18}H_{22}N_2O_2$

[a] The analysis of all new compounds were within ±0.4% of the theoretical value for C, H, N.

Table II. Physical Properties of Preparations **2** and **10–17**

compd	R	R^1	R^2	mp, °C	recryst solvent[a]	% yield	formula[b]
10b	*n*-C_3H_7	H	NO_2	71.5–72.5	A	56	$C_{15}H_{16}N_2O_4$
10f	*n*-$C_{10}H_{21}$	H	NO_2	oil		48	$C_{22}H_{30}N_2O_4$[c]
10g	C_6H_{11}	H	NO_2	73–74	A	60	$C_{18}H_{20}N_2O_4$[c]
10h	$CH_2C_6H_{11}$	H	NO_2	92–93	B	29	$C_{19}H_{22}N_2O_4$[c]
10k	4-MeO-benzyl	H	NO_2	106.5–107	C	64	$C_{20}H_{18}N_2O_5$
11b	*n*-C_3H_7	CH_3	NO_2	62–63	D	64	$C_{16}H_{18}N_2O_4$
12b	*n*-C_3H_7	4-$NO_2C_6H_4$	NO_2	177–178	E/A	22	$C_{21}H_{19}N_3O_4$[c]
13a	H	H	NO_2	>250	F	78	$C_{12}H_{10}N_2O_4$
13b	*n*-C_3H_7	H	NO_2	149–151	B/D	79	$C_{15}H_{16}N_2O_4$
13c	$CH_2CH(CH_3)_2$	H	NO_2	136–137	E/A	68	$C_{16}H_{18}N_2O_4$
13d	*n*-C_5H_{11}	H	NO_2	75–79	E/A	85	$C_{17}H_{20}N_2O_4$
13e	*n*-C_7H_{15}	H	NO_2	91–92	E/A	64	$C_{19}H_{24}N_2O_4$
13f	*n*-$C_{10}H_{21}$	H	NO_2	oil		25	$C_{22}H_{30}N_2O_4$[c]
13g	C_6H_{11}	H	NO_2	163–164	B/D	42	$C_{18}H_{20}N_2O_4$
13h	$CH_2C_6H_{11}$	H	NO_2	191–194	B/D	42	$C_{19}H_{22}N_2O_4$
13i	benzyl	H	NO_2	150–152	C	36	$C_{19}H_{16}N_2O_4$
13k	4-MeO-benzyl	H	NO_2	146–147	C/G	56	$C_{20}H_{18}N_2O_5$
14b	*n*-C_3H_7	CH_3	NO_2	128.5-129.5	B/D	86	$C_{16}H_{18}N_2O_4$
15b	*n*-C_3H_7	4-$NO_2C_6H_4$	NO_2	237–238	C/G	15[d]	$C_{21}H_{19}N_3O_4$
16b	*n*-C_3H_7	CH_3	NH_2	135–136	E/A	91	$C_{16}H_{20}N_2O_2$
17b	*n*-C_3H_7	4-NH_2	NH_2	149–150	C/B	80	$C_{21}H_{23}N_3O_2$
2a	H	H	NH_2	265 dec	H	61	$C_{12}H_{12}N_2O_2$
2b	*n*-C_3H_7	H	NH_2	166–167	E/A	87	$C_{15}H_{18}N_2O_2$
2c	$CH_2CH(CH_3)_2$	H	NH_2	158–160	E	65	$C_{16}H_{20}N_2O_2$
2d	*n*-C_5H_{11}	H	NH_2	92–94	E/A	47	$C_{17}H_{22}N_2O_2$
2e	*n*-C_7H_{15}	H	NH_2	69–71	C/A	86	$C_{19}H_{26}N_2O_2$
2f	*n*-$C_{10}H_{21}$	H	NH_2	81.5–82.5	E/A	44	$C_{22}H_{32}N_2O_2$
2g	C_6H_{11}	H	NH_2	139–140	C	62	$C_{18}H_{22}N_2O_2$
2h	$CH_2C_6H_{11}$	H	NH_2	140–146	C	51	$C_{19}H_{24}N_2O_2$
2i	benzyl	H	NH_2	164–165.5	C/E	83	$C_{19}H_{18}N_2O_2$
2k	4-MeO-benzyl	H	NH_2	147–147.5	C	92	$C_{20}H_{20}N_2O_3$

[a] A, hexane; B, CH_2Cl_2; C, Et_2O; D, diisopropyl ether; E, EtOAc; F, CH_3CN; G, toluene; H, 2-methoxyethanol. [b] The analysis of all new compounds were within ±0.4% of the theoretical value for C, H, N. [c] Not purified. [d] One experiment, not optimized.

ring by an analogous four-membered ring, as in 1,3-cyclobutanedicarboximides.

The basic structures **13–15** were obtained according to our previously described procedures by thermal[22] or UV-induced[23] intramolecular head-to-tail [2 + 2]-cycloaddition of suitably substituted *N*-acryloylacrylimides **10–12** (Scheme III). Upon subsequent reduction of the nitro group, the products **2b,f–h,k**, **16b**, and **17b** were prepared. Intermediate **13k**, bearing a 4-methoxybenzyl residue, also served for the preparation of the corresponding N-unsubstituted imide, which was not accessible in the above manner. The N-protecting group was removed by oxidation with cerium ammonium nitrate and subsequent hydrolysis to give **13a**. This approach enabled us to synthesize a number of further derivatives for structure–activity analysis (Scheme IV). The physical properties of

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Table III. Activity of Imides 1a and 1h toward the Aromatase Enzyme in Vitro Compared with That of 1d and Aminoglutethimide

compd	R	aromatase IC_{50},[a] μmol	inhibition potency
1a	H	1.8	1.05
1*R*,2*S*-(+)-1a	H	1.0	1.9
1*S*,2*R*-(−)-1a	H	777.0	0.002
1h	$CH_2C_6H_{11}$	0.012	158
1*R*,5*S*-(+)-1h	$CH_2C_6H_{11}$	0.005	200
1S,5R-(−)-1h	$CH_2C_6H_{11}$	0.15	12.6
1d	*n*-C_5H_{11}	0.023	83
AG		1.90	1

[a] Androstenedione as substrate.

Table IV. Activity of Imides 2a–h,k toward Aromatase Enzyme in Vitro Compared with That of 1d and Aminoglutethimide

compd	R	aromatase IC_{50},[a] μmol	inhibition potency
2a	H	2.0	0.95
2b	*n*-C_3H_7	0.111	17
2c	$CH_2CH(CH_3)_2$	0.062	30
2d	*n*-C_5H_{11}	0.031	61
2e	*n*-C_7H_{15}	0.049	39
2f	*n*-$C_{10}H_{21}$	0.225	8.5
2g	C_6H_{11}	0.013	140
2h	$CH_2C_6H_{11}$	0.097	20
2k	4-MeO-benzyl	0.24	8
1d	*n*-C_5H_{11}	0.023	83
AG		1.9	1

[a] Androstenedione as substrate.

all new products are summarized in Tables I and II.

Pharmacological Results and Discussion

The new products were tested for inhibition of human placental aromatase by measuring the release of ^3H_2O from [1,2-^{3}H]androstenedione. The respective IC_{50}s, i.e. the concentrations inhibiting steroid production by 50%, were determined and are listed in Tables III and IV. It is evident from these figures that 1h is twice as potent as enzyme inhibitor in vitro as the already published derivative 1d[17] and >150 times better than AG. Interestingly the (+)-enantiomers of 1a[20] and 1h are biologically 780 and 30 times more active than their (−)-antipodes, and have the same absolute configuration as the eutomer of AG.[24]

Among the preparations of type 2 the N-alkylated imides display better aromatase-inhibitory activity than unsubstituted 2a (IC_{50} = 2 μmol). In particular, lengthening the alkyl chain augments the activity by a factor of >50, and maximal activity (IC_{50} = 0.031 μmol) is obtained with the C-5 chain. Derivatives 1d and 2d with the same C-5 chain but different basic structures have comparable IC_{50} values. A further increase in activity over that of preparations 2b and 2c, can be achieved by branching the alkyl substituent. The most active compound in this series with an IC_{50} of 0.013 μmol proved to be *N*-cyclohexyl derivative 2g. Further prolongation of the chain as in 2h, or replacement of the ring by an aromatic function, leads to a slight reduction of activity. These results would appear to indicate that there may be room for a short branched imide substituent at the binding site of the aromatase enzyme.

Subsequently, compounds 2g and 1h and its (+)-enantiomer were tested for selectivity in two new recently developed in vitro models measuring their effects on the production of estrogens (E) and progesterone (P) in hamster ovarian tissue[25] and the production of corticosterone (C) and aldosterone (A) in rat adrenals.[26] The respective IC_{50} values were determined and are listed in Table V. The data clearly show that 1h (IC_{50} = 0.78 μmol) and (+)-1h (IC_{50} = 0.23 μmol) are highly active in inhibiting E production, whereas IC_{50} for P, C, and A are not reached even at the maximum possible concentration (>300 times higher for 1h). Compound 2g (IC_{50} = 0.07 μmol) proved to be the most potent E inhibitor in this group of compounds and was about 200 times more potent than AG. Its effect is also relatively specific, as concentrations more than 2000 times higher are needed to inhibit C, P, and A. These results clearly demonstrate the good selectivity of the new aromatase inhibitors.

Table V. Effects of 1h, 2g, and AG on Steroid Production in Vitro and Aromatase Inhibition in Vivo

compd	steroid production inhibn; IC_{50}, μmol				aromatase inhibition: min. ED, mg/kg po
	E[a]	P[a]	C[b]	A[b]	
1h	0.78	>330	>330	>330	1.5
(+)-1h	0.23	>34	>34	>34	1.0
2g	0.07	140	100	140	0.3
AG	13	60	50	110	2.3

[a] E = estrogens, P = progesterone, assayed in hamster ovaries. [b] C = corticosterone, A = aldosterone, assayed in rat adrenals.

Finally, these same compounds were tested for activity in vivo, and their minimum inhibitory doses for the uterotropic effect of gonadotropin in immature rats were determined (Table V). The resultant data indicate that they are effective as aromatase inhibitors in vivo, are absorbed after oral administration and are also more potent than AG in this biological test system. The superiority of these new types of structures over AG was further demonstrated with racemic 1h in a rat model of estrogen-dependent, DMBA-induced mammary carcinoma. 1h and AG were administered orally, daily for 6 weeks, to tumor-bearing rats. In this experiment 1h exerted a dose-related inhibitory effect on tumor growth, and its activity at the low dose of 31.3 mg/kg was comparable to that of 100 mg/kg AG (Table VI). At the two highest doses used it proved well-tolerated and significantly effective, as more than 80% of tumors initially present responded to the treatment and practically no new tumors developed. Analogous evaluations with (+)-1h and 2g are in progress and will be reported elsewhere.

In conclusion, (1) we have identified new types of structure with improved aromatase-inhibiting properties and selectivity. (2) The derivatization of the imide function in the type 2 series yielded more potent preparations than the unsubstituted analogues. (3) The 1*R*,5*S*-(+)-enantiomers of type 1 imides possess greater activity against human placental aromatase than the 1*S*,5*R*-(−)-isomers. (4) Some of these compounds are effective in vivo. The products (+)-1h and 2g are of interest as potential drugs for endocrine therapy of hormone-dependent tumors, e.g. breast cancer.

Experimental Section

Melting points were determined in open capillary tubes with a Büchi apparatus according to Dr. Tottoli and are uncorrected. TLC of each compound was performed on Merck F 254 silica gel plates and flash column chromatography, if necessary, on Merck silica gel 60 (230–400 mesh). R_f values for characterization of oily compounds were determined with the same solvent systems as for the corresponding preparative chromatography. Elemental

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Table VI. Regression of Established and Suppression of New DMBA-Induced Mammary Tumors in Female Sprague–Dawley Rats

compd	dose, mg/kg ×42 po	no. of tumors[a]	response			new tumors/rat, median (range)
			% CR + % PR	% SD	% P[b]	
1h	control	21	0	9.5	90.5	6 (4–8)
	125.0	23	82.6	0	17.4	0 (0–0)
	62.5	24	91.6	4.2	4.1	0 (0–2)
	31.3	20	15.0	30.0	55.0	3 (0–8)
	15.6	23	4.3	17.4	78.3	3 (0–8)
AG	control	20	0	5.0	95.0	6 (0–8)
	100.0	18	11.2	22.2	66.6	5 (0–7)
	30.0	24	4.25	4.1	91.6	4 (1–8)

[a] Beginning of the treatment. [b] End of the treatment, CR = complete regression, PR = partial regression, SD = stable disease, P = progression.

analyses were within ±0.4% of the theoretical values, except where indicated. The structure of all compounds were confirmed by their IR (Perkin-Elmer 1310 or 298 spectrophotometers) and ^{1}H NMR spectra (Varian HA-100D or Bruker WM-250). The optical rotations were recorded at 20 °C using a Perkin-Elmer 241 polarimeter with a 10-cm cuvette.

1*R*,2*S*-(+)-1-Phenyl-1,2-cyclopropanedicarboxylic Acid (1*R*,2*S*-(+)-3). To a solution of 165.0 g (0.8 mol) of 3[18,19] in 400 mL of ethyl acetate was added dropwise a solution of 194.0 g (1.6 mol) of *R*-(+)-1-phenylethylamine in 400 mL of ethyl acetate followed by a mixture of 1 L of ether and 10 mL of water. This mixture was stirred for 16 h at room temperature and for 3 h in an ice bath. The crystalline salt (153 g) was collected, recrystallized from 4.5 L of ethyl acetate, and yielded 139.0 g (77.4%) of white crystals. From 55.6 g of this salt 30.4 g of 1*R*,2*S*-(+)-3 was obtained: mp 164 °C; $[\alpha]_D$ +185.6° (*c* = 1.043, MeOH); NMR (100 MHz, DMSO-d_6) δ 1.46 (q, 1 H), 1.80 (q, 1 H), 2.17 (q, 1 H), 7.38 (m, 5 H). Anal. ($C_{11}H_{10}O_4$) C, H.

Similarly, with *S*-(−)-1-phenylethylamine 1*S*,2*R*-(−)-3 was obtained: mp 161–165 °C; $[\alpha]_D$ −185.0° (*c* = 1.008, MeOH); (lit.[21] mp 154 °C, $[\alpha]_D$ −185.3°). Anal. ($C_{11}H_{10}O_4$) C, H.

1*R*,5*S*-(+)-3-(Cyclohexylmethyl)-1-phenyl-3-azabicyclo[3.1.0]hexane-2,4-dione (1*R*,5*S*-(+)-4h). A solution of 40.0 g (0.194 mol) of 1*R*,2*S*-(+)-3 in 400 mL of xylene (isomeric mixture) was boiled under a water trap for 5 h and cooled. To this solution of the intermediary anhydride formed during the reaction was added (aminomethyl)cyclohexane (25.2 mL, 0.194 mol), dissolved in 50 mL of xylene. The mixture was boiled for a further 15 h and evaporated to dryness. The residue was crystallized from methanol and yielded 39.7 g (72%) of 1*R*,5*S*-(+)-4: mp 69–70 °C; $[\alpha]_D$ +77.6° (*c* = 1.224, $CHCl_3$); NMR (250 MHz, $CDCl_3$) δ 0.82–1.31 (2 m, 5 H), 1.53–1.80 (m, 6 H), 1.81–1.90 (m, 2 H), 2.74 (q, 1 H), 3.28 (q, 2 H), 7.4 (m, 5 H). Anal. ($C_{18}H_{21}NO_2$) C, H, N.

Similarly, starting from 19.48 g (94.5 mmol) of 1*S*,2*R*-(−)-3, 21.7 g (81.1%) of 1*S*,5*R*-(−)-4h was obtained: mp 67–70 °C; $[\alpha]_D$ −84.4° (*c* = 0.966, MeOH). Anal. ($C_{18}H_{21}NO_2$) C, H, N.

Similarly, racemic **4h** was obtained, mp 64–65 °C. Anal. ($C_{18}H_{21}NO_2$) C, H, N.

1*R*,5*S*-(+)-3-(Cyclohexylmethyl)-1-(4-nitrophenyl)-3-azabicyclo[3.1.0]hexane-2,4-dione (1*R*,5*S*-(+)-5h). A solution of 39.4 g of 1*R*,5*S*-(+)-4h in 75 mL of concentrated sulfuric acid was cooled to 0 °C and 20 mL of nitric acid (65%) added slowly, keeping the temperature below 10 °C. After a further 2 h the reaction mixture was poured onto ice and extracted with ethyl acetate. The organic phase was washed with water, dried, and evaporated. The residue was crystallized from 500 mL of methanol, yielding 20.6 g (45%) of 1*R*,5*S*-(+)-5h: mp 105 °C; $[\alpha]_D$ +91.5° (*c* = 1.099, $CHCl_3$); NMR (250 MHz, $CDCl_3$) δ 0.86–1.33 (2 m, 5 H), 1.52–1.78 (m, 6 H), 1.86–1.98 (m, 2 H), 2.86 (q, 1 H), 3.30 (q, 2 H), 7.64 (d, 2 H), 8.24 (d, 2 H). Anal. ($C_{18}H_{20}N_2O_4$) C, H, N.

Similarly, starting from 217.0 g (0.766 mol) of 1*S*,5*R*-(−)-4h, 117 g (46%) of 1*S*,5*R*-(−)-5h was obtained: mp 103–105 °C; $[\alpha]_D$ −92.7° (*c* = 1.020, $CHCl_3$). Anal. ($C_{18}H_{20}N_2O_4$) C, H, N.

Similarly, racemic **5h** was obtained, mp 125–127 °C. Anal. ($C_{18}H_{20}N_2O_4$) C, H, N.

1*R*,5*S*-(+)-1-(4-Aminophenyl)-3-(cyclohexylmethyl)-3-azabicyclo[3.1.0]hexane-2,4-dione (1*R*,5*S*-(+)-1h). A solution of 80 g (0.33 mol) of 1*R*,5*S*-(+)-5h in 1.6 L of ethyl acetate was hydrogenated at normal pressure in the presence of 2.0 g of 5% Pd/C. After removal of the catalyst, the solvent was distilled off and the residue was crystallized from 350 mL of ethyl acetate, yielding 56.3 g (77.3%) of 1*R*,5*S*-(+)-1h: mp 157–159 °C; $[\alpha]_D$ +88.6° (*c* = 0.542, MeOH); NMR (250 MHz, $CDCl_3$) δ 0.8–1.04 (m, 2 H), 1.04–1.3 (m, 3 H), 1.48–1.83 (m, 8 H), 2.61 (q, 1 H), 3.26 (q, 2 H), 3.73 (bs, 2 H), 6.67 (d, 2 H), 7.16 (d, 2 H). Anal. ($C_{18}H_{22}N_2O_2$) C, H, N.

Similarly 1*S*,5*R*-(−)-1h was obtained: mp 159–160 °C; $[\alpha]_D$ −88.1° (*c* = 0.519, MeOH). Anal. ($C_{18}H_{22}N_2O_2$) C, H, N.

Similarly, racemic (**1h**) was obtained, mp 130–133 °C. Anal. ($C_{18}H_{22}N_2O_2$) C, H, N.

Chromatographic Resolution of Racemic 1h. The resolution of racemic **1h** (0.5 g) was carried out on a 5 × 90 cm column of cellulose triacetate with ethanol–water 95:5. The concentration of the diastereoisomers was measured by a UV spectrophotometer (Shimadzu UV-120-02) in series with a polarimeter (Perkin-Elmer 241MC). The separated products were crystallized as above, yielding 0.251 g (52%) of 1*R*,5*S*-(+)-1h, [$[\alpha]_D$ +86.9° (*c* = 0.53, MeOH)] and 0.11 g (22%) of 1*S*,5*R*-(−)-1h, [$[\alpha]_D$ −87.2° (*c* = 0.45, MeOH)].

4-Aza-2-(4-nitrophenyl)-1,6-heptadiene-3,5-diones 10b,f–h,k, 11b, 12b. Method A. A solution of 76.2 g (0.6 mol) of oxalyl chloride in 500 mL of methylene chloride was added dropwise over a period of 2 h at room temperature to a stirred suspension of 115.8 g (0.6 mol) of 2-(4-nitrophenyl)acrylic acid in 5 mL of DMF and 2.5 L of methylene chloride. When the addition was complete, the mixture was stirred for a further 2 h until the evolution of gas ceased. The resulting solution of **9** was cooled to 0 °C and added dropwise to a precooled solution of 0.6 mol of acrylamides **6h,f–h,k**, **7b**, or **8b** and 121 g (1.2 mol) of triethylamine in 450 mL of methylene chloride. The mixture was stirred for 1.5 h at room temperature and evaporated to dryness. The residue was extracted with 2 L of ether, the filtrate was evaporated, and the title compounds were recrystallized. The ether extract of **10f** was filtered over 1.5 kg of silica gel and gave the title product as yellow oil: IR ($CHCl_3$) 1355, 1660, 1685 cm^{-1}, NMR (100 MHz, $CDCl_3$) δ 1.3 (bs, 19 H), 3.8 (t, 2 H), 5.5–6.5 (m, 5 H), 7.6 (d, 2 H), 8.1 (d, 2 H).

3-*n*-Propyl-1-(4-nitrophenyl)-3-azabicyclo[3.1.1]heptane-2,4-dione (13b). Method B. A solution of 46.1 g (0.16 mol) of 4-aza-2-(4-nitrophenyl)-4-*n*-propyl-1,6-heptadiene-3,5-dione (**10b**) and 0.5 g (2.2 mmol) of 2,6-di-*tert*-butyl-*p*-cresol in 900 mL of 1,3-dichlorbenzene was stirred at 170 °C for 1.5 h. After concentration by evaporation the residue was crystallized from a methylene chloride/diisopropyl ether mixture, yielding the crude **13b** as pale yellow crystals, mp 141–143 °C. The mother liquor was concentrated by evaporation and filtered over silica gel with methylene chloride. After recrystallization the resulting product **13b** yielded white crystals: mp 149–151 °C, IR ($CHCl_3$) 1350, 1685, 1745 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 0.93 (t, 3 H), 1.55–1.71 (m, 2 H), 2.74–2.86 (m, 4 H), 3.28–3.36 (m, 1 H), 3.72 (t, 2 H), 7.29 (d, 2 H), 8.27 (d, 2 H). Anal. ($C_{15}H_{16}N_2O_4$) C, H, N.

Similarly, starting from **10f–h,k** and **12b**, the products **13f–h,k** and **15b** were obtained.

1-(4-Nitrophenyl)-3-azabicyclo[3.1.1]heptane-2,4-dione (13a). A solution of 283 g (0.51 mol) of cerium(IV) ammonium nitrate in 400 mL of water was added dropwise at room temperature to a stirred solution of 50 g (136 mmol) of **13k** in 1.3 L of acetonitrile. When the addition was complete, the mixture was stirred for a further 4 h. The resulting emulsion was concentrated to half the volume and diluted with 2 L of water. The product

formed was filtered with suction, washed with water, and dried. The crude product was then dissolved in 3 L of hot acetonitrile, and the resulting solution was filtered over HYFLO-Supercel and the filtrate was concentrated at 60–70 °C under a water-jet vacuum until crystallization began. The title compound was obtained in the form of brownish crystals: mp >250 °C; IR (KBr) 1355, 1695, and 1720 cm^{-1}; NMR (250 MHz, DMSO-d_6) δ 2.69–3.05 (m, 5 H), 7.42 (d, 2 H), 8.23 (d, 2 H), 10.94 (s, 1 H). Anal. ($C_{12}H_{10}N_2O_4$) C, H, N.

3-Alkyl-1-(4-nitrophenyl)-3-azabicyclo[3.1.1]heptane-2,4-diones 13c–e,i. Method C. Fifteen millimoles of isobutyl iodide, *n*-pentyl iodide, *n*-heptyl bromide, or benzyl bromide was added to a mixture of 2.46 g (10 mmol) of **13a** and 0.36 g (15 mmol) of sodium hydride in 25 mL of DMF. The reaction mixture was then stirred for 2.5 h and evaporated. The residue was dissolved in ethyl acetate, washed with water, dried, and crystallized after evaporation of the solvent.

5-Methyl-1-(4-nitrophenyl)-3-*n*-propyl-3-azabicyclo-[3.1.1]heptane-2,4-dione (14b). Method D. While stirring, a solution of 23.0 g (76 mmol) of **11b** and 0.22 g (1.0 mmol) of 2,6-di-*tert*-butyl-*p*-cresol in 2.3 L of acetone was irradiated for 3 h with a UV lamp (Philips 125 HPK), which was immersed in the reaction solution in a double-walled, water-cooled Pyrex glass shaft. After concentration by evaporation, the residue was recrystallized from methylene chloride/diisopropyl ether mixture, yielding **14b** in the form of white crystals: mp 128.5–129.5 °C; IR ($CHCl_3$) 1350, 1685, 1745 cm^{-1}; NMR (250 MHz, $CDCl_3$) δ 0.93 (t, 3 H), 1.45 (s, 3 H), 1.54–1.72 (m, 2 H), 2.48–2.59 (m, 2 H), 2.82–2.94 (m, 2 H), 3.72 (t, 2 H), 7.30 (d, 2 H), 8.26 (d, 2 H). Anal. ($C_{16}H_{18}N_2O_4$) C, H, N.

3-Alkyl-1-(4-aminophenyl)-3-azabicyclo[3.1.1]heptane-2,4-diones 2a–i,k, 16b, 17b. Method E. A 2–5% alcoholic, ethyl acetate, or 2-methoxyethanol solution of the nitro derivatives **13a–i,k**, **14b**, or **15b** was hydrogenated at normal pressure in the presence of 5% Pd/C. After removal of the catalyst, the solvent was distilled off under vacuum, giving the title products.

Enzyme Preparation and Assay Procedures. The aromatase enzyme was prepared and its activity checked with [1,2-^{3}H]androstenedione as substrate according to the method of Thompson.[1] The assays for inhibition of estrogen biosynthesis in comparison to the synthesis of progesterone in hamster ovaries[25] and corticosterone and aldosterone in rat adrenal[26] tissues were performed exactly as previously described.

In Vivo Aromatase Assay. The effectiveness of compounds as aromatase inhibitors in vivo was tested in immature rats treated with human chorionic gonadotropin and androstenedione and determined from the inhibition of the uterotropic effect according to the method of Steele.[7]

DMBA-Mammary Carcinoma Test. Female Sprague–Dawley rats bearing one to three DMBA-induced mammary tumors of about 1–1.2 mm in diameter were randomized and divided into treatment and control groups of 15 animals each. **1h** and AG were suspended in 0.5% sterile solution of CMC with 20% 1,2-propylene glycol. The total daily dose of the test compounds was given orally in two administrations at 8.00 a.m. and 3.00 p.m. for 42 consecutive days. The body weight, the number of tumors, and the tumor size were determined once weekly until the end of experiment.[27]

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2-Alkoxyadenosines: Potent and Selective Agonists at the Coronary Artery A_2 Adenosine Receptor

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A Langendorff guinea pig heart preparation served for the assay of agonist activity of a series of 24 2-alkoxyadenosines at the A_1 and A_2 adenosine receptors of, respectively, the atrioventricular node (conduction block) and coronary arteries (vasodilation). Activities are low at the A_1 receptor and do not show a clear relationship to the size or hydrophobicity of the C-2 substituent. All the analogues are more potent at the A_2 receptor, activity varying directly with the size and hydrophobicity of the alkyl group. The most potent analogue in this series, 2-(2-cyclohexylethoxy)adenosine, has an EC_{50} of 1 nM for coronary vasodilation and is 8700-fold selective for the A_2 receptor.

Exocyclic substituents on the adenine base profoundly alter the affinity of adenosine for its receptors. N^6-substituted adenosines such as N^6-cyclopentyladenosine[1,2] are potent and very selective agonists at the A_1 adenosine receptor (A_1AR).[3,4] Certain other N-6 substituents containing aryl groups confer selectivity for the A_2AR.[5,6] The availability of large numbers of N^6-substituted adenosines has aided the development of models of the N-6 region of the A_1AR[3,7] and also of the A_2AR.[8,9] In part because the synthesis routes to adenosines substituted at C-2 are more difficult than those at N-6, the information about the C-2 regions of the two types of adenosine receptor is much less detailed. Several early studies examined a number of 2-substituted adenosines as agonists at the A_2AR of the platelet[10,11] and the coronary artery.[12–15] However, most

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