Synthesis and Aromatase Inhibition of 3-Cycloalkyl-Substituted 3-(4-Aminophenyl)piperidine-2,6-diones

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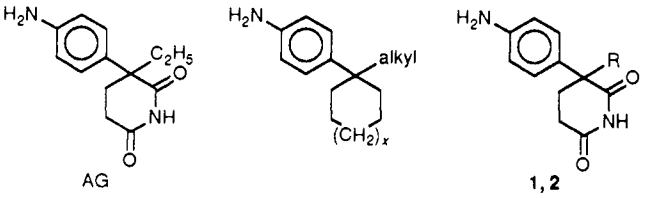
The synthesis of 3-cycloalkyl-substituted 3-(4-aminophenyl)piperidine-2,6-diones is described [cyclopentyl (1), cyclohexyl (2)]. The enantiomers of 2 were separated either by using HPLC on optically active sorbent or by crystallization of the brucine salt of the phthalamic acid of 2. The absolute configuration of the (+)- and (−)-enantiomers of 2 were assigned as *S* and *R*, respectively, by comparing the CD spectra to those of the enantiomers of aminoglutethimide (AG, 3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione). The compounds were tested in vitro for inhibition of human placental aromatase, the cytochrome P_{450}-dependent enzyme which is responsible for the conversion of androgens to estrogens. Compounds 1 and 2 inhibited aromatase by 50% at 1.2 and 0.3 μM, respectively (IC_{50} AG = 37 μM). According to the findings with AG, the (+)-enantiomer of 2 was responsible for the inhibitory activity, being a 240-fold more potent aromatase inhibitor in vitro than racemic AG. On the other hand, (+)-2 displayed a strongly reduced inhibition of desmolase (cholesterol side-chain cleavage enzyme) compared to AG (relative activity = 0.3). Thus (+)-2 is of interest as a potential drug for the treatment of estrogen-dependent diseases, e.g. mammary tumors.

A high percentage of breast carcinomas occurring in postmenopausal women are estrogen-dependent,[1] i.e. their growth is stimulated by estrogens in physiological concentrations. To reduce the stimulatory effect of estrogens on tumor growth, mainly two therapeutic methods are applied today.[2] Antiestrogens bind to the estrogen receptor in the tumor cell and block estrogen action, whereas inhibitors of estrogen biosynthesis reduce the circulating estradiol and estrone levels. The last step of estrogen biosynthesis is catalyzed by the cytochrome P_{450}-dependent enzyme aromatase.[3,4] So far, the only commercially available aromatase inhibitor is aminoglutethimide [AG, 3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione, Chart I]. Though AG has shown clinical benefit in many trials with postmenopausal breast cancer patients,[5] it is far from being an optimal drug due to its lack of specificity and its only moderate tolerability.[6]

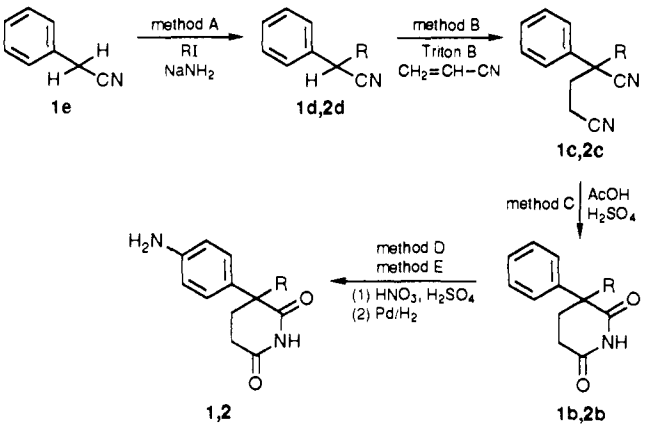
Numerous attempts to optimize AG have been undertaken.[7-11] The replacement of the 4-aminophenyl substituent of AG by a 4-pyridyl group results in a decrease of inhibitory potency toward aromatase but also in an increase of selectivity in that pyridoglutethimide exhibits a strongly reduced inhibitory potency toward the cholesterol side-chain cleavage enzyme (desmolase), the first and rate-limiting enzyme in the overall steroidogenesis.[12] Investigations of our group showed that elongation of the ethyl substituent of AG leads to a dramatic increase in aromatase inhibition without notably influencing desmolase inhibition.[13] In a recent study, we demonstrated that 1-alkyl-substituted 1-(4-aminophenyl)cycloalkanes (Chart I) are more potent aromatase inhibitors in vitro than AG.[14] This structure–activity study suggests that these compounds interact with the aromatase enzyme in a different manner than does AG, the cycloalkyl substituent not mimicking the piperidinedione moiety but rather the 3-ethyl group of AG, with the piperidinedione binding region in the active site of aromatase being left unoccupied. On the basis of this hypothesis, exchange of the 3-ethyl group of AG by cycloalkyl substituents offers a very promising perspective for the further design of new potent aromatase inhibitors of the AG type. This article describes the synthesis of the 3-cycloalkyl-substituted 3-(4-aminophenyl)-piperidine-2,6-diones 1 and 2 (Chart I), separation of the enantiomers of 2, the determination of their absolute configurations, and the evaluation of the inhibitory potency in vitro toward aromatase and desmolase.

Chart I[a]



[a] 1: R = cyclopentyl; 2, (+)-2, (−)-2: R = cyclohexyl.

Chart II[a]



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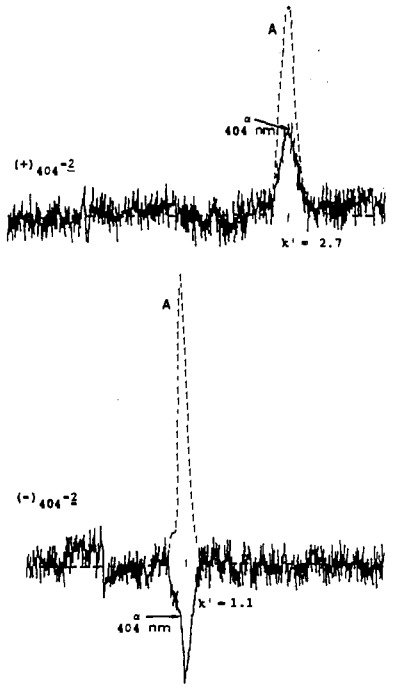
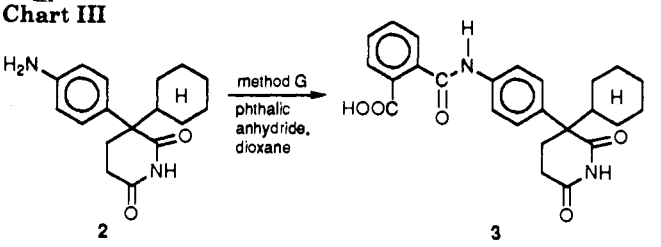


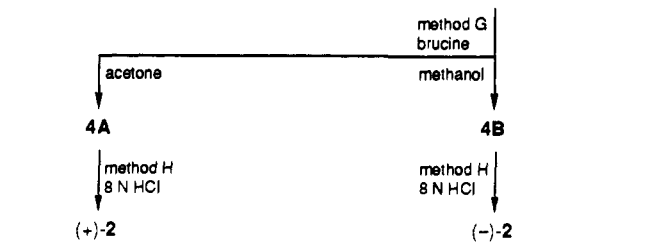
Figure 1. Analytical separation of (+)- and (−)-2 by HPLC. Tris(phenylcarbamoyl)cellulose/SiO_2; EtOH–H_2O, 96:4, $p \equiv 32$ bar; k': capacity factor; A: absorbance at 310 nm; α: rotation angle at 404 nm.

Chemistry

The synthesis of the 3-(4-aminophenyl)-3-cycloalkylpiperidine-2,6-diones (compounds **1** and **2**) started from phenylacetonitrile (**1e**, Chart II). The conversion of the latter compound to the corresponding 2-cycloalkyl-substituted phenylacetonitriles (compounds **1d** and **2d**) was carried out with cycloalkyl iodide and sodamide in benzene (method A). Micheal reaction of compounds **1d** and **2d** with acrylonitrile using Triton B (trimethylbenzylammonium hydroxide; method B) as catalyst and cyclization of the resulting 2-cycloalkyl-2-phenylglutarodinitriles (**1c**, **2c**) with acetic acid and concentrated H_2SO_4 (method C) yielded the corresponding 3-cycloalkyl-3-phenylpiperidine-2,6-diones (**1b**, **2b**). Nitration of **1b** and **2b** was performed according to the method of Hoffmann and Urech[15] using concentrated H_2SO_4 and HNO_3 (method D). Only small amounts of the isomeric 2-cycloalkyl-2-(3-nitrophenyl)glutarimides were found as byproducts,

Chart III





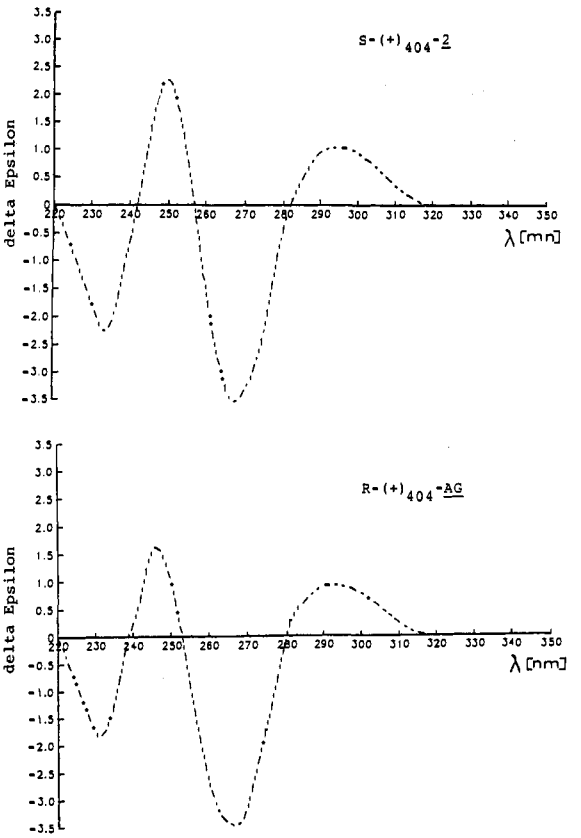


Figure 2. Correlation of the configurations of enantiomerically pure (+)-2 and (+)-AG by circular dichroism (CD); solvent: CH_3CN; $\Delta\epsilon$: differential absorption coefficient in $l \cdot mol^{-1} \cdot cm^{-1}$.

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Table I. Inhibition of Human Placental Aromatase and Bovine Adrenal Desmolase by AG and Compounds 1 and 2

	aromatase		desmolase		
compd	IC_{50},[a,b] μM	RP[c]	% inhibn[d]	IC_{50},[a,e] μM	RP[c,e]
1	1.2	31	59	ND	ND
2	0.3	123	31	67	0.4
(+)-2	0.15	240	19	82	0.3
(−)-2	4.6	8	39	ND	ND
AG	37	1	57	29	1
(+)-AG	19	1.9	67	ND	ND
(−)-AG	370	0.1	36	ND	ND

[a] IC_{50} is the concentration of inhibitor required to give 50% inhibition. The given values are mean values of at least three experiments. The deviations were within ±10%. [b] Concentration of testosterone: 5 μM. [c] Relative potency, calculated from the IC_{50} values and related to AG. [d] Concentration of inhibitor: 25 μM. [e] ND = not determined.

while no ortho compound could be detected. The para nitro compounds **1a** and **2a** were obtained by fractional crystallization in satisfying amounts. Reduction of the latter compounds by catalytic hydrogenation using palladium on charcoal (method E) led to compounds **1** and **2**. The separation of the enantiomers of **2** was performed by HPLC on the optically active sorbent tris(phenylcarbamoyl)cellulose/SiO_2 (method F). Several injections gave milligram quantities of the enantiomerically pure compounds (Figure 1). The antipodes of aminoglutethimide were prepared in the same way.

For the preparation of larger amounts of the enantiomers, a crystallization procedure had to be applied. The enantiomers of AG were separated as described[16] by crystallization of the diastereomeric tartrates. Attempts to prepare the enantiomers of **2** using tartaric acid and other optically active acids failed. The separation was successfully performed, however, using the phthalamic acid derivative **3** (Chart III). Compound **3** was obtained by reaction of **2** with phthalic anhydride (method G). Crystallization of the mixture of the diastereomeric salts obtained by reaction of **3** with brucine afforded the diastereomers **4A** and **4B** (method G).

The cleavage of the phthalamide was performed using 8 N HCl and gave the enantiomers (+)-**2** and (−)-**2** (method H). The absolute configurations of (+)-**2** and (−)-**2** could be determined by circular dichroism based upon the results of Finch and co-workers[16] who assigned *S* configuration to (−)-AG. The correlation of the CD curve of (+)-**2** with the CD curve of (+)-AG demonstrates that these compounds exhibit identical absolute configurations (method I, Figure 2). As the replacement of the ethyl group by a cyclohexyl residue leads to a change in the sequence of substituents according to the Cahn–Ingold–Prelog convention, the *S*-configuration has to be assigned to the dextrorotary enantiomer of the cyclohexyl compound.

Biological Properties

Inhibition of Aromatase and Desmolase. The inhibitory activities of the compounds toward aromatase were determined in vitro using human placental microsomes and [1β,2β-^{3}H]testosterone according to the method of Thompson and Siiteri.[3] The IC_{50} values and the potencies of the derivatives, relative to AG, are given in Table I. Exhibiting activities enhanced by a factor of 31 and 123, respectively, the cycloalkyl-substituted piperidinediones (compounds **1** and **2**) are clearly superior to the ethyl-substituted parent compound AG. According to the finding of Graves and Salhanick,[17] it is (+)-AG which is much more active than (−)-AG. The same phenomenon is observed with compound **2**. Showing a relative potency of 240, (+)-**2** is 30 times as active as its antipode (relative activity of (−)-**2** is 8).

The inhibitory activities of the compounds toward desmolase were determined in vitro using bovine adrenal mitochondria and [26-^{14}C]cholesterol according to the method of Hochberg et al.[18] In this test the cyclopentyl compound shows an activity similar to AG (Table I). In contrast, the cyclohexyl compound **2** is less active an inhibitor of desmolase than is the parent compound. While in the case of AG the (+)-enantiomer is more active than the (−)-enantiomer, the opposite is true for compound **2**. The most active aromatase inhibitor, (+)-**2**, is the least active inhibitor of desmolase.

Discussion

AG, the only aromatase inhibitor which at present is commercially available, has despite of its proven clinical effectiveness major disadvantages in being a relatively weak and less selective aromatase inhibitor. In a previous study we have shown that elongation of the ethyl side chain and introduction of CH_3 residues into the *n*-alkyl chains leads to a strong increase in aromatase inhibition without notably influencing desmolase inhibition.[13] In the study described here, we could demonstrate that aromatase inhibition can be further enhanced by replacing the ethyl group by a cycloalkyl moiety, in particular by a cyclohexyl residue, leading also to a decrease of desmolase inhibition.

It is noteworthy that for the inhibitory activity toward aromatase the dextrorotary enantiomers of both AG and compound **2** are predominantly responsible, whereas concerning inhibition of desmolase the differences of the enantiomers thus are not great for both compounds. Interestingly, it is the (+)-enantiomer of AG which is the more potent inhibitor of desmolase, whereas in the case of the cyclohexyl compound **2**, the active aromatase-inhibiting (+)-enantiomer (+)-**2** is less active in inhibiting desmolase than is the levorotary enantiomer (−)-**2**. Thus, taking into regard the high inhibitory potency toward aromatase (relative activity = 240) and the decreased inhibitory activity toward desmolase (relative activity = 0.3), (+)-**2** is highly superior to racemic AG concerning both activity *and* selectivity.

In addition (+)-**2** proved to be highly effective in vivo concerning the E_2 lowering effect in PMSG-pretreated adult rats and the mammary tumor-inhibiting activity in ovariectomized, testosterone-treated rats.[19] Keeping in mind the side effects of AG originating from the CNS depressive activity of the drug, it is of utmost importance that (+)-**2**, even at very high doses, does not display a CNS depressive effect in rats in the rotarod and motility experiment.[19] These results taken together indicate that (+)-**2** is a highly interesting aromatase inhibitor of the AG type which could be regarded as a promising candidate for

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the treatment of estrogen-dependent diseases, e.g. mammary and endometrium carcinoma.

Experimental Section

General Procedures. TLC of each compound was performed on Merck F 254 silica gel or Merck F 254 neutral Al_2O_3 60 plates, respectively. Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Yields are related to analytically pure product. No effort was made to optimize yields. Elemental analyses were performed by the Mikroanalytisches Laboratorium, Universität Regensburg. All compounds were analyzed for C, H, and N within ±0.40% of the calculated values. The structures of all compounds were confirmed by their IR (Beckman Acculab 3) and ^1H NMR (Varian EM 360 L, 60 MHz, Bruker AW 80, 80 MHz, or Bruker AC 300, 300 MHz) spectra. HPLC equipment: Model 6000 A pump and Model U6K injector from Waters; detectors: absorbance (A) was measured on a ERMA ERC 7210 photometer, rotation angles (α) were measured on a Perkin Elmer PE 241 or PE 241 MC polarimeter; sorbent and column: tris(phenylcarbamoyl)cellulose/SiO_2 (5 μm, 250 × 4.6 mm, Daicel). The differential absorbance (ΔA) was measured on a Jasco Circulardichrograph J-40A.

Method A. 2-Cyclopentyl-2-phenylacetonitrile (1d). To a mixture of phenylacetonitrile (1e; 11.7 g, 0.1 mol) and cyclopentyl iodide (19.6 g, 0.1 mol) in 30 mL of dry benzene was slowly added a suspension of $NaNH_2$ (3.90 g, 0.1 mol) in toluene at 80 °C. After 1 h, the mixture was cooled and water was added. The organic layer was separated, washed with water, and dried ($MgSO_4$). The solvent was removed, and the product crystallized from ethanol to give 8.34 g (45%) of **1d** (mp 51–2 °C). Anal. ($C_{13}H_{15}N$) C, H, N.

2-Cyclohexyl-2-phenylacetonitrile (2d). The reaction was performed with cyclohexyl bromide using dry toluene as solvent. The crude product was purified by column chromatography (SiO_2; petroleum ether (40–60)–ethyl acetate = 4:1) to yield 9.37 g (47%) of **2d** (mp 56–7 °C). Anal. ($C_{14}H_{17}N$) C, H, N.

Method B. 2-Cyclopentyl-2-phenylglutarodinitrile (1c). To a stirred solution of **1d** (19.9 g, 0.1 mol) and Triton B (1.67 g, 0.01 mol, 40% solution in MeOH) in 100 mL of dry dioxane, acrylonitrile (5.31 g, 0.1 mol) in 20 mL of dry dioxane was added dropwise at 100 °C. After refluxing for 48 h, the solvent was removed, and the residue dissolved in ether. The solution was washed with water and dried ($MgSO_4$). The ether was evaporated, and the resulting crude product was purified by column chromatography (SiO_2; CH_2Cl_2–petroleum ether (40–60) = 4:1) to yield 16.4 g (69%) of **1c** (mp 86–8 °C). Anal. ($C_{16}H_{18}N_2$) C, H, N.

2-Cyclohexyl-2-phenylglutarodinitrile (2c). NaOEt and dry toluene were used. Column chromatography (SiO_2; petroleum ether (40–60)–ethyl acetate = 4:1) gave 22.5 g (89%) of **2c** (mp 92–3 °C). Anal. ($C_{17}H_{20}N_2$) C, H, N.

Method C. 3-Cyclopentyl-3-phenylpiperidine-2,6-dione (1b). A suspension of **1c** (23.8 g, 0.1 mol) in 150 mL of concentrated H_2SO_4 and 500 mL of acetic acid was stirred and heated for 6 h at 120 °C. After cooling, the mixture was poured onto ice and extracted with CH_2Cl_2. The organic layer was washed with a saturated Na_2CO_3 solution and water and dried ($MgSO_4$). After removal of the solvent, the resulting crude product was recrystallized from ethyl acetate–ligroin to give 19.8 g (77%) of **1b** (mp 109–11 °C). Anal. ($C_{16}H_{19}NO_2$) C, H, N.

3-Cyclohexyl-3-phenylpiperidine-2,6-dione (2b). The crude product was recrystallized from ethyl acetate–ligroin to yield 20.4 g (75%) of **2b** (mp 139–41 °C). Anal. ($C_{17}H_{21}NO_2$) C, H, N.

Method D. 3-Cyclopentyl-3-(4-nitrophenyl)piperidine-2,6-dione (1a). At −10 °C a mixture of 11.0 g of concentrated H_2SO_4 and 11.0 g of HNO_3 (63%) was slowly added to a suspension of **1b** (25.7 g, 0.1 mol) in 80.0 g of concentrated H_2SO_4. After stirring for 2 h at −10 °C, the reaction mixture was poured onto ice and extracted with CH_2Cl_2. The organic layer was washed with water and dried ($MgSO_4$). After evaporation of the solvent, the crude product was crystallized from toluene to give 27.5 g (91%) of **1a** (mp 159–60 °C). Anal. ($C_{16}H_{18}N_2O_4$) C, H, N.

3-Cyclohexyl-3-(4-nitrophenyl)piperidine-2,6-dione (2a). The crude product was crystallized from EtOH to yield 22.4 g (71%) of **2a** (mp 165–7 °C). Anal. ($C_{17}H_{20}N_2O_4$) C, H, N.

Method E. 3-(4-Aminophenyl)-3-cyclopentylpiperidine-2,6-dione (1). Palladium on charcoal (10%, 0.1 g) was added to a solution of **1a** (3.02 g, 0.01 mol) in 100 mL of EtOH. The suspension was shaken under a hydrogen atmosphere until no more H_2 was accepted. The reaction mixture was filtered. The solvent was removed, and the crude product was recrystallized from toluene to give 1.99 g (73%) of **1** (mp 138–40 °C). Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

3-(4-Aminophenyl)-3-cyclohexylpiperidine-2,6-dione (2). The crude product was purified by column chromatography (SiO_2; petroleum ether (40–60)–ethyl acetate = 1:1) to give 2.84 g (99%) of **2** (mp 199–200 °C). Anal. ($C_{17}H_{22}N_2O_2$) C, H, N.

Method F. (+)- and (−)-3-(4-Aminophenyl)-3-cyclohexylpiperidine-2,6-dione ((+)-2 and (−)-2). For the HPLC separation on tris(phenylcarbamoyl)cellulose/SiO_2, 7.5 mg of **2** were dissolved in 1 mL of ethanol. Repeatedly, 200 μL of this solution was injected (flow rate 1.5 mL/min; p = 32 bar). EtOH (96%) was used as mobile phase. The absorbance was detected at 310 nm, and the rotation angle was detected at 404 nm. The dead time was 5.8 min (determined by using 1,3,5-tri-*tert*-butylbenzene). (−)-2: t = 12.1 min, k' = 1.1; $[\alpha]^{24}_{546}$ = −90 ± 6 (c = 0.38 in MeOH); mp 102–4 °C. (+)-2: t = 20.8 min, k' = 2.7; $[\alpha]^{24}_{546}$ = +90 ± 8 (c = 0.40 in MeOH); mp 103–5 °C.

(+)- and (−)-3-(4-Aminophenyl)-3-ethylpiperidine-2,6-dione ((+)-AG and (−)-AG). The enantiomers of AG were separated in the same way. (−)-AG: t = 12.8 min, k' = 1.2; $[\alpha]^{23}_{589}$ = −157 ± 9 (c = 2.02 in MeOH); mp 114–5 °C. (+)-AG: t = 16.8 min, k' = 1.9; $[\alpha]^{23}_{589}$ = +153 ± 10 (c = 1.42 in MeOH); mp 113–4 °C.

Method G. Diastereomeric Salts of 3-[4-[(2-Carboxybenzoyl)amino]phenyl]-3-cyclohexylpiperidine-2,6-dione (3) with Brucine (4A and 4B). Compound **2** (2.86 g, 0.01 mol) and phthalic anhydride (1.48 g, 0.01 mol) in 100 mL of dioxane were stirred and heated for 2 h at 100 °C. After removing the solvent, the crude product was purified by column chromatography (SiO_2; EtOH–ethyl acetate = 5:1) to yield 3.82 g (88%) of **3** (mp 189–91 °C).

A solution of brucine (3.95 g, 0.01 mol) in 60 mL of acetone–MeOH (2:1) was dropped into a solution of **3** (4.35 g, 0.01 mol) in 70 mL of acetone–MeOH (2:1) at 55 °C. After stirring for 30 min and cooling, the precipitate was filtered off, washed with little amounts of cold solvent, and dried to yield 4.06 g (49%) of brucine salt **B**. Brucine salt **A** (4.23 g, 51%) was isolated from the mother liquor. Two-fold crystallization of brucine salt **A** from acetone and isolation of the precipitate yielded 3.94 g (95%) of **4A** ($[\alpha]^{24}_{546}$ = +9 ± 6 (c = 0.89 in MeOH–$CHCl_3$ = 1:1)).

Brucine salt **B** was suspended in 1 L of MeOH and stirred for several hours while boiling. The precipitate was filtered and dried to give 1.82 g (44%) of **4B** (mp 250–4 °C; $[\alpha]^{24}_{546}$ = −49 ± 6 (c = 0.83 in MeOH–$CHCl_3$ = 1:1)).

(+)- and (−)-3-(4-Aminophenyl)-3-ethylpiperidine-2,6-dione Tartrate Salt ((+)- and (−)-AG Tartrate Salt). AG was resolved via recrystallization of the tartrate salts in MeOH as described.[16] Use of (+)-tartric acid yielded the (+)-tartrate salt of (+)-AG ($[\alpha]^{24}_{589}$ = +85 ± 4 (c = 0.80 in MeOH); lit.:[16] $[\alpha]^{25}_{589}$ = +79 (MeOH)) after six recrystallizations. (−)-Tartaric acid provided the (−)-tartrate salt of (−)-AG ($[\alpha]^{24}_{589}$ = −84 ± 4 (c = 0.80 in MeOH); lit.:[16] $[\alpha]^{25}_{589}$ = −80 (MeOH)).

Method H. (+)-3-(4-Aminophenyl)-3-cyclohexylpiperidine-2,6-dione ((+)-2). Compound **4A** (4.15 g, 5 mmol) was stirred in 8 N HCl at room temperature. After 6 h, the mixture was diluted with H_2O, and 2 N NaOH was added until the pH was 6.0. After extraction with CH_2Cl_2, the organic phase was dried ($MgSO_4$). The solvent was removed, and the crude product was purified by column chromatography (SiO_2; EtOH–ethyl acetate = 5:1) to give 1.00 g (70%) of (+)-**2** (mp 100–2 °C; $P \approx 1$; $[\alpha]^{24}_{546}$ = +101 ± 8 (c = 0.85 in MeOH)).

(−)-3-(4-Aminophenyl)-3-cyclohexylpiperidine-2,6-dione ((−)-2). The procedure yielded 1.00 g (70%) of (−)-**2** (mp 92–5 °C; $P \geq 0.96$; $[\alpha]^{24}_{546}$ = −97 ± 8 (c = 0.84 in MeOH)).

(−)-3-(4-Aminophenyl)-3-ethylpiperidine-2,6-dione ((−)-AG). The (−)-tartrate salt of (−)-AG was treated with aqueous sodium carbonate. The solution was extracted with CH_2Cl_2 to yield (−)-AG (mp 112–4 °C, lit.:[16] mp 114–5 °C; $[\alpha]^{24}_{589}$ = −153 ± 6 (c = 0.80 in MeOH); lit.:[16] $[\alpha]^{25}_{589}$ = −163.6 (MeOH)).

(+)-3-(4-Aminophenyl)-3-ethylpiperidine-2,6-dione ((+)-AG). The compound was isolated in the same way. (+)-AG (mp 108–12 °C; lit.:[16] mp 114–5 °C; $[\alpha]^{24}_{589}$ = +149 ± 6 (c = 0.80 in MeOH); lit.:[16] $[\alpha]^{25}_{589}$ = +163.1 (MeOH)).

Method I. Circular Dichroism Studies. Compounds (+)-**2** and (+)-AG were dissolved in Uvasol acetonitrile (c = 80.3 and 163.5 μmol/L, respectively. The CD spectra were measured from 220 to 350 nm at a temperature of 23 °C. The $\Delta\epsilon$ scales given previously[20,21] show values which are lower because of erroneous calibrations of the dichrograph.

Biological Methods. The preparation of aromatase and desmolase as well as the enzyme assays were performed as described previously.[13]

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Registry No. (±)-1, 140926-09-6; (±)-**1a**, 140926-10-9; (±)-1b, 140926-11-0; (±)-**1c**, 140926-12-1; (±)-1d, 140926-13-2; 1e, 140-29-4; (±)-**2**, 137548-41-5; (+)-**2**, 137623-89-3; (−)-**2**, 137623-91-7; (±)-**2a**, 140926-14-3; (±)-**2b**, 140926-15-4; (±)-**2c**, 140926-16-5; (±)-**2d**, 140926-17-6; (±)-**3**, 140926-18-7; **4A**, 141042-79-7; **4A**·brucine, 141042-80-0; **4B**, 141042-81-1; **4B**·brucine, 141042-82-2; (±)-AG, 55511-45-0; (+)-AG, 55511-44-9; (+)-AG·(+)-tartrate, 57344-88-4; (−)-AG, 57288-03-6; (−)-AG·(−)-tartrate, 57288-04-7; cyclopentyl iodide, 1556-18-9; cyclohexyl bromide, 108-85-0; aromatase, 9039-48-9; desmolase, 37292-81-2.

Supplementary Material Available: ^{1}H-NMR data (Tables II and III) of the synthesized compounds (2 pages). Ordering information is given on any current masthead page.

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Benzodiazepine Receptor Affinity and Interaction of Some *N*-(Indol-3-ylglyoxylyl)amine Derivatives

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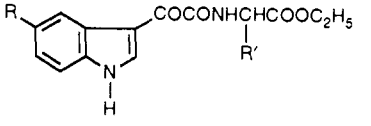
Several derivatives, in which tryptamine, tyramine, and dopamine moieties are linked to the indole nucleus by an oxalyl bridge, were tested for their ability to displace the specific binding of [^{3}H]flunitrazepam from bovine brain membranes. GABA ratio and in vivo tests for the most potent compounds showed they behave as inverse agonists at the benzodiazepine receptor (BzR). To better define the structure-activity relationship (SAR) of this kind of ligand, several phenylethylamine derivatives were synthesized to evaluate their affinity to BzR. Some of these derivatives (**17**, **21**, **24**, **26**, and **30**) were found to exhibit high affinity (K_i = 0.51-0.085 μM) for BzR and possessed a partial agonist activity, although their chemical structure is closely related to tryptamine **2-6**, tyramine **7-11**, and dopamine **12-16** derivatives. A different interaction of these ligands to the receptor site is hypothesized. Moreover, all the prepared 1-methyl derivatives exhibited very low binding affinity to BzR.

The identification of high-affinity, saturable, and stereospecific binding sites for benzodiazepines in the central nervous system[1,2] has prompted an intensive search for an endogenous ligand that physiologically acts on these receptors. Some β-carboline derivatives as esters of the β-carboline-3-carboxylic acid,[3] which exhibited high affinity to the benzodiazepine receptor (BzR), gave rise to a noteworthy interest[4-13] as they could have represented the endogenous ligand. However, the endogenous compound has not yet been isolated. Although the complete biosynthetic pathway of β-carbolines in animals or in man is not known, it seems evident that these compounds are derivatives of tryptophan, tryptamine, or more generally of a (2-aminoethyl)indole structure. With this in mind, in the past we prepared some *N*-(indol-3-ylglyoxylyl)amino acid derivatives **1** containing a more flexible structure but analogous to that of β-carboline.[14]



1

Since there are many suggestions[12,15-17] indicating that

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