L-threonine, and sarcosine were from Wako Pure Chemical Ind. Co., and L-valinamide hydrochloride, L-leucinamide hydrochloride, L-serinamide hydrochloride, L-methioninamide hydrochloride, L-phenylalaninamide, L-tyrosinamide, and L-prolinamide were from Sigma Chemical Co.

General Synthesis for the Congeners 1–3 (Method A). A solution of 4-nitrophenyl *N*-(2-chloroethyl)-*N*-nitrosocarbamate[24] (1.2 mmol) in tetrahydrofuran (4 mL) was added to a solution of amino acid amide (1.0 mmol) in methanol (2 mL). After 30 min, the solutin was concentrated, and the residue was purified by silica gel column chromatography using 8:1 (v/v) $CHCl_3$–CH_3OH as an eluant. The results are summarized in Table I.

General Synthesis for the Congeners 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, and 24 (Method B). 2-Chloroethyl isocyanate (1.2 mmol) was added to a solution of amino acid amide (1.0 mmol) in methanol (1.5 mL). After 30 min, the solution was concentrated, and the residue was recrystallized from alcohol to give the *N*-carbamoyl derivative (see Table II). Sodium nitrite (1.1–1.5 mmol) was added to a solution of the *N*-carbamoyl derivative (1.0 mmol) in 99% formic acid (3 mL) under ice cooling. After 20 min, the solution was treatd with Amberlite IR-120 (H^+) and subsequently concentrated to give an analytically pure product in most cases, except the congeners **5** and **13** which were purified by silica gel column chromatography. The results are summarized in Table III.

Chemical Decomposition of 23. Compound **23** (10 mg) was dissolved in 0.1 M phosphate buffer (pH 7.4, 2 mL), and the solution was settled at ambient temperature. After 7 days, the solution was concentrated, and the residue was developed on an ascending paper chromatogram by 4:6:3 (v/v) acetic acid–1-butanol–water. The paper was sprayed with ninhydrin to visualize the spot (R_f 0.73), which was identified as sarcosinamide by comparison with an authentic sample.

Acknowledgment. This research was supported by the Keio University Koizumi Memorial Fund. The authors thank Dr. John A. Montgomery, Southern Research Institute, for helpful advices. They also thank Saburo Nakada for elemental analyses, Toyomi Sato for determination of chemical decomposition rates, and Shiro Sakakibara for technical assistances.

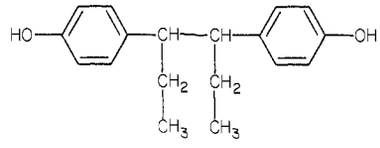
N,N'-Dialkyl-1,2-bis(hydroxyphenyl)ethylenediamines and *N,N'*-Dialkyl-4,5-bis(4-hydroxyphenyl)imidazolidines: Syntheses and Evaluation of Their Mammary Tumor Inhibiting Activity

Erwin von Angerer, Günter Egginger, Gerhard Kranzfelder, Horst Bernhauer, and Helmut Schönenberger*

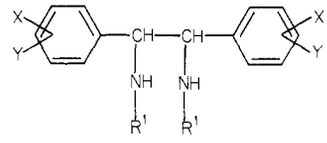
Institut für Pharmazie der Universität Regensburg, Universitätsstrasse 31, D-8400 Regensburg, West Germany.
Received August 13, 1981

Diastereomeric *N,N'*-dialkyl-1,2-bis(hydroxyphenyl)ethylenediamines (**5**) were synthesized and tested for their affinity for the estradiol receptor. Only the (±)-1,2-bis(4-hydroxyphenyl)ethylenediamines with the alkyl groups C_3H_7 [(±)-**5c**, $K_a = 1.1 \times 10^6$)], C_4H_9 [(±)-**5e**, $K_a = 3.6 \times 10^6$], and C_5H_{11} [(±)-**5h**, $K_a = 2.2 \times 10^6$] showed a marked affinity, which is mainly due to the (+) enantiomers [e.g., (+)-**5e**, $K_a = 2.1 \times 10^7$]. No enhancement of affinity by cyclization to imidazolidines [e.g., (±)-*trans*-**7a**, $K_a = 1.2 \times 10^7$] was observed. These compounds [e.g., (±)-, (+)-, and (−)-**5e**], which did not produce any uterine response in the mouse, were able to inhibit weakly the growth of the DMBA-induced mammary carcinoma of the rat. The inhibitory effect of (±)-**5e** against MCF-7 cells, which can be overcome by hexestrol, makes a direct antiestrogenic mode of action probable, since general cytotoxic effects and a central action could be ruled out.

Inhibition of the [^{3}H]estradiol ([^{3}H]E2) receptor interaction in vitro by *N,N'*-dialkyl-1,2-bis(2,6-dichlorophenyl)ethylenediamines (**3**)[1] and evidence of a weak retardation of the growth of the DMBA-induced hormone-dependent mammary adenocarcinoma of the Sprague–Dawley rat by some unsubstituted diphenylethylenediamines (**2**)[2] prompted us to investigate this class of compounds thoroughly. This paper describes the synthesis and some pharmacological results of *N,N'*-dialkyl-1,2-bis(hydroxyphenyl)ethylenediamines (**5a–i**) and attempts to improve their affinity for the E2 receptor by converting them into imidazolidines (**7a–g**) and imidazolidinethione (**9**). The close structural relation of **5** to hexestrol (**1**) is apparent, for *meso*-*N,N'*-dimethyl-1,2-bis(4-hydroxyphenyl)ethylenediamine (**5a**) can be considered as N-isosteric **1**.



1



2, R^1 = alkyl; X, Y = H
3, R^1 = alkyl; X = 2-Cl; Y = 6-Cl
4a–i, R^1 = alkyl; X = H; Y = OCH_3
5a–i, R^1 = alkyl; X = H; Y = OH

Chemistry. The isomeric *N,N'*-dialkyl-1,2-bis(methoxyphenyl)ethylenediamines (**4**; Table I) were synthesized by reductive dimerization of the methoxybenzaldehyde alkylimines with activated aluminum.[1] The resulting mixtures of *meso*- and (±)-**4** were separated by fractional crystallization of the hydrochlorides (**4h,i**) or by Craig countercurrent distribution (Craig CCD) in the solvent system $CHCl_3$/MeOH/HCl or $CHCl_3$/HCl ($K_{(\pm)\text{-}4a\text{-}g} \gg K_{meso\text{-}4a\text{-}g}$; Table II).

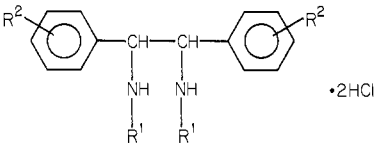
We related the (±) structure to the diastereomers with the larger paramagnetic shift of the α-hydrogen atoms in the ^{1}H NMR spectrum of the hydrochlorides of **4**. In the case of **4c** and **4e**, this result was confirmed by converting

(1) von Angerer, E.; Kranzfelder, G.; Taneja, A. K.; Schönenberger, H. *J. Med. Chem.* **1980**, *23*, 1347.

(2) Schönenberger, H.; Kranzfelder, G.; Hoffmann, E.; Egginger, G.; Schmitt, H.; Taneja, A. K. *Pharmazie* **1976**, *31*, 590.

0022-2623/82/1825-0832$01.25/0

Table I. *N,N'*-Dialkyl-1,2-bis(methoxyphenyl)ethylenediamines (4) and *N,N'*-Dialkyl-1,2-bis(hydroxyphenyl)ethylenediamines (5) Dihydrochlorides



compd	R^1	R^2	mp, °C (recrystn solvent[a]) meso	mp, °C (recrystn solvent[a]) (±)	formula[b]	K_a, M^{-1}
4a	CH_3	4-OCH_3	228 (A)	218 (A)	$C_{18}H_{24}N_2O_2 \cdot 2HCl$	
4b	C_2H_5	4-OCH_3	208 (A)[d]	187 (A)	$C_{20}H_{28}N_2O_2 \cdot 2HCl$	
4c	C_3H_7	4-OCH_3	204 (A)	134 (A)	$C_{22}H_{32}N_2O_2 \cdot 2HCl$	
4d	*i*-C_3H_7	4-OCH_3	249 (A)	153 (A)	$C_{22}H_{32}N_2O_2 \cdot 2HCl$	
4e	C_4H_9	4-OCH_3	194 (A)	180 (A)	$C_{24}H_{36}N_2O_2 \cdot 2HCl$	
(+)-4e	C_4H_9	4-OCH_3	230 (A)		$C_{24}H_{36}N_2O_2 \cdot 2HCl$	
(−)-4e	C_4H_9	4-OCH_3	234 (A)		$C_{24}H_{36}N_2O_2 \cdot 2HCl$	
4f	C_4H_9	3-OCH_3	180 (A)	181 (A)	$C_{24}H_{36}N_2O_2 \cdot 2HCl$	
4g	C_4H_9	2-OCH_3	190 (A)	175 (A)	$C_{24}H_{36}N_2O_2 \cdot 2HCl$	
4h	C_5H_{11}	4-OCH_3	211 (B)	202 (B)	$C_{26}H_{40}N_2O_2 \cdot 2HCl$	
4i	C_6H_{13}	4-OCH_3	130 (B)	191 (B)	$C_{28}H_{44}N_2O_2 \cdot 2HCl$	
5a[e]	CH_3	4-OH	226 (A)	174 (A)	$C_{16}H_{20}N_2O_2 \cdot 2HCl$[f]	inactive
5b[d]	C_2H_5	4-OH	204 (A)	198 (A)	$C_{18}H_{24}N_2O_2 \cdot 2HCl$	$<10^4$
5c	C_3H_7	4-OH	229 (A)	178 (A)	$C_{20}H_{28}N_2O_2 \cdot 2HCl$[g]	1.1×10^6
5d	*i*-C_3H_7	4-OH	174 (A)	184 (A)	$C_{20}H_{28}N_2O_2 \cdot 2HCl$	1.4×10^5
5e	C_4H_9	4-OH	225 (A)	174 (A)	$C_{22}H_{32}N_2O_2 \cdot 2HCl$	3.6×10^6
(+)-5e	C_4H_9	4-OH	168 (A)		$C_{22}H_{32}N_2O_2 \cdot 2HCl$[h]	2.1×10^7
(−)-5e	C_4H_9	4-OH	153 (A)		$C_{22}H_{32}N_2O_2 \cdot 2HCl$[i]	7.0×10^5
5f	C_4H_9	3-OH	205 (C)	208 (C)	$C_{22}H_{32}N_2O_2 \cdot 2HCl$	$<10^4$
5g	C_4H_9	2-OH	208 (A)	220 (C)	$C_{22}H_{32}N_2O_2 \cdot 2HCl$[j]	inactive
5h	C_5H_{11}	4-OH	147 (D)	104 (D)	$C_{24}H_{36}N_2O_2 \cdot 2HCl$	2.2×10^6
5i	C_6H_{13}	4-OH	220 (D)	200 (D)	$C_{26}H_{40}N_2O_2 \cdot 2HCl$	3.2×10^5

[a] A = MeOH/Et_2O; B = MeOH; C = EtOH/Et_2O; D = H_2O. [b] All compounds were analyzed for C, H, and N within 0.4% of the calculated values, except where noted. [c] Association constants of 5 were calculated from data generated by a Lineweaver-Burk analysis using a competitive inhibitor model (see ref 24); *meso*-5a-i did not show any receptor affinity. [d] Reference 6. [e] Reference 5. [f] (±), analyzed as base. C: calcd, 70.50; found, 69.52; N: calcd, 10.29; found, 10.80. [g] Meso; analyzed as base. C: calcd, 73.15; found, 70.29. [h] C: calcd, 61.40; found, 62.20. [i] C: calcd, 61.40; found, 60.29. [j] Meso. C: calcd, 61.40; found, 60.78.

Table II. Craig Countercurrent Distribution (Craig-CCD)[a] of the Diastereomeric Mixtures 4a-g·2HCl[b]

compd	solvent system[c]	K^d *d,l*	K^d meso	β^e	n^f	V^g	$r_{max(dl)}{}^h$	$r_{min}{}^i$	$r_{max(meso)}{}^h$
4a	I[l]	100.00	0.11	909	24	1.6	0	3-17	22
4b	I[l]	50.00	0.15	333	24	1.6	1	4-16	21
4c	I[k]	25.00	1.00	25	96	1.0	3	10-39	48
4d	I[k]	25.00	0.75	33	96	1.0	3	10-44	55
4e	I[k]	100.00	7.70	13	96	1.0	2	5	10
	II[l]	35.71	1.33	27	24	1.6	0	4-6	13
	III[l]	21.74	0.08	267	24	1.6	1	5-13	20
	IV[l]	1.72	0.05	35	24	1.6	10	15-17	21
4f	III[l]	18.52	0.12	157	24	1.6	1	5-12	19
4g	III[l]	50.00	0.15	340	24	1.6	0	4-11	18

[a] Method: fundamental distribution, t = 20 °C. [b] Applied quantity: 1-3 g. [c] I = chloroform/methanol/HCl, 2%, 40:12:28; II = chloroform/1 N HCl, 40:40; III = chloroform/0.1 N HCl, 40:40; IV = chloroform/0.01 N HCl, 40:40. [d] K = partition coefficient. [e] β = separation factor. [f] n = number of transfers. [g] V = milliliters in the upper phase/milliliters in the lower phase. [h] r_{max} = maximum of the distribution curve. [i] Minimum of the distribution curve. [k] V. Metzsch 200-tube CCD train manufactured by H. Kühn, Hospitalstraße 9, Göttingen, BRD (tube capacity = 25 mL lower phase); Hecker 25-tube CCD train manufactured by E. Bühler, Reutlinger Straße 6, Tübingen, BRD (tube capacity = 50 mL lower phase).

these structures with formaldehyde into the imidazolidines and studying the NMR of the latter.[3] The other imidazolidines (6) and the imidazolidinethione (8) were obtained by the reaction of the ethylenediamines 4 with the corresponding aldehydes or CS_2, respectively (Table III). The ether cleavage was accomplished by $AlBr_3$ (**5a-i**) or BBr_3 (**7a-g** and **9**) (Scheme I).

In order to determine which of the isomeric forms of (±)-**5c** had the higher affinity for the E2 receptor, the (+) and (−) enantiomers were synthesized: The primary amines (+)- and (−)-1,2-bis(4-methoxyphenyl)ethylenediamine (12), which were obtained by separation of (±)-12[4] tartrate, were used for the synthesis, because the direct resolution of the racemates **4e** or **5e** failed. Acylation of (+)-12 with butyryl chloride, followed by $LiAlH_4$ reduction, yielded (−)-**4e**, which was converted by $AlBr_3$ to (−)-**5e**. (+)-**5e** was synthesized in an analogous manner.

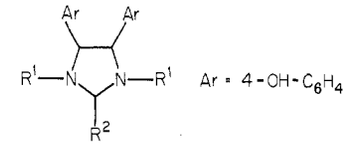
(3) Schönenberger, R.; Sunkel, C.; Schönenberger, H. *Arzneim.-Forsch. (Drug Res.)* **1972**, *22*, 1952.

(4) Vögtle, F.; Goldschmitt, E. *Angew. Chem.* **1973**, *85*, 824.

(5) Schönenberger, H.; Bernhauer, H. *Arch. Pharm. (Weinheim, Ger.)* **1970**, *303*, 804.

(6) Bruce, W. F.; Hanslick, R. S.; Freed, M. E. U.S. Patent 2746959; *Chem. Abstr.* **1957**, *51*, P2052*f*.

Table III. *N,N'*-Dialkyl-4,5-bis(4-hydroxyphenyl)imidazolidines (7) and -imidazolidinethione (9)



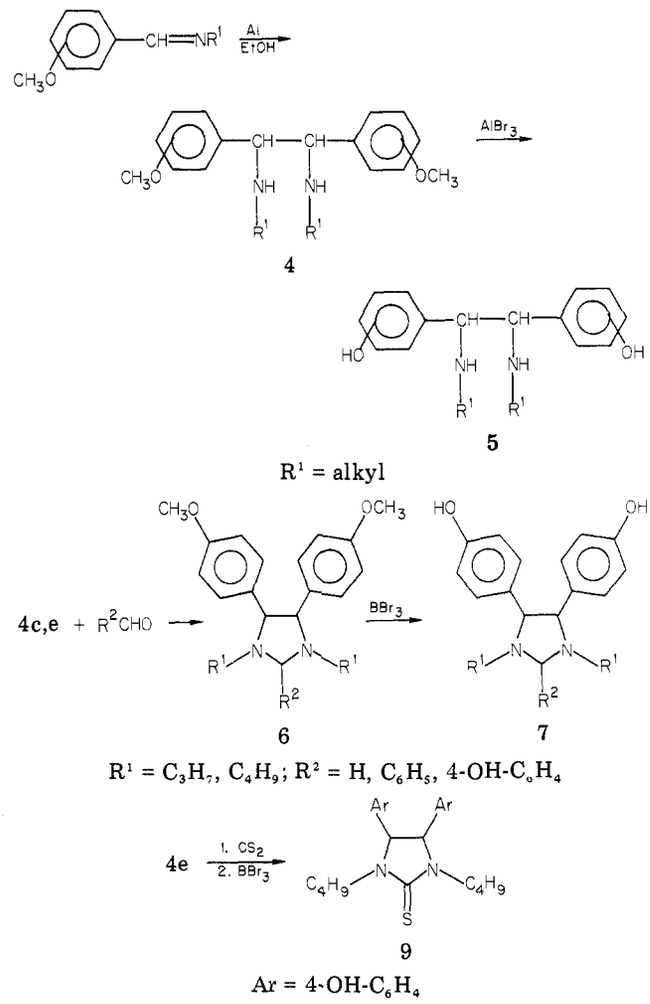
compd	R^1	R^2	config, C_4/C_5	mp, °C (recrystn solvent)	formula	anal.[a]	inhibn,[b] %
7a	C_4H_9	H	(±)-trans	96 dec (Et_2O-ligroin)	$C_{23}H_{32}N_2O_2$	C, H, N	70[c]
7b	C_4H_9	H	(+)-trans	65-68 dec[d] (Et_2O-ligroin)	$C_{23}H_{32}N_2O_2$	C, H, N	3
7c	C_4H_9	H	(−)-trans	126-128 dec[e] (Et_2O-ligroin)	$C_{23}H_{32}N_2O_2$	C, H, N	75
7d	C_4H_9	H	cis	65-70 dec (ligroin)	$C_{23}H_{32}N_2O_2$	H; C[f]	0
7e	C_3H_7	H	(±)-trans	163-165 (Et_2O)	$C_{21}H_{28}N_2O_2$	C, H	66
7f	C_4H_9	C_6H_5	(±)-trans	122-125 (Et_2O-ligroin)	$C_{29}H_{36}N_2O_2$	C, H, N	39
7g	C_4H_9	4-OHC_6H_4	(±)-trans	85-90 dec (Et_2O-ligroin)	$C_{29}H_{36}N_2O_3$	C, H, N	48
9	C_4H_9	=S	(±)-trans	157-159 ($CHCl_3$-ligroin)	$C_{23}H_{30}N_2O_2S$	H, N; C[g]	75

[a] All compounds were analyzed for the elements given within 0.4% of the calculated values, except where noted. [b] The inhibition was determined by the percentage of [^{3}H]E2 bound to the receptor in the presence of 2×10^{-6} M inhibitor ([^{3}H]E2 concentration = 5×10^{-9} M). [c] $K_a = 1.2 \times 10^7$. [d] $[\alpha]^{25}_D$ +32.9° (*c* 1.0, MeOH). [e] $[\alpha]^{25}_D$ −32.3° (*c* 1.0, MeOH). [f] C: calcd, 74.96; found, 72.67. [g] C: calcd, 69.31; found, 68.81.

Biological Properties. All *N,N'*-dialkyl-1,2-bis(hydroxyphenyl)ethylenediamines (**5**) were tested to determine their ability to inhibit the [^{3}H]E2 receptor interaction in vitro. Of these compounds, only the racemic forms showed an affinity for the E2 receptor, in contrast to 1,2-bis(2,6-dichlorophenyl)ethylenediamines (**3**)[1] and derivatives of hexestrol[7] which antagonize [^{3}H]E2 in both diastereomeric forms. The degree of affinity depends on the position of the phenolic hydroxy group (para > meta; ortho inactive) and the *N*-alkyl chain length ($C_4H_9 > C_5H_{11} > C_3H_7 > C_6H_{13} > C_2H_5$; CH_3 inactive). (±)-*N,N'*-Dibutyl-1,2-bis(4-hydroxyphenyl)ethylenediamine [(±)-**5e**] proved to be the most active compound in this series, with an association constant of 3.6×10^6. Ramification of the alkyl chains diminished the effect (**5c** > **5d**). For all compounds, the Lineweaver–Burk plot showed a competitive inhibition. A pronounced difference in the association constants of the enantiomers of (±)-**5e** was observed [(+)-**5e**, 2.1×10^7; (−)-**5e**, 7.0×10^5]; the value for (+)-**5e** nearly reaches the K_a value of the antiestrogen nafoxidine (8.3×10^7). By conversion of (±)-**5e** into *trans*-1,3-dibutyl-4,5-bis(4-hydroxyphenyl)imidazolidine (*trans*-**7a**) and -imidazolidine-2-thione (*trans*-**9**), no major enhancement of receptor affinity was achieved. The enantiomers of (±)-*trans*-**7a** also differed from one other in their affinities for the E2 receptor. Introduction of a phenyl (*trans*-**7f**) or a 4-hydroxyphenyl group (*trans*-**7g**) into the 2-position of trans-**7a** also did not increase its effect. In contrast to the 2,6-dichlorophenyl-substituted imidazolidines,[1] *cis*-**7d** was not able to inhibit [^{3}H]E2 receptor interaction.

In the Allen–Doisy test, the homologous ethylenediamines (±)-**5a–i** and *trans*-**7a** did not exhibit any estrogenic effects up to a dose of 1920 μg/mouse. The lack of estrogenic activity was confirmed using the Dorfman uterine weight test of (±)- and *meso*-**5e** in the mouse. The proof of antiestrogenic properties by inhibition of the estrone-stimulated uterus growth of the immature mouse failed in all cases [(±)-, (+)-, and (−)-**5e**; highest dose, 480 μg/animal]. In this context, the findings of Niederl and

Scheme I



(7) Hartmann, R. W.; Buchborn, H.; Kranzfelder, G.; Schönenberger, H.; Bogden, A. E. *J. Med. Chem.* **1981**, *24*, 1192.

Table IV. [^{3}H]Thymidine Incorporation in Estrogen-Responsive MCF-7 Human Breast Tumor Cells

compd	concn, M	incorporated [^{3}H]thymidine dpm/dish[a]	%
control		13 760 ± 658	100
hexestrol (1)	10^{-8}	16 485 ± 124	20
tamoxifen	10^{-6}	11 225 ± 260	82
(±)-5e	10^{-5}	10 833 ± 246	79
(±)-5e (10^{-5} M) + hexestrol (1)	10^{-8}	13 942 ± 861	101

[a] Mean of six dishes ± SD.

Table V. Growth Inhibition of the DMBA-Induced Mammary Carcinoma of the Rat

compd	dose,[a] mg/kg	no. of animals	increase of tumor area,[b] %
control		10	330
(±)-5e	15	9	121[c]
(+)-5e	20	10	112[c]
(−)-5e	20	10	115[c]
meso-5e	15	10	475[d]
(±)-5i	25	11	132[d]
trans-7a	15	8	105[d]

[a] Administered three times a week (Monday, Wednesday, and Friday), dissolved in arachis oil, sc. [b] Mean values after 28 days of therapy; tumor area on the first day of administration (0%) was larger than 140 mm^2 per animal. [c] $p < 0.05$, determined by U test according to Wilcoxon, Mann, and Withney. [d] Not significant ($p > 0.05$).

Dexter[8] and of Takahashi[9] that *meso*-**5a** has no estrogenic potency are consistent with this compound's lack of receptor affinity.

Although proliferation of MCF-7 human mammary tumor cells is not stimulated by estrogens in vitro,[10] cell growth and DNA polymerase activity are inhibited by antiestrogens, such as tamoxifen or nafoxidine, and reversal of this inhibition can be achieved specifically with 17β-E2.[11] Lippman[12] has shown that estrogens stimulate thymidine kinase activity and tamoxifen inhibits it; therefore, the incorporation of [^{3}H]thymidine into DNA can be used for evaluation of the antiestrogenic potency of a compound. We found that (±)-**5e** was able to inhibit the incorporation of thymidine in MCF-7 cells; this effect was overcome by the simultanous addition of hexestrol (1) (Table IV).

The ethylenediamines *meso*-, (±)-, (+)-, (−)-**5e**, and (±)-**5i** and the imidazolidine *trans*-**7a** were tested on the established DMBA-induced hormone-dependent mammary carcinoma of the Sprague–Dawley rat, which is considered to have many similarities to human breast cancer.[13-15] All compounds except *meso*-**5e** led to a weak inhibition of the tumor growth without a correlation to the different receptor affinities. *meso*-**5e** increased the tumor growth slightly (Table V).

Table VI. Enzyme Activities of DMBA-Induced Mammary Tumor of the Rat After Administration of **5e**[a]

compd	growing tumors	regressing tumors
Dehydrogenase Activity, μM/mL		
(+)-5e	191.7 ± 27.7	101.8 ± 23.7[b]
(−)-5e	183.3 ± 18.7	121.3 ± 30.2[b]
control	200.8 ± 31.4	137.4 ± 18.3[b]
p-Nitrophenylphosphatase Activity, μM/mL		
(+)-5e	39.4 ± 10.1	48.4 ± 8.8[c]
(−)-5e	42.8 ± 8.0	44.9 ± 6.3[c]
control	41.2 ± 8.6	50.7 ± 9.8[c]

[a] Results are expressed as the mean ± SD. [b] $p < 0.05$ (vs. growing tumors) determined by Student's t test. [c] Not significant.

Nicholson and Golder[16] have shown that biochemical parameters can be used as an indicator of hormonal effects on the growth of mammary tumors. In accordance with these results, the tumors regressing after administration of (+)-**5e** and (−)-**5e** exhibited a significantly lower activity of glucose-6-phosphate and 6-phosphogluconate dehydrogenase than growing tumors and a not significant increase of *p*-nitrophenylphosphatase activity. (Table VI).

The lack of estrogenic properties and the nullification of the effect of (±)-**5e** on MCF-7 cells by hexestrol (1) led us to the assumption that the weak tumor growth inhibiting effect of (±)-**5e** and compounds derived from it was possibly due to an antiestrogenic action. As a reason for the antitumor effect of antiestrogens, a direct inhibition of the proliferation stimulation in mammary carcinoma cells by endogenous estrogens, as well as an interference with the E2 or prolactin biosynthesis,[17] has to be discussed. Serum E2 levels were unaffected by administration of (+)-**5e** or (−)-**5e** [3 × 25 (mg/kg)/week; duration 4 weeks], a result similar to that obtained with the antiestrogen tamoxifen.[16] It is known that mammary tumor growth is inhibited by lowering the prolactin level,[18] but the results concerning the influence of antiestrogens on the prolactin level have been confusing.[16,19,20] We were not able to detect a significant change in the amount of serum prolactin in rats treated with (±)-**5e** or *meso*-**5e** (1 × 80 mg/kg), whereas ergocornine (50 μg/kg) reduced the mean prolactin level by 48% ($p < 0.02$). Usually we observe a correlation between antitumor effect and E2 receptor affinity within one class of estrogenic or antiestrogenic compounds;[7] the lack of such dependance in this case raised the question of whether general cytotoxic effects are responsible for the inhibition, as has been described for some estrogens.[21,22] This possiblity was ruled out by in vitro tests on the hormone-independent Walker 256 carcinosarcoma: even at a concentration of 10^{-4} M, none of the tested compounds [(±)-**5e**, *meso*-**5e**, and (±)-**5i**] were able to inhibit the DNA, RNA, or protein biosynthesis or produced a cytocidic effect. These results were also confirmed by in vivo experiments on Ehrlich ascites carcinoma of the mouse: no increase in life span was reached after administration of (±)-**5c** or (±)-**5e** (5 × 25 mg/kg).

(8) Niederl, J. B.; Dexter, M. I. *J. Am. Chem. Soc.* **1948**, *70*, 3071.
(9) Takahashi, I. *J. Chem. Soc. Jpn., Pure Chem. Sect.* **1952**, *73*, 805.
(10) Edwards, D. P.; Murthy, S. R.; McGuire, W. L. *Cancer Res.* **1980**, *40*, 1722.
(11) Edwards, D. P.; McGuire, W. L. *Endocrinology* **1980**, *107*, 884.
(12) Bronzert, D. A.; Monaco, M. E.; Pinkus, L.; Aitken, S.; Lippman, M. E. *Cancer Res.* **1981**, *41*, 604.
(13) Huggins, C.; Briziarelli, G.; Sutton, H. *J. Exp. Med.* **1959**, *109*, 25.
(14) Huggins, E.; Grand, L. C.; Brillantes, F. P. *Nature (London)* **1961**, *189*, 204.
(15) Fiebig, H. H.; Schmähl, D. *Recent Res. Cancer Res.* **1980**, *71*, 80.
(16) Nicholson, R. I.; Golder, M. P. *Eur. J. Cancer* **1975**, *11*, 571.
(17) McGuire, W. L. In "Breast Cancer: Antiestrogens"; Plenum Press: New York and London, 1977; pp 229–230.
(18) Teller, M. N.; Stock, C. L.; Hellman, L.; Mountain, I. M.; Bowie, M.; Rosenberg, B. J.; Boyar, R. M.; Budinger, J. M. *Cancer Res.* **1977**, *37*, 3932.
(19) Jordan, V. C.; Koerner, S.; Robinson, C. *J. Endocrinol.* **1975**, *65*, 151.
(20) Jordan, V. C.; Koerner, S. *J. Endocrinol.* **1976**, *68*, 305.
(21) Dietrich, L. S.; Friedland, M. J. *Cancer Res.* **1961**, *21*, 502.
(22) Lippman, M.; Bolan, G.; Huff, K. *Cancer Res.* **1976**, *36*, 4595.

Discussion

In our studies we were able to show that the E2 receptor affinity of hexestrol can be retained to some extent, if the ethyl groups are replaced by alkylamino groups and the configuration is altered from meso to racemic. Despite the receptor affinity, there was neither a uterotrophic nor an antiuterotrophic effect after administration of (±)-**5e**, the most active compound in this series. On the other hand, we observed a moderate growth inhibition of E2-dependent tumors like DMBA-induced mammary carcinoma of the Sprague–Dawley rat and MCF-7 mammary carcinoma cell culture by (±)-**5e**. There are no hints at general cytotoxic effects or a central mode of action via a decrease of the prolactin level. We tend to assume an estrogen antagonism, because the results obtained from the experiments with the MCF-7 cell line show that the growth inhibition by (±)-**5e** can be overcome by addition of hexestrol. This assumption has to be proved in further studies concerning the influence of **5e** on E2 receptor biosynthesis, depletion of cytoplasmatic E2 receptors, and progesterone receptor biosynthesis.

Experimental Section

Melting points were determined on Büchi melting point apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, Universities of Munich and Regensburg. The structures of all compounds were confirmed by their ^1H NMR spectra (Varian T 60 or EM 360A spectrometer).

General Procedures of Synthesis. Methoxybenzaldehyde Alkylimines. With stirring and cooling in a water bath, the alkylamine (0.33 mol) was added slowly to a solution of the methoxybenzaldehyde (0.30 mol) in a small volume of $CHCl_3$. After the mixture was stirred for 1 h at room temperature, the organic layer was separated and dried over $MgSO_4$. The solvent and starting material were removed in vacuo. The products were pure enough for further reactions. The yields ranged from 80 to 95%.

***N,N'*-Dialkyl-1,2-bis(methoxyphenyl)ethylenediamines (4a–i).** Aluminum foil (21 g, 0.75 g-atom), cut in small pieces, was added to a solution of $HgCl_2$ (3.0 g) in EtOH (25 mL) and heated. When gas evolution had started, a solution of methoxybenzaldehyde alkylimine (0.25 mol) in toluene (250 mL) was added slowly with mechanical stirring. Finally, the mixture was kept for 4 h at 100 °C. HCl (6 N, 100 mL) was added, and 30 min later, the solution was made alkaline by the addition of 6 N NaOH. The organic layer was separated, and the aqueous solution was extracted several times with $CHCl_3$. After the solution was dried ($MgSO_4$), the solvent was removed under reduced pressure, and the *N*-alkylmethoxybenzylamine byproduct was distilled off in vacuo. After the residue was dissolved in $CHCl_3$, the hydrochlorides were precipitated by saturating the solution with HCl gas. The dihydrochlorides of *meso*- and (±)-**4h,i** were separated by fractional crystallization from MeOH/Et_2O; the isomers of **4a–g** were isolated by Craig countercurrent distribution (CCD) (for data see Table II). The latter method yielded pure *meso*-**4a–g**; (±)-**4a–g** were purified by a second CCD using a lower concentration of HCl. The yields were 17–38% for the *meso*-ethylenediamines, 8–42% for the *d,l*-ethylenediamines, and 12–33% for the benzylamines. Melting points and recrystallization solvents are reported in Table I.

***N,N'*-Dialkyl-1,2-bis(hydroxyphenyl)ethylenediamines (5a–i).** $AlBr_3$ (10.7 g, 0.04 mol) in dry benzene (45 mL) was added to **4a–i** (0.01 mol) in the same solvent (20 mL). The mixture was boiled under reflux for 2 h. After the mixture was evaporated to dryness, water was added, and the solution was brought to pH 8–9 by the addition of ammonia. The phenolic ethylenediamines **5** were filtered off and recrystallized from Et_2O, or as hydrochlorides, from MeOH/Et_2O. The yields were between 88 and 96%. For further data, see Table I.

***N,N'*-Dialkyl-4,5-bis(4-methoxyphenyl)imidazolidines (6a–g).** A solution of **4** (0.005 mol) and paraformaldehyde (2.0 g), benzaldehyde, or anisaldehyde (0.005 mol) in $CHCl_3$/benzene (1:1, 30 mL) was refluxed for 15 h. After filtration, the solvent was removed and the residue was recrystallized from EtOH. **6a**: mp 73–74 °C; **6b**: mp 91–92 °C; $[\alpha]^{25}_D$ +30.5° (*c* 0.25, MeOH), synthesized from (–)-**4e**; **6c**: mp 92–93 °C; $[\alpha]^{25}_D$ –30.3° (*c* 0.25, MeOH), synthesized from (+)-**4e**.

Compounds that resisted crystallization (**6d–g**) were chromatographed on alumina (activity I) with elution by CH_2Cl_2/ligroin (1:1). The yields varied from 52 to 90%.

***N,N'*-Dialkyl-4,5-bis(4-hydroxyphenyl)imidazolidines (7a–g).** A solution of **6a–g** or **8** (5 mmol) in dry CH_2Cl_2 (30 mL) was cooled to –70 °C and, after the addition of BBr_3 (2.0 mL, 22 mmol), was stirred for 30 min. The cooling bath was removed and stirring was continued for 15 h. Under external cooling, water (20 mL) was added, and CH_2Cl_2 was removed under reduced pressure. The aqueous solution was made alkaline by the addition of NaOH and was then filtered and brought to pH 9 with 1 N HCl. The precipitate was filtered, washed thoroughly with water, and dried. The yields varied from 63 to 86%.

Special Procedures of Synthesis. *meso*-*N,N'*-Bis(4-methoxybenzylidene)-1,2-bis(4-methoxyphenyl)ethylenediamine (*meso*-13). *meso*-1,2-Bis(4-methoxyphenyl)ethylenediamine[4] (*meso*-**12**, 0.25 mol) and 4-methoxybenzaldehyde (0.5 mol) in MeCN (300 mL) were boiled under reflux for 3 h. After evaporation of 150 mL of solvent, the solution was cooled, and the crystalline product precipitated: yield 117 g (92%); mp 183 °C. The product was used without further purification.

(±)-1,2-Bis(4-methoxyphenyl)ethylenediamine [(±)-12]. In a 1-L flask, *meso*-**13** (55.9 g) was heated with stirring to 200–220 °C until the crystalline solid had been molten completely. The yellow oil was cooled and, after the addition of 3 N H_2SO_4 (300 mL), was distilled by steam until no more aldehyde was obtained. The hot mixture in the distillation flask was filtered; from the filtrate, *meso*-**12** sulfate precipitated as shiny platelets. The filtration residue was treated several times in the same way, until pure (±)-**12** sulfate crystallized from the aqueous solution. The separated (±)-**12** sulfate was treated with NaOH, extracted with $CHCl_3$, and dried (Na_2SO_4). After evaporation of the solvent, (±)-**12** crystallized on addition of Et_2O: yield 25.1 g [90% based on a 1:1 mixture of (±)- and *meso*-**12**]: mp 114 °C; ^1H NMR ($CDCl_3$) δ 1.60 (s, 4 H), 3.78 (s, 6 H), 4.00 (s, 2 H), 6.80, 7.18 (AB, J = 9 Hz, 8 H). Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

(+)- and (–)-1,2-Bis(4-methoxyphenyl)ethylenediamine [(+)- and (–)-12]. An aqueous solution (80 mL) of (±)-**12** (10.9 g, 0.04 mol) and L(+)-tartaric acid (6.0 g, 0.04 mol) was boiled for 10 min. After filtration, the solution was left standing overnight. The L(+)-tartrate of (–)-**12** precipitated and was recrystallized from water. The free base was obtained by treatment with NaOH, extraction with $CHCl_3$, and crystallization from Et_2O, yielding (–)-**12** as white needles: yield 4.73 g (87%); mp 89 °C ($CHCl_3$/Et_2O); $[\alpha]^{25}_{546}$ –134.5° (*c* 1, MeOH). Anal. ($C_{16}H_{20}N_2O_2$) C, H, N. From the mother liquor of the first precipitate, the free base was isolated and, together with a solution of D(–)-tartaric acid (3.5 g, 0.023 mol) in water (30 mL), was boiled for 5 min. While cooling, the D(–)-tartrate of (+)-**12** precipitated and was recrystallized from water. (+)-**12** was then obtained as described above: yield 4.79 g (88%); mp 90 °C ($CHCl_3$/Et_2O); $[\alpha]^{25}_{546}$ +138.4° (*c* 1, MeOH). Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

(+)-*N,N'*-Dibutyl-1,2-bis(4-methoxyphenyl)ethylenediamine [(+)-4e]. Butyryl chloride (2.23 g) in dry Et_2O (20 mL) was added dropwise with stirring to a suspension of (–)-**12** (1.9 g, 0.007 mol) in dry Et_2O (50 mL) containing Et_3N (2.2 g). After the addition, the mixture was boiled under reflux overnight. The precipitate was filtered, washed with Et_2O, and dried in vacuo. This product containing the amide and Et_3N hydrochloride was suspended in dry Et_2O (50 mL) and added with stirring to a suspension of $LiAlH_4$ (2.5 g) in dry Et_2O (100 mL). After boiling for 12 h, the excess of $LiAlH_4$ was destroyed by the dropwise addition of water with cooling. The etheral phase was separated, and the residue was extracted with Et_2O. After evaporation, the residue was dissolved in $CHCl_3$ and saturated with 3 N HCl (20 mL). The organic layer was dried, the solvent was removed, and the obtained hydrochloride was recrystallized from MeOH/Et_2O: yield 71%; mp 228–230 °C; $[\alpha]^{25}_{546}$ +46.2° (*c* 1, MeOH); ^1H NMR (CD_3OD) δ 0.83–2.63 (m, 18 H), 3.83 (s, 6 H), 4.83 (s, 2 H), 6.85–7.43 (m, 8 H). Anal. ($C_{24}H_{36}N_2O_2 \cdot 2HCl$) H, N; C: calcd, 63.01; found, 64.08.

(–)-*N,N'*-Dibutyl-1,2-bis(4-methoxyphenyl)ethylenediamine [(–)-4e]. Treatment of (+)-**12** as in the above procedure

gave (-)-4e as its hydrochloride (67%): mp 234 °C; $[\alpha]^{25}_{546}$ -45.6° (c 1, MeOH); ^{1}H NMR identical with (+)-4e and (±)-4e. Anal. ($C_{24}H_{36}N_2O_2 \cdot 2HCl$) H, N; C: calcd, 63.01; found, 63.66.

(±)-N,N'-Dibutyl-4,5-bis(4-methoxyphenyl)-imidazolidine-2-thione (8). (±)-4e (3.0 g, 7.7 mmol) was dissolved in CS_2 and stirred for 1 day. After evaporation of the solvent, the residue was dissolved in dilute EtOH (50%, 60 mL), and HCl (0.3 mL) was added. The solution was boiled under reflux for 60 h. After the addition of aqueous ammonia, the solution was extracted with ether. After drying and evaporation of the organic phase, etheral HCl was added and the precipitate was filtered. The solvent was removed from the filtrate, and the white solid obtained was dried in vacuo: yield 2.2 g (67%); mp 114-116 °C. The product was used for the ether cleavage without further purification.

Biological Methods. Thymidine Incorporation Study. Chemicals: [*Me*-^{3}H]thymidine (40-60 Ci/mol) was obtained from New England Nuclear Corp. Cell Culture: MCF-7 cells, a gift of Dr. Marc Lippman (NIH, Bethesda, MD), were grown in Falcon plastic flasks in 5% CO_2 in humidified air at 37 °C. The growth medium consisted of Richter's improved minimal essential medium with zinc and without insulin (Associated Biomedic Systems, Buffalo, NY), 10% fetal calf serum (FCS; Gibco), 2.2 g/L $NaHCO_3$, and 40 μg/mL gentamycin (Sigma Chemical Co.). Thymidine incorporation: Cells, growing in log phase, were harvested in 0.02% EDTA in Ca^{2+}/Mg^{2+}-free PBS salt solution and plated in 6-well Linbro dishes (Bellco) in Richter's medium with 10% FCS. The next day the medium was changed to Richter's medium with 5% calf serum stripped of endogenous hormones by 30-min incubation at 45 °C with a dextran-coated charcoal (DCC) pellet (0.25% Norit A, 0.0025% dextran in 0.01 M Tris/HCl, pH 7.4). The medium was changed every 72 h. When the cells were grown preconfluent (60% confluent), the test compounds, dissolved in absolute ethanol, were added; the final ethanol concentration was 0.1%. The incubation time was 60 h; 2 h before harvesting, 1 μCi of [^{3}H]-thymidine was added to each Linbro well. The cells were harvested with EDTA, washed 3 times with PBS, and sonicated (Branson, Cell disruptor B 15). [^{3}H]Thymidine incorporation into the acid-precipitable fraction of the cell sonicate was determined by the addition of 10% Cl_3AcOH and collection of the precipitate by filtering over a 0.45-μm filter (Millipore). The filters were transfered to scintillation vials and counted in a Liquid Scintillation Counter (Beckman LS 8000).

All other applied procedures have already been described in detail and were used without alterations. These methods are: E2-receptor binding assay,[23] Allen-Doisy test,[24] Dorfman uterine weight test,[23], inhibition of the DMBA-induced mammary tumor growth,[23] determination of enzyme activities of tumors,[16] assay of serum 17β-estradiol,[25] assay of prolactin,[26] inhibition of DNA, RNA, and protein syntheses of Walker 256 carcinosarcoma cells in vitro,[24] and growth inhibition of the Ehrlich ascites carcinoma in vivo.[27]

Acknowledgment. The authors thank Dr. M. Lippman, Medicine Branch, NCI, NIH, for the supply of MCF-7 cells, J. Curran, NIH, for providing the prolactin kit, Professor W. Wuttke, MPI for Biophysical Chemistry, Göttingen, BRD, for his advise concerning the prolactin assay, Mrs. M. Horn for technical assistance, and the Deutsche Forschungsgemeinschaft and the Verband der Chemischen Industrie, Fonds der Chemischen Industrie, for financial support.

(23) Hartmann, R. W.; Kranzfelder, G.; von Angerer, E.; Schönenberger, H. *J. Med. Chem.* **1980**, *23*, 841.
(24) Kranzfelder, G.; Schneider, M.; von Angerer, E.; Schönenberger, H. *J. Cancer Res. Clin. Oncol.* **1980**, *97*, 167.
(25) Cameron, E. H. D.; Jones, D. A. *Steroids* **1972**, *20*, 737.
(26) Cassedy, J. M.; Li, G. S.; Spitzer, E. B.; Floss, H. G.; Clemens, J. A. *J. Med. Chem.* **1974**, *17*, 300.
(27) Lettré, H. *Ergeb. Physiol., Biol. Chem. Exp. Pharmakol.* **1950**, *46*, 379.

Novel Orally Active Inhibitors of Passive Cutaneous Anaphylaxis in Rats: *N*-[2-(4-Pyridinyl)-4-pyrimidinyl]ureas and Dialkyl [[[2-(4-Pyridinyl)-4-pyrimidinyl]amino]methylene]malonates

George Y. Lesher,† Baldev Singh,*,† and Zigurd E. Mielens‡

Medicinal Chemistry Department and Department of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, New York 12144. *Received October 8, 1981*

4-Chloro-2-(4-pyridinyl)pyrimidines were treated with alkylamines to afford the corresponding N-substituted amino derivatives. 4-Amino-2-(4-pyridinyl)pyrimidines and their N-substituted analogues were converted to amides, carbamates, aminiomethylenemalonates, and ureas. Many of these compounds were found to have potential antiallergic activity as indicated by the rat passive cutaneous anaphylaxis screen. The most potent compounds were found in the last two series.

The introduction of disodium cromoglycate[1] in 1968 marked a major advancement for the treatment of allergic disease, such as extrinsic bronchial asthma; however, this compound does not have any significant oral activity. Since then, extensive efforts have been made to find more potent and orally effective compounds.[2]

Following the initial discovery of the antiallergic activity of 4-amino-2-(4-pyridinyl)pyrimidine (**4a**), an intermediate required for another project, we embarked on a program to explore this lead. We will describe here some of our work with this novel pyridinyl-substituted pyrimidine system which covers mainly the various substituents on the amino group of **4a** represented by general structure **1**. There is, we believe, only a superficial resemblance between **1** and both **2**[3] and **3**[4] (M + B 22948)[5] because the

† Medicinal Chemistry Department.
‡ Department of Pharmacology.

(1) J. B. L. Howell and R. E. C. Altounyan, *Lancet*, **2**, 539 (1967); J. S. G. Cox, *Nature (London)*, **216**, 1328 (1967); J. S. G. Cox in "Proceedings of Symposium of Disodium Cromoglycate in Allergic Airways Disease", J. Pepys and A. W. Frankland, Eds., Butterworths, London, 1970, pp 13-27; H. Cairns, C. Fitzmaurice, D. Hunter, P. B. Johnson, J. King, T. B. Lee, G. H. Lord, R. Minshull, and J. S. G. Cox, *J. Med. Chem.*, **15**, 583 (1972).
(2) J. P. Devlin, *Annu. Rep. Med. Chem.*, **16**, 61 (1981); J. P. Devlin, *ibid.*, **15**, 59 (1980); see also chapters on pulmonary and antiallergy drugs in previous volumes of *Annu. Rep. Med. Chem.*

0022-2623/82/1825-0837$01.25/0