(2-Chloroethyl)nitrosourea Congeners of Amino Acid Amides

Tetsuo Suami,* Tsuguhiro Kato, Hiroaki Takino, and Takashi Hisamatsu

Department of Applied Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Yokohama 223, Japan.
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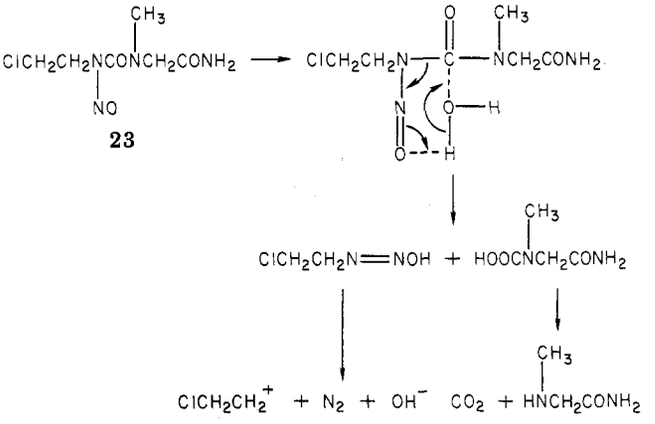
Fourteen (2-chloroethyl)nitrosourea congeners of L-amino acid amides have been synthesized as potential antineoplastic agents. Almost all the congeners tested were found to be highly active against experimental leukemia L1210 in mice. The chemical decomposition rates of the congeners were measured in a buffered solution (pH 7.4) at 37 °C. Acute toxicities of some of the congeners were determined for mice. The congener of sarcosinamide shows the longest half-life ($T_{0.5}$ = 329.7 min) and the lowest toxicity, LD_{50} = 392.0 mg/kg (ip) and 426.6 mg/kg (iv), in this series.

A large number of nitrosoureas have been synthesized and evaluated for antitumor activity against mouse leukemia L1210.[1] Since enhancement of the activity resulted by replacement of the methyl group on the nitrosated nitrogen atom by a 2-chloroethyl group, a wide variety of (2-chloroethyl)nitrosourea derivatives have been synthesized, and the structural dependences on antitumor activity have been studied extensively.[2-14] The (2-chloroethyl)nitrosoureas are currently recognized as an important class of clinically useful antitumor agents.

Except for a few methylnitrosoureas of amino acids[15] and the congener of glycinamide (acetamido-CNU),[16] the (2-chloroethyl)nitrosourea congeners of amino acids and their derivatives have never been studied.

Since L-amino acids are actively transported into mammalian tissues, they might be good carriers of the functional (2-chloroethyl)nitrosoureido group. Also, the alteration of permeability of plasma membranes of mammalian cells may play an important role in the regulation of cell growth and proliferation.[17-19] It has been described in the literature[20] that polyoma virus-transformed 3T3 cells accumulated α-aminobutyric acid, cycloleucine, and L-glutamine about twice as rapidly as 3T3 cells. This has also been demonstrated by other investigators.[21-23] The differences in the permeabilities of some amino acids through the plasma membranes of normal cells and those of transformed cells may provide a rationale for the selectivity in cancer chemotherapy. That is, a proper choice of a suitable amino acid as a carrier of the functional nitrosoureido group might give an antitumor agent that could kill the transformed cells selectively without causing fatal damages in the normal cells.

Scheme I



With this in mind, the (2-chloroethyl)nitrosourea congeners of amino acid amides have been synthesized in an attempt to develop an antitumor agent with clinical potential. The synthesis of the 14 congeners and their antitumor activity against leukemia L1210 in mice are reported here, together with chemical decomposition rates and acute toxicity for mice.

Chemistry. The synthesis of the congeners of amino acid amides involved two different general methods. Method A: Treatment of an amino acid amide with 4-nitrophenyl *N*-(2-chloroethyl)-*N*-nitrosocarbamate[24] yields the congeners of glycinamide (**1**), β-alaninamide (**2**), and

(1) T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, **6**, 669 (1963).
(2) T. P. Johnston, G. S. McCaleb, P. S. Opliger, and J. A. Montgomery, *J. Med. Chem.*, **9**, 892 (1966).
(3) T. P. Johnston, G. S. McCaleb, P. S. Opliger, W. R. Laster, and J. A. Montgomery, *J. Med. Chem.*, **14**, 600 (1971).
(4) T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, **18**, 104 (1975).
(5) T. Machinami, S. Nishiyama, K. Kikuchi, and T. Suami, *Bull. Chem. Soc. Jpn.*, **48**, 3763 (1975).
(6) J. A. Montgomery, G. S. McCaleb, T. P. Johnston, J. G. Mayo, and W. R. Laster, Jr., *J. Med. Chem.*, **20**, 291 (1977).
(7) T. S. Lin, P. H. Fischer, G. T. Shiau, and W. H. Prusoff, *J. Med. Chem.*, **21**, 130 (1978).
(8) T. P. Johnston, G. S. McCaleb, T. Anderson, and D. S. Murison, *J. Med. Chem.*, **22**, 597 (1979).
(9) J. A. Montgomery and H. J. Thomas, *J. Med. Chem.*, **22**, 1109 (1979).
(10) H. P. Lam, A. Begleiter, G. J. Goldenberg, and C. M. Wong, *J. Med. Chem.*, **22**, 200 (1979).
(11) T. Suami, T. Machinami, and T. Hisamatsu, *J. Med. Chem.*, **22**, 247 (1979).
(12) T. Suami, K. Tadano, and W. T. Bradner, *J. Med. Chem.*, **22**, 314 (1979).
(13) A. M. Crider, R. Lamey, H. G. Floss, J. M. Cassady, and W. T. Bradner, *J. Med Chem.*, **23**, 848 (1980).
(14) C. Hansch, A. Leo, C. Schmidt, P. Y. C. Jow, and J. A. Montgomery, *J. Med. Chem.*, **23**, 1095 (1980).
(15) T. Machinami and T. Suami, *Bull. Chem. Soc. Jpn.*, **48**, 1333 (1975).
(16) W. J. Zeller, G. Eisenbrand, and H. H. Fiebig, *J. Cancer Res. Clin. Oncol.*, **95**, 43 (1979).
(17) K. K. Sanford, B. E. Barker, M. W. Woods, R. Parshad, and L. W. Law, *J. Natl. Cancer Inst.*, **39**, 705 (1967).
(18) G. L. Nicolson, *Biophys. Acta*, **458**, 1 (1976).
(19) Y. S. Kim, J. S. Whitehead, B. Siddiqui, and D. Tsao, *ACS Symp. Ser.*, **80**, 295 (1978).
(20) D. O. Foster and A. B. Pardee, *J. Biol. Chem.*, **244**, 2675 (1969).
(21) J. F. Perdue, *J. Cell. Physiol.*, **89**, 729 (]976).
(22) J. E. Lever, *J. Cell. Physiol.*, **89**, 779 (1976).
(23) J. R. Parnes, T. Q. Garvey III, and K. J. Isselbacher, *J. Cell. Physiol.*, **89**, 789 (1976).
(24) H. Nakao, M. Fukushima, F. Shimizu, and M. Arakawa, *Yakugaku Zasshi*, **98**, 1032 (1974).
(25) H. T. Hanson and E. L. Smith, *J. Biol. Chem.*, **175**, 833 (1948).
(26) T. Jakobiec, *Acta Polon. Pharm.*, **23**(2), 115 (1966).
(27) S. Goldschmidt and K. K. Gupta, *Chem. Ber.*, **98**, 2831 (1965).
(28) E. Sondheimer and R. W. Holley, *J. Am. Chem. Soc.*, **76**, 2467 (1954).
(29) C. A. Dekker, S. P. Taylor, and J. S. Fruton, *J. Biol. Chem.*, **180**, 155 (1949).
(30) S. M. McElvain and P. M. Laughton, *J. Am. Chem. Soc.*, **73**, 442 (1951).

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Table I. Physical and Analytical Data for the (2-Chloroethyl)nitrosourea Congeners of Amino Acid Amides (Method A)

	$ClCH_2CH_2N(NO)CO$-R				
no.	R	mp, °C	yield, %	formula	anal.
1	$NHCH_2CONH_2$	125-126 dec	73	$C_5H_9N_4ClO_3$	C, H, N, Cl
2	$NH(CH_2)_2CONH_2$	95-97 dec	59[a]	$C_6H_{11}N_4ClO_3$	C, H, N, Cl
3	$NH(CH_2)_3CONH_2$	102-104 dec	63[b]	$C_7H_{13}N_4ClO_3$	C, H, N, Cl

[a] Yield from *N*-(benzyloxycarbonyl)-β-alaninamide.[25] [b] Yield from methyl 4-amionbutyrate hydrochloride.[26]

Table II. Physical and Analytical Data for the (2-Chloroethyl)urea Derivatives of Amino Acid Amides (Method B)

	$ClCH_2CH_2NHCO$-R					
no.	R	mp, °C	$[\alpha]_D$, deg (*c*, solvent)	yield, %	formula	anal.
4	$NHCH(CH_3)CONH_2$	151-152	+30.6 (1.1, DMF)	66[a]	$C_6H_{12}N_3ClO_2$	C, H, N, Cl
6	$NHCH[CH(CH_3)_2]CONH_2$	197-199	+48.9 (0.5, DMF)	86	$C_8H_{16}N_3ClO_2$	C, H, N, Cl
8	$NHCH[CH_2CH(CH_3)_2]CONH_2$	171-173	−3.7 (0.6, CH_3OH)	76	$C_9H_{18}N_3ClO_2$	C, H, N, Cl
10	$NHCH(CH_2CONH_2)CONH_2$	162-167	+23.5 (0.9, DMF)	66[b]	$C_7H_{13}N_4ClO_3$	C, H, N, Cl
12	$NHCH(CH_2CH_2SCH_3)CONH_2$	150-151	+5.2 (0.4, CH_3OH)	86	$C_8H_{16}N_3ClSO_2$	C, H, N
14	$NHCH(CH_2OH)CONH_2$	131-132	+35.7 (0.6, CH_3OH)	76	$C_6H_{12}N_3ClO_3$	C, H, N, Cl
16	$NHCH[CH(CH_3)OH]CONH_2$	132-134	+17.2 (1.3, CH_3OH)	88[c]	$C_7H_{14}N_3ClO_3$	C, H, N, Cl
18	$NHCH(CH_2C_6H_5)CONH_2$	158-160	+9.8 (0.8, CH_3OH)	84	$C_{12}H_{16}N_3ClO_2$	C, H, N, Cl
20	$NHCH(CH_2C_6H_4OH\text{-}p)CONH_2$	187-189	+0.4 (0.5, DMF)	86	$C_{12}H_{16}N_3ClO_3$	C, H, N, Cl
22	$N(CH_3)CH_2CONH_2$	103-105		56[d]	$C_6H_{12}N_3ClO_2$	C, H, N, Cl

[a] Yield from methyl L-alaninate hydrochloride.[27] [b] Yield from *N*-(benzyloxycarbonyl)-L-asparaginamide.[28] [c] Yield from *N*-(benzyloxycarbonyl)-L-threoninamide.[29] [d] Yield from ethyl sarcosinate hydrochloride.[30]

Table III. Physical and Analytical Data for the (2-Chloroethyl)nitrosourea Congeners of Amino Acid Amides (Method B)

	$ClCH_2CH_2N(NO)CO$-R					
no.	R	mp, °C	$[\alpha]_D$, deg (*c*, solvent)	yield, %	formula	anal.
5	$NHCH(CH_3)CONH_2$	84-85	+58.3 (0.4, CH_3OH)	62	$C_6H_{11}N_4ClO_3$	C, H, N, Cl
7	$NHCH[CH(CH_3)_2]CONH_2$	115-117 dec	+60.7 (0.6, DMF)	80	$C_8H_{15}N_4ClO_3$	C, H, N, Cl
9	$NHCH[CH_2CH(CH_3)_2]CONH_2$	85-87	+18.4 (0.6, DMF)	64	$C_9H_{17}N_4ClO_3$	C, H, N, Cl
11	$NHCH(CH_2CONH_2)CONH_2$	146-148	+37.4 (0.8, DMF)	64	$C_7H_{12}N_5ClO_4$	C, H, N, Cl
13	$NHCH(CH_2CH_2SCH_3)CONH_2$	95-97 dec	+18.7 (0.5, DMF)	71	$C_7H_{15}N_4ClSO_3$	C, H, N
15	$NHCH(CH_2OH)CONH_2$	117-120 dec	+57.6 (0.7, CH_3OH)	66	$C_6H_{11}N_4ClO_4$	C, H, N, Cl
17	$NHCH[CH(CH_3)OH]CONH_2$	129-130 dec	+49.3 (1.3, CH_3OH)	84	$C_7H_{13}N_4ClO_4$	C, H, N, Cl
19	$NHCH(CH_2C_6H_5)CONH_2$	113-115 dec	−44.6 (0.8, DMF)	70	$C_{12}H_{15}N_4ClO_3$	C, H, N, Cl
21	$NHCH(CH_2C_6H_4OH\text{-}p)CONH_2$	136-137 dec	−30.4 (0.5, DMF)	51	$C_{12}H_{15}N_4ClO_4$	C, H, N, Cl
23	$N(CH_3)CH_2CONH_2$	86-88 dec		83[a]	$C_6H_{11}N_4ClO_3$	C, H, N, Cl
24		82-85 dec	−25.5 (0.7, CH_3OH)	70[a,b]	$C_6H_{13}N_4ClO_3$	C, H, N, Cl

[a] The product was extracted with ethyl acetate from the nitrosation solution, which had been neutralized with a $NaHCO_3$ solution. [b] Yield from L-prolinamide.

4-aminobutyramide (**3**) (see Table I). Method B: Carbamoylation of an amino acid amide with 2-chloroethyl isocyanate, followed by conventional nitrosation with sodium nitrite in 99% formic acid, gives the other 11 congeners (see Tables II and III). In the latter method, there are two positions to be nitrosated, but the nitrogen attached to the 2-chloroethyl group is preferentially nitrosated. This has been demonstrated by NMR spectroscopy (see Table IV).

Chemical decomposition rates of the congeners have been determined in a buffered solution (pH 7.4) at 37 °C by the method described in the literature.[31,32] Half-lives of the congeners have been calculated and are listed in Table V. The half-life of the congener of sarcosinamide (**23**) is extremely long ($T_{0.5}$ = 329.7 min) and so is that of the congener of L-prolinamide (**24**) ($T_{0.5}$ = 294.6 min).

The mode of decomposition of a nitrosourea has been studied extensively, and it has been described that the decomposition of *N*-(2-chloroethyl)-*N'*-substituted-*N*-nitrosourea yields (2-chloroethyl) diazohydroxide, which gives rise to the 2-chloroethyl cation, and the corresponding isocyanate.[33-35]

Table IV. ^1H NMR Chemical Shifts[a] and Coupling Constants

	chemical shift (*J*, Hz), δ		
compd	$ClCH_2CH_2$ (t, 2 H)	$ClCH_2CH_2$ (t, 2 H)	CONHCH (d, 1 H)
5	3.48 (6.9)	4.12 (6.9)	9.30 (7.0)
7	3.56 (6.6)	4.20 (6.6)	8.91 (8.1)
9	3.51 (7.1)	4.15 (7.1)	9.31 (8.1)
11	3.45 (7.1)	4.07 (7.1)	9.80 (8.1)
13	3.53 (7.1)	4.17 (7.1)	9.46 (8.1)
15	3.47 (6.1)	4.10 (6.1)	9.03 (6.1)
17	3.21 (7.1)	3.85 (7.1)	8.77 (8.0)
19		4.09 (7.1)	9.39 (8.1)
21		4.07 (7.1)	9.23 (7.6)
23	3.56 (6.1)	4.11 (6.1)	
24	3.57 (6.1)	4.16 (6.1)	

[a] In parts per million downfield from tetramethylsilane. Pyridine-d_5 was used as a solvent, except in the case of **23**, which was determined in $CDCl_3$.

(31) G. P. Wheeler, B. J. Bowden, J. Grimsley, and H. H. Lloyd, *Cancer Res.*, **34**, 194 (1974).
(32) L. C. Panasci, D. Green, R. Nagourney, P. Fox, and P. S. Schein, *Cancer Res.*, **37**, 2615 (1977).

Table V. Antitumor Activity of (2-Chloroethyl)nitrosourea Congeners of L-Amino Acid Amides against Mouse Leukemia L1210[a]

no.	compd	MED,[b] (mg/kg)/ day	mean survival days	ILS,[c] %	60-day survivors[d]	TI[e]	half-life, min
1	glycinamide	16	>60.0	>679.2	5/5	36.4	44.0
2	β-alaninamide	16	>60.0	>733.3	5/5	15.2	61.0
3	4-aminobutyramide	16	>60.0	>733.3	5/5	7.3	72.5
5	alaninamide	8	>60.0	>710.8	5/5	14.3	37.0
7	valinamide	8	>60.0	>757.1	5/5	15.7	44.5
9	leucinamide	24	>60.0	>757.1	5/5	11.4	48.3
11	asparaginamide	8	>42.8	>494.4	2/5	15.7	32.1
13	methioninamide	16	27.2	277.8	0/5	5.9	38.1
15	serinamide	8	>60.0	>757.1	5/5	40.0	19.8
17	threoninamide	8	>60.0	>757.1	5/5	40.0	23.3
19	phenylalaninamide	32	>38.2	>445.7	1/5	3.8	49.9
21	tyrosinamide	16	>35.4	>342.5	2/5	10.0	
23	sarcosinamide	225	>60.0	>710.8	5/5	15.5	329.7
24	prolinamide	64	>25.2	>250.0	1/5	5.6	294.6

[a] Male BDF_1 hybrid mice were inoculated intraperitoneally with 10^6 cells of lymphoid leukemia L1210. Compounds were dissolved in distilled water and were administered intraperitoneally at a volume of 0.1 mL/animal once a day for 3 days from 24 h (day 1) after the tumor implantation. [b] The shown dosages were maximal effective ones. [c] Percentage increase in life span of treated animals compared with control tumor bearers [100(T/C − 1)]. Most of the animals were surviving for 60 days; therefore, we calculated percent ILS including 60-day survivors. [d] Numbers of 60-day survivors per treated mice. [e] Therapeutic index = ratio of maximal effective dose/the dose which gives 30% ILS.

Both **23** and **24** have the tertiary amino group, and their decomposition must follow a unique route. By analogy with the mode of the decomposition of an ordinary nitrosourea, it can be speculated that decomposition of **23** in buffered solution would give the same (2-chloroethyl) diazohydroxide. At the same instance, carbamic acid would be generated, instead of an isocyanate, since the methylamino group of **23** can not produce an isocyanate. The carbamic acid, in turn, gives carbon dioxide and sarcosinamide as decomposition products. This has been deduced from the fact that decomposition of **23** in buffered solution gave sarcosinamide as the sole ninhydrin-positive decomposition product. The mechanistic sequence of the in vitro decomposition of **23** is proposed in Scheme I. It is conceivable that the decomposition might follow an alternative route in vivo.

Biological Results and Discussion

The congeners synthesized in the present study were evaluated for antitumor activity against leukemia L1210 in mice by the method described in the literature.[36,37] The results are shown in Table V, together with the chemical decomposition rates and therapeutic indexes.

Almost all the congeners tested were highly active against leukemia L1210 in mice. The most unique member in this series is the congener of sarcosinamide **23**, producing >710.8% ILS between 96 and 225 mg/kg doses and curing all mice by day 60, except for one case in which a 180 mg/kg dose was administered.

The in vitro half-life of **23** is extremely long compared to those of hitherto known water-soluble (2-chloroethyl)-nitrosoureas. However, the half-life of **24** is also long, and **24** is far less active than **23**. The difference in antitumor activities between **23** and **24** may be attributable to their different modes of decomposition in vivo.

Acute toxicities of **1**, **23**, and **24** were determined for male mice of the BDF_1 hybrid strain by a single intraperitoneal or intravenous injection, followed by observation for 21 days. The median lethal doses (LD_{50}) of the congeners for mice were calculated by the Litchfield–Wilcoxon method,[38] and the results are shown in Table VI. Compound **23** is the least toxic one in this series and shows LD_{50} values of 426.6 mg/kg (iv) and 392.0 mg/kg (ip).

Table VI. LD_{50} Values of (2-Chloroethyl)nitrosourea Congeners for Mice[a]

compd	route	sex	LD_{50}, mg/kg
1	ip	male	21.23 (20.58–21.90)[b]
	iv	male	22.40 (20.90–24.00)
23	ip	male	392.00 (385.00–400.00)
	iv	male	426.60 (346.70–524.80)
24	ip	male	219.60 (204.40–236.40)
	iv	male	195.40 (154.90–246.60)

[a] SLC-BDF_1 mice were used, six males/group, 5 weeks old. The LD_{50} values were calculated according to the Litchfield–Wilcoxon method.[38] [b] Figures in parentheses show 95% fiducial limits. Observation period was 21 days after the ip or iv administration.

The congeners **1**, **2**, and **3** are equally active (>679.2, >733.3, and >733.3% ILS, respectively), and their in vitro half-life increases from 44.0 to 61.0 to 72.5 min in order of their increasing molecular weight. The congener of serinamide (**15**) shows the shortest half-life ($T_{0.5}$ = 19.8 min) and yet is as highly active (>757.1% ILS) as **3**.

In summary, almost all the congeners of amino acid amides tested in the present study are highly active against experimental leukemia L1210 in mice. The congener of sarcosinamide **23** exhibits especially high antitumor activity, low acute toxicity, and extremely long half-life.

Experimental Section

Solutions were concentrated under reduced pressure with a rotary evaporator below 30 °C. Melting points were determined in capillary tubes in a liquid bath and are uncorrected. ^1H NMR spectra were recorded with a Varian Associates EM-390 spectrometer at 90 MHz with reference to tetramethylsilane as internal standards, and chemical shifts are expressed in δ values (parts per million). Analytical results indicated by elemental symbols were within ±0.4% of the theoretical values. As a starting material, glycinamide hydrochloride was purchased from Tokyo Kasei Kogyo Co., β-alanine, 4-aminobutyric acid, L-alanine, L-asparagine,

(33) J. A. Montgomery, R. James, G. S. McCaleb, M. C. Kirk, and T. P. Johnston, *J. Med. Chem.*, **18**, 568 (1975).
(34) G. P. Wheeler, *ACS Symp. Ser.*, **30**, 88 (1976).
(35) J. W. Lown and S. M. S. Chauhan, *J. Org. Chem.*, **46**, 2479 (1981).
(36) J. L. Hartwell, *Cancer Chemother. Rep., Part 1*, **25**, (1962).
(37) T. Hisamatsu and S. Uchida, *Gann*, **18**, 819 (1977).
(38) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).

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.

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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.
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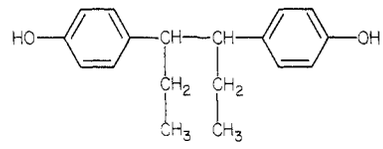
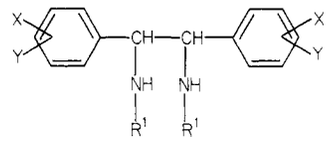
Diastereomeric *N,N'*-dialkyl-1,2-bis(hydroxyphenyl)ethylenediamines (**5**) were synthesized and tested for their affinity for the estradiol receptor. Only the (±)-1,2-bis(4-hydroxyphenyl)ethylenediamines with the alkyl groups C_3H_7 [(±)-**5c**, $K_a = 1.1 \times 10^6$)], C_4H_9 [(±)-**5e**,$K_a = 3.6 \times 10^6$], 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