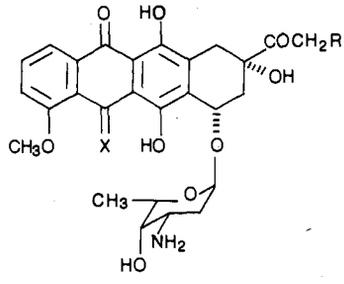
Anthrapyrazole Anticancer Agents. Synthesis and Structure-Activity Relationships against Murine Leukemias

H. D. Hollis Showalter,*† Judith L. Johnson,† Jeanne M. Hoftiezer,† William R. Turner,† Leslie M. Werbel,† Wilbur R. Leopold,‡ Joan L. Shillis,‡ Robert C. Jackson,‡ and Edward F. Elslager‡

Departments of Chemistry and Chemotherapy, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105. *Received May 8, 1986*

Chromophore modification of the anthracenediones related to mitoxantrone (**5**) in an attempt to provide agents with diminished or no cardiotoxicity has resulted in a novel class of DNA binders, the anthrapyrazoles (**9**). Their synthesis was carried out by a two-stage condensation sequence starting from requisite 1,4- or 1,5-dichloro-9,10-anthracenedione precursors. Reaction with a monoalkylhydrazine gave a chloroanthrapyrazole intermediate whose subsequent condensation with primary or secondary alkylamines provided the target "two-armed" anthrapyrazoles. A-ring 7,10-dihydroxy anthrapyrazoles were derived from amine condensation with intermediate 5-chloro-7,10-dihydroxyanthrapyrazoles or, alternatively, from intermediate 5-chloro-7,10-bis(benzyloxy)anthrapyrazoles followed by hydrogenolysis of the benzyl protecting groups to provide the target compounds. Potent in vitro activity was demonstrated against murine L1210 leukemia in vitro (IC_{50} = 10^{-7}–10^{-8} M) as well as against P388 leukemia in vivo over a wide range of structural variants. In general, activity against the P388 line was maximized by (a) basic side chains at N-2 and C-5, (b) two to three carbon spacers between proximal and distal nitrogens of the side chain, and (c) A-ring hydroxylation. Besides having curative activity against the P388 line, the more active compounds were curative against murine B-16 melanoma in vivo. On the basis of their exceptional in vivo anticancer activity, A-ring dihydroxy compounds **71** and **74** reported in this study have been selected for development toward clinical trials.

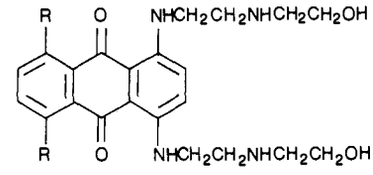
Intercalating agents that exert their action primarily through binding to DNA occupy a prominent position in the treatment of malignant diseases. The anthracycline antibiotics, primarily daunorubicin (**1**) and doxorubicin (**2**), have well-established roles in the treatment of human cancer.[1] Doxorubicin is considered to have the broadest range of clinical utility of all the anticancer drugs in current use. Among several toxicities associated with doxorubicin treatment, a serious chronic toxicity is cumulative dose-related cardiotoxicity that ranges from a delayed and insidious cardiomyopathy to irreversible congestive heart failure.



1: X=O, R=H; daunorubicin
2: X=O, R=OH; doxorubicin
3: X=NH, R=H; 5-iminodaunorubicin
4: X=NH, R=OH; 5-iminodoxorubicin

A great deal of research effort has been directed toward finding new anthracyclines that might retain the excellent broad-spectrum activity of doxorubicin while eliminating its cardiotoxicity.[2] In this regard, the third-generation anthracycline epirubicin is reported to have a reduced risk of cardiotoxicity in animal models. While the clinical data are inconclusive, preliminary findings indicate that epirubicin may have a lower cumulative cardiotoxicity than doxorubicin.[3]

The new synthetic anthracenedione DNA-intercalating agents, mitoxantrone (**5**)[4] and ametantrone (**6**),[5] superficially related to the anthracyclines structurally, have shown to date a relatively low incidence of associated cardiac failure in the clinic. Of these two agents, mitoxantrone has been more thoroughly evaluated. In phase II studies, it has been used primarily for the treatment of breast cancer, the acute leukemias, and certain lymphomas.[6]



5: R=OH; mitoxantrone
6: R=H; ametantrone

Several studies have suggested that anthracycline cardiotoxicity in part may be associated with the formation of reactive oxygen species and subsequent intracellular lipid peroxidation from enzymatic reduction of the quinone chromophore to a semiquinone radical species.[7] Hence, the reports by Acton et al.[8] of the significantly reduced

† Department of Chemistry.
‡ Department of Chemotherapy.

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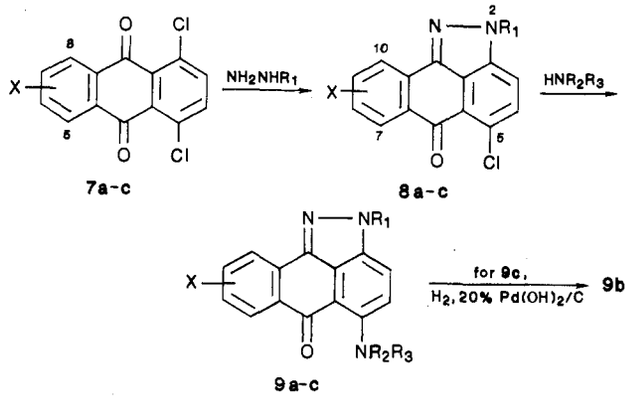
Scheme I[a]



a, X = H; b, X = 7,10-$(OH)_2$; c, X = 7,10-$(OBn)_2$; R_1 = H, alkyl, substituted aminoalkyl; R_2 = alkyl, substituted aminoalkyl. R_3 = H, alkyl

[a] Bn = benzyl.

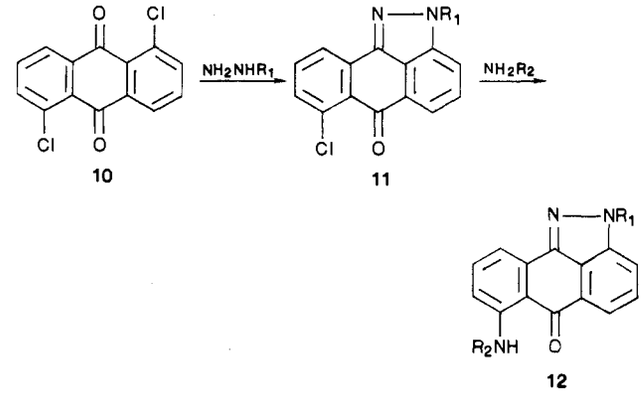
cardiotoxicity in conventional in vitro and in vivo assays by the semisynthetic anthracyclines 5-iminodaunorubicin (**3**) and 5-iminodoxorubicin (**4**) have spurred the search for additional analogues in which similar C-ring chromophore modification might lead to the suppression of redox cycling and radical generation.[9]

On the basis of these findings, we decided to design chromophore-modified anthracenediones related to mitoxantrone (**5**), and the anthra[1,9-*cd*]pyrazol-6(2*H*)-one (hereafter referred to as anthrapyrazole) ring system thus became an initial target. Here, not only is the central quinone moiety being modified to a quasi-iminoquinone but also, in contrast to the chromophore-modified anthracyclines, our strategy incorporates another ring into the chromophore.

Previous reports from these laboratories have delineated design rationale[10] and preliminary synthetic work[11] for selected members of the anthrapyrazole series. In this paper, we report the detailed synthetic studies and the biological evaluation against murine L1210 leukemia in vitro and P388 leukemia and B-16 melanoma in vivo for a large series of anthrapyrazoles possessing deshydroxy or 7,10-dihydroxylation patterns in the A ring and varied substituents on the N-2 and C-5 positions, and a smaller series of A-ring deshydroxy anthrapyrazoles with substituents at the N-2 and C-7 positions (Schemes I and II).

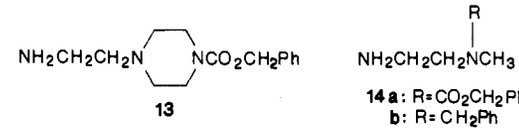
Chemistry. The anthrapyrazoles bearing side-chain functionality at the N-2 and C-5 positions were synthesized by a two-stage condensation sequence starting from requisite 1,4-dichloro-9,10-anthracenedione precursors **7a–c** (Scheme I). Briefly, reaction of **7a–c** with a monoalkylhydrazine gave the 2-substituted 5-chloroanthrapyrazole intermediates **8a–c** whose subsequent condensation with primary or secondary substituted alkylamines gave the "two-armed" anthrapyrazoles **9a–c**. We utilized a similar

Scheme II



route, starting from 1,5-dichloro-9,10-anthracenedione (**10**), to synthesize the 7-chloroanthrapyrazole intermediates **11** and thence target 2,7-disubstituted compounds **12** (Scheme II). Detailed procedures for the synthesis and spectroscopic evaluation of all chloroanthrapyrazole precursors (**8a–c** and **11**) for compounds reported in this study have been published elsewhere.[12]

A number of methods were developed to attach the lower side chains onto the chloroanthrapyrazole precursors. Method A, in which the substrates **8a–c** and **11** were condensed with 5–10 equiv of the lower side chain amine in refluxing pyridine, has precedent in the literature.[13] This was the method of choice for A-ring deshydroxy substrates **8a** and **11**, giving good to excellent yields of products in most cases. While condensations of some 7,10-dihydroxy-5-chloroanthrapyrazoles (**8b**) proceeded satisfactorily under these conditions, we found generally that lowering of the reaction temperature to 80 °C resulted in better yields and purer product (method F). The apparent higher reactivity of **8b** relative to **8a** reflects the added activation of the C-5 chlorine toward aromatic nucleophilic displacement due to polarization of the C-6 carbonyl and N-1 imine moieties through intramolecular hydrogen bonding by the C-7 and C-10 hydroxyls, respectively.[12] For the synthesis of compounds **27** and **45**, it was necessary to protect the secondary nitrogen of the piperazinyl moiety with the carbobenzyloxy (Cbz) group since condensation with the unmasked precursor triamine led to a mixture of products. Hence, we synthesized compound **13** by a procedure similar to the synthesis of **14a** and condensed it with the appropriately elaborated



chloroanthrapyrazole precursors **8a** by utilizing method A. The resultant adducts were then converted to target compounds via 48% HBr hydrolysis of the Cbz group (method C). In a similar fashion, compounds **74** and **98**, which possess the [2-(methylamino)ethyl]amino substituent at C-5, were derived from protected precursor **14a**.[14]

The condensation of **8a–c** with diamino alcohols [e.g., $NH_2(CH_2)_xNH(CH_2)_2OH$, x = 2 or 3] or polyamines [e.g., $NH_2(CH_2)_2NH(CH_2)_2NH_2$, $NH_2(CH_2)_2NH(CH_2)_2NMe_2$, spermine], which in principle could occur either on a

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primary or secondary nitrogen, always occurred selectively on the terminal primary nitrogen when the secondary nitrogen was flanked by two hydrogen-bonding moieties.[15] This unexpected "reverse" selectivity is observed here due to intramolecular inductive and hydrogen-bonding effects, which render the primary nitrogen more nucleophilic. Such functional group interaction has been previously documented for polyamines[16] and has been exploited advantageously in polyamine synthesis.[17]

The synthesis of selected A-ring deshydroxy anthrapyrazoles **9a** was also investigated by two alternate methods. Compound **18** was derived from the condensation of its corresponding chloroanthrapyrazole precursor **8a** with excess diamine in refluxing Me_2SO with KF catalysis (method B), and compounds **39** and **40** were derived via similar condensation in refluxing 2-ethoxyethanol under modified Ullman conditions[18] (method E). Both methods were inferior to method A.

All A-ring deshydroxy and 7,10-dihydroxyanthrapyrazoles synthesized in this study are listed in Tables I and III.

The synthesis of 7,10-dihydroxyanthrapyrazoles **9b** was carried out from directed amine condensation onto diphenolic precursors **8b** in hot pyridine (methods A and F, vide supra) or by hydrogenolysis of benzylated precursors **9c** (method D). Compounds **9c** were derived from **8c** by method A or more conveniently and in higher yield by condensation with an excess of neat amine at 130–135 °C (method G), a reaction previously reported in the anthrapyrazole literature.[14,19] Such benzylated precursors prepared by these methods are shown in Table II and offered the advantage of silica gel chromatographic purification of **9c** prior to a clean and high-yielding hydrogenolysis to **9b** over Pearlman's catalyst.[20] For the synthesis of compound **74** by method D, it was necessary to proceed through the intermediacy of **116**. This required the use of protected diamine **14b**[21] since **14a** possesses marginal stability at 130 °C.

For many of the compounds in Tables I–III, difficulty was encountered in obtaining pure samples for microanalysis, particularly since some compounds tenaciously retained solvents. We did not dry these compounds at higher temperatures because of potential thermal instability. The amounts of solvent needed to rationalize the analytical data and the ^1H NMR evidence for the presence of this solvent are given in the tables. The salt and free base forms of many of the target compounds in Tables I and III were sufficiently insoluble in the assay medium or hygroscopic to preclude an accurate determination of water of hydration by Karl Fischer analysis. For larger lots of certain compounds prepared for secondary evaluation, we found it expedient to allow these to equilibrate in the air at normal room humidity prior to microanalysis.

The color of the target anthrapyrazoles as salts in aqueous solution ranges from orange for A-ring deshydroxy compounds to deep red for compounds with the 7,10-dihydroxylation pattern. The anthrapyrazoles in general display excellent long-term stability over a wide pH range. However, the dihydroxy analogues are oxidatively unstable at alkaline pH (ca. 30% decomposition at 25 °C for 24 h in 0.1 M aqueous NaOH).

Biological Activity. All of the compounds listed in Tables I and III were tested in vitro against murine L1210 leukemia as described by Baguley and Nash.[22] Compounds with basic side chains in either the 2,5 or 2,7 disposition showed potent activity ($IC_{50} = 10^{-7}$–10^{-8} M), although the effects of A-ring hydroxylation seemed to be somewhat deleterious (compare **32**, **76**, **121**). The anthrapyrazoles in this study were less potent than doxorubicin or mitoxantrone.

Among the 90 analogues listed in Tables I and III prepared and tested in vivo against P388 leukemia in mice (ip/ip; D1,5),[23] 68 of the compounds demonstrated a T/C $\geq$ 125% and 20 compounds a T/C > 200% with one or more cures at optimal doses that ranged from 3.12–400 mg/kg per injection.

In evaluating the structure–activity relationships of the anthrapyrazoles against P388 leukemia, several trends are evident. First, as shown in Tables I and III, compounds with side chains in the N-2 and C-5 positions showed considerably more efficacy at the maximum tolerated dose than corresponding 2,7-isomers (**32** vs. **121**, **39** vs. **122**).

A-ring hydroxylation at C-7,10 generally was not associated with increased potency as with the anthracenediones,[4] but in all instances where there were identical substituents at N-2 and C-5, the hydroxylated derivatives were more active (**32** vs. **76**, **33** vs. **80**, **21** vs. **55**, and others). Note that compound **76**, which is congeneric with mitoxantrone, possessed neither the potency nor efficacy of this clinical agent. The 7,10-bis(benzyloxy) precursor of **76**, compound **115** listed in Table II, possessed marginal activity (T/C = 144%) at the maximum tolerated dose (100 mg/kg per injection); hence, most of the compounds in Table II were not tested against the P388 line. On the basis of the above comparisons, we concentrated our efforts primarily toward the synthesis of the A-ring hydroxylated anthrapyrazoles shown in Table I.

For a series of compounds in which the C-5 lower side chain was kept constant (e.g., **48**, **55**, **60**, **93**, **102**), there was a considerable range of activity for N-2 variation. Analogues having side chains with simple alkyl substituents (**48**) showed good activity, but those with terminal nonbasic heteroatoms (e.g., **55**, **60**) gave excellent % T/C values but at high dose levels. Similar efficacy with cures at considerably lower dose levels resulted for compounds with side chains possessing one (**93**) or two (**102**) basic amine functions. The same trend was observed for compounds with the same N-2 upper side chain and variation at C-5. Note that the proximal nitrogen bonded to C-5 is not basic and for optimal activity must be secondary such that hydrogen bonding with the C-6 carbonyl can occur. Compounds in which this nitrogen was tertiary (**87**) were considerably less active than their secondary counterparts (**80**).

For compounds in which both the N-2 and C-5 side chains possessed one or two distal basic amine moieties, there was some variation in activity with the nature of the substituent(s) on the nitrogen. For a series of compounds

(15) The regiochemistry of addition was clearly evident from the ^1H NMR spectrum, in which the C-5 proximal amine proton displayed a downfield D_2O-exchangeable triplet.

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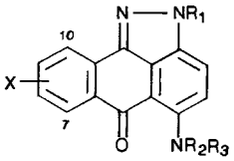
Table I. Activity of 2-Substituted 5-Aminoanthra[1,9-*cd*]pyrazol-6(2*H*)-ones against Murine L1210 Leukemia in Vitro and P388 Leukemia in Vivo



no.	X	R_1	NR_2R_3	method	yield,[a] %	mp, °C	molecular formula[b]	L1210 leukemia[c] in vitro: IC_{50}, M	P388 leukemia in vivo[d] opt. dose, mg/kg per inj	P388 leukemia in vivo[d] net log tumor cell kill	P388 leukemia in vivo[d] % T/C (day 30 surv)
2	(doxorubicin)							6.9×10^{-8}	8.0	5.6	225
5	(mitoxantrone)							1.6×10^{-9}	3.0	>6.8	267 (3/6)
15	H	H	$NHCH_2CH_2NHCH_2CH_2OH$	A	39	251–254[e]	$C_{18}H_{18}N_4O_2 \cdot 1.5HCl \cdot 0.5H_2O$[f]	2.2×10^{-6}	100	−0.8	126
16	H	H	$NHCH_2CH_2NEt_2$	A	27	219–224	$C_{20}H_{22}N_4O \cdot 1.4HCl \cdot 1.1H_2O$[g]	1.5×10^{-6}	400	−1.4	111
17	H	CH_3	$NHCH_2CH_2NHCH_2CH_2OH$	A	26	270–272	$C_{19}H_{20}N_4O_2 \cdot HCl \cdot 0.1H_2O$	7.1×10^{-7}	100	−0.2	136
18	H	CH_3	$NHCH_2CH_2NEt_2$	B	37	260–264[e]	$C_{21}H_{24}N_4O \cdot 1.8HCl \cdot 0.7H_2O$	6.7×10^{-7}	400	−1.5	98
19	H	CH_2CH_2OH	$NH(CH_2)_5CH_3$	A	79	148–150	$C_{22}H_{25}N_3O_2$	inactive	100	−1.6	98
20	H	CH_2CH_2OH	$NHCH_2CH_2CO_2H$	A[h]	87	235–239[e]	$C_{19}H_{17}N_2O_4 \cdot 0.4H_2O$	inactive	25	−1.6	98
21	H	CH_2CH_2OH	$NHCH_2CH_2NHCH_2CH_2OH$	A	71	267–272[e,i]	$C_{20}H_{22}N_4O_3 \cdot 1.6HCl \cdot 0.5H_2O$	1.8×10^{-6}	25	−0.9	125
22	H	CH_2CH_2OH	$NHCH_2CH_2NEt_2$	A	91	199–205[e,j,k]	$C_{22}H_{26}N_4O_2 \cdot 1.7HCl \cdot 0.6H_2O$	8.8×10^{-7}	400	0.8	145
23	H	CH_2CH_2OH	$NH(CH_2)_3NEt_2$	A	60	180–187[l]	$C_{23}H_{28}N_4O_2 \cdot 2HCl \cdot 0.8H_2O$	inactive	200	−1.5	104
24	H	CH_2CH_2OH	$NH(CH_2)_4NEt_2$	A	67	130–135	$C_{24}H_{30}N_4O_2 \cdot 1.2HCl \cdot H_2O$	inactive	300	−1.3	115
25	H	CH_2CH_2OH	$NH(CH_2)_7NEt_2$	A	72	206–208[m]	$C_{27}H_{36}N_4O_2 \cdot HCl$	inactive	100	−1.6	98
26	H	CH_2CH_2OH	$NHCH_2CH_2$-c-$N(CH_2CH_2)_2O$	A	60	260[e,n]	$C_{22}H_{24}N_4O_3 \cdot 1.9HCl \cdot 0.5H_2O$	inactive	25	−1.5	100
27	H	CH_2CH_2OH	$NHCH_2CH_2$-c-$N(CH_2CH_2)_2NH$	C[o]	35	292–297[e]	$C_{22}H_{25}N_5O_2 \cdot 2.1HCl \cdot 0.2H_2O$	inactive	50	−1.3	109
28	H	$CH_2CH_2NH_2$	$NHCH_2CH_2NHCH_2CH_2OH$	A	21	270–272[e]	$C_{20}H_{23}N_5O_2 \cdot 1.7HCl \cdot 0.8H_2O \cdot 0.2C_3H_8O$[p]	8.0×10^{-8}	25	3.7	187
29	H	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_3$	A[q]	56	285–288[e]	$C_{19}H_{20}N_4O_2 \cdot HCl$	7.4×10^{-7}	200	−0.7	127
30	H	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2OH$	A	51	260–261[e]	$C_{20}H_{22}N_4O_3 \cdot 1.1HCl \cdot 0.7H_2O$	7.5×10^{-7}	25	0.1	139
31	H	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2NH_2$	A	44	263–267[e]	$C_{20}H_{23}N_5O_2 \cdot 2HCl \cdot 0.9H_2O$	6.9×10^{-8}	25	4.4	189 (1/5)
32	H	$CH_2CH_2NHCH_2OH$	$NHCH_2CH_2NHCH_2CH_2OH$	A	60	146–148[r,s]	$C_{22}H_{27}N_5O_3 \cdot C_2H_4O_2 \cdot 0.3H_2O$[t]	7.4×10^{-8}	50	5.2	199
33	H	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2NMe_2$	A	29	286–288[e]	$C_{22}H_{27}N_5O_2 \cdot 2.1HCl \cdot 0.8H_2O$	3.2×10^{-8}	6.25	3.1	174
34	H	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2NEt_2$	A	81	272–274[e,u]	$C_{24}H_{31}N_5O_2 \cdot 2HCl \cdot H_2O$[v]	6.0×10^{-8}	12.5	1.8	158
35	H	$CH_2CH_2NEt_2$	$NH(CH_2)_5CH_3$	A	71	176–179[e]	$C_{26}H_{34}N_4O \cdot 1.1HCl \cdot 0.1H_2O$	2.0×10^{-6}	200	−1.4	110
36	H	$CH_2CH_2NEt_2$	$NHCH_2CH_2CO_2H$	A[h]	69	168	$C_{23}H_{26}N_4O_3 \cdot 0.2H_2O$	inactive	12.5	−1.3	117
37	H	$CH_2CH_2NEt_2$	$NHCH_2CH_2NH_2$	A	46	276–279[e]	$C_{22}H_{27}N_5O \cdot 2HCl \cdot 1.8H_2O$[w]	4.6×10^{-8}	25	−1.5	104
38	H	$CH_2CH_2NEt_2$	$NHCH_2CH_2NHMe$	D	61	206–209[e]	$C_{23}H_{29}N_5O \cdot 2.4HCl \cdot H_2O$[x]	2.7×10^{-8}	25	0.7	138
39	H	$CH_2CH_2NEt_2$	$NHCH_2CH_2NHCH_2CH_2OH$	E[y]	29	239–241[e]	$C_{24}H_{31}N_5O_2 \cdot 2HCl \cdot 2.4H_2O$[z]	3.2×10^{-8}	25	2.6	169
40	H	$CH_2CH_2NEt_2$	$NHCH_2CH_2NEt_2$	E	67	272–276[e]	$C_{26}H_{35}N_5O \cdot 2.1HCl \cdot 1.2H_2O$	3.9×10^{-7}	50	−1.4	106
41	H	$CH_2CH_2NEt_2$	$NH(CH_2)_3NEt_2$	A	60	270–272[e]	$C_{27}H_{37}N_5O \cdot 2HCl \cdot 0.2H_2O$	5.2×10^{-7}	25	−1.5	104

42	H	$CH_2CH_2NEt_2$	$NH(CH_2)_4NEt_2$	A	73	243–246[e]	$C_{28}H_{39}N_5O\cdot 2HCl\cdot 0.7H_2O$	6.2×10^{-7}	25	−1.4	107
43	H	$CH_2CH_2NEt_2$	$NH(CH_2)_7NEt_2$	A	65	190–193	$C_{31}H_{45}N_5O\cdot 2HCl\cdot 0.3H_2O$	6.3×10^{-7}	25	−1.5	104
44	H	$CH_2CH_2NEt_2$	$NHCH_2CH_2$-c-$N(CH_2CH_2)_2O$	A	48	288–290[e]	$C_{26}H_{33}N_5O_2\cdot 2HCl\cdot 1.2H_2O$[aa]	4.8×10^{-7}	400	>6.8	211
45	H	$CH_2CH_2NEt_2$	$NHCH_2CH_2$-c-$N(CH_2CH_2)_2NH$	C	83	284–287[e]	$C_{26}H_{34}N_6O\cdot 3.3HBr\cdot 0.1C_2H_4O_2\cdot 1.5H_2O$[t]	5.0×10^{-7}	25	−1.3	109
46	H	$CH_2CH_2NEt_2$	$NHCH_2CH$-c-$N(CH_2CH_2)_2$-NCbz[bb]	A	48	245–246[e]	$C_{34}H_{40}N_6O_3\cdot 2.4HCl$	3.9×10^{-7}	not tested		
47	7,10-$(OH)_2$	CH_3	$NHCH_2CH_2NH_2$	A	38	323–326[e]	$C_{17}H_{16}N_4O_3\cdot 1.1HCl\cdot 0.2H_2O\cdot 0.1C_7H_7NO$[cc]	2.4×10^{-7}	12.5	0.0	138
48	7,10-$(OH)_2$	CH_3	$NHCH_2CH_2NHCH_2$-CH_2OH	A	41	280–284[e]	$C_{19}H_{20}N_4O_4\cdot HCl\cdot 0.6H_2O$	1.5×10^{-7}	100	2.9	174
49	7,10-$(OH)_2$	CH_3	$NHCH_2CH_2NEt_2$	A	58	298[e]	$C_{21}H_{24}N_4O_3\cdot 1.5HCl\cdot 0.8H_2O$	4.5×10^{-7}	400	1.3	152
50	7,10-$(OH)_2$	CH_2Ph	$NHCH_2CH_2$-NMe_2	A	56	239[e,dd]	$C_{25}H_{24}N_4O_3\cdot 1.9HCl\cdot 0.3H_2O$	8.6×10^{-7}	25	−1.5	104
51	7,10-$(OH)_2$	CH_2CH_2OMe	$NHCH_2CH_2NH_2$	F	75	263–268[e,ee]	$C_{19}H_{20}N_4O_4\cdot HCl\cdot 0.3H_2O$	inactive	50	−0.6	127
52	7,10-$(OH)_2$	CH_2CH_2OMe	$NHCH_2CH_2NHCH_2$-CH_2OH	F	72	68–72[ff]	$C_{21}H_{24}N_4O_5\cdot 1.1HCl\cdot 0.3H_2O\cdot 0.2C_3H_8O$[p]	1.6×10^{-6}	100	0.3	140
53	7,10-$(OH)_2$	CH_2CH_2SMe	$NHCH_2CH_2$-$NHCH_2CH_2OH$	F	40	207–209[e]	$C_{21}H_{24}N_4O_4S\cdot 1.7HCl\cdot 0.3H_2O$	inactive	6.25	0.3	140
54	7,10-$(OH)_2$	CH_2CH_2OH	$NHCH_2CH_2NH_2$	F	47	>200[e]	$C_{18}H_{18}N_4O_4\cdot 1.8HCl\cdot 0.5H_2O$[gg]	4.8×10^{-7}	100	6.8	196
55	7,10-$(OH)_2$	CH_2CH_2OH	$NHCH_2CH_2NHCH_2$-CH_2OH	A	24	196–203[e]	$C_{20}H_{22}N_4O_5\cdot HCl\cdot 0.5H_2O$	7.8×10^{-7}	200	6.8	271
56	7,10-$(OH)_2$	CH_2CH_2OH	$NHCH_2CH_2$-NMe_2	F	24	253–255[e]	$C_{20}H_{22}N_4O_4\cdot 1.5HCl\cdot 1.9H_2O$	1.5×10^{-8}	200	6.6	283 (2/5)
57	7,10-$(OH)_2$	CH_2CH_2OH	$NHCH_2CH_2NEt_2$	A	47	215–219[hh]	$C_{22}H_{26}N_4O_4\cdot 1.6HCl\cdot 0.5H_2O$	7.3×10^{-7}	400	4.6	190
58	7,10-$(OH)_2$	CH_2CH_2OH	$NHCH_2CH_2$-c-$N(CH_2CH_2)_2O$	F	41	245–249[e]	$C_{22}H_{24}N_4O_5\cdot 0.5HCl\cdot 0.2H_2O$	1.1×10^{-6}	400	−1.2	122
59	7,10-$(OH)_2$	CH_2CH_2OH	$NHCH_2CH_2NHCH_2$-CH_2NH_2	D	87	180–195[e]	$C_{20}H_{23}N_5O_4\cdot 1.9HCl\cdot 1.1H_2O$	inactive	200	1.2	174
60	7,10-$(OH)_2$	$CH_2CH(OH)CH_2OH$	$NHCH_2CH_2NHCH_2$-CH_2NMe_2	D[ii]	78	168–172	$C_{21}H_{24}N_4O_6\cdot 1.6HCl\cdot H_2O$	2.2×10^{-6}	400	6.7	281 (1/6)
61	7,10-$(OH)_2$	$CH_2CH_2NH_2$	$NHCH_2CH_2NH_2$	A	23	>230[e]	$C_{18}H_{19}N_5O_3\cdot 1.9HCl\cdot 0.9H_2O$	4.8×10^{-7}	12.5	5.6	221
62	7,10-$(OH)_2$	$CH_2CH_2NH_2$	$NH(CH_2)_3NH_2$	A	45	>310[e]	$C_{19}H_{21}N_5O_3\cdot 3HCl\cdot 3.4H_2O$	3.1×10^{-7}	not tested		
63	7,10-$(OH)_2$	$CH_2CH_2NH_2$	$NHCH_2CH_2$-$NHMe$	D	70	259–264[e]	$C_{19}H_{21}N_5O_3\cdot 2.2HCl\cdot 0.5H_2O\cdot 0.01C_2H_4O_2\cdot 0.03C_3H_8O$[p,t]	7×10^{-7}	not tested		
64	7,10-$(OH)_2$	$CH_2CH_2NH_2$	$NHCH_2CH_2NHCH_2$-CH_2OH	D	96	275–280[e]	$C_{20}H_{23}N_5O_4\cdot 2HCl\cdot 0.5H_2O$	5.8×10^{-7}	12.5	>6.7	270 (5/6)
				A	55						
65	7,10-$(OH)_2$	$CH_2CH_2NH_2$	$NH(CH_2)_3NHCH_2$-CH_2OH	A	39	170–175[e]	$C_{21}H_{25}N_5O_4\cdot 2HCl\cdot 1.3H_2O$	8.7×10^{-7}	6.25	3.4	194
66	7,10-$(OH)_2$	$CH_2CH_2NH_2$	$NHCH_2CH_2NHCH_2$-CH_2NMe_2	A	45	245–260[e]	$C_{22}H_{28}N_6O_3\cdot 3.4HCl\cdot H_2O\cdot 0.4C_3H_8O$[p]	9.3×10^{-7}	50	6.4	242
67	7,10-$(OH)_2$	$(CH_2)_3NH_2$	$NHCH_2CH_2NHCH_2$-CH_2OH	D	85	292–294[e]	$C_{21}H_{25}N_5O_4\cdot 2HCl\cdot H_2O$[jj]	1.6×10^{-7}	50	>6.6	285 (5/6)
68	7,10-$(OH)_2$	$(CH_2)_3NH_2$	$NHCH_2CH_2NHCH_2$-CH_2NMe_2	D	84	294[e]	$C_{23}H_{30}N_6O_3\cdot 3HCl\cdot 2H_2O$[kk,ll]	6.4×10^{-7}	25	5.6	228
69	7,10-$(OH)_2$	CH_2CH_2NHMe	$NHCH_2CH_2NHCH_2$-CH_2OH	D	97	180–185[e]	$C_{21}H_{25}N_5O_4\cdot 2HCl\cdot 1.8H_2O$[mm]	4.4×10^{-7}	3.12	>6.8	324 (5/6)
70	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2NH_2$	F	45	272–278[e,nn]	$C_{20}H_{23}N_5O_4\cdot 2HCl\cdot 0.9H_2O$[oo]	1.6×10^{-6}	3.12	4.8	197 (1/5)
71	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NH(CH_2)_3NH_2$	D	83	267–272[e]	$C_{21}H_{25}N_5O_4\cdot 1.8HCl\cdot 0.6H_2O$	9.6×10^{-7}	45	>6.5	294 (4/8)
				F	43						
72	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NH(CH_2)_4NH_4$	F	62	240–245[e]	$C_{22}H_{27}N_5O_4\cdot HCl\cdot 0.4H_2O$[pp]	inactive	100	3.4	173
73	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NH(CH_2)_5NH_2$	F	48	270–275[e]	$C_{23}H_{29}N_5O_4\cdot 1.9HCl\cdot 0.8H_2O$	inactive	100	1.5	150

Table I (Continued)

no.	X	R_1	NR_2R_3	method	yield,[a] %	mp, °C	molecular formula[b]	L1210 leukemia[c] in vitro: IC_{50}, M	P388 leukemia in vivo[d] opt. dose, mg/kg per inj	P388 leukemia in vivo[d] net log tumor cell kill	P388 leukemia in vivo[d] % T/C (day 30 surv)
74	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2$-NHMe	D	91	270–276[e]	$C_{21}H_{25}N_5O_4 \cdot 1.9HBr \cdot 1.2H_2O$	1.4×10^{-7}	12.5	>6.6	277 (6/6)
				C	72						
75	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2$-N(Cbz)Me[bb]	A	35	152–155	$C_{29}H_{31}N_5O_6 \cdot 0.3H_2O$	inactive	not tested		
76	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2NHCH_2$-CH_2OH	F	52	215–225[e,qq]	$C_{22}H_{27}N_5O_5 \cdot 2HCl \cdot 0.8H_2O$[rr]	7.4×10^{-7}	6.25	4.6	206 (1/5)
				D	91						
77	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NH(CH_2)_3NHCH_2$-CH_2OH	F	35	170–180[e]	$C_{23}H_{29}N_5O_5 \cdot 2.1HCl \cdot 0.8H_2O \cdot 0.1C_3H_8O$[p,ss,tt]	1.8×10^{-6}	6.25	>6.8	254 (3/6)
78	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2N$-$(CH_2CH_2OH)_2$	D	61	245–250[e]	$C_{24}H_{31}N_5O_6 \cdot 2HCl$	4.3×10^{-7}	200	>6.6	277 (4/6)
				F	64						
79	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NH(CH_2)_3N$-$(CH_2CH_2OH)_2$	F	53	198–215[e]	$C_{25}H_{33}N_5O_6 \cdot 2.1HCl \cdot 0.3H_2O$	9.2×10^{-7}	100	3.6	194
80	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2$-NMe_2	F	60	278–280[e]	$C_{22}H_{27}N_5O_4 \cdot 1.9HCl \cdot 1.7H_2O$[uu]	2.3×10^{-7}	12.5	>6.6	250 (1/5)
81	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2NEt_2$	F	53	228–231	$C_{24}H_{31}N_5O_4 \cdot 1.9HCl \cdot 1.5H_2O$[vv]	5.1×10^{-7}	50	>6.7	241
82	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2$-c-$N(CH_2CH_2)_2O$	F	47	275–280[e]	$C_{24}H_{29}N_5O_5 \cdot 2.4HCl \cdot 0.8H_2O$	6.5×10^{-7}	50	2.9	177
83	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	c-$N(CH_2CH_2)_2$-NMe	F	40	>153[e]	$C_{23}H_{27}N_5O_4 \cdot 2.2HCl \cdot 0.4H_2O \cdot 0.2C_3H_8O$[p]	4.3×10^{-7}	12.5	3.8	192
84	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2NHCH_2$-CH_2NH_2	F	14	210–215[e]	$C_{22}H_{28}N_6O_4 \cdot HCl \cdot H_2O$	3.3×10^{-7}	25	>6.6	277 (3/6)
85	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NHCH_2CH_2$-$NHCH_2CH_2NMe_2$	F	51	85–95[e]	$C_{24}H_{32}N_6O_4 \cdot 2.4HCl \cdot 1.4H_2O \cdot 0.2C_3H_8O$[p,ww]	7.6×10^{-7}	50	>6.8	254 (4/6)
86	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$NH(CH_2)_3NH$-$(CH_2)_4NH$-$(CH_2)_3NH_2$	F	45	185–200[e]	$C_{28}H_{41}N_7O_4 \cdot 2.8HCl \cdot 0.6H_2O \cdot 0.1C_3H_8O$[p]	inactive	12.5	0.6	145
87	7,10-$(OH)_2$	$CH_2CH_2NHCH_2CH_2OH$	$N(Me)CH_2CH_2$-NMe_2	F	22	>91[e]	$C_{23}H_{29}N_5O_4 \cdot 2HCl \cdot 1.9H_2O$	6.3×10^{-7}	12.5	4.9	188
88	7,10-$(OH)_2$	$(CH_2)_3NHCH_2CH_2OH$	$NH(CH_2)_3NH_2$	F	74	120–130[e]	$C_{22}H_{27}N_5O_4 \cdot 0.1HCl \cdot 0.3H_2O \cdot 0.1C_3H_8O$[p]	1.8×10^{-6}	100	3.4	185
89	7,10-$(OH)_2$	$(CH_2)_3NHCH_2CH_2OH$	$NHCH_2CH_2NHCH_2$-CH_2OH	F	61	100–105	$C_{23}H_{29}N_5O_5 \cdot 0.7H_2O$	inactive	100	>7.0	275 (3/6)
90	7,10-$(OH)_2$	$CH_2CH_2NMeCH_2$-CH_2OH	$NHCH_2CH_2NHCH_2$-CH_2OH	F	37	240[e]	$C_{23}H_{29}N_5O_5 \cdot 1.6HCl \cdot 0.4H_2O$	3.3×10^{-7}	12.5	4.2	203 (1/6)
91	7,10-$(OH)_2$	$CH_2CH_2NMe_2$	$NHCH_2CH_2NH_2$	A	38	300–302[e]	$C_{20}H_{23}N_5O_3 \cdot 1.9HCl \cdot 2.5H_2O$[xx]	2.2×10^{-7}	12.5	>6.6	263 (2/5)
92	7,10-$(OH)_2$	$CH_2CH_2NMe_2$	$NH(CH_2)_3NH_2$	A	81	281–285[e]	$C_{21}H_{25}N_5O_3 \cdot 1.9HCl \cdot 1.4H_2O$	5.4×10^{-7}	12.5	4.7	208
93	7,10-$(OH)_2$	$CH_2CH_2NMe_2$	$NHCH_2CH_2NHCH_2$-CH_2OH	F	77	310–313[e]	$C_{22}H_{27}N_5O_4 \cdot 2.4HCl \cdot 2H_2O$[yy]	1.2×10^{-7}	12.5	6.5	242 (2/5)
94	7,10-$(OH)_2$	$(CH_2)_3NMe_2$	$NHCH_2CH_2NH_2$	A	42	316[e]	$C_{21}H_{25}N_5O_3 \cdot 2HCl \cdot 0.5H_2O \cdot 0.1C_3H_8O$[p,zz]	2.0×10^{-7}	12.5	6.6	230 (1/6)
95	7,10-$(OH)_2$	$(CH_2)_3NMe_2$	$NH(CH_2)_3NH_2$	A	58	>300	$C_{22}H_{27}N_5O_3 \cdot 2HCl \cdot 0.5H_2O$	8.0×10^{-7}	25	4.0	212
96	7,10-$(OH)_2$	$(CH_2)_3NMe_2$	$NHCH_2CH_2NHCH_2$-CH_2OH	A	61	311[e,ab]	$C_{23}H_{29}N_5O_4 \cdot 2HCl \cdot H_2O$	5.9×10^{-7}	12.5	5.4	218
97	7,10-$(OH)_2$	$CH_2CH_2NEt_2$	$NHCH_2CH_2NH_2$	A	55	277–281[e,ac]	$C_{22}H_{27}N_5O_3 \cdot 2HCl \cdot 1.7H_2O$	4.6×10^{-8}	12.5	>7.1	280 (2/5)
98	7,10-$(OH)_2$	$CH_2CH_2NEt_2$	$NHCH_2CH_2NHMe$	C	85	217–220[e]	$C_{23}H_{29}N_5O_3 \cdot 2.3HBr \cdot 2.7H_2O$[ad]	7.4×10^{-6}	25	>7.1	219

99	7,10-$(OH)_2$	$CH_2CH_2NEt_2$	$NHCH_2CH_2N(Me)Cbz^{bb}$	A	31	138–141	$C_{31}H_{35}N_5O_5$	inactive	not tested		
100	7,10-$(OH)_2$	$CH_2CH_2NEt_2$	$NHCH_2CH_2NHCH_2$-CH_2OH	A	69	198–202[ee]	$C_{24}H_{31}N_5O_4{\cdot}2HCl{\cdot}0.7H_2O^{ee}$	1.3×10^{-7}	12.5	>6.8	264
101	7,10-$(OH)_2$	$CH_2CH_2NEt_2$	$NHCH_2CH_2NEt_2$	A	51	290–292[ff]	$C_{28}H_{35}N_5O_3{\cdot}2HCl{\cdot}0.3H_2O$	5.5×10^{-7}	100	3.5	180
102	7,10-$(OH)_2$	$CH_2CH_2NHCH_2$-CH_2NMe_2	$NHCH_2CH_2NHCH_2$-CH_2OH	D	92	168–173[gg]	$C_{24}H_{32}N_6O_4{\cdot}3HCl{\cdot}1.7H_2O$	1.4×10^{-6}	50	>6.8	226
				F	23						
103	7,10-$(OH)_2$	$CH_2CH(OH)CH_2NEt_2$	$NHCH_2CH_2NH_2$	A	59	148–152[hh]	$C_{23}H_{29}N_5O_4{\cdot}2HCl{\cdot}2.8H_2O^{gg}$	inactive	6.25	>6.7	262
104	7,10-$(OH)_2$	$CH_2CH(OH)CH_2NEt_2$	$NHCH_2CH_2NHCH_2$-CH_2OH	A	38	110–120[ii,jj]	$C_{25}H_{33}N_5O_5{\cdot}2.2HCl{\cdot}2.7H_2O$	8.4×10^{-7}	12.5	5.3	223
105	7,10-$(OH)_2$	$CH_2CH(OH)CH_2NEt_2$	$NHCH_2CH_2NEt_2$	A	55	253–255[kk]	$C_{27}H_{37}N_5O_4{\cdot}2HCl{\cdot}1.9H_2O^{ii,ll}$	1.3×10^{-6}	100	1.2	157

[a] Yields were not optimized. [b] Unless otherwise stated, the analyses are within ±0.4% of the theoretical values. [c] See ref 22. [d] Optimum response; carried out by a slight modification (Q04Dx2, ip schedule) of the National Cancer Institute testing protocol.[23] Net kill calculated by method of Schabel et al. detailed in ref 29, except that "cured" mice are included in calculations of % T/C values ≥125 are considered indicative of significant activity. [e] With decomposition. [f] H_2O: calcd, 2.33; found, 2.78. [g] H_2O: calcd, 4.89; found, 3.79. [h] β-Alanine reacted as preformed $(n\text{-Bu})_4N^+$ salt. [i] Free base mp 180–181 °C. [j] Free base mp 119–122 °C. [k] Monohydrochloride salt mp 222–224 °C. [l] Free base mp 145–147 °C. [m] Free base mp 115–119 °C. [n] Free base mp 204–207 °C. [o] Intermediate carbamate synthesized by Method A was not characterized. [p] ^1H NMR indicates the presence of 2-propanol. [q] Pyridine solution saturated with methylamine; reaction carried out in steel bomb at 100 °C. [r] Free base mp 154–156 °C. [s] dihydrochloride mp 270–275 °C. [t] ^1H NMR indicates the presence of acetic acid. [u] Free base mp 132–133 °C. [v] H_2O: calcd, 3.52; found, 0.21. [w] H: calcd, 6.81; found, 6.24. [x] H: calcd, 6.77; found, 6.22. [y] Reaction carried out with catalytic KI. [z] H_2O: calcd, 8.04; found, 9.78. [aa] H: calcd, 6.95; found, 6.34. [bb] Cbz = carbobenzyloxy. [cc] ^1H NMR indicates the presence of DMF. [dd] Free base mp 186–188 °C. [ee] Free base mp 173–175 °C. [ff] Free base mp 163–165 °C. [gg] H_2O: calcd, 2.10; found, 1.40. [hh] Free base mp 197–200 °C. [ii] Dioxolane hydrolysis occurred during formation of hydrochloride salt following hydrogenolysis of **108**. [jj] H_2O: calcd, 3.59; found, 4.48. [kk] H: calcd, 6.39; found, 5.68. [ll] N: calcd, 14.39; found, 13.97. [mm] H: calcd, 5.97; found, 5.33. [nn] Free base mp 140–148 °C. [oo] H_2O: calcd, 3.33; found, 1.86. [pp] H_2O: calcd, 1.46; found, 1.75. [qq] Free base mp 150–153 °C. [rr] H_2O: calcd, 2.73; found, 0.97. [ss] H: calcd, 6.11; found, 5.02. [tt] H_2O: calcd, 2.61; found, 1.68. [uu] H_2O: calcd, 5.83; found, 5.01. [vv] H_2O: calcd, 4.91; found, 3.76. [ww] H_2O: calcd, 4.25; found, 2.39. [xx] H_2O: calcd, 9.08; found, 8.36. [yy] H_2O: calcd, 6.56; found, 7.54. [zz] H_2O: calcd, 1.86; found, 3.01. [ab] Free base mp 125–128 °C. [ac] Free base mp 187–189 °C. [ad] H: calcd, 5.62; found, 5.06. [ae] H_2O: calcd, 2.27; found, 2.74. [af] Free base mp 148–152 °C. [ag] H_2O: calcd, 8.96; found, 9.38. [ah] Free base mp 138–140 °C. [ai] H: calcd, 7.16; found, 6.54. [aj] H_2O: calcd, 5.68; found, 5.21.

in which the C-5 side chain was held constant, good to outstanding activity was observed for a number of N-2 variations (compare **64**, **76**, **93**, **100**, **102**), with smaller or more polar substituents producing curative activity. A similar trend was observed for a series of compounds in which the N-2 upper side chain was held constant and the substituents on the distal nitrogen of the C-5 side chain were varied (e.g., **70**, **74**, **76**, **81**, **85**). Interestingly, ^1H NMR studies show that there is also a pronounced transannular effect on the electron-deficient C-3 and C-4 positions of the anthrapyrazole nucleus by the distal basic moieties on the N-2 and C-5 side chains, respectively. Such an effect would be expected to markedly affect the pK_a values of these amines and hence drug distribution into biological compartments.

For a series of pairs of compounds with otherwise constant structural features in which the number of methylene spacers at N-2 (e.g., **64** vs. **67**, **76** vs. **89**, **93** vs. **96**) or C-5 (e.g., **76** vs. **77**, **78** vs. **79**) was changed from two to three, there was generally little variation in activity, although potency (**76** vs. **89**) and efficacy (**78** vs. **79**) varied widely in some cases. This is in contrast to the anthracenedione series where compounds with more than two carbon atoms separating the nitrogen were either inactive or greatly diminished in activity.[4] With a progression from two to five methylene connectors (compare sequentially compounds **70–73**), there was a marked reduction in both efficacy and potency as the side-chain length was increased beyond three methylene spacers.

In comparing a series of isomeric pairs of compounds in which dissimilar side chains at the N-2 and C-5 positions were inverted (e.g., **64** vs. **70**, **67** vs. **71**, **69** vs. **74**, **77** vs. **89**, **85** vs. **102**), each pair generally displayed comparable efficacy at the maximum tolerated dose. Furthermore, the compounds of each isomeric pair gave similar log P values. This suggests that maximal activity is associated in part with an optimal range of hydrophilic–lipophilic balance.

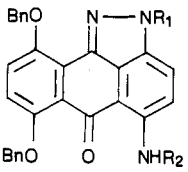
In summary, on the basis of these and previous studies,[11,24] antitumor activity in vivo against P388 leukemia is generally maximized by (a) basic side chains at N-2 and C-5, (b) two to three carbon spacers between proximal and distal nitrogens of the side chain, and (c) A-ring hydroxylation. For constant side chains at N-2 and C-5, activity decreases in the order of 7-OH > 7,10-$(OH)_2$ ≫ 10-OH > H.[25] Curative activity is most frequently associated with (a) A-ring hydroxylation in which 7-OH > 7,10-$(OH)_2$ and (b) basic side chains at N-2 and C-5 with optimal combinations made up of primary–secondary, secondary–secondary, and primary–tertiary distal amine pairs.

Besides having curative activity against P388 leukemia, the more active compounds in Table I were curative against murine B-16 melanoma in vivo.[24] The data for these compounds are given in Table IV. All compounds tested showed T/C > 175% (ip/ip; D1–9), with the most active compounds demonstrating a T/C > 300% and five or more cures at 1.5–25 mg/kg per injection. Additionally, many of the compounds in Table I showed curative activity against M5076 sarcoma and the MX-1 mammary xenograft in nude mice. Other tumors that responded to anthrapyrazole treatment included the Ridgeway osteogenic sarcoma, mammary adenocarcinomas 16/c, 18/c, and 13/c,

(24) Leopold, W. R.; Nelson, J. M.; Plowman, J.; Jackson, R. C. *Cancer Res.* **1985**, *45*, 5532–5539.

(25) We also have synthesized the 7,8,10- and 7,9,10-trihydroxy congeners of compound **76**. Full details of their synthesis and biological activity will be published when unequivocal structural assignments have been made.

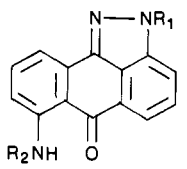
Table II. 7,10-Bis(benzyloxy)-2-substituted-5-aminoanthra[1,9-*cd*]pyrazol-6(2*H*)-ones[a]



no.	R_1	R_2	method	yield,[b] %	mp, °C	molecular formula[c]
106	CH_2CH_2OH	$CH_2CH_2NHCH_2CH_2OH$	A	42	209–214	$C_{34}H_{34}N_4O_5 \cdot 0.3H_2O$
107	CH_2CH_2OH	$CH_2CH_2NHCH_2CH_2NH_2$	A[d]	45	195–197	$C_{34}H_{35}N_5O_4 \cdot 0.3CHCl_3$[e]
108	CH_2-c-$CHCH_2OC(Me_2)O$	CH_2CH_2NH- CH_2CH_2OH	A[f]	29	208–211	$C_{38}H_{40}N_4O_6$
109	$CH_2CH_2NH_2$	$CH_2CH_2N(CH_3)CH_2Ph$	G	37	169–172	$C_{40}H_{39}N_5O_3 \cdot 0.3H_2O$[g]
110	$(CH_2)_2NH_2$	$CH_2CH_2NHCH_2CH_2OH$	G	44	184–193	$C_{34}H_{35}N_5O_4 \cdot H_2O$
111	$(CH_2)_3NH_2$	$CH_2CH_2NHCH_2CH_2OH$	A	31	199–203	$C_{35}H_{37}N_5O_4 \cdot 0.7H_2O$
			G	51		
112	$(CH_2)_3NH_2$	$CH_2CH_2NHCH_2CH_2NMe_2$	A	27	143–160	$C_{37}N_{42}N_6O_3 \cdot 1.7H_2O \cdot 0.4C_3H_8O$[h]
113	CH_2CH_2NHMe	$CH_2CH_2NHCH_2CH_2OH$	G	45	186–189	$C_{35}H_{37}N_5O_4 \cdot 0.2H_2O$
114	$CH_2CH_2NHCH_2CH_2OH$	$(CH_2)_3NH_2$	G	67	202–204	$C_{35}H_{37}N_5O_4 \cdot 0.3H_2O$
			A	65		
115	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2NHCH_2CH_2OH$	G	68	205–208	$C_{36}H_{39}N_5O_5 \cdot 0.3H_2O$
			A	40		
116	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2N(CH_3)CH_2Ph$	G	43	143–145	$C_{42}H_{43}N_5O_4 \cdot 0.2H_2O$
117	$CH_2CH_2NHCH_2CH_2OH$	$CH_2CH_2N(CH_2CH_2OH)_2$	G	48	172–175	$C_{38}H_{43}N_5O_6$
118	$CH_2CH_2NHCH_2CH_2NMe_2$	$CH_2CH_2NHCH_2CH_2OH$	A	27	180–190[i]	$C_{38}H_{44}N_6O_4 \cdot 0.6H_2O$

[a] Bn = benzyl. [b] See footnote *a*, Table I. [c] See footnote *b*, Table I. [d] Reaction carried out in presence of K_2CO_3. [e] ^1H NMR indicates presence of $CHCl_3$. [f] Reaction carried out in presence of $KHCO_3$. [g] H_2O: calcd, 0.84; found, 1.56. [h] H_2O: calcd, 4.55; found, 3.88. [i] With decomposition.

Table III. Activity of 2-Substituted 7-Aminoanthra[1,9-*cd*]pyrazol-6(2*H*)-ones against Murine L1210 Leukemia in Vitro and P388 Leukemia in Vivo



							L1210 leukemia[c] in vitro:	P388 leukemia in vivo[d]		
no.	R_1	R_2	method	yield,[a] %	mp, °C	molecular formula[b]	IC_{50}, M	opt. dose, mg/kg per inj	net log tumor cell kill	% T/C (day 30 surv)
119	CH_3	CH_2CH_2NH-CH_2CH_2OH	A	33	261–262[e,f]	$C_{19}H_{20}N_4O_2 \cdot HCl$	1.2×10^{-6}	200	−1.3	118
120	CH_3	$CH_2CH_2NEt_2$	A	14	216–217[e]	$C_{21}H_{24}N_4O \cdot HCl \cdot 0.2H_2O \cdot 0.1C_3H_8O$[g]	inactive	not tested		
121	CH_2CH_2NH-CH_2CH_2OH	CH_2CH_2NH-CH_2CH_2OH	A	20	285–287[e]	$C_{22}H_{27}N_5O_3 \cdot 2HCl \cdot 0.4H_2O$	3.9×10^{-7}	12.5	−0.3	133
122	$CH_2CH_2NEt_2$	CH_2CH_2NH-CH_2CH_2OH	A	44	212–216[e,h]	$C_{24}H_{31}N_5O_2 \cdot 2HCl \cdot 1.1H_2O$	2.3×10^{-7}	12.5	−1.3	117
123	$CH_2CH_2NEt_2$	$CH_2CH_2NEt_2$	A	64	292–294[e,i]	$C_{26}H_{35}N_5O \cdot 2HCl \cdot 0.3H_2O$	6.0×10^{-7}	50	−1.5	103

[a] See footnote *a*, Table I. [b] See footnote *b*, Table I. [c] See footnote *c*, Table I. [d] See footnote *d*, Table I. [e] With decomposition. [f] Free base mp 144–146 °C. [g] See footnote *p*, Table I. [h] Free base mp 104–107 °C. [i] Free base mp 118–120 °C.

and colon adenocarcinomas 11/a and 36.[24] Tumors found unresponsive to date include the CX-1 and LX-1 human xenografts and colon adenocarcinoma 38.

Studies of the biochemical pharmacology of the anthrapyrazoles by Fry et al.[26] suggest that the anthrapyrazoles may in fact have a low potential for cardiotoxicity relative to doxorubicin. Their data indicated that selected compounds from Table I induce far less superoxide dismutase sensitive oxygen consumption than does doxorubicin when incubated with a rat liver microsomal preparation. These findings are in accord with the polarographic properties of selected A-ring-modified anthrapyrazoles that show a much greater resistance to reduction ($E^1_{1/2}$ = −0.983 to −1.085 V) relative to daunorubicin ($E^1_{1/2}$ = −0.625 V) and mitoxantrone ($E^1_{1/2}$ = −0.775 V).[10] Furthermore, studies by Fagan et al.[27] in which several anthrapyrazoles were compared to doxorubicin for toxicity to cultured fetal mouse hearts suggested that many of the anthrapyrazoles are less cardiotoxic than doxorubicin.

On the basis of their exceptional in vivo anticancer activity, possible lack of cross-resistance with the anthracyclines,[28] and low potential for cardiotoxicity in preclinical models, A-ring dihydroxy compounds **71** and **74** have been selected for development toward clinical trials and have been given the designations CI-942 and CI-937, respectively. Additionally, we have selected an A-ring C-7 mo-

(26) Fry, D. W.; Boritzki, T. J.; Besserer, J. A.; Jackson, R. C. *Biochem. Pharmacol.* **1985**, *34*, 3499–3508.

(27) Fagan, M. A.; Hacker, M. P.; Newman, R. A. *Proc. Am. Assoc. Cancer Res.* **1984**, *25*, 302.

(28) Klohs, W. D.; Steinkampf, R. W.; Havlick, M. J.; Jackson, R. C. *Cancer Res.* **1986**, *46*, 4352–4356.

Table IV. Activity of 2-Substituted 5-Aminoanthra[1,9-*cd*]pyrazol-6(2*H*)-ones against Murine B-16 Melanoma in Vivo[a]

no.	opt. dose, mg/kg per inj	% T/C (day 60 surv)
2 (doxorubicin)	1.0	434 (5/10)
5 (mitoxantrone)	0.6	265 (4/6)
32	12.5	231 (1/9)
55	80	246 (1/10)
64	3.0	306 (6/10)
67	6.25	375 (5/10)
71	8.0	338 (7/10)
74	1.5	357 (5/10)
78	25	342 (6/10)
93	3.0	183
97	3.6	176
100	6.0	189

[a] Optimum response; carried out according to standard NCI protocol.[23] Cured animals are included in calculations of % T/C. % T/C values > 135 indicate significant activity.

nohydroxy compound with the same side chains found in mitoxantrone (**5**) and have designated it as CI-941. The results of more advanced preclinical studies with these compounds as well as current studies on the mechanisms of action of the anthrapyrazoles at the molecular level will be the subject of forthcoming publications.

Experimental Section

General. Melting points were taken on a Thomas-Hoover Unimelt capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined on a Digilab FTS-14 or Nicolet MX-1 FT-IR spectrometer system. Ultraviolet (UV) spectra were taken on a Cary Model 118C recording spectrophotometer. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 90 MHz or at 200 MHz on a Varian EM-390 or XL-200 instrument, respectively. Chemical shifts are reported as δ values (parts per million) downfield from internal tetramethylsilane on samples of ~1%, w/v. Combustion analyses were performed on a Perkin-Elmer 240 elemental analyzer and are reported within ±0.4% of the theoretical values. Water of crystallization was determined by Karl Fischer titration. pK_a values were determined on a Copenhagen Radiometer TTT60 titrator.

Chromatography was carried out with E. Merck products with use of silica gel 60 catalog no. 5760 for TLC, catalog no. 7734 for open column chromatography, and catalog no. 9385 for flash chromatography. All solvents and reagents were reagent grade unless otherwise noted.

Method A. The following preparations are examples of method A.

Example 1. 5-[[2-(Diethylamino)ethyl]amino]-2-[2-[(2-hydroxyethyl)amino]ethyl]anthra[1,9-*cd*]pyrazol-6(2*H*)-one Hydrochloride (34). A mixture of 1.91 g (5 mmol) of 5-chloro-2-[2-[2-hydroxyethyl)amino]ethyl]anthra[1,9-*cd*]pyrazol-6(2*H*)-one[12] and 3.5 mL (25 mmol) of *N,N*-diethylethylenediamine in 5 mL of pyridine was heated at reflux for 6.5 h and then cooled. The resulting precipitate was collected and washed with 2-propanol to afford 1.43 g of **34** as the free base, mp 132–133 °C. The chilled filtrate afforded an additional 0.3 g (81% total yield). A solution of the material in hot 2-propanol was treated with an excess of HCl in 2-propanol and allowed to cool. The precipitate was collected, washed with 2-propanol, and dried at 80 °C (200 mm) to provide 1.58 g of **34**: pK_a (H_2O) = 6.8, 8.4; IR (KBr) 1664, 1606, 1562, 1471, 1403, 720 cm^{-1}; UV (MeOH) λ_{max} 230 nm (ϵ 26 550), 242 (25 420), 296 (8560), 360 (9740), 467 (13 990). Anal. ($C_{24}H_{31}N_5O_2$·2HCl·H_2O) C, H, N, Cl$^-$.

Example 2. 5-[(3-Aminopropyl)amino]-2-[2-(dimethylamino)ethyl]-7,10-dihydroxyanthra[1,9-*cd*]pyrazol-6(2*H*)-one Hydrochloride (92). A mixture of 1.9 g (5 mmol) of 5-chloro-2-[2-(dimethylamino)ethyl]-7,10-dihydroxyanthra[1,9-*cd*]pyrazol-6(2*H*)-one[12] and 1.3 mL (15 mmol) of 1,3-diaminopropane in 13 mL of pyridine was heated in an oil bath at 110 °C for 6 h and then slowly cooled. The precipitate that formed was collected, washed with 2-propanol, and dissolved in hot MeOH. The hot solution was treated with an excess of 2-propanolic HCl. The resulting precipitate was collected by filtration, washed with MeOH, and dried at 40 °C (200 mm) to afford 2.1 g of **92**: ^{1}H NMR [10% D_2O in $(CD_3)_2SO$] δ 1.94 (t, 2, J = 7 Hz), 2.86 (s, 6), 2.93 (t, 2, J = 7 Hz), 3.65 (t, 2, J = 7 Hz), 3.74 (t, 2, J = 6 Hz), 5.02 (t, 2, J = 6 Hz), 6.88 (d, 1, J = 8.9 Hz), 7.30 (d, 1, J = 8.9 Hz), 7.32 (d, 1, J = 9.4 Hz), 8.20 (d, 1, J = 9.3 Hz). The $(CD_3)_2SO$ spectrum shows an exchangeable three-proton broad singlet at δ 8.11, a one-proton broad singlet at δ 10.77, a one-proton triplet at δ 8.93, and two sharp one-proton singlets at δ 8.97 and δ 13.62. IR (KBr) 1672, 1604, 1582, 1211, 825 cm^{-1}. Anal. ($C_{18}H_{18}N_4O_2$·1.5HCl·0.5H_2O) C, H, N, Cl$^-$, H_2O.

Example 3. 2-[2-(Diethylamino)ethyl]-7-[[2-(diethylamino)ethyl]amino]anthra[1,9-*cd*]pyrazol-6(2*H*)-one Hydrochloride (123). A mixture of 2.1 g (6 mmol) of 7-chloro-2-[2-(diethylamino)ethyl]anthra[1,9-*cd*]pyrazol-6(2*H*)-one[12] and 5 mL (36 mmol) of *N,N*-diethylethylenediamine in 20 mL of pyridine was heated at reflux for 28 h and concentrated to dryness. The residue was triturated in 2-propanol to afford 1 g of **123** as the free base, mp 118–120 °C. The filtrate was concentrated, dissolved in CH_2Cl_2, and extracted with 1% HCl. The HCl solution was made basic with dilute NaOH and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed, dried, and concentrated. The residue was recrystallized from 2-propanol to afford 0.8 g of **123** as the free base. The two crops were combined, dissolved in boiling 2-propanol, and treated with an excess of 2-propanolic HCl to afford 1.9 g of **123**: ^{1}H NMR [10% D_2O in $(CD_3)_2SO$] δ 1.1–1.3 (t overlapping t, 12), 3.1–3.4 (m, 10), 3.70 (t, 2, J = 6 Hz), 3.80 (t, 2, J = 7 Hz), 5.02 (t, 2, J = 6 Hz), 7.06 (d, 1, J = 8 Hz), 7.45 (d, 1, J = 7 Hz), 7.63 (t, 1, J = 8 Hz), 7.77 (t, 1, J = 8 Hz), 7.88 (d, 1, J = 7 Hz), 8.15 (d, 1, J = 8 Hz). The $(CD_3)_2SO$ spectrum shows an exchangeable one-proton triplet at δ 10.07 and one-proton broad singlets at δ 10.72 and δ 10.9. IR (KBr) 1654, 1619, 1597, 1267, 706 cm^{-1}; UV (MeOH) λ_{max} 238 nm (ϵ 20 430), 258 (21 600), 296 (7420), 330 (4560), 390 (4150), 475 (11 720). Anal. ($C_{26}H_{35}N_5O$·2.0HCl·0.3H_2O) C, H, N, Cl$^-$.

Method B. 5-[[2-(Diethylamino)ethyl]amino]-2-methylanthra[1,9-*cd*]pyrazol-6(2*H*)-one Hydrochloride (18). A mixture of 1.88 g (7 mmol) of 5-chloro-2-methylanthra[1,9-*cd*]pyrazol-6(2*H*)-one,[12] 1.45 mL (10 mmol) of *N,N*-diethylethylenediamine, 0.14 g of anhydrous KF, and 10 mL of Me_2SO was heated at reflux for 4 h, cooled, diluted with H_2O, and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed twice with brine and with 5% aqueous HCl. The acid layer was washed with CH_2Cl_2, made basic with Na_2CO_3, and extracted with CH_2Cl_2. The dried CH_2Cl_2 layer was clarified with charcoal, filtered, and concentrated. The residue was dissolved in 2-propanol and treated with an excess of 2-propanolic HCl. The resulting solid was collected, washed with 2-propanol and then ether, and then dried at 65 °C (200 mm) to afford 1.1 g of **18**: ^{1}H NMR [10% D_2O in $(CD_3)_2SO$] δ 1.22 (t, 6, J = 7 Hz), 3.22 (q, 4, J = 7 Hz), 3.35 (t, 2, J = 7 Hz), 3.95 (t, 2, J = 7 Hz), 4.24 (s, 3), 7.34 (d, 1, J = 9 Hz), 7.60 (dt, 1, J = 7.5 Hz, 7.4 Hz, 1.4 Hz), 7.79 (dt, 1, J = 7.5 Hz, 7.4 Hz, 1.4 Hz), 8.07 (d, 1, J = 9 Hz), 8.20 (dd, 1, J = 7.7 Hz, ~1 Hz), 8.41 (dd, 1, J = 8.1 Hz, 1 Hz). The $(CD_3)_2SO$ spectrum shows exchangeable one-proton broad singlets at δ 9.11 and δ 10.77. IR (KBr) 1656, 1607, 1563, 716 cm^{-1}. Anal. ($C_{21}H_{24}N_4O$·1.8HCl·0.7H_2O) C, H, N, Cl$^-$, H_2O.

Method C. 2-[2-(Diethylamino)ethyl]-5-[[2-(1-piperazinyl)ethyl]amino]anthra[1,9-*cd*]pyrazol-6(2*H*)-one Dihydrochloride (45). A solution of 2.5 g (4 mmol) of 4-[2-[[2-[2-(diethylamino)ethyl]-2,6-dihydro-6-oxoanthra[1,9-*cd*]-pyrazol-5-yl]amino]ethyl]-1-piperazinecarboxylic acid, phenylmethyl ester (**46**) in 4 mL of 48% HBr and 25 mL of HOAc was heated at 100 °C for 0.5 h, cooled, and filtered. The filter cake was washed successively with HOAc, 2-propanol, and ether and then dried to afford 2.7 g of **45**: IR (KBr) 1657, 1607, 1577, 1562, 716 cm^{-1}. Anal. ($C_{26}H_{34}N_6O$·3.3HBr·1.5H_2O·0.1HOAc) C, H, N, Br$^-$.

4-[2-[[2-[2-(Diethylamino)ethyl]-2,6-dihydro-6-oxoanthra[1,9-*cd*]pyrazol-5-yl]amino]ethyl]-1-piperazinecarboxylic Acid, Phenylmethyl Ester (46). This compound was prepared by the reaction of 5-chloro-2-[2-(diethylamino)-ethyl]anthra[1,9-*cd*]pyrazol-6(2*H*)-one[12] and 4-(2-aminoethyl)-1-piperazinecarboxylic acid, phenylmethyl ester (**13**) (method A, example 1). The crude product was purified by chromatography

over SiO_2 eluting with CH_2Cl_2–MeOH–Et_3N (95:5:0.5) to afford the product (**46**) as a brown oil: TLC [SiO_2; CH_2Cl_2–MeOH–Et_3N (90:10:0.5)] R_f 0.47. A sample was converted to the hydrochloride salt: IR (KBr) 1704, 1656, 1607, 1561, 1244 cm^{-1}. Anal. ($C_{34}H_{40}N_6O_3 \cdot 2.4HCl$) C, H, N, Cl^-.

4-(2-Aminoethyl)-1-piperazinecarboxylic Acid, Phenylmethyl Ester (13). A mixture of 32.3 g (250 mmol) of 1-(2-aminoethyl)piperazine and 27 g (0.25 mmol) of benzaldehyde in 200 mL of toluene was refluxed over a Dean–Stark apparatus for 3 h, chilled to 3 °C, and treated with the slow addition of 39 mL (250 mmol) of benzyl chloroformate. The reaction mixture was maintained at 25 °C for 3 days and then concentrated to an oil. A solution of the oil in 500 mL of MeOH was chilled to 3 °C, treated with 125 mL of 2 N HCl, warmed to 25 °C, and concentrated to remove MeOH. The aqueous concentrate was washed with CH_2Cl_2, made slightly basic with NH_4OH, and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed, dried, concentrated, and chromatographed over SiO_2 with CH_2Cl_2–MeOH–Et_3N (90:10:1) as the eluant to obtain 9.2 g (14%) of the product (**13**) as a brown oil: TLC [SiO_2; CH_2Cl_2–MeOH (4:1)] R_f 0.26; ^1H NMR ($CDCl_3$) δ 1.70 (s, 2, exchanges D_2O), 2.22–2.53 (m, 6), 2.77 (t, 2, J = 6 Hz), 3.35–3.60 (m, 4), 5.10 (s, 2), 7.16–7.35 (m, 5).

Method D. 7,10-Dihydroxy-2-[2-[(2-hydroxyethyl)amino]ethyl]-5-[[2-(methylamino)ethyl]amino]anthra[1,9-*cd*]pyrazol-6(2*H*)-one Hydrobromide (74). A mixture of 20.5 g (30 mmol) of 2-[2-[(2-hydroxyethyl)amino]ethyl]-5-[[2-[methyl(phenylmethyl)amino]ethyl]amino]-7,10-bis(phenylmethoxy)-anthra[1,9-*cd*]pyrazol-6(2*H*)-one[12] in 450 mL of glacial HOAc was hydrogenated over 4.2 g of 20% $Pd(OH)_2/C$[20] at 25 °C and at atmospheric pressure for 4 h, filtered through Celite, and concentrated to 200 mL. The concentrate was treated with 8 mL of 48% HBr, diluted with 150 mL of 2-propanol, and chilled overnight. The precipitate was collected, washed successively with MeOH and then ether, and dried at 50 °C (25 mm) to afford 16 g of **74** as a red orange solid that was 98% pure by HPLC [Zorbax C-1, 10 μm; mobile phase CH_3CN–H_2O–HOAc (60:40:1), 0.005 M in octanesulfonic acid, sodium salt]: pK_a (50% aqueous MeOH) = 6.8, 8.0; log P (pH) = 4.57 (5.8), 4.83 (6.4), 6.48 (7.1), 6.70 (7.5), 6.95 (8.2);[30] ^1H NMR [10% D_2O in $(CD_3)_2SO$] δ 2.66 (s, 3), 3.14 (t, 2, J = 5 Hz), 3.25 (t, 2, J = 5 Hz), 3.50–3.75 (m, 4), 3.90 (t, 2, J = 5 Hz), 6.92 (d, 1, J = 9 Hz), 7.32 (d, 1, J = 9 Hz), 7.36 (d, 1, J = 9 Hz), 8.14 (d, 1, J = 9 Hz). The $(CD_3)_2SO$ spectrum shows an exchangeable one-proton singlet at δ 5.28, a five-proton multiplet at δ 8.55–9.05, and one-proton sharp singlets at δ 8.97 and δ 13.62. IR (KBr) 1661, 1604, 1571, 1277, 1213, 822 cm^{-1}; UV λ_{max} (MeOH) 290 nm (ϵ 7840), 318 (7280), 334 (7680), 387 (3900), 406 (4940), 512 (15720). Anal. ($C_{21}H_{25}N_5O_4 \cdot 1.95HBr \cdot 1.1H_2O$) C, H, N, Br^-, H_2O.

Method E. 2-[2-(Diethylamino)ethyl]-5-[[2-(diethylamino)ethyl]amino]anthra[1,9-*cd*]pyrazol-6(2*H*)-one Hydrochloride (40). A mixture of 1.2 g (3.4 mmol) of 5-chloro-2-[2-(diethylamino)ethyl]anthra[1,9-*cd*]pyrazol-6(2*H*)-one,[12] 1.8 mL (13 mmol) of *N,N*-diethylethylenediamine, 1 mg of anhydrous CuCl, and 30 mL of 2-ethoxyethanol was heated at reflux for 48 h, cooled, filtered, and concentrated to a residue. A solution of the residue in CH_2Cl_2 was washed successively with H_2O, dilute NH_4OH, and brine and then dried. Purification by chromatography over SiO_2 with CH_2Cl_2–MeOH–Et_3N (89:10:1) as the eluant afforded the product, which was dissolved in 2-propanol and treated with excess 2-propanolic HCl. The precipitate was collected and dried to give 1.2 g of **40**: ^1H NMR [10% D_2O in $(CD_3)_2SO$] δ 1.23 (t, 12, J = 7 Hz), 3.1–3.4 (m, 10), 3.72 (t, 2, J = 6 Hz), 3.97 (t, 2, J = 6 Hz), 5.05 (t, 2, J = 6 Hz), 7.43 (d, 1, J = 9.4 Hz), 7.65 (t, 1, J = 7.3 Hz), 7.83 (t, 1, J = 7 Hz), 8.22 (d, 1, J = 9.6 Hz), 8.23 (d, 1, J = 6 Hz). The $(CD_3)_2SO$ spectrum shows an exchangeable one-proton triplet at δ 9.12 and exchangeable one-proton broad singlets at δ 10.93 and δ 11.05. IR (KBr) 1657, 1608, 1563, 1470, 1021, 716 cm^{-1}; UV λ_{max} (MeOH) 231 nm (ϵ 27120), 242 (26320), 288 (8720), 362 (10050), 467 (14460). Anal. ($C_{26}H_{35}N_5O$) C, H, N, Cl^-, H_2O.

Method F. 5-[[2-(Diethylamino)ethyl]amino]-7,10-dihydroxy-2-[2-[(2-hydroxyethyl)amino]ethyl]anthra[1,9-*cd*]pyrazol-6(2*H*)-one Hydrochloride (80). A mixture of 2.2 g (6 mmol) of 5-chloro-7,10-dihydroxy-2-[2-[(2-hydroxyethyl)-amino]ethyl]anthra[1,9-*cd*]pyrazol-6(2*H*)-one,[12] 6.5 mL (60 mmol) of *N,N*-dimethylethylenediamine, and 25 mL of pyridine was stirred at 80 °C for 18 h. The solution was concentrated to a residue, which was triturated in 2-propanol. The solids were filtered, washed successively with 2-propanol and ether, and then dissolved in hot 95% EtOH. The solution was treated with excess 2-propanolic HCl and cooled. The resulting red solid was collected by filtration, washed with 2-propanol, and dried to afford 1.6 g of **80**: IR (KBr) 1660, 1600, 1570, 1455, 1270, 1210, 820 cm^{-1}; UV λ_{max} (MeOH) 291 nm (ϵ 7250), 318 (7460), 334 (7880), 390 (3830), 408 (4570), 515 (16860). Anal. ($C_{22}H_{27}N_5O_4 \cdot 1.9HCl.7H_2O$) C, H, N, Cl^-.

Method G. 2-[2-[(2-Hydroxyethyl)amino]ethyl]-5-[[2-[methyl(phenylmethyl)amino]ethyl]amino]-7,10-bis(phenylmethoxy)anthra[1,9-*cd*]pyrazol-6(2*H*)-one (116). A mixture of 33.2 g (60 mmol) of 5-chloro-2[2-[(2-hydroxyethyl)-amino]ethyl]-7,10-bis(phenylmethoxy)anthra[1,9-*cd*]pyrazol-6-(2*H*)-one[12] and 98.6 g (600 mmol) of *N*-methyl-*N*-(phenylmethyl)-1,2-ethanediamine (**14b**)[21] was stirred in a 132 °C oil bath for 3 h and then cooled to 80 °C where it was diluted with 400 mL of aqueous 2-propanol. The stirred mixture was slowly cooled to 25 °C, and the precipitated solids were collected by filtration, washed successively with H_2O, 2-propanol, and then ether, and dried to afford 27.5 g of crude product. Purification was carried out by chromatography over 1.7 kg of SiO_2 (230–400 mesh) utilizing a gradient elution from 5% to 12% MeOH in CH_2Cl_2. Pure product fractions were concentrated to a solid residue, whose crystallization from MeOH gave 17.4 g (43%) of **116**: mp 140–146 °C; TLC [SiO_2; CH_2Cl_2–CH_3OH–Et_3N (80:20:1)] one major spot, R_f 0.38; ^1H NMR [$(CD_3)_2SO$] δ 2.27 (s, 3), 2.62 (t, 2, J = 6 Hz), 2.71 (t, 2, J = 6 Hz), 3.19 (t, 2, J = 6 Hz), 3.35–3.40 (m, 4; collapses to t, 2, J = 6 Hz at 3.38 with D_2O wash), 3.54–3.65 (s, 2 at 3.62 overlapping q, 2), 4.44 (s, 1, exchanges D_2O), 5.27 (s, 2), 5.36 (s, 2), 7.08–8.00 (m, 19), 9.61 (t, 1, J = 4 Hz, exchanges D_2O; IR (KBr) 1661, 1606, 1578, 1568, 1453, 1266, 736, 697 cm^{-1}. Anal. ($C_{42}H_{43}N_5O_4 \cdot 0.2H_2O$) C, H, N, H_2O.

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Registry No. **8a** (R_1 = $CH_2CH_2NHCH_2CH_2OH$), 91440-33-4; **8a** (R_1 = H), 64598-58-9; **8a** (R_1 = Me), 91441-54-2; **8a** (R_1 = CH_2CH_2OH), 91441-55-3; **8a** (R_1 = $CH_2CH_2NH_2$), 91440-42-5; **8a** (R_1 = $CH_2CH_2NEt_2$), 91440-03-8; **8b** (R_1 = $CH_2CH_2NMe_2$), 91440-50-5; **8b** (R_1 = Me), 91441-58-6; **8b** (R_1 = CH_2Ph), 104739-77-7; **8b** (R_1 = CH_2CH_2OH), 91441-57-5; **8b** (R_1 = $CH_2CH_2NH_2$), 91450-83-8; **8b** (R_1 = $CH_2CH_2NHCH_2CH_2OH$), 91733-40-3; **8b** (R_1 = $CH_2CH_2NEt_2$), 91440-46-9; **8b** (R_1 = $CH_2CH_2NHCH_2CH_2NMe_2$, 91441-16-6; **8b** (R_1 = $CH_2CH(OH)CH_2NEt_2$), 91440-66-3; **8b** (R_1 = CH_2CH_2OMe), 91441-71-3; **8b** (R_1 = CH_2CH_2SMe), 91441-15-5; **8b** (R_1 = $CH_2CH_2NMeCH_2CH_2OH$), 91441-64-4; **8b** (R_1 = $CH_2CH_2CH_2NMe_2$), 91440-72-1; **8c** (R_1 = CH_2CH_2OH), 91733-39-0; **8c** (R_1 = CH_2-c-$CHCH_2OCMe_2O$), 91441-74-6; **8c** (R_1 = $CH_2CH_2NH_2$), 91441-70-2; **8c** (R_1 = $CH_2CH_2CH_2NH_2$), 91441-88-2; **8c** (R_1 = CH_2CH_2NHMe), 91441-91-7; **8c** (R_1 = $CH_2CH_2NHCH_2CH_2OH$), 91441-59-7; **8c** (R_1 = $CH_2CH_2NHCH_2CH_2NMe_2$), 91441-63-3; **11** (R_1 = $CH_2CH_2NEt_2$), 91441-75-7; **11** (R_1 = Me), 104740-54-7; **11** (R_1 = $CH_2CH_2NHCH_2CH_2OH$), 91441-76-8; **13**, 104740-55-8; **15**, 91440-27-6; **15**·xHCl, 91440-28-7; **16**·xHCl, 104739-78-8; **17**, 91440-17-4; **17**·xHCl, 104739-79-9; **18**, 91440-15-2; **18**·xHCl, 91440-16-3; **19**, 104739-80-2; **20**, 104739-81-3; **21**, 91440-19-6; **21**·xHCl, 91440-20-9; **22**, 91440-95-8; **22**·xHCl, 91440-21-0; **23**, 91440-97-0; **23**·xHCl, 104739-82-4; **24**, 91440-98-1; **24**·xHCl, 91440-24-3; **25**, 91440-99-2; **25**·xHCl, 104739-83-5; **26**, 91440-96-9; **26**·xHCl, 91440-22-1; **27**, 104740-39-8; **27**·xHCl, 104739-84-6; **27** (Cbz deriv.), 104740-56-9;

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28, 91440-43-6; 28·xHCl, 91440-44-7; 29, 91441-01-9; 29·xHCl, 104739-85-7; 30, 91440-37-8; 30·xHCl, 91440-38-9; 31, 91441-00-8; 31·xHCl, 104739-86-8; 32, 91440-30-1; 32·AcOH, 91440-31-2; 33, 91440-39-0; 33·xHCl, 91440-40-3; 34, 91440-35-6; 34·xHCl, 104739-87-9; 35, 91451-21-7; 35·xHCl, 104739-88-0; 36, 104739-89-1; 37, 91440-08-3; 37·xHCl, 104739-90-4; 38, 104740-40-1; 38·xHCl, 104739-91-5; 38 (benzyl deriv.), 104740-57-0; 39, 91451-17-1; 39·xHCl, 104739-92-6; 40, 91440-04-9; 40·xHCl, 104739-93-7; 41, 91451-18-2; 41·xHCl, 104739-94-8; 42, 91451-20-6; 42·xHCl, 104778-44-1; 43, 91451-19-3; 43·xHCl, 104739-95-9; 44, 91470-46-1; 44·xHCl, 104739-96-0; 45, 91440-94-7; 45·xHBr, 91440-14-1; 46, 104740-41-2; 46·xHCl, 104739-97-1; 47, 91440-62-9; 47·xHCl, 91440-65-2; 48, 91441-09-7; 48·xHCl, 104739-98-2; 49, 104740-42-3; 49·xHCl, 104739-99-3; 50, 104740-43-4; 50·xHCl, 104740-00-3; 51, 91441-39-3; 51·xHCl, 104740-01-4; 52, 91450-90-7; 52·xHCl, 91450-91-8; 53, 94059-40-2; 53·xHCl, 91440-74-3; 54, 91441-07-5; 54·xHCl, 91440-59-4; 55, 91440-57-2; 55·xHCl, 104740-02-5; 56, 91441-08-6; 56·xHCl, 91440-61-8; 57, 91440-55-0; 57·xHCl, 91440-56-1; 58, 104740-44-5; 58·xHCl, 91440-60-7; 59, 104740-45-6; 59·xHCl, 91451-08-0; 60, 91441-49-5; 60·xHCl, 91450-93-0; 61, 91441-35-9; 61·xHCl, 91450-86-1; 62, 91441-36-0; 62·xHCl, 91450-87-2; 63, 94035-69-5; 63·xHCl, 104740-03-6; 64, 91441-34-8; 64·xHCl, 104740-04-7; 65, 91441-38-2; 65·xHCl, 91450-89-4; 66, 91441-37-1; 66·xHCl, 91450-88-3; 67, 91451-22-8; 67·xHCl, 104740-05-8; 68, 91441-44-0; 68·xHCl, 104740-06-9; 69, 104740-46-7; 69·xHCl, 104740-07-0; 70, 91441-20-2; 70·xHCl, 104740-08-1; 71, 91441-23-5; 71·xHCl, 91440-86-7; 72, 91441-24-6; 72·xHCl, 104740-09-2; 73, 91441-25-7; 73·xHCl, 91440-88-9; 74, 91441-48-4; 74·xHBr, 91450-82-7; 75, 104740-10-5; 76, 91441-19-9; 76·xHCl, 104740-11-6; 77, 91441-27-9; 77·xHCl, 91440-90-3; 78, 91441-31-5; 78·xHCl, 91450-79-2; 79, 91441-32-6; 79·xHCl, 91450-80-5; 80, 91441-21-3; 80·xHCl, 91440-84-5; 81, 91441-22-4; 81·xHCl, 91440-85-6; 82, 91441-26-8; 82·xHCl, 91440-98-0; 83, 91441-29-1; 83, 91440-92-5; 84, 104740-47-8; 84·xHCl, 104740-12-7; 85, 91441-28-0; 85·xHCl, 91440-91-4; 86, 104740-48-9; 86·xHCl, 104740-13-8; 87, 91441-30-4; 87·xHCl, 91440-93-6; 88, 91441-18-8; 88·xHCl, 104740-14-9; 89, 91440-78-7; 90, 91441-17-7; 90·xHCl, 91440-77-6; 91, 91441-05-3; 91·xHCl, 91440-53-8; 92, 104740-49-0; 92·xHCl, 104740-15-0; 93, 91441-04-2; 93·xHCl, 91440-51-6; 94, 91441-47-3; 94·xHCl, 104740-16-1; 95, 91441-14-4; 95·xHCl, 104740-17-2; 96, 91441-13-3; 96·xHCl, 104740-18-3; 97, 91441-02-0; 97·xHCl, 104740-19-4; 98, 91441-03-1; 98·xHBr, 104740-20-7; 98 (Cbz deriv.), 104778-45-2; 99, 104740-21-8; 100, 91451-14-8; 100·xHCl, 104740-22-9; 101, 91440-47-0; 101·xHCl, 104740-23-0; 102, 91441-51-9; 102·xHCl, 91440-76-5; 103, 91441-12-2; 103·xHCl, 104740-24-1; 104, 91441-10-0; 104·xHCl, 91440-67-4; 105, 104740-50-3; 105·xHCl, 104740-36-2; 106, 104740-26-3; 107, 91441-86-0; 108, 91441-73-5; 109, 104740-27-4; 110, 104740-28-5; 111, 91441-87-1; 112, 91441-45-1; 113, 91441-90-6; 114, 104740-29-6; 115, 104740-30-9; 116, 104740-31-0; 117, 104740-32-1; 118, 104740-33-2; 199, 104740-51-4; 119·xHCl, 104740-34-3; 120, 104740-52-5; 120·xHCl, 104740-35-4; 121, 104740-53-6; 121·xHCl, 104740-36-5; 122, 91450-95-2; 122·xHCl, 104740-37-6; 123, 91441-40-6; 123·xHCl, 104740-38-7; $NH_iCH_2CH_2NEt_2$, 100-36-7; $NH_2(CH_2)_3NH_2$, 109-76-2; $NH_2CH_2CH_2NHCH_2CH_2OH$, 111-41-1; $NH(CH_2)_5CH_3$, 111-26-2; $NH_2CH_2CH_2CO_2^-N^+Bu_4$, 104761-05-9; $NH_2(CH_2)_3Net_2$, 104-78-9; $NH_23(CH_2)_4NEt_2$, 27431-62-5; $NH_2(CH_2)_7NEt_2$, 20526-69-6; $NH_2CH_2CH_2$-c-$N(CH_2CH_2)_2O$, 2038-036-1; NH_2CH_3, 74-89-5; $NH_2CH_2CH_2OH$, 141-43-5; $NH_2CH_2CH_2NH_2$, 107-15-3; $NH_2CH_2CH_2NMe_2$, 108-00-9; $NH_2CH_2CH_2NHMe$, 109-81-9; $NH_2(CH_2)_3NHCH_2CH_2OH$, 4461-39-6; $NH_2(CH_2)_2NH(CvH_2)_2NMe_2$, 24229-53-6; $NH_2(CH_2)_4NH_2$, 110-60-1; $NH_2(CH_2)_5NH_2$, 462-94-2; $NH_2CH_2CH_2N(Cbz)Me$, 19023-94-0; $NH_2CH_2CH_2N(CH_2CH_2OH)_2$, 4439-20-7; $NH_2(CH_2)_3N(CH_2CH_2OH)_2$, 4985-85-7; c-$HN(CH_2CH_2)_1NMe$, 109-01-3; $NH_2CH_2CH_2NHCH_2CH_2NH_2$, 111-40-0; $NH_2CH_2CH_2NHCH_2CH_2NMe_2$, 24229-53-6; $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, 71-44-3; $NH(Me)CH_2CH_2NMe_2$, 142-25-6; $NH_2CH_2CH_2N(Me)CH_2Ph$, 14165-18-5; 1-(2-aminoethyl)-piperazine, 140-31-8; benzyl chloroformate, 501-53-1.

2-Phenylindoles. Effect of N-Benzylation on Estrogen Receptor Affinity, Estrogenic Properties, and Mammary Tumor Inhibiting Activity

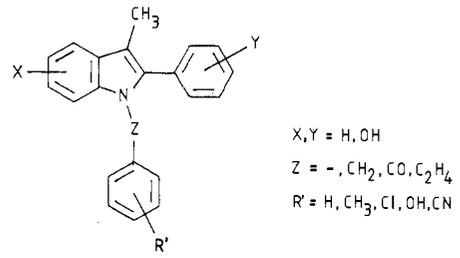
Erwin von Angerer* and Josef Strohmeier

Institut für Pharmazie, Universität Regensburg, D-8400 Regensburg, Federal Republic of Germany. *Received April 21, 1986*

Hydroxy-2-phenylindoles carrying substituted benzyl groups and similar substituents at the nitrogen were synthesized and tested for their ability to displace estradiol from its receptor. All of the derivatives tested exhibited high binding affinities for the calf uterine estrogen receptor, with RBA values ranging from 0.55 to 16 (estradiol 100). The mouse uterine weight tested revealed only low estrogenicity for this class of compounds. Several derivatives showed antiestrogenic activity with a maximum inhibition of estrone-stimulated uterine growth of 40%. Two of the compounds (**6c**, **21c**) were tested for antitumor activity in dimethylbenanthracene- (DMBA-) induced estrogen-dependent rat mammary tumors. Only the 4-cyanobenzyl derivative **21c** was active. After 4 weeks of treatment with 12 mg/kg (6 times/week), the average tumor area was decreased by 57% (control +204%). In vitro, an inhibitory effect of **21b** was only observed with hormone-sensitive MCF-7 breast cancer cells but not with hormone-independent MDA-MB 231 cells. These results make a mode of action involving the estrogen receptor system likely.

In recent years, we paid much attention to the 2-phenylindole system as a new structure for the development of agents for the treatment of estrogen-dependent malignancies.[1-4] In a previous paper,[2] we reported on the influence of structural variations on estrogen receptor affinity, endocrine potency, and antineoplastic activity within a series of *N*-alkyl-2-phenylindoles. Among the compounds studied, several derivatives proved to be very active against experimental hormone-dependent mammary tumors.[1-4] One of these compounds, zindoxifene,[3] is presently undergoing phase I clinical trials.

Chart I



In order to improve the activity in this class of compounds, we introduced aromatic substituents into positions 1 and 3 of the indole nucleus. The latter derivatives have

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