1.66 ppm (s, 3, $=CCH_3$). Anal. ($C_{23}H_{25}ClN_2$) C, H, Cl, N.

2-Chloro-11-(4-methylpiperazino)-5-(2-propylidene)-5*H*-dibenzo[*a*,*d*]cycloheptene (10c) was prepared from 2-chloro-11-(4-methylpiperazino)-5*H*-bicyclo[*a*,*d*]cyclohepten-5-one (**21b**) and isopropyltriphenylphosphonium iodide as outlined above for the preparation of **10a** from **21a**. Column chromatography on silica gel, crystallization from absolute ethanol, and recrystallization from methanol gave **10c** (5%) as light yellow solid: mp 148–150 °C; ^1H NMR (Bruker AM-400) δ 7.69 (d, 1, J = 2.3 Hz, C-1 H), 7.30–7.10 (m, 6, aromatic H), 6.15 (s, 1, C-10 H), 2.99 (m, 4, piperazino C-2 and C-6 H), 2.61 (m, 4, piperazino C-3 and C-5 H), 2.37 (s, 3, NCH_3), 1.69 (s, 3, $=CCH_3$), 1.66 ppm (s, 3, $=CCH_3$). Anal. ($C_{23}H_{25}ClN_2$) C, H, Cl, N.

***trans*-2-Chloro-10,11-dibromo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (19).** Bromine (4.8 g, 0.030 mol) in carbon tetrachloride (10 mL) was added dropwise with stirring to a suspension of 2-chloro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (**18**), mp 158–159 °C (lit.[22] mp 159–160 °C) (6.00 g, 24.9 mmol), in carbon tetrachloride (25 mL). The mixture was stirred at room temperature for 4 h and then washed with aqueous sodium thiosulfate. The washings were extracted with ether (3 × 50 mL), and the ether extracts were combined with the carbon tetrachloride solution. The dried ($MgSO_4$) organic solution was evaporated, and recrystallization of the residue from ethanol gave **19** (6.6 g, 66%): mp 165–167 °C dec; ^1H NMR (JEOL FX-90Q) δ 8.13–8.04 (m, 2, C-4 and C-6 H), 7.56–7.40 (m, 5, aromatic H), 5.72 ppm (d, 2, C-10 and C-11 H). Anal. ($C_{15}H_9Br_2ClO$) C, H, Br, Cl.

11-Bromo-2-chloro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (20b). *trans*-2-Chloro-10,11-dibromo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (**19**) (12.0 g, 30.0 mmol) and sodium hydroxide (3.6 g, 90 mmol) in methanol (250 mL) were boiled for 1.5 h. The solvent was evaporated and the solid residue mixed with water (100 mL). The resulting slurry was extracted with ether. Evaporation of the dried ($MgSO_4$) ethereal extracts gave a mixture (9.2 g, 96%) of 10-bromo-2-chloro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-one (**20a**) and **20b** as a slightly yellow solid: mp 130–135 °C. (lit.[32] mp 127–129 °C). Separation of these isomers by chromatography was not successful, but recrystallization of a portion (1.0 g) from hexanes gave **20b** (0.50 g, 48% from **19**): mp 142–143 °C; ^1H NMR (Bruker AM-400) δ 8.09 (d, 1, J = 1.9 Hz, C-1 H), 7.93 (d, 1, J = 8.5 Hz, C-6 H), 7.84 (d, 1, J = 8.5 Hz, C-4 H), 7.76 (s, 1, C-10 H), 7.61–7.58 (m, 1, C-7 H), 7.56–7.54 (m, 1, C-8 H), 7.51–7.47 (m, 1, C-3 H), 7.44–7.40 ppm (m, 1, C-9 H); ^{13}C NMR δ 192.93 (carbonyl C), 138.63, 138.15, 137.52, 135.72, 134.68, 132.74, 132.05, 130.82, 130.72, 130.02, 129.93, 129.51, 128.88, 123.42 ppm. Anal. ($C_{15}H_8BrClO$) C, H, Br, Cl.

(32) Eur. Pat. Appl. EP 0 035 711, 1981 (to BASF).

2-Chloro-10-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (21a). *N*-Methylpiperazine (7.6 g, 0.076 mol) and potassium *tert*-butoxide (4.4 g, 0.039 mol) were added with stirring to a mixture of 10-bromo- and 11-bromo-2-chloro-5*H*-dibenzo-[*a*,*d*]cyclohepten-5-one (**20a** and **-b**) (12.0 g, 37.5 mmol) suspended in *tert*-butyl alcohol (70 mL), and the stirred mixture was boiled for 4 h. The solvent was evaporated, and the residual tar was mixed with water (200 mL). The resulting slurry was extracted with methylene chloride (5 × 200 mL). Evaporation of the dried ($MgSO_4$) methylene chloride solution gave a mixture (10 g, 79%) of **21a** and 2-chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-one (**21b**) in a ratio of 1.5 to 1, respectively (^1H NMR), as an orange-brown solid. Chromatography on silica gel (500 g) using ethyl acetate–absolute ethanol–triethylamine (100:1.0:0.25) as eluant gave, in the combined earliest fractions (7 × 100 mL), **21a** contaminated with a small amount of **21b**. Evaporation of these fractions and recrystallization of the residue from absolute ethanol gave **21a** (1.5 g, 12%) as bright yellow needles: mp 110–113 °C; ^1H NMR (Bruker AM-400) δ 8.05–8.02 (m, 1, C-9 H), 7.88–7.85 (m, 1, C-6 H), 7.76 (d, 1, J = 9.7 Hz, C-4 H), 7.62–7.51 (m, 2, C-8 and C-7 H), 7.41 (d, 1, J = 2.0 Hz, C-1 H), 7.30–7.26 (m, 1, C-3 H), 6.27 (s, 1, C-11 H), 3.04–2.93 (m, 4, piperazino C-2 and C-6 H), 2.65–2.55 (m, 4, piperazino C-3 and C-5 H), 2.38 ppm (s, 3, NCH_3). Anal. ($C_{20}H_{19}ClN_2O$) C, H, Cl, N.

2-Chloro-11-(4-methylpiperazino)-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (21b). Middle fractions (5 × 100 mL) from the chromatography outlined above contained mixtures of **21a** and **21b**. The combined latest fractions (9 × 100 mL) contained mostly **21b** and were evaporated. Recrystallization of the residue from absolute ethanol gave **21b** (0.90 g, 7%) as bright yellow needles: mp 130–132 °C; ^1H NMR (Bruker AM-400) δ 8.03 (d, 1, J = 2.1 Hz), 7.88 (s, 1), 7.78 (s, 1), 7.43 (m, 4), 6.43 (s, 1, C-10 H), 2.95 (m, 4, piperazino C-2 and C-6 H), 2.65 (m, 4, piperazino C-3 and C-5 H), 2.39 ppm (s, 3, NCH_3). Anal. ($C_{20}H_{19}ClN_2O$) C, H, Cl, N.

Acknowledgment. We thank Professor Thomas M. Harris for help with the NOE experiments. The synthetic work was supported by National Institutes of Health Grant HD-05797. Binding experiments were supported by grants to A.J. from the National Institutes of Health (MH-42894) and from the Medical Research Foundation of Oregon.

Registry No. **9a**, 124380-92-3; **9c**, 124380-93-4; **10a**, 124380-94-5; **10c**, 124380-95-6; **18**, 3973-55-5; **19**, 124380-98-9; **20a**, 124381-00-6; **20b**, 124380-99-0; **21a**, 124380-96-7; **21b**, 124380-97-8; methyltriphenylphosphonium bromide, 1779-49-3; isopropyltriphenylphosphonium iodide, 24470-78-8; *N*-methylpiperazine, 109-01-3.

Potential Antitumor Agents. 59. Structure–Activity Relationships for 2-Phenylbenzimidazole-4-carboxamides, a New Class of "Minimal" DNA-Intercalating Agents Which May Not Act via Topoisomerase II

William A. Denny,* Gordon W. Rewcastle, and Bruce C. Baguley

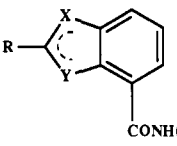
Cancer Research Laboratory, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand.
Received May 11, 1989

A series of substituted 2-phenylbenzimidazole-4-carboxamides has been synthesized and evaluated for in vitro and in vivo antitumor activity. These compounds represent the logical conclusion to our search for "minimal" DNA-intercalating agents with the lowest possible DNA-binding constants. Such "2–1" tricyclic chromophores, of lower aromaticity than the structurally similar 2-phenylquinolines, have the lowest DNA binding affinity yet seen in the broad series of tricyclic carboxamide intercalating agents. Despite very low in vitro cytotoxicities, several of the compounds had moderate levels of in vivo antileukemic effects. However, the most interesting aspect of their biological activity was the lack of cross-resistance shown to an amsacrine-resistant P388 cell line, suggesting that these compounds may not express their cytotoxicity via interaction with topoisomerase II.

Although high DNA binding affinity correlates positively with in vitro cytotoxicity for several series of DNA-intercalating agents,[1,2] this property is also thought to be the factor limiting the penetration of such drugs into multi-

0022-2623/90/1833-0814$02.50/0

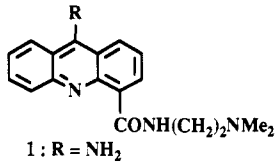
Table I. Physiochemical and Cytotoxic Properties of 2-Arylbenzimidazoles



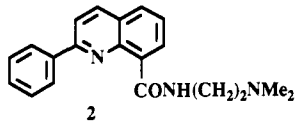
no	X	Y	R	mp, °C	formula	anal.	Rm[a]	log K_{AT}[b]	in vitro, IC_{50}[e], μm P388/W[c]	P388/A[d]	ratio[f]	in vivo P388/W OD[g]	ILS[h]
1	9-aminoacridinecarboxamide						-1.11	7.35	0.011	0.73	66	4.5	98
2	phenylquinolinecarboxamide						-0.01	5.97	1.3			100	91
3	amsacrine						0.18	5.57	0.012	0.89	0.74	13.3	78
4	acridinecarboxamide						-0.20	6.12	0.15	2.02	13	66	91
5	NH	=N—	Ph	192–194	$C_{18}H_{20}N_4O$	C, H, N	-0.26	5.41	22.0	36.2	1.7	100	56
6	=N—	NMe	Ph	213–214	$C_{19}H_{22}N_4O{\cdot}2HCl{\cdot}0.5H_2O$	C, H, N. Cl	-0.55	4.87	40	>40	>1	150	NA[i]
7	—NMe	=N—	Ph	208–210	$C_{19}H_{22}N_4O{\cdot}2HCl$	C,[j] H, N, Cl	-0.36	5.16	20			150	NA
8	NH	=N—	2-furyl	239–243	$C_{16}H_{18}N_4O_2{\cdot}2HCl{\cdot}3H_2O$	C, H, N, Cl	-0.57	5.18	40	40	1.0	150	NA
9	NH	=N—	2-thienyl	125–126	$C_{16}H_{18}N_4OS{\cdot}2HCl{\cdot}3H_2O$	C, H, N, Cl	-0.45	5.48	23.1	25.6	1.1	150	NA
10	NH	=N—	3-thienyl	126–127	$C_{16}H_{18}N_4OS{\cdot}2HCl{\cdot}3H_2O$	C, H, N, Cl	-0.55	5.39	28.2	24.3	0.9	150	NA
11	NH	=N—	2-pyrrolyl	250–255	$C_{16}H_{19}N_5O{\cdot}2HCl$	C, H, N, Cl	-0.79	5.38	23.1	34.2	1.5	150	NA

[a] Rm values were determined as detailed in ref 27 with 4'-(9-acridinylamino)methanesulfonanalide (AMSA) as a standard. [b] log K_{AT} = the binding constant to poly[d(AT)], determined by the ethidium bromide displacement assay, as detailed in ref 14. [c] P388/W: wild-type P388 murine leukemia cell line. [d] P388/A: amsacrine-resistant P388 cell line. [e] IC_{50}: the concentration of drug in μM to inhibit cell growth by 50%, measured in 96-well cultures as described in ref 28. [f] ratio = IC_{50}(P388/A)/IC_{50}(P388/W). [g] OD: optimal dose of drug in mg/kg per day, administered intraperitoneally as a solution in 0.1 mL of 30% v/v ethanol/water on days 1, 5, and 9 after intraperitoneal inoculation of 10^6 P388/W leukemia cells. [h] ILS: percentage increase in lifespan of treated animals (at the optimal dose) compared with tumor-bearing control animals. Values are the mean of two determinations. Average lifespan of control animals was 11 days. [i] Compound inactive (ILS < 20%) at all dose levels up to toxic ones. [j] C out by 0.6%.

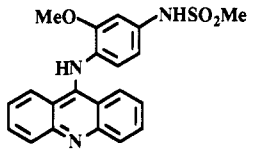
cellular spheroids which are used as models of solid tumors.[3,4] Following our original[5] observation of the high activity of 9-aminoacridinecarboxamide (1), we have recently reported[6-8] the synthesis and biological evaluation of several classes of weakly basic DNA-intercalating antitumor agents with lower DNA binding abilities. In particular, the broad-spectrum activity and relatively low DNA-binding abilities[7-9] of the "2–1" tricyclic phenylquinoline system (e.g. 2) suggested that the binding levels of this type of compound might be lowered even further without loss of biological activity.



1 : R = NH_2
4 : R = H

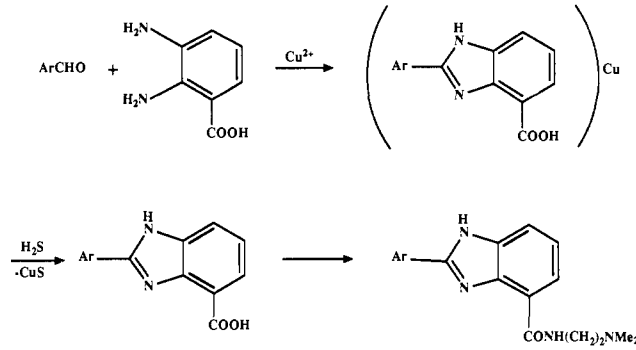


2



3

Scheme I. Synthesis of 2-Arylbenzimidazole-4-carboxamides



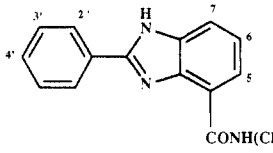
Since the main driving force for intercalative binding are stacking and electronic interactions between the aromatic systems of the DNA basepairs and drug chromophores, we have evaluated additional classes of "2–1" tricyclic carboxamide systems with lower aromaticity than the fully benzenoid phenylquinolines, anticipating that such compounds would have lower levels of binding to DNA. The number of such structures is limited by the requirement, noted previously in work with linear tricyclic carboxamides,[10] for an electronegative O or —N= atom positioned in the chromophore peri to the carboxamide group. On discovering that 2-phenyl-1*H*-benzimidazole-4-carboxamide (5) showed in vivo antitumor activity but had lower DNA binding than 2 (log *K* for binding to poly[d(AT)] was 5.41 and 5.97, respectively), we undertook a structure–activity relationships (SAR) study of the broad class of 2-arylbenzimidazole-4-carboxamides as "minimal" DNA-intercalating antitumor agents and report this work here.

Chemistry

The standard methods of benzimidazole synthesis (PPE- or PPA-induced cyclocondensation of benzoic acids and 1,2-diaminobenzenes[11]) have not been applied to the syn-

(1) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1981**, *24*, 520.
(2) Le Pecq, J.-B.; Xuong, N.-D.; Gosse, C.; Paoletti, C. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 5078.
(3) Kerr, D. J.; Kaye, S. B. *Cancer Chemother. Pharmacol.* **1987**, *19*, 1.
(4) West, C. M. L.; Stratford, I. J. *Cancer Chemother. Pharmacol.* **1987**, *20*, 109.
(5) Atwell, G. J.; Cain, B. F.; Baguley, B. C.; Finlay, G. J.; Denny, W. A. *J. Med. Chem.* **1984**, *27*, 1481.
(6) Denny, W. A.; Atwell, G. J.; Baguley, B. C. *Anti-Cancer Drug Des.* **1987**, *2*, 263.
(7) Atwell, G. J.; Bos, C. D.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1988**, *31*, 1048.
(8) Atwell, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1989**, *32*, 396.
(9) McKenna, R.; Beveridge, A.; Neidle, S.; Denny, W. A. *Mol. Pharmacol.* **1989**, *35*, 720.
(10) Palmer, B. D.; Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1988**, *31*, 707.

Table II. Physicochemical and Cytotoxic Properties of Substituted 2-Phenylbenzimidazole-4-carboxamides



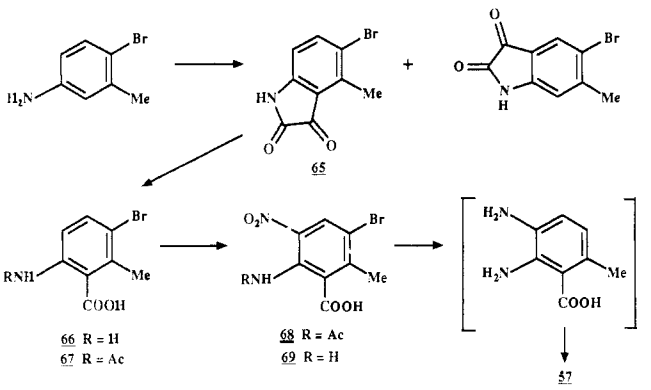
no.	R	mp, °C	formula	anal.	Rm[a]	log K_{AT}[b]	in vitro IC_{50}[e] P388/W[c]	in vitro IC_{50}[e] P388/A[d]	ratio	in vivo P388/W OD[g]	in vivo P388/W ILS[h]
12	2′-CH_3	230–234	$C_{19}H_{22}N_4O{\cdot}2HCl{\cdot}H_2O$	C, H, N	−0.20	5.48	22.9	32.7	1.4	100	34
13	2′-OCH_3	220–223	$C_{19}H_{22}N_4O_2{\cdot}2HCl$	C, H, N, Cl	−0.40	5.51	8.0	12.5	1.6	150	31
14	2′-Cl	203–205	$C_{18}H_{19}ClN_4O{\cdot}2HCl$	C, H, N, Cl	−0.14	5.22	16.0	22.5	1.4	100	30
15	2′-aza	237–240	$C_{17}H_{19}N_5O{\cdot}2HCl{\cdot}H_2O$	C, H, N, Cl	−1.32	5.06	19.0	30.2	1.6	150	26
16	3′-CH_3	161–164	$C_{19}H_{22}N_4O{\cdot}2HCl{\cdot}H_2O$	C, H, N, Cl	−0.10	5.34	9.5	14.4	1.6	150	21
17	3′-OCH_3	184–185	$C_{19}H_{22}N_4O_2{\cdot}2HCl{\cdot}H_2O$	C, H, N, Cl	−0.31	5.43	11.7	24.3	2.1	65	85
18	3′-Cl	155–156	$C_{18}H_{19}ClN_4O{\cdot}2HCl{\cdot}H_2O$	C, H, N, Cl	0.01	6.00	5.9	8.0	1.4	150	NA[i]
19	3′-aza	235–237	$C_{17}H_{19}N_5O{\cdot}2HCl$	C, H, N, Cl	−0.98	5.04	11.7	35.2	3.0	100	22
20	4′-CH_3	177–181	$C_{19}H_{22}N_4O{\cdot}2HCl{\cdot}3H_2O$	C, H, N, Cl	−0.18	5.86	7.8	12.5	1.6	150	33
21	4′-OCH_3	150–154	$C_{19}H_{22}N_4O_5{\cdot}2HCl$	C, H, N, Cl	−0.42	5.62	5.8	12.7	2.2	150	81
22	4′-Cl	154–155	$C_{18}H_{19}ClN_4O{\cdot}2HCl{\cdot}2H_2O$	C, H, N, Cl	0.08	5.52	4.0	7.7	1.9	100	20
23	4′-aza	266–270	$C_{17}H_{19}N_5O{\cdot}2HCl$	C, H	−1.18	5.43	6.6	15.9	2.4	65	NA
24	4′-Ph	253–259	$C_{24}H_{24}N_4O{\cdot}2HCl$	C, H, N, Cl	−0.29	5.72	1.3	2.0	1.5	65	NA
25	4′-NHAc	275–280	$C_{20}H_{23}N_5O_2{\cdot}2HCl{\cdot}1.5H_2O$	C, H, N, Cl	−1.00	5.34	32.5	>40	>1.2	225	47
26	4′-$N(CH_3)_2$	213–217	$C_{20}H_{25}N_5O{\cdot}2HCl{\cdot}0.5H_2O$	C, H, N, Cl	−0.71	6.43	2.6	7.0	2.7	150	NA
27	5-CH_3	255–259	$C_{19}H_{22}N_4O{\cdot}2HCl{\cdot}0.5H_2O$	C, H, N, Cl	−0.40	5.14	18.0	>40	>2.2	225	NA
28	6-CH_3	188–189	$C_{19}H_{22}N_4O{\cdot}3HCl{\cdot}H_2O$	C, H, N, Cl	−0.14	5.06	21.0	22.4	1.1	150	NA
29	7-CH_6	261–264	$C_{19}H_{22}N_4O{\cdot}2HCl$	C, H, N, Cl	−0.23	5.49	18.6	29.3	1.05	225	NA
30	2′,3′-benz	160–170	$C_{22}H_{22}N_4O{\cdot}2HCl{\cdot}H_2O$	C, H, N, Cl	−0.11	5.61	8.9	8.0	0.9	45	NA
31	3′,4′-benz	181–183	$C_{22}H_{22}N_4O{\cdot}2HCl{\cdot}H_2O$	C, H, N, Cl	−0.27	5.19	2.9	4.4	1.5	100	NA
32	2′,3′-(−OCH_2−)	225–230	$C_{19}H_{20}N_4O_3{\cdot}2HCl$	C, H, N, Cl	−0.58	5.95	2.7	4.6	1.7	150	65
33	2′,3′-$(OCH_3)_2$	215–220	$C_{20}H_{24}N_4O_3{\cdot}2HCl{\cdot}H_2O$	C, H, N, Cl	−0.67	5.92	12.5	7.7	0.62	100	32
34	2′,3′,4′-$(OCH_3)_3$	184–187	$C_{21}H_{26}N_4O_4{\cdot}2HCl$	C, H, N, Cl	−0.31	5.37	7.8	23.3	3.0	65	23

[a–i] Footnotes as for Table I.

thesis of 4-carboxylic acid analogues, although a recent report[12] on "phosphonium anhydride" reagents did note a good yield of ethyl 2-(4′-methylphenyl)benzimidazole-4-carboxylate from 4-methylbenzoic acid and ethyl 1,2-diaminobenzoate. In the present work, benzimidazoles **5–34** were prepared by the method of Scheme I, via the oxidative condensation of 1,2-diaminobenzoic acids and aldehydes with cupric ion.[13] Precipitation of the insoluble copper complexes usually occurred immediately at 20 °C, and treatment of this with H_2S gave the required 2-arylbenzimidazole-4-carboxylic acids in good yield (50–90%). The carboxamides of Tables I and II were prepared in high yield from the corresponding acids and *N,N*-dimethylethylenediamine by coupling with 1,1′-carbonyldiimidazole.

Most of the syntheses required 1,2-diaminobenzoic acid, which was prepared from 3-nitrophthalic acid via 2-amino-3-nitrobenzoic acid. (See the Experimental Section). The two isomeric *N*-methyldiaminobenzoic acids required for synthesis of **40** and **41** were prepared by treatment of the corresponding chloronitrobenzoic acids with methylamine under pressure, followed by catalytic reduction. The precursor diaminobenzoic acids for **57–59** were prepared by the isatin route, as outlined in Scheme II, which shows the synthesis of **57**.

Scheme II. Synthesis of 1,2-Diamino-5-methylbenzoic Acid



Results and Discussion

The parent 2-phenylbenzimidazole **5** is considerably more hydrophilic than the corresponding 2-phenylquinoline (**2**, Table I). Although the chromophore is a somewhat stronger base (the pK_a of **5** is 3.42 compared to 2.75 for **2**, measured by UV spectrophotometry[7]), the benzimidazole chromophore of **5** will still be essentially un-ionized at physiological pH. The parent compound **5** shows considerably lower levels of DNA binding than the corresponding 2-phenylquinoline (**2**, Table I), as measured by the ethidium displacement assay,[14] although the benzimidazole and quinoline chromophores have similar aromaticity as determined by their empirical resonance energies (ERE, 203 kJ mol^{-1} for quinoline, 204 kJ mol^{-1} for benzimidazole[15]). In spite of this lower binding, **5** appears to intercalate DNA, as determined by its ability to unwind and rewind closed circular supercoiled DNA[16] with an unwinding angle of 11° (data not shown). In order to more closely delineate the minimum requirements for intercalative binding, a series of derivatives (**8–11**) bearing five-membered heterocyclic rings (thiophene, pyrrole, and furan) at the 2-position of the benzimidazole nucleus were

(11) Kahaoka, Y. S.; Yohemistu, O.; Tanizawa, K.; Bah, Y. *Chem. Pharm. Bull.* **1964**, *12*, 773.
(12) Hendrickson, J. B.; Hussoin, M. S. *J. Org. Chem.* **1989**, *54*, 1144.
(13) Weidenhagen, R.; Weeden, U. *Chem. Ber.* **1938**, *69B*, 2263.
(14) Baguley, B. C.; Falkenhaug, E.-M. *Nucleic Acids Res.* **1978**, *5*, 161.
(15) Cook, M. J.; Katritzky, A. R.; Linda, P. *Adv. Heterocycl. Chem.* **1974**, *22*, 255.
(16) Revet, B. M. J.; Schmir, M.; Vinograd, J. *Nature New Biol.* **1971**, *229*, 10.

evaluated. These heterocycles have varying degrees of aromaticity as determined by their empirical resonance energies but are all less aromatic than benzene (ERE values of 122, 90, and 67 kJ mol^{-1} respectively, compared with 150 kJ mol^{-1} for benzene[15]). However, this was not reflected in the DNA binding, with the 2-heterocycles (**8**, **9**, and **11**) showing similar levels to phenyl compound **5**. Unexpectedly, 3-thienyl compound **10** showed much poorer binding.

Benzimidazoles such as **5** can exist in two tautomeric forms, where the group peri to the carboxamide is either —N= or —NH—. Therefore the two isomeric *N*-methyl compounds (**6** and **7**), where the tautomeric forms are fixed with respect to the side chain, were evaluated. 1-Me compound **7** showed a similar level of binding to **5** (log K = 5.16 and 5.18, respectively), while 3-Me compound **6**[17] bound much more weakly (log K = 4.87), presumably due to increased steric hindrance of the peri methyl group. As noted previously with linear tricyclic carboxamides,[10] where substituents peri to the carboxamide abolished in vivo activity, compound **6** was inactive. Although the methyl group in 1-Me isomer **7** does not hinder DNA binding and in fact fixes the benzimidazole structure in the preferred tautomeric form, it was nevertheless inactive in vivo (Table I). Therefore, phenyl ring SAR studies were carried out on analogues **12–34** of the parent benzimidazole chromophore, with compounds in which the phenyl substituent electronic and lipophilic properties were varied widely. The only relationship seen with DNA binding was a trend toward higher binding for compounds bearing electron-donating groups (e.g., **19** cf. **26**), but this was not quantitative across the whole data set.

All the compounds were evaluated in vitro against both the wild-type P388 leukemia and an amsacrine-resistant line (P388/A).[18] This cell line has a structurally altered topoisomerase enzyme[18] and is highly resistant (20–70-fold) to amsacrine (**3**) and other intercalating agents such as the 9-aminoacridinecarboxamide (**1**) and the corresponding acridinecarboxamide (**4**), which act[19] via topoisomerase II. The parent phenylbenzimidazole (**5**) was much less cytotoxic than any of these compounds toward wild-type P388 leukemia, with an IC_{50} of 22 μM, and was considerably less toxic than even the structurally similar 2-phenylquinolinecarboxamide (**2**, IC_{50} 1.3 μM, Table I). However, in contrast with the abovementioned compounds, there was relatively little difference in the potency of **5** against the P388 and P388/A cell lines (IC_{50} ratio of 1.7), and similarly low ratios (1–3-fold) were found for all the compounds (Tables I and II). These data suggest that the 2-phenylbenzimidazoles do not act via inhibition of topoisomerase II.

Within the phenyl-substituted series, cytotoxicities against the wild-type P388 leukemia varied over a 40-fold concentration range, which is somewhat less than that observed within other classes of tricyclic carboxamides.[7,8,10,20] For the whole data base (compounds **5–34**) there is a weak positive correlation between cytotoxic potency and DNA binding (eq 1).

$$\log (1/IC_{50}) = 0.68\ (\pm 0.34) \log K + 1.24\ (\pm 0.93) \quad (1)$$

$$n = 30 \qquad r = 0.61 \qquad s = 0.31$$

Despite the low absolute in vitro cytotoxicity shown by **5** against the wild-type P388, it had significant in vivo activity (ILS 56%) and reasonable in vivo potency (optimal dose 100 mg/kg per dose for a three-dose schedule, Table I). However, methylation of the benzimidazole nitrogen peri to the carboxamide to give **6** abolished this activity, as did replacement of the phenyl group with heterocycles (compounds **8–11**) or methylation of the 5-, 6-, and 7-positions of the benzimidazole (compounds **27–29**). In contrast, substitution of the phenyl ring at all positions was compatible with in vivo activity. The low but significant activity of the 2′-substituted compounds (**12–14**) is interesting, since it contrasts with the unacceptability of similar ortho substitution in the analogous 2-phenylquinolinecarboxamides.[8] The least acceptable substituents were aza (—N=), again in direct contrast with the phenylquinoline series, where this was the preferred substituent.[8] No clear-cut structure–activity relationships for in vivo activity could be discerned among the phenyl-substituted compounds. The dystherapeutic effects of the aza group (compounds **19** and **23**) and the beneficial effects of the OMe function (compounds **17** and **21**) suggested a correlation with electron-donating ability, but the 4′-NMe_2 derivative (**26**) was inactive and the polymethoxy compounds (**32–34**) did not show enhanced activity. Four of the compounds showing in vivo activity against P388 (**5**, **17**, **20** and **21**) were also evaluated against the Lewis lung carcinoma in vivo, but they were inactive in this system.

Conclusions

The 2-phenylbenzimidazoles discussed here are the logical conclusion of our development of the general class of tricyclic carboxamides as DNA-intercalating antitumor agents with minimal DNA binding. This work began with the discovery of 9-aminoacridine derivative **1**,[5] which showed an association constant (log K, measured by ethidium displacement[14]) of 7.35 for binding to poly[d(AT)]. We then showed[20] that this high level of binding could be significantly lowered by lowering the pK_a of the chromophore to give compound **4** (log K = 6.12) and reduced still further by splitting the chromophore into a "2–1" tricyclic system to give[7] phenylquinoline **5** (log K = 5.97). However, this appeared to be the "minimal" chromophore, since complete removal of one ring to give a quinolinecarboxamide chromophore led to nonintercalating and tumor-inactive compounds.[7]

In this paper we have therefore explored the effects of retaining the overall "2–1" topology of the phenylquinoline system with structures of lower aromaticity and have shown that 2-phenylbenzimidazole **5** retains intercalative binding and in vivo antitumor activity, while binding to poly[d(AT)] nearly 100-fold less strongly (log K = 5.41) than the original 9-aminoacridinecarboxamide (**1**), as measured by the ethidium displacement assay.

Although the compounds show much lower levels of cytotoxicity (IC_{50}s in the micromolar range) than usual for compounds which act as DNA-binding drugs, the fact that IC_{50} still correlates with DNA binding constant (eq 1) suggests the phenylbenzimidazoles do work in this manner. Despite their low cytotoxicity, the compounds show moderate in vivo antileukemic acitivity, although, unlike several previous series of tricyclic carboxamides,[8,20,21] they

(17) For clarity, numbering of compounds is carried out according to the diagram for Table I, although it is recognized that compound **6** is correctly named as *N*-[2-(*N*,*N*-dimethylamino)-ethyl]-1-methyl-2-phenylbenzimidazole-7-carboxamide.

(18) Per, S. R.; Mattern, M. R.; Mirabelli, C. K.; Drake, F. H.; Johnson, R. K. *Mol. Pharmacol.* **1987**, *32*, 17.

(19) Schneider, E.; Darkin, S. J.; Lawson, P. A.; Ching, L.-M.; Ralph, R. K.; Baguley, B. C. *Eur. J. Cancer Clin. Oncol.* **1988**, *24*, 1783.

(20) Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1987**, *30*, 664.

(21) Rewcastle, G. W.; Denny, W. A.; Baguley, B. C. *J. Med. Chem.* **1987**, *30*, 834.

were not active against the Lewis lung carcinoma. The most interesting aspect of the biological activity of the phenylbenzimidazoles is their lack of cross-resistance to the amsacrine-resistant P388 cell line, suggesting the mechanism of cytotoxicity of these compounds either may not involve inhibition of topoisomerase II or may be via the altered enzyme.

Experimental Section

Where analyses are indicated by symbols of the elements, results were within ±0.4% of the theoretical values. Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin. Melting points were determined on an Electrothermal apparatus using the supplied, stem-corrected thermometer and are as read. NMR spectra were measured on a Bruker WP-60 (Me_4Si).

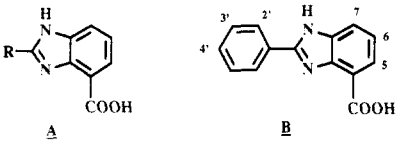
Synthesis of 2-Arylbenzimidazoles: 3-Nitroanthranilic Acid. Ethyl 2-carboxy-3-nitrobenzoate[22] (105 g, 0.44 mol) was heated under reflux for 1 h in $SOCl_2$ (300 mL). Volatiles were removed under reduced pressure, and the residue was dissolved in dry Me_2CO (500 mL) and added slowly to a solution of NaN_3 (57 g, 0.88 mol) in water (300 mL) to keep the temperature below 25 °C. The mixture was stirred vigorously for 1 h and then diluted with water (1 L). The resulting solid acyl azide was collected, washed well with water, and dissolved in a mixture of AcOH (400 mL) and water (150 mL). The resulting solution was heated gently until N_2 evolution began and then for a further 10 min after evolution ceased. Dilution with water gave crude ethyl 3-nitroanthranilate as a yellow solid (71 g, 77% yield). This was suspended in water (400 mL) containing KOH (28 g, 1.5 equiv), and the mixture was heated under reflux until all solids dissolved. The cooled solution was filtered and acidified with AcOH to give 3-nitroanthranilic acid (60 g, 75% yield overall), homogeneous by TLC. A sample crystallized from water as yellow plates, mp 207–209 °C. (lit.[23] mp 208–209 °C).

2-(3′-Methoxyphenyl)-1*H*-benzimidazole-4-carboxylic Acid (47) (Example of General Preparation). A solution of 3-nitroanthranilic acid (5.0 g, 27 mmol) and NaOH (1.2 g, 1.1 equiv) in water (100 mL) was hydrogenated ($Pd/C/H_2$) until the red color was discharged. The solution was filtered and made acidic with AcOH, and a solution of 3-methoxybenzaldehyde (5 g, 37 mmol) in MeOH (200 mL) was added, followed by a solution of cupric acetate (7.5 g, 37 mmol) in water (100 mL). The resulting mixture was stirred vigorously and heated briefly to boiling and then filtered hot. The precipitate was washed with water and dissolved in EtOH (200 mL) containing concentrated HCl (10 mL). A solution of Na_2S (10 g of the nonahydrate, 41 mmol of Na_2S) in water was added (keeping the solution acidic by addition of more HCl if necessary), and the mixture was filtered hot to remove the copper sulfide. The pH of the filtrate was adjusted to 5–6, and it was then diluted with water (100 mL) and concentrated to half-volume to give the crude product. This was collected and triturated with boiling MeOH to give 2-(3′-methoxyphenyl)-benzimidazole-4-carboxylic acid (47; 5.8 g, 80% yield), pure by TLC: ^1H NMR (CD_3SOCD_3) δ 8.07–6.96 (m, 7 H, aromatics), 3.89 (s, 3 H, OMe). A sample was crystallized from DMF as brown crystals, mp 280–281 °C. Anal. in Table III.

Similar preparations using the appropriate substituted benzaldehydes or other heterocyclic aldehydes gave the other 2-arylbenzimidazole-4-carboxylic acids listed in Table III.

Preparation of *N*′-[2-(*N*,*N*-Dimethylamino)ethyl]-2-(3′-methoxyphenyl)-1*H*-benzimidazole-4-carboxamide (17, Table I). Example of General Method. 2-(3′-Methoxyphenyl)-1*H*-benzimidazole-4-carboxylic acid (47; 2 g, 7.6 mmol) was suspended in dry DMF (10 mL), 1,1′-carbonyldiimidazole (2 g, 12 mmol) was added, and the mixture was warmed at 40 °C until gas evolution ceased (ca. 5 min). Excess *N*,*N*-dimethylethylenediamine (2 g) was added, followed after 10 min with 2 mL of water. Volatiles were removed under reduced pressure, and the residue was partitioned between EtOAc and 2 N Na_2CO_3. The organic layer was washed with water and brine and evaporated to give the crude free base. This was dissolved in MeOH, the pH was adjusted to 2–3 with concentrated HCl, and EtOAc was then added at the boil until crystallization began, giving the dihydrochloride salt of compound 17 as white needles (2.5 g, 80% yield), mp 184–185 °C. Anal. in Table II.

Table III. Analytical Data for 2-Substituted Benzimidazole-4-carboxylic Acids



No.	structure	R	mp, °C	formula	anal.
35	A	2-furyl	291–293	$C_{12}H_8N_2O_3$	C, H, N
36	A	2-thienyl	326–328	$C_{12}H_8N_2O_2S$	C, H, N, S
37	A	3-thienyl	330–333	$C_{12}H_8N_2O_2S$	C, H, N, S
38	A	2-pyrryl	>350	$C_{12}H_9N_3O_2$	C, H, N
39	B	H	290–293	$C_{14}H_{10}N_2O_2$	C, H, N
40	B	1-CH_3	154–154.5	$C_{15}H_{12}N_2O_2$	C, H, N
41	B	3-CH_3	314.5–315.5	$C_{15}H_{12}N_2O_2$	C, H, N
42	B	2′-CH_3	244–246	$C_{15}H_{12}N_2O_2$	C, H, N
43	B	2′-OCH_3	278–279	$C_{15}H_{12}N_2O_3$	C, H, N
44	B	2′-Cl	273–274	$C_{14}H_9ClN_2O_2$	C, H, N, Cl
45	B	2′-aza	297–300	$C_{13}H_9N_3O_2$	C, H, N
46	B	3′-CH_3	>320	$C_{15}H_{12}N_2O_2$	C, H, N
47	B	3′-OCH_3	280–281	$C_{15}H_{12}N_2O_3$	C, H, N
48	B	3′-Cl	335–337	$C_{14}H_9ClN_2O_2$	C, H, N, Cl
49	B	3′-aza	320–323	$C_{13}H_9N_3O_2$	C, H, N
50	B	4′-CH_3	>300	$C_{15}H_{12}N_2O_2$	C, H, N
51	B	4′-OCH_3	>310	$C_{15}H_{12}N_2O_3$	C, H, N
52	B	4′-Cl	>300	$C_{14}H_9ClN_2O_2$	C, H, N, Cl
53	B	4′-aza	311–313	$C_{13}H_9N_3O_2$	C, H, N
54	B	4′-Ph	319–320	$C_{20}H_{14}N_2O_2$	C, H, N
55	B	4′-NHAc	>360	$C_{16}H_{13}N_3O_3$	C, H, N
56	B	4′-NMe_2	>360	$C_{16}H_{15}N_3O_2$	C, H, N
57	B	5-CH_3	310–312	$C_{15}H_{12}N_2O_2$	C, H, N
58	B	6-CH_3	298–300	$C_{15}H_{12}N_2O_2$	C, H, N
59	B	7-CH_3	304–306	$C_{15}H_{12}N_2O_2$	C, H, N
60	B	2′,3′-benz	274–277	$C_{18}H_{12}N_2O_2$	C, H, N
61	B	3′,4′-benz	354–358	$C_{18}H_{12}N_2O_2$	C, H, N
62	B	2′,3′-(OCH_2O-)	>330	$C_{15}H_{10}N_2O_4$	C, H, N
63	B	2′,3′-$(OMe)_2$	306–308	$C_{16}H_{14}N_2O_4$	C, H, N
64	B	2′,3′,4′-$(OMe)_3$	304–309	$C_{17}H_{16}N_2O_5$	C, H, N

Preparation of 5-Methyl-2-phenyl-1*H*-benzimidazole-4-carboxylic Acid (57). A suspension of 5-bromo-4-methylisatin[24] (65; 0.11 mol) in 3% aqueous NaOH (2 L) was treated dropwise with 27% H_2O_2 (60 mL). As soon as the mixture was homogeneous it was filtered and acidified to give 5-bromo-6-methylanthranilic acid (66) as a brown solid, which was washed well with water and dried (21 g, 84% yield). A sample was crystallized from aqueous EtOH: mp 149–150 °C; ^1H NMR (CD_3SOCD_3) δ 7.29 (d, J = 9.3 Hz, 1 H, H-4), 6.56 (d, J = 9.3 Hz, 1 H, H-3), 2.34 (s, 3 H, Me). Anal. ($C_8H_8BrNO_2$) C, H, N.

The above crude acid (20.2 g, 0.088 mol) was dissolved in AcOH (300 mL) at 90 °C and Ac_2O (20 mL) was added. The mixture was held at 90–95 °C for 10 min, water (20 mL) was added, and most of the solvent was removed under reduced pressure. The residue was diluted with water, and the precipitate was washed well with water and dried to give 2-(acetylamino)-5-bromo-6-methylbenzoic acid (67; 16.5 g, 70% yield). A sample was crystallized from MeOH: mp 184–185 °C; ^1H NMR (CD_3SOCD_3) δ 9.48 (s, 1 H, CONH), 7.64 (d, J = 8.7 Hz, H-3), 7.30 (d, J = 8.7 Hz, 1 H, H-4), 2.35 (s, 3 H, Me), 1.99 (s, 3 H, COMe). Anal. ($C_{10}H_{10}BrNO_3$) C, H, N, Br. The above *N*-acetate (16.3g, 0.06 mol) was added slowly to well-stirred, fuming HNO_3 (50 mL) at 0–5 °C. The mixture was kept at 5 °C for a further 10 min and poured into ice. The resulting solid was collected and washed well with water to give 2-(acetylamino)-5-bromo-6-methyl-3-nitrobenzoic acid (68; 12.9 g, 68% yield). A sample was crystallized from aqueous EtOH: mp 212–213 °C; ^1H NMR (CD_3SOCD_3) δ 10.06 (s, 1 H, CONH), 8.21 (s, 1 H, H-4), 2.38 (s, 3 H, Me), 1.96 (s, 3 H, COMe). Anal. ($C_{10}H_9BrN_2O_5$) C, H, N, Br.

(22) Hecker, E.; Latrell, R.; Meyer, E. *Chem. Ber.* **1962**, *95*, 985.
(23) Chapman, E.; Stephen, H. *J. Chem. Soc.* **1925**, *127*, 1795.
(24) Rewcastle, G. W.; Denny, W. A.; Baguley, B. C. *J. Med. Chem.* **1987**, *30*, 843.

The above nitroacid (6 g, 19 mmol) and NaOH (1.5 g, 37 mmol) were dissolved in water (20 mL), and the solution was kept at 90 °C for 5 h. The cooled mixture was neutralized with HCl, and the resulting bright yellow precipitate of 5-bromo-6-methyl-3-nitroanthranilic acid (**69**; 5.1 g, 97% yield) was collected and washed with water. A sample was crystallized from EtOAc/petroleum ether as orange needles: mp 205–206 °C; ^{1}H NMR (CD_3SOCD_3) δ 8.29 (s, 1 H, H-4), 7.33 (br s, 2 H, NH_2), 2.45 (s, 3 H, Me). Anal. ($C_8H_7BrN_2O_4$) C, H, N. The crude acid was dissolved in 2.5 equiv of dilute aqueous NaOH and hydrogenated over Pd/C to effect debromination and nitro-group reduction. The resulting crude 2,3-diamino-6-methylbenzoic acid was condensed with benzaldehyde by the method detailed above to give 2-phenyl-5-methyl-1*H*-benzimidazole-4-carboxylic acid (**57**).

A similar sequence from 5-bromo-6-methylisatin[24] gave 2-phenyl-7-methyl-1*H*-benzimidazole-4-carboxylic acid (**59**): mp 304–306 °C; ^{1}H NMR (CD_3SOCD_3) δ (12.10 (s, 1 H, COOH), 8.50–6.90 (m, 6 H, phenyl protons and NH), 7.75 (d, *J* = 7.5 Hz, 1 H, H-5), 7.25 (d, *J* = 7.5 Hz, 1 H, H-6), 2.65 (s, 3 H, Me). 2-Phenyl-6-methyl-1*H*-benzimidazole-4-carboxylic acid (**58**) was prepared similarly from 5-methyl-3-nitroanthranilic acid:[25] mp 298–300 °C; ^{1}H NMR (CD_3SOCD_3) δ 8.90–7.00 (m, 8 H, aromatic protons and NH), 2.52 (s, 3 H, Me).

Preparation of 1-Methyl-2-phenyl-1*H*-benzimidazole-4-carboxylic Acid (40). A solution of 3-chloro-2-nitrobenzoic acid (10 g, 49 mmol) in 10% aqueous methylamine (100 mL) was heated at 100 °C for 4 days in a bomb. The resulting solution was evaporated to dryness under reduced pressure to remove excess methylamine, and the residue was redissolved in water. The solution pH was adjusted to 2–3 with concentrated HCl, and extraction with EtOAc gave the crude product (10 g) as a red solid containing several products by TLC. Repeated crystallization from diethyl ether gave 3-(methylamino)-2-nitrobenzoic acid (2.5 g, 26% yield). A sample was recrystallized from diisopropyl ether as red needles, mp 199.5–180 °C. Anal. ($C_8H_8N_2O_4$) C, H, N. Reduction and coupling with benzaldehyde as above gave 1-methyl-2-phenyl-1*H*-benzimidazole-4-carboxylic acid (**40**), mp 154–154.5 °C. Anal. in Table III. Reaction with *N,N*-dimethylethylenediamine as described above gave the free base of **7** as an oil: ^{1}H NMR ($CDCl_3$) δ 10.20 (t, *J* = 4.9 Hz, 1 H, CONH), 8.16 (dd, *J* = 1.2, 7.5 Hz, 1 H, H-5), 7.83 (m, 2 H, H-2′), 7.55 (m, 3 H, H-3,4′), 7.45 (dd, *J* = 1.2, 8.0 Hz, 1 H, H-7), 7.35 (dd, *J* = 7.5, 8.0 Hz, 1 H, H-6), 3.885 (s, 3 H, NMe), 3.695 and 3.68 (2 t, *J* = 6.5 Hz, 2 H, $CONHCH_2$), 2.64 (t, *J* = 6.5 Hz, 2 H, CH_2NMe_2), 2.33 (s, 6 H, NMe_2).

Similar treatment of 2-chloro-3-nitrobenzoic acid gave the known[26] 2-(methylamino)-3-nitrobenzoic acid, which was used to prepare 3-methyl-2-phenyl-1*H*-benzimidazole-4-carboxylic acid (**41**). Coupling with *N,N*-dimethylethylenediamine as above gave the free base of **6** as an oil: ^{1}H NMR ($CDCl_3$) δ 8.215 (dd, *J* = 1.0, 8.0 Hz, 1 H, H-5), 8.09 (m, 2 H, H-2′), 7.89 (m, 3 H, H-3′,4′), 7.685 (dd, J = 1.0, 7.4 Hz, 1 H, H-7), 7.575 (dd, *J* = 7.4, 8.0 Hz, 1 H, H-6), 7.49 (t, *J* = 5.0 Hz, CONH), 3.95 and 3.965 (2 t, *J* = 6.6 Hz, 2 H, $CONHCH_2$), 2.92 (t, *J* = 6.1 Hz, CH_2NMe_2), 2.64 (s, 6 H, NMe_2).

Acknowledgment. We thank Linley Fray for expert technical help and Margaret Snow for preparation of the manuscript. This work was supported by the Auckland Division of the Cancer Society of New Zealand and by the Medical Research Council of New Zealand.

(25) Cassebaum, H. *J. Prakt. Chem.* **1964**, *23*, 301.

(26) *Dictionary of Organic Compounds*; Buckingham, J., Ed.; Chapman & Hall: New York, 1982.

(27) Cain, B. F.; Atwell, G. J.; Denny, W. A. *J. Med. Chem.* **1976**, *19*, 772.

(28) Finlay, G. J.; Baguley, B. C.; Wilson, W. R. *Eur. J. Cancer Clin. Oncol.* **1986**, *22*, 655.

Analogues of Carbamyl Aspartate as Inhibitors of Dihydroorotase: Preparation of Boronic Acid Transition-State Analogues and a Zinc Chelator Carbamylhomocysteine

David H. Kinder,† Sandra K. Frank,‡ and Matthew M. Ames*

Division of Developmental Oncology Research, Department of Oncology, Mayo Clinic & Foundation, 200 First Street, S.W., Rochester, Minnesota 55905. *Received May 4, 1989*

Dihydroorotase (DHO) catalyzes the conversion of carbamyl aspartate (CA) to dihydroorotate (DO) in the de novo pyrimidine biosynthetic pathway. Few effective inhibitors of DHO have been reported, and thus blockade of this reaction has not been widely pursued as a strategy for development of antitumor agents. Utilizing two mechanism-based strategies, we have designed and prepared potential DHO inhibitor analogues of CA. One strategy replaced the γ-carboxyl moiety of CA with a boronic acid. This substitution yields compounds which form stable charged tetrahedral intermediates and mimic the enzyme–substrate transition state. Preparation of the boronic acid analogues of CA and its carboxylic acid esters focused on a Curtius rearrangement as a key step following a malonic ester synthesis. This was followed by carbamoylation of the free amine under nonaqueous neutral conditions with $Si(NCO)_4$. The ethyl ester was a competitive inhibitor of DHO with an apparent K_i of 5.07 μM, while the nonesterified analogue and the methyl ester were not effective inhibitors. None of the compounds were cytotoxic against L1210 cells in culture. An active-site-directed sulfhydryl-containing zinc chelator was also prepared. This analogue irreversibly inhibited the enzyme, but it also was ineffective in L1210 growth inhibition.

Inhibitors of pyrimidine and purine biosynthetic enzymes are useful antineoplastic agents.[1] Of the pyrimidine biosynthetic enzymes, dihydroorotase (DHO, EC 3.5.2.3, L-5,6-dihydroorotate amidohydrolase) has been least explored as a target for antimetabolite antitumor agents because of the paucity of effective DHO inhibitors. DHO catalyzes conversion of *N*-carbamylaspartic acid (CA) to dihydroorotate (DO, Figure 1) in the third step of de novo pyrimidine biosynthesis. In mammalian cells DHO activity resides on the multifunctional protein abbreviated CAD. The reversible cyclization of CA to DO catalyzed by DHO proceeds through a reactive tetrahedral transition state.[2] Kelly et al.[3] reported that mammalian DHO contains one

†Current address: College of Pharmacy, Washington State University, Pullman, WA 99164.

‡Current address: Abbott Laboratories, Abbott Park, IL 60060.

(1) Kinsler, J. W.; Cooney, D. A. *Adv. Pharm. Chem. Ther.* **1981**, *18*, 273.

(2) Christopherson, R. I.; Jones, M. E. *J. Biol. Chem.* **1980**, *255*, 3358.

0022-2623/90/1833-0819$02.50/0