Inhibition of Nucleic Acid Synthesis in Tumor Cells in Vitro. DNA and RNA synthesis in P388 and HT-29 cell lines was monitored in the presence of drugs, and the rates of inhibition of their synthesis were estimated from the amounts of [2-^{14}C]-uridine and [^{3}H]thymidine into RNA and DNA, respectively, according to the methods described previously.[4]

In brief, for HT-29 colon carcinoma monolayer cells, 10^4 cells in each of five wells per concentration, were incubated with radiolabeled nucleosides (0.5 μCi each) for the last hour of a 2-h drug exposure, following which the cells were aspirated and washed with buffered medium. Cells were trypsinized and counted in the Coulter counter. The cell suspensions were then precipitated onto glass fiber filter discs (Whatman 934H), with 10% trichloroacetic acid. Filters were washed twice with ice-cold 10% trichloroacetic acid and twice with 95% ethanol and dried, and the incorporation of radionucleotides was estimated.

In addition, to investigate the cross contamination of radioactivity from [2-^{14}C]uridine into DNA in these cells, two sets of experiments were set up using a large number of cells (10^6–10^7 cells). After incubation with [2-^{14}C]uridine, the cells were lysed with ice-cold 1% Triton X for 2.5 min. Macromolecules were precipitated with the addition of 10% perchloric acid (5% in the medium) and centrifuged; the pellet in 1% NaCl was treated with an equal volume of phenol reagent, the emulsion was centrifuged at 10000g for 30 min at 4 °C, and the separated layer was adjusted to 1% NaCl and treated with an equal volume of 2-ethoxyethanol to precipitate the nucleic acids. The precipitate was washed with 75% ethanol and dissolved in an aqueous solution containing 2% sodium acetate and 1.5% NaCl. An aliquot was used for estimation of the total radionucleotide incorporation. Ribonuclease, previously heated to 80 °C for 10 min to destroy deoxyribonuclease activity, was added, and the mixture was incubated at 37 °C for 30 min. DNA was thus freed from RNA with the addition of 2-ethoxyethanol as in the above and was found to have minimal radioactivity (8–12% of the total incorporated); the supernatant, which contained all the RNA digest, accounted for the remainder, 87–92%, of the radioactivity found in the above mixture of nucleic acids, demonstrating only a marginal contamination in the DNA from the added [2-^{14}C]uridine.

Acknowledgment. We gratefully acknowledge David P. Rosenbaum, Department of Pharmacology, Boston University School of Medicine, for his assistance in organizing and preparing the manuscript and Mary Anne Guire for conducting the evaluation of in vivo and in vitro tests. The mouse tumors P388 leukemia, P388/Adria, L1210 leukemia, B_{16} melanoma, colon tumor C26 and C38, and human tumor xenografts, e.g., CX-1, MX-1, and LX-1, were obtained either from NCI, Bethesda, MD, or from Arthur D. Little, Cambridge, MA. We are also grateful to Albert Ross and Jacob J. Clement of Arthur D. Little for the supply of many tumor lines in mice and to Dr. Herbert Lazarus of Dana-Farber Cancer Center, Boston, MA, for providing us with a supply of CCRF-CEM cells in culture. The work was supported by the American Cancer Society (Grant CH-34G).

Supplementary Material Available: The metabolism flow sheet (Chart II), in vitro data (Table IV), in vivo data (Tables VIII, IX, and XIII), and figures depicting the electrophoretic analysis of 3′- and 5′-end ^{32}P-labeled DNA fragments are presented (7 pages). Ordering information is given on any current masthead page.

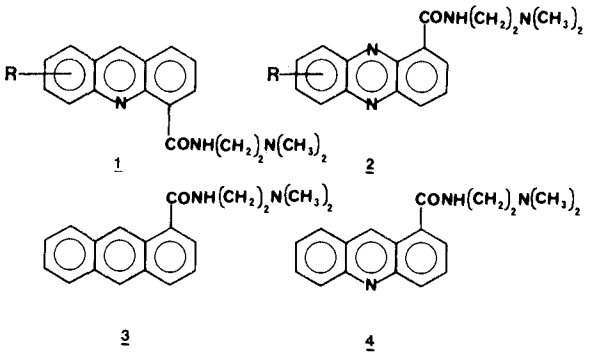
Potential Antitumor Agents. 55. 6-Phenylphenanthridine-4-carboxamides: A New Class of DNA-Intercalating Antitumor Agents

Graham J. Atwell, Bruce C. Baguley, and William A. Denny*

Cancer Research Laboratory, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand.
Received July 13, 1987

Derivatives of the DNA-intercalating agent *N*-[2-(dimethylamino)ethyl]phenanthridine-4-carboxamide (**7**) have been prepared and shown to have moderate in vivo antitumor activity against both the P388 leukemia and Lewis lung carcinoma. This demonstrates that the effective pharmacophore in the broad class of tricyclic carboxamides is not limited to linear tricyclic chromophores. Both **7** and the 6-phenyl derivative **10** have identical DNA binding properties, suggesting that the phenyl ring of **10** is not involved in the DNA intercalation site. A series of phenyl-substituted derivatives of **10** was evaluated. Aza substituents led to compounds with the highest in vivo cytotoxicity and in vivo P388 activity, but the in vivo solid tumor activity of the substituted 6-phenylphenanthridine-4-carboxamides was in general low.

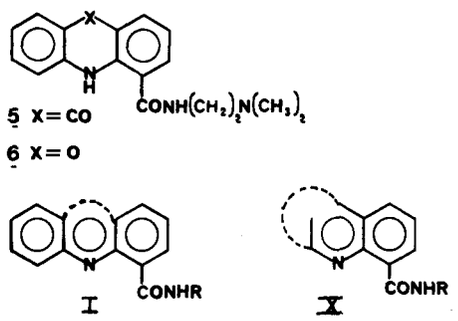
Recent studies[1–4] have identified a number of linear, tricyclic carboxamides as DNA-intercalating antitumor agents. In particular, the weakly basic acridine-4-carboxamides (**1**) and phenazine-1-carboxamides (**2**) have broad-spectrum in vivo activity, with members of both series showing curative effects against both the P388 leukemia and the Lewis lung (LL) carcinoma.[2,3] In such compounds, the constitution and disposition of the carboxamide side chain has been shown to be critical.[2,5] Other work[4] has shown the necessity of having an aromatic nitrogen atom in the chromophore, peri to the carboxamide side chain; the linear, coplanar tricyclic anthracene-1-carboxamide (**3**), acridine-1-carboxamide (**4**), 9-oxoacridan-4-carboxamide (**5**), and phenoxazine-4-carboxamide (**6**) compounds are inactive,[4] although they all bind to DNA by intercalation with very similar binding constants to those of the active compounds **1** and **2**. Thus the



(1) Rewcastle, G. W.; Atwell, G. J.; Chambers, D.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1986**, *29*, 472.
(2) Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1987**, *30*, 664.
(3) Rewcastle, G. W.; Denny, W. A.; Baguley, B. C. *J. Med. Chem.* **1987**, *30*, 843.
(4) Denny, W. A.; Palmer, B. D.; Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C. *J. Med. Chem.*, in press.
(5) Atwell, G. J.; Cain, B. F.; Baguley, B. C.; Finlay, G. J.; Denny, W. A. *J. Med. Chem.* **1984**, *27*, 1481.

0022-2623/88/1831-0774$01.50/0 © 1988 American Chemical Society

effective pharmacophore in the general series of linear, tricyclic carboxamides studied so far can be envisaged as I.



However, compounds that incorporate the essential elements of this pharmacophore are not confined to linear tricyclic structures, and in this paper we report the synthesis and evaluation of a series of angular, tricyclic carboxamides, the phenanthridine-4-carboxamides, which contain the pharmacophore X and which show in vivo antitumor activity.

Chemistry

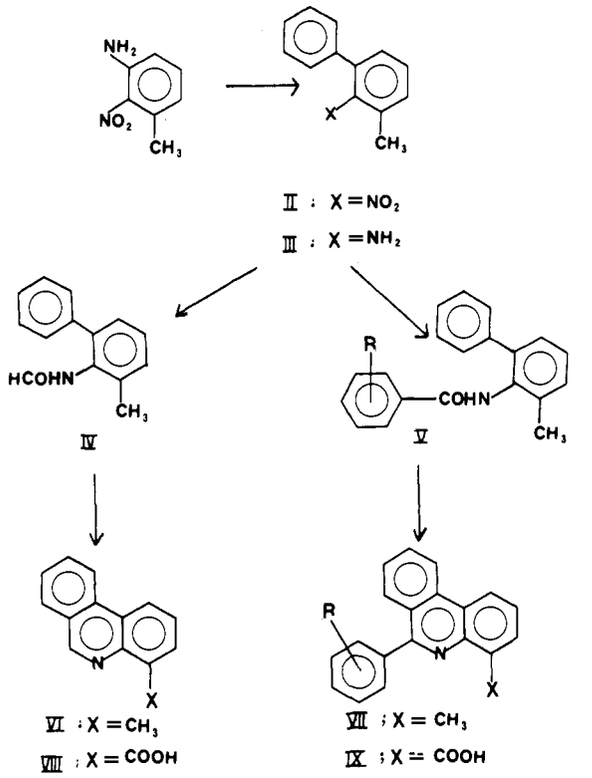
A number of routes are available for the synthesis of phenanthridines from biphenyl derivatives,[6-8] and an approach employing cyclodehydration of acylated 2-aminobiphenyl derivatives (Scheme I) was used here to obtain the required phenanthridine-4-carboxylic acids. Phenylation of 3-methyl-2-nitroaniline under the conditions employed in the Gomberg–Hey reaction[9] gave 3-methyl-2-nitrobiphenyl (II), which was reduced (Fe/H^+) to the aminobiphenyl III. Reaction of this amine with the appropriate benzoyl chloride provided the biphenylylbenzamide derivatives (V), except for the aza compounds, where utilization of a pyridinecarboxylic acid in a phosphorazo coupling[10] was preferred. Ring closure of the benzamides (V) with use of PPA or $POCl_3$/nitrobenzene gave the 4-methyl-6-phenylphenanthridines (VII), and subsequent methyl group oxidation (CrO_3/H_2SO_4 or SeO_2) provided the required 6-phenylphenanthridine-4-carboxylic acids (IX). Phenanthridine-4-carboxylic acid (VIII) was prepared from VI, the formyl derivative of III, by the same method. The acids were coupled with the appropriate amine side chains with use of 1,1′-carbonyldiimidazole[11] to give the required carboxamides of Table I.

Since this route to phenanthridine-6-carboxylic acids is limited to those compounds (VII) bearing phenyl ring substituents capable of withstanding the oxidation step, an alternative synthesis[12] providing the acid precursor at a higher oxidation level was investigated, but was unsuccessful.

Results and Discussion

Twenty-one compounds were prepared and evaluated, and the results are given in Table I. The first three (**7–9**) are a small, homologous series of unsubstituted phenanthridine-4-carboxamides, and the remainder (**10–27**) are 6-phenylphenanthridine-4-carboxamides. We were particularly interested in the effect of the additional phenyl

Scheme I



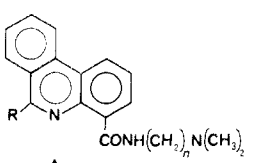
ring on the DNA binding properties of compounds (**10–27**) compared to those of compounds (**7–9**), and derivatives with substituents at each of the different phenyl positions were included in the study.

Drug lipophilicities were measured as before[13] by liquid–liquid chromatography of the compounds in the presence of 0.3% methanesulfonic acid. The parent unsubstituted phenanthridine-4-carboxamide (**7**) had an R_m value of −0.24, virtually identical with that (−0.20) of the corresponding linear isomer, the acridine-4-carboxamide (**1**, R = H).[2] Binding of the compounds to poly[d(AT)] and poly[d(GC)] was measured by the ethidium displacement assay as described previously,[14,15] and the log K values determined show that the unsubstituted phenanthridine-6-carboxamides (**7–9**) have association constants similar to the linear acridine-4-carboxamides[2] and bind much less strongly than the 9-aminoacridine-4-carboxamides.[5] As expected, **7** is a DNA-intercalating agent, showing an ability to unwind and rewind closed circular supercoiled DNA, with an unwinding angle of 12° relative to ethidium bromide. This is identical with the value found for the acridine derivative (**1**, R = H) and indicative of intercalative binding.

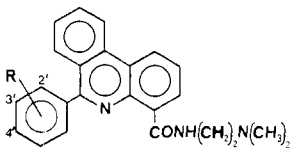
The parent phenanthridinecarboxamide **7** did not show high in vitro potency, with an IC_{50} of 2100 nM against cultured L1210 cells, compared to an IC_{50} of 105 nM for the corresponding acridine-4-carboxamide (**1**, R = H).[2] However, the compound was active in vivo at moderate potency against both the P388 leukemia and the LL carcinoma (Table I). Extending the side chain of **7** gave compounds (**8** and **9**) of similar DNA binding but lowered in vitro potency, and these proved inactive in vitro against both tumor models (Table I). Addition of the phenyl ring provided a much more lipophilic compound (**10**), which

(6) Hollingsworth, B. L.; Petrow, V. *J. Chem. Soc.* **1961**, 3771.
(7) Bartram, C. A.; Harrison, D.; Short, F. W. *J. Chem. Soc.* **1958**, 1158.
(8) Leardini, R.; Tundo, A.; Zanardi, G. *Synthesis* **1985**, 107.
(9) Bachmann, W. E.; Hoffman, R. A. In *Organic Reactions*; Vol. II Adams, R., Ed.; Wiley: New York, 1944, p 224.
(10) Cain, B. F.; Atwell, G. J.; Denny, W. A. *J. Med. Chem.* **1977**, *20*, 987.
(11) Murata, S. *Chem. Lett.* **1983**, 1819.
(12) Vaidya, N. A.; Blanton, C. D., Jr. *J. Org. Chem.* **1982**, *47*, 1777.
(13) Denny, W. A.; Cain, B. F. *J. Med. Chem.* **1978**, *21*, 430.
(14) Baguley, B. C.; Falkenhaug, E.-M. *Nucleic Acids Res.* **1978**, *5*, 161.
(15) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1981**, *24*, 170.

Table I. Physical and Biological Data for Phenanthridinecarboxamides



A



B

no.	formula	R	n	R_m[a]	pK_a[b]	DNA binding, log K[c] AT	DNA binding, log K[c] GC	cytotoxicity: L1210, IC_{50}[d]	in vivo activity: P388 OD[e]	P388 ILS_{max}[f]	LL OD	LL ILS_{max}
7	A	H	2	-0.24	2.95	5.90	5.61	2100	100	73	150	117 (1)[g]
8	A	H	3	-0.14		5.74	5.37	3590	100	NA[h]		NT[i]
9	A	H	4	-0.04		5.64	5.28	3290	100	NA		NT
10	A	Ph	2	0.21	2.75	6.26	6.33	120	45	92	65	126 (1)
11	A	Ph	3	0.25		6.50	6.11	1120	45	24	65	NA
12	A	Ph	4	0.32		6.59	6.40	1060	65	NA	45	NA
13	B	2'-aza		-0.50		5.69	5.77	720	65	20	65	
14	B	2'-Cl		0.27		6.45	6.60	2870	45	NA	NT	
15	B	3'-aza		-0.48		6.35	6.21	13	45	104	65	93 (3)
16		3'-Cl		0.43		6.41	6.56	66	65	88	100	NA
17	B	4'-aza		-0.51		6.90	7.00	14	100	93 (1)	100	NA
18	B	4'-F		0.18		7.14	6.48	43	20	63	45	45 (1)
19	B	4'-Cl		0.22	2.52	6.26	6.24	410	45	34	65	NA
20	B	4'-Br		0.22		7.04	6.76	1010	65	NA	45	NA
21	B	4'-I		0.27		7.33	7.23	2240	65	NA	65	NA
22	B	4'-OMe		0.26	3.26	6.56	6.87	500	65	22	100	NA
23	B	4'-OH		0.02		7.51	6.97	370	65	NA	65	NA
24	B	4'-NO_2		-0.05		6.41	6.54	540	65	65	65	NA
25	B	4'-NH_2		-0.41		6.29	6.57	130	30	27	45	NA
26	B	4'-NHCOMe		0.06		6.13	6.31	54	30	22	45	NA
27	B	4'-$NHSO_2Me$		-0.18		6.24	6.36	350	30	NA	20	NA

[a] R_m values were determined as detailed in ref 20, with use of 4'-(9-acridinylamino)methanesulfonanilide (AMSA) as a standard. [b] pK_a values were determined in aqueous solutions spectrophotometrically, as detailed in ref 20. [c] log K = binding constant to poly[d(A-T)] or poly[d(G-C)], determined by ethidium bromide displacement, see ref 21. [d] IC_{50} = concentration of drug in nanometers to inhibit growth of murine leukemia (L1210) or human colon tumor (HCT-8) cells in culture by 50%, following a 40-h exposure. See ref 22 and 23. [e] 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 leukemia cells or on days 5, 9, and 13 after intravenous inoculation of 10^6 Lewis lung carcinoma cells. See ref 4. [f] ILS_{max} = the percentage increase in lifespan of drug-treated tumor-bearing controls when treated at the optimal dose; values above 20% for P388 and above 40% for Lewis lung are considered statistically significant. [g] Numbers in parentheses indicate the number of animals in a group of six that were long-term survivors (50 days for P388, 60 days for LL). [h] Compound inactive at all dose levels up to toxic ones. [i] Compound not tested.

had very similar DNA binding characteristics. Bindings to poly[d(AT)] and poly[d(GC)] were similar to that of the parent compound **7**, suggesting that the additional phenyl ring is not significantly involved in the DNA interaction. The compound also binds by intercalation, with an unwinding angle of 16°. The pK_a of compound **10** was determined as 2.75, indicating that, like compound **7**, it acts as a monocation, with the chromophore uncharged under physiologic conditions. Despite the very similar DNA binding properties and much greater lipophilicity, the 6-phenylphenanthridinecarboxamide **10** showed much more potent in vitro cytotoxicity than **7**, with an IC_{50} of 120 nM. However, extension of the side chain by one methylene unit to give **11** resulted in a 10-fold drop in potency. Compound **10** also showed good in vivo activity in both tumor systems (Table I), but the homologues **11** and **12** were inactive (compound **11** showed minimal activity in the P388). The results for the two homologous series (**7–9** and **10–12**) demonstrate the importance of the side chain for activity, a criterion noted before with the 9-aminoacridine-4-carboxamides.[5] In both homologous series, addition of one methylene unit (from $(CH_2)_2$ to $(CH_2)_3$) severely reduced in vitro potency (in the case of the 6-phenyl series, by over 1 order of magnitude) and essentially abolished in vivo activity.

The effects of substituents attached to the phenyl ring were then investigated. Substituents on the phenyl ring had some influence on the pK_a (the 4'-OCH_3 derivative **22** had a pK_a of 3.26, while that of the 4'-NO_2 derivative **24** was 2.52), but the effects were too small to alter the charge distribution under physiological conditions, and all the phenyl-substituted derivatives (**11–27**) also exist as monocations. Groups at all three available phenyl positions had little effect on DNA binding, with association constants for the three isomeric Cl derivatives (**14**, **16**, and **19**) being virtually identical. Since the 2'-Cl group on (**14**) would be expected to prevent the phenyl ring from taking up an orientation coplanar with the phenanthridine chromophore, the fact that this compound binds equally as strongly as **10** by intercalation provides further evidence that the intercalation moiety is the phenanthridine, with the phenyl group protruding from the helix. Space-filling molecular models suggest that, if the compounds bind with the carboxamide side chain in the DNA minor groove (as proposed for the 9-aminoacridine-4-carboxamides[16]), with maximum possible overlap of the phenanthridine with the DNA base pairs, the phenyl ring is in an area of considerable bulk tolerance. Binding constants did increase steadily for the 4'-halo series (**18–21**), but the reason for this is not clear.

However, phenyl substituents did have significant effects on in vitro cytotoxicity, with activity falling off steadily for the 4'-halo series (**18–21**). Since electronic and lipophilic effects of these substituents are similar, some bulk intolerance for cytotoxicity at this position seemed pos-

(16) Wakelin, L. P. G.; Atwell, G. J.; Rewcastle, G. W.; Denny, W. A. *J. Med. Chem.* **1987**, *30*, 855.

sible. However, over the entire set of 12 4′-substituted compounds (**10**, **17–27**), the only substituent variable correlating with cytotoxicity at the 5% level was π (eq 1).

$$\log (1/IC_{50}) = -0.47\ (\pm 0.38)\pi - 2.46\ (\pm 0.31) \quad (1)$$

$$n = 12,\ r = 0.62,\ s = 0.51$$

Although the correlation is only moderate, it suggests that more hydrophilic compounds are desirable. However, the high cytotoxicity shown by the three aza derivatives (**13**, **15**, and **17**) cannot be due solely to this lipophilic effect, as can be seen from the residuals calculated from eq 1, and may also reflect electronic properties.

Although the 2′-Cl substituent did not affect DNA binding (see above), bulk in this critical position does seem dystherapeutic, as witnessed by the greatly different cytotoxicity of the 2′-Cl derivative **14** and the isomeric 3′-Cl and 4′-Cl compounds (**16** and **19**, respectively) and between it and the 2-aza derivative **13**.

Our interest was initially drawn to the phenanthridine-4-carboxamides because of the moderate but broad-spectrum in vivo activity shown by the parent compound **7**, and the improvement in in vitro cytotoxicity and in vivo potency shown by the 6-phenyl derivative **10** (Table I). Although the compounds did not possess the exemplary LL activity of the acridine-4-carboxamides,[3] it was of interest to determine to what extent phenyl ring modification could modulate this activity. In the event, the results have been disappointing. While the aza compounds (**13**, **15**, and **17**) all show improved in vitro cytotoxicity and in vivo P388 activity, these more polar compounds are inactive against the LL solid tumor. Of all the phenyl-substituted derivatives, only the 4′-F compound (**18**) shows LL activity, but at a lower level than the parent (**10**).

Conclusions

The nature of the pharmacophore essential for in vivo antitumor activity among tricyclic carboxamides has been further defined, as the structure X. The angular phenanthridine analogue (**7**) of the broad-spectrum acridine-4-carboxamide antitumor agent **1** (R = H) has similar physicochemical properties, DNA binding properties, and in vitro cytotoxicity and also shows broad-spectrum in vivo activity. Addition of a 6-phenyl ring to give **10** does not alter DNA binding properties, suggesting that the appended phenyl ring is not involved in the binding site. This compound also shows broad-spectrum in vivo activity, but at a moderate level and with disappointingly narrow structure–activity relationships for phenyl substituents.

Experimental Section

Where elemental analyses are indicated only by symbols of the elements, results obtained were within ±0.4% of the theoretical. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, New Zealand, under the direction of Professor A. D. Campbell. Melting points were determined on an Electrothermal apparatus with use of the supplied stem-corrected thermometer and are as read.

3-Methyl-2-nitrobiphenyl (II). A suspension of 3-methyl-2-nitroaniline (I) (56 g, 0.37 mol) in boiling water (200 mL) was treated with 12 N HCl (116 mL) and diazotized at 0 °C with $NaNO_2$ (26.2 g, 0.38 mol). Cold benzene (700 mL) was added, and the mixture was vigorously stirred and treated dropwise at 5–10 °C with KOAc (95 g) in water (120 mL). Stirring was continued at 5–10 °C for 4 h and then at room temperature for 24 h. The benzene layer was filtered and washed with 2 N aqueous NaOH and water and evaporated. The resulting oil was extracted with hot petroleum ether (bp 60–80 °C) and treated with charcoal; evaporation of the clarified solution gave the crude product as an orange solid (47.8 g, 61%). Repeated crystallization of a sample from petroleum ether (bp 40–60 °C) provided pure material as colorless needles, mp 85–85.5 °C. Anal. ($C_{13}H_{11}NO_2$) C, H, N.

Table II. Physicochemical Properties for the New Compounds of Table I

no.	mp, °C	formula	analyses
7	147–149	$C_{18}H_{19}N_3O{\cdot}2HCl$	C, H, N
8	147–150	$C_{19}H_{21}N_3O{\cdot}2HCl{\cdot}H_2O$	C,[a] H, N, Cl
9	183–186	$C_{20}H_{23}N_3O{\cdot}HCl$	C, H, N
10	122.5–123.5	$C_{24}H_{23}N_3O$	C, H, N
11	241–242	$C_{25}H_{25}N_3O{\cdot}2HCl$	C, H, N, Cl
12	189–192	$C_{26}H_{27}N_3O{\cdot}2HCl$	C, H, N, Cl
13	183–184	$C_{23}H_{22}N_4O{\cdot}HCl{\cdot}H_2O$	C, H, N, Cl
14	112–115	$C_{24}H_{22}ClN_3O{\cdot}HCl{\cdot}{}^1/_2H_2O$	C, H, N
15	250–252	$C_{23}H_{22}N_4O{\cdot}3HCl$	C, H, N, Cl
16	221–223	$C_{24}H_{22}ClN_3O{\cdot}2HCl{\cdot}H_2O$	C, H, N, Cl
17	243–245	$C_{23}H_{22}N_4O{\cdot}2HCl{\cdot}{}^1/_2H_2O$	C, H, N, Cl
18	226–228	$C_{24}H_{22}FN_3O{\cdot}2HCl$	C, H, N, Cl
19	147–149	$C_{24}H_{22}ClN_3O$	C, H, N
20	141–142	$C_{24}H_{22}BrN_3O$	C, H, N
21	145–147	$C_{24}H_{22}IN_3O{\cdot}2HCl{\cdot}{}^1/_2H_2O$	C, H, N
22	142–146	$C_{25}H_{25}N_3O_2{\cdot}2HCl{\cdot}H_2O$	C, H, N, Cl
23	277–279	$C_{24}H_{23}N_3O_2{\cdot}2HBr$	C, H, N, Br
24	189–190	$C_{24}H_{22}N_4O_3$	C, H, N
25	195–196	$C_{24}H_{24}N_4O$	C, H, N
26	215–216	$C_{26}H_{26}N_4O_2$	C, H, N
27	179–181	$C_{25}H_{26}N_4O_3S{\cdot}2HCl$	C, H, N, Cl

[a] C out by 0.5%.

2-Amino-3-methylbiphenyl (III). The above compound was reduced by use of Fe/H^+ following usual procedures.[17] The residue after filtration of iron species and removal of solvents was extracted with hot petroleum ether to give 2-amino-3-methylbiphenyl in 89% yield. A sample was purified (via the hydrochloride salt) and gave crystals from petroleum ether, mp 63.5–64 °C. Anal. ($C_{13}H_{13}N$) C, H, N.

2-Formamido-3-methylbiphenyl (IV). The above amine (4 g, 0.02 mol) was heated under reflux in excess formic acid for 20 min. Solvent was removed under vacuum, and the residue was crystallized from benzene/petroleum ether as colorless needles, mp 165–166 °C. Anal. ($C_{14}H_{13}NO$) C, H, N.

4-Methylphenanthridine (VI). The above compound (3 g, 0.014 mol) was finely powdered and heated with polyphosphoric acid (25 g) at 150 °C until a clear melt resulted. The melt was treated with excess ice-cold NH_4OH, and the residue was extracted with $CHCl_3$. Evaporation gave a solid, which was crystallized from petroleum ether as white prisms, mp 94–95 °C (lit.[18] mp 95.5 °C). Anal. ($C_{14}H_{11}N$) C, H, N.

Phenanthridine-4-carboxylic Acid (VIII). Powdered CrO_3 (12 g) was added portionwise to a hot stirred solution of 4-methylphenanthridine (3.9 g, 0.02 mol) in a mixture of concentrated H_2SO_4 (30 mL) and water (50 mL), while the temperature was kept at 100–105 °C. After being stirred for a further 30 min, the mixture was cooled and slowly neutralized with just sufficient aqueous NH_3 to precipitate the free acid. This was extracted with hot aqueous KOH and acidified (AcOH) to recover the crude product. Two recrystallizations from EtOH provided pure compound as white prisms, mp 242–243 °C (1.31 g, 29%). Anal. ($C_{14}H_9NO_2$) C, H, N.

***N*-(2-Methyl-6-phenylphenyl)benzamide** (V, R = H). A solution of 2-amino-3-methylbiphenyl (III) (9.15 g, 0.05 mol) in pyridine (15 mL) was treated with benzoyl chloride (1.05 equiv) at 0 °C and then allowed to come to room temperature over 30 min. Addition of water precipitated a solid, which was crystallized from EtOH and then benzene/petroleum ether to give pure product as white prisms (11.6 g, 81%), mp 183–184 °C. Anal. ($C_{20}H_{17}NO$) C, H, N.

Similar reactions with appropriately substituted benzoyl chlorides gave the intermediates (V) listed in Table III. The 4-aza derivative was more conveniently prepared by coupling of the amine (III) with pyridine-4-carboxylic acid, by use of PCl_3 in pyridine.

4-Methyl-6-phenylphenanthridine (VII, R = H). The above *N*-phenylbenzamide (V, R = H) (6.6 g, 0.023 mol) was finely powdered and heated with polyphosphoric acid (50 g) at 150 °C

(17) Koopman, H. *Recl. Trav. Chim. Pays-Bays* **1961**, *80*, 1075.
(18) Kenner, J.; Ritchie, W. H.; Statham, F. S. *J. Chem. Soc.* **1937**, 1169.

Table III. Substituted *N*-Phenylbenzamides (V)

R	mp, °C	formula	analyses
H	183–184	$C_{20}H_{17}NO$	C, H, N
2′-aza	89.5–90	$C_{19}H_{16}N_2O$	C, H, N
2′-Cl	150–151	$C_{20}H_{16}ClNO$	C, H, N, Cl
3′-aza	182–184	$C_{19}H_{16}N_2O$	C, H, N
3′-Cl	146–147	$C_{20}H_{16}ClNO$	C, H, N, Cl
4′-aza	178–179	$C_{19}H_{16}N_2O$	C, H, N
4′-F	173.5–174.5	$C_{20}H_{16}FNO$	C, H, N
4′-Cl	192–193	$C_{20}H_{16}ClNO$	C, H, N
4′-Br	204.5–205.5	$C_{20}H_{16}BrNO$	C, H, N
4′-I	202–204	$C_{20}H_{16}N_2O_3$	C, H, N
4′-OCH_3	201.5–202.5	$C_{21}H_{19}NO_2$	C, H, N

Table IV. Substituted 4-Methyl-6-phenylphenanthridines (VII)

R	mp, °C	formula	analyses
H	114.5–115.5	$C_{20}H_{15}N$	C, H, N
2′-aza	153–154	$C_{19}H_{14}N_2$	C, H, N
2′-Cl	135–136	$C_{20}H_{14}ClN$	C, H, N
3′-aza	168–169	$C_{19}H_{16}N_2$	C, H, N
3′-Cl	149–150	$C_{20}H_{14}ClN$	C, H, N
4′-aza	212–214	$C_{19}H_{14}N_2$	C, H, N
4′-F	164.5–165	$C_{20}H_{14}FN$	C, H, N
4′-Cl	166–167	$C_{20}H_{14}ClN$	C, H, N
4′-Br	179–180	$C_{20}H_{14}BrN$	C, H, N
4′-I	189–190	$C_{20}H_{14}IN$	C, H, N
4′-NO_2	233–235	$C_{20}H_{14}N_2O_2$	C, H, N
4′-OCH_3	178–179	$C_{21}H_{17}NO$	C, H, N

until a clear melt resulted and then for a further 15 min. The melt was treated with excess ice-cold NH_4OH, and the resulting solid was extracted with $CHCl_3$. The residue after evaporation of this was extracted with petroleum ether, and the clarified extract was evaporated to give the crude phenanthridine (5.6 g, 91%), suitable for oxidation. A sample was recrystallized from petroleum ether as colorless prisms, mp 114.5–115.5 °C. Anal. ($C_{20}H_{15}N$) C, H, N.

Similar reactions on the substituted phenylbenzamides (V) gave the corresponding phenanthridines (VII) listed in Table IV. The 4′-OCH_3 derivative was preferably obtained by carrying out the ring closure in $POCl_3$/nitrobenzene (1:2).

6-Phenylphenanthridine-4-carboxylic Acid (IX, R = H). A hot stirred solution of the preceding methylphenanthridine (VII, R = H) (25 g, 0.093 mol) in a mixture of concentrated H_2SO_4 (180 mL) and water (290 mL) was treated portionwise with powdered CrO_3 (75 g), while the temperature was kept at 95–100 °C (cooling may be necessary). The reaction mixture was stirred for a further 30 min, cooled, and diluted with water. The precipitated hydrosulfate salt was collected, washed well with water, and dissolved in hot 2 N aqueous KOH. The solution was filtered, diluted with EtOH, and then slowly neutralized hot with AcOH, allowing the free acid to separate in granular form. This was dissolved in hot CH_2Cl_2, diluted with EtOH, and treated with charcoal. The clarified solution was concentrated at the boiling point until spontaneous crystallization occurred to give the pure acid (IX, R = H) as white prisms (9.4 g, 34%), sufficiently pure for the next step. A sample crystallized from 2-ethoxyethanol had mp 230–231 °C. Anal. ($C_{20}H_{13}NO_2$) C, H, N.

Similar reactions with substituted methylphenanthridines (VII) gave the phenanthridine-4-carboxylic acids (IX) listed in Table V. The more insoluble derivatives were oxidized as a finely divided suspension in H_2SO/H_2O. Some reactions required a higher temperature (110–120 °C), and in many cases the product separated during the course of the reaction. Products were purified by crystallization from CH_2Cl_2/EtOH or DMF/EtOH in overall yields of 18–47%.

Unsuccessful Alternative Synthesis of Phenanthridine-4-carboxylic Acids (IX). *N*-(2-Carboxy-6-phenylphenyl)benzamide was prepared from 2-aminobiphenyl-3-carboxylic acid[19] by benzoylation under Schotten–Baumann conditions and was crsytallized from EtOAc, mp 213–214 °C. Anal. ($C_{20}H_{15}NO_3$) C, H, N. *N*-[2-(Methoxycarbonyl)-6-phenylphenyl]benzamide was prepared by methylation of the above benzamide with CH_2N_2 and was crystallized from petroleum ether as prisms, mp 129 °C. Anal. ($C_{21}H_{17}NO_3$) C, H, N.

The above benzamides were treated with a variety of cyclizing reagents (e.g., PPA/150 °C, PPE/100 °C, nitrobenzene / $POCl_3$/150 °C) in attempts to prepare the phenanthridine acid (IX) directly, but in all cases a nearly quantitative yield of 2,8-diphenyl-3,1-benzoxazin-4(4*H*)-one was obtained.[12] This was crystallized from EtOH as colorless needles, mp 155–157 °C. Anal. ($C_{20}H_{13}NO_2$) C, H, N.

Table V. Substituted 6-Phenylphenanthridine-4-carboxylic Acids (IX)

R	mp, °C	formula	analyses
H	230–231	$C_{20}H_{13}NO_2$	C, H, N
2′-aza	272–273	$C_{19}H_{12}N_2O_2$	C, H, N
2′-Cl	135–137	$C_{20}H_{12}ClNO_2$	C, H, N, Cl
3′-aza	276–277	$C_{19}H_{12}N_2O_2$	C, H, N
3′-Cl	251–253	$C_{20}H_{12}ClNO_2$	C, H, N, Cl
4′-aza	307–309	$C_{19}H_{12}N_2O_2$	C, H, N
4′-F	266–268	$C_{20}H_{12}FNO_2$	C, H, N, F
4′-Cl	283–285	$C_{20}H_{12}ClNO_2$	C, H, N, Cl
4′-Br	277–279	$C_{20}H_{12}BrNO_2$	C, H, N, Br
4′-I	270–272	$C_{20}H_{12}INO_2$	C, H, N, I
4′-NO_2	341–343	$C_{20}H_{12}N_2O_4$	C, H, N
4′-OCH_3	247–248	$C_{21}H_{15}NO_3$	C, H, N

Formation of the Carboxamides of Table I. General Example. *N*-[2-(Dimethylamino)ethyl]phenanthridine-4-carboxamide (Compound 7 of Table I). A mixture of phenanthridine-4-carboxylic acid (1 equiv) and 1,1′-carbonyldiimidazole (2 equiv) in dry DMF (15 mL/g of acid) was stirred at 50 °C until homogeneous. The mixture was cooled to 5 °C (imidazole may separate), *N*,*N*-dimethylethylenediamine (2.5 equiv) was added, and the mixture was kept at 20 °C for 10 min and then concentrated under vacuum to small volume. Addition of water precipitated a solid, which was washed well with water, dried, and extracted with hot petroleum ether. The hot extract was treated with charcoal, filtered, and evaporated to give the crude free base. This was crystallized from MeOH/EtOAc/HCl to give the dihydrochloride, mp 147–149 °C. Anal. (Table II).

The other compounds of Table I were similarly prepared.

***N*-[2-(Dimethylamino)ethyl]-6-(4-aminophenyl)-phenanthridine-4-carboxamide (Compound 25 of Table I).** The 4′-nitro derivative (compound 24 of Table I) was prepared by the method described above. A solution of this compound in 65% aqueous EtOH was reduced by addition of Fe and HCl. After 30 min at reflux, the hot solution was basified with NH_4OH and filtered. The filtrates were evaporated to dryness and extracted with hot EtOAc to give a crude product. Crystallization from aqueous MeOH gave the pure free base, mp 195–196 °C. Anal. (Table II). Subsequent crystallizations from MeOH/EtOAc/HCl gave the trihydrochloride salt of compound **25** as orange-red prisms, mp 186–188 °C.

***N*-[2-(Dimethylamino)ethyl]-6-[4-[(methylsulfonyl)-amino]phenyl]phenanthridine-4-carboxamide (Compound 27 of Table I).** The free base of compound **25** (0.95 g, 2.5 mmol) was dissolved in pyridine (10 mL) and treated with methanesulfonyl chloride (0.25 mL) at 0 °C. The reaction mixture was then stirred at 20 °C for 6 h, during which time the product separated as as yellow solid. This was collected by filtration and recrystallized from DMF/EtOH/H_2O, followed by crystallization from MeOH/EtOAc/HCl to give the dihydrochloride (1.04 g, 82%), mp 179–181 °C. Anal. (Table II).

A similar process using Ac_2O gave compound **26** of Table I.

Acknowledgment. We thank Cherry Grimwade for supervising the animal testing, Claudia Bos for the physicochemical measurements, and Margaret Snow for pre-

(19) Newman, M. S.; Kannan, R. *J. Org. Chem.* **1976**, *41*, 3356.
(20) Atwell, G. J.; Cain, B. F.; Denny, W. A. *J. Med. Chem.* **1977**, *20*, 1128.
(21) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1981**, *24*, 170.
(22) Baguley, B. C.; Nash, R. *Eur. J. Cancer* **1981**, *17*, 671.
(23) Finlay, G. J.; Wilson, W. R.; Baguley, B. C. *Anal. Biochem.* **1984**, *139*, 272.

paring 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.

Registry No. 7, 112421-77-9; 8, 112421-78-0; 9, 112421-79-1; 10, 112421-80-4; 11, 112421-81-5; 12, 112421-82-6; 13, 112421-83-7; 14, 112421-84-8; 15, 112421-85-9; 16, 112421-86-0; 17, 112421-87-1; 18, 112421-88-2; 19, 112421-89-3; 20, 112421-90-6; 21, 112421-91-7; 22, 112421-92-8; 23, 112421-93-9; 24, 112421-94-0; 25, 112421-95-1; 25·3HCl, 112422-04-5; 26, 112421-96-2; 27, 112421-97-3; I, 601-87-6; II, 82617-45-6; III, 14294-33-8; IV, 14294-35-0; V (R = H), 112421-98-4; V (R = 2′-aza), 112422-05-6; V (R = 2′-Cl), 112422-06-7; V (R = 3′-aza), 112422-07-8; V (R = 3′-Cl), 112422-08-9; V (R = 4′-aza), 112422-09-0; V (R = 4′-Cl), 112422-11-4; V (R = 4′-F), 112422-10-3; V (R = 4′-Br), 112422-12-5; V (R = 4′-I), 112422-13-6; V (R = 4′-NO_2), 112422-14-7; V (R = 4′-OCH_3), 112422-15-8; VI, 14294-36-1; VII (R = H), 112421-99-5; VII (R = 2′-aza), 112422-16-9; VII (R = 2′-Cl), 112422-17-0; VII (R = 3′-aza), 112422-18-1; VII (R = 3′-Cl), 112422-19-2; VII (R = 4′-aza), 112422-20-5; VII (R = 4′-Cl), 112422-22-7; VII (R = 4′-F), 112422-21-6; VII (R = 4′-Br), 112422-23-8; VII (R = 4′-I), 112422-24-9; VII (R = 4′-NO_2), 112422-25-0; VII (R = 4′-OCH_3), 112422-26-1; VIII, 104728-15-6; IX (R = H), 112422-00-1; IX (R = 2′-aza), 112422-27-2; IX (R = 2′-Cl), 112422-28-3; IX (R = 3′-aza), 112481-47-7; IX (R = 3′-Cl), 112422-29-4; IX (R = 4′-aza), 112422-30-7; IX (R = 4′-F), 112422-31-8; IX (R = 4′-Cl), 112422-32-9; IX (R = 4′-Br), 112422-33-0; IX (R = 4′-I), 112422-34-1; IX (R = 4′-NO_2), 112422-35-2; IX (R = 4′-OCH_3), 112422-36-3; C_6H_6, 71-43-2; *N*-(2-carboxy-6-phenylphenyl)benzamide, 112422-01-2; *N*-[2-(methoxycarbonyl)-6-phenylphenyl]benzamide, 112422-02-3; 2,8-diphenyl-3,1-benzoxain-4(4*H*)-one, 112422-03-4; *N*,*N*-dimethylethylenediamine, 108-00-9.

Synthesis and Antiulcer Activity of 5,11-Dihydro[1]benzoxepino[3,4-*b*]pyridines

Toshiaki Kumazawa, Hiroyuki Harakawa, Hiroyuki Obase,* Yoshimasa Oiji, Hiroshi Tanaka, Katsuichi Shuto, Akio Ishii, Tetsuo Oka, and Nobuhiro Nakamizo

Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka-ken, 411 Japan. *Received January 23, 1987*

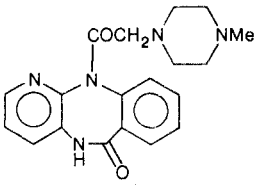
A series of substituted 5,11-dihydro[1]benzoxepino[3,4-*b*]pyridines was synthesized and evaluated for antiulcer activity in water immersion/restrained stress ulcer assay in rats. Structure–activity relationships are described. Most of the tested compounds exhibited low affinity to the muscarinic acetylcholine receptor. The molecular features for the best activities are the 2-(diethylamino)ethylenediamine group at the 5-position of the oxepin ring and an oxepin skeleton rather than a thiepin or a pyran skeleton. Methyl and chlorine substitution on the benzene ring reduced the activity. Compound 11, 5-[[2-(diethylamino)ethyl]amino]-5,11-dihydro[1]benzoxepino[3,4-*b*]pyridine trihydrochloride was selected for further evaluation. Synthesis and antiulcer activity of optically active 11 is described. There were no statistically significant differences between (+)-, (−)-, and (±)-11. Compound 11 showed weak antisecretory activity in pylorus-ligated rats. It is now under clinical evaluation as KW 5805.

Peptic ulcers have been generally thought to result from an imbalance between the aggressive forces of acid and pepsin and the defensive forces of resistance.[1] Consequently, antiulcer therapy has been directed toward these two factors. The histamine (H_2) receptor antagonists, anticholinergics, and antacids are known to lower acid secretion.

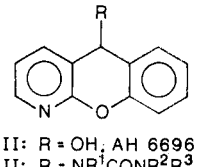
Some tricyclic compounds, I–IV, with a pyridine nucleus have been reported to inhibit gastric acid secretion in animals.[2–5] Pirenzepine is a recently introduced antimuscarinic drug with antiulcer activity.

We have prepared and tested (the objective being novel and potent antiulcer compounds with fewer side effects) a series of 5,11-dihydro[1]benzoxepino[3,4-*b*]pyridine derivatives (V) for antiulcer activity (preventive activity against gastric erosions induced by restraint and water immersion stress) and for anticholinergic effect (muscarinic receptor binding assay).

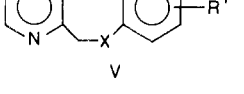
Little work has been reported on the 5,11-dihydro[1]-benzoxepino[3,4-*b*]pyridine structure, and none of the



I: pirenzepine



II: R = OH; AH 6696
III: R = $NR^1CONR^2R^3$
IV: R = $S(CH_2)_nNR^1R^2$



V

compounds possessing gastric antisecretory and/or antiulcer activities have been described.[6]

We found a novel antiulcer agent, 5-[[2-(diethylamino)ethyl]amino]-5,11-dihydro[1]benzoxepino[3,4-*b*]-pyridine trihydrochloride (compound 11) whose pharmacological profile is quite different from the known tricyclic compounds I–IV. The present compound elicits antiulcerogenic effects in various experimental models by mainly acting on the defensive mechanisms.

We report here the synthesis and antiulcer activity of 5,11-dihydro[1]benzoxepino[3,4-*b*]pyridine derivatives V.

Chemistry. The 5,11-dihydro[1]benzepino[3,4-*b*]pyridine derivatives were synthesized by several routes with

(1) Shay, H.; Sun, D. C. H. In *Gastroenterology*; Bockus, H. L., Ed.; W. B. Saunders: Philadelphia, 1974; Vol. 1, p 579.
(2) Eberlein, V. G.; Schmidt, G.; Reuter, A.; Kutter, E. *Arzneim.-Forsch.* **1977**, *27*, 356.
(3) Ashby, P.; Curwain, B. P.; Daly, M. J.; Mclsaac, R. L. *Br. J. Pharmacol.* **1976**, 440P.
(4) Bristol, J. A.; Gold, E. H.; Lovey, R. G. *J. Med. Chem.* **1981**, *24*, 927.
(5) Bristol, J. A.; Gold, E. H.; Gross, I.; Lovey, R. G. *J. Med. Chem.* **1981**, *24*, 1010.
(6) Tagawa, H.; Kubo, S.; Ishikawa, F. *Chem. Pharm. Bull.* **1981**, *29*, 3515.

0022-2623/88/1831-0779$01.50/0 © 1988 American Chemical Society