Tricyclic Heteroaromatic Systems. [1]Benzopyranopyrrol-4-ones and [1]Benzopyrano-1,2,3-triazol-4-ones as Benzodiazepine Receptor Ligands. Synthesis and Structure-Activity Relationships

Vittoria Colotta,† Lucia Cecchi,*,† Fabrizio Melani,† Guido Filacchioni,† Claudia Martini,‡,§ Gino Giannaccini,‡ and Antonio Lucacchini‡

Dipartimento di Scienze Farmaceutiche, Università di Firenze, Via Gino Capponi, 9, 50121 Firenze, Italy, Istituto Policattedra di Discipline Biologiche, Università di Pisa, Via Bonanno, 6, 56100 Pisa, Italy, and Istituto di Chimica Biologica, Università di Parma, Via Gramsci, 14, 43100 Parma, Italy. *Received November 13, 1989*

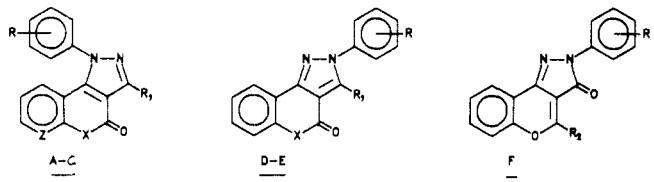
The synthesis, ability to displace [^{3}H]flunitrazepam binding from bovine brain membranes, and GABA ratio of some [1]benzopyranopyrroles **1a–i** and [1]benzopyrano-1,2,3-triazoles **2a,b** are reported. The GABA ratios of some previously synthesized pyrazoloquinolines A and [1]benzopyranopyrazoles C are also presented in order to draw some structure–activity relationships among our benzodiazepine receptor ligands. 1,3-Diarylpyrrole derivatives **1a–h** show similar affinity and efficacy to that of diazepam, while the 1-aryltriazoles **2a,b** have no receptor affinity. Comparison of the latter results with those on previously reported compounds suggests that there are several hydrophobic regions on the benzodiazepine recognition site whose occupation gives rise to different affinity and efficacy.

Introduction

Benzodiazepines (BDZ) are important therapeutic agents which have been the object of intense investigation. These drugs exert their main actions on the central nervous system (anxiolytic, hypnotic, anticonvulsant, and muscle relaxing) by interacting with special neuronal membrane proteins, benzodiazepine receptors (BZR), which are at least partly located in GABA-ergic synapses.

A number of synthetic compounds with diverse structures have been found to have affinity for BZR. The great structural differences among these non-BDZ compounds make it difficult to generalize about the molecular requirements of the recognition site of the receptor itself.

In an effort to shed light on these requirements, some research in our laboratory has been directed in recent years toward the synthesis of BZR ligands containing a six–six–five rigid tricyclic ring system. This program has led to the synthesis of about 100 compounds, all of which have been tested for their ability to displace [^{3}H]flunitrazepam from its specific binding in bovine or rat brain membranes.[1-7] The general structures of the reported compounds are shown.



A–C D–E F

R = H, Me, OMe, halogen
R_1 = Me, Ph
R_2 = H, Me

A: Z = CH; X = NH, NMe (1-arylpyrazolo[4,5-*c*]quinolin-4-ones[1-4])
B: Z = N; X = NMe (1-arylpyrazolo[4,5-*c*][1,8]naphthyridin-4-ones[5])
C: Z = CH; X = O (1-aryl[1]benzopyrano[3,4-*d*]pyrazol-4-ones[6])
D: X = NH (2-arylpyrazolo[4,3-*c*]quinolin-4-ones[1])
E: X = O (2-aryl[1]benzopyrano[4,3-*c*]pyrazol-4-ones[5])
F: 2-aryl[1]benzopyrano[4,3-*c*]pyrazol-3-ones[7])

The binding assays showed that only the 1-arylpyrazolo[4,5-*c*]quinolin-4-ones (A)[1-4] and 1-aryl[1]benzopyrano[3,4-*d*]pyrazol-4-ones (C)[6] have good affinity for the BZR, while all the others showed poor or no receptor affinity.[5,7] Moreover, the 1-aryl[1]benzopyrano[3,4-*d*]pyrazol-4-ones (C) are on the whole better ligands than the isosteres 1-arylpyrazolo[4,5-*c*]quinolin-4-ones (A).[6]

However, the binding assays are not sufficient in themselves to reveal the biological effect of BZR ligands. In fact, the biological responses of compounds which bind to the BZR range from anxiolytic for agonists (benzodiazepine-like activity), anxiogenic for inverse agonists, to a lack of any observable biological effect for antagonists.

This multiplicity of effects elicited by ligands which bind to the BZR may arise from conformational changes effected at the receptor recognition site by various ligands. It follows that structure–activity relationships are meaningful only within the class of chemicals that elicits a similar biological response.

A convenient "in vitro" method with which to roughly divide compounds interacting with the BZR into agonists, inverse agonists, or antagonists is the GABA shift or GABA ratio, i.e. the ratio between the receptor affinity of a ligand measured as the concentration of the displacer able to inhibit 50% of [^{3}H]flunitrazepam binding (IC_{50}) in the absence and in the presence of GABA. The agonist affinity is enhanced, the inverse-agonist affinity is decreased and the antagonist affinity is unaffected by the presence of GABA.[8-10] Although the three groups may overlap, the GABA ratio could be useful for predicting the pharmacological profile of BZR ligands.

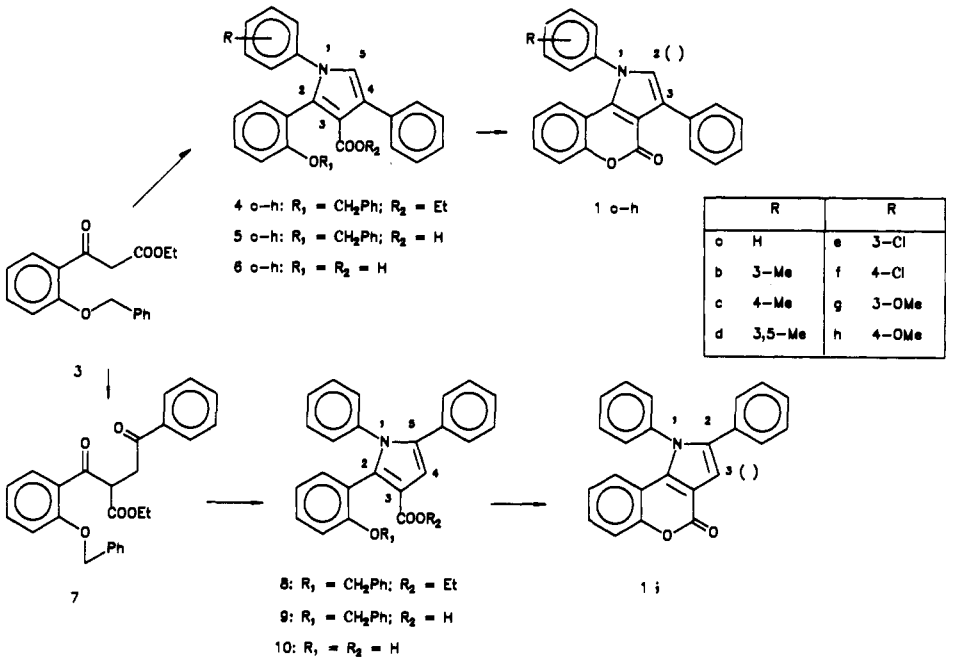
So far our synthetic work has dealt with quinolines,[1-4] naphthyridines,[5] and [1]benzopyrano derivatives[6,7] fused

† Università di Firenze.
‡ Università di Pisa.
§ Università di Parma.

(1) Cecchi, L.; Melani, F.; Palazzino, G.; Filacchioni, G.; Martini, C.; Pennacchi, E.; Lucacchini, A. *Farmaco, Ed. Sci.* **1985**, *40*, 509.
(2) Melani, F.; Cecchi, L.; Palazzino, G.; Filacchioni, G.; Martini, C.; Pennacchi, E.; Lucacchini, A. *J. Med. Chem.* **1986**, *29*, 291.
(3) Melani, F.; Cecchi, L.; Palazzino, G.; Filacchioni, G.; Martini, C.; Pennacchi, E.; Lucacchini, A. *J. Pharm. Sci.* **1986**, *75*, 1175.
(4) Palazzino, G.; Cecchi, L.; Melani, F.; Colotta, V.; Filacchioni, G.; Martini, C.; Lucacchini, A. *J. Med. Chem.* **1987**, *39*, 1737.
(5) Cecchi, L.; Colotta, V.; Filaccioni, G.; Melani, F.; Palazzino, G.; Galli, A. *Farmaco, Ed. Sci.* **1987**, *42*, 671.
(6) Colotta, V.; Cecchi, L.; Filacchioni, G.; Melani, F.; Palazzino, G.; Martini, C.; Giannaccini, G.; Lucacchini, A. *J. Med. Chem.* **1988**, *31*, 1.
(7) Colotta, V.; Cecchi, L.; Melani, F.; Palazzino, G.; Filacchioni, G.; Martini, C.; Giannaccini, G.; Lucacchini, A. *J. Pharm. Sci.* **1989**, *78*, 239.
(8) Doble, A.; Martin, I. L.; Richards, C. *Br. J. Pharmacol.* **1982**, *76*, 238P.
(9) Braestrup, C.; Schniechen, R.; Neff, G.; Nielsen, M.; Petersen, E. N. *Science* **1982**, *216*, 1241.
(10) Mohler, H.; Richards, J. C. *Nature* **1981**, *294*, 763.

0022-2623/90/1833-2646$02.50/0

Scheme I



with the pyrazole ring; at this point of our research we thought it would be of interest to test the influence of the five-membered ring on affinity and efficacy. Thus, taking the most active ligands, i.e. the 1-aryl[1]benzopyranopyrazoles, as lead compounds, we replaced the pyrazole moiety with the pyrrole or 1,2,3-triazole.

We therefore synthesized the 1-aryl[1]benzopyranopyrroles **1** and 1-aryl[1]benzopyrano-1,2,3-triazoles **2**, whose affinity (as measured by the concentration of the compound able to displace a radioligand) and efficacy (as measured by the GABA ratio) is also presented.

Chemistry

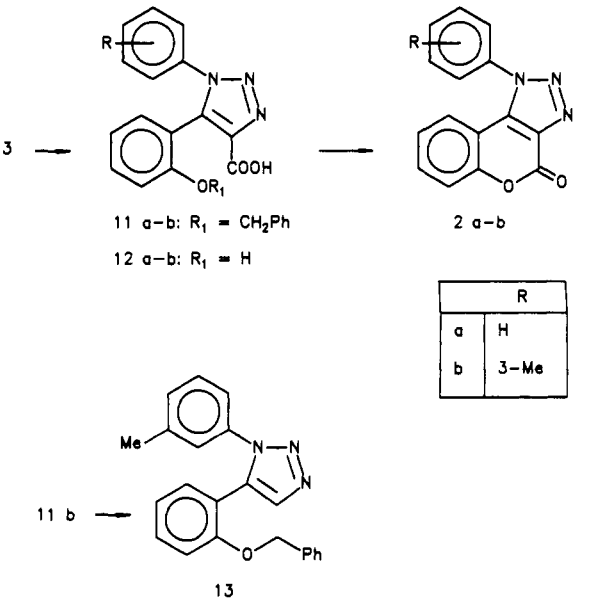
The synthesis of compounds **1a–i** was achieved by following the two synthetic pathways outlined in Scheme I.

By allowing the same starting material, i.e. ethyl 3-[2-(benzyloxy)phenyl]-3-oxopropanoate[11] (**3**), to react with different reagents, either the 1,3- or 1,2-disubstituted tricyclic derivatives **1a–h** or **1i** may be obtained.

Thus, allowing **3** to react with phenacylarylamines[12–15] in the presence of catalytic amounts of the corresponding arylamine hydrobromide and anhydrous zinc chloride gave ethyl 1-aryl-2-[2-(benzyloxy)phenyl]-4-phenylpyrrole-3-carboxylates (**4a–h**). Alkaline hydrolysis of **4a–h**, followed by acidification of the reaction mixture, gave rise to the corresponding acids **5a–h**, which were debenzylated by catalytic hydrogenation to the 1-aryl-2-(2-hydroxyphenyl)-4-phenylpyrrole-3-carboxylic acids (**6a–h**). Heating the latter at reflux with an excess of thionyl chloride yielded the tricyclic compounds **1a–h**.

By allowing **3** to react with phenacyl bromide, the β,γ-diketo ester **7** was prepared. By reacting **7** with aniline, according to Paal–Knorr's pyrrole synthesis, the ethyl 1,2,5-trisubstituted pyrrole-3-carboxylate **8** was obtained. The hydrolysis, debenzylation, and cyclization of **8**, **9**, and **10**, respectively, to **1i** was carried out following the above procedure.

Scheme II



The structures of compounds **1a–i** were confirmed by ^{13}C NMR. The ^{1}H NMR spectra were of no use in assigning the 1,3- or the 1,2-disubstituted tricyclic structure, since both the α- and the β-pyrrole proton have the same chemical shift. In the coupled ^{13}C NMR spectrum of compound **1i**, whose parent compound **8** was prepared by Paal–Knorr's unambiguous synthesis, the β-pyrrole carbon atom appeared as a doublet at 107.36 ppm ($^{1}J_{C3H3}$ = 177 Hz) while the α-pyrrole carbon atom of the corresponding compound **1a** appeared at 128.26 ppm ($^{1}J_{C2H2}$ = 189 Hz). These chemical shift and coupling constant values are consistent with the literature data on α- and β-pyrrole CH.[16,17]

(11) Charlton, J. L.; Lypka, G.; Sayeed, V. *J. Heterocycl. Chem.* **1980**, *17*, 593.
(12) Bischler, A. *Chem. Ber.* **1892**, *25*, 2865.
(13) Buu-Hoi, N. P.; Saint-Ruf, G.; Deschamps, D.; Bigot, P.; Hieu, H. T. *J. Chem. Soc. C* **1971**, 2606.
(14) Colonna, M. *Gazz. Chim. Ital.* **1948**, *78*, 502.
(15) The phenacyl(3-methoxyphenyl)amine was never synthesized. We obtained it following the method reported in ref 12. Mp: 89–90 °C (EtOH). Yield: 35%.
(16) Grehn, L. *Chem. Scr.* **1978**, *13*, 67.

It has been reported that cycloaddition of β-keto esters and substituted phenacylarylamines may give rise both to 1,2,4- and 1,2,5-pyrrole-3-carboxylates.[18,19] However, in our experience, reaction of **3** with substituted phenacylarylamines gave only the isomers 1,2,4-trisubstituted pyrrole-3-carboxylates **4b–h**, since the coupled ^{13}C NMR spectra of the final tricyclic compounds **1b–h** are similar to that of compound **1a**.

The synthesis of the [1]benzopyrano-1,2,3-triazoles **2a,b** is shown in Scheme II.

By reacting **3** with aryl azides[20,21] in sodium ethoxide, 1,2,3-triazole acids **11a,b** were directly obtained, since their corresponding esters were hydrolyzed "in situ" by heating the alkaline mixture after dilution with water followed by acidification. Hydrogenation of compounds **11a,b** and cyclization of **12a,b** led to the tricyclic derivatives **2a,b**. The structures of the latter were assigned on the ^{13}C NMR spectrum of compound **13** which ensued from the thermal decarboxylation of the acid **11b**. In the coupled ^{13}C NMR spectrum of **13** the C4 carbon atom appeared as a doublet ($^1J_{C4H4}$ = 194.2 Hz) at 134.35 ppm. These chemical shift and coupling constant values are in agreement with the literature data on 4-unsubstituted 1,2,3-triazoles.[22]

Binding to the BZR

Compounds of series 1 and 2 were tested for their ability to displace [^3H]flunitrazepam from bovine brain membranes. First, a single concentration of the compounds was examined; this was followed by examination of the IC_{50} values from log–probit plots.

Compounds which showed a low IC_{50} value, i.e. **1a–h**, were further examined "in vitro" to determine their IC_{50} values in the absence and in the presence of GABA (the GABA ratio) as to roughly differentiate agonist, inverse agonist, and antagonist compounds at the BZR recognition sites.

The IC_{50} and GABA ratio of some previously reported compounds of series A and C are also compared in Table I.

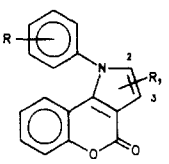
The IC_{50} values and the GABA ratios of diazepam, β-carboline (β-CCE), and Ro 15-1788 as agonist, inverse agonist, and antagonist, respectively, are also included for reference.

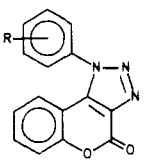
Results and Conclusions

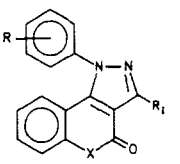
From the results shown in Table I it appears that in the 1-aryl[1]benzopyranopyrazoles the replacement of the pyrazole with the pyrrole moiety gives rise to better BZR ligands. In fact, [1]benzopyranopyrroles **1a–h** are more active than the corresponding [1]benzopyranopyrazoles C-1, C-2, and C-3 and even 1-aryl-3-phenylpyrazoloquinolines A-6 and A-7. These data are marked in compounds **1d** and **1e**, which show the same receptor affinity as diazepam.

On the other hand, the replacement of the pyrazole with the 1-aryl-1,2,3-triazole moiety in the 1-aryl-3-methyl[1]benzopyranopyrazol-4-ones C-1, C-2, and C-3 is detrimental since compounds **2a,b** are devoid of receptor affinity.

Table I. Inhibition of [^3H]Flunitrazepam Binding and GABA Ratio


1 a–i


2 a–b


A, C

no.	R	R_1	X	IC_{50},[a,b] nM	GABA ratio[c]
1a	H	3-Ph		65 ± 3	1.36
1b	3-Me	3-Ph		25 ± 2	1.16
1c	4-Me	3-Ph		172 ± 9	1.10
1d	3,5-Me	3-Ph		8.6 ± 0.7	1.15
1e	3-Cl	3-Ph		10 ± 0.9	1.28
1f	4-Cl	3-Ph		40 ± 3	1.28
1g	3-OMe	3-Ph		48 ± 3	1.16
1h	4-OMe	3-Ph		62 ± 5	1.38
1i	H	2-Ph		30 (34)[d]	
2a	H			0 (34)[d]	
2b	3-Me			18 (34)[d]	
A-1[e,f]	3-OMe	Me	NH	7500 ± 400	0.79
A-2[g]	3-Br	Me	NH	2500 ± 800	0.76
A-3[f]	3-Me	Me	NMe	260 ± 10	0.47
A-4[f]	3-Cl	Me	NMe	1000 ± 200	0.75
A-5[f]	4-Cl	Me	NMe	2700 ± 200	0.38
A-6[h]	3-Me	Ph	NMe	90 ± 5	3.60
A-7[h]	3-Cl	Ph	NMe	70 ± 2	1.36
C-1[i]	3-Me	Me	O	280 ± 10	0.23
C-2[i]	3-Br	Me	O	100 ± 9	0.66
C-3[i]	3-OMe	Me	O	750 ± 30	0.70
diazepam				13 ± 2	1.50
β-CCE				16 ± 0.8	0.75
Ro 15-1788				0.5 ± 0.02	1.00

[a] The tests were carried out with EtOH as solvent. [b] Concentrations necessary for 50% inhibition (IC_{50}) are means ± SEM of five determinations. [c] IC_{50}(compound)/IC_{50}(compound + 10 μM GABA). [d] Percentages of inhibition (*I*%) of [^3H]flunitrazepam binding at 34 μM concentration as shown in bracket. [e] See ref 1. [f] See ref 2. [g] See ref 3. [h] See ref 4. [i] See ref 6.

The important role played by the position and nature of the second substituent on the 1-aryl tricyclic ring system should be noted. When another substituent is present at position 2 (compound **1i**), there is a complete lack of receptor affinity. Instead, the presence of a second phenyl substituent at position 3 gives rise to an increase in affinity.

As regards the efficacy (as measured by the GABA ratio), the size of the second substituent at position 3 of the 1-aryl tricyclic ring system is even more relevant. The GABA ratio of 1-aryl-3-methylpyrazoloquinolines A-1, A-2, A-3, A-4, and A-5 and that of 1-aryl-3-methyl[1]benzopyranopyrazoles C-1, C-2, and C-3 are similar to that of the inverse agonist β-CCE. When the small lipophilic 3-methyl substituent of compounds A-3 and A-4 is replaced with the more lipophilic 3-phenyl (compounds A-6, A-7), we find GABA ratios which are greater than 1 and similar to that of the agonist diazepam. Similar GABA ratio values are shown by 1,3-diaryl[1]benzopyranopyrroles **1a–h** reported here.

In conclusion, it would seem to be of no importance whether the tricyclic ring system contains a quinoline or a benzopyrano moiety, while the presence and the position on the rigid system of some lipophilic substituent is instead significant. The importance of the position of the substituent(s) is suggested by the lack of receptor affinity of compound **1i**, which bears the second lipophilic moiety at position 2, as well as by the similar affinity and efficacy

(17) Begtrup, M. *Acta Chem. Scand.* **1973**, *27*, 3101.
(18) Filacchioni, G.; Porretta, G. C.; Scalzo, M. *Farmaco, Ed. Sci.* **1979**, *34*, 987.
(19) Filacchioni, G.; Porretta, G. C.; Scalzo, M.; Giardi, T. *Farmaco, Ed. Sci.* **1983**, *38*, 619.
(20) Smith, P. A. S.; Boyer, J. H. *Organic Syntheses*; John Wiley and Sons, Inc.: New York, 1963; Collect Vol. 4, p 75.
(21) Dutt, P. K.; Whitehead, H. R.; Wormall, A. *J. Chem. Soc.* **1921**, *119*, 2088. In our experience the 3-methylphenyl azide has been prepared following the method reported in ref 20.
(22) Toppet, S.; Wouters, G.; Smets, G. *Org. Magn. Res.* **1978**, *11*, 578.

of 1,3-diaryl[1]benzopyranopyrroles 1 **d,e** and 1,3-diarylpyrazoloquinolines A-6 and A-7. It follows that in the compounds of series 1 a phenyl moiety at position 2 prevents binding to the receptor site, since it falls in an inaccessible region of the receptor.[23]

Concerning the role played by the five-membered ring, the results shown in Table I give the following affinity trend: pyrrole > pyrazole > triazole.

However the comparison is true only for pyrrole and pyrazole derivatives, since both bear 1,3-diaryl substituents. The lack of affinity of the 1-aryltriazoles may be due to the absence of a lipophilic substituent at position 3. This substitution in the triazole moiety is forbidden: should the triazole ring bear a second substituent at position 3, it would no longer be aromatic and coplanar to the benzopyrano ring. Since coplanarity is a prerequisite for receptor affinity,[23] the 1,3-diaryl-1,2,3-trihydrotriazoles would be inactive.

These findings provide the medicinal chemist with very useful information on these series of rigid BZR ligands; they suggest that on the BZR recognition site there are several hydrophobic areas whose occupation may lead to different affinity and efficacy, as already reported for different kinds of ligands.[23,24]

Experimental Section

Chemistry. All melting points were determined on a Gallenkamp capillary melting point apparatus. The IR spectra were recorded with a Perkin-Elmer 1420 spectrophotometer in Nujol mull. The ^{1}H NMR spectra were run on a Varian EM 360 instrument; chemical shifts are reported in δ (ppm) downfield from internal tetramethylsilane (TMS). The natural abundance ^{13}C NMR spectra were run on a Varian FT-80A spectrometer at 20 MHz in the Fourier transform mode. All samples were recorded in 10 mm o.d. tubes at the probe temperature (30 °C) with a $CDCl_3$ concentration of approximately 10% w/v, which provided the deuterium signal for the field frequency lock. Chemical shifts were measured relative to the central peak of the solvent [$CDCl_3$ = 76.9 ppm) and corrected to internal TMS. Typical acquisition parameters included a spectral width of 5000 Hz, a flip angle of 42°, and an interpulse delay between acquisitions of 510 μs. Chemical shift values were reproducible to better than ±0.05 ppm. The decoupled spectra were obtained without pulse delay. Silica gel plates (Merck F_{254}) and silica gel 60 (Merck, 70–230 mesh) were used for analytical and column chromatography, respectively. The elemental analyses were performed for C, H, N with a Perkin-Elmer 260C elemental analyzer, and results were within ±0.4% of the theoretical values.

The physical data of the newly synthesized compounds are listed in Table II.

Ethyl 1-Aryl-2-[2-(benzyloxy)phenyl]-4-phenylpyrrole-3-carboxylates (4a–h). A suspension of ethyl 3-[2-(benzyloxy)-phenyl]-3-oxopropanoate[11] (3; 40 mmol), phenacylarylamine[12–14] (20 mmol), arylamine hydrobromide (4 mmol), and anhydrous zinc chloride (14 mmol) in dry benzene is refluxed. The reaction is followed by TLC and heating is carried on until the spot of the starting phenacylarylamine has disappeared. The cooled suspension is treated with water (20–30 mL), and the two layers are separated. The organic layer is washed twice with 6 N hydrochloric acid (15 mL each time) and then three times with 5% sodium hydroxide (20 mL each time) and finally twice with water (15 mL each time). The dried (Na_2SO_4), organic layer is brought to dryness and the residue is chromatographed on a silica gel column (eluting system cyclohexane/ethyl acetate/benzene 7:2:1 for **4a–f,h** and *n*-hexane/benzene/dichloromethane 3:4:3 for compound **4g**). The central eluates are evaporated at reduced pressure and the residue is recrystallized.

Table II. Physical Data of Newly Synthesized Compounds

no.	formula	mp, °C	crystn solv	% yield
1a	$C_{23}H_{15}NO_2$	183–184	cyclohexane/AcOEt	45
1b	$C_{24}H_{17}NO_2$	196–197	cyclohexane/AcOEt	70
1c	$C_{24}H_{17}NO_2$	153–154	cyclohexane	45
1d	$C_{25}H_{19}NO_2$	169–170	cyclohexane	50
1e	$C_{23}H_{14}ClNO_2$	212–213	AcOEt	40
1f	$C_{23}H_{14}ClNO_2$	170–171	EtOH	60
1g	$C_{24}H_{17}NO_3$	194–195	cyclohexane/AcOEt	56
1h	$C_{24}H_{17}NO_3$	227–228	acetone	40
2a	$C_{15}H_9N_3O_2$	206–207	EtOH	72
2b	$C_{16}H_{11}N_3O_2$	228–230	AcOEt	65
4a	$C_{32}H_{27}NO_3$	76–78	ligroin/2propanol	75
4b	$C_{33}H_{29}NO_3$	42–43	petroleum ether (30–50 °C)	70
4c	$C_{33}H_{29}NO_3$	90–92	ligroin/diethyl ether	74
4d	$C_{34}H_{31}NO_3$	55–56	petroleum ether (30–50 °C)	60
4e	$C_{32}H_{26}ClNO_3$	oil		35
4f	$C_{32}H_{26}ClNO_3$	127–129	EtOH	65
4g	$C_{33}H_{29}NO_4$	96–97	EtOH	25
4h	$C_{33}H_{29}NO_4$	125–126	EtOH	30
5a	$C_{30}H_{23}NO_3$	176 dec	cyclohexane	50
5b	$C_{31}H_{25}NO_3$	190 dec	cyclohexane	58
5c	$C_{31}H_{25}NO_3$	172 dec	AcOEt	50
5d	$C_{32}H_{27}NO_3$	207 dec	AcOEt	54
5e	$C_{30}H_{22}ClNO_3$	201 dec	cyclohexane/AcOEt	35
5f	$C_{30}H_{22}ClNO_3$	175 dec	EtOH	30
5g	$C_{31}H_{25}NO_4$	190 dec	cyclohexane/AcOEt	50
5h	$C_{31}H_{25}NO_4$	207 dec	cyclohexane/AcOEt	40
6a	$C_{23}H_{17}NO_3$	105 dec	cyclohexane/AcOEt	60
6b	$C_{24}H_{19}NO_3$	83 dec	cyclohexane	80
6c	$C_{24}H_{19}NO_3$	94 dec	cyclohexane	65
6d	$C_{25}H_{21}NO_3$	157 dec	cyclohexane/AcOEt	54
6e	$C_{23}H_{16}ClNO_3$	112 dec	cyclohexane	80
6f	$C_{23}H_{16}ClNO_3$	165 dec	cyclohexane/AcOEt	70
6g	$C_{24}H_{19}NO_4$	105 dec	cyclohexane/AcOEt	85
6h	$C_{24}H_{19}NO_4$	163 dec	cyclohexane/AcOEt	60
11a	$C_{22}H_{17}N_3O_3$	170 dec	cyclohexane/AcOEt	80
11b	$C_{23}H_{19}N_3O_3$	165 dec	AcOEt	65
12a	$C_{15}H_{11}N_3O_3$	202 dec	AcOEt	65
12b	$C_{16}H_{13}N_3O_3$	170 dec	AcOEt	80

Compound **4a** displayed the following. ^{1}H NMR ($CDCl_3$) δ: 0.83 (t, 3 H, CH_3, J = 7 Hz), 3.98 (q, 2 H, CH_2 ester, J = 7 Hz), 4.86 (s, 2 H, CH_2 benzyl), 6.7–7.6 (m, 20 H, 19 benzene protons + α-pyrrole proton).

1-Aryl-2-[2-(benzyloxy)phenyl]-4-phenylpyrrole-3-carboxylic Acid (5a–h). A suspension of the esters **4a–h** (4.5 mmol) and sodium hydroxide (90 mmol) in ethanol (110 mL) and water (40 mL) is refluxed for 48 h. The cooled solution is brought to dryness at reduced pressure. The residue is treated with water (70–80 mL) and washed three times with diethyl ether (20 mL each time). The aqueous layer is acidified with 6 N hydrochloric acid and then extracted three times with chloroform (30 mL each time). The dried (Na_2SO_4), organic extracts are brought to dryness at reduced pressure and the residue is recrystallized.

Compound **5a** displayed the following spectral data. IR cm^{-1}: 3200–2500, 1660. ^{1}H NMR ($CDCl_3$) δ: 4.80 (s, 2 H, CH_2 benzyl), 6.6–7.6 (m, 20 H, 19 benzene protons + α-pyrrole proton).

1-Aryl-2-(2-hydroxyphenyl)-4-phenylpyrrole-3-carboxylic Acid (6a–h). To a solution of benzyloxy acids **5a–h** (3 mmol) in ethyl acetate (200 mL) is added 10% Pd/C (30% of the weight of the benzyloxy derivative). The mixture is hydrogenated in a Parr apparatus at 40 psi for 12–36 h. The reaction is followed by TLC and hydrogenation is carried out until the spot of the starting material has disappeared. Removal of the catalyst and evaporation of the solvent afford a residue which is recrystallized.

Compound **6a** displayed the following spectral data. IR cm^{-1}: 3360, 3200–2400, 1710, 1670. ^{1}H NMR ($CDCl_3$) δ: 6.66 (s, 1 H, α-pyrrole proton), 6.8–7.7 (m, 14 H, benzene protons).

1,4-Dihydro-1-aryl-3-phenyl[1]benzopyrano[4,3-*b*]-pyrrol-4-ones (1a–h). To a suspension of the hydroxyphenyl acids **6a–h** (3 mmol) in dry diethyl ether (30 mL) is added thionyl chloride (4 mL) and the mixture is refluxed. The reaction is followed by TLC and heating is carried on until the spot of the starting material has disappeared. The solvent and the excess of thionyl chloride are distilled off at reduced pressure, and the residue is recrystallized.

Compound **1a** displayed the following spectral data. IR cm^{-1}: 1740. ^{1}H NMR ($CDCl_3$) δ: 6.8–7.0 (m, 2 H, benzene protons),

(23) Villar, H. O.; Uyeno, E. T.; Toll, L.; Polgar, W.; Davies, M. F.; Loew, G. H. *Mol. Pharmacol.* **1989**, *36*, 589.
(24) Tebib, S.; Bourguignon, J. J.; Wermuth, C. G. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 153.

7.06 (s, 1 H, α-pyrrole proton), 7.1–7.9 (m, 12 H, benzene protons). ^{13}C NMR ($CDCl_3$) ppm: 128.26 (C2, d, $^1J_{C2H2}$ = 189 Hz), 107.04 (C-3).

Ethyl 2-[2-(Benzyloxy)benzoyl]-4-phenyl-4-oxobutanoate (7). To a suspension of the potassium salt of β-keto ester **3** (10 mmol) in diethyl ether (200 mL) is added an ethereal solution (20 mL) of phenacyl bromide (10 mmol). The suspension is refluxed for 36 h, cooled, and then washed with water (50 mL). The dried (Na_2SO_4) organic layer is evaporated at reduced pressure to give an oily residue which solidifies upon recrystallization from ethyl acetate/ligroin. Mp: 64–65 °C. Yield: 70%. IR cm^{-1}: 1740, 1690, 1680. ^1H NMR ($CDCl_3$) δ: 1.10 (t, 3 H, CH_3, J = 7 Hz), 3.60 (d, 2 H, CH_2 at position 3, J = 7 Hz), 4.06 (q, 2 H, CH_2 ester, J = 7 Hz), 5.0–5.3 (m, 3 H, CH_2 benzyl + CH at position 2), 6.9–7.6 (m, 11 H, benzene protons), 7.7–8.0 (m, 3 H, benzene protons).

Ethyl 1,5-Diphenyl-2-[2-(benzyloxy)phenyl]pyrrole-3-carboxylate (8). A solution of 7 (5.5 mmol), aniline (5.5 mmol), and aniline hydrobromide (2.75 mmol) in dry ethanol (35 mL) is refluxed for 40 h and the solution is concentrated to a small volume. The resulting precipitate is collected by suction, washed with diethyl ether, and recrystallized from ligroin. Mp: 138–139 °C. Yield: 70%. IR cm^{-1}: 1690. ^1H NMR ($CDCl_3$) δ: 1.10 (t, 3 H, CH_3, J = 8 Hz), 4.14 (q, 2 H, CH_2 ester, J = 8 Hz), 4.8–4.9 (m, 2 H, CH_2 benzyl), 6.7–7.4 (m, 20 H, 19 benzene protons + β-pyrrole proton).

1,5-Diphenyl-2-[2-(benzyloxy)phenyl]pyrrole-3-carboxylic Acid (9). A suspension of 8 (4 mmol) and sodium hydroxide (80 mmol) in water (50 mL) and ethanol (80 mL) is refluxed for 12 h. The mixture is concentrated to a small volume, diluted with water (50 mL), and acidified with 6 N hydrochloric acid. The resulting precipitate is recrystallized from ethanol. Mp: 198 °C dec. Yield: 90%. IR cm^{-1}: 3600–2600, 1670. ^1H NMR (DMSO-d_6) δ: 4.8–5.0 (m, 2 H, CH_2 benzyl), 6.7–7.4 (m, 20 H, 19 benzene protons + β-pyrrole proton).

1,5-Diphenyl-2-(2-hydroxyphenyl)pyrrole-3-carboxylic Acid (10). To a warm solution of **9** (3 mmol) in ethyl acetate (200 mL) is added 10% Pd/C (500 mg). The mixture is hydrogenated in a Parr apparatus at 40 psi for 8 h. Removal of the catalyst and evaporation of the solvent afford a residue which is recrystallized from cyclohexane/ethyl acetate. Mp: 225 °C dec. Yield: 60%. IR cm^{-1}: 3560, 3200–2200, 1680, 1660. ^1H NMR (DMSO-d_6) δ: 6.4–7.3 (m, aromatic protons).

1,4-Dihydro-1,2-diphenyl[1]benzopyrano[4,3-*b*]pyrrol-4-one (1i). To a suspension of **10** (3 mmol) in diethyl ether (30 mL) is added thionyl chloride (4 mL). The mixture is refluxed for 2 h, and then the solvent and the excess of thionyl chloride are distilled off at reduced pressure. The oily residue is taken up with cyclohexane/ethyl acetate and the resulting solid is collected by suction and recrystallized from ethanol. Mp: 224–225 °C. Yield: 70%. IR cm^{-1}: 1720. ^1H NMR ($CDCl_3$) δ: 6.5–7.0 (m, 2 H, benzene protons), 7.05 (s, 1 H, β-pyrrole proton), 7.1–7.7 (m, 12 H, benzene protons); ^{13}C NMR ($CDCl_3$) ppm: 127.42 (C2), 107.36 (C3, d, $^1J_{C3H3}$ = 177 Hz).

1-Aryl-5-[2-(benzyloxy)phenyl]-1,2,3-triazole-4-carboxylic Acids (11a,b). Equimolar amounts (9.8 mmol) of β-keto ester **3**, aryl azide,[20,21] and sodium ethoxide are refluxed in dry ethanol (30 mL). The reaction is followed by TLC and the heating is carried on until the spot of the starting β-keto ester has disappeared. The reaction mixture is concentrated to a small volume at reduced pressure, diluted with water, and refluxed for another hour. The mixture is quenched with ice and water and washed three times with diethyl ether (15–20 mL each time). The aqueous layer is acidified with 6 N hydrochloric acid and extracted three times with chloroform (40 mL each time). The dried (Na_2SO_4), organic layers are evaporated at reduced pressure to give an oily residue which solidifies upon treatment with cyclohexane/ethyl acetate. The solid residue is then recrystallized.

Compound **11a** displayed the following spectral data. IR cm^{-1}: 3200–2300, 1700. ^1H NMR ($CDCl_3$) δ: 4.80 (s, 2 H, CH_2 benzyl), 6.8–7.6 (m, 14 H, benzene protons), 7.7 (br s, 1 H, COOH).

1-Aryl-5-(2-hydroxyphenyl)-1,2,3-triazole-4-carboxylic Acids (12a,b). A solution of **11a,b** (3 mmol) in ethyl acetate (200 mL), to which 10% Pd/C (30% of the weight of compounds **11a,b**) has been added, is hydrogenated in a Parr apparatus at 40 psi for 8 h. Removal of the catalyst and evaporation of the solvent at reduced pressure afford a crude residue which is recrystallized.

Compound **12a** displayed the following spectral data. IR cm^{-1}: 3500–2300, 1730. ^1H NMR ($CDCl_3$) δ: 6.6–7.0 (m, 2 H, benzene protons), 7.1–7.6 (m, 7 H, benzene protons).

1,4-Dihydro-1-aryl[1]benzopyrano[3,4-*d*]-1,2,3-triazol-4-ones (2a,b). To a suspension of **12a,b** (3 mmol) in dry diethyl ether (50 mL) is added thionyl chloride (4 mL) and the mixture is refluxed. The reaction is followed by TLC and heating is carried out until the spot of the starting material has disappeared. The solvent and excess of thionyl chloride are distilled off at reduced pressure and the residue is recrystallized.

Compound **2a** displayed the following spectral data. IR cm^{-1}: 1760. ^1H NMR ($CDCl_3$) δ: 7.1–7.4 (m, 2 H, benzene protons), 7.5–7.8 (m, 7 H, benzene protons).

1-(3-Methylphenyl)-5-[2-(benzyloxy)phenyl]-1,2,3-triazole (13). A solution of **11b** (1.9 mmol) in DMF (20 mL) is refluxed for 10 h. The solvent is evaporated at reduced pressure and the residue is treated with chloroform (30 mL). The solution is washed three times with a 5% solution of sodium bicarbonate (10 mL each time) and then with water (10 mL). The dry (Na_2SO_4) organic layer is evaporated at reduced pressure and the resulting oily residue is taken up with diethyl ether to give a solid which is recrystallized from cyclohexane/ethyl acetate. Mp 107–108 °C. Yield: 50%. ^1H NMR ($CDCl_3$) δ: 2.20 (s, 3 H, CH_3), 4.76 (s, 2 H, CH_2 benzyl), 6.7–7.4 (m, 13 H, benzene protons), 7.80 (s, 1 H, H-4); ^{13}C NMR ($CDCl_3$) ppm: 134.3 (C4, d, $^1J_{C4H4}$ = 194.2 Hz).

Binding Studies. Bovine brains were obtained from a local slaughterhouse and stored at −20 °C after cortex dissection. Membranes were prepared by homogenization in 20 vol of ice-cold 0.32 M sucrose in an Ultraturrax homogenizer for 30 s. The homogenate was centrifuged at 1000*g* for 5 min at 4 °C. The pellet was osmotically shocked by suspension in 20 vol of 50 mM Tris–HCl buffer and centrifuged at 48000*g* for 15 min at 4 °C. The homogenization media contained protease inhibitors according to Martini et al.[25] To remove the endogenous GABA, the membranes were frozen, thawed, and resuspended in 32 vol of 50 mM Tris-citrate buffer, pH 7.4, containing 0.01% Triton X-100 and protease inhibitors. They were then homogenized, incubated for 60 min at 37 °C, and centrifuged for 15 min at 48000*g* at 4 °C. The pellet was resuspended in 50 mM Tris–citrate buffer, pH 7.4, homogenized, and recentrifuged. The washing procedure was repeated three times. BZR binding activity was determined as follows: 100 μL of diluted membranes (0.4–0.5 mg of proteins) were incubated in triplicate with 0.6 nM [^3H]flunitrazepam at 0 °C (90 min) in 50 mM Tris–HCl buffer in a final volume of 500 μL. After incubation, the samples were diluted at 0 °C with 5 mL of the assay buffer and immediately filtered under reduced pressure through glass-fiber filter disks (Whatman GF/B). Afterward the samples were washed with 5 mL of the same buffer, dried, and added to 8 mL of HP Beckman scintillation liquid containing 0.4 mL of a solution 0.01 M KOH in plastic vials.

All the benzopyranopyrrole derivatives were dissolved in EtOH and added to the assay mixture. Blank experiments were carried out to determine the effect of EtOH (2%) on the binding.

Specific binding was obtained by subtracting nonspecific binding from total binding and was approximately 85–90% of the total binding. The amount of nonspecific binding was determined by incubating membranes and [^3H]flunitrazepam in the presence of 10 μM diazepam. The estimation of proteins was based on the method of Lowry et al.,[26] after membrane solubilization with 0.75 N NaOH. Bovine serum albumin was utilized as standard. Four to six concentrations of the compounds in duplicate were added to samples to determine the IC_{50} values in the absence and in the presence of 10 μM GABA, always in parallel experiments. Values shown for the GABA ratio are means of the ratio of IC_{50} without GABA to IC_{50} with GABA, added as determined in five independent experiments.

Registry No. A-1, 107677-12-3; A-2, 107677-15-6; A-3, 98990-72-8; A-4, 98990-73-9; A-5, 98990-75-1; A-6, 109334-77-2; A-7, 109363-30-6; C-1, 110570-19-9; C-2, 110570-23-5; C-3,

(25) Martini, C.; Lucacchini, A.; Ronca, G.; Hrelia, S.; Rossi, C. A. *J. Neurochem.* **1982**, *38*, 15.
(26) Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.

110570-28-0; β-CCE, 74214-62-3; Ro 15-1788, 78755-81-4; **1a**, 127791-79-1; **1b**, 127791-80-4; **1c**, 127791-81-5; **1d**, 127791-82-6; **1e**, 127791-83-7; **1f**, 127791-84-8; **1g**, 127791-85-9; **1h**, 127791-86-0; **1i**, 81263-57-2; **2a**, 127791-87-1; **2b**, 127818-96-6; **3**, 39208-08-7; **4a**, 127791-88-2; **4b**, 127791-89-3; **4c**, 127791-90-6; **4d**, 127791-91-7; **4e**, 127791-92-8; **4f**, 127791-93-9; **4g**, 127791-94-0; **4h**, 127791-95-1; **5a**, 127791-96-2; **5b**, 127791-97-3; **5c**, 127791-98-4; **5d**, 127791-99-5; **5e**, 127792-00-1; **5f**, 127792-01-2; **5g**, 127792-02-3; **5h**, 127792-03-4; **6a**, 127792-04-5; **6b**, 127792-05-6; **6c**, 127818-77-3; **6d**, 127792-06-7; **6e**, 127792-07-8; **6f**, 127792-08-9; **6g**, 127792-09-0; **6h**, 127792-10-3; **7**, 127792-11-4; **8**, 127792-12-5; **9**, 127792-13-6; **10**, 127792-14-7; **11a**, 127792-15-8; **11b**, 127792-16-9; **12a**, 127792-17-0; **12b**, 127792-18-1; **13**, 127792-19-2; diazepam, 439-14-5; phenacyl bromide, 70-11-1; aniline hydrobromide, 542-11-0.

Heterosubstituted Anthracene-9,10-dione Analogues. The Synthesis and Antitumor Evaluation of 5,8-Bis[(aminoalkyl)amino]naphtho[2,3-*b*]thiophene-4,9-diones

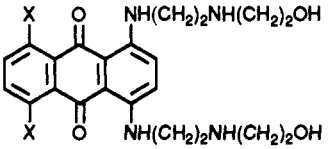
A. Paul Krapcho,* Mary E. Petry, and Miles P. Hacker

Departments of Chemistry and Pharmacology, The University of Vermont, Burlington, Vermont 05405.
Received February 1, 1990

A number of 5,8-bis[(aminoalkyl)amino]naphtho[2,3-*b*]thiophene-4,9-diones have been synthesized and evaluated for antitumor activity against L1210 leukemia both in vitro and in vivo. Two of the congeners exhibited in vivo activities quite comparable to that of mitoxantrone.

The anthracycline antibiotics daunorubicin and doxorubicin have established places in the chemotherapeutic control of cancer.[1] One severe drawback in their use is the risk of severe, dose-related cardiotoxicity.[2] The expression of the cytotoxicity of doxoxrubicin is not well-understood and it would appear that multiple pathways may exist.[3] One rationale which has been utilized for the synthesis of congeners with high antitumor and low cardiotoxic potential is based on the assumption that the cardiotoxicity, at least in part, may be associated with the formation of reactive oxygen species (radical cycling) which attack heart cell membrane lipids. The cardiac tissue, due to its relative lack of endogenous free-radical scavengers, is more susceptible to damage. This has led to the preparation and biological evaluations of chromophore-modified anthracyclines with lower reduction potentials than doxorubicin. Several of the synthetics such as 5-iminodaunorubicin[4] and 5-iminodoxorubicin[5] have been reported to have significantly reduced cardiotoxicies relative to doxorubicin. Xanthone-[6] and thioxanthone-[7] modified anthracyclines have also been studied. Attempts to prepare N^9,N^{10}-dioxides tetrahydrobenzo[*b*]phenazines have been unsuccessful.[8]

The anthracene-9,10-diones ametantrone (**1a**) and mitoxantrone (**1b**) have been shown to have outstanding antitumor activities[9] but a much narrower spectrum of activity in comparison with those of the anthracyclines. Although early studies indicated that mitoxantrone (**1b**) was noncardiotoxic, clinical cardiotoxicity has since been reported.[10]



1a: X = H
b: X = OH

In order to more fully evaluate the structure–activity relationship for ametantrone and mitoxantrone, many 9,10-dione congeners with the sidearms occupying different

(1) (a) Arcamone, F. *Doxorubicin Anticancer Antibiotics: Medicinal Chemistry*; Academic Press: New York, 1981; Vol. 17. (b) Arcamone, F. *Med. Res. Rev.* **1984**, *4*, 153. (c) Arcamone, F. *Cancer Res.* **1985**, *45*, 5995. (d) Crooke, S. T.; Reich, S. D., Eds. *Anthracyclines: Current Status and New Development*; Academic Press: New York, 1980. (e) Beckman, R. A.; McFall, P. J.; Sikic, B. I.; Smith, S. D.; *JNCI, J. Natl. Cancer Inst.* **1988**, *80*, 361. (f) Weiss, R. B.; Sarosy, G.; Clagett-Carr, K.; Russo, M.; Leyland-Jones, B. *Cancer Chemother. Pharmacol.* **1986**, *18*, 185.

(2) Speyer, J. L.; Green, M. D.; Kramer, E. *New Engl. J. Med.* **1988**, *319*, 745.

(3) (a) Fisher, J. F.; Aristoff, P. A. *Prog. Drug Res.* **1988**, *32*, 411. (b) Abdella, B. R. J.; Fisher, *J. Environ. Health Perspect.* **1985**, *64*, 3. (c) *Anthracycline and Anthracenedione-Based Anticancer Agents, Bioactive Molecules*; Lown, J. W., Ed.; Elsevier; Amsterdam, 1988; Vol. 6.

(4) (a) Tong, G. L.; Henry, D. W.; Acton, E. M. *J. Med. Chem.* **1979**, *22*, 36. (b) Lown, J. W.; Chen, H. H.; Plambeck, J. A.; Acton, E. M. *Biochem. Pharm.* **1979**, *28*, 2563. (c) Lown, J. W.; Chen, H. H.; Plambeck, J. A.; Acton, E. M. *Biochem. Pharm.* **1982**, *31*, 574. (d) Myers, C. E.; Muindi, J. R. F.; Zweiter, J.; Sinha, B. K. *J. Biol. Chem.* **1987**, *262*, 11571.

(5) (a) Acton, E. M.; Tong, G. L. *J. Med. Chem.* **1981**, *24*, 669. (b) Bird, D. M.; Boldt, M.; Koch, T. H. *J. Am. Chem. Soc.* **1987**, *109*, 4046.

(6) (a) Lown, J. W.; Sondhi, S. M.; Mandal, S. B.; Murphy, J. *J. Org. Chem.* **1982**, *47*, 4304. (b) Lown, J. W.; Sondhi, S. M. *J. Org. Chem.* **1984**, *49*, 2844. (c) Lown, J. W.; Sondhi, S. M. *J. Org. Chem.* **1985**, *50*, 1413. (d) Charlton, J. L.; Sayeed, V. A.; Lypka, G. N. *Can. J. Chem.* **1982**, *60*, 1996.

(7) Wong, C. M.; Mi, A. Q.; Ren, J.; Lam, H. Y.; Marat, K. *Can. J. Chem.* **1984**, *62*, 1600.

(8) Acton, E. M.; Tong, G. L. *J. Heterocycl. Chem.* **1981**, *18*, 1141.

(9) (a) Murdock, K. C.; Child, R. G.; Fabio, P. F.; Angier, R. B.; Wallace, R. E.; Durr, F. E.; Citarella, R. V. *J. Med. Chem.* **1979**, *22*, 1024. (b) Zee-Cheng, R. K. Y.; Cheng, C. C. *J. Med. Chem.* **1978**, *21*, 291. (c) Wallace, R. E.; Murdock, K. C.; Angier, R. B.; Durr, F. E. *Cancer Res.* **1979**, *39*, 1570. (d) Cheng, C. C.; Zee-Cheng, R. K. Y. in *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier: Amsterdam, 1983; pp 83 and references cited therein. (e) Zee-Cheng, R. K. Y.; Cheng, C. C. *Drugs Future* **1983**, *8*, 229. (f) Shenkenberg, M. D.; Von Hoff, D. D. *Ann. Int. Med.* **1986**, *105*, 67.

(10) (a) Tumminello, F. M.; Leto, G.; Gebbia, N.; Gebbia, V.; Russo, A.; Rausa, L. *Cancer Treat. Rep.* **1987**, *71*, 529. (b) Perkin, W. E.; Schroeder, R. L.; Carrano, R. A.; Imondi, A. R. *Cancer Treat. Rep.* **1984**, *68*, 841. (c) Backmann, E.; Weber, E.; Zbinden, G. *Cancer Treat. Rep.* **1987**, *71*, 361. (d) Tham, P.; Dougherty, W.; Iatropoulos, M. J.; Gordon, G.; James, V. C.; Hall, C.; Noble, J. F. *Am. J. Pathol.* **1987**, *128*, 121.

0022-2623/90/1833-2651$02.50/0