Synthesis and Anticonvulsant Activity of Some New 2-Substituted 3-Aryl-4(3*H*)-quinazolinones

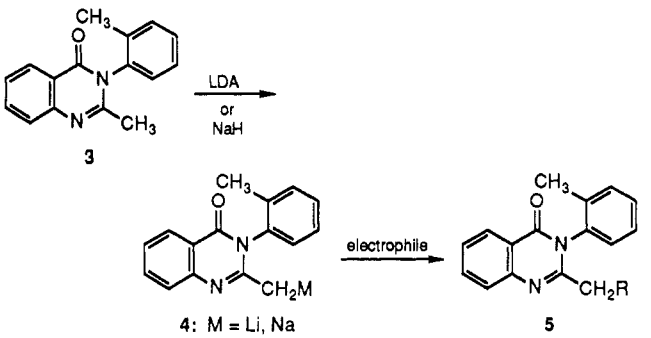
James F. Wolfe,* Terry L. Rathman, Mark C. Sleevi, James A. Campbell, and Thomas D. Greenwood

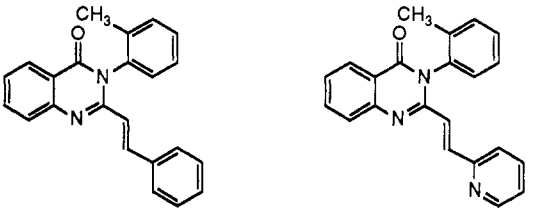
Department of Chemistry and The Harvey W. Peters Research Center for the Study of Parkinson's Disease and Disorders of the Central Nervous System, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.
Received February 24, 1989

A series of 4(3*H*)-quinazolinones structurally related to 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone (methaqualone, **3**) were synthesized and evaluated for anticonvulsant activity. Preliminary screening of these compounds revealed that 2-[2-oxo-2-(4-pyridyl)ethyl]-3-aryl-4(3*H*)-quinazolinones **6l** and **8i**, **8k**, and **8p–r** having a single ortho substituent on the 3-aryl group had the most promising anticonvulsant activity. Compounds **6l** and **8i** possessing 3-*o*-tolyl and 3-*o*-chlorophenyl groups, respectively, showed good protection against MES- and scMet-induced seizures, combined with relatively low neurotoxicity after intraperitoneal administration in mice. They also exhibited low toxicity in tests for determining the mean hypnotic dose (HD_{50}) and the median lethal dose (LD_{50}). Although these compounds were markedly more potent as anticonvulsants when administered orally in mice and rats, they were also more neurotoxic. This neurotoxicity was particularly acute in oral tests with rats, which resulted in marginal protective indices. In drug differentiation tests, compound **6l** was ineffective against seizures induced by bicuculline, picrotoxin, and strychnine, while **8i** showed some protection against picrotoxin-induced seizures.

The sedative–hypnotic (neurotoxic) properties of 4-(3*H*)-quinazolinones are well-documented.[1-13] The prototype sedative–hypnotic in this series is 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone (**3**), also known as methaqualone.[14] In spite of the fact that literally hundreds of quinazolinones related to **3** have been synthesized and tested for central nervous system (CNS) depression and anticonvulsant activity, none of the anticonvulsant drugs currently in use contain the 4(3*H*)-quinazolinone ring system. A persistent problem with such compounds arises from the fact that, to date, nearly every derivative tested in combined neurotoxicity and anticonvulsant screenings has exhibited neurotoxicity values (TD_{50}'s) that are less than, or only slightly higher than, the ED_{50}'s observed in typical anticonvulsant tests, i.e., protection against maximal electroshock (MES)[15] or subcutaneous metrazol (scMet) induced seizures.[16] Consequently, the protective index (PI), corresponding to the value TD_{50}/ED_{50}, is too low to provide sufficient differential between dosages effecting sedation and those leading to protection against seizures. Although previous attempts to uncover useful 4(3*H*)-quinazolinone anticonvulsants had proven unsuccessful, there still existed the possibility that appropriate derivatives of these CNS-active compounds, which obviously cross the blood–brain barrier, might find use as anticonvulsants if the parent ring system could be appropriately functionalized. Among the few reports in the literature of tentative separation of anticonvulsant and sedative properties of 4(3*H*)-quinazolinones, our attention was drawn to an earlier discovery by Boltze[1] that 2-(2-arylethenyl)-3-*o*-tolyl-4(3*H*)-quinazolinones **1** and **2** did indeed exhibit protection against MES-induced seizures.

Scheme I





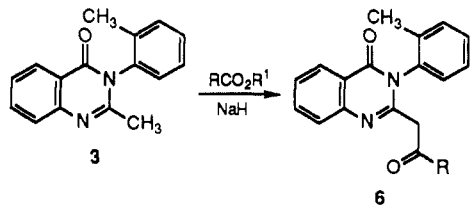
1 2

Thus, it appeared to us that considerable promise for discovering new anticonvulsants might be found through the development of new chemistry for the synthesis of structural analogues of these compounds. Consequently, we focused our initial efforts on metalating the 2-methyl group of **3** with the idea that generation of a lateral carbanion center would provide a site for facile structural modification in ways not previously achieved through traditional syntheses of 4(3*H*)-quinazolinones. Subsequent

(1) Boltze, K. H.; Dell, H. D.; Lehwald, H.; Lorenz, D.; Ruberg-Schweer, M. *Arzneim.-Forsch./Drug Res.* **1963**, *13*, 688.
(2) Gujral, M. L.; Saxona, P. N.; Tiwari, R. S. *Indian J. Med. Res.* **1955**, *43*, 637.
(3) Gupta, C. M.; Bhaduri, A. P.; Khanna, N. W. *Indian J. Chem.* **1969**, *7*, 866.
(4) Ager, I. R.; Harrison, D. R.; Kennewell, P. D.; Taylor, J. B. *J. Med. Chem.* **1977**, *20*, 379.
(5) Lata, A.; Satsangi, R. K.; Srivastava, V. K.; Kishor, K. *Arzneim.-Forsch./Drug Res.* **1982**, *32*, 24.
(6) Hussain, M. I.; Singh, E. *Pharmazie* **1982**, *37*, 408.
(7) Kornet, M. J.; Varia, T.; Beaven, W. *J. Heterocycl. Chem.* **1983**, *20*, 1553.
(8) Vaidya, N. A.; Panos, C. H.; Kite, A., Iturrian, W. B.; Blanton, C. D., Jr. *J. Med. Chem.* **1983**, *26*, 1422.
(9) Hussain, M. I.; Amir, M. *J. Chem. Soc. Pak.* **1984**, *6*, 211.
(10) Buyuktimkin, S. *Arch. Pharmacol.* **1986**, *319* (10), 933 and references cited therein.
(11) Kornet, M. J. *Eur. J. Med. Chem.-Chim. Ther.* **1986**, *21* (6), 529.
(12) Deshpande, N.; Rao, Y. V.; Kandlikar, R. P.; Rao, A. D.; Reddy, V. M. *Indian J. Pharmacol.* **1986**, *18*, 127.
(13) Saksena, R. K.; Yasmeen, R. *Indian J. Chem., Sect. B* **1986**, *25B*, 438.
(14) Kacker, I. K.; Zaheer, S. H. *J. Indian Chem. Soc.* **1951**, *28*, 344.
(15) Tulloch, I. F.; Walter, D. S.; Howe, G. M.; Howe, S. S. *J. Neuropharmacol.* **1982**, *21*, 555.
(16) (a) Anticonvulsant Screening Project, Antiepileptic Drug Development Program, National Institutes of Health, DHEW Publ (NIH) (U.S.) 1978, NIH 78-1093. (b) Porter, R. J.; Cereghino, J. J.; Gladding, G. D.; Hessie, B. J.; Kupferberg, H. J.; Scoville, B.; White, B. G. *Cleveland Clin. Q.* **1984**, *51*, 293. (c) Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. *Epilepsia* **1978**, *19*, 409.

0022-2623/90/1833-0161$02.50/0 © 1989 American Chemical Society

Table I. Acylations of Methaqualone (3) with Various Esters[a]



3 6

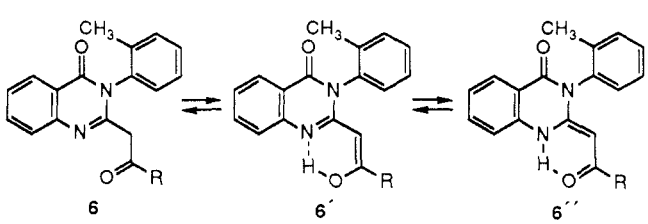
compd	R	yield, %	mp, °C	recryst solvent	anal.
6a	CH_3	61	164–165	*i*-PrOH	C, H, N
6b	CF_3	87	194–195	$CHCl_3$/*i*-PrOH	C, H, N
6c	C_6H_5	80	216–219	*i*-PrOH	C, H, N
6d	*p*-Cl-C_6H_4	74	227–227.5	$CHCl_3$/*i*-PrOH	C, H, N
6e	*p*-MeO-C_6H_4	72	169–170	*i*-PrOH	C, H, N
6f	3,4,5-$(MeO)_3C_6H_2$	67	200–201	$CHCl_3$/hexane	C, H, N
6g	*p*-(CH_3CONH)-C_6H_4	42	272–275 dec	$CHCl_3$	C, H, N
6h	*p*-(CF_3CONH)-C_6H_4	47	278–280	$CHCl_3$	C, H, N
6i	1-adamantyl	81	221–222	*i*-PrOH/$CHCl_3$/hexane	C, H, N
6j	2-pyridyl	80	254–255	$CHCl_3$/hexane	C, H, N
6k	3-pyridyl	70	234–235	$CHCl_3$/hexane	C, H, N
6l	4-pyridyl	85	219–220	$CHCl_3$/*i*-PrOH	C, H, N
6m	$C_2H_5O_2C$	62	191–191.5	*i*-PrOH	C, H, N

[a] IR and ^1H NMR spectra were consistent with the assigned structures. The ^1H NMR spectra of **6a–m** indicated that these compounds exist in $CDCl_3$ solution predominantly in the tautomeric form represented by structure 6″.

reaction of this metalated derivative **4** with various electrophiles would afford new 2-substituted 3-aryl-4(3*H*)-quinazolinones (**5**) (Scheme I). In this paper, we report the preparation and results of pharmacological testing of a series of new 4(3*H*)-quinazolinones resulting from the acylation of **4** with a variety of esters.

Chemistry

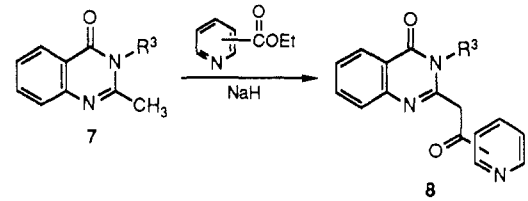
Methaqualone (**3**) was easily converted into its 2-lithiomethyl derivative (**4**, M = Li) by means of lithium diisopropylamide (LDA) in THF–hexane at 0 °C. Reactions of **4** with electrophiles such as alkyl halides, aldehydes, ketones, esters, and diphenyl disulfide afforded substituted derivatives **5**, where R corresponds to the appropriate electrophilic component of the reaction[17] (Scheme I). We also discovered that lateral metalation of **3** could be effected with sodium hydride if the metalation was carried out in the presence of an ester as the electrophilic reactant. In the absence of ester, the metalation was extremely slow. This combined metalation–acylation process permitted the synthesis of a new class of 4(3*H*)-quinazolinones, the 2-(2-oxoalkyl) derivatives, represented by tautomeric structures **6**, 6′, 6″.[17]



6 6′ 6″

Since compounds of type **6** represented a new and untested class of potential anticonvulsants and since the sodium hydride promoted acylation of **3** provided a convenient general method for their synthesis, we set out to prepare a series of such compounds for anticonvulsant assay. Furthermore, in light of the structural similarity of tautomer 6′ to compounds **1** and **2**, it seemed possible that acylation of **3** with benzoate and pyridinecarboxylate esters might provide compounds with similar or superior

Scheme II



7 8

anticonvulsant activity. Pursuant to this goal, **3** was allowed to react in the presence of excess sodium hydride with a variety of esters to afford quinazolinones **6a–m** in good yields. These results are summarized in Table I. The nature of the substituents on the benzoate esters was chosen to assess whether electron-donating and electron-withdrawing groups would affect activity according to the general approach for testing the influence of aromatic substitution on biological activity as suggested by Topliss.[18]

On the basis of initial pharmacological screening of compounds **6a–m**, another series of 3-aryl-4(3*H*)-quinazolinones containing 2-(2-oxo-2-pyridyl)ethyl groups was prepared by reaction of 2-methyl-3-aryl-4(3*H*)-quinazolinones **7** with pyridinecarboxylate esters in the presence of NaH to afford 2-(2-oxo-2-pyridylethyl)-3-aryl-4(3*H*)-quinazolinones **8** (Table II).

Pharmacological Results and Discussion

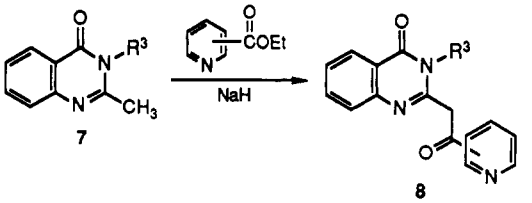
Anticonvulsant activity was evaluated in the maximal electroshock seizure (MES) and subcutaneous metrazol (scMet) tests. The rotorod[19] test was used to evaluate the level of neurotoxicity exhibited by the test compounds. Preliminary pharmacological testing (phase I) of compounds **6a–m** (Table III) revealed that only **6l** (R = 4-pyridyl) showed anticonvulsant activity at doses <300 mg/kg. Moreover, it was the only compound that was active in both MES and scMet screens, showing activity against seizures induced by MES at 100 mg/kg after 0.5 h and protection from pentylenetetrazol-induced seizures at 30 mg/kg after 0.5 h. Four other compounds in this series (**6a**, **6h**, **6j**, and **6k**) showed marginal activity against

(17) Rathman, T. L.; Sleevi, M. C.; Krafft, M. E.; Wolfe, J. F. *J. Org. Chem.* **1980**, *45*, 2169.

(18) Topliss, J. G. *J. Med. Chem.* **1972**, *15*, 1006.

(19) Collier, H. O. J.; Fieller, E. C.; Hall, R. H. *Analyst (London)* **1949**, *74*, 592.

Table II. Acylation of 2-Methyl-3-aryl-4(3*H*)-quinazolinones **7** with Pyridinecarboxylic Acid Esters



compd	R^3	pyridyl	yield, %	mp, °C	recryst solvent	anal.
8a	C_6H_5	2	76	272–273 dec	$CHCl_3$/hexane	C, H, N
8b	C_6H_5	3	72	248–249	*i*-PrOH/$CHCl_3$	C, H, N
8c	C_6H_5	4	62	241–242	$CHCl_3$/hexane	C, H, N
8d	*p*-$CH_3C_6H_4$	2	76	268–269	$CHCl_3$/hexane	C, H, N
8e	*p*-$CH_3C_6H_4$	3	81	267–268	*i*-PrOH/$CHCl_3$	C, H, N
8f	*p*-$CH_3C_6H_4$	4	84	262–263	DMSO/$CHCl_3$	C, H, N
8g	*o*-ClC_6H_4	2	82	240–241	*i*-PrOH/$CHCl_3$	C, H, N
8h	*o*-ClC_6H_4	3	79	235–237	$CHCl_3$/hexane	C, H, N
8i	*o*-ClC_6H_4	4	92	214–215	$CHCl_3$	C, H, N
8j	*m*-ClC_6H_4	4	70	234.5–235	*i*-PrOH/$CHCl_3$	C, H, N
8k	*o*-BrC_6H_4	4	70	214–215	*i*-PrOH/$CHCl_3$	C, H, N
8l	*p*-BrC_6H_4	2	96	312–313	DMSO/acetone	C, H, N
8m	*p*-BrC_6H_4	3	92	269–270	*i*-PrOH/$CHCl_3$/hexane	C, H, N
8n	*p*-BrC_6H_4	4	90	307–308 dec	DMSO/$CHCl_3$	C, H, N
8o	2,6-$Cl_2C_6H_3$	4	72	208–209	*i*-PrOH/$CHCl_3$	C, H, N
8p	*o*-FC_6H_4	4	69	212–213	*i*-PrOH/$CHCl_3$	C, H, N
8q	*o*-$CH_3OC_6H_4$	4	70	229.5–230.5	*i*-PrOH/$CHCl_3$	C, H, N
8r	*o*-IC_6H_4	4	46	221–222	*i*-PrOH/$CHCl_3$	C, H, N

[a] IR and ^1H NMR spectra were consistent with the assigned structures. The ^1H NMR spectra of **8a–r** indicated that these compounds exist in solution predominately in the enol form presented by structure 6″.

Table III. Phase I Anticonvulsant Testing of **6a–m**[a]

	MES[b,f]		scMet[c,f]		tox[d,f]	
compd	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
6a	–	–	–	+ (1/1)	–	–
6h	–	–	+ (1/1)	–	+ (1/4)	–
6j	–	–[e]	+ (1/1)	–	–	–
6k	–	–	+ (1/1)	–	–	–
6l	++ (1/1)	–	+++ (1/1)	+ (1/1)	–	–

[a] Compounds **6b–6g**, **6i**, and **6m** showed no activity or toxicity up to 300 mg/kg. [b] Maximal electroshock seizure test (number of animals protected/number of animals tested). [c] Subcutaneous pentylenetetrazol test. [d] Toxicity (number of animals exhibiting toxicity/number of animals tested). [e] 1/2 protected at 600 mg/kg. [f] Activity and toxicity at 30, 100, and 300 mg/kg are represented by +++, ++, and +, respectively; – denotes no activity or toxicity observed at 300 mg/kg.

scMet-induced seizures. Of these, **6j** (R = 2-pyridyl) and **6k** (R = 3-pyridyl) were also pyridyl-substituted. Consequently, the pyridyl moiety was tentatively identified as a pharmacophoric group, and this prompted us to synthesize a series of pyridyl-substituted quinazolinones (**8a–r**) in which the 3-aryl substituent (R^3) and the position of attachment of the pyridyl residue were varied. The results of phase I testing of these compounds are summarized in Table IV.

Examination of the test data in Tables III and IV permit some preliminary conclusions. It appears that only those compounds that have a single ortho substituent on the 3-aryl group and contain a 4-pyridyl substituent have appreciable activity. Of the compounds screened, **8i** (R^3 = *o*-chlorophenyl) emerged as the most promising candidate, exhibiting good anticonvulsant activity in the MES and scMet tests combined with low neurotoxicity. Four other compounds, **8k** and **8p–r** were also potent anticonvulsants but were much more toxic. Compounds **8i** and **8p** appear to be longer acting anticonvulsants. This is particularly true of **8p**, which exhibits activity in the scMet test at a lower dosage after 4 h than after 30 min.

Compounds **6l**, **8i**, **8k**, **8p**, and **8q** were selected for phase II screening by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS).[16] Accurate ED_{50} and TD_{50} values were determined at the time of peak anticonvulsant and sedative effect. These results, which are presented in Table V, confirm the preliminary screening data and demonstrate that these compounds are most effective against scMet-induced seizures. The most potent anticonvulsant in the scMet test, compound **8i**, showed phase II activity similar to that of phenobarbital (ED_{50} = 13.2 mg/kg) and was superior to other prototype drugs, e.g., mephenytoin (ED_{50} = 30.5 mg/kg), primidone

Table IV. Pharmacological Activity of 2-(2-Oxo-2-pyridylethyl)-3-aryl-4(3*H*)-quinazolinones **8**[c]

	MES[a]		scMet[a]		tox[a]	
compd	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
8b	–	–	+ (1/1)	–	–	–
8c	–	–	–	+ (1/1)	–	–
8h	–	–	+ (1/1)	+ (1/1)	–	+ (1/1)
8i	+ (1/1)	++ (1/1)	+++ (1/1)	++ (1/1)	–	–
8k	++ (1/1)	++ (1/1)	+++ (2/4)	+ (4/4)	+ (1/4)	+ (1/2)
8m	–	–	–	+ (1/1)	–	–
8n	–	–	+ (1/1)	–	+ (2/4)	–
8p	++ (1/1)	++ (1/1)	++ (4/4)	+++ (3/4)	+ (4/4)	–[b]
8q	++ (1/1)	+ (1/1)	+++ (1/4)	+ (1/4)	++ (1/4)	+ (2/4)
8r	++ (1/1)	–	+++ (1/4)	+ (2/4)	+++ (2/4)	+ (4/4)

[a] See footnotes *a*, *b*, and *c*, Table III. [b] 2/2 exhibited toxicity at 600 mg/kg. [c] Compounds **8a**, **8d–g**, **8j**, **8l**, and **8o** showed no activity or toxicity up to 300 mg/kg.

Table V. Phase II Quantification of Anticonvulsant Activity and Neurotoxicity

compd	ED_{50},[a,f] mg/kg ip		TD_{50},[b,f] mg/kg	PI[c]		TPE[d]	
	MES	scMet		MES	scMet	activity	toxicity
6l	148 (112–196)	100 (56–197)	561 (425–733)	3.8	5.6	2.0	2.0
8i	196 (155–240)	14.5 (5.6–24.3)	262 (125–400)	1.3	18	2.5	2.5
8k	136 (87–193)	19 (9.7–29)	>2000	>14.7	>105	1.0	*e*
8p	196 (178–217)	50 (41–70)	832 (552–1201)	4.2	16.6	2.0	1.0
8q	*e*	42 (14–70)	370 (267–471)	*e*	8.8	2.0	0.5

[a] Measured at time of peak effect. [b] Measured at time of peak neurologic deficit. [c] Protective index (TD_{50}/ED_{50}). [d] Time of peak effect, h. [e] Not determined. [f] Values in parentheses are 95% confidence intervals determined by probit analysis.

Table VI. Phase III Toxicity Profiles of **6l** and **8i**

compd	righting reflex (HD_{50}),[a] mg/kg ip	lethality test (LD_{50}),[b] mg/kg ip
6l	*c*	>4000 mg/kg[d]
8i	1181[e] (776–1781)	>3000 mg/kg[f]

[a] HD_{50} refers to the dose which causes loss of the righting reflex in 50% of the mice. [b] LD_{50} refers to the dose that causes death in 50% of the mice within 24 h. [c] 4/8 mice suffered loss of righting reflex at 4000 mg/kg after 2 h; 5/8 at 3000 mg/kg after 4 h. [d] No deaths among eight test animals at 4000 mg/kg after 24 h. [e] Measured at 24 h. [f] 1/8 mice died at 3000 mg/kg after 24 h.

(ED_{50} = 58.6 mg/kg), and ethosuximide (ED_{50} = 130.4 mg/kg).[16b] Compared to methaqualone (**3**) (ED_{50} = 33.5 mg/kg, TD_{50} = 55 mg/kg), **8i** was more effective at preventing scMet-induced seizures and was significantly less neurotoxic.

On the basis of phase II screening, **6l** and **8i** were selected by NINCDS for advanced testing. In phase V drug differentiation evaluation against bicuculline, picrotoxin, and strychnine, the test data for **6l** were disappointing, as no protection was shown against seizures induced by any of these convulsants at doses up to 600 mg/kg. However, **8i** was somewhat effective against picrotoxin-induced seizures, ED_{50} = 153 mg/kg and PI = 1.7.

Phase III toxicity profiles were determined for **6l** and **8i** (Table VI). Difficulty was experienced in clearly defining the mean hypnotic dose (HD_{50}) for these compounds since the time of peak effect and HD_{50} varied with the dose administered (see footnotes in Table VI). Median lethal doses (LD_{50}'s) were in excess of the maximum intraperitoneal (ip) doses that could be administered. Even though accurate HD_{50}'s and LD_{50}'s could not be measured, the data strongly suggest that **6l** and **8i** are relatively nontoxic.

Table VII summarizes the anticonvulsant and neurotoxicity data for **6l** and **8i** relative to oral (po) administration in mice and rats. A comparison of the median neurotoxic doses (TD_{50}'s) and median effective doses (ED_{50}'s) from Tables V and VII for ip vs po administration in mice reveals that these drugs are both more toxic and more potent by the MES and scMet tests after po administration than they are after ip administration. It is also evident from Table VII that in oral tests, these compounds are markedly more toxic and more potent in rats than in mice. Furthermore, there is no significant difference between the toxic dose and the anticonvulsant potencies of **6l** and **8i** po in rats. Thus the PI values are not as favorable as in tests with mice and are low compared to the PI's of the prototype drugs mentioned previously, which range from ca. 4 to 19.

In summary, of the 4(3*H*)-quinazolinones prepared and screened during the course of this study, 2-[2-oxo-2-(4-pyridyl)ethyl]-3-aryl-4(3*H*)-quinazolinones having a single ortho substituent on the 3-aryl moiety showed good anti-scMet activity with low neurotoxicity. Two of these compounds (**6l** and **8i**) selected for advanced testing exhibited severe neurotoxicity and low PI's following oral administration in mice and rats.

Experimental Section

A. General Procedures. Melting points were determined in open glass capillaries using either a Thomas-Hoover or Mel Temp apparatus and are uncorrected. Infrared spectra were recorded on samples as dilute solutions (chloroform or carbon tetrachloride) in matched sodium chloride cells or as potassium bromide pellets with a Beckman IR-20 AX spectrometer. All ^1H NMR spectra were recorded on a JEOL JMN-PS-100 or a Varian EM-390 spectrometer with an internal standard of tetramethylsilane. Mass spectra were taken with a Varian MAT 112 mass spectrometer. Elemental analyses (C, H, N) were performed in this laboratory under the direction of Thomas W. Glass, using a Perkin-Elmer 240 C, H, and N analyzer or by Galbraith Laboratories of Knoxville, TN; the results obtained were all within ±0.4 of the calculated percentages. Analytical thin-layer chromatography (TLC) was performed with Eastman Chromatogram Sheets (silica gel) Type 13181 containing fluorescent indicator. Spots were detected with ultraviolet light or iodine. Column chromatography was performed with Baker 70–230-mesh silica gel in standard gravity fed columns or with ICN >230-mesh silica gel with solvents delivered from a reservoir under a pressure of 10–20 psi. The following experimental procedures are representative of the general procedures used to synthesize all compounds. Experimental data for the methaqualone derivatives **6**

Table VII. Oral Anticonvulsant Activity and Neurotoxicity of **6l** and **8i** in Mice and Rats[a]

compd	test type	ED_{50}, mg/kg		TD_{50}, mg/kg	PI		TPE	
		MES	scMet		MES	scMet	activity	toxicity
6l	po in mice	27.1 (19.5–34.5)	15.0 (11.4–18.5)	67.6 (38.5–128.5)	2.5	4.5	2.0	0.5
6l	po in rats	1.73 (1.27–2.21)	2.57 (2.00–3.10)	2.89 (2.36–3.54)	1.7	1.1	4.0	4.0
8i	po in mice	14.1 (12.1–16.0)	2.53 (1.08–4.47)	19.0 (13.7–25.2)	1.3	7.5	1.0	0.5
8i	po in rats	1.44 (1.17–1.73)	3.09 (2.41–3.81)	2.91 (2.48–3.40)	2.0	.94	4.0	4.0

[a] See Table V footnotes.

and the 2-(2-oxo-2-pyridylethyl)-3-aryl-4(3*H*)-quinazolinones 8 are provided in Tables I and II, respectively.

B. 2-Methyl-3-aryl-4(3*H*)-quinazolinones. The 2-methyl-3-aryl-4(3*H*)-quinazolinones required for this study were prepared according to a modified method of Grimmel, Guenther, and Morgan.[20] The reactions were performed in a three-necked, round-bottom flask equipped with a mechanical stirrer, a reflux condenser fitted with a drying tube, an addition funnel, and a heating mantle. The procedure is described in detail for the preparation of 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone (**3**).

2-Methyl-3-*o*-tolyl-4(3*H*)-quinazolinone (3). To a vigorously stirred suspension of 24.8 g (139 mmol) of *N*-acetylanthranilic acid and 14.8 g (139 mmol) of freshly distilled *o*-toluidine in 450 mL of toluene was added dropwise 9.2 g (69 mmol) of phosphorus trichloride (PCl_3) in 25 mL of toluene over a period of 15 min. When the addition was complete, the reaction mixture was heated at reflux for 3 h and cooled to room temperature and the crude solid collected by filtration. This solid was suspended in 50 mL of water and neutralized with saturated sodium bicarbonate. An additional 250 mL of water was added and the resulting suspension was extracted twice with 200-mL portions of chloroform. The organic extract was dried over $MgSO_4$ and the solvent was removed under reduced pressure to give a viscous oil, which crystallized on standing. The crude product was recrystallized from isopropyl alcohol to yield 22.8 g (66%) of **3**, mp 114–115 °C (lit.[4] mp 115–116 °C).

2-Methyl-3-phenyl-4(3*H*)-quinazolinone (7a). From 14.0 g (150 mmol) of aniline, 27 g (150 mmol) of *N*-acetylanthranilic acid, and 9.6 g (70 mmol) of PCl_3 was obtained 25 g (71%) of **7a**, mp 143–144 °C (lit.[20] mp 145–147 °C).

2-Methyl-3-*p*-tolyl-4(3*H*)-quinazolinone (7b). From 16.0 g (150 mmol) of *p*-toluidine, 27 g (150 mmol) of *N*-acetylanthranilic acid and 9.6 g (70 mmol) of PCl_3 was obtained 27 g (77%) of **7b**, mp 147–148 °C (lit.[20] mp 147–149 °C).

2-Methyl-3-(*o*-chlorophenyl)-4(3*H*)-quinazolinone (7c). From 16.4 g (130 mmol) of *o*-chloroaniline, 21 g (120 mmol) of *N*-acetylanthranilic acid and 7.5 g (55 mmol) of PCl_3 was obtained 20 g (62%) of **7c**, mp 126–127 °C (lit.[1] mp 126–127 °C).

2-Methyl-3-(*m*-chlorophenyl)-4(3*H*)-quinazolinone (7d). From 14.1 g (112 mmol) of *m*-chloroaniline, 20 g (12 mmol) of *N*-acetylanthranilic acid and 7.69 g (56 mmol) of PCl_3 was obtained 22.28 g (73%) of **7d**, mp 132–133 °C (lit.[21] mp 129–131 °C).

2-Methyl-3-(*o*-bromophenyl)-4(3*H*)-quinazolinone (7e). From 7.58 g (44 mmol) of *o*-bromoaniline, 7.84 g (44 mmol) of *N*-acetylanthranilic acid and 9.48 g (69 mmol) of PCl_3 was obtained 4.84 g (29%) of **7e**, mp 143–144 °C (lit.[21,22] mp 148–150 °C and 142–143 °C).

2-Methyl-3-(*p*-bromophenyl)-4(3*H*)-quinazolinone (7f). From 26 g (150 mmol) of *p*-bromoaniline, 27 g (150 mmol) of *N*-acetylanthranilic acid and 10.23 g (75 mmol) of PCl_3 was obtained 42 g (88%) of **7f**, mp 168–169 °C (lit.[1] mp 171–172 °C).

2-Methyl-3-(2,6-dichlorophenyl)-4(3*H*)-quinazolinone (7g). From 18.14 g (112 mmol) of 2,6-dichloroaniline, 20 g (112 mmol) of *N*-acetylanthranilic acid and 7.69 g (56 mmol) of PCl_3 was obtained 11.24 g (33%) of **7g**, mp 138–139 °C (lit.[23] mp 136–137 °C).

2-Methyl-3-(*o*-fluorophenyl)-4(3*H*)-quinazolinone (7h). From 12.45 g (112 mmol) of *o*-fluoroaniline, 20 g (112 mmol) of *N*-acetylanthranilic acid and 7.69 g (56 mmol) of PCl_3 was obtained 20.34 g (71%) of **7h**, mp 118–119.5 °C (lit.[1] mp 116–117 °C).

2-Methyl-3-(*o*-methoxyphenyl)-4(3*H*)-quinazolinone (7i). From 13.79 g (112 mmol) of *o*-anisidine, 20 g (112 mmol) of *N*-acetylanthranilic acid and 7.69 g (56 mmol) of PCl_3 was obtained 21.1 g (71%) of **7i**, mp 132.5–133 °C (lit.[21] mp 130–132 °C).

2-Methyl-3-(*o*-iodophenyl)-4(3*H*)-quinazolinone (7j). From 10 g (46 mmol) of *o*-iodoaniline, 8.13 g (46 mmol) of *N*-acetylanthranilic acid and 3.16 g (23 mmol) of PCl_3 was obtained 7.8 g (47%) of **7j**: mp 134.5–135 °C; ^1H NMR (90 MHz, $CDCl_3$) δ 2.14 (s, 3 H, CH_3), 7.40 (m, 7 H, aromatic), 8.18 (d, J = 8 Hz, 1 H, 5-H); IR ($CHCl_3$) 1690 cm^{-1} (C=O); MS m/e 362 M^+. Anal. ($C_{15}H_{11}N_2OI$) C, H, N.

C. Acylation of 2-Methyl-3-aryl-4(3*H*)-quinazolinones. The apparatus used in these reactions consisted of a three-necked, round-bottom flask equipped with a pressure-equalizing addition funnel, a condenser attached to a water-filled gas buret (or Precision Scientific wet-test meter), a magnetic stirrer, and a heating mantle. The gas buret was separated from the system by a U-shaped drying tube charged with Drierite. To a solution of 10 mmol of 2-methyl-3-aryl-4(3*H*)-quinazolinone in 100–150 mL of anhydrous 1,2-dimethoxyethane (DME) was added 50 mmol of sodium hydride, obtained by washing 2.53 g of a 50% NaH/mineral oil dispersion with hexane. The mixture was heated to reflux and connected to the gas buret. A solution of 10 mmol of the appropriate methyl or ethyl ester dissolved in 50 mL of DME was then added dropwise at a controlled rate, taking care not to allow the reaction to become too vigorous. After addition was complete, the stirred reaction mixture was refluxed until the theoretical amount of hydrogen had evolved. When hydrogen evolution had ceased (2–10 h), the heating mantle was removed and the reaction mixture cooled to room temperature. Glacial acetic acid (3 mL) was then added (**Caution**), followed by 150 mL of cold water, and the mixture was transferred to a separatory funnel. The aqueous slurry was then extracted with chloroform (3 × 100 mL). The extracts were combined, dried over $MgSO_4$, filtered, and concentrated. The resulting residues were purified by recrystallization from appropriate solvents.

All compounds listed in Tables I and II except **6g**, **6h**, and **6m** were prepared by using this procedure. Anhydrous tetrahydrofuran (THF) was substituted for DME in the syntheses of **6g** and **6h** but in all other respects the procedure was the same. Compound **6m** was prepared by inverse addition of 5 mmol of **3** in DME to a refluxing slurry of 25 mmol of NaH and 22 mmol of diethyl oxalate in 140 mL of DME. After the reaction was complete, it was processed as previously described.

D. Pharmacology. Pharmacological testing of the new 4-(3*H*)-quinazolinones as well as methaqualone (**3**) was performed by the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, NINCDS.[16] Preliminary anticonvulsant evaluation (phase I) was conducted with at least three dosage levels (30, 100, 300 mg/kg) and in some cases a fourth dose of 600 mg/kg (see footnotes, Tables III and IV). All tests were performed with either male Carworth Farms number-one mice (in a volume of 0.01 mL/g of body weight) or Sprague–Dawley rats (in a volume of 0.01 mL/g of body weight). Test solutions of all compounds were prepared in 30% polyethylene glycol 400 and animals were dosed intraperitoneally 30 min prior to testing.

The scMet seizure threshold test was conducted by administration of the convulsant dose (CD_{97}) of pentylenetetrazol (85 mg/kg in mice, 70 mg/kg in rats) as a 0.5% solution in the posterior midline. The rotorod test was used to evaluate neurotoxicity in mice. Neurotoxicity in rats was determined by the positional sense test and gait and stance test.

In phase II testing, anticonvulsant activity and neurotoxicity ip in mice were quantitated by determining the median effective dose (ED_{50}) and the median toxic dose (TD_{50}). For the determination of ED_{50} or TD_{50}, groups of 8–16 mice were given a range of doses of the test compounds until at least three points were established in the range of 10–90% seizure protection or minimal neurotoxicity. The data was then plotted and the ED_{50}, TD_{50}, the 95% confidence interval, the slope of the regression line, and the SE of the slope were determined by means of a computer program written by NINCDS. The same procedure was used to evaluate these pharmacological parameters for oral administration of test compounds in mice and rats (phases IV and VI).

The toxicity profiles (phase III) of the test drugs were assessed by administering the TD_{50}, 2× TD_{50} and 4× TD_{50} to different pairs of mice and testing them at 10, 20, and 30 min and at 1, 2, 4, 6, 8, and 24 h for the onset, intensity, and type of overt toxicity to the central and autonomic nervous systems. These observations served as a basis for the subsequent determination of the median hypnotic dose (HD_{50}) and the 2-h median lethal dose (LD_{50}). The HD_{50} corresponds to the median dose at which 50% of the mice tested lose their righting reflex and the LD_{50} represents the median

(20) Grimmel, H. W.; Guenther, A.; Morgan, J. F. *J. Am. Chem. Soc.* **1946**, *68*, 542.
(21) Klosa, J. *J. Prakt. Chem.* **1961**, *286*, 84.
(22) Kozhevnikov, Yu. V.; Pekvunin, P. A. *Nauch. tr. Perm. Farma. Inst.* **1967**, 37; *Chem. Abstr.* **1968**, *69*, 77222f.
(23) Sociele d'Elevage de Recherches et l'Experimentation Pharmaceutique 1968, Fr.M. 6,200; *Chem. Abstr.* **1970**, *72*, 43717t.

dose that causes death in 50% of the test animals within 24 h.

Phase V testing measured the ability of the test compounds to provide protection against seizures induced by ip injection of convulsant doses (CD_{97}) of the following convulsants: bicuculline (2.70 mg/kg), picrotoxin (3.15 mg/kg), and strychnine (1.20 mg/kg). ED_{50} values were determined as in phase II at the time of peak effect.

Acknowledgment. We are pleased to acknowledge the generous financial support of this work by the Harvey W. Peters Research Center for Parkinson's Disease and Disorders of the Central Nervous System Foundation and by the National Institute of Neurological and Communicative Disorders and Stroke, Grant No. NS10197. The authors also wish to thank Gill Gladding and James Stables for providing pharmacological data through the Antiepileptic Drug Development Program, National Institutes of Health.

Registry No. **3**, 72-44-6; **6a**, 73283-07-5; **6b**, 73283-08-6; **6c**, 73283-14-4; **6d**, 73283-15-5; **6e**, 73283-16-6; **6f**, 73283-17-7; **6g**, 73283-19-9; **6h**, 73283-18-8; **6i**, 73283-12-2; **6j**, 73283-09-7; **6k**, 73283-10-0; **6l**, 73283-11-1; **6m**, 56232-60-1; **7a**, 2385-23-1; **7b**, 22316-59-2; **7c**, 340-57-8; **7d**, 340-94-3; **7e**, 4260-20-2; **7f**, 1788-95-0; **7g**, 25509-06-2; **7h**, 1897-87-6; **7i**, 4260-28-0; **7j**, 35289-03-3; **8a**, 73283-25-7; **8b**, 73283-26-8; **8c**, 73283-27-9; **8d**, 73283-29-1; **8e**, 73283-30-4; **8f**, 73283-31-5; **8g**, 73283-21-3; **8h**, 73283-22-4; **8i**, 73283-23-5; **8j**, 123382-21-8; **8k**, 123382-22-9; **8l**, 73283-33-7; **8m**, 73283-34-8; **8n**, 73283-35-9; **8o**, 123382-23-0; **8p**, 123382-24-1; **8q**, 123382-25-2; **8r**, 123382-26-3; CH_3CO_2H, 64-19-7; CF_3CO_2H, 76-05-1; $C_6H_5CO_2H$, 65-85-0; p-$ClC_6H_4CO_2H$, 74-11-3; p-$MeOC_6H_4CO_2H$, 100-09-4; 3,4,5-$(MeO)_3C_6H_2CO_2H$, 118-41-2; p-$(CH_3CONH)C_6H_4CO_2H$, 556-08-1; *N*-acetylanthranilic acid, 89-52-1; *o*-toluidine, 95-53-4; aniline, 62-53-3; *p*-toluidine, 106-49-0; *o*-chloroaniline, 95-51-2; *m*-chloroaniline, 108-42-9; *o*-bromoaniline, 615-36-1; *p*-bromoaniline, 106-40-1; 2,6-dichloroaniline, 608-31-1; *o*-fluoroaniline, 348-54-9; *o*-anisidine, 90-04-0; *o*-iodoaniline, 615-43-0; 1-adamantanecarboxylic acid, 828-51-3; 2-pyridinecarboxylic acid, 98-98-6; 3-pyridinecarboxylic acid, 59-67-6; 4-pyridinecarboxylic acid, 55-22-1; ethyl 2-pyridinecarboxylate, 2524-52-9; ethyl 3-pyridinecarboxylate, 614-18-6; ethyl 4-pyridinecarboxylate, 1570-45-2.

Supplementary Material Available. Complete anticonvulsant and toxicity screening data for all compounds submitted to the National Institute of Health's Antiepileptic Drug Development (ADD) Program protocol is available from the authors.

Structure–Activity Relationship of Anthracyclines in Vitro

D. Hoffmann,*[,†] H. G. Berscheid,[‡] D. Böttger,[‡] P. Hermentin,[†] H. H. Sedlacek,[†] and H. P. Kraemer[†]

Research Laboratories of Behringwerke AG, D-3550 Marburg/Lahn, FRG, and Department of Microbiology, Hoechst AG, D-6230 Frankfurt/Main 80, FRG. *Received December 8, 1988*

The cytotoxic activities of several natural and semisynthetic anthracyclines against L1210 leukemia and two human colon tumor cells (Colon 4, HT 29) in vitro were examined after short (1 h) and long (7 days) incubation times and correlated with the water/octanol partition coefficients and the DNA-binding affinity of the compounds. Analysis of equation in which cytotoxicity against L1210 (1-h incubation) was parabolically related to the partition coefficient revealed an almost exclusive correlation ($r = 0.80$) between the cytotoxicity and the parameters, and this correlation was only slightly improved by addition of DNA-binding affinity ($r = 0.85$). On the other hand, cytotoxic activities displayed after continuous incubation were partially related to both partition coefficients (parabolic dependence) and DNA-binding affinities (linear dependence). In this case the correlation between the activity and partition coefficient ($r = 0.67$) was significantly improved by addition of DNA-binding affinity ($r = 0.90$). Similar results were also obtained for human colon tumor cells although the corresponding correlation coefficients were generally of lower value, indicating that cytotoxic activity of anthracyclines against these primary resistant cells may be influenced by additional factors not yet determined.

Anthracyclines, especially doxorubicin (adriamycin) and daunorubicin, are of high value in today's cancer chemotherapy, showing activity against some types of leukemia, lymphoma, and soft-tissue carcinoma and also, to a lesser extent, against breast and lung cancer.[1] However, because of the dose-limiting cumulative cardiotoxicity of these compounds and their lack of activity against various kinds of tumors, especially those of the gastrointestinal tract, several anthracycline derivatives have been developed in order to overcome these drawbacks. Some of these derivatives are currently being investigated in clinical trials.[2]

Despite considerable effort, the mechanism of action of anthracyclines has not been fully clarified up to now. The cytotoxic activity was first attributed to intercalation of anthracyclines between adjacent DNA base pairs,[3] but other intracellular events, such as induction of DNA breaks,[4] generation of radicals,[5] inhibition of DNA-related enzymes such as DNA and RNA polymerase[6,7] and topoisomerase II,[8] and enhanced lipid peroxidation,[9] have been suggested to be involved in the mechanism of action of these drugs. It remains to be investigated whether the drug actually has to be taken up by cells to exert its cytotoxic activity or whether the cellular membrane is a target for anthracyclines.[10,11]

Based on analysis of the quantitative structure–activity relationships of anthracyclines, a correlation between biological activity and certain isolated parameters such as

[†] Behringwerke AG.
[‡] Hoechst AG.

(1) Carter, S. K. In *Anthracycline Antibiotics in Cancer Therapy*; Muggia, F. M.; Young, C. W.; Carter, S. K., Eds.; Martinus Nijhof Publishers: Boston, 1982; pp 471–494.
(2) Weiss, R. B.; Sarosy, G.; Clagett-Carr, K.; Russo, M.; Leyland-Jones, B. *Cancer Chemother. Pharmacol.* **1986**, *18*, 185.
(3) Calendi, A.; Di Marco, A.; Reggiani, M. *Biochim. Biophys. Acta* **1965**, *103*, 25.
(4) Potmesil, M.; Kirschenbaum, S.; Israel, M.; Levin, M.; Khetarpal, V. K.; Silber, R. *Cancer Res.* **1983**, *43*, 3528.
(5) Bachur, N. R.; Gee, M. J.; Friedman, R. C. *Cancer Res.* **1982**, *42*, 1078.
(6) Zunino, F.; Gambetta, R.; Di Marco, A.; Zaccara, A.; Luoni, G. A. *Cancer Res.* **1975**, *35*, 754.
(7) Di Marco, A.; Casazza, A. M.; Gambetta, R.; Supino, R.; Zunino, F. *Cancer Res.* **1984**, *36*, 1962.
(8) Tewey, K. M.; Rowe, T. C.; Yang, L.; Halligan, B. D.; Liu, L. F. *Science* **1984**, *226*, 466.
(9) Mimnaugh, E. G.; Kennedy, K. A.; Trush, M. A.; Sinha, B. K. *Cancer Res.* **1985**, *45*, 3296.
(10) Tritton, T. R.; Yee, G. *Science* **1982**, *217*, 248.
(11) Tokes, Z. A.; Rogers, K. E.; Rembaum, A. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 2026.

0022-2623/90/1833-0166$02.50/0 © 1989 American Chemical Society