Table IV. Hypolipidemic Effects of Indazolone Derivatives in CF_1 Male Mice at a Dose of 20 mg/(kg day) ip

	% control		
	serum cholesterol, $\bar{X} \pm$ SD		serum triglyceride, $\bar{X}$ + SD:
compd (N = 6)	day 9	day 16	day 16
1	96 ± 6	80 ± 6^a	75 ± 6^a
5	78 ± 5^a	60 ± 6^a	61 ± 4^a
23	92 ± 7	69 ± 5^a	72 ± 5^a
24	98 ± 7	71 ± 6^a	71 ± 6^a
25	92 ± 6	68 ± 5^a	77 ± 6^a
26	92 ± 7	46 ± 4^a	44 ± 3^a
27	79 ± 6^a	73 ± 6^a	77 ± 6^a
28	76 ± 6^a	74 ± 6^a	83 ± 6^b
29	98 ± 7	75 ± 5^a	69 ± 7^a
30	95 ± 8	97 ± 7	81 ± 6^a
31	97 ± 7	83 ± 8	63 ± 5^a
32	96 ± 6	83 ± 7	72 ± 5^a
33	83 ± 5^a	72 ± 7^a	58 ± 6^a
34	76 ± 6^a	75 ± 6^a	50 ± 5^a
35	86 ± 7^b	70 ± 5^a	56 ± 4^a
36	98 ± 7^a	60 ± 5^a	41 ± 3^a
37	87 ± 6^a	79 ± 6^a	76 ± 6^a
39	78 ± 8^a	46 ± 5^a	46 ± 5^a
40	88 ± 5	77 ± 3	70 ± 7
phthalimide^f	63 ± 12	57 ± 7	44 ± 8
clofibrate^g	92 ± 5	87 ± 5	75 ± 6
CM-cellulose^h	100 ± 5^c	100 ± 6^d	100 ± 6^e

$^a p \le 0.001$. $^b p \le 0.010$. ^{c}118 mg %. ^{d}112 mg %. ^{e}137 mg/dL. ^{f}20 mg/kg. ^{g}150 mg/kg. ^{h}1%.

7.47 (m, 4 H, Ar H_4), 4.45 (t, 2 H, NCH_2), 3.06 (t, 2 H, Ch_2CO), 2.17 (s, 3 H, CH_3). Anal. ($C_{11}H_{12}N_2O_2$) C, H, N.

***N*^{1}-Carbethoxy-*N*^{2}-(2-carboxyethyl)indazolone (40).** Compound 18 (400 mg, 1.5 mmol) was dissolved in 4 mL of glacial acetic acid and added dropwise to a cooled solution of CrO_3 (608 mg, 6.01 mmol) in 10 mL of glacial acetic acid and 1 mL of water. After stirring overnight at room temperature, the reaction mixture was poured into water and extracted with ether. The ether extracts were dried (Na_2SO_4) and evaporated in vacuo to afford 362 mg of semisolid, which was column chromatographed on silica gel 60 (CH_2Cl_2–MeOH, 9:1) to afford 300 mg (72%) of product as a semisolid: ^{1}H NMR ($CDCl_3$ Me_4Si) δ 7.60 (m, 4 H, Ar H_4), 4.52 (q, 4 H, OCH_2CH_3, NCH_2), 2.82 (m, 2 H, CH_2COOH), 1.53 (t, 3 H, CH_3).

Assay for Antihyperlipidemic Activity. Compounds **1–5**, **23–37**, **39**, and **40**, as well as clofibrate, were suspended by homogenation in 1% (carboxymethyl)cellulose to deliver 20 mg/kg in 0.2 mL. (Carboxymethyl)cellulose (0.2 mL) was used as a vehicle for the control. Animals were maintained in a group of six, housed in plastic cages on "beta chips", Northeastern Products, and fed Wayne Blox laboratory animal chow ad libitum with water. CF_1 male mice (~25 g) were administered the drugs ip between 9:00 and 11:00 a.m. On days 9 and 16, blood (~1 mL) was obtained by tail bleeding. After centrifugation to obtain serum, 25-μL samples were assayed for total cholesterol by the procedure of Ness et al.[10] Serum triglycerides were assayed by using the commercially available Bio-Dynamics/bmc triglyceride kit on blood collected on the 16th day. By comparison to standards, the milligram percent of cholesterol and milligram per deciliter of triglycerides were calculated. Treated values were expressed (Table IV) as percent of control plus or minus standard deviation. The p values were obtained by using the Student "t" test.

Acknowledgment. This work was supported by National Heart, Lung, and Blood Institute Grant HL25680.

Registry No. **1**, 5686-93-1; **2**, 7364-29-6; **3**, 7364-28-5; **4**, 7364-26-3; **5**, 16105-24-1; **6**, 89438-38-0; **7**, 89438-39-1; **8**, 89438-40-4; **9**, 1848-42-6; **10**, 89438-41-5; **11**, 89438-42-6; **12**, 89438-43-7; **13**, 89438-44-8; **14**, 89438-45-9; **15**, 89438-46-0; **16**, 89438-47-1; **17**, 89438-48-2; **18**, 89438-49-3; **19**, 1848-43-7; **20**, 89438-50-6; **21**, 89438-51-7; **22**, 89438-52-8; **23**, 1848-40-4; **24**, 89438-53-9; **25**, 89438-54-0; **26**, 89438-55-1; **27**, 89438-56-2; **28**, 89438-57-3; **29**, 89438-58-4; **30**, 89438-59-5; **31**, 89438-60-8; **32**, 89438-61-9; **33**, 1848-46-0; **34**, 89438-62-0; **35**, 89438-63-1; **36**, 89438-64-2; **37**, 17049-65-9; **38**, 89438-65-3; **39**, 89438-66-4; **40**, 89438-67-5.

(10) Ness, A. T.; Pastewka, J. V.; Peacock, A. C. *Clin. Chem. Acta* 1964, *10*, 229.

Synthesis of Previously Inaccessible Quinazolines and 1,4-Benzodiazepines as Potential Anticonvulsants[1]

Aqeel A. Fatmi, Niteen A. Vaidya, W. B. Iturrian, and C. DeWitt Blanton, Jr.*

Departments of Medicinal Chemistry/Pharmacognosy and Pharmacology/Toxicology, College of Pharmacy, University of Georgia, Athens, Georgia 30602. *Received November 3, 1983*

A series of 4,6,7,8-tetrasubstituted 3,4-dihydroquinazolines, quinazolines, quinazolin-2-ones, 1,2,3,4-tetrahydroquinazolin-2-ones, and 5,7,8,9-tetrasubstituted 1,4-benzodiazepines have been synthesized by utilizing the Diels–Alder reaction between furan *o*-amino nitriles and various alkyl or aryl vinyl ketone dienophiles to obtain the anthranilic acid precursors. All of the newly synthesized target compounds were evaluated in mice for anticonvulsant activity. Pro- and anticonvulsant action was quantified by the timed intravenous pentylenetetrazol seizure threshold method. Selected compounds were also evaluated for benzodiazepine receptor binding properties and in vivo antagonist potential. Although the compounds lack potency, the data suggest that previously inaccessible substituted analogues may be useful to segregate the proconvulsant, anticonvulsant, and antagonist actions of benzodiazepines and quinazolines.

Quinazoline and 1,4-benzodiazepine derivatives have been found to be biologically versatile compounds.[2,3] It is probable, but not proven, that these actions involve different mechanisms possessing distinct chemical requirements.[4] Exploiting the Diels–Alder reaction (Scheme I) between furan *o*-amino nitriles and various dienophiles to synthesize novel anthranilic acid derivatives opens avenues for the synthesis of a wide variety of previously inaccessible substituted heterocycles[5] that may be useful

(1) Taken in part from the Ph.D. Dissertation of A. A. Fatmi, University of Georgia, Athens, GA, Aug, 1981.

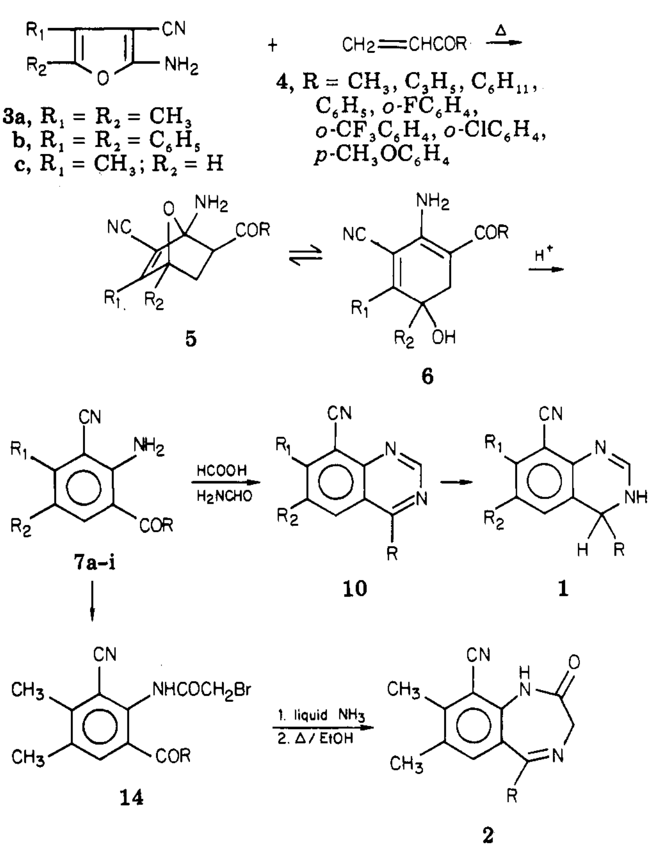
(2) (a) Amin, A. M.; Mehta, D. R.; Samarth, S. S. *Prog. Drug Res.* 1970, *14*, 218. b) Johne, S. *Ibid.* 1982, *26*, 259.

(3) (a) Garatti, S.; Mussini, E.; Randall, O. L. "The Benzodiazepines"; Raven Press: New York, 1973. (b) Randall, L. O.; Kappel, B. In ref 3a; p 27. (c) Sternbach, L. H. *J. Med. Chem.* 1979, *22*, 1. (d) Fryer, R. I. In "The Benzodiazepines: From Molecular Biology to Clinical Practice"; Costa, E., Ed.; Raven Press: New York, 1983; p 7.

(4) (a) Vida, J. A. "Anticonvulsants"; Academic Press: New York, 1977. (b) Randall, L. O.; Shallek, W.; Sternback, L. H.; Ning, R. Y. In "Psychopharmacological Agents"; Gordon, M., Ed.; Academic Press: New York, 1974; Vol. 3, pp 175–281.

0022-2623/84/1827-0772$01.50/0

Scheme I

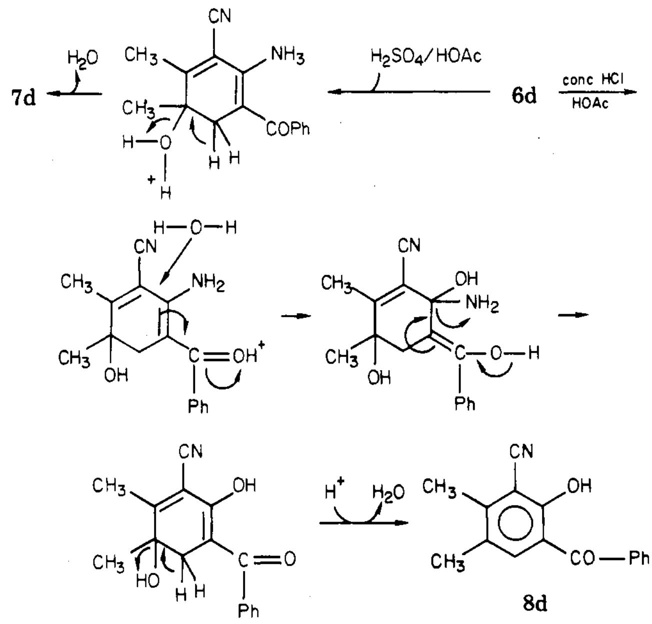


to segregate these pharmacological actions.

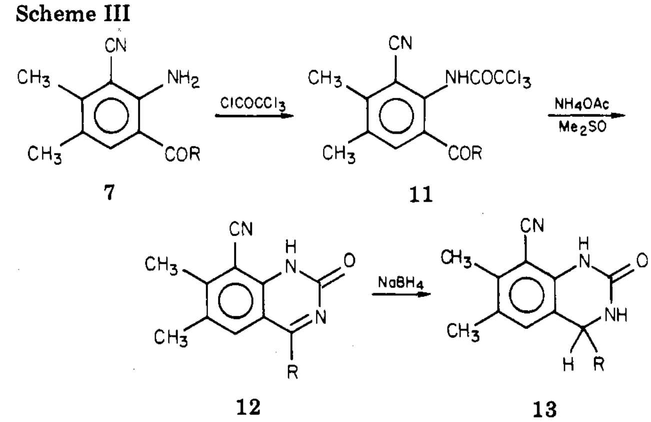
Benzodiazepines and quinazolines both possess an unusually broad spectrum of actions on the seizure process, since some analogues are convulsant, others are anticonvulsant, and a few are even specific antagonists.[3d,6] Desmedt et al.[7] and Masuda et al.[8] recently proposed that selective alterations in various components of the seizure continuum during the maximal metrazol seizure test could be used to screen anticonvulsant drugs and to classify them according to pharmacological mechanism. Desmedt et al.[7] also reviewed the neurological relevance and methods of interpreting the screening results from the metrazol tests.

In this paper, we report the preparation and the evaluation of activity in the seizure process of a series of 3,4-dihydroquinazolines (1) and 1,4-benzodiazepines (2) synthesized from substituted *o*-amino ketones (anthranilonitriles) derived from furan *o*-amino nitrile precursors (Scheme I). We use the timed intravenous metrazol infusion method of Orloff et al.[9] because it provides a rapid quantification of a compound's alteration of metrazol

Scheme II



Scheme III



thresholds and seizure spread but detects both pro- and anticonvulsant effects as well.

Chemistry. 3,4-Dihydroquinazoline (1) and 1,4-benzodiazepine (2) derivatives were prepared from *o*-amino ketones **7** (3-acyl- or 3-benzoylanthranilonitriles). The starting *o*-amino ketones (Table I) were synthesized from the Diels–Alder adducts[5a] obtained with 2-amino-3-cyano-4,5-dimethylfuran **3**[10] and the appropriate alkyl or aryl vinyl ketone **4**. A convenient procedure[11] for the synthesis of all vinyl ketones, except the commercially available methyl vinyl ketone, was followed with slight modification.[12] The adducts **5** and **6** formed by this reaction between **3** and **4** may be isolated and characterized[5a] or used directly in subsequent reactions. The Diels–Alder adducts may be dehydrated in the presence of hydrochloric and acetic acid (1:3) at room temperature[5] to give the *o*-amino ketones (viz., *o*-aminoacetophenones or *o*-aminobenzophenones) (Table I). However, when R in adduct **6d** was an aryl group, a mixture of products was obtained.

(5) (a) Nixon, W. J., Jr.; Garland, J. J.; Blanton, C. D., Jr. *Synthesis* **1980**, 56. (b) Vaidya, N. A.; Blanton, C. D., Jr. *J. Org. Chem.* **1982**, *47*, 1977. (c) Vaidya, N. A.; Nixon, W. J., Jr.; Fatmi, A. A.; Blanton, C. D., Jr. *Ibid.* **1982**, *47*, 2483.

(6) (a) Williams, M. *J. Med. Chem.* **1983**, *26*, 619. (b) Möhler, H.; Richards, J. G. In ref 3d; p 93. (c) Yokoyama, N.; Ritter, B.; Neubert, A. D. *J. Med. Chem.* **1982**, *25*, 338.

(7) Desmedt, L. K. C.; Niemegeers, C. J. E.; Lewi, P. J.; Janssen, P. A. J. *Arnzeim.-Forsch.* **1976**, *26*, 1592.

(8) Masuda, Y.; Shiraishi, Y.; Karasawa, T.; Yoshida, K.; Shimizu, M. *J. Pharm. Dyn.* **1980**, *3*, 526.

(9) (a) Orloff, M. J.; Williams, H. L.; Pfeiffer, C. C. *Proc. Soc. Exp. Biol. Med.* **1949**, *70*, 254. (b) Fingl, E.; McQuarrie, D. G. *Arch. Int. Pharmacodyn.* **1960**, *136*, 17. (c) Swinyard, E. A.; Radhakrishnan, N.; Goodman, L. S. *J. Pharmacol. Exp. Ther.* **1962**, *138*, 337. (d) Iturrian, W. B.; Fink, G. B. *Dev. Psychobiol.* **1969**, *2*, 10.

(10) Gewald, K. *Chem. Ber.* **1966**, *99*, 1002; all furan precursors were prepared by this method.

(11) Gras, J.-L. *Tetrahedron Lett.* **1978**, 2955.

(12) The original procedure employed trioxymethylene (*s*-trioxane) as a reagent in the synthesis of vinyl ketones. In our hands, the use of this reagent consistently resulted in low yields of the vinyl ketone. However, substitution paraformaldehyde for trioxymethylene led to yields ranging from 40 to 90% of the desired vinyl ketones.

Table I. *o*-Amino Ketones (7), 3,4-Dihydroquinazolines (1), and Quinazolines (10)

compd	R	R_1	R_2	mp, °C	yield, %	formula	anal.[a]
7a	CH_3	CH_3	CH_3	195–197[b]	64.2[c]	$C_{11}H_{12}N_2O$	C, H, N
7b	C_3H_5	CH_3	CH_3	139–140	72.8[d]	$C_{13}H_{14}N_2O$	C, H, N
7c	C_6H_{11}	CH_3	CH_3	156–158	79.5[d]	$C_{16}H_{20}N_2O$	C, H, N
7d	C_6H_5	CH_3	CH_3	167–169	89.0[d]	$C_{16}H_{14}N_2O$	C, H, N
7e	*o*-FC_6H_4	CH_3	CH_3	149–151	67.5[e]	$C_{16}H_{13}N_2O$	
7f	*o*-$CF_3C_6H_4$	CH_3	CH_3	195–197	88.5[d]	$C_{17}H_{13}N_2O$	C, H, N
7g	*o*-ClC_6H_4	CH_3	CH_3	174–175	40.9[e]	$C_{16}H_{13}NOCl$	C, H, N
7h	*p*-$CH_3OC_6H_4$	CH_3	CH_3	196–197	55.5[c]	$C_{17}H_{16}N_2O_2$	C, H, N
7i	CH_3	C_6H_5	C_6H_5	206–207.5	56.0[e]	$C_{21}H_{16}N_2O$	C, H, N
7j[f]	CH_3	CH_3	H				
1a	CH_3	CH_3	CH_3	231–234[g]	76.0[h]	$C_{12}H_{13}N_3$	C, H, N
1b	C_3H_5	CH_3	CH_3	216–218	64.6[h]	$C_{14}H_{15}N_3$	C, H, N
1c	C_6H_{11}	CH_3	CH_3	200–203	91.6[i]	$C_{17}H_{21}N_3$	C, H, N
1i	CH_3	C_6H_5	C_6H_5	304–305	35.4[j]	$C_{22}H_{17}N_3$	C, H, N
10a	CH_3	CH_3	CH_3	178–179	73.0[k]	$C_{12}H_{11}N_3$	C, H, N
10d	C_6H_5	CH_3	CH_3	185–187	69.5[l]	$C_{17}H_{13}N_3$	C, H, N
10e	*o*-FC_6H_4	CH_3	CH_3	195–197	77.5[l]	$C_{17}H_{12}N_3F$	C, H, N

[a] The results of the elemental analyses are within ±0.4% of the theoretical values. [b] Literature[5a] mp 194–196 °C. [c] Chloroform–petroleum ether. [d] EtOAc. [e] MeOH. [f] See ref 5c. [g] Literature[5a] mp 230–233 °C. [h] 95% EtOH. [i] Methylene chloride–EtOH. [j] MeOH–DMF. [k] Benzene–hexane. [l] EtOH.

Table II. Trichloroacetamides (11), Quinazolin-2-ones (12), Bromoacetamide (14), and Benzodiazepines (2)

compd	R	mp, °C	yield, %	formula	anal.[a]
11a	CH_3	155–157	97.0[b,c]	$C_{13}H_{11}N_2O_2Cl_3$	
11c	C_6H_{11}	oil	98.7[c]	$C_{18}H_{19}N_2O_2Cl_3$	
11d	C_6H_5	177–179	88.0[b,c]	$C_{18}H_{13}N_2O_2Cl_3$	
11e	*o*-FC_6H_4	189–190	90.0[b,c]	$C_{18}H_{12}N_2O_2Cl_3F$	
12a	CH_3	253–254	77.0[d]	$C_{12}H_{11}N_3O$	C, H, N
12c	C_6H_{11}	232–234	82.0[e]	$C_{17}H_{19}N_3O$	C, H, N
12d	C_6H_5	311–312	89.0[f]	$C_{17}H_{13}N_3O$	C, H, N
12e	*o*-FC_6H_5	299–301	73.5[d]	$C_{17}H_{12}N_3OF$	C, H, N
14a	CH_3	156–158	86.0[e]	$C_{13}H_{13}N_2O_2Br$	C, H, N
14b	C_3H_5	195–197	86.8[c,e]	$C_{16}H_{15}N_2O_2Br$	
14d	C_6H_5	173–174	91.6[g]	$C_{19}H_{15}N_2O_2Br$	C, H, N, Br
14e	*o*-FC_6H_4	167–169	87.4[c,e]	$C_{19}H_{14}N_2O_2BrF$	
2a	CH_3	223–225	91.0[h]	$C_{13}H_{13}N_3O$	C, H, N
2b	C_3H_5	192–194	68.8[h]	$C_{13}H_{15}N_3O$	C, H, N
2d	C_6H_5	214–215	82.5[h]	$C_{18}H_{15}N_3O$	C, H, N
2e	*o*-FC_6H_4	229–231	75.1[e]	$C_{18}H_{14}N_3OF$	C, H, N

[a] The results of elemental analyses are within ±0.4% of the theoretical values. [b] 95% EtOH. [c] Compound utilized in the next step without further purification. [d] Pyridine–EtOH. [e] Methylene chloride–EtOH. [f] Pyridine. [g] EtOH. [h] Acetone.

Since adduct **6d** may behave as an enamine, the presence of an electron-withdrawing benzoyl moiety can facilitate hydrolysis and dehydration to yield to the corresponding phenol (**8d**, Scheme II). Extraction of a methylene chloride solution of the reaction mixture with aqueous sodium hydroxide allowed convenient separation and characterization of the phenol **8d** and *o*-amino ketone **7d**. To avoid formation of the phenolic ketones **8d**, we modified the dehydration procedure by using concentrated sulfuric and acetic acid (1:3) instead of concentrated hydrochloric and acetic acid.[13]

The acylanthranilonitriles (**7a–c,i**) gave 3,4-dihydroquinazolines (**1a–c,i**, Table I) when treated with formamide and formic acid (Scheme I). However, the 3-benzoylanthranilonitriles (**7d,e**) gave quinazolines **10d,e** (Table I). Further reduction to 3,4-dihydroquinazolines did not occur, apparently due to the more highly conjugated nature of these aryl-substituted quinazolines. When **7a** was treated with formamide and acetic acid, in the absence of formic acid, quinazoline **10a** was isolated.

Since **1a** possessed activity and did not possess an amido group often associated with anticonvulsant activity,[1,14] it may be that **1a** underwent in vivo metabolism to give an active product, such as the tetrahydroquinazolin-2-one (**13a**, Scheme III). Based on this possibility, two compounds, **13a** and **13d**, were synthesized via the trichloroacetamido derivatives (**11**, Table II) of appropriate *o*-amino ketones **7**. When intermediates **11** were allowed to react[15] with ammonium acetate in dimethyl sulfoxide, quinazolin-2-ones (**12**, Table II) were obtained. Reduction of **12** with sodium borohydride[16] in ethanol provided the tetrahydroquinazolin-2-ones **13**.

(13) Should the Diels–Alder adduct **6** be poured directly into a dilute hydrochloric acid solution and allowed to stand for a short period, high yields of the phenolic product (e.g., **9**) may be obtained even when R is an alkyl moiety. Thus, the Diels–Alder reaction between furan *o*-amino nitriles and aryl or alkyl vinyl ketones may also be utilized to synthesize substituted *o*-hydroxyacetophenones and *o*-hydroxybenzophenones.

(14) (a) Spinks, A.; Waring, W. S. *Prog. Med. Chem.* **1963**, *3*, 261. (b) Close, W. J.; Spielman, M. A. *Med. Chem. (Wiley)* **1961**, *5*, 1. (c) Isaacson, E. I.; Delgado, J. N. In "Burger's Medicinal Chemistry", Part III; Wolff, M. E., Ed.; Wiley: New York, 1981; p 829. (d) Gagneux, A. R. In "Epileptic Seizures–Behavior–Pain"; Birkmayer, W., Ed.; University Park Press: Baltimore, 1976; p 120.

(15) Yamamoto, M.; Inaba, S.; Yamamoto, H. *Chem. Pharm. Bull.* **1978**, *26*, 1633.

(16) Coombs, R. V.; Danna, R. P.; Denzu, M.; Hardtmann, G. E.; Buegi, B.; Koletar, G.; Koletar, J.; Ott, H. *J. Med. Chem.* **1973**, *16*, 1237.

Table III. Activity of Selected Quinazolines and 1,4-Benzodiazepine Analogues in the Anticonvulsant, Seizure Threshold, and Benzodiazepine (BDZ) Binding Tests[a]

	ADD tests[b]		seizure threshold tests[c]			
compd	sc Met: ED_{50}, mg/kg	rotorod: TD_{50}, mg/kg	dose, mg/kg	MST: % change in iv perfusion	MMT: % change in iv perfusion	BDZ binding: IC_{50}, μM
1a	I[d]	51.5	25	−36	−56	NT[f]
		(38.8–68.6)[e]				
1c	I	>600	300	I	I	I[g]
2a	I	70	30	I	−40	I
2b	I	600	300	I	I	I
2d	370	>1000	100	I	I	3×10^{-6}
			300	+44	I	
2e	48.9	709.9	49	+23	+30	1×10^{-5}
	(18.3–97.7)[e]	(510–996.7)[e]	100	+65	+50	
10a	I	45	30	I	−24	I
12c	260	>600	300	+44	I	I
13a	I	300	75	I	−16	NT
13d	600[h]	>1000	300[h]	+36	+22	NT
		Open Ring Analogues				
7a	300	>600	300	+15	+13	NT[i]
7i	260	>600	300	I	+20	
7b	600	>600	600	+23	+37	
7c	410	>600	300	I	+17	
14a	500	600	100	−14	I	
			300	−22	−17	
14d	480	600	100	+70	I	
			300	+37	+49	
methaqualone	33.5	55	30	+18	+17	
	(28–40)[e]	(47–65)[e]	50[j]	+42	+40	
diazepam	0.17	7.30	0.2	+50	+15	8.9×10^{-9}
	(0.13–0.21)[e]	(4.60–8.70)[e]	1	+60	+40	
desmethyldiazepam	0.28	11.86	0.2	+48	I	9.4×10^{-9}
	(0.26–0.32)[e]	(8.60–15.00)[e]	1[j]	+70	+35	

[a] The following compounds were inactive in the in vivo tests: **1b,i**, **10d,e**, **12a,d,e 7d,g**, and **8d**. Only two compounds (**1a** and **9a**) were active in the ADD maximal electroshock test. [b] Antiepileptic Drug Development Program. [c] Continuous iv perfusion method of Orloff et al.[9] MST is the metrazol seizure threshold to persistent clonus, and MMT is the maximal metrazol threshold to tonic flexion. Thresholds determined at 0.5 h. For all measures shown, changes are in comparison to matched controls, and all controls analyzed together were statistically significant (p <0.05 or less). [d] Inactive at all doses, including 600 mg/kg. [e] 95% confidence limits. [f] Not tested. [g] Inactive at 80 μM. [h] Determined at 4 h. [i] None of the open ring analogues were assayed for BDZ binding. [j] Determined at 1 h.

For synthesis of the substituted benzodiazepines (**2**, Scheme I), the *o*-amino ketones **7** were treated with bromoacetyl bromide in the customary manner.[17] Ammonolysis of the bromoacetamido intermediates (**14**, Table II) in liquid ammonia, followed by reflux in ethanol,[17] gave the corresponding benzodiazepines (**2**, Table II) in good yields.

Biological Results

All of the newly synthesized 3,4-dihydroquinazolines (**1**), quinazolines (**10**), quinazolin-2-ones (**12**), tetrahydroquinazolin-2-ones (**13**), 1,4-benzodiazepines (**2**), and selected intermediates were tested for anticonvulsant activity by the Antiepileptic Drug Development (ADD) Program[18] (administered by the Section on Epilepsy, National Institute of Health, Bethesda, MD). Similarly, selected benzodiazepines were subjected to benzodiazepine receptor binding studies. Most of the compounds were evaluated for alteration in the seizure process by Orloff's method.[9] Selected compounds were evaluated for antagonist potential. All of the tests are described in the Experimental Section.

Methaqualone, diazepam, and desmethyldiazepam were used as reference compounds. Diazepam and methaqualone, unlike phenytoin, ethosuximide, or most antiepileptic drugs, are active in all four of the common anticonvulsant tests: the maximal metrazol, the sc metrazol, the audiogenic seizure, and the maximal electroshock (MES) tests.[4] Desmethyldiazepam is a metabolite of diazepam having comparable anticonvulsant activity.[3b]

The finding that a 6,7,8-trisubstituted 3,4-dihydroquinazoline (**1a**) was apparently active only in the MES test and that a 7,8,9-trisubstituted benzodiazepine (**2e**) was active in the sc Met, but no other, test provided leads that other analogues might provide compounds with more selective antiepileptic action. From a structure–activity standpoint, the compounds were interesting in that they lacked features usually required for central nervous system (CNS) activity.[3,4,14,19] The benzodiazepines are the most potent antiepileptics known,[3d,19] but substitution at the 7-, 8-, and 9-positions remarkedly decreases anticonvulsant potency (Table III). Particularly noteable is compound **2e**, the analogue of Ro 5-3367 (desalkylflurazepam), which is active in the sc metrazol test at 80 μg/kg.[4b] Rather

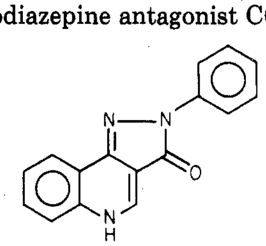
(17) Early, J. V.; Fryer, R. I.; Ning, R. Y. *J. Pharm. Sci.* **1979**, *68*, 845.
(18) (a) Anticonvulsant Screening Project, Antiepileptic Drug Development Program, National Institues of Health, *DHEW Publ. (NIH) (U.S.)* **1978**, *NIH 78-1093*. (b) Swinyward, E. A.; Brown, W. C.; Goodman, L. S. *J. Pharmacol. Exp. Ther.* **1952**, *106*, 319. (c) Kornet, M. J.; Crider, A. M.; Magarian, E. O. *J. Med. Chem.* **1977**, *20*, 405. (d) Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.
(19) Jones, G. L.; Woodbury, D. M. *Drug. Dev. Res.* **1982**, *2*, 333.

surprisingly, the open-ring compounds were about as active as the heterocyclic analogues, but all compounds, with the exception of the leads **1a** and **2e**, lack sufficient anticonvulsant potency. Substitution at the R position introduces several interesting changes in the CNS effects of the analogues. Intraperitoneal injection of 100 mg/kg of compounds **1a**, **2a**, and **10a** produced respiratory depression, coma, and death. The median lethal dose (LD_{50}) for these analogues is about 150 mg/kg, whereas the LD_{50} for **2d** and **2e** was over 2 g/kg.

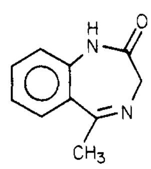
Although **1a** and **10a** produced tremors, none of the analogues synthesized produced convulsions. Compounds **1a**, **2a**, **10a**, **13a**, and **14a** possess a methyl substitution at the R position and all had proconvulsant activity in that they decrease metrazol thresholds in Orloff's[9] continuous perfusion threshold test. Those compounds with an aryl group at R (**2d**,**e**,**13d**, and **14d**) were anticonvulsant or inactive (**10d**,**e** and **12d**,**e**). Thus, with the exception of an amine **7a**, compounds with methyl at R were proconvulsant and those with aryl tended to be anticonvulsant. Although a similar structure–activity relationship (SAR) convulsant/anticonvulsant action has been observed in 1,4-benzodiazepines,[3,6] it also occurs with piperidinediones.[20]

While correlation between in vitro binding and in vivo antimetrazol effects of benzodiazepines is generally good,[6,21,22] any correlation of activity with this series of 7,8,9-trisubstituted analogues is not clear. Most of the compounds were inactive or had low affinity in the binding assay. However, correlation of benzodiazepine antagonism of electroshock-induced convulsions with a low-affinity (10^{-5} M) synaptosomal binding site has been reported.[23] However, compounds **2d** and **2e** do not protect from tonic extension in the maximal electroshock or the maximal metrazol tests. Bowling and DeLorenzo[23] report the following inhibition constants (K_i) from binding studies to "the micromolar receptor": desmethyldiazepam, 73 μM; diazepam, 85 μM; phenytoin, 155 μM.

The proconvulsant actions of **1a** and **2a**, as well as the anticonvulsant effects of **2e**, were blocked by the selective benzodiazepine antagonist CGS-8216 (**15**), but the com-



15



16

pounds failed to reverse the "inverse agonist" action of **15** using an audiogenic seizure test.[21] Furthermore, **2e** alone was inactive against audiogenic seizures, whereas benzodiazepines[4,21] and methaqualone[4] are potent inhibitors of audiogenic seizure. The duration of the antimetrazol action of **2e** was only about 90 min, much briefer than would be expected from a desalkylflurazepam analogue.[4b]

Compound **2d** does not posses benzodiazepine antagonist action, since 300 mg/kg failed to reverse the antimetrazol effects of desmethyldiazepam. Although **2a** was inactive in the binding assay, it has some nonspecific antagonist action in that 30 mg/kg completely reverses the antimetrazol effects of 2 mg/kg of desmethyldiazepam. The convulsant benzodiazepine Ro 5-3663 (**16**) has negligible benzodiazepine receptor affinity[6] and, like **2a**, is a methyl (R) analogue.

It should be noted that **16** and the **2** analogues, unlike most benzodiazepines, do not possess a 7-chloro substitution. Although not proven, it has been suggested that **16** may not be acting through the benzodiazepine receptor.[24] Compound **16** selectively blocks the inhibitory neurotransmitter γ-aminobutyric acid (GABA).[24] Further work is needed to clarify any effects of **2a**,**d**,**e** to the actions of GABA.

Desmedt et al.[7] and Masuda et al.[8] have reviewed the neurological and clinical significance of changes in the clonic and tonic threshold, as well as alterations in the seizure pattern, during the maximal metrazol test that are relevant to the Orloff perfusion method[9] used in this study.

The introduction of a fluorine atom at the ortho position enhanced anticonvulsant action. Compound **2d** was more selective against the threshold for clonic than tonic metrazol seizure, but it was a much less potent anticonvulsant than **2e**, despite better binding. It should be noted that the benzodiazepines are particularly potent in raising the threshold for clonic seizures induced by metrazol, while considerably higher doses are required to block the tonic-extensor seizures of the maximal metrazol (MMS) or maximal electroshock (MES) tests.[8,19] The results from the continuous intravenous (iv) perfusion method support the conclusions of Desmedt et al.[7] and Masuda et al.[8] that anticonvulsant action against clonic and tonic seizures may represent different mechanisms. Compounds **2e**, **13d**, **7a**,**b**, and **14d** elevate both metrazol thresholds, while **2d**, **12c**, and the lower dose of **14d** only elevate the threshold for clonus.

Jones and Woodbury[19] have emphasized the pitfalls of not distinguishing activity in the threshold tests rather than the pattern test of the metrazol seizure during structure–activity relationship studies. Methaqualone raises the thresholds for both clonic and tonic end points of the Orloff's infusion test and, unlike diazepam and desmethyldiazepam, blocks tonic hindlimb extension. Compounds **1a** and **9a**, the only compounds in this series active against MES, also alter the tonic-hindlimb pattern of the maximal metrazol seizure. Although **1a** was active in the maximal electroshock (MES) test, higher doses were proconvulsant. This effect is unusual, but it has also been reported for Ro 5-4864, a 4′-chloro-substituted benzodiazepine.[3] This toxicity was disappointing, since **1a** was a lead compound for the quinazolines of this series.

The data suggest that the broad spectrum of effects of the benzodiazepines and quinazolines on the seizure process can be modified by substituted heterocyclic analogues but that future compounds should have less extensive substitution. Compound **2e** is an unusual compound that warrants further study.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Ind., Atlanta, GA, and results were within ±0.4 of the calculated values unless otherwise noted. Satisfactory IR (Perkin-Elmer 467 grating spectropho-

(20) Fink, G. B.; Juchau, M. R. *J. Pharm. Sci.* **1964**, *53*, 325.
(21) Jensen, L. H.; Petersen, E. N.; Braestrup, C. *Life Sci.* **1983**, *33*, 393.
(22) (a) Paul, S. M.; Syapin, P. J.; Paugh, B. A.; Moneada, V.; Skolnick, P. *Nature (London)* **1979**, *281*, 688. (b) Squires, R. F.; Braestrup, C. *Ibid.* **1977**, *266*, 732. (c) Möhler, H.; Okada, V. T. *Science* **1977**, *198*, 849. (d) Frey, H. H.; Losher, W. *Pharmacologist* **1982**, *25*, 154.
(23) Bowling, A. C.; DeLorenzo, R. J. *Science* **1982**, *216*, 1247.
(24) (a) Speth, R. C.; Wastek, G. J.; Yamamura, H. I. *Life Sci.* **1979**, *24*, 351. (b) Schlosser, W.; Franco, S. *J. Pharmacol. Exp. Ther.* **1979**, *211*, 290. (c) Geller, H. M. *Neurosci. Lett.* **1979**, *15*, 313.

tometer, KBr) and NMR (60-MHz Hitachi Perkin-Elmer R20A high-resolution spectrometer, Me_4Si as internal reference) spectra were obtained for all new compounds. TLC was performed on Eastman chromatogram sheets, Type 13181, coated with silica gel.

2-Amino-3-cyano-4,5-dimethylbenzophenone (7d). 2-Amino-3-cyano-4,5-dimethylfuran (**3a**;[10] 13.7 g, 0.1 mol) was dissolved in 200 mL of *p*-dioxane, treated with phenyl vinyl ketone (**4d**;[11,12] 15.9 g, 0.12 mol), and refluxed for 12 h with continuous stirring.[5a] Removal of the solvent in vacuo afforded an oil, which was treated with anhydrous Et_2O to give a yellow solid. Recrystallization from benzene gave 20.8 g (77.7%) of the intermediate **6d**, mp 159–161 °C. The Diels–Alder adduct **6d** (10 g, 0.037 mol) was suspended in a mixture of 100 mL of glacial HOAc and 33 mL of concentrated sulfuric acid and stirred for 3 h at room temperature. The dark solution was poured in 250 mL of ice-cold water to give a yellow voluminous solid. Recrystallization from EtOAc gave 8.2 g (89.0%) of a yellow solid: TLC (EtOAc) R_f 0.45; mp 167–169 °C; IR (KBr) 3450, 3340, 3000, 2200, 1625, 1545 cm^{-1}; NMR ($CDCl_3$) δ 2.2 (s, 3 H, 4-CH_3), 2.5 (s, 3 H, 5-CH_3), 6.85 (s, 2 H, 2-NH_2), 7.5 (s, 1 H, 6-CH), 7.65 (m, 5 H, aromatic proton). Anal. ($C_{16}H_{14}N_2O$) C, H, N.

The physical properties of **7a–c,e–i**, which were prepared in a similar maner, are included in Table I.

3-Cyano-4,5-dimethyl-2-hydroxybenzophenone (8d). When the Diels–Alder adduct **6d**, (5 g, 0.02 mol) was suspended in 60 mL of HOAc and 20 mL of concentrated hydrochloric acid, the reported[5a] method for dehydration, a mixture was obtained. The TLC (EtOAc) examination of the mixture showed two spots with R_f values of 0.45 and 0.57. The mixture was separated by dissolving it in methylene chloride (50 mL) and then washing it with 5% aqueous NaOH (2 × 5 mL) solution and water. After drying over Na_2SO_4, the methylene chloride solution was evaporated in vacuo to yield a yellow solid. Recrystallization from EtOAc gave a yellow solid: TLC (EtOAc) R_f 0.45; mp 167–169 °C. IR and NMR spectra confirmed the product to be identical with **7d** (59.4% yield).

The NaOH washings were combined, and the pH was adjusted to 2 by 1 N HCl (pH Hydrion paper). A white precipitate, **8**, was collected and recrystallized from methanol–methylene chloride (25% yield): TLC (EtOAc) R_f 0.57; mp 149–150 °C; IR (KBr) 3300, 2220, 1670, 1625, 1600 cm^{-1}; NMR ($CDCl_3$) δ 2.2 (s, 3 H, 4-CH_3), 2.35 (s, 3 H, 5-CH_3), 6.8 (m, 6 H, aromatic protons), 12.5 (s, 1 H, 2-OH). Anal. ($C_{16}H_{13}NO_2$) C, H, N.

3-Cyano-4,5-dimethyl-2-hydroxyacetophenone (9). The furan *o*-aminonitrile **3a**[10] (13.7 g, 0.1 mol) was dissolved in 200 mL of *p*-dioxane, treated with methyl vinyl ketone (8.5 g, 0.12 mol), and refluxed for 12 h with continuous stirring. The reaction mixture was allowed to cool and then poured into 1 L of cold water containing 50 mL of concentrated HCl. After the solution was left standing overnight, a voluminous white precipitate was collected and recrystallized from ethyl acetate–petroleum ether (30–60 °C) to yield 15.5 g (82.0%) of **9**: mp 133–134 °C; IR (KBr) 3000 (br), 2220, 1630 cm^{-1}; NMR (Me_2SO-d_6) δ 2.25 (s, 3 H, 4-CH_3), 2.3 (s, 3 H, 5-CH_3), 2.65 (s, 3 H, $COCH_3$), 7.95 (s, 1 H, 6-CH). Anal. ($C_{11}H_{11}NO_2$) C, H, N.

8-Cyano-4-methyl-6,7-diphenyl-3,4-dihydroquinazoline (1i). Compound **7i** (3.0 g, 9.6 mmol) was heated at 170–180 °C for 4 h with 100 mL of formamide and 30 mL of formic acid.[5a] The dark solution was poured into 1 L of ice-cold water and basified with ammonium hydroxide. The yellow solid was collected and recrystallized from MeOH–DMF to yield 1.1 g (35.4%) of an off-white product: mp 304–305 °C; IR (KBr) 3200, 2215, 1590 cm^{-1}; NMR (Me_2SO-d_6, TFA) δ 1.65 (d, 3 H, 4-CH_3), 5.15 (q, 1 H, 4-CH), 7.05–7.30 (m, 10 H, 6,7-diphenyls), 7.55 (s, 1 H, 5-CH). Anal. ($C_{22}H_{17}N_3$) C, H, N.

The physical properties of **1a–c**, which were prepared in a similar manner, are included in Table I.

8-Cyano-4,6,7-trimethylquinazoline (10a). Compound **7a** (1.88 g, 0.01 mol) was heated at 170–180 °C for 4 h with formamide (50 mL) and HOAc (10 mL). The dark solution was diluted with water (500 mL) to give an orange precipitate. Recrystallization from benzene–hexane gave **10a** in 73.0% yield: mp 178–179 °C; IR (KBr) 3000, 2200, 1620, 1555, 1545 cm^{-1}; NMR ($CDCl_3$) δ 2.15 (s, 3 H, 4-CH_3), 2.25 (s, 3 H, 6-CH_3), 2.35 (s, 3 H, 7-CH_3), 7.05 (s, 1 H, 2-CH), 7.3 (s, 1 H, 5-CH). Anal. ($C_{12}H_{11}N_3$) C, H, N.

8-Cyano-6,7-dimethyl-4-phenylquinazoline (10d). Compound **7d** (5 g, 0.02 mol) was heated at 170–180 °C for 4 h with formamide (120 mL) and formic acid (24 mL). The dark solution was diluted with water (500 mL) to give a light brown precipitate. Recrystallization from EtOH gave **10d** in 69.5% yield: mp 185–187 °C; IR (KBr) 3000, 2200, 1620, 1560, 1540 cm^{-1}; NMR ($CDCl_3$) δ 2.5 (s, 3 H, 6-CH_3), 2.8 (s, 3 H, 7-CH_3), 7.7 (m, 5 H, 4-C_6H_5), 8.15 (s, 1 H, 5-CH), 9.5 (s, 1 H, 2-CH). Anal. ($C_{17}H_{13}N_3$) C, H, N.

The physical properties of **10e**, which was prepared in a similar manner, are included in Table I.

3-Cyano-4,5-dimethyl-2-(2,2,2-trichloroacetamido)acetophenone (11a). A mixture of **7a** (4.1 g, 0.02 mol) and trichloroacetyl chloride (18.2 g, 0.10 mol) was heated at 125–130 °C for 3 h. The excess acid chloride was evaporated in vacuo, and the residue was recrystallized from 95% EtOH to give **11a** in 97% yield: mp 155–157 °C; IR (KBr) 3360, 2200, 1660, 1625, 1600 cm^{-1}; NMR ($CDCl_3$) δ 2.35 (s, 3 H, 4-CH_3), 2.55 (s, 3 H, 5-CH_3), 2.65 (s, 3 H, $COCH_3$), 7.8 (s, 1 H, 6-CH), 11.2 (s, 1 H, 2-NHCO).

The physical properties of **11c–e**, which were prepared in a similar manner, are included in Table II. All were used in subsequent steps without further purification and characterization.

8-Cyano-4,6,7-trimethyl-1*H*-quinazolin-2-one (12a). A mixture of **11a** (5.0 g, 0.014 mol), ammonium acetate (5.38 g, 0.07 mol), and Me_2SO (100 mL) was allowed to stir at room temperature for 24 h and at 75–80 °C for 2 h. The solution was poured into ice–water (300 mL), and the precipitate was collected by filtration, washed with water, and dried. Recrystallization from pyridine–EtOH gave **12a** in 77% yield: mp 253–254 °C; IR (KBr) 3450, 2200, 1665, 1600 cm^{-1}; NMR (Me_2SO-d_6, $CDCl_3$) δ 1.9 (s, 3 H, 4-CH_3), 2.2 (s, 3 H, 6-CH_3), 2.35 (s, 3 H, 7-CH_3), 7.1 (s, 1 H, 5-CH), 8.45 (s, 1 H, NHCO). Anal. ($C_{12}H_{11}N_3O$) C, H, N.

The physical properties of **12c–e**, which were prepared in a similar manner, are included in Table II.

8-Cyano-4,6,7-trimethyl-1*H*-1,2,3,4-tetrahydroquinazolin-2-one (13a). To a solution of **12a** (4 g, 0.019 mol) in 95% EtOH (150 mL) was added, in portions, 1.15 g (0.037 mol) of $NaBH_4$. The mixture was stirred at room temperature for 30 min, after which time the excess $NaBH_4$ was destroyed with HOAc, and the EtOH was evaporated in vacuo. The residue was washed with EtOH, filtered, and recrystallized from pyridine–EtOH to give **13a** in 69.6% yield: mp 277–279 °C; IR (KBr) 3450, 3225, 3110, 2220, 1690, 1600 cm^{-1}; NMR (Me_2SO-d_6, $CDCl_3$) δ 1.8, (d, 3 H, 4-CH_3), 2.3 (s, 3 H, 6-CH_3), 2.45 (s, 3 H, 7-CH_3), 5.4 (q, 1 H, 4-CH), 7.1 (s, 1 H, 5-CH). Anal. ($C_{12}H_{13}N_3O$) C, H, N.

8-Cyano-6,7-dimethyl-4-phenyl-1*H*-1,2,3,4-tetrahydroquinazolin-2-one (13d). Compound **13d** was prepared from **12d** by the procedure described for **13a**. Recrystallization from 95% EtOH gave an 84% yield: mp 239–241 °C; IR (KBr) 3600, 3225, 3100, 2200, 1675 cm^{-1}; NMR (Me_2SO-d_6, $CDCl_3$) δ 2.15 (s, 3 H, 6-CH_3), 2.35 (s, 3 H, 7-CH_3), 5.55 (s, 1 H, 4-CH), 7.4 (m, 5 H, 4-C_6H_5), 7.6 (s, 1 H, 5-CH). Anal. ($C_{17}H_{15}N_3O$) C, H, N.

2-(2-Bromoacetamido)-3-cyano-4,5-dimethylacetophenone (14a). A solution of **7a** (15.5 g, 0.08 mol) in 400 mL of $CHCl_3$ was chilled in an ice bath. Sodium bicarbonate (10 g) and bromoacetyl bromide (27.8 g, 0.13 mol) were added. The mixture was stirred for 24 h, allowing the ice to melt gradually and the temperature of the mixture to achieve room conditions. Solids were removed by filtration, and the solvent was concentrated in vacuo. The residue was recrystallized from $CHCl_3$–pentane to yield colorless needles of **14a** in 86% yield: mp 156–158 °C; IR (KBr) 3320, 2200, 1690, 1600 cm^{-1}; NMR ($CDCl_3$) δ 2.2 (s, 3 H, 4-CH_3), 2.3 (s, 3 H, 5-CH_3), 2.45 (s, 3 H, $COCH_3$), 4.1 (s, 2 H, $COCH_2Br$), 7.7 (s, 1 H, 6-CH), 10.45 s, 1 H, NHCO). Anal. ($C_{13}H_{13}N_2O_2Br$) C, H, N.

The physical properties of **14b,d,e**, which were prepared in a similar manner, are included in Table II.

9-Cyano-1,3-dihydro-5,7,8-trimethyl-2*H*-1,4-benzodiazepin-2-one (2a). To 75 mL of liquid ammonia was added 5 g (0.016 mol) of **14a**. After the solution was refluxed for 3 h, the ammonia was allowed to evaporate. The residue was heated at reflux in 50 mL of EtOH for 3 h. Ethanol was evaporated in vacuo, and 25 mL of acetone was added. This suspension was heated to boiling and filtered to remove inorganic salts. Concentration of the acetone filtrate gave 3.3 g (91%) of **2a**: mp 223–225 °C; IR (KBr) 3200, 3125, 2220, 1680 cm^{-1}; NMR ($CDCl_3$)

δ 2.4 (s, 3 H, 7-CH_3), 2.5 (s, 3 H, 8-CH_3), 2.65 (s, 3 H, 5-CH_3), 4.15 (s, 2 H, 3-CH_2), 7.6 (s, 1 H, 6-CH), 9.55 (s, 1 H, NHCO). Anal. ($C_{13}H_{13}N_3O$) C, H, N.

The physical properties of **2b,d,e**, which were prepared in a similar manner, are included in Table II.

Pharmacological Testing. Methaqualone, diazepam, and desmethyldiazepam were used as the reference compounds in this study, since they have activity in several different anticonvulsant tests and are clinically useful analogues of the compounds we synthesized.

All compounds were suspended in 30% polyethylene glycol 400 and injected intraperitoneally in a volume of 0.01 mL/g of body weight into CAW:CF1 mice. The compounds were evaluated for anticonvulsant action, as well as alterations of the seizure process and threshold.

Anticonvulsant activity was tested by the Antiepileptic Drug Development (ADD) Program administered by the Epilepsy Section (National Institutes of Health, Bethesda, MD) using the Anticonvulsant Screening Project Test Systems.[18] Compounds were tested at four dosage levels (30, 100, 300, and 600 mg/kg) at 30 min and at 4 h with the maximal electroshock (MES) seizure and pentylenetetrazol (sc Met) seizure threshold test for anticonvulsant activity and with the rotorod test to evaluate acute neurotoxicity. Four animals were injected with each dose. An estimate of the ED_{50} and TD_{50} was made with a graphic method.[18d] Compounds **1a** and **2e** were subjected to additional ADD testing to verify the ED_{50} and TD_{50} values and to establish confidence limits as calculated by the method of Litchfield and Wilcoxon.[18d]

Anticonvulsant activity in the MES test is defined as abolition of the hindlimbs' tonic extensor component of the maximal electroshock seizure, which is elicited in mice with a 60-Hz alternating current of 50 mA delivered for 0.2 s via corneal electrodes. The failure to observe even a threshold seizure (a single episode of clonic spasm of 5-s duration) following the subcutaneous administration of the convulsant dose 99 for pentylenetetrazol is considered anticonvulsant activity in the sc Met test.[18b] The complete details of ADD test systems are available.[18a,18c]

The in vitro benzodiazepine binding studies were performed by the Antiepileptic Drug Development Program[18,25] or the Upjohn Co. We assume reports of inactivity are directly comparable. Values for active compounds are reported for three determinations by the method of Möhler and Okada.[6b,22c,d]

Drug-induced alteration of the seizure threshold was evaluated by the modified continuous iv infusion method originally described by Orloff et al.[9] A solution containing 0.5% pentylenetetrazol, 0.9% NaCl, and 0.001% sodium heparin was infused into a lateral tail vein at a rate of 6.31 μL/s with a constant infusion pump. The mice were minimally restrained by the end of the tail during the seizure test.

Threshold for two types of seizure were determined—minimal metrazol seizures and maximal, generalized tonic–clonic seizures. The time of infusion, measured in tenths of a second, until the onset of 3 s of persistent clonus, hindlimb tonic flexion, and tonic extension were recorded and converted to milligrams per kilogram of pentylenetetrazol infused. The thresholds for clonic seizure and tonic flexion were reported as the metrazol seizure threshold (MST) and the maximaal metrazol threshold (MMT), respectively. The maximal seizure pattern—tonic forepaw and/or hindpaw extension—was also evaluated, as suggested by Desmedt et al.,[7] as was the duration of hindlimb tonic flexion.[9d] A dose of the compound to be tested that did not alter the rotorod test was given to groups of four 6-week-old CAW:CF1 mice raised in our laboratory. Littermates given the polyethylene glycol 400 vehicle and tested concurrently serve as controls.

Statistical significance was evaluated by the Student's t test, and the results of significant alterations are expressed as the mean percentage change from all control groups. The metrazol seizure threshold was 41.2 ± 1.7 mg/kg (mean plus or minus SEM) and the maximal metrazol threshold was 99.4 ± 3.7 mg/kg in 52 mice treated only with vehicle.

Antagonist potential was also evaluated by the continuous iv-infusion method. The compounds were tested for the ability to reverse the antimetrazol effects of 2 mg/kg of desmethyldiazepam. The test compounds were administered 10 min before the agonist and 30 min before the test. The selective benzodiazepine antagonist, CGS-8216 (**15**; 3 mg/kg), was used to reverse the actions of the compounds when they were tested as specific agonists. The "inverse agonist" action was evaluated in the audiogenic seizure induced in DBA/2J mice[21] and by the audiosensitization test.[9d]

Acknowledgment. This research was supported in part by the National Institute of Neurological and Communicative Disorders and Stroke (Grant NS 16988). The authors are indebted to Gill Gladding, Antiepileptic Drug Development Program, National Institutes of Health, and to Dr. Paul W. O'Connell, Research Laboratories of the Upjohn Co., for providing pharmacological data. Appreciation is expressed to Ciba-Geigy for providing the CGS 8216 drug and to Julie Warren and J. E. Fisher, Jr., for technical assistance.

Registry No. **1a**, 73318-20-4; **1b**, 89638-32-4; **1c**, 89638-33-5; **1i**, 89638-34-6; **2a**, 89638-50-6; **2b**, 89638-51-7; **2d**, 89638-52-8; **2e**, 89638-53-9; **3a**, 5117-88-4; **4** (R = CH_3), 78-94-4; **4** (R = C_3H_5), 59819-62-4; **4** (R = C_6H_{11}), 2177-34-6; **4** (R = C_6H_5), 768-03-6; **4** (R = o-FC_6H_4), 89638-21-1; **4** (R = o-$CF_3C_6H_4$), 89638-22-2; **4** (R = o-ClC_6H_4), 89638-23-3; **4** (R = p-$CH_3OC_6H_4$), 7448-86-4; **6d**, 89638-54-0; **7a**, 73318-15-7; **7b**, 89638-24-4; **7c**, 89638-25-5; **7d**, 89638-26-6; **7e**, 89638-27-7; **7f**, 89638-28-8; **7g**, 89638-29-9; **7h**, 89638-30-2; **7i**, 89638-31-3; **8d**, 89638-55-1; **9**, 89638-56-2; **10a**, 89638-35-7; **10d**, 89638-36-8; **10e**, 89638-37-9; **11a**, 89638-38-0; **11c**, 89638-39-1; **11d**, 89638-40-4; **11e**, 89638-41-5; **12a**, 89638-42-6; **12c**, 89638-43-7; **12d**, 89638-44-8; **12e**, 89638-45-9; **13a**, 89638-57-3; **13d**, 89638-58-4; **14a**, 89638-46-0; **14b**, 89638-47-1; **14d**, 89638-48-2; **14e**, 89638-49-3; formamide, 75-12-7; trichloroacetyl chloride, 76-02-8; bromoacetyl bromide, 598-21-0.

(25) Braestrup, C.; Squires, R. F. *Proc. Natl. Acad. Sci. U.S.A.* 1977, *74*, 3805.