similarly coupled to **10**, giving **16** [mp 125–127 °C; $[\alpha]_D$ −3.7° (*c* 0.2, $CHCl_3$); yield 89%], which was finally deprotected by hydrogenolysis[7a] in AcOH and trifluoroacetic acid treatment[7b] to produce **5** [mp 194–198 °C dec; $[\alpha]_D$ −7.9° (*c* 0.4, AcOH); TLC R_f 0.39 (A), 0.40 (C),[10] yield 89%].[14]

In Table I is given the phagocytic activity in DDY mice in the carbon clearance assay. An increase in the rate of carbon clearance was observed in mice treated with 1 mg/kg of **1**. In contrast, **3** showed an increase at a dose of 10 mg, thus retaining the activity, though less than **1**. Compounds **4** and **5**, on the other hand, showed activities of the same order as or rather superior to **1** at 1 mg/kg. The enhancing effect on the potency by introduction of the fatty acid residues to **3** is noteworthy.

As shown in Table II, all the three new compounds also displayed some effects in induction of delayed-type hypersensitivity to egg albumin in Hartley guinea pigs. As compared with **1**, **3** was somewhat less potent, while **4** showed the same order of activity at a dose of 1 μg/site. Compound **5** did not so markedly affect the adjuvant potency in this assay system. This last case might deserve more experimental investigation, because **5**, on the other hand, exhibited a potent tumor-suppressive property as can be seen in Table III. In fact, when Meth-A fibrosarcoma in BALB/c mice was used, **5** was shown to be highly effective in suppressing the tumor growth, while compound **1** was entirely inactive. There might possibly be no parallel relationship between the adjuvanticity and the tumor-suppression activity.

Table IV shows the results of an experiment on antiinfectious effect in ICR mice against *Escherichia coli* 22. Compound **3** was moderately active but again slightly less than **1**. Noticeable is that **4** and **5** were both potent comparably to **1**, thus serving satisfactorily as substitutes for **1** in stimulating the antibacterial resistance.

In summary, except for the tumor suppression test, compound **3** represents the minimal active structure essential for eliciting the effects in the immunostimulating assays so far examined. Its derivatives **4** and **5** were found to be capable of increasing resistance to bacterial infection as efficiently as **1**, and **5** proved to possess the unique tumor-suppression ability lacking in **1**. On the basis of these activity profiles, **4** and **5** are now undergoing more detailed examinations for antiinfectious and antitumor effectiveness, respectively.

(14) Anal. Calcd for $C_{30}H_{55}N_3O_8 \cdot 1.5H_2O$: C, 58.80; H, 9.54; N, 6.86. Found: C, 58.72; H, 9.70; N, 6.68. Amino acid analysis: Glu, 1.00; A_2pm, 1.09.

(15) G. Biozzi, B. Benacerraf, and B. N. Halpern, *Br. J. Exp. Pathol.*, **34**, 441 (1953).

(16) In a regression test, **5** also showed a significant effect on regression of the tumor on systemic administration to mice already bearing the tumor. This will be reported in due course.

(17) For convenience in preparation of the suspension, **5** was converted into its HCl salt [mp ~125 °C dec; $[\alpha]_D$ −9.0° (*c* 0.2, AcOH). Anal. Calcd for $C_{30}H_{55}N_3O_8 \cdot HCl \cdot H_2O$: C, 56.27; H, 9.13; N, 6.56; Cl, 5.54. Found: C, 55.88; H, 9.07; N, 6.61; Cl, 5.17].

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2-Arylpyrazolo[4,3-*c*]quinolin-3-ones: Novel Agonist, Partial Agonist, and Antagonist of Benzodiazepines

Sir:

We wish to report on three novel 2-arylpyrazolo[4,3-*c*]quinolin-3-ones, all of which possess extremely high affinity for the benzodiazepine receptors, yet the first (**3a**) is a very potent antagonist of diazepam, the second (**3b**) is a safe antianxiety agent, and the third (**3c**) is a hitherto unknown partial agonist of the benzodiazepine receptor.

In 1977, Squires and Braestrup[1a] and Möhler and Okada[1b] reported on the high-affinity binding sites for benzodiazepines in rat brain tissues. Since that time, the receptors have been found in several mammalian species, including humans.[1b,2] The purpose of the existence of such receptors is not clearly understood, but obviously their function is not merely to receive benzodiazepine derivatives. Rather, they happen to show high affinity for various benzodiazepine derivatives, such as chlordiazepoxide and diazepam, as well as for still unknown endogenous ligands with unknown biological importance.[3] While search for the yet elusive endogenous ligands continues,[4] a number of synthetic compounds with diverse structures have been found to possess high affinity for the benzodiazepine receptors,[5] and some of them were reported to be antagonistic toward the physiological effects of benzodiazepine anxiolytics.[6] It is important to learn the physiological properties and structural requirements of various benzodiazepine–receptor binders (agonists, antagonists, and

(1) (a) R. F. Squires and C. Braestrip, *Nature (London)*, **266**, 732 (1977); (b) H. Möhler and T. Okada, *Science*, **198**, 849 (1977).

(2) (a) Braestrup, R. Albrechtsen, and R. F. Squires, *Nature (London)*, **269**, 702 (1977); (b) C. Braestrup and R. F. Squires, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 3805 (1977); (c) C. Braestrup and R. F. Squires, *Eur. J. Pharmacol.*, **48**, 263 (1978).

(3) There have been a number of reports on putative endogenous ligands for the benzodiazepine receptors. However, they do not rigorously meet the criteria of endogenous ligands. For reviews, see (a) P. J. Marangos, S. M. Paul, F. K. Goodwin, and P. Skolnick, *Life Sci.*, **25**, 1093 (1979), and (b) I. L. Martin, *Trends Neurosci.*, **3**, 299 (1980).

(4) (a) L. G. Davis, H. McIntosh, and D. Reker, *Pharmacol. Biochem. Behav.*, **14**, 839 (1981); (b) J. H. Woolf and J. C. Nixon, *Biochemistry*, **20**, 4243 (1981).

(5) (a) A. S. Lippa, D. Critchett, M. C. Sano, C. A. Klepner, E. N. Greenblatt, J. Coupet, and B. Beer, *Pharmacol. Biochem. Behav.*, **10**, 831 (1979); (b) J. C. Blanchard, A. Boireau, C. Garret, and L. Julou, *Life Sci.*, **24**, 2417 (1978); (c) C. Braestrup, M. Nielsen, and C. E. Olsen, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 2288 (1980); (d) Guérémy, and A. Uzan, *Life Sci.*, **28**, 1439 (1981).

(6) (a) S. S. Tenen and J. D. Hirsch, *Nature (London)*, **288**, 609 (1980); (b) P. J. Cowen, A. R. Green, D. J. Nutt, and I. L. Martin, *ibid.*, **290**, 54 (1981); (c) P. Skolnick, S. Paul, J. Crawley, K. Rice, S. Barker, R. Weber, M. Cain, and J. Cook, *Eur. J. Pharmacol.*, **69**, 525 (1981). (d) J. N. Crawley, P. J. Marangos, S. M. Paul, P. Skolnick, and F. K. Goodwin, *Science*, **211**, 725 (1981). (e) W. Hunkeler, H. Möhler, L. Pieri, P. Polc. E. P. Bonetti, R. Cumin, R. Schaffner, and W. Haefely, *Nature (London)*, **290**, 514 (1981); (f) A. J. Czernick, B. Petrack, C. Tsai, F. R. Granat, R. K. Rinehart, H. J. Kalinsky, R. A. Lovell, and W. D. Cash, "High-Affintiy Occupancy of Benzodiazepine Receptors by the Novel Antagonist CGS 8216", ASPET/PSC Meeting, Calgary, Canada, 1981; *Pharmacologist*, **23**, 160 (1981); (g) P. Bernard, K. Bergen, R. Sobiski, and R. D. Robson, "CGS 8216—An Orally Effective Benzodiazepine Antagonist", ASPET/PSC Meeting, Calgary, Canada, 1981; *Pharmacologist*, **23**, 150 (1981); (h) A. Darragh, M. Scully, R. Lambe, I. Brick, C. O'Boyle, and W. W. Downie, *Lancet*, **2**, 8 (1981); (i) H. Möhler, W. P. Burkard, H. H. Keller, J. G. Richards, and W. Haefely, *J. Neurochem.*, **317**, 714 (1981).

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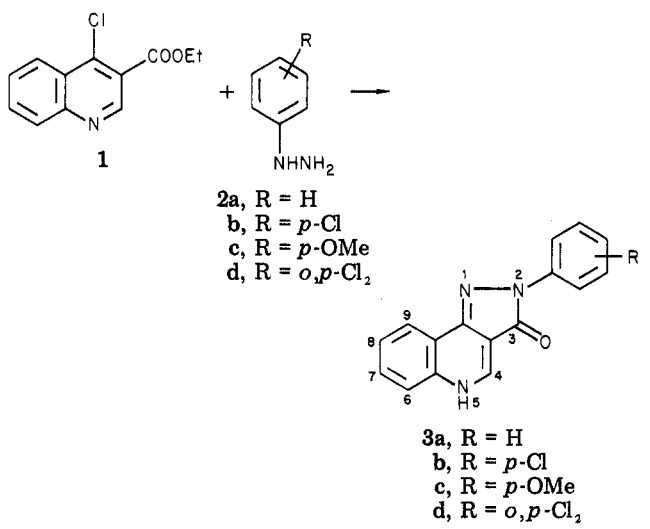
Table I. Biological Activities of 2-Arylpyrazolo[4,3-*c*]quinolin-3(5*H*)-ones (3)

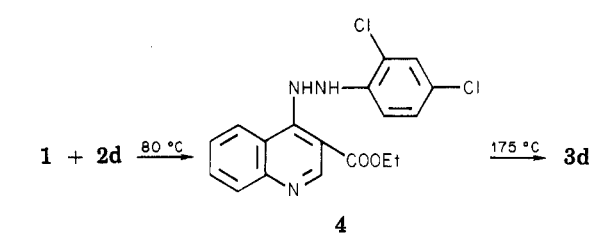
agent	benzodiazepine-receptor binding: in vitro IC_{50},[a,b] nM	benzodiazepine-receptor binding: in vivo ID_{50},[c] mg/kg (95% CL)	RMET:[d] ED_{50}, mg/kg po (95% CL)	diazepam interaction in RMET[d,e]: dose, mg/kg ip	diazepam interaction in RMET[d,e]: no. of animals protected	Cook-Davidson test[f]: dose, mg/kg po	Cook-Davidson test[f]: VI_{30}, mean % change	Cook-Davidson test[f]: FR_{10} control	Cook-Davidson test[f]: FR_{10} treated
		4.7		1	5/6				
		(3.23-7.04) ip		3	1/6				
3a	0.4	7.06	inactive to 30	10	0/6				
		(3.97-13.2) po		20	0/6				
		1.5 ip[g]				1	0.1	67	87
3b	0.6	3.30	0.90			3	10.2	112	217
		(1.56-6.85) po	(0.74-1.09)			10	18.2	81	234
						30	7.5	73	230
						3	6.5	61	98
3c	0.1	0.81	2.81	30	4/6	10	7.8	65	124
		(0.36-1.9) ip	(2.19-3.68)[h]			30	5.7	50	100
		0.91				1	5.9	82	97
diazepam	5.0	(0.57-1.5) ip	4.0[i]			3	6.1	85	131
		5.0				10	3.6	63	206
		(3.7-6.6) po				30	-23.8	76	240

[a] Reference 2b. [b] H. Möhler and T. Okada, *Life Sci.*, **20**, 2101 (1977). [c] R. S. L. Chang and S. H. Snyder, *Eur. J. Pharmacol.*, 48, 213 (1978). [d] Rat metrazol anticonvulsant test. G. Zbinden and L. O. Randall, *Adv. Pharmacol.*, 5, 213-291 (1967). See text for the dosing schedule. [e] Diazepam (5.4 mg/kg po) 1 h before metrazol challenge (24 mg/kg iv) blocked convulsion in 51 out of 54 rats. [f] L. Cook and A. B. Davidson in "The Benzodiazepines", S. Garattini, E. Mussini, and L. O. Randall, Eds., Raven Press, New York, 1973, pp 327-345. See text for the details of schedules. [g] Determined graphically. [h] Calculated from responses at 1, 3, and 10 mg/kg doses. Four out of twelve rats protected at 30 mg/kg, and one out of six rats protected at 100 mg/kg. [i] Raw data not suitable for statistical analysis.

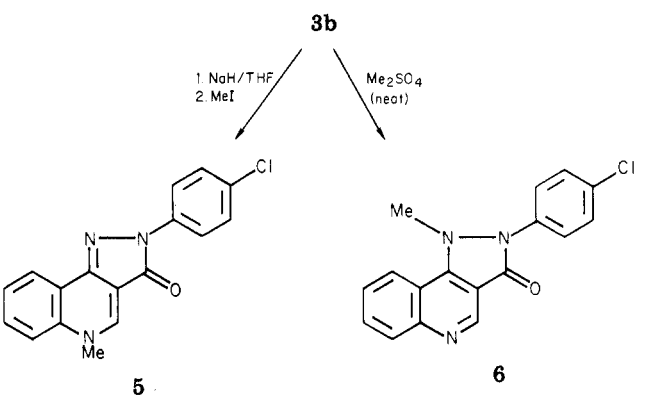
Scheme I



Scheme II



Scheme III



partial agonists) so that the true functions of these receptors may be fully understood. Because of their chemical similarity and biological diversity, the compounds reported here would be very useful for studying the nature of the benzodiazepine receptors; most importantly, they would be invaluable for investigating the modes of interaction between the receptors and various types of ligands.

When ethyl 4-chloroquinoline-3-carboxylate (**1**)[7] was heated with phenylhydrazine (**2a**) in xylene at 120 °C, **3a** was formed. Product **3a** [mp 328–331 °C. Anal. ($C_{16}H_{11}N_3O$) C, H, N] is soluble in aqueous alkali and is reprecipitated as the pH is lowered below 9. Treatment of **1** with substituted phenylhydrazines gave the corresponding products **3b** [mp 324–327 °C. Anal. ($C_{16}H_{10}ClN_3O$) C, H, N], **3c** [mp 268–270 °C. Anal. ($C_{17}H_{13}N_3O_2$) C, H, N], and **3d** [mp >350 °C. Anal. ($C_{16}H_9Cl_2N_3O$) C, H, N] (Scheme I).

Support for the 2-aryl structure of **3** was obtained from the reaction of **1** with the hindered phenylhydrazine derivative **2d**. When the reaction was carried out at 80 °C, a 4-hydrazinoquinoline intermediate **4** [mp 151–153 °C. Anal. ($C_{18}H_{15}Cl_2N_3O_2$) C, H, N] was obtained in good yield. Heating of **4** at a higher temperature (175 °C) in Dowtherm completed the cyclization reaction, obtaining **3d** as the sole product (Scheme II).

Examination of ^{13}C NMR spectra of **3b** and its sodium

(7) E. E. Kaslow and W. R. Clark, *J. Org. Chem.*, 18, 55 (1953).

salt in Me_2SO-d_6 revealed that the chemical shifts of C_3 were identical (δ 161.80 and 161.81, respectively), indicating that the enolate anion is not involved in the sodium salt formation.

Methylation of **3b** gave two *N*-methyl derivatives, **5** [mp 322–323 °C. Anal. ($C_{17}H_{12}ClN_3O$) H, N; C: calcd, 65.92; found, 65.20] or **6** [mp 158–161 °C. Anal. ($C_{17}H_{12}ClN_3O$) C, H, N], depending upon the conditions of the reaction (Scheme III). Chemical shifts of the introduced methyl group in ^{13}C NMR spectra (δ 41.44 and 40.96, respectively) clearly indicated that they were both *N*-methyl groups. Location of the *N*-methyl group in **5** (5-Me) and **6** (1-Me) was assigned from their 4-H chemical shifts (δ 8.86 and 9.33, respectively). The specific tautomeric structure **3** shown in Scheme I was assigned from its ^1H NMR spectrum (in Me_2SO-d_6) in which the 4-H appeared as a doublet [δ 8.86 (J = 6 Hz)] and collapsed to a singlet upon D_2O treatment or irradiation at the low-field exchangeable 5-H signal (δ 12.00).

Comparison of the UV spectra of **3b** with those of **5** and **6** further proved the 5-H structure of **3**. At neutral pH, the UV spectrum of **3b** in EtOH [λ_{max} 385 nm (log ϵ = 3.63)] resembles that of **5** [λ_{max} 383 nm (log ϵ = 3.79)], whereas at higher pH [λ_{max} 350 nm (log ϵ = 4.13)] it resembles that of **6** [λ_{max} 323 nm (log ϵ = 4.13)].

Biological profiles of **3** are summarized in Table I. Compounds **3a–c** have an order of magnitude greater affinity for the benzodiazepine receptors than diazepam. They are well absorbed and cross the blood–brain barrier as evidenced by the in vivo ID_{50} values. In the rat metrazol anticonvulsant test, the compounds were orally administered 1.5 h before the metrazol challenge (24 mg/kg iv). In this test, **3b** was more potent than diazepam, whereas **3a** was completely inactive, and **3c** gave a U-shaped dose–response curve with a peak effect at 10 mg/kg.[8] Diazepam antagonism by **3a** and **3c** was studied also in the rat metrazol model. Diazepam (5.4 mg/kg po) and test compounds (ip) were administered 1 h before the metrazol challenge.[9] In this study, **3a** blocked the anticonvulsant activity of diazepam at 3 mg/kg, and **3c** showed a hint of the antagonism at 30 mg/kg. Antianxiety effect of **3b** was confirmed by the Cook–Davidson behavior test. In the VI_{30} schedule, a milk reinforcement was delivered at an average of 30 s after the response. In the FR_{10} schedule, a milk reinforcement was delivered concomitantly with an electric foot shock following the tenth response, thus creating a conflict situation. A drug-induced decrement in VI_{30} performance was taken as an indication of a neurological deficit. A drug-induced enhancement of performance during the FR_{10} schedule was taken as an indication of antianxiety activity. Data shown in the table suggest substantial anticonflict activity for both **3b** and diazepam at 3, 10, and 30 mg/kg.

It is apparent from these biological data that **3a** is a potent benzodiazepine antagonist,[6f,g] **3b** is diazepam-like both in the anticonvulsant test and in the anticonflict behavioral test, and **3c** acts as an agonist (diazepam-like) at low doses and as an antagonist at higher doses.

All three compounds have very unique potentials as medicinal agents. Possible utilities of a benzodiazepine antagonist have been speculated in recent publications.[6e,g,h] In addition to its solid anxiolytic potency, the real advantage of **3b** over currently marketed benzodiazepines lies in its total lack of neurological deficits, as evidenced by no activity in the rat rotarod test up to a 300 mg/kg po dose.[10] This can also be seen from the VI_{30} data in the Cook–Davidson test.

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(8) See footnote *h* in Table I.
(9) See footnote *e* in Table I.
(10) Unpublished result from this laboratory.

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