to angiotensin I. The presence of 1% Me_2SO in the final incubation mixture caused no statistically significant effect on the renin activity.

Plasma assays utilized the endogenous renin and angiotensinogen. The final incubation mixture (100 μL) contained the following: plasma, 40 μL; maleate buffer, pH 6.0, 0.135 M; EDTA, 3 mM; PMSF, 1.4 mM; Me_2SO, 1%; 8-hydroxyquinoline, 3.4 mM (rat plasma only). Samples were incubated at 37 °C for 2 h and then placed in ice; an aliquot was analyzed for angiotensin I as before.

Bovine cathepsin D (Sigma) and porcine pepsin (Sigma) activities were assessed by the hydrolysis of hemoglobin at pH 3.1 and 1.9, respectively, at 37 °C, and measurements of the absorbance at 280 nm of the supernatant after precipitation with trichloroacetic acid.[30]

Stability Studies. Chymotrypsin (bovine pancrease, Sigma C 4129) and protease (pancreatic, Sigma P 4630) were dissolved at 0.1 mg/mL in 30 mM sodium phosphate/100 mM sodium chloride, pH 6.9. Fresh rat liver, kidney, and intestinal mucosa homogenates were prepared by homogenization of the tissues in 4 volumes of phosphate-buffered saline followed by low-speed centrifugation. The concentrated homogenate was diluted 10-fold in phosphate-buffered saline for incubation with the compounds.

Test compounds **21a**, **22a**, **25**, and **26** were introduced at 0.1 mg/mL by dilution of a 5 mg/mL methanol stock solution (20 μL/mL) into the enzyme solution or homogenate suspension. Aliquots were removed at intervals, quenched by addition of acetonitrile, then centrifuged, and filtered. Analyses of the incubation were carried out by HPLC on a Waters μBondapak analytical C-18 column (30 cm) eluted with a gradient from 20% CH_3CN/80% H_2O/0.1% TFA to 80% CH_3CN/20% H_2O/0.1% TFA over 20 min. Peak detection was by UV absorbance at 214 nM with quantitation using a Perkin-Elmer LCI-100 computing integrator.

Blood Pressure Measurement. The cardiovascular effects of **21a** were assessed in salt-depleted male cynomolgus monkeys (*Macaca fascicularis*) weighing 4–6 kg. Enhancement of base-line plasma renin activity was achieved by feeding the monkeys a low-salt chow and fruit diet in conjunction with two furosemide treatments (5 mg/kg, po; each dose) given 1 week and 1 day before the experiment. On the morning of the experiment, the monkeys were sedated with ketamine (10 mg/kg, im) and anesthetized with an intravenous bolus 15 mg/kg and a 0.1 mg/kg per min maintenance infusion of sodium pentobarbital (Abbott Laboratories, North Chicago, IL) through a leg vein catheter. Blood pressure and heart rate were monitored noninvasively at 2-min intervals by using an arm cuff with microphone and a sphygmomanometer (Narco Biosystems, Houston, TX). Blood samples were collected before and 5, 15, 30, and 60 min following drug administration for the determination of plasma renin activity. Compound **21a** was given as an intravenous bolus in three concentrations in a constant volume. Each animal received only one dose. Data were analyzed by using paired "*t*" test to examine within-group differences, and ANOVA was used to compare differences among groups.

(30) Mycek, M. In *Methods of Enzymology*; Perlmann, G., Lorand, L., Eds.; Academic: New York, 1970; Vol. XIX, p 286.

1,3-Diarylpyrazolo[4,5-*c*]- and -[5,4-*c*]quinolin-4-ones. 4. Synthesis and Specific Inhibition of Benzodiazepine Receptor Binding

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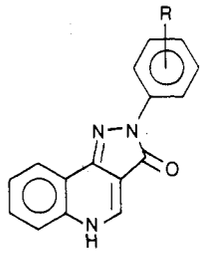
A series of 1,3-diarylpyrazolo[4,5-c]- and -[5,4-c]quinolin-4-ones were prepared and tested for their ability to displace [^{3}H]flunitrazepam from bovine brain membranes. While the 1,3-diarylpyrazolo[4,5-c]quinoline derivatives showed affinity for the receptor site, their [5,4-c] isomers were devoid of binding activity.

The great structural differences among non-benzodiazepine compounds with affinity for the benzodiazepine receptor make it difficult to generalize about the molecular requirements of the recognition site of the receptor itself. Consequently, in an effort to elucidate these requirements, numerous compounds have been synthesized and tested.[1]

Most of the reported structures displayed a fused nitrogen-containing heteroaromatic system as a common feature. In particular, some 2-arylpyrazolo[4,3-*c*]-quinolin-3-ones (CGS series[2]) have been reported to have an extremely high affinity for the benzodiazepine receptor site (see Table I). Following this finding, the synthesis and binding activity of some 2-aryl- and 1-aryl-3-methylpyrazolo[4,5-*c*]quinoline derivatives[3-5] have been reported. In order to provide a better insight into the molecular requirements of the recognition site of the receptor and to provide further structure–activity relationships in our series of pyrazoloquinolines, we are now reporting the synthesis and binding activity of some 1,3-diarylpyrazolo[4,5-*c*]quinolin-4-ones. The 3-methyl was replaced by the phenyl group to provide information on how a bulkier group than methyl would affect the binding potency.

Table I. Benzodiazepine Receptor Binding of CGS Series[2]



CGS a-c

compd	R	IC_{50},[a,b] nM
CGSa	H	0.4
CGSb	4-Cl	0.6
CGSc	4-OCH_3	0.1

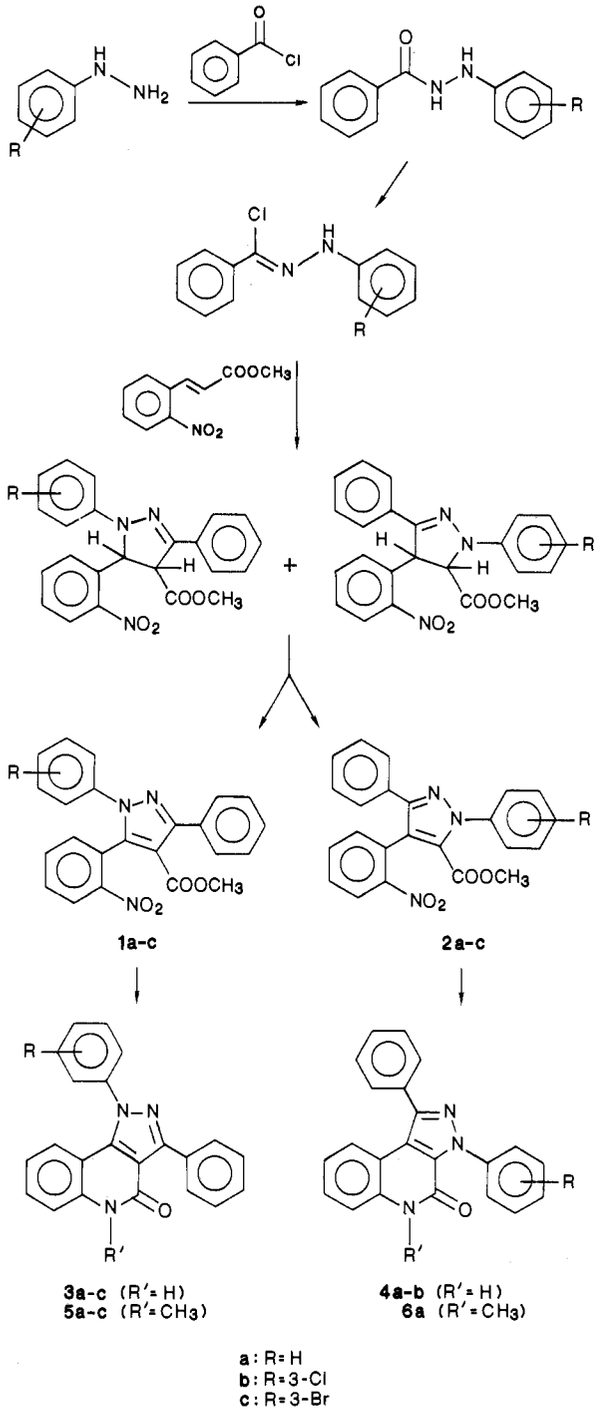
[a] Braestrup, C.; Squire, R. F. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3805. [b] Möhler, H.; Okada, T. *Life Sci.* **1977**, *20*, 2101.

† Dipartimento di Scienze Farmaceutiche.
‡ Istituto Policattedra di Discipline Biologiche.

(1) Williams, M. *J. Med. Chem.* **1983**, *26*, 619.

0022-2623/87/1830-1737$01.50/0

Scheme I



Chemistry. In a first approach, the 1,3-diarylpyrazoloquinolines were prepared as outlined in Scheme I.

The 1,3-dipolar cycloaddition of (α-chlorobenzylidene)arylhydrazines to the *E* isomer of methyl 2-nitrocinnamate gave rise to a mixture of the two isomeric pyrazolines, which were isolated together from the starting materials. We were unable to separate these two isomers because of their very close R_f values. Their identification and ratio ensued from their ^{1}H NMR spectra. The ^{1}H NMR spectra of the mixture of the pyrazolines showed a multiplet at 8.3–6.6 ppm due to the aryl protons, four doublets in the spectral range 6.3–4.3 ppm attributable to the two pyrazoline protons of each isomer, and a singlet at about 4.8 ppm due to the methyl groups of the two isomers. The signals of the methyl groups of the two isomers in fact coincide. Oxidation of the mixture with lead tetraacetate yielded the corresponding triarylpyrazoles, which were separated by column chromatography. The ^{13}C NMR spectra of the triarylpyrazoles **1a–c** and **2a–c** were used in structural characterization.

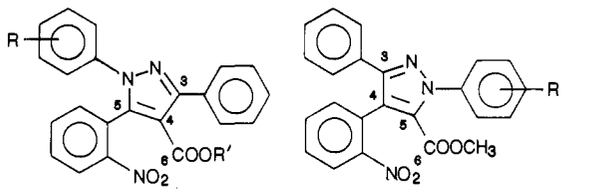
The key tools of this analysis were the chemical shift values of the C-4 pyrazole atoms of the two isomers. An earlier ^{13}C NMR study on pyrazole-4-carboxylate[5] enabled us to know that the chemical shift value of the C-4 pyrazole atom when it bears a carboxylate group is about 110 ppm. Since the overall C-4 chemical shift of compounds **1a–c** fell in this very range, it follows that their C-4 bears a carboxylate group. Thus the structure of methyl 1,3,5-triarylpyrazole-4-carboxylates was attributed to compounds **1a–c**. It followed that the structure of methyl 1,3,4-triarylpyrazole-5-carboxylates ought to be attributed to the isomers **2a–c**. This is in agreement with the C-4 chemical shift values of compounds **2a–c**, which are about 120 ppm (see Table II). In fact, the C-4 of compounds **2a–c** in this case bears an *o*-nitrophenyl group.

Reduction and cyclization of compounds **1a–c** and **2a,b** gave the target compounds 1,3-diarylpyrazolo[4,5-*c*]-quinolin-4-ones (**3a–c**) and 1,3-diarylpyrazolo[5,4-*c*]-quinolin-4-ones (**4a,b**), respectively.

We were unable to obtain 1-phenyl-3-(3-bromophenyl)pyrazolo[5,4-*c*]quinolin-4-one in the pure state since the reduction of the corresponding pyrazole **2c** yielded a partial debromination. Compounds **3a–c** and **4a** were transformed into their 5-*N*-methyl derivatives **5a–c** and **6a**, respectively.

Compounds **3a–c** and **4a,b** and their methyl derivatives **5a–c** and **6a** were tested for their ability to displace [^{3}H]flunitrazepam from bovine brain membranes. From this preliminary binding study it appeared that some 1,3-diarylpyrazolo[4,5-*c*]quinolin-4-ones were more active

Table II. Carbon-13 Data[a] of the Most Significant Carbon Atoms

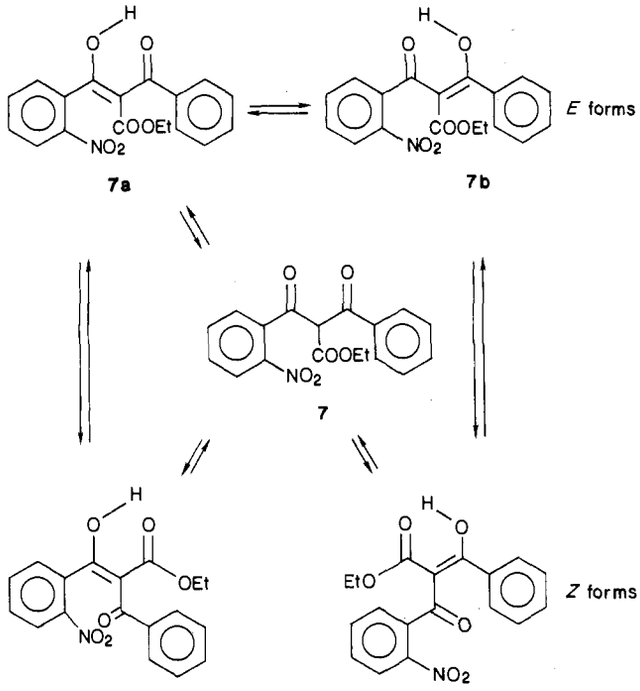


compd	C-3	C-4	C-5	C-6
1a	153.71	110.85	142.86	162.98
1b	153.98	111.44	142.73	162.76
1c	152.72	110.84	143.12	162.37
2a	150.00	121.04	140.19	159.09
2b	150.52	121.55	141.10	158.92
2c[b]	149.77	121.18	140.97	158.55
8a	153.81	111.25	142.76	162.48
8b	154.16	111.44	142.81	162.29
8c[b]	152.81	111.12	143.06	161.79

[a] $CDCl_3$ solvent unless otherwise stated. [b] Me_2SO-d_6.

(2) Yokoyama, N.; Ritter, B.; Neubert, A. D. *J. Med. Chem.* **1982**, *25*, 337.
(3) Cecchi, L.; Melani, F.; Palazzino, G.; Filacchioni, G.; Martini, C.; Pennacchi, E.; Lucacchini, A. *Farmaco, Ed. Sci.* **1985**, *40*, 509.
(4) Melani, F.; Cecchi, L.; Palazzino, G.; Filacchioni, G.; Martini, C.; Pennacchi, E.; Lucacchini, A. *J. Med. Chem.* **1986**, *29*, 291.
(5) Melani, F.; Cecchi, L.; Palazzino, G.; Filacchioni, G.; Martini, C.; Pennacchi, E.; Lucacchini, A. *J. Pharm. Sci.* **1986**, *75*, 1175.

Scheme II

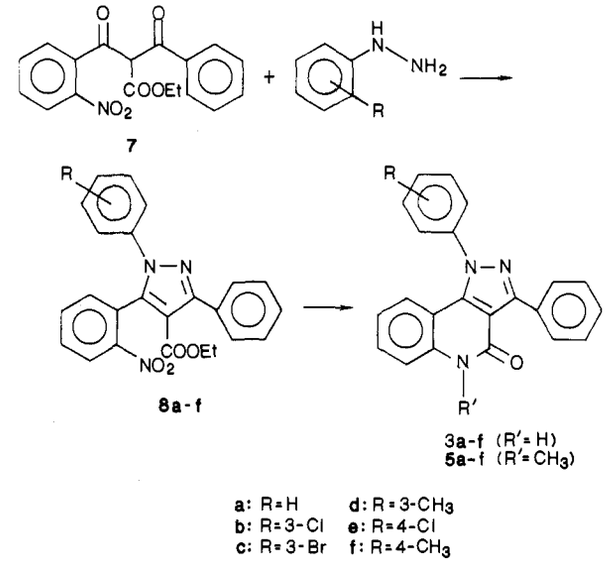


than the corresponding 1-aryl-3-methylpyrazolo[4,5-*c*]-quinolin-4-ones,[3-5] while their [5,4-*c*] isomers were completely devoid of binding activity.

The number of steps in the cycloaddition reaction and the finding that the [5,4-*c*] isomers were devoid of binding activity led us to drop this synthetic pathway. Thus, in order to synthesize significant quantities of 1,3-diarylpyrazolo[4,5-*c*]quinolin-4-ones for further evaluation, we explored an alternative route. Since all the other reported pyrazoloquinolines[3-5] were satisfactorily prepared by reacting ethyl (2-nitrobenzoyl)acetylacetate with arylhydrazines, we thought that the 1,3-diarylpyrazolo[4,5-*c*]quinolines could be obtained with an analogous procedure. Ethyl (2-nitrobenzoyl)benzoylacetate (**7**)[6] was the logical β-diketone to employ. However, it has been reported[7] that unsymmetrical diaryl β-diketones may exist either in the *E* and *Z* forms or in the diketone form, depending on the nature of the substituent and on the solvent. Since the presence of the electron-withdrawing nitro group would stabilize the enol forms, we decided to investigate by ^{1}H and ^{13}C NMR study what conditions would stabilize the **7a** form (Scheme II).

The latter was the sole form that would yield the desired ethyl 1-aryl-3-phenyl-5-(2-nitrophenyl)pyrazole-4-carboxylates. Thus the ^{1}H and ^{13}C NMR spectra of **7** in $CDCl_3$, MeOH-d_4, and alkaline MeOH-d_4 were obtained. In $CDCl_3$, compound **7** was present in both the *E* and *Z* forms (65% and 35%, respectively), meaning a very slow equilibrium between the two forms. On the other hand, in MeOH-d_4, the equilibrium between the *E* and *Z* forms was so fast that in the ^{1}H and ^{13}C NMR spectra of **7** all the signals were broad. The ^{1}H NMR spectrum of **7** in MeOH-d_4 in the presence of NaOH revealed the presence of only one *E* form (CH_2 ester appeared as a sharp quartet at 3.69 ppm). We could not, however, know which of the two *E* forms (**7a** or **7b**) was present. The site of the enolization was readily assigned by a ^{13}C NMR study. In the coupled spectrum, the more downfield signal (197.76 ppm, carbonyl carbon) appeared as a triplet (^3J = 3.4 Hz coupling constant between the carbonyl carbon and the ortho hydrogens of the phenyl ring), meaning that the carbonyl carbon was bound to a phenyl ring which was freely rotating along its σ bond. In agreement with this, the enol carbon signal at 189.04 ppm appeared as a doublet (^3J = 3.4 Hz), because of its coupling solely with an ortho proton of the phenyl ring, meaning that the other ortho position of the phenyl ring was occupied by the nitro group. It follows that the only *E* form present in alkali MeOH-d_4 was the **7a** form. This is the form that, when allowed to react with arylhydrazines, will give rise to ethyl 1-aryl-3-phenyl-5-(2-nitrophenyl)pyrazole-4-carboxylates. Thus the reaction between **7** and arylhydrazines was carried out in methanol. The alkaline medium was provided by the hydrazines themselves.

Scheme III



A sole pyrazole to which the structure of ethyl 1-aryl-3-phenyl-5-(2-nitrophenyl)pyrazole-4-carboxylates (**8a–f**) was attributed (see Scheme III) was obtained from the reaction mixture. Comparison between the ^{13}C NMR data of **1a–c** and **8a–c** confirmed the attributed structures (see Table II). Reduction and cyclization of compounds **8a–f** yielded compounds **3a–f**. The structure of the latter was further confirmed by the identity of the physical and analytical data of compounds **3a–c** with those already prepared.

Compounds **3a–f** were transformed into their 5-*N*-methyl derivatives **5a–f**.

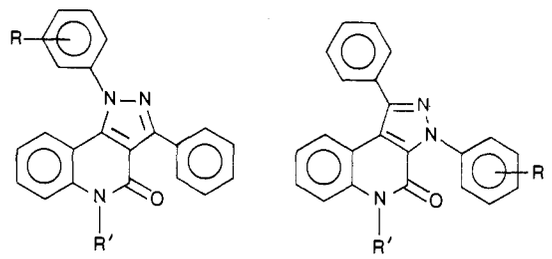
Binding to the Benzodiazepine Receptor. All the synthesized compounds were tested for their ability to displace specific [^{3}H]flunitrazepam binding from bovine brain membranes. First, a single concentration of the compounds to be tested was examined, followed by the examination of the IC_{50} values from log–probit plots of the more active ones. The resulting data are listed in Table III. The physical properties of new compounds are listed in Table IV.

The newly synthesized 1,3-diarylpyrazolo[4,5-*c*]-quinolin-4-one derivatives **3a–f** and **5a–f** interact with the benzodiazepine receptor at micromolar concentrations as indicated by the IC_{50} values listed in Table III. On the other hand, the [5,4-*c*] isomers **4a,b** and **6a** are completely inactive. The IC_{50} values of diazepam and chlordiazepoxide, two classical benzodiazepines in clinical use, are also included as reference compounds.

From the results it appears that a variation of the kind of condensation occurring between the pyrazole and the

(6) Gabriel, S.; Gerhard, W. *Ber. Dtsch. Chem. Ges.* **1921**, *54*, 1613.
(7) Courtot, P.; Le Saint, J.; Pichon, R. *Bull. Soc. Chim. Fr.* **1975**, 2538.

Table III. Inhibition of [^{3}H]Flunitrazepam Binding



3a-c **5a-c** **4a,b** **6a**

compd	R	R′	I%[a] (μM)	IC_{50},[b] μM
3a	H	H		21 ± 2.5
3b	3-Cl	H		3.0 ± 0.1
3c	3-Br	H		1.5 ± 0.1
3d	3-Me	H		3.0 ± 0.2
3e	4-Cl	H		30 ± 1.8
3f	4-Me	H		39 ± 2.1
4a	H	H	10 (34)	
4b	3-Cl	H	8 (34)	
5a	H	Me		3.1 ± 0.09
5b	3-Cl	Me		0.07 ± 0.02
5c	3-Br	Me		0.10 ± 0.009
5d	3-Me	Me		0.09 ± 0.05
5e	4-Cl	Me		1.16 ± 0.1
5f	4-Me	Me		1.1 ± 0.095
6a	H	Me	21 (3.4)	
diazepam				0.025 ± 0.002
chlordiazepoxide				0.79 ± 0.07

[a] Percents of inhibition (I%) of specific [^{3}H]flunitrazepam binding are means ± SEM of five determinations. [b] Concentrations necessary for 50% inhibition (IC_{50}) are means ± SEM of seven determinations.

quinoline moiety dramatically affects the inhibitory potency. The pyrazolo[4,5-*c*]quinolines **3a–f** and **5a–f** showed affinity for the benzodiazepine receptor while their [5,4-*c*] isomers **4a,b** and **6a** are completely devoid of binding activity. It follows that the position of the nitrogens in the heterocyclic system is crucial. In the [4,5-*c*] series, the replacement of the methyl group at position 3[3,4] with the bulkier phenyl group resulted in an enhancement of binding potency. The affinity to the benzodiazepine receptor of the 1,3-diaryl and 1-aryl-3-methyl derivatives tends to vary in the same manner, the binding potency decreasing when the substituent on the 1-phenyl ring is displaced from the meta to the para position or when the 1-phenyl ring carries no substituent.

In agreement with what has been previously[4] observed, the replacement of the hydrogen atom in position 5 with a methyl group led to a significant increase in the ability to displace the radioligand, as shown by the comparison between the IC_{50} values of the 5-NH derivatives **3a–f** and those of the 5-NCH_3 derivatives **5a–f**. For instance, **5a** was about 7 times more potent than its parent compound **3a**, **5b** was about 43 times more potent than **3b**, **5c** was 15 times more potent than **3c**, and so on.

The important role played by the position of the substituent on the 1-phenyl ring[3-5] is also confirmed. In fact, the meta-substituted 1,3-diarylpyrazolo[4,5-*c*]quinolines are the most active.

In conclusion, the replacement of the methyl group in position 3 with the phenyl group and the replacement of the hydrogen in position 5 with a methyl group afforded two compounds, **5b** and **5c**, that showed an inhibition potency that was higher than that of chlordiazepoxide and close to that of diazepam.

Experimental Section

Chemistry. All melting points were determined on a Büchi 510 capillary melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded with a Varian EM 360 instrument; chemical shifts are reported in δ (ppm) downfield from internal Me_4Si. The natural abundance ^{13}C NMR spectra were run on a Varian FT-80A spectrometer at 20 MHz in the Fourier transform mode. All samples were recorded in 10 mm o.d. tubes at the probe temperature (30 °C) with a concentration in $CDCl_3$, MeOH-d_4, or Me_2SO-d_6 of approximately 10% w/v, which provided the deuterium signal for the field frequency lock. Chemical shifts were measured relative to the central peak of the solvent ($CDCl_3$, 76.9 ppm; MeOH-d_4, 49.0 ppm; Me_2SO-d_6, 39.6 ppm) and corrected to internal Me_4Si. Typical acquisition parameters included a spectral width of 5000 Hz, a flip angle of 42°, and an interpulse delay between acquisitions of 510 μs. Chemical shift values were reproducible to better than ±0.05 ppm. The decoupled spectra

Table IV. Physical Properties of the Prepared Compounds

no.	formula	R	R′	mp, °C	solv crystn	yield, % (method)
1a	$C_{23}H_{17}N_3O_4$	H		140–142	EtOH	33
1b	$C_{23}H_{16}ClN_3O_4$	3-Cl		113–115	EtOH	30
1c	$C_{23}H_{16}BrN_3O_4$	3-Br		108–110	EtOH	30
2a	$C_{23}H_{17}N_3O_4$	H		125–127	EtOH	33
2b	$C_{23}H_{16}ClN_3O_4$	3-Cl		118–120	EtOH	30
2c	$C_{23}H_{16}BrN_3O_4$	3-Br		99–101	EtOH	30
3a	$C_{22}H_{15}N_3O$	H	H	>300	DMF/H_2O	52
3b	$C_{22}H_{14}ClN_3O$	3-Cl	H	>300	DMF	59
3c	$C_{22}H_{14}BrN_3O$	3-Br	H	>300	DMF/H_2O	35
3d	$C_{23}H_{17}N_3O$	3-CH_3	H	>300	DMF	56
3e	$C_{22}H_{14}ClN_3O$	4-Cl	H	>300	AcOH	48
3f	$C_{23}H_{17}N_3O$	4-CH_3	H	>300	DMF	65
4a	$C_{22}H_{15}N_3O$	H	H	>300	DMF	69
4b	$C_{22}H_{14}ClN_3O$	3-Cl	H	>300	AcOH	65
5a	$C_{23}H_{17}N_3O$	H	CH_3	193–195	EtOH	20 (A, B)
5b	$C_{23}H_{16}ClN_3O$	3-Cl	CH_3	152–153	EtOH	30 (A)
5c	$C_{23}H_{16}BrN_3O$	3-Br	CH_3	171–173	EtOH	24 (B)
5d	$C_{24}H_{19}N_3O$	3-CH_3	CH_3	127–128	EtOH	13 (A)
5e	$C_{23}H_{16}ClN_3O$	4-Cl	CH_3	238–240	Me_2SO	15 (B)
5f	$C_{24}H_{19}N_3O$	4-CH_3	CH_3	232–235	Me_2SO	20 (B)
6a	$C_{23}H_{17}N_3O$	H	CH_3	172–174	EtOH	19 (A)
8a	$C_{24}H_{19}N_3O_4$	H		138–139	EtOH	60
8b	$C_{24}H_{18}ClN_3O_4$	3-Cl		103–104	EtOH	40
8c	$C_{24}H_{18}BrN_3O_4$	3-Br		109–110	EtOH	41
8d	$C_{25}H_{21}N_3O_4$	3-CH_3		140–142	EtOH	45
8e	$C_{24}H_{18}ClN_3O_4$	4-Cl		146–148	EtOH	47
8e	$C_{24}H_{18}ClN_3O_4$	4-Cl		146–148	EtOH	47
8f	$C_{25}H_{21}N_3O_4$	4-CH_3		157–159	EtOH	71

were obtained without pulse delay. The coupled spectra with nuclear Overhauser effect (NOE) were obtained by putting the decoupler on during a pulse delay of 1.6 s and off during an acquisition time of 0.8 s.

Silica gel plates (Merck F_{254}), silica gel 60 (Merck; 70–230 mesh), and aluminum oxide 90 (Merck; 70–230 mesh) were used for analytical and column chromatography. The elemental analyses were performed for C, H, N with a Perkin-Elmer 260C elemental analyzer, and results were within ±0.4% of the theoretical values.

General Procedure for Preparation of Benzoylhydrazines. The title compounds were obtained by reacting the arylhydrazine hydrochlorides with benzoyl chloride as described in ref 8.

1-Benzoyl-2-phenylhydrazine: white crystals; mp 165–168 °C (EtOH) (lit.[9] mp 168 °C); 50% yield.

1-Benzoyl-2-(3-chlorophenyl)hydrazine: white crystals; mp 148–150 °C (EtOH); 73% yield.

1-Benzoyl-2-(3-bromophenyl)hydrazine: white crystals; mp 154–155 °C (EtOH); 70% yield.

General Procedure for the Preparation of (α-Chlorobenzylidene)arylhydrazines. The title compounds were obtained from benzoylarylhydrazines as described in ref 8 and 10.

1-(α-Chlorobenzylidene)-2-phenylhydrazine: white crystals; mp 124–127 °C (EtOH) (lit.[10] mp 129.5–130.5 °C); 40% yield.

1-(α-Chlorobenzylidene)-2-(3-chlorophenyl)hydrazine: white crystals; mp 91–93 °C (EtOH); 60% yield.

1-(α-Chlorobenzylidene)-2-(3-bromophenyl)hydrazine: white crystals; mp 90–93 °C (EtOH); 40% yield.

General Procedure for 1,3-Dipolar Cycloaddition Reaction. To a solution of 1-(α-chlorobenzylidene)-2-arylhydrazine (3.5 mmol) and methyl *trans*-2-nitrocinnamate (10.6 mmol) in dry benzene (30 mL) was added dropwise a solution of Et_3N (14.2 mmol) in dry benzene (20 mL). The mixture was refluxed for 5 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under vacuum. The residue was chromatographed on a silica gel column with an eluting system of cyclohexane/EtOAc (6:4). Evaporation in vacuo of the solvents of the first eluates yielded a red residue, which consisted of a mixture (50:50) of the isomers of the methyl triarylpyrazolinecarboxylates.[11]

General Procedure for Oxidation of Methyl Triarylpyrazolinecarboxylates. To a solution of the mixture of the pyrazolines (3.3 mmol) in glacial AcOH (5 mL) was added lead tetraacetate (5 mmol), dropwise with stirring. The resulting suspension was heated in a steam bath at 60 °C for 5 min. The reaction was finished when the starting red acetic mixture became bright yellow. The excess of lead tetraacetates was filtered off. The solution was poured into water and adjusted to pH 7 (Na_2CO_3, saturated solution). Extraction with EtOAc and evaporation of the dry (Na_2SO_4) organic solvent gave rise to a residue, which was chromatographed on an aluminum oxide column with an eluting system of cyclohexane/EtOAc (8:2). Evaporation of the solvents of the first eluates gave compounds **2a–c** while compounds **1a–c** were present in the second running eluates.

General Procedure for Preparation of Ethyl 1,3,5-Triarylpyrazole-4-carboxylates 8a–f. A solution of equimolar amounts of **7**,[6] arylhydrazine hydrochloride, and Et_3N in dry MeOH was refluxed for 6 h. Upon cooling, a crystalline precipitate was separated.

General Procedure for Preparation of 1,3-Diaryl-4,5-dihydro-1*H*-pyrazolo[4,5-*c*]quinolin-4-ones 3a–f. To a solution of methyl or ethyl 1,3,5-triarylpyrazole-4-carboxylate (**1a–c** and **8a–f**) (1 g) in EtOAc (200 mL) was added 10% Pd/C (0.4 g). The mixture was hydrogenated in a Parr apparatus at 50 psi for 12 h. The catalyst was filtered off and the solvent evaporated in vacuo. The residue was made up of a mixture of amino derivative and the corresponding 1,3-diarylpyrazoloquinolinone. This mixture was heated in an oil bath over 150 °C for 30 min. The glassy cooled mass was treated with Et_2O, and the solid product was collected.

Compound 3a displayed the following: ^1H NMR (Me_2SO-d_6) δ 11.5 (br m, 1 H, NH), 8.5–6.8 (four m, 14 H, aromatic protons).

General Procedure for Preparation of 1,3-Diaryl-4,5-dihydro-3*H*-pyrazolo[5,4-*c*]quinolin-4-ones 4a,b. The title compounds were obtained by reducing methyl 1,3,4-triarylpyrazole-5-carboxylates **2a–c** under the same conditions as described above. The residue was made up of the title compounds alone except for compound **2c**, which gave rise to a mixture of **4a** and **4c**. The latter was never isolated.

Compound **4a** displayed the following: ^1H NMR (Me_2SO-d_6) δ 11.9 (br s, 1 H, NH), 8.0–7.0 (m, 14 H, aromatic protons).

Preparation of 5-*N*-Methyl Derivatives of 1,3-Diarylpyrazoloquinolin-4-ones 5a–f and 6a. Method A. To a solution of compounds **3a,b,d** and **4a** (0.8 mmol) in DMF (30 mL) were added anhydrous K_2CO_3 (1 mmol) and MeI (1.6 mmol). The mixture was warmed with stirring for 24 h. The solid residue was filtered off from the warm mixture, and the solution was poured into water. The precipitate was collected and chromatographed on a silica gel column with an eluting system of cyclohexane/EtOAc (1:1).

Method B. To a suspension of compounds **3a,c,e,f** (0.8 mmol) in dry MeOH (100 mL) were added triethylmethylammonium hydroxide[12] (16 mmol, 2 N solution in MeOH) and MeI (8.0 mmol) slowly in small portions. The mixture was refluxed for 48 h. The suspension was concentrated in vacuo, diluted with water, and adjusted to pH 9 with NaOH (10% solution). Extraction with EtOAc and evaporation of the dry (Na_2SO_4) organic solvent afforded a residue, which was chromatographed on a silica gel column with an eluting system of cyclohexane/AcOEt (7:3).

Binding Studies. Tritiated flunitrazepam was obtained from New England Nuclear (Dreieichenhain, West Germany) and had a specific activity of 78 Ci/mmol and radiochemical purity >99%. All the other chemicals were reagent grade and obtained from commercial suppliers. Membranes from bovine brains were prepared as described in ref 4. Benzodiazepine receptor binding activity was determined as follows: diluted membranes (0.4–0.5 mg of proteins) were incubated in triplicate with 0.6 nM [^3H]-flunitrazepam at 0 °C (90 min) in 50 mM Tris·HCl buffer in a final volume of 500 μL. After incubation the samples were diluted at 0 °C with 5 mL of the assay buffer and were immediately filtered under reduced pressure through glass fiber filter disks (Whatman GF/B). Afterward the samples were washed with 5 mL of the same buffer, dried, and added to 8 mL of HP Beckman scintillation liquid containing 0.4 mL of a solution of 0.01 M KOH in plastic vials.

All except three of the 1,3-diarylpyrazoloquinoline derivatives were dissolved in ethanol and added to the assay mixture to a final volume of 500 μL. Blank experiments were carried out to determine the effect of ethanol (2%) on the binding. The ethanol-insoluble compounds **4a,b** and **3b** were dissolved in Me_2SO and added to the same mixture. Blank experiments were carried out to determine the effect of Me_2SO (1–2%) on the binding. Specific binding was obtained by subtracting nonspecific binding from total binding and approximated to 85–90% of the total binding. The amount of nonspecific binding was determined by incubating membranes and [^3H]flunitrazepam in the presence of 10 μM diazepam.

The estimation of proteins was based on the method of Lowry et al.[13] after membrane solubilization with 0.75 N NaOH. Bovine serum albumin was utilized as the standard.

The concentrations of the 1,3-diarylpyrazoloquinolines that inhibit specific [^3H]flunitrazepam binding by 50% (IC_{50}) were determined by log–probit analysis with seven concentrations of the displacers, each performed in triplicate.

Registry No. **1a**, 109334-55-6; **1b**, 109334-56-7; **1c**, 109334-57-8; **2a**, 109334-58-9; **2b**, 109334-59-0; **2c**, 109334-60-3; **3a**, 89522-18-9; **3b**, 109334-68-1; **3c**, 109334-69-2; **3d**, 109334-70-5; **3e**, 109334-71-6; **3f**, 109334-72-7; **4a**, 109334-73-8; **4b**, 109334-74-9; **5a**, 109334-75-0; **5b**, 109363-30-6; **5c**, 109334-76-1; **5d**, 109334-77-2; **5e**, 109334-78-3; **5f**, 109334-79-4; **6a**, 109334-80-7; **7**, 109334-61-4; **8a**, 109334-62-5; **8b**, 109334-63-6; **8c**, 109334-64-7; **8d**, 109334-65-8; 8e, 109334-66-9;

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8f, 109334-67-0; PhC(Cl)=NNHPh, 15424-14-3; m-PhC(Cl)=NNHC$_6$H$_4$Cl, 50802-12-5; m-PhC(Cl)=NNHC$_6$H$_4$Br, 109334-48-7; (E)-o-NO$_2$C$_6$H$_4$CH=CHCO$_2$Me, 39228-29-0; PhNHNH$_2$·HCl, 59-88-1; m-ClC$_6$H$_4$NHNH$_2$·HCl, 2312-23-4; m-BrC$_6$H$_4$NHNH$_2$·HCl, 27246-81-7; m-MeC$_6$H$_4$NHNH$_2$·HCl, 637-04-7; p-ClC$_6$H$_4$NHNH$_2$·HCl, 1073-70-7; p-MeC$_6$H$_4$NHNH$_2$·HCl, 637-60-5; Et$_3$MeN$^+$OH$^-$, 109334-81-8; methyl 1,3-diphenyl-5-(2-nitrophenyl)-4-pyrazolinecarboxylate, 109334-49-8; methyl 1-(3-chlorophenyl)-5-(2-nitrophenyl)-3-phenyl-4-pyrazolinecarboxylate, 109334-50-1; methyl 1-(3-bromophenyl)-5-(2-nitrophenyl)-3-phenyl-4-pyrazolinecarboxylate, 109334-51-2; methyl 1,3-diphenyl-4-(2-nitrophenyl)-5-pyrazolinecarboxylate, 109334-52-3; methyl 1-(3-chlorophenyl)-4-(2-nitrophenyl)-3-phenyl-5-pyrazolinecarboxylate, 109334-53-4; methyl 1-(3-bromophenyl)-4-(2-nitrophenyl)-3-phenyl-5-pyrazolinecarboxylate, 109334-54-5.

Discovery and Anticonvulsant Activity of the Potent Metabolic Inhibitor 4-Amino-*N*-(2,6-dimethylphenyl)-3,5-dimethylbenzamide[1]

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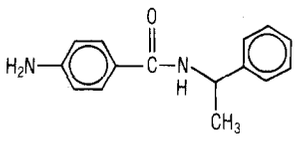
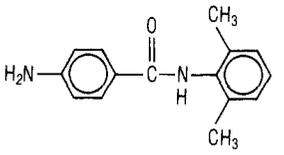
Compound **2** [4-amino-*N*-(2,6-dimethylphenyl)benzamide] is an effective anticonvulsant in several animal models. For example, following oral administration to mice, it antagonized maximal electroshock (MES) induced seizures with an ED_{50} of 1.7 mg/kg. During drug disposition studies with **2**, we found that it was rapidly metabolized by N-acetylation. Thirty minutes after oral administration of 1.7 mg/kg of **2** to mice, plasma concentrations of parent drug and the *N*-acetyl metabolite **5** were 1.09 and 0.41 μg/mL, respectively. Six hours postadministration the concentrations were 0.23 and 0.22 μg/mL, respectively. In order to sterically preclude or diminish the rate of metabolic N-acetylation, we synthesized analogues of **2** possessing either one (**3**) or two (**4**) methyl groups ortho to the 4-amino substituent. Both compounds antagonized MES-induced seizures after administration to mice; oral ED_{50} values for **3** and **4** were 3.5 and 5.6 mg/kg, respectively. Compound **3** was rapidly metabolized by N-acetylation. However, **4** provided exceptionally high and long-lived plasma concentrations of parent drug; no *N*-acetyl metabolite could be detected. While **2** and **3** had no pharmacologically relevant effects on hexobarbital-induced sleeping time in mice, **4** was a potent, dose-dependent potentiator of sleeping time. Oral administration of 375 μg/kg led to a 61% increase in sleeping time relative to control values. Thus, **4** represents one of the most potent potentiators of hexobarbital-induced sleeping time described to date.

The 4-aminobenzamides represent a fertile series for the discovery of anticonvulsants. Compound **1** [4-amino-*N*-(α-methylbenzyl)benzamide] inhibited maximal electroshock (MES) induced seizures in mice, and the ED_{50} following oral administration was 10.3 mg/kg.[2] Compound



1

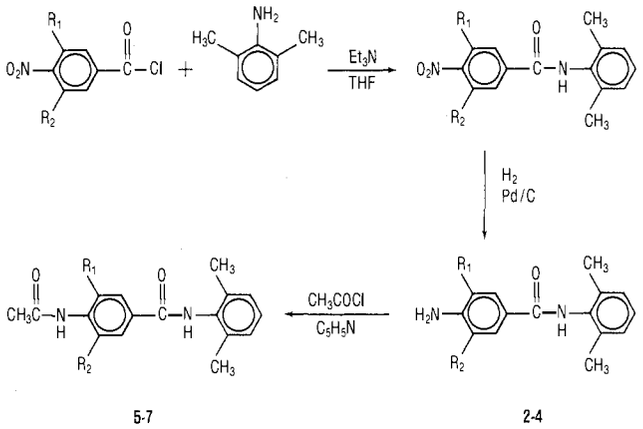


2

1 was resolved, and the racemate and its two enantiomers were evaluated in a variety of anticonvulsant assays. Surprisingly, both enantiomers were less active than the racemate in the horizontal screen test for ataxia; the ED_{50} values were 140, 640, and 94 for the *S* enantiomer, the *R* enantiomer, and the racemate, respectively.[3]

Compound **2** [4-amino-*N*-(2,6-dimethylphenyl)benzamide] was more potent than **1** against MES-induced seizures in mice (oral ED_{50} values = 1.7 and 10.3 mg/kg, respectively).[4,5] In contrast to **1**, compound **2** was unable to antagonize any of the standard chemically induced seizures. Thus, in these animals models, **2** displayed a phenytoin-like profile. On the basis of extensive pharmacological studies, **2** (LY201116)[6] is being developed for treatment of generalized tonic–clonic and partial seizures.

Scheme I



5-7 **2-4**

During metabolism studies involving **2**, we found that this compound was metabolically inactivated by N-

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