June 1989
Volume 78, Number 6

JOURNAL OF PHARMACEUTICAL SCIENCES

A publication of the American Pharmaceutical Association

Synthesis of 1,5-Diaryl-3-methyl-1*H*-pyrazolo[4,5-*c*]isoquinolines and Studies of Binding to Specific Peripheral Benzodiazepine Binding Sites

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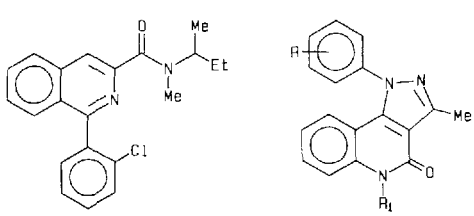
Received May 2, 1988, from the *Dipartimento di Scienze Farmaceutiche, Universita' di Firenze, 50121 Firenze, Italy, and the ‡Istituto Policattedra di Discipline Biologiche, Universita' di Pisa, 56100 Pisa, Italy. Accepted for publication November 16, 1988.

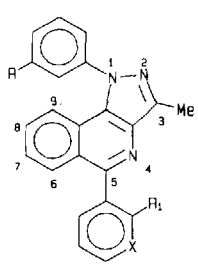
Abstract □ Some 1,5-diaryl-3-methyl-1*H*-pyrazolo[4,5-*c*]isoquinolines were synthesized and tested for their ability to displace [^{3}H]clonazepam or [^{3}H]Ro 5-4864 from their specific binding on the central and peripheral benzodiazepine receptors. None of the tested compounds showed any activity as central binding inhibitors, while most of them were specific as peripheral binding inhibitors, although they were not very potent.

Benzodiazepines were shown to exert their classic clinical action by interacting with the central receptors through the GABA:chloride ion channel macromolecular complex. A second type of recognition site was discovered soon after[1] in the peripheral tissues and in the brain. This second class of benzodiazepine peripheral binding sites, long considered simply as "acceptors", is not linked to the GABA receptor:chloride ion channel complex and differs from the central receptor in physiological function and pharmacological sensitivity. In fact, the peripheral site does bind diazepam (though with lower affinity), Ro 5-4864, and the nonbenzodiazepine PK 11195 (with nanomolar affinity), but does not bind clonazepam; while the central site binds clonazepam and diazepam with high affinity and does not bind Ro 5-4864 and PK 11195. These anatomical and pharmacological differences between central and peripheral benzodiazepine binding sites have been demonstrated to be due to two structurally different receptors.[2]

The physiological role of the peripheral benzodiazepine receptor, if any, is unclear at this time. Nevertheless Mestre and co-workers[3,4] have reported that in cardiac tissue, the peripheral benzodiazepine receptor is associated with voltage-sensitive calcium channels, and PK 11195 may be useful in the treatment of angina and cardiac ischemia.

Following our previous studies on the synthesis and binding of pyrazoloquinoline derivatives with the central benzodiazepine receptor,[5-8] we now report the results of synthesis and binding studies of a series of 1,5-diaryl-3-methyl-1*H*-pyrazolo[4,5-*c*]isoquinolines with the aim of clarifying the structural requirements of the recognition site of the peripheral benzodiazepine receptor. Our approach to the synthesis of the pyrazoloisoquinolines was based on the consideration that one of the requirements affecting the affinity or the selectivity for the receptor is the presence of a planar heteroaromatic system.[6,9-11] Thus, taking as lead compounds PK 11195 and pyrazoloquinolines,[5-8] we constrained the side chain at position 3 of PK 11195 into the pyrazole ring. Moreover, we located the substituent on the 1-phenyl ring at the meta position since, in the central active pyrazoloquinolines, the most active compounds bear a meta substituent. The structural similarities between PK 11195 and pyrazoloquinolines are illustrated below.





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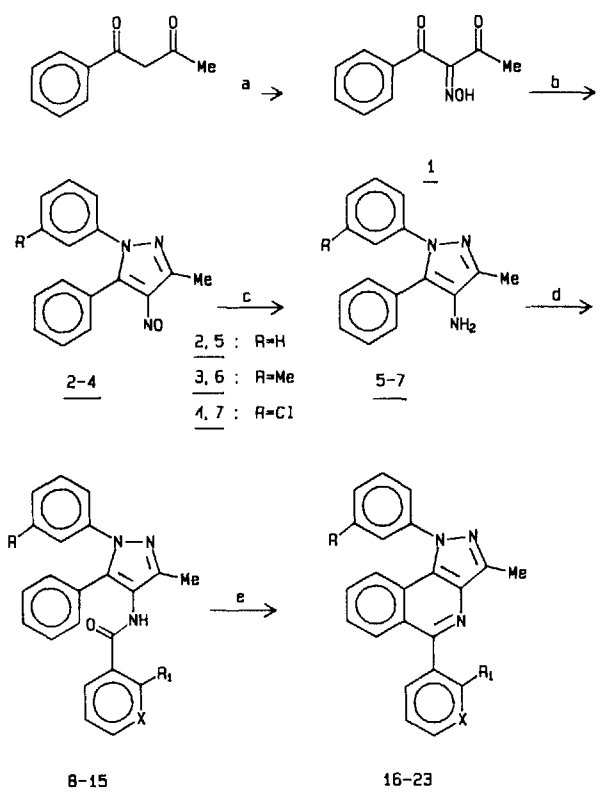
Results and Discussion

Chemistry—The synthesis of the pyrazoloisoquinolines **16–23** and **29–30** was carried out following the pathways illustrated in Schemes I and II. It should be noted that by allowing **1** to react with arylhydrazines, a sole isomer pyrazole was isolated. This is in agreement with the fact that in arylhydrazines only the β-nitrogen has the necessary nucleophilicity to carry out the attack on the electrophilic carbonyl group. Since in unsymmetrical β-diketones the nucleophilic attack could be directed at either of the carbonyl groups, two isomeric structures could be isolated. However, it is reported[12] that in methyl-aryl-β-diketones, the nucleophilic attack is exclusively on the acyl carbonyl group; thus, the structure of 1,5-diaryl- was attributed to **2–4**.

The attributed structures **2–4** were further confirmed by reacting **1** with methylhydrazine. In this case, the α-nitrogen is more nucleophilic than the β-nitrogen because of the electron donor effect of the methyl group. In fact, only one isomer was isolated from the reaction mixture, though a small amount of the other isomer (5%) could be detected spectroscopically (see Scheme II).



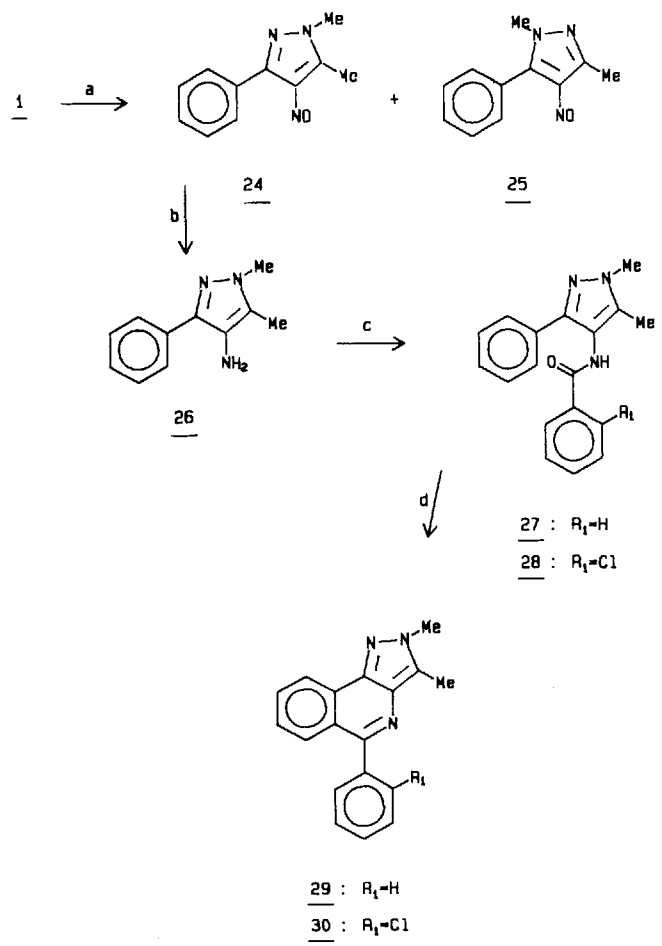
	R	R_1	X
8, 16	H	H	CH
9, 17	H	Cl	CH
10, 18	Me	H	CH
11, 19	Me	Cl	CH
12, 20	Me	Cl	N
13, 21	Cl	H	CH
14, 22	Cl	Cl	CH
15, 23	Cl	Cl	N

[a]:$NaNO_2$ in glacial AcOH. [b]:Arylhydrazines. [c]:H_2/Pd/C.
[d]:Aroylchloride in dry pyridine. [e]:$POCl_3/P_2O_5$

Scheme I

The structure of the main product of the reaction was assigned by ^1H and ^{13}C NMR data. In the ^1H NMR spectrum of the mixture of **24** and **25**, two C-CH_3 signals could be detected; a stronger signal at 2.31 ppm and a weaker one at 2.50 ppm. Since in *N*-substituted pyrazoles position 5 displays a higher electron density than position 3, it follows that a methyl group at position 5 is more shielded than one at position 3. Thus, the structure of 1,5-dimethyl-3-phenyl-4-nitrosopyrazole was assigned to the main product of the reaction **24**. The upfield value of the C-CH_3 signal of 1,5-dimethyl-**24** is also due to the steric interaction with the adjacent N-CH_3 group. The assignment of the structure to the main product **24** is also confirmed by its ^{13}C NMR spectra. In the coupled spectra of **24**, the carbon atom bearing the methyl group (149.66 ppm) appears as an unresolved multiplet because of its long-range couplings with both the C-CH_3 and NCH_3 protons. If it had been 1,3-dimethyl-**25**, the C-CH_3 signal would have appeared as a sharp quartet.

Moreover, to test the influence of the 5-aryl group on the biological activity, we tried to prepare the 5-unsubstituted pyrazoloisoquinoline **31**. Reacting **6** with formaldehyde, using the Pictet–Spengler reaction[13] or the same reaction as modified by Massiot,[14] led either to the 4-dimethylaminopyrazole derivative **32** or to a series of decomposition products. It follows that the pyrazole moiety acts as an electron-withdrawing substituent in the electrophilic substitution of the



[a]:Methylhydrazine. [b]:H_2/Pd/C [c]:Aroylchloride in dry pyridine.
[d]:$POCl_3/P_2O_5$

Scheme II

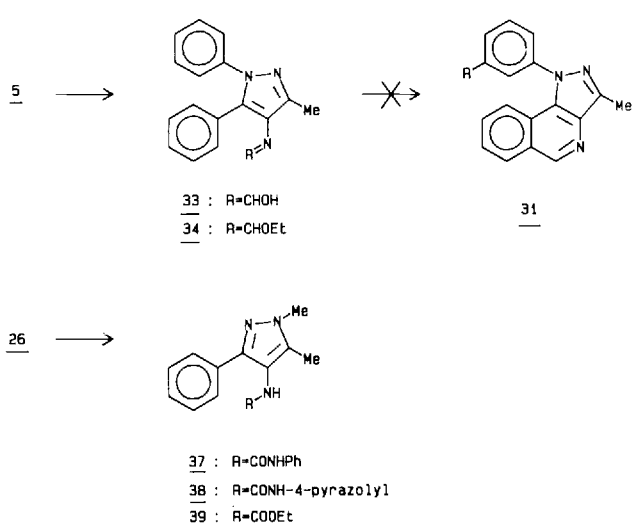
benzene ring. In fact, the cyclization of **16–23** and **29** and **30** is carried out in drastic conditions.

Thus, we reacted **5** with formic acid or ethyl orthoformate to obtain 4-formylamido-**33** and 4-ethoxymethylenamino-**34**, respectively. However, we were unable to cyclize **33** and **34** to **31** because heating them at their melting points led to a series of decomposition products (see Scheme III). It was then thought that **31** could easily be obtained by dechlorination of the corresponding 5-chloroderivative which ensued from the nucleophilic displacement of the OH group of the tautomeric 5-hydroxyderivative. Aromatic and heteroaromatic amine derivatives, such as carbamates and ureas, are known to undergo cyclization reactions to isoquinoline derivatives under various conditions[15,16] (Scheme IV). Thus, 4-aminopyrazoles **5** and **6** were allowed to react with phenylisocyanate to give rise to the *N*-phenyl-*N'*-4-pyrazolyl ureas **35a** and **b**, which we were unable to cyclize to pyrazoloisoquinolines. By heating **35a** and **b** at their melting points, the *N,N'*-4-dipyrazolyl ureas **36a** and **b** were isolated. The latter were also directly prepared by reacting **5** and **6** with ethylisocyanate. By heating **36a** and **b** at their melting points, the starting 4-aminopyrazoles **5** and **6** were recovered.

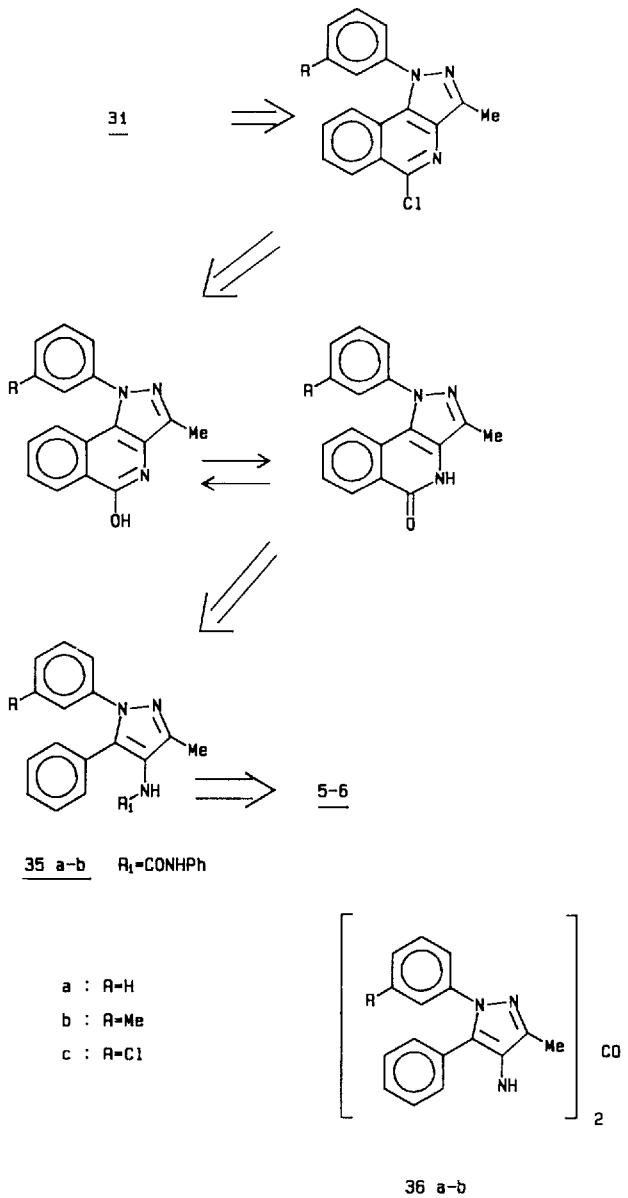
The reaction of **26** (see Scheme III) with phenylisocyanate, ethylisocyanate, and ethyl chlorocarbonate was also a dead end since the reaction products **37–39** never cyclized to pyrazoloisoquinolines.

Binding to the Central and Peripheral Benzodiazepine Receptors—Compounds **16–23** and **29** and **30** were tested for their ability to displace [^{3}H]Ro 5-4864 bound to rat kidney 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.

None of the tested compounds showed any ability to displace [^{3}H]clonazepam from its central binding at a concentration of 60 μM. This concentration was chosen because of the insolubility of the compounds at higher concentration. All the tested compounds showed some ability to displace [^{3}H]Ro 5-4864 from its specific peripheral binding in rat kidney membranes. The resulting data are listed in Table I. From the results it appears that all the tested compounds are, at μM concentration, specific for the peripheral benzodiazepine receptor, although they are not very potent. Nevertheless, the tested compounds could provide information on the molecular requirements of the peripheral benzodiazepine recognition sites. The high IC_{50} value of **29** and **30** suggests that the presence of the 1-aryl moiety enhances the activity



Scheme III



Scheme IV

Table I—Inhibition of [^{3}H]Ro 5-4864 Binding

Compound	R	R_1	X	IC_{50}, $\mu M^{a,b}$
16	H	H	CH	10.5 ± 1.8
17	H	Cl	CH	6.3 ± 0.4
18	Me	H	CH	5.6 ± 0.5
19	Me	Cl	CH	6.2 ± 0.4
20	Me	Cl	N	6.7 ± 0.6
21	Cl	H	CH	5.6 ± 0.6
22	Cl	Cl	CH	7.5 ± 0.6
23	Cl	Cl	N	7.5 ± 0.5
29	—	H	CH	49 ± 2.7
30	—	Cl	CH	48.9 ± 1.2
Diazepam	—	—	—	0.07 ± 0.005
PK 11195	—	—	—	0.004 ± 0.0005
Ro 5-4864	—	—	—	0.008 ± 0.0005

[a] The tests were carried out with EtOH as solvent. [b] Concentrations necessary for 50% inhibition (IC_{50}) are means ± SEM of seven determinations.

in the binding assay. This is in agreement with what had previously been observed for the binding of the pyrazoloquinolines to the central benzodiazepine receptor.[6] The position of a meta-substituent on the 1-phenyl ring also seems to enhance the binding potency. In fact, **16** shows a higher IC_{50} value than its meta-phenyl-substituted derivatives **18** and **21**. This finding is also in agreement with previous observations for the binding of the pyrazoloquinolines to the central benzodiazepine receptor.[5-8] In conclusion, there seems to be some common requirement for the central and peripheral benzodiazepine receptors; that is, there may be some similarity in the recognition sites of the central and peripheral receptors.

Experimental Section

Chemistry—All melting points were determined on a Gallenkamp capillary melting point apparatus and are uncorrected. The ^1H NMR spectra were recorded with a Varian EM 360 instrument; chemical shifts are reported in δ (ppm) downfield from the internal standard 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 $CDCl_3$ or Me_2SO-d_6 of ~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; Me_2SO-d_6, 39.6 ppm) and corrected to the internal standard Me_4Si. Typical acquisition parameters included a spectral width of 5000 Hz, a flip angle of 42°, and an interpulse delay between acquisition of 510 μs. Chemical shift values were reproducible to better than ±0.05 ppm. The decoupled spectra were obtained without pulse delay. Silica gel plates (Merck F_{254}), 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, and N with a Perkin-Elmer 260C elemental analyzer, and results were within ±0.4% of the theoretical values.

Physical data for the newly synthesized compounds are listed in Table II. The ^{13}C NMR chemical shifts and the most significant coupling constants are reported in Table III. In the latter, an arbitrary numbering is used to identify all carbon atoms.

Synthesis of 1-Aryl-3-methyl-4-nitroso-5-phenylpyrazoles 2–4 and 1,5-Dimethyl-3-phenyl-4-nitrosopyrazole 24—To a solution of arylhydrazine or methylhydrazine in glacial AcOH, an equimolecular amount of isonitrosobenzoylacetone[17] was added in a dropwise manner with stirring. The solution was warmed (60 °C) for a few minutes until it became green. The cooled solution was diluted with water and Et_2O, and the whole solution was neutralized with Na_2CO_3. The organic layer was separated, the water solution was extracted three times with Et_2O, and the combined organic extracts were dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue which was recrystallized to give green crystals.

Compound **24** was obtained in the pure state by several recrystallizations. Compound **25** was never isolated; its formation was detected by TLC and by ^1H NMR of the crude mixture.

Synthesis of 1-Aryl-3-methyl-4-amino-5-phenylpyrazoles 5–7 and 1,5-Dimethyl-3-phenyl-4-aminopyrazole 26—To a solution of **2–4** and **25** (0.500 g) in AcOEt (250 mL) was added 10% Pd/C (0.250 g). The mixture was hydrogenated in a Parr apparatus at 50 psi at room temperature for 18 h. The catalyst was filtered off and the solution was evaporated to give a residue which was recrystallized.

Synthesis of 1-Aryl-3-methyl-4-aroylamido-5-phenylpyrazoles 8–15 and 1,5-Dimethyl-3-phenyl-4-aroylamidopyrazoles 27–28—To a solution of **5–7** and **26** (1 g) in dry pyridine (15 mL) an equimolecular amount of aroylchloride was added in a dropwise manner with stirring. The solution was refluxed for 6 h. Pyridine was distilled off under reduced pressure. The residue was dissolved in $CHCl_3$, and washed with a diluted solution of HCl and then with a diluted solution of Na_2CO_3. The organic layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a residue which was recrystallized.

Synthesis of 1,5-Diaryl-3-methyl-1*H*-pyrazolo[4,5-*c*]isoquinolines 16–23 and 2,3-Dimethyl-5-aryl-2*H*-pyrazolo[4,3-*c*]isoquinolines 29–30—To a solution of **8–15** and **27** and **28** (1 g) in $POCl_3$ (15 mL) was added P_2O_5 (6–7 g). The solutions of all the compounds but two were refluxed for 8 h, while those of the 2-chloronicotinoyl derivatives **12** and **15** were refluxed for 48 h. The mixture was diluted with water and ice, neutralized with Na_2CO_3, and extracted

Table II—Physical Data of the Synthesized Compounds

Compound	Formula	mp, °C	Solv. Crystn.	Yield, % (method)	^1H Nuclear Magnetic Resonance Spectral Data[a,b]
2[c]	$C_{16}H_{13}N_3O$	127–128	EtOH	58	7.8–7.3(m, 10H, benzene protons), 2.31(s, 3H, CH_3)[a]
3	$C_{17}H_{15}N_3O$	101–102	EtOH	51	7.8–7.3(m, 9H, benzene protons), 2.36(s, 3H, tolylCH_3), 2.32(s, 3H, CH_3)[a]
4	$C_{16}H_{12}ClN_3O$	107–108	EtOH	55	7.8–7.1(m, 9H, benzene protons), 2.28(s, 3H, CH_3)[a]
5[d]	$C_{16}H_{15}N_3$	105–106	Ligroin	86	7.5–7.0(m, 10H, benzene protons), 2.9(br s, 2H, NH_2), 2.29(s, 3H, CH_3)[a]
6	$C_{17}H_{17}N_3$	84–85	Ligroin	95	7.5–6.7(m, 9H, benzene protons), 2.9(br s, 2H, NH_2), 2.33(s, 3H,tolylCH_3), 2.29(s, 3H, CH_3)[a]
7	$C_{16}H_{14}ClN_3$	86–88	Ligroin	84	7.6–6.9(m, 9H, benzene protons), 3.9(br s, 2H, NH_2), 2.31(s, 3H, CH_3)[a]
8	$C_{23}H_{19}N_3O$	218–219	AcOEt	71	7.9–7.1(m, 16H, 15 benzene protons + NH), 2.32(s, 3H, CH_3)[a]
9	$C_{23}H_{18}ClN_3O$	214–215	AcOEt	91	7.5–7.1(m, 15H, 14 benzene protons + NH), 2.41(s, 3H, CH_3)[a]
10	$C_{24}H_{21}N_3O$	206–207	EtOH	75	7.9–6.9(m, 15H, 14 benzene protons + NH), 2.36(s, 3H, tolylCH_3), 2.31(s, 3H, CH_3)[a]
11	$C_{24}H_{20}ClN_3O$	161–162	EtOH	96	7.8–6.9(m, 14H, 13 benzene protons + NH), 2.42(s, 3H, tolylCH_3), 2.31(s, 3H, CH_3)[a]
12	$C_{23}H_{19}ClN_4O$	127–128	AcOEt	90	9.9(br s, 1H, NH), 8.52(m, 1H, α-pyridine proton), 7.91(m, 1H, α-pyridine proton), 7.7–6.8(m, 10H, 9 benzene protons + β-pyridine proton), 2.28(s, 6H, $2CH_3$)[b]
13	$C_{23}H_{18}ClN_3O$	209–210	AcOEt	75	7.9–7.0(m, 15H, 14 benzene protons + NH), 2.33(s, 3H, CH_3)[a]
14	$C_{23}H_{17}Cl_2N_3O$	179–180	EtOH	96	7.8–7.0(m, 14H, 13 benzene protons + NH), 2.41(s, 3H, CH_3)[a]
15	$C_{22}H_{16}Cl_2N_4O$	156–157	AcOEt	76	8.45(m, 1H, α-pyridine proton), 8.07(m, 1H, α-pyridine proton), 7.9–6.6(m, 11H, 9 benzene protons + β-pyridine proton + NH), 2.37(s, 3H, CH_3)[a]

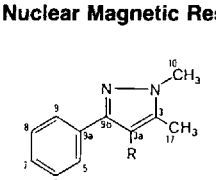
(Continued)

Table II—Continued

Compound	Formula	mp, °C	Solv. Crystn.	Yield, % (method)	^{1}H Nuclear Magnetic Resonance Spectral Data[a,b]
16	$C_{23}H_{17}N_3$	223–224	Ligroin	70	8.3–7.4(m, 14H, benzene protons), 2.83(s, 3H, CH_3)[a]
17	$C_{23}H_{16}ClN_3$	254–255	Ligroin	77	7.9–7.4(m, 13H, benzene protons), 3.82(s, 3H, CH_3)[a]
18	$C_{24}H_{19}N_3$	168–169	EtOH	75	8.3–7.3(m, 13H, benzene protons), 2.71(s, 3H, CH_3), 2.49(s, 3H, tolylCH_3)[a]
19	$C_{24}H_{18}ClN_3$	202–203	AcOEt	79	7.9–7.3(m, 12H, benzene protons), 2.80(s, 3H, CH_3), 2.49(s, 3H, tolylCH_3)[a]
20	$C_{23}H_{17}ClN_4$	227–228	Acetone	67	8.61(m, 1H, α-pyridine proton), 8.1–7.3(m, 10H, 8 benzene protons + β- and α-pyridine protons), 2.79(s, 3H, CH_3), 2.50(s, 3H, tolylCH_3)[a]
21	$C_{23}H_{16}ClN_3$	181–182	EtOH	75	8.3–7.3(m, 13H, benzene protons), 2.80(s, 3H, CH_3)[a]
22	$C_{23}H_{15}Cl_2N_3$	179–180	Acetone	73	7.9–7.4(m, 12H, benzene protons), 2.82(s, 3H, CH_3)[a]
23	$C_{22}H_{14}Cl_2N_4$	231–232	Acetone	51	8.63(m, 1H, α-pyridine proton), 8.9–7.2(m, 10H, 8 benzene protons + β- and α-pyridine protons), 2.79 (s, 3H, CH_3)[a]
24	$C_{11}H_{11}N_3O$	89–90	EtOH	72	8.3–8.0(m, 2H, benzene protons), 7.6–7.3(m, 3H, benzene protons), 3.72(s, 3H, N-CH_3), 2.31(s, 3H, CH_3)[a]
26	$C_{11}H_{13}N_3$	136–137	Ligroin	86	7.9–7.2(m, 5H, benzene protons), 3.76(s, 3H, N-CH_3), 2.8(br s, 2H, NH_2), 2.16(s, 3H, CH_3)[a]
27	$C_{18}H_{17}N_3O$	211–212	AcOEt	88	8.0–7.2(m, 11H, 10 benzene protons + NH), 3.79 (s, 3H, N-CH_3), 2.18(s, 3H, CH_3)[a]
28	$C_{18}H_{16}ClN_3O$	188–189	AcOEt	74	7.8–7.2(m, 10H, 9 benzene protons + NH), 3.79(s, 3H, N-CH_3), 2.24(s, 3H, CH_3)[a]
29	$C_{18}H_{15}N_3$	182–183	Ligroin	75	8.6(m, 1H, benzene proton), 8.1–7.3(m, 8H, benzene protons), 4.14(s, 3H, N-CH_3), 2.71(s, 3H, CH_3)[a]
30	$C_{18}H_{14}ClN_3$	238–239	AcOEt	74	8.6(m, 1H, benzene proton), 7.9–7.4(m, 7H, benzene protons), 4.17(s, 3H, N-CH_3), 2.74(s, 3H, CH_3)[a]
32	$C_{19}H_{21}N_3$	69–71	Ligroin	80	7.4–6.7(m, 9H, benzene protons), 2.65(s, 6H, $N(CH_3)_2$), 2.40(s, 3H, CH_3), 2.22(s, 3H, CH_3)[a]
33	$C_{17}H_{15}N_3O$	121–122	Cyclohexane	81	8.28(s, 1H, CH), 7.5–6.8(m, 11H, 10 benzene protons + NH), 2.32(s, 3H, CH_3)[a]
34	$C_{19}H_{19}N_3O$	69–70	Ligroin	66	7.71(s, 1H, CH), 7.4–7.1(m, 10H, benzene protons), 4.31(q, 2H, CH_2), 2.36(s, 3H, CH_3), 1.32(t, 3H, CH_3)[a]
35a	$C_{23}H_{20}N_4O$	224–225	EtOH	63	7.5–6.9(m, 16H, 15 benzene protons + NH), 6.39(s, 1H, NH), 2.29(s, 3H, CH_3)[a]
35b	$C_{24}H_{22}N_4O$	193–194	Et_2O	94	7.5–6.6(m, 16H, 14 benzene protons + 2 NH), 2.22 (s, 6H, $2CH_3$)[a]
36a	$C_{33}H_{28}N_6O$	310–312	EtOH:H_2O	24 (A), 52 (B)	8.6–7.0(m, 22H, 20 benzene protons + 2 NH), 2.12 (s, 6H, $2CH_3$)[b]
36b	$C_{35}H_{32}N_6O$	310–312	EtOH	29 (A)	7.4–6.7(m, 18H, benzene protons), 6.19(s, 2H, 2NH), 2.23(s, 6H, 2tolylCH_3), 2.18(s, 6H, $2CH_3$)[a]
37	$C_{18}H_{18}N_4O$	207–208	EtOH	95	—
38	$C_{23}H_{24}N_6O$	310–312	EtOH	37 (A), 47 (B)	7.9–7.2(m, 12H, 10 benzene protons + 2NH), 3.75 (s, 6H, $2NCH_3$), 2.11(s, 6H, $2CH_3$)[b]
39	$C_{14}H_{17}N_3O_2$	92–94	Ligroin	70	7.8–7.2(m, 5H, benzene protons), 5.97(br s,1H, NH), 4.18(q, 2H, CH_2), 3.81(s, 3H, N-CH_3), 2.19(s, 3H, CH_3), 1.23(t, 3H, CH_3)[a]

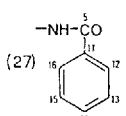
[a] $CDCl_3$. [b] DMSO-d_6. [c] See ref 18. [d] See ref 19.

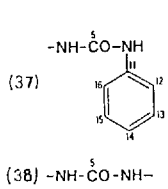
Table III—Carbon-13 Nuclear Magnetic Resonance Shifts[a]



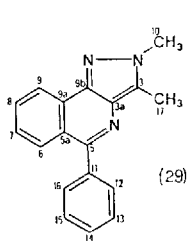
R: (24) –NO

(26)–NH_2





(38) –NH–CO–NH–



(29)

Compound	C-3	C-3a	C-5	C-5a	C-6	C-7	C-8	C-9	C-9a	C-9b	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17
24	149.66	65.50	—	128.76	128.21	129.19	128.21	128.76	130.90	159.39	35.48	—	—	—	—	—	—	10.45
26	133.55	122.77	—	128.24	126.37	126.54	126.37	128.24	127.44	140.13	36.11	—	—	—	—	—	—	8.34
27	133.86	113.39	166.73	128.31[b]	126.78[c]	127.41	126.78[c]	128.31[b]	132.45	145.16	36.45	136.88	128.43[b]	127.16[c]	131.59	127.16[c]	128.43[b]	9.48
29	133.70	77.10	157.47	126.10	126.48	128.04	128.86	121.53	131.54	140.10	37.29	137.14	129.63	128.04	130.03	128.04	129.63	8.95
37	140.20	114.42	154.34	128.35	126.25	127.17	126.25	128.35	133.25	144.44	36.68	137.44	118.25	128.65	121.56	128.65	118.25	9.01
38	137.60	114.96	156.46	128.19	126.19	127.07	126.19	128.19	133.35	144.51	36.64	—	—	—	—	—	—	8.74

[a] ^{13}C–^1H Coupling constants (Hz): **24**, $^3J_{C9bH9}$ = 2.4; **26**, $^3J_{C3H10}$ = 7.1, $^2J_{C3H17}$ =7.1, and $^3J_{C9bH9}$ = 3.3; **27**, $^3J_{C3H10}$ = 7.5, $^2J_{C3H17}$ = 7.5, and $^3J_{C9bH9}$ = 3.8; **37**, $^3J_{C3H10}$ = 8.0, $^2J_{C3H17}$ = 8.0 and $^3J_{C9bH9}$ = 3.3. [b,c] The assignment may be reversed.

three times (100 mL each time) with AcOEt. The organic layer was dried over anhydrous Na_2SO_4 and taken to dryness under reduced pressure. The solid residue was recrystallized.

1-(3-Methylphenyl)-3-methyl-5-phenyl-4-dimethylaminopyrazole (32)—A solution of **6** (0.7 g, 2.7 mmol), 40% folmaldehyde (2 mL, 27 mmol), and 2*M* HCl (6 mL) was refluxed for 2 h. The solution was made alkaline with concentrated ammonia and then extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a residue which was recrystallized.

1,5-Diphenyl-3-methyl-4-formylamidopyrazole (33)—A solution of **5** (1 g) in 99% formic acid (10 mL) was refluxed for 30 min, cooled, diluted with water and ice, combined with Et_2O, and neutralized with $NaHCO_3$. The organic layer was separated, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a residue which was recrystallized.

1,5-Diphenyl-3-methyl-4-ethoxymethyleniminopyrazole (34)—To a solution of **5** (1 g) in dry benzene (40 mL) an equimolecular amount of triethyl orthoformate was added. The mixture was refluxed for 20 h. Evaporation of the solvent under reduced pressure afforded a red oil which when treated with Et_2O gave rise to a solid which was then recrystallized.

Synthesis of *N*-Phenyl-*N'*-(1-aryl-3-methyl-5-phenylpyrazol-4-yl) Ureas 35a and b and *N*-Phenyl-*N'*-(1,5-dimethyl-3-phenylpyrazol-4-yl) Urea 37—To a solution of **5**, **6** and **26** (2 g) in anhydrous benzene (15 mL) an equimolecular amount of phenylisocyanate was added in a dropwise manner with stirring. The mixture was refluxed for 30 min. Upon cooling, a solid mass was formed which was collected, washed several times with Et_2O, and recrystallized.

Synthesis of *N,N'*-(1-Aryl-3-methyl-5-phenylpyrazol-4-yl) Ureas 36a and b and *N,N'*-(1,5-Dimethyl-3-phenylpyrazol-4-yl) Urea 38—(*Method A*) Compounds **35a** and **b** and **37** (1 g) were heated at their melting points in an oil bath for 1 h. The cooled fused mass was suspended in EtOH and recrystallized.

(*Method B*) To a solution of **5**, **6**, and **26** (1 g) in pyridine (10 mL) an equimolecular amount of ethylisocyanate was added. The solution was refluxed for 24 h. The pyridine was distilled off under reduced pressure and the residue was diluted with water and set aside for 2–3 h. The solid was collected, washed with water and with Et_2O, and recrystallized.

1,5-Dimethyl-3-phenyl-4-carbethoxyamidopyrazole (39)—To a solution of **26** (1 g) in dry benzene (50 mL) equimolecular amounts of ethyl chlorocarbonate and triethylamine were added. The mixture was refluxed for 20 h and then cooled, diluted with water, and extracted with Et_2O. The dried (anhydrous Na_2SO_4) organic layer was taken to dryness under reduced pressure to give a red oil which was recrystallized.

Binding to the Central and Peripheral Benzodiazepine Receptors—The ability of **16–23**, **29**, and **30** to displace specific [^{3}H]clonazepam binding to bovine brain membrane (i.e., the inhibition percentage) was performed as described previously.[20] The potency of **16–23**, **29**, and **30** as inhibitors of [^{3}H]Ro 5-4864 binding to peripheral benzodiazepine receptors was determined as previously described.[21] In brief, kidney from adult, male Sprague-Dawley rats was weighed and disrupted in 10 volumes of 0.32 M ice-cold sucrose containing protease inhibitors. The homogenate was centrifuged at 2000 × *g* for 5 min at 4 °C; the pellet (P_1) was discarded and the supernatant was recentrifuged at 48,000 × *g* for 15 min at 4 °C. The membranes were lysed by resuspension in 50 mM Tris-HCl buffer (pH 7.4) containing protease inhibitors and recentrifuged at 50,000 × *g* for 15 min at 4 °C (P_2). Studies of [^{3}H]Ro 5-4864 binding to kidney membrane preparation were performed by incubating aliquots of the P_2 fraction (0.3 mg protein) for 60 min at 0 °C in 500 μL of 50 mM Tris-HCl buffer at pH 7.4/0.6 nM [^{3}H]Ro 5-4864 (77.9 Ci/mmol) in the absence or presence of 5 μM unlabeled diazepam. Incubation was terminated by filtration through Whatman GF/B glass fiber filters under suction. The filters were washed twice with 5 mL of 50 mM Tris-HCl at pH 7.4, and radioactivity was counted in 10-mL of filter counter Packard scintillation cocktail. The IC_{50} values (the concentration of compound inhibiting the specific binding of [^{3}H]Ro 5-4864) was estimated by log-probit analysis.[22]

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