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Communications to the Editor

Synthesis, Binding Studies, and Structure–Activity Relationships of 1-Aryl- and 2-Aryl[1]benzopyranopyrazol-4-ones, Central Benzodiazepine Receptor Ligands

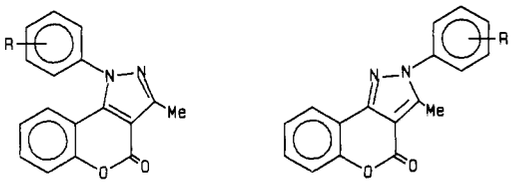
Sir:

Benzodiazepines are important therapeutic agents which have been the object of intense investigation. These drugs exert their main actions, such as anxiolytic, hypnotic, anticonvulsant, and muscle relaxant effects, on the central nervous system, by interacting with special neuronal membrane proteins, the benzodiazepine receptors, which are at least partly located in GABA-ergic synapses. A number of synthetic compounds with diverse structures have been found to have affinity for the benzodiazepine receptors.[1] These non-benzodiazepine compounds with affinity for the benzodiazepine receptors are important tools for finding out the physiological properties and the structural requirements of the recognition site of the receptor itself.

Following our previous research on the syntheses and binding studies on pyrazoloquinoline derivatives interacting with the central benzodiazepine receptors,[2-6] we hereby report the syntheses and binding studies of a series of 1,4-dihydro-1-aryl-3-methyl[1]benzopyrano[3,4-*d*]-pyrazol-4-ones **1** and a series of 2,4-dihydro-2-aryl-3-methyl[1]benzopyrano[4,3-*c*]pyrazol-4-ones **2**.

Both series, **1** and **2**, are isosteres of the previously reported ones since the quinoline NH has been replaced by the oxygen of the [1]benzopyrano moiety.

Chemistry. The syntheses of series **1** and **2** compounds were achieved, as outlined in Scheme I, according to literature methods,[7,8] by cyclizing the arylhydrazones **3a–o** of 3-acetyl-4-hydroxycoumarin either with *p*-toluenesulfonic acid in refluxing xylene or with arylhydrazine hydrochloride in refluxing acetic acid. The amino derivatives **1p–r** were obtained by catalytic reduction of the corresponding nitro compounds **1i–k**.

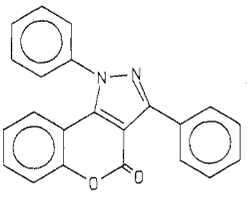


1 a–r

2 a, c, h, m

R			
	a=H	g=2-Cl	m=3-OMe
	b=4-Me	h=3-Br	n=2-OMe
	c=3-Me	i=4-NO_2	o=3, 5-Me
	d=2-Me	j=3-NO_2	p=4-NH_2
	e=4-Cl	k=2-NO_2	q=3-NH_2
	f=3-Cl	l=4-OMe	r=2-NH_2

In our previous report,[5] we synthesized and tested some 1,3-diarylpyrazoloquinoline derivatives, some of which displayed inhibiting potency at nanomolar concentration. Thus, to test the influence of the 3-methyl group in the anchoring to the receptor, we replaced it with the phenyl group, thereby obtaining compound **4**. The latter was prepared by a 1,3-dipolar cycloaddition reaction according to the method of Shawali.[9]



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

The IC_{50} values of diazepam and chlordiazepoxide, two classical benzodiazepines in clinical use, are also included as reference compounds.

From our results it appears that, in agreement with our previous findings on pyrazoloquinoline series,[2] a prerequisite for showing a satifactory inhibiting potency is the presence of an aryl substituent in position 1. Most of the 1-aryl derivatives of series **1** interact with the benzodiazepine receptor with an inhibiting potency which ranks

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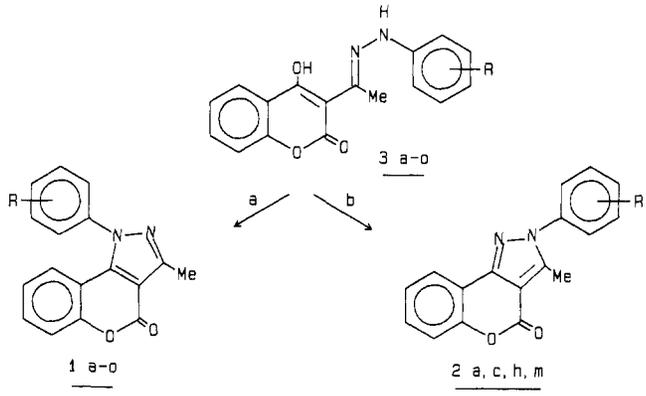
Scheme I[a]



[a] a, *p*-toluenesulfonic acid in xylene; b, arylhydrazine hydrochloride in acetic acid.

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

compd	R	IC_{50},[a,b] μM	compd	R	IC_{50},[a,b] μM
1a	H	0.30 ± 0.02	**1n**	2-OMe	8.00 ± 0.9
1b	4-Me	2.0 ± 0.18	**1o**	3,5-Me_2	0.065 ± 0.008
1c	3-Me	0.28 ± 0.01	**1p**	4-NH_2	1.3 ± 0.83
1d	2-Me	30.0 ± 1.8	**1q**	3-NH_2	3.4 ± 0.25
1e	4-Cl	0.75 ± 0.06	**1r**	2-NH_2	0.63 ± 0.05
1f	3-Cl	0.09 ± 0.008	**2a**	H	3.1 ± 0.02
1g	2-Cl	16 ± 2 (34)[c]	**2c**	3-Me	3.0 ± 0.2
1h	3-Br	0.10 ± 0.009	**2h**	3-Br	25 ± 1.8
1i	4-NO_2	1.00 ± 0.08	**2m**	3-OMe	5.0 ± 0.5
1j	3-NO_2	0.32 ± 0.01[b]	**4**		16 ± 1.8 (34)[c]
1k	2-NO_2	43 ± 2.3	chlordiazepoxide		0.79 ± 0.07
1l	4-OMe	0.70 ± 0.08	diazepam		0.025 ± 0.002
1m	3-OMe	0.75 ± 0.03			

[a] The tests were carried out with EtOH as solvent unless otherwise stated. [b] Concentrations necessary for 50% inhibition (IC_{50}) are means ± SEM of seven determinations. [c] Percentages of inhibition (*I*%) of specific [^{3}H]flunitrazepam binding at 34 μM compound concentration are means ± SEM of five determinations.

between chlordiazepoxide and diazepam, while their 2-aryl isomers **2** show very poor affinity for the benzodiazepine receptor binding sites. That means that the displacement of the 1-aryl substituent from position 1 to position 2 dramatically affects binding potency.

As for the pyrazoloquinoline series,[2-5] the important role played by the position of the substituent on the 1-phenyl ring is also confirmed. In fact, for methylphenyl, chlorophenyl, nitrophenyl, and methoxyphenyl derivatives, the affinity for the benzodiazepine receptor 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 to the ortho position. The fact that the meta-substituted 1-phenyl compounds are the most active is stressed by the binding potency of the 1-(3,5-dimethylphenyl) derivative **1o**, which shows an IC_{50} value of 0.065 μM, about 12 times lower than that of chlordiazepoxide and only 2.6 times higher than that of diazepam.

The varying trends of the amino derivatives **1p–r** may be due to the presence of the basic amino group, which changes the physicochemical properties of the whole molecule.

In disagreement with what has previously been observed,[4] the replacement of the methyl group at position 3 with phenyl results in the complete lack of binding affinity on the part of compound **4**.

In conclusion, it has to be noted that it is the position of the substituent on the 1-phenyl ring, and not its nature, that influences binding potency. In fact, the electron-donor methyl group and the electron-withdrawing nitro group act in the same manner, and changes in potency depend only on their position.

Most important in these new series of compounds interacting with the benzodiazepine receptor is the fact that the isosteric replacement of the quinoline NH with the benzopyrano oxygen brings about an enhancement of the inhibiting potency. Comparison of the IC_{50} values of the most active pyrazoloquinolines[4] with those of the corresponding benzopyranopyrazoles reveals a noteworthy enhancement of binding potency; e.g., compound **1o** has an IC_{50} of 0.065 μM as compared to 3 μM for its corresponding pyrazoloquinoline derivative.

It has been observed that in the pyrazoloquinoline series the replacement of the hydrogen atom in position 5 with a methyl group leads to a significant increase in radioligand displacement ability.[3] However, the lowest IC_{50} value (0.26 μM), that of 5-*N*-methyl-substituted 1-(3-methylphenyl)-pyrazoloquinoline, is the same as the average IC_{50} value of meta-substituted 1-phenyl benzopyranopyrazole, and this shows the advantage of the replacement of the quinoline with the benzopyrano moiety in the planar tricyclic system.

Experimental Section

Chemistry. All melting points were determined on a Gallenkamp 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 of $CDCl_3$ 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) 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 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}) were used for analytical 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. The physical data of new synthesized compounds are listed in Table II.

Synthesis of Arylhydrazones 3a–o of 3-Acetyl-4-hydroxycoumarin. To a mixture of arylhydrazine hydrochloride (4 mmol) in EtOH was added 1 mmol of 3-acetyl-4-hydroxycoumarin.[10] After a few minutes of heating, a colored solid mass was obtained.

Compound **3c** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3 H, Me tolyl), 2.73 (s, 3 H, Me), 6.6–8.1 (m, 8 H, aromatics), 9.0 (br s, 1 H, NH), 15.6 (br s, 1 H, OH).

Synthesis of 1,4-Dihydro-1-aryl-3-methyl[1]benzopyrano[3,4-*d*]pyrazol-4-ones 1a–o and 2,4-Dihydro-2-aryl-3-methyl[1]benzopyrano[4,3-*c*]pyrazol-4-ones 2a,c,h,m. Compounds **1a–o** were prepared from **3a–o** by following the method reported in ref 7.

Compound **1c** displayed the following spectral data: ^{1}H NMR ($CDCl_3$) δ 2.51 (s, 3 H, Me), 2.72 (s, 3 H, Me pyrazole), 6.9–7.7 (m, 8 H, aromatics); ^{13}C NMR ($CDCl_3$) δ 12.70 (Me pyrazole), 106.18 (C-3a), 141.51 (C-9b), 150.57 (C-3), 157.81 (C-4).

Compounds of series **2** were obtained from the corresponding arylhydrazones by following the method described in ref 8.

Compound **2c** displayed the following spectral data: ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3 H, Me), 2.75 (s, 3 H, Me pyrazole), 7.1–7.6 (m, 7 H, aromatics), 8.0–8.3 (m, 1 H, aromatic); ^{13}C NMR ($CDCl_3$) δ 11.76 (Me pyrazole), 106.12 (C-3a), 144.23 (C-3), 148.32 (C-9b), 158.58 (C-4).

Preparation of 1,4-Dihydro-1-(aminophenyl)-3-methyl[1]benzopyrano[3,4-*d*]pyrazol-4-ones 1p–r. To a warm solution

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Table II. Physical Data of New Synthesized Compounds

no.	formula	R	mp, °C	crystn solv	yield, %
1b	$C_{18}H_{14}N_2O_2$	4-Me	191–192	EtOH	78
1c	$C_{18}H_{14}N_2O_2$	3-Me	202–203	EtOH	60
1d	$C_{18}H_{14}N_2O_2$	2-Me	186–187	EtOH	58
1e	$C_{17}H_{11}ClN_2O_2$	4-Cl	208–209	AcOEt	60
1f	$C_{17}H_{11}ClN_2O_2$	3-Cl	213–215	AcOEt	60
1g	$C_{17}H_{11}ClN_2O_2$	2-Cl	209–210	EtOH	55
1h	$C_{17}H_{11}BrN_2O_2$	3-Br	220–221	EtOH	75
1i	$C_{17}H_{11}N_3O_4$	4-NO_2	255–256	DMF	63
1j	$C_{17}H_{11}N_3O_4$	3-NO_2	277–278	DMF	30
1k	$C_{17}H_{11}N_3O_4$	2-NO_2	211–213	EtOH	65
1l	$C_{18}H_{14}N_2O_3$	4-OMe	199–200	AcOEt	52
1m	$C_{18}H_{14}N_2O_3$	3-OMe	202–203	EtOH	95
1n	$C_{18}H_{14}N_2O_3$	2-OMe	202–204	AcOEt	56
1o	$C_{19}H_{16}N_2O_2$	3,5-Me_2	238–240	MeOH	28
1p	$C_{17}H_{13}N_3O_2$	4-NH_2	258–259	DMSO	30
1q	$C_{17}H_{13}N_3O_2$	3-NH_2	228–230	acetone	30
1r	$C_{17}H_{13}N_3O_2$	2-NH_2	232–233	EtOH	83
2c	$C_{18}H_{14}N_2O_2$	3-Me	158–159	EtOH	75
2h	$C_{17}H_{11}BrN_2O_2$	3-Br	208–210	EtOH	35
2m	$C_{18}H_{14}N_2O_3$	3-OMe	184–185	EtOH	30
3b	$C_{18}H_{16}N_2O_3$	4-Me	207–208 dec	EtOH	85
3c	$C_{18}H_{16}N_2O_3$	3-Me	175–176 dec	EtOH	61
3d	$C_{18}H_{16}N_2O_3$	2-Me	163–164 dec	EtOH	82
3e	$C_{17}H_{13}ClN_2O_3$	4-Cl	200–202 dec	AcOEt	35
3f	$C_{17}H_{13}ClN_2O_3$	3-Cl	236–237 dec	EtOH	75
3g	$C_{17}H_{13}ClN_2O_3$	2-Cl	150–151 dec	EtOH	75
3h	$C_{17}H_{13}BrN_2O_3$	3-Br	233–234 dec	EtOH	61
3i	$C_{17}H_{13}N_3O_5$	4-NO_2	263–264 dec	DMF	75
3j	$C_{17}H_{13}N_3O_5$	3-NO_2	248–249 dec	EtOH	70
3k	$C_{17}H_{13}N_3O_5$	2-NO_2	208–210 dec	AcOEt	96
3l	$C_{18}H_{16}N_2O_4$	4-OMe	211–212 dec	AcOEt	57
3m	$C_{18}H_{16}N_2O_4$	3-OMe	187–189 dec	EtOH	55
3n	$C_{18}H_{16}N_2O_4$	2-OMe	144–146 dec	EtOH	75
3o	$C_{19}H_{18}N_2O_3$	3,5-Me_2	216–217 dec	EtOH	40

of **1i–k** (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 24 h. The catalyst was filtered off, and the solution was evaporated to give a residue.

Binding Studies. Tritiated flunitrazepam was obtained from New England Nuclear (Dreieichenhain, West Germany) and had a specific activity of 78 Ci/mmol and a 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 3.

Benzodiazepine receptor binding activity was determined as follows: 100 μL of diluted membranes (0.4–0.5 mg of proteins) was incubated in triplicate with 0.6 nM [^{3}H]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 assay buffer and were immediately filtered under reduced pressure through glass fiber filter disks (Whatman GF/B). Afterwards 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.

The benzopyranopyrazole derivatives, unless otherwise stated, were dissolved in EtOH and added to the assay mixture to a final volume of 500 μL. Blank experiments were carried out to determine the effect of the solvent (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 [^{3}H]flunitrazepam in the presence of 10 μM diazepam.

Protein estimation was based on the method of Lowry et al.[11] after membrane solubilization with 0.75 N NaOH. Bovine serum albumin was utilized as the standard.

The concentrations of the test compounds that inhibited specific [^{3}H]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**, 2764-19-4; **1b**, 110570-18-8; **1c**, 110570-19-9; **1d**, 110570-20-2; **1e**, 110570-21-3; **1f**, 110613-13-3; **1g**, 110570-22-4; **1h**, 110570-23-5; **1i**, 110570-24-6; **1j**, 110570-25-7; **1k**, 110570-26-8; **1l**, 110570-27-9; **1m**, 110570-28-0; **1n**, 110570-29-1; **1o**, 110570-30-4; **1p**, 110570-31-5; **1q**, 110570-32-6; **1r**, 110570-33-7; **2a**, 86100-07-4; **2c**, 110570-34-8; **2h**, 110613-14-4; **2m**, 110570-35-9; **3a**, 2587-10-2; **3b**, 110570-04-2; **3c**, 110570-05-3; **3d**, 110570-06-4; **3e**, 110570-07-5; **3f**, 110570-08-6; **3g**, 110570-09-7; **3h**, 110570-10-0; **3i**, 110570-11-1; **3j**, 110570-12-2; **3k**, 110570-13-3; **3l**, 110570-14-4; **3m**, 110570-15-5; **3n**, 110570-16-6; **3o**, 110570-17-7; **4**, 100008-84-2; 4-$MeC_6H_4NHNH_2$·HCl, 637-60-5; 3-$MeC_6H_4NHNH_2$·HCl, 637-04-7; 2-$MeC_6H_4NHNH_2$·HCl, 635-26-7; 4-$ClC_6H_4NHNH_2$·HCl, 1073-70-7; 3-$ClC_6H_4NHNH_2$·HCl, 2312-23-4; 2-$ClC_6H_4NHNH_2$·HCl, 41052-75-9; 3-$BrC_6H_4NHNH_2$·HCl, 27246-81-7; 4-$O_2NC_6H_4NHNH_2$·HCl, 636-99-7; 3-$O_2NC_6H_4NHNH_2$·HCl, 636-95-3; 2-$O_2NC_6H_4NHNH_2$·HCl, 6293-87-4; 4-$MeOC_6H_4NHNH_2$·HCl, 19501-58-7; 3-$MeOC_6H_4NHNH_2$·HCl, 39232-91-2; 2-$MeOC_6H_4NHNH_2$·HCl, 57396-67-5; 3,5-$Me_2C_6H_3NHNH_2$·HCl, 60481-36-9; 3-acetyl-4-hydroxycoumarin, 2555-37-5.

† Università di Firenze.
‡ Università di Pisa.

Vittoria Colotta,† Lucia Cecchi,*† Guido Filacchioni† Fabrizio Melani,† Giovanna Palazzino† Claudia Martini,‡ Gino Giannaccini‡ Antonio Lucacchini‡

Dipartimento di Scienze Farmaceutiche Università di Firenze, 50121 Firenze, Italy Istituto Policattedra di Discipline Biologiche Università di Pisa, 56100 Pisa, Italy

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Orally Active Hydroxamic Acid Inhibitors of Leukotriene Biosynthesis

Sir:

5-Lipoxygenase has been the subject of intense study since its identification as the first enzyme involved in the biosynthesis of the leukotrienes. Because the leukotrienes have been suggested to be important mediators in a variety of diseases including asthma, arthritis, and psoriasis, inhibition of 5-lipoxygenase is a promising therapeutic target for the development of new, potentially more effective treatments for these conditions.

Simple, stable molecules containing the hydroxamic acid moiety that are potent in vitro inhibitors of 5-lipoxygenase have been identified.[1-5] The hydroxamate unit appears to be required for the inhibition observed with these molecules since replacement by closely related functional groups leads to inactive compounds.[1]

All of the inhibitors that we previously described[1,6] have

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