Tricyclic Heteroaromatic Systems: Synthesis, [^{3}H]Flunitrazepam Brain Membrane Binding Inhibition, and Structure–Activity Relationships of 2,3-Dihydro-2-aryl-4-*R*-[1]benzopyrano[4,3-*c*]pyrazole-3-ones

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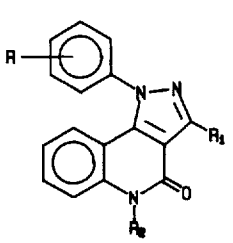
Abstract □ We report the synthesis and binding activity to the central benzodiazepine receptors of some 2,3-dihydro-2-aryl-4-*R*-[1]benzopyrano[4,3-*c*]pyrazole-3-ones, which are isosteres of the CGS series. Although the compounds of the CGS series are potent ligands of the benzodiazepine receptors, none of the isosteres tested showed any significant inhibiting potency. This may be due to the change in electronic properties brought about by the replacement of the NH of the CGS series with an oxygen atom.

We have recently reported on two different classes of benzodiazepine receptor ligands: pyrazolo-quinoline-4-ones (**A**)[1–4] and [1]benzopyrano-pyrazole-4-ones (**B**).[5] The similarities in the structure–activity relationships in the two classes are striking. In both series, the highest inhibiting potency was conferred by the presence of a meta substituent on the 1-phenyl ring. The displacement of the substituent on the 1-phenyl ring from the meta to the para or ortho position resulted in a reduction of binding potency. In both series, the aryl moiety must be placed at position 1; its displacement from the pyrazole nitrogen in position 1 to that in position 2 gave products devoid of binding affinity.[1,5] Moreover, the binding potency of the [1]benzopyrano-pyrazole-4-one series was higher than that of the pyrazolo-quinoline-4-one series,[5] which means that the replacement of the NH with the oxygen atom is advantageous because it brings about an enhancement of binding potency.

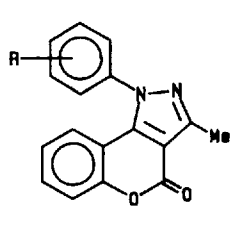
Thus, taking the 3,5-dihydro-2-aryl-2H-pyrazolo-[4,3-c]quinoline-3-one compounds (CGS series),[6] potent ligands of the benzodiazepine receptors, as lead compounds, we report on the synthesis and binding activity of the isosteric 2,3-dihydro-2-aryl-[1]benzopyrano[4,3-*c*]pyrazole-3-ones (series 1) and of their 4-methyl derivatives (series 2).

Experimental Section

Synthesis—Little attention has been paid in the literature to the synthesis of 2-aryl-[1]benzopyrano-pyrazole-3-ones and the methods



A



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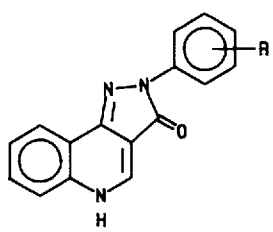
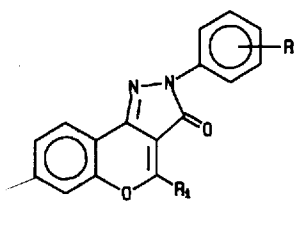


CGS series



1 and 2 series

reported are misleading.[7,8] In fact, from chromone derivatives and phenylhydrazine, 1-phenyl-[1]benzopyrano[3,4-*d*]pyrazole-4-ones are obtained[9,10] by nucleophilic rearrangement. Thus, in a preliminary letter,[10] we reported the first correct and generalized method for obtaining 2-aryl-4-*R*-[1]benzopyrano[4,3-*c*]pyrazole-3-ones. The synthetic pathway followed is outlined in Scheme I. Equimolar amounts of ethyl 3-(2-benzyloxyphenyl)-3-oxopropanoate and arylhydrazine were heated in a 100–120 °C oil bath to give rise to 1-aryl-3-(2-benzyloxyphenyl)pyrazole-5-ones (**3a–d**). Compounds **3a–d** were catalytically reduced to yield the 3-(2-hydroxyphenyl) derivatives **4a–d**. An anhydrous ethanolic solution of the latter was refluxed with ethyl orthoformate and aniline to give the 4-anilinomethylene derivatives **5a–d**. The heating of an alkaline solution of **5a–d**, followed by acidification with hydrochloric acid, yielded a solid which was mainly constituted of 1-aryl-3-(2-hydroxyphenyl)-4-formylpyrazole-5-one (**6**) together with a small amount of the tricyclic compounds **1a–d**. Only the 4-formyl derivative **6a** (R = H) was isolated and characterized. The presence of the other 4-formyl derivatives was detected in TLC and in the ^{1}H NMR spectra. Complete cyclization ensued when the mixture was treated with a few drops of concentrated sulfuric acid and then diluted with water.

When hydrochloric acid is added to an ethanolic solution of **6a** and the solution is heated, the dimer **7a** only is obtained. The latter could also be prepared by allowing **4a** to react with ethyl orthoformate.

Compounds **2a–d** were obtained, through the diacetate **8a–d**, by reacting **4a–d** with acetic anhydride and sodium acetate. Compounds **8a–d** were collected and dissolved in ethanol. An equimolar amount of piperidine was added and the solution was refluxed to yield **2a–d**.

The structures of the synthesized compounds were confirmed by ^{1}H and ^{13}C NMR data (Table I). The ^{13}C chemical shifts and the significant coupling constants are listed in Table II. The assignments are in agreement with those previously reported.[10]

Binding Studies—The ability of compounds of series 1 and 2 to interact with the benzodiazepine receptor sites was investigated by a binding assay, using [^{3}H]flunitrazepam as ligand and membranes from bovine cortical tissues as receptor sources. The results are shown in Table III.

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 360L instrument; chemical shifts are reported in δ (ppm) downfield from the internal standard

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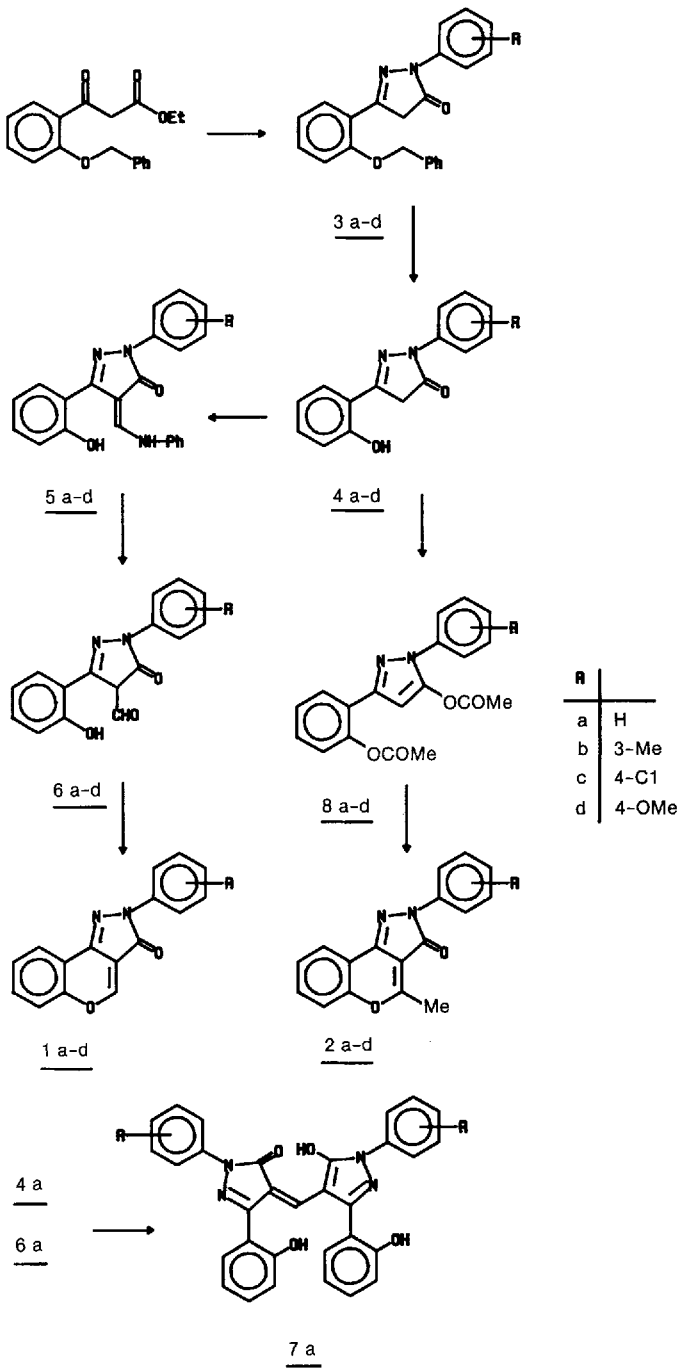


Scheme I

Me$_4$Si. The IR spectra were recorded with a Perkin-Elmer 1420 spectrophotometer in nujol mull. 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 of 30 °C, with a concentration in $CDCl_3$ 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) and corrected to internal Me$_4$Si. 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. The coupled spectra with nuclear Overhauser effect (NOE) were obtained by putting the decoupler on for a pulse delay of 1.6 s and off for an acquisition time of 0.8 s. The coupling constants are reported in Hz. 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. Physical data of the newly synthesized compounds are listed in Table IV.

Table I—Analytical Data of the Newly Synthesized Compounds

Compound	Formula	C	H	N
		Calc/Found	Calc/Found	Calc/Found
1c	$C_{16}H_9ClN_2O_2$	64.76/64.70	3.06/2.95	9.44/9.32
1d	$C_{17}H_{12}N_2O_3$	69.85/69.92	4.15/4.23	9.59/9.99
2c	$C_{17}H_{11}ClN_2O_2$	65.70/65.59	3.58/3.43	9.02/9.17
2d	$C_{18}H_{14}N_2O_3$	70.57/70.69	4.62/4.51	9.15/9.08
3c	$C_{22}H_{17}ClN_2O_2$	70.11/70.28	4.56/4.69	7.44/7.58
3d	$C_{23}H_{20}N_2O_3$	74.17/73.99	5.42/5.31	7.52/7.39
4c	$C_{15}H_{11}ClN_2O_2$	62.83/62.71	3.87/3.75	9.77/9.64
4d	$C_{16}H_{14}N_2O_3$	68.09/68.21	5.01/4.88	9.92/9.81
5c	$C_{22}H_{16}ClN_3O_2$	67.77/67.62	4.15/4.03	10.78/10.92
5d	$C_{23}H_{19}N_3O_3$	71.66/71.48	4.98/4.80	10.90/10.77
6a	$C_{16}H_{12}N_2O_3$	68.56/68.42	4.32/4.18	10.00/9.81
7a	$C_{31}H_{21}N_4O_4$	72.50/72.36	4.13/4.01	10.91/10.76
8c	$C_{19}H_{15}ClN_2O_4$	61.54/61.42	4.09/4.20	7.56/7.42
8d	$C_{20}H_{18}N_2O_5$	65.56/65.42	4.96/4.81	7.65/7.53

1-Aryl-3-(2-benzyloxyphenyl)pyrazole-5-ones (3a–d)—Equimolar amounts of ethyl 3-(2-benzyloxyphenyl)-3-oxopropanoate[11] and arylhydrazine were heated in a 100–120 °C oil bath for ~1–2 h. The fused mass was taken up with a little ethanol, collected by suction, and recrystallized. Compound **3d** displayed the following: ^1H NMR ($CDCl_3$): δ 3.83 (s, 3 H, OMe), 3.93 (s, 2 H, CH_2), 5.13 (s, 2 H, CH_2 benzyl), 6.8–7.6 (m, 10 H, aromatic protons), and 7.7–8.2 ppm (m, 3 H, aromatic protons); IR: 1690 cm^{-1}.

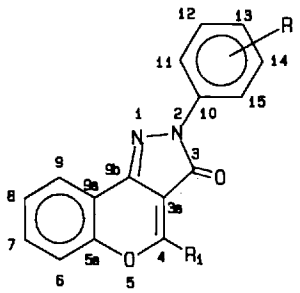
1-Aryl-3-(2-hydroxyphenyl)pyrazole-5-ones (4a–d)—Compounds **3a–d** (2.5 mmol) were dissolved in warm ethyl acetate (250 mL), and 10% Pd/C (30% relative to the weight of **3d**) was added. The mixture was hydrogenated in a Parr apparatus at 20 psi for 2 h. The catalyst was filtered off, the solvent evaporated at reduced pressure, and the solid residue recrystallized. Compound **4d** displayed the following ^1H NMR ($CDCl_3$): δ 3.83 (s, 3 H, OMe), 3.87 (s, 2 H, CH_2), 6.9–7.5 (m, 6 H, aromatic protons), and 7.6–7.9 ppm (m, 2 H, aromatic protons); IR: 1710 cm^{-1}.

1-Aryl-3-(2-hydroxyphenyl)-4-anilinomethylenepyrazole-5-ones (5a–d)—Compounds **4a–d** (2.5 mmol) were suspended in anhydrous ethanol (25 mL) and equimolar amounts of aniline and ethyl orthoformate were added. The resulting yellowish solution was refluxed. In the case of **5a–b**, the heating was carried out for 1 h and the solution was cooled to give a yellow precipitate, while in the case of **5c–d**, a solid was formed after a few minutes of heating. However, even in this case, the heating was carried on for another hour. The yellow precipitate was collected by filtration and recrystallized. Compound **5d** displayed the following: ^1H NMR ($CDCl_3$):δ 3.80 (s, 3 H, OMe), 6.8–7.5 (m, 12 H, 11 aromatic protons $^+$NH), 7.7–8.0 (m, 2 H, aromatic protons), 8.16 (br s, 1 H, CH = N), and 10.5 ppm (br s, 1 H, OH); IR: 1625 and 1655 cm^{-1}.

2,3-Dihydro-2-aryl-[1]benzopyrano[4,3-c]pyrazole-3-ones (1a–d)—Compounds **5a–d** (3 mmol) were dissolved in a 5% solution of sodium hydroxide (30 mL) and heated in an open flask for 2 h. The solution was cooled and acidified with 6 M HCl to give a solid which was mainly made up of the 4-formyl derivatives together with a small amount of **1a–d**. Only the 2-phenyl-4-formyl derivative **6a** was isolated and characterized. Its physicochemical data are listed in Table IV. All the other 4-formyl derivatives were detected in TLC and ^1H NMR spectra. Compound **6a** displayed the following: ^1H NMR ($CDCl_3$): δ 6.8–8.0 (m, 9 H, aromatic protons), 9.94 (s, 2 H, 2 OH), and 10.00 ppm (s, 1 H, formyl); IR: 1620 cm^{-1}.

To complete the cyclization, the crude mixture, made up of the 4-formyl derivative and tricyclic compound, was stirred for 1 min at room temperature with a few drops of concentrated H_2SO_4 and then quickly diluted with water to give a precipitate which was collected by filtration and then recrystallized. Compound **1d** displayed the following: ^1H NMR ($CDCl_3$): δ 3.83 (s, 3 H, OMe), 6.9–7.1 (m, 2 H, aromatic protons), 7.4–7.7 (m, 3 H, aromatic protons), 7.9–8.3 (m, 3 H, aromatic protons), and 8.43 ppm (s, 1 H, H-4); IR: 1235, 1650, and 1670 cm^{-1}.

Table II—^{13}C Chemical Shifts and Significant ^{13}C-^{1}H Coupling Constants of Compounds of 1 and 2 Series[a]

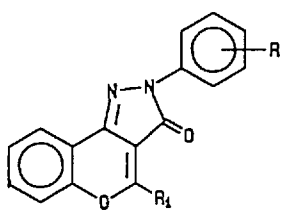


1a : R=R$_1$=H
1b : R=3-CH_3; R$_1$=H
1c : R=4-Cl; R$_1$=H
1d : R=4-OCH_3; R$_1$=H
2a : R=H; R$_1$=CH_3
2b : R=3-CH_3; R$_1$=CH_3
2c : R=4-Cl; R$_1$=CH_3
2d : R=4-OCH_3; R$_1$=CH_3

Compound	Carbon																	
	3	3a	4	5a	6	7	8	9	9a	9b	10	11	12	13	14	15	16	17
1a	160.79	113.38	153.17	151.71	118.41	131.29	126.99	122.91	116.97	140.01	138.79	119.42	128.69	125.08	128.69	119.43	—	—
1b	160.66	113.37	152.92	151.64	118.31	131.14	126.84	122.84	116.94	139.80	138.73	119.92	138.46	128.44	125.82	116.56	21.43	—
1c	160.74	113.25	153.32	151.80	118.48	131.48	127.10	122.96	116.85	140.24	137.48	120.32	128.69	130.10	128.69	130.32	—	—
1d	160.42	113.42	153.00	151.69	118.41	131.15	126.95	122.89	117.07	139.75	132.24	113.94	121.34	157.11	121.34	113.94	55.36	—
2a	161.66	109.04	167.76	151.97	117.88	130.75	126.38	122.56	116.75	140.07	139.09	119.17	128.54	124.68	128.54	119.17	—	16.41
2b	161.67	108.93	168.51	151.94	117.90	130.84	126.50	122.60	116.57	140.20	138.63	120.28	138.44	128.41	125.99	116.89	21.36	16.56
2c	161.65	108.90	168.09	152.04	119.95	130.96	126.51	122.62	116.60	140.34	137.71	120.04	128.53	129.68	128.53	120.04	—	16.51
2d	161.29	109.04	167.69	151.95	117.89	130.63	126.38	122.54	116.81	139.82	132.51	113.78	121.07	156.82	121.07	113.78	55.28	16.44

[a] ^{13}C-^{1}H Coupling constants: 1 series: ^{1}JC4-H4 = 199.0 Hz, ^{2}JC3a-H4 = 7.5 Hz, ^{3}JC9b-H9 = 4.7; 2 series: ^{2}JC4-H17 = 6.6 Hz, ^{3}JC3a-H17 = 3.1 Hz, ^{3}JC9b-H9 = 4.4.

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



Compound	R	R$_1$	I%, μM[a]	IC$_{50}$, μM[b]
1a	H	H	4.8 ± 0.2 (68)	—
1b	3-Me	H	4.7 ± 0.3 (68)	—
1c	4-Cl	H	7.5 ± 0.5 (68)	—
1d	4-OMe	H	8.5 ± 0.7 (68)	—
2a	H	Me	51 ± 0.4 (68)	70 ± 6.0
2b	3-Me	Me	0 (6)	—
2c	4-Cl	Me	2.8 ± 0.1 (68)	—
2d	4-OMe	Me	16.3 ± 1.2 (6)	55 ± 6.0
Chlordiazepoxide			—	0.79 ± 0.07
Diazepam			—	0.025 ± 0.002
CGS 9896			—	0.0006 ± 0.00004

[a] Inhibition percentages (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 four determinations.

4-[1-Phenyl-3-(2-hydroxyphenyl)-5-hydroxy-4-pyrazolylmethylene]-1-phenyl-3-(2-hydroxyphenyl)pyrazole-5-one (7a)—*Method A*—To a hot solution of **4a** (0.6 g, 2.4 mmol) in toluene (40 mL), ethyl orthoformate (0.78 mL, 4.8 mmol) was added in a dropwise manner. The mixture was refluxed for 10 min, and the toluene was distilled off under reduced pressure to leave a brownish oily residue. The latter was taken up with diethyl ether to give an orange solid which was recrystallized.

Method B—Compound **6a** (0.5 g, 1.8 mmol) was suspended in 6 M HCl (15 mL) and ethanol (10 mL) and refluxed for 10–15 min. The orange solid was collected and recrystallized. Compound **7a** displayed the following: ^{1}H NMR (CDCl$_3$): δ 6.6–7.7 (m, 14 H, aromatic protons), 7.8–8.1 (m, 4 H, aromatic protons), 8.15 (s, 1 H, CH=), 9.6 (br s, 2 H, 2 OH), and 17.3 ppm (br s, 1 H, OH pyrazole); IR: 1580 and 1620 cm^{-1}.

Table IV—Physical Data of Newly Synthesized Compounds

Compound	Formula	mp, °C	Crystallization Solvent[a]	Yield, % (method)
1c	$C_{16}H_9ClN_2O_2$	214–215	A	63
1d	$C_{17}H_{12}N_2O_3$	188–189	A	72
2c	$C_{17}H_{11}ClN_2O_2$	205–207	B	78
2d	$C_{18}H_{14}N_2O_3$	180–182	B	90
3c	$C_{22}H_{17}ClN_2O_2$	194–196	C	50
3d	$C_{23}H_{20}N_2O_3$	125–126	C	40
4c	$C_{15}H_{11}ClN_2O_2$	195–196	A	50
4d	$C_{16}H_{14}N_2O_3$	136–137	A	62
5c	$C_{22}H_{16}ClN_3O_2$	216–218	C	88
5d	$C_{23}H_{19}N_3O_3$	168–170	D	78
6a	$C_{16}H_{12}N_2O_3$	107–108	E	70
7a	$C_{31}H_{21}N_4O_4$	194–195	C	50 (A) 74 (B)
8c	$C_{19}H_{15}ClN_2O_4$	93–94	E	76
8d	$C_{20}H_{18}N_2O_5$	90–92	E	35

[a] A = cyclohexane:ethyl acetate; B = ethanol; C = ethyl acetate; D = ethyl acetate:ethanol; E = cyclohexane.

1-Aryl-3-(2-phenylacetate)-5-pyrazole acetates (8a–d)—A mixture of **4a–d** (3 mmol) and anhydrous sodium acetate (3.1 mmol) in acetic anhydride (2 mL) was heated in a 80–100 °C oil bath for 2 h. The solution was cooled and diluted with water (20 mL). Chloroform (20 mL) was added and the mixture was then stirred overnight at room temperature. The two layers were separated, and the organic layer was washed three times with water (15 mL each time). The chloroform solution was dried with anhydrous sodium sulfate and the organic solvent was evaporated at reduced pressure. The resulting residue was recrystallized. Compound **8d** displayed the following: ^{1}H NMR (CDCl$_3$): δ 2.23 (s, 3 H, Me), 2.30 (s, 3 H, Me), 3.83 (s, 3 H, OMe), 6.63 (s, 1 H, pyrazole proton), 6.9–8.7 (m, 7 H, aromatic protons), and 7.8–8.1 ppm (m, 1 H, aromatic proton); IR: 1760, 1775, and 1790 cm^{-1}.

2,3-Dihydro-2-aryl-4-methyl-[1]benzopyrano[4,3-c]pyrazole-3-ones (2a–d)—To a hot solution of **8a–d** (2 mmol) in anhydrous ethanol (3 mL) an equimolar amount of piperidine was added. The

yellow solution was refluxed for 15 min. After cooling, the yellow or orange precipitate formed was collected by suction and recrystallized. Compound **2d** displayed the following: ^{1}H NMR ($CDCl_3$): δ 2.83 (s, 3H, Me), 3.85 (s, 3 H, OMe), 6.9–7.1 (m, 2 H, aromatic protons), 7.4–7.6 (m, 3 H, aromatic protons), and 7.9–8.3 ppm (m, 3 H, aromatic protons); IR: 1245, 1655, and 1685 cm^{-1}.

Binding Studies—Membranes from bovine brains were prepared as described by Melani et al.[2] The ability of **1a–d** and **2a–d** to displace a concentration of 0.6 nM of [^{3}H]flunitrazepam from its specific binding was determined as described by Colotta et al.[5] Compounds **1a–d** and **2a–d** 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 the ethanol (2%) on the binding. All the compounds but two were assayed at a concentration of 68 μM. Compounds **2b** and **2d**, because of their insolubility in the ethanol buffer, were assayed at a concentration of 6 μM.

The percentages for inhibition of [^{3}H]flunitrazepam are reported in Table III. The concentration able to inhibit specific [^{3}H]flunitrazepam binding by 50% (IC_{50}) is reported only for the most active compounds. For **2a**, this concentration was determined by a log-probit plot, while for **2d** it was derived according to the equation of Cheng and Prusoff:[12]

$$K_1 = \frac{IC_{50}}{1 + \frac{[L]}{K_D}} \quad (1)$$

where K_D and K_1 were derived by Lineweaver–Burk analysis of the binding data using [^{3}H]flunitrazepam at various concentrations (0.2–6 nM) in the absence and in the presence of a 6μM concentration of **2d**, as shown in Figure 1. Moreover, the results indicate that **2d**, and most likely the others too, inhibits specific [^{3}H]flunitrazepam binding in a competitive manner.

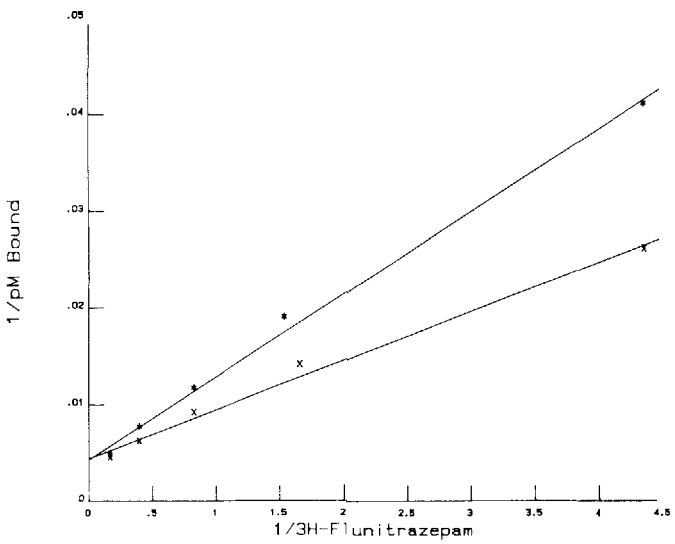


Figure 1—*Lineweaver–Burk analysis of* **2d** *inhibition of specific [^{3}H]-flunitrazepam binding. Compound* **2d** *was incorporated at a concentration of 6 μM in the [^{3}H]flunitrazepam receptor binding assay ([^{3}H]flunitrazepam concentration ranges from 0.2 to 6 nm). The experiments were performed four times and gave the same results.*

Results and Discussion

None of the compounds of series 1 showed any ability to displace [^{3}H]flunitrazepam from its specific binding to bovine brain membrane receptors. On the other hand, some inhibiting activity is shown by the compounds of series 2, although none of them could be considered sufficiently potent to warrant additional studies. These results are quite unexpected, in view both of the close similarity of compounds of series 1 and those of the CGS series, and of our previous findings on pyrazolo-quinoline-4-ones (**A**)[1–4] and [1]benzopyrano-pyrazole-4-ones (**B**).[5]

However the present findings, even if disappointing, allowed us to establish some more structure–activity relationships on the two 4-one tricyclic systems **A** and **B**, on 3-ones 1 and 2, and on the CGS series. Since the only difference between the tested compounds and the CGS series is the presence of an oxygen atom in the former and of an NH in the latter, it follows that the lack of binding activity of the benzopyrano-pyrazole-3-ones should be due to the replacement of the NH of the CGS series with the oxygen atom. It seems evident that the NH of the CGS series is the most important part in the interaction with the receptor.

This implies that in the case of the previously reported series (**A** and **B**), their anchoring to the recognition site of the benzodiazepine receptor is not affected by the replacement of the δ-lactame with the δ-lactone moiety, since in both series the electronic properties of this part of the molecule are almost the same.[13] On the contrary, the replacement of the NH of the CGS series with the oxygen atom turns a cyclic amine into a cyclic ether, giving rise to different electronic properties which may explain the lack of potency of the compounds of series 1 and 2.[13]

References and Notes

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