Tricyclic Heteroaromatic Systems. Pyrazolo[3,4-*c*]quinolin-4-ones and Pyrazolo[3,4-*c*]quinoline-1,4-diones: Synthesis and Benzodiazepine Receptor Activity

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Summary

Some pyrazolo[3,4-*c*]quinoline-4-ones **1–14** and pyrazolo[3,4-*c*]-quinoline-1,4-diones **15–17** were prepared and biologically evaluated for their binding at the benzodiazepine receptor (BZR) in rat cortical membranes. The moderate binding activity of **1–5, 7, 9–10, 13** is attributable to the lack of the optional proton acceptor at position-1, while the inactivity of the 1,4-dione derivatives **15–17** is due to the lack of the essential proton acceptor at position-3. These conclusions confirm the validity of our proposed pharmacophoric model.

Introduction

The continuum of biological effects elicited by the interaction of a BZR ligand with its receptor suggests that, whatever the final effect, the structural requirements for receptor recognition should have some common features [1]. With the aim of understanding what these pharmacophoric descriptors for the anchoring of a chemical compound to the BZR are, some research in our laboratory has been directed toward the synthesis, BZR binding activity, and structure-activity relationship (SAR) studies of some 6,6,5 tricyclic heteroaromatic systems. The SAR studies have led to the formulation of a schematic representation of some optional (a_1 and d) and essential (L_1, L_2, and a_2) pharmacophoric descriptors for receptor recognition of our 6,6,5-tricyclic BZR ligands [2–6] (see Figure 1). In this paper we report on the synthesis, BZR binding activity, and SAR of some pyrazolo[3,4-*c*]quinolines **1–17** (see Chart 1).

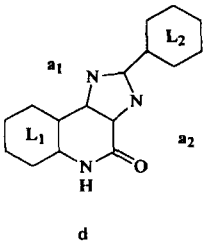


Figure 1. Schematic two-dimensional representation of essential and optional pharmacophoric descriptors of a BZR ligand. Essential pharmacophoric descriptors are: L_1 and L_2 lipophilic areas, and a_2 proton acceptor site. Optional binding sites, only affecting the potency of a ligand, are: d proton donor and a_1 proton acceptor sites.

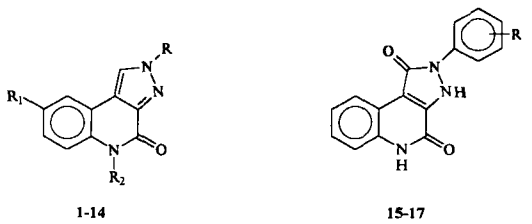
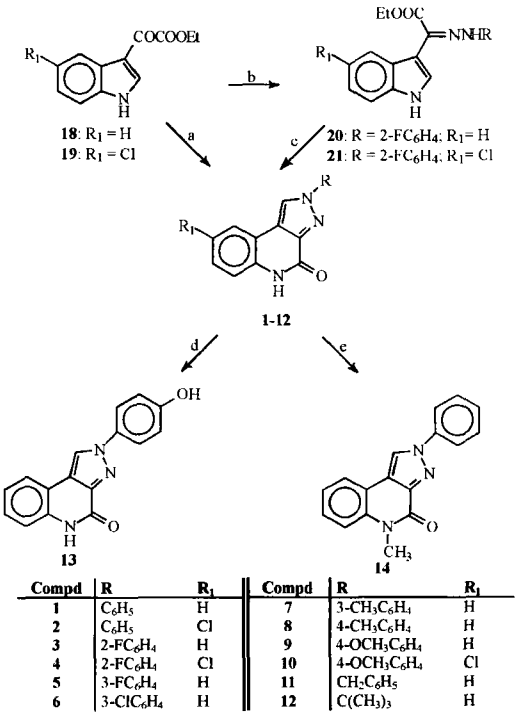


Chart 1

Chemistry

The synthetic pathway which yielded compounds **1–17** is illustrated in Schemes 1–2.

The synthesis of the 2-substituted pyrazolo[3,4-*c*]quinolin-4-ones **1**[7] **–14** is shown in Scheme 1. Most of these tricyclic derivatives, i.e., **1–2, 5–12**, could be obtained by reacting the 3–ethoxalylindoles **18**[8] **–19** with hydrazine hydrochlorides. This one-pot reaction, however, failed to occur using 2-



Compd	R	R_1	Compd	R	R_1
1	C_6H_5	H	7	3-$CH_3C_6H_4$	H
2	C_6H_5	Cl	8	4-$CH_3C_6H_4$	H
3	2-FC_6H_4	H	9	4-$OCH_3C_6H_4$	H
4	2-FC_6H_4	Cl	10	4-$OCH_3C_6H_4$	Cl
5	3-FC_6H_4	H	11	$CH_2C_6H_5$	H
6	3-ClC_6H_4	H	12	$C(CH_3)_3$	H

Scheme 1. a: hydrazine hydrochloride, EtOH/AcOH. b: 2-Fluorophenylhydrazine, EtOH/AcOH. c: EtOH/conc HCl. d: AcOH, 48% HBr. e: CH_3I, NaH, DMF.

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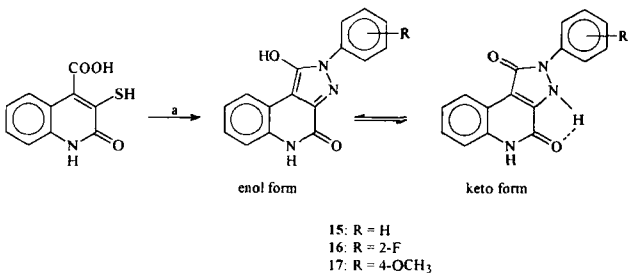
fluorophenylhydrazine hydrochloride. Thus, the final 2-(2-fluorophenyl) derivatives **3** and **4** were prepared following a two-step synthetic pathway. Compounds **18–19** were allowed to react with 2-fluorophenylhydrazine free base to afford the hydrazones **20–21** which were rearranged under acidic conditions to form the tricyclic derivatives **3–4**. Dealkylation of the 2-(4-methoxyphenyl) derivative **9** and *N*-methylation of compound **1** yielded compounds **13** and **14**, respectively.

When screened compounds **1–14** on the whole displayed a weak BZR affinity, which we supposed could be due to the lack of the optional proton acceptor atom at position-1 of the *tricyclic system*. We then tried to enhance the BZR affinity of these pyrazolo[3,4-*c*]quinolines by introducing an exocyclic oxygen atom at position-1. Toward this aim, we prepared compounds **15–17** [9], which were readily obtained by reacting the 2-oxo-3-mercaptoquinoline-4-carboxylic acid[10] with arylhydrazines (Scheme 2).



Scheme 2. a: aryl hydrazine, EtOH.

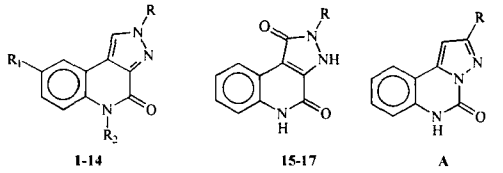
Compounds **15–17** may exist either in the keto or in the enol form. X-ray crystallographic analysis of **15** evidenced the existence of the keto form [9]. We hoped that, at least at the receptor level, **15–17** existed in the aromatic enol form, which would provide both the optional proton acceptor enol at position-1 and the essential nitrogen acceptor at position-3. The BZR binding inactivity of **15–17** suggested that at the receptor level **15–17** existed also in the keto form. The keto form, in fact, provided a strong exocyclic proton acceptor at position-1, but also implied the presence of a proton on the essential nitrogen atom at position-3. The proton on the nitrogen atom at position-3 is able to engage an intramolecular hydrogen bond with the carbonyl oxygen of position-4, thus eliminating the possibility of **15–17** to engage a hydrogen bond with a BZR hydrogen donor in the essential a_2 region.

Results and Discussion

The binding activities of the reported compounds at the BZR in rat cortical membranes were determined by displacement experiments with radiolabeled [^{3}H]flunitrazepam and are listed in Table 1. In Table 1 the K_i values of diazepam and of previously reported 5,6-dihydro-2-phenylpyrazolo[1,5-*c*]quinazolin-5-one[6] **A** are also shown.

Above we have explained the weak BZR affinity of **1–14** and the inactivity of their oxidised derivatives **15–17**. However, some further SAR can be drawn on **1–14**. The non-additive effect of the 8-chloro substituent has previously been

Table 1. Binding activity of pyrazolo[3,4-*c*]quinolines **1–17**.

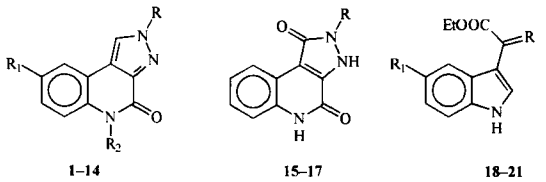


Cpd	R	R_1	R_2	$K_i \pm$ SEM (nM)[a]
1	C_6H_5	H	H	119 ± 14
2	C_6H_5	Cl	H	52 ± 7
3	2-FC_6H_4	H	H	175 ± 21
4	2-FC_6H_4	Cl	H	4405 ± 910
5	3-FC_6H_4	H	H	7902 ± 700
6	3-ClC_6H_4	H	H	>10000
7	3-$CH_3C_6H_4$	H	H	2657 ± 700
8	4-$CH_3C_6H_4$	H	H	>10000
9	4-$OCH_3C_6H_4$	H	H	203 ± 28
10	4-$OCH_3C_6H_4$	Cl	H	608 ± 70
11	$CH_2C_6H_5$	H	H	>10000
12	$C(CH_3)_3$	H	H	>10000
13	4-OHC_6H_4	H	H	601 ± 126
14	C_6H_5	H	CH_3	>10000
15	C_6H_5	H	H	>10000
16	2-FC_6H_4	H	H	>10000
17	4-$OCH_3C_6H_4$	H	H	>10000
A	C_6H_5			59 ± 3.5[b]
diazepam				10.5 ± 1.4

[a] K_i values are means ± SEM of 3–5 separate determinations. [b] Ref.[6].

observed [4–6]. In one case only, compound **2** vs **1**, the presence of 8-halogen enhances binding activity, while the presence of a chlorine atom at position-8, in the case of the 2-(2-fluorophenyl)- and 2-(4-methoxyphenyl) derivatives, causes a decrease in receptor affinity (see Table 1, **4** vs **3** and **10** vs **9**, respectively). The appended 2-phenyl ring does not support any substitution. Only an *ortho*-fluorine atom is tolerated since compound **3** displayed the same order of BZR affinity of the unsubstituted compound **1**. Replacement of the 2-phenyl ring of **1** with aralkyl or alkyl moieties (compounds **11** and **12**, respectively) is deleterious to binding activity. The dramatically reduced affinity of the methylated derivative **14**, compared to that of the parent compound **1**, may be attributed to the steric effect of the methyl substituent and has already been observed [2, 4, 6]. In fact, the steric effect of the methyl substituent at position-5 of **14** is the only one responsible for the BZR inactivity of the compound, since previously reported benzoxazine derivatives [5], devoid of the d proton donor, bind to the BZR with nanomolar affinity [5].

Table 2. Physical data of pyrazolo-quinolines **1–17** and of their parent compounds **18–21.**



1–14 **15–17** **18–21**

Cpd	R	R_1	R_2	mp, °C	solv.[a]	% yield
1[b]	C_6H_5	H	H	>300	A	72
2	C_6H_5	Cl	H	>300	B	64
3	2-FC_6H_4	H	H	>300	A	12
4	2-FC_6H_4	Cl	H	>300	C	13
5	3-FC_6H_4	H	H	>300	D	86
6	3-ClC_6H_4	H	H	>300	C	40
7	3-$CH_3C_6H_4$	H	H	291–292	D	75
8	4-$CH_3C_6H_4$	H	H	>300	D	90
9	4-$OCH_3C_6H_4$	H	H	291–292	D	75
10	4-$OCH_3C_6H_4$	Cl	H	>300	C	84
11	$CH_2C_6H_5$	H	H	215–216	E	55
12	$C(CH_3)_3$	H	H	276–277	A	55
13	4-OHC_6H_4	H	H	>300	C	84
14	C_6H_5	H	CH_3	234–236	E	90
15[c]	C_6H_5			>300	E	55
16	2-FC_6H_4			>300	D	41
17[d]	4-$OCH_3C_6H_4$			285–286	D	22
18[e]	O	H		188–190	A	74
19	O	Cl		242–243	A	49
20	NNH-2FC_6H_4	H		166–167	A	62
21	NNH-2FC_6H_4	Cl		143–144	A	42

[a]Recrystallization solvents: A = ethanol. B = glacial acetic acid/dimethylformamide. C = dimethylformamide. D = glacial acetic acid. E = glacial acetic acid/ethanol. [b]Ref.[7]: mp 310 °C (chloroform). [c]Ref.[9]: mp >260 °C (methanol). [d]Ref.[9]: mp 285 °C. [e]Ref.[8]: mp 189 °C (acetone).

SAR of **1–14** are similar to those of some previously reported tricyclic derivatives [2–6]. It follows that the compounds reported in this paper bind to the BZR in a similar way, according to the proposed pharmacophoric representation shown in Figure 1.

In conclusion, the synthesis and the binding data of the pyrazolo-quinolines **1–17** confirm the validity of our proposed BZR pharmacophoric model. In fact, although the pyrazolo-quinolin-4-ones **1–14** lack the optional proton acceptor at position-1, most of them bind to the BZR, even though with moderate affinity. That confirms that the optional proton acceptor at position-1 only influences the potency of a BZR ligand. On the contrary, the pyrazolo-quinoline-1,4-diones **15–17**, which in their keto form are lacking the essential proton acceptor at position-3, do not bind to the BZR, thus supporting our hypothesis of the necessary presence of a proton acceptor atom in the a_2 region.

Experimental Section

A) Chemistry

Silica gel plates (Merck F_{254}) and silica gel 60 (Merck; 70–230 mesh) were used for analytical and column chromatography, respectively. All melting points were determined on a Gallenkamp melting point apparatus. Microanalyses were performed with a Perkin-Elmer 260 elemental analyser for C, H, N, and the results were within ± 0.4% of the theoretical values. The IR spectra were recorded with a Perkin-Elmer 1420 spectrometer in Nujol mulls and are expressed in cm^{-1}. The ^1H NMR spectra were obtained with a Varian Gemini 200 instrument at 200 MHz. The chemical shifts are reported in δ (ppm) and are relative to the central peak of the solvent. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, and ar = aromatic protons. The physical data of the newly synthesised compounds are shown in Table 2.

General procedure for the synthesis of ethyl 2-(3-indolyl)-2-oxoethanoates **18** [8] **–19**

A solution of ethyl oxalylchloride (7.9 mmol) in anhydrous diethyl ether (5 mL) was added dropwise and under stirring to a cooled solution (0–5 °C) of indole or 5-chloroindole (6.6 mmol) in anhydrous diethyl ether (20 mL) and pyridine (0.7 mL). At the end of the addition (about twenty minutes) the reaction mixture was stirred at 0 °C for 1 h and then at room temperature for another 5 h. The resulting solid was collected and washed with a small amount of diethyl ether and then with water. Compound **19** displayed the following spectral data: ^1H NMR (DMSO-d_6): 1.35 (t, 3H, CH_3,), 4.37 (q, 2H, CH_2), 7.34 (d, 1H, H-6, J = 8.7 Hz), 7.59 (d, 1H, H-7, J = 8.7 Hz), 8.14 (s, 1H, H-4), 8.53 (s, 1H, H-2), 12.56 (s, 1H, NH).– IR: 1730, 3220.

General procedure for the synthesis of 4,5-dihydro-2H-pyrazolo[3,4-c]-quinolin-4-ones **1** [7] **–2, 5–12**

An excess of the suitable hydrazine hydrochloride (10.1 mmol) was added to a suspension of **18** [8] **–19** (4.6 mmol) in absolute ethanol (20 mL) and glacial acetic acid (0.8 mL). The reaction mixture was heated at reflux for 3 h (3 days for compound **12**). The resulting solid (compounds **1–2, 5–10**) was collected, washed with a small amount of diethyl ether, and recrystallized. The solution was concentrated (10 mL), and the resulting solid (**11–12**) was collected, washed with a small amount of ethanol, and then recrystallized. Compounds **1–2, 5–12** displayed the following spectral data:

1 [7]: ^1H NMR (DMSO-d_6): 7.20–7.28 (m, 1H, ar), 7.35–7.52 (m, 3H, ar), 7.61–7.68 (m, 2H, ar), 7.96–8.07 (m, 3H, ar), 9.50 (s, 1H, H-1), 11.50 (s, 1H, NH).– IR: 1665, 3185.

2: ^1H NMR (DMSO-d_6): 7.33–7.53 (m, 3H, ar), 7.62–7.68 (m, 2H, ar), 8.00 (d, 2H, ar, J = 9.5 Hz), 8.08 (d, 1H, H-9, J = 1.9 Hz), 9.55 (s, 1H, H-1), 11.62 (s, 1H, NH).– IR: 1675, 3120.

5: ^1H NMR (DMSO-d_6): 7.21–7.42 (m, 4H, ar), 7.64–7.76 (m, 1H, ar), 7.91–7.96 (m, 3H, ar), 9.54 (s, 1H, H-1), 11.54 (s, 1H, NH).– IR: 1680, 3120.

6: 1H NMR (DMSO-d_6): 7.21–7.29 (m, 1H, ar), 7.34–7.41 (m, 2H, ar), 7.53–7.71 (m, 2H, ar), 7.92–8.15 (m, 3H, ar), 9.57 (s, 1H, H-1), 11.53 (s, 1H, NH).– IR: 1680, 3120.

7: ^1H NMR (DMSO-d_6): 2.46 (s, 3H, CH_3), 7.21–7.40 (m, 4H, ar), 7.51 (t, 1H, ar, J = 7.8 Hz), 7.81–7.99 (m, 3H, ar), 9.47 (s, 1H, H-1), 11.48 (s, 1H, NH).– IR: 1680, 3120.

8: ^1H NMR (DMSO-d_6): 2.40 (s, 3H, CH_3), 7.19–7.45 (m, 5H, ar), 7.90–7.98 (m, 3H, ar), 9.43 (s, 1H, H-1), 11.47 (s, 1H, NH).– IR: 1680, 3120.

9: ^1H NMR (DMSO-d_6): 3.85 (s, 3H, CH_3), 7.15–7.38 (m, 5H, ar), 7.93–7.97 (m, 3H, ar), 9.36 (s, 1H, H-1), 11.45 (s, 1H, NH).– IR: 1685, 3200.

10: ^1H NMR (DMSO-d_6): 3.86 (s, 3H, CH_3), 7.19 (d, 2H, ar, J = 9.0 Hz), 7.34–7.46 (m, 2H, ar), 7.91 (d, 2H, ar, J = 9.0 Hz), 8.07 (d, 1H, H-9, J = 2.1 Hz), 9.43 (s, 1H, H-1), 11.58 (s, 1H, NH).– IR: 1675, 3120.

11: ^1H NMR (DMSO-d_6): 5.63 (s, 2H, CH_2), 7.13–7.21 (m, 1H, ar), 7.33–7.40 (m, 7H, ar), 7.89 (d, 1H, ar, J = 7.8 Hz), 8.80 (s, 1H, H-1), 11.37 (s, 1H, NH).– IR: 1685, 3120.

12: ^1H NMR (DMSO-d_6): 1.66 (s, 9H, $3CH_3$), 7.14–7.22 (m, 1H, ar), 7.31–7.34 (m, 2H, ar), 7.92–7.96 (d, 1H, ar, J = 7.8 Hz), 8.86 (s, 1H, H-1), 11.32 (s, 1H, NH).– IR: 1680, 3120.

General procedure for the synthesis of 2-fluorophenylhydrazones of ethyl 2-(3-indolyl)-2-oxoethanoates **20–21**

An excess of 2-fluorophenylhydrazine (7.1 mmol) was added to a suspension of **18** [8]–**19** (3.2 mmol) in absolute ethanol (15 mL) and glacial acetic acid (0.8 mL). The reaction mixture was heated at reflux until the disappearance of the starting material (TLC monitoring). The yellow solid resulting from the cooled mixture was collected, washed with ethanol/diethyl ether and then recrystallized. Compounds **20–21** displayed the following spectral data:

20: ^{1}H NMR ($CDCl_3$): 1.46 (t, 3H, CH_3), 4.46 (q, 2H, CH_2), 6.88–7.44 (m, 6H, ar), 7.74–7.84 (m, 2H, ar), 8.2 (br s, 1H, NH), 8.38–8.43 (m, 1H, ar), 12.31 (s, 1H, NH).– IR: 1675, 3240, 3400.

21: ^{1}H NMR (DMSO-d_6): 1.40 (t, 3H, CH_3), 4.43 (q, 2H, CH_2), 6.95–7.03 (m, 1H, ar), 7.18–7.36 (m, 3H, ar), 7.50 (d, 1H, ar, J = 8.7 Hz), 7.62 (t, 1H, ar, J = 8.0 Hz), 7.90 (d, 1H, ar, J = 2.5 Hz), 8.25 (s, 1H, ar), 11.60 (s, 1H, NH), 11.98 (s, 1H, NH).– IR: 1670, 3270, 3480.

General procedure for the synthesis of 2-(2-fluorophenyl)-4,5-dihydro-2H-pyrazolo[3,4-c]quinolin-4-ones **3–4**

A suspension of **20–21** (1.1 mmol) in ethanol (30 mL) and concentrated hydrochloric acid (0.3 mL) was heated at reflux until the disappearance of the starting material (TLC monitoring). Upon cooling a solid precipitated which was collected, washed with ethanol and recrystallized. Compounds **3–4** displayed the following spectral data:

3: ^{1}H NMR (DMSO-d_6): 7.19–7.27 (m, 1H, ar), 7.35–7.64 (m, 5H, ar), 7.92–8.06 (m, 2H, ar), 9.21 (s, 1H, H-1), 11.52 (s, 1H, NH).– IR: 1680, 3070, 3150.

4: ^{1}H NMR (DMSO-d_6): 7.31–7.67 (m, 5H, ar), 7.92–8.0 (m, 1H, ar), 8.22 (d, 1H, H-9, J = 2.1 Hz), 9.28 (s, 1H, H-1), 11.62 (br s, 1H, NH).– IR: 1680, 3200.

2-(4-Hydroxyphenyl)-4,5-dihydro-2H-pyrazolo[3,4-c]quinolin-4-one **13**

A solution of the 4-methoxy-phenyl derivative **9** (0.68 mmol) in glacial acetic acid (5 mL) and hydrobromic acid (48%, 5 mL) was heated at reflux for 10 h. Upon cooling a solid precipitated which was collected, washed with water, and recrystallized. The title compound displayed the following spectral data:– ^{1}H NMR (DMSO-d_6): 6.97 (d, 2H, ar, J = 8.9 Hz), 7.20–7.37 (m, 3H, ar), 7.82 (d, 2H, ar, J = 8.9 Hz), 7.95 (d, 1H, ar, J = 7.3 Hz), 9.3 (s, 1H, H-1), 9.92 (s, 1H, OH), 11.44 (s, 1H, NH).– IR: 1670, 3120.

2-Phenyl-4,5-dihydro-5-methyl-2H-pyrazolo[3,4-c]quinolin-4-one **14**

Iodomethane (1.44 mmol, 0.09 mL) and natrium hydride (80% paraffin oil, 0.06 g) were added to a solution of **1** (0.96 mmol) in anhydrous dimethylformamide (15 mL). The mixture was stirred at room temperature for 6 h and then quenched with ice/water (about 30 mL). The resulting solid was collected, washed with water, and recrystallized. The title compound displayed the following spectral data:– ^{1}H NMR (DMSO-d_6): 3.69 (s, 3H, CH_3), 7.30–7.38 (m, 1H, ar), 7.45–7.54 (m, 3H, ar), 7.60–7.67 (m, 2H, ar), 8.05 (m, 3H, ar), 9.50 (s, 1H, H-1).– IR: 1670.

General procedure for the synthesis of 2,3,4,5-tetrahydro-2-aryl-1H-pyrazolo[3,4-c]quinolin-1,4-diones **15–17** [9]

Aryl hydrazine (5.54 mmol) was added to a solution of 1,2-dihydro-2-oxo-3-mercaptoquinoline-2-carboxylic acid [10] (2.26 mmol) in ethanol (20 mL). The mixture was refluxed for 3 h. Evaporation at reduced pressure of the solvent yielded a residue which was treated with hydrochloric acid (2N, 15 mL), collected, and recrystallized. Compound **16** displayed the following spectral data:– ^{1}H NMR (DMSO-d_6): 7.20–7.72 (m, 7H, ar), 8.18 (d, 1H, ar, J = 6.8 Hz), 11.85 (br s, 2H, 2NH).– IR: 1620–1660, 3180.

B) Biochemistry

Crude synaptic membranes were prepared from cerebral cortices of male Sprague-Dawley rats (170–250 g). The tissue was homogenized in 15 vol of ice-cold 0.32 M sucrose, containing 20 mg/ml of phenylmethanesulfonyl fluoride, using a glass-Teflon homogenizer (clearance = 0.15–0.23 mm). The homogenate was centrifuged at 1000 × g for 10 min and the resulting supernatant further centrifuged at 20000 × g for 20 min. The final pellet was resuspended in 15 vol of ice-cold distilled water, dispersed with an Ultra-Turrax sonicator (30% of maximum speed) for 30 s, and centrifuged at 8000 × g for 20 min. The membranes were resuspended once more in distilled water, centrifuged, and frozen at –70 °C.

On the day of the experiment, appropriate amounts of membranes were thawed at room temperature, resuspended (0.5 mg of protein/mL) in 0.05 M of Tris/HCl buffer, at pH 7.4, containing 0.01% (v/v) Triton X-100, incubated at 37 °C for 60 min. The membranes were then washed with two additional resuspension and centrifugation cycles and finally resuspended in cold Tris-HCl buffer to yield 0.2–0.3 mg of protein/assay tube. [^{3}H]Flunitrazepam (83.4 Ci/mmol) binding assays were carried out in ice for 60 min at 1 nM of ligand concentration in a total of 0.5 mL vol. Bound radioactivity was separated by rapid filtration through Whatman GF/B filters using a Brandel cell harvester. Non-specific binding was determined in the presence of 10 mM of diazepam. The IC_{50} values were calculated from displacement curves based on four to six scalar concentrations of the test compound in triplicate, using the ALLFIT computer program [11], and converted to K_i values by application of the Cheng-Prusoff equation[12]. A stock of 1 mM of the test compound was prepared in ethanol. Subsequent dilutions were accomplished in buffer. Ethanol up to a final 1% concentration was seen to affect [^{3}H]flunitrazepam binding only negligibly.

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