Synthesis and Binding Activity of Some Pyrazolo[1,5-*c*]quinazolines as Tools To Verify an Optional Binding Site of a Benzodiazepine Receptor Ligand

Vittoria Colotta, Daniela Catarzi, Flavia Varano, Guido Filacchioni, and Lucia Cecchi*

Dipartimento di Scienze Farmaceutiche, Universita' di Firenze, Via Gino Capponi 9, 50121 Firenze, Italy

Alessandro Galli and Chiara Costagli

Dipartimento di Farmacologia Preclinica e Clinica, Universita' di Firenze, Viale G.B. Morgagni 65, 50134 Firenze, Italy

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The synthesis and binding activity at the benzodiazepine receptor of some 2-substituted pyrazolo[1,5-*c*]quinazolines are reported. The structure−activity relationships and *in vitro* efficacy of the title compounds, which are devoid of the proton acceptor atom at position 1, are similar to those of some previously reported tricyclic heteroaromatic compounds. This suggests that a proton acceptor at position 1 is an optional binding site of a benzodiazepine receptor ligand which only affects potency.

Introduction

Interaction of γ-aminobutyric acid (GABA) with its $GABA_A$ receptor is responsible for inhibitory neurotransmission in the vertebrates. The inhibitory effect of GABA can be modulated by the interaction of diverse chemical structures with allosteric sites. Allosteric $GABA_A$ receptor modulators, such as benzodiazepines which interact with the so-called benzodiazepine receptor (BZR), are widely used as therapeutic agents. The BZR ligands are functionally classified as agonists (positive modulators like the benzodiazepines in clinical use), inverse agonists (negative modulators), and neutral antagonists (binding but without functional consequences). Agonists, antagonists, and inverse agonists bind to the same domain of the receptor protein or at least with different states of the same domain.[1] It follows that all BZR ligands should have certain common characteristics that allow for recognition regardless of the type of efficacy.

An enormous amount of structure−activity relationship (SAR) data available for a large number of diverse structural classes of ligands has resulted in the formulation of several models of the pharamacophore for the BZR binding.[2] The common feature of these models is the attempt to explain ligand efficacy as a function of ligand−receptor interaction at the molecular level, on the basis of changes in the conformation of the receptor from its unoccupied resting state.[3−12]

Recently we proposed a schematic representation for the binding to the BZR of some 6,6,5-tricyclic heteroaromatic compounds in which some essential and optional pharmacophoric descriptors (see Figure 1) were identified.[13−16] The essential pharmacophoric descriptors were thought to be two lipophilic substituents called L_1 and L_2 and a proton acceptor atom designated a_2, while the optional sites, which were not necessary for receptor−ligand interaction but only affect the potency of a ligand, were a proton acceptor site called a_1 and a proton donor site called d. The optional hydrogen-bonding sites a_1 and d are identical with H_2 and A_2 sites, respectively, of Cook's model.[11,12] The hypothesis of the optionality of the proton donor site d is well supported by the synthesis and binding activity of compounds devoid of the proton donor d.[14,15,17−20] In agreement with Cook's report,[11,12] the interaction at point d appears to be important for potent inverse agonist activity but is not a requirement for high affinity *in vitro*. On the contrary, only a few examples[13,16] of 6,6,5-tricyclic compounds not bearing the proton acceptor atom a_1 are known. Thus to verify the hypothesis that the proton acceptor site a_1 is optional, a hypothesis which cannot be based on the binding activity of just a few compounds,[13,16] the synthesis and BZR binding activity of further 1-deaza 6,6,5-tricyclic derivatives, namely, pyrazolo[1,5-*c*]quinazolin-5-ones, are reported.

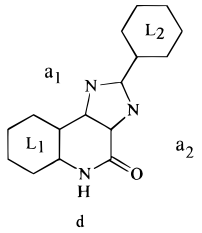

Figure 1. Schematic representation of the essential and optional pharmacophoric descriptors of a BZR ligand. Essential pharmacophoric descriptors: L_1 and L_2, lipophilic areas; a_2, proton acceptor site. Due to the lone pair orientation of the nitrogen and the carbonyl oxygen in the a_2 proton acceptor area, a favorable three-centered hydrogen bond with a proton donor of the receptor site could be engaged. Optional binding sites: d, proton donor area; a_1, proton acceptor region. The model is in agreement with that proposed by Cook[11,12] (d, a_1, and a_2 coincide with Cook's A_2, H_2, and H_1, respectively).

Chemistry

The synthesis of ethyl 5,6-dihydro-5-oxopyrazolo[1,5-*c*]quinazolin-2-carboxylate (**1**),[13] its 9-chloro analog **2**,[21] and 5,6-dihydro-2-phenylpyrazolo[1,5-*c*]quinazolin-5-one (**3**)[13] is reported elsewere.

The synthesis of 2-arylpyrazolo[1,5-*c*]quinazolin-5-ones **4**−**11** is shown in Schemes 1−4. In Scheme 1 the two methods to prepare the 1,3-diaryl-2-propenones **12**−**19**,[22,23] which with hydrazine hydrate yielded the 4,5-dihydro-3,5-diarylpyrazoles **20**−**27**, are illustrated. The choice of method depended on the availability of the starting materials.

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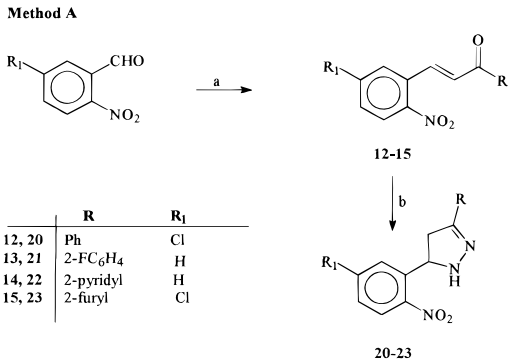
Scheme 1[a]

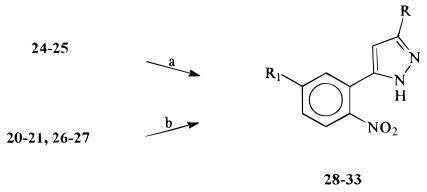


	R	R_1
12, 20	Ph	Cl
13, 21	2-FC_6H_4	H
14, 22	2-pyridyl	H
15, 23	2-furyl	Cl

	R
16, 24	4-MeC_6H_4
17, 25	4-ClC_6H_4
18, 26	2-furyl
19, 27	2-thienyl

[a] (a) $RCOMe/BF_3 \cdot 2MeCOOH$, MeCOOH; (b) $N_2H_4 \cdot H_2O$, EtOH; (c) RCHO/KOH, $MeOH/H_2O$.

Scheme 2[a]

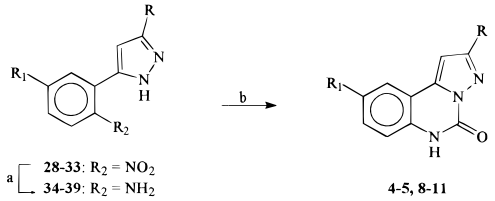


	R	R_1
28	Ph	Cl
29	2-FC_6H_4	H
30	4-MeC_6H_4	H
31	4-ClC_6H_4	H
32	2-furyl	H
33	2-thienyl	H

[a] (a) Method A: $Pb(AcO)_4$/HCl (concd), EtOH at 60 °C; (b) Method B: $Pb(AcO)_4/SiO_2$ at 10 °C.

The 4,5-dihydropyrazoles **20**, **21**, and **24**−**27** were dehydrogenated with lead tetraacetate (see Scheme 2). The crude materials were treated either with concentrated hydrochloric acid and ethanol at 60 °C (method A) or with silica gel at 10 °C (method B) to give the pyrazoles **28**−**33**. It has to be noted that when in 4,5-dihydropyrazoles **20**−**27** the substituent R is a heterocyclic moiety, the dehydrogenation to pyrazoles procedes with low yields (see in Table 3 compounds **32**−**33**). In the case of 4,5-dihydropyrazoles **22** and **23**, only traces of the corresponding (2-nitrophenyl)pyrazoles could be detected in the ^1H NMR spectra of the reaction mixture. Thus the final tricyclic derivatives **6** and **7** were obtained following a different procedure. Catalytic reduction (Pd/C) of the (2-nitrophenyl)pyrazoles **28**−**33** afforded the (2-aminophenyl)pyrazoles **34**−**39** which were cyclized with triphosgene to the tricyclic derivatives **4**, **5**, **8**−**11** (see Scheme 3).

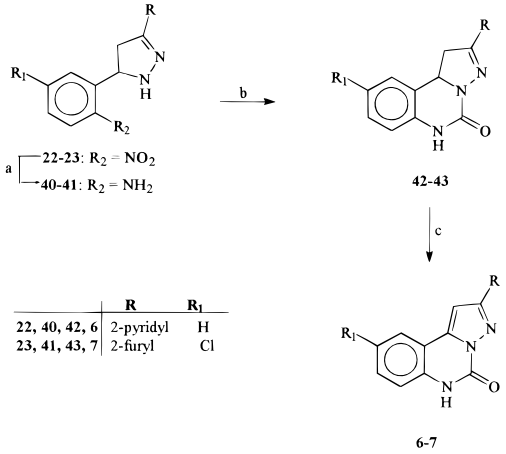
Scheme 3[a]



	R	R_1
28, 34, 4	Ph	Cl
29, 35, 5	2-FC_6H_4	H
30, 36, 8	4-MeC_6H_4	H
31, 37, 9	4-ClC_6H_4	H
32, 38, 10	2-furyl	H
33, 39, 11	2-thienyl	H

[a] (a) H_2/Pd/C, AcOEt; (b) $(CCl_3O)_2CO$, Et_3N, THF.

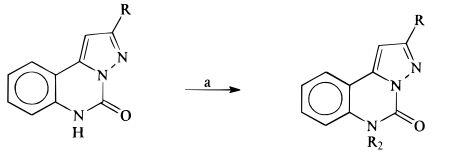
Scheme 4[a]



	R	R_1
22, 40, 42, 6	2-pyridyl	H
23, 41, 43, 7	2-furyl	Cl

[a] (a) H_2/PtO_2, AcOEt; (b) $(CCl_3O)_2CO$, Et_3N, THF; (c) $Pb(AcO)_4$, MeCOOH.

Scheme 5[a]



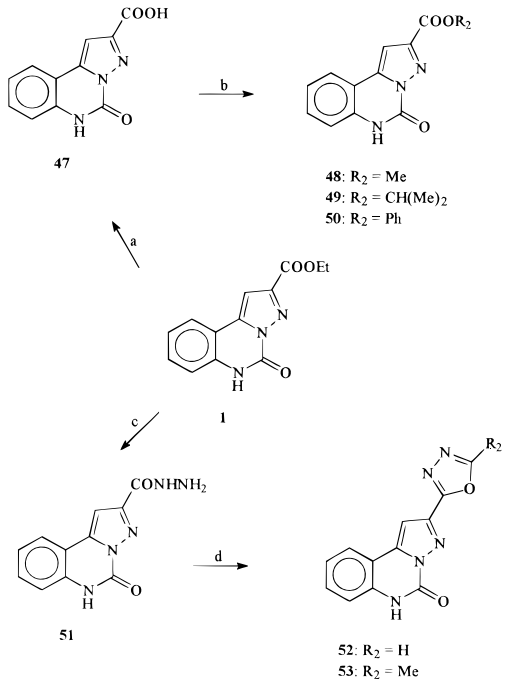
	R	R_2
44	COOEt	Me
45	COOEt	Et
46	Ph	Me

[a] (a) MeI or EtI, NaH, DMF.

The preparation of the 2-pyridyl- and 2-furylpyrazolo-[1,5-*c*]quinazolin-5-ones **6** and **7** was achieved as shown in Scheme 4. Briefly, the 4,5-dihydro-(2-nitrophenyl)-pyrazoles **22** and **23** were catalytically reduced (PtO_2) to the corresponding 4,5-dihydro-(2-aminophenyl)pyrazoles **40** and **41** which with triphosgene yielded the 1,5,6,10b-tetrahydroderivatives **42** and **43**. The latter were dehydrogenated with lead tetraacetate to the final compounds **6** and **7**. Alkylation of compounds **1** and **3** with alkyl iodides yielded the 6-*N*-alkyl derivatives **44**−**46**[21] (see Scheme 5).

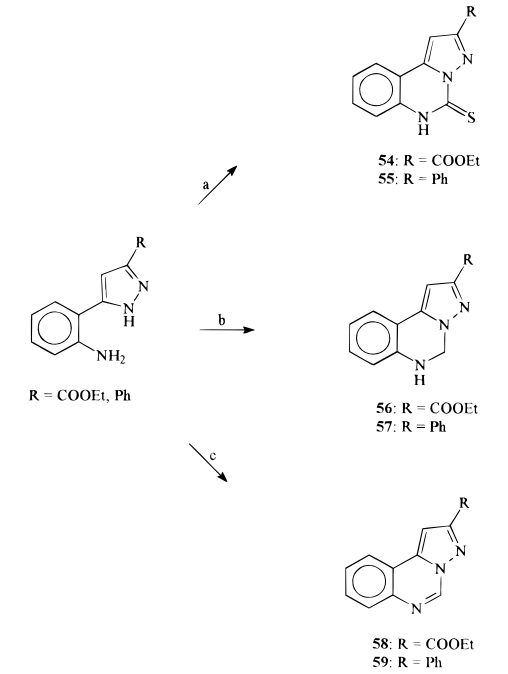
Ester **1** was hydrolyzed to the corresponding acid **47**.[21] The latter, upon treatment with thionyl chloride and alcohol or sodium phenoxide, afforded the esters **48**−**50**[21] (see Scheme 6). On the other hand, reaction of **1** with anhydrous hydrazine gave the hydrazide **51** which with imidates provided the oxadiazolyl derivatives **52**

Scheme 6[a]



[a] (a) HCl/AcOH; (b) $SOCl_2$, R_2OH or PhONa, benzene; (c) N_2H_4, EtOH; (d) $R_2C(NH)OEt \cdot HCl$, pyridine.

Scheme 7[a]

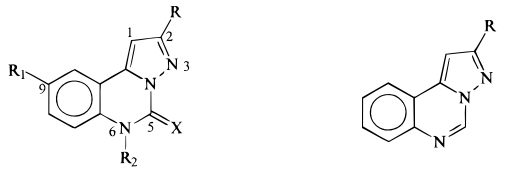


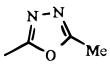
[a] (a) CS_2, pyridine/H_2O; (b) $(CH_2O)_n$, benzene; (c) HCOOH.

and **53**. Finally, treatment of the previously reported ethyl 5-(2-aminophenyl)pyrazole-3-carboxylate[13] or 2-phenyl-5-(2-aminophenyl)pyrazole[13] with either carbon disulfide, paraformaldehyde, or formic acid yielded compounds **54**−**59**, respectively (see Scheme 7).

The chemical structures of the reported compounds have been attributed on the basis of ^{1}H NMR and IR spectra. In particular the 5-thione structure of derivatives **54** and **55** is due to the presence in the IR spectra of the strong stretching bands at 1550 cm^{-1} (C═S) and 3200 cm^{-1} (NH). The presence of the NH is confirmed in the ^{1}H NMR by the singlet at about δ 13 which is present in all the cyclic derivatives bearing the NH group.

Table 1. Binding Activities and GABA Ratios[a]



compd	R	R_1	R_2	X	K_i (nM)[b]	GR
1[c]	COOEt	H	H	O	28 ± 4.9	1.2
2	COOEt	Cl	H	O	50 ± 3.0	1.16
3[c]	Ph	H	H	O	59 ± 3.5	0.99
4	Ph	Cl	H	O	105 ± 7.0	
5	2-FC_6H_4	H	H	O	67 ± 14	
6	2-pyridyl	H	H	O	53 ± 4.1	0.73
7	2-furyl	Cl	H	O	41 ± 2.1	0.94
8	4-MeC_6H_4	H	H	O	9500 ± 800	
9	4-ClC_6H_4	H	H	O	5300 ± 630	
10	2-furyl	H	H	O	16 ± 1.4	0.97
11	2-thienyl	H	H	O	7.4 ± 0.3	1.08
44	COOEt	H	Me	O	>10 000	
45	COOEt	H	Et	O	>10 000	
46	Ph	H	Me	O	>10 000	
47	COOH	H	H	O	>10 000	
48	COOMe	H	H	O	22 ± 1.1	1.07
49	$COOCH(Me)_2$	H	H	O	434 ± 36	
50	COOPh	H	H	O	3147 ± 419	
52		H	H	O	NT[d]	
53		H	H	O	4592 ± 815	
54	COOEt	H	H	S	3287 ± 419	
55	Ph	H	H	S	>10 000	
56	COOEt	H	H	H_2	70 ± 5.6	0.91
57	Ph	H	H	H_2	6573 ± 419	
58	COOEt	H			>10 000	
59	Ph	H			>10 000	
diazepam					10.5 ± 1.4	1.50
β-CCE					4.5 ± 0.7	0.67
Ro 15-1788					2.5 ± 0.3	0.96

[a] GABA ratio (GR): IC_{50}(compound)/IC_{50}(compound + 0.1 mM GABA). [b] K_i values are means ± SEM of three to five separate determinations. [c] Reference 13. [d] NT = not tested because of the insolubility of the compound.

Biochemistry

Compounds **2, 4**−**11**, **44**−**50**, and **52**−**59** were tested for their ability to displace [^{3}H]flunitrazepam (1 nM, K_D = 2.3 nM) from its specific binding sites in rat brain cortical membranes. The BZR affinities of the tested compounds, expressed as K_i, are listed in Table 1 together with the *in vitro* efficacy trends of the most active ones (K_i < 100 nM), expressed by the GABA ratio (GR). In Table 1 the K_i and GR of 2-carbethoxy **1** and 2-phenylpyrazoloquinazolin-5-one **3**[13] are also reported together with the K_i and GR of diazepam, ethyl β-carboline-3-carboxylate (β-CCE), and flumazenil (Ro 15-1788) as representative of an agonist, an inverse agonist, and an antagonist, respectively.

Results and Conclusions

The binding results on the fundamental pyrazolo[1,5-*c*]quinazolin-5-ones **1**−**11** show that these 1-deaza tricyclic derivatives display good BZR affinity with the exception of the 2-(*p*-substituted-phenyl) derivatives **8**

and **9**. Comparison of the BZR affinity of the lead compounds **1** and **3** with those of their 9-chloro analogs **2** and **4** reveals that the 9-chloro substituent halves the binding potency of these series of compounds to the BZR. This behavior is confirmed by the decreased binding activity of the 9-chloro derivative **7** with respect to that of its 9-unsubstituted analog **10**. A nonadditive 9-substituent effect has already been observed.[15,16]

Also the importance of the position of the substituent on the 2-phenyl ring is in agreement with previous findings.[15] In fact, the para-substitution drammatically affects the BZR affinity (see compouds **8** and **9**), while the presence of a fluorine atom in the ortho-position (**5**) or a 2-heteroaryl moiety (**6**, **7**, **10**, and **11**) resulted in an unchanged or increased binding activity. The favorable effect of the ortho-substituent and 2-heteroaryl moiety is also in accordance with previous data.[16] This effect may be explained by the existence of the hydrophilic "pocket 5" proposed by Crippen.[24] This hydrophilic pocket, according to Cook's most plausible alignment of his inclusive model,[11] would accommodate the *o*-fluoro or the heteroatom of the heterocyclic ring. No meta-substituent was introduced on the 2-phenyl moiety since previous data[16] indicate that the *m*-fluoro derivative was less potent than its *o*-fluoro isomer.

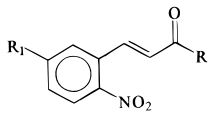
Comparison of the BZR affinities of 2-carboxymethyl **48**, 2-carboxyethyl **1**, 2-carboxyisopropyl **49**, and 2-carboxyphenyl **50** indicates that the smaller the ester group the better the binding activity. The BZR affinity of the bulky bioisoster of the carboxylic ester, i.e., 5-methyl-1,3,4-oxadiazol-2-yl derivative **53,** supports this suggestion. The inactivity of the 6-N-alkylated derivatives **44**−**46** and that of the 2-carboxylic acid **47** are also in accordance with previous data.[16,25]

The chemical modifications performed in the lead compounds **1** and **3** at the level of the carbonyl oxygen at position 5, i.e., compounds **54**, **55**, **57**, and **59**, show the paramount importance of the three-centered hydrogen-bonding formation between the nitrogen at position 3 and the carbonyl oxygen at position 5 with the proton donor of the receptor site. The discrepancy of the BZR affinity of compound **56** devoid of the carbonyl oxygen and with a BZR affinity only 2-fold lower than that of the parent compound **3** seems to be the only exception to this rule.

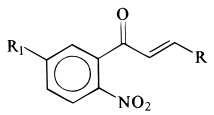
In Table 1 the GRs of the most active compounds are also shown. This *in vitro* classification method is useful for roughly estimating the trend of efficacy of test compounds.[26−28] The GR values (close to or below 1.0) show that the efficacy trend of the tested pyrazoloquinazolines is that of antagonists with only one potential partial inverse agonist (**6**).

In conclusion, the SAR and the GRs on pyrazoloquinazolines **1**−**11**, **44**−**50**, and **52**−**59** are very similar to those of the previously reported 1,2,4-triazolo-quinoxalines[16] and 1,2,4-triazolobenzoxazines.[15] It follows that compounds **1**−**11**, **44**−**50**, and **52**−**59** bind to the BZR in a similar way and that the pharmacophoric descriptors shown in Figure 1 should be the same. Since the compounds reported in this paper are devoid of the proton acceptor a_1, our hypotheses are confirmed as follows: (i) that the proton acceptor a_1 is not essential for the anchoring of inverse agonists/antagonists to the BZR and (ii) that the proton acceptor a_1 is an optional binding site which only affects potency. These conclusions confirm that in the BZR recognition site there is the optional H_2 hydrogen-bonding site proposed by Cook.[11] In fact, Cook's H_2 acceptor site corresponds to our a_1, and likewise it is found to be unnecessary for inverse agonist/antagonist activity.[11]

Table 2. Physical Properties of 1,3-Diarylpropenones



12-15



16-19

compd	R	R_1	mp (°C)	solv[a]	yield (%)
12	Ph	Cl	115−120	A	54
13	2-FC_6H_4	H	125−130	B	82
14[b]	2-pyridyl	H	142−143	A	68
15	2-furyl	Cl	155−158	C	30
16[c]	4-MeC_6H_4	H	128−130	A	93
17[d]	4-ClC_6H_4	H	125−127	A	91
18	2-furyl	H	99−100	A	70
19	2-thienyl	H	94−95	D	78

[a] Recrystallization solvents: A = ethanol, B = ethyl acetate, C = glacial acetic acid, D = methanol. [b] Reference 22: mp 141 °C. [c] Reference 23: mp 134−135 °C. [d] Reference 23: mp 123−124 °C.

Experimental Section

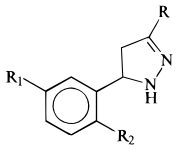
Chemistry. Silica gel plates (Merck F_{254}) and silica gel 60 (Merck; 70−230 mesh) were used for analytical and column chromatography, respectively. All melting points were determined on a Gallenkamp melting point apparatus. Microanalyses were performed with a Perkin-Elmer 260 elemental analyzer for C, H, N, and the results are within ±0.4% of the theoretical values. The IR spectra were recorded with a Perkin-Elmer 1420 spectrometer in Nujol mull 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, dd = double doublet, t = triplet, td = triple doublet, q = quartet, m = multiplet, br = broad, and ar = aromatic protons. The physical data of the newly synthesized compounds are shown in Tables 2−4.

1,3-Diaryl-2-propenones 12−19. Method A. A solution of equimolar amounts (16 mmol) of the suitable 2-nitroaryl aldehyde and aryl methyl ketone in glacial acetic acid (8 mL) was treated with the acetic acid complex of boron trifluoride (48 mmol). The mixture was stirred at room temperature for 5 days. The resulting solid was collected, washed with water, and recrystallized. Following this method, compounds **12**−**15**[22] were prepared. Compound **12** displayed the following: ^1H NMR ($CDCl_3$) 7.34 (d, 1H, H-2, J = 15.7 Hz), 7.50−7.70 (m, 5H, ar), 8.01−8.15 (m, 4H, 3H ar + H-3).

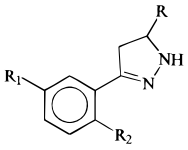
Method B. Compounds **16**−**19**[23] were obtained from equimolar amounts (15 mmol) of 2-nitroacetophenone and the suitable aryl aldehyde following the procedure described[23] to prepare **16** and **17**. Compound **19** displayed the following: ^1H NMR ($CDCl_3$) 6.79 (d, 1H H-2, J = 15.8 Hz), 7.03−7.08 (m, 1H, thienyl proton), 7.24−7.51 (m, 4H, 3H ar + H-3), 7.60−7.79 (m, 2H, ar), 8.17 (dd, 1H, ar, J = 8.1, 1.1 Hz).

4,5-Dihydro-3-aryl-5-(2-nitroaryl)pyrazoles 20−23 and 4,5-Dihydro-3-(2-nitrophenyl)-5-arylpyrazoles 24−27. Hydrazine hydrate (55%, 9.6 mmol) was added to a suspension of **12**−**19** (8 mmol) in ethanol (60 mL). The mixture was refluxed for 1−2 h. The reaction was monitored by TLC. Compounds **20**−**22**, which became solid upon cooling, were collected and recrystallized. Compound **23** was obtained by evaporation at reduced pressure of the solvent, and the resulting oily residue became solid upon treatment with diethyl ether. In the case of **24**−**27**, evaporation of the solvent at reduced pressure yielded an oil which was purified by

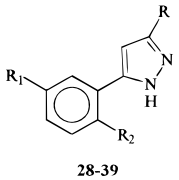
Table 3. Physical Properties of 3,5-Diarylpyrazoles



20-23, 40-41



24-27



28-39

compd	R	R_1	R_2	mp (°C)	solv[a]	yield (%)
20	Ph	Cl	NO_2	142−145	A	90
21	2-FC_6H_4	H	NO_2	86−87	A	87
22	2-pyridyl	H	NO_2	152−154	A	87
23	2-furyl	Cl	NO_2	103−105	B	73
24	4-MeC_6H_4	H	NO_2	139 dec[b]	C	47
25	4-ClC_6H_4	H	NO_2	140−142[b]	C	37
26	2-furyl	H	NO_2	142−144[b]	D	47
27	2-thienyl	H	NO_2	138 dec[b]	E	55
28	Ph	Cl	NO_2	158−160	F	47
29	2-FC_6H_4	H	NO_2	161−163	G	27
30	4-MeC_6H_4	H	NO_2	153−155	H	64
31	4-ClC_6H_4	H	NO_2	161−163	I	35
32	2-furyl	H	NO_2	121 dec	C	25
33	2-thienyl	H	NO_2	105−106	E	19
34	Ph	Cl	NH_2	172−174	J	94
35	2-FC_6H_4	H	NH_2	142−144	J	97
36	4-MeC_6H_4	H	NH_2	179−180	K	98
37	4-ClC_6H_4	H	NH_2	224−225	A	96
38	2-furyl	H	NH_2	127−128	J	80
39	2-thienyl	H	NH_2	176−178	J	87
40	2-pyridyl	H	NH_2	140−141	A	62
41	2-furyl	Cl	NH_2	125−128	L	40

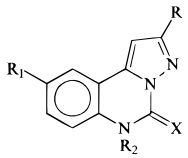
[a] Recrystallization solvents: A = ethanol, B = diethyl ether, C = column chromatography, eluting system cyclohexane/ethyl acetate (7:3), D = column chromatography, eluting system chloroform/ethyl acetate (8:2), E = column chromatography, eluting system chloroform/tetrahydrofuran/cyclohexane (8:1:1), F = column chromatography, eluting system cyclohexane/ethyl acetate (8:2), G = column chromatography, eluting system chloroform/ethyl acetate (9:1), H = column chromatography, eluting system chloroform/ethyl acetate (4:6), I = column chromatography, eluting system cyclohexane/ethyl acetate (1:1), J = benzene, K = cyclohexane/ethyl acetate, L = diethyl ether/ethanol. [b] Picrate melting point.

column chromatography. By treating **24**−**27** with picric acid, the solid picrates were obtained. Compounds **20** and **27** displayed the following ^{1}H NMR spectra: **20** ($CDCl_3$) 2.97 (dd, 1H, H-4, J = 16.8, 10.5 Hz), 3.86 (dd, 1H, H-4, J = 16.8, 10.9 Hz), 5.46 (td, 1H, H-5, J = 10.7, 4.3 Hz), 6.01 (d, 1H, NH, J = 4.3 Hz), 7.36−7.44 (m, 4H, ar), 7.65−7.70 (m, 2H, ar), 7.96−8.05 (m, 2H, ar); **27** ($CDCl_3$) 3.01 (dd, 1H, H-4, J = 16.2, 9.1 Hz), 3.38 (dd, 1H, H-4, J = 16.2, 10.1 Hz), 5.27 (td, 1H, H-5, J = 9.6, 1.8 Hz), 6.27 (d, 1H, NH, J = 1.8 Hz), 6.95−7.05 (m, 2H, ar), 7.22.7.27 (m, 1H, ar), 7.48−7.68 (m, 3H, ar), 7.81 (d, 1H, ar, J = 7.4 Hz).

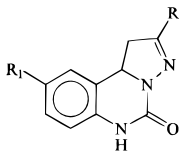
3-Aryl-5-(2-nitroaryl)pyrazoles 28−33. To a solution of **20**, **21**, and **24**−**27** (4.5 mmol) in anhydrous dichloromethane (25 mL) was added dropwise a solution of equimolar amount of lead tetraacetate in anhydrous dichloromethane (25 mL). The mixture was stirred at room temperature for 15 min. Addition of water (70 mL) and elimination of the dark solid gave two layers. The organic layer was washed four times with water (30 mL each time) and dried (sodium sulfate).

Method A. Evaporation at reduced pressure of the solvent yielded an oily residue which was treated with concentrated hydrochloric acid (0.11 mL) and ethanol (20 mL). The mixture became a solution by heating it at 60 °C for 10 min. Chloroform (80 mL) was added to the cooled solution. The solution was washed once with a 2.5% solution of sodium hydrogen carbonate (50 mL) and three times with water (30 mL each time), dried (sodium sulfate), and evaporated at reduced pressure. The resulting oily residue became solid upon standing. Following this method, compounds **30** and **31** were

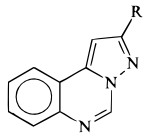
Table 4. Physical Properties of Pyrazolo[1,5-*c*]quinazolines



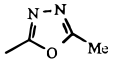
2, 4-11, 44-57



42-43



58-59

compd	R	R_1	R_2	X	mp (°C)	solv[a]	yield (%)
2[b]	COOEt	Cl	H	O	298−300	A	40
4	Ph	Cl	H	O	>310	B	42
5	2-FC_6H_4	H	H	O	290−292	B	74
6	2-pyridyl	H	H	O	301−304	A	48
7	2-furyl	Cl	H	O	300−302	C	55
8	4-MeC_6H_4	H	H	O	295−297	B	98
9	4-ClC_6H_4	H	H	O	>310	B	77
10	2-furyl	H	H	O	304−305	B	61
11	2-thienyl	H	H	O	299−300	B	76
42	2-pyridyl	H	H	O	303−305	D	91
43	2-furyl	Cl	H	O	297 dec	E	79
44[b]	COOEt	H	Me	O	184−185	F	90
45	COOEt	H	Et	O	163−165	A	45
46	Ph	H	Me	O	244−245	D	40
47[b]	COOH	H	H	O	282 dec	G	93
48[c]	COOMe	H	H	O	295−297	H	68
49	$COOCH(Me)_2$	H	H	O	258−259	B	49
50	COOPh	H	H	O	298−300	B	50
51	$CONHNH_2$	H	H	O	>310	D	67
52[d]		H	H	O	>310		71
53		H	H	O	>310	D	36
54	COOEt	H	H	S	258 dec	B	85
55	Ph	H	H	S	309−311	B	72
56	COOEt	H	H	H_2	116−117	I	55
57	Ph	H	H	H_2	120−212	I	20
58	COOEt				155−156	A	80
59	Ph				131−132	H	53

[a] Recrystallization solvents: A = ethanol, B = glacial acetic acid, C = tetrahydrofuran, D = dimethylformamide, E = diethyl ether/ethanol, F = ethyl acetate, G = glacial acetic acid/water, H = methanol, I = cyclohexane. [b] Reference 21: mp not reported. [c] Reference 21: mp 295−297 °C with softening at 290 °C. [d] Due to its insolubility, the compound was not recrystallized. The mp refers to the crude product.

prepared. Compound **30** displayed the following: ^{1}H NMR ($CDCl_3$) 2.38 (s, 3H, CH_3), 6.66 (s, 1H, pyrazole proton), 7.19−7.26 (m, 2H, ar), 7.47−7.65 (m, 4H, ar), 7.68−7.77 (m, 2H, ar).

Method B. Silica gel (30 g) was added to the organic layer. The mixture was allowed to stand in a refrigerating apparatus (10 °C) for 15−60 h. The reaction was monitored by ^{1}H NMR. The silica gel was filtered off and washed several times with ethyl acetate. Evaporation of the solvent at reduced pressure and at a temperature not higher than 30−40 °C yielded an oily residue which was purified by column chromatography. Following this method, compounds **28**, and **29**, **32**, and **33** were prepared. Compound **28** displayed the following: ^{1}H NMR ($CDCl_3$) 6.70 (s, 1H, pyrazole proton), 7.43−7.48 (m, 4H, ar), 7.55−7.62 (m, 2H, ar), 7.72−7.78 (m, 2H, ar).

3-Aryl-5-(2-aminoaryl)pyrazoles 34−39. A mixture of **28**−**33** (1.5 mmol) and 30% (w/w) Pd/C (10%) in ethyl acetate (100 mL) was hydrogenated in a Parr apparatus at 30 psi for 6 h. Elimination of the catalyst and evaporation at reduced pressure of the solvent yielded a residue which was recrystallized. Compound **34** displayed the following: ^{1}H NMR (DMSO-d_6) 6.5 (br s, 2H, NH_2), 6.77 (d, 1H, ar), 7.04 (dd, 1H, ar, J = 8.5, 2.2 Hz), 7.31−7.54 (m, 4H, ar), 7.62 (d, 1H, ar, J = 2.2 Hz), 7.85 (d, 1H, ar, J = 7.0 Hz), 13.4 (br s, 1H, NH).

5,6-Dihydro-2-arylpyrazolo[1,5-*c*]quinazolin-5-ones 4, 5, 8, and 11. Triphosgene (0.56 mmol) and triethylamine (3.4 mmol) were successively added to a solution of **34**−**39** (1.40

mmol) in anhydrous tetrahydrofuran (20 mL). The mixture was stirred at room temperature for 30 min and then diluted with water (50 mL). The resulting solid was collected and recrystallized. Compound **4** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 7.33−7.60 (m, 5H, ar), 7.86 (s, 1H, H-1), 7.98−8.02 (m, 2H, ar), 8.18−8.21 (m, 1H, ar), 12.0 (br s, 1H, NH); IR 3200, 1740.

4,5-Dihydro-3-aryl-5-(2-aminoaryl)pyrazoles 40 and 41. A mixture of **22** and **23** (1.4 mmol) and 10% (w/w) platinum dioxide monohydrate in ethyl acetate (60 mL) was hydrogenated in a Parr apparatus at 25 psi for 18 h. Elimination of the catalyst and evaporation at reduced pressure of the solvent yielded a residue which was recrystallized. Compound **40** displayed the following: ^{1}H NMR ($CDCl_3$) 3.48 (d, 2H, H-4, J = 11.9 Hz), 4.3 (br s, 2H, NH_2), 4.97 (td, 1H, H-5, J = 11.9, 3.7 Hz), 6.08 (d, 1H, NH, J = 3.7 Hz), 6.68−6.76 (m, 2H, ar), 7.09−7.26 (m, 3H, ar), 7.69 (td, 1H, ar, J = 7.9, 1.7 Hz), 7.92 (d, 1H, ar, J = 8.0 Hz), 8.59 (d, 1H, ar, J = 4.8 Hz).

1,5,6,10b-Tetrahydro-2-arylpyrazolo[1,5-*c*]quinazolin-5-ones 42 and 43. The title compounds were obtained from **40** and **41** (1.40 mmol) and triphosgene (0.56 mmol) following the procedure described above to prepare **4**, **5**, **8**, and **11**. Compound **42** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 3.54 (dd, 1H, H-1, J = 17.1, 14.3 Hz), 3.99 (dd, 1H, H-1, J = 17.1, 10.6 Hz), 5.29 (dd, 1H, H-10b, J = 14.3, 10.6 Hz), 6.95−7.07 (m, 2H, ar), 7.23−7.32 (m, 2H, ar), 7.45−7.51 (m, 1H, ar), 7.85−8.07 (m, 2H, ar), 8.68 (d, 1H, ar, J = 4.8 Hz), 9.7 (br s, 1H, NH); IR 3240, 3160, 3100, 1700.

4,5-Dihydro-2-arylpyrazolo[1,5-*c*]quinazolin-5-ones 6 and 7. Lead tetraacetate (1.38 mmol) was added to a warm (60 °C) solution of **42** and **43** (0.69 mmol) in glacial acetic acid (10 mL). The mixture was heated at 60 °C for 30 min in the case of **42** and for 3 h in the case of **43**. The solid obtained upon cooling was collected. A second crop of the title compounds was obtained as follows: The mother solution was diluted with water (15 mL) and extracted with ethyl acetate (20 mL). The organic layer was washed with a 0.1 M solution of hydrochloric acid (20 mL) and water until neutralization, dried (sodium sulfate), and evaporated at reduced pressure. The oily residue became solid upon treatment with diethyl ether. Compound **6** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 7.30−7.39 (m, 2H, ar), 7.45−7.60 (m, 2H, ar), 7.82 (s, 1H, H-1), 7.99 (td, 1H, ar, J = 7.9, 1.7 Hz), 8.21 (d, 2H, ar, J = 7.9 Hz), 8.72 (d, 1H, ar, J = 4.8 Hz), 11.95 (s, 1H, NH); IR 3240, 3180, 3060, 1740.

5,6-Dihydro-6-*N*-alkylpyrazolo[1,5-*c*]quinazolin-5-ones 44−46.[21] Alkyl iodide (1.97 mmol) and sodium hydride (80%, 2.32 mmol) were added to a solution of **1** and **3** (1.16 mmol) in anhydrous dimethylformamide (5 mL). The mixture was stirred at room temperature for 1−2 h. The reaction was monitored by TLC. Ice and water (20 mL) were added, and the mixture was extracted three times with chloroform (30 mL each time). The organic layer was washed three times with water (30 mL each time), dried (sodium sulfate), and evaporated at reduced pressure to afford a residue which was recrystallized. Compound **46** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 3.73 (s, 3H, CH_3), 7.43−7.66 (m, 6H, ar), 7.77 (s, 1H, H-1), 8.04 (d, 2H, ar, J = 7.8 Hz), 8.15 (d, 1H, ar, J = 7.6 Hz); IR 3140, 1700.

5,6-Dihydro-5-oxopyrazolo[1,5-*c*]quinazoline-2-carboxylic Acid 47.[21] The title compound was obained by hydrolysis of **1** (2.56 mmol) as described.[21] Compound **47** displayed the following: ^{1}H NMR (DMSO-d_6) 7.33−7.40 (m, 2H, ar), 7.35−7.57 (m, 1H, ar), 7.65 (s, 1H, H-1), 8.16 (d, 1H, ar, J = 7.5 Hz), 12.1 (br s, 1H, NH), 13.4 (br s, 1H, OH).

5,6-Dihydro-5-oxopyrazolo[1,5-*c*]quinazoline-2-carboxylates 48 and 50.[21] A mixture of **47** (1.74 mmol) in thionyl chloride (10 mL) was refluxed for 5 h. The excess of thionyl chloride was distilled off. The residue acyl chloride was washed with cyclohexane (1 mL). The suitable alcohol (10 mL) or a suspension of sodium phenoxide (1.74 mmol) in anhydrous benzene (20 mL) was then added to the intermediate acyl chloride. The mixture was refluxed. The esterification reaction was monitored by TLC, and heating was continued until the starting acid had disappeared. The cooled mixture afforded a solid which was recrystallized. Compound **49** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.37 (d, 6H, $2CH_3$, J = 6.2 Hz), 5.23 (q, 1H, CH, J = 6.2 Hz), 7.28−7.39 (m, 2H, ar), 7.52−7.60 (m, 1H, ar), 7.68 (s, 1H, H-1), 8.17 (d, 1H, ar, J = 7.8 Hz); IR 3140, 1760, 1730.

5,6-Dihydro-5-oxopyrazolo[1,5-*c*]quinazoline-2-hydrazide (51). Anhydrous hydrazine (5.8 mmol) was added to a boiling suspension of **1** (1.9 mmol) in absolute ethanol (15 mL). The mixture was refluxed for 1 h. The resulting solid was collected and recrystallized. Compound **51** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 4.57 (br s, 2H, NH_2), 7.27−7.38 (m, 2H, ar), 7.51−7.58 (m, 2H, 1H ar + H-1), 8.11 (d, 1H, ar, J = 7.41 Hz), 8.5 (br s, 1H, NH), 12.0 (br s, 1H, NH); IR 3380, 3320, 3100, 1750, 1720.

5,6-Dihydro-2-(1,3,4-oxadiazolyl)pyrazolo[1,5-*c*]quinazolin-5-ones 52 and 53. The suitable ethyl imidate hydrochloride (2.88 mmol) was added to a mixture of **51** (1.44 mmol) in pyridine (5 mL). The mixture was refluxed for 2−3 h. The solid was collected and, in the case of **53**, recrystallized. Because of its insolubility, compound **52** could not be recrystallized; however, it was pure enough to be characterized. Compound **53** displayed the following: ^{1}H NMR (DMSO-d_6) 2.65 (s, 3H, CH_3), 7.31−7.41 (m, 2H, ar), 7.55−7.62 (m, 1H, ar), 7.68 (s, 1H, H-1), 8.22 (d, 1H, ar, J = 7.8 Hz), 12.0 (br s, 1H, NH).

Ethyl 5,6-Dihydro-5-thioxopyrazolo[1,5-*c*]quinazoline-2-carboxylate (54) and 5,6-Dihydro-2-phenylpyrazolo-[1,5-*c*]quinazoline-5-thione (55). Carbon disulfide (4 mL) was added to a solution of ethyl 5-(2-aminophenyl)pyrazole-2-carboxylate[13] or 3-phenyl-5-(2-aminophenyl)pyrazole[13] (0.92 mmol) in water (1 mL) and pyridine (5 mL). The mixture was refluxed for 3−5 h. The reaction was monitored by TLC, and the heating was continued until the starting material had disappeared. Evaporation at reduced pressure of the solvent yielded a residue which was collected, washed with diethyl ether (15 mL), and recrystallized. Compound **54** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.39 (t, 3H, CH_3, J = 7.1 Hz), 4.43 (q, 2H, CH_2, J = 7.1 Hz), 7.41−7.50 (m, 1H, ar), 7.63−7.69 (m, 2H, ar), 7.86 (s, 1H, H-1), 8.26 (d, 1H, ar, J = 8.5 Hz), 13.53 (s, 1H, NH); IR 3200, 3140, 1720, 1550.

Ethyl 5,6-Dihydropyrazolo[1,5-*c*]quinazoline-2-carboxylate (56) and 5,6-Dihydro-2-phenylpyrazolo[1,5-*c*]-quinazoline (57). A mixture of the suitable (2-aminophenyl)-pyrazole[13] (1.3 mmol) and paraformaldehyde (1.45 mmol) in benzene (5 mL) was refluxed for 4−5 h. Evaporation of the solvent at reduced pressure yielded a residue which was purified by column chromatography, eluting system chloroform/acetonitrile, 8:2. Evaporation of the second eluates at reduced pressure afforded a residue which was recrystallized. Compound **56** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.32 (t, 3H, CH_3, J = 7.1 Hz), 4.30 (q, 2H, CH_2, J = 7.1 Hz), 5.42 (s, 2H, CH_2), 6.75−6.90 (m, 3H, 2H ar + NH), 7.14−7.21 (m, 2H, 1H ar + H-1), 7.61 (d, 1H, ar, J = 7.6 Hz); IR 3380, 1740.

Ethyl Pyrazolo[1,5-*c*]quinazoline-2-carboxylate (58) and 2-Phenylpyrazolo[1,5-*c*]quinazoline (59). A solution of the suitable (2-aminophenyl)pyrazole[13] (1.3 mmol) in formic acid (2 mL) was refluxed for 30 min. Addition of water (30 mL) to the cooled solution yielded a solid which was collected and recrystallized. Compound **58** displayed the following spectral data: ^{1}H NMR (DMSO-d_6) 1.38 (t, 3H, CH_3, J = 7.1 Hz), 4.41 (q, 2H, CH_2, J = 7.1 Hz), 7.70−7.85 (m, 3H, 2H ar + H-1), 7.92−7.99 (m, 1H, ar), 8.43 (d, 1H, ar, J = 8.0 Hz), 9.49 (s, 1H, H-5); IR 3140, 3060, 1740.

Biochemistry. Crude synaptic membranes were prepared from cerebral cortices of male Sprague−Dawley rats (170−250 g) according to Zukin et al.[29] Tissue was homogenized in 15 vol of ice-cold 0.32 M sucrose, containing 20 μg/mL 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 supernatant and the soft upper layer of the pellet were collected together and centrifuged at 48000*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 Tris-HCl buffer, at pH 7.4, containing 0.01% (v/v) Triton X-100, incubated at 37 °C for 60 min, and centrifuged at 48000*g* for 20 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 ligand concentration in a total 0.5 mL volume. Bound radioactivity was separated by rapid filtration through Whatman GF/B filters using a Brandel cell harvester. Nonspecific binding was determined in the presence of 10 μM diazepam. The IC_{50} values were calculated from displacement curves based on four to six scalar concentrations of the test compounds in triplicate, using the ALLFIT computer program,[30] and converted to K_i values by application of the Cheng−Prusoff equation.[31] The GABA ratios[26] of the compounds with the lowest K_i values were calculated by measuring, in the same experiment, the IC_{50} value of each compound in the absence and presence of 0.1 mM GABA. A stock 1 mM solution of the test compounds was prepared in 50% ethanol. Subsequent dilutions were accomplished in buffer. Ethanol up to a final 5% concentration was seen to affect [^{3}H]-flunitrazepam binding negligibly (≤3%).

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