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Synthesis, benzodiazepine receptor affinity and in vivo testing of 3-aryl-4,7-dihydro-6-($N^{1'}$-alkylpyrazol-3'- or 5'-yl)pyrazolo[1,5-*a*]pyrimidin-7-ones

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Summary — The synthesis of a series of 3-aryl-4,7-dihydro-6-($N^{1'}$-alkylpyrazol-3'- or 5'-yl)pyrazolo[1,5-*a*]pyrimidin-7-ones and their in vitro biological evaluation as ligands for benzodiazepine receptor (BzR) are described. The in vitro activities, as determined by an analysis of GABA (γ-aminobutyric acid) shift ratios, and binding affinities of these compounds to BzR are compared in terms of the electronic, lipophilic and steric effect changes of their substituents either at the 3-position or at the $N^{1'}$ of 6-(pyrazol-3'(5')-yl) moiety. The most interesting compounds were tested in vivo.

benzodiazepine receptor binding studies / in vitro agonism–antagonism profiles / in vivo anticonvulsant evaluation

Introduction

Since their discovery as high-affinity ligands for Benzodiazepine receptor (BzR), the interest in CGS series, β-carbolines, imidazopyridines, pyrazoloquinolines, imidazoquinoxalines and ethazolate [1–5] has continued to grow mainly because they exhibit pharmacological selectivity, lacking some of the effects found in classical Bz ligands [6–8].

In fact most of the drugs in clinical use, such as Diazepam, are full agonist-type ligands and exhibit anticonvulsant sedative and muscle relaxant effects together with anxiolytic properties [9–12]. In contrast, partial agonists are considered to be likely to afford anxiolytic activity without the undesired side effects [11, 13–14]. In addition, a need is felt for inverse agonists/antagonists which enhance neuronal firing in the CNS without proconvulsant/convulsant activity (treatment of barbiturate alcohol-induced CNS depression, treatment of hepatic encephalopathy, therapeutic support for cognition enhancement).

Our program has been designed to identify novel compounds with potential anxyolitic activity, having a pyrazolo[1,5-*a*]pyrimidine nucleus that has excited interest in the field of non-benzodiazepine anticonvulsant agents [15–16], and thus our synthetic work has dealt with pyrazolo[1,5-*a*]pyrimidines bearing a pyrazole ring at the 6-position [17]. In our previous paper we identified the essential structural features for BzR recognition and in this new structural class of high-affinity ligands for Bz binding site the 3-phenyl-4,7-dihydro-6-(1'*H*-pyrazol-3'-yl)pyrazolo[1,5-*a*]pyrimidine-7-one **10a** (table I) was chosen as lead molecule. A subsequent structural optimization involving the introduction of various substituents at various positions on the phenyl ring or its replacement with α- and β-thiophene was performed **10b–10g** (table I).

The substituents at the 3-position were chosen on the basis of their relative lipophilicity and the obtained new ligands display a continuum of activity ranging from an agonist to an antagonist through an inverse agonist profile. Small modifications in the structures of these molecules can cause a shift from one pharmacological class to another, so it is reasonable to assume that all three classes of BzR ligands (agonist, antagonist, inverse agonist) bind the same location in the receptor complex, according to the recent hypothesis of an inclusive pharmacophoric model [18, 19]. In this model the agonist requirements

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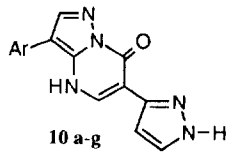
Table I. In vitro data of 3-aryl-4,7-dihydro-6-(*1'H*-pyrazol-3'-yl)pyrazolo[1,5-*a*]pyrimidin-7-ones [17].



10 a-g

Compound	*Ar*	*Inhibition (%)*[a]	*K_i (nM)*[b]	*GABA ratio*[c]
10a	C_6H_5	94.0 ± 10	326.3 ± 37	2.20
10b	2'-$OCH_3C_6H_4$	96.0 ± 8.8	77.7 ± 2.2	0.80
10c	3'-$OCH_3C_6H_4$	97.2 ± 8.0	91.5 ± 2.7	3.07
10d	2'-ClC_6H_4	93.3 ± 7.4	178.5 ± 3.6	1.20
10e	3'-ClC_6H_4	90.0 ± 9.2	95.4 ± 2.4	1.70
10f	2'-Thienyl	94.3 ± 6.2	533 ± 17	1.14
10g	3'-Thienyl	100 ± 8.5	70.7 ± 3.7	0.97

[a]Percent of inhibition of specific [^{3}H]flunitrazepam binding at 10 μM concentration are means ± SEM of five determinations (the tests were carried out using DMSO as solvent). [b]K_i values are means ± SEM of five determinations. [c]GABA ratio = IC_{50} compound/IC_{50} compound + 10 μM GABA performed in five independent experiments.

consist of two hydrogen bond donor sites (H_1, H_2), two lipophilic zones (L_1, L_2) and an additional lipophilic pocket L_3, whose full occupation resulted in a full agonist spectrum of activity. Three areas of negative steric interaction (S_1, S_2, S_3) between the ligand and receptor binding protein have also been defined. The structural requirements for the inverse agonist site are instead identified in a narrow lipophilic pocket, a hydrogen bond donor site (H_1) and a hydrogen bond acceptor site (A_2).

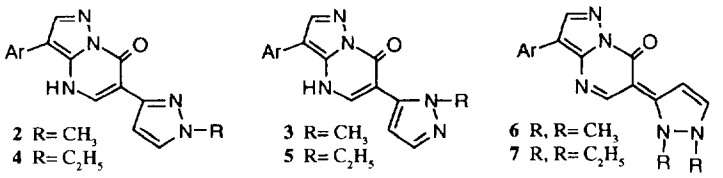
The interaction between ligands and receptor binding sites produces a different functional state of this complex, corresponding to different conformations of the protein complex. The differences in efficacy of the agonists and inverse agonists may be in relationship with the total or partial interaction at the different BzR GABA-A isoforms: that produces a full agonist and partial agonist or inverse partial agonist respectively [20].

On the other hand, the different intrinsic efficacies related to the different levels of receptor activation may be the consequence of receptor density (spare receptor capacity) or efficiency of signal transduction or the result of the combinations of both of these effects [13, 20, 21].

Because of the marked differences of the biological profiles of the 3-aryl-4,7-dihydro-6-(1'*H*-pyrazol-3'-yl)pyrazolo[1,5-*a*]pyrimidin-7-ones **10a–g** depending on the substitution at the 3-position, it seemed useful to discuss the role of this substitution with concomitant modifications at the 6-position. We thought it interesting to first study the role of the N–H group belonging to the pyrazole moiety on affinity and efficacy.

We report herein the synthesis of the 3-aryl-4,7-dihydro-6-(*N*1'-alkylpyrazol-3'- or 5'-yl)pyrazolo[1,5-*a*]-pyrimidin-7-ones whose parent compounds 3-aryl-4,7-dihydro-6-(1'*H*-pyrazol-3'-yl)pyrazolo[1,5-*a*]-pyrimidin-7-ones **10a–g** showed affinity values in a range from 70 up to 500 nM (table II). The affinity (as measured by the concentration of the compounds able to displace a radioligand) and efficacy (as measured by the GABA ratio) are also reported. Compounds showing the highest affinity, as well as the most interesting behavioral properties (from inverse agonism to full agonism) which can be foreseen by GABA ratio values (tables I and II), were tested in vivo in mice for their ability to prevent the seizures induced by pentylenetetrazole. The results of these studies are shown in tables III and IV together with data coming from classical Bz anticonvulsants and anxiolytics like Diazepam and classical antagonist-type ligands as Flumazenil.

Table II. In vitro data of new 3-aryl-4,7-dihydro-6-($N^{1'}$-alkylpyrazol-3' or 5'-yl)pyrazolo[1,5-*a*]pyrimidin-7-ones and 3-aryl-4,7-dihydro-6-($N^{1'}$,$N^{2'}$-dialkylpyrazol-3'-yliden)pyrazolo[1,5-*a*]pyrimidin-7-one ligands at the BzR.



Compound	*Ar*	*R*	*Inhibition (%)*[a]	K_i *(nM)*[b]	*GABA ratio*[c]
2a	C_6H_5	CH_3	99.0 ± 7.5	41 ± 3.7	1.31
3a	C_6H_5	CH_3	95.0 ± 2.5	270 ± 19	–
4a	C_6H_5	C_2H_5	99.0 ± 6.2	90 ± 1.3	–
5a	C_6H_5	C_2H_5	48.0 ± 3.6	–	–
6a	C_6H_5	CH_3	43.0 ± 3.8	–	–
7a	C_6H_5	C_2H_5	38.0 ± 2.8	–	–
2b	2'-$OCH_3C_6H_4$	CH_3	100 ± 4.3	67.9 ± 1.20	1.62
3b	2'-$OCH_3C_6H_4$	CH_3	77.6 ± 2.7	–	–
5b	2'-$OCH_3C_6H_4$	C_2H_5	90.0 ± 5.2	1110 ± 180	2.39
2c	3'-$OCH_3C_6H_4$	CH_3	98.0 ± 3.6	61.6 ± 2.2	0.79
3c	3'-$OCH_3C_6H_4$	CH_3	89.1 ± 2.5	404 ± 3.7	1.08
4c	3'-$OCH_3C_6H_4$	C_2H_5	95.0 ± 4.3	5.1 ± 0.5	1.58
5c	3'-$OCH_3C_6H_4$	C_2H_5	92.0 ± 4.2	15.6 ± 1.8	1.67
7c	3'-$OCH_3C_6H_4$	C_2H_5	23.4 ± 8.9	–	–
2d	2'-ClC_6H_4	CH_3	79.8 ± 3.2	1880 ± 316	2.3
3d	2'-ClC_6H_4	CH_3	11.5 ± 0.8	–	–
2e	3'-ClC_6H_4	CH_3	95.8 ± 4.1	27.35 ± 0.9	2.05
3e	3'-ClC_6H_4	CH_3	93.1 ± 3.5	162.2 ± 2.6	1.33
4e	3'-ClC_6H_4	C_2H_5	98.4 ± 4.6	23.3 ± 1.3	1.96
5e	3'-ClC_6H_4	C_2H_5	95.0 ± 2.8	130.0 ± 11.2	1.05
2f	2'-Thienyl	CH_3	100 ± 6.7	403 ± 16	1.34
3f	2'-Thienyl	CH_3	54.4 ± 2.7	–	–
2g	3'-Thienyl	CH_3	100 ± 5.7	69.3 ± 5.5	1.85
3g	3'-Thienyl	CH_3	56.8 ± 3.6	–	–
4g	3'-Thienyl	C_2H_5	98 ± 6.5	29.5 ± 2.5	2.05
5g	3'-Thienyl	C_2H_5	86 ± 4.7	843 ± 43	1.82

[a]Percent of inhibition of specific [^{3}H]flunitrazepam binding at 10 μM concentration are means ± SEM of five determinations (the tests were carried out using DMSO as solvent). [b]K_i values are means ± SEM of five determinations. [c]GABA ratio = IC_{50} compound/IC_{50} compound + 10 μM GABA.

Table III. Miorelaxant and anticonvulsant effects of some 3-aryl-4,7-dihydro-6-(N^1-alkylpyrazol-3' or 5'-yl)pyrazolo[1,5-*a*]-pyrimidin-7-ones in comparison with diazepam.

Treatment	*mg/kg po*	*n*	*Number of falls from Rota-rod*	*Non convulsant*		*Lethality*
				n	%	
CMC 1%	0.1 mL	121	0.30 ± 0.06	14/121	11.6%	3/121
Diazepam	0.1	21	0.33 ± 0.10	10/21	47.6%[a,b]	–
	0.3	33	0.54 ± 0.20	29/33	87.8%[a,b]	–
	1	15	0.73 ± 0.28	15/15	100%[c]	–
	3	10	1.50 ± 0.45[a]	10/10	100%[c]	–
	5	32	2.42 ± 0.44[c]	32/32	100%[c]	–
2a	100	20	0.45 ± 0.17	1/20	5%	1/20
	300	18	0.16 ± 0.09	5/18	27.8%	1/18
5c (ip)	10	16	0.25 ± 0.14	4/16	25%[a]	–
	30	15	0.86 ± 0.23[b]	6/15	40%[a]	1/15
	100	8	0.50 ± 0.26	2/8	25%	–
10c	3	16	0.31 ± 0.15	3/16	18.75%[a]	–
	10	28	0.57 ± 0.13[a]	10/28	35%[c]	–
	30	23	0.65 ± 0.17[a]	4/23	17.4%	1/23
	100	15	0.66 ± 0.18[a]	5/15	33.3%[a]	1/15
	300	18	0.5 ± 0.18	9/18	50%[c]	–
10e	0.1	8	0.37 ± 0.18	1/8	12.5%	–
	0.3	16	0.25 ± 0.14	4/16	25%	–
	1	16	0.62 ± 0.22	5/16	31.25%[a]	–
	3	16	0.43 ± 0.15	5/16	31.2%[a]	–
	10	15	0.53 ± 0.16	3/15	20%	–
	30	20	0.45 ± 0.13	3/20	15%	2/20
	100	21	0.52 ± 0.16	4/21	19%	1/21
	300	17	0.35 ± 0.14	4/17	23.55%	–
10g	10	8	0.12 ± 0.12	1/8	12.55%	–
	30	15	0.4 ± 0.16	4/15	26.7%	–
	100	15	0.4 ± 0.13	4/15	26.7%	–
	300	18	0.27 ± 0.13	3/18	16.7%	–

[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ versus carboxymethylcellulose (CMC) 1% (Kruskal–Wallis test for Rota-rod and chi-square test (χ^2) for convulsions). Treatment was performed 30 min and Rota-rod test 5 min before penthylenetetrazole (PTZ) (75 mg/Kg sc) treatment. Mice were observed for 30 min after PTZ injection.

Chemistry

The synthesis of the final compounds was achieved following a described procedure [17]. By allowing ethyl 7-dimethylaminovinyl pyrazolo[1,5-*a*]pyrimidin-6-carboxylates **1a–g** to react with methyl or ethyl hydrazine, either 3-aryl-4,7-dihydro-6-(N^1-methyl or ethyl pyrazol-3'(5')-yl)pyrazolo[1,5-*a*]pyrimidin-7-ones **2a–g**, **3a–g**, **4a,c,e,g**, **5a,b,c,e,g** were obtained (scheme 1). Through a reaction pathway that we previously investigated [22], alkylhydrazine reacts with the enaminic carbon atom to give two possible linear intermediates, depending on which nitrogen atom belonging to the reagent carries out the nucleophilic attack. The subsequent intramolecular attack of

Table IV. Effect of flumazenil on anticonvulsant activity of 3-aryl-4,7-dihydro-6-(*N*'-alkylpyrazol-3' or 5'-yl)pyrazolo[1,5-*a*]-pyrimidin-7-ones in comparison with diazepam.

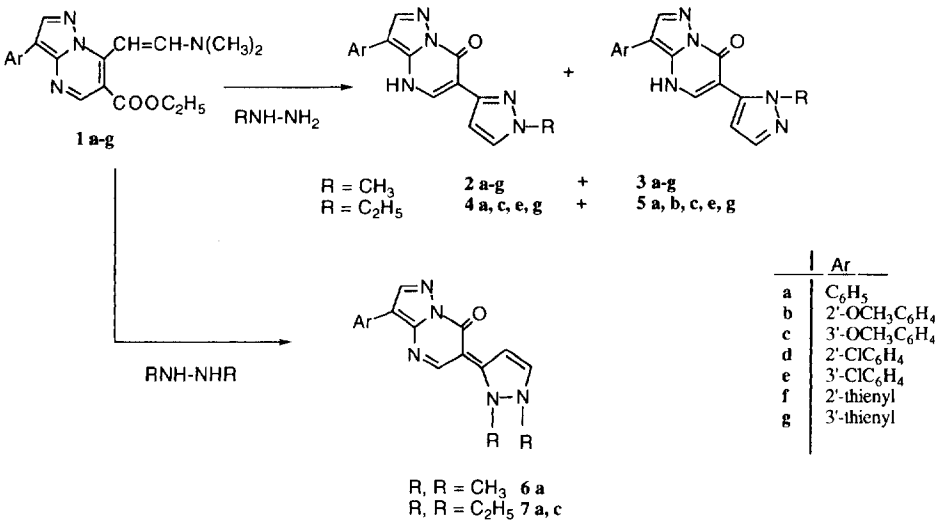
Treatment	*mg/kg po*	*n*	*Number of falls from Rota-rod*	*Non convulsant*		*Lethality*
				n	%	
Diazepam	0.3	27	0.51 ± 0.20	26/27	96.3%[a]	–
Flumazenil (ip)	0.3	11	0.09 ± 0.09	4/11	36.3%[b]	1/11
	1	20	0.70 ± 0.18	6/20	30%[b]	–
	10	12	0.25 ± 0.13	2/12	16.6%	1/12
	30	18	0.33 ± 0.14	2/18	11.1%	–
	100	15	0.40 ± 0.16	0/15	0%	–
Flumazenil	1	20	0.45 ± 0.18	13/29	65%[c]	–
+ Diazepam	0.3					
Flumazenil	10	12	0.17 ± 0.11	7/12	58.3%[d]	–
+ Diazepam	0.3					
Flumazenil	30	12	0.45 ± 0.21	3/12	25%[c]	–
+ Diazepam	0.3					
Flumazenil	100	14	0.46 ± 0.21	2/14	14.3%[c]	–
+ Diazepam	0.3					
Flumazenil	30	17	0.70 ± 0.22	4/17	23.5%	–
+ 10c	10					
Flumazenil	100	14	0.36 ± 0.13	4/14	28.5%	–
+ 10c	10					
Flumazenil	1	18	1.00 ± 0.24	8/18	44.4%	–
+ 10c	100					
10g	300	15	0.67 ± 0.19	6/15	40%[c]	–
+ Diazepam	0.3					

[a]$P < 0.01$, [b]$P < 0.05$ versus CMC 1% po, [c]$P < 0.001$, [d]$P < 0.05$ versus diazepam 0.3 mg/kg po. Pretreatment was performed 40 min, the treatment 30 min and the Rota-rod test 5 min before penthylenetetrazole (PTZ) (75 mg/kg sc) treatment. Mice were observed for 30 min after PTZ injection.

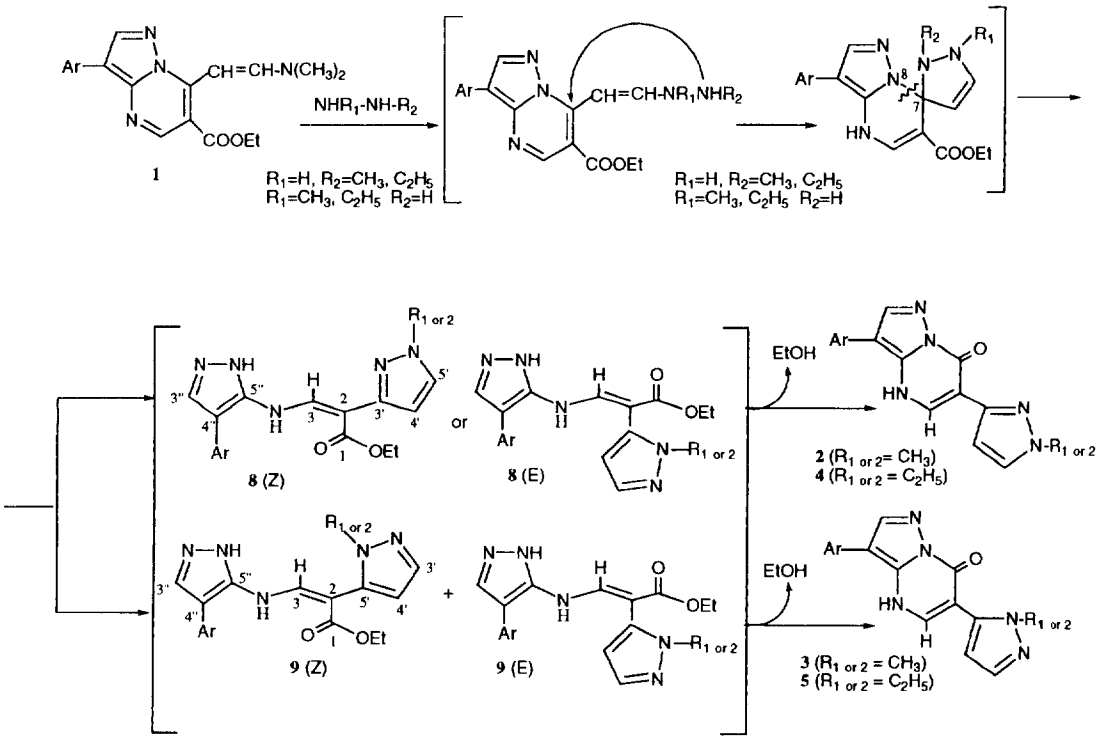
the terminal nitrogen (NH_2 or NHR) to the C-7 electron-poor carbon atom causes the closure of a second pyrazole ring. The cleavage of the C_7–N_8 bond produces a mixture of open-chain intermediate isomers (scheme 2). The subsequent reaction between the ethoxycarbonyl group and the endocyclic N_8 (belonging to the original pyrazole moiety), as shown in scheme 2, affords the newly condensed pyrimidine nucleus, yielding the final products **2**–**5**. By column chromatography the pure isomers were isolated.

As regards the regioisomers **2** and **3**, the relative amount of the latter depends on the nature of the substituents at the 3-position. When the reaction is run in acetic acid solution the regioisomers **3** are generally obtained in low yield. In order to circumvent this drawback the same reaction was performed in ethanol under acidic catalysis for compounds **3b** and **3d**. According to an already described procedure the formed open chain intermediates were separated by column chromatography (scheme 2). Only the slowest running band, consisting of (*Z*) and (*E*) **9b** or (*Z*) and (*E*) **9d** was used. On heating in acetic acid the above mixture cyclized to give the expected compounds **3b** and **3d** respectively.

The reaction between 7-dimethylaminovinylderivatives and dialkylhydrazine (*N*,*N*'-dimethyl or diethyl-



Scheme 1.



Scheme 2. Compounds **3b** and **3d** were also obtained by using a mixture of (*Z*)/(*E*) **9b** and (*Z*)/(*E*) **9d** respectively as reported in the experimental section.

hydrazine) afforded only one product whose spectral data are in accordance with the structure of $N^{1'},N^{2'}$-dialkylpyrazol-(3'(*E*)-yliden)pyrazol[1,5-*a*]pyrimidin-7-ones (**6a** and **7a,c**) (scheme 1). On the basis of the chemical shift value of H-4' (6.76–7.04) and considering the anisotropy effects of the carbonyl group, it can be reasonably assumed that the *E* geometry is to be attributed to compounds **6a** and **7a,c** [22]. Unfortunately both isomers were not available for comparison to confirm the validity of this empirical NMR correlation.

Results and discussion

Biochemistry

The methylderivatives of the **2** series (table II) have affinity values better than the respective '*N*-unsubstituted' compounds **10a–g** (table I) except for the regioisomer **2d**. On the contrary, the methylderivatives belonging to the **3** series have affinity values lower than those of the **2** series to such an extent that the BzR recognition is lost (**3b, 3d, 3f, 3g**). An analogous trend can be observed for ethylderivatives of the **4** series which have the same different affinity degree in comparison to the **5** regioisomers; it is particularly noteworthy to compare the affinity values of compounds **4c** and **5c** with those of **2c** and **3c**. However, as it appears in table II, the ethylderivatives of the **4** series generally have affinity values better than the corresponding methylderivatives of the **2** series. These observations might suggest that the variety of K_i values in the **2–5** series is due to the nature and the position of the alkyl substituents or probably to the spatial orientation of the pyrazole moiety bearing the alkyl group with regard to the pyrazolo[1,5-*a*]pyrimidine rigid structure. This observation is supported by the lack of BzR recognition of the dialkyl derivatives of the **6** and the **7** series where the dialkylated pyrazole moiety is bound to the pyrazolo[1,5-*a*]pyrimidine nucleus through a double bond which causes a stiffening of the molecule that is totally unfavourable to BzR interaction.

In addition, as shown in table II, the nature or the position of the alkyl group is not related to the GABA ratio values (that are known to give valuable indications as to the behavioural properties of the BzR ligands). Similarly neither the nature nor the position of the substituents on the 3-phenyl ring are related to the behavioural profiles of the ligands.

The continuum of activity of these ligands as illustrated by the GABA shifts of the 3-aryl-4,7-dihydro-6-($N^{1'}$-methyl or ethyl pyrazol-3'(5')-yl)pyrazolo[1,5-*a*]-pyrimidin-7-ones ranging, with gradual variety and efficacy, from full to partial agonist or from full to partial inverse agonist, supports the hypothesis of an inclusive pharmacophoric model, since small modifications in the structures cause a shift from one pharmacological class to another [19, 23, 24]. As can be observed in the **c**- and **e**-series, the GABA values shift from inverse agonism (ie, **2c**) to full agonism (ie, **10c**) (ie, **2e**) through antagonism (ie, **3c**) (ie, **5e**). The different behavioral properties among analogous compounds belonging to a series **a–g** could be related to an interrelationship between the 3 and 6 positions.

In order to explain the high affinity values of the new 3-aryl-4,7-dihydro-6-($N^{1'}$-alkylpyrazol-3'- or 5'-yl)-pyrazolo[1,5-*a*]pyrimidin-7-ones together with the high flexibility in terms of behavioural profiles of these ligands, we suggest that the groups involved in the interaction with the receptor sites H_1 and H_2 could be the lone pair of electrons on the nitrogen atom N_1 and N_4 respectively. On the other hand, it may be more reasonable to assume that the hydrogen bond donor site H_2 interacts with the closer lone pair of N_4 rather than with the remote $N_{1'}/N_{2'}$ of the pyrazole moiety. The lipophilic areas should accept the 3-phenyl ring with its substituents; furthermore, these substituents could occupy the L_3 additional lipophilic cleft when the ligand shifts as a consequence of the substitution at the 6-pyrazolyl moiety. This latter could cause the lack of interaction with A_2 and could be a hindrance to the shifting of the ligand toward the L_3 (figs 1 and 2). In fact full occupation of L_3 by BzR ligands, as reported in the literature, leads to full agonist activity whereas its partial occupation results in a partial agonist response [19, 25, 26].

Indeed, the interaction with the hydrogen bond acceptor site A_2 on the receptor protein complex, whose essential role for the anchoring of inverse agonist/antagonist ligand to the BzR is controversial for many authors [27–29], could involve the N–H group of the 6-pyrazolyl moiety or the O–H group (of the enolic form) bound to the C_7 (**2c**, **10b**, **10g**). The enolic form of the tautomeric equilibrium involving the N_4HC_7O groups could strenghten the hypothesis of both the interactions of C_7–OH with A_2 and N_4 with H_2.

In this proposed allignment, the C_2 carbon atom should be close to the negative steric interaction area S_1, which could explain the lack of BzR recognition of the 2-phenyl 4,7-dihydro-6-(1'*H*-pyrazol-3'-yl)pyrazolo[1,5-*a*]pyrimidin-7-one [17].

For the purpose of supporting our hypothesis about the groups involved in the BzR interaction, and of investigating the suitable substitutions at the 3- and 6-positions that give favourable size and shape to the molecule for partial occupation of L_3, a molecular modelling study with the synthesis of analogous compounds is currently in progress.

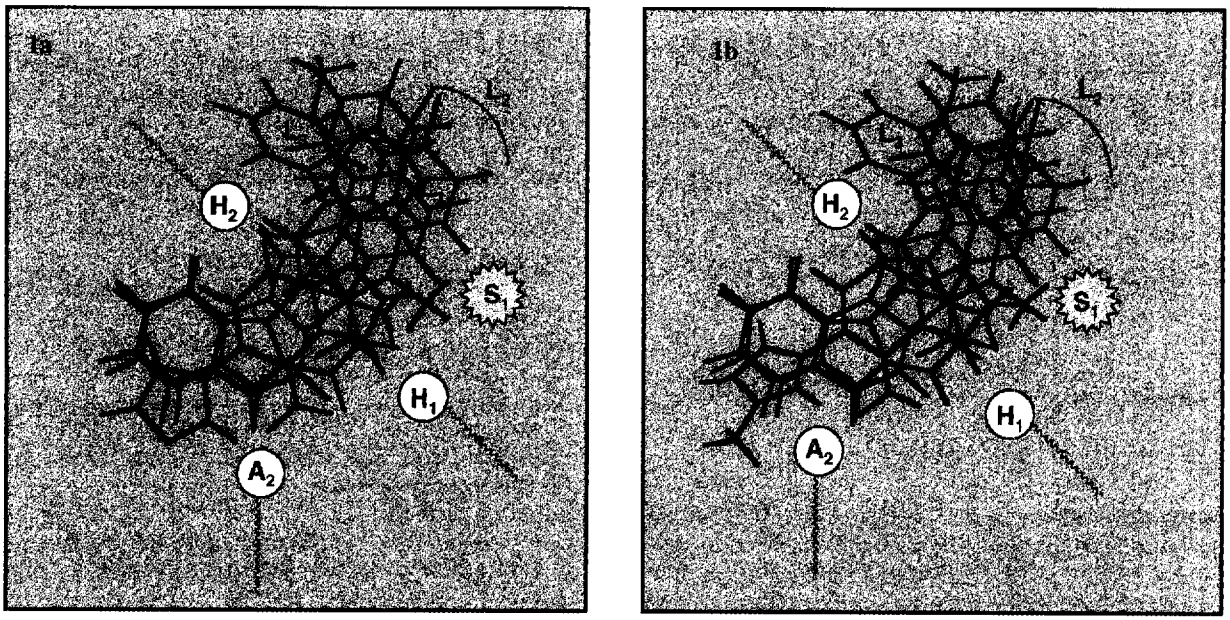


Fig 1. Schematic representation of the inclusive pharmacophore model for the BzR [19]. The sites H_1 and H_2 designate hydrogen bond donator sites on the receptor protein, A_2 the hydrogen bond acceptor site. L_1, L_2 and L_3 represent the lipophilic pockets, the latter out of the plane. Receptor descriptor S_1 is a region of negative steric repulsion. The lone pairs of electrons appear cyano. (**a**) Superposition of **10c** (green, full agonist) with CGS-9896 (magenta), diazepam (black), pyridodiindole (blue). Interaction with the lipophilic pocket L_1, L_2 and/or L_3 with H_1 and H_2 is required for agonist activity. (**b**) Superposition of **2c** (green, inverse agonist/antagonist) with CGS-9896 (magenta), diazepam (black), pyridodiindole (blue). Interaction with the lipophilic pocket L_1 as well as with H_1 and A_2 is required for potent inverse agonist activity.

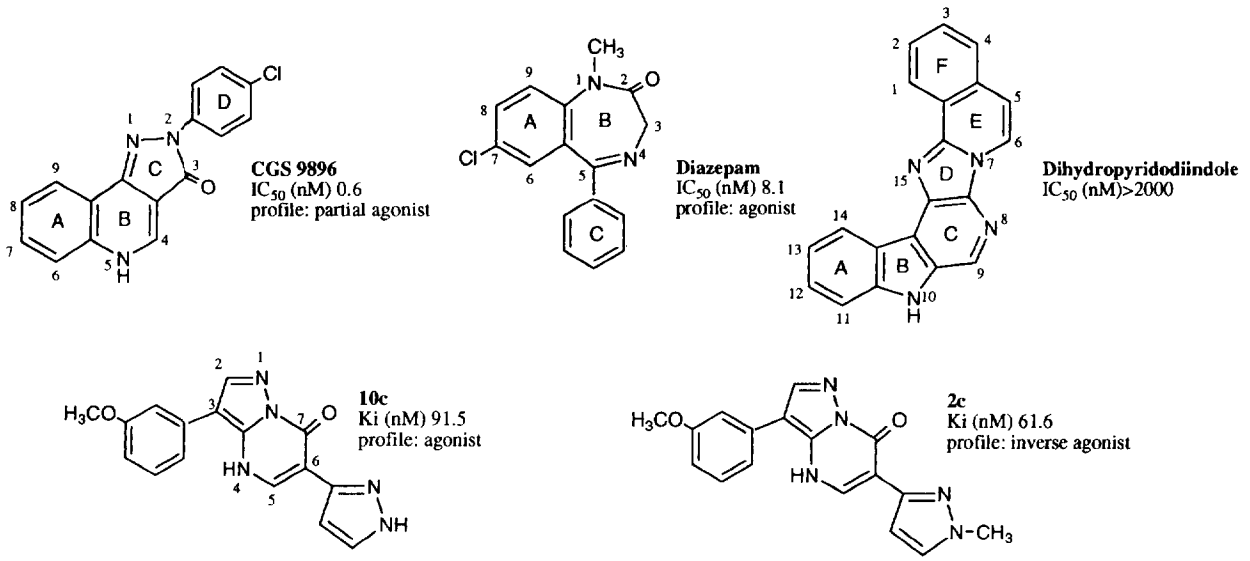


Fig 2. The BzR ligands used in the receptor modeling.

Pharmacology

Compounds used for in vivo studies were chosen because of their agonist profile (**2a**, **5c**, **10c**, **10e**) or antagonist behaviour (**10g**) and for the favourable dual concept K_i/GABA ratio. These compounds were evaluated for their anticonvulsant and miorelaxant activity in mice in 2–8 doses (table III). The animals were carefully observed for their gross behaviour for 30 min after drug administration. No behavioral alterations were evident following the administration of each of the tested substances when the animals were in their home cages. However, when they were tested on the rota rod, **5c** and **10c** showed a slight, but significant, impairment of motor coordination at the central doses (table III).

Diazepam was used as a reference molecule. The substance was dose-dependently effective both in protecting the mice from pentylentetrazole (PTZ)-induced convulsions and in impairing animal performance in the rota rod test (table III).

Neither the prototype **2a**, nor the antagonist **10g** had a protective effect from convulsions caused by PTZ, while the agonists **5c** and **10e** showed bell-shaped dose–response protection curves. Compound **10c**, which from binding studies appeared to be a full agonist on the benzodiazepine recognition site, showed a dual dose–response curve, bell-shaped at doses from 3 to 30 mg/kg and linear for higher doses.

Flumazenil has been demonstrated to act as an agonist–antagonist on the benzodiazepine recognition site [30–32], and in the present experiments had, at low doses (0.3 and 1 mg/kg ip), a statistically significant protective effect against PTZ-induced convulsions, while at high doses (100 mg/kg ip) it was proconvulsant. Nevertheless, it was able to antagonize dose-dependently the protective effect of 0.3 mg/kg po of Diazepam (table IV). When Flumazenil was administered 10 min before **10c**, two different effects were observed. The protective effect of 10 mg/kg po of **10c** was diminished, although not significantly, by 30 or 100 mg/kg ip of Flumazenil, while that of 100 mg/kg po of **10c** was raised by 1 mg/kg ip of Flumazenil. This may explain the peculiar dose–response relationship showed by **10c**, which presumably acts on two different sites at low or at high doses. Finally, **10g**, which appears to act as an antagonist on the benzodiazepine recognition site, at a dose of 300 mg/kg po was able to antagonize significantly the protective effect of 0.3 mg/kg po of Diazepam, thus confirming its antagonist action.

In any case, the GABA ratio values of the tested compounds are in accordance with in vivo results, except for compound **10c** whose full agonist profile (K_i = 91.5 nM, GR = 3.07) is not completely antagonized by Flumazenil.

Conclusions

The present study demonstrates that the affinity values and intrinsic activity of these ligands to BzR cannot be correlated solely with electronic or steric effects of the substituents at the 3-phenyl ring. The consideration of any substituent effect (ie lipophilic, electronic, steric) without concomitant consideration of the alkyl substituent at the 6-pyrazole is inadequate as a predictor of the pharmacological profile (GABA ratio values) for the 3-aryl-4,7-dihydro-6-(N^1'-alkylpyrazol-3'(5')-yl)pyrazolo[1,5-*a*]pyrimidin-7-ones ligands. Further work is needed to better define the role of the groups involved in the BzR interaction.

Experimental protocols

Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian Gemini 200 instrument; chemical shifts are reported in ppm high-frequency and coupling constants in Hz. Silica gel plates (Merck F_{254}) were used for analytical TLC. Solvents were removed under reduced pressure. Microanalyses were performed with a Perkin Elmer Model 240 C Elemental Analyzer and values are within ±0.4% of the theoretical values. Solvents were removed under reduced pressure. Compounds **1a–g** were synthesized according to the published procedures [17].

General procedure for preparing 3-aryl-4,7-dihydro-6-(N^1'-alkylpyrazol-3'(5')-yl)pyrazolo[1,5-a]pyrimidin-7-ones ***2a–g, 3a–g, 4a,c,e,g, 5a–c,e,g***

Alkylhydrazine (10 mmol) was added to a solution of ethyl 7-dimethylaminovinylpyrazolo[1,5-*a*]pyrimidin-6-carboxylates **1a–g** (10 mmol) and sodium acetate (24 mmol) in acetic acid (50 mL). The solution was refluxed under magnetic stirring; the disappearance of starting materials was monitored by TLC analysis.

Compounds **2a–g** were obtained together with the regioisomer **3a–g** and **4a,c,e,g** with **5a–c,e,g** respectively. The crude yields of these reactions were in the range 55–68%. A 1 g quantity of each mixture was generally separated by column chromatography (silica gel column 3.0 x 60 cm).

3-Phenyl-4,7-dihydro-6-(N^1'-methylpyrazol-3'-yl)pyrazolo[1,5-a]-pyrimidin-7-one ***2a*** *and 3-phenyl-4,7-dihydro-6-(N^1'-methyl-pyrazol-5'-yl)pyrazolo[1,5-a]pyrimidin-7-one* ***3a***

By column chromatography ($CHCl_3$:MeOH, 10:2.5 v/v as eluent) to give **2a** (0.56 g) and **3a** (0.38 g), respectively.

2a: white crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.89 (s, 3H, N^1-CH_3), 6.90 (d, $J_{H4'-H5'}$ = 2.1 Hz, 1H, H-4'), 7.32–7.37 (m, 1H, ArH), 7.46–7.54 (m, 2H, ArH_2), 7.62–7.66 (m, 2H, ArH_2), 7.74 (d, $J_{H5'-H4'}$ = 2.1 Hz, 1H, H-5'), 8.28 (s, 2H, H-2, H-5), 12.55 (bs, 1H, NH, exchangeable). Anal $C_{16}H_{13}N_5O$ (C, H, N).

3a: white crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.79 (s, 3H, N^1-CH_3), 6.29 (d, $J_{H4'-H5'}$ = 2.1 Hz, 1H, H-4'), 7.14–7.20 (m, 1H, ArH), 7.36–7.45 (m, 3H, ArH_2, H-3'), 8.04–8.11 (m, 3H, H-2, ArH_2), 8.30 (s, 1H, H-5), 12.68 (bs, 1H, NH, exch). Anal $C_{16}H_{13}N_5O$ (C, H, N).

3-Phenyl-4,7-dihydro-6-(N1'-ethylpyrazol-3'-yl)pyrazolo[1,5-a]-pyrimidin-7-one ***4a*** *and 3-phenyl-4,7-dihydro-6-(N1'-ethylpyrazol-5'-yl)pyrazolo[1,5-a]pyrimidin-7-one* ***5a***
By column chromatography ($CHCl_3$:MeOH, 10:1.5 v/v as eluent) to give **4a** (0.48 g) and **5a** (0.38 g), respectively.

4a: white crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 1.45 (t, 3H, N^1'-CH_2CH_3), 4.22 (q, 2H, N^1'-CH_2CH_3), 7.08 (d, $J_{H4'-H5'}$ = 1.50 Hz, 1H, H-4'), 7.19–7.21 (m, 1H, ArH), 7.39–7.47 (m, 2H, ArH_2), 7.79 (d, $J_{H5'-H4'}$ = 1.50 Hz, 1H, H-5'), 8.11–8.15 (m, 2H, ArH_2), 8.43 (s, 1H, H-2), 8.73 (s, 1H, H-5), 12.54 (bs, 1H, NH, exch). Anal $C_{17}H_{15}N_5O$ (C, H, N).

5a: white crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 1.28 (t, 3H, N^1'-CH_2CH_3), 4.10 (q, 2H, N^1'-CH_2CH_3), 6.23 (d, $J_{H4'-H3'}$ = 1.50 Hz, 1H, H-4'), 7.12–7.18 (m, 1H, ArH), 7.35–7.42 (m, 2H, ArH_2), 7.48 (d, $J_{H3'-H4'}$ = 1.50 Hz, 1H, H-3'), 8.00 (s, 1H, H-2), 8.12–8.15 (m, 2H, ArH_2), 8.30 (s, 1H, H-5), 12.66 (bs, 1H, NH, exch). Anal $C_{17}H_{15}N_5O$ (C, H, N).

3-(2'-Methoxyphenyl)-4,7-dihydro-6-(N1'-methylpyrazol-3'-yl)pyrazolo[1,5-a]pyrimidin-7-one ***2b*** *and 3-(2'-methoxyphenyl)-4,7-dihydro-6-(N1'-methylpyrazol-5'-yl)pyrazolo[1,5-a]-pyrimidin-7-one* ***3b***
By column chromatography ($CHCl_3$:MeOH, 10:1 v/v as eluent) to give **2b** (0.55 g) and **3b** (0.20 g), respectively.

2b: light yellow crystals, mp 275–276 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.83 (s, 3H, OCH_3), 3.93 (s, 3H, N^1'-CH_3), 6.89 (d, $J_{H4'-H5'}$ = 2.24 Hz, 1H, H-4'), 7.07–7.18 (m, 2H, ArH_2), 7.31–7.49 (m, 2H, ArH_2), 7.73 (d, $J_{H5'-H4'}$ = 2.24 Hz, 1H, H-5'), 8.11 (s, 1H, H-2), 8.29 (s, 1H, H-5). Anal $C_{17}H_{15}N_5O_2$ (C, H, N).

3b: light yellow crystals, mp 265–266 °C, ^{1}H-NMR (DMDO–d_6) δ ppm: 3.78 (s, 3H, N^1'-CH_3), 3.83 (s, 3H, OCH_3), 6.36 (d, $J_{H4'-H3'}$ = 1.83 Hz, 1H, H-4'), 7.02–7.14 (m, 2H, ArH_2), 7.34–7.43 (m, 2H, ArH_2), 7.47 (d, $J_{H3'-H4'}$ =1.83 Hz, 1H, H-3'), 7.92 (s, 1H, H-2), 8.13 (s, 1H, H-5), 12.22 (bs, 1H, NH, exch). Anal $C_{17}H_{15}N_5O_2$ (C, H, N).

Alternative synthesis of ***3b***
Methylhydrazine (10 mmol) was added to a stirred solution of 6-ethyl-7-(2-dimethylaminovinyl)pyrazolo[1,5-*a*]pyrimidin-6-carboxylate **1b** (10 mmol) in ethanol (30 mL) containing acetic acid (1 mL). The reaction mixture was refluxed until the disappearance of the starting material; evaporation of the solvent gave a solid mainly consisting (^{1}H-NMR spectrum) of a mixture of the isomeric compounds **9b**(*Z*) and **9b**(*E*) with traces (ca 5%) of compound **8b**(*Z*). Column chromatography with $CHCl_3$:MeOH, 10:1 as eluent afforded the separation of **8b** from the mixture of the diasteroisomers **9b**(*Z*) and **9b**(*E*).

The first material eluted was ethyl 2-(1'-methylpyrazol-3'-yl)-3-(4"-ortho-methoxyphenyl pyrazol-5"-ylamino)propenoate **8b**: ^{1}H-NMR ($CDCl_3$) δ ppm: 1.17 (t, 3H, OCH_2CH_3), 3.91 (s, 3H, N^1'-CH_3), 4.18 (q, 2H, OCH_2CH_3), 6.90 (d, $J_{H4'-H5'}$ =2.3 Hz, 1H, H-4'), 7.05–7.14 (m, 4H, ArH_4), 7.30 (d, $J_{H5'-H4'}$ = 2.3 Hz, 1H, H-5'), 8.11 (s, 1H, H-3"), 8.24 (d, 1H, $J_{H3'-NH'}$ = 14 Hz, 1H, H-3), 11.0 (d, $J_{H3'-NH'}$ = 14 Hz, 1H, NH, exch). Anal $C_{19}H_{21}N_5O_3$ (C, H, N).

The second material eluted was a mixture of ethyl 2-(1'-methylpyrazol-5'-yl)-3-(4"-ortho-methoxyphenyl pyrazol-5"-ylamino)propenoate **9b**(*Z*) and **9b**(*E*) which was used without further purification: ^{1}H-NMR ($CDCl_3$) δ ppm: 1.18–1.27 (dt, OCH_2CH_3), 3.54 (s, N^1'-CH_3), 3.71 (s, N^1'-CH_3), 3.99 (bs, OCH_3), 4.19–4.22 (dq, OCH_2CH_3), 6.12 (d, 1H, H-4'(*Z*)), 6.20 (d, 1H, H-4'(*E*)), 6.90–7.03 (m, ArH), 7.23–7.33 (m, ArH + H-3'(*Z*)), 7.54–7.66 (m, H-3'(*E*), H-3"(*E* + *Z*), H-3(*Z*)), 8.51 (d, *J* = 12.9 Hz, H-3(*E*)), 9.05 (d, *J* = 12.9 Hz, NH(*E*), exch), 10.28 (d, NH(*Z*), exch).

The treatment of the mixture of **9b**(*Z*) and **9b**(*E*) with acetic acid (20 mL) containing sodium acetate (0.4 g) under reflux for 1 h, gave the same final product **3b** as a pale yellow solid in 86% yield.

3-(2'-Methoxyphenyl)-4,7-dihydro-6-(N1'-ethylpyrazol-5'-yl)pyrazolo[1,5-a]pyrimidin-7-one ***5b***
Light yellow crystals from ethyl acetate, mp 248–249 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 1.34 (t, 3H, N^1'-CH_2–CH_3), 3.82 (s, 3H, OCH_3), 4.08 (q, 2H, N^1'-CH_2CH_3), 6.34 (d, $J_{H4'-H3'}$ = 1.81 Hz, 1H, H-4'), 7.04–7.18 (m, 2H, ArH_2), 7.34–7.48 (m, 2H, ArH_2), 7.49 (d, $J_{H3'-H4'}$ = 1.81 Hz, 1H, H-3'), 7.92 (s, 1H, H-2), 8.13 (s, 1H, H-5). Anal $C_{18}H_{17}N_5O_2$ (C, H, N).

In the raw reaction mixture a second substance was detected by TLC and ^{1}H-NMR. Unfortunately, because of its scarcity the isolation and characterization was impossible, even if it is reasonable to assume that it is the regioisomer 3-(2'-methoxyphenyl)-4,7-dihydro-6-(N1'-ethylpyrazol-3'-yl)pyrazolo[1,5-*a*]-pyrimidin-7-one.

3-(3'-Methoxyphenyl)-4,7-dihydro-6-(N1'-methylpyrazol-3'-yl)pyrazolo[1,5-a]pyrimidin-7-one ***2c*** *and 3-(3'-methoxyphenyl)-4,7-dihydro-6-(N1'-methylpyrazol-5'-yl)pyrazolo[1,5-a]-pyrimidin-7-one* ***3c***
By column chromatography ($CHCl_3$:MeOH, 10:1 v/v as eluent) to give **2c** (0.58 g) and **3c** (0.33 g), respectively.

2c: white crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.84 (s, 3H, OCH_3), 3.91 (s, 3H, N^1'-CH_3), 6.76–6.81 (m, 1H, ArH), 7.03 (d, $J_{H4'-H5'}$ = 1.91 Hz, 1H, H-4'), 7.30–7.38 (m, 1H, ArH), 7.60–7.68 (m, 2H, ArH_2), 7.72 (d, $J_{H5'-H4'}$ = 1.91 Hz, 1H, H-5'), 8.37 (s, 1H, H-2), 8.64 (s, 1H, H-5), 12.56 (bs, 1H, NH, exch). Anal $C_{17}H_{15}N_5O_2$ (C, H, N).

3c: white crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.79 (s, 3H, N^1'-CH_3), 3.83 (s, 3H, OCH_3), 6.28 (d, $J_{H4'-H3'}$ = 1.6 Hz, 1H, H-4'), 6.72–6.75 (m, 1H, ArH), 7.30–7.34 (m, 1H, ArH), 7.44 (d, $J_{H3'-H4'}$ = 1.6 Hz, 1H, H-3'), 7.67–7.71 (m, 2H, ArH_2), 8.05 (s, 1H, H-2), 8.31 (s, 1H, H-5). Anal $C_{17}H_{15}N_5O_2$ (C, H, N).

3-(3'-Methoxyphenyl)-4,7-dihydro-6-(N1'-ethylpyrazol-3'-yl)-pyrazolo[1,5-a]pyrimidin-7-one ***4c***
By addition of water to the acetic acid solution compound **4c** was obtained, which was then recrystallized from isopropanol (1.0 g). White crystals, mp 257 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 1.42 (t, 3H, N^1'-CH_2CH_3), 3.84 (s, 3H, OCH_3), 4.18 (q, 2H, N^1'-CH_2CH_3), 6.82–6.93 (m, 2H, ArH, H-4'), 7.18–7.38 (m, 3H, ArH_3), 7.76 (d, $J_{H5'-H4'}$ = 1.89 Hz, 1H, H-5'), 8.28 (s, 2H, H-2, H-5). Anal $C_{18}H_{17}N_5O_2$ (C, H, N).

3-(3'-Methoxyphenyl)-4,7-dihydro-6-(N1'-ethylpyrazol-5'-yl)-pyrazolo[1,5-a]pyrimidin-7-one ***5c***
After eliminating the solvent under reduced pressure the residual solid (**5c**) was recrystallized from ethanol/water (0.67 g). White crystals, mp 237 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 1.31 (t, 3H, N^1'-CH_2CH_3), 3.83 (s, 3H, OCH_3), 4.05 (q, 2H, N^1'-CH_2CH_3), 6.30 (d, $J_{H4'-H3'}$ = 1.64 Hz, 1H, H-4'), 6.88–6.93 (m, 1H, ArH), 7.22–7.26 (m, 2H, ArH_2), 7.36–7.40 (m, 1H, ArH), 7.51 (d, $J_{H3'-H4'}$ = 1.64 Hz, 1H, H-3'), 7.91 (s, 1H, H-2), 8.32 (s, 1H, H-5), 12.67 (bs, 1H, NH). Anal $C_{18}H_{17}N_5O_2$ (C, H, N).

3-(2'-Chlorophenyl)-4,7-dihydro-6-(N1'-methylpyrazol-3'-yl)-pyrazolo[1,5-a]pyrimidin-7-one ***2d*** *and 3-(2'-chlorophenyl)-4,7-dihydro-6-(N1'-methylpyrazol-5'-yl)pyrazolo[1,5a]pyrimidin-7-one* ***3d***

By column chromatography ($CHCl_3$:MeOH, 10:1 v/v as eluent) to give **2d** (0.43 g) and **3d** (0.20 g), respectively.

2d: white crystals, mp 291–293 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.89 (s, 3H, N^1'-CH_3), 6.93 (d, $J_{H4'-H5'}$ = 1.83 Hz, 1H, H-4'), 7.33–7.48 (m, 3H, ArH_3), 7.58–7.62 (m, 1H, ArH), 7.71 (d, $J_{H5'-H4'}$ = 1.83 Hz, 1H, H-5'), 8.19 (s, 1H, H-2), 8.39 (s, 1H, H-5). Anal $C_{16}H_{12}N_5OCl$ (C, H, N).

3d: light-yellow crystals, mp 279–280 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.77 (s, 3H, N^1'-CH_3), 6.36 (d, $J_{H4'-H3'}$ = 1.75 Hz, 1H, H-4'), 7.44–7.67 (m, 5H, ArH_4, H-3'), 7.99 (s, 1H, H-2), 8.14 (s, 1H, H-5), 12.83 (bs, 1H, NH, exch). Anal $C_{16}H_{12}N_5OCl$ (C, H, N).

Alternative synthesis of ***3d***

Methylhydrazine (10 mmol) was added to a stirred solution of ethyl 3-(2-chlorophenyl)-7-(2-dimethylaminovinyl)pyrazolo-[1,5-*a*]pyrimidin-6-carboxylate **1d** (10 mmol) in ethanol (30 mL) containing acetic acid (1 mL). The reaction mixture was refluxed until the disappearance of the starting material; evaporation of the solvent gave a solid mainly consisting (^{1}H-NMR spectrum) of a mixture of the isomeric compounds **9d**(*Z*) and **9d**(*E*) with traces (ca 10%) of compound **8d**(*E*). Column chromatography with $CHCl_3$:MeOH, 10:1 as eluent afforded the separation of **8d** from the mixture of the diasteroisomers **9d**(*Z*) and **9d**(*E*).

The first material eluted was ethyl 2-(1'-methylpyrazol-3'-yl)-3-(4"-ortho-chlorophenyl pyrazol-5"-ylamino)propenoate **8d**: ^{1}H-NMR (DMSO–d_6) δ ppm: 1.26 (t, 3H, OCH_2CH_3), 3.66 (s, 3H, N^1'-CH_3), 4.20 (q, 2H, OCH_2CH_3), 6.70 (d, $J_{H4'-H5'}$ = 2.0 Hz, 1H, H-4'), 7.42–7.67 (m, 5H, ArH_4, H-5'), 7.91 (s, 1H, H-3"), 8.45 (d, 1H, $J_{H3-NH'}$ = 13.6 Hz, 1H, H-3), 11.00 (d, $J_{H3-NH'}$ = 13.6 Hz, 1H, NH exch), 12.70 (1H, NH exch). ^{13}C-NMR (DMSO–d_6) δ ppm: 16.24 (OCH_2CH_3), 39.00 (N^1'-CH_3), 60.89 (OCH_2CH_3), 96.60 (C-2), 106.22 (C-4"), 107.54 (C-4'), 129.45 (C-5'), 130.88 (ArC-H), 131.68 (ArC-H), 131.89 (ArC-H), 132.13 (ArC-H), 132.26 (ArC-H), 133.70 (C-3'), 134.40 (C-3), 140.74 (C-3"), 148.34 (ArC-Cl), 149.76 (C-5"), 168.18 (C1=O). Anal $C_{18}H_{18}N_5OCl$ (C, H, N).

The stereochemical assignment was based on the value of $^3J_{Cl-H3}$ (^{13}C-NMR) and on the chemical shift of H-3 (^{1}H-NMR). In fact the C=O carbon atom resonating δ 168.18 appears as a doublet of triplets $^3J_{Cl-H3}$ = 7.0 Hz, therefore it is reasonable to suppose that this value corresponds to $^3J_{cis}$. Furthermore, considering the anisotropy effects of the C=O group, the *E* configuration could be supported by the deshielded chemical shift value (δ 8.55) of H-3.

The second material eluted was a mixture of ethyl 2-(1'-methylpyrazol-5'-yl)-3-(4"-orthochlorophenyl pyrazol-5"-yl-amino)propenoate **9d**(*Z*) and **9d**(*E*) which was used without further purification: ^{1}H-NMR (DMSO–d_6) δ ppm: 1.15–1.28 (dt, OCH_2CH_3), 3.60 (s, N^1'-CH_3), 3.70 (s, N^1'-CH_3), 4.12–4.22 (dq, OCH_2CH_3), 6.08 (d, 1H, H-4'(*Z*)), 6.19 (d, 1H, H-4'(*E*)), 7.22–7.74 (m, ArH + H-3'(*E*), H-3"(*E* + *Z*), H-3(*Z*)), 8.40 (d, H-3(*E*)), 10.24 (d, NH(*E* + *Z*), exch), 10.28 (d, NH(*Z*) exch).

The treatment of the mixture of **9d**(*Z*) and **9d**(*E*) with acetic acid (20 mL) containing sodium acetate (0.4 g) under reflux for 1 h, gave the same final product **3d** as a light yellow solid in 76% yield.

3-(3'-Chlorophenyl)-4,7-dihydro-6-(N1'-methylpyrazol-3'-yl)-pyrazolo[1,5-a]pyrimidin-7-one ***2e*** *and 3-(3'-chlorophenyl)-4,7-dihydro-6-(N1'-methylpyrazol-5'-yl)pyrazolo[1,5-a]pyrimidin-7-one* ***3e***

By column chromatography ($CHCl_3$:MeOH, 10:1 v/v as eluent) to give **2e** (0.55 g) and **3e** (0.40 g), respectively.

2e: white crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.90 (s, 3H, N^1'-CH_3), 7.01 (d, $J_{H4'-H5'}$ = 1.7 Hz, 1H, H-4'), 7.19–7.24 (m, 1H, ArH), 7.41–7.48 (m, 1H, ArH), 7.71 (m, 1H, ArH), 7.93–7.99 (m, 1H, ArH), 8.16 (d, $J_{H5'-H4'}$ = 1.7 Hz, 1H, H-5'), 8.42 (s, 1H, H-2), 8.61 (s, 1H, H-5). Anal $C_{16}H_{12}N_5OCl$ (C, H, N).

3e: white crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.79 (s, 3H, N^1'-CH_3), 6.28 (d, $J_{H4'-H3'}$ = 1.67 Hz, 1H, H-4'), 7.18–7.20 (m, 1H, ArH), 7.37–7.46 (m, 2H, ArH, H-3'), 8.04–8.10 (m, 2H, ArH, H-2). Anal $C_{16}H_{12}N_5OCl$ (C, H, N).

3-(3'-Chlorophenyl)-4,7-dihydro-6-(N1'-ethylpyrazol-3'-yl)-pyrazolo[1,5-a]pyrimidin-7-one ***4e*** *and 3-(3'-chlorophenyl)-4,7-dihydro-6-(N1'-ethylpyrazol-5'-yl)pyrazolo[1,5-a]pyrimidin-7-one* ***5e***

By column chromatography ($CHCl_3$:MeOH, 10:1.5 v/v as eluent) to give **4e** (0.42 g) and **5e** (0.30 g), respectively.

4e: white crystals, mp 228–230 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 1.44 (t, 3H, N^1'-CH_2CH_3), 4.20 (q, 2H, N^1'-CH_2CH_3), 6.99 (d, $J_{H4'-H5'}$ = 1.75 Hz, 1H, H-4'), 7.21–7.24 (m, 1H, ArH), 7.41–7.48 (m, 1H, ArH), 7.76 (d, $J_{H5'-H4'}$ = 1.75 Hz, 1H, H-5'), 7.95–7.99 (m, 1H, ArH), 8.16 (s, 1H, ArH), 8.41 (s, 1H, H-2), 8.62 (s, 1H, H-5), 12.51 (bs, 1H, NH, exch). Anal $C_{17}H_{14}N_5OCl$ (C, H, N).

5e: white crystals, mp 176 °C dec, ^{1}H-NMR (DMSO–d_6) δ ppm: 1.31 (t, 3H, N^1'-CH_2CH_3), 4.03 (q, 2H, N^1'-CH_2CH_3), 6.32 (d, $J_{H4'-H3'}$ = 1.63 Hz, 1H, H-4'), 7.36–7.73 (m, 5H, ArH_4, H-3'), 7.96 (s, 1H, H-2), 8.39 (s, 1H, H-5), 12.97 (bs, 1H, NH, exch). Anal $C_{17}H_{14}N_5OCl$ (C, H, N).

3-(2'-Thienyl)-4,7-dihydro-6-(N1'-methylpyrazol-3'-yl)pyrazolo[1,5-a]pyrimidin-7-one ***2f*** *and 3-(2'-thienyl)-4,7-dihydro-6-(N1'-methylpyrazol-5'-yl)pyrazolo[1,5-a]pyrimidin-7-one* ***3f***

By column chromatography ($CHCl_3$:MeOH, 10:1.5 v/v as eluent) to give **2f** (0.55 g) and **3f** (0.40 g), respectively.

2f: white crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.78 (s, 3H, N^1'-CH_3), 6.38 (d, $J_{H4'-H5'}$ = 2.6 Hz, 1H, H-4'), 7.21–7.24 (m, 1H, thienyl), 7.38–7.40 (m, 1H, thienyl), 7.48 (d, $J_{H5'-H4'}$ = 2.6 Hz, 1H, H-5'), 7.57–7.60 (m, 1H, thienyl), 7.96 (s, 1H, H-2), 8.21 (s, 1H, H-5), 12.78 (bs, 1H, NH, exch). Anal $C_{14}H_{11}N_5OS$ (C, H, N).

3f: white crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.91 (s, 3H, N^1'-CH_3), 6.91 (d, $J_{H4'-H3'}$ = 2.08 Hz, 1H, H-4'), 7.23–7.24 (m, 1H, thienyl), 7.37–7.39 (m, 1H, thienyl), 7.58–7.61 (m, 1H, thienyl), 7.75 (d, $J_{H3'-H4'}$ = 2.08 Hz, 1H, H-3'), 8.19 (s, 1H, H-2), 8.30 (s, 1H, H-5), 12.52 (bs, 1H, NH, exch). Anal $C_{14}H_{11}N_5OS$ (C, H, N).

3-(3'-Thienyl)-4,7-dihydro-6-(N1'-methylpyrazol-3'-yl)pyrazolo-[1,5-a]pyrimidin-7-one ***2g***

From the reaction mixture a precipitate was obtained which was then recrystallized from dimethylformamide (0.90 g). White crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.89 (s, 3H, N^1'-CH_3), 6.88 (d, $J_{H4'-H5'}$ = 2.0 Hz, 1H, H-4'),

7.56–7.58 (m, 1H, thienyl), 7.72–7.76 (m, 3H, thienyl, H-2, H-5'), 8.29 (s, 1H, H-5), 12.42 (bs, 1H, NH, exch). Anal $C_{14}H_{11}N_5OS$ (C, H, N).

3-(3'-Thienyl)-4,7-dihydro-6-(N1'-methylpyrazol-5'-yl)pyrazolo-[1,5-a]pyrimidin-7-one ***3g***
By addition of water to the acetic acid solution a precipitate was obtained which was then recrystallized from ethanol (0.60 g). White crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 3.76 (s, 3H, $N^{1'}$-CH_3), 6.35 (d, $J_{H4'-H3'}$ = 1.8 Hz, 1H, H-4'), 7.47 (d, $J_{H3'-H4'}$ = 1.8 Hz, 1H, H-3'), 7.56–7.59 (m, 1H, thienyl), 7.69–7.71 (m, 1H, thienyl), 8.81–8.82 (m, 1H, thienyl), 7.98 (s, 1H, H-2), 8.35 (s, 1H, H-5), 12.6 (bs, 1H, NH, exch). Anal $C_{14}H_{11}N_5OS$ (C, H, N).

3-(3'-Thienyl)-4,7-dihydro-6-(N1'-ethylpyrazol-3'-yl)pyrazolo-[1,5-a]pyrimidin-7-one ***4g***
From the reaction mixture a precipitate was obtained which was then recrystallized from dimethylformamide (0.57 g). White crystals, mp > 300 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 1.42 (t, 3H, $N^{1'}$-CH_2CH_3), 4.18 (q, 2H, $N^{1'}$-CH_2CH_3), 6.88 (d, $J_{H4'-H5'}$ = 1.71 Hz, 1H, H-4'), 7.54–7.56 (m, 1H, thienyl), 7.75–7.79 (m, 3H, 2H thienyl, H5'), 8.29 (s, 2H, H-2, H-5). Anal $C_{15}H_{13}N_5OS$ (C, H, N).

3-(3'-Thienyl)-4,7-dihydro-6-(N1'-ethylpyrazol-5'-yl)pyrazolo-[1,5-a]pyrimidin-7-one ***5g***
By addition of water to the acetic acid solution a precipitate was obtained which was then recrystallized from ethanol/water (0.5 g). White crystals, mp 282–284 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 1.35 (t, 3H, $N^{1'}$-CH_2CH_3), 4.06 (q, 2H, $N^{1'}$-CH_2CH_3), 6.34 (d, $J_{H4'-H3'}$ = 1.65 Hz, 1H, H-4'), 7.54 (d, $J_{H3'-H4'}$ = 1.65 Hz, 1H, H-3'), 7.55–7.57 (m, 1H, thienyl), 7.72–7.75 (m, 1H, thienyl), 7.81–7.82 (m, 1H, thienyl), 7.98 (s, 1H, H-2), 8.37 (s, 1H, H-5), 12.62 (bs, 1H, NH, exch). Anal $C_{15}H_{13}N_5OS$ (C, H, N).

General procedure for preparing 3-aryl-4,7-dihydro-6-(N1',N2'-dialkylpyrazol-3'-yliden)pyrazolo[1,5-a]pyrimidin-7-ones ***6a, 7a, 7c***

Dialkylhydrazine (10 mmol) was added to a solution of ethyl 7-dimethylaminovinylpyrazolo[1,5-*a*]pyrimidin-6-carboxylates **1a–g** (10 mmol) and sodium acetate (24 mmol) in acetic acid (50 mL). The solution was refluxed under magnetic stirring; the disappearance of starting materials was monitored by TLC analysis.

3-Phenyl-4,7-dihydro-6-(N1',N2'-dimethylpyrazol-3'-yliden)-pyrazolo[1,5-a]pyrimidin-7-one (E) ***6a***
After evaporation of the solvent the residue was dissolved in $CHCl_3$ and treated with an aqueous solution of $NaHCO_3$. The organic phase was dried on Na_2SO_4; the solvent was removed in vacuo and the yellow residue recrystallized from ethanol. Light yellow crystals (1.76 g, 58%), mp 252 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 4.02 (s, 3H, N-CH_3), 4.24 (s, 3H, N-CH_3), 7.04 (d, $J_{H4'-H5'}$ = 2.7 Hz, 1H, H-4'), 7.34–7.38 (m, 1H, ArH), 7.47–7.55 (m, 2H, ArH_2), 7.71–7.75 (m, 2H, ArH_2), 8.20 (s, 1H, H-2), 8.42 (s, 1H, H-5), 8.61 (d, $J_{H5'-H4'}$ = 2.7 Hz, 1H, H-5'). Anal $C_{17}H_{15}N_5O$ (C, H, N).

3-Phenyl-4,7-dihydro-6-(N1',N2'-diethylpyrazol-3'-yliden)pyrazolo-[1,5-a]pyrimidin-7-one (E) ***7a***
Following the above-described procedure a yellow residue was obtained. Crystals from ethanol (1.99 g, 60%), mp 199–202 °C with decomposition. ^{1}H-NMR (DMSO–d_6) δ ppm: 1.15 (t, 3H, N-CH_2CH_3), 1.51 (t, 3H, N-CH_2CH_3), 4.36 (q, 2H, N-CH_2CH_3), 4.63 (q, 2H, N-CH_2CH_3), 6.76 (d, $J_{H4'-H5'}$ = 2.9 Hz, 1H, H-4'), 7.12–7.19 (m, 1H, ArH), 7.34–7.41 (m, 2H, ArH_2), 7.97–8.06 (m, 4H, ArH_2, H-5', H-2*), 8.22 (s, 1H, H-5*). Anal $C_{19}H_{19}N_5O$ (C, H, N). The attribution can be reversed in the cases indicated by *.

3-(3'-Methoxyphenyl)-4,7-dihydro-6-(N1',N2'-diethylpyrazol-3'-yliden)pyrazolo[1,5-a] pyrimidin-7-one (E) ***7c***
After evaporation of the solvent the sticky residue was treated with aceton and filtered. Light yellow crystals from isopropanol (3.22 g, 88%), mp 246–247 °C, ^{1}H-NMR (DMSO–d_6) δ ppm: 1.25 (t, 3H, N-CH_2CH_3), 1.51 (t, 3H, N-CH_2CH_3), 3.80 (s, 3H, OCH_3), 4.49–4.64 (m, 4H, N-CH_2CH_3, N-CH_2CH_3), 6.67–6.72 (m, 1H, ArH), 6.93 (d, $J_{H4'-H5'}$ = 3.1 Hz, 1H, H-4'), 7.22–7.31 (m, 1H, ArH), 7.65–7.81 (m, 2H, ArH_2), 8.09 (s, 1H, H-2), 8.31 (s, 1H, H-5), 8.59 (d, $J_{H5'-H4'}$ = 3.1 Hz, 1H, H-5'). Anal $C_{20}H_{21}N_5O_2$ (C, H, N).

Biochemistry

In vitro methods
The binding affinity of compounds was tested for the ability to displace [^{3}H]flunitrazepam (at 0.2 nM, K_d = 1.8 nM) from its specific binding in bovine brain membranes obtained as previously described [33]. The inhibition of the specific binding was determined in the presence of a single concentration (10 μM) of the potential displacing agent. Then the IC_{50} values of the most active compounds were calculated from the displacement curves by log probit analysis. From the latter, K_i, used to define the BzR affinity, and the GABA ratio, which according to several authors [34, 35] generally predicts the expected behavioral properties of a BzR ligand, were derived.

Pharmacology

In vivo methods
Male Swiss Webster mice (22–28 g) were used. Fifteen mice were housed per cage. The cages were taken to the experimental room 24 h before the experiment, for acclimatisation. The animals were fed with a standard laboratory diet and tap water ad libitum.

Drugs. The following drugs were used: Diazepam (Valium 10, Roche), Flumazenil (RO 15-1788, Roche) and Pentylentetrazole (Sigma). Drug concentrations were prepared in CMC (1%) (because of the insolubility of the tested subtances) in such a way that the necessary dose could be injected in a volume of 10 mL/kg.

Rota-rod test [36]. The apparatus consists of a base platform and a rotating rod of 3 cm diameter with a non-slippery surface. This rod is placed at a height of 15 cm from the base. The rod, 30 cm in length, is divided into 5 equal sections by 6 disks. The integrity of motor coordination was assessed at a rotating speed of 24 rpm, counting the number of falls from the rod in 30 s, 25 min after treatment. The statistical analysis was performed by means of the Kruskal–Wallis test.

Assessment of the anticonvulsant activity. The test was performed by using Pentylenetetrazole (Sigma) at a dose (75 mg/kg sc) which induced generalized tonic–clonic seizures in 88.4% of control mice. The animals were treated po with the substances or with Diazepam 30 min before administration of PTZ and observed for the following 30 min. The number of the

mice that did not show convulsion was counted, and the percentage of non-convulsant treated mice was compared to that of controls by means of the χ^2 test.

Molecular modelling

The computer models of the ligands were obtained using the AM1 routine in MOPAC. The graphic presentations were obtained using Insight II software by Molecular Simulation Inc. The structures used in the receptor modelling belong to three different structural families: Diazepam, CGS 9896 and Dihydropyridodiindole; this latter was used as negative control (fig 2). The first position L_1 corresponds to the D ring centroid of the CGS 9896 and the fused benzene ring (A) of the Diazepam. The second and the third point (H_1 and H_2) correspond to the lone pairs of electrons which extend from the N_8 of Dihydropiridodiindole to the carbonyl oxygen atoms belonging to CGS 9896 and Diazepam, and from N_1 of CGS 9896 to N_4 of Diazepam respectively.

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