3-Aryl-[1,2,4]triazino[4,3-*a*]benzimidazol-4(10*H*)-ones: Tricyclic Heteroaromatic Derivatives as a New Class of Benzodiazepine Receptor Ligands

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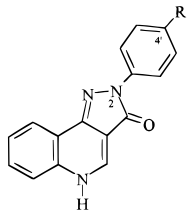
A series of 3-substituted [1,2,4]triazino[4,3-*c*]benzimidazoles **V** were prepared and tested at the central benzodiazepine receptor (BzR). These compounds were designed as rigid analogues of the previously described *N*-benzylindolylglyoxylylamide derivatives **IV**. The title compounds **V** showed an affinity which depended directly on the presence of the N(10)-H group and an aromatic ring at position 3. Some of them elicited a 2- or 3-fold higher affinity with respect to that of the indolylglyoxylylamide derivatives **IV** (R = H). The GABA ratio and [^{35}S]-*tert*-butylcyclophosphorothionate binding data revealed an efficacy profile of partial inverse agonists/antagonists for compounds **1c,e,f,j,k**, and of a partial agonist for **2c**. This last compound proved to be effective in antagonizing pentylenetetrazole-induced seizures in mice. Attempts were made to interpret the structure−affinity relationships of compounds **V** in the light of possible tautomeric equilibria involving the ligands.

Introduction

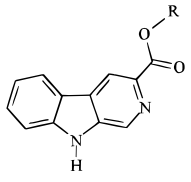
Benzodiazepine receptor (BzR) ligands allosterically modulate the action of GABA on neuronal chloride ion flux, thus eliciting a wide variety of pharmacological actions ranging in a continuum from full agonists (anxiolytic, sedative/hypnotic, and anticonvulsant activities) to inverse agonists (proconvulsant and anxiogenic activities), via antagonists, which do not exhibit per se any pharmacological effects but can antagonize the action of both agonists and inverse agonists.[1−3]

To this day, the structure of the BzR remains unresolved.[4,5] A wide variety of compounds with a chemical structure different from that of benzodiazepines have been synthesized and tested[6−16] to identify BzR ligands displaying high potency and the desired efficacy profile. Among them, the most interesting classes are 2-arylpyrazoloquinolines (**I**),[6,10] β-carbolines (**II**),[8] and pyridodiindoles (**III**).[11] A comprehensive pharmacophore model for BzR ligands has recently been proposed by Cook et al.[17] which rationalizes the binding affinity and the efficacy profile of many chemically heterogeneous classes of compounds in terms of hydrophobic and hydrogen bond interactions.

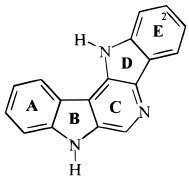
A useful contribution to the refinement of Cook's model came also from some structure−activity relationships (SARs) and molecular modeling studies of a new class of BzR ligands, the *N*-benzylindolylglyoxylylamide



I

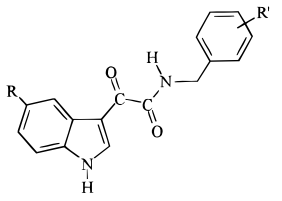


II

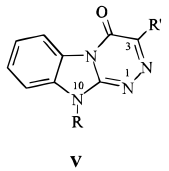


III

derivatives (**IV**), which were hypothesized to assume a pseudoplanar arrangement mimicking the β-carboline system.[18] However, compounds **IV** were generally found to be less potent than ligands of classes **I**−**III**, probably owing to their greater conformational flexibility.



IV



V

We recently started a wide research program aimed at devising new BzR ligands characterized by a higher degree of rigidity with respect to the *N*-benzylindolyl

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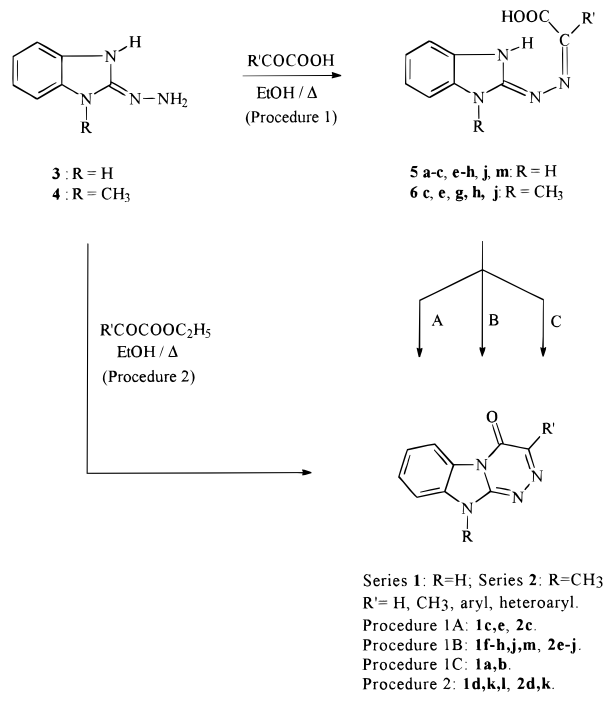
Scheme 1[a]



[a] Reagents: (A) fusion; (B) ethanol, gaseous HCl, reflux; (C) glacial acetic acid, reflux.

glyoxylylamides **IV**. The early results of our studies are reported in the present paper, where we describe the synthesis and biological evaluation of a series of [1,2,4]-triazino[4,3-*a*]benzimidazole derivatives **V** designed as geometrically constrained analogues of the indolylglyoxylyl system of compounds **IV**. Figure 1 shows a superimposition of the molecular models of ligands of types **I**, **III,** and **V** (R′ = phenyl), in accordance with their hypothesized common mode of interaction with the BzR.

Chemistry

The triazinobenzimidazole derivatives **1a**−**m** and **2c**−**e,g,h,j,k** were prepared essentially following two general procedures. Procedure 1 involved the reaction of 2-hydrazinobenzimidazole **3** or **4**[19] with the appropriate glyoxylic acid in ethanol to give the intermediate substituted (benzimidazol-2-ylhydrazono)acetic acids **5a**−**c,e**−**h,j,m** and **6c,e,g,h,j** which were purified by suspension in hot methanol, as the recrystallization process always led to partial cyclization of the products (Scheme 1, Table 1, Supporting Information). The cyclization of these acids was obtained using one of the following methods (Scheme 1, Table 2): (A) by heating the acids at a temperature 30−40 °C above their melting point for 10 min; (B) by saturating with anhydrous hydrogen chloride a suspension of the acid in absolute ethanol at 0 °C, then refluxing the reaction mixture for 4−7 h; (C) by refluxing the acids for 1 h in glacial acetic acid.

In procedure 2, compounds **3** and **4** were reacted with the appropriate glyoxylic acid ethyl ester in refluxing ethanol to give the target compounds **1d,k,l** and **2d,k** directly (Scheme 1, Table 2, Supporting Information).

The product **1i** was obtained from the corresponding dimethoxy derivative **1h** by demethylation with BBr_3[20] in anhydrous dichloromethane (Table 2, Supporting Information).

Compounds **1c**,[21]**d**,**g**,**h** were allowed to react with methyl iodide in absolute ethanol in the presence of sodium ethylate. Compound **1c** gave an approximately 1:2 mixture of **2c** and the 1-methylated product **7**, while **1d**,**g**,**h** gave almost exclusively the 1-methylated products **8**−**10**, isomers of **2d**,**g**,**h**, respectively (Scheme 2, Supporting Information).

Results and Discussion

Binding Studies. The binding affinities of the triazinobenzimidazoles **1a**−**m** and **2c**−**e,j,k** at the BzR were determined in bovine brain membranes by displacement experiments with the radiolabeled antagonist [^{3}H]Ro 15-1788.[22] The in vitro efficacy profile of the most active compounds was measured by the GABA ratio[23−25] and by the [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]-TBPS) binding shift.[26−28] The results of the in vitro assays, listed in Table 3, are here summarized.

Affinity depends directly on the nature of the substituents at positions 10 and 3. The benzimidazole N(10) has to be unsubstituted for a high affinity, as its

Scheme 2

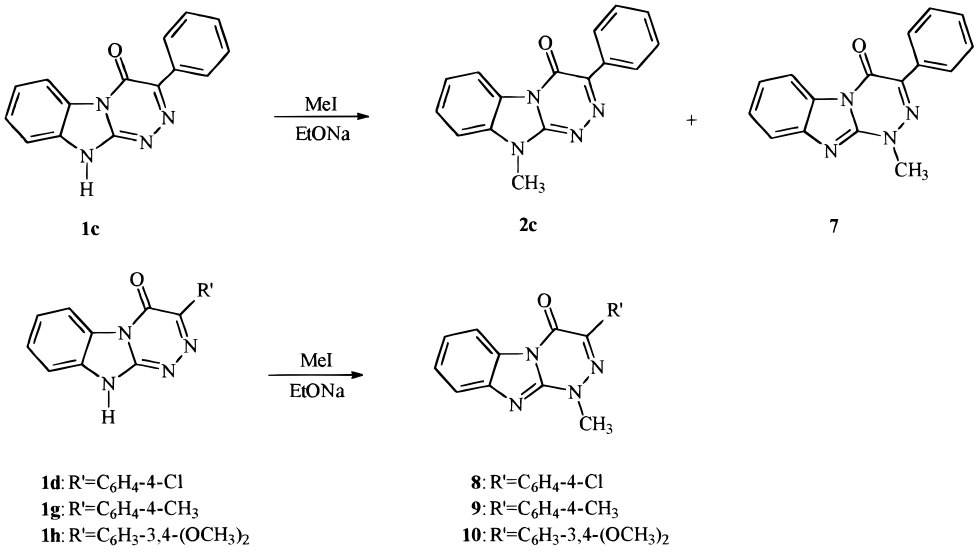
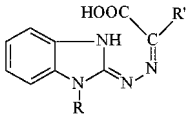


Table 1. Physical Properties of Benzimidazol-2-ylhydrazone Derivatives **5a**−**c**,**e**−**h**,**j**,**m** and **6c**,**e**,**g**,**h**,**j**



no.	R	R′	yield (%)	mp (°C)	formula[a]
5a	H	H	79	186−189	$C_9H_8N_4O_2$
5b	H	CH_3	70	220−222	$C_{10}H_{10}N_4O_2$[b]
5c	H	C_6H_5	78	247−250	$C_{15}H_{12}N_4O_2$
5e	H	C_6H_4-4-OCH_3	66	278−280	$C_{16}H_{14}N_4O_3$
5f	H	C_6H_4-4-OH	93	256−259	$C_{15}H_{12}N_4O_3$
5g	H	C_6H_4-4-CH_3	87	>300	$C_{16}H_{14}N_4O_2$
5h	H	C_6H_3-3,4-$(OCH_3)_2$	67	299−301	$C_{17}H_{15}N_4O_4$
5j	H	fur-2-yl	98	>300	$C_{13}H_{10}N_4O_3$
5m	H	thien-3-yl	66	228−231	$C_{13}H_{10}N_4O_2S$
6c	CH_3	C_6H_5	80	211−214 dec	$C_{16}H_{14}N_4O_2$
6e	CH_3	C_6H_4-4-OCH_3	56	194−197	$C_{17}H_{16}N_4O_3$
6g	CH_3	C_6H_4-4-CH_3	73	195−198	$C_{17}H_{16}N_4O_2$
6h	CH_3	C_6H_3-3,4-$(OCH_3)_2$	70	210−211	$C_{18}H_{18}N_4O_4$
6j	CH_3	fur-2-yl	66	213−215	$C_{14}H_{12}N_4O_3$

[a] Elemental analyses for C, H, N were within ±0.4% of the calculated values. [b] This product is described in refs 49 and 50 with mp 291 and >360 °C, respectively, very different from the one reported here. The IR, ^{1}H NMR, and MS spectral data for our product are fully consistent with the structure assigned (Supporting Information).

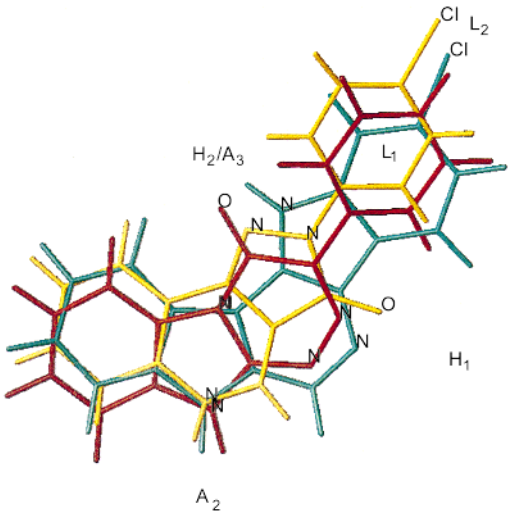
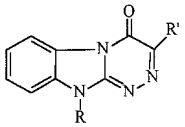


Figure 1. Overlay of the triazinobenzimidazole derivative **1c** of type **V** (red) on the 4′-chlorophenylpyrazoloquinoline derivative[6] of type **I** (yellow) and the 2-chloropyridodiindole derivative[30] of type **III** (cyan). Labels A_2, A_3, H_1, H_2, L_1, and L_2 refer to BzR subsites in accordance with Cook's pharmacophore model.[17] Molecular models of the ligands were obtained as described in the Experimental Section.

methylation dramatically lowers affinity (compare series **2** with series **1**). The only *N*(10)-methyl derivative with an appreciable affinity was **2c**, with a K_i value of 860 nM. These results are consistent with the role of a hydrogen bond donor postulated for the *N*(10)-H group at the BzR A_2 site (see pharmacophore model in Figure 1).Within series **1**, an aromatic ring at position 3 (**1c,e,f,i**−**m**) seemed to be necessary for a high affinity, since the 3-H- and 3-CH_3-substituted compounds **1a**,**b**, respectively, were devoid of any affinity. According to Cook's model,[17] this aryl ring might be involved in a hydrophobic interaction with the lipophilic site L_1 of the BzR (in Figure 1 the 3-phenyl group of **1c** matches the fused benzene ring E of pyridodiindoles **III** and the pendant phenyl ring of 2-arylpyrazoloquinolines **I**). Compared with the previously investigated *N*-benzylindolylglyoxylylamides unsubstituted at the indole 5 position (**IV**, R = H),[18] these more rigid 3-aryltriazinobenzimidazoles **V** exhibited a 2- or 3-fold improvement in terms of potency, in agreement with the rationale leading to their design.

It must be noted that, in compounds of series **1**, potency was highly sensitive to the type of substitution on the 3-phenyl ring. Insertion of a methyl or a chlorine, two substituents with opposite electronic effects, at the 4′ position of **1c** to give **1g** or **1d**, respectively, abolished affinity. These findings cannot be related to an excessive steric bulk of the 4′-substituent, as **1e**,**f**, which bear a similarly small-sized group at this position (methoxyl

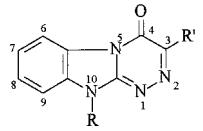
Table 2. Physical Properties of Triazinobenzimidazole Derivatives **1a**−**m** and **2c**−**e**,**g**,**h**,**j**,**k**



no.	R	R′	reaction procedure	yield (%)	recryst solv	mp (°C)	formula[a]
1a	H	H	1C	36	MeOH	288−290	$C_9H_6N_4O$
1b	H	CH_3	1C	30	DMF	>300	$C_{10}H_8N_4O$[b]
1c	H	C_6H_5	1A	53	DMF	>300	$C_{15}H_{10}N_4O$[c]
1d	H	C_6H_4-4-Cl	2	30	DMF	>300	$C_{15}H_9ClN_4O$
1e	H	C_6H_4-4-OCH_3	1A	39	DMF	>300	$C_{16}H_{12}N_4O_2$
1f	H	C_6H_4-4-OH	1B	37	DMF	>300	$C_{15}H_{10}N_4O_2$
1g	H	C_6H_4-4-CH_3	1B	46	DMF	>300	$C_{16}H_{12}N_4O$
1h	H	C_6H_3-3,4-$(OCH_3)_2$	1B	61	DMF	>300	$C_{17}H_{14}N_4O_3$
1i	H	C_6H_3-3,4-$(OH)_2$		91	DMF	>300	$C_{15}H_{10}N_4O_3$
1j	H	fur-2-yl	1B	35	EtOH	298−300	$C_{13}H_8N_4O_2$
1k	H	thien-2-yl	2	43	EtOH	>300	$C_{13}H_8N_4OS$
1l	H	5-methylthien-2-yl	2	37	DMF	>300	$C_{14}H_{10}N_4OS$
1m	H	thien-3-yl	1B	32	EtOH	>300	$C_{13}H_8N_4OS$
2c	CH_3	C_6H_5	1A	37	DMF	220−221	$C_{16}H_{12}N_4O$
2d	CH_3	C_6H_4-4-Cl	2	30	DMF	271−272	$C_{16}H_{11}ClN_4O$
2e	CH_3	C_6H_4-4-OCH_3	1B	35	DMF	241−242	$C_{17}H_{14}N_4O_2$
2g	CH_3	C_6H_4-4-CH_3	1B	36	EtOH	257−259	$C_{17}H_{14}N_4O$
2h	CH_3	C_6H_3-3,4-$(OCH_3)_2$	1B	92	DMF	220−221	$C_{18}H_{16}N_4O_3$
2j	CH_3	fur-2-yl	1B	31	DMF	294−295	$C_{14}H_{10}N_4O_2$
2k	CH_3	thien-2-yl	2	19	benzene	246−248	$C_{14}H_{10}N_4OS$

[a] Elemental analyses for C, H, N were within ±0.4% of the calculated values. [b] Lit. refs 49 and 50. [c] Lit. ref 21.

Table 3. Inhibition of [^{3}H]Ro 15-1788 Specific Binding to Bovine Brain Membranes by Triazinobenzimidazole Derivatives **1a**−**l** and **2c**−**e**,**j**,**k**



no.	R	R′	% inhibn[a] (10 μM)	K_i[b] (nM)	GABA ratio[c]	[^{35}S]TBPS binding with GABA[d] (% clonazepam)
1a	H	H	11	ND[e]		
1b	H	CH_3	26	ND		
1c	H	C_6H_5	97	53 ± 2	1.20	35 ± 5
1d	H	C_6H_4-4-Cl	34	ND		
1e	H	C_6H_4-4-OCH_3	89	44 ± 3	1.25	5 ± 3
1f	H	C_6H_4-4-OH	99	74 ± 5	0.87	15 ± 5
1g	H	C_6H_4-4-CH_3	56	ND		
1h	H	C_6H_3-3,4-$(OCH_3)_2$	17	ND		
1i	H	C_6H_3-3,4-$(OH)_2$	99	170 ± 8	1.20	
1j	H	fur-2-yl	93	56 ± 4	0.98	25 ± 4
1k	H	thien-2-yl	99	13 ± 3	0.97	28 ± 3
1l	H	5-methylthien-2-yl	83	115 ± 11	0.96	
1m	H	thien-3-yl	85	107 ± 10	1.20	
2c	CH_3	C_6H_5	92	860 ± 60	1.70	66 ± 7
2d	CH_3	C_6H_4-4-Cl	30	ND		
2e	CH_3	C_6H_4-4-OCH_3	53	ND		
2j	CH_3	fur-2-yl	68	ND		
2k	CH_3	thien-2-yl	63	ND		
Ro 15-1788				0.90 ± 0.05	0.90	15 ± 5
clonazepam				0.85 ± 0.02	1.97	100 ± 10

[a] Percentages of inhibition of specific [^{3}H]Ro 15-1788 binding at 10 μM compound concentration are means ± SEM of five determinations. [b] K_i values are means ± SEM of three determinations. [c] GABA ratio = (K_i without GABA)/(K_i with GABA). [d] The effects of the compounds at 0.5 μM on TBPS binding were normalized with respect to the corresponding action of clonazepam. The data represent the means ± standard error of three separate experiments. [e] Not determined.

or hydroxyl), exhibited K_i values of 44 and 74 nM, respectively. No less surprising was the fact that two substituents at the 3′ and 4′ positions of the phenyl ring can be associated with submicromolar affinity or lack of affinity, depending on whether they are two hydroxyls (**1i**) or methoxyls (**1h**), respectively.

The lack of affinity shown by compounds **1d**,**g**,**h** was unexpected on the basis of the alignment reported in Figure 1, where the 4′ position of triazinobenzimidazole **1c** matches the 2 position of pyridodiindole **III**. If this latter position bears a chlorine, a methyl, or a methoxyl, the affinity of the resulting pyridodiindole derivative does not change significantly.[29,30] Extending our comparisons to 2-arylpyrazoloquinolines **I**, it is well-known that hydrogen, chlorine, and methoxyl at the 4′ position of the pendant phenyl ring (spatially close to the 4′ position of **1c** in Figure 1) are reported to confer subnanomolar affinity,[6] while the effects on potency produced by a 4′-methyl unfortunately have not been disclosed in the literature, although this structure was included in two patents.[31,32]

The GABA ratio values for the most active compounds showed a trend of the efficacy profile of partial inverse agonists/antagonists for series **1** (values ranging between 0.87 and 1.25) and of a partial agonist for the 10-methyl derivative **2c**, with a value of 1.70.

Compounds **1c,e,f,j,k** and **2c** were also assayed with the [^{35}S]TBPS shift test,[33,34] which confirmed the efficacy profile predicted by the GABA ratio values, since compounds **1c,e,f,j,k** showed [^{35}S]TBPS shift values ranging between 5 and 35, similar to the value of 15 obtained for the antagonist Ro 15-1788, and compound **2c** showed a value of 66, intermediate between those of the full agonist clonazepam (fixed here at a value of 100) and Ro 15-1788. The agonist activity of the *N*(10)-methyl derivative **2c**, unable to form the hydrogen bond at the A_2 subsite (Figure 1), is not in contradiction with Cook's model,[17] which considers the interaction at the A_2 subsite a necessary requirement only for inverse agonist activity.

Table 4. Biological Activity of Selected Triazinobenzimidazole Derivatives

no.	anticonvulsant action: ED_{50} (mg/kg)[a]	proconvulsant action[b]	diazepam antagonism[c]
1c	no effect[d]	no effect[d]	no effect[d]
1e	no effect[d]	no effect[d]	no effect[d]
1k	no effect[d]	no effect[d]	no effect[d]
2c	60[e]	no effect[d]	no effect[d]
diazepam	0.4[e]		
Ro 15-1788	no effect[f]	no effect[f]	0.3[e]
β-CCM	no effect[f]	19.4[e]	0.02[e]

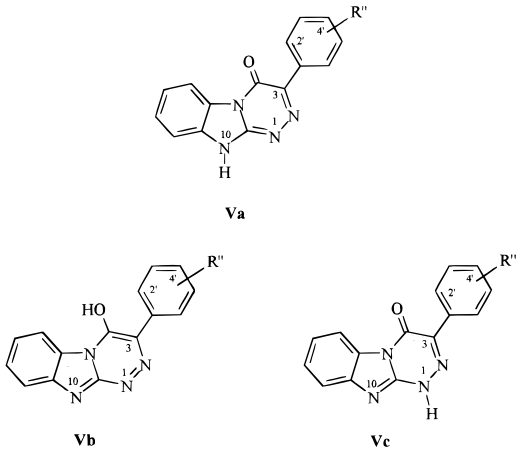
[a] Dose necessary to antagonize the convulsant action of PTZ (80 mg/kg, sc) in 50% of mice. [b] Dose necessary to induce convulsions in 50% of the mice that had previously been given a subconvulsant dose of PTZ (40 mg/kg, sc). [c] Dose necessary to antagonize the anticonvulsant effect of diazepam (2.5 mg/kg, ip) in mice that had been given a convulsant dose of PTZ (80 mg/kg, sc). [d] The highest concentration of the compounds tested was 250 mg/kg. [e] Values represent the means of at least three determinations (≤20% differences between experiments). [f] The highest dose administered of Ro 15-1788 and β-CCM (3-carbomethoxy-β-carboline[51]) was 100 and 30 mg/kg, respectively.

Pharmacological Studies. The pharmacological profile of compounds **1c,e,k** and **2c** was evaluated by in vivo assays carried out as described previously.[35] The results obtained (Table 4) showed no in vivo efficacy for compounds **1c,e,k**, which were neither anticonvulsant nor proconvulsant, nor did they antagonize the anti-PTZ action of diazepam. Their lack of in vivo efficacy might be due either to a poor penetration through the blood−brain barrier or to an unfavorable pharmacokinetics.

Compound **2c** showed an anticonvulsant activity with an ED_{50} of 60 mg/kg and a complete lack of diazepam

antagonism or proconvulsant action. For this compound, the in vivo activity was consistent with the in vitro results, as the GABA ratio of 1.70 and the TBPS shift of 66 were predictive of an in vitro partial agonist efficacy.

NMR- and Methylation Reaction-Based Studies of Tautomeric Equilibria. The puzzling SARs observed in the series of triazinobenzimidazoles **1** could not be rationalized in terms of substituent effects or by comparison with structures of classic BzR ligands (see molecular alignment in Figure 1). Therefore, NOE difference experiments were performed on **1c,d,g,h** in DMSO-d_6, to investigate tautomeric equilibria involving these compounds which could be potentially related to their binding affinities. Upon irradiation of the hydrogen of **1c** at δ 14.28, NOE enhancements were observed for H(9) at δ 7.67 and H(2′) at δ 8.07 of the fused benzene and pendant phenyl rings, respectively. The NOE effect observed for H(9) is consistent with its proximity to the N(10)-H of the imidazole moiety (structure **Va**), while the NOE enhancement observed for H(2′) can be attributed to the enolic tautomer **Vb** featuring a hydroxy group at the 4 position. A similar pattern of NOE effects was obtained from the 4′-methyl derivative **1g**.



Va **Vb** **Vc**

When the same experiments were performed on the 4′-chloro and 3′,4′-dimethoxy derivatives **1d**,**h**, none of the above NOE effects were detected, suggesting that these compounds exist only in the tautomeric form **Vc** featuring the N(1)-H on the triazinone nucleus.

The tautomeric forms of **1c,d**,**h** could explain the binding affinities observed, since the N(10)-H function is an essential pharmacophore element (see Figure 1). However, the tautomeric behavior exhibited by **1g** (devoid of affinity), which appeared to be identical to that of **1c** (highly potent), seems not to be in agreement with this assumption.

The lack of correlation between the binding affinities observed and the tautomeric equilibria which emerged from the NMR studies in DMSO solution prompted us to investigate this problem from another point of view, in an indirect, even if less rigorous, way. Taking into account that methylation products might have furnished useful information about the tautomer(s) prevalently present in solution, methylation reactions were carried out on compounds **1c,d,g,h** with methyl iodide in hot ethanol, in the presence of sodium ethylate (Scheme 2). Compound **1c** gave, in an approximately 1:2 ratio, both the 10-methylated product **2c** and the 1-methylated **7**, while compounds **1d,g,h** gave almost exclusively the 1-methylated products **8**−**10**, respectively. Though we are aware that the product of a chemical reaction depends not only on thermodynamic but also on kinetic factors, the results of these methylations seemed to suggest that for **1c** the forms mainly involved in the tautomeric equilibria are **Va** (active) and **Vc** (inactive), while for compounds **1d,g,h** the equilibrium seemed mostly to involve the inactive form **Vc**.

Although the conditions of the binding studies and those of the NMR and methylation experiments are quite different, the methylation reaction results are in agreement with our binding hypothesis postulating the N(10)-H function (tautomer **Va**) as an essential pharmacophore element and with the binding affinity data.

Conclusions

Sound SARs could not be deduced by simple qualitative analysis of the results obtained for this new class of BzR ligands. Insertion of an electron-donating group (OCH_3, OH, CH_3) at the 4′ position of the 3-phenyl ring of **1c** gave either highly potent products (**1e**,**f**) or a compound devoid of any affinity (**1g**). Also the insertion at the same 4′ position of a group with opposite electronic effects such as a chlorine (**1d**) completely abolished affinity.

When the NMR- and methylation-based studies of tautomeric equilibria are taken into consideration, contrasting results are again observed. While NMR studies revealed the same tautomeric equilibria (**Va**,**b**) for **1c** (active) and **1g** (inactive) and a different tautomer (**Vc**) for inactive **1d**,**h**, methylation reactions suggested the equilibrium form **Va** only for **1c** and the tautomer form **Vc** for **1d**,**g**,**h**.

Although at present we do not know the reasons for these conflicting results, the higher affinity shown by some 3-aryltriazinobenzimidazoles **V** with respect to the *N*-benzylindolylglyoxylylamides **IV** (R = H) confirmed the rationale of our design, which was that constraining the indolylglyoxylyl fragment would enhance the binding affinity.

Experimental Section

Chemistry. Melting points were determined using a Reichert Köfler hot-stage apparatus and are uncorrected. Infrared spectra were obtained on a PYE/UNICAM model PU 9561 spectrophotometer in Nujol mulls. Nuclear magnetic resonance spectra were recorded in DMSO-d_6 solutions with a Bruker AC 200 or a Varian CFT-20 spectrometer using TMS as an internal standard. Mass spectra were obtained on a Hewlett-Packard 5988 A spectrometer using a direct injection probe and an electron beam energy of 70 eV. Magnesium sulfate was always used as the drying agent. Evaporations were made in vacuo (rotating evaporator). Analytical TLC was carried out on Merck 0.2-mm precoated silica gel aluminum sheets (60 F-254). Silica gel 60 (230−400 mesh) was used for column chromatography. Elemental analyses were performed by our Analytical Laboratory and agreed with theoretical values to within ±0.4%.

3-Substituted [1,2,4]Triazino[4,3-*a*]benzimidazol-4(10*H*)-ones 1a−c,e−h,j,m and 2c,e,g,h,j (Procedure 1). A solution of 2-hydrazinobenzimidazole **(3)**[19] or 2-hydrazino-1-methylbenzimidazole **(4)**[19] (4 mmol) and the appropriate glyoxylic acid[36−40] (4.4 mmol) in 10 mL of ethanol was refluxed for 2 h. After cooling, the precipitate which formed was collected to give the substituted (benzimidazol-2-ylhydrazono)acetic acids

5a−c,e−h,j,m and **6c,e,g,h,j**, which were purified by suspension in hot methanol (Table 1, Supporting Information). They can be cyclized by means of the following methods.

Method A. The acid derivatives **5c,e** and **6c** (2 mmol) were heated at a temperature of 30−40 °C above their melting points for 10 min.

Method B. A suspension of the acid derivatives **5f−h,j,m** and **6e,g,h,j** (2 mmol) in 5 mL of absolute ethanol, at 0 °C, was saturated with anhydrous HCl and then refluxed for 4−7 h, monitoring the reaction by TLC analysis. The reaction mixture was evaporated to dryness and the solid residue was treated with saturated sodium hydrogen carbonate aqueous solution.

Method C. A suspension of the acid derivatives **5a,b** (2 mmol) in 20 mL of glacial acetic acid was refluxed for 1 h. The solution obtained was evaporated to dryness and the oily residue was filtered on a silica gel chromatographic column (eluting system: toluene:chloroform = 1:1).

All the crude solid products obtained were purified by recrystallization from the appropriate solvent (Table 2, Supporting Information).

3-Substituted [1,2,4]Triazino[4,3-*a*]benzimidazol-4(10*H*)-ones 1d,k,l and 2d,k (Procedure 2). A solution of **3** or **4** (4 mmol) and the appropriate glyoxylic acid ethyl ester[39,41,42] (4.4 mmol) in 10 mL of absolute ethanol was refluxed for 2−11 h (TLC analysis). After cooling, the precipitate which formed was collected and purified by recrystallization from the appropriate solvent (Table 2, Supporting Information).

3-(3′,4′-Dihydroxyphenyl)-[1,2,4]triazino[4,3-*a*]benzimidazol-4(10*H*)-one (1i). A solution of boron tribromide (6 mmol) in 1 mL of anhydrous dichloromethane was added dropwise, at −10 °C, under stirring and in a nitrogen atmosphere, to a suspension of the dimethoxy derivative **1h** (0.9 mmol) in 8 mL of the same solvent. Stirring was continued for 30 min at −10 °C and for 1 h at room temperature. The reaction mixture was carefully added dropwise to 5 mL of methanol at −10 °C. The suspension obtained was evaporated to dryness and the solid residue was treated with saturated sodium hydrogencarbonate aqueous solution. The crude product **1i** was collected and purified by recrystallization (Table 2, Supporting Information).

Reaction of 3-Phenyl-[1,2,4]triazino[4,3-*a*]benzimidazol-4(10*H*)-one (1c) with Methyl Iodide: 3-Phenyl-1-methyl-[1,2,4]triazino[4,3-*a*]benzimidazol-4(1*H*)-one (7) and 3-Phenyl-10-methyl-[1,2,4]triazino[4,3-*a*]benzimidazol-4(10*H*)-one (2c). A solution of methyl iodide (0.4 mL, 7 mmol), sodium ethoxide (0.16 g, 7 mmol of sodium in 15 mL of absolute ethanol), and **1c** (0.35 g, 1.4 mmol) was refluxed for 12 h. After cooling, the precipitate which formed was collected and purified by recrystallization from DMF to give 0.20 g (yield 52%) of pure **7**, mp 196−197 °C. Anal. Calcd for $C_{16}H_{12}N_4O$ (Supporting Information).

The reaction mother liquor was evaporated to dryness and the residue obtained was suspended in water and neutralized with dilute hydrochloric acid to give crude **2c**, which was recrystallized from DMF (0.110 g, yield 29%).

Reaction of 3-(4′-Chlorophenyl)-[1,2,4]triazino[4,3-*a*]-benzimidazol-4(10*H*)-one (1d), 3-(4′-Methylphenyl)-[1,2,4]-triazino[4,3-*a*]benzimidazol-4(10*H*)-one (1g), and 3-(3′,4′-Dimethoxyphenyl)-[1,2,4]triazino[4,3-*a*]benzimidazol-4(10*H*)-one (1h) with Methyl Iodide: 3-(4′-Chlorophenyl)-1-methyl-[1,2,4]triazino[4,3-*a*]benzimidazol-4(1*H*)-one (8), 3-(4′-Methylphenyl)-1-methyl-[1,2,4]triazino[4,3-*a*]benzimidazol-4(1*H*)-one (9), and 3-(3′,4′-Dimethoxyphenyl)-1-methyl-[1,2,4]triazino[4,3-*a*]benzimidazol-4(1*H*)-one (10). A solution of methyl iodide (0.4 mL, 7 mmol), sodium ethoxide (0.16 g, 7 mmol of sodium in 15 mL of absolute ethanol), and **1d**, **1g,** or **1h** (1.4 mmol) was refluxed for 5 h. After cooling, the precipitate which formed was collected and purified by recrystallization from DMF to give pure **8**, **9,** or **10**, respectively. **8**: yield 81%; mp 194−195 °C. Anal. Calcd for $C_{16}H_{11}N_4ClO$ (Supporting Information). **9**: yield 79%; mp 186−188 °C. Anal. Calcd for $C_{17}H_{14}N_4O$ (Supporting Information). **10**: yield 81%; mp 188−190 °C. Anal. Calcd for $C_{18}H_{16}N_4O_3$ (Supporting Information).

From the mother liquor it was not possible to isolate the 10-methyl isomer **2d**, **2g,** or **2h**, respectively, of which only traces were present (TLC analysis).

Binding Studies. [^{3}H]Ro 15-1788 (specific activity 83.2 Ci/mmol) and [^{35}S]TBPS (specific activity 80 Ci/mmol) were obtained from DuPont de Nemours, New England Nuclear Division (Dreieichenhaim, Germany). All other chemicals were of reagent grade and obtained from commercial suppliers.

Bovine cerebral cortex membranes were prepared in accordance with ref 43. The membrane preparations were subjected to a freeze−thaw cycle, washed by suspension, centrifuged in 50 mM tris-citrate buffer pH 7.4 (T1), and then used in the binding assay. Protein concentration was assayed by the method of Lowry et al.[44]

[^{3}H]Ro 15-1788 and [^{35}S]TBPS binding studies were performed as previously reported.[35]

In Vivo Studies. Proconvulsant, anticonvulsant, and diazepam antagonism actions were performed as previously reported.[35]

NMR-Based Studies of Tautomeric Equilibria. Samples were prepared by dissolving compounds **1c,d,g,h** in DMSO-d_6. ^{1}H NMR spectra and NOE difference experiments were recorded on a Bruker AMX-500 spectrometer, at 300 K. Signals were referenced to the residue solvent signal at 2.5 ppm.

Molecular Modeling. Molecular modeling was performed using the software package SYBYL[45] running on a Silicon Graphics R8000 Indigo 2 workstation. Models of the 4′-chlorophenylpyrazoloquinoline derivative of type **I** and the 2-chloropyridodiindole derivative of type **III** correspond to crystal structures retrieved from the October 1995 release (5.10 version for UNIX platforms) of the Cambridge Structural Database[46] (codes COVLOO and JOJHIZ, respectively). The model of triazinobenzimidazole **1c** was constructed using the SYBYL fragment library and geometry-optimizing the trial conformation by the semiempirical quantum-mechanics AM1 method[47] available within the MOPAC program.[48] MOPAC was run under default settings, using the keyword "MMOK". Superimpositions of the molecular models were performed as described in previous works.[18,35]

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Supporting Information Available: Tables containing the IR, ^{1}H NMR, and MS spectral data of compounds **5a−c,e−h,j,m** and **6c,e,g,h,j** (Table 1), compounds **1a−l** and **2c−e,g,h,j,k** (Table 2), and compounds **7−10** (Table 3). This material is available free of charge via the Internet at http://pubs.acs.org.

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