Eur J Med Chem (1996) 31, 259–272

Benzodiazepine receptor ligands. Synthesis and pharmacological evaluation of 3-, 7- and 8-substituted [5,1-*c*][1,2,4]benzotriazines and 5-oxide derivatives. Part I

G Guerrini[1], A Costanzo[1]*, F Bruni[1], S Selleri[1], L Casilli[1], L Giusti[2], C Martini[2], A Lucacchini[2], P Malmberg Aiello[3], A Ipponi[3]

[1]*Dipartimento di Scienze Farmaceutiche, Università degli Studi di Firenze, Via Gino Capponi 9, 50121 Florence;*
[2]*Istituto Policattedra di Discipline Biologiche, Università delgi Studi di Pisa, Via Bonanno 6, 56100 Pisa;*
[3]*Dipartimento di Farmacologia Preclinica e Clinica Aiazzi-Mancini, Università degli studi di Firenze, Viale Morgagni 11, 50121 Florence, Italy*

(Received 3 October 1995; accepted 27 November 1995)

Summary — A new series of 3-, 7- and 8-substituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides and a series of pyrazolo[5,1-*c*]-[1,2,4]benzotriazines were synthesized and their benzodiazepine receptor affinities were evaluated in vitro. A study of structure–affinity relationships within the series is briefly discussed, considering the role of various substituents at the 3-, 7- and 8-positions and the role of N^5-oxide. Compounds **1b**, **1c**, **1cR**, **4c**, **4cR**, **9d**, **12d** and **12dR** were evaluated in vivo for their anticonvulsant effects.

pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide / pyrazolo[5,1-*c*][1,2,4]benzotriazine / benzodiazepine receptor / anticonvulsant activity / receptor binding

Introduction

Benzodiazepines (BZs) are the drugs of choice in the pharmacotherapy of anxiety and related emotional disorders, insomnia and epilepsies; they are also used as myorelaxants and as premedication in anesthesia.

The mechanism of action of BZs was discovered in 1974 and since then the benzodiazepine receptor (BZR) has been widely investigated. The BZR is part of the $GABA_A$/chloride channel supramolecular complex (GABA = γ-aminobutyric acid) and is composed of α, β and γ subunits in various combinations [1].

Bz ligands act as allosteric modulators of the $GABA_A$–receptor complex and elicit full agonist, inverse agonist or antagonist properties and some intermediate points within that spectrum. These ligands can enhance (agonists) or reduce (inverse agonists) the GABA-induced chlorine ion flux, or have no influence on the $GABA_A$-gated chlorine current (antagonists). Whilst the agonists have non-specific central nervous system (CNS) depressant effects, the partial agonists are particularly interesting because of the expected lower incidence of undesired side effects [2], eg, sedation, tolerance and addition.

In addition to the classical 1,4-BZs several other structurally distinct families of compounds show affinity for the BZR complex. These non-BZ ligands include a 5- or 6-membered azole ring condensed with an aromatic or heteroaromatic moiety. The resulting molecule has an approximately planar shape.

Several papers [3–9] have reported the molecular requirements for the agonist, antagonist and inverse agonist activity. From comparison of various ligands belonging to chemically different classes, a pharmacophoric model for the agonist and inverse agonist activity has been proposed. These findings can aid the molecular design and synthesis of new more anxioselective compounds.

Continuing our investigations on tricyclic heterocycles containing a pyrazole moiety with potential CNS activity, the synthesis and evaluation of the BZR affinity of a series of pyrazolo[5,1-*c*][1,2,4]-benzotriazine 5-oxides are reported here. These compounds are related to the pyrazolo[4,3-*c*]quinolin-3-ones of the CGS [10] series by their similar size and shape, and should possess the requirements for interaction with BZR.

Our previous paper [11] reported the synthesis of the parent compound pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide **1b** and some of its 3-, 7- and 8-substituted derivatives.

*Correspondence and reprints

For the purpose of systematically evaluating the effects of different substituents on receptor affinity and pharmacological activity, a new series of 7- and 8-derivatives were synthesized, with a variety of different lipophilic and electronic natures and amounts of steric hindrance of the substituent. Moreover, a series of pyrazolo[5,1-*c*][1,2,4]benzotriazines (**R** series) were prepared by reduction of 5-oxides, in order to evaluate the role of the *N*-oxide group on receptor affinity.

Chemistry

The new compounds described here are listed in table I, together with some of the previously reported 5-oxide derivatives [11], which are useful for the chemical and biological discussion.

A procedure described in a preceding paper [11] was used to obtain the pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides from suitable 1-(2-nitrophenyl)-5-aminopyrazoles, which cyclized to the triazine system in 10% sodium hydroxide at room temperature. The ethyl 1-(2-nitro-4- or 5-methylphenyl)-5-aminopyrazole-4-carboxylates **2** and **3** and their 4-unsubstituted aminopyrazoles **2'** and **3'** were prepared first [11–13]. Under basic conditions the aminopyrazoles **2**, **2'** and **3'** cyclized to give the expected 7-methyl- and 8-methylpyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides **2a**, **b**, **3b**, respectively, while the aminopyrazole **3** gave only an intractable tar.

Using a previously described procedure [11] the acid **3a** was indirectly obtained via 1-(2-nitro-5-methylphenyl)-5-aminopyrazol-4-carboxyamide **3"**, which cyclized to give 3-carbamoyl-8-methylpyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide **3e"**. The latter afforded the desired acid **3a** when treated with sodium nitrite and sulphuric acid.

In order to synthesize the pyrazolo[5,1-*c*][1,2,4]-benzotriazine 5-oxides with different substituents at the 8-position, we exploited the aromatic nucleophilic substitution of chlorine in the 8-chloro derivatives **4a** and **4b** [11]. Thus, compounds **4a** and **4b** were reacted with a series of nucleophilic agents to give the 8-derivatives **6b**, **7a**, **8b**, **9a**, **b**, **10a**, **11a** and **17b**.

When the acid **4a** was reacted with sodium thiomethoxide in refluxing ethanol, the 8-methylthio derivative **9a** and its oxidized sulphur derivatives **10a** and **11a** were obtained. These were separated by column chromatography and identified by IR and ^{1}H-NMR spectra. The same reaction performed under nitrogen flow afforded compound **9a** only.

The treatment of **1b** with conc nitric acid/sulphuric acid afforded a mixture of 3-nitro and 3,7-dinitro derivatives, **1e** and **14e**, respectively, which were separated by recrystallization. Compound **14e** was obtained as the only product, using fuming nitric acid.

The ethyl esters were prepared by treatment of **2a**, **3a**, **7a** and **9a** with diethylcarbonate/sulphuric acid or ethyl alcohol/sulphuric acid. The 3-bromo derivatives were obtained by treatment of **2–4b**, **6b**, **9b**, **16bR** and **19bR** with a solution of bromine in chloroform.

In order to extend the study of structure–activity relationships between 7- and 8-substituted 5-oxides, we decided to synthesize 3-bromopyrazolo[5,1-*c*]-[1,2,4]benzotriazine 5-oxides bearing an amino group at the 7-position and an amino and/or a nitro group at the 8-position.

The synthesis of the desired compounds is shown in scheme 1.

The 7-amino and 8-aminopyrazolo[5,1-*c*][1,2,4]-benzotriazines, **15bR** and **18bR** were found to be the key reagents to obtain the target compounds. Treatment of the 7-nitropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide **14b** [11] with tin/conc hydrochloric acid afforded compound **15bR** as the only product and in good yield (treatment with zinc/acetic acid yielded a complex mixture of reduced products).

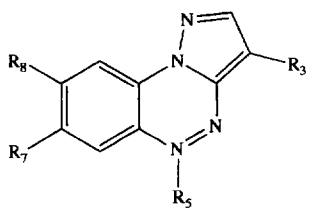
The synthesis of the 8-amino derivative **18bR** was more difficult than its isomer. We again used the nucleophilic aromatic substitution of chlorine at the 8-position of benzotriazine moiety.

The reaction of **4b** [11] with hydrazine hydrate in tetramethylenoxide at 50 °C afforded the 8-hydrazinyl derivative **17b**, which by reduction with zinc/acetic acid yielded the desired 8-aminopyrazolo[5,1-*c*][1,2,4]benzotriazine **18bR**. An attempt to obtain **18bR** directly by reaction of **4b** [11] with sodium amide/liquid ammonia was unsuccessful.

Since the direct oxidation of 7- and 8-amino derivatives **15bR**, **16bR**, **18bR** and **19bR** did not give the target compounds, it was necessary to bypass this obstacle to synthesis. The protection of the amino group by acetylation, and the subsequent bromination of the 3-position, yielded the 7- and 8-acetylamino-3-bromo derivatives, **16dR** and **19dR**, which, upon oxidation, gave the desired 5-oxide derivatives, **16d** and **19d** respectively.

Oxidation of 7-aminopyrazolo[5,1-*c*][1,2,4]benzotriazine **15bR** by treatment with various oxidizing agents (oxone [14], acetic anhydride/hydrogen peroxide) actually gave 7-nitropyrazolo[5,1-*c*][1,2,4]benzotriazine **14bR** selectively, and the subsequent oxidation at 5-position occurred very slowly. Moreover, oxidation of the 7-acetylamino derivative **16bR** led only to a mixture of two inseparable *N*-oxides in different amounts. The structures of the two isomers were assigned by comparison of the ^{1}H-NMR spectral data of the mixture with those of the 7-substituted 5-oxides and with the corresponding reduced compounds. In the ^{1}H-NMR spectra of pyrazolo[1,5-*c*][1,2,4]benzo-

Table I. Chemical data for pyrazolo[5,1-*c*][1,2,4]benzotriazines and oxides.



R_3 =	
COOH	**a**
H	**b**
COOR	**c**
Br	**d**
NO_2	**e**
CN	**e'**
$CONH_2$	**e"**

Compound[a]	R_7	R_8	*Formula (MW)*	*Mp (°C) (recrystallization solvent)*	*Method of reduction*[b]
1e	H	H	$C_9H_5N_5O_3$ (231.17)	261–262 (EtOH/AcOH)	
1bR	H	H	$C_9H_6N_4$ (170.16)	189–190 (H_2O)	A/B
1cR	H	H	$C_{11}H_8N_4O_2$ (228.20)	177–178 (EtOH)	A
1dR	H	H	$C_9H_5N_4Br$ (248.97)	199–200 (EtOH/H_2O)	A
1eR	H	H	$C_9H_5N_5O_2$ (215.17)	255–256 (EtOH)	A
2a	CH_3	H	$C_{11}H_8N_4O_3$ (244.22)	250–251 (EtOH)	
2b	CH_3	H	$C_{10}H_8N_4O$ (200.22)	196–198 (EtOH)	
2c	CH_3	H	$C_{13}H_{12}N_4O_3$ (272.27)	180–181 (EtOH)	
2d	CH_3	H	$C_{10}H_7N_4OBr$ (278.97)	234–235 (EtOH)	
2bR	CH_3	H	$C_{10}H_8N_4$ (184.22)	166–167 (EtOH/H_2O)	A
3a	H	CH_3	$C_{11}H_8N_4O_3$ (244.22)	> 300 (H_2O/AcOH)	
3b	H	CH_3	$C_{10}H_8N_4O$ (200.22)	167–168 (EtOH)	
3c	H	CH_3	$C_{13}H_{12}N_4O_3$ (272.27)	195–196 (EtOH)	
3d	H	CH_3	$C_{10}H_7N_4OBr$ (278.97)	215–216 (EtOH)	
3e"	H	CH_3	$C_{11}H_9N_5O_2$ (243.23)	278–279 (EtOH/AcOH)	
3bR	H	CH_3	$C_{10}H_8N_4$ (184.22)	132–133 (EtOH)	A
4d	H	Cl	$C_9H_4N_4OClBr$ (299.85)	243–244 (EtOH)	
4bR	H	Cl	$C_9H_5N_4Cl$ (204.61)	169–170 (EtOH)	A
4cR	H	Cl	$C_{12}H_9N_4O_2Cl$ (276.63)	> 300 (EtOH)	A
5bR	Cl	H	$C_9H_5N_4Cl$ (204.61)	155–156 (EtOH)	A
6b	H	$OCH_2CH_2OCH_2CH_3$[c]	$C_{13}H_{14}N_4O_3$ (274.28)	85–86 (*i*-Propylether)	
6d	H	$OCH_2CH_2OCH_2CH_3$[c]	$C_{13}H_{13}N_4O_3Br$ (353.08)	135–136 (*i*-Propanol)	
7a	H	Morpholine	$C_{14}H_{13}N_5O_4$ (315.29)	278–279 (EtOH)	
7c	H	Morpholine	$C_{16}H_{17}N_5O_4$ (343.36)	256–257 (MeOH)	
8b	H	NEt_2	$C_{13}H_{15}N_5O$ (257.24)	192–193 (Column)[d]	
8c	H	NEt_2	$C_{16}H_{19}N_5O_3$ (329.23)	182–183 (Ethylacetate)	
8cR	H	NEt_2	$C_{16}H_{19}N_5O_2$ (313.40)	123–124 (EtOH)	A
9a	H	SCH_3	$C_{11}H_8N_4O_3S$ (276.24)	272–273 (EtOH)	
9b	H	SCH_3	$C_{10}H_8N_4OS$ (232.22)	217–218 (EtOH)	
9c	H	SCH_3	$C_{13}H_{12}N_4O_3S$ (304.24)	202–203 (EtOH)	
9d	H	SCH_3	$C_{10}H_7N_4OSBr$ (311.02)	226–227 (EtOH)	
9dR	H	SCH_3	$C_{10}H_7N_4SBr$ (295.02)	176–177 (*i*-Propanol)	A
10a	H	$SOCH_3$	$C_{11}H_8N_4O_4S$ (292.24)	276–277 (Column)[e]	
11a	H	SO_2CH_3	$C_{11}H_8N_4O_5S$ (308.24)	291–293 (Column)[e]	
12cR	H	OEt	$C_{14}H_{14}N_4O_3$ (286.36)	175–176 (EtOH)	A
12dR	H	OEt	$C_{11}H_9N_4OBr$ (293.03)	179–180 (*i*-Propanol)	A

Table I. *(Continued.)*

Compound[a]	R_7	R_8	*Formula (MW)*	*Mp (°C) (recrystallization solvent)*	*Method of reduction*[b]
14e	NO_2	H	$C_9H_4N_6O_5$ (276.16)	281–282 (Acetic acid)	
14bR	NO_2	H	$C_9H_5N_5O_2$ (215.17)	240–241 (*i*-Propanol)	A
14cR	NO_2	H	$C_{12}H_9N_5O_4$ (287.27)	> 300 (*i*-Propanol)	A
15d	NH_2	H	$C_9H_6N_5OBr$ (279.98)	255–256 (Methoxyethanol)	
15bR	NH_2	H	$C_9H_7N_5$ (185.18)	214–215 (EtOH)	C
15dR	NH_2	H	$C_9H_5N_5Br$ (263.98)	234–235 (EtOH/H_2O)	
16d	NHAc	H	$C_{11}H_8N_5O_2$ Br(322.03)	> 300 (Methoxyethanol)	
16bR	NHAc	H	$C_{11}H_9N_5O$ (227.23)	264–265 (EtOH)	
16dR	NHAc	H	$C_{11}H_8N_5OBr$ (306.03)	267–268 (EtOH)	
17b	H	$NHNH_2$	$C_9H_8N_6O$ (216.19)	230–231 (EtOH/H_2O)	
18d	H	NH_2	$C_9H_6N_5OBr$ (279.98)	> 300 (Methoxyethanol)	
18bR	H	NH_2	$C_9H_7N_5$ (185.18)	284–285 (EtOH/H_2O)	B
18dR	H	NH_2	$C_9H_6N_5Br$ (263.98)	> 300 (EtOH/H_2O)	
19d	H	NHAc	$C_{11}H_8N_5O_2Br$ (322.03)	> 300 (EtOH/H_2O)	
19bR	H	NHAc	$C_{11}H_9N_5O$ (227.23)	298–299 (EtOH)	
19dR	H	NHAc	$C_{11}H_8N_5OBr$ (306.03)	278–279 (EtOH/H_2O)	

[a]All compounds: **a** R_3 = COOH; **b** R_3 = H; **c** R_3 = COOR (R = Me for compounds **1**, R = Et for compounds **2**, **3** and **7–9**); **d** R_3 = Br; **e** R_3 = NO_2; **e'** R_3 = CN; **e"** R_3 = $CONH_2$. See reference [11] for compounds **1a–d**, **1e'** and **1e"** (R_7 = R_8 = H), **4a–c**, **4e'** and **4e"** (R_7 = H, R_8 = Cl), **5a–c** (R_7 = Cl, R_8 = H), **12a–d** (R_7 = H, R_8 = OEt), **13a–c** (R_7 = H, R_8 = OH), **14a–d** (R_7 = NO_2, R_8 = H). [b]Method of reduction: A = triethylphosphite; B = zinc/acetic acid; C = tin/conc hydrochloric acid. [c]$OCH_2CH_2OCH_2CH_3$ = ethylethylenedioxo. [d]Toluene/ethylacetate 8:2. [e]Toluene/ethylacetate/acetic acid 8:2:1.

triazine 5-oxides and their reduced derivatives, it appears that the H-3 proton is more influenced by the presence of an oxygen atom than the other protons (H-2, H-6, H-8 and H-9). For example, the H-3 proton of 7-chloropyrazolo[5,1,-*c*][1,2,4]benzotriazine 5-oxide [11] appears at 6.81 ppm, while the same proton is shifted to 7.45 ppm in its reduced derivative. Thus, the structure of 5-oxide **16b** was assigned to the compound present in greater amounts in the mixture. The chemical shifts of all its protons agree with chemical shifts of protons of the directly obtained 7-substituted 5-oxides; the chemical shift of H-3, which appears at 6.90 ppm as a doublet ($J_{H3\text{-}H2}$ = 2.20 Hz), is diagnostic.

The structure of a 4-oxide **16b'** was assigned to the other compound of the mixture as the most probable. In the ^{1}H-NMR spectra of **16b'** the doublet of H-3 is shifted (7.28 ppm, $J_{H3\text{-}H2}$ = 2.19 Hz with respect to 6.90 ppm of 5-oxide **16b**) and the doublet ($J_{H6\text{-}H8}$ = 2.00 Hz) of H-6 is strongly influenced (7.82 ppm with respect to 8.84 ppm of the 5-oxide **16b**). Since the other protons (H-2, H-8 and H-9) have minor shifts, oxidation at the 1-position can be ruled out.

Finally, treatment of **16dR** with *meta*-chloroperbenzoic acid afforded the target compound **16d**, showing that bromine is required in the 3-position to selectively orientate the oxidation on N^5.

As regards the other isomers 8-aminopyrazolo[5,1-*c*][1,2,4]benzotriazine **18bR** and 8-acetylaminopyrazolo[5,1-*c*][1,2,4]benzotriazine **19bR**, the oxidation with the same oxidizing agents always gave a mixture of 5- and 4-oxides. (Compounds **18b'** and **19b'** were inseparable and were identified by ^{1}H-NMR as previously stated.) Acylation at the 8-amino group and bromination at the 3-position was again necessary before oxidation with hydrogen peroxide/acetic acid/acetic anhydride. The 8-nitro derivative was never obtained from **18bR**.

Compounds **16dR**, **16d**, **19dR** and **19d** were hydrolyzed to the desired compounds **15dR**, **15d**, **18dR** and **18d** by treatment with diluted hydrochloric acid. Unfortunately, oxidation of 8-amino compounds **18b**,

18bR, **18d** and **18dR** never gave the desired 8-nitro derivatives, thus confirming the higher reactivity of the 7-position towards oxidation.

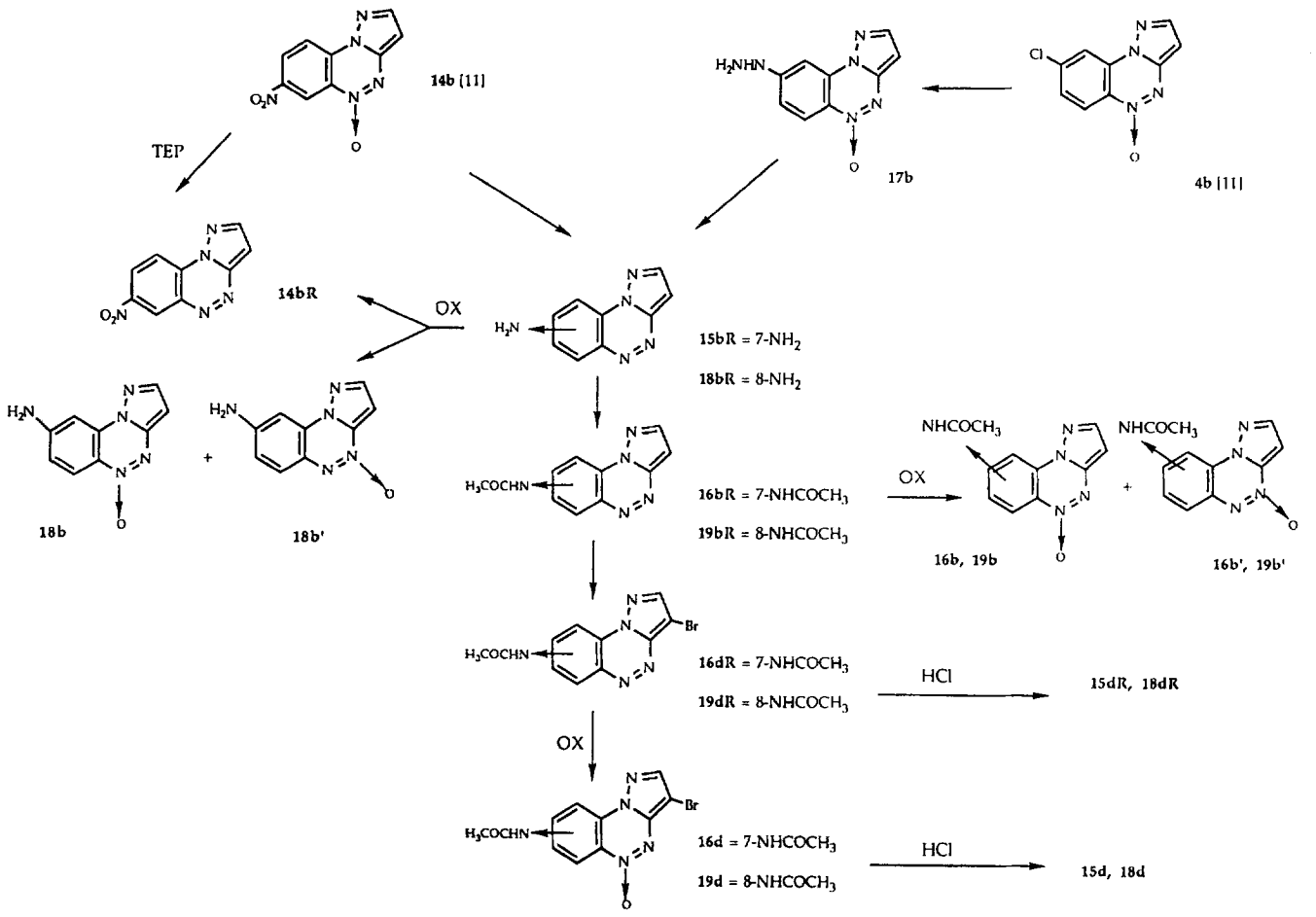
Finally, some 5-oxides (**1–5b**, **14b**, **1c**, **4c**, **8c**, **12c**, **14c**, **1d**, **9d**, **12d**, **1e**) were treated with triethylphosphite, to selectively reduce the *N*-oxide group. All the pyrazolo[1,5-*c*][1,2,4]benzotriazines prepared are listed in table I.

Biological and pharmacological results and discussion

Binding studies

The ability of pyrazolo[5,1-*c*][1,2,4]benzotriazines and their 5-oxides to interact with the benzodiazepine receptor site was evaluated by their ability to displace [^{3}H]flunitrazepam (FNZ) (at 0.2 nM, K_d = 1.8 nM) from its specific binding in bovine brain membranes. The compounds were tested at a concentration of 10 μM in the presence of 2% ethanol (or DMSO) to dissolve the pyrazolo[5,1-*c*][1,2,4]benzotriazines and 5-oxides. IC_{50} values were determined for the most active compounds and K_i values were then derived. GABA ratio values were evaluated as an in vitro indicator of the agonist, inverse agonist or antagonist properties, according to different authors [15–17]. Binding data for all compounds are shown in table II.

Compounds **3d**, **4c**, **9c**, **9d**, **12c** and **12d** possess good affinity for BZR (K_i range 35–93 nM) with an efficacy trend typical of partial agonists and agonists (GABA from 1.0 to 1.9). By comparison of the tested compounds with the parent compound **1b**, which shows no affinity for BZR, the following observations arise.



Scheme 1.

Table II. BZR ligand affinity of pyrazolo[5,1-*c*][1,2,4]benzotriazines and 5-oxides.

Compound	R_3	R_7	R_8	*% Inhibition*[a]	K_i *(nM)*[b]	*GABA ratio*[c]
1a	COOH	H	H	2	–	–
1b	H	H	H	0	–	–
1c	COOMe	H	H	93 ± 9	583	1.97
1d	Br	H	H	62 ± 5.9	–	–
1e	NO_2	H	H	0	–	–
1e'	CN	H	H	20	–	–
2c	COOEt	Me	H	10	–	–
2d	Br	Me	H	61.5	–	–
3c	COOEt	H	Me	100	106	2.67
3d	Br	H	Me	99.5	53.2	1.29
4b	H	H	Cl	64	–	–
4c	COOEt	H	Cl	99.8 ± 5	35	1.91
4d	Br	H	Cl	98	120	1.31
4e'	CN	H	Cl	96 ± 1	342	1.37
4e"	$CONH_2$	H	Cl	27	–	–
5b	H	Cl	H	0	–	–
5c	COOEt	Cl	H	12 ± 3	–	–
6b	H	H	$OCH_2CH_2OCH_2CH_3$[d]	45 ± 4.7	–	–
6d	Br	H	$OCH_2CH_2OCH_2CH_3$[d]	94 ± 6	145.5	2.05
7c	COOEt	H	Morpholine	48.5	–	–
8b	H	H	NEt_2	30 ± 6	–	–
8c	COOEt	H	NEt_2	85 ± 7.9	660	1.73
9b	H	H	SMe	55	–	–
9c	COOEt	H	SMe	99.7 ± 9	77	1.23
9d[e]	Br	H	SMe	93.6 ± 5.4	93.6	1.09
12b	H	H	OEt	38	–	–
12c[e]	COOEt	H	OEt	91.6 ± 8.5	81.8	1.61
12d	Br	H	OEt	98 ± 8.5	36.9	1.14
13c	COOEt	H	OH	93 ±8	227.7	2.0
14b	H	NO_2	H	2 ± 0.5	–	–
14c	COOEt	NO_2	H	19.7	–	–
14d	Br	NO_2	H	13 ± 1.5	–	–
14e	NO_2	NO_2	H	0	–	–
15d	Br	NH_2	H	90	539.4	1.73
16d	Br	NHAc	H	24.15	–	–
18d	Br	H	NH_2	49.5	–	–
19d	Br	H	NHAc	42	–	–
1bR	H	H	H	9	–	–
1cR	COOMe	H	H	78 ± 8	2130	1.25
4cR	COOEt	H	Cl	93.5	281.3 ± 25	1.7
8cR	COOEt	H	NEt_2	42	–	–
9dR	Br	H	SMe	93	104.6 ± 7	0.8
12cR	COOEt	H	OEt	95.5	192,5 ± 16	1.7
12dR	Br	H	OEt	90.4	477.5 ± 38	1.8
14cR	COOEt	NO_2	H	4.9	–	–
15dR	Br	NH_2	H	67.8	–	–
16dR	Br	NHAc	H	0	–	–
18dR	Br	H	NH_2	75.5	–	–
19dR	Br	H	NHAc	79.4	3578.6	1.86
DAZ					10 ± 1.1	1.5

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

Pyrazole ring 3-substitutions
A substitution at the 3-position with either a ethoxycarbonyl group or bromine is required in order to obtain compounds that bind to the BZR. This suggestion is supported by the lack of receptor affinity of **1b**, **1a**, **1e** and **1e'**, which bear a hydrogen atom or hydrophilic group or electron-withdrawing group at the 3-position. Moreover, the 3-ethoxycarbonyl derivatives show an efficacy trend towards full agonist (eg, **3c** with respect to **3d** and **4c** with respect to **4d**).

Benzotriazine moiety with 7- and 8-substitutions
As indicated in table II, introduction of chlorine or another small lipophilic group (such as methylthio-, ethoxy-, methyl-) at the 8-position of the benzotriazine moiety in the parent compound **1b** appears necessary for binding to BZR. Furthermore, the concomitant presence of a suitable substitution at the 3-position seems to be very important for enhancing the receptor affinity (**3d**, **4c**, **9c**, **9d**, **12c** and **12d**). Moreover, the replacement of the chlorine with a bulky lipophilic substituent like a long etheric chain (**6d**), a diethylamino group (**8c**) or a morpholine ring (**7c**) reduces or eliminates the receptor affinity even though there is a suitable substitution at the 3-position. The position of the lipophilic substituent on the benzotriazine moiety was found to be extremely important; the 7-derivatives exhibit a reduced or insignificant receptor affinity (**2d**, **2c** and **5c**). Thus, the displacement of the substituent from the 8-position to the 7-position is unfavourable.

When the 8-position is replaced by an amino group (**18d**) or by another hydrophilic substituent, a decrease in receptor affinity was observed. In contrast with the general trend of the 7-substituted compounds, only the 7-amino derivative **15d** shows affinity for BZR.

In general the BZR affinity of 5-oxides is better than the reduced derivatives.

Pharmacophore model
From the various proposed models [3–5, 9], common features of the agonist pharmacophore for BZR binding have emerged. This pharmacophore model consists of: two hydrogen bond donor sites, H_1 and H_2; three lipophilic regions L_1, L_2 and L_3, whose occupation is required for a full agonist activity, whereas the occupation of L_1 and L_2 regions gives partial agonist profile [18]; and two regions of repulsive steric interaction called S_1 and S_2, which reduce receptor–ligand affinity.

On the basis of this receptor map, from a qualitative point of view, it may be that our compounds interact at the receptor site with the N-1 and N-4 by means of a hydrogen bond involving the H_1 and H_2 donor sites respectively. It is plausible that the 5-oxide group and the 3-ethoxycarbonyl group can reinforce N-4 binding. This is confirmed by the decrease in affinity of the reduced compounds.

Moreover, the reduced affinity for BZR of the 8-derivatives with bulky substituents, **6d**, **8c** and **7c**, and the lack of affinity of the 7-substituted ones, **2c** and **5c**, might be explained by the fact that these substituents interact with the repulsive regions S_2. It can be assumed that the lipophilic regions L_1 and L_2 are occupied by the aromatic ring of benzotriazine moiety and by a small lipophilic group at the 8-position. This substituent may be located in an area which corresponds to the *para* position of the 2-aryl CGS series [9, 19].

The L_3 region should not be involved; this would explain the partial agonist trend that we generally observed with the GABA ratio value.

Pharmacological results

Compounds **1b**, **1c**, **1cR**, **4c**, **4cR**, **9d**, **9dR**, **12d** and **12dR** were evaluated for their anticonvulsant and myorelaxant effects in three to five doses ranging from 3 to 300 mg/kg po (see table III). During the 30 min prior to the test, the animals were carefully observed for gross behaviour in their home-cages. No behavioural alterations were evident following the administration of each of the substances studied. One indication of absorption of all the compounds, except **9d** and **9dR**, after po administration was the intense yellow colour of the animals' urine, which was the same colour as the compounds under study. None of the compounds induced myorelaxation or sedation in mice, as demonstrated by rotarod testing, while all of them, except the prototype **1b**, showed a statistically significant protective effect from convulsions caused by pentylenetetrazole (PTZ). Compounds **1c**, **4cR**, **9d**, **9dR** and **12dR** showed bell-shaped dose–response curves, while the dose–response relationships for compounds **1cR**, **4c** and **12** appeared linear in the dose ranges used. In particular, **12**d was the most effective in reducing the percentage of convulsant animals (36.8% at 300 mg/kg po).

For the compounds that showed a low GABA ratio value, **9d**, **9dR** and **12d**, 0.8, 1.09 and 1.14 respectively, a further in vivo study was performed (table IV). These compounds were administered in combination with diazepam (DAZ) and were evaluated for their ability to antagonize the anticonvulsant activity of the latter, in comparison with Flumazenil (RO 15-1788, GABA ratio 1.1, IC_{50} 1.82 nM, Anexate, Roche). Flumazenil is considered to be an antagonist at the central BZ recognition site and shows, per se, an anticonvulsant effect. Nevertheless it can significantly reduce the effect of DAZ. This is probably due to the fact that it is actually an agonist/antagonist [20–22]. It is interesting to note that **9d** and **9dR**, which showed

bell shaped dose–response curves (table III), were significantly effective in reducing the anticonvulsant action of DAZ at doses at which they had lost their protective effect per se. The reduction induced by **12d**, which had a linear dose–response curve, did not reach statistical significance. A possible explanation of the bell-shaped curves obtained could be that compounds **9d** and **9dR** act as agonist/antagonist drugs, ie, in an opposite manner on BZR sites, like Flumazenil.

Compound **4c**, which showed a high GABA ratio value (1.91) and a low K_i value (35 nM), was also evaluated for its anxiolytic effect in rats and in comparison with a significantly anxiolytic dose of DAZ (Valium, Roche 1 mg/kg ip) in Plus–Maze testing. Neither the percentage entries in the open arms nor the cumulative time spent in the open arms of **4c**-treated rats (100 mg/kg po) differed from controls.

Conclusion

The parent compound **1b**, which had no affinity to BZR, does not show in vivo activity. Compounds **9d** and **9dR** (3-bromo-8-methylthio-substituted) show a modest in vivo activity as anticonvulsant, but significantly antagonize the anticonvulsant action of DAZ.

Table III. Myorelaxant and anticonvulsant effect of some pyrazolo[5,1-*c*][1,2,4]benzotriazines and 5-oxides in comparison with DAZ.

Treatment	*mg/kg po*	*n*	*Number of falls from rotarod*	*Non-convulsant*		*Lethality*
				n	*%*	
CMC 1%	0.1 mL	82	0.29 ± 0.07	8/82	9.7	3/82
DAZ	0.1	21	0.33 ± 0.10	10/21	46.7**	–
	0.3	27	0.51 ± 0.20	26/27	96.3**	–
	1	15	0.73 ± 0.28	15/15	100**	–
	3	1	1.5 ± 0.45**	10/10	100**	–
	5	33	2.42 ± 0.44***	30/30	100**	–
1b	100	17	0.11 ± 0.08	2/17	11.7	3/17
1c	30	16	0.5 ± 0.18	2/16	12.5	–
	100	15	0.17 ± 0.09	4/17	23.5*	–
	300	16	0.43 ± 0.15	17/16	6.245	–
1cR	30	15	0 ± 0.0	0/15	0	–
	100	16	0 ± 0.0	4/16	25*	–
	300	15	0.2 ± 0.1	4/15	26.6*	–
4c	30	20	–	3/20	15	–
	100	32	0.5 ± 0.15	9/32	28.1**	–
	300	20	0.33 ± 0.18	5/20	25*	–
4cR	10	10	0.3 ± 0.15	1/10	10	–
	30	17	0.11 ± 0.08	4/17	23.5*	1/17
	100	16	0.06 ± 0.06	5/16	31.2**	1/16
	300	16	0.31 ± 0.15	3/16	18.7	1/4
9d	30	18	0.27 ± 0.13	3/18	16.6	–
	100	18	0.38 ± 0.14	4/18	22*	–
	300	18	0.055 ± 0.055	3/18	16.6	–
9dR	3	18	0.16 ± 0.09	2/18	11.1	–
	10	20	0.2 ± 0.11	5/20	25*	–
	30	17	0.35 ± 0.11	5/17	29.4**	3/17
	100	20	0.45 ± 0.18	5/20	25*	–
	300	17	0.23 ± 0.1	1/17	5.8	2/17
12d	10	16	0.56 ± 0.15	2/16	12.5	1/16
	30	19	0.2 ± 0.13	4/19	21*	–
	100	25	–	7/25	28**	–
	300	19	0.15 ± 0.08	7/19	36.8**	–
12dR	3	17	0.35 ± 0.14	1/17	11.76	–
	10	16	0.12 ± 0.08	4/16	25*	1.16
	30	16	0.38 ± 0.15	4/16	25*	1/16
	100	18	0.16 ± 0.09	5/18	27.7**	1/18
	300	15	0.46 ± 0.21	2/15	13.3	1/15

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus carboxymethylcellulose (CMC) treated mice.

Table IV. Ability of some pyrazolo[1,5-*c*][1,2,4]benzotriazines and 5-oxides to antagonize DAZ activity.

Treatment	*mg/kg po*	*n*	*Number of falls from rotarod*	*Non-convulsant*	
				n	*%*
Flumazenil	0.3 ip	11	0.09 ± 0.09	4/11	36.3*
	1 ip	20	0.7 ± 0.17*	6/20	30*
Flumazenil + DAZ	1 ip 0.3	20	0.45 ± 0.18	13/29	65**
9d + DAZ	100 0.3	15	0.73 ± 0.20	15/15	100
9d + DAZ	300 0.3	7	0.0 ± 0.0	5/7	71.4*
9dR + DAZ	100 0.3	13	0.38 ± 0.31	11/13	84.6
9dR + DAZ	300 0.3	21	0.28 ± 0.12	15/21	71.4*
12d + DAZ	100 0.3	14	0.57 ± 0.20	13/14	92.85
12d + DAZ	300 0.3	22	0.54 ± 0.12	18/22	81.8

*$P < 0.05$, **$P < 0.01$ versus DAZ (0.3 mg/kg) treated mice.

Therefore it is conceivable that, for these compounds, the antagonist properties are dominant, also according to GABA ratio values, 1.09 and 0.8, respectively.

Compound **12d** (3-bromo-8-ethoxy-substituted) shows modestly higher anticonvulsant activity than the other compounds (36.8% at 300 mg/kg po). On the other hand, even though its GABA ratio value is 1.14, **12d** does not present antagonist properties (see table IV). Thus, for **12d** it is plausible to assume a partial agonist profile with low efficacy.

Compound **4c** (3-carbethoxy-8-chloro-substituted) has the best affinity to BZR (K_i = 35 nM) with trend towards full-agonist (GABA ratio 1.91). It shows modest anticonvulsant activity, which is lower than **12d**, considering that their K_i are comparable: 35 nM for **4c** and 36.5 nM for **12d**. Compound **4c** does not have any anxiolytic properties.

These in vivo results, which seem to be in contrast with binding data, may be attributed to bioavailability problems. Compound **12d** probably has more favourable chemical and features of lipophilic and steric hindrance causing it to have in vivo activity.

The BZR affinity of the 5-oxides is better than reduced derivatives and does not correspond with the best activity in in vivo tests, where the anticonvulsant effects of the two series are comparable.

Experimental protocols

Chemistry

The structures of all compounds were supported by their IR spectra (KBr pellets in Nujol mulls, Perkin-Elmer 681 spectrophotometer) and ^{1}H-NMR data (measured with a Varian Gemini at 200 MHz; chemical shifts are expressed in δ (ppm) using DMSO-d_6 or $CDCl_3$ as solvent). Melting points were determined with a Gallenkamp apparatus and were uncorrected. Elemental analyses were performed by the laboratories of Dipartimento Farmaco-Chimico-Tecnologico of University of Siena, Italy, with a Perkin-Elmer, model 240C, Elemental Analyzer and the results are within ±0.4% of theoretical values. The purity of samples was determined by means of TLC, which was performed using Machery-Nagel Duren, Alugram silica-gel plates.

*General procedure for synthesis of **2** and **3***

The products were prepared as previously described [11], using 2-nitro-4-methyl- [12] or 2-nitro-5-methylphenylhydrazine [13] and ethyl 2-cyano-3- ethoxypropeneate.

*Ethyl 1-(2-nitro-4-methylphenyl)-5-aminopyrazol-4-carboxylate **2**.* This was obtained from 2-nitro-4-methylphenylhydrazine. Yellow crystals, yield 75%; mp 187–188 °C after recrystallization from ethanol. IR ν cm^{-1}: 3420–3300, 1680, 1530; ^{1}H-NMR ($CDCl_3$) δ: 7.82 (d, 1H, H-3'); 7.70 (s, 1H, H-3); 7.54 (dd, 1H, H-5'); 7.45 (d, 1H, H-6'); 5.20 (bs, 2H, NH_2); 4.28 (q, 2H, CH_2); 2.50 (s, 3H, 4'-CH3); 1.35 (t, 3H, CH_3). Anal $C_{13}H_{14}N_4O_4$ (C, H, N).

*Ethyl 1-(2-nitro-5-methylphenyl)-5-aminopyrazol-4-carboxylate **3**.* This was obtained from 2-nitro-5-methylphenylhydrazine. Yellow crystals, yield 70%; mp 132–133 °C after recrystallization from ethanol/water. IR ν cm^{-1}: 3440–3300, 1700; ^{1}H-NMR ($CDCl_3$) δ: 7.98 (d, 1H, H-3'); 7.78 (s, 1H, H-3); 7.42 (m, 2H, H-4', H-6'); 5.15 (bs, 2H, NH_2); 4.30 (q, 2H, CH_2); 2.50 (s, 3H, 4'-CH_3); 1.35 (t, 3H, CH_3). Anal $C_{13}H_{14}N_4O_4$ (C, H, N).

*General procedure for synthesis of **2'** and **3'***

A suspension of **2** or **3** (1.00 g) in 15 mL conc hydrochloric acid was refluxed for 12 h. After cooling at room temperature, the respective hydrochlorides were filtered. Upon dissolution in water and neutralization with conc ammonia, the 5-aminopyrazoles **2'** or **3'** were isolated.

*1-(2-Nitro-4-methylphenyl)-5-aminopyrazole **2'***. The aminopyrazole is a liquid and was characterized as the hydrochloride. White crystals, yield 90%; mp 272–273 °C after recrystallization from isopropyl alcohol. IR ν cm^{-1}: 2560–2500; ^{1}H-NMR (DMSO-d_6) δ: 8.10 (m, 4H, H-3' and NH_3^+); 7.80 (d, 1H, H-3); 7.73 (m, 2H, H-5', H-6'); 5.68 (d, 1H, H-4); 2.50 (s, 3H, CH_3). Anal $C_{10}H_{11}N_4O_2Cl$ (C, H, N).

*1-(2-Nitro-5-methylphenyl)-5-aminopyrazole **3'***. Cream crystals, yield 70%; mp 250–251 °C after recrystallization from ethanol. IR ν cm^{-1}: 3300–3180; ^{1}H-NMR ($CDCl_3$) δ: 8.08 (m, 1H, H-3'); 7.60 (m, 3H, H-4', H-6' and H-3'); 6.35 (s, 2H, NH_2); 5.62 (d, 1H, H-4); 2.50 (s, 3H, CH_3). Anal $C_{10}H_{10}N_4O_2$ (C, H, N).

*1-(2-Nitro-5-methylphenyl)-5-aminopyrazole-4-carbonitrile **3''***
This was obtained from 2-nitro-5-methylphenylhydrazine and 2-ethoxy-1,1-ethenedicarbonitrile, according to the described procedure [11]. Yellow crystals, yield 70%; mp 173–174 °C after recrystallization from ethanol/water. IR ν cm^{-1}: 3440–3340; ^{1}H-NMR ($CDCl_3$) δ: 8.04 (d, 1H, H-3'); 7.65 (s, 1H, H-2); 7.49 (dd, 1H, H-4'); 7.38 (d, 1H, H-6'); 4.49 (bs, 2H, NH_2); 2.53 (s, 3H, CH_3). Anal $C_{11}H_9N_5O_2$ (C, H, N).

*1-(2-Nitro-5-methylphenyl)-5-aminopyrazole-4-carboxiamide **3'''***
This was obtained from **3''** by treatment with conc sulphuric acid according to [11]. Yellow crystals, yield 35%; mp 182–183 °C after recrystallization from water. IR ν cm^{-1}: 3440, 3360, 3200,1650; ^{1}H-NMR (DMSO-d_6) δ: 7.98 (d, 1H, H-3'); 7.85 (s, 1H, H-2); 7.52 (m, 2H, H-4' and H-6'); 7.40 (bs, 1H, NH); 6.85 (bs, 1H, NH); 6.41 (bs, 2H, NH_2); 2.47 (s, 3H, CH_3). Anal $C_{11}H_{11}N_5O_3$ (C, H, N).

*General procedure for synthesis of **2a**, **2b**, **3b** and **3e''***
A suspension of 5 mmol of the appropriate 5-aminopyrazole in 40 mL of 10% sodium hydroxide was kept at room temperature for 24 h. The precipitate was filtered and purified by recrystallization.

*3-Carboxy-7-methylpyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide **2a***. This was obtained from **2**. Yellow crystals, yield 97%. IR ν cm^{-1}: 3000–2900, 1720, 1570; ^{1}H-NMR ($CDCl_3$) δ: 8.58 (d, 1H, H-6); 8.38 (m, 2H, H-2, H-9); 7.88 (dd, 1H, H-8); 2.61 (s, 3H, CH_3).

*7-Methylpyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide **2b***. This was obtained from **2'**. Yellow crystals, yield 88%. IR ν cm^{-1}: 1560; 1H-NMR ($CDCl_3$) δ: 8.35 (d, 1H, H-6); 8.30 (d, 1H, H-9); 8.08 (d, 1H, H2); 7.75 (dd, 1H, H-8); 6.74 (d, 1H, H3); 2.60 (s, 3H, CH_3).

*8-Methylpyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide **3b***. This was obtained from **3'**. Yellow crystals, yield 85%. IR ν cm^{-1}: 1550; ^{1}H-NMR ($CDCl_3$) δ: 8.42 (d, 1H, H-6); 8.20 (d, 1H, H-9); 8.08 (d, 1H, H-2); 7.42 (dd, 1H, H-7); 6.71 (d, 1H, H-3); 2.62 (s, 3H, CH_3).

*3-Carbamoyl-8-methylpyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide **3e''***. This was obtained from **3''**. Yellow crystals, yield 98%. IR ν cm^{-1}: 3400, 3180, 1650, 1570; ^{1}H-NMR (DMSO-d_6) δ: 8.57 (s, 1H, H-2); 8.36 (d, 1H, H-6); 8.23 (d, 1H, H-9); 7.66 (m, 2H, H-7 and NH); 7.15 (bs, 1H, NH); 2.64 (s, 3H, CH_3).

*3-Carboxy-8-methylpyrazolo[5,1-c[1,2,4]benzotriazine 5-oxide **3a***
This was prepared according to [11] by treatment of **3e''** with conc sulphuric acid and sodium nitrite. Yellow crystals, yield 98%. IR ν cm^{-1}: 2700–2600, 1680, 1570; ^{1}H-NMR (DMSO-d_6) δ: 8.58 (s, 1H, H-2); 8.35 (d, 1H, H-6); 8.23 (d, 1H, H-9); 7.63 (dd, 1H, H-7); 2.63 (s, 3H, CH_3).

*8-Ethylethylenedioxopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide **6b***
Compound **4b** (0.1 mmol) [11] was dissolved in a mixture of ethoxyethanol (20 mL) and 10% sodium hydroxide (10 mL) and was stirred at room temperature for 2 days. The solution was evaporated in vacuo and the residue recrystallized. Yellow crystals, yield 45%. IR ν cm^{-1}: 1570, 1250, 1090; ^{1}H-NMR ($CDCl_3$) δ: 8.47 (d, 1H, H-6); 8.10 (d, 1H, H-2); 7.74 (d, 1H, H-9); 7.20 (dd, 1H, H-7); 6.71 (d, 1H, H-3); 4.36 (t, 2H, $ArOCH_2CH_2O$); 3.90 (t, 2H, $ArOCH_2CH_2O$); 3.63 (q, 2H, CH_2); 1.28 (t, 3H, CH_3).

*3-Carboxy-8-morpholinopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide **7a***
A suspension of **4a** (1 mmol) [11] in 2-ethoxyethanol (15 mL) was treated with 5 mL morpholine and maintained at 60 °C. The residue obtained after evaporation of solvent was acidified with acetic acid and the precipitate filtered and recrystallized. Yellow crystals, yield 79%. IR ν cm^{-1}: 3200, 2600, 1710,1560; ^{1}H-NMR (DMSO-d_6) δ:12.70 (s, 1H, OH); 8.51 (d, 1H, H-2); 8.22 (d, 1H, H-6); 7.42 (m, 2H, H-7, H-9); 3.80 (m, 4H, OCH_2CH_2); 3.69 (m, 4H, NCH_2CH_2).

*8-Diethylaminopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide **8b***
A suspension of **4b** (0.7 mmol) [11] in 10 mL diethylamine and 5 mL 10% sodium hydroxide was stirred at 60 °C for 2 days. The solution was then evaporated in vacuo and the residue purified by column chromatography (toluene/ethylacetate 8:2 as eluent, faster eluted band). Yellow crystals, yield 38%. IR ν cm^{-1}: 1540; ^{1}H-NMR ($CDCl_3$) δ: 8.33 (d, 1H, H-6); 8.03 (d, 1H, H-2); 7.20 (d, 1H, H-9); 6.87 (dd, 1H, H-7); 6.60 (d, 1H, H-3); 3.58 (q, 4H, $N(CH_2)_2$); 1.30 (t, 6H, $N(CH_2CH_3)_2$.

*3-Ethoxycarbonyl-8-diethylaminopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide **8c***
To a solution of **4c** (0.7 mmol) in 2-ethoxyethanol (15 mL), diethylamine (20 mL) was added and the mixture was stirred at 40 °C for 4 days. After evaporation of the solution, the residue was treated with diethyl ether, filtered and purified. Yellow crystals, yield 66%. IR ν cm^{-1}: 1760, 1570; ^{1}H-NMR ($CDCl_3$) δ: 8.45 (d, 1H, H-2); 8.30 (d, 1H, H-6); 7.19 (d, 1H, H-9); 6.90 (dd, 1H, H-7); 4.42 (q, 2H, CH_2CH_3); 3.57 (q, 4H, $N-(CH_2)_2$); 1.40 (t, 3H, CH_2CH_3); 1.30 (t, 6H, $N(CH_2CH_3)_2$.

*3-Carboxy-8-methylthiopyrazolo[5,1-cl[1,2,4]benzotriazine 5-oxide **9a***
A suspension of **4a** (0.5 mmol) [11] in ethanol (150 mL) was reacted with sodium thiomethoxide (1.75 mmol) at reflux under nitrogen flow for 5 days. The reaction was monitored by TLC (toluene/ethyl acetate/acetic acid 8:2:1 as eluent). After dilution and acidification with diluted hydrochloric acid, the precipitate obtained was filtered and recrystallized. Yellow crystals, yield 80% IR ν cm^{-1}: 2600, 1670, 1560; ^{1}H-NMR (DMSO-d_6) δ: 12.50 (bs, 1H, OH); 8.58 (s, 1H, H-2); 8.30 (d, 1H, H-6); 8.00 (d, 1H, H-9); 7.64 (dd, 1H, H-7); 2.72 (s, 3H, SCH_3).

*8-Methylthiopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide **9b***
To a solution of **4b** (1.0 mmol) [11] in 25 mL of methoxyethanol, was added sodium thiomethoxide (2.0 mmol) and the

reaction was maintained at room temperature for 48–72 h. The precipitate was filtered and recrystallized. Yellow crystals, yield 78%. IR ν cm^{-1}: 1570; ^{1}H-NMR ($CDCl_3$) δ: 8.38 (d, 1H, H-6); 8.10 (d, 1H, H-2); 8.00 (d, 1H, H-9); 7.40 (dd, 1H, H-7); 6.72 (d, 1H, H-3); 2.68 (s, 3H, SCH_3).

3-Carboxy-8-methylsulphoxypyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***10a*** *and 3-carboxy-8-methysulphonylpyrazolo-[5,1-c][1,2,4]benzotriazine 5-oxide* ***11a***

These products were obtained by treatment of **4a** [11] with sodium thiomethoxide in refluxing ethanol without nitrogen flow. The compounds **10a** and **11a** were chromatographed over a silica-gel column eluting with toluene/ethyl acetate/acetic acid (8:2:1).

Compound ***10a***. Yellow crystals. IR ν cm^{-1}: 3400, 2600, 1740, 1550, 1120; ^{1}H-NMR (DMSO-d_6) δ: 8.74 (s, 1H, H-2); 8.57 (d, 1H, H-6); 8.10 (d, 1H, H-9); 7.80 (dd, 1H, H-7); 2.76 (s, 3H, $SOCH_3$).

Compound ***11a***. Yellow crystals IR ν cm^{-1}: 3300, 2600, 1700, 1560, 1310–1140; ^{1}H-NMR (DMSO-d_6) δ: 12.50 (bs, 1H, OH) 9.06 (d, 1H, H-6); 8.95 (d, 1H, H-9); 8.89 (d, 1H, H-2); 8.42 (dd, 1H, H-7); 3.55 (s, 3H, SO_2CH_3).

8-Hydrazinylpyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***17b***

To a solution of **4b** [11] (0.9 mmol, 200 mg) and THF (10 mL), was added hydrazine hydrate (3.0 mL). The reaction was kept at 50–60 °C for 48–72 h and a precipitate was obtained; this was filtered, washed with a small amount of ethanol and recrystallized. Orange crystals; yield 45%. IR ν cm^{-1}: 3380–3300; ^{1}H-NMR (DMSO-d_6) δ: 8.80 (bs, 1H, NH); 8.18 (d, 1H, H-2); 8.10 (d, 1H, H-6); 7.42 (d, 1H, H-9); 6.98 (dd, 1H, H-7); 6.68 (d, 1H, H-3); 4.72 (bs, 2H, NH_2).

3-Nitropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***1e***

To a solution of **1b** [11] (4.0 mmol) dissolved in cooled conc sulphuric acid (10 mL), was added dropwise conc nitric acid (1.0 mL); the red solution was stirred at 0 °C for 2 h. Addition of ice/water yielded a solid mixture of **1e** and **14e** (as a minor product; toluene/ethyl acetate/acetic acid 8:2:1 as eluent) which was filtered and purified by recrystallization from acetic acid. Compound **1e** was obtained pure as yellow crystals; yield 47%. IR ν cm^{-1}: 1570, 1500, 1340; ^{1}H-NMR (DMSO-d_6) δ: 9.12 (s, 1H, H-2); 8.50 (m, 2H, H-6 and H-9); 8.25 (t, 1H, H-7); 7.92 (t, 1H, H-8).

Evaporation of the recrystallization solvent of **1e** gave **14e**.

3,7-Dinitropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***14e***

This compound was obtained, as single product, from **1b** [11] in the same manner as **1e**, using fuming nitric acid (5.0 mL). Yellow crystals. Yield 80%; IR ν cm^{-1}: 1570, 1530, 1330; ^{1}H-NMR ($CDCl_3$) δ: 9.24 (s, 1H, H-2); 9.18 (d, 1H, H-6); 8.92 (dd, 1H, H-8); 8.69 (d, 1H, H-9).

3-Ethoxycarbonyl-7-methylpyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***2c***

A suspension of 0.38 mmol of the acid **2a** in 10 mL diethylcarbonate and 2.0 mL conc sulphuric acid was refluxed for 5 h. After evaporation, the residue was treated with a small amount of ethanol, filtered and recrystallized. Yellow crystals; yield 64%. IR ν cm^{-1}: 1730, 1570; ^{1}H-NMR ($CDCl_3$) δ: 8.50 (s, 1H, H-6); 8.32 (m, 2H, H-2 and H-9); 7.84 (dd, 1H, H-8); 4.45 (q, 2H, CH_2); 2.60 (s, 3H, 7-CH_3); 1.42 (t, 3H, CH_3).

3-Ethoxycarbonyl-8-methylpyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***3c***

A suspension of 0.38 mmol of the acid **3a** in anhydrous ethanol (50 mL) and conc sulphuric acid (2.0 mL) was refluxed for 12 h. After evaporation, the residue was treated with a small amount of ethanol, filtered and recrystallized. Yellow crystals; yield 70%. IR ν cm^{-1}: 1700, 1570; ^{1}H-NMR ($CDCl_3$) δ: 8.50 (s, 1H, H-2); 8.43 (d, 1H, H-6); 8.23 (d, 1H, H-9); 7.49 (dd, 1H, H-7); 4.46 (q, 2H, CH_2); 2.65 (s, 3H, 8-CH_3); 1.42 (t, 3H, CH_3).

3-Ethoxycarbonyl-8-morpholinopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***7c***

This compound was obtained from the acid **7a**, in the same manner as **2c**. Yellow crystals; yield 56%. IR ν cm^{-1}: 1695, 1560; ^{1}H-NMR (DMSO-d_6) δ: 8.56 (s, 1H, H-2); 8.22 (d, 1H, H-6); 7.42 (m, 2H, H-7 and H-9); 4.32 (q, 2H, CH_2); 3.78 (t, 4H, OCH_2CH_2); 3.58 (t, 4H, NCH_2CH_2); 1.33 (t, 3H, CH_3).

3-Ethoxycarbonyl-8-methylthiopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***9c***

A suspension of 0.38 mmol of the acid **9a** in anhydrous ethanol (20 mL) containing 5.0 mL conc sulphuric acid was refluxed for 2 days until the starting material disappeared in TLC (toluene/ethyl acetate 8:2 as eluent). After cooling the precipitate was filtered and recrystallized. Yellow crystals; yield 45%. IR ν cm^{-1}: 1710, 1560; 1H-NMR (DMSO-d_6) δ: 8.62 (s, 1H, H-2); 8.30 (d, 1H, H-6); 7.98 (d, 1H, H-9); 7.65 (dd, 1H, H-7); 4.35 (q, 2H, CH_2); 2.78 (s, 3H, SCH_3); 1.38 (t, 3H, CH_3).

General procedure for synthesis of ***2d, 3d, 4d, 6d, 9d, 16dR*** *and* ***19dR***

The appropriate pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (0.60 mmol) was dissolved in 5 mL chloroform to which an equimolar amount of bromine was slowly added. A solution or precipitate was obtained, and after the normal workup, the residue was recrystallized from the appropriate solvent.

3-Bromo-7-methylpyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***2d***. This was obtained from **2b**. Yellow crystals, yield 95%. IR ν cm^{-1}: 1550; ^{1}H-NMR ($CDCl_3$) δ: 8.36 (d, 1H, H-6); 8.26 (d, 1H, H-9); 8.06 (s, 1H, H-2); 7.79 (dd, 1H, H-8); 2.59 (s, 3H, CH_3).

3-Bromo-8-methylpyrazolo[5,1-c][1,2,41benzotriazine 5-oxide ***3d***. This was obtained from **3b**. Yellow crystals, yield 95%. IR ν cm^{-1}: 1550; ^{1}H-NMR ($CDCl_3$) δ: 8.42 (d, 1H, H-6); 8.16 (d, 1H, H-9); 8.06 (s, 1H, H-2); 7.45 (dd, 1H, H-7); 2.65 (s, 3H, CH_3).

3-Bromo-8-chloropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***4d.*** This was obtained from **4b** [11]. Yellow crystals, yield 58%. IR ν cm^{-1}: 1550; ^{1}H-NMR ($CDCl_3$) δ: 8.50 (d, 1H, H-6); 8.38 (d, 1H, H-9); 8.10 (s, 1H, H-2); 7.62 (dd, 1H, H-7).

3-Bromo-8-ethylethylenedioxopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***6d***. This was obtained from **6b**. Yellow crystals, yield 90%. IR ν cm^{-1}: 1570; ^{1}H-NMR ($CDCl_3$) δ: 8.44 (d, 1H, H-6); 8.05 (s, 1H, H-2); 7.69 (d, 1H, H-9); 7.20 (dd, 1H, H-7); 4.36 (t, 2H, $ArOCH_2CH_2$); 3.88 (t, 2H, $ArOCH_2CH_2$); 3.62 (q, 2H, CH_2); 1.2 (t, 3H, CH_3).

3-Bromo-8-methylthiopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***9d***. This was obtained from **9b**. Yellow crystals, yield 89%. IR ν cm^{-1}: 1570; ^{1}H-NMR ($CDCl_3$) δ: 8.35 (d, 1H, H-6); 8.04

(s, 1H, H-2); 7.96 (d, 1H, H-9); 7.40 (dd, 1H, H-7); 2.65 (s, 3H, SCH_3).

3-Bromo-7-acetylaminopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***16dR***. This was obtained from **16bR**. Yellow crystals, yield 70%. IR ν cm^{-1}: 3280, 1720; ^{1}H-NMR (DMSO-d_6) δ:10.62 (s, 1H, NH); 9.02 (d, 1H, H-6); 8.56 (s, 1H, H-2); 8.42 (d, 1H, H-9); 8.16 (dd, 1H, H-8); 2.20 (s, 3H, CH_3).

3-Bromo-8-acetylaminopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***19dR***. This was obtained from **19bR**. Yellow crystals, yield 80%. IR ν cm^{-1}: 3380, 1690; ^{1}H-NMR (DMSO-d_6) δ:10.95 (s, 1H, NH); 8.90 (d, 1H, H-9); 8.62 (m, 2H, H-2 and H-6); 7.78 (dd, 1H, H-7); 2.20 (s, 3H, CH_3).

Preparation of pyrazolo[5,1-c][1,2,4]benzotriazines ***1bR–5bR, 14bR, 15bR, 18bR, 1cR, 4cR, 8cR, 12cR, 14cR, 1dR, 9dR, 12dR and 1eR***. *Methods of reduction*

Method A. A solution of the appropriate 5-oxide **1–5b**, **14b**, **1c**, **4c**, **8c**, **12c**, **14c**, **1d**, **9d**, **12d** or **1e** (1.0 mmol) in toluene (15 mL) containing 3.0 mL triethylphosphite was refluxed until the starting material disappeared in TLC. After evaporation, the residue was treated with a few millilitres of diethyl ether or petroleum ether at 40–60 °C, filtered and recrystallized from a suitable solvent.

Method B. A suspension of suitable 5-oxide derivative **1b** or **18b** (1.0 mmol) in acetic acid (15 mL for **1b** and 20 mL acetic acid/ethanol solution 1:1 for **18b**), was magnetically stirred at room temperature. A large excess of zinc dust (8.0 mmol in three portions) and 2 × 10 mL acetic acid were added. The reaction was monitored by TLC; the zinc residue was filtered off and the solution was evaporated in vacuo. The residue was treated with water, filtered and recrystallized from an appropriate solvent.

Method C. A suspension of **14b** (1.34 mmol), in 15 mL conc hydrochloric acid was cooled at 0 °C and treated with 0.8 g of tin under stirring. After 5 min a white precipitate of tin(IV) chloride showed the end of reduction, which was also confirmed by TLC. The suspension was made alkaline with 3 N sodium hydroxide until dissolution of the white tin(IV) chloride and precipitation of **15bR** as a red residue. The latter was filtered and recrystallized from ethanol (more crude **15bR** was obtained by extraction of the aqueous solution with ethyl ether).

Pyrazolo[5,1-c][1,2,4]benzotriazine ***1bR***. This was obtained from **1b** [11] by *Methods A* (yield 65%) and *B* (yield 90%). TLC eluent chloroform; cream crystals; ^{1}H-NMR ($CDCl_3$) δ: 8.65 (d, 1H, H-9); 8.50 (d, 1H, H-6); 8.22 (d, 1H, H-2); 7.98 (t, 1H, H-8); 7.75 (t, 3H, H-7); 7.42 (d, 1H, H-3).

7-Methylpyrazolo[5,1-c][1,2,4]benzotriazine ***2bR***. This was obtained from **2b** by *Method A*. TLC eluent chloroform/ethyl acetate 8:2; yellow crystals; yield 38%. ^{1}H-NMR ($CDCl_3$) δ: 8.41 (m, 1H, H-6 and H-9); 8.20 (d, 1H, H-2); 7.78 (dd, 1H, H-8); 7.40 (d, 1H, H-3); 2.65 (s, 3H, CH_3).

8-Methylpyrazolo[5,1-c][1,2,4]benzotriazine ***3bR***. This was obtained from **3b** by *Method A*. TLC eluent chloroform/ethyl acetate 8:2; yellow crystals; yield 38%. ^{1}H-NMR ($CDCl_3$) δ: 8.50 (d, 1H, H-6); 8.28 (d, 1H, H-9); 8.20 (d, 1H, H-2); 7.78 (dd, 1H, H-7); 7.38 (d, 1H, H-3); 2.68 (s, 3H, CH_3).

8-Chloropyrazolo[5,1-c][1,2,4]benzotriazine ***4bR***. This was obtained from **4b** [11] by *Mehod A*. TLC eluent toluene/ethyl acetate 8:2; yellow crystals; yield 63%. ^{1}H-NMR ($CDCl_3$) δ: 8.54 (d, 1H, H-6); 8.44 (d, 1H, H-9); 8.22 (d, 1H, H-2); 8.66 (dd, 1H, H-7); 7.42 (d, 1H, H-3).

7-Chloropyrazolo[5,1-c][1,2,4]benzotriazine ***5bR***. This was obtained from **5b** [11] by *Method A*. TLC eluent toluene/ethyl acetate 8:2; ellow crystals; yield 85%. ^{1}H-NMR ($CDCl_3$) δ: 8.63 (d, 1H, H-6); 8.45 (d, 1H, H-9); 8.22 (d, 1H, H-2); 7.89 (dd, 1H, H-8); 7.45(d, 1H, H-3).

7-Nitropyrazolo[5,1-c][1,2,4]benzotriazine ***14bR***. This was obtained from **14b** [11] by *Method A*. TLC eluent toluene/ethyl acetate 8:2; yellow crystals; yield 70%. IR ν cm^{-1}: 1530, 1360; ^{1}H-NMR ($CDCl_3$) δ: 9.53 (d, 1H, H-6); 8.80 (dd, 1H, H-8); 8.61 (d, 1H, H-9); 8.34 (d, 1H, H-2); 7.56 (d, 1H, H-3).
This product was also prepared from **15bR** by oxidation in the same manner as **18b** and **18b'**.

7-Aminopyrazolo[5,1-c][1,2,4]benzotriazine ***15bR***. This was obtained from **14b** [11] by *Method C*. TLC eluent chloroform/ethyl acetate 7:3; red crystals; yield 70%. IR ν cm^{-1}: 3460, 3360-3220; ^{1}H-NMR (DMSO-d_6) δ: 8.25 (m, 2H, H-2 and H-9); 7.59 (d, 1H, H-3); 7.44 (m, 2H, H-6 and H-8); 5.99 (bs, 2H, NH_2).

8-Aminopyrazolo[5,1-c][1,2,4]benzotriazine ***18bR***. This was obtained from **17b** by *Method B*. TLC eluent ethyl acetate/cyclohexane 2:1; yellow crystals; yield 60%. IR ν cm^{-1}: 3380–3180; ^{1}H-NMR (DMSO-d_6) δ: 8.28 (d, 1H, H-2); 8.20 (d, 1H, H-6); 7.25 (m, 4H, H-3, H-9 and NH_2); 7.15 (dd, 1H, H-7).

Methyl pyrazolo[5,1-c][1,2,4]benzotriazine-3-carboxylate ***1cR***. This was obtained from **1c** [11] by *Method A*. TLC eluent toluene/ethyl acetate 8:2; yellow crystals; yield 45%. IR ν cm^{-1}: 1710; ^{1}H-NMR ($CDCl_3$) δ: 8.78 (d, 1H, H-6); 8.68 (s, 1H, H-2); 8.55 (d, 1H, H-9); 8.08 (t, 1H, H-7); 7.88 (t, 1H, H-8); 4.10 (s, 3H, CH_3).

Ethyl 8-chloropyrazolo[5,1-c][1,2,4]benzotriazine-3-carboxylate ***4cR***. This was obtained from **4c** [11] by *Method A*. TLC eluent toluene/ethyl acetate/acetic acid 8:2:1; yellow crystals; yield 87%. IR ν cm^{-1}: 1740; ^{1}H-NMR ($CDCl_3$) δ: 8.70 (m, 2H, H-2 and H-6); 8.66 (d, 1H, H-9); 7.80 (dd, 1H, H-7); 4.55 (q, 2H, CH_2); 1.53 (t, 3H, CH_3).

Ethyl 8-diethylaminopyrazolo[5,1-c][1,2,4]benzotriazine-3-carboxylate ***8cR***. This was obtained from **8c** by *Method A*. TLC eluent ethyl acetate/cyclohexane 2:1; yellow crystals; yield 66%. IR ν cm^{-1}: 1700; ^{1}H-NMR ($CDCl_3$) δ: 8.58 (s, 1H, H-2); 8.38 (d, 1H, H-6); 7.26 (d, 1H, H-9); 7.14 (dd, 1H, H-7); 4.55 (q, 2H, CH_2CH_3); 3.62 (q, 4H, $N(CH_2)_2$); 1.47 (t, 3H, CH_2CH_3); 1.33 (t, 6H, $N(CH_2CH_3)_2$.

Ethyl 8-ethoxypyrazolo[5,1-c][1,2,4]benzotriazine-3-carboxylate ***12cR***. This was obtained from **12c** [11] by *Method A*. TLC eluent toluene/ethyl acetate 8:2; yellow crystals; yield 70%. IR ν cm^{-1}: 1720, 1250; ^{1}H-NMR ($CDCl_3$) δ: 8.67 (s, 1H, H-2); 8.60 (d, 1H, H-6); 7.80 (d, 1H, H-9); 7.38 (dd, 1H, H-7); 4.55 (q, 2H, CH_2CH_3); 4.35 (q, 2H, OCH_2CH_3); 1.56 (t, 3H, CH_2CH_3); 1.48 (t, 6H, $OCH_2CH_3)_2$.

Ethyl 7-nitropyrazolo[5,1-c][1,2,4]benzotriazine-3-carboxylate ***14cR***. This was obtained from **14c** [11] by *Method A*. TLC eluent chloroform/cyclohexane/ethylacetate 8:2.5:2; yellow crystals; yield 40%. IR ν cm^{-1}: 1730, 1570, 1350; ^{1}H-NMR

($CDCl_3$) δ: 9.62 (s, 1H, H-6); 8.85 (d, 1H, H-8); 8.70 (m, 2H, H-9 and H-2); 4.55 (q, 2H, CH_2); 1.50 (t, 3H, CH_3).

3-Bromopyrazolo[5,1-c][1,2,4]benzotriazine ***1dR***. This was obtained from **1d** [11] by *Method A*. TLC eluent toluene/ethyl acetate 8:2; yellow crystals; yield 78%; ^{1}H-NMR ($CDCl_3$) δ: 8.68 (d, 1H, H-6); 8.45 (d, 1H, H-9); 8.20 (s, 1H, H-2); 7.99 (t, 1H, H-7); 7.78 (t, 1H, H-8).

3-Bromo-8-methylthiopyrazolo[5,1-c][1,2,4]benzotriazine ***9dR***. This was obtained from **9d** by *Method A*. TLC eluent ethyl acetate/cyclohexane 2:1; yellow crystals; yield 83%; ^{1}H-NMR ($CDCl_3$) δ: 8.45 (d, 1H, H-6), 8.19 (s, 1H, H-2); 8.05 (d, 1H, H-9); 7.55 (dd, 1H, H-7); 2.67 (s, 3H, SCH_3).

3-Bromo-8-ethoxypyrazolo[5,1-c][1,2,4]benzotrinzine ***12dR***. This was obtained from **12d** [11] by *Method A*. TLC eluent toluene/ethyl acetate 8:2; yellow crystals; yield 72%. IR ν m^{-1}: 1230; ^{1}H-NMR ($CDCl_3$) δ: 8.53 (d, 1H, H-6); 8.19 (s, 1H, H-2); 7.71 (d, 1H, H-9); 7.30 (dd, 1H, H-7); 4.31 (q, 2H, CH_2); 1.56 (t, 3H, CH_3).

3-Nitropyrazolo[5,1-c][1,2,4]benzotriazine ***1eR***. This was obtained from **1e** by *Method A*. TLC eluent ethyl acetate/cyclohexane 2:1; yellow crystals; yield 89%. IR ν cm^{-1}: 1550, 1310; ^{1}H-NMR ($CDCl_3$) : 8.86 (m, 2H, H-6 and H-2); 8.60 (d, 1H, H-9); 8.18 (t, 1H, H-7); 7.98 (t, 1H, H-8).

7-Acetylaminopyrazolo[5,1-c][1,2,4]benzotriazine ***16bR***
A solution of **15bR** (1.0 mmol) in acetic acid (10 mL) and acetic anhydride (10 mL) was refluxed until the starting material disappeared in TLC (chloroform/ethyl acetate 7:3 as eluent). After cooling and dilution the precipitate was filtered and recrystallized. Yellow crystals; yield 59%. IR ν cm^{-1}: 13300, 1680; ^{1}H-NMR (DMSO-d_6) δ: 10.61 (s, 1H, NH); 8.98 (d, 1H, H-6); 8.42 (m, 2H, H-9 and H-2); 8.16 (dd, 1H, H-8); 7.60 (d, 1H, H-3); 2.19 (s, 3H, CH_3).

8-Acetylaminopyrazolo[5,1-c][1,2,4]benzotriazine ***19bR***
This was obtained from **18bR** in the same manner as **16bR**. TLC eluent cyclohexane/ethyl acetate 2:1; yellow crystals; yield 75 %. IR ν cm^{-1}: 3280, 1700; ^{1}H-NMR (DMSO-d_6) δ:10.87 (bs, 1H, NH); 8.97 (d, 1H, H-9); 8.56 (d, 1H, H-6); 8.41 (d, 1H, H-2); 7.79 (dd, 1H, H-7); 7.51 (d, 1H, H-3); 2.20 (s, 3H, CH_3).

3-Bromo-7-acetylaminopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***16d***
To a solution of **16dR** (0.5 mmol) in chloroform (20 mL), an excess of *m*-chloroperbenzoic acid (MCPBA) was added. The reaction was kept at 60 °C and monitored by TLC (dichloromethane/methanol 10:0.5 as eluent). The orange precipitate obtained was filtered and recrystallized. Yield 45%. IR ν cm^{-1}: 3360, 1690, 1550; ^{1}H-NMR (DMSO-d_6) δ: 10.62 (bs, 1H, NH); 8.83 (d, 1H, H-6); 8.40 (s, 1H, H-2); 8.35 (d, 1H, H-9); 8.18 (dd, 1H, H-8); 2.15 (s, 3H, CH_3).

3-Bromo-8-acetylaminopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***19d***
To a solution of **19dR** (0.8 mmol) in acetic acid (30 mL), were added acetic anhydride (15 mL) and hydrogen peroxide (6.0 mL). The solution was refluxed for 8 h and the yellow precipitate obtained was filtered and recrystallized. Yield 83%. IR ν cm^{-1}: 3340, 1700; ^{1}H-NMR (DMSO-d_6) δ: 10.90 (bs, 1H, NH); 8.82 (d, 1H, H-9); 8.41 (s, 1H, H-2); 8.40 (d, 1H, H-6); 7.69 (dd, 1H, H-7); 2.19 (s, 3H, CH_3).

8-Aminopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***18b*** *and 8-aminopyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide* ***18b'***
These compounds were obtained from **18bR** using various oxidizing agents: oxone as described in [14], or hydrogen peroxide/acetic anhydride and/or acetic acid in the same manner as **19d**. A mixture of **18b** and **18b'** was obtained; **18b** was purified by preparative TLC (chloroform/acetonitrile 1:1 as eluent), while **18b'** (present in a small amount) was only identified in the reaction mixture, by ^{1}H-NMR spectroscopy.

Compound ***18b***. Mp 281–282 °C. IR ν cm^{-1}: 3460, 3330, 3240, 1570; ^{1}H-NMR (DMSO-d_6) δ: 8.14 (d, 1H, H-2); 8.08 (d, 1H, H-6); 7.20 (d, 1H, H-9); 7.15 (bs, 2H, NH_2); 6.90 (dd, 1H, H-7); 6.67 (d, 1H, H-3).

Compound ***18b'***. ^{1}H-NMR (DMSO-d_6) δ: 8.26 (d, 1H, H-2); 7.65 (d, 1H, H-6); 7.30 (d, 1H, H-9); 7.15 (d, 1H, H-3); 6.99 (dd, 1H, H-7); 6.72 (bs, 2H, NH_2).

8-Acetylaminopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***19b*** *and 8-acetylaminopyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide* ***19b'***
These compounds were obtained from **19bR** by oxidation with various oxidizing agents in the same manner as **18b** and **18b'**. Compound **19b** was purified from the mixture by recrystallization from ethanol, while **19b'** (present in a small amount) was only identified in the reaction mixture by ^{1}H-NMR spectroscopy.

Compound ***19b***. Mp 284–286 °C. IR ν cm^{-1} 3360, 1700, 1570; ^{1}H-NMR (DMSO-d_6) δ: 10.84 (s, 1H, NH); 8.84 (d, 1H, H-9); 8.40 (d, 1H, H-6); 8.26 (d, 1H, H-2); 7.65 (dd, 1H, H-7); 6.86 (d, 1H, H-3); 2.19 (s, 3H, CH_3).

Compound ***19b'***. ^{1}H-NMR (DMSO-d_6) δ: 10.68 (s, 1H, NH); 8.81 (d, 1H, H-9); 8.32 (d, 1H, H-2); 7.92 (d, 1H, H-6); 7.72 (dd, 1H, H-7); 7.29 (d, 1H, H-3); 2.21 (s, 3H, CH_3).

7-Acetylaminopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***16b*** *and 7-acetylaminopyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide* ***16b'***
These compounds were obtained from **16bR** by oxidation with various oxidizing agents in the same manner as **19b** and **19b'**. Compounds **16b** and **16b'** were not separable and were only identified by ^{1}H-NMR spectroscopy of the reaction mixture.

Compound ***16b***. ^{1}H-NMR (DMSO-d_6) δ: 10.84 (s, 1H, NH); 8.84 (d, 1H, H-6); 8.32 (m, 2H, H-2 and H-9); 8.14 (dd, 1H, H-8); 6.88 (d, 1H, H-3); 2.19 (s, 3H, CH_3).

Compound ***16b'***. ^{1}H-NMR (DMSO-d_6) δ: 10.45 (s, 1H, NH); 8.44 (d, 1H, H-2); 8.26 (d, 1H, H-9); 7.90 (dd, 1H, H-8); 7.20 (d, 1H, H-6); 7.28 (d, 1H, H-3); 2.21 (s, 3H, CH_3).

General procedure for synthesis of ***15d***, ***18d***, ***15dR*** *and* ***18dR***
A suspension of **16d**, **19d**, **16dR** or **19dR** (100 mg, 0.310 mmol) in 50 mL of 6 N hydrochloric acid was refluxed for 2 days. After cooling, the residue was treated with 10% sodium hydroxide and the precipitate was filtered and recrystallized.

3-Bromo-7-aminopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***15d***. This was obtained from **16d**. TLC eluent chloroform/ethyl acetate 7:3; orange crystals; yield 55%. IR ν cm^{-1} 3500–3380, 1570; ^{1}H-NMR (DMSO-d_6) δ: 8.31 (s, 1H, H-2); 8.15 (d, 1H, H-9); 7.45 (d, 1H, H-6); 7.38 (dd, 1H, H-8); 6.22 (bs, 2H, NH_2).

3-Bromo-8-aminopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***18d***. This was obtained from **19d**. TLC eluent chloroform/ethyl acetate 7:3; yellow crystals; yield 75%. IR ν cm^{-1} 3420, 3380–3260; ^{1}H-NMR (DMSO-d_6) δ: 8.30 (s, 1H, H-2); 8.10 (d, 1H, H-6); 7.25 (s, 2H, NH_2); 7.15 (d, 1H, H-9); 6.90 (dd, 1H, H-7).

3-Bromo-7-aminopyrazolo[5,1-c][1,2,4]benzotriazine ***15dR***. This was obtained from **16dR**. TLC eluent chloroform/ethyl acetate 7:3; orange crystals; yield 55%. IR ν cm^{-1} 3340–3220; ^{1}H-NMR (DMSO-d_6) δ: 8.40 (s, 1H, H-2); 8.29 (d, 1H, H-9); 7.61 (d, 1H, H-6); 7.58 (dd, 1H, H-8); 6.20 (bs, 2H, NH_2).

3-Bromo-8-aminopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***18dR***. This was obtained from **19dR**. TLC eluent dichloromethame/methanol 10:0.5; yellow crystals; yield 80%. IR ν cm^{-1} 3420, 3360–3240; ^{1}H-NMR (DMSO-d_6) δ: 8.41 (s, 1H, H-2); 8.21 (d, 1H, H-6); 7.38 (s, 2H, NH_2); 7.21 (d, 1H, H-9); 7.08 (dd, 1H, H-7).

Pharmacology

In vitro inhibition of [^{3}H]flunitrazepam binding
[^{3}H]Flunitrazepam binding assays on bovine celebral cortex were carried out as described in the literature [23].

Pharmacological methods
The experiments were carried out on adult male Swiss–Webster mice, body weight 22–28 g, and Wistar rats, body weight 200–210 g, from the Morini S Polo breeding farm. Fifteen mice or four rats were housed per cage. The cages were taken to the experimental room 24 h before the experiment, for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum. After administration of substances, the animals' gross behaviour was observed for 30 min.

Rotarod test
The apparatus for the rotarod test [24] 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 30 cm long rod is divided into five equal sections by six 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 using PTZ (Sigma) at a dose (75 mg/kg sc) that induced convulsion in 90.3% of control mice. The animals were treated po with the substances under test or with DAZ 30 min before administration of PTZ and observed thereafter for 30 min. The number of mice that did not develop convulsions was counted and the percentage of non-convulsant-treated mice was compared to that of controls by means of the χ^2 test.

Plus–maze test
The apparatus consists of an elevated (52 cm from the floor) cross-shaped, metal platform with two (opposite) open arms and two arms protected by walls of the same material (50 × 10 × 40 cm). The base of each of the four arms was covered with black rubber in order to allow the animals to walk on a non-slippery surface. The anxiolytic effect of the substances was assessed by placing the rats in the middle of the maze facing one of the open arms 30 min after the pharmacological treatment, and letting them free to explore the platform for 5 min. The percentage entries in the open arm over the total number of entries and the cumulative time spent in the open arms were evaluated.

References

1 Doble A, Martin IL (1992) *TIPS* 13, 76–81
2 Haefely W, Martin JR, Schoch P (1990) *TIPS* 11, 452–456
3 Hollinshead SP, Trudell ML, Skolnick P, Cook JM (1990) *J Med Chem* 33, 1062–1069
4 Arauzo HD, Evoniuk GE, Skolnick P, Cook JM (1991) *J Med Chem* 34, 1754–1756
5 Zhang W, Koehler KF, Harris B, Skolnick P, Cook JM (1994) *J Med Chem* 37, 745–757
6 Martin MJ, Trudell ML, Arauzo HD et al (1992) *J Med Chem* 35, 4105–4117
7 Allen MS, Yun-Chou T, Trudell ML et al (1990) *J Med Chem* 33, 2343–2357
8 Trudell Ml, Lifer SL, Yun-Chou T et al (1990) *J Med Chem* 33, 2412–2420
9 Zhang W, Koehler KF, Zhang P, CooK JM (1995) *Drug Design Discovery* 12, 193–248
10 Yokoyama N, Ritter B, Neubert AD (1982) *J Med Chem* 25, 337–339
11 Costanzo A, Guerrini G, Bruni F, Selleri S (1994) *J Heterocycl Chem* 31, 1369–1376
12 Lindley JM, McRobbie IM, Meth-Chan O, Suschitzky H (1980) *J Chem Soc, Perkin Trans I* 982–994
13 Mangini A, Colonna M (1938) *Gazz Chim Ital* 68, 708–717
14 Zabrowski DL, Moormann AE, Beck KR Jr (1988) *Tetrahedron Lett* 29, 4501–4504
15 Sanger DJ, (1985) *Life Sci* 36, 1503–1513
16 Skerritt JH, Johnston GAR, Braestrup C, (1982) *Eur J Pharmacol* 86, 299–301
17 Braestrup C, Nielsen M, Honorè T, Jensen L, Petersen EN (1983) *Neuropharmacology* 22, 1451–1457
18 Da Settimo A, Primofiore G, Da Settimo F *et al* (1994) *Drug Design Discovery* 11, 307–328
19 Fryer RJ, Zhang P, Rios R, Zi-Qing G, Basile AS, Skolnick P (1993) *J Med Chem* 36, 1669–1673
20 Dantzer R, Perio A (1982) *Eur J Pharmacol* 81, 655–658
21 Hart MY, Sander JWAS, Meinardi H, Nutt DJ, Shorvon SD (1991) *The Lancet* 337, 744
22 Rathouska J, Kubova H, Mares P, Vorlicek J (1993) *Pharmacol J Bioch Behav* 44, 581–586
23 Colotta V, Cecchi L, Catarzi D et al (1993) *Il Farmaco* 48, 1051–1063
24 Vaught JL, Pelley K, Costa LG, Setler P, Enna SG (1985) *Neuropharmacology* 24, 211–216