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Laboratory note

Benzodiazepine receptor ligands – Part II. Synthesis and biological evaluation of pyrazolo[5,1-*c*][1,2,4]benzotriazine 4-oxide

Annarella Costanzo[a]*, Gabriella Guerrini[a], Fabrizio Bruni[a], Giovanna Ciciani[a], Silvia Selleri[a], Silvia Cappelletti[a], Barbara Costa[b], Claudia Martini[b], Antonio Lucacchini[b]

[a]*Dipartimento di Scienze Farmaceutiche, Università degli Studi di Firenze, Via Gino Capponi 9, 50121 Firenze, Italy*
[b]*Istituto Policattedra di Discipline Biologiche, Università degli Studi di Pisa, Via Bonanno 6, 56100 Pisa, Italy*

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Abstract – A new series of 3-, 8-substituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 4-oxides **3** were synthesized and their benzodiazepine receptor (BZR) affinities were evaluated in vitro in comparison with their 5-oxide isomers **2**. The 4-oxide compounds **3c,m,n,o** showed a better receptor affinity than their corresponding 5-oxide isomers, with an efficacy trend of antagonist/partial inverse agonist. From a structure–affinity relationship point of view some insight in the role played by N-4 and N-oxide is gained.
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pyrazolo[5,1-*c*][1,2,4]benzotriazine 4-oxide / benzodiazepine receptor / receptor binding / antagonist profile / partial inverse agonist profile

1. Introduction

As part of a program directed towards the search for non-benzodiazepine (non-BZ) ligands to benzodiazepine receptor (BZR), we recently identified a series of pyrazolo[5,1-*c*][1,2,4]benzotriazines **1** and 5-oxide derivatives **2**, which showed a different profile to the BZR and in vivo test [1, 2]. From binding data we found that the substances with good affinity for BZR (K_i range 35–93 nM) were the 5-oxide compounds, bearing at the 3-position bromine or ethoxycarbonyl group and at the 8-position chlorine or small lipophilic group (such as ethoxy-, methyl-). The pharmacological profile of these compounds, evaluated by GABA ratio (GR), was from agonist (GR 1.61–2.67) for 3-ethoxycarbonyl derivatives, to partial agonist for 3-bromo derivatives (GR 1.14–1.31).

On the basis of the proposed agonist pharmacophore models [3–7], consisting of two hydrogen bond donor sites (H_1 and H_2), three lipophilic regions (L_1, L_2 and L_3) and two regions of repulsive steric interaction (S_1 and S_2), we postulated 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. The 5-oxide group and the ethoxycarbonyl group can reinforce N-4 binding. Both the lipophilic regions L_1 and L_2 could be occupied by the aromatic ring of benzotriazine moieties and by small lipophilic groups at the 8-position, respectively. The partial agonist trend generally observed suggests that the L_3 region should not be involved [2].

In order to evaluate the role of N-4 and N-oxide groups on the receptor affinity and then confirm the proposed ligand–receptor interaction hypothesis, the synthesis of pyrazolo[5,1-*c*][1,2,4]benzotriazine 4-oxides **3**, isomers of the previously reported 5-oxides **2** [1, 2], are reported.

2. Chemistry

In order to directly obtain the 4-oxide derivatives, we have tried to isomerize our 5-oxides, as reported in the literature for acyclic azoxycompounds [8, 9]. However, the attempts carried out to isomerize the 5-oxides failed. In fact **2a,f,h**, if treated with sulphuric acid, gave only the reduced compounds **1a,f,h** (see footnotes to *table I*). The chemical behaviour of the azoxycompounds, by treating with acid agents, is also reported by other authors [10].

*Correspondence and reprints

On the other hand, in our previous paper [2], the oxidation of 8-amino, 8-acetylamino and 7-acetylamino[5,1-*c*][1,2,4]benzotriazines led us to identify the 4-oxide derivatives by ^{1}H-NMR spectroscopy, in the reaction mixture with its 5-oxide isomer. These pyrazolo[5,1-*c*][1,2,4]benzotriazine 4-oxides represent the only examples reported in literature.

Consequently, the oxidation of pyrazolo[5,1-*c*]-[1,2,4]benzotriazines **1** appeared as the only possible route to obtain the desired 4-oxide compounds **3** (see *figure 1*). The pyrazolo[5,1-*c*][1,2,4]benzotriazine **1a** and some 3-, 7- and 8-substituted derivatives were treated with various oxidizing agents: oxone, hydrogen peroxide/acetic anhydride and/or acetic acid.

Some observations about the influence of substituents, from oxidation of the pyrazolo[5,1-*c*]-[1,2,4]benzotriazine system, arise.

The substitution at the 3-position selectively orientates the oxidation at N-5, independently from oxidizing agents used; in fact the oxidation of **1b,c,i** (see footnotes at *table I*) afforded only the 5-oxide derivatives **2b,c,i**, previously described [1, 2].

The 3-unsubstituted pyrazolo[5,1-*c*][1,2,4]benzotriazines (**1a,d,e,g,h** [2], **1l**), if treated with oxone or hydrogen peroxide/acetic acid always gave a mixture of 4- and 5-oxides (**2a**, **3a**, **2d,e**, **3d,e**, **2g,h**, **3g,h**, **2l** and **3l**), these latter in greater amounts. The 4-oxides **3a,d,e** were separated from their 5-oxide isomers by chromatography, while this method was unsuccessful for the 8-substituted 4-oxides **3g,h,l** and they were identified only by ^{1}H-NMR in reaction mixture.

As reported in [2] the structure of 4-oxide derivatives was assigned by comparison of the ^{1}H-NMR spectral data of **3** with those of the corresponding 5-oxide derivatives **2**, and deoxyderivatives **1**. The chemical shift of the H-3 proton is diagnostic, in fact it appears to be more influenced by the presence of an oxygen atom than the other protons (H-2, H-9, H-8 or H-7). For example the H-3 proton of 8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine [2] **1h**, appears at 7.42 ppm while the same protons are shifted at 6.75 ppm in its 5-oxide derivative **2h** [1, 2] and at 7.22 ppm in its N-oxide isomer **3h**. Moreover the chemical shift of the H-6 proton is strongly influenced

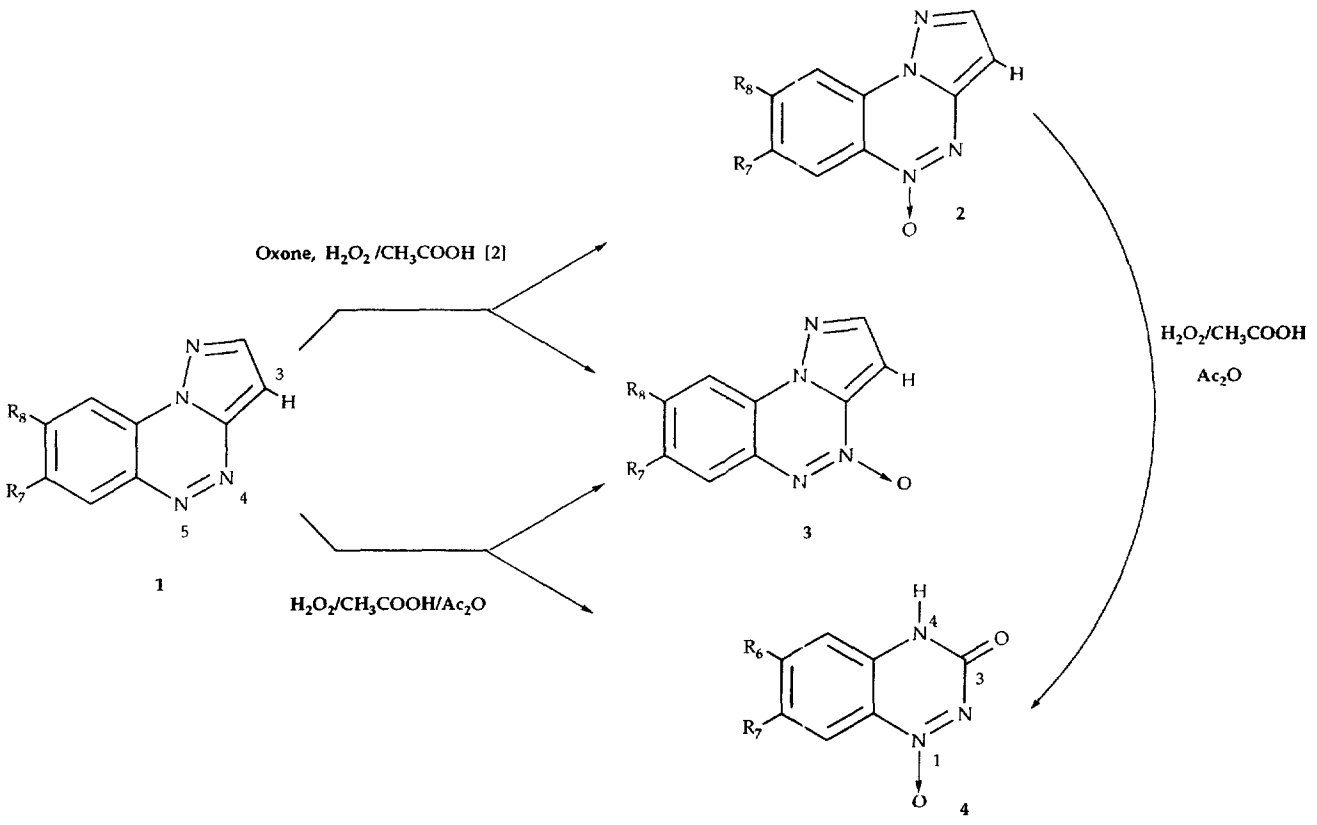
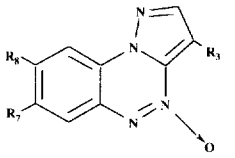


Figure 1.

Table I. Chemical data for pyrazolo[5,1-*c*][1,2,4]benzotriazine 4-oxides **3**.



Compound[a]	R3	R7	R8	Formula (MW)	M.p. (°C) (recrystallization solvent)[b]
3a	H	H	H	$C_9H_6N_4O$ (186.16)	133–134 (Isopropyl ether)
3c	Br	H	H	$C_9H_5N_4OBr$ (264.97)	186–187 (EtOH)
3d	H	CH_3	H	$C_{10}H_8N_4O$ (200.22)	203–205 (Cyclohexane)[c]
3e	H	Cl	H	$C_9H_5N_4OCl$ (220.61)	247–249 (Cyclohexane)[c]
3g	H	H	CH_3	$C_{10}H_8N_4O$ (200.22)	164–166 (EtOH)
3h	H	H	Cl	$C_9H_5N_4OCl$ (220.61)	156–157 (EtOH)
3l	H	H	OEt	$C_{11}H_{10}N_4O_2$ (230.92)	178–179 (Cyclohexane)
3m	Br	H	CH_3	$C_{10}H_7N_4OBr$ (280.01)	186–187 (EtOH)
3n	Br	H	Cl	$C_9H_4N_4OClBr$ (299.85)	206–607 (CHX/*i*-Pr_2O)[d]
3o	Br	H	OEt	$C_{11}H_9N_4O_2Br$ (309.03)	217–218 (*i*-Propanol)
3p	Br	Br	OEt	$C_{11}H_{10}N_4O_2Br_2$ (388.84)	230–231 (Cyclohexane)

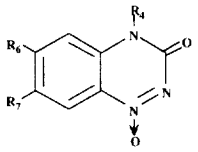
[a]See [1, 2] for compounds of series **2** (5-oxides): **2a** ($R_3 = R_8 = R_7 = H$), **2b** ($R_3 = NO_2$, $R_8 = R_7 = H$), **2c** ($R_3 = Br$, $R_8 = R_7 = H$), **2d** ($R_3 = R_8 = H$, $R_7 = CH_3$), **2e** ($R_3 = R_8 = H$, $R_7 = Cl$), **2f** ($R_3 = R_8 = H$, $R_7 = NO_2$), **2g** ($R_3 = R_7 = H$, $R_8 = CH_3$), **2h** ($R_3 = R_7 = H$, $R_8 = Cl$), **2i** ($R_3 = COOEt$, $R_7 = H$, $R_8 = Cl$), **2l** ($R_3 = R_7 = H$, $R_8 = OEt$) and for series **1** (reduct compounds) **1a** ($R_3 = R_8 = R_7 = H$), **1b** ($R_3 = NO_2$, $R_8 = R_7 = H$), **1c** ($R_3 = Br$, $R_8 = R_7 = H$), **1d** ($R_3 = R_8 = H$, $R_7 = CH_3$), **1e** ($R_3 = R_8 = H$, $R_7 = Cl$), **1f** ($R_3 = R_8 = H$, $R_7 = NO_2$), **1g** ($R_3 = R_7 = H$, $R_8 = CH_3$), **1h** ($R_3 = R_7 = H$, $R_8 = Cl$), **1i** ($R_3 = COOEt$, $R_7 = H$, $R_8 = Cl$) and **1l** ($R_3 = R_7 = H$, $R_8 = OEt$): new compound, see experimental protocols. [b]If purified by column chromatography, eluent: ethyl acetate/cyclohexane 1:2. [c]If purified by column chromatography, eluent: cyclohexane/ethyl acetate/isopropyl ether 2:1:0.5. [d]Cyclohexane/isopropyl ether.

by the displacement of the oxygen atom (8.50 ppm of the 5-oxide derivative **2h**, with respect to 7.90 ppm of the N-oxide isomer **3h**). Since the other protons (H-2, H-7, H-9) have minor shifts, the oxidation at the 1-position can be ruled out and the 4-oxide structure is confirmed for isomer **3h**. A similar trend appears in the spectra of the other compounds belonging to the **3** series.

Finally, the desired 4-oxide compounds **3a,d,e,g,h,l** were easily obtained by treatment of the corresponding pyrazolo[5,1-*c*][1,2,4]benzotriazines **1** with hydrogen peroxide/acetic anhydride/acetic acid after a long refluxing time (*table I* and *figure 1*). Fortunately under these conditions, the performed 4-oxides are stable, while the 5-oxide isomers decomposed, after demolition of the pyrazole ring, to give the 3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxides **4a,d,e,g,h,l** (see *table II*). These latter compounds, that were less soluble than the corresponding 4-oxides, were easily separated. Under the same conditions the 7-nitropyrazolo[5,1-*c*][1,2,4]benzotriazine **1f** yielded only the 5-oxide derivative **2f**, which gave 7-nitro-3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide **4f** by heating at reflux for a long time, and the 4-oxide derivative was identified only in trace by TLC.

Since the formation of 3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide was experimentally evaluated to derive from 5-oxide compounds, a presumed demolition mechanism was postulated (in fact in the reaction mixture of 4- and 5- oxides, monitored by TLC, the

Table II. Chemical data for 3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxides **4**.



Compound[a]	R4	R6	R7	Formula (MW)	M.p. (°C) (recrystallization solvent)
4a	H	H	H	$C_7H_5N_3O_2$ (163.13)	223–224 d (MeOH)
4a'	CH_3	H	H	$C_8H_7N_3O_2$ (177.15)	236–237 d (EtOH)
4d	H	H	CH_3	$C_8H_7N_3O_2$ (177.15)	224–225 d (EtOH)
4e	H	H	Cl	$C_7H_4N_3O_2Cl$ (197.55)	239–240 d (*i*-Propanol)
4f	H	H	NO_2	$C_7H_4N_4O_4$ (208.10)	220–221 d (Ethyl acetate)
4g	H	CH_3	H	$C_8H_7N_3O_2$ (177.15)	237–238 d (EtOH)
4g'	CH_3	CH_3	H	$C_9H_9N_3O_2$ (190.17)	199–200 d (EtOH)
4h	H	Cl	H	$C_7H_4N_3O_2Cl$ (197.55)	238–239 d (EtOH)
4h'	CH_3	Cl	H	$C_8H_6N_3O_2Cl$ (211.59)	240–241 d (H_2O)
4l	H	OEt	H	$C_9H_9N_3O_2$ (207.17)	237–238 d (EtOH)

[a]See [11] for compounds **4a**, **4a'** and **4e**.

5-oxide compound vanishes and there appears the spot of a new product). Probably, under the strong reaction condition, the 5-oxide function permits the generation of a dioxidated intermediate (e.g. 1,5-dioxide) which makes the pyrazole ring susceptible to demolition.

The structures of the 3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxides **4** were assigned by ^{1}H-NMR and IR spectral data, chemical properties and have been further supported by elemental analyses and literature data [11]. In the IR spectra of these acid compounds, (soluble in sodium hydrogen carbonate) three bands appear at about 3240, 1690 and 1530 cm^{-1}, attributable to NH, CO and N-oxide groups respectively. In the ^{1}H-NMR spectra only aromatic protons of the benzotriazine moiety appear, while the signal of a proton, exchangeable with deuterium oxide, is not always revealed. Compounds **4a** and **4e** have identical chemical properties and melting points as the 3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxides reported in the literature and obtained by another synthetic route [11].

The presence of the NH group was confirmed by synthesis of the 4-methyl-3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide **4a'** [11], **4g'** and **4h'** by reaction of **4a**, **4g** and **4h** with iodomethane.

Since the synthesis of the 3-ethoxycarbonyl derivative 4-oxide was impossible, the pyrazolo[5,1-*c*]-[1,2,4]benzotriazine 4-oxide **3a** and its 8-substituted derivatives **3g** and **3h** were converted into 3-bromo derivatives, **3c**, **3m** and **3n**, useful to complete the structure–activity relationship study. To obtain these 3-bromo derivatives it was necessary to work with an excess of bromine in chloroform and to add silver acetate as catalyst, differently from the 5-oxide compounds. Under these conditions the 8-ethoxypyrazolo [5,1-*c*][1,2,4]benzotriazine 4-oxides **3l** yielded a mixture of 3- and 3,7-dibromoderivatives **3o** and **3p**, purified by TLC.

All the new 4-oxides **3** are listed in *table I*. These compounds, with some of the previously reported pyrazolo[5,1-*c*][1,2,4]benzotriazines **1** and 5-oxides **2** [1, 2], are useful for the chemical and biological discussion.

3. Biological results and discussion

3.1. Binding studies

The ability of pyrazolo[5,1-*c*][1,2,4]benzotriazine 4-oxides **3** to interact with the BZR 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% of ethanol to dissolve the pyrazolo[5,1-*c*][1,2,4]benzotriazine 4-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 studies [12–14]. Binding data for all new compounds are shown in *table III*.

Compounds **3m**, **3n** and **3o** possess better affinity for BZR (K_i range 10.5–15.6 nM) than their 5-oxide isomers **2m**, **2n** and **2o** (K_i 53.2, 120.0 and 36.9 nM respectively) [2]. The efficacy trend of the new compounds **3** was typical for the partial inverse agonist/antagonist (GABA ratio range 0.75–0.85). The compounds 3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxides, **4a,a',e,g,h,h',l** show no affinity for BZR. Comparing the tested compounds with the corresponding 5-oxide isomers, the following observations arise in order to better define the proposed pharmacophore model [2].

Table III. (a) BZR ligand affinity of pyrazolo[5,1-*c*][1,2,4]benzotriazine 4-oxides **3** and 5-oxides **2**.

Compound[a]	R3	R7	R8	% inhibition[b]	K_i (nM)[c]	GABA ratio[d]
2a	H	H	H	0	–	–
3a	H	H	H	15 ± 0.80	–	–
2c	Br	H	H	62 ± 5.30	–	–
3c	Br	H	H	86.7 ± 7.30	712.4 ± 6.00	0.88 ± 0.05
2m	Br	H	CH_3	99.5 ± 8.30	53.2 ± 4.40	1.29 ± 0.10
3m	Br	H	CH_3	98 ± 7.00	10.51 ± 0.80	0.75 ± 0.03
2n	Br	H	Cl	98 ± 6.52	120.0 ± 7.91	1.31 ± 0.11
3n	Br	H	Cl	88 ± 6.80	13.56 ± 1.04	0.85 ± 0.02
2o	Br	H	OEt	98 ± 8.52	36.9 ± 3.20	1.14 ± 0.08
3o	Br	H	OEt	100 ± 6.10	15.6 ± 0.95	0.84 ± 0.04

[a]For series **2** see [2].

(b) BZR ligand affinity of 3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxides **4**.

Compound	R4	R6	R7	% inhibition[b]	K_i (nM)[c]	GABA ratio[d]
4a	H	H	H	17 ± 1.00	–	–
4a'	CH_3	H	H	6.4 ± 0.32	–	–
4e	H	H	Cl	0	–	–
4g	H	CH_3	H	3.5 ± 0.51	–	–
4h	H	Cl	H	0	–	–
4h'	CH_3	Cl	H	42 ± 2.83	–	–
4l	H	OEt	H	0	–	–

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

The parent compound 4-oxide **3a**, as its 5-oxide isomer **2a**, lacks receptor affinity; this behaviour confirms the importance of substitution on the pyrazolo[5,1-*c*][1,2,4]benzotriazine system, to interact with the BZR site. On the other hand, between the compounds bearing a bromine at the 3-position, **2c** and **3c**, only the 4-oxide derivative **3c** displays moderate BZR affinity (K_i 712.4 nM) and an efficacy trend of antagonist/partial inverse agonist (GR 0.88).

Even in this new series of 4-oxide derivatives **3** the concomitant presence of a substituent at the 8-position appears to be important to increase the BZR affinity, in agreement with the previous finding on 5-oxides compounds **2** [2]. However, from the data listed in *table III*, the most important observation is that the displacement of the N-oxide group from the 5-position to the 4-position increases the BZR affinity by 2–9 times.

The N-oxide group in the 5-oxide series **2** is thought to reinforce the interacion of N-4 at the receptor site [2]; this same N-oxide group in the 4-oxide series **3** seems to be the one which is directly interested in the interaction with the donor site (H_1/H_2) on the receptor protein.

Another observation arising from *table III* is that the GABA ratio value of the new compounds **3m,n,o**, with high affinity to BZR, suggests that these could function as antagonist/partial inverse agonist. From the literature it is known that for antagonist/inverse agonist efficacies a liphophilic group, a proton donor group and a proton acceptor group are necessary [6, 15]. Since our new compounds lack the proton donor group, it may be suggested that this group seems to be unnecessary for antagonist/inverse agonist activity, in agreement with other studies [16, 17].

The synthesis of 3-substituted compounds, different from 3-bromine derivatives, was not possible and therefore the importance of various substituents in this new series of 4-oxides **3** on receptor affinity and pharmacological profile was not evaluated. Moreover the lack of affinity to BZR of 3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxides **4** might confirm the importance of pyrazole N-1 for receptor interaction; in fact this atom is absent in the **4** series, different from pyrazolo[5,1-*c*][1,2,4]benzotriazines **1** and their 5- and 4-oxide derivatives (series **2** and **3** respectively).

4. Experimental protocols

4.1. Chemistry

The structures of all compounds were supported by the 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 the Dipartimento Farmaco-Chimico-Tecnologico of the University of Siena, Italy, with a Perkin-Elmer, model 240C, Elemental Analyzer and results (C, H, N) 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. The column chromatography was performed using Machery-Nagel 5160 Duren MN-Kieselgel 60 silica-gel.

*4.1.1. 8-Ethoxypyrazolo[5,1-c][1,2,4]benzotriazine **1l***

A suspension of **2l** [1] (1.0 mmol) in acetic acid (20 mL) and hydrochloric acid (1.0 mL) was magnetically stirred at room temperature. A large excess of zinc-dust (8.0 mmol in three portions) and 2 x 10 mL of acetic acid were added. The reaction was monitored by TLC; the zinc residue was filtered off and the final solution was evaporated in vacuo. The residue was treated with water, filtered and recrystallized. Yellow crystals, yield 53%; m.p. 151–152 °C after recrystallization from ethanol. ^{1}H-NMR ($CDCl_3$) δ: 8.50 (d, 1H, H-6); 8.18 (d, 1H, H-2); 7.73 (d, 1H, H-9); 7.35 (m, 2H, H-7, H-3); 4.32 (q, 2H, CH_2); 1.56 (t, 3H, CH_3). Anal. $C_{11}H_{10}N_4O_2$ (C, H, N).

*4.1.2. General procedure for the synthesis of pyrazolo[5,1-c]-[1,2,4]benzotriazine 4-oxides **3a,d,e,g,h,l** and 3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxides **4a,d–h,l***

Compounds **1a,d–h** [2] and **1l** (0.80 mmol) were refluxed in a solution of acetic acid (30 mL), acetic anhydride (15 mL) and hydrogen peroxide (20 mL) for 5–8 h. The solution turned from dark yellow to lemon yellow and a precipitate was obtained. This consisted mainly of demolition product **4** that was filtered and purified by recrystallization or by treatment with sodium hydroxide and then with hydrochloric acid. The desired 4-oxide compound **3** remained in solution that was then evaporated in vacuo and the residue, washed with sodium hydroxide, recrystallized or purified by chromatography column (compounds **3a,d,e** were obtained from oxidation mixture with corresponding 5-oxides **2a,d,e** [1, 2] and separated by column chromatography).

*4.1.3. Pyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide **3a***

This compound was obtained from **1a** [2], after evaporation of the solution. Yellow crystals, yield 30%. ^{1}H-NMR ($CDCl_3$) δ: 8.36 (dd, 1H, H-9); 8.12 (d, 1H, H-2); 8.00 (dd, 1H, H-6); 7.76 (dt, 1H, H-8); 7.66 (dt, 1H, H-7); 7.22 (d, 1H, H-3).

*4.1.4. 7-Methylpyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide **3d***

This compound was obtained from **1d** [2], after evaporation of the solution. Yellow crystals, yield 40%. ^{1}H-NMR ($CDCl_3$) δ: 8.24 (d, 1H, H-9); 8.10 (d, 1H, H-2); 7.76 (d, 1H, H-6); 7.56 (dd, 1H, H-8); 7.22 (d, 1H, H-3); 2.57 (s, 3H, CH_3).

*4.1.5. 7-Chloropyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide **3e***

This compound was obtained from **1e** [2], after evaporation of the solution. Yellow crystals, yield 35%. ^{1}H-NMR ($CDCl_3$) δ: 8.30 (d, 1H, H-9); 8.12 (d, 1H, H-2); 7.96 (d, 1H, H-6); 7.80 (dd, 1H, H-8); 7.24 (d, 1H, H-3).

*4.1.6. 8-Methylpyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide **3g***

This compound was obtained from **1g** [2], after evaporation of the solution. Yellow crystals, yield 45% ^{1}H-NMR ($CDCl_3$) δ: 8.15 (d, 1H, H-9); 8.10 (d, 1H, H-2); 7.82 (d, 1H, H-6); 7.48 (dd, 1H, H-7); 7.20 (d, 1H, H-3); 2.60 (s, 3H, CH_3).

4.1.7. 8-Chloropyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide ***3h***

This compound was obtained from **1h** [2], after evaporation of the solution. Yellow crystals, yield 38%. ^{1}H-NMR ($CDCl_3$) δ: 8.34 (d, 1H, H-9); 8.12 (d, 1H, H-2); 7.90 (d, 1H, H-6); 7.62 (dd, 1H, H-7); 7.22 (d, 1H, H-3).

4.1.8. 8-Ethoxypyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide ***3l***

This compound was obtained from **1l**, after evaporation of the solution. Yellow crystals, yield 43%. ^{1}H-NMR ($CDCl_3$) δ: 8.10 (d, 1H, H-2); 7.88 (d, 1H, H-6); 7.71 (d, 1H, H-9); 7.20 (m, 2H, H-7, H-3); 4.26 (q, 2H, CH_2); 1.55 (t, 3H, CH_3).

4.1.9. General procedure for synthesis of ***3c***, ***3m***, ***3n*** *and* ***3p***

To a solution of a suitable pyrazolo[5,1-*c*][1,2,4]benzotriazine 4-oxide (0.50 mmol) in chloroform (50 mL) an excess of bromine (1.0 mL) and silver acetate (1.0 g) were added. The reaction was kept at 25–30 °C and monitored by TLC. The yellow precipitate of silver bromine was filtered off and the solution was evaporated in vacuo. The residue of bromo-derivatives was purified by recrystallization.

4.1.10.3-Bromopyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide ***3c***

This compound was obtained from **3a**. Yellow crystals, yield 48%. ^{1}H-NMR ($CDCl_3$) δ: 8.28 (d, 1H, H-9); 8.08 (s, 1H, H-2); 7.92 (d, 1H, H-6); 7.73 (m, 2H, H-8, H-7).

4.1.11. 3-Bromo-8-methylpyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide ***3m***

This compound was obtained from **3g**. Dark yellow crystals, yield 35%. ^{1}H-NMR ($CDCl_3$) δ: 8.09 (m, 2H, H-9, H-2); 7.81 (d, 1H, H-6); 7.45 (dd, 1H, H-7); 2.20 (s, 3H, CH_3).

4.1.12. 3-Bromo-8-chloropyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide ***3n***

This compound was obtained from **3h**. Yellow crystals, yield 38%. ^{1}H-NMR ($CDCl_3$) δ: 8.28 (d, 1H, H-9); 8.10 (s, 1H, H-2); 7.86 (d, 1H, H-6); 7.58 (dd, 1H, H-7).

4.1.13. 3,7-Dibromo-8-ethoxypyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide ***3p***

This compound was obtained from **3l**. Yellow crystals, yield 45%. ^{1}H-NMR ($CDCl_3$) δ: 8.11 (s, 1H, H-2); 8.09 (s, 1H, H-6); 7.62 (s, 1H, H-9); 4.30 (q, 2H, CH_2); 1.60 (t, 3H, CH_3).

4.1.14. 3-Bromo-8-ethoxypyrazolo[5,1-c][1,2,4]benzotriazine 4-oxide ***3o***

This compound was obtained from **3l** (80 mg), using 0.35 mL of bromine and 0.4 g of silver acetate in chloroform (50 mL), with **3p** and was separated from this latter compound by column chromatography (toluene/ethyl acetate, 8:2 as eluent): **3p** faster runner banding, **3o** second runner banding. Yellow crystals from isopropanol, yield 43%. ^{1}H-NMR ($CDCl_3$) δ: 8.06 (s, 1H, H-2); 7.84 (s, 1H, H-6); 7.66 (s, 1H, H-9); 7.20 (dd, 1H, H-7); 4.30 (q, 2H, CH_2); 1.60 (t, 3H, CH_3).

4.1.15. 3-Oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide ***4a*** *[11]*

This compound was obtained from **1a** [2] as precipitated product. Yellow crystals, yield 58%; IR ν cm^{-1}: 3410, 1670; ^{1}H-NMR (DMSO-d_6) δ: 12.6 (bs, 1H, NH); 8.14 (d, 1H, H-8); 7.84 (t, 1H, H-6); 7.38 (m, 2H, H-7, H-5).

4.1.16. 7-Methyl-3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide ***4d***

This compound was obtained from **1d** [2] as precipitated product. Yellow crystals, yield 60%; IR ν cm^{-1}: 3400, 1680; ^{1}H-NMR (DMSO-d_6) δ: 7.99 (d, 1H, H-8); 7.65 (dd, 1H, H-6); 7.28 (d, 1H, H-5); 2.30 (s, 3H, CH_3).

4.1.17. 7-Chloro-3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide ***4e*** *[11]*

This compound was obtained from **1e** [2] as precipitated product. Yellow crystals, yield 48%; IR ν cm^{-1}: 1710; ^{1}H-NMR (DMSO-d_6) δ: 8.15 (d, 1H, H-8); 7.86 (dd, 1H, H-6); 7.36 (d, 1H, H-5).

4.1.18. 7-Nitro-3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide ***4f***

This compound was obtained from **1f** [2] as single product in the solution. After evaporation, the residue was washed with ether and then recrystallized. Red crystals, yield 48%; IR ν cm^{-1}: 1710; ^{1}H-NMR (DMSO-d_6) δ: 13.1 (bs, 1H, NH); 8.77 (d, 1H, H-8); 8.55 (dd, 1H, H-6); 7.51 (d, 1H, H-5).

4.1.19. 6-Methyl-3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide ***4g***

This compound was obtained from **1g** [2] as precipitated product. Yellow crystals, yield 43%; IR ν cm^{-1}: 3340, 1680; ^{1}H-NMR (DMSO-d_6) δ: 10.6 (bs, 1H, NH); 8.02 (d, 1H, H-8); 7.17 (m, 2H, H-7, H-5); 2.42 (s, 3H, CH_3).

4.1.20. 6-Chloro-3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide ***4h***

This compound was obtained from **1h** [2] as solution in a mixture with **3h**. After evaporation the residue was treated with ethyl acetate, which dissolved compound **3h**, while compound **4h** was filtered and recrystallized. Yellow crystals, yield 38%; IR ν cm^{-1}: 1720; ^{1}H-NMR (DMSO-d_6) δ: 12.8 (bs, 1H, NH); 8.11 (d, 1H, H-8); 7.38 (m, 2H, H-7, H-5).

4.1.21. 6-Ethoxy-3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide ***4l***

This compound was obtained from **1l** in the same manner as **4h**; yield 50%; IR ν cm^{-1}: 3400, 1700; ^{1}H-NMR (DMSO-d_6) δ: 8.05 (d, 1H, H-8); 6.90 (dd, 1H, H-7); 6.72 (d, 1H, H-5); 4.15 (q, 2H, CH_2); 1.35 (t, 3H, CH_3).

4.1.22. General procedure for synthesis of ***4a'*** *[11],* ***4g'***,***h'***

To the suitable 3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide (0.50 mmol) dissolved in anhydrous dimethylformamide (10 mL), iodomethane (0.05 mL, 1:1.5) and anhydrous potassium carbonate (70 mg) were added. The reaction was monitored by TLC (ethyl acetate/cyclohexane 2:1 as eluent). A solution or a precipitate was obtained and after the normal workup, the residue was recrystallized from the suitable solvent.

4.1.23. 4-Methyl-3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide ***4a'*** *[11]*

This compound was obtained from **4a**; yellow crystals, yield 40%; IR ν cm^{-1}: 1680; ^{1}H-NMR ($CDCl_3$) δ: 8.35 (d, 1H, H-8); 7.84 (t, 1H, H-6); 7.39 (m, 2H, H-7, H-5); 3.74 (s, 3H, N-CH_3).

4.1.24. 4,6-Dimethyl-3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide ***4g'***

This compound was obtained from **4g**; yellow crystals, yield 45%; IR ν cm^{-1}: 1680; ^{1}H-NMR ($CDCl_3$) δ: 8.21 (d, 1H, H-8); 7.18 (m, 2H, H-7, H-5); 3.70 (s, 3H, N-CH_3); 2.58 (s, 3H, CH_3).

4.1.25. 6-Chloro-4-methyl-3-oxo-3,4-dihydro-1,2,4-benzotriazine 1-oxide ***4h'***

This compound was obtained from **4h**; yellow crystals, yield 46%; IR ν cm^{-1}: 1680; ^{1}H-NMR ($CDCl_3$) δ: 8.28 (d, 1H, H-8); 7.39 (d, 1H, H-5); 7.32 (dd, 1H, H-7); 3.70 (s, 3H, N-CH_3).

4.2. Pharmacology

4.2.1. In vitro inhibition of [^{3}H]-flunitrazepam binding

[^{3}H]-flunitrazepam binding assays on bovine celebral cortex were carried out as described in [18].

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