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Benzodiazepine receptor ligands III. Synthesis and biological evaluation of 2- and/or 3-substituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides

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Abstract

A new series of 2- and/or 3-substituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides and their 8-chloro derivatives were synthesized, and their benzodiazepine receptor (BZR) affinities were evaluated in vitro in comparison to lead compound 3-ethoxycarbonyl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**29**) [1,2]. None of the new compounds showed significant affinity for BZR. On the basis of a pharmacophore/receptor model suggested for lead compound **29**, some hypotheses to explain the inactivity of new derivatives are discussed.

Keywords: 2- and/or 3-substituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide; Benzodiazepine receptor; Receptor binding

1. Introduction

In our studies on tricyclic heterocycles containing a pyrazole moiety with potential CNS activity, we focused on central benzodiazepine receptor (BZR) ligands [1,2]. In addition to the classic 1,4-benzodiazepines (1,4-BZs), a large number of ligands belonging to different chemical families have been shown to bind with high affinity to the $GABA_A$–BZR. BZ ligands act as allosteric modulators of the $GABA_A$/chloride channel supramolecular complex and have been classified as agonists, inverse agonists and antagonists if they enhance, reduce or have no influence on the $GABA_A$-gated chloride current.

Agonists exhibit anxiolytic, anticonvulsant, muscle relaxant and sedative/hypnotic activity, the inverse agonists cause the opposite behavioral effects, such as anxiogenesis and proconvulsant action, the antagonists inhibit the action of both agonists and inverse agonists.

Recently, we reported the synthesis and evaluation of the BZR affinity of a series of pyrazolo[5,1-*c*][1,2,4]-benzotriazines and corresponding 5-oxides 3-, 7- and 8-substituted with a variety of groups different for lipophilic and electronic features and amounts of steric hindrance [2]. From binding data we found that the best ligand was the 3-ethoxycarbonyl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (K_i 35 nM), even if compounds bearing at the 3-position bromine and at the 8-position also a chlorine or a small lipophilic group such as ethoxy- or methyl group showed good affinity. The pharmacological profile of these new ligands, evaluated by GABA-ratio (GR), was from agonists for 3-ethoxycarbonyl derivatives (GR 1.61–2.67) to partial agonists for 3-bromine derivatives (GR 1.14–1.31) [2].

As part of a wider program of SAR studies, in order to explain the BZR binding of the new pyrazolo[5,1-*c*]-[1,2,4]benzotriazine ligands, it is very interesting to focus the role of the substituent at 2- and/or 3-position on receptor affinity and on intrinsic activity in the presence of a favorable substituent at the 8-position.

The importance of the rotable aromatic rings in BZ-ligands to recognize and activate BZR and the rela-

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tionship between the full agonism spectrum activity and the presence on the ligands of aromatic rings interacting at lipophilic pocket L_3, have recently been outlined [3–5].

In this paper, the synthesis and evaluation of BZR affinity of a new series of pyrazolo[5,1-*c*][1,2,4]-benzotriazine 5-oxides and their 8-chloro derivatives substituted at the 2- and the 3-positions with aromatic or heteroaromatic groups with different lipophilic, steric, and electronic features, are reported. These aromatic rings might change the affinity and intrinsic activity of this class of BZR ligands.

In addition to the 2-aromatic and 2-heteroaromatic derivatives, the 2-methyl- and 2-ethoxycarbonyl-5-oxide derivatives were also synthesized to verify if the 2-position of pyrazole, not previously investigated by us, tolerates any kinds of substituents.

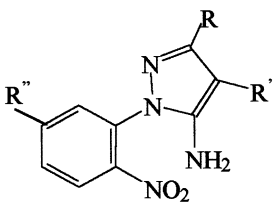
2. Chemistry

The new compounds described here, are listed in Tables 1 and 2.

In order to synthesize the 2- or 3-substituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides, the suitable 5-aminopyrazoles were obtained following two different synthetic methods (see Schemes 1 and 2). The aminopyrazoles **1**–**6**, **8**–**28** and the iminopyrazole **7**, were cyclized to the triazine system (**1a**–**28a**) in 10% sodium hydroxide following a known procedure [1,2].

2-Phenyl-3-cyanopyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**1a**), 2-phenyl-3-cyano-8-chloropyrazolo[5,1-*c*]-[1,2,4]benzotriazine 5-oxide (**2a**), and 2-methyl-3-cyano-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**9a**), after a normal work up to obtain the corresponding amides (**1b**, **2b** and **9b**) and acids (**1c**, **2c** and **9c**), were

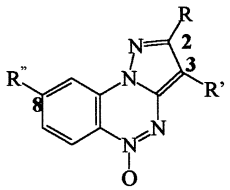
Table 1
Chemical data for 5-aminopyrazoles



Comp. [a]	R	R′	R″	Formula (MW)	M.p.(°C) (recrystallization solvent) yield
1	Ph	CN	H	$C_{16}H_{11}N_5O_2$ (305.31)	174–174 (*i*-propanol) 30%
2	Ph	CN	Cl	$C_{16}H_{11}N_5O_2Cl$ (339.75)	234–235 (ethanol) 35%
3	Ph	H	Cl	$C_{15}H_{11}N_4O_2Cl$ (314.74)	180–182 (ethanol/water) 68%
4	2-CH_3OPh	H	Cl	$C_{16}H_{13}N_4O_3Cl$ (344.74)	153–155 (ethanol/water) 38%
5	2-Thienyl	H	Cl	$C_{13}H_9N_4O_2SCl$ (320.75)	200–201 (ethanol) 50%
6	3-Thienyl	H	Cl	$C_{13}H_9N_4O_2SCl$ (320.75)	213–214 (ethanol) 70%
7 [b]	2-Furyl	H,H	Cl	$C_{13}H_9N_4O_3Cl$ (304.69)	211–212 (ethanol) 30%
8	COOEt	Ph	Cl	$C_{18}H_{15}N_4O_4Cl$ (386.80)	192–193 (ethanol) 58%
9	Me	CN	Cl	$C_{11}H_8N_5O_2Cl$ (277.67)	186–187 (ethanol/water) 50%
10	Me	H	Cl	$C_{10}H_9N_4O_2Cl$ (252.65)	157–158 (water) 45%
11	H	Ph	H	$C_{15}H_{12}N_4O_2$ (280.3)	153–154 (lit. 153–155) (ethanol) 64%
12	H	Ph	Cl	$C_{15}H_{11}N_4O_2Cl$ (314.74)	168–169 (ethanol/water) 50%
13	H	2-FPh	H	$C_{15}H_{11}N_4O_2F$ (298.28)	133–134 (ethanol/water) 60%
14	H	2-FPh	Cl	$C_{15}H_{10}N_4O_2ClF$ (332.72)	129–130 (ethanol/water) 69%
15	H	3-FPh	H	$C_{15}H_{11}N_4O_2F$ (298.28)	166–167 (ethanol/water) 72%
16	H	3-FPh	Cl	$C_{15}H_{10}N_4O_2ClF$ (332.72)	174–175 (ethanol/water) 70%
17	H	4-FPh	H	$C_{15}H_{11}N_4O_2F$ (298.28)	141–142 (ethanol/water) 80%
18	H	4-FPh	Cl	$C_{15}H_{10}N_4O_2ClF$ (332.72)	128–129 (ethanol/water) 72%
19	H	2-ClPh	H	$C_{15}H_{11}N_4O_2Cl$ (314.74)	132–133 (ethanol/water) 79%
20	H	2-ClPh	Cl	$C_{15}H_{10}N_4O_2Cl_2$ (349.18)	129–130 (ethanol/water) 80%
21	H	3-ClPh	H	$C_{15}H_{11}N_4O_2Cl$ (314.74)	155–156 (ethanol/water) 67%
22	H	3-ClPh	Cl	$C_{15}H_{10}N_4O_2Cl_2$ (349.18)	156–157 (ethanol/water) 64%
23	H	4-ClPh	H	$C_{15}H_{11}N_4O_2Cl$ (314.74)	153–154(lit. 154–155)(ethanol/water)80%
24	H	4-ClPh	Cl	$C_{15}H_{10}N_4O_2Cl_2$ (349.18)	147–148 (ethanol/water) 72%
25	H	3,4-diClPh	H	$C_{15}H_{10}N_4O_2Cl_2$ (349.18)	142–143 (ethanol/water) 63%
26	H	3,4-diClPh	Cl	$C_{15}H_9N_4O_2Cl_3$ (383.62)	179–180 (ethanol/water) 73%
27	H	2-Py	H	$C_{14}H_{11}N_5O_2$ (281.28)	162–164 (ethanol/water) 22%
28	H	2-Py	Cl	$C_{14}H_{10}N_5O_2Cl$ (315.72)	148–150 (ethanol/water) 39%

[a] For compounds **11** and **23** see Ref. [19].
[b] Isolated as iminoderivative.

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



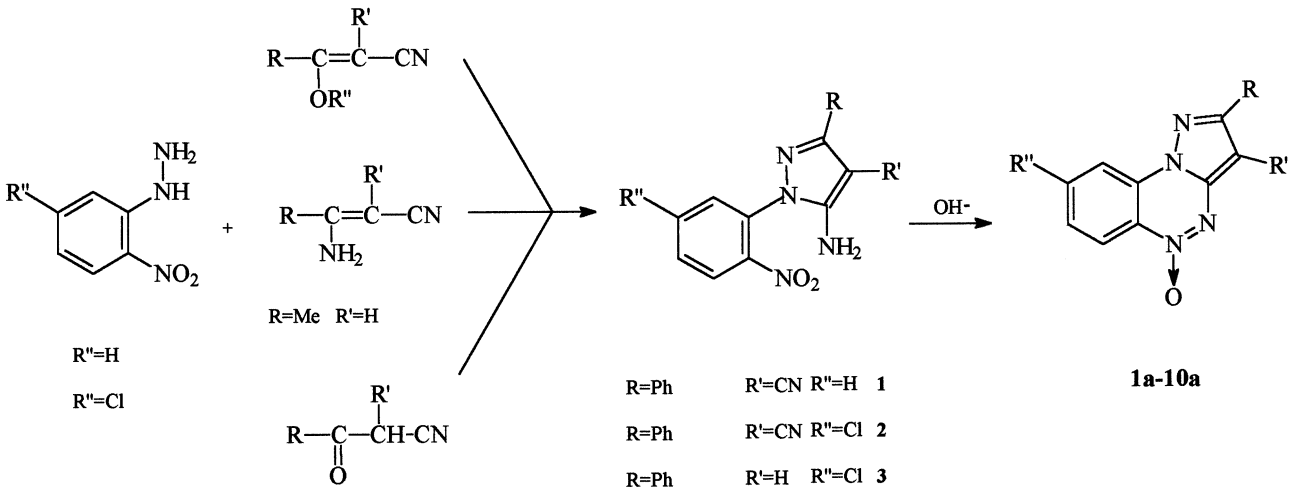
Comp. [a]	R (**2**)	R′ (**3**)	R″ (**8**)	Formula (MW)	M.p. (°C) (recrystallization solvent) yield
1a	Ph	CN	H	$C_{16}H_9N_5O$ (287.29)	239–240 (methoxyethanol) 77%
2a	Ph	CN	Cl	$C_{16}H_8N_5OCl$ (321.75)	281–282 (methoxyethanol) 62%
1b	Ph	$CONH_2$	H	$C_{16}H_{11}N_5O_2$ (305.21)	271–272 (methoxyethanol) 50%
2b	Ph	$CONH_2$	Cl	$C_{16}H_{10}N_5O_2Cl$ (339.73)	273–274 (methoxyethanol) 38%
1c	Ph	COOH	H	$C_{16}H_{10}N_4O_3$ (306.28)	253–255 (acetic acid 50%) 25%
2c	Ph	COOH	Cl	$C_{16}H_9N_4O_3Cl$ (340.80)	255–256 (methoxyethanol) 70%
1d	Ph	COOEt	H	$C_{18}H_{14}N_4O_3$ (334.33)	239–240 (ethanol) 40%
2d	Ph	COOEt	Cl	$C_{18}H_{13}N_4O_3Cl$ (368.78)	190–191 (ethanol) 50%
3a	Ph	H	Cl	$C_{15}H_9N_4OCl$ (296.72)	268–269 (methoxyethanol) 69%
3e	Ph	Br	Cl	$C_{15}H_8N_4O_2ClBr$ (375.74)	293–294 (methoxyethanol) 95%
4a	2-CH_3OPh	H	Cl	$C_{16}H_{11}N_4O_2Cl$ (326.73)	282–284 (methoxyethanol) 74%
4f	2-CH_3O-5-BrPh	Br	Cl	$C_{16}H_9N_4O_2ClBr_2$ (484.55)	269–270 (methoxyethanol) 50%
5a	2-thienyl	H	Cl	$C_{13}H_7N_4OSCl$ (302.73)	232–233 (methoxyethanol) 65%
5e	2-thienyl	Br	Cl	$C_{13}H_6N_4OSClBr$ (381.53)	257–258 (methoxyethanol) 55%
5g	3,4,5-triBrthien-2-yl	Br	Cl	$C_{13}H_3N_4OSClBr_4$ (618.34)	262–263 (ethanol) 30%
6a	3-thienyl	H	Cl	$C_{13}H_7N_4OSCl$ (302.73)	235–236 (methoxyethanol) 65%
6e	3-thienyl	Br	Cl	$C_{13}H_6N_4OSClBr$ (381.53)	244–245 (methoxyethanol) 53%
7a	2-furyl	H	Cl	$C_{13}H_7N_4O_2Cl$ (286.69)	223–224 (ethanol) 85%
7f	3-Brfur-2-yl	Br	Cl	$C_{13}H_5N_4O_2ClBr_2$ (444.25)	260–261 (methoxyethanol) 45%
7h	3-Brfur-2-yl	H	Cl	$C_{13}H_6N_4O_2ClBr$ (365.64)	226–227 (ethanol) 20%
8a	COOH	Ph	Cl	$C_{16}H_9N_4O_3Cl$ (340.80)	285–286 (ethanol) 77%
8d	COOEt	Ph	Cl	$C_{18}H_{13}N_4O_3Cl$ (368.78)	214–215 (ethanol) 78%
9a	Me	CN	Cl	$C_{11}H_6N_5OCl$ (259.65)	239–240 (isopropanol) 46%
9b	Me	$CONH_2$	Cl	$C_{11}H_8N_5O_2Cl$ (295.69)	283–284 (methoxyethanol) 40%
9c	Me	COOH	Cl	$C_{11}H_7N_4O_3Cl$ (278.65)	288–290 (isopropanol) 37%
9d	Me	COOEt	Cl	$C_{13}H_{11}N_4O_3Cl$ (306.60)	200–201 (isopropanol) 70%
10a	Me	H	Cl	$C_{10}H_7N_4OCl$ (234.63)	199–200 (isopropanol) 60%
10e	Me	Br	Cl	$C_{10}H_6N_4OClBr$ (313.54)	206–209 (isopropanol) 65%
11a	H	Ph	H	$C_{15}H_{10}N_4O$ (262.28)	216–217(lit. 213–214)(ethanol) 82%
12a	H	Ph	Cl	$C_{15}H_9N_4OCl$ (296.72)	229–230 (methoxyethanol) 50%
13a	H	2-FPh	H	$C_{15}H_9N_4OF$ (280.27)	228–229 (ethanol) 92%
14a	H	2-FPh	Cl	$C_{15}H_8N_4OClF$ (314.71)	229–230 (ethanol) 73%
15a	H	3-FPh	H	$C_{15}H_9N_4OF$ (280.27)	235–236 (ethanol) 75%
16a	H	3-FPh	Cl	$C_{15}H_8N_4OClF$ (314.71)	244–245 (ethanol) 50%
17a	H	4-FPh	H	$C_{15}H_9N_4OF$ (280.27)	243–244 (methoxyethanol) 86%
18a	H	4-FPh	Cl	$C_{15}H_8N_4OClF$ (314.71)	263–264 (methoxyethanol) 78%
19a	H	2-ClPh	H	$C_{15}H_9N_4OCl$ (296.72)	203–204 (methoxyethanol) 78%
20a	H	2-ClPh	Cl	$C_{15}H_8N_4OCl_2$ (331.16)	247–248 (methoxyethanol) 56%
21a	H	3-ClPh	H	$C_{15}H_9N_4OCl$ (296.72)	236–237 (methoxyethanol) 70%
22a	H	3-ClPh	Cl	$C_{15}H_8N_4OCl_2$ (331.16)	256–257 (methoxyethanol) 54%
23a	H	4-ClPh	H	$C_{15}H_9N_4OCl$ (296.72)	240–241(lit. 236–37) (methoxyethanol) 70%
24a	H	4-ClPh	Cl	$C_{15}H_8N_4OCl_2$ (331.16)	269–270 (methoxyethanol) 70%
25a	H	3,4-diClPh	H	$C_{15}H_8N_4OCl_2$ (331.16)	249–251 (methoxyethanol) 77%
26a	H	3,4-diClPh	Cl	$C_{15}H_7N_4OCl_3$ (365.60)	280–238 (methoxyethanol) 76%
27a	H	2-Py	H	$C_{14}H_9N_5O$ (263.62)	251–252 (methoxyethanol) 84%
28a	H	2-Py	Cl	$C_{14}H_8N_5OCl$ (297.70)	274–275 (methoxyethanol) 43%

[a] For compounds **11a** and **23a** see Ref. [19].

converted into the ethyl ester with thionyl chloride and absolute ethanol (**1d**, **2d** and **9d**). Compounds **3a**–**7a** and **10a** were transformed in 3-bromoderivatives, required by the SAR study. By treatment with a solution of an equimolar amount of bromine in chloroform, compound **3e** from **3a**, the dibromoderiva-

R=Ph R'=CN R''=Me [13]

R=Me R'=CN R''=Et [14]



R''=H

R''=Cl

R=Me R'=H

R=Ph R'=H [15]

R=2-MeOPh R'=H [15]

R=2-Thienyl R'=H [15]

R=3-Thienyl R'=H [15]

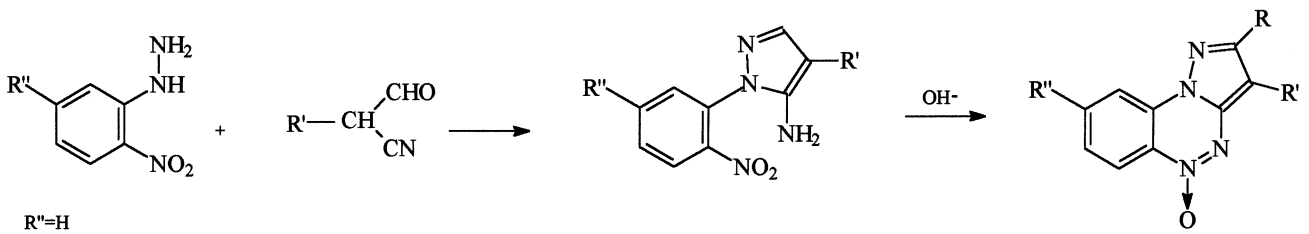
R=2-Furyl R'=H [16]

R=COOEt R'=Ph [17]

R=Ph	R'=CN	R''=H	**1**
R=Ph	R'=CN	R''=Cl	**2**
R=Ph	R'=H	R''=Cl	**3**
R=2-MeOPh	R'=H	R''=Cl	**4**
R=2-Thienyl	R'=H	R''=Cl	**5**
R=3-Thienyl	R'=H	R''=Cl	**6**
R=2-Furyl	R'=H	R''=Cl	**7**
R=COOEt	R'=Ph	R''=Cl	**8**
R=Me	R'=CN	R''=Cl	**9**
R=Me	R'=H	R''=Cl	**10**

1a-10a

Scheme 1.



R''=H

R''=Cl

R'=Ph [18]

R'=2-FPh

R'=3-FPh

R'=4-FPh

R'=2-ClPh [18]

R'=3-ClPh [18]

R'=4-ClPh [18]

R'=3,4-Cl_2Ph [18]

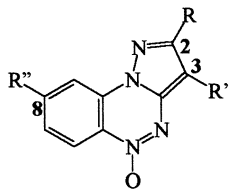
R'=2-Py

R'=Ph	R''=H	**11**
R'=Ph	R''=Cl	**12**
R'=2-FPh	R''=H	**13**
R'=2-FPh	R''=Cl	**14**
R'=3-FPh	R''=H	**15**
R'=3-FPh	R''=Cl	**16**
R'=4-FPh	R''=H	**17**
R'=4-FPh	R''=Cl	**18**
R'=2-ClPh	R''=H	**19**
R'=2-ClPh	R''=Cl	**20**
R'=3-ClPh	R''=H	**21**
R'=3-ClPh	R''=Cl	**22**
R'=4-ClPh	R''=H	**23**
R'=4-ClPh	R''=Cl	**24**
R'=3,4-diClPh	R''=H	**25**
R'=3,4-diClPh	R''=Cl	**26**
R'=Py	R''=H	**27**
R'=Py	R''=Cl	**28**

11a-28a

Scheme 2.

Table 3
BZR ligand affinity of pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides



Comp	R (**2**)	R′ (**3**)	R″ (**8**)	% Inhibition[a]	K_i (nM)[b]	GR[c]
1a	Ph	CN	H	3 ± 0.15		
2a	Ph	CN	Cl	3.9 ± 0.16		
1d	Ph	COOEt	H	6.1 ± 0.52		
2d	Ph	COOEt	Cl	10 ± 0.91		
3a	Ph	H	Cl	0		
3e	Ph	Br	Cl	0		
4a	2-CH_3OPh	H	Cl	0		
4f	2-CH_3O-5-BrPh	Br	Cl	0		
5e	2-thienyl	Br	Cl	47.2 ± 2.79		
6e	3-thienyl	Br	Cl	0		
7a	2-furyl	H	Cl	31.7 ± 2.52		
7f	3-Brfur-2-yl	Br	Cl	0		
8d	COOEt	Ph	Cl	0		
9a	Me	CN	Cl	25 ± 1.90		
9d	Me	COOEt	Cl	61 ± 4.80		
10a	Me	H	Cl	41 ± 2.70		
10e	Me	Br	Cl	35 ± 1.80		
11a	H	Ph	H	62 ± 6.4		
12a	H	Ph	Cl	36.7 ± 2.8		
13a	H	2-FPh	H	0		
14a	H	2-FPh	Cl	0		
15a	H	3-FPh	H	0		
16a	H	3-FPh	Cl	0		
17a	H	4-FPh	H	8.6 ± 0.72		
18a	H	4-FPh	Cl	6 ± 0.42		
19a	H	2-ClPh	H	11 ± 0.93		
20a	H	2-ClPh	Cl	5.1 ± 0.40		
21a	H	3-ClPh	H	0		
22a	H	3-ClPh	Cl	0		
23a	H	4-ClPh	H	10.4 ± 0.73		
24a	H	4-ClPh	Cl	1 ± 0.05		
25a	H	3,4-diClPh	H	0		
26a	H	3,4-diClPh	Cl	4 ± 0.25		
27a	H	2-Py	H	20 ± 1.3		
28a	H	2-Py	Cl	35 ± 2.10		
29[d]	H	COOEt	Cl	99.8 ± 5.0	35 ± 2.0	1.91
30[d]	H	Br	Cl	98 ± 3.2	120 ± 4.5	1.31
31[d]	H	CN	Cl	96 ± 1.0	342 ±	1.37

[a] % Inhibition of specific [^{3}H]Ro15-1788 ([^{3}H]Flunitrazepam for **29**, **30**, **31**) binding at 10 μM concentration are means ± SEM of five determinations.
[b] K_i values are means ± SEM of five determinations.
[c] GR (GABA ratio) = IC_{50} (compound)/ + IC_{50} (compound + 10 μM GABA).
[d] Refs. [1,2].

tive **4f** from **4a**, compound **5e** from **5a**, **6e** from **6a**, and **10e** from **10a** were obtained. Tetrabromoderivative **5g** from **5a** was obtained, if the reaction was carried out with a molar ratio 1/5 (substrate/bromine). Compound **7a** treated with either an equivalent amount or an excess of bromine gave a mixture of three brominated compounds in both cases: the 2-(3-bromofur-2-yl)-3-bromo-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**7f**), the 2-(3-bromofur-2-yl)-8-chloropyrazolo-[5,1-*c*][1,2,4]benzotriazine 5-oxide (**7h**) (separates by column chromatography) and traces of 2-(fur-2-yl)-3-bromo-8-chloro-pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide, only identified by spectroscopical means in the reaction mixture.

3. Biological results and discussion

The BZR binding affinity of pyrazolo[5,1-*c*][1,2,4]-benzotriazine 5-oxides was evaluated by their ability to displace [^{3}H]Ro15-1788 from its specific binding in bovine brain membranes.

Binding data for all new compounds and of the reference compounds (**29**, **30**, **31**) [2] are listed in Table 3.

As shown in Table 3, the new synthesized pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides display insignificant or no affinity for BZR. From these results the following observations arise.

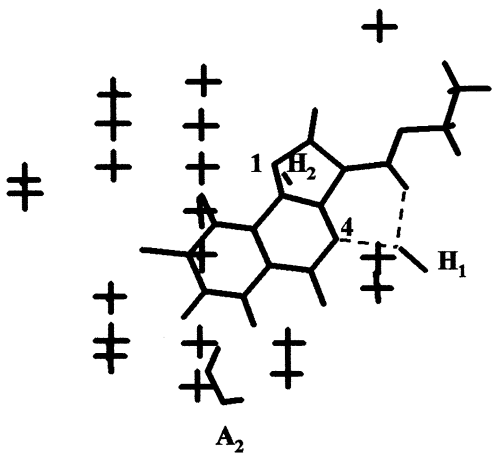


Fig. 1. Interaction of 3-ethoxycarbonyl-8-chloropyrazolo[5,1-*c*][1,2,4]-benzotriazine 5-oxide (**29**), at the proposed qualitative model for recognition at the BZR. Hydrogen bonds involving H_1 and H_2 by means N–4/CO and N-1 are labeled.

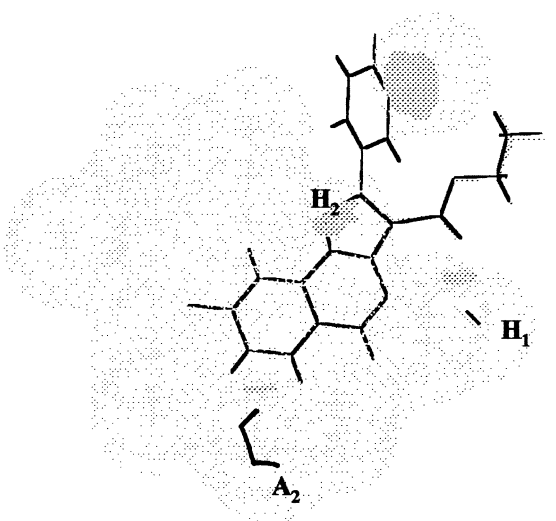


Fig. 2. Superimposition of 2-phenyl-3-ethoxycarbonyl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**2d**) (black line) and (**29**) (gray line). The hydrogen bond sites and an overlay between the 2-phenyl ring of **2d** and a steric region of the BZR protein are evidenced.

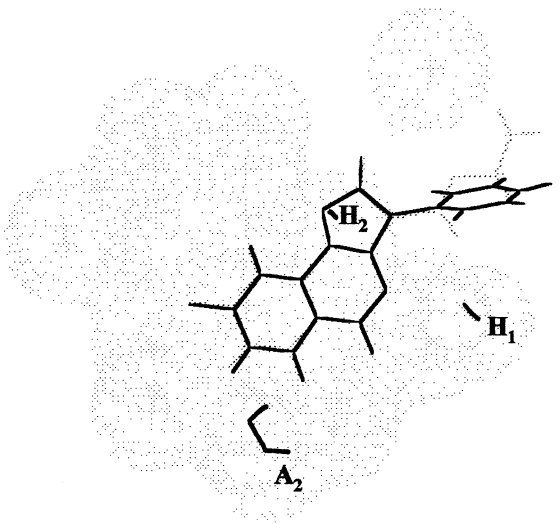


Fig. 3. Overlay of 3-phenyl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**12a**) (black line) and (**29**) (gray line). The lack of ethoxycarbonyl group at the 3-position does not support the hydrogen bond by H_1/N-4 and causes the loss of receptor affinity.

The introduction of a substituent as methyl-, ethoxycarbonyl-, heteroaryl- and aryl- at the 2-position (**1a**, **2a**, **1d**, **2d**, **3a**, **3e**, **4a**, **4f**, **5e**, **6e**, **7f**, **8d**, **9a**, **9d**, **10a** and **10e**) is generally unfavorable for BZR recognition. This is also true for those compounds bearing, at the 3-position, a bromine or ethoxycarbonyl group (**1d**, **2d**, **3e**, **4f**, **5e**, **6e**, **7f**, **9d** and **10e**) which were found to be the suitable substituents for receptor binding (see reference compounds **29**, **30**, **31** and Ref. [2]).

The replacement of the 3-ethoxycarbonyl group in the lead compound **29** by a phenyl ring (**11a**, **12a**) or *ortho*-, *meta*-, *para*-substituted phenyl ring with electron-withdrawing atoms (**13a**–**25a**) or by a 2-pyridine (**27a**–**28a**) eliminates the receptor recognition, even though the planar tricyclic moiety of pyrazolo[5,1-*c*]-[1,2,4]benzotriazine 5-oxide is unchanged.

Comparison of BZR affinity of **29**, **30** and **31** with new compounds suggests that the lack of affinity of these latter compounds could be due to a steric problem and/or an inefficient formation of the hydrogen bond with the receptor protein.

In the attempt to explain the better fitting of **29** with BZR, a qualitative pharmacophore/receptor model was carried out by active analog approach [6]. A total of 12 molecules were selected as the training set. These compounds span a great range of affinity values to BZR (1 nM $< K_i <$ 1000 nM) and belong to chemically different classes such as β-carboline, diazepines, pyrazoloquinolines, pyrazolopyrimidines. Among the molecules of the training set used for building the qualitative BZR model, there is the lead molecule 3-ethoxycarbonyl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**29**). From this qualitative model, three hydrogen bond

points and areas of negative steric interaction have been characterized, as previously reported. These pharmacophoric descriptors can be designated as H_1 and H_2 for the hydrogen donor sites, S_1 and S_2 for the repulsive steric interaction regions and L_1, L_2 and L_3 for the lipophilic regions [3,7–12] (see Figs. 1–3 for the sake of clarity).

As shown in Fig. 1, the affinity of 29 ($K_i = 35$ nM, GR = 1.91 [2]) may be explained since it interacts at the receptor site with N-1 and N-4 by means of hydrogen bond involving H_2 and H_1 donor sites, respectively. Moreover, the hydrogen bond N-4/H_1 is also reinforced by the presence of the carbonyl group of the estereal chain, forming a three-centered hydrogen bond (hydrogen bond length CO/H_1 2.10 Å). Furthermore, the 5-oxide group can reinforce N-4 binding. The alignment of ligand **29** in the pharmacophore/receptor model shows that the ethoxycarbonyl group seems to occupy the lipophilic region L_1/L_2.

According to this model, the above suggested hypotheses to explain the inactivity of new compounds are plausible.

In fact, choosing compounds **2d** and **12a** as representative of the series of the 2- or 3-arylpyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides and overlaying them on compound **29**, the following remarks on the SAR can be made:

- The presence of an overlay between the 2-phenyl ring of **2d** and a steric hindrance region of the BZR protein causes a distortion of the molecule with loss of alignment for N-4/H_1 and CO/H_1, required for hydrogen bond formation (Fig. 2).
- In the superimposition of **12a** and **29**, although the hindrance of the 3-phenyl ring and the ethoxycarbonyl group are almost the same, the loss of receptor affinity may be explained by the lack of an ethoxycarbonyl group which exhibits a supporting function to N-4/H_1 hydrogen bond formation. Moreover, the 3-phenyl ring, which would cause the shift of the whole molecule, does not allow the 5-oxide/H_1 hydrogen bond to be formed to give its contribution to the receptor affinity (Fig. 3). On the other hand, this situation is permitted when a less hindering group, such as a bromine, is present in the 3-position, as in 3-bromo-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**30**) (see Table 3).

In conclusion, structure–activity relationships (SARs) indicate that these pyrazolo[5,1-*c*][1,2,4]-benzotriazine ligands bind to BZR with N-1 and N-4 by means of a hydrogen bond involving the H_2 and H_1 donor sites, respectively, the substituent at 3-position, critical for steric hindrance and lipophilic feature, fits into lipophilic region L_1/L_2. In particular, if this substituent is an ethoxycarbonyl group, as in the lead molecule **29**, the lone pair orientation of carbonyl oxygen of the estereal chain can reinforce the receptor binding by means of a three-centered hydrogen bond. The 5-oxide group also has an important role to reinforce this hydrogen bond, as in compound **30**.

The substituent at the 2-position can interact with a steric hindrance region and does not permit the ligand interaction with the receptor protein.

Current studies are now in progress to verify the validity of the above proposed pharmacophore model and in particular to confirm the fitting of the 3-substituent in the lipophilic pockets (L_1/L_2). Moreover, to better define the features, size and location of these lipophilic pockets (L_1/L_2), the synthesis of new pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides bearing in the 3-position estereal, ethereal, ketonic group or isoster rings to esteric function, are now in progress.

4. Experimental

4.1. Chemistry

The structure 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 are uncorrected. Elemental analyses were performed by the laboratories of Dipartimento Farmaco-Chimico-Tecnologico of the University of Siena, Italy, with a Perkin–Elmer, model 240C, Elemental Analyzer and their results are within $\pm 0.4\%$ of theoretical values. The purity of samples was determined by means of TLC, which was performed using Machery–Nagel Duren, Alugram silica-gel plates.

4.2. General procedure for synthesis of 3-oxopropanenitriles

A suspension of 50% sodium hydride in mineral oil was added to anhydrous toluene (300 ml) in a 1 l round bottomed flask. After addition of tert-amyl alcohol (2 ml) the mixture was heated at 70°C and a solution of a commercially available suitable acetonitrile derivative (200 mmol) and ethyl formate (200 mmol, 17 ml) in anhydrous toluene (50 ml), was added dropwise over a period of 60 min. After 6 h of stirring and heating at 70–80°C, the resulting solid was allowed to stand overnight. The mixture was treated with ice-cold water (400 ml) and the aqueous phase was separated and washed with diethyl ether.

Acidification with conc. hydrochloric acid caused the separation of an oil which was extracted with diethyl ether. The extracts were dried on anhydrous sodium sulfate, evaporated to dryness and a solid residue was obtained.

4.3. 2-(2′-Fluorophenyl)-3-oxopropanenitrile

This compound was obtained from 2-(2′-fluorophenyl)acetonitrile. Yellow crystals, yield 74%; m.p. 130°C after recrystallization from ethanol/water; IR ν (cm^{-1}): 2210, 1650; ^1H NMR (DMSO-d_6): δ 12.20 (s, 1H, OH, exch.), 7.84 (s, 1H, CHO), 7.72 (s, 1H, CH), 7.20–7.46 (m, 4H, Ph). *Anal.* C_9H_6NOF (C, 1H, N).

4.4. 2-(3′-Fluorophenyl)-3-oxopropanenitrile

This compound was obtained from 2-(3′-fluorophenyl)acetonitrile. Yellow crystals, yield 77%; m.p. 154°C after recrystallization from ethanol/water; IR ν (cm^{-1}): 2210, 1650; ^1H NMR (DMSO-d_6): δ 12.40 (s, 1H, OH, exch.), 8.16 (s, 1H, CHO), 7.76 (s, 1H, CH), 7.06–7.59 (m, 4H, Ph). *Anal.* C_9H_6NOF (C, 1H, N).

4.5. 2-(4′-Fluorophenyl)-3-oxopropanenitrile

This compound was obtained from 2-(4′-fluorophenyl)acetonitrile. Yellow crystals, yield 80%; m.p. 150–151°C after recrystallization from ethanol/water; IR ν (cm^{-1}): 2210, 1650; ^1H NMR (DMSO-d_6): δ 12.25 (s, 1H, OH, exch.), 8.08 (s, 1H, CHO), 7.65–7.76 (m, 2H, Ph, CH), 7.53–7.58 (m, 1H, Ph), 7.20–7.47 (m, 2H, Ph). *Anal.* C_9H_6NOF (C, 1H, N).

4.6. 2-(2-Pyridyl)-3-oxopropanenitrile

This compound was obtained from 2-(2-pyridyl)-acetonitrile. Yellow crystals, yield 67%; m.p. 184–188°C after recrystallization from ethyl acetate/cyclohexane; IR ν (cm^{-1}): 2190, 1630; ^1H NMR (DMSO-d_6): δ 9.08 (s, 1H, CHO), 8.28–8.34 (m, 1H, H-6 Py), 8.04–8.16 (m, 1H, H-5 Py), 7.18–7.34 (m, 2H, H-3 and H-4 Py). *Anal.* $C_8H_6N_2O$ (C, 1H, N).

*4.7. General procedure for the synthesis of **1**, **2**, **9** and **10***

A solution of suitable 5-nitrophenylhydrazine or 2-nitro-5-chlorophenylhydrazine (4.5 mmol), phenylmethoxymethylenemalononitrile [13], or ethylethoxymethylenemalononitrile [14], or 3-aminocrotononitrile (5.4 mmol) in ethanol (60 ml) with acetic acid as catalyst, was refluxed for 12 h and monitored by TLC. The final solution was evaporated and the residue was washed with diethyl ether and recrystallized by suitable solvent.

*4.8. 1-(2-Nitrophenyl)-3-phenyl-5-aminopyrazole-4-carbonitrile (**1**)*

From 2-nitrophenylhydrazine and phenylmethoxymethylenemalononitrile. Yellow crystals; TLC eluent cyclohexane/ethyl acetate 2/1; IR ν (cm^{-1}): 3405–3238, 2217, 1360; ^1H NMR ($CDCl_3$): δ 8.10 (d, 1H, H-3′), 7.75 (m, 3H, H-4′, H-5′, H-6′ and 2H 3-Ph), 7.40 (m, 3H, 3-Ph), 4.58 (s, 2H, NH_2).

*4.9. 1-(2-Nitro-5-chlorophenyl)-3-phenyl-5-aminopyrazole-4-carbonitrile (**2**)*

From 2-nitro-5-chlorophenylhydrazine and phenylmethoxymethylenemalononitrile. Orange crystals; TLC eluent cyclohexane/ethyl acetate 2/1; IR ν (cm^{-1}): 3480–3100, 2210, 1370; ^1H NMR ($CDCl_3$): δ 8.08 (d, 1H, H-3′), 7.90 (m, 2H, 3-Ph), 7.68 (m, 2H, H-4′ and H-6′), 7.45 (m, 3H, 3-Ph), 4.51 (s, 2H, NH_2).

*4.10. 1-(2-Nitro-5-chlorophenyl)-3-methyl-5-aminopyrazole-4-carbonitrile (**9**)*

From 2-nitro-5-chlorophenylhydrazine and ethylethoxymethylenemalononitrile. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3410–3330, 2219; ^1H NMR ($CDCl_3$): δ 8.04 (d, 1H, H-3′), 7.64 (dd, 1H, H-4′), 7.62 (d, 1H, H-6′), 4.45 (bs, 2H, NH_2), 2.30 (s, 3H, CH_3).

*4.11. 1-(2-Nitro-5-chlorophenyl)-3-methyl-5-aminopyrazole (**10**)*

From 2-nitro-5-chlorophenylhydrazine and 2-aminocrotononitrile. Yellow crystals; TLC eluent toluene/ethyl acetate 8/2; IR ν (cm^{-1}): 3320–3280, 1360; ^1H NMR ($CDCl_3$): δ 7.94 (d, 1H, H-3′), 7.64 (dd, 1H, H-6′), 7.48 (d, 1H, H-4′), 5.50 (s, 1H, CH), 3.55 (bs, 2H, NH_2), 2.20 (s, 3H, CH_3).

*4.12. General procedure for the synthesis of **3**–**8**, **11**–**28***

A solution of the suitable aroylacetonitrile [15,16] or ethyl 2-oxo-3-phenyl-3-cyanopropionate [17] or 3-oxopropanenitrile 2-substituted (0.01 mmol) [18] and 2-nitro-5-chlorophenylhydrazine or 2-nitrophenylhydrazine (0.01 mmol), were dissolved in ethanol (100 ml) with conc. hydrochloric acid as catalyst. Compounds **27** and **28** were obtained from suitable oxopropanenitrile (0.91 mmol) using acetic acid as catalyst and the reaction time was 60 h. The final solution was evaporated to dryness and the residue, neutralized with 10% sodium hydroxide, was filtered and purified by a suitable solvent.

*4.13. 1-(2-Nitro-5-chlorophenyl)-3-phenyl-5-amino-pyrazole (**3**)*

From benzoylacetonitrile and 2-nitro-5-chlorophenylhydrazine. Yellow crystals; TLC eluent carbon tetrachloride/methanol 10/0.5; IR ν (cm^{-1}): 3420–3280, 1360; ^1H NMR ($CDCl_3$): δ 7.98 (d, 1H, H-3′), 7.75 (m, 3H, H-2, H-6 of 3-Ph, H-6′), 7.52 (dd, 1H, H-4′), 7.38 (m, 3H, H-3, H-4 and H-5 of 3-Ph), 6.02 (s, 1H, H-4), 3.68 (s, 2H, NH_2).

*4.14. 1-(2-Nitro-5-chlorophenyl)-3-(2-methoxyphenyl)-5-aminopyrazole (**4**)*

From 2-methoxybenzoylacetonitrile and 2-nitro-5-chlorophenylhydrazine. Yellow crystals; TLC eluent cyclohexane/ethyl acetate 2/1; IR ν (cm^{-1}): 3410–3300, 1340; ^1H NMR ($CDCl_3$): δ 7.92 (m, 2H, H-3′ and H-6 of 3-Ph), 7.74 (d, 1H, H-6′), 7.50 (dd, 1H, H-4′), 7.30 (t, 1H, H-4 of 3-Ph), 6.95 (t, 2H, H-3 and H-5 of 3-Ph), 6.30 (s, 1H, H-4), 3.90 (s, 3H, OCH_3), 3.68 (s, 2H, NH_2).

*4.15. 1-(2-Nitro-5-chlorophenyl)-3-(2-thienyl)-5-amino-pyrazole (**5**)*

From 2-thenoylacetonitrile and 2-nitro-5-chlorophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3400–3300, 1340; ^1H NMR ($CDCl_3$): δ 8.00 (d, 1H, H-3′), 7.71 (d, 1H, H-6′), 7.55 (dd, 1H, H-4′), 7.31 (dd, 1H, H-3″ thio), 7.25 (m, 1H, H-5″ thio.), 7.04 (t, 1H, H-4″ thio.), 5.95 (s, 1H, H-4), 3.65 (s, 2H, NH_2).

*4.16. 1-(2-Nitro-5-chlorophenyl)-3-(3-thienyl)-5-amino-pyrazole (**6**)*

From 3-thenoylacetonitrile and 2-nitro-5-chlorophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate 8/2; IR ν (cm^{-1}): 3400–3300, 1350; ^1H NMR ($CDCl_3$): δ 7.98 (d, 1H, H-3′), 7.72 (d, 1H, H-6′), 7.55 (m, 2H, H-4′ and H-2″ thio.), 7.45 (dd, 1H, H-5″ thio.), 7.32 (dd, 1H, H-4″ thio.), 5.95 (s, 1H, H-4), 3.65 (s, 2H, NH_2).

*4.17. 1-(2-Nitro-5-chlorophenyl)-3-(2-furyl)-4H-5-iminopyrazole (**7**)*

From 2-furoylacetonitrile and 2-nitro-5-chlorophenylhydrazine and isolated as iminopyrazole. Orange crystals; TLC eluent cyclohexane/ethyl acetate 1/2; IR ν (cm^{-1}): 3300, 1330; ^1H NMR ($CDCl_3$): δ 12.61(s, 1H, NH), 8.15 (d, 1H, H-3′), 8.03 (d, 1H, H-6′), 7.79 (d, 1H, H-3″ fur.), 6.95 (d, 1H, H-5″ fur.), 6.88 (dd, 1H, H-4′), 6.67 (dd, 1H, H-4″ fur.), 3.80 (s, 2H, CH_2).

*4.18. Ethyl 1-(2-Nitro-5-chlorophenyl)-4-phenyl-5-aminopyrazole-3-carboxylate (**8**)*

From 2-nitro-5-chlorophenylhydrazine and ethyl 2-oxo-3-phenyl-3-cyanopropionate. Yellow crystals; TLC eluent chloroform/methanol 10/1; IR ν (cm^{-1}): 3440–3360, 1710; ^1H NMR (DMSO-d_6): δ 8.25 (d, 1H, H-3′), 8.00 (d, 1H, H-6′), 7.90 (dd, 1H, H-4′), 7.25–7.35 (m, 5H, 4-Ph), 5.55 (bs, 2H, NH_2), 4.18 (q, 2H, CH_2), 1.15 (t, 3H, CH_3).

*4.19. 1-(2-Nitrophenyl)-4-phenyl-5-aminopyrazole (**11**)*

From 2-phenyl-3-oxopropanenitrile and 2-nitrophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate 2/1; IR ν (cm^{-1}): 3440–3300, 1360; ^1H NMR ($CDCl_3$): δ 8.00 (d, 1H, H-3′), 7.70 (m, 4H, H-3, H-5′, H-6′ and 1H of 4-Ph), 7.45 (m, 4H, 4-Ph), 7.26 (t, 1H, H-4′), 3.88 (s, 2H, NH_2).

*4.20. 1-(2-Nitro-5-chlorophenyl)-4-phenyl-5-amino-pyrazole (**12**)*

From 2-phenyl-3-oxopropanenitrile and 2-nitro-5-chlorophenylhydrazine. Yellow crystals; TLC eluent cyclohexane/ethyl acetate 2/1; IR ν (cm^{-1}): 3440–3300, 1360; ^1H NMR ($CDCl_3$): δ 7.98 (d, 1H, H-3′), 7.70 (m, 2H, H-3, H-6′), 7.55 (dd, 1H, H-4′), 7.42 (m, 5H, 4-Ph), 3.88 (s, 2H, NH_2).

*4.21. 1-(2-Nitrophenyl)-4-(2-fluorophenyl)-5-amino-pyrazole (**13**)*

From 2-(2-fluorophenyl)-3-oxopropanenitrile and 2-nitrophenylhydrazine. Orange crystals; TLC eluent carbon tetrachloride/methanol 10/0.5; IR ν (cm^{-1}): 3420–3380, 1360; ^1H NMR (DMSO-d_6): δ 8.11 (d, 1H, H-3′), 7.50–7.80 (m, 5H, H-3, H-4′, H-5′, H-6′ and 1H of 4-Ph), 7.26 (m, 3H, 4-Ph), 5.53 (s, 2H, NH_2).

*4.22. 1-(2-Nitro-5-chlorophenyl)-4-(2-fluorophenyl)-5-aminopyrazole (**14**)*

From 2-(2-fluorophenyl)-3-oxopropanenitrile and 2-nitro-5-chlorophenylhydrazine. Yellow crystals; TLC eluent carbon tetrachloride/methanol 10/0.5; IR ν (cm^{-1}): 3300–3160, 1360; ^1H NMR ($CDCl_3$): δ 8.00 (d, 1H, H-3′), 7.73 (m, 2H, H-3, H-6′), 7.58 (dd, 1H, H-4′), 7.47 (t, 1H, 4-Ph), 7.23(m, 3H, 4-Ph), 3.89 (s, 2H, NH_2).

*4.23. 1-(2-Nitrophenyl)-4-(3-fluorophenyl)-5-amino-pyrazole (**15**)*

From 2-(3-fluorophenyl)-3-oxopropanenitrile and 2-nitrophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}):

3300–3200, 1360; ^{1}H NMR (DMSO-d_6): δ 8.11 (d, 1H, H-3′), 7.77 (m, 4H, H-3, H-4′, H-5′, H-6′), 7.37 (m, 3H, 4-Ph), 6.98 (t, 1H, 4-Ph), 5.72 (s, 2H, NH_2).

4.24. 1-(2-Nitro-5-chlorophenyl)-4-(3-fluorophenyl)-5-aminopyrazole ***(16)***

From 2-(3-fluorophenyl)-3-oxopropanenitrile and 2-nitro-5-chlorophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3300–3180, 1360; ^{1}H NMR (DMSO-d_6): δ 8.15 (d, 1H, H-3′), 7.83 (m, 3H, H-3, H-4′, H-6′), 7.38 (m, 2H, 4-Ph), 6.99 (m, 2H, 4-Ph), 5.87 (s, 2H, NH_2).

4.25. 1-(2-Nitrophenyl)-4-(4-fluorophenyl)-5-aminopyrazole ***(17)***

From 2-(4-fluorophenyl)-3-oxopropanenitrile and 2-nitrophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3300–3180, 1360; ^{1}H NMR (DMSO-d_6): δ 8.10 (d, 1H, H-3′), 7.77 (m, 4H, H-3, H-4′, H-5′, H-6′), 7.56 (m, 2H, 4-Ph), 7.21 (m, 2H, 4-Ph), 5.56 (s, 2H, NH_2).

4.26. 1-(2-Nitro-5-chlorophenyl)-4-(4-fluorophenyl)-5-aminopyrazole ***(18)***

From 2-(4-fluorophenyl)-3-oxopropanenitrile and 2-nitro-5-chlorophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3390–3360, 1360; ^{1}H NMR (DMSO-d_6): δ 8.14 (d, 1H, H-3′), 7.89 (d, 1H, H-6′), 7.81 (m, 2H, H-3, H-4′), 7.54 (m, 2H, 4-Ph), 7.24 (m, 2H, 4-Ph), 5.72 (s, 2H, NH_2).

4.27. 1-(2-Nitrophenyl)-4-(2-chlorophenyl)-5-aminopyrazole ***(19)***

From 2-(2-chlorophenyl)-3-oxopropanenitrile and 2-nitrophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3420–3360, 1360; ^{1}H NMR (DMSO-d_6): δ 8.08 (d, 1H, H-3′), 7.83 (m, 3H, H-4′, H-5′, H-6′), 7.54 (s, 1H, H-3), 7.41 (m, 4H, 4-Ph), 5.43 (s, 2H, NH_2).

4.28. 1-(2-Nitro-5-chlorophenyl)-4-(2-chlorophenyl)-5-aminopyrazole ***(20)***

From 2-(2-chlorophenyl)-3-oxopropanenitrile and 2-nitro-5-chlorophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate/acid acetic 8/2/1; IR ν (cm^{-1}): 3400–3300, 1360; ^{1}H NMR (DMSO-d_6): δ 8.13 (d, 1H, H-3′), 7.90 (d, 1H, H-6′), 7.79 (dd, 1H, H-4′), 7.59 (s, 1H, H-3), 7.54 (dd, 1H, 4-Ph), 7.34 (m, 3H, 4-Ph), 5.60 (s, 2H, NH_2).

4.29. 1-(2-Nitrophenyl)-4-(3-chlorophenyl)-5-aminopyrazole ***(21)***

From 2-(3-chlorophenyl)-3-oxopropanenitrile and 2-nitrophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3420–3400; ^{1}H NMR (DMSO-d_6): δ 8.11 (d, 1H, H-3′), 7.80 (m, 4H, H-3, H-4′, H-5′, H-6′), 7.47 (m, 3H, 4-Ph), 7.21 (dd, 1H, 4-Ph), 5.73 (s, 2H, NH_2).

4.30. 1-(2-Nitro-5-chlorophenyl)-4-(3-chlorophenyl)-5-aminopyrazole ***(22)***

From 2-(3-chlorophenyl)-3-oxopropanenitrile and 2-nitro-5-chlorophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3290–3160; ^{1}H NMR (DMSO-d_6): δ 8.15 (d, 1H, H-3′), 7.88 (d, 1H, H-6′), 7.80 (m, 2H, H-3, H-4′), 7.47 (m, 3H, 4-Ph), 7.22 (dd, 1H, 4-Ph), 5.87 (s, 2H, NH_2).

4.31. 1-(2-Nitrophenyl)-4-(4-chlorophenyl)-5-aminopyrazole ***(23)***

From 2-(4-chlorophenyl)-3-oxopropanenitrile and 2-nitrophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3400–3290, 1360; ^{1}H NMR (DMSO-d_6): δ 8.10 (d, 1H, H-3′), 7.76 (m, 4H, H-3, H-4′, H-5′, H-6′), 7.48 (m, 4H, 4-Ph), 5.66 (s, 2H, NH_2).

4.32. 1-(2-Nitro-5-chlorophenyl)-4-(4-chlorophenyl)-5-aminopyrazole ***(24)***

From 2-(4-chlorophenyl)-3-oxopropanenitrile and 2-nitro-5-chlorophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3460–3375; ^{1}H NMR (DMSO-d_6): δ 8.14 (d, 1H, H-3′), 7.88 (d, 1H, H-6′), 7.79 (m, 2H, H-3, H-4′), 7.49 (m, 4H, 4-Ph), 5.82 (s, 2H, NH_2).

4.33. 1-(2-Nitrophenyl)-4-(3,4-dichlorophenyl)-5-aminopyrazole ***(25)***

From 2-(3,4-dichlorophenyl)-3-oxopropanenitrile and 2-nitrophenylhydrazine. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3420–3380; ^{1}H NMR (DMSO-d_6): δ 8.12 (d, 1H, H-3′), 7.55–7.89 (m, 4H, H-3, H-4′, H-5′, H-6′ and 3H of 4-Ph), 5.83 (s, 2H, NH_2).

4.34. 1-(2-Nitro-5-chlorophenyl)-4-(3,4-dichlorophenyl)-5-aminopyrazole ***(26)***

From 2-(3,4-dichlorophenyl)-3-oxopropanenitrile and 2-nitro-5-chlorophenylhydrazine. Orange crystals; TLC

eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3480–3390, 1360; ^1H NMR (DMSO-d_6): δ 8.15 (d, 1H, H-3′), 7.54–7.88 (m, 3H, H-3, H-4′, H-6′ and 3H of 4-Ph), 5.97 (s, 2H, NH_2).

*4.35. 1-(2-Nitrophenyl)-4-(2-pyridyl)-5-aminopyrazole (**27**)*

From 2-(2-pyridyl)-3-oxopropanenitrile and 2-nitrophenylhydrazine. Yellow crystals; TLC eluent ethyl acetate/chloroform 7/3; IR ν (cm^{-1}): 3300–3240; ^1H NMR (DMSO-d_6): δ 8.49 (d, 1H, H-3 Py), 8.09 (m, 2H, H-3, H-3′), 7.78 (m, 5H, H-5 and H-6 Py, H-4′, H-5′, H-6′), 7.08 (t, 1H, H-4 Py), 6.78 (s, 2H, NH_2).

*4.36. 1-(2-Nitro-5-chlorophenyl)-4-(2-pyridyl)-5-aminopyrazole (**28**)*

From 2-(2-pyridyl)-3-oxopropanenitrile and 2-nitro-5-chlorophenylhydrazine. Yellow crystals; TLC eluent ethyl acetate/chloroform 7/3; IR ν (cm^{-1}): 3280–3240, 1360; ^1H NMR (DMSO-d_6): δ 8.50 (d, 1H, H-3 Py), 8.14 (m, 2H, H-3, H-3′), 7.94 (d, 1H, H-6′), 7.76 (m, 3H, H-5 and H-6 Py, H-4′), 7.10 (t, 1H, H-4 Py), 6.90 (s, 2H, NH_2).

*4.37. General procedure for the synthesis of **1a**–**28a***

A suspension of 1.00 mmol of the suitable 5-aminopyrazole (**1**–**28**) in 30 ml of 10% sodium hydroxide and few ml of diglyme, was stirred for 12–24 h. The precipitate was filtered and purified by recrystallization.

*4.38. 2-Phenyl-3-cyanopyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**1a**)*

From compound **1** at 60–80°C. Yellow crystals; TLC eluent cyclohexane/ethyl acetate 2/1; IR ν (cm^{-1}): 2225, 1552; ^1H NMR ($CDCl_3$): δ 8.55 (m, 2H, H-6, H-9), 8.15 (m, 3H, H-8 and 2-Ph), 7.78 (t, 1H, H-7), 7.52 (m, 3H, 2-Ph).

*4.39. 2-Phenyl-3-cyano-8-chloropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**2a**)*

From compound **2** at 60–80°C. Yellow crystals; TLC eluent cyclohexane/ethyl acetate 2/1; IR ν (cm^{-1}): 2210, 1560; ^1H NMR ($CDCl_3$): δ 8.50 (m, 2H, H-6, H-9), 8.20 (m, 2H, 2-Ph), 7.70 (dd, 1H, H-7), 7.55 (m, 3H, 2-Ph).

*4.40. 2-(2-Methoxyphenyl)-8-chloropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**4a**)*

From compound **4** at 50°C. Yellow crystals; TLC eluent cyclohexane/ethyl acetate 2/1; IR ν (cm^{-1}): 1550, 1250, 1020; ^1H NMR (DMSO-d_6): δ 8.42 (m, 2H, H-6, H-9), 8.18 (dd, 1H, 2-Ph), 7.72 (dd, 1H, H-7), 7.45 (t, 1H, 2-Ph), 7.15 (m, 3H, H-3, 2-Ph), 3.92 (s, 3H, OCH_3).

*4.41. 2-(2-Thienyl)-8-chloropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**5a**)*

From compound **5** at room temperature (r.t.). Yellow crystals; TLC eluent toluene/cyclohexane/*i*-propyl ether 2/1/0.5; IR ν (cm^{-1}): 1570; ^1H NMR ($CDCl_3$): δ 8.47 (m, 2H, H-6, H-9), 7.60 (dd, 1H, H-3′ thio.), 7.58 (dd, 1H, H-7), 7.42 (dd, 1H, H-5′ thio.), 7.16 (t, 1H, H-4′ thio.), 6.92 (s, 1H, H-3).

*4.42. 2-(3-Thienyl)-8-chloropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**6a**)*

From compound **6** at r.t. Yellow crystals; TLC eluent toluene/ethyl acetate 8/2; IR ν (cm^{-1}): 1570; ^1H NMR ($CDCl_3$): δ 8.48 (d, 1H, H-6), 8.42 (d, 1H, H-9), 7.87 (dd, 1H, H-2′ thio.), 7.64 (dd, 1H, H-5′ thio.), 7.54 (dd, 1H, H-7), 7.46 (dd, 1H, H-4′ thio.), 6.90 (s, 1H, H-3).

*4.43. 2-(2-Furyl)-8-chloropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**7a**)*

From compound **7** at 30–40°C. Dark yellow crystals; TLC eluent ethyl acetate/cyclohexane/*i*-propyl ether 2/1/0.5; IR ν (cm^{-1}): 1570; ^1H NMR ($CDCl_3$): δ 8.49 (d, 1H, H-6), 8.45 (d, 1H, H-9), 7.59 (d, 1H, H-3′ fur.), 7.57 (dd, 1H, H-7), 6.99 (d, 1H, H-5′ fur.), 6.98 (s, 1H, H-3), 6.58 (dd, 1H, H-4′ fur.).

*4.44. 2-Carboxy-3-phenyl-8-chloropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**8a**)*

From compound **8** at 50°C. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 2800–2500, 1700, 1550; ^1H NMR (DMSO-d_6): δ 8.48 (m, 2H, H-6, H-9), 7.85 (dd, 1H, H-7), 7.55–7.45 (m, 6H, Ph and OH).

*4.45. 2-Methyl-3-cyano-8-chloropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**9a**)*

From compound **9** at r.t. Yellow crystals; TLC eluent toluene/ethyl acetate 8/2; IR ν (cm^{-1}): 2225, 1570; ^1H NMR ($CDCl_3$): δ 8.46 (d, 1H, H-6), 8.36 (d, 1H, H-9), 7.46 (dd, 1H, H-7), 2.60 (s, 3H, CH_3).

*4.46. 2-Methyl-8-chloropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**10a**)*

From compound **10** at r.t. Yellow crystals; TLC eluent toluene/ethyl acetate 8/2; IR ν (cm^{-1}): 1560; ^1H

NMR ($CDCl_3$): δ 8.48 (d, 1H, H-6), 8.34 (d, 1H, H-9), 7.50 (dd, 1H, H-7), 6.55 (s, 1H, H-3), 2.55 (s, 3H, CH_3).

4.47. 3-Phenylpyrazolo[5,1-c][1,2,4]benzotriazine-5-oxide ***(11a)***

From compound **11** at 80–90°C. Red crystals; TLC eluent chloroform/methanol 10/1; IR ν (cm^{-1}): 1570; ^1H NMR ($CDCl_3$): δ 8.58 (d, 1H, H-6), 8.40 (m, 2H, H-9 and 3-Ph), 7.98 (m, 3H, H-2, H-8, 3-Ph), 7.62 (t, 1H, H-7), 7.40 (m, 3H, 3-Ph).

4.48. 3-Phenyl-8-chloropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***(12a)***

From compound **12** at 40–50°C. Red crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1560; ^1H NMR ($CDCl_3$): δ 8.50 (d, 1H, H-6), 8.40 (m, 2H, H-2, H-9), 7.95 (d, 2H, 3-Ph), 7.45 (m, 4H, H-7, 3-Ph).

4.49. 3-(2-Fluorophenyl)pyrazolo[5,1-c][1,2,4]-benzotriazine- 5-oxide ***(13a)***

From compound **13** at 80–90°C. Red crystals; TLC eluent carbon tetrachloride/methanol 10/0.5; IR ν (cm^{-1}): 1560; ^1H NMR ($CDCl_3$): δ 8.58 (m, 2H, H-2, H-9), 8.47 (d, 1H, H-6), 8.29 (t, 1H, 3-Ph), 7.99 (t, 1H, H-8), 7.66 (t, 1H, H-7), 7.28 (m, 3H, 3-Ph).

4.50. 3-(2-Fluorophenyl)-8-chloropyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide ***(14a)***

From compound **14** at 40–50°C. Red crystals; TLC eluent carbon tetrachloride/methanol 10/0.5; IR ν (cm^{-1}): 1560; ^1H NMR ($CDCl_3$): δ 8.50 (m, 3H, H-2, H-6, H-9), 8.25 (t, 1H, 3-Ph), 7.60 (dd, 1H, H-7), 7.31 (m, 3H, 3-Ph).

4.51. 3-(3-Fluorophenyl)pyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide ***(15a)***

From compound **15** at 80–90°C. Red crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1570; ^1H NMR (DMSO-d_6): δ 8.86 (s, 1H, H-2), 8.43 (m, 2H, H-6, H-9), 8.12 (t, 1H, H-8), 7.83 (m, 3H, H-8, 3-Ph), 7.56 (m, 1H, 3-Ph), 7.17 (t, 1H, 3-Ph).

4.52. 3-(3-Fluorophenyl)-8-chloropyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide ***(16a)***

From compound **16** at 40–50°C. Orange crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1570; ^1H NMR (DMSO-d_6): δ 8.91 (s, 1H, H-2), 8.44 (m, 2H, H-6, H-9), 7.85 (m, 3H, H-7, 3-Ph), 7.56 (m, 1H, 3-Ph), 7.20 (t, 1H, 3-Ph).

4.53. 3-(4-Fluorophenyl)pyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***(17a)***

From compound **17** at 80–90°C. Red crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1580; ^1H NMR (DMSO-d_6): δ 8.79 (m, 2H, 3-Ph), 8.42 (m, 2H, H-6, H-9), 8.08 (m, 3H, H-8, 3-Ph), 7.77 (t, 1H, H-7), 7.36 (m, 2H, 3-Ph).

4.54. 3-(4-Fluorophenyl)-8-chloropyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide ***(18a)***

From compound **18** at 40–50°C. Red crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1570; ^1H NMR (DMSO-d_6): δ 8.83 (s, 1H, H-2), 8.42 (m, 2H, H-6, H-9), 8.09 (m, 2H, 3-Ph), 7.80 (dd, 1H, H-7), 7.37 (m, 2H, 3-Ph).

4.55. 3-(2-Chlorophenyl)pyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***(19a)***

From compound **19** at 80–90°C. Orange crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1570; ^1H NMR (DMSO-d_6): δ 8.62 (s, 1H, H-2), 8.45 (m, 2H, H-6, H-9), 8.14 (t, 1H, H-8), 7.75 (t, 1H, H-7), 7.64 (m, 2H, 3-Ph), 7.49 (m, 2H, 3-Ph).

4.56. 3-(2-Chlorophenyl)-8-chloropyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide ***(20a)***

From compound **20** at 40–50°C. Orange crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1560; ^1H NMR (DMSO-d_6): δ 8.65 (s, 1H, H-2), 8.44 (m, 2H, H-6, H-9), 7.80 (dd, 1H, H-7), 7.74 (dd, 1H, 3-Ph), 7.66 (dd, 1H, 3-Ph), 7.49 (m, 2H, 3-Ph).

4.57. 3-(3-Chlorophenyl)pyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***(21a)***

From compound **21** at 80–90°C. Orange crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1570; ^1H NMR (DMSO-d_6): δ 8.88 (s, 1H, H-2), 8.43 (m, 2H, H-6, H-9), 8.10 (m, 3H, H-8, 3-Ph), 7.79 (t, 1H, H-7), 7.54 (t, 1H, 3-Ph), 7.41 (dd, 1H, 3-Ph).

4.58. 3-(3-Chlorophenyl)-8-chloropyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide ***(22a)***

From compound **22** at 40–50°C. Red crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1570; ^1H NMR (DMSO-d_6): δ 8.93 (s, 1H, H-2), 8.44 (m, 2H, H-6, H-9), 8.05 (m, 2H, 3-Ph), 7.79 (dd, 1H, H-7), 7.53 (t, 1H, 3-Ph), 7.42 (dd, 1H, 3-Ph).

*4.59. 3-(4-Chlorophenyl)pyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**23a**) [19]*

From compound **23** [19] at 80–90°C. Orange crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1560; ^1H NMR (DMSO-d_6): δ 8.84 (s, 1H, H-2), 8.43 (m, 2H, H-6, H-9), 8.09 (m, 3H, H-8, 3-Ph), 7.77 (t, 1H, H-7), 7.58 (m, 2H, 3-Ph).

*4.60. 3-(4-Chlorophenyl)-8-chloropyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide (**24a**)*

From compound **24** at 40–50°C. Red crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1560; ^1H NMR (DMSO-d_6): δ 8.90 (s, 1H, H-2), 8.42 (m, 2H, H-6, H-9), 8.10 (m, 2H, 3-Ph), 7.80 (dd, 1H, H-7), 7.63 (m, 2H, 3-Ph).

*4.61. 3-(3,4-Dichlorophenyl)pyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**25a**)*

From compound **25** at 80–90°C. Red crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1570; ^1H NMR (DMSO-d_6): δ 8.91 (s, 1H, H-2), 8.43 (m, 2H, H-6, H-9), 8.28 (d, 1H, 3-Ph), 7.98 (m, 2H, H-8, 3-Ph), 7.75 (m, 2H, H-7, 3-Ph).

*4.62. 3-(3,4-Dichlorophenyl)-8-chloropyrazolo[5,1-c]-[1,2,4]benzotriazine 5-oxide (**26a**)*

From compound **26** at 40–50°C. Orange crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 1565; ^1H NMR (DMSO-d_6): δ 8.95 (s, 1H, H-2), 8.45 (m, 2H, H-6, H-9), 8.30 (d, 1H, 3-Ph), 8.05 (dd, 1H, 3-Ph), 7.80 (m, 2H, H-7, 3-Ph).

*4.63. 3-(2-Pyridyl)pyrazolo[5,1-c][1,2,4]benzotriazine-5-oxide (**27a**)*

From compound **27** at 80–90°C. Red crystals; TLC eluent ethyl acetate/chloroform 7/3; IR ν (cm^{-1}): 1560; ^1H NMR ($CDCl_3$): δ 8.83 (s, 1H, H-2), 8.60 (m, 2H, H-6 and Py), 8.44 (d, 1H, Py), 8.33 (d, 1H, H-9), 7.97 (t, 1H, H-8), 7.77 (t, 1H, Py), 7.68 (t, 1H, H-7), 7.21 (t, 1H, Py).

*4.64. 3-(2-Pyridyl)-8-chloropyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**28a**)*

From compound **28** at 40–50°C. Red crystals; TLC eluent ethyl acetate/chloroform 7/3; IR ν (cm^{-1}): 1560; ^1H NMR ($CDCl_3$): δ 8.85 (s, 1H, H-2), 8.65 (d, 1H, Py), 8.49 (m, 2H, H-6, H-9), 8.30 (dd, 1H, H-7), 7.80 (t, 1H, Py), 7.60 (d, 1H, Py), 7.22 (t, 1H, Py).

*4.65. General procedure for the synthesis of **1b**, **2b** and **9b***

The products were prepared according to a previously described procedure [1,2] by treatment with conc. sulfuric acid of compounds **1a**, **2a** and **9a**, respectively.

*4.66. 2-Phenyl-3-carbamoylpyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**1b**)*

From compound **1a**. Yellow crystals; TLC eluent toluene/ethyl acetate 8/2; IR ν (cm^{-1}): 3420, 1650, 1540; ^1H NMR (DMSO-d_6): δ 8.48 (m, 2H, H-6, H-9), 8.15 (t, 1H, H-8), 7.95 (m, 2H, 2-Ph), 7.78 (m, 2H, H-7, NH), 7.52 (m, 4H, 2-Ph, NH).

*4.67. 2-Phenyl-3-carbamoyl-8-chloropyrazolo[5,1-c]-[1,2,4]benzotriazine 5-oxide (**2b**)*

From compound **2a**. Yellow crystals; TLC eluent cyclohexane/ethyl acetate 2/1; IR ν (cm^{-1}): 3420, 1670, 1550; ^1H NMR (DMSO-d_6): δ 8.48 (m, 2H, H-6, H-9), 7.96 (m, 2H, 2-Ph), 7.84 (dd, 1H, H-7), 7.75 (s, 1H, NH), 7.52 (m, 4H, 2-Ph, NH).

*4.68. 2-Methyl-3-carbamoyl-8-chloropyrazolo[5,1-c]-[1,2,4]benzotriazine 5-oxide (**9b**)*

From compound **9a**. Yellow crystals; TLC eluent chloroform/methanol 10/1; IR ν (cm^{-1}): 3440–3340, 1660, 1570; ^1H NMR (DMSO-d_6): δ 8.46 (d, 1H, H-6), 8.38 (d, 1H, H-9), 7.80 (dd, 1H, H-7), 7.62 (s, 1H, NH), 7.02 (s, 1H, NH), 2.65 (s, 3H, CH_3).

*4.69. General procedure for the synthesis of **1c**, **2c** and **9c***

The products were prepared following an already known method [1,2] by treatment with conc. sulfuric acid and sodium nitrite of compounds **1b**, **2b** and **9b**, respectively.

*4.70. 2-Phenyl-3-carboxypyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide (**1c**)*

From compound **1b**. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid/ 8/2/1; IR ν (cm^{-1}): 1720; ^1H NMR (DMSO-d_6): δ 8.46 (m, 3H, H-6, H-9 and 2-Ph), 8.14 (m, 2H, H-8, 2-Ph), 7.82–7.70 (m, 3H, H-7, 2-Ph), 7.52 (m, 1H, 2-Ph).

*4.71. 2-Phenyl-3-carboxy-8-chloropyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide (**2c**)*

From compound **2b**. Yellow crystals; TLC eluent ethyl acetate/chloroform 7/3; IR ν (cm^{-1}): 3300–3140,

1690, 1545; ^{1}H NMR (DMSO-d_6): δ 12.90 (s, 1H, OH), 8.48 (m, 2H, H-6, H-9), 7.82 (m, 3H, H-7, 2-Ph), 7.50 (m, 3H, 2-Ph).

4.72. 2-Methyl-3-carboxy-8-chloropyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide ***(9c)***

From compound **9b**. Yellow crystals; TLC eluent toluene/ethyl acetate/acetic acid 8/2/1; IR ν (cm^{-1}): 3300–3100, 1720, 1560; ^{1}H NMR (DMSO-d_6): δ 12.00 (s, 1H, OH), 8.44 (d, 1H, H-6), 8.36 (d, 1H, H-9), 7.80 (dd, 1H, H-7), 2.62 (s, 3H, CH_3).

4.73. General procedure for the synthesis of ***1d***, ***2d***, ***8d*** *and* ***9d***

Compounds **1c**, **2c**, **8a** and **9c** (100 mg) were added to thionyl chloride (1.00 ml) and refluxed for 30 min. The final solution was evaporated to dryness and 10 ml of anhydrous ethanol were added; the reaction was monitored by TLC. The precipitate was filtered and purified by recrystallization.

4.74. 2-Phenyl-3-ethoxycarbonylpyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide ***(1d)***

From compound **1c**. Yellow crystals; TLC eluent toluene/ethyl acetate 8/2; IR ν (cm^{-1}): 1720, 1550; ^{1}H NMR ($CDCl_3$): δ 8.60 (d, 1H, H-6), 8.51 (d, 1H, H-9), 8.36 (d, 2H, 2-Ph), 8.08 (m, 3H, 2-Ph), 7.79 (m, 2H, H-7, H-8), 4.40 (q, 2H, CH_2), 1.41(t, 3H, CH_3).

4.75. 2-Phenyl-3-ethoxycarbonyl-8-chloropyrazolo-[5,1-c][1,2,4]benzotriazine 5-oxide ***(2d)***

From compound **2c**. Yellow crystals; TLC eluent ethyl acetate/chloroform 7/3; IR ν (cm^{-1}): 1688, 1540, 1105; ^{1}H NMR ($CDCl_3$): δ 8.50 (m, 2H, H-6, H-9), 7.80 (m, 2H, 2-Ph), 7.55 (m, 4H, H-7, 2-Ph), 4.40 (q, 2H, CH_2), 1.35 (t, 3H, CH_3).

4.76. 2-Ethoxycarbonyl-3-phenyl-8-chloropyrazolo-[5,1-c][1,2,4]benzotriazine 5-oxide ***(8d)***

From compound **8a**. Yellow crystals; TLC eluent chloroform/methanol 10/1; IR ν (cm^{-1}): 1720, 1560; ^{1}H NMR (DMSO-d_6): δ 8.48 (m, 2H, H-6, H-9), 7.86 (dd, 1H, H-7), 7.58–7.44 (m, 5H, Ph), 4.35 (q, 2H, CH_2), 1.35 (t, 3H, CH_3).

4.77. 2-Methyl-3-ethoxycarbonyl-8-chloropyrazolo-[5,1-c][1,2,4]benzotriazine 5-oxide ***(9d)***

From compound **9c**. Yellow crystals; TLC eluent chloroform/methanol 10/1; IR ν (cm^{-1}): 1690, 1560; ^{1}H NMR ($CDCl_3$): δ 8.46 (d, 1H, H-6), 8.38 (d, 1H, H-9), 7.58 (dd, 1H, H-7), 4.35 (q, 2H, CH_2), 2.70 (s, 3H, CH_3), 1.35 (t, 3H, CH_3).

4.78. General procedure for the synthesis of ***3e***, ***4f***, ***5e***, ***5g***, ***6e***, ***7f***, ***7h***, *and* ***10e***

Compounds **3a**, **4a**, **5a**, **6a**, **7a** and **10a** (0.21 mmol) were each dissolved in 15 ml of chloroform to which an equimolar amount of bromine was slowly added. From compound **5a** (0.21 mmol), an excess of bromine (1 mmol) gave the tetrabromo derivative **5g**. From compound **7a** a mixture of bromo derivatives **7f** and **7h** has always been obtained; they were separated by column chromatography.

The solution was stirred for 2 h at r.t. After evaporation the residue was recrystallized from the suitable solvent.

4.79. 2-Phenyl-3-bromo-8-chloropyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide ***(3e)***

From compound **3a**. Yellow crystals; TLC eluent toluene/ethyl acetate 8/2; IR ν (cm^{-1}): 1550; ^{1}H NMR ($CDCl_3$): δ 8.45 (m, 2H, H-6, H-9), 8.10 (m, 2H, 2-Ph), 7.55 (m, 4H, H-7, 2-Ph).

4.80. 2-(2-Methoxy-5-bromophenyl)-3-bromo-8-chloro-pyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***(4f)***

From compound **4a**. Yellow crystals; TLC eluent cyclohexane/ethyl acetate 2/1; IR ν (cm^{-1}): 1550; ^{1}H NMR (DMSO-d_6): δ 8.45 (m, 2H, H-6, H-9), 7.78 (m, 2H, H-7, 2-Ph), 7.55 (d, 1H, 2-Ph),7.25 (d, 1H, 2-Ph), 3.85 (s, 3H, CH_3).

4.81. 2-(2-Thienyl)-3-bromo-8-chloropyrazolo[5,1-c]-[1,2,4]benzotriazine 5-oxide ***(5e)***

From compound **5a**. Yellow crystals; TLC eluent toluene/ethyl acetate 8/2; IR ν (cm^{-1}): 1590; ^{1}H NMR ($CDCl_3$): δ 8.48 (d, 1H, H-6), 8.42 (d, 1H, H-9), 8.06 (d, 1H, H-3′ thio.), 7.58 (dd, 1H, H-7), 7.51 (d, 1H, H-5′ thio.), 7.21 (t, 1H, H-4′ thio.).

4.82. 2-(3,4,5-Tribromothien-2-yl)-3-bromo-8-chloro-pyrazolo[5,1-c][1,2,4]benzotriazine 5-oxide ***(5g)***

From compound **5a**. Orange crystals; TLC eluent toluene/ethyl acetate 8/2; IR ν (cm^{-1}): 1560; ^{1}H NMR ($CDCl_3$): δ 8.50 (d, 1H, H-6), 8.39 (d, 1H, H-9), 7.64 (dd, 1H, H-7).

4.83. 2-(3-Thienyl)-3-bromo-8-chloropyrazolo[5,1-c]-[1,2,4]benzotriazine 5-oxide ***(6e)***

From compound **6a**. Yellow crystals; TLC eluent toluene/ethyl acetate 8/2; IR ν (cm^{-1}): 1560; ^{1}H NMR

($CDCl_3$): δ 8.48 (d, 1H, H-6), 8.42 (d, 1H, H-9), 8.28 (dd, 1H, H-2′ thio.), 7.86 (dd, 1H, H-5′ thio.), 7.58 (dd, 1H, H-7), 7.48 (dd, 1H, H-4′ thio.).

*4.84. 2-(3-Bromofur-2-yl)-3-bromo-8-chloropyrazolo-[5,1-c][1,2,4]benzotriazine 5-oxide (**7f**)*

From compound **7a**. From column chromatography (eluent toluene/*i*-propyl ether 8/1) fast band running. Dark yellow crystals; IR ν (cm^{-1}): 1560; ^1H NMR ($CDCl_3$): δ 8.50 (m, 2H, H-6 and H-9), 7.60 (dd, 1H, H-7), 7.34 (d, 1H, H-5′ fur.), 6.56 (d, 1H, H-4′ fur.).

*4.85. 2-(3-Bromofur-2-yl)-8-chloropyrazolo-[5,1-c][1,2,4]benzotriazine 5-oxide (**7h**)*

From compound **7a**. From column chromatography (eluent toluene/*i*-propyl ether 8/1) third band running. Yellow crystals; IR ν (cm^{-1}): 1560; ^1H NMR ($CDCl_3$): δ 8.48 (m, 2H, H-6 and H-9), 7.55 (dd, 1H, H-7), 6.95 (d, 1H, H-5′ fur.), 6.92 (s, 1H, H-3), 6.50 (d, 1H, H-4′ fur.).

*4.86. 2-Methyl-3-bromo-8-chloropyrazolo[5,1-c][1,2,4]-benzotriazine 5-oxide (**10e**)*

From compound **10a**. Yellow crystals; TLC eluent toluene/ethyl acetate 8/2; IR ν (cm^{-1}): 1590; ^1H NMR ($CDCl_3$): δ 8.46 (d, 1H, H-6), 8.31 (d, 1H, H-9), 7.53 (dd, 1H, H-7), 2.50 (s, 3H, CH_3).

5. Biochemistry

[^{3}H]Ro15-1788 (at 0.2–0.4 nM, $K_d = 0.65$ nM, specific activity 87 Ci $mmol^{-1}$) binding assays on bovine cerebral cortex were carried out as described in the literature [20].

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