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Short communication

Synthesis, local anesthetic activity and QSAR studies for a set of *N*-[2-(alkylamino)ethyl]benzotriazol-*x*-yl acetamides

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Summary — A set of *N*-[2-(alkylamino)ethyl]benzotriazol-*x*-yl acetamides were synthesized and tested for local anesthetic activity. The compounds were designed by varying independently the hydrophobicity and size of the side chains. Anesthetic activity was assessed by different tests using lidocaine as a reference: rabbit corneal and mouse tail anesthesia. These two anesthetic activities were correlated with calculated log *P* values and significant linear dependences were observed. The three most potent compounds of the series were evaluated in the rat sciatic nerve block assay and their acute toxicity in mice was also assessed. Compound **4b** (*N*-[2-(diethylamino)ethyl]benzotriazol-2-yl acetamide), which has an anesthetic activity comparable to that of lidocaine, was also characterized by a more favorable therapeutic index.

benzotriazole / anesthetic activity / acute toxicity / QSAR

Introduction

Many compounds are known to display both local anesthetic and antiarrhythmic activity [1]. Indeed several drugs, such as lidocaine, can be effectively applied in either of these two clinical uses. This fact justifies the practice of screening newly synthesized molecules designed as potential antiarrhythmic agents by simple local anesthetic activity assays [2]. Following such an approach, we recently prepared a series of *N*-[2-(alkylamino)ethyl]benzotriazol-*x*-yl acetamides **3a–f** and **4a–f** (scheme 1), which we believed to possess both local anesthetic and antiarrhythmic properties. The compounds in fact fulfil the requirements of the pharmacophore scheme proposed by Löfgren [3], because they feature an aromatic system, an intermediate polar chain and an aminic moiety ionizable under physiological pH. The role of lipophilicity in determining local anesthetic activity has been well established [4]. It has also been proposed that the molar refractivity (describing the volume and the polarizability of the molecule) is the property that best explains the variation of the local anesthetic activity [2]. In order to understand which physicochemical properties modulate anesthetic activity, the compounds of our data set have been designed by selecting side chains whose lipophilicity and molar refractivity vary independently.

Subsequent *in vivo* pharmacological evaluations have enabled us to identify a few members of the series exhibiting significant local anesthetic activities, which will be tested as antiarrhythmic agents. Synthesis, local anesthetic activity, acute toxicity and QSAR studies for the investigated set of benzotriazole derivatives are reported herein.

Chemistry

The synthetic route used for the preparation of the compounds reported in table I is outlined in scheme 1. The reaction of benzotriazole with ethyl chloroacetate in absolute ethanol and sodium ethoxide gave a mixture of 1- and 2-substituted isomers [5], generally

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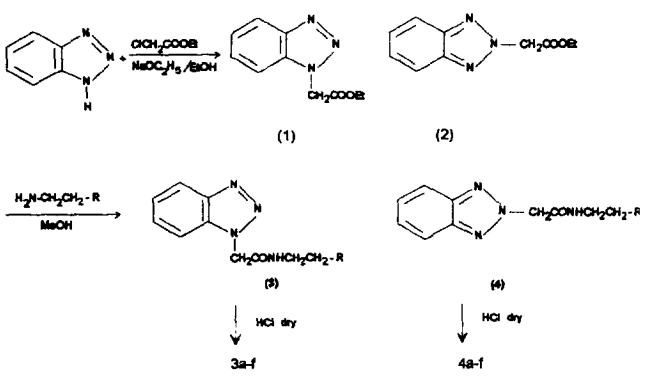


3 or 4	R
a	$-N(CH_3)_2$
b	$-N(CH_2CH_3)_2$
c	-N
d	-N
e	-N O
f	-N N-H

Scheme 1.

with an overall yield of 85%. The two isomers were separated by chromatography on a silica-gel column using *n*-hexane/diethyl ether 1:1 v/v as eluent; the faster moving 2-substituted isomer was generally obtained in lower yield. The ethylacetate derivatives **1** and **2** were converted into the amino derivatives **3** and **4** by reaction with the appropriate amines in methanol solution. The free bases obtained were converted into their corresponding hydrochlorides (**3a–f** and **4a–f**) by the usual methods. All the final products were further purified by crystallization from the appropriate solvent.

The compounds listed in table I were characterized by UV and ^{1}H-NMR spectroscopy. The UV spectra showed the presence of an aromatic system for all compounds. The UV spectra of the 1-substituted isomers were characterized by two absorption peaks at 250–260 nm and 279–280 nm (log ε = 3.81 and 3.68). In contrast, the 2-substituted compounds showed a single absorption peak ranging from 276–278 nm [5].

The differences in the chemical shift values of the protons in the ^{1}H-NMR spectra of the series of 1- and 2-substituted benzotriazoles confirm the contrasting π-electron delocalization of the two systems. Indeed, the benzotriazol-2-yl derivatives showed a greater molecular symmetry. The aromatic protons of the benzotriazole ring of compound **4a**, taken as an example, appear as two doublets of doublets (dd) at δ 7.89, J = 9.5 and 3.2 Hz (H-4 and H-7) and δ 7.42, J = 9.5 and 3.2 Hz (H-5 and H-6); the *N*-alkylaminoethylacetamido group of the side chain [aCH$_2$CONH-bCH$_2$-cCH$_2$N(CH$_3$)$_2$] is characterized by the following chemical shifts: δ 5.59 (2H, s, aCH$_2$), 3.44 (2H, t, J = 7.5 Hz, bCH$_2$), 2.83 (2H, t, J = 7.5 Hz, cCH$_2$) and 2.3 (6H, s, N(CH$_3$)$_2$). Larger differences occurred in the benzotriazol-1-yl derivatives (*eg*, **3b**) in which the aromatic proton of the benzotriazole moiety, on the basis of the distributions of the resonance of the hybrids, appeared as two doublets of doublets at δ 8.05 (H-4) and 7.75 (H-7) J = 8.5 and 1.1 Hz, and two doublets of triplets at δ 7.60 (H-6) and 7.47 (H-5) J = 8.5 and 1.1 Hz. The chemical shifts of the side chain were the following: δ 5.49 (2H, s, aCH$_2$), 3.39 (2H, t, J = 7.5 Hz, bCH$_2$), 2.67 (2H, t, J = 7.5 Hz, cCH$_2$) and 2.2 (6H, s, N(CH$_3$)$_2$).

Similar ^{1}H-NMR data, consistent with the described structures, were found for all the other benzotriazole derivatives.

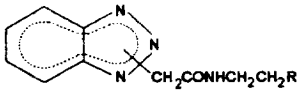
Pharmacology

All the compounds were tested *in vivo* for their anesthetic activity by different tests: corneal anesthesia in the rabbit and mouse tail anesthesia (table II). Rat sciatic nerve block anesthesia, ip acute toxicity and the therapeutic index of the more active compounds were also determined (fig 1–3). The synthesized compounds were always compared for their activity with lidocaine, taken as a reference drug.

QSAR studies

Regression analysis and calculation of overall octanol/water log P (CLOGP) and molar refractivity (MR) parameters [6] were performed by using the C-QSAR program [7]. The CLOGP values refer to non-ionized species. For correlation purposes, the biological data listed in table II were converted into logarithmic scales. Surface anesthetic activity (log CORN) was expressed as the logarithm of percentage of the area under the curve (AUC) with respect to lidocaine used as reference drug. Infiltration anesthesia was expressed as log $1/IC_{50}$. The log CORN, log $1/IC_{50}$, CLOGP and MR values are listed in table III. In addition to the F-test, the 'leave-one-out' method [8] was applied to evaluate the significance of the correlations.

Table I. Physicochemical properties of benzotriazole derivatives **3a–f** and **4a–f**.



			1-Substituted benzotriazole 3a–f				*2-Substituted benzotriazole 4a–f*			
R	*Formula*[a]	*MW*	*Comp*	*Yield (%)*	*Mp (°C)*	*Recrystallization solvent*[b]	*Comp*	*Yield (%)*	*Mp (°C)*	*Recrystallization solvent*[b]
$-N(CH_3)_2$	$C_{12}H_{17}N_5O\cdot HCl$	283.47	**3a**	85	206–210	b	**4a**	93	164–167	b
$-N(C_2H_5)_2$	$C_{14}H_{21}N_5O\cdot HCl$	311.47	**3b**	82	102–103	b	**4b**	80	128–129	b
	$C_{14}H_{19}N_5O\cdot HCl$	309.47	**3c**	65	207–208	a + b	**4c**	90	238–239	a + b
	$C_{15}H_{21}N_5O\cdot HCl$	323.47	**3d**	62	150–151	a + b	**4d**	87	240–241	a + b
	$C_{14}H_{19}N_5O_2\cdot HCl$	325.46	**3e**	85	206–207	a + b	**4e**	90	174–175	a + b
	$C_{14}H_{20}N_6O\cdot 2HCl$	360.12	**3f**	55	213–214	a + b	**4f**	65	108–109	a + b

[a]Satisfactory microanalyses obtained: C, H, N values are within ± 0.3% of the theoretical values; [b]crystallization solvents: a) ethyl alcohol; b) diethyl ether.

Table II. Rabbit corneal and mouse tail anesthetic activities.

Compound	*Corneal anesthesia*[a]	*Mouse tail anesthesia*[b]
3a	1.2 ± 1.1	9.6 (± 0.34) x 10^{-2}
3b	13.2 ± 5.6	6.9 (± 0.30) x 10^{-2}
3c	15.4 ± 4.0	5.6 (± 0.38) x 10^{-2}
3d	24.4 ± 8.3	3.1 (± 0.37) x 10^{-2}
3e	2.0 ± 0.9	5.6 (± 0.34) x 10^{-2}
3f	0.9 ± 1.0	1.6 (± 0.42) x 10^{-1}
4a	1.5 ± 0.6	5.0 (± 0.36) x 10^{-2}
4b	62.8 ± 9.2	1.0 (± 0.43) x 10^{-2}
4c	116.0 ± 10.0	1.3 (± 0.37) x 10^{-2}
4d	98.0 ± 11.2	9.8 (± 0.31) x 10^{-3}
4e	2.0 ± 1.5	1.0 (± 0.52) x 10^{-1}
4f	3.0 ± 2.0	1.2 (± 0.43) x 10^{-1}
Lidocaine HCl[c]	100	1.6 (± 0.43) x 10^{-2}

[a]All compounds were in aqueous solution at 2% concentration. The values, expressed as percentage of the anesthetic activity of lidocaine (= 100), are means ± SE of three determinations. [b]IC_{50} values expressed as mol/l. [c]Lidocaine hydrochloride was used for comparison.

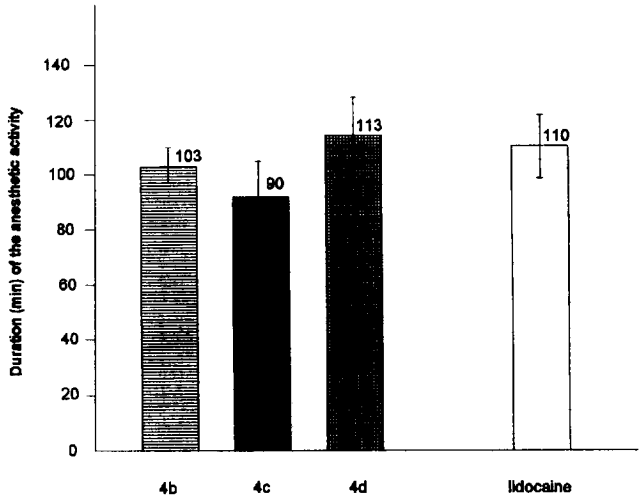


Fig 1. *In vivo* duration of local anesthetic activity (rat sciatic nerve block) of lidocaine and compounds **4b**, **4c** and **4d**. Each rat received 0.2 ml of the 2% anesthetic solution. The values are means ± SE of three determinations.

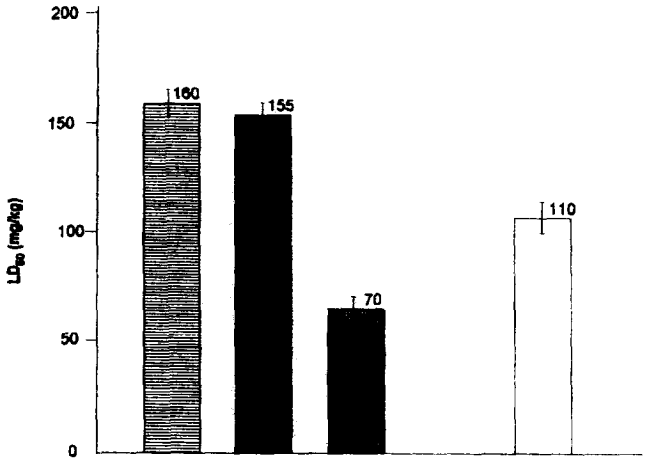


Fig 2. Acute toxicity (ip) in male mice of lidocaine and compounds **4b**, **4c** and **4d**.

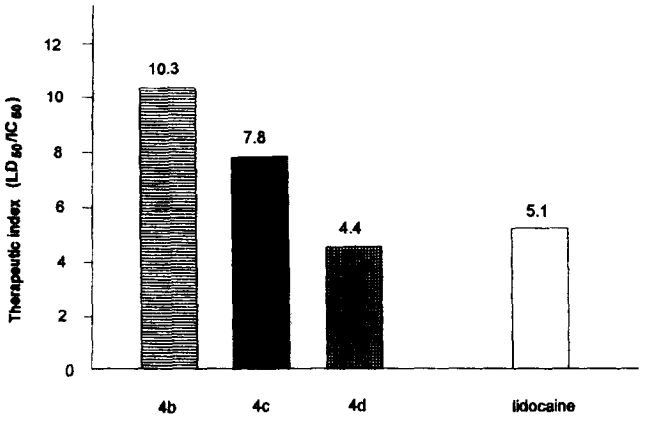


Fig 3. Therapeutic index of lidocaine and compounds **4b**, **4c** and **4d** evaluated as ratio between LD_{50} and IC_{50} (mouse tail test expressed in mg/kg).

Results and discussion

Table II summarizes the results of the surface and infiltration anesthesia assays. In both tests, the 2-substituted benzotriazoles turned out to be more effective than the 1-isomers. Compounds **4b**, **4c** and **4d** stand out in the data set for their remarkably high activities (these values are comparable to or even superior to those determined for lidocaine).

It appears that the position and the nature of the side chains affect surface and infiltration activities in a similar way. This is confirmed by the existence of a non-negligible degree of correlation between log CORN and log $1/IC_{50}$ values ($r^2 = 0.791$).

In order to highlight the physicochemical properties which influence surface and infiltration anesthesia, QSAR studies were carried out. We employed the

Table III. Anesthetic activity and overall CLOGP and MR values employed in the QSAR studies.

Compound	*log CORN*		*log 1/IC₅₀*		*CLOGP*	*MR*
	Observed	*Calculated*[a]	*Observed*	*Calculated*[b]		
3a	0.08	0.08	1.02	0.94	0.86	6.94
3b	1.12	1.18	1.16	1.48	1.76	7.87
3c	1.19	1.07	1.25	1.42	1.67	7.69
3d	1.39	1.75	1.51	1.76	2.23	8.15
3e	0.30	0.20	1.25	1.00	0.96	7.84
3f	–0.04	–0.07	0.80	0.86	0.73	8.06
4a	0.18	0.49	1.30	1.14	1.19	6.94
4b	1.80	1.58	2.00	1.68	2.09	7.87
4c	2.06	1.47	1.89	1.62	2.00	7.69
4d	1.99	2.16	2.01	1.96	2.56	8.15
4e	0.30	0.61	1.00	1.20	1.29	7.84
4f	0.48	0.33	0.92	1.06	1.06	8.06
Lidocaine HCl	2.00	–	1.80	–	–	–

[a]Values calculated according to equation [1]. [b]Values calculated according to equation [2].

CLOGP and MR parameters as molecular descriptors (see table III). An appropriate selection of the aminic moieties while we were planning the syntheses of the benzotriazoles allowed us to avoid correlation between hydrophobicity and size of the side chains (r^2 between CLOGP and MR is only 0.163).

The CLOGP and MR values reported in table III show that the two series of isomers have identical MR values. In contrast, the CLOGP values of the 2-substituted benzotriazoles are 0.33 units higher than the corresponding 1-isomers.

The surface (log CORN) and infiltration (log $1/IC_{50}$) anesthetic activities were correlated with CLOGP and significant linear dependences were found (equations [1] and [2]).

$$\log \text{CORN} = 1.22(\pm 0.31)\text{CLOGP} - 0.97(\pm 0.51) \quad [1]$$

$n = 12$, $r_f^2 = 0.882$, $r_{cv}^2 = 0.854$, $s = 0.281$, $F_{1,10} = 74.54$

$$\log 1/IC_{50} = 0.60(\pm 0.26)\text{CLOGP} + 0.42(\pm 0.42) \quad [2]$$

$n = 12$, $r_f^2 = 0.728$, $r_{cv}^2 = 0.627$, $s = 0.231$, $F_{1,10} = 26.69$

In the above equations, n is the number of data points, r_f^2 and r_{cv}^2 are the squared correlation coefficients associated with the calibration and cross-validation models respectively [8], s is the standard error from the reported calibration equation, and the numbers in parentheses are the 95% fiducial limits. The fact that the r_{cv}^2 values are reasonably close to the r_f^2 values supports the significance of both equations.

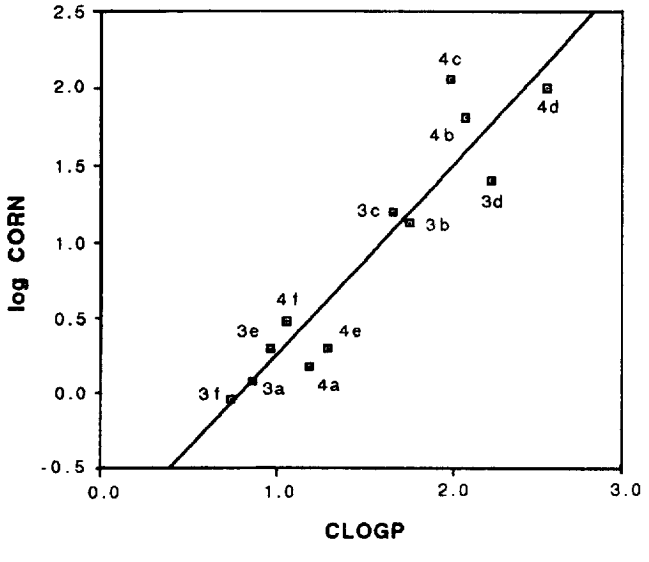


Fig 4. Plot of log CORN *versus* CLOGP values.

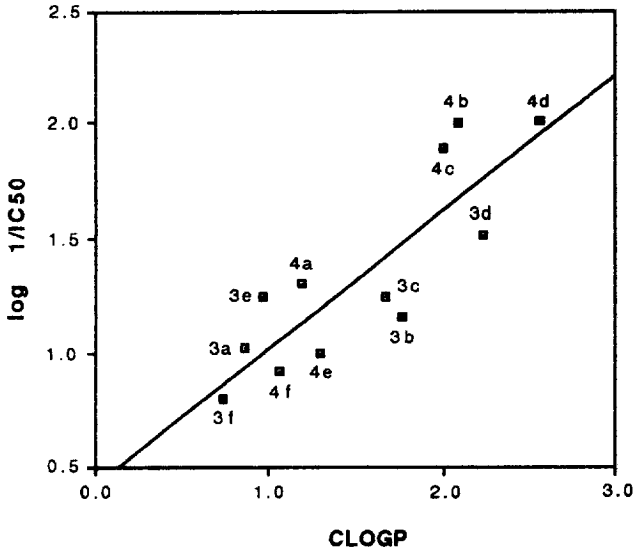


Fig 5. Plot of log $1/IC_{50}$ *versus* CLOGP values.

Table III lists the calculated log CORN and log $1/IC_{50}$ values according to equations [1] and [2]. Plots of log CORN and log $1/IC_{50}$ against CLOGP are shown in figures 4 and 5, respectively.

Interestingly, the two subsets of 1- and 2-substituted benzotriazoles could be merged together in equations [1] and [2] without the need for additional descriptors for isomerism. The observed slight differences in activity within pairs of isomers, with the 2-substituted isomer generally being the most active, are evidently accounted for by the above-mentioned small difference in CLOGP values.

If CLOGP is replaced by MR in equations [1] and [2], the r_f^2 indices decrease dramatically (0.156 and 0.028, respectively). This finding clearly indicates that the size of aminic moiety is totally unimportant for anesthetic activity. We are currently using equations [1] and [2] in the design of new benzotriazole anesthetic agents.

Due to their fairly good anesthetic activities, additional investigations were conducted on **4b**, **4c** and **4d**. The duration of the local anesthetic activity of these compounds was evaluated in the rat sciatic nerve block assay. According to the results reported in figure 1, the three compounds showed a very similar anesthetic profile to that of the reference drug lidocaine.

Finally, the acute toxicity and therapeutic index of **4b**, **4c** and **4d** were determined in mice. From the corresponding LD_{50} and LD_{50}/IC_{50} values shown in figures 2 and 3, it is evident that **4b** is characterized by the most favorable ratio between toxicity and

mouse tail anesthetic activity (its therapeutic index is significantly higher than that measured for lidocaine). Due to its interesting pharmacological properties, **4b** appears to us to be most promising compound for further pharmacological investigations.

Experimental protocols

Chemistry

All compounds gave satisfactory elemental analysis (C, H, N) within ±0.3% of the theoretical values and were characterized by UV and ^{1}H-NMR spectroscopy. Melting points, were determined with a Kofler apparatus and are uncorrected. Chromatographic separations were performed on a silica-gel column (Kieselgel 40, 0.063–0.200 mm, Merck). Analytical thin-layer chromatography (TLC) was carried on Merck silica gel-60 F-254 glass-backed plates and visualized by UV.

UV spectra were taken on a Beckman DU-40 spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker WM250 instrument using CD_3OD as a solvent and Me_4Si as an internal standard. Chemical shifts are reported in parts per million (δ). The signals were designated as follows: dd, doublet of doublets; s, singlet; d, doublet; t, triplet. The spectra obtained were consistent with the described structures.

General procedure for the preparation of compounds 3a–f and 4a–f

In a typical general procedure, to 0.01 mol of the appropriate benzotriazolylethylacetate derivative (**1** and **2**), prepared according to reference [5] and dissolved in anhydrous methanol (50 ml), was added the appropriate amine (0.01 mol) dropwise. The reaction mixture was kept under reflux with magnetic stirring for 8–12 h and monitored by TLC, until the starting material was disappeared. After cooling the solvent was removed under reduced pressure and the residue was purified by silica-gel column chromatography using mixtures of diethylether/methanol in an appropriate ratio as required by each product. Further purification was obtained by crystallization from the appropriate solvent (table I). The hydrochloride salts were prepared from the amines using dry HCl in an anhydrous Et_2O/EtOH mixture (2:1). Compounds **3f** and **4f** were obtained as dihydrochlorides; the assigned formulae were supported by argentometric titration of Cl ion.

Pharmacology

Corneal anesthesia

Male New Zeland rabbits (Morini S, Polo d'Enza, Reggio Emilia, weighing 2.5–2.7 kg) were used. Local surface anesthesia [9] was evaluated by determining the number of stimuli to the cornea every 3 min. This was effected rhythmically with a Frey's horse-hair, in order to produce the blink reflex. If the reflex did not occur after 100 stimulations, anesthesia was considered total. At the beginning of the experiment care was taken to ascertain that this reflex was normal in both eyes of the rabbits used. The aqueous solutions (2%) of the compounds studied were dropped onto the conjunctival sac so that the space between the eyelids contained a clearly visible film of solution for the set time of 3 min. Lidocaine solution (2%) was used for comparison.

Mouse tail anesthesia

Male Swiss mice (Nossan, Correzzana, Milan, weighing 18–20 g) were used. The test was performed according to the method of Bianchi [10] in which the aqueous anesthetic solution (0.1 ml) is injected sc about 1 cm from the root of the tail. Fifteen minutes after injection, the pain reflex of all the injected animals was tested applying a small artery clip to the zone where the compound was injected. The proportion of animals which do not show the usual pain reflex within 30 s was noted for each dose. Lidocaine solutions were used for comparison. IC_{50} values were calculated for each compound by probit analysis using a computer program [11].

Rat sciatic nerve block

This test was performed according to Al-Saadi and Sneider [12]. Triplicate sets of three groups of three male Wistar rats (Nossan, Correzzana, Milan, weighing 180–200 g) were used. Each rat received an injection (0.2 ml) of the aqueous anesthetic solution (2%) into the posterior aspect of the femur head. A positive effect of the drug resulted in a complete loss of motor control of the injected limb. In order to assess the duration of the effect, the animals were observed from the time of onset of the motor paralysis at 5 min intervals for the first 30 min, and at 15 min intervals after that up to the first sign of motor activity.

Acute toxicity

The ip acute toxicity of the most active compounds **4b**, **4c** and **4d** was determined in male Swiss mice (Nossan, Correzzana, Milan, weighing 18–20 g) 7 d after treatment. LD_{50} values were calculated for each compound by probit analyisis using a computer program [11].

Acknowledgments

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