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Antiinflammatory and antinociceptive activities of some benzotriazolylalkanoic acids

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Abstract

Sets of benzotriazol-1/2-ylalkanoic acids (**1**, **2**, **3**) and benzotriazol-1-yloxyalkanoic acids (**4**, **5**) were prepared and tested for antiinflammatory activity; when significant activity was observed also the antinociceptive activity was explored. While the acids of structure **1**, **4** and **5** were devoid of antiinflammatory action, most 2-(benzotriazol-1/2-yl)propionic acids (**2**, **3**) exhibited significant activity as antiinflammatory and antinociceptive agents, with compound **2c** and **3a** being the most active in the two assays, respectively. The dextrorotatory enantiomer of **2c** ((+)-**2c**) was also prepared and found to be practically as active as the racemic mixture, though some differences in the steepness of the dose–response curves were observed.

Keywords: Benzotriazolyl alkanoic acids; Benzotriazolyloxyalkanoic acids; Antiinflammatory agents; Antinociceptive agents

1. Introduction

We have been interested from long time in the investigation of the pharmacological potentialities of benzotriazole derivatives, particularly of benzotriazolyl alkanoic acids [1], among which we have found choleretic [2–4] and auxinic (growth enhancing) [5–9] activities.

The isosterism between benzotriazole and naphtalene rings suggests that also antiinflammatory and analgesic activities could be found in suitably substituted benzotriazolyl acetic and propionic acids, as analogues of the well known naproxen [(*S*)-6-methoxy-α-methyl-2-naphtalene acetic acid] [10] and 6-methoxy-2-naphtalene acetic acid (6-MNA), the active metabolite of nabumetone [11,12].

Therefore, we deemed worthy to investigate the antiinflammatory and antinociceptive activities of sets of benzotriazolyl- (**1**–**3**) and benzotriazolyloxy-alkanoic (**4**–**5**) acids. While the first groups of compounds are related to the cited naphtalene alkanoic acids, the latter two recall the very active aryloxyalkanoic acids, which finally led to the ibuprofen discovery [13], and also the more recent MR 714 (2-[4-(2′,4′-difluorophenyl)phenoxy]propionic acid), whose ulcerogenetic action is practically nil [14].

Compounds of structures **2**, **3** and **5** contain a chiral carbon and it is known that for most 'profen' type antiinflammatory agents the *S*-enantiomers are responsible for activity. However, racemic mixtures are commonly used in therapy, because the inactive *R* enantiomers can be converted in vivo into the active ones.

This is not the case of 2-(6-methoxynaphtalene)propionic acid, whose D-form (naproxen) is almost 30 times more active than the L-form in the inhibition of carrageenan induced rat paw edema [10].

Thus we deemed interesting to prepare and test also one pure enantiomer of compound **2c** (which in preliminary experiments resulted the most active in the antiedema assay) in order to investigate the possible difference of activity versus the racemic mixture.

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2. Chemistry

Of the required benzotriazolylalkanoic acids of Scheme 1, only the 2-(6-methyl-benzotriazol-1-yl)propionic acid (**3c**) has not yet been described, whereas **1a**, **2a**, **3a** [1] and **1b**, **2b**–**2e**, **3b** [15] were previously described by us.

When 5-substituted benzotriazoles are alkylated with the suitable ethyl haloalkanoate, three isomeric esters are formed. Chromatography on silica allows the easy separation of the non-basic 2,5-substituted isomer from the two basic 1,5- and 1,6-substituted ones. The further separation of the two latter isomers, either as esters or as free acids, results very difficult and in fact was pursued only for the methoxy derivative **3b** [15].

Therefore, compound **3c** was obtained through the sequence of Scheme 2.

3,4-Dinitrotoluene was reacted with the sodium salt of D,L-alanine in DMSO; the catalytic reduction of the nitro group and the diazotization of the resulting diamino compound should be effected in strictly controlled conditions (see Section 3) to avoid the otherwise very easy formation of 3,5-dimethyl-3,4-dihydro-2*H*-quinoxalin-2-one and of its N-nitroso derivative.

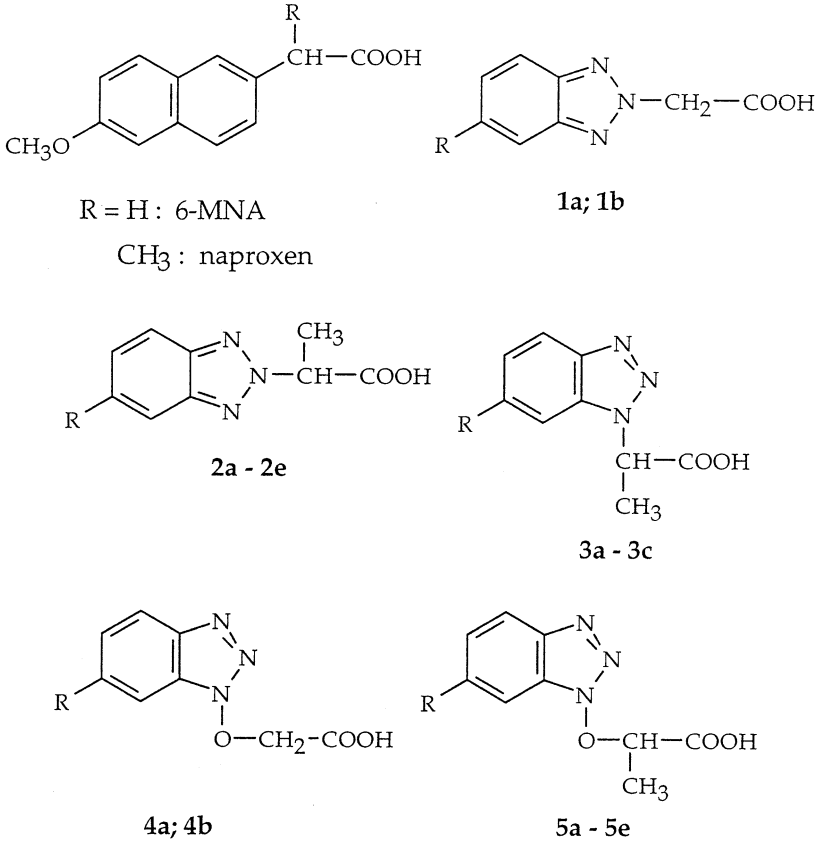
The nucleophilic displacement of the 3-nitro group of 3,4-dinitrotoluene was proved through the formation of a different compound, namely the isomeric *N*-(4-methyl-2-nitrophenyl)alanine (**10**), by reacting the sodium salt of D,L-alanine with 4-methyl-2-nitrochlorobenzene. From **10**, the isomeric 2-(5-methyl-benzotriazol-1-yl)propionic acid was obtained (Scheme 3).

UV spectra of the two isomers **3c** and **12** were quite different and conforming respectively to the known spectra of 1,6- and 1,5-dimethylbenzotriazoles [16,17].

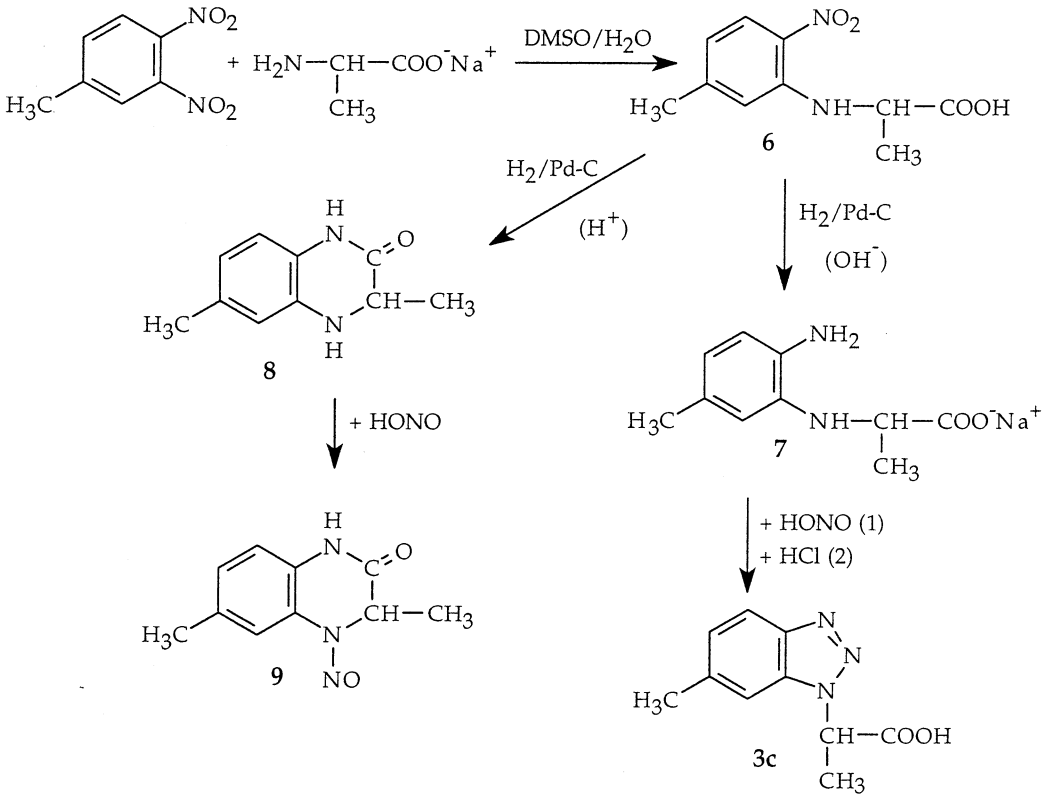
In order to prepare one single enantiomer of compound **2c**, 5-methyl-1*H*-benzotriazole was reacted for a short time with (*S*)-(−)-2-bromopropionic acid in the presence of 2 equiv. of sodium hydroxyde (Scheme 4).

Through a S_N2 mechanism the (*R*)-(+)-2-(5-methyl-benzotriazol-2-yl)propionic acid should be expected, however, due to the effect of the neighboring carboxylate group, the alkylation reaction could proceed also with retention of (*S*)-configuration [18,19] and the two mechanisms could even operate competitively.

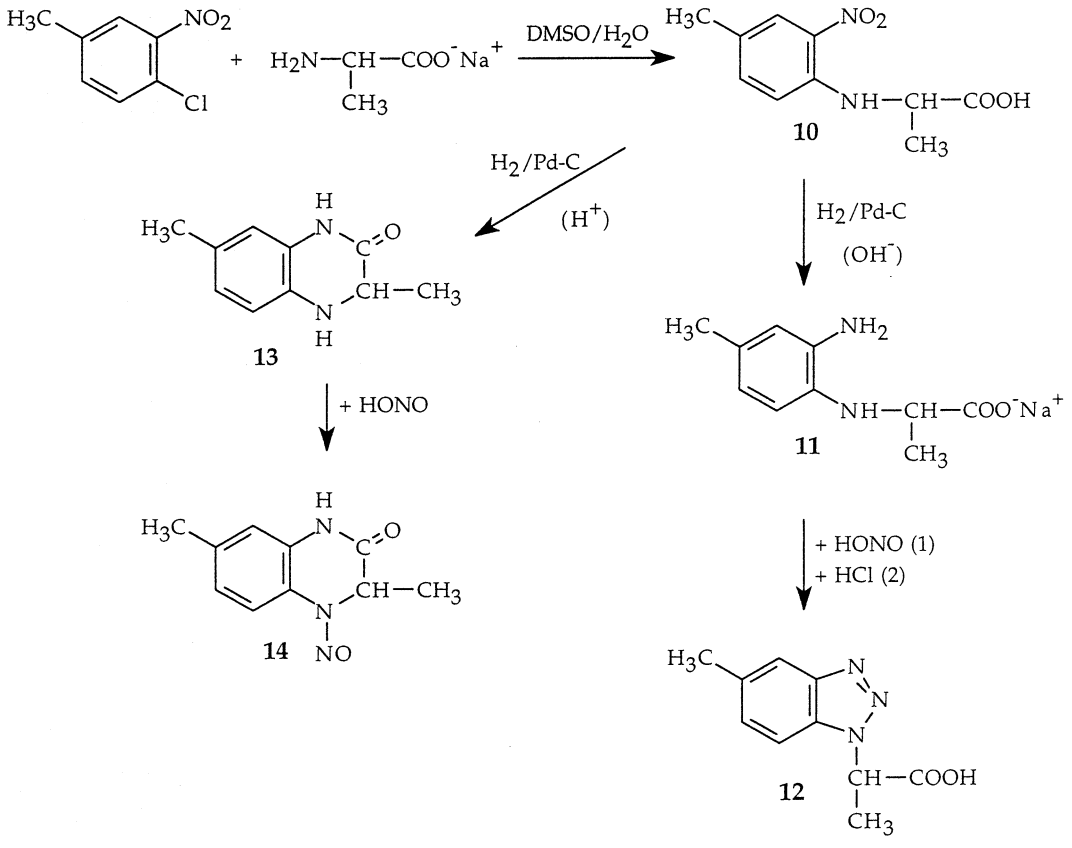
The required non basic 2-(5-methyl-benzotriazol-2-yl)propionic acid was separated from 2-(5-methyl-benzotriazol-1-yl)propionic acid and 2-(6-methyl-benzotriazol-1-yl)propionic acid by converting the latter two into



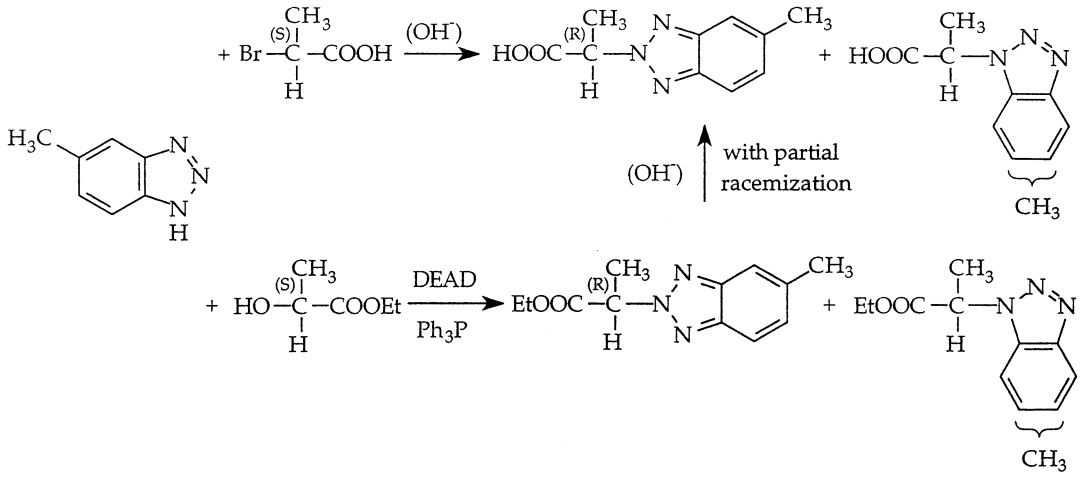
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 4.

the ether insoluble hydrochlorides by means of dry hydrogen chloride in dry ether.

Thus a dextro-rotatory 2-(5-methyl-benzotriazol-2-yl)propionic acid ((+)-**2c**) was obtained, whose enantiomeric purity was established through the ^{1}H NMR spectrum of the salt formed with (1*R*,2*R*)-(+)-1,2-diphenylethylenediamine, as suggested by Fulwood and Parker [20] for chiral carboxylic acids.

This spectrum exhibits a single quartet and a single doublet, respectively, for the CH and methyl group of the propionic chain, while the spectrum of the corresponding salt of racemic **2c** exhibits two well separate quartet for the CH and two, partially superimposed, doublets for the methyl group.

For an unequivocal determination of the absolute configuration of (+)-**2c**, the preparation of the pure (*R*)-enantiomer was attempted by condensing 5-methyl-1*H*-benzotriazole with ethyl (*S*)-(−)-lactate in Mitsunobu conditions [21,22], which are supposed to produce the inversion of configuration of the chiral carbon. However, when the obtained ethyl 2-(5-methyl-benzotriazol-2-yl)propionate was hydrolyzed, a dextro-rotatory acid was obtained whose specific rotation was quite lower than expected.

The ^{1}H NMR spectrum of the corresponding salt with (1*R*,2*R*)-(+)-1,2-diphenylethylenediamine indicates the presence of two diastereoisomeric salts in the ratio of 64 and 36%, respectively.

Similarly the condensation of 5-methyl-1*H*-benzotriazole with methyl (*R*)-(+)-lactate in Mitsunobu conditions yielded a levo-rotatory acid whose salt with (1*R*,2*R*)-(+)-1,2-diphenylethylenediamine exhibited in the ^{1}H NMR spectrum the presence of the above diastereoisomeric salts but in the inverted ratio of 30 and 70%, respectively.

These results suggest either that in these cases the Mitsunobu reaction does not follow a single path [22] or that, even in the mild conditions which were used for the ester hydrolysis, a consistent racemization can occur. The last possibility is contrasting with what has been observed by other Authors for the hydrolysis of (*R*)- or (*S*)-2-phenoxypropionic esters [23,24] in similar experimental conditions.

Assuming that the most abundant component of the above mixtures are, respectively, the expected (*R*) and (*S*) enantiomer, the position of corresponding quartet due to CH group allows to establish the (*R*)-configuration of compound (+)-**2c** and, therefore, its formation through a pure S_N2 mechanism (Scheme 5).

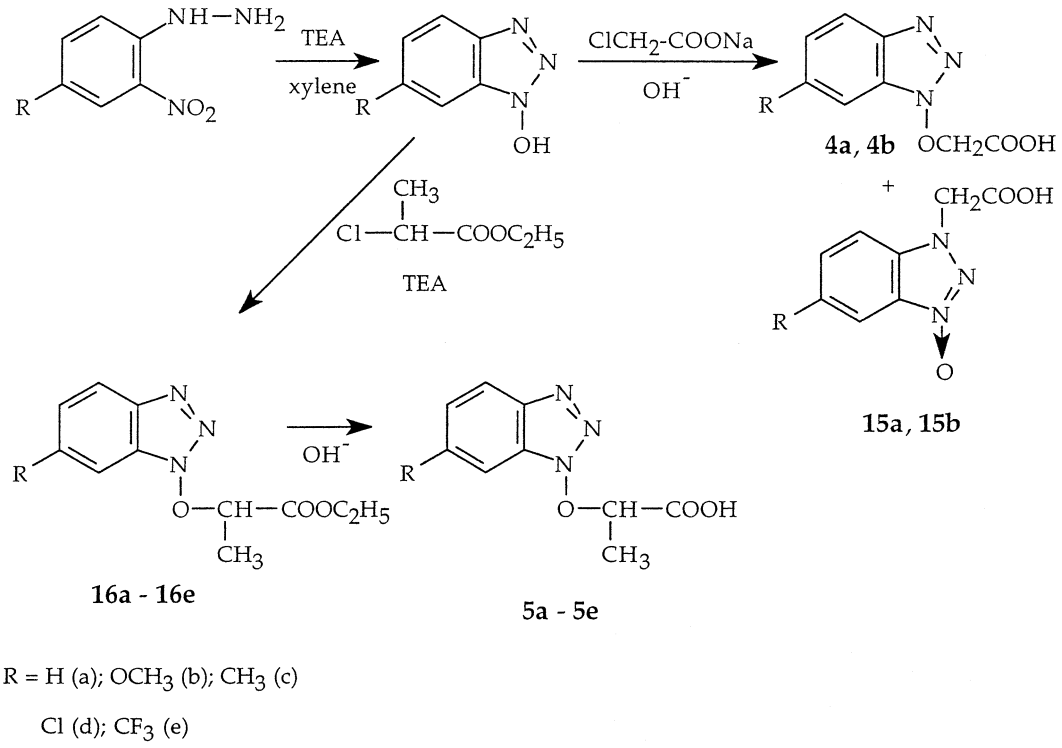
The benzotriazol-1-yloxyacetic acids (**4**) were obtained, in only moderate yields, by the alkylation of suitable substituted 1-hydroxybenzotriazole with chloroacetic acid in alkaline medium. Beside the expected acids **4a** and **4b**, isomeric acids were isolated, which were considered as N-oxides (**15a**, **15b**) on the base of their peculiar UV spectra [25].

The formation of N-oxides of N-alkylated benzotriazole was already observed by us [26] and by other Authors [27] when 1-hydroxybenzotriazole was treated, respectively, with epichloridrin and with dialkylaminoalkyl chlorides.

The required 6-substituted-1-hydroxybenzotriazoles were obtained through the base (triethylamine (TEA)) catalyzed cyclization of 2-nitro-4-substituted-phenylhydrazines. This method represents a valid alternative to that previously described by Brady and Reynolds [28] and successively extended by Koenig and Geiger [29].

The benzotriazol-1-yloxy propionic acids (**5**) were prepared by hydrolyzing the corresponding esters, which, in turn, were obtained in a one pot reaction, from 2-nitro-4-substituted-phenylhydrazines, TEA and ethyl 2-chloropropionate in xylene solution.

The isomeric N-oxides were not isolated, since their formation through a N-alkylation could have been hampered by steric hindrance.



Scheme 5.

Structures of all compounds were supported by elemental analyses results and spectral data (UV and ^{1}H NMR).

3. Experimental

3.1. Chemistry

Melting points were determined by the capillary method on a Büchi apparatus and are uncorrected.

Elemental analyses were performed with CE EA 1110 CHNS-O instrument and the results obtained for the indicate elements were within $\pm 0.4\%$ of the calculated values.

UV spectra were recorded on a Perkin–Elmer model 550S spectrophotometer; ^{1}H NMR were taken on a Varian Gemini 200 spectrometer, using $CDCl_3$ or d_6-DMSO as solvent with TMS as internal standard.

Optical activity was measured in ethanol solution, with Perkin–Elmer 241 MC polarimeter (sodium lamp, $\lambda = 589$ nm; tube length 10 cm).

Preparation and characteristics of most benzotriazolyl alkanoic acids of Scheme 1 have been already described [1,15].

3.1.1. D,L-N-(5-methyl-2-nitrophenyl)alanine (6)

3,4-Dinitrotoluene (5 g, 27 mmol) was dissolved in 2 ml of DMSO and mixed with a solution of D,L-alanine (2.45 g, 27 mmol) in 4.5 ml of 6 N sodium hydroxide; the mixture was heated for 30 min at 150 °C. After cooling water was added and the unreacted dinitrotoluene was removed with dichloromethane; after acidification the solution was extracted again with dichloromethane. The solvent was removed and the residue was chromatographed on silica eluting with dichloromethane. From the first fractions, 3.01 g (49.7%) of **6** were obtained; m.p. 108–110 °C. Analysis for $C_{10}H_{12}N_2O_4$.

^{1}H NMR (d_6-DMSO): δ 1.5 (d, 3H, CH–CH_3); 2.3 (s, 3H, CH_3–Ar); 4.5 (q, 1H, CH); 6.5 (d, 1H arom); 6.8 (s, 1H arom); 8.0 (d, 1H arom); 8.2 (d, 1H, NH; collapses after D_2O exchange); 13.3 (s, 1H, COOH; exchanges with D_2O).

3.1.2. D,L-2-(6-methylbenzotriazol-1-yl)propionic acid (3c)

The above compound **6** (1.5 g, 6.7 mmol) was dissolved in ethanol, added with 3.3 ml of 2 N NaOH and was hydrogenated in the presence of 5% palladium on activated carbon at room temperature and atmospheric pressure. The catalyst and the solvent were removed and the residue was dissolved in 15 ml of water. A 1 N solution of sodium nitrite (7.5 ml) was added and, after cooling to 0–5 °C and under vigorous stirring, 7 ml of 1 N HCl were dropped slowly. Stirring was continued for 30 min in the cold and for 1 h further at room temperature.

The solution was acidified and extracted with dichloromethane. The crude extract was chromatographed on silica eluting with dichloromethane containing 0.5% of

methanol. A pure acid **3c** (0.47 g, 34%), melting at 180–182 °C, was obtained. Analysis (C, H, N) for $C_{10}H_{11}N_3O_2$.

UV (ethanol): λ_{max} (nm) 231; 263; 274sh.

^{1}H NMR (d_6-DMSO): δ 1.92 (d, 3H, CH–CH_3); 2.40 (s, 3H, CH_3–Ar); 5.9 (q, 1H, CH); 7.26 (m, 1H arom); 7.60 (m, 1H arom); 7.92 (d, 1H arom); 13.4 (s, 1H, COOH exchangeable with D_2O).

3.1.3. 2,7-Dimethyl-1-nitroso-1,2-dihydroquinoxalin-4H-3-one (**9**)

D,L-*N*-(5-methyl-2-nitrophenyl)alanine (1g, 4.5 mmol) dissolved in ethanol (or in ethanol acidified with 1.5 ml of 6 N HCl) was reduced in the presence of 5% palladium on activated carbon. After removing the catalyst and the solvent the residue was taken up either with 15 ml of acetic acid/water (1:1, v/v) or with 15 ml of water plus 0.7 ml 6 N HCl and diazotized at ~ 0 °C with 0.36 g (5.2 mmol) of sodium nitrite in 6 ml of water.

In both cases a white precipitate (0.77 g, 84% yield) was obtained; m.p. 218–220 °C with evolution of reddish vapor. Analysis (C, H, N) for $C_{10}H_{11}N_3O_2$.

^{1}H NMR ($CDCl_3$): δ 1.35 (d, 3H, CHCH_3); 2.41 (s, 3H, CH_3–Ar); 5.63 (q, 1H, CH); 6.95 (d, 1H arom); 7.15 (d, 1H arom); 7.75 (s, 1H arom); 9.55 (s, 1H, NH exchangeable with D_2O).

3.1.4. D,L-N(4-methyl-2-nitrophenyl)alanine (**10**)

A mixture of 4-methyl-2-nitrochlorobenzene (5 g, 29 mmol), D,L-alanine (2.6 g, 29 mmol), 5.3 ml of 6 N NaOH solution and 2 ml DMSO were heated for 1 h at 150 °C. After cooling the reaction mixture was worked up as described for compound **6**, obtaining 1.74 g (27%) of **10** melting at 143–145 °C. Analysis (C, H, N) for $C_{10}H_{12}N_2O_4$.

^{1}H NMR (d_6-DMSO): δ 1.5 (d, 3H, CH–CH_3); 2.3 (s, 3H, CH_3–Ar); 4.5 (q, 1H, CH); 6.9 (d, 1H arom); 7.4 (m, 1H arom); 7.9 (m, 1H arom); 8.3 (d, 1H, NH, exchangeable with D_2O), 13.2 (s, 1H, COOH, exchangeable with D_2O).

3.1.5. D,L-2-(5-methylbenzotriazol-1-yl)propionic acid (**12**)

D,L-*N*-(4-methyl-2-nitrophenyl)alanine (1 g, 4.5 mmol) was reduced catalytically (5% Pd on activated carbon) in ethanol solution added with 2.2 ml of 2 N NaOH. After the hydrogen absorption was terminated, the solution was worked up as described for the preparation of **3c**.

Compound **12** was obtained as off-white crystals melting at 173–177 °C. Analysis (C, H, N) for $C_{10}H_{11}N_3O_2$.

UV (ethanol): λ_{max} (nm): 255; 260sh; 288.

^{1}H NMR (d_6-DMSO): δ 1.90 (d, 3H, CH–CH_3); 2.38 (s, 3H, CH_3–Ar); 5.91 (q, 1H, CH); 7.4 (m, 1H arom); 7.71 (d, 1H arom); 7.83 (m, 1H arom); 13.4 (s, 1H, COOH, exchangeable with D_2O).

3.1.6. 2,6-Dimethyl-1-nitroso-1,2-dihydroquinoxalin-4H-3-one (**14**)

The reduction of D,L-*N*-(4-methyl-2-nitrophenyl)alanine in acidic solution and the subsequent diazotization in acidic condition as described for compound **9** gave rise to a white precipitate (84% yield) melting at 222–225 °C with evolution of reddish vapor. Analysis (C, H, N) for $C_{10}H_{11}N_3O_2$.

^{1}H NMR ($CDCl_3$): δ 1.33 (d, 3H, CHCH_3); 2.37 (s, 3H, CH_3–Ar); 5.64 (q, 1H, CH); 6.88 (s, 1H arom); 7.0 (d, 1H arom); 7.78 (d, 1H arom); 9.82 (m, 1H, NH, exchangeable with D_2O).

3.1.7. R-(+)-2-(5-Methyl-benzotriazol-2-yl)propionic acid ((+)-**2c**)

For this preparation the commercially available (*S*)-(−)-2-bromopropionic acid ($[\alpha]_D^{24} = -30°$, $c = 3.5\%$ in ethanol) was used.

To a solution of 5-methyl-1*H*-benzotriazole (1.33 g, 10 mmol) in 20 ml of 1 N NaOH (20 mmol) (*S*)-(−)-2-bromopropionic acid (1.53 g, 10 mmol) was added. The solution was heated at 90 °C for 30 min. After cooling the unreacted methylbenzotriazole was extracted with dichloromethane (0.45 g); the solution was acidified to pH 2 and thoroughly extracted with dichloromethane. The extract was washed with water, dried and evaporated. The residue was dissolved in dry ether and treated, drop-by-drop, with an ethereal solution of dry hydrogen chloride until no more precipitate was formed. The clear solution was separated from the precipitated hydrochlorides and evaporated to dryness under reduced pressure. The residue was dissolved in the smallest volume of dry ether and treated, again, with an ethereal solution of dry hydrogen chloride; this operation was repeated until no more precipitate was formed. After removing the solvent the residue was crystallized from dry ether giving 280 mg (20.6% yield) of the expected compound; m.p. 143–146 °C; $[\alpha]_D^{24} = +3.4°$ ($c = 1.8\%$ in ethanol). Analysis (C, H, N) for $C_{10}H_{11}N_3O_2$.

^{1}H NMR ($CDCl_3$): δ 2.06 (d, 3H; CH–CH_3); 2.49 (s, 3H, CH_3Ph); 5.73 (q, 1H; CH–CH_3); 7.25 (dd, 1H arom); 7.61 (s, 1H arom); 7.78 (d, 1H arom); 9.9 (s, 1H, COOH; exchangeable with D_2O).

- ^{1}H NMR ($CDCl_3$) of the salt of (+)-**2c** with (1*R*,2*R*)-(+)-1,2-diphenylethylenediammine (ratio acid:-diamine = 2:1; conc. 0.1 M): δ 1.78 (d, 6H; 2CH–CH_3); 2.42 (s, 6H, $2CH_3$–Ph); 4.38 (s, 2H; 2Ph–CH–NH_3^+); 5.15 (q, 2H; $2CH$–CH_3); 6.84 (d, 4H arom); 7.10–7.30 (m, 8H arom); 7.48 (s, 2H arom); 7.63 (d, 2H arom); 9.06 (s, 6H, $2NH_3^+$).
- ^{1}H NMR ($CDCl_3$) of the salt between racemic **2c** and (1*R*,2*R*)-(+)-1,2-diphenylethylenediammine (ratio

acid:diamine = 2:1; conc. 0.1 M): δ 1.78–1.87 (an apparent symmetric t, due to two partially superimposed d, 6H, 2CH–CH_3); 2.40 (s, 6H, 2$CH3$–Ph); 4.37 (s, 2H; 2Ph–CH–NH_3^+); 5.11–5.25 and 5.26–5.40 (2q, 1 H each; 2CH–CH_3); 6.80–7.68 (m, 16H arom); 8.78 (s, 6H, 2NH_3^+).

3.1.8. Attempts to prepare (*R*)-(+)- and (*S*)-(−)-2-(5-methylbenzotriazol-2-yl)propionic acid through a Mitsunobu condensation

For these preparations the commercially available ethyl (*S*)-(−)-lactate ($[\alpha]_D^{24} = -11°$, neat) and methyl (*R*)-(+)-lactate ($[\alpha]_D^{24} = +8.2°$, neat) were used.

(a) To a solution of 5-methyl-1*H*-benzotriazole (1.33 g; 10 mmol), (*S*)-(−)-ethyl lactate (1.18 g; 10 mmol) and triphenylphosphine (2.62 g; 10 mmol) in 15 ml of dry tetrahydrofuran (THF), a solution of diethyl azodicarboxylate (1.92 g; 11 mmol) in 7 ml of THF was added drop-by-drop. The mixture was stirred at room temperature, under a nitrogen atmosphere, for 8 h. The solvent was removed under reduced pressure and the residue was treated with 30 ml of a mixture of dry ether–hexane (1:1) in order to separate the triphenylphosphine oxide. The solution was evaporated to dryness and the residue treated with 20 ml of ether–hexane mixture to separate some more triphenylphosphine oxide; this treatment may be repeated.

After removing the solvent an oily residue was obtained, which was chromatographed on silica gel (1:15) eluting with dichloromethane to eliminate most of the formed diethyl hydrazodicarboxylate. The oil obtained from the forerunning fractions was chromatographed several times on silica gel (1:25) to separate the ethyl 2-(5-methylbenzotriazol-2-yl)propionate from the ethyl 2-(5/6-methylbenzotriazol-1-yl)propionate.

Finally 360 mg (15.4% yield) of pure ester were obtained; $[\alpha]_D^{24} = +6.8°$ ($c = 1.03\%$ in ethanol).

^{1}H NMR ($CDCl_3$): δ 1.22 (t, 3H; CH_3–CH_2); 2.04 (d, 3H; CH–CH_3); 2.49 (s, 3H; CH_3–Ph); 4.22 (q, 2H; CH_3–CH_2); 5.65 (q, 1H; CH–CH_3); 7.24 (dd, 1H arom); 7.62 (s, 1H arom); 7.77 (d, 1H arom).

Hydrolysis. The above ester (220 mg; 0.94 mmol) was dissolved in THF (3 ml) and treated with 2 ml of 1 N NaOH solution + 1 ml of water; the mixture was stirred at room temperature for 2 h. The organic solvent was removed at r.t. under reduced pressure, water was added (7 ml) and the unreacted ester was extracted with ether. The water solution was acidified with 1 ml of 2 N HCl and extracted again with ether; 140 mg of acid (m.p. 141–144 °C; $[\alpha]_D^{24} = +0.8°$, $c = 1\%$ in ethanol) were obtained, whose ^{1}H NMR spectrum was coincident with that of acid described in point Section 3.1.7).

^{1}H NMR ($CDCl_3$) of the salt with (1*R*,2*R*)-(+)-1,2-diphenylethylenediamine was practically coincident with that of the racemic acid salt, differing only for the two quartet at δ 5.10–5.25 and 5.25–5.40 corresponding, respectively, to about 1.28 H and 0.72 H; the lateral peaks of the triplet (two partially superimposed d) at δ 1.72–1.85 are unsimmetrical, but do not allow a ratio evaluation.

(b) In the same way, starting from methyl (*R*)-(+)-lactate, a levo-rotatory methyl 2-(5-methylbenzotriazol-2-yl)propionate was obtained: $[\alpha]_D^{24} = -17°$ (c 1.1% in ethanol).

^{1}H NMR ($CDCl_3$): δ 2.03 (d, 3H, CH–CH_3); 2.49 (s, 3H, CH_3–Ph); 3.74 (s, 3H, OCH_3); 5.68 (q, 1H, CH–CH_3); 7.24 (dd, 1H arom); 7.63 (s, 1H arom); 7.78 (d, 1H arom).

The hydrolysis of the above ester yielded an acid melting at 141–144 °C; $[\alpha]_D^{24} = -1.6$ ($c = 1.2\%$ in ethanol). The ^{1}H NMR spectrum was coincident with that of the acid described in point Section 3.1.7.

^{1}H NMR ($CDCl_3$) of the salt with (1*R*,2*R*)-(+)-1,2-diphenylethylenediamine differed from that of the salt of racemic **2c** only for the two quartet at δ 5.14–5.28 and 5.28–5.44, corresponding, respectively, to about 0.6 and 1.4 H; the lateral peaks of the triplet at δ 1.70–1.85 exhibit an inverted asymmetry with respect to the salt described under (a).

3.1.9. 6-Substitutes-1-hydroxybenzotriazoles

4-Substituted-2-nitrophenylhydrazine (20 mmol) was dissolved in xylene (40 ml) and added with 6 g ($\sim$60 mmol) of TEA; the solution was refluxed under nitrogen for 3 h. The solvent was removed under reduced pressure and the residue was triturated with 1 N hydrochloric acid, filtered and washed with the same acid and then with water. The product was crystallized from ethanol.

1-Hydroxybenzotriazole; m.p. 158–160 °C (lit. [30] 157–158 °C); yield 62%. 6-Methoxy-1-hydroxybenzotriazole, m.p. 168–170 °C, yield 81% (lit. [29] m.p. 172–173 °C, yield 6%!).

3.1.10. (6-Substituted-benzotriazol-1-yl)oxyacetic acids (*4a*, *4b*)

6-Substituted-1-hydroxybenzotriazole (18.5 mmol) was dissolved in ethanol (50 ml) and treated with 1.75 g (18.5 mmol) of chloroacetic acid and 1.48 g (37 mmol) of sodium hydroxide. The solution was refluxed for 3 h and then evaporated to dryness under reduced pressure and the residue dissolved in water. The solution was extracted with ether and then acidified with 1 N HCl and the precipitate was collected and washed with water. The product was crystallized from water (R = H) or from ethanol (R = CH_3O).

Compound **4a**, m.p. 170.5–171.5 °C, yield 39%. Analysis (C, H, N) for $C_8H_7N_3O_3$.

Compound **4b**, m.p. 172 °C (dec.), yield 43%. Analysis (C, H, N) for $C_9H_9N_3O_4$.

*3.1.11. 6-Substituted-benzotriazol-3-yl acetic acids 1-oxydes (**15a, 15b**)*

The acid solutions from which compounds **4a** and **4b** have been filtered out, were evaporated to dryness under reduced pressure. The residues were dried under vacuum and then extracted with absolute ethanol; the solvent was removed and the new residues were crystallized from ethanol.

Compound **15a**, m.p. 222 °C (dec.), yield 4.2%. Analysis (C, H, N) for $C_8H_7N_3O_3$.

UV (ethanol): λ_{max} (nm) 271.5; 281; 322.5.

Compound **15b**, m.p. 254 °C (dec.), yield 14.3%. Analysis (C, H, N) for $C_9H_9N_3O_4$.

UV (ethanol): λ_{max} (nm) 274.5; 283.5; 336.5.

*3.1.12. Ethyl 2-(6-substituted-benzotriazol-1-yloxy)propionates (**16a–16e**)*

To the xylene solution of the suitable 4-substituted-2-nitrophenylhydrazine (20 mmol in 40 ml), 6 g (60 mmol) of TEA and 2.7 g of ethyl 2-chloropropionate were added. The solution was refluxed for 3 h under a stream of nitrogen; after cooling the precipitate was filtered and the solution was evaporated to dryness under reduced pressure. The residue was partitioned between dichloromethane and 1 N hydrochloric acid. The organic phase was washed with water, dried with sodium sulfate and evaporated to dryness. The obtained viscous oil was chromatographed on silica, eluting with dichloromethane and then distilled in vacuo (0.03 torr) or crystallized.

Yields and characteristics of esters **16a–16e** are collected in Table 1a.

*3.1.13. 2-(6-Substituted-benzotriazol-1-yloxy)propionic acids (**5a–5e**)*

Esters **16a–16e** (5–8 mmol) were dissolved in ethanol (15–24 ml) and added with 3 N NaOH solution (2.5–4 ml); the solution was refluxed for 1.5 h and then evaporated almost to dryness. The residue was dissolved in water and extracted with ether to remove the unreacted ester. The alkaline solution was acidified with 1 N hydrochloric acid and extracted again with ether. The acids **5a–5e** were crystallized from the solvent indicated in Table 1a, where yields and characteristics are also reported.

3.2. Pharmacology

The 17 acids of Scheme 1 were screened in vivo for their antiinflammatory activity; compounds active in this test were assayed also for antinociceptive activity.

Male albino Sprague–Dawley rats (120–150 g) and male Swiss mice (15–25 g) were used. Animals were fasted for 15 h before treatment, but had always free access to water.

All compounds were administered orally by a gastric tube, as finely homogenized suspension in 0.5% carboxymethylcellulose (CMC) (1 ml/100 g body weight), at the initial dose of 200 mg/kg. Compounds which exhibited a statistically significant activity at this dose were further tested at doses decreasing by a factor of 2, always in the same volume of 0.5% CMC. Controls received the same volume of CMC dispersion.

3.2.1. Anti-inflammatory activity

The carrageenan-induced paw edema test [31] was performed in groups of five rats. Sixty minutes after administering the test compound, 0.1 ml of 1% carrageenan suspension in saline was injected into the plantar surface of the right hind paw of each rat. Paw volume, as determined by measuring the volume of water displaced after immersing paw up to the lateral malleolus level, was recorded immediately after the carrageenan injection and again 2 h later. The difference between these two values was taken as edema volume. The percent inhibition of the edema of treated rats with respect to control animals was calculated and compared

Table 1a
2-(6-Substituted-benzotriazol-1-yloxy)propionic acids (**5a–5e**) and their ethyl esters (**16a–16e**)

Comp.	R	Formula [a]	M.p. (°C) b.p. (°C/torr)	Solvent [b]	Yield (%)
5a	H	$C_9H_9N_3O_3$	136.5–137.5	A	87 [c]
5b	OCH3	$C_{10}H_{11}N_3O_4$	142.5–144	B	82 [c]
5c	CH3	$C_{10}H_{11}N_3O_3$	107–109	B	88 [c]
5d	Cl	$C_9H_8ClN_3O_3$	133–134	B	63 [c]
5e	CF3	$C_{10}H_8F_3N_3O_3$	88.5–90	C	80 [c]
16a	H	$C_{11}H_{13}N_3O_3$	85–90/0.02		45
16b	OCH3	$C_{12}H_{15}N_3O_4$	100–110/0.03		51
16c	CH3	$C_{12}H_{15}N_3O_3$	100–110/0.03		50
16d	Cl	$C_{11}H_{12}ClN_3O_3$	53–54	C	53
16e	CF3	$C_{12}H_{12}F_3N_3O_3$	49.5–50.5	C	40

[a] All compounds were analyzed for C, H, N and the results were within ±0.4 % of the calculated values.
[b] A, ethanol. B, dry ether. C, dry ether–petroleum ether.
[c] Yields of acids **5** from the hydrolysis of esters **16**.

Table 1b
Analytical results of new compounds and corresponding intermediates

Comp.	Formula	%C		%H		%N	
		Found	Calc.	Found	Calc.	Found	Calc.
6	$C_{10}H_{12}N_2O_4$	53.71	53.57	5.28	5.39	12.64	12.49
3 c	$C_{10}H_{11}N_3O_2$	58.81	58.53	5.48	5.40	20.32	20.48
9	$C_{10}H_{11}N_3O_2$	58.71	58.53	5.56	5.40	20.31	20.48
10	$C_{10}H_{12}N_2O_4$	53.41	53.57	5.45	5.39	12.60	12.49
12	$C_{10}H_{11}N_3O_2$	58.21	58.53	5.55	5.40	20.71	20.48
14	$C_{10}H_{11}N_3O_2$	58.24	58.53	5.61	5.40	20.34	20.48
(+) 2 c	$C_{10}H_{11}N_3O_2$	58.35	58.53	5.65	5.40	20.13	20.48
4 a	$C_8H_7N_3O_3$	50.04	49.74	3.89	3.65	22.04	21.75
4 b	$C_9H_9N_3O_4$	48.58	48.43	4.21	4.06	18.73	18.83
15 a	$C_8H_7N_3O_3$	49.50	49.74	3.86	3.65	21.82	21.75
15 b	$C_9H_9N_3O_4$	48.12	48.43	4.14	4.06	18.92	18.83
5 a	$C_9H_9N_3O_3$	51.92	52.17	4.52	4.38	20.28	20.28
5 b	$C_{10}H_{11}N_3O_4$	50.81	50.63	4.69	4.68	17.67	17.71
5 c	$C_{10}H_{11}N_3O_3$	54.08	54.29	5.02	5.01	19.17	18.99
5 d	$C_9H_8ClN_3O_3$	44.72	44.73	3.37	3.33	17.25	17.39
5 e	$C_{10}H_8F_3N_3O_3$	43.78	43.64	3.05	2.93	15.22	15.27
16 a	$C_{11}H_{13}N_3O_3$	55.98	56.16	5.32	5.57	17.86	17.86
16 b	$C_{12}H_{15}N_3O_4$	54.52	54.33	5.39	5.70	15.79	15.84
16 c	$C_{12}H_{15}N_3O_3$	58.01	57.82	6.24	6.07	16.78	16.86
16 d	$C_{11}H_{12}ClN_3O_3$	48.81	48.99	4.61	4.49	15.51	15.58
16 e	$C_{12}H_{12}F_3N_3O_3$	47.72	47.53	3.92	3.99	13.97	13.86

with that produced by indomethacin (6 mg/kg p. o.), used as reference drug.

3.2.2. Analgesic activity

The writhing test [32] was performed in groups of six mice. One hour after the administration of the test compound, 0.01 ml/g of a 0.6% acetic acid solution was injected intraperitoneally in each mouse. The writhing movements of each animal were counted for 15 min (between the fifth and 20th min after the injection of the irritant). The antinociceptive effect was expressed as the percent reduction of writhing number compared with the control group. Indomethacin (6 mg/kg, p.o.) was used as reference drug.

4. Results and discussion

The results of the antiinflammatory and antinociceptive assays are summarized in Table 2.

Four out of the 16 test compounds exhibited significant inhibition of the carrageenan-induced rat paw edema at the oral dose of 200 mg/kg, with activity decreasing in the order **2c** > **2a** > **3b** > **3a**. The most active compound **2c** displayed a significant inhibition of the paw edema in doses ranging from 50 to 200 mg/kg, with a percent inhibition going from 35 to 51%. Compound **2a** was still active at 100 mg/kg, whereas compounds **3b** and **3a** did not show statistically significant activity at such a dose. The dextro-rotatory form of compound **2c** was somewhat more active than the racemic form in the dose range from 50 to 200 mg/kg and was still active at 25 mg/kg. Another difference between the two forms is the steepness of the dose response curve of the dextro-rotatory compound, which passes from a 27% inhibition at 25 mg/kg to a 61% at 50 mg/kg and remains at this level of activity when the dose is increased further up to 200 mg/kg.

Different explanations could be advanced for these results; however, we deem that a sound interpretation can be formulated only when the levo-rotatory form of compound **2c** will be available for testing.

Indomethacin used as reference drug gave a 65% inhibition of paw edema at the oral dose of 6 mg/kg.

It is noteworthy that compounds of structure **1**, **4** and **5** were practically devoid of antiedema activity; thus the benzotriazolyloxyalkanoic acids differ strikingly from the mentioned highly active aryloxypropionic acids.

The proximity of the ramification of alkanoic chain to the heterocyclic ring seem to be responsible for the activity, probably by forcing the carboxyl group out of the plane of the benzotriazole ring.

All the compounds displaying antiedema activity were also active in the antinociceptive assay; however, in the latter test the most active compound was **3a** which was the least active as antiinflammatory agent. Compound **3a** protected mice against the acetic acid-induced writhing in the dose range from 12.5 to 200 mg/kg.

Evidently in this set of compounds the structural requirements for antinociceptive and antiinflammatory activities are somewhat different.

Table 2
Antiinflammatory and antinociceptive activities of compounds **1**–**5**

Comp.	Tested dose (mg/kg p.o.)	Antiinflammatory activity [a] (% edema inhibition)	Antinociceptive activity [b] (% reduction of writhings)
1a	200	0	nt
Table 1b	200	0	nt
2a	200	45*[c]	28*[c]
	100	27**[c]	23**[c]
	50	8	14
2b	200	10	nt
2c	200	51* [c]	57* [c]
	100	45* [c]	42* [c]
	50	35* [c]	30* [c]
	25	15	17
(+)-**2c**	200	58* [c]	47* [c]
	100	63* [c]	45* [c]
	50	61* [c]	44* [c]
	25	27** [c]	18
	12.5	10	nt
2d	200	5	nt
2e	200	0	nt
3a	200	23** [c]	63* [c]
	100	0	59*[c]
	50	nt	40*[c]
	25	nt	32*[c]
	12.5	nt	21**[c]
	6.25	nt	8
3b	200	39* [c]	51* [c]
	100	12	19** [c]
	50	nt	8
3c	200	nt	45* [c]
	100	nt	41* [c]
	50	nt	4
4a	200	0	nt
4b	200	0	nt
5a	200	0	nt
5b	200	0	nt
5c	200	0	nt
5d	200	12	nt
5e	200	0	nt
Indome thacin	6	65* [c]	72* [c]

[a] Carrageenan-induced paw edema test (on group of five rats).
[b] Acetic acid induced writhings test (on group of six mice).
[c] Statistical significance versus control group evaluated by the Student's t-test: *$P < 0.01$, **$P < 0.05$; nt, not tested.

It is worth noting that the racemic and dextro-rotatory forms of compound **2c** present some small differences also in the antinociceptive activity, though these can be hardly considered statistically significant. For (+)-**2c** the protective action reaches a 44% level at 50 mg/kg and remains unchanged when the dose is increased up to 200 mg/kg, while for the racemic form a gradual increase of activity from 30 to 57% is observed when the dose is increased from 50 to 200 mg/kg.

Moreover, the dextro-rotatory form of compound **2c**, at the highest dose used (200 mg/kg), produced a mild diarrhoea in mice; such an effect (which was not observed in rats used for the antiinflammatory assay) could have influenced the antinociceptive activity of this compound.

Concluding, it appears worthwhile to extend the study to other benzotriazol-1/2-ylalkanoic acids in order to define an appropriate structure–activity correlation for this kind of compounds.

Moreover, compound **2c**, which exhibits good activity as antiinflammatory and antinociceptive agent, deserves further investigations in order to verify any possible ulcerogenic action and the capability to discriminate between the inducible cyclooxygenase (COX-2) and the constitutive one (COX-1).

The importance of the selectivity for COX-2 for developing antiinflammatory agents devoid of the usual gastro-intestinal side effects is well established [33–35]. However, it was recently reported that COX-2 may be important for homeostasis in health and disease [36];

therefore, in particular situations the safety of selective COX-2 inhibitors remains questionable [37–39].

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