# Preparation and local anaesthetic activity of benzotriazinone and benzoyltriazole derivatives

**ARTICLE** *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · DECEMBER 1999

Impact Factor: 3.45 · DOI: 10.1016/S0223-5234(99)00126-9

*Eur. J. Med. Chem. 34 (1999) 1043–1051*



**Original article**

# Preparation and local anaesthetic activity of benzotriazinone and benzoyltriazole derivatives

Giuseppe Caliendo[a]*, Ferdinando Fiorino[a], Paolo Grieco[a], Elisa Perissutti[a], Vincenzo Santagada[a], Rosaria Meli[b], Giuseppina Mattace Raso[b], Angelina Zanesco[c], Gilberto De Nucci[c]

[a]*Dipartimento di Chimica Farmaceutica e Tossicologica, Università degli Studi di Napoli "Federico II" Via D. Montesano 49, 80131 Napoli, Italy*
[b]*Dipartimento di Farmacologia Sperimentale, Università degli Studi di Napoli "Federico II" Via D. Montesano 49, 80131 Napoli, Italy*
[c]*Cartesius Analytical Unit, Department of Pharmacology, Biomedical Sciences Institute, University of Sao Paulo, Sao Paulo, Brazil*

(Received 19 April 1999; revised 6 July 1999; accepted 15 July 1999)

**Abstract** – Two sets of benzotriazinone and benzoyltriazole derivatives were prepared and tested for local anaesthetic activity in comparison with lidocaine. Several of the prepared compounds exhibited a fairly good activity comparable or superior to that of lidocaine. The presence of a benzotriazinone or a benzoyltriazole moiety as an aromatic system was quite profitable for both the intensity and duration of activity. The acute toxicity in mice of the four most potent compounds of the series was also assessed. Compound **1b,** which has an anaesthetic activity comparable to that of lidocaine, was also characterized by a more favourable therapeutic index. All compounds were tested in vitro to evaluate their negative chronotropic action in isolated rat right atria. © 1999 Éditions scientifiques et médicales Elsevier SAS

**benzotriazinone / benzoyltriazole / local anaesthetic / negative chronotropic action**

## 1. Introduction

In previous articles we described the synthesis of two sets of N-[2-(alkylamino)ethyl]benzotriazol-X-yl-acetamides and of N-[2-(alkylamino)ethyl]benzotriazol-X-yl-isobutyramides designed as local anaesthetic agents [1, 2]. The compounds of two sets fulfill the requirements of the pharmacofore scheme proposed by Löfgren [3] because they are provided with an aromatic system, an intermediate chain and an aminic moiety ionizable under physiological pH. They have been assayed in vivo for their local anaesthetic activity. Some of the investigated compounds showed anaesthetic activities comparable with or higher than those exhibited by the reference drug lidocaine.

Considering these results and the important role played by the aromatic system in the interaction with a corresponding hydrophobic region, in order to increase the activity and to evaluate the influence on activity by the different aromatic systems, the benzotriazole nucleus was replaced by a 1,2,3-benzotriazin-4(3H)-one and by a 4-benzoyl-1,2,3-triazole ring, thereby achieving general structures **1**, **2** and **3** (*figure 1*).

The N-alkylamino groups of the intermediate acetylacetamido side chain are those previously reported [1, 2] displaying the highest anaesthetic activity: dimethylamino, diethylamino, pyrrolidine and piperidine substituents.

Herein we report the synthesis of a series of 1,2,3-benzotriazinones (**1a–d**) and of 4-benzoyl-1,2,3-triazole (**2a–d** and **3a–d**) derivatives (*tables I* and *II*). The synthesized compounds were first tested as local anaesthetics with different in vivo assays and, successively considering the relationships between local anaesthetic ($Na^+$ channel block) and antiarrhythmic activities [4, 5], all compounds were preliminary tested to evaluate their negative chronotropic action in rat isolated right atria.

*Correspondence and reprints

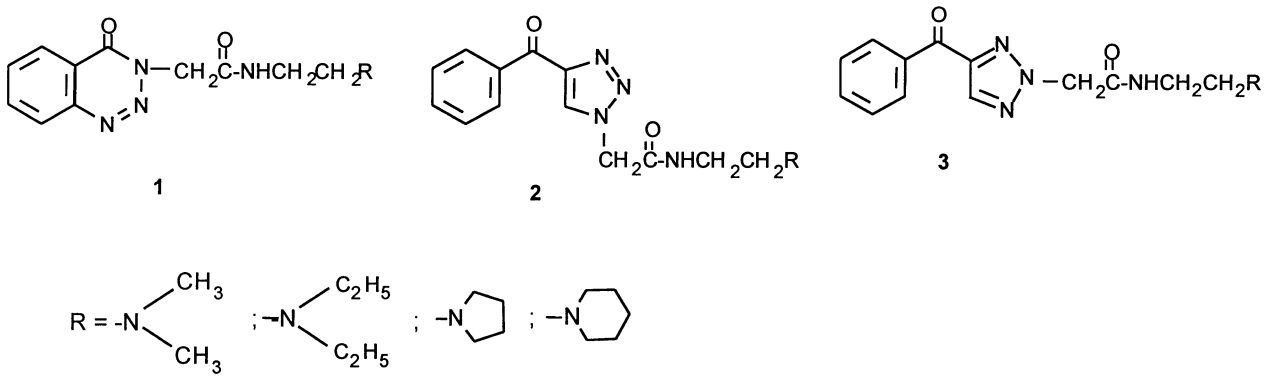
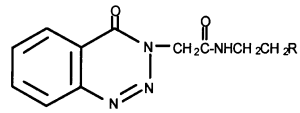
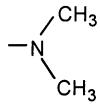
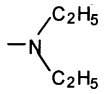
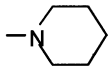



**Figure 1.** General structure of considered compounds.

**Table I.** Physicochemical properties of 1,2,3-benzotriazinone derivatives **1a–d**.



| R | Formula* | MW | Compound | M.p. (°C) | Cryst.** Solvent | Yield (%) |
|---|---|---|---|---|---|---|
|  | $C_{13}H_{17}N_5O_2$ | 275.31 | **1a** | 145–146 | a + b | 60 |
|  | $C_{15}H_{21}N_5O_2$ | 303.37 | **1b** | 155–156 | a | 55 |
|  | $C_{15}H_{19}N_5O_2$ | 301.35 | **1c** | 180–181 | a + b | 66 |
|  | $C_{16}H_{21}N_5O_2$ | 315.38 | **1d** | 169–170 | a | 52 |

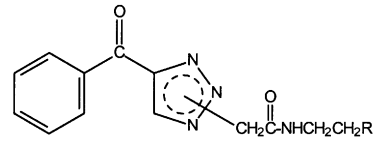
*Satisfactory microanalyses obtained: C, H, N values are within ± 0.4% of theoretical values. **Crystallization solvents: a) diethyl ether; b) ethyl alcohol.

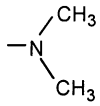
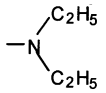
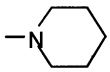
## 2. Chemistry

Compounds of general formula **1** reported in *figure 1* were synthesized as illustrated in *figure 2*. They derived from condensation of a 1,2,3-benzotriazin-4(3H)-one ring (**4**) with ethyl bromoacetate in butan-2-one in the presence of potassium carbonate. The ethyl acetate derivatives (**5**) were converted into the amide derivatives (**1**) by reaction in methyl alcohol solution with the appropriate amines.

The final compounds **1a–d,** reported in *table I*, were purified by cromatography on a silica gel column and further by crystallization from an appropriate solvent (yields ranging between 52–66%).

**Table II.** Physicochemical properties of 4-benzoyl-1,2,3-triazole derivatives **2a–d** and **3a–d**.



| R | Formula* | MW | 1-substituted 4-benzoyl-1,2,3-triazoles | | | 2-substituted 4-benzoyl-1,2,3-triazoles | | |
|---|---|---|---|---|---|---|---|---|
| | | | Compound** | M.p. (°C) | Yield (%) | Compound** | M.p. (°C) | Yield (%) |
|  | $C_{15}H_{19}N_5O_2$ | 301.35 | **2a** | 132–133 | 70 | **3a** | 96–97 | 60 |
|  | $C_{17}H_{23}N_5O_2$ | 329.40 | **2b** | 136–137 | 40 | **3b** | 99–100 | 45 |
|  | $C_{17}H_{21}N_5O_2$ | 327.39 | **2c** | 151–153 | 50 | **3c** | 98–99 | 55 |
|  | $C_{18}H_{23}N_5O_2$ | 341.41 | **2d** | 159–161 | 40 | **3d** | 94–95 | 50 |

*Satisfactory microanalyses obtained: C, H, N values are within ± 0.4% of theoretical values. **All compounds were crystallized by methyl alchol/diethyl ether.

The 4-benzoyl-1,2,3-triazole derivatives (**2a–d** and **3a–d**) were synthesized following the steps outlined in *figure 3*.

The parent compound, 4-benzoyl-1,2,3-triazole (**8**), was obtained in 95% yield by a modified method already described [6, 7]. Oxidation of the phenylethynylcarbinol (**6**) with chromium trioxide and concentrated sulphuric acid produced phenyl ethynyl ketone (**7**) which was successively treated with $NaN_3$ in anhydrous dimethylacetamide providing the expected compound. Reaction of 4-benzoyl-1,2,3-triazole with ethyl bromoacetate and potassium carbonate in butan-2-one gave a mixture of the triazole isomers 1- and 2- with an overall yield of 80% [8]. The two isomers were separated by column chromatography on silica gel using diethyl ether/*n*-hexane (7:3 v/v) as eluent. The faster moving 2- isomer, ethyl 4-(benzoyl)-1,2,3-triazole-2-acetate (**10**), was collected with a higher yield (65%) with respect to the slower moving 1-substituted isomer (**9**) (35%). The ethyl acetate derivatives (**9** and **10**) were converted into the corresponding amide derivatives **2** and **3** by reaction in methyl alcohol solution with the appropriate amines.

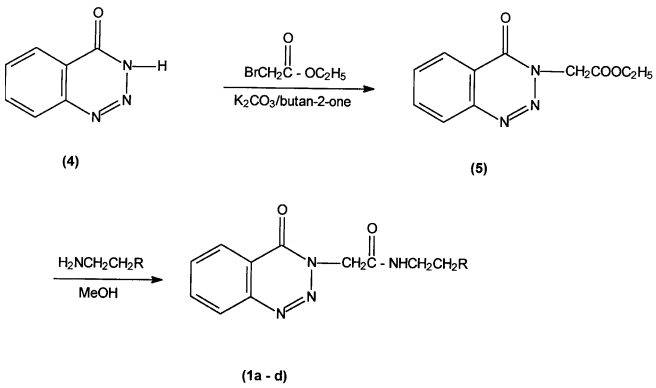



**Figure 2.** Synthesis of **1a**–**d**.

All the final products (**2a–d** and **3a–d**) reported in *table II* were further purified by crystallization from a mixture of diethyl ether and methyl alcohol (yields ranging between 40–70%).

The synthesized compounds listed in *tables I* and *II* were characterized by $^1$H-NMR spectroscopy whose data were fully consistent with the described structures.

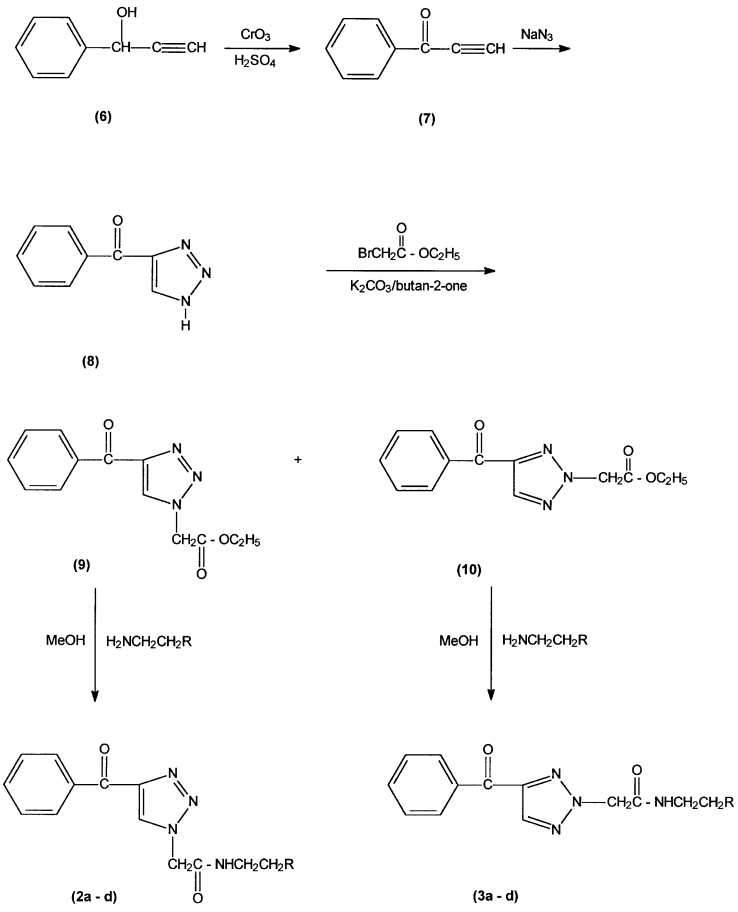



**Figure 3.** Synthesis of 4-benzoyl-1,2,3-triazole derivatives.

$^1$H-NMR of compounds **2a–d** and **3a–d** ($CDCl_3$) differentiated clearly between 1- and 2- substituted 4-benzoyl-1,2,3-triazole derivatives. In fact there is a difference in chemical shift values among the protons in the 5- position of the benzoyltriazole ring in the series of 1- and 2- isomers. The triazole proton of the 1- isomer appears always as a singlet at lower field with respect to the position of the same proton of analogues 2-substituted (chemical shift values ranging between 8.45–8.70 δ and 8.20–8.35 δ, respectively) according with literature [8, 9]. Physicochemical data of all the final compounds are summarized in *tables I* and *II*.

## 3. Pharmacology

The compounds reported in *tables I* and *II* were tested in vivo for their anaesthetic activity by different tests: corneal anaesthesia in the rabbit, mouse tail anaesthesia (*table III*) and rat sciatic nerve block anaesthesia (*table IV*). The ip acute toxicity and the therapeutic index of the more active compounds were also determined (*table V*). Successively the synthesized compounds were tested in vitro to evaluate their negative chronotropic action in rat isolated right atria (*table VI*). The synthesized compounds were always compared for their activity with lidocaine, taken as reference drug.

## 4. Results and discussion

*Table III* summarizes the results of the surface and infiltration anaesthesia assays performed on the 1,2,3-benzotriazinone (**1a–d**) and on the 4-benzoyl-1,2,3-triazole (**2a–d** and **3a–d**) derivatives. As for the 1,2,3-benzotriazinone derivatives (**1a–d**), in both tests, the results indicate that the structures endowed with highest activity are **1b** and **1d**, with values similar to that measured for lidocaine.

**Table III.** Rabbit corneal and mouse tail anaesthetic activities.

| Compound | Corneal anaesthetic[a] | Mouse tail anaesthetic[b] |
|---|---|---|
| **1a** | inactive | 5.4 (± 0.38)$10^{-2}$ |
| **1b** | 74.3 ± 9.3 | 0.69 (± 0.31)$10^{-2}$ |
| **1c** | 12.1 ± 3.9 | 2.6 (± 0.43)$10^{-2}$ |
| **1d** | 73.8 ± 8.9 | 1.2 (± 0.42)$10^{-2}$ |
| **2a** | inactive | 4.0 (± 0.36)$10^{-2}$ |
| **2b** | 4.7 ± 1.5 | 3.0 (± 0.37)$10^{-2}$ |
| **2c** | 3.2 ± 2.1 | 3.0 (± 0.33)$10^{-2}$ |
| **2d** | 30.1 ± 5.6 | 3.6 (± 0.35)$10^{-2}$ |
| **3a** | 22.5 ± 4.5 | 3.0 (± 0.29)$10^{-2}$ |
| **3b** | 105.0 ± 3.5 | 1.4 (± 0.25)$10^{-2}$ |
| **3c** | 48.5 ± 9.1 | 3.0 (± 0.31)$10^{-2}$ |
| **3d** | 92.3 ± 8.0 | 1.4 (± 0.28)$10^{-2}$ |
| lidocaine HCl[c] | 100 | 0.68 (± 0.39)$10^{-2}$ |

[a]All compounds were in aqueous solution at 2% concentration. The values expressed as % of the anaesthetic 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.

As for the 4-benzoyl-1,2,3-triazole derivatives (**2a–d** and **3a–d**), the results indicate that the nature and the position of the intermediate acetylacetamido side chain on the benzoyltriazole nucleus have significant influence on activity. In both tests the 2-substituted isomers appear to be more active than the 1-substituted derivatives. Compounds **3b** and **3d** stand out in the data set for their high activities (these values are comparable to those determined for lidocaine). It appears that the position and the nature of the side chain affect surface and infiltration activities in a similar way.

**Table IV.** Duration of local anaesthetic activity in rat sciatic nerve block.

| Compound | Duration[a] (min) | |
|---|---|---|
| | 1% | 2% |
| **1a** | 25 (± 10.5) | 45 (± 17.0) |
| **1b** | 77 (± 6.8) | 160 (± 18.2) |
| **1c** | inactive | n.t. |
| **1d** | 90 (± 3.6) | 160 (± 15.3) |
| **2a** | 40 (± 15.0) | 72 (± 6.9) |
| **2b** | 35 (± 11.0) | 63 (± 6.8) |
| **2c** | inactive | n.t. |
| **2d** | 40 (± 12.0) | 72 (± 7.0) |
| **3a** | 75 (± 7.0) | 150 (± 17.2) |
| **3b** | 117 (± 8.0) | 210 (± 16.9) |
| **3c** | 25 (± 12.3) | 50 (± 6.5) |
| **3d** | 70 (± 6.6) | 180 (± 20.3) |
| lidocaine HCl | 65 (± 10.7) | 117 (± 11.0) |

[a]In vivo duration of local anaesthetic activity in rat sciatic nerve block (each rat received 0.2 mL of 1% and 2% anaesthetic solution, n.t. = not tested. The values are means ± SE of three determinations.

**Table V.** Acute toxicity in mouse ($LD_{50}$) and therapeutic index of selected compounds **1b**, **1d**, **3b** and **3d**.

| Compound | $LD_{50}$[a] | $IC_{50}$ | Therapeutic index[b] $LD_{50}/IC_{50}$ |
|---|---|---|---|
| **1b** | 6.09 (± 0.49)$10^{-2}$ M | 0.69 (± 0.31)$10^{-2}$ M | 8.83 |
| **1d** | 5.24 (± 0.38)$10^{-2}$ M | 1.2 (± 0.42)$10^{-2}$ M | 4.37 |
| **3b** | 8.69 (± 0.53)$10^{-2}$ M | 1.4 (± 0.25)$10^{-2}$ M | 6.21 |
| **3d** | 4.46 (± 0.35)$10^{-2}$ M | 1.4 (± 0.28)$10^{-2}$ M | 3.19 |
| lidocaine HCl | 5.60 (± 0.39)$10^{-2}$ M | 0.68 (± 0.39)$10^{-2}$ M | 8.23 |

[a]Molar concentration of the injected solution (v = 0.2 mL/20 g body weight). [b]Evaluated as ratio between $LD_{50}$ and $IC_{50}$ (mouse tail test) expressed in mg/kg.

For all considered compounds, with respect to the nature of the intermediate acetylacetamido side chain, analogues with a terminal diethylamino group or a piperidine ring displayed the highest activity in accordance with previous results [1, 2]. Reducing the size from a six to a five-membered ring resulted in an improvement of anaesthetic activity.

In order to evaluate the duration of the local anaesthetic activity, additional investigations were conducted by rat sciatic nerve block assay. In accordance with the previous results, when a 2% solution was used, as reported in *table IV*, several compounds (**1b**, **1d**, **3a**, **3b**, and **3d**) exhibited a better performance in blocking the rat sciatic nerve with respect to lidocaine (160, 160, 150, 210 and 180 min for motor activity recovering, respectively, versus 117 min for lidocaine).

Finally, the acute toxicity and therapeutic index of the more active compounds in all anaesthetic tests (**1b**, **1d**,

**Table VI.** Maximum response to different compounds in rat isolated right atria compared to lidocaine.

| Compound | Maximum response (beats/min) | *n* |
|---|---|---|
| **1a** | –15 ± 6 | 6 |
| **1b** | –42 ± 8 | 6 |
| **1c** | –52 ± 8 | 6 |
| **1d** | –106 ± 27 | 5 |
| **2a** | –33 ± 13 | 6 |
| **2b** | –55 ± 12 | 6 |
| **2c** | –72 ± 5 | 6 |
| **2d** | –107 ± 27 | 6 |
| **3b** | –67 ± 9 | 6 |
| **3c** | –135 ± 31 | 6 |
| **3d** | –152 ± 12 | 6 |
| lidocaine | –180 ± 35 | 6 |

**3b**, and **3d)** were determined in mice. From the corresponding $LD_{50}$ and $LD_{50}/IC_{50}$ values shown in *table V* it is evident that **1b** is characterized by the most favourable ratio between toxicity and mouse tail anaesthetic activity (its therapeutic index is slightly higher than that measured for lidocaine).

These studies showed that the presence of a piperidine ring as a terminal N-alkylamino group gives an increase in toxicity, particularly evident on compound **3d**.

In conclusion, a comparison between the pharmacological profile of benzotriazinone and benzoyltriazole derivatives versus that of the above mentioned benzotriazole counterpartes [1, 2] clearly shows that the replacement of the benzotriazole moiety, found in the preceeding derivatives, by a benzotriazinone or by a benzoyltriazole ring, seems to have a little influence on the anaesthetic activity. These results might be ascribed to an analogue lipophilic, steric, and electronic complementarity between the benzotriazole residue compared to the benzotriazinone or to the benzoyltriazole moiety.

As regards the results of chronotropic activity reported in *table VI*, all compounds induced a negative chronotropic effect in rat isolated right atria at high doses, in a concentration range greater than μM. The intrinsic activity of different compounds in decreasing spontaneous beating rate in isolated right atria was distinct. Lidocaine was used as the pattern drug to compare the intrinsic activity of all compounds; in fact it is well known that lidocaine has local anaesthetic as well antiarrhythmic actions

In series **1**, compound **1d** determines a larger magnitude of maximum response compared to drugs **1b**, **1c**, and **1a,** whereas drug **1a** was less effective in producing a negative chronotropic response among these compounds (*figure 4* and *table VI*). In series **2**, compound **2d** had higher intrinsic activity compared to compounds **2a**, **2b**, and **2c** (*figure 5*). The intrinsic activity of compounds **2a** and **2b** were similar.

The intrinsic activity of compound **3d** was the best of all studied compounds. Compound **3c** showed similar **3d** intrinsic activity even if less, whereas compound **3b** induced the lowest activity of the series (*figure 6* and *table VI*).

A comparison between 'in vivo' anaesthetic activity and 'in vitro' negative chronotropic activity data displayed a similar pattern. In fact, clear negative chronotropic action was displayed by compound **1d** and **3d**, which bear a piperidine ring, but also compound **2d**, which was poorly active on anaesthetic activity, showed an appreciable negative chronotropic action.

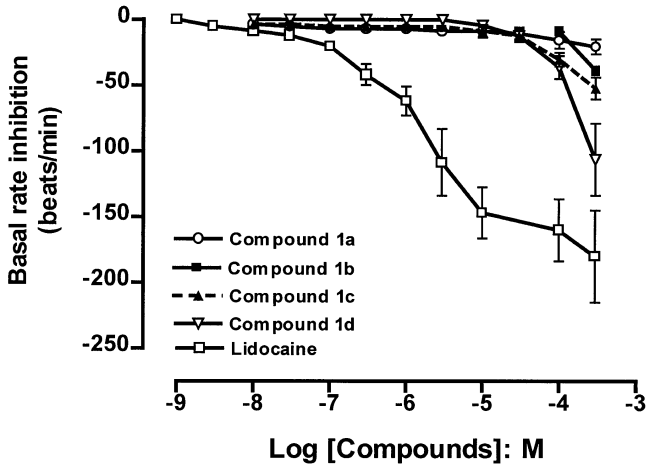



**Figure 4.** Negative chronotropic response for the compounds **1a**, **1b**, **1c**, **1d**, and lidocaine in isolated right atria from rats. Data are means ± SEM for 5–6 experiments.

## 5. Experimental protocols

### *5.1. Chemistry*

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Structures described were supported by $^1$H-NMR spectra and microanalytical data. $^1$H-NMR spectra were recorded on a Bruker WM 500 spectrometer using DMSO and $CDCl_3$ as solvent; chemical shifts (δ) are reported as follows: s, singlet; d,

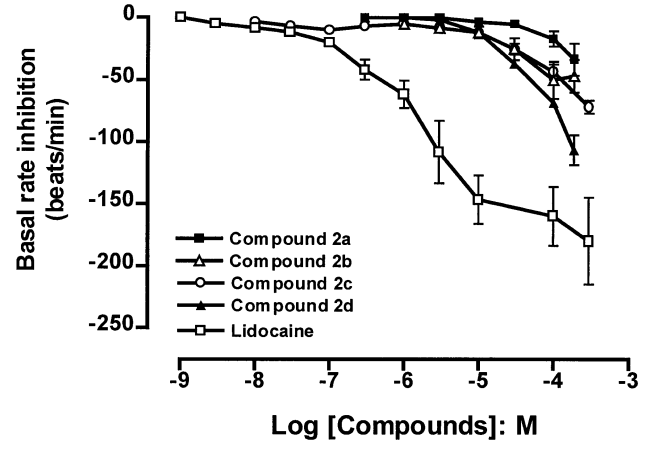



**Figure 5.** Negative chronotropic response for the compounds **2a**, **2b**, **2c**, **2d**, and lidocaine in isolated right atria from rats. Data are means ± SEM for 6 experiments.

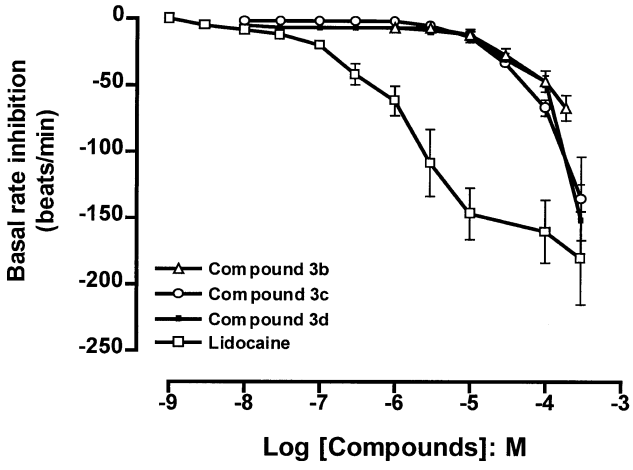



**Figure 6.** Negative chronotropic response for the compounds **3b**, **3c**, **3d**, and lidocaine in isolated right atria from rats. Data are means ± SEM for 6 experiments.

doublet; t, triplet; m, multiplet. The spectra obtained confirmed the proposed structures. All pure compounds gave a satisfactory analysis (C, H, N) within ± 0.4% of theoretical values.

Analytical TLC was performed on precoated silica-gel (0.2 mm GF 254, E Merck); the spots were located by UV (254 nm) light. Evaporation was performed in vacuo. Anhydrous sodium sulfate was used as a drying agent. Crude products were routinely passed through columns of silica gel (0.05–0.20 mm, Carlo Erba). Analytical data, melting points and crystallization solvents are reported in *tables I* and *II*.

*5.1.1. 3-Carbethoxymethyl-1,2,3-benzotriazin-4(3H)-one* (**5**)

To a magnetically stirred solution of 1,2,3-benzotriazin-4(3H)-one (**4**) (0.01 mol) and ethyl bromoacetate (0.01 mol) in butan-2-one (50 mL) was added $K_2CO_3$ (0.01 mol). The reaction mixture was heated under reflux for 10 h and monitored by TLC. After cooling, the butan-2-one was removed under reduced pressure and the residue was diluted with $H_2O$ and extracted with $CHCl_3$. The organic layer was dried and evaporated to dryness. The crude product **5** was purified by crystallization from methyl alcohol/diethyl ether. Yield 68%, m.p. 114–116 °C.$^1$H-NMR ($CDCl_3$) δ = 8.37 (d, 1H, Ar-H, *J* = 7.5 Hz), 8.20 (d, 1H, Ar-H, *J* = 7.5 Hz), 7.99 (t, 1H, Ar-H, *J* = 7.5 Hz), 7.82 (t, 1H, Ar-H), 5.19 (s, 2H, -$NCH_2$), 4.28 (m, 2H, $OCH_2$), and 1.30 ppm (t, 3H, $CH_3$).

*5.1.2. General procedure for the preparation of compounds* **1a–d**

To 0.01 mol of 3-carbethoxymethyl-1,2,3-benzotriazin-4(3H)-one (**5**) 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 had disappeared. After cooling the solvent was removed under reduced pressure and the residue was purified by silica-gel column chromatography using mixtures of diethyl ether/methanol 9:1 v/v. Further purification was obtained by crystallization from the appropriate solvent (*table I*).

Yields ranging between 52–66%. Spectral data of title compound **1a**: $^1$H-NMR ($CDCl_3$), δ = 8.33 (d, 1H, Ar-H, *J* = 7.5 Hz), 8.15 (d, 1H, Ar-H, *J* = 7.5 Hz), 7.94 (t, 1H, Ar-H, *J* = 7.5 Hz), 7.78 (t, 1H, Ar-H, *J* = 7.5 Hz), 5.11 (s, 2H, $CH_2$-CO), 3.39 (t, 2H, NH-$CH_2$, *J* = 7.5 Hz), 2.47 (t, 2H, $CH_2$-N, *J* = 7.5 Hz) and 2.24 ppm (s, 6H, $N(CH_3)_2$). Similar $^1$H-NMR data occur in all derivatives of general formula **1**.

*5.1.3. Phenyl ethynyl ketone* (**7**)

A solution of chromium trioxide (0.10 mol) in water (30 mL) and concentrated sulphuric acid (8.5 mL) was slowly added to a stirred and cooled solution of phenylethynylcarbinol (**6**) (0.15 mol) in acetone (50 mL). The reaction mixture was carried out at 4 °C under a nitrogen atmosphere. After stirring for 7 h, water was added to dissolve the precipitated chromium salts and the product was extracted with chloroform. Evaporation of the organic solution gave a yellow solid which was crystallized from aqueous methanol to give 16.6 g (85%) of **7** as pale yellow needles. The physical data are in agreement with those given in ref. [6].

*5.1.4. 4-benzoyl-1H-1,2,3-triazole* (**8**)

To a stirred and heated solution of $NaN_3$ (0.10 mol) in anhydrous dimethylacetamide (80 mL), phenyl ethynyl ketone **7** (0.10 mol) dissolved in anhydrous dimethylacetamide (80 mL) was slowly added. The reaction mixture was kept at 100 °C for 2 h. After stirring for a further 12 h at room temperature, evaporation of the solvent under reduced pressure gave a liquid residue which was diluted with water. The aqueous layer was acidified (pH = 5) with 10% HCl and extracted with ether (3 × 200 mL). The combined organic layers were dried and evaporated to give a solid residue which was purified by crystallization from ethyl alcohol: 16.4 g (95%) of **8**. The physical data are in agreement with those given in ref. [7].

*5.1.5. 1-carbethoxymethyl-4-benzoyl-1H-1,2,3-triazole* (**9**) *and 2-carbethoxymethyl-4-benzoyl-2H-1,2,3-triazole* (**10**)

Anydrous $K_2CO_3$ (0.04 mol) was added to a solution of 4-benzoyl-1,2,3-triazole (**8**) (0.04 mol) and ethyl bromoacetate (0.04 mol) in 50 mL of butan-2-one. The mixture was refluxed for 12 h and monitored by TLC. After cooling, the butan-2-one was removed under reduced pressure and the residue was diluted with $H_2O$ and extracted with $CHCl_3$. The organic layer was washed with 2 M NaOH, dried and evaporated to dryness.

The obtained residue, containing 1- and 2-substituted isomers was finally fractionated by column chromatography using diethyl ether/n-hexane, 7:3 v/v, as eluent. Further purification of the isolated 1- and 2- isomers by crystallization from diethyl ether gave the final products **9** and **10**. Characterization by $^1$H-NMR spectra showed that the first compound to be eluted was the 2-substituted 1,2,3-triazole (compound **10**, yield 65%, m.p. 55–57 °C), whereas the 1-substituted isomer was eluted successively (compound **9**, yield 35%, m.p. 132–133 °C). **9**: $^1$H-NMR (DMSO), δ = 9. (s, 1H, H-triaz), 8.50 (d, 2H, Ar-H, *J* = 7.5 Hz), 7.68 (t, 1H, Ar-H, *J* = 7.5 Hz), 7.55 (t, 2H, Ar-H *J* = 7.5 Hz), 5.53 (s, 2H, $CH_2$-CO), 4.27 (q, 2H, $CH_2O$, *J* = 7.5Hz) and 1.28 (t, 2H, $CH_3$, *J* = 7.5 Hz). **10**: $^1$H-NMR (DMSO), δ = 8 (s, 1H, H-triaz), 8.58 (d, 2H, Ar-H, *J* = 7.5 Hz), 7.70 (t, 1H, Ar-H, *J* = 7.5 Hz), 7.58 (t, 2H, Ar-H, *J* = 7.5 Hz), 5.61 (s, 2H, $CH_2$-CO), 4.26 (q, 2H, $CH_2O$, *J* = 7.5 Hz) and 1.25 (t, 2H, $CH_3$, *J* = 7.5 Hz).

*5.1.6. General procedure for the preparation of compounds* **2a–d** *and* **3a–d**

To 0.01 mol of the appropriate ethyl-4-benzoyl-1,2,3-triazolacetate derivative (**9** or **10**) dissolved in anhydrous methanol was added dropwise the appropriate amine (0.01 mol). The reaction mixture was kept under reflux with magnetic stirring for 8–12h and monitored by TLC, until the starting material had disappeared. After cooling, the solvent was removed by filtration under reduced pressure and the residue was purified by silica gel column chromatography using methanol as eluent. Further purification was obtained by crystallization from a mixture of methyl alcohol/diethyl ether. Yields ranging between 40 and 70%. Spectral data of the title compound **2a**: $^1$H-NMR ($CDCl_3$), δ = 8 (s, 1H, H-triaz), 8.27 (d, 2H, Ar-H, *J* = 7.5 Hz), 7.62 (t, 1H, Ar-H, *J* = 7.5 Hz), 7.51 (t, 2H, Ar-H, *J* = 7.5 Hz), 5.14 (s, 2H, $CH_2$-CO), 3.35 (t, 2H, NH-$CH_2$, *J* = 7.5 Hz), 2.40 (t, 2H, $CH_2$-$N(CH_3)_2$, *J* = 7.5 Hz) and 2.17 ppm (s, 6H, $N(CH_3)_2$). Similar $^1$H-NMR data occur in all derivatives of the general formula **2**. Spectral data of the title compound **3a**: $^1$H-NMR ($CDCl_3$), δ = 8.31 (s, 1H, H-triaz), 8.27 (d, 2H, Ar-H, *J* = 7.5 Hz), 7.62 (t, 1H, Ar-H, *J* = 7.5 Hz), 7.52 (t, 2H, Ar-H, *J* = 7.5 Hz), 5.28 (s, 2H, $CH_2$-CO), 3.33 (t, 2H, NH-$CH_2$, *J* = 7.5 Hz), 2.35 (t, 2H, $CH_2$-$N(CH_3)_2$, *J* = 7.5 Hz) and 2.15 ppm (s, 6H, $N(CH_3)_2$). Similar $^1$H-NMR data occur in all derivatives of the general formula **3**.

*5.2. Pharmacology*

*5.2.1. Corneal anaesthesia*

Local anaesthetic activity was evaluated in male New Zealand rabbits (Harlan-Nossan, Correzzana, Milan, weighing 2.4–2.8 kg) as local surface anaesthesia [10], by determining every 3 min the number of stimuli to the cornea, effected rhythmically with a Frey's horse-hair, necessary to produce the blink reflex. If the reflex did not occurr after 100 stimulations, anaesthesia 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. All compounds were dissolved in 0.1 N HCl and the solution buffered to pH 6–7. 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.

*5.2.2. Mouse tail anaesthesia*

Male Swiss mice (Harlan-Nossan, Correzzana, Milan, weighing 18–20 g) were used. The test was performed according to the method of Bianchi [11] in which the aqueous anaesthetic solution (0.1 mL) is injected subcutaneously about 1 cm from the base of the tail. Fifteen minutes after injection, the pain reflex of all injected animals was tested by applying a small artery clip to the zone where the compound was injected. The proportion of animals which did 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 [12].

*5.2.3. Rat sciatic nerve block*

This test was performed according to Al-Saadi and Sneider [13] to determine conduction anaesthesia and its duration. Triplicate sets of three groups of three male Wistar rats (Harlan-Nossan, Correzzana, Milan, weighing 180–200 g) were used. Each rat received an injection (0.2 mL) of the aqueous anaesthetic solution (1% and 2%) into the posterior side 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 10 min intervals over time, up to the first sign of motor activity.

*5.2.4. Acute toxicity*

The ip acute toxicity of the most active compounds **1b**, **1d**, **3b**, and **3d** was determined in male Swiss mice (Harlan-Nossan, Correzzana, Milan, weighing 18–20 g) 7 days after treatment. $LD_{50}$ values were calculated for each compound by probit analysis using a computer program [12].

*5.2.5. Chronotropic activity: functional assay using isolated rat right atria*

This test was performed according to Hughes and Smith [14]. Wistar rats (250–300 g) were anesthetized with halothane and the hearts were rapidly removed and placed in oxygenated Krebs Henseleit buffer (KHB). The right atria were removed and mounted in a water jacketed tissue chamber (20 mL volume) containing KHB, pH 7.3–7.5, at 37 °C and gassed with 95% $O_2$/5% $CO_2$. The composition of the KHB was (mM): NaCl, 124; KCl, 4.75; $MgSO_4$, 1.30; $CaCl_2$, 2.25; $NaHCO_3$, 25.0; $NaH_2PO_4$, 0.6; dextrose, 10.0; sodium ascorbate, 0.3.

*5.2.6. Construction and analyses of concentration-response curves*

Concentration-response curves for all compounds were constructed by the cumulative variation of agonist concentration at one-half log unit increments [15]. All concentration-response data were evaluated for a fit to a logistic function in the form:

$$E = E_{max}/((1 + (10^c/10^x)^n) + \Phi),$$

where E is the increase in rate above basal; $E_{max}$ is the maximum response that the agonist can produce; c is the logarithm of the $EC_{50}$, the concentration of agonist that produces half-maximal response; x is the logarithm of the concentration of agonist; the exponential term, n, is a curve fitting parameter that defines the slope of the concentration-response line, and $\Phi$ is the response observed in the absence of added agonist. Nonlinear regression analyses to determine the parameters $E_{max}$, log $EC_{50}$ and n were done using GraphPad Prism (GraphPad Software, San Diego, CA) with the constraint that $\Phi = 0$.

## Acknowledgements

The NMR spectral data were provided by Centro di Ricerca Interdipartimentale di Analisi Strumentale, Università degli Studi di Napoli “Federico II”. The assistance of staff is gratefully appreciated.

## References

[1] Caliendo G., Di Carlo R., Greco G., Grieco P., Meli R., Novellino E., Perissutti E., Santagada V., Eur. J. Med. Chem. 30 (1995) 603–608.

[2] Caliendo G., Greco G., Grieco P., Mattace Raso G., Meli R., Novellino E., Perissutti E., Santagada V., Eur. J. Med. Chem. 31 (1996) 99–110.

[3] Löfgren N., Studies on Local Anaesthetics, Hoeggstrom, Stockholm, 1948.

[4] Morgan P.H., Mathison I.W., J. Pharm. Sci. 65 (1976) 635–648.

[5] Heymans L., Le Therizien L., Godfroid J., J. Med. Chem. 23 (1980) 184–193.

[6] Bowden K., Heilbron I.M., Jones E.R.H., Weedon B.C.L., J. Chem. Soc. Part I (1946) 39–45.

[7] Nesmeyanov A.N., Rybinskaya M.I., Dolk. Akad. Nauk. SSSR 158 (1964) 408–410.

[8] Biagi G., Ferretti M., Livi O., Scartoni V., Lucacchini A., Mazzoni M., Il Farmaco Ed. Sc. 41 (1986) 388–400.

[9] Livi O., Biagi G., Ferretti M., Lucacchini A., Barili P.L., Eur. J. Med. Chem. -Chim. Ther. 18 (1983) 471–475.

[10] Regnier J., Bull. Sci. Pharm. 30 (1923) 580–586.

[11] Bianchi C., Br. J. Pharmacol. 11 (1956) 104–106.

[12] Tallarida R.J., Murray R.B., Manual of Pharmacological Calculations with Computer Programs, 2nd Edition, Springer, New York, 1981.

[13] Al Saadi D., Sneider W.E., Arzneim. -Forsch. 41 (1991) 195–198.

[14] Hughes I.E., Smith J.A., J. Pharm. Pharmacol. 30 (1978) 124–126.

[15] Van Rossum J.M., Arch. Int. Pharmacodyn. Ther. 143 (1963) 299–33.