Available online at www.sciencedirect.com
SCIENCE DIRECT®
Il Farmaco 58 (2003) 845–849
IL FARMACO
www.elsevier.com/locate/farmac

# 2-Aryl-3-phenylamino-4,5-dihydro-2h-benz[g]indazoles with analgesic activity

Silvia Schenone [a,*], Olga Bruno [a], Angelo Ranise [a], Chiara Brullo [a], Francesco Bondavalli [a], Walter Filippelli [b], Filomena Mazzeo [b], Annalisa Capuano [b], Giuseppe Falcone [b]

[a] *Dipartimento di Scienze Farmaceutiche, Facoltà di Farmacia dell'Università degli Studi di Genova, Viale Benedetto XV, 3, 16132, Genova, Italy*
[b] *Dipartimento di Medicina Sperimentale, sezione di Farmacologia 'L. Donatelli', Facoltà di Medicina e Chirurgia, II Università degli Studi di Napoli, Via Costantinopoli 16, 80138, Napoli, Italy*

Received 8 November 2002; accepted 18 February 2003

**Abstract**

A series of 2-aryl-3-phenylamino-4,5-dihydro-2H-benz[g]indazoles was synthesized and tested for antiarrhythmic, local anaesthetic and analgesic activity. The title compounds showed a good antinociceptive activity.


*Keywords:* 2-Aryl-3-phenylamino-4,5-dihydro-2H-benz[g]indazoles; Analgesic activity

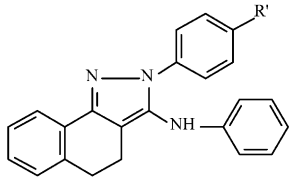
## 1. Introduction

The pyrazole ring is present in a large number of compounds endowed of pharmacological activity, either as isolated ring or fused with other mono or policyclic systems.

In particular examples of differently substituted dihydro-benz[g]indazolic compounds are present in the literature showing antimicrobial [1–3] antiallergic [4] and non-estrogenic contraceptive activity [5]. More recently also tyrosine kinase inhibitors [6,7], dopamine receptors ligands [8], antiinflammatory and analgesic [9] compounds have been reported quite extensively in the patent literature.

Being involved in a study on pyrazole derivatives, with the purpose to perform a large program of biological screening, we focused our attention on some fused pyrazolic compounds and in particular on pyrano-pyrazoles [10,11] and on dihydro-benz[g]indazoles [12,13], bearing an arylamino pyrazole moiety. Some terms of these series showed interesting activity profiles, in particular analgesic, antiinflammatory and antipyretic, typical of the well-known pyrazolone derivatives, but also antiarrhythmic activity, previously found by us in non fused pyrazole derivatives [14].

We have recently synthesized compounds **1** [12] which showed appreciable analgesic activity, but lower than that of indomethacin; in some terms of the series (R′ = F, $CH_3$) local anaesthetic and antiarrhythmic activities were present.

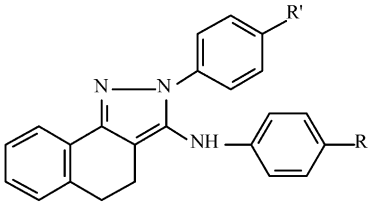



**1**

To better understand the SAR of these type of compounds, we synthesized a new series of 3-arylamino-4,5-dihydro-2H-benz[g]indazoles **2**, bearing together with the previously studied substituents on the phenyl ring in position 2, a methyl, electron-donating, or a fluorine, weak electron-attracting substituent in para position on the arylamino moiety.

* Corresponding author.
*E-mail address:* schensil@unige.it (S. Schenone).

0014-827X/03/$ - see front matter 
doi:10.1016/S0014-827X(03)00142-3




2 a-l

The synthesis of compounds **2a**–**l** is outlined in Scheme 1. Reaction of 1-tetralone (**3**) with NaH in anhydrous DMF followed by addition of *p*-tolyl or *p*-fluoro-phenylisothiocyanate afforded the corresponding oxocarbothioamides **4a**–**b** in very good yields as reported previously [12].

Reaction of **4a**–**b** with the appropriate p-substituted arylhydrazine at reflux, in presence of a catalytical amount of acetic acid gave the 2-aryl-3-phenylamino-4,5-dihydro-2H-benz[g]indazoles (**2a**–**l**) (Table 1) in good yields as crystal materials.

Compounds **2a**–**l** were submitted to a preliminary screening for analgesic, antiarrhythmic and local anaesthetic activities.

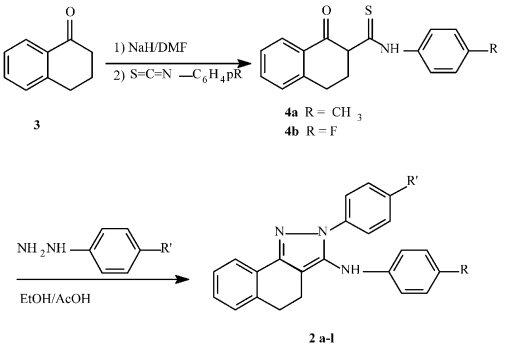
## 2. Experimental

### 2.1. Chemistry

Starting materials were purchased from Aldrich-Italia (Milan).

Melting points were determined with a Büchi B 540 apparatus and are uncorrected. IR spectra were measured in $CHCl_3$ with a Perkin-Elmer 398 spectrophotometer. $^1H$ NMR spectra were recorded in $CDCl_3$ solution on a Varian Gemini 200 (200 MHz) instrument, chemical shifts are reported as $\delta$ (ppm) relative to TMS as internal standard; $J$ in Hz. $^1H$ patterns are described using the following abbreviations: s = singlet, t = triplet, q = quartet, m = multiplet.

All compounds were tested for purity by TLC (Merk, Silica gel 60 $F_{254}$, $CHCl_3$ as eluant).




Scheme 1.

Table 1
Yields and melting points of compounds **2a**–**l**

| Comp. | R | R' | Yield (%) | M.p. (°C) |
|---|---|---|---|---|
| **2a** | $CH_3$ | $CH_3$ | 70 | 181–182 |
| **2b** | $CH_3$ | $OCH_3$ | 65 | 192–193 |
| **2c** | $CH_3$ | F | 70 | 210–211 |
| **2d** | $CH_3$ | Cl | 72 | 207–208 |
| **2e** | $CH_3$ | Br | 79 | 206–207 |
| **2f** | $CH_3$ | $CF_3$ | 83 | 150–151 |
| **2g** | F | $CH_3$ | 71 | 188–189 |
| **2h** | F | $OCH_3$ | 40 | 201–202 |
| **2i** | F | F | 67 | 151–152 |
| **2j** | F | Cl | 70 | 182–183 |
| **2k** | F | Br | 78 | 205–206 |
| **2l** | F | $CF_3$ | 87 | 196–197 |

Analyses for C, H, N were within $\pm$0.3% of the theoretical value.

#### 2.1.1. General procedure for N-aryl-1-oxo-1,2,3,4-tetrahydronaphtalene-2-carbothioamides (*4a*–*4b*)

To a cold solution of 1-tetralone (**3**) (14.62 g, 0.100 mol) in dry dimethylformamide (DMF, 60 ml), a 60% sodium hydride dispersion in mineral oil (4.25 g, 0.105 mol) was added and the resulting mixture was stirred at room temperature (r.t.) until the hydrogen evolution subsided (15 min). The proper arylisothiocyanate (15.36 g, 0.103 mol for **4a**, 15.77 g, 0.103 mol for **4b**) was then added and the mixture was stirred at r.t. for 4 h, poured cautiously into cold water (500 ml) and extracted with petroleum ether (b.p. 40–70 °C). The aqueous solution was cooled and acidified with 3 M HCl (pH 4–5).

The yellow solid precipitated was filtered, washed with water and finally crystallized from absolute ethanol.

**4a**: light yellow crystals, yield 80%, m.p. 154–155 °C. IR: 3300 and 1674 $cm^{-1}$ (NH, CO). $^1H$ NMR: 2.36 (s, 3H, $CH_3$), 2.60–3.40 (m, 4H, $2CH_2$), 3.70–3.85 (m, 1H, CH), 7.20–7.60 (2m, 7H Ar), 8.01–8.10 (m, 1H, H-8), 10.62 (s, 1H, NH, disappears with $D_2O$). *Anal.* Calc. for $C_{18}H_{17}NOS$: C, 73.19; H, 5.80; N, 4.74. Found: C, 73.34; H, 5.75; N, 4.77%.

**4b**: light yellow crystals, yield 87%, m.p. 148–149 °C. IR: 3299 and 1669 $cm^{-1}$ (NH, CO). $^1H$ NMR: 2.60–3.35 (m, 4H, $2CH_2$), 3.70–3.85 (m, 1H, CH), 7.00–7.80 (2m, 7H Ar), 8.00–8.10 (m, 1H, H-8), 10.70 (s, 1H, NH, disappears with $D_2O$) *Anal.* Calc. for $C_{17}H_{14}NOSF$: C, 68.21; H, 4.71; N, 4.68. Found: C, 68.16; H, 5.03; N, 4.64%.

#### 2.1.2. General procedure for 2-aryl-3-phenylamino-4,5-dihydro-2H-benz[g]indazoles (*2a*–*l*)

To a solution of the relevant carbothioamide (10 mmol) in absolute ethanol (25 ml) was added the proper arylhydrazine ( $\sim$15 mmol) (if necessary liberated with alkali from the corresponding hydrochloride, extracted

with diethyl ether and isolated under reduced pressure). A small amount of glacial acetic acid (5 drops) was added and the mixture was refluxed for 8 h.

By standing in a refrigerator a solid crystallized, which was filtered and recrystallized from absolute ethanol.

Yields and melting points are reported in Table 1.

**2a**: light brown crystals, IR: 3412 $cm^{-1}$ (NH). $^1H$ NMR: 2.28 and 2.37 (2s, 6H, $2CH_3$), 2.57 and 2.94 (2t, $J = 6.0$, 4H, $2CH_2$), 5.20 (s, 1H, NH, disappears with $D_2O$), 6.60–8.00 (5m, 12H Ar). *Anal.* Calc. for $C_{25}H_{23}N_3$: C, 82.16; H, 6.34; N, 11.50. Found: C, 81.85; H, 6.47; N, 11.73%.

**2b**: light brown crystals, IR: 3412 $cm^{-1}$ (NH). $^1H$ NMR: 2.27 (s, 3H, $CH_3$), 2.55 and 2.95 (2t, $J = 6.0$, 4H, $2CH_2$), 3.80 (s, 3H, $OCH_3$), 5.20 (s, 1H, NH, disappears with $D_2O$), 6.60–8.05 (6m, 12H Ar). *Anal.* Calc. for $C_{25}H_{23}N_3O$: C, 78.71; H, 6.08; N, 11.04. Found: C, 78.53; H, 6.27; N, 11.04%.

**2c**: light brown crystals, IR: 3413 $cm^{-1}$ (NH). $^1H$ NMR: 2.28 (s, 3H, $CH_3$), 2.57 and 2.91 (2t, $J = 7.6$, 4H, $2CH_2$), 5.15 (s, 1H, NH, disappears with $D_2O$), 6.60–8.00 (5m, 12H Ar). *Anal.* Calc. for $C_{24}H_{20}N_3F.1/3\ H_2O$: C, 76.78; H, 5.55; N, 11.19. Found: C, 77.04; H, 5.65; N, 11.51%.

**2d**: light brown crystals, IR: 3412 $cm^{-1}$ (NH). $^1H$ NMR: 2.20 (s, 3H, $CH_3$), 2.55 and 2.93 (2t, $J = 7.4$, 4H, $2CH_2$), 5.21 (s, 1H, NH, disappears with $D_2O$), 6.60–8.00 (5m, 12H Ar). *Anal.* Calc. for $C_{24}H_{20}N_3Cl$.: C, 74.70; H, 5.22; N, 10.89. Found: C, 74.59; H, 5.28; N, 10.92%.

**2e**: light brown crystals, IR: 3412 $cm^{-1}$ (NH). $^1H$ NMR: 2.29 (s, 3H, $CH_3$), 2.56 and 2.93 (2t, $J = 7.0$, 4H, $2CH_2$), 5.20 (s, 1H, NH, disappears with $D_2O$), 6.60–8.00 (5m, 12H Ar). *Anal.* Calc. for $C_{24}H_{20}N_3Br$.: C, 66.98; H, 4.68; N, 9.76. Found: C, 66.75; H, 4.80; N, 9.90%.

**2f**: light brown crystals, IR: 3412 $cm^{-1}$ (NH). $^1H$ NMR: 2.28 (s, 3H, $CH_3$), 2.57 and 2.93 (2t, $J = 7.0$, 4H, $2CH_2$), 5.20 (s, 1H, NH, disappears with $D_2O$), 6.60–8.00 (5m, 12H Ar). *Anal.* Calc. for $C_{25}H_{20}N_3F_3$.: C, 71.59; H, 4.81; N, 10.02. Found: C, 71.38; H, 4.61; N, 10.01%.

**2g**: light brown crystals, IR: 3174 $cm^{-1}$ (NH). $^1H$ NMR: 2.37 (s, 3H, $CH_3$), 2.54 and 2.93 (2t, $J = 6.0$, 4H, $2CH_2$), 5.25 (s, 1H, NH, disappears with $D_2O$), 6.63–8.05 (5m, 12H Ar). *Anal.* Calc. for $C_{24}H_{20}N_3F$: C, 78.03; H, 5.46; N, 11.37. Found: C, 77.74; H, 5.70; N, 11.22%.

**2h**: light brown crystals, IR: 3197 $cm^{-1}$ (NH). $^1H$ NMR: 2.54 and 2.94 (2t, $J = 6.5$, 4H, $2CH_2$), 3.81 (s, 3H, $OCH_3$), 5.25 (s, 1H, NH, disappears with $D_2O$), 6.63–7.98 (5m, 12H Ar). *Anal.* Calc. for $C_{24}H_{20}N_3OF$: C, 74.79; H, 5.23; N, 10.90. Found: C, 74.47; H, 5.15; N, 10.96%.

**2i**: light brown crystals, IR: 3412 $cm^{-1}$ (NH). $^1H$ NMR: 2.55 and 2.93 (2t, $J = 6.1$, 4H, $2CH_2$), 5.20 (s, 1H, NH, disappears with $D_2O$), 6.61–7.98 (5m, 12H Ar). *Anal.* Calc. for $C_{23}H_{17}N_3F_2$: C, 73.98; H, 4.59; N, 11.25. Found: C, 73.75; H, 4.85; N, 11.15.

**2j**: light brown crystals, IR: 3412 $cm^{-1}$ (NH). $^1H$ NMR: 2.54 and 2.93 (2t, $J = 7.5$, 4H, $2CH_2$), 5.21 (s, 1H, NH, disappears with $D_2O$), 6.64–8.00 (5m, 12H Ar). *Anal.* Calc. for $C_{23}H_{17}N_3ClF$: C, 70.86; H, 4.40; N, 10.78. Found: C, 70.67; H, 4.42; N, 10.92%.

**2k**: light brown crystals, IR: 3412 $cm^{-1}$ (NH). $^1H$ NMR: 2.53 and 2.92 (2t, $J = 7.3$, 4H, $2CH_2$), 5.20 (s, 1H, NH, disappears with $D_2O$), 6.60–7.97 (5m, 12H Ar). *Anal.* Calc. for $C_{23}H_{17}N_3BrF$: C, 63.61; H, 3.95; N, 9.68. Found: C, 63.59; H, 3.75; N, 9.74%.

**2l**: light brown crystals, IR: 3412 $cm^{-1}$ (NH). $^1H$ NMR: 2.56 and 2.94 (2t, $J = 7.8$, 4H, $2CH_2$), 5.22 (s, 1H, NH, disappears with $D_2O$), 6.62–8.00 (5m, 12H Ar). *Anal.* Calc. for $C_{24}H_{17}N_3F_4.1/2H_2O$: C, 66.66; H, 4.20; N, 9.72. Found: C, 66.56; H, 4.32; N, 9.76%.

### 2.2. Pharmacology

The following experimental procedures were employed:

#### 2.2.1. Analgesic activity

The acetic acid writhing test was used on mice [15]. Groups of ten mices (weight 20–25 g) of both sexes, pregnant females excluded, were administered orally by gavage in 1% methylcellulose suspension, using a single dose of 50 mg/kg or scalar doses of 12.5, 25 and 50 mg/kg for the most active compounds. Indomethacin (Sigma Aldrich SrL, Milano, Italy) was used as reference drug in all the tests for comparison purposes at the dose of 5 mg/kg.

Thirty minutes later the animals were injected intraperitoneally with 0.25 ml/mouse of 0.5% acetic acid solution and writhes were counted during the following 25 min. The mean number of writhes for each experimental group and percentage inhibition compared to the control group were calculated. The experimental results are listed in Table 2.

The estimation of $ED_{50}$ values was obtained using the Litchfield and Wilcoxon I formula, by means of the computer program PHARM-PCS [7] [16].

#### 2.2.2. Antiarrhythmic activity

Ventricular fibrillation induced by aconitine hydrochloride, according to method suggested by Marmo, [17], was used to study antiarrhythmic activity of test compounds on groups of ten albino rats (weight 200–250 g) of both sexes, pregnant females excluded. Aconitine hydrochloride (15 μg/kg/i.v./min, Sigma Aldrich Srl, Milano, Italy) was injected in the control group and appearance time of extrasystoles and death time were determined. The same procedure was applied either in the groups treated with the reference compound

Table 2
Acetic acid writhing test: analgesic activity

| Comp. | Dose (mg/kg) | Mean number of writhes in 25 min period after treatment ±SE | Inhibition % relative to controls |
|---|---|---|---|
| Control | acetic acid 0.5% | 45.8±5.3 | |
| Indomethacin | 5 | 21.7±4.2 | 52.6 |
| **2a** | 50 | 23.6±3.9 | 48.5 |
| **2b** | 50 | 24.3±5.1 | 46.9 |
| **2c** | 12.5 | 26.0±3.6 | 43.2 |
| | 25 | 24.3±5.1 | 46.9 |
| | 50 | 22.9±4.2 | 50.0 |
| **2d** | 50 | 24.6±3.7 | 46.3 |
| **2e** | 50 | 23.1±4.1 | 49.5 |
| **2f** | 12.5 | 25.1±4.3 | 45.2 |
| | 25 | 24.7±3.8 | 46.0 |
| | 50 | 22.6±2.9 | 50.6 |
| **2g** | 50 | 25.1±6.3 | 45.2 |
| **2h** | 50 | 25.6±3.2 | 44.1 |
| **2i** | 12.5 | 23.8±2.9 | 48.0 |
| | 25 | 22.9±5.4 | 50.0 |
| | 50 | 21.9±3.8 | 52.2 |
| **2j** | 50 | 24.0±2.9 | 47.6 |
| **2k** | 12.5 | 25.7±3.8 | 43.8 |
| | 25 | 23.5±4.2 | 48.7 |
| | 50 | 22.3±4.7 | 51.3 |
| **2l** | 12.5 | 26.1±3.1 | 43.0 |
| | 25 | 24.2±5.0 | 47.2 |
| | 50 | 22.5±5.1 | 50.8 |

(quinidine, 25 mg/kg p.o. Sigma Aldrich Srl, Milano, Italy) or in the test compounds (50 mg/kg p.o). No compound showed appreciable activity.

*2.2.3. Local anaesthetic activity*

According to Bianchi method [18], pain stimulation was elicited in mice by Dieffenbach tweezers application for 10 s on the tail root, in groups of ten mice (weight 20–25 g) of both sexes, pregnant females excluded. The efficacy of local anaesthetic activity was expressed as percent of animals anaesthetized 5 and 30 min after infiltration of the test compound (0.2 ml of 0.1% glycofurol solution) into the tail root respect to lidocaine (Sigma Aldrich Srl, Milano, Italy) at the same dose. No compound showed appreciable activity.

## 3. Result and discussion

As a general and preliminary consideration compounds **2a**–**l** showed a different pharmacological profile than that of the analogues **1**.

In fact only a weak antiarrhithmic activity and no local anaesthetic activity was found in **2**, whereas a good analgesic activity turned out to be present in most of the newly synthesized derivatives (Table 2).

Compounds **1**, having substituent only in position 2 of the phenyl ring, showed a fair analgesic activity. The insertion of another substituent in position 3 of the arylamino group of compounds **2**, produced a strongly increase of analgesic activity and, in the same time, reduced all other activities present in the series **1**.

The presence of an electron-withdrawing group on the phenyl ring in 2, associated with either a methyl or a fluorine substituent on the arylamino moiety produced the more active compounds of the series: in fact antalgic activity was clearly evident in **2c**, **2f**, **2k** and **2l** (50.0, 50.6, 51.3 and 50.8% of inhibition), showing an $ED_{50}$ of 41.37 (18.32–93.44), 37.86 (15.35–93.35), 33.86 (12.44–92.14) and 40.87 (18.31–93.43) mg/kg respectively. The most active compound of the series resulted to be **2i**, bearing *p*-fluorine substituents on both the phenyl rings, which produced an inhibition of 52.2% associated to an $ED_{50}$ of 33.856 (12.44–92.14) mg/kg.

The achievement of compounds **2** with this interesting analgesic activity is a good starting point for future work in this area of research and in this direction we are going on in our laboratory.

## References

[1] R.W. Chesnut, D.F. Haslam, N.N. Durham, K.D. Berlin, Mechanism of biological action of a new benzindazole compound, Can. J. Biochem. 50 (1972) 516–523.
[2] K. Ramalingam, L.F. Wong, K.D. Berlin, R.A. Brown, R. Fisher, J. Blunk, N.N. Durham, Synthesis and antimicrobial activity of azasteroid-type compounds and related systems. Effect of hydrophilic and lipophilic groups on activity, J. Med. Chem. 20 (1977) 664–669.

[3] A. Zayed, J. Metri, S. El Hawary, Reactions of 6,7-benzindazole-3-carboxylic acid hydrazide. Synthesis of some heterocycles of potential biological activity, Egypt J. Chem. 24 (1981) 389–396.
[4] M.T. Di Parsia, C. Suarez, M.J. Vitolo, V.E. Marquez, B. Beyer, C. Urbina, I. Hurtado, Synthesis and study of the potential antiallergic activity of some pyrazole derivatives, J. Med. Chem. 24 (1981) 117–119.
[5] D.A. Habeck, W.J. Houlihan, US Patent 3,842,088 (1974); C.A., 82 (1975), 43412w.
[6] L.D. Arnold, Y. Xu, T. Barlozzari, PTC Int. Appl. WO 99 17,769 (1999); C.A., 130 (1999), 282067d.
[7] K.J. Doyle, P. Rafferty, R.W. Steele, D.J. Wilkins, M. Hockley, L.D. Arnold, A.M. Ericsson, PTC Int. Appl. WO 00 27,822 (2000); C.A., 132 (2000), 347566d.
[8] I. Collins, M. Rowley, W.B. Davey, F. Emms, R. Marwood, S. Patel, S. Patel, A. Fletcher, I.C. Ragan, P.D. Leeson, A.L. Scott, T. Broten, 3-(1-Piperazinyl)-4,5-dihydro-1H-benzo[g]indazoles: high affinity ligands for the human dopamine $D_4$ receptor with improved selectivity over ion channels, Bioorg. Med. Chem. 6 (1998) 743–753.
[9] S.M. Sakya, Eur. Pat. Appl. EP 1,142,889; C.A., 135 (2001), 288779n.
[10] O. Bruno, A. Ranise, S. Schenone, F. Bondavalli, M. D'Amico, M. Falciani, L. Berrino, F. Rossi, 3-(Arylamino)-6,7-dihydro-6-methylpyrano[4,3-c]pyrazol-4(1H or 2H)-ones with antipyretic, analgesic, antiarrhythmic, hypotensive and other activities, Farmaco 48 (1993) 1697–1708.
[11] O. Bruno, S. Schenone, A. Ranise, F. Bondavalli, G. Falcone, W. Filippelli, I. Marabese, 2-Aryl-6-methyl-3-phenylamino-6,7-dihydropyrano[4,3-c]pyrazol-4(2H)-ones with analgesic, Farmaco 51 (1996) 721–724.
[12] S. Schenone, O. Bruno, A. Ranise, F. Bondavalli, M. D'Amico, W. Filippelli, G. Falcone, V. De Novellis, 2-Aryl-3-phenylamino-4,5-diydro-2H-benz[g]indazoles with antiarrhythmic and local anaesthetic activities, Farmaco 50 (1995) 179–182.
[13] S. Schenone, O. Bruno, A. Ranise, F. Bondavalli, G. Falcone, W. Filippelli, B. Rivaldi, *N*-Substituted 3-(arylamino)-4,5-dihydro-2H-benz[g]indazol-2-yl acetamides with anti-inflammatory and analgesic activities, Farmaco 55 (2000) 383–388.
[14] O. Bruno, A. Ranise, F. Bondavalli, P. Schenone, M. D'Amico, M. Falciani, L. Berrino, F. Rossi, *N*-Substituted 4,5(3)-diphenyl-3(5)-pyrazoleamines with antipyretic, antiarrhythmic, hypotensive and other activities, Farmaco 48 (1993) 967–977.
[15] J.E. Davies, J.E. Kellett, J.C. Pennington, The anti-inflammatory and analgesic effects of noverdan, a novel, non steroidal agent, Arch. Int. Pharmacodyn. Ther. 221 (1976) 274–282.
[16] J.T. Litchfield, F. Wilcoxon, A simplified method of evaluating dose-effect experiments, J. Pharmacol. Exper. Ther. 96 (1949) 99–113.
[17] E. Marmo, E. Lampa, F. Rossi, A.P. Caputi, S. Chiappa, C. Vacca, L. Giordano, P. Pedone, Ricerche sperimentali sulla specificità ed aspecificità di un β-adrenolitico: il bunitrololo, Arch. Sci. Med. 135 (1978) 15–56.
[18] C. Bianchi, A simple new quantitative method for testing local anaesthetics, Br. J. Pharmacol. 11 (1956) 104–106.