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Synthesis and pharmacological properties of benzisothiazole/benzimidazole derivatives with acidic groups

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Abstract

The synthesis and the pharmacological evaluation of benzisothiazole and benzimidazole tetrazolyl- and carboxyl- derivatives **1**–**6** are described. Structural modification was aimed at investigating the influence of two isosteric substituents (tetrazolyl- and carboxyl-) on the title benzofused heterocycles. The antiphlogistic, antipyretic and analgesic activities have been investigated in in vivo experimental models. Additional investigations have been performed in vitro to study the antiplatelet and spasmolytic activity of the compounds synthetized. All the compounds produced peripheral analgesic effects, but were less effective in hot plate test. The tetrazole and the carboxylic benzisothiazole derivatives **2** and **3** proved to be the most effective drugs within the series, exhibiting maximal inhibition of writhes with a potency 3-fold higher than that of acetaminophen. Only compound **5** provided indication for a central analgesic activity since it was active in hot plate test although with a low potency. The findings obtained in these in vivo and in vitro studies indicate that these compounds do not share the same mechanism of action of acetaminophen. All of the compounds under study present lower acute toxicity than acetaminophen when orally administered in mice. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Benzisothiazole/benzimidazole; In vivo and in vitro pharmacological activities; Analgesic activity

1. Introduction

Proceeding with the study of the pharmacological properties of benzisothiazolyl-tetrazoles and analogous -alkanoic acids, in this work the investigation was extended to some new benzofused derivatives tested for their analgesic/antiphlogistic/antipyretic activity [1,2].

In detail, our research during recent years was addressed to the study of compounds bearing, on a benzisothiazole moiety an acidic group namely a carboxyl function or a tetrazole group, isosteric of the former. In the previous part of this research, we carried out the synthesis and the pharmacological evaluation of a series of 5-(benzo[*d*]isothiazolyl)tetrazoles analogues of benzo[*d*]isothiazolylalkanoic acids which have been reported to be active as antipyretics and, moderately, as analgesics. No significant anti-inflammatory activity was detected [1].

In the present work, in order to gain a better insight into the pharmacological profile of benzofused tetrazolyl- and carboxyl- derivatives, we have synthetized isothiazole and imidazole derivatives **1**–**6** (Fig. 1). Compound **3**, not known in literature, and compound **4**, never investigated for analgesic/antipyretic/antiphlogistic activities, have been considered for the phenyl group on the acidic moiety of **1** and **2** previously described [1]. Structural modification in compounds **5** and **6**, by replacing the benzo[*d*]isothiazole with a benzimidazole heterocycle, was aimed at elucidating the main pharmacophoric components.

Analgesic/antipyretic/antiphlogistic activities of the molecules under study were tested in vivo and compared to acetaminophen used as reference drug since it is endowed with preferential antipyretic/analgesic activity. Additional investigations were performed in vitro to study the antiplatelet activity of the compounds in

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order to achieve details on their mechanism of action [3].

2. Chemistry

Compounds **1**–**6** were obtained from the appropriately substituted nitriles as shown in Fig. 2. Alkaline hydrolysis, followed by acidification provided the carboxylic acids **1**, **3** and **5**, while reaction with NaN_3 in the DMF and in the presence of NH_4Cl afforded the tetrazole derivatives **2**, **4** and **6**.

The key intermediate nitrile for compounds **1** and **2** was obtained in one step from benzo[*d*]isothiazol-3-ylacetonitrile according to the method previously reported by us [2]. Benzo[*d*]isothiazol-3-ylphenylacetonitrile necessary to synthesize compounds **3** and **4** (here newly described) was obtained as previously described by Plazzi et al. [4] from 3-chloro-benzo[*d*]isothiazole and phenylacetonitrile. 1*H*-Benzimidazol-2-ylacetonitrile was commercially available. Compound **5**, previously reported as intermediate in the preparation of DNA minor groove binder [5] was prepared by us, in a different way from the cited authors, applying our general alkaline hydrolysis and improving the yield (from 77 to 93%). Compound **6**, reported in German Patents (no data available) as uricosuric/analgesic agent [6,7], was synthetized following the general method, by heating with NaN_3 and NH_4Cl in DMF. Because of the amphoteric nature of the final product, in this case, a buffered aqueous solution was necessary to precipitate the desired compound. The experimental details for the procedure to obtain novel compound **4**, compounds **5** and **6**, are given, together with their characterization data, in Section 4.

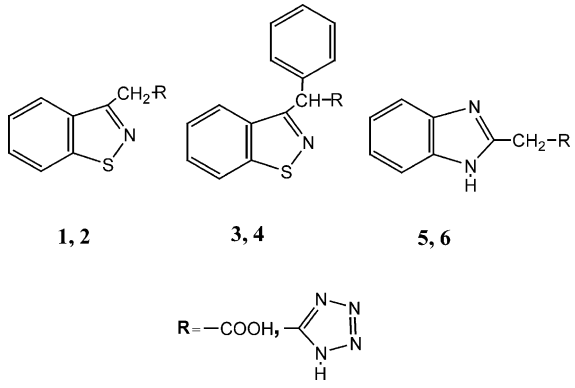

Fig. 1. Structure of compounds **1**–**6**.

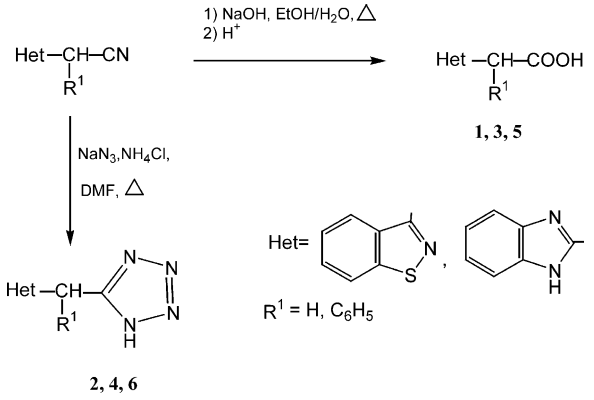

Fig. 2. Scheme of synthesis of compounds **1**–**6**.

3. Biological evaluation

The pharmacological properties of the benzisothiazole/benzimidazole derivatives were tested in suitable experimental models in order to evaluate their antiphlogistic, antipyretic and analgesic activities. Accordingly, their acute anti-inflammatory and antipyretic activities were studied in rat paw oedema and rat *Escherichia coli* derived LPS-induced pyrexia. The antinociceptive property was examined in both writhing and hot plate tests in mice. The ethical guidelines for investigation of experimental pain in conscious animals were followed and all of the tests were carried out according to the EEC ethical regulation (EEC Council 86/609; D.L.27/01/1992, No.116).

The test compounds were suspended in 0.5% methylcellulose and were orally administered at the dosage of 100 mg/kg to groups of eight rats 1 h before the subplantar injection of the phlogogen carrageenan or the intraperitoneal injection of the pyretogen LPS in rats. The compounds were also orally administered at doses from 1 to 400 mg/kg to groups of eight mice 1 h before the application of the chemical noxious stimulus and at 100 mg/kg in the hot plate test. Acetaminophen was used as reference drug while control animals received an equivalent volume of vehicle alone (10 ml/kg body weight). Furthermore, all the compounds were screened for their platelet antiaggregating property in guinea-pig platelet aggregation induced by ADP and arachidonic acid in vitro and their possible spasmolytic activity was ascertained by considering their inhibitory actions on the contractile response to acetylcholine, histamine and KCl in guinea-pig isolated ileum. For these in vitro experiments the compounds were initially dissolved in DMSO and then diluted with buffer solution so that the final concentration of DMSO (0.5%) was devoid of any biological effect.

Male guinea-pigs (300–350 g), Wistar female rats (180–200 g) and Swiss female mice (30–35 g) were fasted for 18 h with free access to water. They were kept in proper cages to prevent coprophagy at constant environment conditions (21 ± 1 °C; 40 ± 5% relative humidity) during the night preceding each experiment.

4. Experimental

4.1. Chemistry

Melting points (°C) were determined with a Buchi 512 (Buchi, Flawil, Switzerland) and are uncorrected. New compounds were analyzed in our analytical laboratory, on a Carlo Erba 1106 Elemental Analyzer (Carlo Erba Milan, Italy) for C, H and N. IR spectra were recorded (KBr pellets) on a Jasco FT-IR 300E spectrophotometer (Jasco Ltd., Tokyo, Japan). All reactions were monitored by TLC on F_{254} silica gel precoated sheets (Merck) using acetic acid/ethanol/water = 1:2:2 for compounds **1**, **3**, **5** and petroleum ether/ethyl acetate = 3:1 for **2**, **4**, **6** as eluents. The purified compounds each showed a single spot. Spectral IR data were consistent with the assigned structure in all cases and the reported wavenumbers are given in cm^{-1}. The found values for C, H, N elemental analysis were $\pm 0.4\%$ of the theoretical ones.

^1H NMR spectra of the synthetized compounds, in DMSO-d_6 solutions, were recorded on a Bruker AC 300 instrument at 298 K. Chemical shifts are reported as δ (ppm) relative to TMS as internal standard.

4.1.1. 5-[Benzo[d]isothiazol-3-yl-(phenyl)-methyl]tetrazole (4)

To a solution of benzo[*d*]isothiazol-3-ylphenylacetonitrile (0.01 mol) in *N*,*N*-dimethylformamide (40 ml), ammonium chloride (0.03 mol) and sodium azide (0.03 mol) were added. The mixture, heated at 127–130 °C, was vigorously stirred for 5 h. After cooling, it was concentrated under reduced pressure and the residue was stirred with water (50–60 ml) and alkalized with 2 N NaOH. A pitchy residue was eliminated and the solution acidified with 2 N HCl, afforded a crystalline residue which was crystallized from ethanol/water. White solid (77%), $C_{15}H_{11}N_5S$ (293.35); m.p. 201–202 °C; IR *v* max = 3120–2380 (N–H), 1555 (C=N), 1230, 1112, 1047 (substituted tetrazole).

Table 1
^1H NMR spectral data of compounds **1**–**6**

Comp.	^1H NMR (DMSO-d_6): δ (ppm)
1	10.55 (br s, 1H, COOH); 8.19 (d, 1H, H-7); 8.12 (d, 1H, H-4); 7.61 (t, 1H, H-6); 7.51 (t, 1H, H-5); 4.17 (s, 2H, CH_2)
2	8.24–8.18 (m, 2H, H-4,7); 7.64 (t, 1H, H-6); 7.54 (t, 1H, H-5); 4.88 (s, 2H, CH_2)
3	10.34 (br s, 1H, COOH); 8.19 (d, 1H, H-7); 8.11 (d, 1H, H-4); 7.59 (t, 1H, H-6); 7.47 (t, 1H, H-5,2′,6′); 7.31 (m, 3H, H-3′,4′,5′); 5.87 (s, 1H, CH)
4	8.23 (d, 1H, H-7); 8.07 (d, 1H, H-4); 7.61 (t, 1H, H-6); 7.49–7.45 (m, 3H, H-5,2′,6′); 7.37–7.26 (m, 3H, H-3′,4′,5′); 6.80 (s, 1H, CH)
5	10.25 (br s, 1H, COOH); 7.45–7.42 (m, 2H, H-4,7); 7.10–7.07 (m, 2H, H-5,6); 4.05 (s, 2H,CH_2)
6	7.53–7.50 (m, 2H, H-4,7); 7.18–7.15 (m, 2H, H-5,6); 4.59 (s, 2H, CH_2)

4.1.2. (1H-Benzimidazol-2-yl)acetic acid (5)

1*H*-Benzimidazol-2-ylacetonitrile (0.02 mol) was added to a solution of water (40 ml) and ethanol (16 ml) containing sodium hydroxide (0.06 mol) and refluxed until no more ammonia was evolved. The reaction mixture was evaporated to dryness under reduced pressure and the resulting residue was dissolved in water. The solution, after treatment with decolourizing carbon, was acidified with acetic acid. After standing for a long time (30 h) the solid crude product was filtered off. It was purified by dissolution in sodium hydoxide aqueous solution (2%) and reprecipitation by acidification with acetic acid. White solid (93%), $C_9H_8N_2O_2$ (176.17); m.p. 121 °C dec.; IR *v* max = 3393 (N–H), 1644 (C=O), 1612 (C=N).

4.1.3. 5-[(1H-Benzimidazol-2-yl)methyl]tetrazole (6)

To a solution of 1*H*-benzimidazol-2-ylacetonitrile (0.01 mol) in *N*,*N*-dimethylformamide (20 ml), ammonium chloride (0.03 mol) and sodium azide (0.03 mol) were added. The reaction mixture was heated for 5 h at 127 °C, with stirring. After standing overnight at room temperature the precipitated sodium chloride was filtered off and the filtrate was concentrated under reduced pressure. The residue, added with water (100 ml), was buffered with ammonium chloride saturated aqueous solution. The resulting precipitate was collected by filtration, dried and crystallized from water. White solid (86%), $C_9H_8N_6$ (200.19); m.p. 130 °C dec.; IR *v* max = 3160–2210 (N–H), 1575 (C=N), 1228, 1110, 1020 (substituted tetrazole).

^1H NMR spectral data of compounds **1**–**6** are reported in Table 1.

4.2. Pharmacology

4.2.1. In vivo experiments

Antiphlogistic, analgesic and antipyretic activities were evaluated following the experimental procedures already described [8]. Briefly, rat paw oedema was induced through subplantar injection of carrageenan, and fever was produced in rats with intraperitoneal injection of *E. coli* lipopolysaccharide (LPS). The pharmacological activities of the compounds were expressed as the percentage of inhibition calculated from the difference in the response between the treated and the control group at the time the maximum noxious effect occurred. Antinociceptive activity was investigated by studying the dose–response relationship (range of administration from 1 to 400 mg/kg o.s.) in writhing test performed in mice through acetic acid intraperitoneal injection and by examining the analgesic action in mice

hot plate test (at 100 mg/kg o.s.) according to Sheardown et al. [9]. Antinociceptive potency in writhing test was calculated by linear regression analysis of the dose–response curve and it was expressed as ID_{50} value, the dose of compound that reduces responses by 50% relative to control value. After placing the mice on a hot plate maintained at 55 °C, the latency to licking of the front paws was measured in each mouse. In any case the mice were removed from the hot plate after 30 s (cutoff time). After this predrug trial, mice were treated with vehicle, test compounds (100 mg/kg o.s.) and retested. The percentage of analgesia was calculated with the following formula: (postdrug latency − predrug latency/cutoff time − predrug latency) × 100.

4.2.1.1. Acute toxicity. LD_{50} values were determined by means of probit analysis [10] with mortality counts taken in a 24 h period after intraperitoneal administration of the compounds in groups of eight mice.

4.2.2. In vitro experiments

The antiplatelet activity was evaluated on male guinea-pig platelet rich plasma (PRP) following an experimental procedure already described [3]. The blood containing sodium citrate (3.8% w/v; 9:1 blood:sodium citrate) was centrifuged (10 min at 1000 rpm) to obtain PRP and the remaining blood was recentrifuged (10 min at 3600 rpm) to produce platelet poor plasma (PPP). Platelet aggregation was performed in an aggregometer (Aggrecorder PA 3220, A. Menarini, Firenze, Italy) following the Born's turbidimetric method [11]. PRP (250 μl) was preincubated at 37 °C for 5 min with solvent, the compounds under study or the reference drug acetaminophen (from 1 μM to 1 mM) before the addition of the platelet aggregatory agents (25 μl) used at concentrations sufficient to achieve maximum aggregation (3 μM ADP or 50 μM arachidonic acid). The antiplatelet activity of test compounds and acetaminophen was determined by comparing the maximum aggregation obtained in the presence and in the absence of the compounds and it was expressed as percent inhibition with respect to control.

The spasmolytic activity of the compounds was studied in guinea-pig segments of terminal ileum set up in organ bath containing modified Krebs solution (mM composition: NaCl 134, KCl 3.4, $CaCl_2$ 2.8, KH_2PO_4 1.3, $NaHCO_3$ 16, $MgSO_4$ 0.6, glucose 7.7) bubbled with 95% O_2–5% CO_2 and warmed to 37 °C. The contractile responses to acetylcholine, histamine and KCl were isometrically recorded in the absence and in the presence of the examined compounds (1–10 μM).

Table 2
Analgesic effect of compounds **1**–**6** and acetaminophen against acetic acid induced writhing in mice (values expressed as mean ± SEM of eight determinations)

Comp.	**ID_{50}** (mg/kg os)	Maximal inhibition % mean ± SEM
1	23	62 ± 16 **
2	72	89 ± 17 **
3	81	78 ± 31 **
4	74	54 ± 8 **
5	>200	45 ± 12 *
6	6	52 ± 7 **
Acetaminophen	208	90 ± 17 **

* $P<0.05$.
** $P<0.01$ in statistical analysis with Student's t-test versus controls.

4.3. Statistical analysis

The results were expressed as mean ± SEM and the means were compared using Student's t-test, P-value < 0.05 or < 0.01 being considered as statistically significant or highly significant, respectively.

5. Results and discussion

5.1. In vivo experiments

No significant, or slight, antiphlogistic/antipyretic activity was displayed by the compounds tested at 100 mg/kg o.s. in rat carrageenan paw oedema activity (from 7 to 36% inhibition versus control group) or in rat *E. coli* induced fever. The reference drug acetaminophen at 200 mg/kg o.s. failed to exhibit anti-inflammatory activity and produced a significant antipyretic effect (about 45% inhibition versus control group). All compounds produced a significant reduction of writhings induced in mice by i.p. acetic acid injection with respect to those observed in the vehicle-treated animals. The analgesic potency was estimated by considering the dose–antinociceptive response relationship in writhing test. Compounds **2** and **3** proved to be the most effective drugs within the series exhibiting maximal inhibition of writhes (89 and 78%) with a comparable potency (ID_{50} = 72 and 81 mg/kg, respectively) which was 3-fold higher than that of acetaminophen (ID_{50} = 208). Tetrazole derivative **6** displayed the highest antinociceptive potency but was scarcely effective (Table 2). In the pain model represented by mice hot plate test, all of the compounds produced poor antinociceptive effect (data not shown). Only compound **5** significantly prolonged the reaction time to the application of the thermal stimulus in comparison with the vehicle yielding the maximal inhibition (35%) at 100 mg/kg o.s.. Only for acetaminophen a LD_{50} value was estimated (300 mg/kg o.s.), whilst for all the other compounds no lethal effect was detected up to 800 mg/kg o.s..

5.2. In vitro experiments

All of the compounds under study were ineffective on AA-induced guinea-pig platelet aggregation up to 1 mM, unlike acetaminophen which completely prevented aggregatory response at this concentration. Only the compounds **2** and **3** exhibited a slight antiplatelet effect against ADP aggregation, producing about 30% inhibition of the maximal aggregation as the reference compound acetaminophen. On guinea pig isolated ileum the compounds as well as acetaminophen, up to 10 μM, failed to modify the basal tone and to inhibit histamine, acetylcholine or KCl contractile responses (data not shown).

The new compounds developed as structural analogues of the two benzisothiazole derivatives (**1**, **2**), endowed with analgesic activity coupled with scarce antipyretic/antiinflammatory action [1], display only antinociceptive activity of interest. The most effective analgesics were the tetrazole (**2**) and the carboxylic (**3**) benzisothiazole derivatives, which exhibited antinociception of peripheral nature since they prevented algesic response in the inflammatory pain produced by acetic acid i.p. injection, but were less effective in hot plate test, which can be considered a reliable model for studying central antinociceptive activity [9]. The dose–response results indicate that these compounds possess 3-fold higher analgesic potency than acetaminophen, but they do not seem to share the same mechanism of action as it is suggested by comparing the findings obtained in the other in vivo and in vitro studies herein reported. Indeed the two compounds failed to produce in vivo significant antipyretic effect and they were ineffective in vitro against AA-induced aggregation at variance with acetaminophen described as antipyretic and analgesic agent endowed with in vitro antiaggregating action [12,13].

For benzimidazolyltetrazole **6** described as uricosuric/analgesic agent in previous reports [6,7], low analgesic activity was ascertained in this study. The replacement of tetrazole moiety (derivative **6**) with the carboxyl group (derivative **5**) caused a marked drop in analgesic potency, suggesting that these two substituents, notwithstanding the strict correlation between their physicochemical properties [2], do not confer bioisosteric behaviour when inserted on this benzimidazole scaffold. It is noteworthy that, in spite of the weak potency, only the carboxyl derivative **5** was able to produce equiactive antinociception in the two pain tests performed providing indication for a central analgesic activity. Finally, all of the compounds under study presented a lower acute toxicity than acetaminophen when orally administered in mice. In conclusion, the new benzofused derivatives proved to be mild analgesic agents but with a better safety profile than the reference drug and they could provide information to design more active substances useful as analgesics.

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