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Synthesis and Biological Evaluation of New 4,5-Disubstituted-Thiazolyl Amides, Derivatives of 4-Hydroxy-Piperidine or of 4-N-Methyl Piperazine

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Abstract: 4,5-disubstituted-thiazolyl amides, derivatives of 4-hydroxy-piperidine and of 4-N-methyl piperazine, were synthesized and tested as anti-inflammatory agents. Log P values were theoretically calculated and experimentally determined. These compounds were tested for antioxidant activity, as hydroxyl radical scavengers and for their ability to interact with stable 1,1-diphenyl-2-picryl hydrazyl free radical (DPPH). The effect of the synthesized compounds on inflammation, using the carrageenin induced mice paw edema model was studied. Both anti-inflammatory and antioxidant activities depended on some structural characteristics of the synthesized compounds.

Keywords: Thiazolyl amides, anti-inflammatories, antioxidants, lipoxygenase inhibitors.

Introduction

Various non-steroidal anti-inflammatory drugs (NSAIDs) are in widespread clinical use for the treatment of inflammation diseases. However, despite their great number, their mechanism of action as

well as their precise therapeutic activities are still under investigation. Furthermore, almost all of them present a number of unwanted, often serious, side effects as consequence of interference with the arachidonic cascade [1]. It is well known that free radicals play an important role in the inflammatory process [2]. Superoxide anion radicals, hydrogen peroxide and hydroxyl radicals, produced by activation of phagocytes, are considered to be involved in inflammation and tissue destruction. Free radicals are also involved in the biosynthesis of prostaglandins, important mediators of inflammation [2]. Compounds with antioxidant properties are generally expected to protect against inflammation.

Several substituted thiazolyl derivatives [3,4] are reported to possess anti-inflammatory and/or antioxidant activities. This paper is an extension of previous work on the synthesis of thiazole derivatives with structures justifying anti-inflammatory activity.

Results and Discussion

The structures of the synthesized compounds are given in Figure 1 and the general method used to prepare the final compounds is shown in Figure 2.

Figure 1. Structures of synthesized compounds

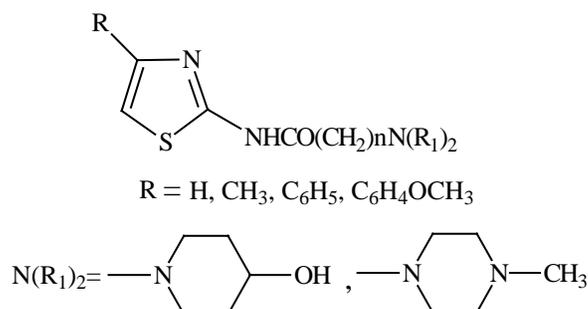
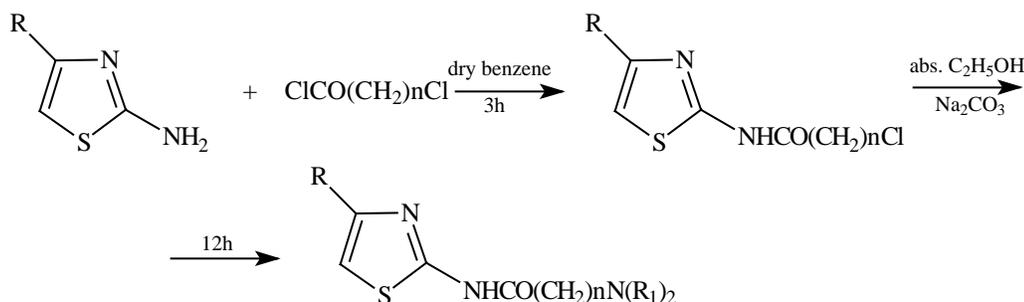
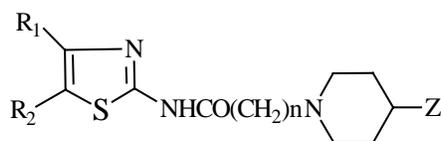


Figure 2. Synthetic procedure



Overall the reactions proceeded smoothly in good yields. The structure of the synthesized compounds and their physicochemical properties are shown in Table 1. The amides were identified both by elemental analyses as well as by their spectroscopic analyses.

Table 1. Characterization data of the synthesized amides

| N | R ₁ | R ₂ | Z | n | m.p. °C | MS | Mol. Structure ^b |
|-----------------|-----------------------|--|-----------------|---|--------------------|-------|---|
| 1 | H | H | OH | 1 | 85-86 | 241 | C ₁₂ H ₁₄ N ₃ O ₂ S |
| 2 | CH ₃ | H | OH | 1 | 89-92 | 254 | C ₁₁ H ₁₆ N ₃ O ₂ S |
| 3 | Ph | H | OH | 1 | 89.5-92 | 316 | C ₁₆ H ₁₈ N ₃ O ₂ S |
| 4 | Ph | CH ₃ (CH ₂) ₁₃ | OH | 1 | 79-80 | 512 | C ₃₀ H ₄₆ N ₃ O ₂ S |
| 5 | Ph-OCH ₃ | H | OH | 1 | 115-117 | 346 | C ₁₇ H ₂₀ N ₃ O ₄ S |
| 6 | CH ₂ COOEt | H | OH | 1 | 79-82 ^c | 326 | C ₁₄ H ₂₀ N ₃ O ₄ S |
| 7 | H | H | OH | 2 | semisolid | 255 | C ₁₁ H ₁₆ N ₃ O ₂ S |
| 8 | CH ₃ | H | OH | 2 | 148-150 | 269 | C ₁₂ H ₁₉ N ₃ O ₂ S |
| 9 | Ph | H | OH | 2 | 227-8 | 331 | C ₁₇ H ₂₁ N ₃ O ₂ S |
| 10 | Ph-OCH ₃ | H | OH | 2 | 193-5 | 360 | C ₁₈ H ₂₂ N ₃ O ₂ S |
| 11 | Ph | CH ₃ (CH ₂) ₁₃ | OH | 2 | semisolid | 526 | C ₃₁ H ₄₈ N ₃ O ₂ S |
| 12 | CH ₂ COOEt | H | OH | 2 | 116-8 | 343 | C ₁₅ H ₂₃ N ₃ O ₄ S |
| 13 ^a | H | H | CH ₃ | 1 | 225-228 | 240 | C ₁₃ H ₁₆ N ₃ OS |
| 14 ^a | Ph | H | CH ₃ | 1 | semisolid | 316 | C ₁₇ H ₂₀ N ₃ OS |
| 15 ^a | CH ₂ COOEt | H | CH ₃ | 1 | semisolid | 326 | C ₁₅ H ₂₂ N ₃ O ₃ S |
| 16 ^a | Ph-OCH ₃ | H | CH ₃ | 2 | 248-50 | 382.5 | C ₁₅ H ₂₂ N ₃ O ₃ S |

Notes: ^a Piperazinyl – ring; ^b Elemental analyses for molecular formula; ^c m.p for hydrochloride salt

Physicochemical parameters, e.g. log P have been calculated theoretically, as an expression of lipophilicity (Table 2). The *in vivo* antiinflammatory activity of the synthesized compound was determined using carrageenin induced mice paw edema (26.1-64.3 %) (see Table 2). The compounds have also been screened for their *in vitro* reducing activity towards the free stable radical DPPH (15.6-26.6 %) and for their hydroxyl free radical scavenging activity (44.6-100 %) (Table 3). For the 4-OH-piperidinyl amides the inhibition on the soybean LOX has been performed (77.5-100 %). The preliminary results reveal that the tested compounds exhibit in generally good biological activity. The results are discussed in terms of structural characteristics. The presence of a R₁ = phenyl group seems to be crucial for high activity *in vivo*. The length of the chain also plays an important role. The derivatives with n = 1 are stronger inhibitors. The nature of the alicyclic amine is an important feature, since the 4-N-methyl piperazinyl derivatives possess higher *in vivo* results.

Table 2. Lipophilicity values a) experimentally performed R_M values; b) theoretically calculated lipophilicity values using: i) Suzuki-Kudo's method ii) the clog P program from Biobyte. *In vivo* carrageenin rat paw edema % inhibition after 3.5 h (CPE %).

| N | R_M | logPsk | Clog P | CPE% |
|----|---------------|--------|---------|------|
| 1 | -0.494± 0.03 | -4.287 | -0.34 | 52.3 |
| 2 | nt | -1.878 | 0.159 | 59 |
| 3 | -0.578± 0.031 | -0.434 | 1.758 | 62.6 |
| 4 | 0.911± 0.037 | 6.322 | 6.322 | 44.2 |
| 5 | -0.490± 0.031 | -1.062 | 1.777 | 45 |
| 6 | nt | -2.473 | -0.133 | 33.4 |
| 7 | -0.504± 0.021 | -1.751 | -0.143 | 26.1 |
| 8 | -0.534± 0.037 | -0.365 | 0.212 | 27.5 |
| 9 | -0.589± 0.039 | 1.081 | -1.0955 | 47.5 |
| 10 | -0.504± 0.024 | 0.453 | 1.974 | 57 |
| 11 | 0.894± 0.033 | 6.5 | 8.684 | 32.2 |
| 12 | nt | -0.958 | 0.014 | 59.6 |
| 13 | nt | -3.747 | 1.102 | 61 |
| 14 | nt | -1.969 | 3.2 | 64.3 |
| 15 | nt | -2.161 | 1.309 | 47.9 |
| 16 | nt | 0.97 | 3.219 | 38.5 |

Table 3. Biological testing results. *In vitro* effects of the examined and reference drugs (in % at 0.1 mmol/L and 0.2 mmol/L) on reducing ability (DPPH), on hydroxyl radical scavenging activity (\cdot OH) and on soybean lipoxygenase activity (LOX).

| N | DPPH (0.1 mmol/L) 20min | DPPH (0.1 mmol/L) 1hr | DPPH (0.2 mol/L) 1 hr | \cdot OH | LOX |
|---|-------------------------------|-----------------------------|-----------------------------|------------|-----|
| 1 | 25.4 | 24.6 | 23.5 | 97.2 | no |
| 2 | 13.5 | 17.3 | 18.1 | 100 | 100 |
| 3 | nt | nt | nt | 75.3 | 100 |
| 4 | 15.6 | 29.8 | 17.5 | 78.4 | 100 |
| 5 | no | 27.6 | no | 44.6 | 100 |
| 6 | nt | nt | nt | no | 100 |
| 7 | nt | nt | nt | no | 100 |
| 8 | nt | nt | nt | nt | nt |
| 9 | 23.7 | 25.1 | 36 | no | 100 |

Table 3. Cont.

| N* | DPPH (0.1 mmol/L) 20min | DPPH (0.1 mmol/L) 1hr | DPPH (0.2 mol/L) 1 hr | ·OH | LOX |
|------|-------------------------------|-----------------------------|-----------------------------|------|------|
| 10 | 26.7 | 32 | 35.3 | 95.9 | 100 |
| 11 | 17.8 | 17.7 | 16.1 | 40 | 100 |
| 12 | 13.3 | 14 | 20.6 | 87 | 77.5 |
| ASA | 80.5 | – | – | – | – |
| NDGA | 94 | – | – | – | 91 |

Conclusions

We have presented a facile route for the formation of new 4,5-disubstituted thiazolyl amides, possessing anti-inflammatory activity. These compounds also present mild antioxidant activity. They highly compete with DMSO for hydroxyl radical and strongly inhibit LOX. A phenyl group in R₁, the length of the chain and a value of n = 1 are all significant structural features for the activity observed.

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Experimental

General

Melting points were obtained with a MELTEMP II capillary apparatus (LAB. Devices Holliston, MA, USA) and are reported without correction. Infrared spectra (Nujol mulls) were recorded on a Perkin Elmer 597 spectrophotometer. UV-Vis were determined on a Perkin Elmer 554 UV-Vis spectrophotometer. Proton NMR spectra were obtained on a Bruker AW 80 apparatus at 80 MHz and are reported in ppm downfield from tetramethylsilane (TMS). Analyses indicated by the symbols of elements were within 0.4 % of theoretical values. Mass spectra (MS) were determined on a VG-250 instrument (VG Labs. Tritech, England) with the ionization energy maintained at 70 eV. The reactions were monitored by TLC on silica gel 60 F₂₅₄ (Merck). All reagents were obtained from commercial sources. 2-Aminothiazole, 2-amino-4-methylthiazole, 2-amino-4-phenylthiazole and 2-amino-4-(4-methoxyphenyl)thiazole were synthesized as described previously [5].

General synthetic procedure for the synthesis of the 2-chloroacetamido- and 3-chloropropionylamido thiazoles [5].

To a solution of 2-aminothiazole or suitable 4-substituted aminothiazole (0.02 mol) in dry benzene a cooled solution of chloroacetyl or 3-chloropropionyl chloride (0.033 mol) in dry benzene (7.5 mL) was added dropwise. The reaction mixture was refluxed in a water bath at 80 °C for 3 h. Benzene and excess 3-chloropropionyl chloride were removed by distillation. The residue was washed with aqueous sodium bicarbonate (5 % w/v) followed by cold water. The crude product was dried and crystallized from ethanol.

General synthetic procedure for the synthesis of the 2-(N-substituted aminoacetamido)/3-(N-substituted aminopropionamido) thiazoles [5].

A mixture of 2-chloroacetamido or 3-chloropropionamido thiazole (0.006mol), amine (4-OH-piperidine or N-methyl-piperazine, 0.7 mol), absolute ethanol (15 mL) and anhydrous sodium carbonate (1.48 g) was heated under reflux in a water bath for 12 h. The excess of amine and ethanol was removed by distillation and the residue was treated with 5 % sodium bicarbonate solution to remove acid impurities, filtered, washed with water and dried. It was crystallized from ethanol (95 %) to give white crystals.

Preparation of the hydrochlorides of 2-(N-substituted- aminoacetamido) / 3-(N-substituted-amino-propionamido) thiazoles.

A solution of the base in anhydrous ethanol was saturated with dry hydrogen chloride gas. The salt was filtered off, washed with dry ether and recrystallized from absolute ethanol to give white crystals. The structure of all compounds was identified both by elemental analyses as well as by spectroscopic analysis (IR, ¹H-NMR, MS).

Spectral Data: ¹H-NMR (CDCl₃, DMSO) for representative compounds (δ):

Compound **1**: 1.94-2.16 (d, 4H, piperidyl C3-H), 2.157 (s, 2H, COCH₂), 3.2 (s, 1H, piperidyl C4-H), 3.2-3.38 (d, 4H, piperidyl NCH₂), 6.4 (d, 1H, thiazolyl C5-H), 6.86 (d, 1H, thiazolyl C4-H). Compound **9**: 1.71-1.89 (d, 4H, piperidyl C3-H), 2.49-2.53 (m, 8H, NCH₂, COCH₂CH₂), 7.42-7.45 (m, 3H, phenyl C3,4,5-H), 7.46-7.48 (m, 2H, phenyl C-2,6-H), 9.05 (b, 1H, NH). Compound **16**: 2.78 (s, 3H, NCH₃), 2.78-3.53 (m, 8H, piperidyl), 3.35 (s, 2H, COCH₂), 3.71 (s, 3H, OCH₃), 6.96-6.99 (d, 2H, phenyl C2,6-H), 7.51 (s, 1H, thiazolyl C5), 7.8-7.89 (d, 2H, phenyl C3,5-H); IR cm⁻¹: 3190-3230, 1680, 1620

*Biological assays**1. Physicochemical Studies [4]*

Lipophilicity is an important factor affecting the distribution and fate of drug molecules. Increased lipophilicity is correlated with increased biological activity and more rapid metabolism. Theoretical calculations of lipophilicity as $\log P$ and Suzuki-Kudo's method [10] were performed (Table 2). The program CLOG P [11], has been designed to calculate the lipophilicity of a molecule using the additivity method. Reversed phase TLC (RPTLC) was performed on silica gel plates impregnated with 55 (v/v) liquid paraffin in light petroleum ether. Mobile phase: methanol/water mixture (70/30, v/v) containing 2 % aqueous ammonia (27 %). The plates were developed in closed chromatography tanks saturated with the mobile phase at 24 °C. Spots were detected under UV light or by iodine vapours. R_M values were determined from the corresponding R_f values (from ten individual measurements) using the equation $R_M = \log [(1/R_f) - 1]$. For results see Table 2.

2. Inhibition of carrageenin induced paw oedema [4]

All tested compounds were suspended in water with few drops of Tween-80 and ground in a mortar before use. Groups of 5-6 AKR mice weighing 20-30 g were used. A single dose of 0.2 mmol/Kg or indomethacin (0.11 mmol/Kg) was administered intraperitoneally i.p., simultaneously to the administration of the phlogistic agent : carrageenin (2%, 0.05 ml was injected intradermally i.d. into the right foot pad, the left serving as control). Results are given in Table 2.

3. Competition of the tested compounds with DMSO for hydroxyl radicals [6]

The hydroxyl radicals generated by the Fe^{3+} /ascorbic acid system were detected by the determination of formaldehyde produced from the oxidation of DMSO. The reaction mixture contained EDTA (0.1mM), Fe^{3+} (167 μ M, as a 1:2 mixture with EDTA) and DMSO (33mM) in phosphate buffer (50 mM, pH 7.4) and the tested compounds (final concentration 1mM - final volume of the samples 1 mL). Ascorbic acid (150 μ L, 10 mM in phosphate buffer) was added at the end in order to initiate the reaction. The mixture was incubated at 37 °C for 30 min. The reaction was stopped by the addition of trichloroacetic acid (250 μ L, 17.5 % w/v) and the formaldehyde formed was detected spectrophotometrically at 412 nm by the method of Nash [7].

Interaction of the synthesized compounds with DPPH [8]

To a solution of DPPH (0.1 mM) in absolute ethanol, an equal volume of the compounds dissolved in ethanol was added (0.1 mM). A control solution containing ethanol was also used. After 20 and 60 mins at room temperature, absorbance was recorded at 517 nm. For the results see Table 3.

4. Soybean lipoxygenase inhibition [9]

The conversion of sodium linoleate to 13-hydroxy-peroxylinoleic acid at 234 nm, was recorded and compared with appropriate standard, see Table 3.

Each *in vitro* experiment was performed at least in triplicate and the standard deviation in absorbance was less than ± 10 %. Acetyl salicylic acid (ASA) and/or nor-dihydroguaeretic acid (NDGA) were used as standards.

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