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Short communication

Synthesis of some triazolyl-antipyrine derivatives and investigation of analgesic activity

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Abstract – The synthesis of some triazolyl-antipyrine derivatives starting from 4-chloroacetamidoantipyrine and 3-(aryloxyalkyl)-4-ethyl/phenyl-5-mercapto-1,2,4-triazoles is described. The chemical structures of the compounds were elucidated by IR, ^{1}H-NMR and mass spectral studies. These compounds were tested for analgesic activity. © 2001 Éditions scientifiques et médicales Elsevier SAS

antipyrine / triazoles / analgesic activity

1. Introduction

Antiinflammatory, analgesic, sedative and antipyretic properties of antipyrine and also 3-pyrazolin-5-one derivatives [1–7], and different pharmacological effects of triazole derivatives [8–11] prompted the synthesis of the title compounds incorporating the two structures in a single molecule.

For this purpose, 4-chloroacetamidoantipyrine and 3-(aryloxyalkyl)-4-ethyl/phenyl-5-mercapto-1,2,4-triazoles were reacted to synthesize 4-[(3-(aryloxyalkyl)-4-ethyl/phenyl-1,2,4-triazol-5-yl)thioacetamido]antipyrine derivatives.

Taking literature findings as a basis and considering that our compounds, which carry the antipyrine residue, should also possess analgesic activity, we tested the activity of our compounds in vivo.

2. Chemistry

In the present work, 4-chloroacetamidoantipyrine (**I**) was prepared for the first time by reacting 4-aminoantipyrine with chloroacetyl chloride in accordance with the method described in the literature [12–14] (*figure 1*).

The condensation of aryloxyacetohydrazides/aryloxy-2-propionohydrazides with suitable ethyl/phenyl isothiocyanate resulted in the formation of 1-(aryloxyacetyl/2-propionyl)-4-ethyl/phenyl-3-thiosemicarbazides (**II**) [10, 15–17].

The thiosemicarbazides, on refluxing with 2 N sodium hydroxide solution, were cyclized into their corresponding 3-(aryloxyalkyl)-4-ethyl/phenyl-5-mercapto-1,2,4-triazoles (**III**) [15–17]. Treatment of these triazoles with equimolar quantities of 4-chloroacetamidoantipyrine in the presence of anhydrous potassium carbonate resulted in the formation of the title compounds (**1**–**8**) (*table I*).

Analytical and spectral data (IR, ^{1}H-NMR, MS-(FAB$^+$)) confirmed the structures of **1**–**8**.

3. Results, discussion and conclusions

The reaction of 4-chloroacetamidoantipyrine (**I**) with 3-(aryloxyalkyl)-4-ethyl/phenyl-5-mercapto-1,2,4-triazoles (**III**), resulted in 4-[(3-(aryloxyalkyl)-4-ethyl/phenyl-1,2,4-triazol-5-yl)thioacetamido]anti-

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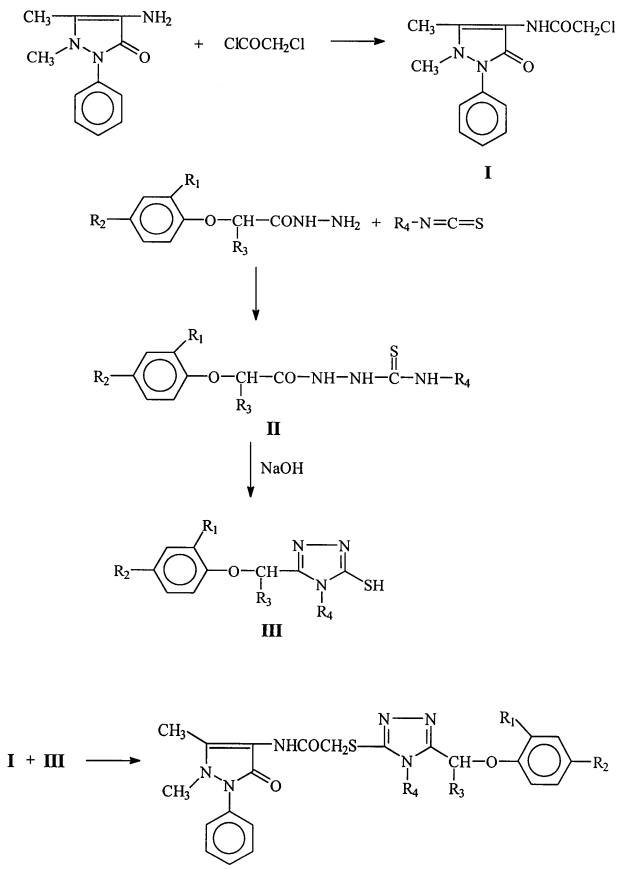

Figure 1. Preparation of the compounds.

pyrine derivatives (**1**–**8**) (*figure 1*, *table I*). The structures of these compounds were confirmed by IR, ^{1}H-NMR and mass spectral data.

IR data were very informative. Two C=O functions showed strong bands in the expected regions: amide C=O at 1699–1690 cm^{-1}, and pyrazolone C=O at 1649–1643 cm^{-1}.

^{1}H-NMR spectral data were also consistent with the assigned structures. C–CH_3 and N–CH_3 protons of the pyrazolone moiety appeared at 1.90–2.10 and 3.00–3.10 ppm, while $COCH_2$ protons and the NHCO proton appeared at 4.00–4.10 and 9.50–9.55 ppm, respectively. Aromatic protons and *N*-ethyl, *O*-alkyl protons were observed in the expected regions.

All compounds had both central and peripheral antinociceptive activities and their activities were greater than that of dipyrone administered at the same dose. High MPE% values were obtained in three central antinociceptive activities, the highest central activities were observed with **1**, **7**, **8** which contain chloride in their structures (*table II*).

The compounds were tested for antinociceptive activity because all of them contained the antipyrine structure. Actually, since it has been reported that aspirin-like drugs [18], especially dipyrone [19] and paracetamol [20], also show a central analgesic effect, we tested both the central and peripheral antinociceptive activity of our compounds. Four methods were used. The peripheral activity was evaluated using the acetic acid-induced stretching test [21]. The tail clip test to pressure, tail flick test to radiant heat and hot plate test were used to test the central antinociceptive activity.

4. Experimental

4.1. Chemistry

Melting points were determined using a Gallenkamp apparatus and are uncorrected. Spectroscopic data were recorded by the following instruments. IR, Shimadzu IR-435 spectrophotometer; ^{1}H-NMR, Bruker 250 MHz, JEOL JNM-EX 90a FT 90 MHz spectrometer; MS, fast

Table I. Physicochemical data of the compounds.

Number	R_1	R_2	R_3	R_4	M.p. (°C)	Yield (%)	Mol. formula	MW
1	Cl	H	H	C_2H_5	192	89	$C_{24}H_{25}ClN_6O_3S$	513
2	CH_3	H	H	C_2H_5	193	85	$C_{25}H_{28}N_6O_3S$	492.6
3	CH_3	H	H	C_6H_5	206	92	$C_{29}H_{28}N_6O_3S$	540.6
4	H	CH_3	H	C_2H_5	72	78	$C_{25}H_{28}N_6O_3S$	492.6
5	H	H	CH_3	C_2H_5	45	70	$C_{25}H_{28}N_6O_3S$	492.6
6	OCH_3	H	CH_3	C_6H_5	56	75	$C_{30}H_{30}N_6O_4S$	570.6
7	H	Cl	CH_3	C_2H_5	62	77	$C_{25}H_{27}ClN_6O_3S$	527
8	H	Cl	CH_3	C_6H_5	175	82	$C_{29}H_{27}ClN_6O_3S$	575.1

Table II. Analgesic activities of the compounds.

	Tail clip test to pressure MPE%	Tail flick test to radiant heat MPE%	Hot plate test MPE%	HOAc stretching, number
control ($n=7$)	17.29 ± 7.56	11.88 ± 7.99	0.80 ± 0.30	33.00 ± 1.41
1 ($n=7$)	100.00 ± 0.00*	88.63 ± 19.16*	52.43 ± 11.07*	0.29 ± 0.19*
2 ($n=7$)	100.00 ± 0.00*	82.14 ± 14.11*	19.00 ± 6.98*	0.14 ± 0.10*
4 ($n=7$)	100.00 ± 0.00*	96.43 ± 6.16*	10.43 ± 1.43*	0.00 ± 0.00*
5 ($n=7$)	68.00 ± 14.67*	43.67 ± 17.30*	8.46 ± 3.80*	0.00 ± 0.00*
6 ($n=7$)	82.14 ± 12.08*	87.83 ± 19.06*	8.36 ± 3.80*	0.00 ± 0.00*
7 ($n=7$)	100.00 ± 0.00*	100.00 ± 0.00*	8.36 ± 3.02*	0.14 ± 0.10*
8 ($n=7$)	97.14 ± 7.55*	89.57 ± 10.41*	13.61 ± 5.87*	0.14 ± 0.10*
dipyrone ($n=7$)	58.00 ± 11.13*	76.29 ± 11.10*	22.93 ± 9.91*	0.57 ± 0.29*

* $P<0.05$.

atom bombardment mass spectra (FAB-MS) were obtained using a VG Quattro mass spectrometer. Microanalytical data were obtained by the Microanalytical Section of Service Centre (CNRS, Ecole Normale de Chimie de Montpellier, France).

4.1.1. General procedure

4.1.1.1. Preparation of 4-chloroacetamidoantipyrine (I)

Chloroacetyl chloride (30 mmol) and triethylamine (30 mmol) were added to a solution of 4-aminoantipyrine (30 mmol) in anhydrous benzene and the mixture was treated as described in the literature [12–14].

4.1.1.2. Preparation of 1-(aryloxyacetyl/2-propionyl))-4-ethyl/phenyl-3-thiosemicarbazides (II)

Equimolar quantities of hydrazide (20 mmol) and ethyl/phenyl isothiocyanate (20 mmol) in 25 mL of absolute ethanol were refluxed on a steam bath for 3–5 h. The resulting solid was filtered and recrystallized from ethanol.

4.1.1.3. Preparation of 3-(aryloxyalkyl)-4-ethyl/phenyl-5-mercapto-1,2,4-triazoles (III)

Suitable substituted thiosemicarbazides (20 mmol) were dissolved in 2 N sodium hydroxide and the resulting solution was heated under reflux for 3 h. The solution was cooled and acidified to pH 2–3 with water and recrystallized from ethanol (yield 72%).

4.1.2. Preparation of 4-[(3-(aryloxyalkyl)-4-ethyl/phenyl-1,2,4-triazol-5-yl)thioacetamido] antipyrines (1–8)

A mixture of the 4-chloroacetamidoantipyrine (**I**) (30 mmol), appropriate triazoles (**III**) (30 mmol) and anhydrous potassium carbonate in acetone was refluxed for 4 h. The mixture was filtered, the filtrate was evaporated until dryness. The residue was recrystallized from ethanol.

4.1.2.1. 4-[(3-(2-Chlorophenoxymethyl)-4-ethyl-1,2,4-triazol-5-yl)thioacetamido]antipyrine (1)

IR (KBr, cm^{-1}): 1690 (C=O, amide), 1645 (C=O, pyrazolone).

^{1}H-NMR (DMSO-d_6, δ): 1.35 (3H, t ($J=7.1$ Hz), CH_3), 2.05 (3H, s, CH_3–C), 3.00 (3H, s, CH_3–N), 4.05 (2H, m, N–CH_2), 4.15 (2H, s, CO–CH_2), 5.40 (2H, s, CH_2O), 6.90–7.55 (9H, m, aromatic protons), 9.50 (1H, s, NHCO).

4.1.2.2. 4-[(3-(2-Methylphenoxymethyl)-4-ethyl-1,2,4-triazol-5-yl)thioacetamido] antipyrine (2)

IR (KBr, cm^{-1}): 1695 (C=O, amide), 1648 (C=O, pyrazolone).

^{1}H-NMR (DMSO-d_6, δ): 1.15 (3H, t ($J=7.1$ Hz), CH_3–C), 1.90 (3H, s, CH_3), 2.05, (3H, s, CH_3–Ph), 3.00 (3H, s, CH_3–N), 4.00 (2H, m, N–CH_2), 4.10 (2H, s, $COCH_2$), 5.25 (2H, s, CH_2O), 6.70–7.45 (9H, m, aromatic protons), 9.50 (1H, s, NHCO).

4.1.2.3. 4-[(3-(2-Methylphenoxymethyl)-4-phenyl-1,2,4-triazol-5-yl)thioacetamido]antipyrine (3)

IR (KBr, cm^{-1}): 1692 (C=O, amide), 1649 (C=O, pyrazolone).

^{1}H-NMR (DMSO-d_6, δ): 1.90 (3H, s, CH_3), 2.15 (3H, s, CH_3–Ph), 3.10 (3H, s, CH_3–N), 4.15 (2H, s, $COCH_2$), 5.20 (2H, s, CH_2O), 6.80–7.60 (14H, m, aromatic protons), 9.55 (1H, s, NHCO).

*4.1.2.4. 4-[(3-(4-Methylphenoxymethyl)-4-ethyl-1,2,4-triazol-5-yl)thioacetamido]antipyrine (**4**)*

IR (KBr, cm^{-1}): 1697 (C=O, amide), 1645 (C=O, pyrazolone).

^{1}H-NMR (DMSO-d_6, δ): 1.25 (3H, t ($J = 7.1$ Hz), CH_3), 2.00 (3H, s, CH_3), 2.25 (3H, s, CH_3–Ph), 3.00 (3H, s, CH_3–N), 4.00 (2H, q, N–CH_2), 6.90–7.60 (9H, m, aromatic protons), 9.50 (1H, s, NHCO).

MS (FAB^+): m/z: 493 [M+1], 515 [M+sodium].

*4.1.2.5. 4-[(3-(Phenoxy-1-ethyl)-4-ethyl-1,2,4-triazol-5-yl)thioacetamido]antipyrine (**5**)*

IR (KBr, cm^{-1}): 1692 (C=O, amide), 1648 (C=O, pyrazolone).

^{1}H-NMR (DMSO-d_6, δ): 1.15 (3H, t ($J = 7.00$ Hz), CH_3), 1.65 (3H, d ($J = 6.36$ Hz), CH_3), 2.00 (3H, s, CH_3), 3.00 (3H, s, CH_3–N), 4.00 (2H, q, CH_2–N), 4.10 (2H, s, $COCH_2$), 5.80 (1H, q, CH–O), 6.85–7.50 (10H, m, aromatic protons), 9.50 (1H, s, NHCO).

MS (FAB^+): m/z: 493 [M+1], 531 [M+potassium].

*4.1.2.6. 4-[(3-(2-Methoxyphenoxy-1-ethyl)-4-phenyl-1,2,4-triazol-5-yl)thioacetamido]antipyrine (**6**)*

IR (KBr, cm^{-1}): 1690 (C=O, amide), 1643 (C=O, pyrazolone)

^{1}H-NMR (DMSO-d_6, δ): 1.60 (3H, d ($J = 6.51$ Hz), CH_3–C–O), 2.10 (3H, s, CH_3), 3.05 (3H, s, CH_3–N), 3.65 (3H, s, OCH_3), 4.10 (2H, s, $COCH_2$), 5.40 (1H, q, CH–O), 6.65–7.55 (14H, m, aromatic protons), 9.50 (1H, s, NHCO).

MS (FAB^+): m/z: 571 [M+1], 593 [M+sodium].

*4.1.2.7. 4-[(3-(4-Chlorophenoxy-1-ethyl)-4-ethyl-1,2,4-triazol-5-yl)thioacetamido]antipyrine (**7**)*

IR (KBr, cm^{-1}): 1699 (C=O, amide), 1647 (C=O, pyrazolone)

^{1}H-NMR (DMSO-d_6, δ): 1.15 (3H, t ($J = 7.1$ Hz), CH_3), 1.65 (3H, d ($J = 6.8$ Hz), CH_3–C–O), 2.00 (3H, s, CH_3), 3.00 (3H, s, CH_3–N), 4.00 (2H, m, N–CH_2), 4.10 (2H, s, $COCH_2$), 5.80 (1H, q, CH–O), 6.70–7.55 (9H, m, aromatic protons), 9.50 (1H, s, NHCO).

*4.1.2.8. 4-[(3-(4-Chlorophenoxy-1-ethyl)-4-phenyl-1,2,4-triazol-5-yl)thioacetamido]antipyrine (**8**)*

IR (KBr, cm^{-1}): 1691 (C=O, amide), 1645 (C=O, pyrazolone).

^{1}H-NMR (DMSO-d_6, δ): 1.55 (3H, d ($J = 6.5$ Hz), CH_3–C–O), 2.00 (3H, s, CH_3–C), 3.00 (3H, s, CH_3–N), 4.05 (2H, s, $COCH_2$), 5.50 (1H, q, CH–O), 6.60–7.50 (14H, m, aromatic protons), 9.50 (1H, s, NHCO).

MS (FAB^+): m/z: 576 [M+1], 597 [M+sodium].

4.2. Pharmacology

Albino mice weighing 30–35 g were used in the present study. The laboratory temperature was maintained at 20±1°C under a 12 h light dark schedule. Before experimentation mice were allowed 1 week of adaptation. They were used only once. The study was approved by the Committee of Ethics at Osmangazi University Medical School.

The animals were divided into 15 groups. Each group included seven animals.

All compounds were dissolved in DMSO and given per os to the animals at 100 mg kg^{-1} doses in approximately 0.1 mL volume. Control animals received 0.1 mL DMSO p.o.

The tail clip test [22, 23], tail flick test to radiant heat [24], hot plate test [25, 26] and writhing test [21, 27] induced by acetic acid were performed 60 min after administration of the compounds or vehicle (DMSO for the control group).

4.2.1. Mouse tail clip test

A pressure-standardized artery clip was placed approximately 2 cm from the base of the tail and only the mice that responded to the clip placement by turning or biting at the clip within 20 s were used in this test.

4.2.2. Tail flick test to radiant heat

This test was done with a beam of high-intensity light focused on the dorsal surface of the tail. The response latency between the onset of the radiant heat stimulus and the movement of the tail out of the light beam of the apparatus (MAY, 9604-A Tail Flick Unit Commat, Ankara, Turkey) was determined. The light intensity was set to provide a predrug response time of 2–4 s. A cut-off of 20 s was used in order to prevent damage to the tail.

4.2.3. Hot plate test

A glass cylinder (16 mm high, 16 mm diameter) was used to keep the mouse on the heated surface of the plate which was kept at a temperature of 55±0.5 °C using a thermoregulated water circulating pump. The latency period until the mouse licked a foot or jumped was registered by a stopwatch (cut-off time 45 s).

The results were expressed as the percentage of the maximal possible effect (%MPE)

$$\%\text{MPE} = [(\text{post drug latency} - \text{-predrug latency})/ (\text{cut-off time} - \text{predrug latency})] \times 100$$

4.2.4. Writhing test

The abdominal constrictor test was performed by the i.p. application of 0.6% acetic acid (60 mg kg^{-1}) and stretching movements (arching of the back, development of tension in the abdominal muscles, elongation of the body and extension of forelimbs) were counted in a period of 10 min starting 5 min after the i.p. administration of acetic acid.

All tests were conducted between 9 and 12 a.m.

All results were expressed as means ±S.D. Statistical comparisons were performed using Student's test.

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