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1,4,5-Trialkyl Imidazole System Anti-inflammatory Properties of New Substituted Derivatives

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In an investigation of the anti-inflammatory properties of five-membered ring nitrogen-containing heterocyclic compounds, two series of derivatives of imidazole were prepared by altering the sites of substitution and by joining aliphatic chains to the nitrogen atom in the 1 position of the imidazole ring. Some of them were more potent inhibitors of carragenan-induced edema than indomethacin. An electron spin resonance study indicated that these compounds possess anti-radical activity.

Keywords imidazole; anti-inflammatory activity; free radical; scavenger; structure–activity relationship

Oxygenated free radicals are known to be involved in inflammatory pathology.[1−3] Although their mechanisms of action are complex, they are thought to play a role in the enhanced vascular permeability, the destruction of endothelial cells and the inactivation of various enzyme systems that occur during inflammation.[4] Methimazole or 1-methylimidazole-2(3*H*)-thione (MIT), the active metabolite of carbimazole, has been found to be a potent scavenger of OH radicals.[5] We report here the synthesis of new derivatives of 1,4,5-trialkylimidazole (Fig. 1), which were expected to possess anti-radical activity. The anti-inflammatory activity of these compounds was examined.

Synthesis The 1,4,5-trialkyl imidazoles were prepared by condensation of α-hydroxyketones with alkylthioureas in 1-hexanol. The reaction occurs through an ionic mechanism (Fig. 2). The α-hydroxyketones were obtained by reacting esters with sodium.[7,8] The radical anion obtained dimerizes to form an α-diketone. Keto-enol tautomerism in the acid medium, gives rise to the α-hydroxyketone (Fig. 3a). The alkyl thioureas were prepared in the conventional way[9,10] by the action of ammonium hydroxide on alkyl isothiocyanate in the presence of ethanol according to the following reaction scheme (Fig. 3b). The compounds prepared can be divided into two main groups depending on the nature of the substituents R^1 and R^2, *i.e.*, 1) the substituents R^2 in positions 4 and 5 of the imidazole ring are methyl groups, while R^1 in position 1 on the nitrogen is altered (compounds **1** to **5**); 2) different substituents R^2 with R^1 a methyl group (compounds **6** and **7**).

Results and Discussion

The anti-inflammatory activity was evaluated *in vivo* by measuring the inhibition of carrageenan-induced edema in the hind paw of the rat (results were compared to the reference drug indomethacin) and *in vitro* by measuring the degradation of ox synovial fluid. The results are listed in Table I. At a dose of $30\,mg \cdot kg^{-1}$ all compounds were found to have marked anti-inflammatory activity *in vivo*. The most active compound in the first group (compound **3**) reduced edema by 77%, which is greater that produced by the same dose of indomethacin (53%), and slightly higher than that obtained with methimazole

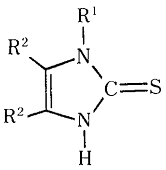


Fig. 1

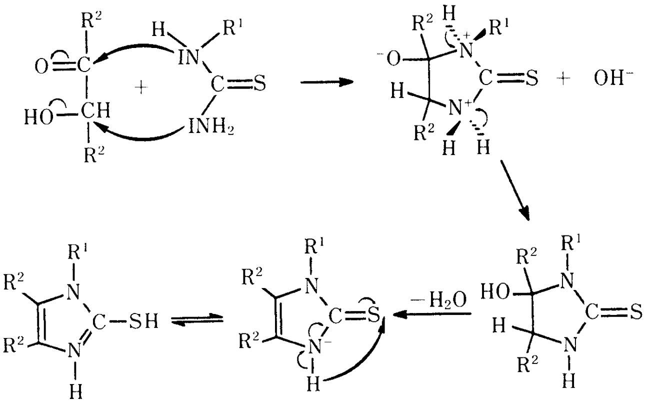
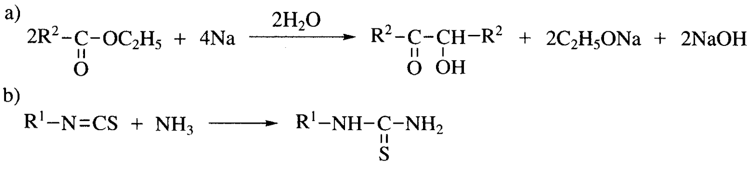


Fig. 2



a)
$$2R^2-\underset{\overset{\|}{O}}{C}-OC_2H_5 + 4Na \xrightarrow{2H_2O} R^2-\underset{\overset{\|}{O}}{C}-\underset{\overset{|}{OH}}{CH}-R^2 + 2C_2H_5ONa + 2NaOH$$

b)
$$R^1-N{=}CS + NH_3 \longrightarrow R^1-NH-\underset{\overset{\|}{S}}{C}-NH_2$$

Fig. 3

Table I. Anti-inflammatory Activity of Synthesized Compounds

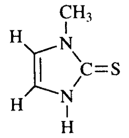
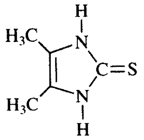
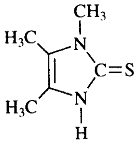
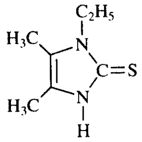
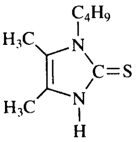
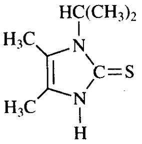
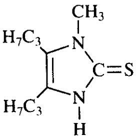
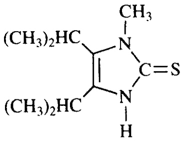
Compounds	No.	Carrageenan test inhibition (%)	Synovial fluid degradation inhibition (%)	Relative activity to that of indomethacin	
		Dose (30 mg/kg)	Concentration (80 μM)	Edema	Synovial fluid
		76	57	1.40	0.84
	**1**	14	25	0.26	0.37
	**2**	53	30	0.99	0.44
	**3**	77	38	1.44	0.56
	**4**	71	31	1.34	0.45
	**5**	25	55	0.47	0.80
	**6**	73	41	1.37	0.59
	**7**	34	18	0.63	0.26
Indomethacin		53	68	1	1

Table II. Inhibition of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Signal

Compound	Concentration for 50% inhibition		Relative activity
	μg/100 μl	mM	
1	12.80	1.004	0.72
2	18.30	1.280	0.57
3	21.00	1.340	0.54
4	22.50	1.380	0.53
5	18.70	1.098	0.66
6	25.00	1.261	0.57
7	28.00	1.412	0.51
Methimazole	11.80	1.033	0.70
α-Tocopherol	31.50	0.731	1.00

Results are mean values of 3 experiments.

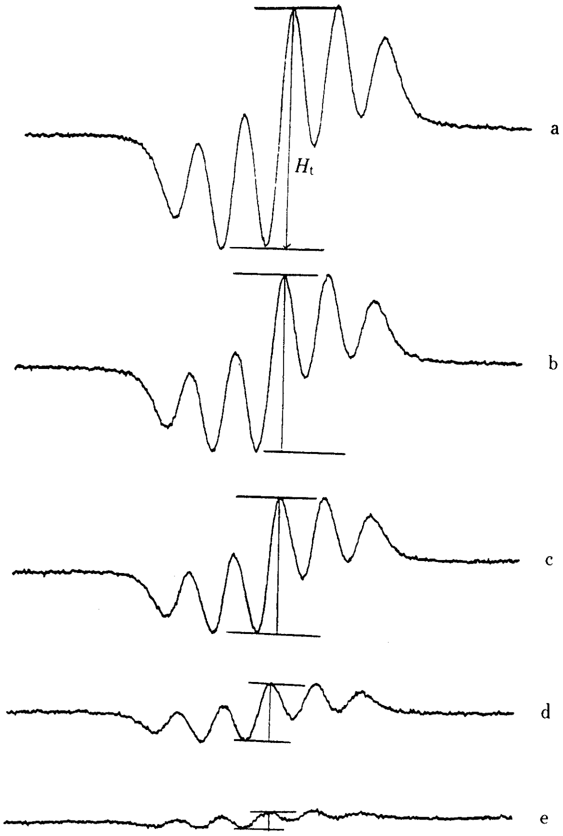


Fig. 4. ESR Spectrum of DPPH Radical after Addition of 0, 5, 20, 60 or 100 μg of 4,5-Diisopropyl-1-methyl-2-thioimidazole (**7**), in 200 μl of Ethanol

(76%). In the first group of compounds, there was a progressive increase in activity from $R^1 = H$ to C_2H_5, and then a fall in activity with further increase in chain length. In the second group, the derivative with the more bulky substituent R^2 had the lower anti-inflammatory activity. Similar results were also observed in the *in vitro* test. Small substituents on the nitrogen conferred high biological activity, which declined with increasing size of the substituent.

The anti-radical activity was evaluated with respect to the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) by electron spin resonance spectroscopy in ethanol solution. Figure 4 illustrates (for compound **7**) the direct relationship between the reduction in signal intensity and the amount of substance. Table II lists the concentrations giving to a 50% reduction in signal. All the compounds tested appeared to be better radical scavengers than the reference agent, α-D-tocopherol. It is also noteworthy that the anti-radical activity increased with increase in chain length of the substituent R^1 up to C_3 (compounds **1** to **4**), with a fall in activity with further increase in chain length (compound **5**). Similar observations were made for the two compounds in group 2. Compound **6** was more

TABLE III. Physical and Spectral Data for Intermediate Compounds

Intermediate compounds	Formula	Yield (%)	HRMS Calcd (Found)	mp (°C)	bp (°C)	IR (cm^{-1})
Propionoin	$C_6H_{12}O_2$	56	116.16 (115.98)		65 (12 mmHg)	σC=O: 1710 σC–OH: 3380 σCH_3: 2940
Isopropylthiourea	$C_4H_{10}N_2S$	86	118.19 (118.14)	170		σC=S: 1620, 1155 σNH: 3160, 2960 σCH: 2880
Butylthiourea	$C_5H_{12}N_2S$	70	132.22 (132.05)	81		σC=S: 1620, 1135 σNH: 3150 σCN: 1370

TABLE IV. Physical Data for Synthesized Compounds

No.	R^1	R^2	Formula	Yield (%)	HRMS Calcd (Found)	mp (°C)	IR (m^{-1}) (KBr)	^{1}H-NMR (δ in $CDCl_3$)
1	H	CH_3	$C_5H_8N_2S$	25	128.12 (127.70)	>300	σNH: 3145, 3089, 2781, 2674 σCH: 2933 σC=C: 1661 σC=S: 1201	11.5 (1H, s, SH), 3.36 (1H, s, NH), 1.98 (3H, s, CH_3–C)
2	CH_3	CH_3	$C_6H_{10}N_2S$	42	142.13 (142.29)	218	σNH: 3158, 3082, 2734, 2513 σCH_3: 2237, 2919 σC=C: 1652 σC=S: 1141, σC–N: 1388	11.8 (1H, s, SH), 2 (3H, s, CH_3–C), 3.5 (3H, s, N–CH_3)
3	C_2H_5	CH_3	$C_7H_{12}N_2S$	40	156.15 (155.88)	186	σNH: 3160, 3088, 2736 σC–H: 2969, 2929 σC=C: 1658 σC=S: 1141, σC–N: 1388	12 (1H, s, SH), 2 (3H, s, CH_3–C), 4 (2H, q, N–CH_2), 1.3 (3H, t, CH_3–C)
4	C_4H_9	CH_3	$C_9H_{16}N_2S$	35	184.17 (184.21)	134	σNH: 3166, 3087, 2742 σC–H: 2958, 2928 σC=C: 1652 σC–N: 1375	12 (1H, s, SH), 3.9 (2H, t, N–CH_2), 2 (3H, s, CH_3), 1.45 (2H, m, C=CH_2), 1.65 (2H, m, CH_2–C), 0.96 (3H, t, CH_3)
5	$CH(CH_3)_2$	CH_3	$C_8H_{14}N_2S$	45	170.16 (170.82)	190	σNH: 3160, 3092, 2732 σC–H: 2965, 2944 σC=C: 1652, σC=N: 1372	11.75 (1H, s, SH), 5.3 (1H, m, CH), 2.07 (3H, s, CH_3–C=C), 1.49 (3H, d, $CH(CH_3)_2$
6	CH_3	C_3H_7	$C_{10}H_{18}N_2S$	42	198.18 (198.12)	140	σNH: 3156, 3084, 2722 σCH_3: 2958, 2933 σCH_2: 2871 σC=C: 1653, σC=S: 1151	12.05 (1H, s, SH), 3.6 (3H, s, N–CH_3), 2.46 (3H, t, CH_3–C–C), 1.45 (2H, m, C–CH_2–C), 0.95 (2H, m, C–CH_2–C)
7	CH_3	$CH(CH_3)_2$	$C_{10}H_{18}N_2S$	45	198.18 (198.12)	220	σNH: 3143, 3089, 2738 σCH_3: 2929 σCH: 2868 σC=C: 1635, 1623	11.40 (1H, s, SH), 3.60 (3H, s, N–CH_3), 3.02 (1H, m, $CH(CH_3)_2$), 1.26 (3H, d, $CH(CH_3)_2$)

active than compound **7** with a larger substituent R^2 on positions 4 and 5 of the imidazole ring.

Experimental

All melting points were determined on a Koffler block and are uncorrected. Elemental analyses were carried out by the Central Microanalysis Department of the CNRS at Vernaison (Rhone, France). Infra red (IR) spectra were recorded in a Beckman 4250 (spectrometer for dispersion in potassium bromide. ^{1}H-NMR spectra were recorded in solution in deuterochloroform on a Bruker 200 MHz spectrogrameter with tetramethylsilane (TMS) as internal reference. The free radical scavenger activity was determined on a Bruker ESP 300E spectrometer. The physical properties and spectral data of these compounds are summarized in Table IV.

Anti-inflammatory Activity The compounds employed, carrageenan, hypoxanthine, and xanthine oxidase, were obtained from Sigma Chimie (France).

Inhibition of Carrageenan-Induced Edema in the Rat Hind Paw The carrageenan-induced edema test provides a measure of the initial phase of acute inflammation.[11] Groups of 7 rats received a gastric intubation of the various compounds (30 mg·kg^{-1}) 30 min before injection of 0.05 ml of an aqueous solution of 1% carrageenan into the pad of the hind paw. The edematous reaction was measured 3 h later by plethysmography in an Appelex 7150 apparatus. Two control groups were injected with saline, and two other groups received the reference drug, indomethacin at the same dose (30 mg·kg^{-1}). The anti-inflammatory activity was expressed as the percent reduction in edema with respect to the controls.

Degradation of Ox Synovial Fluid Ox synovial fluid was obtained by sterile needle puncture from the knee joint of healthy oxen. The test was carried out as described by McCord,[12] as modified by Puig–Parellada and Planas.[13] The synovial fluid was centrifuged at 12000 *g* for 60 min at 4 °C. It was further centrifuged for 30 min prior to use.

Synovial fluid (5 ml) was degraded in the following system: 0.05 ml of EDTA (0.1 M), and 0.5 ml of hypoxanthine (10 mM). After preincubation for 30 min at 37°C, the enzyme reaction was started by addition of xanthine oxidase (0.10 U in 0.05 ml). The final pH was adjusted to 7.5. The relative viscosity was measured by recording the number of seconds required for a given volume (0.7 ml) to fall under gravity through a needle from a plastic syringe. The viscosity measurements were replicated five times for each reaction mixture. Four different solutions were prepared for each compound. The results were expressed as the percent inhibition of degradation of synovial fluid for each compound at a dose of 80 μM. The same dose of indomethacin was used as the reference.

Anti-radical Activity The anti-radical activity was determined by electron spin resonance (ESR) spectroscopy with respect to the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, Sigma) in solution in ethanol according to Uchida *et al.*,[14] as modified by Devaux *et al.*[15] Spectra were recorded in a Bruker ESP 300E spectrometer. A solution of the compound under test in ethanol (100 μl) was added to 100 μl of a solution of DPPH (3×10^{-3} M). After homogenization by agitation for 10 s, 200 μl of the solution were placed in a special flat cuvette. Exactly 60 s later, the spectrum of the hyperfine structure of the DPPH radical was recorded usind the following parameters: klystron frequency = 9.77 GHz, field = 3487.20 gauss, power = 8 mW, response time = 5 ms, amplitude modulation = 0.14 gauss, gain = 5.10^4, sweep time = 5.24 s, room temperature.

The anti-radical activity was calculated for each compound from the following relationship:

$$I = (H_t - H_x)/H_t \times 100$$

where I = percent inhibition of DPPH radical, H_t = maximum height (peak to peak) of radical signal from control (200 μl of pure ethanol), and H_x = maximum height (peak to peak) of the signal obtained with the test compound. For each compound an activity curve was obtained by plotting the percent radical inhibition against the final concentration (μg/100 μl) of compound in the cuvette. The concentration (μg/100 μl) leading to 50% inhibition was then calculated. Methimazole was employed as reference agent, as it has been shown to have a scavenging action towards DPPH.[16]

α-Hydroxyketones Isobutyroin, Propionoin: Sodium metal (36 g) in 75 ml of xylene was heated to boiling with vigorous stirring. The powdered sodium was then mixed with 600 ml of anhydrous diethyl ether, and 1 mol of ethyl propionoate (102 g = 115 ml) was added dropwise from a bromine dropping funnel. The heat of the reaction caused the ether to boil. Crystals of hydrated sodium sulfate ($Na_2SO_4 \cdot 10H_2O$) formed after rapid cooling and addition of 210 g of 2 N sulfuric acid. The solution of propionoin in ether was mixed with 50 ml of 20% sodium carbonate to remove traces of propanoic acid. The crude propionoin was purified by distillation under reduced pressure. Experimental data are listed in Table III.

Isobutyroin used was a product of Aldrich (reference 24594-1).

Alkylthioureas Isopropylthiourea, Butylthiourea: Butyl isothiocyanate (0.065 mol, 8 ml) in 15 ml of ethanol was mixed with 0.10 mol of ammonium hydroxide (7.5 ml). The mixture was heated to boiling for 10 min. After cooling, the solvent was evaporated. Recrystallization of the solid residue from ethanol afforded butylthiourea.

Isopropylthiourea was prepared in the same way from isopropyl isothiocyanate (0.065 mol, 7.76 ml). Experimental data are listed in Table III.

4,5-Dimethylimidazole-2(3*H*)-thione (1) 3-Hydroxy-2-butanone (acetoin, from Fluka) (0.11 mol, 8.8 g), of thiourea (0.10 mol, 7.6 g) and 50 ml of 1-hexanol were placed in a round-bottomed flask equipped with a reflux condenser. After refluxing for 6 h, the flow of coolant was stopped and the mixture was heated until its volume was reduced to a third. The crystals formed on cooling were collected, washed with water and recrystallized from ether to give **1**.

Compounds **2** to **7** [1,4,5-trimethylimidazole-2(3*H*)-thione (**2**), 1-ethyl-4,5-dimethylimidazole-2(3*H*)-thione (**3**), 1-butyl-4,5-dimethylimidazole-2(3*H*)-thione (**4**), 1-isopropyl-4,5-dimethylimidazole-2(3*H*)-thione (**5**), 1-methyl-4,5-dipropylimidazole-2(3*H*)-thione (**6**), and 1-methyl-4,5-diisopropylimidazole-2(3*H*)-thione (**7**)] were prepared in the same way as **1**. The spectral data are given in Table IV.

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