6-(HYDROXYPHENYL)IMIDAZO[2,1-*b*]THIAZOLES AS POTENTIAL ANTIINFLAMMATORY AGENTS: EFFECTS ON HUMAN NEUTROPHIL FUNCTIONS

Aldo ANDREANI[a1,*], Mirella RAMBALDI[a2], Alberto LEONI[a3], Alessandra LOCATELLI[a4], Rita MORIGI[a], Serena TRANIELLO[b1], Alessio CARIANI[b], Olivia RIZZUTI[b] and Susanna SPISANI[b2]

[a] *Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy; e-mail: [1] aldoandr@alma.unibo.it, [2] mirella@alma.unibo.it, [3] aleoni@alma.unibo.it, [4] aleloc@alma.unibo.it*

[b] *Department of Biochemistry and Molecular Biology, University of Ferrara, Via L. Borsari 46, 44100 Ferrara, Italy; e-mail: [1] trs@unife.it, [2] sps@unife.it*

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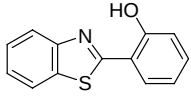
Synthesis of 6-(hydroxyphenyl)imidazo[2,1-*b*]thiazoles related to levamisole from the corresponding methoxy derivatives is reported. These compounds were tested on *in vitro* neutrophil activation, namely locomotion induced by chemoattractants, superoxide generation and lysozyme degranulation triggered by agonists. These functions were evaluated after cell pulse with different concentrations of the drugs. Several derivatives showed significant inhibitory effects, in some cases more potent than the parent compound.

Key words: Phenols; Antiinflammatory agents; Imidazo[2,1-*b*]thiazoles; Levamisole; NSAIDs; Neutrophil functions.

The hydroxyphenyl group of salicylic acid has perhaps inspired the design of some of the subsequent antiinflammatory/antirheumatic/antiaggregatory agents containing this group as well as the decision to test structures known for different pharmacological activities[1–9]. We just mention the paper by Dewhirst[10], who described structure–activity relationships in a series of 63 phenols endowed with prostaglandin cyclooxygenase inhibitory activity. One of the most active compounds was 2-(2-hydroxyphenyl)benzothiazole (**1**). On the basis of these considerations and taking



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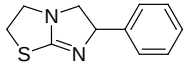

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into account that imidazothiazoles related to levamisole **2** have been reported as antiinflammatory agents[11–15] and that levamisole itself is an antirheumatic drug[16–22] acting on neutrophils[23], we planned the synthesis of hydroxyphenylimidazo[2,1-*b*]thiazoles in order to test their effects on human neutrophil functions.

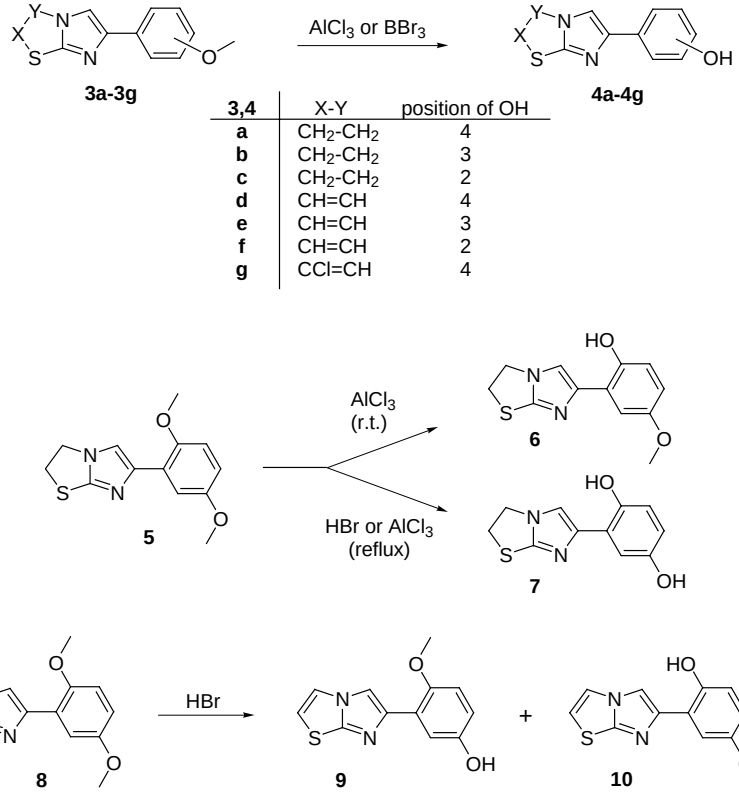
It has been ascertained that nonsteroidal antiinflammatory drugs (NSAIDs) show *in vitro* effects on neutrophil functions, considering the role of these cells in the inflammatory process[24]. In fact, neutrophils are the principal cell type of the cellular infiltrate and present an important defense component of the immune system involved in the ingestion and degradation of microorganisms. On the other hand, neutrophils are considered harmful as mediators of tissue damage[25] since the activation of these cells leads to generation of reactive species and extracellular release of granule constituents which participate in the propagation and maintenance of acute and chronic inflammation. Suppression of neutrophil functions may control the inflammatory response and has been implicated in the mechanisms of action of some NSAIDs since inhibition of constitutive and inducible cyclooxygenases does not seem to completely justify their antiinflammatory effects (for a review see ref.[26]). Some NSAIDs inhibit neutrophil activation by inflammatory stimuli, such as C5a, LTB_4, or formyl–methionine–leucine–phenylalanine (FMLP), by interfering with early events of signal transduction[27–30]. Indomethacin, salicylic acid and acetylsalicylic acid were found to inhibit superoxide generation by human neutrophils exposed to phorbol 12-myristate 13-acetate (PMA) in a whole-cell system[31] while prednisolone inhibited elastase release from neutrophils exposed to FMLP. Moreover, NSAIDs, such as acetylsalicylic acid, indomethacin, phenylbutazone, ibuprofen and naproxen, have long been known to inhibit platelet aggregation by acting on the cyclooxygenase enzyme[32,33]. The trend of measuring the effects on neutrophil functions for the evaluation of new potential antiinflammatory agents is supported by a recent paper on 2-substituted 3-chloro-1,4-naphthoquinone derivatives[34].

The present paper is connected to our previous communication[35] reporting synthesis of ring-opened indomethacin analogs inhibiting biological functions related to human neutrophils at nanomolar concentrations. The new 6-(hydroxyphenyl)imidazo[2,1-*b*]thiazoles described here (**4a**–**4g**, **6**, **7**, **9**, **10**, Scheme 1) were tested for *in vitro* neutrophil activation induced by different stimuli which act either through specific cell-surface receptors (*e.g.*, FMLP) or by postreceptor signaling (*e.g.*, PMA). Chemotaxis, superoxide anion generation and lysozyme degranulation were evaluated after cell pulse with different concentrations of the drugs.

RESULTS AND DISCUSSION

Chemistry

The hydroxy compounds **4a**–**4g**, **6**, **7**, **9** and **10** were prepared from the corresponding methoxy derivatives. The starting methoxy derivatives have been previously described, except 2-chloro-6-(4-methoxyphenyl)imidazo-[2,1-*b*]thiazole (**3g**) which was prepared by the same procedure[36,37]. The first attempts of ether cleavage were performed on imidazo[2,1-*b*]-thiazolines **3a**–**3c** by refluxing with anhydrous aluminum chloride in xylene for 5 h (method *A*) and furnished the expected phenols **4a**–**4c**. The same reaction was successfully employed for the synthesis of compounds **4e** and **4g** from the respective methoxy derivatives **3**, whereas, if the ether **5** was subjected to the same treatment, a complex mixture was obtained.



3,4	X-Y	position of OH
a	CH_2-CH_2	4
b	CH_2-CH_2	3
c	CH_2-CH_2	2
d	CH=CH	4
e	CH=CH	3
f	CH=CH	2
g	CCl=CH	4

SCHEME 1

When the reaction was performed at room temperature, only one methoxy group was cleaved to give compound **6**. The expected 2,5-dihydroxyphenyl derivative **7** was obtained after a short reflux with aluminum chloride or using azeotropic (48%, 8.8 M) hydrobromic acid (method *B*). When attempts to demethylate compound **8**, bearing a double bond in the 2,3 position, by this method was done, a mixture dihydroxy derivative **10** and a partially cleaved derivative **9** was obtained. Only the dihydroxy derivative **10** was obtained with the use of boron tribromide (method *C*). If compound **3f** was subjected to the methods *A* and *B*, formation of compound **4f** was not observed. We failed to prepare **4f** also with iodotrimethylsilane prepared *in situ* from chlorotrimethylsilane and sodium iodide according to the literature[38]. Compounds **4d**, **4f** were prepared from the respective methoxy compounds **3d**, **3f** by method *C*. The above methods were not tested systematically for each compound and therefore the yields reported in the experimental section could be improved.

The spectroscopic data of compounds **4a**–**4g**, **6**, **7**, **9** and **10** are in agreement with the assigned structures. The hydroxy groups of compounds **4a**–**4g**, **6**, **7**, **9** and **10** give a broad band in the range 2 500–3 300 cm^{-1}. In ^1H NMR spectra, imidazothiazoles **4d**–**4g**, **9** and **10** show a typical aromatic pattern with two doublets at ≈7 ppm (H-2) and ≈8 ppm (H-3) whereas the 2,3-dihydro analogs **4a**–**4c**, **6** and **7** show an aliphatic pattern with two triplets at ≈3.9 and 4.2 ppm. In order to establish the structure of the monohydroxyphenyl derivatives **6** and **9**, some NOE experiments were performed. If the hydroxy group of compound **6** was irradiated (10.46 ppm), NOE was observed at the H-5 of imidazothiazole (7.80 ppm) and at the H-3 of the phenyl ring (6.75 ppm); moreover, if the methoxy group (3.69 ppm) was irradiated, NOE was observed with the protons adjacent to position 5 of the phenyl ring (6.64 ppm (H-4) and 7.24 ppm (H-6)). The opposite situation was encountered when we repeated the same experiments on compound **9**: the irradiation of the hydroxy group (8.96 ppm) gave NOE only at the phenyl ring (6.63, 7.59 ppm) and the irradiation of the methoxy group (3.84 ppm) gave enhancement at the H-3 of the phenyl ring (6.90 ppm) and at the H-5 of imidazothiazole (8.16 ppm). These data suggest that the hydroxy group is in position 2 in compound **6** and in position 5 in compound **9**.

Biological Activity

In the first step of our study, we tested whether compounds **4a**–**4g**, **6**, **7**, **9**, **10** and levamisole were chemotaxins for human neutrophils, secretagogue

agents or were able *per se* to trigger superoxide anion production. None of the molecules was able to mediate these activities. In the second step, we tested a possible influence of the compounds on neutrophil functions.

In order to study the initial event of inflammation, we analyzed the influence of the prepared compounds and levamisole on random locomotion *in vitro*. All compounds showed slight effects: however, these were not statistically significant ($p > 0.05$) at the concentrations tested (10^{-7} and 10^{-5} M). Conversely, when the same compounds were tested for their ability to modulate directed migration induced by casein (Table I), a significant inhibition of chemotaxis was observed in the presence of compounds **4c**, **7**, **9** and levamisole. This inhibition was concentration-dependent but was greater with levamisole at 10^{-5} M ($p < 0.01$) than with the hydroxyphenyl derivatives. On the other hand, compound **4c** was able to inhibit about 40% even at 10^{-7} M. The chemotactic motility in response to FMLP and in the presence of the tested compounds showed that compounds **7** and **10** at the higher concentration had the strongest inhibiting effect followed by **4c**, **4d**, **4g** which showed a trend similar to that found for levamisole (Table I). The other compounds shown were weaker inhibitors ($p < 0.05$) at the same concentration. It is to be pointed out that compounds **4c**, **4d**, **7**, **9** and **10**, at the lower concentration displayed behavior analogous to that of levamisole with about 40% ($p < 0.05$) inhibitory effect. These data suggest that compounds **4c**, **7**, **9** and levamisole probably act at the same level, since they caused similar responses not depending on the chemoattractants used to activate neutrophil chemotaxis.

Compounds **4a**–**4g**, **6**, **7**, **10** and levamisole were not able to affect PMA- or FMLP-induced respiratory burst (not shown) at the two concentrations tested. Compound **9** was the only one showing a moderate inhibitory effect (35% at 10^{-5} M, FMLP, $p = 0.05$).

Testing the imidazothiazoles for their ability to influence PMA- and FMLP-dependent lysozyme release, an inhibitory effect was seen for several derivatives (Table I). The inhibition of the lysozyme release induced by PMA decreased significantly ($p < 0.01$) when the compounds added were **4a** and **7** without a dose-effect relationship. Levamisole was unable to reduce degranulation. If the agonist was FMLP, compound **4c**, followed by **4b** and **9**, were the most potent inhibitors ($p < 0.01$) even at the lower dose tested, a behavior very close to that of levamisole. The other compounds shown were less effective ($p < 0.05$); however, with the exception of compound **10**, all the other derivatives showed a higher inhibitory effect at 10^{-7} M.

It is evident that the tested 6-(hydroxyphenyl)imidazo[2,1-*b*]thiazoles **4a**–**4g**, **6**, **7**, **9** and **10** are not toxic (measurement of cell viability, see Experimen-

TABLE I
Activity of compounds **4a**–**4g**, **6**, **7**, **9** and **10** on human neutrophil functions in comparison with levamisole **2** (only the significant values are reported)

Compound	Concentration mol/l	FMLP-induced chemotaxis %	Casein-induced chemotaxis %	PMA-induced lysozyme secretion , %	FMLP-induced lysozyme secretion, %
2	10^{-7}	60	80	105	60
	10^{-5}	50	44	102	60
4a	10^{-7}	75		59	74
	10^{-5}	65		59	81
4b	10^{-7}	69			65
	10^{-5}	70			76
4c	10^{-7}	61	56		50
	10^{-5}	48	54		53
4d	10^{-7}	61		87	72
	10^{-5}	53		73	90
4e	10^{-7}	79			
	10^{-5}	75			
4f	10^{-7}	78			
	10^{-5}	80			
4g	10^{-7}	83		74	
	10^{-5}	56		70	
6	10^{-7}	80		67	70
	10^{-5}	67		67	79
7	10^{-7}	62	76	60	67
	10^{-5}	30	73	54	83
9	10^{-7}	57	77	65	62
	10^{-5}	67	70	64	71
10	10^{-7}	60		75	87
	10^{-5}	34		77	73

tal) and possess different spectra of biological activities regarding the parameters related to neutrophil functions. Levamisole displayed good levels of inhibition in both chemotaxis stimulated by either FMLP or casein (in a dose-dependent manner) and in degranulation induced by FMLP. The present derivatives, with the exception of **9** which gave about 35% inhibition, did not affect neutrophil-generated superoxide. Nevertheless, almost all the compounds inhibited the degranulation induced by either the receptor agonist FMLP or the postreceptor signalling PMA (with some differences in potency depending on the stimulus used) as well as chemotaxis activated by FMLP. Compounds **7** and **9** appeared to be endowed with a wide range of activity. They maintain the inhibitory power of levamisole, being in some cases more active; for example both compounds in PMA-induced degranulation and compound **7** also in FMLP-induced chemotaxis. Compound **4c** is as potent as levamisole in FMLP-triggered degranulation and induced a 40% inhibition at the lower concentration in casein-activated chemotaxis.

In conclusion, some derivatives display interesting properties as inhibitors of human neutrophil functions and, for this reason, may be considered as potential antiinflammatory agents. They could block recruitment of neutrophils into inflammatory lesions and control neutrophil degranulation, showing a protective effect against the direct injury to the target tissue derived from enzyme release.

EXPERIMENTAL

Chemistry

Melting points are uncorrected. IR spectra were taken on a Perkin–Elmer 683 spectrophotometer (wavenumbers in cm^{-1}). ^1H NMR spectra were recorded on a Varian Gemini (300 MHz) machine, and were referenced to solvent signals (dimethyl sulfoxide-d_6). Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Thin layer chromatography was done on pre-coated TLC plates Bakerflex (silica gel IB2-F) using mixtures of petroleum ether and acetone as eluents. Silica gel 60 (Merck) was used for column chromatography.

2-Chloro-6-(4-methoxyphenyl)imidazo[2,1-*b*]thiazole (**3g**)

2-Amino-5-chlorothiazole (6.7 g, 50 mmol) was dissolved in acetone (100 ml) and treated with 2-bromo-1-(4-methoxyphenyl)ethanone (11.4 g, 50 mmol). The reaction mixture was refluxed for 2 h and the resulting precipitate was collected by filtration. This intermediate 2-(2-imino-5-chlorothiazol-3-yl)-1-(4-methoxyphenyl)ethanone was treated, without further purification, with 2 M solution of hydrobromic acid (400 ml) and refluxed for 1 h. The solution thus obtained was basified with 5 M solution of ammonium hydroxide and the result-

ing free base was crystallized from ethanol to give pure **3g** (7.9 g, 60%); m.p. 204–208 °C. For $C_{12}H_9ClN_2OS$ (264.7) calculated: 54.44% C, 3.43% H, 10.58% N; found: 54.72% C, 3.19% H, 10.71% N. IR: 995, 1 030, 1 250, 1 545. ^{1}H NMR: 3.77 s, 3 H (CH_3O); 6.97 d, 2 H, *J* = 9 (arom. H); 7.76 d, 2 H, *J* = 9 (arom. H); 8.12 s, 1 H (H-3); 8.28 s, 1 H (H-5).

Demethylation with Anhydrous Aluminum Chloride (Method *A*) – General Procedure

A mixture of the starting methoxy derivative (8 mmol) and xylene (100 ml) was treated with anhydrous aluminum chloride (2.7 g, 20 mmol). The reaction mixture was refluxed for 5 h and evaporated under reduced pressure. Water was added to the residue and the mixture was basified with 2 M solution of ammonium hydroxide. The resulting precipitate was collected by filtration and crystallized from ethanol to give yields of 55–60%. Compound **6** could be obtained after 48 h at room temperature and compound **7** after 40 min reflux.

6-(4-Hydroxyphenyl)imidazo[2,1-b]thiazoline (**4a**). Yield 58%, m.p. 280–282 °C. For $C_{11}H_{10}N_2OS$ (218.3) calculated: 60.53% C, 4.62% H, 12.83% N; found: 60.65% C, 4.88% H, 13.04% N. IR: 820, 1 270, 1 490, 1 545. ^{1}H NMR: 3.86 t, 2 H, *J* = 7 (H-2); 4.16 t, 2 H, *J* = 7 (H-3); 6.72 d, 2 H, *J* = 8 (arom. H); 7.48 d, 2 H, *J* = 8 (arom. H); 7.49 s, 1 H (H-5); 9.35 s, 1 H (OH).

6-(3-Hydroxyphenyl)imidazo[2,1-b]thiazoline (**4b**). Yield 56%, m.p. 237–239 °C. For $C_{11}H_{10}N_2OS$ (218.3) calculated: 60.53% C, 4.62% H, 12.83% N; found: 60.35% C, 4.33% H, 12.75% N. IR: 965, 1 220, 1 475, 1 575. ^{1}H NMR: 3.88 t, 2 H, *J* = 7 (H-2); 4.19 t, 2 H, *J* = 7 (H-3); 6.58 m, 1 H (arom. H); 7.09 m, 3 H (arom. H); 7.63 s, 1 H (H-5); 9.32 s, 1 H (OH).

6-(2-Hydroxyphenyl)imidazo[2,1-b]thiazoline (**4c**). Yield 60%, m.p. 165–169 °C. For $C_{11}H_{10}N_2OS$ (218.3) calculated: 60.53% C, 4.62% H, 12.83% N; found: 60.84% C, 4.58% H, 12.64% N. IR: 750, 830, 1 250, 1 580. ^{1}H NMR: 3.80 t, 2 H, *J* = 7 (H-2); 4.15 t, 2 H, *J* = 7 (H-3); 6.90 m, 3 H (arom. H); 7.62 m, 1 H (arom. H); 7.72 s, 1 H (H-5); 11.13 s, 1 H (OH).

6-(3-Hydroxyphenyl)imidazo[2,1-b]thiazole (**4e**). Yield 59%, m.p. 207–211 °C. For $C_{11}H_8N_2OS$ (216.3) calculated: 61.09% C, 3.73% H, 12.95% N; found: 61.37% C, 3.94% H, 12.66% N. IR: 1 155, 1 175, 1 215, 1 590. ^{1}H NMR: 6.66 m, 1 H (arom. H); 7.20 m, 3 H (arom. H); 7.25 d, 1 H, *J* = 4.4 (H-2); 7.91 d, 1 H, *J* = 4.4 (H-3); 8.14 s, 1 H (H-5); 9.43 s, 1 H (OH).

2-Chloro-6-(4-hydroxyphenyl)imidazo[2,1-b]thiazole (**4g**). Yield 60%, m.p. 185–189 °C. For $C_{11}H_7ClN_2OS$ (250.7) calculated: 52.70% C, 2.81% H, 11.17% N; found: 52.91% C, 2.63% H, 10.94% N. IR: 1 240, 1 260, 1 550, 1 610. ^{1}H NMR: 6.78 d, 2 H, *J* = 8 (arom. H); 7.63 d, 2 H, *J* = 8 (arom. H); 8.03 s, 1 H (H-3); 8.25 s, 1 H (H-5); 9.49 s, 1 H (OH).

6-(2-Hydroxy-5-methoxyphenyl)imidazo[2,1-b]thiazoline (**6**).Yield 58%, m.p. 227–230 °C. For $C_{12}H_{12}N_2O_2S$ (248.3) calculated: 58.05% C, 4.87% H, 11.28% N; found: 57.86% C, 5.06% H, 11.49% N. IR: 1 155, 1 200, 1 265, 1 500. ^{1}H NMR: 3.69 s, 3 H (CH_3O); 3.93 t, 2 H, *J* = 7 (H-2); 4.25 t, 2 H, *J* = 7 (H-3); 6.64 dd, 1 H, *J* = 3, *J* = 8 (arom. H-4); 6.75 d, 1 H, *J* = 8 (arom. H-3); 7.24 d, 1 H, *J* = 3 (arom. H-6); 7.80 s, 1 H (H-5); 10.46 s, 1 H (OH).

6-(2,5-Dihydroxyphenyl)imidazo[2,1-b]thiazoline (**7**). Yield 60%, m.p. 188–190 °C. For $C_{11}H_{10}N_2O_2S$ (234.3) calculated: 56.40% C, 4.30% H, 11.96% N; found: 56.69% C, 4.12% H, 12.12% N. IR: 965, 1 195, 1 335, 1 480. ^{1}H NMR: 3.94 t, 2 H, *J* = 7 (H-2); 4.26 t, 2 H, *J* = 7 (H-3); 6.45 dd, 1 H, *J* = 3, *J* = 8 (arom. H-4); 6.62 d, 1 H, *J* = 8 (arom. H-3); 7.07 d, 1 H, *J* = 3 (arom. H-6); 7.71 s, 1 H (H-5); 8.72 s, 1 H (OH); 10.29 s, 1 H (OH).

Demethylation with Hydrobromic Acid (Method *B*) – General Procedure

A mixture of the starting methoxy derivative (2 mmol) and 20 ml of azeotropic aqueous hydrobromic acid (8.8 M) was refluxed for 18 h. The mixture was diluted with water (40 ml) and treated with 15% (5 M) ammonium hydroxide until pH 6 was reached. Then the mixture was extracted with methylene chloride, dried and evaporated under reduced pressure. The crude product thus obtained was purified by column chromatography (hexane–butan-2-one, 70 : 30).

6-(2,5-Dihydroxyphenyl)imidazo[2,1-b]thiazoline (**7**). Yield 30%. The sample was in all aspects identical with the compound prepared by method *A*.

6-(5-Hydroxy-2-methoxyphenyl)imidazo[2,1-b]thiazole (**9**). Yield 20%, m.p. 165–169 °C. For $C_{12}H_{10}N_2O_2S$ (246.3) calculated: 58.52% C, 4.09% H, 11.37% N; found: 58.81% C, 3.86% H, 11.49% N. IR: 1 160, 1 195, 1 275, 1 495. ^{1}H NMR: 3.84 s, 3 H (CH_3O); 6.63 dd, 1 H, *J* = 3, *J* = 8 (arom. H-4); 6.90 d, 1 H, *J* = 8 (arom. H-3); 7.23 d, 1 H, *J* = 4.4 (H-2); 7.59 d, 1 H, *J* = 3 (arom. H-6); 7.92 d, 1 H, *J* = 4.4 (H-3); 8.16 s, 1 H (H-5); 8.96 s, 1 H (OH).

6-(2,5-Dihydroxyphenyl)imidazo[2,1-b]thiazole (**10**). Yield 20%, m.p. 197–200 °C. For $C_{11}H_8N_2O_2S$ (232.3) calculated: 56.88% C, 3.47% H, 12.06% N; found: 57.04% C, 3.62% H, 11.88% N. IR: 1 260, 1 325, 1 485, 1 530. ^{1}H NMR: 6.54 dd, 1 H, *J* = 3, *J* = 8 (arom. H-4); 6.71 d, 1 H, *J* = 8 (arom. H-3); 7.26 d, 1 H, *J* = 3 (arom. H-6); 7.28 d, 1 H, *J* = 4.4 (H-2); 7.96 d, 1 H, *J* = 4.4 (H-3); 8.22 s, 1 H (H-5); 8.77 s, 1 H (OH); 10.20 s, 1 H (OH).

Demethylation with Boron Tribromide (Method *C*) – General Procedure

A solution of the starting methoxy derivative (3 mmol) in methylene chloride (40 ml) was treated under nitrogen at 0–5 °C with 1 M solution of boron tribromide in methylene chloride (4 ml, 4 mmol). The mixture was stirred overnight at room temperature, treated with 1 M solution of sodium hydrogencarbonate (pH 6), stirred for additional 10 min and extracted with methylene chloride. After the usual procedures, the crude product was crystallized from a mixture of acetone and petroleum ether.

6-(4-Hydroxyphenyl)imidazo[2,1-b]thiazole (**4d**). Yield 42%, m.p. 242–245 °C. For $C_{11}H_8N_2OS$ (216.3) calculated: 61.09% C, 3.73% H, 12.95% N; found: 60.98% C, 4.02% H, 13.05% N. IR: 1 165, 1 280, 1 540, 1 605. ^{1}H NMR: 6.79 d, 2 H, *J* = 8 (arom. H); 7.25 d, 1 H, *J* = 4.4 (H-2), 7.63 d, 2 H, *J* = 8 (arom. H); 7.93 d, 1 H, *J* = 4.4 (H-3); 8.06 s, 1 H (H-5); 9.50 s, 1 H (OH).

6-(2-Hydroxyphenyl)imidazo[2,1-b]thiazole (**4f**). Yield 45%, m.p. 170–173 °C. For $C_{11}H_8N_2OS$ (216.3) calculated: 61.09% C, 3.73% H, 12.95% N; found: 61.15% C, 3.73% H, 12.78% N. IR: 1 020, 1 240, 1 255, 1 570. ^{1}H NMR: 6.88 m, 2 H (arom. H); 7.12 t, 1 H, *J* = 8 (arom. H); 7.31 d, 1 H, *J* = 4.4 (H-2); 7.87 d, 1 H, *J* = 8 (arom. H); 7.99 d, 1 H, *J* = 4.4 (H-3); 8.29 s, 1 H (H-5); 10.97 s, 1 H (OH).

Biology

Dextran and Ficoll–Paque were purchased from Pharmacia (Uppsala, Sweden). Levamisole, FMLP, PMA, cytochalasin B, ferricytochrome C, bovine serum albumin, superoxide dismutase and *Micrococcus lysodeicticus* were obtained from Sigma Chemical Co. (St. Louis (MO), U.S.A.); filters for the chemotactic chamber were from Millipore (Roma, Italy) and casein "Hammarsten" was from Merck (Darmstadt, Germany). Sterile 96-well microtitre plates with flat-bottomed wells were from Falcon Microtest III, Becton–Dickinson Labware (Milano,

Italy). NADH was from Fluka. A 10^{-2} M FMLP stock solution in dimethyl sulfoxide (DMSO) was diluted before use in Krebs–Ringer-phosphate containing 0.1% (w/v) D-glucose (KRPG), pH 7.4. A PMA stock solution of 1 mg/ml in DMSO was diluted before use with KRPG. A casein stock solution 10 mg/ml in KRPG was diluted before use with KRPG containing 1 mg/ml of bovine serum albumin (KRPG-A). Stock solutions (10^{-2} M) of the tested compounds and levamisole in DMSO were diluted before use with KRPG. DMSO used did not interfere with any of the biological assays performed.

Cell Preparation

Cells were obtained from the blood of healthy subjects and neutrophils were purified employing the standard techniques of dextran sedimentation, centrifugation on Ficoll–Paque and hypotonic lysis of contaminating red cells. The cells were washed twice, resuspended in KRPG to obtain a final concentration of $50 \cdot 10^6$ cells/ml and kept at room temperature until used. The percentage of neutrophils was 98–100% pure and >99% viable as determined by Trypan blue exclusion test.

Random Locomotion

Random locomotion was evaluated with a 48-well microchemotaxis chamber, by estimating the distance in micrometers which the leading front of the cell migrated, using the method of Zigmond and Hirsch[39] after 90 min incubation at 37 °C. A 3-μm pore-size filter separated upper and lower compartments. The actual control for random locomotion was 40 ± 4 μm SE from 15 separate experiments.

Chemotaxis

Chemotaxis was studied by adding the chemoattractant to the lower compartment. The chemotactic factors used were FMLP (10^{-8} M in KRPG-A) and casein (2 mg/ml in KRPG-A). The actual control of chemotaxis was 95 ± 4 μm or 83 ± 2 μm SE, from 15 separate experiments, induced by 10^{-8} M FMLP and 2 mg/ml casein, respectively.

Superoxide Anion Production

The superoxide anion was measured by the superoxide dismutase-inhibitable reduction of ferricytochrome[40] modified for microplate-based assays. The tests were carried out in a final volume of 200 μl containing $4 \cdot 10^5$ neutrophils, 100 nmol cytochrome C and KRPG. At zero time, the stimulant was added and the plates were incubated in a microplate reader (Ceres 900, Bio-TeK instruments, INC) with the compartment T set at 37 °C. The absorbance was recorded at wavelengths of 550 and 468 nm. Differences in absorbance at the two wavelengths were used to calculate the O_2^- produced in nmol, using a millimolar absorption coefficient for cytochrome C of 15.5. The stimulants employed were PMA (100 ng/ml in KRPG) and FMLP (10^{-6} M in KRPG). Neutrophils were preincubated with cytochalasin B 5 μg/ml for 5 min prior to activation by FMLP.

Enzyme Assay

The release of neutrophil granule enzymes was evaluated by determining lysozyme activity[40], modified for microplate-based assays. $3 \cdot 10^6$ cells were incubated in microplates wells

in the presence of the stimulus for 15 min at 37 °C. The plates were then centrifuged for 5 min at 400 *g* and the lysozyme was quantified nephelometrically by the lysis rate of cell-wall suspension of *Micrococcus lysodeikticus*. The reaction rate was measured with a microplate reader at 465 nm. The enzyme was expressed as a net percentage of the total enzyme content released by 0.1% Triton X-100. The total enzyme activity was 85 ± 1 μg/1 · 10^7 cells/min. Spontaneous release was less than 10%. The degranulating agents used were PMA (0.1 μg/ml) and FMLP (10^{-6} M) in KRPG. Cells were preincubated with cytochalasin B (5 μg/ml) for 15 min prior to activation by FMLP. Assays were currently done in triplicate for each experimental condition. Cells were incubated with 10^{-7} and 10^{-5} M of the compounds under test for 10 min before the addition of stimulus. The actual control of lysozyme release was or 30 ± 2% or 45 ± 4%/3 · 10^6 cells/15 min from 15 separate experiments, induced by 100 ng/ml PMA and 10^{-6} M FMLP, respectively.

Measurement of Cell Viability

In order to assess possible cytotoxic effects of the tested compounds, the cytoplasmic marker enzyme, lactate dehydrogenase (LDH), was determined by measuring the rate of oxidation of NADH. The absorbance change was followed at 340 nm (ref.[41]) (data not shown).

Treatment with the Compounds under Test

When required, 10^{-7} and 10^{-5} M solutions of compounds **4a**–**4g**, **6**, **7**, **9**, **10** and levamisole were added to neutrophils 10 min before the incubation step for cell functionality.

Statistical Analysis

The nonparametric Wilcoxon test was used in the statistical evaluation of differences between groups.

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REFERENCES

1. Ozaki Y.: *Chem. Pharm. Bull.* **1992**, *40*, 954.
2. Bendele A. M., Benslay D. N., Hom J. T., Spaethe S. M., Ruterbories K. J., Lindstrom T. D., Lee S. J., Naismith R. W.: *J. Pharmacol. Exp. Ther.* **1992**, *260*, 300.
3. Mullican M. D., Wilson M. W., Connor D. T., Kostlan C. R., Schrier D. J., Dyer R. D.: *J. Med. Chem.* **1993**, *36*, 1090.
4. Schrier D. J., Baragi V. M., Connor D. T., Dyer R. D., Jordan J. H., Imre K. M., Lesch M. E., Mullican M. D., Okonkwo G. C., Conroy M. C.: *Prostaglandins* **1994**, *47*, 17.
5. Unangst P. C., Connor D. T., Cetenko W. A., Sorenson R. J., Kostlan C. R., Sircar J. C., Wright C. D., Schrier D. J., Dyer R. D.: *J. Med. Chem.* **1994**, *37*, 322.
6. Fukumoto S., Terashita Z., Ashida Y., Terao S., Shirashi M.: *Chem. Pharm. Bull.* **1996**, *44*, 749.
7. Yamamoto S., Jiang H., Kato R.: *Pharmacology* **1994**, *48*, 273.

8. Krause M., Rouleau A., Stark H., Luger P., Lipp R., Garbar M., Schwartz J. C., Schunack W.: *J. Med. Chem.* **1995**, *38*, 4070.
9. Rouleau A., Garbar M., Ligneau X., Mantion C., Lavie P., Advenier C., Lecomte J. M., Krause M., Stark H., Schunack W., Schwartz J. C.: *J. Pharmacol. Exp. Ther.* **1997**, *281*, 1085.
10. Dewhirst F. E.: *Prostaglandins* **1980**, *20*, 209.
11. Paolini J. P., Lendvay L. J.: J. *Med. Chem.* **1969**, *12*, 1031.
12. Powers L. J., Fogt S. W., Ariyan Z. S., Rippin D. J., Heilman R. D., Matthews R. J.: *J. Med. Chem.* **1981**, *24*, 604.
13. Andreani A., Bonazzi D., Rambaldi M., Fabbri G., Rainsford K. D.: *Eur. J. Med. Chem.* **1982**, *17*, 271.
14. Isomura Y., Ito N., Sakamoto S., Homma H., Abe T., Kubo K.: *Chem. Pharm. Bull.* **1983**, *31*, 3179.
15. Palagiano F., Arenare L., Luraschi E., de Caprariis P., Abignente E., D'Amico M., Filippelli W., Rossi F.: *Eur. J. Med. Chem.* **1995**, *30*, 901.
16. Hunneyball I. M.: *Prog. Drug Res.* **1980**, *24*, 101.
17. Veys E. M., Mielants H., Verbruggen G., Dhondt E., Goethals L., Cheroutre L.: *J. Rheumatol.* **1981**, *8*, 45.
18. Dayrens P., Ivanoff B., Cussac M., Fontanges R.: *Arzneim. Forsch.* **1983**, *33*, 372.
19. Wildfeuer A.: *Arzneim. Forsch.* **1983**, *33*, 780.
20. Huck F., de Medicis R., Lussier A., Dupuis G., Federlin P.: *J. Rheumatol.* **1984**, *11*, 605.
21. Veys E. M., Mielants H., Verbruggen G.: *Clin. Exp. Rheumatol.* **1987**, *5*, 111.
22. Nielsen O. H., Ahnfelt-Ronne I., Elmgreen J.: *Agents Actions* **1989**, *26*, 233.
23. Nielsen O. H., Ahnfelt-Ronne I., Elmgreen J.: *Pharmacol. Toxicol. (Copenhagen)* **1988**, *62*, 322.
24. Raghoebar M., Tiemessen H. L., van den Berg W. B., van Ginneken C. A.: *Pharmacology* **1989**, *39*, 350.
25. Berradia N., Marchand-Arvier M., Humbert J. C., Vigneron C.: *J. Pharm. Pharmacol.* **1988**, *40*, 806.
26. Kankaanranta H., Moilanen E., Vapaatalo H.: *Naunyn–Schmiedeberg's Arch. Pharmacol.* **1994**, *350*, 685.
27. Crowell R. F., van Epps D. E.: *Inflammation* **1990**, *14*, 163.
28. Partsch G., Schwarzer C., Eberl R.: *J. Rheumatol.* **1990**, *17*, 583.
29. Kankaanranta H., Moilanen E., Vapaatalo H.: *Inflammation* **1991**, *15*, 137.
30. Gonzales-Alvaro I., Carmona L., Diaz-Gonzales F., Gonzales-Amaro R., Mollinedo F., Sanchez-Madrid F., Laffon A., Garcia-Vicuna R.: *J. Rheumatol.* **1996**, *23*, 723.
31. Umeki S.: *Biochem. Pharmacol.* **1990**, *40*, 559.
32. Adeyemi E. O., Chadwick V. S., Hodgson J. F.: *J. Pharm. Pharmacol.* **1990**, *42*, 487.
33. Petrusewicz J., Turowski M., Foks H., Pilarski B., Kaliszan R.: *Life Sci.* **1995**, *56*, 667.
34. Lien J. C., Huang L. J., Wang J. P., Teng C. M., Lee K. H., Kuo S. C.: *Bioorg. Med. Chem.* **1997**, *5*, 2111.
35. Andreani A., Leoni A., Locatelli A., Morigi R., Rambaldi M., Gehret J. C., Traniello S., Cariani A., Spisani S.: *Collect. Czech. Chem. Commun.* **1999**, *64*, 299.
36. Andreani A., Rambaldi M., Bonazzi D., Lelli G., Bossa R., Galatulas I.: *Eur. J. Med. Chem.* **1984**, *19*, 219.
37. Andreani A., Rambaldi M., Bonazzi D., Bossa R., Galatulas I.: *Arch. Pharm. (Weinheim, Ger.)* **1985**, *318*, 1003.

38. Olah G. A., Narang S. C., Gupta B. G. B., Malhotra R.: *J. Org. Chem.* **1979**, *44*, 1247.
39. Zigmond S. H., Hirsch J. G.: *J. Exp. Med.* **1973**, *137*, 387.
40. Torrini I., Pagani Zecchini G., Paglialunga Paradisi M., Lucente G., Mastropietro G., Gavuzzo E., Mazza F., Pochetti G., Traniello S., Spisani S.: *Biopolymers* **1996**, *39*, 327.
41. Bergmeyer H. U. in: *Methods of Enzymatic Analysis* (H. U. Bergmeyer, Ed.), 3rd ed., Vol. 3, p. 118. Verlag Chemie, Weinheim 1983.