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2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole derivatives: New potent inhibitors of fMLP-induced neutrophil chemotaxis

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Abstract—It is well known that both acute and chronic autoimmune inflammatory disorders arise following a breakdown in control of neutrophil activation and recruitment. In the search for new anti-inflammatory agents, we synthesized some new 2-phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole derivatives and tested them in vitro in order to evaluate their ability to interfere with human neutrophil functions. All tested compounds showed strong inhibition of fMLP-OMe-induced chemotaxis, although they appeared unable to block degranulation and the fMLP-OMe-induced respiratory burst, and were inactive in binding experiments.

Inflammation is the immune system's first response to infection or irritation. The white blood cells (leukocytes) extravasate from the capillaries into tissue and continue as phagocytes, picking up bacteria and cellular debris. If the injurious agent persists, or the control of cellular recruitment breaks down, both acute and chronic autoimmune inflammatory disorders, such as asthma, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease, will ensue. In recent years, remarkable efforts have been made in order to clarify the complex regulation pathways involved in acute inflammation, during which neutrophils are the main cells infiltrated. Their recruitment to sites of inflammation depends upon a gradient of locally produced chemotactic factors. The bacterial peptide N-formyl-methionyl-leucyl-phenylalanine (fMLP) has been identified as potent leukocyte chemoattractant.[1,2] It acts by binding classical G-protein-coupled receptors, first identified in 1976 and then classified as high-affinity (FPR) or low-affinity (FPRL1, FPR-like 1) fMLP receptors.[3,4] Downstream of these, a number of signalling systems are activated. The intracellular FPR-signalling cascade includes activation of phosphoinositide 3-Kinases (PI3Ks), phospholipase A (PLA), phospholipase D (PLD) and mitogen activated protein kinases (MAPKs).[5] In recent years, many academics, medicinal chemists and pharmaceutical research divisions have been involved in the search for new molecules able to interfere in neutrophil upregulation in order to exploit their therapeutic potential.

In this context, we recently synthesized a number of pyrazolyl-ureas (see Fig. 1), beginning from the interesting intermediate **1** (see Scheme 1). These compounds inhibited the IL8-induced, but not the fMLP-induced, neutrophil chemotaxis at nanomolar concentration.[6a–c]

Synthetic rearrangement of the same intermediate **1** gave new interesting 2-phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*] pyrazoles, which were preliminarily tested in chemotaxis

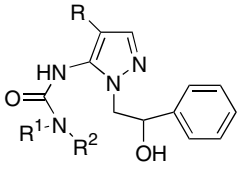

Figure 1. General structure of N-Pyrazolyl-N′alkyl/benzyl/phenyl-ureas, potent inhibitors of IL8-induced neutrophil chemotaxis.[6a–c]

Keywords: 2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazoles; Neutrophil inflammation; Neutrophil chemotaxis; *N*-Formyl-methionyl-leucyl-phenylalanine (fMLP); fMLP receptors (FPRs).
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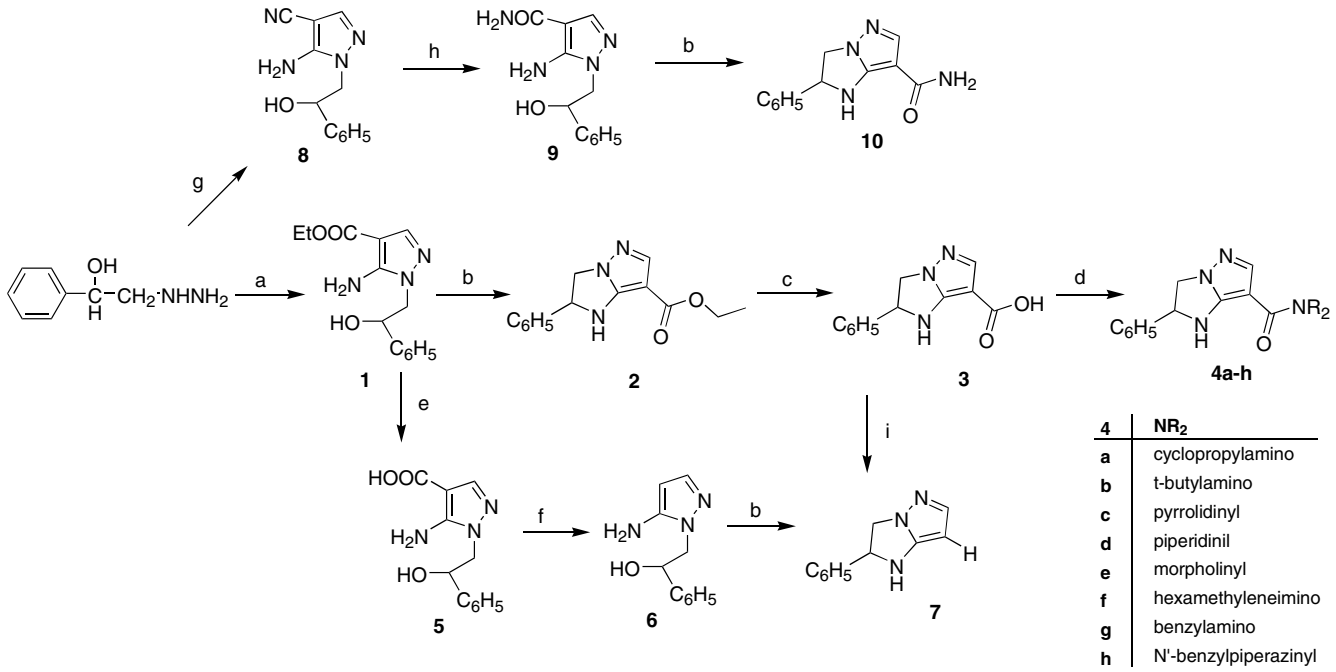

Scheme 1. Synthesis of title compounds. Reagents and conditions: (a) Ethyl ethoxymethylenecyanoacetate, anhyd toluene, 70–80 °C, 8 h; (b) Concd. H_2SO_4, rt, 15 min.; then, aq. NH_3, 0 °C; (c) 2M NaOH, 120 °C, 2h; then, CH_3COOH until pH 5.5; (d) Anhyd. DMF, excess amines, anhyd Et_3N, DPPA, 30–60 °C,12 h; (e) 3.5 M NaOH, ethanol, reflux 4 h then 1M HCl until pH 5.5; (f) Heating at 190 °C until complete development of CO_2; (g) Ethoxymethylenemalononitrile, anhyd ethanol, 70–80 °C, 6 h; (h) 2M NaOH, ethanol/water (50%), reflux, 2 h; (i) Heating at 190 °C until complete development of CO_2.

assays. The positive results obtained in these tests prompted us to develop a new series of 7-substituted derivatives, since no imidazo[1,2-*b*]pyrazoles have yet been reported as anti-inflammatory agents.

Here we report the synthesis of 2-phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazoles **2**, **3**, **4a**–**h**, **7** and **10**, and the results of a preliminary biological study aimed at evaluating their ability to interfere in neutrophil activation and recruitment.

The synthetic methods used to obtain the title compounds are reported in Scheme 1. Compound **1**, obtained from 2-hydrazino-1-phenylethanol with ethyl ethoxymethylenecyanoacetate as previously reported,[7] was treated with concentrated sulfuric acid at 0 °C to give the 2-phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxylic acid ethyl ester (**2**).[8] Compound **3**[9] was prepared by hydrolysis in an alkaline medium of compound **2**. Since compound **2** reacts slightly with primary or secondary amines to give amido derivatives, we prepared compounds **4a**–**h** by reaction of compound **3** in anhydrous dimethylformamide (DMF) with an excess of the suitable amine in the presence of anhydrous triethylamine and diphenylphosphorylazide (DPPA).[10] Several modes of reaction are available to DPPA, depending upon the co-reactant and reaction conditions.[11] In this case, the Curtius rearrangement was not observed because the coupling of the excess amine to the intermediate carboxy-diphenylphosphorazidate prevented formation of the carboxy-azide.

The intermediate compounds **5** and **6** were prepared by hydrolysis and subsequent decarboxylation of compound **1**, as previously reported.[12] Treatment with concentrated sulfuric acid yielded the 2-phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole (**7**).[13] The same compound can be obtained from compound **3** by decarboxylation at high temperature.

Starting from 2-hydrazino-1-phenylethanol, we obtained the intermediate 5-amino-1-(2-hydroxy-2-phenylethyl)-1*H*-pyrazole-4-carbonitrile (**8**) by condensation with ethoxymethylenemalononitrile.[14] Compound **8** was then hydrolysed in an alkaline ethanol/water solution to 5-amino-1-(2-hydroxy-2-phenylethyl)-1*H*-pyrazole-4-carboxamide (**9**),[15] which was finally cyclized to 2-phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxamide (**10**) by the same procedure used for compounds **2** and **7**.[16]

The anti-inflammatory properties of compounds **2**, **3**, **4a**–**h**, **7** and **10** were determined as their ability to inhibit functions such as superoxide anion (O_2^-) production, granule enzyme release and chemotaxis, in neutrophils activated by fMLP-OMe (a synthetic derivative of fMLP endowed with the same chemoattractant activity) following the methods already reported[17] and summarised here.[18a–d]

The antagonist data (percentage activity) were obtained by comparing nmoles of O_2^- production, the percentage of lysozyme released and the chemotactic index (% C.I.) in the absence (100%) and in the presence of the compounds tested. Due to their complete inactivity in superoxide anion production, as well as in lysozyme release, we report here only the results of the influence of increasing concentrations of these compounds on

chemotaxis. These effects are expressed as dose-dependent curves (Fig. 2), induced by 10 nM fMLP-OMe, and as antagonist concentrations inhibiting fMLP-OMe-induced chemotaxis by 50% (IC_{50}) (Table 1). Data were compared with positive (cyclosporine A, CSA)[19,20] and negative (DMSO, blank) controls. Functional experiments ($n = 6$) were calculated by nonlinear regression analysis using the equation for a sigmoid concentration response curve (Graph Pad Prism, San Diego, CA, USA).

In addition, competition binding experiments were carried out[18e] to establish the relative ability of the synthesized compounds to compete for [^{3}H]-fMLP binding, following previously reported methods.[21]

The inhibitory binding constant, K_i, values were also calculated from the IC_{50} values according to the Cheng and Prusoff equation.[22]

All tested compounds were found to be ineffective as antagonists in superoxide anion production, as well as in granule enzyme release (data not shown).

However, the results of chemotaxis experiments were extremely interesting. In fact, all compounds inhibited fMLP-OMe-induced neutrophil chemotaxis in a dose-dependent manner, as evidenced by the curves in Figure 2.

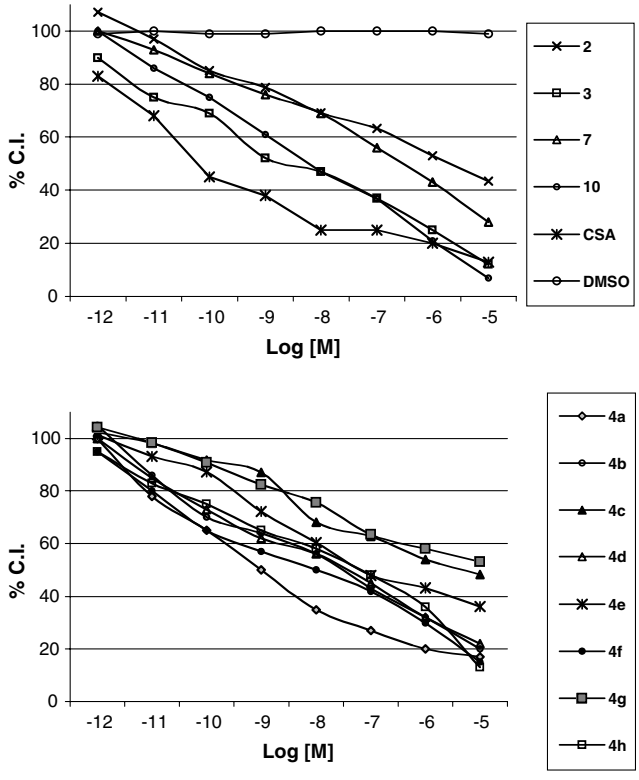

Figure 2. Effect of compounds **2**, **3**, **7**, **10, CSA** (positive control), **DMSO** (negative control) and **4a**–**h** on fMLP-OMe-activated neutrophil chemotaxis. Data are expressed as a percentage of the C.I. (chemotactic index). Each value represents the mean of six separate experiments carried out in duplicate. SEs are within 10% of the mean value.

Table 1. IC_{50} values of compounds **2**, **3**, **4a**–**h**, **7**, **10**, **CSA** (positive control) and **DMSO** (negative control) in fMLP-OMe-induced chemotaxis

Compound	IC_{50} (nM)
2	2400 ± 220
3	1.16 ± 0.10
7	29 ± 3
10	3.67 ± 0.42
4a	0.48 ±0.05
4b	7.52 ± 0.72
4c	9000 ± 850
4d	1.48± 0.15
4e	300 ± 32
4f	1.01 ± 0.09
4g	10,000 ± 1500
4h	27 ± 2
CSA	0.047 ± 0.006
DMSO	>10,000

The most active compounds in this respect were found to be **4a**, **4f**, **4d**, **3** and **10**, with IC_{50}s of 0.48, 1.01, 1.48, 1.16 and 3.67 nM, respectively (Table 1). On the other hand, compounds **4b**, **4h** and **7** were also shown to be active, with IC_{50}s ranging from 7.52 to 29 nM. An order of potency of **4e** (300 nM) > **2** > **4c** > **4g** (10,000 nM) was found for the remaining compounds.

In binding studies, fMLP-OMe was found to be the most potent compound ($K_i = 42 \pm 5$ nM), but not all of the compounds tested were efficacious in displacing [^{3}H]-fMLP from its specific binding sites (Table 2). This is not surprising if we hypothesise that the formylpeptide receptor undergoes conformational changes dependent on the type of cellular response that it must evoke.[23] In addition, it has long been known that the transduction pathway underlying the chemotactic response is different from those responsible for O_2^- production or lysozyme release.[24,25] However, the existence of at least three formylpeptide receptor subtypes has been demonstrated in humans. It has also been reported that some of these receptor subtypes show different affinity values for fMLP[26,27] so we can theorize that the different antagonist activity exerted by the molecules towards the various neutrophil responses could be the consequence of their interaction with different states and/or different subtypes of the FPR. There are many compounds which are able to inhibit neutrophil responses, and these act by impairing some of the different steps in these transduction pathways.[28] However, the major limitation in their use as therapeutic agents for the treatment of inflammation-related diseases is that these molecules are not selective and may inhibit other cellular responses at the same time. Nevertheless, the

Table 2. [^{3}H]-fMLP competition binding experiments on human neutrophils using the tested compounds **2**, **3**, **4a**–**h**, **7** and **10.** Data are taken from a series of three independent experiments

Compound	K_i (nM)
fMLP	42 ± 5
2, **3**, **4a**–**h**, **7**, **10**	>10,000

Non-specific binding was determined in the presence of 10 μl fMLP.

development of receptor antagonists of neutrophil stimulators, able to transiently inhibit cellular responses, should improve knowledge about leukocyte chemoattractant functions and could be of clinical relevance. Additional studies are therefore planned to further explore this topic.

Little information on SAR can be obtained owing to the small number of compounds. At the moment it seems reasonable to affirm that the presence of a tertiary amide or carboxyethyl ester in position 7 results in less active compounds (see compounds **4c**, **4e** and **2**), with compound **4d** (piperidinyl-amide) as the exception. The introduction in the amide group of a benzyl is detrimental (see compound **4g**) but, if a piperazine is employed as a spacer between the benzyl and carbonyl groups (see compound **4h**), only a slight reduction in activity is observed. On the other hand, the steric hindrance of the substituent in position 7 is not a decisive factor in determining the activity, as the most active compounds (**4a** and **4f**) show.

In conclusion, further investigation is required as many structural modifications have been planned in order to obtain more information for SAR studies.

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8. 2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxylic acid ethyl ester. (**2**) Preparation: Compound **1** (2.7 g, 10 mmol) was dissolved in concentrated sulfuric acid (10 mL) at 0 °C and the mixture was stored at room temperature for 15 min. Then, iced water (600 mL) was added and the solution was made neutral with NH_4OH solution; the white solid obtained was filtered, washed with water and recrystallized from absolute ethanol. Yield: 80%. mp 162–163 °C. IR (KBr) cm^{-1}: 3325 (NH), 1660 (CO). ^1H NMR ($CDCl_3$): δ 1.32 (t, $J = 7.0$, 3H, CH_3CH_2), 4.01 (near t, 1H, H_3), 5.05 (br s, 1H, NH, disappears with D_2O), 5.47 (near t, 1H, H_2), 7.27–7.45 (m, 5H, Ar), 7.72 (s, 1H, H_6). Anal calcd for $C_{14}H_{15}N_3O_2$: C, 65.35; H, 5.88; N, 16.33. Found: C, 65.35; H, 5.88; N, 16.43.
9. 2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxylic acid (**3**). Preparation: A solution of compound **2** (2.57 g, 10 mmol) in 2 M NaOH (30 mL) was stirred at 120 °C for 4 h. After cooling, iced water was added and then acetic acid was added until pH 5. The ivory solid obtained was filtered, washed with water and recrystallized from $CHCl_3$/absolute ethanol (1:1). Yield: 1.81 g (79%). mp 188–189 °C. IR (KBr) cm^{-1}: 3400–2500 (OH, NH), 1650 (CO). ^1H NMR (DMSO-d_6): δ 3.8 (near t, 1H, H_3), 4.61 (near t, 1H, H_3), 5.40–5.50 (m, 2H, H_2 + NH, 1H disappears with D_2O), 7.30–7.50 (m, 5H, Ar), 7.54 (s, 1H, H_6), 11.50–12.00 (br s, 1H, COOH, disappears with D_2O). Anal calcd for $C_{12}H_{11}N_3O_2$: C, 62.87; H, 4.84; N, 18.33. Found: C, 62.81; H, 5.08; N, 18.43.
10. 2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazoles 7-substituted **4a**–**h**. General procedure: To a solution of compound **3** (2.3 g, 10 mmol) in anhyd DMF (10 mL) the suitable amine (20 mmol), triethylamine (2 mL) and DPPA (3.4 g, 12 mmol) were added. The mixture was stirred at 30–60 °C for 12 h. After cooling, iced water (200 mL) was added and the solution was made acidic with 1 M HCl. The crude solids obtained were filtered, washed with water and, if necessary, purified by chromatography on neutral Al_2O_3 ($CHCl_3$ as eluent). Finally, the white solids obtained were recrystallized from absolute ethanol.
2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxylic acid cyclopropylamide (**4a**): Yield: 71%. mp 205–206 °C. IR (KBr) cm^{-1}: 3290 (NH), 1630 (CO). ^1H NMR ($CDCl_3$): δ 0.56 (d, $J = 6.0$, 2H, CH_2-cycl), 0.76 (d, $J = 6.0$, 2H, CH_2-cycl), 2.67–2.81 (m, 1H, CH-cycl), 3.94, (near t, 1H, H_3), 4.51 (near t, 1H, H_3), 5.42 (near t, 1H, H_2), 5.47 (br s, 1H, *NH*CO, disappears with D_2O), 5.93 (br s, 1H, NH), 7.22–7.56 (m, 6H, 5Ar + H_6), Anal calcd for $C_{15}H_{16}N_4O$: C, 67.15; H, 6.01; N, 20.88. Found: C, 66.86; H, 6.14; N, 20.87.
2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxylic acid *tert*-butylamide (**4b**):Yield: 51%. mp 195–197 °C. IR (KBr) cm^{-1}: 3220 (NH), 1640 (CO). ^1H NMR ($CDCl_3$): δ 1.44 (s, 9H, $3CH_3$), 3.96 (near t, 1H, H_3), 4.52 (near t, 1H, H_3), 5.20 (br s, 1H, *NH*CO, disappears with D_2O), 5.40–5.50 (m, 2H, H_2 + NH, 1H disappears with D_2O), 7.26–7.40 (m, 5H, Ar), 7.43 (s, 1H, H_6). Anal calcd for $C_{16}H_{20}N_4O$: C, 67.58; H, 7.09; N, 19.70. Found: C, 67.41; H, 7.08; N, 19.43.
Pyrrolidin-1-yl-(2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazol-7-yl)methanone (**4c**): Yield: 50%. mp 198–200 °C. IR (KBr) cm^{-1}: 3280 (NH), 1600 (CO). ^1H NMR ($CDCl_3$): δ 1.89–2.03 (m, 4H, $2CH_2$-pyrr), 3.58–3.74 (m, 4H, $2CH_2N$-pyrr), 3.98 (near t, 1H, H_3), 4.55 (near t, 1H, H_3), 5.43–5.58 (m, 2H, H_2 + NH, 1H disappears with D_2O), 7.26–7.40 (m, 5H, Ar), 7.57 (s, 1H, H_6). Anal calcd for $C_{16}H_{18}N_4O$: C, 68.06; H, 6.43; N, 19.84. Found: C, 67.87; H, 6.61; N, 20.02.
Piperidin-1-yl-(2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*] pyrazol-7-yl)methanone (**4d**): Yield: 52%. mp 198–200 °C. IR (KBr) cm^{-1}: 3230 (NH), 1600 (CO). ^1H NMR ($CDCl_3$): δ 1.58–1.64 (m, 6H, $3CH_2$-pip), 3.60–3.65 (m, 4H, $2CH_2N$-pip), 3.95 (near t, 1H, H_3), 4.53 (near t, 1H, H_3), 5.40–5.52

(m, 2H, H_2 + NH, 1H disappears with D_2O), 7.28–7.40 (m, 5H, Ar), 7.45 (s, 1H, H_6). Anal calcd for $C_{17}H_{20}N_4O$: C, 68.89; H, 6.80; N, 18.90. Found: C, 69.02; H, 6.90; N, 19.11.

Morpholin-4-yl-(2-phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]-pyrazol-7-yl)methanone (**4e**): Yield: 65%. mp 202–204 °C. IR (KBr) cm^{-1}: 3230 (NH), 1610 (CO). ^1H NMR ($CDCl_3$): δ 3.64–3.77 (m, 8H, $4CH_2$-morph.), 3.96 (near t, 1H, H_3), 4.54 (near t, 1H, H_3), 5.40–5.52 (m, 1H, H_2) 5.58 (br s, 1H, NH, disappears with D_2O), 7.28–7.40 (m, 5H, Ar), 7.44 (s, 1H, H_6). Anal calcd for $C_{16}H_{18}N_4O_2$: C, 64.41; H, 6.08; N, 18.78. Found: C, 64.10; H, 6.09; N, 18.89.

Azepan-1-yl-(2-phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazol-7-yl)methanone (**4f**): Yield: 74%. mp 142–143 °C. IR (KBr) cm^{-1}: 3260 (NH), 1600 (CO). ^1H NMR ($CDCl_3$): δ 1.6–1.85 (m, 8H, $4CH_2$-hex), 3.50–3.80 (m, 4H, $2CH_2N$-hex), 3.98 (near t, 1H, H_3), 4.55 (near t, 1H, H_3), 5.40–5.55 (m, 2H, H_2 + NH, 1H disappears with D_2O), 7.26–7.41 (m, 5H, Ar), 7.49 (s, 1H, H_6). Anal calcd for $C_{18}H_{22}N_4O$: C, 69.65; H, 7.14; N, 18.05. Found: C, 69.45; H, 7.06; N, 17.89.

2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole-7-carboxylic acid benzylamide (**4g**): Yield: 48%. mp 232–234 °C. IR (KBr) cm^{-1}: 3300 (NH), 1630 (CO). ^1H NMR ($CDCl_3$): δ 3.98 (near t, 1H, H_3), 4.50–4.65 (m, 3H, H_3 + CH_2Ar), 5.35 (br s, 1H, NH, disappears with D_2O), 5.40–5.54 (m, 1H, H_2), 5.90 (br s, 1H, *NH*CO, disappears with D_2O), 7.27–7.40 (m, 10H, Ar), 7.48 (s, 1H, H_6). Anal calcd for $C_{19}H_{18}N_4O$: C, 71.68; H, 5.70; N, 17.60. Found: C, 71.55; H, 5.76; N, 17.69.

(4-Benzyl-piperazin-1-yl)-(2-phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazol-7-yl)methanone (**4h**): Yield: 75%. mp 161–162 °C (dec). IR (KBr) cm^{-1}: 3270 (NH), 1590 (CO). ^1H NMR ($CDCl_3$): δ 2.49–2.51 (m, 4H, $2CH_2$-pip), 3.55 (s, 2H, CH_2Ar), 3.68–3.80 (m, 4H, $2CH_2N$-pip), 3.97 (near t, 1H, H_3), 4.55 (near t, 1H, H_3), 5.34–5.50 (m, 2H, H_2 + NH, 1H disappears with D_2O), 7.30–7.40 (m, 10H, Ar), 7.45 (s, 1H, H_6). Anal calcd for $C_{23}H_{25}N_5O$: C, 71.29; H, 6.50; N, 18.02. Found: C, 70.97; H, 6.57; N, 18.01.

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13. 2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole (**7**). Method b: Starting from compound **6** (2.03 g, 10 mmol), the same procedure for compound **2** was used. Yield: 65%. Method i: Compound **3** was heated at 190 °C until complete development of CO_2. The crude was dissolved in $CHCl_3$, washed twice with saturated $NaHCO_3$ solution and dried ($MgSO_4$). After solvent evaporation, the pale solid obtained was recrystallized from absolute ethanol. Yield: 74%. mp 136 °C. IR (KBr) cm^{-1}: 3170 (NH). ^1H NMR ($CDCl_3$) : δ 3.89 (near t, 1H, H_3), 4.12 (br s, 1H, NH, decreases with D_2O), 4.45 (near t, 1H, H_3), 5.25 (near t, 1H, H_2), 5.33 (d, J = 1.8, 1H, H_7), 7.16–7.40 (m, 6H, 5Ar + H_6). Anal. calcd for $C_{11}H_{11}N_3$: C, 71.33; H, 5.99; N, 22.69. Found: C, 71.49; H, 5.87; N, 22.73.
14. 5-Amino-1-(2-hydroxy-2-phenylethyl)-1*H*-pyrazole-4-carbonitrile (**8**). Preparation: To a solution of 2-hydrazino-1-phenylethanol (6.08 g, 40 mmol) in absolute ethanol (50 mL), ethoxymethylenemalononitrile (4.88 g, 40 mmol) was added and the reaction mixture was refluxed for 6 h. The solvent was concentrated until 50% of the initial volume and cooled. The yellow solid obtained was filtered and recrystallized from absolute ethanol. Yield: 63%. mp 180–181 °C. IR (KBr) cm^{-1}: 3438, 3339, 3199 (OH, NH_2), 2223 (CN). ^1H NMR ($CDCl_3$): δ 3.15 (br s, 1H, OH, disappears with D_2O), 3.98–4.27 (m, 2H, CH_2N), 4.80 (br s, 2H, NH_2, disappears with D_2O), 5.08–5.18 (m, 1H, *CH*OH), 7.26–7.45 (m, 5H, Ar), 7.46 (s, 1H, H_3). Anal. calcd for $C_{12}H_{12}N_4O$: C, 63.15; H, 5.30; N, 24.55. Found: C, 62.99; H, 5.25; N, 24.60.
15. 5-Amino-1-(2-hydroxy-2-phenylethyl)-1*H*-pyrazole-4-carboxamide (**9**). Preparation: To a solution of compound **8** (2.28 g, 10 mmol) in absolute ethanol (10 mL) 2M NaOH (10 mL) was added, and the mixture was refluxed for 2 h. After cooling, the white solid obtained was filtered, washed with water and recrystallized from ethanol. Yield: 65%. mp 228 °C. IR (KBr) cm^{-1}: 3389 (OH), 3299, 3169 (NH_2), 1643 (CO) ^1H NMR ($CDCl_3$): δ 3.80–3.90 (m, 2H, CH_2N), 4.80–4.90 (m, 1H, *CH*OH), 5.61 (d, J = 4, 1H, OH, disappears with D_2O), 5.98 (s, 2H, NH_2, disappears with D_2O), 6.60 (br s, 2H, $CONH_2$, disappears with D_2O), 7.10–7.35 (m, 5H, Ar), 7.56 (s, 1H, H_3). Anal. calcd for $C_{12}H_{14}N_4O_2$: C, 58.53; H, 5.73; N, 22.75 Found: C, 58.55; H, 5.66; N, 22.94.
16. 2-Phenyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole 7-carboxamide (**10**). Preparation: Starting from compound **9** (2.46 g, 10 mmol), the same procedure for compound **2** was used. Yield: 41%. mp 248–249 °C. IR (KBr) cm^{-1}: 3450, 3156 (NH, NH_2), 1665 (CO). ^1H NMR ($CDCl_3$) δ: 3.67 (near t, 1H, H_3), 4.46 (near t, 1H, H_3), 5.31 (near t, 1H, H_2), 6.70 (br s, 1H, NH, disappears with D_2O), 6.95 (s, 2H, $CONH_2$, disappears with D_2O), 7.21-7.40 (m, 5H, Ar), 7.56 (s, 1H, H_6). Anal. calcd for $C_{12}H_{12}N_4O$. H_2O: C, 58.53; H 5.70; N, 22.75. Found: C, 58.63; H, 5.40; N, 22.67.
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8. (a) *Neutrophil preparation:* Neutrophils were obtain from the blood of healthy subjects and cells were purified employing standard techniques, and were resuspended in Krebs-Ringer-phosphate containing 0.1% w/v glucose (KRPG), pH 7.4.; (b) *Random locomotion and chemotaxis* were evaluated using a 48-well microchemotaxis chamber (BioProbe, Milan Italy), estimating the distance in micrometres which the leading edge of the cell migrated.; (c) O_2^- *production* was measured by superoxide dismutase-inhibitable reduction of ferricytochrome c, modified for microplated-based assays.; (d) *Release of neutrophil granule enzymes* was evaluated by determining lysozyme activity, modified for microplate-based assays. Lysozyme was quantified nephelometrically by the rate of lysis of a cell wall suspension of *Micrococcus lysodeikticus*.; (e) *Receptor binding assays:* In competition experiments, carried out to determine K_i values, 10 nM of [^{3}H]fMLP was incubated with 100 µl of human neutrophils (5×10^6) and at least 6–8 different concentrations of the examined compounds at 37 °C for 15 min. Non-specific binding was measured in the presence of 10 µM fMLP and was about 20% of total binding. Bound and free radioactivity was separated by rapid filtration through Whatman GF/C glass-filters, which were washed with ice-cold buffer. The filter-bound radioactivity was measured by scintillation spectrometer with an efficiency of 57%. (f) *Preparation of the tested compounds*: 10^{-2} M in dimethylsulfoxide (DMSO) of fMLP-OMe and the tested compounds were diluted in buffer before use. At the concentrations used, DMSO did not interfere with any of the biological assays performed.
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