Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3696–3701

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

Olga Bruno,[a,*] Chiara Brullo,[a] Francesco Bondavalli,[a] Angelo Ranise,[a] Silvia Schenone,[a] Maria Sofia Falzarano,[b] Katia Varani[c] and Susanna Spisani[b]

[a]*Dipartimento di Scienze Farmaceutiche, Università di Genova, Viale Benedetto XV, 3, 16132, Genova, Italy*
[b]*Dipartimento di Biochimica e Biologia Molecolare, Università degli Studi di Ferrara, via Luigi Borsari, 46, 44100 Ferrara, Italy*
[c]*Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Ferrara, Via Fossato di Mortara 17/19, 44100 Ferrara, Italy*

Received 14 February 2007; revised 11 April 2007; accepted 12 April 2007
Available online 19 April 2007

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

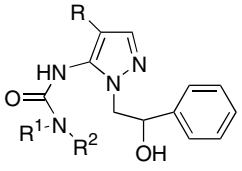
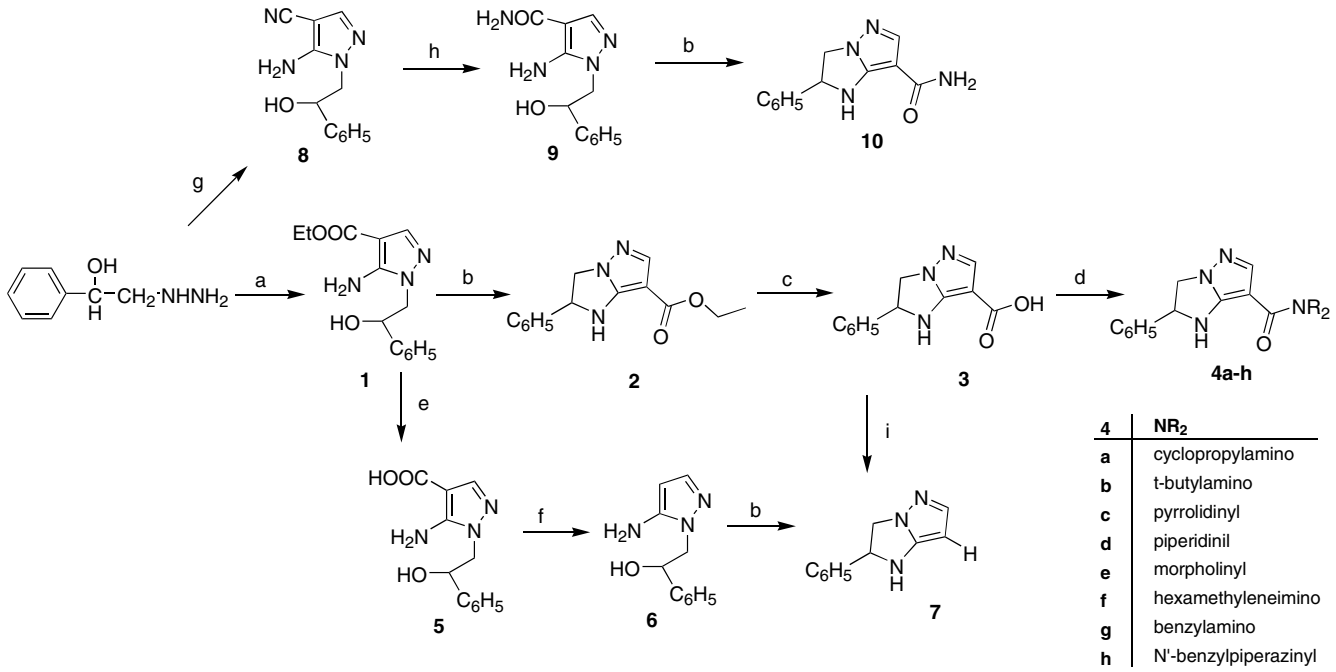


Figure 1. General structure of N-Pyrazolyl-N′alkyl/benzyl/phenyl-ureas, potent inhibitors of IL8-induced neutrophil chemotaxis.[6a–c]

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).
* Corresponding author. Tel.: +39 010 3538367; fax: +39 010 3538358; e-mail: obruno@unige.it

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.04.036



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