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Synthesis of *N*-Pyridinyl(methyl)-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamides and analogues and their anti-inflammatory activity in mice and rats

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Abstract

The topical anti-inflammatory activity of a series of *N*-pyridinyl(methyl)1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamides, analogues of roquinimex, has been evaluated by measuring their inhibitory effect in the phorbol myristate acetate (PMA)-induced mouse ear swelling test, used as a screening test.

All the eight carboxamides tested (**9**–**16**) exhibited significant inhibitory activity at 0.4 and 0.2 mM kg^{-1}. The most potent compound, the 6-bromo derivative **12**, induced a 73% inhibition at 0.2 mM kg^{-1}. Pharmacomodulation was carried out by heterocycle opening and molecular simplification leading to pentafluorobenzoylacetamide **17**, pentafluorocinnamamides **18** and **19**, and pentafluorobenzaldimines **20** and **21**. All the five compounds exerted a reduction in swelling (49–63% at 0.2 mM kg^{-1}) comparable with ibuprofen (56%). Anti-inflammatory activity of the most efficient compounds was evaluated by carrageenan-induced rat paw oedema inhibition. The pentafluorobenzaldimine **20** showed the highest activity with an inhibition percentage of 85% at 0.2 mM kg^{-1}.

Introduction

Classical non-steroidal anti-inflammatory drugs are not always effective at controlling chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease and psoriasis at concentrations compatible with their gastrointestinal and renal toxicity. In these pathologies, tumour necrosis factor-α (TNF-α) has been shown to be one of the main cytokines recruiting activated immune and inflammatory cells to the site of lesions, thereby amplifying and perpetuating the inflammatory state (Brennan et al 1995). That is why, for perhaps the first time since cyclooxygenase, researchers of inflammatory disease have been provided with a validated target on which they could focus their efforts.

A new class of anti-inflammatory compounds that inhibit TNF-α production are the cytokine suppressing anti-inflammatory drugs, typified by the 5-(4-fluorophenyl)-4-(4-pyridinyl)imidazole SKF-86002 (**1**, Figure 1) with an ID50 value of approximately 32 mg kg^{-1} (Black et al 1997).

We described previously the synthesis, anti-inflammatory activity and TNF-α inhibitory activity of *N*-(4,6-dimethylpyridin-2-yl)aryl and heteroarylcarboxamides (Lang et al 1995; Robert et al 1995; Vernhet et al 1997). The furan-2-carboxamide **2** was the most interesting derivative in that series, with an inhibition percentage of 87% at 0.4 mmol kg^{-1} in carrageenan-induced rat paw oedema. It also inhibited

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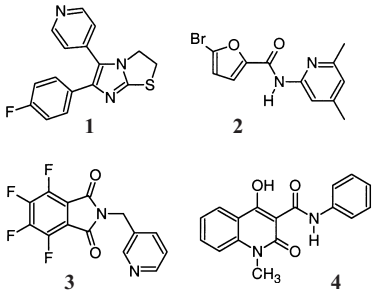
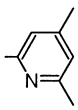
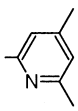
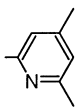
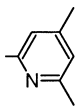
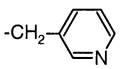
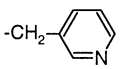
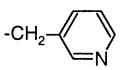
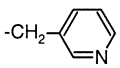


Figure 1 Chemical structures of anti-inflammatory compounds inhibiting TNF-α production.

Table 1 Inhibition of phorbol myristate acetate-induced mouse ear oedema of *N*-pyridinyl(methyl)-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamides **9–16**.

Compound	X	R_1	R	Inhibition (%)[a] Dose (mM·kg^{-1}) 0.2	0.4
9	H	H		62±6	68±4
10	H	CH_3		44±2	60±6
11	Cl	H		60±2	75±2
12	Br	H		73±3	92±4
13	H	H		59±4	80±4
14	H	CH_3		58±6	60±5
15	Cl	H		69±2	80±2
16	Br	H		55±1	73±6
Ibuprofen				56±4	86±6

[a] Inhition is expressed as the mean of five measures.

TNF-α production with an IC50 inferior to that of thalidomide: approximately 70 vs 200 μM (Sampaio et al 1991).

More recently we studied a series of *N*-azaaryl-(alkyl)phthalimides incorporating amino (alkyl)pyridines (Collin et al 1998). *N*-(3-pyridinylmethyl)-tetrafluorophthalimide **3** was found to be the most potent TNF-**a** production inhibitor (IC50 = 6 μM), and the most efficient in-vivo anti-inflammatory compound in phorbol myristate acetate (PMA)-induced mouse ear oedema test (63% at 0.2 mM kg^{-1}) and in carrageenan-induced rat paw oedema (ID50 = 0.14 mmol kg^{-1}).

Roquinimex (**4**), a 1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamide, could represent another possible lead compound for the above purpose. Interest in this TNF-α inhibitor for the treatment of inflammatory bowel disease and psoriasis has been claimed (Anonymous 1997).

On the basis of these findings we have focused on synthesizing a series of *N*-pyridinyl(methyl)-1, 2-di-hydro-4-hydroxy-2-oxoquinoline-3-carboxamides (**9**–**16**, Table 1). Preliminary pharmacomodulation was carried out by nitrogen and homocycle substitution and then by heterocycle opening followed by molecular simplification leading to a β-phenyl-β-ketoamide (**17**), cinnamamides (**18**, **19**) and arylimines (**20**, **21**).

Due to their poor solubility in water and ethanol, the effect of most of these compounds on the in-vitro production of TNF-α by activated peritoneal macrophages could not be quantified as described previously (Collin et al 1998). Their anti-inflammatory activity was then evaluated in PMA-induced mouse ear oedema assay (Carlson et al 1985), considered to be a relevant model of human psoriasis, used as a screening test. The most potent compounds were tested using the carrageenan-induced rat paw oedema model (Winter et al 1962).

Materials and Methods

Chemistry

Melting points were determined on a Tottoli-Büchi apparatus and were uncorrected. Structures were supported by data from IR, ^{1}H NMR and mass spectra. IR spectra were recorded on a Perkin Elmer-Paragon PC 1000 spectrometer as potassium bromide discs. ^{1}H NMR spectra were recorded on a Bruker AC 250 spectrometer(250fs,25MHz)using$CDCl_3$ord$_6$-$(CH_3)_2SO$ as solvent. Chemical shifts refer to tetramethylsilane, which was used as internal reference; coupling constants

are in Hz. Mass spectra were recorded on a double beam Varian Mat 112 spectrometer; ionization energy 70 eV. Analytical TLC was performed on precoated silica-gel aluminium plates (0.2 mm, GF254, E Merck). Spots were located by UV illumination. Sodium sulfate or phosphorus pentoxide was used as the drying agent. Column chromatography was conducted on silica gel (Kieselgel 60, 70–230 mesh, E Merck) and for delicate separations a preparative centrifugally-accelerated thin layer chromatography (Chromatotron 7924 T, Harrisson Research, Palo Alto, CA) was used.

Starting materials were purchased from Aldrich Chimie (St Quentin-Fallavier, France), Acros (Noisy-le-Grand, France) or Interchim (Montluçon, France).

Synthesis of intermediary ethyl quinoline-3-carboxylates

*Ethyl 1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxylate (**5**). Method i.*
Condensation of diethyl malonate and isatoic anhydride in the presence of sodium hydride in dry dimethylformamide led to compound **5** (Hayashi et al 1993; Ismaili 1995). Yield: 62%; mp 205°C (lit. 204°C). IR (KBr), ν (cm^{-1}) 1690 (νC=O ester), 1650 νC=O lactam). ^{1}H NMR ($CDCl_3$) δ 1.53 (t, J = 7.07, 3H, CH_3), 4.53 (q, J = 7.08, 2H, CH_2), 7.19–8.09 (m, 4H, Ar-H), 11.65 (s, 1H, NH), 14.30 (s, 1H, OH).

*Ethyl 1,2-dihydro-4-hydroxy-1-methyl-2-oxoquinoline-3-carboxylate (**6**). Method i.*
Yield: 69%; mp 103°C (lit. 105°C). IR (KBr), ν (cm^{-1}) 1690 (νC=O ester), 1670 (νC=O lactam). ^{1}H NMR ($CDCl_3$) δ 1.43 (t, J = 7.12, 3H, CH_3-CH_2), 3.64 (s, 3H, N-CH_3), 4.48 (q, J = 7.11, 2H, CH_2), 7.21–8.18 (m, 4H, Ar-H), 14.19 (s, 1H, OH).

*Ethyl 6-chloro-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxylate (**7**). Method i.*
Yield: 57%. IR (KBr), ν (cm^{-1}) 1685 (νC=O ester), 1620 (νC=O lactam). ^{1}H NMR ($DMSOd_6$) δ 1.33 (t, J = 7.10, 3H, CH_3-CH_2), 4.36 (q, J = 7.57, 2H, CH_2), 7.32 (d, J = 8.8, 1H, Ar-8H), 7.70 (dd, J = 8.8, 2.3, 1H, Ar-7H), 7.92 (d, J = 2.3, 1H, Pyr-4H), 11.72 (s, 1H, NH), 14.30 (s, 1H, OH).

*Ethyl 6-bromo-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxylate (**8**). Method i.*
Yield: 73%. IR (KBr), ν (cm^{-1}) 1685 (νC=O ester), 1620 (νC=O lactam). ^{1}H NMR ($DMSOd_6$) δ 1.34 (t, J = 7.15, 3H, CH_3-CH_2), 4.34 (q, J = 7.20, 2H, CH_2), 7.27 (d, J = 8.8, 1H, Ar-8H), 7.79 (dd, J = 8.8, 2.4, 1H, Ar-7H), 8.18 (d, J = 2.3, 1H, Pyr-4H), 11.73 (s, 1H, NH), 14.25 (s, 1H, OH).

Synthesis of 1,2-dihydroquinoline-3-carboxamides

N-*(4,6-Dimethylpyridin-2-yl)-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamide (**9**). Method ii.*
A mixture of 2-amino-4,6-dimethylpyridine (0.8 g, 6.6 mmol) and ethyl 1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxylate (**5**, 0.5 g, 2.2 mmol) in xylene (20 mL) was refluxed for 2 h. The solution was cooled in an ice bath for 1 h. The resultant precipitate was collected, washed with xylene, diethyl ether and recrystallized from tetrahydrofurane–hexane (80:20) (0.53 g, 78%); mp 315°C. IR (KBr), ν (cm^{-1}) 1685 (νC=O amide), 1620 (νC=O lactam), 1575 (δNH). EI-MS m/z (%) 309 (M, 9), 122 (100), 95 (15). ^{1}H NMR ($DMSOd_6$) δ 2.36 (s, 3H, 4-CH_3), 2.43 (s, 3H, 6-CH_3), 6.96–8.04 (m, 6H, Ar-H, Pyr-5H, Pyr-3H), 12.18 (s, 1H, OH, CONH or NH), 12.82 (s, 1H, OH, CONH or NH), 15.38 (s, 1H, OH, CONH or NH).

N-*(4,6-Dimethylpyridin-2-yl)-1,2-dihydro-4-hydroxy-1-methyl-2-oxoquinoline-3-carboxamide (**10**). Method ii.*
Yield: 75%; mp 232°C. IR (KBr), ν (cm^{-1}) 1655 (νC=O amide), 1595 (νC=O lactam), 1545 (δNH). EI-MS m/z (%) 322 (M, 58), 122 (100), 95 (18). ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, 4-CH_3), 2.46 (s, 3H, 6-CH_3), 3.70 (s, 3H, N-CH_3), 6.78 (s, 1H, Pyr-5H), 7.85 (s, 1H, Pyr-3H), 7.30–8.20 (m, 4H, Ar-H), 12.80 (s, 1H, OH or CONH), 15.20 (s, 1H, OH or CONH).

N-*(4, 6-Dimethylpyridin-2-yl)-6-chloro-1, 2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamide (**11**). Method ii.*
Yield: 63%; mp 323°C. IR (KBr), ν (cm^{-1}) 1670 (νC=O amide), 1625 (νC=O lactam), 1580 (δNH). EI-MS m/z (%) 345 (M+2, 3), 343 (M, 9), 122 (100), 95 (17). ^{1}H NMR (CF_3COOD) δ 2.69 (s, 3H, 4-CH_3), 2.84 (s, 3H, 6-CH_3), 7.38 (s, 1H, Pyr-5H), 7.48 (d, J = 8.9, 1H, Ar-8H), 7.51 (s, 1H, Pyr-3H), 7.86 (dd, J = 8.7, 2.6, 1H, Ar-7H), 8.34 (d, J = 1.9, 1H, Ar-5H).

N-*(4, 6-Dimethylpyridin-2-yl)-6-bromo-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamide (**12**). Method ii.*
Yield: 65%; mp 328°C. IR (KBr), ν (cm^{-1}) 1690 (νC=O amide), 1620 (νC=O lactam), 1565 (δNH). EI-MS m/z (%) 388 (M+2, 23), 386 (M, 24), 122 (100), 95 (14). ^{1}H NMR (CF_3COOD) δ 2.68 (s, 3H, 4-CH_3), 2.83 (s, 3H, 6-

CH_3), 7.38 (s, 1H, Pyr-5H), 7.41 (m, 1H, Ar-8H), 7.50 (s, 1H, Pyr-3H), 7.98 (m, 1H, Ar-7H), 8.49 (m, 1H, Ar-5H).

N-*(3-Methylpyridinyl)-1, 2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamide (**13**). Method ii.*
Yield: 80 %; mp 267°C. IR (KBr), ν (cm^{-1}) 1660 νC=O amide), 1615 νC=O lactam), 1560 (δNH). EI-MS m/z (%) 295 (M, 100), 188 (19), 161 (27), 107 (100), 92 (29). ^{1}H NMR ($DMSOd_6$) δ 4.66 (d, J = 6.05, 1H, CH_2), 7.29–8.63 (m, 8H, Ar-H, Pyr-H), 10.75 (m, 1H, CONH), 11.92 (s, 1H, OH or NH), 11.94 (s, 1H, OH or NH).

N-*(3-Methylpyridinyl)-1, 2-dihydro-4-hydroxy-1-methyl-2-oxoquinoline-3-carboxamide (**14**). Method ii.*
Yield: 88 %; mp 162°C. IR (KBr), ν (cm^{-1}) 1675 νC=O amide), 1595 (νC=O lactam), 1555 (δNH). EI-MS m/z (%) 309 (M, 20), 292 (12), 175 (49), 107 (95), 92 (15). ^{1}H NMR ($CDCl_3$) δ 3.68 (s, 3H, N-CH_3), 4.67 (d, J = 5.97, 1H, CH_2), 7.29–8.64 (m, 8H, Ar-H, Pyr-H), 10.79 (m, 1H, CONH), 14.90 (s, 1H, OH).

N-*(3-Methylpyridinyl)-6-chloro-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamide (**15**). Method ii.*
Yield: 80 %; mp 266°C. IR (KBr), ν (cm^{-1}) 1660 νC=O amide), 1605 (νC=O lactam), 1560 (δNH). EI-MS m/z (%) 331 (M + 2, 20), 329 (M, 56), 107 (100), 92 (14). ^{1}H NMR ($DMSOd_6$) δ 4.66 (d, J = 6.05, 1H, CH_2), 7.40 (d, J = 8.7, 1H, Ar-8H), 7.42 (m, 1H, Pyr-5H), 7.73 (dd, J = 8.8, 2.4, 1H, Ar-7H), 7.81 (m, 1H, Pyr-4H), 7.93 (d, J = 2.4, 1H, Ar-5H), 8.50 (m, 2H, Pyr-2H, Pyr-6H).

N-*(3-Methylpyridinyl)-6-bromo-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamide (**16**). Method ii.*
Yield: 81 %; mp 264°C. IR (KBr), ν (cm^{-1}) 1690 νC=O amide), 1615 (νC=O lactam), 1565 (δNH). EI-MS m/z (%) 374 (M + 2, 33), 372 (M, 32), 107 (100). ^{1}H NMR (CF_3COOD) δ 5.05 (m, 1H, CH_2), 7.47 (m, 1H, Ar-8H), 7.93 (m, 1H, Ar-7H), 8.15 (m, 1H, Pyr-5H), 8.43 (m, 1H, Ar-7H), 8.76 (m, 1H, Pyr-4H), 8.82–9.01 (m, 2H, Pyr-2H, Pyr-6H).

Synthesis of β-ketoamide 17

N-*(4,6-Dimethylpyridin-2-yl) pentafluorobenzoylacetamide (**17**). Method iii.*
2-Hydroxypyridine (0.28 g, 2.9 mmol) was added to a solution of 2-amino-4,6-dimethylpyridine (1.7 g, 14 mmol) and ethyl pentafluorobenzoylacetate (1.4 mL, 7 mmol) in dry toluene (30 mL). The mixture was refluxed for 6 h. The solvent was removed under reduced pressure. Chromatography of the crude oily product on a preparative centrifugally-accelerated thin layer chromatograph using dichloromethane–ethanol (95:5) as eluent yielded 2.08 g (83 %) of pure compound; mp 134°C. IR (KBr), ν (cm^{-1}) 3280 (νOH), 3220 (νNH), 1660 (νC=O), 1530 (δNH), 1235 (combined NH/CN). EI-MS m/z (%) 358 (M, 100), 195 (21), 163 (21), 122 (100), 106 (12). ^{1}H NMR ($DMSOd_6$) δ 2.29 (s, 3H, 4-CH_3), 2.33 (s, 3H, 6-CH_3), 4.17 (s, 1H, CH-CO), 6.10 (s, 1H, Pyr-5H), 7.80 (s, 1H, Pyr-3H), 10.73 (s, 1H, CONH), 11.56 (s, 1H, OH).

Synthesis of cinnamamides

N-*(4,6-Dimethylpyridin-2-yl)pentafluorocinnamamide (**18**). Method iv.*
A solution of pentafluorocinnamic acid (1.36 g, 5.7 mmol), 2-amino-4,6-dimethylpyridine (0.69 g, 5.7 mmol) and triethylamine (2.4 mL, 17.1 mmol) in dry 1,2-dichloroethane (60 mL) was cooled in an ice bath. Phenyl dichlorophosphate (0.85 mL, 5.7 mmol) was added dropwise. The mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure. The residue was purified by column chromatography using dichloromethane–ethanol (98:2) as eluent. Crystallization from diisopropyl ether afforded 1.81 g of pure product. Yield: 81 %; mp 154°C. IR (KBr), ν (cm^{-1}) 3420 (νNH), 1700 (νC=O), 1535 (δNH), 1430 (combined NH/CN). EI-MS m/z (%) 342 (M, 100), 221 (28), 193 (60), 147 (83), 122 (55), 77 (9). ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, 4-CH_3), 2.43 (s, 3H, 6-CH_3), 6.80 (s, 1H, Pyr-5H), 6.84 (d, J = 15.8, 1H, C = CH-CO), 7.74 (d, J = 15.9, 1H, CH=C-CO),7.98 (s, 1H, Pyr-3H), 8.66 (s, 1H, CONH).

N-*(3-Methylpyridinyl)pentafluorocinnamamide (**19**). Method iv.*
Yield: 75 %; mp 94 °C. IR (KBr), ν (cm^{-1}) 3305 (νNH), 1670 (νC=O), 1535 (δNH), 1430 (combined NH/CN). EI-MS m/z (%) 328 (M, 100), 221 (84), 193 (80), 107 (53), 92 (25). ^{1}H NMR ($CDCl_3$) δ 4.50 (s, 1H, CH_2), 6.72 (d, J = 16.0, 1H, C=CH-CO), 6.96 (s, 1H, CONH), 7.22 (m, 1H, Pyr-5H), 7.57 (d, J = 16.0, 1H, CH=C-CO), 7.65 (m, 1H, Pyr-4H), 8.43 (m, 2H, Pyr-6H, Pyr-2H).

Synthesis of imines

N-*(3-Methylpyridinyl)pentafluorobenzaldimine (**21**). Method v.*
Sodium sulfate (5 g, 35 mmol) was added to a solution of pentafluorobenzaldehyde (3.92 g, 20 mmol) and 3-

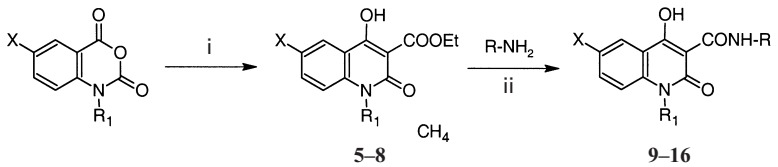


Figure 2 Synthetic pathway to *N*-pyridinyl(methyl)1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamides. Reagents: i. diethyl malonate, sodium hydride, DMF, 130°C, 2.5 h; ii. xylene, reflux, 2 h.

(aminomethyl)pyridine (2.03 mL, 20 mmol) in dry 1,2-dichloroethane (15 mL). The mixture was refluxed for 3 h. The solvent was removed under reduced pressure. The solid collected was recrystallized from ethyl acetate (4.8 g, 84%); mp 98°C. IR (KBr), ν (cm^{-1}) 1665 (νC=N). EI-MS m/z (%) 286 (M, 17), 180 (10), 119 (62), 92 (100), 65 (60), 39 (30). ^{1}H NMR ($CDCl_3$) δ 4.91 (s, 1H, CH_2), 7.30 (m, 1H, Pyr-5H), 7.70 (m, 1H, Pyr-4H), 8.54 (m, 1H, Pyr-6H), 8.56 (s, 1H, CH=N), 8.60 (m, 1H, Pyr-2H).

Method vi.
A solution of pentafluorobenzaldehyde (3.92 g, 20 mmol) and 3-(aminomethyl)pyridine (2.03 ml, 20 mmol) in benzene (30 mL) was refluxed for 6 h using a Dean-Stark trap to separate water. After removal of the solvent under reduced pressure, the crude oily imine was dissolved in the minimum of ethyl acetate and crystallized by slow addition of petroleum ether. Beige crystals (5.4 g; 95%) were obtained.

N-*(4,6-Dimethylpyridin-2-yl)pentafluorobenzaldimine* (***20***). *Method vi.*
Yield: 72%; mp 86°C. IR (KBr), ν (cm^{-1}) 1605 (νC=N). EI-MS m/z (%) 300 (M, 10), 107 (100). ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, 4-CH_3), 2.53 (s, 3H, 6-CH_3), 6.95 (s, 1H, Pyr-5H), 7.02 (s, 1H, Pyr-3H), 9.31 (s, 1H, CH=N).

Pharmacology

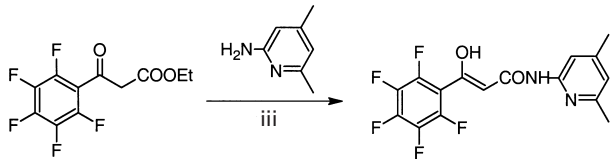
Acute phorbol ester-induced mouse ear-swelling test.
Introduction of mouse ear oedema was based on the method of Carlson et al (1985) with some modifications. Groups of five male Swiss mice (20–25 g) were fasted 24 h before the experiments and maintained in suitable environmental conditions throughout the experiments. Phorbol-12-myristate-13-acetate (PMA) was dissolved in aqueous ethanol 80% at a concentration of 250 μg mL^{-1} and 10 μL was applied topically to the anterior and posterior surfaces on the right ear of each mouse. Left ear (control) received the vehicle (10 μL aqueous ethanol 80%). The compounds under study were orally administered 1 h before PMA application (treated animals). Ear thickness was measured with a model micrometer gauge (Oditest Kroeplin) 3.5 h after PMA treatment. Ear oedema was calculated by subtracting the thickness of the left ear (vehicle) from the thickness of the right ear (treatment) and was expressed as an increase in ear thickness. The percentage of inhibition of the inflammatory reaction was determined for each animal by comparison between ear oedema of treated and non-treated animals.

Carrageenan-induced rat paw oedema.
Anti-inflammatory activity against carrageenan-induced rat paw oedema was assayed in adult male Wistar CF rats (180–220 g) according to the method of Winter et al (1962) with slight modifications. The drugs were orally administered 1 h before injection of 0.05 mL of a 1% suspension of carrageenan saline into the subcutaneous tissue of one hind paw. The other hind paw was injected in the same way with 0.05 mL of a saline solution. Rats were fasted 24 h before the experiment and water (1.5 mL/100 g body weight) was orally administered twice before injections (20 h and 4 h). The volume of both hind paws of the control and treated animals was measured with a plethysmograph, 3 h after injection. Rats were kept in the same experimental conditions. The inhibition percentage of the inflammatory reaction was determined for each animal by comparison with controls, and calculated by the formula $I(\%) = 100 \times (1 - dt/dc)$, where dt is the difference in paw volume in the drug-treated group and dc the difference in paw volume in the control group.

Results and Discussion

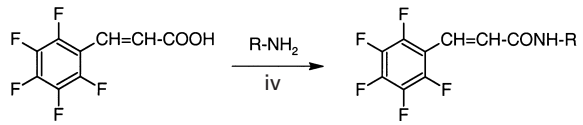
Chemistry

N-Pyridinyl(methyl)-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamides (**9–16**) were prepared by aminolysis of the corresponding esters in refluxing xylene, as outlined in Figure 2 and according to methods described by Clemence et al (1988). As previously ob-



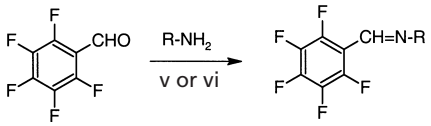
17

Figure 3 Synthesis of *N*-(3-methylpyridinyl)β-ketoamide **17**. Reagent: iii. 2-hydroxypyridine, toluene, reflux, 6 h.



18, 19

Figure 4 Synthesis of *N*-pyridinyl(methyl)cinnamamides. Reagent: iv. phenyl dichlorophosphate, TEA, 1.2-dichloroethane, room temperature, 24 h.



20, 21

Figure 5 Synthesis of *N*-pyridinyl(methyl)benzaldimines. Reagents: v. sodium sulfate, 1,2-dichloroethane, reflux, 3 h; vi. benzene, Dean Stark, reflux, 6 h.

Table 2 Inhibition of phorbol myristate acetate-induced mouse ear oedema of *N*-pyridinyl(methyl)amides **17–19** and imines **20** and **21**.

Compound	R	Inhibition (%)[a] Dose (mM·kg^{-1}) 0.2	0.4
17	-CO-CH$_2$-CONH-	63±3	82±2
18	-CH=CH-CONH-	60±3	86±1
19	-CH=CH-CONH-CH$_2$-	62±4	79±4
20	-CH=N-	52±9	79±3
21	-CH=N-CH$_2$-	49±6	78±5
Ibuprofen		56±4	86±6

[a] Inhibition is expressed as the mean ± of five measures.

served (Collin et al 1999), yields were ruled by nucleophilicity of the starting amines: β-picolylamine afforded compounds **13–16** in 80–88 % yields whereas 6-amino-2,4-lutidine led to compounds **9–12** in only 63–78 % yields. The synthesis of intermediary ethyl 1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxylates (**5–8**) was carried out by condensing corresponding isatoic anhydrides with diethyl malonate in the presence of sodium hydride (Hayashi et al 1993; Ismaili 1995).

Aminolysis of ethyl pentafluorobenzoylacetate in refluxing toluene in presence of 2-hydroxypyridine as a bifunctional catalyst (Openshaw & Whittaker 1968) led to β-ketoamide **17** in good yield (83 %) (Figure 3). For this compound, ^{1}H NMR data were in favour of a preponderant (> 95 %) enol structure.

N-Pyridinyl(methyl)pentafluorocinnamamides (**18** and **19**) were synthesized by condensation of corresponding acids with appropriate amines, as outlined in Figure 4, using the method which afforded the best results in previous studies (Collin et al 1999): phenyl dichlorophosphate activation of the carboxylic acid in the presence of triethylamine in 1,2-dichloroethane, at room temperature.

N-Pyridinyl(methyl)pentafluorobenzaldimines (**20** and **21**) were obtained by reacting pentafluorobenzaldehyde with appropriate amines (Figure 5). The use of a Dean Stark to separate formed water provided compound **21** in better yield (95 %) than the sodium sulfate method (84 %).

Pharmacology

All the quinoline-3-carboxamides exhibited significant activity in the PMA-induced ear swelling test at 0.4 and 0.2 mM kg^{-1} (Table 1. *N*-Methylation led to less active molecules **10** and **14**. Among the four 7-halogenated derivatives (**11**, **12**, **15** and **16**), **12** was the most potent (inhibition percentage of 73 % at 0.2 mM kg^{-1} and 92 % at 0.4 mM kg^{-1}), showing higher activity than ibuprofen (56 % at 0.2 mM kg^{-1} and 86 % at 0.4 mM kg^{-1}). In the series of *N*-pyridinyl(methyl)amides and imines incorporating a pentafluorophenyl moiety (Table 2), all

Table 3 Inhibition of carrageenan-induced rat paw oedema by the most potent compounds (0.2 mM kg^{-1}).

Compound	Inhibition (%)[a]
12	15 ± 8
13	52 ± 2
15	45 ± 4
17	36 ± 1
18	40 ± 2
19	47 ± 2
20	85 ± 6
21	58 ± 3
Ibuprofen	50 ± 1

[a]Inhibition is expressed as the mean ± s.e.m. of six measurements.

compounds were practically equipotent, with an inhibition percentage superior to 77% at 0.4 mM kg^{-1}.

To confirm the high potency as anti-inflammatory agents of compounds **12**, **13**, **15**, and **17–21** (inhibition percentage superior or equivalent to 80% at 0.4 mM kg^{-1} in the PMA-induced ear oedema test) they were evaluated in the carrageenan-induced rat paw oedema model (Table 3). Although quinoline-3-carboxamide **12** was the most efficient in the PMA mouse ear oedema, astonishingly it did not exert a significant inhibitory effect on the carrageenan paw oedema, at least not at 0.2 mM kg^{-1} (15%). The other compounds all kept a high level of activity (**13**, **15**, **18**, **19**, **20**, **21**: 45–85% at 0.2 mM kg^{-1}), except compound **17** (36%). Pentafluorobenzaldimine **20** induced the most significant reduction in paw thickness (85%) and its level of activity compared favourably with ibuprofen (50%).

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