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New *N*-pyridinyl(methyl)-indole-2- and 3-(Alkyl)carboxamides and Derivatives Acting as Systemic and Topical Inflammation Inhibitors

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A series of novel *N*-substituted-(indol-2-yl)carboxamides (12–18) and (indol-3-alkyl)carboxamides (25–31) were synthesized and evaluated as inhibitors of the inflammation process. Pharmacomodulation at the level of the amidic nitrogen by incorporation of the previously described pharmacophoric moieties 6-aminolutidine, β-picolylamine, 4-aminopyridine and piperazine was investigated; only two compounds (12) and (31) exhibited significant (~40%) inhibitory effect in the carrageenan-induced rat paw edema after oral administration of a dose of 0.1 mM kg^{-1}. Replacement of the indole core by indazole failed to increase activity. Incorporation of an alkyl chain spacer led to more efficient compounds (46–52) especially in the indolepropanamide sub-series. Determination of the efficiency of the most active compounds on topical inflammation, by measuring reduction of ear thickness in the acute tetradecanoyl phorbol acetate (TPA)-induced mouse ear swelling assay, confirmed the high potency of propanamides (49) and (51) after oral administration: ID_{50} = 0.041 ± 0.013 and 0.042 ± 0.016 mM kg^{-1} respectively. The less toxic propanamide (51) exerted a high level of inhibitory activity after topical application of 2 × 100 μg/ear: 78 ± 2%.

Keywords: Amino(methyl)pyridines; Non acidic and non steroidal anti-inflammatory drugs; (Derivatives of) 2 and 3-indol(alkyl)carboxamides; 3-indazolcarboxamides

INTRODUCTION

In previous works we have synthesized and evaluated *N*-(4,6-dimethylpyridin-2-yl)heteroarylcarboxamides,[1] *N*-(pyridin-3-ylmethyl)phthalimides[2] and structurally related compounds. It was established that the level of activity of these novel non acidic anti-inflammatory agents could be enhanced by introduction of halogen atoms in the homocycle of the arylcarbonyl moiety. 5-Bromofuran-2-carboxamide **I**[1] is a highly efficient inhibitor of carrageenan-induced rat paw edema (ID_{50}: 105 μM kg^{-1}); moreover, it exerts a marked anti-oedematous effect (95%) on PLA_2-induced rat brain oedema at an IP dose of 12.5 μM kg^{-1}. Tetrafluorophthalimide **II**[2] is a potent TNFα production inhibitor (IC_{50}: 6 μM) which inhibits systemic (rat paw edema) and topical (ear edema) inflammation after oral administration: ID_{50} = 140 μM kg^{-1} (Figure 1).

These encouraging results prompted us to carry out pharmacomodulation in the indole series.[3] We report here on results obtained with *N*-substituted-indole-2-carboxamides **12–18** and some novel indole-3-carboxamides **22–31** and alkanamides **46–52**. The consequence of replacing indole by an indazole (**35**, **40**) and the amidic function by an imine one (**53**) was also determined.

MATERIALS AND METHODS

Chemistry

Instrumentation

Melting points (m.p.) were determined on a Tottoli-Büchi apparatus and were uncorrected. Structures

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Abbreviations: NSAI, non steroidal anti-inflammatory; ID, inhibitory dose; TPA, tetradecanoyl phorbol acetate

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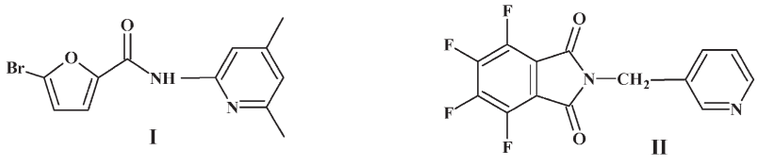


I II

FIGURE 1 Anti-inflammatory agents I and II.

were supported by IR and ^{1}H-NMR data. IR spectra were recorded on a Perkin–Elmer Paragon PC 1000 spectrometer as potassium bromide discs or as a film on NaCl plates. ^{1}H-NMR spectra were recorded on a Bruker AC 250 spectrometer (250 MHz) using $CDCl_3$ or $(CH_3)_2SO$-d_6 as solvent. Chemical shifts δ (ppm) refer to tetramethylsilane used as internal reference; coupling constants are in Hz. Analytical TLC was performed on precoated silica-gel aluminium (0.2 mm, GF254, E. Merck) and for delicate separations, a preparative centrifugally accelerated TLC (Chromatotron 7924 T, Harrisson Research, Palo Alto, CA) was used. Microanalyses for C, H and N were performed using a Perkin Elmer C, H, N 240 apparatus; the analytical results were within ±0.4% of the theoretical values.

Synthesis

Method **a**: Ethyl (indol-2-yl)carboxylate **2**

This ester was prepared by refluxing (indol-2-yl)carboxylic acid **1** in ethanol in the presence of gaseous HCl. Yield: 70%. m.p.: 122–123°C (lit.:[4] 120–121°C).

Method **b**: Ethyl (3-bromoindol-2-yl)carboxylate **7**

To ester **2** (0.94 g; 5 mmol) in 50 mL of DMF was added with stirring NBS (1.07 g; 6 mmol) in 20 ml of DMF at 0°C. After overnight stirring at 0°C, the mixture was poured on to cold water, acidified with HCl (10%) and extracted with ethyl acetate. The combined extracts were washed with saturated $NaHCO_3$ and NaCl and dried (Na_2SO_4). Evaporation of solvent followed by chromatography (hexane/ethyl acetate) afforded **2** as a yellow solid. Yield: 84%. m.p.: 148°C. IR (KBr) ν cm^{-1}: 3296 (NH), 1687 (C = O). ^{1}H-NMR (d_6-DMSO) ppm: 1.40 (t, 3H, CH_3, J = 7.02 Hz), 4.40 (q, 2H, CH_2, J = 7.02 Hz), 7.20 (dd, 1H, H^5, J, J′ = 7.92 Hz), 7.40 (dd, H, H^6, J, J′ = 7.92 Hz), 7.50 (d, 1H, H^7, J = 7.92 Hz), 7.60 (d, 1H, H^4, J = 7.92 Hz).

Method **c**: Ethyl (3-bromo-1-methylindol-2-yl)-carboxylate **8**

Ethyl-(3-bromoindol-2-yl)carboxylate **7** (0.4 g, 1.65 mmol) was dissolved in dry DMF and NaH (0.066 g, 1.65 mmol) was added. When no more gas evolved, methyl iodide (0.1 mL, 1.50 mmol) was added and the reaction mixture was stirred 1 h at room temperature. After addition of water, the aqueous phase was extracted with ethyl ether and, the organic layers were washed with saturated NaCl solution. Crystallization from hexane afforded **8** as a white solid. Yield: 77%. m.p.: 60°C. IR (KBr) ν cm^{-1}: 1704 (C = O). ^{1}H-NMR (d_6-DMSO) ppm: 1.41 (t, 3H, CH_3, J = 7.00 Hz), 4.03 (s, 3H, NCH_3), 4.44 (q, 2H, CH_2, J = 7.00 Hz), 7.28 (dd, 1H, H^5, J, J′ = 7.60 Hz), 7.47 (dd, 1H, H^6, J, J′ = 7.60 Hz), 7.60 (d, 1H, H^7, J = 7.60 Hz), 7.70 (d, 1H, H^4, J = 7.60 Hz).

Method **d**: Ethyl [1-(4-fluorobenzyl)indol-2-yl)]-carboxylate **4**

A mixture of **2** (1 g, 5.3 mmol) and cesium carbonate in dry acetonitrile was refluxed for 2 h with stirring. 4-Fluorobenzylchloride (0.76 g, 5.3 mmol) was then added and after 2 h further stirring at reflux, acetonitrile was evaporated in vacuo. The oily residue was crystallized in methanol to afford a white solid. Yield: 80%. m.p.: 74°C. IR (KBr) ν cm^{-1}: 1710 (C = O). ^{1}H-NMR (d_6-DMSO) ppm: 1.30 (t, 3H, CH_3, J = 7.30 Hz), 4.30 (q, 2H, CH_2, J = 7.30 Hz), 5.87 (s, 2H, CH_2), 7.18 (dd, 1H, H^5, J, J′ = 7.90 Hz), 7.10–7.20 (m, 4H, Ph), 7.36 (dd, 1H, H^6, J, J′ = 7.90 Hz), 7.40 (s, 1H, H^3), 7.60 (d, 1H, H^7, J = 7.90 Hz), 7.76 (d, 1H, H^4, J = 7.90 Hz).

Method **e**: [1-(4-Fluorobenzyl)-3-bromoindol-2-yl]carboxylic Acid **11**

A mixture of **9** (1 g, 2.65 mmol), ethanol (10 mL) and 5 M aqueous NaOH (2 mL) was refluxed with stirring for 1 h, cooled to room temperature and acidified with 1 M aqueous HCl. The precipitate was then filtered, washed with cold water and dried in vacuo to afford the acid as a white solid. Yield: 90%. m.p.: 207–208°C (dec.). IR (KBr) ν cm^{-1}: 2925 (OH), 1675 (C = O). ^{1}H-NMR (d_6-DMSO) ppm: 5.87 (s, 2H, CH_2), 7.12–7.15 (m, 4H, Ph), 7.20 (dd, 1H, H^5, J, J′ = 7.60 Hz), 7.40 (dd, 1H, H^6, J, J′ = 7.60 Hz), 7.63 (d, 1H, H^7, J = 7.60 Hz), 7.70 (d, 1H, H^4, J = 7.60 Hz), 13.70 (s, 1H, OH).

Method **f**: *N*-(4,6-dimethylpyridin-2-yl)-(3-bromo-1-methylindol-2-yl)carboxamide **16**

A solution of the acid **10** (0.350 g, 1.37 mmol), triethylamine (0.2 mL, 1.37 mmol) and 2-amino-4,6-dimethylpyridine (0.167 g, 1.37 mmol) was cooled to 0°C. Phenyl dichlorophosphate was then added dropwise and the mixture was stirred at room

temperature for 24 h. Evaporation of the solvent and purification of the residue by column chromatography (CH_2Cl_2) afforded the carboxamide **16**. Yield: 50%. m.p.: 145°C. IR (KBR) ν cm^{-1}: 1675 (C = O). ^{1}H-NMR (d_6-DMSO) ppm: 2.37 (s 3H, γCH_3), 2.43 (s, 3H, αCH_3), 3.89 (s, 3H, NCH_3), 6.95 (s, 1H, pyr H^5), 7.20 (dd, 1H, H^5, J, J′ = 7.00 Hz), 7.42 (dd, 1H, H^6, J, J′ = 7.00 Hz), 7.56 (d, 1H, H^7, J = 7.00 Hz), 7.66 (d, 1H, H^4, J = 7.00 Hz), 7.94 (s, 1H, pyr. H^3), 10.80 (s, 1H, NH). Elemental analysis ($C_{17}H_{16}BrN_3O$) C, H, N.

Method **g**: *N*-(4-methylpiperazinyl)-[5-bromo-1-(4-fluorobenzyl)indol-3-yl]Carboxamide **29**

A mixture of 5-bromo-1-[(4-fluorobenzyl)indol-3-yl]carboxylic acid (1 g, 2.84 mmol), dichloromethane (30 mL), 2-chloro-*N*-methylchloropyridinium iodide (0.8 g, 2.87 mmol), triethylamine (1 mL, 7.17 mmol) and 1-methylpiperazine (0.6 mL, 2.87 mmol) was refluxed for 20 min. The solvent was evaporated and the crude product was purified by column chromatography (CH_2Cl_2/ethanol: 95/5) to give pure **29** as a light yellow oil. Yield: 73%. IR ν cm^{-1}: 1607 (C = O). ^{1}H-NMR (d_6-DMSO) ppm: 2.24 (s, 3H, NCH_3), 2.36–2.40 (m, 4H, pip. CH_2), 3.65–3.69 (m, 4H, pip. CH_2), 5.49 (s, 1H, CH_2), 7.15–7.22 (m, 2H, Ph), 7.32–7.38 (m, 3H, H^6 and Ph), 7.55 (d, 1H, H^7, J = 8.80 Hz), 7.90 (d, 1H, H^4, J = 1.85 Hz), 8.05 (s, 1H, H^2). Elemental analysis ($C_{21}H_{21}BrFN_3O$) C, H, N.

Method **h**: *N*-(4,6-dimethylpyridin-2-yl)-(1-methylindol-2-yl)Carboxamide **12**

Triphenyl phosphine (1.15 g, 4.4 mmol), bromotrichloromethane (1.74 g, 8.8 mmol) and (1-methylindol-2-yl)carboxylic acid (0.770 g, 4.4 mmol) were dissolved in dry THF. 2-Amino-4,6-dimethylpyridine (1.075 g, 8.8 mmol) was then added and the reaction mixture was refluxed for 5 h. After filtration and evaporation of THF, the residue was purified by column chromatography (CH_2Cl_2) to afford **12**. Yield: 24%. m.p.: 117°C. IR (KBr) ν cm^{-1}: 3420 (NH), 1660 (C = O). ^{1}H-NMR (d_6-DMSO) ppm: 2.35 (s, 3H, γCH_3), 2.44 (s, 3H, αCH_3), 4.10 (s, 3H, NCH_3), 6.91 (s, 1H, pyr. H^5), 7.16 (dd, 1H, H^5, J, J′ = 7.60 Hz), 7.35 (dd, 1H, H^6, J, J′ = 7.60 Hz), 7.53 (s, 1H, H^3), 7.60 (d, 1H, H^7, J = 7.60 Hz), 7.70 (d, 1H, H^4, J = 7.60 Hz), 7.90 (s, 1H, pyr. H^3), 10.67 (s, 1H, NH). Elemental analysis ($C_{17}H_{17}N_3O$) C, H, N.

Methods **i** And **j**

Acids **23** and **24** were obtained by oxidation of the corresponding aldehydes **20** and **21**[3] produced from Vilsmeyer-Hack formylation ($POCl_3$, DMF) and N^1-alkylation (methods **c** and **d**) of 5-bromoindole **19**.

Method **k**: *N*-(4-methylpiperazin-1-yl)-(5-bromo-1-methylindol-3-yl)carboxamide **28**

N,*N*′-Carbonyldiimidazole (0.57 g, 3.5 mmol) was added gradually to a solution of **10** (0.9 g, 3.5 mmol) in dry THF (20 mL). The mixture was stirred for 1 h at room temperature. 1-Methylpiperazine (0.39 mL, 3.5 mmol) was added and stirring continued for 3 days. The solvent was evaporated and the amide was purified using preparative centrifugally accelerated thin layer chromatography (CH_2Cl_2/ethanol: 98/2) to afford pure **28** as an orange oil. Yield: 84%. IR ν cm^{-1}: 1599 (C = O). ^{1}H-NMR (d_6-DMSO) ppm: 2.26 (s, 3H, pip. NCH_3), 2.37–2.41 (m, 4H, pip. CH_2), 3.65–3.69 (m, 4H, pip. CH_2), 3.86 (s, 3H, NCH_3), 7.38 (dd, 1H, H^6, J, J′ = 8.70 and 1.90 Hz), 7.52 (d, 1H, H^7, J = 8.70 Hz), 7.83 (s, 1H, H^2), 7.90 (d, 1H, H^4, J = 1.90 Hz). Elemental analysis ($C_{15}H_{18}BrN_3O$) C, H, N.

Method **l**: *N*-(Piperazin-1-yl)-[5-bromo-1-(4-fluorobenzyl)indol-3-yl]Carboxamide **31**

A mixture of **30** (0.45 g, 0.88 mmol), ethanol (4 mL), 5% palladium on activated carbon (120 mg) and ammonium formate (225 mg, 3.57 mmol) dissolved in the minimum of water was heated at 60°C for 5 h. The suspension was filtered and the solvent evaporated. The crude product was purified by preparative centrifigally accelerated thin layer chromatography (CH_2Cl_2/ethanol: 95/5) to afford **31** as a pale yellow oil. Yield: 86.5%. IR ν cm^{-1}: 3431 (NH), 1603 (C = O). ^{1}H-NMR (d_6-DMSO) ppm: 3.16 (m, 4H, pip. CH_2), 3.84 (m, 4H, pip. CH_2), 5.50 (s, 2H, CH_2), 7.15–7.25 (m, 3H, H^4 and 2H Ph), 7.57 (m, 1H, H^7), 7.77 (m, 1H, H^6), 8.07 (s, 1H, H^2). Elemental analysis ($C_{20}H_{19}BrFN_3O$) C, H, N.

Method **m**: *N*-(4,6-dimethylpyridin-2-yl)-2-nitrophenylacetamide **37**

To a suspension of **36** (2.5 g, 13.8 mmol) and DMF (0.3 mL) in 1,2-dichloroethane (DCE) (14 mL) was added dropwise $SOCl_2$ (1.2 mL, 16.56 mmol) at 35–40°C over a period of 15 min. The mixture was stirred at this temperature for 2 h. After cooling to 20°C, a solution of 2-amino-4,6-dimethylpyridine (5.06 g, 41.4 mmol) in DCE (14 ml) was added dropwise over a period of 30 min and the mixture was stirred at 20–25°C for 2 h. The precipitate was filtered off and washed with DCE. The filtrate was successively washed with aqueous 1 M HCl and water. The combined organic layers were dried (Na_2SO_4), filtered and evaporated to dryness. The crude product was purified by chromatography on silica gel (CH_2Cl_2/:ethanol: 98/2) to afford **37**. Yield: 28%. m.p.: 202–203°C. IR (KBr) ν cm^{-1}: 3421 (NH), 1635 (C = O). ^{1}H-NMR ($CDCl_3$) ppm: 2.20 (s, 3H, γCH_3), 2.32 (s, 3H, αCH_3), 3.99 (s, 2H, CH_2), 6.65 (s, 1H, pyr. $H^{5'}$), 7.41 (m, 2H, H^4 and H^6), 7.55 (ddd, 1H, H^5, J, J′, J″ = 7.50, 7.40, 1.30 Hz), 7.72 (s, 1H, pyr. H^3), 8.03 (dd, 1H, H^3, J, J′ = 8.0 and 1.30 Hz); 8.39 (s,1H, NH).

Method **n**: *N*-(4,6-dimethylpyridin-2-yl)-2-acetamidophenylacetamide **38**

Hydrogenation of **37** (0.52 g, 2.17 mmol) in the presence of 5% Pd-C and acetic anhydride

(0.82 mL, 8.67 mmol) in toluene (20 mL) was carried out with vigorous stirring at atmospheric pressure, for 2 h. The catalyst was removed by filtration and the filtrate was evaporated in vacuo. The residue was washed with hexane to afford **38**. Yield: 93%. m.p.: 155–156°C. IR (KBr) ν cm^{-1}: 3250 (NH), 1699 and 1675 (C = O). ^{1}H-NMR ($CDCl_3$) ppm: 2.29 (s, 3H, γCH_3), 2.36 (s, 3H, αCH_3), 2.44 (s, 3H, $COCH_3$), 3.77 (s, 2H, CH_2), 6.79 (s, 1H, pyr. H^5), 7.11 (dd, 1H, H^5, J, J′ = 7.20 Hz), 7.30 (m, 1H, H^6), 7.36 (dd, 1H, H^4, J, J′ = 7.20 Hz), 7.89 (d, 1H, H^3, J^3 = 7.20 Hz), 7.94 (s, 1H, pyr. H^3), 9.65 (s, 1H, NH), 10.82 (s, 1H, NHAc).

METHOD **o**: 1-ACETYL-*N*-(4,6-DIMETHYLPYRIDIN-2-YL)INDAZOLE-3-CARBOXAMIDE **39**

Tert-butyl nitrite (0.1 ml, 0.88 mmol) was added dropwise to a solution of **38** (0.25 g, 0.80 mmol) and acetic anhydride (0.26 ml, 2.5 mmol) in toluene (20 ml) at 90–95°C. After being stirred at this temperature for 40 min, the solution was evaporated. The residue was dissolved in chloroform and washed with an aqueous solution of 5% potassium carbonate. The combined organic layers were dried (Na_2SO_4), filtered and evaporated to afford **39**. Yield: 39%. ^{1}H-NMR (d_6-DMSO) ppm: 2.40 (s, 3H, γCH_3), 2.49 (s, 3H, αCH_3), 2.89 (s, 3H, $COCH_3$), 6.83 (s, 1H, pyr. H^5), 7.50 (dd, 1H, H^5, J, J′ = 7.90 Hz), 7.63 (dd, 1H, H^6, J, J′=7.90 Hz), 8.10 (s, 3H, pyr. H^3), 8.50 (m, 2H, H^4 and H^7), 9.32 (s, 1H, NH).

METHOD **p**: *N*-(4,6-DIMETHYLPYRIDIN-2-YL)-1*H*-INDAZOLE-3-CARBOXAMIDE **40**

A suspension of **39** (0.1 g, 0.32 mmol) and NaOH was stirred at 60°C for 30 min. An aqueous solution of 1 M HCl was then added and the mixture stirred at room temperature for 1 h. The precipitate was collected, washed with water and crystallized from ethanol to afford **40**. Yield: 98%. m.p.: 228°C. IR (KBr) ν cm^{-1}: 3270 (NH), 1677 (C = O). ^{1}H-NMR (d_6-DMSO) ppm: 2.40 (s, 3H, γCH_3), 2.49 (s, 3H, αCH_3), 6.88 (s, 1H, pyr. H^5), 7.33 (dd, 1H, H^5, J, J′ = 8.0 Hz), 7.45 (dd, 1H, H^6, J, J′ = 8.0 Hz), 7.58 (dd, 1H, H^7, J, J′ = 8.0 Hz), 8.18 (s, 3H, pyr. H^3); 8.47 (d, 1H, H^4, J = 8.0 Hz), 10.01 (s, 1H, amide NH), 13.79 (s, 1H, NH). Elemental analysis ($C_{15}H_{14}N_4O$) C, H, N.

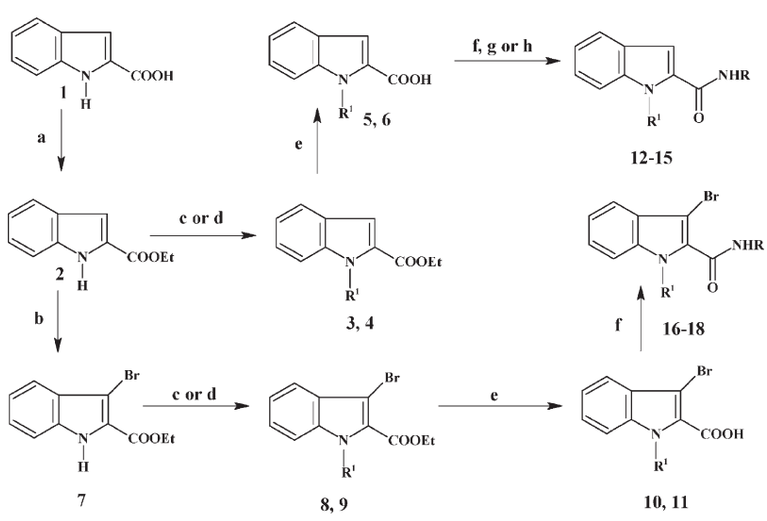
METHOD **q**: *N*-(3-PYRIDINYLMETHYL)-1*H*-INDOLE-3-CARBOXIMINE **53**

A solution of 1*H*-indole-3-carboxaldehyde (2.00 g, 13.80 mmol) in toluene (20 mL) was placed in a 100 mL three-necked round-bottomed flask equipped with a thermometer and a Dean-Stark Separator. 3-Picolylamine (1.49 g, 13.80 mmol) was added in one portion and the mixture was stirred at 105°C for 2 h. The precipitate was filtered off to afford **53**. Yield: 96%. m.p.: 180–181°C. IR (KBr) ν cm^{-1}: 3100 (NH), 1630 (C = N). ^{1}H-NMR (d_6-DMSO) ppm: 4.79 (s, 2H, CH_2), 7.11–7.24 (m, 2H, pyr. H^4 and H^6), 7.42–7.49 (m, 2H, H^5 and H^6), 7.82 (d, 1H, H^7, J = 7.90 Hz), 7.87 (m, 1H, pyr. H^5), 8.25 (d, 1H, H^4, J = 7.00 Hz), 8.50 (s, 1H, CH = N), 8.67 (m, 2H, H^2 and pyr. H^2), 11.60 (s, 1H, NH). Elemental analysis ($C_{15}H_{13}N_3$) C, H, N.

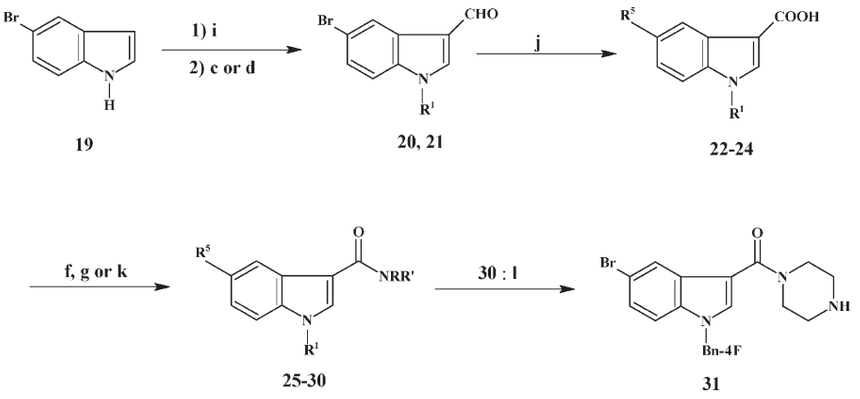
Pharmacology

Carrageenan-induced Rat-paw Oedema

Anti-inflammatory activity against carrageenan-induced rat-paw oedema was assayed in adult male Wistar CF rats weighing 180–220 g, according to the method of Winter *et al.*[5] with slight



SCHEME 1 Synthesis of 2-indolecarboxamides. (**a**) EtOH, HCl, reflux; (**b**) NBS, DMF, 0°C; (**c**) NaH, DMF, CH_3I, RT; (**d**) Cs_2CO_3, CH_3CN, 4F-BnCl, reflux; (**e**) 1 M NaOH, EtOH reflux; (**f**) RNH_2, DCP, Et_3N, CH_2Cl_2, RT; (**g**) RNH_2, 2-chloro-*N*-methylpyridinium iodide, CH_2Cl_2, reflux; (**h**) RNH_2, Ph_3P, $BrCCl_3$, THF, reflux.



SCHEME 2 Synthesis of 3-indolecarboxamides. (**i**) $POCl_3$, DMF, 10°C; (**j**) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, THF, RT; (**f**) RNH_2, DCP, Et_3N, CH_2Cl_2, RT; (**g**) RNH_2, 2-chloro-*N*-methylpyridinium iodide, CH_2Cl_2, reflux; (**k**) CDI, THF, RT, (**l**) $HCOONH_4$, Pd/C, $EtOH/H_2O$, 70°C.

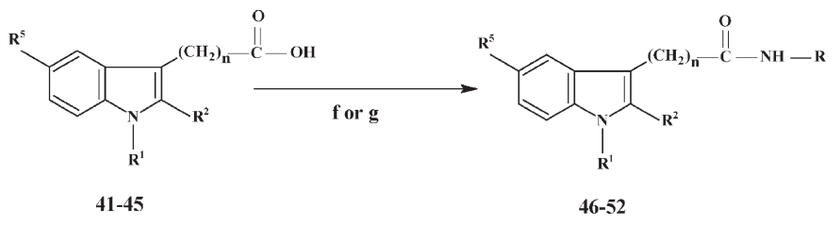
modifications. The drugs were orally administered 1 h before the injection of 0.05 mL of a 1% suspension of carrageenan saline into the subcutaneous tissue of one hind paw. The other hind paw was injected in the same way with 0.05 mL of a saline solution. Rats were fasted 24 h before the experiment, and water (1.5 mL/100 g body weight) was orally administered twice (20 and 4 h before injections). The volume of both the hind paws of control and treated animals was measured with a plethysmograph 3 h after injection. Rats were kept under the same experimental conditions. The percentage inhibition 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 d*t* is the difference in paw volume in the drug-treated group and d*c* the difference in the control group.

TPA-induced Mouse-ear Oedema (Orally Administered Drugs)

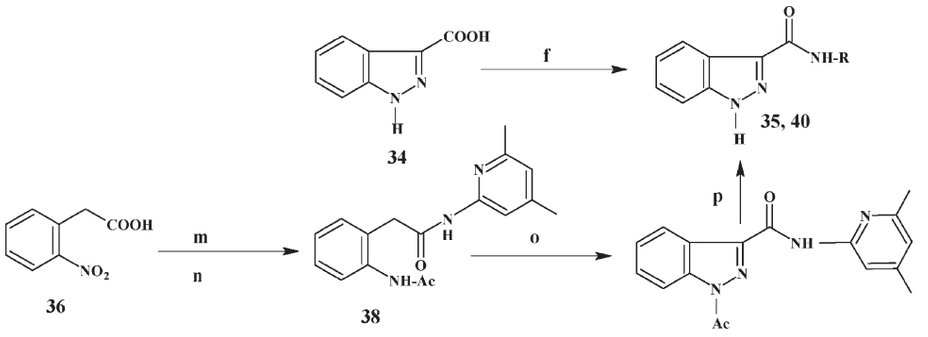
Induction of mouse-ear oedema was based on the method of Carlson *et al.*[6] with some modifications. Groups of five male Swiss mice weighing 20–25 g were fasted 24 h before the experiments and maintained in suitable environmental conditions throughout the experiments. TPA (tetradecanoyl phorbol acetate) was dissolved in 80% aqueous ethanol at a concentration of 250 $\mu g\,mL^{-1}$; 10 μL^{-1} was applied topically to the anterior and posterior surfaces of the right ear of each mouse. The left ear (control) received the vehicle (10 µL of 80% aqueous ethanol). The compounds studied were orally administered 1 h before the TPA application. Ear thickness was measured with a model micrometer gauge (Oditest Kroeplin) 3 h and 30 min after treatment. Ear oedema, calculated by subtracting the thickness of the left ear (vehicle) from the thickness of the right ear (PMA), was expressed as an increase in ear thickness. The percentage of inhibition of the inflammatory reaction was determined for each animal by the comparison of ear oedema in treated and non-treated animals.

TPA-induced Mouse-ear Oedema (Topically Applied Drugs)

Groups of five male Swiss mice weighing 20–25 g were briefly anaesthetised with ether for ear application. TPA was dissolved in 80% aqueous ethanol at a concentration of 250 $\mu g\,mL^{-1}$; 10 µL was applied topically to the anterior and posterior



SCHEME 3 Synthesis of 3-indolealkanamides. (**f**) RNH_2, DCP, Et_3N, CH_2Cl_2, RT; (**g**) RNH_2, 2-chloro-*N*-methylpyridinium iodide, CH_2Cl_2, reflux.



SCHEME 4 Synthesis of 3-indazolecarboxamides. (**f**) RNH_2, DCP, Et_3N, CH_2Cl_2, RT; (**m**) 1) $SOCl_2$, DCE, DMF, 35–40°C, 2) 2-amino-4,6-dimethylpyridine, DCE, 20°C; (**n**) H_2/Pd-C, Ac_2O, toluene, RT; (**o**) t-BuONO, Ac_2O, toluene, 90–95°C; (**p**) 1) 2.8 M NaOH, reflux, 2) HCl, RT.

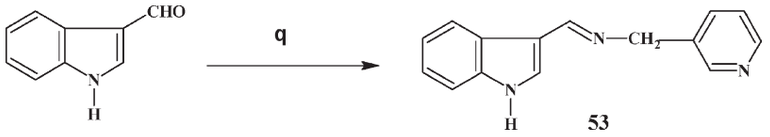
surface of the right ear of each mouse. The left ear (control) received the vehicle (10 μL of 80% aqueous ethanol). A solution of 100 μg or 500 μg of drug in 10 μL of ethanol was applied to the inner surface of the right ear of treated animals, and 10 μL of vehicle (ethanol) to the contralateral ear as the control. These applications were made 30 min before TPA application, and then again 5 min later. Ear thickness was measured with a model micrometer gauge (Oditest Kroeplin) 3 h and 30 min after treatment. Ear oedema, calculated by subtracting the thickness of the left ear (vehicle) from the thickness of the right ear (TPA), was expressed as an increase in ear thickness. The percentage of inhibition of the inflammatory reaction was determined for each animal by the comparison of ear oedema in treated and non-treated animals.

RESULTS AND DISCUSSION

Chemistry

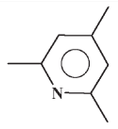
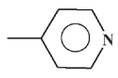
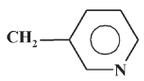
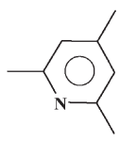
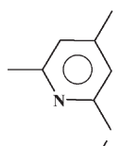
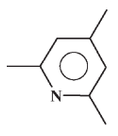
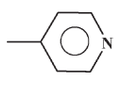
5-Bromoindole **19**, (indol-2-yl)carboxylic acid **1**, (5-methoxy-2-methylindol-3-yl)acetic acid **43** and 3-(indol-3-yl)propionic acid **44** were purchased from Sigma Aldrich (Saint-Quentin, Fallavier, France). (1-Methylindol-2-yl)carboxylic acid **5** was obtained from the same supplier or synthesized by N^1-methylation of ethyl (indol-2-yl)carboxylate **2** (method c, Scheme 1) followed by hydrolysis of **3** (method e). Indazole-3-carboxylic acid **34** can be prepared starting from isatine[7] via heterocyclization of 2-hydrazinophenylglyoxylic acid but yield remains low (32%). The method described by M. FERRARI *et al.*,[8] starting from 2-acetyl-1-phenylhydrazine afforded this acid in moderate yield (42%). The non-commercially available (5-bromo-1-methylindol-3-yl)carboxylic acid **23**[3] and the corresponding 1-(4-fluorobenzyl) analogue **24**[3] were obtained by oxidation of aldehydes **20** and **21** according to M. L. CURTIN *et al.*[9] (methods i and j, Scheme 2). 3-(5-Fluoroindol-3-yl)propionic acid **44**[10] was prepared in an overall yield of 25% from 4-fluoroaniline by the Japp-Klingemann reaction followed by hydrolysis of the diester and C-2 decarboxylation by the couple Cu/*N*-methylpyrrolidinone. 1-(4-Fluorobenzylindol-3-yl)acetic acid **42**[11] was obtained in an overall yield of 52%, by benzylation (NaH, DMSO) of the ethyl ester of **41** followed by alkaline hydrolysis; the corresponding amide **47**[11] was prepared by method g in 71% yield *N*-(pyridin-4-yl)-(indol-3-yl)acetamide **46**[3] was obtained from the acid **41** after DCC activation.

The synthetic routes to targeting indol-2 and 3-carboxamides and 3-alkanamides are outlined in Schemes 1, 2 and 3. The starting acids were activated via phosphoric anhydride, acyloxypyridinium salt, acyloxy phosphonium salt or imidazolide formation (methods f, g, h and k). Preliminary N-substitution was necessary in the (indol-2-yl)carboxamide series to avoid self-condensation and exclusive formation of 6*H*, 13*H*-pyrazino [1,2-*a*: 4,5-*a*′]di-indol-6,13-dione.[1] Attempted 3-bromination of the amides **12**–**15** failed; this reaction was best carried out at the level of ethyl (indol-2-yl)carboxylate **7** (93% yield) before N^1-substitution (method d). In the (indol-3-yl)carboxamide series,



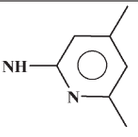
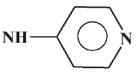
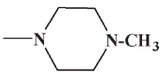
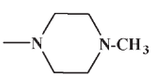
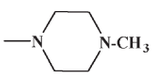
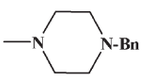
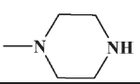
SCHEME 5 Synthesis of 3-indolecarbaldimine 53. (**q**) 3-picolylamine, toluene, reflux.

TABLE I Physicochemical properties and anti-oedema effect of (indol-2-yl)carboxamides

No.	R^1	R^3	R	Method yield (%)	m. p. (°C) Solvent	Inh. % at 0.1 mM kg^{-1}
12	CH_3	H		**h**:24	117 [a]	38 ± 11
13	CH_3	H		**g**:12	145 [b]	NA
14	CH_3	H		**g**:53	125 [b]	29 ± 10
15	4F-Bn	H		**f**:41	128 [b]	NA
16	CH_3	Br		**f**:50	145 [b]	NA
17	4F-Bn	Br		**f**:70	151 [b]	NA
18	CH_3	Br		**f**:25	190 [c]	NA
Ibuprofen at 0.1 mM kg^{-1}						42 ± 4

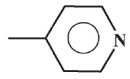
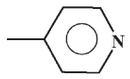
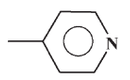
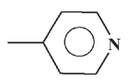
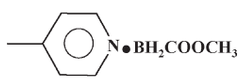
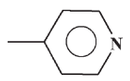
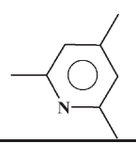
Crystallization solvents: [a]isopropyl ether; [b]dichloromethane; [c]diethyl ether. NA = not active.

TABLE II Physicochemical properties and anti-oedema effect of (indol-3-yl)carboxamides

No.	R^1	R^5	R, R′	Method yield (%)	m. p. (°C) Solvent	Inh. % at 0.1 mM kg^{-1}
25	CH_3	H		**g**:16	146 [a]	NA
26	CH_3	H		**f**:35	200 [a]	NA
27	CH_3	H		**f**:65	89 [a]	NA
28	CH_3	Br		**k**:84	oil	32 ± 5
29	4F-Bn	Br		**g**:73	oil	35 ± 10
30	4F-Bn	Br		**g**:62	115 [b]	26 ± 6
31	4F-Bn	Br		**l**:86	Oil	43 ± 8

Crystallization solvents: [a]dichloromethane/ethanol; [b]diethylether. NA = not active

TABLE III Physicochemical properties and anti-oedema effect of (indol-3-yl)alkylcarboxamides

No.	R^1	R^2	R^5	n	R	Method yield %	m.p. (°C) Solvent	inh % at 0.1 mM kg^{-1}	ID_{50} mM kg^{-1}
46	H	H	H	1		**g**:58	230[a]	41 ± 11	
47	4F-Bn	H	H	1		**g**:71	140–142[b]	65 ± 11	0.085 ± 0.021
48	H	CH_3	OCH_3	1		**g**:50	203[c]	38 ± 12	
49	H	H	H	2		**f**:76	100[d]	95 ± 3	0.044 ± 0.011
50	H	H	H	2		11	135[c]	84 ± 9	0.049 ± 0.014
51	H	H	F	2		**g**:51	150–155[e]	86 ± 8	0.032 ± 0.010
52	H	H	F	2		**f**:23	102[c]	25 ± 13	

Crystallization solvents: [a]ethanol; [b]diisopropyl ether; [c]dichloromethane; [d]petroleum ether, [e]ethyl acetate.

selective $N^{4'}$-deprotection of the piperazinyl amide **30** by the couple $HCOONH_4$/Pd on C (method l) afforded **31** in excellent yield (86%).

Methods and yields obtained in the three series are gathered in Tables I, II and III.

Due to their low nucleophilicity, 6-amino-2,4-lutidine and 4-aminopyridine did not react with the acyl chloride of **34**; only the more basic β-picolylamine afforded the corresponding amide **35** (Scheme 4). Access to amide **40** was achieved by carrying out amidification of 2-nitrophenylacetic acid, followed by heterocyclisation of the nitroso derivative of **38**, according to Yoshida *et al.*[12]

As we previously obtained fair pharmacological results in the series of pentafluorobenzaldimines,[13] the N-(3-pyridinylmethyl) derivative **53** in the indole-3-carbaldimine series was prepared; it was obtained in excellent yield (96%) by azeotropic distillation (Dean Stark) in refluxing toluene (method q) (Scheme 5).

Pharmacology

Effect in the Carrageenan Paw Oedema (CPO) Test

The anti-inflammatory activity of the synthesized compounds was determined in terms of their ability to inhibit foot pad oedema in rats after induction by subcutaneous injection of carrageenan into the plantar surface of the right hind paw. Compounds of the indole-2-carboxamide series were first evaluated in this assay at 0.4 mM kg^{-1}; only compounds **12**, **13** and **14** exhibited significant activity: 84 ± 7, 47 ± 8 and 55 ± 8% inhibition, respectively. As illustrated in Table I, at 0.1 mM kg^{-1}, only *N*-(4,6-dimethylpyridin-2-yl)-(1-methylindol-2-yl)carboxamide **12** exerted moderate inhibition, 38 ± 11%. Evaluation of its ID_{50} gave 0.14 ± 0.02 mM kg^{-1}. Introduction of a 4-fluorobenzyl group at N^1 of the indole exerted a detrimental effect: **12** → **15**. Examination of the bromo derivatives **16**, **17** and **18** showed that, in that case, at least at carbon 3, bromine afforded no positive effect.

No increase in activity was observed when the carboxamido grouping was fixed at C^3; in the piperazinyl subseries (**27**–**31**), N^4-debenzylation of **30**, leading to **31**, induced a slight enhancement of inhibitory activity, 26 and 43% respectively at 0.1 mM kg^{-1} (Table II).

In the (indol-3-yl)acetamide series (Table III), a comparative study with the previously examined compound **46** showed the favourable effect of a 4-fluorobenzyl group at N^1 and no effect of simultaneous substitution at positions 2 and 5 (compounds **47** and **48**). In the propanamide series, we observed that lengthening of the alkanamide chain induced a marked increase in activity (**46** → **49**) with percentage inhibition of 41 ± 11 and 95 ± 3% at 0.1 mM kg^{-1} respectively. As compound

TABLE IV Inhibition of TPA-induced oedema in mice

N°	ID_{50} mM kg^{-1} after oral administration	Inhibition % after topical application	
		2 × 100 μg	2 × 500 μg
49	0.041 ± 0.016	38 ± 3	67 ± 3
50	0.082 ± 0.055		
51	0.042 ± 0.016	78 ± 2	90 ± 2
Dexamethasone		96 ± 2	100

49 exerted toxic effects at 0.4 mM kg^{-1}, pharmacomodulation was carried out which showed that its toxicity could be markedly attenuated by introduction of a fluorine atom at C^5 or a methoxycarbonylborane grouping in the pyridinyl nucleus, amides **50** and **51** respectively. In the present work, it was observed that replacement of the 4-aminopyridine of **51** by 6-aminolutidine, another pharmacophoric moiety, (leading to **52**), exerted a detrimental effect: the percentage inhibition was lowered from 95 ± 3% to 25 ± 13% respectively.

Contrary to the corresponding pentafluorobenzaldimine,[13] imine **53** exerted but a very low inhibitory activity (19 ± 9%) at 0.1 mM kg^{-1}.

Effect in the Acute TPA-induced Mouse Ear Swelling Test

As psoriatic skin shares many of the pathologic features of phorbol ester-treated mouse skin[14]—including elevated levels of arachidonic acid metabolism products, inflammatory cells and cell proliferation—the effect of the most active amides (**12**, **31**, **49**, **50** and **51**) was evaluated in a model of topical inflammation: the acute TPA-induced mouse ear swelling test.[15] After oral administration of 0.2 mM kg^{-1}, the percentage inhibition by amides **12** and **31** was 54 ± 2 and 57 ± 2%, respectively. The level of activity was enhanced in the propanamide series; the ID_{50} values of **49**, **50** and **51** were 0.041 ± 0.013, 0.082 ± 0.055 and 0.042 ± 0.016 mM kg^{-1}, respectively.

Compounds **49** and **51** also proved to be highly efficient in mouse ear thickness reduction after topical application of 2 × 500 μg with 67 ± 3 and 90 ± 2% respectively. Although less potent than dexamethasone, they remained significantly active at 2 × 100 μg: 96 ± 2, 38 ± 3 and 78 ± 2%, respectively. The most potent compound, **51**, is now being evaluated in our laboratory in a mouse model of multiple TPA-induced chronic inflammation,[16] considered to be relevant for human psoriasis (Table IV).

Propanamide **51** constitutes the most efficient non acidic NSAI compound ever discovered in the series of N-pyridinyl heteroarylalkanamides (ID_{50} in CPO: 9.1 mg kg^{-1}). The favourable effect exerted by a 4-fluorobenzyl group at the indolic nitrogen in **47** (by comparison with the unsubstituted counterpart **46**)[3] prompts us to study the effect of N^1-substitution using **51** as a lead compound.

Acknowledgements

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