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Synthesis and Evaluation of Disubstituted N^1- and N^3-Imidazolidin-2-ones Acting as Potential Immunosuppressive Agents

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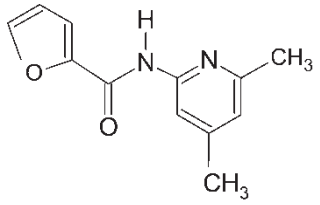
New N^1-mono and N^1, N^2-disubstituted imidazolidin-2-one with a significant immunosuppressive activity have been discovered. Among the 17 synthesized and tested compounds, five of them showed maximal inhibition of proliferation of concanavallin A (Con A)- stimulated splenocytes at 90 μM, identical to that obtained with cyclosporin A (CsA) at 5 μM, an optimal concentration.

Keywords: Ureas; Imidazolidin-2-ones; Immunosuppressive drugs

INTRODUCTION

Many immunosuppressive[1] drugs have been described which are now used to control unwanted immune responses in a variety of therapeutic uses, for example, type I diabetes mellitus,[2] arthritis,[3–5] dermatological diseases such as psoriasis,[6] systemic lupus erythematosus[7] and control of allograft rejection by inhibition of T-lymphocyte-dependent immune responses to donor antigens.[1,8] Today, according to their mechanisms of action, there are five groups of immunosuppressants:[1] regulators of gene expression (glucocorticoids), alkylating agents (cyclophosphamide), kinase and phosphatase inhibitors (cyclosporin A, and apparented compounds, sirolimus, tacrolimus), inhibitors of *de novo* purine synthesis (mycophenolate mofetil, methotrexate), and inhibitors of *de novo* pyrimidine synthesis (brequinar, leflunomide)[9–11].

However, most compounds exhibit important specific toxicities, as nephrotoxicity and hematoxicity which should be considered in long-term strategies of immunosuppression. This is particularly true in the field of transplantation, where these deleterious side effects impair patient and graft survival. So, the challenge for the future is the discovery of new immunosuppressive compounds with a better therapeutic index and more than only an "anti-rejection" activity, a possible application to induce tolerance to allografts



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Structure of compound **1**

In a previous work concerning new antiinflammatory drugs, furanecarboxamide **1** was identified as a lead compound.[12] Additional studies on this molecule showed that it was also able to exhibit a potent immunosuppressive activity.[13] These results prompted us to synthesize some derivatives of **1**, such as other heteroarylcarboxamides, ureas and cyclic derivatives of ureas with an imidazolidin-2-one structure. We present in this paper the synthesis and pharmacological evaluation of some new N^1-mono and N^1, N^2-disubstituted imidazolidin-2-ones (Scheme 1).

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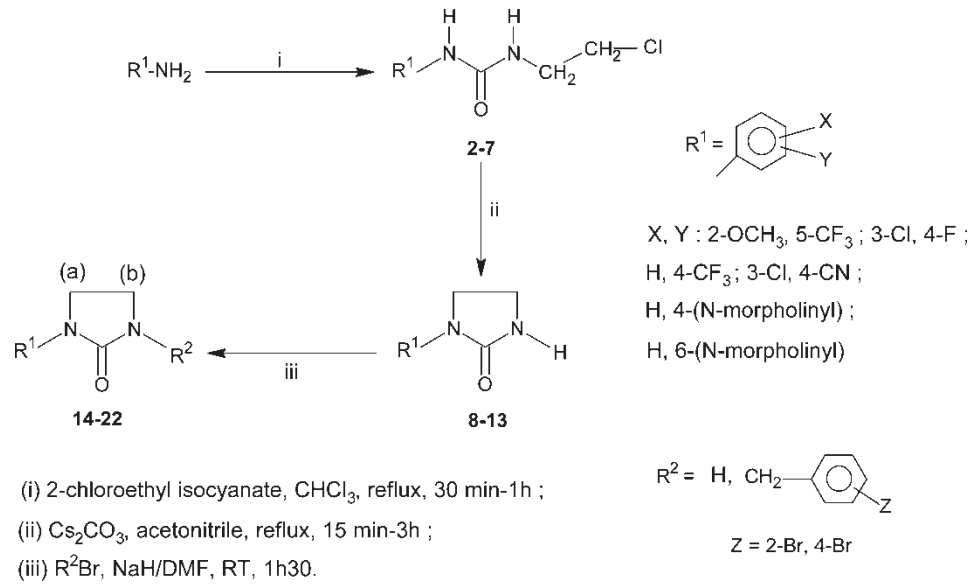


SCHEME 1 Chemical pathways for the synthesis of ureas **2**–**7**, mono-*N*-substituted and di-*N*-substituted imidazolidin-2-ones substituted **8**–**13** and **14**–**22**. (i) 2-chloroethyl isocyanate, $CHCl_3$, reflux, 30 min–1 h; (ii) Cs_2CO_3, acetonitrile, reflux, 15 min–3 h; (iii) R^2Br, NaH/DMF, RT, 1 h30.

MATERIALS AND METHODS

General

All reagents and solvents were general purpose grade. Melting points were determined on a Tottoli-Büchi apparatus (Büchi, Flawil, Switzerland) and are uncorrected. Structures of the described compounds were supported by IR, ^{1}H-NMR and microanalytical data. IR spectra were run with KBr pellets on a Perkin-Elmer FT-IR Paragon 1000 grating infrared spectrometer (Perkin-Elmer, St-Quentin-en-Yvelines, France). ^{1}H-NMR spectra were recorded on a Bruker AC 250 spectrometer (250 MHz) (Bruker, Wissembourg, France), using $CDCl_3$ as a solvent; chemical shifts (δ) are reported in parts per million (ppm), from internal Me_4Si. Purification of synthesized compounds were made using columns of silica gel (Silica gel 60, 70–230 mesh, E. Merck, Darmastadt, Germany), with appropriate solvents. Anhydrous Na_2SO_4 was always used as the drying agent. Chemicals were purchased from Sigma-Aldrich Fluka (St Quentin Fallavier, France), Lancaster Synthesis (Bischheim, France) or Avocado (La Tour du Pin, France).

Chemistry

The synthesis of mono *N*-substituted imidazolidin-2-ones **8**–**13** and di-*N*-substituted imidazolidin-2-ones **14**–**22** from ureas **2**–**7** is shown in Scheme 1.

1-(2-Chloroethyl)-3-(2-methoxy-5-trifluoromethylphenyl)urea (2)

To a solution of 2-methoxy-5-trifluoromethylaniline (3 g, 15.69 mmol.) in chloroform (100 mL) was added 2-chloroethyl isocyanate (1.35 mL, 15.69 mmo). The mixture was refluxed for 30 min, and then the solvent was removed under reduced pressure. The crystalline residue was recrystallized from diethyl ether to give compound **2** as a white powder (3.70 g, 80%), m.p. 140°C.; IR (KBr) cm^{-1}, 3381 (NH), 1660 (NC*O*N); ^{1}H NMR ($CDCl_3$) δ ppm 3.63–3.73 (m, 4H, CH_2-CH_2), 3.92 (s, 3H, OCH_3), 5.27 (bs, 1H, $NHCH_2$), 6.90 (d, JH^3H^4 = 8.5 Hz, 1H, H^3), 7.04 (s, 1H, H^6), 7.25 (d, JH^3H^4 = 8.5 Hz, 1H, H^4), 8.45 (s, 1H, R^1NH). Anal. ($C_{11}H_{12}ClF_3N_2O_2$) C, H, N.

Ureas **3**–**7** were also synthesized according to this procedure, with a reflux time of 30 min–1 h.

1-(2-Chloroethyl)-3-(3-chloro-4-fluorophenyl)urea (3)

This compound was obtained as a white powder after recrystallization from diethyl ether (87%), m.p. 116°C. IR (KBr) cm^{-1}, 1646 (NC*O*N), 1057 (C—F), 823 (C—Cl); ^{1}H NMR ($CDCl_3$) δ ppm 3.44 (t, J = 5.5 Hz, 2H, CH_2Cl), 3.69 (d, J = 5.5 Hz, J′ = 5.5 Hz, 2H, NHC*H*$_2$), 6.53 (t, J′ = 5.5 Hz 1H, N*H*CH_2), 7.26–7.35 (m, 2H, H^2 and H^5), 7.79 (dd, JH^2H^6 = 2.1 Hz, JH^6H^5 = 6.8 Hz, 1H, H^6), 8.91 (s, 1H, R^1NH). Anal. ($C_9H_9Cl_2FN_2O_2$) C, H, N.

1-(2-Chloroethyl)-3-(4-trifluoromethylphenyl)urea (4)

Recrystallized from diethyl ether (75%), m.p. 132°C. IR (KBr) cm^{-1}, 3356 (NH), 1641 (NC*O*N); ^{1}H NMR ($CDCl_3$) δ ppm 3.59–3.70 (m, 4H, CH_2—CH_2), 5.66 (bs, 1H, N*H*CH_2), 7.30 (s, 1H, R^1NH), 7.40 (d, JH^2H^3 = JH^6H^5 = 8.5 Hz, 2H, H^2 and H^6), 7.50 (d, 2H, H^3 and H^5). Anal. ($C_{10}H_{10}ClF_3N_2O$) C, H, N.

1-(2-Chloroehtyl)-3-(3-chloro-4-cyanophenyl)-urea (**5**)

Obtained as a white powder after recrystallization from diethyl ether (62%), m.p. 131°C. IR (KBr) cm^{-1}, 3327 (NH), 2228 (CN), 1656 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.60–3.70 (m, 4H, CH_2—CH_2), 6.01 (bs, 1H, $NHCH_2$), 7.34 (dd, $JH^6H^5 = 8.6$ Hz, $JH^6H^2 = 2.0$ Hz, 1H, H^6), 7.52 (d, 1H, H^5), 7.71 (d, 1H, H^2), 8.13 (s, 1H, R^1NH). Anal.($C_{10}H_9Cl_2N_3O$) C, H, N.

1-(2-Chloroehtyl)-3-[4-(4-morpholino)phenyl]-urea (**6**)

Crystalline powder obtained after recrystallization from diisopropyl ether (86%), m.p. 146°C. IR (KBr) cm^{-1}, 3329 (NH), 1640 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.01 (s, 4H, CH_2NCH_2), 3.44 (m, 2H, CH_2Cl), 3.68 (m, 2H, $NHCH_2$), 3.74 (s, 4H, CH_2OCH_2), 6.34 (bs, 1H, $NHCH_2$), 6.86 (d, $JH^2H^3 = JH^6H^5 = 8.6$ Hz, 2H, H^2 and H^6), 7.29 (d, 2H, H^3 and H^5), 8.43 (s, 1H, R^1NH). Anal. ($C_{13}H_{18}ClN_3O_2$) C, H, N.

1-(2-Chloroethyl)-3-[2-(4-morpholino)phenyl]-urea (**7**)

Obtained after recrystallization from diethyl ether (82%), m.p. 149°C. IR (KBr) cm^{-1}, 3330 (NH), 1641 (NCON), ^1H NMR ($CDCl_3$) δ ppm 2.88 (m, 4H, CH_2NCH_2), 3.58–3.70 (m, 4H CH_2—CH_2), 3.86 (m, 4H, CH_2OCH_2), 5.77 (bs, 1H, $NHCH_2$), 7.00–7.19 (m, 3H, H^3, H^4 and H^5), 7.52 (s, 1H, R^1NH), 7.91 (dd, $JH^6H^5 = 8.0$ Hz, $JH^6H^4 = 1.5$ Hz, 1H, H^6). Anal. ($C_{13}H_{18}ClN_3O_2$) C, H, N.

1-(2-Methoxy-5-trifluoromethylphenyl)imidazolidin-2-one (8)

To a solution of urea **2** (2 g, 6.74 mmol) in acetonitrile (50 mL) was added cesium carbonate (3 g, 6.74 mmol). The mixture was refluxed for 3.5 h and after filtration, the filtrate solvent was removed under reduced pressure. Crystallization of the oily residue from diethyl ether gave compound **8** as a white powder (0.96 g, 55%), m.p. 120°C. IR (KBr) cm^{-1}, 1690 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.59 (t, $JH^aH^b = 7.3$ Hz, 2H, H^b), 3.88 (t, 2H, H^a), 3.91 (s, 3H, OCH_3), 5.31 (s, 1H, NH), 6.96 (d, $JH^3H^4 = 8.6$ Hz, 1H, H^3), 7.51 (d, 1H, H^4), 7.64 (s, 1H, H^6). Anal. ($C_{11}H_{11}F_3N_2O_2$) C, H, N.

The other imidazolidinones **9**–**13** were synthesized according to this general procedure.

1-(3-Chloro-4-fluorophenyl)imidazolidin-2-one (**9**)

Recrystallized from diethyl ether as a white powder (87%), m.p. 161°C. IR (KBr) cm^{-1}, 1687 (NCON), 1025 (CF), 760 (CCl); ^1H NMR ($CDCl_3$) δ ppm 3.43 (t, $JH^aH^b = 8.0$ Hz, 2H, H^b), 3.87 (t, 2H, H^a), 7.16 (s, 1H, NH), 7.37–7.50 (m, 2H, H^5 and H^6), 7.89 (m, 1H, H^2). Anal. ($C_9H_8ClFN_2O$) C, H, N.

1-(4-Trifluoromethylphenyl)imidazolidin-2-one (**10**)

Obtained as a white crystalline powder by recrystallization from diethyl ether (70%), m.p. 170°C. IR (KBr) cm^{-1}, 1703 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.63 (t, $JH^aH^b = 7.2$ Hz, 2H, H^b), 3.93 (t, 2H, H^a), 5.89 (s, 1H, NH), 7.58 (d, $JH^2H^3 = JH^5H^6 = 8.9$ Hz, 2H, H^2 and H^6), 7.66 (d, 2H, H^3 and H^5). Anal. ($C_{10}H_9F_3N_2O$) C, H, N.

1-(3-Chloro-4-cyanophenyl)imidazolidin-2-one (**11**)

Recrystallized from diethylether (86%), m.p. 140°C. IR (KBr) cm^{-1}, 2224 (CN), 1718 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.67 (t, $JH^aH^b = 7.4$ Hz, 2H, H^b), 3.99 (t, 2H, H^a), 7.58 (s, 1H, NH), 7.64 (dd, $JH^6H^5 = 8.8$ Hz, $JH^6H^2 = 2.1$ Hz, 1H, H^6), 7.90 (d, $JH^5H^6 = 8.8$ Hz, 1H, H^5,), 8.04 (d, 1H, H^2). Anal. ($C_{10}H_8ClN_3O$) C, H, N.

1-[2-(4-Morpholino)phenyl]imidazolidin-2-one (**12**)

Recrystallized from diisopropyl ether (57%), m.p. 77°C. IR (KBr) cm^{-1}, 1684 (NCON); ^1H NMR ($CDCl_3$) δ ppm 2.87 (t, J = 4.6 Hz, 4H, CH_2NCH_2), 3.86 (t, J = 4.6 Hz, 4H, CH_2OCH_2), 3.98 (t, $JH^aH^b = 8.5$ Hz, 2H, H^b), 4.32 (t, 2H, H^a), 6.97 (ddd, $JH^6H^4 = 1.3$ Hz, $JH^5H^4 = JH^3H^4 = 7.1$ Hz, 1H, H^4), 7.16 (m, 2H, H^3 and H^5), 7.52 (bs, 1H, NH), 8.19 (d, $JH^6H^5 = 8.1$ Hz, 1H, H^6). Anal. ($C_{13}H_{17}N_3O_2$) C, H, N.

1-[4-(4-morpholino)phenyl]imidazolidin-2-one (**13**)

Recrystallized from diisopropyl ether (80%) as a quite white powder, m.p. 185°C. IR (KBr) cm^{-1}, 1692 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.07 (t, J = 4.8 Hz, 4H, CH_2NCH_2), 3.80 (t, $JH^aH^b = 8.3$ Hz, 2H, H^b), 3.85 (t, J = 4.8 Hz, 4H, CH_2OCH_2), 4.35 (t, 2H, H^a), 6.85 (d, $JH^2H^3 = JH^6H^5 = 8.9$ Hz, 2H, H^2 and H^6), 7.20 (d, 2H, H^3 and H^5). Anal. ($C_{13}H_{17}N_3O_2$) C, H, N.

1-(2-Methoxy-5-trifluoromethylphenyl)-3-(4-bromobenzyl)imidazolidin-2-one (14)

Imidazolidinone **8** (0.8 g, 3.73 mmol) was dissolved in DMF (2 mL) at 0°C. Sodium hydride (0.45 g of a 60% dispersion in mineral oil, 11.19 mmol.) was added, the mixture was stirred for 15 min at 0°C and then 4-bromobenzyl bromide (0.93 g, 3.73 mmol.) was added. The mixture was stirred at room temperature for 30 min, water (50 mL) was added, and the resulting mixture washed with diethylether (3 × 80 mL). The organic fractions were collected, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Recrystallisation of the obtained residue from diethyl ether gave 0.51 g of compound **14** as a white powder (24%), m.p. 96°C. IR (KBr) cm^{-1}, 1706 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.35 (m, 2H, H^b), 3.76 (m, 2H, H^a), 3.90

(s, 3H, OCH_3), 4.42 (s, 2H, CH_2Ph), 6.99 (d, $JH^4H^3 = 8.5\,Hz$, 1H, H^3), 7.23 (d, $JH^{3'}JH^{2'} = JH^{5'}H^{6'} = 8.5\,Hz$, 2H, $H^{2'}$ and $H^{6'}$), 7.47–7.51 (m, 3H, $H^{3'}$, $H^{5'}$ and H^4), 7.67 (s, 1H, H^6). Anal. ($C_{18}H_{16}BrF_3N_2O_2$) C, H, N.

The other disubstituted imidazolidinones **15**–**22** were synthesized according to the procedure described for the synthesis of compound **14**.

1-(2-Methoxy-5-trifluoromethylphenyl)-3-(2-bromobenzyl)imidazolidin-2-one (**15**)

Recrystallized from diethyl ether (40%), m.p. 98°C. IR (KBr) cm^{-1}, 1690 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.47 (t, $JH^aH^b = 7.2\,Hz$, 2H, H^b), 3.80 (t, 2H, H^a), 3.91 (s, 3H, OCH_3), 4.62 (s, 2H CH_2Ph), 7.01 (d, $JH^4H^3 = 8.7\,Hz$, 1H, H^3), 7.18 (dd, $JH^{3'}H^{4'} = JH^{4'}H^{5'} = 7.4\,Hz$, 1H, $H^{4'}$), 7.34 (dd, 1H, $H^{5'}$), 7.48 (m, 2H, $H^{3'}$ and $H^{6'}$), 7.58 (d, 1H, H^4), 7.68 (s, 1H, H^6). Anal. $C_{18}H_{16}BrF_3N_2O_2$) C,H,N.

1-(3-Chloro-4-fluorophenyl)-3-(4-bromobenzyl)-imidazolidin-2-one (**16**)

Recrystallized from diethyl ether as a white powder (36%), m.p. 102°C. IR (KBr) cm^{-1}, 1703 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.37 (t, $JH^aH^b = 7.6\,Hz$, 2H, H^b), 3.77 (t, 2H, H^a), 4.43 (s, 2H, CH_2Bn), 7.11 (dd, $JH^5H^6 = 9.0\,Hz$, $JH^5F = 9.0\,Hz$, 1H, H^5), 7.20 (d, $JH^{3'}H^{2'} = JH^{5'}H^{6'} = 8.4\,Hz$, 2H, $H^{2'}$ and $H^{6'}$), 7.44 (ddd, $JH^2H^6 = 2.9\,Hz$, $JH^6H^5 = 9.0\,Hz$, $JH^6F = 4.0\,Hz$, 1H, H^6), 7.49 (d, 2H, $H^{3'}$ and $H^{5'}$), 7.68 (dd, $JH^2F = 6.4\,Hz$, 1H, H^2). Anal. $C_{16}H_{13}BrClFN_2O$) C, H, N.

1-(3-Chloro-4-fluorophenyl)-3-(2-bromobenzyl)-imidazolidin-2-one (**17**)

Recrystallised from diethyl ether (45%), m.p. 144°C. IR (KBr) cm^{-1}, 1702 (NCON), ^1H NMR ($CDCl_3$) δ ppm 3.48 (t, $JH^aH^b = 7.2\,Hz$, 2H, H^b), 3.79 (t, 2H, H^a), 4.63 (s, 2H, CH_2Ph), 7.11 (dd, $JH^5H^6 = 9.0\,Hz$, $JH^5F = 9.0\,Hz$, 1H, H^5), 7.19 (ddd, $JH^{4'}H^{3'} = JH^{4'}H^{5'} = 7.9\,Hz$, $JH^{4'}H^{2'} = 1.9\,Hz$, 1H, $H^{4'}$), 7.32 (ddd, $JH^{5'}H^{6'} = 7.1\,Hz$, $JH^{3'}H^{5'} = 1.1\,Hz$, 1H, $H^{5'}$), 7.41 (dd, $JH^{4'}H^{2'} = 1.9\,Hz$, 1H, $H^{6'}$), 7.46 (ddd, $JH^2H^6 = 2.8\,Hz$, $JH^6F = 4.0\,Hz$, 1H, H^6), 7.59 (dd, 1H, $H^{3'}$), 7.69 (dd, $JH^2F = 6.4\,Hz$, 1H, H^2). Anal. ($C_{16}H_{13}BrClN_3O$) C, H, N.

1-(3-Chloro-4-cyanophenyl)-3-(4-bromobenzyl)-imidazolidin-2-one (**18**)

Recrystallised from diethyl ether (46%), m.p. 194°C. IR (KBr) cm^{-1}, 1705 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.43 (t, $JH^aH^b = 7.6\,Hz$, 2H, H^b), 3.83 (t, 2H, H^a), 4.45 (s, 2H, CH_2Ph), 7.19 (d, $JH^{3'}H^{2'} = JH^{5'}H^{6'} = 8.3\,Hz$, 2H, $H^{2'}$ and $H^{6'}$), 7.50 (d, 2H, $H^{3'}$ and $H^{5'}$), 7.60 (s, 2H, H^5 and H^6), 7.84 (s, 1H, H^2). Anal. ($C_{17}H_{13}BrClN_3O$) C, H, N.

1-(3-Chloro-4-cyanophenyl)-3-(2-bromobenzyl)-imidazolidin-2-one (**19**)

Recrystallized from diethyl ether (37%), m.p. 180°C.. IR (KBr) cm^{-1}, 1710 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.53 (t, $JH^aH^b = 7.3\,Hz$, 2H, H^b), 3.84 (t, 2H, H^a), 4.65 (s, 2H, CH_2Ph), 7.20 (ddd, $JH^{4'}H^{5'} = JH^{4'}H^{3'} = 7.8\,Hz$, $JH^{4'}H^{6'} = 1.8\,Hz$, 1H, $H^{4'}$), 7.33 (ddd, $JH^{5'}H^{6'} = 7.6\,Hz$, 1H, $H^{5'}$), 7.40 (dd, 1H, $H^{6'}$), 7.60–7.62 (m, 3H, H^5, H^6 and $H^{3'}$), 7.85 (s, 1H, H^2). Anal. ($C_{17}H_{13}BrClN_3O$) C, H, N.

1-(2-(4-Morpholino)phenyl)-3-(4-bromobenzyl)-imidazolidin-2-one (**20**)

White powder recrystallized from diisopropyl ether (40%), m.p. 144°C. IR (KBr) cm^{-1}, 1646 (NCON)); ^1H NMR ($CDCl_3$) δ ppm 2.98 (m, 4H, CH_2NCH_2), 3.86 (m, 6H, H^b and CH_2OCH_2), 4.30 (t, $JH^aH^b = 8.5\,Hz$, 2H, H^a), 4.92 (s, 2H, CH_2Ph), 6.93 (m, 2H, H^3 and H^5), 7.02 (d, $JH^5H^6 = 7.8\,Hz$, 1H, H^6), 7.09 (d, $JH^{5'}H^{6'} = JH^{2'}H^{3'} = 8.3\,Hz$, 2H, $H^{2'}$ and $H^{6'}$), 7.20 (m, 1H, H^4), 7.35 (d, 2H, $H^{3'}$ and $H^{5'}$). Anal. ($C_{20}H_{22}BrN_3O_2$) C, H, N.

1-[4-(4-Morpholino)phenyl]-3-(4-bromobenzyl)-imidazolidin-2-one (**21**)

Recrystallized from diethyl ether (28%), m.p. 172°C.. IR (KBr) cm^{-1}, 1659 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.10 (t, J = 4.7 Hz, 4H, CH_2NCH_2), 3.35 (t, $JH^aH^b = 7.8\,Hz$, 2H, H^b), 3.86 (t, J = 4.7 Hz, 4H, CH_2OCH_2), 4.31 (t, 2H, H^a), 4.53 (s, 2H, CH_2Ph), 6.88 (d, $JH^2H^3 = JH^6H^5 = 8.8\,Hz$, 2H, H^2 and H^6), 7.07 (d, 2H, H^3 and H^5), 7.27 (d, $JH^{2'}H^{3'} = JH^{5'}H^{6'} = 8.3\,Hz$, 2H, $H^{2'}$ and $H^{6'}$), 7.50 (d, 2H, $H^{3'}$ and $H^{5'}$). Anal. ($C_{20}H_{22}BrN_3O_3$) C, H, N.

1-[4-(4-Morpholino)phenyl]-3-(2-bromobenzyl)-imidazolidin-2-one (**22**)

Recrystallized from diisopropyl ether (57%), m.p. 156°C. IR (KBr) cm^{-1}, 1665 (NCON); ^1H NMR ($CDCl_3$) δ ppm 3.10 (t, J = 4.7 Hz, 4H, CH_2NCH_2), 3.46 (t, $JH^aH^b = 7.7\,Hz$, 2H, H^b), 3.87 (t, J = 4.7 Hz, 4H, CH_2OCH_2), 4.35 (t, 2H, H^a), 4.73 (s, 2H, CH_2Ph), 6.86 (d, $JH^2H^3 = JH^6H^5 = 8.9\,Hz$, 2H, H^2 and H^6), 7.04 (d, 2H, H^3 and H^5), 7.17 (ddd, $JH^{4'}H^{6'} = 1.6\,Hz$, $JH^{3'}H^{4'} = 7.9\,Hz$, $JH^{4'}H^{5'} = 7.8\,Hz$, 1H, H^{4s}), 7.33 (ddd, $JH^{5'}H^{6'} = 7.6\,Hz$, $JH^{3'}H^{5'} = 1.1\,Hz$, 1H, $H^{5'}$), 7.52 (dd, 1H, $H^{6'}$), 7.58 (dd, 1H, $H^{3'}$). Anal. ($C_{20}H_{22}BrN_3O_2$) C, H, N.

Pharmacology

Drugs

All imidazolidinone derivatives were solubilized in DMSO to a stock concentration of 50 mM and further diluted in RPMI medium (Sigma) for *in vitro* experiments. Final concentrations of DMSO never exceeded 0.2%. Cyclosporin A (CsA) (Tocris, Illkirch, France) was dissolved in absolute ethanol containing 2% tween 80 and further diluted in RPMI medium for *in vitro* experiments.

Splenocyte Proliferation

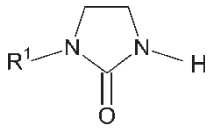
Female C57/BL6 mice (Janvier, Laval, France) 8.9 weeks old were used for experiments. The mice were exsanguinated and their spleens were excised and placed in sterile Petri dishes containing HBSS (Sigma, St Quentin Fallavier, France) medium. Spleens were forcefully flushed with HBBS using a syringe and the spleen suspension was then treated with buffer containing 0.02 M Tris–HCl and 0.14 M NH_4Cl to lyse red blood cells. Cells were washed twice with HBSS medium and subsequently suspended in RPMI medium complemented with 1% L-glutamine (Gibco BRL, Paisley, Scotland) and 10% heat inactivated FCS (Sigma) and 50 μM M-mercaptoethanol (Sigma). Spleen cells were seeded at densities of 1.5×10^5/well in U-bottom 96-well culture plates containing the imidazolidinone derivatives (30 μM and 100 μM) or CsA and cultured at 37°C in 5% CO_2 in a final volume of 150 μL of complete RPMI medium. Cell proliferation was assessed in sextuplicate, after 72 h of culture, by MTT method based on the tetrazolium salt reduction in the presence of mitochondrial dehydrogenases. Absorbance was determined at 570 nm with a microplate reader (Dynex Technologies, Guyancourt, France).

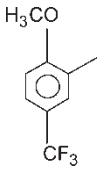
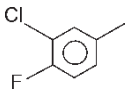
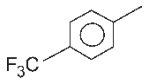
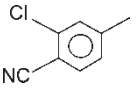
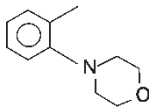
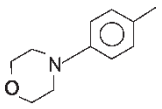
RESULTS AND DISCUSSION

In preliminary work, some monosubstituted imidazolidin-2-ones were synthesized by a "one-pot" method, without isolation of intermediate ureas. However, it was very difficult to purify the expected compound, and corresponding yields were very poor. So, we have preferred a more efficient two-step method, including isolation and characterization of ureas **2**–**7** and then cyclisation into the imidazolidin-2-ones **8**–**13**.

The effect of drugs on mouse splenocytes proliferation was examined in order to determine the immunosuppressive potential with a rapid, low-cost *in vitro* test. Freshly isolated spleen cells were stimulated with 1 μg/mL mitogen, ConA, for 72 h in the presence of different doses of drugs. Splenocytes were also treated with CsA (5 μM) as a positive control. The results are shown in Tables I and II. Among the 17 tested compounds, seven were active. Five of them, **11**, **14**, **15**, **16**, **20** showed maximal inhibition of proliferation at 90 μM, identical to that obtained with the optimal concentration (5 μM) of CsA, and two, **9** and **10** gave a lower percentage inhibition: 47 and 67% respctively. Generally speaking, it seems that N^3-substitution (R^2) by a 4-bromobenzyl group was favourable; for example mono substituted compound **8** was inactive, whereas the corresponding **14** and **15** disubstituted

TABLE I Inhibition of the mouse splenocytes ConA-induced proliferation by mono-*N*-substituted imidazolidin-2-ones **8**–**13**



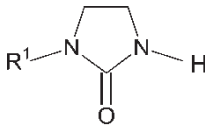
Compound	R^1	Percentage inhibition at 90 μM	Percentage inhibition at 30 μM
8		i	i
9		47	ne
10		i	i
11		100	ne
12		26	2
13		i	i
Ciclosporin A (5 μM)		100	

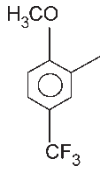
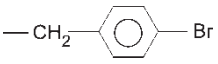
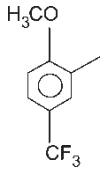
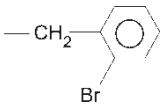
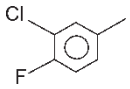
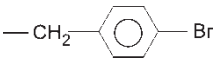
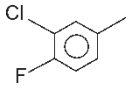
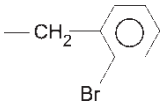
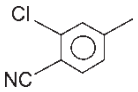
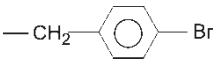
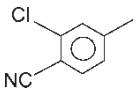
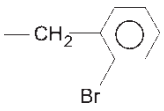
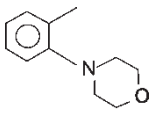
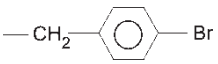
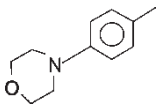
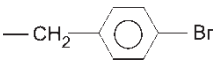
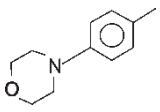
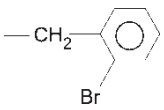
i: inactive; ne: not evaluated.

derivatives had a high level of inhibition of splenocytes proliferation (100%), at 90 μM. Nevertheless, in the case of compound **11**, a N^3-benzylation reaction had a significant deleterious effect: the 4-bromobenzyl derivative **18** was less active than **11** and the 2-bromobenzyl analogue **19** totally inactive. Comparison of the percentage inhibition by compounds **20** and **21** (100% and 19%) point out the favourable effect of a morpholinyl moiety positioned *ortho* (instead of *para*) to the phenyl group (R^1).

In summary, our data firstly demonstrated *in vitro* immunosuppression by imidazolidinone derivatives by showing inhibition of splenocytes proliferation after ConA stimulation. Pharmacomodulation allowed access to several active compounds. Other pharmacological tests *in vitro*, on human T cells, and *in vivo*, in animal models, need to be performed in order to confirm this immunosuppressive activity.

TABLE II Inhibition of the mouse splenocytes ConA-induced proliferation by di-N^1, N^3-substituted imidazolidin-2-ones **14**–**22**



Compound	R^1	R^2	Percentage inhibition at 90 μM	Percentage inhibition at 30 μM
14			100	97
15			100	ne
16			100	i
17			i	i
18			67	ne
19			i	i
20			100	100
21			19	7
22			16	3

i: inactive; ne: not evaluated.

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