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Short communication

Synthetic *N*-pyridinyl(methyl)-indol-3-ylpropanamides as new potential immunosuppressive agents

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Abstract

Several *N*-pyridinyl(methyl)-indol-3-ylpropanamides were synthesized and pharmacological evaluations of their immunosuppressive potential were performed. Among thirteen compounds tested in vitro on murine T proliferation, three showed interesting inhibiting activity. For the most active compound (propanamide **18**), immunosuppressive activity was documented both in vitro on human T lymphocytes proliferation and in vivo on mice delayed-type hypersensitivity. These experimental data demonstrated that these compounds hold potential as immunosuppressive agents.

Keywords: *N*-Pyridinyl(methyl)-indol-3-ylpropanamides; Immunosuppression; T lymphocytes

1. Introduction

The major immunosuppressive drugs in clinical use today are cyclosporin A (CsA), rapamycin, FK506, and mycophenolate mofetil. These drugs act mainly in targeting T cell activation by different mechanisms [1] and their use either alone or in combination has significantly improved graft survival [2–5]. However, these agents are less than perfect as their therapeutic activity is often associated with significant side effects including nephrotoxicity, neurotoxicity, infection, cancer, hyperlipidemia and hypertension that limit their clinical use [6–10]. Furthermore, chronic rejection is not satisfactorily controlled with current immunosuppressants [11]. Thus there is a pressing need for novel immunomodulatory drugs to develop alternative therapeutics and intense screening efforts of natural and synthetic compounds are therefore ongoing in our laboratory to identify new immunosuppressants [12,13]. In the present study, we investigated the potential immunosuppressive effects of new synthetic *N*-pyridinyl (methyl)-indol-3-ylpropanamides. In previous studies, we described the synthesis and biological evaluation of *N*-[pyridinyl (methyl)-N^1-substituted-indol-3-yl]alkanamides (Scheme 1), identified as novel non acidic anti-inflammatory agents [14–17]. In this series, the indol-3-ylpropanamides exhibited an anti-edematous effect after oral and topical administrations in the tetradecanoyl phorbol acetate (TPA)-induced mouse ear-swelling test. Surprisingly, results of pharmacological screenings revealed that some of these compounds significantly inhibited splenocyte proliferation in vitro. These results prompted us to carry out some pharmacomodulations in these series and to investigate the potential immunosuppressive effects of these new synthetic compounds. We thus analyzed the in vitro effects of *N*-pyridinyl(methyl)-indol-3-ylpropanamides on

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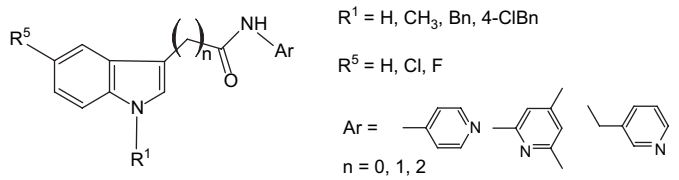


Scheme 1. General structure of indol-3-ylalkanamides.

proliferation of mouse and human lymphocytes induced by mitogens (concanavalin A (ConA) and phytohemagglutinin (PHA), respectively). The most active compound, **18**, was further studied in vivo on a delayed-type hypersensitivity (DTH) induced in mice by sheep red blood cells (SRBC). Our data clearly demonstrate its immunosuppressive activity.

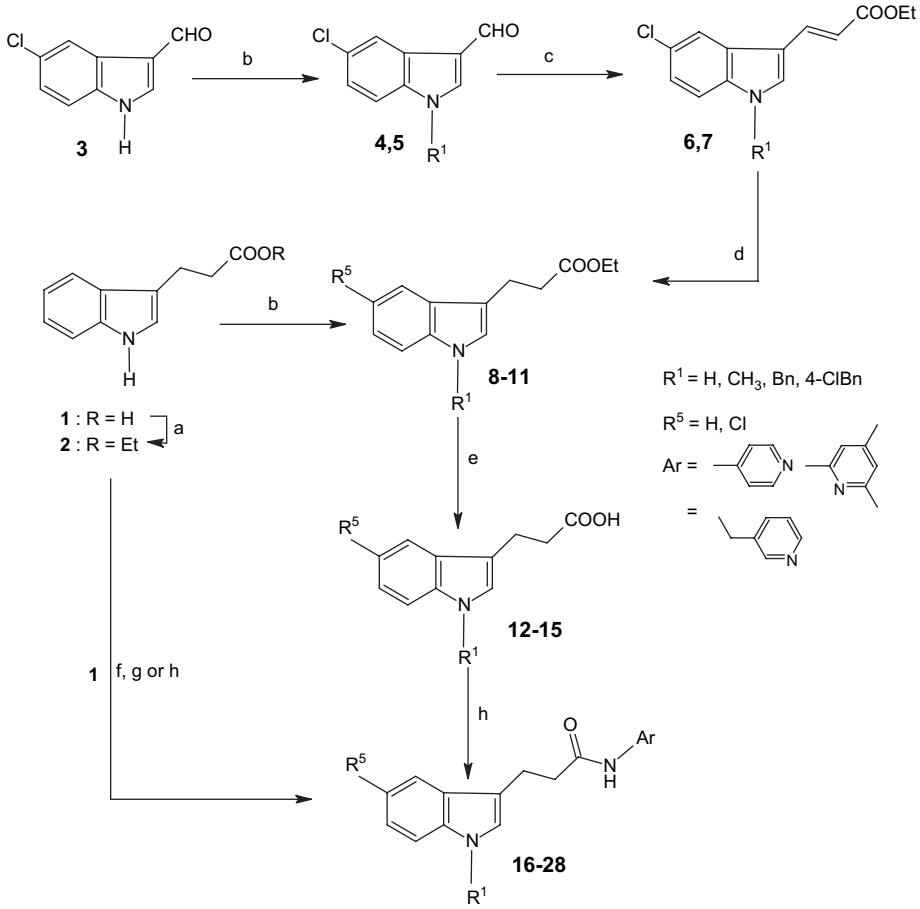
2. Chemistry

The target *N*-pyridinyl(methyl)-indol-3-ylpropanamides **16–28** were synthesized as shown in Scheme 2. The key (indol-3-yl)propanoic acids **12–15** were obtained after saponification of the corresponding esters **8–11** (method e). For the non-substituted compounds at R^5, the esters **8** and **9** were prepared from the commercial (indol-3-yl)propanoic acid **1** by esterification (method a) [16], followed by a N^1-substitution carried out after metallation by Cs_2CO_3 in CH_3CN (method b). For the 5-chloro congeners, the N^1-substitution of 5-chloro(indol-3-yl)carboxaldehyde **3** in the presence of the same couple Cs_2CO_3/CH_3CN, followed by a Wittig–Horner reaction (method c), afforded the (*E*)-propenoates **6** and **7** which were transformed into the corresponding propanoates **10** and **11** by catalytic reduction (method d).

Amidification of the acids **1**, **12–15** was performed after activation by phenyl dichlorophosphate (method f), triphenylphosphine/bromotrichloromethane (method g) or 2-chloro-1-methylpyridinium iodide (method h), leading to the propanamides **16–28**.

3. Biological results

Effects of *N*-pyridinyl(methyl)-indol-3-ylpropanamides **16–28** on splenocyte cell proliferation were examined. Freshly isolated murine splenocytes were stimulated with 1 μg/mL ConA for 72 h in the presence of different doses of target propanamides. Three series were studied: *N*-pyridin-4-yl, *N*-4,6-dimethylpyridin-2-yl and *N*-pyridin-3-ylmethyl

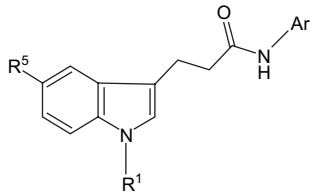


Scheme 2. (a) EtOH, 0.6 M HCl, reflux, 92%; (b) (1) Cs_2CO_3, MeCN, reflux (2) R^1I or R^1Cl; (c) $(EtO)_2POCH_2CO_2Et$, NaH, THF, rt; (d) H_2, Raney Ni, THF, rt; (e) 2 M NaOH, EtOH, reflux; (f) phenyl dichlorophosphate 4-aminopyridine, CH_2Cl_2, rt; (g) Ph_3P, $BrCCl_3$, THF, 2-amino-4,6-dimethylpyridine, reflux; (h) 2-chloro-1-methylpyridinium iodide, RNH_2, Et_3N, CH_2Cl_2, reflux.

(*N*-β-picolyl) series. As shown in Table 1, among the thirteen tested compounds some exerted a moderate to potent inhibitory activity. In the *N*-β-picolyl series, all the compounds except **28**, showed a moderate activity (37–55% inhibition at 90 μM). The introduction of a chlorine atom at R^5 position with the simultaneous presence of a 4-chlorobenzyl group at N^1, exerted a detrimental effect (compound **28**). For other compounds **24**–**27**, it was difficult to establish a structure–activity relationship. In *N*-(4,6-dimethylpyridin-2-yl) series, N^1-4-chlorobenzylation and the presence of a chlorine atom at R^5 were not favourable; only the non-substituted propanamide **20** exerted a significant antiproliferative effect: 76% at 90 μM. The highest inhibition was obtained in the *N*-pyridin-4-yl series with propanamide **18** which showed a strong inhibition (87%) of proliferation at 90 μM, whereas other compounds **16**, **17**, and **19** were not active. In contrast to the *N*-(4,6-dimethylpyridin-2-yl) series, the unsubstituted compound **16** was inactive and N^1-4-chlorobenzylation was favourable (compound **18**). The antiproliferative effect of propanamide **18** was not due to direct toxicity since 95% of the cells were still viable after 48 h incubation with 100 μM of **18** (data not shown).

On the basis of screening data, propanamide **18** was subjected to more in-depth characterization. We first investigated its effect on proliferation of murine splenocytes and human peripheral blood lymphocytes (PBL) using a large range of doses in order to confirm its antiproliferative activity on T cells. Murine splenocytes were stimulated as described above with ConA and freshly isolated PBL were stimulated with

Table 1
Splenocytes proliferation assay data for the indolpropanamide compounds **16**–**28**



Compound	R^1	R^5	Ar	Splenocytes proliferation inhibition % ± SEM at 90 μM[a]	Splenocytes proliferation inhibition % ± SEM at 30 μM[a]
16	H	H		Inactive	Inactive
17	Bn	H		Inactive	Inactive
18	4-ClBn	H		87 ± 1.7	19 ± 3.2
19	CH_3	Cl		Inactive	Inactive
20	H	H		76 ± 1.2	22 ± 2.8
21	Bn	H		Inactive	Inactive
22	4-ClBn	H		Inactive	Inactive
23	4-ClBn	Cl		Inactive	Inactive
24	H	H		38 ± 2.3	38 ± 2.2
25	Bn	H		38 ± 1.5	20 ± 3.6
26	4-ClBn	H		55 ± 2.7	27 ± 1.3
27	CH_3	Cl		37 ± 1.6	22 ± 2.3
28	4-ClBn	Cl		Inactive	Inactive

[a] Cell assay results of one representative experiment out of three performed.

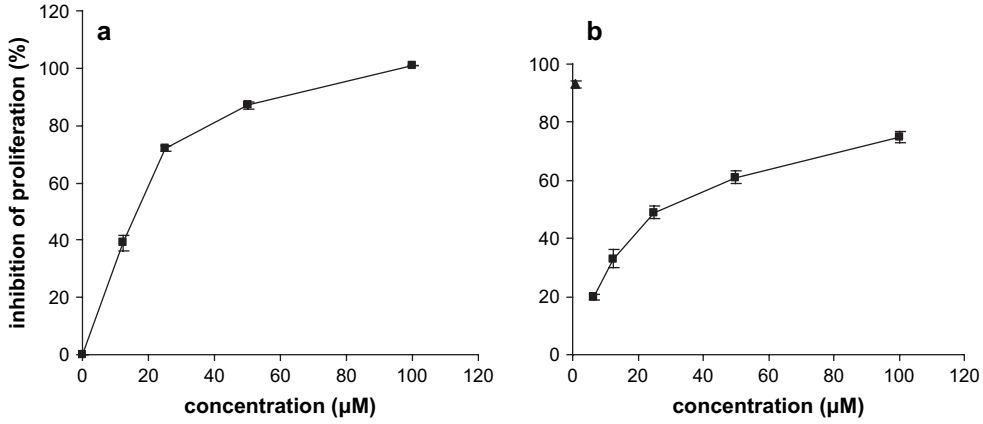


Fig. 1. Effect of compound **18** on T cell proliferation. Murine splenocytes (a) or human PBL (b) were stimulated with either ConA or PHA in the presence of increasing concentrations of compound **18** (filled squares) or CsA at 5 μM (filled triangle). Mean ± SEM of three independent experiments is presented.

0.3 μg/mL PHA for 48 h in the presence of different doses of **18**. As shown in Fig. 1, we found that it inhibited mitogen-dependent T cell proliferation in a dose-dependent manner on both murine splenocytes ($IC_{50} = 17$ μM) (Fig. 1a) and human PBL ($IC_{50} = 25$ μM) (Fig. 1b) proliferation. These data confirmed its immunosuppressive potential.

Finally, we examined the effect of this indolylpropanamide in vivo on delayed-type hypersensitivity (DTH) reaction. Experiments were performed as previously described with slight modifications [18]. Animals were sensitized with 5×10^6 SRBC by I.V. and challenged four days later by injection of SRBC into the hind footpad. Compounds were administrated orally from the day of priming until the day of challenge. Fig. 2 shows the inhibition of DTH footpad swelling in mice treated with compound **18** at 12.5, 25, and 50 mg/kg. Compound **18** significantly inhibited the DTH response in a dose-dependent manner and at the highest dose, inhibition (69%) was comparable to that of CsA (73.9%) at the same dose (data not shown). These results demonstrated that *N*-(pyridin-4-yl)indolylpropanamide **18** exerted significant immunosuppressive effects in vivo.

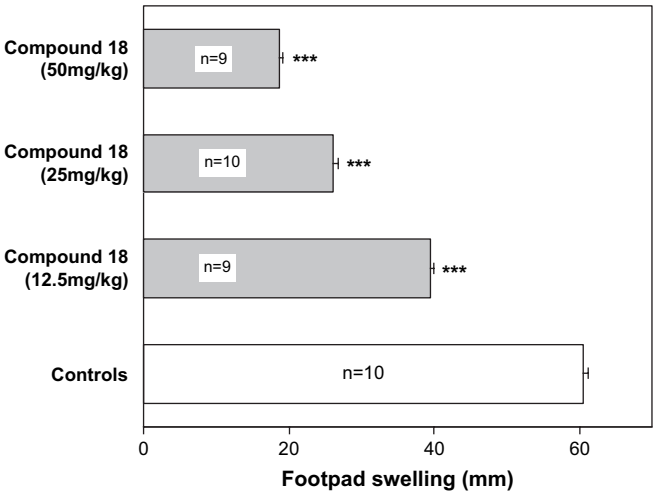


Fig. 2. Effect of compound **18** on DTH response. Each bar represents the mean difference ± SEM in swelling between the two hind footpads, one injected with saline, the other with SRBC, measured 24 h after challenge. Compounds were administered daily per os from the time of priming until SRBC challenge. The number of mice (*n*) in each group is indicated; $^{***}p < 0.001$ compared to the control group.

4. Discussion and conclusion

In this study we highlighted the immunosuppressive effect of indol-3ylpropanamide compounds. Among them, compound **18** was the most active in vitro to inhibit murine and human lymphocytes proliferation induced by mitogens. We also provided evidence that it exerted a significant inhibition of DTH in the mouse (Fig. 2), a response that is mainly due to the proliferation of Th1 lymphocytes [19]. Since the *N*-pyridin-4-yl series had previously been evaluated for anti-inflammatory activity in a mouse ear-swelling test [17], we checked whether their immunosuppressive effect correlated with their anti-inflammatory potential. Indeed, compound **18** displayed a significant inhibition of inflammation but was not the most active in the tested series. However, our data do not support such a correlation since the most active compound in the mouse ear-swelling test (corresponding to compound **17** in our study) was devoid of any significant immunosuppressive effect (Table 1). Our results thus suggest that the anti-inflammatory and immunosuppressive activities of our compounds resulted from the inhibition of distinct pathways. In this respect, it will be interesting to test whether compound **18** inhibits production and/or response of several cytokines involved in Th1 response, like IL-2 or IFNγ. In-depth studies on the mechanism of action will allow us to evaluate if compound **18** acts like cyclosporin A or FK506 by inhibiting IL-2 production or by other mechanism, i.e by inhibiting IL-2 response on T lymphocytes like rapamycin.

The fact that we obtained a significant in vivo activity per os is of great interest since it demonstrated that our compound was orally available. Moreover during the 5-day administration we saw no acute side effect (death of mice or significant weight loss) in treated mice compared to control untreated group. Thus, all these in vivo results deeply increase the attractiveness of this new class of compounds.

In addition to compound **18** of the *N*-pyridin-4-yl series, splenocyte screening test also allowed us to select two other compounds with interesting activity: amides **20** and **26** in *N*-(4,6-dimethylpyridin-2-yl) and *N*-β-picolyl series, respectively. The fact that N^1-4-chlorobenzylation, necessary for emergence of activity in the pyridin-4-yl series (compound **18**), induces a loss of inhibitory activity in the 4,6-dimethylpyridin-2-yl series (**20** → **22**) leads to hypothesize that **18** and **20** could exert their antiproliferative effects by different mechanisms, and prompts us to explore, by in-depth biochemical studies, the transduction pathways that are inhibited by the different compounds.

We provide evidence for the first time that *N*-pyridinyl (methyl)-indol-3-ylpropanamides display potentially interesting immunosuppressive activities both in vitro and in vivo. These compounds may thus represent an interesting alternative for the prevention and treatment of graft rejection and autoimmune diseases.

5. Experimental protocols

5.1. Synthesis

Commercially available solvents and chemicals were used for syntheses without further purification. Reactions were monitored by analytical TLC. It was performed on pre-coated silica gel aluminium plates (0.2 mm, GF_{254}, E. Merck). Spots were located by UV illumination. Crude products were passed through short silica gel columns (silica gel 60, 70–230 mesh, E. Merck). Melting points (m.p.), determined on a Tottoli–Büchi apparatus, were uncorrected. The structure of the products described was confirmed by IR and ^{1}H NMR. IR spectra were run with pellets or a film on NaCl plates using a Perkin–Elmer Paragon PC 1000 spectrometer. ^{1}H NMR spectra were recorded on a Bruker AC250 spectrometer (250 MHz). Chemical shifts are expressed in parts per million (ppm, δ scale) using tetramethylsilane as the internal standard and Me_2SO-d_6 as solvent. When peak multiplicities are reported, the following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), br (broad). Coupling constants are reported in hertz (Hz). Elemental analyses (C, H, N) were performed on a Perkin–Elmer CHN 2400 apparatus and all analyzed compounds are within ±0.4% of the theoretical value. Ethyl 3-(indol-3-yl)propanoate (**2**) was obtained by esterification of **1** (method a) [16]. Propanamides **16**, **20** and **24** have been previously prepared by methods f [14], g [20] and h [16], respectively.

The following experimental procedures are illustrative of the general procedures used to afford the target molecules.

5.1.1. Method b: (5-Chloro-1-methylindol-3-yl)-carboxaldehyde (*4*)

To a solution of 5-chloroindol-3-ylcarboxaldehyde **3** (2.5 g, 13.92 mmol) in dry CH_3CN (25 mL) was added Cs_2CO_3 (9.0 g, 27.84 mmol). The suspension was stirred for 2 h under reflux and cooled at room temperature. Iodomethane (1.7 mL, 27.84 mmol) was then added and the reaction mixture was stirred at room temperature for 30 min. The precipitate was filtered and washed with CH_3CN. The filtrate was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 and the organic layer was washed with water, dried (Na_2SO_4) and concentrated to obtain **4** as a white powder. Yield: 92%; m.p. (dec.): 116–117 °C; IR (KBr, cm^{-1}): ν(C=O) 1660; ^{1}H NMR (DMSO-d_6, ppm): δ 3.93 (s, 3H, CH_3), 7.39 (dd, 1H, H^6, $J = 8.8$, 2.1 Hz), 7.66 (d, 1H, H^7), 8.11 (d, 1H, H^4), 8.38 (s, 1H, H^2), 9.93 (s, 1H, CHO).

5.1.2. Method c: Ethyl 3-(5-chloro-1-methylindol-3-yl)-prop-2-enoate (*6*)

To a suspension of NaH (0.78 g, 19.52 mmol) in dry THF (40 mL) was progressively added ethyl diethylphosphonoacetate (4.38 g, 19.52 mmol). The reaction mixture was stirred until cessation of gas evolution and a solution of (5-chloro-1-methylindol-3-yl)carboxaldehyde **4** (2.52 g, 13.01 mmol) in anhydrous THF (40 mL) was then added. The solution was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue obtained was treated with CH_2Cl_2 (200 mL) and washed with water (2 × 100 mL), dried (Na_2SO_4) and evaporated. Purification by column chromatography on silica gel, eluting with dichloromethane, afforded **6** as a white powder. Yield: 58%; m.p. (dec.): 112–113 °C; IR (KBr, cm^{-1}): ν(C=O) 1695, ν(C=C) 1628; ^{1}H NMR (DMSO-d_6, ppm): δ 1.30 (t, 3H, CH_3, $J = 7.3$ Hz), 3.86 (s, 3H, N–CH_3), 4.20 (q, 2H, OCH_2), 6.39 (d, 1H, CH–CO, $J = 16.2$ Hz), 7.32 (dd, 1H, H^6, $J = 8.5$, 1.8 Hz), 7.60 (d, 1H, H^7), 7.84 (d, 1H, CH), 7.97 (d, 1H, H^4), 8.05 (s, 1H, H^2).

5.1.3. Method d: Ethyl 3-(5-chloro-1-methylindol-3-yl)-propanoate (*10*)

Ethyl 3-(5-chloro-1-methylindol-3-yl)prop-2-enoate **6** (1.3 g, 4.93 mmol) was dissolved in dry THF (150 mL) and a suspension of Raney nickel (1 g) was quickly added. The reaction mixture was purged three times with hydrogen and was stirred at room temperature overnight. The inorganic layer was filtered, washed with THF and the filtrate was removed under reduced pressure to obtain **10** as a yellow oil. Yield: 99%; IR (KBr, cm^{-1}): ν(C=O) 1733; ^{1}H NMR (DMSO-d_6, ppm): δ 1.19 (t, 3H, CH_3, $J = 7.3$ Hz), 2.65 (t, 2H, CH_2–CO, $J = 7.3$ Hz), 2.95 (t, 2H, CH_2), 3.76 (s, 3H, N–CH_3), 4.05 (q, 2H, O–CH_2), 7.16 (dd, 1H, H^6, $J = 8.5$, 1.8 Hz), 7.22 (d, 1H, H^4), 7.43 (d, 1H, H^7), 7.62 (s, 1H, H^2).

5.1.4. Method e: 3-(5-Chloro-1-methylindol-3-yl)propanoic acid (*14*)

A mixture of ethyl 3-(5-chloro-1-methylindol-3-yl)propanoate **10** (1.70 g, 6.40 mmol), ethanol (10 mL) and 2 M aqueous NaOH (10 mL) was refluxed with stirring for 2 h, cooled to room temperature and acidified with 2 M aqueous HCl. The precipitate was then filtered, washed with cold water and dried (P_2O_5). By recrystallization from diisopropylic ether, a pure white powder was obtained. Yield: 79%; IR (KBr, cm^{-1}): ν(O–H) 3100–2600, ν(C=O) 1712; ^{1}H NMR (DMSO-d_6, ppm): δ 2.59 (t, 2H, CH_2–CO, $J = 7.3$ Hz), 2.93

(t, 2H, CH_2), 3.76 (s, 3H, N–CH_3), 7.16 (dd, 1H, H^6, $J = 8.8$, 1.8 Hz), 7.22 (s, 1H, H^2), 7.44 (d, 1H, H^7), 7.61 (d, 1H, H^4).

*5.1.5. Method h: N-(Pyridin-4-yl)-3-(5-chloro-1-methylindol-3-yl)propanamide (**19**)*

To a solution of 3-(5-chloro-1-methylindol-3-yl)propanoic acid **14** (0.40 g, 1.68 mmol) in dry CH_2Cl_2 (50 mL), were added 2-chloro-1-methylpyridinium iodide (0.429 g, 1.68 mmol), triethylamine (0.60 mL, 4.20 mmol) and 4-aminopyridine (0.174 g, 1.85 mmol). The mixture was refluxed for 12 h and after cooling, washed with water, dried over Na_2SO_4 and concentrated. The crude was purified by chromatography on silica gel using dichloromethane–ethanol 95:5 as the eluent. Trituration in diisopropylic ether afforded **19** as a white powder. Yield: 61%; m.p. (dec.): 165–166 °C; IR (KBr, cm^{-1}): ν(NH) 3246, ν(C=O) 1719; ^{1}H NMR (DMSO-d_6, ppm): δ 2.73 (t, 2H, CH_2–CO, $J = 7.9$ Hz), 3.03 (t, 2H, CH_2), 3.76 (s, 3H, N–CH_3), 7.16 (dd, 1H, H^6, $J = 8.8$, 2.4 Hz), 7.23 (s, 1H, H^2), 7.44 (d, 1H, H^7), 7.59 (d, 2H, pyr. H^3, H^5, $J = 5.9$ Hz), 7.66 (d, 1H, H^4), 8.44 (d, 2H, pyr. H^2, H^6), 10.32 (s, 1H, NH). Anal. $C_{17}H_{16}ClN_3O$ (C, H, N).

*5.1.6. N-(Pyridin-4-yl)-3-(1-benzylindol-3-yl)-propanamide (**17**)*

Yield: 69%; m.p. (dec.): 140–141 °C; IR (KBr, cm^{-1}): ν(NH) 3290, ν(C=O) 1690; ^{1}H NMR (DMSO-d_6, ppm): δ 2.76 (t, 2H, CH_2–CO, $J = 7.0$ Hz), 3.08 (t, 2H, CH_2), 5.38 (s, 2H, N–CH_2), 7.15 (m, 7H, H^5, H^6, Bn), 7.31 (s, 1H, H^2), 7.43 (d, 1H, H^7, $J = 7.9$ Hz), 7.60 (m, 3H, H^4, pyr. H^3, H^5), 8.44 (d, 2H, pyr. H^2, H^6, $J = 4.9$ Hz), 10.33 (s, 1H, NH). Anal. $C_{23}H_{21}N_3O$ (C, H, N).

*5.1.7. N-(Pyridin-4-yl)-3-[1-(4-chlorobenzyl)indol-3-yl]-propanamide (**18**)*

White powder; Yield: 64%; m.p. (dec): 137–138 °C; IR (KBr, cm^{-1}): ν(NH) 3280, ν(C=O) 1695; ^{1}H NMR (DMSO-d_6, ppm): δ 2.76 (t, 2H, CH_2–CO, $J = 7.7$ Hz), 3.07 (t, 2H, CH_2), 5.38 (s, 2H, N–CH_2), 7.05 (dd, 1H, H^6, $J = 7.9$, 7.6 Hz), 7.13 (dd, 1H, H^5, $J = 7.6$, 7.1 Hz), 7.17 (d, 2H, Bn. H^2, H^6, $J = 8.4$ Hz), 7.31 (d, 2H, Bn. H^3, H^5), 7.32 (s, 1H, H^2), 7.42 (d, 1H, H^7), 7.60 (d, 2H, pyr. H^3, H^5, $J = 5.0$ Hz), 7.63 (d, 1H, H^4), 8.44 (d, 2H, pyr. H^2, H^6), 10.33 (s, 1H, NH). Anal. $C_{23}H_{20}ClN_3O$ (C, H, N).

*5.1.8. N-(4,6-Dimethylpyridin-2-yl)-3-(1-benzylindol-3-yl)-propanamide (**21**)*

White powder, yield: 52%; m.p. (dec.): 110–111 °C; IR (KBr, cm^{-1}): ν(NH) 3273, ν(C=O) 1669. ^{1}H NMR (DMSO-d_6, ppm): δ 2.29 (s, 3H, γ-CH_3), 2.37 (s, 3H, α-CH_3), 2.77 (t, 2H, CH_2–CO, $J = 7.0$ Hz), 3.04 (t, 2H, CH_2), 5.37 (s, 2H, N–CH_2), 6.82 (s, 1H, pyr. H^5), 7.12 (m, 7H, H^5, H^6, Bn), 7.32 (s, 1H, H^2), 7.42 (d, 1H, H^7, $J = 7.6$ Hz), 7.63 (d, 1H, H^4, $J = 7.0$ Hz), 7.82 (s, 1H, pyr. H^3), 10.40 (s, 1H, NH). Anal. $C_{25}H_{25}N_3O$ (C, H, N).

*5.1.9. N-(4,6-Dimethylpyridin-2-yl)-3-[1-(4-chlorobenzyl)-indol-3-yl]propanamide (**22**)*

Oil, yield: 58%; IR (NaCl, cm^{-1}): ν(NH) 3270, ν(C=O) 1680; ^{1}H NMR (DMSO-d_6, ppm): δ 2.28 (s, 3H, γ-CH_3), 2.37 (s, 3H, α-CH_3), 2.77 (t, 2H, CH_2–CO, $J = 7.3$ Hz), 3.04 (t, 2H, CH_2), 5.38 (s, 2H, N–CH_2), 6.82 (s, 1H, pyr. H^5), 7.09 (m, 4H, H^5, H^6, Bn. H^2, H^6), 7.23 (d, 2H, Bn. H^3, H^5, $J = 8.4$ Hz), 7.32 (s, 1H, H^2), 7.41 (d, 1H, H^7, $J = 7.9$ Hz), 7.63 (d, 1H, H^4, $J = 7.3$ Hz), 7.82 (s, 1H, pyr. H^3), 10.39 (s, 1H, NH). Anal. $C_{25}H_{24}ClN_3O$ (C, H, N).

*5.1.10. N-(4,6-Dimethylpyridin-2-yl)-3-[5-chloro-1-(4-chlorobenzyl)indol-3-yl]propanamide (**23**)*

White powder, yield: 27%; m.p. (dec.): 90–91 °C; IR (KBr, cm^{-1}): ν(NH) 3207, ν(C=O) 1663; ^{1}H NMR (DMSO-d_6, ppm): δ 2.28 (s, 3H, γ-CH_3), 2.37 (s, 3H, α-CH_3), 2.74 (t, 2H, CH_2–CO, $J = 7.3$ Hz), 3.02 (t, 2H, CH_2), 5.39 (s, 2H, CH_2), 6.82 (s, 1H, pyr. H^5), 7.12 (d, 2H, Bn. H^2, H^6, $J = 8.5$ Hz), 7.15 (dd, 1H, H^6, $J = 8.5$, 1.8 Hz), 7.23 (d, 2H, Bn. H^3, H^5), 7.41 (s, 1H, H^2), 7.45 (d, 1H, H^7), 7.71 (d, 1H, H^4), 7.81 (s, 1H, pyr. H^3), 10.39 (s, 1H, NH). Anal. $C_{25}H_{23}Cl_2N_3O$ (C, H, N).

*5.1.11. N-[(Pyridin-3-yl)methyl]-3-(1-benzylindol-3-yl)-propanamide (**25**)*

White powder, yield: 78%; m.p. (dec.): 124–125 °C; IR (KBr, cm^{-1}): ν(NH) 3311, ν(C=O) 1642; ^{1}H NMR (DMSO-d_6, ppm): δ 2.53 (t, 2H, CH_2–CO, $J = 7.5$ Hz), 3.01 (t, 2H, CH_2), 4.31 (d, 2H, NH–CH_2, $J = 5.8$ Hz), 5.37 (s, 2H, N–CH_2), 7.02 (dd, 1H, H^5, $J = 7.9$, 7.0 Hz), 7.12 (dd, 1H, H^6, $J = 7.9$, 7.0 Hz), 7.27 (m, 7H, H^2, pyr. H^5, Bn), 7.42 (d, 1H, H^7), 7.55 (m, 2H, H^4, pyr. H^4), 8.43 (m, 3H, NH, pyr. H^2, pyr. H^6). Anal. $C_{24}H_{23}N_3O$ (C, H, N).

*5.1.12. N-[(Pyridin-3-yl)methyl]-3-[1-(4-chlorobenzyl)-indol-3-yl]propanamide (**26**)*

Yield: 78%; m.p. (dec.): 121–122 °C; IR (KBr, cm^{-1}): ν(NH) 3334, ν(C=O) 1643; ^{1}H NMR (DMSO-d_6, ppm): δ 2.56 (t, 2H, CH_2–CO, $J = 7.5$ Hz), 3.00 (t, 2H, CH_2), 4.31 (d, 2H, $NHCH_2$, $J = 5.9$ Hz), 5.37 (s, 2H, NCH_2), 7.04 (dd, 1H, H^5, $J = 8.0$, 7.6 Hz), 7.13 (dd, 1H, H^6, $J = 8.0$, 7.2 Hz), 7.20 (d, 2H, Bn. H^2, H^6, $J = 8.8$ Hz), 7.30 (dd, 1H, pyr. H^5, $J = 8.0$, 4.8 Hz), 7.38 (d, 2H, Bn. H^3, H^5), 7.41 (d, 1H, H^7), 7.55 (d, 1H, pyr. H^4), 7.59 (d, 1H, H^4), 8.46 (m, 3H, NH, pyr. H^2, H^6). Anal. $C_{24}H_{22}ClN_3O$ (C, H, N).

*5.1.13. N-[(Pyridin-3-yl)methyl]-3-(5-chloro-1-methylindol-3-yl)propanamide (**27**)*

White powder, Yield: 82%; m.p. (dec.): 106–107 °C; IR (KBr, cm^{-1}): ν(NH) 3334, ν(C=O) 1643; ^{1}H NMR (DMSO-d_6, ppm): δ 2.51 (t, 2H, CH_2–CO, $J = 7.2$ Hz), 2.96 (t, 2H, CH_2), 3.75 (s, 3H, NCH_3), 4.31 (d, 2H, NH–CH_2, $J = 6.0$ Hz), 7.14 (dd, 1H, H^6, $J = 8.4$, 2.0 Hz), 7.16 (s, 1H, H^2), 7.32 (dd, 1H, pyr. H^5, $J = 7.6$, 4.8 Hz), 7.44 (d, 1H, H^7), 7.52 (d, 1H, pyr. H^4), 7.63 (d, 1H, H^4), 8.45 (m, 3H, NH, pyr. H^2, H^6). Anal. $C_{18}H_{18}ClN_3O$ (C, H, N).

*5.1.14. N-[(Pyridin-3-yl)methyl]-3-[5-chloro-1-(4-chlorobenzyl)indol-3-yl)propanamide (**28**)*

White powder, yield: 75%; m.p. (dec.): 168–169 °C; IR (KBr, cm^{-1}): ν(NH) 3280, ν(C=O) 1641; ^1H NMR (DMSO-d_6, ppm): δ 2.54 (t, 2H, CH_2–CO, $J = 7.6$ Hz), 2.98 (t, 2H, CH_2), 4.30 (d, 2H, NH–CH_2, $J = 5.8$ Hz), 5.39 (s, 2H, N–CH_2), 7.12 (dd, 1H, H^6, $J = 8.8$, 1.8 Hz), 7.19 (d, 2H, Bn. H^2, H^6, $J = 8.5$ Hz), 7.29 (dd, 1H, pyr. H^5, $J = 7.9$, 4.6 Hz), 7.36 (s, 1H, H^2), 7.38 (d, 2H, Bn. H^3, H^5), 7.46 (d, 1H, H^7), 7.53 (d, 1H, pyr. H^4), 7.66 (d, 1H, H^4), 8.45 (m, 3H, NH, pyr. H^2, H^6). Anal. $C_{24}H_{21}Cl_2N_3O$ (C, H, N).

5.2. Pharmacological activity

5.2.1. Splenocytes proliferation assay

All compounds were solubilized in DMSO and further diluted in RPMI medium (Sigma, St Quentin Fallavier, France) complemented with 1% L-glutamine (Gibco BRL, Paisley, Scotland) and 10% heat inactivated FCS (Sigma) referred as complete medium. Splenocytes were isolated from two spleens of 8-week-old female C57BL/6 (Janvier, Laval, France) mice. Spleens were aseptically harvested and homogenized in a Petri dish containing HBSS medium (Sigma) and splenocytes suspension was hemolysed by buffer containing 20 mM Tris–HCl and 140 mM NH_4Cl. Cells were washed twice with RPMI, subsequently suspended in complete RPMI medium and seeded at densities of 1.5×10^5/well in U-bottom 96-well culture plates (Falcon). Cells were incubated with 1 μg/mL concanavalin A (Sigma) in the presence of several concentrations of studied compounds and cultured at 37 °C in 5% CO_2 in a final volume of 150 μL of complete RPMI medium supplemented with 50 μM mercaptoethanol. Cell proliferation was assessed in sextuplicate after 72 h of culture, by colorimetric detection. Briefly, cells were incubated with 12.5 μg/well of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 4 h at 37 °C. Formazan products were solubilized by 100 μL of lysis buffer (dimethylformamide (1V), SDS 20% (2V), pH 4.7) and overnight incubation at 37 °C. Cell growth was assessed using a MRX microplate reader (Dynex Technologies, Chantilly, USA) with the test wavelength at 570 nm and expressed as optical density (OD) values. The inhibition of splenocytes proliferation was expressed as inhibitory rate [(OD value of control untreated cells − OD value of treated cells)/OD value of control untreated cells group] × 100.

5.2.2. Peripheral blood lymphocyte cells proliferation

Compound **18** was solubilized in DMSO and cyclosporin A (CsA) (Tocris, Illkirch, France) was dissolved in absolute ethanol containing 2% Tween 80. This solution was further diluted in complete RPMI medium. PBL were isolated from 50 to 100 mL fresh blood from healthy donors by Ficoll-Hypaque gradient centrifugation (Eurobio, Les Ulis, France) and washed twice in RPMI medium. Freshly isolated PBL were seeded at densities of 1×10^5/well in U-bottom 96-well culture plates (Falcon). Cells were incubated with 0.3 μg/mL phytohemagglutinin (PHA) (Sigma) in the presence of **18** (6.25–100 μM) 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 48 h of culture by measuring [^3H] thymidine uptake (1 μCi/well) for the last 6–8 h. Cells were harvested using an automated cell harvester, and incorporated radioactivity was assessed by a beta counter.

5.2.3. Delayed-type hypersensitivity

Compound **18** was solubilized in olive oil and CsA was dissolved in olive oil solution containing 3% absolute ethanol. Groups of five female BALB/C mice (Janvier, Laval, France), 8–9 weeks of age, were injected with 5×10^6 sheep red blood cells (SRBC) (BioMérieux, Marcy-l'Etoile, France) in 200 μL PBS into the tail vein. Four days after immunisation, mice were tested for DTH with a subinflammatory challenging dose ($3–4 \times 10^8$ SRBC suspended in 40 μL) injected into the right hind footpad. The left footpad was used as a control and injected with PBS alone. The size of both hind footpads was measured with an "Oditest" (Kroeplin, Germany) 24 h after challenge. DTH reaction was determined by the difference in size between the SRBC- and PBS-injected footpads. Mice were daily treated orally for five days with different doses of compound **18**, from the time of priming until the SRBC challenge. The DTH inhibition index was calculated as the ratio of DTH reaction in the treated animal versus the DTH reaction in control mice. Results are expressed as mean ± SEM. All results were compared statistically using Dunnett unpaired multiple test.

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