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Synthesis of *N*-aryl-3-(indol-3-yl)propanamides and their immunosuppressive activities

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ABSTRACT

N-Aryl-3-(indol-3-yl)propanamides were synthesized and their immunosuppressive activities were evaluated. This study highlighted the promising potency of 3-[1-(4-chlorobenzyl)-1*H*-indol-3-yl]-*N*-(4-nitrophenyl)propanamide **15** which exhibited a significant inhibitory activity on murine splenocytes proliferation assay in vitro and on mice delayed-type hypersensitivity (DTH) assay in vivo.

Cyclosporin A (CsA), tacrolimus (FK506), and sirolimus (rapamycin) are known to be potent immunosuppressive agents. They act by inhibiting T lymphocyte proliferation during immune response.[1] All three compounds are current chemotherapeutic agents, limiting chronic rejection after organ transplantation and improving lifetime of grafts receivers, and successfully used for treatment of autoimmune diseases in clinic.[2] However, these molecules suffer from several side effects such as nephrotoxicity, neurotoxicity, infection, cancer, hyperlipidemia, and hypertension.[3] Thus, the search for new immunosuppressants with a comparable efficacy but with lower toxicity is an important task for medicinal chemistry.

We previously described a series of *N*-pyridinyl(methyl)-(indol-3-yl)propanamides with a promising immunosuppressive activity.[4,5] Our initial structure–activity investigations showed the importance of the benzyl moiety and the nature of the pyridine ring for immunosuppressive potential and this study disclosed compound **1** as a promising lead (Fig. 1). This compound exerted a potent inhibitory activity on murine splenocytes proliferation (87% inhibition at 90 μM, 19% inhibition at 30 μM, compared to 90% inhibition obtained with CsA at 0.5 μM). Previous experiments[5] highlighted its antiproliferative activity in vitro on T lymphocytes (IC_{50} = 17 μM) and a significant immunosuppressive effect in vivo in a model of delayed-type hypersensitivity (DTH) in mouse. We also showed its non-toxicity and its oral bioavailability.

Previously, compound **1** was found to decrease IL2-induced T lymphocyte proliferation by inhibiting preferentially JAK3 kinase over JAK2 (IC_{50} = 52.0 μM and 133.6 μM, respectively).[6] Moreover, compound **1** significantly prolonged rat heart allograft survival demonstrating its in vivo immunosuppressive potential.

The Janus kinases (JAKs), consisting of JAK1, JAK2, JAK3, and TYK2, are an important family of cytoplasmic tyrosine kinases as a consequence of their essential role in cytokine signal transduction.[7] Janus Kinase 3 is a particularly attractive target for therapeutic intervention in the treatment of autoimmune disorders, inflammatory diseases, and organ transplant rejection because, unlike other JAK family members that are widespread, JAK3 expression is restricted to haematopoietic cells.[8] A number of inhibitors of JAK3 have already been described.[9]

Herein, we will report our continuous work consisting of synthesis and immunosuppressive activity evaluation of 3-[1-(4-chlo-

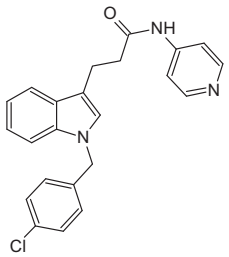

Figure 1. Chemical structure of lead compound **1**.

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robenzyl)-1*H*-indol-3-yl]propanamide derivatives in order to explore the effect of *N*-aryl substitution with compound **1** as the initial lead.

Synthesis of *N*-aryl-3-(indol-3-yl)propanamides **6–15** was performed, as previously described,[4,5] in four or in five steps from commercially available 3-(1*H*-indol-3-yl)propanoic acid **2** (Scheme 1).

To obtain the target amides, the first step was the esterification of 3-(1*H*-indol-3-yl)propanoic acid **2** in ethanol–hydrogen chloride medium at reflux. Ester **3** was further reacted with 4-chlorobenzyl chloride in the presence of Cs_2CO_3 in anhydrous acetonitrile to afford the corresponding *N*-(4-chlorobenzyl)indole derivative **4**. Subsequent hydrolysis under basic conditions followed by the amidation of the resulting propanoic acid **5** with Mukaiyama reagent in refluxing CH_2Cl_2 gave the *N*-aryl-3-(indol-3-yl)propanamides **6–15** in low to moderate yields (17–58%) depending on the nature of the aromatic amines.

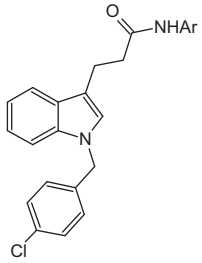
All starting amines were commercially available except 4-aminoquinoline that was prepared by treatment of the 4-quinolone with $POCl_3$ at reflux, in a first step, to afford 4-chloroquinoline in quantitative yield.[10] Nucleophilic displacement of the chlorine atom was performed in the presence of ammonia in phenol by following a literature procedure.[11]

Moreover, catalytic reduction of the nitro functional group of compound **15**, using palladium charcoal in THF at room temperature, yielded amino derivative **16**.

The new *N*-aryl-3-(indol-3-yl)propanamides **6–16** were tested in vitro for their inhibitory activity on concanavalin A (ConA)-induced T cell proliferation with compound **1** as the standard. Freshly isolated murine spleen cells were stimulated with 1 μg/mL ConA for 72 h in the presence of two different doses of the tested compound.[12] The pharmacological results are summarized in Table 1.

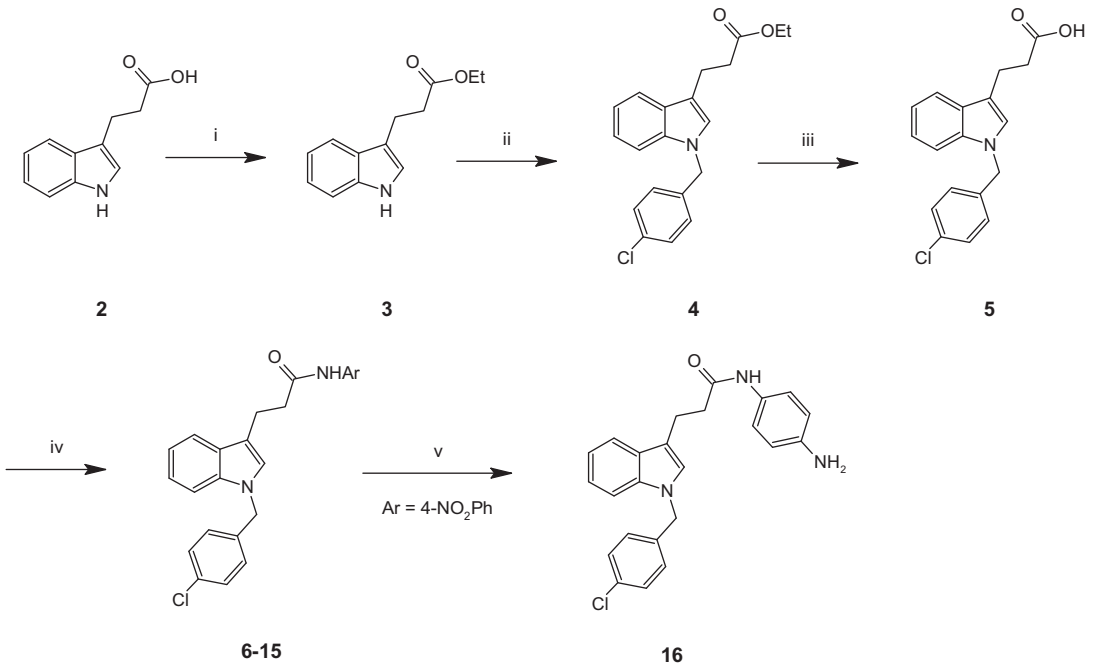
To understand the incidence of the *N*-aryl substitution in the pharmacological activity, diverse alternatives to the 4-pyridyl group (compound **1**) were studied. Replacement of the heteroaromatic ring by the phenyl ring (compound **12**) or by 4-substituted phenyls revealed that the presence of a polar and electro-withdrawing group (compounds **13** and **15**) was a more suitable modification for getting an active entity except for **14** bearing a trifluoromethyl moiety. Indeed, the amino analog **16** displayed no activity but stimulated the proliferation response of cells (data not shown).

Table 1
Splenocytes proliferation assay data for the *N*-aryl-3-(indol-3-yl)propanamide compounds **1**, **6–16**



Compounds	Ar	Splenocytes proliferation	
		Inhibition% ± SEM at 90 μM[a]	Inhibition% ± SEM at 30 μM[a]
1	Pyridin-4-yl	87 ± 1.7	19 ± 3.2
6	Pyridin-3-yl	40 ± 1.6	28 ± 2.8
7	Pyridin-2-yl	26 ± 1.4	na
8	Quinolin-4-yl	37 ± 1.6	20 ± 2.0
9	Quinolin-5-yl	na	na
10	Quinolin-6-yl	86 ± 1.2	na
11	Isoquinolin-5-yl	17 ± 2.1	na
12	Phenyl	na	na
13	4-Cyanophenyl	78 ± 1.0	30 ± 1.2
14	4-Trifluoromethyl-phenyl	18 ± 1.7	nd
15	4-Nitrophenyl	92 ± 1.5	87 ± 1.7
16	4-Aminophenyl	na	na

na = not active.
nd = not determined.
[a] Cell assay results of one representative experiment out of three performed.



Scheme 1. Reagents and conditions: (i) EtOH, HCl, reflux, 95%; (ii) Cs_2CO_3, 4-ClPhCH$_2$Cl, CH_3CN, reflux, 81%; (iii) NaOH 1 N, EtOH, reflux, 94%; (iv) Et_3N, CNMPI, Ar-NH_2, CH_2Cl_2, reflux, 17–58%; (v) H_2 (10 bars), Pd/C 10%, THF, rt, 45%.