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Tetrahydropyridine derivatives with inhibitory activity on the production of proinflammatory cytokines: Part 1

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ABSTRACT

We investigated proinflammatory cytokine TNFα production inhibitors in order to develop novel anti-inflammatory agents. According to the results, we found that **17**, a pyrrole derivative possessing a tetrahydropyridine group at the β-position, showed potent inhibitory activity in vitro (inhibition of lipopolysaccharide (LPS) induced TNFα production in human whole blood, IC_{50} = 1.86 μM) and in vivo (inhibition of LPS induced TNFα production in mice, ID_{50} = 5.98 mg/kg).

Proinflammatory cytokines such as TNFα and IL-1β,[1] are associated with the onset of inflammatory diseases as well as several autoimmune diseases,[2] including rheumatoid arthritis (RA),[3] toxic shock syndrome, osteoarthritis, and inflammatory bowel disease.[4,5] The recent success of anti-cytokine biological agents has demonstrated clinical benefits in the treatment of inflammatory diseases.[6] However, due to the well known disadvantages common to these protein-based therapies, such as high cost and subcutaneous or intravenous administration, orally active small molecules that can effectively act as anti-cytokine agents would clearly be of additional benefit to patients.[5]

The p38 mitogen-activated protein kinase (MAPK) pathway has been proven to play a central role in the regulation of the proinflammatory cytokines, TNFα and IL-1β.[7] The discovery of a series of triaryl-imidazoles as p38 inhibitors, as exemplified by SB203580[7,8] (Fig. 1), was seminal. The clinical proof of concept in rheumatoid arthritis was achieved with VX-745[9] and BIRB-795,[10] which validated the MAP kinase pathway as a useful mechanism for intervention in inflammatory disease.

Merck reported that L-167307 with an imidazole ring of SB203580 modified to a pyrrole ring, showed more potent inhibitory activity in vivo in a rat adjuvant-induced arthritis model (rat AA model).[11] These results suggested that the pyrrole ring could be explored as a possible alternative to the imidazole scaffold.

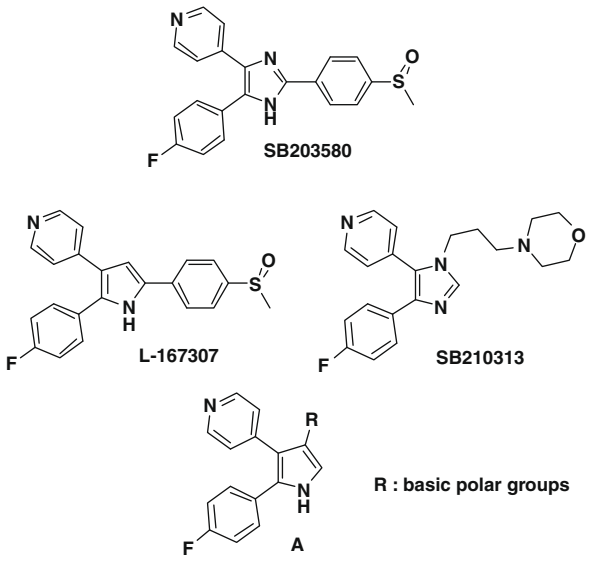

Figure 1.

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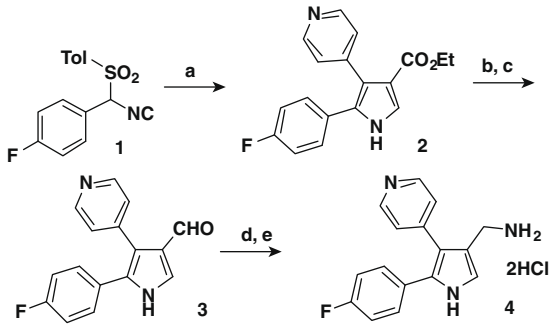
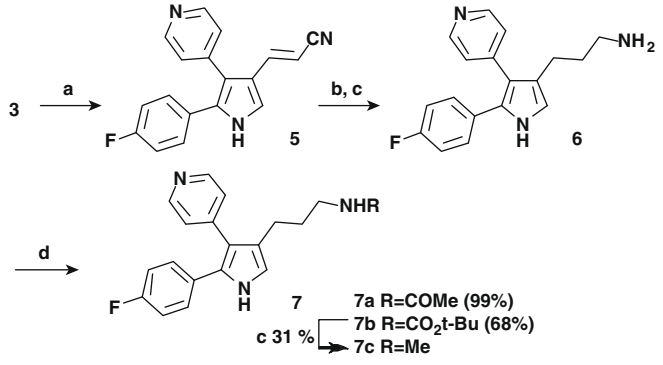
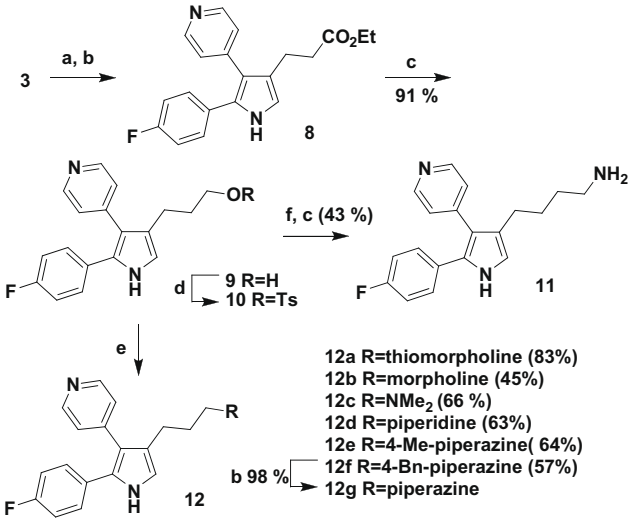


Scheme 1. Reagents and conditions: (a) *n*-BuLi, LiBr, THF, −45 °C then ethyl (2*E*)-3-pyridin-4-ylacrylate, 89%; (b) DIBAL, THF, 0 °C then rt, 99%; (c) MnO_2, DMSO, 50 °C, 69%; (d) $MeONH_2$·HCl, Et_3N, MeOH, reflux, 77%; (e) H_2, 10% Pd/C, MeOH/10% HCl (1:1), 68%.



Scheme 2. Reagents and conditions: (a) NaH, THF, 0 °C then diethylphosphonoacetonitrile, rt, 68%; (b) H_2, 10% Pd/C, MeOH, 81%; (c) $LiAlH_4$, THF, 60 °C, 94%; (d) acyl anhydride, THF, rt.



Scheme 3. Reagents and conditions: (a) NaH, THF, 0 °C then triethyl phosphonoacetate, rt, 60%, 73%; (b) H_2, 10% Pd/C, EtOH, rt, 73%; (c) $LiAlH_4$, THF, 60 °C; (d) Ts_2O, Et_3N, CH_2Cl_2, rt, 53%; (e) secondary amine, K_2CO_3, acetonitrile, reflux; (f) KCN, DMF, 100 °C, 42%.

On the other hand, SmithKline reported that SB210313, an imidazole derivative, which substituted the basic polar group at the N-1-position, showed an anti-inflammatory effect.[12]

On the basis of these reports, we investigated simple diaryl-pyrrole derivatives possessing basic polar groups at β-position (**A**) which were expected to increase potency in vitro and in vivo. Herein, we report our initial results, which have led to the identification of a unique and potent pyrrole-based inhibitor of the pro-inflammatory cytokine TNFα, **17**.

The preparation of **4**, **6** and **11**, each of which has a distal amino alkyl group at the β-position of the pyridylpyrrole, and their derivatives are summarized in Schemes 1–3. The key intermediate **3** was provided from α-(*p*-toluene-sulfonyl)-4-fluorobenzylisonitrile **1**,[13] which was condensed with ethyl (2*E*)-3-pyridin-4-ylacrylate to give pyrrole ester **2**.[14] Reduction of the ester by diisobutylaluminum hydride (DIBAL) gave a corresponding alcohol and subsequent oxidation by MnO_2 provided the aldehyde **3**.

The 3-methylaminopyrrole **4** was synthesized by treatment of **3** with *O*-methyl hydroxylamine hydro-chloride to give oxime, and subsequent catalytic hydrogenation was carried out to afford **4** (Scheme 1). We next prepared the 3-(3-amino)propylpyrrole **6** by a Horner–Emmons reaction of **3**, and subsequent hydrogenation of olefin and a nitrile reduction with lithium aluminum hydride ($LiAlH_4$). Several amide derivatives, **7a** and **7b** from **6**, were prepared by using corresponding anhydrides. Reduction of the *N*-*tert*-butoxy carbonyl derivative **7b** with $LiAlH_4$ gave *N*-methyl derivative **7c** (Scheme 2).

The 3-(4-amino)butylpyrrole **11** was obtained as follows (Scheme 3). In the same manner as described above, a Horner–Emmons reaction of **3** and subsequent reductions gave the alcohol **9**. The corresponding tosylate **10** was converted to a nitrile, which was then reduced to obtain **11**. As well, the tosylate **10** was converted by displacement with a desired secondary amine to dialkylaminopropyl derivatives **12a–f**. Catalytic hydrogenation of **12f** gave the piperazine derivative **12g**.

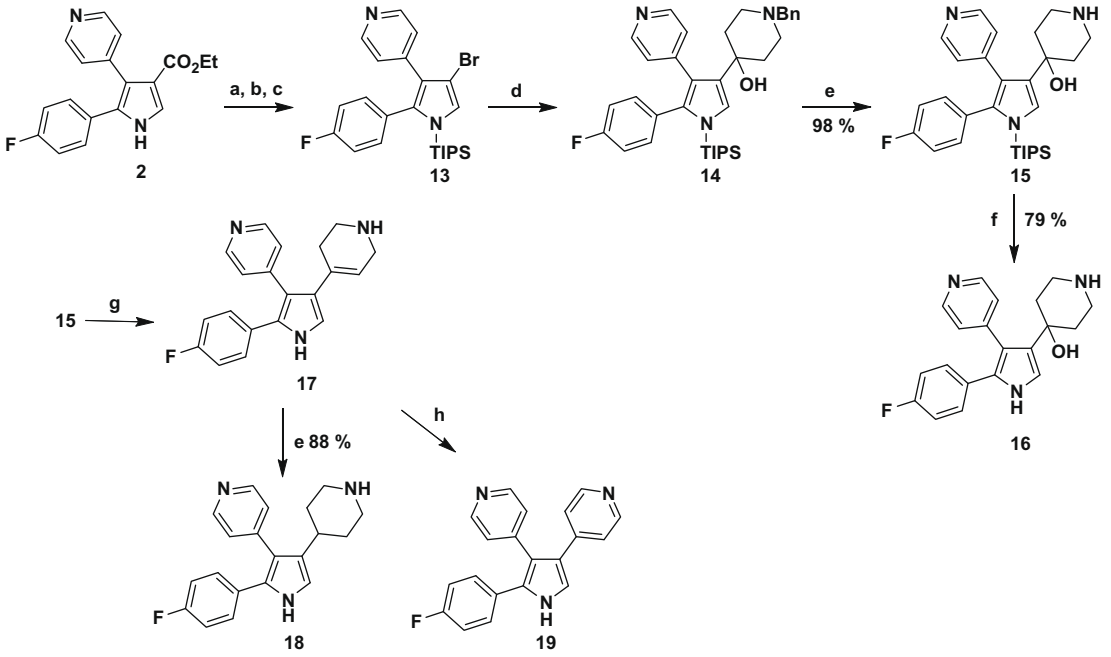
We now describe the syntheses of pyridyl pyrrole derivatives possessing a cyclicaminoalkyl group at the β-position of the pyrrole, which are shown in Scheme 4. Removal of the ethoxycarbonyl group of **2** by an acidic condition and the subsequent protection of the pyrrole nitrogen by a triisopropylsilyl (TIPS) group[15] followed by selective β-bromination using *N*-bromo succinimide (NBS) gave **13**. Introduction of a cyclicamino group to the β-position of the pyrrole ring was carried out by bromine-lithium exchange followed by 1,2-addition with 1-benzyl-4-piperidin-1-one. Debenzylation and subsequent deprotection of the TIPS group on the obtained *tert*-alcohol derivative **14** was performed to give **16**. Dehydroxylation of the *tert*-alcohol in **15** was carried out in excellent yield by exposure to trifluoroacetic acid (TFA) concurrently with deprotection of the TIPS group to give the tetrahydropyridine derivative **17**. Compound **17** was further transformed to the piperidine **18** and the pyridyl derivative **19**.

The designed and synthesized 2-(4-fluorophenyl)-3-(4-pyridyl)pyrrole derivatives were evaluated in terms of their inhibitory activities in LPS induced TNFα production in human whole blood.[16] SB203580 was used as a reference for a comparison of the in vitro potencies of the new analogues (Table 1).

Compound **6**, possessing a 3-aminopropyl group ($n = 3$) at the β-position of the pyridylpyrrole, was proven to be more effective than **4** ($n = 1$), having an aminomethyl group, **11** ($n = 4$) having a 4-aminobutyl group and SB-203580. Therefore, for inhibitory activity the most suitable linker connecting the amino group and pyrrole ring was the propyl group.[17]

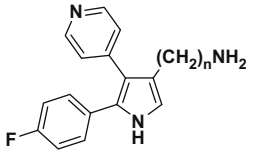
Then, we carried out a replacement of the amino moiety of **6** (Table 2).

The corresponding amido analogues (**7a**) resulted in a loss of activity. Furthermore, in aminoalkyl analogues such as **7c** and **12c**, which possess methyl and dimethyl groups, respectively, the increasing bulkiness of the substituent led to decreased potency. We next examined various cyclicaminopropyl derivatives, such as **12a**, **b** and **d**–**g**, which showed no potencies. These results indicate that the nitrogen atom of the aminopropyl substituent should



Scheme 4. Reagents and conditions: (a) AcOH, H_2SO_4, H_2O, 100 °C, 99%; (b) *n*-BuLi, THF, −78 °C then TIPSOTf, rt, quant.; (c) NBS, THF, −78 °C then rt, 43%; (d) *n*-BuLi, THF, −78 °C then 1-benzylpiperidin-4-one, −78 °C then rt, 60%; (e) H_2, 10% Pd/C, EtOH; (f) TBAF, THF, rt, 79%; (g) TFA, CH_2Cl_2, rt then TBAF, THF, rt, 93%; (h) 10% Pd/C, K_2CO_3, xylene/MeOH (25: 2), 140 °C, 53%.

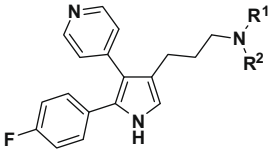
Table 1
Activities of aminoalkyl derivatives



Compd	N	IC_{50}[a] (μM)
SB203580		6.40
4	1	>30
6	3	4.76
11	4	13.8

[a] Inhibition of LPS induced TNFα production in human whole blood. *N* = 3–4.

Table 2
Activities of aminopropyl derivatives



Compd	R^1	R^2	IC_{50}[a] (μM)
6	H	H	4.76
7a	COMe	H	>30
7c	Me	H	14.7
12a	Thiomorphorine		>30
12b	Morphorine		>30
12c	Me	Me	22.4
12d	Piperidine		28.4
12e	4-Methylpiperazine		>30
12f	4-Benzylpiperazine		>30
12g	Piperazine		>30

[a] Inhibition of LPS induced TNFα production in human whole blood. *N* = 3–4.

be located at a length of 4 atoms from the β-position of the pyrrole, should have basicity and should be sterically unhindered, in order to show inhibitory activity.

Based on the information above, we designed further pyrrole derivatives possessing a cyclicaminoalkyl group at the β-position (Table 3) to fix the lone-pair on the nitrogen atom.

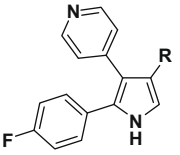
The 4-hydroxypiperidine analogue **16** unexpectedly showed no potency, while the tetrahydropyridine analogue **17** and its saturated analogue, piperidine derivative **18**, showed good inhibitory activity (IC_{50} = 1.86 and 2.98 μM, respectively). The derivative **19**, possessing pyridine, which is a more planar and more oxidized substituent compared to tetrahydropyridine, showed however no potency.

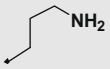
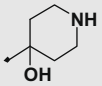
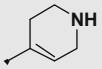
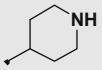
We evaluated the in vivo efficacy of these derivatives (**16**–**19**) in LPS induced TNFα production in mice.[18] The analogue **17** showed the most effective activity (ID_{50} = 5.98 mg/kg), which was more than four times as potent as that of SB203580. However, the analogue **18** showed several times lower activity than **17**.

From these results, the tetrahydropyridine group which has basic nitrogen and an olefin bond at the β-position of the pyrrole ring showed good inhibitory activities in vitro and in vivo. The results of the in vivo assay were reflected in those of the in vitro assay and we found that the tetrahydropyridine group was the effective group in this template.

In order to develop new anti-inflammatory agents, we synthesized and evaluated derivatives possessing basic polar functional groups at the β-position of the pyrrole ring. The analogue **17**, which has a tetrahydropyridine group, showed good inhibitory activity in LPS induced TNFα production in vitro (human whole blood) and in vivo (mice) compared to that of SB203580. Based on these results, we plan to further investigate pyridylpyrrole possessing tetrahydropyridine groups.

Table 3
In vitro and in vivo activities of piperidine, tetrahydropyridine, and pyridine derivatives



Compds	R	IC_{50}[a] (μM)	ID_{50}[b] (mg/kg)
SB203580		6.40	27.9
6		4.76	–[c]
16		>30	−2.7%[d]
17		1.86	5.98
18		2.98	17.9
19		>30	24.5%[e]

[a] Inhibition of LPS induced TNFα production in human whole blood. $N = 3–4$.
[b] Inhibition of LPS induced TNFα production in mice. $N = 5$.
[c] Not tested.
[d] % Inhibition at 20 mg/kg.
[e] % Inhibition at 50 mg/kg.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.094.

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16. Fresh blood was collected aseptically in the presence of heparin by venipuncture from healthy adult volunteers. The subjects did not have any apparent inflammatory conditions and had taken no drug for at least 7 days prior to blood collection. Written informed consent was obtained from all volunteers before the experiments. Blood aliquots of 988 μL were mixed with either 2 μL of a test compound solution or 2 μL of DMSO, and with 10 μL of 1.0 mg/mL LPS (dissolved in PBS, final concentration: 10 μg/mL) in Eppendorf tubes. The compounds were dissolved and diluted to the appropriate concentrations with DMSO. The compound solutions were prepared immediately before use. The mixture was incubated at 37 °C for 6 h. Control assays were carried out in additional tubes by mixing blood with 2 μL of DMSO and 10 μL of PBS instead of the test compounds and LPS (negative control), respectively. After the incubation was finished, the mixture was immediately chilled at 4 °C and centrifuged at 15,300*g* for 5 min, and the plasma was stored at −20 °C until the assay. The levels of cytokines in the plasma were determined by using commercially available immunoassay kits. The percent inhibition of cytokine production by the compounds was calculated by the following equation: Percent inhibition of cytokine production = {1 − (concentration of cytokine in the reaction mixture of test compound − concentration of cytokine in the reaction mixture of negative control)/(concentration of cytokine in the reaction mixture of control − concentration of cytokine in the reaction mixture of the negative control)} × 100.
17. We tried to synthesize an analogue possessing an aminoethyl group ($n = 2$), but it was too unstable a compound to isolate.
18. TNFα production was induced in mice by the procedure of Griswold et al. (*J. Immunol. Methods* **1996**, *195*, 1–5.) with a slight modification. In brief, mice were deprived of food overnight, but not water. The next day, these mice were orally administered with test compounds suspended in 0.5% CMC aqueous solution at 10 mL/kg except normal and control mice that were administered with 0.5% CMC aqueous solution. Thirty minutes later, LPS solution dissolved in physiological saline was intravenously injected at 0.45-mg/10 mL/kg except for the normal mice, which were injected with physiological saline at 10 mL/kg. One hour later, blood samples were taken from the mice from the vena cava inferior into a syringe containing heparin sodium. After centrifugation of the blood samples at 13,230*g* for 3 min at 4 °C, plasma samples were taken immediately and frozen at −20 °C until measurement of the concentration of TNFα in the plasma sample. The concentrations of TNFα in the plasma samples were measured with commercially available ELISA kits. The percent inhibition of TNFα production was obtained by the following equation: Percent inhibition = {1 − (concentration of TNFα in plasma sample of mice administrated test compounds − mean concentration of TNFα in the plasma samples of normal mice)/(concentration of TNFα in the plasma samples in control mice − mean concentration of TNFα in the plasma samples of normal mice)} × 100.