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Investigations of SCIO-469-like compounds for the inhibition of p38 MAP kinase

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ABSTRACT

The p38 MAP kinase is implicated in the release of the pro-inflammatory cytokines TNFα and IL-1b. Inhibition of cytokine release may be a useful treatment for inflammatory conditions such as rheumatoid arthritis and Crohn's disease. A new lead structure for p38 MAP kinase inhibition was identified. Herein, we report the SAR of this new class of p38 inhibitors.

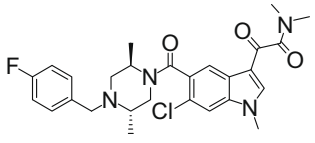
The p38 mitogen-activated protein (MAP) kinase is a member of the serine/threonine kinase superfamily that includes extracellular signal regulated kinase-2 (ERK2) and c-Jun N-terminal kinase (JNK). Activation of p38 under a variety of conditions (such as external stimuli, inflammatory cytokines, heat, and UV light) results in the bis-phosphorylation of the Ser-Thr amino acids in the activation loop.[1] Subsequently, activation of other downstream kinases and transcription factors leads to mRNA stabilisation and an increase or decrease in the expression of certain target genes.[2] To date, four splice variants of p38 MAPK are known (p38α, p38β, p38γ, and p38δ). Although the role of the ubiquitously expressed isoform p38α during inflammation has been defined, the functions of the other isoforms are not well understood.

The discovery that p38 MAPK is a member of the stress-activated signal transduction pathway and experiments with small-molecule p38 inhibitors (SB-203580) validated this kinase as an important anti-inflammatory therapeutic target. Numerous analogues of SB-203580, as well as a variety of alternate scaffolds, have been reported as potent and selective inhibitors of p38 MAPK.[3] In addition, several of these inhibitors were shown to be effective as anti-inflammatory agents when evaluated in animal models of acute and chronic diseases.

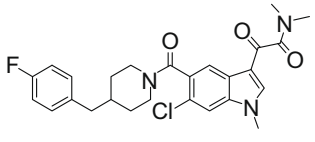
Several companies have reported preliminary human clinical results for p38 MAPK inhibitors. Scios Inc. is a leader in this field, with the advancement of SCIO-469 into Phase II human clinical trials for the treatment of pain, multiple myeloma, and rheumatoid arthritis. SCIO-469 modestly inhibits LPS-induced TNFα production in human whole blood with an IC_{50} = 300 nM. SCIO-469 is a potent p38α inhibitor with >1000-fold selectivity versus ERK2, JNK1, and LCK.

A distinctive feature of the indole amide class of p38 inhibitors relative to others (e.g., pyridinylimidazoles, diarly ketones) is the ability to achieve high selectivity toward the p38α isoform versus the p38β isoform. SX-011 has 10-fold p38α/β selectivity whereas the azaindole derivative of SX-011 shows 249-fold p38α/β selectivity and is one of the most highly selective p38α inhibitors reported (Fig. 1).

The proposed binding mode for SCIO-469 involves interaction of the C5 carbonyl interacting with p38 Met109 via a hydrogen bond.[4] The lipophilic benzylpiperazine is proposed to occupy the selectivity pocket of the kinase. The ortho substituent on the indole ring may be instrumental in achieving the high p38α selectivity. This prediction is based on the proposal that the ortho substituent occupies a hydrophobic pocket that is near the Ala40 residue in



SCIO-469



SX-011

Figure 1. p38 inhibitors SCIO-469 and SX-011.

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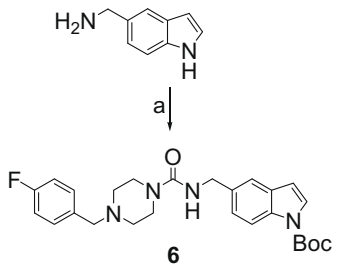
p38α. Because p38β contains a larger, more hydrophilic serine residue at the analogous position, this isoform was proposed to be less likely to accommodate the ortho substituent, which leads to increased selectivity towards p38α versus p38β. The proposed binding orientation of this class of inhibitors was confirmed recently.[5]

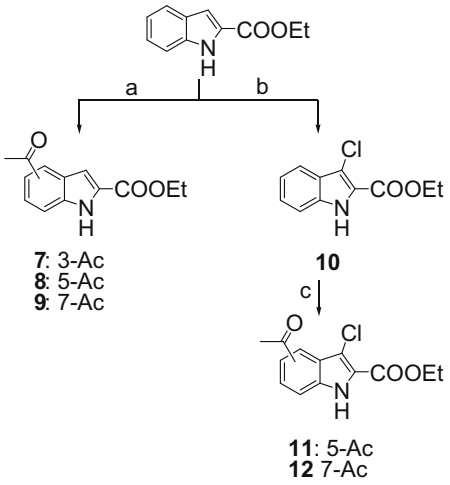
Scios Inc. reported structure–activity relationship (SAR) studies of a series of indole-based heterocyclic inhibitors.[6] The inhibitory activities of indole-3-, indole-4-, indole-5-, indole-6-, and indole-7-carboxamides were studied. In order to close the gaps of these study and based on modeling results, we investigated some synthetic modifications of the Scios template. Here, we report a new lead structure of p38 inhibitors derived from SCIO-469.

The first attempted synthetic approach to obtain the urea derivative is outlined in Scheme 1. 5-Amino-1-methyl-1*H*-indole (**3**) was prepared in good yields with previously described methods.[7] For the urea formation, we adopted the procedure from Knölker et al. Therefore, **3** was reacted with di-*tert*-butydicarbonate, with 4-dimethylaminopyridine (DMAP) as catalyst, to form in situ an isocyanate, which was treated with 4-fluorobenzylpiperazine to obtain (4-(4-fluorobenzyl)-*N*-(1-methyl-1*H*-indole-5-yl)piperazine-1-carboxamide (**4**) in excellent yields. The introduction of the oxalylamide residue was accomplished by standard procedures. Because urea has a fixed binding angle, we wished to synthesize the urea with a benzylic CH_2 group before the indole nucleus. Starting from commercially available 5-(aminomethyl)-1*H*-indole, we could only obtain the Boc-protected urea (**6**) (Scheme 2). Attempts to gently cleave the protecting group resulted also in cleavage at the benzylic group of the molecule.

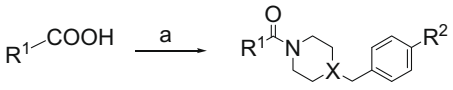
Subsequently, we prepared a set of heterocyclic 2-amides. The synthesis of the amides was done under standard conditions with *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) and DMAP in dichloromethane to activate the carboxylic acids (Scheme 4).[8] The acetyl-substituted indoles were obtained by Friedel–Crafts acylation of ethyl 1*H*-indole-2-carboxylate or ethyl 3-chloro-1*H*-indole-2-carboxylate (**10**) (Scheme 3). In this manner, the different isomers were generated in the same ratio and could be separated *via* column chromatography (silica gel) with dichloromethane as eluent. The produced esters (**7**–**9**, **11**, **12**) were saponified with sodium hydroxide in ethanol and subsequently converted to the corresponding amides using the standard procedure (Scheme 4). 4-Bromo-1*H*-indole-2-carboxylic acid, which is required to obtain the amide **16**, was synthesized accordingly to the described procedure.[9] Benzofuran-2-carboxylic acid, which is required for amides **20** and **21**, was prepared analogous to the method from Ashram.[10] The precursor for amide **19** was obtained by previ-



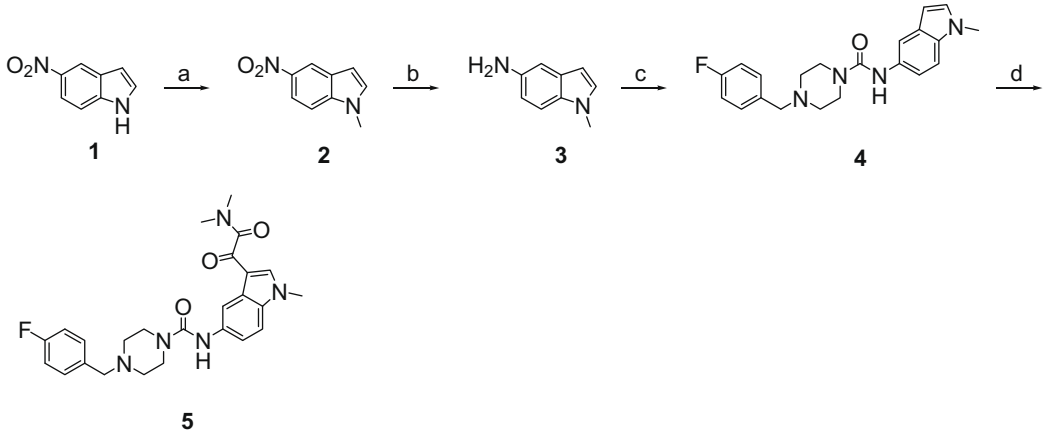
Scheme 2. Synthesis of *tert*-butyl 5-((4-(4-fluorobenzyl)piperazine-1-carboxamido)methyl)-1*H*-indole-1-carboxylate (**6**). Reagents and conditions: (a) 1—(Boc_2)O, DMAP, CH_2Cl_2, rt, 20 min; 2—4-fluorobenzylpiperazine, 40 °C, 14 h, 38%.



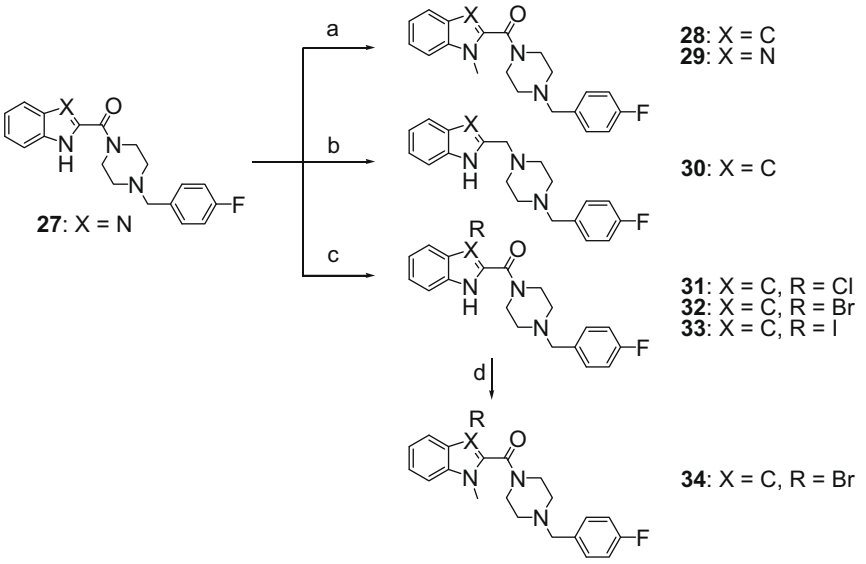
Scheme 3. Synthesis of the acetyl-substituted indole-2-carboxylates. Reagents and conditions: (a) AcCl, $AlCl_3$, CH_2Cl_2, reflux, 10 h; (b) NCS, acetone, rt, 1 h; (c) AcCl, $AlCl_3$, CH_2Cl_2, reflux, 10 h.



Scheme 4. Synthesis of heterocyclic 2-amides. Reagents and conditions: (a) EDAC, DMAP, 4-fluorobenzylpiperazine or benzylpiperazine or benzylpiperidine, CH_2Cl_2, rt, 14 h.[8]



Scheme 1. Synthesis of the urea derivatives **4** and **5**. Reagents and conditions: (a) dimethyl carbonate, potassium carbonate, DMF, reflux, 2 h, 96%; (b) Pd/C 10%, EtOH, 40 °C, 4 h, 85%; (c) 1—(Boc_2)O, DMAP, CH_2Cl_2, rt, 20 min; 2—4-fluorobenzylpiperazine, 40 °C, 14 h, 77%; (d) 1—oxalyl chloride, CH_2Cl_2, rt, 3 h; 2—dimethylamine, CH_2Cl_2, rt, 30 min, 94%.



Scheme 5. Derivatization of amides. Reagents and conditions: (a) 1—NaH, THF, 0 °C, 1 h; 2—MeI, rt, 12 h, 83% (**28**), 96% (**29**); (b) $LiAlH_4$, THF, reflux, 3 h, 49%; (c) NCS or NBS or NIS, acetone, rt, 1 h, 95–97%; (d) 1—NaH, THF, 0 °C, 1 h; 2—MeI, rt, 12 h, 90%.

ously described procedures (Table 1).[11] As outlined in Scheme 5, some derivatizations of the obtained amides were executed.

N-Methylation of (4-(4-fluorobenzyl)piperazine-1-yl)(1*H*-indole-2-yl)methanone (**13**) was accomplished with sodium hydride and methyl iodide in tetrahydrofuran to yield compound **28**. The synthesis for the 1*H*-benzo[*d*]imidazole amide (**27**) began with benzene-1,2-diamine, which was reacted with methyl 2,2,2-trichloroacetimidate to obtain 2-(trichloromethyl)-1*H*-benzo[*d*]imidazole according to the procedure of Venable et al.[12]

Subsequent reaction with 4-fluorobenzylpiperazine resulted in the amide formation. The N-methylation of **27** was accomplished analogous to the indole amide. The reduction of the carbonyl function was carried out with lithium aluminum hydride in refluxing tetrahydrofuran to obtain 2-((4-(4-fluorobenzyl)piperazin-1-yl)-methyl)-1*H*-indole (**30**). We were able to chlorinate, brominate, and iodinate the amides in high yields with a simple and fast synthesis using *N*-chlorosuccinimide, *N*-bromosuccinimide, and *N*-iodosuccinimide, respectively, in acetone at room temperature.

According to the developed method,[13] 25 compounds were tested for anti-p38 activity at concentrations ranging from 10^{-5} to 10^{-8} M. Pyridinyl-imidazole SB-203580 was used as a reference compound, and the optimized ATP concentration at which the test was performed was 100 μM. Briefly, the assay involved the immobilization of the kinase substrate ATF-2 (activating transcription factor-2) on microtiter plates, addition of the kinase reaction mixture, and measurement of substrate phosphorylation by a two-step antigen-antibody reaction in which primary antibody binds to the doubly phosphorylated (Thr^{69} and Thr^{71}) ATF-2 and acts as antigen for the secondary antibody. The secondary antibody is conjugated to alkaline phosphatase, which is able in the last step to dephosphorylate 4-nitrophenolphosphate disodium salt (4-NPP). 4-Nitrophenol was detected by an ELISA reader at 405 nm.

The biological test results (Table 2) show that the ureas **4** and **5** were inactive against p38 MAPK. This result arose from the fixed binding angle of the urea group. The urea **6**, which was extended with a CH_2 group to make the indole nucleus freely rotatable, also showed no inhibition. This finding can be explained by the presence of a bulky Boc-group that cannot be cleaved synthetically.

In the heterocyclic 2-amide series, the following SAR can be concluded. The *p*-fluoro substituent improved the inhibitory activity (e.g., compounds **13** and **14**). Comparison of indole-2-amides **14** and **15** reveals that the piperidine-amides are more potent than the corresponding piperazine-amides. Another improvement in the inhibitory activity was achieved through N-methylation. The *N*-methylated derivative (**28**) was three times more potent than the unmethylated compound (**13**). In general, substituents at the 3-position (compounds **22**, **31**, **32**, and **33**) were not preferred compared with the unsubstituted analogues. All 2-furanyl- (**17**, **18**),

Table 1
Synthesized heterocyclic 2-amides

Compound	R^1	X	R^2	Yield (%)
13	2-Indolyl	N	F	61
14	2-Indolyl	N	H	31
15	2-Indolyl	C	H	80
16	4-Bromo-2-indolyl	N	F	72
17	2-Furanyl	N	F	44
18	2-Furanyl	N	H	42
19	Imidazo[1,2-*a*]pyridine-2-yl	N	F	56
20	2-Benzofuranyl	N	F	16
21	2-Benzofuranyl	C	H	37
22	3-Acetyl-2-indolyl	N	F	27
23	5-Acetyl-2-indolyl	N	F	64
24	7-Acetyl-2-indolyl	N	F	94
25	5-Acetyl-3-chloro-2-indolyl	N	F	30
26	7-Acetyl-3-chloro-2-indolyl	N	F	63

Table 2
Inhibition of p38 MAPK by heterocyclic 2-amides

Compound	IC_{50} ± SEM[a] [μM]	Compound	IC_{50} ± SEM[a] [μM]
4	28%[b]	**23**	45%[b]
5	12%[b]	**24**	3.61 ± 0.98
6	8%[b]	**25**	6.51 ± 0.35
13	3.20 ± 1.04	**26**	2.46 ± 0.18
14	5.77 ± 1.56	**27**	24%[b]
15	1.09 ± 0.28	**28**	1.36 ± 0.34
16	34%[b]	**29**	32%[b]
17	35%[b]	**30**	25%[b]
18	20%[b]	**31**	7.57 ± 0.13
19	33%[b]	**32**	6.46 ± 0.37
20	8.42 ± 1.21	**33**	7.47 ± 0.88
21	1.75 ± 0.32	**34**	2.24 ± 0.35
22	24%[b]		

[a] % Number of determinations were three.
[b] % Inhibition at 10 μM.

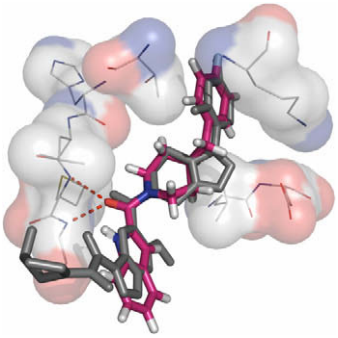


Figure. 2. Proposed binding mode for **15** (pink) in overlay with the ligand from pdb-ID 2qd9 (grey; 1-[5-[[3-(2,4-difluorophenyl)-6,8-dihydro-5*H*-imidazo[5,1-*c*]pyrazin-7-yl]carbonyl]-6-methoxy-3*aH*-pyrrolo[5,4-*b*]pyridin-3-yl]-2-[(3*R*)-3-hydroxypyrrolidin-1-yl]ethane-1,2-dione. After geometric optimization in the MMFF94 force field, the molecule has been docked in the p38 binding pocket by using the software AutoDock 4.0.[14] The picture was generated using PyMOL.

imidazo[1,2-*a*]pyridin-2-yl- (**19**), and 1*H*-benzo[*d*]imidazol-2-yl- (**27**, **29**) derivatives were inactive against p38 MAPK. Whereas 2-benzofuranyl-derivatives (**20**, **21**) were equipotent to the indole-amides. The acetyl-group on the indole nucleus was best tolerated at the 7-position (compare compounds **22**, **23**, and **24**) A chloro-substituent at the 3-position of an indole further improved the inhibitory activity (compound **26**).

In Figure 2, the suggested binding mode of compound **15** (pink) is displayed. Noteworthy is the similarity with the binding mode of the ligand from pdb-ID 2qd9 (grey). Compound **15** forms hydrogen bonds to the hinge region (Met^{109} and Gly^{110}) of p38 MAPK. The position is equally to the ligand from the X-ray structure and shows that either the 4- or the 7-position of our heterocyclic 2-amides should be substituted with the oxalic amide residue, which improve the inhibitory activity in SCIO-469.

First investigations of the inhibitory activities and structure–activity relationships of heterocyclic 2-amide inhibitors for p38 MAPK were made. Novel inhibitors for p38 were identified that should be optimized in further studies.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.01.023.

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8. *General synthesis of heterocyclic 2-amides:* To a solution of 1.00 equiv of the corresponding carboxylic acid in 15 mL absolute dichloromethane was added at room temperature 1.40 equiv *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride, 1.40 equiv triethylamine, 0.20 equiv 4-dimethylaminopyridine and 1.00 equiv 4-fluorobenzylpiperazine. The mixture was stirred for 14 h at room temperature. Following it was quenched with water and extracted three times with dichloromethane. The organic layer was dried over sodium sulfate and evaporated. If necessary the raw product was purified through column chromatography. The identity and purity of the target compounds were evaluated by ^{1}H, ^{13}C NMR, MS, IR, mp and HPLC. All experimental procedures and analytical data are given in Supporting Information.
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