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N-Phenyl-*N*-purin-6-yl Ureas: The Design and Synthesis of P38α MAP Kinase Inhibitors

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Abstract—The design, synthesis and SAR of a series of 2,6,9-trisubstituted purine inhibitors of p38α kinase is reported. Synthetic routes were devised to allow for array synthesis in which all three points of diversity could be facilely explored. The binding of this novel series to p38α kinase, which was predicted to have several key interactions in common with **SB-203580**, was confirmed by X-ray crystallography of **19** (p38 $IC_{50} = 82$ nM).

Enbrel, a soluble TNF-α receptor, and Remicade, an antibody to TNF-α have demonstrated clinical efficacy in the treatment of rheumatoid arthritis (RA) and inflammatory bowel disease (IBD).[1] These protein agents, which act by blocking the action of TNF-α, are significant therapeutic advances for the treatment of these debilitating conditions. p38 is a member of the intracellular family of mitogen activated protein kinases, implicated in the phosphorylation cascade leading to the release of TNF-α and other cytokines. The inhibition of p38 kinase has been proposed as an alternative small molecule approach to block the action of TNF-α.[2] As a small molecule approach would enjoy the advantages of more convenient oral administration, p38α inhibition has received widespread attention as a therapeutic target. Several research teams have undertaken the challenge to identify p38α inhibitors suitable for clinical development and recently multiple p38 kinase inhibitors have advanced into clinical trials as potential therapies for the treatment of RA.[3] These include, **SB-242235**, a member of the pyridinyl-imidazole class of selective p38 inhibitors, as well as, **VX-745** and **BIRB 796**, compounds from alternate structural classes.[4]

Our group has had an on going effort utilizing database screening and structure-based design strategies to identify novel p38α inhibitors. Having demonstrated that the inhibition of P450 seen with **SB-203580** and analogues was a result of the 4-pyridyl group,[5] we were particularly interested in replacing the pyridyl group with an amide carbonyl as an alternate H-bond acceptor. Hence, we were intrigued by the publication of a Vertex Pharmaceuticals patent application claiming ureidopyridines as p38 inhibitors.[6] Molecular modeling experiments performed with compound **A**[7] suggested that the urea carbonyl could form a H-bond donor-acceptor interaction with the backbone amide NH of M109 that would mimic the H-bond of the N-1 adenine of ATP and is analogous to that seen for the 4-pyridyl nitrogen of **SB-203580**.[8] Additionally, in this model one of the

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terminal urea nitrogen NH groups of **A** could mimic the H-bond of the N-6 amino group of adenine to the amide carbonyl of H107 (Fig. 1).

This communication describes our efforts towards the synthesis and SAR studies of a novel purine series of p38α inhibitors that were designed based upon the model shown in Figure 2. The purine template was chosen in order to allow for a third point of diversity (R3) fit into the ATP triphosphate binding site, an area known to tolerate diverse substitutions. The preparation of the required trisubstituted purines was accomplished utilizing a strategy, which relied on the different reaction preferences of halogen atoms at C2 and C6 of purine.[9] Schemes 1–4 summarize four strategies for the preparation of target **B** analogues.

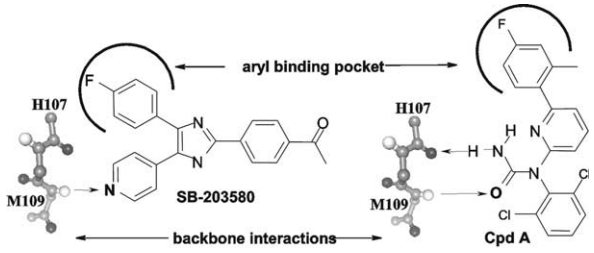


Figure 1. Structures of **SB-203580** and compound **A**.

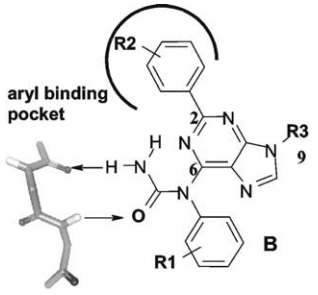
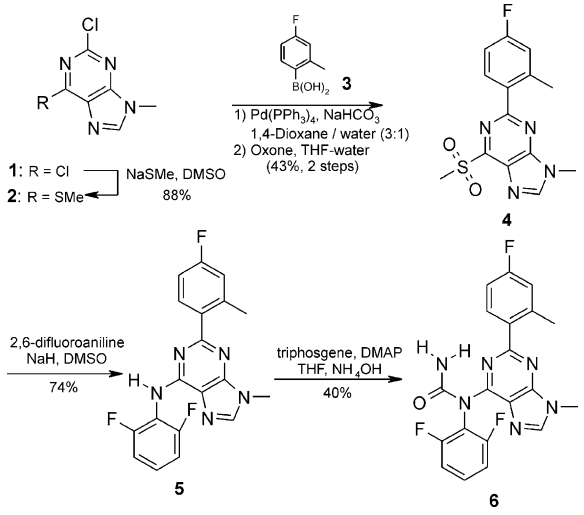
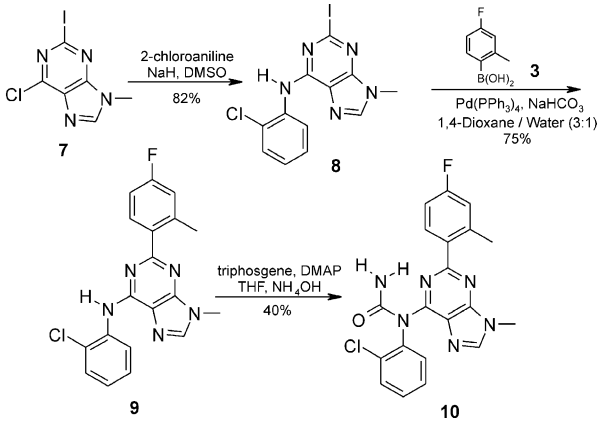


Figure 2. Structure of p38 Inhibitors **B**.

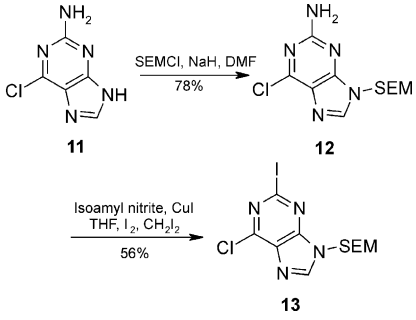


Scheme 1.

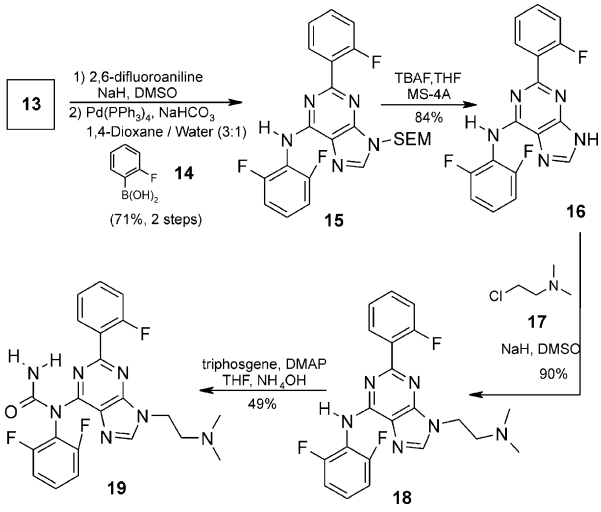
Our point of departure for the synthesis is 2,6-dichloro-9-methyl purine (**1**, Scheme 1) which was readily available from the methylation of 2,6-dichloropurine.[10] Selective displacement of chlorine at C6 with sodium methylsulfide afforded **2**. Palladium mediated Suzuki cross-coupling of the remaining chlorine with **3** and oxidation with oxone provided sulfone **4**. Substitution with 2,6-difluoroaniline followed by treatment with triphosgene and ammonium hydroxide then furnished urea **6**. This scheme allows for the introduction of differently substituted anilines at C6 late in the synthesis and was utilized to synthesize compounds **20**–**23**.



Scheme 2.



Scheme 3.



Scheme 4.

In order to optimize the series, late introduction of substituents at C2 was achieved utilizing a synthetic method described in Scheme 2. The preparation of urea **10** started with 6-chloro-2-iodo-9-methyl purine (**7**) which was easily constructed from 2-amino-6-chloro purine following a literature procedure.[11] Selective displacement of the chlorine of **7** with 2-chloroaniline afforded **8**. Palladium catalyzed Suzuki cross-coupling of iodide **8** with **3** provided **9**. Compound **9** could also be obtained with a similar yield by carrying the reaction sequence in the reverse order (Suzuki coupling, then displacement with 2-chloroaniline). Treatment of **9** with triphosgene and ammonium hydroxide then furnished urea **10**. This strategy, which permits the independent introduction of different substituents at either C2 or C6, was applied to prepare compounds **24**–**35**.

Encouraged by the success of manipulating substituents at C2 and C6, we explored the introduction of variations at N9 late in the synthetic sequence using a protection/deprotection sequence. To this end, compound **13** was prepared from 2-amino-6-chloro purine (**11**, Scheme 3) employing a two-step procedure; protection of N9 with trimethylsilylethoxymethyl chloride (SEMCl) followed by conversion of NH_2 at C2 to iodine. Compound **13**, whose structure was unambiguously confirmed by X-ray crystallography shown in Figure 3, is an versatile building block, providing access to variable substitution at C2, C6 and N9. The chlorine at C6 is more reactive toward nucleophilic substitutions, while in contrast, the iodine at C2 is more reactive in coupling reactions catalyzed by palladium(0).[12]

The utility of **13** was demonstrated through the synthesis of two N-9 substitution analogues (Scheme 4). Displacement of chlorine with 2,6-difluoroaniline followed by Suzuki cross-coupling of iodine with 2-fluorophenylboronic acid (**14**) afforded **15**. The two step sequence could also be reversed (Suzuki cross-coupling followed by displacement of chlorine) to produce **15** in a comparable yield. Removal of SEM (TBAF, THF, reflux, 4A-MS) afforded disubstituted purine **16**. Reaction with alkyl chloride **17** furnished **18** exclusively, which was transformed into urea **19**. This synthetic procedure was employed to construct compound **36** as well.

Table 1 summarizes the p38 inhibition results.[13] Group **R1** was the first to be investigated. Compound **20**, the unsubstituted aniline, is essentially inactive. p38 inhibition is improved with halogen substituents (**6**, **10**, **21**, **23**, and **24**), but not with the substitutions of electron donating groups (e.g., methoxy, **22**). Inhibitor **10** has an *ortho* chlorine, and it is eighty fold more active than **23**, indicating a strong preference for *ortho* versus *para* substitution. Variation of **R2** was also found to be important for optimizing p38 kinase activity. The unsubstituted analogue (**25**) displays modest potency ($IC_{50} = 0.51\ \mu M$) while *meta* fluoro substitution is not well tolerated (**27** and **30**), *ortho* substitution is, with every compound (**26**, **31**, and **33**) improved relative to **25**. In terms of the substituent characteristics, halogen is 6-fold more potent than methyl. For the purpose of improving physio-chemical properties of the inhibitors, two R3 substitutions were prepared with the optimal R1 and R2. Both compounds (**19** and **36**) proved to be potent p38 inhibitors, exhibiting IC_{50} values comparable to the N9 methyl group derivative (**26**). Noteworthy is improved aqueous solubility of **19** ($>20\ \mu M$) versus **26** ($1\ \mu M$).[14] This may suggest that the N9 position of the purine can be used to adjust physio-chemical properties without compromising p38 potency.

In order to confirm the binding mode of this novel class with p38 proposed in Figure 2, the X-ray structure of **19** bound to p38α was determined from crystals soaked with a solution of the inhibitor (Fig. 4). We were pleased to find that the proposed model accurately predicted several key features of the crystallographically determined binding mode. These features include: (1) an intra-molecular hydrogen bond from the urea group to N1 of the purine which positions the urea co-planar

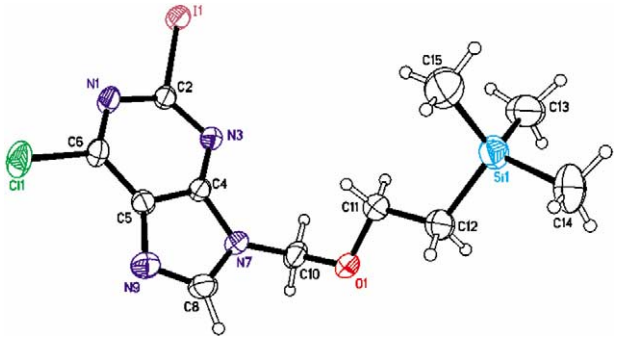
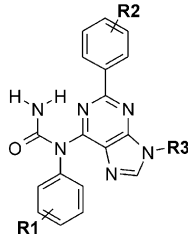


Figure 3. Crystal structure of compound **13**.

Table 1. Results of SAR studies around the purine template



Compd	Method (scheme)	R1	R2	R3	IC_{50} (μM)
20	1	H	2-Me, 4-F	Me	>16.7
6	1	2,6-di-F	2-Me, 4-F	Me	0.128
10	2	2-Cl	2-Me, 4-F	Me	0.123
21	1	4-F	2-Me, 4-F	Me	1.4
22	1	2-OMe	2-Me, 4-F	Me	>16.7
23	1	4-Cl	2-Me, 4-F	Me	9.8
24	2	2,4,6-tri-F	2-Me, 4-F	Me	0.163
25	2	2,6-di-F	H	Me	0.51
26	2	2,6-di-F	2-F	Me	0.036
27	2	2,6-di-F	3-F	Me	5
28	2	2,6-di-F	4-F	Me	1
29	2	2,6-di-F	2,4-di-F	Me	0.026
30	2	2,6-di-F	3,5-di-F	Me	>16.7
31	2	2,6-di-F	2-Cl	Me	0.03
32	2	2,6-di-F	3-Cl	Me	0.55
33	2	2,6-di-F	2-Me	Me	0.14
34	2	2,6-di-F	3-Me	Me	0.35
35	2	2,6-di-F	4-Me	Me	2.1
36	4	2,6-di-F	2-F	Bn	0.016
19	4	2,6-di-F	2-F	2-di-Me-amino-Et	0.082

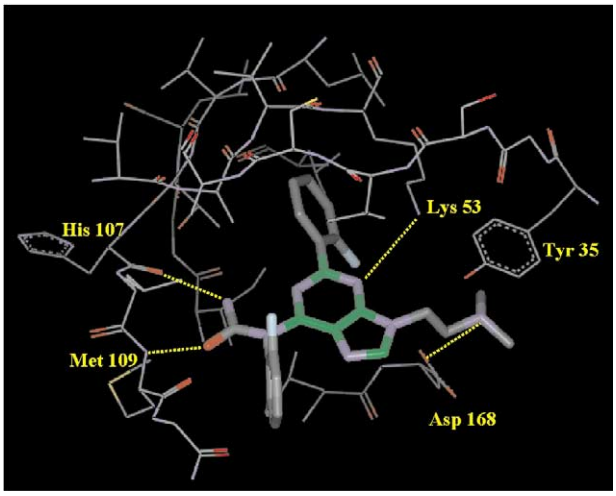


Figure 4. Compound **19** bound in p38 MAP kinase.

with purine, (2) the hydrogen bond donor-acceptor between the kinase backbone **M109** amide NH and **H107** amide carbonyl to the urea which replicates the interaction of adenine N1 and the C6 NH2 of ATP that is conserved across all kinases,[15] (3) the binding of the C2 fluorophenyl ring into a hydrophobic pocket which imparts selectivity for p38,[16] and (4) the projection of the dimethylaminoethyl group (**R3**) into the phosphate binding pocket. The propeller-like arrangement of the two aryl rings attached to the central purine deserves comment as it may shed some light on the SAR for **R1** and **R2**. The preference for *ortho* substitution of these aryls can be partially understood to the extent it will enhance the conformational bias towards the crystallographically observed propeller conformation. However, for the **R1** group a steric clash with the N7 of adenine already forces this conformation and the more important feature of *ortho* substitution may be that it directs the attached residues back into the lipophilic pocket instead of solvent. Additional notable features are acid-base pairings of Lys53 with N-3 of the purine and Asp168 with the dimethylamino group and the π–cation interactions of the 2-fluorophenyl and dimethyl groups with Lys53 and Tyr35, respectively. While the pronounced electronic requirement (compare **20** to **21** and **10** to **22**), is not readily understood, it may be related to effects on the H-bonding donor–acceptor properties of the urea.

In summary, a number of potent p38 inhibitors have been prepared based upon this novel use of a purine template. The proposed binding mode for this novel series of inhibitors is consistent with the crystallography results obtained from inhibitor **19**/p38α. Additional novel p38 inhibitors designed from this model will be reported in due course.

Acknowledgements

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