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Synthesis and SAR of 4-substituted-2-aminopyrimidines as novel c-Jun N-terminal kinase (JNK) inhibitors

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ABSTRACT

The development of a series of novel 4-substituted-2-aminopyrimidines as inhibitors of c-Jun N-terminal kinases is described. The synthesis, in vitro inhibitory values for JNK1, and the in vitro inhibitory value for a c-Jun cellular assay are discussed. Optimization of microsomal clearance led to the identification of **9c**, whose kinase selectivity is reported.

Type 2 diabetes is a metabolic disorder that accounts for 120 million patients worldwide and the number is likely to grow to greater than 366 million by the year 2030.[1] Patients with type 2 diabetes are insulin resistant, a condition in which the body fails to respond to insulin properly.

The c-Jun N-terminal protein kinases (JNKs) are a family of serine/threonine protein kinases and members of the mitogen-activated protein kinase (MAPK) family. JNK1 has recently emerged as an attractive target for diabetes therapy, since JNK1 is believed to play a key role in linking obesity and insulin resistance.[2] JNK1 disrupts the insulin signaling cascade via phosphorylation of the insulin receptor substrate (IRS-1) at serine[307], which leads to the degradation of IRS-1. JNK1$^{-/-}$ mice show marked reduction in both plasma glucose and insulin concentrations relative to their wild-type littermates, and thus are protected from diet-induced obesity.[2b] In addition, JNK1 activity is elevated in adipocytes of type 2 diabetic patients.[3] Inhibitors of JNK1 can potentially increase insulin sensitivity, and therefore could be useful as therapeutics for the treatment of type 2 diabetes. Therefore, considerable efforts have been directed toward the identification of JNK1 inhibitors suitable for clinical development.[4]

We wish to report here our efforts toward the identification of 4-substituted-2-aminopyrimidines as novel ATP competitive, pan-JNK inhibitors.[5] A high-throughput screening (HTS) of the Pfizer compound collection identified compound **1** (Fig. 1) as an ATP-competitive JNK1 inhibitor. This hit was extremely attractive based on it's high ligand efficiency,[6] and promising selectivity against an initial panel of kinases. JNK1 inhibitors need to be cell permeable to reach the intracellular targets, therefore a phospho c-Jun cell-based ELISA assay was also utilized to test the compounds cellular potency.[7] In this assay, compound **1** was moderately active with an IC_{50} of 460 nM. The above properties motivated us to initiate hit-to-lead chemistry to explore the activity of this class of compounds as JNK1 inhibitors.

1
JNK1 K_i = 26 nM
LE = 0.44
ELogD = 4.42
HLM Cl = 116 μL/min/kg

Figure 1. Initial hit from high-throughput screening.

Unfortunately, **1** was rapidly cleared in vitro (human liver microsomes (HLM) Cl = 116 μL/min/kg). Metabolite ID studies revealed that metabolism was occurring exclusively on the alkylamino portion of the molecule. Our strategy was thus to dramatically reduce the lipophilicity (ELog*D* = 4.42) of this hit,

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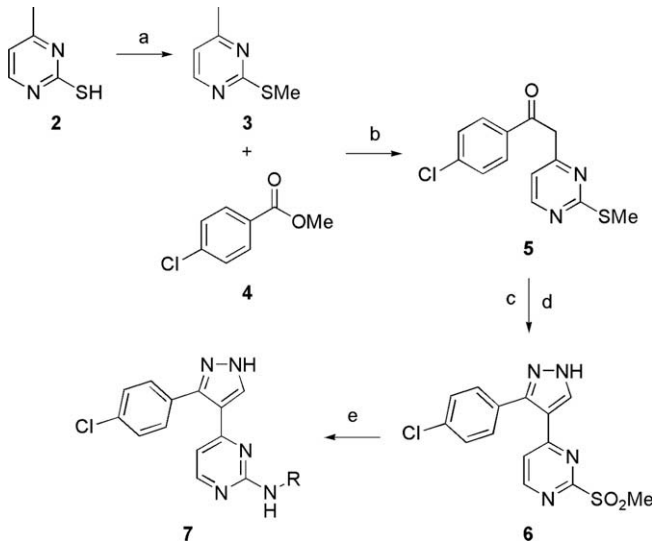
while maintaining or improving the ligand efficiency (LE = 0.44). More specifically, we wished to expediently and efficiently vary the alkylamino portion of the molecule.

The final list of compounds for synthesis was chosen by taking a list of ~1500 potential virtual compounds (potential products utilizing commercially available primary amines) and applying the following filters–cLog$D$ < 3, PSA < 140 Å^2, MW < 450, Pass Ro5[8] and predicted to be stable in HLM (calculation based on in-house HLM data). The resulting ~200 potential virtual compounds were then docked (utilizing proprietary in-house software) into a co-crystal structure of compound **1** with JNK1 and the top 50 compounds chosen for synthesis.

The initial route to these targets was via the 2-methylsulfonylpyrimidine intermediate **6**.[9] This intermediate was accessed in a straight forward fashion following the four step protocol below (Scheme 1). 2-Mercapto-4-methyl pyrimidine **2** was alkylated with iodomethane to afford 2-mercapto-4-methyl pyrimidine **3**.[10] Deprotonation and reaction of the anion of **3** with methyl 4-chlorobenzoate **4** gave ketone **5** in good yield.[10] Ketone **5** was treated with dimethylformamide dimethylacetal followed by hydrazine hydrate yielding the required pyrazole.[11] Oxidation of the methylsulfide intermediate to the required methylsulfonylpyrimidine **6** was achieved in a straightforward fashion.[12]

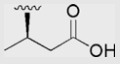
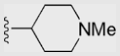
The final S_NAr step was optimized under a variety of conditions, utilizing cyclopentylamine as our partner of choice.[13] Initially it was found that performing this reaction in dioxane under microwave irradiation (137 °C, 10 min) afforded the required product (**7**, where R = cyclopentyl) in a 77% yield. Unfortunately, when using this protocol with a small test set of amines and amine hydrochlorides, it was immediately apparent that heterogeneity hindered the reaction progress in some instances. Further optimization showed that this reaction could be performed in 2-propanol (175 °C, 10 min) with no diminution in yield. Due to the enhanced solubility of the reagents and the higher reaction temperature, this optimized protocol showed greater generality over a more diverse set of amine monomers.

Unfortunately, a number of amine monomers failed to give any pure final target, due to either their lack of reactivity (presumably due to steric hindrance and/or unfavorable electronics) or instability under the reaction conditions. We therefore attempted to increase the reactivity of the pyrimidine electrophile by choosing to access these final targets via the 2-chloropyrimidine intermediate, which was accessed via a similar synthetic sequence as before. This route provided access to the majority of the remaining ~50 compounds assigned for synthesis.



Scheme 1. Reagents and conditions: (a) NaOH, H_2O, EtOH, MeI, rt, 16 h, 95%; (b) LiHMDS, THF, 0 °C, 2 h, 89%; (c) $(MeO)_2CHNMe_2$, PhMe, reflux, 16 h then $H_2NNH_2{\cdot}H_2O$, EtOH, rt, 2 h, 84%; (d) *m*CPBA, CH_2Cl_2, rt, 16 h, 84%; (e) RNH_2, ^{i}PrOH, μW, 175 °C, 30 min.

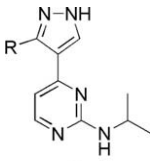
Table 1
Enzymatic and cellular activity of JNK inhibitors **1** and **7**

Compound	R	JNK1 K_i (nM)	Pc-Jun IC_{50} (nM)	HLM (μL/min/kg)
2b	Me	2060	NT	81
7b	^{i}Pr	48	920	106
7c	c-C_3H_5	151	840	96
1	c-C_5H_9	26	460	116
7d	c-C_6H_{11}	27	1100	156
7e		315	NT	<6
7f		1570	NT	14

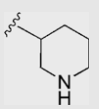
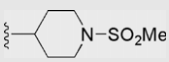
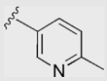
All biological data are mean values of multiple experiments and thus shows the reader that these have a higher accuracy than just providing data from one experiment.
NT, not tested.

All the compounds were tested in our JNK1 enzymatic inhibition assay as well as a cell-based assay measuring inhibition of TNFα stimulated phosphorylation of c-Jun in HepG2 cells.[7b] The ef-

Table 2
Enzymatic and cellular activity of JNK inhibitors **8**



8

Compound	R	JNK1 K_i (nM)	Pc-Jun IC_{50} (nM)	HLM (μL/min/kg)
8a	H	2730	NT	21
8b		381	3500	21
8c		43	357	34
8d		169	814	18
8e		530	NT	14
8f		101	221	12
8g		162	6020	61

All biological data are mean values of multiple experiments and thus shows the reader that these have a higher accuracy than just providing data from one experiment.
NT, not tested.

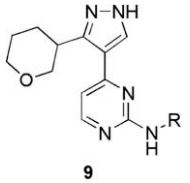
fect of different alkylamino substitution was studied first, and these results are summarized in Table 1. Attempts to reduce the microsomal clearance by reducing MW either led to lower enzymatic potency (e.g., **7a**) or no significant reduction in clearance (e.g., **7b**). Alternatively, reduction in lipophilicity led to improved microsomal clearance, but at the expense of enzymatic potency (e.g., **7e** and **7f**), reduced in vitro permeability (e.g., **7e**–CaCo-2 AB = 0.3 and BA = 13.2 $\times$ 10^{-6} cm/s) or increased hERG affinity (e.g., **7f**–Dofetilide K_i = 1020 nM). It is also interesting to note that a drop-off in potency was observed in moving from the JNK1 enzymatic assay to the cellular c-Jun assay. In some cases (e.g., **7c**–CaCo-2 AB = 28.5 and BA = 15.0 $\times$ 10^{-6} cm/s) this was not due to poor in vitro permeability.

The above SAR indicated that the 4-chlorophenyl moiety was so lipophilic that charged and/or extremely polar aminoalkyl substituents were required to obtain reasonable Log*D* values, thus compromising permeability or increasing hERG affinity. It was thus decided that alternatives to the initial 4-chlorophenyl moiety were required, which was achieved using synthetic schemes similar to the one shown in Scheme 1.

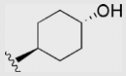
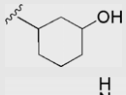
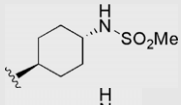
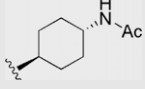
As summarized in Table 2, successful replacements for the 4-chlorophenyl moiety were obtained. Dramatic reduction in MW and lipophilicity led to a large reduction in enzymatic potency (e.g., **8a**), but with much improved microsomal clearance. Of the tetrahydropyran isomers tested, **8c** was the most potent, and microsomal clearance was still good. Interestingly, sulfonamide **8f** displayed moderate enzymatic potency, only a small drop-off in cellular potency and low clearance.

At this point, we chose to fix the 3-tetrahydropyranyl moiety (as seen in **8c**), due to it's improved rat microsomal (RLM) clearance vs sulfonamide **8f** (RLM Cl = 18 μL/min/mg for **8c** and 52 μL/min/mg for **8f**). The effect of different alkylamino substitution was once again studied with this new less lipophilic 3-tetrahydropyran moiety, and these results are summarized in Table 3. Alkyl

Table 3
Enzymatic and cellular activity of JNK inhibitors **8c** and **9**



9

Compound	R	JNK1 K_i (nM)	Pc-Jun IC_{50} (nM)	HLM (μL/min/kg)
8c	^{i}Pr	43	357	40
9b	c-C_5H_9	12	245	89
9c		12	98	<15
9d		36	209	15
9e		9	114	11
9f		28	299	<8

All biological data are mean values of multiple experiments and thus shows the reader that these have a higher accuracy than just providing data from one experiment.
NT, not tested.

Table 4
Kinase selectivity profile of JNK inhibitor **9c**

Kinase	IC_{50} (μM)	Kinase	IC_{50} (μM)	Kinase	IC_{50} (μM)
JNK1	0.012[a]	GSK3β	1.9	IR	>30
JNK2	0.017[a]	SAPK3	6.2	MAPKAP-K2	>30
JNK3	0.016[a]	IKKb	13	MEK1	>30
CDK2/cyclinA	0.59	MAPK2	13	p70S6K	>30
MKK7β	0.86	MKK6	15	PDK1	>30
MKK4	0.92	SAPK2b	15	PKCΘ	>30
SAPK4	1.2	AMPK	>30	SAPK2a	>30

[a] Values are inhibitor constant K_i.

substituents led to good enzymatic potency (e.g., **9a** and **9b**), but increased microsomal clearance. Cyclohexanol moieties provided good enzymatic and cellular potency along with reduced clearance (e.g., **9c**). Cyclohexylamine substituents also provided a good balance of enzymatic and cellular potency with low clearance (e.g., **9e**).

The selectivity data of **9c** against a panel of protein and lipid kinases are listed in Table 4. This compound is a pan-JNK inhibitor with an estimated margin of at least 100-fold over other MAP kinases (MKK1, MKK4, MKK7β and p38MAPKαγδ), >27-fold over glycogen synthase kinase-3β, and 65-fold over cyclin-dependent kinase-2.

JNK inhibitor **9c** attenuated the effects of elevated fatty acids on induction of insulin resistance both in vitro and in vivo and the results of this have been reported elsewhere.[7b]

In summary, we have identified a series of 4-substituted-2-aminopyrimidines as potent JNK inhibitors with good cellular activity. Optimization of in vitro clearance was achieved by a reduction in lipophilicity and also by more evenly distributing polarity throughout the molecule. This exercise led to the identification of **9c**, which showed good selectivity across a panel of diverse protein and lipid kinases. Further work demonstrating the utility of this tool compound for treating diabetes and/or obesity has been reported previously.[7b]

Acknowledgments

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