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B-Raf kinase inhibitors: Hit enrichment through scaffold hopping

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ABSTRACT

In continuation of our efforts toward hit identification and optimization for a B-Raf kinase project, we have employed a scaffold hopping strategy. The original HTS hit scaffold pyrazolo[1,5-*a*]pyrimidine was replaced with different thienopyrimidine and thienopyridine scaffolds to append the optimal pharmacophore moieties in order to generate novel B-raf kinase inhibitors with desirable potency and properties. This strategy led to the identification of additional lead compound **11b** which had good enzyme and cell potency, while maintaining selectivity over a number of kinases.

The RAS-RAF-MEK signal transduction pathway plays a key role in tumor biology.[1] A V600E mutation of the B-Raf isoform induces constitutive activation of this kinase in the ERK pathway that increases cell proliferation and cell survival. Inhibitors of B-Raf could be used in the treatment of melanomas, colorectal cancer, and other Ras related human cancers.[2] A number of small molecule B-Raf inhibitors have been disclosed in the recent past. As detailed in our earlier communications,[3] work in our laboratories commenced with a high throughput screen[4] (HTS) that resulted in the identification of pyrazolo[1,5-*a*]pyrimidine-3-carboxylate **1**. Structure–activity relationships determined by modifying different regions of the hit molecule, combined with structure based design to incorporate kinase hinge region interacting groups, resulted in lead compound **2** which exhibited significant improvement in enzyme and cellular potency.

From the lead generation work carried out on compound **1**, it was clear that the hinge region interaction provided by the 4-pyridyl ring of compound **2** and the hydrophobic interaction provided by the 3-trifluoromethyl substituted benzamide form the key pharmacophores for the binding of the ligand (Fig. 1). The bicyclic core pyrazolo[1,5-*a*]pyrimidine acts as the scaffold positioning these moieties, providing required directionality. The pyrazolo[1,5-*a*]pyrimidine scaffold has been extensively used in the design of the ATP competitive kinase inhibitors since it mimics the bicyclic heterocycle core of ATP. Different research groups in our organization[5] and elsewhere[6] have successfully exploited other bicyclic scaffolds like thienopyrimidines and cyanothienopyridines in the design of kinase inhibitors. This prompted us to evaluate other bicyclic scaffolds shown in Figure 2 during the course of our lead generation effort for the B-raf project.

A quick survey of the literature (Table 1) on the different proposed scaffolds indicated that these scaffolds offered a further competitive advantage for providing novel B-raf kinase inhibitors. In this communication we detail our efforts to synthesize the designed analogs using these scaffolds and the observed potency and selectivity.

Syntheses of analogs with scaffolds A–D incorporating the required pharmacophores are detailed in Schemes 1–3.[7] As shown in Scheme 1, for the synthesis of analog **7b** with scaffold A, sequential Suzuki reactions were employed to install the required *N*-phenyl-3-(trifluoromethyl)benzamide and 4-pyridyl groups.

In the case of analog **11b** that incorporates scaffold B, the 4-pyridyl group was installed at an earlier stage followed by cyclocondensation to give intermediate **9**.

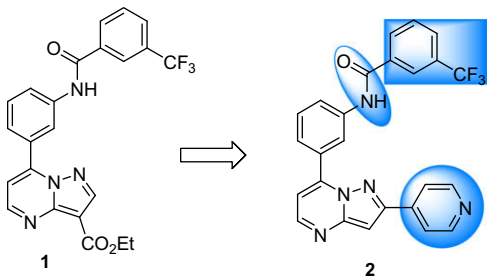

Figure 1. Hit to lead optimization of pyrazolo[1,5-*a*]pyrimidine as a B-raf kinase inhibitor. Key pharmacophores are highlighted in red.

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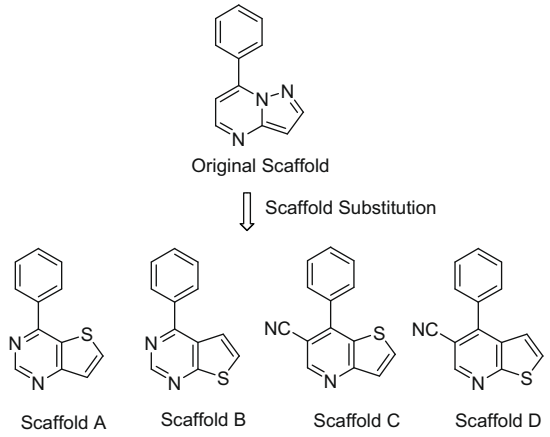
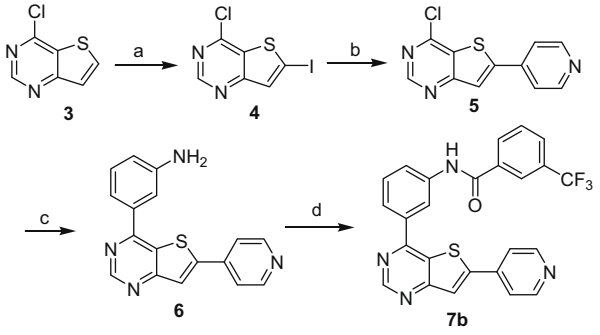


Figure 2. Scaffold substitutions for 7-phenylpyrazolo[1,5-*a*]pyrimidine.

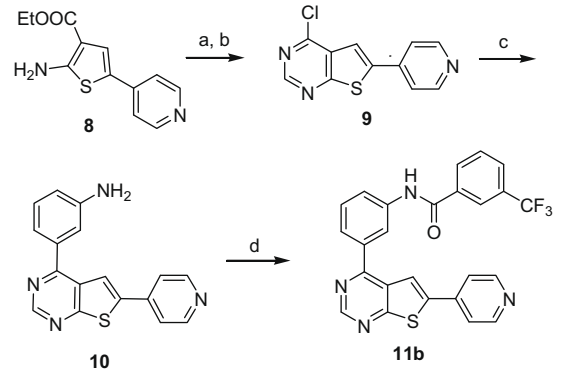
Table 1
Literature search results for different scaffolds

Scaffold	No. of hits in SciFinder[a]
7-Phenylpyrazolo[1,5-*a*]pyrimidine (original scaffold)	6449
4-Phenylthieno[3,2-*d*]pyrimidine (scaffold A)	63
4-Phenylthieno[2,3-*d*]pyrimidine (scaffold B)	1508
7-Phenylthieno[3,2-*b*]pyridine-6-carbonitrile (scaffold C)	261
4-Phenylthieno[2,3-*b*]pyridine-5-carbonitrile (scaffold D)	1243

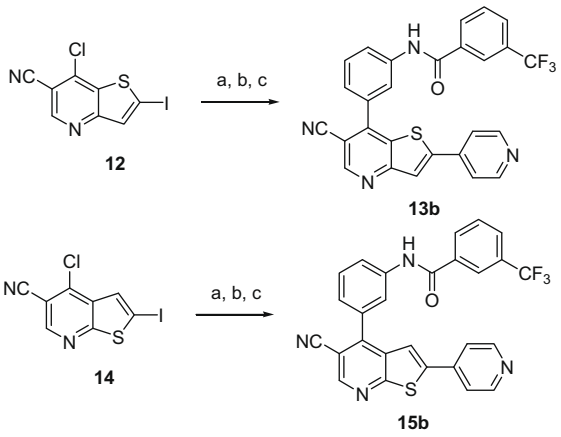
[a] Search carried out with rings locked to prevent further ring fusion; data reflect the search results as of 01/25/2010 (includes examples from our work).



Scheme 1. Reagents: (a) LDA/THF, I_2, 69%; (b) 4-pyridylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, DME, 50%; (c) 3-aminophenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, DME, 70%; (d) (3-trifluoromethyl)benzoyl chloride, Et_3N, CH_2Cl_2, 88%.



Scheme 2. Reagents: (a) formamidine acetate, methoxyethanol, 88%; (b) thionyl chloride, DMF, 93%; (c) 3-aminophenylboronic acid Na_2CO_3, $Pd(PPh_3)_4$, DME, 67%; (d) 3-(trifluoromethyl)benzoyl chloride, Et_3N, CH_2Cl_2, 90%.



Scheme 3. Reagents: (a) 4-pyridylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, DME; (b) 3-aminophenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, DME; (d) (3-trifluoromethyl)benzoyl chloride, Et_3N, CH_2Cl_2.

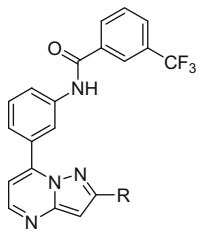
Analogs **13b** and **15b** incorporating scaffolds C and D were prepared using the same synthetic sequence employed for analog **7b** starting from appropriate bishalides **12** and **14**.[5a]

Since scaffolds A–D incorporate larger sulfur atom in the five-membered ring, both 3-pyridyl (compounds **7a**, **11a**, **13a** and **15a**) and 4-pyridyl analogs (compounds **7b**, **11b**, **13b** and **15b**) were explored as the pharmacophore targeting the hinge interaction with the protein. The 3-pyridyl analogs with scaffolds A–D were synthesized by following the synthetic routes analogous to those shown in the above schemes. The synthesized analogs were evaluated for their enzyme activity against B-Raf kinase. The results are shown in Table 2. As seen from the results, thienopyrimidine scaffolds A and B (compounds **7b** and **11b**) were very well tolerated compared to the cyano thienopyridine scaffolds C and D (compounds **13b** and **15b**). Among the thienopyrimidine analogs, scaffold B (thieno[2,3-*d*]pyrimidine, compound **11b**) was found to be more active than scaffold A (thieno[3,2-*d*]pyrimidine compound **7b**). Although the sulfur containing ring is larger in these scaffolds compared to pyrazole ring in the original lead **2**, the core was well accommodated and the 4-pyridyl ring was probably still able to invoke the key hinge interaction. As seen before with the pyrazolo[1,5-*a*]pyrimidine scaffold, the 3-pyridyl analogs (compounds **7a** and **11a**) were much inferior compared to the 4-pyridyl analogs.

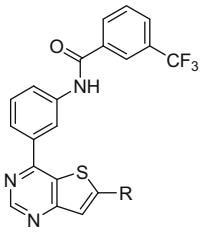
Our observed SAR can be rationalized from the docking studies using these different scaffolds. As shown in Figure 3, compound **11b** (green) has a proposed binding pose that retains the crucial interactions of the benzamide and pyridine pharmacophores with the protein and overlays well with the original pyrazolopyrimidine compound **2b** (green). Compound **7b** that incorporates scaffold A, changes the orientation of the 4-pyridyl ring due to the position of the large S atom resulting in loss of hinge interaction with Cys 532. In our predicted binding model, the cyano thienopyridine scaffolds C and D (not shown in Fig. 3) could not be docked favorably due to the limited space available around the cyano group. The cyano group cannot be accommodated without significant movement of the side chain Lys 483.

Thieno[2,3-*d*]pyrimidine analog **11b** was further profiled for selectivity and its ability to inhibit cell proliferation. As shown in Table 3, compound **11b** exhibited the ability to inhibit growth in a variety of tumor cell lines. To validate that the anti-proliferative effect observed with compound **11b** is indeed related to the inhibition of B-Raf, the compounds ability to inhibit p-MAPK was determined in A375 cell line. The compound had a comparable IC_{50} to that of cell proliferation. Compound **11b**, in spite of its hinge inter-

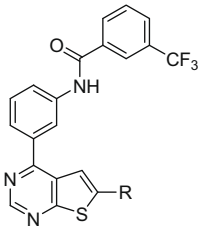
Table 2
B-Raf kinase activity of thienopyrimidines and cyano thienopyridines



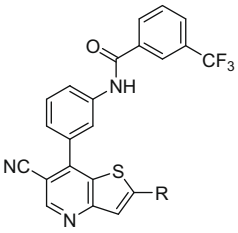
Lead Scaffold
2a: R = 3-pyridyl
2 : R = 4-pyridyl



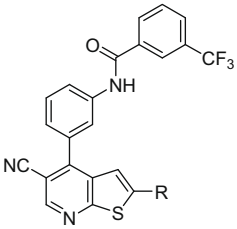
Scaffold A
7a: R = 3-pyridyl
7b: R = 4-pyridyl



Scaffold B
11a: R = 3-pyridyl
11b: R = 4-pyridyl



Scaffold C
13a: R = 3-pyridyl
13b: R = 4-pyridyl



Scaffold D
15a: R = 3-pyridyl
15b: R = 4-pyridyl

Scaffold	Class	Examples	IC_{50}[a] (μM)
Lead	Pyrazolo[1,5-*a*]pyrimidine	**2a**	>10
		2	0.032 ± 0.009
Scaffold A	Thieno[3,2-*d*]pyrimidine	**7a**	>10
		7b	0.54 ± 0.156
Scaffold B	Thieno[2,3-*d*]pyrimidine	**11a**	1.11 ± 0.22
		11b	0.08 ± 0.018
Scaffold C	Cyanothieno[3,2-*b*]pyridine	**13a**	>10
		13b	5.94 ± 0.87
Scaffold D	Cyanothieno[2,3-*b*]pyridine	**15a**	>10
		15b	>10

[a] Values are means of two or more experiments.

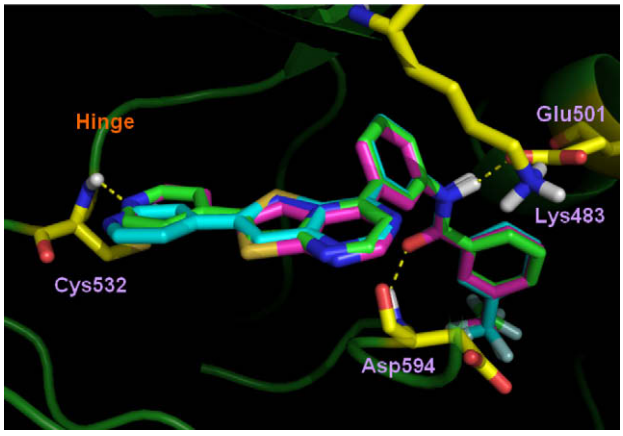


Figure 3. Binding models of compounds **2b**, **7b** and **11b**. The carbon atoms of compounds **2b**, **7b** and **11b** are colored as green, cyan and magenta, respectively. The predicted H-bonds were indicated by yellow dotted lines.

Table 3
Cell growth inhibition data

Tumor cell lines	Compound **11b** IC_{50}[a] (μM)
A375 (B-Raf mutant)	0.23 ± 0.12
LoVo (K-ras mutant)	0.3 ± 0.035
HT29 (B-Raf mutant)	0.15 ± 0.082
CaCo-2 (wild type)	1.1 ± 0.44
WM-266-4 (B-Raf mutant)	3.1 ± 0.53

[a] Values are means of two or more experiments.

action in the ATP binding pocket of B-Raf enzyme, was found to be highly selective against a number of kinases including CDK4, IKK-2, AKT, m-Tor, ITK, PKC-theta, BTK, SRC, LYN, PDK1, LCK, PI3Kα (IC_{50}: >10 μM). Employing fluorescence spectroscopy techniques, compound **11b** exhibited a single digit nanomolar K_D from the changes in the endogenous tryptophan fluorescence of the enzyme upon inhibitor binding at the emission and excitation wavelengths of 465 nm and 295 nm, respectively.

In summary, we have successfully employed a scaffold hopping strategy starting from the pyrazolopyrimidine scaffold identified as a B-Raf inhibitor from HTS. The alternate thieno[2,3-*d*]pyrimidine scaffold showed good cellular potency and excellent selectivity over a number of kinases, providing a novel lead for further exploration.

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