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# Discovery of highly potent and selective type I B-Raf kinase inhibitors

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ABSTRACT

A series of pyrazolo[1,5-α]pyrimidine analogs has been prepared and found to be potent and selective B-Raf inhibitors. Molecular modeling suggests they bind to the active conformation of the enzyme.


The extracellular signal-regulated Mitogen-Activated Protein (MAP) kinase pathway plays an important role in cell survival, growth, and proliferation.[1] B-Raf, a key component in this cascade, is mutationally activated in approximately 8% of all human cancers with the highest incidence (66%) in malignant melanomas.[2] It has thus received considerable attention as the target of small molecule inhibitors as potential cancer therapeutics.[3]

In our ongoing efforts to develop B-Raf inhibitors we identified the lead pyrazolo[1,5-*a*]pyrimidine-3-carboxylate **1**[4] (B-Raf kinase $IC_{50}$ = 1.5 μM) (Fig. 1), which binds in the same manner as sorafenib,[3a,5] a type II kinase inhibitor. Subsequently, a Letter[3b] appeared describing a series of novel B-Raf inhibitory tri-aryl imidazoles lacking a large hydrophobic group which occupies the DFG-out pocket.[5] We envisioned these tri-aryl imidazoles binding to the active conformation of B-Raf as type I kinase inhibitors. This was confirmed by a recent report from the same group.[3c] This led us to investigate the design of a type I B-Raf inhibitor based on the pyrazolo[1,5-α]pyrimidine core.

Analogous to the triaryl imidazoles, our initial work involved putting pyridyl and phenolic moieties onto the 2 and 3 positions of the pyrazolo[1,5-α]pyrimidine (Fig. 2). The analogs of **2** and **3** overlay well in CHEM3D, which suggests **3** may be able to bind to B-Raf in the same manner as do tri-aryl imidazoles. Molecular modeling[6] also suggested a good fit of **3** into the ATP binding pocket of the active conformation of B-Raf.

It was found that aryl or heteroaryl groups on C-7 position of the pyrazolopyrimidine core improved activity, particularly basic amines appended to these aryl or heteroaryl further enhanced enzyme and cell potency.[7] Our exploration of the SAR resulted in the potent new lead 3-(3-hydroxyphenyl)-2-(pyridin-4-yl)pyrazolo[1,5-*a*]pyrimidine **4**, (Fig. 3, B-Raf kinase[8] $IC_{50}$ = 0.9 nM). It also showed good activity against proliferation of tumor cell lines with $B\text{-}Raf^{V600E}$ including A375 ($IC_{50}$ = 0.300 μM) and HT29 ($IC_{50}$ = 0.270 μM) while it is much less potent against a wild-type B-Raf cell line CaCo-2 ($IC_{50}$ = 1.3 μM). The synthesis of **4** (Scheme 1) started from 2-(3-methoxyphenyl)acetonitrile **8**. Condensation of **8** and methyl isonicotinate (**9**) followed by reaction with phosphorus oxychloride afforded chloroacrylonitrile **11**, which then reacted with hydrazine to give aminopyrazole **12**. The annulation of **12** and enaminone **6** followed by a Buchwald coupling with diazabicyclo[3.2.1]octanyl (**A1**) afforded compound **14**. Finally, demethylation with boron tribromide provided compound **4**.

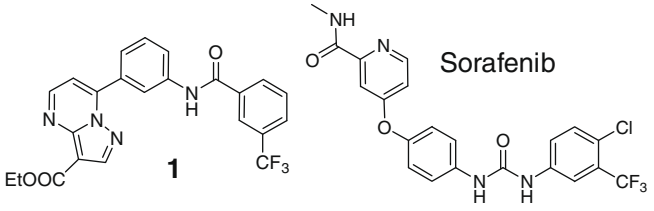


**Figure 1.** B-Raf inhibitor **1** and sorafenib.

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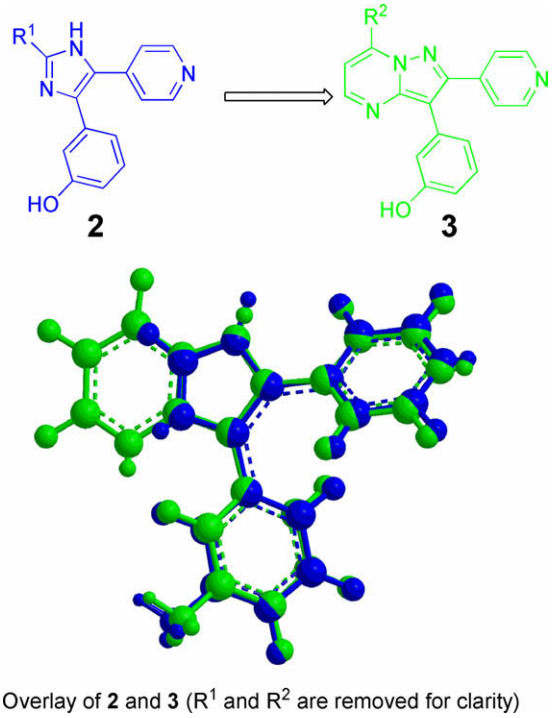



**Figure 2.** Design of a B-Raf inhibitor with pyrazolopyrimidine core.

To further optimize **4**, we prepared a number of analogs (Table 1) by following the same synthetic sequence. It was found that the phenolic hydrogen is crucial for the activity as compounds with an anisole moiety are much less potent (**14**, **16**). An increase in potency was observed when a halogen atom was introduced next to the hydroxyl groups (**15**, **16**). Analogs (**17**–**19**) with a short linker between the phenyl and bicycles were also prepared and found to be inferior to analogs without the linker. The replacement of the **A1** moiety with a diazabicyclo[2.2.1]heptanyl group (Table 2) resulted in the improvement of both enzyme and cell activities (**4** vs **25**). Anisole derivatives (**33**–**38**) were much less potent than corresponding phenolic compounds (**20**, **22**, **24**, **29**, **30**), which further confirmed the importance of the phenolic hydrogen. Tertiary amines ($R^4$ = alkyl) showed better cell potency than secondary amines ($R^4$ = H) while being almost equipotent in the enzyme assay. Ethyl and acetyl groups (**31**, **32**) were deleterious to cell potency and a methyl group was optimal for the tertiary amines. When a small *ortho*-group ($R^5$) was introduced into the phenyl linker, a set of extremely potent compounds (**21**–**23** and **26**–**28**) resulted. The B-Raf $IC_{50}$ of these analogs were below the test limit (<0.32 nM) and compound **28** had an A375 cell $IC_{50}$ less than 0.010 μM.

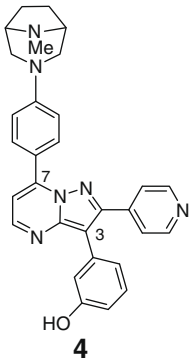
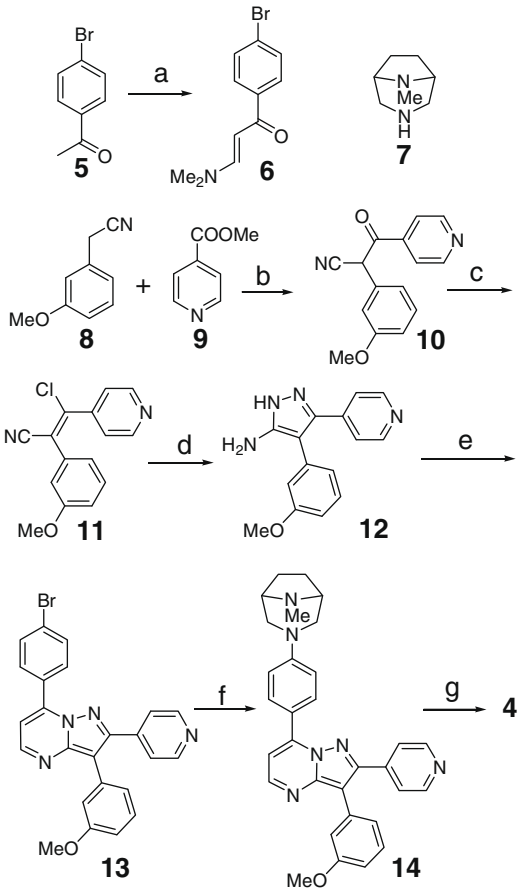
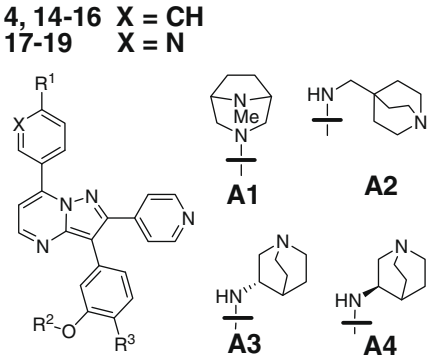



**Figure 3.** Lead compound **4**.




**Scheme 1.** Reagents and conditions: (a) DMF-DMA, reflux, 4 d, 70%; (b) NaOEt, EtOH, reflux, 3 h, 34%; (c) $POCl_3$, 80 °C, 18 h, 57%; (d) $N_2H_4$, EtOH, reflux, 6.5 h, 94%; (e) **6**, HOAc, heat, 41%; (f) **7**, cat. BINAP and $Pd_2(dba)_3$, NaOtBu, 100 °C, 20 h, 49%; (g) $BBr_3$, rt, 1 h, 83%.

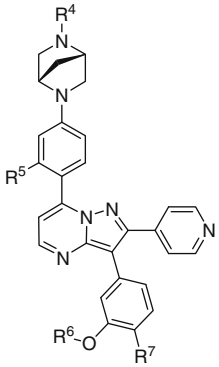
**Table 1**
Activities of pyrazolopyrimidine analogs




| Compd | $R^1$ | $R^2$ | $R^3$ | B-Raf $IC_{50}$ (nM) | A375 Cell $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| **4** | **A1** | H | H | 0.9 | 0.300 |
| **14** | **A1** | Me | H | 17 | 1.900 |
| **15** | **A1** | H | Cl | <0.32 | 0.170 |
| **16** | **A1** | Me | Cl | 9.7 | 0.630 |
| **17** | **A2** | H | H | 2.0 | 0.930 |
| **18** | **A3** | H | H | 1.5 | 0.370 |
| **19** | **A4** | H | H | 1.0 | 0.385 |

Illustrated in Figure 4 is a binding model of compound **28** in complex with B-Raf.[6] In the model, the pyridyl nitrogen forms a hydrogen bond with the hinge region residue Cys532.[3c] The

**Table 2**
Activities of pyrazolopyrimidines with diazabicyclo[2.2.1]heptanyl



| Compd | $R^4$ | $R^5$ | $R^6$ | $R^7$ | B-Raf $IC_{50}$ (nM) | A375 Cell $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|
| **20** | H | H | H | H | 0.49 | 0.300 |
| **21** | H | Me | H | H | <0.32 | 0.065 |
| **22** | H | F | H | H | <0.32 | 0.023 |
| **23** | H | Cl | H | H | <0.32 | 0.016 |
| **24** | H | H | H | F | <0.32 | 0.150 |
| **25** | Me | H | H | H | 0.38 | 0.077 |
| **26** | Me | Me | H | H | <0.32 | 0.031 |
| **27** | Me | F | H | H | <0.32 | 0.012 |
| **28** | Me | Cl | H | H | <0.32 | <0.010 |
| **29** | Me | H | H | F | <0.32 | 0.076 |
| **30** | Me | Me | H | F | <0.32 | 0.015 |
| **31** | Et | H | H | F | 0.51 | 0.200 |
| **32** | Ac | H | H | F | 0.50 | 0.210 |
| **33** | H | H | Me | H | 12 | 1.100 |
| **34** | H | F | Me | H | 0.44 | 0.504 |
| **35** | H | H | Me | F | 8.7 | 1.200 |
| **36** | Me | H | Me | H | 8.2 | 0.760 |
| **37** | Me | H | Me | F | 21 | 6.000 |
| **38** | Me | Me | Me | F | 1.0 | 0.933 |

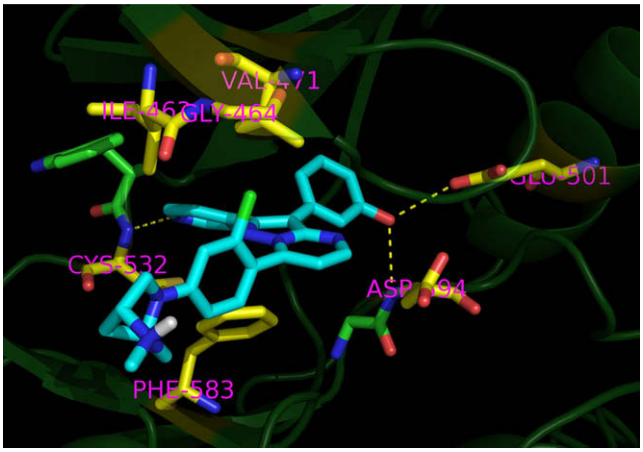



**Figure 4.** Docked model of compound **28** in the active site of B-Raf.

hydroxyl group makes two hydrogen bonds to the side chain of Glu501 and the backbone NH of Asp 594, which may make an important contribution to the affinity. The model also indicated that the pyrazolo[1,5-*a*]pyrimidine core sits in a hydrophobic pocket formed by Phe583 and Val471 with a possible π–π stacking interaction with Phe583. The chlorine atom occupies another small hydrophobic pocket defined by Ile463, Gly464, and Val471, which may explain the potency enhancement brought by the substituents *ortho* to the pyrazolopyrimidine core.

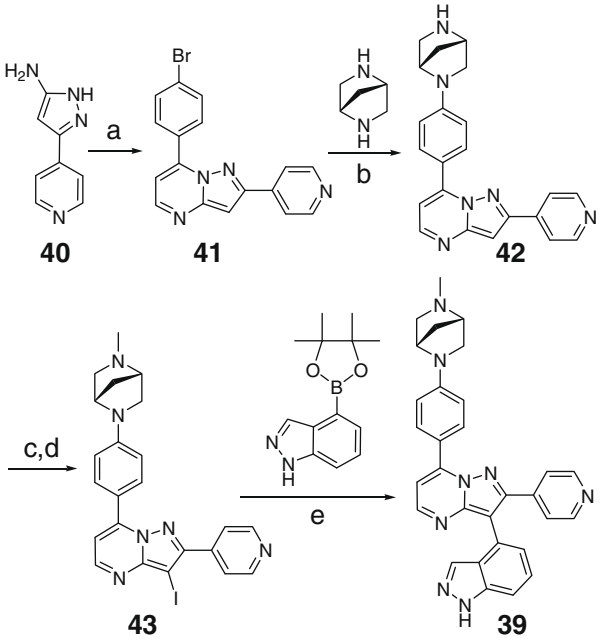
The selectivity profile of compound **25** was assessed against a panel of 24 protein kinases (Table 3). It shows at least 182-fold selectivity for all kinases tested. The most potent off target activity was seen for p38α, PKCβ, and VEGFR2 with $IC_{50}$s less than 0.3 μM.

**Table 3**
The selectivity profile of compound **25**

| Kinase | $IC_{50}$[a] |
|---|---|
| ABL1 | 0.563 |
| Aurora B | 25.5 |
| CDK1 | 28.4 |
| CDK2 | 26.4 |
| CHK | 18.6 |
| CK1γ1 | 9.51 |
| ERK2 | >50 |
| FYN | 1.94 |
| GCK | 4.10 |
| HCK | 1.24 |
| IKKα | >50 |
| IKKβ | >50 |
| LYN | 1.76 |
| MET | 3.10 |
| MK2 | 17.1 |
| p38α | 0.069 |
| PDGFRα | 6.56 |
| PKA | 1.79 |
| PKCα | 2.46 |
| PKCβ | 0.119 |
| ROCK1 | 17.6 |
| RSK1 | 9.71 |
| SRC | 0.675 |
| VEGFR2 | 0.222 |

[a] μM, ATP concentration = $K_{m,\ ATP}$ of the tested kinase.

While some analogs such as **25**–**28** showed excellent cell activity, it was found that these compounds were labile in microsomes. Metabolite identification studies indicated that the phenol was glucuronidated in liver microsomes. Therefore, a search for a phenol replacement was undertaken,[9] which led to the discovery of compound **39** containing an indazole group. Compound **39** was prepared from 3-(pyridin-4-yl)-1*H*-pyrazol-5-amine **40** in five steps (Scheme 2). Although compound **39** is slightly less potent (B-Raf kinase $IC_{50}$ = 1.2 nM; A375 $IC_{50}$ = 0.202 μM) than the phenol




**Scheme 2.** Reagents and conditions: (a) **6**, HOAc, 100 °C, 15 h, 57%; (b) cat. BINAP and $Pd_2(dba)_3$, $NaOBu^t$, 100 °C, 2 h, 57%; (c) HCHO (aq), $NaBH(OAc)_3$, DMF, rt, 1 h, 97%; (d) NIS, $HOAc/CH_2Cl_2$, rt, 30 min, 69%; (e) cat. $Pd(PPh_3)_4$, $Na_2CO_3$, 130 °C (μW), 1 h, 43%.

analog **25**, it shows excellent microsomal stability ($t_{1/2}$ >30 min, nude mice). Moreover, it also has a better selectivity profile (>245-fold for the 24-kinase panel, data not shown) compared to analog **25**.

In summary, a series of pyrazolopyrimidine B-Raf inhibitors has been designed and evaluated. SAR studies resulted in an extremely potent analog, compound **28**, possessing an optimal *ortho* substituent on the phenyl linker and a novel *N*-methyl-diazabicyclo[2.2.1]heptanyl headpiece. Modeling studies suggest that it binds to the active conformation of B-Raf as type I kinase inhibitors. These compounds also show good kinase selectivity profiles. An indazole was introduced to replace the metabolically labile phenol group and further optimization of the indazole analogs is ongoing and will be reported in the future.

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