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Novel pyrazolopyrimidines as highly potent B-Raf inhibitors

Martin J. Di Grandi [a,*], Dan M. Berger [a], Darrin W. Hopper [a], Chunchun Zhang [a], Minu Dutia [a], Alejandro L. Dunnick [a], Nancy Torres [a], Jeremy I. Levin [a], George Diamantidis [a], Christoph W. Zapf [a], Jonathan D. Bloom [a], YongBo Hu [b], Dennis Powell [a], Donald Wojciechowicz [c], Karen Collins [c], Eileen Frommer [c]

[a] Chemical Sciences, Wyeth Research, 401 North Middletown Road, Pearl River, NY 10965, USA
[b] Structural Biology and Computational Chemistry, Wyeth Research, 401 North Middletown Road, Pearl River, NY 10965, USA
[c] Discovery Oncology, Wyeth Research, 401 North Middletown Road, Pearl River, NY 10965, USA

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ABSTRACT

A novel series of pyrazolo[1,5-*a*]pyrimidines bearing a 3-hydroxyphenyl group at C(3) and substituted tropanes at C(7) have been identified as potent B-Raf inhibitors. Exploration of alternative functional groups as a replacement for the C(3) phenol demonstrated indazole to be an effective isostere. Several compounds possessing substituted indazole residues, such as **4e**, **4p**, and **4r**, potently inhibited cell proliferation at submicromolar concentrations in the A375 and WM266 cell lines, and the latter two compounds also exhibited good therapeutic indices in cells.

The Ras-MAP kinase pathway has been implicated in tumor progression for a variety of human cancers. The Raf kinases, which are components of this cascade, are serine/threonine kinases that activate Mek1/2. Mutant B-Raf containing a V600E substitution causes aberrant constitutive activation of this pathway and has high occurrence in several human cancers.[1] As such, chemotherapeutic inhibition of mutant B-Raf offers a viable means for treating cancer.[2] Recently we disclosed the preliminary characterization of pyrazolo[1,5-*a*]pyrimidine-3-carboxylates (Fig. 1; **1**) as B-Raf inhibitors.[3] Further enhancements to this class of compounds (**2**) were able to boost the in vitro potency of these non-hinge region binders but modest cellular potency remained an issue.[4] Exploration of substituents at the C(2) position provided analogs that could form a hydrogen bond to the hinge region of B-Raf.[5] In an effort to further improve the potency and physical properties of these compounds,[4] we next explored C(2), C(3) disubstituted pyrazolopyrimidines with smaller C(7) substituents to reduce molecular weight. In this paper we describe a novel series of pyrazolopyrimidines incorporating a C(7) tropane moiety (Fig. 1; **3** and **4**) to afford structurally unique compounds with improved potency. Further modification around the core, based on the more highly substituted **5**, led to the preparation of fused tropanone analogs **6**. An important distinction to be noted is that while compounds of series **2** bind the inactive conformation of B-Raf, compounds **3–6** comprise a structural class that targets its active conformation.[6]

The chemistry to prepare these analogs is shown in Scheme 1. Commercially available tropanone **7** was converted in three steps to enaminone **8**.[7] Condensation of this reagent with aminopyrazole **9a**[8] in hot AcOH gave pyrazolopyrimidine **10a**.[9] Selective functionalization with *N*-iodosuccinimide yielded C(3) iodide **10b**, which upon exposure to a variety of boronic acids or esters afforded the arylated products **3** (Table 1; $R^1 = CO_2C_2H_5$; $R^2 = OCH_3$; R^3 = variable). In some instances, these carbamates were further modified to produce anisoles or phenols bearing different R^1 groups on the tropane. For instance phenols **3c–e** (Table 1) were prepared from their corresponding anisoles by exposure to BBr_3. Trimethylsilyl iodide (TMSI) was used to remove the *N*-carboethoxy group from **3a** and **3b** to yield products **3f** and **3g** and subsequent demethylation with BBr_3 gave secondary aminophenols **3h** and **3i**. *N*-Ethyl compound **3j** was synthesized by alkylating **3g** with C_2H_5I in the presence of K_2CO_3. From the appropriate starting materials, phenols **3k–m** were synthesized in like manner followed by deprotection of the methoxy group with BBr_3. Finally, acetamide **3n** and sulfonamide **3o** were prepared from **3b** in three steps: boron tribromide to deprotect the anisole residue, followed by TMSI mediated *N*-carboethoxy group cleavage, and subsequent treatment with acetyl chloride (in the presence of triethylamine as base) to provide **3n**, or methanesulfonyl chloride to provide **3o**.

* Corresponding author.
E-mail address: digranm@pfizer.com (M.J. Di Grandi).

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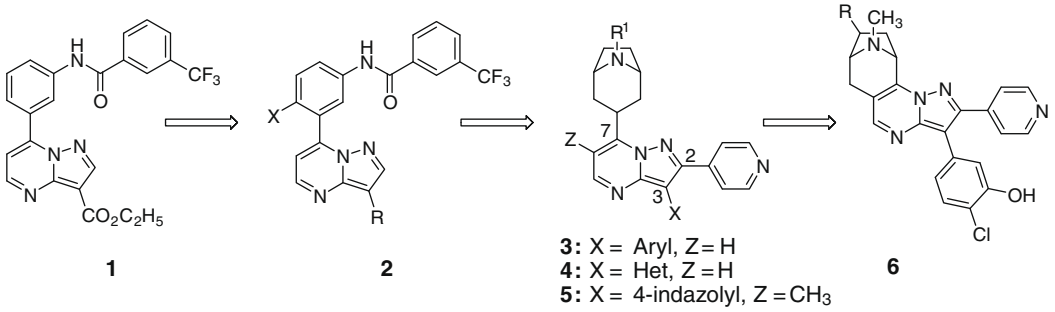
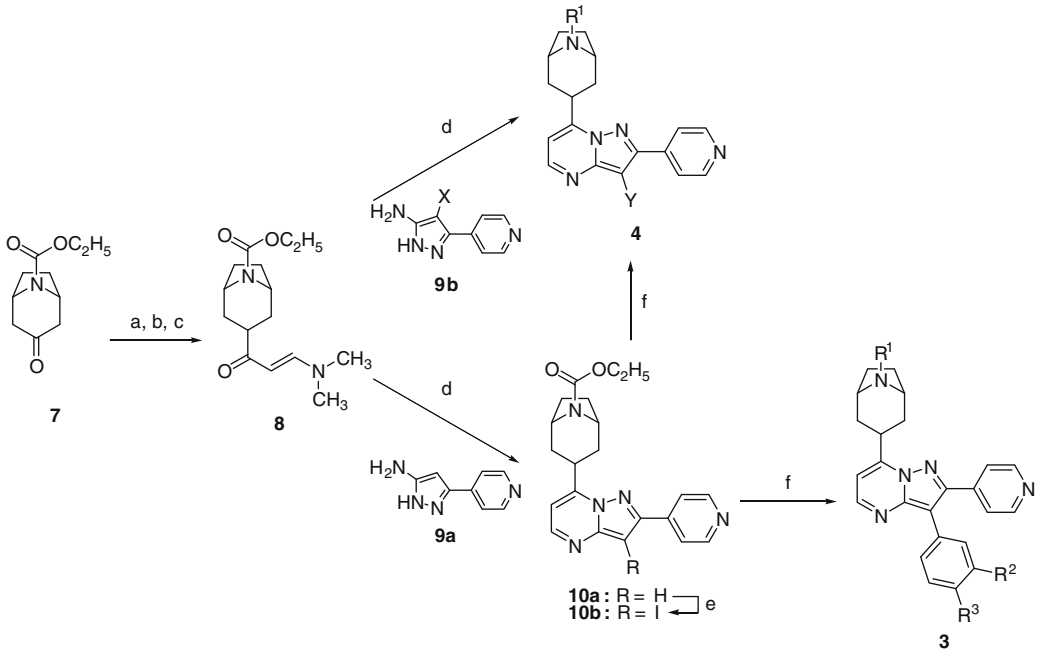


Figure 1. The evolution of B-Raf inhibitors at Wyeth.



Scheme 1. Reagents and conditions: (a) TosMic, KOtBu, −10 °C, 4 h; (b) MeMgBr, 5 °C, 3 h; (c) DMF-DMA, 100 °C, 3 d; (d) AcOH, 80–100 °C; (e) NIS, rt, overnight; (f) $Pd(PPh_3)_4$, $ArB(OR)_2$, aq Na_2CO_3, DME, 80 °C to **3**; $Pd(PPh_3)_4$, $HetB(OR)_2$, aq Na_2CO_3, DME, 80 °C to **4**.

The final products **4** bearing C(3) heterocycles (Tables 2 and 3; $R^1 = CO_2C_2H_5$; Y = heterocycles) were prepared by Suzuki coupling of a heterocyclic boronic acid or ester with iodide **10b** or by direct condensation of **8** with the more elaborate aminopyrazoles **9b** (X = heterocycles).[7,10] Subsequent reaction of these carbamates afforded additional analogs. Thus, compound **4s** (Table 3) was prepared by TMSI induced deprotection of **4r**. Similarly, reaction of **4e**, **4p**, **4t**, or **4v** with TMSI followed addition of the appropriate alkylating reagent in the presence of K_2CO_3 afforded compounds **4k**, **4m**, **4o**, **4q**, **4u**, and **4w**. Compound **4n** was synthesized from the free amine derived from **4e** by reductive amination with acetone in the presence of $NaBH(OAc)_3$. Compounds **4g–j** and **4l** were assembled by acylation of the free amine derived from **4e** with the appropriate reagent and **4f** was derived from **4l** by Boc deprotection with TFA.[7]

The preparation of the C(6) methyl analog **5** commenced with the reaction of enaminone **11** with the aminopyrazole **9b** (X = 4-indazoyl) in hot AcOH to give the desired pyrazolopyrimidine (Scheme 2). The fused analogs **6** (Table 4) required a slight variation of this scheme. Accordingly, tropanones **12a**[11] were treated with dimethylformamide dimethylacetal to give the expected enaminones **12b**, which upon condensation with the fully substituted aminopyrazole **9b** (X = 3-OH-4-Cl–C_6H_3) afforded the desired fused compounds **6**.

With in vitro IC_{50}s <0.1 μM, all compounds presented in Table 1 are effective B-Raf inhibitors[12,13] but typically the phenols were 17- to 87-fold more potent than their methyl ether counterparts (compare **3a**:**3c**; **3b**:**3d**; **3f**:**3h**; **3g**:**3i**; **3j**:**3k**). Additionally, consistent with published data,[6] incorporation of a chlorine atom *ortho* to either the –OH or $–OCH_3$ group generally increased in vitro potency almost 10-fold (for instance, **3f**:**3g**; **3h**:**3i**), presumably due to favorable hydrophobic interaction between this atom and the binding pocket of the enzyme. Though chlorine could be replaced with methyl (**3d**:**3e**) or fluorine (**3k**:**3m**) to give very potent analogs, CN was far less active, apparently being too large (**3k**:**3l**). With respect to the tropane moiety, a diverse set of substituents on nitrogen was tolerated (**3d**, **3i**, **3k**, **3n**, **3o**) and with the exception of the surprisingly potent amine **3g** (compared to analogs **3b**, **3j**), all tropane derivatives with comparable substitution on the C(3) aryl ring were essentially equipotent.

A depiction of **3d** docked into the DFG-in conformation of the active site of B-Raf is shown in Figure 2. The phenolic OH group forms two hydrogen bonds, one to the Glu501 residue, and one to the NH group of Asp594. Additionally, the C(3) pyridine residue