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Design and synthesis of *N*-(4-aminopyridin-2-yl)amides as B-Raf^{V600E} inhibitors

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ABSTRACT

B-Raf^{V600E} was an effective target for the treatment of human cancers. Based on a pan-Raf inhibitor TAK-632, a series of *N*-(4-aminopyridin-2-yl)amide derivatives were designed as novel B-Raf^{V600E} inhibitors. Detailed structure–activity studies of the compounds revealed that most of the compounds displayed potent enzymatic activity against B-Raf^{V600E}, and good selectivity over B-Raf^{WT}. One of the most promising compound **4l** exhibited potent inhibitory activity with an IC_{50} value of 38 nM for B-raf^{V600E}, and displayed antiproliferative activities against colo205 and HT29 cells with IC_{50} values of 0.136 and 0.094 μM, respectively. It also displayed good selectivity on both enzymatic and cellular assays over B-Raf^{WT}. These inhibitors may serve as lead compounds for further developing novel B-Raf^{V600E} inhibitors as anticancer drugs.

The Ras-Raf-Mek-Erk signaling pathway plays a critical role in regulating cell growth, proliferation and differentiation.[1] Among the serine/threonine kinases Raf (A-Raf, B-Raf, and C-Raf) family, the B-Raf aberrant activation or constitutively activating mutation has been identified in various cancers.[2,3] The single point mutation $Val^{600} \rightarrow Glu^{600}$ accounts for more than 90% of the B-Raf oncogenic mutants. Thus, B-Raf^{V600E} became as an effective target for the treatment of human cancers harboring this mutation.[4]

Many classes of B-Raf small molecule inhibitors have been identified.[5] Two selective inhibitors vemurafenib (**1**) and dabrafenib (**2**) have been approved by US FDA (Fig. 1),[6,7] and shown significant efficacy against metastatic and unresectable melanoma bearing B-Raf^{V600E} mutation in clinical.[8,9] However, intrinsic and acquired resistance in clinic limits the therapeutic benefit of current used drugs.[10,11] For instance, a number of colorectal cancer patients who were detected to harbor B-Raf^{V600E} mutation also display inherent resistance against vemurafenib.[12] As acquired resistance increasingly observed in the clinic, novel selective B-Raf^{V600E} inhibitors are still desirable to not only suppress resistance but also display reduced side effects. Drug discovery efforts directed toward new B-Raf inhibitors alone or in combination with EGFR kinase inhibitors to overcome this clinical resistance have been published by our group.[13,14]

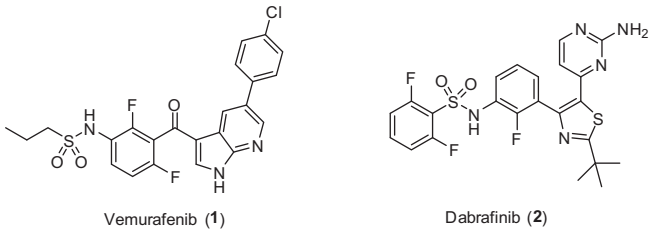

Figure 1. The chemical structures of FDA-approved B-Raf inhibitors vemurafenib and dabrafinib.

As part of our continuous efforts to identify new molecules that could target B-Raf^{V600E} mutant, a series of *N*-(4-aminopyridin-2-yl) amide derivatives were designed as novel B-Raf^{V600E} inhibitors by structural modifications based on a pan-Raf inhibitor TAK-632[15] under the 'atomic economy' principle (Fig. 2). *N*-(4-Aminopyridin-2-yl)amide scaffold was designed to bind to the hinge region instead of 1,3-benzothiazole amide of TAK-632, and substituted phenylamide targeted the hydrophobic back pocket of B-Raf. Some *N*-(4-aminopyridin-2-yl)amide derivatives were found as potent inhibitors of B-Raf^{V600E}. In this Letter, we report the synthesis,

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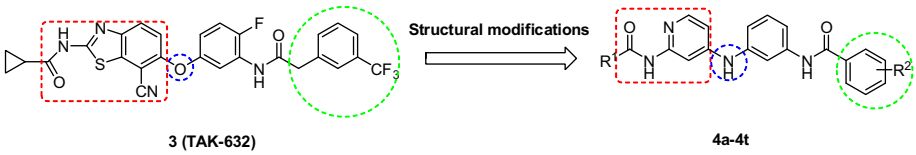
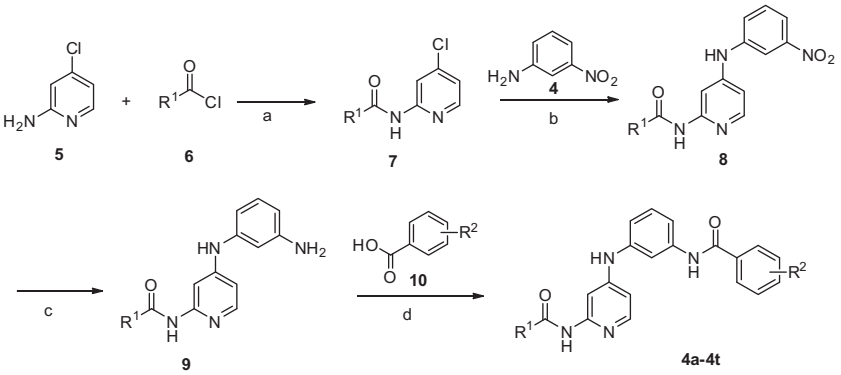


Figure 2. Design of *N*-(4-aminopyridin-2-yl)amide derivatives as B- Raf^{V600E} inhibitors.



Scheme 1. Reagents and conditions: (a) Et_3N, DCM, overnight, rt, 60–90%; (b) $Pd(OAc)_2$, Binap, Cs_2CO_3, dioxane, 90 °C, overnight, 55–75%; (c) H_2, Pd/C, MeOH, rt, overnight, 60–85%; (d) HATU, DIEA, DMF, rt, overnight, 70–90%.

structure–activity relationships (SAR) and antiproliferative activity studies of *N*-(4-aminopyridin-2-yl)amide-based B-Raf^{V600E} inhibitors.

The synthesis of target compounds **4a**–**4t** is outlined in Scheme 1. Briefly, the amide **7** was afforded by amide condensation of 4-chloropyridin-2-amine (**5**) with freshly prepared acyl chloride **6**. A Buchwald–Hartwig coupling reaction with amide **7** and 3-nitroaniline (**4**) provided substituted 4-aminopyridin **8**. Reduction of the intermediate **8** with hydrogen in the presence of Pd/C gave the key intermediate **9**, which was coupled with variable substituted benzoic acid **10** to get the desired compounds **4a**–**4t** by using 1-[bis(dimethylamino) methylene]-1*H*-1,2,3-triazolo[4,5-*b*] pyridinium 3-oxidhexa fluorophosphate (HATU) and diisopropylethylamine (DIEA) in anhydrous DMF at room temperature. All desired compounds were given satisfactory analytical and spectroscopic data in accordance with their depicted structure.

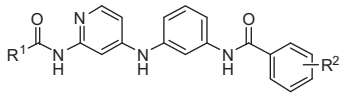
Kinase inhibitory activities of the designed compounds against B-Raf^{V600E} and B-Raf^{WT} were evaluated by using a well established FRET-based Z′-Lyte assay.[13,14] FDA-approved drug **1** as well as TAK-632 were used as positive controls to validate the screening conditions. As shown in Table 1, vemurafenib and TAK-632 both potently suppressed the B-Raf^{V600E} with IC_{50} values of 59 nM and 15 nM, respectively, which were similar to the reported data.[6,15]

Our designed initial lead **4a** with a trifluoromethyl group at the *meta* position on phenylamide was discovered to exhibit potent B-Raf^{V600E} kinase inhibitory activity with an IC_{50} of 0.141 μM, indicating this novel scaffold might be a good starting point for development of potent inhibitors to target B-Raf^{V600E} (Table 1). Therefore, extensive structure activity studies have been conducted around this new scaffold. Initial investigation revealed that the position of trifluoromethyl on phenylamide is essential for maintaining kinase inhibitory activity, since *ortho*- and *para*-trifluoromethyl (**4b** and **4c**) totally abolished their suppressing effect on B-Raf^{V600E} kinase (IC_{50} >10 μM). Next, we replaced CF_3 with other electronegative groups on meta-position, the results were summarized in Table 1. It showed that compounds **4d**–**4f** featured with Cl, NO_2, CN suffered a significant decrease in biochemical potential to different extent with IC_{50} values of 0.785, 1.512 and 3.53 μM. Further exploration suggested that introduction of various lipophilic groups at the meta-position instead of CF_3 can exerted great effect on kinase inhibition. Compounds **4g** and **4h** with relatively small lipophilic substituents displayed less potent or comparable potency to **4a**, While compounds with increasing size of lipophilic substituents such as ethyl, isopropyl, *t*-butyl, cyclopropyl, 2-methyl-2-propanenitrile, cyclopropanecarbonitrile (**4h**–**4m**), exhibited 1.8- to 10.8-folds improvement on kinase inhibitory activities. Additionally, we investigated whether cyclopropyl is the optimal group in the hinge-binding moiety *N*-(4-aminopyridin-2-yl)amide. It was found that with the increasing size of cycloalkyl (**4r**–**4t**), the corresponding compounds' potency decreased gradually. The same trend was observed in alkyl analogues, with compound **4n** harboring a methyl group being the most potent with IC_{50} value of 0.05 μM, in contrast to compound **4q** with *t*-butyl with IC_{50} value of 1.03 μM.

Compounds **4i**–**4n** stood out in the above structure activity studies for they exhibited comparable potency to vemurafenib and TAK-632. We further determined the selectivity of these B-Raf^{V600E} inhibitors over B-Raf^{WT}. The results are summarized in Table 2. It was shown that compounds **4i**–**4n** inhibited B-Raf^{WT} with IC_{50} values of 0.148, 0.240, 0.453, 0.476, 2.031 and 1.200 μM, respectively, which are 11.4, 12.0, 12.9, 12.5, 25.7 and 24-folds greater than IC_{50} values on the according B-Raf^{V600E}. While the ratios for vemurafenib and TAK-632 are only 2.0 and 5.1-folds, which indicate the good selectivity of these B-Raf^{V600E} inhibitors over B-Raf^{WT}. To our knowledge, **4m** is one of the best selective B-Raf^{V600E} inhibitors over B-Raf^{WT}.

The antiproliferative activities of **4i**–**4n** were examined against Colo205 and HT29 cells harboring B-Raf^{V600E} mutation. The results were summarized in Table 2. The compounds displayed comparable or slightly weak antiproliferative effects against B-Raf^{V600E} mutated cancer cells to that of vemurafenib and TAK-632. Compounds **4k** and **4l** potently inhibited the growth of Colo205 and HT29 cells with nanomolar magnitude, which were equally potent to vemurafenib. Furthermore, the compounds (**4i**–**4n**) displayed less potent activity against HCT116 harboring B-Raf^{WT} than Colo205 and HT29 cells, which are consistent with the kinase

Table 1
Inhibitory activities of compounds **4a**–**4t** against B-Raf^{V600E}



Compds	R^1	R^2	B-Raf^{V600E} (IC_{50}, μM)
4a		*m*-CF_3	0.141
4b		*o*-CF_3	>10
4c		*p*-CF_3	>10
4d		*m*-Cl	0.785
4e		*m*-NO_2	1.512
4f		*m*-CN	3.53
4g		*m*-CH_3	1.21
4h		*m*-CH_2CH_3	0.11
4i		*m*-$CH(CH_3)_2$	0.013
4j		*m*-$C(CH_3)_3$	0.020
4k		*m*-	0.035
4l		*m*-CN	0.038
4m		*m*-CN	0.079
4n	CH_3	*m*-	0.050
4o	CH_2CH_3	*m*-	0.125
4p	$CH(CH_3)_2$	*m*-	0.420
4q	$C(CH_3)_3$	*m*-	1.03
4r		*m*-	0.182
4s		*m*-	0.434
4t		*m*-	0.563
Vemurafenib	–	–	0.059
TAK-632	–	–	0.015

Table 2
Kinase and cell inhibitory activities of compounds **4i**–**4n**

Compds	Kinase inhibition (IC_{50}, μM)		WT: V600E	Cell growth inhibition (IC_{50}, μM)		
	B-Raf^{V600E}	B-Raf^{WT}		Colo205 (B-Raf^{V600E})	HT29 (B-Raf^{V600E})	HCT116 (B-Raf^{WT})
4i	0.013	0.148	11.4	0.444	0.462	1.055
4j	0.020	0.240	12.0	0.787	0.991	1.385
4k	0.035	0.453	12.9	0.111	0.092	0.221
4l	0.038	0.476	12.5	0.136	0.094	1.091
4m	0.079	2.031	25.7	0.725	0.791	1.351
4n	0.050	1.200	24.0	0.131	0.236	0.367
Vemurafenib	0.059	0.119	2.0	0.044	0.156	14.58
TAK-632	0.015	0.077	5.1	0.025	0.033	0.362

inhibition results. For example, compound **4l** inhibited colo205 and HT29 cells with IC_{50} values of 0.136 and 0.094 μM, which is almost 12 times more selective than that of HCT116.

The B-Raf^{V600E} kinase inhibitory activity of compounds **4k** and **4l** were further validated by using western-blot analysis. As shown in Figure 3, compounds **4k** and **4l** both displayed dose-dependent inhibition against the autophosphorylation of B-Raf^{V600E} in

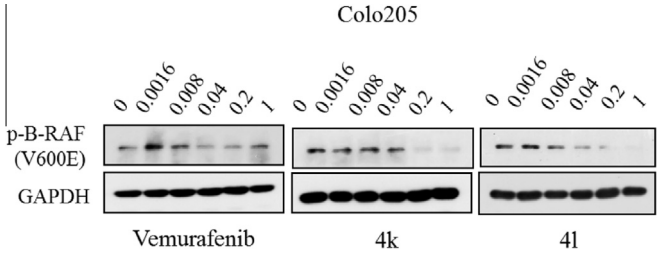


Figure 3. Compounds **4k** and **4l** dose-dependently inhibited the autophosphorylation of B-Raf^{V600E} in Colo205 cells.

Colo205 cells. The results further support strong target inhibition of the newly designed compounds.

In summary, a series of *N*-(4-aminopyridin-2-yl)amide derivatives are designed and synthesized as new B-raf^{V600E} inhibitors. The compounds **4i**–**4n** displayed good inhibitory activities against B-Raf^{V600E}, and good selectivity over B-Raf^{WT}, which are better than the control drugs vemurafenib and TAK-632. Compound **4l** was the most promising compound with the potent inhibitory activity for B-raf^{V600E} (IC_{50} = 38 nM), and good antiproliferative activity against colo205 (IC_{50} = 0.136 μM) and HT29 (IC_{50} = 0.094 μM), respectively. Therefore, compound **4l** might be a promising lead compound for development of novel selective B-raf^{V600E} inhibitors overcoming the clinical acquired resistance.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.04.076.

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