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Identification and synthesis of *N*-(thiophen-2-yl) benzamide derivatives as BRAF^{V600E} inhibitors

Yunfeng Xie [a,†], Xianjie Chen [b,†], Jie Qin [c,†], Xiangqian Kong [d], Fei Ye [d], Yuren Jiang [a,*], Hong Liu [d], Hualiang Jiang [b,d,*], Ronen Marmorstein [c], Cheng Luo [d,*]

[a] College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, China
[b] School of Chemistry and Chemical Engineering, Nanjing National Laboratory of Microstructures, Nanjing University, Nanjing 210093, China
[c] The Wistar Institute, Philadelphia, PA 19104, USA
[d] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

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ABSTRACT

The V600E BRAF kinase mutation, which activates the downstream MAPK signaling pathway, commonly occurs in about 8% of all human malignancies and about 50% of all melanomas. In this study, we employed virtual screening and chemical synthesis to identify a series of *N*-(thiophen-2-yl) benzamide derivatives as potent BRAF^{V600E} inhibitors. Structure–activity relationship studies of these derivatives revealed that compounds **b40** and **b47** are the two most potent BRAF^{V600E} inhibitors in this series.

Ras/RAF/MEK/ERK signal transduction cascades play critical roles in transducing signals from extracellular growth factors to the nucleus and participate in many cellular programs, such as cell proliferation, differentiation, and survival.[1–3] These cascades are intimately involved in many human cancers mainly because a large number of oncogenic mutations have been frequently identified in rapidly growing fibrosarcoma (RAF) family members,[4] including ARAF, BRAF, and CRAF.[5] RAF protein kinases play central roles in the MAPK signaling pathway and have been shown to be critical in mediating cell proliferation, differentiation, and survival.[6,7] Among the three paralogs of RAF, oncogenic mutations in BRAF are the most frequently observed in human cancers.[4] The BRAF gene is located on human chromosome 7q24 and encodes a cytosolic serine–threonine protein kinase that is expressed in many human cell types.[8] The BRAF oncogene is mutated in approximately 8% of all human tumors, and especially in melanoma (~50%), papillary thyroid (~50%), ovarian (~25%), and colorectal (~12%) cancer.[6–8] The most common BRAF mutation is the replacement of valine with glutamic acid at position 600 (V600E), which accounts for over 90% of all BRAF mutations in cancers and aberrantly drives the activation of the MAPK signaling pathway, thus facilitating malignant transformation.[7,9–12] Thus, BRAF^{V600E} has emerged as a promising therapeutic cancer target.[5,13,14]

To date, various inhibitors of BRAF have been evaluated in clinical trials, such as CEP-32496, LGX-818, ARQ-736, and RG-7256 in phase I clinical trials. DCC-2036 has been tested in phase II clinical trials, dabrafenib has been tested in phase III clinical trials, regorafenib is in pre-registration, and vemurafenib has been made publicly available.[15] However, recent data indicate that patients eventually develop significant drug resistance to these inhibitors[16,17] or suffer severe side effects.[18] Therefore, the development of novel, potent BRAF^{V600E} inhibitors that may that may not suffer from these limitations is of significant importance.

High-throughput screening and structure based virtual screening (SBVS) are two screening methods frequently used by medicinal chemists. Indeed, most of the currently available BRAF kinase inhibitors have been identified by these two complementary approaches.[19–21] In our previous work, a series of 2-phenyl-5-vinylfuran derivatives were identified as potent novel BRAF^{V600E} inhibitors based on SBVS and chemical optimization.[22] In the present study, *N*-(thiophen-2-yl) benzamide derivatives are reported as another series of BRAF^{V600E} selective inhibitors. In particular, compounds **b40** and **b47** in this series exhibit submicromolar inhibitory activities against the BRAF^{V600E} kinase.

Molecular docking methods and SBVS are commonly used approaches in hit identification.[23] To find more potent compounds with novel scaffolds toward BRAF^{V600E}, a hierarchical virtual

* Corresponding authors. Tel.: +86 731 88879616; fax: +86 731 88859988 (Y.J.); tel.: +86 21 50806600; fax: +86 21 50807088 (H.J. and C.L.).
E-mail addresses: jiangyr@mail.csu.edu.cn (Y. Jiang), hljiang@mail.shcnc.ac.cn (H. Jiang), cluo@mail.shcnc.ac.cn (C. Luo).
† These authors contributed equally to this work.

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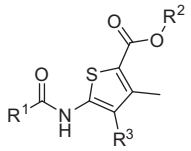
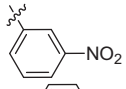
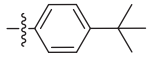
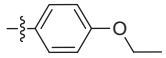
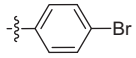
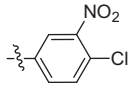
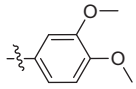
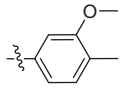
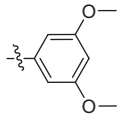
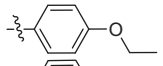
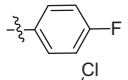
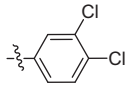
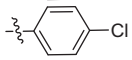
Table 1
$BRAF^{V600E}$ inhibition activity data for compounds **a1**–**a22**



Compound	R^1	R^2	R^3	Inhibition (%) (at 2 μM)	IC_{50} (μM)
[a]**22f**[22]					**0.47**
a1		$-CH_2CH_3$	CN	52	2.01
a2		$-CH_2CH_3$	CN	42	ND
a3		$-CH_2CH_3$	CN	NA	ND
a4		$-CH_2CH_3$	CN	36	ND
a5		$-CH_2CH_3$	CN	46	ND
a6		$-CH_2CH_3$	CN	69	1.16
a7		$-CH_2CH_3$	CN	NA	ND
a8		$-CH_2CH_3$	CN	51	2.16
a9		$-CH_2CH_3$	CN	32	ND
a10		$-CH_2CH_3$	CN	NA	ND
a11		$-CH_3$	CN	NA	ND
a12		$-CH_3$	CN	NA	ND
a13		$-CH_3$	CN	NA	ND
a14		$-CH(CH_3)_2$	CN	NA	ND
a15		$-CH(CH_3)_2$	CN	NA	ND
a16		$-CH(CH_3)_2$	CN	42	ND
a17		$-CH_2CH_3$	CN	NA	ND
a18		$-CH_2CH_3$	$-COOCH_2CH_3$	NA	ND
a19		$-CH_2CH_3$	$-COOCH_3$	NA	ND

(*continued on next page*)

Table 1 (*continued*)

Compound	R^1	R^2	R^3	Inhibition (%) (at 2 μM)	IC_{50} (μM)
a20		$-CH_2CH_3$	$-COOCH_2CH_2CH_3$	NA	ND
a21		$-CH_2CH_3$	$-CONH_2$	NA	ND
a22		$-CH_2CH_3$	$-CONH_2$	26	ND

NA = not active; ND = not determined.
[a] Compound **22f** from Ref. 22 was assayed as control.

screening process was initiated. First, the SPECS database, which contains more than 200,000 chemicals (http://www.specs.net), was filtered using drug-like criteria[24] to create a focused library containing about 50,000 theoretically drug-like small molecules. Next, the compounds were docked into the ATP-binding site of the $BRAF^{V600E}$ kinase (PDB entry: 3OG7[25]) using the GLIDE[26] program in standard precision mode. The top 2000 compounds were submitted for further evaluation using the GLIDE extra precision mode. The top 500 compounds were then retained for structural diversity analysis. Finally, 30 compounds from 38 manually classified groups were purchased and evaluated for their ability to inhibit the enzymatic activity of $BRAF^{V600E}$. An ELISA-based MEK phosphorylation assay, which was performed according to our previous work,[22] revealed that the *N*-(thiophen-2-yl) benzamide derivative, **a1**, was the most potent $BRAF^{V600E}$ kinase inhibitor with an IC_{50} value of about 2.01 μM (Table 1). Since few studies demonstrated that *N*-(thiophen-2-yl) benzamide derivatives were potential $BRAF^{V600E}$ kinase inhibitors,[4,27–30] **a1** was chosen for further studies.

To explore the structure–activity relationship (SAR) of **a1**, a similarity-based analogue search was performed in the SPECS database, and another 21 compounds were selected based on the search results from the vendor database and their inhibitory activities against $BRAF^{V600E}$ were assessed. As shown in Table 1, the $BRAF^{V600E}$ inhibitory activities of **a2** and **a6** were better than **a11** and **a14**, indicating that better inhibition could be obtained by R^2 substitution of an ethyl group. However, **a17**, containing a furan-2-carboxamide showed a loss of inhibitory enzyme potency, highlighting the superior performance of a substituted phenyl group at R^1. In addition, comparison of the activities of **a8**, **a16**, **a4** and **a22** revealed that double substitution at the *ortho*- and *para*-positions of the phenyl group was desirable. Moreover, substitution of the cyano group at R^3 with an ester or amide group exhibited substantially reduced activity (e.g., compounds **a18**–**a21**). Compound **a22**, which features a 4-chloro phenyl group at R^1 and an amide substitution at R^3, retained some inhibitory activity against $BRAF^{V600E}$.

To better understand the molecular mechanism of $BRAF^{V600E}$ inhibition by these compounds, the most potent inhibitor (**a6**) was docked to the active site of $BRAF^{V600E}$.[22] The docking results suggest that a hydrophobic interaction may occur between the ethoxy group of the phenyl ring of **a6** and the hydrophobic pocket formed by S535, F583, and C532 of $BRAF^{V600E}$ (Fig. 1). A π–π stacking interaction is also predicted to occur between the phenyl ring of **a6** and F583 of $BRAF^{V600E}$. This modeling also predicts a hydrophobic interaction between the inhibitor thiophen ring and L514 of $BRAF^{V600E}$, a hydrogen bond formed by the oxygen atom of the amide between the phenyl and thiophen rings of **a6** with the hydroxyl of T529 of $BRAF^{V600E}$, and the inhibitor ester group of the thiophen ring extended toward the activation loop, yielding favorable interactions with D594, I527, and L505 of $BRAF^{V600E}$. Another hydrogen bond is predicted to form between the nitrogen atom of K483 of $BRAF^{V600E}$ and the oxygen atom of the ester group of the

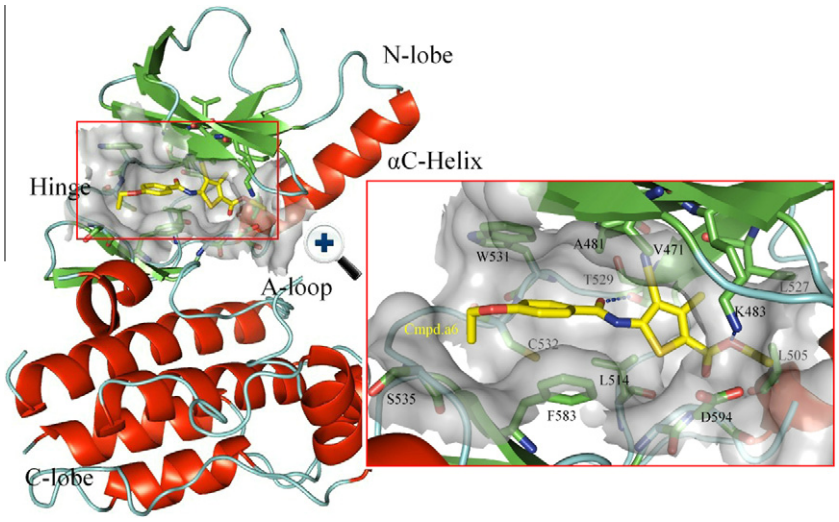
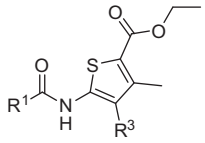
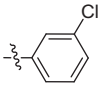
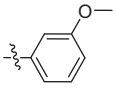
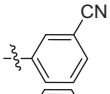
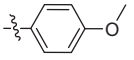
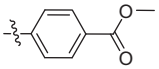
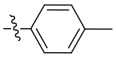
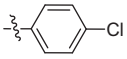
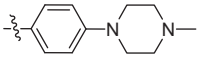
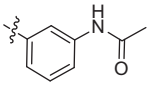
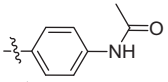
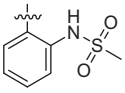
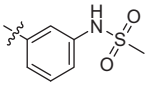
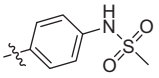
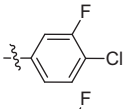
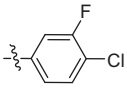
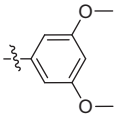


Figure 1. Docking results of compound **a6** with $BRAF^{V600E}$ kinases. Left panel: $BRAF^{V600E}$ kinase in the active conformation (PDBID: 3OG7). The **a6** molecules that occupy ATP bonding sites are shown as sticks in yellow. Right panel: the close-up view of the key interactions between compound **6** and 3OG7 Hydrogen bonds are shown as dotted blue lines.

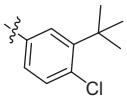
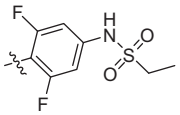
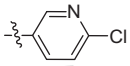
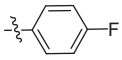
Table 2
$BRAF^{V600E}$ inhibition activity data for compounds **b23**–**b47**



Compound	R_1	R_3	Inhibition (%)		IC_{50} (μM)
			2 μM	1 μM	
b23		–CN	51	ND	ND
b24		–CN	57	ND	ND
b25		–CN	50	ND	ND
b26		–CN	13	ND	ND
b27		–CN	40	ND	ND
b28		–CN	15	ND	ND
b29		–CN	54	ND	ND
b30		–CN	48	ND	ND
b31		–CN	60	ND	ND
b32		–CN	NA	ND	ND
b33		–CN	ND	17	ND
b34		–CN	ND	NA	ND
b35		–CN	ND	NA	ND
b36		–CN	ND	NA	ND
b37		–CN	ND	NA	ND
b38		–CN	59	ND	ND
b39		–CN	36	ND	ND
b40		–CN	85	60	0.77

(*continued on next page*)

Table 2 (*continued*)

Compound	R_1	R_3	Inhibition (%)		IC_{50} (μM)
			2 μM	1 μM	
b41		–CN	60	ND	ND
b42		–CN	ND	34	ND
b43		–CN	ND	39	ND
b44		–CN	ND	20	ND
b45		–CN	ND	22	ND
b46		–CN	ND	10	ND
b47		$–CONHC_6H_6$	87	66	0.63

NA = not active; ND = not determined.

inhibitor. Moreover, the cyano group of the inhibitor is predicted to occupy a small hydrophobic pocket created by A481, V471, and K483 of $BRAF^{V600E}$.

Based on the docking result of **a6** with $BRAF^{V600E}$, another set of 25 **a1** analogs were synthesized using the condensation reaction (see Supplementary data); the bioactivity data of these analogs are listed in Table 2. Comparable activity between **a2** and **b23** indicates that both electron-donating groups (EDG) and electron-withdrawing groups (EWG) are well tolerated at the *ortho*-position of the phenyl ring. Compounds **a4** and **b24**–**b27** showed that substitution of a hydrogen atom by a chlorine atom at the *meta*-position correlates with better inhibition activity. In addition, when the phenyl group in the *meta*-position was substituted by chlorine (**b24**), the resulting compound showed more potency compared with compounds with replacements using other elements (e.g., **a4**, **b25**–**b27**.) The activity data of **a5**, **a6**, and **b28**–**b31** indicate that the inhibitory activities were improved when the *para*-position of the phenyl ring was substituted by ethoxyl or chlorine. However, when chlorine was substituted by bromine, inhibition potency was lost. In these compounds, the difference in influence between EWG and EDG was not obvious. Inhibitory activity was entirely lost when a piperazine ring was introduced at the *para*-position of the phenyl ring (**b32**). Compounds **b33**–**b37**, **b42**, and **b46** were designed to possess the potential to form hydrogen bonds. However, consistent with our docking results, no significant improvement in inhibition potency was achieved by these compounds, indicating that the phenyl group may point into solvent (Fig. 2). Based on the aforementioned SAR data, double substitution of the phenyl group may improve inhibitory activity. Thus, compounds **b38**–**b41**, which possess diverse double substitutes, were synthesized. Compound **b40** had an IC_{50} value of about 0.77 μM. From the docking result in Figure 1, a small vacuity of the pocket around the cyano group was proposed. To further verify this hypothesis, **b47** was designed and synthesized. Interestingly, **b47** possessed the most potent

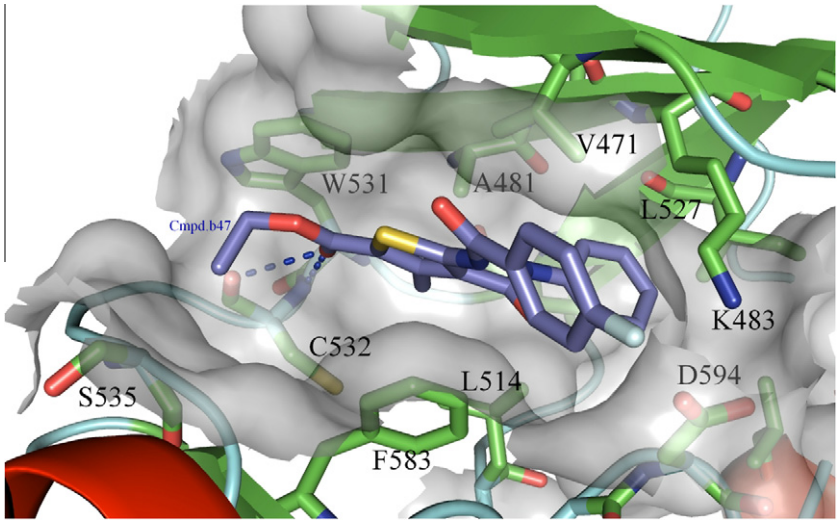


Figure 2. Docking results of compound **b47** with $BRAF^{V600E}$ kinase. Compound **b47** and salt bridges are shown in blue.

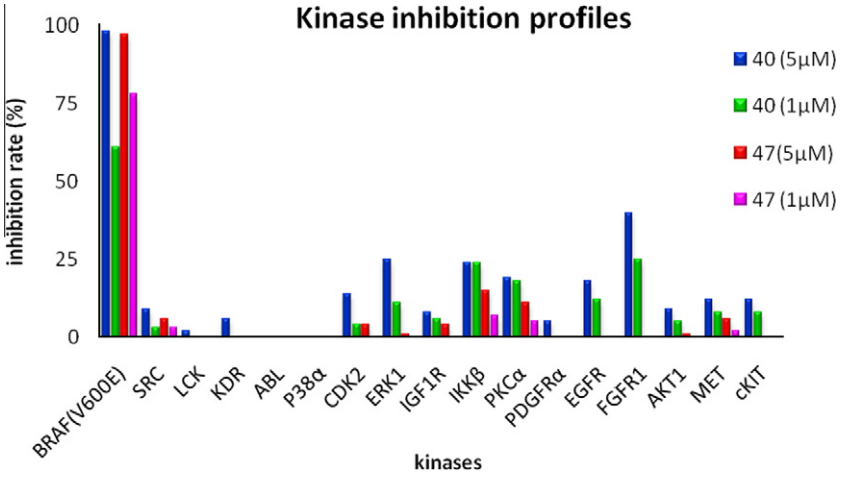


Figure 3. Kinase inhibition profiles of compounds **b40** and **b47** against a panel of 16 protein kinases compared with $BRAF^{V600E}$.

inhibitory activity against $BRAF^{V600E}$ with an IC_{50} value of 0.63 μM. Upon close inspection of the putative binding mode of **b47**, a distinct binding mode within the active site of $BRAF^{V600E}$ was predicted (Fig. 2), wherein an inverted orientation was observed to remarkably strengthen the geometric and chemical features of **b47** with the receptor, to better accommodate $BRAF^{V600E}$. This finding suggests that **b47** may represent a novel chemotype with acceptable biological potency for oncogenic $BRAF^{V600E}$ inhibition.

To exploit the selectivity profile of this novel chemotype, the specificity and selectivity of the two most potent inhibitors against BRAF (**b40** and **b47**) were cross-screened over a small panel of 16 other kinases. The results shown in Figure 3 reveal significant levels of $BRAF^{V600E}$ inhibitor selectivity against this small pool of tyrosine- and serine/threonine-kinases.

In summary, a series of *N*-(thiophen-2-yl) benzamide derivatives have been developed as novel $BRAF^{V600E}$ kinase inhibitors utilizing SBSV and chemical optimization. Two of these compounds (**b40** and **b47**) exhibited submicromolar inhibitory activities, with the potential for future development as $BRAF^{V600E}$—selective kinase inhibitors for cell-based and clinical studies.

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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.2013.02.072.

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