# Novel Potent BRAF Inhibitors: Toward 1 nM Compounds through Optimization of the Central Phenyl Ring

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BRAF, a serine/threonine specific protein kinase that is part of the MAPK pathway and acts as a downstream effector of RAS, is a potential therapeutic target in melanoma. We have developed a series of small-molecule BRAF inhibitors based on a 1*H*-imidazo[4,5-*b*]pyridine-2(3*H*)-one scaffold (ring A) as the hinge binding moiety and a number of substituted phenyl rings C that interact with the allosteric binding site. The introduction of various groups on the central phenyl ring B combined with appropriate A- and C-ring modifications afford very potent compounds that inhibit  $V^{600E}$ BRAF kinase activity in vitro and oncogenic BRAF signaling in melanoma cells. Substitution on the central phenyl ring of a 3-fluoro, a naphthyl, or a 3-thiomethyl group improves activity to yield compounds with an IC<sub>50</sub> of 1 nM for purified  $V^{600E}$ BRAF and nanomolar activity in cells.

## Introduction

The RAF family of protein kinases are serine/threonine specific protein kinases that are key players in the mitogen activated protein (MAP) kinase pathway. These proteins act immediately downstream of Ras to conduct extracellular signals from the cell membrane to the nucleus via a cascade of phosphorylation events, thereby regulating cell growth, proliferation, and differentiation in response to growth factors, cytokines, and hormones.1 The v-Raf murine sarcoma viral oncogene homologue B1  $(BRAF^{a})^{2}$  is frequently mutated to generate active forms in human cancer, with the most frequent mutation occurring at position 600 as a glutamic acid for valine substitution (V600E; 90%).<sup>3</sup> V600EBRAF is ~500-fold more active than the wild-type protein,<sup>4</sup> and in cutaneous malignant melanoma, these mutations occur with a frequency of 50-70%. BRAF mutations are also present in a range of other human cancers, particularly thyroid (30%), colorectal (10%), and ovarian (35%) cancers.<sup>3</sup> Importantly, inhibition of BRAF signaling blocks cancer cell proliferation and induces apoptosis, validating <sup>V600E</sup>BRAF as a therapeutic target<sup>5</sup> and offering opportunities for anticancer drug development.

The interest in developing new drugs targeting the MAP kinase pathway has increased considerably, and inhibitors are currently being tested in preclinical and clinical trials.<sup>6</sup> Despite the fact that early detection and improved surgical techniques has led to increased survival of melanoma patients, the prognosis of patients with disseminated disease remains disappointing. Several small molecule inhibitors acting at different levels in the MAPK pathway have been described, such as PD0325901<sup>7</sup> and AZD6244,<sup>8</sup> which target MEK, the downstream target of

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BRAF IC<sub>50</sub> = 23 nM and SRB GI<sub>50</sub> = 4.2  $\mu$ M

**Figure 1.** Structure of the pyridoimidazolone BRAF inhibitor 1 and its proposed binding mode with BRAF.

BRAF, and PLX4032<sup>9</sup> and RAF265,<sup>10</sup> which target <sup>V600E</sup>BRAF. Some of these drugs have progressed to clinical evaluation.<sup>11,12</sup>

We have previously described the development of new  $^{V600E}$ BRAF inhibitors based on a disubstituted pyrazine scaffold,  $^{13,14}$  and more recently we reported on a novel series of BRAF inhibitors, exemplified by 1*H*-imidazo[4,5-*b*]pyridine-2(3*H*)-one **1** (see Figure 1), which are based on an innovative hinge-binding fragment. <sup>15</sup> The proposed binding mode is shown in Figure 1 and is based on an analogy to the crystal structure of BRAF with sorafenib, which was previously resolved by us.<sup>4</sup>

We demonstrated good activity with these inhibitors against purified V600EBRAF, but these compounds were only weakly active at inhibiting cell proliferation, with GI<sub>50</sub> values of over  $1 \ \mu M.^{15}$  The compounds we previously reported contain an unsubstituted phenyl ring B. To improve the potency of these compounds on both the isolated enzyme and on cell lines driven by oncogenic BRAF, we anticipated that appropriate functionalization of the phenyl ring B would lead to the desired gain in potency in cells. Assuming a binding mode similar to that of sorafenib, an examination of this cocrystal structure revealed a space in the lipophilic pocket adjacent to the adenine binding site formed in the DFG-out conformation (BP-I pocket),<sup>16</sup> which would be available to accommodate these ring B substitutions. These modifications might simultaneously provide a potential means of increasing the selectivity of inhibitors toward mutant BRAF (see Table 1).

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<sup>&</sup>lt;sup>*a*</sup> Abbreviations: BRAF, V-RAF murine sarcoma viral oncogene homologue B1; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; ERK, extracellular regulated kinase; Boc, *tert*-butoxycarbonyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; DCM, dichloromethane; DMF, dimethylformamide; DMSO, dimethylsulfoxide; atm, atmosphere.





| Compound number | Central phenyl ring | <sup>V600E</sup> BRAF IC <sub>50</sub> (µM) | pERK IC <sub>50</sub> (µM) | SRB GI50 (µM) |
|-----------------|---------------------|---|----------------------------|---------------|
| 1               |                     | 0.023                                       | 2.39                       | 4.2           |
| 21a             | S N                 | 0.001                                       | 0.61                       | 0.47          |
| 21b             |                     | 0.025                                       | 5.5                        | 6.2           |
| 21c             |                     | 0.023                                       | 59                         | 1.79          |
| 21d             | F                   | 0.008                                       | 2.17                       | 1.53          |
| 21e             |                     | 0.030                                       | 0.559                      | 0.47          |
| 21f             |                     | 0.106                                       | >100                       | ND            |
| 21g             | N A                 | 10  | 20.7                       | 5.34          |
| 21h             | V V                 | 0.056                                       | 3.9                        | 2             |
| 21i             | CF <sub>3</sub>     | 0.022                                       | 3.58                       | 0.93          |
| 21j             |                     | 6.5   | >100                       | 5.1           |
| 21k             | F                   | 0.047                                       | 34                         | ND            |
| 211             | CI<br>V             | 0.021                                       | 1.33                       | 2             |
| 21m             |                     | 0.037                                       | 4.72                       | 5.9           |
| 26              |                     | 0.090                                       | 11.0                       | 11.9          |





R<sub>1</sub>, R<sub>2</sub> = independently H, CH<sub>3</sub>

In this paper, we show that several ring B modifications improve the activity of this class of inhibitor on both purified <sup>V600E</sup>BRAF in vitro and on oncogenic BRAF signaling in cells.

#### Chemistry

The target compounds 21a-y, 22a-f, 23a-c, 24a,b, and 25a,b were obtained via the two routes described in Scheme 1. In route A, the A–B system is first fully synthesized, whereafter ring C is attached. Route B relies on the introduction of ring C before the formation of the 1H-imidazo[4,5-*b*]pyridine-2(3*H*)- one A ring. Route A has the key advantage that it generates final compounds with different C rings readily and within a few steps from advanced amino intermediates such as 2a-e, 3d,e, and 4 summarized in Figure 2. Route B, developed due to the difficulties encountered in protecting the amino group on the

phenyl ring B of some intermediates 9, generates final compounds in only three steps from intermediates 5a/f-m.

Whichever route is used, the first step is a coupling reaction between 2-amino-4-chloro-3-nitropyridine and an appropriately functionalized 4-aminophenol or 4-amino-1-naphthol. Only intermediate **4** required 4-chloro-3-nitro-2-*N*-methylaminopyridine intermediate **17**, whose synthesis has already been described.<sup>15</sup> Substituted nitrophenols **6b**–**d/h**,**i** were commercially available; intermediates **6a**, **6f**,**g** and **7j** were synthesized. Substitution of the fluorine atom in the 3-fluoro-4nitrophenol afforded the corresponding 3-thiomethyl– (**6a**), 3-methoxy– (**6f**),<sup>17</sup> and 3-dimethylamino-4-nitrophenol (**6g**)<sup>18</sup> intermediates (Scheme 2). 3-Phenyl-4-aminophenol **7j** was synthesized in three steps by Avenova's method<sup>19</sup> via a









R<sub>3</sub> = H for 6a, 6d, 6f,g/i, 7a, 7d, 7f,g/i,j, 8a/d R<sub>4</sub> = H for 6b,c and 7b,c

<sup>*a*</sup> Reagents and conditions: (a) NaSMe, K<sub>2</sub>CO<sub>3</sub>, DMF; (b) NaOMe, MeOH, reflux; (c) (CH<sub>3</sub>)<sub>2</sub>NH·HCl, K<sub>2</sub>CO<sub>3</sub>, DMSO/H<sub>2</sub>O, 100 °C; (d) Fe, NH<sub>4</sub>Cl, EtOH/H<sub>2</sub>O, reflux; (e) Pd/C, H<sub>2</sub>, RT, (f) Boc<sub>2</sub>O, InCl<sub>3</sub>, 35 °C.

Scheme 3. Synthesis of Key Intermediates  $2a-e^{a}$ 



R<sub>3</sub> = H for 7a, 7f,g/i,j/l,m, 8a/d R<sub>4</sub> = H for 7b,c/k

<sup>*a*</sup> Reagents and conditions: (a) 2-amino-4-chloro-3-nitropyridine, tBuOK, DMF, 70 °C; (b) 2-amino-4-chloro-3-nitropyridine, NaH, DMSO, 100 °C; (c) Boc<sub>2</sub>O, THF; (d) Pd/C, H<sub>2</sub>, RT; (e) Fe, NH<sub>4</sub>Cl, EtOH/H<sub>2</sub>O for **10a**; (f) (Cl<sub>3</sub>CO)<sub>2</sub>CO, pyridine, THF; (g) TFA.

Scheme 4. Synthesis of Key Intermediates  $3d-e^{a}$ 



compounds **d** R<sub>3</sub>= H R<sub>4</sub>= F compounds **e** Naphthyl

<sup>a</sup> Reagents and conditions: (a) EtOCOCl, pyridine, THF; (b) TFA; (c) MeI, NaH, THF; (d) EtONa, EtOH, microwave condition.

Scheme 5. Synthesis of Key Intermediate  $4^{a}$ 



<sup>a</sup> Reagents and conditions: (a) 4-(N-Boc-amino)naphthol, tBuOK, DMF, 70 °C; (b) H<sub>2</sub>, Pd/C, EtOH; (c) triphosgene, pyridine, THF; (d) TFA.

Diels-Alder cycloaddition of (*Z*)-4-benzylidene-2-phenyl-2-oxazolin-5-one with Danishefsky's diene.

Substituted *p*-nitrophenol compounds **6b**–**d/f**–**i** were reduced by catalytic hydrogenation over Pd/C to provide *p*-aminophenols **7b**–**d/f**–**i**. Reduction of **6a** required the use of iron as the thiomethyl group poisoned Pd/C and Raney nickel catalysts. Ammonium chloride<sup>20</sup> was preferred to acetic acid<sup>21</sup> as a proton source because the latter reaction gave 20% of the undesired corresponding acetamide. Subsequent protection of the amino group as required for route A was readily achieved using Boc<sub>2</sub>O and catalytic InCl<sub>3</sub> (1 mol %)<sup>22</sup> at 35 °C to generate **8a** and **8d**.

Coupling of the functionalized phenols with 2-amino-4chloro-3-nitropyridine is presented in Scheme 3. NaH in DMSO is preferred to tBuOK in dry DMF, as 4-(2-amino-3-nitropyridin-4-ylamino) substituted phenols were formed as side products (10 to 100%) in the latter case. The use of NaH provided only the expected ether-linked compounds 9a-c/e-m in high yields and purities. Compounds 10a/d were synthesized in a similar way.

Protection of the amino group of intermediates 9a-c/e was carried out using Boc<sub>2</sub>O in THF at room temperature. However, the presence of bulky groups at the 3-position of the ring B (see Figure 1 for numbering) and the electronic effects of the substituents led to slow reactions for compounds 91 and 9m and a total lack of reactivity for compound 9a. The use of InCl<sub>3</sub> (1 mol %) considerably improved the yield to 90% after 4 h at 35 °C for 9a. From this stage on, key intermediates 2a-e, 3d, e, and 4 were obtained by following a similar synthetic route described previously by Niculescu-Duvaz et al.<sup>15</sup> (Schemes 3, 4, and 5). Reduction of the 3-nitropyridine group of protected intermediates 10a-e gave intermediates 12a-e. Cyclisation of the diamino pyridyl derivative with triphosgene, followed by

Table 2. Combinations of Various Substituted Phenyl Rings C with the Optimal Rings B

| Compound<br>number | Ring C          | Ring B <sup>a</sup> | <sup>V600E</sup> BRAF IC <sub>50</sub><br>(µM) | pERK IC <sub>50</sub> $(\mu M)$ | SRB GI <sub>50</sub><br>(µM) |
|--------------------|-----------------|---------------------|--|---------------------------------|------------------------------|
| 21a                |                 | SMe                 | 0.001  | 0.61                            | 0.47                         |
| 21d                | $ /$ $CF_3$     | F                   | 0.008  | 2.17                            | 1.53                         |
| 21e                | - CI            | Naphthyl            | 0.030  | 0.559                           | 0.47                         |
| 21n                |                 | SMe                 | 0.001  | 1.3                             | 1.06                         |
| 21p                |                 | OMe                 | 0.016  | 29.4                            | >100                         |
| <b>2</b> 1q        |                 | F                   | 0.012  | 3.45                            | 1.61                         |
| 21t                | - \/            | Naphthyl            | 0.006  | 1.91                            | 0.32                         |
| 210                | •               | SMe                 | 0.012  | 3.06                            | 2.6                          |
| 21r                |                 | F                   | 0.007  | 1.29                            | 1.15                         |
| 21u                | - F             | Naphthyl            | 0.037  | 0.214                           | 0.29                         |
| 21s                | SCF3            | F                   | 0.007  | 4.15                            | 1.19                         |
| 21v                | F               | Naphthyl            | 0.22   | >100                            | 5.5                          |
| 21w                | F F             | Naphthyl            | 0.079  | 19.3                            | 3.9                          |
| 21x                | K               | Naphthyl            | 0.014  | 0.346                           | 0.291                        |
| 21y                | CF <sub>3</sub> | Naphthyl            | 0.638  | 1.57                            | 0.89                         |

<sup>*a*</sup> All substitutions occurred in position 3 of the phenyl ring B with the exception of inhibitor **21p** substituted in position 2.

deprotection of the ring B amino group with trifluoroacetic acid, afforded intermediates 2a-e (Scheme 3).

Intermediates **3d**,**e** were obtained in four steps from compounds **12d** and **12e**.<sup>15</sup> First the diamino-intermediates **12d**,**e** were acylated in position N1 with ethyl chloroformate, after which the Boc amino group protection was removed with TFA to give intermediates **15d**,**e**. Selective methylation of the carbamate nitrogen using MeI and NaH, followed by an intramolecular cyclization using microwave irradiation produced imidazolones **3d** and **3e**, which are methylated at position N1 (Scheme 4).

The synthesis of 3-*N*-methylated intermediate **4** is similar to the synthesis described previously for the common intermediates  $2\mathbf{a}-\mathbf{e}$ , the only difference being the first step coupling of 4-chloro-3-nitro-2-*N*-methylaminopyridine  $17^{15}$  and 4-(*N*-Bocaminonaphthol) (Scheme 5).

The final compounds **21b**–**e**/**n**–**y** were obtained after coupling key intermediates **2a**–**e**, **3e**, and **4** with several substituted aryl isocyanates in dry THF at room temperature (Scheme 6). Compounds **24a**,**b** with a methylene amide linker and compounds **25a**,**b** with an amide linker resulting from condensation of the corresponding substituted benzoyl chloride or pheny-lacetyl chloride with intermediates **2e** and **3e** in dioxane in the presence of triethylamine under an inert atmosphere at 80 °C for 20 h (Scheme 7).

As the protection of some 4-(4-substituted aminophenoxy)-3-nitropyridin-2-amines failed when using the conventional procedure, route B was explored and the formation of ring A was performed as the last step (Scheme 8). Thus, the rings C were introduced by coupling intermediates **9a/f**—**m** with 4-chloro3-trifluoromethylphenyl isocyanate in DCM at room temperature to form 5a/f-m. The subsequent nitro group reduction was performed using conditions previously described for the starting substituted nitrophenol, i.e., Pd/C catalyst, H<sub>2</sub>, or iron with NH<sub>4</sub>Cl (9a) to provide the corresponding 2,3-diaminopyridin-4-yloxy intermediate 11a/f-m. Finally, formation of the imidazolone ring was performed using triphosgene with pyridine in THF at room temperature to afford compounds 21a/f-m.

Oxidation of the thiomethyl group of 21a using oxone in water at 0 °C yielded the corresponding sulfoxide compound 26.

## **Results and Discussion**

Our aim was to improve the potency and the cellular activity of **1** by exploring the influence of modifications to ring B. To compare the biological data from this modified series with the unsubstituted **1**, we kept constant the 1*H*-imidazo[4,5-*b*]pyridine-2(3H)-one ring A, which binds to the hinge region, the urea linker B–C and the 3-trifluoromethylphenyl substituted ring C, which interact with the DFG-pocket (BP-III and BP-IV).<sup>16</sup>

The biological activities of all compounds were tested in vitro using three assays: (a) the determination of the  $IC_{50}$  against the isolated <sup>V600E</sup>BRAF enzyme (IC<sub>50</sub>, BRAF), (b) the determination of the growth inhibition by SRB (GI<sub>50</sub>, SRB) of WM266.4 human melanoma cells that are driven by mutant BRAF, (c) compounds were also tested for cellular inhibition of the target pathway (IC<sub>50</sub> of ERK phosphorylation in WM266.4 melanoma cells-IC<sub>50</sub>, pERK)<sup>23</sup> (see Table 1).

**Modifications on Ring B.** All the modifications of ring B yield compounds that inhibit oncogenic BRAF except substitu-





| Compound | Ring C | R1, R2              | V600EBRAF             | pERK IC <sub>50</sub> | SRB GI <sub>50</sub> |
|----------|--------|---------------------|-----------------------|-----------------------|----------------------|
| number   |        | (Ring A)            | IC <sub>50</sub> (µM) | (µM)                  | (µM)                 |
| 21e      |        | H, H                | 0.030                 | 0.559                 | 0.47                 |
| 22b      |        | CH <sub>3</sub> , H | 0.023                 | 0.77                  | 1.04                 |
| 23a      | CI     | H, CH₃              | 0.156                 | 3.14                  | 2.3                  |
| 21u      |        | H, H                | 0.037                 | 0.214                 | 0.29                 |
| 22c      |        | CH <sub>3</sub> , H | 0.017                 | 0.81                  | 0.49                 |
| 23b      | F      | H, CH <sub>3</sub>  | 0.167                 | 2.0                   | 0.73                 |
| 21x      |        | H, H                | 0.014                 | 0.346                 | 0.291                |
| 22d      |        | CH <sub>3</sub> , H | 0.037                 | 1.06                  | 1.13                 |
| 23c      |        | H, CH₃              | 0.43                  | 46                    | >100                 |
| 21t      |        | H, H                | 0.006                 | 1.91                  | 0.32                 |
| 22e      | CF3    | CH₃, H              | 0.010                 | 0.60                  | 0.81                 |
| 22f      | SCF3   | CH₃, H              | 0.024                 | 1.46                  | 1.2                  |

tion in the 3-position with a phenyl (**21j**) or a dimethylamino group (**21g**) that lead to inactive compounds (see Table 1). The replacement of the phenyl with a naphthyl ring is beneficial.

The introduction of a methyl or a fluoro group at position 3 (21m, 21d) confers greater potency to the compounds than the same substitution at position 2 (21c, 21k). Despite good activity against the <sup>V600E</sup>BRAF enzyme (0.023 and 0.047  $\mu$ M compared to 0.037 and 0.008  $\mu$ M, respectively) a drop of 12- to 16-fold in potency on pERK is observed. Unexpectedly, the reverse is seen with a methoxy group (the 2-methoxy compound 21b is more potent on IC<sub>50</sub> BRAF and IC<sub>50</sub> pERK than its 3-methoxy analogue 21f), although no improvement is achieved with respect to the parent compound 1 with an unsubstituted central phenyl ring.

In general, hydrophobic groups such as halogens, especially fluorine, are beneficial for potency on <sup>V600E</sup>BRAF (BRAF IC<sub>50</sub> = 0.023  $\mu$ M for a chlorine **211** and 0.008  $\mu$ M for a fluorine **21d**). However, inhibition of pERK activity in cells (IC<sub>50</sub> ppERK) and inhibition of proliferation (SRB GI<sub>50</sub>) remain above 1  $\mu$ M. The presence of a trifluoromethyl group (**21i**) enhances the activity on SRB by 4.5-fold to afford a submicromolar compound at 0.93  $\mu$ M.

Two ring B modifications show considerable overall improvements compared to **1**. A 3-thiomethyl substituent on phenyl ring B improves the BRAF potency to an IC<sub>50</sub> of 1 nM (**21a**) and shows good activity on cells with pERK IC<sub>50</sub> at 0.61  $\mu$ M

and SRB GI<sub>50</sub> at 0.47  $\mu$ M. The use of the naphthyl system as ring B (**21e**), despite having a modest negative effect on BRAF potency (BRAF IC<sub>50</sub> of 30 nM), sharply increases the cellular activity of these compounds (pERK IC<sub>50</sub> of 0.56  $\mu$ M and SRB GI<sub>50</sub> of 0.47  $\mu$ M).

Isosteric replacements of the 3-thiomethyl substituent with other heteroatom groups did not lead to equipotent or more potent compounds. A methoxy group (**21f**) decreased drastically the cell activity to greater than 100  $\mu$ M, even though it showed a relatively good BRAF activity at 0.106  $\mu$ M. A dimethylamino substituent (**21g**) is not tolerated. The oxidation of the thiomethyl group of **21a** to a methylsulfonyl group (**26**) also reduces both the cellular activity and the activity against BRAF. Similarly, replacing the naphthyl group by a 2,3-dimethylphenyl ring (**21h**) is detrimental.

These results clearly show that the modification of the phenyl middle ring can greatly enhance the activity of the compounds. The substitution of the phenyl B ring in position 3 leads to more potent inhibitors than in position 2. Hydrophobic groups enhance the activity on cells providing submicromolar compounds in pERK and SRB assays; the most promising groups are 3-thiomethyl, 3-fluorine, or replacement of the phenyl ring with naphthyl.

**Variation of Ring C.** We investigated the effect of ring C when combined with the most beneficially substituted middle ring B, following our improvements to the potency of **1** against

Table 4. Compounds with Amide or Methylene Amide Linker B-C

| Compound | Compound  | V600E<br>BRAF     | PERK IC <sub>50</sub> | SRB GI50 |
|----------|---|-------------------|-----------------------|----------|
| number   |   | $IC_{50} (\mu M)$ | (µM)                  | (µM)     |
| 24a      |   | >10               | >100                  | ND       |
| 24b      |   | >10               | >100                  | 3.1      |
| 25a      |   | 5.2               | 31                    | 12       |
| 25b      | SCF3<br>SCF3<br>O<br>V<br>N<br>N<br>O<br>N<br>N<br>N<br>O<br>N<br>N<br>O<br>N<br>N<br>O<br>N<br>N<br>O<br>N<br>N<br>O<br>N<br>N<br>O<br>N<br>N<br>O<br>N<br>N<br>O<br>N<br>N<br>N<br>O<br>N<br>N<br>N<br>N<br>N<br>N<br>N<br>N<br>N<br>N<br>N<br>N<br>N | 4.6               | 18.4                  | 7.6      |

<sup>V600E</sup>BRAF and also on cells by modification of the phenyl ring B. We selected C rings that had been identified as active,<sup>15</sup> and we combined them with the 3-fluoro, 3-thiomethylphenyl, and naphthyl rings B (see Table 2).

Replacement of the chlorine atom of ring C for a hydrogen atom does not affect the activity against isolated <sup>V600E</sup>BRAF for inhibitors with a 3-fluoro- (**21q**) or a 3-thiomethyl (**21n**) substituted phenyl ring B, but the potency on cells decreases by approximately 1.5-fold. In contrast, the naphthyl compound



Figure 3. Graph showing the linear relationship between pERK  $IC_{50}$  and SRB  $GI_{50}$ .

**21t** shows an enhancement on both BRAF  $IC_{50}$  and SRB  $GI_{50}$ , but the inhibition of pERK decreases by more than 3-fold.

Replacement of the trifluoromethyl group with the more lipophilic trifluoromethylthio group (21s) has no effect on the potency on the isolated enzyme or on cellular assays. However, replacing the trifluoromethyl group by a *tert*-butyl group (21x) (naphthyl substituted ring B) enhances the potency on all three assays by almost 2-fold.

Compounds with a 3-trifluoromethyl-6-fluoro phenyl ring C are less active in cellular potency assays for the 3-fluoro **21r** and 3-thiomethyl **21o** compounds despite having a good activity against <sup>V600E</sup>BRAF. In contrast, the combination of this ring with naphthyl ring B (**21u**) increases the potency on cells. Increasing the bulk on position 2 by introducing a morpholino group leads to a decrease of the activity in all three assays.

Among the different rings C investigated, the 3-trifluoromethyl-6-fluorophenyl or a 3-*tert*-butylphenyl group when combined with a naphthyl middle ring achieve the greatest potency against <sup>V600E</sup>BRAF, cellular ERK phosphorylation, and cell growth inhibition.

*N*-Methylation of Ring A. We have shown previously that methylation of the pyridoimidazolone improves the pharmacokinetic (PK) profile of our compounds by eliminating an H-bond donor while preserving good <sup>V600E</sup>BRAF and cellular activities.<sup>15</sup> We therefore combined the naphthyl ring B with the methylated pyridoimidazolone ring A and with different rings C.

Of the compounds containing 4-chloro-3-trifluoromethylphenyl **22b**, a 3-trifluoro-6-fluoro-methylphenyl **22c** or a 3-*tert*-



Figure 4. Kinase selectivity of compounds 21n, 21u, and 21x. Compounds were tested against a panel of 69 kinases at the MRC National Centre for Protein Kinase Profiling. The color bar represents percent activity relative to the control.

butylphenyl **22d** ring C, the methylation on the N1 position is tolerated with an effectively unchanged activity on <sup>V600E</sup>BRAF, despite some detrimental effect on cellular activity compared to the nonmethylated counterparts **21e**, **21u**, and **21x** (see Table 3). The 3-trifluoromethylphenyl ring C (**22e**) combined with the *N*1-methylated ring A is active with only a slight loss of potency in the SRB assay. Again, a 3-trifluorothiomethylphenyl ring C (**22f**) has no positive effect on cellular assays. Similarly, a loss of cellular activity is notable for the 3-fluoro-substituted ring B compound **22a** (not shown, BRAF IC<sub>50</sub> of 0.01  $\mu$ M, pERK IC<sub>50</sub> of 21  $\mu$ M, and SRB GI<sub>50</sub> of 100  $\mu$ M) compared to its nonmethylated counterpart **21d**.

Methylation at the N3 position is detrimental with a significant drop of potency in all three assays (**23a**, **23b**, and **23c**). The N3 of the imidazolone, facing the hinge backbone,<sup>15</sup> does not tolerate a substitution. These results suggest that the bulkier naphthyl ring B subtly shifts the position of the hinge-binding group A with respect to the published unsubstituted compounds and possibly that hydrogen bonding of NH-3 of the imidazolone with the hinge occurs to a certain extent for these compounds.

**B**-**C** Linker Modifications. To improve the bioavailability of these BRAF inhibitors, we synthesized compounds with one less NH-bond donor in the B-C linker (Table 4). Unfortunately, the replacement of the urea linker for a methylene amide or an amide leads to inactive compounds (cf 24a vs 21t and 25b vs 22f).

Linear Relationship between pERK  $IC_{50}$  and SRB  $GI_{50}$ . Linear regression analysis of the pERK  $IC_{50}$  and SRB  $GI_{50}$ 



**Figure 5.** Selective inhibition of a mutant BRAF-driven human melanoma cell line displayed with **22c**. Growth inhibition of WM266.4 (BRAF mutant) and WM1361 (NRAS mutant) melanoma cell lines following a 5 day exposure to sorafenib (A) or **22c** (B) and analysis by SRB assay.

values of 29 of the inhibitors synthesized shows a high correlation (eq 1), as the regression coefficient *r* is 0.87 ( $r^2 = 0.757$ ) (see Figure 3). This analysis was performed using TSAR.<sup>24</sup>

$$SRB(GI_{50}) = 2.41pERK(IC_{50}) - 0.77$$
  
 $n = 29 \quad s = 3.80 \quad f = 81.21$  (1)

These data indicate that there is a significant correlation between the SRB  $GI_{50}$  and the pERK  $IC_{50}$ . This finding provides evidence that the inhibition of growth of the mutant BRAF driven cells is an on-target effect.

Selectivity Studies. Our inhibitors were elaborated into the BPI pocket in an attempt to provide selectivity for mutant BRAF compared to other similar kinases. In this, we were successful for the three compounds (21n, 21u, and 21x) we submitted to the MRC National Centre for Protein Kinase Profiling and which were assessed at a single concentration of 1  $\mu$ M. The three compounds are highly selective, hitting only a few kinases against the assessed panel of 69 (Figure 4). Src, Lck, and p38 (MAP) kinases are common hits with the three compounds, with 21u showing selectivity toward the  $\beta$  isoform of p38 with respect to the  $\alpha$  one.

Not surprisingly, the same three proteins have been shown to be sensitive to ligands that elaborate into the BPI pocket. This is the case of the recently released cocrystal structure of nilotinib<sup>25</sup> with p38 $\beta$ , and of the ones of imatinib with Src<sup>26</sup> and Lck.<sup>27</sup> In all those structures, the methylated central ring fits into the BPI pocket. Doramapimod, an inhibitor with similarity to some of our compounds, is a highly selective inhibitor of p38 MAP kinase<sup>28,16</sup> and presents a naphthyl moiety interacting with the BP1 pocket.

A cell selectivity experiment was also carried out. Growth inhibition for sorafenib and **22c** was performed against the WM266.4 (BRAF mutant) and WM1361 (NRAS mutant) cell lines (Figure 5). While no differential was observed in the case

Scheme 6. Formation of the B-C Urea Linker; Synthesis of Final Compounds via Route A



Scheme 7. Synthesis of Compounds with Amide and Methylene Amide Linker B-C via Route A



of sorafenib, a 6-fold difference is observed between the two cell lines for **22c**.

## Conclusions

Following the development of a series of V600EBRAF inhibitors based on a novel hinge-binding fragment,<sup>15</sup> we have extended their scope by modifying the central phenyl ring. Our investigations show that ring B modification leads to a spectrum of low nanomolar inhibitors of V600EBRAF. In a number of examples, notably by ring B functionalization with fluoro, thiomethyl groups, or replacement with a naphthyl, highly potent inhibitors are obtained with IC<sub>50</sub> values up to an order of magnitude lower than those for analogues with unsubstituted phenyl rings B. Seven compounds have an  $IC_{50}$  on  $^{V600E}BRAF$ of less than 10 nM, and compounds 21a and 21n, each substituted with a thiomethyl group, notably achieve a <sup>V600E</sup>BRAF IC<sub>50</sub> of 1 nM. Moreover, this 3-thiomethyl substitution, as for the naphthyl group, increases the potency on cells greatly with GI<sub>50</sub>s in the nanomolar range. <sup>V600E</sup>BRAF inhibition in cells, measured with the pERK assay, is also submicromolar for some of the reported compounds and correlates with the cell growth inhibition; this strongly supports the mode of action of the compounds in this series. We have shown that these compounds inhibit both the <sup>V600E</sup>BRAF isolated enzyme and the enzyme in cellular assays and that this effect translates to potent inhibition of the growth of a mutant BRAF dependent melanoma cell line. Several of these compounds achieve over 10-fold increase in cellular potency compared to the reported BRAF inhibitor sorafenib. Furthemore, we have shown that elaborating the phenyl middle ring into the BPI pocket provides protein kinase and cell selectivities. These promising data warrant further in vivo investigation of this series.

#### **Experimental Section**

**General Information.** All starting materials, reagents, and solvents for reactions were reagent grade and used as purchased. Chromatography solvents were HPLC grade and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) analysis using Merck silica gel 60 F-254 thin layer plates. Flash column chromatography was carried out on

Scheme 8. Access to Final Compounds 21a/f - m and 26 via Route B<sup>a</sup>



<sup>*a*</sup> Reagents and conditions: (a) 4-chloro-3-trifluoromethylphenylisocyanate in DCM or THF at room temperature, Ar atm; (b) Pd/C,  $H_2$ , RT or Fe, NH<sub>4</sub>Cl in EtOH/H<sub>2</sub>O, reflux for **9a**; (c)(Cl<sub>3</sub>CO)<sub>2</sub>CO, pyridine, THF, room temperature; (d) oxone, 0 °C.

Merck silica gel 60 (0.015-0.040 mm) or in disposable Isolute Flash Si and Si II silica gel columns. Preparative TLC was performed on either Macherey-Nagel [809 023] precoated TLC plates SIL G-25 UV<sub>254</sub> or Analtech [2015] precoated preparative TLC plates, 2000  $\mu$ m with UV<sub>254</sub>. LC-MS analyses were performed on a Micromass LCT/Water's Alliance 2795 HPLC system with a Discovery 5  $\mu$ m, C18, 50 mm × 4.6 mm i.d. column from Supelco at a temperature of 22 °C using the following solvent system: solvent A, methanol; solvent B, 0.1% formic acid in water, at a flow rate of 1 mL/min. Gradient starting with 10% A/90% B from 0 to 0.5 min then 10% A/90% B to 90% A/10% B from 0.5 to 6.5 min and continuing at 90% A/10% B up to 10 min. From 10 to 10.5 min, the gradient reverted back to 10% A/90% B and was held until 12 min. UV detection was at 254 nm, and ionization was positive or negative ion electrospray. The molecular weight scan range was 50-1000. Samples were supplied as 1 mg/mL in DMSO or methanol with 3  $\mu$ L injected on a partial loop fill. NMR spectra were recorded on a Bruker DPX 250 MHz or a Bruker Advance 500 MHz spectrometer. The signal of the deuterated solvent was used as internal reference. Chemical shifts ( $\delta$ ) are given in ppm and are referenced to residual, not fully deuterated solvent signal (e.g., DMSO-d<sub>5</sub>). Coupling constants (J) are given in Hz. The purity of the final compounds was determined by HPLC as described above and is 95% or higher unless specified otherwise.

Procedure A: 1-(4-(2,3-Dihydro-2-oxo-1H-imidazo[4,5-b]pyridin-7-yloxy)-2-(methylthio) phenyl)-3-(4-chloro-3-(trifluoromethyl)phenyl)urea (21a). 1-(4-(2,3-Diaminopyridin-4-yloxy)-2-(methylthio)phenyl)-3-(4-chloro-3-(trifluoromethyl)phenyl)urea 11a (1.52 g, 3.1 mmol) was dissolved in dry THF (41 mL), and pyridine (2 mL) was added and the solution was cooled at 0 °C. Triphosgene (3.1 mmol) in dry THF (20 mL) was added dropwise. The reaction mixture was stirred for 15 min more at 0 °C and at room temperature for 16 h. The solvent was evaporated and the residue washed with water to provide after filtration the title compound (1.55 g, 97%) as a brown powder. <sup>1</sup>H NMR  $\delta$ : 2.45 (s, 3H, CH<sub>3</sub>), 6.42 (d, 1H, J smd 4.2, H<sub>Pv</sub>), 6.99 (d, 1H, J smd 8.4, H<sub>arom</sub>), 7.19 (s, 1H, H<sub>arom</sub>), 7.63 (s, 2H, H<sub>arom</sub>), 7.78 (s, 2H, H<sub>arom</sub>), 8.12 (s, 1H, Harom), 8.26 (s, 1H, NHurea), 9.91 (s, 1H, NHurea), 11.25 (s, 1H, NH<sub>Py</sub>), 11.44 (bs, 1H, NH<sub>Py</sub>). <sup>13</sup>C NMR δ: 15.7, 105.7, 113.2, 116.4, 116.5, 117.3, 121.7, 122.2, 122.7, 123.8, 124.4, 131.9, 132.0, 133.1, 139.4, 141.1, 145.4, 146.8, 150.5, 152.6, 154.1. LC-MS: m/z 510  $([M + H]^+, 100)$ . HRMS: m/z calcd for  $C_{21}H_{15}ClF_3N_5O_3S$   $([M + H]^+, 100)$ . H]<sup>+</sup>): 510.0611; found: 510.0614.

Compounds **21f**-**m** were synthesized in a similar manner. The data are available in Supporting Information.

Procedure B: 1-(4-(2,3-Dihydro-2-*oxo*-1*H*-imidazo[4,5-*b*]pyridin-7-yloxy)-3-methoxyphenyl)-3-(4-chloro-3-(trifluoromethyl)phenyl)urea (21b). 7-(4-Amino-2-methoxyphenoxy)-1*H*-imidazo[4,5-b]pyridin-2(3*H*)-one 2b (16 mg, 0.06 mmol) and corresponding substituted arylisocyanate (1.1 equiv) in anhydrous THF were stirred at room temperature for 14 h. The solvent was evaporated and the solid residue was washed with Et<sub>2</sub>O to afford after filtration the title compound (28 mg, 98%) as a pale-yellow powder. <sup>1</sup>H NMR  $\delta$ : 3.75 (s, 3H, CH<sub>3</sub>), 6.05 (d, 1H, J = 5.8, H<sub>Py</sub>), 7.05 (d, 1H, J =8.7, H<sub>arom</sub>), 7.26 (dd, 1H, J = 8.6 and J = 1.9, H<sub>arom</sub>), 7.56 (d, 1H, J = 8.8, H<sub>arom</sub>), 7.60 (d, 1H, J = 5.8, H<sub>Py</sub>), 7.65 (d, 1H, J = 2.1, H<sub>arom</sub>), 7.90 (m, 1H, H<sub>arom</sub>), 8.28 (d, 1H, J = 2.4, H<sub>arom</sub>), 10.02 (bs, 2H, NH<sub>urea</sub>), 10.82 (bs, 1H, NH<sub>Py</sub>), 10.98 (bs, 1H, NH<sub>Py</sub>). <sup>13</sup>C NMR  $\delta$ : 55.7, 104.3, 106.2, 111.0, 112.5, 116.8, 122.2, 122.4, 123.2, 124.0, 126.5, 131.7, 136.2, 138.8, 140.3, 151.2, 151.5, 153.1, 153.8, 156.2, 162.3. LC-MS: m/z 494 ([M + H]<sup>+</sup>, 100). HRMS: m/z calcd for C<sub>21</sub>H<sub>15</sub>ClF<sub>3</sub>N<sub>5</sub>O<sub>4</sub> ([M + H]<sup>+</sup>): 494.0837; found: 494.0836.

Compounds 21c-e/n-y; 22a-f; 23a-c were synthesized in a similar manner. The data are available in Supporting Information.

Procedure C: N-(4-(2-oxo-2,3-dihydro-1H-imidazo[4,5-b]pyridin-7-vl-oxv)naphthalen-1-vl)-aminocarbonvlmethvlene-(3-trifluoromethyl-phenyl) (24a). To 7-(4-aminonaphthalen-1-yloxy)-1Himidazo[4,5-b]pyridin-2(3H)-one 2e (50 mg, 0.17 mmol) in anhydrous dioxane (7 mL), triethylamine (150  $\mu$ L), and 2-(3-(trifluoromethyl)phenyl)acetyl chloride (0.20 mmol) were added under stirring and nitrogen atmosphere and heated for 20 h at 80 °C. The solvent was evaporated under vacuum and the solid residue dissolved in 10 mL of EtOAc and washed successively with brine  $(2 \times 10 \text{ mL})$ , a solution of citric acid, and brine again. The organic solution was evaporated under vacuum, and the solid residue recrystallized from EtOAc to afford the title compound (59 mg, 67%). <sup>1</sup>H NMR  $\delta$ : 3.97 (s, 2H, CH<sub>2</sub>), 6.24 (d, 1H, J = 5.9, H<sub>Py</sub>), 7.26 (d, 1H, J = 8.2,  $H_{arom}$ ), 7.58–7.74 (m, 7H,  $H_{arom} + H_{Py}$ ), 7.81 (s, 1H,  $H_{arom}$ ), 7.98 (d, 1H, J = 8.2, H<sub>arom</sub>), 8.12 (d, 1H, J = 8.4, H<sub>arom</sub>), 10.29 (s, 1H, NH<sub>amide</sub>), 11.38 (s, 1H, NH<sub>Pv</sub>), 11.44 (s, 1H, NH<sub>Pv</sub>). LC-MS: m/z 479 ( $[M + H]^+$ , 100). HRMS: *m*/*z* calcd for C<sub>25</sub>H<sub>17</sub>F<sub>3</sub>N<sub>4</sub>O<sub>3</sub> ([M +H]<sup>+</sup>) 479.1331; found 479.1318.

Compounds **24b**, **25a**,**b** were synthesized in a similar manner. The data are available in the Supporting Information.

1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(2-(methylsulfonyl)-4-(2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*b*]pyridin-7-yloxy)phenyl)urea (26). To a slurry of compound 21a 1-(4-(2,3-dihydro-2-oxo-1*H*-imidazo[4,5-*b*]pyridin-7-yloxy)-2-(methylthio)phenyl)-3-(4chloro-3-(trifluoromethyl)phenyl)urea (35 mg, 0.07 mmol) in MeOH (700  $\mu$ L) was added slowly a solution of oxone (5 equiv) in water (900  $\mu$ L). The mixture maintained at 0 °C was stirred 5 h and then neutralized with a saturated solution of NaHCO<sub>3</sub> to pH 8. The mixture was diluted in water and then filtered to provide the expected sulfone as a powder (22 mg, 60%). <sup>1</sup>H NMR  $\delta$ : 3.32 (s, 3H, CH<sub>3</sub>), 6.58 (d, 1H, *J* smd 5.8, H<sub>Py</sub>), 7.51 (dd, 1H, *J* smd 8.9 and *J* smd 2.7, H<sub>arom</sub>), 7.54 (d, 1H, *J* smd 2.7, H<sub>arom</sub>), 7.64 (d, 1H, *J* smd 8.7, H<sub>arom</sub>), 7.70 (d, 1H, *J* = 8.1, H<sub>arom</sub>), 7.83 (d, 1H, *J* = 5.8, H<sub>Py</sub>), 8.11 (m, 1H, H<sub>arom</sub>), 8.14 (d, 1H, *J* = 8.9, H<sub>arom</sub>), 8.64 (s, 1H, NH<sub>urea</sub>), 10.29 (s, 1H, NH<sub>urea</sub>), 11.21 (s, 1H, NH<sub>Py</sub>), 11.45 (bs, 1H, NH<sub>Py</sub>). <sup>13</sup>C NMR  $\delta$ : 42.7, 106.7, 113.7, 116.9, 118.5, 121.6, 123.0, 123.8, 125.2, 126.3, 126.6, 130.2, 131.9, 133.3, 139.0, 141.3, 143.9, 147.2, 149.7, 151.9, 154.1. LC-MS: *m*/*z* 542 ([M + H]<sup>+</sup>, 100). HRMS: *m*/*z* calcd for C<sub>21</sub>H<sub>15</sub>ClF<sub>3</sub>N<sub>5</sub>O<sub>5</sub>S ([M + H]<sup>+</sup>): 542.0507; found: 542.0503.

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Supporting Information Available: Experimental protocols and analytical data for final compounds 21f-m, 21c-e/n-y, 22a-f, 23a-c, 24b, 25a,b, and all intermediates 2a-e, 3d,e, 4, 5a/f-m, 6a/f/g, 7a-d/f-j, 8a/d, 9a-c/e-m, 10a-e, 11a/f-m, 12a-e, 13a-e, 14d,e, 15d,e, 16d,e, 18, 19, 20, and protocols for biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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