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Design and synthesis of 6,6-fused heterocyclic amides as raf kinase inhibitors

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ABSTRACT

Compounds belonging to several scaffolds—quinazolines, quinolines and quinoxalines—were designed and synthesized as Raf kinase inhibitors. Scaffolds were assessed for in vitro $Braf^{V600E}$ inhibition, and overall kinase selectivity. Pharmacokinetic parameters for one of the scaffolds were also determined.

The Ras-mitogen activated protein kinase (MAPK) signaling pathway was the first signaling pathway elucidated from the cell membrane to the nucleus.[1] The MAPK signaling pathway consists of the Ras/Raf/MEK/ERK signal transduction cascade which is a vital mediator of a number of cellular activities including growth, proliferation, survival and other aspects of cellular behavior that can contribute to the transformed phenotype, making it an attractive pathway to target in several cancer types. The three Raf isoforms (Raf-1 or c-Raf, A-Raf and B-Raf) are all able to interact with Ras and activate the MAP kinase pathway.[2–5]

Inhibition of the Raf/MEK/ERK pathway at the level of Raf kinases is expected to be effective against tumors driven by this pathway. It has been shown that the B-Raf mutation V600E in skin nevi is a critical step in the initiation of melanocytic neoplasia.[6] Furthermore, activating mutations in the kinase domain of B-Raf occur in roughly 66% of malignant melanomas, 40–70% of papillary thyroid carcinomas, 12% of colon carcinomas and 14% of liver cancers.[5,7–9] The many effects of Raf kinases on cancer cell growth and survival, together with the high prevalence of mutation in melanoma, for which there is no good treatment, make Raf an attractive target for anticancer therapy.

The first reported RAF inhibitor, BAY43-9006 (Sorafenib),[10–14] while effective in renal cell carcinoma (RCC), has shown a lack of efficacy in patients expressing the $Braf^{V600E}$ mutation suggesting its mechanism of action is through inhibition of VEGFR rather than RAF.[15,16] Recently PLX-4032[17] has been launched as a Raf inhibitor. However, several other RAF inhibitors (e.g. GSK2118436)[18] are in clinical trials and have shown evidence of clinical benefit. Our group has previously disclosed the benzimidazole amide series containing orally available and potent Raf inhibitors.[19,20] In particular, the 3-*t*-butylphenylaminobenzimidazole amide **1** (Fig. 1) potently inhibited $Braf^{V600E}$ and the phosphorylation of the downstream target ERK in the SKMEL-28 cell line with an EC_{50} of 0.3 μM. To further explore the structure activity relationship of the benzimidazole core for inhibition of $Braf^{V600E}$, the quinazolines, quinolines and quinoxaline amide series (Fig. 1) were synthesized[21] and compared to the benzimidazole amide series. In addition to the

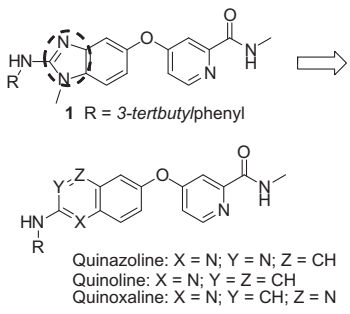

Figure 1. 6,6-Fused heterocyclic pyridyl amides designed as analogues of the benzimidazole pyridyl amide.

Abbreviations: MAPK, Ras-mitogen activated protein kinase; VEGFR, vascular endothelial growth factor receptor; CSFR1, colony stimulating factor-1 receptor; RTK's, receptor tyrosine kinases; STK's, serine threonine kinases; TK, tyrosine kinases; KDR, kinase domain receptor.

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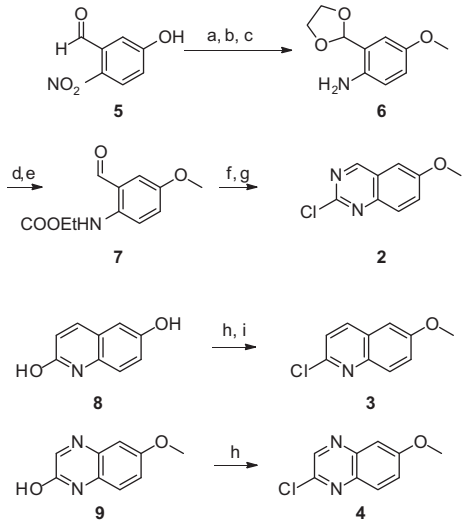
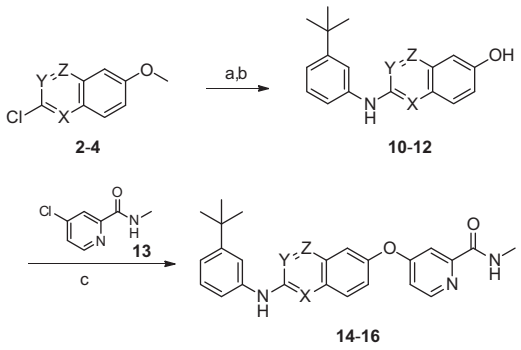
in vitro Braf^{V600E} SAR, pharmacokinetic profiles for a representative example will be presented.

For all the scaffolds, acquiring the key intermediate 2-chloro-6-methoxyheterocycles (**2–4**) was essential. A survey of literature procedures for the preparation of appropriately substituted quinazolines rapidly led us to the conclusion that it would be necessary to develop alternate synthetic routes for both the intermediates and final products (Scheme 1). Methylation of 5-hydroxy-2-nitrobenzaldehyde **5** provided the corresponding methyl ether. Protection of the aldehyde with glycol in the presence of tosic acid afforded the dioxalane, which upon reduction yielded **6** in 60–70% yield. Treatment with ethylchloroformate followed by deprotection afforded the precursor **7**. Ring closure was effected using condensed ammonia under forcing conditions, followed by chlorination to afford the key intermediate 2-chloro-6-methoxy quinazoline (**2**). 2-Chloro-6-methoxyquinoline (**3**) was obtained from commercially available **8** by selective chlorination at the 2-position of the heterocyclic ring followed by methylation. Synthesis of 2-chloro-6-methoxy quinoxaline (**4**) required only chlorination of **9**. Introduction of anilines was effected under S_NAr conditions for all the above mentioned scaffolds (Scheme 2). O-Arylation was then performed using KHMDS and chloro-pyridylacetamide[22] (**13**) in DMF as solvent.

Table 1 highlights the structure–activity relationship of the aniline and amide functionalities in the quinazoline series. As observed in the benzimidazole series[19] ortho substituents (**17–19**) on the aniline led to significant loss of affinity >1.0 μM, offering no advantage over the unsubstituted phenyl analog (**1a**) against Braf^{V600E}. Meta-and para substituents (14, **19–24**) were in general similarly potent compared to meta- and para-substituents in the benzimidazole series (e.g., **1**). In particular, the 3-OCF_3 substituted phenyl **23** had the best affinity in the meta-substituted sub-series. For substituents in the para-position of the phenyl ring, affinity appeared to improve as the hydrophobic nature of the substituents increased (**25–30**). However, larger groups in the para-position of the phenyl ring such as phenoxy (**31**) and 3-pyridyl (**32**) showed a decrease in affinity indicating size/shape restrictions in this area of the binding pocket. The similar binding affinity of phenoxy **31** and O–Me **29** analogs suggests an intricate balance between hydrophobicity and size/shape. Interestingly, appendage of solubilizing groups on the amide led to degradation of potency which was counter to the SAR seen in the benzimidazole series.

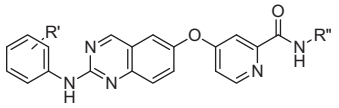


Scheme 1. Synthesis of intermediates **2–4**. Reagents and conditions: (a) K_2CO_3, MeI, DMF; (b) Glycol, tosic acid; (c) PtO_2, H_2, EtOH; (d) EtOCOCl, THF; (e) concd HCl; (f) NH_3 at 0–130 °C; (g) $POCl_3$, 95–100 °C, 4 h; (h) $POCl_3$, THF, 65 °C; (i) K_2CO_3, MeI, DMF.



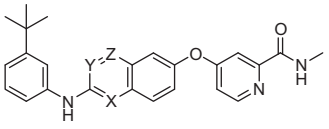
Scheme 2. Reagents and conditions: (a) 3-*t*-Butylaniline, EtOH, 80 °C; (b) 48% HBr, 140 °C, microwave, 6 min; (c) KHMDS, K_2CO_3, DMF, microwave, 170 °C, 6 min.

Table 1
Structure–activity relationship of the quinazoline series



Compound	R′	R″	Braf^{V600E} IC_{50} (μM)[23]
1	—	—	0.12
1a	H	Me	2.2
17	2-Br	Me	24.0
18	2-Et	Me	1.3
19	2-OPh	Me	5.3
20	3-Cl	Me	0.66
21	3-Br	Me	0.18
14	3-*t*-Bu	Me	0.045
22	3-CF_3	Me	0.64
23	3-OCF_3	Me	0.07
24	3-*i*-Pr	Me	0.15
25	4-F	Me	0.60
26	4-Br	Me	0.31
27	4-CF_3	Me	0.067
28	4-*i*-Pr	Me	0.001
29	4-OMe	Me	0.39
30	4-OCF_3	Me	0.08
31	4-OPh	Me	0.60
32	4-(3)-Pyridyl	Me	25.0
33	4-Br	2-Hydroxy-ethylamino	8.6
34	4-Br	Piperidinyl-ethyl	1.3

Table 2
Comparison of the 6,6-heterocyclic series



Compound	X	Y	Z	Braf^{V600E} IC_{50} (μM)
1	—	—	—	0.12
16	N	CH	N	0.025
15	N	CH	CH	0.026
14	N	N	CH	0.045

Table 2 compares the three scaffolds to the benzimidazole **1**. The biochemical assay data indicated quinazoline **14** to be equipotent with **1** whilst quinoline **15** and quinoxazoline **16** are roughly sixfold more potent than benzimidazole **1**.

In order to understand the binding mode for the quinazoline series, **14** was docked in the active site of the public domain crystal

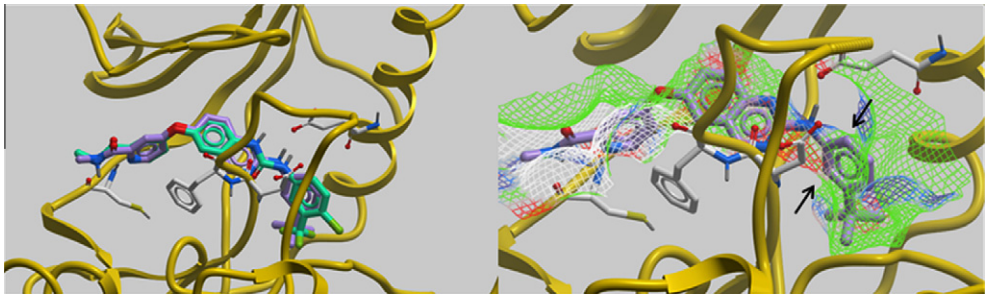


Figure 2. Binding site model for compound **14** (in purple), derived by docking into the crystal structure of B-Raf (PDB accession code 1UWH). The left picture shows a cartoon representation of the kinase with selected residues in stick model (Glu501, Cys532, Phe593 and Asp594) and the co-crystallized Sorafenib in green. The right picture zooms in and displays the extra radius binding surface around **14**, colored by surface properties: red = hydrogen-bond donor, blue = hydrogen-bond acceptor, green = hydrophobic surface, white = aromatic surface. Ortho positions in optimal van-der-Waals contact with the protein are indicated with arrows.

structure published for B-Raf (PDB accession code 1UWH).[24] Figure 2 (left) shows the overlap of the docking model with the co-crystallized conformation of Sorafenib. The model suggests a very similar binding mode when comparing **14** with Sorafenib.[25] Specific interactions between **14** and the B-Raf protein include hydrogen bonds to (1) the backbone NH and C=O of Cys532 in the hinge region through the pyridyl-amide moiety, (2) the backbone NH of Asp594 through the quinazoline nitrogen, and (3) the side chain COO– of Glu501 through the aniline NH. This model uses the 'DFG-out' conformation of the protein where the substituted aniline displaces Phe593, causing it to swing out and interact with the aromatic systems in the hinge region and the selectivity pocket. The extra radius surface model in Figure 2 (right) shows that the ortho carbons of the aniline are in optimal van-der-Waals contact with the binding pocket, providing a rationale for the drop in affinity with substitution in that position. The model indicates there is space available for meta- and para-substitution, in accordance with the observed SAR.

While all three series yielded potent $Braf^{V600E}$ inhibitors in the biochemical assay, none of these inhibited phosphorylation of ERK in cells (SKMEL-28, Raf^{V600E} EC_{50} >10 μM). This observation was consistent with the majority of the compounds from our original benzimidazole series. This discrepancy between biochemical and cellular potency could be due to permeability and solubility limitations. Compounds from all series were tested in the Caco2 assay and shown to have poor permeability (P_{app} A–B $<1 \times 10^{-6}$ cm/s). Solubility at pH7 was measured and shown to be <1 μM. In addition, it should be noted that in the biochemical assay, the purified kinase domain of $Braf^{V600E}$ is not representative of the full length protein in cells where $Braf^{V600E}$ exists as a complex with chaperones, cytoskeleton, phosphatases and kinases.[23]

The kinase profiles of three representative compounds, **14–16** were determined for 50 kinases including RTKS, STKs, TKs and AGC kinases. The compounds exhibited a fairly narrow kinase profile, inhibiting four RTKs (CSFR1, Flt3, KDR and cABL) out of 50 kinases with an IC_{50} <1 μM. The pharmacokinetic properties for one of the prototypes in the quinazoline series, **24,** were determined. Following a single 20-mg/kg oral administration to female mice in 15% captisol, **24** exhibited a clearance of 53.1 mL/min/kg, a volume of distribution of 4717 mL/kg, a half-life of 468 min, and an oral bioavailability of 35%.

In conclusion, we developed three series of biochemically potent $Braf^{V600E}$ inhibitors. Lack of cellular potency of these compounds discouraged us from continuing further work on these series.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.12.112.

References and notes

1. Dumaz, N.; Marais, R. *J. Biol. Chem.* **2003**, *278*, 29819.
2. Weber, C. K.; Slupsky, J. R.; Herrmann, C.; Schuler, M.; Rapp, U. R.; Block, C. *Oncogene* **2000**, *19*, 169.
3. Pritchard, C. A.; Samuels, M. L.; Bosch, E.; McMahon, M. *Mol. Cell. Biol.* **1995**, *15*, 6430.
4. Minamoto, T.; Mai, M.; Ronai, Z. e. *Cancer Detect. Prev.* **2000**, *24*, 1.
5. Yuen, S. T.; Davies, H.; Chan, T. L.; Ho, J. W.; Bignell, G. R.; Cox, C.; Stephens, P.; Edkins, S.; Tsui, W. W.; Chan, A. S.; Futreal, P. A.; Stratton, M. R.; Wooster, R.; Leung, S. Y. *Cancer Res.* **2002**, *62*, 6451.
6. Pollock, P. M.; Harper, U. L.; Hansen, K. S.; Yudt, L. M.; Stark, M.; Robbins, C. M.; Moses, T. Y.; Hostetter, G.; Wagner, U.; Kakareka, J.; Salem, G.; Pohida, T.; Heenan, P.; Duray, P.; Kallioniemi, O.; Hayward, N. K.; Trent, J. M.; Meltzer, P. S. *Nat. Genet.* **2003**, *33*, 19.
7. Davies, H.; Bignell, G. R.; Cox, C.; Stephens, P.; Edkins, S.; Clegg, S.; Teague, J.; Woffendin, H.; Garnett, M. J.; Bottomley, W.; Davis, N.; Dicks, E.; Ewing, R.; Floyd, Y.; Gray, K.; Hall, S.; Hawes, R.; Hughes, J.; Kosmidou, V.; Menzies, A.; Mould, C.; Parker, A.; Stevens, C.; Watt, S.; Hooper, S.; Wilson, R.; Jayatilake, H.; Gusterson, B. A.; Cooper, C.; Shipley, J.; Hargrave, D.; Pritchard-Jones, K.; Maitland, N.; Chenevix-Trench, G.; Riggins, G. J.; Bigner, D. D.; Palmieri, G.; Cossu, A.; Flanagan, A.; Nicholson, A.; Ho, J. W. C.; Leung, S. Y.; Yuen, S. T.; Weber, B. L.; Seigler, H. F.; Darrow, T. L.; Paterson, H.; Marais, R.; Marshall, C. J.; Wooster, R.; Stratton, M. R.; Futreal, P. A. *Nature* **2002**, *417*, 949.
8. Kimura, E. T.; Nikiforova, M. N.; Zhu, Z.; Knauf, J. A.; Nikiforov, Y. E.; Fagin, J. A. *Cancer Res.* **2003**, *63*, 1454.
9. Brose, M. S.; Volpe, P.; Feldman, M.; Kumar, M.; Rishi, I.; Gerrero, R.; Einhorn, E.; Herlyn, M.; Minna, J.; Nicholson, A.; Roth, J. A.; Albelda, S. M.; Davies, H.; Cox, C.; Brignell, G.; Stephens, P.; Futreal, P. A.; Wooster, R.; Stratton, M. R.; Weber, B. L. *Cancer Res.* **2002**, *62*, 6997.
10. Smith, R. A.; Barbosa, J.; Blum, C. L.; Bobko, M. A.; Caringal, Y. V.; Dally, R.; Johnson, J. S.; Katz, M. E.; Kennure, N.; Kingery-Wood, J.; Lee, W.; Lowinger, T. B.; Lyons, J.; Marsh, V.; Rogers, D. H.; Swartz, S.; Walling, T.; Wild, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2775.
11. Khire, U. R.; Bankston, D.; Barbosa, J.; Brittelli, D. R.; Caringal, Y.; Carlson, R.; Dumas, J.; Gane, T.; Heald, S. L.; Hibner, B.; Johnson, J. S.; Katz, M. E.; Kennure, N.; Kingery-Wood, J.; Lee, W.; Liu, X.-G.; Lowinger, T. B.; McAlexander, I.; Monahan, M.-K.; Natero, R.; Renick, J.; Riedl, B.; Rong, H.; Sibley, R. N.; Smith, R. A.; Wolanin, D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 783.
12. Wood, J. E.; Wild, H.; Rogers, D. H.; Lyons, J.; Katz, M. E.; Caringal, Y. V.; Dally, R.; Lee, W.; Smith, R. A.; Blum, C. L. PCT Int. Appl.: WO 9852559, 1998, p 53.
13. Dumas, J. *Exp. Opin. Ther. Pat.* **2001**, *11*, 405.
14. Kumar, C. C.; Madison, V. *Exp. Opin. Emerg. Drugs* **2001**, *6*, 303.
15. Wilhelm, S. M.; Carter, C.; Tang, L.; Wilkie, D.; McNabola, A.; Rong, H.; Chen, C.; Zhang, X.; Vincent, P.; McHugh, M.; Cao, Y.; Shujath, J.; Gawlak, S.; Eveleigh, D.; Rowley, ; Liu, L.; Adnane, L.; Lynch, M.; Auclair, D.; Taylor, I.; Gedrich, R.; Voznesensky, A.; Riedl, B.; Post, L. E.; Bollag, G.; Trail, P. A. *Cancer Res.* **2001**, *64*, 7099.
16. Eisen, T.; Ahmad, T.; Flaherty, K. T.; Gore, M.; Kaye, S.; Marias, R.; Gibebns, I.; Hackett, S.; James, M.; Schuster, L. M.; Nathanson, K. L.; Xia, C.; Simantov, R.; Schwartz, B.; Poulin-Costello, M.; O'Dwyer, P. J.; Ratain, M. J. *Br. J. Cancer* **2006**, *95*, 581.
17. Flaherty, K. T.; Puzanov, I.; Kim, K. B.; Ribas, A.; McArthu, G. A.; Sosman, J.; O'Dwyer, P. J.; Lee, R. J.; Grippo, J. F.; Nolop, K.; Chapman, P. B. *N. Eng. J. Med.* **2010**, *363*, 809.
18. Kefford, R.; Arkenau, H.; Brown, M. P.; Millward, M.; Infante, J. R.; Long, G. V.; Ouellet, D.; Curtis, M.; Lebowitz, P. F.; Falchook, G. S. Annual ASCO meeting, Chicago, IL, 2010, abstract 8503.

19. Ramurthy, S.; Subramanian, S.; Aikawa, M.; Payman, A.; Costales, A.; Dove, J.; Fong, S.; Jansen, J. M.; Levine, B.; Ma, S.; McBride, C. M.; Michaelian, J.; Pick, T.; Poon, D. J.; Girish, S.; Shafer, C. M.; Stuart, D.; Sung, L.; Renhowe, P. A. *J. Med. Chem.* **2008**, *51*, 7049.
20. Ramurthy, S.; Aikawa, M.; Amiri, P.; Costales, A.; Hashash, A.; Jansen, J. M.; Lin, S.; Ma, S.; Renhowe, P. A.; Shafer, C. M. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3286.
21. Ramurthy, S.; Renhowe, P. A.; Subramanian, S. U.S. Pat. Appl. Publ. 2005, p 42. US 20050085482.
22. Synthetic methodology and characterization data for **2** and **3** are included in the Supplementary data.
23. Information on kinetic assays included in the Supplementary data.
24. Ligands were docked in the protein model from the 1UWH structure downloaded from the PDB. The docking program Glide SP was used through Maestro 8.0 (by Schrödinger, LLC). Selected poses for each of the ligands were further minimized with the Embrace routine in Maestro, to allow optimization of the ligands and selected residues. The forcefield OPLS_2005 was used with the solvent model for water and the LBFGS minimizer in energy difference mode. Residues in a 6 Å shell were allowed to move except for their C-α atoms which were restrained in a separate shell with a force constant of 200. That same force constant was applied to an additional 4 Å shell outside the 6 Å shell.
25. Wan, P. T. C.; Garnett, M. J.; Roe, S. M.; Lee, S.; Niculescu-Duvaz, D.; Good, V. M.; Jones, C. M.; Marshall, C. J.; Springer, C. J.; Barford, D.; Marais, R. *Cell* **2004**, *116*, 855.