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Design and synthesis of 5,6-fused heterocyclic amides as Raf kinase inhibitors

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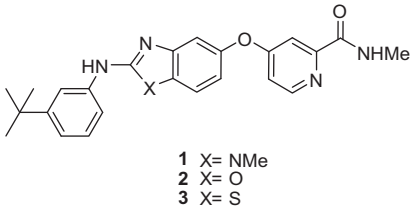
ABSTRACT

Two scaffolds based on 5,6-fused heterocyclic backbones were designed and synthesized as Raf kinase inhibitors. The scaffolds were assessed for in vitro pan–Raf inhibition, activity in cell proliferation and target modulation assays, and pharmacokinetic parameters.

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 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 a very attractive target for anticancer therapy.



1 X= NMe
2 X= O
3 X= S

Figure 1. 5,6-Fused heterocyclic cores explored for Raf inhibition.

The first reported Raf inhibitor, BAY43-9006 (Sorafenib,)[10–14], while effective in 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] However, several other Raf inhibitors (PLX-4032[17] GSK2118436[18]) are now in clinical trials and have shown indications of clinical benefit. Our group has previously disclosed the benzimidazole amide series as orally available potent Raf inhibitors.[19] In particular, the 3-*t*-butylphenylbenzimidazole 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. The benzoxazole and benzothiazole amide series (**2** and **3**, respectively) were synthesized and compared to the lead benzimidazole scaffold to

Abbreviations: MAPK, ras-mitogen activated protein kinase; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; CSF-1, colony stimulating factor-1 receptor; RTK's, receptor tyrosine kinases; STK's, serine threonine kinases; Lck, Leukocyte specific protein tyrosine kinase.

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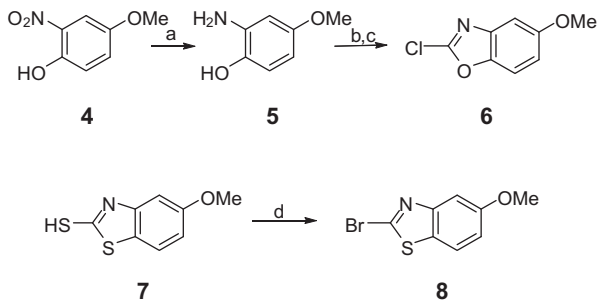
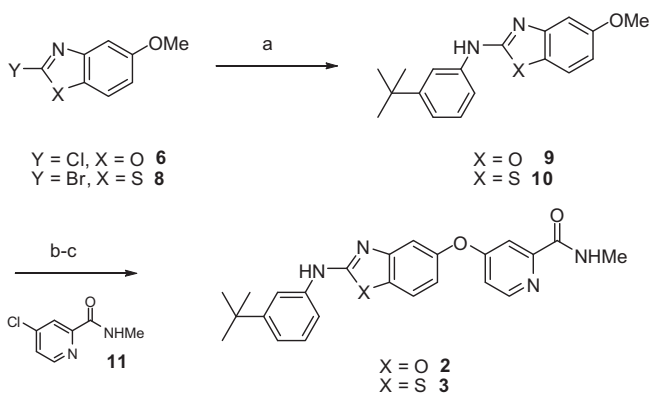


Scheme 1. Synthesis of benzoxazole intermediate **6** and benzothiazole intermediate **8**. Reagents and conditions: (a) H_2, 10% Pd/C, MeOH; (b) potassium ethylxanthate, KOH, EtOH, reflux, 18h; (c) $SOCl_2$, DMF, 5 min; (d) bromine, CH_2Cl_2, rt.



Scheme 2. Reagents and conditions: (a) 3-*t*-butylaniline, NMP, 200 °C, microwave, 7 min; (b) 48% HBr, 140 °C, microwave, 6 min; (c) KHMDS, K_2CO_3, DMF, 170 °C microwave 7 min.

further explore the structure activity relationship of additional heterocycle ring systems against Raf. Benzoxazoles were reported first by our group[20] and they were also developed by Potashman et al. as VEGFR inhibitors.[21] In addition to the in vitro Raf SAR, pharmacokinetic profiles for representative examples will also be presented in this communication.

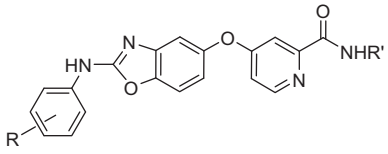
The synthetic[22] routes to key intermediates (**6** and **8**) for the benzoxazole and benzothiazole scaffolds are depicted in Scheme 1. The synthesis of **6** began with the commercially available nitrophenol **4** which was reduced by hydrogenation to give aminophenol (**5**). Compound **5** was treated with ethyl potassium xanthate to afford the sulfuryl benzoxazole, which was then chlorinated to afford **6**. Bromination of commercially available mercapto benzothiazole **7** yielded the desired intermediate **8** required for the synthesis of the benzothiazole core.

With the key intermediates for both scaffolds in hand, introduction of an aniline was effected by S_NAr conditions using microwave to afford **9** and **10** (Scheme 2). Demethylation with 48% HBr followed by O-arylation using KHMDS and 4-chloro-pyridylacetamide (**11**)[23] in DMF in the microwave gave the desired compounds **2** and **3.**[24]

An evaluation of the structure activity relationship of the aniline for the benzoxazole series was undertaken (Table 1). As observed in the benzimidazole series, the 3-tert-butyl group was important for in vitro potency and inhibition of phosphorylation of ERK.[19] It was also determined that compounds with ortho substituents (**12**–**14**) were less potent than compounds with substituents at the meta or para positions. Compounds substituted at the meta position (**2**, **15 and 16**) potently inhibited both c-Raf and mutant-B-Raf. In addition, two analogs (**2** and **16**) were tested in a target modulation assay and inhibited ERK phosphorylation in the 1.0 μM range which is three-fold less potent than the lead benzimidazole (**1**). Finally, the para position of the aniline tolerated a large range of substituents ranging from halogens to aliphatics to oxygen and nitrogen linked moieties (**17–24**). Only the appendage of a piperazine to the ring (**25**) led to degradation of in vitro potency.

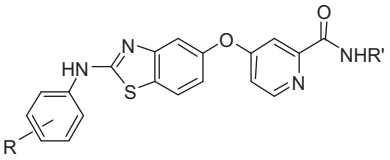
In the exploration of the aniline SAR for the benzothiazole series, we focused on the meta and para positions (Table 2, **3**, **28–33**), to obtain the best combination of in vitro and cellular potency, consistent with what we learned from the benzoxazole series. We found that the SAR tracked well between the two series. The

Table 1
Structure–activity relationship of the benzoxazole scaffold



Compd	R	R′	c-Raf IC_{50} (μM)	$Braf^{V600E}$ IC_{50} (μM)	p-ERK SKMEL28 EC_{50} (μM)25
1			—	0.12	0.28
2	3-*t*-Bu	Me	0.001	0.006	1.0
12	2-F	Me	0.20	0.58	—
13	2-Cl	Me	0.12	0.26	—
14	2-Et	Me	0.11	0.34	—
15	3-CF_3	Me	0.027	0.11	—
16	3-*i*-Pr	Me	0.003	—	1.0
17	4-Cl	Me	0.019	0.021	—
18	4-Br	Me	0.003	0.025	10
19	4-Et	Me	0.001	0.005	7.0
20	4-*i*-Pr	Me	0.001	0.006	—
21	4-*n*-Bu	Me	0.002	0.011	—
22	4-OCF_3	Me	0.002	0.012	—
23	4-OPh	Me	0.053	0.68	—
24	4-NMe_2	Me	0.012	0.13	—
25	4-N-Et-piperazine	Me	7.3	17	—
26	3-*i*-Pr	H	0.004	0.004	3.7
27	3-*i*-Pr	Morpholinoethyl	0.14	0.2	—

Table 2
Structure–activity relationship of the benzothiazole scaffold



Compd	R	R′	c-Raf IC_{50} (μM)	$Braf^{V600E}$ IC_{50} (μM)	p-ERK SKMEL28 EC_{50} (μM)
BAY43-9006					
1			–	0.12	0.28
3	3-*t*-Bu	Me	0.001	0.004	2.1
28	4-Cl	Me	0.004	0.015	–
29	4-Br	Me	0.002	0.007	>10
30	4-Me	Me	0.007	0.026	–
31	4-*n*-Bu	Me	0.009	0.065	–
32	4-*i*-Pr	Me	0.003	0.019	3.2
33	4-OCF_3	Me	0.004	0.018	–
34	3-*t*-Bu	H	0.001	0.002	2.9
35	4-Br	Morpholinoethyl	0.041	0.24	>10

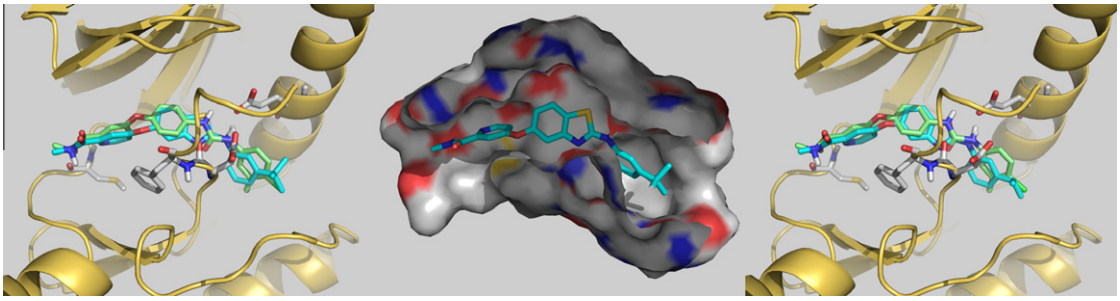


Figure 2. Binding site models for compounds **3** (left, in cyan) and **2** (right, in cyan) derived by docking into the crystal structure of B-Raf (PDB accession code 1UWH). The left and right pictures show cartoon representations of the kinase with selected residues in stick model (Glu501, Cys532, Phe593, Asp594) and the co-crystallized BAY43-9006 in green. The middle picture shows a surface representation, of the docking model for **3** with the surface colored by atom type (red = oxygen, blue = nitrogen, yellow = sulfur, grey = carbon/hydrogen).

effect of altering the amide substituent was investigated for both benzoxazole and benzothiazole series. The primary carboxamides (**26**, **34)** were found to be equipotent to the methyl amide analogs (**2**, **3**) and showed a similar effect in the cellular target modulation (3.7 μM for **26** and 2.9 μM for **34**). Incorporation of the solubilizing 2-morpholinoethyl group (**27**, **35**) gave a >30-fold loss in $Braf^{V600E}$ potency in both series, indicating that bulkier groups are not tolerated at this position.

While both series yielded extremely potent Raf inhibitors, neither series inhibited phosphorylation of ERK (SKMEL-28, Raf^{V600E}) as well as our original benzimidazole series. The discrepancy between biochemical and cellular potency does not appear to be due to permeability limitations. Compounds from both series were tested in the Caco2 assay and shown to have good permeability (P_{app} A and B in the range of 10–20 $\times$ 10^{-6} cm/s). A working hypothesis could be that in the biochemical assay, purified kinase domain of Raf is not representative of the full length protein in cells where Raf exists as a complex with chaperons, cytoskeleton, phosphatases and kinases.[25]

The kinase profiles of two representative compounds, **3** and **16**, were tested against 50 kinases including receptor tyrosine kinases (RTKS), serine/threonine kinases (STKs), tyrosine kinases (TKs) and AGC kinases. The compounds exhibited a fairly narrow kinase profile, inhibiting five RTKs (CSFR1, Flt3, KDR, cABL and Ret) out of 50 kinases with an IC_{50} <1 μM.

In order to understand the binding mode for these series, **2** and **3** were docked[26] in the active site of the public domain crystal structure published for B-Raf (PDB accession code 1UWH) Figure 2 shows the overlap of the docking models with the co-crystallized conformation of BAY43-9006. The model suggests a very similar binding mode when comparing **2** or **3** with BAY43-9006.[27] Specific interactions of the benzothiazole and benzoxazole compounds in the B-Raf model 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 benzothiazole or benzoxazole nitrogen, and 3) the sidechain COO– of Glu501 through the aniline NH.

These models use the 'DFG-out' conformation of the protein where the substituted anilines take the place of Phe593, which swings out and interacts with the aromatic systems in the hinge region and the selectivity pocket. The surface model in Figure 2 shows that the space available at the meta and para positions of the aniline is larger than the space around the ortho position which could be the reason the ortho-substituted analogues show a reduction in potency.

Addition of a 2-morpholinoethyl group on the pyridyl-amide side of the molecules (**27**, **35**) leads to a reduction in potency. Evaluation of these compounds in the docking model shows that there isn't enough space to accommodate the morpholinoethyl group in a low-energy conformation.

The pharmacokinetic properties for the two series were examined. Following a single 20 mg/kg oral administration to female mice in 15% captisol, benzothiazole **30** exhibited a clearance of 14.1 mL/min/kg, a low volume of distribution (1042 mL/kg), a short

half-life (80 min), and an oral bioavailability of 90%. On the other hand, benzoxazole **19** in the same formulation exhibited a low clearance (0.93 mL/min/kg), very low volume of distribution (82 mL/kg), a short half-life (32 min) and an oral bioavailability of 33%.

In conclusion, we developed two novel series of potent Raf inhibitors with acceptable pharmacokinetic properties. The cellular potency for these series was lower than for our previously disclosed benzimidazole series and therefore further work on the benzoxazoles and benzothiazoles was discontinued.

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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.2011.04.023.

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24. Synthetic methodology and characterization data for 2 and 3 are included in the Supplementary data.
25. Information on kinetic assays included in the Supplemental Material.
26. 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.
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