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Article

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Identification of 1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)urea Hydrochloride (CEP-32496), a Highly Potent and Orally Efficacious Inhibitor of V-RAF Murine Sarcoma Viral Oncogene Homologue B1 (BRAF) V600E

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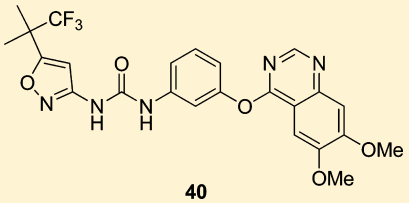
Supporting Information

ABSTRACT: The Ras/RAF/MEK/ERK mitogen-activated protein kinase (MAPK) signaling pathway plays a central role in the regulation of cell growth, differentiation, and survival. Expression of mutant $BRAF^{V600E}$ results in constitutive activation of the MAPK pathway, which can lead to uncontrolled cellular growth. Herein, we describe an SAR optimization campaign around a series of quinazoline derived $BRAF^{V600E}$ inhibitors. In particular, the bioisosteric replacement of a metabolically sensitive *tert*-butyl group with fluorinated alkyl moieties is described. This effort led directly to the identification of a clinical candidate, compound **40** (CEP-32496). Compound **40** exhibits high potency against several $BRAF^{V600E}$-dependent cell lines and selective cytotoxicity for tumor cell lines expressing mutant $BRAF^{V600E}$ versus those containing wild-type BRAF. Compound **40** also exhibits an excellent PK profile across multiple preclinical species. In addition, significant oral efficacy was observed in a 14-day $BRAF^{V600E}$-dependent human Colo-205 tumor xenograft mouse model, upon dosing at 30 and 100 mg/kg BID.



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INTRODUCTION

The Ras/RAF/MEK/ERK mitogen-activated protein kinase (MAPK) signaling pathway plays a central role in the transduction of signals from cell surface receptors to the nucleus, regulating both cell proliferation and survival.[1] The RAF members of this pathway consist of the serine/threonine kinases ARAF, BRAF, and CRAF (also known as RAF1), which phosphorylate and activate downstream MEK1/2. A number of activating mutations of the *BRAF* gene have been identified, with a single point mutation (Val600 → Glu) accounting for more than 90% of those described. Constitutively activated $BRAF^{V600E}$ is 500 times more active than wild-type BRAF and is found to be present in approximately 7% of all cancers, being most frequently associated with melanoma (50−70%), papillary thyroid (∼50%), colorectal (∼15%), and ovarian (∼30%) carcinomas.[2] The presence of $BRAF^{V600E}$ in certain tumors is correlated with increased malignancy and decreased response to chemotherapy.[3] It is therefore not surprising that $BRAF^{V600E}$ has emerged as a target of considerable interest, based on the rationale that inhibition of $BRAF^{V600E}$ should result in clinical benefit for patients with carcinomas expressing this enzyme.[4] As such, numerous reports describing small-molecule inhibitors of $BRAF^{V600E}$ have emerged,[5] with several investigational drugs, including RAF265 (Chiron/Novartis, **1**, Figure 1) and vemurafenib (PLX-4032, Daiichi Sankyo/Roche, **2**, Figure 1) having advanced into human clinical trials.[6−9]

Some time ago, we initiated our own efforts to identify novel inhibitors of $BRAF^{V600E}$. To identify appropriate chemical starting points, we employed the KINOME*scan* technology platform to screen the Ambit compound collection. The utilization of KINOME*scan* allows for the rapid screening of large compound collections across several hundred individual human kinase targets (including mutants of known therapeutic value).[10] This technology represents an extremely powerful tool for aiding in the identification of hit series for any given kinase target and

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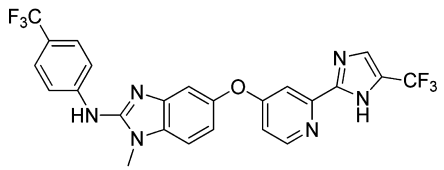
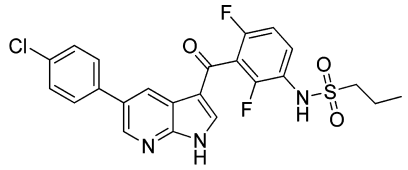


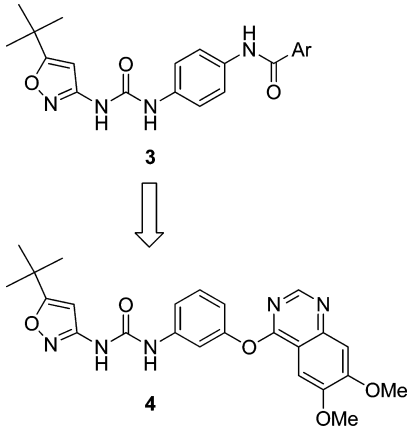
1; Raf-265



2; Vemurafenib

Figure 1. Structures of known RAF inhibitors.

for gaining a greater understanding of the kinome selectivity profile of such hits.[11] Analysis of the data set obtained from screening of the Ambit internal compound collection across the KINOME*scan* panel led to the identification of several hit series exhibiting high affinity for $BRAF^{V600E}$. One such series is represented by the diaryl amide derivative **3** (Figure 2), and an

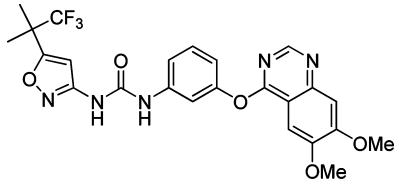


3

4

Figure 2. Evolution of the 4-quinazolinyloxy-diaryl urea series of b-Raf^{V600E} inhibitors.

initial hit to lead the optimization campaign described elsewhere led to the identification of 4-quinazolinyloxy-diaryl urea derivative **4**.[12] Herein we describe further optimization of the 4-quinazolinyloxy-diaryl urea series, in particular SAR around the left-hand aryl urea moiety. This effort ultimately led to the discovery of the clinical candidate compound **40** (CEP-32496),[13] a highly potent and orally efficacious inhibitor of $BRAF^{V600E}$.

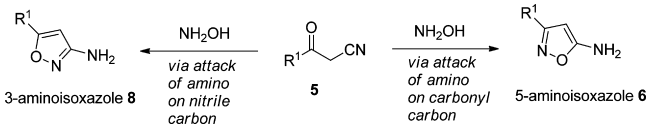


40 (CEP-32496)

CHEMISTRY

All compounds described herein were prepared as illustrated in Schemes 2−5. Final derivatives incorporating substituted isoxazole urea moieties were obtained *via* the corresponding intermediate isoxazole carbamates. These isoxazole carbamates were obtained from the corresponding aminoisoxazoles, which in turn were prepared from substituted ketonitriles **5** (Schemes 1 and 2). Reaction of substituted ketonitriles with hydroxylamine can result in the formation of regioisomeric 3- or 5-aminoisoxazoles (Scheme 1). Results summarized by Takase et al.[14]

Scheme 1. Formation of Aminoisoxazoles from Ketonitriles



3-aminoisoxazole **8** — **5** — 5-aminoisoxazole **6**

indicated a general preference for formation of 5-aminoisoxazoles **6** (or their corresponding hydrolysis products) resulting from initial attack of the hydroxylamine nitrogen on the keto group of the ketonitrile. Nevertheless, these workers found specific conditions (initial reaction at pH ∼ 8 at 60−100 °C, followed by ring closure under acidic conditions at 70−100 °C) under which, when R^1 = *tert*-butyl, attack of the hydroxylamine nitrogen on to the nitrile is favored, leading to formation of the 3-aminoisoxazole product. However, under these conditions the 5-amino product still prevailed for R^1 = Me, isopropyl, and Ph, suggesting a considerable steric contribution in the case of R^1 = *tert*-butyl. Consistent with the expectations from these previously reported results,[14] treatment of several ketonitriles (R^1 = cyclopentyl, **5b**; $C(CF_3)Me_2$, **5f**; $C(CH_2F)_2Me$, **5j**; and *tert*-butyl) with hydroxylamine in the presence of an extra equivalent of hydroxide resulted in predominant formation of 5-aminoisoxazoles (Scheme 2). In our hands, 5-aminoisoxazoles were also the predominant products when the starting pH was adjusted to ∼7.5 for R^1 = isopropyl (**5a**), $CFMe_2$ (**5c**), CF_2Me (**5d**), $C(CN)Me_2$ (**5g**), and cyclobutyl-1-CF_3 (**5i**). Some combination of lower steric demand, compared with *tert*-butyl, together with increased electrophilicity of the ketonitrile carbonyl when electron-withdrawing groups (F, CN) are present, presumably contributes to disfavoring formation of the 3-amino isomer under these conditions. This is despite the similarity to the initial conditions in which the 3-amino isomer was ultimately formed when R^1 = *tert*-butyl.[14] For a couple of the above R^1 groups ($C(CF_3)Me_2$, **5f**, and $C(CH_2F)_2Me$, **5j**), initial reaction in the presence of excess $NaHCO_3$ in aqueous MeOH followed by cyclization under acidic conditions resulted in good yields of the corresponding 3-aminoisoxazoles. Interestingly, these conditions also provided a good yield of the 3-amino regioisomer when R^1 = cyclopropyl-1-CF_3. Comparing this result to those described above for R^1 = cyclobutyl-1-CF_3, the latter bicarbonate conditions seem to be particularly suitable for favoring initial events (presumably reaction of the hydroxylamine nitrogen with the nitrile group of the ketonitrile) that lead ultimately to the 3-aminoisoxazole regiochemistry. 3-Aminoisoxazole derivatives **8h** (R^1 = isopropyl), **8i** (R^1 = cyclopentyl), and **8j** (R^1 = $CFMe_2$), inaccessible *via* the aforementioned procedures, were prepared *via* alternate routes involving protection of the ketonitrile carbonyl moiety, as described in Scheme 3. Protection of the carbonyl of **5a** and **5b** *via* conversion to the 1,3-dioxolane moiety gave intermediates **10a** and **10b**, respectively. Subsequent reaction of **10a** and **10b** with hydroxylamine yielded exclusively the required amidoxime

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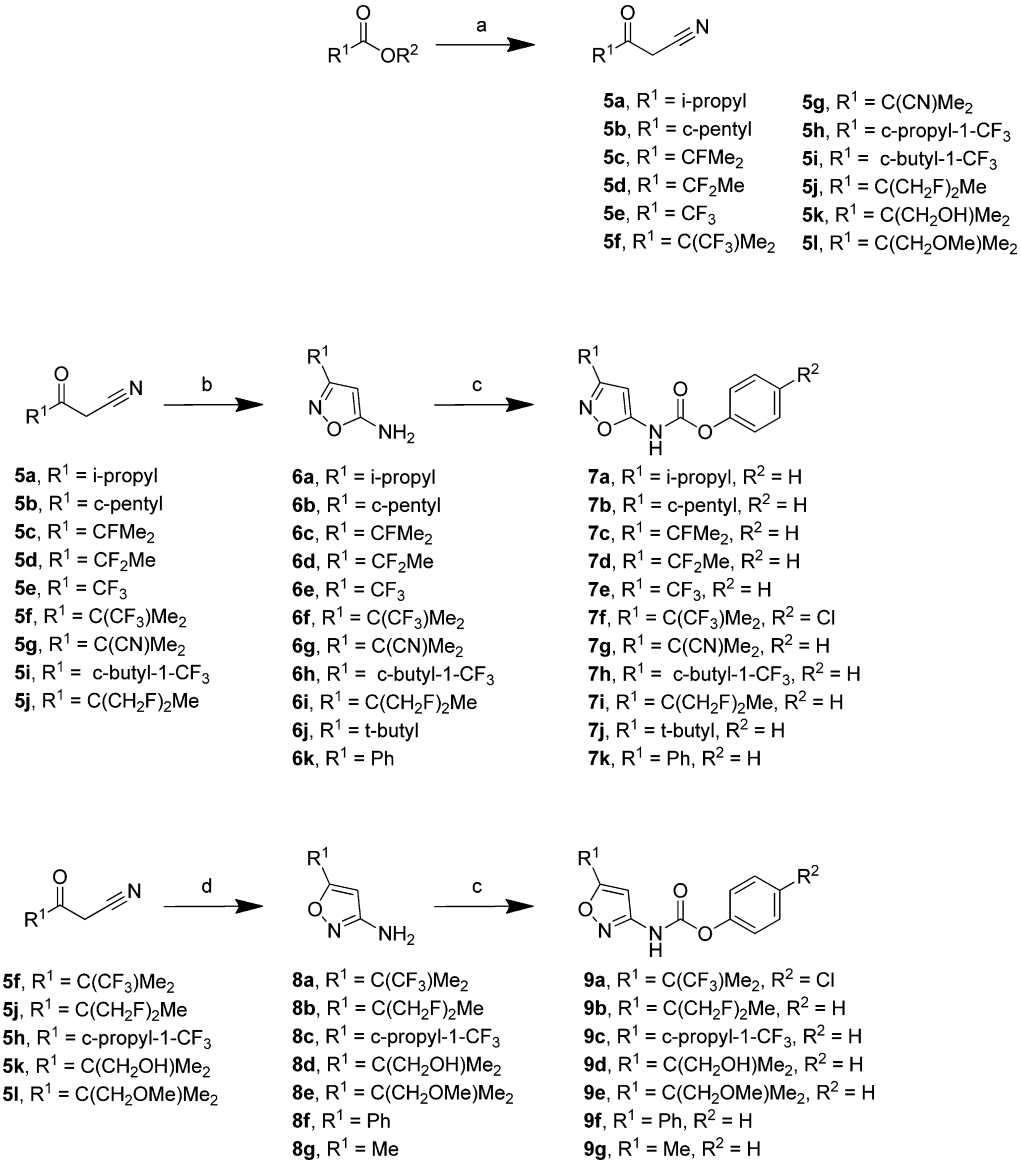
Scheme 2. Synthesis of Isoxazol-5-yl-carbamates 7a–k and Isoxazol-3-yl-carbamates 9a–g[a]



[a]Reagents and conditions: (a) NaH, MeCN, THF, 75 °C, 15 h, 29–93%; (b) hydroxylamine hydrochloride, NaOH, water, 100 °C, 2.5 h, or hydroxylamine sulfate, NaOH, water/EtOH, 80 °C, 15 h; or (for **6e**) (i) $TMSCHN_2$, Et_2O, rt, 15 h, (ii) hydroxylamine hydrochloride, NaOMe, MeOH, 60 °C, 15 h, (iii) HCl, aq EtOH, 60 °C, 2 h; 11–100%; (c) PhOC(O)Cl or 4-Cl-PhOC(O)Cl, K_2CO_3, THF, rt, 33–100%; (d) (i) hydroxylamine sulfate, $NaHCO_3$, water/MeOH, 65 °C, or hydroxylamine hydrochloride, NaOH, water/EtOH, 65 °C, (ii) conc HCl, reflux, 26–71%.

intermediates that upon treatment with concentrated HCl underwent carbonyl deprotection followed by cyclization to give the required 3-aminoisoxazole derivatives **8h** and **8i**, respectively. All attempts to convert ketonitrile **5c** to the corresponding 1,3-dioxolane derivative failed. As a result, **5c** was converted to the corresponding methyl enol ether **11** *via* treatment with (trimethylsilyl)diazomethane. Subsequent reaction of **11** with hydroxylamine followed by heating in the presence of HCl gave the desired 3-aminoisoxazole derivative **8j**, albeit in low yield (13%). The reason for the lower than expected yield was not clearly apparent; however, enough **8j** was prepared to be utilized in the next step. In a similar manner, an attempt was made to gain access to 5-(trifluoromethyl)-isoxazole-3-amine. Thus, ketonitrile **5e** was converted first to 4,4,4-trifluoro-3-methoxybut-2-enenitrile *via* treatment with (trimethylsilyl)diazomethane. However, subsequent treatment with hydroxylamine followed by heating in the presence of HCl failed to yield any of the desired 5-(trifluoromethyl)isoxazole-3-amine. Rather, for reasons that are unclear, only the corresponding 3-(trifluoromethyl)isoxazole-5-amine (**6e**) was obtained albeit in low yield (11%). All 5-aminoisoxazoles (**6**) and 3-aminoisoxazoles (**8**) were subsequently converted to the corresponding isoxazole carbamates **7** and **9**, respectively, *via* reaction with either phenyl chloroformate or 4-chlorophenyl chloroformate. The pyrazole carbamates required for the preparation of the pyrazole ring containing urea derivatives were prepared as described in Scheme 4. The reaction of substituted ketonitriles with a variety of hydrazines in refluxing EtOH gave 5-aminopyrazoles **12** in moderate to good yields. Subsequent treatment of 5-aminopyrazoles **12** with phenyl chloroformate

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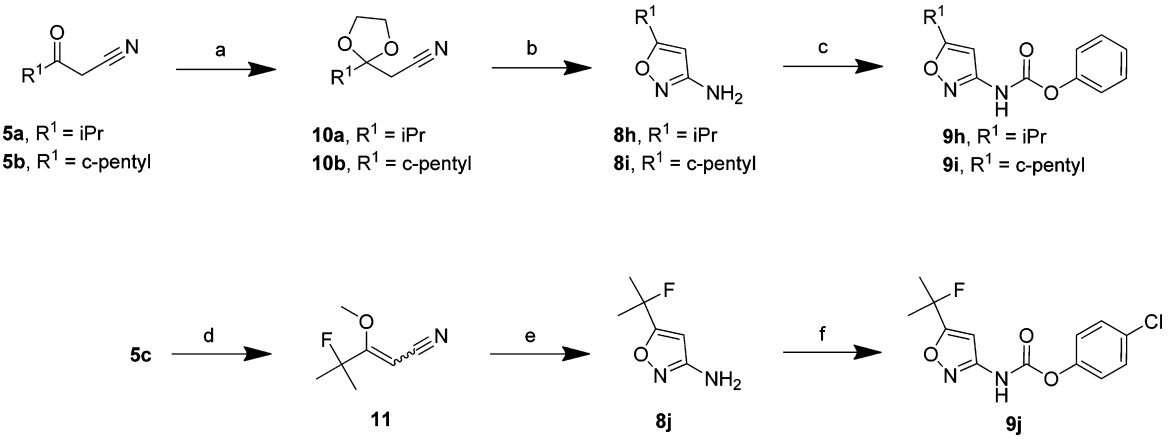
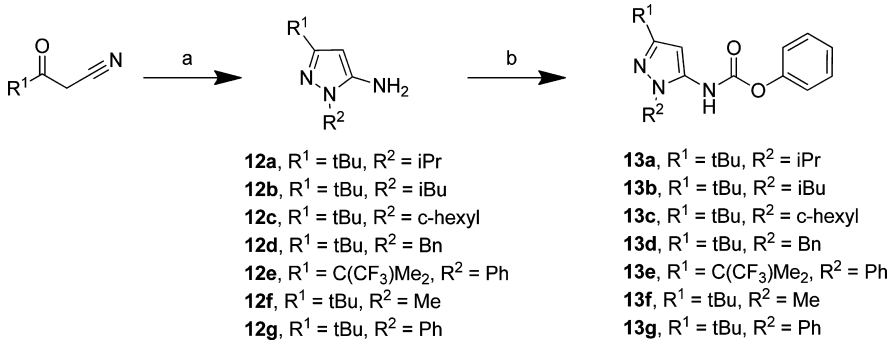
Scheme 3. Synthesis of Isoxazol-3-yl-carbamates 9h–j[a]



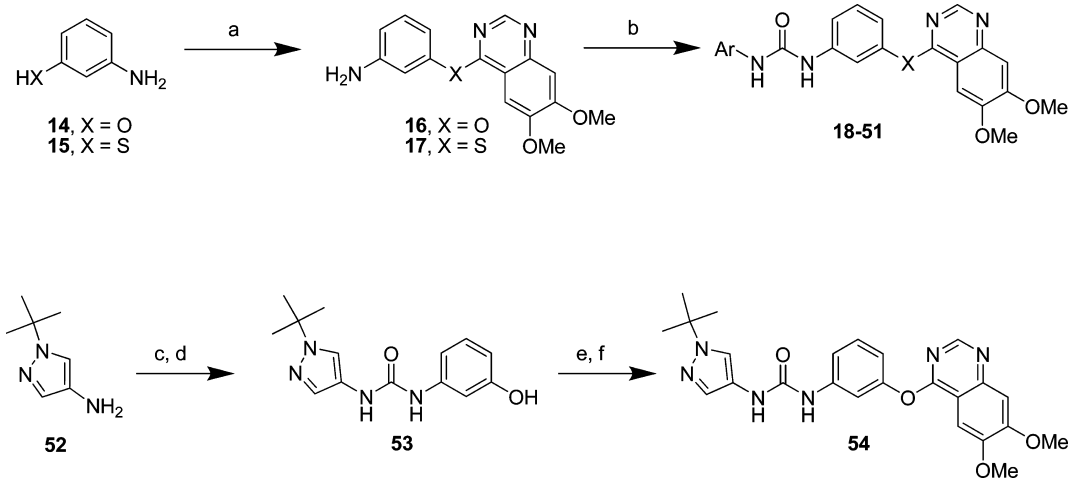
[a]Reagents and conditions: (a) ethylene glycol, TMSCl, DCM, reflux, 15 h, 57–78%; (b) (i) hydroxylamine hydrochloride, NH_3/MeOH, 8-hydroxyquinoline, 70 °C, 15 h, (ii) conc HCl, EtOH, reflux, 68–69% (over two steps); (c) PhOC(O)Cl, K_2CO_3, THF, rt, 77–89%; (d) $TMSCHN_2$, Et_2O, rt, 15 h, 90%; (e) (i) hydroxylamine hydrochloride, NaOMe, MeOH, 70 °C, 16 h, (ii) conc HCl, 80 °C, 30 min, 13%; (f) 4-Cl-PhOC(O)Cl, K_2CO_3, THF, rt, 100%.

Scheme 4. Synthesis of 1*H*-Pyrazol-5-yl-carbamates 13a–g[a]



[a]Reagents and conditions: (a) R^2NHNH_2, EtOH, reflux, 36–51%; (b) PhOC(O)Cl, K_2CO_3, DCM or THF, 0 °C or rt, 30–100%.

Scheme 5. Synthesis of Quinazoline Derivatives 18–51 and 54[a]



[a]Reagents and conditions: (a) X = O, (i) Cs_2CO_3, THF, rt, 30 min, (ii) 4-chloro-6,7-dimethoxyquinazoline, 50 °C, 24 h, 100%; X = S, (i) NaH, THF, rt, 30 min, (ii) 4-chloro-6,7-dimethoxyquinazoline, rt, 15 h, 85%; (b) ArNCO, DMF, 50 °C; or **7a–k** or **9a–j** or **13a–g**, DIEA, cat. DMAP, THF, rt or 50 °C, 5–75%; (c) PhOC(O)Cl, K_2CO_3, THF, rt, 15 h, 89%; (d) 3-aminophenol, THF, 120 °C, 2 h, 20%; (e) (i) Cs_2CO_3, THF, rt, 1 h, (ii) 4-chloro-6,7-dimethoxyquinazoline, 55 °C, 15 h, 43%.

and potassium carbonate in THF or CH_2Cl_2 gave the required pyrazole carbamates **13**. The desired urea compounds **18–51** and **54** were prepared as described in Scheme 5. 3-Aminophenol (**14**) was reacted with cesium carbonate in THF to generate the

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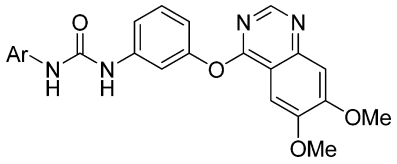
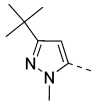
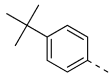
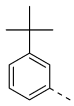
Table 1. SAR of Quinazoline-Derived BRAF^{V600E} Inhibitors: Variation of the Aryl Urea Moiety



Compound	Ar	BRAF^{V600E} K_d (nM)[a,b]	A375 pMEK IC_{50} (nM)[a,c]	A375 Proliferation IC_{50} (nM)[a,d]	$S(10)$ Score[e]
4		73	121	328	0.286
18		51	101	183	0.293
54		75	1131	>15000	0.162
19		87	336	4267	0.297
20		96	290	3492	0.172
21		36	333	1771	0.179

[a]Results are presented as mean values from experiments performed in duplicate. Variability between individual values is less than 2-fold. [b]Competition binding assay performed at rt for 1 h. K_d values were determined using eleven serial 3-fold dilutions. [c]Phosphorylation assay in A375 cells (expressing BRAF^{V600E}). Cells were incubated with test compound for 2 h. [d]Cell Titer Blue proliferation assay in A375 cells (expressing BRAF^{V600E}). Cells were incubated with test compound for 72 h. [e]Compounds were screened at 10 μM concentration against the KINOME*scan* panel consisting of 290 individual wild-type kinases (mutants being excluded from this analysis). Selectivity $S(10)$ scores represent the fraction of kinase targets exhibiting >90% competition (measured as <10% of control experiment).

corresponding cesium phenoxide, which was coupled *in situ* with 4-chloro-6,7-dimethoxyquinazoline to give **16** in quantitative yield. Similarly, 3-aminobenzenethiol (**15**) was treated first with sodium hydride in THF, followed by 4-chloro-6,7-dimethoxyquinazoline to give **17** in high yield. Direct coupling of **16** with 4-*tert*-butylphenyl isocyanate gave the 4-oxyquinazoline derivative **20**, whereas 4-(dimethylamino)pyridine-catalyzed coupling of **16** with phenyl 3-*tert*-butylphenylcarbamate gave compound **21**. 4-(Dimethylamino)pyridine-catalyzed coupling of **16** with isoxazole carbamates **7a**−**k** and **9a**−**j** gave the corresponding 4-oxyquinazoline derivatives **18** and **22**−**41**. Overall, yields for the final coupling step proved reasonable; however, for the case of the trifluoromethyl substituted derivative **31**, the recovery was low (5%) for reasons that are unclear. The 4-(dimethylamino)pyridine-catalyzed coupling of **17** with isoxazole carbamates **7c** and **9a**−**c** gave the corresponding 4-thioquinazoline derivatives **42**−**45**. In an analogous fashion, coupling of **16** with pyrazole carbamates **13a**−**g** gave the corresponding pyrazole containing 4-oxyquinazoline derivatives **46**−**51**. Pyrazole containing 4-oxyquinazoline derivative **54** was prepared in three steps from 1-*tert*-butyl-1*H*-pyrazol-4-amine **52**.

RESULTS AND DISCUSSION

As part of our SAR campaign, all compounds described (Tables 1 and 2) were profiled *in vitro* for activity against BRAF^{V600E}, both in a biochemical binding assay and for their ability to inhibit BRAF^{V600E}-mediated phosphorylation of MEK (pMEK formation) in the A375 cell line. In addition, each compound was further profiled for its ability to inhibit the proliferation of A375 cells over a 72 h period. As part of this profiling process, particular emphasis was placed on the potencies observed in the A375 cellular assays. The A375 cell line is dependent on signaling through BRAF^{V600E}, and the observation of activity in these assays, coupled with suitable ADME properties, would be considered a reasonable predictive measure of activity in appropriate *in vivo* models of BRAF^{V600E}-driven disease. We were also interested in obtaining a preliminary understanding of the

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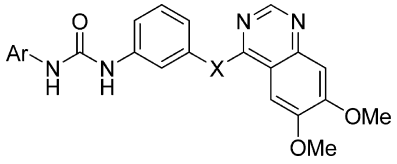
Table 2. SAR of Quinazoline-Derived BRAF^{V600E} Inhibitors 22−51



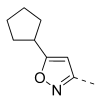
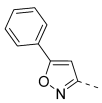
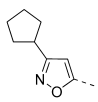
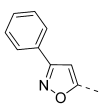
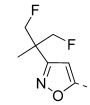
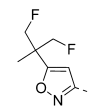
Compound	Ar	X	BRAF^{V600E} K_d (nM)[a,b]	A375 pMEK IC_{50} (nM)[a,c]	A375 Proliferation IC_{50} (nM)[a,d]	*S*(10) Score[e]
22		O	678	>15000	>15000	0.062
23		O	19	355	527	0.214
24		O	37	134	488	0.228
25		O	136	956	>15000	0.052
26		O	31	340	2956	0.186
27		O	122	1134	4375	0.190
28		O	1454	>15000	7069	0.052
29		O	49	649	>15000	0.172
30		O	151	324	1554	0.169
31		O	78	2229	5154	0.131
32		O	76	645	694	0.203
33		O	44	155	125	0.228
34		O	132	339	1985	0.424
35		O	66	174	549	0.310
36		O	129	308	2243	0.286
37		O	48	332	6163	0.217
38		O	29	276	493	0.241
39		O	45	109	145	0.286
40		O	14	82	78	0.245
41		O	27	183	117	0.217

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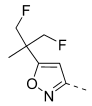
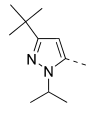
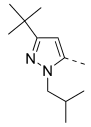
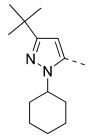
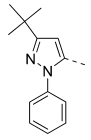
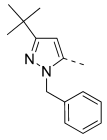
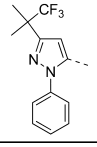
Table 2. continued

Compound	Ar	X	BRAF^{V600E} K_d (nM)[a,b]	A375 pMEK IC_{50} (nM)[a,c]	A375 Proliferation IC_{50} (nM)[a,d]	$S(10)$ Score[e]
42		S	50	93	223	0.248
43		S	74	142	>15000	0.210
44		S	31	469	222	0.183
45		S	39	84	1471	0.228
46		O	85	505	543	0.252
47		O	101	681	1163	0.214
48		O	174	>1667	>15000	0.162
49		O	295	21	ND[f]	0.400
50		O	175	480	1869	0.159
51		O	271	62	ND[f]	0.376

[a]Results are presented as mean values from experiments performed in duplicate. Variability between individual values is less than 2-fold. [b]Competition binding assay performed at rt for 1 h. K_d values were determined using eleven serial 3-fold dilutions. [c]Phosphorylation assay in A375 cells (expressing BRAF^{V600E}). Cells were incubated with test compound for 2 h. [d]Cell Titer Blue proliferation assay in A375 cells (expressing BRAF^{V600E}). Cells were incubated with test compound for 72 h. [e]Compounds were screened at 10 μM concentration against the KINOME*scan* panel consisting of 290 individual wild-type kinases (mutants being excluded from this analysis). Selectivity $S(10)$ scores represent the fraction of kinase targets exhibiting >90% competition (measured as <10% of control experiment). [f]Not determined due to biphasic dose–response curves. Complete inhibition was observed at 5000 nM.

selectivity profile across the human kinome. This was facilitated by use of the KINOME*scan* technology platform.[10] Compounds prepared were screened at 10 μM concentration across a diverse panel of 290 individual wild-type kinases. Selectivity for each compound is represented by a selectivity $S(10)$ score, which equals the fraction of kinase targets assayed exhibiting >90% competition (measured as <10% of control experiment), with lower $S(10)$ scores indicating greater selectivity.[10a]

Structure–Activity Relationships. The previously identified *tert*-butyl-isoxazole urea-containing quinazoline derivative **4** (Table 1) exhibited high binding affinity for BRAF^{V600E} (K_d = 73 nM), and potent inhibition of both BRAF^{V600E}-mediated pMEK activity (IC_{50} = 121 nM) and proliferation (IC_{50} = 328 nM) in A375 cells.[12] Compound **4** also proved moderately selective across the panel of 290 kinases, exhibiting an $S(10)$ score of 0.286. An initial scan of alternate aryl urea moieties (Table 1) indicated that the isoxazole regioisomer of **4**, the 3-(*tert*-butyl)isoxazole-5-urea derivative **18**, was equipotent in both binding and cellular assays and had a very similar $S(10)$ score. Substitution of the isoxazole moiety for an *N*-1-(*tert*-butyl)-pyrazole-4-urea motif gave compound **54**, which also exhibited good binding affinity for BRAF^{V600E} and a somewhat improved kinome selectivity profile. However, compound **54** proved to be essentially inactive in the A375 cell proliferation assay. Compared to **54**, the related *N*-1-methyl-3-(*tert*-butyl)pyrazole-5-urea derivative **19** proved more potent in both the A375 pMEK and proliferation assays; however, potency in the proliferation assay was still 10-fold lower than that observed with the lead compound **4**. Incorporation of either a 4-(*tert*-butyl)phenyl urea (**20**) or a 3-(*tert*-butyl)phenyl urea moiety (**21**) led to a modest improvement in kinome selectivity. However, although potent in both the BRAF^{V600E} binding and A375 pMEK cellular assays, these compounds again proved 5- to 10-fold less active than lead compound **4** in the A375 proliferation assay. Due to the promising cellular activity observed with the isomeric (*tert*-butyl)isoxazole-containing derivatives **4** and **18**, we decided to investigate the effect of varying the *tert*-butyl moiety to other functional groups. A range of different substituents were incorporated as summarized in Table 2 (compounds **22**–**41**). Overall, it was observed that potency for BRAF^{V600E} was governed by both the size and lipophilicity of this substituent. For example, substitution of the *tert*-butyl (**4**) for the smaller methyl group (**22**) led to a loss in both binding affinity (K_d = 678 nM) and cellular potency (IC_{50} > 15000 nM in both assays). Compound **22** also exhibited reduced potencies across the broader panel of kinase targets, as indicated by an $S(10)$ score of 0.062. Incorporation of the larger phenyl moiety, exemplified by compounds **25** and **28**, also led to much reduced BRAF^{V600E} binding affinity and cellular potencies compared to the corresponding *tert*-butyl containing analogues **4** and **18**, respectively. Again both compounds **25** and **28** exhibited much reduced affinity across the broader panel of

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kinases, with the $S(10)$ score being 0.052 for both. Overall, it was found that incorporation of substituents of similar size and lipophilicity to the *tert*-butyl group gave analogues with a more optimal potency profile. For instance, compared to **4**, the 5-isopropylisoxazol-3-yl derivative (**23**) and the 5-cyclopentylisoxazol-3-yl derivative (**24**) exhibited very similar potencies toward $BRAF^{V600E}$ in both binding and cellular assays. However, incorporation of the more polar hydroxyl-bearing *tert*-butyl group (**29**), although potent in the $BRAF^{V600E}$ binding assay (K_d = 49 nM), surprisingly led to much reduced cellular potencies. Other more polar substituents such as alkylamine-bearing *tert*-butyl groups were also incorporated; however, incorporation of such groups resulted in significantly lower affinity for $BRAF^{V600E}$ (data not shown). Also worth noting is the observation that the initial (*tert*-butyl)isoxazolyl isomers **4** and **18** exhibited a very similar potency profile, but such comparable activity of isoxazole regioisomers did not prove to be universal. Activity differences between corresponding isoxazole isomeric pairs depended upon the exact nature of the isoxazole substituent. For instance, compared with the corresponding 5-substituted isoxazol-3-yl isomers, the 3-isopropylisoxazol-5-yl (**26**) and the 3-cyclopentylisoxazol-5-yl derivatives (**27**) both exhibited reduced potencies in cells, particularly with respect to the A375 proliferation assay.

As described, the (*tert*-butyl)isoxazole-containing derivatives **4** and **18** exhibited good potency against $BRAF^{V600E}$. However, *in vitro* metabolite profiling studies on compound **4** indicated that, in both rat and human liver microsomes, hydroxylation of the *tert*-butyl moiety and subsequent oxidation to the carboxylate occurred to a significant degree (data not shown).[15] As we had already demonstrated that the corresponding hydroxylated *tert*-butyl-containing derivative (**29**) exhibits much reduced cellular activity compared to the nonhydroxylated analogue **4**, we were interested in finding replacements for the *tert*-butyl group that would both be well tolerated by $BRAF^{V600E}$ yet be less prone to such oxidative metabolic processes. It was clear from earlier SAR that the incorporation of polar groups was not an attractive option. However, we expected that the selective incorporation of fluorine would lead to more stable derivatives with similar potency profiles. A carbon−fluorine bond is often used as a bioisosteric replacement for a corresponding carbon−hydrogen bond, due to its similar size and lipophilicity. More importantly, due to the high electronegativity of fluorine, carbon−fluorine bonds are much stronger and exhibit a differential dipole-moment compared to the corresponding carbon−hydrogen bond. As such, the strategic introduction of a carbon−fluorine bond into a bioactive molecule can often have positive effects on overall metabolic stability, while maintaining the desired potency profile at the target of interest.[16] With this in mind, we prepared a number of fluoroalkyl substituted isoxazole derivatives (compounds **31**−**36** and **38**−**45**). Direct replacement of the *tert*-butyl of **18** with the likely more metabolically stable trifluoromethyl group (**31**) resulted in loss of cellular potency, even though binding affinity remained comparable to that observed with **18**. Replacement of one (**32**) or two (**33**) fluoro groups (of **31**) with methyl, thereby increasing both the size and lipophilicity of this motif, resulted in the restoration of cellular activity, with the 2-fluoropropyl-containing derivative (**33**) proving equipotent to **18**. The 2-(trifluoromethyl)propyl derivative (**35**) also proved potent in both binding and cellular assays. However, incorporation of the slightly larger (trifluoromethyl)cyclobutyl group (**36**) led to a reduction in activity in the A375 proliferation assay, again suggesting that the size of the isoxazole substituent is important for optimal activity. On the basis of earlier observations, wherein differences in activity were observed between corresponding isoxazole isomeric pairs, we also investigated replacement of the *tert*-butyl group of isoxazol-3-yl derivative **4** with select fluorine-containing alkyl motifs. Again, key differences between isoxazole regioisomers were observed. For instance, compared to **33**, the corresponding 5-(2-fluoropropan-2-yl)isoxazol-3-yl derivative **38** proved approximately 2- and 4-fold less potent in the A375 pMEK and proliferation assays, respectively. In contrast, both isoxazole-3-yl derivatives **39** and **40** proved significantly more potent in cells than the corresponding isoxazol-5-yl isomers **34** and **35**, respectively. In fact, the 5-(1,1,1-trifluoro-2-methylpropan-2-yl)-isoxazol-3-yl derivative **40** proved one of the most potent compounds in this series, exhibiting potent binding ($BRAF^{V600E}$ K_d = 14 nM) and cellular activity (pMEK IC_{50} = 82 nM and A375 proliferation IC_{50} = 78 nM), with activity in the proliferation assay being approximately 4-fold greater than that observed with the *tert*-butyl analogue **4**.

The analogues described thus far contain an oxygen atom linking the quinazoline ring with the central phenyl moiety. We had previously observed that replacement of the oxygen linker of **4** with the slightly larger and more lipophilic sulfur atom not only yielded a derivative that proved equipotent in both the binding and pMEK cellular assays but also exhibited an improved $S(10)$ score (0.18 vs 0.29).[12] However, for some of the more potent derivatives described herein, replacement of oxygen with sulfur did not result in greatly improved selectivity profiles, and in certain cases, decreased activity was observed in the A375 proliferation assay (compare **43** with **40**, and **45** with **33**). The complete loss of proliferation activity for **43** could not be readily explained. However, a similar drop-off in proliferation activity was observed for certain other closely related sulfur-linked analogues (data not shown).

In addition to the described isoxazole urea-containing derivatives, we had earlier identified a related 3-*tert*-butyl-1*H*-pyrazol-5-yl urea derivative **19** that displayed modest potency toward $BRAF^{V600E}$ (K_d = 87 nM, pMEK IC_{50} = 336 nM). A number of additional pyrazole-containing analogues were prepared, represented by compounds **46**−**51**. Overall, SAR around the pyrazole *N*-1 substituent indicated that incorporation of larger alkyl moieties such as isopropyl (**46**), isobutyl (**47**), or cyclohexyl (**48**), although increasingly more selective as represented by decreasing $S(10)$ scores, proved less potent in both the pMEK and proliferation cell assays. Only upon incorporation of a phenyl group (**49**) did we observe robust pMEK cell activity (IC_{50} = 21 nM), although less than optimal activity was observed in the A375 proliferation assay. In addition, **49** exhibited decreased selectivity ($S(10)$ = 0.40). Although significant effort was applied toward the optimization of the pyrazole series, a suitable balance between potency, selectivity, and PK profile ultimately proved elusive.

The rat PK profile for selected analogues is illustrated in Table 3. Male Sprague−Dawley rats were dosed with compound either *via* intravenous (iv) or oral (po) administration (n = 2 and 3 animals for the iv and po arms, respectively), and PK parameters were calculated, including clearance (CL), volume of distribution (V_d), terminal half-life ($t_{1/2}$), AUC (iv and po), and absolute oral bioavailability (%F). As predicted, replacement of the isoxazole *tert*-butyl substituent with an appropriate fluoro-containing alkyl motif led to improved metabolic stability, as reflected by decreased *in vivo* clearance rates. This trend was further supported by rat liver microsome stability data. For

Table 3. Rat Pharmacokinetic[a] and Rat Liver Microsomal Stability Data[b] for Select Compounds

compd	CL [(mL/min)/kg][c]	V_d (L/kg)[c]	$t_{1/2}$ (h)	$AUC_{0-\infty}$(po) (μM·h)[d]	C_{max}(po) (μM)[d]	t_{max}(po) (h)[d]	F (%)[d]	RLM % remaining (at 60 min)
4	(7.3, 17.6)	(0.5, 2.5)	19.2	17.0 ± 5.8	11.0 ± 2.0	0.8 ± 0.3	49 ± 17	46
18	(9.8, 11.9)	(1.0, 1.5)	8.2	1.9 ± 1.4	0.7 ± 0.5	0.8 ± 0.3	6 ± 4	51
33	(4.0, 4.1)	(0.6, 0.6)	4.7	42.0 ± 19.2	7.9 ± 3.5	2.0 ± 0	48 ± 22	82
35[e]	ND[f]	ND[f]	ND[f]	40.7 ± 12.2	4.7 ± 4.0	1.7 ± 0.6	ND[f]	ND[f]
38	(2.9, 3.1)	(0.5, 0.5)	3.5	127.0 ± 23.3	21.1 ± 4.0	2.0 ± 0	100 ± 20	92
40[g]	(3.4, 5.0)	(1.6, 2.0)	7.8	74.4 ± 9.7	6.5 ± 1.5	2.0 ± 0	94 ± 12	81
49	(3.1, 5.2)	(0.3, 0.3)	4.2	25.9 ± 5.4	6.7 ± 4.6	1.3 ± 0.6	33 ± 7	ND[f]

[a]Dosed to Sprague–Dawley rats. The parameters were calculated using plasma concentration–time data for individual animals, n = 2 animals (iv arm) and 3 animals (po arm). Dosed iv at 1 mg/kg as a solution in 3:1 PEG400/water. Dosed po at 10 mg/kg as a solution in Pharmatek #6 (Hot Rod Chemistry vehicle series; Pharmatek Incorporated, San Diego, CA). [b]Results are presented as mean values from experiments performed in duplicate. Compound concentration used was 1 μM. Compounds were incubated at 37 °C. [c]Data reported for both individual animals (n = 2). [d]Mean values and standard deviation reported (n = 3). [e]Dosed po (10 mg/kg) only. [f]ND = not determined. [g]Dosed as a free base.

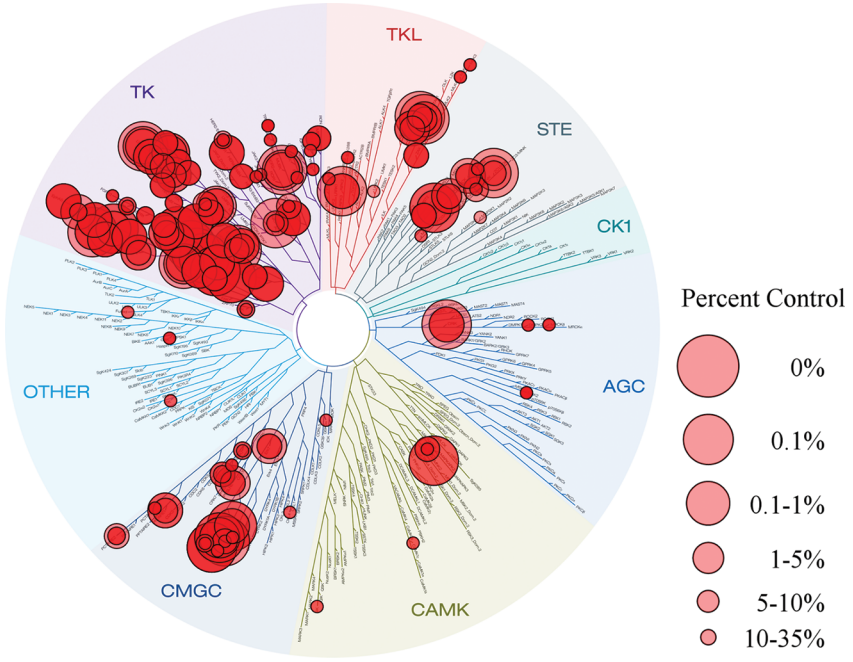

Figure 3. KINOME*scan* tree spot graphic illustrating the selectivity profile for compound **40** versus a panel of 290 wild-type kinase targets (plotted as percent of control at 10 μM concentration, where 0 and 35% of control equals 100 and 65% competition, respectively). Images courtesy of KINOME*scan* (http://www.kinomescan.com) and used with permission from KINOME*scan*, a division of DiscoveRx Corporation.

example, in a rat liver microsome stability assay, the fluorinated derivatives **33**, **38**, and **40** exhibit approximately 2-fold improvement in metabolic stability when compared with **4**, as reflected by measured percent remaining of parent at 60 min (Table 3). A second observation from the data in Table 3 is that while their clearance rates and volumes of distribution remain equal, the isoxazol-3-yl-containing urea isomers typically exhibited significantly improved absorption profiles compared to the corresponding isoxazol-5-yl-containing urea isomers. For instance, the 5-*tert*-butyl-isoxazol-3-yl isomer **4** exhibited significantly improved oral exposure ($AUC_{0-\alpha}$ = 17 μM·h; %F = 49) compared to the 3-*tert*-butyl-isoxazol-5-yl isomer **18** ($AUC_{0-\alpha}$ = 1.9 μM·h; %F = 6). A similar trend was observed between the 2-fluoropropyl-isoxazole isomeric pairs **33** and **38** and the 2-(trifluoromethyl)propyl-isoxazole isomeric pairs **35** and **40**, with the isoxazol-3-yl isomer exhibiting the greatest exposure after oral dosing in each case.

Selectivity and Further Pharmacokinetic Profiling of 40. Due to the combination of high potency for $BRAF^{V600E}$ in both binding and cellular assays coupled with excellent oral exposure in rat, **40** was chosen for further profiling. Compound **40** proved modestly selective when screened at 10 μM concentration across a KINOME*scan* panel of 290 diverse wild-type kinases, as indicated by the selectivity $S(10)$ score of 0.245 (Figure 3). Corresponding K_d determinations versus select kinase targets are presented in Table 4. In addition to $BRAF^{V600E}$, **40** exhibits high binding affinity for both wild-type BRAF and related CRAF, as well as certain receptor tyrosine kinases of known therapeutic utility, such as Abl-1, c-Kit, Ret, PDGFR-β, and VEGFR-2. However, **40** proved selective for the RAF members of the MAPK signal transduction pathway, as no significant affinity was observed for other key kinases of the MAPK pathway, including MEK-1, MEK-2, ERK-1, and ERK-2. This suggests that the observed cellular activity was driven primarily through inhibition of $BRAF^{V600E}$, which is further supported by the observation that **40** exhibited selective cytotoxicity for tumor cell lines expressing mutant BRAF versus those expressing wild-type BRAF (Table 5). When screened across a panel of tumor cell lines expressing $BRAF^{V600E}$ (A375, SK-MEL-28, Colo-205,

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Table 4. Select Kinase K_d Determinations for Compound 40

kinase	K_d (nM)[a]	kinase	K_d (nM)[a]
$BRAF^{V600E}$	14	JAK-1	>10000
wt BRAF	36	JAK-2	4700
CRAF	39	JAK-3	>10000
Abl-1	3	c-Kit	2
ALK	>10000	LCK	2
Aurora A	>10000	MEK-1	7100
Aurora B	>10000	MEK-2	8300
CSF-1R	9	c-Met	513
EGFR	22	PDGFR-β	2
EPHA2	14	PIK3-CA	>10000
ERK-1	>10000	PIK3-CB	>10000
ERK-2	>10000	PLK-1	>10000
IGFR-1	>10000	Ret	2
INSR	>10000	VEGFR-2	8

[a]Competition binding assays performed at rt for 1 h. K_d values were determined using eleven serial 3-fold dilutions. Results are presented as mean values from experiments performed in duplicate. Variability between individual values is less than 2-fold.

Table 5. Cell Titer Blue Viability of Compound 40 across Human Tumor Cell Lines Expressing $BRAF^{V600E}$ or Wild-Type BRAF[a]

cell line ($BRAF^{V600E}$)	EC_{50} (nM)	cell line (wt BRAF)	EC_{50} (nM)
A375 (homozygous)	78	HCT116	669
Colo-205 (heterozygous)	36	Hs578T	2736
Colo-679 (homozygous)	211	LNCaP	6631
SK-MEL-28 (homozygous)	454	DU145	2911
HT-144 (homozygous)	228	PC-3	6257

[a]Results are presented as mean values from experiments performed in duplicate. Cells were cultured in 0.5% serum overnight prior to incubation with test compound for 72 h. This was followed by addition of Cell Titer Blue (Promega, Madison, WI) to detect viable cells after 3 h incubation with reagent.

Colo-679, and HT-144) versus a panel expressing only wild-type BRAF (HCT116, Hs578T, LNCaP, DU145, and PC-3), those cell lines harboring mutant $BRAF^{V600E}$ proved significantly more sensitive to treatment with **40**. The only wild-type cell line in which **40** had appreciable activity was the HCT116 cell line, a colonic epithelial line with a known RAS mutation and wild-type p53, a combination previously shown to confer sensitivity to inhibition of the MAPK pathway.[17] Compound **40** also exhibited good stability in mouse, dog, monkey, and human liver microsomal preparations (with measured intrinsic clearance values of <23 (μL/min)/mg and $t_{1/2}$ > 60 min in all assays; data not shown), thus predicting low phase I hepatic clearance in these species. Metabolite profiling of **40** in rat, dog, monkey, and human liver microsomes indicates that both mono O-demethylation (of the quinazoline methoxy groups) and oxidation of the (1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl moiety occur to some extent (data not shown). However, as indicated above, the measured *in vitro* clearance rates of such oxidative processes remain low across all species tested. In line with these observations, **40** exhibits an exceptional PK profile in mouse, dog, and cynomolgus monkey, as illustrated in Table 6. For example, administration of **40** to beagle dogs (single dose of 1 mg/kg iv and 10 mg/kg po) resulted in low clearance (CL = 5.0 (mL/min)/kg) and excellent bioavailability (%F = 100). Similarly, in cynomolgus monkey, the administration of **40** (single dose of 1 mg/kg iv and 10 mg/kg po) led to high oral exposure due to low clearance (CL = 6.7 mL/min/kg) and excellent bioavailability (%F = 100). Compound **40** also exhibits a favorable CYP450 inhibition profile, with measured IC_{50} values greater than 10 μM versus the CYP1A2, 2C9, 2D6, and 3A4 isoforms and an IC_{50} = 3.4 μM versus 2C19 (data not shown). In addition, as a preliminary measure of potential cardiotoxicity, **40** was assessed for activity against the hERG potassium ion channel in a patch-clamp assay.[18] Compound **40** does not significantly inhibit the hERG channel, exhibiting <10% inhibition at 10 μM concentration (data not shown).

In Vivo Efficacy and Pharmacodynamic Profiling of 40. Compound **40** was further evaluated for antitumor efficacy and tolerability in several $BRAF^{V600E}$-driven human carcinoma xenograft mouse models, including both Colo-205 and A375.[19] Data from the Colo-205 xenograft study is presented here (Figure 4). Athymic nude mice bearing established Colo-205 tumor xenografts were dosed orally with **40** at 10, 30, and 100 mg/kg twice daily (BID) over a 14-day period. All doses were well tolerated, with no mortality or significant body weight loss (<5% relative to vehicle matched controls) observed during treatment or up to 1 week post dosing. Dosing at 10 mg/kg BID had little effect on tumor growth compared to the control group. However, increasing the dose to either 30 or 100 mg/kg BID resulted in significant efficacy. The 30 mg/kg dose group exhibited tumor stasis and a 40% incidence of partial tumor regressions (PRs) (p < 0.008), whereas the 100 mg/kg BID dose group exhibited both tumor stasis and an 80% incidence of PRs (p < 0.0001). The observed efficacy is correlated with an *in vivo* pharmacodynamic−pharmacokinetic end point as illustrated in Figure 5. Oral administration of **40** to Colo-205 tumor xenograft-bearing mice resulted in significant inhibition of pMEK in tumor cell lysates. For instance, a single 30 mg/kg (po) dose of **40** led to a 50 and 75% inhibition of normalized pMEK in tumor lysates at the 2 and 6 h postdose time point, respectively (p < 0.03), while a 55 mg/kg (po) dose resulted in a 75% to 57% (p < 0.03) inhibition of pMEK at 2 through 10 h post administration, with normalization to baseline by

Table 6. Pharmacokinetics Profile of Compound 40 in Mouse,[a] Dog,[b] and Monkey[c] Following Intravenous (iv) or Oral Dosing (po)[d]

species	CL (mL/min/kg)	V_d (L/kg)	$t_{1/2}$ (h)	$AUC_{0-\infty}$(po) (μM·h)	C_{max}(po) (μM)	t_{max}(po) (h)	F (%)
mouse	ND[e]	ND[e]	ND[e]	394 ± 131	35.6 ± 6.7	1.3 ± 0.6	ND[e]
dog	5.0 ± 1.0	4.5 ± 0.5	12 ± 2.8	55.1 ± 6.4[f]	5.2 ± 0.6	2.0 ± 1.0	100 ± 14
monkey	6.7 ± 3.3	2.5 ± 0.6	4.9 ± 0.9	92.2 ± 15.4	4.3 ± 0.5	4.0 ± 2.3	100 ± 43

[a]Athymic nude mice (n = 3 animals), dosed at 100 mg/kg po only in Pharmatek #6 (Hot Rod Chemistry vehicle series; Pharmatek Incorporated, San Diego, CA). [b]Beagle dogs (n = 3 animals), dosed iv at 1 mg/kg as a solution in 22% HPβCD, and po at 10 mg/kg as a solution in 22% HPβCD. [c]Male Cynomolgus monkeys (n = 3 animals), dosed iv at 1 mg/kg as a solution in 22% HPβCD, and po at 10 mg/kg as a solution in 22% HPβCD. [d]The parameters were calculated using plasma concentration−time data for individual animals, and mean values are reported with standard deviations. [e]ND = not determined. [f]Reported as AUC_{0-24h}.

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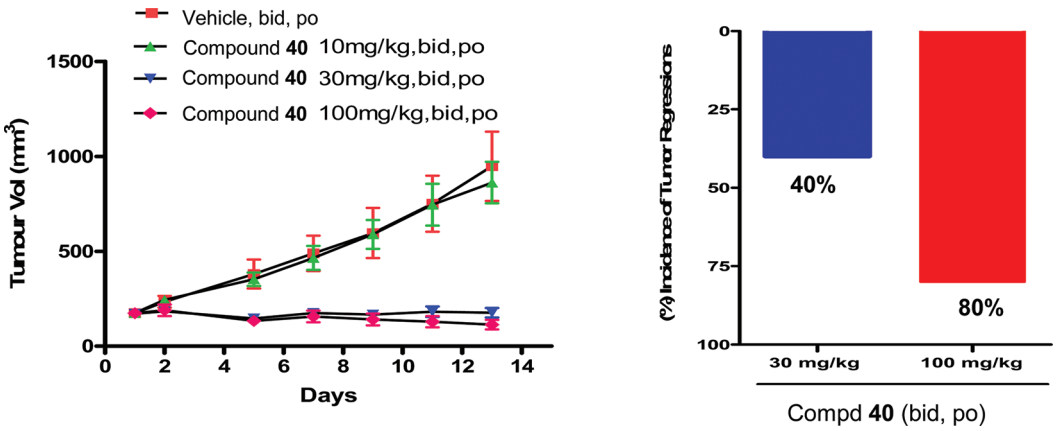

Figure 4. Efficacy results upon oral dosing of compound **40** at 10, 30, and 100 mg/kg BID (dosed in 22% HPβCD) for 14 days to athymic nu/nu nude mice bearing established Colo-205 human colon tumor subcutaneous xenografts with the $BRAF^{V600E}$ genotype (10 animals per treatment group). A dose response relationship (left panel) for complete Colo-205 tumor growth inhibition and incidence of tumor regressions (right panel) was observed. Mice were monitored for signs of morbidity (behavior and body weight loss), and tumors were measured 3 times per week. Statistical significance ($p < 0.05$) for antitumor efficacy, based upon tumor growth inhibition relative to vehicle-treated controls, for both the 30 and 100 mg/kg groups was reached by day 5 of administration and extended to the termination of the study, with a final significance for tumor growth inhibition of $p < 0.008$ (30 mg/kg group) and $p < 0.0001$ (100 mg/kg group).

24 h (Figure 5a). In general, the magnitude and duration of normalized pMEK inhibition by **40** was consistent with plasma and tumor levels over the dose range and time course of the pharmacodynamic study (Figure 5b).

Molecular Modeling Studies of Compound 40 with $BRAF^{V600E}$. In order to gain a better understanding of how the described quinazoline-derived inhibitors might bind to $BRAF^{V600E}$, we carried out docking experiments into the ligand binding site of the previously reported cocrystal structures of sorafenib (Figure 6d) bound to a DFG-out form of $BRAF^{V600E}$ (PDB ID: 1uwj) and a DFG-out form of wt-BRAF (PDB ID: 1uwh).[20] Select compounds, including **22**, **30**, and **40** docked to the DFG-out mode in a consistent manner, and all docking trials scored compound **40** the best. The predicted binding mode of these compounds to DFG-out $BRAF^{V600E}$ is illustrated with compound **40** (Figure 6a). In this binding mode the quinazoline N-1 nitrogen participates in a hydrogen bonding interaction with the NH of Cys532 of the hinge domain. In addition, the quinazoline core is in close proximity to three aromatic residues, namely Trp531, Phe583, and Phe595 (of the DFG motif), which could participate in appropriate $\pi-\pi$ or $\pi-\sigma$ aromatic binding interactions with the quinazoline ring system. In a similar manner, Phe595 could also interact with the central phenyl ring of **40**. The urea moiety of **40** participates in two key hydrogen bonding interactions with the protein: one between the urea oxygen and the NH of Asp594 (of the DFG motif); the other between one or both of the urea NH's and the carboxylate of Glu501 (of the α-C Helix). Finally, the 5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol moiety sits in a hydrophobic pocket formed between the α-C and α-E helices and the N-terminal regions of the DFG motif and the catalytic loop, with the 1,1,1-trifluoro-2-methylpropan-2-yl group in close proximity to the residues Val504, Leu505, Thr508, Ile513, and Leu567. The model predicts that along with the expected hydrophobic interactions with the residues described, the CF_3 group can interact electrostatically with Thr508 via a hydrogen bonding interaction between a fluorine atom and the OH of Thr508 (F–H distance ~2.97 Å). This interaction with Thr508 may contribute to the enhanced binding affinity observed with **40** relative to **4**. In an attempt to rationalize certain SAR observations, other compounds were docked into the $BRAF^{V600E}$ protein, including **22**, which contains an isoxazole bearing the much smaller methyl group at the 5-position of the isoxazol-3-yl ring. Compound **22** scored lower in these docking experiments, presumably due to less efficient hydrophobic interactions within the hydrophobic pocket (data not shown), which may explain the observed reduction in the binding affinity of **22** to $BRAF^{V600E}$. Compounds such as **30**, which also exhibits reduced binding affinity compared to **40**, has a methoxy group in place of the CF_3. Compound **30** docked with this methoxy group pointing away from Thr508 and is therefore is unable to interact with this residue (Figure 6c). Figure 6b allows comparison between the binding mode of **40** and sorafenib. As one might expect, overall, these ligands occupy similar space within the binding site, with similar positioning of the urea moieties and interaction of both quinazoline N-1 of **40** and the pyridyl nitrogen of sorafenib with the same residue (the NH of Cys532) located on the hinge of the protein.

CONCLUSION

An optimization campaign around the initial lead 5-*tert*-butyl-isoxazol-3-yl urea-containing quinazoline **4** was described. Targeted replacement of the metabolically sensitive *tert*-butyl moiety with a variety of alkyl fluoride motifs led to the identification of 5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl urea-containing quinazoline derivative **40**. Compared to **4**, compound **40** exhibited improved cellular potency against the $BRAF^{V600E}$-dependent A375 cell line and much improved oral exposure in rat by virtue of reduced *in vivo* clearance. Compound **40** also exhibited excellent oral exposure in other preclinical species. In addition, **40** proved selective for the RAF kinase members of the MAPK signaling cascade and exhibited selective cytotoxicity for tumor cell lines expressing mutant $BRAF^{V600E}$ versus those containing wild-type BRAF. Compound **40** also demonstrated significant oral efficacy in a 14-day $BRAF^{V600E}$-dependent human Colo-205 tumor xenograft mouse model, when dosed 30 and 100 mg/kg BID. These observations, coupled with excellent druglike properties and a favorable tolerability profile, led to the selection of **40** as a clinical candidate, which represents a new opportunity for the treatment of mutant $BRAF^{V600E}$-driven human carcinomas.

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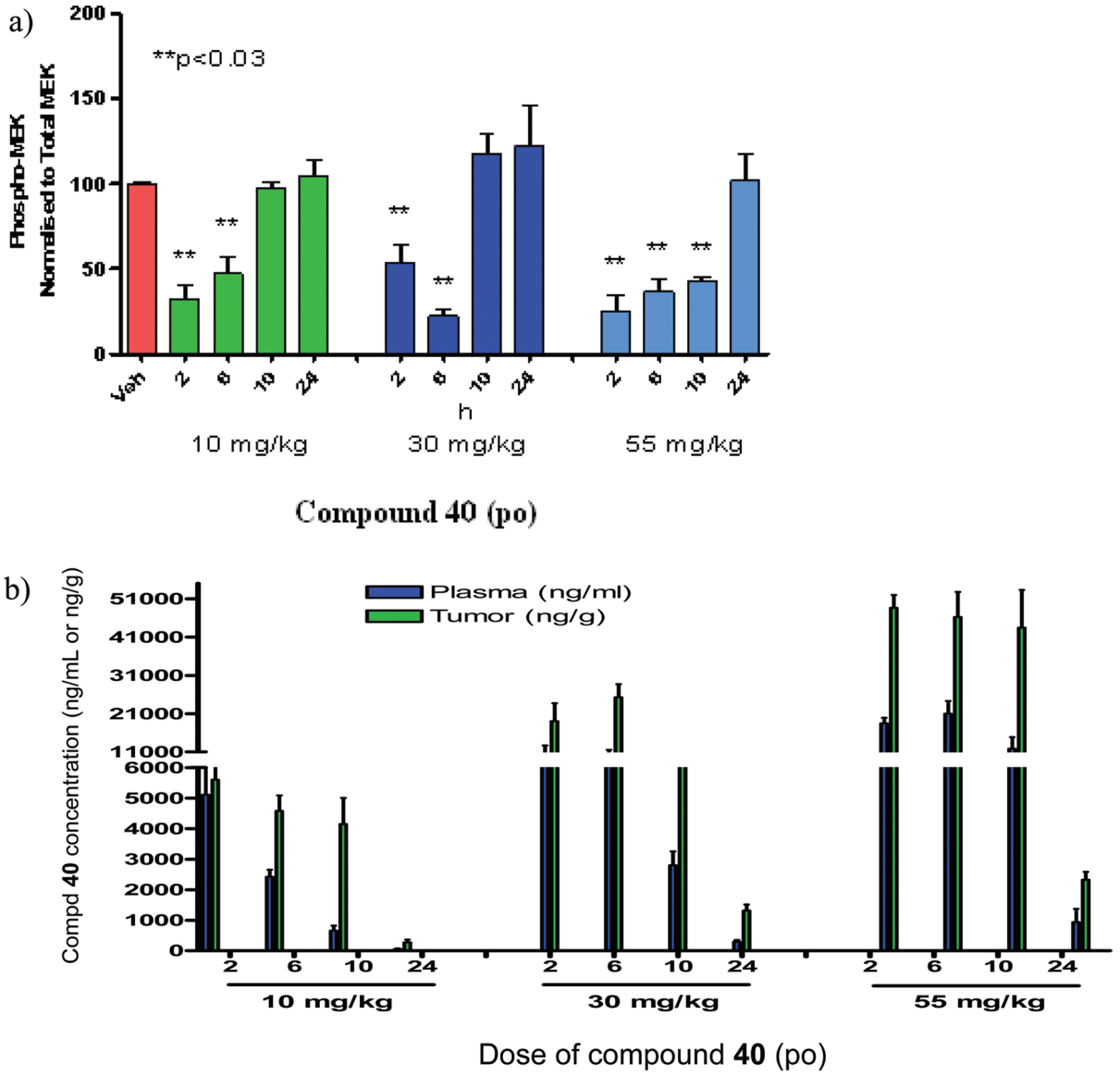

Figure 5. (a) In vivo inhibition of normalized MEK phosphorylation in Colo-205 tumor xenograft-bearing mice over a 24 h period following a single oral dose of either vehicle (22% HPβCD) or compound **40** (10, 30, and 55 mg/kg, dosed in 22% HPβCD), and (b) corresponding compound levels in both plasma and tumor samples. At appropriate time points over a 24 h period, animals were sacrificed and plasma and tumor samples were collected and lysates prepared. Amounts of total MEK and pMEK in tumor lysates were measured by immunoblotting and ELISA methods. Compound levels in plasma and tumor tissue samples were analyzed and quantitated by LC-MS/MS. Values shown are mean + SEM from two studies; n = 3 tumor-bearing mice per time point per study.

EXPERIMENTAL SECTION

Chemistry. *General Methods.* All commercially available solvents and reagents were used as received. Where appropriate, reactions were carried out under an argon atmosphere. Flash column chromatography was conducted under medium pressure either on silica (Merck silica gel 40−63 μM) or on prepacked silica gel cartridges (Biotage), and eluents were monitored by UV light (λ = 254 nm). ^{1}H NMR spectra were recorded using a Bruker Avance 300 MHz NMR spectrometer unless stated otherwise. Chemical shifts (δ) are reported as parts per million (ppm) relative to tetramethylsilane (internal standard), and coupling constants (J) are reported in Hz. The following abbreviations are used for multiplicities: s = singlet; br s = broad singlet; d = doublet; t = triplet; q = quartet; m = multiplet. Low resolution mass spectra (MS) were obtained as electrospray ionization (ESI) mass spectra, which were recorded on a Shimadzu HPLC/MS instrument using reverse-phase conditions (acetonitrile/water, containing 0.05% acetic acid). Preparative reverse-phase HPLC was performed using Varian HPLC systems with either a Varian Pursuit XRs 10 μm Diphenyl 250 mm × 50.0 mm column (eluent flow rate 95 mL/min) or a Phenomenex Luna 10 μm C18(2) 100 Å 250 × 21.20 mm column (eluent flow rate 30 mL/min), eluting with acetonitrile/water, containing 0.05% acetic acid. The purity of all final compounds was determined to be ≥95% by reverse-phase LC-MS, which was performed on a Shimadzu LC-MS 2010 EV (ESI probe) using a Phenomenex Luna 5 μm C18(2) 100 Å 250 mm × 4.60 mm column, eluting with a gradient of 10−95% acetonitrile in water (containing 0.05% acetic acid) over 30 min (at rt), with a flow rate of 1 mL/min and visualized using a wavelength of 254 nm.

1-(5-tert-Butylisoxazol-3-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea Hydrochloride (4). A stirred mixture of 3-aminophenol (4.36 g, 40 mmol) and 5-*tert*-butyl-3-isocyanatoisoxazole (6.64 g, 40 mmol) in degassed THF (300 mL) was heated at 50 °C for 15 h. After cooling to rt, the reaction was concentrated under reduced pressure and the resulting foam purified by silica gel flash chromatography (eluting with 25% to 75% EtOAc in hexanes) to afford

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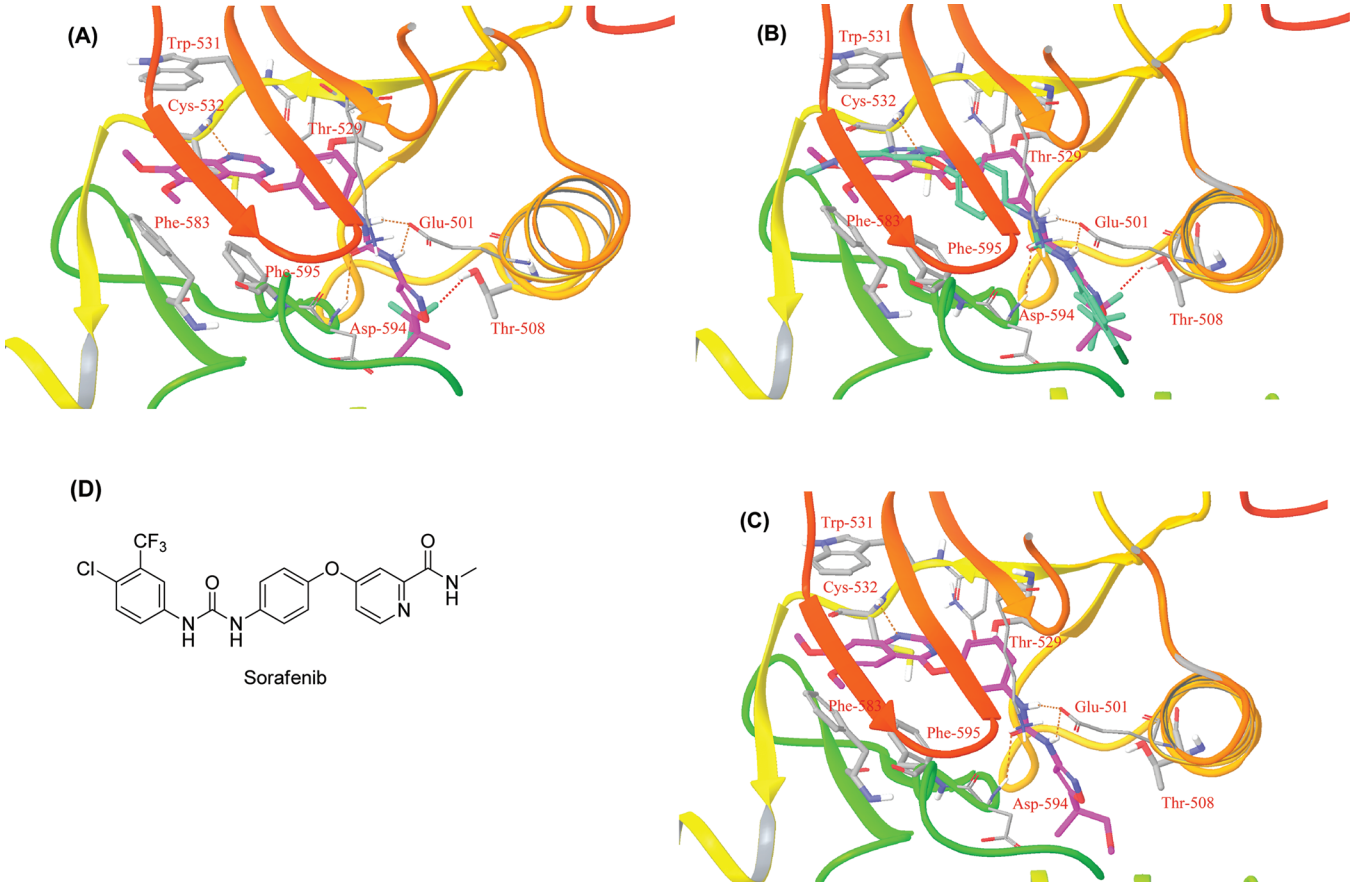

Figure 6. (a) Binding mode of the isoxazol-3-yl urea compounds, based on docking experiments with DFG-out $BRAF^{V600E}$, illustrated with compound **40** (carbon-magenta). The urea forms two hydrogen bonding interactions: one between the urea-O and the NH of Asp594 (DFG motif); the other between the urea NH's and the carboxylate of Glu501 (α-C helix). A hydrogen bond (electrostatic interaction) between one of the fluorines of the CF_3 and the Thr508 OH may contribute a key binding interaction. (b) Comparison of the binding mode of sorafenib (carbon-light green) from the X-ray structure and compound **40** (carbon-magenta) from the docking studies. (c) Subtle changes in the binding mode of compound **30** (carbon-magenta). (d) Chemical structure of sorafenib.

1-(5-*tert*-butylisoxazol-3-yl)-3-(3-hydroxyphenyl)urea as a solid (8.81 g, 80%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.39 (s, 1H), 9.37 (s, 1H), 8.69 (s, 1H), 7.01−7.06 (m, 2H), 6.78 (d, J = 6.0 Hz, 1H), 6.49 (s, 1H), 6.41 (d, J = 6.0 Hz, 1H), 1.29 (s, 9H). LC-MS (ESI) m/z 275 $[M + H]^+$. To a slurry of potassium *tert*-butoxide (6.73 g, 60 mmol) in THF (300 mL) was added 1-(5-*tert*-butylisoxazol-3-yl)-3-(3-hydroxyphenyl)-urea (8.25 g, 30 mmol), and the solution was stirred at rt for 1 h, at which point 4-chloro-6,7-dimethoxyquinazoline (6.74 g, 30 mmol) was added, followed by potassium carbonate (4.1 g, 30 mmol). After stirring at rt for 72 h, the reaction was concentrated under reduced pressure and the resulting solid was partitioned between EtOAc and water. The organic layer was separated, dried over $MgSO_4$, filtered, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 15% to 100% EtOAc in hexanes) to give 1-(5-*tert*-butylisoxazol-3-yl)-3-(3-(2-chloro-6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea as a white solid. The solid was dissolved in EtOAc (50 mL), and 4 M HCl in 1,4-dioxane (5 mL, 20 mmol) was added and stirred at rt for 30 min. The mixture was concentrated under reduced pressure to afford the title compound as a solid (6.29 g, 42%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.72 (s, 1H), 9.44 (s, 1H), 8.73 (s, 1H), 7.60−7.65 (m, 2H), 7.38−7.45 (m, 2H), 7.29 (d, J = 9.0 Hz, 1H), 6.98 (d, J = 9.0 Hz, 1H), 6.48 (s, 1H), 4.02 (s, 3H), 4.00 (s, 3H), 1.28 (s, 9H). LC-MS (ESI) m/z 464 $[M + H]^+$.

*Preparation of Substituted 3-Oxopropanenitriles. General Procedure A. 4-Methyl-3-oxopentanenitrile (**5a**).* A stirred suspension of sodium hydride (1.95 g, 60% dispersion in mineral oil, 48.75 mmol) in THF (25 mL) was heated to 75 °C. To this was added a mixture of methyl isobutyrate (3.19 g, 31.25 mmol) and acetonitrile (2.56 mL, 48.75 mmol) dropwise over the course of 45 min. The resulting pale yellow suspension was heated at 70 °C for a further 15 h. After cooling to rt, the reaction mixture was poured into water (150 mL) and the resulting solution was extracted with diethyl ether (2 × 100 mL). The aqueous layer was separated, acidified to pH 2 with aqueous 2 M HCl, and extracted with diethyl ether (2 × 100 mL). The combined ether layers were dried over $MgSO_4$ and then concentrated under reduced pressure to afford the title compound as a yellow oil, which did not require further purification (2.71 g, 78%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.53 (s, 2H), 2.81 (septet, J = 6 Hz, 1H), 1.21 (d, J = 6 Hz, 6H).

*3-Cyclopentyl-3-oxopropanenitrile (**5b**).* Prepared from methyl cyclopentanecarboxylate (4.0 g, 31.25 mmol) according to General Procedure A to afford the title compound as a yellow oil (3.97 g, 93%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.5 (s, 2H), 3.02−3.13 (m, 1H), 1.62−1.95 (m, 8H).

*4-Fluoro-4-methyl-3-oxopentanenitrile (**5c**).* Prepared from ethyl 2-fluoroisobutyrate (10 g, 74.62 mmol) according to General Procedure A to afford the title compound as a yellow oil (8 g, 83%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.82 (s, 2H), 1.54 (d, J = 21 Hz, 6H).

*4,4-Difluoro-3-oxopentanenitrile (**5d**).* Prepared from ethyl 2,2-difluoropropionate (3 g, 22 mmol) according to General Procedure A. The crude product was purified by silica gel flash chromatography (eluting with 10%−40% EtOAc in hexanes) to afford the title compound as a yellow oil (1 g, 31%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.95 (s, 2H), 1.86 (t, J = 19 Hz, 3H).

*4,4,4-Trifluoro-3-oxobutanenitrile (**5e**).* Prepared from methyl 2,2,2-trifluoroacetate (10.24 g, 80 mmol) according to General Procedure

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A to afford the title compound as an oil (9.08 g, 83%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.93 (s, 2H).

*5,5,5-Trifluoro-4,4-dimethyl-3-oxopentanenitrile (**5f**).* The reaction was carried out in two separate batches, employing 5 g (32 mmol) of 3,3,3-trifluoro-2,2-dimethylpropionic acid in each batch. To a stirred solution of 3,3,3-trifluoro-2,2-dimethylpropionic acid (5 g, 32 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C was added dropwise a solution of (trimethylsilyl)diazomethane (18 mL of a 2 M solution in diethyl ether, 35 mmol) (gas evolution observed). The resulting yellow solution was allowed to warm to rt and stirred for a further 48 h. Additional (trimethylsilyl)diazomethane (5 mL of a 2 M solution in diethyl ether, 10 mmol) was added, and stirring was continued for a further 5 h, whereupon a further amount of (trimethylsilyl)diazomethane (6 mL of a 2 M solution in diethyl ether, 12 mmol) was added. After stirring for a further 15 h, the reaction mixture was concentrated under reduced pressure (keeping bath temperature below 30 °C). The resulting oil was redissolved in diethyl ether (200 mL), washed with saturated aqueous $NaHCO_3$ solution (100 mL), separated, and dried over $MgSO_4$. Filtration followed by concentration under reduced pressure (keeping bath temperature below 30 °C) afforded methyl 3,3,3-trifluoro-2,2-dimethylpropanoate as an oil. Product from both batches was combined to afford methyl 3,3,3-trifluoro-2,2-dimethylpropanoate as a yellow oil (7.69 g, 71%), which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.86 (s, 3H), 1.40 (s, 6H). Methyl 3,3,3-trifluoro-2,2-dimethylpropanoate (7.69 g, 45 mmol) was treated according to General Procedure A to afford the title compound as a yellow oil (4.27 g, 37%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.77 (s, 2H), 1.43 (s, 6H).

*2,2-Dimethyl-3-oxopentanedinitrile (**5g**).* Prepared from ethyl 2-cyano-2-methylpropanoate (3.0 g, 21.25 mmol) according to General Procedure A to afford the title compound as a yellow oil (1.40 g, 48%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.98 (s, 2H), 1.60 (s, 6H).

*3-Oxo-3-(1-(trifluoromethyl)cyclopropyl)propanenitrile (**5h**).* Prepared from methyl 1-(trifluoromethyl)cyclopropane carboxylate (2.0 g, 11.9 mmol) according to General Procedure A. The crude product was purified by silica gel flash chromatography (eluting with 5%–40% EtOAc in hexanes) to afford the title compound as an oil (1.03 g, 49%). LC-MS (ESI) m/z 178 $[M + H]^+$.

*3-Oxo-3-(1-(trifluoromethyl)cyclobutyl)propanenitrile (**5i**).* To a stirred solution of 1-trifluoromethylcyclobutane-1-carboxylic acid (10 g, 59.5 mmol) in MeOH (50 mL) was added concentrated sulfuric acid (3 mL), and the resulting mixture was heated at 75 °C for 15 h. After cooling to rt, the solvent was removed under reduced pressure and the residue was partitioned between EtOAc and water. The organic phase was separated and the aqueous solution was further extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and then concentrated under reduced pressure (water bath at 20 °C) to afford methyl 1-(trifluoromethyl)cyclobutanecarboxylate as an oil, which did not require further purification (4.5 g, 42%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.76 (s, 3H), 2.50–2.60 (m, 2H), 2.35–2.45 (m, 2H), 1.91–2.04 (m, 2H). Methyl 1-(trifluoromethyl)cyclobutanecarboxylate (2 g, 11 mmol) was treated according to General Procedure A to afford the title compound as a yellow oil (1.68 g, 80%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.76 (s, 2H), 2.39–2.65 (m, 4H), 1.95–2.15 (m, 2H).

*5-Fluoro-4-(fluoromethyl)-4-methyl-3-oxopentanenitrile (**5j**).* To a stirred suspension of 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoic acid (10.06 g, 75 mmol) in MeOH at rt was added dropwise (trimethylsilyl)diazomethane (50 mL of a 2 M solution in diethyl ether, 100 mmol). The reaction mixture was stirred at rt for 15 h. The mixture was concentrated under reduced pressure, and the residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$ solution. The organic layer was separated, dried over $MgSO_4$, and then concentrated under reduced pressure to afford methyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate as an oil, which did not require further purification (3.79 g, 34%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.81 (d, J = 13.8 Hz, 2H), 3.67 (s, 3H), 3.60 (d, J = 13.8 Hz, 2H), 2.89 (br s, 2H), 0.96 (s, 3H). To a solution of methyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate (13.0 g, 88 mmol) and 2,6-lutidine (26.79 g, 250 mmol) in CH_2Cl_2 at −78 °C was added dropwise trifluoroacetic anhydride (50.0 g, 177 mmol). The reaction mixture was stirred for 2 h at −78 °C, after which the reaction was allowed to warm slowly to rt and was stirred for an additional 2 h. The mixture was partitioned between CH_2Cl_2 (200 mL) and 3% aqueous HCl solution (200 mL). The organic layer was separated, washed with 3% aqueous HCl solution (200 mL), dried over $MgSO_4$, and then concentrated under reduced pressure to give an oil. The oil was dissolved in THF (50 mL) and cooled to 0 °C. Tetrabutylammonum fluoride (200 mL of a 1 M solution in THF, 200 mmol) was added dropwise, and the reaction mixture was stirred at rt for 15 h. The mixture was concentrated under reduced pressure and the residue dissolved in CH_2Cl_2 (400 mL), which was washed with brine (2 × 200 mL), dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by vacuum distillation to afford methyl 3-fluoro-2-(fluoromethyl)-2-methylpropanoate as an oil (2.89 g, 22%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.33–4.66 (m, 4H), 3.67 (s, 3H), 1.14 (s, 3H). Methyl 3-fluoro-2-(fluoromethyl)-2-methylpropanoate (5.21 g, 34.2 mmol) was treated according to General Procedure A to afford the title compound as an oil (4.41 g, 80%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.67–4.72 (m, 2H), 4.45–4.52 (m, 2H), 3.80 (s, 2H), 1.27 (s, 3H).

*5-Hydroxy-4,4-dimethyl-3-oxopentanenitrile (**5k**).* A solution of methyl 3-hydroxy-2,2-dimethylpropanoate (5.00 g, 38 mmol), *N*,*N*-diisopropylethylamine (7.30 g, 57 mmol), and *tert*-butyldimethylchlorosilane (6.80 g, 45 mmol) in DMF (70 mL) was stirred at rt for 12 h. The reaction solution was partitioned between water and diethyl ether. The combined organic layers were washed with water and then brine and then dried over $MgSO_4$. Concentration under reduced pressure afforded methyl 3-(*tert*-butyldimethylsilyloxy)-2,2-dimethylpropanoate as a colorless oil, which did not require further purification (9.34 g, 100%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.64 (s, 3H), 3.55 (s, 2H), 1.13 (s, 6H), 0.85 (s, 9H), 0.00 (s, 6H). Methyl 3-(*tert*-butyldimethylsilyloxy)-2,2-dimethylpropanoate (6.0 g, 24.39 mmol) was treated according to General Procedure A and then purified by silica gel flash chromatography (eluting with 33% EtOAc in petroleum ether) to afford the title compound as a yellow oil (1 g, 29%). ^{1}H NMR ($CDCl_3$, 300 MHz,): δ 3.76 (s, 2H), 3.61 (s, 2H), 1.19 (s, 6H).

*5-Methoxy-4,4-dimethyl-3-oxopentanenitrile (**5l**).* Prepared from methyl 3-methoxy-2,2-dimethylpropanoate[21] (8 g, 54.7 mmol) according to General Procedure A to afford the title compound as a yellow oil (2.5 g, 29%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.72 (s, 2H), 3.32–3.33 (m, 5H), 1.18 (s, 6H).

*Preparation of Substituted Aminoisoxazoles. General Procedure B. 3-Isopropylisoxazol-5-amine (**6a**).* To a stirred solution of sodium hydroxide (238 mg, 5.95 mmol) and compound **5a** (600 mg, 5.41 mmol) in a mixture of water (5 mL) and EtOH (5 mL) was added hydroxylamine sulfate (977 mg, 5.95 mmol). The reaction mixture was adjusted to pH 7.5 with aqueous 1 M sodium hydroxide solution and then heated at 80 °C for 15 h. After cooling to rt, the solvent was removed under reduced pressure. The resulting solid was partitioned between CH_2Cl_2 (50 mL) and water (50 mL), and the organic layer was separated, washed with brine (50 mL), dried over $MgSO_4$, and then concentrated under reduced pressure to afford the title compound as a cream solid, which did not require further purification (530 mg, 78%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.00 (s, 1H), 4.39 (br s, 2H), 2.89 (septet, J = 6 Hz, 1H), 1.23 (d, J = 6 Hz, 6H). LC-MS (ESI) m/z 127 $[M + H]^+$.

*Preparation of Substituted Aminoisoxazoles. General Procedure C. 3-Cyclopentylisoxazol-5-amine (**6b**).* To a stirred solution of compound **5b** (645 mg, 4.7 mmol) and sodium hydroxide (386 mg, 9.65 mmol) in water (4.7 mL) was added hydroxylamine hydrochloride (343 mg, 4.94 mmol), and the resulting mixture was heated at 100 °C for 2.5 h. After cooling to rt, the mixture was diluted with $CHCl_3$ and the organic layer was separated. The aqueous layer was further extracted with $CHCl_3$, and the combined organic layers were dried over $MgSO_4$ and then concentrated under reduced pressure to afford the title compound as a colorless solid, which did not require further purification (680 mg, 95%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.09 (br s, 2H), 4.93 (s, 1H), 2.94–3.02 (m, 1H), 1.73–2.00 (m, 2H), 1.62–1.73 (m, 6H). LC-MS (ESI) m/z 153 $[M + H]^+$.

*3-(2-Fluoropropan-2-yl)isoxazol-5-amine (**6c**).* Prepared from compound **5c** (6.0 g, 47 mmol) according to General Procedure B to afford the title compound as a light yellow solid (4.83 g, 71%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.19 (s, 1H), 4.48 (br s, 2H), 1.68 (d, J = 21 Hz, 6H). LC-MS (ESI) m/z 145 $[M + H]^+$.

*3-(1,1-Difluoroethyl)isoxazol-5-ylamine (**6d**).* Prepared from compound **5d** (100 mg, 0.75 mmol) according to General Procedure B to afford the title compound as a colorless solid (100 mg, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.30 (s, 1H), 4.70 (s, 2H), 2.00 (t, J = 19 Hz, 3H). LC-MS (ESI) m/z 149 $[M + H]^+$.

*3-(Trifluoromethyl)isoxazol-5-amine (**6e**).* (Trimethylsilyl)-diazomethane (21 mL of a 2 M solution in diethyl ether, 42 mmol) was added dropwise to a stirred solution of compound **5e** (3.79 g, 26 mmol) in diethyl ether (25 mL) at 0 °C. The resulting mixture was allowed to slowly warm to rt and was stirred for 15 h. The mixture was concentrated under reduced pressure to afford 4,4,4-trifluoro-3-methoxybut-2-enenitrile as an oil, which did not require further purification (3.93 g, 100%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.00 (s, 1H), 4.16 (s, 3H). To a stirred solution of hydroxylamine hydrochloride (2.88 g, 41.5 mmol) in MeOH (20 mL) at 0 °C was added solid sodium methoxide (2.24 g, 41.5 mmol), and the resulting suspension was stirred at rt for 15 min. The suspension was cooled to 0 °C, and then 4,4,4-trifluoro-3-methoxybut-2-enenitrile (3.93 g, 26 mmol) was added dropwise and the mixture was allowed to slowly warm to rt. The mixture was then heated to 60 °C for 15 h. The colorless precipitate was removed by filtration, and the filtrate was concentrated under reduced pressure to afford a solid. The solid was redissolved in EtOH (25 mL) and the solution acidified to pH = 1 with 37% aqueous HCl and then stirred at 60 °C for 2 h. After cooling to rt, the mixture was concentrated under reduced pressure and the residue partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$ solution. The organic layer was separated, and the aqueous layer was further extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 5% EtOAc in CH_2Cl_2) to afford the title compound as a solid (446 mg, 11%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.31 (s, 1H), 5.03 (br s, 2H). LC-MS (ESI) m/z 153 $[M + H]^+$.

*3-(1,1,1-Trifluoro-2-methylpropan-2-yl)isoxazol-5-amine (**6f**).* Prepared from compound **5f** (524 mg, 2.9 mmol) according to General Procedure C to afford the title compound as a solid (150 mg, 27%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.19 (s, 1H), 4.50 (br s, 2H), 1.54 (s, 6H). LC-MS (ESI) m/z 195 $[M + H]^+$.

*2-(5-Aminoisoxazol-3-yl)-2-methylpropanenitrile (**6g**).* Prepared from compound **5g** (500 mg, 3.68 mmol) according to General Procedure B to afford the title compound as a colorless solid (130 mg, 23%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.22 (s, 1H), 4.58 (br s, 2H), 1.72 (s, 6H). LC-MS (ESI) m/z 152 $[M + H]^+$.

*3-(1-(Trifluoromethyl)cyclobutyl)isoxazol-5-amine (**6h**).* Prepared from compound **5i** (500 mg, 2.6 mmol) according to General Procedure B to afford the title compound as a colorless solid (210 mg, 39%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.04 (s, 1H), 4.55 (br s, 2H), 2.40–2.59 (m, 4H), 1.90–2.09 (m, 2H). LC-MS (ESI) m/z 207 $[M + H]^+$.

*3-(1,3-Difluoro-2-methylpropan-2-yl)isoxazol-5-amine (**6i**).* To a stirred solution of compound **5j** (1.00 g, 6.2 mmol) and sodium hydroxide (0.27 g, 6.8 mmol) in EtOH (5 mL) and water (5 mL) at rt was added a solution of hydroxylamine sulfate (1.12 g, 6.8 mmol) in water (5 mL). To the mixture was added additional sodium hydroxide until the pH was 8–9, and the mixture was heated at 100 °C for 2 h. After cooling to rt, the mixture was partitioned between CH_2Cl_2 and water. The organic layer was separated and the aqueous layer re-extracted with additional CH_2Cl_2. The combined organic layers were washed with water, separated, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 100% hexanes to 50% EtOAc in hexanes) to afford the title compound as a solid (191 mg, 17%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.16 (s, 1H), 4.54–4.68 (m, 2H), 4.32–4.50 (m, 4H), 1.37 (s, 3H). LC-MS (ESI) m/z 177 $[M + H]^+$.

*3-tert-Butylisoxazol-5-amine (**6j**).* Prepared from pivaloylacetonitrile (1 g, 7.90 mmol) according to General Procedure C to afford the title compound as a colorless solid (1.07 g, 97%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.00 (s, 1H), 4.79 (br s, 2H), 1.33 (s, 9H). LC-MS (ESI) m/z 141 $[M + H]^+$.

*3-Phenylisoxazole-5-amine (**6k**).* Prepared from benzoylacetonitrile (707 mg, 4.9 mmol) according to General Procedure C. The crude product was purified by silica gel flash chromatography (eluting with 20–40% EtOAc in hexanes) to afford the title compound as a solid (456 mg, 58%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.70–7.76 (m, 2H), 7.40–7.47 (m, 3H), 5.49 (s, 1H), 4.52 (s, 2H). LC-MS (ESI) m/z 161 $[M + H]^+$.

*Preparation of Substituted Isoxazole Carbamates. General Procedure D. Phenyl 3-Isopropylisoxazol-5-ylcarbamate (**7a**).* To a stirred mixture of compound **6a** (250 mg, 1.98 mmol) and potassium carbonate (634 mg, 4.59 mmol) in THF (6 mL) was added phenyl chloroformate (341 mg, 2.18 mmol). The reaction mixture was stirred at rt for 3.5 h, and then additional phenyl chloroformate (341 mg, 2.18 mmol) was added and stirring was continued for a further 15 h. The resulting mixture was partitioned between CH_2Cl_2 (50 mL) and water (50 mL), and the organic layer was separated, washed with brine (50 mL), dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 4–40% EtOAc in hexanes) to afford the title compound as a colorless solid (330 mg, 68%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.76 (br s, 1H), 7.40–7.45 (m, 2H), 7.18–7.31 (m, 3H), 6.07 (s, 1H), 3.02 (septet, J = 6 Hz, 1H), 1.28 (d, J = 6 Hz, 6H). LC-MS (ESI) m/z 247 $[M + H]^+$.

*Phenyl 3-Cyclopentylisoxazol-5-ylcarbamate (**7b**).* Prepared from compound **6b** (675 mg, 4.44 mmol) according to General Procedure D to afford the title compound as a colorless solid (528 mg, 50%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.21 (br s, 1H), 7.39–7.44 (m, 2H), 7.26–7.30 (m, 1H), 7.21 (d, J = 9 Hz, 2H), 6.06 (s, 1H), 3.12 (m,1H), 1.99–2.06 (m, 2H), 1.63–1.76 (m, 6H). LC-MS (ESI) m/z 273 $[M + H]^+$.

*Phenyl 3-(2-Fluoropropan-2-yl)isoxazol-5-ylcarbamate (**7c**).* Prepared from compound **6c** (4.83 g, 33.54 mmol) according to General Procedure D to afford the title compound as a colorless solid (6.04 g, 68%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.80 (br s, 1H), 7.39–7.45 (m, 2H), 7.18–7.32 (m, 3H), 6.27 (s, 1H), 1.74 (d, J = 21 Hz, 6H). LC-MS (ESI) m/z 265 $[M + H]^+$.

*Phenyl 3-(1,1-Difluoroethyl)isoxazol-5-ylcarbamate (**7d**).* Prepared from compound **6d** (100 mg, 0.68 mmol) according to General Procedure D to afford the title compound as an oil (141 mg, 77%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.02 (s, 1H), 7.40–7.45 (m, 2H), 7.18–7.32 (m, 3H), 6.38 (s, 1H), 2.03 (t, J = 19 Hz, 3H). LC-MS (ESI) m/z 269 $[M + H]^+$.

*Phenyl 3-(Trifluoromethyl)isoxazol-5-ylcarbamate (**7e**).* To a stirred solution of compound **6e** (446 mg, 2.93 mmol) in THF (6 mL) at rt were added triethylamine (1.1 mL, 8.2 mmol), phenyl chloroformate (0.88 mL, 7.03 mmol), and 4-(dimethylamino)pyridine (357 mg, 2.93 mmol). The reaction mixture was stirred at rt for 3 h. The mixture was filtered through a pad of Celite, and then the filtrate was concentrated under reduced pressure. The residue was redissolved in CH_2Cl_2, washed with brine, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 20% EtOAc in hexanes) to afford the title compound as a colorless solid (269 mg, 33%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.99 (br s, 1H), 7.39–7.42 (m, 2H), 7.02–7.35 (m, 3H), 6.70 (s, 1H). LC-MS (ESI) m/z 273 $[M + H]^+$.

*4-Chlorophenyl 3-(1,1,1-Trifluoro-2-methylpropan-2-yl)isoxazol-5-ylcarbamate (**7f**).* Prepared from compound **6f** (150 mg, 0.77 mmol) and 4-chlorophenyl choloroformate (412 mg, 2.15 mmol) according to General Procedure D to afford the title compound as a colorless solid (210 mg, 78%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.72 (br s, 1H), 7.39 (d, J = 12 Hz, 2H), 7.16 (d, J = 12 Hz, 2H), 6.27 (s, 1H), 1.57 (s, 6H). LC-MS (ESI) m/z 349 $[M + H]^+$.

*Phenyl 3-(2-Cyanopropan-2-yl)isoxazol-5-ylcarbamate (**7g**).* Prepared from compound **6g** (130 mg, 0.861 mmol) according to General Procedure D to afford the title compound as a colorless solid (93 mg, 40%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.82 (br s, 1H), 7.41–7.46

(m, 2H), 7.32 (m, 1H), 7.18−7.21 (m, 2H), 6.29 (s, 1H), 1.83 (s, 6H). LC-MS (ESI) m/z 272 $[M + H]^+$.

*Phenyl 3-(1-(Trifluoromethyl)cyclobutyl)isoxazol-5-ylcarbamate (**7h**).* Prepared from compound **6h** (210 mg, 1 mmol) according to General Procedure D to afford the title compound as a colorless solid (320 mg, 98%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.17 (br s, 1H), 7.10−7.54 (m, 5H), 6.08 (s, 1H), 2.50−2.70 (m, 4H), 1.90−2.10 (m, 2H). LC-MS (ESI) m/z 327 $[M + H]^+$.

*Phenyl 3-(1,3-Difluoro-2-methylpropan-2-yl)isoxazol-5-ylcarbamate (**7i**).* Prepared from compound **6i** (190 mg, 1.08 mmol) according to General Procedure D to afford the title compound as a solid (0.319 g, 100%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.83 (s, 1H), 7.17−7.45 (m, 5H), 6.23 (s, 1H), 4.68−4.74 (m, 2H), 4.49−4.58 (m, 2H), 1.40 (s, 3H). LC-MS (ESI) m/z 297 $[M + H]^+$.

*Phenyl 3-tert-Butylisoxazol-5-ylcarbamate (**7j**).* Prepared from compound **6j** (620 mg, 4.43 mmol) according to General Procedure D to afford the title compound as a colorless solid (1.02 g, 89%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.81 (br s, 1H), 7.42−7.47 (m, 2H), 7.23−7.32 (m, 3H), 6.05 (s, 1H), 1.27 (s, 9H). LC-MS (ESI) m/z 261 $[M + H]^+$.

*Phenyl 3-Phenylisoxazol-5-ylcarbamate (**7k**).* Prepared from compound **6k** (456 mg, 2.85 mmol) according to General Procedure D to afford the title compound as a colorless solid (675 mg, 84%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.77−7.81 (m, 3H), 7.40−7.47 (m, 5H), 7.19−7.32 (m, 3H), 6.54 (s, 1H). LC-MS (ESI) m/z 281 $[M + H]^+$.

*5-(1,1,1-Trifluoro-2-methylpropan-2-yl)isoxazol-3-amine (**8a**).* Compound **5f** (3 g, 16.76 mmol), hydroxylamine sulfate (3.30 g, 20.11 mmol), and $NaHCO_3$ (3.52 g, 41.90 mmol) in a mixture of 10% MeOH in water (60 mL) were heated at 65 °C for 15 h. After cooling to rt, an additional 30 mL of 10% MeOH in water was added and the mixture was divided into 9 × 10 mL batches. Each batch was adjusted to pH 1 with concentrated HCl and placed into a 20 mL volume microwave vial fitted with a stirrer bar. After sealing, each batch was placed in a Biotage Microwave Synthesizer and heated (with stirring) at 140 °C for 5 min (maximum internal pressure attained was 7 bar). Each batch was cooled and neutralized with saturated aqueous $NaHCO_3$ solution. All processed batches were combined and then concentrated under reduced pressure, and the aqueous solution was extracted with 10% isopropanol in $CHCl_3$ (3 × 150 mL). The combined organic layers were washed with brine (200 mL), dried over $MgSO_4$, and then concentrated under reduced pressure to afford the title compound as a pale yellow solid, which did not require further purification (2.34 g, 71%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.80 (s, 1H), 3.98 (br s, 2H), 1.53 (s, 6H). LC-MS (ESI) m/z 195 $[M + H]^+$.

*5-(1,3-Difluoro-2-methylpropan-2-yl)isoxazol-3-amine (**8b**).* A stirred mixture of compound **5j** (483 mg, 3 mmol), hydroxylamine sulfate (590 mg, 3.6 mmol), and $NaHCO_3$ (700 mg, 8.3 mmol) in MeOH (1 mL) and water (10 mL) was heated at 60 °C for 8 h. The reaction was adjusted to pH =1 with 10% aqueous HCl, and the resulting mixture was heated at 60 °C for a further 3 h. After cooling to rt, the mixture was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$ solution. The organic layer was separated, and the aqueous layer was further extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and then concentrated under reduced pressure to afford the title compound as a solid, which did not require further purification (289 mg, 55%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.76 (s, 1H), 4.58−4.60 (m, 2H), 4.48−4.51 (m, 4H), 1.37 (s, 3H). LC-MS (ESI) m/z 177 $[M + H]^+$.

*5-(1-(Trifluoromethyl)cyclopropyl)isoxazol-3-amine (**8c**).* Prepared from compound **5h** (1 g, 5.65 mmol) according to the procedure described for **8a** to afford the title compound as a pale yellow solid (687 mg, 64%). LC-MS (ESI) m/z 193 $[M + H]^+$.

*2-(3-Aminoisoxazol-5-yl)-2-methylpropan-1-ol (**8d**).* A stirred solution of compound **5k** (1 g, 7.90 mmol), 96% sodium hydroxide (374 mg, 9.35 mmol), and hydroxylamine hydrochloride (650 mg, 9.35 mmol) in a mixture of EtOH (100 mL) and water (100 mL) was heated at 60 °C for 22 h. Concentrated HCl (3 mL) was added, and the mixture was heated at 80 °C for 1 h. The reaction mixture was cooled to rt and then concentrated under reduced pressure. The residue was partitioned between $CHCl_3$ and 7 M aqueous sodium hydroxide solution, and the organic layer was separated, washed with water, and then dried over Na_2SO_4 and then concentrated under reduced pressure. The residue was purified by recrystallization from diethyl ether to afford the title compound as a colorless solid (600 mg, 49%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.64 (s, 1H), 3.65 (s, 2H), 2.30 (br s, 2H), 1.31 (s, 6H). LC-MS (ESI) m/z 157 $[M + H]^+$.

*5-(1-Methoxy-2-methylpropan-2-yl)isoxazol-3-amine (**8e**).* Prepared from compound **5l** (1 g, 6.5 mmol) according to the procedure described for **8d**. Purified by silica gel flash chromatography (eluting with a mixture of petroleum ether and EtOAc) to afford the title compound as a colorless solid (350 mg, 32%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.60 (s, 1H), 3.39 (s, 2H), 3.32 (s, 3H), 2.94 (br s, 2H), 1.28 (s, 6H). LC-MS (ESI) m/z 171 $[M + H]^+$.

*5-Phenylisoxazol-3-amine (**8f**).* Prepared from benzoylacetonitrile (1.45 g, 10 mmol) according to the procedure described for **8d**. Purified by silica gel flash chromatography (eluting with 20−40% EtOAc in hexanes) to afford the title compound as a colorless solid (420 mg, 26%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.72 (d, J = 9 Hz, 2H), 7.39−7.69 (m, 3H), 6.20 (s, 1H), 4.01 (br s, 2H). LC-MS (ESI) m/z 161 $[M + H]^+$.

*5-Isopropylisoxazol-3-amine (**8h**).* To a stirred solution of hydroxylamine hydrochloride (6.3 g, 91.7 mmol) in MeOH (2.5 mL) was added ammonia (15.7 mL of a 7 M solution in MeOH, 110 mmol), and the resulting suspension was stirred at rt for 30 min. 8-Hydroxyquinoline (290 mg, 2 mmol) was added, followed by a solution of compound **10a** (3.38 g, 22 mmol) in MeOH (2.5 mL). The mixture was heated at 70 °C for 15 h. After cooling to rt, the mixture was filtered and the filtrate concentrated under reduced pressure and reconcentrated three times from toluene to afford *N*′-hydroxy-2-(2-isopropyl-1,3-dioxolan-2-yl)acetimidamide as a yellow solid, which did not require further purification (3.9 g, 94%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.01 (br s, 2H), 3.94−4.05 (m, 4H), 2.44 (s, 2H), 1.99 (m, 1H), 0.95 (d, J = 12 Hz, 6H). LC-MS (ESI) m/z 189 $[M + H]^+$. *N*′-Hydroxy-2-(2-isopropyl-1,3-dioxolan-2-yl)acetimidamide (1.8 g, 9.57 mmol) was dissolved in EtOH (12 mL) and acidified to pH 1 with 37% aqueous HCl. The mixture was placed into a 20 mL microwave vial fitted with a stirrer bar. After sealing, the reaction mixture was placed in a Biotage Microwave Synthesizer and heated (with stirring) at 120 °C for 30 min. After cooling to rt, the mixture was concentrated under reduced pressure and the residue diluted with CH_2Cl_2. Saturated aqueous $NaHCO_3$ solution was added until the mixture became basic (pH = 11) and the organic layer separated. The aqueous layer was further extracted with CH_2Cl_2, and the organic layers were combined, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 20−100% EtOAc in hexanes) to afford the title compound as a solid (819 mg, 68%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.52 (s, 1H), 3.89 (br s, 2H), 2.94 (m, 1H), 1.27 (d, J = 12 Hz, 6H). LC-MS (ESI) m/z 127 $[M + H]^+$.

*5-Cyclopentylisoxazol-3-amine (**8i**).* Prepared from compound **10b** (1.5 g, 8.3 mmol) according to the procedure described for compound **8h**, to afford the title compound as a colorless solid (875 mg, 69%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.52 (s, 1H), 3.86 (br s, 2H), 3.06 (m, 1H), 2.04 (m, 2H), 1.62−1.75 (m, 6H). LC-MS (ESI) m/z 153 $[M + H]^+$.

*5-(2-Fluoropropan-2-yl)isoxazol-3-amine (**8j**).* To MeOH (10 mL) at rt was added portionwise sodium metal (145 mg, 6.30 mmol). After all of the metal had dissolved, the reaction mixture was cooled to 0 °C and hydroxylamine hydrochloride (438 mg, 6.30 mmol) was added in one portion. The reaction mixture was stirred for 15 min. A solution of compound **11** (500 mg, 3.50 mmol) in MeOH (3 mL) was added, and the mixture was heated at 70 °C for 16 h. Concentrated HCl (0.8 mL, 9.6 mmol) was added and the reaction mixture stirred at 80 °C for 30 min. After cooling to rt, the mixture was concentrated under reduced pressure to give an orange foam which was dissolved in water (50 mL) and the solution adjusted to pH 10 using aqueous 1 M sodium hydroxide solution. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL), and the combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography

(eluting with 12−100% EtOAc in hexanes) to afford the title compound as a cream solid (64 mg, 13%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.82 (s, 1H), 4.08 (br s, 2H), 1.71 (d, J = 21 Hz, 6H). LC-MS (ESI) m/z 145 $[M + H]^+$.

*4-Chlorophenyl 5-(1,1,1-Trifluoro-2-methylpropan-2-yl)isoxazol-3-ylcarbamate (**9a**).* Prepared from compound **8a** (123 mg, 0.63 mmol) and 4-chlorophenyl chloroformate (180 mg, 0.95 mmol) according to General Procedure D to afford the title compound as a colorless solid (85 mg, 39%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.83 (br s, 1H), 7.38 (d, J = 9 Hz, 2H), 7.15 (d, J = 9 Hz, 2H), 6.82 (s, 1H), 1.59 (s, 6H). LC-MS (ESI) m/z 349 $[M + H]^+$.

*Phenyl 5-(1,3-Difluoro-2-methylpropan-2-yl)isoxazol-3-ylcarbamate (**9b**).* Prepared from compound **8b** (287 mg, 1.6 mmol) according to General Procedure D to afford the title compound as a solid (358 mg, 76%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.17 (br s, 1H), 7.39−7.44 (m, 2H), 7.26 (m, 1H), 7.17−7.20 (m, 2H), 6.81 (s, 1H), 4.63−4.71 (m, 2H), 4.47−4.55 (m, 2H), 1.42 (s, 3H). LC-MS (ESI) m/z 297 $[M + H]^+$.

*Phenyl 5-(1-(Trifluoromethyl)cyclopropyl)isoxazol-3-ylcarbamate (**9c**).* Prepared from compound **8c** (687 mg, 3.58 mmol) according to General Procedure D to afford the title compound as a colorless solid (727 mg, 65%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.34 (br s, 1H), 7.40−7.47 (m, 2H), 7.20−7.31 (m, 3H), 6.80 (s, 1H), 1.45−1.56 (m, 4H). LC-MS (ESI) m/z 313 $[M + H]^+$.

*Phenyl 5-(1-Hydroxy-2-methylpropan-2-yl)isoxazol-3-ylcarbamate (**9d**).* Prepared from compound **8d** (100 mg, 0.64 mmol) according to General Procedure D to afford the title compound as a colorless solid (120 mg, 68%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.30 (br s, 1H), 7.42−7.43 (m, 2H), 7.26 (m, 1H), 7.18−7.21 (m, 2H), 6.65 (s, 1H), 3.67 (s, 2H), 1.98 (br s, 1H), 1.32 (s, 6H). LC-MS (ESI) m/z 277 $[M + H]^+$.

*Phenyl 5-(1-Methoxy-2-methylpropan-2-yl)isoxazol-3-ylcarbamate (**9e**).* Prepared from compound **8e** (30 mg, 0.176 mmol) according to General Procedure D to afford the title compound as an oil (50 mg, 98%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.04 (br s, 1H), 7.42−7.43 (m, 2H), 7.31 (m, 1H), 7.18−7.21 (m, 2H), 6.63 (s, 1H), 3.45 (s, 2H), 3.33 (s, 3H), 1.35 (s, 6H). LC-MS (ESI) m/z 291 $[M + H]^+$.

*Phenyl 5-Phenylisoxazol-3-ylcarbamate (**9f**).* Prepared from compound **8f** (428 mg, 2.67 mmol) according to General Procedure D to afford the title compound as a colorless solid (599 mg, 80%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.92 (br s, 1H), 7.77−7.80 (m, 2H), 7.40−7.51 (m, 6H), 7.20−7.32 (m, 2H), 7.12 (s, 1H). LC-MS (ESI) m/z 281 $[M + H]^+$.

*Phenyl 5-Methylisoxazol-3-ylcarbamate (**9g**).* Prepared from 5-methylisoxazol-3-amine **8g** (490 mg, 5.0 mmol) according to General Procedure D to afford the title compound as a solid (425 mg, 39%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.14 (s, 1H), 7.41−7.46 (m, 2H), 7.29 (d, J = 6 Hz, 1H), 7.21 (m, 2H), 6.47 (s, 1H), 2.38 (s, 3H). LC-MS (ESI) m/z 219 $[M + H]^+$.

*Phenyl 5-Isopropylisoxazol-3-ylcarbamate (**9h**).* Prepared from compound **8h** (816 mg, 6.5 mmol) according to General Procedure D to afford the title compound as a solid (1.24 g, 77%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.05 (br s, 1H), 7.38−7.45 (m, 2H), 7.18−7.30 (m, 3H), 6.55 (s, 1H), 3.06 (m, 1H), 1.30 (d, J = 12 Hz, 6H). LC-MS (ESI) m/z 247 $[M + H]^+$.

*Phenyl 5-Cyclopentylisoxazol-3-ylcarbamate (**9i**).* Prepared from compound **8i** (875 mg, 5.75 mmol) according to General Procedure D to afford the title compound as a colorless solid (1.4 g, 89%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.97 (br s, 1H), 7.39−7.44 (m, 2H), 7.18−7.29 (m, 3H), 6.54 (s, 1H), 3.16 (m, 1H), 2.04−2.10 (m, 2H), 1.58−1.78 (m, 6H). LC-MS (ESI) m/z 273 $[M + H]^+$.

*4-Chlorophenyl 5-(2-Fluoropropan-2-yl)isoxazol-3-ylcarbamate (**9j**).* Prepared from compound **8j** (40 mg, 0.278 mmol) and 4-chlorophenyl chloroformate (54 mg, 0.28 mmol) according to General Procedure D to afford the title compound as a colorless solid (83 mg, 100%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.11 (br s, 1H), 7.36−7.40 (m, 2H), 7.12−7.17 (m, 2H), 6.83 (s, 1H), 1.76 (d, J = 21 Hz, 6H). LC-MS (ESI) m/z 299 $[M + H]^+$.

*2-(2-Isopropyl-1,3-dioxolan-2-yl)acetonitrile (**10a**).* A stirred mixture of compound **5a** (3.12 g, 28 mmol), ethylene glycol (4.7 mL, 84 mmol), and chlorotrimethylsilane (10.6 mL, 84 mmol) in CH_2Cl_2 (50 mL) was heated at 40 °C for 15 h. After cooling to rt, aqueous 5% $NaHCO_3$ (50 mL) was added. The organic layer was separated and the aqueous phase further extracted with diethyl ether. The combined organic layers were dried over $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 20−40% EtOAc in hexanes) to afford the title compound as a colorless oil (3.38 g, 78%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.16−4.21 (m, 2H), 3.99−4.07 (m, 2H), 2.69 (s, 2H), 2.07 (m, 1H), 0.96 (d, J = 12 Hz, 6H).

*2-(2-Cyclopentyl-1,3-dioxolan-2-yl)acetonitrile (**10b**).* Prepared from compound **5b** (2 g, 14.6 mmol) according to the procedure described for compound **10a** to afford the title compound as an oil (1.5 g, 57%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.15−4.23 (m, 2H), 4.01−4.12 (m, 2H), 2.72 (s, 2H), 2.36 (m, 1H), 1.45−1.81 (m, 8H).

*4-Fluoro-3-methoxy-4-methylpent-2-enenitrile (**11**).* To a stirred solution of compound **5c** (1 g, 7.75 mmol) in diethyl ether (150 mL) at 0 °C was added dropwise (trimethylsilyl)diazomethane (4.65 mL of a 2.0 M solution in diethyl ether, 9.30 mmol). After warming to rt, the reaction mixture was stirred for a further 15 h. The reaction mixture was concentrated under reduced pressure to afford the title compound as a yellow oil, which did not require further purification (1 g, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.89 (s, 1H), 4.23 (s, 3H), 1.47 (d, J = 21 Hz, 6H).

*Preparation of Substituted Aminopyrazoles. General Procedure E. 3-tert-Butyl-1-isopropyl-1H-pyrazol-5-amine Hydrochloride (**12a**).* A stirred solution of isopropylhydrazine hydrochloride (500 mg, 4.54 mmol) and 4,4-dimethyl-3-oxopentanenitrile (679 mg, 5.44 mmol) in EtOH (5 mL) was heated under reflux for 15 h. After cooling to rt, the reaction mixture was concentrated under reduced pressure and the solid residue was recrystallized from a mixture of diethyl ether and petroleum ether to afford the title compound as a colorless solid (500 mg, 51%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.55 (s, 1H), 5.05 (br s, 2H), 3.62 (m, 1H), 1.68 (d, J = 6.4 Hz, 6H), 1.43 (s, 9H). LC-MS (ESI) m/z 182 $[M + H]^+$.

*3-tert-Butyl-1-isobutyl-1H-pyrazol-5-amine Hydrochloride (**12b**).* Prepared from isobutylhydrazine hydrochloride (1 g, 8 mmol) according to General Procedure E to afford the title compound as a colorless solid (800 mg, 43%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 14.06 (br s, 1H), 6.93 (br s, 2H), 5.52 (s, 1H), 3.92 (m, 2H), 2.16 (m, 1H), 1.26 (s, 9H), 0.83 (m, 6H). LC-MS (ESI) m/z 196 $[M + H]^+$.

*3-tert-Butyl-1-cyclohexyl-1H-pyrazol-5-amine Hydrochloride (**12c**).* Prepared from cyclohexylhydrazine hydrochloride (1.5 g, 9.96 mmol) according to General Procedure E to afford the title compound as a colorless solid (1.0 g, 39%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 13.24 (br s, 1H), 7.02 (br s, 2H), 5.52 (s, 1H), 4.30 (m, 1H), 1.63−1.98 (m, 7H), 1.10−1.40 (m, 12H). LC-MS (ESI) m/z 222 $[M + H]^+$.

*1-Benzyl-3-tert-butyl-1H-pyrazol-5-amine (**12d**).* Prepared from benzylhydrazine (977 mg, 8.0 mmol) according to General Procedure E. The crude product was purified by silica gel flash chromatography (eluting with 5% to 25% EtOAc in hexanes) to afford the title compound as a solid (666 mg, 36%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.21−7.32 (m, 3H), 7.09 (d, J = 9 Hz, 2H), 5.17 (s, 1H), 5.07 (s, 2H), 5.04 (s, 2H), 1.15 (s, 9H). LC-MS (ESI) m/z 230 $[M + H]^+$.

*1-Phenyl-3-(1,1,1-trifluoro-2-methylpropan-2-yl)-1H-pyrazol-5-amine (**12e**).* To a stirred solution of compound **5f** (1 g, 5.58 mmol) in 50% aqueous EtOH (25 mL) was added phenylhydrazine sulfate (1.15 g, 5.58 mmol), and the reaction mixture was heated at 80 °C for 15 h. After cooling to rt, the reaction mixture was concentrated under reduced pressure and the residue was partitioned between EtOAc and aqueous 1 M sodium hydroxide. The organic layer was separated and the aqueous layer was further extracted multiple times with EtOAc. The combined organic layers were dried over $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 7−60% EtOAc in hexanes) to afford the title compound as a yellow solid (590 mg, 40%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.30−7.59 (m, 5H), 5.53 (s, 1H), 5.37 (br s, 2 H), 1.45 (s, 6 H). LC-MS (ESI) m/z 270 $[M + H]^+$.

*Preparation of Substituted Pyrazole Carbamates. General Procedure F. Phenyl 3-tert-Butyl-1-isopropyl-1H-pyrazol-5-ylcarbamate (**13a**).* To a stirred mixture of phenyl chloroformate (170 mg, 1.1 mmol) and potassium carbonate (210 mg, 1.5 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added dropwise a solution of compound **12a** (220 mg, 1 mmol) and *N*,*N*-diisopropylethylamine (130 mg, 1 mmol) in CH_2Cl_2 (2 mL), and the reaction mixture was stirred at 0 °C for 3 h. The mixture was filtered, and the filtrate was washed with water and brine and then dried over $MgSO_4$. Concentration under reduced pressure gave a solid, which was recrystallized from diethyl ether to afford the title compound as a colorless solid (300 mg, 100%). LC-MS (ESI) m/z 302 $[M + H]^+$.

*Phenyl 3-tert-Butyl-1-isobutyl-1H-pyrazol-5-ylcarbamate (**13b**).* Prepared from compound **12b** according to General Procedure F to afford the title compound as a solid. LC-MS (ESI) m/z 316 $[M + H]^+$.

*Phenyl 3-tert-Butyl-1-cyclohexyl-1H-pyrazol-5-ylcarbamate (**13c**).* Prepared from compound **12c** (260 mg, 1 mmol) according to General Procedure F to afford the title compound as a solid (300 mg, 88%). LC-MS (ESI) m/z 342 $[M + H]^+$.

*Preparation of Substituted Pyrazole Carbamates. General Procedure G. Phenyl 3-tert-Butyl-1-benzyl-1H-pyrazol-5-ylcarbamate (**13d**).* To a stirred mixture of compound **12d** (666 mg, 2.91 mmol) and potassium carbonate (522 mg, 3.78 mmol) in THF (10 mL) at rt was added dropwise a solution of phenyl chloroformate (499 mg, 3.2 mmol) in THF (5 mL). The reaction mixture was stirred at rt for 15 h. The mixture was partitioned between water and CH_2Cl_2 and the organic layer was separated, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 100% hexanes to 50% EtOAc in hexanes) to afford the title compound as a solid (565 mg, 56%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.20 (br s, 1H), 7.10−7.43 (m, 10H), 6.14 (s, 1H), 5.29 (s, 2H), 1.22 (s, 9H). LC-MS (ESI) m/z 350 $[M + H]^+$.

*Phenyl 1-Phenyl-3-(1,1,1-trifluoro-2-methylpropan-2-yl)-1H-pyrazol-5-ylcarbamate (**13e**).* Prepared from compound **12e** (590 mg, 2.2 mmol) according to General Procedure G to afford the title compound as a solid (748 mg, 87%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.02−7.55 (m, 11H), 6.65 (br s, 1H), 1.60 (s, 6H). LC-MS (ESI) m/z 390 $[M + H]^+$.

*Phenyl 3-tert-Butyl-1-methyl-1H-pyrazol-5-ylcarbamate (**13f**).* Prepared from 3-*tert*-butyl-1-methyl-1*H*-pyrazol-5-amine **12f** (1.0 g, 6.5 mmol) according to General Procedure G to afford the title compound as a solid (530 mg, 30%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.20 (s, 1H), 7.40−7.49 (m, 2H), 721−7.30 (m, 3H), 6.06 (s, 1H), 3.66 (s, 3H), 1.25 (s, 9H). LC-MS (ESI) m/z 274 $[M + H]^+$.

*Phenyl 3-tert-Butyl-1-phenyl-1H-pyrazol-5-ylcarbamate (**13g**).* Prepared from 3-*tert*-butyl-1-phenyl-1*H*-pyrazol-5-amine **12g** (2.00 g, 9.3 mmol) according to General Procedure G to afford the title compound as a solid (1.3 g, 42%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.00 (s, 1H), 7.42−7.57 (m, 4H), 7.33−7.44 (m, 3H), 7.08−7.23 (m, 3H), 6.37 (s, 1H), 1.30 (s, 9H). LC-MS (ESI) m/z 336 $[M + H]^+$.

*3-(6,7-Dimethoxyquinazolin-4-yloxy)aniline (**16**).* To a stirred slurry of cesium carbonate (28.20 g, 86 mmol) in THF (300 mL) at rt was added 3-aminophenol (4.83 g, 43 mmol). After stirring for 30 min at rt, 4-chloro-6,7-dimethoxyquinazoline (10 g, 43 mmol) was added and the reaction mixture was heated at 50 °C for 24 h. After cooling to rt, the mixture was partitioned between EtOAc and 1 M aqueous sodium hydroxide. The organic layer was separated, washed with brine, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 1% MeOH in CH_2Cl_2) to afford the title compound as a solid (12.7 g, 100%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.55 (s, 1H), 7.51 (s, 1H), 7.37 (s, 1H), 7.09 (m, 1H), 6.38−6.49 (m, 3H), 5.30 (br s, 2H), 3.99 (s, 3H), 3.97 (s, 3H). LC-MS (ESI) m/z 298 $[M + H]^+$.

*3-(6,7-Dimethoxyquinazolin-4-ylthio)aniline (**17**).* To a stirred slurry of sodium hydride (3.52 g of a 60% dispersion in mineral oil, 88 mmol) in THF (500 mL) at rt was added portionwise 3-aminobenzenethiol (10 g, 80 mmol). After stirring for 30 min at rt, 4-chloro-6,7-dimethoxyquinazoline (18 g, 80 mmol) was added and the reaction mixture was stirred at rt for 15 h. The mixture was partitioned between EtOAc and water, and the organic layer was separated, washed with brine, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by trituration with diethyl ether to afford the title compound as a solid (21.3 g, 85%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.70 (s, 1H), 7.33 (s, 1H), 7.31 (s, 1H), 7.13 (t, J = 7.6 Hz, 1H), 6.81 (s, 1H), 6.67−6.74 (m, 2H), 5.35 (br s, 2H), 3.98 (s, 3H), 3.97 (s, 3H). LC-MS (ESI) m/z 314 $[M + H]^+$.

*Preparation of Quinazolin-4-yloxy Phenylurea Derivatives. General Procedure H. 1-(3-tert-Butylisoxazol-5-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**18**).* To a stirred solution of compound **16** (90 mg, 0.3 mmol), *N*,*N*-diisopropylethylamine (78 μL, 0.45 mmol), and 4-(dimethylamino)pyridine (1.8 mg, 0.015 mmol) in THF (1.5 mL) was added compound **7j** (118 mg, 0.45 mmol). The reaction mixture was heated at 50 °C for 2.5 h. After cooling to rt, the mixture was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by preparative reverse-phase HPLC, and the obtained solid was triturated with diethyl ether to afford the title compound as a colorless solid (41 mg, 29%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.19 (s, 1H), 9.04 (s, 1H), 8.57 (s, 1H), 7.56−7.59 (m, 2H), 7.39−7.44 (m, 2H), 7.30 (d, J = 9 Hz, 1H), 6.98 (d, J = 9 Hz, 1H), 6.04 (s, 1H), 3.99 (s, 6H), 1.25 (s, 9H). LC-MS (ESI) m/z 464 $[M + H]^+$.

*1-(3-tert-Butyl-1-methyl-1H-pyrazol-5-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**19**).* Prepared from compound **13f** (123 mg, 0.45 mmol) and compound **16** (89 mg, 0.30 mmol) according to General Procedure H. The crude product was purified by silica gel flash chromatography (eluting with 100% hexanes to 100% EtOAc in hexanes) to afford the title compound as a solid (102 mg, 71%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.20 (s, 1H), 8.56−8.58 (m, 2H), 7.53 (m, 2H), 7.50 (m, 2H), 7.30 (m, 1H), 6.95 (m, 1H), 6.08 (s, 1H), 3.99 (s, 6H), 3.54 (s, 3H), 1.25 (s, 9H). LC-MS (ESI) m/z 477 $[M + H]^+$.

*1-(4-tert-Butylphenyl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**20**).* To a stirred solution of compound **16** (89 mg, 0.3 mmol) in DMF (3 mL) at rt was added 4-*tert*-butylphenyl isocyanate (54 μL, 0.3 mmol), and the mixture was heated at 50 °C for 4 h. After cooling to rt, the mixture was partitioned between EtOAc and water. The organic layer was separated and further washed with brine. The organic layer was separated, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 25% to 100% EtOAc in hexanes) to afford the title compound as a solid (54 mg, 38%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.83 (s, 1H), 8.65 (s, 1H), 8.57 (s, 1H), 7.60 (s, 1H), 7.56 (s, 1H), 7.21−7.40 (m, 7H), 6.91 (d, J = 6 Hz, 1H), 3.99 (s, 6H), 1.25 (s, 9H). LC-MS (ESI) m/z 473 $[M + H]^+$.

*1-(3-tert-Butylphenyl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**21**).* A mixture of 3-*tert*-butylaniline (447 mg, 3 mmol), potassium carbonate (828 mg, 6 mmol), phenyl chloroformate (1.13 mL, 9 mmol), and 4-(dimethylamino)pyridine (36 mg, 0.30 mmol) in THF (15 mL) was stirred at rt for 15 h. The reaction mixture was diluted with EtOAc, filtered, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 5−15% EtOAc in hexanes) to afford phenyl 3-*tert*-butylphenylcarbamate as a solid (458 mg, 57%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.14 (s, 1H), 7.59 (s, 1H), 7.10−7.49 (m, 7H), 7.08 (d, J = 9 Hz, 1H), 1.25 (s, 9H). LC-MS (ESI) m/z 270 $[M + H]^+$. Phenyl 3-*tert*-butylphenylcarbamate (114 mg, 0.42 mmol) and compound **16** (90 mg, 0.3 mmol) were allowed to react according to General Procedure H. The crude product was purified by silica gel flash chromatography (eluting with 25−100% EtOAc in hexanes) to afford the title compound as a solid (83 mg, 59%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.83 (s, 1H), 8.70 (s, 1H), 8.57 (s, 1H), 7.61 (s, 1H), 7.57 (s, 1H), 7.36−7.50 (m, 3H), 7.14−7.31 (m, 3H), 6.86−7.05 (m, 2H), 4.00 (s, 6H), 1.28 (s, 9H). LC-MS (ESI) m/z 473 $[M + H]^+$.

*1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-methylisoxazol-3-yl)urea (**22**).* Prepared from compound **9g** (98 mg, 0.42 mmol) and compound **16** (89 mg, 0.3 mmol) according to General Procedure H to afford the title compound as a solid (31 mg, 25%). ^{1}H NMR

(DMSO-d_6, 300 MHz): δ 9.53 (s, 1H), 9.01 (s, 1H), 8.56 (s, 1H), 7.57 (s, 1H), 7.55 (s, 1H), 7.35−7.45 (m, 2H), 7.26 (d, J = 9 Hz, 1H), 6.97 (d, J = 6 Hz, 1H), 6.51 (s, 1H), 3.99 (s, 6H), 2.35 (s, 3H). LC-MS (ESI) m/z 422 $[M + H]^+$.

1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-isopropylisoxazol-3-yl)urea (**23**). Prepared from compound **9h** (110 mg, 0.45 mmol) and compound **16** (90 mg, 0.3 mmol) according to General Procedure H to afford the title compound as a colorless solid (79 mg, 59%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.57 (s, 1H), 9.01 (s, 1H), 8.56 (s, 1H), 7.57 (s, 2H), 7.38−7.43 (m, 2H), 7.27 (d, J = 9 Hz, 1H), 6.99 (d, J = 9 Hz, 1H), 6.49 (s, 1H), 4.00 (s, 6H), 3.00 (m, 1H), 1.22 (d, J = 12 Hz, 6H). LC-MS (ESI) m/z 450 $[M + H]^+$.

1-(5-Cyclopentylisoxazol-3-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**24**). Prepared from compound **9i** (130 mg, 0.48 mmol) and compound **16** (95 mg, 0.32 mmol) according to General Procedure H to afford the title compound as a colorless solid (81 mg, 53%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.58 (s, 1H), 9.03 (s, 1H), 8.56 (s, 1H), 7.57 (s, 2H), 7.38−7.43 (m, 2H), 7.26 (d, J = 9 Hz, 1H), 6.97 (d, J = 9 Hz, 1H), 6.50 (s, 1H), 4.00 (s, 6H), 3.11 (m, 1H), 1.64−1.66 (m, 2H), 1.18−1.20 (m, 6H). LC-MS (ESI) m/z 476 $[M + H]^+$.

1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-phenylisoxazol-3-yl)urea (**25**). Prepared from compound **9f** (126 mg, 0.45 mmol) and compound **16** (90 mg, 0.3 mmol) according to General Procedure H to afford the title compound as a colorless solid (47 mg, 32%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.75 (s, 1H), 9.08 (s, 1H), 8.58 (s, 1H), 7.86 (d, J = 6 Hz, 2H), 7.51−7.87 (m, 4H), 7.40−7.51 (m, 2H), 7.21−7.31 (m, 3H), 7.00 (d, J = 6 Hz, 1H), 4.00 (s, 6H). LC-MS (ESI) m/z 484 $[M + H]^+$.

1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(3-isopropylisoxazol-5-yl)urea (**26**). Prepared from compound **7a** (89 mg, 0.36 mmol) and compound **16** (89 mg, 0.30 mmol) according to General Procedure H to afford the title compound as a colorless solid (25 mg, 19%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.30 (br s, 1H), 9.14 (s, 1H), 8.56 (s, 1H), 7.57 (s, 2H), 7.39−7.44 (m, 2H), 7.31 (m, 1H), 6.99 (m, 1H), 5.99 (s, 1H), 4.00 (s, 6H), 2.90 (septet, J = 6 Hz, 1H), 1.19 (d, J = 6 Hz, 6H). LC-MS (ESI) m/z 450 $[M + H]^+$.

1-(3-Cyclopentylisoxazol-5-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**27**). Prepared from compound **7b** (124 mg, 0.45 mmol) and compound **16** (104 mg, 0.35 mmol) according to General Procedure H to afford the title compound as a colorless solid (75 mg, 45%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.18 (s, 1H), 9.05 (s, 1H), 8.56 (s, 1H) 7.57 (s, 2H), 7.39−7.56 (m, 2H), 7.29 (d, J = 9 Hz, 1H), 6.98 (d, J = 9 Hz, 1H), 5.95 (s, 1H), 4.00 (s, 6H), 3.03 (m, 1H), 1.93−1.99 (m, 2H), 1.61−1.69 (m, 6H). LC-MS (ESI) m/z 476 $[M + H]^+$.

1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(3-phenylisoxazol-5-yl)urea (**28**). Prepared from compound **7k** (126 mg, 0.45 mmol) and compound **16** (90 mg, 0.3 mmol) according to General Procedure H to afford the title compound as a colorless solid (63 mg, 43%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.40 (s, 1H), 9.14 (s, 1H), 8.50 (s, 1H), 7.74−7.83 (m, 2H), 7.48−7.83 (m, 7H), 7.42 (d, J = 9 Hz, 1H), 7.00 (d, J = 9 Hz, 1H), 6.56 (s, 1H), 4.00 (s, 6H). LC-MS (ESI) m/z 484 $[M + H]^+$.

1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-(1-hydroxy-2-methylpropan-2-yl)isoxazol-3-yl)urea (**29**). Prepared from compound **9d** (41 mg, 0.15 mmol) and compound **16** (30 mg, 0.10 mmol) according to General Procedure H. The crude product was purified by preparative silica gel TLC (eluting with 10% MeOH in CH_2Cl_2) to afford the title compound as a colorless solid (30 mg, 63%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.57 (br s, 1H), 8.99 (br s, 1H), 8.56 (s, 1H), 7.56−7.58 (m, 2H), 7.38−7.42 (m, 2H), 7.25 (m, 1H), 6.97 (m, 1H), 6.49 (s, 1H), 4.95 (br s, 1H), 3.99 (s, 3H), 3.98 (s, 3H), 3.43 (s, 2H), 1.20 (s, 6H). LC-MS (ESI) m/z 480 $[M + H]^+$.

1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-(1-methoxy-2-methylpropan-2-yl)isoxazol-3-yl)urea (**30**). Prepared from compound **9e** (50 mg, 0.17 mmol) and compound **16** (50 mg, 0.16 mmol) according to General Procedure H. The crude product was purified by preparative silica gel TLC (eluting with 10% MeOH in CH_2Cl_2) to afford the title compound as a colorless solid (38 mg, 49%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.57 (br s, 1H), 9.01 (br s, 1H), 8.56 (s, 1H), 7.57−7.58 (m, 2H), 7.38−7.42 (m, 2H), 7.25 (m, 1H), 6.97 (m, 1H), 6.50 (s, 1H), 3.99 (s, 6H), 3.38 (s, 2H), 3.23 (s, 3H), 1.24 (s, 6H). LC-MS (ESI) m/z 494 $[M + H]^+$.

1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(3-(trifluoromethyl)isoxazol-5-yl)urea (**31**). Prepared from compound **7e** (124 mg, 0.45 mmol) and compound **16** (104 mg, 0.35 mmol) according to General Procedure H to afford the title compound as a colorless solid (9 mg, 5%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.90 (br s, 1H), 9.30 (br s, 1H), 8.59 (br s, 1H), 7.20−7.80 (m, 5H), 7.06 (br s, 1H), 6.50 (s, 1H), 4.09 (s, 6H). LC-MS (ESI) m/z 476 $[M + H]^+$.

1-(3-(1,1-Difluoroethyl)isoxazol-5-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**32**). Prepared from compound **7d** (141 mg, 0.52 mmol) and compound **16** (156 mg, 0.52 mmol) according to General Procedure H to afford the title compound as a colorless solid (66 mg, 27%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.70 (s, 1H), 9.17 (s, 1H), 8.56 (s, 1H), 7.56−7.57 (m, 2H), 7.39−7.45 (m, 2H), 7.33 (m, 1H), 7.00 (m, 1H), 6.27 (s, 1H), 3.99 (s, 3H), 3.98 (s, 3H), 2.00 (t, J = 19 Hz, 3H). LC-MS (ESI) m/z 472 $[M + H]^+$.

1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(3-(2-fluoropropan-2-yl)isoxazol-5-yl)urea (**33**). Prepared from compound **7c** (95 mg, 0.36 mmol) and compound **16** (89 mg, 0.3 mmol) according to General Procedure H, to afford the title compound as a colorless solid (63 mg, 45%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.50 (br s, 1H), 9.15 (s, 1H), 8.56 (s, 1H), 7.57−7.58 (m, 2H), 7.40−7.45 (m, 2H), 7.32 (m, 1H), 7.00 (m, 1H), 6.14 (s, 1H), 4.00 (s, 3H), 3.99 (s, 3H), 1.67 (d, J = 21 Hz, 6H). LC-MS (ESI) m/z 468 $[M + H]^+$.

1-[3-(1,3-Difluoro-2-methylpropan-2-yl)isoxazol-5-yl]-3-[3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl]urea (**34**). Prepared from compound **7i** (158 mg, 0.5 mmol) and compound **16** (119 mg, 0.4 mmol) according to General Procedure H. The crude product was purified by silica gel flash chromatography (eluting with 40−50% EtOAc in hexanes) to afford the title compound as a solid (115 mg, 58%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.33 (s, 1H), 9.00 (s, 1H), 8.55 (s, 1H), 7.55−7.58 (m, 3H), 7.39 (s, 1H), 7.26 (d, J = 6 Hz, 2H), 6.24 (s, 1H), 4.72−4.76 (m, 2H), 4.56−4.57 (m, 2H), 3.98 (s, 6H), 1.30 (s, 3H). LC-MS (ESI) m/z 500 $[M + H]^+$.

1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(3-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-5-yl)urea (**35**). Prepared from compound **7f** (104 mg, 0.3 mmol) and compound **16** (89 mg, 0.3 mmol) according to General Procedure H. The crude product was purified by silica gel flash chromatography (eluting with 100% CH_2Cl_2 to 50% EtOAc in CH_2Cl_2) to afford the title compound as a colorless solid (80 mg, 52%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.44 (s, 1H), 9.11 (s, 1H), 8.56 (s, 1H), 7.56−7.58 (m, 2H), 7.40−7.45 (m, 2H), 7.31 (m, 1H), 7.01 (m, 1H), 6.18 (s, 1H), 4.00 (s, 6H), 1.24 (s, 6H). LC-MS (ESI) m/z 518 $[M + H]^+$.

1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(3-(1-(trifluoromethyl)cyclobutyl)isoxazol-5-yl)urea (**36**). Prepared from compound **7h** (147 mg, 0.45 mmol) and compound **16** (89 mg, 0.30 mmol) according to General Procedure H. The crude product was purified by silica gel flash chromatography (eluting with 100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford the title compound as a colorless solid (81 mg, 51%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.50 (br s, 1H), 9.11 (s, 1H), 8.56 (s, 1H), 7.56 (d, J = 2.3 Hz, 2H), 7.29−7.44 (m, 3H), 7.00 (d, J = 7.9 Hz, 1H), 6.08 (s, 1H), 3.99 (s, 6H), 2.50−2.56 (m, 4H), 2.02 (m, 2H). LC-MS (ESI) m/z 530 $[M + H]^+$.

1-(3-(2-Cyanopropan-2-yl)isoxazol-5-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**37**). Prepared from compound **7g** (90 mg, 0.332 mmol) and compound **16** (89 mg, 0.30 mmol) according to General Procedure H. The crude product was purified by silica gel flash chromatography (eluting with 100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford the title compound as a colorless solid (55 mg, 39%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.51 (br s, 1H), 9.12 (br s, 1H), 8.57 (s, 1H), 7.56−7.57 (m, 2H), 7.31−7.45 (m, 3H), 7.01 (m, 1H), 6.27 (s, 1H), 4.00 (s, 6H), 1.68 (s, 6H). LC-MS (ESI) m/z 475 $[M + H]^+$.

1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-(2-fluoropropan-2-yl)isoxazol-3-yl)urea (**38**). Prepared from compound **9j** (90 mg, 0.302 mmol) and compound **16** (90 mg, 0.302 mmol) according to General Procedure H. The crude product was purified by silica gel flash chromatography (eluting with 100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford the title compound as a colorless solid

(37 mg, 26%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.75 (br s, 1H), 9.04 (br s, 1H), 8.56 (s, 1H), 7.56−7.58 (m, 2H), 7.40−7.41 (m, 2H), 7.29 (m, 1H), 7.00 (m, 1H), 6.86 (s, 1H), 4.00 (s, 6H), 1.72 (d, J = 21 Hz, 6H). LC-MS (ESI) m/z 468 $[M + H]^+$.

*1-[5-(1,3-Difluoro-2-methylpropan-2-yl)isoxazol-3-yl]-3-[3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl]urea (**39**).* A mixture of compound **9b** (89 mg, 0.3 mmol), compound **16** (89 mg, 0.3 mmol), and 4-(dimethylamino)pyridine (30 mg, 0.25 mmol) in THF (6 mL) was stirred at rt for 15 h. The reaction mixture was partitioned between CH_2Cl_2 and water, and the organic layer was separated, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 70−95% EtOAc in hexanes) to afford the title compound as a solid (55 mg, 31%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.70 (s, 1H), 9.02 (s, 1H), 8.57 (s, 1H), 7.57−7.58 (m, 2H), 7.38−7.43 (m, 2H), 7.27 (d, J = 9 Hz, 1H), 6.98 (d, J = 9 Hz, 1H), 6.77 (s, 1H), 4.71 (m, 2H), 4.56 (m, 2H), 4.00 (s, 3H), 3.98 (s, 3H), 1.28 (s, 3H). LC-MS (ESI) m/z 500 $[M + H]^+$.

*1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)urea Hydrochloride (**40**).* To a solution of compound **16** (3.52 g, 11.9 mmol) in THF (140 mL) were added compound **9a** (4.1 g, 11.8 mmol) and 4-dimethylaminopyridine (100 mg, 0.8 mmol), and the solution was stirred at rt for 15 h before heating at 45 °C for a further 24 h. After cooling to rt, the mixture was concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 20−100% EtOAc in hexanes) to afford 1-(3-(6,7-dimethoxyquinazolin-4-yloxy)-phenyl)-3-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)urea as a colorless solid (4.55 g, 75%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.74 (s, 1H), 9.01 (s, 1H), 8.56 (s, 1H), 7.56−7.59 (m, 2H), 7.38−7.41 (m, 2H), 7.26 (m, 1H), 6.98 (m, 1H), 6.87 (s, 1H), 3.99 (s, 6H), 1.54 (s, 6H). LC-MS (ESI) m/z 518 $[M + H]^+$. To a stirred solution of 1-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)urea (3.49 g, 6.75 mmol) in CH_2Cl_2 (150 mL) at 0 °C was added slowly a 1 M solution of HCl in diethyl ether (13.50 mL, 13.50 mmol). A colorless precipitate was observed. After stirring for a further 5 min, the mixture was concentrated under reduced pressure. The resulting solid was triturated with diethyl ether,then filtered and dried, to afford the title compound as a colorless solid (3.25 g, 87%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.00 (br s, 1H), 9.73 (br s, 1H), 8.83 (s, 1H), 7.66 (s, 1H), 7.62 (dd, J = 2.1, 2.1 Hz, 1H), 7.48 (s, 1H), 7.43 (dd, J = 8.1, 8.1 Hz, 1H), 7.31 (m, 1H), 7.00 (m, 1H), 6.87 (s, 1H), 4.03 (s, 3H), 4.02 (s, 3H), 1.54 (s, 6H). LC-MS (ESI) m/z 518 $[M + H]^+$.

*1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-(1-(trifluoromethyl)-cyclopropyl)isoxazol-3-yl)urea (**41**).* Prepared from compound **9c** (112 mg, 0.36 mmol) and compound **16** (90 mg, 0.3 mmol) according to General Procedure H to afford the title compound as a solid (107 mg, 69%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.73 (s, 1H), 9.10 (s, 1H), 8.56 (s, 1H), 7.58 (s, 1H), 7.57 (s, 1H), 7.41 (t, J = 8.1 Hz, 1H), 7.39 (s, 1H), 7.26 (d, J = 6 Hz, 1H), 6.98 (d, J = 6 Hz, 1H), 6.85 (s, 1H), 3.99 (s, 3H), 3.98 (s, 3H), 1.56−1.41 (m, 4H). LC-MS (ESI) m/z 516 $[M + H]^+$.

*Preparation of Quinazolin-4-ylthio Phenylurea Derivatives. General Procedure I. 1-[5-(1,3-Difluoro-2-methylpropan-2-yl)-isoxazol-3-yl]-3-[3-(6,7-dimethoxyquinazolin-4-ylthio)phenyl]urea (**42**).* A mixture of compound **9b** (89 mg, 0.3 mmol), compound **17** (94 mg, 0.3 mmol), and 4-(dimethylamino)pyridine (30 mg, 0.25 mmol) in THF (6 mL) was stirred at rt for 15 h. The reaction mixture was partitioned between CH_2Cl_2 and water, and the organic layer was separated, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 70−95% EtOAc in hexanes) to afford the title compound as a solid (48 mg, 31%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.71 (s, 1H), 9.02 (s, 1H), 8.70 (s, 1H), 7.85 (s, 1H), 7.45−7.54 (m, 2H), 7.28−7.36 (m, 3H), 6.78 (s, 1H), 4.72 (m, 2H), 4.56 (m, 2H), 3.99 (s, 6H), 1.29 (s, 3H). LC-MS (ESI) m/z 516 $[M + H]^+$.

*1-(3-(6,7-Dimethoxyquinazolin-4-ylthio)phenyl)-3-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)urea (**43**).* Prepared from compound **9a** (161 mg, 0.46 mmol) and compound **17** (144 mg, 0.46 mmol) according to General Procedure I to afford the title compound as a colorless solid (134 mg, 55%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.77 (s, 1H), 9.04 (s, 1H), 8.70 (s, 1H), 7.86 (s, 1H), 7.28−7.54 (m, 5H), 6.89 (s, 1H), 3.99 (s, 6H), 1.54 (s, 6H). LC-MS (ESI) m/z 534 $[M + H]^+$.

*1-(3-(6,7-Dimethoxyquinazolin-4-ylthio)phenyl)-3-(5-(1-(trifluoromethyl)cyclopropyl)isoxazol-3-yl)urea (**44**).* Prepared from compound **9c** (112 mg, 0.36 mmol) and compound **17** (95 mg, 0.3 mmol) according to General Procedure I to afford the title compound as a solid (108 mg, 68%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.73 (s, 1H), 9.01 (s, 1H), 8.69 (s, 1H), 7.84 (s, 1H), 7.52 (m, 1H), 7.45 (t, J = 9 Hz, 1H), 7.35 (s, 1H), 7.34 (s, 1H), 7.29 (d, J = 6 Hz, 1H), 6.86 (s, 1H), 3.99 (s, 6H), 1.45−1.56 (m, 4H). LC-MS (ESI) m/z 532 $[M + H]^+$.

*1-(3-(6,7-Dimethoxyquinazolin-4-ylthio)phenyl)-3-(3-(2-fluoropropan-2-yl)isoxazol-5-yl)urea (**45**).* Prepared from compound **7c** (253 mg, 0.96 mmol) and compound **17** (200 mg, 0.64 mmol) according to General Procedure I. The crude product was purified by trituration with MeOH to afford the title compound as a colorless solid (142 mg, 46%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.43 (br s, 1H), 9.13 (br s, 1H), 8.70 (s, 1H), 7.84 (s, 1H), 7.57 (m, 1H), 7.46 (m, 1H), 7.30−7.35 (m, 3H), 6.16 (s, 1H), 3.99 (s, 6H), 1.67 (d, J = 21 Hz, 6H). LC-MS (ESI) m/z 484 $[M + H]^+$.

*1-(3-tert-Butyl-1-isopropyl-1H-pyrazol-5-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**46**).* A stirred solution of compound **13a** (150 mg, 0.50 mmol), *N*,*N*-diisopropylethylamine (80 mg, 0.62 mmol), and compound **16** (92 mg, 0.31 mmol) in THF (1 mL) was heated at 60 °C for 15 h. After cooling to rt, the reaction mixture was partitioned between CH_2Cl_2 and saturated aqueous Na_2CO_3. The organic phase was separated, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 2−4% MeOH in CH_2Cl_2) followed by preparative reverse-phase HPLC to afford the title compound as a colorless solid (62 mg, 40%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.08 (s, 1H), 8.57 (s, 1H), 8.43 (s, 1H), 7.55−7.59 (m, 2H), 7.37−7.40 (m, 2H), 7.24 (m, 1H), 6.93 (m, 1H), 6.00 (s, 1H), 4.35 (m, 1H), 4.00 (s, 3H), 3.99 (s, 3H), 1.34 (d, J = 6.4 Hz, 6H), 1.24 (s, 9H). LC-MS (ESI) m/z 505 $[M + H]^+$.

*1-(3-tert-Butyl-1-isobutyl-1H-pyrazol-5-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**47**).* Prepared from compound **13b** (150 mg, 0.47 mmol) and compound **16** (92 mg, 0.31 mmol) according to the procedure described for compound **46** to afford the title compound as a pale yellow solid (60 mg, 38%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.09 (s, 1H), 8.57 (s, 1H), 8.49 (s, 1H), 7.61 (m, 1H), 7.57 (m, 1H), 7.37−7.41 (m, 2H), 7.24 (m, 1H), 6.94 (m, 1H), 6.01 (s, 1H), 4.00 (s, 3H), 3.99 (s, 3H), 3.71 (d, J = 7.6 Hz, 2H), 2.07 (m, 1H), 1.20 (s, 9H), 0.83 (d, J = 6.4 Hz, 6H). LC-MS (ESI) m/z 519 $[M + H]^+$.

*1-(3-tert-Butyl-1-cyclohexyl-1H-pyrazol-5-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**48**).* Prepared from compound **13c** (200 mg, 0.58 mmol) and compound **16** (80 mg, 0.26 mmol) according to the procedure described for compound **46** to afford the title compound as a pale yellow solid (55 mg, 39%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.10 (s, 1H), 8.57 (s, 1H), 8.47 (s, 1H), 7.57−7.60 (m, 2H), 7.37−7.41 (m, 2H), 7.24 (m, 1H), 6.93 (m, 1H), 6.01 (s, 1H), 4.00 (s, 3H), 3.99 (s, 3H), 3.94 (m, 1H), 1.62−1.82 (m, 8H), 1.24−1.35 (m, 2H), 1.24 (s, 9H). LC-MS (ESI) m/z 545 $[M + H]^+$.

*1-(3-tert-Butyl-1-phenyl-1H-pyrazol-5-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**49**).* Prepared from compound **13g** (151 mg, 0.45 mmol) and compound **16** (89 mg, 0.30 mmol) according to General Procedure H. The crude product was purified by silica gel flash chromatography (eluting with 100% hexanes to 85% EtOAc in hexanes) to afford the title compound as a solid (68 mg, 42% yield). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.13 (s, 1H), 8.55 (s, 1H), 8.47 (s, 1H), 7.52−7.55 (m, 6H), 7.36−7.40 (m, 3H), 7.15 (s, 1H), 6.92 (d, J = 7.9 Hz, 1H), 6.36 (s, 1H), 4.00 (s, 6H), 1.25 (s, 9H). LC-MS (ESI) m/z 539 $[M + H]^+$.

*1-(1-Benzyl-3-tert-butyl-1H-pyrazol-5-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)urea (**50**).* Prepared from compound **13d** (105 mg, 0.30 mmol) and compound **16** (90 mg, 0.30 mmol) according to General Procedure H to afford the title compound as a

solid (50 mg, 30%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.00 (s, 1H), 8.59 (br s, 2H), 7.58−6.93 (m, 11H), 6.16 (s, 1H), 5.20 (br s, 2H), 3.98 (s, 6H), 1.21 (s, 9H). LC-MS (ESI) m/z 553 $[M + H]^+$.

*1-(3-(6,7-Dimethoxyquinazolin-4-yloxy)phenyl)-3-(1-phenyl-3-(1,1,1-trifluoro-2-methylpropan-2-yl)-1H-pyrazol-5-yl)urea (**51**).* Prepared from compound **13e** (104 mg, 0.27 mmol) and compound **16** (89 mg, 0.3 mmol) according to the procedure described for compound **46** to afford the title compound as a solid (103 mg, 64%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.28 (s, 1H), 8.55−8.59 (m, 2H), 7.34−7.57 (m, 9H), 7.17 (d, J = 9 Hz, 1H), 6.94 (d, J = 9 Hz, 1H), 6.55 (s, 1H), 4.02 (s, 6H), 1.56 (s, 6H). LC-MS (ESI) m/z 593 $[M + H]^+$.

*1-(1-tert-Butyl-1H-pyrazol-4-yl)-3-(3-hydroxyphenyl)urea (**53**).* To a stirred solution of 1-*tert*-butyl-1*H*-pyrazol-4-amine (995 mg, 7.16 mmol) in THF (20 mL) were added phenyl chloroformate (1.25 g, 8.02 mmol) and potassium carbonate (1.32 g, 9.52 mmol), and the reaction mixture was stirred at rt for 15 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with brine. The organic layer was separated, dried over $MgSO_4$, and then concentrated under reduced pressure to afford phenyl 1-*tert*-butyl-1*H*-pyrazol-4-ylcarbamate as a solid which did not require further purification (1.65 g, 89%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.85 (1H, s), 7.37−7.45 (m, 5H), 7.16−7.19 (m, 2H), 1.60 (s, 9H). To a solution of phenyl 1-*tert*-butyl-1*H*-pyrazol-4-ylcarbamate (782 mg, 3.02 mmol) in THF (10 mL) was added 3-aminophenol (329 mg, 3.02 mmol), and the mixture was heated in a sealed vial at 120 °C for 2 h. After cooling to rt, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc, and the solution was washed with water and brine. The organic layer was dried over $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (eluting with 2−20% MeOH in CH_2Cl_2) to afford the title compound as a solid (169 mg, 20%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.25 (s, 1H), 8.48 (s, 1H), 8.20 (s, 1H), 7.80 (s, 1H), 7.39 (s, 1H), 6.98−7.03 (m, 2H), 6.77 (d, J = 7.9 Hz, 1H), 6.35 (d, J = 7.9 Hz, 1H), 1.49 (s, 9H). LC-MS (ESI) m/z 275 $[M + H]^+$.

*1-(1-tert-Butyl-1H-pyrazol-4-yl)-3-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl) Urea (**54**).* A mixture of compound **53** (169 mg, 0.62 mmol) and cesium carbonate (403 mg, 1.24 mmol) in THF (8 mL) was stirred at rt for 1 h. 4-Chloro-6,7-dimethoxyquinazoline (138 mg, 0.62 mmol) was added, and the mixture was heated at 55 °C for 15 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by preparative reverse-phase HPLC to afford the title compound as a colorless solid (122 mg, 43%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.86 (s, 1H), 8.55 (s, 1H), 8.40 (s, 1H), 7.80 (s, 1H), 7.59 (s, 1H), 7.54 (s, 1H), 7.35−7.40 (m, 3H), 7.23 (d, J = 9 Hz, 1H), 6.88 (d, J = 6 Hz, 1H), 3.98 (s, 6H), 1.50 (s, 9H). LC-MS (ESI) m/z 463 $[M + H]^+$.

Biology. *Kinase Competition Binding Assays.* KINOME*scan* competition binding assays (www.kinomescan.com) were performed as described previously.[10] Kinases were produced displayed on T7 phage or by expression in HEK-293 cells and tagged with DNA. Binding reactions were performed at rt for 1 h, and the fraction of kinase not bound to test compound was determined by capture with an immobilized affinity ligand and quantitation by quantitative PCR. Each kinase was tested individually against each compound. K_d values were determined using eleven serial 3-fold dilutions and presented as mean values from experiments performed in duplicate. Variability between individual values was less than 2-fold.

A375 Cell Phospho-MEK Inhibition Assay. In order to quantify cellular inhibition of $BRAF^{V600E}$, the A375 human melanoma cell line which carries this activating mutation was acquired from the American Type Culture Collection. The cells were seeded at 50,000 per well in a 96-well plate in DMEM (Dulbecco's Modification of Eagle's Medium) (Mediatech) with 10% of Fetal Calf Serum (Omega Scientific) and allowed to attach for at least 6 h. The cells were then washed once with PBS (phosphate buffered saline) and incubated overnight in DMEM with 0.5% of serum. Compounds of interest were added at various concentrations to the cells, and the final DMSO concentration was maintained at 0.5%. The cells were incubated with compounds for 2 h before being washed and lysed using cell extraction buffer (Invitrogen, # FNN0011). Phosphor-MEK Elisa assays were carried out per manufacture's specifications (Biosources, KHO0321). Total MEK Elisa assays from the same lysates were also run in parallel and used for normalization of the phosphor- signal (Biosource, KHO0291). IC_{50} values were derived using a 9-point curve fitted with Igor Pro (WaveMetrics, Inc.) and are presented as mean values from experiments performed in duplicate. Variability between individual values was less than 2-fold.

A375 Cell Proliferation Assay. A375 cells were seeded at 10,000 cells per well in DMEM with 10% fetal calf serum and allowed to attach. The cells were washed with PBS and switched to DMEM with 0.5% of serum and incubated overnight. The test compounds were then added at various concentrations with a final DMSO concentration of 0.5% and incubated for 72 h. At the end of incubation, a Cell Titer Blue (Promega, Madison, WI) was added per instructions, and incubation was continued for 3 h. Remaining viable cells were quantified by measuring the strength of the fluorescence signal using SoftMax Pro (excitation at 560 nm and emission at 590 nm). IC_{50} values were derived using a 9-point curve fitted with Igor Pro (WaveMetrics, Inc.) and are presented as mean values from experiments performed in duplicate. Variability between individual values was less than 2-fold.

Cellular Cytotoxicity Assays. For a comparison of activity in mutated versus wild-type BRAF cell lines, A375, Colo-679, SK-MEL-28, HT-144, Colo-205, HCT 116, Hs578T, LNCaP, DU 145, and PC-3 cell lines were obtained from and cultured in media recommended by ATCC (Manassas, VA). All cell lines were MAP (Mouse Antibody Production) and mycoplasma-tested and deemed contaminant-free prior to *in vitro* and *in vivo* studies. Cells were cultured in 0.5% serum overnight prior to incubation with test compound for 72 h, followed by addition of Cell Titer Blue (Promega, Madison, WI) to detect viable cells after 3 h incubation with reagent. Concentration−response curves were generated from the fluorometric product, and EC_{50} values were determined. Each experiment was conducted in duplicate and repeated at least once.

Microsomal Stability Determinations. Pooled liver microsomes for rat, dog, cynomolgus monkey (In Vitro Technologies, Baltimore, MD), and human (CellzDirect, Dallas, TX) (0.5 mg/mL), 0.1 M phosphate buffer pH 7.4, 6.5 mM $MgCl_2$, and test compound (1 μM; 0.2% final DMSO concentration) were premixed prior to the addition of NADPH (1 mM) to initiate the reaction. A minus NADPH control and testosterone positive control were included. All incubations were carried out at 37 °C. All incubations were performed in duplicate. Aliquots (50 μL) were taken at 0, 5, 15, 30, and 60 min and added to 200 μL acetonitrile containing an internal standard to quench the reaction. Samples were diluted with 100 μL 50/50 acetonitrile/water, vortexed, and centrifuged at 36,000 rpm for 10 min at 4 °C to precipitate the protein. The sample supernatants were analyzed by LC-MS/MS. The t = 0 time point normalized peak area was set to 100%, and the natural log of the percent compound remaining was plotted versus time. The elimination rate constant (k_{el}) was determined by linear regression. Half-life ($t_{1/2}$) and intrinsic clearance (Cl_{int}) were calculated using the following equations: $t_{1/2} = 0.693/k_{el}$ and CL_{int} (μL/min/mg protein) = $0.693V/t_{1/2}$, where V is expressed as μL/mg protein. All microsomes were stored at −80 °C before use.

CYP450 Inhibition Assays. Pooled human liver microsomes (male and female) were purchased from CellzDirect, Dallas, TX (lot HMMC-PL020). The CYP450 inhibition assay was multiplexed, such that individual CYP substrates at 5-fold stock concentration were used, including testosterone (375 μM), diclofenac (80 μM), dextromethorphan (40 μM), mephenytoin (255 μM), and phenacetin (390 μM). Equivolume amounts of the five substrates were mixed to prepare the pooled substrate stock solution. Individual control CYP inhibitors at 5-fold stock concentration were used, including ketoconazole (2.5 μM), sulfaphenazole (10 μM), quinidine (1 μM), ticlopidine (1 μM), and furafylline (0.6 μM). Equivolume amounts of the five control inhibitors were mixed to prepare the pooled inhibitor stock solution. Eight test inhibitor concentrations (0.31, 0.62, 1.25, 2.5, 5, 10, 20, and 40 μM in DMSO; 0.2% final DMSO concentration) were incubated with human liver microsomes (0.25 mg/mL) and NADPH (1 mM) in the presence of the five pooled CYP probe substrates for 20 min at 37 °C. The five pooled CYP inhibitors were screened as positive

controls in parallel with test compounds. Reactions were terminated by the addition of 3:1 acetonitrile/water containing 0.05% formic acid and an internal standard and centrifuged, and the formation of the five CYP isoform-specific metabolites was monitored by LC-MS/MS.

hERG Inhibition Assay. Assays were performed at Aviva Biosciences (San Diego, CA). Aviva's CHO cell line, which stably expresses hERG channels, was employed. Cells were cultured in DMEM/F12 containing 10% FBS (fetal bovine serum), 1% penicillin/streptomycin, and 500 μg/mL Geneticin. Before testing, cells were harvested using Accumax (Innovative Cell Technologies). For electrophysiology experiments, whole cell recordings were performed using PX7000A (Molecular Devices) with Aviva's SealChip technology. An external solution containing 0.1% DMSO (vehicle) was applied to the cells to establish a baseline. After allowing the current to stabilize for 3−10 min, the test article was applied. Test article solutions and cells were kept in test solution until the effect of the test article reached steady state, to a maximum of 12 min. Next, 1 μM of cisapride (positive control) was added. Finally, washout with external solution was performed until the recovery current reached steady state. Data analysis was performed using DataXpress (Molecular Devices), Clampfit (Molecular Devices), and Origin (OriginLab) software.

Rodent Pharmacokinetic Studies. Pharmacokinetic (PK) studies were conducted in accordance with the ILAR Guide for the Care and Use of Laboratory Animals and the USDA Animal Welfare Act and Cephalon Inc. IACUC guidelines. In pharmacokinetic assays, precatheterized (jugular vein) male Sprague−Dawley rats (Charles River, Hollister, CA, 250−350 g, n = 2 (iv) and 3 (po) animals) or female athymic nude mice (Charles River; San Diego, CA, 20−25 g, n = 3 animals) were administered a single dose of compound either iv or po formulated in either a screening vehicle (Pharmatek #6 of the Hot Rod Chemistry vehicle series; Pharmatek, Inc., San Diego, CA) or a safety vehicle, 3:1 PEG400/water or 22−50% HPβCD, at various doses (calculated as free base equivalents), following a minimum of 2 days acclimation to the vivarium. Blood samples were collected at 5 (iv only), 15, and 30 min and 1, 2, 4, 6, and 24 h into K3EDTA tubes and kept on ice until centrifugation. Following centrifugation, plasma was pipetted into 96 well plates and stored at −20 °C for LC-MS analysis. Compound levels in plasma were quantitatively analyzed by LC-MS/MS (API 4000-Qtrap, Applied Biosystems) following protein precipitation with acetonitrile containing an internal standard, and PK parameters were calculated from the normalized LC-MS/MS peak areas using a noncompartmental model with WinNonlin version 5.2, using the linear trapezoidal estimation method. The mean parameters were calculated using plasma concentration−time data for individual animals.

Dog and Monkey Pharmacokinetic Studies. Compound **40** was administered to nondrug-naive male cynomolgus monkeys weighing between 2.6 and 3.4 kg and to beagle dogs weighing between 10.3 and 11.9 kg. Animals received a single 10 mg/kg po dose and a single 1 mg/kg IV dose (n = 3 animals) in a crossover design with a minimum 7 day washout period separating successive phases of dosing. All oral doses were free base equivalents formulated in 22% HPβCD administered *via* nasogastric gavage at a fixed volume of 3 mL/kg (monkeys) and 1 mL/kg (dogs). Intravenous doses were prepared in the same vehicle and administered as bolus injection *via* saphenous vein at a fixed volume of 0.5 mL/kg. Animals were fasted overnight prior to dosing and through approximately 4 h post dose for a total fasting time of 18−23 h. During all phases of dosing, blood samples for pharmacokinetic profiling were collected *via* a femoral vein immediately prior to dosing and at preselected time points through 24 h post dose. Concentrations of compound **40** in plasma samples were determined by LC-MS/MS following acetonitrile precipitation of proteins. PK parameters were calculated from the normalized LC-MS/MS peak areas using a noncompartmental model with WinNonlin version 5.2, using the linear trapezoidal estimation method. The mean parameters were calculated using plasma concentration−time data for individual animals.

Mouse Tumor Xenograft Efficacy Studies. All efficacy studies were conducted in accordance with the ILAR Guide for the Care and Use of Laboratory Animals and the USDA Animal Welfare Act and Cephalon Inc. IACUC guidelines. The Colo-205 cell line was obtained from ATCC (Manassas, VA). Six to eight week old athymic nu/nu nude mice (Charles River; San Diego, CA, 20−25 g) were inoculated subcutaneously with Colo-205 tumor cells (1×10^6/mouse) in the right flank. Upon reaching an average tumor volume of 150−200 mm^3 (10−12 days post implantation), animals were randomized into treatment groups (n = 10 mice/group). Each group was dosed orally for 14 days with either vehicle only (22% HPβCD) or with compound **40** at 10, 30, or 100 mg/kg twice daily (BID), and each dose of drug was given in a volume of 0.1 mL per 20 g of body weight, adjusted for the body weight of the animal. Tumor volumes were measured three times weekly using vernier calipers, and volumes were calculated using the following formula: tumor volume (mm^3) = $W^2(L/2)$, where W = width and L = length in mm. The reported incidence of partial tumor regressions refers to the percent of tumor bearing mice showing a reduction in tumor volume (from initial tumor volume) in 3 or more successive tumor measurements, culminating in tumors whose final volume is smaller than the starting tumor volume but that are still palpable, measurable tumors. Mice were monitored for signs of morbidity (behavioral abnormalities and body weight loss). Upon discontinuation of dosing, animals were evaluated for a period of 7−10 days to assess any potential residual side effects of treatment.

Mouse Tumor Pharmacodynamic−Pharmacokinetic Studies. Studies were conducted in accordance with the ILAR Guide for the Care and Use of Laboratory Animals and the USDA Animal Welfare Act and Cephalon Inc. IACUC guidelines. Athymic nude mice bearing Colo-205 subcutaneous tumors were treated with a single oral dose of vehicle (22% HPβCD) or compound **40** (10, 30, and 55 mg/kg, dosed in 22% HPβCD). At appropriate time points over a 24 h period, animals were sacrificed and plasma and tumor samples were collected and lysates prepared. Amounts of total MEK and pMEK in tumor lysates were measured by immunoblotting and ELISA methods. Compound levels in plasma and tumor tissue samples were analyzed and quantitated by LC-MS/MS. Values given are mean ± SEM from two studies, n = 3 tumor-bearing mice per time point per study.

Computational Chemistry: Ligand Docking Studies. The computational work was carried out using the Schrödinger/Maestro molecular modeling package (Maestro Version 9.1.107, Schrödinger, LLC, New York, NY). The essential steps in the current docking experiment were (1) preparation of two DFG-out BRAF structures from the Protein Data Bank (PDB) (PDB ID: 1uwh, 1uwj),[20] using the Maestro protein preparation workflow, (2) creation of a grid around the ligand, (3) preparation of ligands **4**, **22**, **25**, **30**, and **40** using the LigPrep module, (4) use of Glide/XP docking to keep the top 10 binding poses for each compound, and (5) selection of the binding mode using our previously described knowledge based approach.[22]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The complete list of the 290 individual wild-type kinase targets used for the KINOME*scan* selectivity profiling of the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

ADME, absoption−distribution−metabolism−excretion; BRAF, V-RAF murine sarcoma viral oncogene homologue B1; CYP450, cytochrome P450; ERK, extracellular regulated kinase; hERG, human ether-à-go-go related gene; HPβCD, 2-hydroxypropyl-beta-cyclodextrin; iv, intravenous; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular regulated kinase kinase; PK, pharmacokinetic; po, per os; RAF, rapidly growing fibrosarcoma; SAR, structure−activity relationship

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