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Discovery of a covalent inhibitor of KRAS^{G12C} (AMG 510) for the treatment of solid tumors

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Keywords: RAS pathway; GDP-KRAS; switch II pocket; structure-based design; cryptic pocket; cysteine alkylation; atropisomerism

ABSTRACT: KRAS^{G12C} has emerged as a promising target in the treatment of solid tumors. Covalent inhibitors targeting the mutant cysteine-12 residue have been shown to disrupt signaling by this long-"undruggable" target, however clinically viable inhibitors have yet to be identified. Here, we report efforts to exploit a cryptic pocket (H95/Y96/Q99) we identified in KRAS^{G12C} to identify inhibitors suitable for clinical development. Structure-based design efforts leading to the identification of a novel quinazolinone scaffold are described, along with optimization efforts that overcame a configurational stability issue arising from restricted rotation about an axially chiral biaryl bond. Biopharmaceutical optimization of the resulting leads culminated in the identification of **AMG 510**, a highly potent, selective, and well-tolerated KRAS^{G12C} inhibitor currently in Phase I clinical trials (NCT03600883).

INTRODUCTION

Mutations in the *RAS* oncogene are the most common activating mutation in human cancer, occurring in 30% of human tumors.¹ Although the *RAS* gene family comprises three isoforms (*KRAS*, *HRAS*, and *NRAS*), 85% of *RAS*-driven cancers are caused by mutations in the *KRAS* isoform,² with mutations occurring most frequently in solid tumors such as lung adenocarcinoma, pancreatic ductal carcinoma, and colorectal carcinoma.¹ Amongst *KRAS*-mutant tumors, 80% of all oncogenic mutations occur within codon 12, with the most common mutations being *p.G12D* (41%), *p.G12V* (28%), and *p.G12C* (14%).³

In growth factor signaling pathways, the KRAS protein functions as a molecular switch, regulating proliferation by alternating between a guanosine diphosphate (GDP)-bound inactive form and a guanosine triphosphate (GTP)-bound active form capable of engaging downstream effector proteins to elicit a pro-proliferative response. Codon 12 mutations impair the regulated cycling between these two forms by disrupting the association of GTPase-activating proteins (GAPs), impairing the inactivation of KRAS and leading to the accumulation of the pro-proliferative form. Despite being one of the first oncogenes identified, three decades of effort has failed to identify clinically useful inhibitors of the KRAS protein.⁴ Two features of KRAS confound its tractability as a drug target: (1) KRAS binds to GDP and GTP with picomolar affinity, severely hindering efforts to develop nucleotide-competitive inhibitors, and (2) the KRAS protein lacks other deep surface hydrophobic pockets, thwarting efforts to identify high-affinity allosteric inhibitors.¹

In 2013, Shokat and coworkers reported a novel strategy aimed at overcoming these challenges that used a covalent inhibitor to target the reactive cysteine-12 of KRAS^{G12C,5} They envisioned that covalent modification of Cys12 would allow for persistent disruption of KRAS^{G12C}-driven pro-proliferative signaling by allowing relatively low-affinity, non-covalent interactions to selectively template the formation of a covalent bond between the inhibitor and KRAS, resulting in the permanent inactivation of the adducted protein. In addition to providing a means of overcoming the poor "druggability" of KRAS, it was hoped that this strategy might also allow for the selective growth inhibition of *KRAS*-mutant cells (i.e., tumors) while sparing non-mutant cells (i.e., normal tissue), potentially overcoming the anticipated toxicological challenges posed by non-selective inhibition of *KRAS*-driven cell growth.

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Figure 1. Comparison of the GDP-KRAS^{G12C} binding modes of ARS-1620 (blue, PDB: 5V9U)⁶ and the internally identified indole lead 1 (pink, PDB: 6P8Z)⁷ The tetrahydroisoquinoline portion of indole 1 engages a "cryptic pocket" (magenta) not exploited by prior inhibitors, which is induced by sidechain rotation of H95 and comprises the residues Y96, H95, and Q99. Inhibitor activity on downstream ERK phosphorylation (p-ERK IC₅₀, 2 h incubation) is indicated.

After significant optimization efforts, early hits identified by the Shokat group were modified to provide a tool compound suitable for in vivo applications, ARS-1620 (Figure 1).^{8,9} A cocrystal structure of ARS-1620 with KRAS^{G12C} confirmed that ARS-1620 covalently bound to cysteine-12 of the GDP-bound form of KRAS, with the quinazoline core of the molecule occupying an allosteric pocket (the "switch II pocket" (S-IIP)) located beneath the effector protein-engaging switch II loop region of the protein. While this adduct demonstrated promising anti-proliferative activity against KRAS p.G12Cmutant tumors both in vitro and in vivo,⁹ further improvements in cellular potency appeared necessary to deliver a therapeutically useful molecule. Here, we describe efforts at Amgen to discover and develop a targeted covalent inhibitor of KRAS^{G12C} suitable for clinical application.

RESULTS AND DISCUSSION

Prior to the Shokat lab's initial disclosure of their efforts targeting KRAS^{G12C}, we had independently initiated our own research program to identify covalent inhibitors of KRAS^{G12C}, driven by the same strategic considerations. To support our initial screening efforts and provide access to focused libraries of cysteine-reactive compounds, we entered into a collaboration with Carmot Therapeutics. Using Carmot's Chemotype Evolution platform,¹⁰ we identified a series of selective covalent inhibitors of KRAS^{G12C} that were optimized-via structurebased design and additional rounds of targeted library synthesis-to provide advanced lead 1, which potently inactivated KRASG12C in biochemical and cellular assays.7

Co-crystallization of 1 with GDP-KRASG12C revealed compound 1 to adopt a novel binding mode relative to ARS-1620, with the tetrahydroisoquinoline ring of 1 occupying a previously unexploited cryptic pocket on the surface of KRAS unveiled by rotation of the histidine-95 sidechain and comprising portions of H95, Y96, and Q99 (Figure 1).¹¹ Although engagement of this cryptic pocket led to a multi-fold enhancement in cellular potency relative to ARS-1620, compound 1 suffered from very high clearance and low oral bioavailability in rodent model systems, making it unsuitable for in vivo use. We therefore sought alternative means of exploiting the H95/Y96/Q99 cryptic pocket that might deliver leads with improved pharmaceutical properties.

Superposition of the binding modes of indole lead 1 and ARS-1620 (Figure 2) suggested that substitution of the quinazolinone nitrogen (N1) of ARS-1620 might provide an alternative means of accessing the H95 cryptic pocket, and thus of generating new, enhanced-potency inhibitors of KRAS^{G12C}.

Such a strategy was expected to face some challenges: our prior efforts to modify the quinazoline core of ARS-1620 had shown us that perturbation of the hydrogen bond between N1 and the H95 sidechain of KRAS typically led to significant losses in functional activity. Nevertheless, as this strategy promised to identify new alternatives to the metabolically labile 1, we pursued this approach, anticipating that a successful hybrid molecule would not only deliver enhanced potency (via effective engagement of the cryptic pocket)¹² but also improved ADME properties.



Overlay of the GDP-KRASG12C-bound Figure 2. conformations of ARS-1620 (blue) and indole lead 1 (pink), suggesting potential access to the Y96/H95/Q99 cryptic pocket by substitution of the N1 position of the ARS-1620 scaffold. Conceptual hybrid structure shown at right.

To test this hypothesis, we prepared a series of phthalazine analogues, wherein the C4 position (Y) of the phthalazine core (X = N, Y = C) was substituted with a range of aryl substituents (Table 1). We used a pair of assays to gauge the ability of the resulting analogs to disrupt KRAS-mediated signaling: (1) a cell-free AlphaScreenTM assay,¹³ which measured inhibition of SOS1-catalyzed GDP/GTP exchange by monitoring the disruption of the interaction between GTP-KRASG12C and the Ras binding domain (RBD) of c-RAF, and (2) a cell-based phospho-ERK1/2 immunoassay (MSD),14 which detected decreased ERK phosphorylation resulting from disrupted upstream KRAS signaling. Phenyl substitution of C4 (2) led to an analogue ~10-fold less potent than ARS-1620 in our exchange assay (IC₅₀ = 20.1 μ M) and 20-fold less potent in our p-ERK cellular assay (IC₅₀ = 58 μ M).

Co-crystallization of compound 2 with GDP-KRAS^{G12C} confirmed that compound 2 adopted a similar binding mode to ARS-1620, but with the newly introduced C4 phenyl substituent occupying the H95/Y96/O99 cryptic pocket, as designed (Figure 3a). The C4 phenyl group, however, failed to make many non-covalent contacts with the cryptic pocket residues (e.g., Y96), prompting us to examine whether further ACS Paragon Plus Environment contacts with the cryptic pocket residues that would lead to enhanced potency.



 Table 1. Biochemical and cellular activity of hybrid scaffolds

 designed to access the Y96/H95/Q99 cryptic pocket

-	Cmpd	Х	Y	R	Exchange IC ₅₀ (µM) ^a	p-ERK IC ₅₀ (μM) ^b
	ARS- 1620	СН	N		0.939	0.831
	2	Ν	C	Ì	20.1	58.0
	3	N	С	Me	5.71	3.33
	4	Ν	С	CI CI	3.52	3.53
	5	N	С	Et	0.903	2.58
	6	Ν	C	MeO	9.15	8.05
	7	N	С	c-Pr	1.55	7.15
	8	N	С	i-Pr	0.683	1.80
	9	CO	N	i-Pr	0.101	0.335

^{*a*} SOS1-catalyzed GDP/GTP exchange (AlphaScreenTM, KRAS^{G12C} / c-RAF Ras binding domain), 5 min incubation.^{15 *b*} p-ERK1/2 immunoassay (MSD), EGF-stimulated MIA PaCa-2 cells, 2 h incubation.¹⁶ All data represent $n \ge 2$.

To test this hypothesis, a series of increasingly large orthosubstituents were added to the phenyl ring of **2** (see compounds **3–8**, Table 1) with the aim of enhancing contacts with Y96. Encouragingly, methyl substitution (**3**) provided a 4-fold increase in biochemical potency and 16-fold increase in cellular activity, and ethyl substitution (**5**) led to further gains in potency (exchange IC₅₀ = 0.903 μ M, p-ERK IC₅₀ = 2.6 μ M). Isopropyl substitution (**8**) proved optimal, however, demonstrating biochemical and cellular IC₅₀ values on a par with **ARS-1620**.

In an effort to further enhance the potency of these initial leads, we also examined the effect of changing the phthalazine core of **8**. Notably, quinazolinone **9** (X = CO, Y = N; Table 1) was found to offer a significant improvement in biochemical and cellular potency relative to **8**, with the resulting compound proving \sim 3–9 times more potent than **ARS-1620** in in vitro assays.¹⁷



Figure 3. X-ray crystallography confirms hybrid scaffolds **2** (**a**; tan, PDB: 6PGO) and **9** (**b**; violet, PDB: 6PGP) access the cryptic pocket. Additional van der Waals contacts between the isopropyl moiety of **9** and H95, Y96, and Q99 account for the increased biochemical and cellular potency of **9**.

Co-crystallization of compound 9 with GDP-KRAS^{G12C} (Figure 3b) confirmed 9 to adopt a binding mode in which the isopropylphenyl substituent was positioned in close contact with the Y96, H95, and Q99 residues of the cryptic pocket and one in which the isopropylphenyl and phthalazine rings were nearly orthogonally oriented. Although compound 9 consisted of a mixture of atropisomers about the biaryl C-N bond (due to restricted rotation about the bis-ortho-substituted biaryl linkage), only the R-atropisomer¹⁸ showed significant occupancy of the switch II pocket crystallographically. Separation of the R- and S-atropisomers by chiral chromatography confirmed (R)-9 to be significantly more potent than the corresponding S-atropisomer.¹⁹ Accordingly, subsequent studies were performed with the atropisomer which oriented its larger ortho substituent toward the Y96/H95/Q99 cryptic pocket (typically the R-atropisomer) (Table 2).

Although (R)-9 proved an exceptionally potent inhibitor of KRAS^{G12C} signaling (p-ERK IC₅₀ = 0.130 μ M), the GDP-KRAS^{G12C} co-crystal structure suggested several prospects for further optimization: (1) substitution of the piperazine C2 position appeared to offer an opportunity to enhance activity through additional contacts with C12, E62, and Y96, and (2) replacement of the fluorophenol "tail" of (R)-9 appeared to offer an opportunity to form more extensive contacts with lipophilic residues (e.g., V9 and I100) and to modulate polar interactions with R68 or D69. Additionally, despite the promising potency profile of (R)-9, PK studies revealed no measurable oral bioavailability in BALB/c mice. Low membrane permeability (2 µcm/s in a Madin–Darby Canine Kidney (MDCK) cell transwell permeability assay) and relatively poor aqueous solubility (Table 2) were viewed as likely contributing factors. Therefore, C2 and C7 modifications were also viewed as potential opportunities to modulate the biopharmaceutical properties of (R)-9 to enhance the bioavailability of future analogues.



Table 2. Optimization of the piperazine substituent (R^2) and lipophilic "tail" (R^1) regions of (R)-9 enhances potency & bioavailability

Cmpd	R ^{1a}	R ²	X	p-ERK IC ₅₀ MIA PaCa-2 (µM) ^b	Viability IC ₅₀ MIA PaCa-2 A549 (µM) ^c	$\begin{array}{c} Mu \mid H \ hep \\ CL_{int} \\ (\mu L/min/10^6 \\ cells) \end{array}$	MDCK Permeabili ty A–B (µcm/s)	HT Solubility (μΜ, PBS FaSSIF 0.01 N HCl)	$\begin{array}{c} \text{Mouse CL } (L/h/kg) \\ \text{Vss } (L/kg) t_{1/2} \\ (h) \% F^d \end{array}$
(R)- 9	HO	Н	СН	0.130	0.033 10.3	195 56	2	0 191 0	0.3 0.8 3.4 <0.5
10	OH e	Н	СН		0.017 2.7	l		0 71 0	
(<i>R</i>)-11	N-NH f	Н	СН	0.008	0.004 1.7	180 121	4	0 218 0	4.2 5.3 2.8 <0.5
(<i>R</i>)-12	, F	Н	СН	0.080	0.022 6.4	415 197	7	0 344 0	
(<i>R</i>)-13	, F	Me	СН	0.047	0.006 5.8	356 166	10	0 260 0	1.6 1.4 0.9 12
(<i>R</i>)-14		Me	СН	0.220	0.018 7.4	397 317	6	0 255 0	
15	F ₃ C	Me	СН	0.442	0.040 7.4	270 148	3	0 218 0	
(<i>R</i>)- 16	HO	Me	СН	0.028	0.014 15.3	263 113	1	0 226 0	
(<i>R</i>)-17	HO	Н	N	0.069	0.011 11.5	109 32	6	14 204 14	0.8 0.5 1.5 1.3
(<i>R</i>)-18	HO	Me	N	0.044	0.005 14.2	119 38	9	9 184 7	0.4 0.4 1.3 33
(<i>R</i>)-19	F	Me	Ν	0.066	0.005 7.9	236 135	20	11 224 0	3.6 2.3 0.6 9
(R)- 20		Me	N	0.056	0.016 7.1	319 205	12	0 175 0	5.4 4.8 0.6 12
(<i>R</i>)- 21	НО	Me	N	0.146	0.013 12.2	288 82	18	88 299 57	1.7 0.8 0.5 10

^a Unless noted, separable C7 biaryl bond atropisomers were not observed. ^b p-ERK1/2 immunoassay (MSD), 2 h incubation. ^c Viability assessed at 72 h by CellTiter-Glo[®] luminescence assay (Promega) in MIA PaCa-2 (p.G12C) and A549 (p.G12S) cell lines. All data represent $n \ge 2$. ^d IV/PO dosing in BALB/c mice (vehicle, IV: 1 mg/kg, DMSO; PO: 10 mg/kg, 1% Tween 80, 2% HPMC, 97% water). ^e Mixture of four atropisomers (isopropylphenyl and naphthol biaryl bonds). f Single indazole biaryl bond atropisomer. g Mixture of isopropylphenyl atropisomers.

We began our SAR efforts around (R)-9 by replacing its C7 fluorophenol moiety with larger groups designed to make additional contacts with residues in the "tail" sub-pocket. While we continued to employ an ERK phosphorylation assay as our primary screen for KRAS-based cell activity, we also profiled new compounds in CellTiter-Glo®-based proliferation assays²⁰ using KRAS p.G12C (MIA PaCa-2) and KRAS p.G12S (A549) cells to confirm that growth-inhibitory activity was specific to *p.G12C*-mutant cells and sparing of those in which Gly12 was instead mutated to serine (G12S). Naphthol (10) and indazole (11) substituents did indeed show enhanced activity in ERK phosphorylation and viability assays (Table 2), however these analogues continued to demonstrate poor aqueous solubility, low MDCK permeability, and, in the case of indazole 11, no improvement in oral bioavailability.

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In an effort to enhance MDCK permeability, we examined the effect of removing the phenolic moiety from (R)-9 to provide fluorophenyl 12. Interestingly, this modification led to no loss in activity in either the p-ERK or viability assays, and a more than 3-fold increase in MDCK permeability. Good MDCK permeability was also retained in a related analog wherein a piperazine C2-methyl substituent was introduced (13). Notably, methylpiperazine 13 not only demonstrated further improvements in cellular activity (p-ERK $IC_{50} = 47 \text{ nM}$) but also measurable mouse oral bioavailability (12%). Attempts to replace the fluorophenyl tail of 13 with larger lipophilic substituents (e.g., o-chlorophenyl (14) or otrifluorotolyl (15)) proved detrimental, however, and led to reduced cellular activity and MDCK permeability. Likewise, while re-introduction of the phenolic moiety into 13 (to provide fluorophenol 16) again had minimal impact on cellular activity, membrane permeability was dramatically reduced ($P_{AB} = 1$ μ cm/s).

With the phenolic functionality of (R)-9 and (R)-16 emerging as a key contributor to the reduced MDCK permeability of these analogues, we also decided to examine an alternative strategy to mitigate the adverse effect of this group. As de-solvation of the phenol during membrane diffusion was hypothesized to add to the energetic cost of membrane permeation, we investigated whether nitrogen incorporation at the C8 position of the quinazolinone ring could enhance MDCK permeability by providing an internal hydrogen-bond acceptor to satisfy the adjacent phenolic donor during membrane permeation.²¹ Gratifyingly, azaquinazolinones 17 and 18 both demonstrated not only significantly enhanced MDCK permeability relative to their non-aza congeners (9 and 16), but also significantly improved aqueous solubility. While the oral bioavailability of compound remained disappointing 17 (1.3%),azaquinazolinone 18 demonstrated significantly enhanced oral bioavailability (33%) and excellent cellular activity (p-ERK $IC_{50} = 44 \text{ nM}$; MIA PaCa-2 viability $IC_{50} = 5 \text{ nM}$).

While further improvements in MDCK permeability could be achieved by removing the phenolic functionality from compound 18 (see compounds 19 & 20), the resulting compounds suffered from reduced bioavailability and increased in vivo clearance relative to 18. Similarly, although removal of the fluorine substituent from 18 (to provide phenol 21) enhanced both MDCK permeability and aqueous solubility, such benefits came at the cost of reduced potency, metabolic

stability, and oral bioavailability. Thus, due to its unique balance of promising pharmacokinetic profile and excellent potency, azaquinazolinone 18 became our new lead for subsequent optimization.

Disappointingly, we soon discovered a new challenge with compound 18, originating in the axial chirality of its isopropylphenyl-quinazolinone biaryl bond. Although the sterically congested environment about this bond dramatically restricted rotation, giving rise to separable atropisomers, it unfortunately did not *completely* restrict rotation about this bond. Slow interconversion of the *R*- and *S*-atropisomers occurred at 25 °C with a half-life of 8 days and an interconversion free energy barrier (ΔG^{\ddagger}) of 26 kcal/mol,²² behavior which promised to greatly complicate the development of (R)-18 as a pure substance (Figure 4).



Figure 4. Compound 18 atropisomers are not configurationally stable at 25 °C.

Strategies for addressing atropisomerism in drug discovery have previously been reviewed,²³ however, and drawing upon this guidance, we initiated a trio of strategies to identify a lead molecule with atropisomer configurational properties suitable for drug development needs. These strategies included (1) raising the energetic barrier to atropisomer interconversion $(\Delta G^{\ddagger} > 30 \text{ kcal/mol})$ to allow for the development of a single, pure atropisomer, (2) lowering the interconversion barrier (ΔG^{\ddagger} < 20 kcal/mol) to allow for the development of a freely interconverting mixture of atropisomers, or (3) symmetrizing the cryptic pocket substituent to avoid the generation of an axis of chirality.

We used two methods to assess the atropisomer interconversion free-energy barriers of our lead molecules: slowly interconverting atropisomers were assessed using timecourse ¹H NMR experiments, fitting atropisomer ratio information with the Eyring equation to determine ΔG^{\ddagger} ; rapidly interconverting atropisomers, in contrast, were assessed using the VT NMR method described in Figure 5.24

We first explored methods to further restrict rotation about the isopropylphenyl-quinazolinone biaryl bond, probing the effect of ortho-substituent steric demand on the rotational freeenergy barrier. While replacement of isopropyl substituent with ACS Paragon Plus Environment (22) did little to alter the atropisomer

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interconversion barrier (and led to some loss in activity, see Table 3), introduction of a *tert*-butyl group (23) led to significantly increased configurational stability, with minimal loss in activity. Bis-ortho substitution (24) proved optimal, however, both locking of rotation about the biaryl bond (ΔG^{\ddagger} > 30 kcal/mol) and leading to enhanced biochemical and cellular activity (p-ERK $IC_{50} = 28$ nM). The isopropyl group in 24 proved crucial to this effect, as its replacement with a nonbranched ethyl substituent (25) led to a dramatic reduction in the biaryl rotational barrier ($\Delta G^{\ddagger} = 24$ kcal/mol), as well as some loss in biochemical potency.



Figure 5. VT NMR method for determining atropisomer interconversion free energy barriers (ΔG^{\ddagger}). Coalescence of the H5¹H NMR resonance was monitored at elevated temperature, and the coalescences temperature (T_c) and chemical shift difference (Δv ; slow exchange limit) were used for calculation of ΔG^{\ddagger} . (Compensation applied for minor temperature dependence in the H5 chemical shift.) ¹H spectra (DMSO- d_6) for compound 27 are depicted. At 298 K, methylpiperazine conformational exchange causes H5 line broadening, which is averaged out at T > 320 K.

We next examined whether replacement of the isopropylphenyl ring of (R)-18 with less sterically demanding groups could lower the rotational barrier sufficiently to restore free rotation about the biaryl bond. Replacing the isopropylphenyl group with an analogous isopropylpyridine (c.f., compound 26) meaningfully reduced the rotational barrier $(\Delta G^{\ddagger} = 23.5 \text{ kcal/mol})$, however the resulting atropisomers remained metastable ($t_{1/2} \sim 5$ h at 25 °C) and unsuitable for further development. Contraction of the pendant six-membered ring to a five-membered ring (c.f., pyrazole 27) further reduced the rotational barrier ($\Delta G^{\ddagger} = 21.5$ kcal/mol) but failed to fully free rotation about the biaryl bond and led to moderate losses in biochemical and cellular potency. Introducing a sulfur atom at the open ortho-position of the five-membered ring (c.f., 28), however both restored free rotation about the biaryl bond and ACS Paragon Plus Environment

restored biochemical and cellular activity. Interestingly, attempts to fully restrict biaryl bond rotation by bis-orthosubstitution of five-membered ring cryptic pocket substituents (c.f., 29) were unsuccessful, contrasting with this strategy's success in the context of six-membered rings substituents (c.f., (R)-24). These studies hence reveal biaryl bond rotation to be influenced by a complex interplay of factors including substituent size, substitution pattern, and ring size.



Table 3. Atropisomer stability and KRAS^{G12C} activity as a function of cryptic pocket arene identity and substitution pattern

Cmpd	\mathbf{R}^{a}	Exchange IC ₅₀ (µM) ^b	p-ERK IC ₅₀ (µM) ^c	Interconve rsion barrier $(\Delta G^{\ddagger},$ kcal/mol) ^d
(R)- 18	j-Pr	0.051	0.044	26.01
22	c-Pr	0.225	0.109	25.01
(R)- 23	t-Bu	0.117	0.051	>30 ²
(R)- 24	i-Pr	0.025	0.028	>30 ²
25	Et Me	0.127	0.025	>24 ²
26	i-Pr	0.083	0.053	23.5 ²
27	i-Pr	0.164	0.218	21.5 ²
28	i-Pr N	0.081	0.063	17.5 ²
(R)- 29	Me	0.053	0.127	25.8 ¹
30	Me	0.306	0.199	NA
31	Et	0.068	0.036	NA



^{*a*} Atropisomer configuration assigned by co-crystallization with GDP-KRAS^{G12C}, except for compound **29** (configuration assigned by analogy: more potent isomer assigned *R*-configuration). ^{*b*} SOS1-catalyzed GDP/GTP exchange (AlphaScreenTM, KRAS^{G12C} / c-RAF Ras binding domain), 5 min incubation. ^{*c*} p-ERK1/2 immunoassay (MSD), 2 h incubation. All data represent $n \ge 2$. For separable atropisomers, data is reported for the more potent *R*-atropisomer, as depicted. ^{*d*} Atropisomer interconversion barrier (ΔG^{\ddagger}) as determined by ^{*l*}time-course NMR experiment or ²VT NMR.

As a final strategy to avoid configurationally unstable biaryl bond atropisomers, we examined the properties of symmetrically bis-ortho-substituted cryptic pocket substituents. Although bis-methyl substitution (**30**) failed to afford a potent lead, bis-ethyl (**31**), cyclopropyl (**32**), and isopropyl (**33**) substitution all afforded compounds with good biochemical and cellular activity and no potential to generate rotational atropisomers.

Although all three strategies to address the axial chirality/configurational stability issue were ultimately successful in identifying potent KRAS^{G12C} inhibitors suitable for subsequent development, the resulting leads demonstrated quite distinct pharmacological profiles when dosed orally (10 mg/kg) in BALB/c mice (Table 4). Whereas (*R*)-24 showed moderate oral bioavailability (21%) and good in vivo target coverage (4.5× coverage of the p-ERK IC₅₀ at C_{max}),²⁵ compound 28 demonstrated much more modest in vivo target coverage, and compounds 31–33 failed to show appreciable in vivo target coverage, even at C_{max}, possibly due in part to the reduced oral bioavailability of these analogues. (*R*)-24 thus became our lead candidate for subsequent pharmacodynamic and efficacy testing in xenograft models.

To test the effect of (R)-24 on KRAS signaling in vivo, (R)-24 was dosed orally in athymic nude mice that had been

subcutaneously implanted with MIA PaCa-2 T2 human tumor cells (homozygous *KRAS p.G12C*). Serum and tumor samples were collected two hours post-dosing, revealing dose-proportional exposure of (*R*)-24 across administered doses (10–100 mg/kg), with maximal suppression of downstream ERK phosphorylation achieved at doses as low as 30 mg/kg (Figure 6a).²⁶ Significantly, at this timepoint, the 30 mg/kg dose group showed unbound plasma and total tumor exposures (3 and 11 nM, respectively) considerably lower than the in vitro p-ERK IC₅₀ (28 nM; determined after a 2 h incubation), providing an initial indication that covalent inactivation of KRAS^{G12C} could have durable downstream effects, even in the absence of circulating drug.

Table 4. Mouse PK profiles^a of compounds with atropisomer configurational properties suitable for development

Cmpd	CL (L/h/kg)	PPB (f _u) ^b	t _{1/2} (h)	%F	10 mg/kg C _{max,u} ^c / p- ERK IC ₅₀
(R)- 24	2.7	0.03	0.5	21	4.5
28	2.2	0.02	1.1	22	1.5
31	3.3	0.03	0.5	8	0.8
32	3.3	0.08	0.5	7	0.6
33	2.3	0.03	0.8	13	0.8

 a IV/PO dosing in BALB/c mice (vehicle, IV: 1 mg/kg, DMSO; PO: 10 mg/kg, 1% Tween 80, 2% HPMC, 97% water). b PPB by ultracentrifugation. c Maximum unbound plasma concentration (C_{max} • f_u)

Greatly encouraged by this result, we subsequently profiled (*R*)-**24** in a nude mouse xenograft efficacy study using the same MIA PaCa-2 T2 cell line employed in the prior study. Dosed orally once daily (10–100 mg/kg) over two weeks following tumor establishment, (*R*)-**24** fully suppressed tumor growth at a dose of 30 mg/kg and elicited tumor regression at doses of \geq 60 mg/kg (Figure 6b).²⁷ A time-course PK study of (*R*)-**24** in nude mice (30 mg/kg, PO) again confirmed results from our earlier PK/PD study, showing that in vivo coverage of the in vitro 2 h p-ERK IC₅₀ for <2–3 h was sufficient to achieve tumor growth stasis (Figure 6c).



Figure 6. (*R*)-24 inhibits ERK1/2 phosphorylation in *KRAS p.G12C* tumors (MIA PaCa-2 T2) and causes tumor regression at QD PO doses of \geq 60 mg/kg. **a**. Mice bearing MIA PaCa-2 T2 tumors were given a single oral dose of vehicle (black bar), (*R*)-24 (blue bars), or **ARS-1620** (red bar); tumors were harvested two hours later and assessed for p-ERK levels. Plasma and tumor concentrations of (*R*)-24 are indicated by red triangles and blue open circles, respectively. Data presented as percent of control versus vehicle \pm SEM (n = 3/group). ****p < 0.0001; *p < 0.05 by one-way Anova followed Dunnett's post-hoc analysis. **b**. Effect of (*R*)-24 on MIA PaCa-2 T2 tumor growth in nude mice (QD PO dosing). Data represent mean tumor volume \pm SEM (n = 10/group). ****p < 0.0001 by RMANOVA followed by Dunnett's post-hoc analysis, #p < 0.0001 regression by paired T-test. **c**. Mean unbound plasma ACS Paragon Plus Environment

concentrations + SEM (n = 3) following 30 mg/kg PO dosing in nude mice. p-ERK $IC_{50,u}$ = in vitro cellular $IC_{50} \cdot f_{u,media}$ (fraction unbound in cell culture media).



Table 5. Biopharmaceutical optimization of (R)-24 significantly enhances bioavailability

Cmpd	R	X	Y	logD (pH 7.4) PSA (Å ²) ^a	p-ERK IC ₅₀ MIA PaCa-2 (µM) ^b	Viability IC ₅₀ MIA PaCa-2 A549 (µM) ^c	$\begin{array}{c} Mu \mid H \\ hep \ CL_{int} \\ (\mu L/min/ \\ 10^6 \\ cells) \end{array}$	MDCK Permea bility A–B (µcm/s)	HT Solubility (μM, PBS FaSSIF 0.01 N HCl)	Mouse CL (L/h/kg) Vss (L/kg) t1/2 (h) %F ^d
(R)- 24	Cl	СН	СН	3.6 89	0.028	0.002 11.9	102 30	15	12 204 9	$1.4 \mid 0.8 \mid 0.9 \mid 4-12^{e}$
(R)- 34	Cl	Ν	СН	2.4 102	0.011	0.001 34.1	75 11	1	46 381 500	3.9 1.4 0.4 <0.5
(S) -35 ^f	Cl	СН	Ν	2.4 102	0.065	0.003 32.4	96 5	2	335 435 534	1.4 0.4 0.4 <0.5
(R)- 36	Cl	Ν	Ν	1.7 115	0.038	0.003 >50	139 5	1	499 500 421	3.5 1.1 0.3 <0.5
(R)- 37	F	СН	СН	3.0 89	0.090	0.011 11.6	111 37	22	41 164 24	2.0 1.0 0.5 <0.5
(R)- 38	F	Ν	СН	2.0 102	0.068	0.005 36.5	36 9	6	423 492 499	1.6 0.7 0.5 22–40
(S) -39 ^f	F	СН	Ν	2.0 102	0.142	0.016 49.1	100 9	1	426 477 484	3.7 1.1 0.3 15
(R)- 40	F	Ν	Ν	1.2 115	0.128	0.009 >50	65 5	<1	495 500 500	4.7 1.7 0.3 10

^a logD (pH 7.4) and PSA (polar surface area) calculated using ACD/Percepta (ACD/Labs, Toronto, Canada) ^b p-ERK1/2 immunoassay (MSD), 2 h incubation. ^c Viability assessed at 72 h by CellTiter-Glo[®] luminescence assay (Promega) in MIA PaCa-2 (p.G12C) and A549 (*p.G12S*) cell lines. All data represent $n \ge 2$. ^{*d*} IV/PO dosing in BALB/c mice (vehicle, IV: 1 mg/kg, DMSO; PO: 10 mg/kg, 1% Tween 80, 2% HPMC, 97% water). ^e Crystalline polymorphs. ^f Presence of the pyridyl nitrogen changes IUPAC substituent priority for naming purposes; the isopropyl substituent remains oriented toward the cryptic pocket, as in other analogues.

It came as a great disappointment to us, then, when we subsequently found crystalline polymorphic forms of (R)-24 to have significantly reduced oral bioavailability (4-12%) relative to that of the amorphous form used in our earlier studies (Table 5), impairing our ability to achieve suitable plasma exposures in future studies. As reduced bioavailability coincided with dramatically reduced solubility in biorelevant media (<0.004 mg/mL (crystalline forms) vs. >0.108 mg/mL (amorphous form) in FaSSGF, FaSSIF, PBS), we subsequently prioritized efforts to identify (R)-24 analogues that demonstrated superior aqueous solubility. Two strategies dominated these efforts: (1) reducing the lipophilicity of (R)-24 by changing the quinazolinone C6 halogen substituent, and (2) increasing the polar surface area of (R)-24 by introducing an additional nitrogen atom into the cryptic pocket ring (Table 5).

Initial efforts focused on nitrogen atom incorporation into the cryptic pocket ring. Replacement of the carbon atoms adjacent to the isopropyl or methyl substituents with nitrogen atoms (cf., compounds 34 and 35, respectively) significantly enhanced aqueous solubility without appreciably altering KRAS activity. Such substitution also substantially reduced MDCK permeability, however, affording molecules with no measurable oral bioavailability (BALB/c mice). Although bis-nitrogen substitution (36) likewise further enhanced aqueous solubility, the resulting pyrimidine analogue similarly suffered from low permeability and no measurable oral bioavailability.

We next investigated whether reducing the lipophilicity of the quinazolinone C6 substituent could enhance aqueous solubility while preserving cellular activity. While replacing

the C6 chloro substituent of (R)-24 with a fluoro substituent (compound 37) led to a modest loss of activity in cellular assays (e.g., p-ERK IC₅₀ = 90 nM), the change also resulted in a modest increase in aqueous solubility (~3-fold) and no adverse impact on membrane permeability ($P_{AB} = 22 \ \mu \text{cm/s}$). Unfortunately, (R)-37 failed to show any measurable oral bioavailability (%F Combining C6 fluoro substitution with nitrogen <0.5). incorporation in the cryptic pocket arene ring (compounds 38-40), however, overcame the consistently low bioavailabilities seen with prior analogues. Although nitrogen atom incorporation again led to significantly reduced MDCK permeabilities for all three compounds, this combination of features led to dramatically enhanced aqueous solubilities $(>423 \mu M \text{ in all biorelevant media})$. From these studies, (R)-38 emerged as the standout molecule, showing good activity in cellular assays (p-ERK $IC_{50} = 68 \text{ nM}$), moderate permeability $(P_{AB} = 6 \ \mu cm/s)$, and exceptional oral bioavailability (22–40%) as crystalline form).

Gratifyingly, (R)-38 demonstrated dose-proportional plasma exposure when dosed orally (0.3-100 mg/kg) in our nude mouse MIA PaCa-2 xenograft model, and significantly suppressed ERK phosphorylation at doses $\geq 10 \text{ mg/kg}$ (Figure 7a).²⁶ To further interrogate the PK/PD relationship for (*R*)-38, we also conducted a time-course pharmacodynamic study in this xenograft model (Figure 7b). This study supported earlier results with (R)-24 and (R)-38, showing that, while maximal suppression of ERK phosphorylation was achieved 60-120 min post-dosing, peak (R)-38 plasma and tumor exposures were achieved 30 min post-dosing, and by the 120 min timepoint, only low concentrations of (R)-38 remained in circulation. ACS Paragon Plus Environment

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A time-course study of KRAS^{G12C} ligand occupancy in the MIA PaCa-2 xenograft model shed further light on this PK/PD relationship (Figure 7c). Using protein immunocapture, trypsin digestion, and mass spectrometry, KRAS^{G12C} covalent modification in tumor cells recovered from treated mice was monitored over time, revealing suppression of ERK

phosphorylation to closely mirror the extent of covalent modification of KRAS^{G12C,26} Covalent modification of KRAS^{G12C} was nearly complete 120 min post-dosing, at which point minimal (R)-**38** remained in circulation.



Figure 7.²⁸ p-ERK (**a**) dose-response and (**b**) time-course (MSD) data with plasma and tumor exposures for compound (*R*)-**38**. Mice bearing MIA PaCa-2 T2 tumors were given a single oral dose of either vehicle (black bars) or (*R*)-**38** (blue bars) and harvested at the indicated time. Data represent percent of control versus vehicle \pm SEM (n = 3/group). Plasma and tumor concentrations of (*R*)-**24** are indicated by red triangles and blue open circles, respectively. ****p < 0.0001; ***p < 0.001; *p < 0.05 by one-way Anova followed Dunnett's post-hoc analysis. **c**. p-ERK POC (MSD) and percent KRAS^{G12C} covalent modification (MS) time-course in MIA PaCa-2 T2 mouse xenografts. Markers represent mean p-ERK1/2 levels (percent of basal ERK1/2) \pm SEM and mean %covalent modification \pm SEM (n = 3/group).

Encouraged by these promising pharmacodynamic effects, (*R*)-**38** was profiled in a mouse xenograft efficacy study employing MIA PaCa-2 T2 (*p.G12C*) tumor cells. Once-daily oral dosing (10–100 mg/kg) achieved 86% tumor growth inhibition (TGI) at 10 mg/kg and produced significant tumor regression at doses of \geq 30 mg/kg (Figure 8a). Unbound plasma exposures from this study (Figure 8b) again illustrated that durable growth responses could be achieved in the absence of

continuous drug exposure given the covalent inhibitor mechanism of action. At a dose of 10 mg/kg, plasma coverage of the in vitro p-ERK IC₅₀ was only sustained for ~1 h, and circulating, unbound concentrations of (*R*)-**38** had dropped below 1 nM 8 hours post dosing. Nonetheless, 1 h coverage of the in vitro p-ERK IC₅₀ proved sufficient to achieve near-stasis in tumor growth, and in vitro IC₅₀ coverage for as little as 2 h (30 mg/kg dose) was sufficient to cause tumor regression.



Figure 8.²⁸ Compound (*R*)-**38** dosed orally once daily results in regression of *KRAS p.G12C* tumor xenografts. **a**. Effect of compound (*R*)-**38** on MIA PaCa-2 T2 xenograft growth in nude mice (QD PO dosing). Data represent mean tumor volume \pm SEM (n = 10/group). ***** **p** < 0.0001 by Dunnett's post-hoc analysis; #**p** < 0.05 regression by paired T-test. **b**. Mean unbound plasma concentrations \pm SEM (n = 2/group). **p**-ERK IC_{50,u} = in vitro cellular IC₅₀ • **f**_{u,media} (fraction unbound in cell culture media).

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Figure 9. X-ray crystal structure of compound (*R*)-**38** bound to GDP-KRAS^{G12C} (cyan, PDB: 60IM)

Delighted by the promising pharmacodynamic effect of (R)-38 in our mouse models, we undertook further characterization of (R)-38 to assess its potential as a possible development candidate. Co-crystallization with KRAS^{G12C} revealed (R)-38 to adopt a similar binding mode to prior quinazolinone leads, with the quinazolinone core of (R)-38 occupying the KRAS switch II pocket and the acrylamide moiety making a covalent bond with C12 (Figure 9). The (S)-methyl piperazine ring of (R)-38 adopted a twist-boat conformation, with the C2 methyl substituent making close contacts with C12 and Y96. Critically, the isopropyl substituent of the pyridyl ring again made close contacts with Y96, H95, and Q99, largely filling the cryptic pocket revealed by sidechain rotation of the H95 residue and contributing to the exceptional potency of this molecule. Nitrogen substitution of the cryptic pocket arene ring was not found to adversely impact the configurational stability of the potent R-atropisomer, which was calculated to have a racemization $t_{1/2}$ of >180 years at 25 °C ($\Delta G_{rotation}^{\ddagger}$ > 31 kcal/mol; Table 6).

 Table 6.
 Additional in vitro and PK characterization of compound (R)-38

Atropisomer interconversion barrier $(\Delta G^{\ddagger}, \text{kcal/mol})^a$	>31
$\begin{tabular}{l} Rat CL (L/h/kg) Vss (L/kg) \\ t_{1/2} (h) \%F^b \end{tabular}$	3.4 2.0 0.5 28
$\label{eq:log_cl} \begin{array}{ c c c c c c c c c c c c c c c c c c c$	2.2 0.73 0.4 34
PPB Mu R D Hu (0.25 µM, UC, f _u)	0.06 0.05 0.17 0.09
KRAS ^{G12C} k _{inact} /K _I (M ⁻¹ s ⁻¹) ^d	$9,900 \pm 1,800$
5 mM GSH t _{1/2} (min) ^e	200 min

^{*a*} Determined by NMR kinetic data. ^{*b*} IV/PO dosing in Sprague-Dawley rat (vehicle, IV: 1 mg/kg, DMSO; PO: 10 mg/kg, 1% Tween 80, 2% HPMC, 97% water). ^{*c*} IV/PO dosing in Beagle dog (vehicle, IV: 1 mg/kg, 10% DMAC, 10% EtOH, 30% propylene glycol, 50% water; PO: 10 mg/kg, 1% Tween 80, 2% HPMC, 97% water). ^{*d*} k_{inact}/K₁ was determined from time-course relative %bound data at varied inhibitor concentrations, as assessed by mass spectrometry.^{28,31} ^{*e*} t_{1/2} determined by parent depletion (MS detection) in 5 mM GSH, 37 °C, pH 7.4 aqueous phosphate buffer.³²

Rat and dog pharmacokinetic parameters for (R)-**38** were in line with those observed in mouse, with reasonable oral bioavailability observed in all species. Although terminal half-ACS Paragon lives for (*R*)-**38** were relatively short in all species, this was not viewed as a liability, given our findings from prior pharmacodynamic studies that durable covalent inhibition of KRAS^{G12C} could be achieved without continuous drug exposure. Such persistent PD effects are in line with those expected for covalent inhibitors, where duration of target inhibition is determined not by the duration of target IC₅₀ coverage but rather by the resynthesis rate of the covalently inactivated target protein.²⁹ Given KRAS's long protein half-life (~22 h),³⁰ rapid covalent inactivation allows for durable pharmacodynamic effects even in the face of relatively short inhibitor plasma half-lives.

Although mass spectrometric studies³¹ established (*R*)-**38** to have a second-order rate constant for covalent inactivation of KRAS^{G12C} (k_{inact}/K_1) of 9,900 M⁻¹s⁻¹, rapid inactivation of KRAS was not indicative of promiscuous cysteine reactivity. Incubation of (*R*)-**38** under conditions mimicking intracellular glutathione levels (5 mM GSH, 37 °C, pH 7.4)³² revealed (*R*)-**38** to have a GSH conjugation half-life of 200 min. Cysteineproteome profiling (MS) further revealed (*R*)-**38** to be highly selective for covalent modification of KRAS^{G12C} of 6451 cysteine-containing peptides profiled, only the KRAS^{G12C} C12 peptide was found to be modified.²⁸

Based on the compelling pharmacological profile of (R)-38 and its marked ability to regress *KRAS p.G12C*-mutant tumors in vivo, promising biopharmaceutical properties, and excellent tolerability in preclinical toxicological models,³³ we nominated (R)-38 for clinical development as AMG 510. AMG 510 entered human clinical trials in August 2018, and a phase I/II trial evaluating its safety, tolerability, pharmacokinetic properties, and efficacy in *KRAS p.G12C* mutant tumors is currently ongoing (NCT03600883³⁴). Early results from this study have confirmed AMG 510 to demonstrate excellent tolerability and promising antitumor activity when administered as a monotherapy to patients with advanced *KRAS p.G12C*-mutant solid tumors.³⁵ Dose-expansion efforts are currently ongoing.

CONCLUSIONS

In conclusion, by exploiting a previously unrecognized H95/Y96/Q99 cryptic pocket in GDP-KRAS^{G12C}, we were able to create highly potent and selective covalent inhibitors of KRAS^{G12C}. A collaboration with Carmot Therapeutics⁷ enabled us to rapidly screen cysteine-reactive libraries and investigate the potential of this cryptic pocket. Structure-based design efforts facilitated our discovery of a guinazolinone-based scaffold, which exploited this pocket for enhanced potency and provided ADME properties for suitable further optimization. Optimally leveraging the H95/Y96/Q99 cryptic pocket required us to address axial chirality and configurational stability issues, which were overcome by optimization of the cryptic pocketengaging arene moiety. Further refinement of the resulting leads to address permeability, solubility, and oral bioavailability challenges then led to our discovery of AMG 510. AMG 510 has shown great promise in the treatment of KRAS p.G12C-mutant tumors both pre-clinically and clinically. Efforts to further demonstrate the clinical potential of AMG 510 are currently underway.

EXPERIMENTAL SECTION

General synthetic procedures. All materials were obtained from commercial suppliers and used without further purification unless otherwise noted. Anhydrous solvents were obtained from Sigma-Aldrich and used directly. Reactions

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involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. Silica gel chromatography was performed using pre-packed silica gel cartridges (RediSep® Rf, Teledyne ISCO). Reverse-phase HPLC purification was performed using Gilson (Middleton, WI) workstations. NMR spectra were acquired on Bruker Avance 400, 500, or 600 MHz spectrometers equipped with 5 mm BBFO probes. All final compounds were purified to >95% purity as determined by LC-MS using an Agilent 1100 or 1260 multi-wavelength detector (215 nm detection) and an Advanced Materials Technology HALO C18 column (50×3.0 mm, 2.7 µm) at 40 °C with a 2.0 mL/min flow rate using a 5-95% gradient of acetonitrile/water with 0.1% trifluoracetic acid over 1.5 min. Low-resolution MS data were obtained concurrently with UV chromatography using an Agilent G1956B MSD SL, 6120B, 6130B, or 6140A quadrupole MS in positive electrospray ionization mode. Resolved atropisomers were purified to >95% ee (or de) using a Thar 80, 200, or 350 preparative SFC. Enantiomeric, and diastereomeric excesses were determined by SFC (Waters Acquity UPC2 or Agilent 1260 Infinity analytical systems).

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Synthesis of AMG 510 [(R)-38]. Step 1. 2,6-Dichloro-5fluoronicotinamide. Oxalvl chloride (2 M solution in DCM, 11.9 mL, 23.8 mmol) and DMF (0.05 mL) were sequentially added to 2,6-dichloro-5-fluoro-nicotinic acid (4.0 g, 19.1 mmol, AstaTech, Inc.) in DCM (48 mL), and the resulting mixture was stirred at ambient temperature for 16 h. The reaction mixture was then concentrated in vacuo, and the residue was dissolved in 1,4-dioxane (48 mL) and cooled to 0 °C. Ammonium hydroxide solution (28-30% NH₃ basis, 3.6 mL, 28.6 mmol) was slowly added, and the resulting mixture was stirred at 0 °C for 30 min. The mixture was then concentrated in vacuo, and the residue diluted with 1:1 EtOAc/heptane, agitated for 5 min, and filtered. The filtrate was concentrated to half-volume and re-filtered. The combined collected solids were washed with heptane and dried overnight in a reduced-pressure oven (45 °C) to provide 2,6-dichloro-5-fluoronicotinamide (2.0 g, 50%) yield). ¹H NMR (400 MHz, DMSO- d_6) δ 8.23 (d, J = 7.9 Hz, 1 H) 8.09 (br s, 1 H) 7.93 (br s, 1 H). ¹⁹F NMR (376 MHz, DMSO d_6) δ : -122.39 (s, 1 F). m/z (ESI, +ve ion): 208.9 (M+H)⁺.

2,6-Dichloro-5-fluoro-N-((2-isopropyl-4-2. Step methylpyridin-3-yl)carbamoyl)nicotinamide. To an icecooled slurry of 2,6-dichloro-5-fluoronicotinamide (5.0 g, 23.9 mmol) in THF (20 mL) was slowly added oxalyl chloride (2 M solution in DCM, 14.4 mL, 28.8 mmol). The resulting mixture was stirred at 75 °C for 1 h, then allowed to cool and concentrated in vacuo to half volume. The concentrate was cooled to 0 °C and diluted with THF (20 mL). A solution of 2isopropyl-4-methylpyridin-3-amine (3.59 g, 23.9 mmol) in THF (10 mL) was added dropwise via cannula. The resulting mixture was stirred at 0 °C for 1 h, diluted with a 1:1 mixture of brine and saturated aqueous ammonium chloride, and extracted with EtOAc $(3\times)$. The combined organic extracts were dried over anhydrous sodium sulfate and concentrated in vacuo to provide 2,6-dichloro-5-fluoro-N-((2-isopropyl-4methylpyridin-3-yl)carbamoyl)nicotinamide, which was used without further purification. m/z (ESI, +ve ion): 385.1 (M+H)⁺.

Intermediate, Step 2. 2-Isopropyl-4-methylpyridin-3amine: To a slurry of 3-amino-2-bromo-4-picoline (360 mg, 1.9 mmol; Combi-Blocks, Inc.) in THF (4 mL) was added [1,1'bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with DCM (79 mg, 0.10 mmol). The resulting slurry was deoxygenated with argon, then 2-propylzinc bromide (0.5 M solution in THF, 5.4 mL, 2.7 mmol) was added. The resulting solution was stirred at 60 °C for 17 h, then the reaction m), 3.88–4.07 (1 H, m), 3.78–3.87 (1 H, m), 3.59–3.74 (1 H, ACS Paragon Plus Environment

was allowed to cool to ambient temperature. Water (10 mL) and 1 N NaOH solution (20 mL) were sequentially added, and the resulting mixture was extracted with EtOAc $(2\times)$. The combined organic extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatographic purification of the residue (silica gel; 0-15% MeOH/DCM) provided 2-isopropyl-4-methylpyridin-3-amine (284 mg, 98% yield) as a brown solid. ¹H NMR (400 MHz, DMSO- d_6) δ ppm 7.66 (d, J = 4.6 Hz, 1 H), 6.78 (d, J = 4.8 Hz, 1 H), 4.72 (br s, 2 H), 3.14-3.25 (m, 1 H), 2.08 (s, 3 H), 1.14 (d, J = 6.8 Hz, 6 H). m/z (ESI, +ve ion): 151.1 (M+H)⁺.

3. 7-Chloro-6-fluoro-1-(2-isopropyl-4-Sten methylpyridin-3-yl)pyrido[2,3-d]pyrimidine-2,4(1H,3H)dione. To an ice-cooled solution of 2,6-dichloro-5-fluoro-N-((2-isopropyl-4-methylpyridin-3-yl)carbamoyl)nicotinamide (9.2 g, 24.0 mmol) in THF (40 mL) was slowly added KHMDS (1 M solution in THF, 50.2 mL, 50.2 mmol). The ice bath was removed, and the resulting mixture was stirred for 40 min at ambient temperature. Saturated aqueous ammonium chloride solution was added, and the resulting mixture was extracted with EtOAc $(3\times)$. The combined organic extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatographic purification of the residue (silica gel; 0–50%) 3:1 EtOAc-EtOH/heptane) provided 7-chloro-6-fluoro-1-(2isopropyl-4-methylpyridin-3-yl)pyrido[2,3-d]pyrimidine-

2,4(1H,3H)-dione (9.24 g, quantitative yield). ¹H NMR (400 MHz, DMSO-d₆) δ 12.27 (br s, 1 H), 8.48–8.55 (m, 2 H), 7.29 $(d, J = 4.8 \text{ Hz}, 1 \text{ H}), 2.87 (quin, J = 6.6 \text{ Hz}, 1 \text{ H}), 1.99-2.06 (m, J = 6.6 \text{ Hz}), 1.99-2.06 (m, J = 6.6 \text$ 3 H), 1.09 (d, J = 6.6 Hz, 3 H), 1.01 (d, J = 6.6 Hz, 3 H). ¹⁹F NMR (376 MHz, DMSO- d_6) δ –126.90 (s, 1 F). m/z (ESI, +ve ion): 349.1 (M+H)+.

4. 4,7-Dichloro-6-fluoro-1-(2-isopropyl-4-Step methylpyridin-3-yl)pyrido[2,3-d]pyrimidin-2(1H)-one.

Phosphorus oxychloride (1.63 mL, 17.5 mmol) was added, dropwise, to a solution of 7-chloro-6-fluoro-1-(2-isopropyl-4methylpyridin-3-yl)pyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (4.7 g, 13.5 mmol) and DIPEA (3.5 mL, 20 mmol) in acetonitrile (20 mL), and the resulting mixture was stirred at 80 °C for 1 h, then cooled to ambient temperature and concentrated in vacuo to provide 4,7-dichloro-6-fluoro-1-(2-isopropyl-4methylpyridin-3-yl)pyrido[2,3-d]pyrimidin-2(1H)-one, which was used without further purification. m/z (ESI, +ve ion): 367.1 $(M+H)^{+}$.

Step 5. (S)-tert-Butyl 4-(7-chloro-6-fluoro-1-(2-isopropyl-4-methylpyridin-3-yl)-2-oxo-1,2-dihydropyrido[2,3-

d]pyrimidin-4-yl)-3-methylpiperazine-1-carboxylate. To an ice-cooled solution of 4,7-dichloro-6-fluoro-1-(2-isopropyl-4methylpyridin-3-yl)pyrido[2,3-*d*]pyrimidin-2(1*H*)-one (13.5 mmol) in acetonitrile (20 mL) was added DIPEA (7.1 mL, 40.3 mmol) followed by (S)-4-Boc-2-methyl piperazine (3.23 g, 16.1 mmol, Combi-Blocks, Inc.). The resulting mixture was warmed to ambient temperature and stirred for 1 h, then diluted with cold saturated aqueous sodium bicarbonate solution (200 mL) and EtOAc (300 mL). The mixture was stirred for an additional 5 min, and the organic layer was collected. The aqueous layer was extracted with additional EtOAc $(1\times)$, and the combined organic extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatographic purification of the residue (silica gel; 0-50% EtOAc/heptane) gave (S)-tert-butyl 4-(7-chloro-6-fluoro-1-(2-isopropyl-4-methylpyridin-3-yl)-2oxo-1,2-dihydropyrido[2,3-d]pyrimidin-4-yl)-3-

methylpiperazine-1-carboxylate (5.71 g, 80% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 8.45-8.51 (1 H, m), 8.31-8.41 (1 H, m), 7.23–7.29 (1 H, m), 4.67–5.01 (1 H, m), 4.09–4.25 (1 H,

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m), 3.04-3.21 (2 H, m), 2.59-2.66 (1 H, m), 1.88-1.98 (3 H, m), 1.43–1.50 (9 H, m), 1.27–1.35 (3 H, m), 1.03–1.10 (3 H, m), 0.94–1.03 (3 H, m). *m/z* (ESI, +ve ion): 531.2 (M+H)⁺.

Step 6. (3S)-tert-Butyl 4-(6-fluoro-7-(2-fluoro-6hydroxyphenyl)-1-(2-isopropyl-4-methylpyridin-3-yl)-2oxo-1,2-dihydropyrido[2,3-d]pyrimidin-4-yl)-3-

methylpiperazine-1-carboxylate. A mixture of (S)-tert-butyl 4-(7-chloro-6-fluoro-1-(2-isopropyl-4-methylpyridin-3-yl)-2-

oxo-1,2-dihydropyrido[2,3-d]pyrimidin-4-yl)-3-

- methylpiperazine-1-carboxylate (4.3 g, 8.1 mmol), (2-fluoro-6hydroxyphenyl)potassium trifluoroborate (2.9 g, 10.5 mmol), potassium acetate (3.2 g, 32.4 mmol) and [1,1'bis(diphenylphosphino)ferrocene]dichloropalladium(II),
- complex with dichloromethane (661 mg, 0.81 mmol) in 1,4dioxane (80 mL) was sparged with nitrogen for 1 min. Deoxygenated water (14 mL) was added, and the resulting mixture was heated at 90 °C for 1 h, then cooled to ambient temperature, diluted with half-saturated aqueous sodium bicarbonate, and sequentially extracted with EtOAc $(2\times)$ and DCM $(1\times)$. The combined organic extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatographic purification of the residue (silica gel; 0-60% 3:1 EtOAc-EtOH/heptane) furnished (3S)-tert-butyl 4-(6-fluoro-7-(2fluoro-6-hydroxyphenyl)-1-(2-isopropyl-4-methylpyridin-3-

yl)-2-oxo-1,2-dihydropyrido[2,3-d]pyrimidin-4-yl)-3-

methylpiperazine-1-carboxylate (4.52 g, 92% yield). ¹H NMR (400 MHz, DMSO- d_6) δ 10.19 (br s, 1 H), 8.38 (d, J = 5.0 Hz, 1 H), 8.26 (dd, J = 12.5, 9.2 Hz, 1 H), 7.23–7.28 (m, 1 H), 7.18 (d, J = 5.0 Hz, 1 H), 6.72 (d, J = 8.0 Hz, 1 H), 6.68 (t, J = 8.9 Hz)Hz, 1 H), 4.77-4.98 (m, 1 H), 4.24 (br t, J = 14.2 Hz, 1 H), 3.93-4.08 (m, 1 H), 3.84 (br d, J = 12.9 Hz, 1 H), 3.52–3.75 (m, 1 H), 3.07–3.28 (m, 1 H), 2.62–2.74 (m, 1 H), 1.86–1.93 (m, 3 H), 1.43–1.48 (m, 9 H), 1.35 (dd, *J* = 10.8, 6.8 Hz, 3 H), 1.26– 1.32 (m, 1 H), 1.07 (dd, J = 6.6, 1.7 Hz, 3 H), 0.93 (dd, J = 6.6, 2.1 Hz, 3 H). ¹⁹F NMR (376 MHz, DMSO-*d*₆) δ –115.6 (s, 1 F), -128.6 (s, 1 F). m/z (ESI, +ve ion): 607.3 (M+H)⁺.

Intermediate, 6. Step (2-Fluoro-6hydroxyphenyl)potassium trifluoroborate: A solution of potassium fluoride (44.7 g, 770 mmol) in water (75 mL) was added to a suspension of (2-fluoro-6-hydroxyphenyl)boronic acid (30 g, 192 mmol, Combi-Blocks, Inc.) in acetonitrile (750 mL). After 2 min of stirring, a solution of L-(+)-tartaric acid (72.2 g, 481 mmol) in THF (375 mL) was added over 10 min. The resulting mixture was mechanically stirred for 1 h. Suspended solids were removed by filtration and washed with a small amount of THF. The combined filtrate was then partially concentrated in vacuo until solids began to precipitate. The filtrate was cooled to -20 °C and stirred for 16 h, then slowly warmed to ambient temperature. 2-Propanol (20 mL) was added, and the precipitated solid was collected by filtration and washed with 2-propanol to provide 27.5 g of solid. The filtrate was again partially concentrated (until precipitation was observed), cooled to -20 °C, and stirred for 20 min. Additional 2-propanol was added, and the precipitated solid was collected by filtration and washed with 2-propanol. The two batches of solid were combined to provide 2-fluoro-6hydroxyphenyl)potassium trifluoroborate (34.6 g, 82% yield). ¹H NMR (400 MHz, DMSO- d_6) δ 8.07 (q, J = 14.7 Hz, 1 H) 6.93 (q, J = 7.5 Hz, 1 H) 6.30-6.38 (m, 2 H).

AMG 510 [(R)-38]. (1R)-6-Fluoro-7-(2-fluoro-6hydroxyphenyl)-1-[4-methyl-2-(1-methylethyl)-3pyridinyl]-4-[(2S)-2-methyl-4-(1-oxo-2-propen-1-yl)-1piperazinyl]-pyrido[2,3-d]pyrimidin-2(1H)-one: Trifluoroacetic acid (25 mL, 320 mmol) was added to a solution

of (3*S*)-*tert*-butyl 4-(6-fluoro-7-(2-fluoro-6-hydroxyphenyl)-1-ACS Paragon Plus Environment

(2-isopropyl-4-methylpyridin-3-yl)-2-oxo-1,2-

dihydropyrido[2,3-d]pyrimidin-4-yl)-3-methylpiperazine-1carboxylate (6.3 g, 10 mmol) in DCM (30 mL), and the resulting mixture was stirred at ambient temperature for 1 h. The mixture was then concentrated in vacuo, and the residue was taken up in DCM (30 mL), cooled to 0 °C, and sequentially treated with DIPEA (7.3 mL, 42 mmol) and a solution of acryloyl chloride (0.849 mL, 10.4 mmol) in DCM (3 mL; added dropwise). The resulting mixture was stirred at 0 °C for 10 min, then diluted with half-saturated aqueous sodium bicarbonate and extracted with DCM $(2\times)$. The combined extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatographic purification of the residue (silica gel; 0–100% 3:1 EtOAc-EtOH/heptane) followed by chiral supercritical fluid chromatography (Chiralpak IC, 30 × 250 mm, 5 µm, 55% MeOH/CO₂, 120 mL/min, 102 bar) provided (1R)-6-Fluoro-7-(2-fluoro-6-hydroxyphenyl)-1-[4-methyl-2-(1-methylethyl)-3pyridinyl]-4-[(2S)-2-methyl-4-(1-oxo-2-propen-1-yl)-1piperazinyl]-pyrido[2,3-*d*]pyrimidin-2(1*H*)-one (AMG 510; (**R**)-38; 2.25 g, 43% yield) as the first-eluting peak. ¹H NMR $(600 \text{ MHz}, \text{DMSO-}d_6) \delta \text{ ppm } 10.20 \text{ (s, 1H)}, 8.39 \text{ (d, } J = 4.9 \text{ Hz},$ 1H), 8.30 (d, J = 8.9 Hz, 0.5H), 8.27 (d, J = 8.7 Hz, 0.5H), 7.27

(q, J = 8.4 Hz, 1H), 7.18 (d, J = 4.9 Hz, 1H), 6.87 (dd, J = 16.2),10.8 Hz, 0.5H), 6.84 (dd, J = 16.2, 10.7 Hz, 0.5H), 6.74 (d, J =8.4 Hz, 1H, 6.68 (t, J = 8.4 Hz, 1H), 6.21 (d, J = 16.2 Hz, 0.5H),6.20 (d, J = 16.2 Hz, 0.5H), 5.76 (d, J = 10.8 Hz, 0.5H), 5.76 (d, J = 10.7 Hz, 0.5 H), 4.91 (m, 1 H), 4.41 (d, J = 12.2 Hz, 0.5 H),4.33 (d, J = 12.2 Hz, 1H), 4.28 (d, J = 12.2 Hz, 0.5H), 4.14 (d, J = 12.2 Hz, 0.5H), 4.02 (d, J = 13.6 Hz, 0.5H), 3.69 (m, 1H), 3.65 (d, J = 13.6 Hz, 0.5H), 3.52 (t, J = 12.2 Hz, 0.5H), 3.27 (d, J = 1J = 12.2 Hz, 0.5H), 3.15 (t, J = 12.2 Hz, 0.5H), 2.72 (m, 1H), 1.90 (s, 3H), 1.35 (d, J = 6.7 Hz, 3H), 1.08 (d, J = 6.7 Hz, 3H), 0.94 (d, J = 6.7 Hz, 3H). ¹⁹F NMR (376 MHz, DMSO- d_6) δ – 115.6 (d, J = 5.2 Hz, 1 F), -128.6 (br. s., 1 F). ¹³C NMR (151 MHz, DMSO-d₆) δ ppm 165.0 (1C), 163.4 (1C), 162.5 (1C), 160.1 (1C), 156.8 (1C), 153.7 (1C), 151.9 (1C), 149.5 (1C), 148.3 (1C), 145.2 (1C), 144.3 (1C), 131.6 (1C), 130.8 (1C), 127.9 (0.5C), 127.9 (0.5C), 127.8 (0.5C), 127.7 (0.5C), 123.2 (1C), 122.8 (1C), 111.7 (1C), 109.7 (1C), 105.7 (1C), 105.3 (1C), 51.4 (0.5C), 51.0 (0.5C), 48.9 (0.5C), 45.4 (0.5C), 44.6 (0.5C), 43.7 (0.5C), 43.5 (0.5C), 41.6 (0.5C), 29.8 (1C), 21.9 (1C), 21.7 (1C), 17.0 (1C), 15.5 (0.5C), 14.8 (0.5C). FTMS (ESI) m/z: [M+H]⁺ Calcd for C₃₀H₃₀F₂N₆O₃ 561.24202; Found 561.24150.

ASSOCIATED CONTENT

Experimental details for in vitro and in vivo studies, additional crystallographic data for compounds 2 and 9, and synthetic procedures for compounds 2-40 can be found in the Supporting Information, available free of charge via the Internet at http://pubs.acs.org.

ACCESSION CODES

Atomic coordinates for the X-ray structures of compound 2 (PDB 6PGO) and 9 (PDB 6PGP) bound to GDP-KRASG12C are available from the RCSB Protein Data Bank (www.rcsb.org).

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Author Contributions

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ABBREVIATIONS

KRAS, Kirsten Rat Sarcoma virus oncogene; GDP, guanosine diphosphate; GTP, guanosine triphosphate; S-IIP, switch II pocket; SOS1, son of sevenless homolog 1 protein; c-RAF, rapidly accelerated fibrosarcoma kinase (cellular homolog); p-ERK, phosphorylated extracellular signal-regulated kinase; MSD, Meso Scale Diagnostics; EGF, epidermal growth factor; PK, pharmacokinetic; MDCK, Madin-Darby Canine Kidney cell; IV, intravenous; PO, per os (oral); SAR, structure–activity relationship; QD, quaque die (once daily); TGI, tumor growth inhibition; FaSSGF, fasted-state simulated gastric fluid; FaSSIF, fasted-state simulated intestinal fluid; PBS, phosphate-buffered saline; MS, mass spectrometry; GSH, glutathione

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(13) For details, see https://www.perkinelmer.com/Content/RelatedMaterials/Brochure s/BRO_AlphaScreen2004.pdf (accessed Sep 7, 2019)

(14)	For	details,	see
https://www.mesosca	le.com/en/pro	ducts/phospho-total-	erk1-2-

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(15) 5-min incubation represented the shortest incubation time that could be practically executed.

(16) 2-h incubation was estimated to be the minimum time required to achieve "complete" (~95%) covalent modification of KRAS^{G12C} based on the GTP hydrolysis rate reported in ref. 8.

(17) Possible reasons for the increased activity of compound **8** include more optimal orientation of the isopropylphenyl substituent in the cryptic pocket (a B3LYP/6-31G* quantum mechanical model predicts compound **8** to adopt a ground-state biaryl torsion angle of 65° , whereas the quinazolinone and phenyl rings are nearly perpendicular in compound **9**, enhancing contacts with the cryptic pocket residues) and enhanced contacts between the quinazolinone carbonyl group and proximal protein residues and water molecules.

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(27) No significant body weight loss was observed at any dose level in this study.

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