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Design and Synthesis of Novel Diaminoquinazolines with *in Vivo* Efficacy for β-Catenin/T-Cell Transcriptional Factor 4 Pathway Inhibition

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We are introducing a novel series of 2,4-diaminoquinazolines as β-catenin/Tcf4 inhibitors which were identified by ligand-based design. Here we elucidate the SAR of this series and explain how we were able to improve key molecular properties such as solubility and cLogP leading to compound **9**. Analogue **9** exhibited better biological activity and improved physical and pharmacological properties relative to the HTS hit **49**. Furthermore, **9** demonstrated good cell growth inhibition against several human colorectal cancer lines such as LoVo and HT29. In addition, treatment with compound **9** led to gene expression changes that overlapped significantly with the transcriptional profile resulting from the pathway inhibition by siRNA knockdown of β-catenin or Tcf4. Subsequently, **9** was tested for efficacy in a β-catenin/RKE-mouse xenograft, where it led to more then 50% decrease in tumor volume.

Introduction

Colorectal cancer is a major health burden. Each year, more than one million new cases are diagnosed worldwide, and 155000 new cases develop in the United States.[1] In Western populations, 50% of people develop colorectal tumors by age 70, and about 10% of them have their tumors progress to malignancy.[2] Consequently, colorectal cancer is the second leading cause of cancer mortality in the United States. Despite the overall improvement in colorectal cancer therapy, the survival outcome remains poor or unsatisfactory. Therefore, there is a high unmet medical need for effective treatment of colorectal cancer. Over the past decade, accumulating evidence points to a role that activation of the canonical Wnt[a] (wingless int-1) signaling plays in a wide range of human cancers, especially in colorectal cancers.[2,3]

First discovered in *Drosophila*,[4] the canonical Wnt signaling pathway is evolutionally conserved in mammalians, *Xenopus*, *Drosophila*, and *Caenorhabditis elegans*.[3] It not only controls many events during embryonic development but also regulates proliferation, morphology, motility, and cell fate at a cellular level. As the key player of the pathway, the tumor suppressor adenomatous polyposis coli (APC) forms a complex with Axin and regulates the stability of β-catenin, another central component of the Wnt signaling pathway. The Wnt pathway is activated by Wnt factors which form a large family of cell-signaling glycoproteins.[3] In the absence of a Wnt signal, cytoplasmic β-catenin is captured by the APC/Axin complex and is phosphorylated by kinases GSK3β (glycogen synthase kinase 3β) and CK1α (casein kinase 1α) in the complex; phosphorylated β-catenin is further ubiquitinated and targeted for rapid proteasomal degradation. When cells are stimulated by Wnt factors, the Wnt signaling cascade is activated so that Axin is recruited to the membrane, resulting in inhibition of the intrinsic kinase activity of the APC complex. As a consequence, stable non-phosphorylated β-catenin accumulates in the cell cytoplasm and subsequently translocates into the nucleus, where it forms a complex with Tcf4 (T-cell transcriptional factor 4) protein and constitutively activates the transcription of a variety of Wnt target genes.[5] A number of genes have been proposed as critical downstream effectors of Wnt signaling in cancer, including *c-myc*,[6] *cyclin* D1,[7] MMP-7,[8,9] *axin2*,[10] and others.

Loss of function in APC has been found in 85% of colorectal cancers, and this marks the initiation step of a neoplastic process toward carcinoma formation.[1] In some cases of colorectal cancer in which APC is wild type, β-catenin is activated by mutations in *axin2*[11,12] or by intragenic mutations that abolish inhibitory phosphorylation sites at the N-terminus of β-catenin;[13] both mutations can prevent β-catenin from degradation. All of these mutations result in accumulation of non-phosphorylated β-catenin, thereby constitutively activating transcription of target genes and ultimately promoting carcinogenesis. Either growth suppression or apoptosis occurs when wild-type APC was introduced into colon cancer cells which have lost APC function, suggesting that these cells have become dependent on elevated β-catenin/Tcf4 signaling.[14]

Taken together, the β-catenin/Tcf4 signaling pathway presents a novel target for therapeutic intervention in colorectal cancer. The SW480 colorectal cancer cell line has a truncation mutation in APC and thus has a constitutively activated

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[a] Abbreviations: Tcf4, T-cell transcriptional factor 4; APC, adenomatous polyposis coli; Wnt, wingless int-1; GSK3, glycogen synthase kinase 3; CK1α, casein kinase 1α; MMP-7, matrix metalloprotease 7; SV40, simian vacuolating virus 40; FF, firefly; R, renilla; siRNA, small interfering RNA; DUSP, dual specificity phosphatase; MAP, mitogen-activated protein; ip, intraperitoneal.

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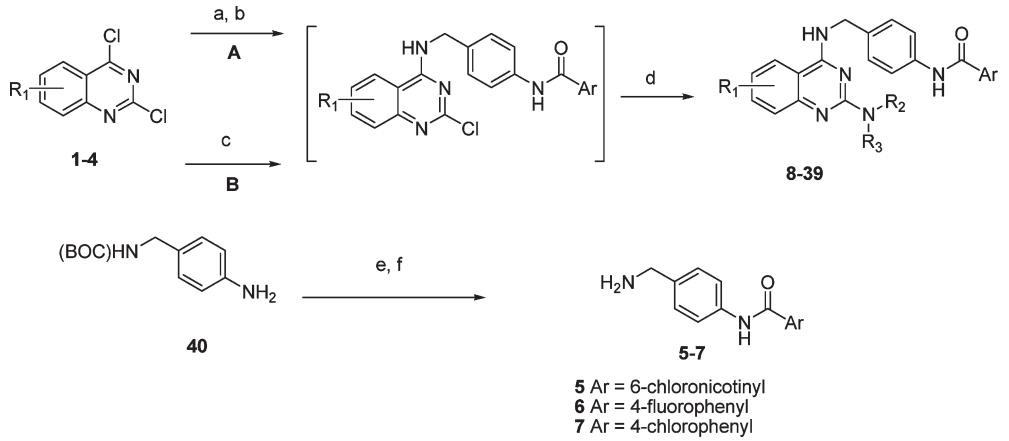
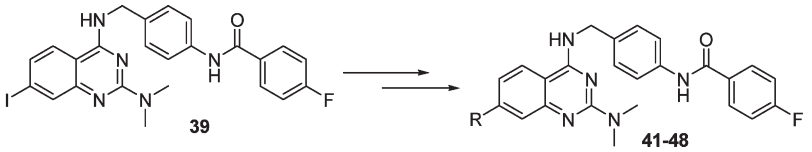
Scheme 1. Synthesis of Quinazoline Analogues **8**−**39**[a]



[a] For structures of **8**−**39**, please see Tables 1−4. Reaction conditions: (a) NEt_3, 4-aminobenzylamine, $CHCl_3$; (b) ArCOCl; (c) **5**−**7**, NEt_3, $CHCl_3$; (d) HNR_5R_4, THF or dioxane, 70 °C, 24 h; (e) ArCOCl, NEt_3; (f) TFA, CH_2Cl_2.

Scheme 2. Synthesis of 7-Substiuted Quinazoline Analogues **41**−**48**[a]



[a] **41**: R = −CN; $ZnCN_2$, $Pd(OAc)_2$, toluene, reflux, 70%. **42**: R = $-COCH_3$; (a) Bu_3Sn-α-ethoxyvinyl, $Pd(PPh_3)_4$, toluene, reflux; (b) HCl, THF reflux, 50% (2 steps). **43**: R = (+/−) $-CH(OH)CH_3$; (a) Bu_3Sn-α-ethoxyvinyl, $Pd(PPh_3)_4$, toluene, reflux; (b) HCl, THF reflux; (c) $NaBH_4$, 40% (3 steps). **44**: R = −CHO; $NaHCO_2$, $Pd(OAc)_2$, CO (1 atm), DMF, 100 °C, 37%. **45**: R = $-CH_2OH$; (a) $NaHCO_2$, $Pd(OAc)_2$, DMF, 100 °C; (b) $NaBH_4$, MeOH, 85% (2 steps). **46**: R = $-CH_2NMe_2$; (a) $NaHCO_2$, $Pd(OAc)_2$, CO (1 atm), DMF, 100 °C; (b) $HNMe_2$, $NaBH_3CN$, AcOH, 23% (2 steps). **47**: R = $-CH{=}CH_2$; Bu_3Sn-vinyl, $Pd(PPh_3)_4$, toluene, reflux, 61%. **48**: R = $-C_2H_5$; (a) Bu_3Sn-vinyl, $Pd(PPh_3)_4$, toluene, reflux; (b) Pd/C, H_2, MeOH, 59% (2 steps).

β-catenin/Tcf4 signaling pathway. We engineered a luciferase reporter with Tcf4 binding sites in its promoter. The Tcf4-luciferase reporter was subsequently integrated into the chromosome of SW480, resulting in two reporter cell lines which were used in a high-throughput screen. Recently, we reported the discovery of a novel class of small molecules that inhibit the β-catenin/Tcf4 pathway built on a quinazoline core.[15] These quinazolines exhibited good solubility, permeability, and oral bioavailability. However, the compounds lacked *in vivo* efficacy. Herein we report a novel and modified class of β-catenin/Tcf4-pathway inhibitors that showed a similarly good *in vitro* profile as the compounds described previously. However, in this new series, we identified a compound that engaged the β-catenin/Tcf4 pathway more effectively than the older analogues and, more importantly, demonstrated *in vivo* efficacy.

Chemistry

The synthesis of the novel 2,4-diamino analogues **8**−**39** is shown in Scheme 1. These were synthesized[20] by the reaction of 2,4-dichloroquinazolines **1**−**4**[21] with 4-aminobenzylamine followed by an *in situ* benzoylation (pathway A) or, alternatively, by reaction with the fully assembled side chains **5**−**7** (pathway B). The side chains **5**−**7** were synthesized in two steps from commercially available *N*-BOC-4-aminobenzylamine **40**, as shown in Scheme 1. Subsequently, amination at the 2-position of the quinazoline moiety was accomplished by heating with the appropriate amines, in THF or dioxane, to yield **8**−**39**.

A variety of 7-substituted 2-dimethylamino analogues **41**−**48** were prepared from the 7-iodo derivative **39**, as depicted in Scheme 2. These 7-substituted quinazolines were synthesized by the palladium-catalyzed cross-coupling reaction of the iodo compound **39** with various organometallic reagents and further functional group interconversion as shown in Scheme 2. The iodo starting material **39** had been obtained earlier as shown in Scheme 1.

Results and Discussion

All of the newly synthesized compounds were evaluated in the Tcf4-luciferase reporter cell lines 33.13 and 22C11, and the selectivity profile was established against the control cell line 5A8. Our Tcf4-luciferase reporter assay cell lines and the control cell line were derived from the SW480 colorectal cancer cell. The 33.13 line was generated by stepwise transfection of SW480 cells with the Tcf4-Firefly luciferase construct (G418-resistant) and then with the Zeocin-resistant SV40-Renilla luciferase construct, while the 22C11 lines was obtained by cotransfection of the Tcf4-Firefly luciferase construct and SV40-Renila luciferase construct under G418 selection. These two reporter lines represent two independent stable clones of the desired integration events. Therefore, assessment in both 33.13 and 22C11 cells provides a better indication of a specific inhibition of the reporter rather than some inhibition that was integration site-dependent. In general, a selective inhibition of the Tcf4-luciferase expression in both 33.13 and 22C11 lines but not in 5A8 was sought during compound evaluation (for details see the Experimental Section).

Compound **49** was identified via HTS and showed potent activity in the β-catenin/Tcf4 reporter assay (Table 5). In addition, this compound showed acceptable TPSA but poor solubility and unfavorable physical properties for oral absorption such as high cLogP.

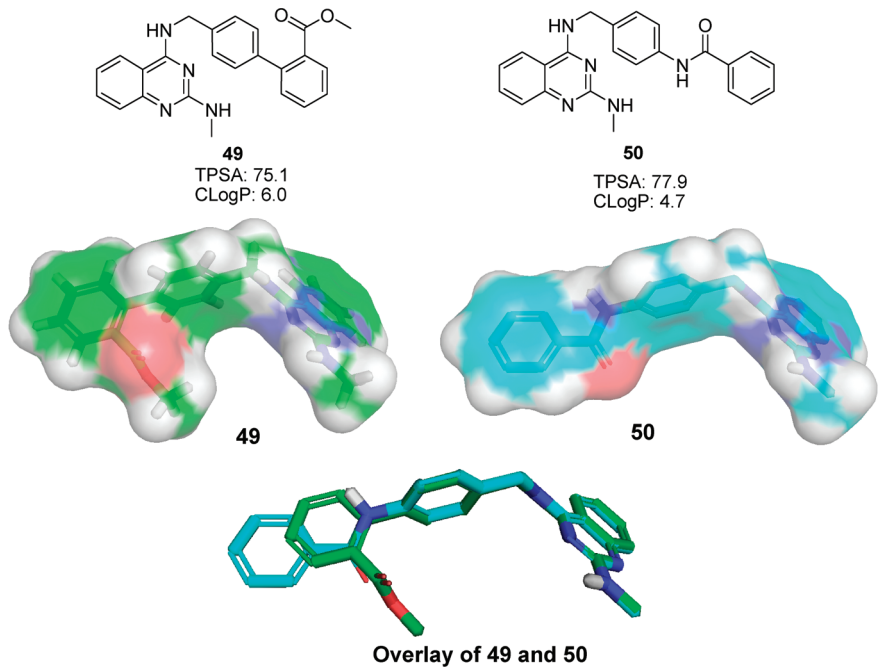


Figure 1. Compound **49** was discovered from HTS. The aminomethylbiphenyl can be replaced with the aminomethylbenzamide substituent in **50** to improve potency and properties.

Furthermore, compound **49** exhibited poor metabolic stability, which was caused by the hydrolysis of the methyl ester. Ligand-based design led us to a new lead series where the biphenyl substituent in **49** was replaced with a 4-benzamido group (e.g., **50**). Figure 1 shows the overlay of compounds **49** and **50**. As one can see, the two structures have similar molecular shapes, and the putative structure **50** offered an avenue for novelty, better cLogP (6.0 for compound **49** vs 4.6 for compound **50**), and, potentially, increased metabolic/chemical stability, improved aqueous solubility, and a springboard from which to develop more potent inhibitors of the β-catenin/Tcf4 pathway.

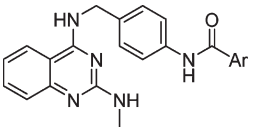
Table 1 summarizes the IC_{50} values of compounds **8**−**16** in the Tcf4-luciferase reporter assay. Among the various 2-methylamino-substituted quinazoline derivatives **8**−**16**, the 4-halogen-substituted aryl derivatives **8** and **10** and the 6-chloronicotinoyl compound **9** showed cell potencies below 1 μM in the reporter cell lines 33.13 and 22C11. The 4-chloro regioisomer **10** was found to be 4-fold more potent than its corresponding 3-chloro analogue **14** in the 33.13 cell line. Similarly, the 6-chloronicotinoyl derivative **9** was marginally more potent than the 2-chloropicolinyl compound **12**. In comparison with **49**, compound **9** had improved water solubility (Table 1). Similarly, the 4-fluorophenyl derivative **8** was the most potent compound in this series and showed modest selectivity over the 5A8 cell line. The other 4-substituted analogues such as $-CF_3$ (**13**), $-CN$ (**15**), and $-CH_3$ (**16**) exhibited IC_{50} values of > 1 μM in all of the above-mentioned three cell lines. From the subset of analogues **8**−**10** with below 1 μM potency, we determined good microsomal stability for **10** and moderate stability for **8** and **9**. In addition, **8** and **10** demonstrated good PAMPA permeability, accompanied by low solubility, while **9** showed poor permeability and moderate solubility.

However, these initial results encouraged us to probe the effect of the methyl substituent in the 5-, 6-, 7-, and 8-positions of the quinazoline ring in compounds **8**−**10** in order to find out which position is most suited for the introduction substitutents, to improve potency, stability, and solubility.

Several 5-, 6-, 7-, and 8-methyl-substituted quinazoline derivatives **17**−**28** were prepared according to Scheme 1, and their *in vitro* activities are tabulated in Table 2. Methyl groups were introduced at the 5-, 6-, 7-, and 8-position of the quinazoline core, with varying "Ar" substituents. It is evident from Table 2 that introduction of a methyl group to the quinazoline core 5-, 6-, 7-, or 8-position can lead to improved *in vitro* potency compared to the unsubstituted compounds **8**−**10**. Among the various methyl-substituted derivatives, 5-methyl derivatives **20** and **24** exhibited the lowest potency. When the aryl moiety was 6-chloronicotinyl (derivatives **26**−**28**), the 5-, 6-, and 7-methyl derivatives **26**−**28** were equipotent with each other against 33.13 and 22C11. However, when the aryl moiety was 4-fluorophenyl, the 7-methyl (**18**) and the 6-methyl (**19**) derivatives showed the best potency against 33.13 and 22C11, which was followed by the 8-methyl analogue (**17**), establishing the potency order as 7-Me > 6-Me > 8-Me. For the 4-chlorophenyl analogues **21**−**24**, the 7-methyl compound **22** showed the best potency against 33.13. Unfortunately, the introduction of a methyl group had, in most cases, no impact on the selectivity against the 5A8 cell line, which was around 3−9-fold for all of the analogues, except **21**, which showed 30-fold selectivity against 5A8. However, all of the methyl analogues had low solubility, and no improvement of microsomal stability or PAMPA permeability was observed, with the exception of **18**, when compared to the demethyl derivatives **8**−**10**. Compound **18** exhibited good microsomal stability but poor solubility and permeability.

The data in Table 2 showed that methyl substitution in the 7-position led to the best potency in the 33.13 reporter line for compounds **18**, **22**, and **26**. In this subset, only the 4-fluoroaryl analogue **18** showed good microsomal stability. Therefore, **18** was chosen for further optimization. This SAR study also showed that the 7-position is best suited for studying the introduction of other functional groups, in order to improve properties, as shown in Table 4.

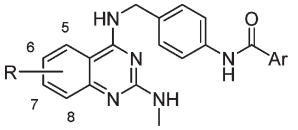
Table 1. Biological Data and Physical Properties for the Arylamido Analogues **8**−**16**



		IC$_{50}$ (nM)[a]					
compd	Ar =	33.13	22C11	5A8	sol, pH = 7.4 (μg/mL)[b]	micr stab (rat), $t_{1/2}$ (min)[c]	PAMPA (pe × 10^{-6} cm/s)[d]
8	4-fluorophenyl	413	424	> 778	1	21	4.2
9	6-chloronicotinyl	596	690	> 2984	23	17	0
10	4-chlorophenyl	771	1188	> 2194	1	30	1.4
11	4-biphenyl	941	> 2720	> 5440	1	16	1.2
12	2-chloropicolinyl	972	931	> 5139	16	0	0
13	4-CF$_3$-phenyl	1038	1120	> 1384	1	30	5.3
14	3-chlorophenyl	2771	2274	> 5982	16	15	3
15	4-cyanophenyl	3647	5642	> 12241	6	10	0.2
16	4-toluoyl	> 25158	> 25158	> 25158	2	16	5.8

[a] The values are an average of at least two separate determinations with a typical variation of less than ±30%. [b] Aqueous solubility at pH = 7.4.[16] [c] Rat microsomes $t_{1/2}$ (min).[17] [d] The effective permeability PAMPA was measured as described by Kerns et al.[18]

Table 2. Biological Data and Physical Properties for Alkyl-Substituted Quinazolines **17**−**28**



			IC$_{50}$ (nM)[a]					
compd	R =	Ar =	33.13	22C11	5A8	sol, pH = 7.4 (μg/mL)[b]	micr stab (rat), $t_{1/2}$ (min)[c]	PAMPA (pe × 10^{-6} cm/s)[d]
17	8-methyl	4-fluorophenyl	942	1113	> 3078	1	10	4.2
18	7-methyl	4-fluorophenyl	299	329	> 752	0	> 30	0.0
19	6-methyl	4-fluorophenyl	415	469	> 3009	10	6	1.3
20	5-methyl	4-fluorophenyl	1080	1716	> 3009	2	15	2.6
21	8-methyl	4-chlorophenyl	410	405	> 11569	1	11	0
22	7-methyl	4-chlorophenyl	270	389	> 832	1	18	0.0
23	6-methyl	4-chlorophenyl	595	840	> 4019	11	6	2.4
24	5-methyl	4-chlorophenyl	492	829	> 2894	0	12	0.9
25	8-methyl	6-chloronicotinyl	541	848	> 4366	2	2	0.2
26	7-methyl	6-chloronicotinyl	414	650	> 3850	7	8	0.3
27	6-methyl	6-chloronicotinyl	483	348	> 1444	3	13	0.0
28	5-methyl	6-chloronicotinyl	471	573	> 1203	1	22	1.4

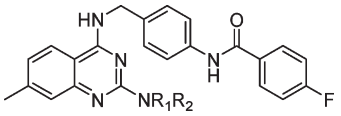
[a] The values are an average of at least two separate determinations with a typical variation of less than ±30%. [b] Aqueous solubility at pH = 7.4.[16] [c] Rat microsomes $t_{1/2}$ (min).[17] [d] The effective permeability PAMPA was measured as described by Kerns et al.[18]

Next, in order to investigate the effect of different amino substituents at the 2-position of the quinazoline core, a variety of amines were introduced on the most potent methyl derivative **18**, and the results are tabulated in Table 3. It is interesting to note that compounds such as **29** and **31**−**33**, bearing tertiary amino groups in the 2-position, exhibited similar potency against 33.13 and a comparable selectivity against 5A8 cell line. Analogues such as **29**, **31**, and **33** showed good PAMPA permeability and poor solubility at physiological pH. The piperazine containing analogue **35** had good selectivity, solubility, and modest PAMPA permeability. However, compound **35** showed poor plasma levels when administered at 50 mpk, po, in female nude mice. This may be due to low absorption or a high first-pass metabolism. Moreover, analogues **29**−**38** showed poor rat microsomal stability, possibly due to the formation of an oxidative metabolite which was identified by LC/MS during *in vitro* microsomal incubation studies.

However, most of the analogues **29**−**38** in this series exhibited better selectivity (33.13 vs 5A8) compared to the methylamino analogue **18**. Therefore, it seemed prudent to obtain further SAR for this series, carrying a tertiary amino group in the 2-position.

In order to boost the potency and to improve the solubility, several polar and nonpolar groups were introduced at the 7-position of the quinazoline moiety. The results are tabulated in Table 4. The presence of iodo (**39**), cyano (**41**), acetyl (**42**), and polar groups (analogues **43**−**46**) is not tolerated. However, the presence of nonpolar substituents such as vinyl (**47**) or ethyl (**48**) groups is well tolerated. Especially compound **47** exhibited good potency in both 33.13 and 22C11 cell lines (239 and 337 nM, respectively) and very good selectivity

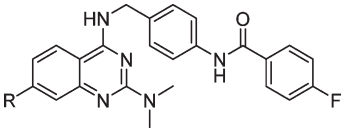
Table 3. Biological Data and Physical Properties for C-2-Substituted Analogues **29**−**38** in the 4-Fluorophenyl Series



		IC$_{50}$ (nM)[a]					
compd	NR_1R_2 =	33.13	22C11	5A8	sol, pH = 7.4 (μg/mL)[b]	micr stab (rat), $t_{1/2}$ (min)[c]	PAMPA (pe × 10^{-6} cm/s)[d]
29	dimethylamine	454	593	> 2910	1	12	1.6
30	*n*-propylamine	506	645	> 2818	1	6	0.0
31	azetidine	300	506	> 2831	1	7	1.5
32	pyrrolidine	378	659	> 2744	1	2	0.5
33	piperidine	291	524	> 2662	0	3	2.0
34	piperazine	322	620	> 2656	22	5	0.1
35	(*R*)-3-methylpiperazine	305	374	> 4013	27	4	0.4
36	3,3-dimethylpiperazine	561	662	> 3858	> 100	3	0.02
37	*N*-methylpiperazine	676	1008	> 9028	1	3	4.4
38	morpholine	636	1779	> 10604	1	6	0

[a] The values are an average of at least two separate determinations with a typical variation of less than ±30%. [b] Aqueous solubility at pH = 7.4.[16] [c] Rat microsomes $t_{1/2}$ (min).[17] [d] The effective permeability PAMPA was measured as described by Kerns et al.[18]

Table 4. Biological Data and Physical Properties for 7-Substiuted Quinazoline Analogues **39** and **41**−**48**



		IC$_{50}$ (nM)[a]					
compd	R =	33.13	22C11	5A8	sol, pH = 7.4 (μg/mL)[b]	micr stab (rat), $t_{1/2}$ (min)[c]	PAMPA (pe × 10^{-6} cm)[d]
39	I	4192	6814	> 18472	> 100		0.0
41	−CN	1479	2138	> 62900	0	11	5.6
42	$-COCH_3$	2330	2592	> 20244	1	2	0.7
43	(+/−) $-CH(OH)CH_3$	1900	2012	> 20162	18	6	0.1
44	−CHO	884	895	> 17940	1	3	0
45	$-CH_2OH$	855	811	> 10374	4	22	0.0
46	$-CH_2NMe_2$	1222	1046	> 9166	55	3	0.4
47	$-CH{=}CH_2$	239	337	> 11042	1	6	1.1
48	$-C_2H_5$	335	431	> 2604	4	6	0.3

[a] The values are an average of at least two separate determinations with a typical variation of less than ±30%. [b] Aqueous solubility at pH = 7.4.[16] [c] Rat microsomes $t_{1/2}$ (min).[17] [d] The effective permeability PAMPA was measured as described by Kerns et al.[18]

against the 5A8 cell line. However, analogue **47** had poor solubility, poor rat microsomal stability, and poor PAMPA permeability.

Among the various compounds prepared in this series, based on potency, selectivity (against 5A8), and solubility, analogues **9** and **19** were chosen for further *in vitro* evaluation.

Compounds **9**, **19**, and **49** were tested in a cell growth inhibition assay with four different colon cancer cell lines (33.13, HT29, DLD1, and LoVo) to investigate whether these compounds could inhibit cell growth by inhibiting the β-catenin/Tcf4 pathway. Listed in Table 5 are IC$_{50}$ values of compounds **9**, **19**, and **49**. Notably, all of these three compounds inhibited growth of the reporter line 33.13 at concentrations similar to those at which they inhibited the Tcf4-luciferase reporter activity. In addition, they all displayed potent inhibitory activity against three other colon cancer cell lines.

To test if these lead compounds specifically inhibited growth of cells that are dependent on activated β-catenin signaling, we used the cell line RK3E, an adenovirus E1A-immortalized epithelial cell line derived from neonatal rat kidney that has been transformed by a mutant β-catenin protein, β-cat/RK3E.[19] This mutant β-catenin gene harbors a mutation at the GSK3β phosphorylation site at the N-terminus of β-catenin, resulting in stabilization of the β-catenin protein. The same RK3E line transformed by a mutant K-ras called Kras/RK3E was used as a comparison. All three compounds **9**, **19**, and **49** were about two to three times more potent against the β-cat/RK3E than the Kras/RK3E line, indicating a selective growth inhibition of the cells that have a mutant β-catenin protein by these compounds (Table 5).

Oligonucleotide arrays were used to determine global transcriptional responses, using Human Genome U133 Plus 2.0 Oligonucleotide Arrays, to siRNA gene knockdown

Table 5. Biological Activity of Compounds **9**, **19**, and **49**

	IC_{50} (nM)[a]									
	reporter assay			colon cell growth inhibition				cell differential assay		
compd	22C11	33.13	5A8	33.13	HT29	DLD1	LoVo	β-cat	Kras	Kras/β-cat
49	561	455	>6273	385	594	1086	850	1037	3036	2.9
9	690	596	>2984	648	1375	1739	1400	749	2096	2.8
19	469	415	>3009	447	767	620	750	912	2021	2.1

[a] The values are an average of at least two separate determinations with a typical variation of less than ±30%.

to determine pathway-specific biomarkers and their correlation with compound activity in HT29 colon cancer cells. Pathway-responsive genes were identified by transcriptional profiling of triplicate cell samples 48 h following siRNA-mediated knockdown of either β-catenin or Tcf4 mRNA in comparison to a combination of mock transfection or Non-targeting siRNA controls. Specific knockdown of siRNA target gene mRNA was confirmed for both β-catenin and Tcf4 (57% and 53% decrease, respectively) as shown in Supporting Information Figure 1. Following targeted siRNA knockdown of β-catenin or Tcf4 in HT29 cells, modulated 310 or 193 tiled gene probe sets were modulated from 1.8- to 13-fold ($P < 0.05$), respectively. Of the 435 unique tiled probe sets modulated by either β-catenin or Tcf4 siRNA knockdown, 66% were modulated more than 1.5-fold by both pathway-specific knockdowns, demonstrating the anticipated overlap in pathway activity. These genes thereby represent pathway-specific transcriptional biomarkers to compare with compound activity in HT29 cells.

Compound-responsive transcription was similarly identified by comparison of mRNA levels in HT29 cells treated for 24 h with either **9** or **19** relative to vehicle controls. Compound treatment generated a robust transcriptional response with 1229 or 1028 unique genes modulated by either **9** or **19** by minimally 3-fold ($P < 0.05$; Table 2). Approximately 75% of the genes were modulated in common by the two compounds and are candidate biomarkers of target activity. Cluster analysis of normalized mRNA expression levels[20] identified sets of genes either modulated in common or preferentially by the individual compounds (Figure 2 and Supporting Information Figure 2). Both compounds led to a generally common decreased gene expression that reflected the strong antiproliferative activity of these compounds including multiple cyclins (CCNB1, CCNA2), phosphatases (CDC25B, CDC25C), and transcription factors (FoxM1) involved in G2/M cell cycle progression. The compound induced-gene expression had a component in common including induction of antiproliferative stress activated genes (ATF3, GADD34, HSP40) and induction of multiple DUSP phosphatases (DUSP1, DUSP5) that both inactivate proliferation-related MAP kinases.

Compound-mediated alterations of the expression of the 435 probe sets modulated by either β-catenin and/or Tcf4 siRNA targeted knockdown were interrogated to determine correlation in pathway-specific gene expression and candidate antagonist activity. Compound treatment with **9** or **19** resulted in a common minimal 50% induction or repression for 125 pathway-induced and 99 pathway-repressed probe sets, respectively. Overall, 52% of the pathway-specific transcription signature responded in a similar direction following treatment with the β-catenin antagonists. The pathway and compound coordinately modulated transcription was normalized to mean expression, and matrix cluster analysis is shown in Figure 3. All control samples including mock transfection, nontargeting oligonucleotides, and vehicle treated cells clustered on a unique node clearly distinct from gene expression in the compound-treated or pathway siRNA knockdown samples. Analogue **9** clustered closer to the samples following pathway knockdown and more closely recapitulated the β-catenin knockdown profile relative to **19**. While the mechanism of action, the kinetics, and the degree of resultant protein inhibition differ between the siRNA-mediated and small molecule antagonists, the overlap in transcriptional signatures provides additional evidence of β-catenin pathway-specific targeting.

Compared to compound **19**, **9** exhibited gene expression profile changes more similar to those mediated by the biological pathway knockdown. Therefore, we wanted to set the stage for *in vivo* studies by evaluating routes of administration. However, in subsequent exposure studies compounds **9** and **19** were each dosed orally at 50 mg/kg in nude mice; only poor plasma and tumor levels of drug substance were observed for both. These findings could be rationalized by the poor PAMPA permeability of **9**, leading to poor absorption, and by the poor solubility and poor metabolic stability of **19**, which could lead to either poor absorption or high absorption and high clearance. Hence, in order to conduct a proof of concept study, we settled on the ip (intraperitoneal) route of administration for an *in vivo* efficacy study with compound **9** in a β-cat/RK3E tumor xenograft model (Figure 3).

When compound **9** was administered once a week at increasing doses by ip injections, tumor growth inhibition was observed in a dose-dependent manner. Up to 66% suppression of tumor growth was achieved when 150 mg/kg body weight was dosed to mice, compared to the vehicle control tumors. In addition, a daily low dose of 18.75 mg/kg by ip for 7 days also led to more than 50% decrease of tumor volume. At the maximal tolerated dose (150 mg/kg once a week), several animals died with no other visible signs of mortality. However, at all other efficacious doses we observed neither deaths nor weight loss or other signs of toxicity.

This *in vivo* study demonstrated that colon cancer cells, whose growth is dependent on activated β-catenin/Tcf4 activity, could be inhibited by intervention with a small molecule. However, the series requires further optimization to obtain an orally bioavailable drug candidate that is efficacious against other colon cancer xenografts, such as HT29 and LoVo. Clearly, future compounds will need to address crucial PK issues in order to bring forward a successful β-catenin/Tcf4 inhibitor. We will report studies with further optimized compounds that exhibit better PK properties and potency in the near future.

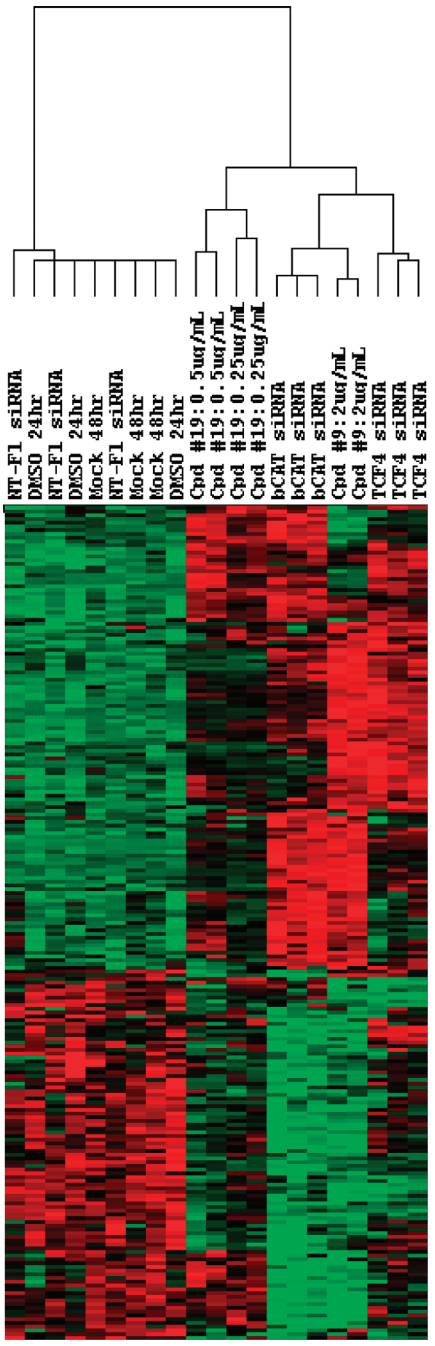


Figure 2. Overlap in pathway and compound-mediated gene expression. Genes modulated in common direction following pathway knockdown (β-catenin or Tcf4 siRNA) or compound treatment **9** or **19** were selected and mRNA expression levels Z normalized to mean expression and clustered by the method of Eisen.[20] Red and green represent mRNA expression levels higher or lower, respectively, to mean expression. All mock, Non-targeting oligo and vehicle-treated controls clustered on a distinct node, while both compound-treated and siRNA knockdown samples coclustered.

Conclusions

A series of novel diaminoquinazoline derivatives have been prepared and evaluated as inhibitors of the β-catenin/Tcf4 signaling pathway. Structural modifications were targeted primarily at improving the pharmaceutical and pharmacokinetic properties of the lead compound **49**. Replacement of the biphenyl methyl ester group solved the issue of hydrolytic instability and cLogP, which resulted in acceptable metabolic stability and solubilty. Compounds such as **9** demonstrated *in vivo* efficacy in a β-catenin/RK3E mouse xenograft model. This is the first example which offers a proof of concept that inhibiting the β-catenin/Tcf4 signaling pathway might be a viable therapy for treating colon cancer. Further gene profiling analysis demonstrated that compound treatment caused changes in the pathway-specific gene expression profile similar to those by siRNA knockdown of β-catenin or Tcf4, suggesting that these compounds exerted their antiproliferative effects by inhibiting the β-catenin/Tcf4 pathway specifically.

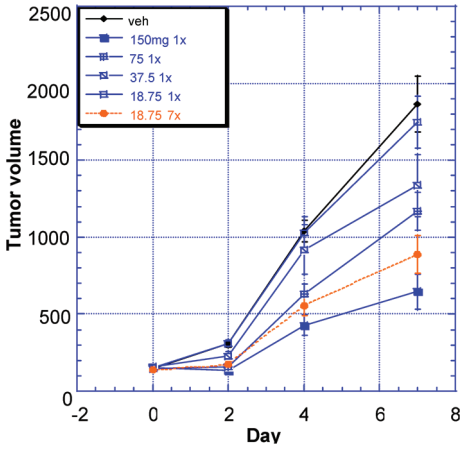


Figure 3. Compound **9** inhibits *in vivo* growth of a β-catenin/RK3E tumor xenograft. Nude mice bearing subcutaneous β-catenin/RK3E tumors were dosed ip with vehicle and compound **9** at indicated doses once a week and at 18.75 mg/kg once daily for 7 days.

Experimental Section

General Methods. Melting points were determined in an open capillary tube on a Meltemp melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined with a Bruker DPX-400 spectrometer at 400 MHz. Chemical shifts δ are reported in parts per million (δ) relative to residual chloroform (7.26 ppm), TMS (0 ppm), or dimethyl sulfoxide (2.49 ppm) as an internal reference with coupling constants (J) reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Electrospray (ES) mass spectra were recorded in positive or negative mode on a Micromass platform spectrometer. Electron impact (EI) and high-resolution mass spectra were obtained on a Finnigen MAT-90 spectrometer. Combustion analyses were obtained using a Perkin-Elmer Series II 2400 CHNS/O analyzer. The purity of compounds **8**−**30**, **35**, **36**, **39**, and **41**−**48** was determined by combustion analysis. The purity of compounds **31**−**34**, **37**, and **38** was determined by analytical LC/MS using an Agilent 1100 LC, equipped with an Agilent MSD mass spectrometer, and an Aquasil C18 column, using MeCN/H_2O eluent at 0.8 mL/min flow (containing 0.1% formic acid), 5.5 min gradient 0% MeCN to 100% MeCN, monitoring UV absorption at 254 nm. The purity of compounds **8**−**39** and **41**−**48** was found to be >95%. Reverse-phase HPLC purifications were performed on a Gilson preparative HPLC system controlled by Unipoint software using a Phenomenex Gemini 100 × 30.0 mm. Thin-layer chromatography (TLC) was performed on Merck PLC prescored plates $_{60}F_{254}$. The terms "concentrated" and "evaporated" refer to removal of solvents using a rotary evaporator at water aspirator pressure with a bath temperature equal to or less than 40 °C. Unless otherwise noted, reagents were obtained from commercial sources and were used without further purification.

General Procedure for the Preparation of 2,4-Diaminoquinazoline Analogues. Procedure A (Step1). To a stirred solution of 4-aminobenzylamine (122 mg, 1 mmol) and triethylamine (202 mg, 2 mmol) in $CHCl_3$ (5 mL) at 20 °C was added (1 mmol) of the appropriately substituted 2,4-dichloroquinazoline, and the mixture was stirred for a minimum of 3 h. After TLC showed the complete disappearance of the 2,4-dichloroquinazoline, aroyl chloride (1 mmol) in $CHCl_3$ (1 mL) was added slowly. The reaction mixture was allowed to stir overnight. At the end, the reaction mixture was quenched with $CHCl_3$ (50 mL) and water (15 mL). The reaction mixture was washed with water, and the chloroform layer was dried over $MgSO_4$. After removal of $MgSO_4$ by filtration and evaporation of solvents the crude product was purified by column chromatography with hexane/CH_2Cl_2/EA to give the 2-chloroquinazolines in yields between 50% and 95%.

Procedure A (Step 2). The appropriately substituted 2-chloroquinazoline derivatives (1 mmol) obtained by procedure A (step 1) was taken up either in a sealed tube or in a round-bottom flask and was suspended in THF (5 mL) or dioxane. (If the reactant amine is monomethylamine or dimethylamine, a sealed tube was used, and for other amines, a round-bottom flask can be used.) The appropriate amine was added, and the mixture was heated over 16 h to 100 °C or alternatively heated for 40 min to 120 °C using a microwave. After reaction was completed, the solvents were removed *in vacuo*, and the crude compound was purified by silica gel column chromatography by using CH_2Cl_2/MeOH/NH_3 mixtures as eluent to give the diaminoquinazolines in yields between 65% and 95%.

Procedure B (Step 1). To a stirred suspension of *N*-[4-(aminomethyl)phenyl]-4-fluorobenzamide (1.40 g, 5.73 mmol) and triethylamine (2 mL) in THF (20 mL) at room temperature was added 7-methyl-2,4-dichloroquinazoline (1.22 g, 5.73 mmol) dissolved in $CHCl_3$ (10 mL), and the mixture was kept stirring for a minimum of 3 h. After TLC showed the complete disappearance of the 7-methyl-2,4-dichloroquinazoline, $CHCl_3$ (250 mL) and water (25 mL) were added. The layers were separated, the aqueous layer was extracted twice with $CHCl_3$ (25 mL), and the combined organic layers were dried over $MgSO_4$. After removal of $MgSO_4$ by filtration and evaporation of solvents the crude product was purified by column chromatography with hexane/CH_2Cl_2/EA to give *N*-(4-{[(2-chloro-7-methylquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide (1.80 g, 73% yield). MS (ESI) *m*/*z* 421.2.

Procedure B (Step 2). *N*-(4-{[(2-Chloro-7-methylquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide (1 mmol) obtained by procedure B (step 1) was taken up either in a sealed tube or in a round-bottom flask and was suspended in an appropriate solvent (THF, 5 mL, or dioxane, DMF, 2-propanol, etc., 5 mL). (If the reactant amine is monomethylamine or dimethylamine, a sealed tube was used, and for other amines, a round-bottom flask can be used.) The appropriate amine or amine hydrochloride was added, and the mixture was heated under stirring over 2−16 h to 100−120 °C. After the reaction was completed, the solvents were removed in vacuum, and the crude compound was purified by preparative HPLC (high-pressure liquid chromatography) using ACN/water/NH_3 or TFA gradients as eluent or column chromatography with CH_2Cl_2/MeOH/NH_3 to give the diaminoquinazolines in yields between 65% and 95%.

Preparation of 4-Fluoro-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]benzamide (8). 4-Fluoro-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]benzamide was prepared starting from 2,4-dichloroquinazoline, 4-aminobenzylamine, and 4-fluorobenzoyl choride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from 2,4-dichloroquinazoline (0.38 g, 1.9 mmol), 100 mg (55% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 2.81 (d, J = 4.4 Hz, 3H), 4.69 (d, J = 5.0 Hz, 2H), 6.45 (m, 1H), 7.03 (t, J = 7.2 Hz), 7.28 (m, 1H), 7.36 (m, 4H), 7.48 (t, J = 7.2 Hz, 1H), 7.69 (d, J = 8.1 Hz, 2H), 8.03 (m, 3H), 8.38 (s, 1H), 10.25 (s, 1H) ppm; MS (ESI) *m*/*z* 402.1. Anal. Calcd for $C_{23}H_{20}FN_5O$ (%): C, 68.81; H, 5.02; F, 4.73; N, 17.45. Found: C, 69.10; H, 4.83; N, 17.16.

Preparation of 6-Chloro-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]nicotinamide (9). 6-Chloro-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]nicotinamide was prepared starting from 2,4-dichloroquinazoline, 4-aminobenzylamine, and 6-chloronicotinoyl chloride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from 2,4-dichloroquinazoline (0.15 g, 0.75 mmol), 140 mg (95% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 2.81 (d, J = 5.0 Hz, 3H), 4.70 (d, J = 5.0 Hz, 2H), 6.47 (br s, 1H), 7.29−7.24 (m, 1H), 7.39−7.35 (m, 2H), 7.50−7.47 (m, 1H), 7.71−7.67 (m, 4H), 8.04−8.0 (m, 2H), 8.47−8.30 (m, 2H), 8.93 (d, J = 3.0 Hz, 1H), 10.47 (s, 1H) ppm; MS (ESI) *m*/*z* 419.1. Anal. Calcd for $C_{22}H_{19}ClN_6O$ (%): C, 63.08; H, 4.57; N, 20.06. Found: C, 63.31; H, 4.84; N, 20.25.

Preparation of 4-Chloro-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]benzamide (10). 4-Chloro-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]benzamide was prepared starting from 2,4-dichloroquinazoline, 4-aminobenzylamine, and 4-chlorobenzoyl chloride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from 2,4-dichloroquinazoline (100 mg, 0.5 mmol), 20 mg (41% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 2.49 (d, J = 5.0 Hz, 3H), 4.71−4.66 (m, 2H), 6.51−6.41 (m, 1H), 7.04 (t, J = 7.8 Hz, 1H), 7.29−7.22 (m, 1H), 7.39 (d, J = 8.8 Hz, 2H), 7.49 (t, J = 7.8 Hz, 1H), 7.61 (d, J = 9.1 Hz, 2H), 7.69 (d, J = 8.8 Hz, 2H), 7.97 (d, J = 9.1 Hz, 2H), 8.01 (d, J = 7.8 Hz, 1H), 8.40 (s, 1H), 10.29 (s, 1H) ppm; MS (ESI) *m*/*z* 418.1. Anal. Calcd for $C_{23}H_{20}ClN_5O$ (%): C, 66.10; H, 4.82; N, 16.76. Found: C, 65.97; H, 5.06; N, 16.80.

Preparation of *N*-[4-({[2-(Methylamino)quinazolin-4-yl]amino}methyl)phenyl]biphenyl-4-carboxamide (11). *N*-[4-({[2-(Methylamino)quinazolin-4-yl]amino}methyl)phenyl]biphenyl-4-carboxamide was prepared starting from 2,4-dichloroquinazoline, 4-aminobenzylamine, and biphenyl-4-carbonyl chloride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from 2,4-dichloroquinazoline (0.17 g, 0.85 mmol), 50 mg (49% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 2.81 (d, J = 5.0 Hz, 3H), 4.72−4.67 (m, 2H), 6.53−6.43 (m, 1H), 7.07−7.01 (m, 1H), 7.52−7.22 (m, 7H), 7.86−7.73 (m, 6H), 8.08−8.00 (m, 3H), 8.45−8.33 (s, 1H), 10.28 (s, 1H) ppm; MS (ESI) *m*/*z* 460.2. Anal. Calcd for $C_{29}H_{25}N_5O$ (%): C, 75.80; H, 5.48; N, 15.24. Found: C, 76.03; H, 5.73; N, 15.13.

Preparation of 2-Chloro-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]isonicotinamide (12). 2-Chloro-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]isonicotinamide was prepared starting from 2,4-dichloroquinazoline, 4-aminobenzylamine, and 2-chloroisonicotinoyl chloride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from 2, 4-dichloroquinazoline (0.18 g, 0.9 mmol), 40 mg (24% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 9.36 (br s, 1H), 9.31 (t, J = 3.0 Hz, 1H), 8.34 (d, J = 9.0 Hz, 1H), 8.27 (d, J = 9.0 Hz, 1H), 7.92 (d, J = 9.0 Hz, 2H), 7.85−7.79 (m, 1H), 7.65 (d, J = 12.0 Hz, 1H), 7.58−7.53 (m, 2H), 7.38−7.31 (m, 3H), 7.14−7.10 (m, 1H), 6.72−6.66 (m, 1H), 4.76 (d, J = 5.0 Hz, 2H), 2.83 (d, J = 5.0 Hz, 3H) ppm; MS (ESI) *m*/*z* 419.8. Anal. Calcd for $C_{22}H_{19}ClN_6O$ (%): C, 63.08; H, 4.57; N, 20.06. Found: C, 63.21; H, 4.78; N, 20.16.

Preparation of *N*-[4-({[2-(Methylamino)quinazolin-4-yl]amino}-methyl)phenyl]-4-(trifluoromethyl)benzamide (13). *N*-[4-({[2-(Methylamino)quinazolin-4-yl]amino}methyl)phenyl]-4-(trifluoromethyl)-benzamide was prepared starting from 2,4-dichloroquinazoline, 4-aminobenzylamine, and 4-trifluoromethylbenzoyl chloride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from 2,4-dichloroquinazoline (0.19 g, 1.0 mmol), 50 mg (43% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 3.07 (d, J = 5.0 Hz, 3H), 4.65−4.63 (m, 2H), 6.62−6.53 (m, 1H), 7.41−7.32 (m, 4H), 7.62−7.51 (m, 4H), 7.79−7.64 (m, 4H), 7.94−7.89 (m, 2H) ppm; MS (ESI) m/z 452.2. Anal. Calcd for $C_{24}H_{20}F_3N_5O$ (%): C, 63.85; H, 4.47; N, 15.51. Found: C, 63.56; H, 4.65; N, 15.43.

Preparation of 3-Chloro-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]benzamide (14). 3-Chloro-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]benzamide was prepared starting from 2,4-dichloroquinazoline, 4-aminobenzylamine, and 3-chlorobenzoyl chloride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from 2,4-dichloroquinazoline (100 mg, 0.5 mmol), 30 mg (30% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 3.05 (d, J = 5. 0 Hz, 3H), 4.81−4.76 (m, 2H), 5.89 (br s, 1H), 7.11−7.04 (m, 1H), 7.44−7.38 (m, 6H), 7.56−7.49 (m, 2H), 7.61−7.58 (m, 2H), 7.76−7.72 (m, 1H), 7.91−7.82 (m, 2H) ppm; MS (ESI) m/z 418.1. Anal. Calcd for $C_{23}H_{20}ClN_5O$ (%): C, 66.10; H, 4.82; N, 16.76. Found: C, 65.82; H, 5.05; N, 16.53.

Preparation of 4-Cyano-*N*-[4-({[2-(methylamino) quinazolin-4-yl]amino}methyl)phenyl]benzamide (15). 4-Cyano-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]benzamide was prepared starting from 2,4-dichloroquinazoline, 4-aminobenzylamine, and 4-cyanobenzoyl chloride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from 2,4-dichloroquinazoline (190 mg, 1.0 mmol), 30 mg (16%yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 3.08−3.06 (m, 3H), 4.67−4.65 (m, 2H), 6.47−6.41 (m, 1H), 7.22−7.16 (m, 1H), 7.40−7.36 (m, 2H), 7.69−7.53 (m, 5H), 7.79−7.73 (m, 4H), 7.92−7.89 (m, 2H) ppm; MS (ESI) m/z 409. Anal. Calcd for $C_{24}H_{20}N_6O$ (%): C, 70.57; H, 4.94; N, 20.58. Found: C, 70.29; H, 5.20; N, 20.45.

Preparation of 4-Methyl-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]benzamide (16). 4-Methyl-*N*-[4-({[2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]benzamide was prepared starting from 2,4-dichloroquinazoline, 4-aminobenzylamine, and 4-methylbenzoyl choride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from of 2,4-dichloroquinazoline (500 mg, 2.5 mmol), 200 mg (40% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 2.50 (s, 3H), 3.35 (d, J = 3.5 Hz, 3H), 4.44 (d, J = 5.0 Hz, 2H), 7.36−7.24 (m, 6H), 7.90−7.68 (m, 6H), 8.97−8.92 (m, 1H), 10.14 (s, 1H) ppm; MS (ESI) m/z 398.2. Anal. Calcd for $C_{24}H_{23}N_5O$ (%): C, 72.52; H, 5.83; N, 17.62. Found: C, 72.41; H, 6.09; N, 17.90.

Preparation of 4-Fluoro-*N*-[4-({[8-methyl-2-(methylamino)-quinazolin-4-yl]amino}methyl)phenyl]benzamide (17). 4-Fluoro-*N*-[4-({[8-methyl-2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]benzamide was prepared starting from 8-methyl-2,4-dichloroquinazoline, 4-aminobenzylamine, and 4-fluorobenzoyl chloride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from 500 mg (2.35 mmol) of 8-methyl-2,4-dichloroquinazoline, 274 mg (28% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 2.40 (s, 3H), 2.83 (d, J = 4.8 Hz, 3H), 4.68 (d, J = 5.8 Hz, 2H), 6.39 (m, 1H), 6.92 (t, J = 7.8 Hz, 1H), 7.35 (m, 4H), 7.68 (d, J = 8.6 Hz, 2H), 7.85 (d, J = 7.8 Hz, 1H), 8.02 (m, 2H), 8.28 (s, 1H), 10.22 (s, 1H) ppm; MS (ESI) m/z 416.1. Anal. Calcd for $C_{24}H_{22}FN_5O$ (%): C, 69.38; H, 5.34; N, 16.86. Found: C, 69.53; H, 5.58; N, 16.57.

Preparation of 4-Fluoro-*N*-[4-({[7-methyl-2-(methylamino)-quinazolin-4-yl]amino}methyl)phenyl]benzamide (18). 4-Fluoro-*N*-[4-({[7-methyl-2-(methylamino)quinazolin-4-yl]amino}methyl)phenyl]benzamide was prepared by amination of *N*-(4-{[(2-chloro-7-methylquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide (250 mg, 0.6 mmol) and 2 M methylamine in THF following procedure B (step 2). After purification by HPLC and solvent removal the final product (150 mg, 76% yield) was isolated. ^{1}H NMR (DMSO) δ 2.34 (s, 3H), 2.79 (d, J = 4.5 Hz, 3H), 4.67 (d, J = 5.6 Hz, 2H), 6.47 (s, 1H), 6.88 (m, 1H), 7.08 (s, 1H), 7.36 (m, 4H), 7.69 (d, J = 8.3 Hz, 2H), 7.91 (d, J = 8.3 Hz, 1H), 8.02 (m, 2H), 8.32 (s, 1H), 10.23 (s, 1H) ppm; MS (ESI) m/z 416.2. Anal. Calcd for $C_{24}H_{22}FN_5O$ (%): C, 69.38; H, 5.34; N, 16.86. Found: C, 69.24; H, 5.52; N, 16.93.

Preparation of 4-Fluoro-*N*-[4-({[6-methyl-2-(methylamino)-quinazolin-4-yl]amino}methyl)phenyl]benzamide (19). 4-Fluoro-*N*-[4-({[6-methyl-2-(methylamino)quinazolin-4-yl]amino}methyl)-phenyl]benzamide was prepared starting from 6-methyl-2,4-dichloroquinazoline, 4-aminobenzylamine, and 4-fluorobenzoyl chloride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from 410 mg (2.0 mmol) of 6-methyl-2,4-dichloroquinazoline, 200 mg (58% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 2.34 (s, 3H), 2.81 (d, J = 5.0 Hz, 3H), 4.68 (d, J = 5.0 Hz, 3H), 6.49 (br s, 1H), 7.23−7.19 (m, 1H), 7.38−7.33 (m, 3H), 7.71−7.68 (m, 2H), 7.86 (s, 1H), 8.06−8.00 (m, 2H), 8.49−8.30 (br s, 1H), 10.25 (s, 1H) ppm; MS (ESI) m/z 416.2. Anal. Calcd for $C_{24}H_{22}FN_5O$ (%): C, 69.38; H, 5.34; N, 16.86. Found: C, 69.21; H, 5.63; N, 16.75.

Preparation of 4-Fluoro-*N*-[4-({[5-methyl-2-(methylamino)-quinazolin-4-yl]amino}methyl)phenyl]benzamide (20). 4-Fluoro-*N*-[4-({[5-methyl-2-(methylamino)quinazolin-4-yl]amino}methyl)-phenyl]benzamide was prepared starting from 5-methyl-2,4-dichloroquinazoline (426 mg, 2 mmol), 4-aminobenzylamine, and 4-fluorobenzoyl chloride following procedure A (step 1) to give the 4-fluoro-*N*-[4-({[2-chloro-5-methylquinazolin-4-yl]-amino}methyl)phenyl]benzamide (490 mg, 58% yield). The intermediate product (250 mg, 0.6 mmol) from step 1 was aminated with monomethylamine following procedure A (step 1) to obtain the final product (98 mg, 40% yield). mp 228−230 °C; ^{1}H NMR (DMSO) δ 2.50 (s, 3H), 2.78 (s, 6H), 4.69 (d, J = 5.8 Hz, 2H), 6.43 (s, 1H), 6.82 (d, J = 6.8 Hz, 1H), 7.11 (m, 1H), 7.30−7.43 (m, 5H), 7.69 (d, J = 7.8 Hz, 2H), 8.02 (m, 2H), 10.23 (s, 1H) ppm; MS (ESI) m/z 416.3. Anal. Calcd for $C_{24}H_{22}FN_5O$ (%): C, 69.38; H, 5.34; N, 16.86. Found: C, 69.42; H, 5.43; N, 16.90.

Preparation of 4-Chloro-*N*-[4-({[8-methyl-2-(methylamino)-quinazolin-4-yl]amino}methyl)phenyl]benzamide (21). 4-Chloro-*N*-[4-({[8-methyl-2-(methylamino)quinazolin-4-yl]amino}methyl)-phenyl]benzamide was prepared starting from 8-methyl-2,4-dichloroquinazoline, 4-aminobenzylamine, and 4-chlorobenzoyl chloride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 1) to yield the final product. Starting from 500 mg (2.35 mmol) of 8-methyl-2,4-dichloroquinazoline, 320 mg (32% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 2.40 (s, 3H), 2.83 (d, J = 4.6 Hz, 3H), 4.68 (d, J = 6.0 Hz, 2H), 6.40 (m, 1H), 6.92 (t, J = 7.8 Hz, 1H), 7.35 (m, 4H), 7.60 (d, J = 7.8 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 7.8 Hz, 1H), 7.97 (d, J = 8.8 Hz, 2H), 8.29 (s, 1H), 10.27 (s, 1H) ppm; MS (ESI) m/z 432.1. Anal. Calcd for $C_{24}H_{22}ClN_5O$ (%): C, 66.74; H, 5.13; N, 16.21. Found: C, 66.53; H, 5.35; N, 16.11.

Preparation of 4-Chloro-*N*-[4-({[7-methyl-2-(methylamino)-quinazolin-4-yl]amino}methyl)phenyl]benzamide (22). 4-Chloro-*N*-[4-({[7-methyl-2-(methylamino)quinazolin-4-yl]amino}methyl)-phenyl]benzamide was prepared starting from 7-methyl-2,

4-dichloroquinazoline (500 mg, 3.0 mmol), 4-aminobenzylamine, and 4-chlorobenzoyl chloride following procedure A (step 1) to give 4-chloro-*N*-[4-({[2-chloro-7-methylquinazolin-4-yl]amino}methyl)phenyl]benzamide (845 mg, 82% yield). The product (200 mg, 0.46 mmol) from step 1 was aminated with monomethylamine hydrochloride to obtain the final product (150 mg, 35% yield). mp 264−266 °C; ^{1}H NMR (DMSO) δ 2.34 (s, 3H), 2.80 (d, J = 4.3 Hz, 3H), 4.67 (d, J = 5.8 Hz, 2H), 6.44 (s, 1H), 6.88 (dd, J = 1.8, 8.3 Hz, 1H), 7.08 (s, 1H), 7.35 (d, J = 8.3 Hz, 2H), 7.60 (d, J = 8.3 Hz, 2H), 7.69 (d, J = 8.6 Hz, 2H), 7.91 (d, J = 8.6 Hz, 1H), 7.07 (d, J = 8.6 Hz, 2H), 8.32 (s, 1H), 10.28 (s, 1H) ppm; MS (ESI) m/z 432.2. Anal. Calcd for $C_{24}H_{22}ClN_5O$ (%): C, 66.74; H, 5.13; N, 16.21. Found: C, 66.96; H, 5.34; N, 16.11.

Preparation of 4-Chloro-*N*-[4-({[6-methyl-2-(methylamino)-quinazolin-4-yl]amino}methyl)phenyl]benzamide (23). 4-Chloro-*N*-[4-({[6-methyl-2-(methylamino)quinazolin-4-yl]amino}methyl)-phenyl]benzamide was prepared starting from 6-methyl-2, 4-dichloroquinazoline, 4-aminobenzylamine, and 4-chlorobenzoyl chloride following procedure A (step 1). The intermediate product from (step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from 6-methyl-2,4-dichloroquinazoline (700 mg, 3.5 mmol), 400 mg (61% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 2.49 (s, 3H), 3.08−3.03 (d, J = 5.0 Hz, 3H), 5.91−5.85 (m, 1H), 6.82−6.76 (m, 1H), 7.30−7.24 (m, 4H), 7.50−7.43 (m, 4H), 7.84−7.78 (m, 2H), 7.99−7.97 (m, 1H), 12.10 (s, 1H) ppm; MS (ESI) m/z 432.3. Anal. Calcd for $C_{24}H_{22}ClN_5O$ (%): C, 66.74; H, 5.13; N, 16.21. Found: C, 66.53; H, 5.36; N, 16.46.

Preparation of 4-Chloro-*N*-[4-({[5-methyl-2-(methylamino)-quinazolin-4-yl]amino}methyl)phenyl]benzamide (24). 4-Chloro-*N*-[4-({[5-methyl-2-(methylamino)quinazolin-4-yl]amino}methyl)-phenyl]benzamide was prepared starting from 5-methyl-2, 4-dichloroquinazoline (426 mg, 2 mmol), 4-aminobenzylamine, and 4-chlorobenzoyl chloride following procedure A (step 1) to give the 4-chloro-*N*-[4-({[2-chloro-5-methylquinazolin-4-yl]-amino}methyl)phenyl]benzamide (155 mg, 18% yield). The product (120 mg, 0.28 mmol) from step 1 was aminated with monomethylamine to obtain the final product (71 mg, 60% yield). ^{1}H NMR (DMSO) δ 2.80 (m, 6H), 4.72 (m, 2H), 6.90 (d, J = 7.6 Hz, 1H), 7.17 (d, J = 7.6 Hz, 1H), 7.38 (m, 1H), 7.59 (d, J = 8.3 Hz, 2H), 7.60 (d, J = 8.3 Hz, 2H), 7.70 (d, J = 9.0 Hz, 2H), 7.97 (d, J = 8.3 Hz, 2H), 10.29 (s, 1H) ppm; MS (ESI) m/z 432.3. Anal. Calcd for $C_{24}H_{22}ClN_5O$ (%): C, 66.74; H, 5.13; N, 16.21. Found: C, 66.53; H, 5.45; N, 16.34.

Preparation of 6-Chloro-*N*-[4-({[5-methyl-2-(methylamino)-quinazolin-4-yl]amino}methyl)phenyl]nicotinamide (25). 6-Chloro-*N*-[4-({[5-methyl-2-(methylamino)quinazolin-4-yl]amino}methyl)-phenyl]nicotinamide was prepared starting from 8-methyl-2, 4-dichloroquinazoline (639 mg, 3.0 mmol), 4-aminobenzylamine, and 6-chloronicotinoyl chloride following procedure A (step 1) to give 6-chloro-*N*-[4-({[2-chloro-8-methylquinazolin-4-yl]amino}-methyl)phenyl]nicotinamide (490 mg, 37% yield). The product (200 mg, 0.46 mmol) from step 1 was aminated with monomethylamine hydrochloride following procedure B (step 2) to obtain the final product (66 mg, 33% yield). mp 233−236 °C; ^{1}H NMR (DMSO) δ 2.79 (m, 6H), 4.70 (d, J = 5.3 Hz, 2H), 6.42 (s, 1H), 6.82 (d, J = 7.0 Hz, 1H), 7.11 (d, J = 8.6 Hz, 1H), 7.32 (t, J = 7.7 Hz, 1H), 7.42 (d, J = 8.3 Hz, 2H), 7.69 (d, J = 8.6 Hz, 1H), 7.70 (d, J = 8.3 Hz, 2H), 8.34 (dd, J = 3.0, 8.8 Hz, 1H), 8.93 (d, J = 2 Hz, 1H), 10.45 (s, 1H) ppm; MS (ESI) m/z 432.3. Anal. Calcd for $C_{23}H_{21}ClN_6O$ (%): C, 63.81; H, 4.89; N, 19.41. Found: C, 63.62; H, 5.03; N, 19.23.

Preparation of 6-Chloro-*N*-[4-({[7-methyl-2-(methylamino)-quinazolin-4-yl]amino}methyl)phenyl]nicotinamide (26). (2-*N*-Dimethylamino-7-methylquinazolin-4-yl)(4-nitrobenzyl)amine (540 mg, 1.67 mmol) was dissolved in THF (30 mL), Pd−C (10% wet wt) (100 mg) was added, and the mixture was hydrogenated under 1 atm of pressure for 24 h. When completed MeOH (500 mL) was added, and the mixture was filtered over Celite. The solvent were removed in vacuum to obtain (4-aminobenzyl)(2-methylamino-7-methyl-quinazolin-4-yl)amine, which was suspended in THF (10 mL) and NEt_3 (0.4 mL) and cooled to 0 °C. 6-Chloronicotinoyl chloride (280 mg, 1.59 mmol) was added. After completion 1 N NaOH (0.5 mL) was added, and the layers were separated. The organic layer was extracted twice with THF/ethyl acetate (10 mL), and the combined organic layers were dried over $MgSO_4$. The crude material was purified by column chromatography using CH_2Cl_2/MeOH/NH_3 to give product (230 mg, 31% yield). ^{1}H NMR (DMSO) δ 2.33 (s, 3H), 2.79 (d, J = 4.5 Hz, 3H), 4.67 (d, J = 5.8 Hz, 2H), 6.38 (m, 1H), 6.87 (d, J = 7.8 Hz, 1H), 7.07 (m, 1H), 7.36 (d, J = 7.8 Hz, 2H), 7.69 (m, 3H), 7.90 (d, J = 7.8 Hz, 1H), 8.29 (s, 1H), 8.34 (d, J = 7.8 Hz, 1H), 8.93 (s, 1H) ppm; MS (ESI) m/z 433.2. Anal. Calcd for $C_{23}H_{21}ClN_6O$ (%): C, 63.81; H, 4.89; N, 19.41. Found: C, 64.10; H, 5.05; N, 19.32.

Preparation of 6-Chloro-*N*-[4-({[6-methyl-2-(methylamino)-quinazolin-4-yl]amino}methyl)phenyl]nicotinamide (27). 6-Chloro-*N*-[4-({[6-methyl-2-(methylamino)quinazolin-4-yl]amino}methyl)-phenyl]nicotinamide was prepared starting from 6-methyl-2, 4-dichloroquinazoline (639 mg, 3.0 mmol), 4-aminobenzylamine, and 6-chloronicotinoyl chloride following procedure A (step 1) to give the 6-chloro-*N*-[4-({[2-chloro-8-methylquinazolin-4-yl]-amino}methyl)phenyl]nicotinamide (490 mg, 37% yield). The product (200 mg, 0.46 mmol) from step 1 was aminated with monomethylamine hydrochloride following procedure B (step 2) to obtain the final product (90 mg, 43% yield). mp 233−236 °C; ^{1}H NMR (DMSO) δ 2.51 (s, 3H), 2.95 (s, 3H), 4.76 (br s, 2H), 7.45−7.40 (m, 2H) 7.65−7.61 (m, 1H) 7.78−7.68 (m, 3H), 8.22−8.16 (m, 1H), 8.39−8.35 (m, 1H), 8.95 (m, 1H), 10.18 (br s, 1H), 9.83−9.70 (m, 1H) 10.58 (s, 1H), 12.30 (br s, 1H) ppm; MS (ESI) m/z 432.3. Anal. Calcd for $C_{23}H_{21}ClN_6O$ (%): C, 63.81; H, 4.89; N, 19.41. Found: C, 63.52; H, 5.07; N, 19.47.

Preparation of 6-Chloro-*N*-[4-({[8-methyl-2-(methylamino)-quinazolin-4-yl]amino}methyl)phenyl]nicotinamide (28). 6-Chloro-*N*-[4-({[8-methyl-2-(methylamino)quinazolin-4-yl]amino}methyl)-phenyl]nicotinamide was prepared starting from 8-methyl-2, 4-dichloroquinazoline, 4-aminobenzylamine, and 6-chloronicotinoyl chloride following procedure A (step 1). The intermediate product from step 1 was aminated using monomethylamine following procedure A (step 2) to yield the final product. Starting from 8-methyl-2,4-dichloroquinazoline (213 mg, 0.1 mmol), 250 mg (23% yield) of the final product was isolated. ^{1}H NMR (DMSO) δ 2.40 (s, 3H), 2.83 (d, J = 4.8 Hz, 3H), 4.70 (d, J = 5.5 Hz, 2H), 6.42 (s, 1H), 6.93 (m, 1H), 7.37 (m, 3H), 7.69 (m, 3H), 7.86 (d, J = 7.8 Hz, 1H), 8.31 (s, 1H), 8.34 (dd, J = 2,5 ; 8.3 Hz, 1H), 8.93 (d, J = 2.5 Hz, 1H), 10.46 (s, 1H) ppm; MS (ESI) m/z 433.2. Anal. Calcd for $C_{23}H_{21}ClN_6O$ (%): C, 63.81; H, 4.89; Cl, 8.19; N, 19.41; O, 3.70. Found: C, 64.03; H, 5.04; N, 19.62.

Preparation of *N*-[4-({[2-(Dimethylamino)-7-methylquinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (29). *N*-[4-({[2-(Dimethylamino)-7-methylquinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide was prepared by amination of *N*-(4-{[(2-chloro-7-methylquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide (150 mg, 0.36 mmol) with 2 M dimethylamine hydrochloride in 2-propanol following procedure B (step 2). After purification by column chromatography and solvent removal the final product (110 mg, 71% yield) was obtained as a white solid. ^{1}H NMR (DMSO) δ 2.36 (s, 3H), 2.63 (s, 6H), 4.68 (d, J = 5.5 Hz, 2H), 6.93 (m, 1H), 7.15 (m, 1H), 7.36 (m, 4H), 7.69 (d, J = 8.5 Hz, 2H), 7.95 (d, J = 8.1 Hz, 1H), 8.02 (m, 2H), 10.23 (s, 1H) ppm; MS (ESI) m/z 430.3. Anal. Calcd for $C_{25}H_{24}FN_5O$ (%): C, 69.91; H, 5.63; F, 4.42; N, 16.31. Found: C, 69.73; H, 5.87; N, 16.23.

Preparation of 4-Fluoro-*N*-[4-({[7-methyl-2-(propylamino)-quinazolin-4-yl]amino}methyl)phenyl]benzamide (30). 4-Fluoro-*N*-[4-({[7-methyl-2-(propylamino)quinazolin-4-yl]amino}methyl)-phenyl]benzamide was prepared by amination of *N*-(4-{[(2-chloro-7-methylquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide (150 mg, 0.36 mmol) and propylamine in THF

following procedure B (step 2). After purification by HPLC and solvent removal, the final product (150 mg, 76% yield) was obtained as an off-white solid. mp 170−174 °C; ^{1}H NMR (DMSO) δ 0.89 (m, 3H), 1.52 (m, 2H), 2.44 (s, 3H), 3.36 (m, 3H), 4.76 (d, J = 5.5 Hz, 2H), 7.25 (m, 2H), 7.37 (m, 4H), 7.73 (d, J = 8.3 Hz, 2H), 7.97 (m, 1H), 8.02 (m, 2H), 8.20 (m, 1H), 10.28 (s, 1H) ppm; MS (ESI) m/z 444.1. Anal. Calcd for $C_{26}H_{26}FN_5O$ (%): C, 70.41; H, 5.91; N, 15.79. Found: C, 70.24; H, 6.03; N, 15.85.

Preparation of *N*-(4-{[(2-Azetidin-1-yl-7-methylquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide (31). *N*-(4-{[(2-Azetidin-1-yl-7-methylquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide was prepared by amination of *N*-(4-{[(2-chloro-7-methylquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide (42 mg, 0.1 mmol) with azetidine hydrochloride and NEt_3 in dioxane following procedure B (step 2). After purification by HPLC and solvent removal, the final product (20 mg, 74% yield) was obtained as an off-white solid. Analytical LC/MS using an Aquasil C18 column, ACN/H_2O eluent at 0.8 mL/min flow (containing 0.1% formic acid), 5.5 min gradient 0% ACN to 100% ACN, monitored by UV absorption at 254 nm showed >99.5% purity. MS (ESI) m/z 442.2.

Preparation of 4-Fluoro-*N*-(4-{[(7-methyl-2-pyrrolidin-1-ylquinazolin-4-yl)amino]methyl}phenyl)benzamide (32). 4-Fluoro-*N*-(4-{[(7-methyl-2-pyrrolidin-1-ylquinazolin-4-yl)amino]methyl}-phenyl)benzamide was prepared by amination of *N*-(4-{[(2-chloro-7-methylquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide (42 mg, 0.1 mmol) and pyrrolidine in dioxane following procedure B (step 2). After purification by HPLC and solvent removal, the final product (16 mg, 56% yield) was obtained as an off-white solid. Analytical LC/MS using an Aquasil C18 column, ACN/H_2O eluent at 0.8 mL/min flow (containing 0.1% formic acid), 5.5 min gradient 0% ACN to 100% ACN, monitored by UV absorption at 254 nm showed >99.5% purity. MS (ESI) m/z 456.5.

Preparation of 4-Fluoro-*N*-(4-{[(7-methyl-2-piperidin-1-ylquinazolin-4-yl)amino]methyl}phenyl)benzamide (33). 4-Fluoro-*N*-(4-{[(7-methyl-2-piperidin-1-ylquinazolin-4-yl)amino]methyl}-phenyl)benzamide was prepared by amination of *N*-(4-{[(2-chloro-7-methylquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide (42 mg, 0.1 mmol) and piperidine in dioxane following procedure B (step 2). After purification by HPLC and solvent removal, the final product (11 mg, 38% yield) was obtained as an off-white solid. Analytical LC/MS using an Aquasil C18 column, ACN/H_2O eluent at 0.8 mL/min flow (containing 0.1% formic acid), 5.5 min gradient 0% ACN to 100% ACN, monitored by UV absorption at 254 nm showed >99.5% purity. MS (ESI) m/z 470.6.

Preparation of 4-Fluoro-*N*-(4-{[(7-methyl-2-piperazin-1-ylquinazolin-4-yl)amino]methyl}phenyl)benzamide (34). 4-Fluoro-*N*-(4-{[(7-methyl-2-piperazin-1-ylquinazolin-4-yl)amino]methyl}-phenyl)benzamide was prepared by amination of *N*-(4-{[(2-chloro-7-methylquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide (42 mg, 0.1 mmol) and piperazine in dioxane following procedure B (step 2). After purification by HPLC and solvent removal, the final product (21 mg, 72% yield) was obtained as an off-white solid. Analytical LC/MS using an Aquasil C18 column, ACN/H_2O eluent at 0.8 mL/min flow (containing 0.1% formic acid), 5.5 min gradient 0% ACN to 100% ACN, monitored by UV absorption at 254 nm showed 96.0% purity. MS (ESI) m/z 471.6.

Preparation of 4-Fluoro-*N*-{4-[({7-methyl-2-[(3*R*)-3-methylpiperazin-1-yl]quinazolin-4-yl}amino)methyl]phenyl}benzamide (35). 4-Fluoro-*N*-{4-[({7-methyl-2-[(3*R*)-3-methylpiperazin-1-yl]quinazolin-4-yl}amino)methyl]phenyl}benzamide was prepared by amination of *N*-(4-{[(2-chloro-7-methylquinazolin-4-yl)amino]-methyl}phenyl)-4-fluorobenzamide (25 mg, 0.06 mmol) and 2-(*R*)-methylpiperazine in dioxane following procedure B (step 2). After purification by HPLC and solvent removal, the final product (19 mg, 64% yield) was obtained as an off-white solid. ^{1}H NMR (DMSO) δ 0.98 (d, J = 6.5 Hz, 3H), 2.34 (m, 5H), 2.54 (m, 1H), 2.69 (m, 1H), 2.86 (d, J = 11.9 Hz, 1H), 4.56 (d, J = 11.9 Hz, 2H), 4.63 (t, J = 5.3 Hz, 2H), 6.89 (d, J = 8.3 Hz, 1H), 7.07 (s, 1H), 7.36 (m, 4H), 7.69 (d, J = 8.0 Hz, 2H), 7.91 (d, J = 8.6 Hz, 1H), 8.02 (m, 2H), 8.43 (t, J = 5.3 Hz, 1H), 10.22 (s, 1H) ppm; MS (ESI) m/z 485.6. Anal. Calcd for $C_{28}H_{29}FN_6O$ (%): C, 69.40; H, 6.03; F, 3.92; N, 17.34; O, 3.30. Found: C, 69.15; H, 6.14; N, 17.17.

Preparation of *N*-[4-({[2-(3,3-Dimethylpiperazin-1-yl)-7-methylquinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (36). 4-*N*-[4-({[2-(3,3-Dimethylpiperazin-1-yl)-7-methylquinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide was prepared by amination of *N*-(4-{[(2-chloro-7-methylquinazolin-4-yl)-amino]methyl}phenyl)-4-fluorobenzamide (150 mg, 0.36 mmol) and 2,2-dimethylpiperazine in THF following procedure B (step 2). After purification by HPLC and solvent removal, the final product (105 mg, 59% yield) was obtained as an off-white solid. ^{1}H NMR (DMSO) δ 0.98 (s, 6H), 2.34 (s, 3H), 2.79 (m, 2H), 3.52 (s, 2H), 3.70 (m, 2H), 4.62 (d, J = 5.5 Hz, 2H), 6.89 (dd, J = 1.5, 8.3 Hz, 1H), 7.06 (s, 1H), 7.35 (m, 4H), 7.60 (d, J = 8.6 Hz, 2H), 7.91 (d, J = 8.3 Hz, 1H), 8.02 (m, 2H), 8.45 (t, J = 5.5 Hz, 1H), 10.22 (s, 1H) ppm; MS (ESI) m/z 499.3. Anal. Calcd for $C_{29}H_{31}FN_6O$ (%): C, 69.86; H, 6.27; N, 16.86. Found: 69.63; H, 6.52; N, 17.01.

Preparation of 4-Fluoro-*N*-[4-({[7-methyl-2-(4-methylpiperazin-1-yl)quinazolin-4-yl]amino}methyl)phenyl]benzamide (37). 4-Fluoro-*N*-[4-({[7-methyl-2-(4-methylpiperazin-1-yl)quinazolin-4-yl]amino}methyl)phenyl]benzamide was prepared by amination of *N*-(4-{[(2-chloro-7-methylquinazolin-4-yl)amino]methyl}-phenyl)-4-fluorobenzamide (42 mg, 0.1 mmol) and 1-methylpiperazine in dioxane following procedure B (step 2). After purification by HPLC and solvent removal, the final product (20 mg, 67% yield) was obtained as an off-white solid. Analytical LC/MS using an Aquasil C18 column, ACN/H_2O eluent at 0.8 mL/min flow (containing 0.1% formic acid), 5.5 min gradient 0% ACN to 100% ACN, monitored by UV absorption at 254 nm showed >99.5% purity. MS (ESI) m/z 485.6.

Preparation of 4-Fluoro-*N*-(4-{[(7-methyl-2-morpholin-4-ylquinazolin-4-yl)amino]methyl}phenyl)benzamide (38). 4-Fluoro-*N*-(4-{[(7-methyl-2-morpholin-4-ylquinazolin-4-yl)amino]methyl}-phenyl)benzamide was prepared by amination of *N*-(4-{[(2-chloro-7-methylquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide (42 mg, 0.1 mmol) and morpholine in dioxane following procedure B (step 2). After purification by HPLC and solvent removal, the final product (17 mg, 60% yield) was obtained as an off-white solid. Analytical LC/MS using an Aquasil C18 column, ACN/H_2O eluent at 0.8 mL/min flow (containing 0.1% formic acid), 5.5 min gradient 0% ACN to 100% ACN, monitored by UV absorption at 254 nm showed >99.5% purity. MS (ESI) m/z 472.5.

Preparation of *N*-[4-({[2-(Dimethylamino)-7-iodoquinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (39). Step 1: Synthesis of Ethyl {[(3-Iodophenyl)amino]carbonothioyl}carbamate. To a solution of 3-iodoaniline (11.4 g, 52 mmol) in CH_2Cl_2 (150 mL) was added a solution of ethyl isothiocyanatoformate (6.14 mL, 52 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred at 20 °C for 2 h and concentrated under reduced pressure to give an off-white solid (18.2 g, 100% yield). MS (ESI) m/z 351.1.

Step 2: Synthesis of 2-(Ethylthio)-7-iodoquinazolin-4(3*H*)-one. To a solution of ethyl {[(3-iodophenyl)amino]carbonothioyl}carbamate (18.2 g, 52 mmol) in acetone (200 mL) was added dropwise EtI (4.16 mL, 52 mmol) and K_2CO_3 (21.5 g, 156 mmol). The resulting mixture was stirred at room temperature overnight and filtered through a pad of Celite. The filtration was concentrated in vacuum, and the residue was dissolved in CH_2Cl_2 (200 mL). The solution was washed with water and brine and dried over ($MgSO_4$). Evaporation of solvent gave a yellow oil (19.0 g), which was dissolved in phenyl ether (200 mL). The mixture was heated at 200 °C overnight and cooled to 20 °C, during which time a lot of white precipitate was formed. The mixture was diluted with hexanes and filtered to

give the title compound as an off-white solid (10.8 g, 63% yield). MS (ESI) *m*/*z* 333.1.

Step 3: Synthesis of 7-iodoquinazoline-2,4(1*H*,3*H*)-dione. To a solution of 2-(ethylthio)-7-iodoquinazolin-4(3*H*)-one (10.8 g, 32.5 mmol) in ethanol (40 mL) was added 6 N HCl (40 mL). The mixture was heated at 80 °C overnight and then cooled to 20 °C, during which time a lot of white precipitate was formed. The resulting solid was collected by filtration to give an off-white solid (8.97 g, 96% yield). MS (ESI) *m*/*z* 289.0.

Step 4: Synthesis of 2,4-Dichloro-7-iodoquinazoline. $POCl_3$ (10 mL) was added to 7-iodoquinazoline-2,4(1*H*,3*H*)-dione (3.78 g, 13.1 mmol), followed by addition of *N*,*N*-dimethylaniline (1 mL). The resulting mixture was heated at 115 °C for 6 h. After cooling to 20 °C, most of $POCl_3$ was removed by distillation under reduced pressure. The residue was poured into ice−water; ammonium hydroxide was added to adjust the pH to 5−7. The mixture was extracted several times with CH_2Cl_2, and the combined extracts were washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (EtOAc/CH_2Cl_2 = 5:95) to give 2,4-dichloro-7-iodoquinazoline as an off-white solid (3.56 g, 84% yield). mp 163 °C; MS (ESI) *m*/*z* 324.9.

Step 5: Synthesis of *N*-(4-{[(2-Chloro-7-iodoquinazolin-4-yl)amino]methyl}phenyl)-4-fluorobenzamide. To a solution of *N*-[4-(aminomethyl)phenyl]-4-fluorobenzamide (537 mg, 2.2 mmol) in CH_2Cl_2 (10 mL) was added Et_3N (0.84 mL, 6.0 mmol), followed by addition of 2,4-dichloro-7-iodoquinazoline (648 mg, 2.0 mmol). The resulting mixture was stirred at room temperature for 20 h, during which time a lot of precipitate was formed. The resulting solid was collected by filtration and washed with a small amount of EtOAc and water to give the expected product as an off-white solid (745 mg, 70% yield). MS (ESI) *m*/*z* 533.0.

Step 6: Synthesis of *N*-[4-({[2-(Dimethylamino)-7-iodoquinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (39). To a solution of *N*-(4-{[(2-chloro-7-iodoquinazolin-4-yl)amino]-methyl}phenyl)-4-fluorobenzamide (532 mg, 1 mmol) in THF (2 mL) was added dimethylamine solution (40% in water, 0.6 mL, 5 mmol). The resulting mixture was heated at 100 °C in a sealed tube for 26 h and then cooled to 20 °C. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography (CH_2Cl_2:CH_3OH = 90:10) to give an off-white solid (487 mg, 90% yield). ^{1}H NMR (DMSO) δ 3.23 (s, 6H), 4.75 (d, J = 6.0 Hz, 2H), 7.37 (m, 4H), 7.72 (d, J = 8.5 Hz, 1H), 7.81 (d, J = 8.3 Hz, 1H), 8.02 (m, 3H), 8.10 (s, 1H), 10.0 (s, 1H), 10.26 (s, 1H), 11.48 (s, 1H) ppm; MS (ESI) *m*/*z* 542.1. Anal. Calcd for $C_{24}H_{21}FIN_5O$ (%): C, 53.25; H, 3.91; N, 12.94. Found: C, 53.07; H, 4.17; N, 12.76.

Preparation of *N*-[4-({[7-Cyano-2-(dimethylamino)quinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (41). To a solution of *N*-[4-({[2-(dimethylamino)-7-iodoquinazolin-4-yl]amino}-methyl)phenyl]-4-fluorobenzamide (252 mg, 0.46 mmol) in DMF 9 (mL) were added $Zn(CN)_2$ (66 mg, 0.56 mmol) and $Pd(PPh_3)_4$ (26 mg, 5 mol %). The resulting mixture was heated at 80 °C under N_2 for 5 h. Upon completion, the reaction mixture was cooled to 20 °C and poured into cold water. The resulting solid was collected by filtration, purified by HPLC, and then converted to the HCl salt to give an off-white solid (156 mg, 70% yield). mp 150 °C; ^{1}H NMR (DMSO) δ 3.28 (s, 6H), 4.78 (d, J = 5.6 Hz, 2H), 7.36 (t, J = 8.5 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H), 7.73 (d, J = 9.3 Hz, 2H), 7.88 (d, J = 8.8 Hz, 1H), 8.02 (d, J = 9.1 Hz, 1H), 8.03 (d, J = 8.5 Hz, 1H), 8.24 (s, 1H), 8.53 (d, J = 8.1 Hz, 1H), 10.29 (s, 1H), 10.36 (s, 1H), 12.08 (s, 1H) ppm; MS (ESI) *m*/*z* 441.1. Anal. Calcd for $C_{25}H_{21}FN_6O$ (%): C, 68.17; H, 4.81; N, 19.08. Found: C, 67.95; H, 5.03; N, 19.03.

Preparation of *N*-[4-({[7-Acetyl-2-(dimethylamino)quinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (42). To a solution of *N*-[4-({[2-(dimethylamino)-7-iodoquinazolin-4-yl]amino}-methyl)phenyl]-4-fluorobenzamide (400 mg, 0.74 mmol) in DMF (5 mL) was added $PdCl_2(PPh_3)_2$ (26 mg, 5 mol %) as catalyst, followed by addition of tributyl(1-ethoxyvinyl)tin (0.27 mL, 0.81 mmol). The resulting mixture was heated at 90 °C under nitrogen for 4 h. Upon completion, the reaction mixture was cooled to 20 °C and then poured into cold water. The resulting solid was collected by filtration. The solid was dissolved in MeOH (3 mL) and 6 N HCl (0.5 mL) and heated at 70 °C for 3h. The mixture was cooled to 20 °C and concentrated in vacuum. The resulting residue was purified by flash chromatography (CH_2Cl_2:CH_3OH = 90:10) to give an off-white solid (168 mg, 50% yield). mp 195 °C; ^{1}H NMR (DMSO) δ 2.67 (s, 3H), 3.28 (s, 6H), 4.78 (d, J = 5.8 Hz, 2H), 7.36 (t, J = 9.0 Hz, 2H), 7.42 (d, J = 8.3 Hz, 2H), 7.74 (d, J = 8.3 Hz, 2H), 7.96 (d, J = 8.3 Hz, 1H), 8.03 (m, 2H), 8.38 (s, 1H), 8.51 (d, J = 8.3 Hz, 1H), 10.30 (s, 1H), 10.34 (s, 1H), 11.91 (s, 1H) ppm; MS (ESI) *m*/*z* 458.2. Anal. Calcd for $C_{26}H_{24}FN_5O_2$ (%): C, 68.26; H, 5.29; N, 15.31. Found: C, 68.14; H, 5.45; N, 15.23.

Preparation of *N*-[4-({[2-(Dimethylamino)-7-(1-hydroxyethyl)-quinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (43). To a solution of *N*-[4-({[7-acetyl-2-(dimethylamino)quinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (84 mg, 0.18 mmol) in MeOH (5 mL) was added $NaBH_4$ (14 mg, 0.36 mmol). The resulting mixture was stirred at 20 °C for 6 h. The reaction mixture was concentrated under reduced pressure, and the residue was treated with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic phases were washed with brine and dried over $MgSO_4$. The solvent was removed, and the residue was purified by HPLC and converted to the HCl salt to give an off-white solid (71 mg, 80% yield). mp 232 °C; ^{1}H NMR (DMSO) δ 1.37 (d, J = 6.3 Hz, 3H), 3.24 (s, 6H), 4.77 (d, J = 5.8 Hz, 2H), 4.86 (m, 1H), 5.56 (d, J = 3.8 Hz, 1H), 7.37 (m, 5H), 7.73 (d, J = 9.1 Hz, 2H), 7.82 (s, 1H), 8.03 (m, 2H), 8.28 (d, J = 8.8 Hz, 1H), 10.04 (s, 1H), 10.29 (s, 1H), 11.62 (s, 1H) ppm; MS (ESI) *m*/*z* 460.2. Anal. Calcd for $C_{26}H_{26}FN_5O_2$ (%): C, 67.96; H, 5.70; N, 15.24. Found: C, 68.12; H, 5.84; N, 15.15.

Preparation of *N*-[4-({[2-(Dimethylamino)-7-formylquinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (44). To a solution of *N*-[4-({[2-(dimethylamino)-7-iodoquinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (417 mg, 0.75 mmol) in DMF 3 (mL) were added HCO_2Na (102 mg, 1.5 mmol) and $PdCl_2(PPh_3)_2$ (26 mg, 5 mol %). The resulting mixture was heated at 100 °C under CO (gas, 1 atm) for 5 h. Upon completion, the reaction mixture was cooled to 20 °C and poured into cold water. The resulting solid was collected by filtration and purified by HPLC to give an off-white solid (123 mg, 37% yield). ^{1}H NMR (DMSO) δ 3.26 (s, 6H), 4.80 (d, J = 5.8 Hz, 2H), 7.34−7.42 (m, 4H), 7.73 (d, J = 8.6 Hz, 2H), 7.92 (s, 1H), 8.02 (m, 2H), 8.18 (s, 1H), 8.48 (d, J = 7.5 Hz, 1H), 10.14 (s, 1H), 10.28 (s, 1H), 10.79 9s, 1H) ppm; MS (ESI) *m*/*z* 444.1. Anal. Calcd for $C_{25}H_{22}FN_5O_2$ (%): C, 67.71; H, 5.00; N, 15.79. Found: C 67.53; H, 5.18; N, 15.55.

Preparation of *N*-[4-({[2-(Dimethylamino)-7-(hydroxymethyl)-quinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (45). To a solution of *N*-[4-({[2-(dimethylamino)-7-formylquinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (100 mg, 0.23 mmol) in MeOH (5 mL) was added $NaBH_4$ (17 mg, 0.46 mmol). The resulting mixture was stirred at 20 °C for 2 h. The reaction mixture was concentrated under reduced pressure, and the residue was treated with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic phases were washed with brine and dried over $MgSO_4$. The solvent was removed, and the residue was purified by HPLC and converted to the HCl salt to give an off-white solid (94 mg, 85% yield). mp 158 °C; ^{1}H NMR (DMSO) δ 3.25 (s, 6H), 4.66 (s, 2H), 4.76 (d, J = 5.8 Hz, 2H), 5.61 (s, 1H), 7.37 (m, 5H), 7.73 (d, J = 8.3 Hz, 2H), 7.81 (s, 1H), 8.03 (m, 2H), 8.29 (d, J = 8.3 Hz, 1H), 10.06 (t, J = 5.8 Hz, 1H), 10.29 (s, 1H), 11.65 (s, 1H) ppm; MS(ESI) *m*/*z* 446.2. Anal. Calcd for $C_{25}H_{24}FN_5O_2$ (%): C, 67.40; H, 5.43; N, 15.72. Found: C, 67.52; H, 5.68; N, 15.96.

Preparation of *N*-{4-[({2-(Dimethylamino)-7-[(dimethylamino)-methyl]quinazolin-4-yl}amino)methyl]phenyl}-4-fluorobenzamide (46). To a solution of *N*-[4-({[7-(aminomethyl)-2-(dimethylamino)quinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide HCl salt (110 mg, 0.21 mmol) in MeOH (2 mL) were added formaldehyde (37%, 68 μL, 0.84 mmol), $NaBH_3CN$ (13 mg, 0.21 mmol), and $ZnCl_2$ (14 mg, 0.10 mmol). The resulting mixture was stirred at 20 °C overnight. The mixture was filtered and washed with methanol. The resulting filtrate was concentrated in vacuum and subjected to HPLC separation. The product was converted to the HCl salt, which was an off-white solid (68 mg, 60% yield). mp 94 °C; ^{1}H NMR (DMSO) δ 2.73 (s, 6H), 3.26 (s, 6H), 4.40 (s, 2H), 4.77 (d, J = 5.6 Hz, 2H), 7.37 (m, 4H), 7.73 (d, J = 9.1 Hz, 2H), 7.87 (s, 1H), 8.02 (d, J = 6.0 Hz, 1H), 8.04 (d, J = 6.0 Hz, 1H), 8.44 (s, 1H), 10.29 (s, 1H), 10.89 (s, 1H), 11.98 (s, 1H) ppm; MS (ESI) m/z 473.3. Anal. Calcd for $C_{27}H_{29}FN_6O$ (%): C, 68.62; H, 6.19; N, 17.78. Found: C, 68.46; H, 6.49; N, 17.83.

Synthesis of *N*-[4-({[2-(Dimethylamino)-7-vinylquinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (47). To a solution of *N*-[4-({[2-(dimethylamino)-7-iodoquinazolin-4-yl]amino}-methyl)phenyl]-4-fluorobenzamide (541 mg, 1 mmol) in DMF (5 mL) was added $PdCl_2(PPh_3)_2$ (35 mg, 5 mol %) as catalyst, followed by addition of tributyl(vinyl)tin (0.35 mL, 1.2 mmol). The resulting mixture was heated at 90 °C under nitrogen for 3 h. Upon completion, the reaction mixture was cooled to room temperature and then poured into cold water. The resulting solid was collected by filtration and then purified by flash chromatography (CH_2Cl_2:CH_3OH = 90:10) to give an off-white solid, which was treated with HCl in methanol to form the HCl salt as an off-white solid (292 mg, 61% yield). mp 145 °C; ^{1}H NMR (DMSO) δ 3.33 (s, 6H), 4.74 (d, J = 5.8 Hz, 2H), 5.50 (d, J = 11.6 Hz, 1H), 6.04 (d, J = 18.1 Hz, 1H), 6.85 (dd, J = 18.1, 11.6 Hz, 1H), 7.37 (m, 4H), 7.51 (s, 1H), 7.61 (s, 1H), 7.72 (d, J = 8.6 Hz, 2H), 8.02 (m, 2H), 8.21 (d, J = 7.5 Hz, 1H), 10.27 (s, 1H), 11.61 (s, 1H) ppm; MS (ESI) m/z 442.1. Anal. Calcd for $C_{26}H_{24}FN_5O$ (%): C, 70.73; H, 5.48; N, 15.86. Found: C, 70.47; H, 5.64; N, 15.95.

Preparation of *N*-[4-({[2-(Dimethylamino)-7-ethylquinazolin-4-yl]amino}methyl)phenyl]-4-fluorobenzamide (48). To a solution of *N*-[4-({[2-(dimethylamino)-7-vinylquinazolin-4-yl]amino}-methyl)phenyl]-4-fluorobenzamide (239 mg, 0.5 mmol) in MeOH (10 mL) was added Pd/C (10%) catalyst (48 mg). The resulting mixture was hydrogenated at room temperature for 2 h. The reaction mixture was filtered through a pad of Celite and washed with methanol, and the resulting filtrate was concentrated under reduced pressure. The residue was subjected to HPLC separation to give an off-white solid, which was converted to the HCl salt as an off-white solid (229 mg, 96% yield). mp 270 °C; ^{1}H NMR (DMSO) δ 1.24 (t, J = 7.3 Hz, 3H), 2.77 (q, J = 7.3 Hz, 2H), 3.25 (s, 6H), 4.77 (d, J = 6.6 Hz, 2H), 7.36 (m, 5H), 7.62 (s, 1H), 7.73 (d, J = 8.6 Hz, 2H), 8.03 (dd, J = 9.4, 5.8 Hz, 2H), 8.25 (d, J = 8.3 Hz, 2H), 10.0 (t, J = 6.6 Hz, 1H), 10.28 (s, 1H), 11.58 (s, 1H) ppm; MS (ESI) m/z 444.2. Anal. Calcd for $C_{26}H_{26}FN_5O$ (%): C, 70.41; H, 5.91; N, 15.79. Found: C, 70.23; H, 6.12; N, 15.53.

Reporter Assay. The SW480 colorectal cancer cell line has a truncation mutation in APC and thus has a constitutively activated β-catenin/Tcf4 signaling pathway, which can be monitored by a luciferase reporter with Tcf4 binding sites in its promoter. Our Tcf4-luciferase reporter assay utilizes three different cell lines that were derived from SW480 by transient transfection with the following luciferase reporter constructs: (1) Tcf4-Luc, a promoter containing Tcf4 binding sites that drive firefly luciferase in the pGL3 vector, to measure the activity of β-catenin/Tcf4; (2) SV40-Luc, SV40 promoter driving firefly luciferase in the pGL3 vector, serving as a control for nonspecific inhibitors; (3) SV40-R-Luc, SV40 promoter driving Renilla luciferase in the pGL3 vector, serving as an internal control for cell number and toxicity. After transfection, stable cell lines were established by selection to contain the above luciferase reporters integrated into their chromosomes as follows: 33.13 cell line (Tcf4-Luc and SV40-R-Luc), 22C11 cell line (Tcf4-Luc and SV40-R-Luc), and 5A8 cell line (SV40-Luc and SV40-R-Luc). The 22C11 and 5A8 cell lines were grown in the presence of 500 μg/mL G418, and 33.13 was grown in the presence of 500 μg/mL G418 + 125 μg/mL Zeocin to maintain the integrated reporters. The cells were plated at 1×10^4 cells/well in solid white 96-well plates. After overnight incubation at 37 °C, 5% CO_2, compounds were added at titrated concentrations between 10 ng/mL and 10 μg/mL. The luciferase signal was detected using Promega's Dual-Glo luciferase assay system. After 24 h of incubation with compound, culture media were removed by aspiration and 75 μL of fresh medium was added into each well. Dual-Glo luciferase reagent (75 μL) was subsequently added per well, mixing with shaking for 10 min on a plate shaker. The firefly luminescence was measured on a CCD camera imaging device or a luminometer. After quantifying the firefly luminescence, 75 μL of Dual-Glo Stop & Glo reagent (Promega) was added to each well, and the plate was mixed by shaking for 10 min. R luciferase was then quantified using a CCD camera or a luminometer. The luminescence signal from each well was expressed as percent of control for both firefly and Renilla luciferase activity. The ratio of FF/R was then calculated and averaged between duplicate plates. Data were plotted using the LSW Data Analysis program (model 33) to determine the IC_{50} of each compound. A compound was considered positive only if it selectively inhibited the reporter activity in 33.13 and 22C11 but did not inhibit reporter activity in 5A8.

Colon Carcinoma Cell Growth Inhibition Assay. The colon cell growth inhibition assay was an *in vitro* assay that helped to determine if the lead compound inhibited the β-catenin/Tcf4 signaling pathway as indicated by growth inhibition of colon cell lines at concentrations similar to its IC_{50} in the reporter assay or if its activity was through another mechanism of action. The colon cell growth inhibition assay used four different colon cancer cell lines: HT29, DLD1, LoVo, and the SW480-derived 33.13 line which was one of the reporter cell lines carrying the integrated Tcf4-luciferase reporter. A compound of interest would be inhibitory at the concentrations similar to its IC_{50} in the reporter assay. The assay readout was based on Promega's Cell Titer-Glo luminescent cell viability assay. This is a homogeneous method to determine the number of viable cells in culture based on the quantification of ATP present. ATP signals the presence of metabolically active cells and can be measured with this system. Since monooxygenation of luciferin is catalyzed by luciferase in the presence of Mg^{2+}, ATP, and molecular oxygen, the resulting luminescence can be quantified. All four lines are grown in DMEM + 10% FBS + penicillin/streptomycin. Cells were trypsinized and plated at 2×10^3 cells/well in opaque 96-well plates. Compounds were added 24 h later so that concentration curves could be generated starting at 10 μg/mL with nine subsequent 2-fold dilutions. The plates were incubated with compound for 3 days, at which time the cells were lysed with Promega's Cell Titer-Glo reagent. The resulting luminescence was read on a CCD camera imaging device or a luminometer.

Differential Cell Assay. The RK3E differential adherent assay is an *in vitro* assay which helped to determine if the lead compound selectively inhibited the β-catenin/Tcf4 signaling pathway. This assay used rat kidney epithelial cells transformed with either mutant β-catenin or K-ras to look for a differential effect of compound between the two lines. A compound of interest would be inhibitory against the β-catenin transformed line but not against the Kras transformed line. This assay used two cell lines which were kindly provided by Dr. B. Vogelstein's laboratory: a transformed RK3E subclone expressing an activated K-rasG12 V (KRAS) and a transformed RK3E subclone expressing a mutant β-catenin harboring the S33Y mutation, which stabilizes β-catenin protein (β-cat). The assay readout is

based on Promega's Cell Titer-Glo luminescent cell viability assay as described above. Both lines were grown in DMEM + 10% FBS + penicillin/streptomycin. Cells were plated at 2×10^4 cells/well in opaque 96-well plates. Twenty-four hours later, compounds were added so that a concentration curve could be generated starting at 10 μg/mL with nine subsequent 2-fold dilutions. Cells were incubated with compound for 3 days, at which time the cells were lysed and luminescence was recorded.

Xenograft Tumor Efficacy Studies. Female nude mice (Charles River Laboratories) bearing β-cat/RK3E cells inoculated subcutaneously on the flank at 5 million cells per mouse were staged and randomized into treatment groups ($n = 15$ for vehicle; $n = 12$ for each compound treatment group). Mice were dosed on day 0 by a single ip injection with vehicle or compound **9** at 18.75, 37.5, 75, and 150 mg/kg formulated in 0.5% methhocel/0.4% Tween. In addition, compound **9** was administered at 18.75 mg/kg once daily for 7 days by ip. Tumor growth was monitored for 1 week.

siRNA Knockdown. HT29 cells were seeded at 5×10^6 in a T25 flask in DMEM/10% FBS and grown overnight at 37 °C with 5% CO_2. Media were removed and cells treated with 100 nmol of targeting siRNA (Dharmacon) or fluorescently labeled nontargeting BLOCK-IT oligo (Invitrogen). siRNA oligos and Lipofectamine 2000 (Invitrogen) lipid were suspended in Opti-MEM (Invitrogen) and mixed following the manufacturer's instructions. Cells were treated with 1.3 mL of the lipid/oligonucleotide mixtures for 4 h prior to addition of 1.3 mL of DMEM/20% FBS and grown overnight. Cells were provided fresh DMEM/10% FBS media and incubated for an additional 48 h at 37 °C with 5% CO_2 prior to harvesting and RNA purification.

Oligonucleotide-Array mRNA Expression Profiling. Purification of RNA and amplification of biotin-labeled cRNA were as described previously.[21] For each sample, 10 μg of fragmented biotin-labeled cRNA was hybridized to Human Genome U133 Plus 2.0 GeneChip Oligonucleotide Arrays (Affymetrix) and scanned with an GeneChip scanner 3000 (Affymetrix) using buffers and conditions recommended by the manufacturer. Fluorescent data were collected and converted to gene-specific signal intensities using MicroArray Suite 4.0 software and converted to mRNA frequency by comparison to the spiked standard curve and normalized to the mean total frequency across all samples.[22] For analysis, mean mRNA frequency values of replicate samples were determined for each of the experimental groups. Genes were initially filtered to remove those probes not called present by the MAS4 software in all samples of at least one cohort. Mean mRNA frequency values were subsequently compared between experimental groups to identify genes with an average fold change typically greater than 2-fold ($P < 0.05$).

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Supporting Information Available: Additional transcriptional profiling data (Figures 1 and 2). This material is available free of charge via the Internet at http://pubs.acs.org.

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