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2,4-Diamino-quinazolines as inhibitors of β-catenin/Tcf-4 pathway: Potential treatment for colorectal cancer

Zecheng Chen [a,*], Aranapakam M. Venkatesan [a], Christoph M. Dehnhardt [a], Osvaldo Dos Santos [a], Efren Delos Santos [a], Semiramis Ayral-Kaloustian [a], Lei Chen [b], Yi Geng [b], Kim T. Arndt [b], Judy Lucas [b], Inder Chaudhary [c], Tarek S. Mansour [a]

[a] Wyeth Research, Chemical Sciences, 401 N. Middletown Road, Pearl River, NY 10965, USA
[b] Wyeth Research, Oncology, 401 N. Middletown Road, Pearl River, NY 10965, USA
[c] Wyeth Research, Drug Metabolism, 401 N. Middletown Road, Pearl River, NY 10965, USA

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ABSTRACT

The synthesis and SAR of a series of 2,4-diamino-quinazoline derivatives as β-catenin/Tcf-4 inhibitors are described. This series was developed by modifying the initial lead **1**, which was identified by screening of our compound library and found to inhibit the β-catenin/Tcf-4 pathway. Replacement of the biphenyl moiety in compound **1** with the *N*-phenylpiperidine-4-carboxamide chain as in **2**, resulted in a number of new analogues, which are potent inhibitors of the β-catenin/Tcf-4 pathway. Compound such as **16k** exhibited good cellular potency, solubility, metabolic stability and oral bioavailability.

Colorectal cancer is the second leading cause of cancer deaths in the United States, and nearly 150,000 patients are diagnosed with colorectal cancer and over 55,000 Americans die of this disease each year.[1] Besides surgical resection, current therapy for colon cancer relies on traditional cytotoxic agents with limited success. However, there is still a high unmet medical need for effective treatment of colorectal cancer. Over the past decades, accumulating evidence has suggested that activation of the canonical Wnt signaling is implicated in a wide range of human cancers, especially in colorectal cancers.[2,3]

The canonical Wnt signaling pathway was first discovered in Drosophila.[4] This signaling pathway is evolutionally conserved in mammalians, *Xenopus*, Drosophila and *Caneorhabditis elegans*.[3] It controls many events during embryonic development and regulates proliferation, morphology, motility and cell fate at a cellular level. Within the pathway, the tumor suppressor Adenomatous Polyposis Coli (APC) in complex with Axin is required to regulate the stability of β-catenin, a central component of the Wnt signaling pathway.[5] In the absence of a Wnt signal, cytoplasmic β-catenin is captured by APC and the resulting Axin complex is phosphorylated by kinases GSK3β and CK1α. The phosphorylated β-catenin is then ubiquitinated and targeted for rapid proteosomal degradation. In the presence of Wnt factors, the Wnt signaling cascade is activated so that Axin is recruited to the membrane, thereby the intrinsic kinase activity of the APC complex is inhibited. As a consequence, stable non-phosphorylated β-catenin accumulates in the cytoplasm and subsequently translocates into the nucleus, where it binds to Tcf-4 (T-cell transcriptional factor-4) protein and activates the transcription of a variety of Wnt target genes.[6] Numerous candidate genes have been proposed as critical downstream effectors of Wnt signaling in cancer, including *c-myc*,[7] *cyclin* D1,[8] and *axin2*,[9] etc.

Notably, most of colorectal cancers have loss of function in APC which initiates a neoplastic process towards carcinoma formation.[1] In some cases of colorectal cancer in which APC is wild type, β-catenin can be activated by mutations in *axin2*[10,11] or by intragenic mutations that abolish inhibitory phosphorylation sites at the N-terminus of β-catenin.[12] Under both situations β-catenin is no longer degraded. All these mutations result in accumulation of non-phosphorylated β-catenin, thereby constitutively activating transcription of genes, and ultimately promoting carcinogenesis. Introduction of wild type APC into cells which have lost APC function has been shown to result in either growth suppression or apoptosis, suggesting that these cells have become dependent on elevated β-catenin/Tcf-4 signaling.[5a,13]

Taken together, the β-catenin/Tcf-4 signaling pathway presents a novel target for potential therapeutic intervention of colorectal cancer. We herein report our medicinal chemistry efforts, which led to identification of 2,4-diamino-quinazoline derivatives that inhibit the β-catenin/Tcf-4 pathway.

Compound **1** was identified[14] as a potent inhibitor of β-catenin/Tcf-4 through screening of our compound library. However, the use

* Corresponding author.
E-mail addresses: chenzecheng@hotmail.com, chenz1@wyeth.com (Z. Chen).

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of this compound was limited due to a variety of issues including poor aqueous solubility, poor oral exposure, and metabolic instability. During the course of SAR exploration of this diamino-quinazoline series it was discovered that replacement of the biphenyl moiety with a *N*-phenylpiperidine-4-carboxamide was feasible (e.g., **1** vs **2**, Fig. 1) with minimal loss of potency (IC_{50} for 22C11 cell line: 0.60 μM vs 0.74 μM). The resulting amides offered several advantages including: (1) increased metabolic/chemical stability; (2) improved aqueous solubility; (3) a springboard from which to develop more potent inhibitors of the β-catenin/Tcf-4 pathway.

In an effort to improve potency, initial modifications were made at the 2-position of the quinazoline by preparing different amino substituted derivatives. Analogues **7a–k** were synthesized by the method outlined in Scheme 1. 2-Amino-4-methylbenzoic acid was heated with urea to gave the 7-methylquinazoline-2,4-dione **3**.[15,16] Chlorination of **3** with $POCl_3$ resulted in the dichloride **4**. Assembly of the side chain was achieved by selective substitution of 4-Cl with **5** (readily prepared from the commercially available ethyl 1-benzylpiperidine-4-carboxylate by a three step process as depicted in Scheme 1). Replacement of 2-Cl with different amino substitutions under microwave irradiation conditions resulted in compounds **7a–k** (Table 1) in good to moderate yields.

All the compounds were tested in a cell-based reporter assay: two cell lines (Tcf22C11 and Tcf33.13) where luciferase expression is driven by multimerized Tcf-4 binding sites and one control cell line (SV5A8) where luciferase expression is driven by the SV40 promoter.[17] The desired profile of a compound is to selectively inhibit the reporter activity in Tcf22C11 and Tcf33.13 but not inhibit the reporter activity in SV5A8.

The effect of different amino substitutions is summarized in Table 1. Among the different amino substitutions screened, the dimethylamino and pyrrolidinyl substitutions turned out to be the optimal. The potency against 22C11 cell line for compound **2** (dimethylamino substitution) and **7c** (pyrrolidinyl substitution) were found to be 0.74 μM and 0.66 μM, respectively, both below 1 μM. Piperidinyl substitution (**7k**) was also tolerated. Compound **7a** (ethylamino substitution) exhibited moderate potency against 22C11 and 33.13 with IC_{50} 1.38 and 1.13 μM, respectively, but showed very limited selectivity against the control cell line 5A8

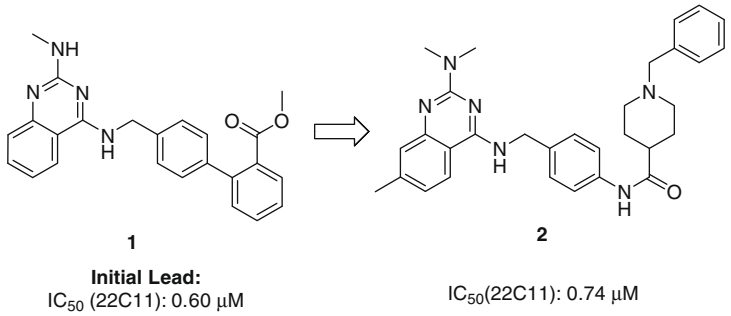
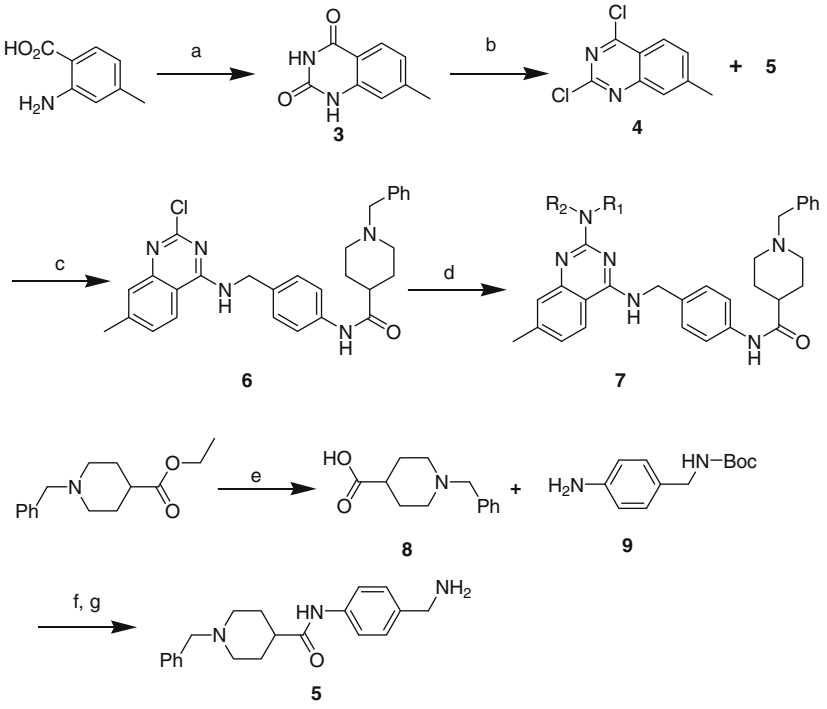


Figure 1.



Scheme 1. Reagents and conditions: (a) urea, 180 °C, 2 h, 85%; (b) $POCl_3$, reflux, 16 h, 45%; (c) **5** (1.2 equiv), Et_3N (3 equiv), CH_2Cl_2, rt, 12 h, 74%; (d) R_1R_2NH (10 equiv), THF, 100 °C, 30 min, microwave irradiation (300 W), 45–90%; (e) KOH (3 equiv), MeOH, 60 °C, 3 h, 99%; (f) (1) $(COCl)_2$ (1.5 equiv), DMF (cat.), CH_2Cl_2, 1 h; (2) Et_3N (3 equiv), **9** (1.0 equiv), CH_2Cl_2, rt, 12 h, 93%; (g) TFA, CH_2Cl_2, 0 °C to rt, 2 h, 99%.

Table 1
Activity of amino substituents at the 2-position of the quinazoline ring[a]

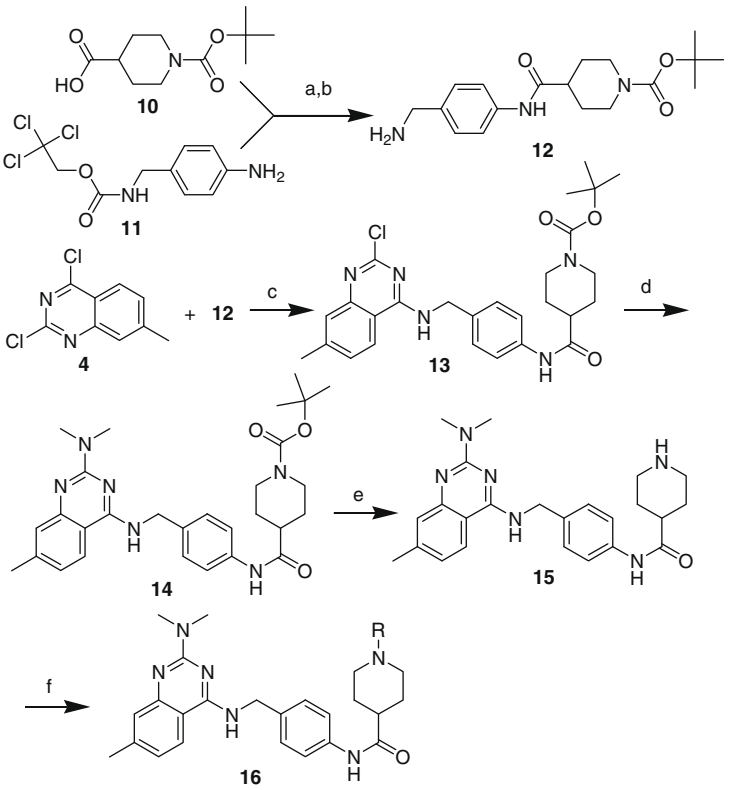
Compds	R_1R_2N	22C11 IC_{50} (μM)	33.13 IC_{50} (μM)	5A8 IC_{50}[b] (μM)
2	Me_2N	0.74	0.76	>7.05
7a	EtNH	1.38	1.13	>2.14
7b	Azetidinyl	1.00	0.80	>8.50
7c	Pyrrolidinyl	0.66	0.79	>7.79
7d	Azepanyl	1.04	0.96	>6.88
7e	$Me_2N(CH_2)_2NMe$	2.26	1.44	>8.83
7f	$Me_2N(CH_2)_3NMe$	1.41	1.31	>8.62
7g	4-Methylpiperazinyl	1.58	1.32	>17.7
7h	4-Ethylpiperazinyl	1.26	1.12	>13.0
7i	(*s*)-3-Methyl-piperzinyl	1.92	1.46	>8.86
7j	3-(2-Hydroxyethyl)-piperazinyl	2.27	2.12	>8.42
7k	4-Pyrrolidinyl-piperidinyl	0.96	0.85	>8.09

[a] Values are means of at least two experiments.
[b] The number reported behind the '>' sign indicates the minimal dose at which cells started to be killed due to toxicity, as indicated by the Renilla luciferase signal.

(IC_{50} >2.14 μM), which indicated some off-target toxicity. Replacement of the pyrrolidinyl group by the azetidinyl (**7b**) or azepanyl (**7d**) led to a slight decrease in potency. A variety of piperazine substitutions (**7g–j**) resulted in a decrease in potency, so did the linear diamino groups (**7e–f**).

In comparison with **1**, compound **2** had an improved water solubility (43 μg/mL at pH 7.4, and >100 μg/mL at pH 3.0) and was found to have an oral bioavailability of 41% in female nude mice (25 mg/kg, po).

The in vivo metabolite identification studies in nude mouse of compound **2** indicated that the N-debenzylation was the major metabolic pathway. To address this metabolic liability, replacing the benzyl group by a variety of substitutions was explored. The new analogues **16a–p** were prepared by a modified route as outlined in Scheme 2, and their in vitro potencies are shown in Table 2. Introduction of a fluorine atom in the phenyl ring resulted in a significant increase in potency in both the 22C11 and 33.13 cell lines when compared to compound **2**. Among the three F substituted analogues (**16k, 16l** and **16m**), the 4-F substituted compound **16k** is the most potent with an IC_{50} value of 0.22 μM against the 22C11 cell line, which is about threefold more potent than compound **2**. The 2-F substituted compound (**16l**) is less potent than the 3-F analogue (**16m**) against the 22C11 cell line, but in the 33.13 cell line **16l** and **16m** are equally potent. As in the case of the fluoro substitution, introduction of 4-Cl in the phenyl ring also led to an increase in potency (**16n**, IC_{50} 0.39 μM). The disubstituted analogue **16p**, with a 2-Cl-4-F phenyl ring, exhibited comparable activity to **16k** against the 33.13 cell line, however, **16p** is less potent than **16k** against the 22C11 cell line. Introduction of chlorine on the pyridine ring resulted in an increase in potency in both cell



Scheme 2. Reagents and conditions: (a) **11** (1 equiv), Et_3N (3 equiv), HOBt (1.5 equiv), EDCI (1.5 equiv), THF, rt, 88%; (b) Zn powder, HOAc, rt, 1 h, 95%; (c) **12** (1.2 equiv), Et_3N (3 equiv), CH_2Cl_2, rt, 12 h, 84%; (d) Me_2NH (10 equiv), THF, 100 °C, 30 min, microwave irradiation (300 W), 90%; (e) TFA, CH_2Cl_2, 0 °C to rt, 2 h, 96%: (f) R_3R_4CO or ArCHO (2 equiv), $ZnCl_2$ (1.5 equiv), $NaBH_3CN$ (1.5 equiv), THF, rt, 2 h, 56–78%.

Table 2
Activity of different substitutions on the piperidine ring[a]

Compds	R	22C11 IC_{50} (μM)	33.13 IC_{50} (μM)	5A8 IC_{50}[b] (μM)
16a	Me	1.92	1.67	>17.3
16b	*i*Pr-CH_2	0.65	0.96	>10.5
16c	*n*-Butyl	1.47	1.17	>10.5
16d	Cyclohexyl	0.75	0.81	>8.32
16e	Boc	0.76	0.85	>4.82
16f	2-Furanylmethyl	1.83	1.93	>20
16g	2-Imidazolylmethyl	>20	>20	>20
16h	4-Pyridinylmethyl	1.18	0.90	>8.07
16i	4-Methylbenzyl	0.47	0.50	>4.15
16j	4-Methoxybenzyl	0.52	0.45	>6.18
16k	4-Fluorobenzyl	0.22	0.19	>4.82
16l	2-Fluorobenzyl	0.53	0.32	>6.67
16m	3-Fluorobenzyl	0.33	0.35	>4.17
16n	4-Chlorobenzyl	0.39	0.32	>3.18
16o	(5-Chloropyridin-2-yl)methyl	0.57	0.41	>5.40
16p	2-Chloro-4-fluorobenzyl	0.52	0.21	>5.91

[a] Values are means of at least two experiments.
[b] The number reported behind the '>' sign indicates the minimal toxic dose at which cells started to be killed due to toxicity, as indicated by the Renilla luciferase signal.

Table 3
Plasma and tumor concentrations of compounds **2** and **16k**

Compds	Dose (po, mg/kg)	Plasma conc. 8 h (ng/mL)	Tumor conc. 8 h (ng/mL)	Tumor/plasma ratio
2	200	2973	90,736	40
16k	50	440	70,306	220

lines (**16o** vs **16h**). Imidazole substitution was not tolerated, and led to a dramatic loss in potency (IC_{50} >20 μM for **16g**). Replacing phenyl with furanyl resulted in a loss in potency in both cell lines (**16f** vs **2**). In addition to aromatic substitutions, some alkyl substitutions were also explored. Interestingly, the linear alkyl substitutions such as methyl (**16a**, IC_{50} 1.92 μM) and *n*-butyl (**16c**, IC_{50} 1.47 μM) led to a loss in potency, but the branched or cyclic alkyl groups such as *iso*-butyl (**16b**, IC_{50} 0.57 μM) and cyclohexyl (**16d**, IC_{50} 0.75 μM) are both better tolerated. Carbamate analogue **16e** is also tolerated with an IC_{50} value of 0.76 μM.

As shown in Table 3, the exposure studies showed that the plasma concentration of compound **2** in nude mouse ($n = 4$) is 2973 ng/mL at 8 h following a single oral dose (200 mg/kg), while the tumor concentration is 90,736 ng/mL, with a favorable tumor/plasma ratio of 40. Compound **16k** also exhibited good exposure in blood and in tumor at 8 h after a single po dose (50 mg/kg). Compound **16k** was preferentially distributed to the tumor with a tumor/plasma ratio of 220. The pharmacokinetic studies in female nude mice showed that both compounds exhibited reasonable bioavailability (41% for **2**, and 46% for **16k**, respectively).

The in vivo efficacy of compounds **2** and **16k** was then assessed in nude mice bearing HT29 or HCT116 tumors grown subcutaneously. In this experiment, tumor volume was measured after po dose of 25, 50, 100, or 200 mg/kg once daily for 17 days. Only 20–35% tumor growth inhibition was observed for the compound **2** at the end, after a 200 mg/kg oral dose. No significant tumor growth inhibition was found by using compound **16k** at a 50 mg/kg po dose even though **16k** is threefolds more potent than **2,** against the 22C11 and 33.13 cell lines. These results were surprising given the excellent exposures of compounds **2** and **16k** in tumor bearing nude mice (Table 3). Another related experiment showed that there were no major pathway-related gene changes in **2** and **16k** treated tumors, while these changes were observed in in vitro gene chip analyses. The reason for this disconnection is not clear.

In summary, initial SAR exploration of the lead diamino-quinazoline has led to a number of potent in vitro inhibitors of the β-catenin/Tcf-4 pathway with improved in vivo pharmacokinetic properties. Compound **16k** exhibited improved potency (IC_{50} 0.22 μM against the 22C11 cell line and 0.19 μM against the 33.13 cell line), good solubility, permeability and oral bioavailability (46%), and showed excellent blood and tumor exposure, following oral dosage. However, neither compound **2** nor **16k** demonstrated significant in vivo efficacy in HT29 and HCT116 xenograft models. Further optimization in potency and PK properties of this series is ongoing, and the results will be reported in due course.

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