Discovery of *N*-(4-Methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine, a Potent Apoptosis Inducer and Efficacious Anticancer Agent with High Blood Brain Barrier Penetration

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As a continuation of our structure−activity relationship (SAR) studies on 4-anilinoquinazolines as potent apoptosis inducers and to identify anticancer development candidates, we explored the replacement of the 2-Cl group in our lead compound 2-chloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (**6b**, EP128265, MPI-0441138) by other functional groups. This SAR study and lead optimization resulted in the identification of *N*-(4-methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine (**6h**, EP128495, MPC-6827) as an anticancer clinical candidate. Compound **6h** was found to be a potent apoptosis inducer with EC_{50} of 2 nM in our cell-based apoptosis induction assay. It also has excellent blood brain barrier penetration, and is highly efficacious in human MX-1 breast and other mouse xenograft cancer models.

Introduction

Currently, cancer is the second leading cause of death in the United States and could become the most common cause in the future.[1] Cancer treatment utilizing chemotherapy agents, including paclitaxel and docetaxel, has been a mainstay.[2] Recent advancements have come with the discovery and development of small molecule targeted therapies. These include the BCR-ABL kinase inhibitor imatinib for chronic myeloid leukemia (CML) and gastrointestinal stromal tumor (GIST[a]),[3] epidermal growth factor receptor (EGFR) kinase inhibitors gefitinib and erlotinib for nonsmall cell lung cancer (NSCLC),[4] the multikinase inhibitor sorafenib for kidney and liver cancers,[5] an inhibitor of EGFR and HER-2 kinases lapatinib ditosylate for breast cancer,[6] a multikinase inhibitor sunitinib for advanced renal cell carcinoma (RCC) and imatinib refractory/intolerant GIST,[7] a proteasome inhibitor bortezomib for multiple myeloma,[8] and an inhibitor of mTOR (mammalian target of rapamycin) temsirolimus for advanced kidney cancer.[9]

Unfortunately, relatively little progress has been made for the treatment of neurologic cancers. One of the reasons is that the blood brain barrier (BBB) effectively prevents many traditional and newer anticancer drugs from entering the brain parenchyma.[10] For example, the EGFR inhibitor erlotinib was reported to have cerebrospinal fluid (CSF) penetration of 7% (CSF/plasma),[11] and EGFR inhibitors gefitinib and erlotinib have been reported to show limited activity in clinical trials in brain cancers.[12] In addition, the brain concentration of another kinase inhibitor, lapatinib, was reported to be low as determined by whole-body autoradiography.[13] Currently, there are only a few treatment options available for malignant glioma including surgery, radiotherapy and DNA-damaging chemotherapies such as temozolomide.[14]

Apoptosis is known to play an important role in tumor biology.[15] Improper or excessive inhibition of apoptosis could lead to unchecked cell proliferation as well as resistance to cancer treatment.[16] It has been well-established that anticancer agents like the camptothecins, such as topotecan and irinotecan,[17] and vinca alkaloids, such as vincristine and vinblastine,[18] kill tumors at least partially through induction of apoptosis.[19] Hence, many novel approaches to activate and promote apoptosis in cancer cells[20] via targeting regulators of apoptosis are currently being explored.[21] We therefore have developed a cell-based Anticancer Screening Apoptosis Program (ASAP) high throughput screening (HTS) assay to identify apoptosis inducers through measurement of caspase activation.[22,23]

We have previously reported the discovery of several novel series of potent apoptosis inducers, including *N*-phenyl nicotinamides (**1a**),[24] gambogic acid (**1b**),[25] 4-aryl-4*H*-chromenes (**1c**),[26] 3-aryl-5-aryl-1,2,4-oxadiazoles (**1d**),[27] 4-anilino-2-(2-pyridyl)pyrimidines (**1e**),[28] and *N*-phenyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-amines (**1f**)[29] using our HTS assays (Chart 1). More recently, we reported the discovery of N^4-(4-methoxyphenyl)-N^4-methyl-N^2-((*E*)-3,7-dimethylocta-2,6-dienyl)quinazoline-2,4-diamine (**6a**) as a potent apoptosis inducer using our cell- and caspase-based HTS assay and the identification of 2-chloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (**6b**), as a highly potent apoptosis inducer with *in vivo* anticancer activity and high BBB penetration.[30] In our quest to optimize this class of anticancer agents, the 2-Cl group in **6b**, a potential liability due to its chemical reactivity, was replaced by other functional groups. Herein we report that this SAR study and lead optimization resulted in the identification of *N*-(4-methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine (**6h**, EP128495, MPC-6827, Azixa), an anticancer clinical candidate with potent

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[a] Abbreviations: SAR, structure−activity relationship; GIST, gastrointestinal stromal tumor; EGFR, epidermal growth factor receptor; BBB, blood brain barrier; CSF, cerebrospinal fluid; ASAP, Anticancer Screening Apoptosis Program; HTS, high throughput screening; iv, intravenous; AUC, area under the concentration−time curves; HBD, hydrogen bond donor; N+O, nitrogen plus oxygen; HBA, hydrogen bond acceptors; ADMET, absorption, distribution, metabolism, excretion and toxicity.

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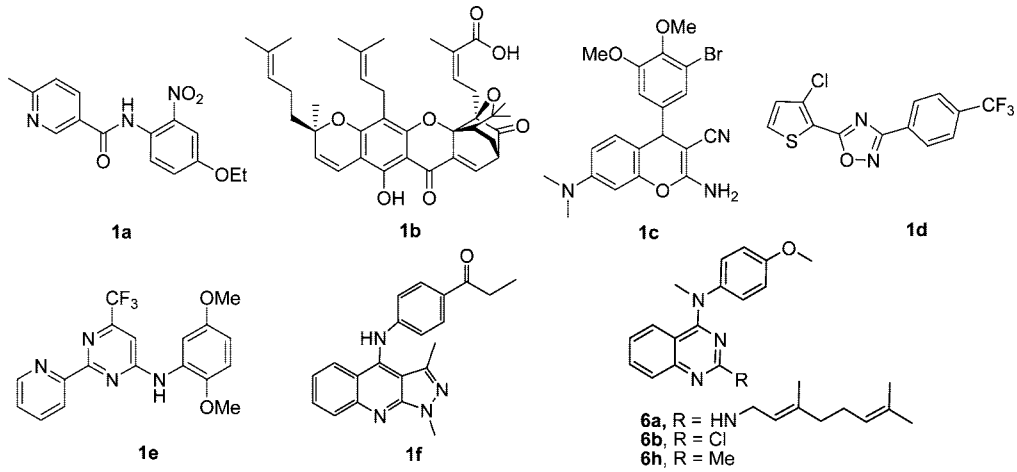
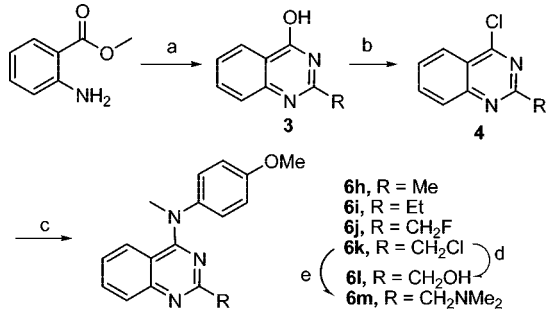
Chart 1

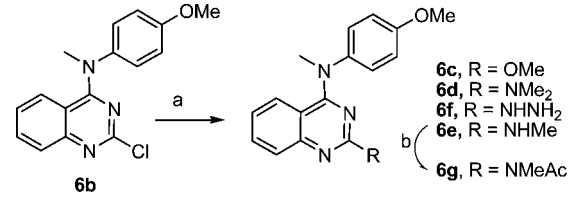


Scheme 1[a]



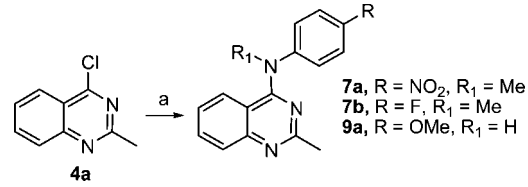
[a] Conditions: (a) RCN (**2**), HCl(g); (b) $POCl_3$, DIPEA, toluene; (c) 4-MeO-PhNHMe (**5a**), IPA, HCl; (d) NaOH; (e) $NHMe_2$.

Scheme 2[a]



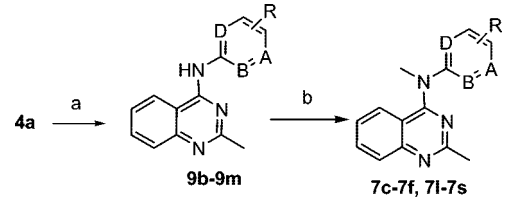
[a] Conditions: (a) **6c**, NaOMe; **6d**, $NHMe_2$; **6e**, NH_2Me; **6f**, NH_2NH_2; (b) AcCl, Et_3N, CH_2Cl_2.

Scheme 3[a]



[a] Conditions: (a) **7a**, 4-NO_2−PhNHMe, NaH, DMF; **7b**, 4-F-PhNHMe, NaAc, THF/H_2O; **9a**, 4-MeO-$PhNH_2$, IPA, HCl.

Scheme 4[a]



[a] Conditions: (a) $ArNH_2$ (**8**), NaAc, THF/H_2O; (b) MeI, NaH, DMF.

apoptosis inducing activity, high BBB penetration, good pharmacokinetics and high efficacies in several *in vivo* anticancer models.

Results and Discussion

Chemistry. Compounds **6h**−**6k** were prepared in three steps as shown in Scheme 1. 4-Chloro-2-methyl-quinazoline (**4a**)[31] was prepared via reaction of 2-aminobenzoic acid methyl ester and acetonitrile (**2a**) in the presence of HCl to produce 2-methyl-quinazolin-4-ol (**3a**), followed by treatment of **3a** with freshly distilled $POCl_3$ in anhydrous toluene and diisopropylethylamine (Scheme 1). Reaction of **4a** with *N*-methyl-4-methoxyaniline (**5a**) in anhydrous isopropanol (IPA) in the presence of concentrated HCl produced *N*-(4-methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine (**6h**) as its hydrochloride salt. In a similar fashion, compounds **6i**−**6k** were prepared in three steps via replacing acetonitrile with propiononitrile, fluoroacetonitrile and chloroacetonitrile, respectively (Scheme 1). Compounds **6l**, **6m** were prepared via reaction of **6k** with sodium hydroxy and dimethylamine, respectively (Scheme 1).

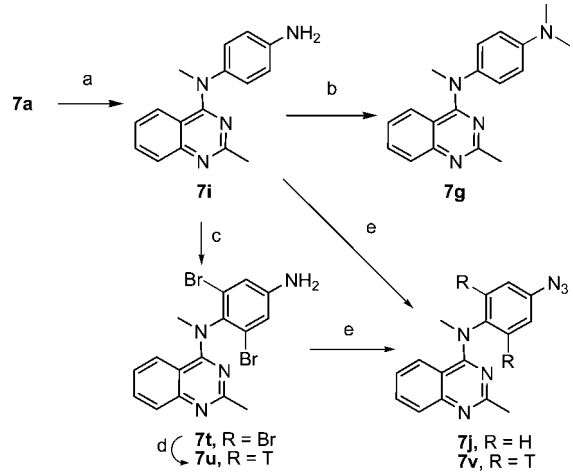
Compounds **6c**−**6f** were prepared via displacement of the chloro group in **6b** with the corresponding nucleophiles ($NaOMe$, $NHMe_2$, NH_2Me, NH_2NH_2); reaction of **6e** with acetyl chloride subsequently produced the amide **6g** (Scheme 2).

Compounds **7a** and **7b** were prepared from reaction of **4a** with the corresponding *N*-Me-aniline **5b** and **5c** in the presence of a base such as NaH or NaAc. Compound **9a** was prepared from reaction of **4a** with 4-methoxyaniline **8a** (Scheme 3).

N-(4-Ethoxyphenyl)-2-methylquinazolin-4-amine (**9b**) was prepared via reaction of **4a** with 4-ethoxyaniline **8b**. Reaction of **9b** with methyl iodide in the presence of sodium hydride produced *N*-(4-ethoxyphenyl)-*N*,2-dimethylquinazolin-4-amine (**7c**) (Scheme 4). In a similar fashion the following compounds were prepared. Reaction of **4a** with substituted aniline **8c**−**8g**; or substituted pyridyl-amine (**8i**, **8j**); or substituted pyrimidyl-amine **8k** or pyrazinyl-amine **8l** produced compounds **9c**−**9m**. Reaction of **9c**−**9m** with methyl iodide in the presence of sodium hydride smoothly produced compounds **7d**−**7f** and **7l**−**7s** (Scheme 4, and see Tables 2 and 3 for structures). The 4-hydroxyl analogue **7h** was prepared from demethylation of **6h** via treatment with boron tribromide.

4-Amino compound **7i** was prepared from hydrogenation of 4-nitro analogue **7a**. Treatment of **7i** with formaldehyde and sodium cyanoborohydride produced the dimethylamino analogue **7g**. Reaction of **7i** with acetic anhydride produced the acetamide

Scheme 5[a]



[a] Conditions: (a) H_2, Pd/C, EtOH; (b) HCHO, sodium cyanoborohydride; (c) Br_2, AcOH; (d) T_2, Pd/C, EtOH; (e) $NaNO_2$, HCl, then NaN_3.

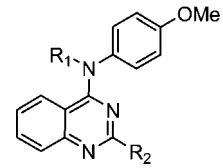
7k. The azido analogue **7j** was prepared via reaction of **7i** with sodium nitrite in aqueous HCl followed by treatment with sodium azide. The corresponding tritium labeled analogue **7v** was prepared in three steps. Reaction of **7i** with bromine produced the dibromo analogue **7t**, which was treated with tritium gas under hydrogenation conditions to produce the ditritium analogue **7u**. Diazotization of **7u** followed by treatment with sodium azide, using procedures similar to the preparation of the cold azido analogue **7j**, produced tritium analogue **7v** (Scheme 5).

HTS and SAR Studies. The apoptosis inducing activities of 4-anilinoquinozalines were measured using our proprietary cell- and caspase-based HTS assay in human breast cancer cells T47D, human colorectal carcinoma cells HCT116 and hepatocellular carcinoma cancer SNU-398 cells as described previously.[24] In brief, the cancer cells were placed in a 384-well microtiter plate containing various concentration of test compound and incubated at 37 °C for 24 h. After incubation, the samples were treated with a fluorogenic substrate *N*-(Ac-DEVD)-*N'*-ethoxycarbonyl-R110. The caspase activation activity (EC_{50}) is summarized in Tables 1−3.

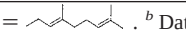
We have found that the methyl group on the nitrogen linker was essential for the apoptosis inducing activity, and substitution in the 6- and 7-positions of the quinazoline core structure resulted in decreased apoptosis inducing activity.[30] Since the Cl group in the 2-position of our lead compound **6b** could be a potential liability due to its chemical reactivity, we expanded our SAR study by replacing the 2-Cl group with other functional groups. As expected, the Cl group in **6b** was replaced by nucleophiles to produce compounds **6c**−**6f**. These analogues in general were less active than **6b** in the caspase activation assay. The 2-OMe analogue **6c** and the 2-NHMe analogue **6e**, with a small hydrophobic group at the 2-position, were the more potent ones giving EC_{50} values of 4 and 8 nM, respectively. The more polar hydrazino analogue **6f** was the least potent one of this cluster of analogues, with an EC_{50} value of 23 nM while the amide **6g** was more active with an EC_{50} value of 9 nM.

Since methyl is a small hydrophobic group with good chemical stability, we decided to replace the 2-chloro group in **6b** with a methyl group. Compound **6h** was found to be highly potent with an EC_{50} value of 2 nM, a potency similar to that of **6b** in the caspase activation assay. Other alkyl and substituted alkyl groups were also introduced in the 2-position, including CH_2F, CH_2Cl, Et, CH_2OH and CH_2NMe_2 (**6i**−**6m**). Several of these compounds were also highly potent in our caspase activation assay. For example, the 2-CH_2F analogue **6j** and 2-CH_2OH analogue **6l** had potencies similar to that of **6h**. Similarly as observed above, compounds with large or polar groups in the 2-position, such as **6m**, were much less potent as inducers of apoptosis. Compound **9a**, with a hydrogen in the linker nitrogen instead of a Me group as in **6h**, was found to have an EC_{50} value of 6400 nM, which is >3000-fold less active than **6h**, confirming our previous observation that a methyl group on the linker nitrogen was critical for the apoptosis inducing activity of **6h** and related compounds.[30]

Table 1. SAR of 4-(4-Methoxyanilino)quinazolines in the Caspase Activation Assay

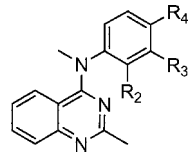


			EC_{50} (μM)[b]		
entry	R_1	R_2	T47D	HCT116	SNU-398
6a	Me	NHR[a]	0.29 ± 0.020	0.46 ± 0.098	0.27 ± 0.03
6b	Me	Cl	0.002 ± 0.0001	0.002 ± 0.0001	0.002 ± 0.0001
6c	Me	OMe	0.004 ± 0.0002	0.004 ± 0.0005	0.003 ± 0.0002
6d	Me	NMe_2	0.015 ± 0.001	0.043 ± 0.006	0.033 ± 0.004
6e	Me	NHMe	0.008 ± 0.001	0.009 ± 0.002	0.005 ± 0.0003
6f	Me	$NHNH_2$	0.023 ± 0.004	0.036 ± 0.007	0.020 ± 0.002
6g	Me	NMeAc	0.009 ± 0.0004	0.009 ± 0.001	0.010 ± 0.001
6h	Me	Me	0.002 ± 0.0001	0.003 ± 0.0002	0.002 ± 0.0003
6i	Me	Et	0.009 ± 0.0002	0.013 ± 0.001	0.008 ± 0.001
6j	Me	CH_2F	0.002 ± 0.0002	0.002 ± 0.0001	0.001 ± 0.0001
6k	Me	CH_2Cl	0.048 ± 0.007	0.048 ± 0.011	ND[c]
6l	Me	CH_2OH	0.002 ± 0.0002	0.004 ± 0.0001	0.002 ± 0.0001
6m	Me	CH_2NMe_2	1.8 ± 0.06	2.6 ± 0.02	2.0 ± 0.09
9a	H	Me	6.4 ± 0.38	6.1 ± 0.32	5.3 ± 0.04

[a] R =  . [b] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM). [c] ND, not determined.

We then explored the SAR of compound **6h** through altering the substitutions in the anilino ring (Table 2), while keeping the 2-methyl-quinazoline ring as a "fixed" unit. The 4-nitro analogue **7a** and 4-fluoro analogue **7b** were >100-fold less potent than **6h**, indicating that an electron-withdrawing group was not preferred, in agreement with what we reported previously.[30] The 4-OEt **7c**, 4-$OCHF_2$ **7d**, 4-SMe **7e** and 4-NMe_2 **7g** analogues were all highly potent, only slightly less active than **6h**, indicating that a small hydrophobic and electron-donating group was preferred at the 4-position. The 4-$OCHF_2$ group in **7d**, a potentially more metabolically stable group, was introduced since the demethylation metabolite of **6h**, the 4-OH analogue **7h**, was significantly less active (see below). The basic 4-NMe_2 group in **7g** was intended to increase the aqueous solubility of the compound by using suitable salt forms. Interestingly, the 4-Et analogue **7f** was about 10-fold less potent than **6h**, indicating that a heteroatom was preferred in that position. The 4-OH analogue **7h** and 4-NH_2 analogue **7i** were >40-fold less potent than **6h**, indicating that a hydrophilic group was not preferred at the 4-position. The 4-azido analogue **7j** was found to have good activity with an EC_{50} value of 16 nM, which allowed us to make a tritium labeled version **7v** that was used to identify tubulin as the primary molecular target of these apoptosis inducing 4-anilinoquinazolines.[32] The aromatic di-substituted analogues **7l**−**7n** were found to be highly potent with EC_{50} values of 2−10 nM. The electron-withdrawing fluoro group in **7l** and **7m** could increase the metabolic stability of the phenyl ring.

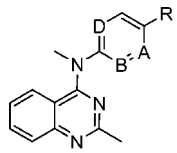
Table 2. SAR of *N*,2-Dimethyl-4-anilinoquinazolines in the Caspase Activation Assay



entry	R_2	R_3	R_4	EC_{50} (μM)[a] T47D	HCT116	SNU-398
7a	H	H	NO_2	0.74 ± 0.05	1.3 ± 0.01	0.62 ± 0.01
7b	H	H	F	0.42 ± 0.001	0.63 ± 0.001	0.39 ± 0.038
7c	H	H	OEt	0.004 ± 0.0001	0.004 ± 0.0004	0.004 ± 0.001
7d	H	H	$OCHF_2$	0.009 ± 0.0002	0.011 ± 0.002	0.010 ± 0.001
7e	H	H	SMe	0.004 ± 0.0004	0.003 ± 0.0004	0.003 ± 0.0003
7f	H	H	Et	0.031 ± 0.003	0.026 ± 0.002	0.021 ± 0.002
7g	H	H	NMe_2	0.016 ± 0.002	0.019 ± 0.004	0.019 ± 0.002
7h	H	H	OH	0.086 ± 0.007	0.13 ± 0.007	0.088 ± 0.015
7i	H	H	NH_2	0.18 ± 0.02	0.25 ± 0.01	0.17 ± 0.03
7j	H	H	N_3	0.011 ± 0.002	0.012 ± 0.001	0.011 ± 0.001
7k	H	H	NHAc	0.059 ± 0.003	0.083 ± 0.009	0.066 ± 0.006
7l	H	F	OMe	0.002 ± 0.0001	0.002 ± 0.0001	0.002 ± 0.0001
7m	F	H	OMe	0.004 ± 0.0001	0.004 ± 0.0003	0.004 ± 0.001
7n	H	OMe	OMe	0.010 ± 0.001	0.014 ± 0.0002	0.013 ± 0.001

[a] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM).

Table 3. SAR of *N*,2-Dimethyl-4-arylaminoquinazolines in the Caspase Activation Assay



entry	A	B	D	R	EC_{50} (μM)[a] T47D	HCT116	SNU-398
7o	N	C	C	OMe	0.011 ± 0.001	0.015 ± 0.001	0.010 ± 0.001
7p	C	N	C	OMe	0.016 ± 0.001	0.044 ± 0.007	0.018 ± 0.001
7q	N	C	C	NMe_2	0.015 ± 0.001	0.033 ± 0.006	0.020 ± 0.002
7r	N	C	N	OMe	0.053 ± 0.003	0.075 ± 0.008	0.049 ± 0.002
7s	C	N	N	OMe	0.15 ± 0.008	0.15 ± 0.008	0.13 ± 0.004

[a] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM).

We also explored the replacement of the anilino group in **6h** by pyridylamino and other heterocyclic-amino groups (Table 3). The pyridyl analogues **7o**−**7q** were 5−8-fold less potent than **6h** but still highly active with EC_{50} values in the 11−16 nM range. These pyridyl analogues, especially compound **7q**, were intended to be more hydrophilic and, thus, more soluble in aqueous media than **6h**. Unfortunately, the pirazinyl analogue **7r** and pyrimidyl analogue **7s** were about 25- and 75-fold less active than **6h**, respectively. Overall, these data indicated that the more hydrophobic phenyl ring was preferred over the nitrogen containing heterocycles.

The activities of these compounds toward the HCT116 and SNU-398 cells were roughly parallel to their activity toward T47D cells. Compound **6h** was highly active in HCT116 and SNU-398 cells with EC_{50} values of 3 nM and 2 nM, respectively, similar to its high activity in T47D cells. Compound **9a** was much less potent with EC_{50} values of 6100 nM and 5300 nM in HCT116 and SNU-398 cells, respectively. This further confirmed the importance of the *N*-Me group for apoptosis inducing activity.[30]

Selected compounds were further evaluated in the traditional growth inhibition assay to confirm that potent compounds in the ASAP assay can inhibit cancer cell growth. The growth inhibition assays in T47D, HCT116 and SNU-398 cell lines were run in a 96-well microtiter plate as described previously.[24] Briefly, the cancer cells in a 96-well microtiter plate were treated with serial concentrations of test compound for 48 h. The survival profiles of the cells were quantitated using the CellTiter-Glo (Promega, Madison, WI) according to the manufacturer's protocol. The GI_{50} values are summarized in Table 4. Compound **6h** was found to be one of the most potent inhibitors of cancer cell growth among the compounds tested, with GI_{50} values of 3−6 nM in T47D, HCT116 and SNU-398 cells. Compounds **6c** and **6i** also were highly active with GI_{50} values similar to that of **6h**. Compound **9a** was much less potent in the growth inhibition assay, with GI_{50} values of 4200 to 6500 nM. This again confirmed that the *N*-Me group was important for both the apoptosis inducing and growth inhibiting activity of **6h** and related compounds.[30]

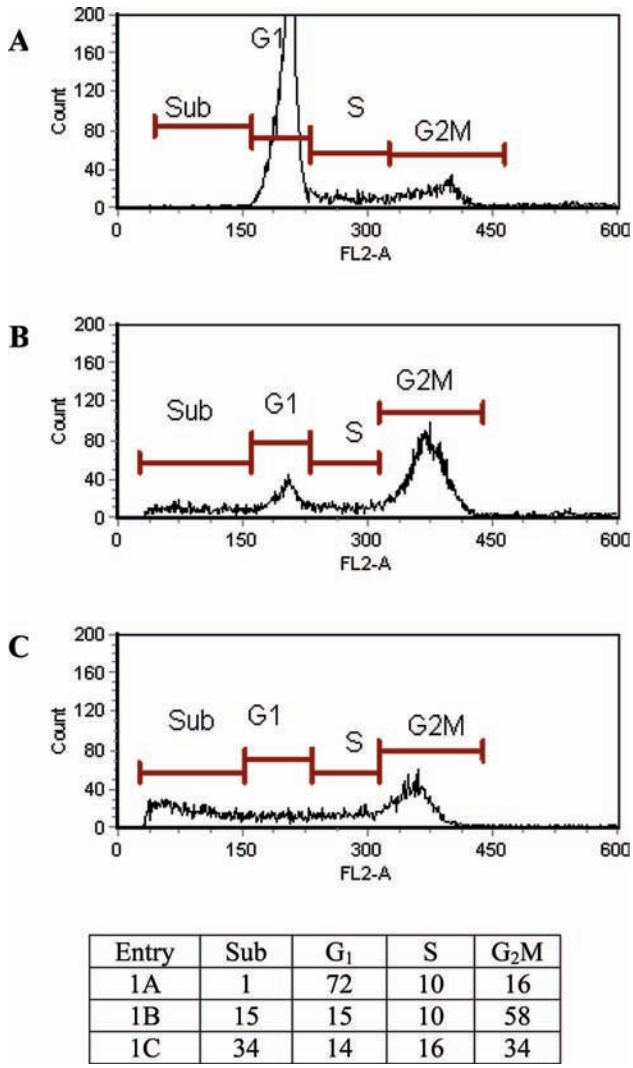
Table 4. Inhibition of Cell Growth of 4-Arylaminoquinazolines

entry	GI_{50} (μM)[a] T47D	HCT116	SNU-398
6c	0.006 ± 0.001	0.006 ± 0.001	0.003 ± 0.001
6h	0.006 ± 0.001	0.006 ± 0.001	0.003 ± 0.0003
6i	0.007 ± 0.001	0.012 ± 0.002	0.008 ± 0.001
9a	5.2 ± 0.60	6.5 ± 0.79	4.2 ± 0.51
7o	0.039 ± 0.013	0.046 ± 0.010	0.019 ± 0.003
7s	0.76 ± 0.03	1.0 ± 0.04	0.39 ± 0.05

[a] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM).

Characterization of 6h and Related Compounds. Utilizing the tritium labeled compound **7v**, the primary molecular target of **6h** has been identified as tubulin.[32] Mechanistically, **6h** was found to inhibit tubulin polymerization with potency equal to that of vinblastine and to bind at or near the colchicine site.[32] Compound **6h** was also found to be effective in multi drug resistant cancer cell lines including P388/ADR, NCI/ADR-RES, MCF-7/MX, MCF-7/VP, with similar GI_{50} values against cancer cell lines and drug resistant cancer cell lines,[32] suggesting that it may be useful for the treatment of drug resistant cancers.

The apoptosis inducing activity of **6h** was also confirmed in a flow cytometry assay. T47D cells were treated with 5 nM of **6h** for 24 or 48 h, stained with propidium iodide, and analyzed by flow cytometry. An increase in the G_2/M DNA content in cells treated with compound **6h** was observed, as shown in



Entry	Sub	G_1	S	G_2M
1A	1	72	10	16
1B	15	15	10	58
1C	34	14	16	34

Figure 1. Drug-induced apoptosis in T47D cells as measured by flow cytometric analysis. The *x*-axis is the fluorescence intensity, and the *y*-axis is the number of cells with that fluorescence intensity. A: Control cells showing most of the cells in G_1 phase of the cell cycle. B: Cells treated with 5 nM of compound **6h** for 24 h showing most of the cells arrested in G_2/M phase. C: Cells treated with 5 nM of compound **6h** for 48 h showing a progression from G_2/M to cells with subdiploid DNA content, which are apoptotic cells with fragmented nuclei.

Figures 1B and 1C. The subdiploid DNA content (apoptotic sub-G_1 area) of cells increased from 1% in the control cells (Figure 1A) to 15% upon compound treatment for 24 h (Figure 1B) and 34% after 48 h treatment (Figure 1C), indicating the presence of apoptotic cells which have undergone DNA degradation and nuclear fragmentation. Previously we reported that when Jurkat cells were treated with 10 nM of **6h** for 18 h, 68% of the cells were apoptotic with subdiploid DNA content as measured by flow cytometry. The apoptotic cells were decreased to 11% in the presence of a specific caspase inhibitor, confirming the apoptotic nature of the subdiploid DNA content.[32]

Importantly, compound **6h** was found to be highly active in several *in vivo* anticancer models. Figure 2 shows that **6h** was highly efficacious in a MX-1 breast tumor model. No tumor growth was observed when **6h** was dosed at 7.5 and 10 mg/kg once weekly for three weeks. At 5 mg/kg **6h** also effectively inhibited the growth of MX-1 tumors. In addition, compound **6h** also significantly ($p < 0.05$) inhibited tumor growth in B16-

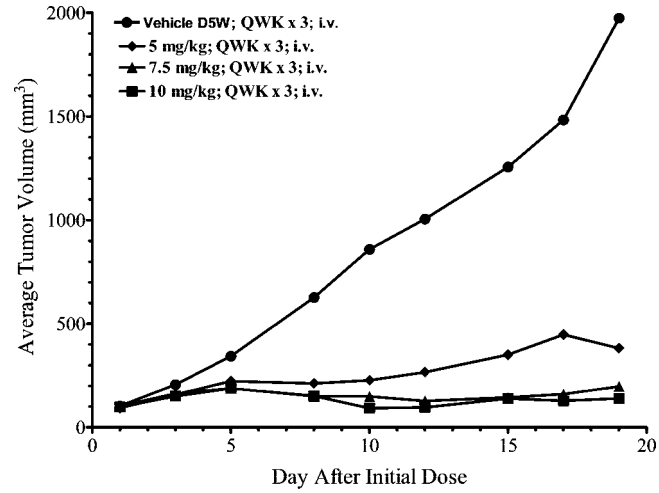
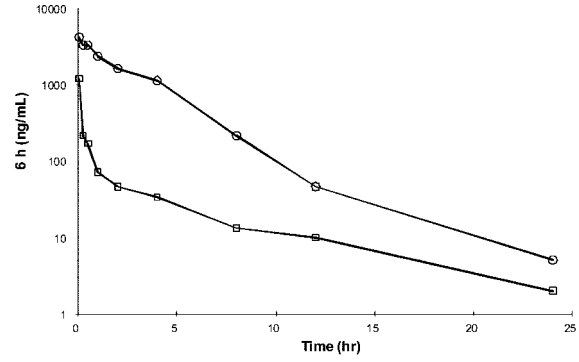


Figure 2. Compound **6h** inhibited the growth of established (~100 mm^3) MX-1 tumor xenografts in Crl:Nu/Nu-nuBR mice. Compound **6h** was dosed iv at 5, 7.5 or 10 mg/kg once weekly for three weeks.



Species	Dose (mg/kg)	Brain $AUC_{(0-\infty)}$	Plasma $AUC_{(0-\infty)}$
C57BL/6 Mice	2.5	11459	686

Figure 3. PK of compound **6h** in brain and plasma after a single iv dose of 2.5 mg/kg in mice. Median brain concentrations (open circles) and median plasma concentrations (open squares).

F1 allograft and OVCAR-3, MIAPaCa-2, MCF-7, HT-29 and MDA-MB-435 xenograft models as reported previously.[32]

Using plasma from blood samples and homogenized whole brain samples, the brain to plasma ratio of the area under the concentration−time curves (AUC) of **6h** after a single intravenous (iv) dose of 2.5 mg/kg in mice was determined via LC−MS/MS to be about 16 (Figure 3), indicating that **6h** maintained the high BBB penetration observed for **6b**.[30] In comparison, many anticancer drugs have been reported to have low BBB penetrations, including erlotinib,[11] lapatinib,[13] imatinib (CSF penetration of 5%),[33] gefitinib,[34] ABT-751 (CSF penetration of 1.1%),[35] oxaliplatin, cisplatin, and carboplatin (CSF penetration of 2.0%, 3.6%, and 3.8%).[36] Since currently there are few active treatments available for brain cancer and one of the reasons is limited BBB penetration of many anticancer drugs,[10] the high BBB penetration of **6h** suggests that it could potentially be developed as an effective treatment for brain cancer.

The brain/plasma AUC ratios of several compounds related with **6h** also were measured using the same method at dose of 2.5 mg/kg (iv) and the results are summarized in Table 5. It has been suggested that hydrogen bond donor (HBD) of <3, ClogP of 2−5 and molecular weight (MW) of <500 are preferred for potential BBB penetration.[37] In addition, two simple rules

Table 5. The Brain/Plasma Ratio of AUC of 4-Arylaminoquinazolines

entry	brain/plasma ratio of AUC	MW	no. of HBA	no. of HBD	# of N+O	ClogP[a]	EC_{50} (nM) T47D[b]
6h	16.0	279	4	0	4	4.18	2
7o	5.8	280	5	0	5	3.61	11
6d	4.0	308	5	0	5	4.59	15
7r	1.9	281	6	0	6	2.82	53
7i	0.90	264	4	2	4	3.02	59
7k	0.74	306	5	1	5	3.27	180

[a] Calculated using ChemDraw. [b] Data from Tables 1, 2 and 3.

have been proposed that if the number of nitrogen plus oxygen (N+O) is five or less in a molecule, then it has a high chance of entering the brain; and if log P − (N+O) > 0, then log BB (brain/blood) is positive.[38] Introduction of more polar groups as well as nitrogen and oxygen into the structure of **6h** therefore was expected to reduce BBB penetration and the brain/plasma AUC ratio. Compound **6d**, with the 2-Me group replaced by a more polar NMe_2 group and one additional nitrogen than that of **6h**, reduced the brain/plasma AUC ratio from 16 to 4.0. Similarly, introduction of 1 or 2 more nitrogens via replacing the phenyl group in **6h** with a pyridyl (**7o**) or pyrazinyl (**7r**) decreased the brain/plasma AUC ratio to 5.8 and 1.9, respectively. These data confirmed that, in this series of compounds, increasing the number of hydrogen bond acceptors (HBA) or the total number of N+O resulted in less BBB penetration. Replacing the 4-OMe group in the phenyl ring by a more polar group such as the amino group in **7i** and acetamido in **7k** also reduced the ratio to 0.90 and 0.74, respectively, indicating that introduction of HBD effectively reduced the BBB penetration. Our data are in agreement with the low BBB penetration of 4-anilinoquinazoline based kinase inhibitors due to large numbers of N+O and HBD in the structures, including gefitinib (**10a**, 7 N+O, 1 HBD), erlotinib (**10b**, 7 N+O, 1 HBD) and lapatinib (**10c**, 8 N+O, 2 HBD), as well as ABT-751 (**10d**, 7 N+O, 3 HBD), which like **6h** is a tubulin inhibitor that binds to the colchicine site[35] (Chart 2). Compound **6h** appears to be optimized for BBB penetration while other potent compounds (Table 5) with less BBB penetration could potentially be developed for peripheral indications.

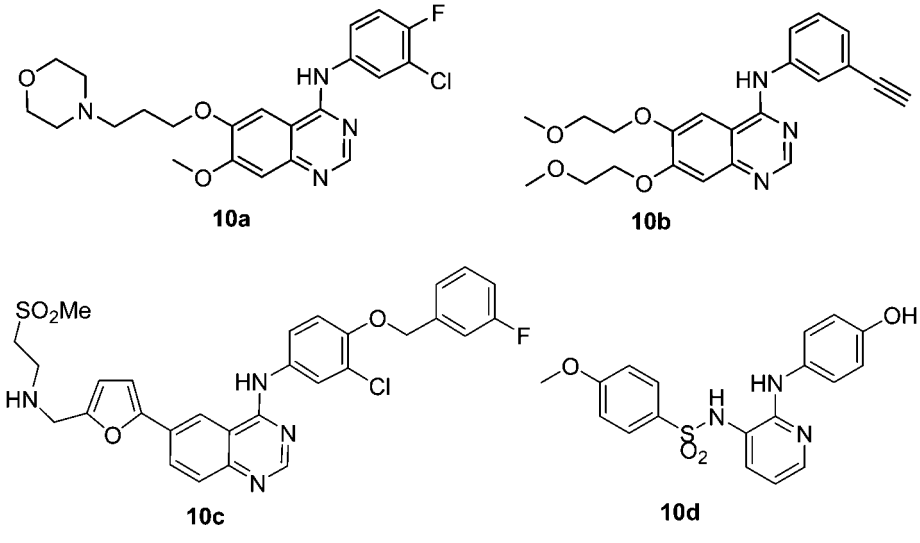
In conclusion, through SAR studies and lead optimization, compound **6h** has been identified as an anticancer development candidate. In comparison with our original lead **6b**, compound **6h** does not have the potentially reactive 2-Cl, but still maintains the desirable properties of a potent apoptosis inducing agent with good BBB penetration and high efficacy in multiple anticancer *in vivo* models. The primary molecular target of **6h** has been identified as tubulin by using a tritium labeled photoaffinity labeling reagent. From our extensive SAR and ADMET (absorption, distribution, metabolism, excretion and toxicity) lead optimizations efforts, we discovered *N*-(4-methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine (**6h**, EP128495, MPC-6827, Azixa), as an anticancer clinical candidate with potent apoptosis inducing activity. Azixa is currently under clinical development and reports concerning its progress in the clinic will be published in due course.

Experimental Section

General Methods and Materials. Commercial-grade reagents and solvents obtained from Acros, Aldrich, Apin Lancaster, TCI or VWR were used without further purification except as indicated. All reactions were stirred magnetically; moisture-sensitive reactions were performed under argon in oven-dried glassware. Thin-layer chromatography (TLC), usually using ethyl acetate/hexane as the solvent system, was used to monitor reactions. Solvents were removed by rotary evaporation under reduced pressure; where appropriate, the compound was further dried using a vacuum pump. The ^{1}H NMR spectra were recorded at 300 MHz. All samples were prepared as dilute solutions in deuteriochloroform ($CDCl_3$) with v/v 0.05% tetramethylsilane (TMS). Chemical shifts are reported in parts per million (ppm) downfield from TMS (0.00 ppm), and *J* coupling constants are reported in hertz. Purity of tested compounds **6c**−**6m**, **7a**−**7s** and **9a** was determined by elemental analysis, HPLC or SFC to be >95% pure. Elemental analyses were performed by Numega Resonance Laboratories, Inc. (San Diego, CA). LCMS was run in the ESI mode using an Xterra MS C18 (Waters) 4.6 × 50 mm 5 μm column; HPLC purity was performed using a 4.6 × 150 mm Xterra C18 5 μm column. Both LCMS and LC were reverse phase with an acetonitile/water containing 0.01%v/v TFA gradient. SFC was run using a normal phase Luna 5u silica 4.6 × 250 mm column. Mobile phase was liquid CO_2 with methanol as the modifying solvent. A gradient was used in which the concentration of methanol was ramped from 5% to 50% over a course of 12 min with a 200 bar back-pressure at 40 °C. Human breast cancer cells T47D, human colorectal carcinoma cells HCT116 and hepatocellular carcinoma cancer cells SNU-398 were purchased from the American type Culture Collection (Manasas, VA).

2-Methylquinazolin-4-ol (3a). To a solution of 2-aminobenzoic acid methyl ester (15.0 g, 99.3 mmol) in 500 mL of acetonitrile in a 1000 mL three necked round bottomed flask equipped with a mechanical stirrer, condenser and a dispersion tube was bubbled

Chart 2



through HCl gas for 15 min with vigorous stirring, and the resulting mixture was refluxed overnight, then cooled to room temperature. The resulting precipitates were collected by filtration. The solid was dissolved in water (250 mL), the solution was neutralized with saturated $NaHCO_3$ (100 mL), and the precipitates were collected by filtration. The solid was washed with water (50 mL), dried at room temperature for 8 h and dried further at 70 °C under high vacuum overnight to give **3a** as white solid (14.32 g, 90%). ^{1}H NMR (DMSO-d_6) 12.21 (s, broad, 1H), 8.06−8.09 (m, 1H), 7.74−7.79 (m, 1H), 7.56−7.59 (m, 1H), 7.43−7.45 (m, 1H), 2.35 (s, 3H).

4-Chloro-2-methylquinazoline (4a). A mixture of **3a** (10.0 g, 62.6 mmol) in 190 mL of anhydrous toluene and anhydrous diisopropylethylamine (DIPEA, 17.0 mL, 97.4 mmol) in a 500 mL round bottomed flask equipped with a condenser and a drying tube was refluxed for 1 h. To this warm solution was added freshly distilled $POCl_3$ (9.0 mL, 97 mmol) and the mixture was heated at 80 °C for 2 h, and cooled to room temperature. The mixture was diluted with 500 mL of ethyl acetate, washed with 200 mL of ice cold water, 200 mL of saturated $NaHCO_3$, 200 mL of water, citric acid (1 N, 4 × 200 mL), 200 mL of water, 200 mL of saturated $NaHCO_3$ and 200 mL of saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to obtain an off-white solid (9.69 g, 87%). ^{1}H NMR ($CDCl_3$): 8.21−8.25 (m, 1H), 7.89−7.99 (m, 2H), 7.66 (ddd, J = 8.7, 6.6 and 1.8 Hz, 1H), 2.87 (s, 3H).

***N*-(4-Methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine hydrochloride (6h).** To a solution of **4a** (2.31 g, 12.9 mmol) and *N*-methyl-4-methoxyaniline (**5a**, 2.00 g, 14.6 mmol) in 35 mL of anhydrous isopropanol (IPA) was added 0.6 mL of concentrated HCl, and the mixture was stirred at room temperature overnight. The yellow precipitates were collected by filtration, washed with cold isopropanol, and dried at room temperature for 3 h under house vacuum. The solid was further dried under high vacuum at 70 °C for 48 h to remove residual isopropanol, provided compound **6h** as yellow solids (2.90 g, 71%). ^{1}H NMR ($CDCl_3$): 8.53 (dd, J = 8.1 and 0.6 Hz, 1H), 7.7 (ddd, J = 8.4, 7.2 and 1.2 Hz, 1H), 7.22 (m, 2H), 7.13 (ddd, J = 8.7, 7.2 and 1.2 Hz, 1H), 7.05 (m, 2H), 6.76 (d, J = 8.7 Hz, 1H), 3.91 (s, 3H), 3.78 (s, 3H), 2.96 (s, 3H). Anal. ($C_{17}H_{17}N_3O$·HCl) C, H, N.

The following compounds were prepared by a procedure similar to that described for the preparation of compound **6h**.

2-Ethyl-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (6i). Compound **6i** was prepared from 4-chloro-2-ethylquinazoline and **5a** and was isolated as a solid (70%). ^{1}H NMR ($CDCl_3$): 7.76 (d, J = 8.4 Hz, 1H), 7.55−7.49 (m, 1H), 7.13−7.09 (m, 2H), 7.03−6.89 (m, 4H), 3.83 (s, 3H), 3.60 (s, 3H), 2.97 (q, J = 7.5 Hz, 2H), 1.44 (t, J = 7.8 Hz, 3H). HRMS calcd for $C_{18}H_{20}N_3O$ (M + H^+), 294.1608; found, 294.1597.

2-Fluoromethyl-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (6j). Compound **6j** was prepared from 4-chloro-2-fluoromethylquinazoline and **5a** and was isolated as a solid (67%). ^{1}H NMR ($CDCl_3$): 7.87−7.84 (m, 1H), 7.60−7.54 (m, 1H), 7.14−7.10 (m, 2H), 7.04−7.01 (m, 2H), 6.95−6.91 (m, 2H), 5.60 (s, 1H), 5.44 (s, 1H), 3.85 (s, 3H), 3.60 (s, 3H). HRMS calcd for $C_{17}H_{17}FN_3O$ (M + H^+), 298.1357; found, 298.1343.

2-Chloromethyl-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (6k). Compound **6k** was prepared from 4-chloro-2-chloromethylquinazoline and **5a** and was isolated as a solid (65%). ^{1}H NMR ($CDCl_3$): 7.82 (d, J = 8.7 Hz, 1H), 7.59−7.53 (m, 1H), 7.15−7.12 (m, 2H), 7.03−7.00 (m, 2H), 6.95−6.91 (m, 2H), 4.73 (s, 2H), 3.85 (s, 3H), 3.62 (s, 3H). HRMS calcd for $C_{17}H_{17}ClN_3O$ (M + H^+), 314.1062; found, 314.1042.

***N*-(4-Methoxyphenyl)-2-methylquinazolin-4-amine (9a).** Compound **9a** was prepared from **4a** and 4-methoxyaniline and was isolated as a solid (60%). ^{1}H NMR (DMSO-d_6): 11.28 (brs, 1H), 8.73 (d, J = 8.4 Hz, 1H), 8.06 (t, J = 7.5 Hz, 1H), 7.85−7.78 (m, 2H), 7.66 (d, J = 9 Hz, 2H), 7.06 (d, J = 8.7 Hz, 2H), 3.81 (s, 3H), 2.60 (s, 3H). HRMS calcd for $C_{16}H_{16}N_3O$ (M + H^+), 266.1295; found, 266.1289.

2-Methoxy-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (6c). To a solution of 2-chloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (**6b**, 50 mg, 0.167 mmol) in 2 mL methanol was added sodium methoxide (500 μL, 25% by wt. in methanol). The solution was stirred at 80 °C for 1 h, and it was diluted with 50 mL ethyl acetate. The solution was washed with water, dried and concentrated. The residue was purified using small silica column (acetate and hexane, 1:9) to give **6c** as a white solid (22 mg, 54%). ^{1}H NMR ($CDCl_3$): 7.89 (d, J = 8.4 Hz, 1H), 7.53 (ddd, J = 8.7, 5.4 and 2.4 Hz, 1H), 7.19- 7.14 (m, 2H), 6.99−6.93 (m, 2H), 6.90−6.85 (m, 2H), 4.14 (s, 3H), 3.86 (s, 3H), 3.64 (s, 3H). Anal. ($C_{17}H_{17}N_3O_2$) C, H, N.

***N*^{4}-(4-Methoxyphenyl)-*N*^{2},*N*^{2},*N*^{4}-trimethylquinazolin-2,4-diamine (6d).** A mixture of **6b** (150 mg, 0.5 mmol) and 2.0 M dimethylamine in methanol (2.0 mL, 4 mmol) in a sealed tube was stirred at 70−80 °C overnight. The mixture was filtered, and the filtration was concentrated by vacuum. The residue was mixed with ethyl acetate (10 mL) and was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by chromatography on silica gel with ethyl acetate and hexane (1:9) as eluent, yielding **6d** as a solid (128 mg, 83%). ^{1}H NMR ($CDCl_3$): 7.44 (d, J = 7.8 Hz, 1H), 7.36−7.30 (m, 1H), 7.11−7.08 (m, 2H), 6.90−6.85 (m, 3H), 6.65−6.59 (m, 1H), 3.82 (s, 3H), 3.51 (s, 3H), 3.30 (s, 6H). Anal. ($C_{18}H_{20}N_4O$) C, H, N.

***N*^{4}-(4-Methoxyphenyl)-*N*^{2},*N*^{4}-dimethylquinazolin-2,4-diamine (6e).** Compound **6e** was prepared from reaction of **6b** with 2.0 M methylamine in THF using a procedure similar to that of **6d** and was isolated as a solid (53.7%). ^{1}H NMR ($CDCl_3$): 7.45 (d, J = 7.8 Hz, 1H), 7.39−7.33 (m, 1H), 7.11−7.07 (m, 2H), 6.90−6.87 (m, 3H), 6.69−6.64 (m, 1H), 4.95 (brs, 1H), 3.82 (s, 3H), 3.50 (s, 3H), 3.11 (d, J = 5.1 Hz, 3H). HRMS calcd for $C_{17}H_{19}N_4O$ (M + H^+), 295.1560; found, 295.1542.

2-Hydrazino-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (6f). To a solution of **6b** (100 mg, 0.33 mmol) in 2 mL of 1,4-dioxane was added 0.4 mL of hydrazine. The reaction mixture was stirred at room temperature overnight. The solvent was removed by evaporation, and the residue was diluted with ethyl acetate (20 mL), washed with saturated $NaHCO_3$ aq, brine, dried over Na_2SO_4, filtered and concentrated by vacuum, yielding **6f** as yellow solids (20 mg, 21%). ^{1}H NMR ($CDCl_3$): 7.52−7.49 (m, 1H), 7.44−7.39 (m, 1H), 7.12−7.08 (m, 2H), 6.93−6.88 (m, 3H), 6.77−6.71 (m, 1H), 6.01 (brs, 1H), 4.11 (brs, 2H), 3.83 (s, 3H), 3.50 (s, 3H). Anal. ($C_{16}H_{17}N_5O$) C, H, N.

***N*^{4}-(4-Methoxyphenyl)-*N*^{2}-acetyl-*N*^{2},*N*^{4}-dimethylquinazolin-2,4-diamine (6g).** To a solution of **6e** (100 mg, 0.34 mmol) in 5 mL of dichloromethane were added triethylamine (0.071 mL, 0.51 mmol) and acetyl chloride (0.036 mL, 0.51 mmol) followed by 2 mg of DMAP at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The solvent was removed by evaporation. The residue was dissolved in ethyl acetate (20 mL), washed with water and brine, dried over Na_2SO_4, filtered and concentrated by vacuum. The crude product was purified by chromatography on silica gel with ethyl acetate, hexane and methanol (1:3 to 1:1:0.05) as eluent, yielding **6g** as a white solid (36 mg, 32%). ^{1}H NMR ($CDCl_3$): 7.70−7.67 (m, 1H), 7.56−7.52 (m, 1H), 7.17−7.14 (m, 2H), 6.97−6.93 (m, 4H), 3.86 (s, 3H), 3.57 (s, 6H), 2.52 (s, 3H). Anal. ($C_{19}H_{20}N_4O_2$) C, H, N.

2-Hydroxymethyl-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (6l). To a solution of **6k** (67 mg, 0.19 mmol) in 1,4-dioxane (3 mL) was added 2 N NaOH aqueous solution (1 mL). The mixture was heated at 80 °C for 24 h and then was cooled to room temperature. The reaction mixture was diluted with ethyl acetate (20 mL) and then was washed with water and dried with $NaSO_4$. The solvent was evaporated, and the residue was purified by column chromatography on silica gel with ethyl acetate and hexane (1:1) as eluent, yielding **6l** as a solid (25 mg, 44%). ^{1}H NMR ($CDCl_3$): 7.78−7.75 (m, 1H), 7.59−7.53 (m, 1H), 7.15−7.12 (m, 2H), 7.02−7.00 (m, 2H), 6.94−6.91 (m, 2H), 4.79 (s, 2H), 3.85 (s, 3H), 3.59 (s, 3H). HRMS calcd for $C_{17}H_{18}N_3O_2$ (M + H^+), 296.1382; found, 296.1389.

2-(Dimethylaminomethyl)-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (6m). Compound **6m** was prepared from reaction of **6k** and dimethylamine by a procedure similar to that of compound **6l** and was isolated as a solid (56%). ^{1}H NMR (DMSO-d_6): 7.71 (d, J = 8.7 Hz, 1H), 7.60 (t, J = 8.4 Hz, 1H), 7.20 (d, J = 8.4 Hz, 2H), 7.09 (t, J = 8.1 Hz, 1H), 7.00−6.96 (m, 3H), 3.78 (s, 3H), 3.63 (s, 2H), 3.50 (s, 3H), 2.33 (s, 6H). Anal. ($C_{19}H_{22}N_4O$) C, H, N.

2,*N*-Dimethyl-*N*-(4-nitrophenyl)quinazolin-4-amine (7a). To a solution of **4a** (1.40 g, 7.84 mmol) and *N*-methyl-4-nitroaniline (1.09 g, 7.16 mmol) in 20 mL of dimethylformamide cooled to 0 °C was added sodium hydride (0.6 g, 60 oil suspension, 15 mmol). The reaction mixture was stirred at 0 °C for 1 h and quenched by adding 200 μL of water. It was diluted with 150 mL of ethyl acetate, washed with water (100 mL × 3) and saturated NaCl, dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (30% ethyl acetate/hexanes) to give **7a** (1.41 g, 67%). ^{1}H NMR ($CDCl_3$): 8.14 (m, 2H), 7.91 (m, 1H), 7.71 (ddd, J = 8.4, 6.6 and 1.8 Hz, 1H), 7.19−7.32 (m, 2H), 7.05 (m, 2H), 3.76 (s, 3H), 2.82 (s, 3H). Anal. ($C_{16}H_{14}N_4O_2$) C, H, N.

***N*-(4-Fluorophenyl)-*N*,2-dimethylquinazolin-4-amine (7b).** A mixture of **4a** (178 mg, 1.00 mmol) and *N*-methyl-4-fluoroaniline (125 mg, 2.52 mmol) and sodium acetate (248 mg, 3.02 mmol) in 6 mL of solvent (THF:water = 1:1) was stirred at 70 °C for 1 h. The reaction mixture was diluted with 30 mL of ethyl acetate. The organics were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by chromatography on silica gel with ethyl acetate and hexane (1:5) as eluent, yielding **7b** as a solid (125 mg, 46.8%). ^{1}H NMR ($CDCl_3$): 7.76 (dd, J = 8.3 and 0.9 Hz, 1H), 7.58−7.52 (m,1H), 7.16−7.00 (m, 6H), 3.60 (s, 3H), 2.73 (s, 3H). HRMS calcd for $C_{16}H_{15}FN_3$ ($M + H^+$), 268.1252; found, 268.1239.

The following intermediates were prepared by a procedure similar to that described for the preparation of compound **7b**.

***N*-(4-Ethoxyphenyl)-2-methylquinazolin-4-amine (9b).** Compound **9b** was prepared from **4a** and 4-ethoxyaniline and was isolated as a solid (93%). ^{1}H NMR ($CDCl_3$): 7.83 (m, 1H), 7.81 (m, 1H), 7.42 (m, 1H), 7.65−7.71 (m, 2H), 7.46 (ddd, J = 8.4, 6.9 and 1.5 Hz, 1H), 7.37 (s, broad, 1H), 6.92−6.97 (m, 2H), 4.06 (q, J = 6.9 Hz, 2H), 2.68 (s, 3H), 1.43 (t, J = 6.9 Hz, 3H).

***N*-(4-Difluoromethoxyphenyl)-2-methylquinazolin-4-amine (9c).** Compound **9c** was prepared from **4a** (450 mg, 2.52 mmol) and 4-difluoromethoxyphenylamine (0.32 mL, 2.52 mmol) and was isolated as a solid (713 mg, 94%). ^{1}H NMR ($CDCl_3$): 7.87−7.76 (m, 5H), 7.51 (t, J = 8.4 Hz, 1H), 7.40 (brs, 1H), 7.19 (d, J = 8.7 Hz, 2H), 6.76−6.27 (m, 1H), 2.71 (s, 3H).

***N*-(4-Methylthiophenyl)-2-methylquinazolin-4-amine (9d).** Compound **9d** was prepared from **4a** (178 mg, 1.00 mmol) and 4-methylthioaniline (139 mg, 1.00 mmol) and was isolated as a solid (273 mg, 97%). ^{1}H NMR ($CDCl_3$): 7.86−7.82 (m, 2H), 7.79−7.73 (m, 3H), 7.50−7.45 (m, 2H), 7.34−7.26 (m, 2H), 2.7 (s, 3H), 2.51 (s, 3H).

***N*-(4-Ethylphenyl)-2-methylquinazolin-4-amine (9e).** Compound **9e** was prepared from **4a** (100 mg, 0.56 mmol) and 4-ethylaniline (0.07 mL, 0.56 mmol) and was isolated as a solid (134 mg, 91%). ^{1}H NMR ($CDCl_3$): 7.85−7.78 (m, 2H), 7.76−7.71 (m, 3H), 7.49 (t, J = 7.5 Hz, 1H), 7.36 (brs, 1H), 7.24 (s, 2H), 2.70−2.69 (m, 5H), 1.26 (t, J = 7.5 Hz, 3H).

***N*-(3-Fluoro-4-methoxyphenyl)-2-methylquinazolin-4-amine (9f).** Compound **9f** was prepared from **4a** (150 mg, 0.84 mmol) and 3-fluoro-4-methoxyaniline (118 mg, 0.84 mmol) and was isolated as a solid (90 mg, 34%). ^{1}H NMR ($CDCl_3$): 7.89−7.75 (m, 4H), 7.53−7.48 (m, 1H), 7.38−7.34 (m, 2H), 7.0 (t, J = 8.7 Hz, 1H), 3.92 (s, 3H), 2.71 (s, 3H).

***N*-(2-Fluoro-4-methoxyphenyl)-2-methylquinazolin-4-amine (9g).** Compound **9g** was prepared from **4a** and 2-fluoro-4-methoxyaniline and was isolated as a solid (79%). ^{1}H NMR ($CDCl_3$): 8.54 (m, 1H), 7.83 (m, 2H), 7.65 (m, 1H), 7.50 (m, 1H), 7.47 (s, broad, 1H), 6.74−6.81 (m, 2H), 3.83 (s, 3H), 2.70 (s, 3H).

***N*-(3,4-Dimethoxyphenyl)-2-methylquinazolin-4-amine (9h).** Compound **9h** was prepared from **4a** (178.6 mg, 1.0 mmol) and 3,4-dimethoxyaniline (153 mg, 1.0 mmol) and was isolated as off white solids (295 mg, 100%). ^{1}H NMR ($CDCl_3$): 7.85−7.70 (m, 4H), 7.51−7.46 (m, 1H), 7.38 (brs, 1H), 7.16 (dd, J = 8.7 and 2.1 Hz, 1H), 6.90 (d, J = 8.7 Hz, 1H), 3.95 (s, 3H), 3.91 (s, 3H), 2.69 (s, 3H).

***N*-(6-Methoxypyridin-3-yl)-2-methylquinazolin-4-amine (9i).** Compound **9i** was prepared from **4a** and 3-amino-6-methoxypyridine and was isolated as a solid (32%). ^{1}H NMR ($CDCl_3$): 8.51 (d, J = 1.8 Hz, 1H), 8.12 (m, 1H), 7.72−7.89 (m, 3H), 7.49 (m, 2H), 6.81 (d, J = 8.7 Hz, 1H), 3.89 (s, 3H), 2.68 (s, 3H).

***N*-(5-Methoxypyridin-2-yl)-2-methylquinazolin-4-amine (9j).** Compound **9j** was prepared from **4a** and 2-amino-5-methoxypyridine and was isolated as a solid (42%). ^{1}H NMR ($CDCl_3$): 8.43 (m, 1H), 7.72−7.92 (m, 2H), 7.46−7.56 (m, 2H), 7.32−7.40 (m, 2H), 3.88 (s, 3H), 2.74 (s, 3H).

***N*-(6-Dimethylaminopyridin-3-yl)-2-methylquinazolin-4-amine (9k).** Compound **9k** was prepared from **4a** (182 mg, 1.02 mmol) and 3-amino-6-dimethylaminopyridine (140 mg, 1.02 mmol) and was isolated as a pale yellow solid (60 mg, 21%). ^{1}H NMR ($CDCl_3$): 8.43 (d, J = 2.4 Hz, 1H), 7.95 (dd, J = 9.3 and 2.7 Hz, 1H), 7.84−7.72 (m, 3H), 7.47 (t, J = 8.1 Hz, 1H), 7.16 (brs, 1H), 6.61 (d, J = 9.0 Hz, 1H), 3.12 (s, 6H), 2.65 (s, 3H).

***N*-(5-Methoxypyrazin-2-yl)-2-methylquinazolin-4-amine (9l).** Compound **9l** was prepared from **4a** (150 mg, 0.84 mmol) and 2-amino-5-methoxypyrazine (105 mg, 0.84 mmol) and was isolated as a solid (106 mg, 47%). The crude product was used as is in the next step.

***N*-(5-Methoxypyrimidin-2-yl)-2-methylquinazolin-4-amine (9m).** Compound **9m** was prepared from **4a** (343 mg, 1.92 mmol) and 2-amino-5-methoxypyrimidine (240 mg, 1.92 mmol) and was isolated as a solid (283 mg, 55%). ^{1}H NMR ($CDCl_3$): 8.69 (dd, J = 8.1 and 1.5 Hz, 1H), 8.43 (s, 2H), 7.73−7.67 (m, 1H), 7.60 (d, J = 7.2 Hz, 1H), 7.49−7.43 (m, 1H), 3.95 (s, 3H), 2.58 (s, 3H).

***N*-(4-Methylthiophenyl)-*N*,2-dimethylquinazolin-4-amine (7e).** To a solution of **9d** (263 mg, 0.94 mmol) in DMF (4 mL) at 0 °C was added sodium hydride (56.4 mg, 1.40 mmol, 60% oil dispersion) and followed by methyl iodide (0.09 mL, 1.4 mmol). The mixture was stirred at 0 °C for 1 h, then allowed to warm to room temperature and stirred for an additional 2 h. The reaction mixture was diluted with ethyl acetate (15 mL), washed with saturated $NaHCO_3$ aq, brine, dried over Na_2SO_4, filtered and concentrated by vacuum. The residue was purified by chromatography on silica gel with ethyl acetate and hexane (1:2 to 1:1) as eluent, yielding 120 mg of **7e** (40.7%). ^{1}H NMR ($CDCl_3$): 7.76 (d, J = 9.0 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.24−7.19 (m, 2H), 7.10−6.97 (m, 4H), 3.59 (s, 3H), 2.74 (s, 3H), 2.48 (s, 3H). HRMS calcd for $C_{17}H_{18}N_3S$ ($M + H^+$), 296.1223; found, 296.1207.

The following compounds were prepared by a procedure similar to that described for the preparation of compound **7e**.

***N*-(4-Ethoxyphenyl)-*N*,2-dimethylquinazolin-4-amine (7c).** Compound **7c** was prepared from **9b** and methyl iodide and was isolated as a solid (67%). ^{1}H NMR ($CDCl_3$): 7.71−7.74 (m, 1H), 7.51 (ddd, J = 8.1, 6.6 and 1.5 Hz, 1H), 7.09 (m, 2H), 6.95−7.04 (m, 2H), 6.86−6.92 (m, 2H), 4.04 (q, J = 6.9 Hz, 2H), 3.58 (s, 3H), 2.72 (s, 3H), 1.44 (t, J = 6.9 Hz, 3H). HRMS calcd for $C_{18}H_{20}N_3O$ ($M + H^+$), 294.1608; found, 294.1595.

***N*-(4-Difluoromethoxyphenyl)-*N*,2-dimethylquinazolin-4-amine (7d).** Compound **7d** was prepared from **9c** (710 mg, 2.36 mmol) and methyl iodide (1.03 mL, 16.5 mmol) and was isolated as a solid (301 mg, 40.8%). ^{1}H NMR ($CDCl_3$): 7.77 (dd, J = 8.4 and 0.9 Hz, 1H), 7.59−7.53 (m, 1H), 7.17−7.10 (m, 4H), 7.06−6.99 (m, 2H), 6.78−6.29 (m, 1H), 3.62 (s, 3H), 2.75 (s, 3H). HRMS calcd for $C_{17}H_{16}F_2N_3O$ ($M + H^+$), 316.1263; found, 316.1245.

***N*-(4-Ethylphenyl)-*N*,2-dimethylquinazolin-4-amine (7f).** Compound **7f** was prepared from **9e** (122 mg, 0.46 mmol) and methyl iodide (0.2 mL, 3.3 mmol) and was isolated as a solid (39 mg, 52%). ^{1}H NMR ($CDCl_3$): 7.74 (d, J = 8.1 Hz, 1H), 7.54−7.49 (m, 1H), 7.19 (d, J = 8.4 Hz, 2H), 7.09−6.92 (m, 4H), 3.61 (s, 3H),

2.73−2.63 (m, 5H), 1.26 (d, J = 7.5 Hz, 3H). HRMS calcd for $C_{18}H_{20}N_3$ (M + H^+), 278.1659; found, 279.1640.

***N*-(3-Fluoro-4-methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine (7l).** Compound **7l** was prepared from **9f** (250 mg, 0.88 mmol) and methyl iodide (0.39 mL, 6.2 mmol) and was isolated as a solid (32 mg, 12%). ^{1}H NMR ($CDCl_3$): 7.76 (d, J = 8.4 Hz, 1H), 7.59−7.53 (m, 1H), 7.09−6.82 (m, 5H), 3.91 (s, 3H), 3.58 (s, 3H), 2.73 (s, 3H). HRMS calcd for $C_{17}H_{17}FN_3O$ (M + H^+), 298.1357; found, 298.1343.

***N*-(2-Fluoro-4-methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine (7m).** Compound **7m** was prepared from **9g** and methyl iodide and was isolated as a solid (51%). ^{1}H NMR ($CDCl_3$): 7.76 (d, J = 8.1 Hz, 1H), 7.55 (ddd, J = 8.1, 6.3 and 1.8 Hz, 1H), 6.98−7.11 (m, 3H), 6.66−6.76 (m, 2H), 3.83 (s, 3H), 3.54 (s, 3H), 2.73 (s, 3H). HRMS calcd for $C_{17}H_{17}FN_3O$ (M + H^+), 298.1357; found, 298.1346.

***N*-(3,4-Dimethoxyphenyl)-*N*,2-dimethylquinazolin-4-amine (7n).** Compound **7n** was prepared from **9h** (288 mg, 0.98 mmol) and methyl iodide (0.094 mL, 1.5 mmol) and was isolated as off white solids (70 mg, 23%). ^{1}H NMR ($CDCl_3$): 7.75−7.72 (m, 1H), 7.56−7.50 (m, 1H), 7.05−6.94 (m, 2H), 6.85 (d, J = 7.5 Hz, 1H), 6.76−6.71 (m, 2H), 3.92 (s, 3H), 3.78 (s, 3H), 3.61 (s, 3H), 2.73 (s, 3H). Anal. ($C_{16}H_{14}N_4O_2$) C, H, N.

***N*-(6-Methoxypyridin-3-yl)-*N*,2-dimethylquinazolin-4-amine (7o).** Compound **7o** was prepared from **9i** and methyl iodide and was isolated as a solid (28%). ^{1}H NMR ($CDCl_3$): 8.03 (d, J = 2.7 Hz, 1H), 7.77 (m, 1H), 7.56 (ddd, J = 8.1, 6.3 and 1.8 Hz, 1H), 7.38 (dd, J = 8.7 and 3.0 Hz, 1H), 7.01 (m, 2H), 6.76 (d, J = 9.0 Hz, 1H), 3.96 (s, 3H), 3.59 (s, 3H), 2.73 (s, 3H). HRMS calcd for $C_{16}H_{17}N_4O$ (M + H^+), 281.1404; found, 281.1400.

***N*-(5-Methoxypyridin-2-yl)-*N*,2-dimethylquinazolin-4-amine (7p).** Compound **7p** was prepared from **9j** and methyl iodide and was isolated as a solid (10%). ^{1}H NMR ($CDCl_3$): 8.31 (d, J = 3.3 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.58 (ddd, J = 8.4, 6.6 and 1.5 Hz, 1H), 7.13 (dd, J = 9.0 and 3.3 Hz, 1H), 6.99−7.10 (m, 2H), 6.82 (d, J = 9.0 Hz, 1H), 3.87 (s, 3H), 3.70 (s, 3H), 2.76 (s, 3H). HRMS calcd for $C_{16}H_{17}N_4O$ (M + H^+), 281.1404; found, 281.1384.

***N*-(6-Dimethylaminopyridin-3-yl)-*N*,2-dimethylquinazolin-4-amine (7q).** Compound **7q** was prepared from **9k** (45 mg, 0.16 mmol) and methyl iodide (0.016 mL, 0.24 mmol) and was isolated as a pale yellow solid (22 mg, 47%). ^{1}H NMR ($CDCl_3$): 8.07 (d, J = 2.4 Hz, 1H), 7.63 (dd, J = 8.4 and 0.9 Hz, 1H), 7.56−7.51 (m, 1H), 7.27−7.18 (m, 2H), 7.05−7.00 (m, 1H), 6.50 (d, J = 9.3 Hz, 1H), 3.55 (s, 3H), 3.12 (s, 6H), 2.72 (s, 3H). HRMS calcd for $C_{17}H_{20}N_5$ (M + H^+), 294.1720; found, 294.1693.

***N*-(5-Methoxypyrazin-2-yl)-*N*,2-dimethylquinazolin-4-amine (7r).** Compound **7r** was prepared from **9l** (106 mg, 0.40 mmol) and methyl iodide and was isolated as a solid (63 mg, 56%). ^{1}H NMR ($CDCl_3$): 8.06 (d, J = 1.2 Hz, 1H), 7.85−7.81 (m, 1H), 7.74 (d, J = 1.5 Hz, 1H), 7.65−7.60 (m, 1H), 7.17−7.05 (m, 2H), 3.94 (s, 3H), 3.70 (s, 3H), 2.78 (s, 3H). HRMS calcd for $C_{15}H_{16}N_5O$ (M + H^+), 282.1356; found, 282.1355.

***N*-(5-Methoxypyrimidin-2-yl)-*N*,2-dimethylquinazolin-4-amine (7s).** Compound **7s** was prepared from **9m** (170 mg, 0.64 mmol) and methyl iodide and was isolated as a solid (141 mg, 50%). ^{1}H NMR ($CDCl_3$) 8.12 (s, 2H), 7.91 (d, J = 8.4 Hz, 1H), 7.74−7.69 (m, 1H), 7.38−7.34 (m, 1H), 7.30−7.24 (m, 1H), 3.85 (s, 3H), 3.78 (s, 3H), 2.83 (s, 3H). Anal. ($C_{15}H_{15}N_5O\cdot 0.7H_2O$) C, H, N.

***N*-(4-Hydroxyphenyl)-*N*,2-dimethylquinazolin-4-amine (7 h).** To a solution of **6h** (106 mg, 0.336 mmol) in dichloromethane (10 mL) at −78 °C was added slowly boron tribromide (1 M in CH_2Cl_2, 0.75 mL) under argon. The cold bath was removed, and the reaction mixture was allowed to warm up slowly to 10 °C in 1.5 h. The reaction mixture was quenched with water (10 mL), basified with 2 N NaOH to pH = 10, and extracted with ethyl acetate (2 × 25 mL). The ethyl acetate extracts were dried and evaporated to give a light brown residue. The crude was purified by column chromatography (SiO_2, ethyl acetate:hexanes/15−50%) to give **7h** as a white solid (5 mg, 6%). ^{1}H NMR (acetone-d_6): 8.74 (s, 1H), 7.75 (m, 1H), 7.67 (m, 1H), 7.20−7.18 (m, 3H), 7.12 (m, 1H), 7.04−6.99 (m, 2H), 3.64 (s, 3H), 2.79 (s, 3H). HRMS calcd for $C_{16}H_{16}N_3O$ (M + H^+), 266.1295; found, 266.1279.

***N*-(4-Aminophenyl)-*N*,2-dimethylquinazolin-4-amine (7i).** A mixture of **7a** (200 mg, 0.68 mmol) in 25 mL of ethyl acetate was hydrogenated over palladium on carbon (70 mg) at 50 psi for 4 h, and the reaction mixture was filtered through a pad of Celite and concentrated. The resulting crude product was purified by chromatography (50% ethyl acetate/hexane) to give **7i** as a solid (140 mg, 78%). ^{1}H NMR ($CDCl_3$): 7.71 (m, 1H), 7.51 (ddd, J = 8.4, 6.9 and 1.5 Hz, 1H), 7.09 (m, 1H), 6.93−7.05 (m, 3H), 6.68 (m, 2H), 3.74 (s, broad, 2H), 3.56 (s, 3H), 2.71 (s, 3H). HRMS calcd for $C_{16}H_{17}N_4$ (M + H^+), 265.1455; found, 265.1436.

***N*-(4-Dimethylaminophenyl)-*N*,2-dimethylquinazolin-4-amine (7g).** To a solution of **7i** (14 mg, 0.053 mmol) in methanol (1.5 mL), 37% aqueous formaldehyde (0.5 mL) and 10 μL of glacial acetic was added sodium cyanoborohydride (15 mg, 0.24 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was quenched by adding 50 μL of 1 N HCl. The mixture was diluted with 50 mL of ethyl acetate, washed with saturated sodium bicarbonate, and followed by saturated sodium chloride. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (25% ethyl acetate/hexanes) on silica gel to give **7g** as a solid (12 mg, 80%). ^{1}H NMR ($CDCl_3$): 7.71 (m, 1H), 7.50 (ddd, J = 8.4, 6.9 and 1.5 Hz, 1H), 7.03−7.09 (m, 3H), 6.95 (ddd, J = 8.1, 6.6 and 0.9 Hz, 1H), 6.70 (m, 2H), 3.57 (s, 3H), 2.99 (s, 6H), 2.71 (s, 3H). HRMS calcd for $C_{18}H_{21}N_4$ (M + H^+), 293.1768; found, 293.1755.

***N*-(4-Azidophenyl)-*N*,2-dimethylquinazolin-4-amine (7j).** To a solution of **7i** (20 mg, 0.076 mmol) in 1.2 mL of 1 N HCl was added 100 μL of methanol and the mixture cooled to 0 °C. One drop of concentrated HCl was added, and the solution was stirred at 0 °C for 0.25 h. To the solution was added dropwise a solution of sodium nitrite (25 mg, 0.36 mmol) in 200 μL of water. The reaction mixture was stirred for 0.5 h, then was added a solution of sodium azide (25 mg, 0.38 mmol) in 300 μL of water followed by an additional batch of sodium azide (25 mg, 0.38 mmol). The reaction mixture was stirred at 0 °C for 1 h, diluted with 50 mL of ethyl acetate, and washed with saturated sodium bicarbonate followed by saturated sodium chloride. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (20−25% ethyl acetate/hexanes) on silica gel to give **7j** as a solid (20 mg, 90%). ^{1}H NMR ($CDCl_3$): 7.76 (m, 1H), 7.56 (ddd, J = 8.1, 6.3 and 1.8 Hz, 1H), 7.13 (m, 2H), 6.98−7.13 (m, 4H), 3.61 (s, 3H), 2.74 (s, 3H). HRMS calcd for $C_{16}H_{15}N_6$ (M + H^+), 291.1360; found, 291.1347.

***N*-(4-Acetamidophenyl)-*N*,2-dimethylquinazolin-4-amine (7k).** To a solution of **7i** (28 mg, 0.11 mmol) in 2 mL of dichloromethane with triethylamine (50 μL, 0.36 mmol) cooled at 0 °C was added acetic anhydride (50 μL, 0.53 mmol), followed by a few crystals of 4-dimethylaminopyridine, and the mixture was allowed to warm to room temperature. The reaction mixture was stirred for 0.5 h and 25 mL of ethyl acetate was added. The solution was washed with saturated $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude was purified by column chromatography (80% ethyl acetate/hexane) to give **7k** (32.5 mg, 100%). ^{1}H NMR ($CDCl_3$): 7.75 (d, J = 8.1 Hz, 1H), 7.5−7.57 (m, 3H), 6.94−7.12 (m, 4H), 3.60 (s, 3H), 2.72 (s, 3H), 2.42 (s, 3H). HRMS calcd for $C_{18}H_{19}N_4O$ (M + H^+), 307.1560; found, 307.1553.

Supporting Information Available: Table of elemental analysis and HPLC data for the targeted compounds **6c**−**6m**, **7a**−**7s** and **9a**. Caspase activation assays, growth inhibition assays, tubulin inhibition assay, cell cycle analysis, MX-1 tumor model studies of **6h**, and determination of brain/plasma AUC ratio of **6h** and related analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

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