Discovery of 2-Chloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (EP128265, MPI-0441138) as a Potent Inducer of Apoptosis with High In Vivo Activity

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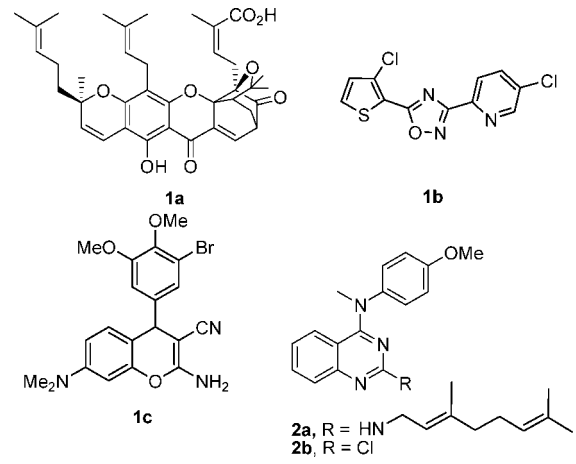
Using a live cell, high-throughput caspase-3 activator assay, we have identified a novel series of 4-anilinoquinazolines as inducers of apoptosis. In this report, we discuss the discovery of 2-chloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine, compound **2b** (EP128265, MPI-0441138) as a highly active inducer of apoptosis (EC_{50} for caspase activation of 2 nM) and as a potent inhibitor of cell proliferation (GI_{50} of 2 nM) in T47D cells. Compound **2b** inhibited tubulin polymerization, was effective in cells overexpressing ABC transporter Pgp-1, and was efficacious in the MX-1 human breast and PC-3 prostate cancer mouse models. In contrast to the SAR of 4-anilinoquinazolines as EGFR kinase inhibitors, the methyl group on the nitrogen linker was essential for the apoptosis-inducing activity of 4-anilinoquinazolines and substitution in the 6- and 7-positions of the quinazoline core structure decreased potency.

Introduction

Apoptosis or programmed cell death is a process that organisms use to eliminate excessive cells and to control cell numbers.[1] Caspases, a family of cysteine proteases, plays a critical role for the initiation as well as execution of apoptosis.[2] It has been well documented that improperly regulated apoptosis can lead to many diseases. Excessive apoptosis could be the cause of debilitating damage from acute diseases such as stroke, heart attack, or liver failure, as well as from neurodegenerative diseases,[3] with several caspase inhibitors currently in clinical trials for the treatment of liver injury.[4] Abnormal inhibition of apoptosis, such as overexpression of antiapoptotic Bcl-2 proteins, could result in unchecked cell proliferation as well as cause cancer treatment failure.[5,6] In addition, many chemotherapeutics are known to induce apoptosis. Therefore, promoting apoptosis has been suggested as a promising strategy for cancer drug discovery.[7] Several novel approaches to promote apoptosis are currently being explored, including the discovery and development of Bcl-2 inhibitors,[8] inhibitors of MDM2-p53 interaction,[9] inhibitors of XIAP,[10] Smac mimetics[11] and activators of procaspase-3.[12]

We have been interested in the discovery and development of apoptosis inducers as potential anticancer agents and have developed a cell-based anticancer screening apoptosis platform (ASAP[a]) using novel fluorescent caspase-3 substrates.[13] We have reported the discovery and structure−activity relationship (SAR) studies of several novel series of apoptosis inducers as well as the identification of their molecular targets,[14] including gambogic acid (**1a**) as a fast and potent apoptosis inducer[15] and the transferrin receptor as its molecular target,[16] 3-aryl-5-aryl-1,2,4-oxadiazoles (**1b**) as tumor selective apoptosis inducers[17] and tail interacting protein (TIP47), an insulin growth factor II (IGF II) receptor binding protein as its molecular target,[18] as well as 4-aryl-4*H*-chromenes (**1c**) as potent apoptosis inducers[19] and vascular disrupting agents.[20] In this study, we report the discovery of N^4-(4-methoxyphenyl)-N^4-methyl-N^2-((*E*)-3,7-dimethylocta-2,6-dienyl)quinazoline-2,4-diamine (**2a**) as a novel apoptosis inducer utilizing our cell- and caspase-based apoptosis high-throughput screening (HTS) assay. SAR and target identification studies demonstrate that 2-chloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (**2b**, EP128265, MPI-0441138, Chart 1) is an inhibitor of tubulin polymerization and a potent inducer of apoptosis with significant in vivo activity.

Chart 1



Results and Discussion

Chemistry. N^4-(4-Methoxyphenyl)-N^4-methyl-N^2-(*E*)-3,7-dimethylocta-2,6-dienyl)quinazoline-2,4-diamine (**2a**) was obtained commercially and its structure was confirmed by ^{1}H NMR and MS. 2-Chloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (**2b**) was prepared from reaction of 2,4-dichloroquinazoline (**3a**) with *N*-methyl-4-methoxyaniline (**4a**). Simi-

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[a] Abbreviations: ASAP, anticancer screening apoptosis platform; SAR, structure activity relationship; TIP47, tail interacting protein; IGF II, insulin growth factor II; HTS, high-throughput screening; BBB, blood−brain barrier; AUC, concentration−time curves.

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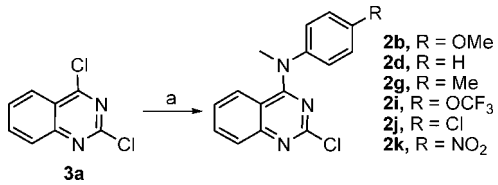
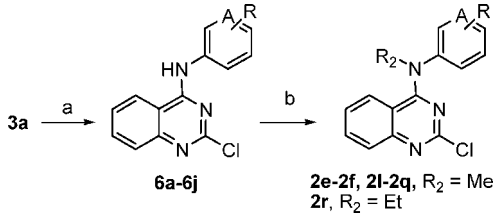
Scheme 1[a]



[a] Conditions: (a) 4-R-PhNMeH (**4a**−**f**), IPA, HCl.

Scheme 2[a]



[a] Conditions: (a) $ArNH_2$ (**5a**−**i**), NaAc, THF/H_2O; (b)MeI or EtI, NaH, DMF.

larly, compounds **2d**, **2g**, and **2i**−**2k** were prepared from reaction of **3a** with the corresponding 4-substituted *N*-Me-aniline **4b**−**4f** (Scheme 1). *N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (**2c**), 2-chloro-6,7-dimethoxy-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (**2s**), 2,6-dichloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (**2t**), and 2,7-dichloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (**2u**) were prepared similarly via reaction of aniline **4a** with 4-chloroquinazoline (**3b**), 2,4-dichloro-6,7-dimethoxyquinazoline (**3c**), 2,4,6-trichloroquinazoline (**3d**), and 2,4,7-trichloroquinazoline (**3e**), respectively. Substituted 2,4-dichloroquinazolines **3d**−**3e** were prepared from the corresponding substituted anthranilic acids via reaction with potassium cyanate, followed by treatment of the substituted quinazoline-2,4-diones with phosphorus oxychloride.[21] 4-(4-Methoxyphenoxy)quinazoline (**2v**) was prepared via reaction of **3b** with 4-methoxyphenol.[22] 2-Chloro-*N*-(4-methoxybenzyl)-*N*-methylquinazolin-4-amine (**2w**) was prepared via reaction of **3a** with *N*-methyl-4-methoxybenzylamine. 2-Chloro-*N*-(4-hydroxyphenyl)-*N*-methylquinazolin-4-amine (**2h**) was prepared from demethylation of compound **2b** via treatment with BBr_3.

2-Chloro-*N*-(4-methoxyphenyl)quinazolin-4-amine (**6a**) was prepared via reaction of **3a** with 4-methoxyaniline **5a**. Similarly, reaction of **3c** with **5a** produced 2-chloro-6,7-dimethoxy-*N*-(4-methoxyphenyl)quinazolin-4-amine (**6b**). Reaction of **6a** with ethyl iodide in the presence of sodium hydride produced 2-chloro-*N*-ethyl-*N*-(4-methoxyphenyl)quinazolin-4-amine (**2r**) (Scheme 2). Similarly, reaction of **3a** with substituted aniline **5b**−**5h** and 6-methoxypyridin-3-amine (**5i**) produced compounds **6c**−**6j**. Reaction of **6c**−**6j** with methyl iodide in the presence of sodium hydride produced compounds **2e**−**2f** and **2l**−**2q**.

HTS and SAR Studies. Compound **2a** was identified as an inducer of apoptosis from our cell- and caspase-based HTS assay[23] and found to have good activity in T47D cells with an EC_{50} value of 290 nM. The HTS assay also was used for SAR study of analogues of **2a** as described previously.[23] Compound **2a** has a large hydrophobic group at the 2-position of the quinazoline, with a ClogP value of 6.58, which was considered undesirable for a potential drug. We initiated the exploration of the SAR of **2a** via replacing the large hydrophobic group at the 2-position with smaller functional groups. Compounds **2b** and **2c**, with a Cl or H group at the 2-position, were found to be much more active than **2a** (Table 1). Compound **2b** was found to have an EC_{50} value of 2 nM in our caspase activation assay in T47D cells, which was about 3-fold and >100-fold more potent than **2c** and **2a**, respectively, suggesting that a small group at the 2-position was preferred for apoptosis inducing activity. In addition, compound **2b** and **2c** have ClogP values of 4.51 and 3.67, respectively, which are 2−3 units less than that of **2a** and are expected to have improved aqueous solubility.

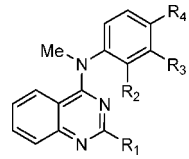
By maintaining the Cl group at the 2-position of quinazoline, we explored the SAR of substitution in the anilino ring. Compound **2d** was observed to be 100-fold less active than **2b**, indicating that the 4-OMe group was important for the apoptosis inducing activity of **2b**. The 4-OEt analogue **2e** and 4-Me analogue **2g** were >2-fold and >7-fold less active than **2b**, and the OPh analogue **2f** was not observed to be active in vitro up to 10000 nM, indicating that there was a size limited pocket at the 4-position of the anilino ring. The 4-OH analogue **2h** was >40-fold less active than **2b**, indicating that a hydrophilic group at the 4-position was less preferred than hydrophobic group. The 4-OCF_3 analogue **2i**, 4-Cl analogue **2j**, and 4-NO_2 analogue **2k** were >20-, > 30-, and >350-fold less active than **2b**, respectively, suggesting that electron withdrawing groups are not preferred. The pyridyl analogue **2q** was less active than **2b** but still was highly active in vitro with an EC_{50} value of 17 nM, indicating that a pyridyl group could be used to replace the phenyl group. Compound **2p** has a ClogP value of 3.84, which is almost one unit less than that of **2b** and might increase its aqueous solubility.

Compound **2l**, with an OMe group at the 3-position, was observed to be >10-fold less active than **2b**, indicating that the position of the 4-OMe group was important for in vitro activity. Compound **2m**, with the OMe group moved to the 2-position, was >2000-fold less active than **2b**, and even >20-fold less active than the nonsubstituted analogue **2d**, indicating that a 2-OMe group contributes negatively to activity. The 3,4-dimethoxy analogue **2n** was a potent compound and was only 4-fold less active than **2b**. The 3,4-methylenedioxy analogue **2o** was 3-fold less potent than **2n**. The 2,3-dimethoxy analogue **2p** was >20-fold less active than the 3-methoxy analogue **2l**, confirming that the 2-methoxy group contributed negatively to activity, probably due to some unfavorable interaction with its target.

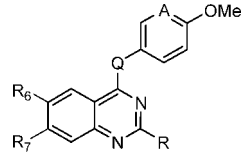
The SAR of the linker nitrogen was also explored. Compound **6a**, with no substitution at the linker nitrogen, was not active in our caspase activation assay up to 10000 nM and >5000-fold less active than **2b**, indicating that the methyl group is essential for the apoptosis inducing activity. Similarly, compound **6b** was not observed to be active in vitro up to 10000 nM. The *N*-ethyl analogue **2r** was about 20-fold less active than **2b**, suggesting a size limited pocket in that position. In addition, compound **2v**, with the *N*-Me linker in **2c** replaced by an O linker, was not active up to 10000 nM and >1000 fold less active than **2c**, confirming the importance of a small alkyl group in the linker for apoptosis inducing activity. The benzyl analogue **2w** was >30-fold less active than **2b**, indicating that a more rigid NMe linker was preferred over the flexible $NMeCH_2$ linker. The critical role of a small alkyl group at the linker nitrogen for the apoptosis inducing activity of **2b** and related 4-anilinoquinazolines could be due to the alkyl group causing a change in the conformation of the molecules or the alkyl group contributing an important hydrophobic interaction with its molecular target, or a combination of both.

We also explored the SAR of compounds with substitutions at the 6- and 7-positions of the quinazoline. The 6,7-dimethoxy

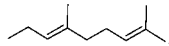
Table 1. SAR of 4-Arylaminoquinazolines in the Caspase Activation Assay



entry	R_1	R_2	R_3	R_4	EC_{50} (μM) [b] T47D	EC_{50} (μM) [b] HCT-116
2a	NHR[a]	H	H	OMe	0.29 ± 0.020	0.46 ± 0.098
2b	Cl	H	H	OMe	0.002 ± 0.0001	0.002 ± 0.0001
2c	H	H	H	OMe	0.006 ± 0.0002	0.008 ± 0.0001
2d	Cl	H	H	H	0.21 ± 0.007	0.30 ± 0.010
2e	Cl	H	H	OEt	0.005 ± 0.0002	0.005 ± 0.0003
2f	Cl	H	H	OPh	>10	>10
2g	Cl	H	H	Me	0.015 ± 0.001	0.021 ± 0.004
2h	Cl	H	H	OH	0.085 ± 0.003	0.12 ± 0.003
2i	Cl	H	H	OCF_3	0.049 ± 0.001	0.059 ± 0.003
2j	Cl	H	H	Cl	0.068 ± 0.002	0.072 ± 0.004
2k	Cl	H	H	NO_2	0.77 ± 0.07	1.4 ± 0.05
2l	Cl	H	OMe	H	0.025 ± 0.002	0.031 ± 0.002
2m	Cl	OMe	H	H	4.44 ± 0.071	>10
2n	Cl	H	OMe	OMe	0.009 ± 0.0001	0.015 ± 0.0001
2o	Cl	H	OCH_2O		0.031 ± 0.002	0.065 ± 0.005
2p	Cl	OMe	OMe	H	0.68 ± 0.017	1.11 ± 0.055



entry	R_6	R_7	R	Q	A	EC_{50} (μM) [b] T47D	EC_{50} (μM) [b] HCT-116
2q	H	H	Cl	NMe	N	0.017 ± 0.004	0.029 ± 0.007
2r	H	H	Cl	NEt	C	0.039 ± 0.005	0.049 ± 0.004
2s	OMe	OMe	Cl	NMe	C	0.59 ± 0.030	0.97 ± 0.14
2t	Cl	H	Cl	NMe	C	0.010 ± 0.003	0.013 ± 0.002
2u	H	Cl	Cl	NMe	C	0.30 ± 0.011	0.35 ± 0.028
2v	H	H	H	O	C	>10	>10
2w	H	H	Cl	$NMeCH_2$	C	0.068 ± 0.004	0.12 ± 0.006
6a	H	H	Cl	NH	C	>10	>10
6b	OMe	OMe	Cl	NH	C	>10	>10
colchicine	NA	NA	NA	NA	NA	0.009 ± 0.001	0.015 ± 0.004

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

analogue **2s** was about 300-fold less active than **2b**, indicating that substitutions at the 6,7-position of quinazoline are not preferred. Interestingly, the 6-chloro analogue **2t** was only 5-fold less active than **2b**, while the 7-Cl analogue **2u** was >100-fold less active than **2b**, suggesting that substitution at the 6-position was more tolerated than the 7-position.

The activities of compounds tested toward the human nonsmall cell lung cancer cell line HCT-116 were roughly parallel to their activity toward T47D cells. Compound **2b** was also highly active in HCT-116 cells with an EC_{50} value of 2 nM. Compounds **2f**, **2v**, **6a**, and **6b** were not observed to be active in T47D or in HCT-116 cells.

Selected compounds were also tested by the traditional growth inhibition assay to confirm that the active compounds can inhibit cancer cell growth. The growth inhibition assays in T47D and HCT-116 cells were run in a 96-well microtiter plate as described previously.[23] In brief, T47D and HCT-116 cells were exposed continuously to test compound for 48 h at 37 °C. CellTiter-Glo reagent (Promega) was added, the samples were mixed by agitation, incubated at room temperature for 10−15 min, and then read using a luminescent plate reader (model Spectrafluor Plus Tecan Instrument). The GI_{50} was defined as 50% inhibition of cell proliferation. GI_{50} values, in comparison with their EC_{50} values, are summarized in Table 2.

Compound **2b** was found to be the most potent inhibitor of cancer cell growth among the compounds tested, with GI_{50} of 1−2 nM in T47D and HCT-116 cells. The compounds that are more active in the apoptosis induction assay, as measured by caspase activation, also are more potent in the growth inhibition assay, indicating a good correlation between our cell-based apoptosis induction assay and the traditional cell growth inhibition assay. Additionally, P388 mouse leukemia cells that overexpress the ABC transporter Pgp-1 (P388-ADR)[24] were tested for resistance to compound **2b** in comparison with the tubulin interacting agents vinblastine and docetaxel. Compound **2b** displayed similar growth inhibition in P388 and P388-ADR cell lines with IC_{50} values of 2.1 and 2.2 nM, respectively (Table 3). Vinblastine was less active in the P388-ADR cell line, with a shift in IC_{50} values from 0.6 to 27 nM. Docetaxel was also less active in

Table 2. Comparison of Caspase Activation Activity and Inhibition of Cell Proliferation Activity of 4-arylaminoquinazolines

	T47D		HCT116	
	EC_{50}[a] (μM)	GI_{50}[b](μM)	EC_{50}[a] (μM)	GI_{50}[b](μM)
2a	0.29 ± 0.020	0.85 ± 0.16	0.46 ± 0.098	0.35 ± 0.049
2b	0.002 ± 0.0001	0.001 ± 0.0005	0.002 ± 0.0001	0.002 ± 0.0001
2q	0.017 ± 0.004	0.037 ± 0.002	0.029 ± 0.007	0.040 ± 0.002

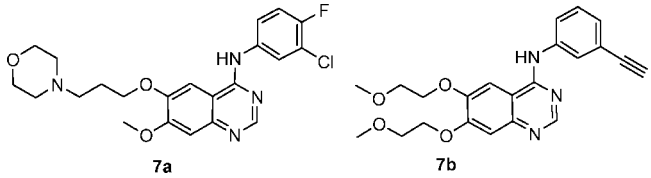
[a] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM). [b] Data are the mean of three experiments and are reported as mean ± standard error of the mean (SEM).

Table 3. Cytotoxicity of Compound **2b** in Non-drug-resistant P388 Cells and in Cells Overexpressing the ABC Transporter Pgp-1 (P388-ADR)

	IC_{50} (nM)[a]		
cell line	**2b**	vinblastine	docetaxel
P388	2.1 ± 0.2	0.6 ± 0.2	2 ± 0.7
P388/ADR (MDR-1)	2.2 ± 0.2	27 ± 60	277 ± 117

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

Chart 2



the Pgp-1 overexpressing cell line, shifting its IC_{50} values from 2 to 277 nM. These results show that different from that of vinblastine and docetaxel, the activity of compound **2b** is not affected by overexpression of Pgp-1, suggesting that **2b** might not be a substrate for Pgp-1.

We noted that compound **2b** and several of the well-known kinase inhibitors, including compounds **7a** (Iressa) and **7b** (Tarceva) (Charts 1 and 2), are all substituted 4-anilinoquinazolines. 4-Anilinoquinazolines have been extensively explored as kinase inhibitors,[25,26] and it has been reported that substitutions at the 2-position or the linker nitrogen of 4-anilinoquinazolines are not preferred, while substitutions at the 6,7-positions are beneficial for kinase inhibiting activity.[27] For example, methylation of the *N*-linking nitrogen of 4-anilinoquinazoline and 4-(3-bromoanilino)-6,7-dimethoxyquinazoline led to 300-fold and 6000-fold drop of EGFR kinase inhibiting activity.[28,29] In addition, it was reported that introduction of a Cl group to the 2-position of the quinazoline ring of 4-(3-chloroanilino)-6,7-dimethoxyquinazoline resulted in a 10000-fold loss of activity, and 4-(3-chloroanilino)quinazoline was >100-fold less active than 4-(3-chloroanilino)-6,7-dimethoxyquinazoline as EGFR kinase inhibitor.[30] Therefore, the SAR of the 2-position and linker nitrogen, as well as substitutions at the 6- and 7-positions of 4-anilinoquinazolines as apoptosis inducers was significantly different from the reported SAR of 4-anilinoquinazolines as EGFR kinase inhibitors.

One of the most striking differences in the SAR of EGFR kinase inhibitors and the apoptosis inducers reported here is with the methyl group at the linker nitrogen. The methyl group was found to be critical for the apoptosis inducing activity of 4-anilinoquinazolines, while methylation at the linker nitrogen of anilinoquinazolines have been reported to be highly disfavored for their activity as EGFR kinase inhibitors. It has been reported that *N*-methylation caused the anilino ring to move completely out of the plane.[27]The critical role of the methyl group in **2b** suggested that a nonplanar structure of the anilino group vs the quinazoline ring was essential for the apoptosis inducing activity of these compounds. The differences in SAR suggest that these compounds should interact with different targets, which was confirmed in our target identification studies. Applying a photoaffinity agent, the molecular target of compound **2b** and related analogues has been identified as β-tubulin.[31]

Several crystal structures of 4-anilinoquinazoline-based inhibitors binding to kinases have been determined. These structures showed that the quinazoline ring binds to the hinge region and the N1 of the quinazoline functions as a hydrogen acceptor and contributes to a hydrogen bonding, and the NH group in the anilino group does not contribute to hydrogen bonding.[32,33] Interestingly, 4-(3-hydroxyanilino)-6,7-dimethoxyquinazoline was found to bind to CDK2 with the anilino group essentially coplanar with the quinazoline ring system, while 4-(3-methylsulfanylanilino)-6,7-dimethoxyquinazoline was found to bind to p38 kinase, with the anilino group out of plane of the quinazoline ring system, indicating that structurally similar 4-anilinoquinazolines can bind to different kinases with different conformations.[34] Our SAR has shown that the methyl group at the linker nitrogen of 4-anilinoquinazolines is essential for the apoptosis inducing activity, and the SAR of 4-anilinoquinazolines as EGFR kinase inhibitors has shown that *N*-methylation of the linker nitrogen is highly undesirable due to noncoplanarity of the two rings. However, because 4-anilinoquinazolines and 4-arylaminoquinazolines have been found to bind to many different kinases, including c-Src/Abl,[26b] aurora A and B,[26c] CDK2, and P38,[34] some of these inhibitors might tolerate a *N*-Me at the linker nitrogen. In fact, it has been reported that *N*-methyl-4-anilino-6,7-dimethoxyquinazoline is a potent inhibitor (IC_{50} of 500 nM) of colony stimulating factor-1 receptor (CSF-1R) tyrosine kinase inhibitor, while the corresponding 4-anilino-6,7-dimethoxyquinazoline is not active in the same assay up to 50000 nM.[35] Therefore, it might be possible to combine the SAR of 4-anilinoquinazolines as apoptosis inducers with the SAR for kinase inhibitors to prepare compounds that are active as both apoptosis inducers and kinase inhibitors.[36] These multifunctional ligands might have advantages over single functional ligands for the treatment of cancer because combination treatments for cancer are commonly practiced and have been found in general to be more effective than single agent treatment.

Characterization of Compound 2b. Compound **2b** has been found to induce apoptosis in tumor cells as indicated by a variety of markers of apoptosis.[31] For example, **2b** treatment induced DNA fragmentation in several cells, including prostate carcinoma cells Du145 and 22Rv1, colorectal carcinoma cells Caco-2, and breast carcinoma cells MDA-MB-231. Induction of DNA fragmentation by **2b** was accompanied by caspase-3 activation and PARP cleavage, two other markers of apoptosis.[31] Compound **2b** was found to inhibit tubulin polymerization, with an IC_{50} of less than 400 nM, which is similar to the potency of colchicine (500 nM) under the same assay conditions. Similar to compound **2b**, colchicine was found to be a potent apoptosis inducer, with an EC_{50} value of 9 nM in our assay (Table 1). These data corroborate with the target identification that

inhibition of tubulin polymerization is the primary mechanism of action of **2b** for induction of apoptosis. The potential binding site of **2b** at tubulin was determined by using a fluorescent labeled colchicine in a tubulin binding competition assay. Compound **2b** was found to compete with fluorescent labeled colchicine for binding to tubulin, with a potency similar to that of nonlabeled colchicine, indicating that **2b** binds at or near the colchicine site of tubulin.

Importantly, compound **2b** was found to be highly active in the MX-1 breast cancer model, a model that is not specific for certain agents and is widely used for testing common chemotherapeutics such as camptothecin derivatives[37] and paclitaxel derivatives.[38] The MX-1 in vivo experiment was performed as described previously.[20b] Compound **2b** inhibited tumor growth dose dependently and produced >95% tumor growth inhibition with once weekly intravenous (iv) dosing at 10 mg/kg (Figure 1A) and is well tolerated with maximum body weight decrease of <10% for the 10 mg/kg dose. The maximum tolerated dose (MTD) of **2b** was determined to be 25 mg/kg when dosed once weekly, providing a good therapeutic index (TI) of 2.5. Compound **2b** also produced 90% tumor growth inhibition in the MX-1 model when dosed once every day for 5 days for 2 weeks at a dose of 2.5 mg/kg (Figure 1B). In addition, **2b** significantly inhibited the growth of human PC-3 prostate cancer xenografts in nude mice ($P = 0.003$, Figure 1C).

Significantly, compound **2b** was found to have high blood−brain barrier (BBB) penetration. 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 **2b** after iv dosing was determined via LC-MS/MS to be about 30 (Figure 2), suggesting that compound **2b** and related analogues might have good anticancer activity against brain tumors, for which there are currently few treatment options.[39] The high BBB penetration of **2b** was predicted based on its structure, which has a low molecular weight of 300, four hydrogen bond acceptors, and no hydrogen bond donor, features that are known to favor brain exposure of small molecules.[40] In comparison, it was reported that the cerebrospinal fluid (CSF) AUC was 7% of that of plasma for the structurally related 4-anilinoquinazloline kinase inhibitor **7b**,[41] which has three more hydrogen bond acceptors and one more hydrogen bond donator than that of **2b**.

In conclusion, using a cell- and caspase-based HTS assay, we discovered a series of 4-anilinoquinazolines as potent apoptosis inducers. The SAR of 4-anilinoquinazolines as apoptosis inducers was different from the reported SAR of 4-anilinoquinazolines as EGFR kinase inhibitors. Through SAR studies, compound **2b** has been identified as a potent apoptosis inducer with excellent activity in the MX-1 and PC-3 mouse xenograft cancer models. The primary molecular target of **2b** appears to be tubulin. Additional SAR studies and lead optimization of **2b** will be presented in future publications.

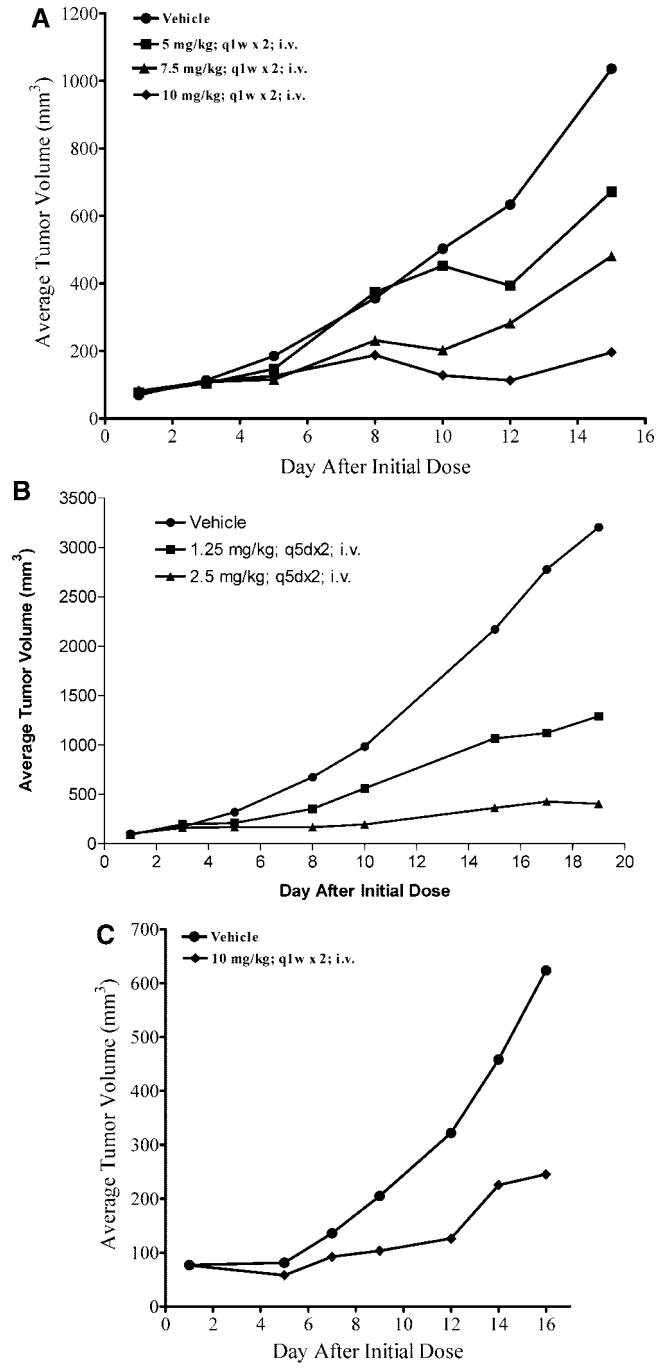
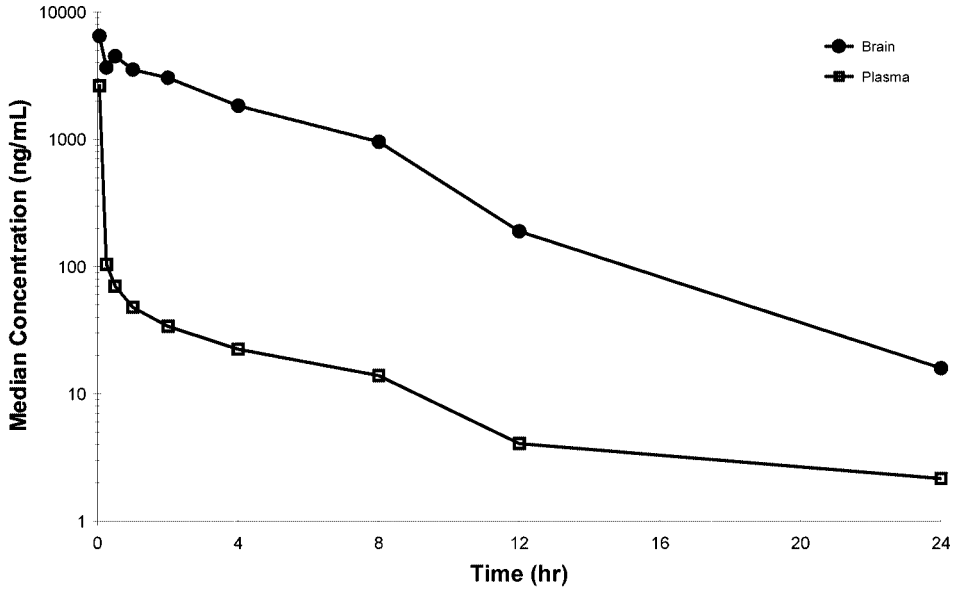


Figure 1. Compound **2b** inhibited the growth of established (~100 mm^3) MX-1 (A) and (B), and PC-3 (C) tumor xenografts in Crl:Nu/Nu-nuBR mice. Compound **2b** was dosed iv once weekly for two weeks at 5, 7.5, or 10 mg/kg (MX-1 model, A), once a day for 5 days for two weeks at 1.25 or 2.5 mg/kg (MX-1 model, B), and once weekly for two weeks at 10 mg/kg (PC-3 model, C), respectively.

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 ^1H 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. 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 mm × 50 mm 5 u column; HPLC purity was performed using 4.6 mm × 150 mm Xterra C18 5 u column. Both LCMS and LC were reverse phase with a acetonitile/water containing 0.01% v/v TFA gradient at a flow rate of 0.5 mL/min. SFC was run using a normal phase Phenomenex Luna 5 u silica 4.6 mm × 250 mm column. Mobile phase was liquid CO_2 with methanol as the modifying solvent. A gradient was used in which



Species	Dose (mg/kg)	Brain AUC	Plasma AUC
C57BL/6 Mice	2.5	20563	669

Figure 2. Concentration of compound **2b** in brain and plasma after a single iv dose of 2.5 mg/kg in mice.

the concentration of methanol was ramped from 5% to 50% over a course of 12 min with a 200 bar column back-pressure at 40 °C at a flow rate of 3 mL/min. Human breast T47D and human small cell lung HCT-116 tumor cell lines were purchased from the American Type Culture Collection (Manasas, VA). Compound **2a** was obtained commercially (Tripos, St. Louis MO), and the structure was confirmed by ^{1}H NMR and MS. Compound **2v** was prepared from 4-chloroquinazoline and 4-methoxyphenol according to reported procedure.[22]

2-Chloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (2b). A solution of 2,4-dichloroquinazoline (**3a**, 300 mg, 1.51 mmol) and *N*-methyl-4-methoxyaniline (**4a,** 248 mg, 1.81 mmol) in 5 mL isopropanol with a drop of concentrated HCl was stirred at room temperature for 8 h. The mixture was filtered, and the solid was washed with isopropanol, then dried under vacuum to give **2b** as a white powder (260 mg, 87%). ^{1}H NMR ($CDCl_3$) 8.66 (dd, J = 8.4 and 0.9, 1H), 7.75 (ddd, J = 8.1, 7.5 and 0.9, 1H), 7.26−7.19 (m, 3H), 7.14 (ddd, J = 8.1, 7.5 and 0.9, 1H), 7.06 (dd, J = 6.9 and 2.4, 2H), 6.75 (d, J = 8.7, 1H), 3.91 (s, 3H), 3.81 (s, 3H). Anal. ($C_{16}H_{16}ClN_3O$) C, H, N.

The following compounds were prepared by a procedure similar to that described for the preparation of compound **2b**.

***N*-(4-Methoxyphenyl)-*N*-methylquinazolin-4-amine (2c).** Compound **2c** was prepared from 4-chloroquinazoline and **4a** and was isolated as a white powder (79%). ^{1}H NMR ($CDCl_3$) 8.81 (s, 1H), 7.81 (d, J = 8.1, 1H), 7.57 (ddd, J = 8.1, 5.4 and 2.7, 1H), 7.09−7.14 (m, 2H), 7.03−7.06 (m, 2H), 6.9−6.93 (m, 2H). Anal. ($C_{16}H_{15}C_{l2}N_3O$) C, H, N.

2-Chloro-*N*-methyl-*N*-phenylquinazolin-4-amine (2d). Compound **2d** was prepared from **3a** and *N*-methylaniline and was isolated as a white powder (80%). ^{1}H NMR ($CDCl_3$) 7.76 (dd, J = 8.7 and 1.5, 1H), 7.56 (ddd, J = 8.1, 6.6 and 1.5, 1H), 7.46−7.35 (m, 3H), 7.24−7.20 (m, 2H), 6.98 (ddd, J = 8.7, 6.6 and 1.5, 1H), 6.90 (dd, J = 8.7 and 1.5, 1H), 3.65 (s, 3H). Anal. ($C_{15}H_{12}ClN_3 \cdot 0.2H_2O$) C, H, N.

2-Chloro-*N*-(4-methylphenyl)-*N*-methylquinazolin-4-amine (2g). Compound **2g** was prepared from **3a** and *N*-methyl-4-methylaniline and was isolated as a white powder (84%). ^{1}H NMR ($CDCl_3$) 8.69 (d, J = 8.4, 1H), 7.75 (dd, J = 8.1 and 7.5, 1H), 7.39 (d, J = 7.8, 2H), 7.25 (d, J = 7.8, 2H), 7.13 (d, J = 8.2, 1H), 6.74 (d, J = 8.7, 1H), 3.81 (s, 3H), 2.49 (s, 3H). HRMS calcd for $C_{16}H_{14}ClN_3$ (M + H^+), 284.0954; found, 284.0952.

2-Chloro-*N*-methyl-*N*-(4-trifluoromethoxyphenyl)quinazolin-4-amine (2i). Compound **2i** was prepared from **3a** and *N*-methyl-4-trifluoromethoxyaniline and was isolated as a white powder (44%). ^{1}H NMR ($CDCl_3$) 7.93 (dd, J = 8.4 and 0.6, 1H), 7.61(ddd, J = 8.4, 4.5 and 1.2, 1H), 7.29−7.22 (m, 4H), 7.06 (ddd, J = 8.4, 4.5 and 1.2, 1H), 6.91 (d, J = 8.7, 1H), 3.65 (s, 3H). Anal. ($C_{16}H_{11}ClF_3N_3O$) C, H, N.

2-Chloro-*N*-(4-chlorophenyl)-*N*-methylquinazolin-4-amine (2j). Compound **2j** was prepared from **3a** and *N*-methyl-4-chloroaniline, and was isolated as a white powder (50%). ^{1}H NMR ($CDCl_3$) 8.66 (d, J = 8.4, 1H), 7.78 (ddd, J = 8.1, 7.5 and 2.4, 1H), 7.57 (d, J = 8.7, 2H), 7.28 (d, J = 8.7, 2H), 7.19 (ddd, J = 8.1, 7.5 and 2.4, 1H), 6.79 (d, J = 8.4, 1H), 3.83 (s, 3H). Anal. ($C_{15}H_{11}C_{l2}N_3$) C, H, N.

2-Chloro-*N*-(4-nitrophenyl)-*N*-methylquinazolin-4-amine (2k). Compound **2k** was prepared from **3a** and *N*-methyl-4-nitroaniline and was isolated as a white powder (12%). ^{1}H NMR ($CDCl_3$) 8.24 (d, J = 8.7, 2H), 7.81 (dd, J = 8.1 and 2.4, 1H), 7.68 (ddd, J = 8.1, 7.5 and 2.4, 1H), 7.28 (d, J = 8.7, 2H), 7.18 (ddd, J = 8.1, 7.5 and 2.4, 1H), 7.07 (d, J = 7.8, 1H), 3.75 (s, 3H). HRMS calcd for $C_{15}H_{11}ClN_4O_2$ (M + H^+), 315.0649; found, 315.0652.

2-Chloro-*N*-(4-methoxybenzyl)-*N*-methylquinazolin-4-amine (2w). Compound **2w** was prepared from **3a** and *N*-methyl-4-methoxybenzylamine, and was isolated as a white powder (60%). ^{1}H NMR ($CDCl_3$) 7.93 (dd, J = 8.4 and 1.2, 1H), 7.78 (dd, J = 8.4 and 1.5, 1H), 7.68 (ddd, J = 8.4, 7.5 and 1.5, 1H), 7.34−7.26 (m, 3H), 6.96−6.92 (m, 2H), 4.94 (s, 2H), 3.83 (s, 3H), 3.31 (s, 3H). HRMS calcd for $C_{17}H_{16}ClN_3O$ (M + H^+), 314.1055; found, 314.1053.

2-Chloro-*N*-(4-methoxyphenyl)quinazolin-4-amine (6a). Compound **6a** was prepared from **3a** and 4-methoxyaniline (**5a**) and was isolated as an off-white powder (77%). ^{1}H NMR ($CDCl_3$) 10.25 (s, 1H), 8.58 (d, J = 8.4, 1H), 7.88 (t, J = 7.2, 1H), 7.71−7.63 (m, 4H), 7.03−6.99 (m, 2H), 3.79 (s, 3H). Anal. ($C_{15}H_{12}ClN_3O \cdot 0.8H_2O$) C, H, N.

2-Chloro-6,7-dimethoxy-*N*-(4-methoxyphenyl)quinazolin-4-amine (6b). Compound **6b** was prepared from 2,4-dichloro-6,7-dimethoxyquinazoline (**3c**) and **5a** and was isolated as an off-white

powder (10%). ^{1}H NMR ($CDCl_3$) 8.10 (s, 1H), 7.70−7.68 (m, 2H), 7.23 (s, 1H), 6.96−6.94 (m, 2H), 4.07 (s, 3H), 3.99 (s, 3H), 3.85 (s, 3H). HRMS calcd for $C_{17}H_{15}ClN_3O_3$ (M + Na^+), 368.0778; found, 368.0781.

2-Chloro-*N*-(6-methoxypyridin-3-yl)quinazolin-4-amine (6i). A mixture of **3a** (61 mg, 0.31 mmol), 6-methoxypyridin-3-amine (40 mg, 0.32 mmol), and sodium acetate (38 mg, 0.46 mmol) in 3 mL of solvent (THF:water/1:1) was stirred at 60 °C for 45 min. The reaction mixture was diluted with 25 mL of ethyl acetate, and it was washed with saturated NaCl, dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified by chromatography (40% ethyl acetate/hexanes) on silica gel to give **6i** as a solid (86 mg, 98%). ^{1}H NMR ($CDCl_3$) 8.38 (d, J = 3.0, 1H), 8.07 (dd, J = 8.7 and 3.0, 1H), 7.89−7.79 (d, J = 8.4, 1H), 7.86 (m, 2H), 7.59−7.53 (m, 2H), 6.84 (d, J = 8.7, 1H), 3.96 (s, 3H).

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

2-Chloro-6,7-dimethoxy-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (2s). Compound **2s** was prepared from **3c** and **4a** and was isolated as a white solid (60%). ^{1}H NMR ($CDCl_3$) 8.19 (s, 1H), 7.32−7.26 (m, 2H), 7.09−7.07 (m, 2H), 6.16 (s, 1H), 4.03 (s, 3H), 3.87 (s, 3H), 3.75 (s, 3H), 3.32 (s, 3H). Anal. ($C_{18}H_{18}ClN_3O_3 \cdot 0.6H_2O$) C, H, N.

2,6-Dichloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (2t). Compound **2t** was prepared from 2,4,6-trichloroquinazoline (**3d**) and **4a** and was isolated as a yellow solid (30%). ^{1}H NMR ($CDCl_3$) 7.66 (d, J = 8.7, 1H), 7.49 (dd, J = 8.7 and 2.1, 1H), 7.18−7.12 (m, 2H), 7.02−6.96 (m, 2H), 6.78 (dd, J = 2.1 and 0.6, 1H), 3.88 (s, 3H), 3.61 (s, 3H). Anal. ($C_{16}H_{13}Cl_2N_3O$) C, H, N.

2,7-Dichloro-*N*-(4-methoxyphenyl)-*N*-methylquinazolin-4-amine (2u). Compound **2u** was prepared from 2,4,7-trichloroquinazoline (**3e**) and **4a** and was isolated as a light-yellow solid (40%). ^{1}H NMR ($CDCl_3$) 7.70 (d, J = 2.4, 1H), 7.16−7.11 (m, 2H), 6.98−6.92 (m, 3H), 6.80 (d, J = 9.3, 1H), 3.86 (s, 3H), 3.60 (s, 3H). Anal. ($C_{16}H_{13}Cl_2N_3O$) C, H, N.

2-Chloro-*N*-(6-methoxypyridin-3-yl)-*N*-methylquinazolin-4-amine (2q). To a solution of **6i** (19.4 mg, 0.068 mmol) in 1 mL of DMF cooled at 0 °C was added methyl iodide (100 μL, 1.61 mmol), followed by sodium hydride (60% oil suspension, 5 mg, 0.13 mmol). The mixture was stirred at 0 °C for 1 h, then allowed to warm to room temperature and stirred for 1 h. The reaction mixture was quenched by adding 50 μL of water and diluted with 25 mL of ethyl acetate. The solution was washed with water (25 mL × 3), saturated NaCl, dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by chromatography (20% ethyl acetate/hexanes) to give **2q** as a solid (14.3 mg, 70%). ^{1}H NMR ($CDCl_3$) 8.06 (d, J = 2.7, 1H), 7.57−7.79 (m, 1H), 7.60 (ddd, J = 8.1, 6.6 and 1.2, 1H), 7.44 (dd, J = 8.7 and 2.7, 1H), 7.09 (ddd, J = 8.1, 6.6 and 1.2, 1H), 6.99−7.02 (m, 1H), 6.82 (dd, J = 8.7 and 0.6, 1H), 3.97 (s, 3H), 3.61 (s, 3H). Anal. ($C_{15}H_{13}ClN_4O \cdot 0.2H_2O$) C, H, N.

The following compounds were prepared by a procedure similar to that described for the preparation of compound **2q**.

2-Chloro-*N*-(4-ethoxyphenyl)-*N*-methylquinazolin-4-amine (2e). Compound **2e** was prepared from **6c** and methyl iodide and was isolated as a solid (28%). ^{1}H NMR ($CDCl_3$) 7.73 (m, 1H), 7.55 (m, 1H), 7.12 (m, 2H), 7.00 (m, 1H), 6.93 (m, 3H), 4.07 (q, J = 7.2, 2H), 3.61 (s, 3H), 1.46 (t, J = 7.2, 3H). Anal. ($C_{17}H_{16}ClN_3O$) C, H, N.

2-Chloro-*N*-(4-phenoxyphenyl)-*N*-methylquinazolin-4-amine (2f). Compound **2f** was prepared from **6d** and methyl iodide and was isolated as a solid (41%). ^{1}H NMR ($CDCl_3$) 7.74−7.77 (m, 1H), 7.59 (ddd, J = 8.4, 6.6 and 1.5, 1H), 7.36−7.42 (m, 2H), 7.10−7.20 (m, 3H), 7.03−7.10 (m, 5H), 6.97−7.00 (m, 1H), 3.64 (s, 3H). HRMS calcd for $C_{21}H_{16}ClN_3O$ (M + H^+), 362.1060; found, 362.1062.

2-Chloro-*N*-(3-methoxyphenyl)-*N*-methylquinazolin-4-amine (2l). Compound **2l** was prepared from **6e** and methyl iodide and was isolated as a solid (60%). ^{1}H NMR ($CDCl_3$) 7.74−7.76 (m, 1H), 7.57 (ddd, J = 8.4, 6.0 and 1.8, 1H), 7.32 (t, J = 7.8, 1H), 6.98−7.03 (m, 2H), 6.89 (dd, J = 8.1 and 2.4, 1H), 6.75−6.81 (m, 2H), 3.65 (s, 3H), 3.37 (s, 3H). Anal. ($C_{16}H_{14}ClN_3O$) C, H, N.

2-Chloro-*N*-(2-methoxyphenyl)-*N*-methylquinazolin-4-amine (2m). Compound **2m** was prepared from **6f** and methyl iodide and was isolated as a solid (72%). ^{1}H NMR ($CDCl_3$) 7.72 (d, J = 8.1, 1H), 7.54 (ddd, J = 8.4, 6.6 and 1.5, 1H), 7.20 (dd, J = 8.4 and 1.8, 1H), 6.87−7.04 (m, 4H), 3.67 (s, 3H), 3.56 (s, 3H). Anal. ($C_{16}H_{14}ClN_3O \cdot 0.7H_2O$) C, H, N.

2-Chloro-*N*-(3,4-dimethoxyphenyl)-*N*-methylquinazolin-4-amine (2n). Compound **2n** was prepared from **6g** and methyl iodide and was isolated as a solid (54%). ^{1}H NMR ($CDCl_3$) 7.72−7.75 (m, 1H), 7.57 (ddd, J = 8.4, 6.6 and 1.5, 1H), 7.01 (ddd, J = 8.7, 6.9 and 1.2, 1H), 6.88−6.96 (m, 2H), 6.73−6.81 (m, 2H), 3.94 (s, 3H), 3.80 (s, 3H), 3.63 (s, 3H). HRMS calcd for $C_{17}H_{16}ClN_3O_2$ (M + Na^+), 352.0829; found, 352.0832.

2-Chloro-*N*-(3,4-methylenedioxyphenyl)-*N*-methylquinazolin-4-amine (2o). Compound **2o** was prepared from **6j** and methyl iodide and was isolated as a solid (66%). ^{1}H NMR ($CDCl_3$) 7.73−7.76 (m, 1H), 7.58 (m, 1H), 7.07 (m, 2H), 6.82 (d, J = 8.4, 1H), 6.72 (m, 1H), 6.68 (m, 1H), 6.06 (s, 2H), 3.59 (s, 3H). HRMS calcd for $C_{17}H_{16}ClN_3O_2$ (M + H^+), 314.0696; found, 314.0755.

2-Chloro-*N*-(2,3-dimethoxyphenyl)-*N*-methylquinazolin-4-amine (2p). Compound **2p** was prepared from **6h** and methyl iodide and was isolated as a solid (71%). ^{1}H NMR ($CDCl_3$) 7.74 (d, J = 8.4, 1H), 7.53−7.59 (m, 1H), 7.12 (t, J = 8.4, 1H), 6.94−7.01 (m, 3H), 6.87 (dd, J = 8.1 and 1.5, 1H), 3.89 (s, 3H), 3.56 (s, 3H). Anal. ($C_{17}H_{16}ClN_3O_2 \cdot 0.3H_2O$) C, H, N.

2-Chloro-*N*-ethyl-*N*-(4-methoxyphenyl)quinazolin-4-amine (2r). Compound **2r** was prepared from **6a** and ethyl iodide and was isolated as a solid (58%). ^{1}H NMR ($CDCl_3$) 7.69−7.72 (m, 1H), 7.53 (ddd, J = 8.1, 6.9 and 1.5, 1H), 7.09−7.14 (m, 2H), 6.94−6.70 (m, 3H), 6.83−6.87 (m, 1H), 4.13 (q, J = 7.2, 2H), 3.87 (s, 1H), 1.30 (t, J = 6.9, 3H). Anal. ($C_{17}H_{16}ClN_3O$) C, H, N.

2-Chloro-*N*-(4-hydroxyphenyl)-*N*-methylquinazolin-4-amine (2h). To a solution of **2b** (100 mg, 0.33 mmol) in 30 mL dichloromethane cooled at −20 °C was added slowly 60 μL of BBr_3 (0.67 mmol). The reaction mixture was stirred at −20 °C for 2 h. It was warmed to room temperature and stirred for another 2 h. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with cold 5% sodium bicarbonate. The organic phase was dried and concentrated. The residue was purified by chromatography using ethyl acetate and hexane (1:3) to give **2h** as a solid (57 mg, 57%). ^{1}H NMR ($CDCl_3$) 7.65−7.56 (m, 2H), 7.04−6.87 (m, 5H), 3.59 (s, 3H). HRMS calcd for $C_{15}H_{12}ClN_3O$ (M + H^+), 286.0747; found, 286.0747.

Supporting Information Available: Table of elemental analysis and HPLC data for the targeted compounds **2b**−**2w** and **6a**−**6b**. Also included are experimentals for intermediates **6c**−**6h** and **6j**, procedures for caspase activation assay, growth inhibition assay, tubulin inhibition assay, colchicine binding competition assays as well as MX-1 and PC-3 tumor model studies of **2b**, and determination of brain/plasma AUC ratio of **2b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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