Identification of Potent Water Soluble Purine-Scaffold Inhibitors of the Heat Shock Protein 90

Huazhong He,[†,¶] Danuta Zatorska,[†,¶] Joungnam Kim,[†,¶] Julia Aguirre,[†] Laura Llauger,[†] Yuhong She,[†] Nian Wu,[†] Robert M. Immormino,[‡] Daniel T. Gewirth,[‡,⊥] and Gabriela Chiosis*[,†,§]

Department of Medicine and Program in Molecular Pharmacology and Chemistry, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, and Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Received August 13, 2005

Hsp90 is a chaperone protein that allows cancer cells to tolerate the many components of dysregulated pathways. Its inactivation may result in targeting multiple molecular alterations and, thus, in reverting the transformed phenotype. The PU-class, a purine-scaffold Hsp90 inhibitor series, has been reported to be potent and selective against Hsp90 both in vitro and in vivo models of cancer. Here, a series of this class was synthesized and evaluated as inhibitors of the chaperone. The structure−activity relationship and selectivity for tumor Hsp90 of compounds within the series is presented. The study identifies water soluble derivatives (>5 mM in PBS pH 7.4) of nanomolar potency ($IC_{50} \sim 50$ nM) in cellular and animal models of cancer. Binding affinities of these compounds for Hsp90 correlate well with their biological activities. When administered in vivo to mice bearing MDA-MB-468 human breast cancer xenografted tumors, these agents result in pharmacologically relevant concentrations and, accordingly, in modulation of Hsp90−client proteins in tumors.

Introduction

A recent trend in cancer therapy has been to develop agents that "target" a single molecular alteration. As most cancers are a result of multiple transformation-specific regulatory alterations, targeting one abnormality may be insufficient in reversing the transformed phenotype. Chaperones are proteins that allow cancer cells to tolerate the components of dysregulated pathways that otherwise would be lethal; thus, their inactivation may result in targeting multiple molecular alterations.[1] One such chaperone with important roles in maintaining cell-specific transformation is the heat shock protein 90 (Hsp90).[2] Its biological role is mediated by the ability of the chaperone to interact with client substrates. Binding and release of Hsp90 substrates is regulated by the activity of the N-terminal ATPase domain, which binds and hydrolyses ATP to mediate a series of association−dissociation cycles between Hsp90 and client proteins.[3] The activity of Hsp90 is further regulated by binding of cochaperones which promote the interconversion of the ATP- and ADP-bound states and modulate the formation of client-specific complexes.[4] Although Hsp90 is highly expressed in both normal and transformed cells, its usefulness as a therapeutic target results from its distinctive activation in normal and stressed environments. Recent evidence suggests that in a number of tumor cell lines, Hsp90 may be exclusively complexed with cochaperones in a state of high affinity for ATP/ADP or ligands of this regulatory pocket (i.e., ATPase inhibitor drugs), whereas in normal tissues, Hsp90 may exist primarily in a latent, uncomplexed, low-affinity state.[5] The shift in equilibrium between the "latent" and "activated" states may be dictated by the amount of "stress" on the system, i.e., mutated and dysregulated proteins, hypoxia, low nutrient concentration environment, in other words, the amount and degree of transformation in the cells.[2d] These suggest that is possible to interfere with Hsp90 function (i.e., by inhibiting its ATPase activity) and disrupt only critical chaperone functions in a transformed cell without affecting normal cell functions. Therefore, Hsp90 ATPase inhibitor concentrations may be identified that will affect cancer cells without being toxic to normal cells. Moreover, the ATP-regulatory pocket of Hsp90 is unique. While bound to Hsp90 the nucleotide adopts a bent shape found only in ATPases belonging to the GHKL family (G = DNA gyrase subunit B, H = Hsp90, K = histidine kinases, and L = MutL). These enzymes share the same left-handed $\beta-\alpha-\beta$ fold, called the Bergerat fold. This fold is not observed in the high-affinity binding sites of kinases (which adopt a P-loop motif) or in other chaperones such as Hsp70 (which adopts an actin fold).[6] These observations suggest that it is possible to discover compounds of high selectivity for Hsp90 by identifying those that specifically bind to Hsp90 via the N-terminal ATPase pocket. Making use of this specific fold, we were able to design a class of purine-scaffold derivatives (PU-class, Figure 1) with Hsp90 inhibitory activities.[7] The first synthesized derivative of this class, **1** (PU3, see Figure 1), bound Hsp90 with moderate affinity and exhibited biological activities in the 50 μM concentration range.[8] Further efforts focused at improving the potency of this agent have led

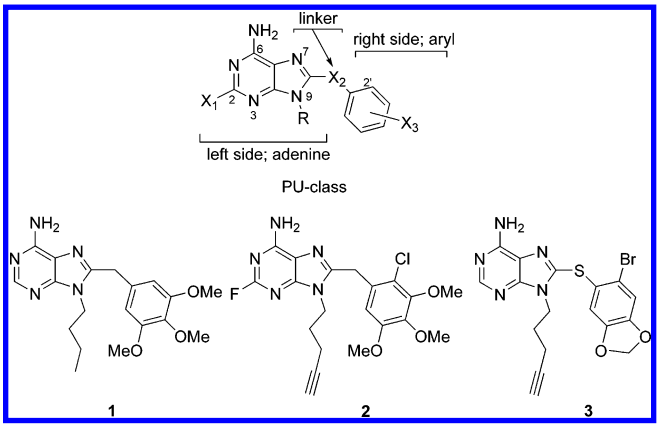
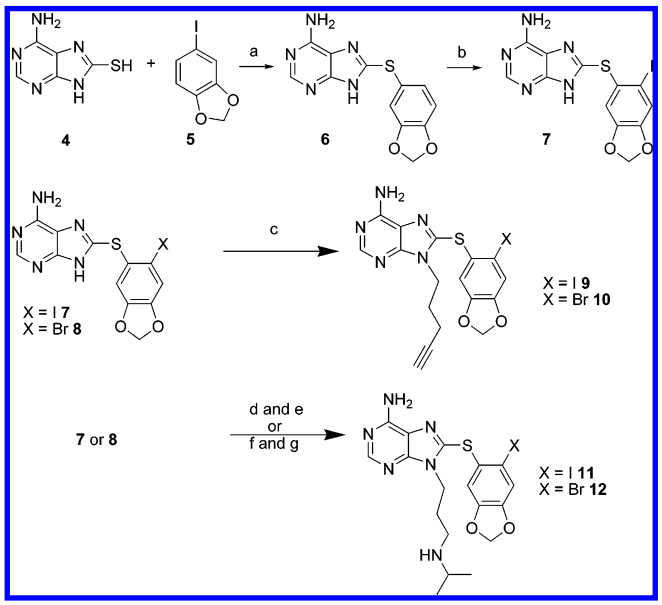


Figure 1. Schematic representation of the purine-scaffold Hsp90 inhibitor class, and its representative derivatives, compounds **1**, **2**, and **3**.

* Corresponding author. Phone: 212-639-8929. Fax: 212-794-6236. E-mail: chiosisg@mskcc.org.
† Program in Molecular Pharmacology and Chemistry, Memorial Sloan-Kettering Cancer Center.
‡ Department of Biochemistry, Duke University Medical Center.
⊥ Hauptman-Woodward Research Institute, Buffalo, NY 14203.
§ Department of Medicine, Memorial Sloan-Kettering Cancer Center.
¶ These authors equally contributed to the work.

10.1021/jm0508078 CCC: $33.50
Published on Web 12/08/2005

Scheme 1[a]



[a] Reagents and conditions: (a) NaO*t*-Bu, neocuproine, CuI, DMF, 110 °C, 58%; (b) NIS, CF_3COOH, acetonitrile, rt, 55%; (c) pent-4-ynyl tosylate, Cs_2CO_3, DMF, 80 °C, 55−65%; (d) N-Boc-protected 3-isopropylamino-propyl tosylate, Cs_2CO_3, DMF, 80 °C, 25%; (e) CF_3COOH/CH_2Cl_2, rt, 98%; (f) $BrCH_2CH_2CH_2OH$, PPh_3, DBAD, toluene/CH_2Cl_2, rt; (g) *i*-$PrNH_2$, 1,4-dioxane, 100 °C.

to the synthesis of several compounds with improved activity in both biochemical and cellular assays.[9] One such compound, **2** (PU24FCl, Figure 1), is a selective inhibitor of tumor Hsp90 and exhibits antitumor activities in both in vitro and in vivo models of cancer.[5b] The biological effects of **2** are demonstrated in the 2−6 μM concentration range. Recently we have disclosed the synthesis of several 8-arylsulfanyl, -sulfoxyl, and -sulfonyl adenine derivatives of the PU-class.[10] The effort has identified derivative **3** (PU-H58, Figure 1) as a specific tumor Hsp90 inhibitor exhibiting its biological effects in the high nanomolar concentration range. Here we present the synthesis and evaluation of several novel PU-class derivatives designed by the rational assembly of favorable substituents identified from our previous SAR efforts.

Results and Discussions

Chemistry. Previous chemistry efforts have identified both a methylene and a sulfur linker ($X_2 = CH_2$ or S, Figure 1) as permissive for activity.[9,10] These also suggested 4,5-methylenedioxy as a favorable substituent when accompanied by bromine in C2′ on the right side aryl (see Figure 1).[10] Thus, present modifications maintain the permissive characteristics and focus at further modifications meant to increase potency and water solubility.

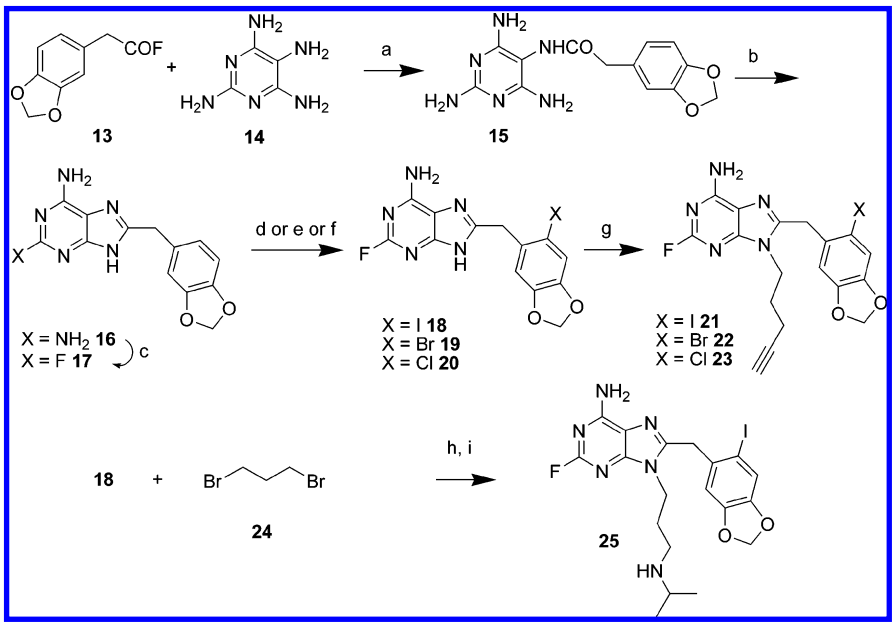
For derivatives containing a sulfur linker ($X_2 = S$, Figure 1), synthesis was carried out using a method previously described by He et al and employed the copper catalyzed coupling of 8-mercaptoadenine (**4**) with the aryliodide **5** (Scheme 1).[11] The reaction occurred in anhydrous DMF at 110 °C under nitrogen to generate the product **6** in 58% yield. Aryliodide **5** was generated from the commercially available amine by its conversion via the diazonium salt to the corresponding iodide,[12] while 8-mercaptoadenine was obtained by condensation of 3,4,5-triaminopyrimidine with CS_2.[10,13] The 8-arylsulfanyl adenine **6** was further halogenated selectively at position 2 of the aryl moiety using NIS as a source of electrophilic iodine and TFA as a catalyst,[10,14] to result in derivative **7**. The active species for this iodination is probably the in situ formed iodine trifluoroacetate that can act as a very reactive electrophile, allowing iodination at room temperature.[14] The bromo derivative **8** was obtained as previously described, by coupling **4** with 5-bromo-6-iodo-benzo[1,3]dioxole.[10] These arylsulfanyl adenines were further alkylated at the position 9-*N* with the tosylate of pent-4-ynyl alcohol in the presence of Cs_2CO_3 to give derivatives **9** and **10**.[15] The choice of pent-4-ynyl comes from its being previously determined to be favored by the Hsp90 pocket.[9a] To increase the water solubility profile of these compounds we probed the introduction of a chain containing an amine functionality instead of pent-4-ynyl. Previous attempts to introduce such chains have led to compounds that retained binding to Hsp90 but were surprisingly inactive in cellular models, likely due to reduced membrane permeability potential.[9a] Preserving the favorable linearity of the first three carbon atoms of the pent-4-ynyl chain[9] and replacing the terminal alkyne with isopropylamine, derivatives **11** and **12** were synthesized. The 3-isopropylamino-propyl chain was introduced by alkylation of **7** with the tosylate of the Boc-protected 3-isopropylpropanol under basic conditions (Cs_2CO_3), followed by TFA induced Boc deprotection[16] to yield **11**. Alternatively, the Mitsunobu conditions[10,17] were utilized to react **8** with 3-bromo-1-propanol. This product was further alkylated by reacting with excess isopropylamine[18] to result in derivative **12**.

If the linker was methylene ($X_2 = CH_2$, Figure 1), synthesis commenced by coupling 2,4,5,6-tetraaminopyrimidine (**14**) with the acid fluoride of the corresponding carboxylic acid (Scheme 2).[9a] The acid fluoride **13** was generated by treating 3,4-(methylenedioxy)phenylacetic acid with cyanuric fluoride and pyridine in CH_2Cl_2.[9a,19] Following a quick water wash, the resulting acid fluoride was used in the next step without further purification. The amide **15** resulting from the pyrimidine **14**−acid fluoride **13** coupling was cyclized to compound **16** by heating in alcoholic NaOMe.[9a] Transformation of the C2-amino group to fluorine ($X_1 = NH_2$ to F) was conducted by a modified Schiemann diazotization−fluorodediazoniation of the amino derivative in HF/pyridine in the presence of $NaNO_2$[20] to yield the corresponding adenine derivatives **17**. We and others[9] have previously determined that fluorine in this position in general augmented the potency of the resulting purines if the linker was methylene ($X_2 = CH_2$, Figure 1), likely by increasing the hydrogen donor ability of C6 NH_2. No beneficial effect was observed by F's presence in this position if the linker was sulfur ($X_2 = S$, Figure 1).[9c,10] Further selective halogenation of **17** using either of NIS, NBS, or NCS[14,21] led to the corresponding iodo, bromo, and chloro derivatives **18**−**20**. These were alkylated with pent-4-ynyl tosylate in the presence of Cs_2CO_3 to result in derivatives **21**−**23**. For the synthesis of the water soluble derivative **25**, the iodo derivative **18** was first alkylated with 1,3-dibromopropane in the presence of Cs_2CO_3. Interestingly, formation of dimer was not detected in this reaction. The resulting bromine was further alkylated in the presence of excess isopropylamine to give **25**.

For derivatives bearing 3,4-methoxy substituents on the right side aryl (**31**−**33**), synthesis was carried out in a manner similar to that of derivatives **21**−**23**, using as starting points both the cyanuric fluoride **26** obtained from 3,4-dimethoxyphenylacetic acid and the tetraaminopyrimidine **14** (Scheme 3).

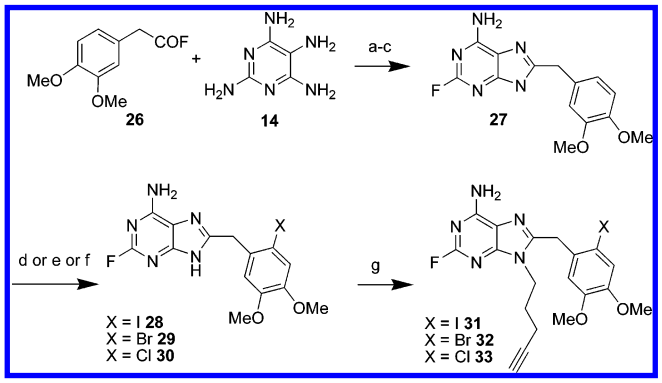
Biological Testing. Compounds synthesized above were tested in a biochemical assay and also in cellular assays that probe for cellular fingerprints of Hsp90 inhibition.[2,10] The biochemical assay tests competitive binding of compounds to

Scheme 2[a]



[a] Reagents and conditions: (a) K_2CO_3, DMAP, DMF, 120 °C; (b) NaOMe, MeOH, *i*-BuOH, 105 °C; (c) HF/pyridine, $NaNO_2$, rt, 50%; (d) NCS, DMF, rt, 50%; (e) NBS, DMF, rt, 40%; (f) NIS, CF_3COOH, DMF, rt, 20%; (g) pent-4-ynyl tosylate, Cs_2CO_3, DMF, 50 °C, 30−90%; (h) Cs_2CO_3, DMF, 50 °C, 95%; (i) *i*-$PrNH_2$, rt, 90%.

Scheme 3[a]



[a] Reagents and conditions: (a) K_2CO_3, DMAP, DMF, 120 °C, 90%; (b) NaOMe, MeOH, *i*-BuOH, 105 °C, 85%; (c) HF/pyridine, $NaNO_2$, rt, 50%; (d) NIS, CF_3COOH, DMF, rt, 20%; (e) NBS, DMF, rt, 54%; (f) NCS, DMF, rt, 50%; (g) pent-4-ynyl tosylate, Cs_2CO_3, DMF, 50 °C, 50−90%.

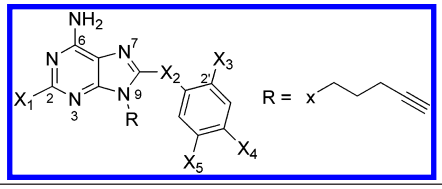
Hsp90 found in cell-specific complexes and uses a fluorescence polarization method.[22,26] When cell lysates are used instead of recombinant protein, the assay measures binding to average Hsp90 population found in cell-specific complexes.[5b]

The cellular assays measure two specific biological effects observed upon addition of known Hsp90 inhibitors to cancer cells: (a) degradation of the tyrosine kinase Her2[23] and (b) mitotic block in retinoblastoma gene product (Rb)-defective cells.[24] Overexpression of the receptor tyrosine kinase Her2 in SKBr3 breast cancer cells leads to Akt activation which in turn promotes cell survival. Hsp90 uniquely stabilizes Her2 via interaction with its kinase domain, and an Hsp90 inhibitor induces Her2 degradation by disrupting the Her2/Hsp90 association.[23] Her2 inactivation/degradation in SKBr3 is detrimental to the cell and leads to its death. Thus Her2 degradation in these cells is a functional read-out of Hsp90 inhibition. Correlation between Her2 degradation and cytotoxicity is indicative of selective biological effect in this cell via Hsp90. We have previously reported a fast microtiter immunoassay able of quantifying cellular levels of Her2 following drug treatments.[25,26] This assay is used here to differentiate the Her2-degradative potential of the above-synthesized purines. Hsp90 inhibitors are also known to cause cells lacking functional Rb to progress normally through G1 and arrest in mitosis.[24] Thus, another assay used here to test cellular Hsp90 inhibition relies on assessing the antimitotic potential of synthesized purines. The assay is a microtiter immunoassay and uses an antibody against a mitotically phosphorylated form of nucleolin to detect cells in mitosis.[10,26]

In addition, the cytotoxicity of these agents against SKBr3 and MDA-MB-468 breast cancer cells was determined. A selected number of derivatives were also tested for possible toxicity against normal lung fibroblast cells (MRC-5).

Several observations may be inferred from these tests. First, in the 2-halo-4,5-methylenedioxy series, activity decreased in the order I, Br, Cl (see **9** vs **10** and **21** vs **22** vs **23**, Table 1 and **11** vs **12**, Table 2). Second, no significant difference was observed in activity when comparing methylene to sulfur linker (X_2 = CH_2 vs S, Figure 1) derivatives that carry identical substituents on the right side aryl (see **9** vs **21** and **10** vs **22**, Table 1; **11** vs **25**, Table 2). Third, substitution of the pent-4-ynyl chain with 3-isopropylamino-propyl had no unfavorable effect on the biological activity of these derivatives while substantially increasing their water solubility (see **9** vs **11**, **10** vs **12** and **21** vs **25**). Fourth, it is likely that the 2-halo-4,5-methylenedioxy series accommodates the right side aryl moiety into the Hsp90 hydrophobic pocket differently from the 2-halo-4,5-dimethoxy series. This is suggested by the diminished or complete lack of biological activity observed in the 2-halo-4,5-dimethoxy series and also by the fact that, contrary to the 2-halo-4,5-methylenedioxy series, activity in this series decreased in the order Cl, Br, I (see **33** vs **32** vs **31**, Table 1). Such trend was previously observed in the 2-halo-3,4,5-trimethoxy derivative series.[9a] Fifth, trends in binding affinity for Hsp90 in the two cancer cell lines translated well into Hsp90-related biological activities in those cells. To exemplify, in the breast cancer cell line SKBr3, Hsp90 binding translated into inhibition of growth and Her2 degradation, both biological effects occurring with similar IC_{50}'s. Likewise, in the breast cancer cell line

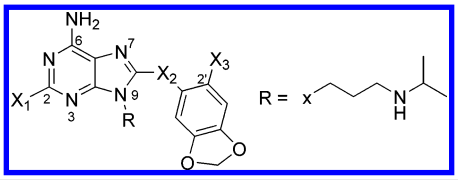
Table 1. Substituent Effects on the Activity of 9-(Pent-4-ynyl)-adenine Derivatives as Inhibitors of Hsp90



					SKBr3			MDA-MB-468		
cpds	X_1	X_2	X_3	X_4, X_5	EC_{50} Hsp90[a]	IC_{50} growth inhibition[a]	IC_{50} Her2 degradation[a]	EC_{50} Hsp90[a]	IC_{50} growth inhibition[a]	IC_{50} antimitotic[a]
9	H	S	I	$-OCH_2O-$	10.8 ± 2	90 ± 2.0	100 ± 10	10.9 ± 1	170 ± 10	184 ± 11
10	H	S	Br	$-OCH_2O-$	50.4 ± 4	300 ± 50	365 ± 45	77.1 ± 3	560 ± 66	530 ± 44
21	F	CH_2	I	$-OCH_2O-$	22.3 ± 2	90 ± 30	90 ± 10	23.7 ± 1	150 ± 10	180 ± 15
22	F	CH_2	Br	$-OCH_2O-$	56.5 ± 2	215 ± 55	210 ± 10	30.5 ± 4	350 ± 30	310 ± 30
23	F	CH_2	Cl	$-OCH_2O-$	77.2 ± 1	250 ± 30	300 ± 15	51.2 ± 5	440 ± 15	450 ± 20
31	F	CH_2	I	OMe, OMe	>15000	>50000	>50000	>15000	>50000	>50000
32	F	CH_2	Br	OMe, OMe	>15000	55000 ± 2300	>50000	>15000	>50000	>50000
33	F	CH_2	Cl	OMe, OMe	4600 ± 20	20600 ± 6700	30000 ± 1000	4500 ± 70	27300 ± 1200	>50000

[a] All values are in nanomol per liter and represent the average of $n = 3 \pm$ standard deviation.

Table 2. Effects of a Water-Solubilizing Chain at Position 9-*N* on the Activity of PU-Class Derivatives as Inhibitors of Hsp90



				SKBr3			MDA-MB-468		
cpds	X_1	X_2	X_3	EC_{50} Hsp90[a]	IC_{50} growth inhibition[a]	IC_{50} Her2 degradation[a]	EC_{50} Hsp90[a]	IC_{50} growth inhibition[a]	IC_{50} antimitotic[a]
11	H	S	I	16.1 ± 1	50 ± 5	50 ± 6	10.2 ± 3	58 ± 2.5	70 ± 5.5
12	H	S	Br	38.8 ± 3	142 ± 22	205 ± 15	55.4 ± 1	270 ± 20	375 ± 24
25	F	CH_2	I	50.4 ± 4	45 ± 6	80 ± 10	39.1 ± 1	90 ± 5.0	100 ± 20

[a] All values are in nanomol per liter and represent the average of $n = 3 \pm$ standard deviation.

Table 3. Selectivity for Transformed versus Normal Cells in a Series of Purine-Scaffold Hsp90 Inhibitors

cpds	EC_{50} Hsp90 lung[a]	EC_{50} Hsp90 heart[a]	EC_{50} Hsp90 SKBr3[a]	lung/ SKBr3	heart/ SKBr3	IC_{50} MRC-5[a]	MRC-5/ SKBr3
11	2200 ± 400	6000 ± 200	16.1 ± 1	136	370	1000 ± 14	~20
12	5200 ± 210	13300 ± 210	38.8 ± 3	134	343	ND	ND
25	2400 ± 240	6900 ± 150	50.4 ± 4	48	140	>5000	>50

[a] All values are in nanomol per liter and represent the average of $n = 3 \pm$ standard deviation.

MDA-MB-468, Hsp90 binding correlated with growth inhibition and antimitotic activity (see Tables 1 and 2).

The two most active derivatives, **11** and **25**, were further tested for specificity toward transformed cells (Table 3). Binding affinities of these compounds for average population Hsp90 complexes found in normal tissues (lung and heart) and, in addition, their cytotoxicities against MRC-5 normal lung fibroblast cells were determined. Compounds were found to bind Hsp90 from normal tissues with 2-log weaker affinities when compared to that of Hsp90 from SKBr3 cells (columns 6 and 7, Table 3). This specificity translated into over 50-fold selectivity (column 8, Table 3) in inhibiting the growth of transformed cells compared to that of cultured normal fibroblast cells. No significant cell death was observed in the purine-scaffold treated MRC-5 cells even at the highest tested concentrations.

When formulated as hydrochloric or phosphoric acid salts, derivatives **11**, **12**, and **25** were soluble in phosphate buffered saline (PBS) pH 7.4 to form 10−40 mM stocks, suggesting that their water solubility was above 5 mg/mL. This is a substantial improvement over compounds **2** and **3** that were not water soluble at these concentrations and required administration in a mixture of 1:1:1 DMSO/EtOH/water.[5b] To date, the only other reported Hsp90 inhibitor to be water soluble is the geldanamycin derivative, 17DMAG.[27a]

To probe if in vivo administration of these agents resulted in pharmacologically relevant concentrations, we used mice xenografted with MDA-MB-468 human breast cancer tumors. Previously, we have reported a tumor accumulation profile for derivative **2**.[5b] Administered intraperitoneally to mice bearing MCF-7 breast cancer xenografted tumors, **2** accumulated inside tumors to micromolar concentrations, and these levels were sustained at 24 h postadministration. Similar tumor retention profiles were later demonstrated for both 17AAG and 17DMAG, suggesting this behavior to be a consequence of a higher affinity state of Hsp90 in tumors.[27] A similar tumor retention profile was observed here with **12**—at 24 h, the biologically active concentration of 300 nM (~IC_{50}, see Table 2) was seen in tumors at the administered dose of 25 mg/kg (Figure 2A). An increase in dose to 50 mg/kg or higher, led to concentrations of **12** in tumors above the IC_{90} value. At 24 h postadministration, **12** could not be detected in plasma (not shown). Compound **12**

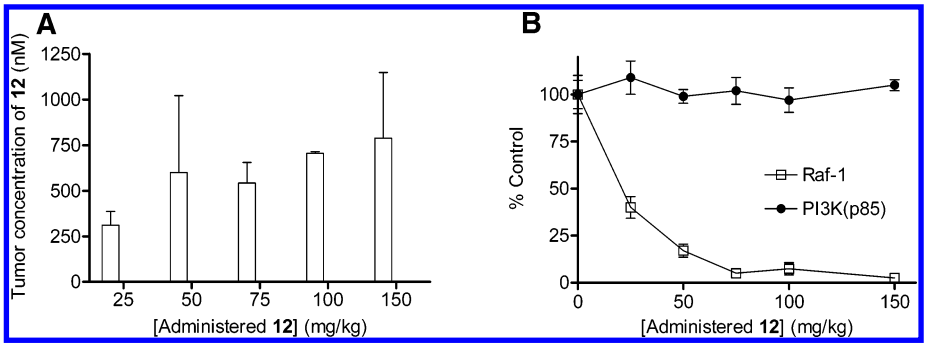


Figure 2. Pharmacodynamic effects of derivative **12**. The phosphate salt of **12** was administered intraperitoneally to MDA-MB-468 tumor-bearing mice at the indicated doses. Mice ($n = 2$) were sacrificed at 24 h postadministration, and tumors were harvested. Tumors were halved and processed accordingly. Inhibitor levels were analyzed by LC−MS/MS (A) while protein levels analyzed by Western blot (B). Data are presented as (A) drug concentration in the tumor at 24 h postadministration and (B) percent control = protein expression in treated mice/protein expression in control mice (administered PBS only) × 100 and plotted against administered dose of **12**.

was further tested for its effects on pharmacodynamic markers, such as Raf-1 kinase. Hsp90 stabilizes the kinase and maintains it in a ready-to-be-activated conformation. Inhibition of Hsp90 leads to the disruption of the complex and further ubiquitinylation and degradation of Raf-1 by the proteasome.[2] Thus Raf-1 degradation in these tumors is a functional read-out of Hsp90 inhibition. We have previously shown for **2** a correlation between compound tumor accumulation profile and its effects on Raf-1 protein.[5b] In concordance, **12** induced a dose-dependent degradation of Raf-1, with maximal effects seen at 50 mg/kg (Figure 2B). The compound had no effect on PI3 kinase (p85 unit) expression, protein unaffected by Hsp90 inhibitors.[2,8]

Conclusions

In summary, we have presented the synthesis and evaluation of several novel purine-class Hsp90 inhibitors. The study has aided in the identification of derivatives with nanomolar biological activities in both cellular and animal models of cancer. It also extended our understanding into the structural elements that allow for high affinity binding to Hsp90. In this regard, it highlighted the importance of substituents on the right side aryl moiety. These have a major impact not only on orienting the moiety inside the hydrophobic pocket but also on reducing the rotational freedom around the C−S or C−C bond and, thus, on alleviating entropic penalties upon binding. In addition, the study has identified position 9-*N* as permissive for the attachment of functionalities that increase water solubility. SAR studies in the series have revealed a high correlation between binding to Hsp90 and biological activities, suggesting Hsp90 inhibition as the major mode of action of these compounds. As previously seen with lead purine-skeleton Hsp90 inhibitors, a tumor retention profile is also characteristic for these novel derivatives. Pharmacologically relevant concentrations are maintained in tumors at 24 h, leading to extended modulation of Hsp90−client proteins in tumors. The higher affinity that these compounds exhibit for tumor cell as compared to normal cell Hsp90 is a likely explanation for the behavior.

Experimental Section

Hsp90 Competition Assay.[22,26] Fluorescence polarization measurements were performed on an Analyst AD instrument (Molecular Devices, Sunnyvale, CA). Measurements were taken in black 96-well microtiter plates (Corning no. 3650). The assay buffer (HFB) contained 20 mM HEPES (K) pH 7.3, 50 mM KCl, 5 mM $MgCl_2$, 20 mM Na_2MoO_4, 0.01% NP40. Before each use, 0.1 mg/mL bovine gamma globulin (BGG) (Panvera Corporation, Madison, WI) and 2 mM DTT (Fisher Biotech, Fair Lawn, NJ) were freshly added. GM-BODIPY was synthesized as previously reported[22a] and was dissolved in DMSO to form 10 μM solutions. Cell lysates were prepared rupturing cellular membranes by freezing at −70 °C and dissolving the cellular extract in HFB with added protease and phosphotase inhibitors. Organs were harvested from a healthy mouse and homogenized in HFB. Saturation curves were recorded in which GM-BODIPY (5 nM) was treated with increasing amounts of cellular lysates. The amount of lysate that resulted in polarization (mP) readings corresponding to 20 nM recombinant Hsp90α was chosen for the competition study. For the competition studies, each 96-well contained 5 nM fluorescent GM, cellular lysate (amounts as determined above and normalized to total Hsp90 as determined by Western blot analysis using as standard Hsp90 purified from HeLa cells (Stressgen no. SPP-770) and tested inhibitor (initial stock in DMSO) in a final volume of 100 μL. The plate was left on a shaker at 4 °C for 24 h, and the FP values in mP were recorded. EC_{50} values were determined as the competitor concentrations at which 50% of the fluorescent GM was displaced.

Cell Culture. The human breast cancer cell lines SKBr3 and MDA-MB-468 were a gift from Dr. Neal Rosen (MSKCC). Cells were maintained in 1:1 mixture of DME/F12 supplemented with 2 mM glutamine, 50 units/mL penicillin, 50 units/mL streptomycin, and 10% heat-inactivated fetal bovine serum (Gemini Bioproducts no. 100-10b) and incubated at 37 °C, 5% CO_2. Normal lung fibroblast cells were obtained from the American Type Culture Collection (Manassas, VA) maintained in minimum essential medium (MEM) adjusted to contain 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 0.1 mM nonessential amino acids, 1.0 mM sodium pyruvate, 10% fetal bovine serum, 1% penicillin and streptomycin and incubated at 37 °C, 5% CO_2.

Growth Assays. Growth inhibition studies were performed using the sulforhodamine B assay as previously described.[28] In summary, experimental cultures were plated at 6000, 8000, and 10 000 cells/well for SKBr3, MDA-MB-468, and MRC-5, respectively, in microtiter plates (Nunc no. 167008). One column of wells was left without cells to serve as the blank control. Cells were allowed to attach overnight. The following day, growth medium having either drug or DMSO (or PBS for **11**, **12**, and **25**) at twice the desired initial concentration was added to the plate in triplicate and was serially diluted at a 1:1 ratio in the microtiter plate. After 72 h of growth, the cell number in treated versus control wells was estimated after treatment with 50% trichloroacetic acid and staining with 0.4% sulforhodamine B in 1% acetic acid. The IC_{50} was calculated as the drug concentration that inhibits cell growth by 50% compared with control growth.

Her2 Assay.[25,26] SKBr3 cells were plated in black, clear-bottom microtiter plates (Corning no. 3603) at 3000 cells/well in growth medium (100 μL) and allowed to attach for 24 h at 37 °C and 5% CO_2. Growth medium (100 μL) with drug or vehicle (DMSO) was carefully added to the wells, and the plates were placed at 37 °C

and 5% CO_2. Following 24 h of incubation with drugs, wells were washed with ice-cold Tris buffer saline (TBS) containing 0.1% Tween 20 (TBST) (200 μL). A house vacuum source attached to an eight-channel aspirator was used to remove the liquid from the plates. Further, methanol (100 μL at −20 °C) was added to each well, and the plate was placed at 4 °C for 10 min. Methanol was removed by washing with TBST (2 × 200 μL). After further incubation at room temperature for 2 h with SuperBlock (Pierce 37535) (200 μL), anti-Her-2 (c-erbB-2) antibody (Zymed Laboratories no. 28-0004) (100 μL, 1:200 in SuperBlock) was placed in each well. The plate was incubated overnight at 4 °C. For control wells, 1:200 dilution of a normal rabbit IgG (Santa Cruz no. SC-2027) in Superblock was used. Each well was washed with TBST (2 × 200 μL) and incubated at room temperature for 2 h with an anti-rabbit HRP-linked antibody (Sigma, A-0545) (100 μL, 1:2000 in SuperBlock). Unreacted antibody was removed by washing with TBST (3 × 200 μL), and the ECL Western blotting reagent (Amersham no. RPN2106) (100 μL) was added. The plate was immediately read in an Analyst AD plate reader (Molecular Devices). Each well was scanned for 0.1 s. Readings from wells containing only control IgG and the corresponding HRP-linked secondary antibody were set as background and deducted from all measured values. Luminescence readings resulted from drug-treated cells versus untreated cells (vehicle treated) were quantified and plotted against drug concentration to give the IC_{50} values as the concentration of drug that caused 50% decrease in luminescence.

Antimitotic Assay.[26] Black, clear-bottom microtiter 96-well plates (Corning Costar no. 3603) were used to accommodate experimental cultures. MDA-MB-468 cells were seeded in each well at 8000 cells per well in growth medium (100 μL) and allowed to attach overnight at 37 °C and 5% CO_2. Growth medium (100 μL) with drug or vehicle (DMSO) was gently added to wells, and the plates were incubated at 37 °C and 5% CO_2 for 24 h. Wells were washed with ice-cold TBST (2 × 200 μL). A house vacuum source attached to an eight-channel aspirator was used to remove the liquid from the 96-well plates. Ice-cold methanol (100 μL) was added to each well, and the plate was placed at 4 °C for 5 min. Methanol was removed by suction and plates were washed with ice-cold TBST (2 × 200 μL). Plates were further incubated with SuperBlock blocking buffer (Pierce no. 37535) (200 μL) for 2 h at room temperature. The Tg-3 antibody (gift of Dr. Peter Davies, Albert Einstein College of Medicine) diluted 1:200 in SuperBlock was placed in each well (100 μL) except the control column that was treated with control antibody (mouse IgM, NeoMarkers, NC-1030-P). After 72 h, wells were washed with ice-cold TBST (2 × 200 μL). The secondary antibody (goat antimouse IgM, Southern-Biotech no. 1020-05) was placed in each well at 1:2000 dilution in SuperBlock and incubated on a shaker at room temperature for 2 h. Unreacted antibody was removed by washing the plates with ice-cold TBST (3 × 200 μL) for 5 min on a shaker. The ECL Western Blotting detection reagents 1 and 2 in a 1:1 mix (100 μL) were placed in each well and the plates were read immediately in an Analyst AD plate reader (Molecular Devices). Luminescence readings were imported into SOFTmax PRO 4.3.1. Antimitotic activity was defined as a concentration-dependent increase in luminescence readings in compound-treated wells as compared to DMSO-only treated wells. Luminescence readings resulting from drug-treated cells versus untreated cells (vehicle treated) were quantified and plotted against drug concentration to give the IC_{50} values as the concentration of drug that caused a doubling in luminescence.

Tumor Analysis. Tumors were harvested at the indicated time and immediately flash frozen in liquid nitrogen. One-half of each tumor was homogenized in SDS lysis buffer (50 mM Tris pH 7.4, 2% SDS). Protein concentrations were determined using the BCA kit (Pierce) according to the manufacturer's instructions. Protein lysates (20−100 μg) were electrophoretically resolved on denaturing 7% SDS−PAGE, transferred to nitrocellulose membrane, and probed with the following primary antibodies: anti-Raf-1 (Santa Cruz) and anti-PI3K (p85) (Upstate Biotechnologies). Membranes were then incubated with a 1:5000 dilution of a peroxidase-conjugated corresponding secondary antibody. ECL (Amersham Life Science, Inc.) was performed according to the manufacturer's instructions. Blots were visualized by autoradiography and the protein quantified using BioRad Gel Doc 1000 software. The second half of each tumor was processed for LC−MS/MS analysis. Tumors were rinsed with saline isotonic solution and dried with gauze. A selected portion of tumor was weighed and then homogenized in mobile phase (acetonitrile (ACN)/0.1% formic acid = 1.2/2.8, v/v). Haloperidol was added as internal standard. Compounds were extracted in methylene chloride. The organic layer was separated, then speedily dried under vacuum and reconstituted in the mobile phase. Concentrations of compound **12** were determined and quantitated by a multiple reaction monitoring (MRM) mode (*m*/*z* 467−407.8) using a tandem high-performance liquid chromatography−mass/mass spectrometry (HPLC−MS/MS) at the Analytical Core Facility of Memorial Sloan-Kettering Cancer Center. The API 4000 LC−MS/MS (Applied Biosystems) was coupled with a Shimadzu LC system and a 96-well plate autosampler. A Gemini C18 column (5μ particle size, 50 mm × 4.6 mm i.d.) was used for the LC separation. The analyte was eluted under an isocratic condition for 4 min at a flow rate of 0.4 mL/min.

General Chemical Procedures. All commercial chemicals and solvents are reagent grade and were used without further purification. The identity and purity of each product was characterized by MS, HPLC, TLC, and NMR. ^{1}H NMR/^{13}C NMR spectra were recorded on a Bruker 400 MHz instrument. Low-resolution mass spectra (MS) were recorded in the positive or negative ion mode under electrospray ionization (ESI). High-performance liquid chromatography analyses were performed on a Waters 2996 instrument with a photodiode array detector (read at 254 nm) and a reversed-phase column (Higgins; HAISIL HL C18 5μm) (method a) and, additionally, a Waters 2695 separation module with a Waters 996 photodiode array detector and a Waters micromass ZQ and a reversed-phase column (Varian; Microsorb 100−5 C18 150 × 2) (method b). Method a: 0.1% TFA in water/acetonitrile at the indicated ratio. Method b: 0.05% TFA in water/0.04% TFA in acetonitrile at the indicated gradient. Characterization data for previously unknown compounds were determined from a single run with isolated yields. Reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates and visualized with UV light. Column chromatography was performed using silica gel (Fisher 170−400 mesh). Analytical thin-layer chromatography (TLC) was performed on E. Merck precoated silica gel 60 F_{254}. All reactions were conducted under inert atmosphere.

8-Mercaptoadenine (4). A mixture of 4,5,6-triaminopyrimidine sulfate (5.35 g, 24 mmol), $NaHCO_3$ (10.08 g, 120 mmol), H_2O (90 mL), CS_2 (14.43 mL, 20.88 g, 240 mmol), and EtOH (45 mL) was refluxed for 3 days. After cooling, the excess CS_2 was evaporated in vacuo. Solids were filtered off, and HOAc (4 mL) was added to the filtrate. The product as a white precipitate was collected, dried (4.0 g, 100% yield), and further used without additional purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.02 (s, 1 H), 12.02 (s, 1 H), 8.05 (s, 1 H), 6.73 (bs, 2 H); MS *m*/*z* 168.0 $(M - H)^+$.

5-Iodo-benzo[1,3]dioxole (5).[29,30] Benzo[1,3]dioxol-5-ylamine (5 g, 36.46 mmol) was dissolved in concentrated HCl (32 mL) and ice (96 g). To it, a chilled solution of $NaNO_2$ (5.3 g, 76.8 mmol) in water (30 mL) was added over a 15 min period. The mixture was stirred for an additional 10 min. Urea (1.66 g, 27.6 mmol) and 10 min later, KI (17.9 g) in water (20 mL) were further added, and the mixture was stirred overnight. Following completion, the reaction mixture was extracted in CH_2Cl_2, dried over $MgSO_4$, and filtered through a silica gel column using hexane as eluent to provide the desired aryliodide (6.9 g, 76.7% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11−7.08 (m, 2H), 6.56 (d, J = 8.0 Hz, 1H), 5.91 (s, 2H).

5-Bromo-6-iodo-benzo[1,3]dioxole.[30] To a solution of 5-bromobenzo[1,3]dioxole (10.1 g, 50 mmol) in AcOH (75 mL) was added ICI (10.1 g, 62.5 mmol) in AcOH[31] (12.5 mL). The reaction mixture was stirred at 80 °C for 18 h and then poured into H_2O and extracted twice with CH_2Cl_2. The organic extracts were washed consequently with aqueous $NaHSO_3$, $NaHCO_3$, and H_2O, dried over $MgSO_4$, and

then evaporated in vacuo. The reaction crude was purified by silica gel chromatography (hexanes) to give the desired iodo compound (3.8 g, 23.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (s, 1H), 7.07 (s, 1H), 5.97 (s, 2H).

8-(Benzo[1,3]dioxol-5-ylsulfanyl)adenine (6). 8-Mercaptoadenine (100 mg, 0.60 mmol), neocuproine hydrate (14 mg, 0.06 mmol), CuI (11 mg, 0.06 mmol), NaO-*t*-Bu (115 mg, 1.2 mmol), 5-iodo-benzo[1,3]dioxole (770 mg, 1.8 mmol), and DMF (4 mL) were charged in a nitrogen box. The vessel was sealed with Teflon tape, placed in an oil bath (110 °C), and magnetically stirred for 24 h. The solvent was removed under high vacuum and the crude purified by column chromatography on silica gel (EtOAc/CH_2Cl_2/MeOH at 2:2:1) to afford **6** (99.6 mg, 58% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (s, 1H), 7.72 (bs, 2H), 7.17 (s, 1H), 7.10 (d, J = 8.2 Hz, 1H), 7.00 (d, J = 8.0 Hz, 1H), 6.09 (s, 2H); MS m/z 288.1 $(M + H)^+$.

8-(6-Iodo-benzo[1,3]dioxol-5-ylsulfanyl)adenine (7). A solution of **6** (201 mg, 0.7 mmol), acetonitrile (8.75 mL), TFA (270 μL, 400 mg), and NIS (945 mg, 4.2 mmol) was stirred at room temperature for 24 h. The solvent was removed under high vacuum and the crude purified by column chromatography on silica gel (EtOAc/CH_2Cl_2 at 1:1 then EtOAc/CH_2Cl_2/MeOH at 2:2:1) to yield **7** (159 mg, 55% yield). ^{1}H NMR (400 MHz, $CDCl_3$/methanol-d_4) δ 8.18 (s, 1H), 7.44 (s, 1H), 7.18 (s, 1H), 6.07 (s, 2H); MS m/z 413.9 $(M + H)^+$.

8-(6-Bromo-benzo[1,3]dioxol-5-ylsulfanyl)adenine (8). 8-Mercaptoadenine (602 mg, 3.6 mmol), neocuproine hydrate (81 mg, 0.36 mmol), CuI (69 mg, 0.36 mmol), NaO-*t*-Bu (692 mg, 7.2 mmol), 5-bromo-6-iodo-benzo[1,3]dioxole (3.53 g, 10.8 mmol), and anhydrous DMF (24 mL) were charged in a nitrogen box. The vessel was sealed with Teflon tape, placed in an oil bath (110 °C), and magnetically stirred for 24 h. The solvent was removed under high vacuum and the crude purified by column chromatography on silica gel (EtOAc/CH_2Cl_2/MeOH at 2:2:1) to provide the product (1.29 g, 97%). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.07 (s, 1H), 7.28 (s, 1H), 7.15 (s, 1H), 7.08 (bs, 2H), 6.13 (s, 2H); MS m/z 366.0 $(M + H)^+$.

8-(6-Iodo-benzo[1,3]dioxol-5-ylsulfanyl)-9-(pent-4-ynyl)adenine (9). A solution of **7** (40 mg, 96.8 μmol), Cs_2CO_3 (31.5 mg, 96.8 μmol), and pent-4-ynyl tosylate (28 mg, 114 μmol) in anhydrous DMF (1 mL) was stirred at 80 °C for 30 min. The solvent was removed under high vacuum and the crude purified by preparatory thin-layer chromatography to give the desired product (25 mg, 53.9%). ^{1}H NMR (400 MHz, $CDCl_3$/methanol-d_4) δ 8.23 (s, 1H), 7.38 (s, 1H), 7.04 (s, 1H), 6.05 (s, 2H), 4.32 (t, J = 7.3 Hz, 2H), 2.33−2.31 (m, 2H), 2.12−2.04 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$/methanol-d_4) δ 151.1, 149.6, 149.2, 147.5, 125.7, 119.4, 113.6, 102.4, 93.8, 82.1, 69.4, 42.8, 28.1, 15.8; MS m/z 480.0 $(M + H)^+$. HPLC: (a) 98.5% (65% water/35% acetonitrile); (b) 97.7% (from 35% to 95% acetonitrile).

3-(*tert*-Butoxycarbonyl-isopropyl-amino)-propyl tosylate. A solution of 3-bromo-1-propanol (5 g, 0.036 mol) in isopropylamine (9 mL, 0.11 mol) was heated overnight at 50 °C with stirring. Solvent was removed under vacuum to give the product, 3-isopropyl-amino-propanol, as a white solid. To this were added di-*tert*-butyl dicarbonate (10 g, 0.05 mol) and triethylamine (11 mL, 0.08 mol), and the resulting solution was stirred at room temperature overnight. Following solvent removal, the crude was purified by column chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2/acetone at 3:1) to provide the 3-(*tert*-butoxycarbonyl-isopropyl-amino)-propanol (5.8 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93 (bs, 1H), 3.58 (m, 2H), 3.33 (m, 2H), 1.67 (m, 2H), 1.48 (s, 9H), 1.16 (d, J = 6.9 Hz, 6H); MS m/z 218.1 $(M + H)^+$. A solution of 3-(*tert*-butoxycarbonyl-isopropyl-amino)-propanol (3.5 g, 0.016 mol), *p*-toluenesulfonyl chloride (3.7 g, 0.019 mol), and pyridine (1.6 mL, 0.019 mol) in CH_2Cl_2 (50 mL) was stirred overnight at room temperature. Following solvent removal, the product (2.3 g, 40%) was isolated by column chromatography on silica gel (hexanes/CH_2Cl_2/EtOAc at 5:4:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.2 Hz, 2H), 4.06−4.03 (m, 3H), 3.09 (t, J = 6.5 Hz, 2H), 2.45 (s, 3H), 1.91−1.87 (m, 2H), 1.42 (s, 9H), 1.08 (d, J = 6.7 Hz, 6H); MS m/z 372.2 $(M + H)^+$.

8-(6-Iodo-benzo[1,3]dioxol-5-ylsulfanyl)-9-(3-isopropylamino-propyl)adeni ne (11). A solution of **7** (125 mg, 303 μmol), 3-(*tert*-butoxycarbonyl-isopropyl-amino)-propyl tosylate (269 mg, 726 μmol), and Cs_2CO_3 (99 mg, 303 μmol) in anhydrous DMF (2 mL) was stirred at 80 °C for 24 h. The solvent was removed under vacuum and the crude purified by preparatory thin-layer chromatography on silica gel ($CHCl_3$/MeOH/NH_4OH at 10:1:0.5) to afford the 9-N-alkylated compound. This was placed in TFA (1 mL) at 0 °C for 1.5 h to remove the Boc protecting group and yield **11** (30 mg, 19.3% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.29 (s, 1H), 6.88 (s, 1H), 6.10 (bs, 2H), 5.96 (s, 2H), 4.29 (t, J = 7.0 Hz, 2H), 2.75−2.69 (m, 1H), 2.58 (t, J = 6.8 Hz, 2H), 2.02−1.95 (m, 2H), 1.03 (d, J = 6.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.6, 152.9, 151.6, 149.2, 148.9, 146.2, 127.9, 120.1, 119.2, 112.2, 102.2, 91.1, 48.7, 43.9, 41.7, 30.3, 22.9; MS m/z 513.2 $(M + H)^+$. HPLC: (a) 98.9% (65% water/35% acetonitrile); (b) 95.0% (from 20% to 40% acetonitrile).

8-(6-Bromo-benzo[1,3]dioxol-5-ylsulfanyl)-9-(3-isopropylamino-propyl)ade nine (12). A solution of **8** (513 mg, 1.4 mmol), PPh_3 (808 mg, 3.08 mmol), 3-bromo-1-propanol (253 mg, 165 μL, 1.82 mmol), DBAD (1612 mg, 7 mmol) in toluene (43.8 mL), and CH_2Cl_2 (8.75, mL) was stirred at room temperature for 20 min. The reaction mixture was loaded to a silica gel column ($CHCl_3$ then $CHCl_3$/EtOAc/hexanes/*i*-propyl alcohol at 4:4:2:1) to provide the 9-N-alkylated compound, 8-(6-bromo-benzo[1,3]dioxol-5-ylsulfanyl)-9-(3-bromo-propyl)adenine) (142.6 mg, 21% yield). A solution of this product (142.6 mg, 0.29 mmol) in 1,4-dioxane (12 mL) and *i*-propylamine (3 mL) was stirred at 100 °C for 2.5 h. The solvent was removed under vacuum and the crude purified by preparatory thin-layer chromatography on silica gel ($CHCl_3$/MeOH/NH_4OH at 10:1:0.5 then EtOAc/CH_2Cl_2/MeOH at 2:2:1) to afford **12** (51 mg, 8% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.04 (s, 1H), 6.81(s, 1H), 6.48 (bs, 2H), 5.94 (s, 2H), 4.29 (t, J = 7.0 Hz, 2H), 2.74−2.68 (m, 1H), 2.57 (t, J = 6.8 Hz, 2H), 2.02−1.95 (m, 2H), 1.02 (d, J = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.8, 152.9, 151.5, 148.8, 148.0, 145.2, 123.8, 120.0, 116.7, 113.2, 112.2, 102.3, 48.6, 43.8, 41.7, 30.2, 22.8; MS m/z 465.0 $(M + H)^+$. HPLC: (a) 99.1% (65% water/35% acetonitrile); (b) 98.0% (from 20% to 40% acetonitrile).

8-(Benzo[1,3]dioxol-5-ylmethyl)-2-aminoadenine (16). 2,4,5,6-Tetraaminopyrimidine sulfate (6 g, 25 mmol) was dissolved in hot NaOH (3.6 g, 90 mmol) in water (36 mL). The solution was slowly cooled to result in the formation of yellow crystals of free base (**14**). These were collected, dried, and further used without additional purification. To a solution of 3,4-(methylenedioxy)-phenylacetic acid (3.0 g, 16.6 mmol) in CH_2Cl_2 (19 mL) under inert atmosphere were added cyanuric fluoride (2.2 mL, 16.7 mmol) and pyridine (1.5 mL, 18.3 mmol). The reaction mixture was stirred for 1.5 h at room temperature and then diluted in CH_2Cl_2 (200 mL) and washed once with water (5 mL). The organic layer was removed to yield the acid fluoride **13**. To this crude were added **14** (3.3 g, 23.85 mmol), K_2CO_3 (3.95 g, 28.62 mmol), DMAP (0.21 g, 1.75 mmol), and DMF (65 mL). The reaction was heated with stirring at 120 °C for 2 h. Following cooling, the solids were filtered off and washed with MeOH. Solvent was removed in vacuo to yield the crude amide **15** (3.5 g, 69% yield). MS m/z 303.1 $(M + H)^+$. To this material were added isobutyl alcohol (12 mL) and a 25% solution of NaOMe in MeOH (12 mL), and the resulting solution was heated at 105 °C for 4.5 h. Following cooling, the pH of the solution was adjusted to 7 by addition of concentrated HCl. The solvent was removed under vacuum and the crude purified by silica gel column chromatography ($CHCl_3$/7N NH_3 in MeOH at 5:1) to yield **16** (1.4 g, 25.7%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.85 (s, 1H), 6.83 (d, J = 8.0 Hz, 1H), 6.72 (d, J = 8.0 Hz, 1H), 6.59 (s, 2H), 5.95 (s, 2H), 5.59 (s, 2H), 3.89 (s, 2H); MS m/z 285.0 $(M + H)^+$.

8-Benzo[1,3]dioxol-5-ylmethyl-2-fluoroadenine (17). To a cooled (0 °C) solution of **16** (1.48 g, 5.2 mmol) in HF/pyridine (3.64 mL), $NaNO_2$ (0.47 g, 6.76 mmol) was slowly added. The reaction was

brought to room temperature and further stirred for 1 h. Following dilution with CH_2Cl_2 (38 mL), the excess HF was quenched by stirring for an additional 1 h with $CaCO_3$ (0.95 g) and water (5 mL). The mixture was dried in vacuo and subsequently purified by silica gel column chromatography ($CHCl_3$/MeOH/NH_4OH at 5:1:0.5) to yield **17** (0.9 g, 60% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (bs, 2H), 6.94−6.90 (m, 3H), 6.81 (d, J = 8.0 Hz, 1H), 6.03 (s, 2H), 4.06 (s, 2H); MS m/z 288.0 $(M + H)^+$.

2-Fluoro-8-(6-iodo-benzo[1,3]dioxol-5-ylmethyl)adenine (18). A solution of **17** (50 mg, 0.17 mmol), NIS (94 mg, 0.4 mmol), and TFA (20 mg, 13.4 μL, 0.17 mmol) in CH_2Cl_2 (200 μL) was stirred at room temperature overnight. After solvent removal, the desired product **18** (6 mg, 8.5%) was purified by silica gel column chromatography ($CHCl_3$/EtOAc at 9:1 to 4:6). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.6 (bs, 2H), 7.38 (s, 1H), 6.95 (s, 1H), 6.03 (s, 2H), 4.12 (s, 2H); MS m/z 414.1 $(M + H)^+$.

2-Fluoro-8-(6-bromo-benzo[1,3]dioxol-5-ylmethyl)adenine (19). A solution of **17** (45 mg, 0.157 mmol) and NBS (56 mg, 0.314 mmol) in DMF (0.5 mL) was stirred at room temperature for 1.5 h. Following solvent removal, product (20 mg, 34.8%) was collected through silica gel column purification ($CHCl_3$/EtOAc at 9:1 to 4:6). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.51 (bs, 2H), 7.21 (s, 1H), 6.98 (s, 1H), 6.06 (s, 2H), 4.13 (s, 2H); MS m/z 366.0 $(M + H)^+$.

2-Fluoro-8-(6-chloro-benzo[1,3]dioxol-5-ylmethyl)adenine (20). A solution of **17** (20 mg, 0.07 mmol) and NCS (35.6 mg, 0.27 mmol) in anhydrous DMF (0.4 mL) was stirred at room temperature for 2.5 h. Following solvent removal, the product (11 mg, 48.8%) was collected through silica gel column purification ($CHCl_3$/EtOAc at 9:1 to 5:5). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.40 (bs, 2H), 6.97 (s, 1H), 6.89 (s, 2H), 5.97 (s, 2H), 4.04 (s, 2H); MS m/z 322.1 $(M + H)^+$.

2-Fluoro-8-(6-iodo-benzo[1,3]dioxol-5-ylmethyl)-9-(pent-4-ynyl)adenine (21). A solution of **18** (6 mg, 0.0145 mmol), Cs_2CO_3 (5 mg, 0.0145 mmol), and pent-4-ynyl tosylate (4.5 mg, 0.189 mmol) in anhydrous DMF (200 μL) was stirred at 60 °C for 1.5 h. Following solvent removal, product (5.9 mg, 84.9%) was collected through silica gel column purification (EtOAc/hexanes/$CHCl_3$/i-PrOH at 10:20:20:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (s, 1H), 6.59 (s, 1H), 5.94 (s, 2H), 5.83 (bs, 2H), 4.26 (s, 2H), 4.11 (t, J = 7.4 Hz, 2H), 2.26−2.19 (m, 2H), 2.00 (t, J = 2.5 Hz, 1H), 1.98−1.94 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.9, 148.9, 147.8, 131.5, 118.8, 109.4, 101.9, 88.1, 82.3, 69.9, 42.3, 39.2, 28.2, 15.9; MS m/z 480.0 $(M + H)^+$. HPLC: (a) 95.5% (60% water/40% acetonitrile); (b) 95.0% (from 35% to 55% acetonitrile).

2-Fluoro-8-(6-bromo-benzo[1,3]dioxol-5-ylmethyl)-9-(pent-4-ynyl)adenine (22). A solution of **19** (20 mg, 55 μmol), Cs_2CO_3 (18 mg, 55 μmol), and pent-4-ynyl tosylate (17 mg, 72 μmol) in anhydrous DMF (138 μL) was stirred at 60 °C for 2 h. Following solvent removal, the product (13 mg, 54.7%) was collected through silica gel column purification (EtOAc/hexanes/$CHCl_3$/i-PrOH at 10:20:20:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (s, 1H), 6.60 (s, 1H), 6.15 (bs, 2H), 5.96 (s, 2H), 4.28 (s, 2H), 4.13 (t, J = 7.5 Hz, 2H), 2.25−2.21 (m, 2H), 2.00 (t, J = 2.6 Hz, 1H), 1.98−1.92 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.6, 156.0, 152.6, 150.2, 147.5, 127.6, 116.4, 114.1, 112.5, 109.5, 101.6, 81.9, 69.5, 41.9, 33.7, 27.8, 15.5; MS m/z 432.0 $(M + H)^+$. HPLC: (a) 99.0% (60% water/40% acetonitrile); (b) 98.5% (from 35% to 55% acetonitrile).

2-Fluoro-8-(6-chloro-benzo[1,3]dioxol-5-ylmethyl)-9-(pent-4-ynyl)adenine (23). A solution of **20** (11 mg, 0.034 mmol), Cs_2CO_3 (11 mg, 0.034 mmol), and pent-4-ynyl tosylate (10.5 mg, 0.044 mmol) in anhydrous DMF (85 μL) was stirred at 50 °C for 1 h. Following solvent removal, the product (4.2 mg, 31.9%) was collected through silica gel column purification (EtOAc/hexanes/$CHCl_3$/i-PrOH at 10:20:20:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (s, 1H), 6.61 (s, 1H), 5.98 (bs, 2H), 5.96 (s, 2H), 4.27 (s, 2H), 4.13 (t, J = 7.5 Hz, 2H), 2.24−2.10 (m, 2H), 2.00−1.91 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 1597, 158.0, 156.3, 150.6, 147.7, 147.2, 126.2, 125.3, 110.0, 102.0, 82.3, 69.9, 42.2, 31.4, 28.1, 15.8; MS m/z 388.1 $(M + H)^+$. HPLC: (a) 98.1% (65% water/35% acetonitrile); (b) 97.0% (from 35% to 45% acetonitrile).

2-Fluoro-8-(6-iodo-benzo[1,3]dioxol-5-ylmethyl)-9-(3-isopropylamino-prop yl)adenine (25). A solution of **18** (300 mg, 0.726 mmol), Cs_2CO_3 (285 mg, 0.87 mmol), and 1,3-dibromopropane (370 μL, 3.63 mmol) in anhydrous DMF (5 mL) was stirred at 50 °C for 2 h. Following solvent removal, product (330 mg, 85%) was collected through silica gel column purification ($CHCl_3$ then EtOAc/hexanes/$CHCl_3$/i-PrOH at 4:2:4:0.4). MS m/z 534.0 $(M + H)^+$. To this product, i-$PrNH_2$ (10 mL) was added in excess, and the resulting solution was stirred at room temperature for 1 h. Excess amine was removed and product (230 mg, 75%) collected through silica gel column purification ($CHCl_3$/EtOAc/i-PrOH/NH_4-OH at 4:4:2:0.3). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (s, 1H), 6.59 (s, 1H), 5.94 (s, 2H), 5.89 (bs, 2H), 4.25 (s, 2H), 4.11 (t, J = 7.0 Hz, 2H), 2.73−2.60 (m, 1H), 2.55 (t, J = 6.8 Hz, 1H), 1.93−1.86 (m, 2H), 1.03−1.02 (d, J = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, methanol-$d4$) δ 160.0, 158.4, 157.2, 152.4, 151.3, 149.4, 148.4, 133.1, 118.7, 110.6, 102.4, 88.5, 42.8, 40.1, 38.8, 27.6, 19.4; MS m/z 513.2 $(M + H)^+$. HPLC: (a) 98.5% (60% water/40% acetonitrile); (b) 97.2% (from 20% to 50% acetonitrile).

2-Amino-8-(3,4-dimethoxy-benzyl)adenine. Starting from 3,4-dimethoxyphenylacetic acid (3.0 g, 15.3 mmol) and 2,4,5,6-tetraaminopyrimidine (4.1 g, 29 mmol) and following the procedure for the synthesis of **16**, the desired product was obtained (1.8 g, 39.2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.90 (s, 1H), 6.85 (d, J = 8.0 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 6.44 (bs, 2H), 5.51 (bs, 2H), 3.88 (s, 2H), 3.70 (s, 3H), 3.68 (s, 3H); MS m/z 301.2 $(M + H)^+$.

2-Fluoro-8-(3,4-dimethoxy-benzyl)adenine (27). Starting from 2-amino-8-(3,4-dimethoxy-benzyl)adenine (0.66 g, 2.2 mmol) and following the procedure for the synthesis of **17**, the desired product was obtained (0.34 g, 51%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (bs, 2H), 7.03 (s, 1H), 6.94 (d, J = 8.3 Hz, 1H), 6.84 (d, J = 8.6 Hz, 1H), 4.07 (s, 2H), 3.80 (s, 3H), 3.77 (s, 3H); MS m/z 304.0 $(M + H)^+$.

2-Fluoro-8-(2-iodo-4,5-dimethoxy-benzyl)adenine (28). A solution of **27** (50 mg, 0.165 mmol), NIS (74 mg, 0.33 mmol), and TFA (18.8 mg, 12.7 μL, 0.165 mmol) in acetonitrile (120 μL) was stirred at room temperature for 24 h. Following solvent removal, the product (12 mg, 16.9%) was collected through silica gel column purification ($CHCl_3$/MeOH/AcOH at 80:1:0.5 to 30:1:0.5). MS m/z 430.1 $(M + H)^+$.

2-Fluoro-8-(2-bromo-4,5-dimethoxy-benzyl)adenine (29). A solution of **27** (65 mg, 0.226 mmol) and NBS (80 mg, 0.45 mmol) in DMF (0.75 mL) was stirred at room temperature for 2.5 h. Following solvent removal, the product (8.2 mg, 53.6%) was collected through silica gel column purification ($CHCl_3$/MeOH/AcOH at 80:1:0.5 to 30:1:0.5). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.13 (s, 1H), 7.09 (s, 1H), 6.80 (bs, 2H), 4.26 (s, 2H), 3.84 (s, 3H), 3.78 (s, 3H); MS m/z 382.0 $(M + H)^+$.

2-Fluoro-8-(2-chloro-4,5-dimethoxy-benzyl)adenine (30). A solution of **27** (40 mg, 0.132 mmol) and NCS (77.8 mg, 0.58 mmol) in anhydrous DMF (0.7 mL) was stirred at room temperature for 5.5 h. Following solvent removal, the product (22 mg, 49.4%) was collected through silica gel column purification ($CHCl_3$/EtOAc at 8:2 to 4:6). MS m/z 338.0 $(M + H)^+$.

2-Fluoro-8-(2-iodo-4,5-dimethoxy-benzyl)-9-(pent-4-ynyl)-adenine (31). A solution of 2-fluoro-8-(2-iodo-4,5-dimethoxy-benzyl)adenine (12 mg, 0.028 mmol), Cs_2CO_3 (9 mg, 0.028 mmol), and pent-4-ynyl tosylate (8.6 mg, 7 μL, 0.036 mmol) in anhydrous DMF (80 μL) was stirred at 50 °C for 1 h. Following solvent removal, the product (13.7 mg, 99%) was collected through silica gel column purification ($CHCl_3$/EtOAc/hexanes/i-PrOH at 20:10:20:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (s, 1H), 6.65 (s, 1H), 5.94 (bs, 2H), 4.29 (s, 2H), 4.13 (t, J = 7.3 Hz, 2H), 3.87 (s, 3H), 3.73 (s, 3H), 2.26−2.22 (m, 2H), 2.00 (t, J = 2.6 Hz, 1H), 1.97−1.90 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 153.2, 151.3, 150.0, 149.1, 130.9, 121.9, 112.6, 88.5, 82.5, 70.0, 56.4, 56.2, 42.6, 39.2, 28.4, 16.1; MS m/z 496.2 $(M + H)^+$. HPLC: (a) 99.9% (60% water/40% acetonitrile); (b) 96.8% (from 35% to 55% acetonitrile).

2-Fluoro-8-(2-bromo-4,5-dimethoxy-benzyl)-9-(pent-4-ynyl)-adenine (32). A solution of 8-(2-bromo-4,5-dimethoxy-benzyl)-2-fluoroadenine (13 mg, 0.034 mmol), Cs_2CO_3 (11 mg, 0.034 mmol), and pent-4-ynyl tosylate (10 mg, 9 μL, 0.044 mmol) in anhydrous DMF (80 μL) was stirred at 60 °C for 30 min. Following solvent removal, the product (8.2 mg, 53.6%) was collected through silica gel column purification ($CHCl_3$/EtOAc/hexanes/*i*-PrOH at 20:10:20:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.67 (s, 1H), 5.92 (bs, 2H), 4.31 (s, 2H), 4.14 (t, J = 7.4 Hz, 2H), 3.88 (s, 3H), 3.75 (s, 3H), 2.25−2.20 (m, 2H), 1.99 (t, J = 2.6 Hz, 1H), 1.96−1.89 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.0, 158.3, 156.7, 153.4, 151.3, 149.4, 127.2, 117.3, 115.9, 114.5, 113.2, 82.7, 70.2, 56.62, 56.56, 42.7, 34.3, 30.1, 28.5, 16.3; MS *m*/*z* 447.9 $(M + H)^+$. HPLC: (a) 99.0% (60% water/40% acetonitrile); (b) 98.8% (from 35% to 55% acetonitrile).

2-Fluoro-8-(2-chloro-4,5-dimethoxy-benzyl)-9-(pent-4-ynyl)-adenine (33). A solution of 8-(2-chloro-4,5-dimethoxy-benzyl)-2-fluoroadenine (22 mg, 0.065 mmol), Cs_2CO_3 (21 mg, 0.065 mmol), and pent-4-ynyl tosylate (20 mg, 17.3 μL, 0.085 mmol) in anhydrous DMF (170 μL) was stirred at 50 °C for 2 h. Following solvent removal, the product (14 mg, 53.8%) was collected through silica gel column purification ($CHCl_3$/EtOAc/hexanes/*i*-PrOH at 20:10:20:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (s, 1H), 6.67 (s, 1H), 6.01 (bs, 2H), 4.31 (s, 2H), 4.14 (t, J = 7.5 Hz, 2H), 3.87 (s, 3H), 3.75 (s, 3H), 2.24−2.20 (m, 2H), 2.01−1.99 (m, 1H), 1.97−1.88 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 1596, 157.9, 156.3, 152.3, 148.9, 146.0, 124.9, 112.7, 82.3, 69.9, 56.3, 56.2, 42.2, 31.2, 28.1, 15.9; MS *m*/*z* 404.1 $(M + H)^+$. HPLC: (a) 95.1% (65% water/35% acetonitrile); (b) 96.7% (from 35% to 45% acetonitrile).

Acknowledgment. This work was supported by AACR-Cancer Research and Prevention Foundation, the Susan G. Komen Breast Cancer Foundation, Mr. William H. Goodwin and Mrs. Alice Goodwin, and the Commonwealth Cancer Foundation for Research and the Experimental Therapeutics Center of Memorial Sloan-Kettering Cancer Center.

Supporting Information Available: Results from spectral data, HPLC, and LC−MS analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Mosser, D. D.; Morimoto, R. I. Molecular chaperones and the stress of oncogenesis. *Oncogene* **2004**, *23*, 2907−2918.

(2) (a) Kamal, A.; Thao, L.; Sensintaffar, J.; Zhang, L.; Boehm, M. F.; Fritz, L. C.; Burrows, F. J. Therapeutic and diagnostic implications of Hsp90 activation. *Trends Mol. Med.* **2004**, *10*, 283−290. (b) Neckers, L. Hsp90 inhibitors as novel cancer chemotherapeutic agents. *Trends Mol. Med.* **2002**, *8*, S55−S61. (c) Workman, P.; Maloney, A. HSP90 as a new therapeutic target for cancer therapy: the story unfolds. *Expert Opin. Biol. Ther.* **2002**, *2*, 3−24. (d) Chiosis, G.; Vilenchik, M.; Kim, J.; Solit, D. Hsp90: the vulnerable chaperone. *Drug Discovery Today* **2004**, *9*, 881−888.

(3) (a) Panaretou, B.; Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone in vivo. *EMBO J.* **1998**, *17*, 4829−4836. (b) Obermann, W. M.; Sondermann, H.; Russo, A. A.; Pavletich, N. P.; Hartl, F. U. In vivo function of Hsp90 is dependent on ATP binding and ATP hydrolysis. *J. Cell Biol.* **1998**, *143*, 901−910.

(4) (a) Wegele, H.; Muller, L.; Buchner, J. Hsp70 and Hsp90-a relay team for protein folding. *Rev. Physiol. Biochem. Pharmacol.* **2004**, *151*, 1−44. (b) Prodromou, C.; Pearl, L. H. Structure and functional relationships of Hsp90. *Curr. Cancer Drug Targets* **2003**, *5*, 301−323.

(5) (a) Kamal, A.; Thao, L.; Sensintaffar, J.; Zhang, L.; Boehm, M. F.; Fritz, L. C.; Burrows, F. J. A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. *Nature* **2003**, *425*, 407−410. (b) Vilenchik, M.; Solit, D.; Basso, A.; Huezo, H.; Lucas, B.; He, H.; Rosen N.; Spampinato, C.; Modrich, P.; Chiosis, G. Targeting wide-range oncogenic transformation via PU24FCl, a specific inhibitor of tumor Hsp90. *Chem. Biol.* **2004**, *11*, 787−797.

(6) Chene, P. ATPases as drug targets: learning from their structure. *Nat. Rev. Drug Discovery* **2002** *9*, 665−673.

(7) Chiosis, G.; Lucas, B.; Huezo, H.; Solit, D.; Basso, A.; Rosen, N. Development of purine-scaffold small molecule inhibitors of Hsp90. *Curr. Cancer Drug Targets* **2003**, *3*, 363−368.

(8) Chiosis, G.; Timaul, M. N.; Lucas, B.; Munster, P. N.; Zheng, F. F.; Sepp-Lorenzino, L.; Rosen, N. A small molecule designed to bind to the adenine nucleotide pocket of Hsp90 causes Her2 degradation and the growth arrest and differentiation of breast cancer cells. *Chem. Biol.* **2001**, *8*, 289−299.

(9) (a) Chiosis, G.; Lucas, B.; Shtil, A.; Huezo, H.; Rosen, N. Development of a purine-based novel class of Hsp90 binders that inhibit the proliferation of cancer cells and induce the degradation of Her2 tyrosine kinase. *Bioorg. Med. Chem.* **2002**, *10*, 3555−3564. (b) Chiosis, G.; Rosen, N. PCT Int. Appl. W. O. 20020236075, 2002. (c) Kasibhatla, S. R.; Hong, K.; Zhang, L.; Biamonte, M. A.; Boehm, M. F.; Shi, J.; Fan, J. PCT Int. Appl. W. O. 2003037860, 2003. (d) Dymock, B.; Barril, X.; Beswick, M.; Collier, A.; Davies, N.; Drysdale, M.; Fink, A.; Fromont, C.; Hubbard, R. E.; Massey, A.; Surgenor, A.; Wright, L. Adenine derived inhibitors of the molecular chaperone HSP90−SAR explained through multiple X-ray structures. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 325−328.

(10) Llauger, L.; He, H.; Kim, J.; Aguirre, J.; Rosen, N.; Peters, U.; Davies, P.; Chiosis, G. 8-Arylsulfanyl and 8-arylsulfoxyl adenine derivatives as inhibitors of the heat shock protein 90. *J. Med. Chem.* **2005**, *48*, 2892−2905.

(11) He, H.; Llauger, L.; Rosen, N.; Chiosis, G. General method for the synthesis of 8-arylsulfanyl adenine derivatives. *J. Org. Chem.* **2004**, *69*, 3230−3232.

(12) Manka, J. T.; McKenzie, V. C.; Kaszynski, P. Azo group-assisted nucleophilic aromatic substitutions in haloarene derivatives: preparation of substituted 1-iodo-2,6-bispropylthiobenzenes. *J. Org. Chem.* **2004**, *69*, 1967−1971.

(13) Naidis, F. B.; Kolesova, M. B.; Aleksander, K. L.; Smirnova, N. V.; Pernikoza, V. G. Method of obtaining 8-bromoadenine. *Izobreteniya* **1995**, *1*, 242−243.

(14) Castanet, A.-S.; Colobert, F.; Broutin, P.-E. Mild and regioselective iodination of electron-rich aromatics with *N*-iodosuccinimide and catalytic trifluoroacetic acid. *Tetrahedron Lett.* **2002**, *43*, 5047−5048.

(15) Tipson, R. S. On esters of *p*-toluenesulfonic acid. *J. Org. Chem.* **1944**, *9*, 235−241.

(16) Tarbell, D. S.; Yamamoto, Y.; Pope, B. M. New method to prepare *N*-*t*-butoxycarbonyl derivatives and the corresponding sulfur analogues from di-*tert*-butyl dicarbonate or di-*tert*-butyl dithiol dicarbonates and amino acids. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 730−732.

(17) (a) Mitsunobu, O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* **1981**, *1*, 1−28. (b) Lucas, B.; Rosen, N.; Chiosis, G. Facile synthesis of 9-alkyl-8-benzyl-9*H*-purin-6-ylamine derivatives. *J. Comb. Chem.* **2001**, *3*, 518−520.

(18) Salvatore, R. N.; Nagle, A. S.; Jung, K. W. Cesium effect: high chemoselectivity in direct N-alkylation of amines. *J. Org. Chem.* **2002**, *67*, 674−683.

(19) Olah, G. A.; Nojima, M.; Kerekes, I. Synthetic methods and reactions; IV.[1] Fluorination of carboxylic acids with cyanuric fluoride. *Synthesis* **1973**, *8*, 487−488.

(20) (a) Fukuhara, T.; Yoneda, N.; Suzuki, A. A facile preparation of fluoropyridines from aminopyridines via diazotization and fluorodediazoniation in HF or HF-pyridine solutions. *J. Fluorine Chem.* **1988**, *38*, 435−438. (b) Thompson, A. M.; Showalter, H. D. H.; Denny, W. A. Synthesis of 7-substituted 3-aryl-1,6-naphthyridin-2-amines and 7-substituted 3-aryl-1,6-naphthyridin-2(1H)-ones via diazotization of 3-aryl-1,6-naphthyridine-2,7-diamines. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1843−1852.

(21) (a) Day, R. A.; Blake, J. A.; Stephens, C. E. Convenient and improved halogenation of 3,5-diarylisoxazoles using *N*-halosucinimides. *Synthesis* **2003**, *10*, 1586−1590. (b) Tanemura, K.; Suzuki, T.; Nishida, Y.; Satsumababayashi, K.; Horaguchi, T. Halogenation of aromatic compounds by *N*-chloro-, *N*-bromo-, and *N*-iodosuccinimide. *Chem. Lett.* **2003**, *32*, 932−934.

(22) (a) Llauger-Bufi, L.; Felts, S. J.; Huezo, H.; Rosen, N.; Chiosis, G. Synthesis of novel fluorescent probes for the molecular chaperone Hsp90. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3975−3978. (b) Kim, J.; Felts, S.; Llauger, L.; He, H.; Huezo, H.; Rosen, N.; Chiosis, G. Development of a fluorescence polarization assay for the molecular chaperone Hsp90. *J. Biomol. Screening* **2004**, *9*, 375−381.

(23) Mimnaugh, E. G.; Chavany, C.; Neckers, L. Polyubiquitination and proteasomal degradation of the p185c-erbB-2 receptor protein-tyrosine kinase induced by geldanamycin. *J. Biol. Chem.* **1996**, *271*, 22796−22801.

(24) Srethapakdi, M.; Liu, F.; Tavorath, R.; Rosen, N. Inhibition of Hsp90 function by ansamycins causes retinoblastoma gene product-dependent G1 arrest. *Cancer Res.* **2000**, *60*, 3940−3946.

(25) Huezo, H.; Vilenchik, M.; Rosen, N.; Chiosis, G. Microtiter cell-based assay for detection of agents that alter cellular levels of Her2 and EGFR. *Chem. Biol.* **2003**, *10*, 629−634.

(26) Chiosis, G.; Rosen, N.; Huezo, H. Assays for identification of bioactive compounds that interact with heat shock protein 90. PCT Int. Appl. W. O. 2005012482, 2005.
(27) (a) Eiseman, J. L.; Lan, J.; Lagattuta, T. F.; Hamburger, D. R.; Joseph, E.; Covey, J. M.; Egorin, M. J. Pharmacokinetics and pharmacodynamics of 17-demethoxy 17-[[(2-dimethylamino)ethyl]amino]-geldanamycin (17DMAG, NSC 707545) in C.B-17 SCID mice bearing MDA-MB-231 human breast cancer xenografts. *Cancer Chemother. Pharmacol.* **2005**, *55*, 21−32. (b) Banerji, U.; Walton, M.; Raynaud, F.; Grimshaw, R.; Kelland, L.; Valenti, M.; Judson, I.; Workman, P. Pharmacokinetic-pharmacodynamic relationships for the heat shock protein 90 molecular chaperone inhibitor 17-allylamino, 17-demethoxygeldanamycin in human ovarian cancer xenograft models. *Clin. Cancer Res.* **2005**, *11*, 7023−7032.
(28) Kelland, L. R.; Sharp, S. Y.; Rogers, P. M.; Myers, T. G.; Workman, P. DT-diaphorase expression and tumor cell sensitivity to 17-allylamino-17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. *J. Natl. Cancer Inst.* **1999**, *91*, 1940−1949.
(29) Krishna Mohan, K. V. V.; Narender, N.; Kulkarni, S. J. Simple and regioselective oxyiodination of aromatic compounds with ammonium iodide and oxone. *Tetrahedron Lett.* **2004**, *45*, 8015−8018.
(30) Orito, K.; Hatakeyama, T.; Takeo, M.; Suginome, H. Iodination of alkyl aryl ethers by mercury(II) oxide-iodine reagent in dichloromethane. *Synthesis* **1995**, *10*, 1273−1277.
(31) Hubig, S. M.; Jung, W.; Kochi, J. K. Cation radicals as intermediates in aromatic halogenation with iodine monochloride: Solvent and salt effects on the competition between chlorination and iodination. *J. Org. Chem.* **1994**, *59*, 6233−6244.

JM0508078