Evaluation of 8-Arylsulfanyl, 8-Arylsulfoxyl, and 8-Arylsulfonyl Adenine Derivatives as Inhibitors of the Heat Shock Protein 90

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Hsp90 is a chaperone protein with important roles in maintaining transformation and in elevating the survival and growth potential of cancer cells. Currently there is an increasing interest in developing inhibitors of this protein as anticancer therapeutics. One of such inhibitors, the purine-scaffold class, has been reported to be potent and selective against Hsp90 both in vitro and in vivo models of cancer. Here, a series of 8-arylsulfanyl, -sulfoxyl, and -sulfonyl adenine members of the purine 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. Our results suggest that 8-arylsulfanyl adenine derivatives are good inhibitors of chaperone activity, whereas oxidation of the sulfides to sulfoxides or sulfones leads to compounds of decreased activity. The study identifies derivative **11v** as the most potent Hsp90 inhibitor of the purine-scaffold series published to date (EC_{50} = 30 nM), and also as the compound of this class with highest selectivity for tumor vs normal cell Hsp90 (700 to 3000-fold). Most rewardingly, this work has allowed for the identification of Hsp90 inhibitors with selective affinities for Hsp90-client protein complexes, derivatives that may represent useful pharmacological tools in dissecting Hsp90-regulated processes.

Introduction

The chaperone heat shock protein 90 (Hsp90) is an emerging target in cancer treatment due to its important roles in maintaining transformation and in increasing the survival and growth potential of cancer cells.[1] Hsp90 function is regulated by a pocket in the N-terminal region of the protein that binds and hydrolyzes ATP.[1a] Occupancy of this pocket by high affinity ligands prevents the dissociation of Hsp90 client proteins from the chaperone complex, and as a consequence, the trapped proteins do not achieve their mature functional conformation and are degraded by the proteasome. Protein clients of Hsp90 are mostly kinases, steroid receptors, and transcriptional factors involved in driving multistep malignancy and, in addition, mutated oncogenic proteins required for the transformed phenotype. Examples include Her2, Raf-1, Akt, Cdk4, cMet, mutant p53, ER, AR, mutant BRaf, Bcr-Abl, Flt-3, Polo-1 kinase, HIF-1 alpha, and hTERT.[1c−e] Degradation of these proteins by Hsp90 inhibitors leads to cell-specific growth arrest and apoptosis in cancer cells in culture and to tumor growth inhibition or regression in animal models. One such inhibitor, **1** (17-allyl-aminodesmethoxy-geldanamycin; 17AAG, see Figure 1) has entered clinical trials in cancer patients in the US and UK and has shown early evidence of therapeutic activity when administered alone or in combination with docetaxel.[2] Despite these early promising results, **1** has several potential limitations. Most prominent are its limited solubility and cumbersome formulation. It also exhibits dose and schedule dependent liver toxicity believed to be caused by the benzoquinone functionality.[2a] A structurally unrelated natural product, **2** (radicicol; RD, see Figure 1) has biological activity similar to that of **1** but is not hepatotoxic,[3] yet no derivative of this class has made it into clinic. Making use of the peculiar bent shape of Hsp90 inhibitors and of existent Hsp90 crystal data, we were able to design a class of purine-scaffold derivatives with Hsp90 inhibitory activities.[4] The first synthesized derivative of this class, **3** (PU3, see Figure 2), bound Hsp90 with moderate affinity and elicited cellular effects that mimic **1** addition.[5] Preliminary efforts focused at improving the potency of this agent have mostly focused on modifying the left side of the scaffold (see Figure 2) and have led to the synthesis of several compounds with improved activity in both biochemical and cellular assays.[6] One such compound, **4** (PU24FCl, Figure 2), is a potent and selective inhibitor of tumor Hsp90 and exhibits antitumor activities in both in vitro and in vivo models of cancer.[7] Other researchers have adopted our purine-scaffold and furthered structure−activity relationship (SAR) studies, their efforts resulting in compounds with higher potency over **4** in in vitro models of cancer.[8,9] Although a significant

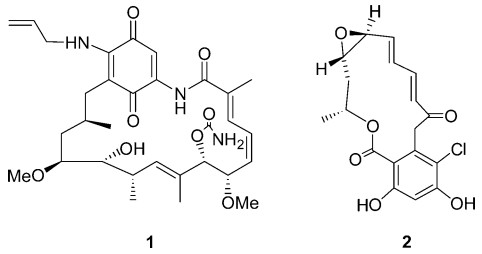

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Figure 1. Natural product Hsp90 inhibitors.

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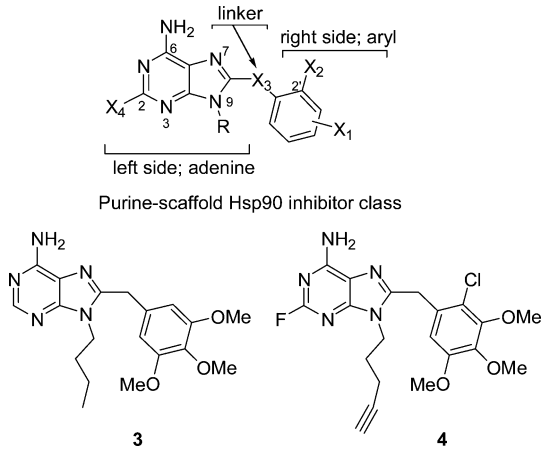


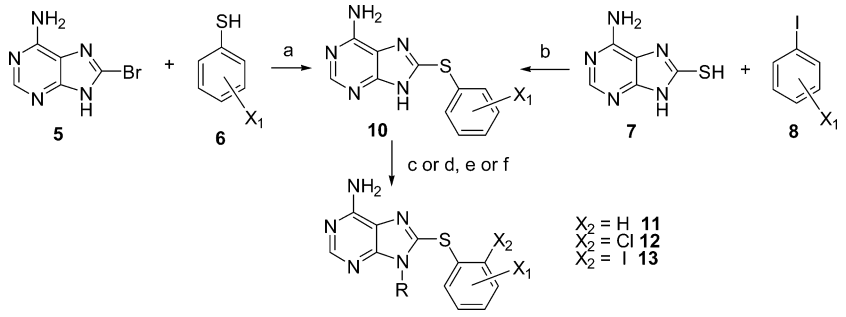
Figure 2. Schematic representation of the purine-scaffold Hsp90 inhibitor class, and its representative derivatives, compounds **3** and **4**.

number of derivatives has been created by these combined efforts, the nature and position of substituents on the right side aryl moiety (X_1 and X_2, Figure 2) has not been sufficiently investigated. A crystal structure of the purine derivative **3** in complex with Hsp90α[8] proposes that its right side aromatic moiety is involved in π-stacking interactions with a phenylalanine residue. It also suggests that the ligand establishes close hydrophobic interactions with the pocket in this region, and thus subtle modifications on the aryl moiety may lead to a dramatic SAR. A more detailed analysis of favorable substituents in this area is thus warranted. Identification of such substitutions is unfortunately not straightforward using the existent chemical methodology when X_3 (see Figure 2) is methylene. The use of phenyl acetic acid derivatives[6] in the formation of the purine scaffold limits the number of alternatives as few such reagents are commercially available. Our efforts[4,6] paralleled by work Kasibhatla et al.[9] have focused on changing the nature of the linker between the purine and aryl moieties (X_3, Figure 2), searching for compounds with retained activity while chemically more flexible. Attempts to change the nature of the linker from carbon to oxygen or nitrogen have led to compounds with diminished biological activity.[6,9] Interestingly, introduction of sulfur in this position resulted in compounds with retained activity against Hsp90.[9] As a higher diversity of reagents is available for building the S-linker (X_3 = S) derivatives, this observation prompts for a detailed exploration of favorable X_1 and X_2 substitutions on the phenyl moiety. Here we present the synthesis of such 8-arylsulfanyl, -sulfoxyl, and -sulfonyl adenine derivatives, respectively, and their structure−activity relationship as inhibitors of Hsp90.

Results and Discussions

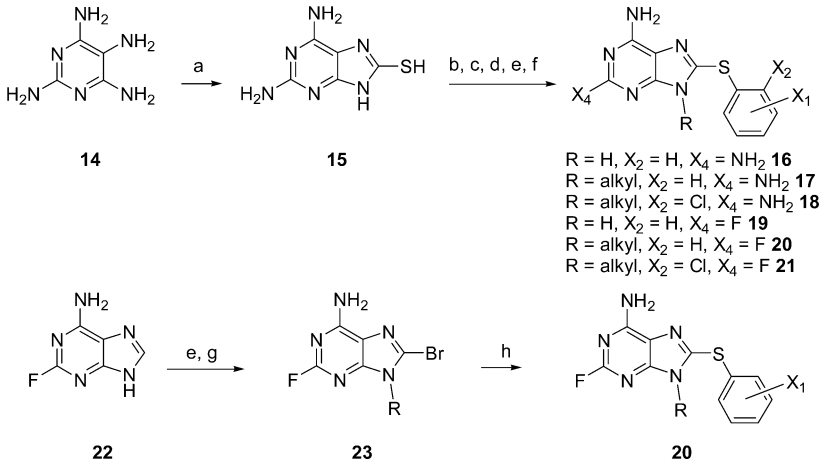
Chemistry. Formation of a sulfur link between adenine and the phenyl ring (X_3 = S, Figure 2) may be obtained either by nucleophilic attack of the arylthiolate anion on 8-bromoadenine (Step a, Scheme 1)[9,10] or by the copper-catalyzed coupling of aryliodides with mercaptoadenine (Step b, Scheme 1).[11] Our method for the formation of 8-arylsulfanyl adenine derivatives (**10**) from 8-mercaptoadenine (**7**) and aryl iodides (**8**) used CuI/neocuproine as catalyst and NaO*t*-Bu/DMF as the base/solvent combination.[11c] The reaction occurred in anhydrous DMF at 110 °C under nitrogen to generate the products in good yields. If bromine or chlorine were present on the aryl moiety, the coupling required milder basic condition and utilized Na_3PO_4 or K_2CO_3 instead of NaO*t*-Bu. Although less attractive due to thiophenols' limited commercial availability, stench, and tendency to quickly oxidize, coupling of 8-bromoadenine (**5**) with thiophenols **6** in the presence of a base has also been used. When not commercially available, thiophenols could be generated by a modified Leuckart thiophenol reaction[12] starting from the corresponding arylamine. In a first step, the arylamines were converted via the aryl diazonium salt to aryl xanthates which afforded thiophenols on reduction with $LiAlH_4$ or on warming with a base solution. The 8-arylsulfanyl adenines **10** obtained in these coupling reactions were further alkylated at the position 9-*N* with butyl, pent-4-ynyl, or 2-isopropoxy-ethyl chains. The presence of these chains at the 9-*N* position was previously determined to be favored by the Hsp90 pocket.[6] Their introduction was carried out using a Mitsunobu type reaction[13b] between the alcohol and the respective 8-arylsulfanyl adenines **10** in toluene/CH_2Cl_2 to result in the corresponding 9-*N*-alkyl-8-arylsulfanyl adenines **11**. Alternatively, derivatives **10** were alkylated using tosylates of the corresponding alcohols[14] in the presence of Cs_2CO_3 or K_2CO_3 in DMF. Formation of the 3-*N* (5−30%) and 7-*N* (1−10%) isomers was observed by these two procedures; however, these byproducts could be removed by column chromatography.[6,13b] Treatment of **10** with base and an alkylating agent tended to result in higher percentage of 3- and 7-*N* isomers compared to alcohol treatment under the Mitsunobu conditions. If further introduction

Scheme 1[a]



[a] Reagents and conditions: (a) K_2CO_3, DMF, 90−120 °C, 29−85%; (b) NaO*t*-Bu, K_2CO_3 or Na_3PO_4, neocuproine, CuI, DMF, 110 °C, 40−80%; (c) ROH, PPh_3, DBAD, toluene:CH_2Cl_2, rt, 40−70%; (d) ROTs, Cs_2CO_3 or K_2CO_3, DMF, 80 °C, 10−80%; (e) HCl, *t*-BuOOH, MeOH, reflux, 31−66%; (f) NIS, CF_3COOH, acetonitrile, rt, 69%. Method A: a, c; Method B: a, c, e; method C: b, d; Method D: a, f, c.

Scheme 2[a]



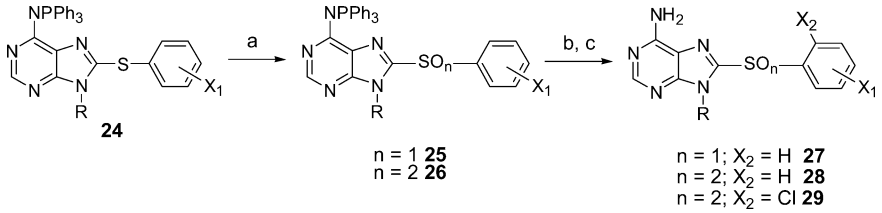
[a] Reagents and conditions: (a) CS_2, $NaHCO_3$, H_2O/EtOH, reflux, 97%; (b) ArI, NaO*t*-Bu, CuI, ethylene glycol, 130 °C, 60%; (c) HF-pyridine, $NaNO_2$, −40 °C to room temperature, 15−20%; (d) ROH, PPh_3, DBAD, toluene:CH_2Cl_2, rt, 50%; (e) ROTs, Cs_2CO_3, DMF, 80 °C, 50%; (f) HCl, *t*-BuOOH, 1,4-dioxane or *i*-PrOH, 80 °C, 20−70%; (g) NBS, DMF, rt, 80%; (h) ArSH, K_2CO_3, DMF, 130 °C, 85%. Method E: a, b, c, d; Method F: a, b, c, d, f; Method G: a, b, e, f, c; Method H: e, g, h.

of chlorine on the aryl moiety was desired (X_2 = Cl, Figure 2), the 9-*N*-alkyl-8-arylsulfanyl adenines **11** were subjected to positive chlorine species generated in situ from HCl/*t*-BuOOH to result in derivatives **12**.[15] It is believed that chlorination in this reagent conditions proceeds via the formation of *tert*-butylhypochlorite. The reaction resulted preferentially in ortho-chlorinated products. If iodine was further added to the aryl moiety (X_2 = I, Figure 2), derivatives **10** were reacted with *N*-iodosuccinimide in trifluoroacetic acid followed by 9-*N* alkylation under the Mitsunobu conditions to generate derivatives **13**.[16] The active species for this iodination is probably the in situ formed iodine trifluoroacetate that can act as a very reactive electrophile, allowing iodination in 1 h at room temperature.

Several derivatives were prepared by the introduction of fluorine at position C2 of the adenine moiety (Scheme 2). We and others[6,8,9] have previously determined that fluorine in this position in general increased the solubility and/or potency of the resulting purines. For the preparation of such derivatives, synthesis commenced with the condensation of the commercially available 2,4,5,6-tetraaminopyrimidine sulfate (**14**) with carbon disulfide in a refluxing solution of $NaHCO_3$ in aqueous EtOH.[17] The resulting 2-amino-8-mercaptoadenine (**15**) was further coupled with aryl iodides in the presence of NaO*t*-Bu and CuI in ethylene glycol[11b] to give 2-amino-8-arylsulfanyl adenines **16**. The reaction did not proceed using the method published by He et al.[11c] as **15** did not dissolve in DMF or most organic solvents for that matter. Unsaturated chains at position 9-*N* were introduced at this stage to result in the 2-amino-9-*N*-alkyl-8-arylsulfanyl adenines **17**. The reaction required the use of K_2CO_3 or Cs_2CO_3 and an alkylating agent in DMF due to the poor solubility of **16** in the Mitsunobu reaction solvent. If further, introduction of chlorine at X_2 on the aryl moiety was desired, compounds **17** were subjected to HCl/*t*-BuOOH to result preferentially in ortho-chlorinated compounds (**18**). Transformation of the C2-amino group to fluorine (X_4 = NH_2 to F) was conducted by diazotization−fluorodediazoniation of the amino derivative in HF/pyridine in the presence of $NaNO_2$[6,18] to yield the corresponding 2-fluoro-9-*N*-alkyl-8-arylsulfanyl adenine derivatives **21**. This reaction gave significantly higher yields over the previously published method[9] using HBF_4/*iso*-amyl nitrite, likely to be due at least in part to the more anhydrous nature of this solvent system which reduces the proportion of hydrolysis. Synthesis of derivatives containing both an unsaturated chain at 9-*N* (i.e. pent-4-ynyl) and fluorine at the C2 position of the adenine moiety (X_4 = F) required a different strategy to avoid fluorine addition to the triple bond. Compound **16** was first subjected to introduction of fluorine at C2 to result in the corresponding 2-fluoro-8-arylsulfanyl adenine **19**. Surprisingly, conducting the reaction at room-temperature resulted in addition of fluorine at both C2 and C6 of the purine moiety. This problem was averted by lowering the reaction temperature to −40 °C. Addition of fluorine at position C2 significantly increased the solubility of these purines in organic solvents. Thus, further alkylation could be easily conducted using the Mitsunobu reaction in toluene:CH_2Cl_2 to result in derivatives **20**. Introduction of chlorine (X_2 = Cl), again with HCl/*t*-BuOOH, led to formation of **21**. Alternatively, synthesis of 2-fluoro-9-*N*-alkyl-8-arylsulfanyl adenines **20** was started with the commercially available 2-fluoroadenine (**22**) (Scheme 2). This reagent was first 9-*N* alkylated by Cs_2CO_3/tosylate treatment followed by 8-bromination with *N*-bromosuccinimide (NBS)[19] to result in 2-fluoro-9-*N*-alkyl-8-bromo adenine **23**. Its coupling with thiophenols generated the 2-fluoro-9-*N*-alkyl-8-arylsulfanyl adenines **20**.

Syntheses of 8-arylsulfoxyl adenine derivatives **27** and 8-arylsulfonyl adenine derivatives **28** (Scheme 3) were previously reported by Llauger et al.[20] Briefly, synthesis started with the preparation of C6-NH_2 triphenylphosphine protected[21] 9-*N*-alkyl-8-arylsulfanyl adenine derivatives **24** in a two-step (alkylation−protection) one-pot reaction from the corresponding 8-arylsulfanyl adenines **10** using the Mitsunobu conditions[13b] followed by addition of an excess of PPh_3 and di-*tert*-butyl azodicarboxylate (DBAD). Oxidation of **24** with OXONE in the presence of alumina[22] allowed for monitoring the reaction to either sulfoxide (**25**) or sulfone (**26**). Deprotection of the C6-NH_2 triphenylphosphine group was conveniently conducted in refluxing AcOH/EtOH[21] to result in good yields in the correspond-

Scheme 3[a]



[a] Reagents and conditions: (a) OXONE, alumina, CH_2Cl_2, rt, 35−70%; (b) AcOH, EtOH, reflux, 60−70%; (c) HCl, *t*-BuOOH, MeOH, 80 °C, 43%.

ing 8-arylsulfoxyl adenine derivatives **27** and 8-arylsulfonyl adenine derivatives **28**. The latter could be chlorinated at C′2 (X_2 = Cl) by HCl/*t*-BuOOH treatment to result in derivatives **29**.

Biological Testing. Compounds synthesized above were tested in a biochemical assay and also in cellular assays that probe for cellular fingerprints of Hsp90 inhibition.[1] The biochemical assay tests competitive binding of compounds to recombinant Hsp90α protein and also Hsp90 found in cell specific complexes and uses a fluorescence polarization method.[7,23] When using cell lysates instead of recombinant protein, the assay measures binding to average Hsp90 population found in cell specific complexes.[7]

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[24] and (b) mitotic block in RB-defective cells.[25] 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.[24] We have previously reported a fast microtiter immunoassay able of quantifying cellular levels of Her2 following drug treatments.[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.[25] 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.[27] This antibody (Tg-3), originally described as a marker of Alzheimer's disease, is highly specific for mitotic cells, Tg-3 immunofluorescence being >50-fold more intense in mitotic cells than in interphase cells.[28]

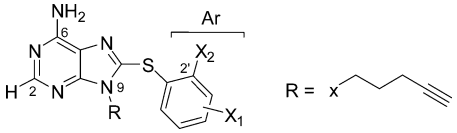
In addition, the cytotoxicity of these agents against SKBr3 breast cancer cells was determined. A selected number of most active purines was also tested for possible toxicity against a normal cell line, renal proximal tubular epithelial cells (RPTEC).[7]

Structure−Activity Relationships. A comprehensive analysis of substituents on the right side aryl moiety (X_1 and X_2) was conducted to determine favorable combinations (Table 1). In a first permutation, the effect of a single substituent on activity was studied. As previously reported for derivatives containing a methylene linker[6,8,9] (X_3 = CH_2, Figure 2), monosubstitution in the meta-position was also favored here over ortho- and para-positions (for methoxy substituent, **11e1** vs **11a** vs **11f**). Effects were substituent-dependent − methoxy (**11a**) and hydroxymethyl (**11c**) were not allowed in the ortho-position while trifluoromethoxy (**11b**) retained activity. Chlorine (**11g**), methoxy (**11f**), and methylcarbonyl (**11h**) were permitted in the para-position; however, a larger and more rigid substituent such as pyrrolyl (**11i**) abolished activity. For a two-substituent analysis, derivatives with combinations 2,4; 2,5; and 3,5 were synthesized. A preference for 2,5-substituents was evident, with the 2-iodo-5-methoxy combination (**11q**) most favored as previously reported by Kasibhatla et al.[9] Changing methoxy into trifluoromethyl at position 5 decreased activity by 10-fold (**11o** vs **11r**). In an iso-subtituent series of 2,5-derivatives, methoxy (**11o**) was favored over chlorine (**11n**), which was better than methyl (**11m**). In a series in which position 5 was kept constant as methoxy and position 2 was varied, activity changed in the order: methoxy = chlorine (**11o** and **12a**) < bromine (**11p**) < iodine (**11q**). These compounds exhibited similar cellular potencies in the 1 to 4 μM range and were the only ones of the 2,5-series to block MDA-MB-468 cells in mitosis (Table 1, column 7). For a three-substituent analysis, derivatives with combinations 2,4,5 and 3,4,5 were synthesized. Not surprisingly, as observed in the methylene linker series,[6] 3,4,5-trimethoxy (**11w1**) was a favorable combination of whose activity was increased by addition of chlorine in ortho-position (**12b**) and almost abolished by larger moieties such as iodine (**13**). Building onto the 2,5-position led to **11v**, a nanomolar potency compound in both biochemical and cellular assays (Table 1; $EC_{50}^{Hsp90\alpha}$ = 30 ± 0.5 nM, IC_{50}^{Her2} = 300 ± 5 nM; IC_{50}^{SKBr3} = 200 ± 10 nM). This compound is the most potent purine-scaffold derivative published to date. Derivative **11q** previously reported to degrade Her2 in the breast cancer cell line MCF7 (a cell line with medium expression of Her2) with an IC_{50} of 300 nM,[9] was less active in the Her2 overexpressing cell line SKBr3, an IC_{50} of 810 ± 30 nM being determined in our hands (Table 1).

Interestingly, for many derivatives presented in Table 1, moderate Hsp90α binding affinity translated into Her2 degradation potential and inhibition of growth in SKBr3 cells but did not result in antimitotic activity (**11b**, **11e1**, **11g**, **11k**, **11n**, **11r**, **11u**, and **11w1**). In three separate cases (**11j**, **11m**, and **13**), no cellular activity was observed despite moderate binding, possibly due to poorer permeability profiles of these compounds into cells.

We tested further if previous observations made in the methylene linker series translated to the sulfur linker series (X_3 = CH_2 vs S, Figure 2). For this purpose, iso-

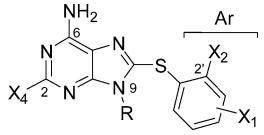
Table 1. Effect of 8-Aryl Substituents on the Activity of 9-(Pent-4-ynyl)-8-arylsulfanyl- Adenines as Inhibitors of Hsp90



compd	method	Ar	EC_{50} Hsp90α[a]	IC_{50} Her2[a]	IC_{50} SKBr3[a]	antimitotic
11a	C	2-OMe-Ph	34.9 ± 4.4	>400	108 ± 10	−
11b	C	2-OCF_3-Ph	5.6 ± 0.6	97.0 ± 6.3	86.0 ± 4.2	−
11c	C	2-CH_2OH-Ph	85.1 ± 24.2	>400	>500	−
11d	C	2-Cl-Ph	42.2 ± 21.0	195 ± 13	122 ± 20	−
11e1	A	3-OMe-Ph	0.97 ± 0.01	28.3 ± 6.3	36.2 ± 1.6	−
11f	C	4-OMe-Ph	19.1 ± 5.4	75.0 ± 8.8	41.4 ± 4.8	−
11g	C	4-Cl-Ph	3.4 ± 1.1	44.7 ± 8.3	30.2 ± 3.7	−
11h	C	4-COMe-Ph	2.7 ± 1.0	>150	>150	−
11i	C	4-Pyrrolyl-Ph	>100	311 ± 21	121 ± 20	−
11j	C	2,4-OMe_2-Ph	2.0 ± 0.2	>150	>150	−
11k	C	2,4-Me_2-Ph	8.6 ± 1.2	39.2 ± 2.9	31.8 ± 3.4	−
11l	C	2,4-Cl_2-Ph	37.5 ± 2.3	355 ± 15	>500	−
11m	C	2,5-Me_2-Ph	13.4 ± 7.9	147 ± 21	467 ± 50	−
11n	C	2,5-Cl_2-Ph	2.1 ± 0.7	26.5 ± 2.1	44.1 ± 3.9	−
11o	A	2,5-OMe_2-Ph	0.19 ± 0.02	3.2 ± 0.3	1.9 ± 0.1	+
12a	B	2-Cl-5-OMe-Ph	0.20 ± 0.06	3.5 ± 0.7	3.2 ± 0.8	+
11p	C	2-Br-5-OMe-Ph	0.07 ± 0.004	1.45 ± 0.15	1.1 ± 0.01	+
11q	A	2-I-5-OMe-Ph	0.05 ± 0.005	0.81 ± 0.03	0.57 ± 0.12	+
11r	C	2-Cl-5-CF_3-Ph	3.7 ± 0.1	35.9 ± 2.7	21.5 ± 2.1	−
11s	C	3,5-Cl_2-Ph	30.4 ± 4.4	60.5 ± 12.7	144 ± 31	−
11t	C	3,5-Me_2-Ph	10.6 ± 0.1	>100	34.4 ± 4.5	−
11u	C	2,4,5-Cl_3-Ph	8.5 ± 0.1	58.0 ± 1.4	48.4 ± 2.5	−
11v	C	2-Br-4,5-(OCH_2O)-Ph	0.03 ± 0.005	0.3 ± 0.05	0.2 ± 0.01	+
11w1	A	3,4,5-OMe_3-Ph	1.5 ± 0.3	22.3 ± 0.8	27.2 ± 2.3	−
12b	B	2-Cl-3,4,5-OMe_3-Ph	0.18 ± 0.05	9.1 ± 2.6	9.4 ± 0.9	+
13	D	2-I-3,4,5-OMe_3-Ph	3.1 ± 0.4	100.0 ± 10	>75	−

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

Table 2. Structure−Activity Relationship Evaluation in a Series of 2-X_4-9-*N*-Alkyl-8-Arylsulfanyl Adenines

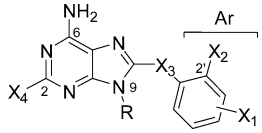


compd	method	X_4	R	Ar	EC_{50} Hsp90α[a]	IC_{50} Her_2[a]	IC_{50} SKBr3[a]	antimitotic
11e1	A	H	pent-4-ynyl	3-OMe-Ph	0.97 ± 0.1	28.3 ± 6.3	36.2 ± 1.6	−
11e2	A	H	butyl	3-OMe-Ph	4.6 ± 0.6	53.7 ± 1.5	37.7 ± 2.1	−
11e3	A	H	2-isopropoxy-ethyl	3-OMe-Ph	2.8 ± 0.5	47.6 ± 4.8	42.3 ± 2.7	−
11w1	A	H	pent-4-ynyl	3,4,5-OMe_3-Ph	1.5 ± 0.3	22.3 ± 0.8	27.2 ± 2.3	−
11w2	A	H	butyl	3,4,5-OMe_3-Ph	3.1 ± 0.7	88.5 ± 17.6	65.4 ± 3.8	−
11w3	A	H	2-isopropoxy-ethyl	3,4,5-OMe_3-Ph	3.4 ± 1.5	65.3 ± 5.7	66.7 ± 5.7	−
20a	E	F	pent-4-ynyl	3,4,5-OMe_3-Ph	1.5 ± 0.6	20.9 ± 2.5	18.2 ± 2.8	−
20b	H	F	2-isopropoxy-ethyl	3,4,5-OMe_3-Ph	2.5 ± 0.2	23.5 ± 1.7	41.2 ± 9.3	−
12d	B	H	butyl	2-Cl-3,4,5-OMe_3-Ph	0.85 ± 0.6	22.8 ± 1.2	14.7 ± 3.6	−
17a	-[b]	NH_2	pent-4-ynyl	3,4,5-OMe_3-Ph	>100	>150	>150	−
21a	F	F	pent-4-ynyl	2-Cl-3,4,5-OMe_3-Ph	0.12 ± 0.03	1.3 ± 0.4	1.8 ± 0.2	+
21b	G	F	2-isopropoxy-ethyl	2-Cl-3,4,5-OMe_3-Ph	0.16 ± 0.05	2.2 ± 0.6	3.3 ± 1.5	+
21c	G	F	butyl	2-Cl-3,4,5-OMe_3-Ph	2.4 ± 0.1	26.3 ± 2.1	20.7 ± 1.3	−
12c	B	H	2-isopropoxy-ethyl	2,4-Cl_2−5-OMe-Ph	5.0 ± 1.1	58.3 ± 2.2	16.1 ± 0.7	−
12e	B	H	2-isopropoxy-ethyl	2,6-Cl_2−3,4,5-OMe_3-Ph	2.1 ± 0.3	13.8 ± 3.3	10.1 ± 0.6	−
10r	-[c]	H	H	2-Cl-5-CF_3-Ph	90.7 ± 10.7	314 ± 15	130 ± 5	+
10u	-[c]	H	H	2,4,5-Cl_3-Ph	15.4 ± 2.2	47.8 ± 3.2	36.0 ± 0.2	+
10n	-[c]	H	H	2,5-Cl_2-Ph	85.1 ± 8.8	122 ± 8	190 ± 15	+

[a] All values are in μM and represent the average of $n = 3 \pm$ standard deviation. [b] Obtained by a, b, d; Scheme 2. [c] Obtained by b; Scheme 1.

series with the 9-*N* alkyl chain as sole variable were synthesized (Table 2). Three well-studied chains,[6,8,9] butyl, pent-4-ynyl, and 2-isopropoxy-ethyl were chosen for the case study. In the methylene linker series, butyl was found to be least favored while appending the pent-4-ynyl resulted in highest potency. Activity in the sulfur linker series declined in a similar fashion, irrespective of substituents on the aryl moiety (**11e2** < **11e3** < **11e1** and **11w2** < **11w3** < **11w1**). Addition of fluorine at position C2 of the adenine moiety (X_4 = F, Figure 2) was also probed. Previous observations made in the methylene linker series[6] pointed to an increase in solubility and potency by exchanging the hydrogen at this position with fluorine and complete abolishment of activity when the position is occupied by an amino group. In the sulfur series case, however, addition of

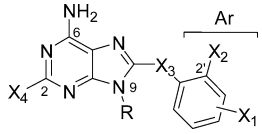
Table 3. Effect of Sulfur's Oxidation State on the Activity of 9-Alkyl-8-Arylsulfanyl Adenines as Inhibitors of Hsp90



compd	X_3	R	Ar	EC_{50} Hsp90α[a]	IC_{50} Her2[a]	IC_{50} SKBr3[a]	antimitotic
12d	S	butyl	2-Cl-3,4,5-OMe_3-Ph	0.85 ± 0.6	22.8 ± 1.2	14.7 ± 3.6	−
29	SO_2	butyl	2-Cl-3,4,5-OMe_3-Ph	4.8 ±d 1.1	12.4 ± 2.8	36.7 ± 3.7	−
27a	SO	pent-4-ynyl	3-OMe-Ph	1.5 ± 0.7	31.8 ± 0.6	25.3 ± 2.6	−
27b	SO	2-isopropoxy-ethyl	3-OMe-Ph	3.5 ± 0.3	70.3 ± 0.6	63.4 ± 2.3	−
27c	SO	butyl	3-OMe-Ph	4.7 ± 0.9	57.2 ± 4.4	53.0 ± 3.6	−
27d	SO	butyl	3,4,5-OMe_3-Ph	76.5 ± 1.9	110.3 ± 15.7	43.9 ± 3.7	−
27e	SO	2-isopropoxy-ethyl	3,4,5-OMe_3-Ph	2.3 ± 0.3	43.9 ± 4.5	66.2 ± 2.8	−
28a	SO_2	pent-4-ynyl	3-OMe-Ph	>50	157 ± 20	41.2 ± 1.9	−
28b	SO_2	2-isopropoxy-ethyl	3-OMe-Ph	>50	170 ± 12	57.6 ± 5.1	−
28c	SO_2	butyl	3-OMe-Ph	>50	125 ± 11	57.2 ± 4.9	−
28d	SO_2	butyl	3,4,5-OMe_3-Ph	>50	315 ± 28	85.8 ± 6.1	−
28e	SO_2	pent-4-ynyl	3,4,5-OMe_3-Ph	>50	105 ± 15	68.4 ± 5.7	−
28f	SO_2	2-isopropoxy-ethyl	3,4,5-OMe_3-Ph	>50	>500	68.8 ± 2.9	−

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

Table 4. Parallel Analysis of Linker's Effect on Activity ($X_3 = CH_2$ vs S)



compd	X_3	X_4	R	Ar	EC_{50} Hsp90 SKBr3[a]	EC_{50} Hsp90 MDA-MB-468[a]	IC_{50} Her2[a]	IC_{50} SKBr3[a]	antimitotic
11w1	S	H	pent-4-ynyl	3,4,5-OMe_3-Ph	4.2 ± 0.2	2.8 ± 0.7	22.3 ± 0.8	27.2 ± 2.3	−
30	CH_2	H	pent-4-ynyl	3,4,5-OMe_3-Ph	3.6 ± 0.3	3.5 ± 0.4	14.7 ± 2.7	40.5 ± 4.8	+
11w3	S	H	2-isopropoxy-ethyl	3,4,5-OMe_3-Ph	5.3 ± 1.7	4.3 ± 0.6	65.3 ± 5.7	66.7 ± 5.7	−
31	CH_2	H	2-isopropoxy-ethyl	3,4,5-OMe_3-Ph	5.1 ± 0.9	5.8 ± 0.5	28.7 ± 6.2	71.1 ± 0.7	+
20a	S	F	pent-4-ynyl	3,4,5-OMe_3-Ph	2.0 ± 0.7	2.2 ± 0.3	20.9 ± 2.5	18.2 ± 2.8	−
32	CH_2	F	pent-4-ynyl	3,4,5-OMe_3-Ph	1.6 ± 0.5	1.4 ± 0.3	10.5 ± 1.5	16.8 ± 2.4	+
21a	S	F	pent-4-ynyl	2-Cl-3,4,5-OMe_3-Ph	0.09 ± 0.03	0.29 ± 0.03	1.3 ± 0.4	1.8 ± 0.2	+
4	CH_2	F	pent-4-ynyl	2-Cl-3,4,5-OMe_3-Ph	0.17 ± 0.09	0.23 ± 0.02	1.5 ± 0.5	1.8 ± 0.4	+

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

fluorine did not seem to affect binding to Hsp90α nor cellular activity in the Her2 degradation and growth inhibition assays (**20a** vs **11w1**, and **20b** vs **11w3**). As anticipated, biological activity was abolished by the introduction of NH_2 in this position ($X_4 = NH_2$) (**17a**).[6] Introduction of chlorine in the ortho-position of the aryl ring (X_2 = Cl) increased potency (3-fold in **21c** vs **11w2**), as seen in the methylene series.[6] Adding both fluorine at position C2 of adenine (X_4 = F) and chlorine in the ortho-position of the aryl moiety (X_2 = Cl) increased activity by over 10-fold (**21a** vs **11w1**, and **21b** vs **11w3**). Antimitotic activity was observed in these series only for the high potency compounds **21a** and **21b** (Table 2, column 9). Derivatives **12c**, **12e**, and **21c** although exhibited good Her2 degradation and growth inhibition potentials, had no antimitotic activity.

The nonalkylated series (compounds **10**, Scheme 1) showed no significant potency in the binding assay or the cellular assays probing for Her2 degradation and growth inhibition (not shown). Paradoxically, **10n**, **10r**, and **10u** were active in the Tg-3 assay at concentrations that did not induce other Hsp90-related events (see Table 2). These compounds have a common substituent pattern on the aryl moiety, with chlorine in position 2 (X_2 = Cl) while position 5 is occupied by a variable substituent (X_1 = variable in C′5). As previously observed in the methylene class ($X_3 = CH_2$), the 3- and 7-*N* isomers obtained as byproducts from alkylation of **10** did not exhibit any notable binding to Hsp90.[6]

The oxidation state of sulfur in the linker was also modified to probe its effect on activity (Table 3). Several sulfoxides and sulfones were synthesized for this purpose. Sulfones (**28a**−**f**) were in general inactive in the binding assay and the Her2 degradation assay but retained modest cytotoxicity, likely via a different mechanism. Sulfoxides of the 3-methoxyphenyl series (**27a**−**c**) were of comparable activity to their corresponding sulfides (**27a** vs **11e1**, **27b** vs **11e3**, **27c** vs **11e2**). No correlation was found in the 3,4,5-methoxyphenyl series (**27d** vs **11w2**, and **27e** vs **11w3**). Addition of chlorine to position C2′ (X_2 = Cl) of the 3,4,5-methoxyphenyl series resulted in sulfones of retained activity (**29** vs **12d**).

To gain a better understanding on the effect of substituting the methylene linker for sulfur ($X_3 = CH_2$ vs S), we subjected several of these derivatives to a parallel analysis. In general, no significant differences in activity between the S and CH_2 series were observed (Table 4). The most potent of the analyzed pairs (**21a** vs **4**) behaved practically identical in the Hsp90-related assays ($EC_{50}^{Hsp90\ SKBr3} = 90 \pm 30$ nM, $EC_{50}^{Hsp90\ MDA-MB-468} = 290 \pm 30$ nM, $IC_{50}^{Her2} = 1300 \pm 40$ nM; $IC_{50}^{SKBr3} = 1800 \pm 200$ nM vs $EC_{50}^{Hsp90\ SKBr3} = 170 \pm 90$ nM, $EC^{50Hsp90\ MDA-MB-468} = 230 \pm 20$ nM, $IC_{50}^{Her2} = 1500 \pm$

Table 5. Selectivity for Transformed vs Normal Cells in a Series of Purine-Scaffold Hsp90 Inhibitors

compd	EC_{50} Hsp90 brain[a]	EC_{50} Hsp90 lung[a]	EC_{50} Hsp90 heart[a]	EC_{50} Hsp90 SKBr3[a]	brain/SKBr3	lung/SKBr3	heart/SKBr3	IC_{50} RPTEC[a]	RPTEC/SKBr3
11w1	ND	ND	ND	4.2 ± 0.2	ND	ND	ND	120 ± 15	4.4
31	ND	ND	ND	3.6 ± 0.3	ND	ND	ND	>200	>100
11o	30.5 ± 2.7	>75	66.3 ± 24.4	0.16 ± 0.04	190	>500	414	31.4 ± 5.2	16.5
11p	14.4 ± 3.3	27.6 ± 1.5	29.5 ± 1.7	0.13 ± 0.05	110	212	226	8.1 ± 1.6	7.4
11q	30.5 ± 5.1	29.7 ± 3.3	26.7 ± 3.1	0.03 ± 0.01	1010	990	890	3.3 ± 0.2	4.1
11v	40.2 ± 25.3	14.7 ± 1.7	65.3 ± 9.9	0.02 ± 0.06	2000	735	3265	4.1 ± 0.9	20.5
12a	44.9 ± 14.1	9.6 ± 3.4	18.9 ± 0.2	0.31 ± 0.06	144	31	61	28.7 ± 5.3	9
12b	42.4 ± 4.9	>75	18.2 ± 2.1	0.33 ± 0.05	128	>200	55	54.5 ± 4.2	5.8
21a	26.9 ± 7.7	217 ± 50	53.1 ± 20.5	0.09 ± 0.03	298	2400	590	28.1 ± 2.2	15.6
21b	76.7 ± 30.3	>75	26.7 ± 8.1	0.47 ± 0.02	163	160	57	47.2 ± 1.9	14.3

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

50 nM; $IC_{50}^{SKBr3} = 1800 \pm 400$ nM). One paradoxical observation was however made in series with lower binding affinity for Hsp90. While the methylene bridge compounds ($X_3 = CH_2$) exhibited antimitotic activity (**30**, **31**, and **33**), their corresponding sulfur bridge derivatives (**11w1**, **11w3**, and **20a**) ($X_3 = S$), although similar in Hsp90α binding affinity and Her2 degradation potential, were inactive in the Tg-3 assay. Differential cell uptake or possible compound metabolism (i.e. S-linker oxidation) is not the base for this discrepancy; both the S- and CH_2-linker compounds inhibited the growth of SKBr3 and MDA-MB-468 cells with similar potencies (not shown). In addition, these compounds were not differentiated by binding affinities toward average population Hsp90 complexes present in SKBr3 and MDA-MB-468 cells (Table 4, columns 6 and 7). These compounds may have selective affinities for Hsp90-client specific complexes that regulate selective pathways in the two cancer cell lines. Since our binding assays are not as sophisticated as to detect client-specific binding in cellular mixtures, the mechanism causing such differences in behavior remains to be elucidated.

Several active derivatives were tested for specificity toward transformed cells (Table 5). Binding affinities of selected compounds for average population Hsp90 complexes found in normal tissues (brain, lung, and heart) and in addition, their cytotoxicities against RPTEC normal cells were determined. Compounds were found to bind Hsp90 from normal tissues with 1- to 3-log weaker affinities when compared to Hsp90 from SKBr3 cells (30−140-fold for the least selective compound **12a** to 730−3200-fold for derivative **11v**). This specificity translated into 5 to 100-fold selectivity (column 10, Table 5) in inhibiting the growth of transformed cells compared to cultured normal epithelial cells (RPTEC tested). No significant cell death was observed in the purine-scaffold treated RPTEC cells even at the highest tested concentrations.

Conclusions

Synthesis of several 8-arylsulfanyl, -sulfoxyl, and -sulfonyl adenine derivatives has allowed for detailed analyses of permissible modifications of the right-side aryl moiety and for the examination of structure−activity relationships of these derivatives as inhibitors of Hsp90. Our results suggest that 8-arylsulfanyl adenine derivatives retain the activity of the methylene linker compounds ($X_3 = CH_2$) and are chemically flexible allowing for extensive SAR studies. Oxidation of the sulfur linker to sulfoxides or sulfones, however, leads to a decrease in activity. The study identifies substituents in positions 2,4,5 as most favored in regard to binding into the Hsp90 ATP-pocket and warrants further explorations in these series. It also discovers derivative **11v** as the most potent Hsp90 inhibitor of the purine-scaffold series published to date, and also the compound of these series with highest selectivity for tumor vs normal cell Hsp90. Most rewardingly, this work has allowed the identification of Hsp90 inhibitors that may have selective affinities for certain Hsp90-client protein complexes. Derivatives with high Hsp90 binding affinity such as **11o**, **11p**, **11q**, **11v**, **12a**, **12b**, **21a**, and **21b** (EC_{50}s from 30 to 300 nM) induce Her2 degradation and inhibition of growth in SKBr3 cells and also exhibit antimitotic activity in MDA-MB-468 cells, these events occurring with similar potencies. However, among the moderate affinity binders, derivatives were identified that degrade Her2 with corresponding potencies but do not affect at similar concentrations the cell cycle distribution in RB-defective cells. The Hsp90 client protein of whose inactivation by Hsp90 inhibitors is responsible for the block of these cells in mitosis is currently unknown.[25] It is conceivable that selective disruption of complexes between Hsp90 and this protein may be the basis for the differential activity shown by purine-scaffold compounds in RB-positive and RB-defective cells. Due to the selectivity profile, these derivatives may be useful pharmacological tools in dissecting Hsp90-regulated processes and in identifying the mitotic checkpoint protein regulated by Hsp90. Thus, a parallel analysis of cells treated with **20a** and **32** might be useful in elucidating these phenomena.

Experimental Section

Hsp90 Competition Assay. Fluorescence polarization measurements were performed on an Analyst AD instrument (Molecular Devices, Sunnyvale, CA). Measurements were taken in black 96-well microtiter plates (Corning # 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[23a] and was dissolved in DMSO to form 10 μM solutions. Recombinant Hsp90α was purchased from Stressgen Bioreagents (cat. No. SPP-776), (Victoria, Canada). 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. To verify that murine Hsp90 in transformed cells has similar affinity to human transformed cell Hsp90, we tested binding of these agents to Hsp90 from Baf3 cells, a murine hematopoietic cell line, and verified that indeed the two species Hsp90s have similar affinities for ATP-pocket

ligands. Thus, we conclude that the use in binding experiments of murine normal organs instead of normal human epithelial cells is acceptable. It is also representative of a closer-to-reality system as the mouse organs are truly normal while the cultured normal epithelial cells are engineered to grow in tissue culture settings. 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# 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 7 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 #100-10b) and incubated at 37 °C, 5% CO_2.

Growth Assays. Growth inhibition studies were performed using the sulforhodamine B assay as previously described.[29] In summary, experimental cultures were plated in microtiter plates (Nunc #167008). One column of wells was left without cells to serve as the blank control. Cells were allowed to attach overnight. On the following day, growth medium having either drug or DMSO 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. Normal human renal proximal tubular epithelial (RPTEC) cells were purchased pre-seeded in 96-well plates (Clonetics, CC-3190). Upon receipt, cells were placed in a humidified incubator at 37 °C, 5% CO_2, and allowed to equilibrate for 3 h. Media was removed by suction and replaced with fresh media provided by the manufacturer. Cells were then treated with either drugs or DMSO for 72 h, and the IC_{50} values were determined as described above.

Her2 Assay. SKBr3 cells were plated in black, clear-bottom microtiter plates (Corning #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 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 RT for 2 h with SuperBlock (Pierce 37535) (200 μL), anti-Her-2 (c-erbB-2) antibody (Zymed Laboratories #28-004) (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 #SC-2027) in Superblock was used. Each well was washed with TBST (2 × 200 μL) and incubated at RT 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#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 EC_{50} values as the concentration of drug that caused 50% decrease in luminescence.

Antimitotic Assay. Black, clear-bottom microtiter 96-well plates (Corning Costar #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 the 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 #37535) (200 μL) for 2 h at RT. The Tg-3 antibody (gift of Dr. 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 Anti-Mouse IgM, SouthernBiotech #1020−05) was placed in each well at 1:2000 dilution in SuperBlock and incubated on a shaker at RT 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 1:1 mix (100 μL) was 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.

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, IR, and NMR. ^1H NMR/^{13}C NMR spectra were recorded on a Bruker 400 MHz instrument. Low-resolution mass spectra (MS) were recorded in the positive ion mode under electron-spray ionization (ESI). High performance liquid chromatography analyses were performed on a Waters 2996 instrument with a photodiode array detector (read at 265 nm) and a reverse-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 reverse-phase column (Varian; Microsorb 100−5 C18 150 × 2) (methods (b) and (c)). Method (a): 0.1% TFA in water-acetonitrile in the indicated ratio; method (b): 0.05% TFA in water-0.04% TFA in acetonitrile; method (c): 0.05% TFA in water-0.04% TFA in acetonitrile gradient (35% acetonitrile over 18 min, 35−95% acetonitrile over 6 min, 95% acetonitrile over 9 min). Infrared spectra (IR) were obtained on a Perkin-Elmer FT-IR model 1600 spectrometer. 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) or alumina (Fisher 60−325 mesh). Oxidation reactions with OXONE were carried out in the presence of the Fisher alumina (A540; 80−200 mesh). Analytical thin-layer chromatography (TLC) was performed on E. Merck precoated silica gel 60 F_{254}. Waters Sep-Pak Vac 6 cm^3 (500 mg) C18 cartridges were used for the purification of compounds **16**. All reactions were conducted under inert atmosphere except of those in aqueous media.

3,4,5-Trimethoxy-benzenethiol (6).[30] To 3,4,5-trimethoxyaniline (2 g, 10.9 mmol) at 0 °C was added a concentrated solution of HCl (3 mL, 0.27 mL/mmol) and H_2O (7.7 mL) followed by $NaNO_2$ (932 mg, 13.1 mmol). The resulting solution

was poured over potassium ethyl xanthogenate (5.35 g, 32.7 mmol) in H_2O (6.2 mL) and stirred at 50 °C for 40 min. The reaction mixture was brought to room temperature, diluted with EtOAc (80 mL), and washed with 10% NaOH, followed by H_2O until the pH reached 7. The organic fraction was dried over Na_2SO_4 and the solvent evaporated under high vacuum. The residue was purified by column chromatography on silica gel (CH_2Cl_2) to furnish the xanthogenate intermediate (1.82 g, 58% yield). This was taken up in anhydrous THF (30 mL). To the resulting solution was slowly added $LiAlH_4$ (1 g, 25 mmol), and the mixture was stirred for 1 h at reflux temperature. Following cooling to room temperature, the reaction was quenched with ice cold water (50 mL) and 10% H_2SO_4 (5 mL) and extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4 and evaporated to give the desired thiophenol (1.18 g, 93% yield). ^{1}H NMR ($CDCl_3$) δ 6.53 (s, 2H), 3.84 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3), 3.46 (s, 1H, SH).

Procedures for the Formation of 8-Arylsulfanyladenine Derivatives 10: Scheme 1, Synthetic Step (b). 8-Mercaptoadenine (**7**) (50.2 mg, 0.30 mmol), neocuproine hydrate (6.8 mg, 0.03 mmol), CuI (5.7 mg, 0.03 mmol), NaO-*t*-Bu (57.6 mg, 0.6 mmol), the corresponding aryl iodide (0.90 mmol), and anhydrous DMF (2 mL) were charged in a nitrogen box. The reaction vessels were sealed with Teflon tape, placed in an oil bath (110 °C) and magnetically stirred for 24 h. The reaction mixture was then cooled to room temperature and DMF was removed in vacuo. The crude material was purified by silica gel flash chromatography eluting with a gradient of $CHCl_3$:NH_4OH at 10:0.5 to $CHCl_3$:MeOH:NH_4OH at 10:1:0.5 to afford the desired product.

8-(2-Trifluoromethoxy-phenylsulfanyl)adenine (10b). Yield, 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.46 (s, 2H), 7.39−7.31 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.4, 145.8, 131.7, 129.5, 128.5, 121.5, 121.3, 118.8; MS *m/z* 327.9 $(M + H)^+$.

8-(4-Acetyl-phenylsulfanyl)adenine (10h). Yield, 75%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13, 7.91−7.89 (d, 2H), 7.43−7.41 (d, 2H), 7.36 (s, 2H), 2.53 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.0, 152.7, 139.6, 135.3, 129.2, 128.3, 26.7; MS *m/z* 285.07 $(M-H)^-$.

8-(4-Pyrrol-1-yl-phenylsulfanyl)adenine (10i). Yield, 78%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.1 (s, 1H), 7.63−7.61 (d, 2H), 7.53 (s, 2H), 7.37 (s, 2H), 7.22 (s, 2H), 6.26 (s, 2H), 5.74 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.4, 132.5, 131.5, 120.1, 118.9, 110.9, 40.1, 39.9, 39.7, 39.5, 39.3, 39.1, 38.8; MS *m/z* 308.9 $(M + H)^+$.

8-(2,4-Dichloro-phenylsulfanyl)adenine (10*l*). Yield, 44%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.74−7.73 (d, 1H), 7.41−7.38 (q, 1H), 7.33 (s, 2H), 7.24−7.22 (d, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.7, 152.3, 141.5, 134.7, 133.4, 132.9, 132.3, 131.1, 129.4, 128.4, 127.7, 127.2; MS *m/z* 311.9 $(M + H)^+$.

8-(2,5-Dimethyl-phenylsulfanyl)adenine (10m). Yield, 76%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.18−7.09 (m, 5H), 2.27 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.3, 136.2, 130.7, 129.5, 20.4, 19.8; MS *m/z* 271.9 $(M + H)^+$.

8-(2,5-Dichloro-phenylsulfanyl)adenine (10n). Yield, 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.59−7.57 (d, 1H), 7.40−7.38 (m, 3H), 7.24 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.8, 152.2, 141.3, 134.3, 132.5, 131.4, 130.9, 129.6, 128.9; MS *m/z* 311.9 $(M + H)^+$. HPLC: (a) 98.5% (65% water − 35% acetonitrile); (b) 99.0%.

8-(2-Bromo-5-methoxy-phenylsulfanyl)adenine (10p). Yield, 66%. ^{1}H NMR (400 MHz, Acetone-d_6/ DMSO-d_6) δ 8.12 (s, 1H), 7.58−7.56 (d, J = 8.79 Hz, 1H), 7.26 (s, 2H), 6.86−6.83 (dd, J = 8.69, J = 2.42, 1H), 6.72 (s, 1H), 3.66 (s, 3H); ^{13}C NMR (100 MHz, Acetone-d_6/ DMSO-d_6) δ 159.2, 152.7, 133.8, 114.4, 55.4; MS *m/z* 351.9 $(M + H)^+$.

8-(3,5-Dichloro-phenylsulfanyl)adenine (10s). Yield, 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.55 (s, 1H), 7.45 (s, 2H), 7.37 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.8, 152.2, 136.5, 134.7, 127.7, 127.3; MS *m/z* 311.9 $(M + H)^+$.

8-(2,4,5-Trichloro-phenylsulfanyl)adenine (10u). Use K_2CO_3 as base. Yield, 56%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1H), 8.00 (s, 1H), 7.60 (s, 1H), 7.37 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 132.5, 132.3, 132.2, 131.4, 131.2, 130.8; MS *m/z* 345.9 $(M + H)^+$. HPLC: (a) 99.9% (65% water − 35% acetonitrile); (b) 99.4%.

8-(7-Bromo-2,3-dihydro-benzo[1,4]dioxin-6-ylsulfanyl)-adenine (10v). Yield, 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (s, 1H), 7.35 (s, 1H), 7.19 (s, 2H), 7.04 (s, 1H), 6.10 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.0, 149.0, 147.8, 113.2, 112.9, 102.6; MS *m/z* 366.0 $(M + H)^+$.

Formation of **10a**, **10c**, **10d**, **10f**, **10g**, **10j**, **10k**, **10r**, and **10t** has followed a similar protocol. Synthesis and characterization of these derivatives has been described elsewhere.[11c]

Scheme 1, Synthetic Step (a): 8-(3-Methoxy-phenylsulfanyl)adenine (10e). Adenine (1.1 g, 8.1 mmol) was added to a solution of bromine (1.7 mL, 33.1 mmol) in water (244 mL), and the resulting mixture was stirred overnight at room temperature. The solvent was evaporated to dryness, and the brominated product **5** was used further without additional purification. Separately, K_2CO_3 (2.25 g, 16.3 mmol) was added to a solution of 3-methoxy-benzenethiol (1.5 mL, 11.8 mmol) in DMF (30 mL), and the reaction mixture was stirred for 1 h at room temperature. To it, a solution of **5** in DMF (81 mL) was dropwise added. The temperature was raised to 120 °C and stirring continued for an additional 14 h. Following cooling and solvent evaporation under high vacuum, the crude material was purified by column chromatography on silica gel ($CHCl_3$:MeOH 97:3) to give **10e** (1.9 g, 85% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 (bs, 1H, NH), 8.16 (s, 1H, H-2), 7.34 (m, 3H), 7.05−6.94 (m, 3H, Ar and NH_2), 3.79 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.0, 154.8, 152.6 (C-2), 133.2, 130.5, 122.3, 115.7, 113.6, 55.3 (OCH_3); MS (EIS) *m/z* 273.9 $(M + 1)^+$.

8-(2,5-Dimethoxy-phenylsulfanyl)adenine (10o). Follow the procedure for **10e**. Yield, 29%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.28 (bs, 1H, NH), 8.08 (s, 1H, H-2), 7.24 (bs, 2H, NH_2), 7.01 (m, 1H), 6.88 (m, 1H), 6.61 (bs, 1H), 3.74 (s, 3H, OCH_3), 3.63 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.9, 159.1, 155.0, 153.5, 153.1, 152.6 (C-2), 150.6, 142.1, 116.0, 113.1, 112.7, 56.5 and 55.5 (OCH_3); MS (EIS) *m/z* 304.1 $(M + 1)^+$.

8-(3,4,5-Trimethoxy-phenylsulfanyl)adenine (10w). Follow the procedure for **10e** except the reaction temperature is 90 °C instead of 120 °C. Yield, 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13 (bs, 1H, NH), 8.09 (s, 1H, H-2), 7.26 (bs, 2H, NH_2), 6.87 (s, 2H), 3.75 (s, 6H, OCH_3), 3.66 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.9, 152.0 (C-2), 138.1, 124.6, 109.9, 60.1 and 56.1 (OCH_3); MS (EIS) *m/z* 334.0 $(M + 1)^+$.

Scheme 1, Synthetic Step (c): Alkylation via the Mitsunobu reaction was conducted as previously described.[13b] Briefly, to a solution of the sulfide **10** (1 equiv) in toluene:CH_2Cl_2 (6 mL/mmol) (5:1) were added 1.3 equiv of the corresponding alcohol, 2.2 equiv of PPh_3, and 5 equiv of di-*tert*-butyl azodicarboxylate (DBAD). The reaction mixture was stirred at room temperature for 30 min, concentrated in vacuo, and taken up in CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated under high vacuum. The crude material was purified by column chromatography on silica gel eluting with $CHCl_3$:Hexanes:EtOAc:*i*-PrOH (2:2:1:0.1) to give the 9-*N*-alkylated products **11** in 40−70% yields.

9-(Pent-4-ynyl)-8-(3-methoxy-phenylsulfanyl)adenine (11e1). Yield, 70%. IR (film) ν_{max} 3300−2835, 1641, 1590, 1573, 1479, 1320 (S); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H, H-2), 7.24 (t, J = 8.3 Hz, 1H), 6.96 (dd, J = 2 and 4 Hz, 1H), 6.83 (m, 1H), 6.11 (bs, 2H, NH_2), 4.30 (t, J = 7.3 Hz, 2H, NCH_2), 3.76 (s, 3H, OCH_3), 2.24 (dt, J = 2.5 and 6.9 Hz, 2H), 1.98 (m, 3H, CH and CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.2, 154.7, 153.2 (C-2), 151.5, 145.3, 132.3, 130.4, 122.7, 120.0, 116.2, 113.8, 82.4 (C quaternary), 69.4 (CH), 55.4 (OCH_3), 42.9 (NCH_2), 28.2, 16.0; MS (EIS) *m/z* 340.1 $(M + 1)^+$. HPLC: (a) 99.0% (70% water − 30% acetonitrile); (c) 99.0%.

9-(Pent-4-ynyl)-8-(2,5-dimethoxy-phenylsulfanyl)adenine (11o).[9] Yield, 45%. HPLC: (a) 95.0% (70% water − 30% acetonitrile); (c) 97.3%.

9-(Pent-4-ynyl)-8-(3,4,5-trimethoxy-phenylsulfanyl)adenine (11w1).[9] Yield, 33%. HPLC: (a) 98.4% (70% water − 30% acetonitrile); (c) 99.0%.

9-Butyl-8-(3-methoxy-phenylsulfanyl)adenine (11e2).[9] Yield, 50%. HPLC: (a) 99.5% (70% water − 30% acetonitrile); (c) 99.0%.

9-Butyl-8-(3,4,5-trimethoxy-phenylsulfanyl)adenine (11w2). Yield, 50%. IR (film) ν_{max} 3313−2932, 1649, 1582, 1498, 1406 (S); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H, H-2), 6.71 (s, 2H), 5.65 (s, 2H, NH_2), 4.20 (t, J = 7.6 Hz, 2H, NCH_2), 3.83 (s, 3H, OCH_3), 3.80 (s, 6H, CH_3), 1.70 (m, 2H), 1.32 (six, J = 7.4 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.0, 154.1, 153.3 (C-2), 151.9, 146.5, 139.0, 125.4, 120.3, 109.4, 61.3 and 56.7 (OCH_3), 44.0 (NCH_2), 32.1, 20.4, 14.0 (CH_3); MS (EIS) *m/z* 390.1 $(M + 1)^+$. HPLC: (a) 99.2% (70% water − 30% acetonitrile); (c) 99.0%.

9-(2-Isopropoxy-ethyl)-8-(3-methoxy-phenylsulfanyl)-adenine (11e3). Yield, 35%. IR (film) ν_{max} 3315−2869, 1642, 1591, 1573, 1478, 1320 (S); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H, H-2), 7.22 (t, J = 8.3 Hz, 1H), 6.95 (m, 2H), 6.81 (dd, J = 2 and 8.3 Hz, 1H), 5.98 (s, 2H, NH_2), 4.42 (t, J = 6 Hz, 2H, NCH_2), 3.76 (s, 3H, OCH_3), 3.69 (t, J = 6 Hz, 2H), 3.46 (six, J = 6.1 Hz, 1H, CH), 1.03 (d, J = 6.1 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1, 154.7, 153.1 (C-2), 151.5, 146.3, 133.0, 130.3, 122.6, 120.1, 115.9, 113.7, 72.2 (CH), 65.4, 55.3 (OCH_3), 43.9 (NCH_2), 21.8 (CH_3); MS (EIS) *m/z* 360.1 $(M + 1)^+$. HPLC: (a) 99.4% (70% water − 30% acetonitrile); (c) 99.0%.

9-(2-Isopropoxy-ethyl)-8-(3,4,5-trimethoxy-phenylsulfanyl)adenine (11w3). Yield, 49%. IR (film) ν_{max} 3312−2934, 1651, 1582, 1498, 1309 (S); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H, H-2), 6.66 (s, 2H), 6.04 (s, 2H, NH_2), 4.36 (t, J = 6 Hz, 2H, NCH_2), 3.74 (s, 3H, OCH_3), 3.73 (s, 6H, CH_3), 3.61 (t, J = 6 Hz, 2H), 3.38 (m, 1H, CH), 0.81 (d, J = 5.8 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.6, 153.6, 152.9 (C-2), 151.4, 147.0, 138.4, 125.5, 120.0, 108.8, 72.2 (CH), 65.4, 60.8 and 56.2 (OCH_3), 43.8 (NCH_2), 21.8 (CH_3); MS (EIS) *m/z* 420.1 $(M + 1)^+$. HPLC: (a) 98.3% (70% water − 30% acetonitrile); (c) 99.0%.

9-(Pent-4-ynyl)-8-(2-iodo-3,4,5-trimethoxy-phenylsulfanyl)adenine (13). A mixture of **10w** (135 mg, 0.4 mmol), *N*-iodosuccinimide (192 mg, 0.8 mmol), and trifluoroacetic acid (31 μL, 0.4 mmol) in acetonitrile (1.6 mL) was stirred for 1 h at room temperature. Following solvent removal, the reaction crude material was washed with an aqueous solution of sodium bisulfite, extracted in CH_2Cl_2, and dried on anhydrous sodium sulfate. The crude solid was added to a solution of PPh_3 (160 mg, 0.6 mmol), DBAD (325 mg, 1.4 mmol), and 4-pentyn-1-ol (35 μL, 0.4 mmol) in toluene:CH_2Cl_2 (8.3:1.7 mL), and the reaction mixture was stirred at room temperature for 30 min. Following solvent removal, the solid mass was taken up in CH_2Cl_2 washed with brine and dried over anhydrous Na_2SO_4. The crude material was purified by column chromatography on silica gel eluting with $CHCl_3$:Hexanes:EtOAc:*i*-PrOH (2:2:1:0.1) to give **13** (43 mg, 20% yield). IR (film) ν_{max} 3299−2936, 1644, 1595, 1471, 1370 (S); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H, H-2), 6.73 (s, 1H), 6.11 (bs, 2H, NH_2), 4.33 (t, J = 7.2 Hz, 2H, NCH_2), 3.89 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 2.26 (dt, J = 2.4 and 7 Hz, 2H), 2.02 (m, 2H), 1.98 (t, J = 2.4 Hz, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.7, 154.3, 154.1, 153.2 (C-2), 151.5, 145.9, 142.1, 131.0, 120.2, 111.0, 89.4, 82.3 (C quaternary), 69.5 (CH), 61.0−60.9 and 56.2 (OCH_3), 43.1 (NCH_2), 28.4, 16.1; MS (EIS) *m/z* 526.0 $(M + 1)^+$. HPLC: (a) 98.7% (70% water − 30% acetonitrile); (c) 95.0%.

Scheme 1, Synthetic Step (d): A mixture of 8-arylsulfanyl adenine **10** (100 μmol), Cs_2CO_3 (100 μmol), and pent-4-ynyl 4-methylbenzenesulfonate (120 μmol) in DMF (1.3 mL) under nitrogen protection was heated at 80 °C for 30 min. Following solvent removal, the crude material was purified by preparatory TLC with $CHCl_3$:MeOH:NH_4OH at 10:1:0.5 or $CHCl_3$:MeOH:AcOH at 10:1:0.5 to provide the corresponding 9-alkyl-8-arylsulfanyladenine derivatives **11**.

9-(Pent-4-ynyl)-8-(2-methoxy-phenylsulfanyl)adenine (11a). Yield, 50%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (s, 1H), 7.32−7.28 (m, 1H), 7.22−7.20 (m, 1H), 6.93−6.91 (m, 2H), 5.73 (s, 2H), 4.35−4.31 (t, 2H), 3.84 (s, 3H), 2.27−2.22 (dt, 2H), 2.07−2.00 (m, 2H), 1.96−1.95 (t, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.4, 154.3, 152.9, 151.5, 145.9, 132.0, 129.7, 121.5, 120.1, 119.5, 111.4, 82.5, 69.2, 56.0, 42.9, 28.3, 16.0; MS *m/z* 340.1 $(M + H)^+$. HPLC: (a) 98.2% (75% water − 25% acetonitrile); (b) 97.7%.

9-(Pent-4-ynyl)-8-(2-trifluoromethoxy-phenylsulfanyl)-adenine (11b). Yield, 40%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.35−7.33 (m, 2H), 7.26−7.22 (m, 2H), 5.77 (s, 2H), 4.35−4.31 (t, 2H), 2.26−2.22 (m, 2H), 2.05−1.98 (m, 2H), 1.96−1.95 (t, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.6, 153.3, 151.6, 147.1, 143.7, 131.8, 129.4, 127.7, 125.6, 121.7, 121.3, 120.3, 82.2, 69.4, 42.9, 28.3, 15.9; MS *m/z* 393.9 $(M + H)^+$. HPLC: (a) 99.5% (75% water − 25% acetonitrile); (b) 99.1%.

9-(Pent-4-ynyl)-8-(2-hydroxymethyl-phenylsulfanyl)-adenine (11c). Yield, 56%. ^{1}H NMR (400 MHz, $CDCl_3$/MeOD-d_4) δ 8.19 (s, 1H), 7.68 (d, 1H), 7.53−7.50 (t, 2H), 7.36−7.32 (t, 1H), 4.85 (s, 2H), 4.37−4.33 (t, 2H), 2.35−2.31 (m, 2H), 2.11−2.04 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD-d_4) δ 151.8, 150.8, 147.8, 144.1, 134.6, 130.1, 128.7, 128.4, 126.1, 81.9, 69.3, 62.3, 42.6, 27.8, 15.5; MS *m/z* 340.0 $(M + H)^+$. HPLC: (a) 99.3% (75% water − 25% acetonitrile); (b) 99.7%.

9-(Pent-4-ynyl)-8-(2-chloro-phenylsulfanyl)adenine (11d). Yield, 22%.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.45−7.43 (d, 1H), 7.24−7.13 (m, 3H), 5.68 (s, 2H), 4.35−4.31 (m, 2H), 2.27−2.23 (m, 2H), 2.05−1.98 (m, 2H), 1.97−1.96 (t, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.6, 153.4, 151.7, 144.2, 133.9, 131.4, 131.0, 130.2, 123.0, 127.7, 82.3, 69.5, 43.1, 28.4, 16.1; MS *m/z* 344.0 $(M + H)^+$. HPLC: (a) 98.5% (75% water − 25% acetonitrile); (b) 99.0%.

9-(Pent-4-ynyl)-8-(4-methoxy-phenylsulfanyl)adenine (11f). Yield, 77%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.48−7.46 (d, 2H), 6.91−6.89 (d, 2H), 5.75 (s, 2H), 4.32−4.28 (2H), 3.80 (s, 3H), 2.28−2.25 (m, 2H), 2.02−2.05 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.7, 134.4, 115.2, 82.5, 69.4, 55.4, 42.7, 28.1, 16.1; MS *m/z* 340.1 $(M + H)^+$. HPLC: (a) 98.3% (75% water − 25% acetonitrile); (b) 98.8%.

9-(Pent-4-ynyl)-8-(4-chloro-phenylsulfanyl)adenine (11 g). Yield, 21%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.41−7.39 (d, 2H), 7.34−7.31 (d, 2H), 5.68 (s, 2H), 4.33−4.29 (t, 2H), 2.28−2.24 (m, 2H), 2.05−1.97 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.3, 152.9, 151.6, 145.7, 134.8, 132.6, 129.8, 129.1, 120.1, 82.4, 69.5, 42.9, 28.2, 16.0; MS *m/z* 344.1 $(M + H)^+$. HPLC: (a) 98.0% (75% water − 25% acetonitrile); (b) 97.1%.

9-(Pent-4-ynyl)-8-(4-acetyl-phenylsulfanyl)adenine (11h). Yield, 48%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.91−7.89 (d, 2H), 7.42−7.39 (d, 2H), 5.70 (s, 2H), 4.34−4.30 (t, 2H), 2.58 (s, 3H), 2.26−2.22 (m, 2H), 2.04−1.96 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.7, 153.6, 151.6, 143.7, 138.3, 136.1, 129.3, 129.0, 82.3, 69.5, 43.0, 28.3, 26.6, 16.0; MS *m/z* 352.0 $(M + H)^+$. HPLC: (a) 99.6% (75% water − 25% acetonitrile); (b) 99.8%.

9-(Pent-4-ynyl)-8-(4-pyrrol-1-yl-phenylsulfanyl)adenine (11i). Yield, 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.55−7.53 (d, 2H), 7.39−7.37 (d, 2H), 7.07−7.06 (d, 2H), 6.36−6.35 (d, 2H), 5.76 (s, 2H), 4.35−4.31 (t, 2H), 2.29−2.25 (m, 2H), 2.05−1.98 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.3, 152.8, 151.6, 146.3, 140.8, 133.1, 126.8, 121.1, 119.0, 111.1, 82.4, 69.5, 42.8, 28.2, 16.0; MS *m/z* 375.0 $(M + H)^+$. HPLC: (a) 99.0% (75% water − 25% acetonitrile); (b) 99.6%.

9-(Pent-4-ynyl)-8-(2,4-dimethoxy-phenylsulfanyl)adenine (11j). Yield, 10%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.42−7.40 (d, 1H), 6.52−6.49 (m, 2H), 5.54 (s, 2H), 4.35−4.32 (t, 2H), 3.82 (s, 3H), 3.79 (s, 3H), 2.29−2.25 (m, 2H), 2.09−2.02 (m, 2H), 1.99−1.98 (t, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3, 153.9, 152.4, 151.6, 135.8, 105.6, 99.5, 82.7, 69.2, 56.0, 55.5, 42.7, 28.3, 16.1; MS *m/z* 370.0 $(M + H)^+$. HPLC: (a) 99.2% (75% water − 25% acetonitrile); (b) 98.3%.

9-(Pent-4-ynyl)-8-(2,4-dimethyl-phenylsulfanyl)adenine (11k). Yield, 54%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.25−7.23 (d, 1H), 7.08 (s, 1H), 6.98−6.96 (d, 1H), 5.96 (s, 2H), 4.28−4.25 (t, 2H), 2.40 (s, 3H), 2.30 (s, 3H), 2.28−2.24 (m, 2H), 2.05−1.98 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ

154.2, 152.5, 151.6, 146.7, 140.0, 139.3, 133.0, 131.8, 127.8, 125.6, 119.9, 82.5, 69.3, 42.7, 28.1, 21.0, 20.6, 16.0; MS *m/z* 338.0 (M + H)$^+$. HPLC: (a) 97.7% (75% water − 25% acetonitrile); (b) 97.1%.

9-(Pent-4-ynyl)-8-(2,4-dichloro-phenylsulfanyl)-adenine (11*l*). Yield, 44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.48−7.47 (d,1H), 7.19−7.18 (m, 2H), 5.69 (s, 2H), 4.35−4.32 (t, 2H), 2.29−2.25 (m, 2H), 2.07−2.00 (m, 2H), 1.98−1.97 (t, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.2, 150.6, 144.7, 136.2, 135.4, 133.8, 129.9, 127.8, 127.6, 81.6, 69.1, 42.6, 27.8, 15.3; MS *m/z* 377.9 (M + H)$^+$. HPLC: (a) 98.7% (75% water − 25% acetonitrile); (b) 99.1%.

9-(Pent-4-ynyl)-8-(2,5-dimethyl-phenylsulfanyl)adenine (11m). Yield, 47%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.16−7.14 (d, 1H), 7.11 (s, 1H), 7.06−7.04 (d, 1H), 5.98 (s, 2H), 4.29−4.26 (t, 2H), 2.39 (s, 3H), 2.27−2.24 (m, 5H), 2.04−1.97 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.3, 152.6, 151.6, 146.3, 136.8, 136.6, 132.8, 130.8, 129.8, 129.1, 120.0, 82.4, 69.3, 42.7, 28.1, 20.8, 20.2, 16.0; MS *m/z* 338.0 (M + H)$^+$. HPLC: (a) 99.8% (75% water − 25% acetonitrile); (b) 99.9%.

9-(Pent-4-ynyl)-8-(2,5-dichloro-phenylsulfanyl)-adenine (11n). Yield, 48%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.36−7.34 (d, 1H), 7.19−7.17 (q, 1H), 7.14−7.13 (d, 1H), 6.19 (s, 2H), 4.37−4.34 (t, 2H), 2.29−2.25 (m, 2H), 2.08−2.01 (m, 2H), 1.98−1.97 (t, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.9, 153.5, 151.5, 142.8, 133.5, 133.2, 131.9, 131.0, 130.1, 128.9, 82.1, 69.5, 43.1, 28.4, 16.0; MS *m/z* 377.8 (M + H)$^+$. HPLC: (a) 99.7% (75% water − 25% acetonitrile); (b) 99.8%.

9-(Pent-4-ynyl)-8-(2-bromo-5-methoxy-phenylsulfanyl)adenine (11p). Yield, 60%.^{1}H NMR (400 MHz, $CDCl_3$/MeOD-d_4) δ 8.24 (s, 1H), 7.58−7.56 (d, J = 8.79 Hz, 1H), 6.89 (d, J = 2.89 Hz, 1H), 6.85−6.82 (dd, J = 8.80 Hz, J = 2.87 Hz, 1H), 4.37−4.33 (t, J = 7.40 Hz, 2H), 3.76 (s, 3H), 2.31−2.27 (dd, J = 6.99 Hz, J = 2.55 Hz, 2H), 2.09−2.02 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD-d_4) δ 159.1, 152.3, 150.7, 145.2, 133.9, 132.0, 118.3, 115.5, 115.4, 81.7, 69.2, 55.1, 42.7, 27.9, 15.5; MS *m/z* 418.1 (M + H)$^+$. HPLC: (a) 99.5% (75% water − 25% acetonitrile); (b) 99.8%.

9-(Pent-4-ynyl)-8-(2-chloro-5-trifluoromethyl-phenylsulfanyl)adenine (11r). Yield, 40%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.59−7.57 (m, 2H), 7.51−7.48 (d, 1H), 5.86 (s, 2H), 4.39−4.36 (t, 2H), 2.29−2.23 (m, 2H), 2.07−2.04 (m, 2H), 1.97−1.96 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.6, 153.5, 151.6, 143.1, 132.7, 130.7, 128.2, 125.8, 120.5, 82.1, 69.5, 43.1, 28.4, 16.0; MS *m/z* 412.0 (M + H)$^+$. HPLC: (a) 99.0% (75% water − 25% acetonitrile); (b) 98.7%.

9-(Pent-4-ynyl)-8-(3,5-dichloro-phenylsulfanyl)-adenine (11s). Yield, 30%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.29−7.28 (m, 3H), 5.96 (s, 2H), 4.35−4.31 (t, 2H), 2.29−2.25 (m, 2H), 2.06−1.98 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.8, 153.5, 151.6, 143.4, 135.8, 134.6, 128.4, 128.1, 82.3, 69.6, 42.9, 28.2, 15.9; MS *m/z* 377.8 (M + H)$^+$. HPLC: (a) 98.4% (75% water − 25% acetonitrile); (b) 97.9%.

9-(Pent-4-ynyl)-8-(3,5-dimethyl-phenylsulfanyl)adenine (11t). Yield, 36%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.03 (s, 2H), 6.92 (s, 1H), 5.99 (s, 2H), 4.31−4.28 (t, 2H), 2.27 (s, 6H), 2.25−2.21 (m, 2H), 2.00−1.93 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.6, 153.0, 151.6, 145.9, 139.4, 130.3, 130.2, 128.6, 120.0, 82.5, 69.3, 42.9, 28.1, 21.2, 16.0; MS *m/z* 338.1 (M + H)$^+$. HPLC: (a) 99.8% (75% water − 25% acetonitrile); (b) 99.6%.

9-(Pent-4-ynyl)-8-(2,4,5-trichloro-phenylsulfanyl)adenine (11u). Yield, 46%. ^{1}H NMR (400 MHz, $CDCl_3$/MeOD-d_4) δ 8.26 (s, 1H), 7.63 (s, 1H), 7.50 (s, 1H), 4.38−4.35 (t, J = 7.3 Hz, 2H), 2.32−2.28 (m, 2H), 2.09−2.02 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$/MeOD-d_4) δ 154.4, 152.7, 150.9, 143.8, 134.1, 133.7, 133.4, 131.9, 131.3, 129.4, 81.8, 69.4, 42.9, 28.1, 15.7; MS *m/z* 411.9 (M + H)$^+$. HPLC: (a) 98.5% (75% water − 25% acetonitrile); (b) 97.1%.

9-(Pent-4-ynyl)-8-(6-bromo-benzo[1,3]dioxol-5-ylsulfanyl)adenine (11v). Yield, 48%.^{1}H NMR (400 MHz, $CDCl_3$/MeOD-d_4) δ 8.22 (s, 1H), 7.17 (s, 1H), 7.00 (s, 1H), 6.06 (s, 2H), 4.35−4.31 (t, J = 7.26 Hz, 2H), 4.12 (s, 2H), 2.33−2.30 (m, 2H), 2.08−2.05 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$/MeOD-d_4) δ 150.9, 149.7, 148.0, 146.9, 121.3, 119.1, 113.8, 113.4, 102.4, 81.9, 69.3, 42.7, 28.0, 15.6; MS *m/z* 432.0 (M + H)$^+$. HPLC: (a) 98.7% (75% water − 25% acetonitrile); (b) 98.9%.

2,6-Diamino-8-mercaptoadenine (15). To a solution of 2,4,5,6-tetraaminopyrimidine sulfate (**14**) (1 g, 4.1 mmol) in EtOH (7.6 mL) and H_2O (15.3 mL) were added CS_2 (2.5 mL, 41.6 mmol) and $NaHCO_3$ (1.7 g, 20.2 mmol). The resulted mixture was refluxed for 24 h. Following cooling to room temperature, the solution was acidified with AcOH to pH 4−5. The formed yellow precipitate was filtered, washed three times with H_2O, and dried on high vacuum. The product was obtained as a pale-yellow powder (720 mg, 97% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.37 (bs, 1H, NH), 11.55 (bs, 1H, SH), 6.28 (bs, 2H, NH_2), 5.84 (bs, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.9 (C-4), 160.3 (C-2), 151.9 (C-8), 148.1 (C-6), 101.4 (C-5); MS (EIS) *m/z* 182.9 (M + 1)$^+$.

2-Amino-8-(3,4,5-trimethoxy-phenylsulfanyl)adenine (16). A solution of **15** (500 mg, 2.74 mmol), CuI (53.3 mg, 0.27 mmol), NaO*t*-Bu (544 mg, 5.49 mmol), and 2-iodo-3,4,5-trimethoxybenzene (1.72 g, 5.73 mmol) in ethylene glycol (8.2 mL) was heated to 130 °C and stirred under inert atmosphere for 15 h. Following cooling to room temperature, the crude material was purified by column chromatography on silica gel ($CHCl_3$:MeOH at 97:3). Excess ethylene glycol was removed by passing the mixture through a short Sep-Pak C18 cartridge and eluting with H_2O:MeOH at 4:1 to afford **16** (580 mg, 60% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.49 (bs, 1H, NH), 6.80 (bs, 1H, H-2), 6.72 (s, 2H), 5.80 (bs, 2H, NH_2), 3.72 (s, 6H, OCH_3), 3.63 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.2, 155.4, 153.9, 153.2, 137.2, 127.8, 114.8, 107.7, 60.0 and 56.0 (OCH_3); MS (EIS) *m/z* 348.8 (M + 1)$^+$.

2-Amino-9-butyl-8-(3,4,5-trimethoxy-phenylsulfanyl)-adenine (17c). To a solution of **16** (101 mg, 0.28 mmol) in DMF (0.7 mL) were added Cs_2CO_3 (94 mg, 0.28 mmol) and butyl 4-methylbenzenesulfonate (86 mg, 0.38 mmol). The reaction mixture was heated to 80 °C and stirred for 5 h. Following solvent removal, the residue was purified by column chromatography on silica gel with $CHCl_3$:Hexanes:EtOAc:MeOH at 2:2:1:0.1 to afford **17c** (46 mg, 42% yield). MS (EIS) *m/z* 405.2 (M + 1)$^+$.

2-Amino-9-(2-isopropoxy-ethyl)-8-(3,4,5-trimethoxy-phenylsulfanyl)adenine (17b). Following the general method for the preparation of **17c**, a mixture of Cs_2CO_3 (280 mg, 0.86 mmol), 2-isopropoxy-ethyl 4-methylbenzenesulfonate (289 mg, 1.2 mmol), and **16** (300 mg, 0.86 mmol) in DMF (2.1 mL) afforded **17b** (182 mg, 49% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.62 (s, 2H), 5.73 (bs, 2H, NH_2), 4.93 (bs, 2H, NH_2), 4.26 (t, J = 6.4 Hz, 2H, NCH_2), 3.80 (s, 3H, OCH_3), 3.78 (s, 6H, CH_3), 3.58 (t, J = 6.4 Hz, 2H), 3.45 (quin, J = 6.1 Hz, 1H, CH), 1.04 (d, J = 6.1 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.5, 160.0, 158.8, 155.4, 153.6, 141.8, 128.6, 107.3, 72.1 (CH), 65.4, 60.8 and 56.2 (OCH_3), 43.3 (NCH_2), 21.9 (CH_3); MS (EIS) *m/z* 435.1 (M + 1)$^+$.

2-Amino-9-(pent-4-ynyl)-8-(3,4,5-trimethoxy-phenylsulfanyl)adenine (17a). Following the general method for the preparation of **17c**, a mixture of Cs_2CO_3 (72 mg, 0.22 mmol), pent-4-ynyl 4-methylbenzenesulfonate (70 mg, 0.29 mmol), and **16** (50 mg, 0.14 mmol) in DMF (0.35 mL) afforded **17a** (54 mg, 90% yield). IR (film) ν_{max} 3304−2937, 1632, 1585, 1497, 1408, 1232, 1126; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.62 (s, 2H), 5.44 (bs, 2H, NH_2), 4.74 (bs, 2H, NH_2), 4.15 (t, J = 7.4 Hz, 2H, NCH_2), 3.82 (s, 3H, OCH_3), 3.79 (s, 6H, CH_3), 2.21 (dt, J = 2.5 and 7.1 Hz, 2H), 1.97 (t, J = 2.5 Hz, 1H, CH), 1.93 (quin, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1, 155.4, 153.7, 153.2, 141.1, 138.0, 127.0, 115.1, 107.4, 82.7 (C quaternary), 60.8 and 56.2 (OCH_3), 60.2 (CH), 42.4 (NCH_2), 28.0, 16.0; MS (EIS) *m/z* 415.1 (M + 1)$^+$. HPLC: (a) 99.0% (70% water − 30% acetonitrile); (c) 98.5%.

9-Butyl-8-(2-chloro-3,4,5-trimethoxy-benzenesulfanyl)-adenine (12d). To a cooled mixture (0 °C) of **11w2** (14 mg, 36 μmol) and concentrated HCl (18 μL, 0.5 mL/mmol) in MeOH (1 mL) was added a 70% aqueous solution of *tert*-butyl hydroperoxide (*t*-BuOOH) (30 μL, 0.2 mmol). The mixture was first stirred at 0 °C for 5 min and then at 80 °C overnight. Following

solvent evaporation, the product was purified by column chromatography on silica gel ($CHCl_3$:Hexanes:EtOAc:*i*-PrOH 2:2:1:0.5) to afford compound **12d** (10 mg, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H, H-2), 7.72 (s, 1H), 5.90 (bs, 2H, NH_2), 4.22 (t, J = 7.5 Hz, 2H, NCH_2), 3.90 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 1.72 (m, 2H), 1.33 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.7, 153.0 (C-2), 152.9, 151.9, 151.2, 145.8, 144.2, 125.1, 122.0, 123.0, 111.6, 61.6 and 56.7 (OCH_3), 44.2 (NCH_2), 32.2, 20.3, 14.0 (CH_2); MS (EIS) *m/z* 424.0 (M + 1)$^+$. HPLC: (a) 98.5% (70% water − 30% acetonitrile); (c) 97.2%.

9-(Pent-4-ynyl)-8-(2-chloro-3,4,5-trimethoxy-benzenesulfanyl)adenine (12b).[9] Following the general method for the preparation of **12d**, **11w1** afforded **12b**. Yield, 21%. HPLC: (a) 95.1% (70% water − 30% acetonitrile); (c) 95.0%.

9-(2-Isopropoxy-ethyl)-8-(2,4-dichloro-5-methoxy-benzenesulfanyl)adenine (12c). Following the general method for the preparation of **12d**, **11e3** afforded **12c**. Yield, 53%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H, H-2), 7.44 (s, 1H), 7.09 (s, 1H), 5.55 (bs, 2H, NH_2), 4.48 (t, J = 5.6 Hz, 2H, NCH_2), 3.81 (s, 3H, OCH_3), 3.74 (t, J = 5.6 Hz, 2H), 3.49 (d, J = 6.1 Hz, 1H, CH), 1.04 (d, J = 6.1 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1 (C-2), 153, 147, 131.0, 130.2, 126.9, 123.6, 116.0, 72.3 (CH), 65.6, 56.5 (OCH_3), 44.1 (NCH_2), 21.8 (CH_2); MS (EIS) *m/z* 428.0 (M + 1)$^+$. HPLC: (a) 90.2% (70% water − 30% acetonitrile); (c) 91.0%.

9-(2-Isopropoxy-ethyl)-8-(2,6-dichloro-3,4,5-trimethoxy-benzenesulfanyl)adenine (12e). Following the general method for the preparation of **12d**, **11w3** afforded **12e**. Yield, 44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H, H-2), 5.60 (bs, 2H, NH_2), 4.49 (t, J = 5.5 Hz, 2H, NCH_2), 3.99 (s, 3H, OCH_3), 3.90 (s, 6H, OCH_3), 3.76 (t, J = 5.5 Hz, 2H), 3.52 (m, J = 6.1 Hz, 1H, CH), 1.11 (d, J = 6.1 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.7, 151.9, 149.7, 130.0, 124.1, 72.5 (CH), 65.8, 61.4 and 61.2 (OCH_3), 44.0 (NCH_2), 21.9 (CH_2); MS (EIS) *m/z* 487.9 (M + 1)$^+$. HPLC: (a) 98.0% (60% water − 40% acetonitrile); (c) 98.0%.

2-Amino-9-butyl-8-(2-chloro-3,4,5-trimethoxy-phenylsulfanyl)adenine (18c). Following the general method for the preparation of **12d**, **17c** afforded **18c**. Yield, 38%. ^{1}H NMR (400 MHz, $CHCl_3$) δ 6.45 (s, 1H), 5.61 (bs, 2H, NH_2), 4.82 (bs, 2H, NH_2), 4.06 (t, J = 7.5 Hz, 2H, NCH_2), 3.91 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 1.65 (m, 2H), 1.30 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CHCl_3$) δ 160.0, 155.3, 153.5, 152.6, 150.5, 142.8, 140.1, 127.3, 119.8, 115.5, 108.8, 61.2 and 56.2 (OCH_3), 43.3 (NCH_2), 31.7, 19.8, 13.6 (CH_2); MS (EIS) *m/z* 439.2 (M + 1)$^+$.

2-Amino-9-(2-isopropoxy-ethyl)-8-(2-chloro-3,4,5-trimethoxy-phenylsulfanyl)adenine (18b). Following the general method for the preparation of **12d**, **17b** afforded **18b**. Yield, 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (bs, 2H, NH_2), 7.61 (bs, 2H, NH_2), 6.81 (s, 1H, H-2), 4.21 (t, J = 5.2 Hz, 2H, NCH_2), 3.80 and 3.76 (2s, 9H, OCH_3), 3.57 (t, J = 5.2 Hz, 2H), 3.43 (m, J = 6 Hz, 1H, CH), 0.94 (d, J = 6 Hz, 6H, CH_3); ^{13}C NMR (400 MHz, DMSO-d_6) δ 150.3, 150.0, 149.6, 142.8, 140.9, 125.8, 119.5, 116.9, 110.8, 108.0, 71.2 (CH), 54.6, 61.0−60.8 and 56.3 (OCH_3), 43.7 (NCH_2), 21.7 (CH_2); MS (EIS) *m/z* 469.1 (M + 1)$^+$.

9-(Pent-4-ynyl)-8-(2-chloro-5-methoxy-phenylsulfanyl)adenine (12a). Following the general method for the preparation of **12d**, **11e1** afforded **12a**. Yield, 31%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H, H-2), 7.24 (t, J = 8.2 Hz, 1H), 6.95 (d, J = 2 Hz, 1H), 6.83 (dd, J = 2 and 8.2 Hz, 1H), 6.11 (bs, 2H, NH_2), 4.30 (t, J = 7.3 Hz, 2H, NCH_2), 3.76 (s, 3H, OCH_3), 2.24 (dt, J = 2.5 and 7 Hz, 2H), 1.99 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 155.5, 154.5, 153.2 (C-2), 132.0, 130.7, 125.4, 116.8, 114.6, 82.3, 69.5 (CH), 55.3 (OCH_3), 43.1 (NCH_2), 28.4, 16.0; MS (EIS) *m/z* 374.0 (M + 1)$^+$. HPLC: (a) 96.0% (70% water − 30% acetonitrile); (c) 95.5%.

9-Butyl-8-(2-chloro-3,4,5-trimethoxy-benzenesulfonyl)-adenine (29). Following the general method for the preparation of **12d**, a mixture of *t*-BuOOH (30 μL, 0.22 mmol), HCl (18 μL, 0.5 mL/mmol) and **28d** (15 mg, 0.11 mmol) in MeOH (1 mL) afforded **29** (7 mg, 43% yield). IR (film) ν_{max} 3317−2873, 1648, 1594, 1573, 1479, 1312, 1149, 1112 (SO), 1013 (SO); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H, H-2), 7.71 (s, 1H), 5.89 (bs, 2H, NH_2), 4.57 (t, J = 7.8 Hz, 2H, NCH_2), 4.00−387 (3s, 9H, OCH_3), 1.85 (m, 2H), 1.40 (m, J = 7.5 Hz, 2H), 0.95 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 155.2 (C-2), 151.9, 151.0, 150.8, 148.5, 145.8, 131.1, 121.0, 119.0, 109.5, 61.4−61.3 and 56.6 (OCH_3), 44.9 (NCH_2), 32.4, 20.0, 13.6; MS (EIS) *m/z* 456.1 (M + 1). HPLC: (a) 99.7% (70% water − 30% acetonitrile); (c) 99.0%.

Method for the Fluorination of the Adenine Moiety at C2: 2-Fluoro-9-butyl-8-(2-chloro-3,4,5-trimethoxy-phenylsulfanyl)adenine (21c). To a cooled solution (0 °C) of **18c** (11.3 mg, 0.02 mmol) in HF/pyridine (18 μL, 0.7 mL/mmol) was slowly added $NaNO_2$ (2.2 mg, 0.03 mmol). The resulted mixture was stirred at room temperature for 1 h and then quenched by stirring for 1 h with 14 mg of $CaCO_3$ in CH_2Cl_2 (75 μL). The crude material was taken up in CH_2Cl_2, washed with water, and dried over anhydrous Na_2SO_4. Following solvent removal, the residue was purified on a preparative silica gel plate ($CHCl_3$:Hexanes:EtOAc:*i*-PrOH at 2:2:1:0.1) to afford 21c (1.9 mg, 17% yield). IR (film) ν_{max} 3318−2953, 1657, 1604, 1583, 1479, 1385, 1111, 1015; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.72 (s, 1H), 5.83 (bs, 2H, NH_2), 4.18 (t, J = 7.5 Hz, 2H, NCH_2), 3.92 (s, 3H, OCH_3), 3.89 (s, 3H, CH_3), 3.74 (s, 3H, CH_3), 1.72 (m, 2H), 1.32 (m, 2H), 0.92 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1, 158.0, 156.1, 152.5, 150.8, 143.9, 124.6, 111.2, 61.2 and 56.3 (OCH_3), 43.9 (NCH_2), 31.7, 29.7, 19.7 (CH_3); MS (EIS) *m/z* 442.2 (M + 1)$^+$. HPLC: (a) 95.9% (60% water − 40% acetonitrile); (c) 98.0%.

2-Fluoro-9-(2-isopropoxy-ethyl)-8-(2-chloro-3,4,5-trimethoxy-phenylsulfanyl)adenine (21b). Following the method for the preparation of **21c**, **18b** afforded **21b**. Yield, 11%. IR (film) ν_{max} 3322−2870, 1648, 1603, 1584, 1478, 1384, 1110, 1013; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 2H), 6.14 (bs, 2H, NH_2), 4.42 (t, J = 5.5 Hz, 2H, NCH_2), 3.88−3.89 and 3.76 (3s, 9H, OCH_3), 3.71 (t, J = 5.5 Hz, 2H), 3.50 (quin, J = 6 Hz, 1H, CH), 1.04 (d, J = 6 Hz, 6H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.0, 156.1 (J_{C-F} = 20 Hz), 152.4, 150.7, 143.7, 128.6, 125.4, 122.0, 111.3, 72.2 (CH), 65.3, 61.2 and 56.3 (OCH_3), 44.1 (NCH_2), 21.8 (CH_3); MS (EIS) *m/z* 471.9 (M + 1)$^+$. HPLC: (a) 98.6% (60% water − 40% acetonitrile); (c) 95.0%.

2-Fluoro-8-(3,4,5-trimethoxy-phenylsulfanyl)adenine (19). To a cooled solution (−40 °C) of **16** (303 mg, 0.87 mmol) in HF/pyridine (0.4 mL, 0.5 mL/mmol) was slowly added $NaNO_2$ (68 mg, 0.95 mmol), and the mixture was stirred for 15 min at −30 °C. The reaction was quenched by stirring for 1 h at room temperature with $CaCO_3$ (380 mg) in CH_2Cl_2 (2 mL). The slurry was taken up in CH_2Cl_2, washed with water, and dried over anhydrous Na_2SO_4. Following solvent removal, the product was further used without additional purification (120 mg, 39% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.34 (bs, 1H, NH), 7.77 (bs, 2H, NH_2), 6.83 (s, 2H), 3.76 (s, 6H, OCH_3), 3.67 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.6, 157.6, 153.4, 109.2, 60.0 and 56.1 (OCH_3); MS (EIS) *m/z* 352.0 (M + 1)$^+$.

2-Fluoro-9-(pent-4-ynyl)-8-(3,4,5-trimethoxy-phenylsulfanyl)adenine (20a). Following the general method for the preparation of **11e1**, a mixture of DBAD (365 mg, 1.55 mmol), PPh_3 (181 mg, 0.68 mmol), 4-pentnyn-1-ol (39 μL, 0.41 mmol), and **19a** (120 mg, 0.34 mmol) afforded **20a** (68.5 mg, 48% yield). IR (film) ν_{max} 3303−2941, 1664, 1582, 1499, 1359 (S), 1128, 1002; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.65 (s, 2H), 6.03 (bs, 2H, NH_2), 4.20 (t, J = 7.5 Hz, 2H, NCH_2), 3.80 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3), 2.18 (dt, J = 2.6 and 7 Hz, 2H), 1.92 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.0, 156.1 (J_{C-F} = 20 Hz), 153.8, 153.1, 146.3, 138.8, 124.7, 118.1, 109.1, 82.3 (C quaternary), 69.5 (CH), 60.9 and 56.3 (OMe_3), 43.0 (NCH_2), 28.0, 16.0; MS (EIS) *m/z* 418.1 (M + 1)$^+$. HPLC: (a) 97.2% (70% water − 30% acetonitrile); (c) 98.0%.

2-Fluoro-9-(pent-4-ynyl)-8-(2-chloro-3,4,5-trimethoxy-phenylsulfanyl)adenine (21a). Following the general method for the preparation of **12d** but using 1,4-dioxane as solvent, **20a** afforded **21a**. Yield, 19%. IR (film) ν_{max} 3307−2850, 1649,

1605, 1584, 1478, 1385, 1110, 1013; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81 (s, 1H), 6.16 (bs, 2H, NH_2), 4.33 (t, J = 7.4 Hz, 2H, NCH_2), 3.91 and 3.90 (2s, 6H, OCH_3), 3.77 (s, 3H, CH_3), 2.27 (dt, J = 2.6 and 7 Hz, 2H), 2.03 (m, J = 7 Hz, 2H), 1.98 (t, J = 2.6 Hz, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.1, 155.8 (J_{C-F} = 20 Hz), 152.6, 150.9, 146.0, 144.3, 123.4, 122.6, 117.3, 112.0, 82.0 (C quaternary), 69.7 (CH), 61.2 and 56.3 (OCH_3), 43.3 (NCH_2), 28.1, 16.0; MS (EIS) m/z 452.1 $(M + 1)^+$. HPLC: (a) 96.2% (60% water − 40% acetonitrile); (c) 95.0%.

2-Fluoro-8-bromo-9-(2-isopropoxy-ethyl)adenine (23). To a solution of 2-fluoroadenine (**22**) (20 mg, 0.13 mmol) in DMF (0.33 mL) were added at room-temperature Cs_2CO_3 (43 mg, 0.13 mmol) and 2-isopropoxy-ethyl 4-methylbenzenesulfonate (44 mg, 0.17 mmol). The reaction mixture was heated to 80 °C and stirred for 5 h. The solvent was evaporated and the residue purified by column chromatography on silica gel ($CHCl_3$:MeOH at 98:2) to afford 2-fluoro-9-(2-isopropoxy-ethyl)-adenine (19 mg, 61% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.72 (bs, 2H, NH_2), 4.17 (t, J = 5.4 Hz, 2H, NCH_2), 3.67 (t, J = 5.4 Hz, 2H), 3.49 (m, J = 6.0 Hz, 1H, CH), 0.98 (d, J = 6.0 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.6, 157.5, 156.1 (J_{C-F} = 21 Hz), 150.8 (J_{C-F} = 20 Hz), 141.6, 116.9, 70.9 (CH), 65.1, 43.4 (NCH_2), 21.8 (CH_3); MS (EIS) m/z 438.0 $(M - 1)^-$. *N*-bromosuccinimide (382 mg, 2.1 mmol) was added to a solution of 2-fluoro-9-(2-isopropoxy-ethyl)adenine (257 mg, 1.1 mmol) in DMF (6 mL), and the resulting mixture was stirred for 11 h at room temperature. Following solvent removal under high vacuum, the crude material was purified by column chromatography on silica gel (EtOAc:MeOH:NH_4-OH at 18:1:0.2) to furnish **23** (84 mg, 25% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 5.59 (bs, 2H, NH_2), 4.41 (t, J = 5.9 Hz, 2H, NCH_2), 3.81 (t, J = 5.8 Hz, 2H), 3.55 (m, J = 6.1 Hz, 1H, CH), 1.08 (d, J = 6.1 Hz, 6H, CH_3); MS (EIS) m/z 299.9 $(M + 2)^+$.

2-Fluoro-9-(2-isopropoxy-ethyl)-8-(3,4,5-trimethoxy-phenylsulfanyl)adenine (20b). To 3,4,5-trimethoxythiophenol (**6**) (35 mg, 0.17 mmol) in DMF (1 mL) was added K_2CO_3 (44 mg, 0.31 mmol), and the resulting solution was stirred for 1 h at room temperature. Following the addition of **23** (50 mg, 0.16 mmol), the reaction mixture was heated to 130 °C and stirred for 4 h. The solvent was removed under high vacuum and the crude material purified by column chromatography on silica gel (Hexanes:CH_2Cl_2:EtOAc at 2:1:2) to afford **20b** (27 mg, 40% yield). IR (film) ν_{max} 3322−2868, 1649, 1582, 1498, 1367, 1127, 1004; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.64 (s, 2H), 6.25 (bs, 2H, NH_2), 4.30 (t, J = 5.9 Hz, 2H, NCH_2), 3.75 (s, 3H, OCH_3), 3.74 (s, 6H, OCH_3), 3.59 (t, J = 5.9 Hz, 2H), 3.41 (quin, J = 6 Hz, 1H, CH), 0.97 (d, J = 6 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1, 156.2 (J_{C-F} = 20.5 Hz), 153.7, 147.2, 138.5, 125.4, 118.0, 108.9, 72.7 (CH), 65.2, 60.8 and 56.3 (OCH_3), 44.0 (NCH_2), 21.8; MS (EIS) m/z 438.1 $(M + 1)^+$. HPLC: (a) 98.1% (60% water − 40% acetonitrile); (c) 96.0%.

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Supporting Information Available: Spectral data, HPLC, and LC-MS analyses are available free of charge via the Internet at http://pubs.acs.org.

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