Orally Active Purine-Based Inhibitors of the Heat Shock Protein 90

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Orally active Hsp90 inhibitors are of interest as potential chemotherapeutic agents. Recently, fully synthetic 8-benzyladenines and 8-sulfanyladenines such as **4** were disclosed as Hsp90 inhibitors, but these compounds are not water soluble and consequently have unacceptably low oral bioavailabilities. We now report that water-solubility can be achieved by inserting an amino functionality in the N(9) side chain. This results in compounds that are potent, soluble in aqueous media, and orally bioavailable. In an HER-2 degradation assay, the highest potency was achieved with the neopentylamine **42** (HER-2 IC_{50} = 90 nM). In a murine tumor xenograft model (using the gastric cancer cell line N87), the H_3PO_4 salts of the amines **38**, **39**, and **42** induced tumor growth inhibition when administered orally at 200 mg/kg/day. The amines **38**, **39**, and **42** are the first Hsp90 inhibitors shown to inhibit tumor growth upon oral dosage.

Introduction

Heat Shock Protein 90 (Hsp90) is a molecular chaperone that maintains the proper conformation of many "client" proteins.[1] Inhibition of Hsp90 causes these client proteins to adopt aberrant conformations, and these abnormally folded proteins are rapidly eliminated by the cell via ubiquitinylation and proteasome degradation. Interestingly, the list of Hsp90 client proteins includes a series of notorious oncogenes. Four of them are clinically validated cancer targets: HER-2/neu (Herceptin (trastuzumab)), Bcr-Abl (Gleevec (imatinib mesylate)), the estrogen receptor (tamoxifen), and the androgen receptor (Casodex (bicalutamide)), while the others play a critical role in the development of cancer. Some of the most sensitive Hsp90 clients are involved in growth signaling (Raf-1, Akt, cdk4, Src, Bcr-Abl, etc.). In contrast, few tumor suppressor genes, if any, seem to be clients of Hsp90,[1,2] and consequently, inhibition of Hsp90 has an overall antiproliferative effect. In addition, some client proteins are involved in other fundamental processes of tumorigenesis, namely apoptosis evasion (e.g. Apaf-1, RIP, Akt), immortality (e.g. hTert), angiogenesis (e.g. VEGFR, Flt-3, FAK, HIF-1), and metastasis (c-Met).

The various client proteins are not equally responsive to Hsp90 inhibitors, and some undergo degradation at lower concentrations of the inhibitor, or with faster kinetics, depending on the cell line. The more sensitive clients are usually those involved in growth signaling, but some mutated proteins found in tumor cells (mutant p53, Gleevec-resistant Bcr-Abl)[3] are particularly dependent on Hsp90 to preserve their conformation and function. This unique feature sensitizes tumor cells to Hsp90 inhibitors, and when these factors converge, they confer on Hsp90 inhibitors notable anticancer properties in vitro and in vivo.

A remarkable advantage of targeting Hsp90 lies in the simultaneous depletion of multiple oncogenic proteins, thereby attacking several pathways necessary for cancer development and reducing the likelihood of the tumor acquiring resistance to the Hsp90 inhibitor.[2b,2c] Another striking feature of Hsp90 is that it occurs in an activated form in cancer cells, and in a latent form in normal cells.[4] This provides an opportunity to specifically target cancer cells with inhibitors selective for the activated form,[4] such as the natural product geldanamycin (**1a**, Figure 1) and its derivatives. What distinguishes the activated and latent forms of Hsp90 at a molecular level is not well understood. It is clear, however, that the activity of Hsp90 is regulated by a highly sophisticated process involving at a minimum (1) Hsp90 dimerization, (2) formation of multiprotein complexes with numerous co-chaperones, and (3) ATP/ADP binding, ATP hydrolysis being essential for the chaperone cycle and function.[1] The chaperoning function of Hsp90 can be "switched off" by inhibiting its ATP-ase activity. The nucleotides ADP and ATP can bind to two sites, one located close to the N-terminal, the other close to the C-terminal. The natural products geldanamycin (Figure 1, **1a**) and radicicol (**2**) bind to the N-terminal domain, while novobiocin binds to the C-terminal domain.[5]

Many of the natural-product-derived Hsp90 inhibitors exhibit pharmaceutical deficiencies. For example, the semisynthetic

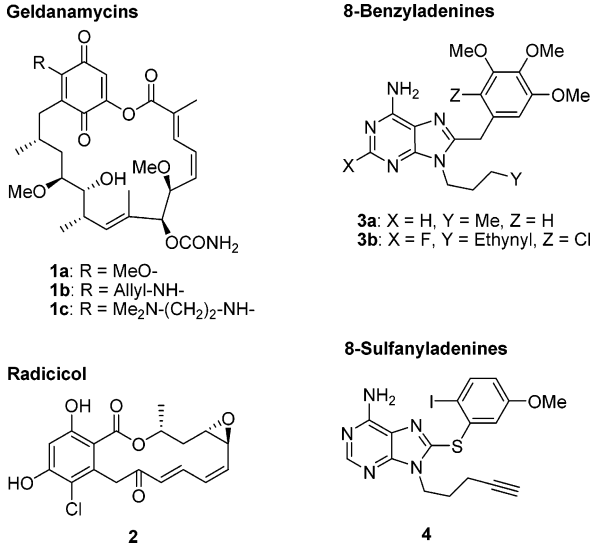

Figure 1. Hsp90 inhibitors.

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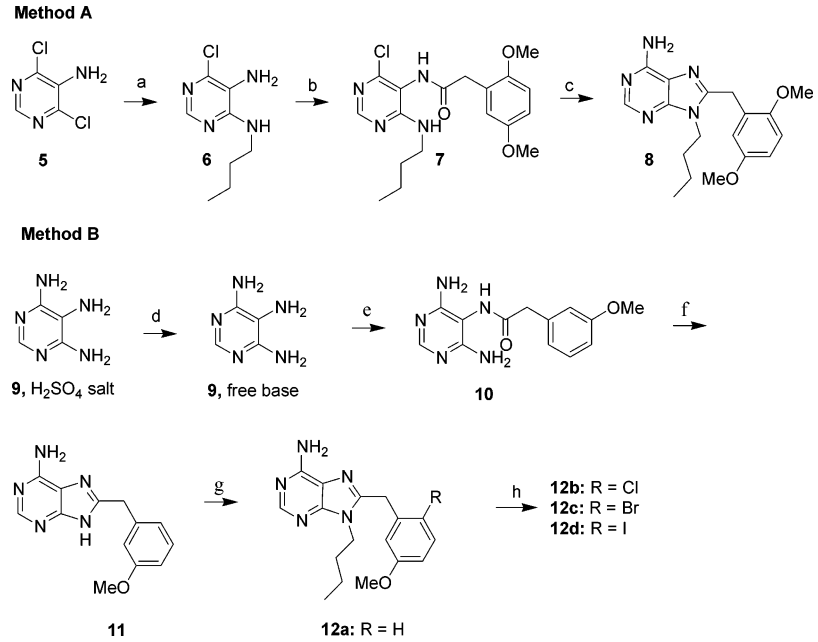
Scheme 1. Preparation of 8-Benzyladenines[a]



[a] Reagents and conditions: (a) $BuNH_2$, Et_3N, *n*-BuOH, 90 °C, 16 h (83%); (b) 2,5-dimethoxyphenylacetic acid, TsCl, Et_3N, DCE, 40 °C, 16 h (71%); (c) NH_3, MeOH, 120 °C, 3 d (80%); (d) NaOH, H_2O, 0−5 °C (67%); (e) 3-methoxyphenylacetyl chloride, NMP, 40−50 °C (98%); (f) MeONa, n-BuOH, reflux, 2 h (75%); (g), BuI, Cs_2CO_3, DMF, rt, 16 h (54%); (h) SO_2Cl_2, Br_2, or NIS (31−57%).

inhibitor 17-allylamino,17-desmethoxy-geldanamycin (17-AAG, **1b**), currently in phase II clinical trials, is expensive to manufacture, difficult to formulate,[6] and at present administered only parenterally. Although the 17-dimethylaminoethylamino analogue (17-DMAG, **1c**) is more soluble, it exhibits all of the side effects of 17-AAG as well as gastrointestinal hemorrhaging in preclinical toxicity studies.[7] Radicicol (**2**, Figure 1), another natural product Hsp90 inhibitor, is poorly water-soluble and is inactive in tumor xenograft models. Semisynthetic oxime derivatives of radicicol provide better solubility and substantially improved the pharmacological profile in murine models, but are still limited to intravenous administration.[8]

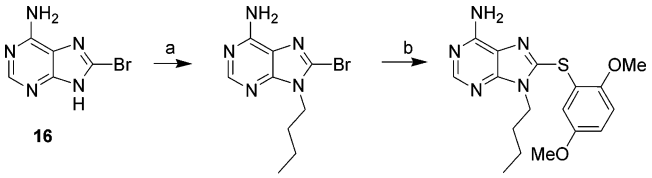
Fully synthetic, orally active inhibitors of Hsp90 have been sought in order to provide more flexible dosing schedule options and to possibly avoid the side-effects of the natural product inhibitors. Recently, several novel nonnatural product Hsp90 inhibitors were reported.[1b,9−11] The structures of these inhibitors were designed using the crystal structures of Hsp90 in complex with ATP, geldanamycin, or radicicol. The 8-benzyladenines such as PU3 (**3a**)[9] were designed to adopt the same C-shaped conformation as geldanamycin[12] with the adenine ring pointing to the adenine-binding site (hinge region), and the trimethoxybenzene ring emulating the H-bond accepting nature of the quinone ring of geldanamycin.[13] The recently obtained[10c] crystal structure of Hsp90 in complex with **3a** confirmed that the purine ring occupies the position normally occupied by ADP/ATP, but the benzene ring points in a direction opposite to the predicted one, to form a π-stacking interaction with Phe138. Nevertheless, **3a** inhibits Hsp90 (HER-2 degradation assay, HER-2 IC_{50} = 40 μM) and afforded a valuable starting point for further optimization. Structure−activity studies based on **3a** led to the more active PU24FCl (**3b**, HER-2 IC_{50} = 1.7 μM)[9] which was subsequently also cocrystallized[10] with Hsp90. When **3b** was formulated in DMSO/EtOH/phosphate-buffered saline 1:1:1 and administered intraperitoneally to mice bearing MCF-7 xenograft tumors, it induced at 100−300 mg/kg down-regulation of HER-2 and Raf-1, a pharmacodynamic response consistent with Hsp90 inhibition, and at 200 mg/kg it significantly repressed tumor growth.[9b] Very high doses (500−1000 mg/kg) of **3b** were required to observe a similar pharmacodynamic response upon oral administration,[9b] and no 8-benzyladenine has been reported to inhibit tumor growth by the oral route. In our hands, **3b** proved to be too insoluble to be effectively formulated and delivered orally. So far, despite extensive SAR studies to improve potency and pharmaceutics properties, Hsp90 inhibitors have not demonstrated activity in animal models of human cancer (xenografts) when administered orally.

The discovery of the 8-benzyladenines led us[14] and others[15] to design 8-sulfanyladenines, exemplified by 8-(2-iodo-5-methoxy-phenylsulfanyl)-9-pent-4-ynyl-9*H*-purin-6-ylamine (**4**). Compound **4** exhibited excellent potency in several cell-based assays but was poorly soluble in water and did not have sufficient oral bioavailability in clinically acceptable formulations. We now provide details on the SAR studies that led to the discovery of **4** and further expand the SAR studies with the introduction of solubilizing groups in the N(9) side-chain, which provide water-solubility, oral bioavailability, and subsequently, oral efficacy in tumor xenograft models.

Chemistry

Our strategy for improving the activity of purine-based Hsp90 inhibitors was to independently optimize the substituents on the benzene ring of 8-benzyladenines and the nature of the linker spanning between the benzene and the purine rings. We would then combine the preferred structural elements emerging from both optimization runs and select for compounds exhibiting acceptable pharmaceutical properties. This plan allowed us to take full advantage of the known methods for the preparation of 8-benzylpurines, although in some cases refinements proved to be necessary. The 8-benzyladenines were synthesized by either of the two methods illustrated in Scheme 1. The first method followed a sequence closely related to the one described by Drysdale et al.[10] and started from commercial 4,6-dichloro-5-aminopyrimidine **5**, which was treated with butylamine, acylated with the appropriate phenacyl chloride, and cyclized to afford the adenine **8**. The second method was similar to the

Scheme 2. Preparation of 8-(Arylsulfanyl)adenine **18**[a]



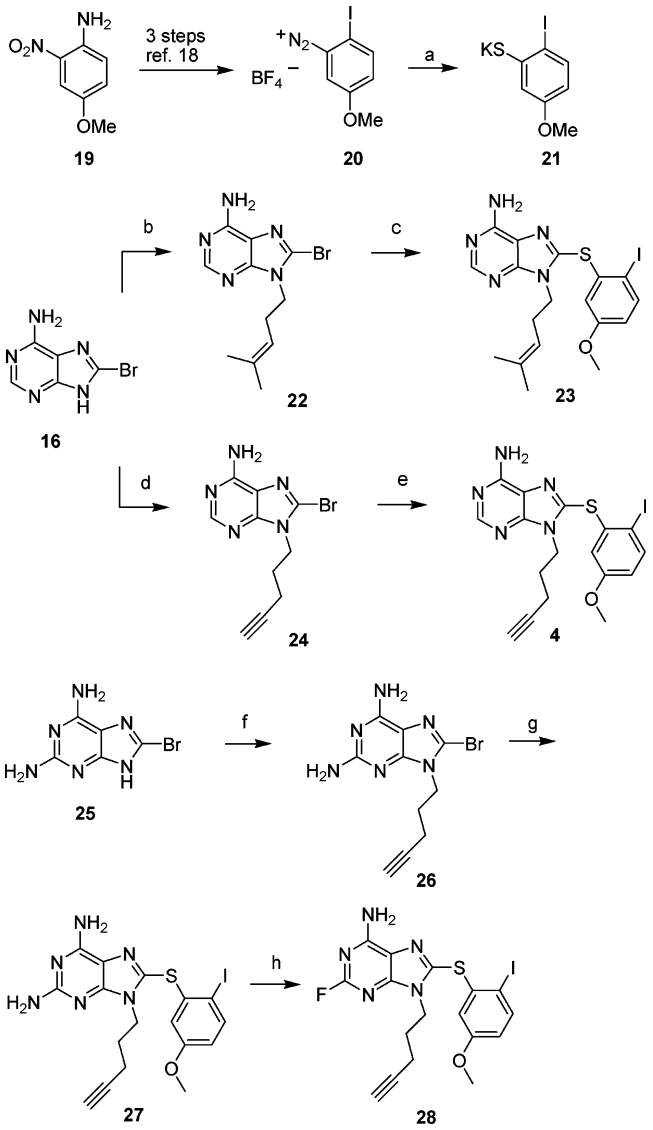
[a] Reagents and conditions: (a) BuI, Cs_2CO_3, DMF, rt, 16 h (62%); (b) 2,5-dimethoxybenzenethiol, 150 °C, 4 h (69%).

one of Chiosis et al.[9] and started with 4,5,6-triaminopyrimidine **9**, which was acylated and cyclized to give the adenine **11**. The final alkylation gave predominantly the desired N(9)-alkyl isomer **12a**, together with a minor regioisomer which was removed by chromatography (regioselectivity = 5:1 by ^{1}H NMR analysis of the crude product). The anisole **12a** was halogenated using standard reagents (SO_2Cl_2, Br_2, NIS/AcOH). One improvement in the synthetic sequence pertained to the acylation step. The published method[9a] involves acylation of 4,5,6-triaminopyrimidine hemisulfate (**9**) in aqueous solution (**9** is soluble only in water at pH ≥ 7) and required, in our hands, several equivalents of the appropriate acyl fluoride to compensate for the accompanying hydrolysis of the reagent. We found that the free base of **9** was readily isolated as needles by neutralizing and cooling to 0−5 °C an aqueous solution of the commercial 4,5,6-triaminopyrimidine hemisulfate. The free base proved to be soluble in *N*-methyl-2-pyrrolidone (NMP) and could be efficiently acylated in this solvent with a single equivalent of acyl chloride to give the desired amide **10**, which precipitated as its HCl salt. The use of DMF as a solvent was less satisfactory, since it gave rise to a competitive formylation of the 5-NH_2 group via a Vilsmeier−Haack type of reaction. Bases such as Et_3N were best avoided, to prevent overacylation.[16] The symmetry of the ^{1}H NMR spectrum of **10** indicated that the acylation had occurred selectively at the 5-position. Finally, the cyclization of **10** to the desired purine **11** was carried out with MeONa in refluxing n-BuOH, a minor deviation from the original MeOH, but which provided more forceful and generally applicable conditions.

A different approach was necessary to investigate the effect of the linker between the purine and the benzene ring. The compounds with a sulfur atom as a linker were prepared according to the example shown in Scheme 2. 8-Bromoadenine (**16**)[17] was alkylated to give a 2:1 mixture of the N(9)- and N(3)-alkylated isomers, from which the desired N(9)-butyl isomer **17** could be isolated by chromatography. Displacement of the bromine atom with the desired thiophenolate gave the 8-sulfanyladenine **18**.

A similar approach was used to generate 8-(benzenesulfanyl)-adenines carrying an iodo substituent on the benzene ring via the convergent synthesis shown in Scheme 3. The nitroaniline **19** was converted in three known steps (1. $NaNO_2$, KI. 2. H_2N-NH_2/Fe. 3. $NaNO_2$, HBF_4) to the diazonium salt **20**, which gave after a two-step Leuckart synthesis (4. $EtOCS_2K$, 5. KOH) the thiophenolate **21**.[18] The purification of **21** proved to be challenging. Dissolution of **21** in MeOH and precipitation with EtOAc gave a low recovery (<20%) of the desired thiophenolate, while neutralization and chromatography was complicated by the odor of the thiophenol and its tendency to oxidize to the corresponding disulfide. The highest overall yield for the conversion of **20** to **23** was achieved by hydrolyzing the intermediate xanthate with 2 equivalents of KOH in MeOH, concentrating the reaction mixture, and using it without removing the excess of potassium salts. Alkylation of 8-bromoadenine (**16**) with homoprenyl bromide gave a 2:1 mixture of the desired N(9)- vs. N(3)-alkylated products, and the desired isomer **22** was isolated by chromatography. Coupling the thiophenolate **21** with the 8-bromoadenine **22** gave the sulfanyladenine **23**. Similarly, the 8-bromoadenine **16** was alkylated with 5-chloro-1-pentyne to give **24** and coupled to the thiophenolate **21** to provide the pentyne analogue **4**. The 2-fluoro-8-sulfanyladenine **28** was prepared by a conceptually similar route starting from 2,6-diamino-8-bromopurine (**25**) and using a Balz-Schiemann reaction (*iso*-AmONO/HBF_4) to replace the 2-NH_2 group by a fluorine substituent.

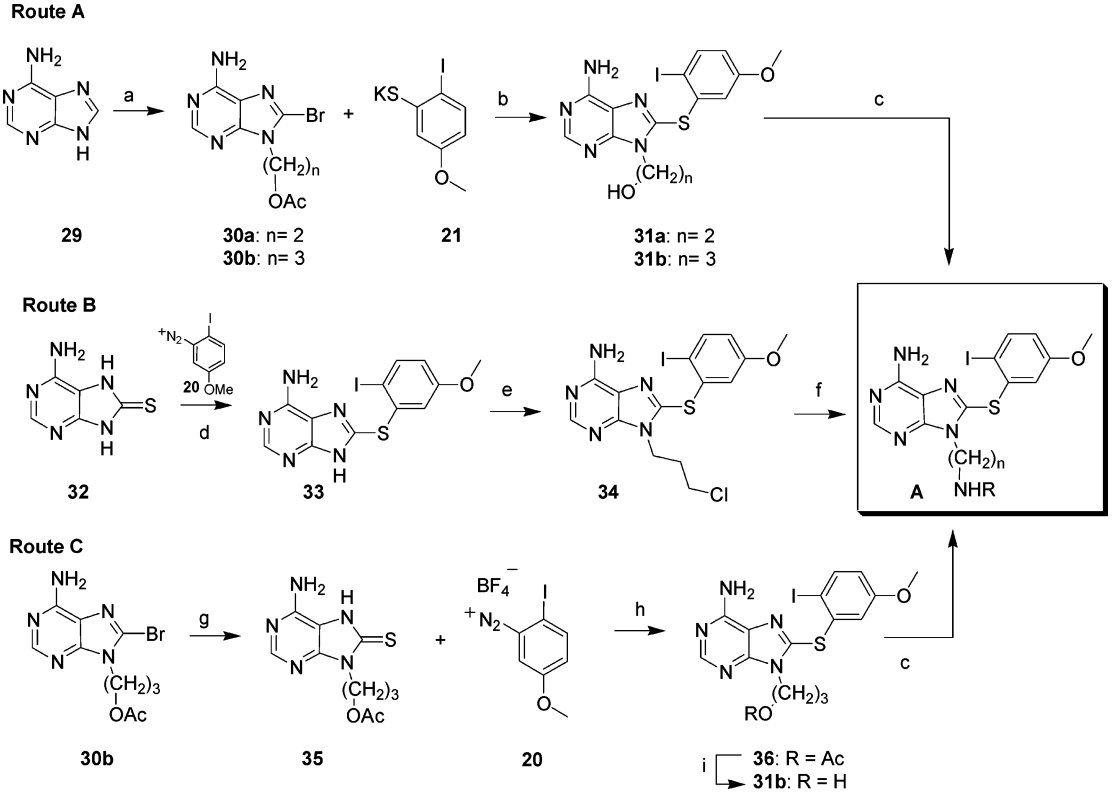
Scheme 3. Preparation of 8-Sulfanyladenines **23**, **4**, and **28**[a]



[a] Reagents and conditions: (a) $EtOCS_2K$, −40 °C to room temperature, 30 min, then KOH, MeOH, 40 °C, 2 h; (b) 5-bromo-2-methyl-pent-2-ene, Cs_2CO_3, DMF, rt, 3 h (38%); (c) **21**, DMF, 100 °C, 16 h (15%); (d) 5-chloro-1-pentyne, Cs_2CO_3, DMF, 70 °C, 3 h (25%); (e) **21**, DMF, 70 °C, 16 h (71%); (f) 5-chloro-1-pentyne, Cs_2CO_3, DMF, 85 °C, 16 h (66%); (g) **21**, DMF, 100 °C, 16 h (43%); (h) *iso*-AmONO, HBF_4, THF, −20 °C to +40 °C, 10 min (13%).

This route suffered from two drawbacks. First, the alkylation was not regioselective and required a tedious chromatographic separation. Second, the thiophenolate **21** was malodorous, difficult to purify, and if used without chromatographic purification, gave results which were very sensitive to its purity. One improvement (Scheme 4, route A) consisted of starting from

Scheme 4. Preparation of Water-Soluble 8-Sulfanyladenines of Generic Structure **A**[a]



[a] Reagents and conditions: (a) AcO−$(CH_2)_2$−Br, Cs_2CO_3, DMF, 45 °C, 5 h (67%) or AcO−$(CH_2)_3$−Cl, Cs_2CO_3, DMF, 70 °C, 12 h (88%), then Br_2, AcOH, THF, H_2O (34−81%); (b) **21**, DMF, 50 °C, 16 h; then K_2CO_3, MeOH (37%); (c) MsCl, Et_3N, dioxane, rt, 30 min; then amine, 55 °C, 16 h (45−75%); (d) **21**, DMF, −60° to room temperature, 2h (26%); (e) Br−$(CH_2)_3$−Cl, Cs_2CO_3, DMF, 50 °C, 2 h (21%); (f) amine, 90−110 °C, 16 h, (67−73%); (g) thiourea, n-BuOH, reflux, 3 h (88%)—see also ref 19; (h) **20**, DMSO, rt, 24 h (51%); (i) K_2CO_3, MeOH (37%).

pure adenine (**29**) which underwent alkylation solely at N(9). We chose as alkylating agents either Br−$(CH_2)_2$−OAc or Cl−$(CH_2)_3$−OAc, in which the masked hydroxyl group provided a handle for further functionalization. Unlike the unsubstituted adenine, the alkylated product was easily brominated at C(8) to give **30** (n = 2, 3). The bromine atom of **30** was then displaced with the potassium thiophenolate **21**, and the acetyl protecting group was cleaved in situ to give the 8-sulfanyladenine **31**. The hydroxy group of **31** was mesylated and displaced with amines to give the corresponding *N*-alkylamines of generic structure A (Scheme 4).

This route, however, still required the disagreeable preparation of the thiophenolate **21**. We therefore investigated the direct treatment of the diazonium salt **20** with the anion of 8-thionoadenine **32**, which already contained the desired sulfur atom (Scheme 4, route B), thus avoiding the preparation of **21**. We synthesized **32** by condensation of 4,5,6-triaminopyrimidine with thiourea,[19] and treatment of **32** with the diazonium salt **20** to give the desired adduct **33**. The yield (unoptimized) was low, and the subsequent alkylation of **33** with Br−$(CH_2)_3$−Cl still gave a 2:1 mixture of N(9)- and N(3)-regioisomers. Despite these problems, the route was effective in generating small amounts of amines of formula **A**. However, an improved route was needed to prepare gram amounts of **A**. A third route was selected (Scheme 4, route C) which eliminated the use of the thiophenolate **21** and gave only one regioisomer. Bromoadenine **30b** (route A) was converted with thiourea to the 8-thionoadenine **35**,[19] which was coupled directly with the diazonium salt **20** to give the desired, known adduct **36**.[19] Finally, the same standard manipulations of the side-chain (deacetylation, mesylation, amination) gave the desired amines of generic formula **A**. Routes A and C gave similar overall yields and were used interchangeably, but the latter was consistently reproducible and avoided the use of **21**.

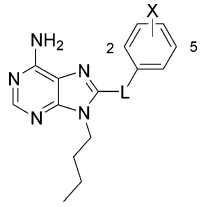
Results and Discussion

The potency of the compounds was assessed using a HER-2 degradation assay, which was described in detail elsewhere.[20] Briefly, compounds were incubated for 16 h with MCF-7 cells, a breast cancer cell line expressing on its surface medium levels of the Hsp90 client HER-2. Inhibition of Hsp90 induces the degradation of HER-2, which was monitored with a combination of phycoerythrin-labeled antibody and flow cytometry. This assay is highly reproducible, with 17-AAG (**1b**) consistently giving an HER-2 IC_{50} of 12.9 ± 0.3 nM, wherein the error refers to the standard error of the mean (SEM).

We first investigated the effect of substituents on the benzene ring. The 2,5-dimethoxy substitution pattern emerged as more potent than the prototypic 3,4,5-trimethoxy pattern of **3a** and **3b** (Table 1, entries 1, 2). Replacing the 2-MeO group by Cl marginally decreased the activity (entry 3), but replacing it with Br or I led to an increase in activity (entries 4, 5). We next investigated the effect of the linker. The compounds with a NH or O as linker are inactive,[9] and it was assumed that only the CH_2 linker could be tolerated. However, upon introduction of an S linker (Table 1, entry 4), we were gratified to observe that the sulfur atom was not only tolerated, but was superior to the original CH_2 linker (Table 1, entry 5).[14]

We next optimized the N(9) side-chain and screened over 100 analogues of the 2,5-dimethoxybenzyl adduct **8**.[21] The homoprenyl side-chain emerged as an equipotent alternative to the already disclosed[9] pent-4-ynyl side-chain, both analogues having an HER-2 IC_{50} = 1.5 μM. Thus, having separately

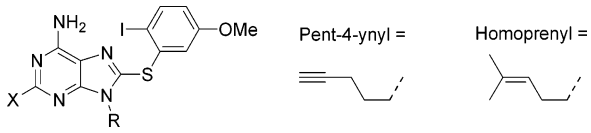
Table 1. Optimization of the Benzene Ring Substituents[a]



entry	ID	L	X	HER-2 IC_{50} [μM]
1	**3a**	CH_2	3,4,5-triMeO	40
2	**8**	CH_2	2,5-diMeO	12
3	**12b**	CH_2	2-Cl, 5-MeO	20
4	**12c**	CH_2	2-Br, 5-MeO	8.0
5	**12d**	CH_2	2-I, 5-MeO	5.0
6	**18**	S	2,5-diMeO	3.5

[a] All values represent the average of at least three independent observations. The standard errors of the mean (SEM) are 6−11% of the mean value.

Table 2. Combinations of the Preferred Structural Elements[a]



entry	ID	X	R	HER-2 IC_{50} [μM]
1	**4**	H	pent-4-ynyl	0.28
2	**23**	H	homoprenyl	0.37
3	**28**	F	pent-4-ynyl	0.36

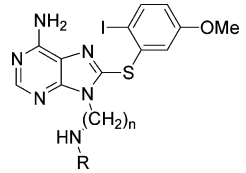
[a] All values represent the average of at least three independent observations. The standard errors of the mean (SEM) are 6−11% of the mean value.

optimized the benzene ring substituents (2-iodo-5-methoxy), the linker (−S−), and the side-chain (homoprenyl or pent-4-ynyl), we examined the combination of these preferred structural features (Table 2). The homoprenyl analogue **23** and the pent-4-ynyl analogue **28** had similar potencies (HER-2 $IC_{50} \approx 0.3$ μM, entries 1, 2). However, the addition of a 2-F substituent on the adenine ring, an operation known to be favorable in the 8-benzyladenine series,[9] did not bring additional activity to the 8-sulfanyl series (entry 3).

Although these compounds exhibited improvements in potency over previously reported Hsp90 inhibitors, they proved to be poorly water-soluble, especially with the 2-iodo substituent. This hampered their formulation and rendered them insufficiently orally bioavailable. We therefore sought to incorporate ionizable amino groups in the N(9) side-chain of the inhibitor. The introduction of the amino group not only improved the water solubility but also increased the potency. The highest potencies were obtained when the amino N atom was separated by two or three methylene units from the purine ring and was further substituted with a bulky alkyl group (Table 3). The most active compound in the three-atom linker series proved to be the *tert*-butylamine **40** (HER-2 $IC_{50} = 140 \pm 15$ nM, entry 6), while in the two-atom linker series the neopentylamine **42** (HER-2 $IC_{50} = 90 \pm 10$ nM, entry 8) showed optimal activity.

The amines **37**−**42** were tested for their ability to inhibit cell-growth, using a previously described assay[20] to quantify cell proliferation. In brief, MCF-7 breast cancer cells were incubated for 5 days with the compound and then treated with MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium). The MTS reagent is reduced only by metabolically active cells to the formazan dye, and the number of live cells was deduced by spectrophotometry (490 nM). The MTS IC_{50} was defined as the concentration of Hsp90

Table 3. Effect of Amino Group in Side-Chain



entry	ID	*n*	R	HER-2 IC_{50} [μM][a]	MTS IC_{50} [μM][b]
1	**3b**			1.7	1.2
2	**4**			0.29	0.7
3	**37**	3	Et_2CH-	0.21	0.2
4	**38**	3	EtMeCH-	0.21	0.2
5	**39**	3	*i*-Pr-	0.18	0.6
6	**40**	3	*t*-Bu-	0.14	0.2
7	**41**	2	*i*-Bu-	0.10	0.2
8	**42**	2	*t*-$BuCH_2$-	0.09	0.5

[a] For the HER-2 degradation assay, the values represent the average of at least three independent observations, and the standard errors of the mean (SEM) are 6−11% of the mean value. [b] For the growth inhibition assay, the values represent the average of at least three independent observations, and the standard errors of the mean (SEM) are 9−21% of the mean value.

inhibitor that gave rise to 50% less new live cells compared to an untreated culture. In this assay, the control 17-AAG (**1b**) had an MTS IC_{50} of 32 ± 4 nM, and the standard error of the mean (SEM) associated with this assay ranged from 9 to 21% of the mean value. The 8-(sulfanyl)adenines proved to be inhibit cell growth (Table 3), with MTS IC_{50} values typically in the 200−500 nM range, which is roughly within 1 logarithmic unit of the "gold standard" 17-AAG.

The selectivity of **42** for Hsp90 over other ATP-binding proteins was assessed with a panel of human kinases (Aurora-A, CHK2, IKKα, MAPK1, MAPK2, MEK1, PDK1, Plk3, PI-3K, c-Raf, c-Src), none of which were significantly inhibited at 10 μM.

Perhaps the most important feature of these compounds, besides their potency, was their dramatically increased water solubility. Once converted to their H_3PO_4 salt, these amines provided excellent water solubility (>10 mg/mL) and were readily administered in standard aqueous solutions. For animal studies, we chose to formulate the compounds in a phosphatidylcholine/water dispersion.[22] The pharmacokinetic properties of these compounds were determined in Balb/C mice (Table 4) as described previously.[20] When the compounds were administered orally at 100 mg/kg, peak plasma concentrations (C_{max}) between 4.8 and 9.7 μg/mL (10−19 μM) were achieved. The plasma concentrations peaked at $T_{max} = 30$ min, indicating rapid absorption, and dropped below the detection limit (0.5 μg/mL, 1 μM) after 1−4 h to give, when integrated over a 4 h period, AUC values of 240 to 680 min·μg/mL (equivalent to 8−22 μM·h). The effect of the solubility on the oral bioavailability was striking, and the %F increased from <10% for pentyne **4** (data not shown) to 14−97% for the amines **37**−**42**. When administered intravenously at 10 mg/kg, the amines **37**−**42** were cleared at the rate of 33−131 mL/min/kg, which is quite high compared to the total liver blood flow (90 mL/min/kg for mice). We did not determine, however, if the clearance was due to metabolism, distribution, or low protein binding. By analogy with the structurally related adenine **3b**,[9b] it is also possible that the inhibitors **37**−**42** accumulate in the tumor to concentrations exceeding those in the plasma. Despite their high clearance at 10 mg/kg, the oral bioavailability of compounds **39**, **40**, and **42** at 100 mg/kg was equal to or greater than 50%, suggesting that the clearance was saturated at 100 mg/kg.

This is to our knowledge the first time that pharmacologically relevant concentrations of Hsp90 inhibitors were achieved via

Table 4. Pharmacokinetic Parameters of Selected Amines

		iv parameters			po parameters				
entry	compound	Cl [mL/min/kg]	$T_{1/2}$ [h]	V_{ss} [L/kg]	C_{max} [μg/mL]	T_{max} [h]	AUC [min·μg/mL]	$T_{1/2}$ [h]	%*F* [%]
1	**37**	69	0.5	2.4	5.8	0.5	590	0.6	42
2	**38**	33	1.3	3.4	4.8	0.5	380	0.7	28
3	**39**	62	4.2	22	4.7	0.5	450	1.5	55
4	**40**	131	1.6	24	6.7	0.5	680	0.9	97
5	**41**	47	0.3	0.4	6.7	0.5	290	0.3	14
6	**42**	64	0.4	1.6	9.5	0.5	760	0.5	50

[a] The compounds were formulated as H_3PO_4 salts in a phosphatidylcholine/water dispersion and delivered intravenously (iv) at 10 mg/kg or orally (po) at 100 mg/kg. Plasma concentrations were measured at six time points over 4 h, and the pharmacokinetic parameters were determined using noncompartmental methods (WinNonlin Professional, Version 4.1). The terminal half-life was calculated using 3−4 data points.

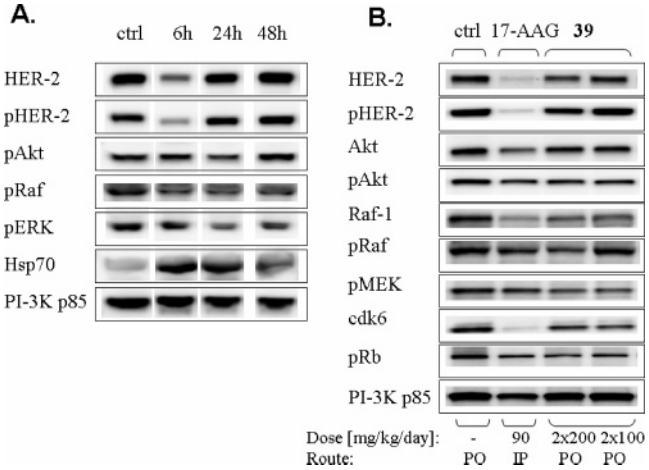


Figure 2. (a) Levels of Hsp90 clients, Hsp70, and PI-3K p85 in murine A549 tumor xenografts following a single oral administration of **41**·H_3PO_4 at 200 mg/kg. (b) Levels of Hsp90 clients and PI-3K in murine N87 tumor xenografts 24 h after a three-day course of 17-AAG (intraperitoneally, 1 × 90 mg/kg/day) or **39**·H_3PO_4 (orally, 2 × 200 or 2 × 100 mg/kg/day).

the oral route, and the results compiled in Table 4 suggested that these inhibitors may be orally active. For instance, a C_{max} = 5.8 μg/mL (**37**) corresponds to a concentration of 12 μM, which is approximately 50-fold higher than the concentrations required to either induce HER-2 degradation in MCF-7 cells (HER-2 IC_{50} = 0.21 μM) or to inhibit the proliferation of MCF-7 cells (MTS IC_{50} = 0.2 μM). The plasma concentration of the amines **37**−**42** remained above 1 μM, the detection limit, for 1−4 h.

The amines **37**−**42** provided a good combination of potency, ease of formulation, and bioavailability but displayed relatively high clearance values in mice. We next verified the ability of the arbitrarily chosen amine **41** to induce the degradation of Hsp90 clients in vivo. Nude mice were implanted with A549 lung cancer cells, a cell line dependent on the Hsp90 clients Raf-1 and Akt for cell proliferation, and were administered a single oral dose of **41**·H_3PO_4 (200 mg/kg). The mice were sacrificed at 6, 24, or 48 h, the tumors were harvested, and Hsp90 client proteins were visualized by Western blot. The levels of the Hsp90 clients HER-2 and pHER-2 significantly decreased at 6 h, and then gradually reached their normal value after 24−48 h (Figure 2a). The levels of the Hsp90 clients pAKT and pRaf and the downstream kinase pERK decreased less dramatically and were lowest at 24 h. Upregulation of the chaperone Hsp70, a response characteristic of Hsp90 inhibition, was evident and lasted 24−48 h. As expected, the kinase PI-3K, which is not an Hsp90 client, was unaffected. These pharmacodynamic data underscore an added benefit of targeting Hsp90, since exposing tumor cells to an Hsp90 inhibitor for a few hours is sufficient to induce the degradation of the client

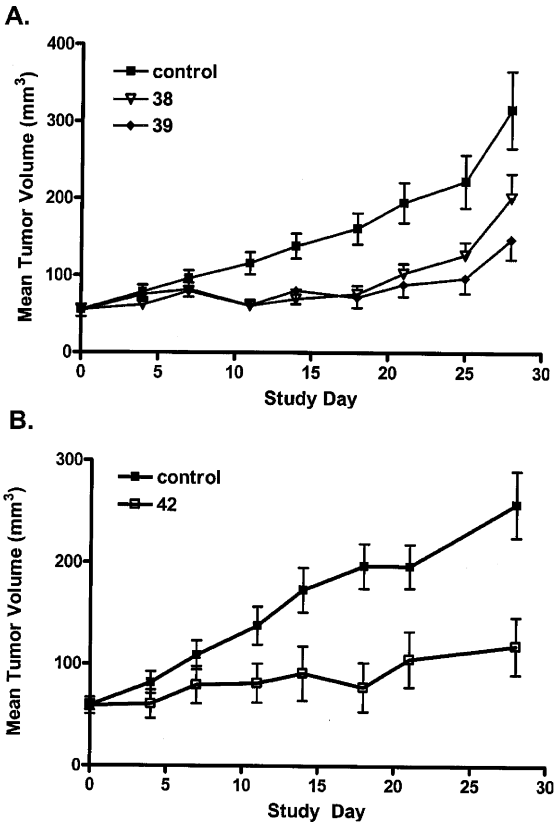


Figure 3. Tumor growth inhibition in murine N87 xenografts models induced by (a) inhibitors **38**·H_3PO_4 and **39**·H_3PO_4 delivered orally (1 × 200 mg/kg/day, 5 days/week) or (b) inhibitor **42**·H_3PO_4 delivered orally (2 × 100 mg/kg/day, 5 days/week). Error bars = SEM.

proteins. Once degraded, those client proteins require 6−48 h to accumulate back to their normal levels, and even if the Hsp90 inhibitor is rapidly cleared as **41**, its pharmacological effect can be long lasting. This behavior differs significantly from that of most ATP-competitive kinase inhibitors which, once cleared, allow their target to immediately resume its function.[23]

The pharmacodynamic effect of amine **39** was examined in a N87 xenograft model (Figure 2b), N87 being a stomach cancer cell line expressing high HER-2 levels. Mice were administered **39**·H_3PO_4 orally at two different regimens (2 × 100 or 2 × 200 mg/kg/day) for 3 days and were sacrificed 24 h after the last dosing. Oral administration of **39**·H_3PO_4 at 2 × 200 mg/kg/day induced the degradation of the Hsp90 clients Akt, pAkt, Raf-1, pRaf, cdk6, and pRb to levels comparable to those obtained with 17-AAG injected intraperitoneally once daily at 90 mg/kg/day. The levels of HER-2 and pHER-2 decreased only partially, probably reflecting the fact that HER-2 and pHER-2 are degraded and re-expressed faster (<24 h) than other Hsp90 clients. A **39**·H_3PO_4 dose of 2 × 100 mg/kg/day was still

effective at degrading Akt, pAkt, Raf-1, pMEK, cdk6, and pRb but promoted little or no degradation of HER-2, pHER-2, and pRaf.

Next, the ability of a subset of amines (**38**, **39**, and **42**) to repress tumor growth was examined in murine xenograft models using the N87 stomach cancer cell line, which grew in mice more reproducibly than the A549 cell line. Compounds **38**·H_3PO_4 and **39**·H_3PO_4 were delivered orally at 200 mg/kg/day (once daily, 5 days/week), in the same experiment (Figure 3a). Tumor growth inhibition was observed for both compounds but with a lower statistical significance for **38**·H_3PO_4 ($p = 0.07$) compared to **39**·H_3PO_4 ($p = 0.03$). At these doses, neither mortality nor weight loss was observed. Similarly, the compound most active in the HER-2 degradation assay, **42**·H_3PO_4, was tested in a separate experiment (Figure 3b), at 200 mg/kg/day but with a different schedule (2 × 100 mg/kg/day, 5 days/week), and also showed statistically significant ($p = 0.02$) tumor growth inhibition and no overt toxicity.

Conclusion

The chaperone Hsp90 is a target of interest for the treatment of cancer because of its central regulatory role. Inhibition of Hsp90 induces the degradation of several client proteins and shuts down multiple oncogenic pathways, which in turn affects a number of critical steps implicated in the genesis of a tumor (proliferation, angiogenesis, acquired immortality, evasion of apoptosis, and metastasis). The simultaneous modulation of various oncogenic effects should reduce the likelihood of the tumor acquiring resistance to Hsp90 inhibitors.[2b,2c] In addition, the existence of an activated form of Hsp90 in cancer cells offers the possibility to develop inhibitors selective for malignant cells. We optimized the purine-based inhibitors of Hsp90, reaching compounds as potent as 90 nM (**42**) in the HER-2 degradation assay and 200 nM in in vitro growth inhibition assays. The introduction of an amino group in the side-chain dramatically improved their aqueous solubility (>10 mg/kg for their H_3PO_4 salts), which greatly facilitated their formulation for oral delivery. In mice, the oral bioavailability of the amines **37**−**42** ranged from 14 to 97%. These amines reached high plasma concentrations ($C_{max} = 10-19$ μM; oral dose of 100 mg/kg) but were cleared rapidly (Cl = 33−131 mL/min/kg; intravenous dose of 10 mg/kg). When administered orally to mice bearing A549 tumor xenografts (200 mg/kg), **41**·H_3PO_4 induced the pharmacodynamic response expected from Hsp90 inhibitors: degradation of the client proteins HER-2, pHER-2, pAKT, and pRaf and upregulation of Hsp70. Similarly, in a murine N87 xenograft model, oral administration of **39**·H_3PO_4 (2 × 200 mg/kg/day) induced the degradation of Hsp90 clients but not of PI-3K. Furthermore, in the N87 model, the H_3PO_4 salts of set of amines **38**, **39**, and **42** inhibited tumor growth orally at 200 mg/kg/day. These are the first Hsp90 inhibitors reported to inhibit tumor growth upon oral administration, but high doses are currently necessary. Further work is necessary to improve the potency and clearance of these compounds and to examine alternate xenograft models.

Experimental Section

Pharmacokinetic, Pharmacodynamic, and Pharmacology. Six to eight week old nu/nu athymic female mice were maintained in sterilized filter topped cages in a room with a 12-h light/12-h dark photoperiod at a temperature of 21−23 °C and a relative humidity of 50 ± 5%. Irradiated pelleted food and autoclaved deionized water were provided ad libitum. Animals were identified by the use of individually numbered ear tags. Experiments were carried out under institutional guidelines for the proper and humane use of animals in research established by the Institute for Laboratory Animal Research (ILAR).

The test compounds were formulated (3−20 mg/mL) in a phospholipon 90G/water dispersion.[22] The dispersion was prepared with 86 g of sucrose and 53 g of phospholipon, brought to 1000 g with water, homogenized for 15 min, microfluidized at 14 000−16 000 psi (F12Y interaction chamber), and stored at 5 °C. The test compounds were administered either orally by gavage at 5−20 mL/kg or intravenously. For pharmacokinetic studies, three mice were administered the inhibitor orally and three others intravenously. Six blood samples were collected with the final sample collected 4 h postdosing, and plasma concentrations were measured by HPLC. The data were analyzed using noncompartmental methods, and the terminal half-life was estimated using 3−4 data points (WinNonlin Professional, Version 4.1).

Tumor fragments (approximately 2 mm^3) or 5×10^6 tumor cells were inoculated subcutaneously in the right or left flank of the animal. Mice with established tumors (50−200 mm^3) were selected for study ($n = 6-10$/treatment group). Tumor dimensions were measured using calipers, and tumor volumes were calculated using the equation for an ellipsoid sphere $(l \times w^2)/2 = mm^3$, where l and w refer to the larger and smaller dimensions collected at each measurement. The vehicle alone was administered to control groups. For pharmacology studies, animals were dosed 5 days per week (Monday through Friday) for four to six consecutive weeks. Animals were weighed, and the tumors were measured twice per week. Mice were followed until tumor volumes in the control group reached approximately 1000 mm^3 and were sacrificed by CO_2 euthanasia. In those rare instances in which there was a clear outlier (over two standard deviations from the mean), that outlier was removed. The mean tumor volumes of each group were calculated. The change in mean treated tumor volume was divided by the change in mean control tumor volume, multiplied by 100, and subtracted from 100% to give the tumor growth inhibition for each group.

Chemistry. The ^1H NMR and ^{13}C NMR spectra were recorded on a 400 MHz spectrometer. Analytical HPLC chromatograms were obtained using a C18 column (5 μm; 4.6 mm × 150 mm). A gradient was applied between solvent A (0.1% TFA in H_2O) and solvent B (0.05% TFA in CH_3CN) increasing the proportion of B linearly from 5% to 100% over 7 min (conditions I), 12 min (conditions II), or 15 min (conditions III) with a constant flow rate of 1 mL/min. The samples were diluted to 0.1 mg/mL in MeOH, and the injection volumes were 10 μL. The column was not heated, and UV detection was effected at 254 nm. Purities were determined by HPLC at 254 nM.

***N*⁴-Butyl-6-chloropyrimidine-4,5-diamine (6).** A suspension of 4,6-dichloro-5-aminopyrimidine (**5**) (4.0 g, 24.4 mmol) in n-BuOH (35 mL) was treated with $BuNH_2$ (2.7 mL, 26.8 mmol, 1.1 equiv) and Et_3N (4.1 mL, 29.3 mmol, 1.2 equiv) and heated to 90 °C for 16 h. Evaporation, workup (EtOAc/water/brine), and drying (Na_2SO_4) gave the crude title compound as a light yellow solid (4.0 g, 83%). HPLC Purity: 99.8%. $t_R = 5.08$ min (Conditions I). mp: 81−82 °C. ^1H NMR (DMSO-d_6) δ 7.73 (s, 1H), 6.76 (t, $J = 4.8$ Hz, 1H), 5.00 (s. 2H), 3.37 (q, $J = 7.0$ Hz, 2H), 1.52 (quint., $J = 7.4$ Hz, 2H), 1.33 (sext., $J = 7.4$ Hz, 2H), 0.83 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (DMSO-d_6) δ 152.5, 146.2, 137.0, 123.8, 41.0, 31.3, 20.1, 14.2.

9-Butyl-8-(2,5-dimethoxy-benzyl)-9*H*-purin-6-ylamine (8). A solution of 2,5-dimethoxyphenylacetic acid (6.46 g, 24.7 mmol, 1.1 equiv) in 1,2-dichloroethane (100 mL) was treated with TsCl (4.7 g, 24.7 mmol, 1.1 equiv) and Et_3N (9.4 mL, 67.2 mmol, 3.0 equiv) at room temperature for 20 min. Then, N^4-butyl-6-chloropyrimidine-4,5-diamine (4.5 g, 22.4 mmol, 1.0 equiv) was added, and the solution was stirred at 40 °C for 16 h. Workup (EtOAc/water/brine) and drying (Na_2SO_4) gave *N*-(4-butylamino-6-chloropyrimidin-5-yl)-2-(2,5-dimethoxy-phenyl)-acetamide (**7**) as a crude (6.0 g, 71%) which was used without further purification.

A solution of *N*-(4-butylamino-6-chloro-pyrimidin-5-yl)-2-(2,5-dimethoxy-phenyl)-acetamide (**7**) (2.5 g, 6.6 mmol) in methanolic ammonia (7 M NH_3 in MeOH, 30 mL) was heated in a stainless steel cylinder to 120 °C for 3 days. Flash chromatography (EtOAc/

hexane) gave the title compound as a white solid (1.8 g, 80%). t_R = 5.55 min (Conditions I). mp: 126−130 °C. ^{1}H NMR ($CDCl_3$) δ 8.34 (s, 1H), 6.86 (d, J = 8.9 Hz, 1H), 6.80 (dd, J = 8.9 & 3.0 Hz, 1H), 6.70 (d, J = 3.0 Hz, 1H), 5.64 (br. s, 2H), 4.25 (s, 2H), 4.11 (t, J = 7.7 Hz, 2H), 3.84 (s, 3H), 3.72 (s, 3H), 1.63 (quint., J = 7.6 Hz, 2H), 1.32 (sext., J = 7.7 Hz, 2H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$:CD_3OD 5:1) δ 154.5, 153.5, 151.7, 151.5, 151.0, 150.4, 124.6, 117.8, 116.5, 112.6, 111.4, 55.7, 55.5, 42.7, 31.7, 27.9, 19.8, 13.4.

4,5,6-Triaminopyrimidine (9). 4,5,6-Triaminopyrimidine sulfate (50 g, 224 mmol) was dissolved in aq 1 M NaOH (500 mL, 2.2 equiv), and the pH was adjusted to 7 with 4 M HCl. The solution was cooled to T_{int} = 0−5 °C, whereupon the 4,5,6-triaminopyrimidine crystallized out of solution as a free base. The crystals were collected by filtration, washed with ice-cold water, and dried to give the title compound as white needles (18.8 g, 67%). mp: 256−258 °C (dec) (lit: 246 °C, dec).[24] ^{1}H NMR (DMSO-d_6) δ 7.47 (s, 1H), 5.57 (br. s. 4H), 3.72 (br. s, 2H).

N-(5,6-diamino-pyrimidin-4-yl)-2-(3-methoxy-phenyl)-acetamide hydrochloride (10). 4,5,6-Triaminopyrimidine (**9**) (6.25 g, 50 mmol) was dissolved in *N*-methyl-2-pyrrolidone (NMP, 70 mL) at 70 °C. The solution was cooled to room temperature, and treated with 3-methoxyphenylacetyl chloride (9.2 g, 50 mmol, 1.0 equiv) for 3 h at 50 °C, whereupon the desired compound precipitated as its HCl salt. The precipitate was collected, washed with EtOAc and acetone, and dried to give the title compound as a white solid (15.2 g, 98%). HPLC Purity: 97.4%. t_R = 4.13 min (Conditions I). mp: 286−288 °C. ^{1}H NMR (DMSO-d_6) δ 9.22 (s, 1H), 8.20 (s, 1H), 6.75−7.58 (br. s, 4H), 7.21 (t, J = 7.9 Hz, 1H), 6.95 (d, J = 1.3 Hz, 1H), 6.90 (d, J = 7.6 Hz, 1H), 6.80 (dd, J = 7.6 & 1.3 Hz, 1H), 3.74 (s, 2H), 3.73 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.9, 159.4, 156.4 (2C), 147.9, 137.7, 129.3, 122.8, 116.2, 112.2, 93.9, 55.4, 41.8

8-(3-Methoxy-benzyl)-9*H*-purin-6-ylamine (11). A solution of crude *N*-(5,6-diamino-pyrimidin-4-yl)-2-(3-methoxy-phenyl)-acetamide hydrochloride (**10**) (17.6 g, 57 mmol) and MeONa (12.3 g, 227 mmol, 4.0 equiv) in n-BuOH (150 mL) was heated to reflux for 2 h, cooled to room temperature, and neutralized with 2 M HCl. Brine was added, which gave a biphasic mixture. Concentration of the organic layer afforded the title compound as a solid (10.7 g, 75%). t_R = 4.70 min (Conditions II). mp: 252−254 °C ^{1}H NMR (DMSO-d_6) δ 8.06 (s, 1H), 7.43 (br. s, 1H), 7.21 (t, J = 7.9 Hz, 1H), 7.05 (s, 2H), 6.93 (s, 1H), 6.87 (d, J = 7.6 Hz, 1H), 6.79 (dd, J = 8.1 & 2.3 Hz, 1H), 4.09 (s, 2H), 3.73 (s, 3H).^{13}C NMR (DMSO-d_6) δ 159.8, 155.5, 152.3, 151.6, 150.8, 139.3, 130.0, 121.4, 119.0. 115.0, 112.4, 55.5, 35.5.

9-Butyl-8-(3-methoxy-benzyl)- 9*H*-purin-6-ylamine (12a). A mixture of 8-(3-methoxy-benzyl)-9*H*-purin-6-ylamine (**11**) (0.50 g, 2.2 mmol), BuI (0.30 mL, 2.65 mmol, 1.2 equiv), Cs_2CO_3 (1.43 g, 4.4 mmol, 2.0 equiv), and DMF (2.5 mL) was stirred at room temperature for 16 h. Flash chromatography (MeOH:CH_2Cl_2 5:95) gave the title compound as a white solid (370 mg, 54%). HPLC Purity: 91.0%. t_R = 6.92 min (Conditions II). mp: 163−165 °C. ^{1}H NMR ($CDCl_3$:CD_3OD 5:1) δ 8.13 (s, 1H), 7.16 (t, J = 7.9 Hz, 1H), 6.73−6.67 (m, 3H), 4.13 (s, 2H), 3.95 (t, J = 7.7 Hz, 2H), 3.68 (s, 3H), 1.48 (quint., J = 7.7 Hz, 2H), 1.20 (sext., J = 7.5 Hz, 2H), 0.78 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$:CD_3OD 5:1) δ 159.9, 154.7, 152.0, 150.9, 150.7, 136.6, 129.9, 120.8, 117.8, 114.5, 112.3, 55.07, 42.9, 34.2, 31.5, 19.8, 13.4. HRMS: calcd for $C_{17}H_{22}N_5O$ $(MH)^+$ m/z 312.1819, found 312.1817.

9-Butyl-8-(2-chloro-5-methoxy-benzyl)-9*H*-purin-6-ylamine (12b). A solution of 9-butyl-8-(3-methoxy-benzyl)-9*H*-purin-6-ylamine (**12a**) (100 mg, 0.32 mmol) in THF (4 mL) was treated with SO_2Cl_2 (78 μL, 0.96 mmol, 3.0 equiv) at room temperature for 2 h. Workup and preparative TLC (MeOH:CH_2Cl_2 10:90) gave the title compound (60.2 mg, 54%). mp: 138−139 °C. HPLC Purity: 92.4%. t_R = 7.77 min (Conditions II). ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1H), 7.30 (d, J = 8.8 Hz, 1H), 6.75 (dd, J = 8.8 & 3.0 Hz, 1H), 6.67 (d, J = 3.0 Hz, 1H), 6.26 (s, 2H), 4.32 (s, 2H), 4.04 (t, J = 7.7 Hz, 2H), 3.67 (s, 3H), 1.62 (quint., J = 7.7 Hz, 2H), 1.30 (sext., J = 7.5 Hz, 2H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 157.1, 153.6, 150.9, 149.8, 148.4, 133.1, 128.8, 123.4, 117.3, 114.7, 112.5, 54.0, 41.5, 30.4, 30.2,18.6, 12.2. HRMS: calcd for $C_{17}H_{21}N_5ClO$ $(MH)^+$ m/z 346.1429, found 346.1426

8-(2-Bromo-5-methoxy-benzyl)-9-butyl-9*H*-purin-6-ylamine (12c). A solution of 9-butyl-8-(3-methoxy-benzyl)-9*H*-purin-6-ylamine (**12a**) (35 mg, 0.11 mmol) in AcOH (1 mL) was treated with Br_2 (1 M in AcOH, 0.2 mL, 2 equiv) at room temperature for 16 h. Evaporation and preparative TLC (MeOH:CH_2Cl_2 10:90) gave the title compound (24.8 mg, 57%), HPLC Purity: 99.5%. t_R = 7.62 min (Conditions I). mp: 158−159 °C. ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H), 7.46 (d, J = 8.6 Hz, 1H), 6.70 (d, J = 2.9 Hz, 1H), 6.66 (dd, J = 8.8 & 2.9 Hz, 1H), 6.34 (s, 2H), 4.31 (s, 2H), 4.04 (t, J = 7.7 Hz, 2H), 3.73 (s, 3H), 1.62 (quint., J = 7.8 Hz, 2H), 1.31 (sext., J = 7.6 Hz, 2H), 0.86 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 159.2, 155.0, 152.4, 151.2, 149.9, 136.4, 133.5, 118.7, 116.3, 114.5, 114.3, 55.4, 42.9, 34.3, 31.9, 19.9, 13.7.

9-Butyl-8-(2-iodo-5-methoxy-benzyl)-9*H*-purin-6-ylamine (12d). A solution of 9-butyl-8-(3-methoxy-benzyl)-9*H*-purin-6-ylamine (**12a**) (1.24 g, 4.0 mmol) in AcOH (50 mL) was treated with NIS (2.79 g, 12 mmol, 3.0 equiv) at room temperature for 16 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL), washed with aq Na_2CO_3, aq $Na_2S_2O_3$, and brine. Drying (Na_2SO_4), evaporation, and flash chromatography (MeOH:CH_2Cl_2 5:95 → 10:90) gave the title compound as a white solid (0.53 g, 31%). HPLC Purity: 91.0%. t_R = 7.35 min (Conditions II). mp: 172−174 °C. ^{1}H NMR ($CDCl_3$) δ 8.36 (s, 1H), 7.77 (d, J = 8.7 Hz, 1H), 6.67 (d, J = 3.0 Hz, 1H), 6.61 (dd, J = 8.7 & 3.0 Hz), 5.67 (s, 2H), 4.33 (s, 2H), 4.06 (t, J = 7.7 Hz, 2H), 3.71 (s, 3H), 1.66 (quint., J = 7.4 Hz, 2H), 1.37 (sext., J = 7.6 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 160.3, 154.7, 152.5, 151.4, 150.3, 140.1, 139.8, 118.8, 116.1, 114.8, 88.8, 55.4, 43.1, 39.5, 32.0, 20.0, 13.7. HRMS: calcd for $C_{17}H_{21}N_5ClO$ $(MH)^+$ m/z 438.0785, found 438.0790.

8-Bromo-9-butyl-9*H*-purin-6-ylamine (17). A mixture of 8-bromoadenine (**16**) (1.87 g, 8.69 mmol),[17] 1-iodobutane (1.19 mL, 10.4 mmol, 1.2 equiv), Cs_2CO_3 (3.4 g, 10.4 mmol, 1.2 equiv), and DMF (200 mL) was stirred at room temperature for 16 h. Workup (EtOAc/H_2O, brine), drying (Na_2SO_4) and flash chromatography (MeOH:$CHCl_3$ 2:98 → 5:95) gave the title compound as a white solid (1.46 g, 62%). mp: 152−154 °C. ^{1}H NMR (CD_3OD) δ 8.18 (s, 1H), 4.24 (t, J = 7.4 Hz, 2H), 1.82 (quint., J = 7.4 Hz, 2H), 1.38 (sext., J = 7.5 Hz, 2H), 0.98 (t, J = 7.4 Hz, 3H).

9-Butyl-8-(2,5-dimethoxy-phenylsulfanyl)-9*H*-purin-6-ylamine (18). A solution of 2,5-dimethoxy-benzenethiol (251 mg, 1.48 mmol, 8.0 equiv) in DMF (3 mL) was treated with NaH (60% in oil, 29.5 mg, 0.74 mmol, 4.0 equiv) at room temperature for 10 min. When gas evolution ceased, 8-bromo-9-butyl-9*H*-purin-6-ylamine (**17**) (50 mg, 0.18 mmol, 1.0 equiv) was added, and the mixture was heated to 150 °C for 4 h. Workup (EtOAc/H_2O, brine), drying (Na_2SO_4) and preparative TLC (MeOH/CH_2Cl_2 5:95) gave the title compound (46 mg, 69%). HPLC Purity: 90.0%. t_R = 8.63 min (Conditions III). ^{1}H NMR (DMSO-d_6) δ 8.15 (s, 1H), 7.41 (s, 2H), 7.04 (d, J = 8.9 Hz, 1H), 6.85 (d, J = 8.9 Hz, 1H), 6.46 (s, 1H), 4.15 (t, J = 7.3 Hz, 2H), 3.76 (s, 3H), 3.60 (s, 3H), 1.64 (quint., J = 7.4 Hz, 2H), 1.16 (sext., J = 7.5 Hz, 2H), 0.79 (t, J = 7.4 Hz, 3H). HRMS: calcd for $C_{17}H_{22}N_5O_2S$ $(MH)^+$ m/z 360.1489, found 360.1494.

2-Iodo-5-methoxy-benzenethiophenol, Potassium Salt (21). The title compound was prepared by a modification of the published procedure.[18b] A solution of $EtOCS_2K$ (100 g, 625 mmol, 1.1 equiv) in acetone (1.3 L) was cooled to an internal temperature T_{int} of −20 °C (T_{bath} = −40 °C), and 2-iodo-5-methoxybenzenediazonium tetrafluoroborate (**20**) (191 g, 549 mmol, 1.0 equiv)[18] was added over 10 min. The reaction vessel was removed from the cold bath, and when T_{int} reached +15 °C, N_2 evolved. The reaction was stirred for 30 min at room temperature, and the gelatinous, insoluble KBF_4 was filtered off. The filtrate was concentrated, worked-up (EtOAc/water/brine), and concentrated to give crude dithiocarbonic acid *O*-ethyl ester *S*-(2-iodo-5-methoxy-phenyl) ester, as a brown oil (200 g) which was used without further purification. ^{1}H NMR (DMSO-d_6) δ 7.82 (d, J = 8.8 Hz, 1H), 7.22 (d, J = 3.0 Hz, 1H),

6.71 (dd, $J = 8.8$ & 3.0 Hz, 1H), 4.62 (q, $J = 7.0$ Hz, 2H), 3.80 (s, 3H), 1.34 (t, $J = 7.0$ Hz, 3H).

The crude dithiocarbonic acid *O*-ethyl ester *S*-(2-iodo-5-methoxyphenyl) ester (40 g) was diluted in THF (300 mL). Separately, KOH (12.6 g, 2 equiv) was dissolved in MeOH (300 mL). The two solutions were combined, heated to 40 °C for 2 h, and thoroughly evaporated. The solid was scraped from the sides of the flask, stirred in Et_2O (500 mL) for 1 h, and filtered. The solid was finely ground, and the ether wash was repeated. Filtration and drying under vacuum at room temperature gave potassium 2-iodo-5-methoxybenzenethiolate (**21**) as a crude brown powder (38.5 g). The crude material still contained the excess of potassium salts, and ^{1}H NMR analysis indicated the presence of two impurities, tentatively assigned as $KSCO_2Me$ (61 mol %) and $KSCO_2Et$ (6 mol %). ^{1}H NMR (DMSO-d_6) δ 7.30 (d, $J = 8.8$ Hz, 1H), 6.97 (d, $J = 3.0$ Hz, 1H), 5.83 (dd, $J = 8.8$ & 3.0 Hz, 1H), 3.58 (s, 3H).

8-Bromo-9-(4-methyl-pent-3-enyl)-9*H*-purin-6-ylamine (22). A mixture of 8-bromoadenine (**16**) (3.0 g, 14 mmol),[17] 5-bromo-2-methyl-2-pentene (2.25 mL, 16.8 mmol, 1.2 equiv), and Cs_2CO_3 (9.1 g, 28 mmol, 2.0 equiv) in DMF (15 mL) was stirred at room temperature for 3 h, filtered, and evaporated. Flash chromatography (MeOH:CH_2Cl_2 5:95 → 10:90) gave the title product as a solid (1.56 g, 38%). mp: 159−162 °C. ^{1}H NMR ($CDCl_3$:CD_3OD 5:1) δ 8.15 (s, 1H), 5.03 (t, $J = 7.5$ Hz, 1H), 4.10 (t, $J = 7.2$, 2H), 2.41 (q, $J = 7.2$ Hz, 2H), 1.53 (s, 3H), 1.32 (s, 3H). ^{13}C NMR ($CDCl_3$:CD_3OD 5:1) δ 154.1, 152.6, 150.7, 135.9, 127.5, 119.4, 118.5, 44.3, 28.0, 25.4, 17.2.

8-(2-Iodo-5-methoxy-phenylsulfanyl)-9-(4-methyl-pent-3-enyl)-9*H*-purin-6-ylamine (23). A mixture of 8-bromo-9-(4-methyl-pent-3-enyl)-9*H*-purin-6-ylamine (**22**) (2.7 g, 9.1 mmol) and crude potassium 2-iodo-5-methoxy-benzenethiophenolate (**21**) (8.3 g, 27.3 mmol, 3.0 equiv) in DMF (50 mL) was stirred at 140 °C for 3 h and then at 100 °C for 16 h. Filtration, evaporation, and flash chromatography (MeOH:CH_2Cl_2 5:95) gave the title product as a solid (0.64 g, 15%). mp: 168−169 °C. $t_R = 8.90$ min (Conditions II). ^{1}H NMR ($CDCl_3$) δ 8.39 (s, 1H), 7.72 (d, $J = 8.7$ Hz, 1H), 6.72 (d, $J = 2.7$ Hz, 1H), 6.58 (dd, $J = 8.7$ & 2.7 Hz, 1H), 5.85 (br. s, 2H), 5.15 (t, $J = 7.3$ Hz, 1H), 4.25 (t, $J = 7.4$ Hz, 2H), 3.69 (s, 3H), 2.50 (quint., $J = 7.3$ Hz, 2H), 1.66 (s, 3H), 1.44 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 160.4, 154.9, 153.4, 151.4, 145.0, 140.4, 138.7, 136.0, 120.4, 119.1, 116.2, 115.0, 87.2, 55.4, 43.8, 28.6, 25.7, 17.6. Anal. ($C_{18}H_{20}IN_5OS$): C, H, N.

8-Bromo-9-pent-4-ynyl-9*H*-purin-6-ylamine (24). A mixture of 8-bromoadenine (**16**) (10.7 g, 50 mmol),[17] 5-chloro-1-pentyne (6.16 g, 60 mmol, 1.2 equiv) and Cs_2CO_3 (32.5 g, 100 mol, 2.0 equiv) in DMF (70 mL) was stirred at 70 °C for 3 h, filtered, and evaporated. Flash chromatography (MeOH:CH_2Cl_2 5:95 ◊ 10:90) gave the title product as a solid (3.5 g, 25%). $t_R = 5.03$ min (Conditions II). mp: 195−196 °C. ^{1}H NMR (DMSO-d_6) δ 8.14 (s, 1H), 7.39 (s, 2H), 4.20 (t, $J = 7.3$ Hz, 2H), 2.82 (t, $J = 2.6$ Hz, 1H), 2.25 (td, $J = 2.6$ & 7.0 Hz, 2H), 1.94 (quint., $J = 7.3$ Hz, 2H). ^{13}C NMR ($CDCl_3$/CD_3OD 5:1) δ 154.2, 152.8, 150.9, 127.3, 119.5, 82.1, 69.5, 43.6, 28.0, 15.8.

8-(2-Iodo-5-methoxy-phenylsulfanyl)-9-pent-4-ynyl-9*H*-purin-6-ylamine (4). A mixture of 8-bromo-9-pent-4-ynyl-9*H*-purin-6-ylamine (**24**) (3.5 g, 12.5 mmol), t-BuOK (2.8 g, 25.0 mmol, 2.0 equiv) and 2-iodo-5-methoxybenzenethiol (7.5 g, 27.5 mmol, 2.2 equiv) in DMF (30 mL) was stirred at 70 °C for 16 h, filtered, and evaporated. Flash chromatography (MeOH:CH_2Cl_2 5:95 → 10:90) gave the title product as a solid which was recrystallized from MeOH to give 2 crops (2.34 and 1.80 g, combined yield 71%). $t_R = 7.82$ min (Conditions II). mp: 171−172 °C. ^{1}H NMR (DMSO-d_6) δ 8.18 (s, 1H), 7.79 (d, $J = 8.7$ Hz, 1H), 7.50 (br. s, 2H), 6.70 (dd, $J = 8.7$ & 2.8 Hz, 1H), 6.49 (d, $J = 2.8$ Hz, 1H), 4.22 (t, $J = 7.1$ Hz, 2H), 3.62 (s, 3H), 2.77 (d, $J = 2.5$ Hz, 1H), 2.20 (td, $J = 6.9$ & 4.5 Hz, 2H), 1.87 (quint., $J = 7.1$ Hz, 2H). ^{13}C NMR ($CDCl_3$:CD_3OD 5:1) δ 160.5, 154.6, 152.7, 151.1, 146.1, 140.8, 136.4, 119.7, 118.5, 116.1, 90.1, 82.2, 69.6, 55.5, 43.1, 28.3, 15.9. Anal. ($C_{17}H_{16}IN_5OS$): C, H, N.

8-Bromo-9-pent-4-ynyl-9*H*-purine-2,6-diamine (26). A mixture of 8-bromo-9*H*-purine-2,6-diamine (**25**) (600 mg, 2.63 mmol),[25] Cs_2CO_3 (1.94 g, 5.95 mmol, 2.2 equiv), 5-chloro-pent-1-yne (0.56 mL, 5.26 mmol, 2.0 equiv), and DMF (5 mL) was heated to 85 °C for 16 h. Workup and evaporation gave the title compound as a crude solid which was used without further purification (510 mg, 66%). ^{1}H NMR (DMSO-d_6) δ 6.80 (s, 2H), 5.95 (s, 2H), 3.98 (t, $J = 7.3$ Hz, 2H), 2.81 (t, $J = 2.6$ Hz, 1H), 2.22 (td, $J = 7.1$ & 2.7 Hz, 2H), 1.96 (quint., $J = 7.2$ Hz, 2H).

8-(2-Iodo-5-methoxy-phenylsulfanyl)-9-pent-4-ynyl-9*H*-purine-2,6-diamine (27). A mixture of 8-bromo-9-pent-4-ynyl-9*H*-purine-2,6-diamine (**26**) (500 mg, 1.59 mmol), 2-iodo-5-methoxy-benzenethiol (1.34 g, 5.07 mmol, 3 equiv), t-BuOK (475 mg, 4.23 mmol, 2.5 equiv), and DMF (7 mL) was heated to 100 °C for 16 h. Workup and chromatography (MeOH:DCM 1:100 → 1:10) gave the title compound as a solid (350 mg, 43%). $t_R = 7.85$ min (Conditions II). ^{1}H NMR (DMSO-d_6) δ 7.73 (d, $J = 8.6$ Hz, 1H), 7.05−6.95 (br. s., 2H), 6.64 (dd, $J = 8.6$ & 2.9 Hz, 1H), 6.24 (d, $J = 2.9$ Hz, 1H), 6.02 (s, 2H), 4.01 (t, $J = 7.2$ Hz, 2H), 3.59 (br. s, 3H), 2.70 (t., $J = 2.6$ Hz, 1H), 2.14 (td, $J = 7.2$ & 2.6 Hz, 2H), 1.80 (quint., $J = 7.3$ Hz, 2H).

2-Fluoro-8-(2-iodo-5-methoxy-phenylsulfanyl)-9-pent-4-ynyl-9*H*-purin-6-ylamine (28). A mixture of 8-(2-iodo-5-methoxy-phenylsulfanyl)-9-pent-4-ynyl-9*H*-purine-2,6-diamine (**27**) (79 mg, 0.165 mmol) and 48% aq HBF_4 (0.5 mL) in THF (0.5 mL) was treated at −20 °C with *iso*-amyl nitrite (22 μL, 0.165 mmol). The reaction mixture was allowed to reach rt and was further heated to 40 °C for 10 min. Workup (DCM/aq K_2CO_3) and preparative plate TLC purification (EtOAc/Hexane 1:4) gave the title compound as a solid (10 mg, 13%). $t_R = 9.43$ min (Conditions II). ^{1}H NMR ($CDCl_3$) δ 7.72 (d, $J = 8.7$ Hz, 1H), 6.70 (d, $J = 2.9$ Hz, 1H), 6.59 (dd, $J = 8.7$ & 2.9 Hz 1H), 6.11−5.87 (br. s, 2H), 4.25 (t, $J = 7.3$ Hz, 2H), 3.69 (s, 3H), 2.25 (td, $J = 7.1$ & 2.7 Hz, 2H), 2.02 (quint., $J = 7.2$ Hz, 2H), 1.97 (t, $J = 2.6$ Hz, 1H).

Acetic Acid 2-(6-Amino-8-bromo-purin-9-yl)-ethyl Ester (30a). A mixture of adenine (60.0 g, 444 mmol), Cs_2CO_3 (223 g, 686 mmol, 1.54 equiv), AcO−CH_2−CH_2−Br (75.8 mL, 686 mmol, 1.54 equiv), and DMF (187 g) was stirred at 45 °C (T_{int}) for 5 h, at which point the reaction was complete as judged by analytical HPLC of both the supernatant and the solid residues. The DMF was evaporated, and the residue was added to a mixture of AcOH (50 mL, 888 mmol, 2 equiv), water (100 mL), and ice (100 g). The solid was filtered, washed with 100 mL ice-cold water, and dried on a rotary evaporator under high vacuum to give acetic acid 2-(6-amino-purin-9-yl)-ethyl ester as a white powder (62.4 g, 67%). The HPLC trace was clean, but the product was still contaminated with traces of cesium bicarbonate (and/or carbonate), as evidenced by an effervescence upon addition to an AcOH/AcONa buffer. mp: 175−177 °C. ^{1}H NMR (DMSO-d_6) δ 8.13 (s, 1H), 8.12 (s, 1H), 7.20 (s, 2H), 4.37 (br. s, 4H), 1.92 (s, 3H).^{13}C NMR (DMSO-d_6) δ 170.5, 156.4, 152.9, 150.1, 141.5, 119.1, 62.5, 42.6, 21.0.

An acetic acid buffer was prepared with AcONa (131 g, 1.6 mol), AcOH (96 mL, 1.6 mol), and H_2O (800 mL). Acetic acid 2-(6-amino-purin-9-yl)-ethyl ester (16.6 g, 75 mmol) was dissolved in a mixture of the AcOH buffer (100 mL), MeOH (30 mL), and THF (30 mL) using magnetic stirring at room temperature. Bromine (7.0 mL, 136 mmol, 1.8 equiv) was added over 1 min, and the stirring was stopped. The desired product, acetic acid 2-(6-amino-8-bromo-purin-9-yl)-ethyl ester (**30a**), partially crystallized out of solution. After 1 h, the crystals were collected by filtration, washed (H_2O), and air-dried to give the desired bromide (11.6 g, 51%) as purple prisms. The aqueous filtrate was combined with $CHCl_3$ (500 mL). The mixture was stirred, and the pH of the aqueous layer was monitored with a pH meter. The excess bromine was quenched with concentrated aq NH_4OH (25 mL; final pH = 7) and then hydrazine monohydrate (3 mL, final pH = 9). Note that the excess Br_2 can also be neutralized with $Na_2S_2O_3$, which is experimentally easier. The brown color of Br_2 disappeared, and the yellow organic layer was separated. The aqueous layer was washed with 300 mL $CHCl_3$, and the combined organic layers were concentrated to give additional acetic acid 2-(6-amino-8-bromo-purin-9-yl)-ethyl ester (**30a**) as a yellow powder (6.7 g, 30%). The combined yield was 81%. mp: 150−152 °C. ^{1}H NMR (DMSO-d_6) δ 8.13 (s, 1H), 7.38

(s, 2H), 4.35 (br. s, 4H), 1.89 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.5, 152.5, 150.9, 150.0, 128.6, 119.2, 61.7, 44.0, 21.0.

Acetic Acid 3-(6-amino-8-bromo-purin-9-yl)-propyl Ester (30b). The title compound was prepared by alkylation of adenine in two steps (i. AcO−$(CH_2)_3$−Cl. ii. Br_2) as reported previously.[19]

2-[6-Amino-8-(2-iodo-5-methoxy-phenylsulfanyl)-purin-9-yl]-ethanol (31a). A solution of acetic acid 2-(6-amino-8-bromo-purin-9-yl)-ethyl ester (**30a**) (16.5 g, 55 mmol) and potassium 2-iodo-5-methoxy-benzenethiolate (**21**) (33 g, 2 equiv) in DMF (600 mL) was heated to 50 °C for 16 h. The reaction mixture was concentrated, dissolved in MeOH, and treated with a catalytic amount K_2CO_3 for 3 h at 50 °C to cleave the acetyl group in situ. The mixture was concentrated again and stirred in a mixture of water and Et_2O for 16 h. The desired alcohol, which was soluble neither in Et_2O nor in water, was recovered by filtration. Washing with ether and drying gave 2-[6-amino-8-(2-iodo-5-methoxy-phenylsulfanyl)-purin-9-yl]-ethanol (**31a**) as an orange-brown powder (9 g, 37%). Note that the coupling step was very clean if performed with pure potassium thiophenolate but gave variable results with crude potassium thiophenolate. The outcome depended on the exact batch of thiophenolate used. In some cases, addition of a small amount of concentrated HCl (0.1−0.2 equiv) was sometimes necessary to adjust the pH. If it was too alkaline, cleavage of the acetyl group occurred and other byproducts were observed. If it was too acidic, the thiophenolate dimerized quickly to the disulfide ArSSAr. mp: 184−187 °C. ^{1}H NMR (DMSO-d_6) δ 8.18 (s, 1H), 7.76 (d, J = 8.7 Hz, 1H), 7.42 (s, 2H), 6.68 (dd, J = 8.7 & 2.9 Hz, 1H), 6.56 (d, J = 2.9 Hz, 1H), 4.99 (t, J = 5.6 Hz, 1H), 4.26 (t, J = 5.7 Hz, 2H), 3.70 (q, J = 5.7 Hz, 2H), 3.67 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 160.3, 155.9, 153.6, 151.3, 144.0, 140.6, 140.0, 120.3, 116.3, 115.1, 87.5, 59.4, 55.8, 46.5.

3-[6-Amino-8-(2-iodo-5-methoxy-phenylsulfanyl)-purin-9-yl]-propan-1-ol (31b). A solution of crude acetic acid 3-[6-amino-8-(2-iodo-5-methoxy-phenylsulfanyl)-purin-9-yl]-propyl ester[19] (**36**, 20 g) in MeOH (250 mL) was treated with K_2CO_3 (500 mg) at 50 °C for 1 h. Filtration and concentration afforded the title compound as a white solid (14 g, 64.5%) identical by ^{1}H and ^{13}C NMR to the known compound.[19]

8-(2-Iodo-5-methoxy-phenylsulfanyl)-9*H*-purin-6-ylamine (33). A suspension of finely ground 6-amino-7,9-dihydro-purine-8-thione (**32**) (12 g, 71 mmol)[19] in DMF (250 mL) was cooled to −60 °C and treated portionwise with 2-iodo-5-methoxybenzenediazonium tetrafluoroborate (39.2 g, 112 mmol, 1.6 equiv). The reaction mixture was allowed to warm to room temperature and was stirred at room temperature for another 2 h. The reaction mixture was neutralized to pH = 7 with aq NaOH and evaporated. Water was added, and the pH was adjusted to 10 with aq NaOH. The aqueous layer was washed with CH_2Cl_2 (3 × 100 mL) and neutralized with HCl 6 N, whereupon the title compound precipitated. The solid was collected and dried under vacuum to afford the crude title compound as a yellow powder which was used without further purification (9.6 g, HPLC Purity: 77%, yield corrected for low purity: 26%). t_R = 6.05 min (Conditions II). mp: 254−156 °C. ^{1}H NMR (DMSO-d_6) δ 8.13 (s, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.38 (br. s, 2H), 6.71 (d, J = 8.4 Hz, 1H), 6.62 (s, 2H), 3.65 (s, 3H).

9-(3-Chloro-propyl)-8-(2-iodo-5-methoxy-phenylsulfanyl)-9*H*-purin-6-ylamine (34). A mixture 6-amino-7,9-dihydro-purine-8-thione (**33**) (10 g, 25 mmol), 1-bromo-3-chloropropane (5.0 mL, 50 mmol, 2.0 equiv), Cs_2CO_3 (89 g, 27.5 mmol, 1.1 equiv), and DMF (600 mL) was stirred at 50 °C for 2 h. Workup and chromatography (CH_2Cl_2:EtOAc:Et_3N 100:50:5) gave a 1.5:1 mixture of regioisomers, which was recrystallized twice from CH_2Cl_2:MeOH 100:5 (100 mL) to give 2.5 g of the pure title compound (2.5 g, 21%). t_R = 7.93 min (Conditions II). ^{1}H NMR ($CDCl_3$) δ 8.36 (s, 1H), 7.73 (d, J = 8.7 Hz, 1H), 6.72 (d, J = 2.8 Hz, 1H), 6.59 (dd, J = 8.7 & 2.8 Hz, 1H), 6.07 (br. s, 2H), 4.38 (t, J = 7.1 Hz, 2H), 3.68 (s, 3H), 3.55 (t, J = 7.1 Hz, 2H), 2.27 (quint., J = 6.7 Hz, 2H).

General Procedure for the Preparation of Amines 37−42. The ethyl alcohol **31a** or propyl alcohol **31b** (4 mmol) was dissolved in hot anhydrous 1,4-dioxane (40 mL). The solution was cooled to 25−40 °C and treated with Et_3N (3.0 equiv) and MsCl (1.5 equiv) for 30 min. The mixture was concentrated, dissolved in the appropriate amine (20 mL), and heated to 55 °C for 16 h in a pressure vessel, whereupon the desired material precipitated and was collected by filtration. The crude amine was purified either by recrystallization from refluxing MeOH or by flash chromatography (0−5% MeOH in Et_3N:hexane:DCM:EtOAc 2:100:200:100) to give the desired amine in 45−75% yield. (Note that if the chloropropane **36** was used instead of the corresponding mesylate, the aminations were performed at 90−110 °C for 16 h to give the desired amines in 67−73% yield after flash chromatography.)

The purified amine (2 mmol) was dissolved in refluxing EtOH (30 mL) and with very vigorous agitation a H_3PO_4 solution (0.84 M in EtOH, 2.3 mL, 1 equiv) was added in one portion. The salt precipitated or crystallized immediately. After cooling, filtration gave the desired phosphate in typically 80−85% yield as a fine powder or white needles, which were dried on high vacuum.

9-[3-(1-Ethyl-propylamino)-propyl]-8-(2-iodo-5-methoxy-phenylsulfanyl)-9*H*-purin-6-ylamine (37). Free Base: t_R = 6.28 min (Conditions I). mp: 154−156 °C. ^{1}H NMR ($CDCl_3$/CD_3OD 3:1) δ 8.02 (s, 1H), 7.56 (d, J = 8.8 Hz, 1H), 6.71 (d, J = 2.9 Hz, 1H), 6.46 (dd, J = 8.8 & 2.9 Hz, 1H), 4.14 (t, J = 6.8 Hz, 2H), 3.52 (s, 3H), 2.56 (t, J = 7.0 Hz, 2H), 2.40 (quint., J = 5.9 Hz, 1H), 1.96 (quint., J = 7.0 Hz, 2H), 1.96 (quint., J = 7.1 Hz, 4H), 0.75 (t, J = 7.5 Hz, 6H). ^{13}C NMR ($CDCl_3$:CD_3OD 3:1) 160.4, 154.7, 152.5, 150.9, 145.7, 140.6, 136.0, 119.4, 118.4, 115.9, 90.0, 60.1, 55.3, 42.6, 41.2, 28.1, 23.6 (2C), 9.2 (2C). **H_3PO_4 Salt:** t_R = 6.36 min (Conditions II). mp: 205−208 °C. ^{1}H NMR (D_2O) δ 8.02 (s, 1H), 7.66 (d, J = 8.6 Hz, 1H), 6.81 (d, J = 2.8 Hz, 1H), 6.62 (dd, J = 8.8 & 2.8 Hz, 1H), 4.18 (t, J = 6.7 Hz, 2H), 3.59 (s, 3H), 2.85 (t, J = 8.0 Hz, 2H), 2.80 (quint., J = 6.0 Hz, 1H), 2.01 (quint., J = 7.3 Hz, 2H), 1.47 (quint., J = 7.1 Hz, 4H), 0.75 (t, J = 7.5 Hz, 6H). ^{13}C NMR (D_2O) δ 160.0, 154.3, 152.4, 150.1, 146.5, 141.0, 135.1, 119.0, 118.7, 116.2, 90.6, 60.4, 55.6, 41.7, 41.1, 25.6 (2C), 21.5, 8.1 (2C). Anal. ($C_{20}H_{27}IN_6O_S \cdot H_3PO_4$) C, H, N.

9-(3-*sec*-Butylamino-propyl)-8-(2-iodo-5-methoxy-phenylsulfanyl)-9*H*-purin-6-ylamine (38). Free Base: t_R = 5.94 min (Conditions I). mp: 151−152 °C. ^{1}H NMR (CD_3OD) δ 8.20 (s, 1H), 7.83 (d, J = 8.6 Hz, 1H), 6.96 (d, J = 2.7 Hz, 1H), 6.75 (dd, J = 2.7 & 8.6 Hz, 1H), 4.35 (m, 2H), 3.73 (s, 3H), 2.70−2.64 (m, 3H), 2.07 (m, 2H), 1.58 (m, 1H), 1.34 (m, 1H), 1.06 (d, J = 5.8 Hz, 3H), 0.92 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$:CD_3OD 3:1) 160.4, 154.6, 152.5, 150.9, 145.8, 140.6, 136.1, 119.4, 118.3, 115.9, 89.9, 55.3, 54.3, 42.9, 41.4, 29.1, 28.5, 18.3, 9.9. **H_3PO_4 Salt:** t_R = 6.10 min (Conditions I). mp: 208−212 °C. ^{1}H NMR (D_2O) δ 8.00 (s, 1H), 7.66 (d, J = 8.6 Hz, 1H), 6.83 (d, J = 2.8 Hz, 1H), 6.60 (dd, J = 8.6 & 2.8 Hz, 1H), 4.16 (t, J = 6.8 Hz, 2H), 3.58 (s, 3H), 2.88−2.81 (m, 3H), 1.99 (quint., J = 6.7 Hz, 2H), 1.51−1.50 (m, 1H), 1.14 (sept., J = 7.1 Hz, 1H), 1.05 (d, J = 6.1 Hz, 3H), 0.77 (t, J = 7.1 Hz, 3H). ^{13}C NMR (D_2O) δ 160.0, 154.1, 152.1, 150.1, 146.8, 141.1, 135.0, 119.0, 118.9, 116.3, 90.9, 55.7, 55.6, 41.6, 41.0, 25.6, 25.4, 14.8, 8.7. Anal. ($C_{19}H_{25}IN_6O_S \cdot H_3PO_4$) C, H, N.

8-(2-Iodo-5-methoxy-phenylsulfanyl)-9-(3-isopropylamino-propyl)-9*H*-purin-6-ylamine (39). Free Base: t_R = 5.61 min (Conditions II). mp: 164−166 °C. ^{1}H NMR (CD_3OD) δ 8.24 (s, 1H), 7.86 (d, J = 8.7 Hz, 1H), 6.87 (d, J = 2.7 Hz, 1H), 6.64 (dd, J = 8.7 & 2.7 Hz, 1H), 4.42 (t, J = 6.6 Hz, 2H), 3.77 (s, 3H), 3.37−3.33 (m, 1H), 3.08 (t, J = 6.6 Hz, 2H), 2.24 (quint., J = 6.6 Hz, 2H), 1.34 (d, J = 6.5 Hz, 6H). ^{13}C NMR ($CDCl_3$:CD_3OD 3:1) 160.4, 154.6, 152.5, 150.9, 145.8, 140.6, 136.1, 119.4, 118.3, 115.9, 89.8, 55.3, 48.6, 43.0, 41.3, 29.0, 21.5 (2C). **H_3PO_4 Salt:** t_R = 4.46 min (Conditions I). mp: 233−237 °C. ^{1}H NMR (D_2O) δ 8.12 (br. s, 1H), 7.83 (d, J = 8.8 Hz, 1H), 7.00 (d, J = 2.9 Hz, 1H), 6.75 (dd, J = 8.8 & 2.9 Hz, 1H), 4.27 (t, J = 6.9 Hz, 2H), 3.66 (s, 3H), 3.20 (sept., J = 6.6 Hz, 1H), 2.94 (t, J = 7.9 Hz, 2H), 2.10 (quint., J = 7.4 Hz, 2H), 1.16 (d, J = 6.6 Hz, 6H). ^{13}C NMR (D_2O) δ 159.9, 154.1, 152.2, 150.1, 146.8, 141.0, 134.9, 119.2, 119.0, 116.4, 91.2, 55.6, 50.6, 41.7, 41.0, 25.7, 18.1 (2C). Anal. ($C_{18}H_{23}IN_6O_S \cdot H_3PO_4$) C, H, N.

9-(3-*tert*-Butylamino-propyl)-8-(2-iodo-5-methoxy-phenylsulfanyl)-9*H*-purin-6-ylamine (40). Free Base: t_R = 5.87 min (Conditions II). mp: 165−166 °C. ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1H), 7.70 (d, J = 8.7 Hz, 1H), 6.69 (d, J = 2.7 Hz, 1H), 6.55 (dd, J = 8.7 & 2.7 Hz, 1H), 5.90 (br. s, 2H), 4.30 (t, J = 6.6 Hz, 2H), 3.66 (s, 3H), 2.50 (t, J = 6.6 Hz, 2H), 1.96 (quint., J = 6.6 Hz, 2H), 1.05 (s, 9H). ^{13}C NMR ($CDCl_3$:CD_3OD 3:1) 160.5, 154.8, 152.7, 151.1, 145.9, 140.8, 136.4, 119.6, 118.4, 116.1, 90.0, 55.4, 51.4, 41.7, 38.7, 29.9, 27.9 (3C). **H_3PO_4 Salt:** t_R = 4.77 min (Conditions I). mp: 249−253 °C. ^{1}H NMR (D_2O) δ 8.03 (s, 1H), 7.65 (d, J = 8.8 Hz, 1H), 6.86 (d, J = 2.9 Hz, 1H), 6.61 (dd, J = 8.8 & 2.9 Hz, 1H), 4.17 (t, J = 6.8 Hz, 2H), 3.60 (s, 3H), 2.82 (t, J = 8.0 Hz, 2H), 2.01 (quint., J = 7.4 Hz, 2H), 1.15 (s, 9H). ^{13}C NMR (D_2O) δ 159.9, 154.2, 152.3, 150.0, 146.7, 141.0, 134.9, 119.0, 118.9, 116.3, 91.0, 57.1, 55.5, 41.1, 38.4, 26.1, 24.7 (3C). Anal. ($C_{19}H_{25}IN_6O_S \cdot H_3PO_4 \cdot 0.08EtOH$) C, H, N.

8-(2-Iodo-5-methoxy-phenylsulfanyl)-9-(2-isobutylamino-ethyl)-9*H*-purin-6-ylamine (41). Free Base: t_R = 6.10 min (Conditions II). mp: 155−158 °C. ^{1}H NMR ($CDCl_3$/CD_3OD 3:1) δ 8.09 (s, 1H), 7.64 (d, J = 8.8 Hz, 1H), 6.77 (d, J = 2.9 Hz, 1H), 6.53 (dd, J = 8.7 & 2.9 Hz, 1H), 4.22 (t, J = 6.5 Hz, 2H), 3.60 (s, 3H), 2.83 (t, J = 6.5 Hz, 2H), 2.27 (d, J = 6.9 Hz, 2H), 1.56 (non., J = 6.7 Hz, 1H), 0.73 (d, J = 6.6 Hz, 6H). ^{13}C NMR (DMSO-d_6) 160.4, 156.0, 153.8, 151.3, 143.5, 140.7, 140.0, 120.2, 115.8, 114.9, 87.1, 57.4, 55.8, 48.8, 43.8, 28.4, 21.0 (2C). **H_3PO_4 Salt:** t_R = 5.10 min (Conditions I). mp: 214−216 °C. ^{1}H NMR (D_2O) δ 8.12 (s, 1H), 7.79 (d, J = 8.3 Hz, 1H), 6.95 (br. s, 1H), 6.73 (d, J = 8.3 Hz, 1H), 4.51 (m, 2H), 3.65 (s, 3H), 3.36 (m, 2H), 2.83 (d, J = 6.6 Hz, 2H), 1.89 (sept., J = 6.4 Hz, 1H), 0.87 (d, J = 6.2 Hz, 6H). ^{13}C NMR (D_2O) δ 160.1, 153.9, 152.1, 150.6, 146.7, 141.2, 134.8, 119.3, 119.0, 116.6, 91.1, 55.6, 54.7, 46.1, 40.0, 25.5, 19.0 (2C). Anal. ($C_{18}H_{23}IN_6O_S \cdot H_3PO_4$) C, H, N.

9-[2-(2,2-Dimethyl-propylamino)-ethyl]-8-(2-iodo-5-methoxy-phenylsulfanyl)-9*H*-purin-6-ylamine (42). Free Base: t_R = 5.13 min (Conditions II). mp: 156−158 °C. ^{1}H NMR ($CDCl_3$/CD_3OD 3:1) δ 8.21 (s, 1H), 7.74 (d, J = 8.7 Hz, 1H), 6.87 (d, J = 2.9 Hz, 1H), 6.63 (dd, J = 8.7 & 2.9 Hz, 1H), 4.32 (t, J = 6.4 Hz, 2H), 3.71 (s, 3H), 2.95 (t, J = 6.4 Hz, 2H), 2.31 (s, 2H), 0.83 (s, 9H). ^{13}C NMR ($CDCl_3$:CD_3OD 3:1) 160.5, 154.6, 152.7, 151.1, 146.5, 140.7, 136.9, 119.6, 118.3, 115.9, 89.7, 61.9, 55.4, 49.5, 43.9, 31.4, 27.5 (3C). **H_3PO_4 Salt:** t_R = 5.13 min (Conditions II). mp: 162−164 °C. ^{1}H NMR (D_2O) δ 8.10 (s, 1H), 7.75 (d, J = 8.7 Hz, 1H), 6.89 (d, J = 2.8 Hz, 1H), 6.68 (dd, J = 8.7 & 2.8 Hz, 1H), 4.51 (t, J = 5.9 Hz, 2H), 3.64 (s, 3H), 3.37 (t, J = 5.9 Hz, 2H), 2.84 (s, 2H), 0.96 (s, 9H). ^{13}C NMR (D_2O) 160.1, 154.2, 152.2, 150.4, 147.3, 141.2, 135.0, 119.2, 119.0, 116.5, 91.0, 59.3, 55.6, 47.0, 40.2, 29.9, 26.3 (3C). Anal. ($C_{19}H_{25}IN_6O_S \cdot H_3PO_4$) C, H, N.

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References

(1) For recent reviews, see: (a) Kamal, A.; Boehm, M. F.; Burrows, F. J. Therapeutic and diagnostic implications of Hsp90 activation. *Trends Mol. Med.* **2004**, *10*, 283−290. (b) Dymock, B. W.; Drysdale, M. J.; McDonald, E.; Workman, P. Inhibitors of Hsp90 and other chaperones for the treatment of cancer. *Expert Opin. Ther. Pat.* **2004**, *14*, 837−847. (c) Isaacs, J. S.; Wanping, X.; Neckers, L. Heat shock protein 90 as a molecular target for cancer therapeutics. *Cancer Cell* **2003**, *3*, 213. (d) Maloney, A.; Workman, P. Hsp90 as a new therapeutic target for cancer therapy: the story unfolds. *Expert Opin. Biol. Ther*. **2002**, *2*, 3−24 (e) Richter, K.; Buchner, J. Hsp90: Chaperoning signal transduction. *J. Cell. Physiol.* **2001**, *188*, 281−290.

(2) For lists of client proteins: (a) Pratt, W. B.; Toft, D. O. Regulation of signaling protein function and trafficking by the hsp90/hsp70-based machinery. *Exp. Biol. Med.* **2003**, *228*, 111−133. (b) Workman, P. Combinatorial attack on multistep oncogenesis by inhibiting the Hsp90 molecular chaperone. *Cancer Lett.* **2004**, *206*, 149−157. (c) Zhang, H.; Burrows, F. Targeting multiple signal transduction pathways through inhibition of Hsp90. *J. Mol. Med.* **2004**, *82*, 488−499.

(3) Gorre, M. E.; Ellwood-Yen, K.; Chiosis, G.; Rosen, N.; Sawyers, C. L. BCR-ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR-ABL chaperone heat shock protein 90. *Blood* **2002**, *100*, 3041−3044.

(4) (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) Workman, P. Altered states: selectively drugging the Hsp90 cancer chaperone. *Trends Mol. Med.* **2004**, *10*, 47−51.

(5) Yun, B.-G.; Huang, W.; Leach, N.; Hartson, S. D.; Matts, R. L. Novobiocin induces a distinct conformation of Hsp90 and alters Hsp90-cochaperone-client interactions. *Biochemistry* **2004**, *43*, 8217−8229.

(6) The NCI clinical protocol consists of injecting a DMSO solution of 17-AAG.

(7) (a) Glaze, E. R.; Smith, A. C.; Johnson, D. W.; McCormick, D. L.; Brown, A. B.: Levin, B. S.; Krishnaraj, R.; Lyubimov, A.; Egorin, M. J.; Tomaszewski, J. E. Dose range-finding toxicity studies of 17-DMAG. *Proc. Am. Assoc. Cancer. Res.* **2003**, *44*, 162−162. (b) Eiseman, J. L.; Lan, J.; Lagatutta, 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.

(8) (a) Ikuina, Y.; Amishiro, N.; Miyata, M.; Narumi, H.; Ogawa, H.; Akiyama, T.; Shiotsu, Y.; Akinaga, S.; Murakata, C. Synthesis and antitumor activity of novel *O*-carbamoylmethyloxime derivatives of radicicol. *J. Med. Chem.* **2003**, *46*, 2534−2541. Furthermore, radicicol and its oximes contain an oxirane ring which has been viewed as a liability for stability and toxicity, prompting the synthesis of cycloproparadicicol: (b) Yang, Z. Q.; Geng, X.; Solit, D.; Pratilas, C. A.; Rosen, N.; Danishefsky, S. J. New efficient synthesis of resorcinylic macrolides via ynolides: establishment of cyclopropa-radicicol as synthetic feasible preclinical anticancer agent based on Hsp90 as the target. *J. Am. Chem. Soc.* **2004**, *126*, 7881. (b) Yang, Z.-Q.; Danishefsky, S. J. A concise route to benzofused macrolactones via ynolides: cycloproparadicicol. *J. Am. Chem. Soc.* **2003**, *125*, 9602−9603.

(9) (a) Chiosis, G.; Lucas, B.; Shtil, A.; Huezo, H.; Rosen, N. Development of a purine-scaffold novel class of Hsp90 binders that inhibit the proliferation of cancer cells and induce the degradation of HER-2 tyrosine kinase. *Bioorg. Med. Chem. Lett.* **2002**, *10*, 3555−3564. (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. (c) Chiosis, G.; Rosen, N. Small molecule composition for binding to Hsp90. WO 0236075, 2002.

(10) (a) Drysdale, M. J.; Dymock, B. W.; Barril-Alonso, X.; Workman, P. 3,4-Diarylpyrazoles and their use in the therapy of cancer. WO 03/055860 A1, 2003. (b) Wright, L.; Barril, X.; Dymock, B.; Sheridan, L.; Surgenor, A.; Beswick, M.; Drysdale, M.; Collier, A.; Massey, A.; Davies, N.; Fink, a.; Fromont, C.; Aherne, W.; Boxall, K.; Sharp, S.; Workman, P.; Hubbard, R. Structure−activity relationships in purine-based inhibitor binding to Hsp90 isoforms *Chem. Biol.* **2004**, *11*, 775−785. (c) 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. (d) Dymock, B. W.; Barril, X.; Brough, P. A.; Cansfield, J. E.; Massey, A.; McDonald, E.; Hubbard, R. E.; Surgenor, A.; Roughley, S. D.; Webb, P.; Workman, P.; Wright, L.; Drysdale, M. J. Novel, potent small-molecule inhibitors of the molecular chaperone Hsp90 discovered through structure-based design *J. Med. Chem.* **2005**, *48*, 4212−4215. Structure of Hsp90 in complex with **3a**: pdb code 1UY6, and with **3b**: pdb code 1UYF.

(11) Clevenger, R. D.; Blagg, B. S. J. Design, synthesis, and evaluation of a radicicol and geldanamycin chimera, radamide. *Org. Lett.* **2004**, *6*, 4459−4462.

(12) 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*, 371−376.

(13) The benzene ring of **3a** was not designed to have exactly the same orientation as the quinone ring of geldanamycin. Rather, the trimethoxybenzene moiety was designed to point in the same general direction and form a hydrogen bond with Lys112, an amino acid which forms a hydrogen bond with the quinone ring of geldanamycin.

(14) Kasibhatla, S. R.; Zhang, L.; Boehm, M. F.; Fan, J.; Hong, K.; Shi, J.; Biamonte, M. A. Purine Analogues Having Hsp90 Inhibiting Activity. WO 3037860, 2003.
(15) Llauger, L.; He, H.; Kim, J.; Aguirre, J.; Rosen, N.; Peters, U.; Davies, P. Chiosis, G. Evaluation of 8-arylsulfanyl, 8-arylsulfoxyl, and 8-arylsulfonyl adenine derivatives as inhibitors of the heat shock protein 90. *J. Med. Chem.* **2005**, *48*, 2892−2905.
(16) The free base of 2,3,5,6-tetraaminopyrimidine, a key intermediate in the production of 2-fluoroadenines, could also be obtained from the commercial sulfate salt and was acylated in NMP by a similar process.
(17) Janeba, Z.; Holy, A.; Masojidkova, M. Synthesis of acyclic nucleoside and nucleotide analogues derived from 6-amino-7*H*-purin-8(9*H*)-one. *Collect. Czech. Chem. Commun.* **2000**, *65*, 1126−1144.
(18) (a) Ma, C.; Liu, X.; Li, X.; Flippen-Anderson, J.; Yu, S.; Cook, J. M. Efficient asymmetric synthesis of biologically important tryptophan analogues via a palladium-mediated heteroannulation reaction. *J. Org. Chem.* **2001**, *66*, 4525−4542. (b) Flynn, B. L.; Verdier-Pinard, P.; Hamel, E. A. Novel palladium-mediated coupling approach to 2,3-disubstituted benzo[*b*]thiophenes and its application to the synthesis of tubulin binding agents. *Org. Lett.* **2001**, *3*, 651−654.
(19) Biamonte, M. A.; Shi, J.; Hurst, D.; Hong, K.; Boehm, M. F.; Kasibhatla, S. R. Preparation of 8-(arylsulfanyl)adenines with diazonium salts under mild, aerobic conditions. *J. Org. Chem.* **2005**, *70*, 717−720.
(20) Le Brazidec, J.-Y.; Kamal, A.; Busch, D.; Thao, L.; Zhang, L.; Timony, G.; Grecko, R.; Trent, K.; Lough, R.; Salazar, T.; Khan, S.; Burrows, F.; Boehm, M. F. Synthesis and biological evaluation of a new class of geldanamycin derivatives as potent inhibitors of Hsp90. *J. Med. Chem.* **2004**, *47*, 3865−3873.
(21) Prepared in a manner analogous to **8** (Scheme 1, route A) but replacing BuI with other alkylating agents.
(22) Purified soy-derived L-α-phosphatidylcholine (Phospholipon 90H) was purchased from American Lecithin Company.
(23) Not every kinase inhibitor has a short-lasting effect. Some are irreversible inhibitors or have slow off-rate kinetics, while others can sequester the kinase into inactive dimers, causing their internalization. For examples, see: (a) Tsou, H.-R.; Overbeek-Klumpers, E. G.; Hallett, W. A.; Reich, M. F.; Floyd, M. B.; Johnson, B. D.; Michalak, R. S.; Nilakantan, R.; Discafani, C.; Golas, J.; Rabindran, S. K.; Shen, R.; Shi, X.; Wang, Y.-F.; Upeslacis, J.; Wissner, A. Optimization of 6,7-disubstituted-4-(arylamino)quinoline-3-carbonitriles as orally active, irreversible inhibitors of human epidermal growth factor receptor-2 kinase activity. *J. Med. Chem.* **2005**, *48*, 1107−1131. (b) Wood, E. R.; Truesdale, A. T.; McDonald, O. B.; Yuan, D.; Hassell, A.; Dickerson, S. H.; Pennisi, C.; Horne, E.; Lackey, K.; Allgood, K. J.; Rusnak, D. W.; Gilmer, T. M.; Shewchuk, L. A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. *Cancer Res.* **2004**, *15*, 6652−6659. (c) Lichtner, R. B.; Menrad, A.; Sommer, A.; Klar, U.; Schneider, M. R. Signaling-inactive epidermal growth factor receptor/ligand complexes in intact carcinoma cells by quinazoline tyrosine kinase inhibitors. *Cancer Res.* **2001**, 61, 5790−5795.
(24) Childress, S. J.; McKee, R. L. Chloroaminopyrimidines. *J. Am. Chem. Soc.* **1950**, *72*, 4271−4272.
(25) (a) Beaman, A. G.; Gerster, J. F.; Robins, R. K. Potential purine antagonists. XXVIII. The preparation of various bromopurines. *J. Org. Chem.* **1962**, *27*, 986−990. (b) Holy, A.; Günter, J.; Dvorakova, H.; Masojidkova, M.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. Structure-antiviral activity relationship in the series of pyrimidine and purine *N*-[2-(2-phosphonomethoxy)ethyl] nucleotide analogues. 1. Derivatives substituted at the carbon atoms of the base. *J. Med. Chem.* **1999**, *42*, 2064−2086.

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