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Article

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Discovery of (2*S*)-1-[4-(2-{6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)sulfanyl]-9*H*-purin-9-yl}ethyl)piperidin-1-yl]-2-hydroxypropan-1-one (MPC-3100), a Purine-Based Hsp90 Inhibitor

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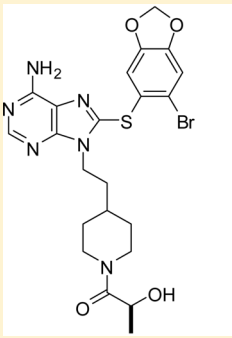
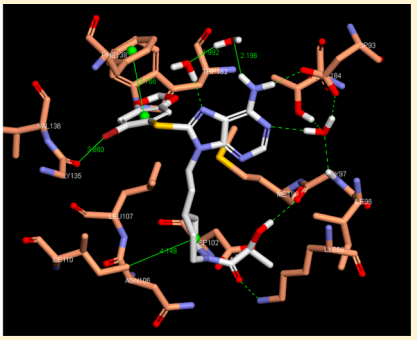
Supporting Information

ABSTRACT: Modulation of Hsp90 (heat shock protein 90) function has been recognized as an attractive approach for cancer treatment, since many cancer cells depend on Hsp90 to maintain cellular homeostasis. This has spurred the search for small-molecule Hsp90 inhibitors. Here we describe our lead optimization studies centered on the purine-based Hsp90 inhibitor **28a** containing a piperidine moiety at the purine N9 position. In this study, key SAR was established for the piperidine N-substituent and for the congeners of the 1,3-benzodioxole at C8. These efforts led to the identification of orally bioavailable **28g** that exhibits good in vitro profiles and a characteristic molecular biomarker signature of Hsp90 inhibition both in vitro and in vivo. Favorable pharmacokinetic properties along with significant antitumor effects in multiple human cancer xenograft models led to the selection of **28g** (MPC-3100) as a clinical candidate.





INTRODUCTION

Heat shock proteins (HSPs) are some of the most abundant intracellular proteins and play critical roles as molecular chaperones in essential cellular functions such as (1) protein maturation, folding, and prevention of aggregation, (2) sorting and translocation, (3) stabilization of proteins involved in signal transduction, and (4) apoptosis and proteasomal degradation.[1−3] Hsp90 is one of the major HSPs and exists as a homodimer. Each monomer is composed of three domains: an N-terminal ATP-binding domain, a middle domain, and a C-terminal dimerization domain.[4,5] Hsp90 is unique among HSPs because it associates with cochaperones (e.g., Hsp70, Hsp40, cdc37, p23) to form a multichaperone complex that interacts with a large number of cofactors (e.g., HIP, HOP, immunophilins). Because Hsp90 is ubiquitously expressed in both normal and tumor tissue, it was believed that inhibition of the Hsp90 pathway would affect normal cellular function and lead to general toxicity. As a consequence, Hsp90 was not immediately recognized as a viable target for cancer treatment.[6] Evidence supporting the therapeutic potential of Hsp90 inhibition was provided by reports that Hsp90 is overexpressed in numerous cancer cell types.[7] However, direct evidence for Hsp90 therapeutic value came from the observation that v-Src-mediated transforming activity was abolished by the natural product geldanamycin (**1a**) via disruption of Src-Hsp90 protein complexes.[8] This seminal work revealed a critical role of the Hsp90 chaperone machinery in the stability of v-Src and also showed that c-Src is less affected than v-Src by inhibition of Hsp90. Since then, over 200 proteins, known collectively as client proteins, were found to depend on the Hsp90 system for their function and stability and most of these client proteins are involved in signal transduction, including kinases (e.g., Her2, AKT, Bcr-Abl, Cdk4, B-raf), hormone receptors (e.g., androgen, estrogen, glucocorticoid receptors), and transcription factors (e.g., mutant p53, HIF-1α). Importantly, many of these are involved in oncogenesis and contribute to the six well-defined hallmarks of cancer.[9] Not surprisingly, a number of Hsp90 client proteins have received attention as potential targets for cancer therapy, and this approach has been successful.[10] However, the development of drug resistance resulting from target kinase mutations, feedback signaling loops, and alternative survival pathway activation has become a significant therapeutic challenge.[11] In contrast, the simultaneous targeting of multiple signaling pathways via inhibition of the Hsp90 chaperone system has the potential to be both more efficacious and less vulnerable to resistance mechanisms.[12] An important aspect of Hsp90 as a cancer target is the differential effect of Hsp90 inhibitors on cancer cells. In animal models, several different Hsp90 inhibitor classes have shown selective

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pharmacology in tumor cells and strong anticancer effects at nontoxic levels. Although a complete understanding of the origins of selectivity requires additional studies, mounting evidence suggests that oncogene addiction,[3,13] synthetic lethality,[14] activation,[15] and post-translational modification of Hsp90 in tumors might account for this unique observation.[16,17]

In contrast to cancer, where Hsp90 inhibition is sought, induction of Hsp90 activity has been proposed as a potential treatment for neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's, and amyotrophic lateral sclerosis (Lou Gehrig's disease). These diseases are associated with accumulation of misfolded proteins, which are toxic to neuronal cells. It has been suggested that enhancing protein-folding capacity by pharmacological activation of transcription factor heat shock protein 1 (HSF1) could be a promising strategy for the treatment of neurodegenerative diseases. Paradoxically, because of feedback regulation, Hsp90 inhibitors induce Hsp90 protein levels via HSF1 activation and therefore may have utility in the treatment of neurodegenerative diseases.[18,19]

Taken together, it is now evident that Hsp90 is a bona fide cancer target, and the development of Hsp90 inhibitors has become one of the most active areas of research not only for cancer chemotherapy but also potentially for treatment of neurodegenerative disease.

Sixteen Hsp90 inhibitors have entered clinical development as novel anticancer agents.[20−25] All of these inhibitors target the N-terminal, ATP-binding site of Hsp90 and effectively block the essential ATPase activity. The first-in-class inhibitor, 17-AAG (**1b**), a geldanamycin analogue with improved pharmacological properties over the parent geldanamycin, is being evaluated in phase II−III clinical trials by several sponsors (Figure 1). However, 17-AAG (**1b**) presents significant barriers to development, including poor aqueous solubility, P-glycoprotein (P-gp) multidrug resistance efflux pump substrate activity, limited oral bioavailability, and hepatotoxicity which is believed to be a consequence of its benzoquinone moiety and the formation of a drug−glutathione (GSH) adduct upon redox activation.[26,27] The more water-soluble geldanamycin analogues 17-DMAG (**1c**) and IPI-504 (**1d**), a prodrug of 17-AAG (**1b**), have solved some of the aforementioned limitations and are being evaluated in clinical trials.[20] Radicicol (**2a**) is a resorcinol-containing natural product inhibitor of Hsp90.[28] Because of its potent Hsp90 inhibition and resultant antiproliferative activity in cells, this natural product has garnered widespread interest in the synthetic community, and several total syntheses have been reported.[29] However, lack of potency in animal models, believed to be a result of the reactive epoxide and 1,6-dienone moieties, has precluded further development. Efforts to modify the radicicol (**2a**) core, specifically by replacement of the epoxide ring and attenuation of the 1,6-dieneone reactivity, have led to the identification of more stable cyclopropyl- (**2b**) and oxime-radicicol analogues **3a,b** with improved in vitro potency.[25,28,29] These compounds, however, remain in the preclinical development stage. As a way of overcoming the limitations presented by natural product Hsp90 inhibitors, synthetic hybrids[30] of geldanamycin (**1a**) and radicicol (**2a**) have been developed with the aid of structure-based drug design, and the chimeric inhibitors, radester (**4**) and radanamycin (**5**), are promising new leads.[25,31]

The identification and optimization of entirely synthetic, small molecule inhibitors of Hsp90 have been undertaken by several research groups as a way of imparting more druglike

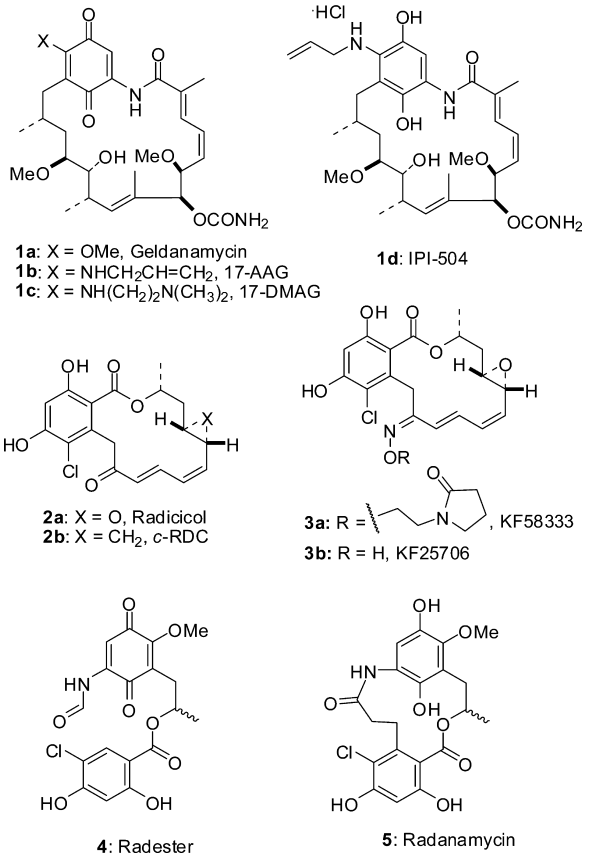

Figure 1. Natural product and natural-product-derived Hsp90 inhibitors.

properties (Figures 2 and 3). The first synthetic Hsp90 inhibitor, purine derivative PU3 (**7**), originated from the Chiosis laboratory[32] and was designed to bind the N-terminal ATP-binding pocket of Hsp90. PU3 (**7**) adopts the C-type conformation reminiscent of geldanamycin (**1a**), and subsequent structure-based optimization resulted in the discovery of the potent, water-soluble inhibitor PU-H71 (**8**),[33] currently being evaluated in phase I clinical trials.[20] A similar purine-based inhibitor **9** was developed by Conforma Therapeutics (now Biogen Idec).[34] A common structural feature of the optimized purine-based inhibitors **8** and **9** is the sulfur atom tethering the purine C8 position and the substituted aryl ring. The PU class (**6**)[32] has received attention by several research groups and has served as a guide for design of new Hsp90 inhibitors. Key PU-class structural features were utilized during the design of BIIB021 (**10**).[35] In this instance, however, binding elements were rationally and systematically "rotated" around the purine ring, resulting in a unique purine derivative in which the aryl group is connected via the N9 position as opposed to the N8 position. BIIIB021 (**10**) was advanced into phase I−II clinical trials. Finally, several synthetic non-purine small molecules **15−20** encompassing diverse structural features have been identified through HTS, fragment-based, and structure-based drug design approaches,[20−24,36,37] and these inhibitors are being evaluated in clinical settings (Figure 3).

We believed that the inherent activity of the purine scaffold, in concert with new synthetic methodologies, would provide a good starting point for the synthesis of novel analogues,[38] and as such, our Hsp90 drug discovery program was also based on

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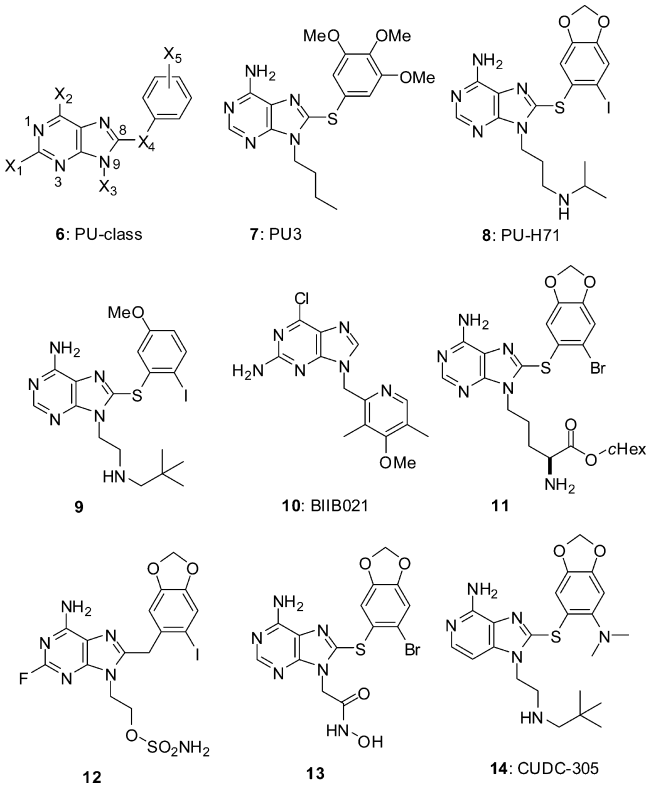


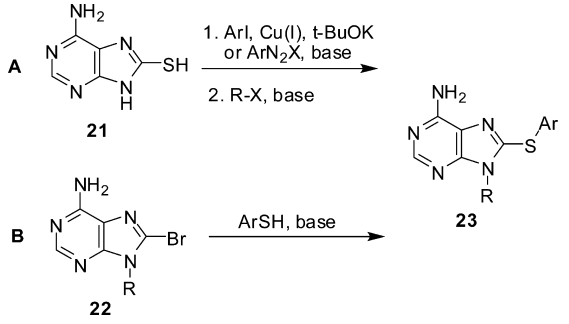
Figure 2. Selected purine-based and purine-like Hsp90 inhibitors.

the PU class. A shared feature of the majority of purine-based inhibitors (Figure 2) is the presence of pendent hydrophilic N9 substituents. This modification, intended to improve aqueous solubility and other physical properties, demonstrated the utility of the N9 substituent for modulation of molecular properties. Similarly, we investigated the effects of various N9 substituents on ligand potency and ADME properties. Herein we report a novel series of purine-based Hsp90 inhibitors, their synthesis via new C−S bond formation methodology, and the biological evaluations leading to the identification of **28g** (MPC-3100) as a phase I clinical candidate.[39]

CHEMISTRY

Synthetic routes for preparation of 9-*N*-alkyl-8-arylsulfanyladenines **23** are categorized into two classes (Scheme 1).[32,40]

Scheme 1



Method A involves a two-step procedure consisting of Cu(I)-catalyzed coupling of 8-mercaptoadenine (**21**) with aryl iodides (ArI),[41] followed by N-alkylation with alkyl halides or mesylates (RX).[33] Alkylation typically resulted in a ∼2: 1 mixture of N9 and N3 regioisomers. Alternatively, aryldiazonium salts (ArN_2X) could be used instead of aryl iodides (ArI) as coupling partners during C−S bond formation.[42] Method B utilizes *N*-alkylbromoadenines **22**, which can be prepared regioselectively in high yields.[34,43] Displacement of the bromide in **22** with arylthiols (ArSH) proceeds under mild conditions to provide **23** as single regioisomers. A significant drawback to method B, however, is the propensity of arylthiols toward disulfide formation.

The synthetic approach for our initial SAR studies was based on method A. However, instead of utilization of difficult to source aryl iodides, more readily available aryl bromides were desirable, and as a consequence, a Pd(0)-catalyzed coupling of 8-mercaptoadenine (**21**)[42] with aryl bromides **24a−g** (Scheme 2) was developed using catalytic Pd_2dba_3, the ligand xantphos, a base (K_2CO_3 or Cs_2CO_3) in dioxane. For the Pd-catalyzed reaction, we chose ortho-substituted aryl bromides **24a−g** (X = Br, CN) as coupling partners, since early SAR studies revealed the critical nature of the 8-aryl group ortho-substituent (X) for

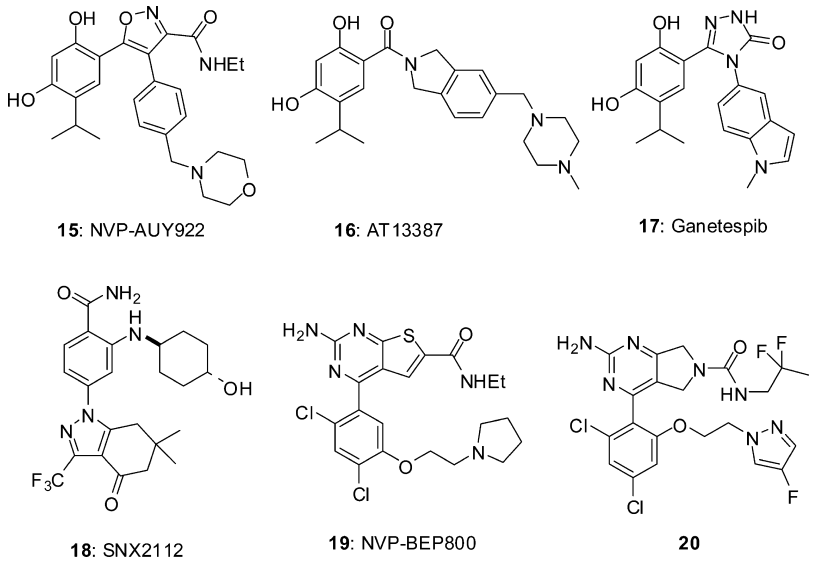


Figure 3. Selected examples of structurally diverse non-purine Hsp90 inhibitors.

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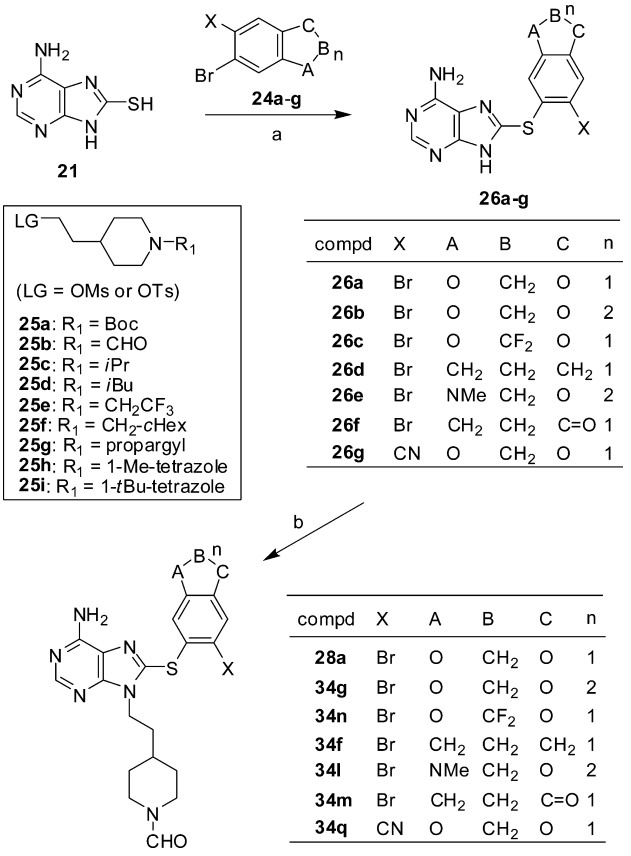
Scheme 2[a]



compd	X	A	B	C	n
26a	Br	O	CH_2	O	1
26b	Br	O	CH_2	O	2
26c	Br	O	CF_2	O	1
26d	Br	CH_2	CH_2	CH_2	1
26e	Br	NMe	CH_2	O	2
26f	Br	CH_2	CH_2	C=O	1
26g	CN	O	CH_2	O	1

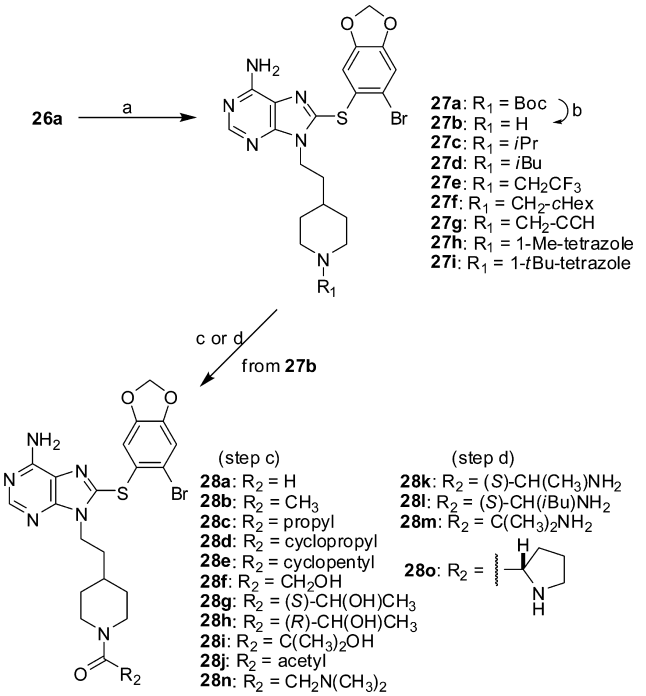
compd	X	A	B	C	n
28a	Br	O	CH_2	O	1
34g	Br	O	CH_2	O	2
34n	Br	O	CF_2	O	1
34f	Br	CH_2	CH_2	CH_2	1
34l	Br	NMe	CH_2	O	2
34m	Br	CH_2	CH_2	C=O	1
34q	CN	O	CH_2	O	1

[a]Reagents and conditions: (a) **24a–g**, Pd_2dba_3, xantphos, K_2CO_3, dioxane, 100 °C, 15–45%; (b) **25b**, Barton's base, 80 °C, DMF, 20–51%.

Hsp90 inhibition.[43,58] This synthetic approach eliminates the need for installation of the key ortho-substituent at a later stage. Although yields were modest, this approach allowed the use of readily available ortho-substituted aryl bromides **24a–g** and was used to prepare key intermediates **26a–g** in a rapid fashion. Moreover, the coupling of aryl dibromides **24a–f** (X = Br) with **21** proceeded without formation of undesired bis-arylsulfanyl products. With 8-arylsulfanyladenines **26a–g** in hand, alkylation with **25b** was carried out in the presence of Barton's base. The desired N9 regioisomers **28a**, **34g**, **34n**, **34f**, **34l**, **34m**, and **34q** were obtained in 20–51% yield after removal of the minor N3 regioisomers (structures not shown) by silica gel chromatography or preparative scale RP-HPLC. In a similar manner, analogues **27a** and **27c–i** were synthesized using **25a** and **25c–i** as alkylating agents. The *N*-Boc group of **27a** was removed using TFA, and the resulting free piperidine analogue **27b** was converted into *N*-acylpiperidine analogues **28a–j** and **28n** via EDCI-mediated coupling with the appropriate carboxylic acid (step c, Scheme 3). Incorporation of *N*-Boc protected amino acids in the EDCI coupling sequence afforded **28k** and **28l–o** (step d, Scheme 3).

The synthesis of **25a–i** is shown in Scheme 4. Compounds **30a,b** are commercially available or can be conveniently prepared from **29** by *N*-Boc protection or N-formylation, respectively. Mesylation of the resulting alcohols **30a,b** afforded compounds **25a,b**. Reductive alkylation[44,45] of **29** with acetone, isobutyraldehyde, or cyclohexanecarboxaldehyde provided **30c,d,f** which were then converted to the mesylates **25c,d,f**.

Scheme 3[a]



[a]Reagents and conditions: (a) **25a** or **25c–i**, Barton's base, 80 °C, DMF, 20–51%; (b) TFA, CH_2Cl_2, rt, >90%; (c) EDCI, HOBt, DMF, R_2CO_2H (R_2 = H, CH_3, propyl, cyclopropyl, cyclopentyl, CH_2OH, (*S* or *R*)-$CH(OH)CH_3$, $C(CH_3)_2OH$, acetyl, $CH_2N(CH_3)_2$); (d) EDCI, HOBt, DMF–THF, rt, R_2CO_2H (R_2 = (*S*)-$CH(CH_3)NHBoc$, (*S*)-$CH(iBu)NHBoc$, $C(CH_3)_2NHBoc$ or *N*-Boc-L-proline), then 4 M HCl or TFA.

Alternatively, N-alkylation of **29** with the appropriate alkyl halide could also be used to prepare these intermediates. N-Alkylation of **29** with CF_3CH_2I or propargyl bromide under the standard procedure[46] followed by mesylation afforded **25e** and **25g**. Synthesis of tetrazole derivatives **25h,i** was achieved employing known synthetic methods.[47] Finally, thioureas **31a** and **31b**, derived from **29** and methyl- or *tert*-butyl isothiocyanate, were converted to tetrazoles **25h,i** with NaN_3 and $HgCl_2$ followed by tosylation.

Although method A proved adequate for initial SAR studies, separation of the undesired N3 regioisomers and low overall yields presented major hurdles to more extensive SAR studies. Therefore, method B (Scheme 1) was adopted for synthesis of later-stage targets. N9-substitued bromoadenines **33a,b** were readily prepared in high overall yield by literature procedures.[34,43] The reaction sequence involved alkylation of adenine (**32**) with alkylating agents **25a,b** (Scheme 7) and bromination in acetic acid buffer. During the alkylation step a small amount (<10%) of N3 isomers were produced but were easily removed by trituration after the bromination step. Our strategy for the synthesis of arylthiols (ArSH) utilized 3-ethylhexyl 3-mercaptopropanoate (**52**, Scheme 5),[48] a known thiol surrogate, as a means of preventing oxidative disulfide formation of the arylthiol intermediates. The synthetic utility of **52** as a thiol surrogate has been demonstrated previously for synthesis of benzothiazoles and biaryl compounds,[48b,c] and a similar strategy has been applied to the synthesis of aryl sulfoxides.[49] Described below is the successful application of the thiol surrogate strategy to the synthesis of structurally diverse 9-*N*-alkyl-8-arylsulfanyladenines (Schemes 5–9).

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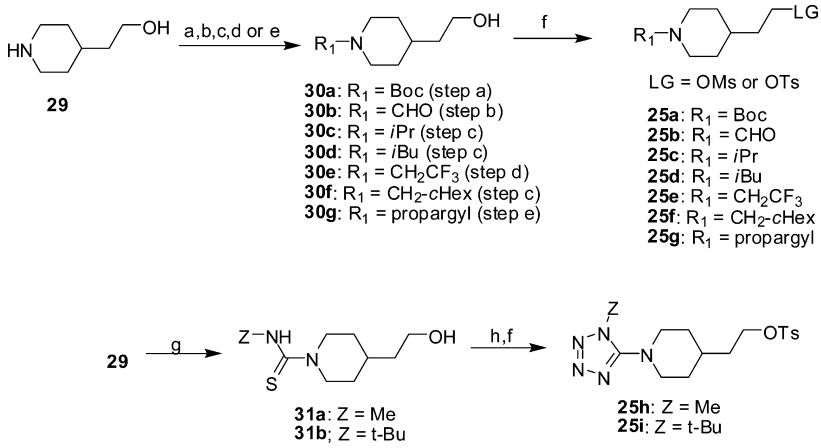
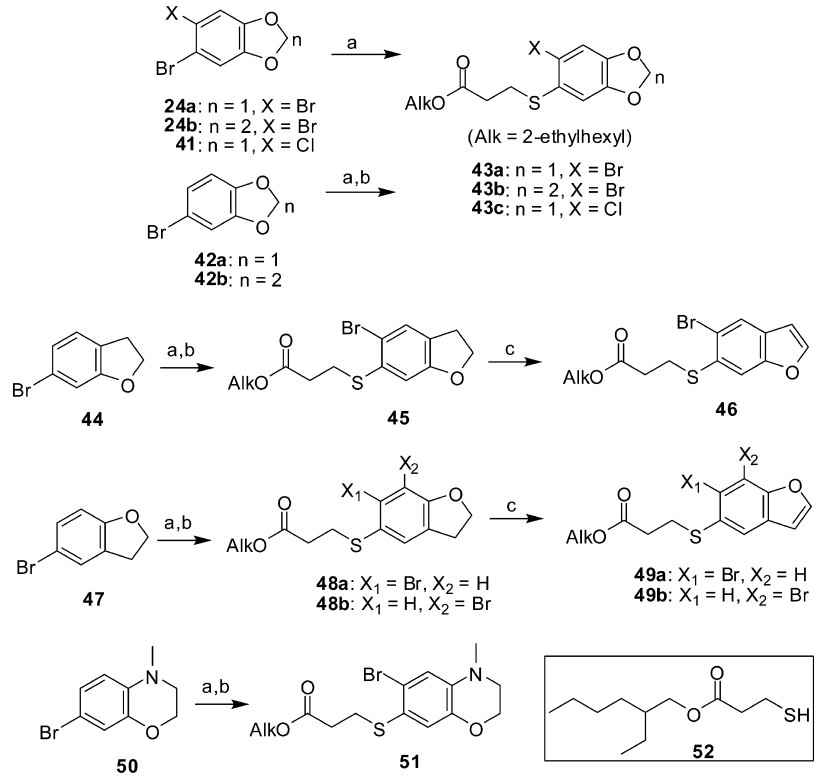
Scheme 4[a]



[a]Reagents and conditions: (a) Boc_2O, NEt_3, CH_2Cl_2; (b) HCO_2Me, NaOH, MeOH; (c) acetone, *i*-Bu-CHO, or *c*-Hex-CHO, $NaBH(OAc)_3$, CH_2Cl_2 (or $NaCNBH_3$, AcOH, MeOH−THF); (d) CF_3CH_2I, K_2CO_3, CH_3CN, reflux or CF_3CH_2OMs, NEt_3, THF; (e) propargyl bromide, Hünig's base, THF; (f) MsCl, or TsCl, NEt_3 (or DMAP), CH_2Cl_2, rt, 30−95%; (g) SCN-Me or SCN-*t*-Bu, CH_2Cl_2, rt, 80−85%; (h) NaN_3, $HgCl_2$, NEt_3, DMF, 41−50%.

Scheme 5[a]



[a]Reagents and conditions: (a) **52**, Pd_2dba_3 (5−10 mol %), xantphos (10−20 mol %), Hünig's base, dioxane, 100 °C, 10 h, >80%; (b) NBS, $HBF_4{\cdot}Et_2O$, AcCN, −15 to 5 °C, >70%; (c) DDQ, 1,4-dioxane, 100 °C, ∼90%.

The detailed reaction sequence for synthesis of aryl sulfides is given in Scheme 5. Aryl sulfides **43a−c** were prepared in a single step using a Pd(0)-catalyzed coupling of aryl dihalides **24a,b** and **41** with **52**. Compounds **43a,b** could also be obtained from aryl bromides **42a,b** via a two-step procedure involving Pd(0)-catalyzed coupling and ortho-bromination.[50] Synthesis of aryl sulfide **45** was achieved in two steps from the known bromodihydrobenzofuran **44**[51] via Pd(0)-catalyzed coupling of **52** and subsequent ortho-bromination. Oxidation of dihydrobenzofuran **45** with DDQ afforded benzofuran **46**. An identical procedure was applied to the synthesis of **48a**, a regioisomer of **45**, using commercially available 5-bromo-2,3-dihydrobenzofuran (**47**). In the instances of aryl sulfides **48a** and **48b**, nonselective bromination afforded ∼1:3 mixtures that were oxidized directly with DDQ to provide benzofurans **49a** and **49b**; the undesired regioisomers were removed by preparative RP-HPLC upon reaction with bromoadenine **33**. Similarly, aryl sulfide **51** was obtained from **50** using the two-step sequence of Pd(0)-catalyzed coupling with **52** and ortho-bromination.

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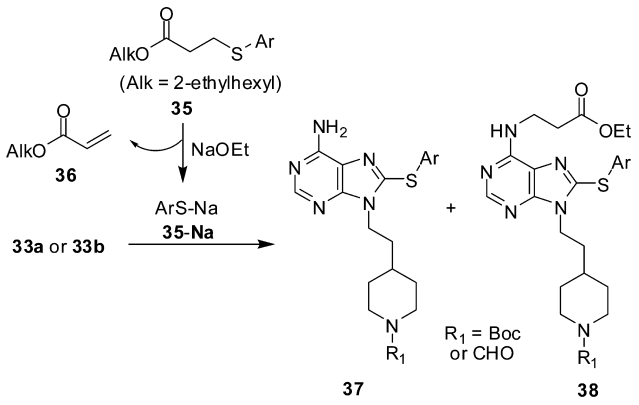
With the requisite aryl sulfides **35** in hand, the stage was set for the critical C−S bond forming reaction (Scheme 6). To this

Scheme 6



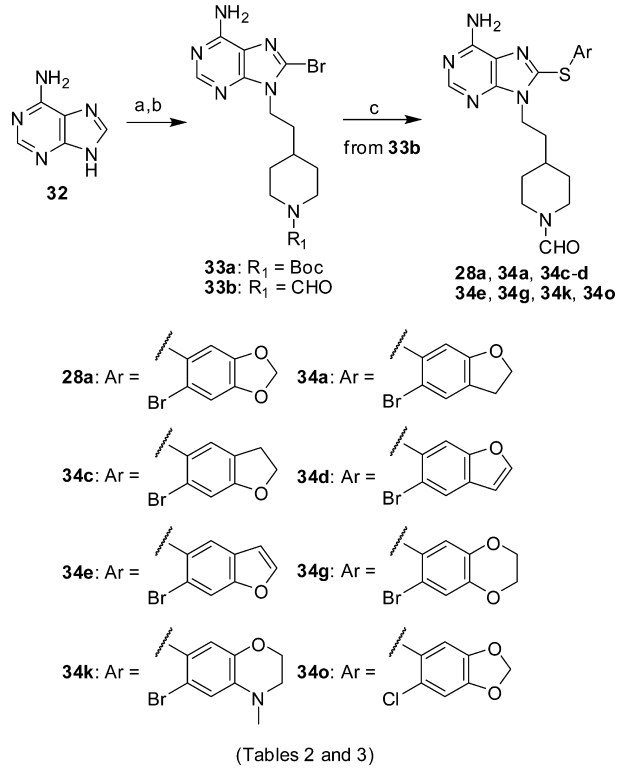
end, a solution of aryl sulfide **35** in THF was treated with NaOEt (2.2 equiv) and the resulting mixture was stirred at ambient temperatures for 2 h. After concentration to dryness, the crude sodium arylthiolate salt (ArSNa, **35**-Na) was diluted with a mixture of THF and ethanol (10/1, v/v), combined with bromoadenine **33a** (or **33b**), and heated at 60−65 °C for several hours. The reaction proceeded cleanly to yield product **37** in moderate to good yield after silica gel chromatography. Concentration of the sodium arylthiolate mixture before addition of **33a,b** is crucial for maximizing the yield of **37**. Otherwise, **37** further reacts with ethyl acrylate **36** (Alk = Et), generated via β-elimination of **35** and transesterification with ethanol, through an aza-Michael reaction resulting in the formation of varying amounts of side product **38**. By use of this protocol, aryl sulfides **43a−c**, **45**, **46**, **48**, **49**, or **51** were converted to the corresponding sodium thiolates (ArSNa), which upon reaction with bromoadenine **33b** afforded *N*-formylpiperidine analogues **28a**, **34a**, **34c**, **34d**, **34e**, **34g**, **34k**, and **34o** in good yields under mild conditions (Scheme 7). Intermediates **27a** and **39a−d** were prepared from **33a** in a similar fashion (Scheme 8). Deprotection of **27a** and **39a−d** with TFA gave piperidine intermediates **27b** and **40a−d**, respectively, which were conveniently transformed into target molecules **28a**, **28f−h**, **34b**, **34g−j**, and **34p** via EDCI-mediated coupling with appropriate carboxylic acids (Scheme 9).

A scalable, straightforward, and chromatography-free procedure for the preparation of **28a** and **28f−h** was also developed. By employment of the stable and readily available 1,3-benzodioxole-5-thiol (**53**), displacement of bromoadenine **33a** followed by bromination with NBS provided **27a**. After removal of the Boc group, **27b** could be converted into **28a** and **28f−h** by EDCI coupling with the appropriate carboxylic acid (Scheme 10).

RESULTS AND DISCUSSION

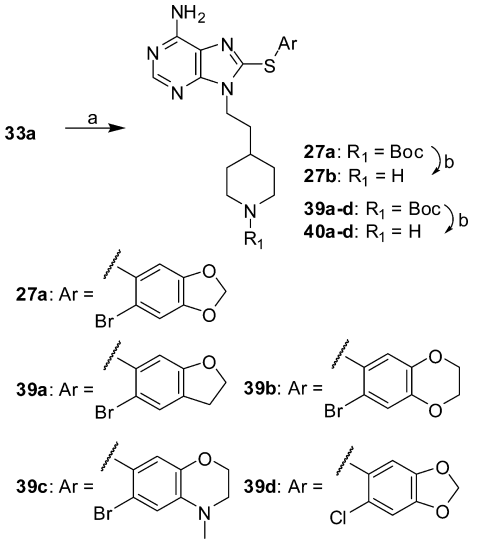
We began our study[52] by looking for a replacement for the N9 propargyl moiety present in inhibitor **54**[43] developed by the Chiosis group. Since phenyl groups can serve as bioisosteres of propargyl groups,[53] analogue **55b** (n = 2) was initially prepared and the in vitro potency was compared to other analogues having various alkyl chain lengths (zero to three carbon atoms). Consistently, phenethyl analogues **55a** (n = 1) demonstrated 3-

Scheme 7[a]



[a]Reagents and conditions: (a) **25a** or **25b**, Cs_2CO_3, DMF, rt, 16 h, ~80%; (b) Br_2, NaOAc−AcOH (pH 4.6), MeOH/THF, rt, 2 h, ~67%; (c) **43a−c**, **45**, **46**, **48**, **49**, or **51**, NaOEt, THF/EtOH (10/1, v/v), 60 °C, 3−10 h, 35−80%.

Scheme 8[a]



[a]Reagents and conditions: (a) **43a−c**, **45**, **51**, NaOEt, THF/EtOH (10/1, v/v), 60 °C, 3−10 h, 35−80%; (b) TFA or 4 M HCl in dioxane, CH_2Cl_2, rt, >90%.

to 6-fold more potent activity than homologues (e.g., **55b**) in the Hsp90 FP (fluorescence polarization) assay. With the two-carbon linking element considered optimal, varying substituents on the aromatic ring were explored, and it was found that electron-withdrawing groups (R = 2′-F, 2′-Cl, 2′-Br) at the aryl 2′-position were most potent giving IC_{50} values in the range

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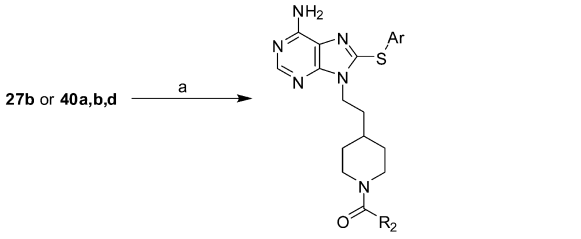
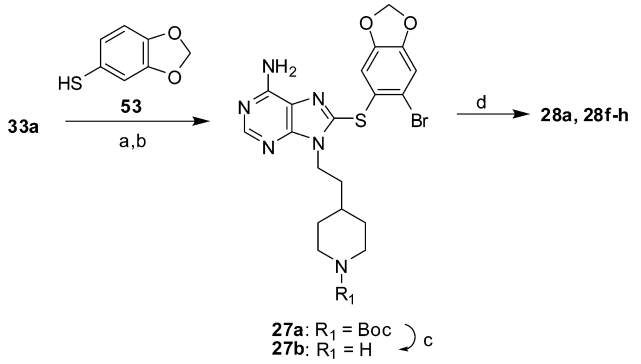
Scheme 9[a]



compd	Ar	R_2	compd	Ar	R_2
28a		H	34g		H
28f		OH	34h		OH
28g	Br	OH	34i	Br	OH
28h		OH	34j		OH
34b	Br	OH	34p	Cl	OH

[a]Reagents and conditions: (a) EDCI, HOBt, DMF−THF, R_2CO_2H (R_2 = H, CH_2OH, (*S* or *R*)-$CH(OH)CH_3$), rt, 10 h, 55−93%.

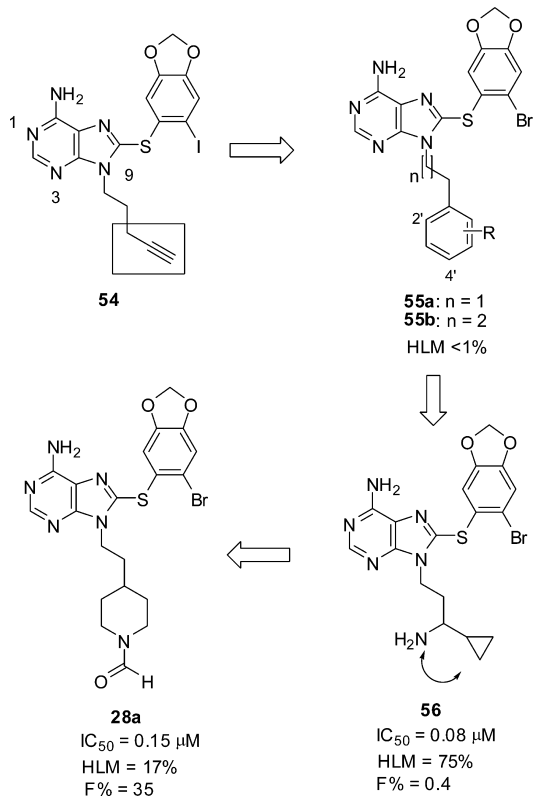
Scheme 10[a]



[a]Reagents and conditions: (a) K_2CO_3, DMF, 120 °C, 16 h, 94%; (b) NBS, AcOH, rt, 73%; (c) TFA, CH_2Cl_2, rt, >90%; (d) EDCI, HOBt, DMF, R_2CO_2H (R_2 = H, CH_2OH, (*S* or *R*)-$CH(OH)CH_3$), rt, 10 h, 55−93%.

30−50 nM in the Hsp90 FP assay. Potency in both the Her2-luciferase degradation and HCT116 antiproliferative assays was commensurate with the Hsp90 FP assay potencies (IC_{50}^{FP} = 0.04−0.05 μM; IC_{50}^{Her2} = 0.05−0.16 μM; IC_{50}^{HCT116} = 0.2−0.5 μM). However, metabolic stability (<1% parent drug remaining after 40 min of incubation with human liver microsomes (HLM)) was found to be a significant liability for these derivatives. Several structural modifications to the pendent aryl group were explored as a means of improving metabolic stability: blocking potential metabolic sites on the aromatic ring and/or benzylic position, reducing log *P* by incorporation of heteroaromatic ring systems (for example, pyridine, pyrrole, and imidazole), and bioisosteric substitution of the phenyl ring with thiophene. Unfortunately, none of these modifications proved to be successful and in most cases resulted in a significant potency loss, and consequently a suitable replacement for the phenethyl system was sought. To this end, the aryl moiety was replaced with an aminomethylcyclopropyl group (**55** → **56**, Scheme 11). Since increasing the cycloaliphatic ring size and modifications to the amino group failed to improve

Scheme 11



potency (IC_{50}^{FP} = 0.08 μM), the PK properties of aminomethylcyclopropyl analogue **56** were evaluated. Despite vastly improved water solubility (>100 μM at pH 7.4 in phosphate buffer) and metabolic stability, **56** demonstrated unacceptably poor PK including high clearance rates and extremely low oral bioavailability (F = 0.4%), presumably a consequence of low cellular permeability.

Efforts to improve oral bioavailability, while preserving inhibitory activity, led to replacement of the aminomethylcyclopropyl moiety in **56** with a 4-piperidine ring (**56** → **28a**, Scheme 11). As part of this strategy, *N*-formyl analogue **28a** was prepared and evaluated for both inhibitory potency and PK properties; the oral bioavailability was much improved (F = 35%) and the potency was acceptable (IC_{50}^{FP} = 0.15 μM; IC_{50}^{Her2} = 0.08 μM; IC_{50}^{HCT116} = 0.43 μM). The piperidine group has been frequently utilized during lead identification and optimization[54] and in this instance resulted in improved oral bioavailability while providing a versatile platform for the design of new analogues. Along these lines, subsequent work was focused on variations on the piperidine N-substituent with the ultimate goal of improving potency and ADME properties.

Unsubstituted piperidine analogue **27b** and *N*-alkyl substituted analogues **27c−f**, where R_1 is *i*-Pr, *i*-Bu, CH_2CF_3, CH_2-*c*-Hex, respectively, were prepared, and their potency was evaluated in the Hsp90 FP assay. As shown in Table 1, this modification resulted in diminished activity compared to **28a** (IC_{50}^{FP} = 0.15 μM), with IC_{50}^{FP} values of 0.47, 0.90, 0.70, 0.60, and 0.24 μM, respectively. On the other hand, *N*-propargyl analogue **27g** displayed similar biochemical (IC_{50}^{FP} = 0.11 μM) and cellular potencies (IC_{50}^{Her2} = 0.10 μM vs 0.07 μM and

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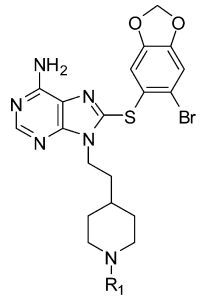
Table 1



Cmpd	R_1	IC_{50} (μM)				[b]HLM(%)
		FP[a]	Her2[a]	HCT116[a]	NCI-ADR-RES[a]	
28a	CHO	0.15	0.07	0.43	2.8	17
27b	H	0.47	0.95	4.6	>100	78
27c	*i*Pr	0.90	0.32	2.58	7.25	nd[c]
27d	*i*Bu	0.70	0.11	nd	1.98	nd
27e	CH_2CF_3	0.60	nd	nd	nd	nd
27f	CH_2-*c*Hex	0.24	0.25	1.00	0.722	10
27g	CH_2-CCH	0.11	0.10	0.74	0.70	10
27h		0.045	0.031	0.062	0.13	0
27i		1.8	nd	nd	nd	nd

[a]Average of at least two experiments. Assay error for FP is ±10% and ±30% for remaining assays. [b]Metabolic stability in human liver microsomes; percent parent remaining after 40 min of incubation. [c]Not determined.

IC_{50}^{HCT116} = 0.74 μM vs 0.43 μM, for **27g** and **28a**) to **28a**, but poor predicted metabolic stability (HLM = 10%) precluded its further evaluation in animals. As part of this SAR study, compound cytotoxicity was also determined on the drug-resistant ovarian tumor cell line NCI-ADR-RES, which overexpresses the P-glycoprotein 1 (P-gp1) drug efflux pump relative to the drug-sensitive parental cell line OVCAR8, which lacks P-gp1 overexpression. Compound potency, as determined by cytotoxicity using the drug-sensitive cell lines HCT116 or OVCAR8, was always similar. As a result, comparison of the cytotoxic potency between NCI-ADR-RES and HCT116 or OVCAR8 was used to indicate whether a test compound was a substrate for the P-gp1 drug efflux pump. Cytotoxicity in the NCI-ADR-RES system was found to be dependent on the steric bulk and lipophilicity of the piperidine nitrogen substituent and on the presence of a hydrogen bond donor atom.[55] Whereas cyclohexylmethyl and propargyl analogues **27f,g** were equipotent in both the HCT116 and NCI-ADR-RES assays, the free piperidine analogue **27b** lost complete potency in the latter assay ($IC_{50}^{NCI\text{-}ADR\text{-}RES} \geq 100$ μM). Similarly, isopropyl analogue **27c** showed a 2- to 3-fold decreased cytotoxicity in NCI-ADR-RES relative to HCT116. For tetrazole analogues **27h** and **27i**, the size of the *N*-alkyl group impacted inhibitory activity; *N*-methyltetrazole **27h** exhibited potent inhibitory activity in both the biochemical (IC_{50}^{FP} = 0.045 μM) and cell-based assays (IC_{50}^{Her2} = 0.031 μM; IC_{50}^{HCT116} = 0.062 μM; $IC_{50}^{NCI\text{-}ADR\text{-}RES}$ = 0.13 μM), whereas *N*-*tert*-butyltetrazole, **27i**, lost significant activity in the FP binding assay (IC_{50}^{FP} = 1.8 μM). Unfortunately, the poor metabolic stability of **27h** precluded further evaluation. Thus, further SAR studies were undertaken via synthesis of *N*-acylpiperidine analogues **28a−o** (Table 2).

As part of this study, other N9 heterocyclic systems were also explored with representative examples illustrated in Scheme 12. The position of the piperidine nitrogen and ring size were modified resulting in 3-piperidine **62**, 2-piperidine **63**, and 3-pyrrolidine **64** analogues that were similar to, or slightly less potent than, the corresponding 4-piperidine analogues (e.g., **63**, R = CHO, IC_{50}^{FP} = 0.18 μM for the *S*-isomer, IC_{50}^{FP} = 0.19 μM, for the *R*-isomer; **28a**, IC_{50}^{FP} = 0.15 μM). The 4-tetrahydropyran derivative **65** showed potency comparable to that of **28a** in the three main assays (IC_{50}^{FP} = 0.057 μM; IC_{50}^{Her2} = 0.09 μM; IC_{50}^{HCT116} = 0.59 μM). Not surprisingly, however, **65** demonstrated very low metabolic stability (HLM = 0%), likely a result of oxidative ring cleavage.[56] In an attempt to improve the metabolic stability of **65**, the known[57] ether bioisostere gem-difluorocyclohexane was incorporated providing **66**. This modification resulted in modestly improved metabolic stability (HLM = 15%) but with concomitant loss in potency ($IC_{50}^{FP} \approx 1.0$ μM). The oxa- and aza-bicyclic heterocyclic analogues **67a,b**, possessing carbon atoms adjacent to the heteroatoms, were also explored as a means of improving the metabolic stability of **65**. And while they were similar in potency to **65**, no improvement in metabolic stability was realized.

For continuation of SAR studies surrounding *N*-acyl analogues **28a−o**, three types of R_2 substituent were examined

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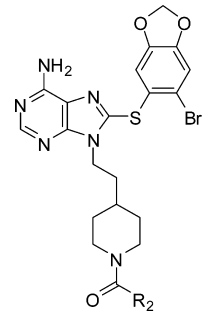
Table 2



Cmpd	R_2	IC_{50} (μM)				[b]HLM
		FP[a]	Her2[a]	HCT116[a]	NCI-ADR-RES[a]	
28a	H	0.15	0.07	0.43	2.8	17
28b	CH_3	0.06	0.09	0.73	3	17
28c	propyl	0.30	nd[c]	0.85	2	0
28d		0.11	0.11	0.65	0.93	1
28e		0.25	0.25	1.3	0.37	0
28f		0.11	0.06	0.38	2.0	54
28g		0.14	0.06	0.54	4.3	42
28h		0.11	0.09	0.64	3.3	58
28i		>5	nd	nd	nd	nd
28j		0.11	0.034	0.27	100	2
28k		0.11	0.20	1.20	100	69
28l		0.068	0.11	0.89	34	44
28m		0.18	0.96	1.6	50	nd
28n		0.07	0.08	0.20	100	nd
28o		0.06	0.19	0.83	89	38

[a]Average of at least two experiments. Assay error for FP is ±10% and ±30% for remaining assays. [b]Metabolic stability in human liver microsomes; percent parent remaining after 40 min of incubation. [c]Not determined.

(Table 2): (1) alkyl or cycloalkyl groups (**28b–e**); (2) alkyl or cycloalkyl groups containing an oxygen functionality (**28f–j**); (3) alkyl or cycloalkyl groups containing an amine functionality (**28k–o**). In the alkyl and cycloalkyl series, alkyl chain length and the ring size were both investigated. Conversion of the formyl group (**28a**, R_2 = H) to an acetyl (**28b**, R_2 = CH_3) resulted in no significant change in potency. Increasing the carbon chain length reduced potency ~2 fold (IC_{50}^{FP} = 0.15 vs 0.29 μM and IC_{50}^{HCT116} = 0.43 vs 0.85 μM, **28a** and **28c**, respectively), and the cyclopropyl analogue **28d** demonstrated potency comparable to that of the lead **28a** in biochemical and cell-based assays. Additionally, **28d** was not a MDR substrate as indicated by equipotency in the NCI-ADR-RES assay, but again, metabolic instability in human microsomal assays obviated further progression of this otherwise promising inhibitor. Inhibitory potency was found to decrease with increasing ring size as illustrated by cyclopentyl analogue **28e**, which was 2- to 3-fold less potent (IC_{50}^{FP} = 0.25 μM; IC_{50}^{Her2} = 0.25 μM; IC_{50}^{HCT116} = 1.3 μM) than the lead **28a**. Incorporation of a primary or secondary hydroxyl group into the R_2 substituent improved metabolic stability (HLM = 42–58% for **28f–h**) while maintaining potency (IC_{50}^{FP} = 0.11–0.14 μM; IC_{50}^{Her2} = 0.06–0.09 μM; IC_{50}^{HCT116} = 0.38–0.64 μM). No significant differences in potency and metabolic stability were observed among the hydroxyl substituted analogues as a group. Although this modification resulted in a moderate decrease in NCI-ADR-RES cytotoxicity, possibly due to hydrogen bonding capacity, they were advanced into PK

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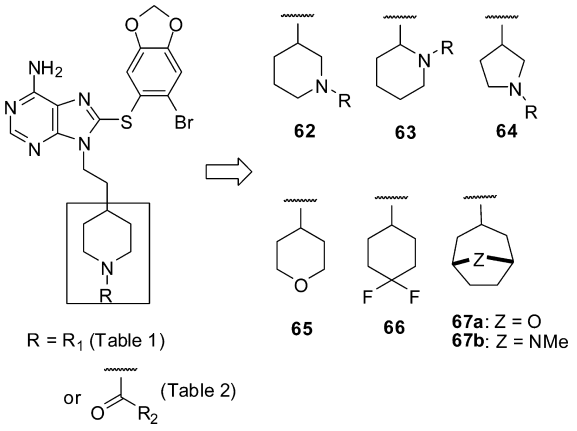
Scheme 12



evaluations given the improved balance between potency and microsomal stability. Interestingly, gem-dimethyl derivative **28i** lost all binding potency ($IC_{50}^{FP} \geq 5\ \mu M$). Propane-1,2-dione analogue **28j**, the oxidized form of **28g** and **28h**, demonstrated improved potency in both the biochemical and cellular assays ($IC_{50}^{FP} = 0.11\ \mu M$; $IC_{50}^{Her2} = 0.034\ \mu M$; $IC_{50}^{HCT116} = 0.27$) while losing all activity against NCI-ADR-RES ($IC_{50}^{NCI\text{-}ADR\text{-}RES} = 100\ \mu M$) and inheriting a poor metabolic profile.

The SAR was extended to amine containing analogues **28k–o**. In general, these analogues exhibited decreased potency in cell-based assays. In particular, the marked loss of potency in the NCI-ADR-RES cell line was observed regardless of substitution on the amino group. Tertiary dimethylamino analogue **28n** ($IC_{50}^{NCI\text{-}ADR\text{-}RES} \geq 100\ \mu M$) and sterically hindered proline analogue **28o** ($IC_{50}^{NCI\text{-}ADR\text{-}RES} = 34\ \mu M$) did not improve the cellular activity, suggesting both low cellular permeability and P-gp substrate susceptibility.

From the SAR studies described above, it was determined that modifications to the N9 piperidine substituent can significantly modulate both the potency and metabolic stability of these purine analogues and we concluded that the *N*-hydroxyacyl moieties present in analogues **28f–h** represented the optimal piperidine N-substituents, imparting the best balance between potency and metabolic stability.

The potential binding mode of these analogues has been examined by molecular modeling methods, and the best docking poses for all active compounds share common features. For example, from molecular modeling with **28g** and the Hsp90 N-terminal, ATP-binding domain reveals an interaction between the N9 piperidine ring and Ile110 side chain. In addition, H-bond donor–acceptor interactions between the lactamide carbonyl and Lys58 ε-amino group and between the lactamide hydroxyl and the Gly97 backbone carbonyl are key ligand–protein interactions (Figure 4).

By employment of N9-optimized analogues **28f–h** and initial lead **28a**, alternatives to the 6-bromo-1,3-benzodioxole moiety were explored next. The binding mode and preference for an *s-trans* conformation for this aryl moiety within the N-terminal ATP-binding pocket of Hsp90 are well documented.[58] As shown in Figure 4, our modeling with **28g** reveals a similar predisposition of the 6-bromo-1,3-benzodioxole moiety and, in addition to the conformational biasing imposed by the ligand bromine atom, points to a π–π interaction and edge-to-edge

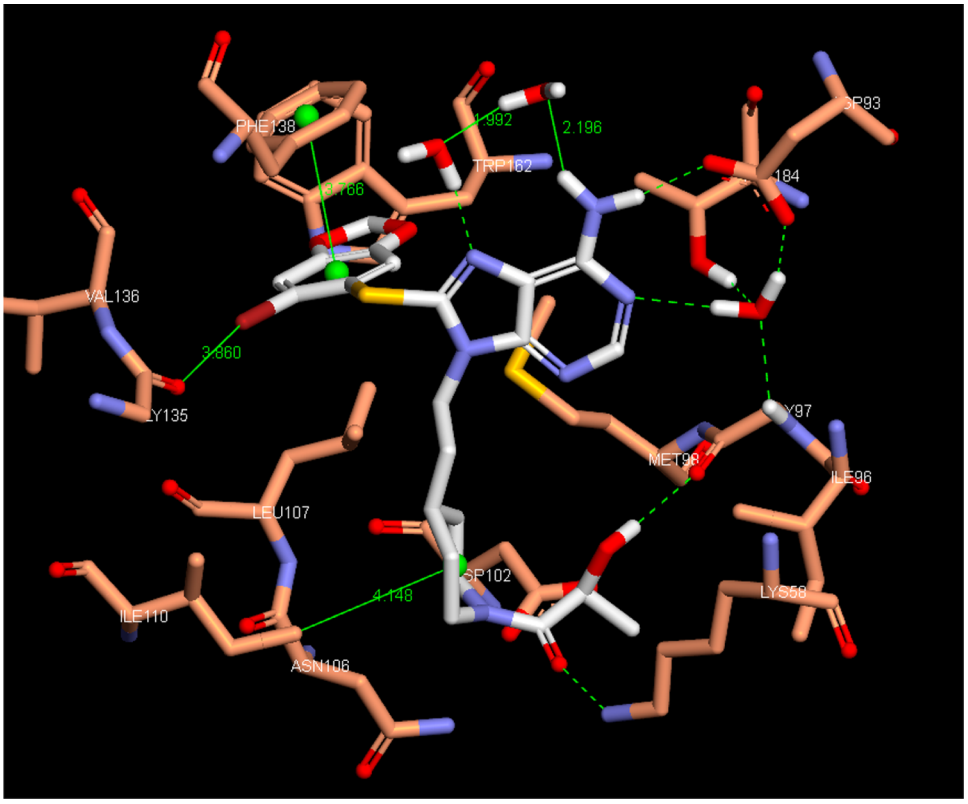


Figure 4. Putative binding mode of **28g** in the ATP-binding site of Hsp90 as determined by molecular modeling with Hsp90 crystal structure (PDB code 1UYI). Protein, ligand, and structured water molecules are rendered with Accelrys DS Visualizer 3.1. Atoms are colored by element: oxygen, red; nitrogen, blue; sulfur, green; ligand carbon, white; protein carbon, gold.

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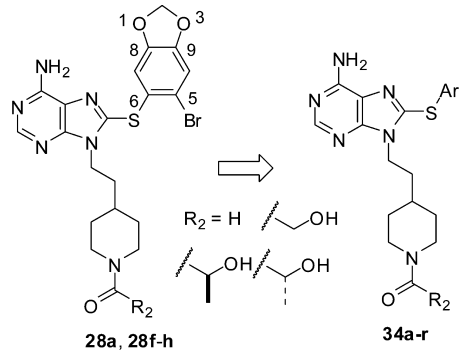
interaction with Phe138 and Trp162 and to a van der Waals interaction with the L107 isobutyl group as being major contributors to favorable binding energetics. Although a great deal of exploratory work has been performed in this region, these studies either were directed at a better understanding of the substituent effects at different positions of the 8-aryl group or were directed at replacement of the 8-aryl group with a benzothiazole ring.[33,34,43,59] On the basis of the information from the literature, we carried out further SAR studies on this region by employing congeners of the 6-bromo-1,3-benzodioxole moiety, including bioisosteres, in order to gain insight into the structural features required for optimal potency and physical properties (Table 3).[60−62]

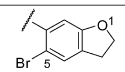
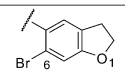
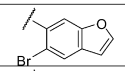
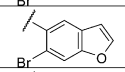
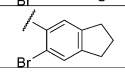
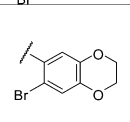
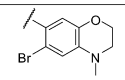
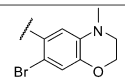
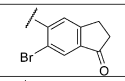
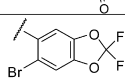
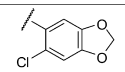
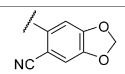
First, a series of dihydrobenzofuran and benzofuran analogues was compared. 5-Bromo-2,3-dihydrobenzofuran **34a** (IC_{50}^{FP} = 0.04 μM) proved to be ∼10 times more potent than 6-bromo-2,3-dihydrobenzofuran **34c** (IC_{50}^{FP} = 0.4 μM) in the FP assay. The results of the Her2 assay (0.01 μM, **34a**, and 0.28 μM, **34c**) and antiproliferative activities on HCT116 cells (0.09 μM, **34a**, and 1.2 μM, **34c**) were consistent with the FP results and supported an on-target mechanism of cytotoxicity. The 5-bromo-2,3-dihydroindole **34b**, (*S*)-2-methyl-*N*-hyroxyacylpiperidine at N9, demonstrated potency similar to that of **34a**. A similar trend was observed with benzofurans **34d** and **34e**, where **34d** was >10-fold more potent than its regioisomer **34e** in all four assays. Replacing both oxygen atoms in the benzodioxole ring with CH_2 provided indane **34f** which was found to be very potent with IC_{50} of 0.02 μM in both the FP and HCT116 antiproliferative assays. It was encouraging that the non-benzodioxole analogues **34a**,**b**, **34d**, and **34f** demonstrated comparable to improved activity relative to the corresponding 1,3-benzodioxole analogues. Noteworthy observations from this segment of the developing SAR were the following: (1) the benzodioxole oxygen atoms were not essential for binding affinity (cf., **28a** vs **34f**); (2) the effects of the oxygen atoms at the 1- or 3-position of the benzodioxole ring were not equal. The oxygen atom at the 3-position (para to the sulfur atom) was detrimental to potency, where in contrast the oxygen atom at the 1-position (meta to the sulfur atom) was tolerated (**34a** vs **34c**; **34d** vs **34e**). While additional studies are needed to better understand this phenomenon, it appears that binding affinity is influenced by both conformational preferences and ring system electronic properties; see, for example, difluororo-1,3-benzodioxole analogue **34n** which lost binding affinity (IC_{50}^{FP} > 5 μM). Interestingly and contrary to our expectations, the metabolic stability of **34n** was found to be very low (HLM = 0%). Since fluorine substitution at the methylene bridge of 1,3-benzodioxole ring systems can prevent oxidative metabolism,[63] difluoro-1,3-benzodioxole analogue **34n** was expected to be metabolically stable.[64] Although metabolite identification is needed to identify the origins of instability, it is tempting to speculate that the increased lipophilicity of **34n** (log *P* = 3.32, 3.42, 4.82, for **28g**, **28a**, **34n**, respectively) is a contributing factor.

To further explore the effects of ring size, benzodioxanes **34g−j** were compared to the corresponding benzodioxoles **28a** and **28f−h**. In general, benzodioxanes **34g−j** had potency similar to that of the corresponding benzodioxoles in the FP assay but were slightly less potent in cell-based assays. Isomeric benzoxazines **34k** and **34l** demonstrated similar SAR trends, as evidenced by dihydrobenzofuran and benzofuran analogues **34a−e**. Thus, benzoxazine **34k**, with the oxygen atom meta to the sulfur atom, was 4- to 6-fold more potent than its isomer

Table 3



28a, **28f-h** **34a-r**

Cmpd	R_2	Ar	IC_{50} (μM)				[b]HLM
			FP[a]	Her2[a]	HCT116[a]	NCI-ADR-RES[a]	
34a	H		0.04	0.01	0.09	1.80	nd
34b			0.034	0.028	0.21	11	21
34c	H		0.4	0.28	1.2	86	5
34d	H		0.059	0.048	0.26	1.1	53
34e	H		0.9	0.66	4.4	7.9	51
34f	H		0.02	nd	0.02	0.23	1
34g	H		0.06	0.049	0.67	2.1	42
34h	CH_2OH		0.09	0.04	0.82	10	45
34i			0.15	0.074	0.47	6.5	45
34j			0.11	0.069	0.92	9.8	35
34k	H		0.065	0.032	0.25	100	nd[c]
34l	H		0.31	0.21	1.5	20	0
34m	H		0.06	0.06	0.27	100	30
34n	H		5	nd	nd	nd	0
34o	H		0.21	0.092	1.4	7.6	31
34p			0.65	0.54	2.7	20	32
34q	H		0.21	0.25	25	nd	81

[a]Average of at least two experiments. Assay error for FP is ±10% and ±30% for remaining assays. [b]Metabolic stability in human liver microsomes; percent parent remaining after 40 min of incubation. [c]Not determined.

34l (IC_{50}^{FP} = 0.065 and 0.31 μM for **34k** and **34l**; IC_{50}^{HCT116} = 0.25 and 1.5 μM for **34k** and **34l**). Indanone **34m**, a nonclassical bioisostere of benzodioxole **28a**,[61] exhibited good potency in the FP (IC_{50}^{FP} = 0.06 μM) and cytotoxicity assays (IC_{50}^{Her2} = 0.06 μM; IC_{50}^{HCT116} = 0.27 μM). The non-benzodioxole analogues were subsequently evaluated for metabolic stability. Inhibitors **34a−c** and **34f**, bearing benzylic methylene protons, and benzoxazines **34k**,**l** exhibited rapid metabolism upon incubation in human microsomes, and

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Table 4. Pharmacokinetic Parameters of 28a, 28f–h, and 34d,g–j in Mice

	iv (2.5 mg/kg)[a]				po (10 mg/kg)[b]			
compd	CL (mL h^{-1} kg^{-1})	V (mL/kg)	$t_{1/2}$ (h)	AUC (h·ng/mL)	C_{max} (ng/mL)	T_{max} (h)	AUC (h·ng/mL)	F (%)
28a	1899	1452	0.5	1316	354	1.0	1808	35
28f	4359	3005	0.5	574	588	0.25	1318	57
28g	1187	2438	1.4	2106	2802	0.25	3214	38
28h	1513	1321	0.6	1653	1429	0.25	2410	37
34g	2585	1053	0.28	967	312	1.0	1175	30
34h	4833	5927	0.85	517	55	0.25	702	34
34i	1712	1070	0.43	1460	564	0.5	1193	20
34j	1639	1347	0.6	1526	1183	1	1960	32
34d	2841	1058	0.3	880	2231	0.5	4612	131

[a]Formulation: DMA/PEG300/EtOH/H_2O (3/40/12/45, v/v/v/v). [b]Formulation: DMA/PEG300 (5/95, v/v).

benzodioxanes **34g–j**, benzofurans **34d,e**, and indanone **34m** displayed stability similar to that of benzodioxoles **28a** and **28f-h**. On the basis of their equivalent potency and metabolic stability to **28a** and **28f–h**, benzodioxanes **34g–j** and benzofuran **34d** were advanced into PK evaluations. Indanone **34m** was excluded from PK analyses because of lack of potency in NCI-ADR-RES ($IC_{50}^{NCI\text{-}ADR\text{-}RES} \geq 100\ \mu M$).

Analogues **34o–q**, where the 1,3-benzodioxole ring bromine atom was replaced with a chloro or cyano, were also examined. Not surprisingly and in accord with previous studies, replacement of Br with Cl led to a ~2-fold decrease in potency.[43,58] Although cyano derivative **34q** showed significantly enhanced metabolic stability (HLM = 81%), the cellular potency dropped unacceptably by 4- to 46-fold ($IC_{50}^{Her2} = 0.25\ \mu M$; $IC_{50}^{HCT116} = 25\ \mu M$).

Ultimately, nine inhibitors, **28a**, **28f–h**, and **34d,g–j** were selected for intravenous (2.5 mg/kg) and oral (10 mg/kg) PK evaluations in Swiss nude mice (Table 4).

Several interesting observations were made as result of these initial PK evaluations. First, 1,3-benzodioxole analogues **28a** and **28f–h** demonstrated favorable PK profiles in comparison to the corresponding 1,4-benzodioxanes **34g–j** (**28a** vs **34g**; **28f** vs **34h**; **28g** vs **34i**; **28h** vs **34j**). Second, compounds **28f** and **34h**, possessing *N*-hydroxyacetyl substituents on the piperidine nitrogen, suffered from high hepatic clearance (>4300 mL h^{-1} kg^{-1})[65] and concomitantly low exposure. Third, inhibitor **28g** displayed superior oral PK profiles with a high C_{max} (2802 ng/mL, ~5.1 μM), good overall exposure (3214 h·ng/mL), and a reasonable hepatic clearance rate. Fourth, most compounds showed rapid absorption following oral administration in mice (T_{max} = 0.25–1 h). Finally, the quantitative oral bioavailability (F > 100%) of benzofuran **34d** was entirely unexpected given the structural similarity of these purine analogues. Oral bioavailability values of >100% have been reported in literature, and the phenomenon has several contributing factors.[66] Regardless of mechanism, the uncharacteristic PK behavior for **34d** led us to eliminate it from further study. For purposes of triaging compounds into human tumor xenograft studies, the mouse PK (100 mg/kg, oral, Table 5) of the eight remaining compounds was evaluated. In the PK study for these analogues, less than expected increases in AUC and C_{max} were observed in general. This effect is possibly due to the dissolution rate or solubility-limited absorption. Most of these analogues belong to BCS (biopharmaceutics classification system) class II. For example, **28g** is modestly soluble (~20 μM at pH 7.4 in phosphate buffer) but highly permeable (>350 x10^{-6} cm/s, PAMPA assay). Issues relating to the solubility and absorption properties of drug candidates are frequently encountered during the drug discovery process, and this compound class could be addressed by formulation.[67] In this instance, formulation in a Captisol (modified cyclodextrin) and phosphate buffer system enhanced oral bioavailability dramatically (e.g., F > 50%, for **28a,f–h** and **34g–j**).

Table 5. Pharmacokinetic Parameters of 28a, 28f–h, and 34g–j (po, 100 mg/kg)[a]

compd	C_{max} (ng/mL)	AUC (h·ng/mL)	Hsp70 induction[b]
28a	10940	63900	47
28f	7780	25100	18
28g	13100	81800	53
28h	8400	50300	24
34g	8540	38000	2.3
34h	7530	18200	1.7
34i	11600	40400	3.2
34j	9300	32600	3.8

[a]Formulation: 40% Captisol in phosphate buffer. [b]Fold over control.

In these PK studies, HSP70 mRNA induction in liver at 6 h after treatment was also measured. HSP70 is robustly upregulated in response to Hsp90 inhibition and is a validated pharmacodynamics biomarker. Induction of HSP70 indicates that the drug is both reaching the intended target site and exerting pharmacological effects via the Hsp90 mechanism. Although the effects of Hsp90 inhibitors on HSP70 induction are expected to be different in tumors and normal cells or tissues,[68,69] this experiment would provide early evidence of target modulation in vivo.

In accord with the previous PK studies (Table 4), **28g** showed the best PK profile with a C_{max} of 13100 ng/mL (~23 μM) and an AUC of 81 800 h·ng/mL with excellent oral bioavailability (97%). The C_{max} was 20- to 60-fold higher than IC_{50} values in representative cancer lines in vitro (Table 6). Initial lead compound **28a** also exhibited a profile fairly similar

Table 6. Antiproliferative Activity for 28g

cell line	IC_{50} (μM)[a]	cell line	IC_{50} (μM)[a]
HT-29 (colon)	0.42	A549 (NSCLC)	0.77
HCT-116 (colon)	0.54	OVCAR8 (ovarian)	0.49
LNCaP (prostate)	0.51	NCI-N87 (gastric)	0.20
DU-145 (prostate)	0.53	BT-474 (breast)	0.55
NCI-H69 (SCLC)	0.15	MV4–11 (leukemia)	0.25

[a]Average of two or more experiments; standard deviation was ±30%.

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to that of **28g**. Benzodioxane analogues **34g–j** had inferior AUC and C_{max} values relative to the corresponding 1,3-benzodioxaole analogues. Interestingly, only 2- to 4-fold induction of liver HSP70 levels was observed for benzodioxane analogues **34g–j** whereas an 18- to 53-fold induction was achieved with 1,3-benzodioxoles **28a** and **28f-h**. This phenomenon may reflect differences in cellular potency or PK properties. However, the similarities in potency and PK of benzodioxole **28f** and benzodioxazines **34g–j**, the former showing 5- to 10-fold higher HSP70 induction than the latter, suggest that other factors are likely in play. Both the PK and HSP70 induction profiles made analogue **28g** the most promising candidate for further development.

With benzodioxole **28g** demonstrating optimal PK properties, its antiproliferative activity against an array of cancer cell lines was profiled and revealed broad spectrum activity with IC_{50} values ranging from 0.1 to 0.8 μM (Table 6). In order to confirm the cellular mode of action of **28g**, HCT-116, NCI-N87, and DU-145 cells were exposed to varying concentrations of **28g** for 24 h and the cell lysates were probed with antibodies to Her2, Akt, and Hsp70. As anticipated, Western blot analysis of **28g** in NCI-N87, HCT-116, and DU-145 cell lysates revealed dose-dependent client protein degradation (Akt and Her2) and Hsp70 induction (Figure 5). Importantly, the IC_{50}

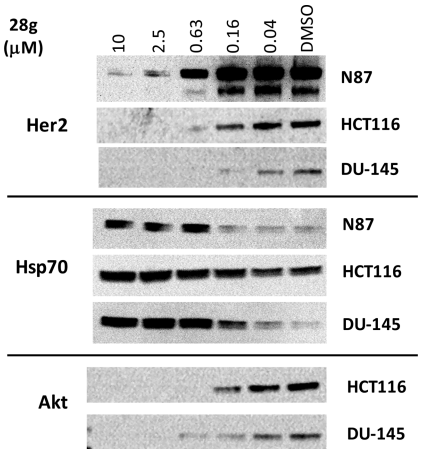

Figure 5. Effect of **28g** on Hsp90 client protein (Her2, Hsp70, and Akt) expression in NCI-N87, HCT116, and DU-145 cell lines by Western blot analyses. Cells were exposed to varying concentrations of **28g** for 24 h, and then cell lysates were probed with Her2-, Hsp70-, and Akt-specific antibodies.

values for client protein degradation (0.1–0.5 μM) were commensurate with cellular cytotoxicity, suggesting a direct relationship between biomarker response and drug exposure. The change in client protein and biomarker levels observed in cells exposed to **28g** provided evidence that the observed cellular cytotoxicity was a result of Hsp90 inhibition.

On the basis of its favorable in vitro profiles and PK properties, **28g** was evaluated in a human xenograft study in mice (Figure 6). In the NCI-N87 gastric cancer xenograft model, **28g** was administered orally for 22 days at doses of 50, 75, 125, or 200 mg/kg and demonstrated dose-related inhibition of tumor growth. The 50 and 75 mg/kg doses resulted in tumor growth inhibition ($p < 0.01$) of 37% and 62%, respectively, and the 125 and 200 mg/kg doses resulted in tumor regression ($p < 0.0001$) of 10% and 48%, respectively. Both the dose level and schedule were well tolerated as evidenced by lack of significant weight loss. Furthermore, robust HSP70 induction in liver and tumor tissue supports on-target, Hsp90-mediated antitumor effects by **28g** in NCI-N87 tumor-bearing mice (Figure 6b).

CONCLUSION

An extensive SAR study aimed at enhancing potency and druglike properties of lead Hsp90 inhibitor **28a** was executed. During the course of these studies, new and improved synthetic methods for C–S bond formation were developed that enabled rapid generation of potential inhibitors and dramatically broadened the scope of the SAR. Through a combination of modifications to the pendent purine N-9 piperidine moiety and congeneric series of 1,3-benzodioxole ring at C8, several potent Hsp90 inhibitors were identified. Pharmacokinetic analyses led to selection of **28g** as the most promising drug candidate. Following oral administration of **28g** to mice (100 mg/kg), the plasma C_{max} (~23 μM) was 20- to 60-fold higher than its IC_{50} values for cancer cell lines in vitro and with good systemic exposure (AUC = 81 800 h·ng/mL). Importantly, inhibitor **28g** showed significant antitumor effects in a human cancer xenograft model with concomitant and robust Hsp70 mRNA induction, a pharmacodynamic marker of Hsp90 inhibition.

Compound **28g** (MPC-3100) has completed a phase I clinical evaluation in refractory, or recurrent, cancer patients. This clinical study demonstrated that **28g** was safe and tolerated when administered at doses below 600 mg per day with the most common side effects being of gastrointestinal origin, including diarrhea, nausea, and vomiting.[70] The results of further clinical evaluation will be reported in due course.

EXPERIMENTAL SECTION

Biology. Binding Assay. Compound binding to purified Hsp90 was monitored using BODIPY-labeled geldanamycin (BODIPY-GM) in a fluorescence polarization assay adapted from the literature method.[71] Compound dilutions (in 100% DMSO) were added to black-bottom 96-well plates (Greiner, 2% DMSO final), and equal volumes of BODIPY-GM (10 nM final) and purified human Hsp90 (Stressgen, SPP-770; 30 nM final) in assay buffer (20 mM HEPES–KOH, pH 7.3, 50 mM KCl, 5 mM $MgCl_2$, 20 mM Na_2MoO_4, 0.01% NP-40, 0.1 mg/mL bovine γ-globulin [Invitrogen, P2045], 2 mM DTT) were added sequentially to yield a final volume of 50 μL. Plates were incubated overnight at room temperature. Parallel and perpendicular fluorescence measurements were read (LJL BioSystems Analyst AD plate reader) with excitation and emission wavelengths of 485 and 530 nm, respectively. Background fluorescence (buffer only) was subtracted, and fluorescence polarization (FP) values, expressed in mP units, were calculated from parallel and perpendicular fluorescence readings as follows: FP = [(parallel − perpendicular)/(parallel + perpendicular)] × 1000. Percent inhibition was calculated by normalizing the FP values to those obtained in parallel reactions containing DMSO and subtracting these normalized values from 100%. Intrinsic compound fluorescence was independently monitored, and FP data points confounded by compound fluorescence were excluded from the analysis.

Her2-Luciferase Degradation Assay. The Her2-luciferase construct was constructed by joining sequences encoding the kinase domain (aa 641–975) of Her2 to those encoding firefly luciferase.[72,73] HCT116 cells stably transfected with the Her2 (kinase domain)-luciferase fusion were seeded into black 96-well plates at 10 000 cells per well in 100 μL (DMEM supplemented with 10% serum) and incubated overnight. Compound dilutions (in 100% DMSO) were added to individual wells (0.4% DMSO final), and plates were incubated for 4 h. Plates were equilibrated to room temperature (5 min), 100 μL of Steady-Glo reagent (Promega no. E2520) was added

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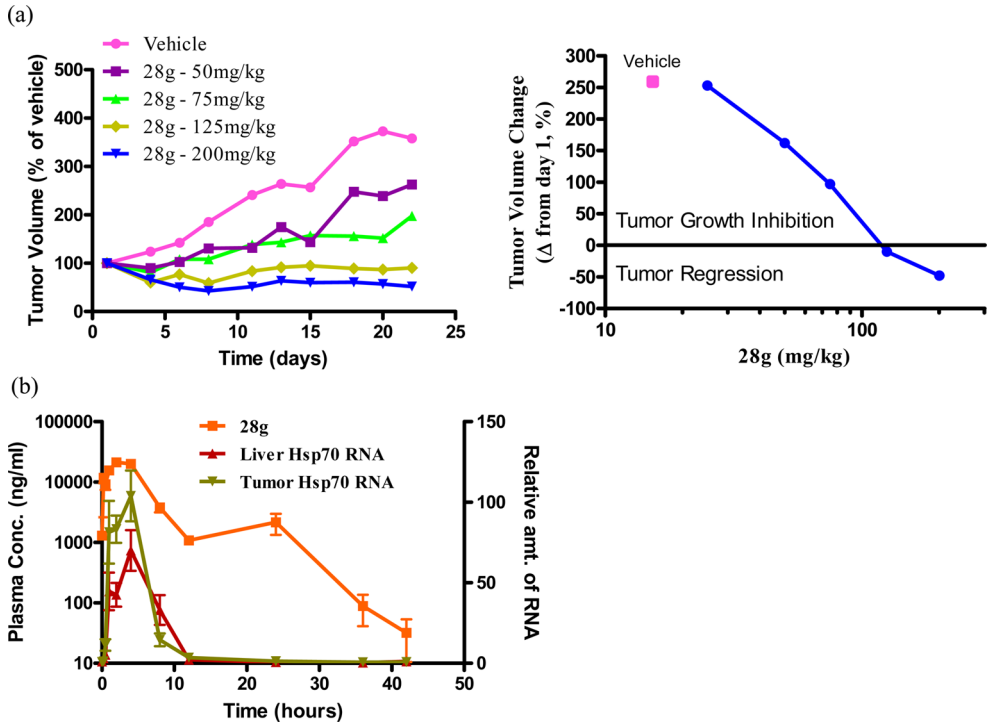

Figure 6. (a) In vivo efficacy data for **28g** (50, 75, 125, or 200 mg/kg, q.d., po 22 days) in the NCI-N87, Her2$^+$ human gastric cancer xenograft model. (b) Change in HSP70 expression level in NCI-N87 tumor-bearing mice. Mice with NCI-N87 tumors (median volume, ~425 mm^3) were given a single oral dose of **28g** (200 mg/kg). Blood, tumor, and liver samples (n = 6) were collected at 0.25, 0.5, 1, 2, 4, 8, 12, 36, and 48 h after dose for determination of compound concentrations in plasma and Hsp70 RNA levels in tumor and liver.

per well, and plates were incubated at room temperature for 5 min. Luminescence was then measured (TopCount, Perkin-Elmer).

Cytotoxicity Assay. Cells were seeded into black 96-well plates at 5000 cells per well in 100 μL (DMEM supplemented with 10% serum) and incubated overnight. Compound dilutions (in 100% DMSO) were added to individual wells (0.4% DMSO final), and plates were incubated for 72 h. Plates were equilibrated to room temperature (5 min). An amount of 50 μL of lysis buffer followed by 50 μL of substrate solution (ATPLite [two-step], Perkin-Elmer, no. 601941) was added to each well, and plates were incubated at room temperature for 5 min. Luminescence was then measured (TopCount, Perkin-Elmer).

Microsomal Stability Assays. Metabolic stability of the test article was assessed in liver microsomes from multiple species (e.g., mouse, rat, dog, monkey, or human) with and without NADPH. Concentrations of test article were determined by high-performance liquid chromatography combined with electrospray ionization tandem mass spectrometry. The test article was incubated in duplicate in the presence of microsomes diluted to 0.5 mg/mL of total protein in 100 mM potassium phosphate buffer (pH 7.4). NADPH-dependent metabolism was initiated with the addition of 1 mM NADPH. Aliquots of sample were removed at 0, 10, 20, and 40 min after NADPH addition, and the reaction was stopped by addition of acetonitrile. Following centrifugation, supernatants were dried under a stream of nitrogen and reconstituted in an appropriate solvent. Samples were quantified by determining peak area from LC−MS/MS analysis. Typically, analytes were ionized by positive-ion electrospray ionization and detected by multiple-reaction monitoring on an ABI Q-Trap mass spectrometer. Peak areas of the duplicates at the various time points were integrated, and the percent of compound remaining at 10, 20, and 40 min was compared to the peak area at 0 min. "No NADPH" control samples were incubated for 40 min and analyzed in the same manner.

PAMPA Permeability Assay. Permeability of a test compound was determined using the double-sink parallel artificial membrane permeability analysis (PAMPA) method (pION Inc.). Donor solutions were prepared at three pH levels of 5.0, 6.2, and 7.4. pION's ASB 7.4 buffer was placed in the permeation plate, and the filter plate was painted with a phospholipid mixture dissolved in dodecane. Test compounds were then added to the filter plate, which was then placed on top of the permeation plate to form the PAMPA sandwich. The sandwich was incubated for 30 min at 37 °C with a mixing speed equivalent to an aqueous boundary layer of 40 μm. Spectra were collected from 250 to 490 nm (Spectra-Max, Molecular Devices). Permeability was calculated from the spectra using pION's software. Verapamil was analyzed at the same time and served as positive control.

Pharamacokinetic Studies. All pharmacokinetic studies of test articles were conducted in mice. Plasma and tissue concentrations of the test article were quantified by LC−ESI-MS/MS using an internal standard and calibration curve with quality control samples. Animals were dosed intravenously or orally. The basic pharmacokinetics of the test article was determined in female CD-1 or Nu$^{\pm}$ mice dosed at 2.5 mg/kg intravenously. The dosing vehicle consisted of DMA/PEG300 (*N*,*N*-dimethylacetamide, polyethylene glycol 300, ethanol, and water in a ratio of 3/40/12/45, respectively). Three or five animals were used per time point, and the time points collected were 5, 15, 30, 60, 120, 240, 480, and 720 minutes after administration. Blood samples were collected through cardiac puncture, and plasma was obtained with EDTA as the anticoagulant. Plasma was submitted for bioanalysis (described below). For oral dosing, the test article was administered via oral gavage formulated in one of the following: DMA/PEG300 (*N*,*N*-dimethylacetamide and polyethylene glycol 300 in a ratio of 5/95 or 10/90), CMC/Tween 80 (2% carboxymethylcellulose, low viscosity with 0.05% Tween 80 in HPLC grade water), or 40% Captisol in phosphate. Concentrations of the test article were determined by adding 10 μL of internal standard to 50 μL of sample and then precipitating protein with 1 mL of acetonitrile. The supernatant was injected onto a reverse-phase HPLC column. Separation was achieved by gradient elution. Analytes were ionized by positive-ion electrospray ionization and detected by multiple-reaction monitoring on an ABI Q-Trap. Peak area ratios of the test article and internal standard were compared to those from a multipoint standard curve spanning a range from 10 to 10 000 ng/mL. To

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confirm accuracy, quality control samples were analyzed in duplicate at four different concentrations. Bioanalytical run acceptance required that at least three-fourths of the calibration standards were within 15% of theoretical and that greater than $^2/_3$ of all quality control samples were within 25% of theoretical. Plasma concentration data were fit with WinNonLin using noncompartmental analysis.

In Vivo Xenograft Study (NCI-N87 Xenograft). Five million NCI-N87 cells were implanted subcutaneously in the right flank of female nude mice (Crl:NU-Fox1nu). When the median tumor volume was approximately >100 mm^3, mice were randomized into 5 cohorts of 10 animals each. Cohort 1 was dosed once daily with vehicle (40% w/w Captisol), cohort 2 with 200 mg/kg, **28g** once daily, cohort 3 with 125 mg/kg, **28g** once daily, cohort 4 with 75 mg/kg, **28g** once daily, and cohort 5 with 50 mg/kg, **28g** once daily. All cohorts were dosed for 22 days. Tumor measurements and body weight determinations were performed a minimum of 2 times weekly.

Chemistry. All solvents and reagents were purchased from commercial sources and used without further purification unless otherwise noted. Reactions were monitored by TLC (thin layer chromatography) using 0.25 mm silica gel 60 F254 plates purchased from EMD chemicals or LC−MS (ESI) using an Agilent 1100 series LC/MSD ion-trap instrument with an Xterra MS C18 (Waters) 4.6 mm × 50 mm, 5 μm column. Purification was performed with either ISCO CombiFlash (Foxy Jr. fraction collector, UA-6 detector) or a reverse phase HPLC (RP-HPLC) on an Agilent 1100 Prep-LC with various columns. ^{1}H NMR spectra were recorded on a Bruker Mercury 400 MHz instrument. Proton chemical shifts are expressed in parts per million (ppm) relative to TMS and calibrated using residual undeuterated solvent as internal reference. High-resolution mass spectra (HRMS) were recorded on an Agilent 6210 TOF LC−MS instrument using electronspray ionization (ESI). GC−MS analysis was performed on an Agilent Technology 6890N. Determination of the enantiomeric purity of chiral compounds was performed on a Chiralcel OJ-RH column, using 100% MeOH with 0.01% TEA (isocratic, 0.5 mL/min). Compound purity was determined by an Agilent HP1100 instrument with a 4.6 mm × 150 mm Xterra C18 3.5 μm column. The flow rate was 1.2 mL/min, and the injection volume was 5 μL. HPLC conditions were as follows: mobile phase A, HPLC grade water (0.01% TFA); mobile phase B, HPLC grade acetonitrile (0.01% TFA); UV detector, 203 and 280 nm; gradient, 95% A/5% B to 0% A/100% B in 10 min, 100% B in 10−11 min, 100% B to 95% A/5% B in 11−13 min, 95% A/5% B in 13−15 min. All final compounds were ≥95% pure by HPLC at both 203 and 280 nm unless otherwise stated.

Intermediates **24a−c,f,g**, **25a**, **29**, **30a,b**, **41**, **42**, **47**, and **50** were purchased from commercial vendors, and **24d,e** were prepared by adapting literature procedures (in Supporting Information). The log *P* value was calculated using Chemdraw software.

8-[(6-Bromo-1,3-benzodioxol-5-yl)sulfanyl]-9*H*-purin-6-amine (26a).[33] To a 250 mL round-bottomed flask was added 8-mercaptoadenine (**21**) (2.00 g, 12.0 mmol), 5,6-dibromobenzo[1,3]-dioxole (**24a**) (6.70 g, 24.0 mmol), Pd_2dba_3 (0.548 g, 0.599 mmol), xantphos (0.693 g, 1.20 mmol), K_2CO_3 (3.31 g, 24.0 mmol), and dry, degassed dioxane (25 mL). The mixture was heated at 100 °C for 16 h under nitrogen, cooled to room temperature, filtered, and washed with a mixture of CH_2Cl_2, EtOAc, and MeOH (2/2/0.5 (v/v/v)). The combined filtrates were concentrated in vacuo. The dark brown residue was purified by chromatography on SiO_2 (CH_2Cl_2/EtOAc/MeOH, 2/2/0.5), and subsequent recrystallization from MeOH provided **26a** (1.4 g, 32%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (s, 1H), 7.38 (s, 1H), 7.23 (s, 1H), 6.12 (s, 2H); LC−MS [M + H$^+$] 365.9; HPLC purity, 94.2% (t_R = 4.20 min).

5-[(6-Amino-9*H*-purin-8-yl)thio]-6-bromoindan-1-one (26f). The title compound was prepared in 24% yield from **21** and **24f** in a manner similar to that described for **26a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.87 (s, 1H), 7.58−7.44 (brs, 2H), 7.14 (s, 1H), 2.95−2.91 (m, 2H), 2.63−2.60 (m, 2H); HRMS (ESI-TOF) calcd for $C_{14}H_{10}BrN_5OS$ [M + H$^+$] 375.9862, found 375.9857.

6-[(6-Amino-9*H*-purin-8-yl)thio]-1,3-benzodioxole-5-carbonitrile (26g). The title compound was prepared in 15% yield from **21** and **24g** in a manner similar to that described for **26a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (s, 1H), 7.59 (s, 1H), 7.29 (s, 1H), 7.26−7.16 (brs, 2H), 6.23 (s, 2H); HRMS (ESI-TOF) calcd for $C_{13}H_8N_6O_2S$ [M + H$^+$] 313.0502, found 313.0509.

***tert*-Butyl 4-{2-[(Methylsulfonyl)oxy]ethyl}piperidine-1-carboxylate (25a).** To a solution of **30a** (15.0 g, 65.4 mmol) in CH_2Cl_2 (100 mL) were added MsCl (11.0 mL, 131 mmol) and NEt_3 (23 mL, 164.0 mmol) at ∼5 °C, and the mixture was stirred at room temperature for ∼16 h. Upon completion, the mixtue was diluted with CH_2Cl_2 (100 mL) and washed with water (100 mL × 2), dried (Na_2SO_4), filtered, and concentrated in vacuo to provide **25a** (20.0 g) in quantitative yield. LC−MS [M + H$^+$] 347.1.

2-(1-Formyl-4-piperidyl)ethyl 4-Methylbenzenesulfonate (25b). To a solution of **30b** (3.00 g, 19.1 mmol) in CH_2Cl_2 (50 mL) were added NEt_3 (8.00 mL, 57.3 mmol) and DMAP (23 mg, 0.19 mmol), followed by TsCl (3.64 g, 19.1 mmol). After being stirred for 10 h at room temperature, the mixture was diluted with CH_2Cl_2, washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification of the residue by column chromatography (SiO_2, EtOAc/hexane, 50−100%) provided **25b** (2.22 g, 37%). LC−MS [M + H$^+$] 311.9.

2-{1-(1-Methyl-1*H*-tetrazol-5-yl)piperidin-4-yl}ethyl 4-Methylbenzenesulfonate (25h). To a solution of **29** (864 mg, 6.70 mmol) in CH_2Cl_2 (22 mL) was added methyl isothiocyanate (490 μL, 6.70 mmol). After being stirred for 10 h at room temperature, the mixture was concentrated in vacuo and the residue was purified by SiO_2 column chromatography (EtOAc) to provide **31a** (1.04 g, 77%). To a mixture of **31a** (1.63 g, 8.07 mmol), $HgCl_2$ (2.41 g, 8.88 mmol), and NaN_3 (1.57 g, 24.2 mmol) in DMF (20 mL) was added NEt_3 (3.37 mL, 24.2 mmol). After being stirred for 10 h at room temperature, the reaction mixture was filtered and the filter cake was washed with CH_2Cl_2. The combined filtrates and washings were washed with brine, dried (Na_2SO_4), filtered, concentrated in vacuo, and the residue purified by SiO_2 chromatography (60% EtOAc in hexane) to afford 2-{1-(1-methyl-1*H*-tetrazol-5-yl)piperidin-4-yl}-ethanol (0.720 g, 42%). LC−MS [M + Na$^+$] 234.0. **25h** (0.417 g, 80%) was obtained from 2-{1-(1-methyl-1*H*-tetrazol-5-yl)piperidin-4-yl}ethanol (0.399 g, 1.42 mmol) in a manner similar to that described for **25a**. HRMS (ESI-TOF) calcd for $C_{16}H_{23}N_5O_3S$ [M + H$^+$] 366.1594, found 366.1593.

4-[2-[6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)sulfanyl]-purin-9-yl]ethyl]piperidine-1-carbaldehyde (28a). To a solution of **26a** (1.00 g, 2.73 mmol) and tosylate **25b** (1.02 g, 3.28 mmol) in DMF (6.8 mL) was added Barton's base (677 μL, 3.28 mmol). After being stirred at 80 °C for 10 h, the mixture was cooled to room temperature and diluted with EtOAc and water. The precipitate was filtered, washed with water and EtOAc, and dried to yield **28a** (400 mg, 29%). The combined filtrates were concentrated in vacuo, and the residue was purified by preparative RP-HPLC to afford additional **28a** (151 mg) and N3 regisomer (281 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.94 (s, 1H), 7.46−7.41 (brs, 2H), 7.39 (s, 1H), 6.81 (s, 1H), 6.09 (s, 2H), 4.18 (t, *J* = 7.2 Hz, 1H), 4.10 (brd, *J* = 13.2 Hz, 1H), 3.62 (brd, *J* = 12.8 Hz, 1H), 2.95 (td, *J* = 12.4, 2.8 Hz, 1H), 2.44 (m, 1H), 1.78−1.67 (m, 2H), 1.61 (q, *J* = 7.2 Hz, 2H), 1.44 (m, 1H), 1.01 (m, 1H), 0.91 (m, 1H); HRMS (ESI-TOF) calcd for $C_{20}H_{21}BrN_6O_3S$ [M + H$^+$] 505.0652, found 505.0662; HPLC purity, 96.7% (t_R = 7.32 min).

8-[(6-Bromo-1,3-benzodioxol-5-yl)sulfanyl]-9-[2-(1-isopropyl-4-piperidyl)ethyl]purin-6-amine (27c). The title compound was prepared from **26a** and **25c** in a manner smilar to that described for **28a**. ^{1}H NMR (400 MHz, CD_3OD) δ 8.23 (s, 1H), 7.27 (s,1H), 7.13 (s, 1H), 6.08 (s, 2H), 4.38−4.30 (m, 2H), 3.62−3.52 (m, 2H), 3.36−3.26 (m, 1H), 3.05−2.98 (m, 2H), 2.96−2.84 (m, 2H), 1.80−0.80 (m, 11H); LC−MS [M + H$^+$] 519.1; HPLC purity, 99.9% (t_R = 6.10 min).

8-[(6-Bromo-1,3-benzodioxol-5-yl)sulfanyl]-9-[2-(1-isobutyl-4-piperidyl)ethyl]purin-6-amine (27d). The title compound was prepared from **26a** and **25d** in a manner smilar to that described for **28a**. ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (s, 1H), 7.30 (s, 1H), 7.10 (s, 1H), 6.10 (s, 2H), 4.40 (t, *J* = 7.2 Hz, 2H), 3.60−3.58 (m, 2H), 3.00−2.90 (m, 2H), 2.15−2.10 (m, 2H), 2.00−1.90 (m, 2H), 1.90−

1.80 (m, 2H), 1.60−1.50 (m, 2H), 1.30−1.20 (m, 2H), 1.10−1.00 (m, 6H); LC−MS [M + H$^+$] 533.1; HPLC purity, 99.9% (t_R = 6.31 min).

8-[(6-Bromo-1,3-benzodioxol-5-yl)sulfanyl]-9-[2-[1-(2,2,2-trifluoroethyl)-4-piperidyl]ethyl]purin-6-amine (27e). The title compound was prepared from **26a** and **25e** in a manner similar to that described for **28a**. ^{1}H NMR (400 MHz, CD_3OD) δ 8.29 (s, 1H), 7.28 (s, 1H), 7.18 (s, 1H), 6.09 (s, 2H), 4.35 (t, J = 7.2 Hz, 2H), 3.56−3.50 (m, 2H), 3.35 (s, 2H), 3.30−3.25 (m, 2H), 2.69−2.59 (m, 3H), 2.00−1.40 (m, 4H); LC−MS [M + H$^+$] 559.0; HPLC purity, 97.0% (t_R = 7.16 min).

8-[(6-Bromo-1,3-benzodioxol-5-yl)sulfanyl]-9-[2-[1-(cyclohexylmethyl)-4-piperidyl]ethyl]purin-6-amine (27f). The title compound was prepared from **26a** and **25f** in a manner similar to that described for **28a**. ^{1}H NMR (400 MHz, CD_3OD) δ 8.20 (s, 1H), 7.10 (s, 1H), 6.08 (s, 2H), 4.35−4.30 (m, 2H), 3.60−3.53 (m, 2H), 2.93−2.84 (m, 4H), 2.16−0.80 (m, 18H); HPLC purity, 99.9% (t_R = 6.75 min).

8-[(6-Bromo-1,3-benzodioxol-5-yl)sulfanyl]-9-[2-(1-prop-2-ynyl-4-piperidyl)ethyl]purin-6-amine (27g). The title compound was prepared from **26a** and **25g** in a manner similar to that described for **28a**. ^{1}H NMR (400 MHz, CD_3OD) δ 8.26 (s, 1H), 7.28 (s, 1H), 7.16 (s, 1H), 6.09 (s, 2H), 4.36 (t, J = 7.6 Hz, 2H), 4.10−4.02 (m, 2H), 3.68 (brd, J = 14.0 Hz, 2H), 3.02 (brt, J = 12.4 Hz, 2H), 2.66 (s, 1H), 2.21 (brd, J = 14.8 Hz, 2H), 1.90−1.81 (m, 2H), 1.62−1.60 (m, 1H), 1.56−1.44 (m, 2H); LC−MS [M + H$^+$] 515.1; HPLC purity, 97.9% (t_R = 6.15 min).

8-[(6-Bromo-1,3-benzodioxol-5-yl)sulfanyl]-9-[2-[1-(1-methyltetrazol-5-yl)-4-piperidyl]ethyl]purin-6-amine (27h). The title compound was prepared from **26a** and **25h** in a manner similar to that described for **28a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (s, 1H), 7.65−7.50 (brs, 2H), 7.40 (s, 1H), 6.85 (s, 1H), 6.10 (s, 2H), 4.22 (t, J = 7.6 Hz, 2H), 3.85 (s, 3H), 3.55 (brd, J = 12.8 Hz, 2H), 2.85 (t, J = 12.8 Hz, 2H), 1.78 (brd, J = 12.8 Hz, 2H), 1.70−1.62 (m, 2H), 1.43 (m, 1H), 1.35−1.22 (m, 2H); HRMS (ESI-TOF) calcd for $C_{21}H_{23}BrN_{10}O_2S$ [M + H$^+$] 559.0982, found 559.0974; HPLC purity, 95.1% (t_R = 7.56 min).

8-[(6-Bromo-1,3-benzodioxol-5-yl)sulfanyl]-9-[2-[1-(1-*tert*-butyltetrazol-5-yl)-4-piperidyl]ethyl]purin-6-amine (27i). The title compound was prepared from **26a** and **25i** in a manner similar to that described for **28a**. ^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (s, 1H), 7.27 (s, 1H), 7.10 (s, 1H), 6.08 (s, 2H), 4.35 (t, J = 7.6 Hz, 2H), 3.14 (brd, J = 12.4 Hz, 2H), 2.92 (t, J = 12.0 Hz, 2H), 1.92 (brd, J = 10.0 Hz, 2H), 1.83 (brq, J = 8.0 Hz, 2H), 1.73 (s, 9H), 1.58−1.42 (m, 3H); HRMS (ESI-TOF) calcd for $C_{24}H_{29}BrN_{10}O_2S$ [M + H$^+$] 601.1452, found 601.1461; HPLC purity, 99.9% (t_R = 8.65 min).

***tert*-Butyl 4-[2-[6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)-sulfanyl]purin-9-yl]ethyl]piperidine-1-carboxylate (27a).** *Method A.* The title compound was prepared from **26a** and **25a** in a manner similar to that described for **28a**. HRMS (ESI-TOF) calcd for $C_{24}H_{29}BrN_6O_4S$ [M + H$^+$] 577.1227, found 577.1279; HPLC purity, 97.3% (t_R = 7.02 min).

Method B. The title compound was prepared from **33a** and aryl sulfide **43a** in a manner similar to that described for **34a**.

Method C. To a solution of **33a** (13.2 g, 31.1 mmol) and 1,3-benzodioxol-5-thiol (**58**) (7.17 g, 46.6 mmol) in DMF (60 mL) was added K_2CO_3 (93.17 mmol, 12.87 g). After being stirred at 100 °C for 3−6 h, the mixture was cooled to room temperature, filtered, and washed with EtOAc. The combined filtrates were diluted with EtOAc (120 mL), washed with water (100 mL × 2), brine (100 mL), dried (Na_2SO_4), filtered, and concentrated to 30 mL. The solid was precipitated by adding hexane (50 mL), filtered, and washed with a 1:2 (v/v) mixture of EtOAc/hexanes to afford the product (14.5 g, 94%). ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H), 7.02 (dd, J = 7.8, 1.9 Hz, 1H), 6.96 (d, J = 1.9 Hz, 1H), 6.80 (d, J = 7.8 Hz, 1H), 5.99 (s, 2H), 4.23 (t, J = 7.4 Hz, 2H), 4.38−3.98 (m, 2H), 2.3−2.15 (m, 2H), 1.82−1.66 (m, 4H), 1.49−1.35 (m, 10H), 1.22−1.1 (m, 2H); LC−MS [M + H$^+$] 499.1. A mixture of the above product (10.0 g, 20.0 mmol) in acetic acid (37 mL) was cooled to 10 °C and treated with NBS (4.28 g, 24.1 mmol) in portions. After the mixture was stirred for 1 h at room temperature, additional NBS (~2 g) was added. The mixture was stirred for 4 h. Upon completion, the mixture was cooled to 15 °C, quenched with aqueous sodium bisulfate (prepared from 11 g of sodium metasulfite and 100 mL of water), stirred at room temperature overnight, filtered, washed with water, and air-dried to give the crude product. The crude was crystallized from methanol to afford **27a** (8.5 g, 73% yield).

8-[(6-Bromo-1,3-benzodioxol-5-yl)sulfanyl]-9-[2-(4-piperidyl)ethyl]purin-6-amine (27b). To a suspension of **27a** (14.0 g, 0.025 mol) in CH_2Cl_2 (82 mL) was added TFA (27 mL, 0.37 mol) at 10 °C. After being stirred for 2 h at room temperature, the mixture was concentrated in vacuo and the residual TFA was removed by azeotropic distillation with AcCN. The remaining oil was dissolved in AcCN (11 mL), and the solution was added to a NH_4OH solution (29%, w/w, 71 mL) at 10 °C. The solid was filtered, washed with water, and air-dried (11.8 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.52−7.43 (brs, 2H), 7.39 (s, 1H), 6.81 (s, 1H), 6.09 (s, 2H), 4.18 (t, J = 7.0 Hz, 2H), 3.23 (d, J = 11.2 Hz, 2H), 3.16 (m, 1H), 2.83−2.70 (m, 1H), 1.86 (d, J = 12.5 Hz, 2H), 1.64 (q, J = 7.0 Hz, 2H), 1.50−1.39 (m, 1H), 1.32−1.20 (m, 2H); HRMS (ESI-TOF) calcd for $C_{19}H_{21}BrN_6O_2S$ [M + H$^+$] 477.0703, found 477.0789; HPLC purity, 99.2% (t_R = 3.95 min).

1-[4-[2-[6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)-sulfanyl]purin-9-yl]ethyl]-1-piperidyl]ethanone (28b). The title compound was prepared by the EDCI coupling method described for the preparation of **28g** using **27b** and AcOH. HRMS (ESI-TOF) calcd for $C_{21}H_{23}BrN_6O_3S$ [M + H$^+$] 519.0809, found 519.0555; HPLC purity, 95.9% (t_R = 7.56 min).

1-[4-[2-[6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)-sulfanyl]purin-9-yl]ethyl]-1-piperidyl]butan-1-one (28c). The title compound was prepared according to the EDCI coupling method described for the preparation of **28g** using **27b** and butyric acid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.16 (s, 1H), 7.44 (brs, 2H), 7.39 (s, 1H), 6.81 (s, 1H), 6.01 (s, 2H), 4.32 (brd, J = 12.4 Hz, 1H), 4.18 (t, J = 6.8 Hz, 2H), 3.81 (brd, J = 12.4 Hz, 1H), 2.86 (brt, J = 10.8 Hz, 1H), 2.39 (brt, J = 10.8 Hz, 1H), 2.24 (t, J = 6.8 Hz, 2H), 1.76−1.65 (m, 2H), 1.69 (q, J = 7.2 Hz, 2H), 1.48 (m, 2H), 1.40 (m, 1H), 1.01 (m, 1H), 0.92 (m, 1H), 0.87 (t, J = 7.6 Hz, 3H); HRMS (ESI-TOF) calcd for $C_{23}H_{27}BrN_6O_3S$ [M + H$^+$] 547.1122, found 547.1109; HPLC purity, 97.0% (t_R = 7.86 min).

[4-[2-[6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)sulfanyl]-purin-9-yl]ethyl]-1-piperidyl]cyclopropylmethanone (28d). The title compound was prepared according to the general EDCI coupling method using cyclopropanecarboxylic acid. HRMS (ESI-TOF) calcd for $C_{23}H_{25}BrN_6O_3S$ [M + H$^+$] 545.0965, found 545.0956; HPLC purity, 99.9% (t_R = 5.10 min).

[4-[2-[6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)sulfanyl]-purin-9-yl]ethyl]-1-piperidyl]cyclopentylmethanone (28e). The title compound was prepared according to the general EDCI coupling method using cyclopentanecarboxylic acid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.49 (s, 1H), 7.05 (s, 1H), 6.06 (s, 2H), 4.50 (d, J = 13.2 Hz, 1H), 4.29 (t, J = 7.2 Hz, 2H), 4.07 (d, J = 14.0 Hz, 1H), 3.07−2.98 (m, 2H), 2.57 (dd, J = 12.9 Hz, 3.1 Hz, 1H), 1.92−1.50 (m, 9H), 1.38−1.25 (m, 4H), 1.22−1.00 (m, 2H); LC−MS [M + H$^+$] 573.2; HPLC purity, 96.6% (t_R = 5.68 min).

1-[4-[2-[6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)-sulfanyl]purin-9-yl]ethyl]-1-piperidyl]-2-hydroxyethanone (28f). The title compound was prepared in 59% yield according to the general EDCI coupling method using glycolic acid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (s, 1H), 7.74−7.58 (brs, 2H), 7.39 (s, 1H), 6.84 (s, 1H), 6.09 (s, 2H), 4.29 (d, J = 12.5 Hz, 1H), 4.19 (t, J = 7.4 Hz, 2H), 4.07 (d, J = 14.4 Hz, 1H), 4.02 (d, J = 14.4 Hz, 1H), 3.60 (d, J = 13.6 Hz, 1H), 2.82 (t, J = 11.7 Hz, 1H), 2.56−2.44 (m, 1H), 1.74−1.67 (m, 2H), 1.61 (q, J = 7.0 Hz, 2H), 1.42 (m, 1H), 1.06 (m, 1H), 0.96 (m, 1H); HRMS (ESI-TOF) calcd for $C_{21}H_{23}BrN_6O_4S$ [M + H$^+$] 535.0758, found 535.0754; HPLC purity, 98.4% (t_R = 7.26 min).

(2*S*)-1-[4-[2-[6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)-sulfanyl]purin-9-yl]ethyl]-1-piperidyl]-2-hydroxypropan-1-one (28g). *General Procedure for EDCI Coupling Method.* To a mixture of **27b** (10.0 g, 21.0 mmol), HOBt (3.54 g, 23.1 mmol), L-lactic acid (2.26 g, 23.1 mmol), and NEt_3 (11.7 mL, 83.9 mmol) was added a mixture of DMF (50 mL) and THF (50 mL) at room temperature,

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followed by EDCI (6.02 g, 31.4 mmol). The mixture was stirred for 10 h at room temperature. Upon compeletion of reaction as judged by HPLC, water (350 mL) was added to the mixture. After being stirred for 1 h, the mixture was filtered, washed with a mixture of water and THF (50 mL, 4/1, v/v), and air-dried to afford a crude product. The crude was purified by repetitive trituration with MeOH (or hot EtOH) to **28g** (7.5 g, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.44 (brs, 2H), 7.39 (s, 1H), 6.81 (s, 1H), 6.09 (s, 2H), 4.76 (d, J = 7.2 Hz, 1H), 4.39 (m, 1H), 4.30 (m, 1H), 4.18 (t, J = 7.2 Hz, 2H), 3.91 (m, 1H), 2.86 (m, 1H), 1.78−1.66 (m, 2H), 1.64−1.56 (m, 2H), 1.43 (m, 1H), 1.18−1.12 (m, 3H), 1.08 (m, 1H), 0.95 (m, 1H); HRMS (ESI-TOF) calcd for $C_{22}H_{25}BrN_6O_4S$ [M + H$^+$] 549.0914, found 549.0913; HPLC purity, 99.0% (t_R = 7.14 min); HPLC (Chiralcel OJ-RH column, 100% MeOH with 0.01% TEA, isocratic, 0.5 mL/min) single peak observed at 11.7 min; no peak at 7.6 min (**28h**).

(2*R*)-1-[4-[2-[6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)-sulfanyl]purin-9-yl]ethyl]-1-piperidyl]-2-hydroxypropan-1-one (28h). The title compound was prepared in 62% yield according to the general EDCI coupling method using *R*-sodium lactate. HRMS (ESI-TOF) calcd for $C_{22}H_{25}BrN_6O_4S$ [M + H$^+$] 549.0914, found 549.0899; HPLC purity, 99.0%; HPLC (Chiralcel OJ-RH column, 100% MeOH with 0.01% TEA, isocratic, 0.5 mL/min) single peak observed at 7.6 min; no peak at 11.7 min (**28f**).

1-[4-[2-[6-Amino-8-(6-bromo-1,3-benzodioxol-5-yl)-sulfanyl]purin-9-yl]ethyl]-1-piperidyl]-2-hydroxy-2-methylpropan-1-one (28i). The title compound was prepared according to the general EDCI coupling method using 2-hydroxy-2-methylproanoic acid. LC−MS [M + H$^+$] 563.1.

1-[4-[2-[6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)-sulfanyl]purin-9-yl]ethyl]-1-piperidyl]propane-1,2-dione (28j). The title compound was prepared according to the general EDCI coupling method using pyruvic acid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.27 (s, 1H), 7.09 (s, 1H), 6.82 (s, 1H), 6.00 (s, 2H), 4.69 (bs, 2H), 4.56 (m, 1H), 4.48 (bd, J = 13.2 Hz, 1H), 4.26 (t, J = 7.6 Hz, 2H), 3.75 (bd, J = 13.6 Hz, 1H), 2.98 (td, J = 10.4, 2.8 Hz, 1H), 2.63 (bd, J = 12.8, 2.8 Hz, 1H), 1.87 (brt, J = 14.0 Hz, 2H), 1.78−1.71 (m, 2H), 1.55 (m, 1H), 1.31−1.20 (m, 2H); HRMS (ESI-TOF) calcd for $C_{22}H_{23}BrN_6O_4S$ [M + H$^+$] 547.0758, found 547.0756; HPLC purity, 97.3% (t_R = 8.09 min).

(2*S*)-2-Amino-1-[4-[2-[6-amino-8-[(6-bromo-1,3-benzodioxol-5-yl)sulfanyl]purin-9-yl]ethyl]-1-piperidyl]propan-1-one (28k). The title compound was prepared in 42% yield according to the general EDCI coupling method using Boc-L-alanine and subsequent deprotection of the Boc group with 4 M HCl in dioxane. ^{1}H NMR (400 MHz, CD_3OD) δ 8.34 (s, 1H), 7.28 (s, 1H), 7.23 (s, 1H), 6.09 (s, 2H), 4.54−4.44 (m, 2H), 4.39 (t, J = 7.4 Hz, 2H), 3.85 (brd, J = 13.2 Hz, 1H), 3.19−3.08 (m, 1H), 2.75−2.63 (m, 1H), 2.00−1.80 (m, 4H), 1.70−1.58 (m, 1H), 1.44 (dd, J = 14.8, 7.0 Hz, 3H), 1.35−1.13 (m, 2H); LC−MS [M + H$^+$] 548.2; HPLC purity, 95.1% (t_R = 6.32 min).

(2*S*)-2-Amino-1-[4-[2-[6-amino-8-[(6-bromo-1,3-benzodioxol-5-yl)sulfanyl]purin-9-yl]ethyl]-1-piperidyl]-4-methylpentan-1-one (28l). The title compound was prepared from **27b** with Boc-L-leucine in a manner similar to that described for **28k**. ^{1}H NMR (400 MHz, CD_3OD) δ 8.34 (s, 1H), 7.28 (s, 1H), 7.22 (s, 1H), 6.09 (s, 2H), 4.58−4.28 (m, 4H), 3.79 (brd, J = 13.2 Hz, 1H), 3.13 (t, J = 12.8 Hz, 1H), 2.76−2.62 (m, 1H), 2.00−1.50 (m, 7H), 1.35−1.10 (m, 3H), 1.08−0.95 (m, 6H); LC−MS [M + H$^+$] = 590.1.

2-Amino-1-[4-[2-[6-amino-8-[(6-bromo-1,3-benzodioxol-5-yl)sulfanyl]purin-9-yl]ethyl]-1-piperidyl]-2-methylpropan-1-one (28m). The title compound was prepared in a manner similar to that described for **28k** using **27b** and *N*-Boc-2-amino-2-methylpropanoic acid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.34 (s, 1H), 7.28 (s, 1H), 7.22 (s, 1H), 6.09 (s, 2H), 4.45−4.16 (m, 4H), 3.10−2.80 (m, 2H), 1.94 (d, J = 12.8 Hz, 2H), 1.84 (q, J = 7.0 Hz, 2H), 1.67−1.65 (m, 7H), 1.32−1.15 (m, 2H); LC−MS [M + H]$^+$ = 562.0.

[4-[2-[6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)sulfanyl]-purin-9-yl]ethyl]-1-piperidyl]-2-(dimethylamino)ethanone (28n). The title compound was prepared according to the general EDCI coupling method using 2-(dimethylamino)acetic acid. HRMS (ESI-TOF) calcd for $C_{23}H_{28}BrN_7O_3S$ [M + H$^+$] 562.4980, found 562.1173; HPLC purity, 99.9% (t_R = 6.28 min).

[4-[2-[6-Amino-8-[(6-bromo-1,3-benzodioxol-5-yl)sulfanyl]-purin-9-yl]ethyl]-1-piperidyl]-[(2*S*)-pyrrolidin-2-yl]methanone (28o). The title compound was prepared in 35% yield in a manner similar to that described for **28k** using **27b** and *N*-Boc-L-proline. ^{1}H NMR (400 MHz, CD_3OD) δ 8.31 (s, 1H), 7.29 (s, 1H), 7.20 (s, 1H), 6.09 (s, 2H), 4.63 (m, 1H), 4.59 (m, 1H), 4.38 (t, J = 8.0 Hz, 2H), 3.87 (m, 1H), 3.40 (m, 1H), 3.13 (m, 1H), 2.73 (m, 1H), 2.49 (m, 1H), 2.10−1.82 (m, 6H), 1.65 (m, 1H), 1.30−1.16 (m, 3H), 0.88 (m, 1H); HRMS (ESI-TOF) calcd for $C_{24}H_{28}BrN_7O_3S$ [M + H$^+$] 574.1231, found 574.1234; HPLC purity, 95.4% (t_R = 6.69 min).

***tert*-Butyl 4-[2-(6-Amino-9*H*-purin-9-yl)ethyl]piperidine-1-carboxylate (33a).** To a mixture of adenine (**32**) (11.1 g, 82.1 mmol) and Cs_2CO_3 (44.60 g, 136.9 mmol) in DMF (100 mL) was added mesylate **25a** (25.2 g, 82.1 mmol). After being stirred for 12 h at room temperature, the mixture was filtered and washed with $CHCl_3$. The combined filtrates were diluted with $CHCl_3$, washed with water and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to afford a 9:1 mixture of N9 and N3 isomers (19.2 g, 82%) (LC/MS [M + H$^+$] 347.1), which was used for the next step without further purification. To a clear solution of the product in NaOAc−AcOH buffer (pH 4.6) and MeOH/THF (60 mL, 1:1 v/v) was added bromine (5.13 mL, 99.8 mmol) dropwise while maintaining the temperature at ∼20 °C. After being stirred for 2 h at room temperature, the mixture was quenched with sodium metabisulfite and the pH of the reaction mixture was adjusted to ∼8 by addition of saturated Na_2CO_3 solution. The product portion was extracted with CH_2Cl_2, washed with H_2O/brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude was purified by crystallization from CH_2Cl_2/hexane to give **33a** (16 g, 67%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.5−7.38 (brs, 2H), 4.15 (t, J = 7.4 Hz, 2H), 3.95−3.84 (m, 2H), 2.74−2.33 (m, 2H), 1.73 (d, J = 12.8 Hz, 2H), 1.67 (q, J = 6.6 Hz, 2H), 1.44−1.34 (m, 10 H), 1.02 (dq, J = 8.2, 3.5 Hz, 2H); HRMS (ESI-TOF) calcd for $C_{17}H_{25}BrN_6O_2$ [M + H$^+$] 425.1295, found 425.1352; HPLC purity, 98.0% (t_R = 5.91 min).

***tert*-Butyl 4-[2-[6-Amino-8-[(5-bromo-2,3-dihydrobenzofuran-6-yl)sulfanyl]purin-9-yl]ethyl]piperidine-1-carboxylate (39a).** To a solution of aryl sulfide **45** (195 mg, 0.470 mmol) in THF (2.4 mL) and EtOH (0.2 mL) was added NaOEt (34 mg). The mixture was stirred for 2 h at room temperature and concentrated in vacuo. The crude sodium thiolate was dissolved in THF (2.4 mL)−EtOH (0.2 mL), and 8-bromopurine **33a** was added. After heating at 70 °C for 10 h, the mixture was cooled to room temperature, diluted with EtOAc, washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification of the residue by column chromatography (SiO_2, EtOAc/hexane, 50−100%) provided **39a** (83 mg, 62%). ^{1}H NMR ($CDCl_3$) δ 8.36 (s, 1H) 7.40 (t, J = 0.8 Hz, 1H), 6.49 (s, 1H), 5.64−5.57 (brs, 2H), 4.56 (t, J = 8.8 Hz, 2H), 4.24 (t, J = 7.2 Hz, 2H), 4.11−3.96 (m, 2H), 3.19 (td, J = 8.8, 0.8 Hz, 2H), 2.68−2.54 (m, 2H), 1.74−1.66 (m, 5H), 1.45 (s, 9H), 1.17−1.04 (m, 2H); HRMS (ESI-TOF) calcd for $C_{25}H_{31}BrN_6O_3S$ [M + H$^+$] 575.1435, found 575.1439; HPLC purity, 96.6% (t_R = 9.15 min).

***tert*-Butyl 4-[2-[6-Amino-8-[(6-bromo-2,3-dihydro-1,4-benzodioxin-7-yl)sulfanyl]purin-9-yl]ethyl]piperidine-1-carboxylate (39b).** The title compound was prepared in 59% yield in a manner similar to that described for **39a**. HRMS (ESI-TOF) calcd for $C_{25}H_{31}BrN_6O_4S$ [M + H$^+$] 591.1384, found 591.1390; HPLC purity, 93.0% (t_R = 8.52 min).

8-[(5-Bromo-2,3-dihydrobenzofuran-6-yl)sulfanyl]-9-[2-(4-piperidyl)ethyl]purin-6-amine (40a). To a solution of of **39a** (77 mg, 0.14 mmol) in CH_2Cl_2 (2 mL) was added TFA (200 μL) at 0 °C, and the mixture was stirred at room temperature for 2 h. Removal of solvent in vacuo and azeotropic removal of residual TFA with toluene gave the title compound (∼100 mg) that was used for the next step without further purificaiton. HRMS (ESI-TOF) calcd for $C_{20}H_{23}BrN_6OS$ [M + H$^+$] 475.0910, found 475.0905.

8-[(6-Bromo-2,3-dihydro-1,4-benzodioxin-7-yl)sulfanyl]-9-[2-(4-piperidyl)ethyl]purin-6-amine (40b). The title compound was prepared in a manner similar to that described for **40a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.52−7.40 (broad s, 2H), 7.28

(s, 1H), 6.69 (s, 1H), 4.28−4.15 (m, 6H), 3.23 (d, J = 12.5 Hz, 2H), 2.82−2.70 (m, 2H), 1.85 (d, J = 12.5 Hz, 2H), 1.62 (q, J = 6.6 Hz, 2H), 1.48−1.36 (m, 1H), 1.33−1.20 (m, 2H); LC−MS [M + H$^+$] 491.2.

4-[2-[6-Amino-8-[(5-bromo-2,3-dihydrobenzofuran-6-yl)-sulfanyl]purin-9-yl]ethyl]piperidine-1-carbaldehyde (34a). To solution of aryl sulfide **45** (235 mg, 0.566 mmol) in THF (2.8 mL) and EtOH (0.28 mL) was added NaOEt (41 mg, 0.57 mmol). The mixture was stirred for ∼2 h at room temperature and concentrated in vacuo. The crude sodium thiolate was dissolved in THF (2.8 mL)−EtOH (0.28 mL), and 8-bromopurine **33b** (100 mg, 0.283 mmol) was added. After being stirred for 10 h at 70 °C, the mixtue was quenched with water and the product portion was extracted with EtOAc. The combined organic layers were washed with water, brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was triturated with EtOAc to provide **34a** (87 mg, 61%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.93 (s, 1H), 7.57 (s, 1H), 7.56−7.46 (brs, 2H), 6.33 (s, 1H), 4.52 (t, J = 8.0 Hz, 2H), 4.17 (t, J = 7.2 Hz, 2H), 4.07 (brd, J = 13.2 Hz, 1H), 3.60 (brd, J = 12.8 Hz, 1H), 3.17 (t, J = 8.0 Hz, 2H), 2.86 (td, J = 12.8, 2.8 Hz, 1H), 2.44 (td, J = 12.4, 3.2 Hz, 1H), 1.76−1.64 (m, 2H), 1.59 (q, J = 6.8 Hz, 2H), 1.39 (m, 1H), 0.97 (qd, J = 12.8, 4.4 Hz, 1H), 0.87 (qd, J = 12.4, 4.4 Hz, 1H); HRMS (ESI-TOF) calcd for $C_{21}H_{23}BrN_6O_2S$ [M + H$^+$] 503.0859, found 503.0857; HPLC purity, 95.4% (t_R = 7.28 min).

(2*S*)-1-[4-[2-[6-Amino-8-[(5-bromo-2,3-dihydrobenzofuran-6-yl)sulfanyl]purin-9-yl]ethyl]-1-piperidyl]-2-hydroxypropan-1-one (34b). The title compound was prepared in 55% yield by the EDCI coupling method described for the preparation of **34j** using **40a** and L-lactic acid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (s, 1H), 7.54 (s, 1H), 6.68 (s, 1H), 4.57 (t, J = 8.0 Hz, 2H), 4.53 (m, 1H), 4.44 (br d, J = 11.6 Hz, 1H), 4.29 (t, J = 7.6 Hz, 2H), 3.97 (br t, J = 14.0 Hz, 1H), 3.23 (t, J = 8.0 Hz, 2H), 2.97 (m, 1H), 2.58 (m, 1H), 1.88−1.76 (m, 2H), 1.75−1.67 (m, 2H), 1.55 (m, 1H), 1.31−1.25 (m, 3H), 1.24−1.04 (m, 2H); HRMS (ESI-TOF) calcd for $C_{23}H_{27}BrN_6O_3S$ [M + H$^+$] 547.1122, found 547.1126; HPLC purity, 93.3% (t_R = 7.25 min).

4-[2-[6-Amino-8-[(6-bromo-2,3-dihydrobenzofuran-5-yl)-sulfanyl]purin-9-yl]ethyl]piperidine-1-carbaldehyde (34c). The title compound was prepared from **33b** and **48** in a manner similar to that described for **34a** except that the purification was performed by preperative RP-HPLC. ^{1}H NMR (400 MHz, CD_3OD) δ 8.28 (s, 1H), 7.99 (s, 1H), 7.60 (s, 1H), 7.19 (s, 1H), 4.67 (t, J = 9.7 Hz, 2H), 4.37 (t, J = 7.2 Hz, 2H), 4.31 (brd, J = 13.2 Hz, 1H), 3.72 (brd, J = 13.6 Hz, 1H), 3.22 (t, J = 9.7 Hz, 2H), 3.10 (td, J = 12.8, 2.8 Hz, 1H), 2.66 (td, J = 12.8, 3.2 Hz, 1H), 1.98−1.85 (m, 2H), 1.79 (q, J = 7.2, Hz, 2H), 1.64 (m, 1H), 1.22 (qd, J = 11.6, 4.8 Hz, 1H), 1.13 (qd, J = 12.0, 4.4 Hz, 1H); HRMS (ESI-TOF) calcd for $C_{21}H_{23}BrN_6O_2S$ [M + H$^+$] 503.0859, found 503.0860; HPLC purity, 99.9% (t_R = 7.29 min).

4-[2-[6-Amino-8-(5-bromobenzofuran-6-yl)sulfanyl-purin-9-yl]ethyl]piperidine-1-carbaldehyde (34d). The title compound was prepared in 65% yield from **33b** and **46** in a manner similar to that described for **34a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 8.07 (s, 1H), 8.05 (d, J = 2.0 Hz, 1H), 7.89 (s, 1H), 7.48−7.43 (brs, 2H), 7.42 (d, J = 0.8 Hz, 1H), 6.95 (dd, J = 2.0, 0.8 Hz, 1H), 4.19 (t, J = 6.8 Hz, 2H), 4.03 (brd, J = 12.4, 1H), 3.55 (br d, J = 13.5 1H), 2.81 (td, J = 12.4, 2.8 Hz, 1H), 2.39 (td, J = 12.4, 2.8 Hz, 1H), 1.67−1.55 (m, 4H), 1.37 (m, 1H), 0.92 (qd, J = 12.4, 4.0 Hz, 1H), 0.83 (qd, J = 12.4, 3.6 Hz, 1H); HRMS (ESI-TOF) calcd for $C_{21}H_{21}BrN_6O_2S$ [M + H$^+$] 501.0728, found 501.0698; HPLC purity, 99.9% (t_R = 7.42 min).

4-[2-[6-Amino-8-(6-bromobenzofuran-5-yl)sulfanyl-purin-9-yl]ethyl]piperidine-1-carbaldehyde (34e). The title compound was prepared from **33b** and **49** in a manner similar to that described for **34a** except that the purification was performed by preperative RP-HPLC. ^{1}H NMR (400 MHz, CD_3OD) δ 8.31 (s, 1H), 8.09 (s, 1H), 8.05 (d, J = 1.2 Hz, 1H), 7.98 (s, 1H), 7.90 (d, J = 2.4 Hz, 1H), 6.94 (dd, J = 2.4, 1.2 Hz, 1H), 4.41 (t, J = 7.6 Hz, 2H), 4.30 (brd, J = 13.6, 1H), 3.71 (brd, J = 13.6 1H), 3.09 (td, J = 12.8, 2.8 Hz, 1H), 2.64 (td, J = 12.4, 2.8 Hz, 1H), 1.95−1.84 (m, 4H), 1.63 (m, 1H), 1.21 (qd, J = 12.0, 4.0 Hz, 1H), 1.13 (qd, J = 12.4, 4.4 Hz, 1H); HRMS (ESI-TOF) calcd for $C_{21}H_{21}BrN_6O_2S$ [M + H$^+$] 501.0728, found 501.0705; HPLC purity, 99.9% (t_R = 7.45 min).

4-[2-[6-Amino-8-(6-bromoindan-5-yl)sulfanylpurin-9-yl]-ethyl]piperidine-1-carbaldehyde (34f). The title compound was prepared from **26d** and **25b** in a manner similar to that described for **28a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.92 (s, 1H), 7.59 (s, 1H), 7.56−7.45 (brs, 2H), 6.91 (s, 1H), 4.16 (t, J = 6.8 Hz, 2H), 4.05 (m, 1H), 3.60 (m, 1H), 2.88−2.81 (m, 3H), 2.69 (t, J = 7.2 Hz, 2H), 2.44 (m, 1H), 1.96 (qn, J = 7.2 Hz, 2H), 1.72−1.61 (m, 2H), 1.61−1.55 (m, 2H), 0.98 (m, 1H), 0.96 (qd, J = 12.4, 4.4 Hz, 1H), 0.85 (qd, J = 11.6, 2.8 Hz, 1H); HRMS (ESI-TOF) calcd for $C_{22}H_{25}BrN_6OS$ [M + H$^+$] 501.10667, found 501.1067; HPLC purity, 99.0% (t_R = 7.7 min).

4-[2-[6-Amino-8-[(6-bromo-2,3-dihydro-1,4-benzodioxin-7-yl)sulfanyl]purin-9-yl]ethyl]piperidine-1-carbaldehyde (34g). The title compound was prepared in 75% yield from **26b** and **25b** in a manner similar to that described for **28a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.93 (s, 1H), 7.62−7.44 (brs, 2H), 7.28 (s, 1H), 6.68 (s, 1H), 4.25−4.15 (m, 6H), 4.09 (d, J = 13.6 Hz, 1H), 3.62 (d, J = 13.2 Hz, 1H), 2.88 (td, J = 12.4, 2.4 Hz, 1H), 2.47 (td, J = 12.4, 2.8 Hz, 1H), 1.76−1.65 (m, 2H), 1.58 (q, J = 7.2 Hz, 2H), 1.39 (m, 1H), 0.99 (qd, J = 12.0, 4.4 Hz, 1H), 0.89 (qd, J = 11.2, 4.0 Hz, 1H); HRMS (ESI-TOF) calcd for $C_{21}H_{23}BrN_6O_3S$ [M + H$^+$] 519.0809, found 519.0797, HPLC purity, 99.9% (t_R = 7.19 min).

1-[4-[2-[6-Amino-8-[(6-bromo-2,3-dihydro-1,4-benzodioxin-7-yl)sulfanyl]purin-9-yl]ethyl]-1-piperidyl]-2-hydroxyethanone (34h). The title compound was prepared in 64% yield by the EDCI coupling method described for the preparation of **34j** using **40b** with 2-hydroxyacetic acid. ^{1}H NMR (400 MHz, DMOS-d_6) δ 8.16 (s, 1H), 7.52−7.42 (brs, 2H), 7.29 (s, 1H), 6.68 (s, 1H), 4.44 (brs, 1H), 4.29 (m, 1H), 4.27−4.15 (m, 6H), 4.07 (d, J = 14.4 Hz, 1H), 4.02 (d, J = 14.4 Hz, 1H), 3.59 (brd, J = 13.2 Hz, 1H), 2.80 (brt, J = 12.0, 1H), 2.50 (m, 1H), 1.72−1.65 (m, 2H), 1.58 (q, J = 6.8 Hz, 2H), 1.38 (m, 1H), 1.04 (m, 1H), 0.94 (m, 1H); HRMS (ESI-TOF) calcd for $C_{22}H_{25}BrN_6O_4S$ [M + H$^+$] 549.0914, found 549.0913; HPLC purity, 95.0% (t_R = 7.03 min).

(2*S*)-1-[4-[2-[6-Amino-8-[(6-bromo-2,3-dihydro-1,4-benzodioxin-7-yl)sulfanyl]purin-9-yl]ethyl]-1-piperidyl]-2-hydroxypropan-1-one (34i). The title compound was prepared in 77% yield by the EDCI coupling method described for the preparation of **34j** using **40b** and L-lactic acid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (s, 1H), 8.14−7.90 (brs, 2H), 7.30 (s, 1H), 6.79 (s, 1H), 4.39 (m, 1H), 4.30 (m, 1H), 4.26−4.17 (m, 6H), 3.91 (m, 1H), 2.85 (m, 1H), 2.45 (m, 1H), 1.77−1.65 (m, 2H), 1.63−1.56 (m, 2H), 1.41 (m, 1H), 1.17−1.15 (m, 3H), 1.09−0.88 (m, 2H); HRMS (ESI-TOF) calcd for $C_{23}H_{27}BrN_6O_4S$ [M + H$^+$] 563.1071, found 563.1071; HPLC purity, 98.3% (t_R = 8.21 min).

(2*R*)-1-[4-[2-[6-Amino-8-[(6-bromo-2,3-dihydro-1,4-benzodioxin-7-yl)sulfanyl]purin-9-yl]ethyl]-1-piperidyl]-2-hydroxypropan-1-one (34j). To a mixture of **40b** (5.0 g, 10.18 mmol) in a 1:1 (v/v) mixture of DMF/THF (45 mL) were added (*R*)-sodium lactate (1.36 g, 12.21 mmol), HOBt (1.60 g, 12.2 mmol), and EDCI (2.90 g, 15.3 mmol) at room temperature, and the mixture was stirred overnight. Upon completion, the mixture was diluted with water (200 mL) and stirred for 15 min at room temperature. The solid was collected by filtration, washed with water, and air-dried to give the crude product that was purified by trituration with MeOH to provide **34j** (89%, 5.15 g). ^{1}H NMR (400 MHz, CD_3OD) δ 8.28 (s, 1H), 7.27 (s, 1H), 7.23 (s, 1H), 4.55 (m, 1H), 4.48 (m, 1H), 4.36 (t, J = 7.4 Hz, 2H), 4.32−4.26 (m, 4H), 4.00 (brd, J = 14.4 Hz, 1H), 3.03 (m, 1H), 2.63 (m, 1H), 1.94−1.85 (m, 2H), 1.85−1.78 (m, 2H), 1.67−1.54 (m, 1H), 1.32 (m, 3H), 1.28−1.1 (m, 2H); HRMS (ESI-TOF) calcd for $C_{23}H_{27}BrN_6O_4S$ [M + H$^+$] 563.1071, found 563.1055; HPLC purity, 99.9% (t_R = 7.21 min).

4-[2-[6-Amino-8-[(6-bromo-4-methyl-2,3-dihydro-1,4-benzoxazin-7-yl)sulfanyl]purin-9-yl]ethyl]piperidine-1-carbaldehyde (34k). The title compound was prepared from **33b** and **51** in a manner similar to that described for **34a**. ^{1}H NMR (DMSO-d_6) δ 8.24 (s, 1H), 7.94 (s, 1H), 6.95 (s, 1H), 6.68 (s, 1H), 4.22−4.15 (m, 4H), 4.12−4.08 (m, 2H), 3.64−3.57 (m, 1H), 3.29−3.26 (m, 2H), 2.95−

2.87 (m, 1H), 2.86 (s, 3H), 1.86−1.40 (m, 5H), 1.34−0.80 (m, 2H); LC−MS [M + H$^+$] 532.1.

4-[2-[6-Amino-8-[(7-bromo-4-methyl-2,3-dihydro-1,4-benzoxazin-6-yl)sulfanyl]purin-9-yl]ethyl]piperidine-1-carbaldehyde (34l). The title compound was prepared from **26e** and **25b** in a manner similar to that described for **28a**. HRMS (ESI-TOF) calcd for $C_{22}H_{26}BrN_7O_2S$ [M + H$^+$] 532.1125, found 532.1119; HPLC purity, 99.9% (t_R = 7.30 min).

4-[2-[6-Amino-8-(6-bromo-1-oxoindan-5-yl)sulfanylpurin-9-yl]ethyl]piperidine-1-carbaldehyde (34m). The title compound was prepared from **26f** and **25b** in a manner similar to that described for **28a**. ^{1}H NMR (400 MHz, CD_3OD) δ 8.32 (s, 1H), 7.97 (s, 1H), 7.95 (s, 1H), 7.34 (s, 1H), 4.36 (t, J = 6.8 Hz, 2H), 4.26 (brd, J = 12.8 Hz, 1H), 3.68 (brd, J = 13.2 Hz, 1H), 3.07−2.99 (m, 3H), 2.70−2.67 (m, 2H), 2.59 (td, J = 12.8, 2.4 Hz, 1H), 1.88−1.74 (m, 4H), 1.57 (m, 1H), 1.15 (m, 1H), 1.06 (m, 1H); HRMS (ESI-TOF) calcd for $C_{22}H_{23}BrN_6O_2S$ [M + H$^+$] 515.0859, found 515.0850; HPLC purity, 98.1% (t_R = 7.03 min).

4-[2-[6-Amino-8-[(6-bromo-2,2-difluoro-1,3-benzodioxol-5-yl)sulfanyl]purin-9-yl]ethyl]piperidine-1-carbaldehyde (34n). The title compound was prepared from **26c** and **25b** in a manner similar to that described for **28a**. HRMS (ESI-TOF) calcd for $C_{20}H_{19}BrF_2N_6O_3S$ [M + H$^+$] 541.0464, found 541.0460; HPLC purity, 96.9% (t_R = 7.50 min).

4-[2-[6-Amino-8-[(6-chloro-1,3-benzodioxol-5-yl)sulfanyl]-purin-9-yl]ethyl]piperidine-1-carbaldehyde (34o). The title compound was prepared in 55% yield from **33b** and **43c** in a manner similar to that described for **34a**. ^{1}H NMR (DMSO-d_6) δ 8.21 (s, 1H), 7.94 (s, 1H), 7.29 (s, 1H), 6.91 (s, 1H), 6.11 (s, 2H), 4.25−4.19 (m, 2H), 4.12−4.05 (m, 1H), 3.66−3.60 (m, 1H), 2.99−2.85 (m, 1H), 1.90−0.95 (m, 8H); HRMS (ESI-TOF) calcd for $C_{20}H_{21}ClN_6O_3S$ [M + H$^+$] 461.1157, found 461.1156; HPLC purity, 99.9% (t_R = 7.11 min).

(2*S*)-1-[4-[2-[6-Amino-8-[(6-chloro-1,3-benzodioxol-5-yl)-sulfanyl]purin-9-yl]ethyl]-1-piperidyl]-2-hydroxypropan-1-one (34p). The title compound was prepared in 80% yield by the EDCI coupling method described for the preparation of **34j** using **40d** and L-lactic acid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (s, 1H), 7.30 (s, 1H), 6.96 (s, 1H), 6.12 (s, 2H), 4.39 (m, 1H), 4.31 (m, 1H), 4.22 (t, J = 7.6 Hz, 2H), 3.92 (m, 1H), 2.88 (m, 1H), 2.48 (m, 1H), 1.78−1.68 (m, 2H), 1.66−1.58 (m, 2H), 1.45 (m, 1H), 1.17−1.12 (m, 3H), 1.10−0.90 (m, 2H); HRMS (ESI-TOF) calcd for $C_{22}H_{25}ClN_6O_4S$ [M + H$^+$] 505.1419, found 505.1428; HPLC purity, 99.8% (t_R = 6.98 min).

6-[6-Amino-9-[2-(1-formyl-4-piperidyl)ethyl]purin-8-yl]-sulfanyl-1,3-benzodioxole-5-carbonitrile (34q). The title compound was prepared in 22% yield from **26g** and **25b** in a manner similar to that described for **28a**. ^{1}H NMR (CD_3OD) δ 8.31 (s, 1H), 7.98 (s, 1H), 7.39 (s, 1H), 7.38 (s, 1H), 6.21 (s, 2H), 4.41 (t, J = 6.8 Hz, 2H), 4.31 (brd, J = 13.6 Hz, 1H), 3.71 (brd, J = 13.2 Hz, 1H), 3.11 (td, J = 12.8, 3.2 Hz, 1H), 2.67 (td, J = 13.2, 3.2 Hz, 1H), 1.99−1.92 (m, 2H), 1.88 (q, J = 7.2 Hz, 2H), 1.65 (m, 1H), 1.21 (qd, J = 12.4, 4.4 Hz, 1H), 1.14 (qd, J = 12.4, 4.0 Hz, 1H); HRMS (ESI-TOF) calcd for $C_{21}H_{21}N_7O_3S$ [M + H$^+$] 452.1499, found 452.1503; HPLC purity, 99.6% (t_R = 11.53 min).

2-Ethylhexyl 3-[(6-Bromo-1,3-benzodioxol-5-yl)sulfanyl]-propanoate (43a). The title compound was prepared in 50% yield from **24a** and **52** in a manner similar to that described for **45**, step 1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.95 (s, 1H), 6.00 (s, 2H), 4.02 (dd, J = 12.8, 6.0 Hz, 1H), 4.00 (dd, J = 12.8, 5.6 Hz, 1H), 3.11 (t, J = 6.8 Hz, 2H), 2.62 (t, J = 6.8 Hz, 2H), 1.57 (m, 1H), 1.39−1.26 (m, 8H), 0.89 (two t, J = 7.2 Hz, 6H); HRMS (ESI-TOF) calcd for $C_{18}H_{25}Br_6O_4S$ [M + H$^+$] 417.0730, found 417.0713.

2-Ethylhexyl 3-[(6-Bromo-2,3-dihydro-1,4-benzodioxin-7-yl)sulfanyl]propanoate (43b). The title compound was prepared in 55% yield from **24b** and **52** in a manner similar to that described for **45**, step 1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (s, 1H), 6.97 (s, 1H), 4.25 (s, 4H), 4.02 (dd, J = 12.8, 6.0 Hz, 1H), 4.00 (dd, J = 12.8, 6.0 Hz, 1H), 3.10 (t, J = 7.6 Hz, 2H), 2.62 (t, J = 7.6 Hz, 2H), 1.56 (m, 1H), 1.39−1.26 (m, 8H), 0.89 (two t, J = 7.2 Hz, 6H).

2-Ethylhexyl 3-[(6-Chloro-1,3-benzodioxol-5-yl)sulfanyl]-propanoate (43c). The title compound was prepared in 92% yield from **41c** and **52** in a manner similar to that described for **45**, step 1. HRMS (ESI-TOF) calcd for $C_{18}H_{25}ClO_4S$ [M + H$^+$] 372.1162, found 372.1162.

2-Ethylhexyl 3-[(5-Bromo-2,3-dihydrobenzofuran-6-yl)-sulfanyl]propanoate (45). *Step 1.* 6-Bromodihydrobenzofuran (**44**) (2.39 g, 12.02 mmol), 3-mercaptopropionic acid 2-ethyhexyl ester (**52**) (2.76 g, 12.6 mmol), Pd_2dba_3 (165 mg, 0.180 mmol), and xantphos (279 mg, 0.481 mmol) were placed in a flask followed by degassed dioxane (40 mL) and Hünig's base (4.2 mL, 0.18 mol) under nitrogen. The mixture was heated at 100 °C for 10 h, cooled to room temperature, diluted with CH_2Cl_2, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (EtOAc/hexane, 0−30%) to afford 3.1 g (83%) of 2-ethylhexyl 3-(2,3-dihydro-1-benzofuran-6-ylthio)propanoate. HRMS (ESI-TOF) calcd for $C_{19}H_{28}O_3$ [M + H$^+$] 337.1832, found 337.1795.

Step 2. To a solution of 2-ethylhexyl 3-(2,3-dihydro-1-benzofuran-6-ylthio)propanoate (500 mg, 1.49 mmol) in AcCN (3 mL) was added HBF_4OEt_2 (204 μL, 1.50 mmol) at −20 °C, followed by NBS (267 mg, 1.50 mmol) portionwise. The reaction mixture was slowly warmed to 5−10 °C, quenched with 10% aqueous $NaHSO_3$ (7 mL), and extracted with Et_2O. The combined extracts were washed with H_2O, brine, and the organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (EtOAc/hexane, 0−30%) to afford **45** (0.455 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (t, J = 0.8 Hz, 1H), 6.76 (s, 1H), 4.59 (t, J = 8.8 Hz, 2H), 4.03 (dd, J = 12.8, 6.0 Hz, 1H), 4.01 (dd, J = 12.8, 6.0 Hz, 1H), 3.19 (td, J = 8.8, 0.8 Hz, 2H), 3.16 (t, J = 7.2 Hz, 2H), 2.67 (t, J = 7.2 Hz, 2H), 1.56 (m, 1H), 1.39−1.25 (m, 8H), 0.89 (two t, J = 6.8 Hz, 6H); GC−MS (EI), m/z 414.

2-Ethylhexyl 3-(5-Bromobenzofuran-6-yl)-sulfanylpropanoate (46). To a solution of **45** (114 mg, 0.274 mmol) in dioxane (1.7 mL) was added DDQ (81 mg, 0.36 mmol), and the mixture was heated at 100 °C. After 10 h, the mixture was cooled to room temperature, diluted with Et_2O, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (EtOAc/hexane, 0−10%) to afford **46** (92 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.60 (d, J = 2.0 Hz, 1H), 7.56 (d, J = 0.8 Hz, 1H), 6.70 (dd, J = 2.0 Hz, 0.8 Hz, 1H), 4.03 (dd, J = 12.8, 6.0 Hz, 1H), 4.01 (dd, J = 12.8, 6.0 Hz, 1H), 3.23 (t, J = 7.6 Hz, 2H), 2.69 (t, J = 7.6 Hz, 2H), 1.59 (m, 1H), 1.42−1.25 (m, 8H), 0.89 (two t, J = 7.6 Hz, 6H); GC−MS (EI), m/z 412.

2-Ethylhexyl 3-[(6-Bromo-4-methyl-2,3-dihydro-1,4-benzoxazin-7-yl)sulfanyl]propanoate (51). The title compound was prepared in 56% yield (two steps) from **50** and **52** in a manner smilar to that described above for **45**. GC−MS (EI), m/z 445.

■ ASSOCIATED CONTENT

Supporting Information

Procedures for molecular modeling, synthesis of compounds **24d−e**, and ^{1}H NMR spectra and HPLC traces for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

17-AAG, 17-allylamino-17-demethoxygeldanamycin; AcCN, acetonitrile; ADME, absorption, distribution, metabolism, and excretion; AKT, protein kinase B; Barton's base, 2-*tert*-butyl-1,1,3,3-tetramethylguanidine; cdc37, cell division cycle 37; CDK4, cyclin-dependent kinase 4; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; 17-DMAG, 17-desmethoxy-17-*N*,*N*-dimethylaminoethylaminogeldanamycin; EDCI, *N*-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; EtOAc, ethyl acetate; FP, fluorescence polarization; Her2, human epidermal growth factor receptor 2; HIF-1α, hypoxia-inducible factor 1α; HIP, Hsp70 interacting protein; HLM, human liver microsome; HOBt, *N*-hydroxybenzotriazole; HOP, Hsp70-Hsp90 organizing protein; HSF1, heat shock protein 1; Hsp90, 90 kDa heat shock protein; HTS, high-throughput screening; Hünig's base, diisopropylethylamine; MDR, multidrug resistance; NSCLC, non-small-cell lung cancer; Pd_2dba_2, tris(dibenzylideneacetone)dipalladium(0); PAMPA, parallel artificial membrane permeability assay; PK, pharmacokinetic; RP-HPLC, reversed-phase high-performance liquid chromatography; SAR, structure–activity relationship; xantphos, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene

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