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(Bis)urea and (Bis)thiourea Inhibitors of Lysine-Specific Demethylase 1 as Epigenetic Modulators

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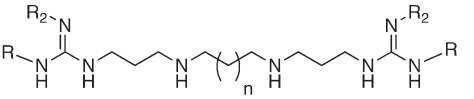
The recently discovered enzyme lysine-specific demethylase 1 (LSD1) plays an important role in the epigenetic control of gene expression, and aberrant gene silencing secondary to LSD1 overexpression is thought to contribute to the development of cancer. We recently reported a series of (bis)guanidines and (bis)biguanides that are potent inhibitors of LSD1 and induce the re-expression of aberrantly silenced tumor suppressor genes in tumor cells in vitro. We now report a series of isosteric ureas and thioureas that are also potent inhibitors of LSD1. These compounds induce increases in methylation at the histone 3 lysine 4 (H3K4) chromatin mark, a specific target of LSD1, in Calu-6 lung carcinoma cells. In addition, these analogues increase cellular levels of secreted frizzle-related protein (SFRP) 2 and transcription factor GATA4. These compounds represent an important new series of epigenetic modulators with the potential for use as antitumor agents.

Introduction

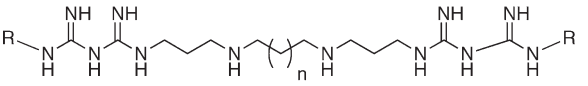
Chromatin architecture is a key determinant in the regulation of gene expression, and this architecture is strongly influenced by post-translational modifications of histones.[1,2] Histone protein tails contain lysine residues that interact with the negative charges on the DNA backbone. These lysine-containing tails, consisting of up to 40 amino acid residues, protrude through the DNA strand and act as a site for post-translational modification of chromatin, allowing alteration of higher-order nucleosome structure.[3] Multiple post-translational modifications of histones can mediate epigenetic remodeling of chromatin, with acetylation being the best characterized process.[4] Transcriptional repression is associated with specific CpG island DNA methylation and recruitment of histone deacetylases (HDACs[a]) to gene promoters that cooperate in the epigenetic silencing of specific genes.[5,6] Normal mammalian cells exhibit an exquisite level of control of chromatin architecture by maintaining a balance between histone acetyltransferase (HAT) and HDAC activity.[7]

In cancer, CpG island DNA promoter hypermethylation in combination with other chromatin modifications, including decreased activating marks and increased repressive marks on histone proteins 3 and 4, have been associated with the silencing of tumor suppressor genes.[8] The important role of promoter CpG island methylation and its relationship to covalent histone modifications has recently been reviewed.[9]



(bis)guanidines **1**

1a (n = 1, R = CH_3, R_2 = H)
1b (n = 5, R = CH_3, R_2 = H)
1c (n = 1, R = CH_3, R_2 = CH_3)
1d (n = 1, R = phenyl, R_2 = phenyl)
1e (n = 5, R = phenyl, R_2 = phenyl)
1f (n = 2, R = R_2 = 3,3-diphenylpropyl, R_2 = H)
1g (n = 2, R = 2,2-diphenylethyl, R_2 = H)



(bis)biguanides **2**

2a (n = 1, R = 2,2-diphenylethyl)
2b (n = 5, R = 2,2-diphenylethyl)
2c (n = 1, R = 3,3-diphenylpropyl)
2d (n = 5, R = 3,3-diphenylpropyl)
2e(n = 2, R = 3,3-diphenylpropyl)
2f (n = 2, R = 2,2-diphenylethyl)

Figure 1. (Bis)guanidine and (bis)biguanides with potent antitrypanosomal activity in vitro.

As was mentioned above, the N-terminal lysine tails of histones can undergo numerous post-translational modifications, including phosphorylation, ubiquitination, acetylation, and methylation.[4,10,11] To date, 17 lysine residues and 7 arginine residues on histone proteins have been shown to undergo methylation,[12] and lysine methylation on histones can signal transcriptional activation or repression depending on the specific lysine residue involved.[13−15] All known histone lysine methyltransferases contain a conserved SET methyltransferase

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[a] Abbreviations: LSD1, lysine-specific demethylase 1; H3K4, histone 3 lysine 4; JmjC, Jumonji C domain-containing demethylase; HAT, histone acetyltransferase; HDAC, histone deacetylase; SET, (Su(var.)3−9 enhancer of zeste; APAO, acetylpolyamine oxidase; SMO, spermine oxidase; SFRP, secreted frizzle-related protein; PCNA, proliferating cell nuclear antigen.

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Table 1. Structures of Compounds **1c**, **2d**, and **3**−**33**, and Inhibition of LSD1 in Vitro Following Treatment with Each Analogue at 10 μM

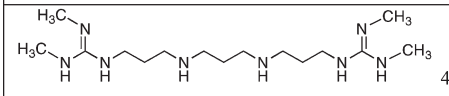
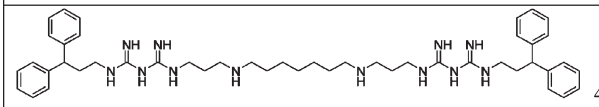
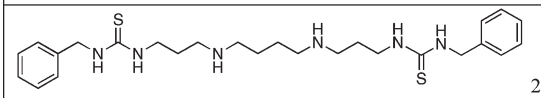
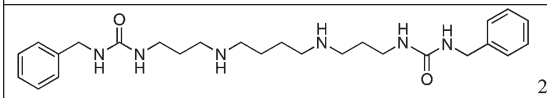
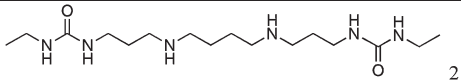
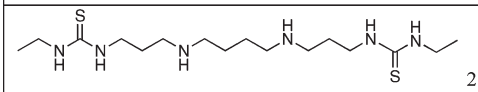
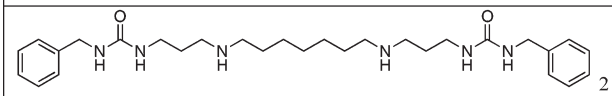
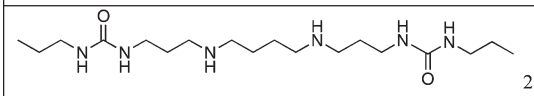
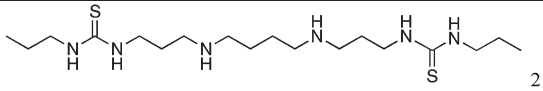
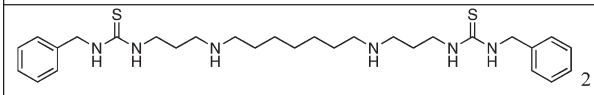
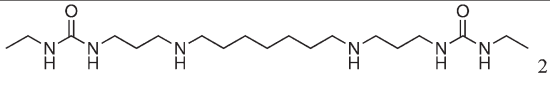
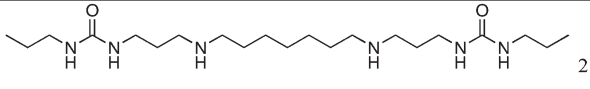
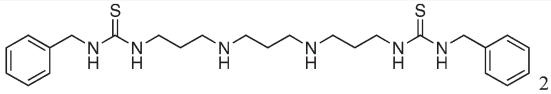
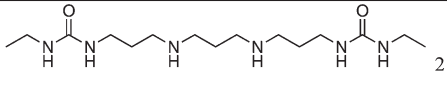
Compound	Compound	%LSD1 activity remaining
 4 HCl	**1c**	14.1 (from ref. 25)
 4 HCl	**2d**	17.2
 2 HCl	**3**	74.8
 2 HCl	**4**	49.5
 2 HCl	**5**	49.2
 2 HCl	**6**	40
 2 HCl	**7**	100
 2 HCl	**8**	79
 2 HCl	**9**	89.6
 2 HCl	**10**	35.9
 2 HCl	**11**	95.9
 2 HCl	**12**	91.5
 2 HCl	**13**	52.1
 2 HCl	**14**	65.5

Table 1. Continued

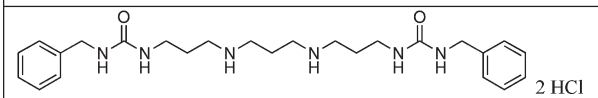
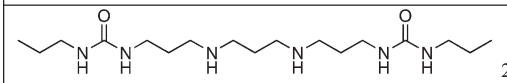
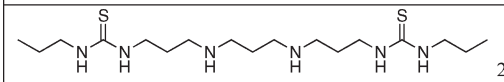
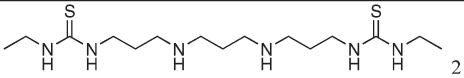
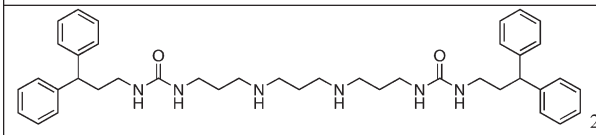
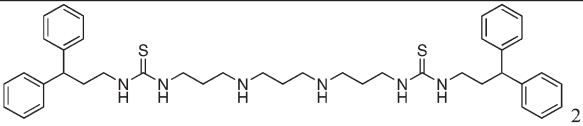
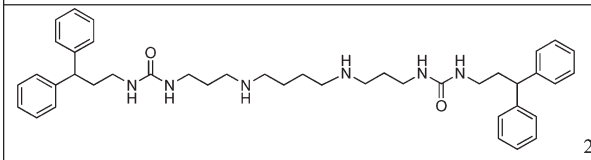
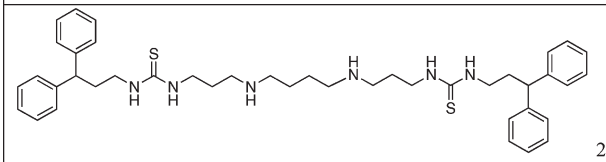
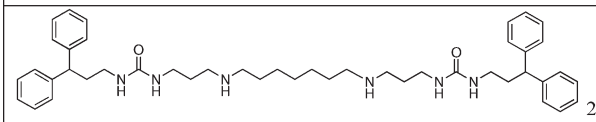
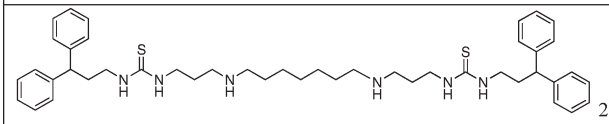
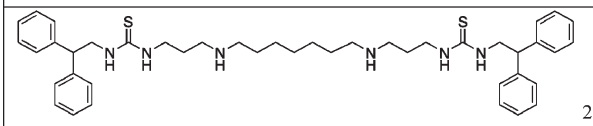
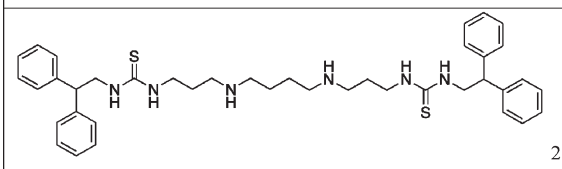
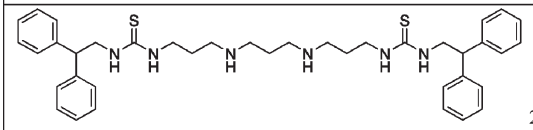
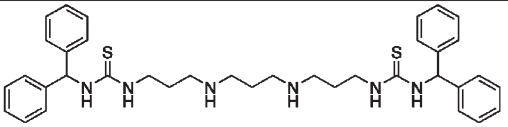
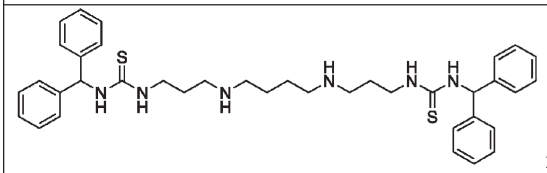
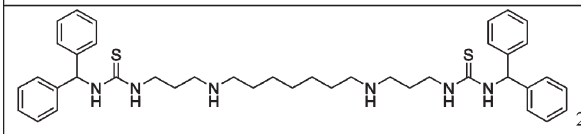
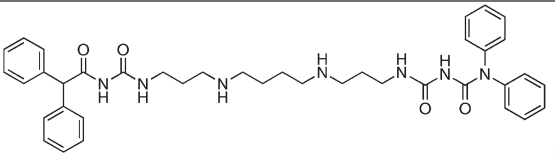
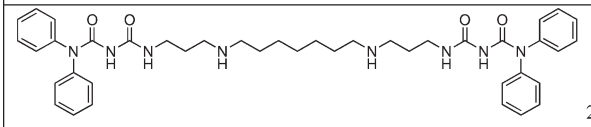
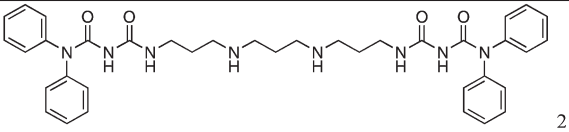
Compound	Compound	%LSD1 activity remaining
 2 HCl	**15**	60.5
 2 HCl	**16**	51.3
 2 HCl	**17**	100
 2 HCl	**18**	36.2
 2 HCl	**19**	92.9
 2 HCl	**20**	92.2
 2 HCl	**21**	74.6
 2 HCl	**22**	88.6
 2 HCl	**23**	51.5
 2 HCl	**24**	77.3
 2 HCl	**25**	19.5
 2 HCl	**26**	17.1

Table 1. Continued

Compound	Compound	%LSD1 activity remaining
 2 HCl	**27**	24.8
 2 HCl	**28**	51.1
 2 HCl	**29**	34.4
 2 HCl	**30**	28.9
 2 HCl	**31**	26.1
 2 HCl	**32**	70
 2 HCl	**33**	91.5

domain, and it has been shown that aberrant methylation of histones due to SET domain deregulation is linked to carcinogenesis.[16] Histone methylation, once thought to be an irreversible process, has recently been shown to be a dynamic process regulated by the addition of methyl groups by histone methyltransferases and removal of methyl groups from mono- and dimethyllysines by lysine specific demethylase 1 (LSD1), and from mono-, di, and trimethyllysines by specific Jumonji C (JmjC) domain-containing demethylases.[10,11,17,18] Additional demethylases in the JmjC demethylase class are continuing to be identified.[19,20] Recent evidence suggests that LSD1 is required for maintenance of global DNA methylation,[21] indicating that the LSD1-mediated demethylation is a general mechanism for transcriptional control.

A key positive chromatin mark found associated with promoters of active genes is histone 3 dimethyllysine 4 (H3K4me2).[22,23] LSD1, also known as BHC110 and KDM1,[10,24] catalyzes the oxidative demethylation of histone 3 methyllysine 4 (H3K4me1) and H3K4me2 and is associated with transcriptional repression.[10] H3K4me2 is a transcription-activating chromatin mark at gene promoters, and demethylation of this mark by LSD1 may prevent expression of tumor suppressor genes important in human cancer.[25] Thus, LSD1 is emerging as an important new target for the development of specific inhibitors as a new class of antitumor drugs.[26]

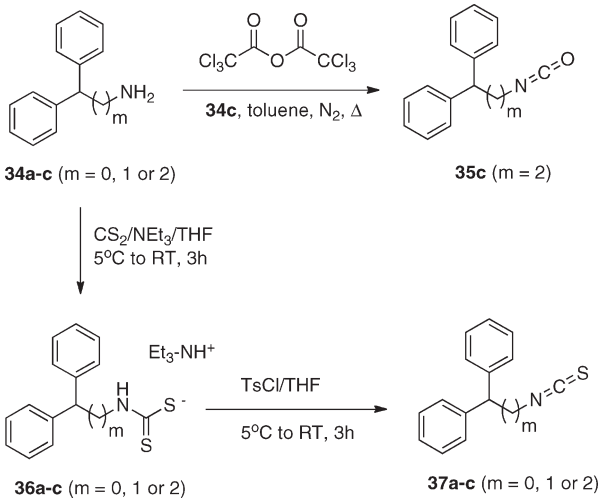
To date, only a few existing compounds have been shown to act as inhibitors of LSD1. The active site structure of LSD1 has considerable sequence homology to monoamine oxidases A and B (MAO A and B) and to N^1-acetylpolyamine oxidase (APAO) and spermine oxidase (SMO).[10,24,27] It has been shown that classical MAO inhibitors phenelzine and tranylcypromine inactivate nucleosomal demethylation by the recombinant LSD1/CoRest complex and increase global levels of H3K4me2 in the P19 cell line.[24,27] The synthetic substrate analogue aziridinyl-K4H3$_{1-21}$ reversibly inhibited LSD1 with an IC_{50} of 15.6 μM, while propargyl-K4H3$_{1-21}$ produced time-dependent inactivation with a K_i of 16.6 μM.[28] Propargyl-K4H3$_{1-21}$ was later shown to inactivate LSD1 through formation of a covalent adduct with the enzyme-bound flavin cofactor.[27,29] McCafferty et al. recently described the synthesis of a series of *trans*-2-arylcyclopropylamine analogues that inhibit LSD1 with K_i values between 188 and 566 μM.[30]

However, in all but one instance, these analogues were 1−2 orders of magnitude more potent against MAO A and MOA B. Most recently, Ueda and co-workers identified small molecule tranylcypromine derivatives that are selective for LSD1 over MAO-A and MAO-B,[31] and Binda et al. described similar tranylcypromine analogues that exhibited partial selectivity between LSD1 and the newly identified histone demethylase LSD2.[32]

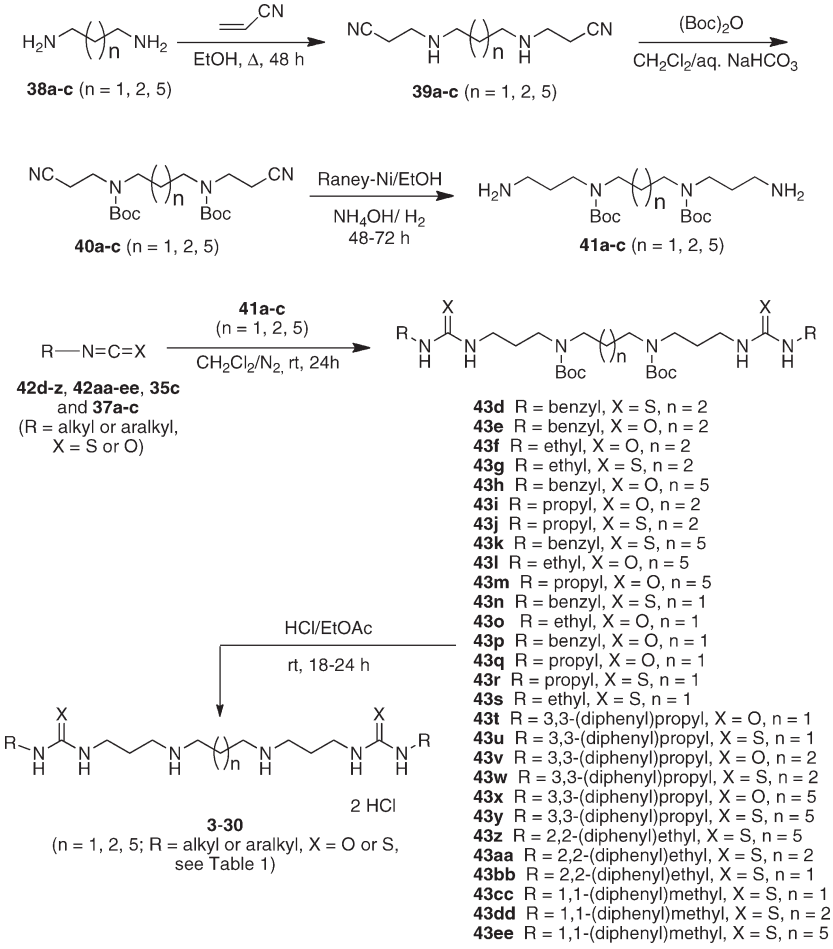
LSD1 was identified in part because its C-terminal domain shares significant sequence homology with the amine oxidases acetylpolyamine oxidase (APAO) and spermine oxidase (SMO).[10,33] Several groups have identified amines, guanidines, or similar analogues that act as selective modulators of these 2 amine oxidases.[33−39] We previously reported the synthesis of a novel series of (bis)guanidines and (bis)biguanides[40] that are potent antitrypanosomal agents in vitro, with IC_{50} values against *Trypanosoma brucei* as low as 90 nM. Because of their structural similarity to guanidine-based inhibitors of APAO and SMO, we sought to determine whether (bis)guanidines **1a**−**g** and (bis)biguanides **2a**−**f** (Figure 1) were inhibitors of LSD1 and whether this inhibition had any influence on selected chromatin marks in tumor cells. Nine of the 13 compounds tested were found to inhibit LSD1 activity by >50% at 1 μM.[25] The two most potent LSD1 inhibitors exhibited noncompetitive kinetics at concentrations up to 2.5 μM. A 48 h exposure of HCT116 human colon carcinoma cells to increasing concentrations of analogues **1c** and **2d** (Figure 1) produced significant global increases in both H3K4me1 and H3K4me2 while not affecting global H3K9-me2 levels. These analogues also induced the re-expression of multiple, aberrantly silenced genes important in the development of colon cancer, including members of the secreted frizzle-related proteins (SFRPs) and the GATA family of transcription factors.

Because of the promising cellular effects of **1c** and **2d**, the synthesis and evaluation of additional analogues was proposed. To access a library of more diverse analogues related to **1c** and **2d**, we adapted our previously published syntheses[40] to produce a series of 30 isosteric

Scheme 1



Scheme 2



43d R = benzyl, X = S, n = 2
43e R = benzyl, X = O, n = 2
43f R = ethyl, X = O, n = 2
43g R = ethyl, X = S, n = 2
43h R = benzyl, X = O, n = 5
43i R = propyl, X = O, n = 2
43j R = propyl, X = S, n = 2
43k R = benzyl, X = S, n = 5
43l R = ethyl, X = O, n = 5
43m R = propyl, X = O, n = 5
43n R = benzyl, X = S, n = 1
43o R = ethyl, X = O, n = 1
43p R = benzyl, X = O, n = 1
43q R = propyl, X = O, n = 1
43r R = propyl, X = S, n = 1
43s R = ethyl, X = S, n = 1
43t R = 3,3-(diphenyl)propyl, X = O, n = 1
43u R = 3,3-(diphenyl)propyl, X = S, n = 1
43v R = 3,3-(diphenyl)propyl, X = O, n = 2
43w R = 3,3-(diphenyl)propyl, X = S, n = 2
43x R = 3,3-(diphenyl)propyl, X = O, n = 5
43y R = 3,3-(diphenyl)propyl, X = S, n = 5
43z R = 2,2-(diphenyl)ethyl, X = S, n = 5
43aa R = 2,2-(diphenyl)ethyl, X = S, n = 2
43bb R = 2,2-(diphenyl)ethyl, X = S, n = 1
43cc R = 1,1-(diphenyl)methyl, X = S, n = 1
43dd R = 1,1-(diphenyl)methyl, X = S, n = 2
43ee R = 1,1-(diphenyl)methyl, X = S, n = 5

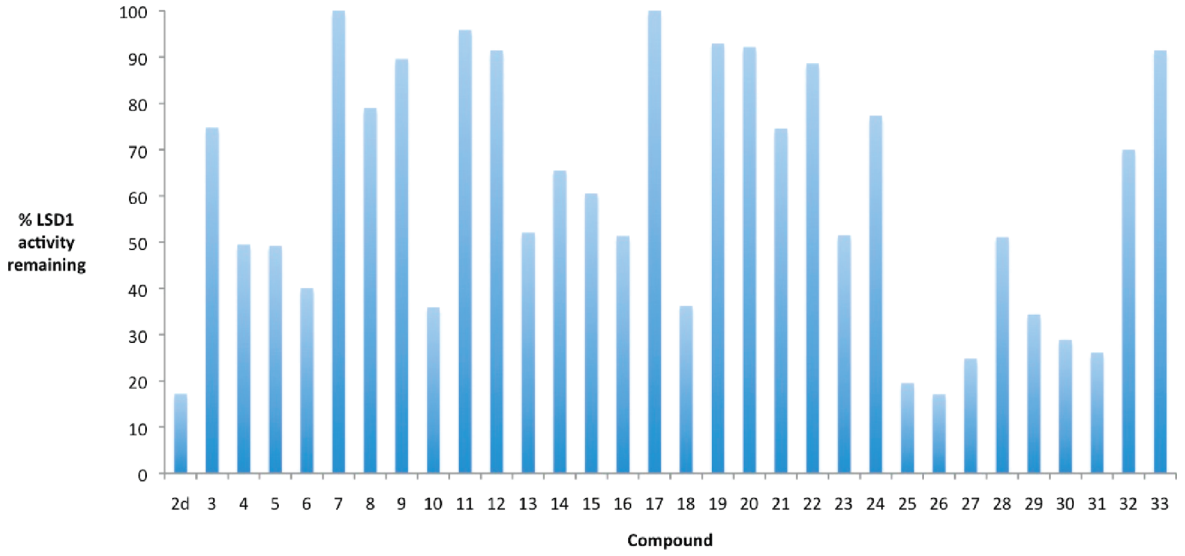
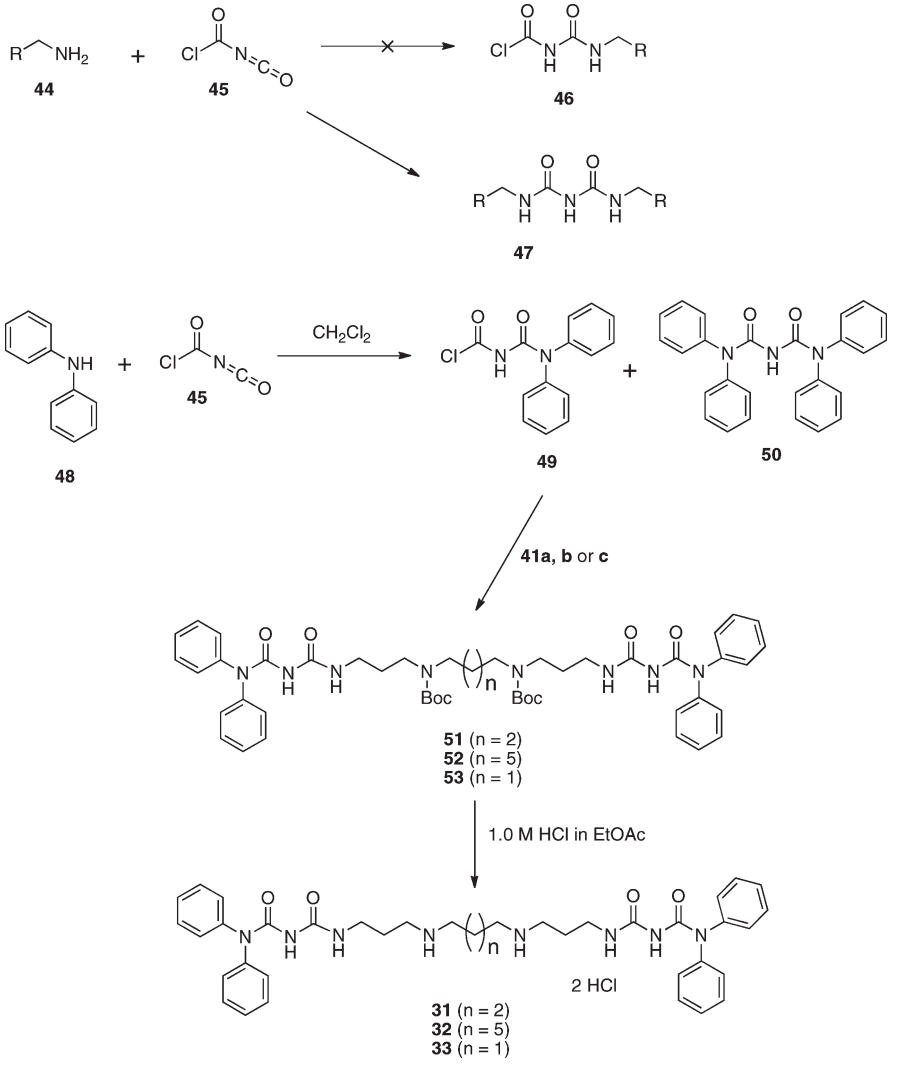


Figure 2. Effect of compounds **2d** and **3**–**33** on LSD1 activity in vitro. Percent of LSD1 activity remaining was determined following treatment with 10 μM of each test compound as determined by the luminol-dependent chemiluminescence method.

Scheme 3



(bis)alkylureas or (bis)alkylthioureas (compounds **3**–**33**, Table 1) and these analogues were evaluated for the ability to inhibit LSD1 and induce increases in global H3K4me2 in vitro.

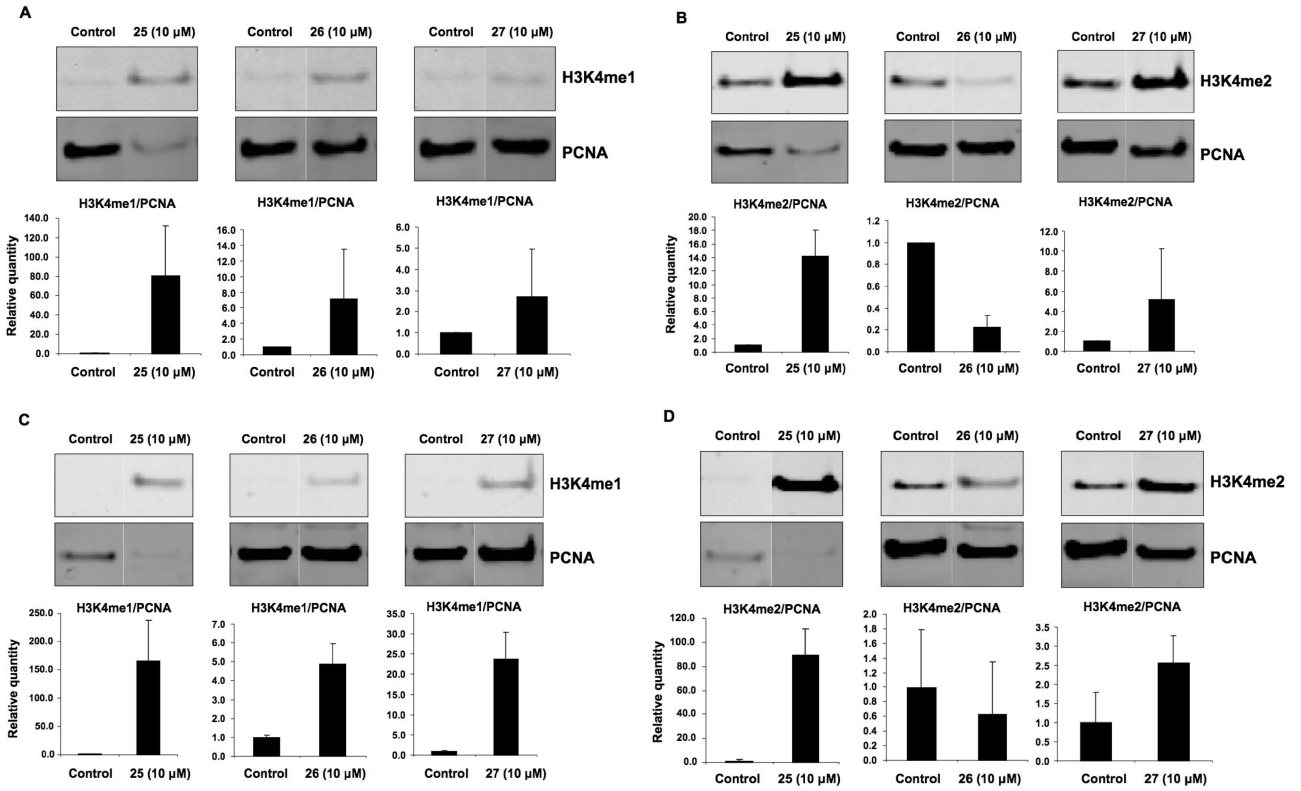


Figure 3. Effect of compounds **25**−**27** on the expression of global H3K4me1 and H3K4me2. Calu-6 human anaplastic nonsmall cell lung carcinoma cells were treated with a 10 μM concentration of **25**, **26**, or **27** for 24 h (A,B) or 48 h (C,D) as described in the Experimental Section. (A,C) Global H3K4me1 expression and (B,D) global H3K4me2 expression. Proliferating cell nuclear antigen (PCNA) was used as a loading control. Shown are Western blot images from a single representative experiment performed in triplicate. Relative protein expression levels were determined by quantitative Western analysis using the Odyssey infrared detection system shown as bar graphs. The results represent the mean of three treatments ± SD. The protein expression level for control samples was set to a value of 1.

Chemistry

Preparation of compounds **3**−**33** depended on the availability of the appropriate isocyanates and isothiocyanates. All of these intermediates were commercially available, with the exception of isocyanate **35c** and isothiocyanates **37a**−**c**, which were synthesized as shown in Scheme 1. Isocyanate **35c** could be made in a single step by reacting the requisite diphenylalkylamine **34c** (m = 2) with trichloroacetic anhydride (toluene, N_2, reflux) for 5 h.[41,42] To produce the corresponding isothiocyanates **37a**−**c** (m = 0, 1 or 2, respectively), amines **34a**−**c** were allowed to react with carbon disulfide in the presence of triethylamine in THF at 5 °C.[43] The reaction was allowed to warm to room temperature, and after 3 h, the intermediate dithiocarbamates **36a**−**c** could be isolated. Reaction of the intermediate dithiocarbamates **36a**−**c** with tosyl chloride in THF then afforded the desired isothiocyanates **37a**−**c**.[43]

To access a library of isosteric urea and thiourea analogues related to **1c** and **2d**, we employed our previously published synthesis[40] of precursor molecules **41a**−**c**, as shown in Scheme 2. The appropriate diamine **38a**, **38b**, or **38c** was (bis)cyanoethylated (acrylonitrile, EtOH, reflux) to afford the corresponding (bis)-cyano intermediates **39a**−**c**. The central nitrogens in **39a**−**c** were then N-Boc protected ($(Boc)_2O$, CH_2Cl_2/Aq. $NaHCO_3$)[44] to form **40a**−**c**, and the cyano groups were reduced (Raney Ni) to yield the desired diamines **41a**−**c**.[40,45] Compounds **41a**−**c** were then reacted with the appropriate isocyanates or isothiocyanates **42d**−**z**, **42aa**−**ee**, **35c**, and **37a**−**c**[42] to produce the corresponding protected (bis)ureas or (bis)thioureas **43d**−**z** and **43aa**−**ee**, followed by acid removal of the N-Boc protection groups (HCl in EtOAc)[44] to afford the desired urea or thiourea products **3**−**30**.

To access isosteric derivatives of the (bis)biguanide lead compound **2d**, the synthetic route outlined in Scheme 3 was devised. Initially, primary amines of general structure **44** were reacted with *N*-chlorocarbonyl isocyanate **45**,[41,46] with the intention of forming the alkyl *N*-chlorocarbonylurea **46**. However, this reaction could not be controlled, even at low temperature, to produce the monoalkylated derivative **46**, but immediately formed the bis-alkylated (bis)biguanide **47**. To form the desired monoalkylated product, it was necessary to use a less reactive secondary amine in the initial step. Thus diphenylamine **48** was added to *N*-chlorocarbonyl isocyanate **45**,[41,46] and the mixture was stirred for 20 min to form a mixture of **49** and the bis-alkylated product **50**. Addition of compounds **41a**,**b**, or **c**[40] to this reaction mixture in the presence of triethylamine produced the bis-N-Boc-protected precursors **51**−**53**, which were separated from **50** by silica gel chromatography. Acid-catalyzed removal of the N-Boc protecting groups[44] in **51**−**53** then afforded the desired target compounds **31**−**33**. Importantly, the syntheses described in Schemes 1−3 can be adapted to produce a wide variety of analogues with chemical diversity in the length of the alkyl chains, and in the terminal alkyl- or aralkyl substituents.

Biological Evaluation

The ability of the target (bis)ureas and (bis)thioureas to inhibit LSD1 was determined in an assay procedure utilizing the recombinant human enzyme. Expression and purification

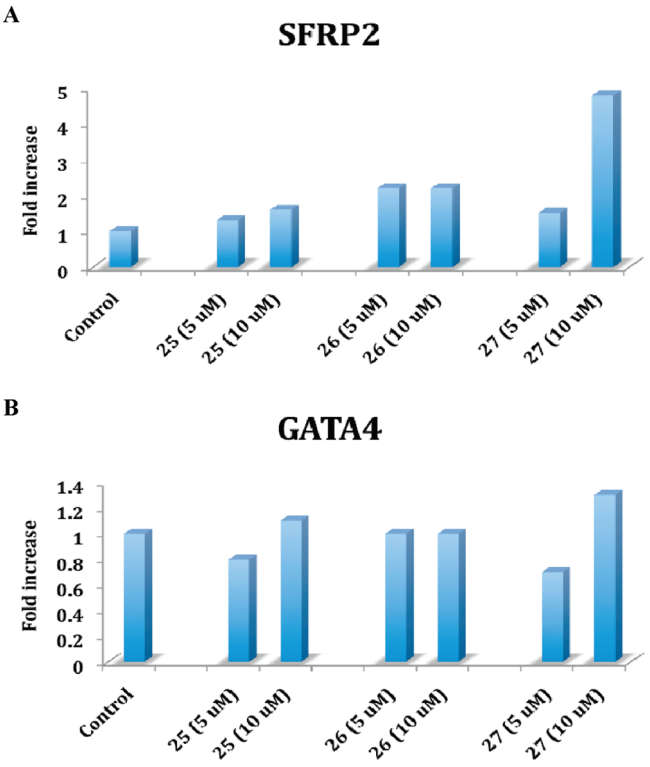


Figure 4. Effect of compounds **25**−**27** on the re-expression of secreted frizzle-related protein 2 (SFRP2, (A)) and the transcription factor GATA4 ((B)) mRNA. Calu-6 human anaplastic nonsmall cell lung carcinoma cells were treated with either a 5 or 10 μM concentration of **25**, **26**, or **27** for 24 h as described in the Experimental Section. cDNA was then synthesized from mRNA, amplified and measured by qPCR. Each data point is the average of three determinations that differed in all cases by 5% or less.

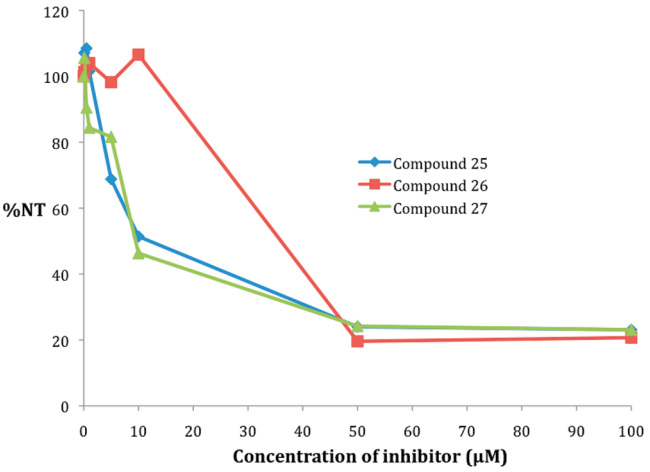


Figure 5. Effect of compounds **25**−**27** on Calu-6 human anaplastic nonsmall cell lung carcinoma cell viability as measured by standard MTS assay. Cells were treated with increasing concentrations of each test compound for 96 h prior to measurement of cell viability. %NT refers to the percent of viable cells remaining at time T (96 h) as compared to the number of cells seeded, N_0. Each data point is the average of three determinations that differed in all cases by 5% or less.

of LSD1 were conducted as previously reported.[10,25] Enzymatic activity of LSD1 in the presence of target compounds was determined using luminol-dependent chemiluminescence to measure the production of H_2O_2, as previously described.[25,33] The results of these experiments are summarized in Table 1 and graphically in Figure 2. As previously observed, compound **2d** at 10 μM reduced LSD1 activity by 82.9%. Among the 31 urea and thiourea isosteres **3**−**33**, six compounds were essentially inactive (i.e., produced $<20\%$ inhibition), while 11 analogues (ureas **4** and **5**, thioureas **6**, **10**, **18**, **25**, **26**, **27**, **29**, and **30** and the disubstituted carbamoylurea **31**) reduced LSD1 activity by 50% or greater at 10 μM concentration (Figure 2). The three most effective LSD1 inhibitors, compounds **25**−**27**, were chosen for additional studies as outlined below. Subsequent experiments were conducted in the Calu-6 human anaplastic nonsmall cell lung carcinoma line because it has a highly reproducible response to epigenetic modulation and because it is known that various tumor suppressor genes are silenced in this line. For synthetic analogues to be effective at the cellular level, any observed decreases in cellular LSD1 activity should be accompanied by an increase in global H3K4me1 and H3K4me2 content. Thus, the ability of compounds **25**, **26**, and **27** to produce increases in global H3K4me1 and H3K4me2 levels was measured as previously described.[10] The results of these studies are shown in Figure 3. At 24 h, analogues **25** and **27** produced significant increases in both H3K4me1 (Figure 3A) and H3K4me2 (Figure 3B), while analogue **26** induced a significant increase in H3K4me1 but decreased the relative amount of H3K4me2. A similar pattern was observed at 48 h (Figure 3C,D). Compound **25** produced the most dramatic increases in H3K4me1 and H3K4me2 at both 24 and 48 h. The reduction in H3K4me2 and corresponding increase in H3K4me1 by **26** at both 24 and 48 h cannot be readily explained and is the subject of continuing investigation. However, this anomalous finding seem to correlate with the observed cytotoxicity of **26** (see below). These data strongly suggest that intracellular inhibition of LSD1 by **25**−**27** leads to significant increases in methylation at the H3K4 chromatin mark. It is noteworthy that in HCT116 human colon tumor cells, compounds **25**−**27** all produced at least a 2-fold increase in global H3K4me2 (data not shown), with the most effective analogue being compound **25** (17.4-fold increase).

The ability of compounds **25**−**27** to induce the re-expression of aberrantly silenced tumor suppressor genes in vitro was next measured using the Calu-6 human lung carcinoma cell line. The tumor suppressor genes SFRP2 and GATA4 were chosen because they are known to be under expressed in human lung cancer and because they are thought to play a role in tumorigenesis when silenced. Thus, the genes coding for these proteins are well-documented LSD1 targets. Cells were treated for 24 h with either a 5 or 10 μM concentration of **25**, **26**, or **27**, after which the levels of secreted frizzle-related protein (SFRP) 2, a soluble modulator of Wnt signaling, and the zinc-finger transcription factor GATA4, were determined by quantitative PCR (qPCR). The results of these studies are shown in Figure 4. All three compounds produced increases in SFRP2 expression that appeared to be dose dependent for **25** and **27** (Figure 4A). Compound **27** produced the largest increase in SFRP2 expression at 10 μM (4.8-fold increase). Compounds **25** and **26** did not produce significant increases in GATA4 levels at 5 and 10 μM (Figure 4B), and compound **27** induced a 1.3-fold increase in GATA4 mRNA protein at 10 μM and had no significant effect at 5 μM (Figure 4B). The increase in GATA4 mRNA caused by 10 μM **27** is reproducible, but is not statistically significant ($P > 0.05$).

The (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) (MTS) reduction assay was used to determine the effects of compounds **25**−**27**

on cell viability in the Calu-6 cell line. Cells were treated with increasing concentrations of each test compound for 96 h prior to measurement of cell viability, and growth inhibition (GI_{50}) values were then determined from the resulting dose−response curve. As seen in Figure 5, compounds **25**, **26**, and **27** produced moderate reduction in cell viability, with GI_{50} values of 10.3, 38.3, and 9.4 μM, respectively.

Discussion

The potential LSD1 inhibitors **3**−**33** were synthesized using pathways that are facile and relatively inexpensive and that can be used to introduce chemical diversity into the resulting urea and thiourea analogues, thus making them suitable for generation of a library of related ureas and thioureas. Our initial series of guanidine and biguanide derivatives[25] represented the first novel small molecule inhibitors of LSD1 with potential for development as therapeutic agents. The current studies suggest that replacement of the imine NH functionality of the terminal guanidine in **1c** with oxygen or sulfur is an allowable isosteric change, and active analogues in both the urea and thiourea series were identified (Figure 2). However, the sulfur isosteric replacement is likely more acceptable because the six best LSD1 inhibitors (**6**, **10**, **18**, and **25**−**27**) were all thioureas. A more bulky aromatic substituent on the terminal nitrogen, as in **25**−**27**, appears to impart greater activity than the smaller alkyl or benzyl substituents found in **6**, **10**, and **18**. There did not appear to be predictable differences in activity between analogues with 3, 4, or 7 carbon central chains, suggesting that this parameter may not have a great influence on inhibition of the enzyme. This is especially apparent among **25**−**27** (terminal N-substituent = 2,2-diphenylethyl), which have 7, 4, and 3 carbon central regions, respectively, but vary in activity by less than 5%. However, by contrast, among compounds **28**−**30** (terminal N-substituent = 1,1-diphenylmethyl), inhibitory potency did appear to be proportional to the length of the internal carbon chain. In addition to the urea and thiourea derivatives, the carbamoylurea **31**, designed as an analogue of **2d**, also produces potent inhibition of LSD1 (73.9% at 10 μM). Additional analogues will need to be synthesized and evaluated to generate a more accurate set of structure/activity relationships for this series of compounds.

The inhibitory effects of **25**−**27** on LSD1 (Figure 2), combined with the observed methylation levels at the H3K4 chromatin mark (Figure 3A−D), strongly suggest that LSD1 is inhibited in the Calu-6 tumor cell line, resulting in increases in the substrates H3K4me1 and H3K4me2. The anomalous reduction in H3K4me2 at 24 and 48 h caused by **26** are unexpected and have yet to be explained. In future studies, we will attempt to elucidate the mechanism of this effect. In addition, the effects of **25**−**27** on other histone demethylases, including LSD2,[47] the Jumonji C (JmjC) domain-containing demethylases,[10,11,17,18] and the recently discovered JmjC demethylase PHF8,[48,49] need to be determined. Additional experiments are being undertaken to determine the inhibitor selectivity of these analogues.

Compounds **25**−**27** were next evaluated for the ability to induce the re-expression of SFRP2 and GATA4 protein, as determined by qPCR from treated Calu-6 human lung carcinoma cells. In the case of SFRP2, all three analogues induced increases of the corresponding mRNA between 1.3- and 4.8-fold (Figure 4A). These increases appeared to be dose-dependent, except in the case of **26**, which induced same level of SFRP2 expression at both 5 and 10 μM. The order of potency in this regard was **27** > **26** > **25**. Compound **27** produced a 1.3-fold increase in GATA4 expression at 10 μM that was not statistically significant and **25**−**27** at all other concentrations produced no effect on GATA4. The observed increases in SFRP2 re-expression following treatment with **25**−**27**, and the increase in GATA4 re-expression induced by 10 μM **27**, are consistent with the previously reported effects of the parent compounds **1c** and **2d**.[25] The disparity in the ability of **25**−**27** to induce SFRP2 expression, but not GATA4 expression, suggests that LSD1 inhibition may have variable effects at different gene promoters. A more detailed account of the effects of **3**−**33** on these and other transcription factors will be reported in a subsequent manuscript.

As discussed above, compounds **25**−**27** proved to be only moderately cytotoxic in the Calu-6 nonsmall cell lung carcinoma line in vitro. Compounds **25** and **27** produced the most prominent reduction in cell viability, exhibiting GI_{50} values of 10.3 and 9.4 μM, respectively. These values are comparable to the GI_{50} value for other epigenetic modulators such as the polyaminohydroxamic acid and polyaminobenzamide HDAC inhibitors developed in our laboratory[50,51] and the parent compound **2d**. In addition, these GI_{50} values are in the range of the histone deacetylase (HDAC) inhibitor MS-275, as measured in three colon tumor cell lines.[52] Compound **26** was significantlty less cytotoxic, exhibiting a GI_{50} value of 38.3 μM. Our data suggests that decreases in H3K4me2 at 24 and 48 h and/or minimal effects on the re-expression of SFRP2 and GATA4 by **26** could account for this reduced cytotoxicity. It is important to note that epigenetic modulators such as those mentioned above are generally used in combination with traditional cytotoxic agents and serve to restore the ability of transformed cells to undergo apoptosis.[53] As such, cytotoxicity is less of an issue as long as the compound produces epigenetic effects in tumor cells that can be exploited by traditional cytotoxic agents. We have recently shown that the LSD1 inhibitor **2d** alone has little effect in vivo on tumor cell growth in an HCT116 human colon carcinoma mouse xenograft model but acts synergistically to limit tumor growth in combination with the DNA methyltransferase inhibitor 5-azacytidine.[54] Additional studies are now being conducted to determine whether isosteres of **2d** such as **25**−**27** also produce a synergistic effect on tumor cell growth in vivo. Additional biological studies, as well as the synthesis and evaluation of additional LSD1 inhibitors in this and other compound libraries, is an ongoing effort in our laboratory.

Experimental Section

All reagents and dry solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI), Sigma Chemical Co. (St. Louis, MO), or Acros Chemical (Chicago, IL) and were used without further purification except as noted below. Pyridine was dried by passing it through an aluminum oxide column and then stored over KOH. Triethylamine was distilled from potassium hydroxide and stored in a nitrogen atmosphere. Methanol was distilled from magnesium and iodine under a nitrogen atmosphere and stored over molecular sieves. Methylene chloride was distilled from phosphorus pentoxide, and chloroform was distilled from calcium sulfate. Tetrahydrofuran was purified by distillation from sodium and benzophenone. Dimethyl formamide was dried by distillation from anhydrous calcium sulfate and was stored under nitrogen. Preparative scale chromatographic procedures were carried out using E. Merck silica gel 60, 230−440 mesh. Thin layer chromatography was conducted on Merck precoated silica gel 60 F-254. Ion exchange chromatography was conducted on

Dowex 1 × 8−200 anion exchange resin. Compounds **41a**−**c**[40,55] were synthesized as previously described.

All ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 mHz spectrometer, and all chemical shifts are reported as δ values referenced to TMS or DSS. Infrared spectra were recorded on a Jasco FT-IR spectrophotometer and are referenced to polystyrene. In all cases, ^{1}H NMR, ^{13}C NMR, and IR spectra were consistent with assigned structures. Mass spectra were recorded on a Kratos MS 80 RFA (EI and CI) or Kratos MS 50 TC (FAB) mass spectrometer. Prior to biological testing, target molecules **3**−**33** were determined to be 95% pure or greater by HPLC chromatography using an Agilent series 1100 high-performance liquid chromatograph fitted with a C18 reversed-phase column.

Synthetic H3K4me2 peptides were purchased from Millipore (Billerica, MA). Calu-6 cells were maintained in RPMI medium, supplemented with 10% fetal bovine serum (Gemini Bio-Products, Woodland, CA) and grown at 37 °C in 5% CO_2 atmosphere.

3,3-Diphenylpropylisocyanate (35c). A 4.24 g (0.020 mol) portion of 3,3-diphenylpropylamine was dissolved in 90 mL of dry toluene in a 250 mL round-bottomed flask under a nitrogen atmosphere, and triphosgene (2.98 g, 0.010 mol) was added to the reaction mixture. The reaction mixture was heated under reflux for 5 h and then cooled to room temperature, at which time an additional 0.5 g of triphosgene was added. The reaction was then stirred for an additional 18 h at room temperature. During this time, the formation of product was monitored by TLC using hexane:ethyl acetate (3:1). When the reaction was complete, activated charcoal (0.50 g) was carefully added into reaction mixture to decolorize the solution, which was stirred for 30 min and filtered. The filtrate was concentrated under reduced pressure to give a light pale-yellow semisolid. A 100 mL portion of *n*-hexane/ethyl ether(1:1 ratio) was then added, and the mixture was stirred for 15 min. The solution was filtered and concentrated to afford 4.23 g of viscous material. The crude product was purified by flash chromatography on silica gel eluted with dichloromethane to furnish 3,3-diphenylpropylisocyanate **35c** as a white solid (1.31 g, 28% yield). ^{1}H NMR ($CDCl_3$): δ 7.38−7.10 (m, 10H, Ar-H), 4.09 (t, 1H, J = 7.2 Hz, $CHPh_2$), 3.27 (t, 2H, J = 6.4 Hz, CH_2NCS), 2.36 (m, 2H, CH_2CH_2). ^{13}C NMR ($CDCl_3$): δ 143.69, 128.94, 128.01, 126.85 (Ar-C), 48.14, 41.51, 36.87 (CH and CH_2).

General Procedure for Preparation of Isothiocyanates 37a−c. 3,3-Diphenylpropylisothiocyanate (37c). In a 250 mL round-bottomed flask under a nitrogen atmosphere, 3,3-diphenylpropylamine **34c** (2.10 g, 0.010 mol) was dissolved in 40 mL of freshly distilled THF, 3.64 g (5.0 mL, 0.036 mol) of triethylamine was added, and the mixture was cooled to 5 °C in an ice bath. Carbon disulfide (0.76 g, 0.96 mL, 0.10 mol) was then added to the reaction mixture via syringe over 20 min. Following addition of carbon disulfide, the mixture was stirred an additional 30 min, warmed to room temperature, and allowed to stir a further 2 h. A ^{1}H NMR of an aliquot (after removing the solvent in vacuo) indicated that conversion to the dithiocarbamate salt **36c** was complete. ^{1}H NMR (DMSO-d_6): δ 8.46 (t, 1H, NH), 7.34−7.12 (m, 8H, Ar-H), 7.06 (t, 2H, Ar-H), 3.94 (t, 1H, $CHPh_2$), 3.34 (m, 2H, CH_2NCS), 3.04 (q, 6H, NCH_2CH_3), 2.24 (m, 2H, CH_2CH_2), 1.20 (t, 6H, NCH_2CH_3).

The reaction mixture from above was recooled in an ice bath, 2.38 g of tosyl chloride (0.012 mol) was added, and the reaction mixture was allowed to stir for 30 min at 5 °C. It was then warmed to room temperature and stirred for an additional 3 h. The solvent was removed in vacuo, the reaction was partitioned between 40 mL of 1.0 N HCl and 150 mL of Et_2O, and the two-phased mixture was stirred for 10 min. The organic layer was separated and the aqueous layer was extracted with a 100 mL portion of Et_2O. The combined organic layers was dried over Na_2SO_4 and concentrated to produce a viscous oil that solidified during vacuum drying. The product was purified by flash chromatography on silica gel (eluted with CH_2Cl_2) to give **37c** as a white solid (1.48 g, 53% based on **34c**, TLC R_f: 0.45 (*n*-hexane/EtOAc, 9:1). ^{1}H NMR ($CDCl_3$): δ 7.32−7.19 (m, 10H, Ar-H), 4.08 (t, 1H, J = 8.0 Hz, $CHPh_2$), 3.44 (t, 2H, J = 6.8 Hz, CH_2NCS), 2.41 (m, 2H, CH_2CH_2). ^{13}C NMR ($CDCl_3$): δ 143.17, 129.08, 127.97, 126.99 (Ar-C), 48.12, 43.66, 35.69 (CH and CH_2).

1,1-Diphenylmethylisothiocyanate (37a). Isothiocyanate **37a** was prepared from 1,1-diphenylethylamine **34a** and carbon disulfide using the procedure described above for the synthesis of **37c**. The product was isolated as a white solid in 70% yield. TLC R_f: 0.90 (*n*-hexane/$MeCO_2Et$, 4:1). ^{1}H NMR ($CDCl_3$): δ 7.40−7.31 (m, 10H, Ar-H), 5.99 (s, 1H, $CHPh_2$). ^{13}C NMR ($CDCl_3$): δ 139.43, 129.18, 128.57, 126.85 (Ar-C), 64.82 (CH).

2,2-Diphenylethylisothiocyanate (37b). Isothiocyanate **37b** was prepared from 1,1-diphenylethylamine **34a** and carbon disulfide using the procedure described above for the synthesis of **37c**. The product was isolated as a white solid in 87% yield. ^{1}H NMR (DMSO-d_6): δ 7.36−7.29 (m, 8H, Ar-H), 7.24−7.20 (t, 2H, J = 7.2 Hz, Ar-H), 4.45 (t, 1H, J = 8.0 Hz, $CHPh_2$), 4.34 (d, 2H, J = 7.6 Hz, CH_2NCS). ^{13}C NMR (DMSO-d_6): δ 1 41.64, 129.31, 128.53, 127.67 (Ar-C), 51.18, 48.95 (CH and CH_2).

General Procedure for Preparation of N-Boc Protected (Bis)-thioureas. 1,12-Bis-{3-[1-(benzyl)thioureado]}-4,9-[*N*-(*tert*-butyl)-oxycarbonyl)]-4,9-diazadodecane (43d). In a 100 mL round-bottom flask, a 0.3 g portion of 4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diaza-1,12-diaminododecane **41b** (0.0008 mol) was dissolved in 20 mL of HPLC grade CH_2Cl_2 under a nitrogen atmosphere and the mixture was cooled to 0 °C. A solution of benzylisothiocyanate (240 mg, 0.0016 mol) in 5 mL of CH_2Cl_2 was then added dropwise with stirring, and the reaction mixture was allowed to stir at room temperature for 5 h. During this time, the formation of product was monitored by TLC (CH_2Cl_2/MeOH/NH_4OH 89:10:1). After completion of the reaction, the CH_2Cl_2 was removed under reduced pressure to produce a viscous colorless oil. The crude product was purified by flash chromatography on silica gel eluted with CH_2Cl_2/MeOH/NH_4OH (94.5:5:0.5 followed by 89:10:1) to furnish pure **43d** (0.46 g, 88% yield) as viscous oil. R_f: 0.46 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.31 (m, 10H, Ar-H), 6.31 (b, 2H, NH), 4.55 (bs, 4H, NCH_2), 3.54 (bs, 4H, NCH_2), 3.20 (bs, 4H, NCH_2), 3.10 (bs, 4H, NCH_2), 1.65 (bs, 4H, CH_2CH_2), 1.46 (bs, 4H, CH_2CH_2), 1.38 (s, 18H, $C[CH_3]_3$).

1,12-Bis-{3-[1-(ethyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43g). Compound **43g** was prepared from 375 mg of **41b** (375 mg, 0.0009 mol) and ethylisothiocyanate according to procedure described above for the synthesis of **43d** to afford **43g** (512 mg, 95%) as viscous oil. R_f: 0.52 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.40 (b, 2H, NH), 6.00 (b, 2H, NH), 3.56 (m, 4H, NCH_2), 3.34 (b, 4H, NCH_2), 3.26 (b, 4H, NCH_2), 3.12 (b, 4H, NCH_2), 1.71 (b, 4H, CH_2CH_2), 1.50 (bs, 4H, CH_2CH_2), 1.40 (s, 18H, $C(CH_3)_3$), 1.20 (t, 6H, J = 7.2 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 80.36 ($[CH_3]_3C$), 46.95, 43.34, 41.19, 38.12, 28.63, 27.31, 26.16 (CH_2), 14.28 (CH_3).

1,12-Bis-{3-[1-(propyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43j). Compound **43j** was prepared from 260 mg of **41b** (0.0007 mol) and *n*-propylisothiocyanate according to procedure described above for the synthesis of **43d** to afford **43j** (380 mg, 96%) as viscous oil. R_f: 0.51 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ3.50−3.36 (b, 8H, NCH_2), 3.28−3.20 (m, 8H, NCH_2), 3.26 (b, 4H, NCH_2) 1.78 (b, 4H, CH_2CH_2), 1.52 (bs, 4H, CH_2CH_2), 1.46 (s, 18H, C-$[CH_3]_3$), 0.73 (t, 6H, J = 7.2 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ80.36 ($[CH_3]_3C$), 46.95, 43.34, 41.19, 38.12, 28.63, 27.31, 26.16 (CH_2), 14.28 (CH_3).

1,15-Bis-{3-[1-(benzyl)thioureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43k). Compound **43k** was prepared from 220 mg of **41c** (0.0005 mol) and benzylisothiocyanate according to procedure described above for the synthesis of **43d** to afford **43k** (360 mg, 96%) as viscous oil. ^{1}H NMR

($CDCl_3$): δ 7.39−7.30 (m, 10H, Ar-H), 4.76 (b, 4H, CH_2Ph), 3.46 (b, 4H, NCH_2), 3.18 (m, 8H, NCH_2), 1.52 (b, 4H, CH_2CH_2), 1.54 (b, 4H, CH_2CH_2), 1.44 (s, 18H, $C(CH_3)_3$), 1.28 (b, 6H, CH_2CH_2).

1,11-Bis-{3-[1-(benzyl)thioureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43n). Compound **43n** was prepared from 291 mg of **41a** (0.0008 mol) and benzylisothiocyanate according to the procedure described above for **43d** to afford **43n** (373 mg, 73%) as viscous oil. R_f: 0.87 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.35 (m, 10H, Ar-H), 4.58 (bs, 4H, N-CH_2), 3.58 (bs, 4H, N-CH_2), 3.21 (b, 4H, N-CH_2), 3.10 (b, 4H, N-CH_2), 1.72 (b, 6H, CH_2CH_2), 1.40 (s, 18H, $C[CH_3]_3$).

1,11-Bis-{3-[1-(propyl)thioureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43r). Compound **43r** was prepared from 291 mg of **41a** (0.0008 mol) and *n*-propylisothiocyanate according to the procedure described above for **43d** to afford **43r** (379 mg, 86%) as viscous oil. R_f: 0.57 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.29 (bs, 1H, NH), 6.44 (s, 2H, NH), 3.43 (bs, 4H, N-CH_2), 3.01−3.15 (b, 12H, N-CH_2), 1.61 (bs, 6H, CH_2CH_2), 1.47 (m, J = 7.2 Hz, 4H, CH_2CH_3), 1.31 (s, 18H, $C[CH_3]_3$), 0.81 (t, J = 7.2 Hz, 6H, CH_2CH_3).

1,11-Bis-{3-[1-(*n*-ethyl)thioureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43s). Compound **43s** was prepared from 291 mg of **41a** (0.0008 mol) and ethylisothiocyanate according to the procedure described above for **43d** to afford **43s** (347 mg, 83%) as viscous oil. R_f: 0.72 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.24 (bs, 2H, NH), 6.22 (bs, 2H, NH), 3.49 (bs, 4H, CH_2N), 3.29 (bs, 4H, N-CH_2), 3.20 (b, 4H, N-CH_2), 3.07 (b, 4H, N-CH_2), 1.62−1.74 (b, 6H, CH_2CH_2), 1.37 (s, 18H, $C[CH_3]_3$), 1.14 (t, 6H, CH_2CH_3).

1,11-Bis-{3-[1-(3,3-diphenylpropyl)thioureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43u). Compound **43u** was prepared from 155 mg of **41a** (0.0004 mol) and **37c** according to procedure described above for the synthesis of **43d** to afford **43u** (290 mg, 81%) as a white solid. R_f: 0.44 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.29−7.15 (m, 22H, Ar-H, and NH), 5.88 (b, 2H, NH), 4.04 (t, 2H, J = 7.6 Hz, $CHPh_2$), 3.53 (b, 4H, NCH_2), 3.28 (b, 4H, NCH_2), 3.23 (b, 4H, NCH_2), 3.12 (b, 8H, NCH_2), 2.36 (q, 4H, J = 8.0 Hz, NCH_2), 1.70 (m, 2H, CH_2CH_2), 1.47 (b, 4H, CH_2CH_2), 1.40 (s, 20H, $C[CH_3]_3$).

1,12-Bis-{3-[1-(3,3-diphenylpropyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43w). Compound **43w** was prepared from 161 mg of **41b** (0.0004 mol) and **37c** according to procedure described above for the synthesis of **43d** to afford **43w** (322 mg, 89%) as a white solid. R_f: 0.52 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.25−7.16 (m, 22H, Ar-H, and NH), 5.88 (b, 2H, NH), 4.02 (t, 2H, J = 8.0 Hz, $CHPh_2$), 3.17 (b, 8H, NCH_2), 3.09 (b, 4H, NCH_2), 2.37 (q, 4H, J = 7.6 Hz, CH_2CH), 1.76−1.65 (m, 8H, CH_2CH_2), 1.41 (s, 18H, $C[CH_3]_3$).

1,15-Bis-{3-[1-(3,3-diphenylpropyl)thioureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43y). Compound **43y** was prepared from 178 mg of **41c** (0.0004 mol) and **37c** according to procedure described above for the synthesis of **43d** to afford **43y** (305 mg, 80%) as a white solid. R_f: 0.57 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.28−7.15 (m, 20H, Ar-H), 5.88 (b, 2H, NH), 4.02 (t, 2H, J = 8.0 Hz, $CHPh_2$), 3.54 (b, 4H, NCH_2), 3.28 (b, 4H, NCH_2), 3.23 (b, 4H, NCH_2), 3.08 (t, 4H, J = 7.2 Hz, NCH_2), 2.36 (q, 4H, J = 7.6 Hz, CH_2CH), 1.69 (bs, 4H, CH_2CH_2), 1.50 (b, 4H, CH_2CH_2), 1.40 (s, 18H, $C[CH_3]_3$), 1.28 (m, 6H, CH_2CH_2).

1,15-Bis-{3-[1-(2,2-diphenylethyl)thioureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43z). Compound **43z** was prepared from 223 mg of **41c** (0.0005 mol) and **37b** according to procedure described above for the synthesis of **43d** to afford **43z** (288 mg, 79%) as a white solid. R_f: 0.68 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.31−7.19 (m, 20H, Ar-H), 5.75 (b, 2H, NH), 4.28 (b, 2H, $CHPh_2$), 4.02 (b, 4H, NCH_2), 3.54 (b, 4H, NCH_2), 3.25 (b, 4H, NCH_2), 3.09 (t, 4H, J = 7.2 Hz, NCH_2), 1.69 (bs, 4H, CH_2CH_2), 1.49 (b, 4H, CH_2CH_2), 1.40 (bs, 18H, $C[CH_3]_3$), 1.24 (m, 6H, CH_2CH_2).

1,12-Bis-{3-[1-(2,2-diphenylethyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43aa). Compound **43aa** was prepared from 161 mg of **41b** (0.0004 mol) and **37b** according to procedure described above for the synthesis of **43d** to afford **43aa** (295 mg, 84%) as a white solid. R_f: 0.60 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.32−7.20 (m, 20H, Ar-H), 5.77 (b, 2H, NH), 4.29 (b, 2H, $CHPh_2$), 4.02 (b, 4H, NCH_2), 3.56 (bs, 4H, NCH_2), 3.26 (bs, 4H, NCH_2), 3.12 (bs, 4H, NCH_2), 1.70 (b, 4H, CH_2CH_2), 1.48 (b, 4H, CH_2CH_2), 1.41 (s, 18H, $C[CH_3]_3$).

1,11-Bis-{3-[1-(2,2-diphenylethyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43bb). Compound **43bb** was prepared from 193 mg (0.0005 mol) of **41b** and **37b** according to procedure described above for the synthesis of **43d** to afford **43bb** (350 mg, 80%) as a white solid. R_f: 0.63 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.32−7.20 (m, 20H, Ar-H), 5.77 (bs, 2H, NH), 4.29 (bs, 2H, $CHPh_2$), 4.02 (bs, 4H, NCH_2), 3.56 (bs, 4H, NCH_2), 3.26 (bs, 4H, NCH_2), 3.12 (t, 4H, J = 7.2 Hz, NCH_2), 1.71 (b, 4H, CH_2CH_2), 1.41 (b, 20H, CH_2 and $C[CH_3]_3$).

1,11-Bis-{3-[1-(1,1-diphenylmethyl)thioureado]}-4,8-[N-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43cc). Compound **43cc** was prepared from 192 mg of **41a** (0.0005 mol) and **37a** according to procedure described above for the synthesis of **43d** to afford **43cc** as a white solid (350 mg, 83%), R_f: 0.63 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.34−7.27 (m, 20H, Ar-H), 6.43 (d, 2H, J = 5.2 Hz, NCH), 6.02 (b, 2H, NH), 3.52 (d, 4H, J = 5.2 Hz, NCH_2), 3.06 (m, 8H, NCH_2), 1.66 (bs, 6H, CH_2CH_2), 1.36 (bs, 18H, $C[CH_3]_3$).

1,12-Bis-{3-[1-(1,1-diphenylmethyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43dd). Compound **43dd** was prepared from 201 mg of **41b** (0.0005 mol) and **37a** according to procedure described above for the synthesis of **43d** to afford **43dd** (380 mg, 89%) as white solid. R_f: 0.60 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.40 (b, 2H, NH), 7.34−7.27 (m, 20H, Ar-H), 6.43 (d, 2H, J = 5.2 Hz, NCH), 6.02 (b, 2H, NH), 3.52 (d, 4H, J = 5.2 Hz, NCH_2), 3.06 (bs, 8H, NCH_2), 1.63 (m, 4H, CH_2CH_2), 1.42 (bs, 4H, CH_2CH_2), 1.36 (s, 18H, $C[CH_3]_3$).

1,15-Bis-{3-[1-(1,1-diphenylmethyl)thioureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43ee). Compound **43ee** was prepared from 223 mg of **41c** and **37a** according to procedure described above for the synthesis of **43d** to afford **43ee** (408 mg, 91%) as a white solid. R_f: 0.77 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.45 (b, 2H, NH), 7.33−7.26 (m, 20H, Ar-H), 6.41 (d, 2H, J = 2.8 Hz, NCH), 6.03 (b, 2H, NH), 3.51 (m, 4H, NCH_2), 3.04 (m, 8H, NCH_2), 1.54 (bs, 4H, CH_2CH_2), 1.45 (b, 4H, CH_2CH_2), 1.35 (bs, 18H, $C[CH_3]_3$), 1.23 (m, 6H, CH_2CH_2).

General Procedure for Preparation of N-Boc Protected (Bis)-ureas. 1,12-Bis-{3-[1-(benzyl)ureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43e). In a 100 mL round-bottom flask, a 0.35 g portion of 4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-1,12-diamino-diazadodecane **41b** (0.0009 mol) was dissolved in 20 mL of HPLC grade CH_2Cl_2 under a nitrogen atmosphere and the mixture was cooled to 0 °C. A solution of benzylisocyanate (0.235 g, 0.0018 mol) in 5 mL of CH_2Cl_2 was then added dropwise with stirring, and the reaction mixture was allowed to stir at room temperature for 24 h. During this time, the formation of product was monitored by TLC (CH_2Cl_2:MeOH:NH_4OH 89:10:1). When the starting material had been consumed, the CH_2Cl_2 was removed under reduced pressure to afford a viscous colorless material. The crude product was purified by flash chromatography on silica gel eluted with CH_2Cl_2:MeOH:NH_4OH (97:2.5:0.5 followed by 94.5:5.0:0.5) to furnish pure **43e** (0.50 g, 86% yield) as viscous oil. R_f: 0.54 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 3.20−3.02 (m, 16H, NCH_2), 1.64 (b, 4H, CH_2CH_2), 1.48 (b, 4H, CH_2CH_2), 1.43 (s, 18H, $C[CH_3]_3$), 1.11 (t, 6H, J = 6.4 Hz, CH_3).

1,12-Bis-{3-[1-(ethyl)ureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43f). Compound **43f** was prepared from 368 mg of **41b** (0.0009 mol) and ethylisocyanate according to the procedure described above for **43e** to afford **43f** (480 mg, 96%) as viscous oil. R_f: 0.54 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 3.20−3.02 (m, 16H, NCH_2), 1.64 (b, 4H, CH_2CH_2), 1.48 (b, 4H, CH_2CH_2), 1.43 (s, 18H, $C[CH_3]_3$), 1.11 (t, 6H, J = 6.4 Hz, CH_3).

1,15-Bis-{3-[1-(benzyl)ureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43h). Compound **43h** was prepared from 230 mg of **41b** (0.0005 mol) and benzylisocyanate according to the procedure described above for **43e** to afford **43h** (350 mg, 96%) as viscous oil. R_f: 0.50 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR (CD_3OD): δ 7.30 (m, 10H, Ar-H), 4.29 (s, 4H, CH_2Ph), 3.21−3.15 (m, 8H, NCH_2), 3.12 (t, 4H, J = 7.2 Hz, NCH_2), 1.70 (bs, 4H, CH_2CH_2), 1.50 (bs, 4H, CH_2CH_2), 1.44 (s, 18H, $C[CH_3]_3$), 1.32 (bs, 6H, CH_2CH_2).

1,12-Bis-{3-[1-(propyl)ureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43i). Compound **43i** was prepared from 260 mg of **41c** (0.0005 mol) and *n*-propylisocyanate according to the procedure described above for **43e** to afford **43i** (356 mg, 94%) as viscous oil. R_f: 0.54 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR (CD_3OD): δ 3.22 (m, 8H, NCH_2), 3.09 (t, 4H, J = 6.4 Hz, NCH_2), 3.05 (t, 4H, J = 7.6 Hz, NCH_2), 1.70 (b, 4H, CH_2CH_2), 1.50 (m, 8H, CH_2CH_2), 1.45 (s, 18H, $C[CH_3]_3$), 0.90 (t, 6H, J = 7.6 Hz, CH_3).

1,15-Bis-{3-[1-(benzyl)ureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43l). Compound **43l** was prepared from 225 mg of **41c** (0.0005 mol) and ethylisocyanate according to the procedure described above for **43e** to afford **43l** (280 mg, 94%) as viscous oil. R_f: 0.37 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR (CD_3OD): δ 3.28−3.12 (m, 8H, NCH_2), 3.10−3.06 (m, 8H, NCH_2), 1.68 (b, 4H, CH_2CH_2), 1.54 (b, 4H, CH_2CH_2), 1.44 (s, 18H, $C[CH_3]_3$), 1.30 (b, 6H, CH_2CH_2), 1.08 (t, 6H, J = 7.2 Hz, CH_2CH_2).

1,15-Bis-{3-[1-(propyl)ureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43m). Compound **43m** was prepared from 225 mg of **41c** (0.0005 mol) and propylisocyanate according to the procedure described above for **43e** to afford **43m** (280 mg, 92%) as viscous oil. R_f: 0.35 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR (CD_3OD): δ 3.25−3.16 (m, 8H, NCH_2), 3.09−3.02 (m, 8H, NCH_2), 1.68 (b, 4H, CH_2CH_2), 1.52 (b, 8H, CH_2CH_2), 1.44 (s, 18H, $C[CH_3]_3$), 1.30 (b, 6H, CH_2CH_2), 0.90 (t, 6H, J = 7.2 Hz, CH_2CH_2).

1,11-Bis-{3-[1-(ethyl)ureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43o). Compound **43o** was prepared from 287 mg of **41a** (0.0007 mol) and ethylisothiocyanate according to the procedure described above for **43e** to afford **43o** (245 mg, 62%) as viscous oil, R_f: 0.63 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 5.59 (bs, 1H, NH), 4.60 (bs, 1H, NH), 3.08−3.31 (m, 16H, N-CH_2), 1.58−1.78 (m, 6H, CH_2CH_2), 1.43 (s, 18H, $C[CH_3]_3$), 1.10 (t, J = 7.2 Hz, 6H, CH_2CH_3).

1,11-Bis-{3-[1-(benzyl)ureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43p). Compound **43p** was prepared from 302 mg of **41a** (0.0008 mol) and benzylisothiocyanate according to the procedure described above for **43e** to afford **43p** (485 mg, 95%) as viscous oil, R_f: 0.63 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.10−7.30 (m, 10H, Ar-H), 4.20 (bs, 4H, $PhCH_2$), 2.98−3.20 (m, 12H, N-CH_2), 1.65 (p, 2H, CH_2CH_2), 1.55 (p, J = 6.4 Hz, 4H, CH_2CH_2), 1.39 (s, 18H, $C[CH_3]_3$).

1,11-Bis-{3-[1-(*n*-propyl)ureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43q). Compound **43q** was prepared from 291 mg of **41a** (0.0008 mol) and *n*-propylisothiocyanate according to the procedure described above for **43e** to afford **43q** (359 mg, 91%) as viscous oil. R_f: 0.63 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 5.60 (bs, 1H, NH), 4.70 (bs, 1H, NH), 3.05−3.28 (m, 16H, N-CH_2), 1.60−1.78 (m, 6H, CH_2CH_2), 1.47 (m, J = 7.2, 4H, CH_2CH_3), 1.42 (s, 18H, $C[CH_3]_3$), 0.88 (t, J = 7.2 Hz, 6H, CH_2CH_3).

1,11-Bis-{3-[1-(3,3-diphenylpropyl)ureado]}-4,8-[*N*-(*tert*-butyl)-oxycarbonyl)]-4,8-diazaundecane (43t). Compound **43t** was prepared from 194 mg (0.0005 mol) of **41a** and **35c** according to the procedure described above for **43e** to afford **43t** (420 mg, 98%) as a viscous oil. R_f: 0.58 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.29−7.15 (m, 20H, Ar-H), 5.50 (b, 2H, NH), 3.96 (t, 2H, J = 8.0 Hz, $CHPh_2$), 3.25 (t, 4H, J = 6.4 Hz, NCH_2), 3.10 (b, 12H, NCH_2), 2.23 (q, 4H, J = 7.2 Hz, NCH_2), 1.72 (b, 2H, CH_2CH_2), 1.61 (b, 4H, CH_2CH_2), 1.42 (s, 18H, $C[CH_3]_3$).

1,12-Bis-{3-[1-(3,3-diphenylpropyl)ureado]}-4,9-[*N*-(*tert*-butyl)-oxycarbonyl)]-4,9-diazadodecane (43v). Compound **43v** was prepared from 193 mg of **41b** (0.0005 mol) and **35c** according to the procedure described above for **43e** to afford **43v** (386 mg, 92%) as viscous oil. ^{1}H NMR ($CDCl_3$): δ 7.29−7.13 (m, 22H, Ar-H, and NH), 5.50 (b, 2H, NH), 3.96 (t, 2H, J = 8.0 Hz, $CHPh_2$), 3.25 (t, 4H, J = 6.4 Hz, NCH_2), 3.10 (m, 12H, NCH_2), 2.24 (b, 4H, CH_2CH_2), 1.60 (b, 4H, CH_2CH_2), 1.42 (s, 22H, CH_2CH_2 and $C[CH_3]_3$).

1,15-Bis-{3-[1-(3,3-diphenylpropyl)ureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43x). Compound **43x** was prepared from 158 mg of **41c** (0.0004 mol) and **35c** according to the procedure described above for **43e** to afford **43x** (310 mg, 95%) as viscous oil. R_f: 0.50 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.30−7.12 (m, 20H, Ar-H), 5.50 (b, 2H, NH), 4.40 (b, 2H, NH), 3.97 (t, 2H, J = 7.2 Hz, $CHPh_2$), 3.25 (t, 4H, J = 6.4 Hz, NCH_2), 3.10 (bs, 12H, NCH_2), 2.26 (q, 4H, J = 8.0 Hz, CH_2CH_2), 1.60 (bs, 4H, CH_2CH_2), 1.42 ((s, 18H, $C[CH_3]_3$), 1.24 (bs, 6H, CH_2CH_2).

General Procedure for Preparation of N-Boc Protected (Bis)-carbamylureas 51−53. 1,12-Bis-{5-[1-(*N*,*N*-diphenyl)carbamyl]-ureado}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (51). A 0.34 g portion of *N*,*N*-diphenylamine **48** (0.34 g, 0.0002 mol) in 5 mL of dry CH_2Cl_2 was added dropwise into a cold solution of *N*-chlorocarbonylisocyanate **45** (0.22 g, 0.0002 mol) in 5.0 mL of CH_2Cl_2, and the reaction was stirred for 30 min under N_2 atmosphere. A solution of **41b** (0.3 g, 0.0008 mol) and NEt_3 (0.3 g, 0.0003 mol) in 10 mL of CH_2Cl_2 was then added via syringe, and the reaction mixture was allowed to stir at room temperature for 18 h. During this time, the progress for formation of product was monitored by TLC (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). The dichloromethane was removed under reduced pressure to produce a viscous material, which was purified by flash chromatography on silica gel eluted with CH_2Cl_2/MeOH/NH_4OH (89:10:1) to furnish pure **51** as a white solid (140 mg, 21%). ^{1}H NMR ($CDCl_3$): δ 8.44 (s, 2H, NH), 7.41−7.39 (m, 8H, Ar-H), 7.29−7.23 (m, 12H, Ar-H), 6.75 (s, 2H, NH), 3.25−3.08 (m, 12H, NCH_2), 1.78−1.69 (m, 6H, CH_2CH_2), 1.43 (s, 18H, $C[CH_3]_3$).

1,15-Bis-{5-[1-(*N*,*N*-diphenyl)carbamyl]ureado}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (52). Compound **52** was made from **48**, **45**, and **41c** according to the procedure described above for the synthesis of **51** to afford pure **52** (140 mg, 21%) as a viscous material. R_f: 0.88 (CH_2Cl_2/MeOH/NH_4OH 89:10:1). ^{1}H NMR ($CDCl_3$): δ 8.40 (s, 2H, NH), 7.37−7.28 (m, 8H, Ar-H), 7.20−7.12 (m, 12H, Ar-H), 6.76 (s, 2H, NH), 3.23−3.06 (m, 12H, NCH_2), 1.72 (m, 4H, CH_2CH_2), 1.39 (bs, 22H, CH_2CH_2 and $C[CH_3]_3$). HRMS (CSI-MS m/z) calcd for $C_{48}H_{62}N_8O_8$ [M^+] = 878.47; found 879.40 [M^+H].

1,11-Bis-{5-[1-(*N*,*N*-diphenyl)carbamyl]ureado}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (53). Compound **55** was made from **48**, **45** and **41a** according to the procedure described above for the synthesis of **51** to afford pure **53** (115 mg, 18%) as a white solid. R_f: 0.90 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 8.44 (s, 2H, NH), 7.41−7.37 (m, 8H, Ar-H), 7.31−7.27 (m, 12H, Ar-H), 6.77 (s, 2H, NH), 3.30−3.12 (m, 12H, NCH_2), 1.75 (t, 4H, J = 6.8 Hz, CH_2CH_2), 1.47 (b, 4H, CH_2CH_2), 1.43 (s, 18H, $C[CH_3]_3$), 1.27 (b, 6H, CH_2CH_2).

General Procedure for Cleavage of N-Boc Protecting Group. 1,12-Bis-{3-[1-(benzyl)thioureado]}-4,9-diazadodecane (3). In a 100 mL round-bottom flask, a 0.4 g portion of **43d** (402 mg, 0.0006 mol) was dissolved in 30 mL of HPLC grade EtOAc under a nitrogen atmosphere, and 4.0 mL of a 1.0 M solution of HCl in EtOAc was added. The reaction mixture was allowed to stir at room temperature for 48 h, during which time the formation of product

was monitored by TLC (CH_2Cl_2/MeOH/NH_4OH 89:10:1 or 78:20:2). The product precipitated as a white crystalline solid during the course of the reaction. When completion of the reaction was confirmed by TLC, the solvent was removed under reduced pressure to produce a white powder. The solid product was stirred with 30 mL of fresh EtOAc, and the solvent was decanted. The solid so obtained was vacuum-dried to give pure **3** as a white solid (315 mg, 95% yield). An analytical sample was obtained by purification on silica gel (CH_2Cl_2:MeOH:NH_4OH 89:10:1). ^{1}H NMR (CD_3OD): δ 7.32−7.10 (m, 10H, Ar-H), 4.67 (s, 4H, CH_2Ph), 3.71 (t, 4H, J = 5.6 Hz, NCH_2), 3.01 (bs, 8H, NCH_2), 1.95 (m, 4H, CH_2CH_2), 1.78 (bs, 4H, CH_2CH_2). MS (CI m/z) calcd for $C_{26}H_{40}N_6S_2$ [M^+] = 500.28; found 501.4 [M^+H].

1,12-Bis-{3-[1-(benzyl)ureado]}-4,9-diazadodecane (4). Compound **4** was prepared from 480 mg (0.0007 mol) of **43e** according to procedure described above for the synthesis of **3** to afford 370 mg (94%) of **4** as a white solid. ^{1}H NMR (D_2O): δ 7.32 (m, 4H, Ar-H), 7.26 (m, 6H, Ar-H), 4.22 (s, 4H, CH_2Ph), 3.16 (t, 4H, J = 6.4 Hz, NCH_2), 2.88 (t, 4H, J = 7.2 Hz, NCH_2), 2.81 (bs, 4H, NCH_2), 1.25 (p, 4H, J = 6.4 and 7.2 Hz, CH_2CH_2), 1.57 (m, 4H, CH_2CH_2). ^{13}C NMR (D_2O): δ 160.89 (C═O), 139.83, 129.00, 127.45, 126.97 (Ar-C), 46.96, 45.12, 43.66, 36.44, 26.71, 22.92 (CH_2).

1,12-Bis-{3-[1-(ethyl)ureado]}-4,9-diazadodecane (5). Compound **5** was prepared from 448 mg (0.0008 mol) of **43f** according to procedure described above for the synthesis of **3** to afford 330 mg (96%) of **5** as a white solid. ^{1}H NMR (D_2O): δ 3.15 (t, 4H, J = 5.6 Hz, N-CH_2), 3.05−2.98 (m, 12H, NCH_2), 1.79 (p, 4H, J = 7.2 Hz, CH_2CH_2), 1.71 (bs, 4H, CH_2CH_2), 1.01 (t, 6H, J = 7.2 Hz, CH_3). ^{13}C NMR (D_2O): δ 160.88 (C═O), 47.06, 45.31, 36.67, 35.27, 26.70, 23.06 (CH_2), 14.63 (CH_3).

1,12-Bis-{3-[1-(ethyl)thioureado]}-4,9-diazadodecane (6). Compound **6** was prepared from 470 mg (0.0008 mol) of **43g** according to procedure described above for the synthesis of **3** to afford 314 mg (87%) of **6** as a white solid. ^{1}H NMR (D_2O): δ 3.51 (bs, 4H, NCH_2), 3.31 (bs, 4H, NCH_2), 3.06 (bs, 4H, NCH_2), 1.93 (p, 4H, J = 6.4 Hz, CH_2CH_2), 1.75 (bs, 4H, CH_2CH_2), 1.12 (t, 6H, J = 6.0 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.38, 153.98 (C═O), 47.13, 45.00, 40.92, 26.13, 23.15 (CH_2), 13.49 (CH_3).

1,15-Bis-{3-[1-(benzyl)ureado]}-4,12-diazapentadecane (7). Compound **7** was prepared from 320 mg (0.0005 mol) of **43h** according to procedure described above for the synthesis of **3** to afford 250 mg (95%) of **7** as a white solid. ^{1}H NMR (D_2O): δ 7.35 (m, 4H, Ar-H), 7.28 (m, 6H, Ar-H), 4.25 (s, 4H, CH_2Ph), 3.18 (t, 4H, J = 5.6 Hz, NCH_2), 2.88 (t, 4H, J = 7.2 Hz, NCH_2), 2.81 (t, 4H, J = 8.0 Hz, NCH_2), 1.76 (p, 4H, J = 7.2 Hz, CH_2CH_2), 1.53 (m, 4H, CH_2CH_2), 1.26 (bs, 6H, CH_2CH_2). ^{13}C NMR (D_2O): δ 160.91 (C═O), 139.83, 129.01, 127.48, 126.99 (Ar-C), 47.78, 44.97, 43.68, 36.47, 27.87, 26.71, 25.64, 25.59 (CH_2).

1,12-Bis-{3-[1-(*n*-propyl)ureado]}-4,9-diazadodecane (8). Compound **8** was prepared from 330 mg (0.0006 mol) of **43i** according to procedure described above for the synthesis of **3** to afford 228 mg (90%) of **8** as a white solid. ^{1}H NMR (D_2O): δ 3.14 (t, 4H, J = 6.4 Hz, NCH_2), 3.00−2.95 (m, 12H, NCH_2), 1.79 (p, 4H, J = 6.4 Hz, CH_2CH_2), 1.70 (bs, 4H, CH_2CH_2), 1.40 (q, 4H, J = 6.4 Hz, CH_2CH_2), 0.79 (t, 6H, J = 7.2 Hz, CH_3). ^{13}C NMR (D_2O): δ 160.98 (C═O), 47.05, 45.31, 42.03, 36.67, 26.70, 23.05, 22.82 (CH_2), 10.77 (CH_3).

1,12-Bis-{3-[1-(*n*-propyl)thioureado]}-4,9-diazadodecane (9). Compound **9** was prepared from 350 mg (0.0006 mol) of **43j** according to procedure described above for the synthesis of **3** to afford 240 mg (86%) of **9** as a white solid. ^{1}H NMR (D_2O): δ 3.59 (b, 4H, NCH_2), 3.23 (b, 4H, NCH_2), 3.07−3.00 (m, 8H, NCH_2), 1.92 (p, 4H, J = 7.2 and 6.4 Hz, CH_2CH_2), 1.75 (b, 4H, CH_2CH_2), 1.57−1.48 (m, 4H, CH_2CH_2), 0.85 (t, 6H, J = 7.2 Hz, CH_3).

1,15-Bis-{3-[1-(benzyl)thioureado]}-4,12-diazapentadecane (10). Compound **10** was prepared from 340 mg (0.0005 mol) of **43k** according to procedure described above for the synthesis of **3** to afford 214 mg (77%) of **10** as a white solid. ^{1}H NMR (D_2O): δ 7.37−7.30 (m, 10H, Ar-H), 4.58 (b, 4H, CH_2Ph), 3.58 (b, 4H, NCH_2), 3.10−2.80 (m, 8H, NCH_2), 1.85 (b, 4H, CH_2CH_2), 1.59 (b, 4H, CH_2CH_2), 1.32 (b, 6H, CH_2CH_2).

1,15-Bis-{3-[1-(ethyl)ureado]}-4,12-diazapentadecane (11). Compound **11** was prepared from 255 mg (0.0004 mol) of **43l** according to procedure described above for the synthesis of **3** to afford 178 mg (89%) of **11** as a white solid. ^{1}H NMR (D_2O): δ 3.16 (t, 4H, J = 7.2 Hz, NCH_2), 3.08 (q, 4H, J = 7.6 Hz, NCH_2), 2.99 (m, 8H, NCH_2), 1.79 (p, 4H, J = 7.2 Hz, CH_2CH_2), 1.62 (bs, 4H, CH_2CH_2), 1.32 (s, 6H, CH_2CH_2), 1.02 (t, 6H, J = 7.2 Hz, CH_3). ^{13}C NMR (D_2O): δ 160.92 (C═O), 47.079, 45.15, 36.67, 35.25, 27.89, 26.69, 25.66 (CH_2), 14.65 (CH_3).

1,15-Bis-{3-[1-(*n*-propyl)ureado]}-4,12-diazapentadecane (12). Compound **12** was prepared from 255 mg (0.0004 mol) of **43m** according to procedure described above for the synthesis of **3** to afford 180 mg (89%) of **12** as a white solid. ^{1}H NMR (D_2O): δ 3.16 (t, 4H, J = 5.6 Hz, NCH_2), 2.99 (m, 12H, NCH_2), 1.79 (p, 4H, J = 7.2 Hz, CH_2CH_2), 1.62 (m, 4H, CH_2CH_2), 1.42 (q, 4H, J = 6.4 Hz, CH_2CH_2), 1.32 (bs, 6H, CH_2CH_2), 0.81 (t, 6H, J = 7.2 Hz, CH_3)). ^{13}C NMR (D_2O): δ 161.03 (C═O), 47.79, 45.14, 42.00, 36.67, 27.89, 26.71, 25.65, 22.86 (CH_2), 10.77 (CH_3).

1,11-Bis-{3-[1-(benzyl)thioureado]}-4,8-diazaundecane (13). Compound **13** was prepared from 373 mg (0.0005 mol) of **43n** according to procedure described above for the synthesis of **3** to afford 302 mg (99%) of **13** as white solid. ^{1}H NMR (DMSO-d_6): δ 9.09 (bs, 2H, NH), 8.21 (t, 2H, NH), 8.00 (bs, 2H, NH), 7.20−7.32 (m, 10H, Ar-H), 4.64 (bs, 4H, N-CH_2), 3.48 (bs, 4H, N-CH_2), 2.97 (bs, 4H, N-CH_2), 2.87 (bs, 4H, N-CH_2), 2.02 (p, 2H, CH_2CH_2), 1.86 (p, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 128.92, 127.91, 127.45 (Ar-C), 47.43, 45.30, 44.60, 41.33, 26.37, 23.01 (CH_2).

1,11-Bis-{3-[1-(ethyl)ureado]}-4,8-diazaundecane (14). Compound **14** was prepared from 245 mg (0.0005 mol) of **43o** according to procedure described above for the synthesis of **3** to afford 178 mg (96%) of **14** as white solid. ^{1}H NMR (DMSO-d_6): δ 9.14 (bs, 2H, NH), 6.00 (bs, 4H, NH), 2.88−3.08 (m, 12H, CH_2N), 2.82 (bs, 4H, CH_2N), 2.02 (bs, 2H, CH_2CH_2), 1.71 (bs, 4H, CH_2CH_2), 0.95 (t, J = 7.2 Hz, 6H, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 159.16 (C═O), 45.25, 44.51, 36.84, 34.80, 27.51, 22.98 (CH_2), 16.34 (CH_3).

1,11-bis-{3-[1-(benzyl)ureado]}-4,8-diazaundecane (15). Compound **15** was prepared from 485 mg (0.0007 mol) of **43p** according to procedure described above for the synthesis of **3** to afford 364 mg (99%) of **15** as white solid. ^{1}H NMR (DMSO-d_6): δ 9.21 (bs, 6H, NH), 7.17−7.30 (m, 10H, Ar-H), 4.19 (s, 4H, N-CH_2), 3.08 (bs, 4H, N-CH_2), 2.94 (bs, 2H, N-CH_2), 2.82 (bs, 4H, N-CH_2), 2.02 (b, 2H, CH_2CH_2), 1.74 (b, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 159.24 (C═O), 141.49, 128.89, 127.63, 127.20 (Ar-C), 45.27, 44.53, 43.57, 37.02, 27.45, 22.93 (CH_2).

1,11-Bis-{3-[1-(*n*-propyl)ureado]}-4,8-diazaundecane (16). Compound **16** was prepared from 359 mg (0.0006 mol) of **43q** according to procedure described above for the synthesis of **3** to afford 303 mg (99%) of **16** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.24 (bs, 6H, NH), 2.82−3.06 (m, 16H, N-CH_2), 2.04 (b, 2H, CH_2CH_2), 1.73 (b, 4H, CH_2CH_2), 1.33 (m, J = 7.2 Hz, 4H, CH_2CH_3), 0.80 (t, J = 7.2 Hz, 4H, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 159.29 (C═O), 45.22, 44.52, 41.88, 36.97, 27.39, 23.78, 22.92 (CH_2), 12.04 (CH_3).

1,11-Bis-{3-[1-(*n*-propyl)ureado]}-4,8-diazaundecane (17). Compound **17** was prepared from 379 mg (0.0006 mol) of **43r** according to procedure described above for the synthesis of **3** to afford 317 mg (99%) of **17** as white solid. ^{1}H NMR (DMSO-d_6): δ 9.46 (b, 2H, NH), 9.16 (b, 2H, NH), 7.82 (b, 2H, NH), 2.85−3.90 (b, 16H, N-CH_2), 1.84 (b, 2H, CH_2CH_2), 1.59 (b, 4H, CH_2CH_2), 1.44 (m, 4H, CH_2CH_3), 0.85 (t, 6H, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 45.30, 44.58, 26.37, 22.92, 22.71, 22.00 (CH_2), 12.10 (CH_3).

1,11-Bis-{3-[1-(ethyl)thioureado]}-4,8-diazaundecane (18). Compound **18** was prepared from 347 mg (0.0006 mol) of **43s** according to procedure described above for the synthesis of **3** to afford 282 mg (99%) of **18** as white solid. ^{1}H NMR (DMSO-d_6): δ 9.10 (bs, 2H, NH), 7.78 (bs, 2H, NH), 7.70 (bs, 2H, NH), 3.43 (bs, 4H,

N-CH_2), 3.32 (bs, 4H, N-CH_2), 2.97 (bs, 4H, N-CH_2), 2.86 (bs, 4H, N-CH_2), 2.02 (b, 2H, CH_2CH_2), 1.83 (b, 4H, CH_2CH_2), 1.02 (t, J = 7.2 Hz, 6H, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 45.27, 44.58, 38.83, 31.99, 26.38, 22.98 (CH_2), 15.12 (CH_3).

1,11-Bis-{3-[1-(3,3-diphenylpropyl)ureado]}-4,8-diazaundecane (19). Compound **19** was prepared from 400 mg (0.0005 mol) of **43t** according to procedure described above for the synthesis of **3** to afford 290 mg (86%) of **19** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.10 (bs, 4H, NH), 7.27−7.21 (m, 16H, Ar-H), 7.18−7.10 (m, 4H, Ar-H), 3.96 (t, 2H, J = 7.2 Hz, $CHPh_2$), 3.02 (t, 4H, J = 6.4 Hz, NCH_2), 2.92 (b, 4H, NCH_2), 2.84 (t, 4H, J = 7.2 Hz, NCH_2), 2.79 (bs, 4H, NCH_2), 2.09 (q, 4H, J = 8.0 Hz, CH_2CH_2), 1.99 (m, 2H, CH_2CH_2), 1.69 (m, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 159.22 (CO), 145.50, 129.08, 128.28, 126.72 (Ar-C), 48.51, 45.25, 44.49, 38.77, 36.88, 36.07, 27.45, 22.95 (CH and CH_2).

1,11-Bis-{3-[1-(3,3-diphenylpropyl)thioureado]}-4,8-diazaundecane (20). Compound **20** was prepared from 260 mg (0.0003 mol) of **43u** according to procedure described above for the synthesis of **3** to afford 205 mg (92%) of **20** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.10 (b, 4H, NH), 7.91 (b, 2H, NH), 7.32−7.14 (m, 20H, Ar-H), 6.10 (b, 2H, NH), 4.04 (t, 2H, J = 7.6 Hz, $CHPh_2$), 3.45 (b, 4H, NCH_2), 3.24 (b, 4H, NCH_2), 2.98 (b, 4H, NCH_2), 2.88 (b, 4H, NCH_2), 2.61 (m, 4H, CH_2CH_2), 2.04 (m, 2H, CH_2CH_2), 1.85 (m, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 145.36, 129.11, 128.31, 126.78 (Ar−C), 48.62, 45.29, 44.60, 42.80, 41.02, 34.97, 26.34, 22.96 (CH and CH_2).

1,12-Bis-{3-[1-(3,3-diphenylpropyl)ureado]}-4,9-diazadodecane (21). Compound **21** was prepared from 370 mg (0.42 mmol) of **43v** according to procedure described above for the synthesis of **3** to afford 285 mg (90%) of **21** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.00 (bs, 4H, NH), 7.21−7.12 (m, 20H, Ar-H, and NH), 3.96 (t, 2H, J = 7.2 Hz, $CHPh_2$), 3.02 (t, 4H, J = 6.4 Hz, NCH_2), 2.84 (t, 4H, J = 6.4 Hz, NCH_2), 2.79 (b, 12H, NCH_2), 2.09 (q, 4H, J = 7.2 Hz, CH_2CH_2), 1.69 (t, 4H, J = 6.4 Hz, CH_2CH_2), 1.63 (b, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 159.27 (C=O), 145.49, 129.08, 128.28, 126.73 (Ar-C), 48.49, 46.50, 45.10, 38.76, 36.88, 36.07, 27.46, 23.23 (CH and CH_2). MS (EI m/z) calculated for $C_{42}H_{56}N_6O_2$ [$M^{+\cdot}$] = 676.45; found 677.40 [M^+H].

1,12-Bis-{3-[1-(3,3-diphenylpropyl)thioureado]}-4,9-diazadodecane (22). Compound **22** was prepared from 260 mg (0.0003 mol) of **43w** according to procedure described above for the synthesis of **3** to afford 205 mg (92%) of **22** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.02 (bs, 4H, NH), 8.02 (b, 2H, NH), 7.30−7.12 (m, 22H, Ar-H, and NH), 4.03 (t, 2H, J = 7.6 Hz, $CHPh_2$), 3.43 (bs, 4H, NCH_2), 3.23 (bs, 4H, NCH_2), 2.85 (b, 8H, NCH_2), 2.24 (m, 4H, CH_2CH_2), 1.84 (b, 4H, CH_2CH_2), 1.67 (b, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 145.34, 129.14, 128.31, 126.79 (Ar-C), 48.58, 46.59, 45.13, 41.18, 34.93, 26.29, 23.26 (CH and CH_2).

1,15-Bis-{3-[1-(3,3-diphenylpropyl)ureado]}-4,12-diazapentadecane (23). Compound **23** was prepared from 290 mg (0.0003 mol) of **43x** according to procedure described above for the synthesis of **3** to afford 225 mg (88%) of **23** as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.94 (bs, 4H, NH), 7.27−7.21 (m, 16H, Ar-H), 7.13−7.10 (m, 4H, Ar-H), 3.96 (t, 2H, J = 7.2 Hz, $CHPh_2$), 3.02 (t, 4H, J = 6.9 Hz, NCH_2), 2.84 (t, 4H, J = 7.2 Hz, NCH_2), 2.77 (bs, 8H, NCH_2), 2.09 (d, 4H, J = 7.2 Hz, CH_2CH_2), 1.69 (t, 4H, J = 6.4 Hz, CH_2CH_2), 1.56 (bs, 4H, CH_2CH_2), 1.21 (bs, 6H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 159.35 (C=O), 145.49, 129.07, 128.28, 126.72 (Ar-C), 48.49, 47.24, 45.09, 38.75, 36.82, 36.07, 28.55, 27.48, 26.37, 25.91 (CH and CH_2).

1,15-Bis-{3-[1-(3,3-diphenylpropyl)thioureado]}-4,12-diazapentadecane (24). Compound **24** was prepared from 287 mg (0.0003 mol) of **43y** according to procedure described above for the synthesis of **3** to afford 230 mg (92%) of **24** as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.87 (bs, 4H, NH), 7.89 (bs, 4H, NH), 7.32−7.25 (m, 16H, Ar-H), 7.18−7.14 (m, 4H, Ar-H), 4.10 (b, 2H, $CHPh_2$), 3.44 (b, 4H, NCH_2), 3.23 (b, 4H, NCH_2), 2.87 (m, 8H, NCH_2), 2.25 (d, 4H, J = 7.6 Hz, CH_2CH_2), 1.83 (t, 4H, J = 7.2 Hz, CH_2CH_2), 1.68 (m, 4H, CH_2CH_2), 1.28 (b, 6H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 145.37, 129.11, 128.30, 126.70 (Ar-C), 48.61, 47.30, 45.15, 41.42, 34.93, 28.58, 26.41, 25.94 (CH and CH_2).

1,15-Bis-{3-[1-(2,2-diphenylethyl)thioureado]}-4,12-diazapentadecane (25). Compound **25** was prepared from 260 mg (0.0003 mol) of **43z** according to procedure described above for the synthesis of **3** to afford 201 mg (90%) of **25** as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.91 (bs, 3H, NH), 7.70 (b, 1H, NH), 7.52 (b, 1H, NH), 7.26 (bs, 16H, Ar-H), 7.16 (bs, 4H, Ar-H), 4.36 (b, 2H, $CHPh_2$), 4.04 (b, 4H, NCH_2), 3.45 (b, 4H, NCH_2), 2.78 (b, 8H, NCH_2), 1.78 (b, 4H, CH_2CH_2), 1.58 (b, 4H, CH_2CH_2), 1.25 (b, 6H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 181.50 (C=S), 143.36, 129.16, 128.62, 127.07 (Ar-C), 50.44, 48.78, 47.30, 45.09, 28.59, 26.40, 26.25, 25.91(CH_2).

1,12-Bis-{3-[1-(2,2-diphenylethyl)thioureado]}-4,9-diazadodecane (26). Compound **26** was prepared from 280 mg (0.0003 mol) of **43aa** according to procedure described above for the synthesis of **3** to afford 214 mg (89%) of **26** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.05 (b, 4H, NH), 7.79 (b, 2H, NH), 7.53 (bs, 2H, NH), 7.28 (bs, 16H, Ar-H), 7.14 (m, 4H, Ar-H), 4.36 (bs, 2H, $CHPh_2$), 4.02 (bs, 4H, NCH_2), 3.42 (bs, 4H, NCH_2), 2.81 (b, 8H, NCH_2), 1.80 (bs, 4H, CH_2CH_2), 1.66 (bs, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 183.29 (C=S), 143.39, 129.16, 128.63, 127.07 (Ar-C), 50.46, 48.76, 46.57, 45.09, 41.21, 26.25, 23.16 (CH and CH_2).

1,11-Bis-{3-[1-(2,2-diphenylethyl)thioureado]}-4,8-diazaundecane (27). Compound **27** was prepared from 330 mg (0.0004 mol) of **43bb** according to procedure described above for the synthesis of **3** to afford 220 mg (79%) of **27** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.13 (b, 4H, NH), 7.77 (bs, 2H, NH), 7.50 (bs, 2H, NH), 7.27 (bs, 16H, Ar-H), 7.16 (bs, 4H, Ar-H), 4.35 (bs, 2H, $CHPh_2$), 4.04 (b, 4H, NCH_2), 3.66 (bs, 4H, NCH_2), 3.42 (bs, 4H, NCH_2), 2.94 (bs, 4H, NCH_2), 2.80 (bs, 4H, NCH_2), 2.01 (b, 2H, CH_2CH_2), 1.79 (bs, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 183.20 (C=S), 143.38, 129.17, 128.63, 127.07 (Ar-C), 50.45, 48.68, 46.24, 44.57, 41.05, 26.28, 22.98 (CH and CH_2).

1,11-Bis-{3-[1-(1,1-diphenylmethyl)thioureado]}-4,8-diazaundecane (28). Compound **28** was prepared from 335 mg (0.0004 mol) of **43cc** according to procedure described above for the synthesis of **3** to afford 227 mg (80%) of **28** as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.90 (b, 4H, NH), 8.29 (b, 2H, NH), 7.40−7.22 (m, 20H, Ar-H), 6.72 (b, 2H, CH), 4.56 (b, NH), 3.52 (b, 4H, NCH_2), 2.97 (m, 8H, NCH_2), 2.02 (b, 2H, CH_2), 1.87 (b, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 183.22 (C=S), 143.43, 129.08, 127.89, 127.56 (Ar-C), 61.28 (CH), 45.37, 44.59, 41.34, 26.33, 23.00 (CH_2).

1,12-Bis-{3-[1-(1,1-diphenylmethyl)thioureado]}-4,9-diazadodecane (29). Compound **29** was prepared from 354 mg (0.0004 mmol) of **43dd** according to procedure described above for the synthesis of **3** to afford 262 mg (87%) of **29** as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.95 (b, 4H, NH), 8.30 (bs, 2H, NH), 7.30 (m, 20H, Ar-H), 6.72 (b, 2H, $CHPh_2$), 3.51 (b, 4H, NCH_2), 2.88 (b, 8H, NCH_2), 1.87 (b, 4H, CH_2CH_2), 1.66 (b, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 183.26 (C=S), 143.42, 129.08, 127.89, 127.57 (Ar-C), 61.30 (CH), 46.60, 45.22, 41.42, 26.38, 23.28 (CH_2).

1,15-Bis-{3-[1-(1,1-diphenylmethyl)thioureado]}-4,12-diazapentadecane (30). Compound **30** was prepared from 390 mg (0.0004 mol) of **43ee** according to procedure described above for the synthesis of **3** to afford 298 mg (89%) of **30** as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.90 (b, 4H, NH), 8.35 (b, 2H, NH), 7.30 (bs, 20H, Ar-H), 6.73 (bs, 2H, $CHPh_2$), 3.51 (bs, 4H, NCH_2), 2.89 (bs, 4H, NCH_2), 2.81 (bs, 4H, NCH_2), 1.87 (bs, 4H, CH_2CH_2), 1.60 (bs, 4H, CH_2CH_2), 1.26 (b, 6H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 183.29 (C=S), 143.45, 129.06, 127.89, 127.55 (Ar-C), 61.30 (CH), 47.32, 45.23, 41.42, 28.59, 26.41, 25.93 (CH_2).

1,12-Bis-{5-[1-(*N*,*N*-diphenyl)carbamyl]ureado}-4,9-diazadodecane (31). Compound **31** was prepared from **51** (130 mg, 0.0002 mol) according to procedure described above for the synthesis of **3** to afford 85 mg of **31** (75%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.08 (bs, 4H, NH), 8.31 (t, 2H, J = 5.6 Hz, NH), 7.86 (s, 2H, NH), 7.41−7.37 (m, 8H, Ar-H), 7.30−7.26

(m, 12H, Ar-H), 3.21 (m, 4H, NCH_2), 2.84 (bs, 8H, NCH_2), 1.81 (m, 4H, CH_2CH_2), 1.67 (bs, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 154.36, 153.97 (C=O), 142.41, 130.19, 128.45, 127.77 (Ar-C), 46.52, 45.13, 37.08, 26.70, 23.26 (CH_2). MS (EI m/z) calcd for $C_{38}H_{46}N_8O_4$ [$M^{+\cdot}$] = 678.36; found 679.32 [M^+H].

1,15-Bis-{5-[1-(*N*,*N*-diphenyl)carbamyl]ureado}-4,12-diazapentadecane (32). Compound **32** was prepared from **52** (90 mg, 0.0001 mol) according to procedure described above for the synthesis of **3** to afford 42 mg of **32** (55%) as a white solid. ^1H NMR (DMSO-d_6): δ 8.92 (b, 4H, NH), 8.32 (bs, 2H, NH), 7.88 (bs, 2H, NH), 7.40−7.31 (m, 20H, Ar-H), 3.21 (bs, 4H, NCH_2), 2.83 (bs, 8H, NCH_2), 1.81 (bs, 4H, CH_2CH_2), 1.60 (bs, 4H, CH_2CH_2), 1.27 (bs, 6H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 154.39, 154.00 (C=O), 142.42, 130.01, 128.44, 127.79 (Ar-C), 45.27, 45.15, 37.04, 28.60, 26.73, 26.42, 25.96(CH_2).

1,11-Bis-{5-[1-(*N*,*N*-diphenyl)carbamyl]ureado}-4,8-diazaundecane (33). Compound **33** was prepared from **53** (110 mg, 0.0001 mol) according to procedure described above for the synthesis of **3** to afford 66 mg of **33** (75%) as a white solid. ^1H NMR (DMSO-d_6): δ 9.14 (bs, 4H, NH), 8.32 (t, 2H, J = 5.6 Hz, NH), 7.87 (s, 2H, NH), 7.42−7.38 (m, 8H, Ar-H), 7.31−7.27 (m, 12H, Ar-H), 3.20 (m, 4H, NCH_2), 2.97 (bs, 4H, NCH_2), 2.84 (bs, 4H, NCH_2), 2.02 (m, 2H, CH_2CH_2), 1.81 (m, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 154.38, 153.98 (C=O), 142.42, 130.21, 128.46, 127.79 (Ar-C), 45.29, 44.56, 37.04, 26.74, 23.03(CH_2).

Expression, Purification, and Demethylase Assay of Recombinant Proteins. Full-length human LSD1 cDNA was subcloned into the pET15b bacterial expression vector (Novagen, Madison, WI) in frame with an N-terminal 6× HIS-tag and transformed into the $BL_{21}(DE_3)$ strain of *Escherichia coli*. Following selection, expression and purification of recombinant LSD1 protein were performed as previously described.[10] Briefly, expression of LSD1-HIS protein was induced by 1 mM IPTG for 6 h at 25 °C. The HIS-tagged protein was purified using Ni-NTA affinity purification resin and column as recommended by the manufacturer (Qiagen, Valencia, CA). Bound protein was eluted by imidazole and the eluate was dialyzed in PBS at 4 °C. Enzymatic activity of LSD1 was examined using luminol-dependent chemiluminescence to measure the production of H_2O_2, as previously described.[33] In brief, LSD1 activity was assayed in 50 mM Tris, pH 8.5, 50 mM KCl, 5 mM MgCl, 5 nmol luminol, and 20 μg/mL horseradish peroxidase with the indicated concentrations of H3K4me2 (1−21 aa) peptide as substrate. The integral values were calibrated against standards containing known concentrations of H_2O_2, and the activities expressed as pmols H_2O_2/mg protein/min.

Western Blotting. Cytoplasmic and nuclear fractions were prepared for Western blot analysis using the NE-PER nuclear and cytoplasmic extraction kit (Pierce, Rockford, IL). Primary antibodies against H3K4me1 and H3K4me2 were from Millipore. The pCNA monoclonal antibody was purchased from Oncogene Research Products (Cambridge, MA). Dye-conjugated secondary antibodies were used for quantification of Western blot results using the Odyssey infrared detection system and software (LI-COR Biosciences, Lincoln, NE).

RNA Isolation and qPCR. RNA was extracted using TRIzol reagents (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized using SuperScript III reverse transcriptase with an oligo(dT) primer (Invitrogen). qPCR was performed using the following primers: *SFRP2* sense, 5′AAG CCT GCA AAA ATA AAA ATG ATG; *SFRP2* antisense, 5′TGT AAA TGG TCT TGC TCT TGG TCT (annealing at 57.4 °C); *GATA4* sense, 5′GGC CGC CCG ACA CCC CAA TCT; *GATA4* antisense, 5′ ATA GTG ACC CGT CCC ATC TCG (annealing at 64 °C). qPCR was performed in a MyiQ single color real-time PCR machine (Bio-Rad, Hercules, CA) with GAPDH as an internal control.

Determination of Cell Viability. Calu-6 human anaplastic nonsmall cell lung carcinoma cells were maintained in culture using RPMI medium plus 10% fetal bovine serum. For the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) (MTS) reduction assay, 4000 cells/well were seeded in 100 μL medium in a 96-well plate and the cells were allowed to attach at 37 °C in 5% CO_2 for one day. The medium was aspirated and cells were treated with 100 μL of fresh medium containing appropriate concentrations of each test compound. The cells were incubated for 4 days at 37 °C in 5% CO_2. After 4 days, 20 μL of the MTS reagent solution (Promega CellTiter 96 Aqueous One Solution cell proliferation assay) was added to the medium. The cells were incubated for another 2 h at 37 °C under 5% CO_2 environment. Absorbance was measured at 490 nm on a microplate reader equipped with SOFTmax PRO 4.0 software to determine the cell viability.

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