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Synthesis and Biological Evaluation of 17β-Hydroxysteroid Dehydrogenase Type 1 (17β-HSD1) Inhibitors Based on a Thieno[2,3-*d*]pyrimidin-4(3*H*)-one Core

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Many breast tumors are hormone-dependent, and estrogens, especially estradiol (E2), have a pivotal role in their growth and development. 17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) is a key enzyme in the biosynthesis of female sex steroids, catalyzing the NADPH-dependent reduction of estrone into biologically active estradiol. In this study, a library of fused (di)cycloalkeno thieno[2,3-*d*]pyrimidin-4(3*H*)-one based compounds was synthesized, and the biological activities against 17β-HSD1 in a cell-free and in a cell-based assay were evaluated. Several thieno[2,3-*d*]pyrimidin-4(3*H*)-one based compounds, at 0.1 and 1 μM test concentrations, were found to be potent 17β-HSD1 inhibitors. For example, 4-(3-hydroxyphenylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2′,3′:4,5]-pyrimido[1,2-*a*]azepine-3-carboxaldehyde (**7f**) is one of the most potent nonsteroidal 17β-HSD1 inhibitors reported to date with 94% inhibition of the recombinant enzyme at 0.1 μM test concentration. Importantly, the majority of these compounds exhibited excellent selectivity over the oxidative isoform 17β-HSD2 and lacked estrogenic effects in an estrogen receptor (ER) binding assay.

Introduction

Breast cancer is the most common cancer in women in the Western countries. More than 180 000 new cases and over 40 000 deaths are expected to occur in the United States in 2008.[1] The majority of breast cancers are initially hormone-dependent (estrogen receptor (ER[a]) positive), and estrogens, especially estradiol (E2), have a crucial role in their development and progression. Lowering the levels of both circulating and tissue E2 by inhibiting the biosynthesis of active sex steroids has emerged as an attractive approach for treating hormone-dependent types of breast cancer.[2] Inhibitors of CYP19 aromatase, the enzyme converting androgens to estrogens, are already used in breast cancer treatment.[3]

17β-Hydroxysteroid dehydrogenase (17β-HSD) enzymes play a crucial role in the synthesis of active female and male sex steroids.[4] They catalyze the NAD(P)(H)-dependent oxidation and reduction of hydroxy or keto groups at the C17 position of androgens and estrogens. At present, 14 mammalian 17β-HSDs have been identified,[5] most of them belonging to the short-chain alcohol dehydrogenase reductase (SDR) superfamily.[6] 17β-HSD type 1 (17β-HSD1) is a key enzyme in female hormonal regulation, converting the less potent estrogen estrone (E1) into the biologically active E2 (Figure 1), and is thus an attractive target for inhibitor development for the prevention and control of breast tumor growth. On the other hand, the reverse inactivating enzymatic reaction is catalyzed by the 17β-HSD2 isoform. 17β-HSD1 is essential for both gonadal and peripheral E2 synthesis and is mainly expressed in female breast tissue, ovaries, and placenta. High 17β-HSD1 expression has been found in malignant breast tissue[7] and immunohistochemical studies suggest that 17β-HSD1 has an important role in the *in situ* E2 production in hormone-dependent breast cancer tumors.[8] 17β-HSD1 has been shown to be an independent prognostic factor in breast cancer.[9] Recently, *in vivo* efficacy of 17β-HSD1 inhibition has been demonstrated. Specific 17β-HSD1 inhibitors were able to reduce hormone-dependent tumor growth in immunodeficient mice inoculated with MCF-7 cells expressing the human recombinant 17β-HSD1 enzyme.[10]

The human 17β-HSD1 is active as a soluble cytosolic homodimer, both monomeric subunits having 327 residues and a molecular mass of 34.9 KDa.[11] 17β-HSD1 has been crystallized with estrogenic[12] and androgenic[13] ligands as well as in the native form[14] and with a known steroid-based inhibitor.[15] In addition, the active site has been studied by molecular modeling[16] and site-directed mutagenesis[17] experiments. As a member of the SDR superfamily,[6] 17β-HSD1 has the highly conserved and catalytically crucial Tyr-Xaa-Xaa-Xaa-Lys sequence and a generally conserved serine in the active site. The ligand-binding cavity of 17β-HSD1 is a narrow tunnel (340 Å^2) where carbon atoms contribute 72% of the

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[a] Abbreviations: E1, estrone; E2, estradiol; ER, estrogen receptor; HSD, hydroxysteroid dehydrogenase; MDS, molecular dynamics simulation; SDR, short-chain alcohol dehydrogenase reductase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; NADH/NADPH, reduced forms of NAD and NADP, respectively; DDQ, 2,3-dichloro-5,6-dicyanobenzoquinone.

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total surface.[12b] Polar regions are located at opposite ends of the otherwise hydrophobic ligand-binding cavity. Superimposition of available crystal structures has revealed variable orientations of several active site residues.[18]

Most of the known inhibitors of 17β-HSD1 are based on modifications of steroidal structures.[19] For example, E2 derivatives substituted at C16[20] or at C15[21] have shown promising 17β-HSD1 inhibitory activity, along with estra-1,3,5(10)-trien-17-ones[22] and D-homoestra-1,3,5(10)-trien-17-ones[23] bearing small substituents at the C2 position. In addition, E2−adenosine hybrid inhibitors, aimed at binding both the ligand- and cofactor-binding domains of 17β-HSD1, have been developed.[15,24] Much less work has been published regarding nonsteroidal molecules as 17β-HSD1 inhibitors. These compounds could benefit from synthetic accessibility, drug-likeness, selectivity, and nonestrogenicity when compared with some of the steroid-based inhibitors. Various phytoestrogens and related compounds have been reported to inhibit multiple isoforms of 17β-HSD,[25] but the lack of selectivity and the potential estrogenic nature of these natural compounds have prohibited their use as such.[26] Substituted 2-benzyltetral-1-ones,[27] benzopyranones,[28] and phenyl ketones[29] are known to show a modest activity against 17β-HSD1. Better activity, however, has been obtained with small biphenolic molecules, for example, with quinoline and naphthalene derivatives[30] and with certain bisphenyl-(tri)azoles and bisphenyloxazoles.[31] Recently, subsequent to our findings on thieno[2,3-*d*]pyrimidin-4(3*H*)-one based inhibitors,[32] a series of benzothienopyrimidinones has been reported to inhibit 17β-HSD1.[18,33]

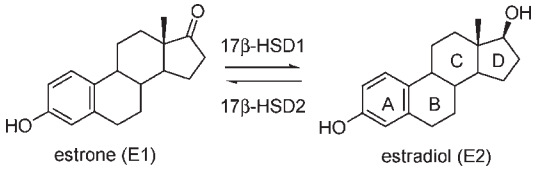


Figure 1. The primary enzymatic reactions catalyzed by 17β-HSD type 1 and type 2.

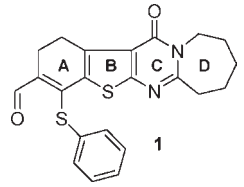


Figure 2. The lead compound 1,2,7,8,9,10,11,13-octahydro-13-oxo-4-(phenylthio)-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (**1**, CAS name) and the labeling of the fused ring system.

In our search for therapeutic agents for hormone-sensitive breast cancer, we have investigated nonsteroidal small molecules as inhibitors of E2 biosynthesis. Based on molecular modeling studies on the active site of 17β-HSD1,[16] a small commercial library of compounds, which were likely to fit and be complementary to the hydrophobic nature of the active site, were chosen for biological screening in order to identify potential nonsteroidal 17β-HSD1 inhibitors. The tetracyclic dicycloalkeno thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivative **1**,[34] bearing an aldehyde and an aromatic thioether moiety in the ring A, was identified as a lead compound (Figure 2). Herein, we report the chemical synthesis and biological activity of 17β-HSD1 inhibitors based on a thieno[2,3-*d*]pyrimidin-4(3*H*)-one core. Furthermore, the selectivity between the 17β-HSD1 and 17β-HSD2 isoforms and possible estrogenicity of these compounds will be discussed.

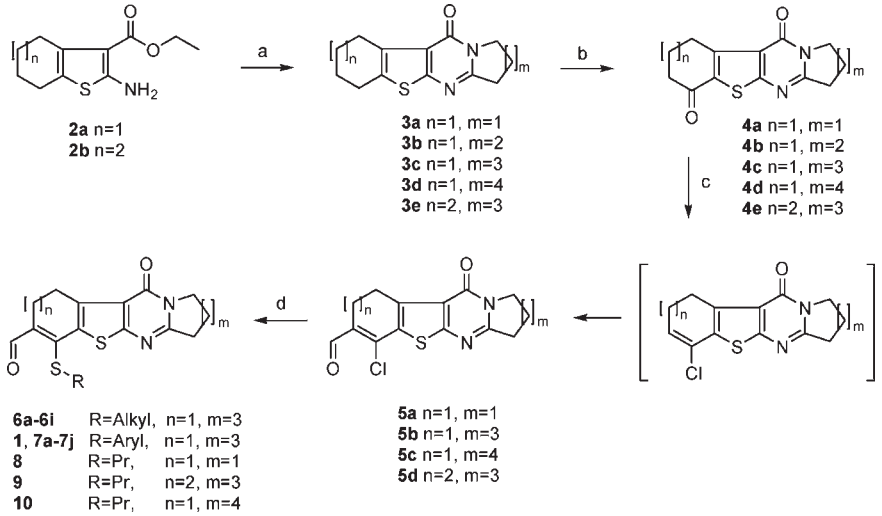
Chemistry

Homologation of the tetracyclic skeleton and variation of the thioether moiety in ring A were chosen for the first

Table 1. Yield (%) of the Oxidation of Thiophene α-Position with PCC−Celite[36] or $K_2S_2O_8$−$CuSO_4$

	oxidant		
	PCC−Celite	$K_2S_2O_8$−$CuSO_4$	$KMnO_4$
4a	52	75	
4b	51	77	20
4c	54	85	
4d	56	78	
4e	24	65	
16a	43	68	
16b	49	70	
27		80	

Scheme 1. The Synthetic Route for the Lead Compound **1** and Its Analogues[a]



[a] Reagents and conditions: (a) $POCl_3$, lactam, 1,2-dichloroethane, reflux, 76−91%; (b) $K_2S_2O_8$, $CuSO_4 \cdot 5H_2O$, MeCN−H_2O, reflux, 65−85%; (c) $POCl_3$−DMF, CH_2Cl_2, 32−83%; (d) RSH, NaOH, THF, 65−93%.

alterations of the lead compound **1**. Although **1** was commercially available in milligram quantities, it was an unknown compound in the chemical literature, and thus an efficient synthetic route needed to be developed for **1** and its derivatives. The dicycloalkeno thieno[2,3-*d*]pyrimidin-4(3*H*)-ones, bearing thioether and aldehyde functionalities at the ring A, were synthesized in four consecutive steps from commercially available starting materials (Scheme 1). First, $POCl_3$-catalyzed cyclization of ethyl 2-amino-4,5,6,7-tetrahydrobenzo-[*b*]thiophene-3-carboxylate (**2a**) or ethyl 2-aminocyclohepta-[*b*]thiophene-3-carboxylate (**2b**) with five- or eight-membered lactams produced the fused tetracyclic dicycloalkeno thieno-[2,3-*d*]pyrimidin-4(3*H*)-ones **3a**−**3e** in 76−91% yield. The oxidation of the thiophene α-position in **3a**−**3e** with $K_2S_2O_8$−$CuSO_4$[35] in MeCN−H_2O (1:1) proceeded with high selectivity and significantly shortened the reaction times and improved the yields of **4a**−**4e** when compared with the previously reported PCC−Celite oxidation (Table 1).[36] In the next step, Vilsmeier haloformylation[37] of the ketones was employed to produce the corresponding β-chlorovinylaldehydes **5a**−**5d**. The Vilsmeier reaction proceeds mainly via a chloroalkene intermediate[38] (colorless and less polar component on TLC, in ^{1}H NMR spectra the alkene proton apparent triplet at ∼5.9 ppm) instead of the direct haloformylation by way of an enaminoketone. With **4a** and **4b**, which have a five- or six-membered ring fused to the pyrimidinone moiety, the amidine α-position (in ring D) is more activated than the carbonyl α-position,[36] and thus the Vilsmeier haloformylation becomes unpropitious as aminomethylenation, chloroalkene formation, and direct haloformylation intervene as competing reactions. For example, with **4a** a moderate 32% yield of β-chlorovinylaldehyde **5a** was isolated when the reaction was performed using DMF as a solvent. In the final step, the introduction of various aliphatic and aromatic thioether side chains to give compounds **1**, **6a**−**6i**, and **7a**−**7j** (Table 2) was done with an addition−elimination reaction of corresponding thiols under basic conditions. The reaction time depended heavily on the nucleophile used, varying from 15 min up to 2 days with the most hindered *t*-butyl group.

All the thiols used were commercially available except for 4-(mercaptoacetyl)morpholine (**13**), which was used in the synthesis of **6i**. Compound **13** was synthesized in three steps starting from morpholine (Scheme 2).[39] The reaction of morpholine with chloroacetyl chloride gave 4-(chloroacetyl)-morpholine (**11**) in 94% yield. For compound **11**, two different sets of ^{1}H NMR data have been previously reported.[39,40] We found our ^{1}H NMR data to be in accordance with those reported by Pielichowski and Popielarz,[40] and we further verified the structure of **11** with ^{13}C NMR and mass spectroscopy. The reaction of **11** with potassium xanthate produced **12** as an intermediate, which was subsequently hydrolyzed without purification. The hydrolysis was first attempted with the published procedure[39] by treating **12** with ammonia (aq) at room temperature. In our hands, compound **13** could not be isolated after the hydrolysis, but the pyrolysis product[41] 4-[(ethylthio)acetyl]morpholine (**14**) was obtained instead, whereas hydrolysis of **12** with 1,3-diaminopropane at 0 °C gave the desired **13** in 67% yield. Also for **13** our ^{1}H NMR data varied from the data reported previously.[39] Again, further analysis of **13** with ^{13}C NMR and mass spectroscopy verified our structure to be correct. In addition, the intermediate **12** was isolated and analyzed.

With the aim of investigating the significance of the aliphatic ring D for the 17β-HSD1 inhibitory activity, tricyclic thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives **15**−**18** were synthesized (Scheme 3). The same four-step synthetic route was employed as for the tetracyclic compounds with the exception that **2a** was reacted with acetamide or *N*-methylacetamide. As described earlier for **4a** and **4b**, the amidine α-position in the dimethyl derivative **16b** is highly activated,[36] and Vilsmeier haloformylation is unfavored, whereas **16a** gave the corresponding β-chlorovinylaldehyde **17** in excellent yield.

Next, modifications in the ring A were investigated. Aromatization of the unsaturated ring A with DDQ in compounds **1** and **6b** produced the corresponding benzothieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives **19a** and **19b** in 84−91% yield (Scheme 4). The subsequent reduction of the aldehyde functionality with $NaBH_4$ in EtOH gave the benzylic alcohols **20a** and **20b**. The aldehyde moiety in compounds **1** and **6b**, with a nonaromatic ring A, could similarly be reduced with $NaBH_4$, but the corresponding allylic alcohols proved to be relatively unstable and were not taken further. The conjugated oxime derivative **21** was prepared by treating **6b** with $NH_2OH \cdot HCl$ under basic conditions. The aldehyde functionality in ring A was also modified in the β-chloro compound **5b** to give corresponding oxime **22**, alcohol **23**, and carboxylic acid **24** derivatives (Scheme 5). The best yield and ease of purification of **24** was obtained with sodium chlorite oxidation. Thioether formation combined with aldol condensation to give the conjugated ketone **25** could be performed as a one-pot reaction.

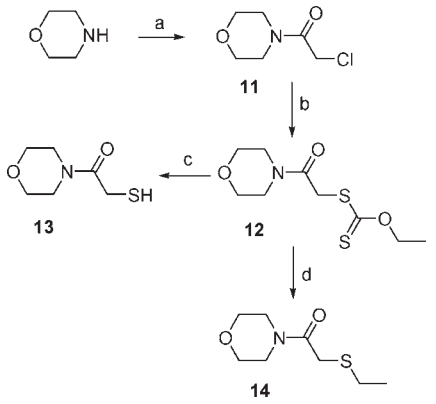
In addition to the thieno[2,3-*d*]pyrimidin-4(3*H*)-one based inhibitors, bicyclic thiophene derivatives **26**−**29** were prepared employing a similar synthetic strategy as for the thieno-[2,3-*d*]pyrimidin-4(3*H*)-one compounds (Scheme 6).

Results and Discussion

The lead compound **1** had already shown auspicious biological activity in a cell-based assay with 41% and 99% inhibition of 17β-HSD1 at 1 and 10 μM concentration, respectively. Nine derivatives of **1** with an aliphatic thioether side chain (**6a**−**6i**) and ten derivatives with an aromatic thioether side chain (**7a**−**7j**) were synthesized, and their biological activity against 17β-HSD1 and 17β-HSD2 was evaluated (Table 2). Most of these compounds, at 0.1 and 1 μM test concentration, showed good to excellent activity against 17β-HSD1 in a cell-free (recombinant enzyme) assay. However, charged substituents like −COOH in **6h** and **7c**, as well as the 2-(4-morpholinyl)-2-oxoethylthio group in **6i**, seemed not to be tolerated, and the inhibitory activity was lost. The most potent compounds found in this study were **7f**, **7d**, **7b**, and **7g** with 94%−86% inhibition of 17β-HSD1 at 0.1 μM concentration.

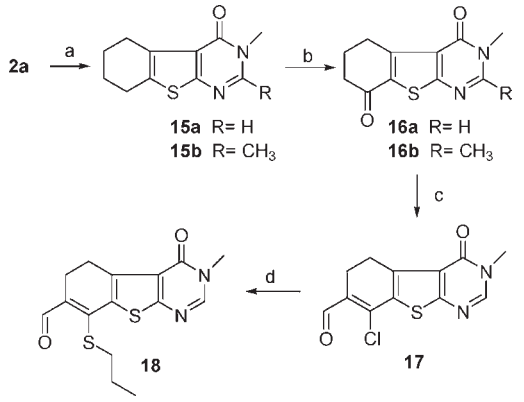
The cyclized intermediates **3a**−**3e** and **4a**−**4e** did not show significant activity against 17β-HSD1 or 17β-HSD2. The activity was somewhat improved with the β-chlorovinylaldehyde intermediates **5a**−**5d**. However, the best inhibitory activity was obtained with compounds bearing a thioether moiety in ring A. Homologation of the dicycloalkeno thieno-[2,3-*d*]pyrimidin-4(3*H*)-one core of potent inhibitor **6b** gave compounds **8**−**10** and **18** with decreased biological activity (Table 3); hence the original thieno[2,3-*d*]pyrimidin-4(3*H*)-one core with a six-membered ring A and a seven-membered ring D was chosen for further modifications. Aromatic ring A did not significantly improve the inhibitory activity of derivatives **19a** and **19b**. Similar inhibitory activity was found with

Scheme 2[a]



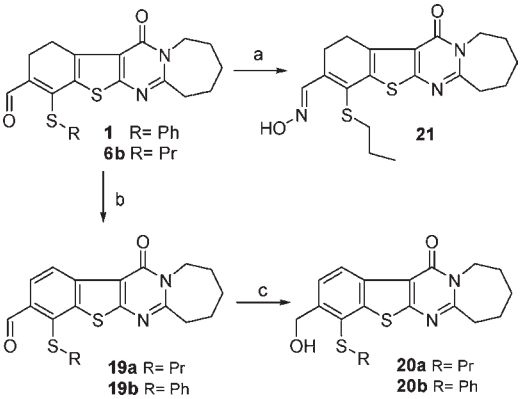
[a] Reagents and conditions: (a) $ClCOCH_2Cl$, Et_2O, 94%; (b) EtOC(S)SK, DMF; (c) 1,3-diaminopropane, dry THF, 0 °C, 67%; (d) NH_3(aq), rt, overnight.

Scheme 3[a]



[a] Reagents and conditions: (a) acetamide or *N*-methylacetamide, $POCl_3$, 1,2-dichloroethane, reflux, 74−85%; (b) $K_2S_2O_8$, $CuSO_4 \cdot 5H_2O$, MeCN−H_2O, reflux, 68−70%; (c) $POCl_3$−DMF, CH_2Cl_2, rt, overnight, 89%; (d) PrSH, NaOH, THF, 91%.

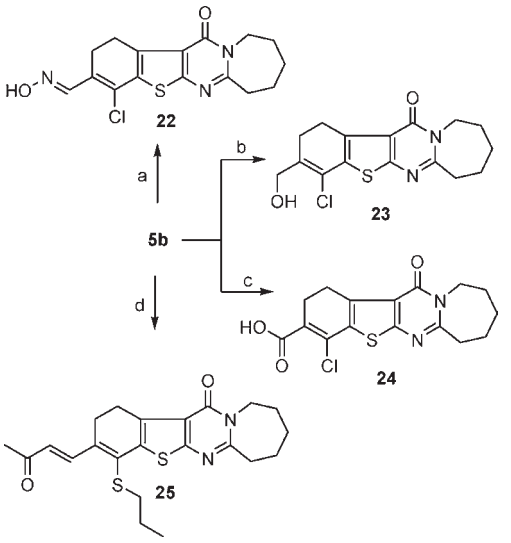
Scheme 4[a]



[a] Reagents and conditions: (a) $NH_2OH \cdot HCl$, AcONa, EtOH, 83%; (b) DDQ, benzene, reflux, 84−91%; (c) $NaBH_4$, THF, 89−92%.

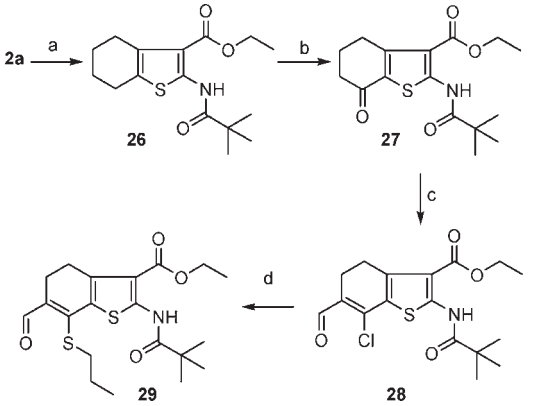
the benzylic alcohols **20a** and **20b**, proving that the potentially labile aldehyde moiety is not essential for the inhibitory activity. The reduction of the aldehyde functionality in **1** and **6b** produced the corresponding allylic alcohols with retained biological activity (data not shown). However, the ring A allylic alcohols were chemically unstable and were rejected as potential drug candidates. Further modifications

Scheme 5[a]



[a] Reagents and conditions: (a) $NH_2OH \cdot HCl$, AcONa, EtOH, 75%; (b) $NaBH_4$, THF, 78%; (c) $NaClO_2$, $NaH_2PO_4 \cdot H_2O$, 2-methyl-2-butene, *t*-BuOH−H_2O, 72%; (d) PrSH, NaOH, acetone, 65%.

Scheme 6[a]

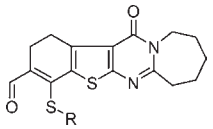


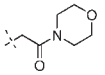
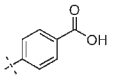
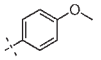
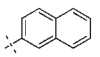
[a] Reagents and conditions: (a) pivaloyl chloride, Et_3N, CH_2Cl_2, 92%; (b) $K_2S_2O_8$, $CuSO_4 \cdot 5H_2O$, MeCN−H_2O, reflux, 80%; (c) $POCl_3$−DMF, CH_2Cl_2, 92%; (d) PrSH, NaOH, THF, 73%.

of the aldehyde functionality in ring A included an introduction of an oxime and α,β-unsaturated ketone (compounds **21** and **25**) to compound **6b**, but neither of these substituents was beneficial (Table 3). The ring A aldehyde functionality was also modified in the β-chlorovinylaldehyde derivative **5b** by introducing oxime, methylenehydroxyl, and carboxy moieties (compounds **22**, **23** and **24**, respectively). These compounds failed to show good inhibitory activity suggesting unfavorable stereoelectronic effects, also emphasizing the importance of the thioether moiety for the biological activity. The bicyclic benzothiophene derivatives **26**−**29** did not show any activity against 17β-HSD1 and 17β-HSD2 in a cell-free assay.

The nature of the thioether moiety at ring A seemed quite strongly to affect the cell permeability of these (di)cycloalkeno thieno[2,3-*d*]pyrimidin-4(3*H*)-one based 17β-HSD1 inhibitors. With few exceptions, the inhibitory activity was retained to a satisfactory level in MCF-7 cells expressing the human recombinant 17β-HSD1 enzyme (Tables 2 and 3), especially at the higher 10 μM test concentration. Compounds **6a**−**6d**, having a simple aliphatic thioether side chain, seemed to best

Table 2. The Influence of the Thioether Substitution on the Biological Activity



		Inhibition% 17β-HSD1 Cell-Free Assay[d]		Inhibition% 17β-HSD1 Cell-Based Assay[e]		Inhibition% 17β-HSD2 Cell-Free Assay[d]	
	-R[a]	0.1 μM	1 μM	1 μM	10 μM	0.1 μM	1 μM
6a		71.3	94.5	66.1	100.0	ni[b]	2.0
6b		72.9	95.2	74.0	100.0	ni	5.3
6c		67.6	93.4	54.1	100.0	ni	7.8
6d		64.7	92.9	71.9	100.0	ni	ni
6e		49.8	89.6	33.6	87.0	ni	1.2
6f		46.6	87.4	40.3	80.5	ni	ni
6g		51.2	82.2	32.7	63.9	ni	ni
6h		0.3	2.8	-[c]	-	-	-
6i		4.0	7.8	3.9	39.4	3.9	3.1
1		63.3	85.1	40.9	98.6	-	-
7a		47.0	80.6	18.9	69.4	8.2	32.1
7b		87.4	91.9	17.3	27.4	17.0	27.4
7c		3.8	22.1	-	-	-	-
7d		89.0	89.8	24.6	71.6	8.6	53.0
7e		58.5	91.8	36.5	76.8	21.7	22.6
7f		94.2	96.5	52.6	84.1	40.9	48.1
7g		85.6	91.3	18.2	90.1	32.6	71.6
7h		79.2	87.2	-	-	10.5	13.7
7i		7.2	30.7	-	-	25.8	62.2
7j		55.2	64.0	-	-	19.0	40.1

[a] The dashed line indicates the point of attachment. [b] No inhibition. [c] Not determined. [d] Human recombinant enzyme. [e] MCF-7 cells stably transfected with 17β-HSD1.

retain their biological activity in a cell-based assay. With compound **7b**, bearing a C4′ fluoro substituent in the aromatic ring, the activity decreased significantly compared with the cell-free assay. The aromatization of the ring A (compounds **19** and **20**) seemed to somewhat reduce the inhibitory activity in the cell-based assay, whereas in the cell-free assay the activity was retained. The results obtained from the cell-based assay raise an interesting point for future investigations concerning the bioavailability or metabolism of these compounds.

In general, all of the 17β-HSD1 inhibitors based on the thieno[2,3-*d*]pyrimidin-4(3*H*)-one core showed good to excellent selectivity over the oxidative isoform 17β-HSD2 (Tables 2 and 3). Compounds **6a**−**6g**, with an aliphatic thioether side chain, failed to show any inhibition of 17β-HSD2 at 0.1 μM. In addition, representative inhibitor structures were chosen, and their binding to estrogen receptors was evaluated. Compounds **1**, **5d**, **6b**, **6e**−**6g**, **7g**, **10**, and **19b** did not show any undesired estrogenicity in an ERα or ERβ binding assay, with EC_{50} values over 1 μM (Supporting Information).

Table 3. The Effect of Homologation of the Dicycloalkeno Thieno[2,3-*d*]pyrimidin-4(3*H*)-one Core and Ring A Modifications on the Biological Activity

	inhibition (%) 17β-HSD1 cell-free assay[c]		inhibition (%) 17β-HSD1 cell-based assay[d]		inhibition (%) 17β-HSD2 cell-free assay[c]	
compd	0.1 μM	1 μM	1 μM	10 μM	0.1 μM	1 μM
3c	8.0	23.2	10.0	45.9	*b*	*b*
4b	*a*	3.8	6.3	35.8	*b*	*b*
5a	3.3	26.1	19.7	38.6	*b*	*b*
5b	32.4	78.6	27.9	78.0	2.5	1.9
8	16.7	67.2	*b*	*b*	*b*	*b*
9	35.2	87.0	43.9	100.0	*a*	*a*
10	63.3	74.7	15.6	57.0	31.4	36.9
18	24.3	55.8	19.5	48.2	12.9	17.4
19a	83.0	90.6	28.5	86.4	3.8	12.7
19b	26.9	88.4	15.1	87.9	*a*	*a*
20a	69.0	85.9	35.6	78.6	13.6	20.6
20b	80.5	87.5	30.5	46.9	30.8	37.3
21	0.1	2.9	*b*	*b*	19.7	41.1
22	4.1	17.9	14.0	15.1	*b*	*b*
23	14.5	39.9	25.9	36.2	*b*	*b*
25	1.1	13.9	8.4	8.9	*b*	*b*

[a] No inhibition. [b] Not determined. [c] Human recombinant enzyme. [d] MCF-7 cells stably transfected with 17β-HSD1.

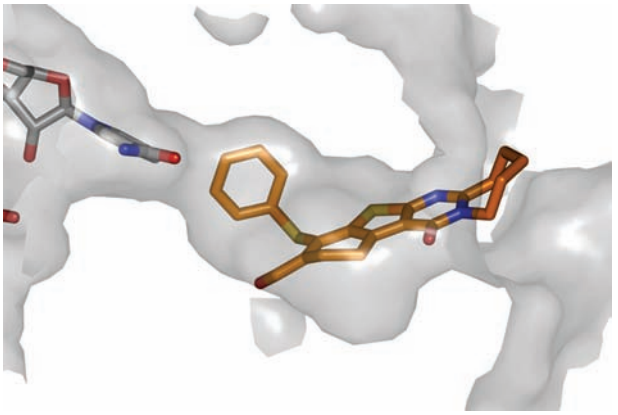


Figure 3. The active site surface around the lead compound **1** (orange). The bulky thioether side chain is facing the cofactor $NADP^+$ and is accommodated by a hydrophobic pocket. The aliphatic seven-membered ring protrudes partially out of the active site.

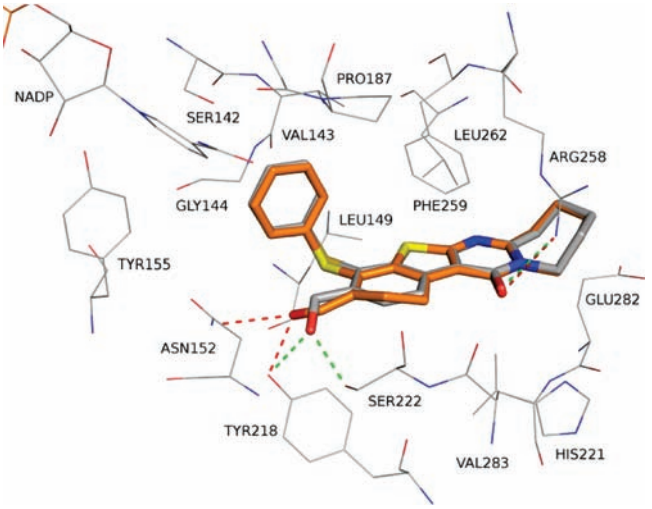


Figure 4. The lead compound **1** (orange) and inhibitor **20b** (gray) docked into the representative structure of the 17β-HSD1 MDS trajectory. Hydrogen bonds from **1** and the inhibitor **20b** are shown as red and green dashed lines, respectively. For clarity, $NADP^+$ and selected active site residues are displayed as thin sticks and hydrogens are omitted.

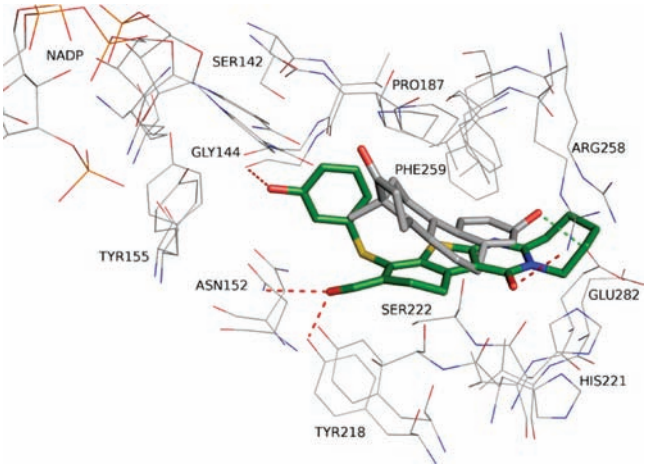


Figure 5. A comparison of the binding modes of the best inhibitor **7f** (green) and E2 (gray). Inhibitor **7f** is docked into the representative structure of the MDS trajectory with the lead compound **1**, and the orientation of E2 is from the relaxed crystal structure of 17β-HSD1. Hydrogen bonds from inhibitor **7f** and E2 are shown as red and green dashed lines, respectively. For clarity, $NADP^+$ and selected active site residues are displayed as thin sticks and hydrogens are omitted. The enzyme structures are superimposed based on the backbone.

We have recently reported the plausible binding mode of 17β-HSD1 inhibitors based on a thieno[2,3-*d*]pyrimidin-4(3*H*)-one core.[42] A crystal structure of 17β-HSD1 (pdb code 1FDT)[12a] was first subjected to molecular dynamics simulation (MDS) to obtain a relaxed enzyme structure with the reduced product E2. The lead compound **1** was docked into the active site of the relaxed enzyme structure and simulated under physiological conditions. The MDS was done in order to mimic the dynamic process of inhibitor binding and to reveal plausible interactions between the active site and **1**. Next, a set of inhibitors, including **6a**−**6i**, **7a**−**7j**, **18**, **19a**, **19b**, **20a**, **20b**, **21**−**25**, and **29**, were docked into the representative structure of the simulation trajectory, resulting in a good alignment. The binding mode of **1** shows an orientation where the ring A aldehyde group is hydrogen bonded to Asn152 and Tyr218 (Figures 3 and 4). Moreover, the pyrimidinone carbonyl forms a hydrogen bond to Arg258. The bulky aromatic

thioether side chain is accommodated by a hydrophobic pocket, formed during the MDS. Aromatization of ring A and reduction of the aldehyde group of **1** results in a more potent inhibitor **20b** in which the hydroxy group serves as an acceptor in the hydrogen bond from Tyr218 and as a donor in the hydrogen bond to Ser222 (Figure 4). Inhibitors **6a**−**6i**, with an aliphatic side chain, adopt a comparable binding mode with **1**. The hydrophobicity of the pocket around the thioether side chain explains the lack of activity of inhibitors **6h** and **7c**, bearing a carboxylic acid substituent. One of the most potent inhibitors in the series, **7f**, has the same interactions as **1**, and an additional hydrogen bond from the phenolic hydroxy group at C3′ of the thiophenyl moiety to the backbone carbonyl at Gly144 (Figure 5), which explains the increase in activity compared with **1**.

Conclusions

17β-HSD1 is an attractive biological target for inhibitor development for treating and preventing hormone-dependent disorders, for example, breast cancer. In this study, several thieno[2,3-*d*]pyrimidin-4(3*H*)-one based compounds were found to be potent and selective 17β-HSD1 inhibitors as well as to lack any undesired estrogenic effects. An efficient synthetic route was developed for the lead compound and subsequently utilized in the synthesis of a molecule library based on a cycloalkeno thieno[2,3-*d*]pyrimidin-4(3*H*)-one core. $K_2S_2O_8$−$CuSO_4$ was shown to be an expedient reagent for the oxidation of the thiophene α-position in these ring structures.

This work shows that good inhibitory activity can be obtained with nonsteroidal and nonphenolic small molecules. For example, 4-(3-hydroxyphenylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2′,3′:4,5]-pyrimido[1,2-*a*]azepine-3-carboxaldehyde (**7f**) is one of the most potent 17β-HSD1 inhibitors reported to date with 94% inhibition of the recombinant enzyme at 0.1 μM test concentration (cell-free assay), and also several other compounds were found to have >80% inhibition. A plausible binding mode, consistent with the structure−activity relationships for the series, was identified for the inhibitors by MDS and docking studies. Most of the compounds showed good inhibitory activity also in a cell-based 17β-HSD1 assay, where the thioether moiety in ring A was shown to influence the cell permeability. Especially compounds **6a**−**6e**, bearing an aliphatic thioether moiety, showed promising activity in the cell-based assay. In general, these compounds did not show notable inhibition of the oxidative isoform 17β-HSD2. The fused dicycloalkeno thieno[2,3-*d*]pyrimidin-4(3*H*)-ones described in this study offer a new template for the design and future development of potent and selective 17β-HSD1 inhibitors.

Experimental Section

Inhibition of 17β-HSD1 and 17β-HSD2 in Cell-Free Assay. 17β-HSD1 and 17-HSD2 recombinant enzyme assays were performed as described previously.[21a] Briefly, 17β-HSD1 and 17β-HSD2 homogenates were prepared as follows: Recombinant baculovirus of 17β-HSD1 and 17β-HSD2 were generated by the Bac to Bac Expression System (Invitrogen). Recombinant bacmid DNAs were transfected to Sf9 insect cells using Cellfectin Reagent (Invitrogen). Cells were harvested 60 h later; the soluble fraction of cell lysates for 17β-HSD1[17] and microsomal fraction for 17β-HSD2 were isolated. Aliquots were stored frozen until determination of enzymatic activity.

Recombinant 17β-HSD1 assay. Recombinant enzyme homogenate (0.1 μg/mL) was incubated in 20 mM KH_2PO_4, pH 7.4, reaction buffer (final volume 0.2 mL), including protease inhibitors (Complete Protease Inhibitor Cocktail tablet, Roche Diagnostics, 1697498), 30 nM E1 (Sigma, E9750) as a substrate, 800 000 cpm/mL [^{3}H]-E1 (PerkinElmer, NET319001MC) as a tracer substrate, and 1 mM β-NADPH (Sigma, N1630) as a cofactor for 30 min at room temperature, in the presence of potential inhibitors at concentrations of 0.1 or 1 μM. Inhibitor stock solutions were prepared in DMSO. The enzyme reaction was stopped by the addition of trichloroacetic acid (1% final concentration). Samples were centrifuged in a microtiter plate at 4000 rpm for 10 min. Supernatants were applied to reverse-phase HPLC on a Waters Symmetry C18 column, equipped with a Waters Sentry Guard column. Isocratic HPLC runs were performed at room temperature at a flow rate of 1 mL/min of CH_3CN−H_2O 48:52 as an eluent. Radioactivity was monitored in the eluate by a Packard Flow scintillation analyzer. Total radioactivity for E1 and E2 were determined in each sample, and percent inhibition of E2 conversion by inhibitor was calculated according to the following formula: inhibition % = [(E2 conversion % of control − E2 conversion % of sample)/E2 conversion % of control]×100.

Recombinant 17β-HSD2 Assay. The assay was performed as described for 17β-HSD1 but with following modifications: 6 μg/mL of recombinant enzyme homogenate, 50 nM E2 (Sigma, E8875) as a substrate, 800 000 cpm/mL [^{3}H]-E2 (PerkinElmer, NET317) as a tracer substrate and 1 mM β-NAD (Sigma N7004) as a cofactor were used.

Inhibition of 17β-HSD1 in a Cell-Based Assay.[33] Briefly, the cell-based assays make use of human MCF-7 breast cancer cells stably transfected with cDNA coding for human 17β-HSD1.[33] These assays were used to evaluate the activity and selectivity of test compounds in a cellular environment. In this assay, the transfected cells were incubated with E1 as a substrate and [^{3}H]-E1 as a tracer substrate in the presence of potential inhibitors at concentrations of 1 and 10 μM for 1 h at 37 °C. The assays were performed in a 96-well plate format with three parallel samples. The analysis of substrate and the end product and percent inhibition of conversion by inhibitor were done as described above in recombinant 17β-HSD1 assay.

Chemistry. The NMR spectra were measured with a Varian Unity INOVA 500 or Varian Mercury$_{Plus}$ 300 spectrometer at 27 °C. The melting points were measured on a Büchi B-545 melting point apparatus and are corrected. The HRMS were measured with Bruker MicroTof$_{LC}$ (ESI) in positive mode using Agilent ESI Tunemix as a calibration solution or with JEOL JMS-SX102 (EI) operating at 70 eV. Silica gel (0.040−0.063 mm) was purchased from Merck. 1,2-Dichloroethane was dried with anhydrous $CaCl_2$ and distilled. DMF and CH_2Cl_2 were distilled from CaH_2. Compounds **2a** and **2b** were purchased from Acros. The purity of the target compounds was ≥95% by analytical HPLC.

General Procedure for the Synthesis of 4a−4e, 16a, 16b, and 27. Compound **3c** (4.0 g, 13.9 mmol), $K_2S_2O_8$ (11.3 g, 41.6 mmol), and $CuSO_4 \cdot 5H_2O$ (10.4 g, 41.6 mmol) in 250 mL of CH_3CN−H_2O (1:1) were heated to reflux for 20 min. The reaction was quenched with ice and extracted with CH_2Cl_2 (3× 50 mL). The organic layer was washed with 10% sodium thiosulfate (30 mL) and brine (30 mL), and dried over Na_2SO_4. After filtration, the solvent was evaporated. Flash chromatography (CH_2Cl_2−EtOAc 5:1 as an eluent) afforded **4c** as a white powder in 85% yield. The analytical data of compounds **4a**−**4d** and **16b** corresponded to previously published data.[36]

3,4,9,10,11,12-Hexahydro-1*H*-cyclohepta[4′,5′]thieno[2′,3′:4,5]-pyrimido[1,2-*a*]azepine-5,14(2*H*,8*H*)-dione (4e). Yield 65%, (white powder); mp 198 °C (EtOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.79−2.02 (m, 8H), 2.83 (m, 2H), 3.06 (m, 2H), 3.56 (m, 2H), 4.36 (m, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 21.4, 25.1, 25.6, 27.7, 27.9, 29.6, 37.8, 41.9, 42.5, 120.8, 137.3, 147.2, 159.4,

162.8, 166.8, 196.6; HRMS (ESI) calculated for $C_{16}H_{19}N_2O_2S$ $[M + H]^+$ 303.1162, found 303.1158.

6,7-Dihydro-3-methyl-[1]benzothieno[2,3-*d*]pyrimidine-4,8(3*H*,5*H*)-dione (16a). Yield 68% (white powder); mp 194 °C (EtOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.25 (m, 2H), 2.68 (m, 2H), 3.30 (m, 2H), 3.59 (s, 3H), 8.08 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 24.0, 25.9, 34.0, 38.3, 122.5, 133.5, 149.1, 149.7, 158.5, 168.5; HRMS (ESI) calculated for $C_{11}H_{11}N_2O_2S$ $[M + H]^+$ 235.0536, found 235.0540.

General Procedure for the Synthesis of 5a−5d, 17, and 28. $POCl_3$ (72.8 mmol, 6.8 mL) was added dropwise into anhydrous DMF (74.9 mmol, 5.8 mL) in anhydrous CH_2Cl_2 (20 mL) at 0 °C under argon. After 30 min, **4c** (3.0 g, 10.4 mmol) in anhydrous CH_2Cl_2 (15 mL) was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 4 days. The reaction was quenched with saturated NaOAc (150 mL) in ice bath (*caution! exothermic*), extracted with CH_2Cl_2 (3 × 50 mL), washed with brine (30 mL) and water (30 mL), and dried with Na_2SO_4. After filtration, the solvent was evaporated. Flash chromatography (CH_2Cl_2−EtOAc 9:1 as an eluent) afforded **5b** as yellow powder in 75% yield.

6-Chloro-1,2,3,8,9-hexahydro-10-oxo-[1]benzothieno[2,3-*d*]-pyrrolo[1,2-*a*]pyrimidine-7-carboxaldehyde (5a). Compound **5a** was synthesized from **4a** using DMF as a solvent. Yield 32%, yellow crystals; mp 225 °C (dec.) (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32 (m, 2H), 2.78 (m, 2H), 3.19 (apparent t, 2H, J = 7.9 Hz), 3.25 (m, 2H), 4.18 (m, 2H), 10.18 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 19.6, 21.8, 22.3, 32.5, 46.6, 120.8, 128.5, 131.0, 139.6, 139.8, 157.6, 161.6, 167.5, 188.6; HRMS (ESI) calculated for $C_{14}H_{12}ClO_2N_2S$ $[M+H]^+$ 307.0303, found 307.0290.

4-Chloro-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (5b). Yield 75%, yellow powder; mp 218 °C (dec.) (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78−1.88 (m, 6H), 2.79 (m, 2H), 3.06 (m, 2H), 3.26 (m, 2H), 4.36 (m, 2H), 10.19 (s, 1H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 21.8, 22.3, 25.0, 27.5, 29.5, 37.6, 42.4, 120.4, 128.5, 131.1, 139.9, 140.2, 158.5, 161.8, 165.6, 188.6; HRMS (EI) calculated for $C_{16}H_{15}ClO_2N_2S$ $[M]^+$ 334.0543, found 334.0531.

4-Chloro-1,2,7,9,10,11,12,14-octahydro-14-oxo-8*H*-[1]benzothieno[2′,3′:4,5]pyrimido[1,2-*a*]azocine-3-carboxaldehyde (5c). From **4d**, yield 60%; mp 229 °C (dec.) (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (m, 2H), 1.60 (m, 2H), 1.85−2.20 (m, 4H), 2.81 (m, 2H), 3.02 (m, 2H), 3.28 (m, 2H), 4.30 (broad s, 2H), 10.21 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 21.9, 22.3, 24.2, 26.1, 28.7, 30.6, 35.8, 42.9, 120.6, 128.5, 131.0, 140.0, 140.1, 158.4, 161.4, 166.1, 188.6; HRMS (ESI) calculated for $C_{17}H_{18}ClO_2N_2S$ $[M + H]^+$ 349.0772, found 349.0698.

5-Chloro-2,3,8,9,10,11,12,14-octahydro-14-oxo-1*H*-cyclohepta-[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-4-carboxaldehyde (5d). From **4e**, yield 83%; yellow powder; mp 112 °C (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.81 (m, 6H), 2.14 (m, 2H), 2.52 (m, 2H), 3.05 (m, 2H), 3.32 (m, 2H), 4.35 (m, 2H), 10.26 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 25.1, 25.5, 27.6, 29.6, 30.02, 30.04, 37.7, 42.5, 121.6, 133.0, 137.3, 141.9, 144.6, 159.0, 162.4, 165.4, 190.3; HRMS (EI) calculated for $C_{17}H_{17}ClO_2N_2S_2$ $[M]^+$ 348.0699, found 348.0706.

8-Chloro-3,4,5,6-tetrahydro-3-methyl-4-oxo-[1]benzothieno-[2,3-*d*]pyrimidine-7-carboxaldehyde (17). Compound **17** was synthesized from **16a** as described above except the crude product was purified by recrystallization from EtOH. Yield 89%; yellow crystals; mp 218 °C (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.82 (m, 2H), 3.30 (m, 2H), 3.59 (s, 3H), 8.02 (s, 1H), 10.22 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 22.2, 22.6, 34.3, 123.1, 129.4, 132.9, 139.75, 139.79, 148.0; HRMS (ESI) calculated for $C_{12}H_{10}ClN_2O_2S$ $[M + H]^+$ 281.0146, found 281.0143.

General Procedure for the Synthesis of 1, 6a−6i, 7a−7j, 8−10, 18, and 29. NaOH (1 M aq solution, 1.5 equiv) was added dropwise to the appropriate thiol (1.5 equiv) in distilled THF (~6 mL/mmol) at 0 °C. The solution was stirred for 5 min, after which the appropriate β-chlorovinylaldehyde intermediate (1 equiv) in THF was added dropwise. The reaction mixture was allowed to reach room temperature and stirred until no starting material was detected on TLC (CH_2Cl_2−EtOAc 9:1 as an eluent). Reaction mixture was poured into a large excess of water (~700 mL/mmol), neutralized with 1 M HCl, and stirred vigorously for 1 h. The precipitated crude product was filtrated followed by purification by recrystallization from a suitable solvent to afford the product. If a clear precipitate is not formed upon the addition of water and 1 M HCl, the product can be alternatively isolated by extraction with CH_2Cl_2 or EtOAc. For compounds **6h**, **7c**, and **7e−7g**, 3.0 equiv of NaOH was used.

1,2,7,8,9,10,11,13-Octahydro-13-oxo-4-(phenylthio)-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (1). Yield 87%, yellow crystals; mp 154 °C (dec.) (EtOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.75−1.83 (m, 6H), 2.86 (m, 2H), 2.99 (m, 2H), 3.28 (m, 2H), 4.32 (m, 2H), 7.19−7.33 (m, 5H), 10.47 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 22.26, 22.36, 25.0, 27.6, 29.5, 37.6, 42.3, 120.2, 127.4, 129.4, 129.5, 133.8, 134.4, 138.7, 139.2, 141.3, 158.5, 161.3, 165.5, 190.7; HRMS (ESI) calculated for $C_{22}H_{21}N_2O_2S_2$ $[M + H]^+$ 409.1039, found 423.1045.

4-(Ethylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2′,3′:4,5]-pyrimido[1,2-*a*]azepine-3-carboxaldehyde (6a). Yield 87%; mp 175 °C (dec.) (EtOH−petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.27 (t, 3H, J = 7.3 Hz), 1.83 (m, 6H), 2.77 (m, 2H), 2.91 (q, 2H, J = 7.3 Hz), 3.06 (m, 2H), 3.22 (m, 2H), 4.36 (m, 2H), 10.49 (s, 1H); HRMS (ESI) calculated for $C_{18}H_{21}N_2O_2S_2$ $[M + H]^+$ 361.1039, found 361.1048.

1,2,7,8,9,10,11,13-Octahydro-13-oxo-4-(propylthio)-[1]benzothieno[2′,3′:4,5]-pyrimido[1,2-*a*]azepine-3-carboxaldehyde (6b). Yield 92%; mp 193 °C (dec.) (EtOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99 (t, 3H, J = 7.3 Hz), 1.63 (q, 2H, J = 7.3 Hz), 1.79 (broad s, 2H), 1.87 (m, 4H), 2.76 (m, 2H), 2.86 (t, 2H, J = 7.3 Hz), 3.07 (m, 2H), 3.22 (m, 2H), 4.36 (m, 2H), 10.49 (s, 1H); HRMS (ESI) calculated for $C_{19}H_{23}N_2O_2S_2$ $[M + H]^+$ 375.1195, found 375.1184.

4-(Butylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2′,3′:4,5]-pyrimido[1,2-*a*]azepine-3-carboxaldehyde (6c). Yield 89% (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.89 (t, 3H, J = 7.3 Hz), 2.81 (m, 2H), 1.59 (m, 2H), 1.79 (broad s, 2H), 1.87 (m, 4H), 2.76 (m, 2H), 2.89 (t, 2H, J = 7.3 Hz), 3.07 (m, 2H), 3.22 (m, 2H), 4.37 (m, 2H), 10.48 (s, 1H); HRMS (ESI) calculated for $C_{20}H_{25}N_2O_2S_2$ $[M + H]^+$ 389.1352, found 389.13656.

4-[(1-Methylethyl)thio)]-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2′,3′:4,5]pyrimido-[1,2-*a*]azepine-3-carboxaldehyde (6d). Yield 68%, yellow crystals; mp 177 °C (dec.) (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (d, 6H, J = 6.7 Hz), 1.73−1.91 (m, 6H), 2.78 (m, 2H), 3.06 (m, 2H), 3.22 (m, 2H), 3.41 (sept, 1H, J = 6.7 Hz), 4.36 (m, 2H), 10.47 (s, 1H); HRMS (ESI) calculated for $C_{19}H_{23}N_2O_2S_2$ $[M + H]^+$ 375.1195, found 375.1212.

4-[(1,1-Dimethylethyl)thio)]-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (6e). Purified by flash chromatography using CH_2Cl_2−EtOAc 1:1 as an eluent. Yield 65%, yellow crystals; mp 151 °C (MeCN); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37 (m, 9H), 1.79 (m, 2H), 1.86 (m, 4H), 2.80 (m, 2H), 3.06 (m, 2H), 3.24 (m, 2H), 4.36 (m, 2H), 10.42 (s, 1H); HRMS (ESI) calculated for $C_{20}H_{25}N_2O_2S_2$ $[M + H]^+$ 389.1353, found 389.1362.

4-(Cyclopentylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]-benzothieno[2′,3′:4,5]-pyrimido[1,2-*a*]azepine-3-carboxaldehyde (6f). Yield 80%, yellow crystals; mp 194 °C (petroleum ether−EtOH, 3:7); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.73 (m, 14H), 2.76 (m, 2H), 3.06 (m, 2H), 3.22 (m, 2H), 3.61 (m, 1H), 4.37 (m, 2H), 10.46 (s, 1H); HRMS (EI) calculated for $C_{21}H_{24}O_2N_2S_2$ 400.1279, found 400.1284.

4-(Cyclohexylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]-benzothieno[2′,3′:4,5]-pyrimido[1,2-*a*]azepine-3-carboxaldehyde (6g). Yield 77%, yellow crystals; mp 209 °C (petroleum

ether−EtOH, 3:7); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25−1.99 (m, 16H), 2.77 (m, 2H), 3.07 (m, 2H), 3.14 (m, 1H), 3.22 (m, 2H), 4.37 (m, 2H), 10.47 (s, 1H); HRMS (EI) calculated for $C_{22}H_{26}O_2N_2S_2$ 414.1436 found 414.1456.

4-[(3-Formyl-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepin-4-yl)thio]propionic acid (6h). Compound **6h** was synthesized as described above except that 3 equiv of NaOH was used. Yield 73%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.75−1.88 (m, 6H), 2.68 (t, 2H, J = 7.0 Hz), 2.76 (m, 2H), 3.07 (m, 2H), 3.16 (t, 2H, J = 7.0 Hz), 3.22 (m, 2H), 4.36 (m, 2H), 10.45 (s, 1H), -*OH* exchanged; HRMS (ESI) calculated for $C_{19}H_{21}N_2O_4S_2$ $[M + H]^+$ 405.0937, found 405.0954.

4-[2-(4-Morpholinyl)-2-oxoethylthio]-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (6i). Purified by flash chromatogaphy using 5% MeOH−CH_2Cl_2 as an eluent. Yellow powder, yield 87%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.75−1.86 (m, 6H), 2.78 (m, 2H), 3.06 (m, 2H), 3.23 (m, 2H), 3.47 (m, 2H), 3.57 (m, 2H), 3.68 (m, 4H), 3.70 (s, 2H), 10.44 (s, 1H); HRMS (ESI) calculated for $C_{22}H_{26}N_3O_4S_2$ $[M + H]^+$ 460.1359, found 460.1349.

4-(4-Methylphenylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (7a). Yield 75%, yellow crystals; mp 211 °C (dec.) (EtOH−petroleum ether 7:3); ^{1}H NMR ($CDCl_3$) δ 1.79 (m, 6H), 2.28 (s, 3H), 2.84 (m, 2H), 2.99 (m, 2H), 3.26 (m, 2H), 4.32 (m, 2H), 7.07 (d, 2H, J = 7.9 Hz), 7.24 (m, 2H), 10.52 (s, 1H); HRMS (ESI) calculated for $C_{23}H_{23}N_2O_2S_2$ $[M + H]^+$ 423.1195, found 423.1180.

4-(4-Fluorophenylthio)-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (7b). Yield 86%; mp 230−231 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.79 (m, 6H), 2.85 (m, 2H), 3.00 (m, 2H), 3.26 (m, 2H), 4.33 (m, 2H), 6.98 (m, 2H), 7.34 (m, 2H), 10.53 (s, 1H); HRMS (ESI) calculated for $C_{22}H_{20}FN_2O_2S_2$ $[M + H]^+$ 427.0945, found 427.0949.

4-[(3-Formyl-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepin-4-yl)thio]benzoic acid (7c). Compound **7c** was synthesized as described above except that 3 equiv of NaOH was used. Yield 82%, yellow crystals; ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.69 (m, 6H), 2.78 (m, 2H), 2.98 (m, 2H), 3.23 (m, 2H), 4.26 (m, 2H), 7.46 (m, 1H), 7.85 (m, 1H), 10.31 (s, 1H), 12.99 (broad s, 1H); HRMS (ESI) calculated for $C_{23}H_{21}N_2O_4S_2$ $[M + H]^+$ 453.0937, found 453.0915.

4-[4-Methoxyphenylthio]-1,2,7,8,9,10,11,13-octahydro-13-oxo-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (7d). Yield 86%, yellow crystals; mp 189 °C (dec.) (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.79 (m, 6H), 2.82 (m, 2H), 3.0 (m, 2H), 3.24 (m, 2H), 3.76 (s, 3H), 4.32 (m, 2H), 6.81 (m, 2H), 7.32 (m, 2H), 10.56 (s, 1H); HRMS (ESI) calculated for $C_{23}H_{23}N_2O_3S_2$ $[M + H]^+$ 439.1145, found 439.1144.

1,2,7,8,9,10,11,13-Octahydro-4-(4-hydroxyphenylthio)-13-oxo-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (7e). Yield 78%, yellow crystals; mp 235 °C (dec.) (benzene−EtOH); ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.67 (m, 6H), 2.69 (m, 2H), 2.99 (m, 2H), 3.13 (m, 2H), 4.26 (m, 2H), 6.75 (m, 2H), 7.28 (m, 2H), 9.77 (s, 1H), 10.41 (s, 1H); HRMS (ESI) calculated for $C_{22}H_{21}N_2O_3S_2$ $[M + H]^+$ 425.0988, found 425.1010.

1,2,7,8,9,10,11,13-Octahydro-4-(3-hydroxyphenylthio)-13-oxo-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (7f). Purified by flash chromatography 10% MeOH−CH_2Cl_2 as an eluent. Yield 70%, yellow crystals; mp 206 °C (dec.) (EtOH); ^{1}H NMR (d_6-DMSO) δ 1.59−1.78 (m, 6H), 2.72 (m, 2H), 2.97 (m, 2H), 3.17 (m, 2H), 4.25 (m, 2H), 6.63 (m, 1H), 6.70 (m, 1H), 7.11 (t, 1H, J = 7.9 Hz), 9.65 (s, 1H), 10.3 (s, 1H); HRMS (ESI) calculated for $C_{22}H_{21}N_2O_3S_2$ $[M + H]^+$ 425.0988, found 425.0991.

1,2,7,8,9,10,11,13-Octahydro-4-(2-hydroxyphenylthio)-13-oxo-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (7g). Purified by flash chromatography using 10% MeOH−CH_2Cl_2 as an eluent. Yield 77%, yellow crystals; mp 214 °C (dec.) (AcOH−H_2O); ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.65 (m, 6H), 2.72 (m, 2H), 2.99 (m, 2H), 3.17 (m, 2H), 4.27 (m, 2H), 6.73 (dt, 1H, J = 1.2, 7.6 Hz), 6.85 (dd, 1H, J = 1.2, 7.9 Hz), 7.10 (m, 2H), 10.27 (s, 1H), 10.38 (s, 1H); HRMS (ESI) calculated for $C_{22}H_{21}N_2O_3S_2$ $[M + H]^+$ 425.0988, found 425.0991.

1,2,7,8,9,10,11,13-Octahydro-13-oxo-4-(4-pyridylthio)-[1]-benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (7h). Yield 80%, yellow crystals; mp 207 °C (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.81 (m, 6H), 2.91 (m, 2H), 3.01 (m, 2H), 3.35 (m, 2H), 4.35 (m, 2H), 7.12 (m, 2H), 8.42 (d, 2H, J = 5.9 Hz), 10.39 (s, 1H); HRMS (ESI) calculated for $C_{21}H_{20}N_3O_2S_2$ $[M + H]^+$ 410.0991, found 410.1009.

1,2,7,8,9,10,11,13-Octahydro-13-oxo-4-(phenylmethylthio)-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (7i). Yield 75%, yellow crystals; mp 182 °C (dec.) (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.85 (m, 6H), 2.61 (m, 2H), 3.10 (m, 4H), 4.03 (s, 2H), 4.38 (m, 2H), 7.04 (m, 2H), 7.23 (m, 3H), 9.91 (s, 1H); HRMS (ESI) calculated for $C_{23}H_{23}N_2O_2S_2$ $[M + H]^+$ 423.1195, found 423.1217.

1,2,7,8,9,10,11,13-Octahydro-4-(2-naphtalenylthio)-13-oxo-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (7j). Yield 81%, yellow crystals; mp 228 °C (dec.) (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78 (m, 6H), 2.91 (m, 4H), 3.21 (m, 2H), 4.30 (m, 2H), 7.38 (dd, 1H, J = 8.8 Hz, 1.8 Hz)), 7.47 (m, 2H), 7.72 (m, 3H), 7.82 (d, 1H, J = 1.8 Hz), 10.57 (s, 1H); HRMS (ESI) calculated for $C_{26}H_{23}N_2O_2S_2$ $[M + H]^+$ 459.1195, found 459.1210.

1,2,3,8,9,10-Hexahydro-10-oxo-6-(propylthio)-[1]benzothieno[2,3-*d*]pyrrolo[1,2-*a*]pyrimidine-7-carboxaldehyde (8). Purified by flash chromatography using EtOAc−CH_2Cl_2 (1:5) as an eluent. Yield 54%, yellow powder; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, 3H, J = 7.3 Hz), 1.63 (m, 2H), 2.32 (m, 2H), 2.76 (m, 2H), 2.87 (t, 2H, J = 7.3 Hz), 3.21 (m, 4H), 4.19 (m, 2H), 10.49 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.3, 19.7, 22.2, 22.4, 23.2, 32.6, 38.8, 46.6, 121.3, 135.3, 137.8, 138.4, 143.3, 157.8, 161.1, 167.3, 191.0; HRMS (ESI) calculated for $C_{17}H_{19}N_2O_2S_2$ $[M + H]^+$ 347.0882, found 347.0880.

2,3,8,9,10,11,12,14-Octatahydro-14-oxo-5-(propylthio)-1*H*-cyclohepta-[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-*a*]azepine-4-carboxaldehyde (9). Purified by flash chromatography using EtOAc−CH_2Cl_2 (1:5) as an eluent. Yield 69%, yellow powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (t, 3H, J = 7.3 Hz), 1.53 (m, 2H), 1.81 (broad s, 2H), 1.88 (m, 4H), 2.34 (m, 4H), 2.70 (t, 2H, J = 7.1 Hz), 3.08 (m, 2H), 3.11 (m, 2H), 4.39 (m, 2H), 10.45 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 13.2, 23.5, 25.1, 25.3, 26.2, 27.6, 29.6, 37.1, 37.2, 37.6, 42.4, 121.1, 135.1, 144.1, 145.2, 148.6, 158.7, 161.2, 165.5, 189.7; HRMS (ESI) calculated for $C_{20}H_{25}N_2O_2S_2$ $[M + H]^+$ 389.1352, found 389.1336.

1,2,7,9,10,11,12,14-Octahydro-14-oxo-4-(propylthio)-8*H*-[1]-benzothieno[2′,3′:4,5]pyrimido[1,2-*a*]azocine-3-carboxaldehyde (10). Yield 76%, yellow crystals; mp 172 °C (EtOH−petroleum ether, 7:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, 3H, J = 7.3 Hz), 1.44 (m, 2H), 1.62 (m, 4H), 1.93 (m, 4H), 2.76 (m, 2H), 2.87 (t, 2H, J = 7.3 Hz), 3.02 (m, 2H), 3.22 (m, 2H), 4.30 (m, 2H), 10.49 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.2, 22.1, 22.3, 23.1, 24.2, 26.1, 28.7, 30.6, 35.8, 38.5, 42.8, 121.0, 135.2, 138.2, 138.3, 143.3, 158.5, 160.8, 165.8, 191.0; HRMS (EI) calculated for $C_{20}H_{24}O_2N_2S_2$ $[M]^+$ 388.1279, found 388.1266.

3,4,5,6-Tetrahydro-3-methyl-4-oxo-8-(propylthio)-[1]benzothieno[2,3-*d*]pyrimidine-7-carboxaldehyde (18). Purified by flash chromatography with 10% MeOH−CH_2Cl_2 as an eluent. Yield 81%, yellow powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.00 (t, 3H, J = 7.3 Hz), 1.64 (m, 2H), 2.78 (m, 2H), 2.87 (t, 2H, J = 7.1 Hz), 3.24 (m, 2H), 3.58 (s, 3H), 8.00 (s, 1H), 10.50 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 13.2, 22.1, 22.3, 23.2, 33.9, 38.7, 123.1, 136.9, 137.7, 138.9, 142.8, 147.2, 158.2, 165.6, 191.0; HRMS (ESI) calculated for $C_{15}H_{17}N_2O_2S_2$ $[M + H]^+$ 321.0726, found 321.0740.

General Procedure for the Synthesis of 19a and 19b. Compound **1** (510 mg, 1.25 mmol) and DDQ (370 mg, 1.62 mmol)

were refluxed overnight in dry benzene (45 mL). The reaction mixture was cooled to room temperature and filtered through a short column of silica gel. The silical gel was washed with CH_2Cl_2−EtOAc (9:1) solution, and the combined organic solutions were evaporated. The crude product was purified by recrystallization to give the product.

7,8,9,10,11,13-Hexahydro-13-oxo-4-(propylthio)-[1]benzothieno[2′,3′:4,5]pyrimido-[1,2-*a*]azepine-3-carboxaldehyde (19a). Compound **19a** was prepared as described above except that **6b** was used as a starting material. White crystals, yield 84%; mp 186 °C (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, 3H, J = 7.2 Hz), 1.59 (m, 2H), 1.85 (m, 6H), 2.93 (t, 2H, J = 7.2 Hz), 3.15 (m, 2H), 4.48 (m, 2H), 8.08 (d, 1H, J = 8.2 Hz), 8.66 (dd, 1H, J = 0.6 Hz, J = 8.3 Hz), 10.84 (d, 1H, J = 0.9 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.6, 23.7, 25.2, 27.8, 29.9, 38.1, 40.1, 42.9, 116.5, 125.2, 126.1, 133.6, 135.3, 138.9, 145.1, 158.3, 164.0, 167.7, 192.1; HRMS (ESI) calculated for $C_{19}H_{21}N_2O_2S_2$ [M + H]$^+$ 373.1039, found 373.1048.

7,8,9,10,11,13-Hexahydro-13-oxo-4-(phenylthio)-[1]benzothieno-[2′,3′:4,5]pyrimido[1,2-*a*]azepine-3-carboxaldehyde (19b). White crystals, yield 91%; mp 198 °C (AcOH−H_2O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.86 (m, 6H), 3.12 (m, 2H), 4.47 (m, 2H), 7.19 (m, 5H), 8.16 (d, 1H, J = 8.2 Hz), 8.74 (dd, 1H, J = 8.2 Hz, J = 0.6 Hz), 10.78 (d, 1H, J = 0.9 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 24.8, 27.4, 29.6, 37.8, 42.6, 115.9, 125.5, 125.9, 126.9, 128.4, 129.5, 130.9, 134.7, 135.2, 139.3, 144.3, 157.9, 163.8, 167.5, 191.3; HRMS (ESI) calculated for $C_{22}H_{19}N_2O_2S_2$ [M + H]$^+$ 407.0882, found 407.0821.

General Procedure for the Synthesis of 20a, 20b, and 23. $NaBH_4$ (21 mg, 0.54 mmol) and compound **19a** (135 mg, 0.36 mmol) in THF (20 mL) were stirred at room temperature for 45 min. Water (30 mL) was added, and the solution was made slightly acidic (pH ∼5) with 10% HCl, furnishing the crude product as a white powder. The precipitate was filtered, washed with water, and dried. The product was purified by flash chromatography using CH_2Cl_2−EtOAc (1:1) as an eluent to give **20a** in 92% yield.

8,9,10,11-Tetrahydro-3-(hydroxymethyl)-4-(propylthio)- [1]-benzothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepin-13(7*H*)-one (20a). Yield 92%, white crystals; mp 162 °C (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, 3H, J = 7.3 Hz), 1.58 (m. 2H), 1.87 (m, 6H), 2.32 (t, -*OH*, J = 6.4 Hz), 2.89 (m, 2H), 3.13 (m, 2H), 4.48 (m, 2H), 5.03 (d, 2H J = 6.4 Hz), 7.62 (d, 1H, J = 8.2 Hz), 8.56 (d, 1H, J = 8.2 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.6, 23.6, 25.1, 27.7, 29.8, 37.8, 38.2, 42.6, 64.2, 116.5, 125.2, 126.4, 126.9, 134.0, 142.6, 144.8, 158.2, 162.6, 165.6; HRMS (ESI) calculated for $C_{19}H_{23}N_2O_2S_2$ [M + H]$^+$ 375.1195, found 375.1184.

8,9,10,11-Tetrahydro-3-(hydroxymethyl)-4-(phenylthio)-[1]-benzothieno[2′,3′:4,5]pyrimido[1,2-*a*]azepin-13(7*H*)-one (20b). Compound **20b** was prepared as described above except **19b** was used as a starting material. Yield 89%, white crystals; mp 200 °C (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1,87 (m, 6H), 2.14 (broad s, −*OH*) 3.11 (m, 2H), 4.47 (m, 2H), 4.96 (s, 2H), 7.13 (m, 5H), 7.73 (d, 1H, J = 8.2 Hz), 8.66 (d, 1H, J = 8.2 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 24.9, 27.5, 29.6, 37.6, 42.5, 63.8, 116.1, 123.7, 125.8, 126.3, 126.8, 127.4, 129.3, 134.31, 135.2, 142.7, 144.5, 158.0, 162.5, 165.5; HRMS (ESI) calculated for $C_{22}H_{21}N_2O_2S_2$ [M + H]$^+$ 409.1039, found 409.1032.

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Supporting Information Available: Synthesis of compounds **3a**−**3e**, **15a**, **15b**, **11**−**14**, **21**, **22**, and **24**−**26**, characterization of compounds **23** and **27**−**29**, ^{13}C NMR data for **1**, **6a**−**6i**, **7a**−**7j**, **8**−**10**, and **18**, HPLC purity determinations for the target compounds, and ERα and ERβ binding analysis for compounds **1**, **5d**, **6b**, **6e**−**6g**, **7g**, **10**, and **19b**.This material is available free of charge via the Internet at http://pubs.acs.org.

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