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Discovery of potent and orally bioavailable 17β-hydroxysteroid dehydrogenase type 3 inhibitors

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ABSTRACT

We have previously reported the discovery of a new class of potent inhibitors of 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) derived from benzylidene oxazolidinedione and thiazolidinedione scaffolds. In this study, these analogs were designed, synthesized, and evaluated in a human cell-based assay. The detailed structure–activity relationship (SAR) surrounding this pharmacophore were developed, and consequently a number of compounds from this series demonstrated single-digit nanomolar 17β-HDS3 inhibitory activity in vitro. Subsequent optimization work in pursuit of the improvement of oral bioavailability demonstrated in vivo proof-of-concept by prodrug strategy based on phosphate esters for these 17β-HSD3 inhibitors. When a phosphate ester **16** was administered orally at a high dose of 100 mg/kg, **16** showed approximately two times more potent testosterone (T)-lowering effect against a positive control in the luteinizing hormone-releasing hormone (LH–RH)-induced T production assay. The T-lowering effect continued at ca 10% level of control over 4 h after administration. The nonsteroidal molecules based on this series have the potential to provide unique and effective clinical opportunities for treatment of prostate cancer.

1. Introduction

Prostate cancer is the most frequently diagnosed cancer in men. According to the American Cancer Society, an estimated 192,280 men were diagnosed with prostate cancer in the US during 2009. With an estimated 27,360 deaths in 2009, prostate cancer is the second-leading cause of cancer death in men.[1] For advanced prostate cancer, 10–60% of the patients experience biochemical recurrence associated with treatment failure, and no consensus exists on the optimal therapy.

The human prostate is a hormone-sensitive organ that depends on androgens for growth and development. The regulation of androgen biosynthesis or its action on the androgen receptor is also central to the management of prostate cancer.[2] Production of androgens is controlled at two levels within the central nervous system; in addition, it is controlled locally in peripheral organs that are targeted by the hormones.

The 17β-hydroxysteroid dehydrogenase (17β-HSD) family mediates the activation and deactivation of sex steroids involving redox reactions centered about the C17 area of the steroid backbone (Fig. 1).[3] This family of enzymes has a direct impact on diseases that have been shown to be hormone-dependent, such as prostate and breast cancers. More specifically, both diseases depend on the long-term exposure to potent androgens and estrogens, which are biosynthesized as a result of the action of different types of 17β-HSD enzymes. 17β-Hydroxysteroid dehydrogenase type 3 (17β-HSD3) catalyzes the final step in the steroidgenesis of the potent androgen testosterone (T) by selectively reducing the C17 ketone of 4-androstene-3,17-dione (Δ^4-dione) with NADPH as a cofactor, in testis and prostate tissue.[4] 17β-HSD3 is almost exclusively located in testis, suggesting its potential involvement in gonadal T biosynthesis. 17β-HSD3 is also responsible for pseudohermaphroditism in deficient man but is asymptomatic in deficient women. Since 17β-HSD3 is not found in the ovary, whereas 17β-HSD5 is, it is suggested that the latter

Abbreviations: HSD, hydroxysteroid dehydrogenase; 17β-HSD3, 17β-hydroxysteroid dehydrogenase type 3; T, testosterone; Δ^4-dione, 4-androstene-3,17-dione; E1, estrone; E2, estradiol; AR, androgen receptor; ERα, estrogen receptor alpha; GR, glucocorticoid receptor; LH–RH, luteinizing hormone–releasing hormone; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of NADP; PK, pharmacokinetics; C_{max}, maximum drug concentration; AUC, area under the plasma concentration curve; HMBC, heteronuclear multiple bond connectivity; TBDMS, *tert*-butyl dimethylsilyl; TMS, trimethylsilyl; MMC, magnesium methyl carbonate; DMAP, *N,N*-dimethylaminopyridine; SAR, structure–activity relationship.

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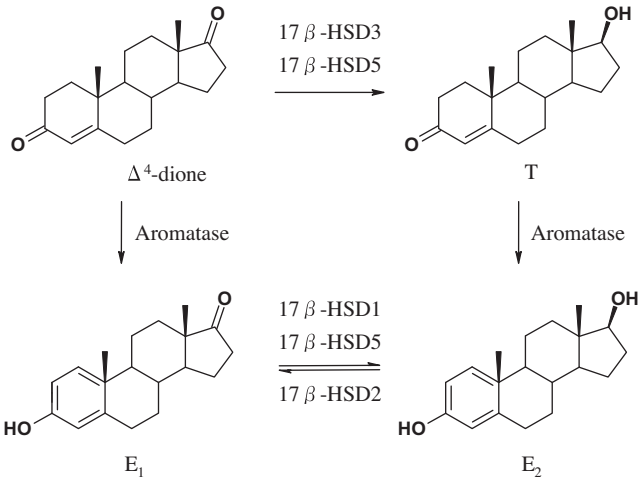


Figure 1. The role of 17β-HSD family in human steroidogenesis.

is involved in the conversion of Δ^4-dione to T in the ovary.[5] In addition, the expression of 17β-HSD3 mRNA in cancerous prostate biopsies was found to be 30-fold higher than in normal tissue.[6] Therefore, the role of 17β-HSD3 in T biosynthesis makes this enzyme an attractive molecular target of a small-molecule inhibitor for the treatment of prostate cancer.[7]

2. Chemistry

We recently described the identification of 3-aryl-5-benzylidene rhodanine **1a** and oxazolidione **2a** as a novel series of 17β-HSD3 inhibitors that were developed in a hit-to-lead optimization effort starting from the hit compound identified in an in-house screening (Fig. 2).[8] Herein, we report the preliminary structure–activity relationship (SAR) studies on these chemotypes and the improvement upon the pharmacokinetic (PK) properties of these compounds, which led to the discovery of orally bioavailable, potent 17β-HSD3 inhibitors.

An efficient protocol was applied to the generation of 3-substituted 5-arylidene rhodanines **1** (Scheme 1). Various commercially available amines (RNH_2, **3**) were used as building blocks to prepare a collection of 3,5-disubstituted rhodanine derivatives **1** in a sequential two-step process combining the Holmberg method, which is based on the reaction between bis(carboxymethyl)trithiocarbonate and primary amines, and the Knoevenagel condensation with a benzaldehyde.[9,10] In all cases, the products were isolated in high purity and in moderate yields after a simple precipitation from ethanol, and the thermodynamically more stable *Z*-isomers predominated (*Z*/*E* ratio $\geqslant$98/2). The *cis-trans* isomers were assigned as previously reported in the literature.[11]

The biaryl-substituted oxazolidione analogs were prepared by the synthetic procedure outlined in Scheme 2. 2-Thioxo-oxazolidinones **6** were prepared by treatment of arylamines **5** with

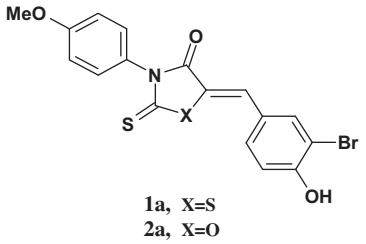
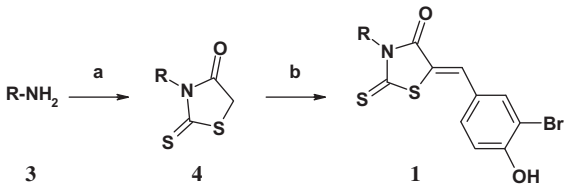
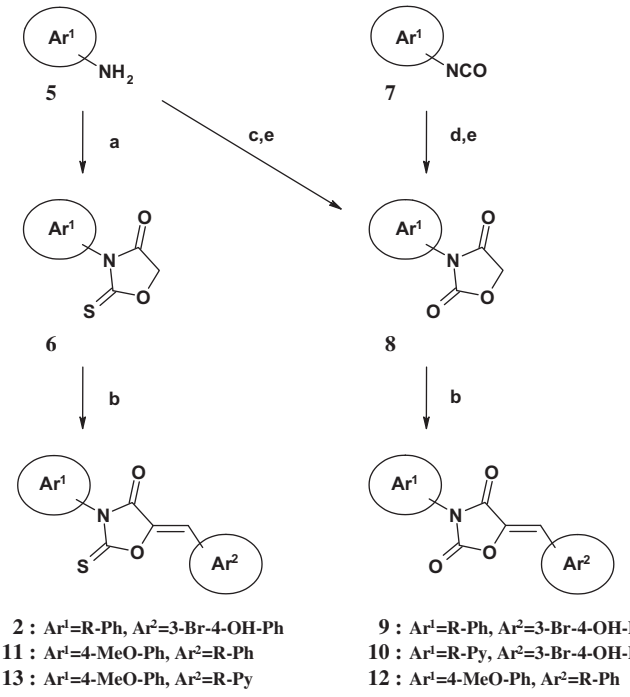


Figure 2. Structure of lead compounds **1a** and **2a**.



Scheme 1. Synthesis of rhodanine analogs **1**. Reagents and conditions: (a) $S{=}C(SCH_2CO_2H)_2$, CDI, THF, reflux, or $S{=}C(SCH_2CO_2H)_2$, Na_2CO_3, water, 60–70 °C, then H_2SO_4, 50 °C, 35–60%; (b) 3-bromo-4-hydroxybenzaldehyde, ammonium acetate or NaOAc, AcOH, reflux, 50–85%.

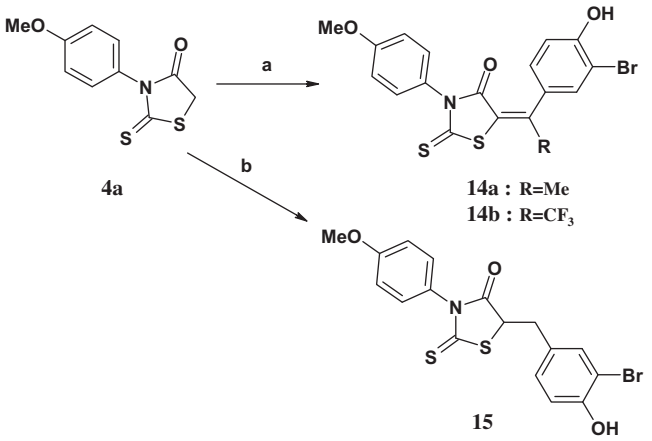


Scheme 2. Synthesis of 3-aryloxazolidinone analogs **2** and **9–13**. Reagents and conditions: (a) $BnSC(S)OCH_2CO_2K$, water, rt, then, Ac_2O, 100 °C, 35–60%; (b) Ar^2CHO, ammonium acetate or β-alanine, AcOH, reflux, 45–95%; (c) $HOCH_2CO_2Me$, CDI, Et_3N, THF, rt 50 °C, crude; (d) $HOCH_2CO_2Me$, DMF, rt 80 °C, crude; (e) NaOMe, toluene, 100 °C, 35–85%.

dithiocarbonate, followed by intramolecular cyclization with acetic anhydride. Arylamines **5** or arylisocyanates **7** were treated with glycolate ester to give the corresponding carbamates. Then, addition of catalytic sodium methoxide in toluene, followed by azeotropic removal of the generated alcohol under reflux, provided the cyclized 3-substituted 2,4-oxazolidinediones **8**. Knoevenagel condensation of the oxazolidinones (**6** and **8**) and aryl aldehydes afforded the corresponding 5-arylidene derivatives **2** and **9–13**.

Substitution on the bridge part was achieved in a similar manner to the unsubstituted analogs, using ketones instead of aldehydes (Scheme 3). The stereoisomers of compounds **14a,b** were determined to be *E*-isomers by heteronuclear multiple bond connectivity (HMBC) experiment (see Supplementary data). Moreover, benzylrhodanine **15**, which is structurally more flexible than compound **1a**, was synthesized by a direct alkylation of compound **4a** with magnesium methyl carbonate (MMC) and a subsequent deprotection of TBDMS in situ.[12]

Finally, a phosphate ester **16** was prepared according to the convenient synthetic route described in Scheme 4, to study bioprecursors of this series for the purpose of an exploration of practical 17β-HSD3 inhibitors. We chose to synthesize a target phos-



Scheme 3. Synthesis of 5-benzylidene and 5-benzyl rhodanes **14** and **15**. Reagents and conditions: (a) 3-Bromo-4-hydroxybenzaldehyde, β-alanine, AcOH, reflux, 50–85%; (b) 3-bromo-4-(*t*-butyldimethylsilyloxy)benzaldehyde, $MeOMgOCO_2Me$, DMF, 80 °C, 42%.

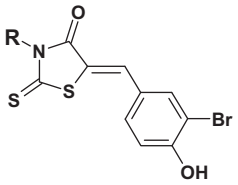
phate-prodrug via a dibenzyl phosphate, as the benzyl group as a protecting group is readily removed under mild conditions. A convenient phosphorylation of the phenols with dibenzyl phosphite proceeded rapidly in one pot and in moderate yield, using dibenzyl phosphate, only reagent quantities of CCl_4, and catalytic amounts of *N,N*-dimethylaminopyridine (DMAP).[13] The benzyl groups on the aryl phosphates were selectively removed with trimethylsilylbromide (TMSBr), providing silyl esters that were subsequently hydrolyzed with H_2O to afford the corresponding phosphate ester **16**.[14]

3. Results and discussion

The analogs were evaluated in a cell based assay, which measured the ability of the compounds to inhibit 17β-HSD3. The IC_{50} values were calculated for the inhibition at each concentration (1, 10, 100 nM, and 1 μM) of each compound, by measured concentration of T converted from Δ^4-dione with HeLa cells expressing the human recombinant 17β-HSD3 enzyme.

The initial result for the inhibition of 17β-HSD3 by our 5-arylidene rhodanines suggested that the nature of the substituent at the 3-position significantly affects the potency in a cell-based assay (Table 1). Replacement of the *p*-methoxyphenyl group with alkyl chains resulted in reduction of the activity, as revealed by a comparison of biological activities between **1a** and **1c–1f**. Additionally, N-unsubstituted rhodanine **1b** led to lack of cellular activity. On the other hand, cyclohexyl derivative **1g** exhibited single-digit nanomolar potency (IC_{50} = 3 nM). However, more extended incorporation of heteroatoms such as oxygen and nitrogen at the 4-position of the cyclohexyl group was found to decrease the potency

Table 1
Biological activity of rhodanine analogs

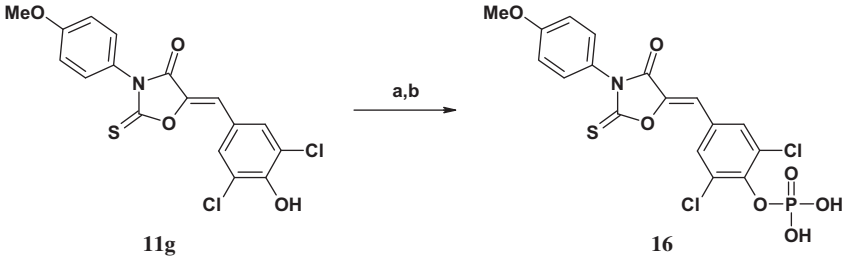


Compound	R	HSD IC_{50}[a] (nM)
1a	4-MeO-Ph	14
1b	H	na[b]
1c	Me	890
1d	allyl	120
1e	propagyl	300
1f	$MeOCH_2CH_2$	190
1g	cyclohexyl	3
1h	tetrahydropyran-4-yl	75
1i	1-methylpiperidin-4-yl	na
1j	4-MeO-$PhCH_2$	90

[a] Human cell-based assay. HeLa cells transiently transfected with 17β-HSD3. The deviations were within ±5%.
[b] Not active.

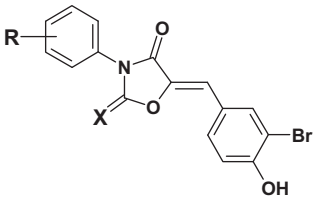
(**1h** and **1i**). A benzyl analog **1j** showed about 6-fold weaker inhibitory activity than the original **1a**. As a result, introduction of sterically bulky and hydrophobic moieties at the 3-position on the rhodanine, which is practically perpendicular to the substituents, enhanced the enzymatic inhibitory activity. The corresponding hydrophobic binding pocket in the enzyme is observed to affect a key interaction with this chemotype.

Next, acceptable diversity of substituents on the *N*-phenyl ring was investigated. The results are summarized in Table 2. With few exceptions, the 17β-HSD3 inhibitory activity was retained to a satisfactory level in a cell-based assay. Concerning the regioisomers of *p*-methoxyphenyl derivative **2a**, *o*-methoxyphenyl derivative **2d** showed reduced activity to about one-fifth of that of compound **2a**; on the other hand, *m*-methoxyphenyl analog **2c** continued to exhibit good potency, as did 3,4-methylenedioxy derivative **2e**. With compounds **2f–2i**, bearing a halo, an alkyl, and a haloalkyl substituent at the C4′ of the aromatic ring, the activity increased significantly compared with compound **2a**. The assay result of an unsubstituted phenyl (**2b**) and a C4′ electro-withdrawing substituent (**2j**) instead of the electro-donating methoxy group (**2a**) suggested that the electrostatic properties of the aromatic ring have no effect against the activity. Replacement of the 2-thioxo group in the hetroring system of compound **2a** to 2-oxo group (**9a**) provided similar potency. In consideration of further investigation concerning the aqueous solubility and the bioavailability, the 2,4-oxazolidinedione derivatives were expected to possess more suitable physicochemical properties than the 2-thioxo analogs. Therefore, water-soluble substituents at the C4′ of the phenyl ring as the



Scheme 4. Synthesis of a phosphate ester derivative **16**. Reagents and conditions: (a) $HP(O)(OBz)_2$, CCl_4, DMAP, DIEA, DMF, −10 °C rt, 61%; (b) Me_3SiBr, CHCl3, rt, then 1 N HCl, rt, 43%.

Table 2
Biological activity of *N*-phenyl analogs



Compound	R	X	HSD IC_{50}[a] (nM)
2a	4-OMe	S	13
2b	H	S	6
2c	3-OMe	S	7
2d	2-OMe	S	60
2e	3,4-OCH_2O-	S	12
2f	4-F	S	2
2g	4-Cl	S	6
2h	4-Me	S	2
2i	4-CF_3	S	3
2j	4-CN	S	19
9a	4-OMe	O	23
9b	4-CO_2H	O	na[b]
9c	4-CO_2Me	O	24
9d	4-C(O)NHMe	O	56
9e	4-C(O)NMe_2	O	65
9f	4-C(O)-piperizine	O	42
9g	4-C(O)-morphorine	O	300
9h	4-NMe_2	O	25
9i	4-NHAc	O	57
9j	4-NHCO-(c-hex)	O	21
9k	4-SMe	O	90
9l	4-SO_2Me	O	900
9m	4-SO_2NH_2	O	na

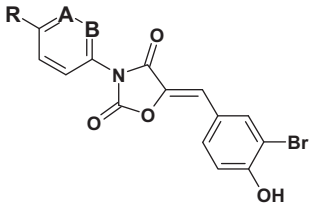
[a] Human cell-based assay. HeLa cells transiently transfected with 17β-HSD3. The deviations were within ±5%.
[b] Not active.

2,4-oxazolidinedione derivatives were sequentially evaluated for the purpose of improvement of undesirable physical properties in this series. Compounds bearing an ester (**9c**), amides (**9d–9f**), an alkylamino (**9h**), retro-amides (**9i** and **9j**), and a thioether (**9k**) were found to show favorable biological activities. However, changed substituents such as the (4-morpholinyl)carbonyl group in **9g**, as well as the methylsulfonyl group in **9l**, seemed not to be tolerated. In addition, introduction of acidic functionalities such as a carboxyl (**9b**) and a sulfamoyl (**9m**) resulted in complete loss of the 17β-HSD3 inhibitory activity.

With further modification on the N-aromatic ring, the pyridyl analogs were studied as shown in Table 3. 3′-Pyridyl derivative **10b** showed more potent inhibitory activity than 2′-pyridyl derivative **10a**. When we introduced several substituents onto the pyridine, a C6′ methoxy and dimethylamino substituent of the 3′-pyridyl ring (**10c** and **10g**) provided a substantial boost in potency, possessing IC_{50} values of 4 nM for each. The compounds **10d–10f**, bearing a fluoro, a chloro, and a methyl substituent at the C6′ of the 3′-pyridyl ring, showed similar activities compared with the unsubstituted **10b**. This SAR trend for the *N*-pyridyl series is different from that observed for the *N*-phenyl series, where the presence of a halo substituent and an alkyl group is likewise beneficial for 17β-HSD3 inhibitory activity.

An influence of the bridge part on the activity was summarized in Table 4. The introduction of a methyl and a trifluoromethyl group at the benzylic position was unfavorable, giving considerably decreased potency due to the predominance of the thermodynamically more stable *E*-isomer (**14a** and **14b**). The substitution at the bridge part made the configuration convert from *Z*-isomer to *E*-isomer, and even the size of the methyl group is critical to the activity. In addition, more flexible benzyl analog **15** was found to be a weak inhibitor, with an IC_{50} value of 90 nM.

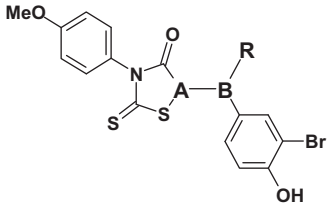
Table 3
Biological activity of *N*-heteroaryl analogs



Compound	R	A	B	HSD IC_{50}[a] (nM)
9a	OMe	CH	CH	23
10a	H	CH	N	74
10b	H	N	CH	22
10c	OMe	N	CH	4
10d	Me	N	CH	67
10e	F	N	CH	21
10f	Cl	N	CH	20
10g	NMe_2	N	CH	4

[a] Human cell-based assay. HeLa cells transiently transfected with 17β-HSD3. The deviations were within ±5%.

Table 4
Biological activity of rhodanine analogs

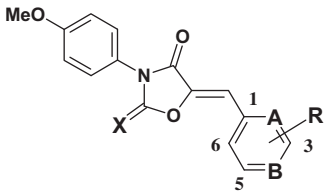


Compound	A–B	R	HSD IC_{50} (nM)[a]
1a	C=C	H	14
14a	C=C	Me	90
14b	C=C	CF_3	na[b]
15	CH–CH	H	90

[a] Human cell-based assay. HeLa cells transiently transfected with 17β-HSD3. The deviations were within ±5%.
[b] Not active.

Finally, our efforts focused on the optimization of the phenol moiety. As an initial result, the phenolic hydroxyl group at the C4′ was found to be essential, because replacement of the hydroxyl group with various functional groups such as hydrogen bonding donor/acceptor (an amino, a carboxyl, an amide, a cyano, and a fluoro substituent) resulted in a precipitous drop in enzymatic activity (data not shown). Next, substitution at in the C3′ and C5′ positions was evaluated. The results are summarized in Table 5. Replacement of the bromine atom with a methyl group (**11b**) was observed to keep the potency. The unsubstituted analog **11a** reduced the activity, and 3′-methoxy substitution (**11c**) was less tolerated. In contrast, the compounds **11d–11g** with mono- or dihalo substituents on the aromatic ring resulted in improvement of the activity; in particular, 3′-chloro-5′-fluoro-4′-hydroxylphenyl derivative **11i** demonstrated the best 17β-HSD3 inhibitory activity in a cell-based assay, possessing an IC_{50} value of 1 nM. Surprisingly, an abrupt drop of potency was observed in the case of X = O, 3′,5′-dichloro derivative **12d** compared with **11g**. In further exploration, introduction of substituents like a cyano in **12a**, a carboxyl in **12b**, and (*N*,*N*-dimethylamino)methyl in **12c**, were found not to be tolerated, and the inhibitory activities were lost. We speculated that the optimal size and the lipophilic effect of R influenced the activity, and that the intermolecular H-bonding between the 4′-hydro-

Table 5
Biological activity of oxazolidinone analogs



Compound	R	A	B	X	HSD IC_{50} (nM)[a]
2a	3-Br, 4-OH	CH	C	S	13
11a	4-OH	CH	C	S	121
11b	3-Me, 4-OH	CH	C	S	20
11c	3-OMe, 4-OH	CH	C	S	270
11d	3-F, 4-OH	CH	C	S	2
11e	3-Cl, 4-OH	CH	C	S	4
11f	2-Cl, 4-OH	CH	C	S	5
11g	3,5-DiCl, 4-OH	CH	C	S	12
11h	3,5DiF, 4-OH	C	CH	S	5
11i	3-Cl-5-F, 4-OH	CH	C	S	1
12a	3-CN, 4-OH	CH	C	O	na[b]
12b	3-CO_2H, 4-OH	CH	C	O	na
12c	3-CH_2NMe_2, 4-OH	CH	C	O	na
12d	3,5DiCl, 4-OH	CH	C	O	na
13a	4-OH	N	C	S	90
13b	3-Br, 4-OH	N	C	S	42

[a] Human cell-based assay. HeLa cells transiently transfected with 17β-HSD3. The deviations were within ±5%.
[b] Not active.

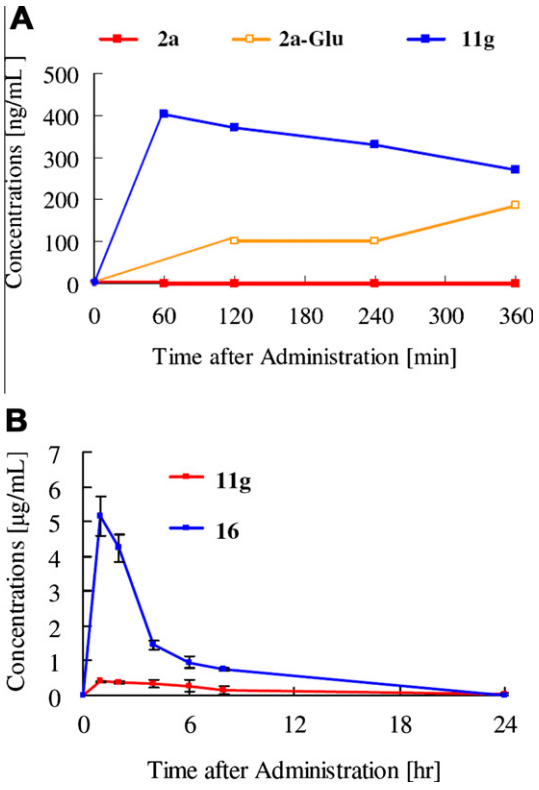


Figure 4. (A) Plasma concentration of compound **2a** and **2a**-glucuronide when dosed **2a**, and compound **11g** when dosed **11g** after oral administration (10 mg/kg) to male SD rats. (B) Plasma concentration of compound **11g** when dosed 11 g or its prodrug **16** after oral administration (10 mg/kg) to male SD rats. Each point with vertical bar represents the mean ± standard error of the mean (SEM) (n = 3).

xyl and the 3′-substituent prevented interaction with a key amino acid residue in the pore region of the enzyme and induced a significant loss of the activity. The 17β-HSD3 inhibitory activity of pyridine derivative **13a** was observed to be reduced to about one-third of that of compound **2a** (IC_{50} = 42 nM). In comparison between **13a** and **13b**, this pyridyl analog was intuitively found to have a similar tendency to the phenyl analog on SAR.

In general, these compounds did not show any undesired inhibition of the oxidative isoenzyme 17β-HSD type 2 or 17β-HSD type 1, which convert the less potent estrogen estrone (E1) into the biologically active estradiol (E2). In selectivity over nuclear receptors such as androgen receptor (AR), estrogen receptor alpha (ERα), and glucocorticoid receptor (GR), intersection with the nuclear receptors was also not observed for these compounds. These chemotypes showed excellent selectivity over 17β-HSD isoenzymes and nuclear receptors (see previous report[8]).

Before examining androgen-lowering effect of the selected original **2a**, we confirmed the oral bioavailability of this compound (Fig. 4). Compound **2a** was obviously not bioavailable in preclinical species, owing to its 'brickdust' nature, with a solubility of less than 1 μg per mL and a high clearance based on glucuronic acid conjugation. Although for 3′,5′-dichloro analog (**11g**), improved oral absorption to the systemic circulation without the glucuronic acid conjugation was observed, the quantity in plasma was apparently not sufficient to enable detection of in vivo efficacy in the LH–RH-induced testosterone production assay.[15] Therefore, we investigated the development of bio-precursor, chemically modified versions of the pharmacologically active agent that must undergo transformation in vivo to release the active drug.

Phosphate ester prodrugs, designed for hydroxyl and amine functionalities of poorly water-soluble drugs with an aim to enhance their aqueous solubility to allow a more favorable oral or parenteral administration, typically display excellent or adequate chemical stability and rapid bioconversion back to the parent drug by phosphatases present in the intestinal brush border or in the liver.[16] Therefore, we studied bioavailability of phosphate esters of selected compounds. The strategy for enhanced bioavailability is depicted in Figure 3. After compound **11g** was converted to the phosphate ester derivative **16**, the oral bioavailability was examined by measuring the plasma concentration of the parent compound **11g**. As shown in Table 6, compound **16** demonstrated a significantly improved oral bioavailability compared to the parent

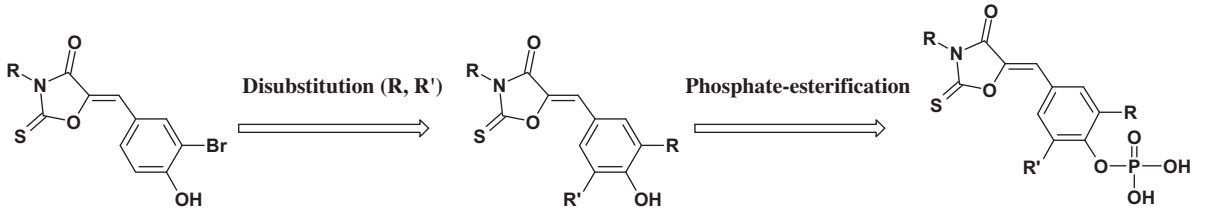


Figure 3. Chemical modification-strategy for enhanced bioavailability.

Table 6
Phamacokinetic parameters of **11g** and **16**

Compound	**11g**	**16**
T_{max} (h)	1	1
C_{max} (μg/mL)	0.40	5.25
AUC (μg h/mL)	4.0	18.8
F (%)	5.5	35.1

T_{max}, C_{max}, and AUC values were calculated from the plasma concentration after 10 mg/kg of **11g** or **16** were administered orally to male SD rats. F values were calculated by comparing the AUC in po study with the dose-corrected AUC values of intravenously administered rat plasma at 0.1 mg/kg.

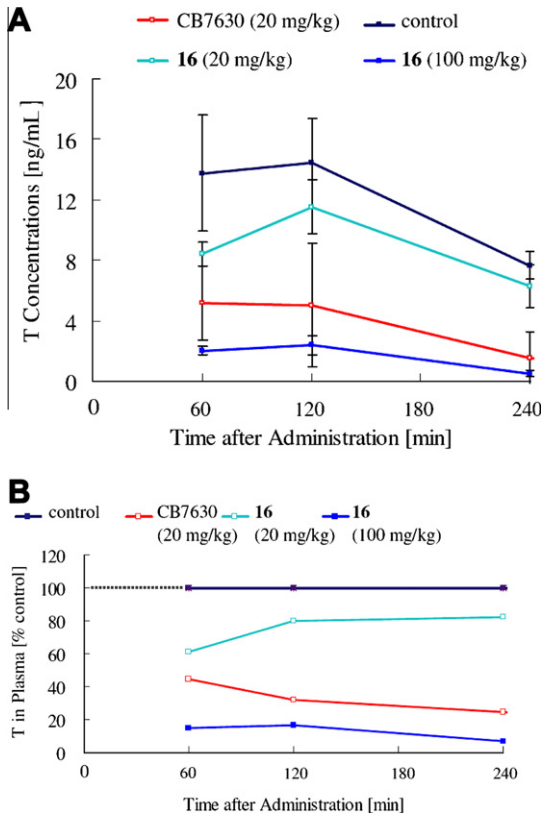


Figure 5. (A) Effects of compound **16** (po, 20 and 100 mg/kg) and a positive control CB7630 (po, 20 mg/kg) on the concentrations of testosterone (T) in plasma, after the administration of LH–RH agonist to male SD rats. Each point with vertical bar represents the mean ± standard error of the mean (SEM) (n = 4 or 5). (B) The effect of each compound on the concentrations of T in plasma is represented as % of control.

phenol **11g**. The pharmacokinetic parameters revealed that the phosphate ester was more druggable than the parent phenol, as indicated by C_{max} and area under the curve $(AUC)_{0-24\ h}$ at 10 mg/kg (**16**; C_{max} = 5.25 μg/mL, $AUC_{0-24\ h}$ = 18.8 μg h/mL versus **11g**; C_{max} = 0.40 μg/mL, $AUC_{0-24\ h}$ = 4.0 μg h/mL).

Based on the results of pre-examination, we used the phosphate ester **16** for in vivo evaluation to demonstrate in vivo proof-of-concept for this series of 17β-HSD3 inhibitors. Compound **16** was evaluated in terms of inhibition of a LH–RH-induced testosterone steroidogenesis. In this in vivo experiment, CB7630 (Abiraterone acetate), which is a steroidal cytochrome P450 17α-hydroxylase-17,20-lyase (CYP17) inhibitor approved by FDA as a drug for the treatment of androgen-dependent prostate cancer, was used a positive control. Compared to CB7630 given orally at a dose of 20 mg/kg to male Sprague–Dawley rats, compound **16** reduced plasma T levels in a dose-dependent manner (Fig. 5). When compound **16** was administered orally at a high dose of 100 mg/kg, **16** showed approximately two times more potent T-lowering effect against the positive control. The T-lowering effect continued at ca 10% level of control over 4 h after administration.

4. Conclusions

17β-HSD3. is an attractive biological target inhibitor for treating and preventing hormone-dependent disorders such as prostate cancer. In our previous work, several rhodanine- and oxazolidinone-based compounds were found to display not only potent and selective inhibition but also reduced androgenic effects.[7] An efficient synthetic route was developed for the seed compound and subsequently utilized in the synthesis of molecule libraries based on rhodanine and oxazolidinone cores. The SAR studies on the 3,5-disubstituted rhodanines and oxazolidinones culminated in the discovery of novel, highly potent, and selective inhibitors of 17β-HSD3. For example, 5-(3-chloro-5-fluoro-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-2-thioxo-1,3-oxazolidin-4-one **11i** is one of the most potent 17β-HSD3 inhibitors reported to date, with an IC_{50} value of 1.0 nM (human cell-based assay). Although representative compounds exhibited promising activity profiles, the compounds displayed very low aqueous solubility and poor PK profiles characterized by a lack of oral bioavailability. Therefore, we investigated the development of phosphate ester prodrugs with an aim to enhance their aqueous solubility to allow a more favorable oral administration. In this strategy, prodrug **16** demonstrated a significantly improved oral bioavailability compared to the parent **11g**. When compound **16** was administered orally to male Sprague–Dawley rats, **16** reduced plasma T levels in a dose-dependent manner, and showed approximately two times more potent T-lowering effect against the positive control. The T-lowering effect continued at ca 10% level of control over 4 h after administration. Preliminary biological evaluation in this study suggests that the nonsteroidal molecules based on this series have the potential to provide effective clinical opportunities for treatment of prostate cancer.

5. Experimental section

NMR spectra were recorded on a JEOL EX-270 or Varian Unity Inova 400 spectrometer. Chemical shifts are given in parts per million (ppm) downfield from internal reference tetramethylsilane in δ units. Mass spectra were measured with an LCQ LC/MS (Thermo Fisher Scientific) system for electro-spray ionization. Elemental analysis was performed on a CE Instruments EA1110 elemental analyzer. All solvents and reagents were obtained from commercial sources and were used without further purification. Column chromatography was performed on Merck 230–400 mesh silica gel. Analytical thin layer chromatography (TLC) was performed using Merck 60-F-254 0.25 mm precoated silica gel plates.

5.1. N-Substituted 2-thioxo-1,3-thiazolidin-4-ones 4: general procedure 1

A solution of trithiocarbonyl diglycolic acid (1.0 equiv) and 1,1′-carbonyldiimidazole (2.0 equiv) in THF (10 mL/mmol) was stirred at room temperature for 1.5 h. An amine **3** (1.0 equiv) was then added, and the mixture was refluxed for 4 h. A 2 M HCl solution (30 mL/mmol) was added, and the mixture was extracted with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluted with ethyl acetate/hexane to afford the title product (**4**).

5.1.1. Knoevenagel condensation: general procedure 2

To a solution of 3-substituted 2-thioxo-1,3-thiazolidin-4-one **4** (1.0 equiv) and 3-bromo-4-hydroxybenzaldehyde (1.0 equiv) in acetic acid (4 mL/mmol) was added ammonium acetate (2.0 equiv) or β-alanine (4.0 equiv). Then the mixture was stirred at 120 °C for 4 h. After cooling, the precipitate was collected by filtration and washed with water. The solid was triturated with ethanol to afford the title product (**1**).

5.1.1.1. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-2-thioxo-1,3-thiazolidin-4-one (1a). Compound **1a** was prepared following: general procedures 1 and 2. Yield 86%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.81 (s, 3H), 7.07 (d, J = 9.2 Hz, 2H), 7.13 (d, J = 8.6 Hz, 1H), 7.29 (d, J = 8.9 Hz, 2H), 7.51 (dd, J = 2.2, 8.4 Hz, 1H), 7.72 (s, 1H), 7.87 (d, J = 2.2 Hz, 1H), 11.31 (s, 1H); MS (ESI) m/z 423 (MH), 425 (MH+2); Anal. Calcd for $C_{17}H_{12}BrNO_3S_2$: C, 48.35; H, 2.87; N, 3.32. Found: C, 48.30; H, 2.98; N, 3.31.

5.1.1.2. 5-(3-Bromo-4-hydroxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one (1b). Compound **1b** was prepared from a commercially available reagent, rhodanine **4b** following: general procedure 2. Yield 92%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.11 (d, J = 8.4 Hz, 1H), 7.44 (dd, J = 2.2, 8.6 Hz, 1H), 7.56 (s, 1H), 7.80 (d, J = 2.2 Hz, 1H), 11.31 (brs, 1H), 13.76 (brs, 1H); MS (ESI) m/z 317 (MH), 319 (MH+2); Anal. Calcd for $C_{10}H_6BrNO_2S_2$: C, 37.99; H, 1.91; N, 4.43. Found: C, 37.96; H, 1.91; N, 4.45.

5.1.1.3. 5-(3-Bromo-4-hydroxybenzylidene)-3-methyl-2-thioxo-1,3-thiazolidin-4-one (1c). Compound **1c** was prepared from a commercially available reagent, 3-methyl-2-thioxo-1,3-thiazolidin-4-one **4c** following: general procedure 2. Yield 91%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.39 (s, 3H), 7.12 (d, J = 8.6 Hz, 1H), 7.48 (dd, J = 2.4, 8.6 Hz, 1H), 7.73 (s, 1H), 7.85 (d, J = 2.2 Hz, 1H), 11.31 (s, 1H); MS (ESI) m/z 331 (MH), 333 (MH+2); Anal. Calcd for $C_{11}H_8BrNO_2S_2$: C, 40.01; H, 2.44; N, 4.24. Found: C, 40.21; H, 2.28; N, 3.96.

5.1.1.4. 3-Allyl-5-(3-bromo-4-hydroxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one (1d). Compound **1d** was prepared from a commercially available reagent, 3-allyl-2-thioxo-1,3-thiazolidin-4-one **4d** following: general procedure 2. Yield 54%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 4.64 (d, J = 5.1 Hz, 2H), 5.09–5.20 (m, 2H), 5.77–5.92 (m, 1H), 7.12 (d, J = 8.4 Hz, 1H), 7.50 (dd, J = 2.3, 8.5 Hz, 1H), 7.75 (s, 1H), 7.87 (d, J = 2.2 Hz, 1H), 11.35 (s, 1H); MS (ESI) m/z 357 (MH), 359 (MH+2); Anal. ($C_{13}H_{10}BrNO_2S_2$): Anal. Calcd for $C_{13}H_{10}BrNO_2S_2$: C, 43.83; H, 2.83; N, 3.93. Found: C, 43.86; H, 3.00; N, 3.85.

5.1.1.5. 5-(3-Bromo-4-hydroxybenzylidene)-3-propagyl-2-thioxo-1,3-thiazolidin-4-one (1e). Compound **1e** was prepared following: general procedures 1 and 2. Yield 30%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.26 (t, J = 2.4 Hz, 1H), 4.76 (d, J = 2.4 Hz, 2H), 7.11 (d, J = 8.4 Hz, 1H), 7.49 (dd, J = 2.2, 8.6 Hz, 1H), 7.78 (s, 1H), 7.87 (d, J = 2.2 Hz, 1H), 11.36 (brs, 1H); MS (ESI) m/z 355 (MH), 357 (MH+2); Anal. Calcd for $C_{13}H_8BrNO_2S_2$: C, 44.08; H, 2.28; N, 3.96. Found: C, 44.27; H, 2.13; N, 3.71.

5.1.1.6. 5-(3-Bromo-4-hydroxybenzylidene)-3-(methoxyethyl)-2-thioxo-1,3-thiazolidin-4-one (1f). Compound **1f** was prepared following: general procedures 1 and 2. Yield 31%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.37 (s, 3H), 3.72 (t, J = 5.8 Hz, 2H), 4.36 (t, J = 5.8 Hz, 2H), 5.98 (s, 1H), 7.11 (d, J = 8.6 Hz, 1H), 7.38 (dd, J = 2.2, 8.6 Hz, 1H), 7.59 (s, 1H), 7.63 (d, J = 2.2 Hz, 1H); MS (ESI) m/z 375 (MH), 377 (MH+2); Anal. Calcd for $C_{13}H_{12}BrNO_3S_2$: C, 41.72; H, 3.23; N, 3.74. Found: C, 41.74; H, 3.39; N, 3.65.

5.1.1.7. 5-(3-Bromo-4-hydroxybenzylidene)-3-cyclohexyl-2-thioxo-1,3-thiazolidin-4-one (1g). Compound **1g** was prepared following: general procedures 1 and 2. Yield 43%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.24–1.92 (m, 10H), 4.96–5.05 (m, 1H), 5.91 (s, 1H), 7.11 (d, J = 8.6 Hz, 1H), 7.37 (dd, J = 2.2, 8.4 Hz, 1H), 7.59 (s, 1H), 7.61 (d, J = 2.4 Hz, 1H); MS (ESI) m/z 399 (MH), 401 (MH+2); Anal. Calcd for $C_{16}H_{16}BrNO_2S_2$: C, 48.25; H, 4.05; N, 3.52. Found: C, 48.20; H, 4.16; N, 3.51.

5.1.1.8. 5-(3-Bromo-4-hydroxybenzylidene)-3-(tetrahydropyran-4-yl)-2-thioxo-1,3-thiazolidin-4-one (1h). Compound **1h** was prepared following: general procedures 1 and 2. Yield 35%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.60 (d, J = 12.0 Hz, 2H), 2.60 (dd, J = 8.1, 16.5 Hz, 2H), 3.40 (d, J = 12.0 Hz, 2H), 3.98 (dd, J = 4.2, 11.2 Hz, 2H), 5.10–5.20 (m, 1H), 7.12 (d, J = 8.6 Hz, 1H), 7.47 (dd, J = 2.2, 8.4 Hz, 1H), 7.67 (s, 1H), 7.85 (d, J = 2.2 Hz, 1H), 11.32 (s, 1H); MS (ESI) m/z 401 (MH), 403 (MH+2); Anal. Calcd for $C_{15}H_{14}BrNO_3S_2$: C, 45.01; H, 3.53; N, 3.50. Found: C, 45.04; H, 3.70; N, 3.42.

5.1.1.9. 5-(3-Bromo-4-hydroxybenzylidene)-3-(1-methylpiperidin-4-yl)-2-thioxo-1,3-thiazolidin-4-one (1i). Compound **4i** was prepared following: general procedures 1 and 2. Yield 12%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.85–1.92 (m, 2H), 2.46–2.56 (m, 4H), 2.57 (s, 3H), 2.67–2.78 (m, 2H), 4.09–4.19 (m, 1H), 6.60 (s, 1H), 6.88 (d, J = 8.6 Hz, 1H), 7.33 (d, J = 8.9 Hz, 1H), 7.47 (d, J = 2.2 Hz, 1H); MS (ESI) m/z 414 (MH), 416 (MH+2); Anal. Calcd for $C_{16}H_{17}BrN_2O_2S_2$: C, 46.49; H, 4.15; N, 6.78. Found: C, 46.46; H, 4.15; N, 6.80.

5.1.1.10. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-methoxybenzyl)-2-thioxo-1,3-thiazolidin-4-one (1j). Compound **1j** was prepared following: general procedures 1 and 2. Yield 75%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.72 (s, 3H), 5.16 (s, 2H), 6.88 (d, J = 8.6 Hz, 2H), 7.12 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 8.6 Hz, 2H), 7.49 (dd, J = 1.6, 8.4 Hz, 1H), 7.76 (s, 1H), 7.86 (d, J = 1.6 Hz, 1 Hz), 11.35 (s, 1H); MS (ESI) m/z 437 (MH), 439 (MH+2); Anal. Calcd for $C_{18}H_{14}BrNO_3S_2$: C, 49.55; H, 3.24; N, 3.21. Found: C, 49.54; H, 3.19; N, 3.33.

5.1.1.11. N-Substituted 2-thioxo-1,3-oxazolidin-4-ones (6): general procedure 3. A solution of {[(benzylsulfanyl)carbonothionyl]oxy} acetic acid potassium salt (1.0 equiv) and an aryl amine **5** (1.0 equiv) in water (1 mL/mmol) was stirred at room temperature for 5 h. The mixture was washed with benzyl methyl ether, the aqueous layers acidified with 2 M HCl solution to pH 3. The precipitate was collected by filtration. The solid was extracted with chloroform, and the organic layers were washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and then the residue was treated with acetic anhydride (1.2 equiv) in acetic acid (1.0 mL/mmol) at 100 °C for 2 h. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography, eluted with ethyl acetate/hexane to afford the title product (**6**).

5.1.1.12. N-Substituted 1,3-oxazolidin-2,4-diones (8): general procedure 4–1. To a solution of ethyl glycolate (1.0 equiv) and DMF (1 mL/mmol) was added an isocyanate (0.91 equiv) at 0 °C. The mixture was then stirred at 80 °C for 3 h, and the reaction was quenched by adding water (5 mL/mmol). The precipitate was collected by filtration. The solid was extracted with ethyl acetate, and the organic layers were washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography, eluted with ethyl acetate/hexane to give ethyl [(arylcarbamoyl)oxy]acetate. A mixture of the ethyl [(arylcarba-

moyl)oxy]acetate (1.0 equiv) and sodium methoxide (0.03 equiv) in toluene (2 mL/mmol) was stirred under reflux for 1 h. After cooling, the precipitate was collected by filtration and purified by silica gel column chromatography, eluted with ethyl acetate/hexane to afford the title product (**8**).

5.1.1.13. N-Substituted 1,3-oxazolidin-2,4-diones (8): general procedure 4–2. To a solution of ethyl glycolate (1.0 equiv) in THF (1 mL/mmol) was added 1,1'-carbonyldiimidazole (1.1 equiv) at 0 °C, and the mixture was stirred at room temperature for 0.5 h. A solution of an aryl amine **5** (1.0 equiv) and triethylamine (0.7 equiv) in THF (1 mL/mmol) was then added, and the mixture was stirred under reflux for 5 h. After cooling, the mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography, eluted with ethyl acetate/hexane to afford the title product (**8**).

5.1.1.14. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-2-thioxo-1,3-oxazolidin-4-one (2a). Compound **2a** was prepared following: general procedures 2 and 3. Yield 70%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.82 (s, 3H), 6.94 (s, 1H), 7.08–7.15 (m, 3H), 7.39 (d, J = 8.9 Hz, 2H), 7.82 (dd, J = 2.2, 8.6 Hz, 1H), 8.10 (d, J = 2.2 Hz, 1H), 11.20 (s, 1H); MS (ESI) m/z 407 (MH), 409 (MH+2); Anal. Calcd for $C_{17}H_{12}BrNO_4S$: C, 50.25; H, 2.98; N, 3.45. Found: C, 50.43; H, 2.96; N, 3.27.

5.1.1.15. 5-(3-Bromo-4-hydroxybenzylidene)-3-phenyl-2-thioxo-1,3-oxazolidin-4-one (2b). Compound **2b** was prepared following: general procedures 2 and 3. Yield 66%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.97 (s, 1H), 7.13 (d, J = 8.6 Hz, 1H), 7.47–7.61 (m, 5H), 7.83 (dd, J = 2.0, 8.5 Hz, 1H), 8.11 (d, J = 2.2 Hz, 1H); MS (ESI) m/z 377 (MH), 379 (MH+2); Anal. Calcd for $C_{16}H_{10}BrNO_3S$: C, 51.07; H, 2.68; N, 3.72. Found: C, 51.11; H, 2.71; N, 3.61.

5.1.1.16. 5-(3-Bromo-4-hydroxybenzylidene)-3-(3-methoxyphenyl)-2-thioxo-1,3-oxazolidin-4-one (2c). Compound **2c** was prepared following: general procedures 2 and 3. Yield 45%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.78 (s, 3H), 6.95 (s, 1H), 7.03–7.14 (m, 4H), 7.47 (t, J = 8.5 Hz, 1H), 7.82 (dd, J = 2.2, 8.6 Hz, 1H), 8.11 (d, J = 2.2 Hz, 1H); MS (ESI) m/z 407 (MH), 409 (MH+2); Anal. Calcd for $C_{17}H_{12}BrNO_4S$: C, 50.25; H, 2.98; N, 3.45. Found: C, 50.45; H, 2.82; N, 3.17.

5.1.1.17. 5-(3-Bromo-4-hydroxybenzylidene)-3-(2-methoxyphenyl)-2-thioxo-1,3-oxazolidin-4-one (2d). Compound **2d** was prepared following: general procedures 2 and 3. Yield 66%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.79 (s, 3H), 7.00 (s, 1H), 7.13 (d, J = 8.6 Hz, 2H), 7.26 (d, J = 7.6 Hz, 2H), 7.43 (dd, J = 1.8, 7.7 Hz, 1H), 7.83 (dd, J = 2.2, 8.6 Hz, 1H), 8.11 (d, J = 2.2 Hz, 1H); MS (ESI) m/z 407 (MH), 409 (MH+2); Anal. Calcd for $C_{17}H_{12}BrNO_4S$: C, 50.25; H, 2.98; N, 3.45. Found: C, 50.17; H, 3.15; N, 3.48.

5.1.1.18. 3-(1,3-Benzodioxol-5-yl)-5-(3-bromo-4-hydroxybenzylidene)-2-thioxo-1,3-oxazolidin-4-one (2e). Compound **2e** was prepared following: general procedures 2 and 3. Yield 79%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.14 (s, 2H), 6.95 (s, 1H), 6.93–6.97 (m, 1H), 7.14 (t, J = 8.8 Hz, 1H), 7.66 (d, J = 8.6 Hz, 1H), 7.73 (d, J = 1.9, 12.4 Hz, 1H), 7.83 (dd, J = 2.4, 8.9 Hz, 1H), 8.28 (d, J = 2.7 Hz, 1H); MS (ESI) m/z 421 (MH), 423 (MH+2); Anal. Calcd for $C_{17}H_{10}BrNO_5S$: C, 48.58; H, 2.40; N, 3.33. Found: C, 48.61; H, 2.41; N, 3.31.

5.1.1.19. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-fluorophenyl)-2-thioxo-1,3-oxazolidin-4-one (2f). Compound **2f** was prepared following: general procedures 2 and 3. Yield 44%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.98 (s, 1H), 7.13 (d, J = 8.6 Hz, 1H), 7.39–7.58 (m, 4H), 7.83 (dd, J = 2.2, 8.6 Hz, 1H), 8.11 (d, J = 1.9 Hz, 1H), 11.22 (s, 1H); MS (ESI) m/z 395 (MH), 397 (MH+2); Anal. Calcd for $C_{16}H_9BrFNO_3S$: C, 48.74; H, 2.30; N, 3.55. Found: C, 48.81; H, 2.21; N, 3.51.

5.1.1.20. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-chlorophenyl)-2-thioxo-1,3-oxazolidin-4-one (2g). Compound **2g** was prepared following: general procedures 2 and 3. Yield 46%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.98 (s, 1H), 7.13 (d, J = 8.6 Hz, 1H), 7.51–7.56 (m, 2H), 7.63–7.68 (m, 2H), 7.83 (dd, J = 2.2, 8.6 Hz, 1H), 8.11 (d, J = 1.9 Hz, 1H), 11.23 (s, 1H); MS (ESI) m/z 411 (MH), 413 (MH+2); Anal. Calcd for $C_{16}H_9BrClNO_3S$: C, 46.79; H, 2.21; N, 3.41. Found: C, 46.74; H, 2.32; N, 3.40.

5.1.1.21. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-methylphenyl)-2-thioxo-1,3-oxazolidin-4-one (2h). Compound **2h** was prepared following: general procedures 2 and 3. Yield 32%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.29 (s, 3H), 6.95 (s, 1H), 7.13 (d, J = 8.6 Hz, 1H), 7.35 (brs, 4H), 7.82 (dd, J = 2.0, 8.5 Hz, 1H), 8.11 (d, J = 1.9 Hz, 1H), 11.20 (s, 1H); MS (ESI) m/z 391 (MH), 393 (MH+2); Anal. Calcd for $C_{17}H_{12}BrNO_3S$: C, 52.31; H, 3.10; N, 3.59. Found: C, 52.51; H, 2.94; N, 3.31.

5.1.1.22. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-trifluoromethylphenyl)-2-thioxo-1,3-oxazolidin-4-one (2i). Compound **2i** was prepared following: general procedures 2 and 3. Yield 25%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.01 (s, 1H), 7.15 (d, J = 8.6 Hz, 1H), 7.83–8.05 (m, 5H), 8.12 (d, J = 2.2 Hz, 1H), 11.24 (s, 1H); MS (ESI) m/z 445 (MH), 447 (MH+2); Anal. Calcd for $C_{17}H_9BrF_3NO_3S$: C, 45.95; H, 2.04; N, 3.15. Found: C, 45.98; H, 2.21; N, 3.07.

5.1.1.23. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-cyanophenyl)-2-thioxo-1,3-oxazolidin-4-one (2j). Compound **2j** was prepared following: general procedures 2 and 3. Yield 63%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.01 (s, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 8.1 Hz, 2H), 7.84 (dd, J = 1.4, 8.4 Hz, 1H), 8.09 (d, J = 8.4 Hz, 2H), 8.12 (d, J = 1.9 Hz, 1H); MS (ESI) m/z 402 (MH), 404 (MH+2); Anal. Calcd for $C_{17}H_9BrN_2O_3S$: C, 50.88; H, 2.26; N, 6.99. Found: C, 50.59; H, 2.21; N, 6.92.

5.1.1.24. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-1,3-oxazolidin-2,4-dione (9a). Compound **9a** was prepared following: general procedure 2 and 4–1. Yield 30%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.81 (s, 3H), 6.89 (s, 1H), 7.03–7.12 (m, 3H), 7.41 (d, J = 9.2 Hz, 2H), 7.73 (dd, J = 2.2, 8.6 Hz, 1H), 8.02 (d, J = 2.2 Hz, 1H), 11.04 (s, 1H); MS (ESI) m/z 391 (MH), 393 (MH+2); Anal. Calcd for $C_{17}H_{12}BrNO_5$: C, 52.31; H, 3.10; N, 3.59. Found: C, 52.32; H, 2.96; N, 3.63.

5.1.1.25. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-methoxycarbonylphenyl)-1,3-oxazolidin-2,4-dione (9c). Compound **9c** was prepared following: general procedure 2 and 4–1. Yield 42%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.90 (s, 3H), 6.95 (s, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.70 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.9 Hz, 1H), 8.04 (d, J = 1.7 Hz, 1H), 8.14 (d, J = 8.4 Hz, 2H), 11.09 (s, 1H); MS (ESI) m/z 419 (MH), 421 (MH+2); Anal. ($C_{18}H_{12}BrNO_6$): Anal. Calcd for $C_{17}H_{12}BrNO_5$: C, 52.31; H, 3.10; N, 3.59. Found: C, 52.32; H, 2.96; N, 3.63.

5.1.1.26. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-carboxyphenyl)-1,3-oxazolidin-2,4-dione (9b). A solution of 3-(4-methoxycarbonylphenyl)-1,3-oxazolidin-2,4-dione (1.38 g, 5.20 mmol) and concd HCl–acetic acid (v/v = 1/1, 26 mL) was stirred at 120 °C for 3 h. After cooling to room temperature, the precipitate was filtered off, washed with water, and dried in vacuo to give

3-(4-carboxyphenyl)-1,3-oxazolidin-2,4-dione (0.47 g, 38%); ^{1}H NMR (270 MHz, DMSO-d_6) δ 4.97 (s, 2H), 7.57 (d, J = 8.3 Hz, 2H), 8.09 (d, J = 8.6 Hz, 2H), 13.12 (s, 1H). Compound **9b** was prepared from the intermediate described above, following: general procedure 2. Yield 20%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.95 (s, 1H), 7.09 (d, J = 8.3 Hz, 1H), 7.66 (d, J = 8.6 Hz, 2H), 7.75 (dd, J = 2.0, 8.7 Hz, 1H), 8.03 (d, J = 2.0 Hz, 1H), 8.10 (d, J = 8.6 Hz, 2H), 11.10 (brs, 1H), 13.16 (brs, 1H); MS (ESI) m/z 404 (M), 405 (M+2); Anal. Calcd for $C_{17}H_{10}BrNO_6$: C, 50.50; H, 2.49; N, 3.47. Found: C, 50.25; H, 2.41; N, 3.42.

5.1.1.27. 5-(3-Bromo-4-hydroxybenzylidene)-3-[4-(*N*,*N*-dimethylcarbamoyl)phenyl]-1,3-oxazolidin-2,4-dione (9e). A solution of 3-(4-carboxyphenyl)-1,3-oxazolidin-2,4-dione (93 mg, 0.42 mmol), 50 wt% dimethylamine aqueous solution (48 mg, 1.3 equiv), *N*-methylmorpholine (85 μL, 1.8 equiv), 1-hydroxybenzotriazole (72 mg, 1.3 equiv), and 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide hydrochloride (121 mg, 1.5 equiv) in DMF (10 mL) was stirred at room temperature for 5 h. The mixture was acidified to pH 4, by addition of 10 wt% HCl solution. The mixture was extracted with ethyl acetate. The combined organic layers were washed with saturated sodium hydrogen carbonate solution and brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to give 4-[(*N*,*N*-dimethylcarbamoyl)phenyl]-1,3-oxazolidin-2,4-dione (96 mg, 66%); ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.89 (s, 3H), 3.00 (s, 3H), 4.96 (s, 2H), 7.46–7.58 (m, 4H). Compound **9e** was prepared from an intermediate described above, following: general procedure 2. Yield 34%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.93 (s, 3H), 3.00 (s, 3H), 6.94 (s, 1H), 7.09 (d, J = 8.6 Hz, 1H), 7.57 (brs, 4H), 7.74 (dd, J = 2.0, 8.5 Hz, 1H), 8.03 (d, J = 2.1 Hz, 1H); MS (ESI) m/z 432 (MH), 434 (MH+2); Anal. Calcd for $C_{19}H_{15}BrN_2O_5$: C, 52.90; H, 3.51; N, 6.50. Found: C, 52.75; H, 3.14; N, 6.41.

5.1.1.28. 5-(3-Bromo-4-hydroxybenzylidene)-3-[4-(*N*-methylcarbamoyl)phenyl]-1,3-oxazolidin-2,4-dione (9d). Compound **9d** was prepared with methyl amine in a manner similar to that described for compound **9e**. Yield 54%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.81 (d, J = 4.3 Hz, 3H), 6.94 (s, 1H), 7.09 (d, J = 8.7 Hz, 1H), 7.56–7.61 (m, 3H), 7.66 (d, J = 2.0 Hz, 1H), 7.96 (d, J = 8.3 Hz, 2H), 8.54 (d, J = 4.5 Hz, 1H), 10.68 (s, 1H); MS (ESI) m/z 418 (MH), 420 (MH+2); Anal. Calcd for $C_{18}H_{13}BrN_2O_5$: C, 51.80; H, 3.14; N, 6.72. Found: C, 52.00; H, 2.96; N, 6.67.

5.1.1.29. 5-(3-Bromo-4-hydroxybenzylidene)-3-[4-(piperidin-1-ylcarbonyl)phenyl]-1,3-oxazolidin-2,4-dione (9f). Compound **9f** was prepared with piperidine in a manner similar to that described for compound **9e**. Yield 23%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.52–1.60 (m, 6H), 3.20–3.70 (m, 4H), 6.94 (s, 1H), 7.08 (d, J = 8.6 Hz, 1H), 7.52–7.60 (m, 4H), 7.74 (dd, J = 2.1, 8.3 Hz, 1H), 8.03 (d, J = 1.9 Hz, 1H); MS (ESI) m/z 472 (MH), 474 (MH+2); Anal. Calcd for $C_{22}H_{19}BrN_2O_5$: C, 56.05; H, 4.07; N, 5.95. Found: C, 55.76; H, 4.02; N, 5.88.

5.1.1.30. 5-(3-Bromo-4-hydroxybenzylidene)-3-[4-(morpholin-4-ylcarbonyl)phenyl]-1,3-oxazolidin-2,4-dione (9g). Compound **9f** was prepared with morpholine in a manner similar to that described for compound **9e**. Yield 14%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.20–3.40 (m, 4H), 3.61–3.66 (m, 4H), 6.95 (s, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.60 (s, 4H), 7.75 (dd, J = 1.9, 8.5 Hz, 1H), 8.04 (d, J = 1.6 Hz, 1H), 11.07 (brs, 1H); MS (ESI) m/z 474 (MH), 476 (MH+2); Anal. Calcd for $C_{21}H_{17}BrN_2O_6$: C, 53.28; H, 3.62; N, 5.92. Found: C, 52.93; H, 3.25; N, 5.80.

5.1.1.31. 5-(3-Bromo-4-hydroxybenzylidene)-3-[4-(dimethylamino)phenyl]-1,3-oxazolidin-2,4-dione (9h). Compound **9h** was prepared following: general procedure 2 and 4–2. Yield 41%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.00 (s, 6H), 6.88 (s, 1H), 7.03–7.14 (m, 3H), 7.35 (d, J = 8.1 Hz, 2H), 7.55 (d, J = 8.4 Hz, 1H), 8.64 (s, 1H); MS (ESI) m/z 404 (MH), 406 (MH+2); Anal. Calcd for $C_{18}H_{15}BrN_2O_4$: C, 53.60; H, 3.75; N, 6.95. Found: C, 53.35; H, 3.67; N, 6.90.

5.1.1.32. 3-[4-(Acetylamino)phenyl]-5-(3-bromo-4-hydroxybenzylidene)-1,3-oxazolidin-2,4-dione (9i). To a solution of 3-(4-nitrophenyl)-1,3-oxazolidin-2,4-dione (1.0 g, 4.5 mmol) in methanol (20 mL) was added 5% palladium on carbon (77 mg), and the mixture was stirred under hydrogen atmosphere at room temperature for 3 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated. To a solution of the residue in THF (30 mL) was added activated carbon (50 mg), and then the mixture was stirred for 30 min. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was purified by recrystallization with ethyl acetate/hexane (v/v = 1/1) to give 3-(4-aminophenyl)-1,3-oxazolidin-2,4-dione (0.74 g, 86%); ^{1}H NMR (270 MHz, DMSO-d_6) δ 4.90 (s, 2H), 5.38 (s, 2H), 6.61 (d, J = 8.9 Hz, 2H), 6.97 (d, J = 8.6 Hz, 2H). To a solution of 3-(4-aminophenyl)-1,3-oxazolidin-2,4-dione (100 mg, 0.52 mmol) and triethylamine (110 μL, 1.5 equiv) in chloroform (4 mL) was added acetyl chloride (41 μL, 1.1 equiv) at 5 °C. The mixture was stirred at room temperature for 1.5 h. The reaction mixture was poured into water, extracted with chloroform, and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography, eluted with ethyl acetate/hexane to give 3-(4-acetylaminophenyl)-1,3-oxazolidin-2,4-dione (117 mg, 96%); ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.34 (s, 3H), 4.94 (s, 2H), 7.31 (d, J = 8.9 Hz, 2H), 7.67 (d, J = 8.9 Hz, 2H), 10.05 (s, 1H). Compound **9i** was prepared from an intermediate described above, following: general procedure 2. Yield 40%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.36 (s, 3H), 6.90 (s, 1H), 7.08 (d, J = 8.5 Hz, 1H), 7.41 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 8.9 Hz, 1H), 7.64 (s, 1H), 7.73 (d, J = 8.1 Hz, 2H), 10.08 (s, 1H); MS (ESI) m/z 418 (MH), 420 (MH+2); Anal. Calcd for $C_{18}H_{13}BrN_2O_5$: C, 51.80; H, 3.14; N, 6.72. Found: C, 51.89; H, 3.16; N, 6.67.

5.1.1.33. 5-(3-Bromo-4-hydroxybenzylidene)-3-[4-(cyclohexanecarbonyl)phenyl]-1,3-oxazolidin-2,4-dione (9j). Compound **9j** was prepared with cyclohexanecarbonyl chloride in a manner similar to that described for compound **9i**. Yield 36%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.15–1.90 (m, 10H), 2.31–2.39 (m, 1H), 6.89 (s, 1H), 7.08 (d, J = 8.9 Hz, 1H), 7.39 (d, J = 8.9 Hz, 2H), 7.55 (d, J = 8.4 Hz, 1H), 7.64 (d, J = 1.9 Hz, 1H), 7.74 (d, J = 8.9 Hz, 2H), 10.03 (s, 1H), 10.67 (brs, 1H); MS (ESI) m/z 486 (MH), 488 (MH+2); Anal. Calcd for $C_{23}H_{21}BrN_2O_5$: C, 56.90; H, 4.36; N, 5.77. Found: C, 56.75; H, 3.99; N, 5.68.

5.1.1.34. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-methylthiophenyl)-1,3-oxazolidin-2,4-dione (9k). Compound **9k** was prepared following: general procedure 2 and 4–1. Yield 32%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.87 (s, 3H), 6.92 (s, 1H), 7.06 (d, J = 8.9 Hz, 2H), 7.23 (s, 1H), 7.32–7.38 (m, 3H), 8.12 (d, J = 2.4 Hz, 1H), 10.43 (s, 1H); MS (ESI) m/z 407 (MH), 409 (MH+2); Anal. Calcd for $C_{17}H_{12}BrNO_4S$: C, 50.25; H, 2.98; N, 3.45. Found: C, 50.27; H, 2.80; N, 3.40.

5.1.1.35. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-methylsulfonylphenyl)-1,3-oxazolidin-2,4-dione (9l). To a solution of 3-(4-methylthiophenyl)-1,3-oxazolidin-2,4-dione (0.45 g, 2.0 mmol) in dichloromethane (20 mL) was added *m*-chloroperbenoic acid (0.69 g, 2.0 equiv) at 5 °C. The mixture was stirred at room temperature for 3 h. Saturated sodium thiosulfate solution and saturated sodium hydrogen carbonate solution were added, and the mixture

was extracted with chloroform. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue triturated with diethyl ether to give 3-(4-methylsulfonylphenyl)-1,3-oxazolidin-2,4-dione (0.38 g, 75%); ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.77 (s, 3H), 4.91 (s, 2H), 7.67 (d, J = 8.4 Hz, 2H), 7.81 (d, J = 8.4 Hz, 2H). Compound **9l** was prepared from an intermediate described above, following: general procedure 2. Yield 38%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.79 (s, 3H), 6.94 (s, 1H), 7.08 (d, J = 8.9 Hz, 1H), 7.56 (dd, J = 1.9, 8.4 Hz, 1H), 7.64 (d, J = 1.9 Hz, 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.86 (d, J = 8.4 Hz, 2H); MS (ESI) m/z 439 (MH), 441 (MH+2); Anal. Calcd for $C_{17}H_{12}BrNO_6S$: C, 46.58; H, 2.76; N, 3.20. Found: C, 46.29; H, 2.71; N, 3.33.

5.1.1.36. 5-(3-Bromo-4-hydroxybenzylidene)-3-(4-sulfamoylphenyl)-1,3-oxazolidin-2,4-dione (9m). Compound **9m** was prepared following: general procedure 2 and 4–1. Yield 49%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.93 (s, 1H), 7.09 (d, J = 8.9 Hz, 1H), 7.55 (s, 2H), 7.59–7.72 (m, 4H), 7.98 (d, J = 8.4 Hz, 2H); MS (ESI) m/z 440 (MH), 441 (MH+2); Anal. Calcd for $C_{16}H_{11}BrN_2O_6S$: C, 43.74; H, 2.53; N, 6.38. Found: C, 43.71; H, 2.22; N, 6.36.

5.1.1.37. 5-(3-Bromo-4-hydroxybenzylidene)-3-(pyridine-2-yl)-1,3-oxazolidin-2,4-dione (10a). Compound **10a** was prepared following: general procedure 2 and 4–2. Yield 48%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.02 (s, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.61 (dd, J = 1.4, 6.2 Hz, 1H), 7.67 (d, J = 6.4 Hz, 1H), 7.84 (dd, J = 2.0, 8.5 Hz, 1H), 8.02 (dt, J = 1.8, 7.8 Hz, 1H), 8.12 (d, J = 1.9 Hz, 1H), 8.69 (dd, J = 1.1, 4.9 Hz, 1H); MS (ESI) m/z 362 (MH), 364 (MH+2); Anal. Calcd for $C_{15}H_9BrN_2O_4$: C, 49.87; H, 2.51; N, 7.76. Found: C, 49.52; H, 2.14; N, 7.64.

5.1.1.38. 5-(3-Bromo-4-hydroxybenzylidene)-3-(pyridine-3-yl)-1,3-oxazolidin-2,4-dione (10b). Compound **10b** was prepared following: general procedure 2 and 4–2. Yield 32%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.02 (s, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.74 (dt, J = 6.5, 3.5 Hz, 2H), 7.85 (d, J = 8.6 Hz, 1H), 7.97 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 1.4 Hz, 1H), 8.71 (s, 1H); MS (ESI) m/z 362 (MH), 364 (MH+2); Anal. Calcd for $C_{15}H_9BrN_2O_4$: C, 49.87; H, 2.51; N, 7.76. Found: C, 49.88; H, 2.37; N, 7.80.

5.1.1.39. 5-(3-Bromo-4-hydroxybenzylidene)-3-(6-methoxypyridine-3-yl)-1,3-oxazolidin-2,4-dione (10c). Compound **10c** was prepared following: general procedure 2 and 4–2. Yield 78%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.92 (s, 3H), 7.00 (s, 1H), 7.03 (d, J = 8.9 Hz, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.84 (dd, J = 1.5, 8.8 Hz, 2H), 8.12 (s, 1H), 8.28 (d, J = 2.4 Hz, 1H); MS (ESI) m/z 392 (MH), 394 (MH+2); Anal. Calcd for $C_{16}H_{11}BrN_2O_5$: C, 49.11; H, 2.84; N, 7.16. Found: C, 49.20; H, 2.86; N, 6.93.

5.1.1.40. 5-(3-Bromo-4-hydroxybenzylidene)-3-(6-methylpyridine-3-yl)-1,3-oxazolidin-2,4-dione (10d). Compound **10d** was prepared following: general procedure 2 and 4–2. Yield 67%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.56 (s, 1H), 7.15 (d, J = 8.4 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.55 (dd, J = 2.2, 8.9 Hz, 1H), 7.76–7.80 (m, 2H), 7.86 (d, J = 2.2 Hz, 1H), 8.47 (d, J = 2.4 Hz, 1H); MS (ESI) m/z 376 (MH), 378 (MH+2); Anal. Calcd for $C_{16}H_{11}BrN_2O_4$: C, 51.20; H, 2.96; N, 7.47. Found: C, 51.22; H, 2.78; N, 7.88.

5.1.1.41. 5-(3-Bromo-4-hydroxybenzylidene)-3-(6-fluoropyridine-3-yl)-1,3-oxazolidin-2,4-dione (10e). Compound **10e** was prepared following: general procedure 2 and 4–2. Yield 30%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.03 (s, 1H), 7.14 (d, J = 8.6 Hz, 1H), 7.47 (dd, J = 2.9, 8.9 Hz, 1H), 7.84 (dd, J = 2.9, 8.9 Hz, 1H), 8.12–8.24 (m, 2H), 8.41 (d, J = 1.9 Hz, 1H); MS (ESI) m/z 380 (MH), 381 (MH+2); Anal. Calcd for $C_{15}H_8BrFN_2O_4$: C, 47.50; H, 2.13; N, 7.39. Found: C, 47.25; H, 2.05; N, 7.34.

5.1.1.42. 5-(3-Bromo-4-hydroxybenzylidene)-3-(6-chloropyridine-3-yl)-1,3-oxazolidin-2,4-dione (10f). Compound **10f** was prepared following: general procedure 2 and 4–2. Yield 59%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.03 (s, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.85 (d, J = 1.9 Hz, 1H), 8.05 (dd, J = 2.7, 8.4 Hz, 1H), 8.12 (d, J = 2.2 Hz, 1H), 8.57 (d, J = 2.2 Hz, 1H), 10.30 (s, 1H); MS (ESI) m/z 396 (MH), 398 (MH+2); Anal. Calcd for $C_{15}H_8BrClN_2O_4$: C, 45.52; H, 2.04; N, 7.08. Found: C, 45.25; H, 1.96; N, 6.99.

5.1.1.43. 5-(3-Bromo-4-hydroxybenzylidene)-3-[6-(dimethylamino)pyridine-3-yl]-1,3-oxazolidin-2,4-dione (10g). Compound **10g** was prepared following: general procedure 2 and 4–2. Yield 48%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.07 (s, 6H), 6.75 (d, J = 9.1 Hz, 1H), 6.89 (s, 1H), 7.08 (d, J = 8.8 Hz, 1H), 7.53–7.66 (m, 3H), 8.13 (d, J = 2.3 Hz, 1H), 10.64 (brs, 1H); MS (ESI) m/z 405 (MH), 407 (MH+2); Anal. Calcd for $C_{17}H_{14}BrN_3O_4$: C, 50.49; H, 3.49; N, 10.40. Found: C, 50.14; H, 3.12; N, 10.28.

5.1.1.44. 5-[1-(3-Bromo-4-hydroxyphenyl)ethylidene]-3-(4-methoxyphenyl)-2-thioxo-1,3-thiazolidin-2,4-dione (14a). Compound **14a** was prepared with 1-(3-bromo-4-hydroxyphenyl)ethanone in a manner similar to that described for compound **1a**. Yield 39%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.67 (s, 3H), 3.82 (s, 3H), 7.07 (d, J = 8.9 Hz, 2H), 7.17 (d, J = 8.6 Hz, 1H), 7.25 (d, J = 8.9 Hz, 2H), 7.39 (d, J = 1.6 Hz, 1H), 7.70 (d, J = 2.2 Hz, 1H); MS (ESI) m/z 437 (MH), 439 (MH+2); Anal. Calcd for $C_{18}H_{14}BrNO_3S_2$: C, 49.55; H, 3.24; N, 3.21. Found: C, 49.46; H, 3.26; N, 2.98.

5.1.1.45. 5-[1-(3-Bromo-4-hydroxyphenyl)-2,2,2-trifluoroethylidene)-3-(4-methoxyphenyl)-2-thioxo-1,3-thiazolidin-2,4-dione (14b). A mixture of 2,2,2-Trifluoro-1-(4-methoxyphenyl)ethanone (13.8 g, 67.6 mmol), silver oxide (II) (1.76 g, 0.12 equiv), concd sulfuric acid (6.5 mL), and carbon tetrachloride (130 mL) was cooled to 5 °C. To the mixture was added bromine (11.1 g, 1.03 equiv) at the temperature, and the reaction mixture was stirred at 65 °C for 8 h. After cooling, the mixture was poured into ice and extracted with chloroform. The combined organic layers were washed with saturated sodium hydrogen carbonate solution and brine, and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography, eluted with ethyl acetate/hexane (v/v = 1/3) to give 1-(3-bromo-4-methoxyphenyl)-2,2,2-trifluoroethanone (17.3 g, 90%); ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.03 (s, 3H), 7.00 (d, J = 8.9 Hz, 1H), 8.05 (dd, J = 1.1, 7.8 Hz, 1H), 8.29 (d, J = 1.1 Hz, 1H). To a solution of 1-(3-bromo-4-methoxyphenyl)-2,2,2-trifluoroethanone (7.52 g, 26.6 mmol) in DMF (75 mL) was added lithium chloride (4.0 g, 3.6 equiv). Then the mixture was stirred under reflux for 1 h. After cooling, the volatile materials were removed in vacuo, the residue was dissolved in methanol (100 mL). The mixture was acidified to pH 3, by addition of 1 M HCl in methanol solution. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography, eluted with ethyl acetate/hexane (v/v = 1/3) to give 1-(3-bromo-4-hydroxyphenyl)-2,2,2-trifluoroethanone (4.68 g, 65%); ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.16 (d, J = 8.8 Hz, 1H), 7.93 (d, J = 8.6 Hz, 1H), 8.11 (s, 1H). Compound **14b** was prepared from an intermediate described above, following: general procedure 2. Yield 22%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.77 (s, 3H), 6.93 (d, J = 8.4 Hz, 1H), 7.02 (d, J = 8.9 Hz, 2H), 7.20 (d, J = 8.9 Hz, 2H), 7.28 (d, J = 8.9 Hz, 1H), 7.53 (d, J = 1.9 Hz, 1H); MS (ESI) m/z 491

(MH), 492 (MH+2); Anal. Calcd for $C_{18}H_{11}BrF_3NO_3S_2$: C, 44.09; H, 2.26; N, 2.86. Found: C, 43.69; H, 1.94; N, 2.79.

5.1.1.46. 5-(3-Bromo-4-hydroxybenzyl)-3-(4-methoxyphenyl)-2-thioxo-1,3-oxazolidin-4-one (15). 3-(Methoxyphenyl)-2-thioxo-1,3-oxazolidin-4-one **4a** (0.10 g, 0.48 mmol) and magnesium methyl carbonate (0.95 mL, 1.8 M in DMF solution) were heated at 85 °C for 0.5 h. 3-Bromo-4-(*t*-butyldimethylsilyl)benzyl chloride (0.18 g, 0.54 mmol) was added and the temperature spontaneously rose to 90 °C. After 3 h at 85–90 °C, the reaction mixture was poured, with vigorous stirring, into 10 g of ice and 0.95 mL of concd HCl. The precipitate was filtered off and recrystallized from ethanol (6 mL) and water (10 mL). Yield 42%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.18–3.37 (m, 2H), 3.81 (s, 1H), 5.14 (t, J = 4.5 Hz, 1H), 6.89–7.00 (m, 5H), 7.15 (dd, J = 2.0, 8.5 Hz, 1H), 7.41 (d, 2.2 Hz, 1H); MS (ESI) *m/z* 425 (MH), 427 (MH+2); Anal. Calcd for $C_{17}H_{14}BrNO_3S_2$: C, 48.12; H, 3.33; N, 3.30. Found: C, 48.07; H, 3.15; N, 3.32.

5.1.1.47. 5-(4-Hydroxy-3-methylbenzylidene)-3-(4-methoxyphenyl)-2-tioxo-1,3-oxazolidin-4-one (11a). Compound **11a** was prepared following: general procedure 2 and 3. Yield 50%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.82 (s, 3H), 6.93 (s, 1H), 6.94 (d, J = 8.9 Hz, 2H), 7.09 (d, J = 8.9 Hz, 2H), 7.39 (d, J = 8.9 Hz, 2H), 7.81 (d, J = 8.9 Hz, 2H), 11.33 (s, 1H); MS (ESI) *m/z* 328 (MH); Anal. Calcd for $C_{17}H_{13}NO_4S$: C, 62.37; H, 4.01; N, 4.28. Found: C, 62.14; H, 4.21; N, 4.19.

5.1.1.48. 5-(4-Hydroxy-3-methylbenzylidene)-3-(4-methoxyphenyl)-2-tioxo-1,3-oxazolidin-4-one (11b).

Compound **11b** was prepared following: general procedure 2 and 3. Yield 42%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.50 (s, 3H), 3.82 (s, 3H), 6.87 (s, 1H), 6.96 (d, J = 9.2 Hz, 1H), 7.09 (d, J = 8.9 Hz, 2H), 7.39 (d, J = 8.9 Hz, 2H), 7.66 (s, 1H), 7.68 (d, J = 9.2 Hz, 1H), 10.30 (brs, 1H); MS (ESI) *m/z* 342 (MH); Anal. Calcd for $C_{18}H_{15}NO_4S$: C, 63.33; H, 4.43; N, 4.11. Found: C, 63.08; H, 4.35; N, 4.06.

5.1.1.49. 5-(4-Hydroxy-3-methoxybenzylidene)-3-(4-methoxyphenyl)-2-tioxo-1,3-oxazolidin-4-one (11c).

Compound **11c** was prepared following: general procedure 2 and 3. Yield 96%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.50 (s, 3H), 3.82 (s, 3H), 6.92 (s, 1H), 6.96 (d, J = 8.4 Hz, 1H), 7.09 (d, J = 8.9 Hz, 2H), 7.39 (d, J = 8.9 Hz, 2H), 7.48 (d, J = 8.4 Hz, 1H), 7.53 (s, 1H), 9.96 (brs, 1H); MS (ESI) *m/z* 358 (MH); Anal. Calcd for $C_{18}H_{15}NO_5S$: C, 60.49; H, 4.23; N, 3.92. Found: C, 60.69; H, 4.07; N, 3.64.

5.1.1.50. 5-(3-Fluoro-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-2-tioxo-1,3-oxazolidin-4-one (11d).

Compound **11d** was prepared following: general procedure 2 and 3. Yield 75%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.82 (s, 3H), 6.95 (s, 1H), 7.07–7.13 (m, 3H), 7.36–7.43 (m, 2H), 7.64 (dd, J = 2.2, 8.5 Hz, 1H), 7.73 (dd, J = 2.2, 12.6 Hz, 1H), 10.82 (s, 1H); MS (ESI) *m/z* 346 (MH); Anal. Calcd for $C_{17}H_{12}FNO_4S$: C, 59.12; H, 3.50; N, 4.06. Found: C, 58.84; H, 3.34; N, 4.26.

5.1.1.51. 5-(3-Chloro-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-2-tioxo-1,3-oxazolidin-4-one (11e). Compound **11e** was prepared following: general procedure 2 and 3. Yield 52%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.82 (s, 3H), 6.95 (s, 1H), 7.08–7.16 (m, 3H), 7.39 (d, J = 8.6 Hz, 2H), 7.78 (dd, J = 2.2, 8.6 Hz, 1H), 7.95 (d, J = 2.2 Hz, 1H), 11.13 (s, 1H); MS (ESI) *m/z* 362 (MH); Anal. Calcd for $C_{17}H_{12}ClNO_4S$: C, 56.43; H, 3.35; N, 3.87. Found: C, 56.46; H, 3.52; N, 3.79.

5.1.1.52. 5-(2-Chloro-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-2-tioxo-1,3-oxazolidin-4-one (11f). Compound **11f** was prepared following: general procedure 2 and 3. Yield 40%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.82 (s, 3H), 6.92 (s, 1H), 6.99–7.13 (m, 4H), 7.36–7.43 (m, 2H), 8.04 (d, J = 8.0 Hz, 1H), 10.81 (s, 1H); MS (ESI) *m/z* 362 (MH); Anal. Calcd for $C_{17}H_{12}ClNO_4S$: C, 56.43; H, 3.35; N, 3.87. Found: C, 56.34; H, 3.37; N, 3.64.

5.1.1.53. 5-(3,5-Dichloro-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-2-tioxo-1,3-oxazolidin-4-one (11g). Compound **11g** was prepared following: general procedure 2 and 3. Yield 68%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.82 (s, 3H), 6.96 (s, 1H), 7.10 (d, J = 8.9 Hz, 2H), 7.38 (d, J = 8.9 Hz, 2H), 7.95 (s, 2H); MS (ESI) *m/z* 396 (MH); Anal. Calcd for $C_{17}H_{11}Cl_2NO_4S$: C, 51.52; H, 2.80; N, 3.54. Found: C, 51.54; H, 2.62; N, 3.49.

5.1.1.54. 5-(3,5-Difluoro-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-2-tioxo-1,3-oxazolidin-4-one (11h). Compound **11h** was prepared following: general procedure 2 and 3. Yield 57%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.81 (s, 3H), 6.95 (s, 1H), 7.09 (d, J = 8.9 Hz, 2H), 7.37 (d, J = 8.9 Hz, 2H), 7.66 (d, J = 9.8 Hz, 2H); MS (ESI) *m/z* 364 (MH); Anal. Calcd for $C_{17}H_{11}F_2NO_4S$: C, 56.19; H, 3.05; N, 3.86. Found: C, 55.92; H, 2.98; N, 3.77.

5.1.1.55. 5-(3-Chloro-5-fluoro-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-2-thioxo-1,3-oxazolidin-4-one (11i).

Compound **11i** was prepared following: general procedure 2 and 3. Yield 78%; 1H NMR (270 MHz, DMSO-d_6) δ 3.81 (s, 3H), 6.95 (s, 1H), 7.09 (d, J = 8.9 Hz, 2H), 7.37 (d, J = 8.9 Hz, 2H), 7.75 (dd, J = 1.9, 10.8 Hz, 1H), 8.32 (d, J = 1.9 Hz, 1H); MS (ESI) *m/z* 380 (MH); Anal. Calcd for $C_{17}H_{11}ClFNO_4S$: C, 53.76; H, 2.92; N, 3.69. Found: C, 53.41; H, 2.61; N, 3.57.

5.1.1.56. 5-(3-Cyano-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-1,3-oxazolidin-2,4-dione (12a).

Compound **12a** was prepared following: general procedure 2 and 4–1. Yield 56%; 1H NMR (400 MHz, CD_3OD) δ 3.85 (s, 3H), 6.82 (s, 1H), 7.04–7.08 (m, 3H), 7.41 (dt, J = 2.8, 9.1 Hz, 2H), 7.97 (dd, J = 2.3, 8.7 Hz, 1H), 8.03 (d, J = 2.3 Hz, 1H); MS (ESI) *m/z* 337 (MH); Anal. Calcd for $C_{18}H_{12}N_2O_5$: C, 64.27; H, 3.60; N, 8.33. Found: C, 63.98; H, 3.55; N, 8.26.

5.1.1.57. 5-(3-Carboxy-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-1,3-oxazolidin-2,4-dione(12b).

Compound **12b** was prepared following: general procedure 2 and 4–1. Yield 40%; 1H NMR (400 MHz, DMSO-d_6) δ 3.81 (s, 3H), 6.98 (s, 1H), 7.08–7.12 (m, 3H), 7.42 (dt, J = 9.0, 2.2 Hz, 2H), 7.98 (dd, J = 8.8, 2.3 Hz, 1H), 8.37 (d, J = 2.3 Hz, 1H); MS (ESI) *m/z* 355 (M); Anal. Calcd for $C_{18}H_{13}NO_7$: C, 60.83; H, 3.69; N, 3.94. Found: C, 60.56; H, 3.62; N, 3.85.

5.1.1.58. 5-[3-(*N,N*-dimethylaminomethyl)-4-hydroxybenzylidene]-3-(4-methoxyphenyl)-1,3-oxazolidin-2,4-dione (12c).

To a mixture of *p*-hydroxybenzaldehyde (1.0 g, 8.2 mmol), *N,N*-dimethylmethylene ammonium iodide (1.6 g, 1.0 equiv) in dichloromethane (30 mL) was added anhydrous potassium carbonate (1.7 g, 1.5 equiv). The mixture was stirred under reflux for 16 h. After cooling, the mixture was extracted with chloroform, and the organic layers was washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography, eluted with chloroform/methanol (v/v = 30/1) to give 3-(*N,N*-dimethylaminomethyl)-4-hydroxybenzaldehyde (1.0 g, 70%); 1H NMR (400 MHz, $CDCl_3$) δ 2.37 (s, 6H), 3.73 (s, 2H), 6.91 (d, J = 8.3 Hz, 1H), 7.54–7.55 (m, 1H), 7.70 (dd, J = 2.1, 8.3 Hz, 1H), 8.00–9.50 (brs, 1H), 9.81 (s, 1H). Compound **12c** was prepared from an intermediate described above, following: general procedure 2. Yield 44%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.37 (s, 6H), 3.73 (s, 2H),

3.85 (s, 3H), 6.82 (s, 1H), 6.89 (d, J = 8.4 Hz, 1H), 7.02 (dt, J = 2.8, 9.1 Hz, 2H), 7.41 (dt, J = 2.8, 9.1 Hz, 2H), 7.53 (d, J = 2.2 Hz, 1H), 7.59 (dd, J = 2.2, 8.4 Hz, 1H); MS (ESI) m/z 369 (MH); Anal. Calcd for $C_{20}H_{20}N_2O_5$: C, 65.19; H, 5.48; N, 7.61. Found: C, 64.90; H, 5.43; N, 7.54.

5.1.1.59. 5-(3,5-dichloro-4-hydroxybenzylidene)-3-(4-methoxyphenyl)-1,3-oxazolidin-2,4-dione (12d). Compound **12d** was prepared following: general procedure 2 and 4–1. Yield 70%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.81 (s, 3H), 6.95 (s, 1H), 7.11 (d, J = 8.9 Hz, 2H), 7.39 (d, J = 8.9 Hz, 2H), 7.94 (s, 2H); MS (ESI) m/z 380 (MH); Anal. Calcd for $C_{17}H_{11}Cl_2NO_5$: C, 53.69; H, 2.92; N, 3.69. Found: C, 53.71; H, 2.74; N, 3.64.

5.1.1.60. 5-[(5-Hydroxypyridin-2-yl)methylidene]-3-(4-methoxyphenyl)-2-thioxo-1,3-oxazolidin-4-one (13a).

Compound **13a** was prepared following: general procedure 2 and 3. Yield 34%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.82 (s, 3H), 6.77 (s, 1H), 7.10 (d, J = 8.9 Hz, 2H), 7.28–7.41 (m, 3H), 7.93 (d, J = 8.6 Hz, 1H), 8.32 (d, J = 2.7 Hz, 1H); MS (ESI) m/z 313 (MH); Anal. Calcd for $C_{16}H_{12}N_2O_5$: C, 61.52; H, 3.88; N, 8.97. Found: C, 61.37; H, 3.51; N, 8.88.

5.1.1.61. 5-[(6-Bromo-5-hydroxypyridin-2-yl)methylidene]-3-(4-methoxyphenyl)-2-thioxo-1,3-oxazolidin-4-one (13b).

Compound **13b** was prepared following: general procedure 2 and 3. Yield 69%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.81 (s, 3H), 6.70 (s, 1H), 7.09 (d, J = 8.9 Hz, 2H), 7.38 (d, J = 8.9 Hz, 2H), 7.45 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 8.6 Hz, 1H); MS (ESI) m/z 392 (MH), 394 (MH+2); Anal. Calcd for $C_{16}H_{11}BrN_2O_5$: C, 49.11; H, 2.84; N, 7.16. Found: C, 48.86; H, 2.76; N, 7.11.

5.1.1.62. 5-[3,5-Dichloro-4-(phosphonoxy)benzylidene]-3-(4-methoxyphenyl)-2-thioxo-1,3-oxazolidin-4-one (16).

Compound **11g** (1.00 g, 2.44 mmol) was dissolved in DMF (20 mL) and the solution was cooled to −10 °C. To the solution was added carbon tetrachloride (1.80 g, 5.0 equiv), *N,N*-diisopropylethylamine (0.68 g, 2.1 equiv), *N,N*-dimethyl-4-aminopyridine (31 mg, 0.1 equiv), and dibenzyl phosphite (1.00 g, 1.6 equiv) at the temperature. The mixture was stirred at −10 °C for 0.5 h, and then at room temperature for 1 h. A 0.5 M potassium dihydrogen phosphate solution (13 mL) was added, extracted with chloroform, and the organic layers were washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was recrystallized from ethyl acetate/hexane (v/v = 1/5, 100 mL) to give 5-[3,5-dichloro-4-(phosphonoxy) benzylidene]-3-(4-methoxyphenyl)-2-thioxo-1,3oxazolidin-4-one dibenzyl ester (1.05 g, 61%); ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.86 (s, 3H), 5.25 (d, J = 8.1 Hz, 4H), 6.62 (s, 1H), 7.05 (d, J = 8.9 Hz, 2H), 7.27 (d, J = 8.9 Hz, 2H), 7.35 (brs, 10H), 7.82 (s, 2H). To a solution of 5-[3,5-dichloro-4-(phosphonoxy)benzylidene]-3-(4-methoxyphenyl)-2-thioxo-1,3oxazolidin-4-one dibenzyl ester (1.00 g, 1.41 mmol) in chloroform (80 mL) was added bromotrimethylsilane (2.29 g, 10 equiv) at 0 °C, and the mixture was then stirred at room temperature for 1.5 h. A 1 M HCl solution (20 mL) was added, and the mixture was stirred for 0.5 h. The precipitate was collected by filtration and washed with water. The solid was triturated with chloroform to afford the title product (**16**). Yield 43%; ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.82 (s, 3H), 7.00 (s, 1H), 7.10 (d, J = 8.6 Hz, 2H), 7.39 (d, J = 8.6 Hz, 2H), 8.02 (s, 2H); MS (ESI) m/z 474 (M-1); Anal. Calcd for $C_{17}H_{12}Cl_2NO_7PS$: C, 42.86; H, 2.54; N, 2.94. Found: C, 42.51; H, 2.17; N, 2.82.

5.1.1.63. Inhibition of 17β-HSD type 3 in a cell-based assay. Cellular activity of the compounds was evaluated in HeLa cells, which were transiently transfected with full-length human 17β-HSD type 3 cDNA. HeLa cells expressing human 17β-HSD type 3 were plated on a 96-well plate (1×10^4 cells, 100 μL per well) in Dulbecco's modified Eagle medium (DMEM) containing 5% charcoal stripped fetal bovine serum (FCS), and incubated for 24 h at 37 °C in a 5% CO_2 atmosphere. The cells were pretreated with the compounds for 30 min, followed by incubation with 10 μL of 500 nM substrate (androstenedione) in DMEM media for 120 min at 37 °C in a 5% CO_2 atmosphere. The cell media were harvested, and the testosterone concentration in the media was determined by DELFIA Testosterone Reagents (PerkinElmer), for which measurement was performed on an Ultra microplate detection system (TECAN). Analytical conditions were as follows: Wavelength, 340 nm (excitation) and 612 nm (fluorescence); integration time, 400 μs; lag time, 400 μs. The percent inhibition was calculated relative to an uninhibited control and plotted against the compound concentrations (1, 10, 100 nM, and 1 μM) to generate the IC_{50} results.

5.1.1.64. Inhibition of 17β-HSD type 1 and type 2 in a cell-based assay. The assay was performed in a manner similar to that described for 17β-HSD3 but estrone (E1) for 17β-HSD1 and estradiol (E2) for 17β-HSD2 as a tracer substrate.

5.1.1.65. Cotransfection assay. Cotransfection assays using human AR were performed in HeLa cells. HeLa cells were cultured in the presence of DMEM supplemented with 10% FBS. Cells were seeded 24 h prior to transfection on a 96-well plate. Cells were transiently transfected by the calcium phosphate coprecipitation procedure with the following plasmids: pRShAR, containing the hAR cDNA under control of the RSV-LTR, a reporter plasmid, mouse mammary tumor virus (MMTV)-luciferase (LUC), containing the cDNA for firefly LUC under control of the MMTV long terminal repeat; a β-Gal expression plasmid, pRS-β-Gal, containing the cDNA for bacterial β-galactosidase, and filler DNA. 6 h after transfection, the medium was removed and cells were washed with phosphate-buffered saline (PBS). After aspiration of PBS, medium containing 10% charcoal resin-stripped FBS containing seven incremental concentrations of compound ranging from 10^{-9} to 10^{-5} M was added to the cells. For antagonist experiments, compounds were prepared and diluted in medium containing an EC_{50} concentration of reference agonist (DHT). After 40 h, the medium was removed from the cells, and the cells were lysed with a detergent containing buffer. Luciferase and β-galactosidase activities were measured in cell extracts to determine the level of transcriptional activation. The normalized response was calculated as: luciferase response/β-gal rate, where luciferase response = relative luciferase units (RLU) and β-gal rate = β-gal O.D.415/β-gal incubation time in minutes. For agonist experiments, the effective concentration that produced 50% of the maximum response (EC_{50}) was determined for each compound by interpolation between two concentrations spanning the midpoint of the concentration–response curve. Agonist efficacy for test compounds is calculated as a percent of normalized response relative to the maximum normalized response by the reference agonist (DHT). For antagonist experiments, the concentration of test compound that resulted in 50% of the maximum repression observed (IC_{50}) of the response induced by an EC_{50} of DHT was determined for each compound by interpolation between two concentrations spanning the midpoint of the concentration–response curve. Cotransfection studies with ERα, and GR were carried out in HeLa cells as described above to determine crossreactivity.

5.1.1.66. Rat pharmacokinetics. Male Sprague–Dawley rats were dosed orally with test compound in corn oil or 0.5% methylcellulose (MC) solution, or intravenously with test compound dissolved in saline containing small amount of DMSO. Plasma samples were measured using a selective HPLC/MS/MS method. The HPLC/MS/MS system was operated in selected reaction monitoring (SRM)

mode under optimized conditions for detection of the selected compounds using positive ions formed by atmospheric pressure chemical ionization (APCI). Quantitation was determined using a weighted regression analysis of peak area of analyte to define the plasma concentration vs time profile.

5.1.1.67. Plasma concentration of testosterone in male rats treated with a LH–RH agonist. An LH–RH agonist, leuprorelin (100 ng) was administered intramuscularly (im) to male Sprague–Dawley rats that had been pretreated with two doses of the test compound (20 and 100 mg/kg) and CB7630 (20 mg 1 h earlier. Blood specimens were obtained 1, 2, and 4 h after LH–RH administration in the rats. The plasma concentration of testosterone was measured by gas chromatography-mass spectrometry (GC/MS) in selected ion monitoring (SIM) mode, after conversion of testosterone to the heptafluorobutyric acid ester using heptafluorobutyric acid anhydride (HFBA). The experimental protocol was approved by the local ethics committee for animal studies.

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Supplementary data

Supplementary data (NMR spectroscopic data for compounds **1a** and **14a**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.03.052.

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