Synthesis, Structure−Activity Relationships, and in Vivo Evaluations of Substituted Di-*tert*-butylphenols as a Novel Class of Potent, Selective, and Orally Active Cyclooxygenase-2 Inhibitors. 1. Thiazolone and Oxazolone Series[1]

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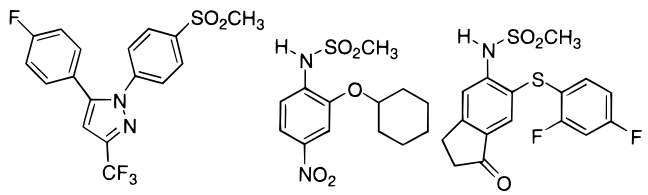
Selective cyclooxygenase-2 (COX-2) inhibitors have been shown to be potent antiinflammatory agents with fewer side effects than currently marketed nonsteroidal antiinflammatory drugs (NSAIDs). Initial mass screening and subsequent structure−activity relationship (SAR) studies have identified **4b** (PD138387) as the most potent and selective COX-2 inhibitor within the thiazolone and oxazolone series of di-*tert*-butylphenols. Compound **4b** has an IC_{50} of 1.7 μM against recombinant human COX-2 and inhibited COX-2 activity in the J774A.1 cell line with an IC_{50} of 0.17 μM. It was inactive against purified ovine COX-1 at 100 μM and did not inhibit COX-1 activity in platelets at 20 μM. Compound **4b** was also orally active in vivo with an ED_{40} of 16 mg/kg in the carrageenan footpad edema (CFE) assay and caused no gastrointestinal (GI) damage in rats at the dose of 100 mg/kg but inhibited gastric prostaglandin E_2 (PGE_2) production in rats' gastric mucosa by 33% following a dose of 100 mg/kg. The SAR studies of this chemical series revealed that the potency and selectivity are very sensitive to minor structural changes. A simple isosteric replacement led to the reversal of selectivity.

Introduction

Prostaglandins are important mediators of inflammation[2] and also provide cytoprotection in the stomach and intestine.[3] Consequently, common nonsteroidal antiinflammatory drugs (NSAIDs) which inhibit cyclooxygenase (COX), a key enzyme involved in prostaglandin production,[2] have had major side effects, such as gastrointestinal (GI) hemorrhage and ulceration.[4] Separation of toxicity from efficacy has been a major focus in the search for new NSAIDs. Recently, it was discovered that COX exists in two isoforms, COX-1 and COX-2,[5] and that the two isoforms are regulated differently: COX-1 is expressed constitutively, whereas COX-2 is transiently upregulated by proinflammatory mediators and downregulated by corticosteroids.[6] These discoveries suggest the possibility that constitutive COX-1 protects the GI tract, whereas inducible COX-2 mediates inflammation. This difference in function provides an opportunity to separate toxicity from efficacy of NSAIDs by selectively inhibiting COX-2. Indeed, in vivo studies with several known selective COX-2 inhibitors, **1** (SC-58125),[7] **2** (NS-398),[8] and **3** (L-745,337)[9] (Chart 1), have shown that they have potent antiinflammatory activity with little GI toxicity.

Prior to the discovery of two isoforms of the cyclooxygenase, our efforts on a dual cyclooxygenase and 5-lipoxygenase inhibitor program[10] had identified a number of potent COX inhibitors. However, some potent COX inhibitors, lacking inhibitory activity toward 5-lipoxygenase, were not fully evaluated under the dual inhibitor strategy. The possibility that these compounds would be selective COX-2 inhibitors was investigated, and a structure−activity relationship (SAR) for selective inhibitors of COX-2 was developed in several series. In this paper, we report the SAR studies on thiazolone and oxazolone series, as well as pharmacological evaluations of selective inhibitors in carrageenan footpad edema (CFE) assay and in a hyperalgesia model. The GI safety profiles of selected compounds are also described.

Chart 1



1 **2** **3**

Mass Screening

The Parke-Davis chemical library was screened for compounds that would inhibit the conversion of [^{14}C]-arachidonic acid by sheep placental COX-2 (oCOX-2) and ram seminal vesicle COX-1 (oCOX-1). Substituted 2,6-di-*tert*-butylphenols with a generic structure of **I** (Chart 2) were identified as potent and selective inhibitors. This chemical class was attractive to us for several reasons: first, substituted di-*tert*-butylphenols are structurally different from the reported selective COX-2 inhibitors (**1**−**3**).[7−9] Second, BF-389,[11] a di-*tert*-butylphenol derivative, has been shown to have potent inhibitory effects toward COX-2 activity in intact cells

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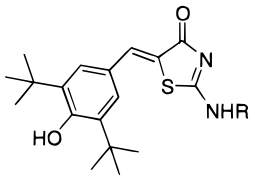
Chart 2



I

R = OH: **4a**
R = OCH_3: **4b** (PD 138387)
R = H: **4f** (CI-1004)

(IC_{50} = 0.09 μM) with a 5-fold selectivity favoring COX-2. However BF-389 was a nonselective and weak COX inhibitor based on purified enzyme assays with IC_{50} values of 24 μM against COX-2 and 12 μM against COX-1. Di-*tert*-butylphenol derivatives **4a**,**b** (PD138387) are more potent and selective for COX-2 versus COX-1 based on enzyme assays (Table 2). Third, several compounds in this class are known antiinflammatory agents, and some of them have a favorable GI safety profile. Compounds **4a**,**b** are congeners of **4f** (CI-1004) which is currently under clinical development as a dual inhibitor of cellular leukotriene and prostaglandin production. Consequently, a great deal of knowledge about the pharmacokinetic and toxicological properties of this series had been accumulated. Therefore, **4a**,**b** were chosen as chemical leads for further SAR studies.

Compound Evaluations

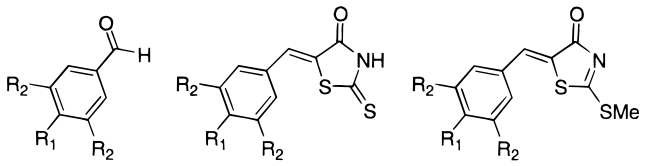
The primary assays for the compounds described in this SAR study were two purified enzyme assays. Recombinant human COX-2 (rhCOX-2) and ram seminal vesicle COX-1 (oCOX-1) were used to measure the inhibitory activity against isolated enzymes in a cell-free environment. Compounds of interest were also tested for their inhibitory effects toward the activity of both enzymes in a cellular environment. Human platelet-rich plasma was used for the evaluation of COX-1 activity and a murine macrophage cell line (J774A.1) for COX-2. Selected compounds were also evaluated in the CFE, in a hyperalgesia model in mice, and in GI safety models for gastric toxicity and inhibition of PGE_2 synthesis in rats. SAR development was primarily guided by biological evaluations in the two purified enzyme assays.

Chemistry

Thiazolones **8a**,**ea**,**eb**,**f**−**h** were prepared according to a procedure reported earlier by Unangst et al.[10a] (Scheme 1). Knovevenagel condensation of aldehydes **5b**,**c**,**e**−**h** with rhodanine gave **6b**,**c**,**e**−**h** in good to excellent yields. Methylation with methyl iodide provided thiazolones **7a**−**c**,**e**−**h** in excellent yields. Reaction of **7a**,**e**−**h** with appropriate amines afforded thiazolones **8a**,**ea**,**eb**,**f**−**h**. Compounds **5**−**7** are listed in Table 1.

Thiazolones **4c**,**d**,**g**,**h**−**n** (Tables 2 and 4; Table 4 is provided as Supporting Information) were synthesized by reaction of thiazolones **9** with appropriate amines. Compounds **4o**,**p** were prepared by acylation and sulfonylation of **4f**, respectively (Scheme 2).

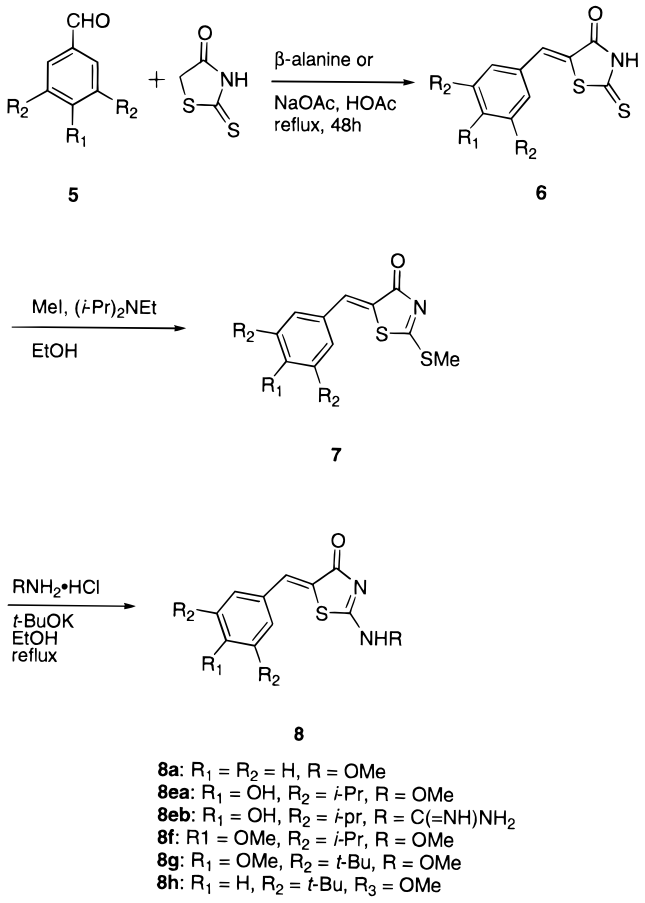
Table 1. Compounds **5**−**7** Shown in Scheme 1



5 **6** **7**

no.	R_1	R_2
6a, **7a**	H	H
5b, **6b**, **7b**	OH	Br
5c, **6c**, **7c**	OH	I
5e, **6e**, **7e**	OH	*i*-Pr
5f, **6f**, **7f**	OMe	*i*-Pr
5 g, **6g**, **7g**	OMe	*t*-Bu
5h, **6h**, **7h**	H	*t*-Bu

Scheme 1. Synthesis of Thiazolone Derivatives



5 **6** **7** **8**

8a: R_1 = R_2 = H, R = OMe
8ea: R_1 = OH, R_2 = *i*-Pr, R = OMe
8eb: R_1 = OH, R_2 = *i*-pr, R = C(=NH)NH_2
8f: R1 = OMe, R_2 = *i*-Pr, R = OMe
8g: R_1 = OMe, R_2 = *t*-Bu, R = OMe
8h: R_1 = H, R_2 = *t*-Bu, R_3 = OMe

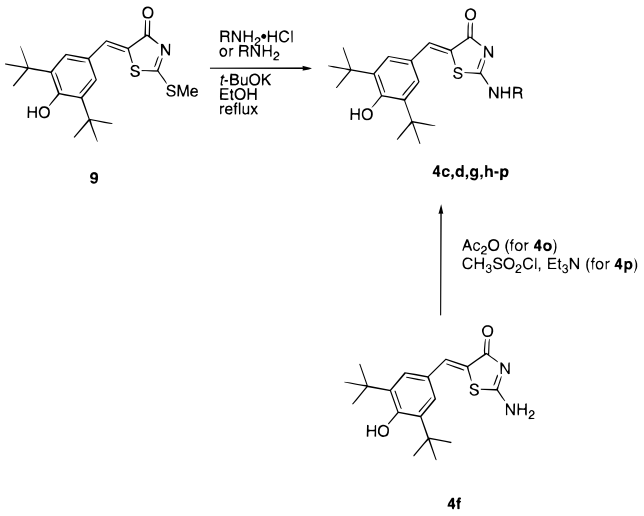
In the case of the oxazolones, reaction of **10** (Scheme 3) with *O*-methylhydroxylamine or hydroxylamine in ethanol at reflux failed to give the desired products. Instead, a ring-opened product **20**[12] was isolated in the reaction of **10** with *O*-methylhydroxylamine. However, reactions in ethanol at room temperature, using 0.8 equiv of amine, did furnish the desired products **11a**−**d** (Table 2), albeit in low yields.

The preparation of **4a**,**b**,**e**,**f**,**q**−**s**, **9**, **10**, **11e**, **12a**−**c**, **13**−**15**, **16a**−**d**, **17a**,**b**, **18a**−**e**, and **19a**,**b** has been reported previously.[13−16]

Results and Discussion

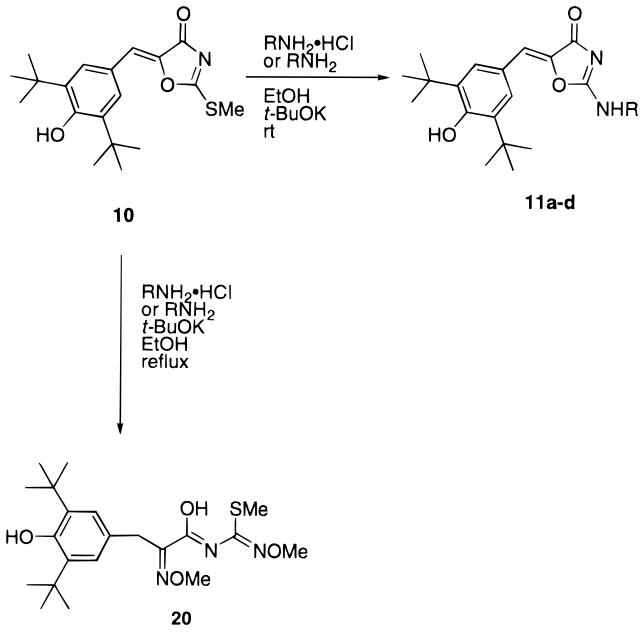
Structure−Activity Relationships and Discussion of the Enzyme Data. COX enzyme catalysis is

Scheme 2. Preparation of Di-*tert*-butylphenol-Derived Thiazolone Analogues



9 **4c,d,g,h-p** **4f**

Scheme 3. Preparation of Oxazolone Derivatives



10 **11a-d** **20**

thought to involve radical intermediates,[17] especially the phenoxy radical formed on a tyrosine residue.[18] Our chemical leads are substituted phenols which are capable of forming phenoxy radicals. Therefore, it is possible that the substituted phenol inhibitors could simply function as radical scavengers. To address this issue, compounds **8g**,**f**, replacing the exchangeable proton with a methyl group, **8h**, deleting the phenolic hydroxy group, and **8a**, removing both hydroxy and *tert*-butyl groups, were synthesized and evaluated in purified enzyme assays and cellular assays. As shown in Tables 2 and 4, against COX-2, **8g** was about 40-fold less active than **4b** and **8f** was essentially inactive. The deshydroxy compounds **8h**,**a** were also inactive against both enzymes. The data demonstrate the importance of the hydroxyl group for the inhibitory activity against COX-2.

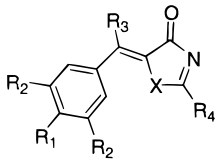
It was clear at the beginning that the inhibitory effect of these substituted di-*tert*-butylphenols, whether by radical scavenging or not, is selective for either COX-2 or COX-1. COX-2 and COX-1 are highly homologous enzymes that use similar catalytic mechanisms, yet **4b** showed good selectivity for COX-2 over COX-1, which suggests that the two *tert*-butyl groups or the thiazolone moiety or the combination of both must be the structural elements which are responsible for the selectivity toward the rhCOX-2 observed with **4b**.

To investigate the substitution effects of R_2, we focused on three selective COX-2 inhibitors (**4b**, **9**, and **12b**) and one selective COX-1 inhibitor (**4e**) and changed the *tert*-butyl groups to other smaller lipophilic groups. The SAR studies reveal that the potency and selectivity requirements of R_2 are dependent upon the nature of R_4. When R_4 is NHOMe or NHC(=NH)NH_2, compounds with both *tert*-butyl (**4b**,**e**) and isopropyl (**8ea**,**eb)** substitutions as R_2 gave potent and selective compounds. When R_4 is SMe or SH, the change of *tert*-butyl groups to any of the following groups: isopropyl (**7e** and **13**), I (**7c**), Br (**7b** and **14**), or OMe (**15**), abolished the activity against both enzymes. Selective inhibition, toward COX-2 or COX-1, favors *tert*-butyl groups as R_2. In the case of **4b** and **8ea**, the potency and selectivity of the two compounds were similar. The *tert*-butyl group still is optimal since **8ea** failed to inhibit COX-2 in the cellular assay. Another reason for favoring *tert*-butyl groups is the prevention of potential *O*-glucuronidation of the phenolic hydroxy group in vivo. It has been observed in series of antiinflammatory dialkylphenols that two *tert*-butyl groups flanking the OH group are required to retain in vivo antiinflammatory potency.[19]

Having established the di-*tert*-butylphenol as optimal, we focused on the SAR around the thiazolone portion of the molecule in this investigation. Isosteric replacement of the sulfur atom with an oxygen atom resulted in the reversal of selectivity. Several thiazolones were selective COX-2 inhibitors; in contrast, the corresponding oxazolones were selective COX-1 inhibitors. This phenomenon was observed when R_4 is NHOMe (**4b** vs **11b**), NHOH (**4a** vs **11a**), NHOEt (**4c** vs **11c**), and NHO-allyl **(4d** vs **11d**), as well as when R_4 is OH (**17a** vs **17b**), SH (**19a** vs **19b**), or SMe (**9** vs **10**). The only exception is when R_4 is NHC(=NH)NH_2, where the trend is reversed: the thiazolone **4e** was much more selective for COX-1 than the corresponding oxazolone **11e**. The reason for the observed differences in activity between thiazolones and oxazolones is not clear.

Isosteric replacement of the sulfur atom with a NMe group abolished the activity or decreased the potency against COX-2 independent of R_4 (**19a** vs **16a**, **9** vs **16b**, **4r** vs **16c**, **4e** vs **16d**). The isosteric replacement of the sulfur atom with a NH group, however, affected the activity and selectivity differently, depending on the nature of R_4. For example, when R_4 is SMe or OH, The change of a sulfur atom to NH (**12a**,**c**) gave inactive compounds against both enzymes (**9** vs **12c**, **17a** vs **12a**). When R_4 is SH, the change of the sulfur atom to a NH group resulted in compound **12b** with increased potency against both enzymes, but the selectivity was decreased (**19a** vs **12b**). The change of R_4 from NHOMe to $NHCNHCNH_2$ also resulted in reversal of selectivity leading to a potent and selective COX-1 inhibitor, **4e**. Compounds with a guanadine group as R_4 are selective COX-1 inhibitors in all cases studied: when X is S, **4e**; O, **11e**; or NMe, **16d**. Here again, the thiazolone and

Table 2. Enzyme and Cellular Activity of Benzylidene Derivatives **4**, **8**−**12**, and **16**−**19** and **1**, **2**, and Indomethacin[a,b,e]



						IC$_{50}$ (μM)		IC$_{50}$ (μM) ± SEM	
compd	X	R_1	R_2	R_3	R_4	rhCOX-2	oCOX-1	COX-2 J774A.1	COX-1 hplatelet
4a	S	OH	*t*-Bu	H	NHOH	1.5	52	0.17 ± 0.10	9.2 ± 2.7
4b	S	OH	*t*-Bu	H	NHOMe	1.7	NA	0.17 ± 0.070	NA
4c	S	OH	*t*-Bu	H	NHOEt	3.2	NA	0.27 ± 0.14	NA
4d	S	OH	*t*-Bu	H	NHO-allyl	2.7	NA	1.6 ± 1.0	NA
4e	S	OH	*t*-Bu	H	NHC(=NH)NH$_2$	21	0.0061	0.43 ± 0.32	0.25 ± 0.50
4f	S	OH	*t*-Bu	H	NH$_2$	41%@10 μM[c]	NA	0.069 ± 0.027	0.60 ± 0.19
4q	S	OH	*t*-Bu	H	NMeOMe	18	51	1.6 ± 0.22	5.8 ± 0.056
4r	S	OH	*t*-Bu	H	NHCN	55	NA	0.23 ± 0.15	NA
8ea	S	OH	*i*-Pr	H	NHOMe	0.57	NA	NA	NA
8eb	S	OH	*i*-Pr	H	NHC(=NH)NH$_2$	1.8	0.22	0.19 ± 0.96	2.7 ± 0.80
9	S	OH	*t*-Bu	H	SMe	1.8	63	1.6 ± 0.19	14.4 ± 5.7
10	O	OH	*t*-Bu	H	SMe	NA[d]	1.6	0.54 ± 0.29	6.4 ± 2.0
11b	O	OH	*t*-Bu	H	NHOMe	NA	7.3	0.79 ± 0.33	NA
11c	O	OH	*t*-Bu	H	NHOEt	79	3.5	0.65 ± 0.081	NA
11d	O	OH	*t*-Bu	H	NHO-allyl	26	4	1.9 ± 0.18	NA
11e	O	OH	*t*-Bu	H	NHC(=NH)NH$_2$	5.1	1.2	0.44 ± 0.17	4.4 ± 1.5
12b	NH	OH	*t*-Bu	H	SH	0.26	1.3	0.083 ± 0.049	0.99 ± 0.26
16d	NMe	OH	*t*-Bu	H	NHC(=NH)NH$_2$	81	0.027	0.75 ± 0.48	1.5 ± 0.45
17a	S	OH	*t*-Bu	H	OH	4.7	42	0.06 ± 0.030	9.3 ± 4.4
17b	O	OH	*t*-Bu	H	OH	34	0.095	0.071 ± 0.051	1.2 ± 0.33
18e	S	OH	*t*-Bu	Me	NHC(=NH)NH$_2$	12	0.46	0.18 ± 0.061	0.59 ± 0.23
19a	S	OH	*t*-Bu	H	SH	1.5	24	0.39 ± 0.23	NA
1						0.31	NA	0.070 ± 0.047	NA
2						0.19	NA	0.022 ± 0.008	3.3 ± 0.87
indomethacin						5.8	0.0122	0.0064 ± 0.001	0.0082 ± 0.001

[a] NA, not active (in purified enzyme assays, NA is defined as less than 20% inhibition at 10 μM; in cellular assays, NA is defined as IC$_{50}$ value greater than 20 μM). [b] Standard errors for the IC$_{50}$ determinations in purified enzyme assays averaged 25% for rhCOX-2 and 14% for oCOX-1. [c] Percent inhibition at 10 μM. [d] Found to be inactive against oCOX-2, not further tested. [e] Enzyme and cellular activity of the following compounds is summarized in Table 4 which is provided as Supporting Information: **4g**−**p**, **4s**, **7b**,**c**,**e**, **8a**,**f**−**h**, **11a**, **12a**,**c**, **13**−**15**, **16a**−**c**, **18a**−**d**, and **19b**.

oxazolone showed marked differences in selectivity: thiazolone **4e** was more than 3400-fold selective, whereas the oxazolone **11e** is only 4-fold selective for COX-1 inhibition.

Within the thiazolone series, we investigated the effect of R_4 in detail. When the proton on the nitrogen was replaced with a methyl group, **4q**, both the potency and selectivity were decreased. We kept the proton and changed the other group on the nitrogen to an array of substituents ranging from electron-donating to electron-withdrawing groups (**4g**−**j**,**b**,**s**,**f**,**r**,**o**,**p**), so that the proton on the nitrogen could have a range of acidity. However, we did not observe any correlation between the potency and electronic nature of the substituents. It seems that the nature of the chemical bond between the nitrogen atom and the substituents attached to it is critical for determining potency and selectivity. An N−O bond gives compounds (**4a**−**d**) which are potent and selective COX-2 inhibitors. N−H bond (**4f**) leads to weak COX-2 inhibition. An N−C (**4r**,**s**,**o**) and N−N (**4g**−**j**) bonds lead to loss of potency against both enzymes. In all cases studied, it is quite clear that the N−O bond is exclusively preferred for potency.

We also studied the effect of bulk on the potency and selectivity within a compound series where X = S, R_1 = OH, R_2 = *t*-Bu, R_3 = H, and R_4 contains an N−O bond. Compounds are potent and selective when R_4 is NHOH or NHOR, where R is lower alkyl (**4a**−**d**). Compounds with bulkier substituents on the oxygen atom (**4l**−**n**) are not active against either enzyme.

The effect of R_3 on potency and selectivity was also examined. In most cases, the methyl substitution abolished activity against both enzymes (**18a**−**d**). When R_4 is NHC(=NH)NH$_2$, the change of H to a methyl group decreased the potency against COX-1 (**4e** vs **18e**).

In summary, potency and selectivity are extremely sensitive to minor changes in chemical structure within this chemical series. The degree of sensitivity might be best illustrated by the results of isosteric replacement studies where the selectivity was completely reversed when a sulfur atom was replaced by an oxygen atom in all the cases studied. The phenolic OH group is essential for potency, and the change of the *tert*-butyl group to other groups mainly affected potency, whereas changes around the thiazolone moiety affected both potency and selectivity.

Enzyme Potency versus Cellular Activity. Evaluation of the cellular and isolated enzyme data revealed no apparent correlation between the two for either potency or selectivity. However, in general, compounds that were selective for COX-2 in the enzyme assays tended to retain their selectivity in the cellular assays (**4a**−**d** and **17a** as examples). In the case of compounds showing selectivity for COX-1 in the enzyme assays, selectivity observed in the isolated enzyme assays was not predictive of selectivity in the cellular assays. For

Table 3. In Vivo Activity of Thiazolones **4b**·choline, **4q**, and Standards **1**, **2**, and Indomethacin

compd	ED_{40} (mg/kg) CFE[a]	ED_{40} (mg/kg) hyperalgesia[b]	lesion frequency (%)	damaged area (%)	ulcer index	PGE_2 synthesis inhibition (% ± SEM)
4b·choline[c]	15.7	0.1	0.00	0.00	0.00	32.8 ± 4.43
4q	8.1	0.06	25.00	0.04	0.91	52.2 ± 14.5
2	0.61	7.4	0.00	0.00	0.00	30.7 ± 12.1
1	>30	0.3	0.00	0.00	0.00	−1.58 ± 13.5
indomethacin	1.6	0.6	75	0.18	13.4	96.5 ± 0.47

[a] Compounds dosed orally, 10 rats/test group. [b] Compounds dosed orally, 8 mice/test group. [c] Compound **4b**·choline is the choline salt of **4b**.

example, the most selective COX-1 inhibitor, **4e**, was almost equally potent in inhibiting the function of both enzymes in cellular assays, and several compounds (**11b**−**d and 17b**) that are selective COX-1 inhibitors in the enzyme assays have the opposite selectivity in cellular assays. Illustrative of the lack of correlation between the purified enzyme and cellular assays, in several instances, compounds (**4f**,**s**) inactive in both enzyme assays could be very potent in the corresponding cellular assays leading to the speculation that these compounds may possess other pharmacological activity (vide infra).

Within this chemical series, compounds **4b**,**c** were identified as the most potent and selective COX-2 inhibitors based on the data from both isolated enzyme and cellular assays. The most potent and selective COX-1 inhibitors, based on the enzyme assay, are **4e**, **17b**, and **16d**. Thus compound **4b** was selected for evaluation in vivo.

In Vivo Evaluations. Selective COX-2 inhibitors, based on both enzyme and cellular assays, were tested in the CFE and hyperalgesia assays. The results are shown in Table 3. Compound **4b**, the most potent and selective inhibitor in vitro, was tested in vivo as its choline salt. The choline salt of **4b** (**4b**·choline) was active in CFE (ID_{40} = 16 mg/kg) and also had potent analgesic activity in the acetic acid writhing test (ED_{40} = 0.1 mg/kg). However, compound **4q**, a weak and less selective COX-2 inhibitor, was more potent than **4b**·choline in CFE (ID_{40} = 8.1 mg/kg) and very potent in the analgesic assay (ED_{40} = 0.06 mg/kg), suggesting that additional pharmacological properties of the substituted di-*tert*-butylphenols may contribute to efficacy in vivo.

Compounds **4b**·choline and **4q**, together with reference standards indomethacin, **1**, and **2**, were further evaluated for their gastric toxicity and inhibition of gastric PGE_2 synthesis in rats (Table 3). Compound **1** was evaluated here even though it did not show activity in CFE in our hands. As expected for selective COX-2 inhibition, compounds **1** and **2** did not cause ulceration at the dose of 100 mg/kg. Compound **2** inhibited PGE_2 production in the rats' gastric mucosa by 31%. Slight enhancement of PGE_2 production was observed with **1**. Indomethacin, on the other hand, caused ulcers in rats with an ulcer index (ulcer index = % frequency × % of gastric area damaged) of 13. No GI damage was detected with **4b**·choline at a dose of 100 mg/kg, although 33% inhibition of PGE_2 production was observed, which is about the same as that observed with **2**. Minimal GI damage was detected with **4q** at a dose of 100 mg/kg, and 52% inhibition of PGE_2 production was observed, slightly higher than observed with **4b**·choline. The gastric toxicity and inhibition of PGE_2 synthesis in vivo observed with **4b**·choline and **4q** are consistent with the potency and selectivity profiles observed with **4b**,**q** in vitro: **4b** is the most selective COX-2 inhibitor in this chemical series and did not show GI toxicity when tested as a choline salt, whereas **4q** is a less selective COX-2 inhibitor and did show some ulceration in rats.

Conclusion

SAR studies on these thiazolone- and oxazolone-derived 2,6-di-*tert*-butylphenol series have shown that potency and selectivity are extremely sensitive to minor changes in chemical structure. The isosteric replacement of a sulfur atom with an oxygen resulted in reversal of selectivity in all cases studied. Several compounds showed good potency and selectivity against COX-2 in both its purified form and a cellular environment. Compound **4b**, the most potent and selective compound identified in this study, was an orally active antiinflammatory agent and lacked GI side effects when tested as a choline salt, further substantiating the hypothesis that the antiinflammatory activity of NSAIDs is derived from inhibition of COX-2 and their GI toxicity from inhibition of COX-1. Compounds reported here represent a new class of selective COX-2 inhibitors that are structurally different from those previously reported. Further improvements on the potency of these compounds could lead to novel and safe antiinflammatory agents with potential therapeutic utilities.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Infrared spectra were recorded using KBr disks on a Nicolet MX-1 FTIR spectrometer. Elemental analyses were performed by Robertson Microlit, Inc. (Madison, NJ) and were within ±0.4% of the theoretical values, unless indicated otherwise. Proton NMR spectra were recorded on a Bruker AM 400-MHz spectrometer, with chemical shifts reported in δ units relative to internal TMS. Mass spectra were obtained by using a Micromass Trio-2000 (APCI), PlatformLC (APCI), or Micromass Trio-2A (EI and CI) mass spectrometer. Reactions were generally run under a nitrogen atmosphere. Organic solutions were concentrated at house vacuum on a rotary evaporator. Flash chromatography was performed with ICN Biomedicals silica gel 60, 63−200 μm, according to the method of Still.[20]

Purified Enzyme Assays. Mass screening of the Parke-Davis compound library was carried out using commercially obtained sheep placental COX-2 (oCOX-2; Cayman Chemical, Ann Arbor, MI). For all the compounds included in the SAR studies, IC_{50} values against purified enzymes were determined using recombinant human COX-2 (rhCOX-2, purified from baculovirus-infected SF-9 cells[21]) and commercially obtained ram seminal vesicle COX-1 (oCOX-1; Cayman Chemical, Ann Arbor, MI). Inhibition assays were conducted in 50 mM phosphate buffer, pH 7.5, containing 2 mM epinephrine as cofactor for the COX-catalyzed peroxidase reaction and 20 μM [^{14}C]arachidonic acid as substrate. rhCOX-2 or oCOX-1 in Tween-20-containing buffer was added in sufficient amount

to convert 20−30% of added substrate to products during 1-min incubation. Reaction products were separated and measured by radiometric HPLC, and percent inhibition was computed by comparison of compound-treated to vehicle control incubations. The concentration of compound causing 50% inhibition (IC_{50}) was estimated using the software package KaleidaGraph, version 3.0.1, running on a Macintosh Centris 650 using operating system 7.1. Percent inhibition versus inhibitor concentration data were fit to the two-parameter equation: % inhibition = 100/(1 + (inhibitor concentration/IC_{50})^{slope}), and best fits for IC_{50} and slope coefficient were determined by least-squares analysis. Standard errors for replicate determinations averaged 25% for rhCOX-2 and 14% for oCOX-1.

Cellular Assays. COX-1 assays utilized platelet-rich plasma (PrP) from NSAID-free normal human volunteers. J774A.1 (J7), a murine macrophage cell line, was used to evaluate COX-2, expression of which was induced by overnight incubation with LPS (1 μg/mL) prior to assay. All tests were performed in serum- and plasma-free medium except PrP, which had a final plasma concentration of 3.1% (autologous). Assays were performed in flat bottom 96-well plates (100 μL of cell preparation, 100 μL of drug dilution). After a 60-min preincubation of cells and test compounds, samples were spiked with 3 μg of arachidonic acid and incubated from from 30 min and the reaction stopped by the addition of 25 μL of 80 μM indomethacin. TxB_2 and PGE_2 concentrations in clarified cell supernatants were determined by EIA from Cayman Chemical Co. (TxB_2) and Assay Designs (PGE_2), both in Ann Arbor, MI. Percent inhibition was determined using the formula: {1 − [(drug − medium)/(maximum − medium)]} × 100, where "drug" is the pg/mL of PGE_2 or TxB_2 (prostanoid) in drug-treated samples, "medium" is the average prostanoid in control samples not treated with exogenous arachidonic acid, and "maximum" is the average prostanoid in samples treated with exogenous arachidonic acid in the absence of any drug sample. IC_{50} values were determined by regression analysis of a plot of percent inhibition (ordinate) versus drug concentration (abscissa) using TableCurve2D (Jandel, San Rafael, CA).

Carrageenan Footpad Edema. Male rats were injected in the right hind paw with 0.1 mL of a 1% solution of carrageenan in saline. Compounds were suspended in vehicle (0.5% hydroxypropyl-methylcellulose (HPMC) containing 0.2% Tween 80) and administered orally (10 mL/kg) 1 h before carrageenan injection. The dose volume was adjusted to 5.0 mL with water. Paw volume was measured by mercury plethysmography 5 h after carrageenan injection. Swelling was compared in the compound- and vehicle-treated groups to obtain percent inhibitions. ID_{40} values were determined by linear regression analysis (see ref 22 for the details). A Student's *t*-test was done on each experimental group (compared to vehicle) to evaluate statistical significance.

Hyperalgesia. Analgesic activity was measured by the acetic acid-induced writhing test as previously described.[23] Male Swiss-Webster mice were fasted for 16 h prior to oral administration of compounds or vehicle (HPMC with Tween 80). Sixty minutes later, each animal was injected intraperitoneally with 0.6% acetic acid in saline (10 mL/kg). Writhing was observed and tallied for 5 min, beginning 7 min after the acetic acid injection. ID_{40} values were determined by linear regression analysis. A two-way ANOVA was used to determine the level of statistical significance for each experimental group.

Gastric Toxicity and Inhibition of PGE_2 Synthesis in Rats. Sprague−Dawley male rats were administered 100 mg/kg of the compounds under study in 1 mL of 1% CMC solution. Four hours later the animals were sacrificed. The stomachs were removed and opened along the greater curvature. Their images were digitized and stored on an optical disk using a 486-based computer equipped with CUE3 system imaging analysis software. Two 6-mm biopsies were taken from a constant region located in each side of the glandular portion of the stomach, and their PGE_2 content was measured using a commercially available ELISA kit (Assay Designs, Inc., Ann Arbor, MI). On the retrieved electronic image, the presence of gastric damage was determined and its extent measured using the CUE3 imaging software. To take into account both the frequency and extent of gastric damage, data were expressed as ulcer index (ulcer index = % frequency × % of gastric area damaged). Indomethacin was also tested and used as a positive control.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-(methoxyamino)thiazol-4-one Choline Salt (4b·choline). A solution of 80% choline in water (0.84 g, 4.1 mmol) was added to a stirred suspension of (*Z*)-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-2-(methoxyamino)thiazol-4-one (**4b**) (1.50 g, 4.1 mmol) in 40 mL of ethanol under an inert atmosphere and heated to reflux. After 1 h the resulting solution was stripped of solvent under reduced pressure to afford a glass, which was crystallized from 10−15 mL of ethyl acetate. The suspension was cooled and the solid filtered off, washed twice with ethyl acetate and then twice with ether, and dried to afford the pure product in two crops (1.7 g, 89% yield): mp 170 °C; IR (KBr) 3420, 2955, 1549 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.39 (s, 18H, 2 × *t*-Bu), 3.10 (s, 9H, $HOCH_2CH_2N(CH_3)_3$), 3.40 (m, 2H, $HOCH_2$-$CH_2N(CH_3)_3$), 3.62 (s, 3H, OCH_3), 3.83 (m, 2H, $HOCH_2CH_2N$-$(CH_3)_3$), 7.04 (s, 1H, H_{vinyl}), 7.24 (s, 2H, 2 × H_{arom}); MS (+APCI) *m*/*z* 362 (parent MH^+). Anal. ($C_{24}H_{39}N_3O_4S$) C, H, N.

General Procedure A for the Preparation of 4c,d,g,l,m,n. To a mixture of **9**[10a] (1 equiv), amine hydrochloride (1.1 equiv), and ethanol (25 mL/1 g of **9**) was added potassium *tert*-butoxide (1.1 equiv). The resultant reaction mixture was refluxed for 2 h, then cooled to ambient temperature, and poured onto ice−water. The precipitate formed was filtered and recrystallized from the appropriate solvent to give the desired product.

General Procedure B for the Preparation of 4h,i,j,k. To a mixture of **9**[10a] (1 equiv), amine (1.5 equiv), and ethanol (25 mL/1 g of **9**) was added potassium *tert*-butoxide (1.5 equiv). The resultant reaction mixture was refluxed for 2 h, then cooled to ambient temperature, and poured onto ice−water. A cloudy red solution was obtained. Upon acidification with concentrated aqueous HCl to pH = 1, a precipitate formed. Filtration and further purification gave the desired product.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-(ethoxyamino)thiazol-4-one (4c). Compound **4c** was prepared by subjecting *O*-ethylhydroxylamine hydrochloride (0.443 g, 4.54 mmol) to general procedure A; 0.69 g (44%) of pure **4c** was obtained as a beige solid after two recrystallizations successively from CH_3OH/CH_3CN and EtOAc/hexanes: IR 3624, 3606, 1689, 1637, 1606, 1593, 1438, 1424, 1241, 1206, 1050 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 3H, *J* = 6.99 Hz, CH_3), 1.41 (s, 18H, 2 × *t*-Bu), 4.05 (q, 2H, *J* = 6.99 Hz, CH_2), 7.35 (s, 2H, 2 × H_{arom}), 7.51 (s, 1H, H_{vinyl}), 7.66 (bs, 1H, OH), 12.05 (bs, 1H, NH); mp 252−254 °C; MS (+APCI) *m*/*z* 377 (MH^+). Anal. ($C_{20}H_{28}N_2O_3S$) C, H, N.

(*Z*)-2-(Allyloxyamino)-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)thiazol-4-one (4d). Compound **4d** was prepared by subjecting *O*-allylhydroxylamine hydrochloride to general procedure A. Crude product, a yellow solid, was triturated with methanol to give 1.2 g (56%) of pure **4d** as a light-yellow solid: ^{1}H NMR (DMSO-d_6) δ 1.41 (s, 18H, 2 × *t*-Bu), 4.54 (dt, 2H, *J* = 5.55, 1.21 Hz, CH_2), 5.21−5.33 (m, 2H, 2 × H_{vinyl}), 5.95−6.05 (m, 1H, 2 × H_{vinyl}), 7.34 (s, 2H, 2 × H_{arom}), 7.53 (s, 1H, H_{vinyl}), 7.67 (bs, 1H, OH), 12.09 (bs, 1H, NH); mp 222−224 °C; MS (+APCI) *m*/*z* 389 (MH^+). Anal. ($C_{21}H_{28}N_2O_3S$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-(pyrrolidin-1-ylamino)thiazol-4-one (4g). Compound **4g** was prepared by subjecting 1-aminopyrrolidine hydrochloride to general procedure A; 1.30 g (78%) of pure **4g** was obtained as a yellow solid after trituration with CH_3CN: IR 3628, 3353, 1703, 1671, 1642, 1618, 1598, 1436, 1422, 1194 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.41 (s, 18H, 2 × *t*-Bu), 1.78 (m, 4H, 2 × CH_2), 2.86 (m, 4H, 2 × CH_2), 7.36 (s, 2H, 2 × H_{arom}), 7.50 (s, 1H, H_{vinyl}), 7.61 (bs, 1H, OH), 11.75 (bs, 1H, NH); mp 265−267 °C; MS (+APCI) *m*/*z* 402 (MH^+). Anal. ($C_{22}H_{31}N_3O_2S$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-(morpholin-4-ylamino)thiazol-4-one (4h). Compound **4h** was prepared by subjecting *N*-aminomorpholine to general proce-

dure B. Crude product, a red solid, was triturated successively with methanol/water and Et_2O/hexanes to give 0.80 g (34%) of pure **4h** as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 1.42 (s, 18H, 2 × *t*-Bu), 2.75 (m, 4H, 2 × CH_2), 3.68 (m, 4H, 2 × CH_2), 7.39 (s, 2H, 2 × H_{arom}), 7.51 (s, 1H, H_{vinyl}), 7.63 (bs, 1H, OH); mp >270 °C; MS (+APCI) *m*/*z* 418 (MH^+). Anal. ($C_{22}H_{31}N_3O_3S$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-hydrazinothiazol-4-one (4i). Compound **4i** was prepared by subjecting hydrozane to general procedure B. Crude product, a yellow solid, was triturated with acetonitrile and filtered, and the filtrate was mixed with water forming a yellow precipitate. Filtration and subsequent drying at 70 °C under vacuum gave 0.51 g (18%) of pure **4i** as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 1.42 (s, 18H, 2 × *t*-Bu), 5.45 (bs, 2H, NH_2), 7.38 (s, 2H, 2 × H_{arom}), 7.47 (s, 1H, H_{vinyl}), 7.57 (bs, 1H, OH); mp 245−246 °C; MS (+APCI) *m*/*z* 348 (MH^+). Anal. ($C_{18}H_{25}N_3O_2S$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-([1,2,4]-triazol-4-ylamino)thiazol-4-one (4j). Compound **4j** was prepared by subjecting 4-amino-1,2,4-triazole to general procedure B. Crude product, a yellow solid, was triturated successively with methanol and CH_3CN/EtOAc to give 0.93 g (42%) of pure **4j** as a beige solid: ^{1}H NMR (DMSO-d_6) δ 1.35 (s, 18H, 2 × *t*-Bu), 7.30 (s, 2H, 2 × H_{phenol}), 7.75 (s, 1H, H_{vinyl}), 7.82 (bs, 1H, OH), 8.76 (s, 2H, 2 × $H_{triazole}$); mp >270 °C; MS (+APCI) *m*/*z* 400 (MH^+). Anal. ($C_{20}H_{25}N_5O_2S$) C, H, N.

(*Z*)-{*N*-[5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl]aminooxy}acetic Acid (4k). Compound **4k** was prepared by subjecting (aminooxy)acetic acid hemihydrochloride to general procedure B. Crude product, a yellow solid, was recrystallized from CH_3CN/H_2O, followed by trituration with dichloromethane to give 2.2 g (66%) of pure **4k** as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 1.44 (s, 18H, 2 × *t*-Bu), 4.57 (s, 2H, CH_2), 7.36 (s, 2H, 2 × H_{arom}), 7.55 (s, 1H, H_{vinyl}), 7.68 (bs, 1H, OH); mp 229−231 °C dec; MS (+APCI) *m*/*z* 332 ($M - OCH_2CO_2H + H^+$). Anal. ($C_{20}H_{26}N_2O_5S$) C, H, N.

(*Z*)-2-(*tert*-Butoxyamino)-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)thiazol-4-one (4l). Compound **4l** was prepared by subjecting *O*-*tert*-butylhydroxylamine to general procedure A. Crude product, a yellow solid, was recrystallized twice from hexanes to give 2.4 g (63%) of pure **4l** as a light-yellow solid: ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H, *O*-*t*-Bu), 1.41 (s, 18H, 2 × *t*-Bu), 7.35 (s, 2H, 2 × H_{arom}), 7.49 (s, 1H, H_{vinyl}), 7.64 (bs, 1H, OH), 12.02 (bs, 1H, NH); mp 177−179 °C; MS (+APCI) *m*/*z* 405 (MH^+). Anal. ($C_{22}H_{32}N_2O_3S$) C, H, N.

(*Z*)-2-(Benzyloxyamino)-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)thiazol-4-one (4m). Compound **4m** was prepared by subjecting *O*-benzylhydroxylamine hydrochloride to general procedure A. Crude product, a yellow solid, was recrystallized successively from EtOAc/hexane, CH_3CN/H_2O, $CH_3OH/CH_3CN/H_2O$, and CH_3CN to give 0.34 g (8%) of pure **4m** as a light-yellow solid: ^{1}H NMR (DMSO-d_6) δ 1.42 (s, 18H, 2 × *t*-Bu), 5.07 (s, 2H, CH_2Ph), 7.30−7.40 (m, 7H, 7 × H_{arom}), 7.52 (s, 1H, H_{vinyl}), 7.68 (bs, 1H, OH), 12.07 (bs, 1H, NH); mp 193−194 °C; MS (+APCI) *m*/*z* 439 (MH^+). Anal. ($C_{25}H_{30}N_2O_3S$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-(phenoxyamino)thiazol-4-one (4n). Compound **4n** was prepared by subjecting *O*-phenylhydroxylamine hydrochloride to general procedure A. Crude product, a yellow solid, was recrystallized twice from acetonitrile/water to give 1.3 g (57%) of pure **4n** as golden plates: ^{1}H NMR (DMSO-d_6) δ 1.42 (s, 18H, 2 × *t*-Bu), 7.05−7.38 (m, 5H, 5 × H_{arom}), 7.40 (s, 2H, 2 × H_{arom}), 7.62 (s, 1H, H_{vinyl}), 7.72 (bs, 1H, OH), 12.45 (bs, 1H, NH); mp 194 °C dec; MS (+APCI) *m*/*z* 425.3 (MH^+). Anal. ($C_{24}H_{28}N_2O_3S$) C, H, N.

(*Z*)-*N*-[5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl]acetamide (4o). To a mixture of **4f**[10a] (0.42 g, 1.1 mmol) and acetic anhydride (10 mL) was added pyridine (5 mL). The resultant reaction solution was stirred at ambient temperature for 90 min forming a precipitate. The reaction solution was diluted with ether (20 mL). Filtration and the subsequent drying under vacuum gave 0.11 g (27%) of **4o** as a yellow solid: IR 3619, 1723, 1695, 1587, 1559, 1438, 1424, 1165 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.42 (s, 18H, 2 × *t*-Bu), 2.23 (s, 3H, CH_3), 7.48 (s, 2H, 2 × H_{arom}), 7.78 (s, 1H, H_{vinyl}), 7.82 (s, 1H, OH), 12.82 (bs, 1H, NH); mp >270 °C; MS (+APCI) *m*/*z* 375 (MH^+). Anal. ($C_{20}H_{26}N_2O_3S \cdot 0.20H_2O$) C, H, N.

(*Z*)-*N*-[5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl]methanesulfonamide (4p). To a mixture of compound **4f**[10a] (1.77 g, 4.79 mmol), Et_3N (2.40 mL, 17.2 mmol), and dichloromethane (60 mL) was added methanesulfonyl chloride (0.450 mL, 5.75 mmol). The solid dissolved rapidly upon addition of methanesulfonyl chloride. The resultant reaction mixture was stirred for 30 min at ambient temperature. TLC indicated that there was still some **4f** left; 0.4 mL of methanesulfonyl chloride was added; 10 min after addition of the additional methanesulfonyl chloride, the reaction solution was diluted with 40 mL of dichloromethane, washed successively with 2% aqueous HCl (2 × 60 mL), brine (2 × 60 mL), and water (2 × 60 mL), and then dried over Na_2SO_4. The dry solution was filtered through a pad of silica gel, and the silica gel was washed with 20−40% of methanol in dichloromethane (2 × 60 mL); the filtrate was concentrated in vacuo to afford a solid. Recrystallization from EtOAC/hexanes followed by trituration with CH_3CN gave 0.3 g (15%) of **4p** as a bright-yellow solid: IR 3580, 1696, 1582, 1440, 1423, 1346, 1196, 965 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 18H, 2 × *t*-Bu), 3.14 (s, 3H, CH_3), 5.77 (s, 1H, OH), 7.40 (bs, 2H, 2 × H_{arom}), 7.83 (s, 1H, H_{vinyl}), 8.79 (bs, 0.54H, NH), 10.25 (bs, 0.46H, NH); mp 235−237 °C; MS (+APCI) *m*/*z* 411 (MH^+). Anal. ($C_{19}H_{26}N_2O_4S_2$) C, H, N.

3,5-Diisopropyl-4-methoxybenzaldehyde (5f). Dimethyl sulfate (3.3 g, 26 mmol) was added to a stirred mixture of 3,5-diisopropyl-4-hydroxybenzaldehyde (4.5 g, 22 mmol) and potassium carbonate (3.9 g, 28 mmol) in 100 mL of acetone under an inert atmosphere and heated to reflux. After 2.5 h the mixture was allowed to cool, and most of the acetone was removed under reduced pressure. The residue was partitioned between 200 mL of water and 200 mL of dichloromethane, and the layers were separated. The aqueous phase was extracted with 100 mL of dichloromethane, and the organic extracts were combined, washed with 0.5 M K_2CO_3, water, and saturated brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure to leave the product as a clear oil (4.9 g) of sufficient purity for the next reaction: ^{1}H NMR (DMSO-d_6) δ 1.23 (d, 12H, 4 × CH_3), 3.31 (septet, 2H, $HCMe_2$), 3.75 (s, 3H, OCH_3), 7.61 (s, 2H, ArH), 9.89 (s, 1H, CHO).

(*Z*)-5-(3,5-Diisopropyl-4-hydroxybenzylidene)-2-thioxothiazolidin-4-one (6e). A mixture of 3,5-diisopropyl-4-hydroxybenzaldehyde (**5e**)[24] (5.7 g, 28 mmol), rhodanine (3.5 g, 25 mmol), sodium acetate (7.5 g), and acetic acid (40 mL) was stirred and heated to reflux under an inert atmosphere. After 16 h the mixture was allowed to cool, then poured into water (500 mL), and stirred vigorously for 1.5−2 h. The precipitate was filtered off, washed three times with water, and then dried. Recrystallization from acetonitrile afforded **6e** (6.8 g, 85%): mp 200−200 °C; IR (KBr) 2962, 1706, 1575, 1431 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.20 (d, 12H, CH_3), 3.34 (m, 2H, $HCMe_2$), 7.27 (s, 2H, ArH), 7.61 (s, 1H, olefin), 9.15 (s, 1H, OH), 13.69 (br s, 1H, NH). Anal. ($C_{16}H_{19}NO_2S_2$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butylbenzylidene)-2-thioxothiazolidin-4-one (6h). Prepared from **5h**[25] and rhodanine by the procedure described in the preparation of **6e**. Recrystallization of the purified product from ethyl acetate gave **11a** in 63% yield: mp 186−188 °C; IR (KBr) 2958, 1696, 1583, 1441 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.32 (s, 18H, CH_3), 7.42 (s, 2H, ArH), 7.55 (s, 1H, olefin), 7.70 (s, 1H, ArH), 13.8 (br s, 1H, NH). Anal. ($C_{18}H_{23}NOS_2$) C, H, N.

(*Z*)-5-(3,5-Diiodo-4-hydroxybenzylidene)-2-thioxothiazolidin-4-one (6c). Prepared from **5c**[26] and rhodanine by the procedure described in the preparation of **6e**. The crude product was triturated in ethanol twice and then diethyl ether twice to give **6c** in 99% yield: mp >285 °C; IR (KBr) 3279,

1720, 1592, 1569, 1455 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.51 (s, 1H, olefin), 7.96 (s, 2H, ArH). Anal. ($C_{10}H_5I_2NO_2S_2$) C, H, N.

(*Z*)-5-(3,5-Diisopropyl-4-methoxybenzylidene)-2-thioxothiazolidin-4-one (6f). A mixture of 3,5-diisopropyl-4-methoxybenzaldehyde (**5f**) (4.9 g, 22 mmol), rhodanine (3.0 g, 22 mmol), and β-alanine (1.6 g, 18 mmol) in acetic acid (50 mL) was stirred and heated to reflux under an inert atmosphere. After 2 h the mixture was cooled, poured into water (300 mL), and stirred vigorously for 0.5 h. The precipitate was filtered off, washed three times with water, and then dried to afford **6f** (6.8 g, 92%): mp 169−170 °C. Recrystallization of a sample from acetonitrile afforded the analytically pure product: mp 172−174 °C; IR (KBr) 3150, 2963, 1698, 1589, 1435 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.22 (d, 12H, CH_3), 3.30 (septet, 2H, $HCMe_2$), 3.72 (s, 3H, SCH_3), 7.37, 2H, ArH), 7.65 (s, 1H, olefin), 13.83 (br s, 1H, NH). Anal. ($C_{17}H_{21}NO_2S_2$) C, H, N.

(*Z*)-5-(3,5-Dibromo-4-hydroxybenzylidene)-2-thioxothiazolidin-4-one (6b). Prepared from **5b**[26] and rhodanine by the procedure described in the preparation of **6f**. The crude product was triturated in ethanol twice and then diethyl ether twice to give **6b** in 89% yield: mp >300 °C; IR (KBr) 1726, 1580, 1473, 1144 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.56 (s, 1H, olefin), 7.78 (s, 2H, ArH). Anal. ($C_{10}H_5Br_2NO_2S_2$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-methoxybenzylidene)-2-thioxothiazolidin-4-one (6g). Prepared from **5g**[27] and rhodanine by the procedure described in the preparation of **6f**. Recrystallization of the purified product from ethanol gave **6g** in 50% yield: mp 229−232 °C; IR (KBr) 3457, 2961, 1692, 1596, 1409 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.41 (s, 18H, CH_3), 3.68 (s, 3H, SCH_3), 7.50 (s, 2H, ArH), 7.68 (s, 1H, olefin), 13.8 (br s, 1H, NH). Anal. ($C_{19}H_{25}NO_2S_2$) C, H, N.

(*Z*)-5-Benzylidene-2-(methylsulfanyl)thiazol-4-one (7a). Iodomethane (1.8 g, 12.9 mmol) was added to a stirred mixture of **6a**[28] (2.0 g, 9.0 mmol) and diisopropylethylamine (1.4 g, 10.6 mmol) in ethanol (50 mL) at room temperature under an inert atmosphere. After 1.5 h the mixture was poured into water (200 mL) and stirred for an additional 2 h. The precipitate was filtered off, washed three times with water, dried, and then chromatographed on a column of silica gel in chloroform to afford the pure **7a** (1.1 g, 52%): mp 147−148 °C; IR (KBr) 3006, 1698, 1601, 1464 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.84 (s, 3H, SMe), 7.50−7.58 (m, 3H, ArH), 7.68 (d, 2H, ArH), 7.86 (s, 1H, olefin). Anal. ($C_{11}H_9NOS_2$) C, H, N.

(*Z*)-5-(3,5-Dibromo-4-hydroxybenzylidene)-2-(methylsulfanyl)thiazol-4-one (7b). Prepared from **6b** and iodomethane by the procedure described in the preparation of **7a**. Recrystallization from dimethylformamide gave **7b** in 86% yield: mp 277 °C dec; IR (KBr) 3282, 1685, 1574, 1463 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.84 (s, 3H, SCH_3), 7.75 (s, 1H, olefin), 7.85 (s, 2H, ArH); MS (+APCI) *m*/*z* 410 (MH^+). Anal. ($C_{11}H_7NO_2S_2Br_2$) C, H, N.

(*Z*)-5-(3,5-Diiodo-4-hydroxybenzylidene)-2-(methylsulfanyl)thiazol-4-one (7c). Prepared from **6c** and iodomethane by the procedure described in the preparation of **7a**. Recrystallization from dimethylformamide gave **7c** in 47% yield: mp 240 °C dec; IR (KBr) 3420, 1684, 1595, 1485 cm^{-1}; ^{1}H NMR (DMSO-d_6 + TFA) δ 2.81 (s, 3H, SCH_3), 7.69 (s, 1H, olefin), 8.01 (s, 2H, ArH); MS (+APCI) *m*/*z* 504 (MH^+). Anal. ($C_{11}H_7I_2NO_2S_2$) C, H, N.

(*Z*)-5-(3,5-Diisopropyl-4-hydroxybenzylidene)-2-(methylsulfanyl)thiazol-4-one (7e). Prepared from **6e** and iodomethane by the procedure described in the preparation of **7a**. Recrystallization from acetonitrile gave **7e** in 78% yield: mp 195−197 °C; IR (KBr) 3429, 2957, 1677, 1577, 1453 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.19 (d, 12H, CH_3), 2.82 (s, 3H, SCH_3), 3.28−3.38 (m, 2H, $HCMe_2$), 7.34 (s, 2H, ArH), 7.80 (s, 1H, olefin), 9.17 (br s, 1H, OH); MS (+APCI) *m*/*z* 336 (MH^+). Anal. ($C_{17}H_{21}NO_2S_2$) C, H, N.

(*Z*)-5-(3,5-Diisopropyl-4-methoxybenzylidene)-2-(methylsulfanyl)thiazol-4-one (7f). Prepared from **6f** and iodomethane by the procedure described in the preparation of **7a**. Recrystallization from acetonitrile gave **7f** in 46% yield: mp 114−116 °C; IR (KBr) 2961, 1700, 1591, 1470 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.22 (d, 12H, CH_3), 2.83 (s, 3H, SCH_3), 3.28 (septet, 2H, $HCMe_2$), 3.72 (s, 3H, OCH_3), 7.45 (s, 2H, ArH), 7.85 (s, 1H, olefin). Anal. ($C_{18}H_{23}NO_2S_2$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-methoxybenzylidene)-2-(methylsulfanyl)thiazol-4-one (7g). Prepared from **6g** and iodomethane by the procedure described in the preparation of **7a**. Recrystallization from acetonitrile gave **7g** in 46% yield: mp 132−134 °C dec; IR (KBr) 2965, 1700, 1599, 1461 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.42 (s, 18H, CH_3), 2.83 (s, 3H, SCH_3), 3.68 (s, 3H, OCH_3), 7.57 (s, 2H, ArH), 7.87 (s, 1H, olefin). Anal. ($C_{20}H_{27}NO_2S_2$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butylbenzylidene)-2-(methylsulfanyl)-thiazol-4-one (7h). Prepared from **6h** and iodomethane by the procedure described in the preparation of **7a**. Recrystallization from ethanol gave **7h** in 45% yield: mp 123−125 °C; IR (KBr) 2963, 1705, 1600, 1474 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.31 (s, 18H, CH_3), 2.82 (s, 3H, SCH_3), 7.48 (s, 2H, ArH), 7.53 (s, 1H, ArH), 7.89 (s, 1H, olefin). Anal. ($C_{19}H_{25}NOS_2$) C, H, N.

(*Z*)-5-Benzylidene-2-(methoxyamino)thiazol-4-one (8a). Potassium *tert*-butoxide (0.37 g, 3.2 mmol) was added to a stirred mixture of **7a** (0.7 g, 3.0 mmol) and methoxylamine hydrochloride (0.27 g, 3.2 mmol) in 20 mL of ethanol under an inert atmosphere. The mixture was heated to reflux for 8 h, then, after being allowed to cool, was poured into 200 mL of water, and was stirred. After approximately 2 h the precipitate was filtered off, washed three times with water, once with ethanol, and once with ether and then dried. The crude product was recrystallized from ethanol and dried to afford the product **8a** (0.3 g, 43%) as fluffy yellow crystals: mp 183−184 °C; IR (KBr) 3170, 3006, 1714, 1643, 1333 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3H, OCH_3), 7.47 (tr, 1H, ArH), 7.53 (tr, 2H, ArH), 7.61 (d, 1H, ArH), 7.63 (s, 1H, olefin) 12.26 (s, 1H, NH); MS (+APCI) *m*/*z* 235 (MH^+). Anal. ($C_{11}H_{10}N_2O_2S$) C, H, N.

(*Z*)-5-(3,5-Diisopropyl-4-hydroxybenzylidene)-2-(methoxyamino)thiazol-4-one (8ea). Prepared from **7e** and methoxylamine hydrochloride by the procedure described in the preparation of **8a**. Recrystallization from dimethylformamide/methanol gave **8ea** in 70% yield: mp 282 °C dec; IR (KBr) 3476, 2951, 1698, 1639, 1591 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.18 (d, 12H, CH_3), 3.34 (m, obscured by water peak, $HCMe_2$), 3.82 (s, 3H, OCH_3), 7.24 (s, 2H, ArH), 7.57 (s, 1H, olefin), 8.90 (s, 1H, OH), 12.06 (s, 1H, NH); MS (+APCI) *m*/*z* 335 (MH^+). Anal. ($C_{17}H_{22}N_2O_3S$) C, H, N.

***N*-[5-(4-Hydroxy-3,5-diisopropylbenzylidene)-4-oxo-4,5-dihydrothiaz-2-yl]guanidine (8eb).** Prepared from **7e** and guanidine hydrochloride by the procedure described in the preparation of **8a**. Trituration in boiling ethyl acetate gave **8eb** in 69% yield: mp 240−241 °C dec; IR (KBr) 3392, 2963, 1653, 1582, 1466 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.17 (d, 12H, CH_3), 3.32 (m, obscured by water peak, $HCMe_2$), 7.24 (s, 2H, ArH), 7.35 (br s, 1H, OH), 7.51 (s, 1H, olefin), 8.25 (br s, ~2H, NH); MS (+APCI) *m*/*z* 347 (MH^+). Anal. ($C_{17}H_{22}N_4O_2S$) C, H, N.

(*Z*)-5-(3,5-Diisopropyl-4-methoxybenzylidene)-2-(methoxyamino)thiazol-4-one (8f). Prepared from **7f** and methoxylamine hydrochloride by the procedure described in the preparation of **8a**. The crude product was first purified by chromatography on silica gel (chloroform/ethyl acetate, 95:5) and then recrystallized from acetonitrile to give **8f** in 16% yield: mp 165−167 °C dec; IR (KBr) 2965, 1692, 1634, 1610 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.22 (d, 12H, CH_3), 3.28 (septet, 2H, $HCMe_2$), 3.71 (s, 3H, $ArOCH_3$), 3.83 (s, 3H, $NOCH_3$), 7.36 (s, 2H, ArH), 7.57 (s, 1H, olefin), 12.19 (s, 1H, NH); MS (+APCI) *m*/*z* 349 (MH^+). Anal. ($C_{18}H_{24}N_2O_3S$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-methoxybenzylidene)-2-(methoxyamino)thiazol-4-one (8g). Prepared from **7g** and methoxylamine hydrochloride by the procedure described in the preparation of **8a**. The crude product was first purified by chromatography on silica gel (chloroform/ethyl acetate, 98:2) and then recrystallized from ethanol to give **8g** in 43% yield: mp 224−225 °C dec; IR (KBr) 2961, 1692, 1638, 1607 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.41 (s, 18H, CH_3), 3.67 (s, 3H, $ArOCH_3$), 3.82 (s, 3H, $NOCH_3$), 7.48 (s, 2H, ArH), 7.58 (s, 1H, olefin), 12.18 (br s, 1H, NH); MS (+APCI) *m*/*z* 377 (MH^+). Anal. ($C_{20}H_{28}N_2O_3S$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butylbenzylidene)-2-(methoxyamino)-thiazol-4-one (8h). Prepared from **7h** and methoxylamine hydrochloride by the procedure described in the preparation of **8a**. Recrystallization from ethanol gave **8h** in 30% yield: mp 210−211 °C dec; IR (KBr) 2963, 1697, 1638, 1607 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.32 (s, 18H, CH_3), 3.83 (s, 3H, OCH_3), 7.42 (s, 2H, ArH), 7.49 (s, 1H, ArH), 7.62 (s, 1H, olefin), 12.21 (s, 1H, NH); MS (+APCI) *m*/*z* 347 (MH^+). Anal. ($C_{19}H_{26}N_2O_2S$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-(methoxyamino)oxazol-4-one (11b). Methoxylamine hydrochloride (0.19 g, 2.3 mmol) in 75 mL of ethanol was cooled in an ice bath and treated with potassium *tert*-butoxide (0.25 g, 2.2 mmol). The mixture was stirred for 15 min and then filtered into a solution of **10**[10a] (1.0 g, 2.9 mmol) in 60 mL of ethanol. The reaction mixture was stirred in a ice bath for 2 h and then for 1 h at room temperature. The reaction mixture was filtered; the filtrate was concentrated to one-half of the original volume and poured onto 450 mL of ice−water. The mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, and then evaporated to an oil. The oil was purified by flash chromatography (elution with 2.5−5% methanol in dichloromethane). Recrystallization from aqueous ethanol gave 0.22 g (29%) of **11b**: mp 204−207 °C; IR 3628, 3608, 1740, 1706, 1328 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.393 and 1.397 (s, 18H, 2 × *t*-Bu), 3.70 and 3.72 (s, 3H, OCH_3), 6.51 and 6.54 (s, 1H, H_{vinyl}), 7.48 and 7.49 (s, 1H, OH), 7.56 (s, 1H, H_{arom}), 7.67 (s, 1H, H_{arom}); MS (EIMS) *m*/*z* 346 (M^+). Anal. ($C_{19}H_{26}N_2O_4$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-(hydroxyamino)oxazol-4-one (11a). Prepared from hydroxylamine hydrochloride (0.38 g, 5.5 mmol), potassium *tert*-butoxide (0.55 g, 4.9 mmol), and **10**[10a] (2.3 g, 6.6 mmol) by the procedure described in the preparation of **11b**, except that the reaction was stirred at room temperature for 5 h. Recrystallization of the purified product from aqueous ethanol gave 0.20 g (13%) of **11a**: mp 220−224 °C; IR 3631, 1752, 1711, 1331, 1239 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.393 and 1.398 (s, 18H, 2 × *t*-Bu), 6.44 and 6.47 (s, 1H, H_{vinyl}), 7.43 and 7.44 (s, 1H, phenol OH), 7.56 (s, 1H, H_{arom}), 7.67 (s, 1H, H_{arom}); 9.61 and 9.69 (s, 1H, NHOH), 11.90 and 12.25 (br s, 1H, NH); MS (EIMS) *m*/*z* 333 (MH^+). Anal. ($C_{18}H_{24}N_2O_4$) C, H, N.

(*Z*)-2-(Allyloxyamino)-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)oxazol-4-one (11d). Prepared from *O*-allylhydroxylamine hydrochloride (0.50 g, 4.6 mmol), potassium *tert*-butoxide (0.49 g, 4.4 mmol), and **10**[10a] (2.0 g, 5.8 mmol) by the procedure described in the preparation of **11b**, except that the reaction mixture was stirred at −40 °C for 15 h. Several recrystallizations of the purified product from aqueous ethanol gave 0.29 g (18%) of **11d**: mp 187−189 °C; IR 3617, 1739, 1705, 1332, 1044 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.39 (s, 18H, 2 × *t*-Bu), 4.40−4.45 (m, 2H, $CH_2CH{=}CH_2$), 5.19−5.38 (m, 2H, $CH_2CH{=}CH_2$), 5.93−6.01 (m, 1H, $CH_2CH{=}CH_2$), 6.51 and 6.54 (s, 1H, ArCH=), 7.48 and 7.49 (s, 1H, OH), 7.55 (s, 1H, H_{arom}), 7.67 (s, 1H, H_{arom}); MS (EIMS) *m*/*z* 372 (M^+). Anal. ($C_{21}H_{28}N_2O_4$) C, H, N.

(*Z*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-(ethoxyamino)oxazol-4-one (11c). Prepared from ethoxylamine hydrochloride (0.45 g, 4.6 mmol), potassium *tert*-butoxide (0.49 g, 4.4 mmol), and **10**[10a] (2.0 g, 5.8 mmol) by the procedure described in the preparation of **11b**, except that the reaction was stirred at −30 °C for 4 h. Several recrystallizations of the purified product from aqueous ethanol gave 0.11 g (7%) of **11c**: mp 214−217 °C; IR 3627, 3614, 1735, 1708, 1328 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.18−1.23 (m, 3H, OCH_2CH_3), 1.39 and 1.40 (s, 18H, 2 × *t*-Bu), 3.90−3.97 (m, 2H, OCH_2CH_3), 6.49 and 6.52 (s, 1H, H_{vinyl}), 7.47 and 7.48 (s, 1H, OH), 7.55 (s, 1H, H_{arom}), 7.68 (s, 1H, H_{arom}); MS (EIMS) *m*/*z* 360 (M^+). Anal. ($C_{20}H_{28}N_2O_4$) C, H, N.

***N*-[3-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1-hydroxy-2-(methoxyimino)propylidene]-*N*′-methoxycarbamidothioic Acid Methyl Ester (20).** Methoxylamine hydrochloride (1.6 g, 19.2 mmol) in 75 mL of ethanol was cooled in ice and treated with potassium *tert*-butoxide (2.1 g, 18.7 mmol). The mixture was stirred for 15 min and then filtered into a solution of **10**[10a] (3.1 g, 8.9 mmol) in 60 mL of ethanol. The reaction mixture was stirred at reflux for 15 h, then cooled, and filtered. The filtrate was condensed about 50% and added to 450 g of ice and water. The precipitated solid was filtered, washed with water, and dissolved in ethyl acetate. The organic solution was washed with brine, dried over Na_2SO_4, and evaporated. The residue was recrystallized from aqueous ethanol to yield 1.8 g (48%) of **20**: mp 123−125 °C; IR 3613, 3363, 1706, 1579, 1054 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.37 (s, 18H, 2 × *t*-Bu), 2.26 (s, 3H, SMe), 3.76 (s, 2H, CH_2), 3.83 (s, 3H, NOMe), 4.04 (s, 3H, NOMe), 5.04 (s, 1H, ArOH), 7.07 (s, 1H, H_{arom}), 7.22 (s, 1H, H_{arom}), 9.76 (bs, 1H, enolic OH); MS (EIMS) *m*/*z* 424 (M^+). Anal. ($C_{21}H_{33}N_3O_4S$) C, H, N.

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Supporting Information Available: Enzyme and cellular activity of compounds **4g**−**p**,**s**, **7b**,**c**,**e**, **8a**,**f**−**h**, **11a**, **12a**,**c**, **13**−**15**, **16a**−**c**, **18a**−**d**, and **19b** (Table 4). This information is available free of charge via the Internet at http://pubs.acs.org.

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(28) Compound **6a** is commercially available from SALOR, Milwaukee, WI.

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