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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. 2. 1,3,4- and 1,2,4-Thiadiazole Series[1]

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Two isoforms of the cyclooxygenase (COX) enzyme have been identified: COX-1, which is expressed constitutively, and COX-2, which is induced in inflammation. Recently, it has been shown that selective COX-2 inhibitors have antiinflammatory activity and lack the GI side effects typically associated with NSAIDs. Initial mass screening and subsequent SAR studies have identified **6b** (PD164387) as a potent, selective, and orally active COX-2 inhibitor. It had IC_{50} values of 0.14 and 100 μM against recombinant human COX-2 and purified ovine COX-1, respectively. It inhibited COX-2 activity in the J774A.1 cell line with an IC_{50} of 0.18 μM and inhibited COX-1 activity in platelets with an IC_{50} of 3.1 μM. The choline salt of compound **6b** was also orally active in vivo with an ED_{40} of 7.1 mg/kg in the carrageenan footpad edema (CFE) assay. In vivo studies in rats at a dose of 100 mg/kg showed that this compound inhibited gastric prostaglandin E_2 (PGE_2) production in gastric mucosa by 77% but caused minimal GI damage. SAR studies of this chemical series revealed that the potency and selectivity are very sensitive to minor structural changes.

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are important therapeutic agents for the treatment of pain and inflammation. However, their therapeutic use is often limited by common side effects, such as gastrointestinal (GI) hemorrhage and ulceration.[2] Therefore, a major challenge of the pharmaceutical industry is to develop drugs that have antiinflammatory activities but lack the toxic side effects associated with currently used NSAIDs. A major mechanism of action of NSAIDs is lowering prostaglandin production through inhibition of cyclooxygenase (COX), a key enzyme in prostaglandin biosynthesis.[3] Because prostaglandins have dual functions, mediation of inflammation[3] and cytoprotection in the stomach and intestine,[4] long-term usage of NSAIDs to relieve the symptoms of inflammation will cause GI damage and ulceration. A possible dissociation of antiinflammatory effects from GI toxicity is suggested by the recent discovery that COX exists in two isoforms, COX-1 and COX-2, which are encoded by two distinct genes.[5] COX-1 is expressed constitutively and is thought to provide cytoprotection; COX-2 expression, however, is transiently upregulated by proinflammatory mediators and downregulated by corticosteroids.[6] This regulated expression suggests that a selective inhibitor of COX-2 might have antiinflammatory properties and lack GI side effects. This hypothesis has been supported by several in vivo studies with known selective COX-2 inhibitors: **1** (SC-58125),[7] **2** (NS-398),[8] **3** (L-745,337),[9] and **4** (PD 138387)[10] (Chart 1).

Prior to the discovery of two isoforms of the COX, our efforts on a dual cyclooxygenase and 5-lipoxygenase inhibitor program[11,12] had identified a number of substituted di-*tert*-butylphenols as potent COX inhibitors, some of which lacked inhibitory activity toward 5-lipoxygenase and were not fully evaluated under the dual inhibitor strategy. The possibility that these compounds would be selective COX-2 inhibitors was investigated and structure−activity relationships (SAR) for selective inhibitors of COX-2 were developed in several series. SAR studies of the chemical series related to **4** have been reported.[10] In this paper we report SAR studies on the 1,3,4- and 1,2,4-thiadiazole-derived di-*tert*-butylphenol series. Also reported are pharmacological evaluations of selective inhibitors in the carrageenan footpad edema (CFE) assay, in a hyperalgesia model, and in a model of GI ulceration.

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 the structures **I** and **II** (Chart 2) were identified as potent and selective inhibitors.

Compound Evaluations

The primary assays for the compounds described in this SAR study were purified enzyme assays. Recombinant human COX-2 (rhCOX-2) and ram seminal

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Chart 1. Known Selective COX-2 Inhibitors

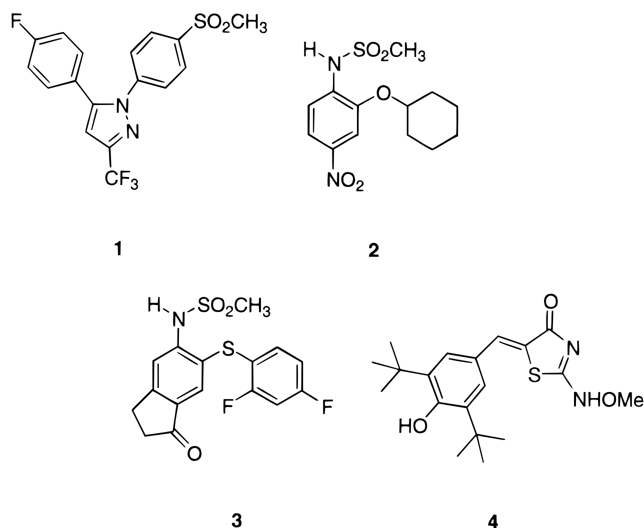
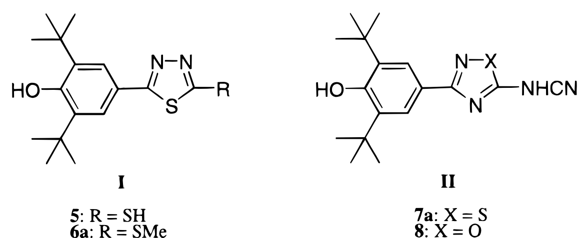


Chart 2. Mass Screening Hits



vesicle COX-1 (oCOX-1) were used to measure 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 model, 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.

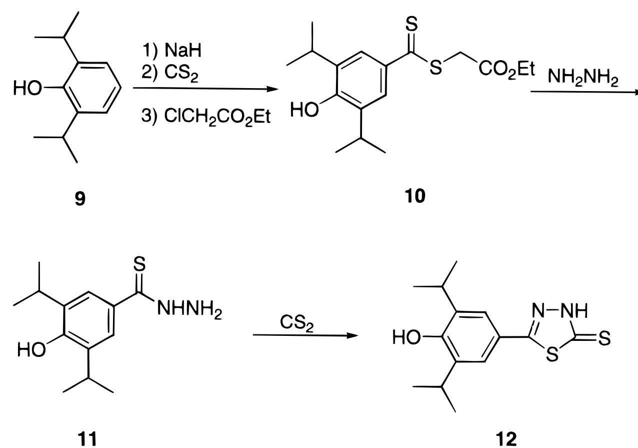
Chemistry

The diisopropylphenol-derived thiadiazolethione **12** was prepared according to the procedure developed for the corresponding di-*tert*-butylphenol derivative (Scheme 1).[12] Condensation of the sodium salt of compound **9** with carbon disulfide followed by alkylation with ethyl chloroacetate gave thioester **10**, which was converted to thiohydrazide **11** by treatment with hydrazine. Condensation of **11** with carbon disulfide produced thiadiazolethione **12** in good yield.

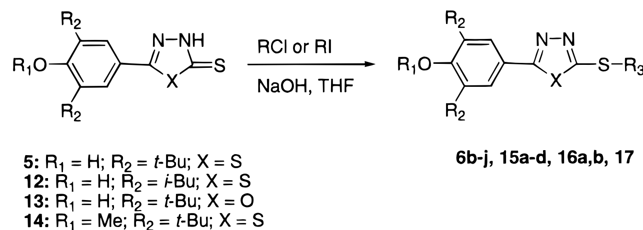
Alkylation of thiadiazolethiones **5**,[12] **12**, **13**,[12] and **14**[12] with sodium hydroxide and alkyl chloride or alkyl iodide gave thiadiazoles **6b**–**j**, **15a**–**d**, **16a,b**, and **17** (Table 1, Scheme 2). Compound **18a** was obtained by oxidation of **6b** with MCPBA. The choline salt of **6b** (**6b**·choline) was made by a standard method.

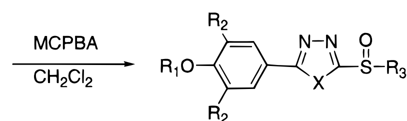
Treatment of sulfone **19**[13] with sodium alkoxide produced the corresponding aryl ethers **20a**–**d** in good yield (Scheme 3). Reaction of sulfone **21**[14] with *O*-methylhydroxylamine in *tert*-butyl alcohol using *tert*-butoxide as the base failed to give the corresponding hydroxylamine derivative; the 1,2,4-thiadiazolone **22** was isolated instead (Scheme 4). Thiadiazoles **24a**,**b** were prepared by acylation and sulfonylation, respectively of 5-aminothiadiazole **23**[14] (Scheme 5). Condensation of **31**[15] with diphenyl cyanocarbonimidate followed by deprotection with acid gave oxadiazole **8** (Scheme 6).

Scheme 1. Synthesis of 1,3,4-Thiadiazole **12**



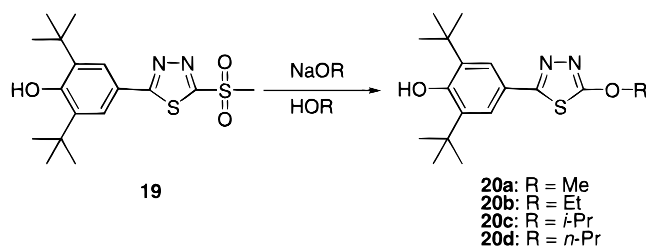
Scheme 2. 1,3,4-Thiadiazole Synthesis





18a: R_1 = H; R_2 = *t*-Bu; X = S; R_3 = Et

Scheme 3. Synthesis of 1,3,4-Thiadiazoles **20a**–**d**

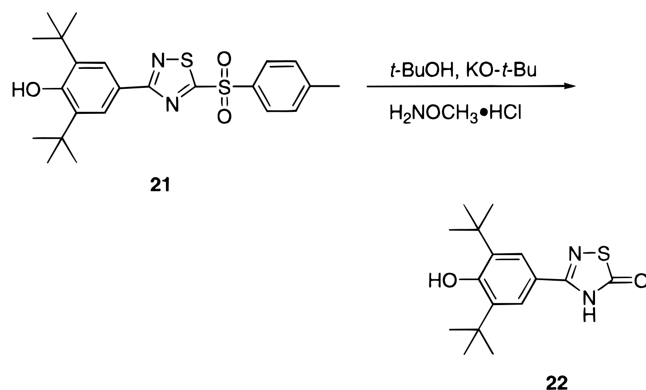


The preparation of **6a**,[12] **7a**,[15] **16c**,[13] **18b**,[13] **19**,[13] **20e**,[12] **23**,[14] **25a**,[12]**b**–**e**,[13] **26a**,**b**,[13] **27**,[12] **28a**,**b**,[16] **29**,[14] and **30a**,[15]**b**[14] has been reported previously.

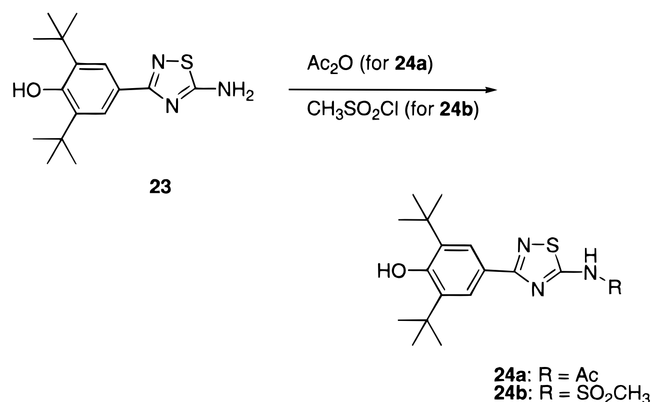
Results and Discussion

Structure–Activity Relationships and Discussion of the Enzyme Data. It is well documentated that COX enzyme catalysis involves radical intermediates[17] such as the phenoxy radical generated on a tyrosine residue.[18] Since our chemical leads are di-*tert*-butylphenols which can form phenoxy radicals and act as radical scavengers, it was of interest to define the importance

Scheme 4. Synthesis of 1,3,4-Thiadiazole **22**



Scheme 5. Synthesis of 1,2,4-Thiadiazoles **24a,b**



of the phenolic hydroxy group for the observed inhibitory activity toward COX. To address this mechanism-related SAR question, methyl ethers **17** and **14** (Table 1), in which the exchangeable protons are replaced with methyl groups, were synthesized and evaluated in the purified enzyme assays. Compared to the corresponding di-*tert*-butylphenols **6a** and **5**, the methyl ethers **17** and **14** had similar potency against COX-2 but were less potent in the COX-1 assay, making them more selective COX-2 inhibitors. These data suggest that the observed inhibitory activity of the two di-*tert*-butylphenols **6a** and **5** in enzyme assays is not due to antioxidant properties of the compounds.

The effects of R_2 substitution were investigated in the 1,3,4-thiadiazole series (Table 1). Changing the *tert*-butyl groups to isopropyl groups abolished the activity against both enzymes (**6a** vs **15a**, **6b** vs **15b**). The two isopropylphenols **15c**,**d** were also inactive against both enzymes, as were the corresponding di-*tert*-butylphenols (**6d**,**e**).

The substitution effects of R_3 were explored in detail in the di-*tert*-butylphenol-derived 1,3,4-thiadiazole series. Amines, ethers, and thioethers were examined. Within the amine series compound **25a** was active and selective for COX-2. Substitutions on the nitrogen all led to decreased potency (**25d**, **26a**, **25e**, **26b**) or decreased selectivity (**25b**,**c**). Compound **25c** was shown to be a selective COX-1 inhibitor. Within the ether series compounds were either nonselective (**20a**,**b**) or inactive (**20c**,**d**). The most potent and selective COX-2 inhibitors were identified among the thioethers. Within this series SAR studies reveal that the size of the R_3 substituent is important for potency and selectivity. When R_3 was SH (**5**), the compound was active but not very selective, slightly favoring COX-2. When R_3 was changed from SH to SMe (**6a**), more than a 30-fold increase in activity against both enzymes was observed, leaving the selectivity essentially unchanged. When R_3 was changed from SMe to SEt (**6b**), the potency against COX-2 remained unchanged ($IC_{50} = 0.14\ \mu M$), but the activity against COX-1 was reduced more than 200-fold, resulting in a potent and selective COX-2 inhibitor. Modification of R_3 from SEt to S-*n*-Pr led to **6d**, which was essentially inactive against both enzymes. Other substitutions, such as S-*i*-Pr (**6e**) or SBn (**6g**), also gave inactive compounds. Several compounds with ionizable groups as part of R_3 (**6h**–**j**) were neither potent nor selective. Compound **6b** (PD 164387) was the most potent ($IC_{50} = 0.14\ \mu M$) and selective (more than 700-fold) COX-2 inhibitor in this series.

In this 1,3,4-thiadiazole series the potency and selectivity are very sensitive to minor structural modifications. For example, the ethyl thioether **6b** was the most potent and selective COX-2 inhibitor in this series, but the corresponding trifluoroethyl thioether **6c** was inactive against both enzymes. The ethyl ether **20b** was more than 40-fold less potent against COX-2 and essentially nonselective, and the sulfoxide **18a** was inactive against both enzymes. Similar changes in activity were observed in the comparison of thioether **6a** with ether **20a** and sulfoxide **18b**. In addition, substitution of NHMe for SMe resulted in a reversal of selectivity affording **25c** as a selective COX-1 inhibitor.

Replacement of two of the aromatic carbon atoms with nitrogens led to two di-*tert*-butylpyrimidine analogues (**28a,b** in Table 1). In comparison with the corresponding di-*tert*-butylphenols, these compounds were more potent against COX-1 and less potent against COX-2 (**6a** vs **28a**, **6b** vs **28b**). Di-*tert*-butylpyrimidine **28a** was a selective COX-1 inhibitor.

With regard to R_3 substitution, the SAR trend observed in the 1,3,4-oxadiazole series (**13**, **16a**–**c**, **26a**,**b**, and **27**) was very similar to that observed in the 1,3,4-thiadiazole series discussed above. Again, the SEt substituent (**16a**) was optimal in terms of potency and selectivity.

In the 1,2,4-thiadiazole series (Table 2) an acidic proton on the side chain seems to be important for potency and selectivity (**22** vs **29**, **7a** vs **23**). The substitution on the nitrogen atom is also important. Among the analogues examined (**23, 7a, 24a**,**b,** and **30a**), compound **7a** was exclusively preferred. It is worth noting that thiadiazole **7a** and oxadiazole **8** showed similar enzyme potency.

The side chain which is preferred for selective COX-2 inhibition in the 1,3,4-thiadiazole series was not preferred in the 1,2,4-thiadiazole series (**6a** in Table 1 vs **30b** in Table 2). The side chain which is preferred for selective COX-2 inhibition in the 1,2,4-thiadiazole series was not preferred in the 1,3,4-thiadiazole series (**7a** in Table 2 vs **25d** in Table 1). The 1,3,4- and 1,2,4-thiadiazole rings clearly are not functionally interchangeable.

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, potent compounds

Scheme 6. Synthesis of 1,2,4-Oxadiazole **8**

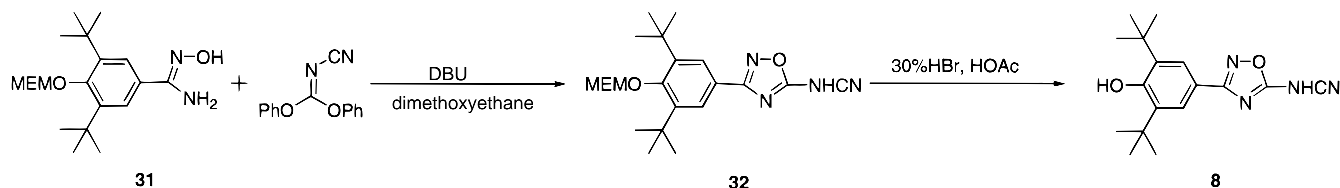


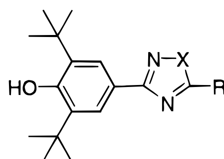
Table 1. Enzyme and Cellular Activity of 1,3,4-Thiadiazoles and 1,3,4-Oxadiazoles[a–c,f]

compd	R_1	R_2	X	Y	R_3	IC_{50} (μM) rhCOX-2	oCOX-1	COX-2 J774A.1	COX-1 hplatelet
5	OH	*t*-Bu	C	S	SH	5.5	20	0.35	NA
6a	OH	*t*-Bu	C	S	SMe	0.15	0.51	0.009	0.18
6b	OH	*t*-Bu	C	S	SEt	0.14	100	0.18	3.1
6c	OH	*t*-Bu	C	S	SCH_2CF_3	NA	NA	NA[d]	NA
6d	OH	*t*-Bu	C	S	S-*n*-Pr	100	NA	NA[d]	4.1
6e	OH	*t*-Bu	C	S	S-*i*-Pr	NA	NA	8.5	13.6
6f	OH	*t*-Bu	C	S	$SCH_2CH_2CO_2Me$	NA	NA	NA[d]	9.9
6 g	OH	*t*-Bu	C	S	SBn	NA	NA	NA	NA
6h	OH	*t*-Bu	C	S	$SCH_2CH_2CO_2H$	NA	NA	NA[d]	NA
6i	OH	*t*-Bu	C	S	$SCH_2CH_2NH_2$	41	36	NA[d]	NA
6j	OH	*t*-Bu	C	S	$SCH_2CH_2NEt_2$	19	NA	NA	NA
13	OH	*t*-Bu	C	O	SH	47	NA	0.92	NA
14	OMe	*t*-Bu	C	S	SH	1.5	NA	0.30	NA
15a	OH	*i*-Pr	C	S	SMe	14@10	25@10	NA	NT
15b	OH	*i*-Pr	C	S	SEt	NA	NA	1.3	NA
15c	OH	*i*-Pr	C	S	S-*n*-Pr	NA	NA	1.5	NA
15d	OH	*i*-Pr	C	S	S-*i*-Pr	NA	NA	NT	NT
16a	OH	*t*-Bu	C	O	SEt	6.5	NA	0.015	1.9
16b	OH	*t*-Bu	C	O	S-*n*-Pr	NA	NA	NA[d]	1.9
16c	OH	*t*-Bu	C	O	SMe	4.1	12	0.14	1.2
17	OMe	*t*-Bu	C	S	SMe	0.34	5.8	0.012	0.77
18a	OH	*t*-Bu	C	S	SOEt	NA	NA	0.23[b]	14.6
18b	OH	*t*-Bu	C	S	SOMe	NA	NA	NT	NA
20a	OH	*t*-Bu	C	S	OMe	3	2.6	NT	0.20
20b	OH	*t*-Bu	C	S	OEt	6.1	3.4	0.07	1.2
20c	OH	*t*-Bu	C	S	O-*i*-Pr	NA	NA	NA[d]	4.5
20d	OH	*t*-Bu	C	S	O-*n*-Pr	NA	NA	NA[d]	3.6
20e	OH	*t*-Bu	C	S	OH	NA	NA	0.24	4.1
25a	OH	*t*-Bu	C	S	NH_2	3.5	34@30	0.11	0.53
25b	OH	*t*-Bu	C	S	$NHNH_2$	1.3	17	3.7	3.2
25c	OH	*t*-Bu	C	S	NHMe	9.6	0.52	0.69	0.30
25d	OH	*t*-Bu	C	S	NHCN	NA	NA	0.033	NA
25e	OH	*t*-Bu	C	S	$NHCNHNH_2$	17	88	0.21	2.8
26a	OH	*t*-Bu	C	O	NHCN	11@10[e]	0@10[e]	0.39	5.8
26b	OH	*t*-Bu	C	O	$NHCNHNH_2$	23	16@30	0.33	2.8
27	OH	*t*-Bu	C	O	OH	NA	NA	NA	3.2
28a	OH	*t*-Bu	N	S	SMe	1.4	0.097	0.026	0.20
28b	OH	*t*-Bu	N	S	SEt	2.0	39@10	0.018	0.68
1						0.31	NA	0.070	NA
2						0.19	NA	0.022	3.3
indomethacin						5.8	0.0122	0.0064	0.0082

[a] NA, not active (in enzyme assays, NA is defined as IC_{50} value greater than 100 μM; in cellular assays, NA is defined as IC_{50} value greater than 20 μM). [b] NT, not tested. [c] When compounds were insoluble at a concentration of 100 μM, the percent inhibition at the highest concentration tested is shown. [d] Found inactive in acetylated J774A.1 cells (Ac-J7), not further tested. [e] Found inactive at 10 μM in the oCOX-2 enzyme assays, not further tested. [f] In the purified enzyme assays, standard errors for the IC_{50} determinations done at least in duplicate averaged 30% for rhCOX-2 and 10% for oCOX-1; In the cellular assays, standard errors for the interday IC_{50} determinations done at least in duplicate averaged 45% for COX-1 and 30% for COX-2.

identified in the COX-2 enzyme assay tended to be more potent in the COX-2 cellular assay (see **6a**, **16a**, **28b** in Table 1 and **22** in Table 2 as examples). In the case of COX-1 there was no clear trend. Consequently, a prediction of selectivity in the cellular assays could not be made based on selectivity observed in the isolated enzyme assays. For example, the most selective COX-2 inhibitor **6b**, which was at least 700-fold selective in the isolated enzyme assays, was only 17-fold selective in cellular assays. Compound **28a** was a selective COX-1 inhibitor in the enzyme assays, but the selectivity was reversed in cellular assays. Compounds inactive in both isolated enzyme assays could be very potent in both cellular assays (**18a**, **20e**, **26a**, **6e**) or could be potent

Table 2. Enzyme and Cellular Activity of 1,2,4-Thiadiazoles and 1,2,4-oxadiazoles[a–d]



compd	X	R	IC$_{50}$ (μM) rhCOX-2	oCOX-1	COX-2 J774A.1	COX-1 hplatelet
7a	S	NHCN	0.047	32	0.026	1.2
8	O	NHCN	0.064	51	1.4	1.2
22	S	OH	1.1	83	0.018	0.43
23	S	NH_2	NA	NA	NT	NT
24a	S	NHAc	NA	NA	NA	NT
24b	S	$NHSO_2Me$	NA	NA	NT	NT
29	S	OMe	100	24	NT	NT
30a	S	$NHCNHNH_2$	27	24	NA	1.7
30b	S	SMe	20@10	16@10	0.26	3.6

[a] NA = Not Active (in enzyme assays, NA is defined as IC$_{50}$ value greater than 100 μM; in cellular assays, NA is defined as IC$_{50}$ value greater than 20 μM). [b] NT, not tested. [c] When compounds were insoluble at a concentration of 100 μM, the percent inhibition at the highest concentration tested is shown. [d] In the purified enzyme assays, standard errors for the IC$_{50}$ determinations done at least in duplicate averaged 30% for rhCOX-2 and 10% for oCOX-1; In the cellular assays, standard errors for the interday IC$_{50}$ determinations done at least in duplicate averaged 45% for COX-1 and 30% for COX-2.

Table 3. In Vivo Activity of Thiadiazoles **6b·**choline, **7a**, and **25d** and Standards **1**, **2**, and Indomethacin[a]

compd	ED$_{40}$ (mg/Kg) CFE[b]	hyperalgesia[c]	lesion frequency (%)	damaged area (%)	ulcer index	PGE$_2$ inhibition (%)
6b·choline	7.1	NT	12.5	0.05	0.64	77.4 ± 3.75
7a	5.8[d]	0.2	87.5	4.91	430	87.7 ± 7.52
25d	7.7	1.3	12.5	0.02	0.25	22.0 ± 8.00
2	0.61	7.4	0	0	0	30.7 ± 12.1
1	>30	0.3	0	0	0	−1.58 ± 13.5
indomethacin	1.6	0.6	75	0.18	13.36	96.5 ± 0.470

[a] In GI safety studies, the rats were dosed at 100 mg/kg. NT, not tested. [b] Compounds dosed orally, 10 rats/test group. [c] Compounds dosed orally, 8 mice/test group. [d] The compound was tested as its choline salt.[15]

only against COX-2 (**25d**, **15b**,**c**). The lack of correlation between isolated enzyme assay and cellular assay results leads to the speculation that these compounds may possess other pharmacological activity besides inhibition of COX-1 and COX-2.

Within this chemical series compounds **6b**, **7a**, and **14** were identified as potent and selective COX-2 inhibitors with consistent data from both isolated enzyme and cellular assays. Compound **25d** was identified as a potent and selective COX-2 inhibitor based on cellular assays. The most potent and selective COX-1 inhibitors based on the isolated enzyme assay are **28a** and **25c**.

When compared to standard COX-2 inhibitors **1** and **2**, compounds **6b** and **7a** showed weak activity against COX-1 in the oCOX-1 enzyme assay with IC$_{50}$ values of 100 and 32 μM, respectively. In cellular assays, compound **6b** was less potent and selective against COX-2 than **2**, and compound **7a** was similar to **2** in terms of potency and selectivity. Activity toward inhibition of COX-1 activity was observed with **6b** (IC$_{50}$ = 3.1 μM), **7a** (IC$_{50}$ = 1.2 μM), and the standard selective COX-2 inhibitor **2** (IC$_{50}$ = 3.3 μM) in the cellular COX-1 assay using human platelet-rich plasma. Compound **25d**, similar to the standard selective COX-2 inhibitor **1**, was potent toward inhibition of COX-2 activity (IC$_{50}$ = 0.033 μM) and was inactive toward inhibition of COX-1 activity.

In Vivo Studies. The three compounds **6b**, **7a**, and **25d** were studied in vivo as the parent or their choline salt (Table 3). Compounds **6b·**choline (the choline salt of **6b**) and **7a** were orally active in CFE with ED$_{40}$ values of 7.1 and 5.8 mg/kg, respectively. Compound **7a** also provided potent analgesic activity (ED$_{40}$ = 0.2 mg/kg). Compound **25d** was also active in CFE (ED$_{40}$ = 7.7 mg/kg) even though it failed to inhibit either COX in the purified enzyme assays.

Compounds **6b·**choline, **7a**, and **25d** were further evaluated for their gastric toxicity and inhibition of PGE$_2$ synthesis in rat gastric mucosa. Results for these three compounds and two known selective COX-2 inhibitors are shown in 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 rat gastric mucosa by 31%, whereas a 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. At a dose of 100 mg/kg, compound **25d** that was inactive toward COX-1 activity in the cellular COX-1 assay caused only 22% inhibition of PGE$_2$ production which is about the same as that observed with **2**. As expected, compound **25d** caused minimal GI damage (ulcer index = 0.25). Compounds **6b·**choline and **7a** that had some activity against COX-1, especially in the cellular COX-1 assay, caused reduction in PGE$_2$ production at a level similar to that of indomethacin (77% for **6b·**choline and 88% for **7a**) in rat gastric mucosa following a dose of 100 mg/

kg. However, compounds **6b**·choline and **7a** had very different effects on gastric damage. Compound **7a** caused more GI damage in rats than indomethacin as indicated by the ulcer index (ulcer index = 430), but the GI damage detected with **6b**·choline was minimal (ulcer index = 0.64).

The results obtained with **25d** support the hypothesis that compounds which selectively inhibit COX-2 activity are antiinflammatory agents and cause less GI damage. The in vivo data obtained with **6b**·choline and **7a** suggest that, at least in the rat, the level of reduction in PGE_2 production is not the sole determinant of GI lesion formation, and other factors must also be involved in evolution of gastric damage. Additional pharmacological properties of the substituted di-*tert*-butylphenols besides the inhibition of COX isoforms or the pharmacological properties of their metabolites may contribute to these effects observed in vivo. For example, compound **6b**·choline or its metabolites could have other pharmacological properties that are beneficial in terms of GI lesions.

Conclusion

SAR studies of these 1,3,4- and 1,2,4-thiadiazole-derived 2,6-di-*tert*-butylphenol series have shown that both in vitro potency and selectivity of COX isoform inhibition are very sensitive to structural changes. Compound **6b** was identified as the most potent and selective COX-2 inhibitor in this series with consistent data from both isolated enzyme assays and cellular assays. Compound **25d** was potent and selective toward inhibition of COX-2 activity in cellular assays, even though it failed to inhibit either enzyme in the isolated enzyme assays. Both **6b**·choline and **25d** were orally active in an acute model of inflammation and caused minimal GI damage. In vivo studies with **25d** support the hypothesis that the antiinflammatory activity of NSAIDs results from inhibition of COX-2 and their GI toxicity from inhibition of COX-1. The in vivo data obtained with **6b**·choline suggest that additional pharmacological properties of this compound besides the inhibition of COX isoforms or the pharmacological properties of its metabolites may contribute to the GI safety profiles observed. Further improvements in potency and selectivity 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.[19]

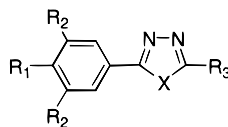
Purified Enzyme Assays. Mass screening of the Parke-Davis compound library was carried out using commercially obtained sheep placental COX-2 (oCOX-2; Cayman Chemical, Ann Arbor, MI). For the compounds included in the SAR studies, IC_{50} values against purified enzymes were determined using recombinant human COX-2 (rhCOX-2; purified from baculovirus infected SF-9 cells[20]) 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 a 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 30% for rhCOX-2 and 10% 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 either untreated or after acetylation with aspirin (Ac-J7) 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 cell preparation, 100-μL drug dilution). After a 60-min preincubation of cells and test compounds, samples were spiked with 3 μg of arachidonic acid and incubated 30 min, and the reaction was 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). Percent inhibition was determined using the formula: $\{1 - [(\text{drug} - \text{medium})/(\text{maximum} - \text{medium})]\} \times 100$, where “drug” is 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). Standard errors for interday determinations averaged 45% for COX-1 and 30% for COX-2.

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 21 for the details). A Student's *t*-test was done on each experimental group (compared to vehicle) to evaluate statistical significance.

Analgesia. Analgesic activity was measured by the acetic acid-induced writhing test as previously described.[22] 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.

Table 4. Physical Data for Thiadiazoles **6c**–**j**, **15a**–**d**, **16a**,**b**, **17**, and **20a**–**c**



compd	R_1	R_2	X	R_3	method	mp (°C)	formula	recryst solvent	yield (%)	analysis
6c	OH	*t*-Bu	S	SCH_2CF_3	A[a]	100–101	$C_{18}H_{23}F_3N_2OS_2$	pentane	35	C, H, N
6d	OH	*t*-Bu	S	S-*n*-Pr	A	107–108	$C_{19}H_{28}N_2OS_2$		89	C, H, N
6e	OH	*t*-Bu	S	S-*i*-Pr	A	93–95	$C_{19}H_{28}N_2OS_2$		97	C, H, N
6f	OH	*t*-Bu	S	$SCH_2CH_2CO_2Me$	A[b]	83–85	$C_{20}H_{28}N_2O_3S_2$		62	C, H, N
6g	OH	*t*-Bu	S	SBn	A	102–104	$C_{23}H_{28}N_2OS_2$		92	C, H, N
6h	OH	*t*-Bu	S	$SCH_2CH_2CO_2H$	A[c]	154–156	$C_{19}H_{26}N_2O_3S_2$	*tert*-butyl methyl ether	62	C, H, N
6i	OH	*t*-Bu	S	$SCH_2CH_2NH_2$	A	131–133	$C_{18}H_{27}N_3OS_2$		71	C, H, N
6j	OH	*t*-Bu	S	$SCH_2CH_2NEt_2$	A	85–88	$C_{22}H_{35}N_3OS_2$		52	C, H, N
15a	OH	*i*-Pr	S	SMe	A	143	$C_{15}H_{20}N_2OS_2$	toluene	67	C, H, N
15b	OH	*i*-Pr	S	SEt	A	124–125	$C_{16}H_{22}N_2OS_2$	toluene	76	C, H, N
15c	OH	*i*-Pr	S	S-*n*-Pr	A	74–75	$C_{17}H_{24}N_2OS_2$	heptane	81	C, H, N
15d	OH	*i*-Pr	S	S-*i*-Pr	A	152–153	$C_{17}H_{24}N_2OS_2$	toluene	82	C, H, N
16a	OH	*t*-Bu	O	SEt	A	125–126	$C_{18}H_{26}N_2O_2S$	hexanes	69	C, H, N
16b	OH	*t*-Bu	O	S-*n*-Pr	A	88–89	$C_{19}H_{28}N_2O_2S$	hexanes	63	C, H, N
17	OMe	*t*-Bu	S	SMe	A	85–88	$C_{18}H_{26}N_2OS_2$		83	C, H, N
20a	OH	*t*-Bu	S	OMe	B	130–132	$C_{17}H_{24}N_2O_2S$	Et_2O/hexanes	56	C, H, N
20b	OH	*t*-Bu	S	OEt	B	110–122	$C_{18}H_{26}N_2O_2S$	hexanes	62	C, H, N
20c	OH	*t*-Bu	S	O-*i*-Pr	B	100–103	$C_{19}H_{28}N_2O_2S$		67	C, H, N

[a] Reaction mixture was refluxed for 18 h. [b] The choline salt of **5** was used as the starting material; no base was used. [c] The choline salt of **5** was used as the starting material; triethylamine was used instead of NaOH.

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.

Method A. 2,6-Di-*tert*-butyl-4-(5-ethylsulfanyl[1,3,4]-thiadiazol-2-yl)phenol (6b). To a solution of **5**[12] (514 mg, 1.59 mmol) in THF (3 mL) cooled at 0 °C was added a solution of 1 N NaOH (1.69 mL) under a nitrogen atmosphere. After the mixture stirred for 15 min, ethyl iodide (140 μL, 1.75 mmol) was added. After stirring at 0 °C for additional 1 h, solvents were stripped off in vacuo affording a yellow solid. Recrystallization from CH_3CN/H_2O gave **6b** as pale-yellow crystals in 79% yield: mp 128–129 °C; IR 3578, 1407, 1378, 1241, 1226, 705 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 18H, 2 × *t*-Bu), 1.49 (t, 3H, *J* = 7.23 Hz, CH_3), 3.36 (q, 2H, *J* = 7.47 Hz, CH_2), 5.56 (s, 1H, OH), 7.70 (s, 2H, 2 × ArH); MS (CI) *m*/*z* 351 (MH^+). Anal. ($C_{18}H_{26}N_2O_1S_2$) C, H, N.

Using this method, compounds **6c**–**j**, **15a**–**d**, **16a**,**b**, and **17** were prepared. The physical data and chemical yields are summarized in Table 4.

2,6-Di-*tert*-butyl-4-(5-ethylsulfanyl[1,3,4]thiadiazol-2-yl)phenol Choline Salt (6b·choline). To a solution of **6b** (2.00 g, 5.71 mmol) in ethanol was added choline carbonate (80%, 1.00 mL, 5.71 mmol). The resulting reaction misture was refluxed for 1 h. After cooling to ambient temperature, the solution was concentrated in vacuo affording a yellow thick oil. Recrystallization twice from EtOAc/MeO-*t*-Bu and EtOAc gave 1.7 g (66%) of pure **6b·**choline as a yellow solid: mp 138–140 °C dec; IR 3436, 2948, 1586, 1394, 1368, 1353, 1221, 712 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.29 (s, 18H, 2 × *t*-Bu), 1.30 (t, 3H, *J* = 7.16 Hz, CH_2CH_3), 3.08 (s, 9H, $RN(CH_3)_3$), 3.12 (q, 2H, *J* = 7.32 Hz, CH_2CH_3), 3.35–3.38 (m, 2H, $HOCH_2CH_2N$), 3.78–3.84 (m, 2H, $HOCH_2CH_2N$), 5.35 (bs, 1H, OH), 7.15 (s, 2H, 2 × ArH); MS (CI) *m*/*z* 351 (MH^+). Anal. ($C_{18}H_{25}N_2O_1S_2$·$C_5H_{14}NO$) C, H, N.

(4-Hydroxy-3,5-diisopropylthiobenzoylsulfanyl)-acetic Acid Ethyl Ester (10). Sodium hydride (60% in mineral oil, 4.40 g, 110 mmol) was washed with pentane (2 × 20 mL) and then mixed with 50 mL of DMF. After the mixture stirred at ambient temperature for 5 min under nitrogen atmosphere, 2,4-diisopropylphenol (18.5 mL, 99.8 mmol) was added dropwise (exothermic reaction and gas evolution). The reaction mixture was stirred at ambient temperature for 15 min; then carbon disulfide (6.00 mL, 102 mL) was added dropwise. The resulting reaction mixture was stirred at 75 °C for 30 min; then ethyl chloroacetate (11.0 mL, 103 mmol) was added at 75 °C. After the addition, the reaction mixture was allowed to cool to ambient temperature and was stirred for 16 h at which time TLC showed that no starting material was left. A mixture of 150 mL of EtOAc and 200 mL of water was added, and the resulting mixture was stirred for 10 min. Two layers were separated, the aqueous layer was extracted with EtOAc (2 × 100 mL), and the combined organic solution was washed with water (2 × 300 mL) and brine (1 × 300 mL) and dried over Na_2SO_4. Concentration in vacuo afforded a viscous oil which upon standing overnight at 4 °C solidified to an orange solid. Trituration with petroleum ether/2-propanol (99:1) gave 21.8 g (64%) of crude product (**10**). A small amount of product was recrystallized from *tert*-butyl methyl ether/hexanes to give pure compound **10**: mp 88–89 °C; IR 3395, 2969, 1719, 1588, 1292, 1192, 1157, 697 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29–1.33 (m, 15H, 2 × $CH(CH_3)_2$ and CH_2CH_3), 3.15 (septet, 2H, *J* = 6.75 Hz, 2 × $CH(CH_3)_2$), 4.21 (s, 2H, CH_2), 4.24 (q, 2H, *J* = 6.99 Hz, CH_2CH_3), 5.27 (s, 1H, OH), 7.26 (s, 2H, 2 × H_{arom}); MS (APCI) *m*/*z* 341.2 (MH^+). Anal. ($C_{17}H_{24}O_3S_2$) C, H.

4-Hydroxy-3,5-diisopropylthiobenzoic Acid Hydrazide (11). A solution of **10** (5.71 g, 16.8 mmol) in 50 mL of ethanol at ambient temperature was treated with hydrazine hydrate (1.60 mL, approximately 27.5 mmol). The reaction solution was stirred for 1.5 h at which time TLC showed the reaction was complete. Water (80 mL) was added, and the resulting suspension was stirred briskly for 15 min. Filtration and water washing followed by air-drying gave 4.11 g (97%) of **11** as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.11 (d, 12H, *J* = 6.84 Hz, 2 × $CH(CH_3)_2$), 3.24 (septet, 2H, *J* = 6.84 Hz, 2 × $CH(CH_3)_2$),

7.40 (s, 2H, 2 × ArH). The material was used in the next step without further purification.

2,6-Diisopropyl-4-(5-mercapto[1,3,4]thiadiazol-2-yl)-phenol (12). To a solution of **11** (4.11 g, 16.3 mmol) in 20 mL of ethanol was added carbon disulfide (2.34 mL, 40.0 mmol). The resulting reaction mixture was refluxed for 1.5 h at which time TLC showed no starting material left. The reaction mixture was cooled to ambient temperature and concentrated in vacuo affording a solid. Recrystallization from toluene gave 3.1 g (65%) of **12** as white fibrous needles: mp 220−221 °C; IR 3430, 3099, 1479, 1306, 1065, 724 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.18 (d, 12H, *J* = 6.75 Hz, 2 × CH(C*H*$_3$)$_2$), 3.32 (septet, 2H, *J* = 6.75 Hz, 2 × C*H*(CH$_3$)$_2$), 3.34 (s, 2H, CH$_2$), 7.31 (s, 2H, 2 × H$_{arom}$), 8.97 (s, 1H, exchangeable proton), 14.54 (s, 1H, exchangeable proton); MS (CI) *m*/*z* 295 (MH$^+$). Anal. ($C_{14}H_{18}N_2O_1S_2$) C, H, N.

2,6-Di-*tert*-butyl-4-(5-ethylsulfinyl[1,3,4]thiadiazol-2-yl)phenol (18a). A solution of **6b** (3.1 g, 8.8 mmol) in 55 mL of CH_2Cl_2 was treated with MCPBA (3.5 g, approximately 9.6 mmol) in one charge at ambient temperature under nitrogen atmosphere. The resulting yellow-amber solution was stirred for 16 h at which time the reaction solution had become very cloudy. The reaction mixture was cooled in an ice−salt bath and filtered; the solids were washed with cold CH_2Cl_2. TLC showed two very well separated spots (ether:hexanes = 3:2). The filtrate was concentrated in vacuo, and the residue was purified by flash chromatography (elution with CH_2Cl_2−1% CH_3OH in CH_2Cl_2). The product with smaller R_f value was isolated. Recrystallization from *tert*-butyl methyl ether/pentane gave 0.81 g (25%) of **18a**: mp 134−135 °C; IR 3520, 2961, 1599, 1385, 1240, 1227, 1028, 704 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.39 (t, 3H, *J* = 7.48 Hz, CH$_3$), 1.49 (s, 18H, 2 × *t*-Bu), 3.21−3.40 (m, 2H, CH$_2$), 5.68 (s, 1H, OH), 7.79 (s, 2H, ArH); MS (CI) *m*/*z* 367 (MH$^+$). Anal. ($C_{18}H_{26}N_2O_2S_2$) C, H, N.

Method B. 2,6-Di-*tert*-butyl-4-(5-propoxy[1,3,4]thiadiazol-2-yl)phenol (20d). Sodium metal (1.4 g, 61 mmol) was dissolved in warm anhydrous 1-propanol (30 mL). To this freshly made solution of sodium 1-propoxide in 1-propanol was added **19**[13] (2.3 g, 6.1 mmol) forming a dark-red solution. After stirring under nitrogen atmosphere at reflux for 3 min, TLC (30% EtOAc in hexanes as eluant) indicated that the starting material had been completely consumed. The reaction solution was cooled to ambient temperature and mixed with brine solution and the pH adjusted to 3 with concentrated aqueous HCl solution. The mixture was extracted with CH_2Cl_2 (3 × 40 mL); the combined organic phase was dried over Na_2SO_4 and then concentrated in vacuo. The crude product was further purified by flash chromatography (20% EtOAc in hexanes as eluant) to gave **20d** as a brown solid in 84% yield: mp 93−95 °C; IR 3587, 1502, 1230, 1146, 957, 705 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.05 (t, 3H, *J* = 7.47 Hz, CH$_3$), 1.47 (s, 18H, 2 × *t*-Bu), 1.88 (h, 2H, *J* = 6.75 Hz, CH$_2$), 4.50 (t, 2H, *J* = 6.51 Hz, CH$_2$), 5.51 (s, 1H, OH), 7.62 (s, 2H, 2 × H$_{arom}$); MS (CI) *m*/*z* 349 (MH$^+$). Anal. ($C_{19}H_{28}N_2O_2S$) C, H, N.

Using method B, compounds **20a**−**c** were prepared. The physical data and chemical yields are summarized in Table 4.

3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-4*H*-[1,2,4]thiadiazol-5-one (22). To a mixture of *O*-methylhydroxylamine hydrochloride (75.5 mg, 0.904 mmol) and potassium *tert*-butoxide in 15 mL of dry *tert*-butyl alcohol was added compound **21**[14] (134 mg, 0.301 mmol). The resulting reaction mixture was refluxed for 5 min. The deep-red color disappeared, and a white cloudy solution was obtained. The reaction mixture was diluted with 10 mL of CH_2Cl_2, washed with brine (2 × 20 mL), dried over Na_2SO_4, and concentrated in vacuo. The solid was triturated with Et_2O/hexanes to gave 63 mg (68%) of **22** as a white solid: mp 251−252 °C; IR 3611, 3588, 1669, 1427, 1243, 792 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.49 (s, 18H, 2 × *t*-Bu), 5.63 (s, 1H, OH), 7.71 (s, 2H, 2 × H$_{arom}$), 11.4 (s, 1H, NH); MS (CI) *m*/*z* 307 (MH$^+$). Anal. ($C_{16}H_{22}N_2O_2S$) C, H, N.

***N*-[3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-[1,2,4]thiadiazol-5-yl]acetamide (24a).** A mixture of **23**[14] (0.40 g, 1.3 mmol) and acetic anhydride (1.3 mL, 1.4 g, 13.8 mmol) in 18 mL of toluene was heated at reflux for 16 h. The precipitated product was filtered and washed with water. Recrystallization of the crude product from aqueous ethanol gave 0.15 g (33%) of **24a**: mp 313−315 °C; IR 3628, 1698, 1662, 1544, 1315 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.43 (s, 18H, *t*-Bu), 2.25 (s, 3H, Me), 7.41 (s, 1 H, OH), 7.99 (s, 2 H, ArH), 13.0 (br s, 1 H, NH); EIMS *m*/*z* 348 (M$^+$). Anal. ($C_{18}H_{25}N_3O_2S$) C, H, N.

***N*-[3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-[1,2,4]thiadiazol-5-yl]methanesulfonamide (24b).** A solution of **23**[14] (0.50 g, 1.6 mmol) in 3 mL of pyridine was cooled in ice and treated dropwise with methanesulfonyl chloride (0.25 mL, 0.37 g, 3.2 mmol). The mixture was stirred at ice-bath temperature for 45 min and then for 16 h at room temperature. An additional 0.45 mL (0.67 g, 5.8 mmol) of methanesulfonyl chloride was added dropwise, and the mixture was stirred for 4 h. The mixture was evaporated, and the residue was dissolved in water. The solution was acidified with 1.0 N HCl, and the precipitated product was filtered and washed with water. Purification of the crude product by flash chromatography (elution with 50% ethyl acetate in hexanes) followed by recrystallization from aqueous ethanol gave 0.20 g (32%) of **24b**: mp 222−224 °C; IR 3616, 1605, 1559, 1289, 1121 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.41 (s, 18H, *t*-Bu), 3.06 (s, 3H, Me), 7.67 (s, 1H, OH), 7.79 (s, 2H, ArH); EIMS *m*/*z* 384 (M$^+$). Anal. ($C_{17}H_{25}N_3O_3S_2$) C, H, N.

[3-[3,5-Bis(1,1-dimethylethyl)-4-[(methoxyethoxy)-methoxy]phenyl]-1,2,4-oxadiazol-5-yl]cyanamide (32). 1,8-Diazabicyclo[5.4.0]undec-7-ene (0.64 g, 4.2 mmol) was added to a stirred solution of **31**[15] (1.0 g, 2.8 mmol) in 30 mL of dimethoxyethane under an inert atmosphere at room temperature and was followed after 5 min by diphenyl cyanocarbonimidate (1.0 g, 4.2 mmol). After 4 h the solvent was removed by rotary evaporator and the residue dissolved in 100 mL of ethyl acetate. The solution was washed with 100 mL of 1 N HCl and then 100 mL of saturated brine and then dried over $MgSO_4$. The solvent was removed under reduced pressure to leave a waxy solid residue which was triturated in cold *tert*-butyl methyl ether to afford 0.9 g (79%) of **32**: mp 167−168 °C; IR (KBr) 2219, 1659 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.42 (s, 18H, CH$_3$), 3.28 (s, 3H, OCH$_3$), 3.55 (m, 2H, CH$_2$), 3.89 (m, 2H, CH$_2$), 4.97 (s, 2H, CH$_2$), 7.76 (s, 2H, aromatic); MS (+APCI) *m*/*z* 403 (MH$^+$). Anal. ($C_{21}H_{30}N_4O_4$) C, H, N.

[3-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-oxadiazol-5-yl]cyanamide (8). 30% HBr in acetic acid (18 mL) was added to a round-bottom flask with **32** (6.0 g, 15 mmol) and stirred at room temperature. After 1 h the solution was poured into 500 mL of ice water, and stirred for 20 min. The precipitate was filtered off, rinsed three times with water, and dried. Recrystallization from acetonitrile afforded 2.2 g (47%) of the crude product. Recrystallization of a sample from acetonitrile afforded pure **8**: mp 275 °C dec; IR 3617, 2960, 2217, 2203, 1669 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.41 (s, 18H, CH$_3$), 7.62 (s, 2H, ArH), 7.81 (bs, 1H, OH); MS (+APCI) *m*/*z* 315 (MH$^+$). Anal. ($C_{17}H_{22}N_4O_2$) C, H, N.

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