Brief Articles

Imidazole−Dioxolane Compounds as Isozyme-Selective Heme Oxygenase Inhibitors

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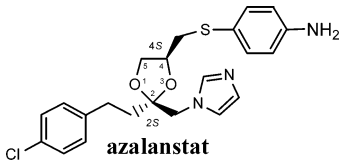
Several imidazole−dioxolane compounds were synthesized and evaluated as novel inhibitors of heme oxygenase (HO). These compounds, which include (2*R*,4*R*)-2-[2-(4-chlorophenyl)ethyl]-2-[(1*H*-imidazol-1-yl)methyl]-4-methyl-1,3-dioxolane (**1**) hydrochloride, are structurally distinct from metalloporphyrin HO inhibitors and lack the aminothiophenol moiety of azalanstat. They were found to be highly selective for the HO-1 isozyme (stress induced) and had substantially less inhibitory potency toward HO-2, the constitutive isozyme. These imidazole−dioxolane compounds are the first of their type known to exhibit this isozyme-selective HO inhibition.

Introduction

Heme oxygenase (HO) isozymes are involved in the biotransformation of heme into biliverdin, releasing ferrous iron (Fe^{2+}) and carbon monoxide (CO) in the process (Figure 1).[1,2] This reaction is the main source of CO in mammals. HO-1 (also known as HSP-32) is the inducible form of the enzyme, and is a stress protein induced by a number of stimuli including heat shock, heme, heavy metals, and reactive oxygen species. HO-2, the constitutive form of the enzyme, is the most prevalent form in most tissues, except the spleen where HO-1 levels are predominant.[3] Although the role of HO in heme catabolism is quite well understood, the physiological role of the CO produced in this process is receiving much attention, as CO is increasingly becoming recognized as a cellular regulator with actions in brain, blood vessels, and immune systems. The availability of drugs that can be used to investigate the CO/HO system has been limited largely to metalloporphyrins such as tin protoporphyrin (SnPP).[4,5] Because of the close structural similarity between heme and the metalloporphyrin HO inhibitors, specificity becomes a problem. In several systems proteins bind heme and employ it for functions such as regulation of enzyme activity {for example, of soluble guanylyl cyclase (sGC)} or as the key component of the active site of an enzyme {for example, nitric oxide synthase (NOS), cytochromes P450 (CYP)}. Accordingly, the use of metalloporphyrins to ascribe various physiological roles to the CO/HO system has been the subject of some criticism because they have been shown to affect the activity of both NOS and sGC.[6,7] Thus, a program in our laboratories is concerned with the design of HO inhibitors that are not based on the porphyrin nucleus and has the objective of obtaining more selective HO inhibitors.

In a previous publication,[8] we described the synthesis and HO inhibitory activity of a series of imidazole−dioxolanes that arose from the lead compound azalanstat.[9] Our initial approach was to perform appropriate synthetic modifications to the structure of azalanstat and develop imidazole-based analogues having enhanced inhibitory potency for HO over other heme-dependent enzymes (such as sGC and NOS), in addition to isozyme-dependent HO selectivity. These aminothiophenol-containing azalanstat analogues were observed to be excellent inhibitors of HO, and most of them showed significantly more potency as inhibitors of the inducible isoform of HO (HO-1) than the constitutive isoform (HO-2). Here we describe the extension of our synthetic program to include a methyl-terminated series of imidazole−dioxolane compounds (**1**−**4**) which were evaluated for their ability to inhibit HO-1 and HO-2. Our results show that these imidazole−dioxolane compounds are much more isozyme-selective than the previously studied azalanstat analogues,[8] as they are excellent inhibitors of HO-1 and virtually devoid of the ability to inhibit HO-2. These features might result in useful therapeutic applications; for example, Fang et al.[10] have described the therapeutic potential of HO-1 inhibition, by targeting HO-1 in tumors, to induce tumor cell apoptosis.



azalanstat

Synthesis. In an effort to evaluate the importance that certain structural features of azalanstat have on the inhibition of HO, we chose to focus initially on the modification of the aminothiophenol moiety. By simply replacing the aminothiophenol functionality by a hydrogen atom, a methyl-terminated series of compounds (**1**−**4**) were synthesized and evaluated for their ability to inhibit HO-1 and HO-2. Further modifications involved the synthesis of compound **5**, which was devoid of the methyl group in the 4-position of the dioxolane ring, and of its dithiolane analogue **6**; the new compounds were investigated for inhibitory activity against HO. The diastereomeric tosylates **7**−**10** were prepared as previously reported.[8] As shown in

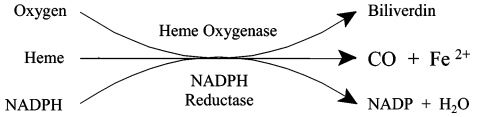


Figure 1. The oxidative degradation of heme in the CO/HO pathway.

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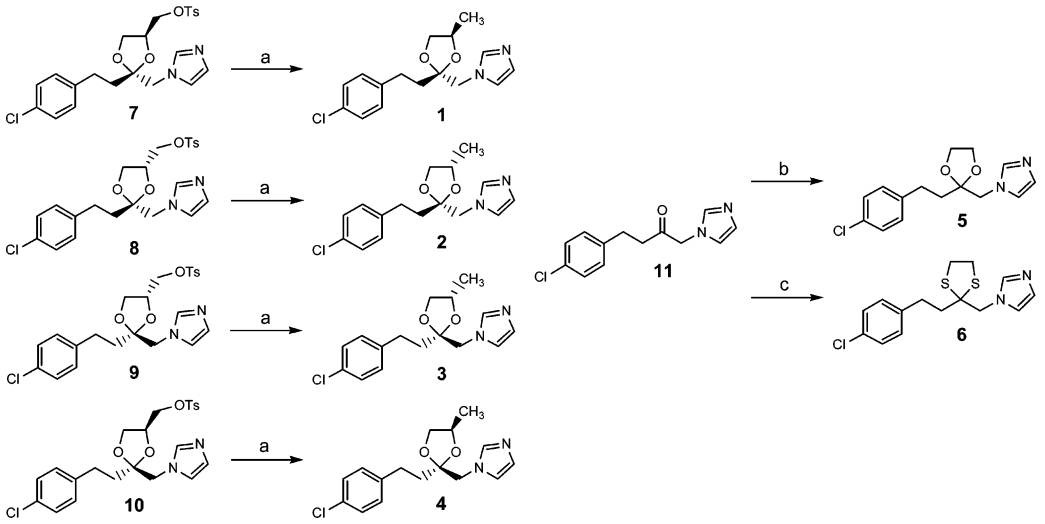
Scheme 1[a]



[a] Reagents and conditions: (a) $LiAlH_4$, THF, reflux; (b) *p*-TsOH·H_2O, ethylene glycol, toluene, reflux; (c) *p*-TsOH·H_2O, 1,2-ethanedithiol, toluene, reflux.

Table 1. Inhibitory Potency and Selectivity of Imidazole−Dioxolane/Dithiolane Compounds against the Activity of Various Enzymes[a]

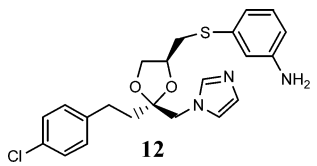
compd	IC_{50} (μM) HO-1 (rat spleen)	IC_{50} (μM) HO-2 (rat brain)	selectivity index rat IC_{50} (HO-2)/ IC_{50} (HO-1)	IC_{50} (μM) nNOS (rat brain)	IC_{50} (μM) sGC (rat lung)	IC_{50} (μM) CYP3A1/3A2 (rat liver)	IC_{50} (μM) CYP2E1 (rat liver)
1	0.8 ± 0.2	305 ± 25	381	362 ± 1	>1000	3.2 ± 0.8	3.6 ± 0.5
2	2.6 ± 0.4	>100	>38	773 ± 426	>500	8 ± 2	6.0 ± 0.1
3	12 ± 4	>100	>8	>500	>500	10 ± 5	20 ± 2
4	20 ± 4	>100	>5	>500	>500	7 ± 3	30 ± 6
5	4 ± 2	>100	>25	182 ± 119	>1000	4 ± 1	5 ± 1
6	4.7 ± 0.6	16 ± 4	3.4	34 ± 12	>500	5.9 ± 0.1	14 ± 6
12	1.0 ± 0.2	35 ± 6	35				
azalanstat	6 ± 1	28 ± 18	4.7				

[a] Assays were performed as indicated in the Experimental Section. Data represent mean IC_{50} values ± standard deviation of replicate experiments. All imidazole−dioxolane compounds were evaluated as HCl salts.

Scheme 1, the methyl-terminated compounds **1**, **2**, **3**, and **4** were obtained by the reduction of tosylates **7**, **8**, **9**, and **10**, respectively, using lithium aluminum hydride in THF.[11] Since the reduction was performed on only one diastereomeric tosylate, only one methyl-terminated diastereomer was produced, thus avoiding the production of a mixture of all four diastereomers which would have resulted by acid-catalyzed acetalation of 4-(4-chlorophenyl)-1-(1*H*-imidazol-1-yl)-2-butanone (**11**) using racemic 1,2-propanediol. The 1,3-dioxolane compound **5** was prepared from ketone **11** by an acid-catalyzed acetalation reaction in toluene using ethylene glycol, according to a procedure similar to that reported by Walker et al.[12] The corresponding 1,3-dithiolane derivative **6** was also prepared in this manner from **11** using 1,2-ethanedithiol. All of the final compounds were isolated/characterized and evaluated as the hydrochloride salts of the structures shown.

Biological Evaluation. The compounds in Table 1 were screened for evidence of their ability to inhibit HO-1 and HO-2 using an in vitro assay for HO in which heme was presented to the enzyme complexed with albumin as described in the Experimental Section. HO-1 was obtained from rat spleen and HO-2 was obtained from rat brain as the microsomal fractions prepared by differential centrifugation; the dominance of HO-1 and HO-2 proteins in the rat spleen and brain, respectively, has been documented.[3,13−15] These particular microsomal preparations were selected in order to use the most native (i.e., closest to in vivo) forms of HO, and hence in order that they would be most relevant to anticipated whole animal studies. Purer forms of HO have been obtained by processes that render the enzymes membrane free; however, this changes the milieu for enzyme activity. Each of the methyl-terminated compounds (**1**−**4**) were potent inhibitors of HO-1, while showing very little activity for HO-2 (Table 1). The results are in contrast to the results obtained in our earlier study[8] in that none of the present compounds were potent inhibitors of HO-2. The selectivity index of these compounds for HO-1 is given in the fourth column.

In contrast to metalloporphyrins such as **SnPP** which show modest selectivity toward HO-2,[16] the methyl-terminated compounds show HO-1 selectivity. The selectivity indexes of compounds **1** and **2** (and presumably **3**−**5**) are also much higher than with the previously studied[8] azalanstat analogues containing the aminothiophenol moiety. As reported in that study, the highest index, namely ∼35, was observed for compound **12**.



Thus, at least two members of the present methyl-terminated series of compounds exhibit enhanced inhibitory selectivity for HO-1 over HO-2 and are in fact the first compounds known to exhibit this essentially isozyme-specific inhibition over a very favorable concentration range. For instance, a representative activity−inhibitor concentration curve showing the difference in potency toward HO-1 (rat spleen) and HO-2 (rat brain) activity exhibited by compound **1** is shown in Figure 2. The

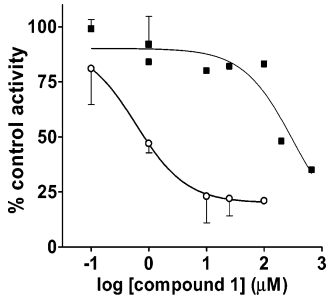


Figure 2. Representative activity−inhibitor concentration curves for the inhibition of HO-1 and HO-2 by **1**. Enzyme activities were determined as described in the Experimental Section. All of the values of activity (ordinate) are expressed as a percentage of the control with no inhibitor present. The values on the abscissa represent the log of the inhibitor concentration in μM. Open circles ○, HO-1 (rat spleen microsomes) and filled squares ■, HO-2 (rat brain microsomes). Inhibitor concentrations that inhibited activity by 50% (IC_{50}) were determined from such nonlinear regression plots (sigmoidal dose−response curve) using GraphPad Prism version 3 and reported as an average ± standard deviation of replicate experiments.

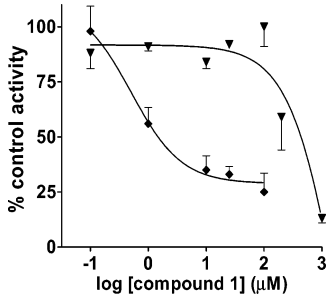


Figure 3. Representative activity−inhibitor concentration curves for the inhibition of HO-1 (human spleen) and HO-2 (rat liver) by **1**. Enzyme activities were determined as described in the Experimental Section. Filled diamonds ◆, HO-1 (human spleen microsomes) and filled triangles ▼, HO-2 (rat liver microsomes).

IC_{50} was 0.8 ± 0.2 μM for HO-1 (rat spleen) and approximately 305 ± 25 μM for HO-2 (rat brain), with a selectivity index ~381. Similar selectivity for HO-1 was observed also for **1** when comparing inhibition of HO isolated from other sources. For example, **1** also showed strong potency toward the activity of HO-1 isolated from human spleen (IC_{50} = 1.5 ± 0.4 μM), while inhibition of HO-2 (predominantly) isolated from rat liver (IC_{50} 532 ± 135 μM) required much higher inhibitor concentrations (Figure 3).

The selectivity between HO-1/HO-2 noticed for **1** was observed also in diastereomers **2**−**4**. As with the aminothiophenol-containing azalanstat series of HO inhibitors studied earlier,[8] the potency and selectivity of these methyl-terminated compounds (**1**−**4**) do appear to be dependent on the diastereomeric configuration of the dioxolane core (which determines 3-D molecular shape). All of the other three diastereomers of **1** were synthesized and evaluated as inhibitors of HO. It is the (2*R*, 4*R*) isomer **1** in the methyl-terminated series that is the most potent toward HO-1 (IC_{50} = 0.8 ± 0.2 μM); this compound is directly comparable to the (2*R*, 4*S*) azalanstat analogues with respect to 3-dimensional shape, compounds which also are the most potent against HO-1 (IC_{50} in the 0.5−2.5 μM range).[8] These methyl-terminated compounds have low potency toward HO-2 (>100 μM); selectivity of these compounds approximately parallels HO-1 inhibitory potency, the order of which is presumably **1** > **2** > **3** > **4**.

Since HO inhibition potency appeared to be related to the orientation of the methyl group, the compound lacking the methyl group, namely **5,** was synthesized and evaluated. Interestingly, compound **5** also showed good potency and selectivity toward inhibition of HO-1 (IC_{50} = 4 ± 2 μM) over HO-2 (IC_{50} > 100 μM). In the case of the dithiolane analogue **6**, the HO-1 inhibitory potency (IC_{50} = 4.7 ± 0.6 μM) was slightly less than that of **5** and there was observed substantially increased potency toward HO-2 (IC_{50} = 16 ± 4 μM), resulting in a drastic reduction in selectivity.

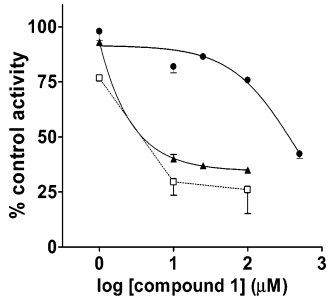


Figure 4. Representative activity−inhibitor concentration curves for the inhibition of various enzymes by **1**. Enzyme activities were determined as described in the Experimental Section. Open squares □, CYP2E1 (rat liver microsomes), filled triangles ▲, CYP3A1/3A2 (rat liver microsomes) and filled circles ●, nNOS (rat brain microsomes).

Previous work on azalanstat and its structural analogues showed potent inhibition of both HO-1 and HO-2 which appeared to be dependent on the position of the amino group on the thiophenol moiety as well as the configurations of the diastereomeric compounds.[8] However, the aminothiophenol moiety is not an absolute requirement for HO-inhibition since from the present study it appears that its removal enhances the selectivity for HO-1 inhibition (compounds **1**−**5**). The aminothiophenol group (or other group in the analogous position) may be an important factor for recognition in the HO-2 system, a fact to keep in mind when designing inhibitors selective for HO-2.

Inhibition of Other Enzymes. Compounds **1** (apparently as a mixture of diastereomers) and **5** have been described in a patent[17] which purports that derivatives of substituted *N*-alkylimidazoles could be useful as anticonvulsant agents. Also, the closely related (2-aryl-4-phenylthioalkyl-1,3-dioxolan-2-ylmethyl)azole derivatives are indicated[18] to be useful as antifungal and antineoplastic agents, presumably owing to the inhibition of lanosterol 14α-demethylase. Thus, we also chose to address the issue of selectivity with respect to other enzymes, in particular, hemoproteins. Since a major drawback of metalloporphyrins is their ability to inhibit nNOS and sGC, selectivity of the imidazole−dioxolane compounds for HO over other heme-dependent enzymes such as nNOS, sGC and cytochromes P450[19] (CYP) was studied also.

With the exception of **6**, the candidate compounds in Table 1 were found to be poor inhibitors of nNOS (with IC_{50} > 400 μM) and all of the compounds had virtually no effect on sGC activity up to 1000 μM. These results are in contrast to those of the metalloporphyrin HO inhibitors, which also interact with the NOS and sGC systems. However, the candidate compounds are potent inhibitors of CYP3A1/3A2 and CYP2E1 (Table 1) obtained from rat liver. For instance, as exemplified for **1** in Figure 4, potent inhibition of CYP3A1/3A2 (IC_{50} = 3.2 ± 0.8 μM) and CYP2E1 (IC_{50} = 3.6 ± 0.5 μM) was observed along with only a weak inhibitory effect on nNOS (IC_{50} = 362 ± 1 μM). The observed P450 inhibition is of significance since cytochromes P450 are responsible for the metabolism of many drugs used in therapy and as experimental tools. Such drugs could be used in conjunction with our novel agents.

Conclusion

We have synthesized several compounds that express excellent selectivity as inhibitors of HO-1 relative to HO-2. In particular, compound **1** is noteworthy for its high potency and selectivity toward HO-1; compound **5**, although not as potent, is prepared relatively easily. As HO-1 is the isozyme that has attracted the most research interest, these compounds are anticipated to become very useful tools in elucidating the physiological roles of HO-1 and carbon monoxide in mammalian and other biological systems. These novel compounds might also have useful therapeutic applications, as indicated earlier, for example, by the studies of Fang et al.[10]

Experimental Section

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer in CD_3OD or D_2O. The signals owing to residual protons in the deuterated solvents were used as internal standards. Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane.[20] Carbon chemical shifts are given relative to CD_3OD: $\delta = 49.00$. High-resolution electrospray mass spectra were recorded on an Applied Biosystems/MDS Sciex QSTAR XL spectrometer with an Agilent HP1100 Cap-LC system. Samples were run in 50% aqueous MeOH at a flow rate of 6 μL/min. Elemental analyses were performed by MHW Laboratories (Phoenix, AZ). Melting points were determined on a Mel-Temp II melting point apparatus and are uncorrected. Optical rotations were measured using an Autopol II automatic polarimeter for solutions in a 1-dm cell at room temperature. Thin-layer chromatography was performed using glass- or aluminum-backed Silica Gel 60 F_{254} plates (Silicycle, Quebec City, Quebec, Canada). Plates were viewed under UV light or by charring after spraying with phosphomolybdic acid (PMA) in EtOH.

Representative procedure for the reduction of tosylates using lithium aluminum hydride to afford **1**−**4**:

(2*R*,4*R*)-2-[2-(4-Chlorophenyl)ethyl]-2-[(1*H*-imidazol-1-yl)-methyl]-4-methyl-1,3-dioxolane Hydrochloride (1·HCl). Under a N_2 atmosphere, a solution of the tosylate **7**[8] (200 mg, 0.42 mmol) in THF (2 mL) was cooled to 0 °C, and a suspension of $LiAlH_4$ (31 mg, 0.83 mmol) in THF (2 mL) was added; the mixture was heated at reflux temperature for 4 h. The mixture was cooled to 0 °C, diluted with Et_2O, and then carefully quenched with wet Et_2O. After dilution with H_2O, the mixture was extracted twice with Et_2O. The combined extracts were washed sequentially with a saturated aqueous solution of Na_2CO_3 and water, dried ($MgSO_4$), and concentrated to a yellow oil. Purification by preparative scale (1-cm thick) thin-layer chromatography (EtOAc) gave 73 mg (0.24 mmol, 57%) of the free base which was dissolved in hot 2-propanol (2 mL), and the solution was treated with a solution of 37% aqueous HCl (40 mg, 0.41 mmol) in 2-propanol (1 mL). The mixture was concentrated and dried under high vacuum. The residue was recrystallized (2-propanol−Et_2O), and the solid was removed by filtration and washed with Et_2O. High-vacuum-drying afforded 85 mg (0.25 mmol, 59%) of **1** as a white solid: mp 172−173 °C; $R_f = 0.24$ (EtOAc); $[\alpha]_D^{22} = -10.2°$ ($c = 2.17$, D_2O); ^{1}H NMR (400 MHz, D_2O): δ 1.24 (d, $J = 6.0$ Hz, 3 H), 1.93−2.08 (m, 2 H), 2.73 (t, $J = 8.2$ Hz, 2 H), 3.51 (t, $J = 8.6$ Hz, 1 H), 3.69−3.78 (m, 1 H), 4.10 (dd, $J = 8.0$, 6.0 Hz, 1 H), 4.43 (d, $J = 2.4$ Hz, 2 H), 7.23 (d, $J = 8.4$ Hz, 2 H), 7.34 (d, $J = 8.4$ Hz, 2 H), 7.49−7.52 (m, 2 H), 8.76 (s, 1 H); ^{13}C NMR (100 MHz, D_2O): δ 17.0, 28.3, 37.5, 54.0, 71.9, 74.7, 108.4, 119.7, 123.7, 128.8, 130.2, 131.5, 136.0, 140.2; HRMS (ES) $[M + H]^+$ Calcd. for $C_{16}H_{20}ClN_2O_2$: 307.1207. Found: 307.1193.

Characterization of the new compounds (**2**−**4**) synthesized following the representative procedure for the reduction of tosylates (shown above for **1**) as outlined in Scheme 1:

(2*R*,4*S*)-2-[2-(4-Chlorophenyl)ethyl]-2-[(1*H*-imidazol-1-yl)-methyl]-4-methyl-1,3-dioxolane Hydrochloride (2·HCl). Beige solid in 64% yield from **8**[8]: mp 148−149 °C; $R_f = 0.21$ (EtOAc); $[\alpha]_D^{22} = +15.1°$ ($c = 1.19$, CD_3OD); ^{1}H NMR (400 MHz, CD_3OD): δ 1.04 (d, $J = 6.0$ Hz, 3 H), 1.95−2.05 (m, 2 H), 2.70−2.85 (m, 3 H), 4.08 (dd, $J = 8.0$, 6.0 Hz, 1 H), 4.26−4.35 (m, 1 H), 4.49 (s, 2 H), 7.22 (d, $J = 8.4$ Hz, 2 H), 7.28 (d, $J = 8.4$ Hz, 2 H), 7.58 (s, 1 H), 7.62 (s, 1 H), 8.96 (s, 1 H); ^{13}C NMR (100 MHz, CD_3OD): δ 18.0, 30.1, 39.2, 54.7, 73.0, 74.7, 109.2, 120.2, 125.4, 129.6, 130.9, 132.8, 137.9, 141.5; HRMS (ES) $[M + H]^+$ Calcd. for $C_{16}H_{20}ClN_2O_2$: 307.1207. Found: 307.1203.

(2*S*,4*S*)-2-[2-(4-Chlorophenyl)ethyl]-2-[(1*H*-imidazol-1-yl)methyl]-4-methyl-1,3-dioxolane Hydrochloride (3·HCl). White solid in 38% yield from **9**[8]: mp 172−173 °C; $R_f = 0.18$ (EtOAc); $[\alpha]_D^{22} = +15.7°$ ($c = 1.65$, D_2O); ^{1}H NMR (400 MHz, D_2O): δ 1.22 (d, $J = 6.0$ Hz, 3 H), 1.92−2.08 (m, 2 H), 2.72 (t, $J = 8.4$ Hz, 2 H), 3.50 (t, $J = 8.6$ Hz, 1 H), 3.67−3.75 (m, 1 H), 4.08 (dd, $J = 8.0$, 6.0 Hz, 1 H), 4.41 (d, $J = 2.0$ Hz, 2 H), 7.22 (d, $J = 8.4$ Hz, 2 H), 7.33 (d, $J = 8.4$ Hz, 2 H), 7.48 (s, 1 H), 7.50 (s, 1 H), 8.74 (s, 1 H); ^{13}C NMR (100 MHz, D_2O): δ 16.9, 28.2, 37.5, 54.0, 71.9, 74.6, 108.4, 119.7, 123.7, 128.8, 130.2, 131.5, 136.0, 140.2; HRMS (ES) $[M + H]^+$ Calcd. for $C_{16}H_{20}ClN_2O_2$: 307.1207. Found: 307.1204.

(2*S*,4*R*)-2-[2-(4-Chlorophenyl)ethyl]-2-[(1*H*-imidazol-1-yl)-methyl]-4-methyl-1,3-dioxolane Hydrochloride (4·HCl). White solid in 51% yield from **10**[8]: mp 152−153 °C; $R_f = 0.21$ (EtOAc); $[\alpha]_D^{22} = -21.0°$ ($c = 1.33$, D_2O); ^{1}H NMR (400 MHz, D_2O): δ 0.97 (d, $J = 6.0$ Hz, 3 H), 1.88−1.98 (m, 2 H), 2.64 (t, $J = 8.2$ Hz, 2 H), 2.73 (t, $J = 8.4$ Hz, 1 H), 4.02 (t, $J = 7.2$ Hz, 1 H), 4.19−4.27 (m, 1 H), 4.37 (s, 2 H), 7.15 (d, $J = 8.4$ Hz, 2 H), 7.25 (d, $J = 8.0$ Hz, 2 H), 7.43 (s, 1 H), 7.44 (s, 1 H), 8.70 (s, 1 H); ^{13}C NMR (100 MHz, D_2O): δ 16.8, 28.5, 37.6, 53.7, 71.8, 74.1, 108.3, 119.5, 123.9, 128.7, 130.1, 131.4, 136.1, 140.2; HRMS (ES) $[M + H]^+$ Calcd. for $C_{16}H_{20}ClN_2O_2$: 307.1207. Found: 307.1207.

2-[2-(4-Chlorophenyl)ethyl]-2-[(1*H*-imidazol-1-yl)methyl]-1,3-dioxolane Hydrochloride (5·HCl). Under a N_2 atmosphere, a mixture of the ketone **11**[8] (400 mg, 1.61 mmol) and *p*-toluenesulfonic acid monohydrate (536 mg, 2.82 mmol, 1.75 eq) in toluene (12 mL) was heated at reflux temperature (Dean−Stark apparatus) for 1.5 h; a solution of ethylene glycol (156 mg, 2.51 mmol, 1.56 eq) in toluene (4 mL) was added and the mixture was heated at reflux temperature for an additional 8.5 h. The mixture was cooled to room temperature and diluted with H_2O, and the mixture was extracted twice with EtOAc. The combined extracts were washed sequentially with aqueous Na_2CO_3 solution and water, dried ($MgSO_4$), and concentrated to a dark brown oil. Purification by flash chromatography on silica gel (EtOAc and then acetone) gave 242 mg (0.83 mmol, 52%) of the free base which was dissolved in hot EtOH (2 mL) and treated with a solution of 37% aqueous HCl (100 mg, 1.02 mmol) in EtOH (1 mL). The mixture was concentrated and dried under high vacuum. Hot EtOAc (10 mL) was added, followed by a minimum amount of hot EtOH to produce a solution. The solution was cooled in the freezer, hexanes (5 mL) added, and the product allowed to crystallize overnight. The solid was removed by filtration and washed with hexanes. High-vacuum-drying afforded 125 mg (0.38 mmol, 24%) of **5** as a white solid: mp 168−169 °C; $R_f = 0.17$ (EtOAc); ^{1}H NMR (400 MHz, D_2O): δ 1.94−2.02 (m, 2 H), 2.64−2.72 (m, 2 H), 3.58−3.68 (m, 2 H), 3.92−4.02 (m, 2 H), 4.41 (s, 2 H), 7.18 (d, $J = 8.0$ Hz, 2 H), 7.29 (d, $J = 8.0$ Hz, 2 H), 7.47 (s, 2 H), 8.72 (s, 1 H); ^{13}C NMR (100 MHz, D_2O): δ 28.3, 37.1, 53.7, 66.1, 108.2, 119.7, 123.7, 128.8, 130.1, 131.4, 136.1, 140.3; HRMS (ES) $[M + H]^+$ Calcd. for $C_{15}H_{18}ClN_2O_2$: 293.1051. Found: 293.1040.

2-[2-(4-Chlorophenyl)ethyl]-2-[(1*H*-imidazol-1-yl)methyl]-1,3-dithiolane Hydrochloride (6·HCl). The title compound was synthesized from ketone **11**[8] by the procedure employed for the synthesis of **5**, except using 1,2-ethanedithiol instead of ethylene glycol, to afford a beige solid in 32% yield after recrystallization (2-propanol): mp 204−205 °C; $R_f = 0.21$ (EtOAc); ^{1}H NMR (400 MHz, CD_3OD): δ 2.17−2.25 (m, 2 H), 2.94−3.06 (m, 4 H), 3.28−3.38 (m, 2 H), 4.65 (s, 2 H), 7.21 (d, $J = 8.4$ Hz, 2 H), 7.28 (d, $J = 8.4$ Hz, 2 H), 7.56 (s, 1 H), 7.81 (s, 1 H), 9.11 (s, 1 H); ^{13}C NMR (100 MHz, D_2O): δ 32.6, 41.6, 43.4, 59.8, 71.2, 119.9, 125.5,

129.6, 131.1, 133.0, 138.2, 141.3; HRMS (ES) $[M + H]^+$ Calcd. for $C_{15}H_{18}ClN_2S_2$: 325.0600. Found: 325.0587.

In Vitro HO Activity Assay. HO activity in rat spleen and brain microsomal fractions was determined by the quantitation of CO formed from the degradation of methemalbumin (heme complexed with albumin).[21,22] Using spleen and brain tissue from Sprague−Dawley rats, microsomal fractions were prepared according to the procedure outlined by Appleton et al.[23] Protein concentration of microsomal fractions was determined by a modification of the Biuret method.[22] Incubations for HO activity analysis were done under conditions for which the rate of CO formation (pmol CO min^{-1} mg $protein^{-1}$) was linear with respect to time and microsomal protein concentration. Briefly, reaction mixtures (150 μL) consisting of 100 mM phosphate buffer (pH 7.4), 50 μM methemalbumin, and 1 mg/mL protein were preincubated with the imidazole−dioxolane compounds (as HCl salts) at final concentrations ranging from 0.1 to 100 μM for 10 min at 37 °C. Reactions were initiated by adding NADPH at a final concentration of 1 mM, and incubations were performed for an additional 15 min at 37 °C. Blanks for which NADPH was omitted were subtracted to correct for CO that might have formed by a route other than HO. Reactions were stopped by instantly freezing the reaction mixture on dry ice, and CO formation was monitored by gas chromatography according to the method described by Vreman and Stevenson.[21]

In Vitro nNOS Assay. The activity of nNOS was measured by monitoring the conversion of ^{14}C-L-arginine into ^{14}C-L-citrulline in rat brain cytosolic fractions according to a modification of previously outlined procedures,[24,25] as described by Kinobe et al.[15]

In Vitro sGC Assay. The activity of sGC in rat lung cytosolic fractions was measured by monitoring the formation of cGMP using a competitive enzyme-linked immunosorbent assay (ELISA), as described by Kinobe et al.[15]

In Vitro CYP3A1/3A2 and CYP2E1 Assay. CYP3A1/3A2-catalyzed erythromycin N-demethylase activity in rat liver microsomal fractions was determined by the spectrophotometric measurement of formaldehyde, whereas the hydroxylation of *p*-nitrophenol by CYP2E1 was determined by the spectrophotometric measurement of 4-nitrocatechol, as described by Kinobe et al.[15]

Analysis of Enzyme Inhibition. Inhibitions of the catalytic activities of HO, nNOS, sGC, CYP2E1, and CYP3A1/3A2 by the imidazole−dioxolane compounds (as HCl salts) studied herein were evaluated by the percentage of control activity of each enzyme remaining in the presence of different concentrations of inhibitors with reference to control reactions. Replicate experiments were performed to generate multiple (two or three) activity−inhibitor concentration curves for each imidazole−dioxolane compound tested. For each curve, an IC_{50} value (inhibitor concentration that decreased enzyme activity by 50%) was calculated from the equation generated from a nonlinear regression plot of the data using a sigmoidal dose−response curve (GraphPad Prism version 3). The IC_{50} values in Table 1 are presented as the mean ± standard deviation from replicate experiments.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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