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Heme Oxygenase Inhibition by α-(1*H*-Imidazol-1-yl)-ω-phenylalkanes: Effect of Introduction of Heteroatoms in the Alkyl Linker

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Several α-(1*H*-imidazol-1-yl)-ω-phenylalkanes were synthesized and evaluated as novel inhibitors of heme oxygenase (HO). These compounds were found to be potent and selective for the stress-induced isozyme HO-1, showing mostly weak activity toward the constitutive isozyme HO-2. The introduction of an oxygen atom in the alkyl linker produced analogues with decreased potency toward HO-1, whereas the presence of a sulfur atom in the linker gave rise to analogues with greater potency toward HO-1 than the carbon-containing analogues. The most potent compounds studied contained a five-atom linker between the imidazolyl and phenyl moieties, whereas the most HO-1-selective compounds contained a four-atom linker between these groups. The compounds with a five-atom linker containing a heteroatom (O or S) were found to be the most potent inhibitors of HO-2; 1-(*N*-benzylamino)-3-(1*H*-imidazol-1-yl)propane dihydrochloride, with a nitrogen atom in the linker, was found to be inactive.

Introduction

A program in our research group is focused on the design and synthesis of selective heme oxygenase (HO) inhibitors. In particular, we are interested in compounds that inhibit the two active isozymes of HO, namely, HO-1 (inducible) and HO-2 (constitutive), especially regarding the role of these HO inhibitors in biology and medicine.[1–4] Notably, inhibitors of HO have been studied for the treatment of hyperbilirubinemia,[5–7] neurodegenerative disorders,[8] certain types of cancer,[9,10] and bacterial and fungal infections;[11] HO inhibitors also provide useful tools for the elucidation of the physiological roles of heme oxygenases. We have identified a number of potent and selective compounds for the inhibition of HO-1.[12–16] The key functional groups of these compounds include an azolyl moiety, a phenyl group, and an alkyl linkage between these. Successful compounds identified to date incorporate a dioxolanyl, a hydroxy, or a carbonyl group in the linker. Herein we describe the effect of introducing heteroatoms in the alkyl linker of α-(1*H*-imidazol-1-yl)-ω-phenylalkanes on heme oxygenase inhibition.

Results and Discussion

Synthesis

The structures of the compounds studied in this work are shown in Table 1. Compounds **1** and **2** were obtained simply by treatment of 1-phenyl-1*H*-imidazole and 1-benzyl-1*H*-imidazole, respectively, with hydrochloric acid in ethanol. The general approach for the synthesis of (phenylalkyl)imidazoles **3**–**6**, (phenoxyalkyl)imidazoles **7**, **8**, and **10**, and {(phenylsulfanyl)alkyl}imidazoles **11**, **12**, and **14** is summarized in Table 2. Compound **3** was obtained by treatment of (2-bromoethyl)benzene with imidazole and potassium carbonate in THF. Compound **4** was obtained by treatment of 1-bromo-3-phenylpropane with imidazole and sodium hydroxide in DMSO. Analogously, compounds **5** and **6** were obtained by treatment of 4-phenylbutyl toluene-4-sulfonate and 5-phenylpentyl toluene-4-sulfonate, respectively, with imidazole and sodium hydroxide in DMSO. The α-(1*H*-imidazol-1-yl)-ω-phenoxyalkanes **7**, **8**, and **10** were obtained by treatment of the corresponding α-bromo-ω-phenoxyalkane with imidazole (in either NaH–THF or NaOH–DMSO), followed by hydrochloride salt formation. The α-(1*H*-imidazol-1-yl)-ω-(phenylsulfanyl)alkanes **11**, **12**, and **14** were obtained by treatment of the corresponding α-bromo-ω-(phenylsulfanyl)alkane with imidazole–NaH–THF, followed by hydrochloride salt formation. 2-(Benzyloxy)-1-(1*H*-imidazol-1-yl)-ethane (**9**) was commercially available. Compound **13** was obtained by treatment of 1-(2-chloroethyl)imidazole with preformed sodium benzylthiolate in ethanol. The secondary amine **15** was prepared by treatment of 1-(3-aminopropyl)imidazole with benzaldehyde in methanol, followed by reduction of the resultant imine with sodium borohydride, and finally dihydrochloride salt formation.

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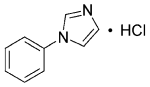
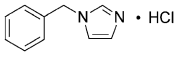
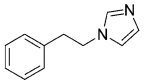
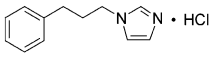
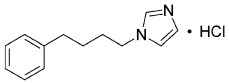
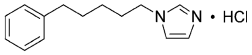
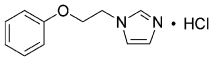
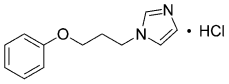
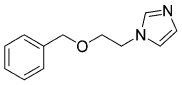
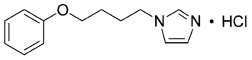
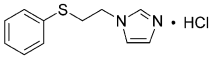
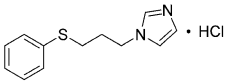
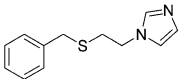
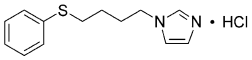
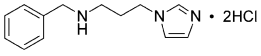
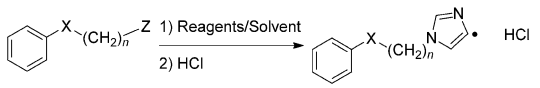
Table 1. Inhibitory potency and selectivity of candidate compounds against HO-1 and HO-2 activity.

Compd	Structure	IC_{50} [μM][a] HO-1 (rat spleen)	HO-2 (rat brain)	SI[b]
1		inactive	inactive	–
2		44 ± 6	> 100	> 2.3
3		72 ± 8	> 100	> 1.4
4		14 ± 3	> 100	> 7.1
5		3.5 ± 0.7	> 100	> 28.6
6		2.8 ± 0.3	20 ± 11	7.1
7		61 ± 20	> 100	> 1.6
8		42 ± 9	> 100	> 2.4
9		32 ± 1	> 100	> 3.1
10		4 ± 1	4.6 ± 0.4	1.2
11		6.0 ± 0.1	> 100	> 16.7
12		2.4 ± 0.1	16 ± 2	6.7
13		4.4 ± 0.7	> 100	> 22.7
14		1.2 ± 0.1	5 ± 2	4.2
15		inactive	inactive	–

[a] Data represent mean values ± SEM of replicate ($n = 4$) experiments. [b] Selectivity index: $IC_{50\,(HO\text{-}2)}/IC_{50\,(HO\text{-}1)}$.

Table 2. General approach for the synthesis of (phenylalkyl)imidazoles **3**–**6**, (phenoxyalkyl)imidazoles **7**, **8**, and **10**, and {(phenylsulfanyl)alkyl}imidazoles **11**, **12**, and **14**.



X	*n*	Z	Reagents/Solvent	Compd
CH_2	1	Br	imidazole, K_2CO_3, THF	**3**[a]
CH_2	2	Br	imidazole, NaOH, DMSO	**4**
CH_2	3	OTs	imidazole, NaOH, DMSO	**5**
CH_2	4	OTs	imidazole, NaOH, DMSO	**6**
O	2	Br	imidazole, NaH, THF	**7**
O	3	Br	imidazole, NaOH, DMSO	**8**
O	4	Br	imidazole, NaOH, DMSO	**10**
S	2	Br	imidazole, NaH, THF	**11**
S	3	Br	imidazole, NaH, THF	**12**
S	4	Br	imidazole, NaH, THF	**14**

[a] Compound **3** was evaluated as the free base.

Biological evaluation

The compounds listed in Table 1 were tested as inhibitors of HO-1 (rat spleen microsomal fraction) and HO-2 (rat brain microsomal fraction) using an in vitro assay for heme oxygenase. Compounds **2**–**6** contain an α-(imidazol-1-yl) moiety, an ω-phenyl moiety, and an alkyl linkage between these. The introduction of oxygen, sulfur, or nitrogen into the alkyl linkage afforded compounds **7**–**10**, **11**–**14**, and **15**, respectively.

The effect of alkyl chain length on HO activity was addressed by the study of compounds **2**–**6**. Increasing the alkyl chain length increased potency in HO-1 inhibition. For example, the methylene and ethylene analogues (compounds **2** and **3**) were found to be weak inhibitors of HO-1, whereas the propylene, butylene, and pentylene analogues (compounds **4**, **5**, and **6**) progressively showed increased inhibitory potency, with pentylene analogue **6** being the most potent ($IC_{50} = 2.8 \pm 0.3$ μM). With respect to HO-2, notable inhibition was not observed in this series until compound **6**, with an alkyl chain length of five carbon units, was examined ($IC_{50} = 20 \pm 11$ μM). The butylene analogue **5** was observed to be the most selective for HO-1 over HO-2. Compound **1**, in which the phenyl and imidazolyl groups are joined directly together, is essentially inactive toward both HO-1 and HO-2.

The effect of introducing oxygen in the alkyl chain on HO activity was determined by the study of compounds **7**–**10**. In general, replacement of a methylene unit with an oxygen atom led to a decrease in HO-1 inhibitory potency. Specifically, analogue **7** ($IC_{50} = 61 \pm 20$ μM) was fourfold less potent than compound **4** ($IC_{50} = 14 \pm 3$ μM). Similarly, the two oxygen-containing structural analogues of compound **5** ($IC_{50} = 3.5 \pm 0.7$ μM), namely compounds **8** ($IC_{50} = 42 \pm 9$ μM) and **9** ($IC_{50} = 32 \pm 1$ μM), were less potent by factors of 12 and 9, respectively. The direct oxygen-containing analogue of **6** ($IC_{50} = 2.8 \pm 0.3$ μM), namely compound **10** ($IC_{50} = 4 \pm 1$ μM), was also less potent with respect to HO-1 inhibition. In accordance with the results obtained in the alkyl series **2**–**6**, significant HO-2 inhibition in the ether series of compounds **7**–**10** was observed only for the compound with a five-atom spacer, namely compound **10**, although this compound is essentially unselective for HO-1 ($IC_{50} = 4 \pm 1$ μM) over HO-2 ($IC_{50} = 4.6 \pm 0.4$ μM).

The effect of introducing sulfur into the alkyl chain on HO activity was determined by the study of compounds **11**–**14**. In contrast to the oxygen-containing compounds, the sulfur-containing compounds (apart from **13**) are more potent HO-1 inhibitors than their carbon analogues. For instance, the thioether **11** ($IC_{50} = 6.0 \pm 0.1$ μM) was twofold more potent than the corresponding propylene compound **4** ($IC_{50} = 14 \pm 3$ μM). The sulfur-containing analogue of **5** ($IC_{50} = 3.5 \pm 0.7$ μM), namely

compound **12** ($IC_{50} = 2.4 \pm 0.1$ μM), was also slightly more active against HO-1, whereas the structural analogue **13** ($IC_{50} = 4.4 \pm 0.7$ μM) was only slightly less active against HO-1 than **5**. The sulfur-containing analogue **14** ($IC_{50} = 1.2 \pm 0.1$ μM) was approximately twice as potent toward HO-1 than the directly comparable alkyl compound **6** ($IC_{50} = 2.8 \pm 0.3$ μM). Regarding HO-2 inhibition, the series of sulfur-containing compounds did show significant activity in the cases of the four- and five-atom linker lengths, and this attribute could be a result of the larger size of the sulfur atom; compounds **12** ($IC_{50} = 16 \pm 2$ μM) and **14** ($IC_{50} = 5 \pm 2$ μM) are both potent HO-2 inhibitors. In general, the compounds with a five-atom linker length are the most potent HO-2 inhibitors (compounds **6**, **14**, and **10**); potency increased in the order $CH_2 < S < O$. Thus, the introduction of heteroatoms in the alkyl linkage may play an important role in the design of HO-2 inhibitors.

Only one nitrogen-containing analogue was studied (compound **15**) owing largely to the fact that it was inactive against both HO-1 and HO-2, even though it contains a spacer length deemed to be desirable as observed in the CH_2, O, and S series of compounds; no other nitrogen-containing analogues were pursued.

Conclusions

The incorporation of a heteroatom into the alkyl chain between the α-(imidazol-1-yl) moiety and the ω-phenyl group imparts dramatic effects on HO activity. Potency in HO-1 inhibition increased in the order $O < CH_2 < S$, and this trend was observed in each of the following three direct comparisons of HO-1 inhibitor potency: **7** < **4** < **11**, **8** < **5** < **12**, and **10** < **6** < **14** (see Table 1). In each of the three series of compounds studied (the CH_2, O, and S series), the compound with the longest spacer in each series, namely compounds **6**, **10**, and **14**, were observed to be the most potent HO-1 and HO-2 inhibitor in that series; however, they were not the most selective HO-1 inhibitors. The data suggest that the most selective HO-1 inhibitor in each respective series contains a four-atom linker between the α-(imidazol-1-yl) moiety and the ω-phenyl group, as observed for compounds **5**, **9**, and **13**. Thus, further modifications to increase the length of the linkage (beyond five atoms) may generate more potent HO-1 and HO-2 inhibitors, although it is doubtful that pronounced HO-1 selectivity will be attained through this approach.

Experimental Section

Chemistry

Materials and methods: 1-Bromo-2-phenoxyethane, 1-bromo-3-phenoxypropane, 1-bromo-4-phenoxybutane, and 1-bromo-2-(phenylsulfanyl)ethane were obtained from Alfa Aesar (Ward Hill, MA, USA). 1-Bromo-3-(phenylsulfanyl)propane and 1-bromo-4-(phenylsulfanyl)butane were prepared as previously reported.[17] 5-Phenylpentyl toluene-4-sulfonate and 4-phenylbutyl toluene-4-sulfonate were prepared by following previously reported procedures.[18] *N*-(2-Chloroethyl)imidazole hydrochloride was obtained from Oakwood Products Inc. (West Columbia, SC, USA). 1-Phenyl-1*H*-imidazole, 1-benzyl-1*H*-imidazole, benzylthiol, 1-(3-aminopropyl)imidazole, 2-(benzyloxy)-1-(1*H*-imidazol-1-yl)ethane (**9**) and all other reagents were obtained from Sigma–Aldrich (Oakville, ON, Canada). Flash column chromatography was performed on Silicycle silica gel (230–400 mesh, 60 Å). Analytical thin-layer chromatography was performed on glass-backed pre-coated Silica Gel 60 F_{254} plates (Silicycle), and the compounds were visualized either by UV illumination (λ 254 nm), or by heating after spraying with phosphomolybdic acid in ethanol. Melting points were determined on a Mel-Temp II apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer in $CDCl_3$ or CD_3OD. The chemical shifts (δ) are reported in ppm relative to tetramethylsilane using the residual solvent peak as an internal reference.[19] High-resolution ESI mass spectra were recorded on an Applied Biosystems/MDS Sciex QSTAR XL mass spectrometer with an Agilent HP1100 Cap-LC system. Samples were run in 50% aqueous methanol at a flow rate of 6 μL min^{-1}. High-resolution EI mass spectra were recorded on a Waters/Micromass GC-ToF instrument.

1-Phenyl-1*H*-imidazole hydrochloride (1): To a solution of 1-phenyl-1*H*-imidazole (310 mg, 2.15 mmol, 1 equiv) in EtOH (2 mL) was added 37% aqueous HCl (262 mg, 2.66 mmol, 1.2 equiv) in EtOH (2 mL) and the mixture was concentrated. High-vacuum drying gave compound **1** as a white solid (380 mg, 98%); mp: 127–128 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.56$–7.69 (m, 3H), 7.71–7.81 (m, 2H), 7.80 (t, $J = 1.6$ Hz, 1H), 8.11 (t, $J = 1.6$ Hz, 1H), 9.52 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 121.9$, 122.7, 123.6, 131.3, 131.5, 135.7, 136.5 ppm; HRMS-ESI: *m/z* $[M-Cl]^+$ calcd for $C_9H_8N_2$: 144.0687, found: 144.0682.

(1*H*-Imidazol-1-yl)phenylmethane hydrochloride (2): To a solution of 1-benzyl-1*H*-imidazole (396 mg, 2.50 mmol, 1 equiv) in EtOH (2 mL) was added 37% aqueous HCl (494 mg, 5.01 mmol, 2 equiv) in EtOH (2 mL) and the mixture was concentrated. High-vacuum drying gave compound **2** as a hygroscopic white solid (423 mg, 2.17 mmol, 87%); mp: 134–135 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta =$ 5.48 (s, 2H), 7.39–7.46 (m, 5H), 7.59 (t, $J = 1.6$ Hz, 1H), 7.65 (t, $J =$ 1.6 Hz, 1H), 9.10 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 53.9$, 121.4, 123.3, 129.6, 130.3, 130.4, 135.5, 136.5 ppm; HRMS-EI: *m/z* $[M-HCl]^+$ calcd for $C_{10}H_{10}N_2$: 158.0844, found: 158.0846.

1-(1*H*-Imidazol-1-yl)-2-phenylethane (3): Under an atmosphere of N_2, a mixture of imidazole (788 mg, 11.57 mmol, 2.1 equiv) and K_2CO_3 (370 mg, 2.68 mmol, 1 equiv) in dry THF (18 mL) was stirred at RT for 10 min. To this mixture was added a solution of (2-bromoethyl)benzene (1.00 g, 5.40 mmol, 1 equiv) in THF (1 mL), and the mixture was heated at reflux for 14 h. The mixture was filtered, and the filtrate was concentrated to afford a clear oil. The oil was dissolved in CH_2Cl_2, and the solution was washed with H_2O (2×). The CH_2Cl_2 layer was then extracted with dilute aqueous HCl (3×). The aqueous extract was then neutralized with solid $NaHCO_3$, and the free base extracted using CH_2Cl_2 (3×). The combined organic extracts were dried (Na_2SO_4) and concentrated. High-vacuum drying gave compound **3** as a clear oil (420 mg, 2.44 mmol, 45%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.05$ (t, $J = 7.0$ Hz, 2H), 4.17 (t, $J =$ 7.0 Hz, 2H), 6.83 (s, 1H), 7.01–7.09 (m, 3H), 7.22–7.34 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 38.0$, 48.6, 118.9, 127.1, 128.7, 128.9, 129.6, 137.2, 137.6 ppm; HRMS-EI: *m/z* $[M]^+$ calcd for $C_{11}H_{12}N_2$: 172.1000, found: 172.0999.

1-(1*H*-Imidazol-1-yl)-3-phenylpropane hydrochloride (4): Under an atmosphere of N_2, a mixture of imidazole (376 mg, 5.52 mmol, 1.1 equiv) and NaOH (221 mg, 5.52 mmol, 1.1 equiv) in DMSO (2 mL) was heated at 70–80 °C with stirring for 1.5 h. To this mixture was added a solution of 1-bromo-3-phenylpropane (1.00 g,

5.02 mmol, 1 equiv) in DMSO (2 mL), and the mixture heated at 70–80 °C with stirring for 13 h. The temperature was slightly elevated, and the DMSO was removed by blowing a stream of air over the reaction mixture. High-vacuum drying left a yellow residue. After dilution with H_2O, the mixture was extracted with benzene (3×50 mL) and the combined organic extracts were washed with brine (2×), dried ($MgSO_4$), and concentrated to give the free base (914 mg, 4.91 mmol, 98%). To a solution of this free base in hot EtOH (3 mL) was added a solution of 37% aqueous HCl (500 mg, 5.08 mmol, 1 equiv) in EtOH (2 mL). The warm mixture was filtered through a syringe filter (0.45 μm), and the filtrate was concentrated and dried under high vacuum. The residue was recrystallized from 2-propanol/Et_2O to give compound **4** as a white solid (1.05 g, 4.71 mmol, 94%); mp: 95–96 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 2.25 (p, 2H), 2.70 (t, J = 7.6 Hz, 2H), 4.29 (t, J = 7.4 Hz, 2H), 7.16–7.22 (m, 3H), 7.22–7.30 (m, 2H), 7.55 (s, 1H), 7.67 (s, 1H), 8.95 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 32.6, 33.4, 50.2, 121.1, 123.3, 127.4, 129.4, 129.6, 136.4, 141.5 ppm; HRMS-ESI: m/z $[M-Cl]^+$ calcd for $C_{12}H_{15}N_2$: 187.1235, found: 187.1242.

1-(1*H*-Imidazol-1-yl)-4-phenylbutane hydrochloride (5): Compound **5** was prepared by a procedure analogous to that used to form **6** below, except that 4-phenylbutyl toluene-4-sulfonate was used instead of 5-phenylpentyl toluene-4-sulfonate, to give the product as a hygroscopic white solid (632 mg, 2.67 mmol, 89%); mp: 88–90 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 1.60–1.71 (m, 2H), 1.87–1.98 (m, 2H), 2.68 (t, J = 7.6 Hz, 2H), 4.28 (t, J = 7.6 Hz, 2H), 7.13–7.21 (m, 3H), 7.22–7.29 (m, 2H), 7.56 (t, J = 1.6 Hz, 1H), 7.65 (t, J = 1.6 Hz, 1H), 8.97 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 29.1, 30.7, 36.0, 50.4, 121.1, 123.3, 127.0, 129.4, 129.5, 136.3, 142.9 ppm; HRMS-ESI: m/z $[M-Cl]^+$ calcd for $C_{13}H_{17}N_2$: 201.1391, found: 201.1390.

1-(1*H*-Imidazol-1-yl)-5-phenylpentane hydrochloride (6): Under an atmosphere of N_2, a mixture of imidazole (612 mg, 9 mmol, 3 equiv) and NaOH (360 mg, 9 mmol, 3 equiv) in DMSO (4 mL) was heated at 70–80 °C with stirring for 1 h. A solution of 5-phenylpentyl toluene-4-sulfonate (954 mg, 3 mmol, 1 equiv) in DMSO (3 mL) was added, and the reaction mixture was stirred at 70–80 °C overnight. The reaction mixture was partitioned between H_2O (100 mL) and EtOAc (30 mL), the aqueous phase was further extracted with EtOAc (20 mL), and then the combined organic phase was washed with H_2O (2×50 mL), dried (Na_2SO_4) and concentrated. The resulting clear oil (626 mg, 2.92 mmol) was dissolved in Et_2O and treated with an excess of ethereal HCl. The solid that separated was collected by filtration and recrystallized from 2-propanol/Et_2O to give compound **6** as a white solid (557 mg, 2.22 mmol, 74%); mp: 86–87 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 1.29–1.41 (m, 2H), 1.63–1.74 (m, 2H), 1.87–1.98 (m, 2H), 2.63 (t, J = 7.4 Hz, 2H), 4.25 (t, J = 7.4 Hz, 2H), 7.10–7.19 (m, 3H), 7.20–7.27 (m, 2H), 7.56 (t, J = 1.6 Hz, 1H), 7.64 (t, J = 1.6 Hz, 1H), 8.95 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 26.6, 31.0, 31.8, 36.5, 50.5, 121.1, 123.3, 126.8, 129.3, 129.4, 136.2, 143.4 ppm; HRMS-ESI: m/z $[M-Cl]^+$ calcd for $C_{14}H_{19}N_2$: 215.1548, found: 215.1545.

1-(1*H*-Imidazol-1-yl)-2-phenoxyethane hydrochloride (7): To a solution of imidazole (1.00 g, 14.70 mmol) in dry THF (20 mL) under an atmosphere of N_2 was added NaH (360 mg, 15.65 mmol), and the mixture was stirred at RT for 1 h. 1-Bromo-2-phenoxyethane (2.00 g, 9.95 mmol) in dry THF (20 mL) was added, and the mixture was stirred at RT overnight. The solvent was removed, 5% aqueous NaOH (50 mL) was added to the residue, and the mixture was extracted with CH_2Cl_2 (2×50 mL) to give a yellow oil. Purification by flash column chromatography on silica gel (EtOAc/hexanes 1:3 *v/v* as eluent) afforded 1-(1*H*-imidazol-1-yl)-2-phenoxyethane (699 mg, 3.71 mmol, 37%). The free base was treated with 37% aqueous HCl (1 equiv) in 2-propanol, and the mixture was concentrated to afford compound **7** as a white solid (488 mg, 2.17 mmol, 22%); R_f = 0.18 (EtOAc/MeOH, 4:1); mp: 85–88 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 4.38 (t, J = 4.8 Hz, 2H), 4.69 (t, J = 4.8 Hz, 2H), 6.95–7.01 (m, 3H), 7.21–7.26 (m, 2H), 7.59 (t, J = 1.6 Hz, 1H), 7.76 (t, J = 1.6 Hz, 1H), 9.08 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 50.2, 67.1, 115.6, 121.0, 122.7, 123.8, 130.6, 137.0, 159.3 ppm; HRMS-EI: m/z $[M-HCl]^+$ calcd for $C_{11}H_{12}N_2O$: 188.0950, found: 188.0958.

General procedure for the formation of (phenoxyalkyl)imidazoles: Under an atmosphere of N_2, a mixture of imidazole (1.00 g, 14.70 mmol, 3 equiv) and NaOH (588 mg, 14.70 mmol, 3 equiv) in DMSO (5 mL) was stirred at 75 °C for 1 h. A solution of the α-bromo-ω-phenoxyalkane (4.90 mmol, 1 equiv) in DMSO (3 mL) was added, and the mixture was stirred at 75–100 °C for 24 h. The solvent was removed, 5% aqueous NaOH (50 mL) was added to the residue, and the mixture was extracted with EtOAc (2×25 mL). The combined organic phase was dried (Na_2SO_4) and concentrated to give a yellow oil. Purification by flash column chromatography on silica gel (EtOAc/hexanes 3:1 *v/v* as eluent) afforded the corresponding α-(1*H*-imidazol-1-yl)-ω-phenoxyalkane. The free base was treated with 37% aqueous HCl (1 equiv) in 2-propanol, and the mixture was concentrated to afford the product.

1-(1*H*-Imidazol-1-yl)-3-phenoxypropane hydrochloride (8): Compound **8** was prepared from 1-bromo-3-phenoxypropane (4.90 mmol, 1 equiv) following the general procedure for the formation of (phenoxyalkyl)imidazoles to afford compound **8** as a white solid (571 mg, 2.39 mmol, 49%); R_f = 0.20 (EtOAc/MeOH, 4:1); mp: 123–124 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 2.34–2.43 (m, 2H), 4.05 (t, J = 5.6 Hz, 2H), 4.51 (t, J = 7.2 Hz, 2H), 6.84–7.96 (m, 3H), 7.21–7.30 (m, 2H), 7.58 (s, 1H), 7.70 (s, 1H), 9.02 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 30.7, 48.2, 65.5, 115.4, 121.1, 122.1, 123.5, 130.5, 136.6, 159.8 ppm; HRMS-EI: m/z $[M-HCl]^+$ calcd for $C_{12}H_{14}N_2O$: 202.1106, found: 202.1109.

1-(1*H*-Imidazol-1-yl)-4-phenoxybutane hydrochloride (10): Compound **10** was prepared from 1-bromo-4-phenoxybutane (4.90 mmol, 1 equiv) following the general procedure for the formation of (phenoxyalkyl)imidazoles to afford the product as a white solid (517 mg, 2.05 mmol, 42%); R_f = 0.18 (EtOAc/MeOH, 4:1); mp: 99–100 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 1.77–1.87 (m, 2H), 2.04–2.16 (m, 2H), 4.02 (t, J = 6.0 Hz, 2H), 4.36 (t, J = 7.2 Hz, 2H), 6.87–6.94 (m, 3H), 7.21–7.29 (m, 2H), 7.58 (t, J = 1.6 Hz, 1H), 7.71 (t, J = 1.6 Hz, 1H), 9.04 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 27.0, 28.2, 50.4, 68.0, 115.5, 121.1, 121.8, 123.3, 130.5, 136.3, 160.2 ppm; HRMS-EI: m/z $[M-HCl]^+$ calcd for $C_{13}H_{16}N_2O$: 216.1263, found: 216.1263.

General procedure for the formation of {(phenylsulfanyl)alkyl}imidazoles: To a mixture of imidazole (3 equiv) in dry THF (10 mL g^{-1} imidazole) under an atmosphere of N_2 was added NaH (2.3 equiv), and the mixture was stirred at RT for 1 h. The corresponding α-bromo-ω-(phenylsulfanyl)alkane (1 equiv) in dry THF (10 mL g^{-1} imidazole) was added, and the mixture was stirred at RT overnight. The solvent was removed, 5% aqueous NaOH (50 mL) was added to the residue, and the mixture was extracted with CH_2Cl_2 (2×50 mL), dried (Na_2SO_4), and then concentrated to give a clear oil. Purification by flash column chromatography on silica gel (EtOAc/MeOH 9:1 *v/v* as eluent) afforded the α-(1*H*-imidazol-1-yl)-ω-(phenylsulfanyl)alkane. The free base was treated with 37% aqueous HCl (1 equiv) in 2-propanol, and the mixture was concentrated to afford the product.

1-(1*H*-Imidazol-1-yl)-2-(phenylsulfanyl)ethane hydrochloride (11): Compound **11** was prepared from 1-bromo-2-(phenylsulfanyl)-ethane (1 g, 4.61 mmol, 1 equiv) following the general procedure for the formation of {(phenylsulfanyl)alkyl}imidazoles to afford the product as a yellow oil (112 mg, 0.47 mmol, 10%); $R_f = 0.10$ (EtOAc/MeOH, 4:1); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 3.50$ (t, $J = 6.0$ Hz, 2H), 4.48 (t, $J = 6.4$ Hz, 2H), 7.20–7.26 (m, 1H), 7.27–7.34 (m, 2H), 7.35–7.40 (m, 2H), 7.47 (s, 1H), 7.64 (s, 1H), 8.95 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 34.7$, 50.1, 121.0, 123.5, 128.1, 130.4, 131.2, 135.2, 136.8 ppm; HRMS-ESI: m/z $[M-Cl]^+$ calcd for $C_{11}H_{13}N_2S$: 205.0799, found: 205.0796.

1-(1*H*-Imidazol-1-yl)-3-(phenylsulfanyl)propane hydrochloride (12): Compound **12** was prepared from 1-bromo-3-(phenylsulfanyl)propane (1.20 g, 4.33 mmol, 1 equiv) following the general procedure for the formation of {(phenylsulfanyl)alkyl}imidazoles to afford the product as a yellow solid (740 mg, 2.90 mmol, 67%); $R_f = 0.30$ (EtOAc/MeOH, 4:1); mp: 70–72 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 2.15$–2.23 (m, 2H), 2.98 (t, $J = 6.8$ Hz, 2H), 4.42 (t, $J = 6.8$ Hz, 2H), 7.18–7.25 (m, 1H), 7.28–7.38 (m, 4H), 7.58 (s, 1H), 7.66 (s, 1H), 9.01 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 30.5$, 30.9, 49.2, 121.2, 123.3, 127.5, 130.2, 130.7, 136.4, 136.6 ppm; HRMS-ESI: m/z $[M-Cl]^+$ calcd for $C_{12}H_{15}N_2S$: 219.0955, found: 219.0953.

1-(Benzylsulfanyl)-2-(1*H*-imidazol-1-yl)ethane (13): Under an atmosphere of N_2, sodium (230 mg, 10.00 mmol, 1 equiv) was added to EtOH (20 mL) to form a NaOEt/EtOH solution. To this solution was added benzylthiol (1.24 g, 10.00 mmol, 1 equiv), and the mixture was heated at reflux with stirring for 0.5 h. The mixture was cooled to RT, and a mixture of *N*-(2-chloroethyl)imidazole hydrochloride (835 mg, 5.00 mmol, 0.5 equiv) and EtOH (10 mL) was added. The reaction mixture was heated at reflux with stirring for 20 h, then cooled to RT. The mixture was filtered and the filtrate concentrated. The resulting residue was dissolved in 10% aqueous HCl, and the solution was washed with EtOAc (3×15 mL). The aqueous phase was basified using NaOH and then extracted with EtOAc. The organic extract was washed sequentially with H_2O and brine, dried (Na_2SO_4), and concentrated. High-vacuum drying gave compound **13** as a clear oil (1.03 g, 4.72 mmol, 94%); $R_f = 0.17$ (EtOAc/MeOH, 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.70$ (t, $J = 6.8$ Hz, 2H), 3.58 (s, 2H), 3.94 (t, $J = 6.8$ Hz, 2H), 6.86 (s, 1H), 7.04 (s, 1H), 7.23–7.29 (m, 3H), 7.30–7.36 (m, 2H), 7.42 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 32.2$, 36.6, 47.0, 118.9, 127.5, 128.8, 129.0, 129.7, 137.3, 137.8 ppm; HRMS-EI: m/z $[M]^+$ calcd for $C_{12}H_{14}N_2S$: 218.0878, found: 218.0871.

1-(1*H*-Imidazol-1-yl)-4-(phenylsulfanyl)butane hydrochloride (14): Compound **14** was prepared from 1-bromo-4-(phenylsulfanyl)butane (1.16 g, 4.73 mmol, 1 equiv) following the general procedure for the formation of {(phenylsulfanyl)alkyl}imidazoles to afford the product as a white solid (380 mg, 1.41 mmol, 30%); $R_f = 0.21$ (EtOAc/MeOH, 4:1); mp: 90–92 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 1.58$–1.68 (m, 2H), 2.00–2.10 (m, 2H), 3.00 (t, $J = 7.0$ Hz, 2H), 4.27 (t, $J = 7.2$ Hz, 2H), 7.15–7.21 (m, 2H), 7.24–7.35 (m, 3H), 7.55 (t, $J = 1.6$ Hz, 1H), 7.64 (t, $J = 1.6$ Hz, 1H), 8.95 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 26.7$, 30.0, 33.5, 50.1, 121.2, 123.3, 127.2, 130.0, 130.5, 136.3, 137.3 ppm; HRMS-ESI: m/z $[M-Cl]^+$ calcd for $C_{13}H_{17}N_2S$: 233.1112, found: 233.1108.

1-(*N*-Benzylamino)-3-(1*H*-imidazol-1-yl)propane dihydrochloride (15): Under an atmosphere of N_2, a mixture of 1-(3-aminopropyl)imidazole (1.25 g, 9.99 mmol, 1 equiv) and benzaldehyde (1.27 g, 11.97 mmol, 1.2 equiv) in MeOH (20 mL) was stirred at RT for 6 h. $NaBH_4$ (1.11 g, 29.34 mmol, 11.7 equiv) was added, and the mixture was stirred at RT overnight. The mixture was concentrated, and the resulting residue was partitioned between EtOAc (30 mL) and H_2O (30 mL). The aqueous phase was extracted further with EtOAc (20 mL). The combined organic phase was washed with H_2O (2× 20 mL), dried (Na_2SO_4), and concentrated. The residue was purified using flash column chromatography on silica gel (EtOAc/MeOH 1:1 *v/v* as eluent) to give the free base (1.46 g, 6.78 mmol, 68%), which was dissolved in 2-propanol (4 mL), and the solution was treated with a solution of 37% aqueous HCl (1.34 g, 13.60 mmol, 2 equiv) in 2-propanol (2 mL). The mixture was concentrated and dried under high vacuum. Recrystallization from 2-propanol (10 mL) gave compound **15** as a hygroscopic white solid (1.63 g, 5.66 mmol, 57%); mp: 144–145 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 2.38$ (p, 2H), 3.16 (t, $J = 7.8$ Hz, 2H), 4.25 (s, 2H), 4.44 (t, $J = 7.2$ Hz, 2H), 7.42–7.50 (m, 3H), 7.53–7.58 (m, 2H), 7.62 (t, $J = 1.6$ Hz, 1H), 7.75 (t, $J = 1.6$ Hz, 1H), 9.09 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 27.9$, 45.3, 47.5, 52.5, 121.4, 123.3, 130.3, 130.7, 131.1, 132.4, 136.7 ppm; HRMS-ESI: m/z $[M-H-2Cl]^+$ calcd for $C_{13}H_{18}N_3$: 216.1500, found: 216.1509.

Biology

In vitro HO activity assays: HO activity in rat spleen (HO-1) and rat brain (HO-2) microsomal fractions was determined by the quantitation of CO formed from the degradation of methemalbumin (heme complexed with albumin).[20,21] Spleen and brain (Sprague–Dawley rats) microsomal fractions were prepared according to the procedure outlined by Appleton et al.[22] Protein concentration of microsomal fractions was determined by a modification of the biuret method.[21] 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^{-1} protein were pre-incubated with the inhibitors 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. Reactions were stopped by instantly freezing the reaction mixture on dry ice, and CO formation was monitored by GC according to the method described by Vreman and Stevenson.[20]

Analysis of enzyme inhibition: The data resulting from the above experiments were plotted as nonlinear regression (sigmoidal dose–response) curves using GraphPad Prism version 3. The values on the abscissa represent the logarithm of inhibitor concentration (in μM), whereas the values of the activity on the ordinate are expressed as a percentage of the control experiments without inhibitor. From these curves, the inhibitor concentration (EC_{50}) at which enzyme activity is halfway between the bottom and top plateau of the curve, as well as the top and the bottom plateau values of the curves, were retrieved by using the same program, and input into the following formula to give the calculated values of the concentration (IC_{50}) of the compound under evaluation, for which the activity of the enzyme is inhibited by 50% relative to control [Eq. (1)].

$$IC_{50} = \frac{EC_{50}}{\frac{bottom - top}{50 - top} - 1} \quad (1)$$

The IC_{50} value reported for each compound is the mean of the values recorded in replicate experiments, and for each of these replicate experiments (consisting of two separate assays) an indi-

vidual IC_{50} value was calculated in the manner described. The IC_{50} values for the replicate experiments were employed to generate the reported standard deviation value.

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[1] L. E. Otterbein, B. S. Zuckerbraun, *Heme Oxygenase—The Elegant Orchestration of its Products in Medicine*, Nova Science Publishers Inc., New York, **2005**.
[2] R. T. Kinobe, R. A. Dercho, K. Nakatsu, *Can. J. Physiol. Pharmacol.* **2008**, *86*, 577 – 599.
[3] M. A. Alaoui-Jamali, T. A. Bismar, A. Gupta, W. A. Szarek, J. Su, W. Song, Y. Xu, B. Xu, G. Liu, J. Z. Vlahakis, G. Roman, J. Jiao, H. M. Schipper, *Cancer Res.* **2009**, *69*, 8017 – 8024.
[4] M. D. Maines, *FASEB J.* **1988**, *2*, 2557 – 2568.
[5] R. J. Wong, V. K. Bhutani, H. J. Vreman, D. K. Stevenson, *NeoReviews* **2007**, *8*, e77 – e84.
[6] R. J. Wong, H. J. Vreman, S. Schulz, F. S. Kalish, N. W. Pierce, D. K. Stevenson, *J. Perinatol.* **2011**, *31*, S35 – S41.
[7] P. Reddy, S. Najundaswamy, R. Mehta, A. Petrova, T. Hegyi, *J. Perinatol.* **2003**, *23*, 507 – 508.
[8] A. Takeda, Y. Itoyama, T. Kimpara, X. Zhu, J. Avila, B. E. Dwyer, G. Perry, M. A. Smith, *Antioxid. Redox Signaling* **2004**, *6*, 888 – 894.
[9] S. K. Sahoo, T. Sawa, J. Fang, S. Tanaka, Y. Miyamoto, T. Akaike, H. Maeda, *Bioconjugate Chem.* **2002**, *13*, 1031 – 1038.
[10] H. Was, J. Dulak, A. Jozkowicz, *Curr. Drug Targets* **2010**, *11*, 1551 – 1570.
[11] L. M. Furci, P. Lopes, S. Eakanunkul, S. Zhong, A. D. MacKerell, A. Wilks, *J. Med. Chem.* **2007**, *50*, 3804 – 3813.
[12] J. Z. Vlahakis, R. T. Kinobe, R. J. Bowers, J. F. Brien, K. Nakatsu, W. A. Szarek, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1457 – 1461.
[13] J. Z. Vlahakis, R. T. Kinobe, R. J. Bowers, J. F. Brien, K. Nakatsu, W. A. Szarek, *J. Med. Chem.* **2006**, *49*, 4437 – 4441.
[14] G. Roman, J. G. Riley, J. Z. Vlahakis, R. T. Kinobe, J. F. Brien, K. Nakatsu, W. A. Szarek, *Bioorg. Med. Chem.* **2007**, *15*, 3225 – 3234.
[15] J. Z. Vlahakis, M. Hum, M. N. Rahman, Z. Jia, K. Nakatsu, W. A. Szarek, *Bioorg. Med. Chem.* **2009**, *17*, 2461 – 2475.
[16] G. Roman, M. N. Rahman, D. Vukomanovic, Z. Jia, K. Nakatsu, W. A. Szarek, *Chem. Biol. Drug Des.* **2010**, *75*, 68 – 90.
[17] F. Gasparrini, M. Giovannoli, D. Misiti, *J. Org. Chem.* **1990**, *55*, 1323 – 1328.
[18] Y.-P. Pang, F. Hong, P. Quiram, T. Jelacic, S. Brimijoin, *J. Chem. Soc. Perkin Trans. 1* **1997**, 171 – 176.
[19] H. E. Gottlieb, V. Kotlyar, A. Nudelman, *J. Org. Chem.* **1997**, *62*, 7512 – 7515.
[20] H. J. Vreman, D. K. Stevenson, *Anal. Biochem.* **1988**, *168*, 31 – 38.
[21] M. N. Cook, K. Nakatsu, G. S. Marks, B. E. McLaughlin, H. J. Vreman, D. K. Stevenson, *Can. J. Physiol. Pharmacol.* **1995**, *73*, 515 – 518.
[22] S. D. Appleton, M. L. Chretien, B. E. McLaughlin, H. J. Vreman, D. K. Stevenson, J. F. Brien, K. Nakatsu, D. H. Maurice, G. S. Marks, *Drug Metab. Dispos.* **1999**, *27*, 1214 – 1219.

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