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Heme oxygenase inhibition by 2-oxy-substituted 1-(1*H*-imidazol-1-yl)-4-phenylbutanes: Effect of halogen substitution in the phenyl ring

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Abstract—A series of 2-oxy-substituted 1-(1*H*-imidazol-1-yl)-4-phenylbutanes comprising imidazole–ketones, imidazole–dioxolanes, and imidazole–alcohols substituted with halogens in the phenyl ring were synthesized and evaluated as novel inhibitors of heme oxygenase which are structurally distinct from metalloporphyrins. The entire library of compounds was found to be highly active, with the bromine- and iodine-substituted derivatives being the most potent. The imidazole–dioxolanes were all selective for the HO-1 isozyme (inducible) and exhibited substantially lower activity toward the HO-2 isozyme (constitutive). The corresponding imidazole–ketones and imidazole–alcohols showed selectivity toward HO-1 to a lesser degree than the similarly substituted imidazole–dioxolanes.

1. Introduction

Heme oxygenases (HOs), comprising two active isozymes HO-1 (inducible) and HO-2 (constitutive), are enzymes responsible for heme degradation in vivo, a process which generates carbon monoxide (CO) whose cellular regulatory actions have recently been acknowledged.[1–3] Inhibitors of these enzymes may prove to be indispensable pharmacological tools for the investigation of the CO/HO system and related physiological pathways. Furthermore, investigation of the mechanism of HO-1's protective effect in acute renal failure,[4] its beneficial involvement in cardiovascular system injuries,[5] or its central role in neurodegenerative diseases[6] significantly depends on the availability of efficient inhibitors of this enzyme. Because the previously used metalloporphyrin-based HO inhibitors lack specificity, the discovery[7] of azalanstat **1** as a compound active toward HO-1 and HO-2 has opened the way for the design of novel, more-selective non-metalloporphyrin inhibitors of these enzymes. Azalanstat itself is a good HO inhibitor and has minimal effects on other enzymes usually inhibited by metalloporphyrins such as nitric oxide synthase (NOS) or soluble guanylyl cyclase (sGC).[8] The initial structure–activity study of a series of azalanstat analogs[9] showed that different degrees of selectivity in the inhibition of HO-1 over HO-2 could be achieved by modifying the aminophenylmercapto moiety in the northeastern region of azalanstat or the diastereomeric configuration of the substituents attached to the dioxolane ring (Fig. 1). Further investigations[10] showed that the removal of the aminophenylmercapto moiety

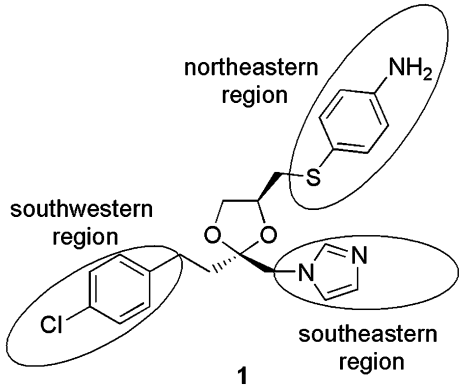

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Figure 1. Topological analysis of azalanstat **1**.

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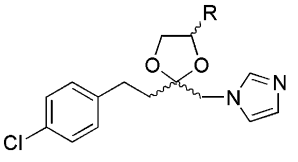
selectively enhanced the inhibition of HO-1 relative to HO-2. As our previous reports have dealt only with compounds featuring the typical chloro substituent in the benzene ring in the southwestern region of azalanstat (Fig. 1), the present work focuses on the investigation of the structure–activity relationship brought about by the modification of the substitution pattern in the benzene ring in imidazole–dioxolanes and structurally related compounds.

2. Results and discussion

2.1. Chemistry

Prompted by the discovery of the selective inhibitory activity of the imidazole–dioxolane **2** (R = H) toward HO-1,[10] a series of analogs bearing various halogen substituents on the benzene ring has been designed for the present study. The imidazole–dioxolane having an unsubstituted benzene ring has also been incorporated in this series. Including two other series of compounds (the related imidazole–ketones and the corresponding imidazole–alcohols) has further broadened the scope of the investigation.



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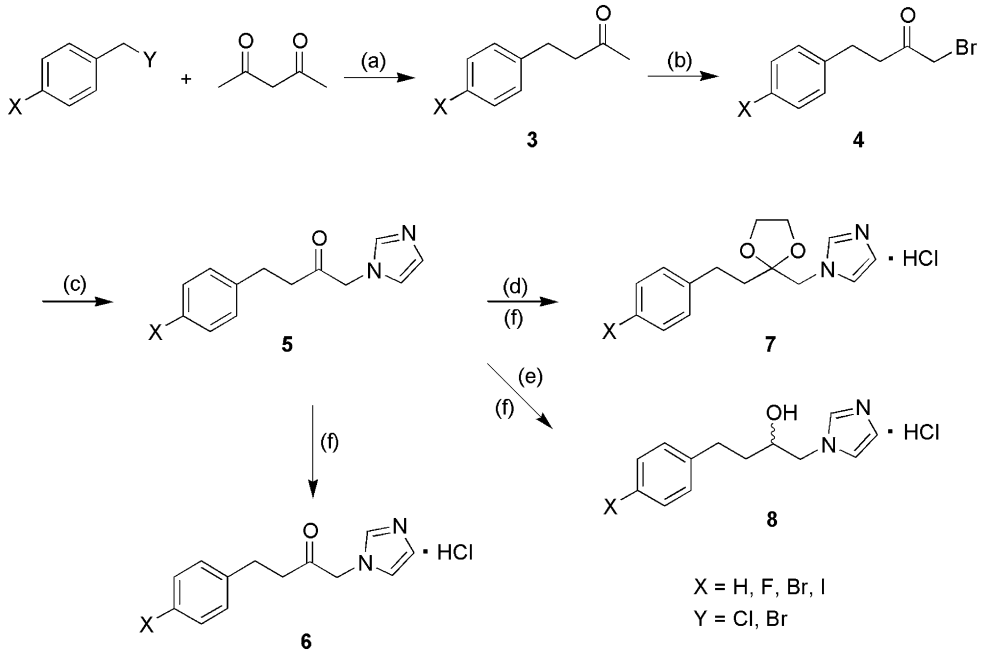
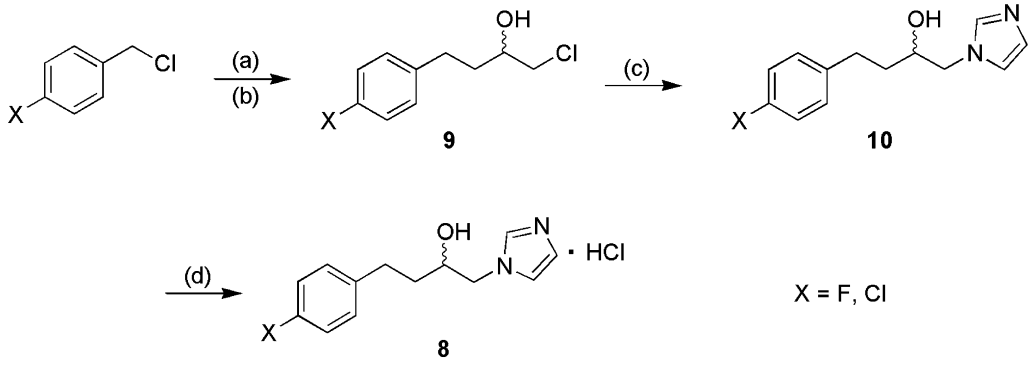
R = H, CH_3

Two different synthetic approaches to the targeted compounds have been considered. The first multi-step reaction sequence, illustrated in Scheme 1, was devised as a general strategy granting access to imidazole–dioxolanes variously substituted in the benzene ring, and debuts with the preparation of 4-aryl-2-butanones **3** by way of a base-catalyzed condensation of the appropriate benzyl halides with 2,4-pentanedione,[11] followed by the bromination of these materials to afford the intermediate 1-bromoketones **4**. The formation of the undesired isomeric 3-bromoketones has been abated by the use of methanol as a solvent instead of the usual halogenated solvents.[12] Alkylation of imidazole with the bromoketones **4** provided easy access to the key intermediate imidazole–ketones **5**, which were subsequently converted into either the corresponding imidazole–dioxolanes upon heating at refluxing temperature in ethylene glycol–toluene in the presence of *p*-toluenesulfonic acid and with continuous azeotropic removal of water, or into the corresponding imidazole–alcohols by reduction with sodium borohydride in methanol. Imidazole–ketones **5** have been characterized as hydrochlorides **6**, whereas the imidazole–dioxolane hydrochlorides **7** and the imidazole–alcohol hydrochlorides **8** have been made available for the biological evaluation from the respective free bases.

The second synthetic pathway (Scheme 2) is based on the methodology of Walker et al.,[13] and was used for the synthesis of imidazole–alcohols **8** only in the case of the commercially available halogen-substituted benzyl halides whose benzylic halogen atom is more reactive than the halogen substituent in the aromatic ring. Treatment of the Grignard reagents derived from either *p*-fluorobenzyl chloride or *p*-chlorobenzyl chloride with



Scheme 1. General approach for the synthesis of imidazole–ketones, imidazole–dioxolanes, and imidazole–alcohols related to azalanstat. Reagents and conditions: (a) anhyd K_2CO_3, MeOH, reflux, 16 h; (b) Br_2, MeOH, rt, 2 h; (c) imidazole, DMF, rt, 1 h; (d) ethylene glycol, *p*-TsOH·H_2O, toluene, reflux, 2 h; (e) $NaBH_4$, MeOH, rt, 3 h; (f) 37% aq HCl, 2-propanol, rt.



Scheme 2. Alternative synthesis of imidazole–alcohols related to azalanstat. Reagents and conditions: (a) Mg, anhyd diethyl ether, reflux, 15 min; (b) (±)-epichlorohydrin, anhyd diethyl ether, reflux, 2 h; (c) imidazole, NaH, anhyd DMF, 70–80 °C, 4.5 h; (d) 37% aq HCl, 2-propanol, rt.

racemic epichlorohydrin led to the intermediate optically inactive 1-chloro-2-butanols **9**, which yielded the imidazole–alcohols **10** as free bases through the N-alkylation of imidazole. The free bases led to the corresponding hydrochlorides **8** upon treatment with hydrochloric acid.

2.2. Biological evaluation

Three different series of compounds, namely imidazole–ketones **6**, imidazole–dioxolanes **7**, and imidazole–alcohols **8**, all as hydrochlorides, were evaluated for their ability to inhibit HO using an in vitro assay for HO in which heme was presented to the enzyme complexed with albumin as described in Section 4. HO-1 was obtained from rat spleen, and HO-2 was obtained from rat brain in 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.[8,14–16] As shown in Table 1, all of the compounds exhibited good potency as inhibitors of HO-1 activity, but only imidazole–ketones **6** and imidazole–alcohols **8** inhibited HO-2 significantly. The selectivity indices given in Table 1, calculated as the ratio of IC_{50} values determined for HO-2 and HO-1 in the case of each compound, have been used to express quantitatively the selectivity of these inhibitors toward the HO isozymes.

Imidazole–ketones **6** have been evaluated for the first time for HO inhibitory activity, and all of them proved to be excellent inhibitors of both HO-1 and HO-2. When compared to azalanstat **1**, the 4-chlorophenyl compound **6e** had similar inhibitory potency for HO-1, but it was the least potent HO-1 inhibitor in this series. The dose–response curves for the iodo-substituted imidazole–ketone **6d**, which exhibited the highest values for the inhibition of both HO-1 and HO-2 while having the best selectivity index in this series, are shown in Figure 2a. However, none of the imidazole–ketones **6** showed any marked selectivity toward any of the two HO isozymes. At the lowest concentration used in the study (0.1 μM), compounds **6a**, **6b**, and **6c** showed almost no inhibition of HO-1, whereas compounds **6d** and **6e** inhibited 44% and 27%, respectively, of the control activity of HO-1. However, a very slight inhibition of HO-1 by compound **6d** was noted upon a 10-fold decrease of its concentration. Even at the highest concentration of the evaluated imidazole–ketones **6**, the greatest inhibition observed was approximately 80%.

Table 1. Inhibitory potency and selectivity of imidazole–ketones **6**, imidazole–dioxolanes **7**, and imidazole–alcohols **8**

Compound	IC_{50} (μM)		Selectivity index IC_{50} (HO-2)/IC_{50} (HO-1)
	Rat spleen (HO-1)	Rat brain (HO-2)	
6a (X = H)	4.0 ± 1.8	11.3 ± 4.7	2.8
6b (X = F)	2.7 ± 0.9	1.9 ± 0.2	1.4
6c (X = Br)	1.7 ± 0.7	9.5 ± 4.6	9.2
6d (X = I)	0.11 ± 0.06	1.8 ± 0.7	5.6
6e (X = Cl)	4.7 ± 0.5	43.1 ± 5.4	16.4
7a (X = H)	0.7 ± 0.4	>100	>143
7b (X = F)	3.8 ± 1.1	>100	>26.3
7c (X = Br)	1.9 ± 0.2	>100	>52.6
7d (X = I)	3.7 ± 0.9	>100	>27
7e (X = Cl)[10]	4.3 ± 2.1	>100	>23.2
8a (X = H)	6.2 ± 0.8	16.0 ± 8.2	2.6
8b (X = F)	1.4 ± 1.1	17.9 ± 11.8	12.8
8c (X = Br)	0.14 ± 0.06	2.6 ± 0.5	18.6
8d (X = I)	0.06 ± 0.03	1.8 ± 1.5	30
8e (X = Cl)	0.5 ± 0.1	4.0 ± 0.6	8
1 (azalanstat)	5.3 ± 2	24.5 ± 2.1	4.6

Data represent mean IC_{50} values ± standard deviation of replicate experiments.

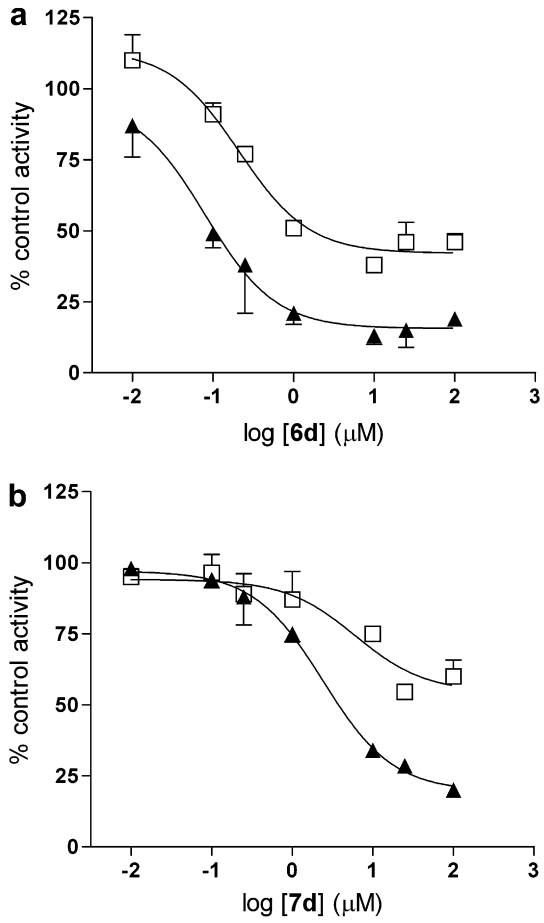


Figure 2. Inhibition of HO-1 and HO-2 by imidazole–ketone **6d** (graph a) and imidazole–dioxolane **7d** (graph b). All values of activity (ordinate) are expressed as a percentage of the control with no inhibitor present. The values on the abscissa represent the log of the inhibitor concentration. Solid triangles (▲), HO-1 (rat spleen microsomes) and open squares (□), HO-2 (rat brain microsomes).

The results for the inhibition of HO-2 parallel those recorded for HO-1, with compounds **6a** and **6c** being almost devoid of any inhibition toward HO-2 at the lowest concentration. Under the same conditions, the rest of the imidazole–ketones **6** inhibited about 10% of the control activity of HO-2, and at the highest concentration (100 μM) all of the compounds in this series inhibited only 60–70% of the control activity of HO-2.

The development of an optimally selective HO inhibitor still remains a problem. Metalloporphyrins show a modest degree of selectivity toward HO-2,[17] but there are certain drawbacks that prevent their use in the characterization of the physiological role of these enzymes. Although all of the imidazole–dioxolanes **7** were potent inhibitors of HO-1, none of these compounds inhibited HO-2 activity significantly in the range of concentrations used in the assay. These results confirm the initial observation[10] of isozyme-selective HO inhibition within this class of compounds, and are consistent with the working hypothesis that the presence of an imidazole ring and a minimally substituted dioxolane moiety in the structure of HO inhibitors enhanced selectivity toward HO-1. The selectivity indices ranged from more than 143 to at least 23 for compounds **7**, and are much higher than those determined for any azalanstat analogs,[9] but comparable with the indices recorded for the methyl-terminated imidazole–dioxolanes **2** (R = CH_3).[10] The dose–response curves (Fig. 2b) for the iodo-substituted imidazole–dioxolane **7d** illustrate the difference in potency toward HO-1 and HO-2. All of the imidazole–dioxolanes did not inhibit significantly HO-1 or HO-2 at the lowest concentration (0.1 μM), whereas the extent of HO-1 inhibition reached 80–85% at the highest concentration of the inhibitor. On the other hand, the inhibition of HO-2 by imidazole–dioxolanes **7** was less than 50% even at 100 μM. Surprisingly, the iodo-substituted imidazole–dioxolane **7d** was not the best HO-1 inhibitor in this series.

Also under scrutiny for the first time as inhibitors of HO, imidazole–alcohols **8** behaved similarly to the structurally related imidazole–ketones **6** in this respect. Compounds **8** inhibited both HO-1 and HO-2 almost to the same extent, their selectivity indexes varying from 2.6 to 30. Again, the iodo-substituted imidazole–alcohol **8d** was the most selective analog in this series, but the bromo-substituted analog **8c**, having an average HO-1 inhibition of 55% and 8% from control at 0.1 and 0.01 μM, respectively, was one of the most potent in this group. HO-2 inhibition in this series ranges from 55% for **8c** to 90% for **8e** at the highest concentration (100 μM).

As far as the substitution pattern in the benzene ring is concerned, no striking differences in potency were noted in the three series of structurally related compounds. It seems that the less electronegative and the bulkier the *para* substituent, the greater the potency toward both HO-1 and HO-2; compounds having a bulky bromo or iodo substituent in the *para* position of the phenyl ring had an improved inhibitory activity than their analogs containing a less bulky atom in that position, and this pattern is reflective also of the variation of the selectivity index of these compounds. The observation[18] that there is a strong correlation between the hydrophilicity of the substituent at the far end in a series of 4-(substituted biphenyl)methylimidazoles and some unidentified residues at the end of the substrate binding loops of $P450_{17\alpha}$ allowed the hypothesis that the ability of the terminal substituent to undergo polar–polar interaction with a hydrogen-bonding group at the active site is responsible for the enhanced inhibition of this enzyme by the bromine-substituted inhibitors compared, for example, with the one exhibited by the fluorine-substituted compounds.[19] The same effect could be responsible for the greater inhibitory action of the iodo- and bromo-substituted compounds reported in this study. Even though the current findings lead to the conclusion that the replacement of chlorine with other halogens or with hydrogen does not result in substantial changes in the HO inhibitory activity of such imidazole-containing compounds, one cannot preclude the possibility of a significant increase or decrease in activity upon dramatic changes in this part of the molecule. The results of the biological evaluation of these compounds seem to

indicate that the southwestern region of compounds **6**, **7**, and **8** is not actively involved in the binding of these inhibitors to HO. Nevertheless, the modification of the southwestern region may become important in the optimization process of a selective inhibitor of HO.

It can be inferred, from the reports[20] of antifungal drugs exerting their action through the coordination of their azole moiety with the protoporphyrin iron atom, that the imidazole ring is essential for the expression of HO inhibitory activity in compounds **6**, **7**, and **8**. Furthermore, a comparative molecular-field analysis study[21] has emphasized the importance of the hydrophobic interactions of the N-1 moiety in these antifungal agents with the active site of an enzyme. The limited up-to-date knowledge on the inhibition of HO by azalanstat and its structurally simpler analogs suggests that the presence of at least one oxygen-containing function strategically located on the hydrophobic backbone of the N-1 substituent is required for the materialization of the inhibitory activity. The replacement of the oxygen atoms of compound **7e** with sulfur yielded an imidazole–dithiolane having a slightly less effect on HO-1, but a significantly less effect on HO-2;[10] this observation is interpreted to mean that the nature of the heteroatom in this five-membered ring on the hydrophobic backbone is relevant for the enhancement of selectivity toward HO-1. The presence of two oxygen atoms in the dioxolane moiety and the bulkiness of the dioxolane ring compared to the size of the carbonyl or hydroxyl functions in compounds **6** or **8**, respectively, may concurrently contribute to the particularly selective inhibition of the two HO isoforms by compounds **7**.

3. Conclusions

Instigated by the discovery of imidazole–dioxolane analogs of azalanstat as selective inhibitors of HO isoforms, the design and synthesis of a series of 2-oxy-substituted 1-(1*H*-imidazol-1-yl)-4-phenylbutanes has been undertaken with the view to explore simultaneously the effect of the substitution pattern in the phenyl ring and the outcome of the replacement of the dioxolane moiety by a secondary alcohol or a carbonyl function on the inhibition of HO. All of the compounds were effective against HO-1, but only the imidazole–ketones and the imidazole–alcohols were active toward HO-2. Thus, the imidazole–dioxolanes were selective toward HO-1, whereas the imidazole–ketones and the corresponding imidazole–alcohols showed selectivity for HO-1 to a lesser degree. The substitution in the phenyl ring did not seem to have a remarkable contribution to the HO inhibition by these compounds; however, the electronegativity of the *para* substituent in the case of the bromo- and iodo-substituted compounds may have improved their effect against both isoforms in terms of potency compared to the typically chloro-substituted analogs. Furthermore, the significance of an oxygen-containing backbone of the N-1 substituent of imidazole for the hydrophobic interaction of an inhibitor with the binding site of the enzyme is supported by the good activity of the entire collection of compounds.

4. Experimental

All chemical reagents were obtained from Sigma–Aldrich and were used without prior purification. Column chromatography was performed on Silicycle silica gel (230–400 mesh, 60 Å). Analytical thin-layer chromatography was performed on glass- or aluminum-backed Silicycle precoated silica gel 60 F_{254} plates, and the compounds were visualized either by UV illumination (254 nm), or by heating after spraying with phosphomolybdic acid in ethanol. Melting points were recorded on a Mel-Temp II apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker Avance 300- and 400-MHz spectrometers. The signals owing to residual protons in the deuterated solvents were used as internal standards for the ^{1}H NMR spectra. The chemical shifts for the carbon atoms are given relative to $CDCl_3$ (δ = 77.16 ppm) or CD_3OD (49.00 ppm). High-resolution mass spectra were obtained on an Applied Biosystems/MDS Sciex QSTAR XL spectrometer equipped with an Agilent HP1100 Cap-LC system. The data for elemental analysis, determined at MHW Laboratories (Pheonix, AZ), were within ±0.4% of theoretical values. 4-(4-Chlorophenyl)-1-(1*H*-imidazol-1-yl)-2-butanone hydrochloride (**6e**)[13] and 1-((2-(2-(4-chlorophenyl)ethyl)-1,3-dioxolan-2-yl)methyl)-1*H*-imidazole hydrochloride (**7e**)[10] were prepared according to the reported procedures.

4.1. General procedure for the synthesis of 4-aryl-2-butanones 3a–d

A mixture of 2,4-pentanedione (200 mg, 206 μL, 2 mmol), the 4-substituted benzyl halide (chloride in the case of **3b**, bromide in all other cases, 2 mmol), and anhydrous potassium carbonate (276 mg, 2 mmol) in methanol (10 mL) was heated at reflux temperature for 16 h. The mixture was then cooled to room temperature, methanol was removed under reduced pressure, and the resulting residue was partitioned between ethyl acetate (10 mL) and water (10 mL). The organic layer was separated, and the aqueous layer was extracted further with ethyl acetate (3× 10 mL). The combined organic phase was washed with water (10 mL), dried over anhydrous Na_2SO_4, and then the solvent was removed under pressure. The resulting oil was chromatographed on a silica gel column using hexanes–ethyl acetate as the mobile phase to give the title compounds.

4.1.1. 4-Phenyl-2-butanone (3a).[22,23] Clear liquid (169 mg, 57%), R_f = 0.63 (hexanes–ethyl acetate 3:1 v/v); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.14 (s, 3H), 2.76 (t, J = 7.2 Hz, 2H), 2.90 (t, J = 7.2 Hz, 2H), 7.15–7.23 (m, 3H), 7.26–7.32 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.8, 30.1, 45.2, 126.2, 128.4, 128.6, 141.1, 208.0; HRMS (EI) Calcd for $C_{10}H_{12}O$: 148.0888 (M^+). Found: 148.0885.

4.1.2. 4-(4-Fluorophenyl)-2-butanone (3b).[24] Clear liquid (177 mg, 54%), R_f = 0.62 (hexanes–ethyl acetate 3:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.14 (s, 3H), 2.74 (t, J = 7.2 Hz, 2H), 2.87 (t, J = 7.2 Hz, 2H), 6.92–7.00

(m, 2H), 7.10–7.17 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.0, 30.2, 45.3, 115.2 (d, $J^2_{C,F} = 21$ Hz), 129.8 (d, $J^3_{C,F} = 8$ Hz), 136.6 (d, $J^4_{C,F} = 3$ Hz), 161.5 (d, $J^1_{C,F} = 242$ Hz), 208; ^{19}F NMR (376 MHz, $CDCl_3$): δ −118.3.

4.1.3. 4-(4-Bromophenyl)-2-butanone (3c).[25] Clear liquid (302 mg, 67%), $R_f = 0.38$ (hexanes–ethyl acetate 3:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.15 (s, 3H), 2.75 (t, $J = 7.2$ Hz, 2H), 2.86 (t, $J = 7.2$ Hz, 2H), 7.07 (d, $J = 8$ Hz, 2H), 7.40 (d, $J = 8.4$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.2, 30.2, 44.9, 120.0, 130.2, 131.7, 140.2, 207.4; HRMS (ESI) Calcd for $C_{10}H_{11}BrONa$: 248.9891 [M+Na$^+$]. Found: 248.9880.

4.1.4. 4-(4-Iodophenyl)-2-butanone (3d). White solid (318 mg, 58%), mp 75–76 °C, $R_f = 0.60$ (hexanes–ethyl acetate 3:1 v/v); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.13 (s, 3H), 2.73 (t, $J = 7.2$ Hz, 2H), 2.83 (t, $J = 7.2$ Hz, 2H), 6.94 (d, $J = 7.8$ Hz, 2H), 7.59 (d, $J = 7.8$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.2, 30.2, 44.9, 91.3, 130.6, 137.6, 140.8, 207.6; HRMS (EI) Calcd for $C_{10}H_{11}IO$: 273.9855 (M$^+$). Found: 273.9853.

4.2. General procedure for the bromination of ketones 3

To a solution of ketone **3** (1 mmol) in methanol (8 mL) stirred at room temperature, a solution of bromine (160 mg, 51.6 μL, 1 mmol) in methanol (1 mL) was added in one portion. The orange reaction mixture was then stirred at room temperature for 2 h, and, after the ketone **3** had been consumed (TLC monitoring, hexanes–ethyl acetate 4:1 v/v), the reaction was quenched by adding a 0.3 M sodium thiosulfate solution (618 μL), and diluted with ethyl acetate (15 mL). The resulting mixture was washed with water (15 mL), the organic layer was separated, and the aqueous layer was extracted further with ethyl acetate (3× 15 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a residue that was chromatographed on a silica gel column using hexanes–ethyl acetate (15:1 v/v) as the mobile phase to give the desired 1-bromo ketones **2**.

4.2.1. 1-Bromo-4-phenyl-2-butanone (4a).[26,27] White solid (131 mg, 58%), mp 37–38 °C, $R_f = 0.53$ (hexanes–ethyl acetate 4:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.98 (t, $J = 6.8$ Hz, 2H), 3.02 (t, $J = 6.8$ Hz, 2H), 3.88 (s, 2H), 7.19–7.24 (m, 3H), 7.28–7.33 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.0, 34.4, 41.6, 126.6, 128.6, 128.7, 140.5, 201.4; HRMS (EI) Calcd for $C_{10}H_{11}BrO$: 225.9993 (M$^+$). Found: 225.9997.

4.2.2. 1-Bromo-4-(4-fluorophenyl)-2-butanone (4b). White solid (159 mg, 65%), $R_f = 0.45$ (hexanes–ethyl acetate 4:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.95 (t, $J = 6.8$ Hz, 2H), 2.99 (m, $J = 6.8$ Hz, 2H), 3.88 (s, 2H), 6.93–7.01 (m, 2H), 7.11–7.18 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.1, 34.4, 41.5, 115.4 (d, $J^2_{C,F} = 21$ Hz), 129.9 (d, $J^3_{C,F} = 8$ Hz), 136.1 (d, $J^4_{C,F} = 3$ Hz), 161.6 (d, $J^1_{C,F} = 243$ Hz), 201.1; ^{19}F NMR (376 MHz, $CDCl_3$): δ −117.8; HRMS (EI) Calcd for $C_{10}H_{10}BrFO$: 243.9902 (M$^+$). Found: 243.9899.

4.2.3. 1-Bromo-4-(4-bromophenyl)-2-butanone (4c). White solid (193 mg, 63%), mp 63–64 °C, $R_f = 0.42$ (hexanes–ethyl acetate 4:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.89 (t, $J = 6.8$ Hz, 2H), 2.96 (t, $J = 6.8$ Hz, 2H), 3.84 (s, 2H), 7.07 (d, $J = 8$ Hz, 2H), 7.41 (d, $J = 8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.3, 34.3, 41.2, 120.2, 130.2, 131.7, 139.4, 201.0; HRMS (EI) Calcd for $C_{10}H_{10}Br_2O$: 303.9098 (M$^+$). Found: 303.9090.

4.2.4. 1-Bromo-4-(4-iodophenyl)-2-butanone (4d). White solid (208 mg, 59%), mp 76–77 °C, $R_f = 0.50$ (hexanes–ethyl acetate 4:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.88 (t, $J = 7.2$ Hz, 2H), 2.96 (t, $J = 7.2$ Hz, 2H), 3.84 (s, 2H), 6.94 (d, $J = 8$ Hz, 2H), 7.60 (d, $J = 8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.4, 34.2, 41.1, 91.6, 130.6, 137.8, 140.1, 201.0; HRMS (EI) Calcd for $C_{10}H_{10}BrIO$: 351.8960 (M$^+$). Found: 351.8963.

4.3. General procedure for the preparation of imidazole–ketones 5 and their hydrochlorides 6

A mixture of bromoketone **4** (0.5 mmol) and imidazole (102 mg, 1.5 mmol) in dry *N*,*N*-dimethylformamide (2 mL) was stirred at room temperature under a nitrogen atmosphere for 1 h. The mixture was then diluted with ethyl acetate (15 mL), and the solution was washed with water (4× 15 mL). The separated organic phase was dried over anhydrous Na_2SO_4, and then the solvent was removed under reduced pressure to afford a residue that was chromatographed on a silica gel column using ethyl acetate–methanol as the mobile phase to give the imidazole–ketones **5** as free bases. The free bases **5** (0.2 mmol) were turned into the corresponding hydrochlorides upon treatment with 37% aqueous HCl (26 mg, 22 μL, 0.26 mmol) in 2-propanol (1 mL). The mixture was then concentrated and dried under high vacuum to afford a residue that was dissolved in the least amount of hot 2-propanol. The solution was cooled at room temperature, and then to −25 °C in a freezer prior to gradual addition of diethyl ether to complete the precipitation of the hydrochlorides **6**, which were collected by filtration and washed with diethyl ether.

4.3.1. 1-(1*H*-Imidazol-1-yl)-4-phenyl-2-butanone (5a).[28] White solid (64 mg, 60%), mp 71–72 °C, $R_f = 0.44$ (ethyl acetate–methanol 8:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.74 (t, $J = 7.2$ Hz, 2H), 2.93 (t, $J = 7.2$ Hz, 2H), 4.61 (s, 2H), 6.78 (s, 1H), 7.06 (s, 1H), 7.15 (d, $J = 7.6$ Hz, 2H), 7.23 (d, $J = 7.2$ Hz, 1H), 7.28–7.33 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.6, 41.1, 55.6, 120.0, 126.5, 128.4, 128.7, 129.6, 137.9, 140.1, 202.7; HRMS (ESI) Calcd for $C_{13}H_{15}N_2O$: 215.1184 [M+H]$^+$. Found: 215.1195.

4.3.2. 1-(1*H*-Imidazol-1-yl)-4-phenyl-2-butanone hydrochloride (6a). White solid (43 mg, 86%), mp 170–171 °C (lit.[29] mp 171–173 °C), $R_f = 0.0$ (ethyl acetate); ^{1}H NMR (400 MHz, D_2O): δ 2.96 (t, $J = 6.8$ Hz, 2H), 3.04 (t, $J = 6.8$ Hz, 2H), 5.27 (s, 2H), 7.27–7.33 (m, 4H), 7.34–7.41 (m, 2H), 7.48 (s, 1H), 8.59 (s, 1H); ^{13}C NMR (100 MHz, D_2O): δ 28.7, 40.6, 57.0, 119.5, 122.9, 126.6, 128.4, 128.8, 135.7, 140.5, 204.7; HRMS

(ESI) Calcd for $C_{13}H_{15}N_2O$: 215.1184 $[M+H]^+$. Found: 215.1195. Anal. Calcd for $C_{13}H_{15}ClN_2O$: C, 62.28; H, 6.03; N, 11.17. Found: C, 62.33; H, 5.85; N, 10.99.

4.3.3. 4-(4-Fluorophenyl)-1-(1*H*-imidazol-1-yl)-2-butanone (5b). White solid (76 mg, 66%), mp 69–70 °C, $R_f = 0.52$ (ethyl acetate–methanol 8:1 v/v); ^1H NMR (400 MHz, $CDCl_3$): δ 2.71 (t, $J = 7.2$ Hz, 2H), 2.90 (t, $J = 7.2$ Hz, 2H), 4.66 (s, 2H), 6.83 (br s, 1H), 6.93–6.99 (m, 2H), 7.08–7.14 (m, 3H), 7.44 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.7, 41.3, 55.8, 115.6 (d, $J^2_{C,F} = 21$ Hz), 120.0, 129.7, 129.9 (d, $J^3_{C,F} = 8$ Hz), 135.8 (d, $J^4_{C,F} = 3$ Hz), 137.9, 161.7 (d, $J^1_{C,F} = 243$ Hz), 202.5; ^{19}F NMR (376 MHz, $CDCl_3$): δ −117.5; HRMS (EI) Calcd for $C_{13}H_{13}FN_2O$: 232.1012 (M^+). Found: 232.1006.

4.3.4. 4-(4-Fluorophenyl)-1-(1*H*-imidazol-1-yl)-2-butanone hydrochloride (6b). White solid (43 mg, 80%), mp 160–162 °C, $R_f = 0.0$ (ethyl acetate); ^1H NMR (400 MHz, D_2O): δ 2.90 (t, $J = 7.2$ Hz, 2H), 3.00 (t, $J = 7.2$ Hz, 2H), 5.26 (s, 2H), 7.01–7.08 (m, 2H), 7.20–7.27 (m, 2H), 7.29 (s, 1H), 7.46 (s, 1H), 8.60 (s, 1H); ^{13}C NMR (100 MHz, D_2O): δ 27.8, 40.6, 57.0, 115.2 (d, $J^2_{C,F} = 21$ Hz), 119.4, 122.9, 129.9 (d, $J^3_{C,F} = 8$ Hz), 135.7, 136.2 (d, $J^4_{C,F} = 3$ Hz), 161.2 (d, $J^1_{C,F} = 240$ Hz), 204.5; ^{19}F NMR (376 MHz, D_2O): δ −118.5; HRMS (ESI) Calcd for $C_{13}H_{14}FN_2O$: 233.1090 $[M+H]^+$. Found: 233.1089. Anal. Calcd for $C_{13}H_{14}ClFN_2O$: C, 58.11; H, 5.25; N, 10.43. Found: C, 58.25; H, 5.17; N, 10.61.

4.3.5. 4-(4-Bromophenyl)-1-(1*H*-imidazol-1-yl)-2-butanone (5c). White solid (111 mg, 76%), mp 79–80 °C, $R_f = 0.50$ (ethyl acetate–methanol 4:1 v/v); ^1H NMR (400 MHz, $CDCl_3$): δ 2.70 (t, $J = 7.2$ Hz, 2H), 2.83 (t, $J = 7.2$ Hz, 2H), 4.65 (s, 2H), 6.81 (br s, 1H), 7.02 (d, $J = 7.6$ Hz, 2H), 7.09 (s, 1H), 7.37–7.40 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.6, 40.9, 55.7, 120.1, 120.4, 130.0, 131.8, 138.0, 139.1, 202.3; HRMS (EI) Calcd for $C_{13}H_{13}BrN_2O$: 292.0211 (M^+). Found: 292.0219.

4.3.6. 4-(4-Bromophenyl)-1-(1*H*-imidazol-1-yl)-2-butanone hydrochloride (6c). White solid (51 mg, 77%), mp 174–175 °C, $R_f = 0.0$ (ethyl acetate); ^1H NMR (400 MHz, D_2O): δ 2.91 (t, $J = 7.2$ Hz, 2H), 3.01 (t, $J = 7.2$ Hz, 2H), 5.27 (s, 2H), 7.18 (d, $J = 8$ Hz, 2H), 7.30 (s, 1H), 7.46–7.50 (m, 3H), 8.61 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 29.4, 41.8, 57.9, 120.8, 121.0, 124.6, 131.5, 132.6, 137.7, 141.2, 201.7; HRMS (ESI) Calcd for $C_{13}H_{14}BrN_2O$: 293.0290 $[M+H]^+$. Found: 293.0279. Anal. Calcd for $C_{13}H_{14}BrClN_2O$: C, 47.37; H, 4.28; N, 8.50. Found: C, 47.60; H, 4.13; N, 8.34.

4.3.7. 1-(1*H*-Imidazol-1-yl)-4-(4-iodophenyl)-2-butanone (5d). Off-white solid (121 mg, 71%), mp 124–125 °C, $R_f = 0.19$ (ethyl acetate); ^1H NMR (400 MHz, $CDCl_3$): δ 2.71 (t, $J = 7.6$ Hz, 2H), 2.86 (t, $J = 7.6$ Hz, 2H), 4.65 (s, 2H), 6.81 (s, 1H), 6.90 (d, $J = 8$ Hz, 2H), 7.09 (s, 1H), 7.39 (s, 1H), 7.60 (d, $J = 8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.0, 40.9, 55.7, 91.8, 120.0, 130.1, 130.6, 137.9, 138.0, 139.8, 202.3; HRMS (EI) Calcd for $C_{13}H_{13}IN_2O$: 340.0073 (M^+). Found: 340.0074.

4.3.8. 1-(1*H*-Imidazol-1-yl)-4-(4-iodophenyl)-2-butanone hydrochloride (6d). Off-white solid (46 mg, 61%), mp 202–203 °C, $R_f = 0.0$ (ethyl acetate); ^1H NMR (400 MHz, CD_3OD): δ 2.90 (t, $J = 6.8$ Hz, 2H), 2.96 (t, $J = 6.8$ Hz, 2H), 5.31 (s, 2H), 7.04 (d, $J = 8$ Hz, 2H), 7.49 (s, 2H), 7.56–7.64 (m, 3H), 8.84 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 29.4, 41.7, 57.9, 91.6, 120.5, 124.7, 131.7, 137.7, 138.7, 141.8, 201.7; HRMS (ESI) Calcd for $C_{13}H_{14}IN_2O$: 341.0151 $[M+H]^+$. Found 341.0147. Anal. Calcd for $C_{13}H_{14}ClIN_2O$: C, 41.46; H, 3.75; N, 7.44. Found: C, 41.59; H, 4.00; N, 7.37.

4.4. General procedure for the preparation of imidazole–dioxolane hydrochlorides 7

A mixture of an imidazole–ketone **5** (0.5 mmol), ethylene glycol (62 mg, 58 μL, 1 mmol), and *p*-toluenesulfonic acid monohydrate (190 mg, 1 mmol) in toluene (20 mL) was heated at reflux temperature under nitrogen until the Dean–Stark trap had filled (1 h). The trap was then emptied, fresh toluene (10 mL) was added to the reaction mixture, and heating at reflux temperature continued for another hour until the trap had refilled. The reaction mixture was then cooled to room temperature, diluted with ethyl acetate (15 mL), and washed sequentially with saturated $NaHCO_3$ solution (15 mL), water (15 mL), and brine (15 mL). The organic layer was dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure to give a residue that was chromatographed on silica gel to afford the dioxolanes as free bases. The hydrochlorides **7** were prepared starting from the corresponding free bases and 37% aqueous HCl (molar ratio 1:1.3) in 2-propanol (1–2 mL) in a manner identical to the one described for the hydrochlorides of the imidazole–ketones **5**.

4.4.1. 1-((2-(2-Phenylethyl)-1,3-dioxolan-2-yl)methyl)-1*H*-imidazole hydrochloride (7a). White solid (90 mg, 61%), mp 164–165 °C, $R_f = 0.0$ (ethyl acetate); ^1H NMR (400 MHz, D_2O): δ 2.00–2.06 (m, 2H), 2.72–2.78 (m, 2H), 3.66 (t, $J = 8$ Hz, 2H), 4.02 (t, $J = 7.2$ Hz, 2H), 4.43 (s, 2H), 7.26–7.31 (m, 3H), 7.34–7.40 (m, 2H), 7.48 (s, 1H), 7.49 (s, 1H), 8.72 (s, 1H); ^{13}C NMR (100 MHz, D_2O): δ 28.6, 37.0, 53.4, 65.8, 108.0, 119.3, 123.4, 126.3, 128.4, 128.8, 135.7, 141.4; HRMS (ESI) Calcd for $C_{15}H_{19}N_2O_2$: 259.1446 $[M+H]^+$. Found: 259.1441. Anal. Calcd for $C_{15}H_{19}ClN_2O_2 \cdot H_2O$: C, 57.60; H, 6.77; N, 8.96. Found: C, 57.79; H, 6.53; N, 8.99.

4.4.2. 1-((2-(2-(4-Fluorophenyl)ethyl)-1,3-dioxolan-2-yl)methyl)- 1*H*-imidazole hydrochloride (7b). White solid (91 mg, 58%), mp 153–154 °C, $R_f = 0.0$ (ethyl acetate); ^1H NMR (400 MHz, D_2O): δ 1.99–2.04 (m, 2H), 2.69–2.75 (m, 2H), 3.64 (t, $J = 6.4$ Hz, 2H), 4.00 (t, $J = 7.2$ Hz, 2H), 4.43 (s, 2H), 7.02–7.09 (m, 2H), 7.22–7.27 (m, 2H), 7.46 (s, 1H), 7.48 (s, 1H), 8.71 (s, 1H); ^{13}C NMR (100 MHz, D_2O): δ 27.8, 37.1, 53.4, 65.8, 108.0, 115.2 (d, $J^2_{C,F} = 21$ Hz), 119.3, 123.4, 129.8 (d, $J^3_{C,F} = 8$ Hz), 135.8, 137.1 (d, $J^4_{C,F} = 3$ Hz),

161.1 (d, $J^{1}_{C,F} = 240$ Hz); ^{19}F NMR (376 MHz, D_2O): δ −118.9; HRMS (ESI) Calcd for $C_{15}H_{18}FN_2O_2$: 277.1352 $[M+H]^+$. Found: 277.1340. Anal. Calcd for $C_{15}H_{18}ClFN_2O_2$: C, 57.60; H, 5.80; N, 8.96. Found: C, 57.86; H, 5.82; N, 8.98.

4.4.3. 1-((2-(2-(4-Bromophenyl)ethyl)-1,3-dioxolan-2-yl) methyl)- 1*H*-imidazole hydrochloride (7c). White solid (110 mg, 59%), mp 205–207 °C, $R_f = 0.0$ (ethyl acetate); ^{1}H NMR (400 MHz, D_2O): δ 1.97–2.03 (m, 2H), 2.66–2.72 (m, 2H), 3.60–3.65 (m, 2H), 3.96–4.01 (m, 2H), 4.42 (s, 2H), 7.16 (d, $J = 8$ Hz, 2H), 7.45–7.49 (m, 4H), 8.72 (s, 1H); ^{13}C NMR (100 MHz, D_2O): δ 28.0, 36.7, 53.3, 65.8, 107.9, 119.2, 119.3, 123.4, 130.2, 131.4, 135.7, 140.5; HRMS (ESI) Calcd for $C_{15}H_{18}{}^{81}BrN_2O_2$: 339.0525 $[M+H]^+$. Found: 339.0510. Anal. Calcd for $C_{15}H_{18}BrClN_2O_2$: C, 48.21; H, 4.86; N, 7.50. Found: C, 48.40; H, 4.73; N, 7.43.

4.4.4. 1-((2-(2-(4-Iodophenyl)ethyl)-1,3-dioxolan-2-yl) methyl)- 1*H*-imidazole hydrochloride (7d). White solid (105 mg, 50%), mp 241–243 °C (dec), $R_f = 0.0$ (ethyl acetate); ^{1}H NMR (400 MHz, CD_3OD): δ 1.94–2.01 (m, 2H), 2.68–2.77 (m, 2H), 3.58–3.67 (m, 2H), 3.93–4.03 (m, 2H), 4.47 (s, 2H), 7.02 (d, $J = 8.4$ Hz, 2H), 7.56 (s, 1H), 7.59–7.64 (m, 3H), 8.93 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 30.0, 38.7, 54.6, 66.9, 91.6, 109.0, 120.4, 125.2, 131.6, 137.8, 138.7, 142.5; HRMS (ESI) Calcd for $C_{15}H_{18}IN_2O_2$: 385.0413 $[M+H]^+$. Found: 385.0406. Anal. Calcd for $C_{15}H_{18}ClIN_2O_2$: C, 42.83; H, 4.31; N, 6.66. Found: C, 42.87; H, 4.38; N, 6.54.

4.5. General procedure for the preparation of imidazole–alcohol hydrochlorides 8 through the reduction of ketones 5

A solution of an imidazole–ketone **5** (0.5 mmol) in methanol (10 mL) was gradually treated with sodium borohydride (57 mg, 1.5 mmol). After the reducing agent had been added, the reaction mixture was further stirred for 3 h, and then the solvent was removed in vacuo to give a solid residue that was partitioned between ethyl acetate (10 mL) and water (10 mL). The aqueous phase was extracted further with ethyl acetate (2× 10 mL), the combined organic phase was dried over anhydrous Na_2SO_4, and then the solvent was removed to give the desired alcohols as free bases. These compounds were turned into the corresponding hydrochlorides **8** upon treatment with 37% aqueous HCl (molar ratio 1:1.3) in 2-propanol (1–2 mL) in a manner similar to the one described for the preparation of the hydrochlorides of the imidazole–ketones **5**.

4.5.1. (±)-1-(1*H*-Imidazol-1-yl)-4-phenyl-2-butanol hydrochloride (8a). White solid (102 mg, 81%), mp 56–57 °C, $R_f = 0.16$ (ethyl acetate); ^{1}H NMR (400 MHz, CD_3OD): δ 1.66–1.80 (m, 1H), 1.82–1.93 (m, 1H), 2.71–2.81 (m, 1H), 2.84–2.94 (m, 1H), 3.86–3.95 (m, 1H), 4.19 (dd, $J = 8.0$ and 14.0 Hz, 1H), 4.41 (dd, $J = 3.0$ and 13.8 Hz, 1H), 7.21–7.38 (m, 5H), 7.64 (s, 1H), 7.70 (s, 1H), 8.99 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 32.5, 37.4, 56.0, 69.9, 120.8, 124.0, 127.1, 129.6, 129.7, 137.0, 143.1; HRMS (ESI) Calcd for $C_{13}H_{17}N_2O$: 217.1341 $[M+H]^+$. Found: 217.1344. Anal. Calcd for $C_{13}H_{17}ClN_2O$: C, 61.78; H, 6.78; N, 11.08. Found: C, 61.68; H, 6.87; N, 10.95.

4.5.2. (±)-4-(4-Bromophenyl)-1-(1*H*-imidazol-1-yl)-2-butanol hydrochloride (8c). White solid (111 mg, 67%), mp 174–175 °C, $R_f = 0.0$ (ethyl acetate); ^{1}H NMR (400 MHz, CD_3OD): δ 1.65–1.76 (m, 1H), 1.78–189 (m, 1H), 2.64–2.74 (m, 1H), 2.77–2.88 (m, 1H), 3.81–3.89 (m, 1H), 4.17 (dd, $J = 8.4$ and 13.6 Hz, 1H), 4.36 (dd, $J = 3.0$ and 13.8 Hz, 1H), 7.16 (d, $J = 8.0$ Hz, 1H), 7.42 (d, $J = 8.4$ Hz, 1H), 7.56 (s, 1H), 7.63 (s, 1H), 8.90 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 31.9, 37.07, 56.2, 70.0, 120.6, 124.1, 131.5, 132.5, 136.9, 142.1; HRMS (ESI) Calcd for $C_{13}H_{16}BrN_2O$: 295.0446 $[M+H]^+$. Found: 295.0432. Anal. Calcd for $C_{13}H_{16}BrClN_2O$: C, 47.08; H, 4.86; N, 8.45. Found: C, 47.19; H, 5.00; N, 8.56.

4.5.3. (±)-1-(1*H*-Imidazol-1-yl)-4-(4-iodophenyl)-2-butanol hydrochloride (8d). White solid (136 mg, 72%), mp 196–197 °C, $R_f = 0.0$ (ethyl acetate); ^{1}H NMR (400 MHz, D_2O): δ 1.64–1.75 (m, 1H), 1.78–1.88 (m, 1H), 2.63–2.73 (m, 1H), 2.75–2.85 (m, 1H), 3.81–3.89 (m, 1H), 4.13 (dd, $J = 8.4$ and 14.6 Hz, 1H), 4.36 (d, $J = 14$ Hz, 1H), 7.03 (d, $J = 8$ Hz, 2H), 7.56 (s, 1H), 7.58–7.65 (m, 3H), 8.92 (s, 1H); ^{13}C NMR (100 MHz, D_2O): 32.0, 37.0, 56.3, 70.0, 91.6, 120.7, 124.1, 131.8, 136.9, 138.7, 142.7; HRMS (ESI) Calcd for $C_{13}H_{16}IN_2O$: 343.0307 $[M+H]^+$. Found: 343.0319. Anal. Calcd for $C_{13}H_{16}ClIN_2O$: C, 41.24; H, 4.26; N, 7.40. Found: C, 41.20; H, 4.44; N, 7.32.

4.6. General procedure for the preparation of alcohols 9

To a suspension of magnesium turnings (168 mg, 6.92 mmol) in diethyl ether (3 mL), stirred under a nitrogen atmosphere, was added a small portion (0.15 mL) of a solution of 4-halobenzyl chloride (6.92 mmol) in diethyl ether (2 mL), followed by a crystal of iodine. The remaining solution of 4-halobenzyl chloride was then added over a period of 15 min, and then the mixture was heated at reflux temperature for 15 min. The resulting Grignard reagent was then cooled to room temperature, and added dropwise, using a syringe, to a solution of (±)-epichlorohydrin (640 mg, 541 μL, 6.92 mmol) in diethyl ether (3 mL) over a period of 10 min. The reaction mixture was then stirred at room temperature for 30 min, then heated at reflux temperature for 2 h, and diluted with water (10 mL) and ethyl acetate (10 mL). Hydrochloric acid (10 mL, 1.0 M) was then added dropwise until all of the solids dissolved. The organic layer was then separated, and the aqueous layer extracted with ethyl acetate (3× 10 mL). The combined organic phase was then washed with water (10 mL), dried over anhydrous Na_2SO_4, and concentrated. The resulting oil was chromatographed on a column of silica gel using hexanes–ethyl acetate as the mobile phase to give the title compounds.

4.6.1. (±)-1-Chloro-4-(4-fluorophenyl)-2-butanol (9b). Clear oil (964 mg, 69%), $R_f = 0.68$ (hexanes–ethyl acetate 1:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.83 (m, 2H), 2.70–2.75 (m, 1H), 2.80–2.83 (m, 1H), 3.51 (dd,

J = 7.1 and 11.1 Hz, 1H), 3.65 (dd, J = 3.2 and 7.8 Hz, 1H), 3.81 (m, 2H), 7.00 (t, J = 8.6 Hz, 2H), 7.18 (t, J = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.9, 35.9, 50.5, 70.4, 115.2 (d, $J^2_{C,F} = 21.2$ Hz), 129.8 (d, $J^3_{C,F} = 7.8$ Hz), 161.4 (d, $J^1_{C,F} = 243.8$); HRMS (EI) Calcd for $C_{10}H_{12}ClFO$: 202.0561 (M^+). Found: 202.0566.

4.6.2. (±)-1-Chloro-4-(4-chlorophenyl)-2-butanol (9e).[13] Golden oil (860 mg, 57%), R_f = 0.18 (hexanes–ethyl acetate 9:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.73–1.90 (m, 2H), 2.20 (br s, 1 H), 2.63–2.73 (m, 1H), 2.76–2.87 (m, 1H), 3.49 (dd, J = 7.0 and 11.0 Hz, 1H), 3.62 (dd, J = 3.2 and 11.2 Hz, 1H), 3.74–3.83 (m, 1H), 7.13 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.2, 35.8, 50.6, 70.6, 128.8, 129.9, 132.0, 139.9; HRMS (EI) Calcd for $C_{10}H_{12}Cl_2O$: 218.0265 (M^+). Found 218.0260.

4.7. General procedure for the preparation of imidazole–alcohol hydrochlorides 8 through the N-alkylation of imidazole with alcohols 9

A dispersion of 60% sodium hydride in mineral oil (960 mg, 24 mmol) was washed twice with hexanes under a nitrogen atmosphere, the solid suspended in dry DMF (5 mL), and added portionwise to a cooled stirred solution of imidazole (1.7 g, 25 mmol) in dry DMF (5 mL). The mixture was brought to room temperature and stirred until the evolution of hydrogen ceased, then warmed at 70–80 °C. A solution of compound **9** (5 mmol) in DMF (5 mL) was then added dropwise, using a syringe, and the reaction mixture was further stirred at 70–80 °C for 4.5 h, then cooled to room temperature. (±)-4-(4-Fluorophenyl)-1-(1*H*-imidazol-1-yl)-2-butanol was isolated by pouring the mixture onto ice (50 g), followed by extraction with ethyl acetate (50 mL). The organic phase was washed with brine (3× 50 mL), dried over anhydrous Na_2SO_4, then the solvent was removed to give a residue from which the desired compound was separated by chromatography on silica gel using ethyl acetate–methanol (4:1 v/v) as the mobile phase. Alternatively, (±)-4-(4-chlorophenyl)-1-(1*H*-imidazol-1-yl)-2-butanol was isolated from the reaction mixture by addition of hexanes (10 mL), followed by addition of ice-cold water until a precipitate started to form. This mixture was then poured in small portions onto ice–water, the separated solid was removed by filtration, washed thoroughly, sequentially with cold water, cold hexanes, and finally with cold water again to give the desired imidazole–alcohol as a free base. In order to prepare the corresponding hydrochlorides **9a** and **9e**, the free bases of imidazole–alcohols were treated with 37% aqueous HCl (molar ratio 1:1.3) in 2-propanol (5–7 mL) in a manner similar to the one described for the preparation of the hydrochlorides of the imidazole–ketones **5**.

4.7.1. (±)-4-(4-Fluorophenyl)-1-(1*H*-imidazol-1-yl)-2-butanol hydrochloride (8b). Colorless solid (839 mg, 62%), mp 86–87 °C, R_f = 0.0 (ethyl acetate); ^{1}H NMR (400 MHz, D_2O): δ 1.65–1.78 (m, 1H), 1.82–1.92 (m, 1H), 2.64–2.75 (m, 1H), 2.77–2.87 (m, 1H), 3.86–3.96 (m, 1H), 4.13 (dd, J = 8.1 and 14.2 Hz, 1H), 4.32 (dd, J = 2.9 and 14.2 Hz, 1H), 7.06 (t, J = 8.9 Hz, 2H), 7.24–7.32 (m, 2H), 7.43 (s, 2H), 8.66 (s, 1H); ^{13}C NMR (100 MHz, D_2O): δ 30.0, 34.9, 54.6, 68.6, 115.1 (d, $J^2_{C,F} = 21.2$ Hz), 119.6, 122.3, 130.0 (d, $J^3_{C,F} = 8.0$ Hz), 137.2 (d, $J^4_{C,F} = 3.0$ Hz), 161.1 (d, $J^1_{C,F} = 241.2$); ^{19}F NMR (376 MHz, D_2O): δ -119.0; HRMS (ESI) Calcd for $C_{13}H_{16}FN_2O$: 235.1247 $[M+H]^+$. Found: 235.1247. Anal. Calcd for $C_{13}H_{16}ClFN_2O$: C, 57.67; H, 5.96; N, 10.35. Found: C, 57.75; H, 5.94; N, 10.49.

4.7.2. (±)-4-(4-Chlorophenyl)-1-(1*H*-imidazol-1-yl)-2-butanol hydrochloride (8e). Colorless solid (775 mg, 54%), mp 138–140 °C, R_f = 0.0 (ethyl acetate); ^{1}H NMR (400 MHz, D_2O): δ 1.70–1.78 (m, 1H), 1.82–1.91 (m, 1H), 2.67–2.74 (m, 1H), 2.78–2.86 (m, 1H), 3.88–3.94 (m, 1H), 4.15 (dd, J = 8.0 and 14.0 Hz, 1H), 4.34 (dd, J = 3.0 and 14.2 Hz, 1H), 7.25 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 7.45 (s, 1H), 7.46 (s, 1H), 8.68 (s, 1H); ^{13}C NMR (100 MHz, D_2O): δ 30.5, 35.0, 54.9, 69.0, 120.0, 122.6, 128.8, 130.4, 131.4, 135.3, 140.5; HRMS (ESI) Calcd for $C_{13}H_{16}ClN_2O$: 251.0945 $[M+H]^+$. Found: 251.0949. Anal. Calcd for $C_{13}H_{16}Cl_2N_2O$: C, 54.37; H, 5.62; N, 9.75. Found: C, 54.56; H, 5.72; N, 9.18.

4.8. Biological evaluation

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).[30,31] Spleen and brain (Sprague–Dawley rats) microsomal fractions were prepared according to the procedure outlined by Appleton et al.[32] Protein concentration of microsomal fractions was determined by a modification of the biuret method.[31] 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 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 gas chromatography according to the method described by Vreman and Stevenson.[30]

The data resulting from the above experiments were plotted as non-linear regression (sigmoidal dose–response) curves using the GraphPad Prism (version 3) computer program. The values on the abscissa represent the logarithm of the inhibitor's 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 value of the concentration (EC_{50}) of the inhibitor at which the enzyme's 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, have been retrieved using the same program, and input in 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 was inhibited by 50% compared to the control.

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

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