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Selective inhibition of heme oxygenase-2 activity by analogs of 1-(4-chlorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole (clemizole): Exploration of the effects of substituents at the *N*-1 position

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ABSTRACT

Several analogs based on the lead structure of 1-(4-chlorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole (clemizole) were synthesized and evaluated as novel inhibitors of heme oxygenase (HO). Many of the compounds were found to be potent and highly selective for the HO-2 isozyme (constitutive), and had substantially less inhibitory activity on the HO-1 isozyme (inducible). The compounds represent the first report of highly potent and selective inhibitors of HO-2 activity, and complement our suite of selective HO-1 inhibitors. The study has revealed many candidates based on the inhibition of heme oxygenases for potentially useful pharmacological and therapeutic applications.

1. Introduction

Currently, there is great interest in gasotransmitters,[1] substances that exist as gaseous compounds dissolved in biological fluids at usual mammalian temperatures and pressures, and that have the ability to regulate a variety of biological functions. Three substances generally have been recognized as gasotransmitters, namely, nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S). Our laboratories have focussed on CO and the enzymes that are responsible for its biosynthesis, namely, the heme oxygenases (HOs). CO is formed in mammals primarily through the action of HO on the substrate heme.[2,3] While most of the heme oxidation occurs in the liver and spleen during the 'housekeeping' degradation of heme, the formation of CO from heme occurs in significant quantities in sites such as blood vessels and the brain. Indeed, the brain, testes, and spleen have the greatest specific activity of HO.[4] HO activity is attributable to two functional isozymes. HO-1 (known also as HSP-32, molecular weight ~32 kD) is induced by various stimuli including heat shock, heavy metals, heme, and reactive oxygen species. HO-2 has a molecular weight of 36.5 kD and is constitutive. The role of the gasotransmitter, CO, as a regulator of cellular processes in the brain, and in circulatory, respiratory, and immune systems has been recognized widely,[3,5] and its anti-inflammatory, anti-proliferative, and anti-apoptotic effects have been demonstrated.[6] The use of HO inhibitors is critical in the elucidation of the physiological functions of the CO/HO system and related physiological pathways.

The most intensively studied and best understood aspect of the CO/HO system is the regulation of HO-1 synthesis, whereas the literature describing a role for HO-2 is relatively sparse. Initially, information on the CO/HO pathway was obtained using metalloporphyrins (such as tin protoporphyrin, SnPP) as HO inhibitors. However, there is a problem with the use of the metalloporphyrin HO inhibitors owing to the lack of selectivity because of the close structural similarity between heme and these inhibitors. We have designed and synthesized several azole-based HO inhibitors that are substantially more-selective than the metalloporphyrins. Many of our compounds were found to be selective for the HO-1 isozyme, and had substantially less inhibitory activity on HO-2.[7–17] In this publication we describe the identification of a highly-selective HO-2 inhibitor and initial exploratory structure–activity relationship (SAR) studies. The availability of selective HO-2 inhibitors, in addition to earlier selective HO-1 inhibitors, will facilitate investigations of the involvement of HO-1 and HO-2 in a variety of physiological roles. To our knowledge the compounds represent the first reported highly selective HO-2 inhibitors.

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2. Results and discussion

From the screening of a compound library, an initial hit for a selective inhibitor of HO-2 was discovered, namely, the benzimidazole, clemizole, whose in vitro IC_{50} against HO-2 (rat brain microsomes) was 3.4 ± 0.3 μM and against HO-1 (rat spleen microsomes) was >100 μM. Here we present the chemical synthesis of candidate compounds and an initial SAR study of analogs of clemizole bearing a selected variety of substituents at the *N*-1 position (see Fig. 1).

2.1. Chemical synthesis

The synthesis of the benzimidazole compounds is shown in Scheme 1 and involved the alkylation of pyrrolidine with 2-(chloromethyl)benzimidazole in ethanol. The alkylation of the resulting benzimidazole derivative was accomplished using two different procedures. Procedure A involved the treatment of the benzimidazole derivative with sodium hydride in tetrahydrofuran, followed by the addition of an alkyl halide and tetra-*n*-butylammonium bromide as phase-transfer catalyst (see Ref. 18). Alternatively, Procedure B involved the treatment of the benzimidazole derivative with sodium hydroxide in dimethyl sulfoxide, followed by the addition of an alkyl halide. The final alkylated benzimidazoles were isolated mainly in the dihydrochloride salt form. Many novel compounds were synthesized using this simple two-step procedure; an attractive feature for large-scale development.

2.2. Biological evaluation

Results for inhibition of HO activity, in vitro, through determining the concentration necessary to decrease enzyme activity by 50% (inhibitory concentration 50%, IC_{50}) are shown in Table 1. Compounds **1**–**12** represent a series of 4-substituted benzyl compounds. The 4-fluoro, 4-bromo, and 4-iodo analogs (**2**–**4**) were found to have essentially the same HO-2 inhibitory activity as the hit clemizole (**1**). In the case of compounds **5**–**12**, having a variety of electron-donating and electron-withdrawing substituents, the least potent compound was found to be the 4-benzyloxy analog **9**. Moreover, compounds **1**–**3** had high selectivity indices for HO-2. In the case of the 3-substituted compounds **13**–**17**, the 3-chloro, 3-bromo, and 3-nitro derivatives were all found to be highly potent inhibitors of HO-2; the least potent was the 3-cyano analog **16**. The 3-nitro compound **17** was found to be the most potent, having a correspondingly high selectivity index. Interestingly, the 2-substituted analogs **18**–**22** were found all to be highly potent inhibitors of HO-2. The dichlorinated analogs **23**–**28** were found to be either highly potent or at least moderately potent towards HO-2 inhibition. Compounds **29**–**31** have different linkers between *N*-1 and the phenyl group. The methylene (**29**) and ethylene (**30**) analogs were found to exhibit essentially the same inhibitory activity; however, the introduction of an oxygen atom in **30** to give **31** resulted in a loss of activity. Compounds **32**–**36** represent a departure from the monophenyl analogs described thus far; compounds **32**–**34** were found to exhibit essentially the same high potency, whereas compound **36**, devoid of a substituent at *N*-1, was found to be inactive.

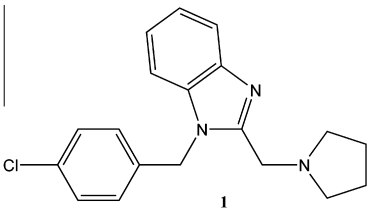

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Figure 1. The structure of clemizole (**1**).

3. Conclusions

We have synthesized several compounds that express high selectivity as inhibitors of HO-2 relative to HO-1. Specifically, compounds **1**–**3**, **5**, **13**–**15**, **17**–**22**, **25**, **28**–**30**, **32**, and **33** are noteworthy for their potency and selectivity towards HO-2. This initial SAR study explored the effects of substituents at the *N*-1 position of 1-(4-chlorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole (clemizole); the introduction of a variety of substituents on the *N*-1 benzyl group was well-tolerated as regards HO-2 inhibitory potency and selectivity. The synthetic protocol for the generation of analogs involved a straightforward two-step process. To our knowledge, the work described represents the first report of highly potent and selective inhibitors of HO-2. This study has provided new potentially useful pharmacological tools for the study of the CO/HO system, and complement our suite of selective HO-1 inhibitors. The compounds could be of use in exploring the role of enzymic products in those cells that are rich in HO-2 activity. These novel compounds might also have useful therapeutic applications.

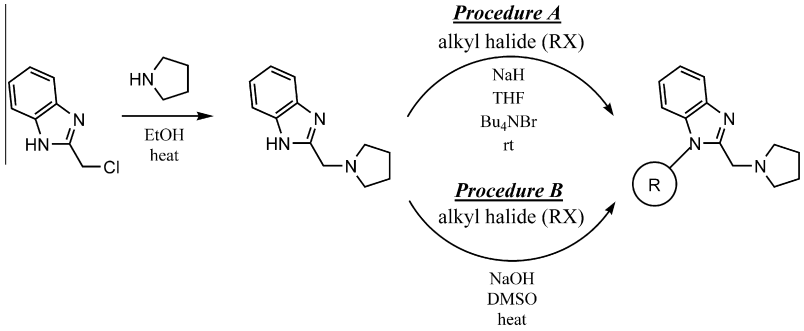
4. Experimental

4.1. General

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 F254 plates (Silicycle), and the compounds were visualized either by UV illumination (254 nm), or by heating after spraying with phosphomolybdic acid in ethanol. Melting points were measured on a Mel-Temp II apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$, CD_3OD, or DMSO-d_6. The chemical shifts are reported in δ (ppm) relative to tetramethylsilane.[19] The compounds synthesized were deemed >95% pure by ^1H NMR analysis. 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 MeOH at a flow rate of 6 μL/min. High-resolution EI mass spectra were recorded on a Waters/Micromass GC-TOF instrument. 2-(Chloromethyl)benzimidazole and all other chemicals were obtained from Sigma–Aldrich.

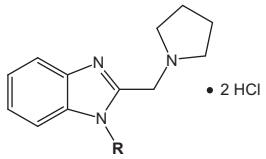
4.2. Representative *Procedure A* for the formation of alkylated benzimidazole derivatives, as outlined in Scheme 1

*1-(4-Chlorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1H-benzimidazole dihydrochloride (**1**).* Under an atmosphere of nitrogen, compound **36** (250 mg, 1.24 mmol, 1 equiv) was dissolved in freshly distilled warm tetrahydrofuran (THF) (2.5 mL), then cooled to 0 °C. To this solution was added 60% NaH in oil (100 mg, 60 mg pure, 2.48 mmol, 2 equiv) and the mixture stirred at 0 °C for 5 min. 4-Chlorobenzyl bromide (255 mg, 1.24 mmol, 1 equiv) was added followed by the addition of tetra-*n*-butylammonium bromide (23 mg, 0.07 mmol, 6 mol %). The mixture was stirred at rt overnight, then diluted with a solution of water (2 drops) in THF (5 mL). The solution was filtered through Celite, and the Celite was washed with THF (50 mL) and then EtOAc (50 mL). The filtrate was concentrated and the remaining residue purified by flash column chromatography on silica gel (EtOAc) to give the free base (195 mg) as an oil (R_f = 0.46, EtOAc). To a solution of the free base in MeOH (2 mL) was added a solution of 37% aqueous HCl (158 mg, 1.60 mmol, 2.7 equiv) in MeOH (2 mL). The mixture was



Scheme 1. Generation of benzimidazole derivatives.

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



Compound	Substituent R	IC_{50} (μM) rat spleen (HO-1)	IC_{50} (μM) rat brain (HO-2)	Selectivity index IC_{50} (HO-1)/IC_{50} (HO-2)
1	4-Cl-$PhCH_2$	>100	3.4 ± 0.3	>29
2	4-F-$PhCH_2$	>100	2.0 ± 0.2	>50
3	4-Br-$PhCH_2$	>100	2.6 ± 0.4	>38
4	4-I-$PhCH_2$	>100	7.6 ± 0.6	>13
5	4-Me-$PhCH_2$	>100	5 ± 1	>20
6	4-*i*-Pr-$PhCH_2$	>100	55 ± 18	>1.8
7	4-OMe-$PhCH_2$	>100	18 ± 5	>5
8	4-SMe-$PhCH_2$	>100	15 ± 3	>6
9[a]	4-OBn-$PhCH_2$	>100	63 ± 23	>1.5
10	4-CN-$PhCH_2$	>100	11 ± 1	>9
11	4-CF_3-$PhCH_2$	>100	15.6 ± 0.4	>6.4
12	4-NO_2-$PhCH_2$	>100	27 ± 5	>3
13	3-Cl-$PhCH_2$	>100	1.65 ± 0.08	>60
14	3-Br-$PhCH_2$	>100	1.5 ± 0.3	>66
15	3-Me-$PhCH_2$	>100	5 ± 1	>20
16	3-CN-$PhCH_2$	>100	15.8 ± 0.3	>6
17	3-NO_2-$PhCH_2$	>100	1.3 ± 0.2	>76
18	2-Cl-$PhCH_2$	>100	4.8 ± 0.4	>20
19	2-Br-$PhCH_2$	42 ± 1	1.6 ± 0.3	26
20	2-Me-$PhCH_2$	76 ± 17	1.8 ± 0.2	42
21	2-CN-$PhCH_2$	>100	4 ± 1	>25
22	2-NO_2-$PhCH_2$	>100	3.5 ± 0.6	>28
23	2,3-diCl-$PhCH_2$	>100	14.7 ± 0.5	>6
24	2,4-diCl-$PhCH_2$	62 ± 10	6.1 ± 0.3	10
25	2,5-diCl-$PhCH_2$	96 ± 5	4 ± 3	24
26	2,6-diCl-$PhCH_2$	>100	9.2 ± 0.2	>10
27	3,4-diCl-$PhCH_2$	>100	17 ± 3	>5
28	3,5-diCl-$PhCH_2$	>100	2.1 ± 0.3	>47
29	$PhCH_2$	>100	3.1 ± 0.7	>32
30	$PhCH_2CH_2$	>100	2.7 ± 0.2	>37
31	$PhOCH_2CH_2$	>100	>100	—
32[b]	$(Ph)_2CH$	>100	4.1 ± 0.1	>24
33	(Naphthalene-2-yl)-CH_2	>100	5 ± 2	>20
34	Cyclohexyl-CH_2	>100	6 ± 1	>16
35	$CH_3CH_2CH_2$	>100	27 ± 5	>3
36	H	>100	>100	—

Data represent mean IC_{50} values ± SD of replicate experiments (n = 4).
[a] Isolated in the free base form.
[b] Isolated in the monohydrochloride form.

concentrated, Et_2O (5 mL) was added, and the mixture concentrated again. High-vacuum drying gave the product (242 mg, 0.61 mmol, 49%) as a white solid; mp 232–234 °C; R_f = 0 (EtOAc); ^{1}H NMR (400 MHz, CD_3OD): δ 2.10–2.26 (m, 4H), 3.55–3.78 (m, 4H), 5.21 (s, 2H), 5.95 (s, 2H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.53–7.68 (m, 3H), 7.92 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 24.0, 48.0, 50.0, 56.5, 114.4, 117.3, 128.0, 128.1, 129.9, 130.4, 134.0, 134.3, 135.1, 135.7, 144.5; HRMS (ESI) $[M-Cl]^+$ Calcd for $C_{19}H_{21}N_3Cl$: 326.1424. Found: 326.1433.

4.3. Representative *Procedure B* for the formation of alkylated benzimidazole derivatives, as outlined in Scheme 1

*1-Benzyl-2-(pyrrolidin-1-ylmethyl)-1H-benzimidazole dihydrochloride (**29**).* Under an atmosphere of nitrogen, compound **36** (511 mg, 2.54 mmol, 1 equiv) was combined with sodium hydroxide (102 mg, 2.54 mmol, 1 equiv) and DMSO (4 mL). The mixture was heated at 75–80 °C for 15 min, cooled to rt, then benzyl bromide (0.30 mL, 434 mg, 2.54 mmol, 1 equiv) was added. The mixture was stirred at rt for 2 h, then at ~60 °C for 21 h. After cooling to rt, a saturated aqueous solution of $NaHCO_3$ was added and the mixture extracted with EtOAc (2×). The combined organic extracts were washed with a saturated aqueous solution of $NaHCO_3$ (2×), then with brine, and dried (Na_2SO_4). The solution was concentrated and the remaining brown oil residue purified by flash column chromatography on silica gel (EtOAc) to give the free base (97 mg, 0.33 mmol) as an oil (R_f = 0.53, EtOAc). To a solution of the free base in MeOH (3 mL) was added a solution of 37% aqueous HCl (98 mg, 0.99 mmol, 3 equiv) in MeOH (3 mL). The mixture was concentrated, Et_2O (5 mL) was added, and the mixture concentrated again. High-vacuum drying gave the product (130 mg, 0.36 mmol, 14%) as a white solid; mp 126–128 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.16 (m, 4H), 3.22–3.50 (m, 2H), 3.50–3.80 (m, 2H), 4.97 (s, 2H), 5.78 (s, 2H), 7.22–7.43 (m, 7H), 7.56–7.62 (m, 1H), 7.76–7.81 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 47.4, 47.7, 53.9, 112.1, 118.0, 123.8, 124.3, 127.1, 127.9, 128.8, 134.1, 135.8, 138.7, 145.4; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{22}N_3$: 292.1813. Found: 292.1810.

4.4. Characterization of the compounds synthesized following the representative procedure for the formation of alkylated benzimidazole derivatives

4.4.1. 1-(4-Fluorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (2)

Compound **2** was prepared using *Procedure B* with **36** and 4-fluorobenzyl bromide as starting materials to give the product (35 mg, 3%) as a beige solid (after recrystallization from 2-propanol); mp 166–168 °C; R_f = 0 (EtOAc); ^{1}H NMR (400 MHz, CD_3OD): δ 2.08–2.22 (m, 4H), 3.50–3.73 (m, 4H), 4.90 (s, 2H), 5.66 (s, 2H), 7.08 (app t, *J* = 8.4 Hz, 2H), 7.20–7.28 (m, 2H), 7.34–7.42 (m, 2H), 7.48–7.55 (m, 2H), 7.74–7.81 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 24.1, 48.1, 50.7, 56.5, 112.5, 116.8, 117.0, 119.7, 125.4 (d, $^2J_{C-F}$ = 67.6 Hz), 130.0 (d, $^3J_{C-F}$ = 8.3 Hz), 132.7 (d, $^4J_{C-F}$ = 3.4 Hz), 136.1, 141.2, 146.3, 163.9 (d, $^1J_{C-F}$ = 246.0 Hz); ^{19}F NMR (376 MHz, CD_3OD): δ −116.9 (t, $^3J_{F-H}$ = 4.3 Hz); HRMS (EI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{21}N_3F$: 310.1720. Found: 310.1711.

4.4.2. 1-(4-Bromobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (3)

Compound **3** was prepared using *Procedure A* with **36** and 4-bromobenzyl bromide as starting materials to give the product (133 mg, 44%) as a beige solid; mp 122–124 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.20 (m, 4H), 3.22–3.50 (m, 2H), 3.50–3.80 (m, 2H), 4.92 (s, 2H), 5.73 (s, 2H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.30–7.37 (m, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.55–7.61 (m, 1H), 7.73–7.81 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 46.8, 47.8, 53.9, 111.7, 118.4, 121.0, 123.4, 124.0, 129.3, 131.7, 134.3, 135.4, 139.7, 145.8; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{21}N_3Br$: 370.0918. Found: 370.0921.

4.4.3. 1-(4-Iodobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (4)

Compound **4** was prepared using *Procedure A* with **36** and 4-iodobenzyl bromide as starting materials to give the product (387 mg, 60%) as a beige solid; mp 141–144 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.20 (m, 4H), 3.20–3.40 (m, 2H), 3.60–3.80 (m, 2H), 4.88 (s, 2H), 5.67 (s, 2H), 7.01 (d, *J* = 8.4 Hz, 2H), 7.28–7.35 (m, 2H), 7.52–7.58 (m, 1H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.72–7.77 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 46.7, 48.0, 54.0, 111.6, 118.6, 123.2, 123.8, 129.3, 132.8, 134.5, 135.9, 137.5, 140.2, 145.9; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{21}N_3I$: 418.0780. Found: 418.0780.

4.4.4. 1-(4-Methylbenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (5)

Compound **5** was prepared using *Procedure A* with **36** and 4-methylbenzyl bromide as starting materials to give the product (648 mg, 87%) as a beige solid; mp 120–125 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 2.14–2.23 (m, 4H), 2.32 (s, 3H), 3.58–3.72 (m, 4H), 5.23 (s, 2H), 5.92 (s, 2H), 7.18–7.24 (m, 4H), 7.55–7.60 (m, 1H), 7.64 (td, *J* = 7.7, 0.9 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.92 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 21.1, 24.0, 47.8, 50.6, 56.4, 114.7, 116.9, 128.1, 128.3 (2C), 130.9, 132.0, 134.3, 134.4, 140.1, 144.2; HRMS (MALDI) $[M-HCl_2]^+$ Calcd for $C_{20}H_{24}N_3$: 306.1970. Found: 306.1973.

4.4.5. 1-(4-Isopropylbenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (6)

Compound **6** was prepared using *Procedure A* with **36** and 4-isopropylbenzyl chloride as starting materials to give the product (155 mg, 31%) as a beige solid; mp 110–112 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15 (d, *J* = 6.8 Hz, 6H), 1.86–2.10 (m, 4H), 2.84 (7-tet, 1H), 3.22–3.44 (m, 2H), 3.57–3.77 (m, 2H), 4.94 (s, 2H), 5.70 (s, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 3.2 Hz, 1H), 7.36 (d, *J* = 3.2 Hz, 1H), 7.62 (dd, *J* = 6.4, 2.8 Hz, 1H), 7.77 (dd, *J* = 6.4, 2.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 23.8, 33.1, 47.2, 47.7, 53.9, 111.9, 118.2, 123.5, 124.1, 126.7, 127.0, 133.3, 134.7, 139.3, 145.6, 148.1; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{22}H_{28}N_3$: 334.2283. Found: 334.2279.

4.4.6. 1-(4-Methoxybenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (7)

Compound **7** was prepared using *Procedure A* with **36** and 4-methoxybenzyl chloride as starting materials to give the product (146 mg, 30%) as a beige solid; mp 116–118 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.15 (m, 4H), 3.28–3.44 (m, 2H), 3.60–3.70 (m, 2H), 3.71 (s, 3H), 4.96 (s, 2H), 5.67 (s, 2H), 6.89 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 8.4 Hz, 2H), 7.31–7.39 (m, 2H), 7.58–7.65 (m, 1H), 7.73–7.79 (m, 1H), 11.68 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 47.2, 47.6, 53.9, 55.1, 112.1, 114.2, 118.1, 123.6, 124.1, 127.6, 128.7, 134.1, 138.9, 145.4, 158.9; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{20}H_{24}N_3O$: 322.1919. Found: 322.1915.

4.4.7. 1-(4-Thiomethylbenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (8)

Compound **8** was prepared using *Procedure A* with **36** and 4-thiomethylbenzyl bromide as starting materials to give the product (117 mg, 66%) as a yellow-white solid; mp 126–129 °C; ^{1}H NMR

(400 MHz, DMSO-d_6): δ 1.83–1.99 (m, 2H), 1.99–2.14 (m, 2H), 2.43 (s, 3H), 3.26–3.43 (m, 2H), 3.61–3.76 (m, 2H), 4.95 (s, 2H), 5.70 (s, 2H), 7.17–7.24 (m, 4H), 7.32–7.38 (m, 2H), 7.57–7.62 (m, 1H), 7.74–7.80 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.6, 22.7, 47.2, 47.7, 54.0, 112.0, 118.2, 123.6, 124.1, 126.1, 127.8, 132.3, 134.2, 138.0, 139.2, 145.6; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{20}H_{24}N_3S$: 338.1690. Found: 338.1694.

4.4.8. 1-(4-Benzyloxybenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole (9)

Compound **9** was prepared using *Procedure A* with **36** and 4-benzyloxybenzyl chloride as starting materials to give the product (144 mg, 29%) as a golden oil isolated in the free base form; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.61–1.73 (m, 4H), 2.45–2.55 (m, 4H), 3.85 (s, 2H), 5.04 (s, 2H), 5.49 (s, 2H), 6.96 (d, J = 8.8 Hz, 2H), 7.12–7.19 (m, 4H), 7.31 (d, J = 6.8 Hz, 1H), 7.36 (app t, J = 7.4 Hz, 2H), 7.39–7.45 (m, 3H), 7.56–7.62 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.2, 46.1, 52.1, 53.5, 69.2, 110.5, 114.8, 118.9, 121.3, 122.1, 127.6, 127.8, 128.3, 128.4, 129.3, 135.5, 137.0, 142.1, 152.1, 157.6; HRMS (ESI) $[M+H]^+$ Calcd for $C_{26}H_{28}N_3O$: 398.2232. Found: 398.2315.

4.4.9. 1-(4-Cyanobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (10)

Compound **10** was prepared using *Procedure A* with **36** and 4-(chloromethyl)benzonitrile as starting materials to give the product (129 mg, 27%) as a white solid; mp 126–130 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.18 (m, 4H), 3.20–3.45 (m, 2H), 3.52–3.80 (m, 2H), 4.90 (s, 2H), 5.86 (s, 2H), 7.33 (app t, J = 3.8 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.53 (dd, J = 5.8, 2.6 Hz, 1H), 7.77 (dd, J = 6.2, 2.6 Hz, 1H), 7.82 (d, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 47.0, 47.8, 53.9, 110.6, 111.6, 118.5, 118.6, 123.4, 124.0, 127.9, 132.7, 134.3, 140.0, 141.7, 146.0; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{20}H_{21}N_4$: 317.1766. Found: 317.1759.

4.4.10. 1-(4-Trifluoromethylbenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (11)

Compound **11** was prepared using *Procedure A* with **36** and 4-trifluoromethylbenzyl bromide as starting materials to give the product (225 mg, 42%) as a white solid; mp 236–237 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.20 (m, 4H), 3.20–3.40 (m, 2H), 3.60–3.80 (m, 2H), 4.90 (s, 2H), 5.85 (s, 2H), 7.28–7.36 (m, 2H), 7.39 (d, J = 8.0 Hz, 2H), 7.53–7.58 (m, 1H), 7.71 (d, J = 8.0 Hz, 2H), 7.74–7.80 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 46.7, 48.0, 53.9, 111.5, 118.8, 123.2, 123.8, 123.9 (quartet, $^1J_{C-F}$ = 263.3 Hz), 125.7 (d, $^3J_{C-F}$ = 3.6 Hz), 127.6, 128.3 (d, $^2J_{C-F}$ = 31.7 Hz), 134.5, 140.4, 140.9, 146.1; ^{19}F NMR (376 MHz, DMSO-d_6): δ −62.0; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{20}H_{21}N_3F_3$: 360.1687. Found: 360.1686.

4.4.11. 1-(4-Nitrobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (12)

Compound **12** was prepared using *Procedure A* with **36** and 4-nitrobenzyl bromide as starting materials to give the product (137 mg, 27%) as an orange solid; mp 132–135 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.00 (m, 2H), 2.00–2.20 (m, 2H), 3.20–3.44 (m, 2H), 3.53–3.80 (m, 2H), 4.90 (s, 2H), 5.91 (s, 2H), 7.28–7.38 (5-tet, 2H), 7.44 (d, J = 8.4 Hz, 2H), 7.51–7.57 (m, 1H), 7.73–7.81 (m, 1H), 8.19 (d, J = 8.8 Hz, 2H), 11.61 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 46.7, 48.0, 53.9, 111.5, 118.8, 123.3, 123.9, 124.0, 128.1, 134.5, 140.4, 143.8, 146.1, 147.0; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{21}N_4O_2$: 337.1664. Found: 337.1663.

4.4.12. 1-(3-Chlorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (13)

Compound **13** was prepared using *Procedure A* with **36** and 3-chlorobenzyl bromide as starting materials to give the product (285 mg, 57%) as an off-white solid; mp 115–120 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.20 (m, 4H), 3.20–3.50 (m, 2H), 3.50–3.80 (m, 2H), 3.50–3.80 (m, 2H), 4.95 (s, 2H), 5.77 (s, 2H), 7.14–7.21 (m, 2H), 7.30–7.41 (m, 5H), 7.53–7.61 (m, 1H), 7.74–7.81 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 46.9, 47.7, 53.9, 111.8, 118.4, 123.6, 124.1, 125.7, 126.9, 127.9, 130.7, 133.4, 134.2, 138.4, 139.5, 145.8; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{21}ClN_3$: 326.1424. Found: 326.1428.

4.4.13. 1-(3-Bromobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (14)

Compound **14** was prepared using *Procedure A* with **36** and 3-bromobenzyl bromide as starting materials to give the product (259 mg, 47%) as a white solid; mp 162–165 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.20 (m, 4H), 3.20–3.52 (m, 2H), 3.52–3.83 (m, 2H), 4.96 (s, 2H), 5.77 (s, 2H), 6.82 (br s, ~1H), 7.21 (d, J = 7.6 Hz, 1H), 7.30 (t, J = 8.2 Hz, 1H), 7.33–7.40 (m, 2H), 7.47–7.53 (m, 2H), 7.54–7.61 (m, 1H), 7.75–7.81 (m, 1H), 11.68 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 47.0, 47.6, 53.9, 111.8, 118.3, 122.1, 123.8, 124.3, 126.1, 129.8, 130.9, 131.0, 134.1, 138.6, 139.1, 145.7; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{21}N_3Br$: 370.0918. Found: 370.0903.

4.4.14. 1-(3-Methylbenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (15)

Compound **15** was prepared using *Procedure A* with **36** and 3-methylbenzyl bromide as starting materials to give the product (207 mg, 44%) as a white solid; mp 121–125 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.85–2.15 (m, 4H), 3.25–3.50 (m, 2H), 3.50–3.80 (m, 2H), 4.95 (s, 2H), 5.72 (s, 2H), 7.01 (d, J = 7.6 Hz, 1H), 7.06–7.14 (m, 2H), 7.22 (t, J = 7.6 Hz, 1H), 7.32–7.40 (m, 2H), 7.55–7.63 (m, 1H), 7.74–7.82 (m, 1H), 7.84 (br s, 1H), 11.73 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.9, 22.7, 47.5, 47.6, 53.9, 112.0, 118.1, 123.7, 124.1, 124.2, 127.5, 128.6, 128.7, 134.2, 135.7, 138.1, 138.9, 145.5; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{20}H_{24}N_3$: 306.1970. Found: 306.1978.

4.4.15. 1-(3-Cyanobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (16)

Compound **16** was prepared using *Procedure A* with **36** and 3-cyanobenzyl chloride as starting materials to give the product (202 mg, 42%) as a yellow-white solid; mp 133–136 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.20 (m, 4H), 3.22–3.52 (m, 2H), 3.52–3.84 (m, 2H), 4.98 (s, 2H), 5.82 (s, 2H), ~6.6 (br s, ~1H), 7.30–7.41 (m, 2H), 7.50–7.65 (m, 3H), 7.73–7.84 (m, 3H), 11.68 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.7, 46.9, 47.7, 54.0, 111.7, 111.8, 118.3, 118.6, 123.7, 124.2, 130.0, 130.8, 131.8, 132.2, 134.0, 137.5, 139.3, 145.9; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{20}H_{21}N_4$: 317.1766. Found: 317.1762.

4.4.16. 1-(3-Nitrobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (17)

Compound **17** was prepared using *Procedure A* with **36** and 3-nitrobenzyl bromide as starting materials to give the product (368 mg, 73%) as an orange solid; mp 130–134 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.20 (m, 4H), 3.20–3.50 (m, 2H), 3.50–3.82 (m, 2H), 4.94 (s, 2H), 5.91 (s, 2H), 7.30–7.39 (m, 2H), 7.55–7.67 (m, 4H), 7.75–7.81 (m, 1H), 8.08–8.13 (m, 1H), 8.13–8.19 (m, 1H), 11.60 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 46.6, 47.9, 53.9, 111.6, 118.6, 121.8, 122.8, 123.5, 124.1, 130.4, 133.6, 134.3, 138.3, 140.0, 146.0, 148.0; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{21}N_4O_2$: 337.1664. Found: 337.1660.

4.4.17. 1-(2-Chlorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (18)

Compound **18** was prepared using *Procedure A* with **36** and 2-chlorobenzyl bromide as starting materials to give the product (310 mg, 63%) as a white solid; mp 203–204 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.84–2.15 (m, 4H), 3.21–3.45 (m, 2H), 3.55–3.80 (m, 2H), 4.88 (s, 2H), 5.78 (s, 2H), 6.72 (d, J = 6.8 Hz, 1H), 7.24 (td, J = 7.5, 0.9 Hz, 1H), 7.28–7.42 (m, 4H), 7.56 (dd, J = 8.0, 0.8 Hz, 1H), 7.79 (d, J = 7.2 Hz, 1H), 9.57 (br s, 1H), 11.66 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 45.6, 47.9, 53.9, 111.4, 118.7, 123.4, 124.0, 127.7, 127.9, 129.6, 129.8, 131.8, 133.1, 134.4, 140.0, 146.2; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{21}ClN_3$: 326.1424. Found: 326.1419.

4.4.18. 1-(2-Bromobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (19)

Compound **19** was prepared using *Procedure A* with **36** and 2-bromobenzyl bromide as starting materials to give the product (431 mg, 78%) as a white solid; mp 238–240 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.20 (m, 4H), 3.18–3.50 (m, 2H), 3.50–3.83 (m, 2H), 4.89 (s, 2H), 5.74 (s, 2H), 6.64 (dd, J = 5.4, 3.8 Hz, 1H), ~7.07 (br s, ~1H), 7.23–7.30 (m, 2H), 7.30–7.40 (m, 3H), 7.69–7.76 (m, 1H), 7.79 (d, J = 7.6 Hz, 1H), 11.67 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.8, 47.9, 48.0, 54.0, 111.5, 118.7, 121.9, 123.5, 124.1, 127.9, 128.3, 129.9, 133.1, 134.3, 134.6, 139.9, 146.2; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{21}N_3Br$: 370.0918. Found: 370.0906.

4.4.19. 1-(2-Methylbenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (20)

Compound **20** was prepared using *Procedure A* with **36** and 2-methylbenzyl chloride as starting materials to give the product (345 mg, 73%) as a pale yellow solid; mp 138–140 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.85–2.10 (m, 4H), 2.42 (s, 3H), 3.20–3.45 (m, 2H), 3.55–3.88 (m, 2H), 4.79 (s, 2H), 5.74 (s, 2H), 6.25 (d, J = 7.6 Hz, 1H), 7.03 (t, J = 7.6 Hz, 1H), 7.19 (t, J = 7.4 Hz, 1H), 7.25–7.43 (m, 3H), 7.47 (d, J = 7.6 Hz, 1H), 7.80 (d, J = 8.0 Hz, 1H), 11.58 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.9, 22.7, 45.5, 47.8, 53.9, 111.7, 118.5, 123.3, 123.9, 124.3, 126.2, 127.4, 130.4, 134.1, 134.7, 135.4, 139.9, 146.1; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{20}H_{24}N_3$: 306.1965. Found: 306.1960.

4.4.20. 1-(2-Cyanobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (21)

Compound **21** was prepared using *Procedure A* with **36** and 2-cyanobenzyl chloride as starting materials to give the product (268 mg, 56%) as a beige solid; mp 138–140 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.17 (m, 4H), 3.22–3.50 (m, 2H), 3.50–3.84 (m, 2H), 4.93 (s, 2H), 5.95 (s, 2H), 6.95 (d, J = 7.6 Hz, 1H), ~7.02 (br s, ~1H), 7.25–7.35 (m, 2H), 7.38 (t, J = 7.4 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.79 (t, J = 7.6 Hz, 1H), 7.93 (t, J = 7.6 Hz, 1H), 11.69 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 46.4, 47.8, 53.9, 110.2, 111.5, 117.2, 118.7, 123.5, 124.1, 127.2, 128.7, 133.7, 133.9, 134.3, 139.3, 140.1, 146.4; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{20}H_{21}N_4$: 317.1766. Found: 317.1761.

4.4.21. 1-(2-Nitrobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (22)

Compound **22** was prepared using *Procedure A* with **36** and 2-nitrobenzyl chloride as starting materials to give the product (25 mg, 5%) as an beige solid; mp 114–116 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 2.10–2.33 (m, 4H), 3.55–3.85 (m, 4H), 5.19 (s, 2H), 6.34 (s, 2H), 6.82–6.92 (m, 1H), 7.50–7.60 (m, 2H), 7.60–7.70 (m, 3H), 7.96 (d, J = 8.0 Hz, 1H), 8.28–8.37 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 24.0, 48.1, 48.6, 56.5, 114.0, 117.8, 127.1, 127.90, 127.97, 129.0, 130.9, 131.0, 134.6, 135.7, 135.8, 145.4, 148.8; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{21}N_4O_2$: 337.1659. Found: 337.1648.

4.4.22. 1-(2,3-Dichlorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (23)

Compound **23** was prepared using *Procedure A* with **36** and 2,3-dichlorobenzyl chloride as starting materials to give the product (306 mg, 57%) as a white solid; mp 163–167 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.70–2.20 (m, 4H), 3.20–3.50 (m, 2H), 3.50–3.80 (m, 2H), 4.84 (s, 2H), 5.81 (s, 2H), 6.51 (d, J = 7.6 Hz, 1H), 7.25 (t, J = 7.8 Hz, 1H), 7.28–7.40 (m, 2H), 7.46 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 8.69 (br s, 1H), 11.63 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 46.1, 47.9, 53.9, 111.4, 118.8, 123.3, 124.0, 125.9, 128.6, 129.8, 132.3, 134.5, 136.0, 146.3; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{20}Cl_2N_3$: 360.1034. Found: 360.1044.

4.4.23. 1-(2,4-Dichlorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (24)

Compound **24** was prepared using *Procedure A* with **36** and 2,4-dichlorobenzyl chloride as starting materials to give the product (236 mg, 44%) as a white solid; mp 252–253 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.15 (m, 4H), 3.18–3.46 (m, 2H), 3.52–3.80 (m, 2H), 4.85 (s, 2H), 5.75 (s, 2H), 6.70 (d, J = 8.4 Hz, 1H), ~7.25 (br s, 1H), 7.27–7.37 (m, 3H), 7.41 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 2.0 Hz, 1H), 7.78 (d, J = 7.2 Hz, 1H), 11.61 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 45.2, 48.0, 53.9, 111.3, 118.9, 123.3, 124.0, 127.9, 129.2, 132.5, 132.8, 133.2, 134.4, 140.4, 146.3; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{20}Cl_2N_3$: 360.1034. Found: 360.1020.

4.4.24. 1-(2,5-Dichlorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (25)

Compound **25** was prepared using *Procedure A* with **36** and 2,5-dichlorobenzyl bromide as starting materials to give the product (441 mg, 82%) as a white solid; mp 192–194 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.20 (m, 4H), 3.20–3.50 (m, 2H), 3.50–3.82 (m, 2H), 4.92 (s, 2H), 5.79 (s, 2H), 6.78 (d, J = 2.4 Hz, 1H), 7.29–7.42 (m, 3H), 7.44 (dd, J = 8.4, 2.4 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.80 (d, J = 7.2 Hz, 1H), 8.44 (br s, 1H), 11.67 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 45.6, 47.8, 53.9, 111.4, 118.7, 123.6, 124.3, 127.6, 129.6, 130.7, 131.5, 132.3, 134.1, 135.3, 139.8, 146.4; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{20}Cl_2N_3$: 360.1034. Found: 360.1050.

4.4.25. 1-(2,6-Dichlorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (26)

Compound **26** was prepared using *Procedure A* with **36** and 2,6-dichlorobenzyl chloride as starting materials to give the product (182 mg, 34%) as a white solid; mp 253–254 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.22 (m, 4H), 3.22–3.50 (m, 2H), 3.50–3.84 (m, 2H), 5.04 (s, 2H), 5.94 (s, 2H), 6.96 (d, J = 8.0 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 7.25 (t, J = 7.6 Hz, 1H), 7.47 (t, J = 8.2 Hz, 1H), 7.55–7.61 (m, 2H), 7.72 (d, J = 8.0 Hz, 1H), 11.62 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 45.0, 48.6, 53.9, 110.7, 119.1, 122.9, 123.8, 129.5, 129.8, 131.5, 133.9, 135.5, 140.6, 146.9; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{20}Cl_2N_3$: 360.1034. Found: 360.1042.

4.4.26. 1-(3,4-Dichlorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (27)

Compound **27** was prepared using *Procedure A* with **36** and 3,4-dichlorobenzyl chloride as starting materials to give the

product (306 mg, 56%) as a white solid; mp 218–220 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.18 (m, 4H), 3.22–3.50 (m, 2H), 3.50–3.82 (m, 2H), 4.94 (s, 2H), 5.76 (s, 2H), 7.19 (dd, J = 8.4, 2.0 Hz, 1H), 7.30–7.40 (m, 2H), 7.54–7.63 (m, 2H), 7.74–7.81 (m, 2H), 7.79 (br s, 1H), 11.63 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 46.3, 47.8, 53.9, 111.6, 118.5, 123.4, 124.0, 127.4, 129.1, 130.5, 130.9, 131.4, 134.2, 137.1, 139.7, 145.9; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{20}Cl_2N_3$: 360.1034. Found: 360.1030.

4.4.27. 1-(3,5-Dichlorobenzyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (28)

Compound **28** was prepared using *Procedure A* with **36** and 3,5-dichlorobenzyl chloride as starting materials to give the product (288 mg, 65%) as a pale yellow solid; mp 144–147 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.20 (m, 4H), 3.21–3.50 (m, 2H), 3.50–3.80 (m, 2H), 4.95 (s, 2H), 5.77 (s, 2H), 7.28–7.33 (m, 2H), 7.33–7.40 (m, 2H), 7.52–7.59 (m, 2H), 7.75–7.81 (m, 1H), 9.79 (br s, 1H), 11.63 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 46.5, 47.7, 53.9, 111.6, 118.5, 123.6, 124.2, 125.9, 127.6, 134.1, 134.4, 139.6, 140.2, 146.0; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{20}Cl_2N_3$: 360.1034. Found: 360.1030.

4.4.28. 1-(2-Phenylethyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (30)

Compound **30** was prepared using *Procedure A* with **36** and (2-bromoethyl)benzene as starting materials to give the product (119 mg, 25%) as a hygroscopic white solid; mp ~70–80 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.10 (m, 4H), 3.09 (t, J = 7.2 Hz, 2H), 3.18–3.42 (m, 2H), 3.42–3.78 (m, 2H), 4.58–4.72 (m, 4H), 7.15–7.30 (m, 5H), 7.30–7.42 (m, 2H), 7.68–7.77 (m, 2H), 11.53 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 34.9, 45.6, 47.4, 53.9, 111.7, 117.8, 123.5, 123.9, 126.7, 128.4, 129.1, 133.9, 137.7, 138.5, 145.1; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{20}H_{24}N_3$: 306.1970. Found: 306.1974.

4.4.29. 1-(2-Phenoxyethyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (31)

Compound **31** was prepared using *Procedure A* with **36** and 2-phenoxyethyl bromide as starting materials to give the product (273 mg, 56%) as a white solid; mp 98–100 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.20 (m, 4H), 3.30–3.50 (m, 2H), 3.60–3.80 (m, 2H), 4.32 (t, J = 4.8 Hz, 2H), 4.93 (t, J = 4.6 Hz, 2H), 5.02 (s, 2H), 6.86 (d, J = 8.0 Hz, 2H), 6.90 (t, J = 7.4 Hz, 1H), 7.24 (t, J = 8.0 Hz, 2H), 7.38 (t, J = 7.6 Hz, 1H), 7.44 (t, J = 7.4 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 8.35 (br s, 1H), 11.69 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.8, 44.0, 47.6, 54.0, 66.0, 112.1, 114.3, 117.8, 121.0, 123.7, 124.1, 129.5, 134.2, 138.5, 145.8, 157.7; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{20}H_{24}N_3O$: 322.1919. Found: 322.1908.

4.4.30. 1-Benzhydryl-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole hydrochloride (32)

Compound **32** was prepared using *Procedure A* with **36** and bromodiphenylmethane as starting materials to give the product (147 mg, 29%) as a white solid; mp 120–124 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.15 (m, 4H), 3.10–3.40 (m, 2H), 3.60–3.90 (m, 2H), 4.86 (s, 2H), 6.55 (d, J = 8.4 Hz, 1H), 6.90 (br s, ~1H), 7.03 (t, J = 7.8 Hz, 1H), 7.17–7.29 (m, 5H), 7.35–7.47 (m, 6H), 7.51 (s, 1H), 7.72 (d, J = 8.4 Hz, 1H), 11.36 (br s, ~1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.8, 49.2, 54.2, 61.8, 112.8, 119.3, 122.3, 123.2, 128.2, 128.4, 128.8, 134.5, 137.6, 141.7, 147.0; HRMS (ESI) $[M-Cl]^+$ Calcd for $C_{25}H_{26}N_3$: 368.2126. Found: 368.2120.

4.4.31. 1-(Naphthalen-2-ylmethyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (33)

Compound **33** was prepared using *Procedure A* with **36** and 2-(bromomethyl)naphthalene as starting materials to give the product (123 mg, 24%) as a white solid; mp 134–138 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–2.24 (m, 4H), 3.20–3.54 (m, 2H), 3.54–3.90 (m, 2H), 4.97 (s, 2H), 5.89 (s, 2H), 7.27–7.36 (m, 2H), 7.38 (d, J = 8.4 Hz, 1H), 7.49 (d, J = 3.2 Hz, 1H), 7.51 (d, J = 3.2 Hz, 1H), 7.61 (d, J = 7.2 Hz, 1H), 7.73–7.82 (m, 2H), 7.85 (dd, J = 6.0, 3.2 Hz, 1H), 7.87–7.95 (m, 2H), 8.39 (br s, 1H), 11.61 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 47.6, 48.0, 54.0, 111.7, 118.5, 123.3, 123.8, 125.0, 125.5, 126.3, 126.5, 127.6, 127.7, 128.5, 132.4, 132.8, 133.5, 134.5, 140.0, 145.9; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{23}H_{24}N_3$: 342.1964. Found: 342.1963.

4.4.32. 1-(Cyclohexylmethyl)-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (34)

Compound **34** was prepared using *Procedure A* with **36** and (bromomethyl)cyclohexane as starting materials to give the product (284 mg, 62%) as a white solid; mp 127–130 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.01–1.21 (m, 5H), 1.40–1.54 (m, 2H), 1.54–1.71 (m, 3H), 1.76–1.89 (m, 1H), 1.89–2.12 (m, 4H), 3.25–3.80 (m, 4H), 4.33 (d, J = 7.2 Hz, 2H), 4.94 (s, 2H), 7.37–7.48 (m, 2H), 7.74–7.81 (m, 1H), 7.81–7.89 (m, 1H), 11.70 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.7, 25.1, 25.7, 29.7, 37.8, 47.1, 50.1, 54.0, 112.4, 117.4, 124.0, 124.3, 134.3, 137.3, 145.0; HRMS (EI) $[M-HCl_2]^+$ Calcd for $C_{19}H_{28}N_3$: 298.2283. Found: 298.2276.

4.4.33. 1-*n*-Propyl-2-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole dihydrochloride (35)

Compound **35** was prepared using *Procedure A* with **36** and 1-bromopropane as starting materials to give the product (227 mg, 58%) as a white solid; mp 190–195 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.93 (t, J = 7.4 Hz, 3H), 1.74–1.87 (m, 2H), 1.87–2.15 (m, 4H), 3.32–3.54 (m, 2H), 3.54–3.80 (m, 2H), 4.49 (t, J = 7.4 Hz, 2H), 4.99 (s, 2H), 7.42–7.54 (m, 2H), 7.81 (d, J = 7.6 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 10.40 (br s, 1H), 11.94 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.9, 22.6, 22.7, 46.3, 46.4, 53.9, 112.5, 116.9, 124.7, 124.8, 133.4, 135.9, 144.3; HRMS (ESI) $[M-HCl_2]^+$ Calcd for $C_{15}H_{22}N_3$: 244.1813. Found: 244.1811.

4.5. Other synthetic procedures

*2-(Pyrrolidin-1-ylmethyl)-1H-benzimidazole (**36**).* Under an atmosphere of nitrogen, 2-(chloromethyl)benzimidazole (1.00 g, 6.00 mmol, 1 equiv) was dissolved in ethanol (20 mL). To this was added pyrrolidine (10 mL, 8.53 g, 119.94 mmol, 20 equiv). The mixture was stirred at rt for 0.5 h, heated at reflux temperature for 2 h, then stirred at rt for an additional 48 h. The mixture was concentrated, diluted with a saturated aqueous solution of $NaHCO_3$ (100 mL), and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic extracts were washed with brine (75 mL), dried (Na_2SO_4), and concentrated. High-vacuum drying gave the product (1.13 g, 5.61 mmol, 94%) as a beige solid; mp 142–143 °C; R_f = 0.13 (EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.65–1.75 (m, 4H), 2.46–2.56 (m, 4H), 3.81 (s, 2H), 7.06–7.16 (m, 2H), 7.35–7.59 (m, 2H), 12.31 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.3, 53.2, 53.7, 111.1, 118.4, 120.9, 121.6, 134.4, 143.0, 152.7; HRMS (ESI) $[M+H]^+$ Calcd for $C_{12}H_{16}N_3$: 202.1344. Found: 202.1349.

4.6. In vitro HO activity assay

HO activity in rat spleen and rat brain microsomal fractions was determined by the quantitation of CO formed from the degradation of methemalbumin (heme complexed with albumin).[20,21] Microsomal fractions of spleen and brain (Sprague–Dawley rats) were prepared according to the procedure outlined by Appleton et al.;[22]

these fractions have been characterized for HO-1 and HO-2 content (see Ref. 9). Protein concentration of microsomal fractions was determined by a modification of the biuret method.[21] Incubations for HO activity analysis were performed under conditions for which the rate of CO formation (pmol CO $\times$ min^{-1} $\times$ 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 freezing the reaction mixture on dry ice, and CO formation was monitored by gas chromatography according to the method described by Vreman and Stevenson.[20]

4.7. Analysis of enzyme inhibition

Potencies of the compounds as inhibitors of HO are reported as their IC_{50} values, which were calculated using the formula $IC_{50} = \frac{EC_{50}}{\frac{bottom-top}{50-top}-1}$. The data resulting from the above experiments were plotted as non-linear regression (sigmoidal dose–response) curves using GraphPad Prism (version 3) software. The values on the abscissa represent the logarithm of the inhibitor concentration (in μM), whereas the values of the activity on the ordinate are expressed as a percentage of the control experiments without inhibitor. Bottom and top refer to the lower and upper plateaus; EC_{50} is the value halfway between the top and bottom plateaus. The IC_{50} value reported for each compound is reported as the mean of the values recorded in replicate experiments, and for each of these replicate experiments 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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