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Microwave-assisted synthesis of new benzimidazole derivatives with lipase inhibition activity

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Abstract

A practical protocol has been used for the synthesis of benzimidazoles. The reaction of iminoester hydrochlorides of phenylacetic with 4,5-dichloro-1,2-phenylenediamine under microwave irradiation leads to the benzimidazole derivatives with good yields and in short reaction times. After the synthesis of benzimidazoles, we synthesized ester and hydrazide derivatives under microwave irradiation with good yields. All compounds were evaluated with regard to pancreatic lipase activity and **3b**, **3c**, **5a** and **6a** showed lipase inhibition at various concentrations.

Keywords

Benzimidazole, iminoester hydrochloride, lipase inhibition, microwave

History

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Introduction

Benzimidazoles are an important group of heterocyclic compounds in the field of medicinal chemistry[1]. Some benzimidazole derivatives with different biological activity such as anticancer[2], antihelmintic[3], antimicrobial[4], antifungal[5], antitubercular[6], anti-allergic[7], antioxidant[8], antihistaminic[9], antitumor[10], anti-inflammatory and analgesic activities[11] have been revealed in the literature. Benzimidazole derivatives exhibit significant activity against several viruses such as HIV[12], herpes (HSV-1)[13] and human cytomegalovirus[12]. In addition, the treatment potency of benzimidazoles in diseases such as ischemia–reperfusion injury[14], hypertension[15], obesity[16] has been recently reported. Because of these reasons, benzimidazole derivatives have been studied by numerous scientists. Obesity is widely recognized as a major public health problem which is caused by an imbalance between energy intake and expenditure. Obesity can be a cause to different serious diseases, including hypertension, hyperlipidemia, arteriosclerosis and type II diabetes[17]. Pancreatic lipase plays a key role for fat digestion. Moreover, pancreatic lipase inhibitors, such as Orlistat, are used as therapeutic agent for curing obesity[18].

In the literature, there are many synthetic routes that are common to the preparation of benzimidazoles. Generally, the condensation of *ortho*-phenylenediamines and carboxylic acids (or their derivatives such as nitriles, imidates and orthoesters) had been widely used for the synthesis of benzimidazole[19,20]. However, many of these procedures are associated with several drawbacks such as expensive reagents, harsh reaction conditions, extended reaction times, the occurrence of side products, unsatisfactory yields and complicated experimental procedures. Also, microwave technology has been used to synthesize benzimidazole derivatives, and important changes have been seen on yield and reaction time[1,21,22]. In this study, synthesis of benzimidazoles has shown that iminoester hydrochlorides could be a useful intermediate in the reaction with 4,5-dichloro-1,2-phenylenediamine under microwave irradiation in methanol and solvent-free conditions. This method can provide a convenient way of synthesizing potential bioactive benzimidazoles. Furthermore, a practical method has been developed for the synthesis of *bis*-benzimidazoles. Iminoester hydrochlorides of phenylacetic acids were used as intermediates in the reaction with 3,3′-diaminobenzidine under microwave irradiation, leading to the products with good yields and short reaction times. This method can be a general technique for the synthesis of *bis*-benzimidazoles. The synthetic path of the target compounds is shown in Scheme 1.

Results and discussion

Iminoester hydrochloride could be a useful intermediate in the reaction with *o*-phenylenediamine under microwave irradiation in methanol for the synthesis of benzimidazole derivatives. In this method, we obtained products within short reaction times and with high yields. In addition, the reaction was carried out catalyst-free under mild conditions. Compounds (**2a**–**c**) were also obtained using conventional heating in methanol[23]. We also synthesized *bis*-benzimidazole from iminoester hydrochloride reaction of the 3,3′-diaminobenzidine under microwave irradiation, leading to the product with good yield. After the synthesis of benzimidazoles, we synthesized esters (**3a**–**c**, **6a**) and hydrazides (**4a**–**c**, **7a**) under microwave irradiation with good yields. Simple alkylation reaction was applied to **2a**–**c**, **6a** with methyl α-bromoacetate in acetone and then, the MeO group was displayed with $NH_2NH_2 \cdot H_2O$ in EtOH. The structures of new compounds were

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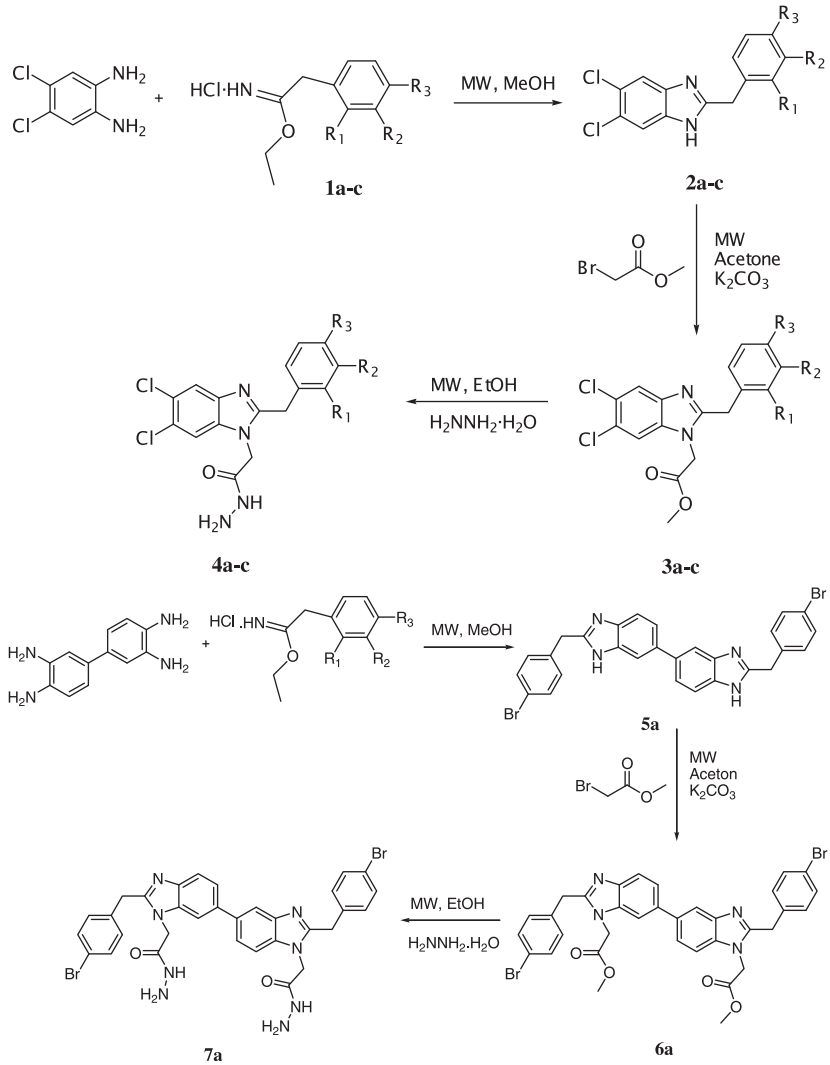
Scheme 1. Synthetic path of the target compounds.



Compound	R_1	R_2	R_3	Compound	R_1	R_2	R_3	Compound	R_1	R_2	R_3
2a	Br	H	H	**3a**	Br	H	H	**4a**	Br	H	H
2b	H	Br	H	**3b**	H	Br	H	**4b**	H	Br	H
2c	H	H	Br	**3c**	H	H	Br	**4c**	H	H	Br
5a	H	H	Br	**6a**	H	H	Br	**7a**	H	H	Br

confirmed by FT-IR (infrared), ^{1}H-NMR, ^{13}C-NMR spectroscopy and mass spectrometry. All the synthesized compounds were screened for their anti-lipase activities.

Anti-lipase activity results

All the compounds were evaluated with regard for pancreatic lipase activity and different concentrations of **3b**, **3c**, **5a** and **6a** showed anti-lipase activities at various concentrations (Table 1). No inhibitory effect was observed in other compounds. Dose-dependent pancreatic lipase activity is shown in Figure 1. Among the compounds tested, **3b** showed the best anti-lipase activity. The compound inhibited pancreatic lipase activity by 84%, 97% and 98% at concentrations of 0.625, 1.25 and 6.25 μg/mL, respectively. Orlistat (Xenical; Hoffmann-La Roche, Segrate, Italy), known pancreatic lipase inhibitor used as anti-obesity drug, showed inhibitory effect by 98%, 99% and 100% at the same concentrations. IC_{50} values of Orlistat and compound **3b** were calculated as 0.04 and 0.17 μg/mL, respectively. Orlistat is the only approved anti-obesity medication[18] but it has some side effects, such as fecal incontinence, flatulence and steatorrhea[24,25]. The synthesized compounds such as **3b** and **6a** can be a good alternative to Orlistat.

Experimental

Melting points were determined in open capillaries on Büchi (Essen, Germany) digital melting point apparatus and were uncorrected. IR spectra were recorded in KBr pellets on a Perkin-Elmer 100 FT-IR spectrophotometer (California). ^{1}H- and ^{13}C-NMR spectra were measured on a Varian 400 spectrometer (Varian, Darmstadt, Germany) using DMSO-d_6 as solvent and TMS as internal standard. Chemical shifts are given in parts per million (ppm), coupling constants *J* in hertz (Hz). Mass spectra (MS) were taken in H-ESI mode on Thermo Quantum Mars (Thermo-scientific, Florida). A monomode CEM-Discover microwave (Linfort, Germany) was used in the standard configuration as delivered, including proprietary software. All experiments were carried out in microwave process vials (30 mL) with control of the temperature by IR detection temperature sensor. The temperature

Table 1. Residual lipase activity of chemical compounds.

	Residual activity (%)	SD (%)
T+	100	±4
3b (0.125 µg)	16	±0.3
3c (0.125 µg)	26	±1
5a (0.125 µg)	58	±1
6a (0.125 µg)	63	±1
Orlistat (0.125 µg)	5	±2.3

T+: porcine pancreatic lipase (PPL) without inhibitor (control).

was computer monitored and maintained constant by a discrete modulation of the delivered microwave power. After completion of the reaction, the vial was cooled to 60 °C by air jet cooling.

*Synthesis of hydrochloride of substitute benzeneimidic acid ethyl ester (**1a–c**)*

To an ice-cooled solution of the substitute benzonitrile (1 mol) in dry EtOH (1.1 mol), dry hydrogen chloride was added until 1.1 mol had been absorbed. The resulting solution was then allowed to stand at 0 °C in the refrigerator for 12 h, after which cold Et_2O was added. The precipitated crystals were filtered off immediately, washed with cold Et_2O and dried in a dessicator.

*General procedure for the synthesis of 5,6-dichloro-2-(substitutedbenzyl)-1H-benzimidazole (**2a–c**) under microwave irradiation*

A mixture of 4,5-dichloro-1,2-phenylenediamine (0.010 mol) and iminoester hydrochlorides (0.013 mol; **1a–c**) in dry methanol (15 mL) was irradiated in closed vessels with the pressure control at 65 °C for 10 min (hold time) at 300 W maximum power. After the completion of the reaction, (monitored by TLC, ethylacetate:hexane, 3:1), the mixture was poured onto water. The precipitate formed was filtered and recrystallized from ethanol–water (1:3) to give pure **2a–c**.

5,6-Dichloro-2-(2-bromobenzyl)-1*H*-benzimidazole (**2a**). Yield (90%); m.p. 228–229 °C; IR (ν_{max}, cm^{-1}): 3422 (NH), 1629 (C=N); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 4.31 (s, 2H, CH_2), 7.28–7.77 (m, 6H, Ar–H) and 12.63 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ (ppm): 32.65 (CH_2), 119.62, 123.79, 124.26, 127.21, 128.77, 129.10, 131.50, 133.23, 134.58 (Ar–C) and 155.24 (C=N). Anal. Calcd for $C_{14}H_9Cl_2BrN_2$: C, 47.23; H, 2.55 and N, 7.87; found: C, 47.20; H, 2.56 and N, 7.89. ESI-MS *m/z* calculated for $C_{14}H_9Cl_2BrN_2$ $[M]^+$ 356.10 and 358.10; found: 356.70 and 358.65.

5,6-Dichloro-2-(3-bromobenzyl)-1*H*-benzimidazole (**2b**). Yield (91%); m.p. 208–209 °C; IR (ν_{max}, cm^{-1}): 3200 (NH), 1627 (C=N); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 4.21 (s, 2H, CH_2), 7.10–7.95 (m, 6H, Ar–H) and 12.64 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ (ppm): 34.89 (CH_2), 113.47, 120.32, 122.39 (2C), 124.48 (2C), 128.76, 130.30, 131.37, 132.32, 132.83, 140.39 (Ar–C) and 156.61 (C=N). Anal. Calcd for $C_{14}H_9Cl_2BrN_2$: C, 47.23; H, 2.55 and N, 7.87; found: C, 47.21; H, 2.59 and N, 7.85. ESI-MS *m/z* calculated for $C_{14}H_9Cl_2BrN_2$ $[M+H]^+$ 356.10 and 358.10; found: 356.78 and 358.67.

5,6-Dichloro-2-(4-bromobenzyl)-1*H*-benzimidazole (**2c**). Yield (95%); m.p. 234–235 °C; IR (ν_{max}, cm^{-1}): 3208 (NH), 1579 (C=N); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 4.10 (s, 2H, CH_2), 7.10–7.71 (m, 6H, Ar–H) and 12.64 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ (ppm): 34.77 (CH_2), 113.25, 120.20, 120.61, 124.58, 131.84 (2C), 132.07 (2C), 134.69, 137.09 (2C), 143.84 (Ar–C) and 156.74 (C = N). Anal. Calcd for $C_{14}H_9Cl_2BrN_2$: C, 47.23; H, 2.55 and N, 7.87; found: C, 47.22; H, 2.58 and N, 7.87. ESI-MS *m/z* calculated for $C_{14}H_9Cl_2BrN_2$ $[M+H]^+$ 357.10 and 359.10; found: 357.78 and 359.10.

*General procedure for the synthesis of methyl [5,6-dichloro-2-(substitutedbenzyl)-1H-benzimidazole-1-yl] acetate (**3a–c**)*

A mixture of compounds **2a–c** (0.01 mol), methyl-α-bromoacetate (0.01 mol) and K_2CO_3 (0.025 mol) in acetone (10 mL) was irradiated in closed vessels with the pressure control at 85 °C for 7 min (hold time) at 300 W maximum power. After the completion of the reaction, (monitored by TLC, ethylacetate:hexane, 3:1), the mixture was poured onto water. The precipitate formed was filtered and recrystallized from acetone:water (1:3) to give pure **3a–c**.

Methyl [5,6-dichloro-2-(2-bromobenzyl)-1*H*-benzimidazole-1-yl] acetate (**3a**). Yield (90%); m.p. 162–163 °C; IR (ν_{max}, cm^{-1}): 1738 (C=O), 1618 (C=N) and 1250 (C–O); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 3.48 (s, 3H, OCH_3), 4.31 (s, 2H, CH_2), 5.30 (s, 2H, N–CH_2) and 7.19–7.89 (m, 6H, Ar-H); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ (ppm): 30.75 (CH_2), 44.48 (N–CH_2), 52.27 (O–CH_3), 112.84, 119.68, 124.10, 124.41, 127.11, 128.56, 129.03, 131.39, 133.19, 133.25, 135.02, 141.42 (Ar–C), 155.44 (C=N) and 167.67 (C=O). Anal. Calcd for $C_{17}H_{13}Cl_2BrN_2O_2$: C, 47.69; H, 3.06 and N, 6.54; found: C, 47.65; H, 3.11 and N, 6.53. ESI-MS *m/z* calculated for $C_{17}H_{13}Cl_2BrN_2O_2$ $[M]^+$ 428.10 and 430.10; found: 428.80 and 430.88.

Methyl [5,6-dichloro-2-(3-bromobenzyl)-1*H*-benzimidazole-1-yl] acetate (**3b**). Yield (93%); m.p. 193–194 °C; IR (ν_{max}, cm^{-1}): 1731 (C=O), 1594 (C=N) and 1227 (C–O); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 3.55 (s, 3H, OCH_3), 4.28 (s, 2H, CH_2), 5.29 (s, 2H, N–CH_2) and 7.24–7.95 (m, 6H, Ar–H); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ (ppm): 32.57 (CH_2), 45.12 (N–CH_2), 52.79 (O–CH_3), 112.75, 120.37, 122.06, 124.83, 128.67, 130.06, 131.03, 132.17, 135.24, 135.75, 139.06, 142.06 (Ar–C), 156.56 (C=N) and 168.39 (C=O). Anal. Calcd for $C_{17}H_{13}Cl_2BrN_2O_2$: C, 47.69; H, 3.06 and N, 6.54; found: C, 47.65; H, 3.11 and N, 6.55. ESI-MS *m/z* calculated for $C_{17}H_{13}Cl_2BrN_2O_2$ $[M]^+$ 428.10 and 430.10; found: 428.80 and 430.91.

Methyl [5,6-dichloro-2-(4-bromobenzyl)-1*H*-benzimidazole-1-yl] acetate (**3c**). Yield (97%); m.p. 152–153 °C; IR (ν_{max}, cm^{-1}): 1738 (C=O), 1591 (C=N) and 1226 (C–O); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 3.64 (s, 3H, OCH_3), 4.23 (s, 2H, CH_2), 4.66 (s, 2H, N–CH_2) and 7.09–7.85 (m, 6H, Ar–H); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ (ppm): 29.11 (CH_2), 40.28 (N–CH_2), 48.25 (O–CH_3), 105.73, 116.31, 116.65, 121.93, 122.41, 125.57 (2C), 127.34 (2C), 129.10, 130.09, 137.02 (Ar–C), 149.80 (C=N) and 162.07 (C=O). Anal. Calcd for $C_{17}H_{13}Cl_2BrN_2O_2$: C, 47.69; H, 3.06 and N, 6.54; found: C, 47.64; H, 3.10 and N, 6.57. ESI-MS *m/z* calculated for $C_{17}H_{13}Cl_2BrN_2O_2$ $[M]^+$ 428.10 and 430.10; found: 428.81 and 430.84.

*General procedure for the synthesis of 2-[5,6-dichloro-2-(substitutedbenzyl)-1H-benzimidazole-1-yl] acetohydrazide (**4a–c**)*

A mixture of compounds **3a–c** (0.01 mol) and hydrazine hydrate (0.025 mol) in absolute ethanol (10 mL) was irradiated in closed vessels with the pressure control at 120 °C for 5 min (hold time) at 300 W maximum power. After the completion of the reaction, (monitored by TLC, ethylacetate:hexane, 3:1), the mixture was cooled to room temperature. The precipitate formed was filtered, washed with excess ethanol and dried over $CaCl_2$ to give pure **4a–c**.

2-[5,6-Dichloro-2-(2-bromobenzyl)-1*H*-benzimidazole-1-yl] acetohydrazide (**4a**). Yield (80%); m.p. 248–249 °C; IR (ν_{max}, cm^{-1}): 3331, 3302 (NH_2), 3173 (NH), 1674 (C=O) and 1588 (C=N); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 4.33 (s, 2H, CH_2), 4.46 (s, 2H, NH_2), 4.83 (s, 2H, N–CH_2), 7.17–7.82 (m, 6H, Ar–H) and 9.51 (s, 1H, –NH); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ (ppm): 31.63 (CH_2), 45.34 (N–CH_2), 112.56, 120.42, 124.49, 125.01, 127.87, 129.34, 129.83, 132.31, 134.10, 134.87, 135.91, 142.32 (Ar–C), 156.58 (C=N) and 166.19 (C=O). Anal. Calcd for

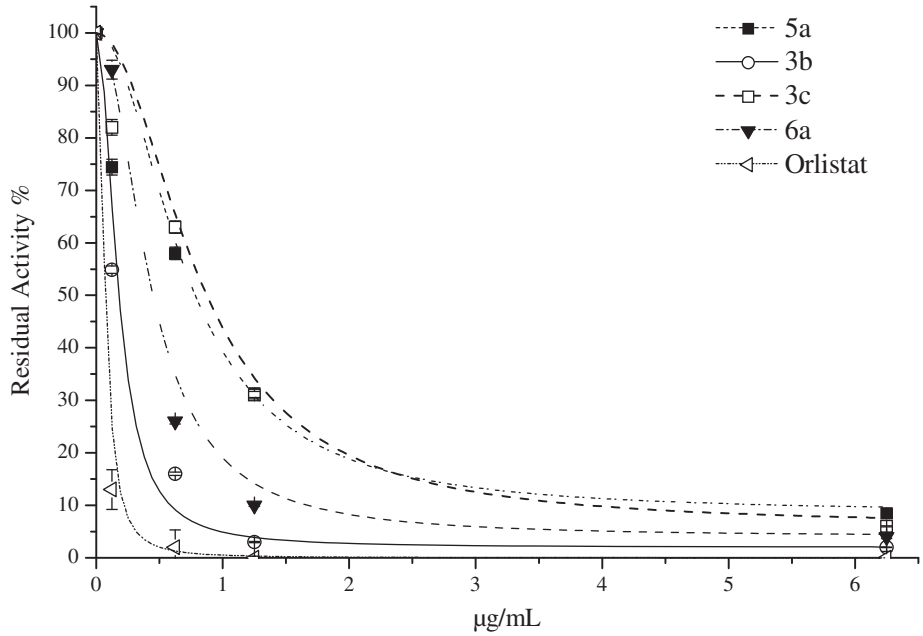


Figure 1. Dose-dependent inhibitory effect of the compounds **3b**, **3c**, **5a** and **6a**. Orlistat was used positive control. All compounds and Orlistat were measured at final concentrations of 0.625, 1.25 and 6.25 µg/mL. Residual activities of compounds are expressed as the mean ± SD in triplicate.

$C_{16}H_{13}Cl_2BrN_4O_1$: C, 44.89; H, 3.06 and N, 13.09; found: C, 44.86; H, 3.07 and N, 13.10. ESI-MS *m/z* calculated for $C_{16}H_{13}Cl_2BrN_4O_1$ $[M+H]^+$ 429.10 and 431.10; found: 429.89 and 431.92.

2-[5,6-Dichloro-2-(3-bromobenzyl)-1*H*-benzimidazole-1-yl] acetohydrazide (**4b**). Yield (83%); m.p. 231–232 °C; IR (ν_{max}, cm^{-1}): 3304, 3150 ($NHNH_2$), 1658 (C=O) and 1550 (C=N); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 4.27 (s, 2H, CH_2), 4.35 (s, 2H, NH_2), 4.91 (s, 2H, N–CH_2), 7.26–7.86 (m, 6H, Arom-H) and 9.53 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ (ppm): 32.72 (CH_2), 45.23 (N–CH_2), 112.50, 120.14, 120.29, 122.07, 124.61, 124.90, 128.79, 130.04, 130.98, 132.30, 135.69, 139.49, 139.60, 142.16 (Ar–C), 156.89 (C=N) and 166.00 (C=O). Anal. Calcd for $C_{16}H_{13}Cl_2BrN_4O_1$: C, 44.89; H, 3.06 and N, 13.09; found: C, 44.86; H, 3.08 and N, 13.11. ESI-MS *m/z* calculated for $C_{16}H_{13}Cl_2BrN_4O_1$ $[M+H]^+$ 429.10 and 431.10; found: 429.02 and 430.98.

2-[5,6-Dichloro-2-(4-bromobenzyl)-1*H*-benzimidazole-1-yl] acetohydrazide (**4c**). Yield (85%); m.p. 264-265 °C; IR (ν_{max}, cm^{-1}): 3310, 3190 ($NHNH_2$), 1678 (C=O), and 1596 (C=N); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 4.23 (s, 2H, CH_2), 4.35 (s, 2H, NH_2), 4.88 (s, 2H, N–CH_2), 7.26 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.51 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.84 (s, 2H, Ar–H) and 9.50 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ (ppm): 32.64 (CH_2), 45.19 (N–CH_2), 112.48, 120.24, 120.34, 124.55, 124.85, 131.74 (2C), 131.86, 135.74, 136.13, 142.14 (Ar–C), 157.00 (C=N) and 165.97 (C=O). Anal. Calcd for $C_{16}H_{13}Cl_2BrN_4O_1$: C, 44.89; H, 3.06 and N, 13.09; found: C, 44.85; H, 3.04 and N, 13.12. ESI-MS *m/z* calculated for $C_{16}H_{13}Cl_2BrN_4O_1$ $[M]^+$ 428.11 and 430.11; found: 428.81 and 430.91.

*General procedure for the synthesis of 2,2′-bis(4-bromobenzyl)-1H,1′H-5,5′-bibenzimidazole under microwave irradiation (**5a**)*

A mixture of 3,3′-diaminobenzidine (0.010 mol) and **1c** (0.026 mol) in dry MeOH (15 mL) was irradiated with microwave at 60 °C for 10 min at 300 W maximum power. After the completion of the reaction, (monitored by TLC, AcOEt:hexane, 3:1), the mixture was poured onto H_2O. The precipitate formed was filtered and recrystallized from EtOH:H_2O (1:3) to give pure compounds, **5a**.

2,2′-*Bis*(4-bromobenzyl)-1*H*,1′*H*-5,5′-bibenzimidazole (**5a**). Yield (90%); m.p. 312–313 °C; IR (ν_{max}, cm^{-1}): 3335 (NH), 1569 (C=N); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 4.30 (s, 4H, $2CH_2$), 7.19–7.56 (m, 14H, Ar–H) and 12.33 (s, 2H, 2NH); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ (ppm): 34.69 ($2CH_2$), 120.44, 121.91, 130.98, 131.15, 131.79, 132.06, 132.15, 135.99, 137.68 (Ar–C) and 154.34 (2C=N). Anal. Calcd for $C_{28}H_{20}Br_2N_4$: C, 58.76; H, 3.52 and N, 9.79; found: C, 58.75; H, 3.54 and N, 9.80. ESI-MS *m/z* calculated $C_{28}H_{20}Br_2N_4$ $[M]^+$ 572.29 and 574.26; found: 572.33, 574.16 and symmetric division signal 286.14.

*General procedure for the synthesis of dimethyl 2,2′-[2,2′-bis(4-bromobenzyl)-1H,1′H-5,5′-bibenzimidazole-1,1′-diyl]diacetate (**6a**)*

A mixture of compounds **5a** (0.01 mol), methyl-α-bromoacetate (0.02 mol) and K_2CO_3 (0.05 mol) in acetone (15 mL) was irradiated in closed vessels with the pressure control at 85 °C for 20 min (hold time) at 300 W maximum power. After the completion of the reaction, (monitored by TLC, ethylacetate:hexane, 3:1), the mixture was poured onto water. The precipitate formed was filtered and recrystallized from acetone:water (1:3) to give pure **6a**.

Yield (85%); m.p. 104–105 °C; IR (ν_{max}, cm^{-1}): 1740 (C=O), 1622 (C=N), 1215, and 1180 (C–O); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 3.34 (s, 6H, $2OCH_3$), 4.22 (s, 4H, $2CH_2$), 5.25 (s, 4H, 2N–CH_2) and 7.11–7.95 (m, 14H, Ar–H); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ (ppm): 34.96 ($2CH_2$), 47.34 (2N–CH_2), 55.11 ($2OCH_3$), 113.29, 119.77, 121.61, 122.63, 124.46, 124.72, 134.13, 138.25, 138.78, 139.21, 144.11, 145.63 (Ar–C), 156.91 (2C=N) and 171.23 (2C=O). Anal. Calcd for $C_{34}H_{28}Br_2N_4O_4$: C, 57.00; H, 3.94 and N, 7.82; found: C, 57.05; H, 3.90 and N, 7.81. ESI-MS *m/z* calculated $C_{34}H_{28}Br_2N_4O_4$ $[M]^+$ 716.42 and 718.41; found: 716.56, 718.71 and symmetric division signal 358.14.

*General procedure for the synthesis of 2,2′-[2,2′-bis(4-bromobenzyl)-1H,1′H-5,5′-bibenzimidazole-1,1′-diyl]diacetohydrazide (**7a**)*

A mixture of compounds **6a** (0.01 mol) and hydrazine hydrate (0.05 mol) in absolute ethanol (10 mL) was irradiated in closed vessels with the pressure control at 125 °C for 13 min (hold time) at 300 W maximum power. After the completion of the reaction, (monitored by TLC, ethylacetate:hexane, 3:1), the mixture was cooled to room temperature. The precipitate formed was filtered, washed with excess ethanol and dried over $CaCl_2$ to give pure **7a**.

Yield (80%); m.p. 185–186 °C; IR (ν_{max}, cm^{-1}): 3292, 3200 ($NHNH_2$), 1658 (C=O), 15 621 (C=N); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 4.25 (s, 4H, $2CH_2$), 4.45 (bs, 4H, $2NH_2$), 4.85 (s, 4H, 2N–CH_2), 7.16–7.85 (m, 14H, Ar–H) and 9.60 (s, 2H, 2NH); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ (ppm): 36.20 ($2CH_2$), 45.33 (2N–CH_2), 109.27, 110.57, 121.25, 122.33, 124.16, 132.13, 132.28, 136.62, 138.10, 139.21, 141.96, 144.03 (Ar–C), 155.34 (2C=N) and 166.33 (2C = O). Anal. Calcd for $C_{32}H_{28}Br_2N_8O_2$: C, 53.65; H, 3.94 and N, 15.64; found: C, 53.63; H, 3.95 and N, 15.65. ESI-MS *m/z* calculated $C_{32}H_{28}Br_2N_8O_2$ $[M]^+$ 716.43 and 718.43; found: 716.60, 718.53 and symmetric division signal 358.23.

Anti-lipase activity

The inhibitory effects of those compounds were evaluated against PPL (15 ng/μL). Lipase activity assay were done according to Verger and Chahinian[26]. Microtiter plates were coated with purified tung oil TAGs. Compounds were mixed with PPL 1:2 (v/v) and incubated for 30 min. The microtiter plates containing purified tung oil, lipase solution and assay buffer (10 mM *Tris*-HCl buffer, pH 8.0, containing 150 mM NaCl, 6 mM $CaCl_2$, 1 mM EDTA and 3 mg/mL β-cyclodextrin) were recorded continuously for 40 min against the buffer alone using microplate reader (SpectraMax M5, Molecular Devices) at 272 nm. The inhibitory activities of those compounds and Orlistat, a positive control against pancreatic lipase, were measured at concentrations of 6.25, 1.25 and 0.625 μg/mL. Residual activities were calculated by comparing to control without inhibitor (T+). The assays were done in triplicate. The IC_{50} value was determined as the concentration of compound that give 50% inhibition of maximal activity.

Conclusion

In conclusion, we have developed a novel and practical method for the synthesis of benzimidazole derivatives under microwave irradiation. This method can provide a convenient way of synthesizing potential bioactive benzimidazoles. This is the first example of benzimidazoles with their inhibitory properties toward pancreatic lipase. The results could be an inspiration for further investigation of potential lipase inhibitors within heterocycles.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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