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Synthesis, acetylcholinesterase, butyrylcholinesterase and tyrosinase ınhibition, and antioxidant studies of 2-[2-(substituted-benzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetohydrazides and methyl 2-[2-(substituted-benzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetates

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In this research study we intend to synthesize six new 2-[2-(substituted-benzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetohydrazides (**8a-f**) and six new methyl 2-[2-(substituted-benzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetates (**7a-f**) with potency inhibiting acetylcholinesterase, butyrylcholinesterase and tyrosinase enzymes and antioxidant activities.

The chemical structures of synthesized compounds were identified by IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis. All these compounds were evaluated for their acetylcholinesterase, butyrylcholinesterase and tyrosinase enzymes inhibitory potentials and antioxidant activities.

Biological activity results revealed that compounds **8f** and **7c** showed the highest tyrosinase inhibition with 52.46 ± 2.67% and 52.32 ± 0.70%, respectively. Compound **8f** had the highest radical scavenging activities with 24.83 ± 1.28% among the compounds whilst compound **8c** showed the lowest activities with 9.62 ± 0.85%. Compound **8f** gave the highest PRAP and FRAP absorbance values with 0.180 ± 0.013 and 0.358 ± 0.009 at 100 μM, respectively.

Key words: benzimidazoles, tyrosinase, acetylcholinesterase, butyrylcholinesterase, antioxidant activity

INTRODUCTION

Today, most of the drugs used in the treatment of diseases are obtained by organic synthesis. The fact that they are more economical than those obtained naturally leads to the rapid development of new compounds through synthesis. In this scope, benzimidazole synthesis, which is an important member of heterocyclic compounds, has become an important area of interest [1]. Some drugs (albendazole, mebendazole, omeprazole, etc.), which bear the benzimidazole ring and have been taken up by the World Health Organization (WHO), are listed in the literature [2-6]. Compounds containing the benzimidazole skeleton also show important pharmacological activities such as antimicrobial [7], antibacterial [8-10], antifungal [11,12], antiviral [13,15], antioxidant [16,17] activities.

Alzheimer Disease (AD) is recognized to impaired cognitive functions such as loss of memory and abnormal social behaviors [18]. AD has become a major health issue in the developing countries due to its rapid growth among elderly people [19]. Even though the pathogenesis of AD has not been established so far, the cholinergic hypothesis is the most accepted theory for this disease due to a deficit in acetylcholine level in the brain of patients. Therefore, treatment of this disease is to increase the acetylcholine level in the brain using acetylcholinesterase (AChE) inhibitor [20]. Tyrosinase is a copper-containing polyphenol oxidase enzyme, which may play an important role in the generation of neuromelanin. Inhibition of tyrosinase is an important target in finding new drugs against Parkinson Disease (PD) [21]. Therefore, the researchers have aimed to find new tyrosinase and cholinesterase enzyme inhibitors against these diseases (AD and PD).

For all the above reasons, in this study, we synthesized a new series of benzimidazole derivatives and evaluate the biological properties (enzyme inhibition and antioxidant) of these compounds for the first time.

EXPERIMENTAL

Measurements

Melting points were determined on a WRS-1B Digital Melting-Point Apparatus and were not corrected. FT-IR spectrums were recorded on a Shimadzu IR-Prestige-21 spectrometer fitted with an Attenuated Total Reflectance (ATR) sampling accessory as neat films. ^{1}H- and ^{13}C-NMR spectrum was acquired on a Varian Inova 400 MR spectrometer. All microwave experiments were

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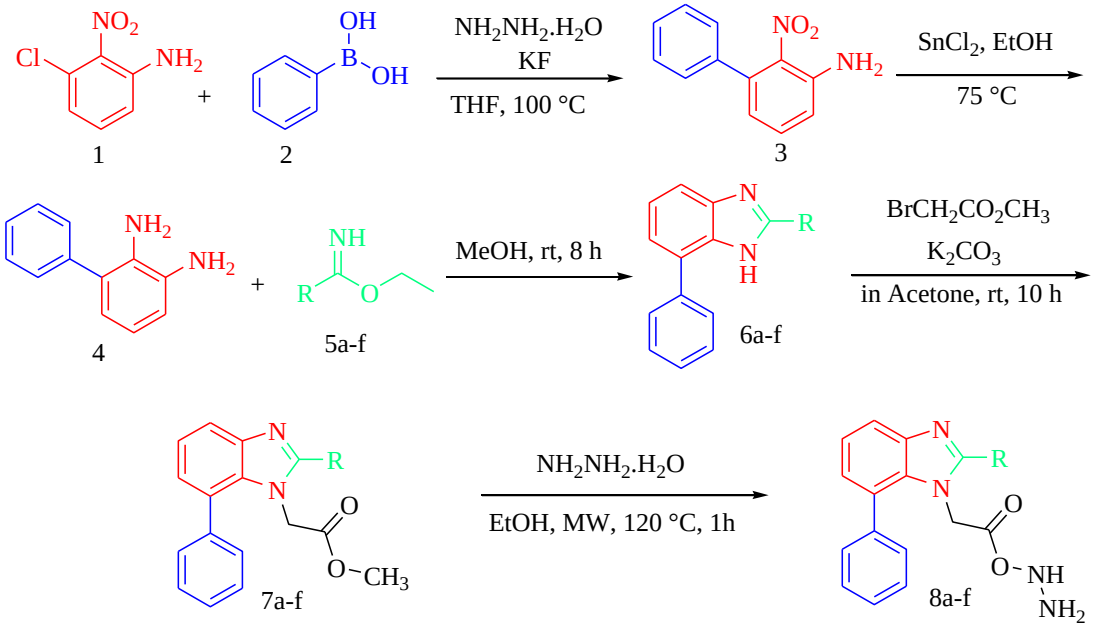
carried out using a monomode Anton Paar Mowave 300 microwave reactor. Elemental analyses were performed on a vario MACRO cube CHNS element analyzer. All reagents and solvents were purchased from commercial suppliers and used without further purification.

Synthesis

Compound **3** and compound **4**, which were used in the synthesis, were prepared by known literature methods [22]. Compound **4** and iminoester hydrochloride derivatives (**5a-f**) reacted in methanol under suitable conditions and benzimidazole derivative compounds (**6a-f**) were synthesized [23]. Esterification of benzimidazole derivative compounds (**7a-f**) was carried out according to the methods described in the literature [24]. The acetohydrazide derivative compounds (**8a-f**) were synthesized by treatment of these ester derivatives (**7a-f**) with hydrazine monohydrate by irradiation at a ceiling temperature of 120 °C and a maximum power of 850W for 60 min. The complete synthesis of the compounds is given in Scheme 1.

Structures of the novel compounds **7a-f** and **8a-f** were elucidated and confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, elemental analysis techniques.



Scheme 1. Synthesis of the compounds, a: 4-chlorobenzyl; b: 3-chlorobenzyl; c: 4-methylbenzyl; d: 3-methylbenzyl; e: benzyl; f: phenyl.

Synthesis of compounds 7a-f: Compounds **7a-f** were synthesized from methyl bromoacetate and compounds **6a-f** in acetone with dry K_2CO_3 under basic conditions at room temperature [24].

Methyl 2-[2-(4-Chlorobenzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetate (7a). Yield 92%, white powder, mp 142-144 °C. IR (ATR, ν_{max}, cm^{-1}): 3047 (aromatic C-H), 2993, 2954, 2931 (aliphatic C-H), 1736 (C=O) 1519, 1489, 1435 (C=C and C=N), 1226, 1010, 748 (C-C, C-N, C-O). ^{1}H NMR spectrum (DMSO-d$_6$, 400 MHz), δ, ppm: 3.49 (3H, s, OCH_3); 4.32 (2H, s, CH_2); 5.20 (2H, s, NCH_2), 7.25-7.38 (6H, m, H Ar); 7.42-7.50 (4H, m, H Ar); 8.04-8.10 (2H, m, H Ar). ^{13}C NMR spectrum (DMSO-d$_6$, 100 MHz, δ (ppm): 32.6 (CH_2); 45.0 (NCH_2); 52.6 (OCH_3); 110.0; 121.3; 123.0; 127.6; 128.7 (2C); 128.8 (2C); 129.4 (2C); 131.0 (3C); 131.7; 135.9; 136.9; 138.5; 140.1 (18C Ar); 153.8 (C=N); 168.6 (C=O). Found, %: C 72.80; H 4.522; N 7.61. $C_{23}H_{19}ClN_2O_2$. Calculated, %: C 70.68; H 4.90; N 7.17.

Methyl 2-[2-(3-Chlorobenzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetate (7b). Yield 95%, dark cream powder, mp 129-131 °C. IR (ATR, ν_{max}, cm^{-1}): 3055 (aromatic C-H), 2993, 2954 (aliphatic C-H), 1736 (C=O) 1597, 1512, 1435 (C=C and C=N), 1211, 1157, 748 (C-C, C-N, C-O). ^{1}H NMR spectrum (DMSO-d$_6$, 400 MHz), δ, ppm: 3.49 (3H, s, OCH_3); 4.34 (2H, s, CH_2); 5.23 (2H, s, NCH_2), 7.20-7.38 (6H, m, H Ar); 7.41-7.50 (4H, m, H Ar); 8.04-8.10 (2H, m, H Ar). ^{13}C NMR spectrum (DMSO-d$_6$, 100 MHz, δ (ppm): 32.7 (CH_2); 45.0 (NCH_2); 52.6 (OCH_3); 110.0; 121.2; 123.0; 127,0 127.6; 127.9; 128.7 (2C); 129.0; 129.4 (2C); 130.7; 131.0; 133.4; 136.9; 138.5; 139.4; 140.1 (18C Ar); 153.6 (C=N); 168.6 (C=O). Found, %: C 71.64; H 4.669; N 7.42. $C_{23}H_{19}ClN_2O_2$. Calculated, %: C 70.68; H 4.90; N 7.17.

Methyl 2-[2-(3-Methylbenzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetate (7c). Yield 88%, light cream powder, mp 143-145 °C. IR (ATR, ν_{max}, cm^{-1}): 3055 (aromatic C-H), 2931 (aliphatic C-H),

1751 (C=O) 1597, 1520, 1427 (C=C and C=N), 1265, 1165, 750 (C-C, C-N, C-O). ^{1}H NMR spectrum (DMSO-d_6, 400 MHz), δ, ppm: 2.22 (3H; s; –CH_3); 3.46 (3H, s, OCH_3); 4.27 (2H, s, CH_2); 5.15 (2H, s, NCH_2), 6.97-7.30 (5H, m, H Ar); 7.33-7.53 (5H, m, H Ar); 8.05-8.10 (2H, m, H Ar). ^{13}C NMR spectrum (DMSO-d_6, 100 MHz, δ (ppm): 21.41 (CH_3); 33.35 (CH_2); 45.05 (NCH_2); 52.54 (OCH_3); 110.98; 121.18; 122.90; 126,20 127.61; 127.66; 128.73 (2C); 128.81; 129.36 (2C); 129.66; 130.95; 136.64; 136.97; 137.99; 138.56; 140.10 (18C Ar); 154.15 (C=N); 168.51 (C=O). Found, %: C 76.27; H 5.435; N 7.47. $C_{24}H_{22}N_2O_2$. Calculated, %: C 77.81; H 5.99; N 7.56.

Methyl 2-[2-(4-Methylbenzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetate (7d). Yield 86%, cream powder, mp 131-133 °C. IR (ATR, ν_{max}, cm^{-1}): 3032 (aromatic C-H), 2924 (aliphatic C-H), 1735 (C=O) 1597, 1512, 1435 (C=C and C=N), 1226, 1011, 750 (C-C, C-N, C-O). ^{1}H NMR spectrum (DMSO-d_6, 400 MHz), δ, ppm: 2.23 (3H; s; –CH_3); 3.48 (3H, s, OCH_3); 4.27 (2H, s, CH_2); 5.14 (2H, s, NCH_2), 7.04-7.16 (4H, m, H Ar); 7.26-7.53 (6H, m, H Ar); 8.06-8.10 (2H, m, H Ar). ^{13}C NMR spectrum (DMSO-d_6, 100 MHz, δ (ppm): 21.04 (CH_3); 33.02 (CH_2); 45.00 (NCH_2); 52.57 (OCH_3); 109.92; 121.15; 122.86; 127,57; 128.71 (2C); 128.96 (2C); 129.37 (2C); 129.44 (2C); 130.95; 133.69; 136.05; 136.96; 138.59; 140.14 (18C Ar); 154.28 (C=N); 168.51 (C=O). Found, %: C 76.74; H 5.580; N 7.47. $C_{24}H_{22}N_2O_2$. Calculated, %: C 77.81; H 5.99; N 7.56.

Methyl 2-[2-(Benzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetate (7e). Yield 92%, white powder, mp 96-98 °C. IR (ATR, ν_{max}, cm^{-1}): 3032 (aromatic C-H), 2924 (aliphatic C-H), 1736 (C=O) 1512, 1427 (C=C and C=N), 1211, 1165, 763 (C-C, C-N, C-O). ^{1}H NMR spectrum (DMSO-d_6, 400 MHz), δ, ppm: 3.46 (3H, s, OCH_3); 4.31 (2H, s, CH_2); 5.17 (2H, s, NCH_2), 7.15-7.30 (6H, m, H Ar); 7.32-7.52 (5H, m, H Ar); 8.04-8.10 (2H, m, H Ar). ^{13}C NMR spectrum (DMSO-d_6, 100 MHz, δ (ppm): 33.32 (CH_2); 45.01 (NCH_2); 52.58 (OCH_3); 109.97; 121.18; 122.90; 127.02; 127,60; 128.72 (2C); 128.89 (2C); 129.09 (2C); 129.37 (2C); 130.96; 136.81; 136.94; 138.56; 140.12 (18C Ar); 154.28 (C=N); 168.51 (C=O). Found, %: C 76.64; H 5.369; N 7.32. $C_{23}H_{20}N_2O_2$. Calculated, %: C 77.51; H 5.66; N 7.86.

Methyl 2-[2-(Phenyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetate (7f). Yield 85%, cream powder, mp 130-131 °C. IR (ATR, ν_{max}, cm^{-1}): 3032 (aromatic C-H), 2924 (aliphatic C-H), 1751 (C=O) 1473, 1442 (C=C and C=N), 1211, 987, 694 (C-C, C-N, C-O). ^{1}H NMR spectrum (DMSO-d_6, 400 MHz), δ, ppm: 3.66 (3H, s, OCH_3); 5.24 (2H, s, NCH_2), 7.32-7.50 (5H, m, H Ar); 7.52-7.73 (6H, m, H Ar); 8.08-8.14 (2H, m, H Ar). ^{13}C NMR spectrum (DMSO-d_6, 100 MHz, δ (ppm): 46.42 (NCH_2); 52.99 (OCH_3); 110.44; 121.75; 123.66; 127.72; 128.76 (2C); 129.32 (2C); 128.40 (2C); 129.53 (2C); 130.26; 130.42; 131.55; 137.40; 138.40; 140.43 (18C Ar); 154.28 (C=N); 168.51 (C=O). Found, %: C 76.01; H 5.445; N 7.41. $C_{22}H_{18}N_2O_2$. Calculated, %: C 77.17; H 5.30; N 8.18.

Synthesis of compounds 8a-f: A mixture of compounds **7a-f** (0.01 mol) and hydrazine monohydrate (0.025 mol) in ethanol (4 ml) in a microwave process vial was added. The mixture was irradiated at a ceiling temperature of 120 °C and a maximum power of 850W for 60 min. The reaction mixture was cooled, the mixture was kept overnight in the refrigerator and precipitated. The crude product was filtered off, dried, and recrystallized from EtOH.

2-[2-(4-Chlorobenzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetohydrazide (8a). Yield 88%, white powder, mp 198-200 °C. IR (ATR, ν_{max}, cm^{-1}): 3309, 3171 (N-H); 3047 (aromatic C-H), 2993 (aliphatic C-H), 1658 (C=O) 1512, 1489, 1442 (C=C and C=N), 1242, 1018, 748 (C-C, C-N, C-O). ^{1}H NMR spectrum (DMSO-d_6, 400 MHz), δ, ppm: 4.30 (2H, s, CH_2); 4.36 (2H, s, NH_2, exchanged with D_2O); 4.80 (2H, s, NCH_2); 7.22-7.50 (10H, m, H Ar); 8.00-8.06 (2H, m, H Ar); 9.50 (1H, s, NH, exchange, D_2O). ^{13}C NMR spectrum (DMSO-d_6, 100 MHz, δ, ppm: 32.59 (CH_2); 45.05 (NCH_2); 109.89; 121.08; 122.76; 127.56; 128.69 (2C); 128.80 (2C); 129.30 (2C); 130.91; 130.98 (2C); 131.64; 136.32; 136.79; 138.57; 140.19 (18 C Ar); 154.05 (C=N); 166.25 (C=O). Found, %: C 67.05; H 4.340; N 14.16. $C_{22}H_{19}N_4O$. Calculated, %: C 67.60; H 4.90; N 14.33.

2-[2-(3-Chlorobenzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetohydrazide (8b). Yield 83%, white powder, mp 179-182 °C. IR (ATR, ν_{max}, cm^{-1}): 3356, 3286, 3178 (N-H); 3047 (aromatic C-H), 2993 (aliphatic C-H), 1712, 1674 (C=O) 1504, 1427, 1396 (C=C and C=N), 1226, 1157, 1010, 748 (C-C, C-N, C-O). ^{1}H NMR spectrum (DMSO-d_6, 400 MHz), δ, ppm: 4.25 (2H, s, NH_2, exchanged with D_2O); 4.33 (2H, s, CH_2); 4.84 (2H, s, NCH_2); 7.22-7.50 (10H, m, H Ar); 8.00-8.06 (2H, m, H Ar); 9.52 (1H, s, NH, exchange, D_2O). ^{13}C NMR spectrum (DMSO-d_6, 100 MHz, δ, ppm: 32.73 (CH_2); 45.10 (NCH_2); 109.96; 121.08; 122.78; 126.99; 127.57; 127.90; 128.68 (2C); 129.05; 129.29 (2C); 130.66; 130.88; 133.40; 136.75; 138.56; 139.85; 140.20 (18 C Ar); 153.85 (C=N); 166.27 (C=O). Found, %: C 66.38; H 4.052; N 13.74.

$C_{22}H_{19}N_4O$. Calculated, %: C 67.60; H 4.90; N 14.33.

2-[2-(3-Methylbenzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetohydrazide (8c). Yield 81%, white powder, mp 152-154 °C. IR (ATR, ν_{max}, cm^{-1}): 3302, 3232 (N-H); 3047 (aromatic C-H), 2993 (aliphatic C-H), 1651, 1604 (C=O) 1512, 1435, 1396 (C=C and C=N), 1234, 1165, 1010, 756 (C-C, C-N, C-O). ^{1}H NMR spectrum (DMSO-d_6, 400 MHz), δ, ppm: 2.23 (3H; s; $–CH_3$); 4.26 (2H, s, CH_2); 4.31 (2H, s, NH_2, exchanged with D_2O); 4.77 (2H, s, NCH_2); 6.98-7.50 (10H, m, H Ar); 8.00-8.08 (2H, m, H Ar); 9.49 (1H, s, NH, exchange, D_2O). ^{13}C NMR spectrum (DMSO-d_6, 100 MHz, δ, ppm: 21.44 (CH_3); 33.38 (CH_2); 45.04 (NCH_2); 109.88; 121.00; 122.67; 126.08; 127.55; 127.68; 128.69 (2C); 128.85; 129.32 (2C); 129.61; 130.87; 136.87; 137.07; 138.05; 138.63; 140.21 (18 C Ar); 154.41 (C=N); 166.29 (C=O). Found, %: C 74.08; H 5.729; N 14.87. $C_{23}H_{22}N_4O$. Calculated, %: C 74.57; H 5.99; N 15.12.

2-[2-(4-Methylbenzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetohydrazide (8d). Yield 91%, white powder, mp 183-184 °C. IR (ATR, ν_{max}, cm^{-1}): 3317, 3163 (N-H); 3032 (aromatic C-H), 2978, 2916 (aliphatic C-H), 1658 (C=O) 1512, 1442, 1396 (C=C and C=N), 1219, 1157, 972, 748 (C-C, C-N, C-O). ^{1}H NMR spectrum (DMSO-d_6, 400 MHz), δ, ppm: 2.23 (3H; s; $–CH_3$); 4.25 (2H, s, CH_2); 4.31 (2H, s, NH_2, exchanged with D_2O); 4.75 (2H, s, NCH_2); 7.05-7.50 (10H, m, H Ar); 8.02-8.08 (2H, m, H Ar); 9.48 (1H, s, NH, exchange, D_2O). ^{13}C NMR spectrum (DMSO-d_6, 100 MHz, δ, ppm: 21.06 (CH_3); 33.04 (CH_2); 45.01 (NCH_2); 109.84; 120.99; 122.65; 126.08; 127.53; 128.69 (2C); 128.87 (2C); 129.32 (2C); 129.52 (2C); 130.87; 134.11; 136.03; 136.86; 138.64; 140.22 (18 C Ar); 154.56 (C=N); 166.29 (C=O). Found, %: C 73.95; H 5.458; N 14.94. $C_{23}H_{22}N_4O$. Calculated, %: C 74.57; H 5.99; N 15.12.

2-[2-(Benzyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetohydrazide (8e). Yield 82%, white powder, mp 150-151 °C. IR (ATR, ν_{max}, cm^{-1}): 3294, 3271 (N-H); 3055 (aromatic C-H), 2939 (aliphatic C-H), 1651 (C=O) 1512, 1472, 1388 (C=C and C=N), 1242, 1165, 918, 694 (C-C, C-N, C-O). ^{1}H NMR spectrum (DMSO-d_6, 400 MHz), δ, ppm: 4.21 (2H, s, NH_2, exchanged with D_2O); 4.30 (2H, s, CH_2); 4.78 (2H, s, NCH_2); 7.15-7.50 (11H, m, H Ar); 8.02-8.06 (2H, m, H Ar); 9.49 (1H, s, NH, exchange, D_2O). ^{13}C NMR spectrum (DMSO-d_6, 100 MHz, δ, ppm: 33.37 (CH_2); 45.02 (NCH_2); 109.87; 121.03; 122.70; 127.01; 127.55; 128.69 (2C); 128.94 (2C); 129.02 (2C); 129.31 (2C); 130.88; 136.83; 137.22; 138.61; 140.20 (18 C Ar); 154.41 (C=N); 166.28 (C=O). Found, %: C 73.02; H 5.246; N 15.42. $C_{22}H_{20}N_4O$. Calculated, %: C 74.14; H 5.66; N 15.72.

2-[2-(Phenyl)-4(7)-phenyl-1*H*-benzimidazol-1-yl]acetohydrazide (8f). Yield 80%, white powder, mp 250-251 °C. IR (ATR, ν_{max}, cm^{-1}): 3302, 3263 (N-H); 3032 (aromatic C-H), 2931 (aliphatic C-H), 1658 (C=O) 1527, 1419, 1388 (C=C and C=N), 1242, 1157, 972, 748 (C-C, C-N, C-O). ^{1}H NMR spectrum (DMSO-d_6, 400 MHz), δ, ppm: 4.40 (2H, s, NH_2, exchanged with D_2O); 4.88 (2H, s, NCH_2); 7.30-7.86 (11H, m, H Ar); 8.08-8.14 (2H, m, H Ar); 9.62 (1H, s, NH, exchange, D_2O). ^{13}C NMR spectrum (DMSO-d_6, 100 MHz, δ, ppm: 46.30 (NCH_2); 110.33; 121.52; 123.36; 127.66; 128.74 (2C); 129.12 (2C); 129.37 (2C); 129.89 (2C); 130.27, 130.47; 131.44; 137.43; 138.50; 140.51 (18 C Ar); 154.15 (C=N); 166.68 (C=O). Found, %: C 74.77; H 5.321; N 16.21. $C_{21}H_{18}N_4O$. Calculated, %: C 73.67; H 5.30; N 16.36.

Enzym assay

Acetylcholinesterase enzyme (AChE) from electric eel, acetylthiocholine iodide (AChI), ascorbic acid (AA), butyrylcholinesterase enzyme (BuChE), butyrylthiocholine iodide (BTCI), 5,5-dithio-bis(2-nitrobenzoic)acid (DTNB), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethanol, galantamine, kojic acid, $_L$-DOPA, methanol, tyrosinase from mushroom, Trisma-base, phoshomolybdic acid, and quercetin (QE) were purchased from Sigma-Aldrich (St. Louis, MO). Experiments of biological activities were performed by Multikan^{Tm} Go Microplate Spectrophotometer.

ENZYME INHIBITION

AChE/BuChE inhibition assays

AChE/BuChE inhibition was examined using the method described by Ingkaninan et al. [25]. All of the compounds were prepared as stock solutions in 20% DMSO. 50 mM Tris-HCl buffer (pH 8.00), 3 mM DTNB (in buffer), 0.2 U/mL AChE/BuChE and compounds at 100 μM were added in a 96-well microplate. The mixtures were incubated for 15 min at 25 °C. After incubation, 15 mM AChI/BTCI was added in a microplate and incubated for 5 min at room temperature. The absorbance was measured at 412 nm using a 96-well microplate reader. Galantamine was used as the positive control. AChE/BuChE inhibition percentage was calculated at 100 μM using the Formula 1. Where $A_{control}$ is the activity of enzyme without compound and $A_{compound}$ is the activity of enzyme with compound at different concentrations. The experiments were carried out in

triplicate and results were expressed as the mean ± standard deviation (SD).

Formula 1. Formula for both enzyme inhibition and scavenging percentage:

$$Inhibition\ \% = \frac{(Acontrol - Acompound)}{Acontrol} \times 100$$

Tyr inhibition assay

The Tyr inhibitions of compounds were investigated using the method described by Masuda et al. [26]. The compounds at 100 µM, 250 U/mL tyrosinase and 100 mM pH 6.8 phosphate buffer solutions were added in a 96-well microplate. The mixtures were incubated for 10 min at room temperature. Then, 3 mM L-DOPA was added and the microplate was further incubated at room temperature for 20 min. Subsequently, the absorbance of dopachrome was measured at 475 nm using a 96-well microplate reader. Kojic acid was used as the positive control. Tyr inhibitions percentage was calculated at 100 µM using Formula 1.

DPPH radical scavenging assay

The DPPH radical scavenging activities were examined using the method described by Blois compared to ascorbic acid as the reference compound [27]. The total volume of assay mixture was 1 mL containing methanolic DPPH solution (0.4 mM) and at 100 µM of compounds. The mixtures were incubated for 30 min at room temperature in the dark. After incubation, the mixtures were measured at 517 nm and scavenging percentage was calculated using Formula 1.

Phosphomolybdenum-reducing antioxidant power (PRAP) assay

PRAP of compounds were examined using phosphomolybdic acid [28]. The total volume of assay mixture was 1 mL containing 10% phoshomolybdic acid solution in ethanol (w/v) and at 100 µM of compounds. The mixtures were incubated for 30 min at 80 ºC. After incubation, the absorbance was measured at 600 nm. PRAP of compounds was expressed absorbance at 100 µM and compared with quercetine as reference compound.

Ferric-Reducing Antioxidant Power (FRAP) Assay

FRAP of compounds was examined using the method described by Oyaizu [29]. 1 mL of various concentrations of the extracts and BHA as the reference compound were added to 1 mL of phosphate buffer (pH 6.6) and 1 mL of 10% (w/v) potassium ferricyanide. The mixtures were incubated at 50 °C for 20 min and then 1 mL of 10% trichloroacetic acid was added. After vigorous shaking, the solutions were mixed with 1 mL distilled water and 150 µL of 0.15% $FeCl_3$. The mixtures were incubated for 30 min at room temperature in the dark. After incubation, the absorbance was measured at 700 nm. FRAP of compounds was expressed absorbance at 100 µM and compared with BHA as reference compound.

RESULTS AND DISCUSSION

Chemistry

Since both the ester derivatives (**7a-f**) and the hydrazide derivatives (**8a-f**) are new, spectral analysis of each compound were made. The spectral data (IR, ^{1}H-NMR, ^{13}C-NMR) results of these new compounds were consistent with the literatures [30-33].

The IR spectra of compounds **7a-f** showed the characteristic bands at 1735-1751 cm^{-1} for ester C=O groups, at 3055-3032 cm^{-1} for aromatic C-H groups. IR absorption band appeared at 1597- 1427 cm^{-1} and 1265-1010 cm^{-1} were assigned to C=C, C=N and C-C, C-N functions in the benzimidazole structure, respectively. In the final compounds, **8a-f**, the hydrazide group is in place of the ester group. So, IR absorption band appeared at 3356-3171 cm^{-1} was assigned to NH atoms.

In case of ^{1}H-NMR spectral analysis of compounds **7a-f**, the methyl and methylene groups have a chemical shift because of their *neighboring protons and have been* appeared as a singlet. The methyl protons (-COOC$\underline{H}_3$) which next to the presence of ester carbonyl, oxygen groups, giving peak in 3.46-3.66 ppm range; the methylene protons (-ArC$\underline{H}_2$-Ph) which next to the presence of substitute phenyl ring and benzimidazole groups, giving peak in 4.27-4.34 ppm range and the methylene protons (-NC$\underline{H}_2$-COO) which next to the presence of ester carbonyl and benzimidazole groups, giving peak in 5.14-5.24 ppm range. Aromatic protons were seen where expected (δ = 6-8 ppm regions). In the compounds **8a-f**, the positions of the protons in the hydrazide group were determined by exchange of deutero-solvent. The ^{1}H-NMR spectra of compounds **8a-f** showed the presence of a singlet at δ = 4.21-4.40 ppm for the hydrazide-NH_2 proton, and hydrazide-NH protons appeared at δ = 9.48–9.62 ppm.

^{13}C-NMR spectrum of compounds **7a-f** and **8a-f**, gives signal at 153.85-154.56 (C=N) and 166.25-166.68 (C=O) ppm and the aromatic field were observed at 109.84–140.51 ppm. All peaks were

observed at expected locations in accordance with literature data [30-33].

The elemental analysis results confirm the carbon, nitrogen, hydrogen and oxygen fractions of the compounds.

Enzyme activity. Enzyme Inhibition

The AChE/BuChE inhibition of compounds was measured by Ingkaninan's assay with galantamine as a standard drug. AChE/BuChE inhibition results of compounds were expressed as inhibition (%) values were presented in Table 1. The solubility of benzimidazole derivative compounds decreases at basic pH. No activity was detected in the solutions prepared for the AChE/BuChE enzyme since the substances precipitated in the pH 8 buffer. The results showed that tested compounds did not inhibit against AChE/BuChE. However, inhibition of compounds and standard drug on tyrosinase enzyme were illustrated in Table 1. The inhibition ratios of tyrosinase by the compounds were measured at the concentrations of 100 µM, as shown in Table 1. **8f** and **7c** exhibited the highest tyrosinase inhibition with 52.46 ± 2.67% and 52.32 ± 0.70%, respectively. In addition, **7a**, **7b**, **7d** and **8d** demonstrated tyrosinase inhibition more than 45.00 % at 100 µM. These results showed that, these compounds have promising candidates for tyrosinase inhibitors.

Table 1. AChE, BuChE and Tyr enzyme inhibition of compounds at 100 µM.

Compounds	AChE at 100 µM (%)	BuChE at 100 µM (%)	Tyr at 100 µM (%) ± SD
7a	Nd	Nd	45.22 ± 0.39
7b	Nd	Nd	49.43 ± 2.04
7c	Nd	Nd	52.32 ± 0.70
7d	Nd	Nd	47.00 ± 0.36
7e	Nd	Nd	43.68 ± 2.63
7f	Nd	Nd	39.63 ± 1.84
8a	Nd	Nd	40.27 ± 1.32
8b	Nd	Nd	35.59 ± 1.68
8c	Nd	Nd	39.36 ± 1.24
8d	Nd	Nd	46.31 ± 1.05
8e	Nd	Nd	41.03 ± 3.87
8f	Nd	Nd	52.46 ± 2.67
Galantamine	88.03 ± 0.31	79.30 ± 0.26	-
Kojic Acid	-	-	85.48 ± 0.40

*nd: no data ** SD: Standart deviation

Table 2. DPPH, PRAP and FRAP activities of compounds **7a-f** and **8a-f** at 100 µM.

Compounds	DPPH at 100 µM (%) ± SD	PRAP at 100 µM (Absorbance) ± SD	FRAP at 100 µM (Absorbance) ± SD
7a	11.25 ± 0.11	0.132 ± 0.002	0.236 ± 0.004
7b	14.50 ± 0.24	0.142 ± 0.007	0.263 ± 0.007
7c	21.42 ± 0.71	0.169 ± 0.010	0.326 ± 0.010
7d	13.50 ± 0.41	0.141 ± 0.004	0.247 ± 0.007
7e	14.71 ± 0.83	0.135 ± 0.010	0.269 ± 0.006
7f	9.26 ± 0.28	0.125 ± 0.006	0.194 ± 0.002
8a	13.48 ± 0.44	0.121 ± 0.003	0.227 ± 0.002
8b	12.26 ± 0.36	0.128 ± 0.005	0.217 ± 0.001
8c	9.62 ± 0.85	0.121 ± 0.001	0.179 ± 0.002
8d	16.05 ± 0.57	0.127 ± 0.004	0.294 ± 0.011
8e	17.27 ± 0.69	0.157 ± 0.009	0.271 ± 0.003
8f	24.83 ± 1.28	0.180 ± 0.013	0.358 ± 0.009
AA	84.02 ± 0.09	-	-
QE	-	0.849 ± 0.022	-
BHA	-	-	1.426 ± 0.014

*SD: Standard deviation

Antioxidant activities

In this work, DPPH radical scavenging activities, PRAP and FRAP reducing antioxidant power assay of compounds have been investigated spectrophotometrically method and their results were presented in Table 2. All the compounds

displayed moderate DPPH radical scavenging activities when compared to AA. **8f** had the highest radical scavenging activities with 24.83 ± 1.28% among the compounds whilst **8c** showed the lowest activities with 9.62 ± 0.85%. PRAP assay was based on the reduction of Mo(VI) to Mo(V) by the compounds. The highest absorbance values of the compounds at 600 nm exhibited a higher reducing antioxidant power. All of the compounds showed the lowest values at 100 μM when compared to quercetin as standard compound. **8f** and **7c** gave the highest absorbance values with 0.180 ± 0.013 and 0.169 ± 0.010 at 100 μg/mL, respectively. The ferric reducing antioxidant power (FRAP) assay was a fast method to determine the antioxidant potential of compounds. . The compounds capacity to reduce Fe^{3+} to Fe^{2+} was determined and the results were shown in Table 2. The results of this study demonstrated that compounds have a moderate ferric reducing power. **8f** has the highest absorbance values with 0.358 ± 0.009 at 100 μM. All of these results showed that, the newly synthesized compounds have moderate antioxidant activities when compared to standard compounds.

CONCLUSION

All the synthesized molecules were achieved in excellent yields using a simple method. The projected structures of synthesized compounds were well supported by the spectral characterization data by IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis.

The antioxidant, AChE/BuChE and tyrosinase inhibition properties of all compounds were evaluated. The tested compounds did not inhibit against AChE/BuChE. The phenyl substituted hydrazide derivative compound **8f** and the 4-methylbenzyl substituted ester derivative compound **7c** showed the highest tyrosinase inhibition with 52.46 ± 2.67% and 52.32 ± 0.70%, respectively. **8f** had the highest radical scavenging activities with 24.83 ± 1.28% among the compounds whilst **8c** showed the lowest activities with 9.62 ± 0.85%. **8f** gave the highest PRAP and FRAP absorbance values with 0.180 ± 0.013 and 0.358 ± 0.009 at 100 μM, respectively. All of these results demonstrated that the newly synthesized compounds have moderate antioxidant and tyrosinase inhibition activities when compared to standard compounds.

In our previous work [23], we have synthesized benzimidazole derivatives **6a-f** compounds, when we evaluated the tyrosine inhibition activity, 4-chlorobenzylsubstitued benzimidazole derivative **6a**, 3-chlorobenzylsubstituted benzimidazole derivative **6b** and 3-methylbenzylsubstitued benzimidazole derivative **6d**, were found to be the most active derivatives. In this study, we evaluated tyrosine inhibition activity of compounds **7a-f** obtained from the esterification of **6a-f** derivatives. The compounds **7a-d** (4-chloro/3-chloro/4-methyl/3-methylbenzylsubstituted compounds) were found to be active. It can be said that the ester derivatives do not show much difference in activity compared to the benzimidazole derivatives. According to the activity results of the hydrazide derivatives **8a-f** compounds, the tyrosine inhibition activity was quite good in the phenyl derivative **8f** compound. The 4-methylbenzyl derivative **8c** showed very low activity. The high activity of the phenyl substituted derivative, compound **8f**, suggested that activity may be high in the aryl derivatives, with reduced activity in the arylalkyl derivative **8a-8e** compounds. Activity evaluation can be done by testing different aryl, heteroaryl derivatives.

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