Synthesis, Characterization and Tyrosinase Inhibitory Properties of Benzimidazole Derivatives[1]

Mert Olgun Karatas[a], Bulent Alici[a], Engin Çetinkaya[b], Çiğdem Bilen[c], Nahit Gençer[c, 2], and Oktay Arslan[c]

[a]*Inonu University, Faculty of Arts and Sciences, Department of Chemistry , Malatya, 44280 Turkey*
[b]*Ege University, Faculty of Science, Department of Chemistry, Izmir, 35100 Turkey*
[c]*Department of Chemistry, Faculty of Art and Sciences, Balikesir University, Balikesir, 10145 Turkey*
Received January 8, 2014; in final form, March 3, 2014

Abstract—1-Alkylbenzimidazole and 1,3-dialkyl benzimidazolium salts were synthesized and characterized by the data of IR, ^{1}H NMR, ^{13}C NMR spectra and elemental analyses. These compounds were investigated as tyrosinase inhibitors. Tyrosinase has been purified from banana by affinity chromatography on a Sepharose 4B gel conjugated with *L*-tyrosine-*p*-aminobenzoic acid. All the synthesized compounds inhibited the tyrosinase activity. Among the compounds studied, 1,4-di(1*H*-benzo[d]imidazol-1-yl)butane was found to be the most active tyrosinase inhibitor (IC$_{50}$ 0.31 mM).

INTRODUCTION

Benzimidazole consists of the fusion of benzene and imidazole. Benzimidazole has been widely used as carbon skeleton for synthesis of N-heterocyclic carbenes (NHC). Benzimidazole derivatives and their NHC's are usually used as ligand for transition metal complexes and these complexes are also used as catalyst in various organic synthesis [1]. Besides using on synthesis of NHC's, various biological activities of benzimidazole derivatives were reported [2]. The biologic potential of benzimidazole can be traced back to 1944. Wooley reported that benzimidazole can act similar to purines to give some biological responses [3]. After this study, biological properties of benzimidazole derivatives were investigated intensively. Benzimidazole bearing bioactive compounds were reported as antihypertensive [4], anti-inflammatory [5], antimicrobial [6], antiviral [7], antioxidant [8], antitumor [9], lipid modulator [10], anticoagulant [11].

Tyrosinase (monophenol or *o*-diphenol, oxygen oxidoreductase, EC 1.14.18.1), also known as polyphenol oxidase (PPO), is a copper-containing monooxygenase that is widely distributed in microorganisms, animals, and plants [12]. Tyrosinase could catalyze two distinct reactions involving molecular oxygen in the hydroxylation of monophenols to *o*-diphenols (monophenolase) and in the oxidation of *o*-diphenols to *o*-quinones (diphenolase) [13]. Due to

the high reactivity, quinines could polymerize spontaneously to form high molecular weight brown pigments (melanins) or react with amino acids and proteins to enhance brown colour of the pigment produced [14, 15]. Previous reports confirmed that tyrosinase not only was involved in melanising in animals, but also was one of the main causes of most fruits and vegetables quality loss during post harvest handling and processing, leading to faster degradation and shorter shelf life [16]. Recently, investigation demonstrated that various dermatological disorders, such as age spots and freckle, were caused by the accumulation of an excessive level of epidermal pigmentation [17, 18]. Tyrosinase has also been linked to Parkinson's and other neurodegenerative diseases [19]. In insects, tyrosinase is uniquely associated with three different biochemical processes, including sclerotization of cuticle, defensive encapsulation and melanisation of foreign organism, and wound healing [20]. These processes provide potential targets for developing safer and effective tyrosinase inhibitors as insecticides and ultimately for insect control. Thus, the development of safe and effective tyrosinase inhibitors is of great concern in the medical, agricultural, and cosmetic industries. However, only a few such as kojic acid, arbutin, tropolone, and 1-phenyl-2-thiourea are used as therapeutic agents and cosmetic products [18, 21].

In this study we synthesized 1-alkylbenzimidazoles and 1,3-dialkyl benzimidazolium salts and their inhibitory properties on PPO activity were evaluated. Imi-

---

[1] The article is published in the original.
[2] Corresponding author: e-mail: ngencer@balikesir.edu.tr.

dazolium salts similar to we synthesized in this study were reported as antimicrobial agents [22] but PPO inhibitory properties of ionic benzimidazole derivatives were investigated first time in this study.

RESULTS AND DISCUSSION

The synthetic procedures employed to obtain the target compounds (4a−m) are depicted in Scheme 1. All new synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR spectroscopic methods and elemental analyses. In the IR spectra of compounds (4a−m), it was possible to see the absorption between 1593 and 1553 cm^{-1} belong to N−C−N bond. In the ^{1}H NMR it was possible to see characteristic NCHN signals between 9.52−9.01 ppm as singlet. Beside these signals, in anthracene skeleton, at position 10 (Fig. 1),
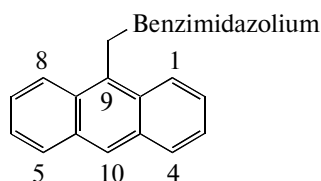
characteristic aromatic proton signals were obtained between 8.95−8.86 ppm as singlet.^{13}C NMR signals and number of peaks were compatible with structure of synthesized compounds. In ^{13}C NMR spectra, characteristic NCN signals were obtained between 141.6 and 162.8 ppm. Furthermore, elemental analysis datas were compatible with synthesizes compounds.

Fig. 1. Anthracene skeleton.
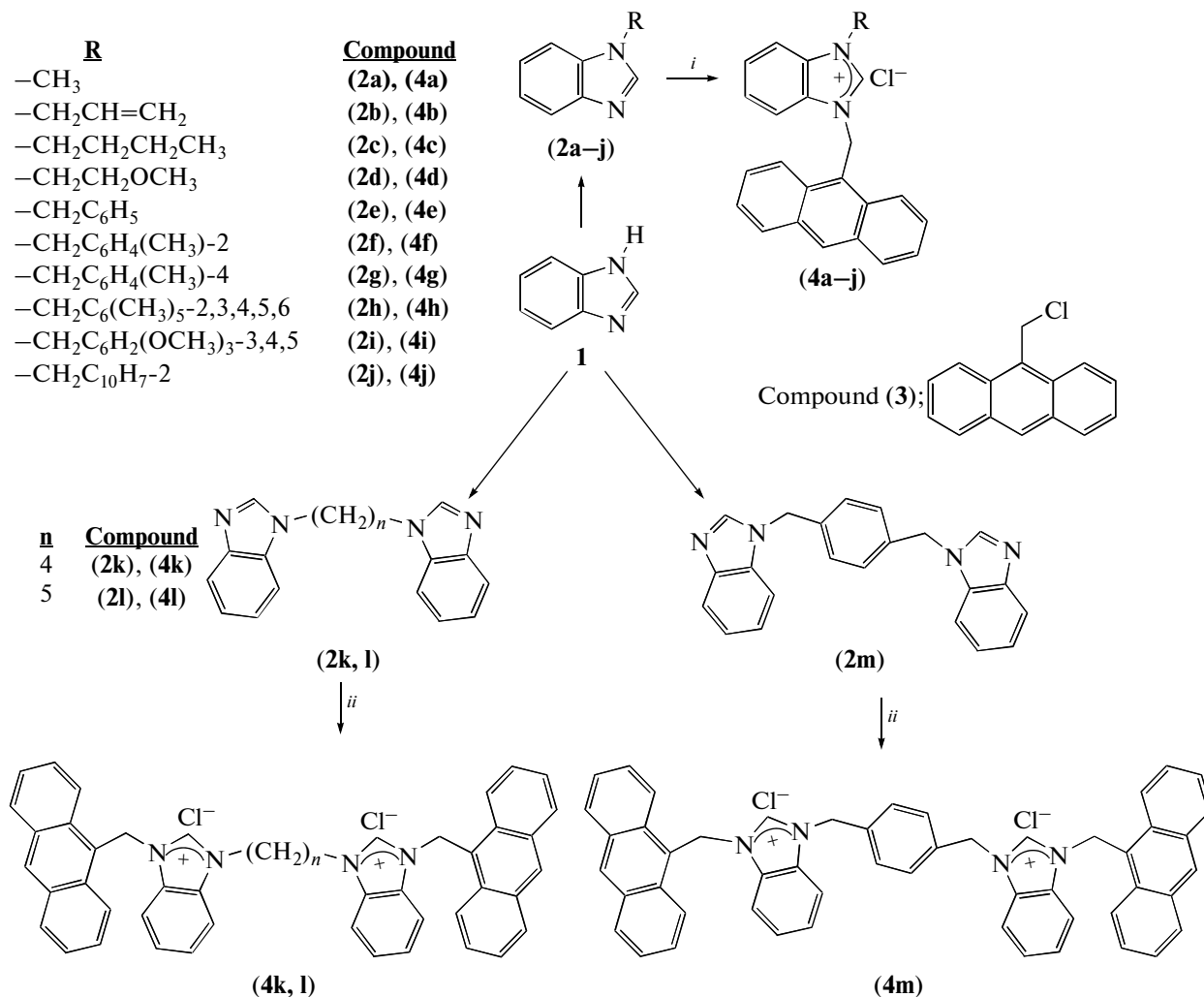
Scheme 1. Synthesized Compounds. Reagents and Conditions: (i) Ethanol, KOH, RX, 8h, reflux (ii) compound (3), 90°C, DMF, 3 days. (iii) 9-(Chlormethyl)anthracene (3), 90°C, DMF, 5 days.

For evaluating the tyrosinase inhibitory activity, all the synthesized compounds were subjected to tyrosinase inhibition assay with catechol as substrate. The result showed that all the synthesized compounds (**4a−m**) inhibited the tyrosinase enzyme activity. IC_{50} values were calculated from inhibition curves obtained with benzimidazole derivatives. The inhibition values of analogues (**4a−m**) against PPO were summarized in table. We have determined the IC_{50} values of 0.31−13.14 mM for the inhibition of banana PPO. According to IC_{50} values, compound (**2k**) was the most effective inhibitor for BPPO (**0.31 mM**). Except compound (5) and compounds (**2a, 2b, 2c, 2d, 4a**), IC_{50} values for 23 compounds were in the range of **0.31−2.56** mM. These results show that aliphatic R groups at position of benzimidazole skeleton decreased PPO inhibitory activity. Inhibition of PPO was investigated using gallic acid as a standard inhibitor, which showed the strongest inhibitory activity with IC_{50} value of 0.03 mM (Fig. 2). Gallic acid (3,4,5-trihydroxybenzoate) has been isolated and identified as a tyrosinase inhibitor from many plants, and its inhibitory mechanism together with those of its ester derivatives has been well studied by Kubo et al. [23−25]. They found that gallic acid inhibited diphenolase activity of mushroom tyrosinase is 100-fold lower than that of kojic acid. The enzymatic browning by a specific inhibitor may involve a single mechanism or may be the result of interplay of two or more mechanisms of inhibitor action.

Enzymatic browning of plants may be delayed or eliminated by removing the reactants, such as oxygen and phenolic compounds, or by using PPO inhibitors. Complete elimination of oxygen from plants during drying is difficult because oxygen is ubiquitous [26]. There are a number of inhibitors, such as sodium metabisulphite [27], ascorbic acid [28], glutathione [29], tropolone [30] decreasing the activity of PPO. Acidulants, such as citric acid can inhibit PPO activity by reducing pH and/or chelating Cu in a food product [31, 32]. Ascorbic acid can also be considered as an effective compound at higher concentrations. The mechanism of ascorbic acid inhibition involves the reduction of quinones generated by PPO [33]. The goal of these studies is determine to the best inhibitor for decreasing the enzymatic browning.

EXPERIMENTAL

All reactions for preparation of benzimidazolium salts were carried out in standard Schlenk-type flasks. Chemicals were purchased from Sigma Aldrich. DMF used as a solvent in the synthesis of benzimidazolium salt was dried by P_2O_5. 9-(Chloromethyl) anthracene (**3**) was used without further purification. Melting points were determined using an Electrothermal-9200 melting point apparatus. FT−IR spectra were recorded on an ATR unit in the range of 400−4000 cm^{-1} using a Perkin Elmer Spectrum 100 Spectrophotometer. ^{1}H NMR and

IC_{50} values for the benzimidazole derivatives tested as tyrosinase inhibitors

Compound	IC_{50} (mM)	Compound	IC_{50} (mM)
(1)	0.50	**(3)**	0.71
(2a)	65.07	**(4a)**	13.14
(2b)	87.40	**(4b)**	0.52
(2c)	11.09	**(4c)**	0.66
(2d)	24.61	**(4d)**	0.38
(2e)	0.97	**(4e)**	0.73
(2f)	0.96	**(4f)**	1.22
(2g)	0.92	**(4g)**	0.66
(2h)	0.63	**(4h)**	0.38
(2i)	2.25	**(4i)**	2.56
(2j)	0.50	**(4j)**	1.29
(2k)	0.31	**(4k)**	0.67
(2l)	0.81	**(4l)**	0.90
(2m)	0.79	**(4m)**	0.50
Gallic acid	0.03		

^{13}C NMR were recorded in DMSO-d_6 using a Bruker AC300P FT spectrometer operating at 300.13 MHz (^{1}H), 75.47 MHz (^{13}C). Chemical shifts (δ) are given in ppm relatively TMS and coupling constants (*J*) are given in Hz. Elementary analysis were done by IBTAM (Inonu University Scientific and Technological Research Central).

Synthesis of 1-alkylbenzimidazole and 1,1'-bisbenzimidazole compounds (2a−m). 1-Alkylbenzimidazole and bisbenzimidazole compounds were synthesized according to the procedure of Ozdemir et al. [34]. Potassium hydroxide (1 mmol) was added to a solution of benzimidazole (1 mmol) in ethanol (20 mL), the mixture was stirred for 1 h at room temperature, and the corresponding alkyl halides was added dropwise and heated for 8 h at 76°C. The mixture was diluted
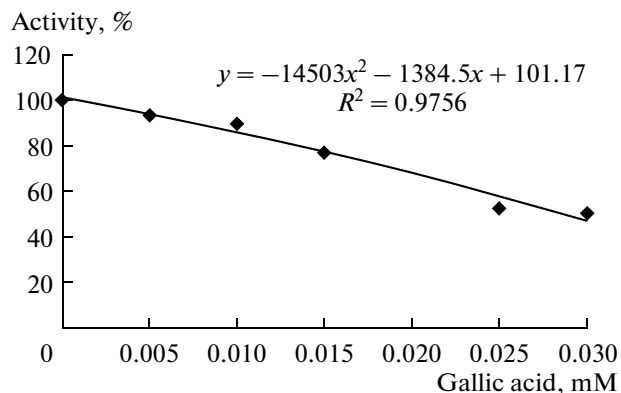
Activity, %

$$y = -14503x^2 - 1384.5x + 101.17$$
$$R^2 = 0.9756$$

Fig. 2. Inhibitory activity of gallic acid on BPPO.

with 30 mL of water and extracted with chloroform (3 × 10 mL). The compounds (**2a–d**) were distilled under reduced pressure and the compounds (**2e–m**) were recrystallized from ethanol-hexane.

Synthesis of benzimidazolium salts (4a–j). 10 mmol 1-alkylbenzimidazole (**2a–j**) was dissolved in 5 mL of dried DMF, then 10 mmol 9-(chloromethyl)anthracene was added into the solution and the mixture was heated for 3 days at 90°C. After cooling the mixture to room temperature, diethyl ether was added, and the precipitate was collected by filtration. The crude product was washed with hexane and then dried under reduced pressure.

1-Methyl-3-(anthracen-9-ylmethyl)benzimidazolium chloride (4a). Yield 81%, mp 255–256°C. Calculated for $C_{23}H_{19}ClN_2$ (%): C 76.98, H 5.34, N 7.81. Found: C 76.88, H 5.62, N 7.65. FT-IR (cm^{-1}): 1563 (C–N). ^{1}H NMR 9.03 (s, 1H, NC**H**N), 8.93 (s, 1H, Ar**H**), 8.42–7.60 (m, 12H, Ar**H**), 6.76 (s, 2H, –C**H**$_2$Ant), 3.91 (s, 3H, N-C**H**$_3$). ^{13}C NMR: 142.0, 132.6, 132.0, 131.6, 131.5, 130.9, 129.9, 128.3, 127.3, 127.2, 126.1, 124.0, 122.6, 114.6, 114.2, 43.7, 33.7.

1-Allyl-3-(anthracen-9-ylmethyl)benzimidazolium chloride (4b). Yield 65%, mp 204–207°C. Calculated for $C_{25}H_{21}ClN_2$ (%): C 78.01, H 5.50, N 7.31. Found: C 77.92, H 5.68, N 7.11. FT-IR (cm^{-1}): 1554 (C–N). ^{1}H NMR: 9.14 (s, 1H, NC**H**N), 8.93 (s, 1H, Ar**H**), 8.42–7.60 (m, 12H, Ar**H**), 6.77 (s, 2H, –C**H**$_2$Ant), 5.94 (ddt, 1H, –CH$_2$–C**H**=H'H", J_{CH_2-CH} 4.8, $J_{CH-H'}$ 6.8, $J_{CH-H''}$ 10.3), 5.27–5.16 (2H, dd, $J_{H-H'}$ 6.8, $J_{H-H'}$ 10.4), 5.03 (d, 2H, –C**H**$_2$CH=CH'H", J_{CH_2-CH} 4.9). ^{13}C NMR: 141.9, 132.3, 131.8, 131.7, 131.6, 131.5, 131.0, 130.0, 128.4, 127.4, 127.3, 126.1, 123.9, 122.5, 119.9, 114.8, 114.5, 49.1, 44.0.

1-(n-Butyl)-3-(anthracen-9-ylmethyl)benzimidazolium chloride (4c). Yield 61%, mp. 241–243°C. Calculated for $C_{26}H_{25}ClN_2$ (%): C 77.89, H 6.29, N 6.9. Found: C 77.82, H 6.51, N 6.88. FT-IR (cm^{-1}): 1564 (C–N). ^{1}H NMR: 9.22 (s, 1H, NC**H**N), 8.92 (s, 1H, Ar**H**), 8.42–7.60 (m, 12H, Ar**H**), 6.76 (s, 2H, –C**H**$_2$Ant), 4.36 (t, 2H, –C**H**$_2$CH$_2$CH$_2$CH$_3$, J 7), 1.69 (five, 2H, –CH$_2$C**H**$_2$CH$_2$CH$_3$, J 7), 1.11 (six, 2H, –CH$_2$CH$_2$C**H**$_2$CH$_3$, J 7), 0.76 (t, 3H, –CH$_2$CH$_2$CH$_2$C**H**$_3$, J 7). ^{13}C NMR : 141.8, 132.2, 131.7, 131.6, 131.5, 130.9, 130.0, 128.3, 127.3, 127.2, 126.1, 123.9, 122.6, 114.7, 114.4, 46.8, 44.0, 31.1, 19.3, 13.6.

1-Methoxyethyl-3-(anthracen-9-ylmethyl)benzimidazolium chloride (4d). Yield 73%, mp 223–226°C. Calculated for $C_{25}H_{23}ClON_2$ (%): C 74.52, H 5.75, N 6.95. Found: C 74.42, H 6.03, N 6.89. FT-IR (cm^{-1}): 1563 (C–N). ^{1}H NMR: 9.11 (s, 1H, NC**H**N), 8.93 (s, 1H, Ar**H**), 8.43–7.60 (m, 12H, Ar**H**), 6.80(s, 2H, –C**H**$_2$Ant), 4.56 (t, 2H, –N–CH$_2$CH$_2$OCH$_3$, J 5), 3.59 (t, 2H, –N–CH$_2$C**H**$_2$OCH$_3$, J 5), 3.05 (s, 3H, –N–CH$_2$CH$_2$OC**H**$_3$). ^{13}C NMR: 142.3, 132.0, 131.9,

131.6, 131.5, 130.9, 130.0, 128.3, 127.3, 127.2, 126.1, 123.9, 122.5, 114.7, 114.1, 69.5, 59.5, 46.5, 44.0.

1-Benzyl-3-(anthracen-9-ylmethyl)benzimidazolium chloride (4e). Yield 70%, mp 234–235°C. Calculated for $C_{29}H_{23}ClN_2$ (%): C 80.08, H 5.33, N 6.44. Found: C 79.71, H 5.42, N 6.53. FT-IR (cm^{-1}): 1565 (C–N), ^{1}H NMR: 9.47 (s, 1H, NC**H**N), 8.94 (s, 1H, Ar**H**), 8.46–8.27 (m, 5H, Ar**H**), 7.97–7.28 (m, 12H, Ar**H**), 6.81 (s, 2H, –C**H**$_2$Ant), 5.63 (s, 2H, –C**H**$_2$Ph). ^{13}C NMR: 142.2, 134.7, 132.4, 131.7, 131.5, 131.4, 131.0, 130.0, 129.3, 129.0, 128.3, 128.2, 127.5, 127.3, 126.1, 123.9, 122.6, 114.9, 114.5, 50.1, 44.0.

1-(2-Methylbenzyl)-3-(anthracen-9-ylmethyl)benzimidazolium chloride (4f). Yield 51%, mp 230–232°C. Calculated for $C_{30}H_{25}ClN_2$ (%): C 80.25, H 5.61, N 6.24. Found: C 79.88, H 5.80, N 6.38. FT-IR (cm^{-1}): 1560 (C–N), ^{1}H NMR: 9.25 (s, 1H, NC**H**N), 8.93 (s, 1H, Ar**H**), 8.47–8.26 (m, 5H, Ar**H**), 7.83–6.85 (m, 11H, Ar**H**), 6.83 (s, 2H, –C**H**$_2$Ant), 5.64 (s, 2H, –C**H**$_2$Ph), 2.13 (s, 3H, Ar-C**H**$_3$). ^{13}C NMR: 142.5, 132.8, 132.4, 131.9, 131.6, 131.5, 131.1, 131.0, 130.0, 128.8, 128.7, 127.5, 127.3, 126.7, 126.1, 123.9, 122.5, 115.0, 114.5, 48.9, 44.2, 19.0.

1-(4-Methylbenzyl)-3-(anthracen-9-ylmethyl)benzimidazolium chloride (4g). Yield 40%, mp 236–237°C. Calculated for $C_{30}H_{25}ClN_2$ (%): C 80.25, H 5.61, N 6.24. Found: C 79.85, H 5.80, N 6.39. FT-IR (cm^{-1}): 1558 (C–N). ^{1}H NMR: 9.41 (s, 1H, NC**H**N), 8.94 (s, 1H, Ar**H**), 8.44–8.25 (m, 5H, Ar**H**), 7.95–7.11 (m, 11H, Ar**H**), 6.79 (s, 2H, –C**H**$_2$Ant), 5.56 (s, 2H, –C**H**$_2$Ph), 2.25 (s, 3H, Ar-C**H**$_3$). ^{13}C NMR: 162.8, 142.0, 138.5, 132.4, 131.7, 131.5, 131.5, 131.0, 130.0, 129.8, 128.3, 128.2, 127.4, 127.3, 126.2, 123.9, 122.5, 114.9, 114.6, 49.9, 44.2, 21.1.

1-(2,3,4,5,6-Pentamethylbenzyl)-3-(anthracen-9-ylmethyl)benzimidazolium chloride (4h). Yield 44%, mp 256–258°C. Calculated for $C_{34}H_{33}ClN_2$ (%): C 80.85, H 6.59, N 5.55. Found: C 80.88, H 6.80, N 5.48. FT-IR (cm^{-1}): 1560 (C–N). ^{1}H NMR: 9.01 (s, 1H, NC**H**N), 8.86 (s, 1H, Ar**H**), 8.48–7.48 (m, 12H, Ar**H**), 6.81 (s, 2H, –C**H**$_2$Ant), 5.69, (s, 2H, –C**H**$_2$Ph), 2.26 (s, 3H, Ar-C**H**$_3$-p position), 2.20 (s, 6H, Ar-C**H**$_3$-o position), 2.08 (s, 6H, Ar-C**H**$_3$-m position). ^{13}C NMR: 141.6, 136.7, 134.0, 133.5, 132.1, 132.0, 131.5, 131.1, 130.8, 130.1, 128.2, 127.2, 127.1, 126.2, 126.1, 123.7, 123.2, 114.6, 114.4, 47.2, 44.5, 17.5, 17.2, 16.8.

1-(3,4,5-Trimethoxybenzyl)-3-(anthracen-9-ylmethyl)benzimidazolium chloride (4i). Yield 69%, mp 230–235°C. Calculated for $C_{32}H_{29}ClO_3N_2$ (%): C 73.20, H 5.57, N 5.34. Found: C 73.18, H 5.80, N 5.38. FT-IR (cm^{-1}): 1593 (C–N). ^{1}H NMR: 9.39 (s, 1H, NC**H**N), 8.95 (s, 1H, Ar**H**), 8.44–7.64 (m, 12H, Ar**H**), 6.77 (s, 2H, Ar**H**), 6.69 (s, 2H, –C**H**$_2$Ant), 5.47 (s, 2H, –C**H**$_2$Ph), 3.60 (s, 6H, Ph-OC**H**$_3$-m positon), 3.58 (s, 3H, Ph-OC**H**$_3$-p position). ^{13}C NMR: 153.4,

141.6, 137.8, 132.3, 131.7, 131.54, 131.51, 131.0, 130.1, 130.0, 128.3, 127.5, 127.3, 126.1, 123.8, 122.4, 114.9, 114.6, 106.1, 60.4, 56.3, 50.2, 44.1.

1-(2-Naphtylmethyl)-3-(anthracen-9-ylmethyl)benzimidazolium chloride (4j). Yield 64%, mp 223−224°C. Calculated for $C_{33}H_{25}ClN_2$ (%): C 81.72, H 5.20. N 5.78. Found: C 81.68, H 5.50, N 5.88. FT-IR (cm^{-1}): 1557 (C−N). ^{1}H NMR: 9.52 (s, 1H, NC**H**N), 8.95 (s, 1H, Ar**H**), 8.48−7.34 (m, 19H, Ar**H**), 6.83 (s, 2H, −C**H**$_2$Ant), 5.80 (s, 2H, −C**H**$_2$Naphtalen). ^{13}C NMR: 142.3, 133.0, 132.4, 132.2, 131.7, 131.6, 131.5, 131.0, 130.0, 129.1, 128.3, 128.2, 128.1, 127.5, 127.3, 127.23, 127.2, 126.2, 125.5, 124.0, 122.6, 114.9, 114.5, 50.3, 44.3.

Synthesis of bisbenzimidazolium salts (4k−m). 5 mmol 1,1'-bisbenzimidazole (**2k−m**) was dissolved in 5 mL dried DMF then 10 mmol 9-(chloromethyl)anthracene was added into the solution and the mixture was heated for 5 days at 90°C. After cooling to room temperature, diethyl ether was added to the mixture and precipitate was collected by filtration. Crude product was washed with acetone and dried under reduced pressure.

1,1'-Bis(anthracen-9-ylmethyl)-3,3'buthylenedibenzimidazolium dichloride (4k). Yield 42%, 255−257°C. Calculated for $C_{48}H_{40}Cl_2N_4$ (%): C 77.51, H 5.42, N 7.53. Found: C 77.48, H 5.80, N 7.68. FT-IR (cm^{-1}): 1558 (C−N). ^{1}H NMR: 9.17 (s, 2H, NC**H**N), 8.90 (s, 2H, Ar**H**), 8.38−7.52 (m, 24H, Ar**H**), 6.74 (s, 4H, −C**H**$_2$Ant), 4.31 (t, 4H, NC**H**$_2$CH$_2$), 1.63 (five, 4H, NCH$_2$C**H**$_2$). ^{13}C NMR: 141.8, 132.1, 131.7, 131.6, 131.4, 130.1, 130.0, 128.2, 127.3, 127.2, 126.1, 123.9, 122.5, 114.7, 114.3, 46.4, 44.1, 26.1.

3,3'-Bis(anthracen-9-ylmethyl)-1,1'penthylenedibenzimidazolium dichloride (4l). Yield 33%, mp: 239−242°C. Calculated for $C_{49}H_{42}Cl_2N_4$ (%): C 77.66, H 5.59, N 7.39. Found: C 77.58, H 5.80, N 7.44. FT-IR (cm^{-1}): 1553 (C−N). ^{1}H NMR: 9.22 (s, 2H, NC**H**N), 8.90 (s, 2H, Ar**H**), 8.42−7.58 (m, 24H, Ar**H**), 6.58 (s, 4H, −C**H**$_2$Ant), 4.25 (t, 4H, NC**H**$_2$CH$_2$CH$_2$−, *J* 7), 2.51 (five, 4H, NCH$_2$C**H**$_2$CH$_2$−, *J* 7), 1.66 (five, 2H, NCH$_2$CH$_2$C**H**$_2$−, *J* 7). ^{13}C NMR: 141.8, 132.1, 131.7, 131.6, 131.4, 130.9, 129.9, 128.3, 127.3, 127.2, 126.1, 123.9, 122.6, 114.7, 114.5, 46.7, 44.0, 28.5, 22.7.

1,1'-Bis(anthracen-9-ylmethyl)-3,3'-(1,4-dimethylenbenzene)dibenzimidazolium dichloride (4m). Yield: 40%, 209−212°C. Calculated for $C_{52}H_{40}Cl_2N_4$ (%): C 78.88, H 5.1, N 7.08. Found: C 78.88, H 5.50, N 7.17. FT-IR (cm^{-1}): 1564 (C−N). ^{1}H NMR: 9.45 (s, 2H, NC**H**N), 8.88 (s, 2H, Ar**H**), 8.46−8.19 (m, 11H, Ar**H**), 7.84−7.18 (m, 17H, Ar**H**), 6.83 (s, 4*H*, −CH$_2$Ant), 5.76 (s, 4H, −C**H**$_2$Ph). ^{13}C NMR: 162.8, 142.5, 132.6, 132.4, 131.7, 131.6, 131.4, 131.0, 129.9, 129.1, 128.1, 127.6, 126.0, 123.9, 129.1, 128.1, 127.6, 126.0, 123.9, 122.4, 115.0, 114.7, 47.8, 44.4.

Purification of tyrosinase. All purification steps were carried out at 25°C. The extraction procedure was adopted from Wesche-Ebeling & Montgomery [35]. The bananas were washed with distilled water three times to prepare the crude extract. 50 g of bananas were cut quickly into thin slices and homogenized in a Waring blender for 2 min using 100 mL of 0.1 M phosphate buffer, pH 7.3 containing 5% polyethylene glycol and 10 mM ascorbic acid. The homogenate was filtered through muslin, the filtrate was centrifuged at 15 000 *g* for 30 min, and the supernatant was collected. A crude protein precipitate was made by adding $(NH_4)_2SO_4$ to 80% saturation. The resulting precipitate was suspended in a minimum volume of 5 mM phosphate buffer and then dialyzed against the same buffer overnight. The enzyme solution was then applied onto the Sepharose 4B-tyrosine-p-amino benzoic acid affinity column [36], pre-equilibrated with 5 mM phosphate buffer, pH 5.0. The affinity gel was extensively washed with the same buffer and then the banana PPO (BPPO) was eluted with 1 M NaCl, 5 mM phosphate, pH 7.0.

Tyrosinase enzyme activity. Enzyme activity was determined according to the method Espin et al. [37] using catechol as a substrate by measuring the increase in absorbance at 420 nm on a Biotek automated recording spectrophotometer. All measurements were performed in duplicate and corrected for the non-enzymatic hydrolysis. Enzyme activity was calculated from the linear portion of the curve. One unit of PPO activity was defined as the amount of enzyme that causes an increase in absorbance of 0.001 unit's min^{-1} for 1 mL of enzyme at 25°C.

Inhibition of tyrosinase enzyme activity. An aliquot of each inhibitor at various final concentrations was added to the standard reaction solution immediately before the addition of enzyme extract. The concentration of inhibitor (benzimidazole derivatives) producing 50% inhibition (IC$_{50}$) was determined from a plot of residual activity against inhibitor concentration using 10 mM catechol as substrate. The activity without inhibitor was taken as a control.

REFERENCES

1. Ozdemir, I., Demir, S., Cetinkaya, B., Gourlaouen, C., Maseras, F., and Bruneau, C., *J. Am. Chem. Soc.*, 2008, vol. 130, pp. 1156−1157.

2. Bansal, Y. and Silakari, O., *Bioorg. Med. Chem.*, 2007, vol. 20, pp. 6208−6236.

3. Wooley, D.W., *J. Bio. Chem.*, 1944, vol. 152, pp. 225−232.

4. Dorsch, D., Cezanne, B., Mederski, W., Tsaklakidis, C., Gleitz, J., and Barnes, C., US Patent no. 7566789B2, 2009.

5. Sabat, M., VanRens, J., Laufersweiler, M.J., Brugel, T.A., Maier, J., Golebiowski, A., De B., Easwaran, V., Hsieh, L.C., Walte, R.L., Mekel, M.J., Evkoki-

mov, A., and Jenusz, M.J., *Bioorg. Med. Chem. Lett.,* 2006, vol. 16, pp. 5973−5977.

6. Desai, R.G. and Desai, K.R., *Bioorg. Med. Chem.,* 2006, vol. 14, pp. 8271−8279.

7. Tamm, I., Nemes, M.M., and Osterhout, S.J., *Exp. Med.*, 1960, vol. 111, pp. 339−349.

8. Cole, E.R., Crank, G., and Salam-Sheikh, A., *J. Agric.; Food Chem.*, 1974, vol. 22, p. 918.

9. Ni, Z.J., Borsenti, P., Brommeier, N., Diebes, A., Poon, D.J., Ng, S., Pecchi, S., Pfister, K., Renhowe, P.A., Ramurthy, S., Wagman, A.S., Bussiere, D.E., Le, V., Zhou, Y., Jansen, J.M., Ma, S., and Gesner, T., *Bioorg. Med. Chem. Lett.*, 2006, vol. 12, pp. 3121−3124.

10. Oballa, R., Bhat, S., Falgueyret, J.D., Guiral, S., Huong, Z., Skorey, K., Tawa, P., and Zhang, L., *Bioorg. Med. Chem. Lett.*, 2010, vol. 20, no. 22, pp. 6366−6369.

11. Havel, N.H., Nar, H., Priepke, H., Ries, U., Stasse, J.M., and Wienen, W., *J. Med. Chem.,* 2002, vol. 45, pp. 1757−1766.

12. Song, K.K., Huang, H., Han, P., Zhang, C.L., Shi, Y., and Chen, Q.X., *Biochem. Biophys. Res. Commun.*, 2006, vol. 342, pp. 1147−1151.

13. Chen, Q.X., Liu, X.D., and Huang, H., *Biochemistry* (Moscow), 2003, vol. 68, pp. 644−649.

14. Kubo, I., Nihei, K.I., and Shimizu, *Bioorg. Med. Chem.,* 2004, vol. 12, pp. 5343−5347.

15. Matsuura, R., Ukeda, H., and Sawamura, M., *J. Agric.; Food Chem.*, 2006, vol. 54, pp. 2309−2313.

16. Yi, W., Cao, R., Peng, W., Wen, H., Yan, Q., Zhou, B., Ma, L., and Song, H., *Eur. J. Med. Chem.*, 2010, vol. 45, pp. 639−646.

17. Gupta, A.K., Gover, M.D., Nouri, K.N., and Taylor, S., *J. Am. Acad. Dermatol.*, 2006, vol. 55, pp. 1048−1065.

18. Thanigaimalai, P., Hoang, T.A.L., Lee, K., Bang, S., Sharma, V.K., Yun, C., Roh, E., Hwang, B., Kim, Y., and Jung, S., *Bioorg. Med. Chem. Lett.*, 2010, vol. 20, pp. 2991−2993.

19. Zhu, Y.J., Zhou, H.T., Hu, Y.H., Tang, J., Su, M., Guo, Y., Chen, Q., and Liu, B., *Food Chem.*, 2011, vol. 124, pp. 298−302.

20. Ashida, M. and Brey, P., *Proc. Natl. Acad. Sci. U.S.A.*, 1995, vol. 92, pp. 10698−10702.

21. Battaini, G., Monzani, E., Casella, L., Santagostini, L., and Pagliarin, R., *J. Biol. Inorg. Chem.*, 2000, vol. 5, pp. 262−268.

22. Hindi, K.M., Panzner, M.J., Tessier, J.A., Cannon, C.L., and Youngs, W.J., *Chem. Rev.,* 2009, vol. 109, pp. 3859−3884.

23. Kubo, I., Kinst-Hori, I., Kubo, Y., Yamagiwa, Y., Kamikawa, T., and Haraguchi, H.J., *Agric. Food Chem.,* 2000, vol. 48, pp. 1393−1399.

24. Kubo, I., Kinst-Hori, I., Nihei, K., Soria, F., Takasaki, M., Calderón, J.S., and Céspedes, C.L.T.Z., *Naturforsch.*, 2003, vol. 58, pp. 719−725.

25. Kubo, I., Chen, Q.X., and Nihei, K., *Food Chem.*, 2003, vol. 81, pp. 241−247.

26. Roudsari, M.H., Signoset, A., and Crovzet, J., *Food Chem.*, 1981, vol. 7, pp. 227−235.

27. Augustin, M.A., Ghazali, H.M., and Hashim, H., *J. Agric.; Food Chem.*, 1985, vol. 36, pp. 1259−1265.

28. Sakiroglu, H., Kufrevioglu, I.O., Kocacaliskan, I., Oktay, M., and Onganer, Y., *J. Agric.; Food Chem.*, 1996, vol. 44, pp. 2982−2986.

29. Jiang, Y., Fu, J., Zauberman, G., and Fuchs, Y., *J. Sci. Food Agric.,* 1999, vol. 79, pp. 950−954.

30. Perez-Gilabert, M. and Garcia-Carmona, F., *J. Agric.; Food Chem,* 2000, vol. 48, pp. 695−700.

31. Richard-Forget, F.C., Groupy, P.M., and Nicholas, J.J., *J. Agric.; Food Chem.*, 1992, vol. 40, pp. 2108−2113.

32. Sayaverde-Soto, L.A. and Montgomery, M.W., *J. Food Sci.*, 1986, vol. 51, pp. 1531−1535.

33. *ACS Symposium Series 600*, Chang, Y.L. and Whitaker, J.R., Eds., Washington, DC: American Chemical Society, 1995, pp. 2−7.

34. Ozdemir, I., Sahin, N., Gok, Y., Demir, S., and Cetinkaya, B., *J. Mol. Catal.*, 2005, vol. 234, pp. 181−185.

35. Wesche-Ebeling, P. and Montgomery, M.W., *J. Food Sci.*, 1990, vol. 55, pp. 1320−1324.

36. Arslan, O., Erzengin, M., Sinan, S., and Ozensoy, O., *Food Chem.*, 2004, vol. 88, pp. 479−484.

37. Espin, J.C., Morales, M., Varon, R., Tudela, J., and Garcia-Canovas, F., *J. Agric.; Food Chem.,* 1995, vol. 43, pp. 2807−2812.