

Novel Benzo[d]imidazole-2(3*H*)-thiones as Potent Inhibitors of the α -Melanocyte Stimulating Hormone Induced Melanogenesis in Melanoma B16 Cells

Jee-Hyun LEE,^a Pillaiyar THANIGAIMALAI,^a Ki-Cheul LEE,^a Seong-Cheol BANG,^a Min-Seok KIM,^a Vinay Kumar SHARMA,^a Cheong-Yong YUN,^b Eunmiri ROH,^b Youngsoo KIM,^b and Sang-Hun JUNG*,^a

^a College of Pharmacy and Institute of Drug Research and Development, Chungnam National University; Daejeon 305–764, Korea; and ^b College of Pharmacy, Chungbuk National University; Cheongju 361–763, Korea.

Received February 16, 2010; accepted April 1, 2010; published online April 6, 2010

In order to determine the optimum size of heterocycle of lead compound **1** (6-methyl-3-phenethyl-3,4-dihydro-1*H*-quinoline-2-thione; $IC_{50}=0.8\ \mu M$) for inhibition of melanogenesis, we have synthesized and evaluated some benzimidazole-2(3*H*)-thiones **5a–e**. The preliminary bioassay has shown that the benzimidazole-2(3*H*)-thione motif of **5** is essential structural unit for their inhibitory activity. Among all thiones **5a–e**, the compound **5d** strongly inhibited the formation of melanin with IC_{50} value of $1.3\ \mu M$.

Key words benzimidazole-2(3*H*)-thione; melanogenesis; inhibitor

Melanin is a heterogeneous biopolymer which is principally responsible for human phenotypic appearance and has major role in protecting human skin from harmful effect of UV-radiation from the sun.¹⁾ Melanin is formed by a series of enzymatic catalyzed reactions in melanocytes.^{2,3)} The biosynthesis of melanin is initiated with oxidation of tyrosine to dopaquinone catalyzed by tyrosinase.⁴⁾ This first step is the rate limiting step in melanogenesis because the remaining reaction sequence can proceed spontaneously at a physiological pH.⁵⁾ However, over expression of tyrosinase lead to induction of hyperpigmentary disorders such as melasma, freckles, age spot, and post-inflammatory melanoderma.^{6,7)} It is also reported to be linked to Parkinson diseases and some other neurodegenerative diseases.^{8,9)} Therefore, the regulation of melanin synthesis by inhibiting the tyrosinase enzyme is the current research topic in context of preventing hyperpigmentation. In this regard, diverse tyrosinase inhibitors have been actively discovered such as kojic acid,^{10,11)} arbutin,¹²⁾ ascorbic acid derivatives,¹³⁾ hydroxylstilbene derivatives like resveratrol^{14–16)} and methyl ester of genistic acid.^{17,18)} However, according to a recent study kojic acid has serious adverse effects such as skin cancer and dermatitis and has been banned as a cosmetic ingredient in many countries.¹⁷⁾ Thus other type of molecules, which inhibit cyclic adenosine monophosphate (cAMP) dependent melanogenesis proteins other than tyrosinase, are gaining attention. Using bioassay system for measuring the amount of melanin formed from melanoma B16 cells upon stimulation of α -melanocyte stimulating hormone (α -MSH), we have screened many different compounds. As a result, 6-methyl-3-phenethyl-3,4-dihydro-1*H*-quinoline-2-thione (Fig. 1, $IC_{50}=0.8\ \mu M$) was discovered as highly potent compound against melanogenesis in melanoma B16 cell line.¹⁸⁾ The mechanism of action of compound **1** is very much unique and important due to its suppression of the melanogenesis without affecting the tyrosinase activity. The preliminary study on the structure–activity relationship (SAR) of **1** has revealed quinazolidine-2-thione in **1** as an essential motif.¹⁸⁾ In order to explore the necessity of 6-membered cyclic thiourea scaffold in **1**, benzo[d]imidazol-2(3*H*)-ones **4** (Fig. 1) containing 5-membered cyclic thiourea were designed,

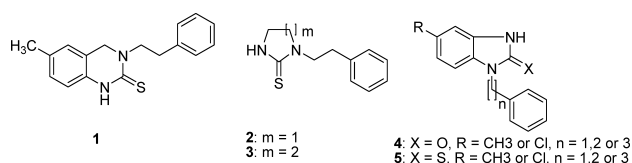
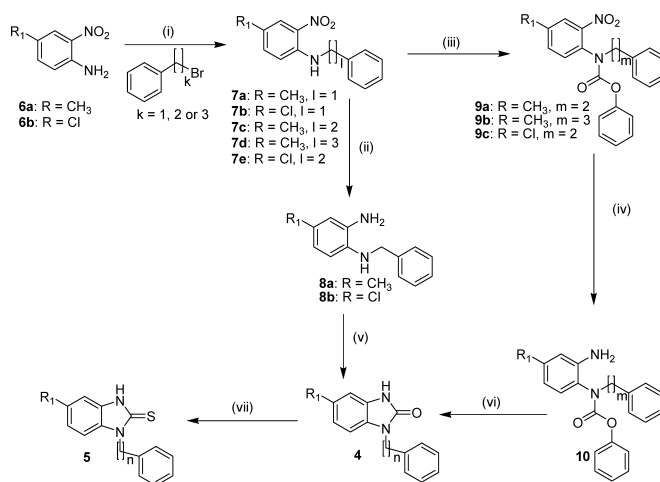


Fig. 1. cAMP Dependent Melanogenesis Inhibitor **1**, **2**, **3** and 1,3-Dihydrobenzo[d]imidazole-2-ones **4** and Their Thiones **5**



Reagents and conditions; (i) pyridine or NaOH; (ii) Fe, acetic acid; (iii) phenylchloroformate, xylene; (iv) H_2 –Pd, MC and ethanol; (v) phenylchloroformate, xylene; (vi) xylene, reflux; (vii) Lawesson's reagent, toluene. Note: $n=1, 2$ or 3. $R=CH_3$ or Cl. Substituents are located in Table 1.

Chart 1. Synthesis of Compounds **4** and **5**

synthesized and evaluated for their inhibitory activity against melanin production in melanoma B16 cells under the stimulation of α -MSH.

Chemistry

Benzo[d]imidazol-2(3*H*)-ones **4** and benzo[d]imidazole-2(3*H*)-thiones **5** were synthesized with the synthetic procedure as shown in Chart 1. In the first step, commercially available amine **6** was treated with appropriate alkyl bromide using pyridine or sodium hydroxide to give an intermediate **7**.^{18,19)} In case of benzylated amines **7**, the reduction reaction

* To whom correspondence should be addressed. e-mail: jungshh@cnu.ac.kr.

was carried out with iron powder in acetic acid at 50–70 °C temperature for 2 h to yield **8**.²⁰ To get the another important intermediate **10**, the phenyl chloroformate was reacted with **7** under refluxing condition for 7 h to achieve **9**,²¹ which was subsequently subjected to reduction using catalytic hydrogenation at ambient temperature to produce **10**.²² Further the diamine **8** was treated with phenyl chloroformate using xylene at refluxing temperature for 33 h to give title compound **4**.²¹ On the other hand, **10** in xylene underwent cyclization at reflux temperature to produce **4**.²¹ Finally, benzo[d]imidazole-2(3*H*)-ones **4** in toluene treated with Lawesson's reagent at reflux temperature to obtain the corresponding thiones **5**.²³

Results and Discussion

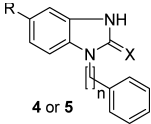
For all the synthesized compounds **4** and **5** the ability to inhibit formation of melanin from melanoma B-16 cells was determined under stimulus of α -MSH (100 nM) for 3 d incubation. Melanoma B16 cell (CRL6323) were obtained from ATCC (Manassas, U.S.A.). Amounts of melanin released into the culture media were determined by measuring absorbance values at 405 nm with synthetic melanin as the standard.²⁴ Data for % inhibition at 10 μ M and IC_{50} values are mean values from 3 to 5 separate experiments as shown in Table 1.

Among the synthesized compounds the benzimidazole-2(3*H*)-thiones **5** shows promising inhibitory activity against melanin production in melanoma B16 cells as compared to kojic acid (IC_{50} =70 μ M) and arbutin (IC_{50} =180 μ M). Interestingly, these compounds **5** have almost comparable activity to **1** (>100% inhibition at 10 μ M, IC_{50} =0.8 μ M). The potent compound **5c** (>100% inhibition at 10 μ M, IC_{50} =1.5 μ M) has almost similar structure to that of **1** with an exception of one methylene unit on heterocyclic motif. This result intimates that the activities of both quinazolidine-2-thione **1** and benzimidazolidine-2-thione **5** are not dependent on variation of ring size. In our previous studies²⁴ we observed that imidazolidine-2-thione analog **2** (42% inhibition at 10 μ M, IC_{50} =>10 μ M) exhibited very low activity unlike six membered cyclic thiourea **3** (93% inhibition at 10 μ M, IC_{50} =2.9 μ M) as shown in Fig. 1. Thus, the benzene ring fused with imidazolidine-2-thione of **5** is an essential motif for their inhibitory activity. Moreover, different position of phenylalkyl substituent on cyclic thiourea unit in **1** and **5** does not affect the activity.

Methyl and chloro substituents at position 5 of benzimidazole-2-thione of **5** do not change activity as shown in the activity of **5a** (>100% inhibition at 10 μ M, IC_{50} =1.7 μ M, $C\log P$ =3.521) and **5b** (>100% inhibition at 10 μ M, IC_{50} =1.9 μ M, $C\log P$ =3.735). Likewise, compound **5c** (>100% inhibition at 10 μ M, IC_{50} =1.5 μ M, $C\log P$ =3.850), **5d** (>100% inhibition at 10 μ M, IC_{50} =1.3 μ M, $C\log P$ =4.229) and **5e** (>100% inhibition at 10 μ M, IC_{50} =2.0 μ M, $C\log P$ =4.064) show similar level of activity. This indicates that the size of side chain and the lipophilicity of compounds **5** do not have much influence on their inhibitory activity.

Unlike benzimidazole-2(3*H*)-thiones **5**, their counterpart benzo[d]imidazole-2(3*H*)-ones **4a–e** gave a very weak inhibitory activity (their IC_{50} values were more than 10 μ M and inhibition was less than 30% at 10 μ M). Thus thiourea unit of **5** is very critical for the activity, which was also observed in our previous studies with analogs of **1**.²⁵

Table 1. Inhibitory Activity of **4** and **5** on Melanogenesis of Melanoma B 16 Cells



Compound	R1	n	X	Inhibition % at 10 μ M	IC_{50} values	$C\log P^{c)}$ μ M
4a	CH ₃	1	O	<10	>100	3.881
4b	Cl	1	O	<10	>10	4.305
4c	CH ₃	2	O	<10	>10	4.210
4d	CH ₃	3	O	<10	>10	4.589
4e	Cl	2	O	<10	>10	4.634
5a	CH ₃	1	S	>100	1.7	3.521
5b	Cl	1	S	>100	1.9	3.735
5c	CH ₃	2	S	>100	1.5	3.850
5d	CH ₃	3	S	>100	1.3	4.229
5e	Cl	2	S	>100	2.0	4.064
1 ^{a)}				>100	0.8	4.289
2 ^{b)}				40	>10	2.381
3 ^{b)}				93	2.9	2.940
Arbutin				—	180	
Kojic acid				—	70	

a) The activity associated with compound **1** was reported in ref. 18. b) The activity associated with compounds **2** and **3** was reported in ref. 24. c) $C\log P$ values were calculated by Chemdraw ver. 9.0.

Conclusion

Taken our studies together, the benzimidazole-2(3*H*)-thione motif of **5** is an essential structural unit for their inhibitory activity. Among all thiones (**5a–e**), the compound **5d** (IC_{50} =1.3 μ M) most strongly inhibits the melanin formation. Therefore benzo[d]imidazole-2(3*H*)-thiones **5** could be quite promising compound for the treatment of hyperpigmentation.

Experimental

General Procedures Melting points (mp) were determined on Electro thermal 1A 9100 MK2 apparatus and are uncorrected. All commercial chemicals were used as obtained and all solvents were purified by the standard procedures prior to use. Thin layer chromatography was performed on E Merck silica gel GF-254 per-coated plates and the identification was done with UV light and colorization with spray 10% phosphomolybdic acid followed by heating. Flash column chromatography was performed with E Merck silica gel (230–400 mesh). IR spectra were recorded with Jasco IR-Report-100 IR spectrometer in cm^{-1} and corrected against peak at 1601 cm^{-1} of polystyrene. NMR spectra were measured against the peak of tetramethylsilane by Varian Unity Inova 400 NMR (400 MHz) spectrometers. High resolution-mass spectrum (HR-MS) was recorded on API2000 mass spectrometer (PE Sciex, Toronto, Canada).

Synthesis of *N*-Benzyl-4-methyl-2-nitrobenzenamine (7a**)** 4-Methyl-2-nitroaniline (**6a**, 3 g, 19.0 mmol) and pyridine (4.78 ml, 59.0 mmol) were dissolved in methylenechloride (150 ml). After stirred for 10 min, benzyl bromide (3.52 ml, 29.0 mmol) was added. The resulting reaction mixture was refluxed for 16 h. After cooled, the reaction mixture was washed with water three times. The organic layer was dehydrated with anhydrous sodium sulfate and solvent was removed under vacuum. The desired compound **7a** was isolated by column chromatography. Brown solid. Yield 90.3%. mp 87.4–88.5 °C. R_f =0.53 (hexane : ethyl acetate=5 : 1). ¹H-NMR (CDCl₃) δ : 8.33 (br, 1H), 7.99 (s, 1H), 7.34–7.17 (m, 6H), 6.72 (d, 1H, J =8.8 Hz), 4.54 (d, 2H, J =5.6 Hz), 2.25 (s, 3H). IR (KBr) cm^{-1} : 3400, 1635, 1530, 1510. HR-MS Calcd for C₁₄H₁₄N₂O₂: 242.1055. Found: 242.1049.

Synthesis of *N*-Benzyl-4-chloro-2-nitrobenzenamine (7b**)** Using the same reaction condition for the preparation of **7a**, 4-chloro-2-nitroaniline (**6b**, 3 g, 17.0 mmol) was treated with benzylbromide (2.48 ml, 20.0 mmol) in the presence of pyridine (1.69 ml, 20.0 mmol) for the preparation of **7b**. Brown solid. Yield 67.1%. mp 78.0–78.2 °C. R_f =0.46 (hexane : ethyl acetate=5 : 1). ¹H-NMR (CDCl₃) δ : 8.42 (br, 1H), 8.20 (m, 1H), 7.47–7.26

(m, 6H), 6.77 (m, 1H), 4.55 (d, 2H, $J=5.6$ Hz). IR (KBr) cm^{-1} : 3400, 1622, 1563. HR-MS Calcd for $\text{C}_{13}\text{H}_{11}\text{ClN}_2\text{O}_2$: 262.0509. Found: 262.0503.

Synthesis of 4-Methyl-2-nitro-*N*-phenethylbenzenamine (7c) To the solution of 4-methyl-2-nitroaniline (**6a**, 5 g, 32 mmol) in toluene, sodium hydroxide (64 mmol) was added and the resulting mixture was stirred for 5 min at room temperature. 2-Bromoethylbenzene (6.73 ml, 48.0 mmol) was added drop wise for 20 min. The resulting mixture was heated at 60–70 °C for 3–4 h. After cooled to ambient condition, solvent was removed under vacuum and the residue was dissolved in dichloromethane and washed with water. The organic layer was dehydrated with anhydrous sodium sulfate and evaporated under vacuum. The desired compound **7c** was isolated by column chromatography. Brown solid. Yield 54.0%. mp 79.4–79.8 °C. $R_f=0.45$ (hexane: ethyl acetate=5:1). $^1\text{H-NMR}$ (CDCl_3) δ : 7.84 (br, 1H), 7.31–6.92 (m, 8H), 3.91 (t, 2H, $J=7.4$ Hz), 3.05 (t, 2H, $J=7.4$ Hz), 2.44 (s, 3H). IR (KBr) cm^{-1} : 2950, 2830, 1635, 1545. HR-MS Calcd for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_2$: 256.1212. Found: 256.1203.

Synthesis of 4-Methyl-2-nitro-*N*-(3-phenylpropyl)benzenamine (7d) Using the same reaction condition for the preparation of **7c**, **7d** was obtained from 4-methyl-2-nitroaniline (**6a**, 3 g, 19.0 mmol) and 1-bromo-3-phenylpropane (3.59 ml, 48.0 mmol). Brown solid. Yield 39.8%. mp 94.9–95.2 °C. $R_f=0.58$ (hexane: ethyl acetate=5:1). $^1\text{H-NMR}$ (CDCl_3) δ : 7.97 (br, 1H), 7.47–7.23 (m, 6H), 6.70 (d, 1H, $J=8.8$ Hz), 3.29 (m, 2H), 2.77 (t, 2H, $J=7.4$ Hz), 2.25 (s, 3H), 2.10 (m, 2H). IR (KBr) cm^{-1} : 3400, 1635, 1541. HR-MS Calcd for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_2$: 270.1368. Found: 270.1361.

Synthesis of 4-Chloro-2-nitro-*N*-phenethylbenzenamine (7e) Using the same reaction condition for the preparation of **7c**, **7e** was obtained from 4-chloro-2-nitroaniline (**6b**, 5 g, 29.0 mmol) and 1-bromo-2-phenylethane (5.94 ml, 43.0 mmol). Orange solid. Yield 38.2%. mp 83.2–84.2 °C. $R_f=0.51$ (hexane: ethyl acetate=5:1). $^1\text{H-NMR}$ (CDCl_3) δ : 8.14 (br, 1H), 7.42–7.20 (m, 6H), 6.79 (d, 1H, $J=9.2$ Hz), 3.51 (m, 2H), 3.00 (t, 2H, $J=7.1$ Hz). IR (KBr) cm^{-1} : 3350, 3100, 3020, 2950, 2830, 1620. HR-MS Calcd for $\text{C}_{14}\text{H}_{13}\text{ClN}_2\text{O}_2$: 276.0666. Found: 276.0659.

Synthesis of *N*¹-Benzyl-4-methylbenzene-1,2-diamine (8a) Compound **7a** (2 g, 8.0 mmol) and Fe powder (4.6 g, 8.0 mmol) were added to the glacial acetic acid (30 ml) the resulting mixture was allowed to heated at 50–70 °C for 2 h. The resulting mixture was filtered and the filter cake was washed with hot ethanol (50 ml) pre-heated at 60–70 °C. The filtrate was evaporated under vacuum and the residue was subjected to column chromatography for separation of **8a**. Violet solid. Yield 51.4%. mp 111.9–112.4 °C. $R_f=0.30$ (hexane: ethyl acetate=5:1). $^1\text{H-NMR}$ (CDCl_3) δ : 7.41–7.13 (m, 5H), 6.38 (m, 2H), 5.87 (d, 1H, $J=7.9$ Hz), 4.21 (s, 2H), 3.67 (br, 1H), 2.17 (s, 3H). IR (KBr) cm^{-1} : 3450, 3360, 3050, 2940, 1710, 1620. HR-MS-electrospray ionization (ESI) Calcd for $\text{C}_{14}\text{H}_{16}\text{N}_2$: 212.1313. Found: 212.1307.

Synthesis of *N*¹-Benzyl-4-chlorobenzene-1,2-diamine (8b) Using the same reaction condition for the preparation of **8a**, compound **8b** was obtained from **7b** (2 g, 7.0 mmol). Dark brown solid. Yield 65.1%. mp 115.3–117.5 °C. $R_f=0.24$ (hexane: ethyl acetate=5:1). $^1\text{H-NMR}$ (CDCl_3) δ : 7.32 (m, 5H), 6.42 (m, 2H), 5.81 (m, 1H), 4.21 (s, 2H), 3.77 (br, 1H). IR (KBr) cm^{-1} : 3350, 3070, 3030, 2970, 2920, 2880, 2850, 1595. HR-MS-ESI Calcd for $\text{C}_{13}\text{H}_{13}\text{ClN}_2$: 232.0767. Found: 232.0752.

Synthesis of Phenyl *N*-(4-Methyl-2-nitrophenyl)-*N*-phenylethylcarbamate (9a) To the mixture of compound **7c** (2 g, 7.0 mmol) in xylene (20 ml) was added phenylchloroformate (2.94 ml, 21.0 mmol). The resulting reaction mixture was refluxed for 7 h. After cooling, xylene was removed under vacuum and the residue was dissolved in dichloromethane and washed with water. The organic layer was dehydrated over anhydrous sodium sulfate and evaporated under vacuum. The residue was purified by column chromatography to give desired compound **9a**. Pale brown solid. Yield 93.8%. mp 110.1–110.3 °C. $R_f=0.18$ (hexane: ethyl acetate=5:1). $^1\text{H-NMR}$ (CDCl_3) δ : 7.62–6.55 (m, 13H), 3.80 (t, 2H, $J=7.6$ Hz), 2.99 (t, 2H, $J=7.6$ Hz), 2.27 (s, 3H). IR (KBr) cm^{-1} : 3100, 2850, 1730. HR-MS Calcd for $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_4$: 376.1423. Found: 376.1418.

Synthesis of Phenyl *N*-(4-Methyl-2-nitrophenyl)-*N*-(3-phenylpropyl)carbamate (9b) Using the same reaction condition for the preparation of **9a**, compound **9b** was obtained from **7d** (1 g, 3.7 mmol). Yellow solid. Yield 93.7%. mp 104.6–105.3 °C. $R_f=0.22$ (hexane: ethyl acetate=5:1). $^1\text{H-NMR}$ (CDCl_3) δ : 7.83–7.04 (m, 13H), 3.78 (m, 2H), 2.68 (t, 2H, $J=8.5$ Hz), 2.44 (s, 3H), 2.07 (m, 2H). IR (KBr) cm^{-1} : 3050, 2920, 2850, 1730. HR-MS Calcd for $\text{C}_{23}\text{H}_{22}\text{N}_2\text{O}_4$: 390.1580. Found: 390.1572.

Synthesis of Phenyl *N*-(4-Chloro-2-nitrophenyl)-*N*-phenylethylcarbamate (9c) Using the same reaction condition for the preparation of **9a**, compound **9c** was obtained from **7e** (1.5 g, 5.0 mmol). Pale brown solid. Yield 90.2%. mp 114.3–115.8 °C. $R_f=0.20$ (hexane: ethyl acetate=5:1). $^1\text{H-NMR}$ (CDCl_3) δ : 7.62–6.55 (m, 13H), 3.80 (t, 2H, $J=7.6$ Hz), 2.99 (t,

2H, $J=7.6$ Hz). IR (KBr) cm^{-1} : 3100, 2850, 1730. HR-MS Calcd for $\text{C}_{21}\text{H}_{17}\text{ClN}_2\text{O}_4$: 396.0877. Found: 396.0879.

Synthesis of Phenyl *N*-(4-Methyl-2-aminophenyl)-*N*-phenylethylcarbamate (10a) Compound **9a** (1.5 g, 3.0 mmol) was dissolved in the co-solvent (30 ml) of ethanol and dichloromethane (1:2). After addition of Pd-C (0.150 g), the mixture was stirred under hydrogen gas (45 psi) for 12 h at room temperature. After filtration through celite pad, the filtrate was evaporated under vacuum to give pure **10a**. Brown solid. Yield 94.0%. mp 127.3–127.8 °C. $R_f=0.15$ (hexane: ethyl acetate=5:1). $^1\text{H-NMR}$ (acetone- d_6) δ : 7.42–6.68 (m, 13H), 4.60 (br, NH_2), 3.65 (m, 2H), 2.92 (t, 2H, $J=8.1$ Hz), 2.21 (s, 3H). IR (KBr) cm^{-1} : 3500, 3330, 3100, 1710, 1620. HR-MS Calcd for $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_2$: 346.1681. Found: 346.1699.

Synthesis of Phenyl *N*-(4-Methyl-2-aminophenyl)-*N*-phenylpropylcarbamate (10b) Using the same reaction condition for the preparation of **10a**, compound **10b** was obtained from **9b** (1 g, 2.0 mmol). Brown oil. Yield 93.5%. $R_f=0.28$ (hexane: ethyl acetate=2:1). $^1\text{H-NMR}$ (acetone- d_6) δ : 8.23–6.71 (m, 13H), 4.82 (br, NH_2), 4.02–3.21 (m, 2H), 2.66 (t, 2H, $J=7.8$ Hz), 2.27 (s, 3H), 2.06 (m, 2H). IR (KBr) cm^{-1} : 3470, 3380, 3050, 2940, 2850, 1715, 1620. HR-MS-ESI Calcd for $\text{C}_{23}\text{H}_{24}\text{N}_2\text{O}_2$: 360.1838. Found: 360.1827.

Synthesis of Phenyl *N*-(4-Chloro-2-aminophenyl)-*N*-phenylethylcarbamate (10c) Using the same reaction condition for the preparation of **10a**, compound **10c** was obtained from **9c** (1 g, 2.0 mmol). Brown solid. Yield 78.6%. mp 119.0–120.8 °C. $R_f=0.53$ (hexane: ethyl acetate=2:1). $^1\text{H-NMR}$ (acetone- d_6) δ : 7.35–6.55 (m, 13H), 5.01 (br, NH_2), 4.11–3.42 (m, 2H), 2.96 (t, 2H, $J=7.9$ Hz). IR (KBr) cm^{-1} : 3450, 3350, 3240, 2940, 1720, 1670, 1630. HR-MS Calcd for $\text{C}_{21}\text{H}_{19}\text{ClN}_2\text{O}_2$: 366.1135. Found: 366.1126.

Synthesis of 1-Benzyl-5-methyl-1*H*-benzo[d]imidazol-2(3*H*)-one (4a) To the solution of diamine **8a** (0.500 g, 2.0 mmol) in xylene (10 ml), phenylchloroformate (0.325 ml, 2.2 mmol) was added in drop wise. The resulting mixture was refluxed for 33 h. After cooling, xylene was removed under vacuum. The residue was dissolved in dichloromethane (100 ml) and extracted with water (70 ml) twice. The organic layer was dehydrated over anhydrous sodiumsulfate and evaporated under vacuum. The residue was subjected to column chromatography for isolation of **4a**. White solid. Yield 52.5%. mp 191.2–192.4 °C. $R_f=0.076$ (hexane: ethyl acetate=2:1). $^1\text{H-NMR}$ (CDCl_3) δ : 9.33 (br, 1H), 7.46–6.66 (m, 8H), 5.07 (s, 2H), 2.34 (s, 3H). IR (KBr) cm^{-1} : 3250, 2900, 1705, 1675. HR-MS Calcd for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}$: 238.1106. Found: 238.1101.

Synthesis of 1-Benzyl-5-chloro-1*H*-benzo[d]imidazol-2(3*H*)-one (4b) Using the same reaction condition for the preparation of **4a**, compound **4b** was obtained from compound **8b** (0.300 g, 0.12 mmol) and phenyl chloroformate (0.17 ml, 1.26 mmol). White solid. Yield 54.3%; mp 185.2–187.2 °C. $R_f=0.15$ (hexane: ethyl acetate=2:1). $^1\text{H-NMR}$ (CDCl_3) δ : 10.2 (br, 1H), 7.31–6.70 (m, 8H), 5.08 (s, 2H). IR (KBr) cm^{-1} : 3250, 2680, 1740–1650, 1620, 1600. HR-MS Calcd for $\text{C}_{14}\text{H}_{11}\text{ClN}_2\text{O}$: 258.0560. Found: 258.0545.

Synthesis of 5-Methyl-1-phenethyl-1*H*-benzo[d]imidazol-2(3*H*)-one (4c) To the solution of compound **10a** (0.500 g, 1.4 mmol) in toluene (20 ml), sodium hydroxide (0.115 g, 2.8 mmol) and water (0.5 ml) were added. The resulting mixture was refluxed for 3 h. After cooled, toluene was removed under vacuum. The residue was dissolved in dichloromethane (100 ml) and extracted with water (70 ml) twice. The organic layer was dehydrated with anhydrous sodiumsulfate and evaporated under vacuum. The residue was subjected to column chromatography for isolation of **10c**. Pale pink solid. Yield 74.4%. mp 139.9–140.7 °C. $R_f=0.15$ (hexane: ethyl acetate=2:1). $^1\text{H-NMR}$ (CDCl_3) δ : 10.6 (br, 1H), 7.24–6.08 (m, 8H), 4.09 (t, 2H, $J=7.6$ Hz), 3.04 (t, 2H, $J=7.6$ Hz), 2.35 (s, 3H). IR (KBr) cm^{-1} : 3250, 2600, 1740, 1600. HR-MS Calcd for $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}$: 252.1263. Found: 252.1247.

Synthesis of 5-Methyl-1-(3-phenylpropyl)-1*H*-benzo[d]imidazol-2(3*H*)-one (4d) Using the same reaction condition for the preparation of **4c**, compound **4d** was obtained from compound **10b** (0.250 g, 6.94 mmol). Pale brown solid. Yield 33.5%. mp 132.1–132.5 °C. $R_f=0.13$ (hexane: ethyl acetate=2:1). $^1\text{H-NMR}$ (CDCl_3) δ : 9.66 (br, NH), 7.26 (m, 6H), 6.93–6.86 (m, 2H), 3.91 (t, 2H, $J=7.2$ Hz), 2.72 (t, 2H, $J=7.8$ Hz), 2.37 (s, 3H), 2.08 (m, 2H). IR (KBr) cm^{-1} : 3260, 2800, 1705, 1650. HR-MS Calcd for $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}$: 266.1419. Found: 266.1410.

Synthesis of 5-Chloro-1-phenethyl-1*H*-benzo[d]imidazol-2(3*H*)-one (4e) Using the same reaction condition for the preparation of **4c**, compound **4e** was obtained from compound **10c** (0.400 g, 1.0 mmol). Pale brown solid. Yield 93.26%. mp 176.1–177.8 °C. $R_f=0.18$ (hexane: ethyl acetate=2:1). $^1\text{H-NMR}$ (CDCl_3) δ : 10.8 (br, 1H), 7.22–6.63 (m, 8H), 4.08 (t, 2H,

$J=7.3$ Hz), 3.03 (t, 2H, $J=7.3$ Hz). IR (KBr) cm^{-1} : 3250, 3000, 1705, 1650. HR-MS Calcd for $\text{C}_{15}\text{H}_{13}\text{ClN}_2\text{O}$: 272.0716. Found: 272.0707.

Synthesis of 1-Benzyl-5-methyl-1H-benzo[d]imidazole-2(3H)-thione (5a) To the solution of compound **4** (0.050 g, 2.10 mmol) in toluene (5 ml), Lawesson's reagent (0.053 g, 1.2 mmol) was added. The resulting mixture was refluxed for 18 h. After cooling, toluene was removed under vacuum. The residue was dissolved in dichloromethane (100 ml) and extracted with water (70 ml) twice. The organic layer was dehydrated with anhydrous sodium sulfate and evaporated under vacuum. The residue was subjected to column chromatography for isolation of **5a**. Pale yellow solid. Yield 50.9%. mp 212.2–212.8 °C. $R_f=0.39$ (hexane:ethyl acetate=2:1). $^1\text{H-NMR}$ (CDCl_3) δ : 10.7 (br, 1H), 7.39–6.91 (m, 8H), 5.52 (s, 2H), 2.38 (s, 3H). IR (KBr) cm^{-1} : 3200, 2850, 1475. HR-MS Calcd for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{S}$: 254.0878. Found: 254.0866.

Synthesis of 1-Benzyl-5-chloro-1H-benzo[d]imidazole-2(3H)-thione (5b) Using the same reaction condition for the preparation of **5a**, compound **5b** was obtained from compound **4b** (0.070 g, 2.70 mmol) and Lawesson's reagent (0.065 g, 1.67 mmol). White solid. Yield 30.9%. mp 213.3–214.5 °C. $R_f=0.68$ (hexane:ethyl acetate=2:1). $^1\text{H-NMR}$ (CDCl_3) δ : 11.0 (br, 1H), 7.32–6.87 (m, 8H), 5.44 (s, 2H). IR (KBr) cm^{-1} : 3200, 3020, 2980, 2900, 2850, 1620. HR-MS Calcd for $\text{C}_{14}\text{H}_{11}\text{ClN}_2\text{S}$: 274.0331. Found: 274.0326.

Synthesis of 5-Methyl-1-(2-phenylethyl)-1H-benzo[d]imidazole-2(3H)-thione (5c) Using the same reaction condition for the preparation of **5a**, compound **5c** was obtained from compound **4c** (0.100 g, 0.0396 mmol) and Lawesson's reagent (0.096 g, 0.023 mmol). White solid. Yield 48.0%. mp 206.6–207.2 °C. $R_f=0.42$ (hexane:ethyl acetate=2:1). $^1\text{H-NMR}$ (CDCl_3) δ : 11.7 (br, 1H), 7.83–6.84 (m, 8H), 4.42 (t, 2H, $J=7.6$ Hz), 3.08 (t, 2H, $J=7.6$ Hz), 2.34 (s, 3H). IR (KBr) cm^{-1} : 3200, 2850, 1470. HR-MS Calcd for $\text{C}_{16}\text{H}_{16}\text{N}_2\text{S}$: 268.1034. Found: 268.1030.

Synthesis of 5-Methyl-1-(3-phenylpropyl)-1H-benzo[d]imidazole-2(3H)-thione (5d) Using the same reaction condition for the preparation of **5a**, compound **5d** was obtained from compound **4d** (0.030 g, 0.0112 mmol) and Lawesson's reagent (0.027 g, 0.0063 mmol). White solid. Yield 53.4%. mp 128.4–129.2 °C. $R_f=0.56$ (hexane:ethyl acetate=2:1). $^1\text{H-NMR}$ (CDCl_3) δ : 7.29–6.83 (m, 8H), 4.30 (t, 2H, $J=7.5$ Hz), 2.77 (t, 2H, $J=7.6$ Hz), 2.39 (s, 3H), 2.17 (m, 2H). IR (KBr) cm^{-1} : 3160–2850, 1515. HR-MS Calcd for $\text{C}_{17}\text{H}_{18}\text{N}_2\text{S}$: 282.1191. Found: 282.1178.

Synthesis of 5-Chloro-1-phenethyl-1H-benzo[d]imidazole-2(3H)-thione (5e) Using the same reaction condition for the preparation of **5a**, compound **5e** was obtained from compound **4e** (0.100 g, 0.0366 mmol) and Lawesson's reagent (0.089 g, 0.0216 mmol). White solid. Yield 65.2%. mp 219.4–220.5 °C. $R_f=0.54$ (hexane:ethyl acetate=2:1). $^1\text{H-NMR}$ (CDCl_3) δ : 11.10 (br, 1H), 7.25–6.72 (m, 8H), 4.46 (t, 2H, $J=7.6$ Hz), 3.13 (t, 2H, $J=7.6$ Hz). IR (KBr) cm^{-1} : 3200, 2750, 1620, 1607, 1500. HR-MS Calcd for $\text{C}_{15}\text{H}_{13}\text{ClN}_2\text{S}$: 288.0488. Found: 288.0480.

Acknowledgment This work was supported by a grant (R01-2007-000-20099-0) and Priority Research Centers Program (2009-0093815) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology.

References and Notes

1) Francisco S., Stefania B., Mauro P., Ghanem G., *Pigment Cell Res.*,

- 19, 550–571 (2006).
- 2) Protá G., *Med. Res. Rev.*, **8**, 525–556 (1988).
- 3) Kuzumaki T., Matsuda A., Wakamatsu K., Ito S., Ishikawa K., *Exp. Cell. Res.*, **207**, 33–40 (1993).
- 4) Hearing V. J., Jimenez M., *Int. J. Biochem.*, **19**, 1141–1147 (1987).
- 5) Halaban R., Patton R. S., Cheng E., Svedine S., Trombetta E. S., Wahl M. L., Ariyan S., Hebert, D. N., *J. Biol. Chem.*, **277**, 14821–14828 (2002).
- 6) Cullán, M. K., “The Pigmentary System: Physiology and Pathophysiology,” ed. by Norlund J. J., Biossy R. E., Hearing V. J., King R. A., Ortonne J. P., Oxford University Press, New York, 1998, pp. 760–766.
- 7) Urabe K., Nakayama J., Hori Y., “The Pigmentary System: Physiology and Pathophysiology,” ed. by Norlund J. J., Boissy R. E., Hearing V. J., King R. A., Ortonne J. P., Oxford University Press, New York, 1998, pp. 909–991.
- 8) Xu Y., Stokes A. H., Roskosi R. J., Vrana K. E., *J. Neurosci. Res.*, **54**, 691–697 (1998).
- 9) Asanuma M., Miyazaki I., Ogawa N., *Neurotox. Res.*, **5**, 165–176 (2003).
- 10) Battaini G., Monzani E., Casella L., Santagostini L., Pagliarin R., *J. Biol. Inorg. Chem.*, **5**, 262 (2000).
- 11) Maeda K., Fukuda M., *J. Pharmacol. Exp. Ther.*, **276**, 765–769 (1996).
- 12) Ros J. R., Rodriguez-Lopes J. N., Garcia-Canovas F., *Biochem. J.*, **295**, 309–312 (1993).
- 13) Ohguchi K., Tanaka T., Ito T., Iinuma M., Matsumoto K., Akao Y., Nozawa Y., *Biosci. Biotechnol. Biochem.*, **67**, 1587–1589 (2003).
- 14) Shin N. H., Ryu S. Y., Choi E. J., Kang S. H., Chang I. M., Min K. R., Kim Y. S., *Biochem. Biophys. Res. Commun.*, **243**, 801–803 (1998).
- 15) Kim Y. M., Yun J., Lee C. K., Lee H., Min K. R., Kim Y., *J. Biol. Chem.*, **277**, 6340–6344 (2002).
- 16) Curto E. V., Kwong C., Hermersdorfer H., Glatt H., Santis C., Virador V., Hearing V. J. Jr., Dooly T. P., *Biochem. Pharmacol.*, **57**, 663–672 (1999).
- 17) Passi S., Nazzaro-Porro M. B., *J. Dermatol.*, **104**, 659–665 (1981).
- 18) Kim Y., Jung S. H., Min K., Park J., Lee J., Korean Patent KR747042 (2007).
- 19) Samuel A., Brown, Carnele J. R., *Synth. Commun.*, **26**, 4065–4080 (1996).
- 20) Boyce R. S., Guo H., Mendenhall K. G., Walter A. O., Wang W., Xiu Y., (Novartis Vaccine and Diagnostic, Inc., U.S.A.) U. S. Patent Appl. Publ. US 20060420 AI 20060420 (2006).
- 21) Gomez-Parra V., Sanchez F., Torres T., *Monatshfte fur Chemie*, **116**, 639–644 (1985).
- 22) Orjales A., Mosguera R., Rodes R., *J. Med. Chem.*, **40**, 586–593 (1997).
- 23) Cava M. P., Levinson M. I., *Tetrahedron*, **41**, 5061–5087 (1985).
- 24) Thanigaimalai P., Lee K. C., Bang S. C., Lee J. H., Yun C. Y., Roh E., Hwang B. Y., Kim Y., Jung S. H., *Bioorg. Med. Chem.*, **18**, 1135–1142 (2010).
- 25) Thanigaimalai P., Lee K. C., Bang S. C., Lee J. H., Yun C. Y., Roh E., Hwang B. Y., Kim Y., Jung S. H., *Bioorg. Med. Chem.*, **18**, 1555–1562 (2010).