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Inhibitory effect of novel tetrahydropyrimidine-2(1*H*)-thiones on melanogenesis

P. Thanigaimalai [a], Ki-Cheul Lee [a], Seong-Cheol Bang [a], Jee-Hyun Lee [a], Cheong-Yong Yun [b], Eunmiri Roh [b], Bang-Yeon Hwang [b], Youngsoo Kim [b], Sang-Hun Jung [a,*]

[a] College of Pharmacy and Institute of Drug Research and Development, Chungnam National University, Daejeon 305-764, Republic of Korea
[b] College of Pharmacy, Chungbuk National University, Cheongu 361-763, Republic of Korea

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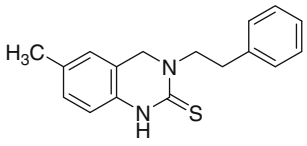
ABSTRACT

The series of imidazoldine-2-thiones **2** and tetrahydropyrimidine-2-thiones **3** were discovered as inhibitor of α-MSH-induced melanin production in melanoma B16 cells. The primary bioassay showed that 1-(4-ethylbenzyl)-tetrahydropyrimidine-2(1*H*)-thione **3e** (>100% inhibition at 10 μM, IC_{50} = 1.2 μM) and 1-(4-*tert*-butylbenzyl)-tetrahydropyrimidine-2(1*H*)-thione **3f** (>100% inhibition at 10 μM, IC_{50} = 0.76 μM) exhibited potent inhibitory effect against α-MSH-induced melanin production. Compounds **3** inhibit the biosynthesis of tyrosinase without affecting its catalytic activity in melanogenesis.

1. Introduction

Melanin is the primary determinant of human skin color, and is a heterogeneous biopolymer which plays an important role in the absorption of free radicals formed in cytoplasm and in protecting human skin from the harmful UV-radiation.[1] Melanin is synthesized in melanocytes through melanogenesis which is a complex pathway involving series of enzymatic reactions.[2–4] Among enzymes involved in the biosynthesis of melanin, tyrosinase is a most critical enzyme that catalyzes the reaction of conversion of L-tyrosine to dopaquinone.[2] Tyosinase, a copper bearing phenolase, is involved in abnormal accumulation of melanin pigments (hyperpigmentation).[5,6] Tyrosinase was reported to be linked in Parkinson diseases and some other neurodegenerative diseases.[7,8] Therefore, tyrosinase inhibitors such as kojic acid,[9] arbutin,[10] ascorbic acid derivatives,[11] hydroxylstilbine derivatives like resveratol[12–14] and methyl ester of genistic acid[15,16] have been well established as important constituents of cosmetic product and depigmenting agents for the treatment of hyperpigmentation.

However, the recent study was reported that the most used kojic acid has serious adverse effects such as skin cancer and dermatitis and has been banned as cosmetic ingredient in many countries.[17] Thus other type of molecules, which inhibit cyclic adenosine monophosphate (cAMP) dependent melanogenesis proteins other than tyrosinase, has gain the attraction. Using the 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, highly potent hypopigmenting activity of 6-methyl-3-phenethyl-3,4-dihydro-1*H*-quinazoline-2-thione (Fig. 1, **1**, IC_{50} = 0.8 μM) was found.[18] This level of activity of **1** is approximately 70 and 180 times more potent compared to those of kojic acid and arbutin in this bioassay system, respectively. The compound **1** suppresses the biosynthesis of tyrosinase, without affecting the activity of this enzyme. Preliminary study on the structure–activity relationship of **1** revealed that quinazolidine-2-thione motif is essential for their inhibition of melanogenesis. In order to explore the role of fused benzene moiety of **1**, imidazolidine-2-thiones **2** and tetrahydropyrimidine-2-thiones **3** (Fig. 1) were designed, synthesized, and evaluated their inhibitory activity for melanin production in melanoma B16 cell under the stimulus of α-MSH. The mechanism of action of these analogs was studied.



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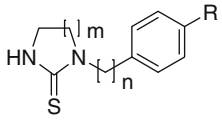

2: m = 1, n = 1, 2 or 3
3: m = 2, n = 1, 2 or 3

Figure 1. cAMP dependent melanogenesis blocker **1** and cyclic thioureas **2** and **3**.

Abbreviation: α-MSH, α-melanostimulating hormone.

* Corresponding author. Tel.: +82 42 821 5939; fax: +82 42 823 6566.
E-mail address: jungshh@cnu.ac.kr (S.-H. Jung).

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2. Chemistry

Imidazolidine-2-thiones **2** and tetrahydropyrimidine-2-thiones **3** were synthesized with the same synthetic procedures as shown in Scheme 1. In the initial step, the commercially available ethylenediamine (**4**) (or propan-1,3-diamine (**5**)) was mono-protected with *tert*-butyloxycarbonyl (*t*Boc) group. Five equivalent of diamine **4** (or **5**) with 1 equiv of Boc_2O in chloroform was stirred for overnight at room temperature. The reaction mixture was filtered and the filtrate was concentrated under vacuum. The resulting residue was dissolved in ethyl acetate and the organic solution was washed with brine and concentrated to give the corresponding mono-protected **6** (or **7**), which was pure enough for next step.[19]

The reductive amination[20] of mono-protected diamine with appropriate aldehyde (**8**) afforded amines **9** (or **10**). The cyclization of **9** (or **10**) to the **11** (or **12**) was performed by treatment with potassium *tert*-butoxide (KO*t*Bu)[21] as summarized in Table 1. According to literature[22] the cyclization of these amines could be facilitated either under basic catalysis or under Lewis acid catalysis. Initial screen showed that strong base was most effective. In the final step, the imidazoidinones **11** (or pyrimidinones **12**) were treated with Lawesson's reagents[23] under refluxing condition in toluene for 12 h to produce corresponding thiones **2** (or **3**) as summarized in Table 1.

3. Pharmacology

For all the synthesized compounds **11**, **12**, **2** and **3** the ability to inhibit formation of melanin from melanoma B-16 cells was determined under stimulus of α-MSH (100 nM) for 3 day incubation. Melanoma B16 cell (CRL6323) were obtained from ATCC (Manassas, USA). Amounts of melanin released into the culture media were determined by measuring absorbance values at 405 nm with synthetic melanin as the standard.[14] Data for % inhibition at 10 μM and IC_{50} values are mean values from 3 to 5 separate experiments as shown in Table 2.

4. Results and discussion

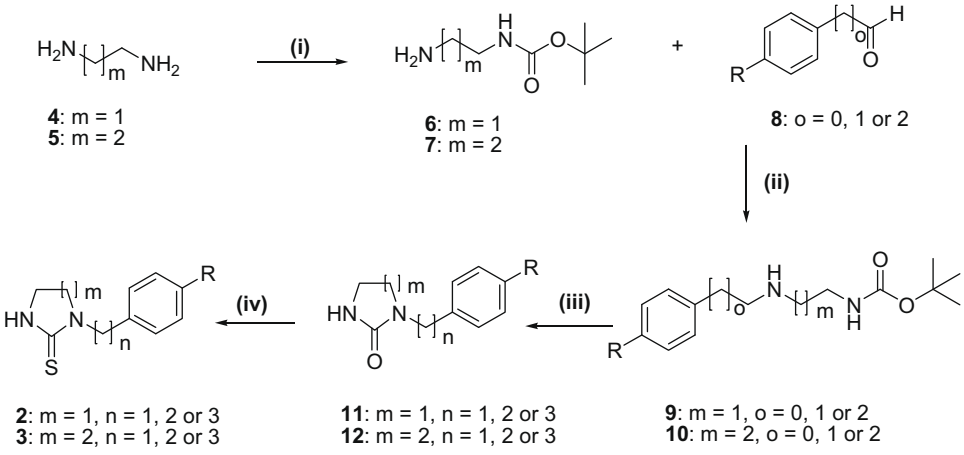
The cyclic thioureas **2b**, **2c**, **2f** and **3b–h** shows remarkably potent inhibitory activity against melanin production in melanoma B16 cells comparing the activity of kojic acid (IC_{50}: 70 μM), arbutin (IC_{50}: 180 μM) and almost similar to **1** (IC_{50} = 0.8 μM). Their IC_{50} values range from 0.8 to 6.1 μM, which are comparable to that of **1**. Especially, compound **3g** (IC_{50}: 2.9 μM) has the partial structure of **1** with omission of methylbenzene unit. Thus benzene ring fused on tetrahydropyrimidine-2-thione of **1** is not essential structural unit for their activity and is obviously an enhancer of the activity by increasing its lipophilicity. Thus tetrahydropyrimidine-2-thione motif appears as novel scaffold for the inhibition of melanin biosynthesis.

However, the cyclic urea compounds **11a–f** and **12a–h** showed very weak activity, although their structures are very similar to those of cyclic thioureas **2** and **3**. Their IC_{50} values are more than 10 μM and less than 30% inhibitory activity at 10 μM. These facts imply that thiourea group in the cyclic thioureas **2** and **3** has a pivotal role for the inhibition of melanin synthesis in the B16 cells. This difference has been recognized in the activity of phenylthiourea (PTU, 58% inhibition at 100 μM) and phenylurea (0% inhibition at 100 μM),[24,25] although mechanism of action of PTU (IC_{50} value for tyrosinase inhibition: 1.8 μM) is quite different from that of the cyclic thioureas **2** and **3** as discussed later. The mechanism of action of PTU has been known as inactivation of tyrosinase through complex formation of copper ion in active site of tyrosinase.

Increase in lipophilicity of cyclic thioureas **2**, 3 enhances the activity of these compounds as indicated with *C* log *P* in Table 1. The compound **3f** shows the strongest activity (IC_{50}: 0.76 μM) and the highest *C* log *P* value (4.437). However, lipophilicity should not be related to difference in activity of cyclic ureas and cyclic thioureas since the activity of **12f** (IC_{50}: >10 μM) is weak in spite of its higher *C* log *P* value (3.259) than those of **3b** (***C* log *P*:** 3.110, IC_{50}: 5.1 μM) and 10 g (*C* log *P*: 2.940, IC_{50}: 2.9 μM). Thus cyclic thiourea motif obviously appears as essential group for inhibition of melanin formation.

The lipophilicity of **2** and **3** is the important factor for the enhancement of activity. The more enhanced activity of pyrimidine-2-thiones **3** compared to those of imidazolidine-2-thiones **2** also can be explained with their increased lipophilicity of **3** as *C* log *P* values of these compounds indicated. The role of phenyl alkyl groups on 1-position of **2** and **3** is the enhancer of their lipophilicity. Increasing size of alkyl group on 4-position of phenyl increases the activity of **2** and **3** as shown in comparison of activity of **2a** and **2b** as well as **3a**, **3b**, **3e**, and **3d**. Increasing the lipophilicity with chloro substituent as shown in compound **3c** (IC_{50}: 2.4 μM) also increases the activity compared to that of **3a** (IC_{50}: >10 μM). Increasing the number of methylene groups between nitrogen and phenyl ring of **2** and **3** also increased the activity. This also considered as enhancing effect of lipophilicity. Introduction of 4-methoxy group on phenyl as shown in **3d** (IC_{50}: 6.1 μM, *C* log *P*: 2.530) decreased the activity and lipophilicity compared to **3b**, **3c**, **3e**, and **3f**.

The above discussion gave the more insight regarding the SAR of **1** related to our current studies. Accordingly, the following struc-



Scheme 1. Synthesis of compounds **2** and **3**. Reagents and conditions: (i) $Boc_2O/CHCl_3$; (ii) $NaBH_4$/TEA; (iii) KO*t*Bu/THF; (iv) Lawesson's reagent/toluene. Substituents for **9**, **10**, **11**, **12**, **2** and **3** are listed in Table 1.

Table 1
The substituents of **11**, **12**, **2** and **3**

Compound No.	*m*	*n*	R	*C* log *P*	Compound No.	*m*	*n*	R	*C* log *P*
11a	1	1	H	0.872	**2a**	1	1	H	2.052
11b	1	1	CH_3	1.371	**2b**	1	1	CH_3	2.551
11c	1	1	Cl	1.585	**2c**	1	1	Cl	2.765
11d	1	1	OCH_3	0.791	**2d**	1	1	OCH_3	1.971
11e	1	2	H	1.201	**2e**	1	2	H	2.381
11f	1	3	H	1.580	**2f**	1	3	H	2.760
12a	2	1	H	1.431	**3a**	2	1	H	2.611
12b	2	1	CH_3	1.930	**3b**	2	1	CH_3	3.110
12c	2	1	Cl	2.144	**3c**	2	1	Cl	3.324
12d	2	1	OCH_3	1.350	**3d**	2	1	OCH_3	2.530
12e	2	1	CH_2CH_3	2.459	**3e**	2	1	CH_2CH_3	3.369
12f	2	1	C $(CH_3)_3$	3.259	**3f**	2	1	C $(CH_3)_3$	4.437
12g	2	2	H	1.760	**3g**	2	2	H	2.940
12h	2	3	H	2.139	**3h**	2	3	H	3.319

Table 2
Inhibitory activity of **11**, **12**, **2**, and **3** on melanogenesis of melanoma B16 cells

Compound No.	% Inhibition at 10 μM	IC_{50} value (μM)	Compound No.	% Inhibition at 10 μM	IC_{50} value (μM)
11a	7	>10	**2a**	35	>10
11b	14	>10	**2b**	64	4.8
11c	10	>10	**2c**	79	4.3
11d	13	>10	**2d**	31	>10
11e	15	>10	**2e**	40	>10
11f	10	>10	**2f**	63	4.2
12a	0	>10	**3a**	42	>10
12b	5	>10	**3b**	80	5.1
12c	26	>10	**3c**	>100	2.4
12d	20	>10	**3d**	60	6.1
12e	29	>10	**3e**	>100	1.3
12f	30	>10	**3f**	>100	0.8
12g	10	>10	**3g**	93	2.9
12h	10	>10	**3h**	>100	2.1
			1		0.8
Kojic acid		70	Arbutin	>100	180

tural modification on **1** was done successfully to get the comparable activity as we observed in our previous studies[18] (1) The bicyclic system as **1** can be replaced to cyclic thiourea derivative **3e**–**f**, (2) chain length also modified with the introduction of bulkiness at *p*-position of phenyl ring **3e**–**f** and (3) we also tried to replace thiourea group to urea one, unfortunately, without any activity. The above points prove fruitful in the relationship of the compound **1** and the current synthesized compounds. Further, on comparing the activity of **1** (IC_{50}: 0.8 μM) with that of **3g**–**h** (IC_{50}: 2.9 μM and 2.1 μM), the level of activity is nearly the same. In fact, all the derivatives **3b**–**f** having bulky groups at *p*-position of phenyl ring showed very comparable activity to **1**. Thus benzene ring fused on tetrahydropyrimidine-2-thione of **1** is obviously enhancement the activity by increasing its lipophilicity. Thus tetrahydropyrimidine-2-thione motif appears as novel scaffold for the inhibition of melanin biosynthesis.

5. Mechanism of action

In defining the mechanism of action of **3**, the effects on tyrosinase activity, on tyrosinase synthesis upon stimulation with α-MSH and on the depigmentation of melanin were evaluated with **3f**. The effects of tyrosinase on melanin production were also studied during α-MSH stimulation with compound **3f**. Compound **3f** showed an inhibitory effect on melanin synthesis in melanoma B16 cells stimulated with α-MSH, an elevator of intracellular cAMP concentration. The amounts of melanin pigments were quite low in the resting cells (2 ± 6 μg/mL) but markedly increased, to 44 ± 4 μg/mL upon exposure to α-MSH for 72 h (Fig. 2A). The dopa oxidation velocity in the presence of **3f** was measured as an indicator of tyrosinase catalytic activity. Compound **3f** did not significantly inhibit the catalytic activity of cell-free tyrosinase (Fig. 2B). Upon exposure to α-MSH alone, levels of tyrosinase in the cells markedly increased from the baseline levels (Fig. 2C). Compound **3f** suppressed α-MSH-induced levels of tyrosinase in a dose-dependent manner (Fig. 2C). However, at concentrations effective for hypopigmentation activity, compound **3f** did not affect the viability of B16 cells (Fig. 2D), ruling out non-specific cytotoxicity. On the basis of these results, the compound **3f** is an inhibitor of α-MSH-induced melanin production without affecting the catalytic activity of tyrosinase. This result was unforeseen considering the well-known tyrosinase inhibitor NPT, because its thiourea moiety plays a key role in complexing the copper ion in the active site of tyrosinase, thereby blocking enzyme activity.[26]

6. Conclusion

In summary, imidazolidine-2-thiones **2** and tetrahydropyrimidine-2-thiones **3** inhibited melanogenesis in α-MSH-induced and melanin production. This implies that benzene ring fused on tetrahydropyrimidine-2-thione of **1** is not essential structural unit for their activity. Thus tetrahydropyrimidine-2-thione motif appears as novel scaffold for the inhibition of melanin biosynthesis. However, the urea analogs **11** and **12** did not show such effects. Among all thiones **2** and **3**, the compound **3f** very effectively inhibits the melanogenesis with an IC_{50} of an around 0.8 μM without affecting the catalytic activity of tyrosinase. These results suggest that tetrahydropyrimidine-2-thiones **3** are quite promising for the treat-

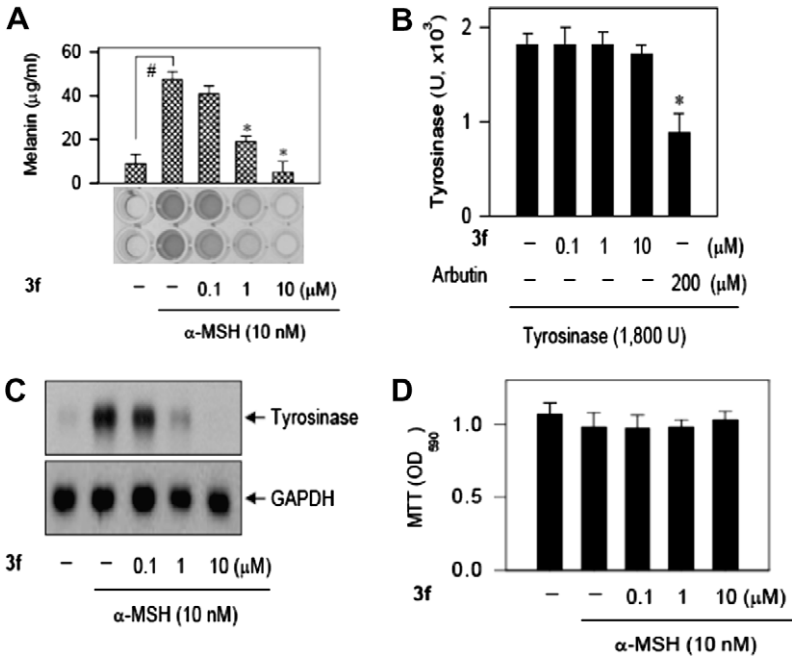


Figure 2. The effects of **3f** on α-MSH-induced melanin production in melanoma B16 cells and its mode of action. (A) The cells were stimulated with α-MSH for 72 h in the presence of **3f**. Melanin content was determined by measuring absorbance at 405 nm against a synthetic melanin standard. Data are expressed as the mean ± SD from three independent experiments. $^{\#}P$ <0.05, versus the media alone-treated group. $^{*}P$ <0.05, versus the α-MSH-treated group. (B) Enzyme sources of tyrosinase were prepared from cells stimulated with α-MSH (10 nM) for 48 h and diluted to a specific activity of 80,000 U/mg of protein, in which one unit of tyrosinase activity was defined as the conversion of 1 nmol dopa to dopachrome/min. The dopa oxidation velocity of cell-free tyrosinase (50 μg) was spectrophotometrically measured in the presence of **3f**. (C) The cells were stimulated with α-MSH for 48 h in the presence of **3f**. Cell extracts were subjected to Western blot analysis with an anti-tyrosinase antibody, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as an internal control. (D) The cells were treated with various concentrations of **3f** for 72 h in the presence of α-MSH. After reacting with MTT solution for 2 h, optical densities were measured at 590 nm.

ment of skin hyperpigmentation. Further exploration of the mechanism of activity of tetrahydropyrimidine-2-thiones **3** in depigmentation is in progress.

7. Materials and methods

7.1. Chemistry

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 precoated 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). A FT-IR spectrum was recorded with Nicolet – 380 models. NMR spectra were measured against the peak of tetramethylsilane by Varian Unity Inova 400 NMR (400 MHz) spectrometers. The ESI-MS mass data were obtained on an 1100-LC-MSD trap spectrometer (Agilent, Santa Clara, CA, USA).

7.2. General synthetic procedure for the preparation of mono-protected amine 6 and 7

A 0.5 M solution of Boc_2O (1 equiv) in $CHCl_3$ was added over a half an hour to a 0.25 M solution of diamine **4** (5 equiv) or **5** in $CHCl_3$ cooled with an ice-bath. The reaction mixture was stirred overnight at room temperature and filtered. The filtrate was concentrated under vacuum and the resulting oil dissolved in ethyl acetate (400 mL) than washed with half-saturated brine (3 × 150 mL), dried (Na_2SO_4) and concentrated reduced pressure to afford the corresponding mono-BOC-protected diamine **6** or **7**.

7.2.1. *tert*-Butyl 2-aminoethylcarbamate (6)

Yield 55%; colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.49 (s, 9H, *tert*-butyl), 2.79 (t, J = 6.4 Hz, 2H, NCH_2), 3.18 (t, J = 6.0 Hz, 2H, NCH_2), 4.91 (br s, 1H, NH). Mass spectra of compound exhibited molecular ion peak at m/z 160 (M^+), 161 (M+1).

7.2.2. *tert*-Butyl 3-aminopropylcarbamate (7)

Yield 50%; Viscous oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9H, *tert*-butyl), 1.61 (m, 2H, $NCH_2CH_2CH_2N$), 2.76 (t, J = 6.8 Hz, 2H, NCH_2C), 3.22 (t, J = 6.0 Hz, 2H, CH_2NBoc). Mass spectra of compound exhibited molecular ion peak at m/z 174 (M^+), 175 (M+1).

7.3. General procedure for the preparation of compounds 9 and 10

To the solution of mono-protected diamine **6** or **7** (1 mmol) in methanol (50 mL) and triethylamine (1 mmol) at 0 °C, the corresponding aldehyde **8** (1 mmol) was added. The reaction mixture was stirred at room temperature for 2 h and then cooled to 0 °C. Sodium borohydride (2 mmol) was added portion wise over a period of 30 min, then the resulting solution was neutralized with 4 M hydrochloric acid (5 mL) and extracted with Et_2O (50 mL). The ethereal phase was discarded and the aqueous phase was neutralized with solid $NaHCO_3$, extracted with ethyl acetate (200 mL), dried over anhydrous Na_2SO_4, concentrated to produce the appropriate analogues **9** or **10**.

7.3.1. *tert*-Butyl 2-(benylamino)ethylcarbamate (9a)

Yield 80%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.49 (s, 9H, *tert*-butyl), 2.75 (t, J = 5.6 Hz, 2H, NCH_2), 3.24 (t, J = 5.6 Hz, 2H, NCH_2), 3.87 (s, NCH_2Ph) 5.02 (br s, 1H, NH), 7.23–7.34 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 250 (M^+), 251 (M+1), 252 (M+2).

7.3.2. *tert*-Butyl 2-(4-methylbenzylamino)ethylcarbamate (9b)

Yield 75%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.49 (s, 9H, *tert*-butyl), 2.31 (s, 3H, CH_3Ph) 2.72 (t, J = 6.0 Hz, 2H, CH_2N), 3.22 (t, J = 6.0 Hz, 2H, CH_2 NBoc), 3.74 (s, 2H, NCH_2Ph), 4.95 (br s, 1H, NH) 7.21 (d, J = 8.2 Hz, 2H, Ar-H),7.30 (d, J = 8.2 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 264 (M^+), 265 (M+1), 266 (M+2).

7.3.3. *tert*-Butyl 2-(4-chlorobenzylamino)ethylcarbamate (9c)

Yield 70%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.49 (s, 9H, *tert*-butyl), 2.73 (t, J = 6.4 Hz, 2H, CH_2N), 3.24 (t, J = 6.4 Hz, 2H, CH_2NBoc), 4.90 (br s, 1H, NH), 7.22–7.30 (m, 4H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 284 (M^+), 286 (M+2).

7.3.4. *tert*-Butyl 2-(4-methoxybenylamino)ethylcarbamate (9d)

Yield 60%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 9H, *tert*-butyl), 2.49 (t, J = 6.4 Hz, 2H, CH_2N), 3.01 (t, J = 6.0 Hz, 2H, CH_2NBoc), 3.58 (s, 3H, OCH_3), 3.70 (s, 2H, NCH_2Ph) 4.31 (br s, 1H, NH) 6.88 (d, J = 8.4 Hz, 2H, Ar-H), 7.19 (d, J = 8.4 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 280 (M^+), 281 (M+1), 282 (M+2).

7.3.5. *tert*-Butyl 2-(phenylethylamino)ethylcarbamate (9e)

Yield 55%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, *tert*-butyl), 2.70 (t, J = 5.6 Hz, 2H, CH_2Ph), 2.77–2.88 (m, 4H, CH_2NCH_2), 3.19 (t, J = 5.6 Hz, 2H, CH_2NBoc), 5.00 (br s, 1H, NH), 7.13–7.30 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 264 (M^+), 265 (M+1), 266 (M+2).

7.3.6. *tert*-Butyl 2-(3-phenylpropylamino)ethylcarbamate (9f)

Yield 55%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H, *tert*-butyl), 1.69 (m, 2H, CCH_2CPh), 2.54–2.61 (m, 6H, $PhCH_2CCH_2NHCH_2$), 3.01 (t, J = 6.4 Hz, 2H, CH_2NBoc), 7.13–7.30 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 278 (M^+), 279 (M+1), 280 (M+2).

7.3.7. *tert*-Butyl 3-(benzylamino)propylcarbamate (10a)

Yield 80%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H, *tert*-butyl), 1.64–1.69 (m, 2H, $NCCH_2CN$), 2.73 (t, J = 6.4 Hz, 2H, CH_2N), 3.22 (t, J = 6.4 Hz, 2H, CH_2NBoc), 3.80 (s, 2H, CH_2Ph), 5.19 (br s, 1H, NH), 7.27–7.32 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 264 (M^+), 265 (M+1), 266 (M+2).

7.3.8. *tert*-Butyl 3-(4-methylbenzylamino)propylcarbamate (10b)

Yield 90%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H, *tert*-butyl), 1.62–1.70 (m, 2H, CCH_2CN), 2.30 (s, 3H, CH_3Ph), 2.69 (t, J = 6.4 Hz, 2H, CH_2N), 3.20 (t, J = 6.0 Hz, 2H, CH_2NBoc), 3.73 (s, 2H, CH_2Ph), 5.31 (br s, 1H, NH), 7.11–7.26 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 278 (M^+), 279 (M+1), 280 (M+2).

7.3.9. *tert*-Butyl 3-(4-chlorobenzylamino)propylcarbamate (10c)

Yield 73%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H, *tert*-butyl), 1.67–1.70 (m, 2H, CCH_2CN), 2.68 (t, J = 6.0 Hz, 2H, CH_2N), 3.20 (t, J = 6.0 Hz, 2H, CH_2NBoc), 3.76 (s, 2H, CH_2Ph), 5.30 (br s, 1H, NH), 7.27–7.33 (m, 4H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 298 (M^+), 300 (M+2).

7.3.10. *tert*-Butyl 3-(4-methoxybenzylamino)propylcarbamate (10d)

Yield 67%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H, *tert*-butyl), 1.49–1.52 (m, 2H, CCH_2CN), 2.43 (t, J = 6.4 Hz, 2H, CH_2N), 2.94 (t, J = 6.0 Hz, 2H, CH_2NBoc), 3.56 (s, 3H, CH_3OPh), 3.70 (s, 2H, NCH_2Ph), 5.74 (br s, 1H, NH), 6.82 (d, J = 8.4 Hz, 2H, Ar-H), (d, J = 8.4 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 294 (M^+), 295 (M+1), 296 (M+2).

7.3.11. *tert*-Butyl 3-(4-ethylbenzylamino)propylcarbamate (10e)

Yield 55%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.22 (t, J = 7.6 Hz, 3H, CH_3), 1.44 (s, 9H, *tert*-butyl), 1.63–1.69 (m, 2H, CCH_2CN), 2.64 (q, J = 5.6 Hz, 2H, $PhCH_2$), 2.71 (t, J = 6.0 Hz, 2H, CH_2N), 3.20 (t, J = 6.0 Hz, 2H, CH_2NBoc), 3.77 (s, 2H, Ar-CH_2N), 7.14 (d, J = 7.6 Hz, 2H, Ar-H), 7.22 (d, J = 8.0 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 292 (M^+), 293 (M+1), 294 (M+2).

7.3.12. *tert*-Butyl 3-(4-*tert*-butylbenzylamino)propylcarbamate (10f)

Yield 57%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 9H, *tert*-butyl), 1.41 (s, 9H, *tert*-butyl), 1.60 (m, 2H, CCH_2CN), 2.71 (t, J = 6.4 Hz, 2H, NCH_2), 3.21 (t, J = 6.0 Hz, 2H, CH_2N), 3.74 (s, 2H, $PhCH_2$), 7.23 (d, J = 7.6 Hz, 2H, Ar-H), 7.33 (d, J = 8.0 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 320 (M^+), 321 (M+1), 322 (M+2).

7.3.13. *tert*-Butyl 3-(phenethylamino)propylcarbamate (10g)

Yield 60%; viscous oil; ^{1}H NMR ($CDCl_3$); δ 1.44 (s, 9H, *tert*-butyl), 1.62 (m, 2H, CCH_2CN), 2.69 (t, J = 6.4 Hz, 2H, CH_2Ph), 2.79 (t, J = 6.4 Hz, 2H, CH_2N), 2.84 (t, J = 6.4 Hz, 2H, CH_2N), 3.19 (t, J = 6.4 Hz, 2H, CH_2NBoc), 5.14 (br s, 1H, NH), 7.11–7.26 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 278 (M^+), 279 (M+1), 280 (M+2).

7.3.14. *tert*-Butyl 3-(3-phenylpropylamino)propylcarbamate (10h)

Yield 55%; viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H, *tert*-butyl), 1.52 (m, 2H, $NCCH_2CN$), 1.62 (m, 2H, $PhCCH_2CN$), 2.47 (m, 4H, CH_2NHCH_2), 2.58 (t, J = 6.0 Hz, 2H, CH_2Ph), 2.95 (t, J = 6.4 Hz, 2H, CH_2NBoc), 7.13–7.30 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 292 (M^+), 293 (M+1), 294 (M+2).

7.4. General procedure for the preparation of compounds 11 and 12

The appropriate amine **9** or **10** (16.90 mmol) charged with tetrahydrofuran (50 mL) in 100 mL round bottom flask. Solid potassium *tert*-butoxide (51.0 mmol) was added and the resulting yellow solution was heated to 60 °C for 3 h. The mixture was cooled to ambient temperature and acidified with aqueous HCl (35 mL, 1 M) and concentrated under reduced pressure. The aqueous residue was extracted with ethyl acetate, organic phase washed twice with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give viscous oil which was solidified on standing. The desired compound was purified by flash column chromatography.

7.4.1. 1-Benzylimidazolidin-2-one (11a)

Yield 71%; yellow solid; mp 124 °C; R_f 0.27 (7:3 ethyl acetate/hexane); IR (neat): 3236, 1677 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.29 (m, 2H, NCH_2CN), 3.42 (m, 2H, $NCCH_2N$), 4.37 (s, 2H, CH_2Ph), 4.53 (br s, 1H, NH) 7.29 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 176 (M^+), 177 (M+1).

7.4.2. 1-(4-Methylbenzyl)imidazolidin-2-one (11b)

Yield 67%; brown solid; mp 148 °C: R_f 0.34 (7:3 ethyl acetate/hexane); IR (neat): 3228, 1681 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3H, CH_3), 3.2 (t, J = 7.2 Hz, 2H, NCH_2CN), 3.45 (t, J = 7.2 Hz, 2H, $NCCH_2N$), 4.32 (s, 2H, CH_2Ph), 4.50 (br s, 1H, NH), 7.18–7.23 (m,

4H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 190 (M^+), 191 (M+1).

7.4.3. 1-(4-Chlorobenzyl)imidazolidin-2-one (11c)

Yield 55%; white solid; mp 154 °C; R_f 0.14 (1:1 ethyl acetate/hexane); IR (neat): 3235, 1678 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.19 (m, 2H, NCH_2CN), 3.41 (m, 2H, $NCCH_2N$), 4.37 (s, 2H, CH_2Ph), 4.41 (br s, 1H, NH), 7.23 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.32 (d, *J* = 8.2 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 210 (M^+), 212 (M+2).

7.4.4. 1-(4-Methoxybenzyl)imidazolidin-2-one (11d)

Yield 60%; yellow solid; mp 130 °C; R_f 0.23 (7:3 ethyl acetate/hexane); IR (neat): 3236, 1677 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.26 (t, *J* = 6.4 Hz, 2H, NCH_2CN), 3.40 (t, *J* = 6.4 Hz, 2H, $NCCH_2N$), 3.8 (s, 3H, OCH_3), 4.34 (s, 2H, CH_2Ph), 4.28 (br s, 1H, NH), 6.82 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.22 (d, *J* = 7.8 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 206 (M^+), 207 (M+1).

7.4.5. 1-Phenylehtylimidazolidin-2-one (11e)

Yield 62%; yellow solid; mp 132 °C; R_f 0.25 (7:3 ethyl acetate/hexane); IR (neat): 3234, 1677 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.87 (t, *J* = 7.2 Hz, 2H, CH_2Ph), 3.08–3.26 (m, 4H, NCH_2CH_2N), 3.50 (t, *J* = 7.6 Hz, 2H, NCH_2CPh), 5.01 (br s, 1H, NH), 7.26–7.31 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 190 (M^+), 191 (M+1).

7.4.6. 1-(3-Phenylpropyl)imidazolidin-2-one (11f)

Yield 55%; yellow oil; R_f 0.20 (6:4 ethyl acetate/hexane); IR (neat) 3227, 1683 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.62–1.71 (m, 2H, $NCCH_2CPh$), 2.83 (t, *J* = 6.4 Hz, 2H, CH_2Ph), 3.12–3.32 (m, 4H, NCH_2CH_2N), 3.81 (t, *J* = 6.4 Hz, 2H, CH_2N), 5.27 (br s, 1H, NH), 7.27–7.43 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 204 (M^+), 205 (M+1).

7.4.7. 1-Benzyltetrahydropyrimidin-2(1*H*)-one (12a)

Yield 84%; orange solid; mp 146 °C; R_f 0.12 (7:3 ethyl acetate/hexane); IR (neat) 3236, 1677 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.90 (m, 2H,CCH_2C), 3.18 (t, *J* = 6.0 Hz, 2H, CH_2N), 3.33 (t, *J* = 5.6 Hz, 2H, CH_2N), 4.56 (s, 2H, NCH_2Ph), 4.96 (br s, 1H, NH), 7.31–7.37 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 190 (M^+), 191 (M+1).

7.4.8. 3-(4-Methylbenzyl)tetrahydropyrimidin-2(1*H*)-one (12b)

Yield 72%; brown solid; mp 108 °C; R_f 0.15 (7:3 ethyl acetate/hexane); IR (neat) 3236, 1677 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.88 (m, 2H, CCH_2C), 2.33 (s, 3H, CH_3Ph), 3.15 (t, *J* = 6.0 Hz, 2H, CH_2N), 3.32 (t, *J* = 6.4 Hz, 2H, CH_2N), 4.51 (s, 2H, NCH_2Ph), 4.83 (br s, 1H, NH), 7.18 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.20 (d, *J* = 8.0 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 204 (M^+), 205 (M+1).

7.4.9. 1-(4-Chlorobenzyl)tetrahydropyrimidin-2(1*H*)-one (12c)

Yield 67%; yellow solid; mp 102 °C; R_f 0.10 (7:3 ethyl acetate/hexane); IR (neat) 3229, 1677 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.89 (m, 2H, CCH_2C), 3.16 (t, *J* = 5.6 Hz, 2H, CH_2NH), 3.33 (t, *J* = 6.0 Hz, 2H, CH_2N), 4.50 (s, 2H, NCH_2Ph), 5.08 (br s, 1H, NH), 7.21–7.28 (m, 4H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 224 (M^+), 226 (M+2).

7.4.10. 3-(4-Methoxybenzyl)tetrahydropyrimidin-2(1*H*)-one (12d)

Yield 50%; yellow solid; mp: 76 °C; R_f 0.11 (7:3 ethyl acetate/hexane); IR (neat) 3233, 1674 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.88 (m, 2H, CCH_2C), 3.13 (t, *J* = 6.0 Hz, 2H, CH_2N), 3.28 (t, *J* = 4.8 Hz, 2H, CH_2N), 3.82 (s, 3H, OCH_3), 4.43 (s, 2H, NCH_2Ph), 5.18 (br s, 1H, NH), 6.82 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.29 (d, *J* = 8.2 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 220 (M^+), 221 (M+1).

7.4.11. 1-(4-Ethylbenzyl)tetrahydropyrimidin-2(1*H*)-one (12e)

Yield 53%; colorless solid; mp 104 °C; R_f 0.10 (7:3 ethyl acetate/hexane); IR (neat) 2926, 2856, 1791, 1651 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (t, *J* = 7.6 Hz, 3H, CH_3), 1.86–1.92 (m, 2H, CCH_2C), 2.63 (q, *J* = 7.6 Hz, 2H, $PhCH_2$), 3.14 (t, *J* = 7.2 Hz, 2H, CH_2N), 3.30 (t, *J* = 6.8 Hz, 2H, CH_2N), 4.85 (s, 2H, $ArCH_2N$), 4.64 (br s, 1H, NH), 7.14 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.18 (d, *J* = 8.0 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 218 (M^+), 219 (M+1).

7.4.12. 1-(4-*tert*-Butylbenzyl)tetrahydropyrimidin-2(1*H*)-one (12f)

Yield 59%; colorless solid; mp 127 °C; R_f 0.36 (ethyl acetate); IR (neat) 2928, 2857, 1641 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 (s, 9H, *tert*-butyl), 1.87–1.93 (m, 2H, CCH_2C), 3.18 (t, *J* = 6.0 Hz, 2H, CH_2N), 3.30 (t, *J* = 6.4 Hz, 2H, CH_2N), 4.51 (s, 1H, Ar-CH_2N), 4.90 (br s, 1H, NH), 7.19 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.19 (d, *J* = 8.4 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 246 (M^+), 247 (M+1).

7.4.13. 3-Phenylethyltetrahydropyrimidin-2(1*H*)-one (12g)

Yield 61%; viscous oil; R_f 0.19 (7:3 ethyl acetate/hexane); IR (neat) 3236, 1678 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.98 (m. 2H, CCH_2C), 2.72 (t, *J* = 7.6 Hz, 2H, CH_2Ph), 3.09 (t, *J* = 6.0 Hz, 2H, CH_2N), 3.25 (t, *J* = 6.4 Hz, 2H, CH_2N), 3.53 (t, *J* = 7.6 Hz, 2H, CH_2N), 5.28 (br s, 1H, NH), 7.38 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 204 (M^+), 205 (M+1).

7.4.14. 3-(3-Phenylpropyl)tetrahydropyrimidin-2(1*H*)-one (12h)

Yield 55%; viscous oil; R_f 0.21 (7:3 ethyl acetate/hexane); IR (neat) 2926, 2859, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.88–190 (m, 4H), 2.60 (t, *J* = 6.4 Hz, 2H, CH_2Ph), 3.16 (t, *J* = 6.0 Hz, 2H, NCH_2), 3.20–3.24 (m, 4H, CH_2NCH_2), 3.72 (t, *J* = 7.2 Hz, 2H, NCH_2), 5.43 (s, 1H, NH), 7.15–7.27 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 218 (M^+), 219 (M+1).

7.5. General procedure for the preparation of compounds 2 and 3

To the solution of urea compounds **11** or **12** in anhydrous toluene (75 mL) was added Lawesson's reagent (1.2 equiv) under nitrogen atmosphere. The resulting solution was refluxed for overnight. Toluene was removed and diluted with water. The aqueous mixture was extracted with dichloromethane. The organic phase was washed twice with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give viscous oil. The crude material was purified by flash column chromatography

7.5.1. 1-Benzylimidazolidine-2-thione (2a)

Yield 50%; white solid; mp 180 °C; R_f 0.29 (4:6 ethyl acetate/hexane); IR (neat) 3205, 2360, 1494, 1452 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.56 (s, 4H, NCH_2CH_2N) 4.8 (s, 2H, CH_2Ph), 5.72 (br s, 1H, NH), 7.31–7.37 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at *m*/*z* 192 (M^+), 193 (M+1), 194 (M+2).

7.5.2. 1-(4-Methylbenzyl)imidazolidine-2-thione (2b)

Yield 50%; white solid; mp 158 °C; R_f 0.41 (4:6 ethyl acetate/hexane); IR (neat) 3215, 2360, 1500, 1477 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.34 (s, 3H, CH_3), 3.54 (s, 4H, NCH_2CH_2N), 4.76 (s, 2H, CH_2Ph), 6.26 (br s, 1H, NH) 7.15 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.24 (d,

J = 8.0 Hz, 2H, Ar-H). Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 206 (M^+), 207 (M+1), 208 (M+2).

7.5.3. 1-(4-Chlorobenzyl)imidazolidine-2-thione (2c)

Yield 45%; white solid; mp 203 °C; R_f 0.22 (4:6 ethyl acetate/hexane); IR (neat) 3258, 2359, 1543, 1464 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.56 (s, 4H, NCH_2CH_2N), 4.72 (s, 2H, NCH_2Ph), 5.88 (br s, 1H, NH), 7.23 (d, J = 8.2 Hz, 2H, Ar-H), 7.33 (d, J = 8.2 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 226 (M^+), 228 (M+2).

7.5.4. 1-(4-Methoxybenzyl)imidazolidine-2-thione (2d)

Yield 40%; brown solid; mp 162 °C; R_f 0.23 (4:6 ethyl acetate/hexane); IR (neat) 3210, 2360, 1510, 1454 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.54 (s, 4H, NCH_2CH_2N), 3.80 (s, 3H, OCH_3), 4.73 (s, 2H, CH_2Ph), 6.25 (br s, 1H, NH), 6.82 (d, J = 8.4 Hz, 2H, Ar-H), 7.26 (d, J = 8.8 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 222 (M^+), 223 (M+1), 224 (M+2).

7.5.5. 1-Phenylehtylimidazolidine-2-thione (2e)

Yield 50%; brown solid; mp 93 °C; R_f 0.30 (4:6 ethyl acetate/hexane); IR (neat) 3297 2360, 1492, 1457 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.82 (t, J = 7.2 Hz, 2H, CH_2Ph), 3.35 (m, 4H, NCH_2CH_2N), 3.45 (t, J = 7.2 Hz, 2H, NCH_2CPh), 4.37 (br s, 1H, NH), 7.28 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 206 (M^+), 207 (M+1), 208 (M+2).

7.5.6. 1-(3-Phenylpropyl)imidazolidine-2-thione (2f)

Yield 55%; brown solid; mp 61 °C R_f 0.30 (4:6 ethyl acetate/hexane); IR (neat) 3224, 2360, 1496, 1456 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.92–1.98 (m, 2H, CCH_2CPh), 2.62 (t, J = 7.6 Hz, 2H, CH_2Ph), 3.53 (t, J = 7.2 Hz, 2H, NCH2), 3.65–3.70 (each m, 4H, NCH_2CH_2N), 5.60 (br s, 1H,NH), 7.27 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 220 (M^+), 221 (M+1), 222 (M+2).

7.5.7. 1-Benzyltetrahydropyrimidine-2(1*H*)-thione (3a)

Yield 60%; brown solid; mp 166 °C; R_f 0.16 (4:6 ethyl acetate/hexane); IR (neat) 3194, 2360, 1536, 1516 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.95–2.05 (m, 2H, CCH_2C), 3.23 (t, J = 6.0 Hz, 2H, CH_2NH), 3.33 (t, J = 6.4 Hz, 2H, CH_2N), 5.18 (s, 2H, NCH_2Ph), 6.38 (br s, 1H, NH), 7.30–7.32 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 206 (M^+), 207 (M+1), 208 (M+2).

7.5.8. 3-(4-Methylbenzyl)tetrahydropyrimidine-2(1*H*)-thione (3b)

Yield 58%; white solid; mp 178 °C; R_f 0.28 (4:6 ethyl acetate/hexane); IR (neat) 3216, 1531, 2360, 1501 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.91–1.97 (m, 2H, CCH_2C), 2.33 (s, 3H, CH_3Ph), 3.23 (t, J = 5.6 Hz, 2H, CH_2NH), 3.32 (t, J = 6.0 Hz, 2H, CH_2N), 5.13 (s, 2H, NCH_2Ph), 6.53 (br s, 1H, NH), 7.23 (d, J = 7.6 Hz, 2H, Ar-H), 7.28 (d, J = 7.6 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 220 (M^+), 221 (M+1), 222 (M+2).

7.5.9. 1-(4-Chlorobenzyl)tetrahydropyrimidine-2(1*H*)-thione (3c)

Yield 50%; Yellow solid; mp 208 °C; R_f 0.23 (4:6 ethyl acetate/hexane); IR (neat) 3212, 2360, 1542, 1487 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.98–2.23 (m, 2H, CCH_2C), 3.22 (t, J = 6.0 Hz, 2H, CH_2NH), 3.33 (t, J = 6.0 Hz, 2H, CH_2N), 5.14 (s, 2H, NCH_2Ph), 6.33 (br s, 1H, NH), 7.29 (m, 4H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 240 (M^+), 242 (M+2).

7.5.10. 3-(4-Methoxybenzyl)tetrahydropyrimidin-2(1*H*)-thione (3d)

Yield 55%; yellow solid; mp 161 °C; R_f 0.17 (4:6 ethyl acetate/hexane); IR (neat) 3210, 1543, 1510, 1452 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.97–2.24 (m, 2H, CCH_2C), 3.22 (t, J = 6.0 Hz, 2H, CH_2N), 3.35 (t, J = 6.4 Hz, 2H, CH_2N), 3.38 (s, 3H, OCH_3), 5.10 (s, 2H, NCH_2Ph), 6.28 (br s, 1H, NH), 6.85 (d, J = 7.6 Hz, 2H, Ar-H), 7.33 (d, J = 8.0 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 236 (M^+), 237 (M+1), 238 (M+2).

7.5.11. 1-(4-Ethylbenzyl)tetrahydropyrimidine-2(1*H*)-thione (3e)

Yield 45%; colorless solid; mp 155 °C; R_f 0.37 (7:3 ethyl acetate/hexane); IR (neat) 3231, 2928, 2854, 1542, 1449 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (t, J = 7.6 Hz, 3H, CH_3), 1.92–1.98 (m, 2H, CCH_2C), 2.60 (q, J = 7.6 Hz, 2H, Ar-CH_2), 3.22 (t, J = 6.0 Hz, 2H, CH_2N), 3.29 (t, J = 6.4 Hz, 2H, CH_2N), 5.11 (s, 2H, Ar-CH_2N), 6.31 (br s, 1H, NH), 7.15 (d, J = 8.4 Hz, 2H, Ar-H), 7.28 (d, J = 8.4 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 234 (M^+), 235 (M+1), 236 (M+2).

7.5.12. 1-(4-*tert*-Butylbenzyl)tetrahydropyrimidine-2(1*H*)-thione (3f)

Yield 45%; colorless solid; mp 187 °C; R_f 0.42 (7:3 ethyl acetate/hexane); IR (neat): 2927, 2856, 1856, 1605, 1458 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (s, 9H, *tert*-butyl), 1.93–2.01 (m, 2H, CCH_2C), 3.24 (t, J = 6.4 Hz, 2H, CH_2N), 3.30 (t, J = 6.0 Hz, 2H, CH_2N), 5.41 (s, 2H, Ar-CH_2), 6.31 (br s, 1H, NH), 7.27 (d, J = 8.4 Hz, 2H, Ar-H), 7.34 (d, J = 8.0 Hz, 2H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 262 (M^+), 263 (M+1), 264 (M+2).

7.5.13. 3-Phenylethyltetrahydropyrimidine-2(1*H*)-thione (3g)

Yield 50%; yellow solid; mp 111 °C; R_f 0.20 (4:6 ethyl acetate/hexane); IR (neat) 3238, 2360, 1531, 1495 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.91- 1.96 (m, 2H), 3.02 (t, J = 6.4 Hz, 2H, $PhCH_2$), 3.18 (t, J = 5.6 Hz, 2H, CH_2N), 3.26 (t, J = 6.0 Hz, 2H, CH_2N), 4.74 (t, J = 7.2 Hz, 2H, $PhCCH_2N$), 6.23 (br s, 1H, NH), 7.29–7,32 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 220 (M^+), 221 (M+1), 222 (M+2).

7.5.14. 3-(3-Phenylpropyl)tetrahydropyrimidine-2(1*H*)-thione (3h)

Yield 68%; brown solid; mp 131 °C; R_f 0.13 (4:6 ethyl acetate/hexane); IR (neat) 3228, 2360, 1541, 1521 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.93–2.03 (m, 4H), 2.67 (t, J = 7.6 Hz, 2H, $PhCH_2$), 3.23–3.30 (m, 4H, NCH_2CCH_2N), 3.90 (t, J = 7.6 Hz, 2H, NCH_2CCPh), 6.16 (br s, 1H, NH), 7.17–7.34 (m, 5H, Ar-H). Mass spectra of compound exhibited molecular ion peak at m/z 234 (M^+), 235 (M+1), 236 (M+2).

7.6. Biological evaluation

7.6.1. Chemicals and antisera

Fetal bovine serum (FBS), Dulbecco's modified Eagle's media and other culture supplements were purchased from Invitrogen (Carlsbad, CA). Antisera against tyrosinase or GAPDH were purchased from Santa Cruz Biotech (Santa Cruz, CA). All other chemicals, including α-MSH, were otherwise purchased from Sigma–Aldrich (St. Louis, MO).

7.6.2. Cell culture

B16 melanoma cells were obtained from American Type Culture Collection (Manassas, USA). The cells were cultured in DMEM (13.4 mg/ml Dulbecco's modified Eagle's medium, 10 mM HEPES, 143 U/ml benzylpenicillin potassium, 100 μg/ml streptomycin sulfate, 24 mM $NaHCO_3$, pH 7.1) containing 10% FBS, and incubated at 37 °C under 5% CO_2 atmosphere.

7.6.3. Melanin quantification

B16 cells were seeded in culture plates, a density of 2.5×10^3 cells per well of 96-well microplates, and incubated at 37 °C under

5% CO_2 atmosphere for 24 h. The cells were then stimulated with α-MSH (10 nM) for 72 h, in the presence of sample. The cells were harvested, and then disrupted in 1 N NaOH-10% dimethylsulfoxide with heating at 80 °C. Melanin contents were measured by absorbance values at wavelength 405 nm with synthetic melanin as a standard.

7.6.4. Measurement of tyrosinase activity

B16 cells were treated with α-MSH (10 nM) alone for 72 h. After washing, the cells were resuspended in sodium phosphate buffer (50 mM, pH 6.8) containing 1% Triton X-100 and phenylmethylsulfonyl fluoride (1 mM), and then subjected to sonication on ice. After centrifugation, supernatants were dialyzed against sodium phosphate buffer and then used as the sources of cell-free tyrosinase. Dopa oxidation activity of tyrosinase was determined as described previously.[14] Briefly, dopa (5 mM) and sample were mixed in sodium phosphate buffer (50 mM, pH 6.8) and finally added with enzyme sources. Initial velocity of dopachrome formation from the reaction mixture was determined by the increase of absorbance values at wavelength 475 nm per min. One unit of tyrosinase activity was defined as the conversion of 1 nmol dopa to dopachrome per min.

7.6.5. Western blot analysis

B16 cells were stimulated with α-MSH (10 nM) for 48 h, in the presence of sample, and then disrupted in a lysis buffer (50 mM Tris, 50 mM NaCl, 0.1% SDS, 1% NP-40, 1 mM phenylmethanesulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, pH 7.4). Equal amounts of the proteins were resolved on SDS-acrylamide gels by electrophoresis and transferred to a polyvinylidene difluoride membrane. Either 5% non-fat milk in PBS containing Tween 20 or 5% BSA in Tris-buffered saline containing Tween 20 was used as the blocking buffer. The blots were usually incubated at 4 °C overnight with primary antisera (dilution); anti-tyrosinase (1:1000), and anti-GAPDH (1:5000). The blots were then incubated with appropriate horseradish peroxidase-conjugated secondary antisera at room temperature for 2–5 h. Immune complexes on the blots were finally visualized by exposure to X-ray film after reacting with an enhanced chemiluminescence's reagent (GE Healthcare, Chalfont St. Giles, UK).

7.6.6. MTT assay

B16 cells were treated with various concentration of sample for 72 h, in the presence of α-MSH (10 nM). The cells were exposed to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT,500 μg/ml) for 2 h. The MTT-formazan complex was dissolved in 100% dimethyl sulfoxide and optical densities were then measured at wavelength 590 nm.

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