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Evaluation of 3,4-dihydroquinazoline-2(1*H*)-thiones as inhibitors of α-MSH-induced melanin production in melanoma B16 cells

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ABSTRACT

Novel 3,4-dihydroquinazoline-2(1*H*)-thiones (QNTs) **1** were found to be potent inhibitors of α-MSH-induced melanin production. The effect of QNTs to inhibit melanin formation in B16 melanoma cells was screened in the presence of α-MSH. In defining the mechanism of activity, the effects on tyrosinase activity, on tyrosinase synthesis and on the depigmentation of melanin were evaluated. QNTs did not affect the catalytic activity of tyrosinase, but rather acted as an inhibitor of tyrosinase synthesis.

1. Introduction

Melanin is a heterogeneous biopolymer and is important in protecting the skin from the harmful effects of sunlight, toxic drugs, and chemicals.[1] Melanin absorbs harmful UV radiation and transforms the energy into heat through an ultrafast internal conversion process.[2] This process keeps the light-induced generation of free radicals at a minimum. However, abnormal deposition of melanin pigments can cause diverse hyperpigmentary disorders, such as melasma, freckles, and age spots.[3,4]

The production of melanin in the melanocytes of hair and skin is strictly controlled by the melanocortin 1 receptor (MC1R), the activity of which is regulated positively by α-melanocyte stimulating hormone (α-MSH) and negatively by agouti signal protein (ASP).[5,6] Stimulation of MC1R by α-MSH results in the activation of adenylyl cyclase and the production of cAMP, which in turn affects the pathways of cAMP-responsive element-binding protein (CREB), MAP kinase, and P13 kinase, resulting in the formation of melanin. Among the steps in this pathway, the CREB component has attracted considerable attention. cAMP leads to the phosphorylation of CREB transcriptional factors, which induces microphthalmia-associated transcription factor (MITF), which, in turn, plays a pivotal role in the expression of the tyrosinase gene.[7,8] Tyrosinase catalyses the rate-limiting steps in the biosynthetic pathway of melanin pigments in melanocytes.[9,10] This key enzyme catalyses the hydroxylation of tyrosine into 3,4-dihydroxyphenylalanine (L-dopa) and the subsequent oxidation to form dopaquinone, a substrate for the synthesis of pheomelanins or eumelanins. Thus, controlling this enzyme has been useful in treating pigmentation disorders and in the development of cosmetic whitening agents. Many tyrosinase inhibitors have been reported, including kojic acid,[11] arbutin,[12] ascorbic acid derivatives,[13] hydroxylstilbene derivatives, like resveratol,[14,15] and genistic acid methyl ester,[16,17] and the hypopigmenting effects of fatty acids.[18] Because of several recently reported adverse effects,[19] such as cytotoxicity, skin cancer, and dermatitis, kojic acid, a frequently used skin whitening agent, has been banned for cosmetic use in many countries. Additionally, compounds that inhibit other melanogenesis signaling proteins have been discovered, including linoleic acid and terrain, which inhibit melanogenesis by down-regulation of tyrosinase expression without changing tyrosinase activity.[20,21]

We attempted to identify, by a random screening process, compounds that inhibited α-MSH-stimulated melanogenesis in B16 melanoma cells. The potent hypopigmentation activity of 6-methyl-3-phenethyl-3,4-dihydro-1*H*-quinazoline-2-thione (Fig. 1, **1a**, IC_{50} = 0.8 μM) was found as a result of these efforts.[22] The potency of **1a** is approximately 80 and 200 times greater than that of kojic acid and arbutin, respectively. Thus, the current study focused on the design and synthesis of its analogs for use as novel

Abbreviations: QNTs, 3,4-dihydroquinazolines-2(1*H*)-thiones; α-MSH, α-melanocyte stimulating hormone.

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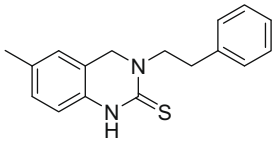


Figure 1. 6-Methyl-3-phenethyl-3,4-dihydro-1*H*-quinazoline-2-thione (**1a**, IC_{50} = 0.8 μM).

whitening agents and as a means of determining structure–activity relationships and mechanism(s) of the observed activity.

2. Chemistry

Target compounds **1a–q** were prepared using the synthetic pathway in Scheme 1. The alkylation of amino groups in **3** was conducted with two different reactions. Direct alkylation of **3a–i** with *o*-nitro benzyl chlorides **2a–d** yielded key intermediates **5a–k**.[22] Intermediates **5l–q** were obtained by reductive amination of **3a**, **3b**, **3e**, **3f**, **3g**, or **3h** with 2-nitro-5-chlorobenzaldehyde (**4**).[23,24] The reduction of nitro compounds **5a–q** with 10% Pd/C under a 30-psi hydrogen atmosphere at room temperature for 5 h yielded intermediates **6a–q**,[22] which were used without further purification. The subsequent cyclization of **6a–q** was performed with carbonyldiimidazole in the presence of triethylamine to yield quinazolones (QNO) **7a–q**.[22] Resulting data concerning the preparation of **7** are summarized in Table 1. Finally these were converted to quinazoline-2-thiones (QNT) **1a–q**, as shown in Table 1, by refluxing overnight with Lawesson's reagent in toluene.[25] The synthesized compounds were purified by flash column chromatography. All QNOs and QNTs exhibited *C*log [P] values of <5.8, as calculated by ChemDraw (v. 9.0) and were fully characterized by spectral analyses.

3. Pharmacology

For QNT **1a–q** and QNO **7a–q**, the ability to inhibit melanin formation in B16 melanoma cells was determined in the presence of α-MSH (100 nM) throughout three-day incubation as shown in Table 2. The amount of melanin released into the culture media was determined by measuring sample absorbance at 405 nm against a synthetic melanin standard[15] (Fig. 2A and B).

The tyrosinase activity was defined as the rate of conversion of 1 nmol of dopa to dopachrome/min. The dopa oxidation of cell-free tyrosinase was measured spectrophotometrically in the presence of QNT **1g** (Fig. 2C).

The cells extracted were subjected to Western blot analysis with an anti-tyrosinase antibody and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as an internal control (Fig. 2D). The optical densities were measured at 590 nm after reacting with MTT solution (Fig. 2E).

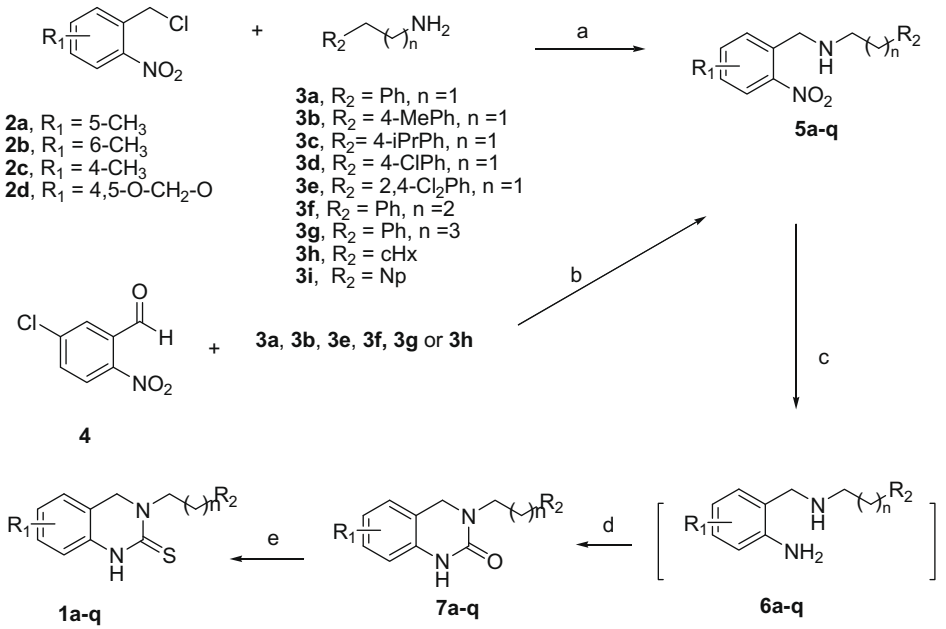
Table 1
The substituents of **7** and **8**

Compd	R_1	R_2	*n*	Compd	R_1	R_2	*N*
7a	6-CH_3	Ph[a]	1	**1a**	6-CH_3	Ph[a]	1
7b	7-CH_3	Ph	1	**1b**	7-CH_3	Ph	1
7c	5-CH_3	Ph	1	**1c**	5-CH_3	Ph	1
7d	6-CH_3	4-CH_3Ph	1	**1d**	6-CH_3	4-CH_3Ph	1
7e	6-CH_3	4-*i*PrPh[b]	1	**1e**	6-CH_3	4-*i*PrPh[b]	1
7f	6-CH_3	4-ClPh	1	**1f**	6-CH_3	4-ClPh	1
7g	6-CH_3	2,4-Cl_2Ph	1	**1g**	6-CH_3	2,4-Cl_2Ph	1
7h	6-CH_3	Ph	2	**1h**	6-CH_3	Ph	2
7i	6-CH_3	Ph	3	**1i**	6-CH_3	Ph	3
7j	6-CH_3	*c*Hx	1	**1j**	6-CH_3	*c*Hx	1
7k	6-CH_3	Np[c]	1	**1k**	6-CH_3	Np[c]	1
7l	6-Cl	Ph	1	**1l**	6-Cl	Ph	1
7m	6-Cl	4-CH_3Ph	1	**1m**	6-Cl	4-CH_3Ph	1
7n	6-Cl	4-ClPh	1	**1n**	6-Cl	4-ClPh	1
7o	6-Cl	Ph	2	**1o**	6-Cl	Ph	2
7p	6-Cl	Ph	3	**1p**	6-Cl	Ph	3
7q	6-Cl	*c*Hx[d]	1	**1q**	6-Cl	*c*Hx[d]	1

[a] Ph = phenyl.
[b] *i*Pr = isopropyl.
[c] Np = 1-naphthyl.
[d] *c*Hx = cyclohexyl.

4. Results and discussion

The activity of both QNOs (**7**) and QNTs (**1**) are summarized in Table 2 with the mean value obtained from 3 to 5 independent



Scheme 1. Synthesis of compounds **1**. Reagents and conditions: TEA, CH_2Cl_2, reflux, overnight; (b) TEA, $NaBH_4$, methanol, reflux, 4 h; (c) 10%Pd/C, 30 psi, rt, 5 h; (d) 1,1-carbonyl diimidazole, THF, reflux, overnight; (e) Lawesson's reagent, toluene, reflux, overnight. Substituents for **7a–q** and **1a–q** are listed in Table 1.

Table 2
In vitro activity of compounds **7** and **1** against α-MSH-induced melanin production in B16 cells

Compd	% Inhibition at 10 μM	IC_{50} (μM)	*C*log *P*[a]	Compd	% Inhibition at 10 μM	IC_{50} (μM)	*C*log *P*[a]
7a	16	>10	3.629	**1a**	>100	0.8	4.289
7b	24	>10	3.629	**1b**	>100	0.8	4.289
7c	18	9.8	3.629	**1c**	>100	1.1	4.289
7d	10	>10	4.128	**1d**	>100	2.7	4.788
7e	6	>10	5.056	**1e**	>100	0.5	5.716
7f	15	>10	4.342	**1f**	>100	4.3	5.002
7g	11	>10	5.055	**1g**	>100	0.5	5.715
7h	22	>10	4.008	**1h**	>100	3.9	4.668
7i	38	>10	4.537	**1i**	>100	0.9	5.197
7j	17	>10	4.711	**1j**	>100	0.8	5.371
7k	5	>10	4.803	**1k**	>100	2.4	5.463
7l	18	>10	4.145	**1l**	>100	2.8	4.503
7m	6	>10	4.644	**1m**	>100	5.5	5.002
7n	7	>10	4.858	**1n**	>100	1.6	5.216
7o	16	>10	4.522	**1o**	>100	0.8	4.882
7p	17	>10	5.053	**1p**	96	5.5	5.411
7q	3	>10	5.227	**1q**	>100	4.3	5.585
Kojic acid		70		Arbutin		180	

[a] *C*log *P* values were calculated by Chemdraw ver. 9.0.

experiments. The IC_{50} of QNTs 1 ranged from 0.5 to 5.5 μM. In general, the activity of QNOs **7a**–**q** (IC_{50}: >10 μM) was very weak. Thus, the thiourea moiety of QNTs appeared to be key for the activity of **1a**–**p**. This trend had been noticed in the activity of phenylthiourea (PTU, 58% inhibition at 100 μM) and phenyl urea (0% inhibition at 100 μM).[26,27] Although the mechanism of PTU activity (IC_{50} value for tyrosinase inhibition: 1.8 μM) was quite different from that of QNTs **1a**–**p**, as discussed later. Because the thiourea moiety of PTU is important for complexing the copper ion in tyrosinase, it was originally considered that the same mechanistic pathway existed for QNTs. However, this was not the case. The role of the thiourea moiety in QNT **1** is unclear.

Positioning the methyl group at 5, 6, or 7 on quinazoline ring of **1** did not alter its activity, as demonstrated with **1a** (IC_{50}: 0.8 μM), **1b** (IC_{50}: 0.8 μM), and **1c** (IC_{50}: 1.1 μM), respectively. Replacement of the 6-methyl group with a chlorine substituent on the quinazoline ring of QNT resulted in a slight variation in activity, as indicated by a comparison of **1a**–**j** and **1l**–**q**. This demonstrates that electronic effects on the quinazoline ring of **1** are detrimental to QNT activity and may indicate that small substituents on the quinazoline ring do not affect QNT activity.

Comparing the relative activities of **1a** (IC_{50}: 0.8 μM), **1d** (IC_{50}: 2.7 μM), **1e** (IC_{50}: 2.7 μM), and **1f** (IC_{50}: 4.3 μM) reveals that substitution at the 4-position of the phenyl group of **1a** with methyl, chloro, or isopropyl substituents decreased the activity slightly. This trend was also apparent in the activity of the chloro-QNT series **1l**, **1m**, and **1n**. However, compound **1g** (IC_{50}: 0.5 μM), with a 2,4-dichloro substitution, retained the same level of activity as **1a**. To determine the optimum size of the aromatic group in **1a**, a 1-naphthyl group was substituted for the phenyl group, as shown in **1k** (IC_{50}: 2.4 μM) with a corresponding decrease in activity. The introduction of substituents or enlargement of the aromatic phenyl group in **1a** was thus not beneficial for QNT activity.

Elongation of the methylene unit connecting the phenyl and quinazoline moieties from two to three units reduced the activity, as indicated by comparison between compounds **1a** and **1h** (IC_{50}: 3.9 μM). However, one additional methylene unit, as in **1i** (IC_{50}: 0.9 μM) resulted in the original level of activity. This trend was reversed in the chloro-QNT series. Compound **1o** (IC_{50}: 0.8 μM) was more potent than **1l** (IC_{50}: 2.8 μM), but **1p** (IC_{50}: 5.5 μM) was less active. Thus, elongation of methylene unit in **1a** only marginally affected activity.

To evaluate the necessity of phenyl group planarity in **1a**, a bulkier cyclohexyl group was introduced as shown in compounds **1j** and **1q**. Compound **1j** (IC_{50}: 0.8 μM) exhibited the same level of activity as **1a**, and the activity of **1q** (**IC_{50}:** 4.3 μM) was similar to that of **1l**. Thus, planarity of the phenyl moiety of **1a** is apparently not required for activity and likely does not participate in binding of receptor molecules. Thus, the *N*-phenyl alkyl groups of QNT **1** may act as controllers of lipophilicity. Although *C*log *P* values were much changed with introduction of substituents on **1a**, the variation in activity is not much recognized by increasing the lipophilicity of these thioureas as indicated in Table. 2. For instance, compounds **1e** (IC_{50}: 0.5 μM, *C*log *P* = 5.716) and **1g** (IC_{50}: 0.5 μM, *C*log *P* = 5.715) showed comparable activity to that of **1a** (IC_{50}: 0.8 μM, *C*log *P* = 4.429). In addition to that, lipophilicity did not differentiate between the activity of urea **7a**–**q** and of thiourea **1a**–**q**, considering the *C*log *P* values and the activity of **7q** (IC_{50}: >10 μM, *C*log *P* = 5.227) and **1a** (IC_{50}: 0.8 μM, *C*log *P* = 4.289). Therefore lipophilicity is not critical factor for the activity of QNT 1.

In defining the mechanism of activity, the effects on tyrosinase activity, on tyrosinase synthesis upon stimulation with α-MSH, and on the depigmentation of melanin were evaluated. Additionally, the effects of tyrosinase on melanin production were studied during α-MSH stimulation with compound **1g**. QNT **1g** showed an inhibitory effect on melanin production in 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). QNT **1g** dose dependently inhibited α-MSH-induced melanin production, with an IC_{50} value of 1.2 μM (Fig. 2A, B). Arbutin, a well-known skin whitener, also inhibited the production of α-MSH-induced melanin, with an IC_{50} value of 190 μM (Fig. 2B).

To define the mode of hypopigmentation activity, the ability of QNT **1g** to influence the catalytic activity of cell-free tyrosinase, prepared from B16 cells stimulated with only α-MSH, was first investigated. The dopa oxidation velocity in the presence of QNT **1g** was measured as an indicator of tyrosinase catalytic activity. Compound **1g** did not significantly inhibit the catalytic activity of cell-free tyrosinase (Fig. 2C). Western blot analysis was then performed to determine the level of tyrosinase. Upon exposure to α-MSH alone, levels of tyrosinase in the cells markedly increased from the baseline levels (Fig. 2D). QNT **1g** suppressed α-MSH-induced levels of tyrosinase in a dose-dependent manner (Fig. 2D). However, at concentrations effective for hypopigmentation activity, compound **1g** did not affect the viability of B16 cells (Fig. 2E), ruling out non-specific cytotoxicity. Thus, QNT **1g** is an efficient

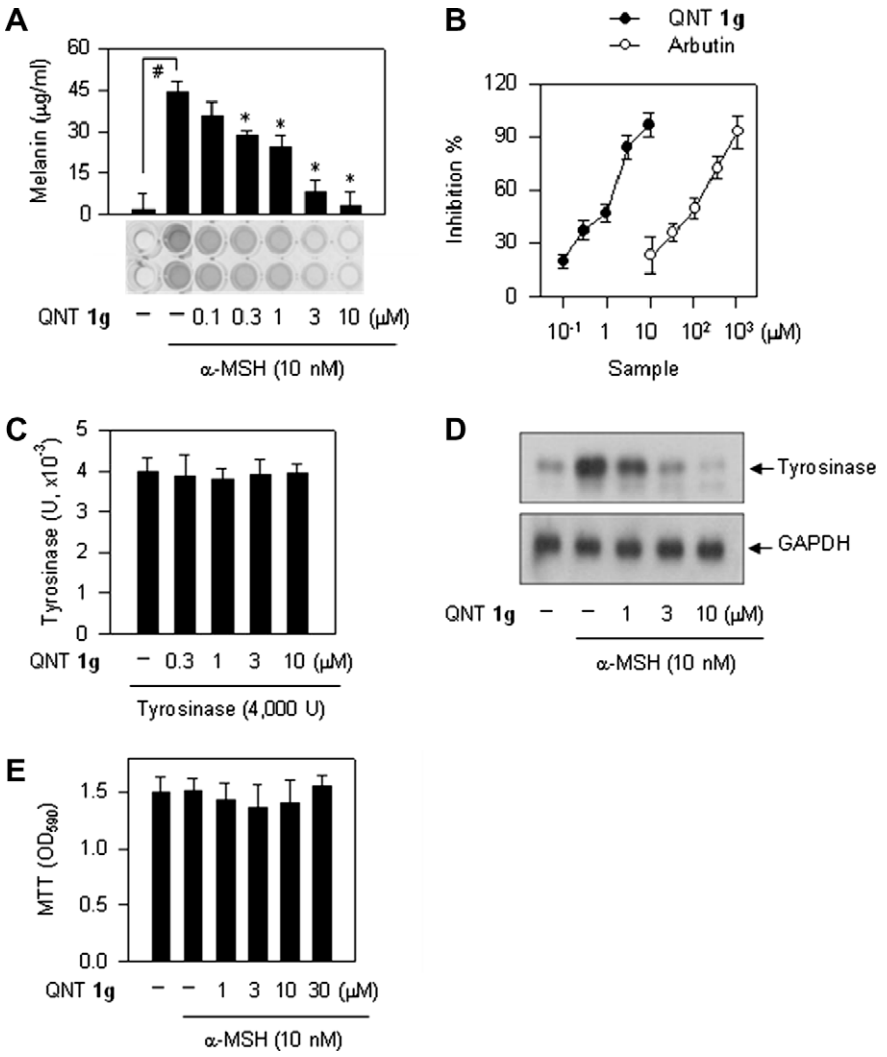


Figure 2. The effects of QNT **1g** on α-MSH-induced melanin production in melanoma B16 cells and its mode of action. (A) The cells were stimulated with α-MSH for 72 h in the presence of QNT **1g**. Melanin content was determined by measuring absorbance at 405 nm against a synthetic melanin standard. 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) The effects of QNT **1g** (solid circle) or arbutin (open circle) on α-MSH-induced melanin production are also shown in terms of percent inhibition. (C) 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 QNT **1g**. (D) The cells were stimulated with α-MSH for 48 h in the presence of QNT **1g**. 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. (E) The cells were treated with various concentrations of QNT **1g** for 72 h in the presence of α-MSH. After reacting with MTT solution for 2 h, optical densities were measured at 590 nm.

inhibitor of α-MSH-induced melanin production and suppressed tyrosinase levels without affecting the catalytic activity of the enzyme. This result was unexpected considering the well-known tyrosinase inhibitor PTU, in which the thiourea moiety plays a key role in complexing the copper ion in the active site of tyrosinase, thereby blocking enzyme activity.[28]

5. Conclusion

In summary, novel QNTs **1** were prepared and identified as potent inhibitors of α-MSH-induced melanin production. Although QNOs **7** are structurally very similar to QNTs **1**, these compounds were nearly inactive. Thus, 6-methyl-3-phenylalkyl-3,4-dihydroquinazoline-2(1*H*)-thione was shown to be an essential scaffold for inhibitory activity of melanin production from B16 melanoma cells stimulated by α-MSH. Although the mechanism of QNT activity requires further exploration, QNTs were shown to block the formation of tyrosinase, without affecting tyrosinase activity. This unusual and potent mechanism suggests that QNTs may be promising whitening agents. Further exploration of the mechanism of QNT activity in depigmentation is in progress.

6. Materials and methods

6.1. 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. High resolution mass spectrum (HRMS) was recorded on API2000 mass spectrometer (PE Sciex, Toronto, Canada).

6.1.1. General procedure for the synthesis of compounds 5 (a–k)

To a corresponding benzyl chloride (1 equiv) in methylene chloride (50 mL) was added the corresponding amines (1.5 equiv) and followed by triethylamine (3 equiv) Then the reaction mixture was allowed to stir for overnight at 55 °C. After the reaction was complete; the reaction mixture was cooled to room temperature and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The product was isolated by flash column chromatography using a mixture of 10–15% ethyl acetate in hexane as an eluent.

6.1.1.1. *N*-(5-Methyl-2-nitrobenzyl)-2-phenylethanamine (5a). Yellow oil; yield 80%; ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H), 2.81 (t, J = 6.8 Hz, 2H), 2.86 (t, J = 6.8 Hz, 2H), 3.98 (s, 2H), 7.01–7.31 (m, 6H), 7.35 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H).

6.1.1.2. *N*-(4-Methyl-2-nitrobenzyl)-2-phenylethanamine (5b). Yellow oil; yield 85%; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H), 2.83 (t, J = 6.4 Hz, 2H), 2.88 (t, J = 6.4 Hz, 2H), 4.00 (s, 2H), 7.19–7.44 (m, 7H), 7.75 (s, 1H).

6.1.1.3. *N*-(2-Methyl-6-nitrobenzyl)-2-phenylethanamine (5c). Yellow oil; yield 80%; ^{1}H NMR ($CDCl_3$) δ 2.46 (s, 3H), 2.83 (t, J = 7.2 Hz, 2H), 2.97 (t, J = 7.2 Hz, 2H), 3.78 (s, 2H), 7.18–7.31 (m, 6H), 7.38 (d, J = 7.6 Hz, 1H), 7.56 (d, J = 8.0 Hz, 1H).

6.1.1.4. *N*-(5-Methyl-2-nitrobenzyl)-2-*p*-tolylethanamine (5d). Yellow oil; yield 73%; ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3H), 2.42 (s, 3H), 2.77 (t, J = 6.8 Hz, 2H), 2.90 (t, J = 6.4 Hz, 2H), 4.02 (s, 2H), 7.09 (s, 4H), 7.15 (d, J = 8.4 Hz, 1H), 7.31 (s, 1H), 7.89 (d, J = 8.4 Hz,1H).

6.1.1.5. 2-(4-Isopropylphenyl)-*N*-(5-methyl-2-nitrobenzyl)ethanamine (5e). Yellow oil; yield 68%; ^{1}H NMR ($CDCl_3$) δ 1.23 (d, J = 4.0 Hz, 6H), 2.43 (s, 3H), 2.86 (t, J = 6.8 Hz, 2H), 2.84 -2.89 (m, 3H), 4.04 (s, 2H), 6.99- 7.17 (m, 5H), 7.34 (s, 1H), 7.90 (d, J = 8.4 Hz, 1H).

6.1.1.6. 2-(4-Chlorophenyl)-*N*-(5-methyl-2-nitrobenzyl)ethanamine (5f). Yellow oil; yield 79%; ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3H), 2.80 (t, J = 6.8 Hz 2H), 2.91 (t, J = 6.4 Hz, 2H), 4.02 (s, 2H), 7.04 (d, J = 8.0 Hz, 1H), 7.10–7.32 (m, 5H), 7.35 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H).

6.1.1.7. 2-(2,4-Dichlorophenyl)-*N*-(5-methyl-2-nitrobenzyl)ethanamine (5g). Yellow oil; yield 73%; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H), 2.86–2.94 (m, 4H), 4.08 (s, 1H), 7.07 (s, 2H), 7.08 (s, 1H), 7.34 (d, J = 8.4 Hz, 2H), 7.89 (d, J = 8.4 Hz, 1H).

6.1.1.8. *N*-(5-Methyl-2-nitrobenzyl)-3-phenylpropan-1-amine (5h). Yellow oil; yield 75%; ^{1}H NMR ($CDCl_3$) δ 1.81–1.87 (m, 2H), 2.42 (s, 3H), 2.67–2.75 (m, 4H), 3.99 (s, 2H), 7.09–7.29 (m, 6H), 7.36 (s, 1H), 7.91 (d, J = 8.4 Hz, 1H).

6.1.1.9. *N*-(5-Methyl-2-nitrobenzyl)-4-phenylbutan-1-amine (5i). Yellow oil; yield 79%; ^{1}H NMR ($CDCl_3$) δ 1.52–1.70 (m, 4H), 2.41 (s, 3H), 2.57–2.67 (m, 4H), 3.98 (s, 1H), 7.16 (d, J = 6.8 Hz, 4H), 7.26 (t, J = 7.6 Hz, 2H), 7.37 (s, 1H), 7.87 (d, J = 8.4 Hz, 1H).

6.1.1.10. *N*-(5-Methyl-2-nitrobenzyl)-2-cyclohexylethanamine (5j). Yellow oil; yield 83%; ^{1}H NMR ($CDCl_3$) δ 1.5–1.99 (m, 11H), 2.13 (t, J = 6.8 Hz, 2H), 2.41 (s, 3H), 2.72 (t, J = 6.8 Hz, 2H), 4.00 (s, 2H), 5.46 (br s, 1H), 7.17 (d, J = 8.4 Hz, 1H), 7.39 (s, 1H), 7.91 (d, J = 8.4 Hz,1H).

6.1.1.11. *N*-(5-Methyl-2-nitrobenzyl)-2-(naphthalen-2-yl)ethanamine (5k). Yellow oil; yield 70%; ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 3H), 3.02 (s, 4H), 4.08 (s, 2H), 7.14 (d, J = 8.0 Hz, 1H), 7.31–7.47 (m, 4H), 7.62 (s, 1H), 7.71–7.81 (m, 3H), 7.86 (d, J = 8.4 Hz, 1H).

6.1.2. General procedure for the synthesis of compounds 5 (l–q)

A mixture of corresponding amine (1 equiv), aldehyde (1 equiv) and triethylamine (2 equiv) was taken in ethanol (50 mL), and cooled to 0 °C. To the reaction mixture was added appropriate amine and allowed to reflux for 4 h. The mixture was cooled to 0 °C and added $NaBH_4$ portion wise over 10 min. The resulting solution was neutralized with 4 M hydrochloric acid (5 mL) and extracted with diethyl ether (50 mL). The ethereal phase was discarded and aqueous phase was neutralized with solid $NaHCO_3$, and extracted with ethyl acetate (200 mL), dried over Na_2SO_4, concentrated to produce an appropriate compound which was purified by flash column chromatography.

6.1.2.1. *N*-(5-Chloro-2-nitrobenzyl)-2-phenylethanamine (5l). Yellow oil; yield 65%; ^{1}H NMR ($CDCl_3$) δ 2.81 (t, J = 6.8 Hz, 2H), 2.91 (t, J = 6.8 Hz, 2H), 4.06 (s, 2H), 7.20–7.36 (m, 6H), 6.56 (s, 1H), 7.91 (d, J = 8.0 Hz, 1H).

6.1.2.2. *N*-(5-Chloro-2-nitrobenzyl)-2-p-tolylethanamine (5m). Yellow oil; yield 68%; ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H), 2.77 (t, J = 6.8 Hz, 2H), 2.94 (t, J = 6.4 Hz, 2H), 4.05 (s, 2H), 7.08–7.10 (s, 4H), 7.33 (d, J = 8.4 Hz, 1H), 7.64 (s, 1H), 7.91 (d, J = 8.4 Hz, 1H).

6.1.2.3. *N*-(5-Chloro-2-nitrobenzyl)-2-(4-chlorophenyl)ethanamine (5n). Yellow oil; yield 61%; ^{1}H NMR ($CDCl_3$) δ 2.77 (t, J = 6.8 Hz, 2H), 2.88 (t, J = 6.8 Hz, 2H), 4.05 (s, 2H), 7.13 (d, J = 8.4 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.4 Hz, 1H), 7.61 (s, 1H), 7.91 (d, J = 8.8 Hz, 1H).

6.1.2.4. *N*-(5-Chloro-2-nitrobenzyl)-3-phenylpropan-1-amine (5o). Yellow oil; yield 67%; ^{1}H NMR ($CDCl_3$) δ 1.81–1.88 (m, 2H), 2.65–2.70 (m, 4H), 4.00 (s, 2H), 7.16 (m, 3H), 7.26 (t, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 1H), 7.67 (s, 1H), 7.92 (d, J = 8.4 Hz, 1H).

6.1.2.5. *N*-(5-Chloro-2-nitrobenzyl)-4-phenylbutan-1-amine (5p). Yellow oil; yield 58%; ^{1}H NMR ($CDCl_3$) δ 1.51–1.71 (m, 4H), 2.61 (m, 4H), 4.02 (s, 2H), 7.16–7.48 (m, 6H), 7.68 (s, 1H), 7.92 (d, J = 8.4 Hz, 1H).

6.1.2.6. *N*-(5-Chloro-2-nitrobenzyl)-2-cyclohexylethanamine (5q). Yellow oil; yield 74%; ^{1}H NMR ($CDCl_3$) δ 1.54–1.90 (m, 11H), 2.14 (t, J = 6.8 Hz, 2H), 2.71 (t, J = 6.8 Hz, 2H), 4.05 (s, 2H), 5.47 (s, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.71 (s, 1H), 7.95 (d, J = 8.8 Hz, 1H).

6.1.3. General procedure for the synthesis of compounds 7 (a–q)

To the amine compound (1 equiv) in tetrahydrofuran (50 mL) was added CDI (1.2 equiv) followed triethylamine (2 equiv) under nitrogen atmosphere and this mixture was allowed to reflux for overnight. After disappearance of starting material, the mixture was neutralized with 2 N HCl, extracted with methylene chloride (100 mL) and dried over anhydrous sodium sulfate. The pure product was isolated by flash column chromatography using 30–40% ethyl acetate in hexane. In order to determine the purity, all compounds were tested HPLC analysis using C18 reverse phase column by the mobile phase of methanol and acetonitrile (1:1).

6.1.3.1. 6-Methyl-3-phenethyl-3,4-dihydroquinazolin-2(1*H*)-one (7a). Brown solid; yield 64%; purity 97.55%; mp 165–167 °C; IR (neat) 3021, 3084, 1650, 1506 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.25 (s, 3H), 2.94 (t, J = 7.2 Hz, 2H), 3.62 (t, J = 6.8 Hz, 2H), 4.30 (s, 2H), 6.53 (d, J = 8.0 Hz, 1H), 6.71 (br s, 1H), 6.76 (s, 1H), 6.94 (d, J = 8.4 Hz, 1H), 7.19–7.31 (m, 5H); HRMS calcd for $C_{17}H_{18}N_2O$ *m/z* 266.1419, found 266.1408.

6.1.3.2. 7-Methyl-3-phenethyl-3,4-dihydroquinazolin-2(1*H*)-one (7b). Brown solid; yield 68%: purity 96.93%; mp 161–164 °C; IR (neat) 3200, 3024, 1662, 1598, 1525 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.29 (s, 3H), 2.95 (t, J = 7.6 Hz, 2H), 3.65 (t, J = 7.6 Hz, 2H), 4.31 (s, 2H), 6.48 (s, 1H), 6.73 (d, J = 7.6 Hz, 1H), 6.83 (d, J = 7.6 Hz, 1H), 7.00 (br s, 1H), 7.22–7.31 (m, 5H); HRMS calcd for $C_{17}H_{18}N_2O$ *m/z* 266.1419, found 266.1409.

6.1.3.3. 5-Methyl-3-phenethyl-3,4-dihydroquinazolin-2(1*H*)-one (7c). Brown solid; yield 68%; purity 95.12%; mp 158–160 °C; IR (neat) 3203, 3056, 1655, 1611, 1455 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.08 (s, 3H), 2.96 (t, J = 7.6 Hz, 2H), 3.68 (t, J = 7.6 Hz, 2H), 4.26 (s, 2H), 6.49 (d, J = 8.0 Hz, 1H), 6.74 (d, J = 7.6 Hz, 1H), 6.98 (br s, 1H), 7.04 (t, J = 8.0 Hz, 1H), 7.24–7.29 (m, 5H); HRMS calcd for $C_{17}H_{18}N_2O$ *m/z* 266.1419, found 266.1408.

6.1.3.4. 6-Methyl-3-(4-methylphenethyl)-3,4-dihydroquinazolin-2(1*H*)-one (7d). Yellow solid; yield 73%; purity 97.07%; mp 132–134 °C; IR (neat) 2924, 2857, 1659, 1503 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.33 (s, 3H), 2.63 (s, 3H), 2.89 (t, J = 7.2 Hz, 2H), 3.62 (t, J = 7.2 Hz, 2H), 4.31 (s, 2H), 6.53 (d, J = 8.0 Hz, 1H), 6.67 (br s, 1H, NH), 6.73 (s, 1H), 6.94 (d, J = 8.0 Hz, 1H), 7.13 (dd, J = 8.0, 8.0 Hz, 4H); HRMS calcd for $C_{18}H_{20}N_2O$ *m/z* 280.1576, found 280.1569.

6.1.3.5. 3-(4-Isopropylphenethyl)-6-methyl-3,4-dihydroquinazolin-2(1*H*)-one (7e). Yellow solid; yield 79%; purity 97.65%; mp 143–145 °C; IR (neat) 3171, 2926, 2856, 1655, 1456 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (d, J = 6.8 Hz, 6H), 2.25 (s, 3H), 2.88 (m, 3H), 3.61 (t, J = 7.6 Hz, 2H), 4.30 (s, 2H), 6.56 (d, J = 8.0 Hz, 1H), 6.74 (s, 1H), 6.96 (d, J = 8.4 Hz, 1H), 7.26 (m, 4H), 7.32 (br s, 1H); HRMS calcd for $C_{20}H_{24}N_2O$ *m/z* 308.1889, found 308.1878.

6.1.3.6. 3-(4-Chlorophenethyl)-6-methyl-3,4-dihydroquinazolin-2(1*H*)-one (7f). Yellow solid; yield 63%; purity 96.0%; mp 140–142 °C; IR (neat) 3193, 2923, 2855, 1660, 1506 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.23 (s, 3H), 2.93 (t, J = 7.2 Hz, 2H), 3.63 (t, J = 7.2 Hz, 2H), 3.43 (s, 2H), 6.59 (d, J = 8.4 Hz, 1H), 6.79 (s, 1H), 7.09 (d, J = 8.4 Hz, 1H), 7.22–7.32 (m, 4H), 7.44 (br s, 1H); HRMS calcd for $C_{17}H_{17}ClN_2O$ *m/z* 300.1029, found 300.1017.

6.1.3.7. 3-(2,4-Dichlorophenethyl)-6-methyl-3,4-dihydroquinazolin-2(1*H*)-one (7g). White solid; yield 69%; purity 95.07%; mp 151–153 °C; IR (neat) 2926, 1855, 1661, 1461 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.43 (s, 1H), 3.14 (t, J = 7.2 Hz, 2H), 3.73 (t, J = 6.8 Hz, 2H), 4.99 (s, 2H), 7.03 (d, J = 8.4 Hz, 1H), 7.46 (m, 2H), 7.60 (br s, 1H), 7.67 (s, 1H), 7.77 (m, 2H), 8.05 (d, J = 8.4 Hz, 1H); HRMS calcd for $C_{17}H_{16}Cl_2N_2O$ *m/z* 334.0640, found 334.0622.

6.1.3.8. 6-Methyl-3-(3-phenylpropyl)-3,4-dihydroquinazolin-2(1*H*)-one (7h). White solid; yield 59%; purity 98.30%; mp 137–139 °C; IR (neat) 3193, 2925, 1662, 1492 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.91–1.99 (m, 2H), 2.27 (s, 3H), 2.67 (t, J = 7.6 Hz, 2H), 3.48 (t, J = 7.6 Hz, 2H), 4.37 (s, 2H), 6.53 (d, J = 8.0 Hz, 1H), 6.66 (br s, 1H), 6.83 (s, 1H), 6.95 (d, J = 6.8 Hz, 1H), 7.16–7.29 (m, 5H); HRMS calcd for $C_{18}H_{20}N_2O$ *m/z* 280.1576, found 280.1559.

6.1.3.9. 6-Methyl-3-(4-phenylbutyl)-3,4-dihydroquinazolin-2(1*H*)-one (7i). White solid; yield 57%; purity 99.20%; mp 129–131 °C; IR (neat) 3193, 2926, 2856, 1665, 1509 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.65–1.71 (m, 4H), 2.24 (s, 3H), 2.65 (t, J = 7.2 Hz, 2H), 3.44 (t, J = 7.2 Hz, 2H), 4.36 (s, 2H), 6.52 (d, J = 8.4 Hz, 1H), 6.65 (br s, 1H), 6.82 (s, 1H), 6.94 (d, J = 8.4 Hz, 1H), 6.96–7.28 (m, 5H); HRMS calcd for $C_{19}H_{22}N_2O$ *m/z* 294.1732, found 294.1721.

6.1.3.10. 6-Methyl-3-(2-cyclohexylethyl)-3,4-dihydroquinazolin-2(1*H*)-one (7j). White solid; yield 69%; purity 97.5%; mp 149–151 °C; IR (neat) 3048, 2915, 2847, 1665, 1501 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92–1.77 (m, 13H), 2.27 (s, 3H), 3.46 (t, J = 7.6 Hz, 2H), 4.38 (s, 2H), 6.53 (d, J = 8.0 Hz, 1H), 6.65 (br s, 1H), 6.85 (s, 1H), 6.9 (d, J = 8.0 Hz, 1H); HRMS calcd for $C_{17}H_{24}N_2O$ *m/z* 272.1889, found 272.1876.

6.1.3.11. 6-Methyl-3-(2-(naphthalen-2-yl) ethyl)-3,4-dihydroquinazolin-2(1*H*)-one (7k). Yellow solid; yield 57%; purity 95.08%; mp 179–181 °C; IR (neat) 2959, 2851, 1663, 1514 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.40 (s, 3H, CH_3), 3.14 (t, J = 7.2 Hz, 2H), 3.73 (t, J = 6.8 Hz, 2H), 4.99 (s, 2H), 7.034 (d, J = 8.4 Hz, 1H), 7.40–7.52 (m, 3H), 7.60 (br s, 1H), 7.67–7.84 (m, 5H), 8.05 (d, J = 8.4 Hz, 1H); HRMS calcd for $C_{21}H_{20}N_2O$ *m/z* 316.1576, found 316.1552.

6.1.3.12. 6-Chloro-3-phenethyl-3,4-dihydroquinazolin-2(1*H*)-one (7l). Yellow solid; yield 56%; purity 95.01%; mp 141–142 °C; IR (neat) 3290, 2924, 2856, 1665, 1604, 1493 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.95 (t, J = 7.6 Hz, 2H), 3.65 (t, J = 7.6 Hz, 2H), 4.35 (s, 2H), 6.59 (d, J = 8.4 Hz, 1H), 6.89 (br s, 1H), 6.91 (m, 2H), 7.10–7.30 (m, 5H); HRMS calcd for $C_{16}H_{15}ClN_2O$ *m/z* 286.0873, found 286.0856.

6.1.3.13. 6-Chloro-3-(4-methylphenethyl)-3,4-dihydroquinazolin-2(1*H*)-one (7m). Yellow solid; yield 72%; purity 97.62%; mp 156–158 °C; IR (neat) 3059, 2925, 2856, 1660, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.31 (s, 3H), 2.89 (t, J = 7.6 Hz, 2H), 3.63 (t, J = 7.6 Hz, 2H), 4.32 (s, 2H), 6.62 (d, J = 8.0 Hz, 1H), 6.80 (br s, 1H), 6.86–6.97 (m, 2H), 7.08 (d, J = 8.4 Hz, 2H), 7.13 (d, J = 8.0 Hz, 2H); HRMS calcd for $C_{17}H_{17}ClN_2O$ *m/z* 300.1029, found 300.1013.

6.1.3.14. 6-Chloro-3-(4-chlorophenethyl)-3,4-dihydroquinazolin-2(1*H*)-one (7n). Yellow solid; yield 56%; purity; 95.02%; mp 139–140 °C; IR (neat) 3190, 2926, 1663, 1595 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.83 (t, J = 7.2 Hz, 2H), 3.84 (t, J = 7.2 Hz, 2H), 4.35 (s, 2H), 6.63 (d, J = 8.0 Hz, 1H), 6.74 (br s, 1H), 6.89–6.96 (m, 2H), 7.10–7.29 (m, 5H); HRMS calcd for $C_{16}H_{14}Cl_2N_2O$ *m/z* 320.0483, found 320.0466.

6.1.3.15. 6-Chloro-3-(3-phenylpropyl)-3,4-dihydroquinazolin-2(1*H*)-one (7o). Yellow solid; yield 66%; purity 96.87%; mp 128–130 °C; IR (neat) 3198, 3057, 2925, 2857, 1666, 1490 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.92–2.00 (m, 2H), 2.56 (t, J = 7.6 Hz, 2H), 3.47 (t, J = 7.6 Hz, 2H), 4.42 (s, 2H), 6.64 (d, J = 8.0 Hz, 1H), 6.70 (br s, 1H), 6.91 (t, J = 7.6 Hz, 1H), 7.03 (d, J = 7.6 Hz, 1H), 7.14–7.27 (m, 5H); HRMS calcd for $C_{17}H_{17}ClN_2O$ *m/z* 300.1029, found 300.1017.

6.1.3.16. 6-Chloro-3-(4-phenylbutyl)-3,4-dihydroquinazolin-2(1*H*)-one (7p). Yellow solid; yield 62%; purity 98.24%; mp 147–148 °C; IR (neat) 3345, 2924, 2856, 1671, 1495 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.66 (t, J = 7. 6 Hz, 4H), 2.51–2.68 (m, 4H), 4.40 (s, 2H), 6.63 (d, J = 7.6 Hz, 1H), 6.78 (br s, 1H), 6.90 (t, J = 7.6 Hz, 1H), 7.00 (d, J = 7.6 Hz, 1H), 7.13–7.18 (m, 5H); HRMS calcd for $C_{18}H_{19}ClN_2O$ *m/z* 314.1186, found 314.1168.

6.1.3.17. 6-Chloro-3-(2-cyclohexylethyl)-3,4-dihydroquinazolin-2(1H)-one (7q). Yellow solid; yield 78%; purity 96.5%; mp 113–115 °C; IR (neat) 3202, 2921, 1667, 1601, 1481 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90–1.93 (m, 13H), 3.45 (t, J = 7.6 Hz, 2H), 4.29 (s, 2H), 6.65 (d, J = 7.6 Hz, 1H), 6.98 (br s, 1H), 7.02 (d, J = 7.6 Hz, 1H),

7.15 (d, J = 7.6 Hz, 1H); HRMS calcd for $C_{16}H_{21}ClN_2O$ *m/z*, 292.1342, found 292.1334.

6.1.4. General procedure for the synthesis of compounds 1 (a–q)

To the carbonyl compounds **7a–q** in anhydrous toluene (75 mL) was added Lawesson's reagent (1.2 equiv) under nitrogen atmosphere. The resulting solution was refluxed for overnight. The toluene was removed and the aqueous mixture was extracted with dichloromethane. The organic phase was washed twice with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give the crude product, which was then purified by flash column chromatography. In order to determine the purity, all compounds have tested HPLC analysis using C18 reverse phase column by the mobile phase of methanol and acetonitrile (1:1).

6.1.4.1. 6-Methyl-3-phenethyl-3,4-dihydroquinazoline-2(1*H*)-thione (1a)[22]. Light yellow solid; yield 47%; purity 96.72%; mp 175–177 °C; IR (neat) 3200, 3020, 2920, 1620, 1550–1480, cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.27 (s, 3H), 3.10 (t, J = 7.7 Hz, 2H), 4.12 (t, J = 7.7 Hz, 2H), 4.36 (s, 2H), 6.60–7.02 (m, 3H), 7.28 (m, 5H), 8.48 (br, NH); HRMS calcd for $C_{17}H_{18}N_2S$ *m/z* 282.1191, found 282.1174.

6.1.4.2. 7-Methyl-3-phenethyl-3,4-dihydroquinazoline-2(1*H*)-thione (1b). White solid; yield 44%; purity 96.12%; mp 181–183 °C; IR (neat) 3200, 3025, 1599, 1525, 1479 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.30 (s, 3H), 3.10 (t, J = 7.6 Hz, 2H), 4.12 (t, J = 7.6 Hz, 2H), 4.36 (s, 2H), 6.49 (s, 1H), 6.79 (d, J = 8.0 Hz, 1H), 6.89 (d, J = 8.0 Hz, 1H), 7.23–7.30 (m, 5H), 7.90 (br s, 1H); HRMS calcd for $C_{17}H_{18}N_2S$ *m/z* 282.1191, found 282.1176.

6.1.4.3. 5-Methyl-3-phenethyl-3,4-dihydroquinazoline-2(1*H*)-thione (1c). White solid; yield 45%; purity 99.20%; mp 128–130 °C; IR (neat) 3199, 3134, 2922, 1616, 1598, 1523 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.03 (s, 3H, Ar-CH_3), 3.12 (t, J = 7.6 Hz, 2H), 4.14 (t, J = 7.6 Hz, 2H), 4.30 (s, 2H), 6.49 (d, J = 8.0 Hz, 1H), 6.80 (d, J = 7.6 Hz, 1H), 7.07 (t, J = 8.0 Hz, 1H), 7.23–7.30 (m, 5H), 7.87 (br s, 1H); HRMS calcd for $C_{17}H_{18}N_2S$ *m/z* 282.1191, found 282.1174.

6.1.4.4. 6-Methyl-3-(4-methylphenethyl)-3,4-dihydroquinazoline-2(1*H*)-thione (1d). White solid; yield 44%; purity 95.95%; mp 135–138 °C; IR (neat) 3198, 2925, 2855, 1711, 1533 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.26 (s, 3H), 2.32 (s, 3H), 3.05 (t, J = 7.6 Hz, 2H), 4.08 (t, J = 7.2 Hz, 2H), 4.36 (s, 2H), 6.54 (d, J = 8.0 Hz, 1H), 6.72 (s, 1H), 6.99 (d, J = 8.4 Hz, 1H), 7.08 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 8.0 Hz, 2H), 7.83 (br s, 1H); HRMS calcd for $C_{18}H_{20}N_2S$ *m/z* 296.1347, found 296.1332.

6.1.4.5. 3-(4-Isopropylphenethyl)-6-methyl-3,4-dihydroquinazoline-2(1*H*)-thione (1e). White solid; yield 54%; purity 95.46%; mp 156–158 °C; IR (neat) 2926, 2855, 1493 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (d, J = 7.2 Hz, 6H), 2.26 (s, 3H), 2.29 (m, 1H), 3.03 (t, J = 7.6 Hz, 2H), 4.09 (t, J = 7.6 Hz, 2H), 4.54 (s, 2H), 6.56 (d, J = 8.0 Hz, 1H), 6.69 (s, 1H), 6.96 (d, J = 8.0 Hz, 1H), 7.13–7.21 (m, 4H), 7.90 (br s, 1H); HRMS calcd for $C_{20}H_{24}N_2S$ *m/z* 324.1660, found 324.1646.

6.1.4.6. 3-(4-Chlorophenethyl)-6-methyl-3,4-dihydroquinazoline-2(1*H*)-thione (1f). White solid; yield 54%; purity 95.15%; mp 157–159 °C; IR (neat) 3193, 2924, 2855, 1708, 1508 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.23 (s, 3H), 3.09 (t, J = 7.6 Hz, 2H), 4.11 (t, J = 7.2 Hz, 2H), 4.36 (s, 2H), 6.55 (d, J = 8.0 Hz, 1H), 6.72 (s, 1H), 6.97 (d, J = 8.4 Hz, 1H), 7.21–7.32 (m, 4H), 7.84 (br s, 1H); HRMS calcd for $C_{17}H_{17}ClN_2S$ *m/z* 316.0801, found 316.0789.

6.1.4.7. 3-(2,4-Dichlorophenethyl)-6-methyl-3,4-dihydroquinazoline-2(1*H*)-thione (1g). White solid; yield 39%; purity 96.23%; mp 160–161 °C; IR (neat) 3195, 2926, 2856, 1595, 1509 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.28 (s, 3H), 3.09 (t, J = 7.6 Hz, 2H), 4.09 (t, J = 7.6 Hz, 2H), 4.35 (s, 2H), 6.54 (d, J = 8.0 Hz, 1H), 6.72–6.84 (m, 2H), 6.97 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 8.4 Hz, 1H), 7.82 (br s, 1H); HRMS calcd for $C_{17}H_{16}Cl_2N_2S$ *m/z* 350.0411, found 350.0402.

6.1.4.8. 6-Methyl-3-(3-phenylpropyl)-3,4-dihydroquinazoline-2(1*H*)-thione (1h). White solid; yield 49%; purity 99.26%; mp 131–133 °C; IR (neat) 3189, 2925, 2856, 1496 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.04–2.13 (m, 2H), 2.26 (s, 3H), 2.73 (t, J = 7.6 Hz, 2H), 3.89 (t, J = 7.6 Hz, 2H), 4.43 (s, 2H), 6.60 (d, J = 8.0 Hz, 1H), 6.79 (s, 1H), 6.96 (d, J = 8.0 Hz, 1H), 7.09–7.28 (m, 5H), 8.24 (br s, 1H); HRMS calcd for $C_{18}H_{20}N_2S$ *m/z* 296.1347, found 296.1328.

6.1.4.9. 6-Methyl-3-(4-phenylbutyl)-3,4-dihydroquinazoline-2(1*H*)-thione (1i). White solid; yield 57%, purity 97.04%; mp 135–136 °C; IR (neat) 3191, 2927, 2857, 1534 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.63–1.87 (m), 2.27 (s, 3H), 2.69 (t, J = 7.2 Hz, 2H), 3.93 (t, J = 7.2 Hz, 2H), 4.44 (s, 2H), 6.54 (d, J = 8.0 Hz, 1H), 6.81 (s, 1H), 6.98 (d, J = 9.2 Hz, 1H), 7.16–7.32 (m, 5H), 7.79 (br s, 1H); HRMS calcd for $C_{19}H_{22}N_2S$ *m/z* 310.1504, found 310.1500.

6.1.4.10. 6-Methyl-3-(2-cyclohexylethyl)-3,4-dihydroquinazoline-2(1*H*)-thione (1j). White solid; yield 60%; purity 98.0%; mp 169–171 °C; IR (neat) 3733, 2922, 2852, 1538 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97–1.80 (m, 13H), 2.28 (s, 3H), 3.94 (t, J = 7.6 Hz, 2H), 4.47 (s, 2H), 6.54 (d, J = 8.4 Hz, 1H), 6.83 (s, 1H), 6.99 (d, J = 8.0 Hz, 1H), 7.79 (br s,1H); HRMS calcd for $C_{17}H_{24}N_2S$ *m/z* 288.1660, found 288.1646.

6.1.4.11. 6-Methyl-3-(2-(naphthalen-2-yl) ethyl)-3,4-dihydroquinazoline-2(1*H*)-thione (1k). White solid; yield 45%; purity 98.08%; mp 199–201 °C; IR (neat) 2922, 1551, 1463 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.23 (s, 3H), 3.25 (t, J = 7.6 Hz, 2H), 4.20 (t, J = 7.6 Hz, 2H), 4.34 (s, 2H), 6.54 (s, 1H), 6.56 (d, J = 8.0 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 7.41–7.46 (m, 3H), 7.68 (m, 2H), 7.78 (m, 2H), 7.91 (br s, 1H); HRMS calcd for $C_{21}H_{20}N_2S$ *m/z* 332.1347, found 332.1334.

6.1.4.12. 6-Chloro-3-phenethyl-3,4-dihydroquinazoline-2(1*H*)-thione (1l). White solid; yield 33%; purity 98.43%; mp 175–177 °C; IR (neat) 2926, 1473 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.10 (t, J = 7.6 Hz, 2H), 4.13 (t, J = 7.6 Hz, 2H), 4.38 (s, 2H), 6.65 (d, J = 8.0 Hz, 1H), 6. 91 (s, 1H), 6.97 (d, J = 8.0 Hz, 1H), 7.17–7.29 (m, 5H), 7.87 (br s, 1H); HRMS calcd for $C_{16}H_{15}ClN_2S$ *m/z* 302.0644, found 302.0627.

6.1.4.13. 6-Chloro-3-(4-methylphenethyl)-3,4-dihydroquinazoline-2(1*H*)-thione (1m). White solid; yield 27%; purity 95.03%; mp 153–154 °C; IR (neat) 3191, 2927, 1499 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.35 (s, 3H), 3.06 (t, J = 7.6 Hz, 2H), 4.08 (t, J = 6.4 Hz, 2H), 4.39 (s, 2H), 6.65 (d, J = 7.6 Hz, 1H), 6.95 (t, J = 8.0 Hz, 2H), 7.08 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 8.4 Hz, 2H), 7.90 (br s, 1H); HRMS calcd for $C_{16}H_{15}ClN_2S$ *m/z* 302.0644, found 302.0627.

6.1.4.14. 6-Chloro-3-(4-chlorophenethyl)-3,4-dihydroquinazoline-2(1*H*)-thione (1n). White solid; yield 42%, purity 98.03%; mp 157–159 °C; IR (neat) 3190, 2926, 2853, 1598 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.08 (t, J = 7.6 Hz, 2H), 4.10 (t, J = 7.6 Hz, 2H), 4.38 (s, 2H), 6.60 (d, J = 8.0 Hz, 1H), 6.89 (s, 1H), 6.98 (d, J = 8.0 Hz, 1H), 7.17–7.29 (m, 4H), 7.91 (br s, 1H); HRMS calcd for $C_{16}H_{14}Cl_2N_2S$ *m/z* 336.0255, found 336.0244.

6.1.4.15. 6-Chloro-3-(3-phenylpropyl)-3,4-dihydroquinazoline-2(1*H*)-thione (1o). White solid; yield 56%; purity 95.04%; mp 166–167 °C; IR (neat) 3171, 2928, 2857, 1723, 1594 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.61–1.80 (m, 2H), 2.69 (t, J = 7.2 Hz, 2H), 3.94 (t, J = 7.2 Hz, 2H), 4.48 (s, 2H), 6.65 (d, J = 8.0 Hz, 1H), 6.80 (s, 1H), 6.85 (d, J = 8.0 Hz, 1H), 6.92–7.29 (m, 5H), 7. 96 (br s, 1H); HRMS calcd for $C_{17}H_{17}ClN_2S$ *m/z* 316.0801, found 316.0792.

6.1.4.16. 6-Chloro-3-(4-phenylbutyl)-3,4-dihydroquinazoline-2(1*H*)-thione (1p). White solid; yield 46%; purity; 97.0%; mp 172–174 °C; IR (neat) 3171, 2928, 2857, 1594 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60–1.80 (m, 4H), 2.67 (t, J = 7.2 Hz, 2H), 3.98 (t, J = 7.2 Hz, 2H), 4.48 (s, 2H), 6.64 (d, J = 8.0 Hz, 1H), 6.85 (s, 1H), 6.91–7.33 (m, 6H), 7.90 (br s, 1H); HRMS calcd for $C_{18}H_{19}ClN_2S$ *m/z* 330.0957, found 330.0943.

6.1.4.17. 6-Chloro-3-(cyclohexylethyl)-3,4-dihydroquinazoline-2(1*H*)-thione (1q). White solid; yield 63%; purity 97.8%; mp 148–150 °C; IR (neat) 3191, 2920, 1605, 1493 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95–1.77 (m, 13H), 3.97 (t, J = 7.6 Hz, 2H), 4.52 (s, 2H), 6.67 (d, J = 7.6 Hz, 1H), 7.01 (s, 1H), 7.17 (d, J = 8.0 Hz, 1H), 7.99 (br s, 1H); HRMS calcd for $C_{16}H_{21}ClN_2S$ *m/z* 308.1114, found 308.11012.

6.2. Biological evaluation

6.2.1. 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).

6.2.2. 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.

6.2.3. 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.

6.2.4. 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. 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/min. One unit of tyrosinase activity was defined as the conversion of 1 nmol dopa to dopachrome/min.

6.2.5. 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).

6.2.6. 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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