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Refinement of the pharmacophore of 3,4-dihydroquinazoline-2(1*H*)-thiones for their anti-melanogenesis activity

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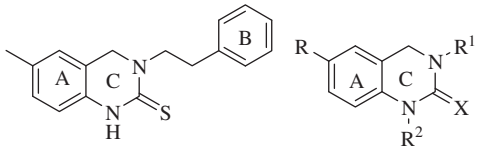
ABSTRACT

In order to define the structural requirements of quinazoline-2(1*H*)-thiones **1** for their inhibitory activity on melanogenesis, a novel series of 3,4-dihydroquinazoline-2(1*H*)-thiones (**3a**–**h**) were prepared and screened for their melanogenesis inhibition on melanoma B16 cell line under the stimulant of α-MSH. The anti-melanogenesis activity of **3** is mainly mediated by the hydrogen bonding ability of thioamide unit in addition to complexation ability of thione and the hydrophobic binding power of side chain substitutions at 3-position. Thus, the pharmacophore of 3,4-dihydroquinazoline-2(1*H*)-thiones for their anti-melanogenesis activity could be refined as 3-hydrophobic substituted quinazolinethione.

In human, skin is the main barrier from external environment, and relies on melanocytes to provide photoprotection by producing melanin through the process known as melanogenesis. Chemically, melanin is heterogeneous biopolymers yielded from the combination of enzymatic and chemical reaction in melanocytes.[1,2] Melanin synthesized from the oxidation of tyrosine to dopaquinone by tyrosinase, which occurs in two steps: hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and then oxidation of the latter to an *o*-quinone (dopaquinone).[3] This first step is the key step in melanogenesis because the reminder of the reaction sequence can proceed spontaneously at physiological pH.[4] Tyrosinase, a copper bearing phenolase, not only involves in abnormal production of melanin (hyperpigmentation)[5,6] but also has been reported to be linked with Parkinson disease and related neurodegenerative diseases.[7,8] In addition, the well known whitening agents such as hydroquinone-*O*-β-glucopyranoside (arbutin)[9–11] and kojic acid[12] are considered as harmful agents due to their undesirable side effects.[13] Taken together the current therapies are considered to be inadequate for the skin treatment despite many compounds have been reported as tyrosinase inhibitor.[14–17] These reports induced researchers to seek new potent tyrosinase inhibitors. Therefore, to meet the current demand for more safer whitening agents, we discovered compound **1**, (6-methyl-3-phenethyl-3,4-dihydro-1*H*-quinozoline-2-thione, IC_{50} = 0.8 μM, Chart 1) that inhibited α-melanocyte stimulated hormone (α-MSH) induced melanogenesis in melanoma B16 cell line.[18] This is one of the important approaches to inhibit melanogenesis by down regulation of tyrosinase expression other than changing the tyrosinase catalytic activity. These results encourage us to go for more detailed structure–activity relationship (SAR) of the compound **1** to get more potent and safer whitening agents. Thus, the current research focused on the design and synthesis of number of derivatives of compound **1**. In our previous studies,[19,20] we discovered the following structural requirements for our lead **1** regarding (i) role of ring A and their substitutions, (ii) optimum size of the ring C (thiourea unit) and (iii) nature of the substituents on ring C. However, the exact requirements of ring C (thiourea unit) and the substituents on ring C in **1** have not been fully explored. Thus, a novel series of quinazolines **2**, **3** and **4** (Chart 1) were synthesized and their inhibitory activity were measured against melanogenesis in melanoma B 16 cells under the stimulant of α-MSH.



1 **2**, **3** and **4**

Chart 1. Melanogenesis inhibitors **1**, **2**, **3** and **4**.

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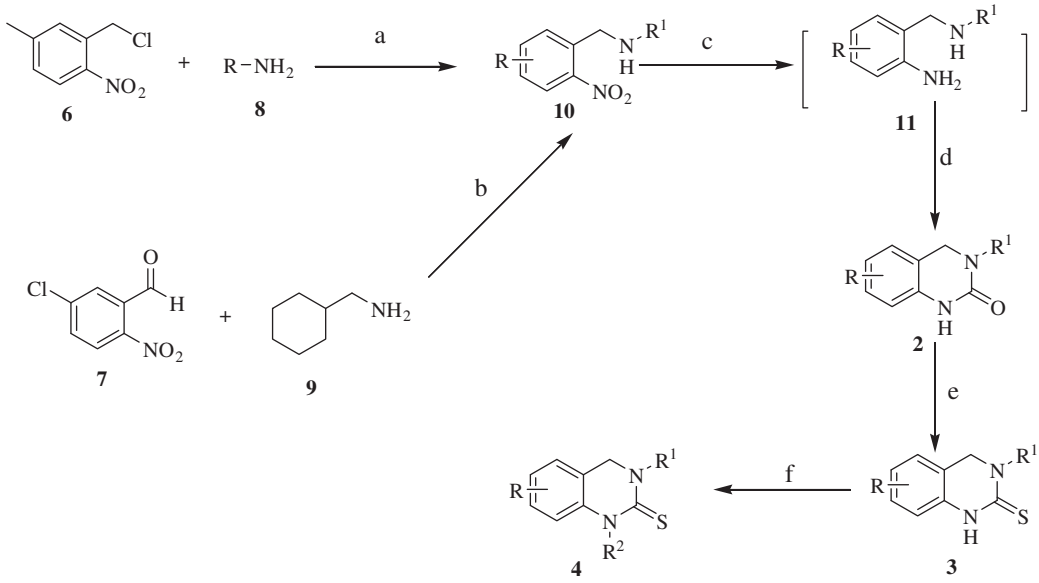
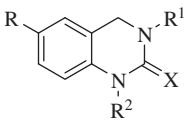


Scheme 1. Preparation of **2**, **3** and **4**. Reagents and conditions: (a) TEA, CH_2Cl_2, reflux, overnight; (b) TEA, $NaBH_4$, methanol, reflux, 4 h; (c) 10% Pd/C, 30-psi, rt, 5 h; (d) 1,1-carbonyldiimidazole, THF, reflux, overnight; (e) Lawesson's reagent, toluene, reflux, overnight; (f) KOH, tetra-(*n*-butyl)ammonium bromide.

Table 1
The substituents of **2**, **3** and **4**



Compd	R	R^1	R^2	X	Yield[b] (%)	Mp (°C)
2a	CH_3	$C(CH_3)_2$	H	O	80	166–167
2b	CH_3	$CH_2C(CH_3)_2$	H	O	83	160–161
2c	CH_3	$CH_2CH_2C(CH_3)_2$	H	O	85	143–144
2d	CH_3	$(CH_2)_5CH_3$	H	O	75	150–152
2e	CH_3	$(CH_2)_6CH_3$	H	O	82	158–160
2f	CH_3	$CH_2C_6H_{11}$	H	O	90	162–163
2g	Cl	$CH_2C_6H_{11}$	H	O	85	166–167
2h[a]	H	H	H	O	95	231–233
3a	CH_3	$C(CH_3)_2$	H	S	60	157–158
3b	CH_3	$CH_2C(CH_3)_2$	H	S	55	168–169
3c	CH_3	$CH_2CH_2C(CH_3)_2$	H	S	63	169–170
3d	CH_3	$(CH_2)_5CH_3$	H	S	68	179–181
3e	CH_3	$(CH_2)_6CH_3$	H	S	70	175–177
3f	CH_3	$CH_2C_6H_{11}$	H	S	55	185–186
3g	Cl	$CH_2C_6H_{11}$	H	S	52	169–161
3h[a]	H	H	H	S	65	199–201
4a	CH_3	C $(CH_3)_2$	CH_3	S	73	129–131
4b	CH_3	$CH_2C(CH_3)_2$	CH_3	S	70	137–139

[a] Compounds **2h** and **3h** are reported with following Refs. 23,25.
[b] The yield of **4**(**a**–**b**) was obtained from the corresponding cyclic thiourea **3**(**a**–**b**), **3**(**a**–**h**) form **2**(**a**–**h**) and **2**(**a**–**h**) from an intermediate **11**.

The detailed chemistry[20] involved in synthesis of **2**, **3** and **4** as shown in Scheme 1. The alkylation of amine **8** with *o*-nitrobenzyl chloride **6** yielded key intermediate **10**. On the other hand, the cyclohexylmethylamine **9** treated with aldehyde **7** to produce **10** by reductive amination.[21,22] This nitro compound **10** was underwent reduction with 10% Pd/C under 30 psi hydrogen atmosphere at room temperature for 5 h to get the intermediate **11**[20] which was used without further purification. Compound **2** obtained by the cyclization of **11** using 1,1-carbonyldiimidazole,[18,23] which was further treated with Lawesson's reagent[24–26] in toluene to get corresponding thio analogs **3**. Finally, N-methylated analog **4** was obtained from **3** using methyl iodide in the presence of potassium hydroxide.[27] Various substituents and the physiochemical properties of **2**, **3** and **4** are indicated in Table 1.

All the above synthesized derivatives were evaluated for their inhibitory activity on melanogenesis[19,20] using α-melanocyte stimulated hormone (α-MSH) induced melanogenesis in melanoma B16 cell line as shown in Table 2. Quinazolinethiones **3a**–**g** (100% inhibition at 10 μM and their IC_{50} values: ranged from 1.2 to 3.0 μM) containing thiourea motif showed strong inhibition against melanogenesis.[20] However, quinazolinones **2a**–**h** containing urea unit do not exhibit the activity. This trend has also been noticed in earlier reports,[28,29] as indicated that phenylthiourea (PTU, 58% inhibition at 100 μM) showed better inhibition than phenylurea (0% inhibition at 100 μM), though the mechanism of PTU activity (IC_{50} value for tyrosinase inhibition: 1.8 μM) was quite different from quinazolinethiones **3** and **4**.

In the first set of experiment, we studied the role of ring B in the SAR of lead **1**. The resulting synthesized compounds **3a** and **3b** by replacing ring B with aliphatic groups as the isopropyl analog **3a** (>100% inhibition at 10 μM, IC_{50} = 1.9 μM, *C* log *P* = 3.030) and isobutyl **3b** (>100% inhibition at 10 μM, IC_{50} = 1.9 μM, *C* log *P* = 3.649) exhibited similar activity to **1**. This study revealed that the phenyl group (ring B) can be replaced by an aliphatic chain to maintain the activity. These results motivated us to investigate the effects of volume or lipophilicity on the activity by varying the sizes of alkyl groups as side chain of **3**. Thus, more bulky aliphatic groups were introduced at this position such as isopentyl **3c** (>100% inhibition at 10 μM, IC_{50} = 3.0 μM, *C* log *P* = 4.178), *n*-hexyl **3d**, (>100% inhibition at 10 μM, IC_{50} = 1.2 μM, *C* log *P* = 4.837), *n*-heptyl **3e** (>100% inhibition at 10 μM, IC_{50} = 1.4 μM, *C* log *P* = 5.366) and cyclohexyl methyl **3f** (>100% inhibition at 10 μM, IC_{50} = 1.6 μM, *C* log *P* = 4.842) as a replacement of ring B. From these results, we observed that there was not much change in activity along with the variation of alkyl chain. These outcomes not only prove that the ring B can be replaced by aliphatic groups but also the chain length of among these groups dose not have influence on the activity of quinazolinethiones. Thus, the side chain of **1** and **3** may have a panhandle effect for proper arrangement of the molecules by their binding to hydrophobic pocket in the binding site rather than controller of lipophilicity (*C* log *P* values in Table 2).

Table 2
The inhibitory activity of **2**, **3** and **4** on melanogenesis using α-melanocyte stimulated hormone (α-MSH) induced melanogenesis in melanoma B16 cell line

Compd	Inhibition at 10 μM (%)	IC_{50}[a] (μM)	*C* log *P*[b]	Compd	Inhibition at 10 μM (%)	IC_{50}[a] (μM)	*C* log *P*[b]
2a	13	>10	2.370	**3a**	>100	1.9	3.030
2b	11	>10	2.989	**3b**	>100	1.9	3.649
2c	15	>10	3.518	**3c**	>100	3.0	4.178
2d	12	>10	4.177	**3d**	>100	1.2	4.837
2e	19	>10	4.706	**3e**	>100	1.4	5.366
2f	18	>10	4.182	**3f**	>100	1.6	4.842
2g	10	>10	4.6984	**3g**	>100	1.2	5.056
2h	11	>10	0.877	**3h**	36	15.8	0.657
1	>100	0.8		**4a**	10	19.0	3.561
Arbutin		180		**4b**	18	17.8	4.180

[a] IC_{50} values are taken as mean from three independent experiments.
[b] *C* log P values were calculated by Chem Draw 9.0 v.

In our previous study[20] we observed the importance of ring B of **1** as well as substitution on ring A for their activity. In order to confirm this fact, we synthesized an analog **3h** (36% inhibition at 10 μM, IC_{50} = 15.8 μM, *C* log *P* = 0.657) with omission of side chain and substitution on ring A of **1**. As expected, the analog **3h** showed very low inhibition compared to **1**. This result strongly implies that its low melanogenesis inhibition may be due to lack of binding to the hydrophobic pocket of putative receptor. This also proves that the panhandle effect of the side chain of **1** and **3** is important for their activity.

Finally, we introduced the methyl group at 1-position of quinazolinethiones. The resulting analogs **4a** (10% inhibition at 10 μM, IC_{50} = 19.0 μM, *C* log *P* = 3.561) and **4b** (18% inhibition at 10 μM, IC_{50} = 17.8 μM, *C* log *P* = 4.180) showed poor activity compared to their corresponding analogs **3a** and **3b**. This is also one of the important finding for SAR of quinazolinethiones. The loss of activities in analogs **4a** and **4b** might be originated from steric congestion at this position for their binding in appropriate region or the loss of hydrogen bonding property of –NH group. Thus, the free 1-NH group of **3** also has an important role for the inhibition of melanogenesis activity in melanoma B16 cells under the stimulant of α-MSH.

We also studied the electronic effect of the substituent at ring A by replacing the methyl group in **3f** with chloro as shown in **3g** (>100% inhibition at 10 μM, IC_{50} = 1.2 μM) dose not alter the activity. This result implies that the 3,4-dihydroquinazoline-2(1*H*)-thiones are only the sole pharmacophore for the melanogenesis inhibition.

In summary, a novel series of 3,4-dihydroquinazoline-2(1*H*)-thiones were prepared, screened for their melanogenesis inhibition on melanoma B16 cell line under the stimulant of α-MSH and structure features responsible have been identified. The SAR studies revealed that the anti-melanogesis activity of **3** is mainly mediated by the hydrogen bonding ability of thioamide unit in addition to complexation ability of thione and the hydrophobic binding power of side chain substitutions at 3-position. Thus, the pharmacophore of 3,4-dihydroquinazoline-2(1*H*)-thiones **1** and **3** for their anti-melanogenesis activity could be refined as 3-hydrophbic substituted quinazolinethione.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.123.

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