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Structure–activity relationship of naphthaldehydethiosemicarbazones in melanogenesis inhibition

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ABSTRACT

2-(Naphthalen-1-ylmethylene)hydrazinecarbothioamide (**14**, IC_{50} = 1.1 μM) was discovered as a highly potent inhibitor of melanogenesis. To define the role of hydrogens (at N1 and N3) and sulfur in **14**, a series of analogs **15a**–**p** were synthesized and evaluated for anti-melanogenic activity using melanoma B16 cells under the stimulus of α-MSH. It was observed that replacement of either of these hydrogens at N1 or N3 by substituents increases the activity significantly. Conversely, concomitant substitutions decrease the inhibitory potency. In addition, the presence of sulfur in thiosemicarbazone is essential for the activity.

Eumelanin and pheomelanin are the two basic types of melanin pigments in human skin and hair. Although melanin has a major role to protect the human skin from sun-related injuries, excess accumulation of melanin in skin can causes diverse hyperpigmentary disorders such as melasma, freckles and age spot.[1,2] Melanin is synthesized by a series of enzymatic catalyzed and chemical reactions.[3,4] Mainly, three melanocyte-specific enzymes such as tyrosinase, tyrosine related protein-1 (TYRP-1) and TYRP-2 are involved in the transformation of tyrosine to melanin pigments.[5] It has been reported that several factors affect the activity of these enzymes and related proteins, which includes melanocyte stimulating hormone (MSH), endothelin-1, basis fibroblast growth factor, and UV-irradiation.[6]

Tyrosinase, a copper bearing multifunctional enzyme, is involved in two rate limiting steps of melanin synthesis, that is, the hydroxylation of tyrosine and oxidation of 2,4-dihydroxyphenylalanine (L-DOPA).[7] Many of the presently known inhibitors act directly or indirectly on this enzyme.[8,9] Chemical agents including natural products (Fig. 1A) such as hydroquinones **1**,[10] kojic acid **2**,[11] tropolone **3**,[12] L-minosine **4**,[13] hydroxylated stilbine derivatives **5**,[14,15] flavonols derivatives **6**,[16] methyl ester of gentisic acid **7**,[17,18] and synthetic agents (Fig. 1B) such as *N*-phenylthiourea derivatives **8**,[19,20] kojic acid derivatives **9**,[21] oxadiazole **10**,[22] oxazolones **11**,[23] tetraketone derivative **12**[24] and 4,4′-dihydroxybiphenyl compound **13**[25] have been reported for the treatment of hyperpigmentation disorders. However, due to adverse effects such as depigmentation,[21] cytotoxicity,[26] paradoxical hyperpigmentation[10] dermatitis and erythema,[27] their use as cosmetic agents is being compromised. Therefore, there is a need to find ideal hypopigmenting agents with fewer or no side effects.

In the course of our studies to discover new hypopigmenting agents, we have previously demonstrated the structural requirements of a series of phenylthiourea (PTU) and thiosemicarbazone analogs[28,29] as the subclass of synthetic melanogenesis inhibitors. Accordingly, π-planar connection to thiourea unit without steric hindrance was identified as a main structural requirement in PTU derivatives.[28] Conversely, in case of thiosemicarbazones the aromatic group of thiosemicarbazones can be replaced with sterically bulky carbocycles. Unlike PTU derivatives, steric bulkiness does not hamper the activity of these thiosemicarbazones. Thus, hydrophobicity of the benzylidene or cyclohexylmethylene group on hydrazine of thiosemicarbazones is determinant factor for their inhibitory activity in melanogenesis rather than planarity.[29] However, the role of hydrogens at N1 and N3, and sulfur in thiosemicarbazone has not been studied yet. In the present study, we selected the compound 2-(naphthalen-1-ylmethylene)hydrazinecarbothioamide (**14**, IC_{50} = 1.1 μM, Fig. 2)[29] as a lead, and investigated the structure–activity relationship by preparing a series of analogs (**15a**–**p**) by replacing either of hydrogens or sulfur (Fig. 2) and evaluating their anti-melanogenesis activity in melanoma B16 cells under the stimulus of α-melanocyte stimulating hormone (α-MSH).

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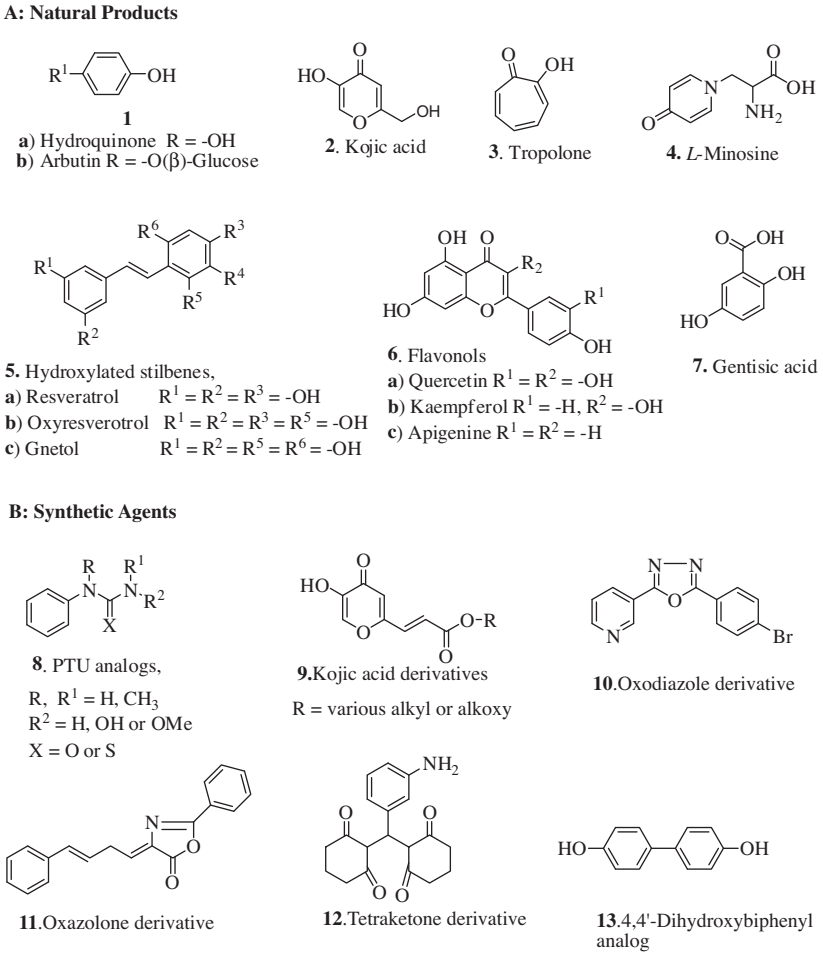


Figure 1. Known natural (A) and synthetic (B) tyrosinase inhibitors.

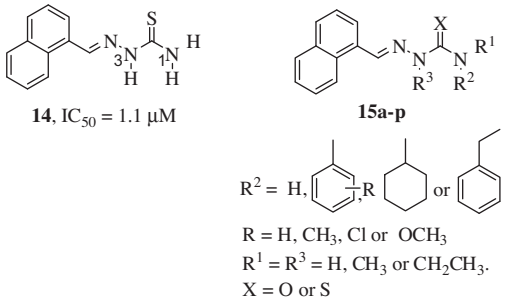
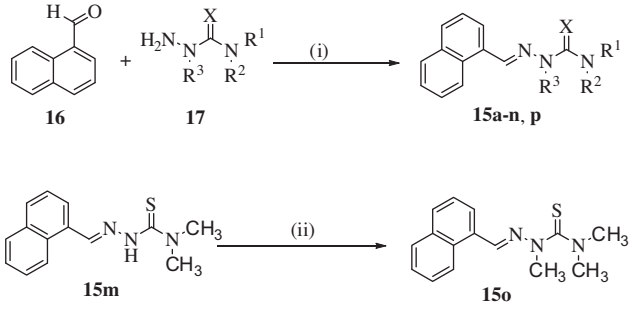


Figure 2. The structures of lead **14** and their newly designed melanogenesis inhibitors **15a–p**.

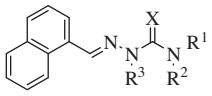


Scheme 1. Synthesis of melanogenesis inhibitors **15a–p**. Regents and condition: (i) ethanol/water (1:1), reflux, 50 min; (ii)CH_3I/KOH, rt. *Note:* Substituents (R^1, R^2, R^3 and X) are indicated in Table 1.

Compounds **15a–p** were prepared as depicted in Scheme 1. Briefly, a hot solution of aldehyde **16** in ethanol was added to the pre-hot solution of appropriate thiosemicarbazide **17**[30–34] in water (equal volume of ethanol) and this mixture was allowed to stir for nearly 50 min at 50–60 °C. The reaction mixture was then cooled to 5–10 °C to precipitate and the resulting thiosemicarbazone compounds **15a–n** were filtered.[35–40] Compound **15o** was synthesized by the treatment of **15m** with methyl iodide in presence of KOH and *tetra*-butyl ammonium bromide in tetrahydrofuran at room temperature for overnight.[41] All thiosemicarbazones **15a–o** were then purified by column chromatography to give the pure compounds. Semicarbazone **15p** was prepared using the same procedure used for the preparation of **15a** with semicarbazide instead of thiosemicarbazide.

As described previously,[42,43] biological assay was performed to determine the ability of compounds **15a–p** to inhibit the formation of melanin in melanoma B16 cells under the stimulus of α-MSH (100 nM) for 3 day incubation. Melanoma B16 cells (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. Data for % inhibition at 10 μM and IC_{50} values are shown in Table 1.

Table 1
Melanogenesis inhibitory activity of **15a–p** in melonama B16 cell line



15a-p

Compound No:	N–R^1	N–R^2	N–R^3	X	% of Inhibition at 10 (μM)[a]	IC_{50} (μM)
15a[37]	$-CH_3$	–H	–H	S	>100	2.00
15b	$-CH_2CH_3$	–H	–H	S	>100	1.10
15c[38]	–Ph	–H	–H	S	>100	0.50
15d	–Ph (4-CH_3)	–H	–H	S	>100	0.58
15e	–Ph(4-Cl)	–H	–H	S	>100	1.90
15f[39]	–Ph(4-OCH_3)	–H	–H	S	>100	0.86
15g	–Ph(3-CH_3)	–H	–H	S	>100	0.45
15h	–Ph(3-OCH_3)	–H	–H	S	>100	0.83
15i	–Ph(2-CH_3)	–H	–H	S	>100	0.49
15j	–Ph(2-OCH_3)	–H	–H	S	>100	0.92
15k	$-CH_2Ph$	–H	–H	S	>100	0.27
15l	$-C_6H_{11}$	–H	–H	S	>100	0.29
15m	$-CH_3$	$-CH_3$	–H	S	>100	0.28
15n	–H	–H	$-CH_3$	S	>100	1.60
15o	$-CH_3$	$-CH_3$	$-CH_3$	S	76	8.60
15p[40]	–H	–H	–H	O	<10	>100
14[29]	–H	–H	–H	S	100	1.10
Kojic acid						70
Arbutin						180

Note: Substituent.
[a] IC_{50} values are taken as a mean from 3–5 independent experiments.

As shown in the Table 1, both kojic acid and arbutin were evaluated in our assay as control. The initial objective of our study was to investigate the role of hydrogens at N1 of **14**. Accordingly, replacement of one of the hydrogens by a methyl group as indicated in the analog **15a** (>100% inhibition at 10 μM, IC_{50} = 2.0 μM) exhibited comparable activity to **14**. Further, we introduced some more substituents like ethyl (**15b**, >100% inhibition at 10 μM, IC_{50} = 1.1 μM) or phenyl (**15c**, >100% inhibition at 10 μM, IC_{50} = 0.5 μM) and observed that phenyl analog **15c** had the most potent activity, among them. Therefore, replacement of *N*1-hydrogen by bulkier phenyl moiety is more appreciable for the activity enhancement.

In an attempt to further improve the potency, we then examined the effect of substituents on phenyl ring of **15c**. Accordingly, analog associate with *p*-substituent methyl (**15d**, >100% inhibition at 10 μM, IC_{50} = 0.58 μM) or chloro (**15e**, >100% inhibition at 10 μM, IC_{50} = 1.90 μM) or methoxy (**15f**, >100% inhibition at 10 μM, IC_{50} = 0.86 μM) exhibited equipotent activity to **15c**. This similar fashion was also observed in the case of *m*- and *o*-substituted analogs as *m*-methyl (**15g**, >100% inhibition at 10 μM, IC_{50} = 0.45 μM), *m*-methoxy (**15h**, >100% inhibition at 10 μM, IC_{50} = 0.83 μM), *o*-methyl (**15i**, >100% inhibition at 10 μM, IC_{50} = 0.49 μM), and *o*-methoxy (**15j**, >100% inhibition at 10 μM, IC_{50} = 0.92 μM). From these studies, it was clearly visualized that the substituent on phenyl ring of **15c** does not have any impact on the activity. On the other hand, replacement of planar phenyl ring with more bulky benzyl or cyclohexyl group resulted in 2–5-fold activity improvement as shown in the analogs **15k** (>100% inhibition at 10 μM, IC_{50} = 0.27 μM) and **15l** (>100% inhibition at 10 μM, IC_{50} = 0.29 μM). This enhancement of the activity might be originated from the increment of bulkiness or basicity at terminal nitrogen (N1) of 14.

In the next experiment, we demonstrated the inhibitory activity by replacing both hydrogens of **14** at N1. The resulting *N*,*N*-dimethyl analog **15m** (>100% inhibition at 10 μM, IC_{50} = 0.28 μM) showed nearly 5 and 10 fold more potent activity than **14** and mono-methyl-substituted **15a**, respectively. This outcome is again emphasizing the fact that the increment of hydrophobicity is important for the activity. Further, the necessity of *N*3-hydrogen in **14** was also explored by replacing with methyl substituent as shown in the analog **15n** (>100% inhibition at 10 μM, IC_{50} = 1.60 μM). This equipotent activity to **14** indicated that *N*3-hydrogen can also be replaced with substituent.

We then investigated the substituents for all hydrogens at a same time. As a result, the activity was dropped to low level as indicated in the analog **15o** (76% inhibition at 10 μM, IC_{50} = 8.60 μM). This low level of activity may occur from the steric congestion of the substituents at both the nitrogens N1 and N3, which block the binding ability of thiosemicarbazide moiety. On the other hand, the activity was totally mediated by the thiosemicarbazide moiety in **14**. As shown in the Table 1, an analog associated with semicarbazide unit in

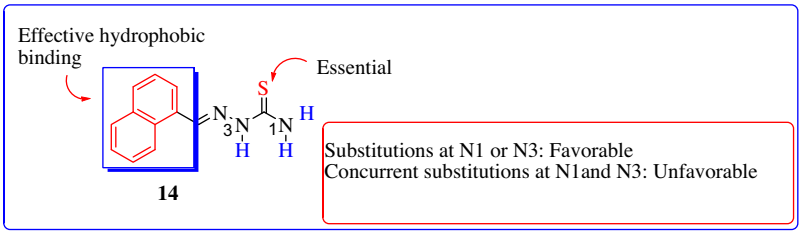


Figure 3. Structure–activity relationships of lead **14**.

15p (<10 inhibition at 10 μM, IC_{50} = >100 μM) exhibited complete loss of activity. Thus, the thiosemicarbazide unit in **14** is critical for the melanogenesis inhibitory activity.

In conclusion, a series of semicarbazides **15a**–**p** were designed, synthesized, and evaluated in for their anti-melanogenesis activity melanoma B16 cells under the stimulus of α-MSH. Systematic structural modifications provided many potent inhibitors with maximum fivefold improvement of activity compared to the lead **14**. The SAR studies indicated that either of these hydrogens at N1 or N3 can be replaced by a substituent. However, the concurrent substituents for all hydrogens are not appreciable. In addition, the thiosemicarbazide unit at **14** mainly mediates the activity. The summary of SARs of **14** has been depicted in Figure 3. A recent mechanistic study shows that these thiosemicarbazones are inhibiting melanogenesis without inhibiting the tyrosinase (data not shown). The detailed mechanism of action directed toward the identification of molecular target of these thiosemicarbazones as hypopigmenting agents are currently ongoing in our lab and will be reported in due course.

Supplementary data

Supplementary data (experimental procedure) associated with this article can be found, in the online version.

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