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# Structural characteristics of thiosemicarbazones as inhibitors of melanogenesis

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ABSTRACT

A series of thiosemicarbazones **2**(**e**–**s**) have been synthesized and studied their structure–activity relationship as melanogenesis inhibitor. Among them, (*Z*)-2-(naphthalen-1-ylmethylene)hydrazinecarbothioamide (**2q**, >100% inhibition at 10 μM, $IC_{50}$ = 1.1 μM, *C* log *P* = 3.039) showed the strongest inhibitory activity. Regarding their structure–activity relationship, the hydrophobic substituents regardless the position on phenyl ring of benzaldehyde thiosemicarbazones enhance the inhibitory activity. Furthermore, the aromatic group of benzaldehydethiosemicarbazones can be replaced with sterically bulky cyclohexyl. Thus, hydrophobicity of the aryl or alkyl group on hydrazine of thiosemicarbazones is determinant factor for their inhibitory activity in melanogenesis rather than planarity.



Tyrosinase, also known as phenoloxidase (PO) a copper bearing bifunctional enzyme is highly present in microorganism, animals, and plants.[1,2] It is the key enzyme involved in the synthesis of melanin through the process called melanogenesis. 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] The first one is the key step in melanogenesis because the reminder of the reaction sequence can proceed spontaneously at physiological pH.[4] Tyrosinase play an important role in human neuromelanin formation in the substantia nigra portion of the brain and it may also contribute to Parkinson disease-related to neurodegeneration.[5,6] In mammals, tyrosinase is primarily responsible for skin pigmentation defects and abnormalities such as flecks.[7] In insects, tyrosinase is uniquely associated with three different biochemical process, including sclerotization of cuticle, defensive encapsulation and melanization of foreign organism, and wound healing.[8] It is possible that inhibition of PO could lead to abrogation of insect defense mechanisms or abnormal body softening, both of which could be used in pest control. Thus, tyrosinase plays a quite significant role in various industries such as medicine,[9] agriculture, and cosmetic products,[10] primarily in relation to hyperpigmentation. The current available therapies are considered to be inadequate for the skin treatment despite many compounds have been reported as tyrosinase inhibitor.[11–14] Therefore, there is an urgent need for new molecules which can effectively be used for the skin treatment. In continuation for the search of good skin whitening agent, researchers have been identified that phenylthiourea (PTU, Chart 1) as one of the best tyrosinase inhibitors[15] with an $IC_{50}$ value of 1.8 μM.[16] Moreover, PTU was reported for the inhibition of tyrosinase enzyme that belongs to catechol oxidase (a type-3 copper protein).[17,18] The crystal structure of phenylthiourea (PTU) bound tyrosinase has not been explored yet. However, the catalytic sites of catechol oxidase and tyrosinase have been known to be almost similar, so we predict that binding mode of PTU at tyrosinase active site may be similar to catechol oxidase. The binding of sulfur atom of this compound to both copper ions in the active site of the enzyme[19] and the van der Waals interactions of phenyl group with the residues lining the hydrophobic cavity (Phe 261, Ile 241, His 244) contribute to the high affinity of the PTU to the enzyme.[19]

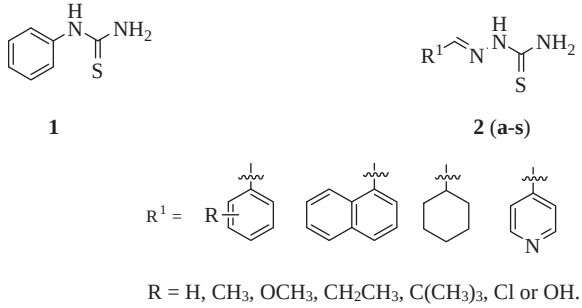


**Chart 1.** Antimelanogenesis agents: phenylthiourea **1** and thiosemicarbazones **2**(**a**–**s**).

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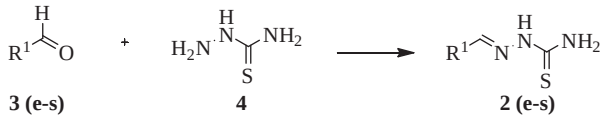



**Scheme 1.** Synthesis of thiosemicarbazone analogs. Reagents and conditions: (i) heat, 20 min, water/ethanol (1:1). Note: $R^1$ = substituents are located in Table 1.

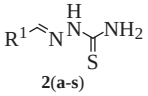
In our previous study,[20] we defined the structural requirements of phenylthiourea derivatives and some benzaldehydethiosemicarbazones **2a**–**d** for their inhibitory activities against melanogenesis in melanoma B16 cells. Interestingly, some of the compounds such as ethyl and *tert*-butyl derivatives of benzaldehydethiosemicarbazone showed very potent activity among the thiourea derivatives. Along with that, the π-planar structure to thiourea unit without steric hindrance in PTU derivatives is required for potent inhibitory activity. However, in case of thiosemicarbazones, a hydrophobic substituent at *p*-position is essential for the same. Thus, the detailed structure–activity relationship of thiosemicarbazones toward their inhibition of melanogenesis in melanoma B16 cells is required. Therefore, in the current study we prepared a series of thiosemicarbazones **2e–s** as shown in Chart 1 and evaluated their inhibitory activity against the formation of melanin in melanoma B16 cells.

All substituted thiosemicarbazones **2** were synthesized by the treatment of appropriate benzaldehyde with thiosemicarbazide as shown in Scheme 1.[20,21] Briefly; a hot (40–50 °C) solution of an appropriate aldehyde **3e–s** in ethanol (30 mL) was added to the pre-heated (40–50 °C) solution of thiosemicarbazide **4** in water (30 mL) and allowed to stir for 10–15 min at same temperature (40–50 °C). The reaction mixture was immediately cooled about to 5–10 °C and resulting title compound (**2e–s**)[22–29]was filtered. All the above-synthesized derivatives were tested for their inhibitory activity of melanogenesis in melanoma B16 cell line[30] as shown in Table 1 rather than tyrosinase itself since the purpose of these inhibitors is to reduce the formation of melanin.

As we discussed earlier,[20] the hydrophobic bulky substituent at *p*-position of benzaldehyde thiosemicarbazones play an important role in enhancing the activity of thiosemicarbazones. As compared to simple benzaldehyde thiosemicarbazone **2a** (<10% inhibition at 10 μM, $IC_{50}$ >0 μM, *C* log *P* = 1.865), the other bulky group containing compounds such as **2b** (64% inhibition at 10 μM, $IC_{50}$ = 6.8 μM, *C* log *P* = 2.364), **2c** (100% inhibition at 10 μM, $IC_{50}$ = 3.4 μM, *C* log *P* = 2.893) and **2d** (100% inhibition at 10 μM, $IC_{50}$ = 2.7 μM, *C* log *P* = 3.69) showed enhanced activity. However, the detailed study is required about the role of hydrophobic and hydrophilic groups at that position. Therefore, in the first set of experiment, we introduced the hydrophobic or hydrophilic groups at the same position as shown in halogen analog **2e** (92% inhibition at 10 μM, $IC_{50}$ = 4.2 μM, *C* log *P* = 2.802), methoxy analog **2f** (42% inhibition at 10 μM, $IC_{50}$ = 15.1 μM, *C* log *P* = 1.902) and hydroxyl analog **2g** (16% inhibition at 10 μM, $IC_{50}$ = 17.5 μM, *C* log *P* = 1.198). Hydrophobic substituted chloro analog enhanced the activity however, polar substituents like methoxy and hydroxy reduced the activity. These results imply that the bulky hydrophobic groups at *p*-position of benzaldehydethiosemicarbazones effectively enhance the inhibitory activity of melanogenesis on melanoma B16 cells.

After confirming the role of substitution at *p*-position of thiosemicarbazone, we shifted our focus toward *m*-substitution effect of thiosemicarbazones on activity. Interestingly, the hydrophobic substituents at *m*-position as shown in **2h** (>100% inhibition at 10 μM, $IC_{50}$ = 2.8 μM, *C* log *P* = 2.364) and **2i** (>100% inhibition at 10 μM, $IC_{50}$ = 2.4 μM, *C* log *P* = 2.802) showed almost same activity as *p*-substituted derivatives. As indicated in *p*-derivatives **2f** and **2g**, the electron donating substituent at *m*-position such as **2j** (40% inhibition at 10 μM, $IC_{50}$ = 15.4 μM, *C* log *P* = 1.920) and **2k** (28% inhibition at 10 μM, $IC_{50}$ = 24 μM, *C* log *P* = 1.198) also showed the poor activity.

**Table 1**
Inhibitory activity of thiosemicarbazones **2a–s** against melanogenesis in melanoma B16 cells




| Entry No. | Compd No. | $R^1$ | Inhibition at 10 μM (%)[b] | $IC_{50}$ values[j] | *C* log *P*[k] |
|---|---|---|---|---|---|
| 1 | **2a**[a] | $C_6H_4$ | <10 | >10 | 1.865 |
| 2 | **2b**[a] | *p*-$(CH_3)C_6H_4$ | 64 | 6.8 | 2.364 |
| 3 | **2c**[a] | *p*-$(CH_2CH_3)C_6H_4$ | >100 | 3.4 | 2.893 |
| 4 | **2d**[a] | *p*-$(C(CH_3)_3)C_6H_4$ | >100 | 2.7 | 3.691 |
| 5 | **2e**[b] | *p*-$(Cl)C_6H_4$ | 92 | 4.2 | 2.802 |
| 6 | **2f**[b] | *p*-$(OCH_3)C_6H_4$ | 42 | 15.1 | 1.920 |
| 7 | **2g**[b] | *p*-$(OH)C_6H_4$ | 16 | 17.5 | 1.198 |
| 8 | **2h**[b] | *m*-$(CH_3)C_6H_4$ | >100 | 2.8 | 2.364 |
| 9 | **2i**[c] | *m*-$(Cl)C_6H_4$ | >100 | 2.4 | 2.802 |
| 10 | **2j**[b] | *m*-$(OCH_3)C_6H_4$ | 40 | 15.4 | 1.920 |
| 11 | **2k**[b] | *m*-(OH) $C_6H_4$ | 28 | 24 | 1.198 |
| 12 | **2l**[d] | *o*-$(CH_3)C_6H_4$ | >100 | 3.4 | 2.364 |
| 13 | **2m** | *o*-$(CH_2CH_3)C_6H_4$ | >100 | 2.7 | 2.899 |
| 14 | **2n**[e] | *o*-$(Cl)C_6H_4$ | >100 | 1.4 | 2.802 |
| 15 | **2o**[f] | *o*-$(OCH_3)C_6H_4$ | 52 | 16.1 | 1.920 |
| 16 | **2p**[g] | *o*-$(OH)C_6H_4$ | 24 | 21 | 1.198 |
| 17 | **2q**[h] | Naphthyl | >100 | 1.1 | 3.039 |
| 18 | **2r** | Cyclohexyl | 81 | 3.4 | 1.987 |
| 19 | **2s**[i] | Pyridyl | 15 | >30 | 1.088 |

*Note:* [a]**2(a–d)**[20], [b]**2(e–h, j–k)**[19], [c]**2i**[23], [d]**2l**[24], [e]**2n**[25], [f]**2o**[26], [g]**2p**[27], [h]**2q**[28] and [i]**2s**[29] are consistent with references. [j]$IC_{50}$ values are taken as mean from three independent experiments. [k]*C* log *P* values are calculated by Chemdraw ultra 9.0v.

Unlike in PTU derivatives,[20] the *o*-methyl substituted thiosemicarbazone **2l** (>100% inhibition at 10 μM, $IC_{50}$ = 3.4 μM, *C* log *P* = 2.364) showed almost comparable activity to *p*-(**2b**) and *m*-(**2h**) substituted derivatives. These results encourage us to go for detail study regarding the substituent effect at *o*-position of thiosemicarbazones on melanogenesis inhibition. Thus, some more analogs with bulky substituents were synthesized such as *o*-ethyl (**2m**, >100% inhibition at 10 μM, $IC_{50}$ = 2.7 μM, *C* log *P* = 2.893) and *o*-chloro analogs (**2n**, >100% inhibition at 10 μM, $IC_{50}$ = 1.4 μM, *C* log *P* = 2.802). As indicated in Table 1, they also turned up with good inhibition against melanogenesis as potent as *p* and *m* bulky substituted analogs. This outcome supports that the substituents at *o*-position did not disturb the 'clamp structure' (H–N–C–N–H)[31] of thiourea unit which has been known as essential structure for activity. However, the electron donating substituents at *o*-position as shown in **2o** (52% inhibition at 10 μM, $IC_{50}$ = 16.1 μM, *C* log *P* = 1.920) and **2p** (24% inhibition at 10 μM, $IC_{50}$ = 21 μM, *C* log *P* = 1.198) exhibited very low inhibition similarly as we discussed above in case of *p*- and *m*-positions.

The above findings suggest that the hydrophobic or bulky substituents at *p*- or *m*- or *o*-position play an important role for the increment of the activity of thiosemicarbazones. Conversely, the hydrophilic or electron donating groups at *p*- or *m*- or *o*-position of thiosemicarbazones were not favorable for the inhibition of melanogenesis.

To find the optimum size of aromatic group in thiosemicarbazones for their inhibitory activity, we extended the phenyl ring in **2a** to naphthyl moiety. Surprisingly, the naphthyl analog **2q** (>100% inhibition at 10 μM, $IC_{50}$ = 1.1 μM, *C* log *P* = 3.039) showed very strong inhibition than **2a**. This result indicated that naphthyl group is more beneficial than a phenyl group due to its larger plane

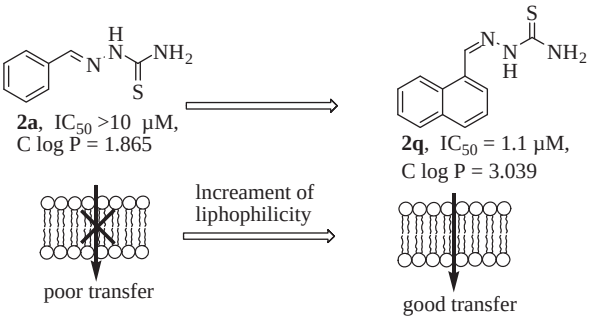



**Figure 1.** The correlation of inhibitory activity of **2a** and **2q** with their liphophilicity on melanoma B16 cell membrane.

size. In addition, the naphthyl group also increases the hydrophobicity. Thus to confirm the necessity of aromatic group, phenyl group of **2a** was replaced with bulky cyclohexyl group as shown in **2r** (81% inhibition at 10 μM, $IC_{50}$ = 3.4 μM, *C* log *P* = 1.987), which also exhibited a strong inhibition. This proves that the aromatic planarity is not important at this position. Thus, the enhancement of activity in naphthyl analog **2q** should result from its increased hydrophobicity. Therefore, we can conclude that hydrophobicity of the substituent on hydrazine of thiosemicarbazones is more important factor for the inhibitory activity in melanogenesis rather than the planarity. Moreover, complete loss of activity in pyridine analog **2s** (15% inhibition at 10 μM, $IC_{50}$ >30 μM, *C* log *P* = 1.088) strengthens our viewpoint on the importance of hydrophobicity rather than hydrophilicity or planarity.

Noticeably, the importance of hydrophobicity becomes more obvious when comparing the *C* log *P* values of **2a–s**. For instance, a simple benzaldehyde thiosemicarbazone (**2a**, <10% inhibition at 10 μM, $IC_{50}$ >10 μM, *C* log *P* = 1.865) has been well known tyrosinase inhibitor[27] with the $IC_{50}$ value of 1.93 μM, but it did not show any notable inhibition in our melanoma B16 cell based bioassay because it may not have enough liphophilicity to penetrate the cell (Fig. 1). Hydroxyl derivatives **2g**, **2k**, and **2p** showed poor activity with the same reason. However, the naphthyl analog **2q** (>100% inhibition at 10 μM, $IC_{50}$ = 1.1 μM, *C* log *P* = 3.039) and other analogs of **2**, containing hydrophobic substituent's showed an excellent inhibitory activity compared to **2a**. Thus, the hydrophobic or bulky groups are important for the effective inhibition of melanogenesis in B16 cells.

In conclusion, we have design, synthesized and tested a series of thiosemicarbazones **2**(**e**–**s**) as inhibitors of melanogenesis for defining their structure–activity relationship (SAR) studies. As a result (*Z*)-2-(naphthalen-1-ylmethylene)hydrazinecarbothioamide (**2q**) proved the best melanogenesis inhibitor among synthesized compounds. Regarding SAR, we observed the two important points. Substituent's with highly hydrophobic property regardless the position of phenyl ring of the thiosemicarbazones is more important for enhancement of the activity than the hydrophilic one. In addition, 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 aryl or alkyl group on hydrazine of thiosemicarbazones is determinant factor for their inhibitory activity in melanogenesis rather than planarity.

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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.2010.08.114.

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