Bioorganic & Medicinal Chemistry Letters 20 (2010) 2991–2993

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structural requirement(s) of *N*-phenylthioureas and benzaldehyde thiosemicarbazones as inhibitors of melanogenesis in melanoma B 16 cells

P. Thanigaimalai [a], Tuan Anh Le Hoang [a], Ki-Cheul Lee [a], Seong-Cheol Bang [a], Vinay K. Sharma [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, South Korea
[b] College of Pharmacy, Chungbuk National University, Cheongu 361-763, South Korea

ARTICLE INFO

Article history:
Received 21 December 2009
Revised 25 January 2010
Accepted 17 February 2010
Available online 20 February 2010

Keywords:
Tyrosinase inhibitor
Melanogenesis inhibitor
Phenylthiourea and benzaldehyde thiosemicarbazone

ABSTRACT

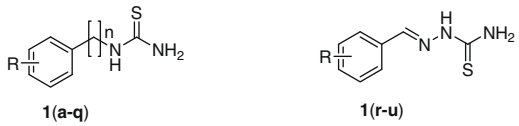
In order to define the structural requirements of phenylthiourea (PTU), a series of thiourea and thiosemicarbazone analogs were prepared and evaluated as inhibitors of melanogenesis in melanoma B16 cells. The most potent analog was 2-(4-*tert*-butylbenzylidene)hydrazinecarbothioamide **(1u)** with an IC_{50} value of 2.7 μM in inhibition of melanogenesis. The structure for potent inhibitory activity of these derivatives are required with the direct connection of π-planar structure to thiourea without steric hinderance in PTU derivatives and the hydrophobic substituent at para position in case of semicarbazones.

In mammals, melanin production is primary responsible for the skin color, and melanin plays vital role in the absorption of free radicals formed in cytoplasm, in protecting human skin from the harmful UV-radiation and in scarvanging chemicals.[1] The biosynthesis of melanin (melanogenesis) is a complex pathway involving enzymatic and chemical reaction in melanocyte cells.[2–5] The melanin synthesized from the conversion of simple amino acid, L-tyrosine, into 3,4-dihydroxyphenylalanine (L-DOPA) and the subsequent oxidation to form dopaquinone by catalyzing the tyrosinase, the membrane bound copper bearing phenolase, which is the critical enzyme for melanin biosynthesis in melanosomes produced by melanocytes.[6–8] In man, defects in the enzyme are related to a number of pathologies such as oculoutaneous albinism. However, the excessive formation and an abnormal aggregation of melanin in different specific parts can cause diverse hyperpigmentary disorders such as melasma, freckles, age spot, and post-inflammatory melanoderma.[9,10] Therefore, the regulation of melanin synthesis by inhibiting the tyrosinase enzyme is a current research topic in the context of preventing hyperpigmentation. In this regard, diverse tyrosinase inhibitors have been actively discovered such as kojic acid,[11] arbutin,[12] ascorbic acid derivatives,[13] hydroxylstilbine derivatives like resveratol[14–16] and methyl ester of genistic acid.[17,18]

N-Phenylthiourea (**1a**, PTU) has long been known as reference inhibitor[19] against tyrosinase with an IC_{50} value of 1.8 μM.[20] This enzyme belongs with catechol oxidase to the type-3 copper protein group.[21,22] Crystal structure of PTU bound catechol oxidase was reported.[21,22] The sulfur atom of this compound binds to both copper ions in the active site of the enzyme.[22] In addition to that interaction with the dicopper center, the van der Waals interactions between the residues lining the hydrophobic cavity (Phe 261, Ile 241, His 244) contribute to the high affinity of the PTU to the enzyme.[22] Although the crystal structure of PTU bound tyrosinase has not been known, the binding mode of this compound would be very much similar[22] since the active site[23] of tyrosinase is highly conserved and has similar hydrophobic cavity with Tyr 98.

To our knowledge, there have been no reports concerning about the structure–activity relationship (SAR) of phenylthiourea analogs. Therefore, we evaluated a series of phenylthiourea derivatives **1a–q** and thiosemicarbazones **1r–u** for their inhibitory activity and studied SAR on melanogenesis. The general structures of PTU and thiosemicarbazone analogs were shown in Chart 1.

The thiourea derivatives **1a–q** are obtained from benzoyl isothiocyanate and commercially available amines as illustrated in Scheme 1. Briefly, the intermediate benzoyl isothiocyanate **3** was



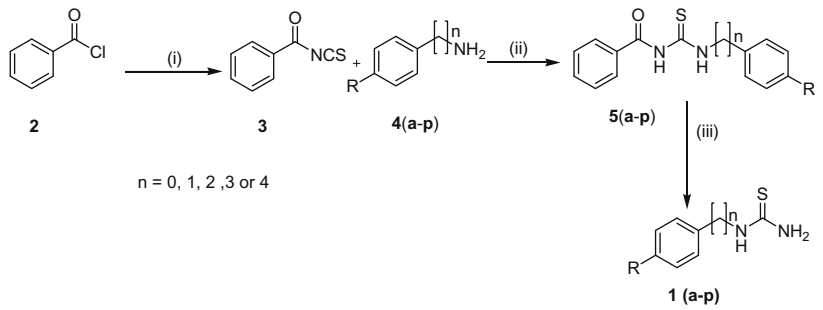
n = 0, 1, 2, 3 or 4.

R = H, CH_3, CH_2CH_3, $C(CH_3)_3$, Cl, CH_2OH or OCH_3

Chart 1. General structure of phenyl thiourea and thiosemicarbazone analogs.

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

0960-894X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.02.067



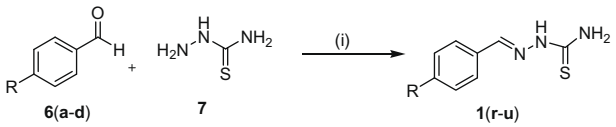
Scheme 1. Synthesis of phenylthiourea analogs. Reagents and conditions: (i) NH_4SCN, 60 °C, 15 min; (ii) 60 °C, 30 min; (iii) 5% NaOH, 70 °C, 20 min. *Note*, R = substituents are located in Table 1.

prepared from benzoyl chloride **2** by treating with ammoniumthiocyanate at reflux for 30 min in acetonitrile.[24] Intermediate **5** was obtained by treating **3** with amines **4** at reflux for 30 min and then was hydrolyzed by hot 5% NaOH for 20 min to give the desired compounds **1a–q**.[24–28] The substituted semithiocarbazones **1r–u** synthesized by the treatment of appropriate benzaldehyde **6a–d** with semicarbazide **7** as shown in Scheme 2.[29,30] All the above-synthesized derivatives were tested for their inhibitory activity of melanogenesis in melanoma B16 cell line[16] as shown in Table 1 rather than tyrosinase itself since the purpose of these inhibitors is to reduce the formation of melanin.

As reported earlier,[20] PTU (**1a**) showed moderate inhibition (84% inhibition at 10 μM, IC_{50} = 5.4 μM, *C* log *P* = 0.745) against melanogenesis. In order to explore the role of substituents on the phenyl ring of PTU, we introduced various substituents at different positions of phenyl ring. The *p*-methyl substituted analog **1b** (83% inhibition at 10 μM, IC_{50} = 5.5 μM, *C* log *P* = 1.244) showed comparable activity with **1a**. Thus, increasing the bulkiness at this position such as 4-ethylphenylthiourea, **1c** (88% inhibition at 10 μM, IC_{50} = 4.9 μM, *C* log *P* = 1.773), 4-*tert*-butylphenylthiourea, **1d** (95% inhibition at 10 μM, IC_{50} = 3.7 μM, *C* log *P* = 2.571) and 4-chlorophenylthiourea, **1e** (84% inhibition at 10 μM, IC_{50} = 4.0 μM, *C* log *P* = 1.764) also exhibited potent inhibition. To examine the effect of hydrophilic or highly electron donating group, we prepared the hydroxyl analog **1f** (70% inhibition at 10 μM, IC_{50} = 6.3 μM, *C* log *P* = 0.073) and the methoxy compound **1g** (80% inhibition at 10 μM, IC_{50} = 5.6 μM, *C* log *P* = 0.847), which also exhibited comparable activity to **1a**. These data suggest that variation of the bulkiness or lipophilicity of substituents at *p*-position of phenyl ring of PTU hardly affect the inhibitory activity, which indicates that these groups do not participate in the interaction of phenylthioureas **1a–g** with the active site of tyrosinase.

Substitution at *m*-position as shown in **1h** (78% inhibition at 10 μM, IC_{50} = 5.0 μM, *C* log *P* = 1.244) gave almost similar effect on the activity as *p*-substitution. Thus, *m*-substitution of PTU dose not disrupt the binding mode of PTU to the active site.

Interestingly, *o*-methyl substituted compound **1i** (>10% inhibition at 10 μM, IC_{50} = >10 μM, *C* log *P* = 1.244) did not show any inhibition. This result made us more curious regarding the substitution at *o*-position and its effect on inhibition in melanogenesis. Thus, we synthesized compounds with bulkier group substitutions



Scheme 2. Synthesis of benzaldehyde thiosemicarbazone analogs. Reagent and conditions: (i) heat, 20 min, H_2O–ethanol. *Note*, R = substituents are located in Table 1.

at this position such as ethyl (**1j**: >10% inhibition at 10 μM, IC_{50} = >10 μM, *C* log *P* = 1.733), *tert*-butyl (**1k**: >10% inhibition at 10 μM, IC_{50} = >10 μM, *C* log *P* = 2.571), methoxy (**1l**: >10% inhibition at 10 μM, IC_{50} = >10 μM, *C* log *P* = 0.847) and hydroxymethyl derivative (**1m**: >10% inhibition at 10 μM, IC_{50} = >10 μM, *C* log *P* = −0.853). These compounds also did not turn up with any inhibition. The above findings lead us to predict that the C-2 substituted phenyl ring may hinder the complex formation between thiourea unit and the metallic center of the active site in enzyme due to the steric hindrance.

In addition to confirm the importance of the direct connection of phenyl to thiourea of PTU, methylene units were inserted between them. The resulting compounds **1n–q** did not exhibit any inhibition against melanogenesis. This implies that increasing bulkiness with sp^3 carbons in this position may prevent the access of thiourea group into the active site and π, π stacking of phenyl group.[23] Thus, direct connection of planar phenyl to thiourea is critical for activity.

For more detailed studies regarding π system of PTU derivatives, thiosemicarbazone derivatives **1r–u** were synthesized as shown in Table 1. The compounds **1r** and **1u** were reported for their inhibitory activity against tyrosinase from *Pieris rapae* (Lepidoptera) larvae,[31] but with very weak inhibition. Accordingly, benzaldehyde thiosemicarbazone (**1r**, >10% inhibition at 10 μM, IC_{50} = >10 μM, *C* log *P* = 1.865) did not show any activity in our bio-

Table 1
Inhibitory activity of thiones **1a–u** against melanogenesis in melanoma B16 cells

Compd	R	*n*	Inhibition at 10 μM	IC_{50} values (μM)	*C* log *P*[b]
1a[a]	4-H	0	84	5.4	0.745
1b[a]	4-CH_3	0	83	5.5	1.244
1c[a]	4-CH_3CH_2	0	88	4.9	1.213
1d[a]	4-$C(CH_3)_3$	0	95	3.7	2.571
1e[a]	4-Cl	0	84	4.0	1.764
1f[a]	4-OH	0	70	6.3	0.073
1g[a]	4-OCH_3	0	80	5.6	0.847
1h[a]	3-CH_3	0	78	5.0	1.244
1i[a]	2-CH_3	0	<10	>10	1.244
1j[a]	2-CH_3CH_2	0	<10	>10	1.213
1k[a]	2-$C(CH_3)_3$	0	<10	>10	2.571
1l[a]	2-OCH_3	0	<10	>10	0.847
1m	2-CH_2OH	0	<10	>10	−0.853
1n[a]	H	1	<10	>10	1.074
1o	H	2	<10	>10	1.403
1p	H	3	<10	>10	1.782
1q	H	4	<10	>10	2.311
1r[a]	H	—	<10	>10	1.865
1s[a]	4-CH_3	—	64	6.8	2.364
1t[a]	4-CH_3CH_2	—	100	3.4	2.893
1u[a]	4-$C(CH_3)_3$	—	100	2.7	3.691

[a] Analytical data of compounds **1a**,[24] **1b**,[24] **1c**,[28] **1d**,[26] **1e**,[24] **1f**,[25] **1g**,[24] **1h**,[24] **1j**,[27] **1k**,[26] **1l**,[24] **1n**,[25] **1r**,[29] **1s**,[29] **1t**,[29] and **1u**,[30] are consistent with references.
[b] *C* log *P* values were calculated by Chemdraw 9.0.

assay system. However, the *p*-methyl substituted analog **1s** (64% inhibition at 10 μM, IC_{50} = 6.8 μM, *C* log *P* = 2.364) showed moderate activity. Interestingly, on increasing the hydrophobicity at this position, the thiosemicarbazone analogs, namely (*E*)-2-(4-ethylbenzylidene)hydrazinecarbothioamide (**1t**, 100% inhibition at 10 μM, IC_{50} = 3.4 μM, *C* log *P* = 2.893) and (*E*)-2-(4-*tert*-butylbenzylidene)hydrazinecarbothioamide (**1u**, 100% inhibition at 10 μM, IC_{50} = 2.7 μM, *C* log *P* = 2.893), showed two folds enhanced activity compared to that of **1a**. These indicate that lipophilicity would be critical factor in case of benzaldehyde thiosemicarbazone analogs. These results also strengthen our viewpoint on the importance of direct connection of π-planar structure to thiourea for activity. The better activity of thiosemicarbazones **1s**–**u** than those of thiourea derivatives **1a**–**q** may be attributed to the 'clamp' structure[31] (H–N–C–N–H) of thiosemicarbazones.

In conclusion, the compounds ethyl and *tert*-butyl derivatives of the benzaldehyde thiosemicarbazone showed the most potent activity among the thiourea derivatives. Along with that, the structure for potent inhibitory activity of these derivatives are required with the direct connection of π-planar structure to thiourea without steric hinderance in PTU derivatives and the hydrophobic substituent at *p*-position in case of semicarbazones.

Acknowledgment

This research was financially supported by a grant (R01-2007-000-20099-0) from the National Research Foundation of Korea

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.067.

References and notes

1. Francisco, S.; Stefania, B.; Mauro, P.; Ghanem, G. *Pigment Cell Res.* **2006**, *19*, 550.
2. Hearing, V. J.; Körner, A. M.; Pawelek, J. M. *J. Invest. Dermatol.* **1982**, *79*, 16.
3. Hearing, V. J.; Jiménez, M. *Int. J. Biochem.* **1987**, *19*, 1141.
4. Prota, G. *Med. Res. Rev.* **1988**, *8*, 525.
5. Kuzumaki, T.; Matsuda, A.; Wakamatsu, K.; Ito, S.; Ishikawa, K. *Exp. Cell Res.* **1993**, *207*, 33.
6. Beradesca, E.; Cameli, N.; Primavera, G.; Carrera, M. *Dermatol. Surg.* **2006**, *32*, 526.
7. Naish, B. S.; Riley, P. A. *Pigment Cell Res.* **1998**, *11*, 94.
8. Hideya, A.; Hirofumi, K.; Masamitsu, I.; Vincent, J. H. *J. Invest. Dermatol.* **2007**, *127*, 751.
9. Cullan, M. K. In *The Pigmentary System: Physiology and Pathophysiology*; Norlund, J. J., Biossy, R. E., Hearing, V. J., King, R. A., Ortonne, J. P., Eds.; Oxford University Press: New York, 1998; pp 760–766.
10. Urabe, K.; Nakayama, J.; Hori, Y. In *The Pigmentary System: Physiology and Pathophysiology*; Norlund, J. J., Biossy, R. E., Hearing, V. J., King, R. A., Ortonne, J. P., Eds.; Oxford University Press: New York, 1998; pp 909–991.
11. Battaini, G.; Monzani, E.; Casella, L.; Santagostini, L.; Pagliarin, R. *J. Biol. Inorg. Chem.* **2000**, *5*, 262.
12. Maeda, K.; Fukuda, M. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 765.
13. Ros, J. R.; Rodriguez-Lopes, J. N.; Garcia-Canovas, F. *Biochem. J.* **1993**, *295*, 309.
14. Ohguchi, K.; Tanaka, T.; Ito, T.; Linuma, M.; Matsumoto, K.; Akao, Y.; Nozawa, Y. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 1587.
15. Shin, N. H.; Ryu, S. Y.; Choi, E. J.; Kang, S. H.; Chang, I. M.; Min, K. R.; Kin, Y. S. *Biochem. Biophys. Res. Commun.* **1998**, *243*, 801.
16. Kim, Y. M.; Yun, J.; Lee, C. K.; Lee, H.; Min, K. R.; Kim, Y. *J. Biol. Chem.* **2002**, *277*, 16340.
17. Curto, E. V.; Kwong, C.; Hermersdorfer, H.; Glatt, H.; Santis, C.; Virador, V.; Hearing, V. J., Jr.; Dooly, T. P. *Biochem. Pharmacol.* **1999**, *57*, 663.
18. Passi, S.; Nazzaro-Porro, M. *Br. J. Dermatol.* **1981**, *104*, 659.
19. DuBois, K. P.; Erway, W. F. *J. Biol. Chem.* **1946**, *165*, 711.
20. Criton, M.; Le Mallay-Harmon, V. *Biorg. Med. Chem. Lett.* **2008**, *18*, 3607.
21. Gerdemann, C.; Eicken, C.; Krebs, B. *Acc. Chem. Res.* **2002**, *35*, 183.
22. Klabunde, T.; Eicken, C.; Sacchettini, J. C.; Krebs, B. *Nat. Struct. Biol.* **1998**, *5*, 1084.
23. Matoba, Y.; Kumagai, T.; Yamamoto, A.; Yoshitsu, H.; Sugiyama, M. *J. Biol. Chem.* **2006**, *281*, 8981.
24. Holla, B. S.; Malini, B.; Rao, B. S.; Sarojini, B. K.; Suchetha Kumari, N. *Eur. J. Med. Chem.* **2003**, *38*, 313.
25. Mitsuo, K.; Masato, S.; Keiko, T.; Tadashi, A. *Tetrahedron Lett.* **2005**, *46*, 5841.
26. Grehn, Leif. *Org. Mass Spectrom.* **1977**, *12*, 267.
27. Anil, K. S.; Suresh, K. P.; Seth, P.; Singh, M. P.; Dikshit, M.; Carpy, A. *Bioorg. Med. Chem.* **2001**, *9*, 2025.
28. Rasmussen, C. R.; Villani, F. J., Jr.; Weaner, L. E.; Reynolds, B. E.; Hood, A. R.; Hecker, L. R.; Nortey, S. O.; Hanslin, A.; Costanzo, M. J., et al *Synthesis* **1998**, *6*, 456.
29. Varsha, J.; Pradeep, M.; Sushil, K.; Stables, J. P. *Eur. J. Med. Chem.* **2008**, *43*, 135.
30. Xie, X. Y.; Jiang, L.; Xue, C. B.; Luo, W. C. *Huaxue Shiji* **2007**, *29*, 34.
31. Xue, C. B.; Zhang, L.; Luo, W. C.; Xie, X. Y.; Jiang, L.; Xiao, T. *Bioorg. Med. Chem.* **2007**, *15*, 2006.