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Analogues of *N*-hydroxy-*N*′-phenylthiourea and *N*-hydroxy-*N*′-phenylurea as inhibitors of tyrosinase and melanin formation

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ABSTRACT

A series of *N*-hydroxy-*N*′-phenylthiourea and *N*-hydroxy-*N*′-phenylurea analogues were prepared and evaluated as inhibitors of tyrosinase and melanin formation. The most active analogue **1** inhibited mushroom tyrosinase with an IC_{50} of around 0.29 μM and also retained a substantial potency in cell culture by reducing pigment synthesis by 78%. Therefore, compound **1** could be considered as a promising candidate for preclinical drug development for skin hyperpigmentation application.

Mammals skin pigmentation results from the production of melanin by melanocytes and its accumulation in the epidermis. Melanin synthesis or melanogenesis is a complex pathway involving enzymatic and chemical reactions, which are restricted to melanosomes, melanocyte-specific organelles containing all components required to synthesise pigment. Among enzymes involved in melanin biosynthesis tyrosinase, a copper-containing, membrane-bound glycoprotein is the most critical and rate-limiting enzyme that catalyzes the first two steps in the biosynthetic pathway: hydroxylation of tyrosine to L-dihydroxyphenylalanine (L-DOPA) and oxidation of L-DOPA to dopaquinone.

Increased production and accumulation of melanin characterize a large number of dermatological disorders, which include acquired hyperpigmentation, such as melasma, freckles, postinflammatory melanoderma, and solar lentigo.[1,2] Many tyrosinase inhibitors find application in cosmetics and pharmaceutical products as a way of preventing overproduction of melanin in epidermis. Hydroquinone is one of the most potent whitening agents first discovered,[3,4] but since its introduction some adverse effects have been recognized. In recent years various tyrosinase inhibitors have been reported such as azelaic acid,[5] ascorbic acid derivatives,[6] arbutin,[7] kojic acid,[8] hydroxystilbene compounds like resveratrol,[9–11] and methyl ester of gentisic acid.[12] Most of the tyrosinase inhibitors are phenol/catechol derivatives, structurally similar to tyrosine or L-DOPA, which act as suicide substrates of tyrosinase.[13]

N-Phenylthiourea (PTU, Fig. 1) was shown to inhibit catechol oxidase enzyme that belongs with tyrosinase to the type-3 copper proteins group. The sulfur atom of the PTU binds to both copper ions in the active site of catechol oxidase and blocks enzyme activity.[14,15] Besides, our interest for chelators agents led us to study the hydroxamic acid group. Hydroxamate molecules, one of the major classes of naturally occurring metal complexing agents, have been thoroughly studied as ligands for different metal ions as Fe(III), Zn(II), and Cu(II).[16,17] The chelation involves the oxygen belonging to the carbonyl moiety and the NHOH groups. Numerous papers

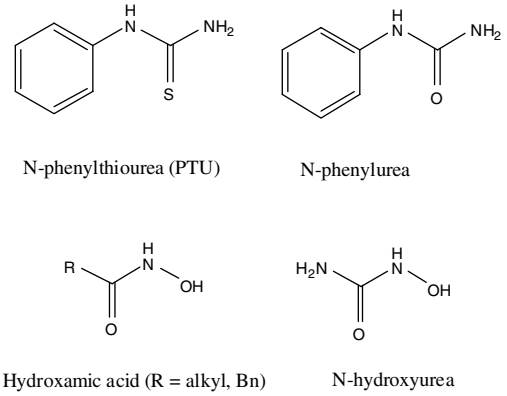

Figure 1. Chemical structures of *N*-phenylurea, *N*-phenylthiourea, hydroxamic acid, and hydroxyurea.

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showed that *N*-hydroxyurea (–NHC(O)NHOH) function seems particularly suited as hydroxamate alternative in metal-chelating ability since it incorporates the C(O)–NHOH group that is necessary to establish the same ideal type of metal chelation. *N*-Hydroxyurea itself, used as antineoplastic drug since 1960s,[18] is known to form a complex with Fe(III) and Cu(II) metal ions.[19]

In the present report, using PTU as starting point, we designed and synthesized new compounds where the primary amino group of PTU was replaced by hydroxylamino derivatives and the sulfur atom was conserved or replaced by an oxygen atom to increase Cu^{2+}-chelating properties of the resulting compounds, thereby generating *N*-hydroxy-*N*′-phenylthiourea and *N*-hydroxy-*N*′-phenylurea derivatives (**1**–**22**, Tables 1–3). These compounds were evaluated on tyrosinase activity in vitro and on melanin production by cultured melanocytes.

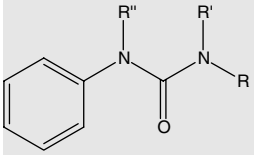
The detailed chemistry of compounds has been previously described by our group in a patent.[20] Briefly, and according to Scheme 1, a number of commercially available phenylisocyanate or phenylisothiocyanate were treated with different *N*-hydroxylamine derivatives in the presence of dimethylformamide, and triethylamine affording *N*-hydroxy-*N*′-phenylthiourea and *N*-hydroxy-*N*′-phenylurea derivatives (**1**–**14**; **18**–**22**). Compounds (**15**–**17**) were prepared according to Scheme 2, by treating phenylcarbamoyl chloride derivatives with *N*-hydroxylamine derivatives in the presence of dimethylformamide, dichoromethane and triethylamine.[21,22]

Then all these derivatives were evaluated on mushroom tyrosinase activity and their ability to inhibit melanin formation by B16 melanoma cell line was investigated. Consistent with previous reports,[23,24] PTU induced a strong inhibition of the tyrosinase activity (IC_{50} = 1.8 μM) in contrast to *N*-phenylurea which showed no inhibition in our assay (Table 1). Interestingly, when the amino group and the sulfur moieties of the PTU were replaced by *N*-hydroxylamine and oxygen, respectively, the resulting compound **1** was more potent to inhibit tyrosinase activity (IC_{50} = 0.29 μM) compared to PTU. Besides, tyrosinase inhibition with compound **1** was more potent than that obtained with the reference tyrosinase inhibitors, kojic acid and hydroquinone, for which the IC_{50} values were, respectively, 75 and 37 μM.

Thus compound **1** was used as benchmark compound to synthesize a series of derivatives in which different substitutions were introduced at the *N*-hydroxyurea moiety (–NH–CO–NHOH) while keeping the phenyl ring unmodified (Table 1). When the terminal NHOH group was methylated on the hydroxyl (**13, 17**) or on the NH (**14, 16**) moiety, the tyrosinase activity was completely lost. Methylation on *N*′ leads to diminished tyrosinase activity, but when NHOH motif was conserved (**15**) the product had a more potent inhibition (IC_{50} = 16 μM) than kojic acid and hydroquinone, but lower than compounds **1** and PTU. When the hydroxyl of the NHOH moiety was silylated with *tert*-butyldimethylsilyl group the resulting compound **22** (IC_{50} = 70 μM) showed an inhibitory activity comparable to kojic acid. Acetylation of the R′ position (compound **18**) had a low inhibitory activity (IC_{50} value of 170 μM) compared to **1**.

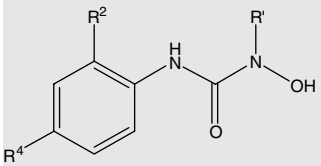
With the exception of the compound **22**, our results indicate that an unsubstituted NHOH moiety is important for inhibition of the tyrosinase activity, suggesting that the chelating ability of *N*-hydroxyurea might be important for a potent inhibition of tyros-

Table 1
Structures (**1, 13–18, 22**), tyrosinase, and melanin-formation inhibition

Compound	Substituent			Tyrosinase inhibition	Melanin production
	R	R′	R″	IC_{50} (μM)	% Inhibition at 100 μM[a]
1	OH	H	H	0.29	78 ± 2.12
13	OMe	H	H	>1000	ni
14	OH	Me	H	>1000	ni
15	OH	H	Me	16	nd
16	OH	Me	Me	>1000	nd
17	OMe	H	Me	>1000	nd
18	OH	Ac	H	170	nd
22	OTBDMSi	H	H	70	ni
N-Phenylthiourea				1.8	58 ± 0.77
N-Phenylurea				>1000	ni
Kojic acid				75	54.3 ± 1.09[b]
Hydroquinone				37	—
Arbutin				—	43.8 ± 0.15

[a] Results are represented as inhibition %, means ± SE of three independent tests.
[b] Kojic acid was tested at 1 mM; ni, no inhibition; nd, not done.

Table 2
Structures (**1**–**12**), tyrosinase and melanin-formation inhibition

Compound	Substituent			Tyrosinase inhibition	Melanin production
	R′	R^4	R^2	IC_{50} (μM)	% Inhibition at 100 μM[a]
1	H	H	H	0.29	78 ± 2.12
2	H	OH	H	41	19 ± 1.31
3	H	OMe	H	32	ni
4	H	OMe	OMe	>1000	ni
5	H	OBu	H	>1000	ni
6	H	OBn	H	6.3	ni
7	H	NO_2	H	2.6	86 ± 0.44
8	Me	NO_2	H	770	82 ± 0.23
9	H	NHCONHOH	H	27	66 ± 1.54
10	H	CF_3	H	4.3	79.3 ± 0.58
11	H	Br	H	2.7	75.5 ± 2.30
12	Me	Br	H	>1000	ni
N-Phenylthiourea, *N*-phenylurea, kojic acid, hydroquinone, arbutin				See Table 1	

[a] Results are represented as inhibition %, means ± SE of three independent tests; ni, no inhibition.