Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2252–2255

Analogues of *N*-hydroxycinnamoylphenalkylamides as inhibitors of human melanocyte-tyrosinase

Sabrina Okombi,[a] Delphine Rival,[b] Sébastien Bonnet,[b] Anne-Marie Mariotte,[a] Eric Perrier[b] and Ahcène Boumendjel[a,*]

[a]*Département de Pharmacochimie Moléculaire, UMR-CNRS 5063, Faculté de Pharmacie de Grenoble, 5 avenue de Verdun, BP 138, 38243 Meylan, France*
[b]*Engelhard-Lyon, 32 rue St Jean de Dieu, 69007 Lyon, France*

Received 19 December 2005; revised 3 January 2006; accepted 6 January 2006
Available online 26 January 2006

Abstract—Melanin play a major role in human skin protection and their biosynthesis is vital. Due to their color, they contribute to the skin pigmentation. Tyrosinase is a key enzyme involved in the first stage of melanin synthesis, catalyzing the transformation of tyrosine to L-dopaquinone. The aim of the present study was to study molecules able to inhibit melanin synthesis through inhibition of tyrosinase and their potential use in treating pigmentation-related disorders. We targeted amides obtained from coupling *p*-hydroxycinnamic acid derivatives with phenylalkylamines. The biological activity was evaluated on human melanocytes by an assay which measures tyrosine-catalyzed L-Dopa oxidation. The most active amides were: *trans*-*N*-caffeoyltyramine, *N*-dihydrocaffeoyltyramine, and *trans*-*N*-dihydro-*p*-hydroxycinnamoyltyramine which induce complete inhibition at 0.1 mM. At the latter concentration, kojic acid, which was used as the reference inhibitor, was inactive.

Skin hyperpigmentation can be dependent on either an increased number of melanocytes or activity of melanogenic enzymes, such as tyrosinase (EC 1.14.18.1).[1,2] The latter plays a critical role in the biosynthesis of melanin and it is considered to be the key enzyme in coloring of skin, hair, eyes, and food browning.[3] Tyrosinase, is a copper-containing enzyme, widely present in mammals, plants and fungi and accepts many phenols and catechols as substrates.[4,5] In mammals, it is involved in the transformation of L-tyrosine to dopaquinone which occurs through two steps: hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), then oxidation of the latter to an *ortho*-quinone (dopaquinone). Dopaquinone is further transformed through several reactions to yield brown to black melanin which is responsible for colour of mammals' skin.[6]

Inhibitors/activators of tyrosinase have become increasingly important in medicinal and cosmetic products. For example, tyrosinase inhibitors are used in depigmentation drugs and cosmetics,[7–10] whereas compounds that increase melanogenesis such as tyrosinase activators may protect human skin from UV irradiation damage.[11]

In this regard, several natural and synthetic compounds acting as tyrosinase inhibitors were reported but only few of them are used as skin-whitening agents due to safety concerns. For example, arbutin and kojic acid were among the most popular before serious side effects came to limit their human use.[12–15]

As recently reviewed by Briganti et al.,[16] it should be highlighted that targeting tyrosinase inhibitors/activators to treat melanogenesis disorders is one of many possible approaches, due to the complex biochemical reaction involved in the melanin synthesis. Moreover, physical therapies such as lasers are now being developed to overcome whitening agents' ineffectiveness.[16]

Effects of wine phenolic compounds such as caffeic acid, ferulic acid, and *p*-coumaric acid have been reported to act as tyrosinase inhibitors.[17] Amides derived from coupling the latter acids with tyramine or dopamine have been isolated from plants or synthesized and have been studied in different therapeutic areas such as anticancer agents,[18,19] antioxidants,[20–23] and as selective NMDA receptor antagonists.[24] In a recent study, Roh et al. have

Keywords: Melanin; Tyrosinase inhibitors; *N*-Hydroxycinnamic acid analogues; Phenylalkylamines; Amides.
* Corresponding author. Tel.: +33 4 76 04 10 06; fax: +33 4 76 04 10 07; e-mail: Ahcene.Boumendjel@ujf-grenoble.fr

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.01.022

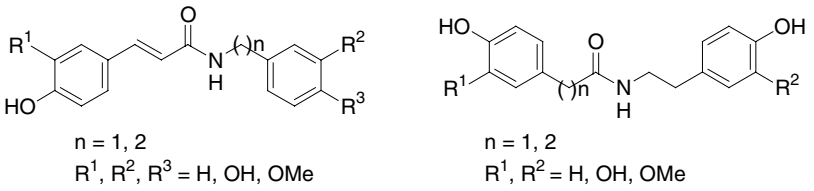


Figure 1. Structures of targeted compounds.

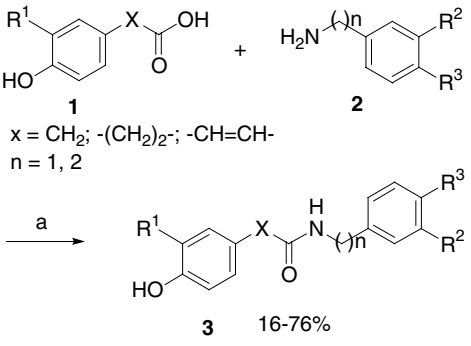
reported melanogenesis-inhibitory effects of the naturally occurring *N*-feruloylserotonin and *N*-(*p*-coumaroyl)serotonin, isolated from Safflower (*Carthamus tinctorius* L.).[25]

In continuing our program aimed to search for polyphenol-derived compounds acting as skin protectors,[26] we investigated amides derived from coupling of: caffeic acid, ferulic acid, *p*-hydroxycinnamic acid, and derivatives with phenylethylamines (Fig. 1). The synthesized compounds were studied for their effect as inhibitors of human melanocyte-tyrosinase and their potential as melanogenesis suppressors.

The amides disclosed in Figure 1 were obtained by coupling of a carboxylic acid (**1**) with a phenylalkylamine (**2**) in DMF in the presence of triethylamine and BOP (benzotriazol-1-yloxy-tris-(dimethylamino)phosphonium hexafluorophosphate) as a coupling agent (Scheme 1).

In the literature, there are two main methods for evaluating tyrosinase inhibitors. The first and the most frequently used is an in vitro assay which uses the commercially available mushroom tyrosinase as a model. The second is a cellular test and measures the tyrosinase activity, either by looking at the intracellular accumulation of melanin or by determining the transformation rate of a tyrosinase substrate. Although, the in vitro test is easy to handle, provides accurate results and allows us to work on the enzymological aspects, however, it has the drawback that the results cannot be exploited for developing products for human use.

Because our ultimate goal was to bring the active compounds to human use, we preferred to relay on the cellular test using human melanocytes obtained from healthy individuals. The inhibitory potency was evaluated by measuring the transformation rate of L-Dopa (tyrosinase substrate) to L-dopaquinone. The latter can be trapped by MBTH (3-methyl-2-benzothiazolinone hydrazone), a chromophore easily quantifiable at 490 nm.[26,27] A compound able to modify tyrosinase activity will be accompanied with a decrease in absorbance at 490 nm compared to the negative test (without molecule) (Table 1).[27,28]



Scheme 1. Reagents and condition: (a) BOP, Et_3N, DMF, 20 h.

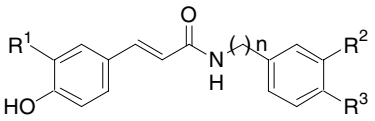
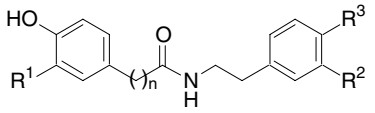
First, the compounds were assessed for their cytotoxicity by measuring melanocyte viability at different concentrations in the range of 10–100 μM (results not shown) and ruled out compounds which alter the cell viability. At the highest concentration (100 μM), kojic acid, which was used as a reference inhibitor, was inactive and therefore it was evaluated at 700 μM.

From the structure–activity point of view, we notice that a higher activity was achieved with amides obtained by coupling tyramine (4-hydroxyphenylethylamine) with caffeic acid, dihydrocaffeic acid, and dihydro-*p*-hydroxycinnamic acid (compounds **4**, **15**, and **17**). Evaluation of free tyramine, dopamine, caffeic acid, ferulic acid, and dihydro-*p*-hydroxycinnamic acid shows weak inhibiting activity, indicating the importance of the amide linkage. Another structural feature is that the amide function should be separated from each phenyl group by two carbons (compound **13** vs **15**). The presence of a catechol entity and preferably at the acid moiety seems to be important, because dopamine derivatives are less active than compounds derived from tyramine.

As mentioned above, one of the reactions catalyzed by the tyrosinase is the oxidation of L-Dopa to *ortho*-dopaquinone by using molecular oxygen and it is obvious that antioxidants may inhibit the oxidation step, without interacting with the tyrosinase. The compounds reported in this study were evaluated at 100 μM for their free radical scavenging activity by using the DPPH method.[29] It revealed that compounds **5**, **7**, and **9** showed the highest free radical scavenging activity (~60% of free radical scavenging activity) and the most active inhibitor (compound **4**) shows 40% of free radical scavenging. These results indicate that in our case there is no evidence for direct correlation between free radical scavenging activity and tyrosinase inhibition. The latter assumption is confirmed by the weak inhibition of *p*-hydroxycinnamic acid and derivatives (caffeic and ferulic acids) which are known for their strong antioxidant activity.

Finally, because our ultimate goal is to bring active compounds to human use, we evaluated the tolerance

Table 1. Inhibition data

Compound	R^1	R^2	R^3	*n*	% inhibition at 100 μM[27]
					
1	OH	H	H	2	nd
2	OMe	H	H	2	19 ± 9
3	OMe	H	OH	2	49 ± 4
4	OH	H	OH	2	100 ± 2
5	OH	OH	OH	2	67 ± 9
6	OMe	OMe	OH	1	40 ± 11
7	OH	OMe	OH	1	nd
8	OH	OMe	OMe	2	nd
9	OMe	OH	OH	2	62 ± 2
10	OMe	OMe	OMe	2	25 ± 5
					
11	OMe	H	OH	1	16 ± 6
12	OMe	OH	OH	1	0 ± 9
13	OH	H	OH	1	34 ± 7
14	OMe	H	OH	2	28 ± 7
15	OH	H	OH	2	94 ± 2
16	OMe	OH	OH	2	42 ± 5
17	H	H	OH	2	96 ± 2
p-OH·cinnamic acid					4 ± 2
Caffeic acid					10 ± 1
Ferulic acid					12 ± 2
Tyramine					23 ± 3
Dopamine					10 ± 2
Kojic acid (evaluated at 700 μM)					20 ± 5

in animals for compounds **4**, **15**, and **17** and found that they do not show any toxicity after oral administration to rats at 2 g/kg dose. In rabbits, topical and eye application of amides **4**, **15**, and **17** did not show any significant irritation.[20]

In summary, we have shown that variation of the substituent pattern and the chain length which separate phenyl rings from the amide linkage alters the inhibitory properties of these compounds. The inhibition data combined with the free radical scavenging activity indicate that the two activities are not directly correlated. The ease of synthesis and animal tolerance make the active compounds potential candidates for treating skin pigmentation related disorders.

References and notes

1. Griffiths, C. E.; Finkel, L. J.; Ditre, C. M.; Hamilton, T. A.; Ellis, C. N.; Voorhees, J. *Br. J. Dermatol.* **1993**, *129*, 415.
2. Kanwar, A. J.; Dhar, S.; Kaur, S. *Dermatology* **1994**, *188*, 170.
3. Perez-Gilabert, M.; Garcia-Carmona, F. *Biochem. Biophys. Res. Commun.* **2001**, *285*, 257.
4. Schoot-Uiterkamp, A. J. M.; Mason, H. S. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 993.
5. van Gelder, C. W. G.; Flurkey, W. H.; Wichers, H. *J. Phytochem.* **1997**, *45*, 1309.
6. Shin, N. H.; Ryu, S. Y.; Choi, E. J.; Kang, S. H.; Chang, I. M.; Min, K.; Kim, Y. *Biochem. Biophys. Res. Commun.* **1998**, *243*, 801.
7. Kligman, A. M.; Willis, I. *Arch. Dermatol.* **1975**, *111*, 40.
8. Alena, F.; Jimbow, K.; Ito, S. *Cancer Res.* **1990**, *50*, 3743.
9. Tasaka, K.; Kamei, C.; Nakano, S.; Takeuchi, Y.; Yamato, M. *Methods Find. Exp. Clin. Pharmacol.* **1998**, *20*, 99.
10. Kim, Y.-J.; Uyama, H. *Cell. Mol. Life Sci.* **2005**, *62*, 1707.
11. Eller, M. S.; Ostrom, K.; Glichrest, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1087.
12. Maeda, K.; Fukuda, M. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 765.
13. Nihei, K.-I.; Kubo, I. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2409.
14. Saruno, R.; Kato, F.; Ikeno, T. *Agric. Biol. Chem.* **1979**, *43*, 1337.
15. Seo, S. Y.; Sharma, V. K.; Sharma, N. *J. Agric. Food Chem.* **2003**, *51*, 2837.
16. Briganti, S.; Camera, E.; Picardo, M. *Pigment Cell. Res.* **2003**, *16*, 101.

17. Gómez-Cordovés, C.; Bartolomé, B.; Vieira, W.; Virador, V. M. *J. Agric. Food Chem.* **2001**, *49*, 1620.
18. Park, J. B.; Schoene, N. *Cancer Lett.* **2003**, *202*, 161.
19. Nesterenko, V.; Putt, K. S.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2003**, *125*, 14672.
20. Ley, J. P. *Int. J. Cosm. Sci.* **2001**, *23*, 35.
21. Khan, K. M.; Maharvi, G. M.; Abbaskhan, A.; Hayat, S.; Khan, M. T. H.; Makhmoor, T.; Choudhary, M. I.; Shaheen, F.; Atta-ur-Rahman *Helv. Chim. Acta* **2003**, *86*, 457.
22. Son, S.; Lewis, B. A. *J. Agric. Food Chem.* **2002**, *50*, 468.
23. Anai, M.; Tashiro, T. JP. Patent 2004175778 A2, 2004; *Chem. Abstr.* **2004**, *141*, 59222.;
24. Tamiz, A. P.; Cai, S. X. I.; Zhou, Z.-L.; Yuen, P.-W.; Schelkun, R. M.; Whittemore, E. R.; Weber, E.; Woodward, R. M.; Keana, J. F. W. *J. Med. Chem.* **1999**, *42*, 3412.
25. Roh, J. S.; Han, J. Y.; Kim, J. H.; Hwang, J. K. *Biol. Pharm. Bull.* **2004**, *27*, 1976.
26. Okombi, S.; Rival, D.; Bonnet, S.; Mariotte, A.-M.; Perrier, E.; Boumendjel, A. *J. Med. Chem.* **2006**, *49*, 329.
27. Winder, A. J.; Harris, H. *Eur. J. Biochem.* **1991**, *198*, 317.
28. *Tyrosinase inhibition:* Human melanocytes were obtained from an abdominal plastic surgery. Melanocytes were seeded in 24-well plates (80,000 cells per well) and were cultivated until confluence. Samples for evaluation were applied in cell culture medium during 24 h at 37 °C. After this time, cell culture medium was taken off and human tyrosinase was extracted from melanocytes by thermal choc lysis. After centrifugation (to eliminate cell membrane), supernatants were incubated with MBTH and L-Dopa during 30 min and then analyzed by spectrophotometry at 490 nm. Inhibition of tyrosinase activity was expressed in percentage of inhibition, compared with negative control (medium without tested molecule). A protein assay was performed on each extracellular medium to correlate the enzymatic activity to a protein content (BCA assay). Inhibition was calculated using the following formula: Inhibition (%) = $100 - [(OD_{sample}/\text{Protein content}_{sample})/(OD_{negative\ control}/\text{Protein content}_{negative\ control})] \times 100$.
29. Blois, M. *Nature* **1958**, *181*, 1199.