Bioorganic & Medicinal Chemistry Letters 21 (2011) 1922–1925

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Structural requirements of (*E*)-6-benzylidene-4a-methyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3*H*)-one derivatives as novel melanogenesis inhibitors

Pillaiyar Thanigaimalai [a], Ki-Cheul Lee [a], Vinay K. Sharma [a], Eeda Vekateswara Rao [a], Eunmiri Roh [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, Cheongju 361-763, South Korea

ARTICLE INFO

Article history:
Received 1 January 2011
Revised 14 February 2011
Accepted 15 February 2011
Available online 18 February 2011

Keywords:
Chalcone
Melanogenesis
Melanoma B16 cells
α-Melanocyte stimulating hormone

ABSTRACT

Chalcone type compound **1a** ((*E*)-6′-benzylidene-4a′-methyl-4′,4a′,7′,8′-tetrahydro-3′*H*-spiro[[1,3]dithiolane-2,2′-naphthalen]-5′(6′*H*)-one) was discovered as an potent inhibitor in melanogenesis. To define its structure–activity relationship, a series of analogs **1b–n**, dithiolane truncated **2a–b** and ring A removed **3a–e** were prepared and evaluated. The electron donating substitution on the phenyl ring (ring C) rather than an electron withdrawing group and dithiolane motif of **1** are needed for the activity enhancement. The scaffold containing both rings A and B associated with α,β-unsaturated system connected to phenyl of **1** was essential for antimelanogenesis.

Melanin is the primary determinant of human skin and hair color, and is heterogeneous biopolymer which plays an important role in prevention of sun-related skin injury. Melanin is produced by melanocytes through a process called melanogenesis in the basal layer of epidermis.[1] However, the excessive formation of melanin in different specific parts of the human skin can causes diverse hyperpigmentary disorders such as melasma, freckles, age spots or liver spots, and actinic damage.[2–7] The biosynthesis of melanin involves series of enzymatic catalyzed and chemical reaction. Among them, tyrosinase has attracted the much attention and it plays important role in melanogenesis. Tyrosinase is a copper bearing enzyme which widely distributed in nature. It catalyze two step pathways involving molecular oxygen in the formation of melanin; the hydroxylation of phenols to *o*-catechols, and the oxidation of *o*-catechols to *o*-quinones, which further polymerize spontaneously at physiological pH into melanin. Tyrosinase is also associated to Parkinson and other neurodegenerative diseases, oxidizing excess dopamine to DOPA quinone, highly reactive compound that induce neuronal damage and cell death. Therefore, the inhibition of tyrosinase is expected to provide effective therapeutic means for the treatment of diseases related to melanogenesis and dermatological disorders. Regarding this context, several inhibitors of melanogenesis have been reported in the literature[8–18] but their usage is being compromised due to their harmful adverse effects and moreover only few of them are successfully inhibiting the melanogenesis in melanoma cell or in skin model. Therefore, the current therapies are considered to be inadequate for these conditions. Taking this consideration into an account, the search for new melanogenesis inhibitor from natural product compounds and synthetic compounds with such activity is still continues.[19–21]

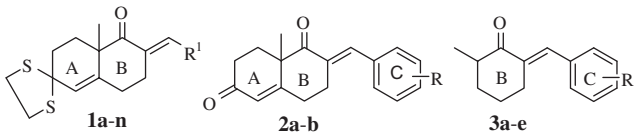
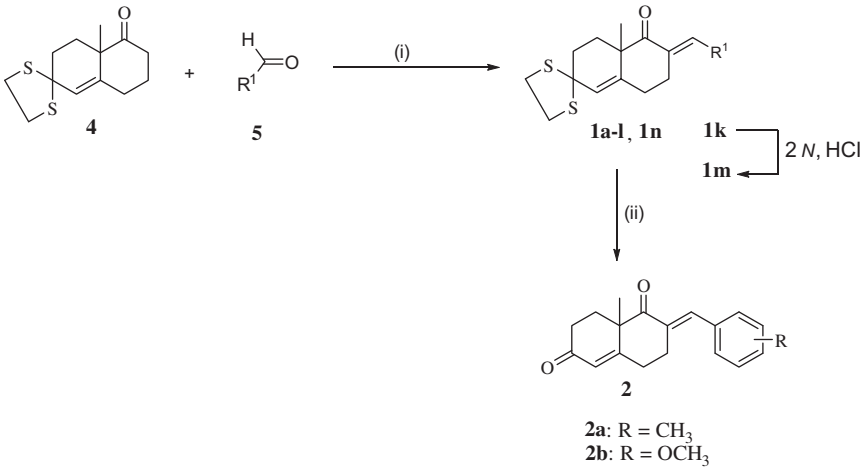


Chart 1. Target structures **1a–n**, **2a–b** and **3a–e** for the antimelanogenesis activity. Note = Substituents are indicated in Tables 1 and 2.

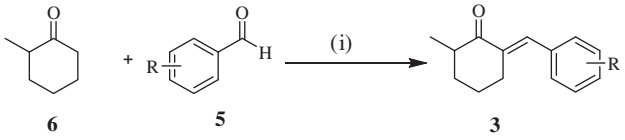
Notably, chalcone derivatives are very versatile as physiologically active and common structure in numerous natural products belonging to the flavonoid family.[22–24] These compounds have been reported to possess several biological activities, such as cytotoxic,[25] anti-malarial,[26] antileishmanial,[27]anti-inflammatory,[28] anti-HIV,[29] antifungal[30] and tyrosinase inhibitors.[31] Having such pharmacological activities, this scaffold has attracted medicinal chemist and prompted us to investigate potent inhibitors of the melaninogenesis. As we engaged to identify the new analog with chalcone type derivatives, compound **1a** (Chart 1 and 85% inhibition at 10 μM, IC_{50} = 10.5 μM) was discovered as melanogenesis inhibitor in melanoma B16 cells under the stimulant of

* Corresponding author.
E-mail address: jungshh@cnu.ac.kr (S.-H. Jung).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.02.060



Scheme 1. Synthesis of the target structures **1** and **2**. Regents and condition: (i) KOH, R^1-CHO (ii) NCS, silver nitrate. Note = substituents (R^1) are indicated in Table 1.



Scheme 2. Synthesis of target structure **3**. Regents and condition: (i) KOH, ethanol/water (90:10), reflux, 12 h; Note = substituents (R) are indicated in Table 2.

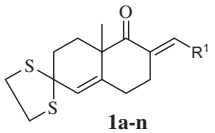
α-melanocyte stimulating hormone (α-MSH) by random screening process. Interestingly, compound **1a** has approximately 8 or 12 times more potent in inhibition than kojic acid and arbutin. Therefore in our present study, we synthesized a series of analogs of **1a** for exploring their preliminary structure–activity relationship on the inhibitory activity of α-MSH-induced melanogenesis in melanoma B16 cell line. The general chemical structures of lead **1a** with their analogs **1b**–**n**, **2a**–**b** and **3a**–**e** have been indicated in Chart 1.

The synthesis of compounds **1**, **2** was outlined in Scheme 1. The expected compound **1** was obtained by the aldol condensation reaction of **4**[32] with various commercial aldehydes **5** in the presence of potassium hydroxide in 90% ethanolic solution under refluxing condition.[33] Subsequently, the conversion of compounds **1** to corresponding compounds **2** by the treatment of *N*-chlorosuccinamide and silver nitrate.[34] The compounds **3** were prepared according to known procedure[35–39] as indicated in Scheme 2.

For all synthesized analogs **1**, **2**, **3** and **4**, the ability to inhibit the formation of melanin from melanoma B16 cells was determined under stimulus of α-MSH (100 nM) for 3 day incubation.[12] The melanoma B16 cells (CRL6323) was 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 mean values from 3 to 5 separate experiments as shown in Tables 1 and 2.

In order to explore the structure activity relationship of **1a** (85% at 10 μM, *C* log *P* = 4.609) in the inhibitory melanogenesis activity, a series of analogs **1b**–**n**, **2a**–**b** and **3a**–**e** were evaluated. Therefore, to investigate the effect of substitution on phenyl ring of **1a**, the various substituents were studied as shown in Table 1. Primarily, the hydrophobic substituents at *p*-position like methyl (**1b**, >100% inhibition at 10 μM, IC_{50} = 17.1 μM, *C* log *P* = 5.108), ethyl

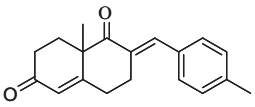
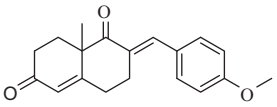
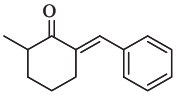
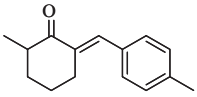
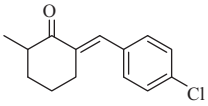
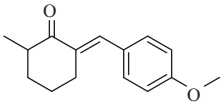
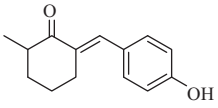
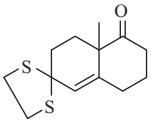
Table 1
Inhibitory activity of **1a**–**n** for their antimelanogenesis in melanoma B16 cell line



Entry No	Compound No	R^1	% of Inhibition at 10[a] (μM)	IC_{50} value (μM)	*C* log *P*[b] value
1	**1a**	C_6H_5	85	10.5	4.609
2	**1b**	4-$(CH_3)C_6H_5$	>100	17.1	5.108
3	**1c**	4-$(CH_2CH_3)C_6H_5$	80	18.5	5.637
4	**1d**	4-$(C(CH_3)_3)C_6H_5$	83	13.5	6.435
5	**1e**	4-$(Cl)C_6H_5$	35	>30	5.322
6	**1f**	3,4-$(Cl)_2C_6H_5$	62	25.5	5.915
7	**1g**	1-Naphthyl	85	11.5	5.783
8	**1h**	(–CH=CH)C_6H_5	75	16.5	5.063
9	**1i**	4-$(OCH_3)C_6H_5$	>100	3.5	4.528
10	**1j**	3,4-$(OCH_3)_2C_6H_5$	43	>30	4.267
11	**1k**	4-$(OCH_2OCH_3)C_6H_5$	92	6.0	4.043
12	**1l**	4-$(OCH_2Ph)C_6H_5$	80	13.5	6.239
13	**1m**	4-$(OH)C_6H_5$	96	4.3	3.942
14	**1n**	4-$(N(CH_3)_3)C_6H_5$	65	7.6	4.744
	Arbutin			120	

[a] The percentage of inhibition values obtained as a mean from 3 to 5 independent experiments.
[b] *C* log *P* values are obtained from Chemdraw 11.0 V.

Table 2
Inhibitory activity of **2a–b**, **3a–e** and **4** for their antimelanogenesis in melanoma B16 cell line

Entry No	Compound No	Structure[a]	% of inhibition at 10[b] (μM)	IC_{50} value (μM)	*C* log *P*[c] value
1	**2a**		60	23	3.312
2	**2b**		72	6.0	2.552
3	**3a**[35]		<10	>30	3.727
4	**3b**		<10	>30	4.226
5	**3c**[36]		<10	>30	4.440
6	**3d**[37]		<10	>30	3.646
7	**3e**[39]		<10	>30	3.06
8	**4**[32]		<10	>30	2.266

[a] The substituents of **3a–e** are directly indicated with complete structure.
[b] The percentage of inhibition values obtained as a mean from 3 to 5 independent experiments.
[c] *C* log *P* values are obtained from Chem Draw 11.0 V.

(**1c**, 80% inhibition at 10 μM, IC_{50} = 18.5 μM, *C* log *P* = 5.637) and *tert*-butyl group (**1d**, 83% inhibition at 10 μM, IC_{50} = 13.5 μM, *C* log *P* = 6.435) were introduced. However, the activity does not altered with the respect of increasing hydrophobic bulky group on the phenyl ring of these analogs, which indicates that the hydrophobic substituent should not be required to enhance the activity. In addition to that, the *p*-chloro (**1e**, 35% inhibition at 10 μM, IC_{50} >30 μM, *C* log *P* = 5.322) and 3,4-dichloro group (**1f** 62% inhibition at 10 μM, IC_{50} = 25.5 μM, *C* log *P* = 5.915) substitutions were noticeably dropped the activity. This suggests that the substituents with electron withdrawing property must have the adverse effect for the activity of **1a**. In the next set of experiment, to examine the effect of size of this planar moiety, phenyl (ring C), was replaced with naphthyl group (**1g**, 85% inhibition at 10 μM, IC_{50} = 11.5 μM, *C* log *P* = 5.783), which results in dramatic loss of activity. Therefore the enlargement of aromatic ring in this region is obviously unfavorable for the effect on anti-melanogenesis activity. Since α,β-unsaturated system in chalcone has an essential role, the extended conjugation with one more vinyl as shown in **1h** (75% inhibition at 10 μM, IC_{50} = 16.5 μM, *C* log *P* = 5.063) was demonstrated. However, it seems that such elongation does not contribute for the activity improvement.

Thus in the next set of experiment, the electron donating groups on phenyl ring (ring C) were introduced. As a result, the activity of *p*-methoxy analog (**1i**, >100% inhibition at 10 μM, IC_{50} = 3.5 μM, *C* log *P* = 4.528) was markedly increased the antimelanogenesis activity. This result encouraged us to prove further, hence the following 3,4-dimethoxy (**1j**, 43% inhibition at 10 μM, IC_{50} >30 μM, *C* log *P* = 4.267), methoxymethoxy (**1k**, 92% inhibition at 10 μM, IC_{50} = 6.0 μM, *C* log *P* = 4.043), benzyloxy (**1l**, 80% inhibition at 10 μM, IC_{50} = 13.5 μM, *C* log *P* = 6.239), hydroxyl (**1m**, 86% inhibition at 10 μM, IC_{50} = 4.3 μM, *C* log *P* = 3.942) and *N*,*N*[1]-dimethylamino (**1n**, 66% inhibition at 10 μM, IC_{50} = 7.6 μM, *C* log *P* = 4.744) derivatives were prepared and evaluated for their inhibitory activity. As results, **1k**, **1m** and **1h** were exhibited comparable activity to each other and more potent than **1a**. From this study, it could be suggested that the electron donating property of the substituents should be favorable to achieve the inhibitors with high potential. However, the inactivity of compound **1j** bearing with 3,4-dimethoxy may due to serious steric hindrance of methoxy at 3-position, which hampers the binding to the putative receptor. Considering the *C* log *P* values of this series **1a–n**, their hydrophobicity do not affect the activity as indicated in Table 1.

In the continuation of SAR study, the beneficial role of dithiolane motif of **1a** was explored by comparison of **2a** (60% inhibition at 10 μM, IC_{50} = 23 μM, *C* log *P* = 3.312) and **2b** (72% inhibition at 10 μM, IC_{50} = 6.0 μM, *C* log *P* = 2.552) with their corresponding analogs **1b** and **1i**. As results, the **2a** and **2b** were nearly twofold reduce the activity. This implies that the bulky dithiolane moiety must be highly favorable for the activity enhancement.

Further, we confirmed the importance of ring A of **1a** by screening a series of monocyclic α,β-unsaturated ketones such as **3a** (<10% inhibition at 10 μM, IC_{50} >30 μM), **3b** (<10% inhibition at 10 μM, IC_{50} >30 μM), **3c** (<10% inhibition at 10 μM, IC_{50} >30 μM), **3d** (<10% inhibition at 10 μM, IC_{50} >30 μM) and **3e** (<10% inhibition at 10 μM, IC_{50} >30 μM). None of them showed any inhibition, which implies that the ring A is very important for the inhibitory activity of **1a**. Additionally, to prove the structural necessity of benzylidene system of **1a**, the activity of compound **4** (<10% inhibition at 10 μM, IC_{50} >30 μM, *C* log *P* = 2.266) was examined. As expectedly, the result was end up with no inhibition even at 100 μM concentration. This strongly suggests that the activity was mainly mediated by both rings A and B associate with α,β-unsaturated system in **1**.

In summary, the compound **1a** was discovered as novel melanogenesis inhibitor in melanoma B16 cells under the stimulant of α-MSH. In order to improve the activity, a series of analogs such as **1b**–**n**, **2a**–**b** and **3a**–**e** were evaluated for their preliminary structure–activity relationship (SAR) study. Accordingly the following structural requirements could be considered for the activity of **1**; (i) the electron donating group on phenyl ring (ring C) rather than electron withdrawing group, (ii) the variation in hydrophobicity (*C* log *P*) does not have an impact on the activity, (iii) the both rings A and B associates with α,β-unsaturated system were mainly important for the activity and (iv) dithiolone moiety must be important for the activity enhancement.

Acknowledgements

This work was supported by a Grant (R01-2007-000-20099-0) and Priority Research Centers Program (2009-0093815) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology.

Supplementary data

Supplementary data (experimental procedure) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.060.

References and notes

1. Hearing, V. J.; Jimenez, M. *Pigment Cell Res.* **1989**, *2*, 75.
2. Lee, N. K.; Son, K. H.; Chang, H. W.; Kang, S. S.; Park, H.; Heo, M. Y.; Kim, H. P. *Arch. Pharmacol. Res.* **2004**, *27*, 1132.
3. Curto, E. V.; Wong, C. K.; Hermersdorfer, H.; Glatt, H.; Santis, C.; Virador, V.; Hearing, V. J.; Dooley, T. P. *Biochem. Pharmacol.* **1999**, *57*, 663.
4. Solano, F.; Briganti, S.; Picardo, M.; Ghanem, G. *Pigment Cell Res.* **2006**, *19*, 550.
5. Meredith, P.; Riesz, J. *Photochem. Photobiol.* **2004**, *79*, 211.
6. Beradesca, E.; Cameli, N.; Primavera, G.; Carrera, M. *Dermatol. Surg.* **2006**, *32*, 526.
7. Yoshimura, K.; Sato, K.; Aiba-Kojima, E.; Matsumoto, D.; Machino, C.; Nagase, T.; Gonda, K.; Koshima, I. *Dermatol. Surg.* **2006**, *32*, 365.
8. Kubo, I.; Nihei, K.; Tsujimoto, K. *Bioorg. Med. Chem.* **2004**, *12*, 5349.
9. Lee, C. W.; Son, E. M.; Kim, H. S.; Xu, P.; Batmunkh, T.; Lee, B. J.; Koo, K. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5462.
10. Tokiwa, Y.; Kitagawa, M.; Raku, T.; Yanagitanib, S.; Yoshino, K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3105.
11. Thanigaimalai, P.; Hoang, T. A. L.; Lee, K. C.; Bang, S. C.; Sharma, V. K.; Yun, C. Y.; Roh, E.; Hwang, B. Y.; Kim, Y.; Jung, S. H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2991.
12. Thanigaimalai, P.; Lee, K. C.; Bang, S. C.; Lee, J. H.; Yun, C. Y.; Roh, E.; Hwang, B. Y.; Kim, Y.; Jung, S. H. *Bioorg. Med. Chem.* **2010**, *18*, 1555.
13. Yamazaki, Y.; Kawano, Y.; Yamanaka, A.; Maruyama, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4178.
14. Nakashima, S.; Matsuda, H.; Oda, Y.; Nakamura, S.; Xu, F.; Yoshikawa, M. *Bioorg. Med. Chem.* **2010**, *18*, 2337.
15. Criton, M.; Mellay-Hamon, V. L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3607.
16. Matsuda, H.; Nakashima, S.; Oda, Y.; Nakamura, S.; Yoshikawa, M. *Bioorg. Med. Chem.* **2009**, *17*, 6048.
17. Park, J. S.; Kim, D. H.; Lee, J. K.; Lee, J. Y.; Kim, D. H.; Kim, H. K.; Lee, H. J.; Kim, H. C. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1162.
18. Okombi, S.; Rival, D.; Bonnet, S.; Mariotte, A. M.; Perrierb, E.; Boumendjel, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2252.
19. Kubo, I.; Kinst-Hori, I.; Kubo, Y.; Yamagiwa, Y.; Kamikawa, T.; Haraguchi, H. *J. Agric. Food. Chem.* **2000**, *48*, 1393.
20. Nihei, K.; Yamagiwa, Y.; Kamikawa, T.; Kubo, I. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 681.
21. Khatib, S.; Nerya, O.; Musa, R.; Samuel, M.; Tamir, S.; Vaya, J. *Bioorg. Med. Chem.* **2005**, *13*, 433.
22. Nowakowska, Z. *Eur. J. Med. Chem.* **2007**, *42*, 125.
23. Akihisa, T.; Tokuda, H.; Hasegawa, D.; Ukiya, M.; Kimura, Y.; Enjo, F.; Suzuki, T.; Nishino, H. *J. Nat. Prod.* **2006**, *69*, 38.
24. Narender, T.; Tanvir, S. K.; Srinivasa Rao, M.; Srivastava, K.; Puri, S. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2453.
25. Modzelewska, A.; Pettit, C.; Achanta, G.; Davidson, N. E.; Huang, P.; Khan, S. R. *Bioorg. Med. Chem.* **2006**, *14*, 3491.
26. Dominguez, J. N.; Leon, C.; Rodrigues, J.; Gamboa de Dominguez, N.; Gut, J.; Rosenthal, P. J. *J. Med. Chem.* **2005**, *48*, 3654.
27. Nielsen, S. F.; Christensen, S. B.; Cruciani, G.; Kharazmi, A.; Liljefors, T. *J. Med. Chem.* **1998**, *41*, 4819.
28. Lee, S. H.; Seo, G. S.; Kim, J. Y.; Jin, X. Y.; Kim, H. D.; Sohn, D. H. *Eur. J. Pharmacol.* **2006**, *532*, 178.
29. Cheenpracha, S.; Karalai, C.; Ponglimanont, C.; Subhadhirasakul, S.; Tewtraku, S. *Bioorg. Med. Chem.* **2006**, *14*, 1710.
30. Svetaz, L.; Tapia, A.; Lopez, S. N.; Furlan, R. L. E.; Petenatti, E.; Pioli, R.; Schmeda-Hirschmann, G.; Zacchino, S. A. *J. Agric. Food. Chem.* **2004**, *52*, 3297.
31. Jun, N.; Hong, G.; Jun, K. *Bioorg. Med. Chem.* **2007**, *15*, 2396.
32. Shah, N.; Scanlan, T. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5199.
33. Thanigaimalai, P.; Hoang, L. T. A.; Lee, K. C.; Sharma, V. K.; Bang, S. C.; Yun, J. H.; Roh, E.; Kim, Y.; Jung, S. H. *Eur. J. Med. Chem.* **2010**, *45*, 2531.
34. Wenkert, E.; Moeller, Peter D. R. *Isr. J. Chem.* **1989**, *29*, 143.
35. Kozlov, N. G.; Basalaeva, L. I. *Russ. J. Org. Chem.* **2009**, *45*, 587.
36. Popkov, S. V.; Talismanov, V. S. *Russ.* **2008**, 8pp. CODEN: RUXXE7 RU 2326878 C1 20080620.
37. Gar, M. M.; Arkhipova, O. N.; Popkov, S. V. *Agrokhimiya* **2009**, *6*, 40.
38. Sane, P. P.; Rao, A. S. *Indian J. Chem.* **1975**, *13*, 1005.
39. Lub, J.; Ten Hoeve, W.; Nijssen, W. P. M.; Diaz, L.; Wegh, R. T. *Liq. Cryst.* **2002**, *29*, 995.