JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 1655–1660

Received May 6, 2003
Accepted July 22, 2003
DOI 10.1211/0022357022250
ISSN 0022-3573

Free radical scavenging and antioxidative activity of melatonin derivatives

Pen-Lin Tsia and Ming-Kuan Hu

Abstract

This article describes the synthesis and antioxidative properties of melatonin derivatives. Tryptamines and cysteinyl or mercaptopropionyl derivatives were deliberately condensed with coupling reagents to give melatonin derivatives **4a–d** and **5a**, **b**. The preliminary evaluation indicated that compound **4c** showed improved scavenging activity compared with vitamin C (IC50 43 μM vs 65 μM, where IC50 is the concentration of the test compound that induced a change of 50% in absorbance during the 30 min observation) on diphenyl-*p*-picrylhydrazyl (DPPH) tests. Derivative **5b**, which possesses the thiolactyl moiety, showed moderate potency compared with melatonin (IC50 235 μM vs 690 μM) in the H_2O_2 scavenging test. Intriguingly, **4c** displayed 2-fold more potency than melatonin (IC50 51 μM vs 125 μM) in scavenging NO in the macrophage model. These results suggested that the cysteinyl-conjugated derivative **4c** may be a suitable lead to further optimize potent antioxidants for certain oxidative stress conditions.

Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine), a highly conserved naturally occurring molecule, is a pineal hormone that regulates circadian rhythms and also protects against oxidative stress-induced tissue damage, lipid peroxidation and apoptosis (Reiter et al 1994; Abuja et al 1997). Recent studies pointed out that melatonin possesses potent hydroxyl and peroxy radical scavenging activity and is a broad-spectrum antioxidant (Chan & Tang 1996). In addition to its direct reactive oxidant scavenging action, melatonin also inhibits peroxynitrite-induced oxidative reactions or direct peroxynitrite inhibition in-vitro (Poeggeler et al 1994).

Investigation of the radioprotective property of systemically administered sulfhydryl compounds, such as WR-2721, *N*-acetylcysteine and cysteamine, has revealed that these sulfhydryl-containin g compounds were shown to offer significant protection against skin damage (Verhey & Sedlacek 1983). On the other hand, some natural thiols may provide a longer window of protection against an excess of reactive oxygen species produced by illness, ageing or UV exposure (Weiss & Landauer 2000; Torres et al 2002). These results suggested that the sulfhydryl pharmacophore was the critical moiety for scavenging of free radicals as a rapid response to the environment. Since melatonin is ubiquitously present in organisms whose metabolism is based on oxygen, it has been speculated that simple modification of the chemical structure of melatonin might provide potent and selective antioxidants for certain free-radical-mediated conditions. In this study, melatonin derivatives were manipulated and their scavenging effects on free radicals and oxygen-derived reactive species were investigated to provide further information on the development of potent antioxidative and radioprotective agents.

Materials and Methods

Chemistry

All chemicals and reagents were commercial materials and were used directly unless otherwise noted. Nomenclature and symbols of abbreviations for some reagents

School of Pharmacy, National Defense Medical Center, 161 Minchuan East Road, Section 6, Taipei 114, Taiwan

Pen-Lin Tsia, Ming-Kuan Hu

Correspondence: M.-K. Hu, School of Pharmacy, National Defense Medical Center, Taipei 114, Taiwan. E-mail: hmk@ndmctsgh.edu.tw

Acknowledgement and funding: The authors are grateful to the National Science Council of the Republic of China for financial support (NSC 91-2623-7-016-003-NU). High-resolution mass spectra by the National Science Council Regional Instruments at National Chenggung University are also appreciated.

used here are: BOP, benzotriazole-1-yloxy-tris(dimethylamino)-phosphonium hexafluorophosphate; DIEA, diisopropylethylamine; DMF, dimethylformamide; EDCI, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide; HOBt, 1-hydroxybenzotriazole monohydrate; LPS, lipopolysaccharide. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini at 300 MHz for ^{1}H and at 75 MHz for ^{13}C. Fast atom bombardment mass spectra (FABMS) and relative high-resolution spectra were obtained on a JEOL JMS-300 spectrometer. Reactions were followed by thin-layer chromatography (TLC) on Merck (0.2 mm) aluminium-packed pre-coated silica gel plates (60 F_{254}). For compound visualization, 4% phosphomolybdic acid in ethanol was employed with heating. Chromatography refers to flash chromatography on silica gel (silica gel 60, 230–400 mesh ASTM, E. Merck). Melting points were recorded on a Thomas Hoover capillary melting point apparatus in open capillary tubes and are uncorrected.

General procedure for amine and acid coupling using EDCI/HOBt or BOP/DIEA as activating reagents

A solution of acid (1.38 mmol, 1.1 eq) and amine (1.25 mmol, 1.0 eq) in DMF (4 mL) was cooled in an ice bath under an inert atmosphere. The mixture was added with the coupling reagent (1.88 mmol, 1.5 eq) and DIEA (2.5 mmol, 2 eq) and stirred for an additional 24 h at room temperature. The resulting mixture was diluted with ethyl acetate (EtOAc) and extracted with water. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to give, after silica gel chromatography, the title compound.

N′-(tert-*Butyloxylcarbonyl)-L-cysteinyltryptamide* (***4a***) Tryptamine (0.4 g, 2.5 mmol, 1.0 eq), and Boc-cysteine (0.61 g, 2.75 mmol, 1.1 eq) were condensed with BOP to yield, after workup and purification on silica gel (EtOAc–*n*-hexane, 1:1 as eluent), a yellow solid (0.26 g, 29%): mp 83–85 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (s, 1H, NH), 7.51–7.35 (m, 2H, Ar-H), 7.04–7.19 (m, 2H, Ar-H), 3.59 (m, 2H, CH_2), 2.94 (s, 2H, β-CH_2), 2.17 (m, 2H, CH_2), 1.80 (1H, s, SH), 1.45 (9H, s, 3 CH_3); EIMS: m/z 363 $[M]^+$; HR-EIMS: exact mass calcd for $C_{18}H_{25}N_3O_3S$ $[M]^+$ 363.1619; found 363.1617.

N′-(tert-*butyloxylcarbonyl)-S-benzyl-L-cysteinyl-tryptamide* (***4b***) Tryptamine (0.2 g, 1.25 mmol, 1.0 eq) and Boc-Cys(Bzl)-OH (0.43 g, 1.38 mmol, 1.1 eq) were condensed with EDC/HOBt to yield, after workup and purification on silica gel (CH_2Cl_2–MeOH, 20:1 as eluent), a pale brown solid (0.47 g, 83%): mp 132–134 °C; Rf = 0.67 (CH_2Cl_2–MeOH, 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (1H, m, NH), 7.60 (d, J = 7.8 Hz, 1H, Ind-H); 7.36 (d, J = 8.1 Hz, 1H, Ind-H), 7.28 (m, 5H, Ar-H), 7.03 (s, 1H, Ind-H) 6.29 (m, 1H, Ind-H), 5.24 (m, 1H, NH), 4.16 (m, 1H, α-CH), 3.69 (d, J = 2.7 Hz, 2H, CH), 3.59(q, J = 6.1 Hz, 2H, CH_2) 2.94 (t, J = 5.6 Hz, 2H, CH_2), 2.71(m, 2H, CH_2), 1.42 (s, 9H, 3CH_3); EIMS: m/z 453 $[M]^+$, HR-EIMS: exact mass calcd for $C_{25}H_{31}N_3O_3S$ $[M]^+$ 453.2088; found 453.2085.

N′-(tert-*butyloxylcarbonyl)-L-cysteinyl-(5-methoxy)-tryptamide* (***4c***) 5-Methoxytryptamine (0.1 g, 0.53 mmol, 1.0 eq) and Boc-cysteine (0.13 g, 0.58 mmol, 1.1 eq) were condensed with BOP to yield, after workup and purification on silica gel (CH_2Cl_2–MeOH, 6:1 as eluent), a yellow solid (0.16 g, 77%): mp 81–83 °C, Rf = 0.32 (EtOAc–*n*-hexane, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (m, 1H, Ind-NH), 7.01 (m, 1H, Ind-H), 6.86 (m, 2H, Ind-H), 5.24 (m, 1H, NH), 4.16 (m, 1H,α-CH), 3.86 (s, 3H, -OCH_3), 3.59 (m, 2H, CH_2), 2.97, 2.90 (s, 2H, CH_2), 2.76 (d, J = 9.84 Hz, 2H, CH_2), 1.70 (s, 1H, SH), 1.45 (s, 9H, 3CH_3); EIMS: m/z 393 $[M]^+$; HR-EIMS: exact mass calcd for $C_{19}H_{27}N_3O_4S$ $[M]^+$ 393.1724; found 393.1721.

N′-(tert-*butyloxylcarbonyl)-S-benzyl-L-cysteinyl-(5-methoxy)tryptamide* (***4d***) 5-Methoxytryptamine (0.1 g, 0.53 mmol, 1.0 eq) and Boc-Cys(Bzl)-OH (0.18 g, 0.58 mmol, 1.1 eq) were condensed with EDC/HOBt to yield, after workup and purification on silica gel (CH_2Cl_2–MeOH, 9:1 as eluent), a brown solid (0.24 g, 94%): mp 93–95 °C; Rf = 0.85 (CH_2Cl_2–MeOH, 6:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (m, 1H, NH), 7.02 (d, J = 4.6 Hz, 1H, Ind-H), 7.01(s, 1H, Ind-H), 7.26–7.28 (m, 5H, Ar-H), 6.88 (dd, J = 2.43, 2.73 Hz, 1H, Ind-H), 6.30 (m, 1H, Ind-H), 5.26 (m, 1H, NH), 4.17 (m, 1H, α-CH), 3.87 (s, 3H, -OCH_3), 3.69 (d, J = 2.9 Hz, 2H, CH_2), 3.58 (q, J = 6.3 Hz, 2H, CH_2), 2.94 (t, J = 6.5 Hz, 2H, CH_2), 2.86–2.73 (m, 2H, CH_2), 1.42 (s, 9H, 3CH_3); FABMS (NBA as matrix): m/z 483.2 $[M+H]^+$; HR FABMS: exact mass calcd for $C_{26}H_{33}N_3O_4S$ $[M+H]^+$ 483.2194; found 483.2191.

2-Mercaptopropionyl-tryptamide (***5a***) Tryptamine (1.0 g, 6.24 mmol, 1.0 eq) and 2-mercaptopropionic acid (0.6 mL, 6.86 mmol, 1.1 eq) were condensed with BOP (4.14 g, 9.36 mmol, 1.5 eq) to yield, after workup and purification on silica gel (CH_2Cl_2–MeOH, 20:1 as eluent), a brown solid (0.26 g, 17%): mp 81–83 °C; Rf = 0.41 (*n*-hexane–EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (s, 1H, NH), 7.62 (d, J = 7.7 Hz, 1H, Ind-H), 7.39 (d, J = 8.1 Hz, 1H, Ind-H), 7.25–7.13 (m, 1H, Ind-H), 7.04 (s, 1H, Ind-H), 6.54 (s, 1H, Ind-H), 3.60 (q, J = 6.0 Hz, 2H, Ind-CH_2), 3.35 (m, 1H, α-CH), 2.99 (t, J = 6.6 Hz, 2H, CH_2), 1.92 (d, J = 8.3 Hz, 1H, SH), 1.51 (d, J = 7.0 Hz, 3H, CH_3); EIMS: m/z 248.3 $[M]^+$, HR-EIMS: exact mass calcd for $C_{13}H_{16}N_2OS$ $[M]^+$ 248.3027; found 248.3022.

2-Mercaptopropionyl-(5-methoxy)tryptamide (***5b***) 5-Methoxytryptamine (0.1 g, 0.53 mmol, 1.0 eq) and 2-mercaptopropionic acid (0.05 mL, 0.58 mmol, 1.1 eq) were condensed with BOP to yield, after workup and purification on silica gel (CH_2Cl_2–MeOH, 6:1 as eluent), a brown solid (0.03 g, 21%), Rf = 0.43 (n-hexane–EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (s, 1H, NH), 7.30 (d, J = 8.7 Hz, 1H, Ind-H), 7.05 (s, 1H, Ind-H), 6.90 (d, J = 8.8 Hz, 1H, Ind-H), 6.56 (s, 1H, Ind-H), 3.88 (s, 3H, -OCH_3), 3.60 (q, J = 5.7 Hz, 2H, CH_2), 3.39 (m, 1H, α-CH), 2.98 (t, J = 6.6 Hz, 2H, CH_2), 1.95 (d, J = 8.1 Hz,

1H, SH), 1.53 (d, J = 7.1 Hz, 3H, CH_3); EIMS: m/z 278.3 $[M]^+$; HR-EIMS: exact mass calcd for $C_{14}H_{18}N_2O_2S$ $[M]^+$ 278.3638; found 278.3635.

Pharmacological evaluation

Free radical scavenging actions
An ethanolic solution of the stable nitrogen-centred free radical DPPH (50 μM) was incubated with a variety of concentrations of the test compounds, and the absorbance was monitored spectrometrically at 517 nm (Mellors & Tappel 1966). The concentration (IC50) of the test compound that induced a change of 50% in absorbance during the 30 min observation was taken as the free radical scavenging potency.

H_2O_2 assay
The method is based on an H_2O_2-dependent, horseradish peroxidase (HRP)-mediated oxidation of phenol red, which results in the appearance of a derivative with an absorbance at 610 nm (Pick & Keisari 1980; Tan et al 2000). HRP was dissolved in phosphate-buffered saline (PBS) to make a final concentration of 6.5 $\mu g\,mL^{-1}$ and the concentration of phenol red in PBS was fixed at 0.1 $\mu g\,mL^{-1}$.

Time course of interaction between melatonin and H_2O_2 The concentration of H_2O_2 was fixed at 25 μM. The melatonin concentration was fixed at 200 μM (the concentration of melatonin that would scavenge roughly 15% of the H_2O_2 as indicated). The IC50 of melatonin is 690 μM.

Measurement of interaction between phenol red and H_2O_2 For the control panel, a mixture containing 500 μL PBS, 480 μL phenol red and 10 μL HRP was kept in the dark for 5 min and then treated with 10 μL of NaOH before it was observed under a UV/visible spectrophotometer.

The reaction mixture consisted of 400 μL PBS, 480 μL phenol red, 100 μL H_2O_2 (25 μM), and 10 μL HRP and kept in the dark for 5 min followed by treating with 10 μL NaOH. These samples were scanned at a wavelength of 320~720 nm and the difference of absorbance between phenol red and oxidized phenol red was observed at 610 nm.

Assay for the tested compound The tested compound was dissolved in ethanol to give a 25 mM stock solution ready to be used with dilution to the gradient concentrations. The reaction mixture consisted of 300 μL PBS, 480 μL phenol red, 100 μL H_2O_2 (25 μM), 10 μL HRP, and 100 μL of the tested compound solution. The resulting mixture was kept in the dark for 5 min and then treated with 10 μL of NaOH to give the final gradient concentrations (e.g. 0, 19, 39, 78, 156, 312, 625, 1250, 2500 μM). The data of absorbance was determined using a Lambda 40 UV/visible spectrophotometer at 610 nm.

Nitric oxide (NO) scavenging test
Determination of nitrite concentration was carried out by the method of Noda et al (1999).

The cell line (RAW 264.7) was incubated in Dulbecco's modified eagle's medium (DMEM) in a 5% CO_2 incuba-

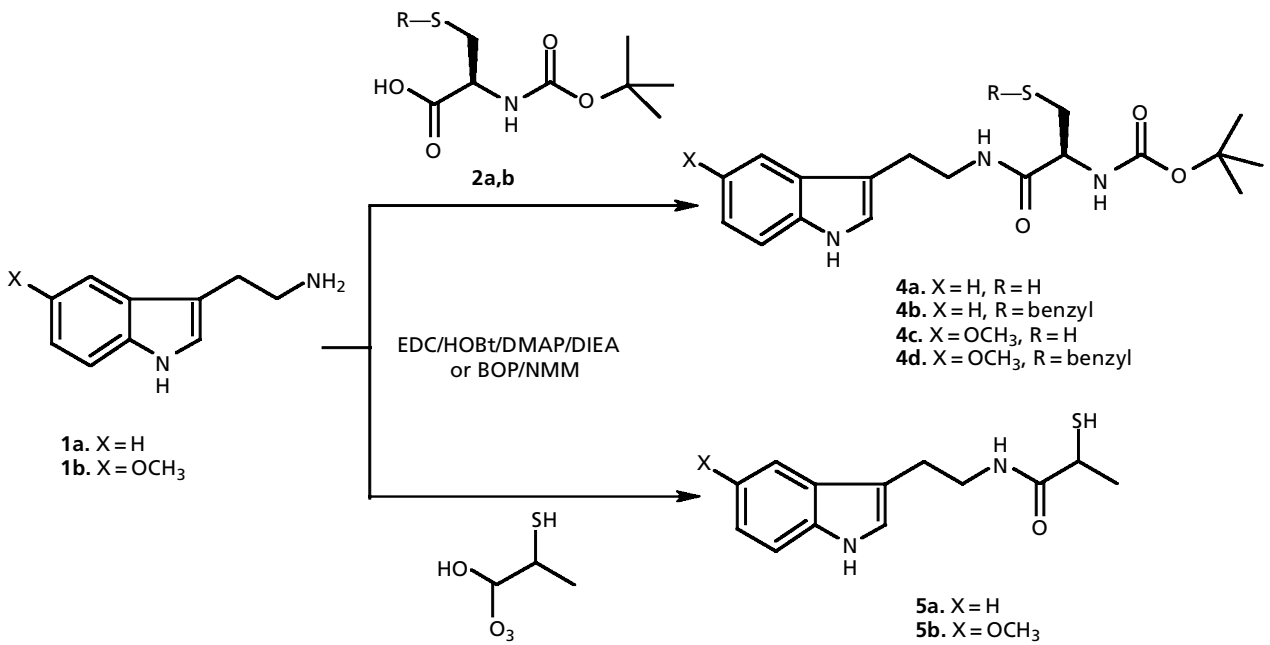


Figure 1 Synthesis of the sulfhydryl-containing melatonin derivatives **4a–d** and **5a**, **b**. EDCI/HOBt or BOP/DIEA were used as activating reagents.

tor (37 °C) and transferred to a 96-well microplate. NO production in culture supernatants was assessed by measuring nitrite, its stable degradation product, using Griess reagent as described by Chen and co-workers (Chen et al 1999). The DMEM medium was changed to phenol-red-free medium before the cells were stimulated for 24 h with 1 μg mL^{-1} LPS. The test compound was dissolved in DMSO as a stock solution. The gradient concentration of the test compound was diluted with DMEM and transferred to the prepared well and incubated in 5% CO_2 conditions. After 24 h, the well was treated with Griess reagent and observed at 550 nm in a microplate reader. $NaNO_2$ was used as standard. The final NO content was calculated as compared with standard curve.

Statistical analysis

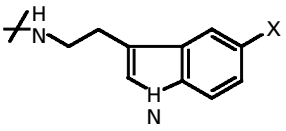
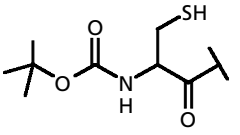
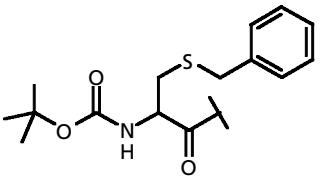
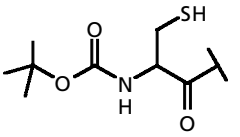
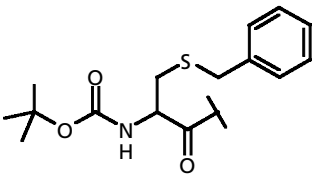
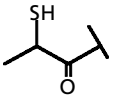
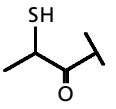
The data were presented as mean ± s.d. for the number of experiments indicated in the legends. The effects of the various synthesised compounds on the biological outcome was statistically examined using a one-way analysis of variance. Dunnett's test was used to compare individual compounds. In all cases, $P < 0.05$ was accepted to denote significance.

Results and Discussion

The synthesis of the sulfhydryl-containing melatonin derivatives **4a**–**d** and **5a**, **b** was carried out by using EDCI/HOBt or BOP/DIEA as activating reagents (Figure 1). The conventional coupling reagent, EDCI, gave moderate yields for the amide formation, while with free sulfhydryl group, compounds **2a** and **3** showed low efficiency for the coupling reaction (yields, 17∼35%) to give **4a** and **5a**, **b** even using BOP as carboxyl-activating reagent. These resulting cysteinyl-containing derivatives of melatonin were characterized by their satisfactory spectra (^{1}H NMR, MS and HR-FABMS).

A series of in-vitro tests on antioxidizing effects were carried out for further access to the selective profile on free-radical scavenging activity. To evaluate and provide direct evidence about the antioxidative reactivity of cysteinyl-containing derivatives of melatonin, the scavenging DPPH free radicals model was used. IC50 values were determined for the tested compounds (a decrease of 50% of absorbance compared with the control). Compound **4c** was more potent than vitamin C (IC50 43 μM vs 65 μM) (Table 1). Structurally, **4c** possesses an extra free thiol conjugated to the melatonin moiety, and this conjugation of the pharmacophores might be critical for its improved scavenging activity. Antioxidative properties of melatonin, in part, may involve a direct effect on scavenging of reactive oxygen species (Zang et al 1998). Therefore, these synthetic compounds were observed for their scavenging effects on H_2O_2. Intriguingly, compounds **4c** and **5a**, **b** showed good to moderate activity against H_2O_2 as compared with melatonin (IC50 235∼468 μM vs 690 μM), but they were around 5 times less potent than vitamin C (Table 2). This is not surprising since vitamin C is a potent

Table 1 Scavenging effects of melatonin derivatives on DPPH radical.

Compound	NH	X	IC50 (μM)
			
4a		H	289 ± 12*
4b		H	615 ± 39
4c		OCH_3	43 ± 7*
4d		OCH_3	1225 ± 21
5a		H	580 ± 17
5b		OCH_3	882 ± 33
Vitamin C	—	—	65 ± 6

Values are mean ± s.d., n = 4. *$P < 0.05$ compared with control.

Table 2 Scavenging effects on H_2O_2 of melatonin derivatives.

Compounds	IC50 (μM)
Melatonin	690 ± 27
Vitamin C	52 ± 8
4a	836 ± 32
4b	1462 ± 68
4c	468 ± 21*
5a	255 ± 17*
5b	235 ± 31*

Values are mean ± s.d., n = 4. *$P < 0.05$ compared with vitamin C.

oxidant that can readily transfer a hydride ion to a suitable oxidant such as H_2O_2.

Melatonin has also been reported to be a scavenger of a number of reactive nitrogen species both in-vitro and in-vivo (Pozo et al 1994; Bettahi et al 1996). NO production in cultured cells was assessed by measuring nitrite. Table 3 summaries the IC50 values of compounds in inhibiting LPS-stimulated accumulation of intracellular NO. The results indicated that **4c** is the most potent (IC50 51 μM) among the tested compounds. Interestingly, compounds **5a**, **b**, which retain a free thiol group, still showed moderate activity against NO production. Considering the above results in view of these concise modifications for melatonin, compounds such as **4c** and **5b** retaining methoxy residue on the indole ring and conjugating a free sulfhydryl moiety displayed improved antioxidant properties. It has been known that melatonin's *O*-methyl and *N*-acetyl residues are not only the basis of amphilicity for its permeability, but are also decisive for its antioxidant properties (Poeggeler et al 2002). Therefore, cysteinyl-conjugated derivative **4c** could be a suitable lead for the development of promising antioxidants for certain free radical-mediated conditions. This report also discloses that the well-known antioxidant, melatonin, can be further modified based on the relative pharmacophore involved in free radical scavenging to develop substantial scavengers for certain diseases.

References

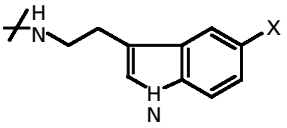
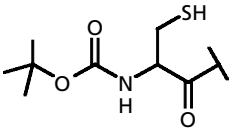
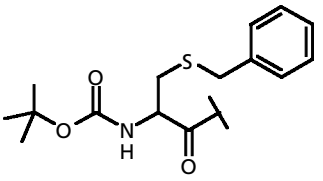
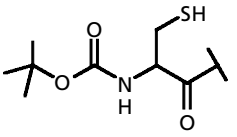
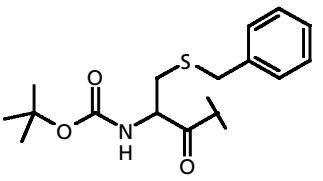
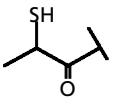
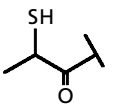
Abuja, P. M., Liebmann, P., Hayn, M., Schauenstein, K., Esterbauer, H. (1997) Antioxidant role of melatonin in lipid peroxidation of human LDL. *FEBS Lett.* **413**: 289–293

Bettahi, I., Pozo, D., Osuna, C., Reiter, R. J., Acuña-Castroviejp, D., Guerrero, J. M. (1996) Melatonin reduces nitric oxide synthase activity in rat hypothalamus. *J. Pineal Res.* **20**: 205–210

Chan, T.-Y., Tang, P.-L. (1996) Characterization of the antioxidant effects of melatonin and related indoleamines in-vitro. *J. Pineal Res.* **20**: 187–191

Chen, C. C., Chiu, K. T., Sun, Y. T., Chen, W. C. (1999) Role of the cyclic AMP-protein kinase A pathway in lipopolysaccharide-induced nitric oxide synthase expression in RAW264.7

Table 3 The inhibition of melatonin derivatives on accumulation of NO_2 in the culture medium bathing RAW 264.7 macrophages exposed to LPS for 24 h.

Compound	NH	X	IC50 (μM)
			
4a		H	220 ± 28
4b		H	541 ± 27
4c		OCH_3	51 ± 6*
4d		H	299 ± 27
5a		H	78 ± 7*
5b		OCH_3	111 ± 9*

Values are mean ± s.d., n = 4. *$P < 0.05$ compared with the control group (Dunnett's test).

macrophage. Involvement of cyclooxygenase-2. *J. Biol. Chem.* **207**: 31559–31564

Mellors, A., Tappel, A. L. (1966) The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. *J. Biol. Chem.* **241**: 4353–4356

Noda, Y., Mori, A., Liburdy, R., Packer, L. (1999) Melatonin and its precursors scavenge nitric oxide. *J. Pineal Res.* **27**: 159–163

Pick, E., Keisari, Y. (1980) A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. *J. Immunol. Methods* **38**: 161–170

Poeggeler, B., Saarela, S., Reiter, R. J., Tan, D.-X., Chen, L.-D., Manchester, L. C., Barlow-Walden, L. R. (1994) Melatonin — a highly potent endogenous radical scavenger and electron donor: new aspects of the oxidation chemistry of this indole accessed *in vitro*. *Ann. NY Acad. Sci.* **738**: 419–420

Poeggeler, B., Thuermann, S., Dose, A., Schoenke, M., Burkhardt, S., Hardeland, R. (2002) Melatonin's unique radical scavenging properties — roles of its functional substituents as revealed by a comparison with its structural analogs. *J. Pineal Res.* **33**: 20–30

Pozo, D., Reiter, R. J., Calvo, J. R., Guerrero, J. M. (1994) Physiological concentrations of melatonin inhibitor nitric oxide synthase in rat cerebellum. *Life Sci.* **55**: 455–460

Reiter, R. J., Tan, D.-X., Poeggeler, B., Menendez-Pelaez, A., Chen, L.-D., Saarela, S. (1994) Melatonin as a free radical scavenger: Implication for aging and age-related diseases. *Ann. NY Acad. Sci.* **719**: 1–12

Tan, D.-X., Manchester, L. C., Reiter, R. J., Plummer, B. F., Limson, J., Weintraub, S. T., Qi, W. (2000) Melatonin directly scavenges hydrogen peroxide: a potentially new metabolic pathway of melatonin biotransformation. *Free Radic. Biol. Med.* **29**: 1177–1185

Torres, J. L., Lozano, C., Julia, L., Sanchez-Baeza, F. J., Anglada, J. M., Centelles, J. J., Cascante, M. (2002) Cysteinyl-flavan-3-ol conjugates from grape procynidins. Antioxidant and antiproliferative properties. *Bioorg. Med. Chem.* **10**: 2497–2509

Verhey, L. J., Sedlacek, R. (1983) Determination of the radioprotective effects of topical applications of MEA, WR-2721, and *N*-acetylcysteine on murine skin. *Radiat. Res.* **93**: 175–183

Weiss, J. F., Landauer, M. R. (2000) Radioprotection by antioxidants. *Ann. NY Acad. Sci.* **899**: 44–60

Zang, L.-Y., Cosma, G., Gardner, H., Vallyathan, V. (1998) Scavenging of reactive oxygen species by melatonin. *Biochim. Biophys. Acta* **1425**: 469–477