Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2561–2565

Synthesis and evaluation of diverse thio avarol derivatives as potential UVB photoprotective candidates

María Amigó,[a] María C. Terencio,[a] Miguel Payá,[a,*] Carmine Iodice[b] and Salvatore De Rosa[b]

[a]*Departamento de Farmacología, Facultad de Farmacia, Universidad de Valencia, Av. Vicente Andrés Estellés s/n, 46100 Burjasot, Valencia, Spain*
[b]*Istituto di Chimica Biomolecolare, CNR, Via Campi Flegrei, 34, 80078 Pozzuoli, Napoli, Italy*

Received 19 December 2006; revised 1 February 2007; accepted 3 February 2007
Available online 7 February 2007

Abstract—Semisynthesis of 13 new thio avarol derivatives (**4**–**16**) and in vitro evaluation on the photodamage response induced by UVB irradiation are described. Their ability to inhibit NF-κB activation and TNF-α generation in HaCaT cells as well as their antioxidant capacity in human neutrophils has also been studied. Among them we have identified two monophenyl thio avarol derivatives (**4**–**5**) lacking cytotoxicity which can be considered promising UVB photoprotective agents through the potent inhibition of NF-κB activation with a mild antioxidant pharmacological profile.

Ultraviolet radiation (UVR) is known for its negative impact on health. The skin, situated at the interface between the body and its environment, directly suffers from the deleterious effects of UV radiation. This UV radiation jeopardizes the integrity of the skin that is critical for cellular homeostasis. The component of the solar UV-light that reaches the earth's surface and that is most responsible for acute and long-term effects is UVB (280–320 nm). Acute effects include erythema, tanning, and inflammatory and immune modulatory changes. Chronic exposure eventually leads to photoaging[1] and photocarcinogenesis.[2,3] In recent years, photochemoprevention has matured into an accepted modality for controlling skin cancer. UVB mediated inflammation that includes the release of growth factors, proinflammatory cytokines, infiltration of inflammatory cells, and radical oxygen species (ROS) production,[4] plays an important role in skin cancer development. Different studies have demonstrated that UV-induced activation of NF-κB-dependent gene transactivation pathways is a critical event for the subsequent development of sunburn reactions in skin.[5,6] Supplementation of antioxidants α-lipoic acid, *N*-acetyl-L-cysteine (NAC), and the flavonoid extract silymarin modulates the activation of the transcription factor NF-κB in HaCaT keratinocytes after exposure to a solar UV simulator.[7,8] These results indicate that some antioxidants can efficiently amend the cellular response to UV radiation through their selective action on NF-κB activation.[9,10] It is well established that NF-κB is activated upon UV irradiation and induces various genes including IL-1 and TNF-α, which subsequently stimulate the signal transduction pathway to activate NF-κB.[11] Cutaneous alterations mediated by UV irradiation through the NF-κB activation pathway could be likewise effectively prevented by blocking NF-κB activation.

Keratinocytes are the major target of UVR and play a central role in the inflammatory and immune modulatory changes observed after UV exposure, at least partly via the UV-induced release of cytokines.[12]

Avarol is a marine sesquiterpenoid hydroquinone with interesting pharmacological properties[13] including anti-inflammatory,[14] antitumor,[15] antioxidant,[16] antiplatelet,[17] anti-HIV,[18] and antipsoriatic[19] effects. Recently, we reported that its derivative 3′-(salicylthio) avarol (**3**) inhibited superoxide generation in stimulated human neutrophils and PGE_2 release in the human HaCaT keratinocyte cell line,[20] offering interesting perspectives as a possible anti-inflammatory or antipsoriatic drug. In this paper, the effects of **3** and 13 new thio avarol

Keywords: Keratinocytes; TNF-α; NF-κB; UVB; Thio avarol derivatives.
* Corresponding author. Tel.: +34 963544946; fax: +34 963544943; e-mail: Miguel.paya@uv.es

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.02.007

derivatives (**4**–**16**) are investigated, with respect to the response induced by UVB irradiation in HaCaT cells. Their ability to inhibit in vitro NF-κB activation and TNF-α generation, as well as their antioxidant capacity in human neutrophils, has also been studied.

Avarol (**1**) was isolated from the sponge *Dysidea avara*,[21] collected in the Bay of Naples, Italy. Avarone (**2**) was obtained by Ag_2O oxidation of avarol, in ethanol, as previously reported.[21] The compound **3** was obtained by adding thiosalicylic acid to a solution of avarone (**2**) in ethanol, as previously reported.[20] Thio derivatives (**4**–**16**) (Fig. 1) were generally obtained by slowly adding the corresponding thio compound dissolved in ethanol to a solution of avarone in ethanol.[22] On the one hand, for the thioglycerol and thioglycolic acid two isomers were obtained with substitution at 3′ (**13** and **15**) and 4′ (**14** and **16**) of the benzoquinone ring. On the other hand, for the thiophenol, *p*-thiocresol, and thioglycol three isomers were obtained with substitution at 3′ (**4**, **7**, and **10**), 4′ (**5**, **8**, and **11**), and in both 3′ and 4′ (**6**, **9**, and **12**). The position of the substituent was determined by the analysis of ^1H NMR spectra. Signals of protons in the benzoquinone ring are doublets in 3′-substituted compounds and singlets in 4′-substituted compounds, whereas 3′,4′-disubstituted compounds show only one signal of proton as singlet. Only chemical shifts of hydroquinone and thio residues are reported because all other signals belonging to the sesquiterpenoid portion were earlier reported.[20,23]

Biological activities of the thio avarol derivatives were evaluated in spontaneously immortalized human keratinocyte cell line (HaCaT)[24] for cellular viability, TNF-α production, and NF-κB activation. Cells were cultured in DMEM supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum at 37 °C in 5% CO_2. For TNF-α experiment cells were seeded in 96-well plate at 2×10^5 cells/mL and in 6-well plate at 4×10^5 cells/mL for NF-κB activation experiment. HaCaT cells were incubated overnight until confluence was reached. Two hours before irradiation, the cells were washed twice in phosphate-buffered saline (PBS) and the compounds were added dissolved in ethanol. Cells were irradiated through a thin film of PBS with a single sublethal UVB dose (40–50 mJ/cm^2) using a LuzChem LZC-5 photoreactor calibrated for UVB irradiation (280–320 nm) with an emission peak centered at 313 nm. After the UVB exposure, fresh culture medium was added to the cells and media were harvested at the appropriated time points. Control conditions consisted of cells pretreated with identical concentrations of ethanol. Final ethanol concentration did not exceed 0.1% and did not affect the experiments. The mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan[25] was used to assess the possible cytotoxic effects of compounds on HaCaT cells after 24 h of irradiation. The concentration of TNF-α in the culture supernatants derived from HaCaT cells 24 h after UVB irradiation[26] was determined by time-resolved fluoroimmunoassay.[27] NF-κB activation was performed 18 h after irradiation[26] by the electrophoretic mobility shift assay (EMSA).[28] Human neutrophils (2.5×10^6 cells/mL) stimulated with 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) were used to evaluate the inhibitory effect on ROS of the thio avarol derivatives studied.[29]

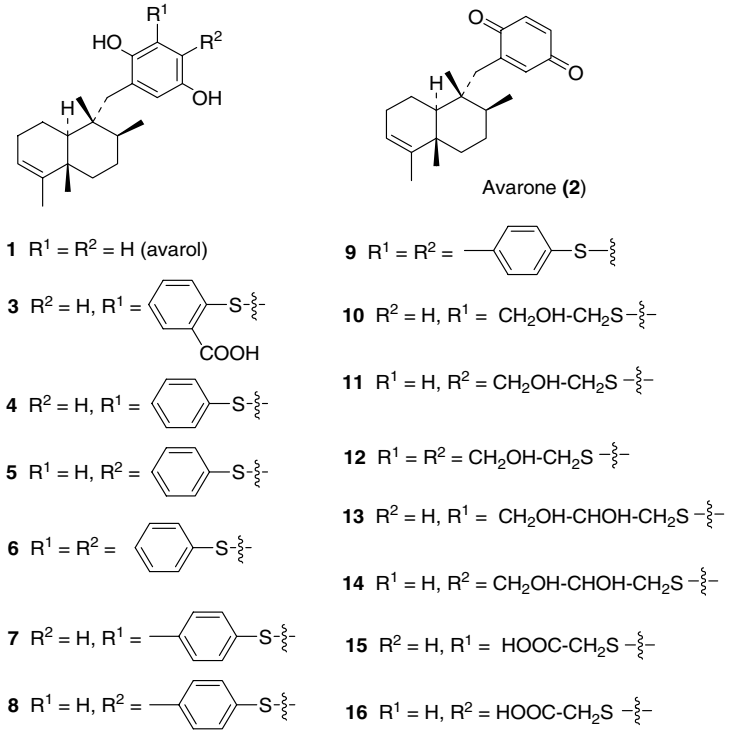

Figure 1. Chemical structures of the thio avarol derivatives.

Table 1. Inhibitory effect of thio avarol derivatives on cellular viability and TNF-α production in UVB irradiated HaCaT cells

Compound	Viability		TNF-α		
	% *I* (10 μM)	% *I* (5 μM)	% *I* (10 μM)	% *I* (5 μM)	IC_{50} (μM)
3	15.7 ± 1.9*	0.6 ± 0.3	ND	76.3 ± 6.4**	3.3 (2.3–5.0)
4	0.0 ± 0.0	0.0 ± 0.0	92.7 ± 2.5**	55.4 ± 3.7**	3.7 (2.5–4.9)
5	0.0 ± 0.0	0.0 ± 0.0	74.3 ± 10.7**	53.6 ± 4.6**	4.2 (2.1–5.6)
6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
10	22.5 ± 2.0*	20.6 ± 2.6*	ND	ND	
11	48.4 ± 1.8**	21.4 ± 1.3*	ND	ND	
12	28.5 ± 3.0**	18.1 ± 1.2*	ND	ND	
13	14.0 ± 2.3	13.6 ± 0.2	ND	ND	
14	18.6 ± 1.5*	12.6 ± 3.8	ND	ND	
15	28.5 ± 3.5**	18.3 ± 1.2*	ND	ND	
16	13.3 ± 1.1	12.4 ± 2.9	ND	ND	
Fraxetin	3.4 ± 2.1	0.0 ± 0.0	68.1 ± 6.6**	58.6 ± 3.7**	2.8 (1.4–8.7)

Data were calculated as inhibitory percentages (% *I*) by taking the values of maximal viability or TNF-α production in drug-free samples as 100%. Values represent means ± SEM ($n = 6$). IC_{50} values and 95% confidence intervals were calculated from at least four significant concentrations for those compounds that reached 50% of inhibition at 10 μM. $^{**}P < 0.01$ and $^{*}P < 0.05$ with respect to the control group.

Table 1 shows the inhibitory effect of thio avarol derivatives (**3**–**16**) on cellular viability and TNF-α production in UVB-irradiated HaCaT cells. The glycyl thio (**10**–**12**), glyceryl thio (**13**–**14**), and glycolyl thio (**15**–**16**) avarol derivatives exerted a significant cytotoxicity at both doses tested, whereas phenyl thio (**4**–**6**) and cresyl thio (**7**–**9**) avarol derivatives showed absence of cytotoxicity. TNF-α production was only determined at concentrations which showed an inhibition of cell viability lower than 10%. Among the noncytotoxic compounds only the monophenyl thio (**4**–**5**) and the salicyl thio (**3**) avarol derivatives were able to inhibit TNF-α production with IC_{50} values very close to the reference compound fraxetin, antioxidant capable to inhibit TNF-α release.[30] Then, we investigated whether the changes in TNF-α production produced by the thio avarol derivatives (**3**–**5**) correlated with changes in NF-κB activation. Nuclear protein extracts from UVB-irradiated HaCaT cells were analyzed for NF-κB-DNA binding activity using a radiolabeled NF-κB-specific oligonucleotide, either in the presence or absence of compounds (**3**–**5**) (Fig. 2). The inhibitory effect of compounds (**3**–**5**) was more potent than proteasome inhibitor MG132 at 10 μM.

In the assay of ROS generation in stimulated human neutrophils, all of these compounds inhibited the generation of oxygen-derived species in a concentration-dependent manner, being the salicyl thio avarol derivative the most potent antioxidant compound (Fig. 3).

The importance of the skin as an immunologic organ is now recognized. In addition to being a mechanical barrier, the skin is critically involved in the regulation of immune responses that can affect the health of the entire organism.[31] Keratinocytes are the major constituent of the epidermis and can synthesize, store, and release different cytokines, including TNF-α.[31,32] The skin is continually exposed to oxidants such as UVR, air pollutants, and chemicals. In order to prevent deleterious oxidative reactions, endogenous antioxidants (both enzymatic and non-enzymatic) are present in the skin. UVB is considered to be the main component of the solar UV irradiation activating NF-κB in HaCaT cells,[8] being the activation of NF-κB significantly enhanced 18 h after UVB irradia-

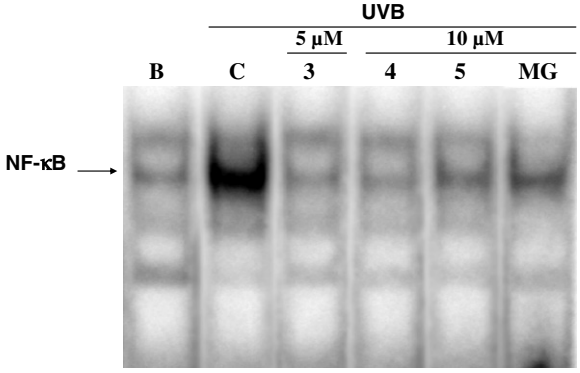

Figure 2. Inhibitory effect of compounds **3**–**5** on NF-κB activation in nuclear extracts of UVB-irradiated (40–50 mJ/cm^2) HaCaT cells. B, non-UVB-irradiated cells. C, UVB irradiated cells. MG, MG132. The figure is representative of three independent experiments.

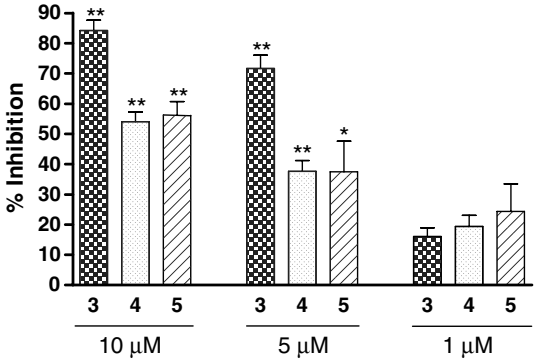

Figure 3. Inhibitory effect of compounds **3**–**5** on radical oxygen species in TPA stimulated human neutrophils. Results show percentages of inhibition. Statistical evaluation included one-way analysis of variance followed by Dunnett's *t* test. Data represent means ± SEM ($n = 6$). $^{**}P < 0.01$ and $^{*}P < 0.05$ with respect to the control group.

tion.[26] Topical application of antioxidants has been used to prevent skin antioxidant loss and photooxidative damage. However, in addition to the capacity of antioxidants to prevent this damage in the skin, growing evidence indicates that they also play a role in modulating the expression of genes whose products are involved in inflammation, aging, and carcinogenesis.[7,8,33]

It is reported that NF-κB activation and translocation is previous to TNF-α production[26] and that not all antioxidants are equally effective inhibitors in UVB-irradiated keratinocytes.[34] The critical event for UVB erythematous damage seems to be the NF-κB-dependent gene transactivation.[10]

We have identified several thio derivatives of avarol, a well-reputed antipsoriatic drug, as effective UVB photoprotective candidates acting mainly through the potent inhibition of NF-κB activation. In addition, these products clearly reduced TNF-α generation in keratinocytes and demonstrated antioxidant properties. These results are according to other studies indicating that ROS are implicated in the induction of TNF-α expression by UVB via NF-κB-dependent pathway.[10,26,34] In this regard it is interesting to note that the best antioxidant properties of compound **3** correlate with the more potent inhibition of NF-κB activation showed by this compound.

Results obtained in the present study show some clear structure–activity relationships. In this regard, monophenyl thio avarol derivatives (**4**–**5**), as well as salicyl thio avarol derivative (**3**), are the only ones that exert a potent UVB photoprotective profile. The disubstituted phenyl thio avarol derivative (**6**) presents a total reduction in activity probably related with the higher steric hindrance of the bulk. The methylation of phenyl thiol moiety in order to get the respective cresyl thio derivatives (**7**–**9**) produces a lack of inhibitory activity. In addition, the presence of glycyl thio/glyceryl thio and glycolyl thio moieties seems to increase the cytotoxicity (**10**–**16**).

In summary, salicyl thio avarol and monophenyl thio avarol derivatives can be considered promising UVB photoprotective agents through the potent inhibition of NF-κB activation and the interference with cellular processes mediated by ROS generation.

Acknowledgments

M.A. was the recipient of a Research Fellowship from the FPU program of Spanish Ministerio de Educación y Ciencia. This work was supported partly by the Grant UV-AE-20060243 from the University of Valencia and by FIS-PI051659.

References and notes

1. Rittie, L.; Fisher, G. J. *Ageing Res. Rev.* **2002**, *1*, 705.
2. Hussein, M. R. *J. Cutan. Pathol.* **2005**, *32*, 191.
3. Matsumura, Y.; Ananthaswamy, H. N. *Toxicol. Appl. Pharmacol.* **2004**, *195*, 298.
4. Halliday, G. M. *Mutat. Res.* **2005**, *571*, 107.
5. Abeyama, K.; Eng, W.; Jester, J. V.; Vink, A. A.; Edelbaum, D.; Cockerell, C. J.; Bergstresser, P. R.; Takashima, A. *J. Clin. Invest.* **2000**, *105*, 1751.
6. Fisher, G. J.; Datta, S. C.; Talwar, H. S.; Wang, Z. Q.; Varani, J.; Kang, S.; Voorhees, J. J. *Nature* **1996**, *379*, 335.
7. Larsson, P.; Ollinger, K.; Rosdahl, I. *Br. J. Dermatol.* **2006**, *155*, 292.
8. Saliou, C.; Kitazawa, M.; McLaughlin, L.; Yang, J. P.; Lodge, J. K.; Tetsuka, T.; Iwasaki, K.; Cillard, J.; Okamoto, T.; Packer, L. *Free Radical Biol. Med.* **1999**, *26*, 174.
9. Mantena, S. K.; Katiyar, S. K. *Free Radical Biol. Med.* **2006**, *40*, 1603.
10. Afaq, F.; Adhami, V. M.; Mukhtar, H. *Mutat. Res.* **2005**, *571*, 153.
11. Tanaka, K.; Hasegawa, J.; Asamitsu, K.; Okamoto, T. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 624.
12. Pupe, A.; Moison, R.; De Haes, P.; van Henegouwen, G. B.; Rhodes, L.; Degreef, H.; Garmyn, M. *J. Invest. Dermatol.* **2002**, *118*, 692.
13. De Rosa, S. In *Natural Products in the New Millennium: Prospects and Industrial Application*; Rauter, A. P., Palma, F. B., Justino, J., Araújo, M. E., Santos, S. P., Eds.; Kluwer Academic Publishers: Dordrecht, 2002; pp 441–461.
14. Ferrándiz, M. L.; Sanz, M. J.; Bustos, G.; Payá, M.; Alcaraz, M. J.; De Rosa, S. *Eur. J. Pharmacol.* **1994**, *253*, 75.
15. Shen, Y. C.; Lu, C. H.; Chakraborty, R.; Kuo, Y. H. *Nat. Prod. Res.* **2003**, *17*, 83.
16. Belisario, M. A.; Maturo, M.; Pecce, R.; De Rosa, S.; Villani, G. R. *Toxicology* **1992**, *72*, 221.
17. Belisario, M. A.; Maturo, M.; Avagnale, G.; De Rosa, S.; Scopacasa, F.; De Caterina, M. *Pharmacol. Toxicol.* **1996**, *79*, 300.
18. Sarin, P. S.; Sun, D.; Thornton, A.; Muller, W. E. G. *J. Natl. Cancer Inst.* **1987**, *78*, 663.
19. Sipkema, D.; Osinga, R.; Schatton, W.; Mendola, D.; Tramper, J.; Wijffels, R. H. *Biotechnol. Bioeng.* **2005**, *90*, 201.
20. Amigó, M.; Terencio, M. C.; Mitova, M.; Iodice, C.; Payá, M.; De Rosa, S. *J. Nat. Prod.* **2004**, *67*, 1459.
21. Minale, L.; Riccio, R.; Sodano, G. *Tetrahedron Lett.* **1974**, *15*, 3401.
22. *Synthesis of 3′-(phenylthio)avarol (**4**), 4′-(phenylthio)avarol (**5**), and 3′,4′-(phenylthio)avarol (**6**).* Thiophenol (100 μL) dissolved in EtOH (5 mL) was added to a solution of avarone (**2**) (100 mg) in EtOH (10 mL) and stirred for 5 min at room temperature. After evaporation of EtOH, the residue was chromatographed on a Si gel column and eluted with petroleum ether/Et_2O (4:1), to give 3′-(phenylthio)avarol (**4**) as the more polar component (65 mg; yield 48.0%): amorphous solid; $[\alpha]_D^{25}$ 7.1° (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 313 (3.36), 433 (1.71); ^{1}H NMR ($CDCl_3$) δ 7.20 (2H, dd, *J* = 7.7 and 7.3 Hz, H-3″ and H-5″), 7.14 (H, d, *J* = 7.7 Hz, H-4″), 7.06 (2H, d, *J* = 7.3 Hz, H-2″ and H-6″), 6.88 (1H, d, *J* = 2.9 Hz, H-4′), 6.73 (1H, d, *J* = 2.9 Hz, H-6′); EIMS *m*/*z* 424 $[M+2]^+$ (0.4), 422 $[M]^+$ (12), 233 (32), 192 (12), 189 (8), 107 (35), 95 (100); HREIMS *m*/*z* 422.2281 (Calcd for $C_{27}H_{34}O_2S$, 422.2279), 4′-(phenylthio)avarol (**5**) (13 mg; yield 9.6%): amorphous solid; $[\alpha]_D^{25}$ 28.7° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 312 (3.43), 430 (2.32); ^{1}H NMR ($CDCl_3$) δ 7.22 (2H, dd, *J* = 7.7 and 7.3 Hz, H-3″ and H-5″), 7.15 (H, d, *J* = 7.7 Hz, H-4″), 7.09 (2H, d, *J* = 7.3 Hz, H-2″ and H-6″), 6.88 (1H, s, H-4′), 6.82 (1H, s, H-6′); EIMS *m*/*z* 424 $[M+2]^+$ (0.5), 422 $[M]^+$ (10), 233 (30), 192 (15), 189 (10), 107 (35), 95 (100); HREIMS *m*/*z* 422.2281 (Calcd for $C_{27}H_{34}O_2S$, 422.2279), and 3′,4′-(phenylthio)avarol (**6**) as the less polar component (6 mg; yield 3.5%): amorphous solid; $[\alpha]_D^{25}$ 7.3° (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 332 (3.52), 433 (1.70), 492 (1.21); ^{1}H NMR ($CDCl_3$) δ 7.16 (6H, m, H-3″, H-4″ and H-5″), 6.97 (4H, m, H-2″ and H-

6″), 6.65 (1H, s, H-6′); EIMS *m/z* 532 $[M+2]^+$ (0.6), 530 $[M]^+$ (12), 233 (30), 192 (12), 189 (8), 107 (35), 95 (100); HREIMS *m/z* 530.2310 (Calcd for $C_{33}H_{38}O_2S_2$, 530.2313).

*Synthesis of 3′-(cresylthio)avarol (**7**), 4′-(cresylthio)avarol (**8**), and 3′,4′-(cresylthio)avarol (**9**).* *p*-Thiocresol (100 μL) dissolved in EtOH (5 mL) was added to a solution of avarone (**2**) (100 mg) in EtOH (10 mL) and stirred for 5 min at room temperature. After evaporation of EtOH, the residue was chromatographed on a Si gel column and eluted with petroleum ether/Et_2O (4:1), to give 3′-(cresylthio)avarol (**7**) as the more polar component (60 mg; yield 43.0 %): amorphous solid; $[\alpha]_D^{25}$ 9.2° (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 312 (3.35), 433 (2.40); ^{1}H NMR ($CDCl_3$) δ 7.01 (2H, d, *J* = 8.3 Hz, H-3″ and H-5″), 6.98 (2H, d, *J* = 8.3 Hz, H-2″ and H-6″), 6.86 (1H, d, *J* = 2.9 Hz, H-4′), 6.70 (1H, d, *J* = 2.9 Hz, H-6′), 2.29 (3H, s, H-7″); EIMS *m/z* 438 $[M+2]^+$ (0.4), 436 $[M]^+$ (10), 232 (98), 191 (25), 107 (40), 95 (100); HREIMS *m/z* 436.2445 (Calcd for $C_{28}H_{36}O_2S$, 436.2442), 4′-(cresylthio)avarol (**8**) (25 mg; yield 18.0%): amorphous solid; $[\alpha]_D^{25}$ 21.7° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 312 (3.76), 430 (2.73); ^{1}H NMR ($CDCl_3$) δ 7.02 (2H, d, *J* = 8.3 Hz, H-3″ and H-5″), 6.98 (2H, d, *J* = 8.3 Hz, H-2″ and H-6″), 6.85 (1H, s, H-3′), 6.80 (1H, s, H-6′), 2.30 (3H, s, H-7″); EIMS *m/z* 438 $[M+2]^+$ (0.4), 436 $[M]^+$ (12), 232 (95), 191 (30), 107 (45), 95 (100); HREIMS *m/z* 436.2445 (Calcd for $C_{28}H_{36}O_2S$, 436.2442), and 3′,4′-(cresylthio)avarol (**9**) as the less polar component (15 mg; yield 8.4%): amorphous solid; $[\alpha]_D^{25}$ 9.9° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 332 (3.57), 433 (1.98), 441 (1.99); ^{1}H NMR ($CDCl_3$) δ 6.98 (4H, d, *J* = 8.3 Hz, H-3″ and H-5″), 6.87 (4H, d, *J* = 8.3 Hz, H-2″ and H-6″), 6.68 (1H, s, H-6′), 2.26 (6H, s, H-7″); EIMS *m/z* 560 $[M+2]^+$ (0.7), 558 $[M]^+$ (10), 232 (55), 191 (25), 107 (40), 95 (100); HREIMS *m/z* 558.2630 (Calcd for $C_{35}H_{42}O_2S_2$, 558.2626).

*Synthesis of 3′-(glycylthio)avarol (**10**), 4′-(glycylthio)avarol (**11**), and 3′,4′-(glycylthio)avarol (**12**).* Thioglycol (200 μL) dissolved in EtOH (5 mL) was added to a solution of avarone (**2**) (100 mg) in EtOH (10 mL) and stirred for 5 min at room temperature. After evaporation of EtOH, the residue was chromatographed on a Si gel column and eluted with petroleum ether/Et_2O/HOAc (7:3:0.1), to give 3′-(glycylthio)avarol (**10**) as the less polar component (25 mg; yield 20.0%): amorphous solid; $[\alpha]_D^{25}$ 7.1° (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 312 (3.54), 433 (2.45); ^{1}H NMR ($CDCl_3$) δ 6.85 (1H, d, *J* = 2.9 Hz, H-4′), 6.63 (1H, d, *J* = 2.9 Hz, H-6′), 3.67 (2H, t, *J* = 5.9 Hz, H-2″); 2.86 (2H, t, *J* = 5.9 Hz, H-1″); EIMS *m/z* 392 $[M+2]^+$ (0.4), 390 $[M]^+$ (10), 343 (5), 258 (8), 196 (18), 189 (28), 107 (30), 95 (100); HREIMS *m/z* 390.2230 (calcd for $C_{23}H_{34}O_3S$, 390.2228), 4′-(glycylthio)avarol (**11**) (40 mg; yield 32.0%): amorphous solid; $[\alpha]_D^{25}$ 11.0° (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 310 (3.61), 433 (2.39); ^{1}H NMR ($CDCl_3$) δ 6.86 (1H, s, H-3′), 6.73 (1H, s, H-6′), 3.73 (2H, t, *J* = 5.9 Hz, H-2″); 2.89 (2H, t, *J* = 5.9 Hz, H-1″); EIMS *m/z* 392 $[M+2]^+$ (0.4), 390 $[M]^+$ (12), 343 (5), 258 (10), 196 (15), 189 (25), 107 (35), 95 (100); HREIMS *m/z* 390.2230 (Calcd for $C_{23}H_{34}O_3S$, 390.2228), and 3′,4′-(glycylthio)avarol (**12**) as the more polar component (6 mg; yield 4.0 %): amorphous solid; $[\alpha]_D^{25}$ 20.4° (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 327 (3.94), 433 (2.28); ^{1}H NMR ($CDCl_3$) δ 6.85 (1H, s, H-6′), 3.66 (2H, t, *J* = 5.9 Hz, H-2″), 3.60 (2H, t, *J* = 5.9 Hz, H-2″), 2.96 (2H, t, *J* = 5.9 Hz, H-1″), 2.92 (2H, t, *J* = 5.9 Hz, H-1″); EIMS *m/z* 468 $[M+2]^+$ (0.6), 466 $[M]^+$ (10), 343 (3), 258 (5), 196 (15), 189 (20), 107 (30), 95 (100); HREIMS *m/z* 466.2214 (Calcd for $C_{25}H_{38}O_4S_2$, 466.2211).

*Synthesis of 3′-(glycerylthio)avarol (**13**) and 4′-(glycerylthio)avarol (**14**).* 1-Thioglycerol (100 μL) dissolved in EtOH (5 mL) was added to a solution of avarone (**2**) (100 mg) in EtOH (10 mL) and stirred for 30 min at room temperature. After evaporation of EtOH, the residue was chromatographed on a Si gel column and eluted with ethyl acetate, to give 3′-(glycerylthio)avarol (**13**) as the less polar component (45 mg; yield 33.4 %): amorphous solid; $[\alpha]_D^{25}$ 8.6° (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 310 (3.18), 433 (2.32); ^{1}H NMR ($CDCl_3$) δ 6.85 (1H, d, *J* = 2.7 Hz, H-4′), 6.63 (1H, d, *J* = 2.7 Hz, H-6′), 3.67 (2H, m, H-2″ and H-3a″); 3.50 (1H, m, H-3b″), 2.77 (1H, dd, *J* = 13.9 and 6.4 Hz, H-1a″), 2.59 (1H, dd, *J* = 13.9 and 7.6 Hz, H-1b″); EIMS *m/z* 422 $[M+2]^+$ (0.4), 420 $[M]^+$ (10), 258 (8), 229 (35), 191 (25), 155 (15), 107 (60), 95 (100); HREIMS *m/z* 420.2330 (Calcd for $C_{24}H_{36}O_4S$, 420.2334), and 4′-(glycerylthio)avarol (**14**) as the less polar component (40 mg; yield 29.7 %): amorphous solid; $[\alpha]_D^{25}$ 32.7° (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 309 (3.86), 433 (3.07); ^{1}H NMR ($CDCl_3$) δ 6.86 (1H, s, H-3′), 6.73 (1H, s, H-6′), 3.72 (2H, m, H-2″ and H-3a″); 3.51 (1H, dd, *J* = 11.2 and 6.30 Hz, H-3b″), 2.87 (1H, dd, *J* = 13.8 and 4.1 Hz, H-1a″), 2.74 (1H, dd, *J* = 13.8 and 8.3 Hz, H-1b″); EIMS *m/z* 422 $[M+2]^+$ (0.4), 420 $[M]^+$ (12), 258 (8), 229 (30), 191 (20), 155 (20), 107 (50), 95 (100); HREIMS *m/z* 420.2330 (Calcd for $C_{24}H_{36}O_4S$, 420.2334).

*Synthesis of 3′-(glycolylthio)avarol (**15**) and 4′-(glycolylthio)avarol (**16**).* Thioglycolic acid (200 μL) dissolved in EtOH (5 mL) was added to a solution of avarone (**2**) (100 mg) in EtOH (10 mL) and stirred for 3 h at room temperature. After evaporation of EtOH, the residue was chromatographed on a Si gel column and eluted with petroleum ether–Et_2O–HOAc (7:3:0.1), to give 3′-(glycolylthio)avarol (**15**) as the less polar component (34 mg; yield 26.3%): amorphous solid; $[\alpha]_D^{25}$ 14.4° (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 312 (4.35), 433 (2.93); ^{1}H NMR ($CDCl_3$) δ 6.88 (1H, d, *J* = 2.9 Hz, H-4′), 6.65 (1H, d, *J* = 2.9 Hz, H-6′), 3.47 (2H, s, H-1″); EIMS *m/z* 406 $[M+2]^+$ (0.4), 404 $[M]^+$ (12), 229 12(35), 191 (25), 107 (60), 95 (100); HREIMS *m/z* 404.2017 (Calcd for $C_{23}H_{32}O_4S$, 404.2021), and 4′-(glycolylthio)avarol (**16**) as the less polar component (28 mg; yield 21.6%): amorphous solid; $[\alpha]_D^{25}$ 14.0° (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max}(log ε) 311 (3.52), 433 (2.20); ^{1}H NMR ($CDCl_3$) δ 6.81 (1H, s, H-3′), 6.65 (1H, s, H-6′), 3.43 (2H, s, H-1″); EIMS *m/z* 406 $[M+2]^+$ (0.4), 404 $[M]^+$ (8), 229 (25), 191 (20), 107 (50), 95 (100); HREIMS *m/z* 404.2025 (Calcd for $C_{23}H_{32}O_4S$, 404.2021).

23. De Rosa, S.; Minale, L.; Riccio, R.; Sodano, G. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1408.
24. Boukamp, P.; Petrussevska, R. T.; Breitkreutz, D.; Hornung, J.; Markham, A.; Fusenig, N. E. *J. Cell Biol.* **1988**, *106*, 761.
25. Borenfreund, E.; Babick, H.; Martin-Alguacil, N. *Toxicol. In Vitro* **1988**, *2*, 1.
26. Lan, C. C.; Yu, H. S.; Wu, C. S.; Kuo, H. Y.; Chai, C. Y.; Chen, G. S. *Br. J. Dermatol.* **2005**, *153*, 725.
27. Pennanen, N.; Lapinjoki, S.; Palander, A.; Urtti, A.; Monkkonen, J. *Int. J. Immunopharmacol.* **1995**, *17*, 475.
28. Amigó, M.; Schalkwijk, J.; Olthuis, D.; De Rosa, S.; Payá, M.; Terencio, M. C.; Lamme, E. *Life Sci.* **2006**, *79*, 2395.
29. Bustos, G.; Ferrándiz, M. L.; Sanz, M. J.; Payá, M.; Alcaraz, M. J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1995**, *351*, 298.
30. Kuo, P. L.; Huang, Y. T.; Chang, C. H.; Chang, J. K. *Int. J. Immunopharmacol.* **2006**, *6*, 1167.
31. Oberyszyn, T. M.; Tober, K. L.; Ross, M. S.; Robertson, F. M. *Mol. Carcinog.* **1998**, *22*, 16.
32. Banno, T.; Gazel, A.; Blumenberg, M. *J. Biol. Chem.* **2004**, *279*, 32633.
33. Jurkiewicz, B. A.; Bissett, D. L.; Buettner, G. R. *J. Invest. Dermatol.* **1995**, *104*, 484.
34. Pupe, A.; Degreef, H.; Garmyn, M. *Photochem. Photobiol.* **2003**, *78*, 68.