Synthesis and Biological Activities of Thio-avarol Derivatives[#]

Boris Pejin, Carmine Iodice, Giuseppina Tommonaro, and Salvatore De Rosa*

Istituto di Chimica Biomolecole CNR, via Campi Flegrei, 34, I-80078 Pozzuoli (Napoli), Italy

Received May 29, 2008

Eleven new thio-avarol derivatives (**3**−**13**) were synthesized. Their antimicrobial, brine shrimp lethality, and free-radical scavenging activities and acetylcholinesterase inhibition, together with 12 already reported semisynthetic thio-avarol derivatives (**14**−**25**), were evaluated. Structure−activity relationships among these thio derivatives were determined.

Avarol is a marine sesquiterpenoid hydroquinone, previously isolated from the marine sponge *Dysidea avara* Schmidt (Dictyoceratida),[1,2] with interesting pharmacological properties[3] including anti-inflammatory,[4] antitumor,[5] antioxidant,[6] antiplatelet,[7] anti-HIV,[8] and antipsoriatic[9,10] effects. Previous studies demonstrated the antioxidant properties of avarol, which inhibits superoxide generation and microsomal lipid peroxidation.[4,6] The biological activities of this compound have been correlated with its redox chemistry and its ability to effect radical production, while the terpenoid moiety plays a marginal role in biological processes.[11] These interesting properties and the previous findings that avarone, the quinone of avarol, reacts toward protein sulfhydryl groups[12] and that 3′-(salicylthio) avarol (**11**) could be a promising antipsoriatic agent[13] prompted us to prepare further sulfhydryl derivatives of avarol and to extend the evaluation to other biological properties. In this paper we report the synthesis of 11 new thio-avarol derivatives (**3**−**13**), the antimicrobial, brine shrimp lethality, and free-radical scavenging activities, and acetylcholinesterase inhibition of these new derivatives together with 12 already described thio derivatives (**14**−**25**).[14] This represents a wide series of thio-avarol derivatives, with different polarities. Brine shrimp lethality[15] was used as an indicator of cytotoxicity. This assay was demonstrated to be in excellent agreement with L5 178y (mouse lymphoma cells) and L12 10 (leukemia cells) assays, using avarol (**1**) and avarone (**2**).[16] The free-radical scavenging assay is a simple test evaluating the potential antioxidant activity of compounds, using 2,2-diphenyl-1-picrylhydrazyl (DPPH).[17,18] Acetylcholinesterase (AChE) inhibition was detected by a TLC bioautographic assay.[19,20] AChE is the enzyme involved in the metabolic hydrolysis of acetylcholine at cholinergic synapses in the central and peripheral nervous system. The abnormal activity of this enzyme is one factor responsible for Alzheimer's disease, the most common cause of senile dementia in later life. AChE inhibitors are still the best drugs currently available for the management of this disease.[21]

Results and Discussion

Avarol (**1**) was isolated from the sponge *Dysidea avara*,[1] collected in the Bay of Naples, Italy. Avarone (**2**) was obtained by Ag_2O oxidation of avarol, in ethanol, as previously reported.[1] Thio derivatives (**3**−**25**) (Figure 1) were generally obtained by slowly adding the corresponding thio compound dissolved in ethanol to a solution of avarone in ethanol.[13] Using 3-mercaptobenzoic acid only one derivative was obtained with substitution at 3′ (**3**) of the benzoquinone ring, as well as with thiosalicylic acid, previously reported (**14**).[22] On the one hand, for the 2-mercaptobenzyl alcohol, thiolactic acid, and 2-mercaptobenzothiazole two isomers were obtained with substitution at 3′ (**4**, **6**, and **8**) and 4′ (**5**, **7**, and **9**), as well as with thiophenol (**15** and **16**, with substitution at 3′ and 4′, respectively), thioglycol (**20** and **21**, with substitution at 3′ and 4′, respectively), thioglycerol (**22** and **23**, with substitution at 3′ and 4′, respectively), and thioglycolic acid (**24** and **25**, with substitution at 3′ and 4′, respectively), previously reported.[14] On the other hand, for *p*-thiocresol three isomers were obtained with substitution at 3′ (**17**), 4′ (**18**), and at both 3′ and 4′ (**19**), as previously reported.[14] Only for the thiocholesterol were four isomers obtained; in fact, in addition to 3′- (**11**), 4′- (**12**), and 3′,4′-

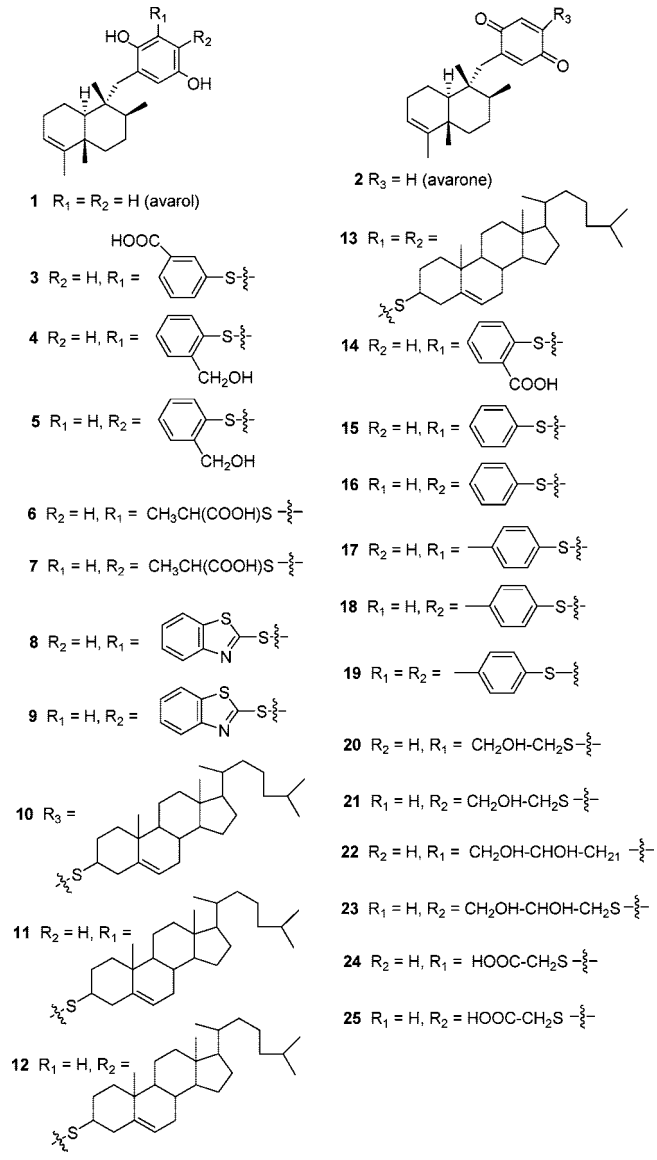

Figure 1. Chemical structures of avarol derivatives.

[#] Dedicated to the memory of Prof. Rodolfo Nicolaus.

* Corresponding author. Tel: +39 081 8675029. Fax: +39 081 8041770. E-mail: sderosa@icmib.na.cnr.it.

10.1021/np800318m CCC: $40.75
Published on Web 11/13/2008

Table 1. Biological Activities of Avarol (**1**), Avarone (**2**), and Thio-avarol Derivatives (**3**−**25**)

	antimicrobial MIC (μg/mL)[a]					
compound	*B. s.*	*M. l.*	*S. c.*	*A. salina* LC_{50} (95% C.L.)[b]	DPPH IC_{50} (μM)[c]	AChE (μg)[d]
1	25	10	N.A.	0.18 (0.32/0.10)	18.0	10
2	10	1.0	N.A.	0.14 (0.32/0.07)	32.1	1
3	10	10	N.A.	14.73 (67.54/4.52)	31.8	1
4	N.A.	N.A.	N.A.	>50	25.3	10
5	N.A.	N.A.	N.A.	10.64 (18.55/5.06)	90.0	>10
6	10	N.A.	N.A.	27.25 (57.41/14.80)	34.6	1
7	10	N.A.	N.A.	>50	35.3	1
8	N.A.	0.01	100	N.A.	29.5	10
9	N.A.	N.A.	N.A.	N.A.	31.2	>10
10	N.A.	N.A.	N.A.	N.A.	N.A.	>10
11	N.A.	N.A.	N.A.	N.A.	31.4	>10
12	N.A.	N.A.	N.A.	N.A.	71.4	>10
13	N.A.	N.A.	N.A.	N.A.	41.5	>10
14	0.01	10	N.A.	15.11 (45.37/6.04)	34.0	1
15	10	N.A.	N.A.	0.97 (1.54/0.11)	10.7	10
16	100	100	N.A.	33.10 (54.53/20.44)	N.A.	N.A.
17	N.A.	N.A.	N.A.	0.23 (0.95/0.10)	63.7	10
18	100	N.A.	N.A.	24.87 (43.23/14.77)	N.A.	N.A.
19	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
20	10	N.A.	N.A.	N.A.	N.A.	N.A.
21	100	N.A.	N.A.	N.A.	N.A.	N.A.
22	10	N.A.	N.A.	1.50 (3.71/0.21)	62.5	10
23	10	N.A.	N.A.	0.23 (1.13/0.12)	71.4	10
24	10	10	N.A.	8.50 (14.85/4.45)	40.5	1
25	0.01	100	N.A.	12.83 (23.70/7.09)	52.3	1
gentamicin	1.0	10				
nystatin			100			
trolox					25.8	
galanthamine						0.01

[a] *B. s.* =*Bacillus subtilis; M. l.* = *Micrococcus luteus; S. c.* = *Saccharomyces cerevisiae.* N.A. = MIC > 100 μg/mL. [b] LC_{50} values are expressed in ppm; 95% C.L.= 95% confidence limits. N.A. = LC_{50} > 100 ppm. [c] Concentration that promotes 50% of DPPH reduction. N.A. = IC_{50} > 100 μM. [d] Amounts given, in μg, are the minimum inhibitory quantities applied on the TLC plates. N.A. = >50 μg.

thiocholesterol avarol (**13**), 4′-thiocholesterol avarone (**10**) was also isolated. The position of the substituent was determined by the analysis of ^{1}H NMR spectra. Signals of protons in the benzoquinone ring are doublets in 3′-substituted compounds and singlets in 4′-substituted compounds, while 3′,4′-disubstituted compounds show only one proton signal as a singlet.

The antimicrobial activity evaluation was carried out using a liquid culture of three bacterial strains (*Escherichia coli*, *Bacillus subtilis*, and *Micrococcus luteus*) and yeast (*Saccharomyces cerevisiae*), by a serial dilution, and the results are reported in Table 1. None of the tested compounds showed effects against the Gram-negative bacterium *E. coli.* Only compound **8** showed good antifungal activity, comparable with that of nystatin, against the yeast *S. cerevisiae*. Further, compound **8** showed high antibacterial activity, more than gentamicin (MIC 10 μg/mL), against the Gram-positive bacterium *M. luteus* (MIC 0.01 μg/mL). Compounds **14** and **25** showed high antibacterial activity, more active than gentamicin (MIC 1.0 μg/mL), against the Gram-positive bacterium *B. subtillis* (MIC 0.01 μg/mL) and were moderately active against *M. luteus*. Compounds **3**, **6**, **7**, **15**, **20**, **22**, **23**, and **24** were moderately active (MIC 10 μg/mL) against *B. subtillis*.

Results obtained in the brine shrimp test (Table 1) showed that all avarol derivatives are less active than avarol (**1**) and avarone (**2**). Only compounds **15**, **17**, and **23** showed interesting cytotoxic activity, with LC_{50} = 0.97, 0.23, and 0.23 ppm, respectively.

Generally, the introduction of a substituent on the hydroquinone ring of the avarol skeleton reduces the antioxidant activity, evaluated by the free-radical scavenging assay using 2,2-diphenyl-1-picryl-hydrazyl (DPPH)[17,18] as a TLC spray reagent. The results are reported in Table 1. Avarol derivatives with substitution at 3′ (**3**, **4**, **6**, **8**, **11**, **15**, **17**, **22**, and **24**) are more active than those with substitution at 4′ (**5**, **7**, **9**, **12**, **16**, **18**, **17**, **23**, and **25**). Avarol (**1**) showed the most potent antioxidant activity, with IC_{50} = 18 μM, while **6**, **9**, and **10** exhibited moderate potencies with IC_{50} = 34, 95, and 98 μM, respectively.

The AChE inhibition tests showed a moderate activity (1 μg) for all avarol derivatives with a carboxylic acid group in the molecule (**3**, **6**, **7**, **24**, and **25**). In comparison, the alkaloid galanthamine used clinically for the treatment of Alzheimer's disease[21] inhibited the enzyme at 0.01 μg. Because most inhibitors of AChE are alkaloids that often possess several side effects,[23] it is important to search for new AChE inhibitors not belonging to this structural class.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP 370 polarimeter, using a 10 cm microcell. UV spectra were obtained on a Varian DMS 90 spectrophotometer. NMR spectra, recorded at the NMR Service of Istituto di Chimica Biomolecolare del CNR (Pozzuoli, Italy) on a Bruker Avance-400 operating at 400 MHz, using an inverse probe fitted with a gradient along the Z-axis, in $CDCl_3$, using the residual $CDCl_3$ resonance at 7.26 ppm as internal references. Only chemical shifts of hydroquinone are reported because all other signals belonging to the sesquiterpenoid portion were earlier reported [1,2] and thio residues are generally well-known.[24] LRMS and HRMS were recorded on a JEOL JMS D-300 and an AEI MS-50, respectively. Column chromatography was carried out on Merck silica gel 60.

Materials. Avarol (**1**) was isolated from the sponge *Dysidea avara*,[1] which was collected in the Bay of Naples, Italy. A voucher specimen is maintained in the collection of the ICB-CNR. Avarone (**2**) was prepared from avarol by oxidation with Ag_2O as previously described.[1] Avarol-3′-thiosalicylate (**14**), avarol-3′-thiophenol (**15**), avarol-4′-thiophenol (**16**), avarol-3′-thiocresol (**17**), avarol-4′-thiocresol (**18**), avarol-3′−4′-dithiocresol (**19**), avarol-3′-thioglycol (**20**), avarol-4′-thioglycol (**21**), avarol-3′-thioglycerol (**22**), avarol-4′-thioglycerol (**23**), avarol-3′-thioglycolate (**24**), and avarol-4′-thioglycolate (**25**) were obtained as previously reported.[13] 3-Mercaptobenzoic acid, 2-mercaptobenzyl alcohol, thiolactic acid, 2-mercaptobenzothiazole, and thiocholesterol were obtained from Sigma-Aldrich (Milano, Italy). Acetylcholinesterase, 1-naphthyl acetate, and the rest of the reagents used in the biological tests were obtained from Sigma Chemicals (St. Louis, MO). Fast Blue B salt was from Fluka (Milano, Italy).

Synthesis of Avarol-3′-thiobenzoate (3). 3-Mercaptobenzoic acid (100 mg) was dissolved in EtOH (5 mL), added to a solution of avarone (**2**) (100 mg) in EtOH (10 mL), and stirred for 2 h at room temperature. After evaporation of EtOH, the residue was chromatographed on a Si gel column and eluted with $CHCl_3$−MeOH (95:5) to give avarol-3′-thiobenzoate (**3**) (77 mg; yield 52%): amorphous solid; $[\alpha]_D$ −0.3 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 224 (5.03), 314 (4.38); ^{1}H NMR ($CDCl_3$) δ 6.90 (H-6′, d, J = 2.7 Hz), 6.76 (H-4′, d, J = 2.7 Hz) and see Supporting Information; EIMS *m/z* 468 $[M + 2]^+$ (0.4), 466 $[M]^+$ (10), 276 (18), 258 (22), 191 (28), 189 (30), 135 (30), 107 (35), 95 (100); HREIMS *m/z* 466.2183 (calcd for $C_{28}H_{34}O_4S$, 466.2178).

Synthesis of 3′-(Benzylthio)avarol (4) and 4′-(Benzylthio)avarol (5). 2-Mercaptobenzyl alcohol (100 mg) was dissolved in EtOH (5 mL), added to a solution of avarone (100 mg) in EtOH (10 mL), and stirred for 2 h at 60 °C. 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). The more polar component was 3′-(benzylthio)avarol (**4**) (28 mg; yield 19%): amorphous solid; $[\alpha]_D$ 0.8 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 238 (4.21), 315 (3.94); ^{1}H NMR ($CDCl_3$) δ 6.77 (H-6′, d, J = 2.9 Hz), 6.67 (H-4′, d, J = 2.9 Hz) and see Supporting Information; EIMS *m/z* 454 $[M + 2]^+$ (0.5), 452 $[M]^+$ (12), 262 (22), 191 (30), 135 (22), 107 (30), 95 (100); HREIMS *m/z* 452.2380 (calcd for $C_{28}H_{36}O_3S$, 452.2385). The less polar component was 4′-(benzylthio)avarol (**5**) (5 mg; yield 4%): amorphous solid; $[\alpha]_D$ 2.4 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 258 (4.16), 332 (3.95); ^{1}H NMR ($CDCl_3$) δ 6.96 (H-6′, s), 6.78 (H-4′, s) and see Supporting Information; EIMS *m/z* 454 $[M + 2]^+$ (0.4), 452 $[M]^+$ (10), 262 (18), 248 (12), 191 (22), 189 (18), 135 (30), 107 (35), 95 (100); HREIMS *m/z* 452.2389 (calcd for $C_{28}H_{36}O_3S$, 452.2385).

Synthesis of Avarol-3′-thiolactate (6) and Avarol-4′-thiolactate (7). Thiolactic acid (100 μL) dissolved in EtOH (5 mL) was added to a solution of avarone (100 mg) in EtOH (10 mL) and stirred for 3 h at 60 °C. After evaporation of EtOH the residue was chromatographed on a Si gel column and eluted with petroleum ether−Et_2O−HOAc (1:1:0.1). Further purification was performed by HPLC (Kromasil C18), using CH_3CN−H_2O (9:1) as a mobile phase, to give avarol-3′-thiolactate (**6**) (11 mg; yield 8%): amorphous solid; $[\alpha]_D$ −4.2 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 213 (4.31), 315 (3.67); ^{1}H NMR ($CDCl_3$) δ 6.85 (H-6′, d, J = 2.7 Hz), 6.67 (H-4′, d, J = 2.7 Hz) and see Supporting Information; EIMS *m/z* 420 $[M + 2]^+$ (0.3), 418 $[M]^+$ (8), 228 (16), 214 (8), 191 (15), 189 (13), 135 (30), 107 (35), 95 (100); HREIMS *m/z* 418.2183 (calcd for $C_{24}H_{34}O_4S$, 418.2178), and avarol-4′-thiolactate (**7**) (23 mg; yield 18%): amorphous solid; $[\alpha]_D$ 11.8 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 279 (4.43), 319 (4.23); ^{1}H NMR ($CDCl_3$) δ 6.85 (H-6′, s), 6.76 (H-4′, s) and see Supporting Information; EIMS *m/z* 420 $[M + 2]^+$ (0.5), 418 $[M]^+$ (10), 228 (14), 214 (10), 191 (20), 189 (10), 135 (30), 107 (30), 95 (100); HREIMS *m/z* 418.2184 (calcd for $C_{24}H_{34}O_4S$, 418.2178).

Synthesis of Avarol-3′-thiobenzothiazole (8) and Avarol-4′-thiobenzothiazole (9). 2-Mercaptobenzothiazole (100 mg) was dissolved in EtOH (5 mL), added to a solution of avarone (100 mg) in EtOH (10 mL), and stirred for 7 h at 60 °C. After evaporation of EtOH the residue was chromatographed on a Si gel column and eluted with petroleum ether−Et_2O (3:2). Further purification was performed by HPLC, using CH_3CN−H_2O (9:1) as a mobile phase, to give avarol-3′-thiobenzothiazole (**8**) (18 mg; yield 12%): amorphous solid; $[\alpha]_D$ −4.6 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 220 (4.90), 277 (4.49), 319 (4.29); ^{1}H NMR ($CDCl_3$) δ 7.00 (H-6′, d, J = 3.0 Hz), 6.84 (H-4′, d, J = 3.0 Hz) and see Supporting Information; EIMS *m/z* 481 $[M + 2]^+$ (1.3), 479 $[M]^+$ (15), 289 (14), 275 (10), 191 (18), 189 (12), 135 (30), 107 (25), 95 (100); HREIMS *m/z* 479.1955 (calcd for $C_{28}H_{33}NO_2S_2$, 479.1952), and avarol-4′-thiobenzothiazole (**9**) (15 mg; yield 10%): amorphous solid; $[\alpha]_D$ 15.1 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 218 (4.91), 274 (4.45), 320 (4.38); ^{1}H NMR ($CDCl_3$) δ 6.98 (H-6′, s), 6.92 (H-4′, s) and see Supporting Information; EIMS *m/z* 481 $[M + 2]^+$ (0.9), 479 $[M]^+$ (10), 289 (18), 275 (8), 191 (15), 189 (15), 135 (25), 107 (20), 95 (100); HREIMS *m/z* 479.1950 (calcd for $C_{28}H_{33}NO_2S_2$, 479.1952).

Synthesis of Avarone-4′-thiocholesterol (10), Avarol-3′-thiocholesterol (11), Avarol-4′-thiocholesterol (12), and Avarol-3′,4′-thiocholesterol (13). Thiocholesterol (100 mg) was dissolved in EtOH (5 mL), added to a solution of avarone (100 mg) in EtOH (10 mL), and stirred for 3 h at 60 °C. After evaporation of EtOH, the residue was chromatographed on a Si gel column and eluted with petroleum ether−Et_2O (9:1) to give, in order of polarity, avarone-4′-thiocholesterol (**10**) as the less polar component (42 mg; yield 18%): amorphous solid; $[\alpha]_D$ −6.1 (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 230 (4.49), 306 (4.12); ^{1}H NMR ($CDCl_3$) δ 6.52 (H-6′, s), 6.37 (H-4′, s) and see Supporting Information; EIMS *m/z* 714 $[M + 2]^+$ (0.4), 712 $[M]^+$ (8), 522 (15), 508 (10), 191 (15), 189 (13), 135 (20), 107 (18), 95 (100); HREIMS *m/z* 712.5257 (calcd for $C_{48}H_{72}O_2S$, 712.5253), avarol-3′,4′-thiocholesterol (**13**) (14 mg; yield 4%): amorphous solid; $[\alpha]_D$ −0.5 (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 232 (4.55), 307 (4.33); ^{1}H NMR ($CDCl_3$) δ 6.80 (H-6′, s) and see Supporting Information; EIMS *m/z* 1116 $[M + 2]^+$ (0.4), 1114 $[M]^+$ (5), 924 (18), 910 (12), 191 (18), 189 (17), 135 (25), 107 (25), 95 (100); HREIMS *m/z* 1114.8575 (calcd for $C_{75}H_{118}O_2S_2$, 1114.8573), avarol-4′-thiocholesterol (**12**) (19 mg; yield 8%): amorphous solid; $[\alpha]_D$ −1.1 (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 234 (4.31), 308 (4.00); ^{1}H NMR ($CDCl_3$) δ 6.82 (H-6′, s), 6.74 (H-4′, s) and see Supporting Information; EIMS *m/z* 716 $[M + 2]^+$ (0.3), 714 $[M]^+$ (6), 524 (10), 510 (8), 191 (18), 189 (12), 135 (20), 107 (25), 95 (100); HREIMS *m/z* 714.5412 (calcd for $C_{48}H_{74}O_2S$, 714.5409), and avarol-3′-thiocholesterol (**11**) as the most polar component (48 mg; yield 21%): amorphous solid; $[\alpha]_D$ −7.7 (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 234 (4.54), 308 (4.12); ^{1}H NMR ($CDCl_3$) δ 6.81 (H-6′, d, J = 2.7 Hz), 6.61 (H-4′, d, J = 2.7 Hz) and see Supporting Information; EIMS *m/z* 716 $[M + 2]^+$ (0.4), 714 $[M]^+$ (8), 524 (8), 510 (8), 191 (15), 189 (15), 135 (23), 107 (20), 95 (100); HREIMS *m/z* 714.5405 (calcd for $C_{48}H_{74}O_2S$, 714.5409).

Biological Assays. Antimicrobial activity was carried out using liquid culture of three bacterial strain, *E. coli* (DSM 498), *B. subtilis* subsp. *Spizizenii* (DSM 347), and *M. luteus* (DSM 348) grown in nutrient broth (Oxoid) at 37 °C, and the yeast *S. cerevisiae* (DSM 70449) grown in medium M186 (peptone 5 g/L, glucose 10 g/L, yeast extract 3 g/L, malt extract 3 g/L) at 37 °C. The MIC was determined by a serial dilution, in duplicate, starting from 100 μg/mL to 0.01 μg/mL. The bacterial and yeast growth was observed after 48 h of incubation.

Cytotoxic activity was evaluated by the brine shrimp (*Artemia salina*) test in triplicate. The compounds were dissolved in DMSO (at least 2 mg/200 μL DMSO) to reach final concentrations of 100, 10, and 1 ppm, in 5 mL of artificial seawater using 10 freshly hatched larvae of *A. salina*.[15] Briefly, for each dose tested, surviving shrimps were counted after 24 h, and the data statistically analyzed by the Finney program,[25] which affords LD_{50} values with 95% confidence intervals.

Free-radical scavenging activity was performed in MeOH, at different concentrations (5, 10, 20, 50, and 100 μM). Solutions of each compounds were prepared and adjusted to 2 mL total volume with 0.7 mL of DPPH−MeOH solution (6 mg/50 mL; 0.1 mM final concentration). The absorbance at 517 nm was determined after 30 min, and the percent free-radical inhibition was calculated and plotted to obtain the IC_{50} value. Trolox, a synthetic antioxidant compound, was used as positive control standard. The IC_{50} value denotes the concentration of compound required to scavenge 50% DPPH free radical.

Acethylcolinesterase inhibition was performed dissolving the samples in MeOH at a concentration of 1 mg/mL. From this main solution was performed a serial dilution in order to obtain lower concentration of samples (0.1; 0.01; 0.001 mg/mL), and 10 μL of each solution was applied to TLC plates to test 10, 1, 0.1, and 0.01 μg of samples to detect the minimum concentration that inhibited AChE. Galanthamine was used as positive control. The assay was carried out as described by Marston et al.[20] Briefly, a stock solution of acetylcholinesterase (1000 U in 150 mL of Tris-hydrochloric acid buffer pH 7.8) was obtained, which was stabilized adding bovine serum albumin (150 mg). A 10 μL aliquot of each solution of the samples was applied to the TLC plates, dried to remove the solvent, and then sprayed with enzyme stock solution. For incubation of the enzyme, the plate was kept at 37 °C for 20 min in a humid atmosphere. For the detection of the enzyme, solutions of 1-naphthyl acetate (250 mg in 100 mL of EtOH) and of Fast Blue B salt (400 mg in 160 mL of distilled H_2O) were mixed and sprayed onto the plate. Acethylcolinesterase inhibition activity was detected by a white spot on a purple background after 1−2 min.

Acknowledgment. This research was supported by CNR-Rome. One of the authors (B.P.) acknowledges an Italian Minister of Foreign Affairs fellowship. The assistance of Mr. V. Mirra is gratefully acknowledged.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Minale, L.; Riccio, R.; Sodano, G. *Tetrahedron Lett.* **1974**, *15*, 3401–3404.
(2) De Rosa, S.; Minale, L.; Riccio, R.; Sodano, G. *J. Chem. Soc., Perkin Trans. 1* **1976**, *1408*, 1414.
(3) De Rosa, S. Mediterranean Marine Organisms as Source of New Potential Drugs. In *Natural Products in the New Millennium: Prospects and Industrial Application*; Rauter, A. P., Palma, F. B., Justino, J., Araújo, M. E., dos Santos, S. P., Eds.; Kluwer Academic Publishers: Dordrecht, 2002; pp 441−461.
(4) Ferrándiz, M. L.; Sanz, M. J.; Bustos, G.; Payá, M.; Alcaraz, M. J.; De Rosa, S. *Eur. J. Pharmacol.* **1994**, *253*, 75–82.
(5) Shen, Y. C.; Lu, C. H.; Chakraborty, R.; Kuo, Y. H. *Nat. Prod. Res.* **2003**, *17*, 83–89.
(6) Belisario, M. A.; Maturo, M.; Pecce, R.; De Rosa, S.; Villani, G. R. *Toxicology* **1992**, *72*, 221–233.
(7) Belisario, M. A.; Maturo, M.; Avagnale, G.; De Rosa, S.; Scopacasa, F.; De Caterina, M. *Pharmacol. Toxicol.* **1996**, *79*, 300–304.
(8) Sarin, P. S.; Sun, D.; Thornton, A.; Müller, W. E. G. *J. Natl. Cancer Inst.* **1987**, *78*, 663–666.
(9) Sipkema, D.; Osinga, R.; Schatton, W.; Mendola, D.; Tramper, J.; Wijffels, R. H. *Biotechnol. Bioeng.* **2005**, *90*, 201–222.
(10) Amigó, M.; Payá, M.; Braza-Boïls, A.; De Rosa, S.; Terencio, M. C. *Life Sci.* **2008**, *82*, 256–264.
(11) De Rosa, S.; De Giulio, A.; Strazzullo, G. *Trends Org. Chem.* **1991**, *2*, 127–141.
(12) Belisario, M. A.; Pecce, R.; Maturo, M.; De Rosa, S. *Toxicology* **1994**, *86*, 89–108.
(13) Amigó, M.; Payá, M.; De Rosa, S.; Terencio, M. C. *Br. J. Pharmacol.* **2007**, *152*, 353–365.
(14) Amigó, M.; Terencio, M. C.; Payá, M.; Iodice, C.; De Rosa, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2561–2565.
(15) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31–34.
(16) Crispino, A.; De Giulio, A.; De Rosa, S.; Strazzullo, G. *J. Nat. Prod.* **1989**, *52*, 646–648.
(17) Takao, T.; Kitatani, F.; Watanabe, N.; Yagi, A.; Sakata, K. *Biosci. Biotechnol. Biochem.* **1994**, *51*, 1780–1783.
(18) Cuendet, M.; Hostettmann, K.; Potterat, O.; Dyatmiko, W. *Helv. Chim. Acta* **1997**, *80*, 1144–1152.
(19) Rhee, I. K.; van de Meent, M.; Ingkaninaa, K.; Verpoorte, R. *J. Chromatogr. A* **2001**, *915*, 217–223.
(20) Marston, A.; Kissling, J.; Hostettmann, K. *Phytochem. Anal.* **2002**, *13*, 51–54.
(21) Scott, L. J.; Goa, K. L. *Drugs* **2000**, *60*, 1095–1122.
(22) Amigó, M.; Terencio, M. C.; Mitova, M.; Iodice, C.; Payá, M.; De Rosa, S. *J. Nat. Prod.* **2004**, *67*, 1459–1463.
(23) U.S. Food and Drug Administration Alert [5/2005].
(24) Pouchert, C. J.; Behnke, J. *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*; Aldrich Chemical Company, Inc., 1993; Vols. 1−3.
(25) Finney D. J. *Probit Analysis*, 3rd ed.; Cambridge University Press: Cambridge, U.K, 1971.

NP800318M