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Design, synthesis, and biological evaluation of new (2*E*,6*E*)-10-(dimethylamino)-3,7-dimethyl-2,6-decadien-1-ol ethers as inhibitors of human and *Trypanosoma cruzi* oxidosqualene cyclase

Ubaldina Galli,[a,*] Simonetta Oliaro-Bosso,[b] Silvia Taramino,[b] Serena Venegoni,[a] Emanuele Pastore,[a] Gian Cesare Tron,[a] Gianni Balliano,[b] Franca Viola[b,*] and Giovanni Sorba[a]

[a]*Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche and Drug and Food Biotechnology Center, Università degli Studi del Piemonte Orientale 'A. Avogadro', Via Bovio 6, 28100 Novara, Italy*
[b]*Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, via Giuria 9, 10125 Torino, Italy*

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Abstract—New dimethylamino truncated squalene ether derivatives containing a different aromatic moiety (phenyl, naphthyl, and biphenyl) or a simple alkyl (*n*-hexylic) group were synthesized as inhibitors of the oxidosqualene cyclase (OSC) and of the sterol biosynthetic pathway. The activity against human OSC was compared with the activity against the OSCs of pathogenic organisms such as *Pneumocystis carinii* and *Trypanosoma cruzi*. The phenyl derivative was the most potent inhibitor of *T. cruzi* OSC.
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Lanosterol synthase (E.C. 5.4.99.7, oxidosqualene cyclase, OSC) plays a pivotal role in the life of most eukaryotic organisms.[1] This enzyme catalyzes the cyclization of 2,3-oxidosqualene (OS) to lanosterol.[2] In this reaction four rings and six bonds are created in a tandem fashion with an impressive stereochemical control to give only one of the 2^8 possible isomers. Lanosterol is then converted into biologically important sterols.[3] OSC has therefore become a fundamental target in medicinal chemistry research and cholesterol-lowering and antifungal compounds acting on this enzymes have been designed and synthesized.[4] This enzyme could be a more advantageous target than the other enzymes of this synthetic pathway. For example, the inhibition of enzymes upstream of OSC should be avoided since it can affect other important biochemical pathways, such as protein prenylation and ubiquinone synthesis.[5] The inhibition of enzymes involved in the downstream steps should also be avoided as toxicity of accumulated intermediates cannot be excluded.[6] Recently a series of OSC inhibitors has been tested against pathogenic protozoa with the aim of developing new antiparasitic agents.[7]

The diseases caused by protozoan parasites are becoming an urgent research topic, not only in tropical regions, but also in once safer countries.[8] These diseases cause high rates of mortality and morbidity, and few drugs are currently available to treat these diseases,[9] therefore the identification of new derivatives endowed with a selective action on protozoan OSC is a hot topic in medicinal chemistry. We focused our attention on the OSC of *T. cruzi*, the protozoan responsible for Chagas' disease.[10] The protein sequence of the *T. cruzi* OSC is known and has important differences compared to human OSC[11] in some amino acids involved in the active site. For example, at position 540 a tyrosine replaces a threonine and this residue appears to be important for cyclization.[11] These differences could therefore be exploited to obtain selective inhibitors.[7,12] In a previous work, we tested different types of squalene derivatives, known to be OSC inhibitors, on the *T. cruzi* OSC. We showed that some phenylthio truncated oxide squalene derivatives were selective for the *T. cruzi* OSC with respect to analogues devoid of a phenyl substituent.[13] The most active and selective phenylthio derivative is

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* Corresponding authors. Tel.: +39 0321 375856; fax: +39 0321 357821 (U.G.); tel.: +39 011 6707698; fax: +39 011 6707695 (F.V.); e-mail addresses: galli@pharm.unipmn.it; franca.viola@unito.it

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shown in Figure 1. In order to verify the effect of the aromatic group on the selectivity for *T. cruzi* OSC, we designed a series of novel truncated dimethylamino squalene ether derivatives containing one of three different aromatic rings (phenyl, naphthyl, and biphenyl) or a simple *n*-hexylic alkyl group (Fig. 1). The new derivatives contain a dimethylamino group, often present in OSC inhibitors, as it is well known that at physiological pH, tertiary amines are protonated and can mimic the carbocationic intermediate at the first steps of OSC cyclization.[14]

The practical synthesis of these new derivatives and their inhibition of OSC and sterol biosynthesis in different biological systems are the subject of the present letter.

The synthesis of the key intermediate **6** and of the final compounds **7**–**10** is summarized in Scheme 1. It was recognized that geraniol **1** could be used as a starting material as it contains two double bonds at the appropriate positions, one of which bears the correct, substrate-like configuration (i.e., *E* configuration). Our first attempt to prepare the intermediate **5**, through a previously established synthetic pathway,[15] by protecting the hydroxyl group with *tert*-butyldimethylsilyl chloride followed by an allylic hydroxylation using catalytic selenium dioxide in the presence of *tert*-butylhydroperoxide and subsequently a conversion to a bromide, failed as it was not possible to brominate the allylic alcohol without an extensive decomposition of the starting material. For this reason, we replaced the silyl-protecting group with an acetate. Geraniol reacts smoothly with Ac_2O yielding the product **2** which was subsequently submitted to an allylic hydroxylation.[16] The expected *E* allylic alcohol **3** was obtained with a yield of 48%. This result was not surprising, since it has been reported that geraniol esters react with selenium dioxide, giving the adduct in higher yields compared to the typical procedure because the ester can participate in the stabilization of the six-membered ring transition state.[17] The allylic alcohol was then brominated using the PPh_3/NBS protocol giving the desired adduct **4** with a 75% yield. The insertion of the dimethylaminoethyl group was accomplished using Rathke's salt[18] with the allylic bromide. The reaction was sluggish and a range of conditions (temperature, base, and solvent) were tested in order to establish the best synthetic protocol. The optimal procedure required the use of 2 equiv of *N*,*N*-dimethylacetamide, 2 equiv of lithium bis(trimethylsilyl)amide in dry THF at room temperature. Two main products were obtained, the desired protected adduct and the hydrolyzed form **5**. For this reason, the mixture was not separated but, after the work-up procedure, was transesterified to give the intermediate **5**, with an overall yield of 36%. The amide was reduced with $LiAlH_4$ to give **6**.[19] From this compound, we synthesized the desired final products **7**–**10** through a Williamson reaction (35–40%).[20] Finally, in order to confirm the important role of the protonatable tertiary amine in the interaction with the OSC, the amide analogue **11** was synthesized starting from **5**.

The inhibition of OSC activity by compounds **6**–**11** was tested in cell-free homogenates of OSC-defective *Saccharomyces cerevisiae* strains expressing the OSC enzymes, respectively, of *T. cruzi*, *Pneumocystis carinii*, and *S. cerevisiae*[21] (Table 1). The inhibition of human OSC was tested in cell-free homogenates of *Pichia pastoris* cells expressing the human OSC enzyme.[22,23] The results showed that the truncated squalene ether derivative **7**, bearing an aromatic phenyl ring, was very active against all the OSCs tested, and was the most active compound against the *T. cruzi* OSC. Comparison of **7** with **10**, the analogue lacking the aromatic moiety, showed that the phenyl ring improves fivefold the inhibition of *T. cruzi* OSC, but does not affect the inhibition of both human and *P. carinii* OSC. When the aromatic substituent is a naphthyl ring (compound **8**) the inhibitory activity at 10 μM fell to almost undetectable levels, except for the *P. carinii* OSC, while with the more flexible biphenyl ring of **9** a moderate activity was detected. The inhibition of the *P. carinii* OSC by compounds **7**–**10** was generally higher than the inhibition of the other OSCs tested and about three times higher than that of the human OSC. The higher inhibition of the *P. carinii* OSC has already been noted with a series of umbelliferone aminoalkyl derivatives.[24] The presence of the tertiary amine was essential for the activity of these truncated squalene ether derivatives since the amide analogue **11** was poorly active at 10 μM against both *T. cruzi* and human OSCs. A tertiary amine is a common moiety of most effective OSC inhibitors[4] possibly because of the interaction with the active site catalytic aspartate, as recently demonstrated in the structure of human OSC crystallized in the presence of the very effective inhibitor Ro48-8071.[25]

The IC_{50} of Table 1 are in agreement with the inhibition of sterol biosynthesis observed in 3T3 fibroblast cell cultures after incubation of [2-^{14}C]acetate either in presence or in absence of the truncated squalene ether derivatives. The incorporation of label into cholesterol and biosynthetic intermediates squalene, 2,3-oxidosqualene

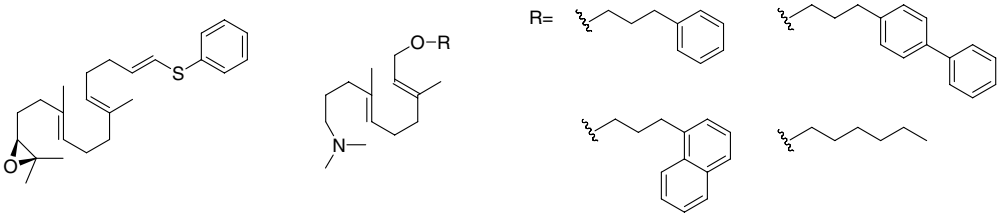
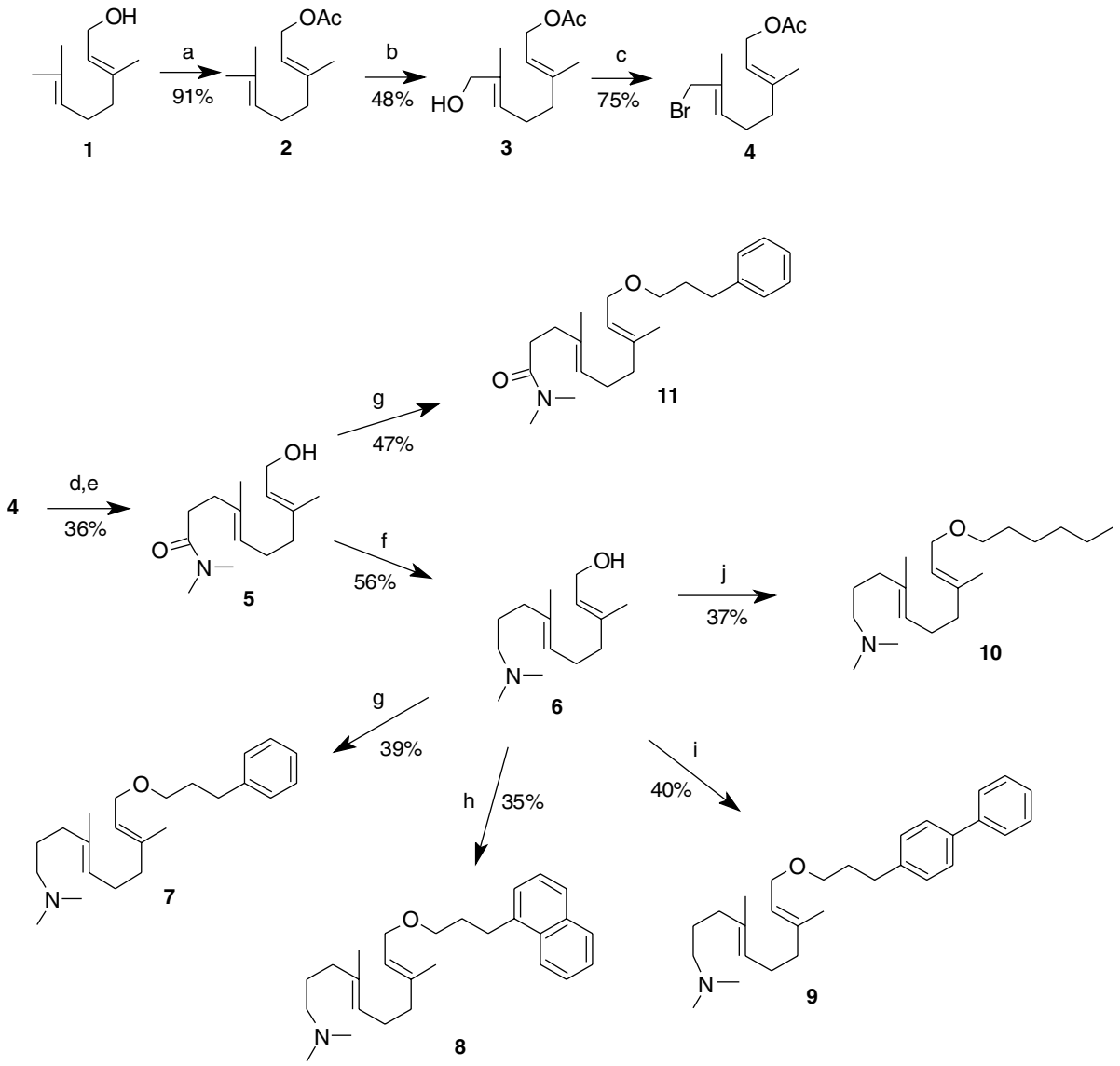


Figure 1. The selective inhibitor of the *T. cruzi* OSC phenylthio truncated oxide squalene derivative (left)[13] and the new truncated dimethylamino squalene ether derivatives (right).



Scheme 1. Reagents and conditions: (a) Ac_2O, TEA, DCM, rt; (b) 70% *t*-BuOOH, SeO_2, DCM, rt; (c) PPh_3, NBS, DCM, −30 °C; (d) *N*,*N*-dimethylacetamide, LHMDS, THF, rt; (e) K_2CO_3, MeOH, rt; (f) $LiAlH_4$, THF, rt; (g), (h), (i), (j) 1-bromo-3-phenylpropane, 1-(3-bromopropyl)naphthalene, 4-(3-bromopropyl)biphenyl, 1-bromohexane, respectively, NaH, DMF, rt.

Table 1. Inhibition of *Trypanosoma cruzi*, *Pneumocystis carinii*, *Saccharomyces cerevisiae*,[21] and human OSCs[23] by compounds **6**–**11**

Compound	IC_{50}[a] (μM)			
	T. cruzi OSC	*P. carinii* OSC	*S. cerevisiae* OSC	Human OSC
6	>10[b]	>10[b]	na[c]	>10[b]
7	0.47	0.33	0.45	1.10
8	>10[b]	10.00	na[c]	>10[b]
9	7.40	1.70	>10[b]	4.50
10	2.70	0.36	1.78	0.85
11	>10[b]	>10[b]	NT[d]	>10[b]

[a] Values are means of two separate experiments, each with duplicate incubations. The maximum deviation from the mean was less than 10%.

[b] IC_{50} not determined as, at the higher concentration tested (10 μM), the inhibition was ⩽20%.

[c] Not active at the higher concentration tested (10 μM).

[d] Not tested.

(OS), 2,3-24,25-dioxidosqualene (DOS), and lanosterol is reported in Table 2, as % of the total radioactivity incorporated.[26] A decrease in cholesterol biosynthesis accompanied by the accumulation of the intermediates OS and DOS, both substrates of OSC, is the typical biosynthetic pattern observed following OSC inhibition.[4d] In the presence of compounds **7** and **10** at a concentration 1 μM, the labeling of cholesterol was reduced by more than fifty percent, while the labeling of DOS and OS was increased by more than 10 and 5 times, respectively. At the same concentration the biphenyl-substituted compound **9** was still able to decrease the labeling of cholesterol by 35% and increase by 10 and 3 times, respectively, the labeling of DOS and OS. As expected, the naphthyl-substituted **8**, the amide **11**, and the geranyl derivative **6** were inactive at 1 μM; When tested at a higher concentration (50 μM), only compound **11** was able to decrease the labeling of cholesterol by more

Table 2. Incorporation of radioactivity in cholesterol and biosynthetic intermediates after incubation of cultured 3T3 fibroblasts with [2-^{14}C]acetate in the presence of 1 μM concentrations of compounds **6**–**11**[26]

Inhibitor (1 μM)	% of total radioactivity incorporated in sterols[a]				
	Cholesterol	Lanosterol	DOS	OS	Squalene
Control in absence of inhibitors	83.3 ± 12.8	29.0 ± 1.5	1.90 ± 0.9	5.70 ± 1.9	6.3 ± 0.9
6	88.2 ± 6.40	2.5 ± 1.8	1.3 ± 0.4	3.90 ± 2.2	4.1 ± 1.9
7	31.3 ± 7.10	5.5 ± 1.6	30.4 ± 7.8	27.9 ± 3.7	5.5 ± 1.7
8	72.7 ± 3.70	5.0 ± 0.4	3.20 ± 0.5	12.5 ± 0.8	6.6 ± 2.0
9	53.2 ± 12.2	6.4 ± 2.3	18.0 ± 6.2	17.5 ± 5.7	4.9 ± 3.2
10	35.5 ± 2.20	7.9 ± 0.8	22.3 ± 2.4	29.6 ± 1.2	4.6 ± 2.5
11	84.6 ± 4.10	3.2 ± 1.2	2.8 ± 0.3	4.60 ± 2.2	4.8 ± 0.4

[a] Values are means ± SD of two separate experiments, each with duplicate incubations.

than 50%, while **6** and **8** reduced the labeling by about 30%.

In summary, we have synthesized, by a simple procedure, new truncated dimethylamino squalene ether derivatives, containing a phenyl, a naphthyl, and a biphenyl aromatic ring. By comparing their inhibition of *T. cruzi*, *P. carinii*, *S. cerevisiae*, and human OSCs with that of a *n*-hexylic derivative, we have shown that the phenyl derivative, differently from the naphthyl and biphenyl, is a more effective inhibitor of the *T. cruzi* OSC.

The new class of compounds and the synthetic strategy adopted could be a good starting point for exploring the effect of substituents in both the phenyl ring and the aminoalkyl moiety, in order to design novel effective inhibitors of *T. cruzi* OSC.

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19. Compound **6**: yellow oil. MS (ESI) *m/z*: 226 $(M+H)^+$;^{1}H NMR (300 MHz, $CDCl_3$): δ 5.35 (br t, J = 6.87 Hz, 1H), 5.07 (br t, J = 6.60 Hz, 1H), 4.10 (d, J = 6.90 Hz, 2H), 2.96 (br s, OH), 2.22 (br s, 8H), 2.08–1.96 (m, 4H), 1.91 (t, J = 7.68 Hz, 2H), 1.64 (s, 3H), 1.55 (br s, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.5, 134.9, 124.4, 124.3, 59.3, 59.1, 45.3, 39.5, 37.2, 26.2, 25.5, 16.3, 16.2.
20. Compound **7**: yellow oil. MS (ESI) *m/z*: 344 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30–7.10 (m, 5-H), 5.33 (t, J = 6.86 Hz, 1H), 5.11 (t, J = 5.76, 1H), 3.97 (d, J = 6.60 Hz, 2H), 3.42 (t, J = 6.30 Hz, 2H), 2.69 (t, J = 7.40 Hz, 2H), 2.30 (br s, 8H), 2.11–1.89 (m, 6H), 1.87 (q, J = 6.33 Hz, 2H), 1.66 (br s, 5H), 1.59 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.1, 140.0, 135.1, 128.6, 128.4, 125.8, 124.1, 121.1, 69.4, 67.4, 59.6, 45.6, 39.7, 37.5, 32.5, 31.5, 26.3, 26.1, 16.6, 16.0. Compound **8**: yellow oil. MS (ESI) *m/z*: 394 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (d, J = 7.44 Hz, 1H), 7.84 (dd, J = 7.14/2.2 Hz, 1H), 7.70 (d, J = 7.68, 1H), 7.52–7.32 (m, 4H), 5.37 (br t, J = 5.49 Hz, 1H), 5.11 (br t, J = 5.76 Hz, 1H), 3.99 (d, J = 6.90 Hz, 2H), 3.48 (t, J = 6.30 Hz, 2H), 3.45 (t, J = 7.60 Hz, 2H), 2.41 (br s, 8H), 2.10–1.90 (m, 8H), 1.66 (br s, 5H), 1.60 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.8, 138.2, 134.1, 133.9, 132.0, 128.8, 126.7, 126.2, 125.8, 125.6, 125.5, 125.0, 124.0, 121.2, 69.6, 67.5, 58.8, 44.5, 39.6, 37.0, 30.8, 29.6, 26.3, 24.4, 16.6, 15.9. Compound **9**: yellow oil. MS (ESI) *m/z*: 420 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58 (d, J = 8.25 Hz, 2H), 7.51 (d, J = 8.28 Hz, 2H), 7.42 (t, J = 7.35, 2H), 7.34–7.32 (m, 1H), 7.27 (d, J = 7.95, 2H), 5.38 (t, J = 6.75 Hz, 1H), 5.12 (t, J = 5.82 Hz, 1 H), 3.99 (d, J = 6.72 Hz, 2H), 3.46 (t, J = 6.42 Hz, 2H), 2.74 (t, J = 7.35 Hz, 2H), 2.25 (br s, 8H), 2.16–1.90 (m, 8H), 1.68 (s, 3H), 1.60 (br s, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 141.3, 141.2, 140.0, 138.8, 134.9, 129.0, 128.8, 127.2, 127.1, 124.3, 121.1, 69.4, 67.4, 59.4, 45.4, 39.7, 37.4, 32.2, 31.5, 26.3, 25.8, 16.6, 16.0. Compound **10**: yellow oil. MS (ESI) *m/z*: 310 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.32 (t, J = 6.60 Hz, 1H), 5.08 (t, J = 6.33, 1H), 3.94 (d, J = 6.87 Hz, 2H), 3.38 (t, J = 6.87 Hz, 2 H), 2.22 (br s, 8H), 2.10–2.01 (m, 4H), 1.95 (t, J = 7.68 Hz, 2H), 1.64 (s, 3H), 1.60–1.50 (m, 7H), 1.36–1.23 (m, 6H), 0.86 (br t, J = 6.87 Hz 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.8, 134.9, 124.2, 121.2, 70.5, 67.3, 59.5, 45.4, 39.6, 37.4, 31.8, 29.9, 26.3, 26.0, 25.8, 22.7, 16.6, 16.0, 14.2. Purity of target compounds was checked by HPLC analysis using a SYNERGI MAX-RP 80A (150 × 4.6 mm, 4 μm particle size) column on a Shimadzu HPLC system. Solvents for the separation were: solvent A: water (0.01% w/v H_3PO_4) ; solvent B: acetonitrile (0.01% w/v H_3PO_4) at a flow rate of 0.7 ml/min and a sample injection volume of 20 μl, λ = 220 nm. For all compounds, eluants A and B were delivered isocratically at a 20:80 ratio. All four compounds displayed a purity of at least 95%.
21. The *S. cerevisiae* strains: SMY8[pBJ1.21], expressing the OSC of *T. cruzi*; SMY8[pSM61.21], expressing the wild-type yeast OSC; SMY8[pBJ4.21], expressing the *P. carinii* OSC were kindly provided by Professor S. P. T. Matsuda [Department of Chemistry and Biochemistry and Cell Biology, Rice University, Houston, TX, USA. For references and cultural conditions see Refs. 11, 13, and 24. The enzymatic activity was determined as described in Refs. 13 and 24.
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26. Incorporation of [2-^{14}C]acetate into cholesterol and biosynthetic intermediates was determined in 3T3 fibroblasts (ATCC CCL 92 3T3 Swiss albino) as previously described in Ref. 4d. Briefly, 7×10^4 cells were suspended and grown for 24 h in 950 μl DMEM containing 10% (v/v) lipid-depleted serum. After a 3 h preincubation in the presence of inhibitor, labeled acetate (1 μCi/well) was added. After further 3 h incubation, the medium was removed and the cells were lysed with 500 μl of 0.1 N NaOH for 30 min. After saponification with 1.5 ml of 10% (w/v) methanolic KOH for 1 h at 80 °C, nonsaponifiable lipids were extracted with petroleum ether and separated on TLC plates (Alufolien Kieselgel 60F254, Merck, Darmstadt, Germany) using *n*-hexane/ethyl acetate (85:15 v/v) as a developing solvent and authentic standards of cholesterol, lanosterol, dioxidosqualene, oxidosqualene, and squalene as reference compounds. Radioactivity in separated bands was measured using a System 200 Imaging Scanner (Hewlett-Packard, Palo Alto, CA, USA).