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DESIGN AND SYNTHESIS OF NEW HYPOCHOLESTEROLEMIC ORGANOSILANES WITH ANTIOXIDANT PROPERTIES

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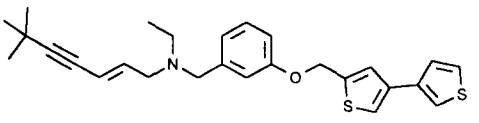
Abstract: (Arylamino)methylsilane derivatives **3** have been prepared and identified as potent inhibitors of human LDL oxidation mediated by copper(II). Combination of this property with the structural requirement of squalene epoxidase inhibitors led to the design and synthesis of the (arylamino)methylsilane derivative **4** which was characterised as the first potent, orally active squalene epoxidase inhibitor with antioxidant properties.

Early epidemiological data indicate a strong association between LDL-cholesterol levels and the incidence of atherosclerosis[1]. More recently, the concept of oxidatively modified LDL as potential atherogenic particles gained momentum since evidence exists to suggest that LDL oxidation may represent an important event in the early steps of atherogenesis[2].

Plasma LDL-cholesterol can be lowered by temporary interruption of cholesterol biosynthesis which leads to an increased activity of hepatic LDL receptors and consequent removal of LDL-cholesterol from the blood plasma[3]. This has been widely illustrated by the use of HMG-CoA reductase inhibitors as cholesterol lowering drugs with proven clinical efficacy[4]. Squalene epoxidase (SE) (EC 1.14.99.7) has also been recently identified as another key enzyme in the biosynthesis of cholesterol[5]. Inhibitors of that particular enzyme could demonstrate advantages over HMG-CoA reductase inhibitors since the latter are involved in other important cellular prenylation processes. The therapeutic potential of SE inhibitors has been confirmed by the ability of NB-598 **1** to decrease cholesterol levels in dogs[6] and, more recently, we have shown that the first silicon-containing SE inhibitor **2** was also a potent orally active cholesterol biosynthesis inhibitor[7] .



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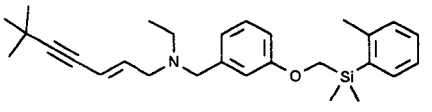

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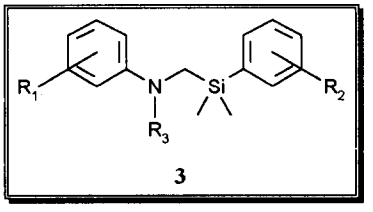
As part of our program directed toward the discovery of new drugs for atherosclerosis, we have focused our attention on the design of compounds which would simultaneously decrease cholesterol levels by inhibiting squalene epoxidase (LDL quantity) and exert an antioxidant activity (LDL quality).

Most of the antioxidant compounds known to protect LDL particles from oxidative stress are essentially electron-rich phenol derivatives[8] (for example, vitamin E or probucol which have demonstrated efficacy in experimental models of atherosclerosis). Nevertheless, the design of potent squalene epoxidase inhibitors with antioxidant properties are not a straightforward goal to reach, SE is a very selective enzyme which only recognizes very lipophilic substrates (squalene) or inhibitors (e.g. compounds **1** or **2**), with a high structural specificity[5,7,9]. Thus, our preliminary attempts to modify known squalene epoxidase inhibitors by introducing electron-rich phenol sub-units led to the identification of potent antioxidant agents, but, unfortunately with a dramatic loss of SE inhibitory potency[10].

In order to address this question, we turned our efforts towards the identification of completely new antioxidant moieties which would be compatible with squalene epoxidase inhibition. Starting from the reported[11] observation that (trimethylsilyl)methyl substitution of aromatic amines induces a dramatic decrease in oxidation potential, we considered that this type of aniline derivative could be potentially active as biological antioxidants. It is noteworthy that some (trialkylsilyl)methylamine derivatives have previously been characterized as potent irreversible MAO inhibitors[12], but to our knowledge, no data has ever been reported concerning their antioxidant properties, especially related to the oxidation of LDL.

We report in this letter the first biological data demonstrating the antioxidant properties of (arylamino)methylsilane derivatives of type **3**, and furthermore, we describe, the synthesis and biological properties of compound **4**, a new SE inhibitor combining for the first time hypocholesterolemic with antioxidant properties.



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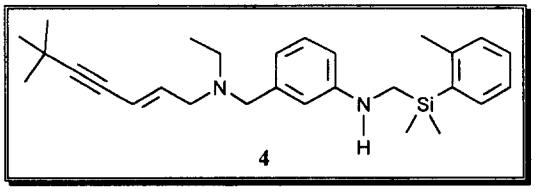

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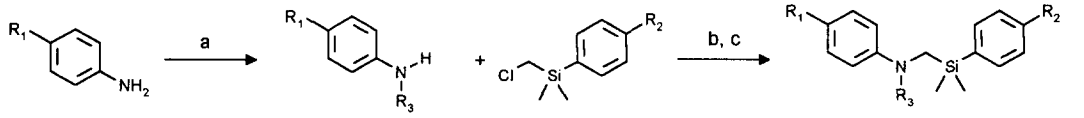
(Arylsilyl)methylaniline derivatives **3a-3e** were first prepared in order to evaluate their potential as antioxidants. Compounds **3a** and **3b** (Table 1) were obtained by condensation of the corresponding trifluoroacetylated aniline derivatives **6a-b** with (chloromethyl)phenyldimethylsilane (Scheme 1) under basic conditions, followed by removal of the trifluoroacetyl activating group with potassium hydroxide in methanol.

N-ethyl substituted silylmethylaniline derivatives **3c-3e** (Table 1) were obtained by condensation of the N-acetylated aniline derivative **6c-6e** with the appropriate chloromethylsilane under the same conditions as described above, followed by reduction of the N-acetyl group with lithium aluminium hydride in tetrahydrofuran (Scheme 1).

Scheme 1[a]



5a-e **6a-b** (R_3=$COCF_3$) **7a-e** **3a-b** (R_3=H)
6c-e (R_3=$COCH_3$) **3c-e** (R_3=Et)

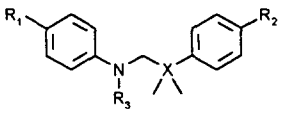
[a] (a) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, r.t., 2h (95%) or $(CH_3CO)_2O$, Et_3N, CH_2Cl_2, r.t., 1h (95%); (b) i. **6a-e**, NaH, DMF, r.t. 1h ii. **7a-e**, 80°C, 4-20h (65-85%); (c) KOH, MeOH, r.t., 2h (100%) or $LiAlH_4$, THF, 0°C, 1h (80-90%).

Compounds **3a-3e** (Table 1) were found to inhibit the *in vitro* oxidation of human LDL mediated by copper(II) in a concentration-dependent manner[13]. In this study, the antioxidative effects of compounds **3a-3e** was compared with the natural antioxidant vitamin E, with probucol and also with the carba-analog[14] derivative **3f**. The results reported in Table 1 show that the (aryldimethyl)silylmethylaniline derivatives **3a** and **3b** are potent inhibitors of copper-induced peroxidation of LDL especially when compared to the results obtained with vitamin E or probucol under the conditions tested. Interestingly enough, **3a** (IC_{50} = 7.8 μM) is much better at protecting LDL from oxidation than its carba-analog **3f** (IC_{50} = 100 μM) demonstrating the importance of the silicon atom on the antioxidant properties of silylmethylanilines.

Moreover, the observations (Table 1) that a trifluoromethyl electron-withdrawing group has a dramatic effect on antioxidant properties only when attached to the aniline aromatic ring but not to the silyl-substituted aromatic ring (compare **3c** with **3d** and **3e**) as well as the superior antioxidant efficacy of compound **3b** (which contains an electron-donating group on the aniline aromatic ring) compared to **3a** are also in accordance with the hypothesis that antioxidant potency, within this series of compounds, is directly related to their oxidation potential[11]. This is to say, the capability of these compounds to protect LDL from oxidative stress seems to be associated with the stability of the cation-radical formed upon oxidation. Therefore, the silylmethylaniline derivatives **3a-3e** are believed to act as "chain-breaking" antioxidants *i.e.* molecules which are oxidised more rapidly than lipids so leading to products which have insufficient reactivity to propagate radical reactions.

Altogether, the results disclosed in Table 1 demonstrate for the first time that (aryldimethyl)silylmethyl derivatives of aniline are lipid peroxidation inhibitors. However, none of compounds **3a-3e** were found to inhibit squalene epoxidase.

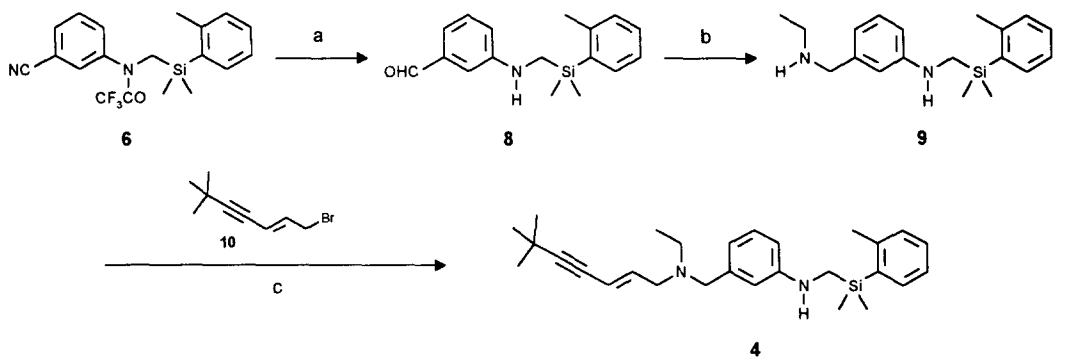
Table 1



Cpd	X	R_1	R_2	R_3	IC_{50} (μM)[13]
3 a	Si	H	H	H	7.8
3 b	Si	OCH_3	H	H	3.0
3 c	Si	H	H	Et	15
3 d	Si	CF_3	H	Et	~100
3 e	Si	H	CF_3	Et	20
3 f	C	H	H	H	~100
Vitamin E	-	-	-	-	10
Probucol	-	-	-	-	5.3

From the data outlined above (Table 1) and from our earlier work demonstrating the potency of compound **2** as a squalene epoxidase inhibitor[7], we decided to prepare the (arylamino)methylsilane derivative of benzylamine **4** as a first prototype which should combine, within the same molecule, SE and lipid peroxidation inhibitory properties. The synthesis of compound **4** was readily achieved in 5 steps according to the method depicted in Scheme 2. Reduction of the intermediate **6** (easily obtained in 75% yield by condensation of the N-trifluoroacetyl derivative of 3-cyano aniline with chloromethyl(2-methylphenyldimethyl)silane) with DiBAl-H in toluene allowed the formation of the aldehyde **8** with concomitant removal of the trifluoroacetyl group. Reductive amination of **8** using ethylamine and sodium borohydride in ethanol gave the expected secondary amine **9**. Treatment of this amine with the allylic bromide[15] derivative **10** in the presence of triethylamine led exclusively to alkylation on the most reactive nitrogen atom to afford the final product **4** in 75% yield[16].

Scheme 2[a]



(a) DiBAl-H, toluene, -20°C, 2h (80%); (b) i. $EtNH_2$, EtOH, 20°C; ii. $NaBH_4$, EtOH, 20°C (85%); (c) Et_3N, DMF, r.t., 8h (75%)

The (arylamino)methylsilane derivative **4** was compared to the (aryloxy)methylsilane derivative **2** (Table 2) as an *in vitro* inhibitor of pig liver microsomal squalene epoxidase[17] and as an inhibitor of cholesterol biosynthesis in Hep-G_2 cells in culture[18]. In addition, these two compounds were also evaluated *in vivo* as inhibitors of cholesterol biosynthesis from [^{14}C] acetate after a single oral administration in female rats[18].

In parallel, the ability of **2** and **4** to protect human LDL from oxidation was compared in two different tests in which oxidative modification of LDL has been induced either by copper ions[13] or by cultured human umbilical vein endothelial cells (Table 2) according to a well established procedure[19].

Table 2

Cpd	S.E. IC_{50} (μM)	Hep G_2 IC_{50} (μM)	rat ED_{50} (mg/kg)	Cu^{++} IC_{50} (μM)	HUVEC IC_{50} (μM)
2	0.03	0.12	3.2	>50	50
4	0.1	0.20	10	20	7

The results disclosed in Table 2 demonstrate that both (aryloxy)methylsilane derivative **2** and (arylamino)methylsilane derivative **4** are potent squalene epoxidase inhibitors which can control cholesterol biosynthesis *in vitro* in Hep-G_2 cells or *in vivo* in rats upon oral administration. Although this data shows that the (arylamino)methylsilane derivative **4** is slightly less potent than the (aryloxy)methylsilane **2** as a cholesterol biosynthesis inhibitor, comparison of both compounds in copper or HUVEC-induced LDL oxidation procedures demonstrates the large superiority of the (arylamino)methylsilane derivative **4** as an antioxidant. It is noteworthy that, when human LDL particles are oxidised by endothelial cells, the protecting effect of compound **4** (IC_{50} = 7 μM) compares very favorably with the result obtained with vitamin E (IC_{50} = 13.6 μM) under the same experimental conditions thus demonstrating the therapeutic potential of such derivatives in protecting human LDL against oxidation.

In conclusion, (arylamino)methylsilanes appear as a new class of promising biological antioxidants as demonstrated here by their ability to protect LDL from oxidative stress. Combining this property with the potency of compound **4** to inhibit cholesterol biosynthesis allows new and promising directions toward the design of new hypocholesterolemic drugs with antioxidant properties. However, the therapeutic potential of antioxidants are not restricted to the problem of atherosclerosis as demonstrated by numerous recent studies (for example ischemia-reperfusion, inflammation, cancer, ageing and neurodegenerative diseases)[20]. Work is in progress to enlarge the scope of application of these new antioxidants and also to elucidate their mechanism of action.

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