Antioxidants Derived from Vitamin E: An Overview

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Abstract: α-tocopherol is a very well-known potent antioxidant and radical scavenger in chemical and biological systems. Its structure has served as starting point for design and synthesis of more potent antioxidant analogues with regard to its potential clinical and nutritional applications in human health. Furthermore, in recent years, intense research has been made not only in the development of hybrid compounds with classical drug moieties in a single molecule, but also in the preparation of label analogues with application in tocopherol metabolism studies. In the present review principal progresses in these aspects are outlined.

Key Words: Vitamin E, antioxidants, synthetic analogues.

INTRODUCTIOIN

Since the early 1960s, α-tocopherol (α-T), a potent antioxidant and radical scavenger in chemical and biological systems, and its analogues have been receiving increasing attention with regard to its potential clinical and nutritional applications in human health [1]. The function of vitamin E (Fig. (**1**)) has been attributed to its capacity to protect the organism against the attack of free radicals by acting as a lipid based radical chain breaking molecule. More recently, alternative non-antioxidant functions of vitamin E have been proposed. These functions include the role of vitamin E in the regulation of cellular signalling and gene activity, the role of proteins that specifically bind and guide α-T to cellular and subcellular destinations, and the metabolism of individual tocopherols. Natural vitamin E is a mixture of eight different forms: α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. Tocotrienols have unsaturated side chain, whereas tocopherols contain a phytyl tail with three chiral centers (Fig. (**1**)). All natural tocopherols in foods have the 2*R,* 4'*R,* 8'*R* stereochemistry in the chiral centers. Commercially available vitamin E consists of either a mixture of naturally occurring tocopherols and tocotrienols which consists of the eight possible tocopherol stereoisomers in equal amounts (*all rac*); or their esters. Further, the bioavailability and bioequivalence of the different forms of vitamin E differ. For instance, although the amount of γ-tocopherol (γ-T) in the diet is higher than that of α-T, the plasma γ-T concentration is only the 10% of that of α-T, which is the most abundant form in plasma. All components of vitamin E are taken up by intestinal cells together with nutritional lipids and released in the lymph within chylomicrons (CM), which suggests the absence of selectivity at this level. After passing through the lymphatic pathway the CM reach the systemic circulation, and are progressively hydrolysed under the action of the endothelial lipoprotein lipase (LPL) present in the target tissue. During this process, a part of vitamin E is released in the plasma and taken up by the cells [2]. The vitamin reaches the liver *via* CM remnants and are taken up mainly *via* low density lipoprotein (LDL) receptor, and a specific protein, alpha-tocopherol transfer protein (α-TTP). α-TTP selectively sorts out α-T from all incoming tocopherols for incorporation into very low density lipoprotein (VLDL) [3]. A large amount of the total secreted VLDL are hydrolysed by LPL and converted to LDL, which becomes the major carrier of vitamin E to the peripheral tissues [4]. Excess amounts of α-T, along with the other absorbed forms of tocopherols, tocotrienols, analogues and synthetic isomers, are metabolised and eliminated through the bile and urine.

The first formal total synthesis of (2*R*, 4'*R*, 8'*R*)-α-T (**1**) was reported by Mayer and Isler and co-workers in 1963 (Fig. (**2**)) [5]. Later, Saucy and Cohen and co-workers have explored different approaches to the total synthesis of **1** [6-14], and in 1981 they described the total synthesis of all eight stereoisomers of α-tocopheryl acetate [15].

Then, much attention has been devoted to the *in vitro* antioxidant effectiveness of individual tocopherols of vitamin E (Fig. (**1**)) and related chain-breaking phenolic antioxidants in order to understand how tocopherols structural differences affects vitamin E activity. To this end, several researchers have prepared a number of tocopherol derivatives, analogues and related phenols, and has been studied their reactions with peroxyl radicals (Fig. (**3**)) [16-28]:

$$ArO\text{-}H + ROO^{\cdot} \rightarrow ArO^{\cdot} + ROOH \quad \text{(equation 1, } k_1\text{)}$$

$$ArO^{\cdot} + ROO^{\cdot} \rightarrow \text{non radical products} \quad \text{(equation 2, } fast\text{)}$$

The results showed that all the tocopherols are exceptionally good chain-breaking antioxidants *in vi*tro. It was observed that the second-order rate constants, k_1, of tocopherols decrease in the order of α > β ~ γ > δ-T. Furthermore, the relative magnitudes of k_1 that is the relative antioxidant activity of α-, β-, γ-, and δ-T, obtained from three different experimental methods agree well with each other [17, 18, 20, 22]. It was found that few tocopherols have higher antioxidant activity than α-T. The factors which enhance the peroxyl radical trapping ability of phenols are

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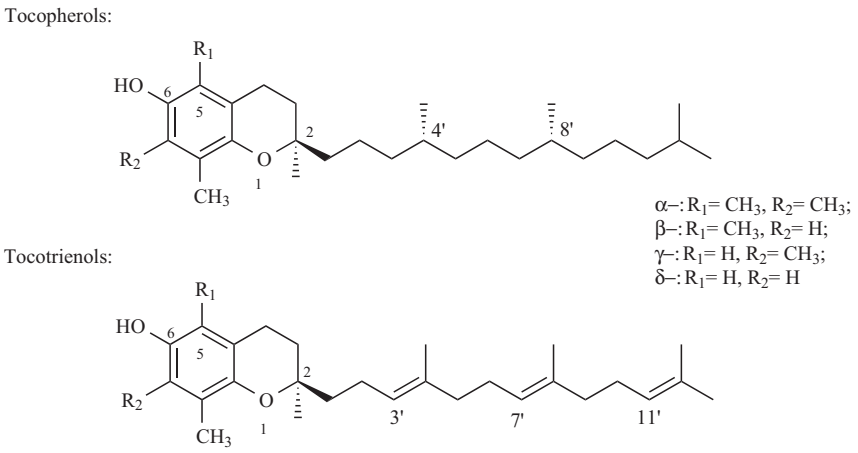


Fig. (1). Vitamin E refers to one or more of the structurally related phenolic compounds named tocopherols and tocotrienols.

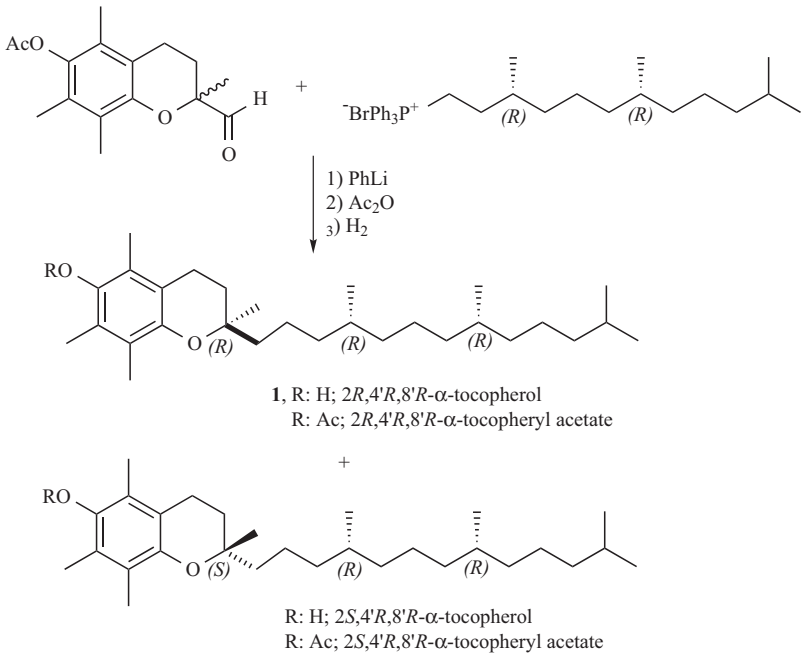
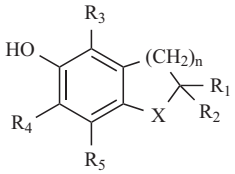
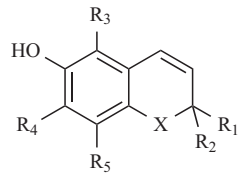
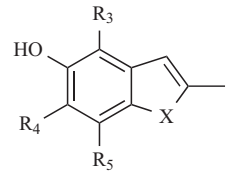
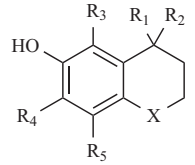


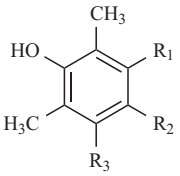
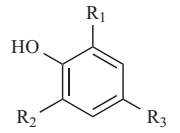
Fig. (2). First total synthesis of (2*R*, 4'*R*, 8'*R*)-α-T by Mayer and Isler and co-workers in 1963.

maximised in α-T: alkyl substitution, respect to hydroxyl moiety, at both *orto* and both *meta* positions and a *para*-alkoxy group held in an orientation which permits stabilisation by interaction of the unpaired electron with the p-type lone pair on the *para* oxygen. Otherwise, α-T is more reactive than β-, γ-, and δ-T because these lack one or more *ortho* methyl groups and such electron-releasing groups stabilise phenoxyl radical and therefore increase k_I values [29]. Although α-T and an α-T model, in which the phytyl side chain is replaced by a methyl group (**5**, Fig. (**3**)), have similar antioxidant activity, the latter compound generally shows little or no vitamin E activity *in vivo*. The phytyl chain appears to be necessary for the phospholipids penetration. In fact, the stereochemistry of the phytyl tail is known to affect the bioactivity of α-T. Among the eight stereoisomers of α-T, the natural isomer, *RRR*-α-T has been shown to be the most bioactive [30]. The isomer with inverted stereochemistry at position 2, has about 30% of the activity of *RRR*-α-T. Membranes "recognise" *RRR*-α-T and that explain its bioactivity.

For related phenols, in a comprehensive survey of the effect of ring substituents on the rate of the reaction (equation 1) [29], the magnitude of the constant for the rate controlling step, k_I, has been shown to be increased by a 4-methoxy group and by methyl groups in the 2,3,5 and 6 positions (antioxidant activity of **35**, k_I=39 x 10^4 $M^{-1}s^{-1}$, and antioxidant activity of **40**, k_I=2.5 x 10^4 $M^{-1}s^{-1}$, Fig. (**3**)) [16]. Most of the differences in k_I values between the various phenols studied can be attributed to the electronic effect of the group that is *para* to the hydroxyl function: on one hand *stereoelectronic effects*-which are concerned with the orientation with respect to the aromatic plane of the p-type lone pair on the heteroatom *para* to the hydroxyl group [20] and on the other hand, *inductive effects*-which are concerned with

Structure	Compd.	n	R_1	R_2	R_3	R_4	R_5	X
	**1** (α-T)	2	CH_3	$C_{16}H_{33}$ [a]	CH_3	CH_3	CH_3	O
	2 (β-T)	2	CH_3	$C_{16}H_{33}$	CH_3	H	CH_3	O
	3 (γ-T)	2	CH_3	$C_{16}H_{33}$	H	CH_3	CH_3	O
	4 (δ-T)	2	CH_3	$C_{16}H_{33}$	H	H	CH_3	O
	5	2	CH_3	CH_3	CH_3	CH_3	CH_3	O
	6	2	H	H	CH_3	CH_3	CH_3	O
	7	2	CH_3	COOH	CH_3	CH_3	CH_3	O
	8	2	CH_3	$COOCH_3$	CH_3	CH_3	CH_3	O
	9	2	CH_3	CH_2COOH	CH_3	CH_3	CH_3	O
	10	2	CH_3	$C_{16}H_{33}$	CH_3	CH_3	H	O
	11	2	CH_3	CH_2COOCH_3	CH_3	CH_3	CH_3	O
	12	2	CH_3	$(CH_2)_2COOH$	CH_3	CH_3	CH_3	O
	13	2	CH_3	$(CH_2)_2COOCH_3$	CH_3	CH_3	CH_3	O
	14	2	CH_3	CH_2OH	CH_3	CH_3	CH_3	O
	15	2	CH_3	OCH_3	CH_3	CH_3	CH_3	O
	16	1	H	CH_3	CH_3	CH_3	CH_3	O
	17	1	CH_3	CH_3	CH_3	CH_3	CH_3	O
	18	1	CH_3	COOH	CH_3	CH_3	CH_3	O
	19	1	CH_3	CH_3	H	CH_3	CH_3	O
	20	1	CH_3	$C_{16}H_{33}$	CH_3	CH_3	CH_3	O
	21	2	H	H	CH_3	CH_3	CH_3	NH
	22	2	H	H	CH_3	CH_3	CH_3	$NC(O)CH_3$
	23	2	H	H	CH_3	CH_3	CH_3	NCH_2CH_3
	24	2	CH_3	$C_{16}H_{33}$	CH_3	CH_3	CH_3	S
	25	2	CH_3	CH_3	CH_3	CH_3	CH_3	S
	26	2	CH_3	H	CH_3	CH_3	CH_3	S
	27	2	H	H	CH_3	CH_3	CH_3	S
	28	2	CH_3	$COOCH_3$	CH_3	CH_3	CH_3	S
	**29**	-	CH_3	CH_3	CH_3	CH_3	CH_3	O
	30	-	CH_3	CH_2COOH	H	CH_3	CH_3	O
	**31**	-	-	-	CH_3	CH_3	CH_3	O
	**32**	-	CH_3	CH_3	CH_3	CH_3	CH_3	S

(Fig. 3. Contd….)

Structure	Compd.	n	R_1	R_2	R_3	R_4	R_5	X
	**33**	-	H	OCH_3	H	-	-	-
	34	-	H	OCH_3	CH_3	-	-	-
	35	-	CH_3	OCH_3	CH_3	-	-	-
	36	-	CH_3	CH_3	CH_3	-	-	-
	37	-	CH_3	CH_3	H	-	-	-
	38	-	H	CH_3	H	-	-	-
	39	-	CH_3	H	CH_3	-	-	-
	40	-	H	H	H	-	-	-
	**41**	-	$(CH_3)_3C$	$(CH_3)_3C$	CH_3O	-	-	-
	42	-	$(CH_3)_3C$	$(CH_3)_3C$	CH_3	-	-	-
	43	-	$(CH_3)_3C$	$(CH_3)_3C$	H	-	-	-

[a] $C_{16}H_{33}$= phytyl

Fig. (3). Tocopherols and synthetic antioxidants.

the inductive effect of groups attached to position 2 of those phenols that have a fused heterocyclic ring. Chromans with alkyl groups or hydrogen at position 2 (**1-6**, **10**, Fig. (**3**)) have small differences in reactivity, are small but certainly real. Burton and co-workers tentatively suggest that these differences are due in part, at least, to changes in the conformation of the heterocyclic ring. That better stabilisation of the phenoxyl radical, should be achieved by decreasing θ, the dihedral angle between the p-type lone pair orbital on O and π-orthogonal orbital of the aromatic plane, (Fig. (**4**)) and hence a larger k_1 [17].

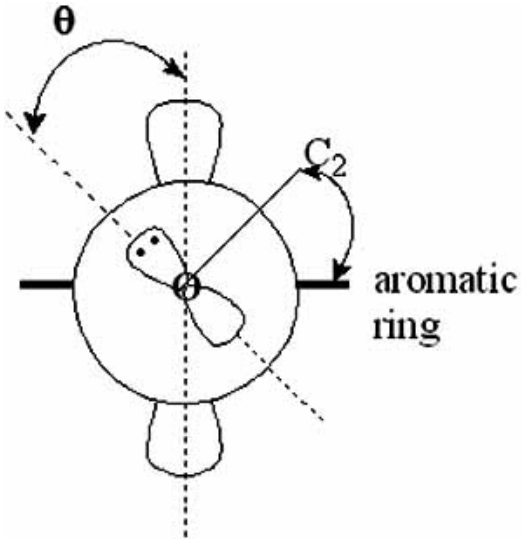


Fig. (4). Dihedral angle between O_1-C_2 bond and aromatic ring plane.

Then, when compared 2,6-di-*tert*-butylphenols, **41-43** (Fig. (**3**)), with the corresponding 2,6-dimethylphenols, **33**, **38**, **40**, (Fig. (**3**)) respectively, it was observed that the former were less reactive. The presence of two ortho tert-butyl groups in the former compounds hinders the approach of the peroxyl radical [20].

For chromans with oxygenated substituents at position 2 (**7**, **8**, Fig. (**3**)) the decreased reactivity toward ROO˙ relative to **1** (Fig. (**3**)) was attributed to the electron-withdrawing carboxyl group, which by the inductive effect impairs the ability of the p-type lone pair on the ring oxygen to participate in the stabilisation of the phenoxyl radical. This effect is reduced when the side chain is increased in one carbon (**11-13**, Fig. (**3**)) [18].

Then, it was expected that the substitution of the oxygen of the chroman ring by nitrogen would lead to better antioxidants than **1** because nitrogen, being less electronegative than oxygen, would be more able to stabilise the neighbouring radical center by conjugative delocalisation of its lone pair of electrons. Therefore, in order to study this fact the tetrahydroquinolines **21-23** (Fig. (**3**)) [18, 31], where synthesised by modifying Svensson and coworkers procedure [32] (Fig. (**5**)). However, an inspection of space-filling models indicates that there will be very severe steric interactions between an equatorial *N*-ethyl group, in compound **23**, and the 8-methyl group. As a consequence, the *N*-ethyl group must adopt the axial position. The nitrogen's lone pair will therefore lie rather close to the plane of the aromatic ring and hence will be in a relatively unfavourable position to stabilise the incipient phenoxyl radical. The amide **22**, which also exhibits severe steric interactions, is less reactive than **23** that can be attributed to the electron-withdrawing effect of the acetyl group.

Detailed structure-activity relationship studies led Ingold and coworkers [18, 20] to synthesise alkylated 5-hydroxydihydrobenzofurans (Fig. (**6**)) [33]. Since 5-membered rings are generally more planar than 6-membered rings it seemed probable that θ (Fig. (**4**)) would be decreased by reducing the heterocyclic ring to this size and would be even better antioxidant than α-T and compound **5** (Fig. (**3**)). For **16** (Fig. (**3**)), the reactivity toward peroxyl radicals is enhanced by a

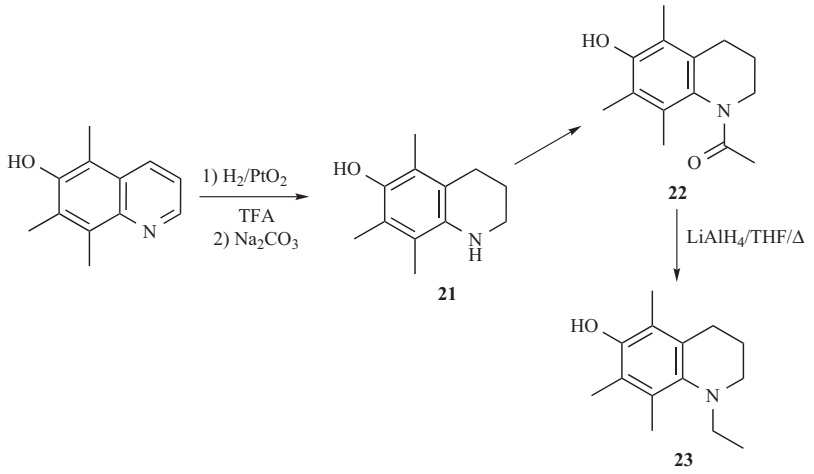


Fig. (5). Synthesis of tetrahydroquinolines **21**-**23** [32].

factor of 1.69 relative to **1** or 1.42 relative to **5**. Thus, alkylated 5-hydroxydihydrobenzofurans were found, as predicted, to be even better antioxidants than the 6-hydroxychromans confirming the stereoelectronic arguments. It

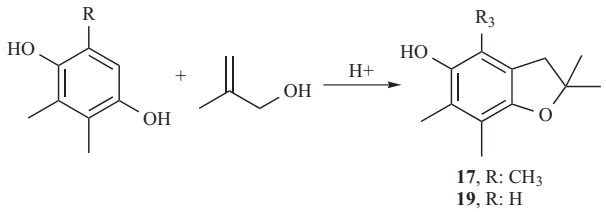


Fig. (6). Synthesis of alkylated 5-hydroxydihydrobenzofurans **17** and **19** [33].

improved the orbital overlapping between the lone pair on the oxygen and the SOMO of the derived phenoxyl radical, resulting an increase in the stabilisation of $ArO^{\bullet}$. Also, they synthesised **20** (Fig. (**3**)) in the *all-rac* form and compared its bioactivity with that of *all-rac*-α-T using the curative myopathy bioassay [34]. The results showed that **20** has 1.5-2.9 times the bioactivity of *all-rac*-α-T [35, 36]. This represents the first time that a systematically designed, man-made compound has at least equaled, and perhaps even outperformed the natural vitamin.

Studies were extended to the sulfur-containing analogue of α-T, namely **24** (Fig. (**3**)), since this compound would also be expected to be an excellent antioxidant. Sulfur is generally considered to be more effective than oxygen at stabilising a neighbouring radical center [37-41]. In a first approach, the only pentamethyl-6-hydroxythiochroman that the authors were able to isolate upon following the general procedure used to synthesise the chroman **5** (Fig. (**3**)) was the thiochroman **32** (Fig. (**3**)), which has quite a different pattern of methyl substitution on the heterocyclic ring. Nevertheless, this compound proved to have ~87% of the peroxyl radical trapping ability of α-T [20]. Then, were synthesised authentic analogues of **5**, i.e. **25** (Fig. (**3**)) and related compounds, as well as 1-thio-α-tocopherol, **24** (Fig. (**3**)) [42, 43]. The preparation of **24** and related compound was first reported by Karrer and Leiser in 1944 [44] and then by Samokhualou in 1982 [45] but after repeating these synthesis Ingold and coworkers [42] found that they could not produce **24**. Moreover, this two-purported synthesis gave identical mixtures of five isomers of **24** which could be separated only by capillary GC. For three of these compounds, the authors showed by GC/MS that the heterocyclic ring had not even been formed [42]. Therefore, they developed a short synthesis of three model 6-hydroxythiochromans **25**-**27** (Fig. (**7**)) being the key intermediate 4-hydroxy-2,3,5-trimethylbenzenethiol.

Unfortunately, the condensation of 4-hydroxy-2,3,5-trimethylbenzenethiol with methyl 3,7,11,15-tetramethylhexadec-2-enoate did not occur under the conditions describe above. Therefore, several attempts to develop a new synthetic route to the desire 1-thio-α-tocopherol were made and finally it was prepared as showed in Fig. (**8**) using the 2-methylfumaric acid derivative as a key-synthon (Fig. (**8**)) [43].

Antioxidant studies showed that 1-thio-α-tocopherol, **24**, is somewhat less reactive toward peroxyl radicals than α-T, as can be seen from their respective nk_1 values of 2.6 x 10^6 $M^{-1}s^{-1}$ and 6.4 x 10^6 $M^{-1}s^{-1}$, respectively [24]. The other 6-hydroxythiochromans are also somewhat less reactive toward peroxyl radicals than the structurally related 6-hydroxychromans.

Considering that many organic fluorine compounds are used as medicinal and agricultural chemicals. As a part of a search for more biologically active analogue of vitamin E, Koyama and co-workers have reported the synthesis of 6-chromanol derivatives (**44**-**46**, Fig. (**9**)) with fluorinated side chains at the 2-position. These compounds result from the ring-closure of trifluoroprenols with trimethylhydroquinone in the presence of zinc chloride (Fig. (**9**)). Later this synthesis was improved [46, 47].

On the other hand, in order to obtain tocopherol-compounds having higher *in vivo* biological activity than α-T, Mukai and coworkers prepared several new tocopherol derivatives that posses different substitutions pattern at *ortho* positions of the OH group and the phytyl side chain at position 2. These authors have measured the reaction rates of tocopherol derivatives and 2,6-di-*tert*-butyl-4-(4-methoxy-

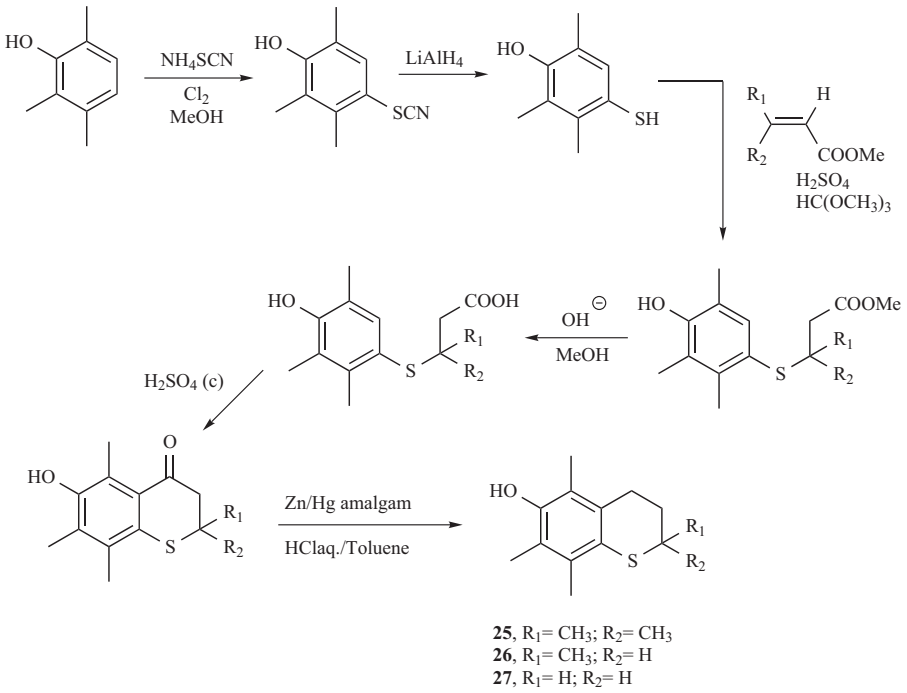


Fig. (7). Synthesis of model 6-hydroxythiochromans **25-27** [42].

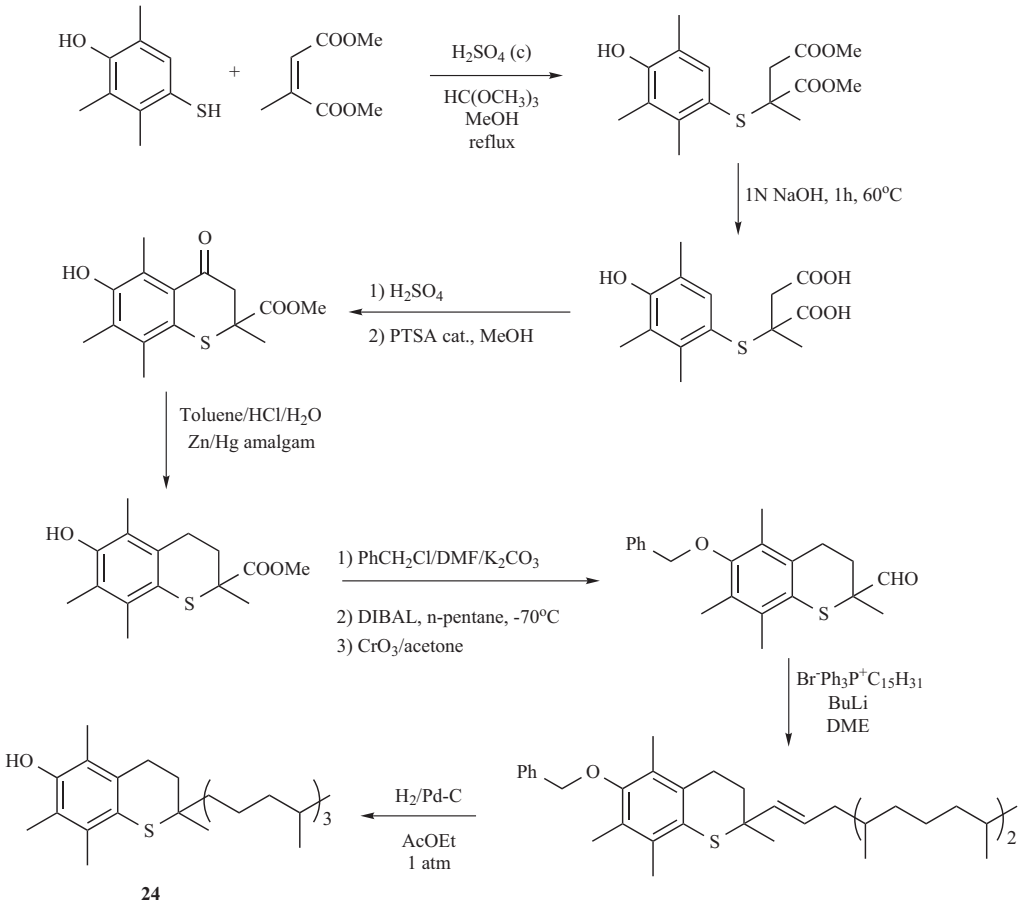
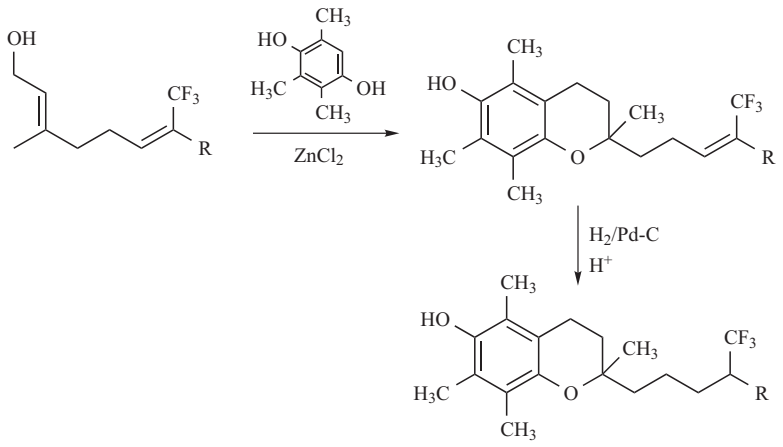


Fig. (8). Synthesis of 1-thio-α-tocopherol [43].



44, R= CH_3
45, R= $CH_2CH_2CH_2CH(CH_3)_2$
46, R= $CH_2CH_2CH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)_2$
47, R= $CH_2CH_2CH=C(CH_3)CH_2CH_2CH=C(CH_3)_2$

Fig. (9). Synthesis of 6-chromanol derivatives with fluorinated side chains at the 2-position [47].

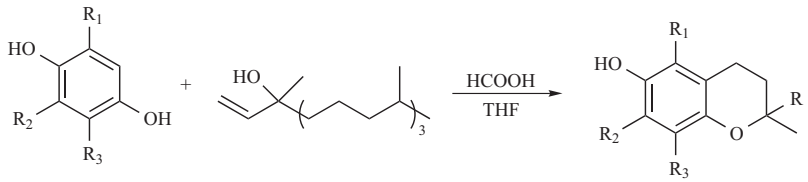
phenyl)phenoxyl radical in ethanolic solution, using stopped-flow spectrophotometry [26]. These derivatives were synthesised from the corresponding substituted hydroquinone and isophytol (**10**, **48**-**52**, Fig. (**10**)).

These results indicated that the antioxidant activity of these tocopherols varies depending on the number of alkyl substituents. Consequently, it can be expected that the antioxidant activity of these tocopherol compounds is related to the total electron-donating character of the alkyl group substituents on the aromatic ring. However, **52** shows 62% higher antioxidant activity than that of β-T, against their expectation. The electronic interaction between the bulky *tert*-butyl group at the C-8 position and the oxygen in position 1 may induce the change in the extent of orbital overlap between the 2p type lone pair on the *para* oxygen atom and the aromatic π electron system and, thus, the change in the second-order rate constants.

In a later work, Mukai and coworkers have synthesised several new tocopherol derivatives **53**-**60** (Fig. (**11**)), which include vitamin K_1-chromanol (**53**), vitamin K_1-chromenol (**54**), ubichromenol (**55**), and ubichromenol (**56**). The oxidation rates of tocopherols by substituted phenoxyl radical were studied spectrophotometrically using the stopped flow technique with tocopherol excess in ethanol [27].

The vitamin K_1-chromanol (**53**) and vitamin K_1-chromenol (**54**) were prepared according to the method of Fujisawa and coworkers [48] and Wilson and coworkers [49], respectively. The synthesis of the ubichromanol (**55**) was performed as the authors previously described [50]. The ubichromenol (**56**) was prepared according to the method of Imada and coworkers [51]. Phenols **59** and **60** were prepared according to the method of Nilsson and coworkers [52]. Meanwhile, phenols **57** and **58** were synthesised by condensation of 2-methyl-2-propen-1-ol to the corresponding alkylhydroquinone, according to a procedure similar to that used by Nilsson and coworkers to prepare phenols **59** and **60** [18, 27].

These results demonstrated that vitamin K_1 derivatives **53** and **54** are 6.9 and 4.5 times as reactive as α-T, which has the highest antioxidant activity already reported among natural tocopherols. Then, it will be interesting to study the biological activity of these compounds. Compounds **57** and **58** with a five-membered heterocyclic ring were also found to be 1.8 and 1.1 times more active than α-T, respectively. The elec-



10, $R_1=R_2= CH_3$; $R_3=$ H; R= phytyl
48, $R_1=R_2=R_3=$ H; R=phytyl
49, $R_1=R_2= CH_2CH_3$; $R_3=$ H; R= phytyl
50, $R_1=R_2= CH(CH_3)_2$; $R_3=$ H; R= phytyl
51, $R_1= CH_3$; $R_2=C(CH_3)_3$; $R_3=$ H; R= phytyl
52, $R_1= CH_3$; $R_2=$ H; $R_3= C(CH_3)_3$; R= phytyl

Fig. (10). Synthesis of tocopherol derivatives that posses different substitution patterns at *ortho* positions of the OH group and phytyl side chain at position 2 [26].

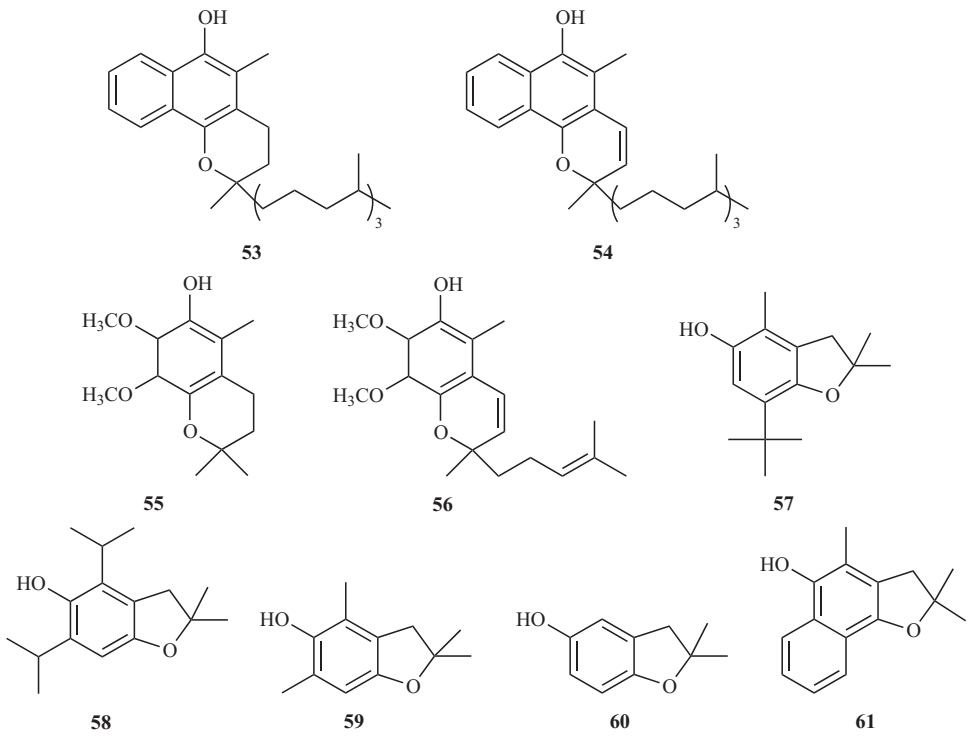


Fig. (11).

tronic and steric interactions between the bulky *tert*-butyl group at the C-7 position and the oxygen in position 1 in compound **57** may increase the extent of orbital overlap between the 2p type lone pair on the *para* oxygen atom and the aromatic π electron system, and, thus, induce the increase in the second-order rate constants [17, 18, 20, 26]. Otherwise, compounds **59** and **60** showed less reactivity than α-T. Further, both **55** and **56** having two methoxy substituents at the aromatic ring are only 10% as reactive as α-T.

The investigation of electronic effects was extended to the determination of the effect of a second fused aromatic ring which would be expected to further delocalise the unpaired electron in the aryloxyl radical $ArO^{\cdot}$ and raise the antioxidant activity. To this end, α-naphthol derivatives **53**, **54** and **61** were studied (Fig. (**11**)) [28]. Known quantitative kinetic methods of autooxidation [53, 20] were used to determine the hydrogen atom donating ability of phenolic antioxidants. The results demonstrated that the incorporation of a second fused aromatic ring causes a remarkable increase in antioxidant activities. Both the benzochroman **53** and benzochromene **54** exhibit absolute rate constant for inhibition (k_{inh}) values 4 times that of α-T, having the conjugated double bond in **54** little effect on the antioxidant activity. This indicates, as might be anticipated, that the second aromatic ring exerts the predominant effect attributed to enhanced electron delocalisation and stabilisation of the phenolic radical in the rate-determining step of antioxidant action. Then, an antioxidant which possesses both the second aromatic ring and the ether oxygen *para* to the hydroxyl incorporated in a five-membered ring as achieved in compound **61** raises the k_{inh} value another 2.5 times over that of compound **53**, and overall its antioxidant activity is 10 times that of α-T in homogeneous solution.

Since much attention is paid to the role of oxygen-derived free radicals in aging processes, in neurodegenerative conditions such as Alzheimer's and Parkinson's diseases, and in neurological injury occurring as the result of stroke and trauma [54-56], the structure of α-T has served as a starting point for analogues. The 2,3-dihydro-1-benzofuran-5-ol analogue **(20**) has shown to be more potent than α-T in the lipid phase and an *in vivo* assay [21, 36]. A water-soluble analogue, Trolox (**7**, Fig. (**3**)), was synthesised by Scott and coworkers [57]. Then, Grisar and coworkers reported an analogue **62** (Fig. (**12**)), which is also hydrophilic, to accumulate

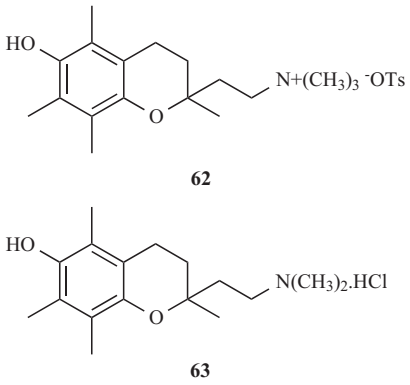


Fig. (12). Structures of hydrophilic antioxidant analogues to α-T.

in heart tissue [58-60], reducing myocardial infarct size following reperfusion [61, 62]. While looking for analogues that sufficiently penetrate the brain, authors focussed on compounds like **63** (Fig. (**12**)) [58, 63] containing a tertiary amino function, which is present in many central nervous system (CNS)-active compounds, such as tranquillizers, antidepressants, and the like. In this regard, it is important to note that a positively charged amino group has an advantage to interact with negatively charged brain lipids. Such an interaction is known to affect inhibition of lipid peroxidation. Further, Grisar and coworkers reported the synthesis (Fig. (**13**)) and evaluation of amino derivatives of 2,3-dihydro-l-benzofuran-5-ol (**66**-**82**) [64]. The key synthetic pathway was the ring contraction of pyran-4-one to furan-3-carboxylate ring using $Tl(NO_3)_3$. When acetyl derivative **64** (Fig. (**13**)) was employed in the ring contraction process the result was poorly reproducible however the *p*-nitrobenzoyl derivative **65** produced the best ring contraction results.

These compounds were first evaluated *in vitro* for the inhibition of lipid autoxidation of rat brain homogenate and for superoxyl radical ($O_2^{\cdot-}$) scavenging. All the 2,3-dihydro-1-benzofuran-5-ol analogues were potent inhibitors of spontaneous lipid peroxidation in rat brain homogenate and showed a strong peroxyl radical-scavenging effect. The benzopyran analogue **63** was significantly less active than the corresponding 5-ring analogues **69**, **71**-**73**, **75**, and **77**. Then, to assess their *in vivo* efficacy, *ex vivo* inhibition of lipid autoxidation was determined. This *ex vivo* evaluation in normal mouse brains revealed **73** and **75** to be more potent than the other analogues in spite of identical antioxidant potencies *in vitro*. The mouse head injury model developed by Hall and co-workers [65] was used to determine pharma-

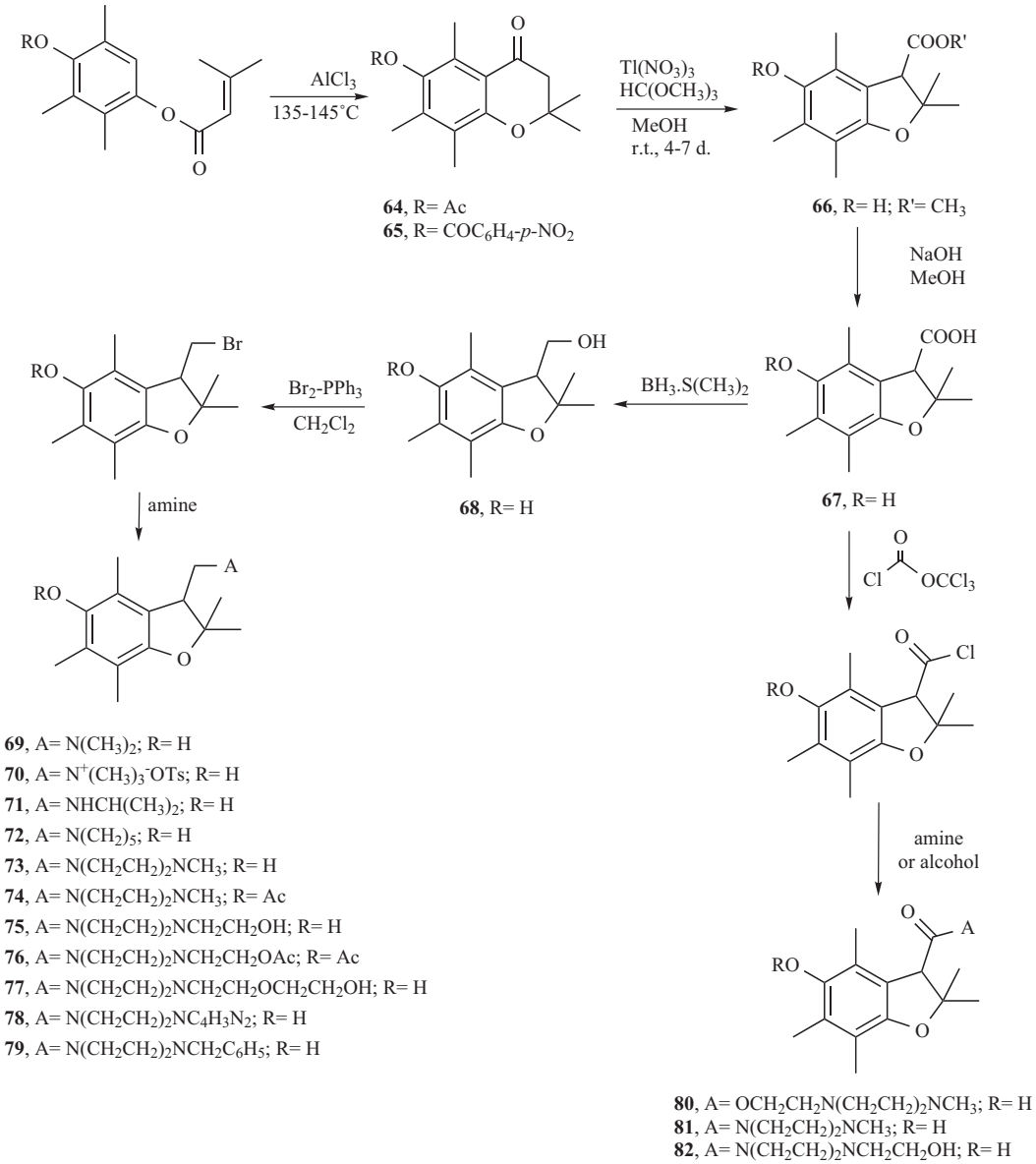


Fig. (13). Synthesis of a series of antioxidant derivatives of 2,3-dihydro-l-benzofuran-5-ol [64].

cologic activity and compound **73** significantly reduced the effect of head injury in mice being selected for further evaluation [66, 67]. Because of these promising results, Grisar and co-workers synthesised additional water-soluble, permanently cationic analogues of α-T and ascorbic acid. Since the α-T analogues have a chiral center at the 2-position, they also synthesised and evaluated both enantiomers (Fig. (**14**)) [68]. Two phosphonium derivatives, **83** and **84** were obtained by reaction of the corresponding halocompound [58, 69] with trimethyl- and triethylphosphine, respectively (Fig. (**14**)). The sulfonium analogue **86** was produced by treatment of the sulfide **85** with methyl *p*-toluenesulfonate in refluxing acetonitrile. Being the sulfide **85** prepared from the halocompound by reaction with excess $NaSCH_3$ in DMF. The acylhydrazinium compounds **87** and **88** were obtained by treatment of the unsymmetrical dimethylhydrazides with methyl *p*-toluenesulfonate in refluxing acetonitrile. Treatment of the resulting tosylates with aqueous NaOH gave the inner salts [68].

The compounds were evaluated *in vitro* for inhibition of spontaneous lipid peroxidation in rat brain homogenate. The phosphonium derivatives **83** and **84** and the sulfonium derivative **86** were as effective as the ammonium derivative **62** (Fig. (**12**)), while the acylhydrazonium derivatives **87** and **88** were somewhat less active. No difference in activity was found among the 2*R* and 2*S* enantiomers of these α-T analogues. The *ex vivo* inhibition of lipid peroxidation in mouse heart homogenates, demonstrated that cardioselectivity may be a common feature of permanently cationic derivatives of α-T, irrespective of the heteroatom involved, and that the molecular geometry at the chiral center is probably not a determining factor for their radical-scavenging as well as cardioselective properties. The pharmacological value of the ammonium derivative **62** as a cardioprotective agent has been demonstrated during the past. Such an effect is also expected for the present compounds which may enhance the choice for selected targets. This and the compartmentalised anti-inflammatory effect, e.g., as in colitis models, remain under further investigation.

In 1997, Niki and co-workers, in order to develop a novel potent radical-scavenging antioxidant, designed the ideal structure of a phenolic compound considering the factors that determine antioxidant potency. Thus, 2,3-dihydro-5-hydroxy-2,2-dipentyl-4,6-di-*tert*-butylbenzofuran (**89**, BO-653, Fig. (**15**)) was synthesised and its antioxidant activity was evaluated against lipid peroxidations *in vitro* model systems [70, 71]. The electron spin resonance study showed that the phenoxyl radical derived from BO-653 was more stable than α-tocopheroxyl radical. BO-653 reduced α-tocopheroxyl radical rapidly, but α-T did not reduce the phenoxyl radical derived from BO-653. However, the chemical reactivity of BO-653 toward peroxyl radical was smaller than that of α-T. This was interpreted as the steric effect of bulky *tert*-butyl groups at both *ortho* positions which hindered the access of peroxyl radical to the phenolic hydrogen. However, the *tert*-butyl substituents increased the stability of BO-653 radical and also lipophilicity, and its antioxidant potency against lipid peroxidation in phosphatidylcholine liposomal membranes was superior to that of α-T. Ascorbic acid reduced the phenoxyl radical derived from BO-653 and spared BO-653 during the oxidation of lipid in the homogeneous solution. On the other hand, ascorbic acid did not spare BO-653 in the

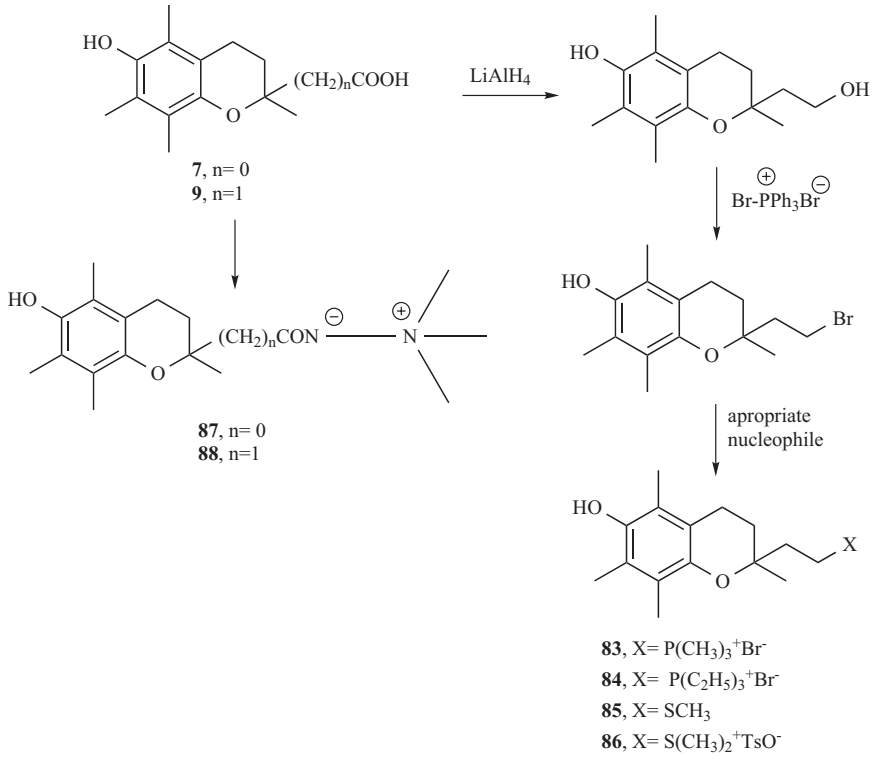
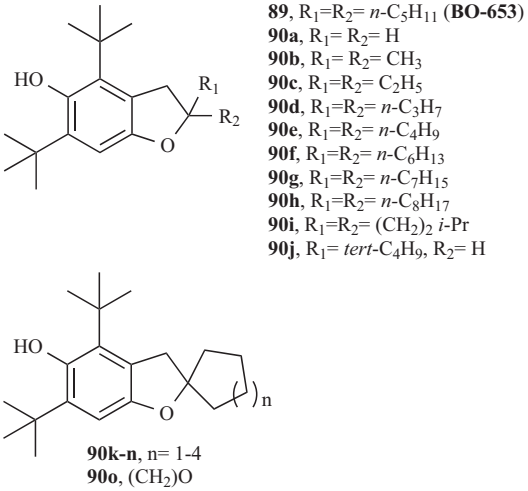


Fig. (14). Synthesis of water-soluble cationic analogues of α-T [68].



89, R_1=R_2= *n*-C_5H_{11} (**BO-653**)
90a, R_1= R_2= H
90b, R_1= R_2= CH_3
90c, R_1=R_2= C_2H_5
90d, R_1=R_2= *n*-C_3H_7
90e, R_1=R_2= *n*-C_4H_9
90f, R_1=R_2= *n*-C_6H_{13}
90g, R_1=R_2= *n*-C_7H_{15}
90h, R_1=R_2= *n*-C_8H_{17}
90i, R_1=R_2= $(CH_2)_2$ *i*-Pr
90j, R_1= *tert*-C_4H_9, R_2= H

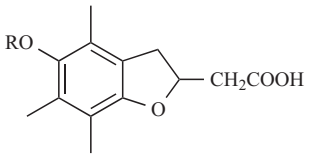
90k-n, n= 1-4
90o, $(CH_2)O$

Fig. (15). Structures of 2,3-dihydro-5-benzofuranol derivatives bearing different alkyl substituents on the 2-position including BO-653.

oxidation of liposomal membranes. It was concluded that BO-653 is a potent novel radical-scavenging antioxidant. Then, further studies were done. Thus, the antioxidant activities of BO-653 against the oxidative modification of low-density lipoprotein (LDL) induced by free radicals were studied. BO-653 was consumed faster than endogenous α-T and inhibited the formation of lipid hydroperoxides, which was observed during the consumption of α-T. Doxylstearic acids incorporated into LDL as spin probes competed with the antioxidants in scavenging radicals. It was found that the efficacy of radical scavenging by α-T became smaller as the radical went deeper into the interior of LDL particle, whereas that by BO-653 did not change. Ascorbic acid in the aqueous phase spared α-T efficiently during oxidation. On the other hand, the sparing effect of ascorbic acid for BO-653 was not remarkable, unlike that for α-T, which implied different locations of radicals derived from BO-653 and α-T within the LDL particle. It was concluded that BO-653 protected LDL from oxidative modification efficiently by scavenging peroxyl radicals and by reducing α-tocopheroxyl radicals and that this novel antioxidant might act as a potent inhibitor of development of atherosclerosis. In fact, exhibits a high affinity for LDL and is well distributed in aortic vessels *in vivo*. In atherosclerosis models of rabbits and mice, BO-653 has shown to be able to suppress the formation of atherosclerotic lesions without untoward side effects. Specifically, there was no reduction of high density lipoprotein levels. This antioxidant provides additional evidence in support of the oxidised-LDL hypothesis, and itself is a promising antioxidant candidate for clinical use [72, 73]. Further studies were done [74-78] and nowadays BO-653 is an antioxidant under development by Chugai Pharmaceuticals for the potential treatment of atherosclerosis and the prevention of restenosis. By November 2001, BO-653 was in phase II trials for restenosis in post-percutaneous transluminal coronary angioplasty in the US, and by April 2002, the compound was in phase I trial for the same indication in Japan [77].

In the same way, other new antioxidants were designed to over come the clinical limitation of vitamin E [79]. The potential drug synthesised consists of 2,3-dihydro-5-benzofuranol derivatives bearing different alkyl substituents on the 2-position, analogues to BO-653 (Fig. (**15**)). In this work, the authors constructed a strategy for screening LDL antioxidants to select the optimal dihydrobenzofuranol. Moreover, it was demonstrated that the selected compound BO-653 possesses a preferable distribution to LDL, the target molecule of the drug design for an antiatherogenic agent.

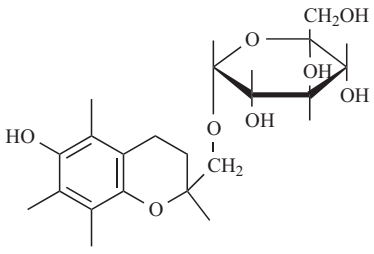
Simultaneously, Raxofelast (**91b**, IRFI016, Fig. (**16**)) and its deacetylated metabolite (**91a**, IRFI015, Fig. (**16**)) emerged from a series of novel compounds designed by Ceccarelli and co-workers with the aim to maximize antioxidant potency of phenols related to α-T [80]. They were prepared *via* a simple two-step route from hydroquinones and 4-bromocrotonates yielding racemic 2,3-dihydro-5-hydroxy-2-benzofuranacetic acids. The antioxidant activity of raxofelast has been convincingly demonstrated in several *in vitro* studies and in various models of ischaemia-reperfusion injury [81-83]. Besides, raxofelast has been shown to exert multiple protective anti-inflammatory effects in different animal models [84-87]. Moreover, several studies in genetically diabetic mice demonstrated that raxofelast should be a potential therapeutic agent on diabetes disease [88-90]. Further studies have been made and currently raxofelast is in the II and III clinical phase development programme (Biomedica Foscama Industria Chimico-Farmaceutica S.p.A., Italy) as potential therapeutic agent against diabetic complications and atherosclerosis.



91a, IRFI015, R= H
91b, IRFI016, R= Ac

Fig. (16). Raxofelast (IRFI016) and its deacetylated metabolite (IRFI015), potential therapeutic agents against diabetic complications and atherosclerosis.

On the other hand, a novel highly hidrosoluble vitamin E derivative, 2-(α-D-glucopyranosyl)methyl-2,5,7,8-tetramethylchroman-6-ol (**92**, TMG, Fig. (**17**)), was prepared from 2-hydroxymethyl-2,5,7,8-tetramethylchroman-6-ol and maltose



92, TMG

Fig. (17). TMG, a novel highly hidrosoluble vitamin E derivative [91].

in a solution containing DMSO by transglycosylation with α-glucosidase from Saccharomyces species. Preliminary results of the radical scavenging activity of TMG were found to be nearly the same as those of α-T, Trolox, and ascorbic acid [91]. Moreover, antioxidant activity of TMG was investigated [92]. Kinetic studies of the inhibition of radical-chain reaction of methyl linoleate in solution demonstrated that the peroxyl radical scavenging activity was not changed by the replacement of phytyl side chain of vitamin E to glucosyl group. TMG acted as an effective inhibitor on lipid peroxidation induced by a water-soluble and a lipid-soluble radical generator, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN), respectively. TMG also showed an excellent antioxidant activity on cupric ion-induced lipid peroxidation of PC-liposomal suspension, and suppressed the oxidation of rat brain homogenate which contained trace level of iron ion. Besides, when human plasma was exposed to either AAPH or AMVN, the accumulation of cholesteryl ester hydroperoxides was retarded by the addition of TMG. Moreover, TMG was evaluated for its ability to inhibit development of atherosclerosis in rabbits [93]. Results obtained indicated that TMG opposes progression of atherosclerosis not only by preventing oxidation of LDL, but also by unknown mechanisms. Even an antioxidant with no uptake by LDL apparently can inhibit development of atherosclerosis despite a very low serum concentration.

Previously, a new phospholipid containing a chromanol structure at its polar head group was synthesised from egg yolk phosphatidylcholine and 2,5,7,8-tetramethyl-6-hydroxy-2-(hydroxyethyl)chroman by transphosphatidylation catalysed by phospholipase D from Streptomyces lydicus [94]. The structure of the product synthesised was shown by spectral analysis to be 1,2-diacyl-glycero-3-phospho-2'-hydroxyethyl-2',5',7',8'-tetramethyl-6'-hydroxychroman. The phosphatidylchromanol (PCh) showed antioxidant activity against radical chain oxidation of methyl linoleate in solution in a manner similar to that of α-T and 2,2,5,7,8-pentamethyl-6-chromanol (**5**, Fig. (**3**)). Also, PCh prove to be useful as a chain-breaking antioxidant in phospholipid membranes [95]. Soon after studies of its activity against oxidative hemolysis of human erythrocytes showed that phosphatidyl group in PCh acts as an excellent carrier of chromanol moiety into cells as well as an anchor within membranes being more efficiently than phytyl group in α-T. The excellent antihemolytic activity of PCh is likely to be caused by its accumulation within erythrocyte membranes.

On the other hand, Testa and co-workers [96] postulate that a potential agent can be derived from Trimetazidine (TMZ, Fig. (**18**)), a well-known drug used in the treatment of angina, by substituting the piperazine nitrogen with moieties derives from tocopherol, flavonoids, coumarin, or cresol. Therefore, twenty-five compounds were examined for their radical scavenging and antioxidant properties (Fig. (**18**)). A method based on the scavenging of the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) has been used to predict the antioxidant activities of these compounds.

The compounds were examined for their potency to inhibit the oxidation of human serum albumin and the lipid peroxidation of synaptosomal lipids. On the other hand, molecular modelling studies were performed. The results shown that the TMZ derivatives having a chroman or a benzofuran group (i.e., compounds **93**, **94**, and **95**) proved to be quite active in the DPPH test. They were also active in inhibiting lipid peroxidation and albumin oxidation. The other compounds containing the 1-hydroxy-2,6-di*tert*-butyl substitution pattern were relatively active in the DPPH test and also relatively good inhibitors of lipid peroxidation.

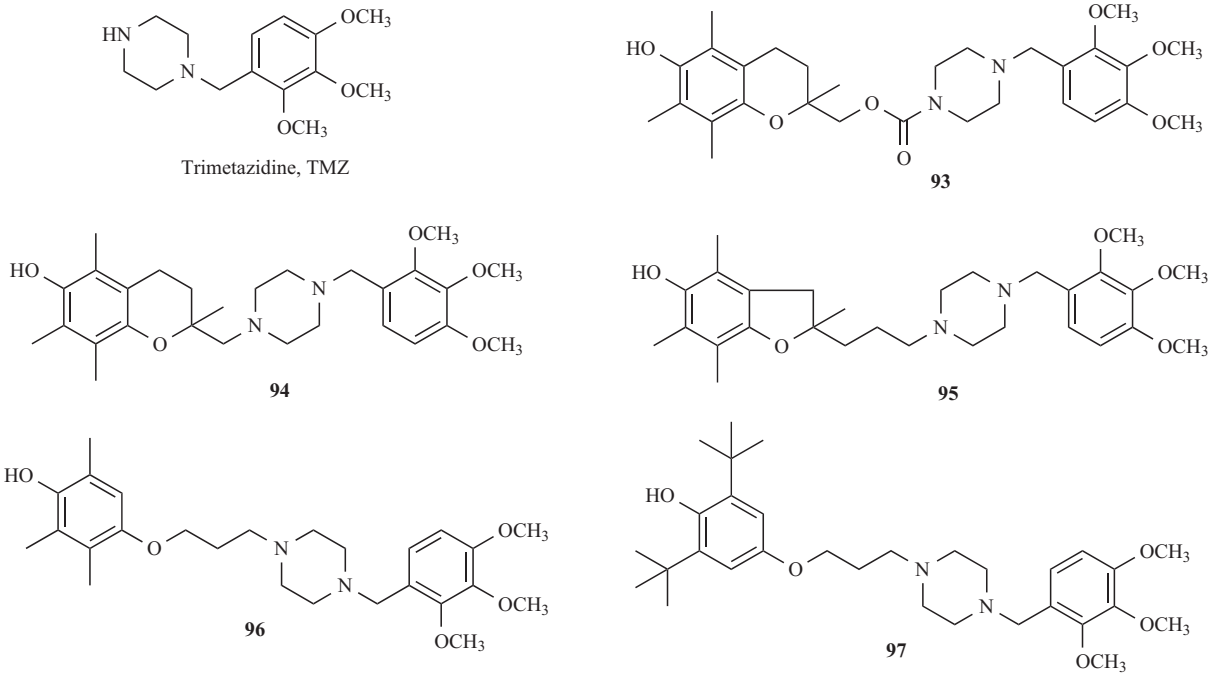


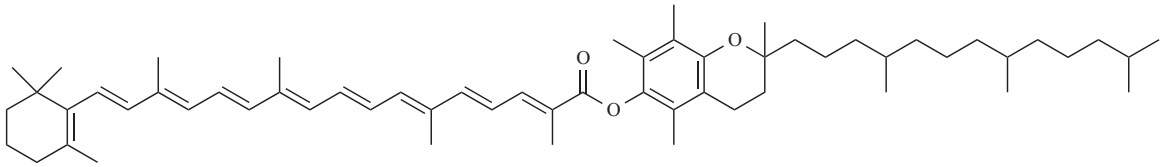
Fig. (18). Some studied Trimetazidine (TMZ) derivatives.

Vitamin E and carotenoids show many similar and complementary properties and protect living tissues against a variety of pathological processes. A mixture of both compounds often exhibits a significantly greater effect than the sum of the individual activities. The synthetic linkage of carotenoids with vitamin E might thus increase the synergistic effects. Sliwka and co-workers [97] therefore esterified β-apo-8'-carotenoic acid with *all-rac*-α-T to give α-tocopheryl-β-apo-8'-carotenoate (**98**, Fig. (**19**)). The carotenoic acid was also connected to α-T *via* glyceryl linker, 1-O-(α-tocopheryl)-3-(β-apo-8'-carotenoyl)-glycerol (**99**), whereas the water-soluble vitamin E analogue, Trolox, was combined with β-apo-8'-carotenoic acid in a diglyceride: 1-(6-hydroxy-2,5,7,8-tetramethylchroman-2-acyl)-3-(β-apo-8'-carotenoyl)-glycerol (**100**) (Fig. (**19**)).

In a later work, [98] Sliwka and coworkers combined these antioxidant substructures (Vitamin E and carotenoids) with a selena fatty acid, which have similar properties. Thus, a carotenoic acid, a selena fatty acid and the vitamin-E derivative Trolox were successively esterified with glycerol to 1-(β-apo-8'carotenoyl)-2-(7-selenaoctanoyl)-3-(6-hydroxy-2,5,7,8-tetramethylchroman-2-acyl)-glycerol (**101**, Fig. (**20**)). This triantioxidant compound revealed, in the DPPH test, an additive effect, consisting of the radical quenching activity of the carotenoid and Trolox. The DPPH test was not sensitive for the Se moiety in the triantioxidant compound.

On the basis of these lines of evidence which suggest that cooperative interactions may occur between carotenoids and tocopherols, Manfredini and co-workers designed FeAOX-6 (**102**, Fig. (**21**)) [99], a novel antioxidant that combines into a single molecule the chroman head of tocopherols and a fragment of lycopene. The ability of FeAOX-6 in inhibiting lipid peroxidation and reactive oxygen species (ROS) production induced by different sources of free radicals (*t*-butyl hydroperoxide, AAPH, and H_2O_2) in arachidonic acid solution and in isolated thymocytes was investigated. Its antioxidant efficiency was also compared with that of α-T, lycopene, and a mixture of the two antioxidants. The results strongly suggest that FeAOX-6 can act as a potent antioxidant, by inhibiting malondialdehyde production and ROS generation in a dose- and a time-dependent manner. In the cell model, the compound also provides a higher antioxidant capacity than α-T and lycopene, alone or in combination, suggesting the possibility of a redox intramolecular cooperation.

Increasingly, attention is being paid to the role that the natural antioxidant, α-T, and its analogues, play in reducing the incidence of heart disease and cancer. One of the drawbacks of using vitamin E for rapid therapeutic use is its extreme insolubility in water. This limitation affects its pharmacokinetics and tissue pharmacodistribution, so Burton and co-workers have synthesised analogs of vitamin E for rapid



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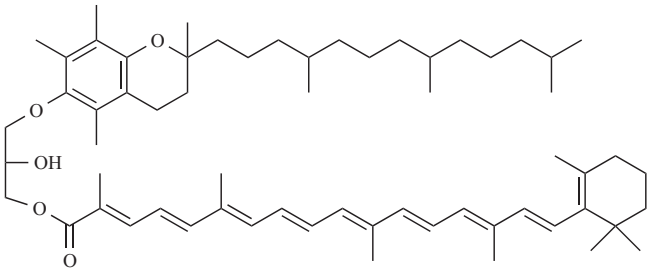

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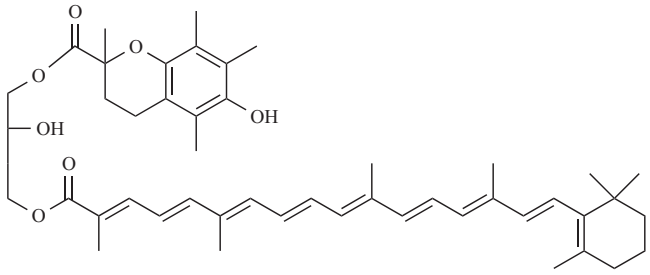

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Fig. (19). Antioxidant agents which combine in their structure tocopherol and carotenoid moieties.

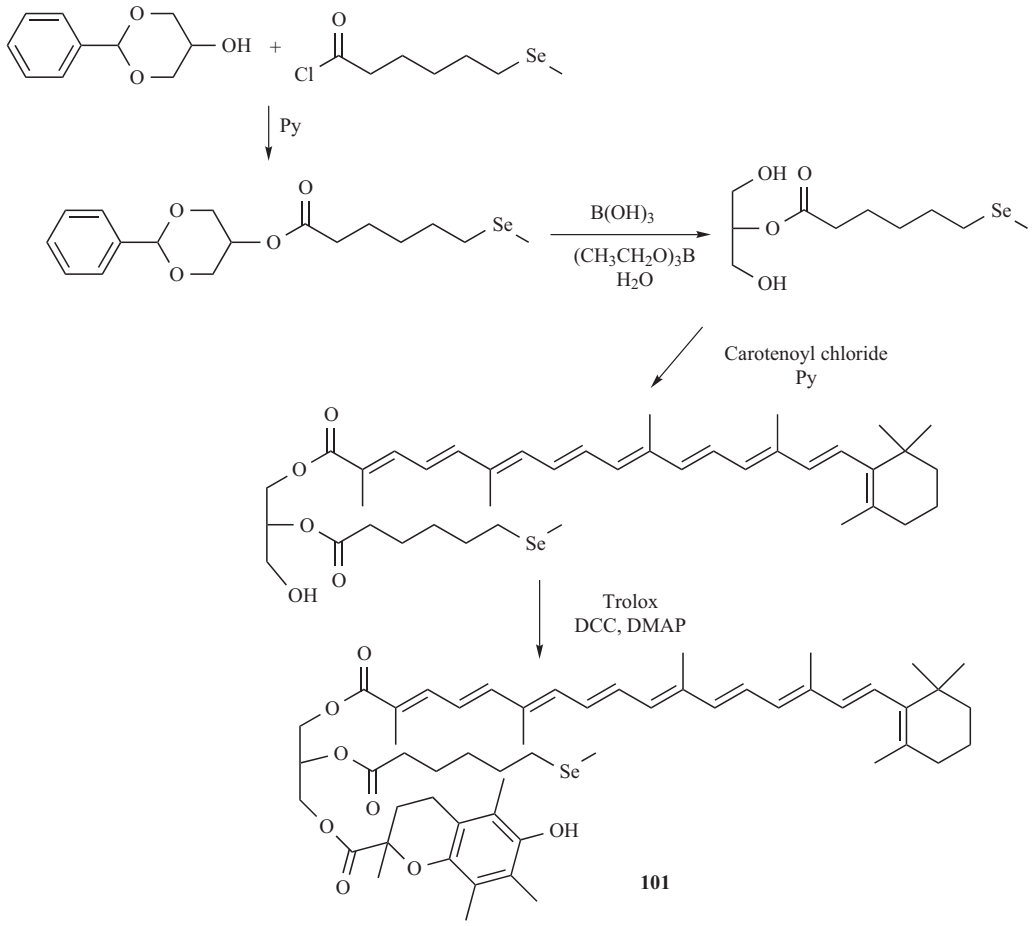
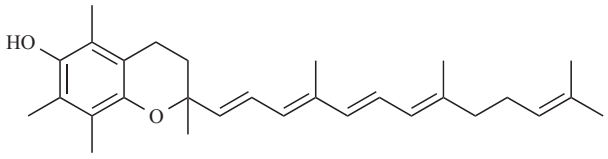


Fig. (20). Synthesis of triantioxidant compound (**101**) [98].

distribution into tissues (i.e. **103-108**, Fig. (**22**)) [100]. Two important factors were considered for the synthetic strategy: i) the derivatisation of the phenolic hydroxyl group of vitamin E with amino acid derivatives *via* an ester bond, and ii) modulation of the nature and length of the phytyl chain expecting to reduce the membrane-philicity of vitamin E and to increase its solubility in aqueous media.



102, FeAOX-6

Fig. (21). FeAOX-6, novel antioxidant that combines into a single molecule the chroman head of tocopherols and a fragment of lycopene.

In comparison to commercially available vitamin E derivatives (α-T, α-tocopheryl acetate, α-tocopheryl succinate and *rac*-Trolox), the new analogs **103**, **106-108** showed antiproliferative activity against the human breast cancer cell line MCF 7. Further work is required to determine why these analogues exert antiproliferative effects in this transformed cell line.

Nixon and co-workers [101] designed a series of phenolic antioxidant ester and amide derivatives of the nonsteroidal antiinflammatory drug naproxen to have both antiinflammatory and cytoprotective activity (Fig. (**23**)). Compounds were evaluated *in vitro* both for antioxidant activity, as assessed indirectly by thiobarbituric acid reactive substance (TBARS) formation in a membrane lipid peroxidation assay, and for antiproliferative activity, as indexed by the inhibition of DNA synthesis in cultured human vascular endothelial cells. Compounds of this series exhibited potent antioxidant activity, but lower than that of Trolox and than that of vitamin E. Structural modifications of the ester or amide substructure did not affect antioxidant activity, but methylation of the phenol resulted in a compound without antioxidant activity. Although indistinguishable in antioxidant activity, the amide derivatives tended to be more potent as antiproliferative agents than the corresponding esters. These preliminary studies demonstrated that the antioxidant-naproxen derivatives represent a novel series of agents that both protect against free-radical damage and possess cytostatic activity in vascular endothelial cells. Studies are in progress to assess the utility of these compounds as potential components of an ocular irrigating solution.

Considerable research effort has been devoted to the development of neuroprotective agents to save neurons from

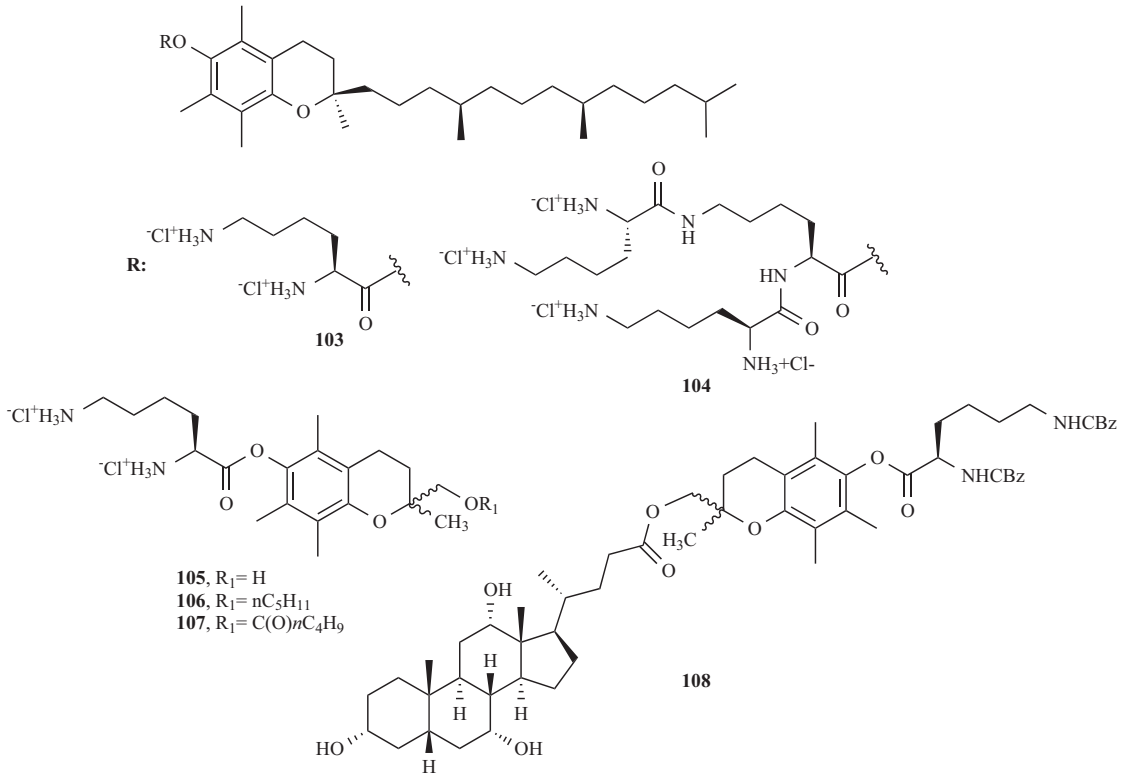


Fig. (22). Analogues of vitamin E with antiproliferative properties.

the biochemical and metabolic consequences of ischemic brain injury. The different neuroprotective strategies are based on the identification of the effectors and the sequence of events that lead to neuronal death. As the mechanisms of neurotoxicity are multifactorial, involving numerous interdependent and sequential processes, the monomodal nature of such therapies may partly explain their lack of clinical efficacy. In this context, new therapeutic strategies focussed on multiple downstream events may provide efficacy and a wider therapeutic window for effective intervention. Chabrier and co-workers [102] have tested the association of nitric oxide synthase (NOS) inhibitors and antioxidants in transient focal ischaemia in rats. They found a synergistic reduction of infarct size when they used a combined treatment of N^G-nitro-L-arginine, a nonselective NOS inhibitor, and the antioxidant/superoxide anion scavenger di-(*tert*-butyl)hydroxybenzoic acid [103]. The combined treatment protected rat brain from the ischemic damage. On the basis of these observations, they have developed a therapeutic concept of combining, in the same molecule, activities inhibiting neuronal NOS and lipid peroxidation. Thus, they described the *in vitro* and *in vivo* neuroprotective properties of BN 80933 (**109**, Fig. (**24**)), a representative compound of this class of agents.

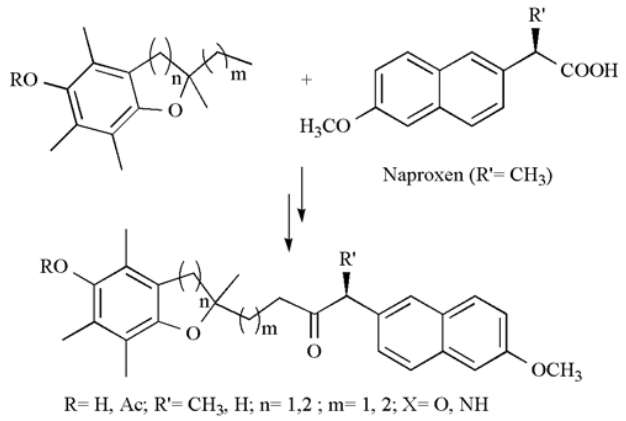
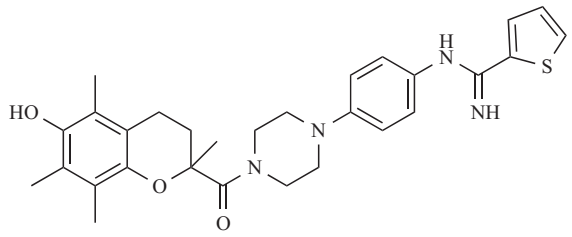


Fig. (23). Antioxidant-Naproxen derivatives with antiinflammatory and cytoprotective activity.

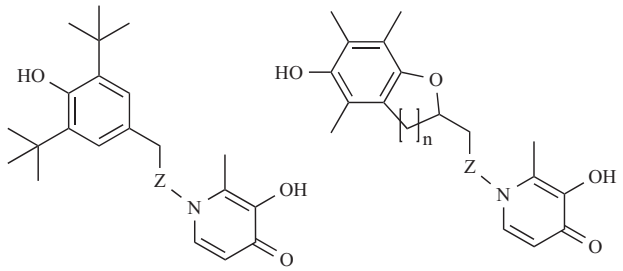


109, BN 80933

Fig. (24). Novel compound, that combine in the same molecule NOS and lipid peroxidation inhibitor substructures, as neuroprotective agent.

All results indicate that BN 80933 represents a class of potentially useful therapeutic agents for the treatment of stroke or trauma and possibly neurodegenerative disorders that involve both NO and ROS. Further studies are in course [104-106].

Simultaneously, Bebbington and co-workers disclosed novel dual action, neuroprotective hybrid molecules, which synergistically combine an iron chelating molecule with an antioxidant, e.g., butylated hydroxytoluene, hydroxybenzofuran or benzopyran derivative (i.e. **110-113**, Fig. (**25**)) [107, 108]. Both antioxidants and iron chelating molecules have shown neuroprotective efficacy in animal models of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and stroke.

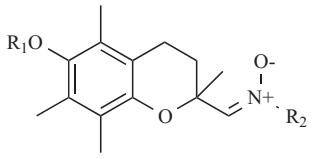


110, Z= $NHCH_2$
111, Z= S

112, n=1, Z= direct bond
113, n=2, Z= $NH(CH_2)_4$

Fig. (25). Neuroprotective hybrid molecules with dual action: iron chelating and antioxidant.

In the same way, Balogh and co-workers prepared nitrone derivatives of Trolox (**114a-f**, Fig. (**26**)) resulting



114a, R_1= H; R_2= *t*-butyl
114b, R_1= H; R_2= *i*-propyl
114c, R_1= Ac; R_2= *t*-butyl
114d, R_1= Ac; R_2= *i*-propyl
114e, R_1= CH_3; R_2= *t*-butyl
114f, R_1= CH_3; R_2= *i*-propyl

Fig. (26). Nitrone derivatives of trolox as neuroprotective agents.

a combination of these two different types of free radical trapping moieties as neuroprotective agents [109]. Their biological evaluation was performed *in vitro* for different relevant free radicals, inhibiting Fe^{2+}-induced lipid peroxidation, and *in vivo* in a permanent middle cerebral artery occlusion model in mice. New compounds exert pharmacological activities comparable to or better than those of Trolox or nitrone-type reference compounds.

On the other hand, within the past decade or less, increasing attention has been given to aspects of vitamin E biokinetics [110-114] and metabolism [115-119]. Essential components in the biokinetics of tocopherol transport and turnover are α-T binding proteins [120, 121]. Of equal interest is compelling evidence that α-T, as mentioned earlier, would have some biological activities that are not dependent on its action as an antioxidant [122]. These new biological activities may involve direct tocopherol-protein contact or be mediated by tocopherol's effect on membrane structure and dynamics. Thus, to investigate the role of the α-TTP would be beneficial, if molecular probes are available to confirm and identify those proteins that recognise α-T as a ligand. To this end, Lei and Atkinson and co-workers have designed analogues that incorporate photosensitive functional groups [123, 124]. The α-TTP preferentially recognises α-T over all of the other tocopherols so this structure has been maintained in the design of a photoaffinity label. Since the stereochemistry at C-2 is also vital to *in vivo* activity it has also been preserved. Work with analogues of α-T where the phytyl side chain has been replaced by straight chain alkanes has demonstrated that methyl substitutions are not mandatory for absorption and activity as antioxidants in rats [125]. This greatly simplifies the synthetic task of making a photoaffinity analogue since preparing a side chain with stereochemically pure methyl groups is considerably more complex. These observations restrict the position for attaching photosensitive groups to the terminus of the phytyl tail and to C-3 or C-4 of the chroman. With these considerations in mind, targets of structure **115** and **116** have been designed as potential photoaffinity label analogues of α-T (Fig. (**27**)). Nowadays the authors, designed and prepared fluorescent analogues of α-T that enable quantitative studies of ligand binding and transfer by α-TTP (**117-120**, Fig. (**27**)) [126]. Thus, compounds have been prepared that allow the investigation of the rate of α-TTP mediated inter-membrane transfer of α-T and to investigate the mechanism of α-TTP function at membranes of different composition.

As mentioned above, the involvement of free radicals in the pathology of human diseases (i.e., intestinal diseases, atherosclerosis, reperfusion injuries, cardiac diseases, neurodegeneration, respiratory disorders, inflammation, diabetes, cancer and aging) is well recognised. Potential therapeutic interventions might include natural antioxidants or synthetic pharmacological agents with antioxidant activity. The importance of vitamins E and C in disease prevention has become widely recognised and is supported by several clinical and epidemiological studies [127]. Taking into account this idea Manfredini and co-workers [128] designed hybrid analogues of these vitamins in the attempt to overcome the above cited drawbacks. In particular, the authors studied molecular combinations of the pharmacophores of the two

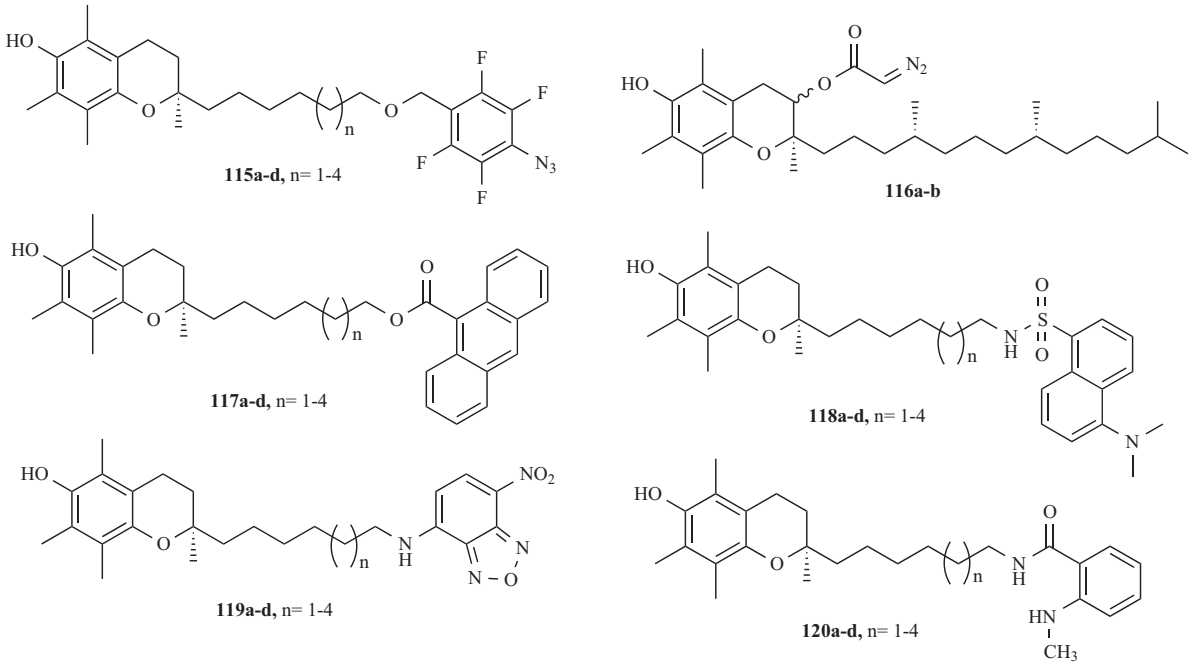


Fig. (27). Potential photoaffinity analogues of α-T (**115** and **116**) and fluorescent analogues of α-T (**117-120**).

vitamins, namely the chromane and the 2,3-dihydroxy-2,3-enono-1,4-lactone rings (Fig. (**28**)).

These compounds were synthesised and investigated for their antioxidant activity by evaluation of their capability to inhibit malondialdehyde (MDA) production in rat liver microsomal membranes. Moreover, in view of the high relevance of myocardial infarct among cause of death, the compounds **121a** (Fig. (**28**)) emerging as promising from the

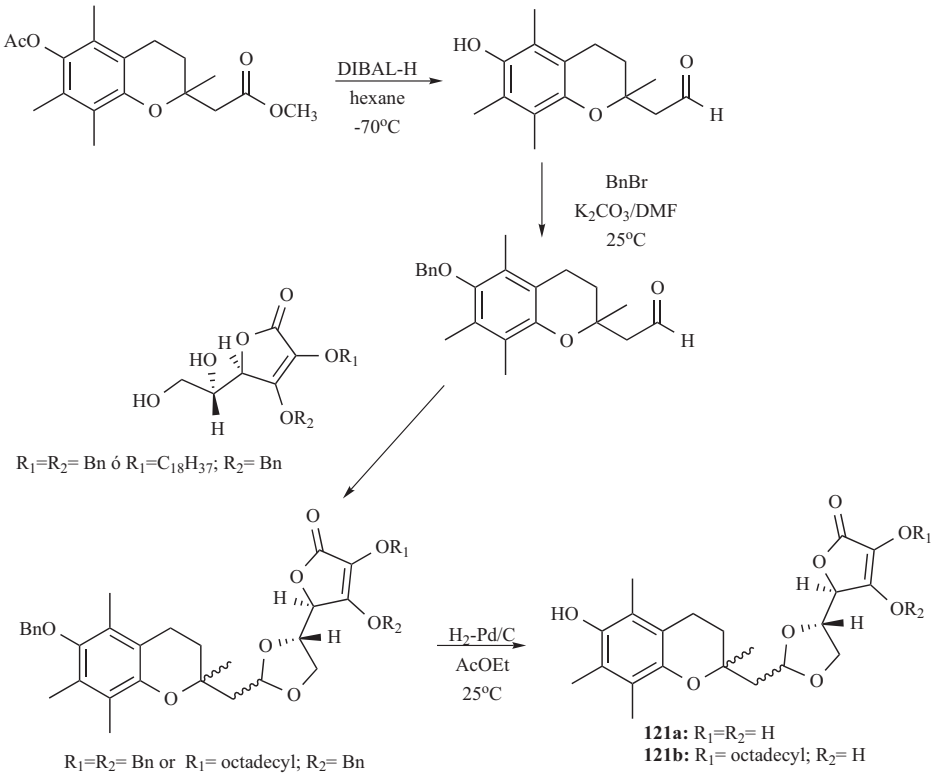


Fig. (28). Hybrid analogues of vitamin E and C.

primary antioxidant test was further evaluated for its capability to reduce ischaemia-reperfusion damage and proved to be effective in preventing damage on isolated rabbit heart. Taken together, these results are of significance for possible therapeutic applications in pathological events in which free radical damage is involved and for the possible extension of this approach to other synergistic antioxidants.

Organoselenium and organotellurium compounds show many interesting antioxidative properties. Selenium and tellurium are less electronegative than oxygen or sulphur thus the electron transfer to reactive alkoxyl or phenoxyl radicals becomes more likely to occur. However, until 2001, 1-seleno-α-tocopherol (**122a**) and 1-telluro-α-tocopherol (**122b**) had not been prepared yet (Fig. (**29**)). Malmström and co-workers [129] decided to synthesise and study the anti oxidant capacity of a series of compound constituted by 2,3-dihydrobenzo[*b*]furan-5-ol and its 1-thio, 1-seleno and 1-telluro analogues (**123a-d**) (Fig. (**29**)).

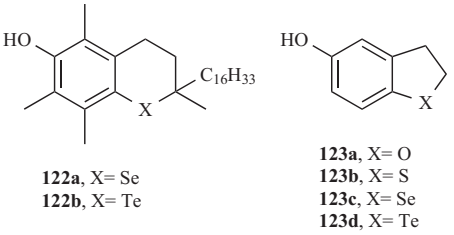


Fig. (29). Organoselenium and organotellurium analogues of α-T.

5-Hydroxy-2,3-dihydrobenzo[*b*]furan (**123a**) was readily prepared according to Wright procedure [130]. However, for the known [131, 132] sulfur derivative **123b** the authors developed an efficient alternative protocol based on homolytic substitution at sulfur (Fig. (**30**)). The authors prepared 5-hydroxy-2,3-dihydrobenzo[*b*]selenophene (**123c**) and 5-hydroxy-2,3-dihydrobenzo[*b*]tellurophene (**123d**) by a tandem $S_{RN}1/S_Hi$ sequence for radical-based, previously described by them (Fig. (**30**)) [133].

Authors evaluated the antioxidative properties of synthesised compounds studying the inhibition of azo-initiated peroxidation of linoleic acid [134]. Results showed that none of the synthetic compounds are as good as α-T as antioxidant, in the absence of a thiol-reducing agent. In fact, organotellurium compound **123d** does not inhibit peroxidation at all, and the organoselenium compound **123c** is a poor inhibitor. They also studied the glutathione peroxidase-like behaviour and the ability to protect liver microsomes subjected to stimulated lipid peroxidation, being **123d** as the compound which has great capacities. With the perspective to obtain antioxidants with similarly good H-atom donating capacity as α-T, but with a catalytic mode of action in the presence of mild reducing agents and glutathione peroxidase-like activity, Engman and co-workers have recently described the syn-

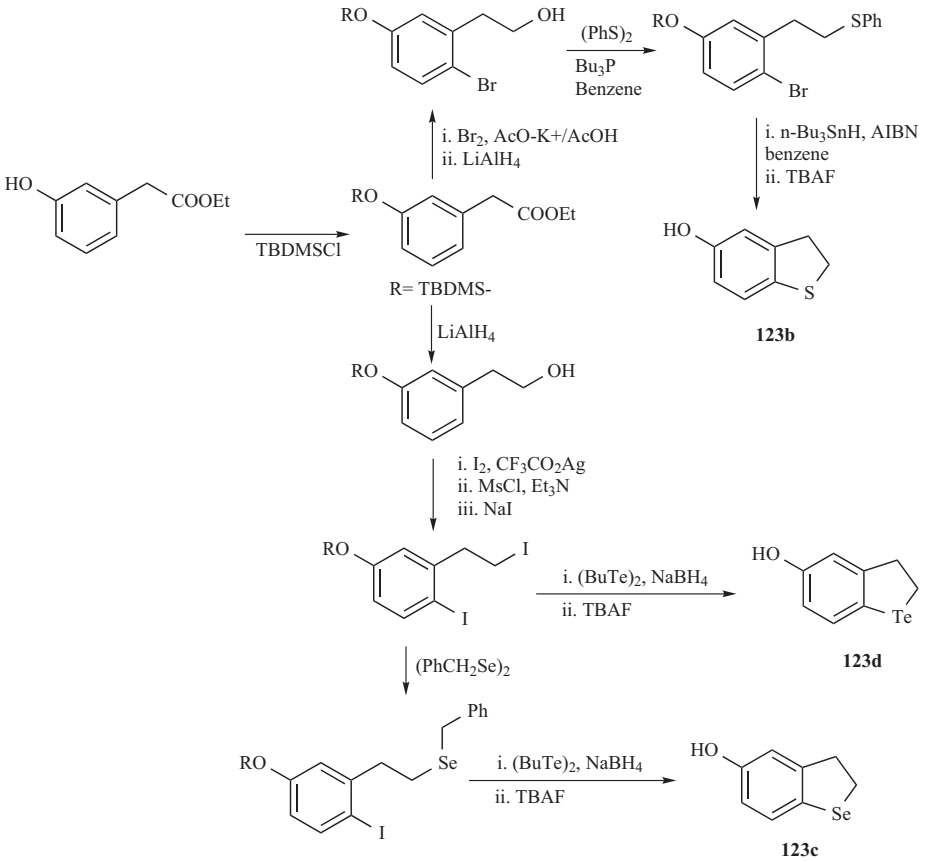
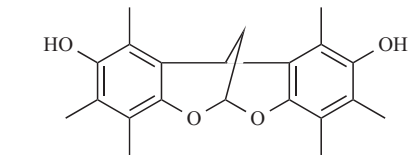


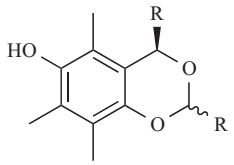
Fig. (30). Synthesis of organoselenium and organotellurium model compounds analogues of α-T.

thesis of the "real" selenium analogue of α-T, **122a** (Fig. (**29**)) [135]. Kinetic studies performed by measuring oxygen uptake of the induced oxidation of styrene in the presence of an antioxidant showed that selenotocopherol (**122a**) was a slightly poorer inhibitor than α-T, in agreement with the Bond Dissociation Enthalpy values. In contrast to simpler selenotocopherol analogues (**123c**), **122a** was not regenerable in the presence of a stoichiometric coreductant in a two-phase lipid peroxidation model.

In order to synthesise novel antioxidants and provide model compounds for studies of the physiologically important tocopherol system, Rosenau and Guille and co-workers decided to synthesise and study the antioxidative properties of "twin chromanol" and 3-oxatocopherol type compounds (**124**, **125a-g**, Fig. (**31**)) [136-139]. Results showed that twin-chromanol had better radical scavenging properties than



twin-chromanol
124

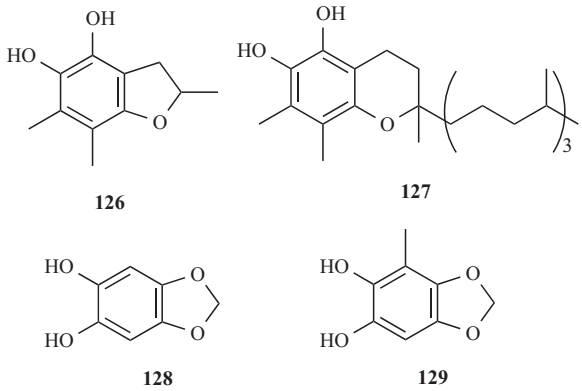


125a, R= CH_3
125b, R= CH_2CH_3
125c, R= $(CH_2)_2CH_3$
125d, R= $CH(CH_3)_2$
125e, R= cyclohexyl
125f, R= phenyl
125g, R= 4-nitrophenyl

Fig. (31). Twin-chromanol and 3-oxa-tocopherol type compounds.

α-T. In addition, twin-chromanol can deliver twice as many reducing equivalents, which makes this compound a promising new candidate as artificial antioxidant in biological systems. In this sense, it was studied the effect of these compounds as well as short chain analogue of α-T, pentamethylchromanol (**5**, Fig. (**3**)), on the bioenergetic functions in mitochondria by testing for their antioxidative potency in rat heart mitochondria (RHM) was studied. Experiments revealed that the bioenergetic parameters of mitochondria were not deteriorated in the presence of these chromanols. Alterations of the bioenergetic parameters were partially prevented in a concentration-dependent manner by preincubating RHM with antioxidants before adding the radical-generating system. In the lower concentration range, twin-chromanol turned out to be more efficient than **5** (Fig. (**3**)), both being far more protective than oxachromanols **125** (Fig. (**31**)). Measurement of protein-bound SH groups and thiobarbituric acid-reactive substances revealed that this protective effect was due to their antioxidative action. Furthermore, HPLC measurements of α-T and α-tocopheryl quinone in rat liver mitochondria demonstrated an α-T-sparing effect of twin-chromanol [140].

Considering certain relevant parameters in the design of synthetic antioxidants, thus, bond dissociation enthalpy (BDE), stability and reactivity of radical formed, and solubility, Wright and co-workers synthesised and characterised a series of new catechol derivatives (i.e. **126-129**, Fig. (**32**)). BDEs were calculated for each and the rate reaction constant with DPPH radical. Since compounds have high reactivity with DPPH· it would consider that they are potentially interesting in biological applications, and further studies to determine toxicity and protective effects are in advance [141].



126 **127**

128 **129**

Fig. (32). Catechol derivatives analogues to α-T.

On the other hand, Koufaki and co-workers encouraged the synthesis of new hybrid compounds combining the pharmacophoric redox moieties of vitamin E and key features responsible for other protective effects, like antiarrhythmic properties of the class I antiarrhythmics procainamide and lidocaine (**130-132**) [142], or with another antioxidant agent like lipoic acid (**133-138**) [143, 144]. These synthesised compounds (Fig. (**33**)) were examined for their antioxidant activity and their protective effects against reperfusion arrhythmias in isolated heart preparations. In addition, these researchers worked on the synthesis of chromanol-catechol hybrids (**139-142**, Fig. (**33**)) and studied its potential as neuroprotective agents [145].

Procainamide analogue **130a** and lidocaine analogues **132a** and **132b** are very strong inhibitors of lipid peroxidation and are also as effective in inhibiting reperfusion arrhythmias, as the standard anti-arrhythmics procainamide and lidocaine. However, the antioxidant properties of the different compounds do not closely parallel their antiarrhythmic effects **132b** is a weaker antioxidant than **132a** but induces more pronounced anti-arrhythmic effects. These data suggest that the efficacy of these compounds in preventing reperfusion arrhythmias could be attributed to their combined effects involving inhibition of free radical mediated damage coupled with antiarrhythmic properties.

All chromanol/lipoic acid hybrid compounds tested are strong inhibitors of lipid peroxidation in rat liver microsomal membranes induced by ferrous ions and ascorbate. Moreover, the new molecules reduced reperfusion arrhythmias. Compound **133d** exhibits anti-lipid peroxidation activity at the nanomolar range. Derivatives **133a** and **133d** totally suppressed reperfusion arrhythmias. The 2- and 5-substituted chromans (**134**, **135**, **137**, **138**) possess the better cardioprotective activity. The series of chromans substituted at positions 2- or 5- by catechol moieties were capable to protect cells from oxidative stress. The 5-substituted chromans **140a**,

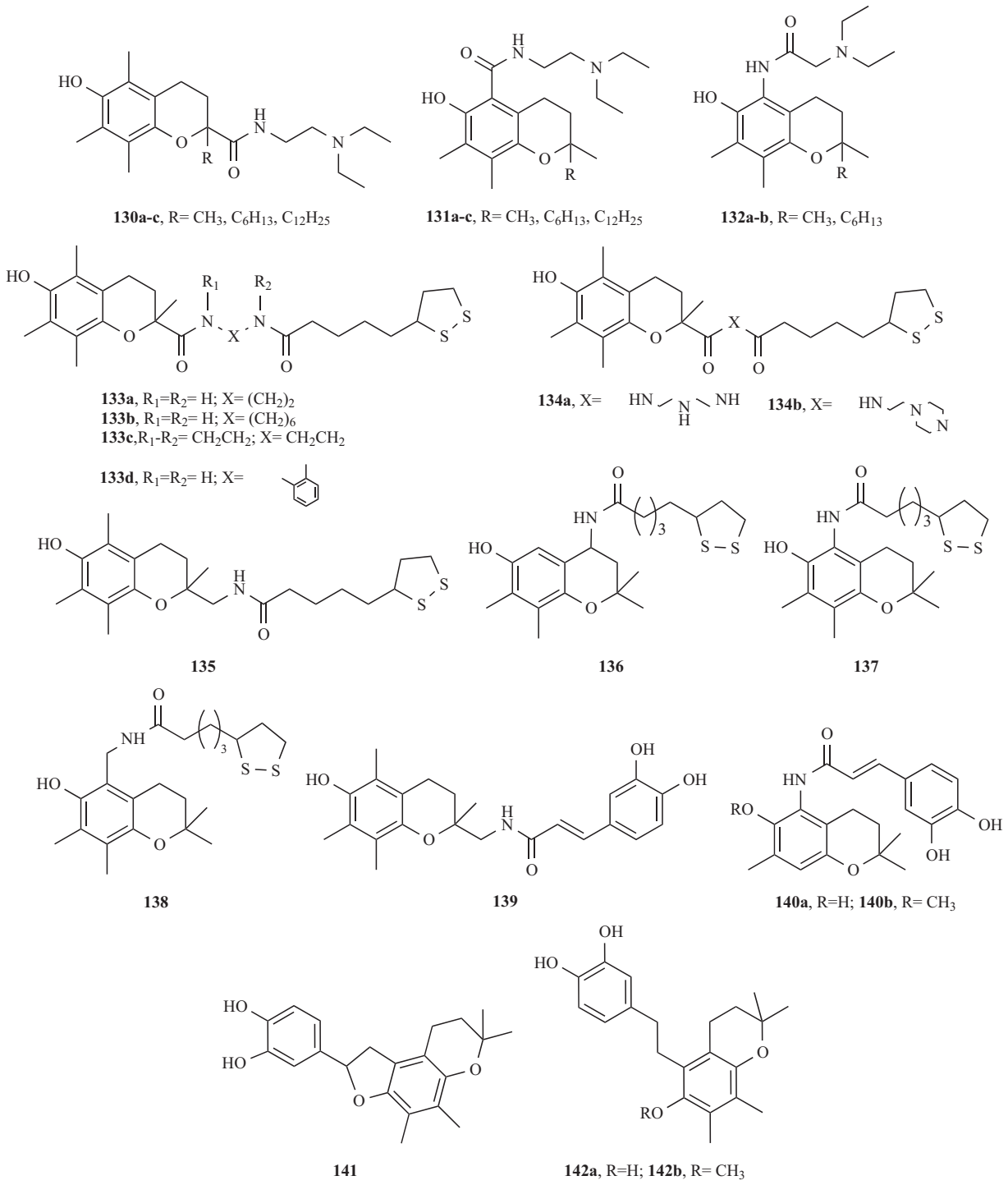
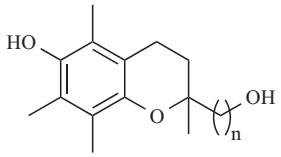


Fig. (33). New hybrid compounds combining the redox moieties of vitamin E and antiarrhythmic substructure (**130-132**), another antioxidant substructure like lipoic acid (**133-138**) and catechol (**139-142**).

140b, and **142a** are very potent against H_2O_2- and glutamate-induced cellular damage. Ethers **141** and **142b** are less potent against DNA damage than the above three analogues but more active than the 2-substituted chromans, and they exhibit strong neuroprotective activity.

At the same time, Muller and co-workers, in an attempt to modulate the neurotoxic properties of activated microglial cells, have undertaken the synthesis and characterisation of the pharmacological properties of a series of tocopherol long chain fatty alcohols (**143a-d**, TFA, Fig. (**34**)) [146]. This



143a-d, TFA, n= 10, 12, 14, 16

Fig. (34). TFA, tocopherol long chain fatty alcohols.

type of activation is observed after brain injury or infection, as well as during the development of neuropathies like Alzheimer's disease, stroke or demyelinating diseases such as multiple sclerosis. After a rapid change in cell morphology, activated microgliocytes produce pro-inflammatory cytokine like tumour necrosis factor-α (TNF-α) as well as the free radicals nitric oxide (·NO) and superoxide anion ($O_2^{\cdot-}$). Therefore, the researchers studied the *in vitro* production of ·NO and TNF-α by activated microglial cells when are co-incubated with the TFAs. The 2-(12-hydroxydodecyl)-2,5,7,8-tetramethyl-chroman-6-ol, the TFA bearing 12 carbon atoms on the side chain (**143b**, n = 12), shows the most potent inhibition of ·NO and TNF-α production by lipopolysaccharide-activated microglia. Further studies on the biological properties, and especially on the signalling cascades affected, of the molecules of the TFA family are necessary to fully characterise their pharmacological potentials.

Recently, Gasco and co-workers described the synthesis and the study of hybrid antioxidant and vasodilating agents as a new class of potential antiatherosclerosis agents (Fig. (**35**)) [147-149]. The products were obtained by joining appropriate antioxidant phenols with either nitrooxy or furoxan moieties as NO releasing entities. Atherosclerosis is the main cause of morbidity and mortality in the western societies relating with oxidative stress and endothelial dysfunction, and both processes would be inhibited by these hybrid molecules. In fact, some of the products behave principally as vasodilators and other as antioxidants (**144**, **145a-b**) and the two properties are relatively balanced in **146a-b** and **147** but further *in vivo* studies should clarify whether some of these products may become preclinical candidates for the treatment of cardiovascular disease underpinned by atheroma.

Simultaneously, our group started to work in the design, synthesis and biological characterisation of hybrid molecules combining the vitamin E structure and ·NO releasing moieties (Fig. (**36**)), to target NO delivery *in vivo* specifically into LDL, as a possible therapeutic strategy to protect LDL from oxidative modifications, contributing for the treatment of atherosclerosis [150, 151].

These hybrid compounds release ·NO, due to the presence of a furoxan or a nitrooxy substructure, inhibiting platelet aggregation and exhibiting vasorelaxation properties. Moreover, they effectively protect LDL from oxidation, combining the tocopherol substructure with affinity to LDL and antioxidant properties of ·NO-donor. Our results show that the new tocopherol analogues represent a new class of ·NO donors having different capacity to release ·NO. These tocopherol derivatives exhibited vasorelaxation properties with derivatives belonging to series c, **150a**, **151a-b** and in a less extent **150b**, displayed greater vasodilating effects. This suggests that these compounds stimulate ·NO signalling pathways in vascular tissue. Moreover, the observed LDL-protective activity of derivative **151b**, suggest the potential use of these compounds for prevention of atherosclerosis disease. These "site specific" observations are significant in view of the fact that the protective effect of typical ·NO donors or antioxidants decrease with time and distances of the biological targets, i.e. LDL. Our observations emphasise the necessity of performing further studies to analyse the LDL protective activity of these compounds *in vivo*.

CONCLUDING REMARKS

Although a considerable work has been performed in the medicinal chemistry field with the design and synthesis of novel antioxidant with different important purposes: i. answer the question, are chain-breaking antioxidant structural features fully optimise in α-tocopherol?; ii. study its metabolism, iii. over come the clinical limitation of vitamin E, iv.

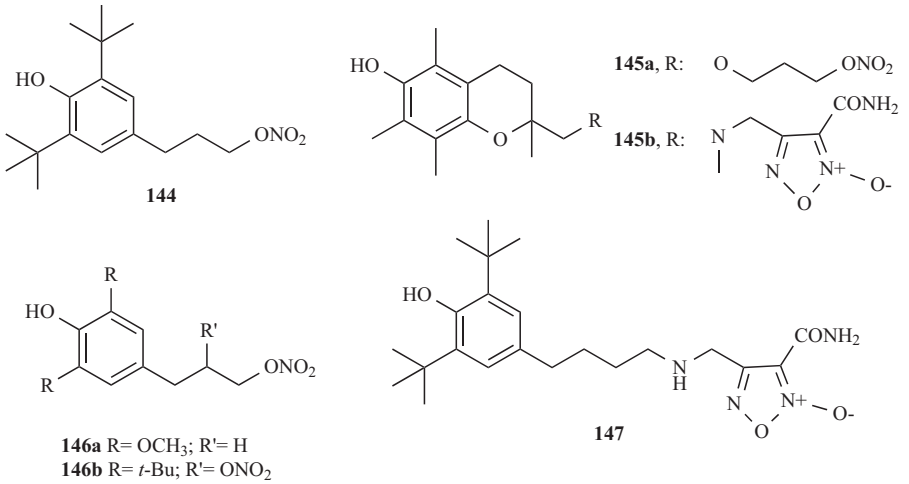


Fig. (35). Hybrid antioxidant and vasodilating agents as a class of potential antiatherosclerosis agents.

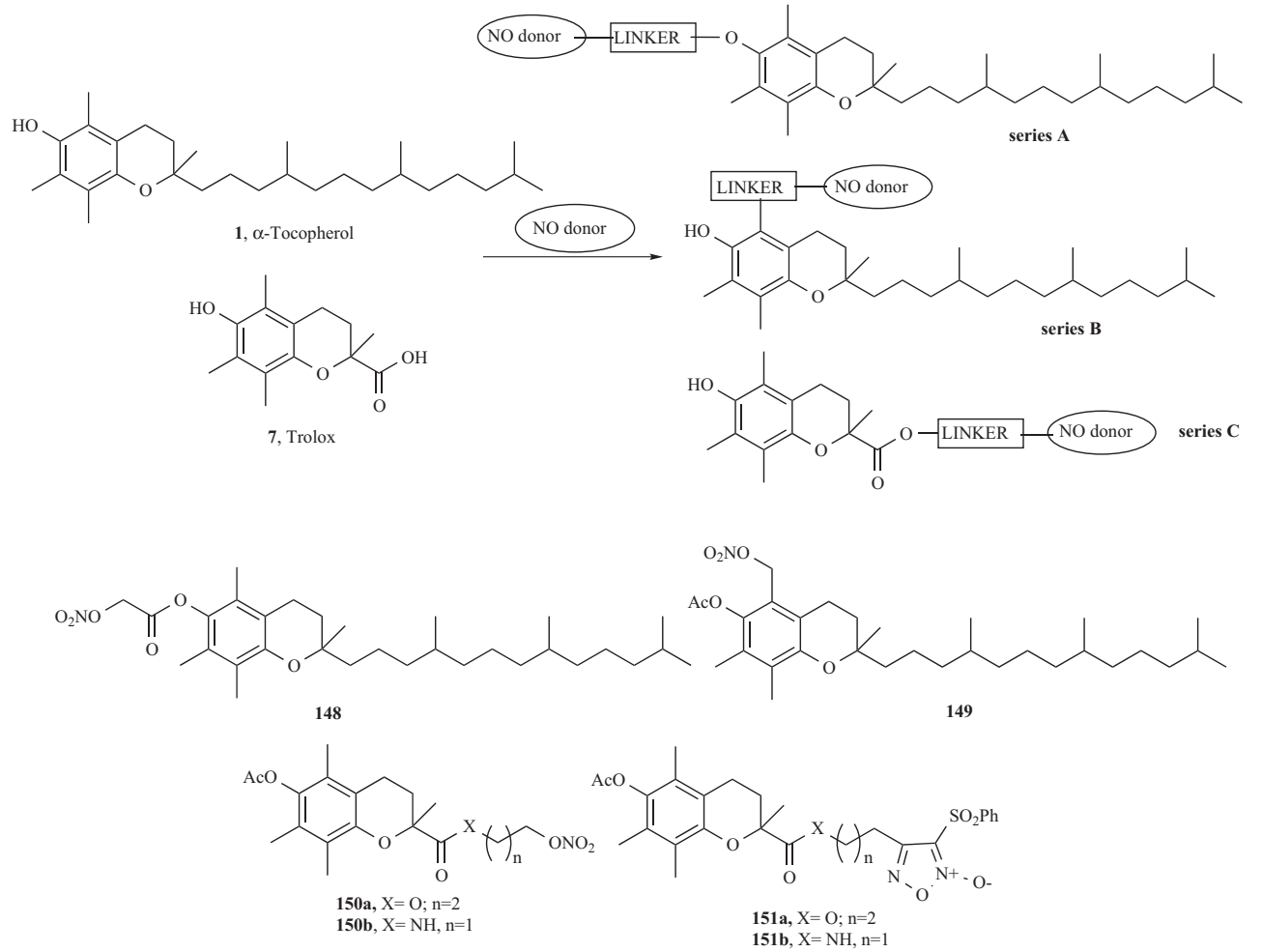


Fig. (36). Hybrid molecules combining the vitamin E structure and ·NO releasing moieties as a possible therapeutic strategy to protect LDL from oxidative modifications for the treatment of atherosclerosis.

design of novel drugs with dual biological properties; further studies could/should still be made.

ABBREVIATIONS

α-T = α-Tocopherol

β-T = β-Tocopherol

γ-T = γ-Tocopherol

δ-T = δ-Tocopherol

CM = Chylomicrons

LPL = Lipoprotein lipase

LDL = Low density lipoprotein

α-TTP = α-Tocopherol transfer protein

VLDL = Very low density lipoprotein

GC = Gas chromatography

RHM = Rat heart mitochondria

AAPH = 2,2'-azobis(2-amidinopropane) dihydrochloride

AMVN = 2,2'-azobis(2,4-dimethylvaleronitrile)

DPPH = 2,2-diphenyl-1-picrylhydrazyl radical

TFA = Tocopherol long chain fatty alcohols

TNF-α = Tumor necrosis factor-α

PCh = Phosphatidylchromanol

NOS = Nitric oxide synthase

MDA = Malondialdehyde

BDE = Bond dissociation enthalpy

Bn = Benzyl

TBARS = Thiobarbituric acid reactive species

ROS = Reactive oxygen species

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Received: 23 August, 2006 Revised: 08 December, 2006 Accepted: 28 December, 2006