Vitamin E analogues as inducers of apoptosis: structure–function relation

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Recent results show that α-tocopheryl succinate (α-TOS) is a proapoptotic agent with antineoplastic activity. As modifications of the vitamin E (VE) molecule may affect its apoptogenic activity, we tested a number of newly synthesised VE analogues using malignant cell lines. Analogues of α-TOS with lower number of methyl substitutions on the aromatic ring were less active than α-TOS. Replacement of the succinyl group with a maleyl group greatly enhanced the activity, while it was lower for the glutaryl esters. Methylation of the free succinyl carbonyl group on α-TOS and Δ-TOS completely prevented the apoptogenic activity of the parent compounds. Both Trolox and its succinylated derivative were inactive. α-Tocotrienol (α-T3 H) failed to induce apoptosis, while γ-T3 H was apoptogenic, and more so when succinylated. Shortening the alphatic side chain of γ-T3 by one isoprenyl unit increased its activity. Neither phytyl nor oleyl succinate caused apoptosis. These findings show that modifications of different functional moieties of the VE molecule can enhance apoptogenic activity. It is hoped that these observations will lead to the synthesis of analogues with even higher apoptogenic and, consequently, antineoplastic efficacy.

Keywords: vitamin E analogues; apoptosis; synthesis; anticancer effect

Current therapies for neoplastic disease, although often effective in causing remission, frequently lead to deleterious and even life-threatening side-effects. Therefore, there is great interest in developing otherwise nontoxic but effective antineoplastic agents, and inducers of apoptosis may fall into this category of agents (Ferreira et al, 2002). In this regard, recent findings suggest that certain analogues of vitamin E (VE), such as α-tocopheryl succinate (α-TOS), may represent a new class of antineoplastic agents with high selectivity for malignant cells and low toxicity. α-Tocopheryl succinate causes apoptotic death of a variety of neoplastic cell lines (Ferriss et al, 1994; Qian et al, 1997; Neuzil et al, 1999; Yamamoto et al, 2000), whereas neither the redox-active α-tocopherol (α-TOH) nor its uncharged ester, α-tocopheryl acetate (α-TOA), is effective (Qian et al, 1997; Neuzil et al, 2001c).

Therefore, the proapoptotic activity appears to be a unique feature of α-TOS, and this agent is also effective in vivo, inhibiting the growth of colon (Weber et al, 2002) and melanoma cancers (Malafa et al, 2001), promoting breast cancer dormancy (Malafa and Neitzel, 2000), and suppressing metastasis (Barnett et al, 2002). These intriguing observations have prompted us to investigate the molecular properties responsible for this interesting and evidently selective (Neuzil et al, 2001b) proapoptotic action of α-TOS.

The apparent selective toxicity of α-TOS towards malignant vs normal cells (Neuzil et al, 2001b, c; Weber et al, 2002) may be related to its negative charge at neutral pH, a notion supported by findings that its apoptotic action is enhanced at acidic pH (Neuzil et al, 2002b). The selective toxicity may thus arise from the fact that pH of the interstitium of most tumours is 6.2–6.5, but in the range of 7.0–7.4 for most normal tissues (Gerweck and Seetharam, 1996). Another possible mechanism by which VE analogues may exert selectivity is because of higher esterase activity of normal compared to malignant cells. Epithelial intestinal cells as well as hepatocytes are known to hydrolyse agents like α-TOS, while Jurkat T lymphoma cells are inefficient, whereby accumulating high levels of the toxic agent (Weber et al, 2003). The importance of the acidic environment is further supported by a recent observation that chlorambucil, a weak acid, exerted more profound antineoplastic effects when pH of the tumour interstitium was lowered (by hyperglycaemia) in an animal model of breast cancer (Kozin et al, 2001).

These findings suggest that modifications of individual functional moieties of VE analogues might modulate proapoptotic activity. We therefore synthesised novel analogues of VE differing from α-TOS in the ester side group, in the number and positions of methyl substitutions on the aromatic ring, and in the alphatic side chain. We show here that these modifications change the original proapoptotic activity of α-TOS, either positively or negatively, depending on the type of modifications and their combination.

MATERIALS AND METHODS

Chemicals

α-T-tocopherol (α-TOH; 1), γ-T-tocopherol (γ-TOH; 3), α-ε-tocopheryl acetate (α-TOA; 5), and α-d-tocopheryl succinate (α-TOS; 6) were from Sigma (Castle Hill, NSW, Australia), α-trolox...
RRR-α-tocopheryl maleate (z-TOM; 7). Brown semisolid; \(^{1}H\) NMR (CDCl\(_3\)) \(\delta\ 0.84 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 6 H), 0.87 (d, J = 6.6 Hz, 6 H), 1.23 (s, 3 H), 1.1 – 1.5 (m, 19 H), 1.5 – 1.6 (m, 2 H), 1.19 (s, 3 H), 2.04 (s, 3 H), 2.58 (s, J = 6.9 Hz, 2 H).\)

RRR-α-tocopheryl 2-methylsuccinate (z-TOM2; 8). Yellow semisolid; \(^{1}H\) NMR (CDCl\(_3\)) \(\delta\ 0.84 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 6 H), 0.87 (d, J = 6.6 Hz, 6 H), 1.23 (s, 3 H), 1.1 – 1.5 (m, 19 H), 1.5 – 1.6 (m, 2 H), 1.19 (s, 3 H), 2.04 (s, 3 H), 2.58 (s, J = 6.9 Hz, 2 H).\)

RRR-α-tocopheryl glutarate (z-TOG; 9). Yellow semisolid; \(^{1}H\) NMR (CDCl\(_3\)) \(\delta\ 0.84 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 6 H), 0.87 (d, J = 6.6 Hz, 6 H), 1.23 (s, 3 H), 1.1 – 1.5 (m, 19 H), 1.5 – 1.6 (m, 2 H), 1.7 – 1.9 (m, 2 H), 1.96 (s, 3 H), 2.04 (s, 3 H), 2.58 (s, J = 6.9 Hz, 2 H).\)

RRR-α-tocopheryl 3-methylglutarate (z-TOS3; 20). Yellow semisolid; \(^{1}H\) NMR (CDCl\(_3\)) \(\delta\ 0.84 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 6 H), 0.87 (d, J = 6.6 Hz, 6 H), 1.23 (s, 3 H), 1.1 – 1.5 (m, 19 H), 1.5 – 1.6 (m, 2 H), 1.19 (s, 3 H), 2.04 (s, 3 H), 2.58 (s, J = 6.9 Hz, 2 H).\)

RRR-α-tocopheryl 3,3-dimethylglutarate (z-TOS3DMG; 11). Yellow semisolid; \(^{1}H\) NMR (CDCl\(_3\)) \(\delta\ 0.84 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 6 H), 0.87 (d, J = 6.4 Hz, 3 H), 1.23 (s, 3 H), 1.1 – 1.5 (m, 19 H), 1.25 (s, 6 H), 1.5 – 1.6 (m, 2 H), 1.7 – 1.9 (m, 2 H), 1.96 (s, 3 H), 2.04 (s, 3 H), 2.58 (s, J = 6.9 Hz, 2 H).\)

RRR-α-tocopheryl 2,2-dimethylglutarate (z-TOS2DMG; 12). Yellow semisolid; \(^{1}H\) NMR (CDCl\(_3\)) \(\delta\ 0.84 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 6 H), 0.87 (d, J = 6.4 Hz, 3 H), 1.23 (s, 3 H), 1.1 – 1.5 (m, 19 H), 1.30 (s, 6 H), 1.5 – 1.6 (m, 2 H), 1.7 – 1.9 (m, 2 H), 1.96 (s, 3 H), 2.04 (s, 3 H), 2.58 (s, J = 6.9 Hz, 2 H), 2.65 (m, 2 H).\)

RRR-δ-tocopheryl succinate (δ-TOS; 17). Yellow semisolid; \(^{1}H\) NMR (CDCl\(_3\)) \(\delta\ 0.84 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 6 H), 0.87 (d, J = 6.6 Hz, 6 H), 1.23 (s, 3 H), 1.1 – 1.5 (m, 19 H), 1.5 – 1.6 (m, 2 H), 1.7 – 1.9 (m, 2 H), 1.96 (s, 3 H), 2.04 (s, 3 H), 2.58 (s, J = 6.9 Hz, 2 H), 2.80 – 2.84 (m, 4 H), 6.6 – 6.7 (m, 2 H).\)

R-γ-tocotrienyl succinate (γ-TTS; 20). Yellow semisolid; \(^{1}H\) NMR (CDCl\(_3\)) \(\delta\ 1.26 (s, 3 H), 1.60 (s, 3 H), 1.68 (s, 3 H), 1.5 – 1.9 (m, 2 H), 1.95 – 2.2 (m, 4 H), 2.01 (s, 3 H), 2.10 (s, 3 H), 2.71 (m, 2 H), 2.75 – 2.95 (m, 4 H), 5.11 (m, 3 H), 6.57 (s, 1 H).\)

Phytol succinate (PYS; 22). Colourless semisolid; \(^{1}H\) NMR (CDCl\(_3\)) \(\delta\ 0.84 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 6 H), 0.87 (d, J = 6.4 Hz, 3 H), 1.1 – 1.5 (m, 19 H), 1.70 (s, 3 H), 1.95 – 2.15 (m, 2 H), 2.6 (m, 4 H), 4.62 (t, J = 6.9 Hz, 2 H), 5.34 (m, 1 H).\)

BCG cultures were grown in the presence of the addition of 200 ng/mL of tocopherol or the respective derivatives. The growth of the cultures was followed by the measurement of optical density at 595 nm. Growth was considered to be enhanced when the optical density of the cultures containing the Vitamin E analogues was higher than the control cultures (20 ng/mL of \(a\)-tocopherol).
Oleyl succinate (OS; 23). Colourless semisolid; $^1$H NMR (CDCl$_3$) $\delta$ 0.88 (t, 3 H), 1.2–1.4 (m, 22 H), 1.62 (m, 2 H), 2.01 (m, 4 H), 2.66 (m, 4 H), 4.09 (t, J = 6.6 Hz, 2 H), 5.35 (m, 2 H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.1, 22.7, 25.8, 2 × 27.2, 28.5 × 28.9, 2 × 29.2, 2 × 29.3, 29.4, 29.5, 29.7, 29.8, 31.9, 65.0, 129.8, 130.0, 172.2, 178.1; ESI-MS (m/z): 367 (M$^+$).

RRR-z-tocopheryl succinyl methyl ester (z-TOSM; 24). Clear colourless oil; $^1$H NMR (CDCl$_3$) $\delta$ 0.85 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.3 Hz, 3 H), 0.87 (d, J = 6.6 Hz, 6 H), 1.24 (s, 3 H), 1.02 – 1.44, (m, 19 H), 1.46 – 1.60 (m, 2 H), 1.74 – 1.82 (m, 2 H), 1.98 (s, 3 H), 2.02 (s, 3 H), 2.09 (s, 3 H), 2.59 (t, J = 6.7 Hz, 2 H), 2.77 (m, 2 H), 2.94 (m, 2 H), 3.72 (s, 3 H); $^{13}$C NMR (CDCl$_3$) 11.8, 12.0, 12.9, 19.6, 19.7, 20.6, 21.0, 22.6, 22.7, 24.4, 24.8, 28.0, 28.8, 28.9, 31.1, 32.7, 32.8, 37.3, 37.4, 39.3, 51.9, 75.0, 77.2, 117.3, 123.0, 124.9, 126.6, 140.4, 149.4, 170.9, 172.6.

RRR-δ-tocopheryl succinyl methyl ester (δ-TOSM; 25). Clear colourless oil; $^1$H NMR (CDCl$_3$) $\delta$ 0.77 (d, J = 7.1 Hz, 3 H), 0.78 (d, J = 6.6 Hz, 3 H), 0.80 (d, J = 6.6 Hz, 6 H), 1.18 (s, 3 H), 0.94 – 1.39, (m, 19 H), 1.41 – 1.51 (m, 2 H), 1.62 – 1.75 (m, 2 H), 2.06 (s, 3 H), 2.63 – 2.68 (m, 4 H), 2.75 – 2.80 (m, 2 H), 3.65 (s, 3 H), 6.55 (d, J = 2.5 Hz, 1 H), 6.60 (d, J = 2.5 Hz, 1 H); $^{13}$C NMR (CDCl$_3$) $\delta$ 16.1, 19.6, 19.7, 21.0, 22.4, 22.6, 22.7, 24.2, 24.4, 24.8, 28.0, 28.9, 29.0, 29.3, 30.9, 32.7, 32.8, 37.3, 37.4, 37.4, 39.4, 40.1, 51.9, 76.1, 77.2, 118.9, 120.9, 121.0, 127.3, 142.3, 149.8, 171.6, 172.6.

Synthesis of z-2-geranylchromanol. 2, 6, 10-trimethyl-10-hydroxy-2, 6, 11-dodecatrienate. Vinyl magnesium bromide (1 M in tetrahydrofuran, 6.4 ml, 6.4 mmol) was added with vigorous stirring under argon at 0 – 5°C to a solution of geranylaceton (1.2 g, 6.2 mmol) in diethylether (100 ml) over 60 min. The reaction mixture was stirred for additional 30 min and acidified with 1M HCl to pH 2, and extracted with ether (3 × C2H$_5$OH) washed with brine (3 × C2H$_5$OH) and dried over Na$_2$SO$_4$. Ether was removed on a rotavapor to yield yellow oil that was used without further purification.

z-2-geranylchromanol (z-T2H; 21) Vinyl alcohol (0.96 g, 4 mmol) in dioxane (2 ml) was added over 1.5 h at 110°C to a stirred solution of 2,3,5-trimethylhydroquinone (0.42 g, 2.8 mmol) and boron trifluoride etherate (0.7 ml, 5.5 mmol) in dioxane (15 ml) under argon. The reaction mixture was extracted with ethyl acetate (3 × 100 ml), and the combined ether extracts washed with brine (3 × 50 ml) and dried over Na$_2$SO$_4$. Ether was removed on a rotavapor to yield yellow oil that was used without further purification.

Apoptosis evaluation

Apoptosis was routinely assessed by the annexin V-binding method, which is based on affinity of annexin V to phosphatidylserine externalised to the outer leaflet of the plasma membrane early in the course of apoptosis (Neuzil et al, 2001a). In brief, suspension cells were harvested by spinning down, while adherent cells were detached by treatment with 2 mM EDTA in PBS and combined with the cells detached during treatment. The cells were then washed with PBS, spun down, resuspended in the binding buffer (10 mM Heps/NaOH, 140 mM NaCl and 25 mM CaCl$_2$, pH 7.4), incubated with 2μl of annexin V – FITC (PharMingen, San Diego, CA, USA), and analysed by flow cytometry (Becton Dickinson, Rutherford, NJ, USA). In some cases, activation of caspase-3 was assessed as follows. Treated cells were washed, permeabilised with 0.02% saponin in PBS, and incubated with an antibody raised against activated caspase-3 (PharMingen) at room temperature for 60 min. The cells were then washed and incubated with secondary IgG conjugated to FITC at room temperature for additional 60 min, washed, and scored for FITC binding in a flow cytometer. The percentage of apoptotic cells or cells with activated caspase-3 was estimated by gating on the population with high fluorescence.

RESULTS AND DISCUSSION

The molecule of VE can be divided into three functionally distinct domains (Figure 1) (Neuzil et al, 2002a). Domain I (the functional domain) is essential for the redox activity of VE analogues, which involves the hydroxyl group in position C6 of the chromanol ring structure. Interestingly, z-TOH does not induce apoptosis (Qian et al, 1997; Neuzil et al, 2001c), nor does it when acetylated at C6 (z-TOA) (Neuzil et al, 2001a). However, succinylation in this position makes z-TOH a strong apoptogen (Neuzil et al, 2001c). Conversion of z-TOH into a charged species may thus play a role
in apoptosis induction (Neuzil et al., 2002b). We therefore investigated the effects of substituting \( \alpha \)-TOS with other dicarboxylic acids in this position. Of these analogues, \( \alpha \)-TOM was more apoptogenic compared to \( \alpha \)-TOS, whereas the other esters tested were less effective than \( \alpha \)-TOS in the order \( \alpha \)-TO2MS \( > \alpha \)-TOG \( > \alpha \)-TO3MG \( > \alpha \)-TO33DMG = \( \alpha \)-TO22DMG (Figures 2 and 3).

Naturally occurring derivatives of \( \alpha \)-TOH differ in the number and position of methyl substitutions on the aromatic ring, that is domain II (signalling domain). These include \( \beta \)-, \( \gamma \)- and \( \delta \)-TOH. All of these agents were largely nonapoptogenic (Neuzil et al., 2002a) (Figure 4). Succinylation made them proapoptotic, although the activity of \( \beta \)-, \( \gamma \)- and \( \delta \)-TOS was lower than that of \( \alpha \)-TOS, with \( \delta \)-TOS being least apoptogenic (Figure 4). Their efficacy in inhibiting protein kinase C also differs, and is not dependent on their antioxidant capacity (Ricciarelli et al., 2001). In addition, the substitution pattern is responsible for the rate of side chain

### Table 1: VE analogues as inducers of apoptosis

| No. | Acronym | Name of analogue               | No. | Acronym | Name of analogue               |
|-----|---------|--------------------------------|-----|---------|--------------------------------|
| 1   | \( \alpha \)-TOH | \( \alpha \)-Tocopherol         | 14  | \( \alpha \)-TroS | \( \alpha \)-Trolox succinate     |
| 2   | \( \beta \)-TOH | \( \beta \)-Tocopherol          | 15  | \( \beta \)-TOS | \( \beta \)-Tocopheryl succinate  |
| 3   | \( \gamma \)-TOH | \( \gamma \)-Tocopherol         | 16  | \( \gamma \)-TOS | \( \gamma \)-Tocopheryl succinate |
| 4   | \( \delta \)-TOH | \( \delta \)-Tocopherol         | 17  | \( \delta \)-TOS | \( \delta \)-Tocopheryl succinate |
| 5   | \( \alpha \)-TOA | \( \alpha \)-Tocopheryl acetate | 18  | \( \alpha \)-T3H | \( \alpha \)-Tocotrienol          |
| 6   | \( \alpha \)-TOS | \( \alpha \)-Tocopheryl succinate | 19  | \( \gamma \)-T3H | \( \gamma \)-Tocotrienol          |
| 7   | \( \alpha \)-TOM | \( \alpha \)-Tocopheryl maleate | 20  | \( \gamma \)-T3S | \( \gamma \)-Tocotrienyi succinate |
| 8   | \( \alpha \)-TO2MS | \( \alpha \)-Tocopheryl 2-methylsuccinate | 21  | \( \alpha \)-T2H | \( \alpha \)-2-Geranylchromanol   |
| 9   | \( \alpha \)-TGG | \( \alpha \)-Tocopheryl glutarate | 22  | PYS | Phytol succinate               |
| 10  | \( \alpha \)-TO3MG | \( \alpha \)-Tocopheryl 3-methylglutarate | 23  | OS | Oleyl succinate                 |
| 11  | \( \alpha \)-TO33DMG | \( \alpha \)-Tocopheryl 3,3 dimethylglutarate | 24  | \( \alpha \)-TOSM | \( \alpha \)-Tocopheryl succinyl methyl ester |
| 12  | \( \alpha \)-TO22DMG | \( \alpha \)-Tocopheryl 2,2-dimethylglutarate | 25  | \( \delta \)-TOSM | \( \delta \)-Tocopheryl succinyl methyl ester |
| 13  | \( \alpha \)-TroH | \( \alpha \)-Trolox             |     |         |                                 |

**Figure 2** Analogues of VE used in this study. The items shown in bold indicate newly synthesised compounds.
Effect of modifications in domain I of the VE molecule on their apoptogenic activity. Jurkat (A), HBT11 (B), MCF7 (C), and MCF7-C3 cells (D) were exposed for 24 h to \( \alpha \)-TOS, \( \alpha \)-TOA, \( \alpha \)-TOM, \( \alpha \)-TOS, \( \alpha \)-TO2MS, \( \alpha \)-TOG, \( \alpha \)-TO3DMG, or \( \alpha \)-TO22DMG (11), and assessed for apoptosis using the annexin V–FITC method.

Figure 4 Effect of modifications in domain II of the VE molecule on their apoptogenic activity. Jurkat (A), HBT11 (B), MCF7 (C), and MCF7-C3 cells (D) were exposed for 24 h to \( \beta \)-TOS, \( \gamma \)-TOS, \( \delta \)-TOS, \( \gamma \)-TOH, \( \delta \)-TOH, \( \alpha \)-TOS, \( \beta \)-TOS, \( \gamma \)-TOS, or \( \delta \)-TOS, and assessed for apoptosis using the annexin V–FITC method.

Figure 5 Effect of methylation of the succinyl moiety of VE succinyl analogues on their apoptogenic activity. Jurkat (A), HBT11 (B), MCF7 (C), and MCF7-C3 cells (D) were exposed for 24 h to \( \alpha \)-TOS, \( \delta \)-TOS, \( \alpha \)-TOSM, or \( \delta \)-TOSM, and assessed for apoptosis using the annexin V–FITC method.

degradation, as \( \gamma \) - and \( \delta \)-TOS are degraded much faster than \( \alpha \) - or \( \beta \)-TOS in cell culture (Birringer et al., 2001).

To address the importance of the free carboxylic group for apoptogenic activity of VE dicarboxylic acid esters with saturated isoprenyl side chains, we esterified \( \alpha \)-TOS and \( \delta \)-TOS on the succinyl moiety. The resulting methyl esters were completely inactive as inducers of apoptosis in all cell lines tested (Figure 5).

Several modifications of the aliphatic side chain (domain II, the hydrophobic domain) are possible. Its desaturation, in the case of \( \alpha \)-TOSM, gives the naturally occurring \( \alpha \)-T3H, which is nonapoptotic (Yu et al., 1999). \( \gamma \)-T3H, however, has a strong apoptogenic activity (Yu et al., 1999), which is further enhanced by its conversion to \( \gamma \)-T3S (Figure 6). Interestingly, a derivative of \( \gamma \)-T3H with the aliphatic side chain shorter by one isoprenyl unit, \( \alpha \)-T2H, exerted higher proapoptotic activity than did \( \gamma \)-T3H itself (Figure 6). Analogues of VE lacking the aliphatic side chain, that is, \( \alpha \)-TroH and \( \alpha \)-TroS did not induce apoptosis, regardless of the succinylation status (Figure 6).

Finally, we synthesised succinyl esters of long-chain fatty acids, that is, PYS and OS. Neither of them caused any signs of apoptosis at up to 100 \( \mu \)M and at times of up to 48 h (Figure 7). These results indicate that presence of an aliphatic group at one end and a chargeable moiety at the other end is not sufficient for apoptosis induction and that presence of the chromanol structure may be essential.

Toxicity of \( \alpha \)-TOS towards cancer cells is known to be governed by apoptotic signalling. To find out whether this form of cell death is also involved in killing by the newly synthesised VE analogues, we exposed Jurkat cells to the agents for increasing time, and analysed the cells for caspase-3 activation, a hallmark of apoptotic signalling. As demonstrated in Figure 8, all new VE analogues, which were shown above to cause annexin V binding in several cancer cell lines, caused time-dependent activation of caspase-3. Assessment of annexin V binding was carried out on adherent and
detached cells pooled together (see Materials and Methods section). It cannot be excluded that some of the cells died by anoikis. However, similar effects were observed with suspension, Jurkat cells. This strongly suggests that apoptotic cell death is the major mechanism by which these novel agents kill cancer cells.

The data presented herein suggest the following conclusions: (i) Forms of VE with saturated aliphatic chains need to be esterified with a dicarboxylic acid to exert apoptogenic activity. (ii) The extent of proapoptotic effect is greatly dependent on the unsaturation and length of the ester group substituent. Thus, the C6 maleyl-substituted analogue showed the highest activity, which was virtually nondetectable in case of the 2,2-dimethyl- and 3,3-dimethylglutaryl substituents. This may suggest that the apparently less water-soluble agents are less bioavailable for the malignant cells tested. Moreover, z-TOM may have higher in vivo effectiveness as it is 10–20-fold more efficient in vitro than the relatively well-studied z-TOS. The latter agent exerts antitumour effects against experimental colon cancer at the pharmacologically relevant blood levels of ~40–50 μM (Weber et al., 2002). We would thus expect z-TOM to be equipotent with z-TOS in vivo at its plasma levels of ~5 μM, that is, similar to those of circulating VE.

**Figure 6** Effect of modifications in domain III of the VE molecule on their apoptogenic activity. Jurkat (A), HBT11 (B), MCF7 (C), and MCF7-C3 cells (D) were exposed for 24 h to z-TOH, z-TOS, γ-T3H, γ-T3S, z-T2H, z-TroH, or z-TroS, and assessed for apoptosis using the annexin V–FITC method.

**Figure 7** Proapoptotic activity of other structural analogues of VE. Jurkat (A), HBT11 (B), MCF7 (C), and MCF7-C3 cells (D) were exposed for 24 h to z-TOS, and for 22 or 48 h to z-TOH, PYS, or OS, and assessed for apoptosis using the annexin V–FITC method.

**Figure 8** Vitamin E analogues induce apoptosis and activate caspase-3. Jurkat cells were exposed for the time shown to 50 μM each of α-TOS, α-TOM, α-TOM2MS, α-TOG, α-TOM3MG, γ-T3H, γ-T3S, or γ-T2H, and assessed for apoptosis extent (A) and caspase-3 activation using the antibody specific for activated caspase-3 and a secondary, FITC-conjugated antibody (B). The inset in (B) shows a typical histogram of flow cytometric evaluation of control cell and cells treated with 50 μM α-TOS for 12 h.
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