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Inhibition of oxidative metabolism of tocopherols with ω-*N*-heterocyclic derivatives of vitamin E

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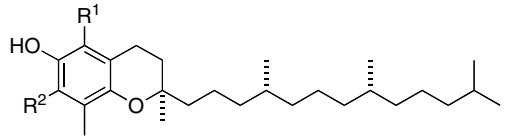
ABSTRACT

The oxidative metabolism of tocopherols and tocotrienols by monooxygenases is a key factor in the plasma and tissue clearance of forms of vitamin E other than α-tocopherol. It is well known that a commonly ingested form of vitamin E, γ-tocopherol, has greatly reduced plasma half-life (faster clearance) than α-tocopherol. The tocotrienols are metabolized even faster than γ-tocopherol. Both γ-tocopherol and α- and δ-tocotrienol possess intriguing biological activities that are different from α-tocopherol, making them potentially of interest for therapeutic use. Unfortunately, the fast clearance of non-α-tocopherols from animal tissues is a significant hurdle to maximizing their effect(s) as dietary supplements. We report here the design and synthesis of *N*-heterocycle-containing analogues of α-tocopherol that act as inhibitors of Cyp4F2, the key monooxygenase responsible for ω-hydroxylation of the side chain of tocols. In particular, an ω-imidazole containing compound, **1**, [(*R*)-2-(9-(1H-imidazol-1-yl)nonyl)-2,5,7,8-tetramethylchroman-6-ol] had an ED_{50} for inhibition of γ-CEHC production from γ-tocopherol of ~1 nM when tested in HepG2 cells in culture. Furthermore, feeding of **1** to mice along with rapidly metabolized δ-tocopherol, resulted in a doubling of the δ-tocopherol/α-tocopherol ratio in liver ($P < 0.05$). Thus, **1** may be a useful adjuvant to the therapeutic use of non-α-tocopherols.

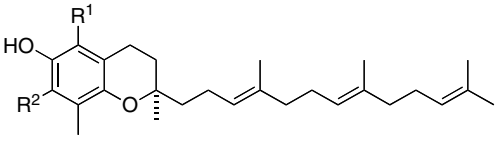
1. Introduction

The tocopherols are understood to be the major lipid soluble antioxidants in mammalian membranes. They act as chain-breaking inhibitors of free radical peroxidation of unsaturated fatty acids. Despite the fact that the diets of most North Americans contain more γ-tocopherol than α-tocopherol, it is predominantly α-tocopherol that is retained in the body due to the actions of a specific binding and transfer protein known as the tocopherol transfer protein (TTP).[1,2] This protein is mostly expressed in liver tissue, binds α-tocopherol specifically,[3,4] and aids the secretion of the vitamin in lipoproteins such as VLDL that then carry the vitamin to remote tissues of the body. Forms of vitamin E other than α-tocopherol are poorly retained by the body and are rapidly metabolized to water-soluble compounds for excretion in urine or transported to bile.[5–7]

The metabolism of non-retained tocopherols (i.e. non-α-tocopherols and tocotrienols, see Fig. 1) is initiated in human cells by a cytochrome P450 monooxygenase, Cyp4F2, and its orthologs in other species. The enzyme activity is referred to as tocopherol-ω-hydroxylase.[8] This enzyme metabolizes all forms of vitamin E by placing a hydroxy group at the terminus of the side chain. This is illustrated in Figure 2 for tocopherols, but is the same for the tocotrienols.



$R^1 = R^2 = CH_3$, α-Tocopherol
$R^1 = CH_3$, $R^2 = H$, β-Tocopherol
$R^1 = H$, $R^2 = CH_3$, γ-Tocopherol
$R^1 = R^2 = H$, δ-Tocopherol



$R^1 = R^2 = CH_3$, α-Tocotrienol
$R^1 = CH_3$, $R^2 = H$, β-Tocotrienol
$R^1 = H$, $R^2 = CH_3$, γ-Tocotrienol
$R^1 = R^2 = H$, δ-Tocotrienol

Figure 1. Structures of naturally occurring tocopherols and tocotrienols.

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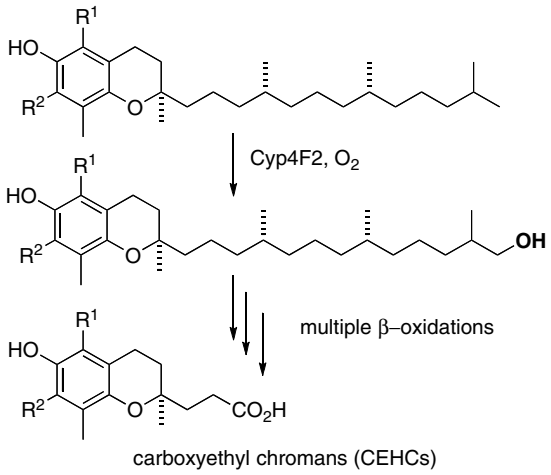


Figure 2. Illustration of the monoxygenase-catalyzed primary ω-oxidation step in the metabolism of tocols (here shown for tocopherols) followed by repeated rounds of β-oxidation that results in the formation of carboxyethyl chromans.

Recently, it has been noted that γ-tocopherol and the tocotrienols have biological activities that are different from that of α-tocopherol. γ-Tocopherol is known to act as an anti-inflammatory, possibly by mechanisms different from that of α-tocopherol[9–17] and to scavenge reactive nitrogen species such as peroxynitrite.[18,19] The tocotrienols have been demonstrated to have significantly different biological activities from the tocopherols[20–22] including inhibition of cholesterol biosynthesis,[23–25] anti-cancer effects,[26–28] and more recently protection against glutamate-induced neurodegeneration in animal models of stroke.[29–32]

To take advantage of the promising biological activities of γ-tocopherol and the tocotrienols requires overcoming their short half-lives of retention in plasma and tissues.[33] The chief reason for their poor bioavailability and short plasma half-lives is their rapid (in comparison to α-tocopherol) oxidative metabolism by the above-mentioned tocopherol-ω-hydroxylase. Any nutritional or chemotherapy involving the use of tocols other than α-tocopherol will suffer from the extremely fast metabolism of these forms of vitamin E. This makes it very difficult to raise plasma levels and thus tissue levels of, for instance, α- or δ-tocotrienol when taken orally. The promising biological activities observed for tocotrienols such as protection from glutamate dependent neurodegeneration in models of stroke would thus benefit enormously by elevating the circulating levels of tocotrienol by inhibiting its metabolism.

We describe here the design, synthesis, and biological testing of α-tocopherol derivatives that act as specific inhibitors of tocopherol-ω-hydroxylase and greatly extend the biological half-lives of non-α-tocopherols and tocotrienols.

2. Results and discussion

2.1. Synthesis

Figure 3 shows the compounds prepared; imidazole, triazole, and benzimidazole derivatives of α-tocopherol, where the isoprenyl side chain has been modified to incorporate the nitrogen heterocycle that is expected to act as a ligand at the heme-iron atom of the Cyp4F2.

The use of nitrogen heterocycles as ligands at heme-iron atoms has been used previously in the design of many agrichemical fungicides.[34–36] There are also some antifungal human therapeutics[37,38] including the imidazoles clotrimazole, miconazole, ketoconazole, and triazoles such as fluconazole. All of these compounds act as heme ligands (and thus inhibitors) of the fungal sterol 14[*a*]-demethylase also known as Cyp51.[37,38] An analogous design principle has been used in the development of inhibitors of retinoic acid metabolism by Cyp2C8 and Cyp26.[39–43] These compounds appear now to have utility in the treatment in retinoid responsive cancers.[44]

The inhibition of tocopherol metabolism is known to occur using the methylenedioxy-containing lignan sesamin,[8,45–49] but this requires micromolar concentrations of sesamin for complete inhibition in cell culture assays versus less than 0.25 μM for compound **1**. Furthermore, sesamin is known to inhibit other enzymes such as fatty acid delta-5-desaturase.[50]

In general, the syntheses followed the first steps of a method we reported earlier[51,52] to form a chromanol that replaces the phytyl side chain with a C9 alkyl extension terminating in an alcohol group. The alcohol is derivatized to the mesylate then displaced with *N*-heterocyclic anions to give compounds **1**–**7**. The process is illustrated below for compound **1** (Scheme 1).

Of the compounds prepared, only the smaller imidazole and triazoles showed inhibitory activity, while the larger benzimidazole**5** and benzotriazoles **6** and **7** had no activity. This can be understood by considering the shape of the active site for CYP4F2 that tends to perform ω-hydroxylations on long chain substrates such as leuko-

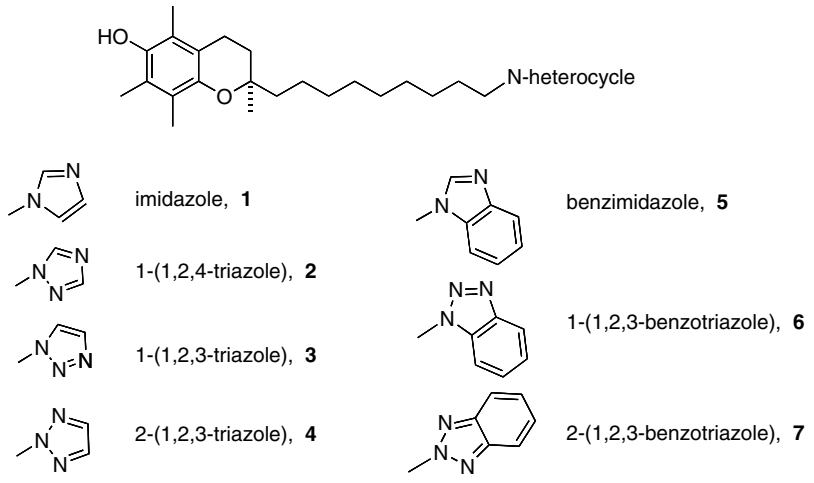
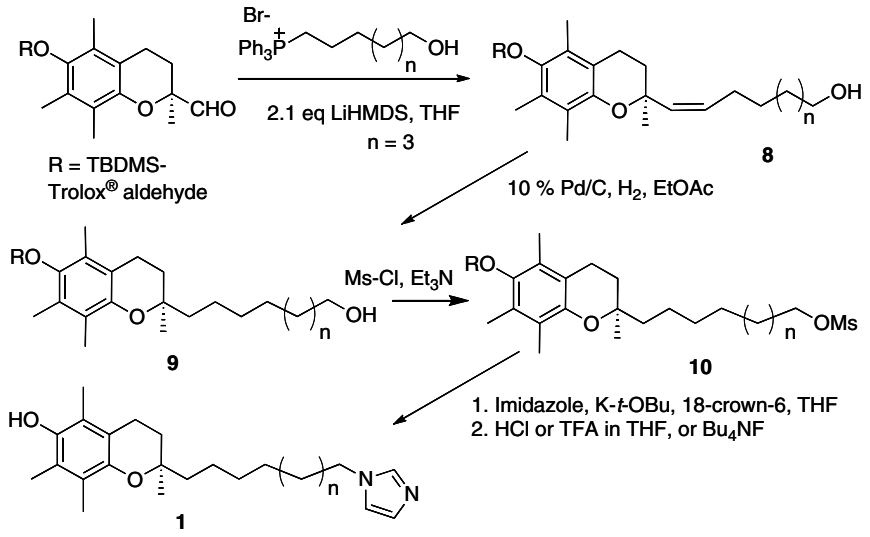


Figure 3. Structures of the *N*-heterocycle-containing chromanols prepared in this work.



Scheme 1. General synthetic method for the synthesis of *N*-heterocycle-containing chromanols, exemplified for **1**, (*R*)-2-(9-(1H-imidazol-1-yl)nonyl)-2,5,7,8-tetramethylchroman-6-ol.

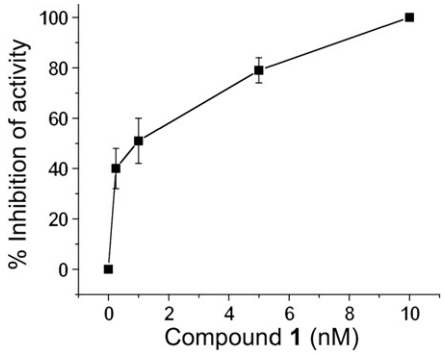


Figure 4. Inhibition of metabolism of γ-tocopherol in HepG2 cultures by imidazole-containing chromanol, **1**.

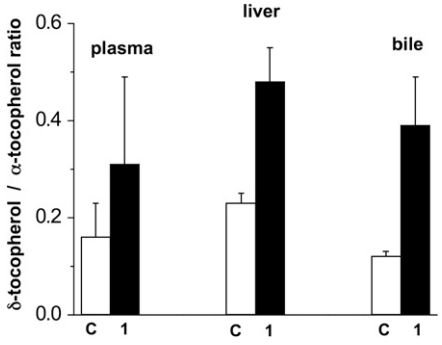


Figure 5. Effect of **1** on the tissue enrichment in δ-tocopherol in mice. Data are means and standard deviation. C = control; **1** = (*R*)-2-(9-(1H-imidazol-1-yl)nonyl)-2,5,7,8-tetramethylchroman-6-ol.

trienes (LTB4) and arachidonic acid.[8,53–55] We prepared the benzimidazole and benzotriazoles because CYP4F2 also shows ethoxycoumarin *O*-deethylase activity,[53] suggesting that larger ring systems might also be acceptable substrates in this series of tocopherol analogues. For compounds **5**–**7**, the benzimidazoles and benzotriazoles may be too large to be bound in the active site, or when bound they have difficulty offering a N-atom as a ligand to the heme-iron.

2.2. Biological assessment

Compounds **1**–**7** were tested in cultured HepG2 liver cells by following the metabolism of γ-tocopherol. Reduction in concentration of 3′- and 5′-carboxychromanol metabolites in the culture media following incubation of the cells with γ-tocopherol and various concentrations of one of compounds **1**–**7** was illustrative of inhibition of the first step of oxidative metabolism (ω-hydroxylation). Cells were pre-incubated with the inhibitor for 4 h, then the media were replaced with fresh media containing 25 μM γ-tocopherol and the inhibitor. After 48 h of incubation the media was analyzed for γ-CEHC. The imidazole analogue **1** showed complete inhibition of γ-tocopherol metabolism at concentrations as low as 250 nanomolar (nM). After titrating this dose, lower we obtained an ED_{50} value—effective dose for 50% inhibition—of approximately 1.0 nM (Fig. 4). Complete inhibition by **1** was also obtained, when cells were exposed to the inhibitor only during a 6 h preincubation period. There was no effect of **1** on total cell protein, and all monolayers appeared normal in morphology. The 1,2,4-triazole **2** had reduced inhibition with 42% inhibition at 20 nM and the 1,2,3-triazole **3** showed only partial inhibition (~36%) at 1.0 μM concentration. None of the benzimidazole or benzotriazole derivatives showed any inhibition of γ-tocopherol metabolism in this assay. These results demonstrate that **1** is a remarkably potent inhibitor of human tocopherol ω-hydroxylase as expressed in human hepatoblastoma cultures.

2.3. Effect of 1 on tissue enrichment in δ-tocopherol in mice in vivo

α-Tocopherol is a poor substrate for tocopherol ω-hydroxylase, thus as expected there was no evidence that α-tocopherol status was affected by feeding δ-tocopherol or **1**. Therefore, data in Figure 5 are presented as the δ-tocopherol/α-tocopherol ratio, where an increase in the ratio reflects enrichment in δ-tocopherol relative to α-tocopherol, a meaningful endpoint.

Feeding of **1** to mice resulted in a doubling in the δ-tocopherol/α-tocopherol ratio in mouse liver ($P < 0.05$), an expected outcome if **1** inhibited the metabolism of δ-tocopherol (a good tocopherol

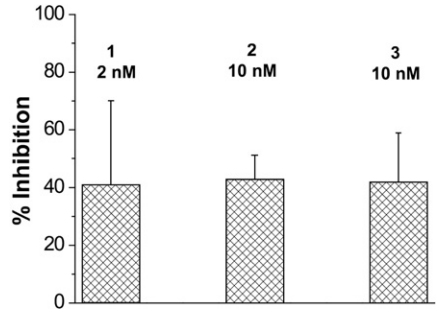


Figure 6. Differing inhibition of CYP3A4 (testosterone-6-hydroxylase) activity by compounds **1**, **2**, and **3** in HepG2/C3A cultures.

ω-hydroxylase substrate) but not α-tocopherol, a poor substrate. In addition, the corresponding ratio in bile was tripled in mice fed **1** ($P < 0.05$), indicating that at least some of the unmetabolized δ-tocopherol was being secreted into bile.

These results clearly demonstrate that **1** is effective in inhibiting tocopherol ω-hydroxylase activity in vivo and in increasing tissue concentrations of tocopherols that are good substrates of that enzyme.

2.4. Cytochrome P450 selectivity of imidazole and triazole compounds: effect on CYP3A4 activity

Ideally the novel imidazole and triazole tocopherol ω-hydroxylase inhibitors would be specific for Cyp4F2 activity, with no effect on other cytochrome P450 enzymes. The major human liver cytochrome P450 is CYP3A4, and its model activity is the 6-hydroxylation of testosterone. The effect of **1**, **2**, and **3** on testosterone 6-hydroxylase activity was determined in HepG2/C3A cell culture, and in commercially available insect cell microsomes expressing only recombinant human CYP3A4 (BD-Gentest, Woburn, MA). All three compounds inhibited testosterone-6-hydroxylase activity in HepG2/C3A cultures as shown in Figure 6. Compound **1** was more potent than either of the triazole compounds **2** or **3**.

2.5. Inhibition of CYP3A4 in microsomes

When tested with CYP3A4 microsomes, 4 μM **1** inhibited testosterone-6-hydroxylase activity by 90%, as shown in Figure 7. The results of studies on CYP3A4 activity, as assessed using testosterone-6-hydroxylase as model activity, show that none of the three compounds tested were specific for tocopherol ω-hydroxylase activity, but rather also inhibited CYP3A4 activity as assessed in two different experimental systems. While not ideal, this situation appears analogous to that of many antifungal imidazole and triazole compounds (e.g. ketoconazole) that are therapeutically effective in humans while also exhibiting anti-CYP3A4 activity unrelated to their therapeutic action.

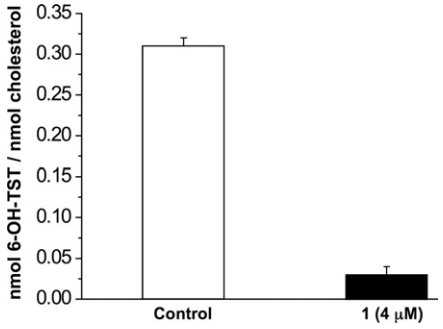


Figure 7. Inhibition of CYP3A4 (testosterone-6-hydroxylase) activity by compound **1** in insect cell microsomes.

3. Summary

The *N*-heterocyclic derivatives of α-tocopherol prepared in this study were designed as potential P450 enzyme inhibitors based on the well-established use of *N*-heterocycles as competitive inhibitors of these monooxygenases in agrochemical and therapeutic fungicides. We were successful in preparing (*R*)-2-(9-(1H-imidazol-1-yl)nonyl)-2,5,7,8-tetramethylchroman-6-ol,**1**, which is an excellent inhibitor of CYP4F2 ($ED_{50} \sim 1$ nM in HepG2 cells) and raised the level of δ-tocopherol in mice when **1** was provided at 500 mg per kg of diet that also contained δ-tocopherol. Our preliminary investigation into the P450 isoform specificity showed that **1** had considerable activity against the major human P450 isoform in liver CYP3A4, but not nearly to the same extent as CYP4F2. Thus, compound **1** can be considered a possible drug candidate that might allow the therapeutic use of tocols that have shown promise in treating disease. This is especially true of the tocotrienols that have very short lifetimes in plasma.

4. Experimental

4.1. Materials

All starting materials for synthesis were purchased from Sigma–Aldrich (Oakville, Ont.) and used without any further purification. (*S*)-Trolox was a kind gift of Dr. Thomas Netscher, DSM Nutritional Products, Basel, Switzerland. Solvents were purchased from Caledon and, where indicated, were dried under argon prior to use. Dichloromethane (CH_2Cl_2), hexane, and triethylamine (Et_3N) were distilled from calcium hydride (CaH_2). Tetrahydrofuran (THF) was dried by distillation over sodium and benzophenone. Dry methanol (MeOH) was obtained by distillation from magnesium and a catalytic amount of iodine.

4.2. Analytical methods

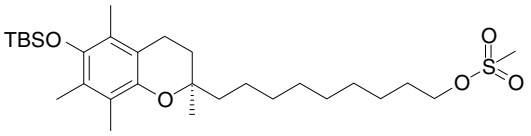
Preparative chromatography was carried out on silica gel (200–300 Å mesh) with the indicated solvent systems. Analytical thin layer chromatography (TLC) was performed on 0.25 mm precoated silica gel 60 Å F-254 plates (Merck). Visualization of the TLC plates was achieved using an ultraviolet (UV) lamp at 254 nm and exposure to iodine vapor, or immersion in 4% H_2SO_4 in methanol followed by heating.

NMR spectra were recorded using a Bruker Advance DPX-300 Digital FT-NMR spectrometer at 300 MHz (^1H) and 75 MHz (^{13}C). Deuterated chloroform (99.8% pure, Cambridge Isotope Laboratories, Inc.) was used as the solvent unless otherwise noted with the internal reference of residual chloroform ($^1H = 7.24$ ppm, $^{13}C = 77.0$ ppm). Chemical shifts are reported in ppm (δ) (multiplicity, number of protons, assignment, and coupling constant in Hz). Multiplicity is designated using the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Mass spectra (MS) were recorded on a Carlo Erba/Kratos GC/MS Concept 1S double focusing mass spectrometer interfaced to a Kratos DART acquisition system and a SUN SPARC workstation. Samples were introduced through a direct inlet system and ions were generated using electron impact (EI) at 70 eV and are reported as m/z values for the parent peak and major fragment ions.

4.3. Synthesis

Details on the synthesis of long chain alcohol **9** have been provided elsewhere.[52]

4.3.1. (*R*)-9-(6-*tert*-Butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethylchroman-2- yl)nonyl methanesulfonate, 10



Five hundred milligrams (1.08 mmol) of (*R*)-9-(6-*tert*-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethylchroman-2-yl)nonanol **9** was dissolved in dry DCM (10 ml) under argon atmosphere, followed by the addition of triethylamine (224 μl), MsCl (mesylchloride) (125 μl) and a catalytic amount of DMAP (dimethylaminopyridine) at 0 °C. After 15 min, the cooling bath was removed and the reaction stirred at rt for 1.5 h. TLC monitoring showed complete conversion in 100% DCM as mobile phase. Extraction with water and DCM afforded crude product that was obtained following evaporation of the solvent under reduced pressure. Column chromatography on silica (100% DCM) gave pure (**7**) in high yields (following high vacuum). (90%, 526 mg, 0.97 mmol).

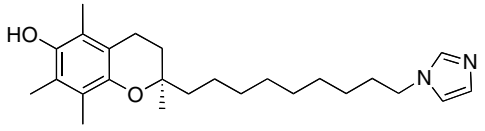
TLC: R_f = 0.50 (100% DCM).

^{1}H NMR ($CDCl_3$); δ 4.24 (t, 2H, C9′–CH_2, J = 6.408), 3.02 (s, 3H, SCH_3), 2.57 (t, 2H, O–CH_2, J = 6.782), 2.12 (s, 3H, Ar–CH_3), 2.09 (s, 3H, Ar–CH_3), 2.07 (s, 3H, Ar–CH3), 1.80 (m, 1H).

^{13}C NMR ($CDCl_3$); δ 145.90, 144.07, 125.85, 123.53, 122.66, 117.51, 74.46, 70.19, 39.58, 37.39, 31.55, 30.11, 29.49, 29.37, 29.14, 29.03, 26.12, 25.42, 23.83, 23.60, 20.91, 18.62, 14.34, 13.41, 11.96, −3.34.

MS[EI$^+$] m/z 540 (M^+, 3%), 444 (13.4%), 97 (56.2%), 57 (100%).

HRMS (EI): calculated for $C_{29}H_{52}O_5SSi$: 540.33047. Found 540.32732.



4.3.2. (*R*)-2-(9-(1H-imidazol-1-yl)nonyl)-2,5,7,8-tetramethylchroman-6-ol, 1

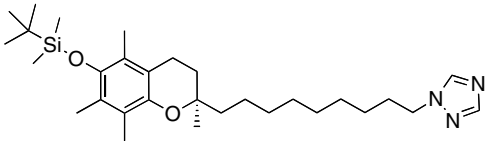
A solution of (*R*)-9-(6-*tert*-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethylchroman-2-yl)nonyl methanesulfonate, **10** (500 mg, 0.924 mmol) in dry ACN (5 ml) was added dropwise at room temperature under argon to a suspension of imidazole (126 mg, 1.849 mmol), KOtBu (207 mg, 1.849 mmol), and 18-crown-6-ether (25 mg, 0.0924 mmol) in dry ACN (10 ml). The mixture was refluxed (90 °C) under argon atmosphere for 15 h. After cooling to room temperature, the resulting white precipitate was filtered off and the remaining organic phase acidified to pH 6 using 10% HCl. The reaction was then diluted with DCM (50 ml), washed several times with H_2O (3 × 30 ml), dried with Na_2SO_4, decanted, and concentrated under reduced pressure to give a brown oil. The crude product was purified by column chromatography (DCM to DCM/MeOH 100:1.5) to yield a light yellow oil (260 mg, 71%). On inspection of this product it was clear that not only had the imidazole added to the chain terminus as expected, but the silyl protecting group had also been removed.

^{1}H NMR ($CDCl_3$); δ 7.48 (s, 1H), 7.07 (s, 1H), 6.91 (s, 1H), 5.30 (s, 1H), 3.91 (t, 2H, J = 7.1 Hz), 2.63 (t, 2H, J = ∼6 Hz), 2.24 (s, 3H), 2.19 (s, 3H), 2.15 (s, 3H), 1.89–1.69 (m, 4H), 1.66–1.53 (m, 2H), 1.49–1.41 (m, 2H), 1.311 (br m, 10H), 1.27 (s, 3H).

^{13}C NMR ($CDCl_3$); δ 145.32, 136.90, 128.91, 122.75, 122.20, 120.21, 118.84, 117.13, 74.35, 53.53, 47.10, 39.53, 31.68, 31.04, 30.11, 29.50, 29.38, 29.09, 26.53, 23.91, 23.62, 20.86, 12.78, 11.89, 11.83.

MS[EI$^+$] for $C_{25}H_{38}N_2O_2$; m/z 398 (M^+, 79%), 387 (13%), 235 (100%), 203 (10%), 179 (9%), 165 (26%). 137 (13%), 123 (10%), 96 (11%).

HRMS (EI): calculated for $C_{25}H_{38}N_2O_2$: 398.29333. Found 398.29336.



4.3.3. (*R*)-1-(9-(6-(*tert*-Butyl-dimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)nonyl)-1H-1,2,4-triazole

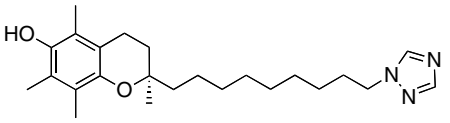
Clear colorless oil. R_f 0.4 (EtOAc). Purified by silica gel column chromatography using 100% Et_2O.

^{1}H NMR ($CDCl_3$); δ 8.04 (s, 1H), 7.94 (s, 1H), 4.15 (t, 2H, J = 7 Hz), 2.56 (t, 2H, J = 6.5 Hz), 2.11 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 1.83 (m, 2H), 1.76 (m, 2H, J = 6.6 Hz), 1.61–1.49 (m, 2H), 1.42 (m, 2H), 1.28 (m, 10H), 1.23 (m, 3H), 1.06 (s, 9H), 0.13 (s, 6H).

^{13}C NMR ($CDCl_3$); δ 151.85, 145.90, 144.06, 125.81, 123.49, 122.63, 117.47, 74.42, 49.69, 39.54, 31.55, 30.07, 29.77, 29.44, 29.32, 28.99, 26.43, 26.12, 23.83, 23.56, 20.90, 18.60, 14.34, 13.41, 11.96, −3.33.

MS[EI$^+$] for $C_{30}H_{51}N_3O_2Si$; m/z 513 (M^+, 37%), 221 (11%), 205 (24%), 149 (12%), 138 (10%), 129 (14%).

HRMS (EI): calculated for $C_{30}H_{51}N_3O_2Si$: 513.37506. Found 513.37517.



4.3.4. (*R*)-2-(9-(1H-1,2,4-Triazol-1-yl)nonyl)-2,5,7,8-tetramethylchroman-6-ol, 2

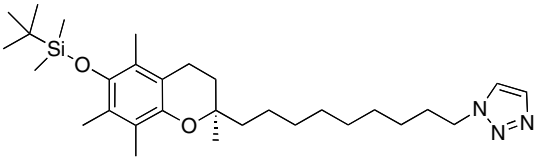
Off-white solid. mp 114–115 °C R_f 0.31 (EtOAc). Purified by silica gel column chromatography using 100% Et_2O.

^{1}H NMR ($CDCl_3$); δ 8.03 (s, 1H), 7.93 (s, 1H), 5.13 (s, 1H), 4.14 (t, 2H, J = 7 Hz), 2.59 (t, 2H, J = 6.5 Hz), 2.16 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 1.86 (m, 2H), 1.76 (m, 2H, J = 6.6 Hz), 1.59–1.49 (m, 2H), 1.43 (m, 2H), 1.28 (m, 10H), 1.23 (m, 3H).

^{13}C NMR ($CDCl_3$); δ 151.73, 144.49, 144.74, 142.76, 122.47, 117.28, 74.43, 49.75, 39.44, 31.58, 30.04, 29.76, 29.43, 29.30, 28.99, 26.42, 23.84, 23.55, 20.78, 12.42, 11.81.

MS[EI$^+$] for $C_{24}H_{37}N_3O_2$; m/z 399 (M^+, 68%), 236 (16%), 205 (12%), 203 (13%), 165 (100%), 121 (11%).

HRMS (EI): calculated for $C_{24}H_{37}N_3O_2$: 399.28858. Found 399.28924.



4.3.5. (*R*)-1-(9-(6-(*tert*-Butyl-dimethylsilyloxy)-2,5,7,8-tetramethylchroman-2- yl)nonyl)-1H-1,2,3-triazole

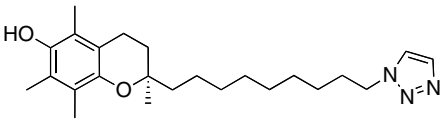
A solution of (*R*)-9-(6-*tert*-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethylchroman-2-yl)nonyl methanesulfonate (110 mg, 0.200 mmol) in dry ACN (2 ml) was added dropwise at room temperature under argon to a suspension of 1H-1,2,3-triazole (27 mg, 0.400 mmol), KOtBu (45 mg, 0.400 mmol), and 18-crown-6-ether (5 mg, 0.020 mmol) in dry ACN (4 ml). The mixture was refluxed (90 °C) under argon atmosphere for 15 h. Upon cooling to room temperature, the resulting white precipitate was filtered off and the remaining organic phase acidified to pH 6 using 10% HCl. The reaction was then diluted with DCM (25 ml), washed several times with H_2O (3 × 20 ml), dried with Na_2SO_4, decanted, and concentrated under vacuum to give a yellow oil. The crude material was purified by column chromatography (DCM to Et_2O) affording the product as a clear, colorless oil (42 mg, 40%) R_f = 0.42 (Et_2O).

^{1}H NMR ($CDCl_3$); δ 7.71 (s, 1H), 7.54 (s, 1H), 4.39 (t, 2H, J = 7.2 Hz), 2.56 (t, 2H, J = 6.7 Hz), 2.11 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 1.92 (m, 2H), 1.79 (m, 2H, J = 6.9 Hz), 1.65–1.49 (m, 2H), 1.45 (m, 2H), 1.32–1.28 (m, 10H), 1.23 (s, 3H), 1.06 (s, 9H), 0.132 (s, 6H).

^{13}C NMR ($CDCl_3$); δ 145.90, 144.06, 133.73, 125.82, 123.52, 123.10, 122.64, 117.50, 74.44, 50.18, 39.55, 31.55, 30.33, 30.07, 29.44, 29.31, 28.97, 26.45, 26.11, 23.82, 23.57, 20.90, 18.60. 14.33, 13.41, 11.96, −3.33.

MS[EI$^+$] for $C_{30}H_{51}N_3O_2Si$; m/z 513 (M^+, 17%), 205 (10%), 129 (10%), 82 (100%).

HRMS (EI): calculated for $C_{30}H_{51}N_3O_2Si$: 513.37506. Found 513.37517.



4.3.6. (*R*)-2-(9-(1H-1,2,3-Triazol-1-yl)nonyl)-2,5,7,8-tetramethylchroman-6-ol, 3

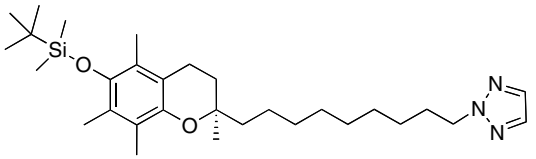
A solution of tetrabutylammonium fluoride (TBAF) (1 M in THF, 250 μL) was added dropwise via syringe to a stirred solution of (*R*)-1-(9-(6-(*tert*-butyl-dimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)nonyl)-1H-1,2,3-triazole (65 mg, 0.126 mmol) in dry THF (5 ml). The mixture was stirred at room temperature for 30 min until starting material was not detected by TLC. The reaction mixture was then quenched with 500 μL of 1 N HCl and diluted with 25 ml of ether, to which an additional 10 ml of H_2O was added. The water phase was extracted again with ether (2 × 25 ml), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was chromatographed on silica gel (Et_2O) to give the pure product (TLC R_f = 0.49, EtOAc) as an off-white/yellow solid, mp 103–106 °C (35 mg, 70%).

^{1}H NMR ($CDCl_3$); δ 7.71 (s, 1H), 7.54 (s, 1 H), 4.55 (s, 1H), 4.39 (t, 2H, J = 7.2 Hz), 2.62 (t, 2H, J = 6.7 Hz), 2.18 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 1.91 (m, 2H), 1.79 (m, 2H, J = 6.9 Hz), 1.65–1.51 (m, 2H), 1.48–1.41 (m, 2H), 1.32–1.28 (m, 10H), 1.24 (s, 3H).

^{13}C NMR ($CDCl_3$); δ 145.55, 144.65, 133.72, 123.12, 122.52, 121.24, 118.71, 117.32, 74.45, 50.20, 39.45, 31.55, 30.33, 30.04, 29.43, 29.29, 28.97, 26.44, 23.82, 23.55, 20.76, 12.29, 11.80, 11.35.

MS[EI$^+$] for $C_{24}H_{37}N_3O_2$ 399.6 (M^+, 68%), 236 (20%) 205 (12%), 203 (21%), 165 (100%), 121 (11%).

HRMS (EI): calculated for $C_{24}H_{37}N_3O_2$: 399.28858. Found 399.28812.



4.3.7. (*R*)-2-(9-(6-(*tert*-Butyl-dimethylsilyloxy)-2,5,7,8-tetramethylchroman-2- yl)nonyl)-2H-1,2,3-triazole

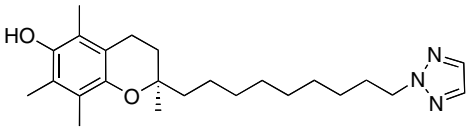
Clear colorless oil. R_f = 0.41 (DCM). Purified by sílica gel column chromatography using 100% DCM to100% Et_2O.

^{1}H NMR ($CDCl_3$); δ 7.60 (s, 2H), 4.46 (t, 2H, J = 7.1 Hz), 2.57 (t, 2H, J = 6.8 Hz), 2.12 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 1.98 (m, 2H, J = 6.7 Hz), 1.74 (m, 2H, J = 7.1 Hz), 1.62–1.52 (m, 2H), 1.50–1.42 (m, 2H), 1.37–1.29 (m, 10H), 1.24 (s, 3H), 1.07 (s, 9H), 0.143 (s, 6H).

^{13}C NMR ($CDCl_3$); δ 145.91, 144.07, 133.79, 125.84, 123.50, 122.67, 117.49, 74.45, 54.88, 39.59, 31.55, 30.11, 29.75, 29.47, 29.35, 29.02, 26.47, 26.13, 23.83, 23.61, 20.91, 18.62, 14.34, 13.41, 11.96, −3.2.

MS[EI$^+$] for $C_{30}H_{51}N_3O_2Si$; m/z 513 (M^+, 83%), 332 (12%), 317 (10%), 279 (20%), 221 (20%), 220, (21%), 168 (44%), 128 (12%).

HRMS (EI): calculated for $C_{30}H_{51}N_3O_2Si$: 513.37506. Found 513.37446.



4.3.8. (*R*)-2-(9-(2H-1,2,3-Triazol-2-yl)nonyl)-2,5,7,8-tetramethylchroman-6-ol, 4

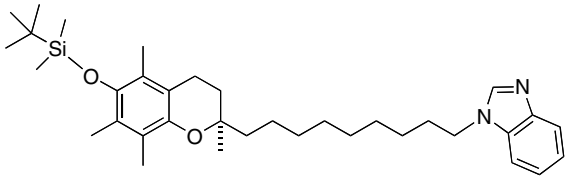
Off-white solid, mp 61–62 °C. R_f = 0.60 (EtOAc) purified by sílica gel column chromatography using 100% Et_2O.

^{1}H NMR ($CDCl_3$); δ 7.61 (s, 2H), 4.45 (t, 2H, J = 7.3 Hz), 4.42 (br s, 1H), 2.62 (t, 2H, J = 6.8 Hz), 2.18 (s, 3H), 2.13 (s, 6H), 2.04–1.94 (m, 2H), 1.88–1.73 (m, 2H), 1.66–1.49 (m, 2H), 1.28 (br s, 10H), 1,24 (s, 3H).

^{13}C NMR ($CDCl_3$); δ 144.58, 133.80, 122.58, 121.18, 118.65, 117.34, 74.47, 54.89, 39.42, 31.56, 30.05, 29.75, 29.43, 29.30, 29.00, 26.45, 23.83, 23.56, 20.77, 12.26, 11.79, 11.32.

MS[EI$^+$] for $C_{24}H_{37}N_3O_2$; m/z 399 (M^+, 36%), 231 (10%), 165 (78%), 122 (20%), 106 (21%), 79 (100%).

HRMS (EI): calculated for $C_{24}H_{37}N_3O_2$: 399.28858. Found 399.28895.



4.3.9. (*R*)-1-(9-(6-(*tert*-Butyl-dimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)nonyl)-1H-benzo[*d*]imidazole

Clear colorless oil. R_f 0.45 (EtOAc). Purified by sílica gel column chromatography using DCM:Et_2O (3:2).

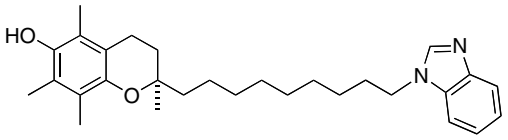
^{1}H NMR ($CDCl_3$); δ 7.91 (s, 1H), 7.85 (m, 1H), 7.44–7.41 (m, 1H), 7.35–7.28 (m, 2H), 4.19 (t, 2H, J = 7.1 Hz), 2.58 (t, 2H, J = 6.5 Hz), 2.14 (s, 3H), 2.11 (s, 3H), 2–10 (s, 3H), 1.94–1.84 (m, 2H), 1.82–1.75 (m, 2H), 1.67–1.51 (m, 2H), 1.53–1.43 (m, 2H), 1.34–1.30 (m, 10H), 1.26 (s, 3H), 1.09 (s, 9H), 0.159 (s, 6H).

^{13}C NMR ($CDCl_3$); δ 145.93, 144.09, 143.90, 142.94, 133.85, 125.84, 123.53, 122.78, 122.67, 122.00, 120.40, 117.52, 109.68,

74.45, 45.10, 39.57, 31.58, 30.10, 29.49, 29.39, 29.12, 26.83, 26.15, 23.86, 23.59, 20.93, 18.63, 14.37, 13.45, 12.00, −3.30.

MS[EI$^+$] for $C_{35}H_{54}N_2O_2Si$; m/z 562 (M^+, 11%), 258 (13%), 230 (17%), 229 (14%), 220 (28%), 205 (100%), 201 (11%), 189 (22%), 187 (19%), 136 (25%), 124 (16%), 122 (20%).

HRMS (EI): calculated for $C_{35}H_{54}N_2O_2Si$: 562.39546. Found 562.39774.



4.3.10. (*R*)-2-(9-(1H-Benzo[*d*]imidazol-1-yl)nonyl)-2,5,7,8-tetramethylchroman-6-ol, 5

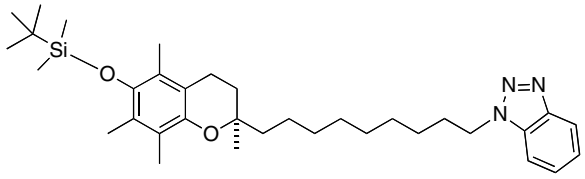
Light yellow oil, R_f = 0.31 (EtOAc). Purified by sílica gel column chromatography using DCM:Et_2O (3:2).

^{1}H NMR ($CDCl_3$); δ 7.90 (s, 1H), 7.84–7.81 (m, 1H), 7.44–7.40 (m, 1H), 7.33–7.29 (m, 2H), 5.15 (br s, 1H), 4.17 (t, 2H, J = 7.1 Hz), 2.62 (t, 2H, J = 6.5 Hz), 2.20 (s, 3H), 2.15, (s, 3H), 2.13 (s, 3H), 1.91–1.85 (m, 2H), 1.81–1.71 (m 2H), 1.66–1.54 (m, 2H), 1.52–1.39 (m, 2H), 1.33–1.28 (m, 10H), 1.24 (s, 3H).

^{13}C NMR ($CDCl_3$); δ 145.48, 144.77, 143.77, 142.89, 133.80, 122.80, 122.48, 122.04, 121.60, 120.36, 119.06, 117.29, 109.68, 74.43, 45.12, 39.42, 31.58, 30.05, 29.81, 29.44, 29.35, 29.09, 26.80, 23.86, 23.56, 20.78, 12.41, 11.81, 11.47.

MS[EI$^+$] for $C_{29}H_{40}N_2O_2$; m/z 448 (M^+, 1%), 437 (1%), 286 (11%), 243 (17%), 229 (25%), 205 (23%), 145 (12%), 131 (35%) 118 (28%), 86 (64%), 84 (100%).

HRMS (EI): calculated for $C_{29}H_{40}N_2O_2$: 448.30898. Found 448.30867.



4.3.11. (*R*)-1-(9-(6-(*tert*-Butyl-dimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)nonyl)-1H-benzo[*d*][1,2,3]triazole

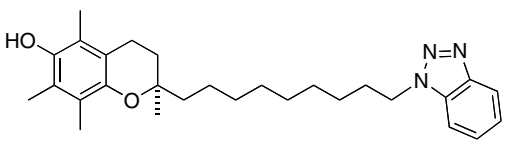
Clear colorless oil. R_f 0.19 (DCM). Purified by silica gel column chromatography using 100% DCM to 100% Et_2O.

^{1}H NMR ($CDCl_3$); δ 8.08 (d, 1H, J = 7.3 Hz), 7.52 (m, 2H), 7.38 (m, 1H), 4.65 (t, 2H, J = 7.1 Hz), 2.57 (t, 2H, J = 6.7 Hz), 2.12 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.03 (m, 2H), 1.92–1.72 (m, 2H), 1.65–1.52 (m, 6H), 1.49–1.36 (br m, 6H), 1.28 (s, 3H), 1.07 (s, 9H), 0.144 (s, 6H).

^{13}C NMR ($CDCl_3$); δ 145.91, 144.08, 132.97, 127.11, 125.82, 123.74, 123.51, 122.65, 120.03, 117.50, 109.34, 74.45, 48.23, 39.57, 31.56, 30.08, 29.70, 29.47, 29.33, 29.04, 26.72, 26.14, 23.84, 23.58, 20.92, 18.62, 14.36, 13.43, 11.97, −3.31.

MS[EI$^+$] for $C_{34}H_{53}N_3O_2Si$; m/z 563 (M^+, 14%), 506 (3%), 259 (6%), 250 (5%), 221 (5%), 205 (4%), 174 (5%), 149 (16%).

HRMS (EI): calculated for $C_{34}H_{53}N_3O_2Si$: 563.39071. Found 563.39020.



4.3.12. (*R*)-2-(9-(1H-benzo[*d*][1,2,3]Triazol-1-yl)nonyl)-2,5,7,8-tetramethylchroman-6-ol, 6

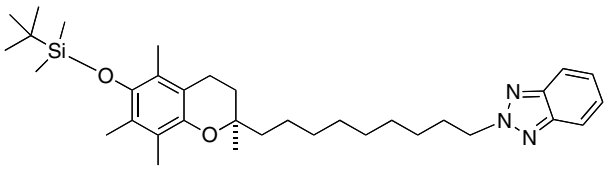
Light yellow oil, TLC R_f = 0.55 (Et_2O). Purified by sílica gel column chromatography using 100% DCM to 100% Et_2O.

^{1}H NMR ($CDCl_3$); δ 8.08 (d, 1H, J = 8.3 Hz), 7.52 (m, 2H), 7.38 (dt, 1H, J_1 = 7.3 Hz, J_2 = 0.9 Hz), 5.31 (s, 1H), 4.65 (t, 2H, J = 7.1 Hz), 2.61 (t, 2H, J = 6.8 Hz), 2.18 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.06–1.97 (m, 2H), 1.86–1.73 (m, 2H, J = 6.8 Hz),1.65–1.51 (m, 2H), 1.47–1.35 (bm, 6H), 1.27 (bs, 6H), 1.24 (s, 3H).

^{13}C NMR ($CDCl_3$); δ 145.51, 144.64, 132.96, 127.13, 123.77, 122.54, 121.22, 120.03, 118.69, 117.33, 108.34, 74.45, 48.25, 39.46, 31.55, 30.04, 29.69, 29.44, 29.30, 29.03, 26.71, 23.81, 23.56, 20.77, 15.27, 12.29, 11.80, 11.35.

MS[EI$^+$] for $C_{28}H_{39}N_3O_2$ m/z 449 (M^+, 41%), 438 (32%), 286 (13%), 205 (12%), 203 (17%), 165 (60%), 146 (13%), 137 (12%), 132 (21%), 120 (28%).

HRMS (EI): calculated for $C_{28}H_{39}N_3O_2$: 449.30423. Found 449.30393.



4.3.13. (*R*)-2-(9-(6-(*tert*-Butyl-dimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)nonyl)-2H-benzo[*d*][1,2,3]triazole

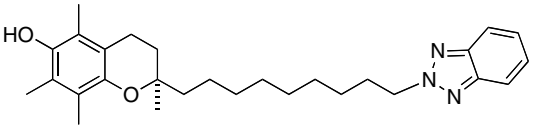
Clear colorless oil. R_f 0.56 (DCM). Purified by sílica gel column chromatography using 100% DCM to100% Et_2O.

^{1}H NMR ($CDCl_3$); δ 7.90 (ddd, 2H, $J_{1(apparent)}$ = 9.6 Hz, $J_{2(apparent)}$ = 3.1 Hz), 7.40 (ddd, 2H, $J_{1(apparent)}$ = 9.6 Hz, J_2 (apparent) = 3.1 Hz), 2.57 (t, 2H, J = 6.7 Hz), 4.75 (t, 2H, J = 7.1 Hz), 2.57 (t, 2H, J = 6.2 Hz), 2.15 (obscured m, 2H, J = 7.2 Hz), 2.13 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 1.92–1.72 (m, 2H, J = 7.2 Hz), 1.66–1.50 (m, 2H), 1.47–1.38 (br m, 6H), 1.30 (br s, 6H), 1.24 (s, 3H).

^{13}C NMR ($CDCl_3$); δ 145.92, 144.30, 126.13, 125.84, 123.50, 122.68, 117.97, 117.49, 74.45, 56.65, 39.59, 31.56, 30.09, 29.48, 29.32, 29.03, 26.57, 26.14, 23.83, 23.60, 20.93, 18.62, 14.35, 13.43, 11.97, −3.31.

MS[EI$^+$] for $C_{34}H_{53}N_3O_2Si$; m/z 563 (M^+, 14%), 428 (13%), 307 (1-%), 243 (11-%), 229 (12%), 205 (30%), 167 (14%), 149 (69%), 132 (10%), 131 (17%), 120 (22%).

HRMS (EI): calculated for $C_{34}H_{53}N_3O_2Si$: 563.39071. Found 563.38909.



4.3.14. (*R*)-2-(9-(2H-benzo[*d*][1,2,3]triazol-2-yl)nonyl)-2,5,7,8-tetramethylchroman-6-ol, 7

Light yellow oil, R_f = 0.26 (DCM). Purified by sílica gel column chromatography using 100% DCM to 100% Et_2O.

^{1}H NMR ($CDCl_3$); δ 7.90 (ddd, 2H, $J_{1(apparent)}$ = 9.6 Hz, $J_{2(apparent)}$ = 3.1 Hz), 7.40 (ddd, 2H, $J_{1(apparent)}$ = 9.6 Hz, $J_{2(apparent)}$ = 3.1 Hz), 4.74 (t, 3H, J = 7.1 Hz), 2.62 (t, 2H, J = 6.7 Hz), ~2.14 (obscured m, 2H), 2.18 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 1.87–1.71 (m, 2H, J = 6.8 Hz), 1.67 (s, 1H), 1.65–1.53 (m, 2H), 1.50–1.35 (br m, 6H), 1.28 (br m, 6H), 1.24 (s, 3H) ^{13}C NMR ($CDCl_3$); δ 145.55, 144.56, 144.27, 126.16, 122.59, 121.14, 118.61, 117.94, 117.35, 74.47, 56.65, 39.41, 31.56, 30.07, 30.02, 29.43, 29.26, 29.01, 26.53, 23.83, 23.55, 20.76, 12.26, 11.79, 11.32.

MS[EI$^+$] for $C_{28}H_{39}N_3O_2$; m/z 449 (M^+, 46%), 399 (13%), 165 (100%), 120 (16%).

HRMS (EI): calculated for $C_{28}H_{39}N_3O_2$: 449.30423. Found 449.30397.

4.4. Biological assessment

4.4.1. Cell culture

Effect of **1**–**7** on metabolism of γ-tocopherol in HepG2/C3A hepatoblastoma cultures was assessed as previously described.[56] Briefly, compounds **1**–**7** and γ-tocopherol stock solutions were prepared in ethanol. Confluent monolayers of HepG2/C3A cells (a human hepatoblastoma cell line) were pre-incubated with varying concentrations of **1**–**7** (or ethanol) for 4 h, followed by addition of media containing γ-tocopherol (25 μM final concentration in media), a good substrate of tocopherol hydroxylase, and replacement of the test compound (or ethanol). After 48 h, culture media were collected and analyzed for ω-oxidation products of γ-tocopherol (the 3′- and 5′-carboxychromanols) by GC–MS, using d_9-3′-α-carboxychromanol (α-CEHC) as internal standard.[8] In a separate design, confluent monolayers were incubated with 1 μM compound **1** for 6 h, then for 24 h with media containing 25 μM γ-tocopherol, but without replacing compound **1**.

In order to assess the effect of **1** on CYP3A4 activity, confluent HepG2 cultures were pre-incubated 4 hr with inhibitor (or ethanol control), followed by addition of 50 μM testosterone. After 48 h incubation, 6-hydroxytestosterone (as the trimethylsilyl ether) was determined in culture media by GC–MS after extraction with ethyl acetate. Data are presented as percent inhibition (means and standard deviation).

4.4.2. Microsomal assays

Insect cell microsomes expressing only recombinant human CYP3A4 (BD-Gentest, Woburn, MA), 20 pmol P450, were incubated 60 min with 200 μM testosterone (50% methanol stock) in the presence or absence of 4 μM **1** and 1 mM NADPH, 0.1 mM potassium phosphate buffer, pH 7.4, 0.25% bovine serum albumin (total reaction volume 0.5 ml). Following the incubation, the reaction mixture was deproteinated with 1.0 ml cold ethanol, extracted with hexane/methyl *tert*-butyl ether (7:1) and analyzed for 6-OH-testosterone and membrane cholesterol as described above. Data are expressed as nmol product per nmol membrane cholesterol.

4.4.3. Animal studies

Two groups of three male FVB/N mice were fed a commercial (Prolab 1000, Agway, Syracuse, NY) ground chow feed enriched with δ-tocopherol (6000 mg per kg diet), with or without 500 mg per kg **1**. Compound **1** (in ethanol) and δ-tocopherol were premixed in Enova diglyceride oil (ADM Lao LLC, Decatur, IL), which was then added dropwise to the ground chow while mixing. After 10 days on diet, mice were fasted for 5 h, euthanized, and tocopherol concentrations determined in plasma, liver and bile, using GC–MS and d_9-α-tocopherol as internal standard.[8] Liver samples (50 mg) were extracted using hexane–isopropanol (3:2), and plasma and bile aliquots were extracted with hexane/methyl *tert*-butyl ether after protein precipitation with ethanol. All animal procedures were approved by the Institutional Animal Care and Use Committee of Cornell University.

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