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Biologically Active Heteroarotinoids Exhibiting Anticancer Activity and Decreased Toxicity

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A series of retinoids, containing heteroatoms in a cyclic ring and called heteroarotinoids, were synthesized, and their biological activity was evaluated using tissue culture lines that have measurable responses to *trans*-retinoic acid (*t*-RA). Transglutaminase (TGase) was assessed in the human erythroleukemia cell line (GMO6141A) as an indicator of differentiation and apoptosis. Proliferation was evaluated in a human cervical cell line, CC-1, which exhibits dose-dependent alterations in growth rate in response to treatment with *trans*-retinoic acid. Activation of nuclear retinoic acid receptors was determined in a reporter cell line established from CC-1. The reporter line, called CC-B, contains a reporter gene controlled by a retinoic acid responsive element (RARE) and a thymidine kinase (tk) promoter. Treatment of the CC-B line with the heteroarotinoids resulted in a dose-responsive and retinoid-dependent regulation of reporter gene expression. The heteroarotinoids exhibited activity in all assays and correlated in a statistically significant manner between assays. RARE transactivation activity in CC-B cells correlated with induction of TGase in GMO6141A ($R = 0.96$) and with a decrease in the growth rate of CC-1 cells ($R = -0.90$). The ability of the selected heteroarotinoids to induce differentation, inhibit proliferation, and activate nuclear receptors demonstrates the chemotherapeutic potential of these agents. In view of the biological activity cited, an *in vivo* toxicity study was conducted on male B6D2F1 mice with three heteroarotinoids, namely **8** [(2*E*,4*E*,6*E*)-3,7-dimethyl-7-(1,2,3,4-tetrahydro-4,4-dimethylthiochroman-6-yl)-2,4,6-heptatrienoic acid], **10** [(2*E*,4*E*,6*E*)-3,7-dimethyl-7-(1,2,3,4-tetrahydro-4,4-dimethylchroman-6-yl)-2,4,6-heptatrienoic acid], and **13** [(*E*)-*p*-[2-(4,4-dimethylchroman-6-yl)propenyl]benzoic acid]. The mice were used with gavage of heteroarotinoids in corn oil [0.1, 0.2, 0.4, or 0.8 mg/kg] and with 0.01 or 0.05 mg/kg of TTNPB (**5**) [(*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid] as reference controls. The target organs affected in the mice by the three heteroarotinoids were those typically associated with *t*-RA (**1**) toxicity. The maximum tolerated dose (MTD) of **13** was 9.4 mg/kg/day, which was equal in toxicity to that of *t*-RA (**1**) and 1000-fold less toxic than TTNPB (**5**). The MTDs of **8** and **10** were 34 and 32 mg/kg/day, respectively, which is 3-fold less toxic than *t*-RA (**1**) and 3000-fold less toxic than TTNPB (**5**). The 3000-fold reduced toxicity, compared with only a 27% reduction biological activity of **8** and **10** with respect to that of TTNPB, observed in our assays indicates a good therapeutic ratio of these heteroarotinoids over the parent compound. The biological activity and reduced toxicity of these heteroarotinoids demonstrate the potential efficacy as anticancer agents.

Introduction

Investigations of the biology and chemistry of retinoids have become highly intensified with the recognition that *trans*-retinoic acid (*t*-RA, **1**)[1–3] and 13-*cis*-retinoic acid (13-*cis*-RA, **2**)[4–7] possess anticancer activity. Potential use of these agents in chemoprevention has been demonstrated by the reversal of the premalignant lesions, oral leukoplakia in patients treated with 13-*cis*-RA, for example.[7] In head and neck cancer, 13-*cis*-RA (**2**) caused decreased incidence of second primary tumors in patients who were free of the disease following surgery or radiation therapy.[8,9] Both *t*-RA (**1**) and 13-*cis*-RA (**2**) have clearly demonstrated potential as chemotherapeutic agents in the treatment of a variety of cancers.[10] The most successful clinical effectivenenss has been observed in the treatment of acute promyelocytic leukemia (APL).[11–15] A combination of 13-*cis*-RA (**2**) with interferon α-2a had demonstrated activity in patients with squamous cell carcinomas of the skin and cervix.[5] It is known that *t*-RA (**1**) is converted *in vitro*[16] to 9-*cis*-retinoic acid (9-*cis*-RA, **3**), which also has anticancer activity.[17]

The clinical effectiveness of *t*-RA (**1**) is limited by significant toxicity.[10] Considerable effort has been

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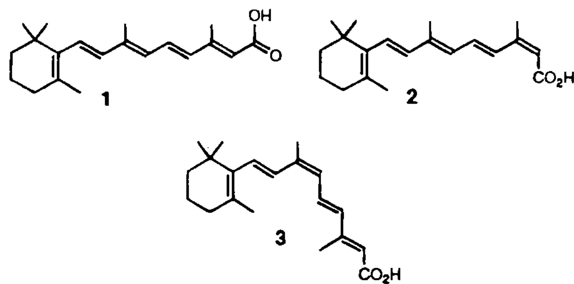
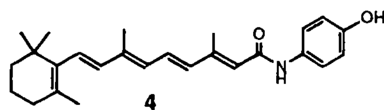
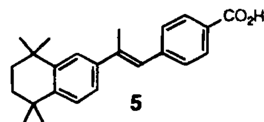
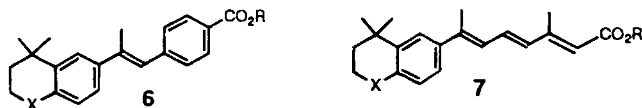


expended to develop synthetic mimics of **1** which will exhibit similar biological effects as **1** but with less toxicity. Fenretinide (**4**) is a synthetic retinoid that possesses few side effects and is in clinical trials as a chemopreventive agent for oral leukoplakia, breast, bladder, and prostate cancer.[18]







Some arotinoids, defined as retinoids with at least one aromatic ring (as **5**-TTNPB), exhibited anticancer activity.[19] However, **5** was extremely toxic. Heteroarotinoids have been defined as systems with one aromatic ring and at least one heteroatom[20] in the partially saturated ring (as retinoids **6** and **7**) with X = O, S, NR, etc. A few reported heteroarotinoids have anticancer activity.[20,21] We have prepared a series of heteroarotinoids **8**−**21** (Table 1) and have evaluated their activity and the toxicity of three active agents.

Retinoid regulation of gene expression leads to several biological activities with anticancer effects.[22−31] For example, retinoid induction of the transglutaminase (TGase) gene is associated with induction of differentiation and apoptosis.[10,32−39] TGases are a group of Ca^{2+}-dependent enzymes that catalyze the post-translational modifications of proteins by introducing ϵ-(γ-glutamyl)-lysine isopeptide bonds.[40,41] Both RAR- and RXR-selective retinoids induce expression of the TGase gene, implicating the RAR/RXR heterodimer in this activity.[42−44]

The biological activities of the compounds herein were tested in several *in vitro* assays to evaluate regulation of gene expression through RARE's, tumor cell growth, and TGase expression. Three active agents (**8**, **10**, and **13**) were selected for toxicity studies in mice. Interestingly, heteroarotinoid **14** displayed better ability than *t*-RA (**1**) to reverse cornification in vaginal smears of vitamin A-deficient, ovariectomized rats while **10** was slightly less effective.[45] This further supports our hypothesis that heteroarotinoids can possess useful anticancer activity.

Table 1. Relative Transcriptional Activation Activities of **8**−**21** Relative to *t*-RA[57]

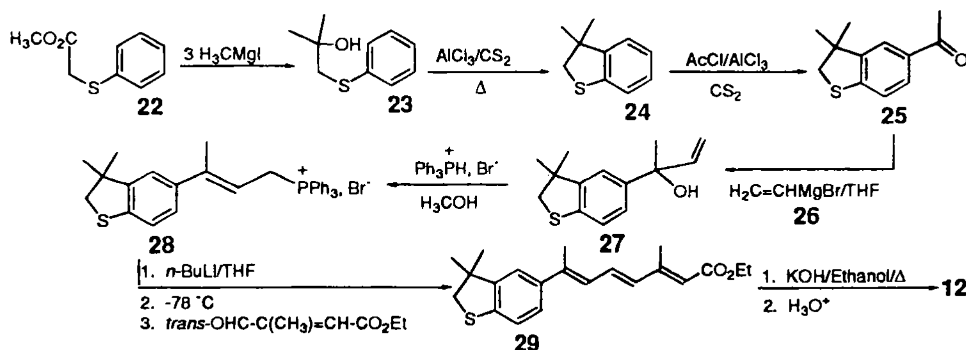
compound	activity	compound	activity
11	56	16	43
19	54	18	40
8	52	14	35.7
15	51	17	35.8
10	50	20	33
12	50	13	31
9	46	21	29

Results and Discussion

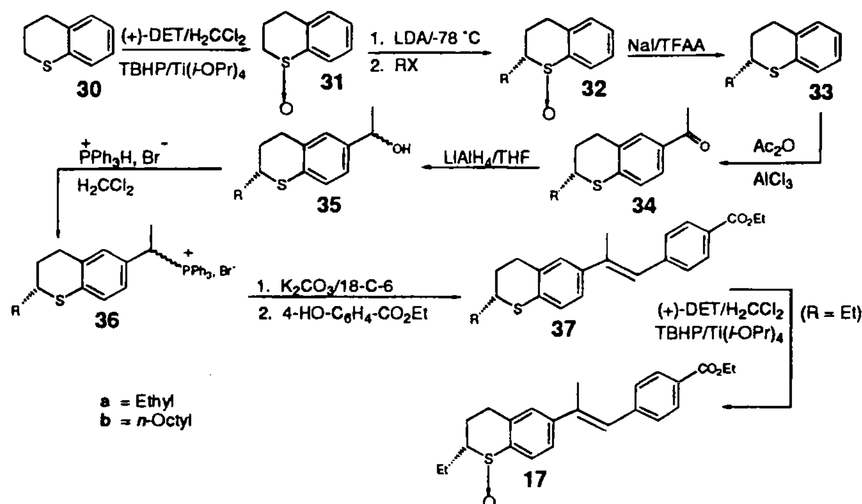
Chemistry. Arotinoids are a class of retinoids found to modulate cellular differentiation and proliferation in types of cells responsive to *t*-RA (**1**).[10] A few retinoidal amides[46] have been diagnosed as being much more active than *t*-RA (**1**) in terms of differentiation-inducing activity on human promyelocytic leukemia cell line HL-60. Conceivably, this may result from the increased ability of amides to rotate and conform to both RAR and RXR receptors. In the present work, the target compounds were **8**−**21** (Table 1) where special cases **18**−**21** have an internal ester linkage which has a lower energy barrier to C−O bond rotation than the amides and will allow greater flexibility and possibly a better fit with the receptor. As a model system, the barrier to rotation of the C−O bond in the ester methyl acetate has been found to be 10−15 kcal/mol while the simple amide counterpart, namely acetamide, has a barrier near 23 kcal/mol.[47]

Selection of the heteroarotinoids **8**−**21** was done on the following basis. Systems **8**−**12** allow a determination of the effect of the heteroatom and type of side group on receptor specificity. Acids **9** and **11**, as well as **8** and **10**, differ only in the heteroatom while **11** and **12** differ in the nature of the side group. Acid **13** and esters **14** and **15** permit an assessment of receptor activity by acid versus ester while esters **14** and **15** differ in heteroatoms. Systems **16** and **17** are void of a geminal dimethyl group, but differences in activity due to the size of the lipophilic group adjacent to the heteroatom can be assessed. Heteroarotinoids **18**−**21** have flexible connecting ester linkages which may enable the system to better "fit" the receptor. Esters **18** and **19** differ only in the heteroatom, while **18** and **20** have different terminal groups. Inclusion of the thiosemicarbazone **20** resulted, in part, from prior knowledge that certain thiosemicarbazones exhibited good anticancer activity.[48] The connecting linkage in the novel **21** is highly hindered and reversed from that in **18**−**20**. Consequently, C−O rotation in **21** may be somewhat restricted, and thus the receptor binding

Scheme 1



Scheme 2



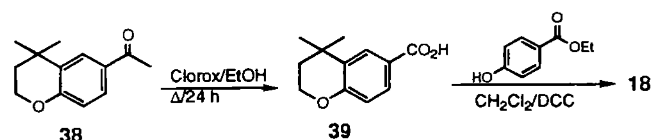
capability might be altered in either a positive or negative fashion.

The syntheses of **9**–**12** were developed as follows. Acids **9** and **10** have been reported[49] as have **11** and certain relatives.[50,51] However, unknown acid **12** was prepared from **22** via the reaction sequence of **22** → **23** → **24** → **25** → **26** → **27** → **28** → **29** → **12** as shown (Scheme 1). The yields for each step were average to very good and were highly dependent upon conditions.

Acid **13** and esters **14** and **15** are known.[20,21,50] Esters **16** and **17**, which have no geminal dimethyl group, were synthesized starting from **30** via the reaction sequence as illustrated in Scheme 2, namely **30** → **31** → **32** → **33** → **34** → **35** → **36** → **37** → **17**. The intermediates and yields with respect to each step for R = ethyl and *n*-octyl, respectively, were **31** (89%), **32a/32b** (80%; 40%), **33a/33b** (98%; 98%), **34a/34b** (72%, qt), **35a/35b** (86%; 80%), **36a/36b** (96%; 98%), **37/16** (91%; 79%), and **17** (64%). Chiral **31** has been reported, but few properties were recorded.[52,53] α-Alkylation to the chiral sulfoxide center gave, after chromatography, light oils for **32a/32b** (R = ethyl and *n*-octyl, respectively). A modified Sharpless procedure[54] for the oxidation of **37** afforded a good conversion to **17**.

Experience with simple, related model systems strongly inferred that removal of the oxygen from **32a/32b** was critical for later steps in the synthesis. Near quantitative yields of both the ethyl and *n*-octyl derivatives **33a/33b** were realized when **32a/32b** were treated with NaI/trifluoroacetic anhydride in acetone. Acetylation of **33a/33b** to give **34a/34b** was smooth with acetic anhydride in preference to acetyl chloride. Reduction of the

Scheme 3

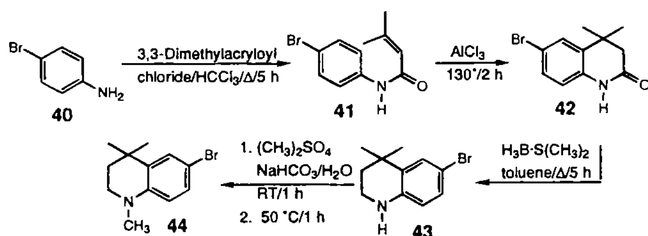


carbonyl group in **34a/34b** to yield **35a/35b** was also facile under somewhat standard conditions with $LiAlH_4$. Acid-catalyzed phosphorylation as shown gave the corresponding phosphonium salt **36a/36b** in high yields in both examples. All intermediates **31**–**35** were utilized as quickly as possible after preparation as some deterioration occurred upon standing. Such degradation was minimized by storage in a freezer. Salts **36a/36b**, although apparently stable, were also used at once and subjected to the conditions outlined to generate the esters **37/16** (R = ethyl and *n*-octyl). These esters were also best stored in the dark and in a freezer. The ethyl analog **37** (R = ethyl) was oxidized under our modified Sharpless conditions to chiral sulfoxide **17**, which is the first chiral heteroarotinoid reported.

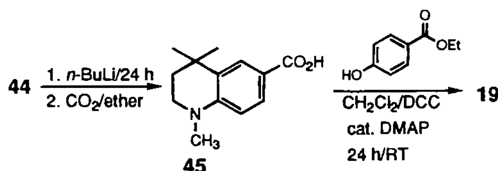
Flexible heteroarotinoids **18**–**21** required special methods for each case. Synthon **38**, in an ethanolic solution, was oxidatively cleaved[55] with Clorox to the corresponding carboxylic acid **39** (Scheme 3).[51,55] Esterification with ethyl 4-hydroxybenzoate, DCC, and a catalytic amount of DMAP gave diester **18** (83%).

The preparation of the nitrogen analog **19** was initially attempted via a Michael type addition with 4-bromoaniline (**40**, Scheme 4) and ethyl acrylate but was unsuccessful, possibly due to the electron-with-

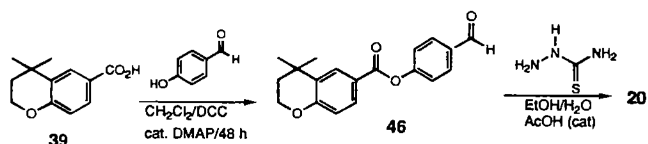
Scheme 4



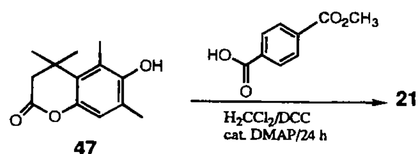
Scheme 5



Scheme 6



Scheme 7



drawing nature of the bromine substituent. *N*-Acylation was achieved, however, with **40** and 3,3-dimethylacryloyl chloride in chloroform at reflux and led to **41** (87%) which required careful recrystallization to avoid polymerization.

Cyclization of **41** to the lactam **42** required an unusually high temperature. Amide **41** was carefully melted, and the temperature was raised to 130 °C at which time solid, anhydrous $AlCl_3$ was added portionwise over a 2-h period in the absence of solvent. Recrystallization of the crude product gave **42** (65%). Reduction of the carbonyl group in **42** was accomplished with $H_3B{:}S(CH_3)_2$ in boiling toluene with a high conversion to **43** (90%). *N*-Methylation of **43** led to derivative **44** (86%) uder the conditions shown.

Transmetalation of **44** occurred with *n*-BuLi in ether, and the salt was subsequently carbonated with solid CO_2 in ether to give the carboxylic acid **45** (30%) (Scheme 5). Efforts to increase the yield of **45** included the addition of TMEDA during transmetalation, but **19** was not obtained. Esterification of **45** gave **19** (43%).

The synthesis of thiosemicarbazone **20** (Scheme 6) required the preparation of aldehyde **46** (43%), which was obtained using the previously cited carboxylic acid **39** and 4-hydroxybenzaldehyde as illustrated. Condensation of aldehyde **46** with thiosemicarbazide easily afforded thiosemicarbazone **20** (83%).

The coumarol **47**[56] was esterified (Scheme 7) in the manner similar to that used to obtain **18** and **19**. The two methyl groups on the benzene ring in **21** serve to decrease the flexibility of the ester functional group by hindering the rotation of the C−O bond. Because the steric effect was expectedly large in **21**, the rotational

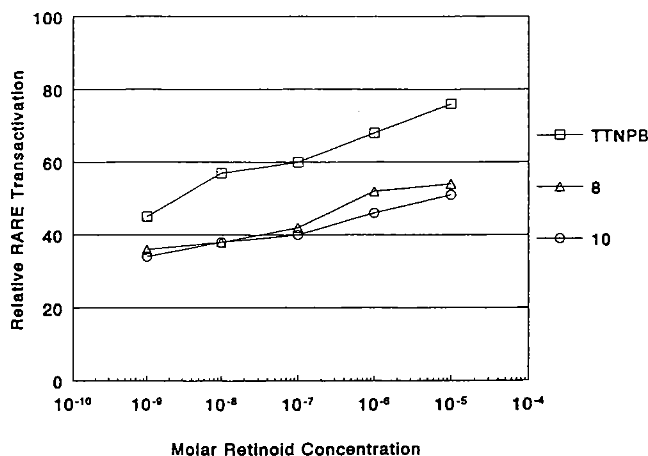


Figure 1. Dose−response transactivation of an RARE. The CC-B cell line was treated with a range of concentrations of the retinoids indicated. After 48 h of treatment, the CAT activity was quantitated, and RARE transactivation was derived as the ratio of activities in treated over control cultures. The results are presented as transactivation relative to that observed in CC-B cultures treated with *t*-RA.

barrier (ΔG^*) was determined, via a variable temperature NMR analysis, to be between 15−16 kcal/mol at 26 °C (see the Experimental Section). This barrier is sufficient to markedly restrict rotation around the C−O ester bond but not large enough to prevent such rotation at room temperature.

Biology. An *in vitro* model system which has demonstrated a linear dose-dependent response to retinoids was chosen to evaluate the biological activities of the heteroarotinoids.[57] This model uses a cervical carcinoma cell line (CC-B) which contains integrated copies of a reporter plasmid that is regulated by the retinoic acid receptor element (RARE) from the RARβ gene. The specific RARE has been shown to be regulated by the RAR/RXR heterodimer and RXR/RXR homodimer in the presence of *t*-RA.[25] Treatment of CC-B with retinoids or heteroarotinoids results in dose-responsive increased expression of the reporter gene, chloramphenical acetyl transferase (CAT). Figure 1 illustrates a dose−response increase in CAT activity in the CC-B in response to treatment with a range of concentrations of **8** and **10**. Since **8** and **10** were approximately 73−77% as active as TTNPB (which also induced a dose−response effect), the approach is an appropriate method to assess and compare the biological activities.

The dose of 10^{-6} M was chosen for screening the other heteroarotinoids since this concentration exhibited the greatest differential in RARE transactivation by individual retinoids and is pharmacologically relevant. All agents tested did induce RARE transactivation (Table 1) in this model within the range 29−56% of that of *t*-RA (**1**). Thioacid **11** exhibited the highest activity at 56% of the *t*-RA (**1**) control. In general, compounds containing a sulfur heteroatom exhibited greater activities than their oxygen-containing counterparts (compare **11** versus **9** and **15** versus **14** and **18** as well as **8** versus **10**). Systems with an aryl group in the side chain displayed greater activity when associated with a sulfur heteroatom (compare **11** versus **8** and **12**), but the reverse situation seems to persist with the oxygen analogs (compare **10** versus **9, 13,** and **14**). Interestingly, the systems with a five-membered ring exhibited greater activity than the six-membered compounds for both

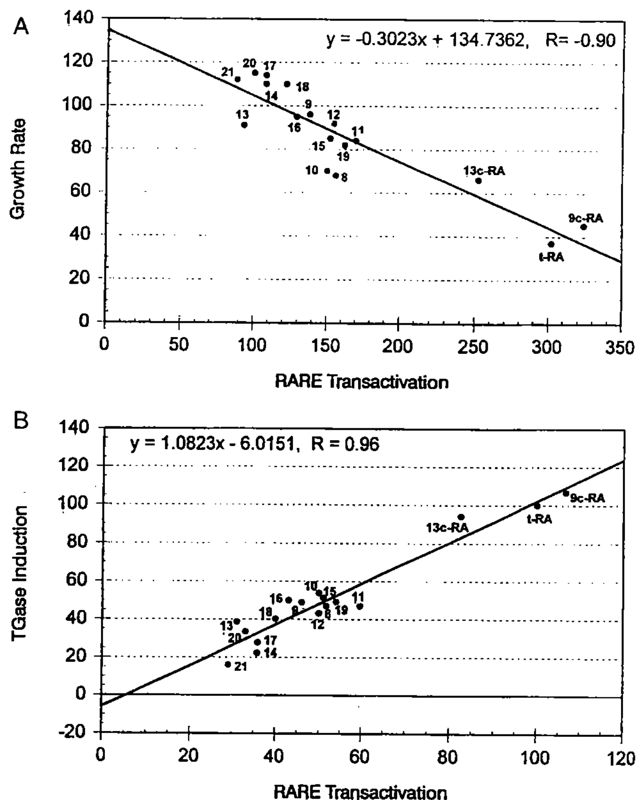


Figure 2. Correlation of RARE transactivation with biological activity. The degree of RARE transactivation is plotted against the growth rate (panel A) and the induction of TGase activity (panel B). In panel A, all values represent the percent increase in activity above basal levels induced by retinoid and heteroarotinoid treatment. In panel B, the results are presented as values relative to *t*-RA (**1**). Linear regression analysis (Harvard Graphics, version 3.0 software) was used to draw the lines and to determine the correlation coefficients.

oxygen- and sulfur-containing analogs (compare **9** with **13** and **14** and compare **11** with **15**). It is quite important to note that sulfur-containing heteroarotinoids have demonstrated greater activity over the oxygen counterparts both in the ornithine decarboxylase culture (ODC) assay[51] in mice as well as in the tracheal organ culture (TOC) assay in hamsters.[20]

One property of retinoids which shows promise for anticancer activity[58-68] is the inhibition of tumor cell growth.[1,10] Generally, the effects of retinoids on such growth is dependent upon the nature of the retinoid and the cells involved. Both growth stimulation and growth inhibition have been reported in the same cell line depending upon the concentration of retinoid.[57-61] The CC-B cell line exhibited statistically significant growth stimulation when treated with 1×10^{-9} M *cis*-9-RA but marked growth inhibition at 10^{-6}–10^{-5} M *cis*-9-RA.[57] At 10^{-6} M used for screening purposes, both stimulation and inhibition of growth were induced by heteroarotinoids. The degree of growth effects correlated linearly ($R = -0.90$) with the degree of activity observed in the transactivation assay (Figure 2, panel A). In general, agents which increased growth rate were poor RARE transactivators and those which decreased growth rate were better RARE transactivators.

The importance of concentration and type of retinoid utilized in determining growth effects is further illustrated in Figure 3. At concentrations of 10^{-6} M and higher, 9-*cis*-RA was a more effective growth inhibitor than agent **15**, but at lower concentrations, the reverse

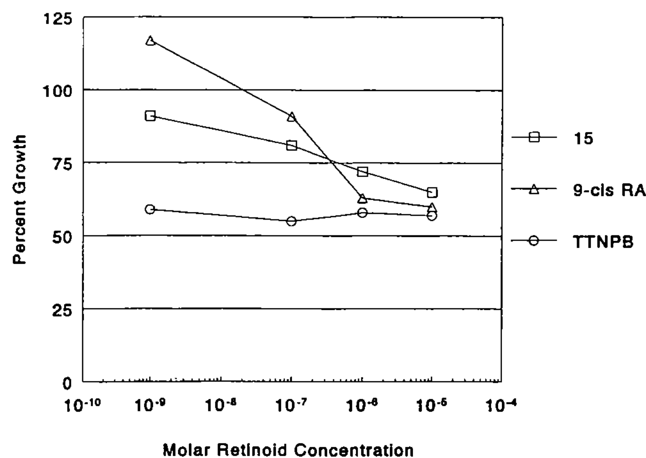


Figure 3. Dose-dependent regulation of CC-B growth. The percentage of cells counted in cultures treated with the indicated retinoids for 7 days in relation to the number of cells in the control cultures is plotted against the concentration of agent utilized.

was true. The control TTNPB was the most effective at all concentrations. Given the complexity of interaction of the retinoid receptor dimers and the various RAREs found in the genome, it is likely that the RARE used in this study provides only a small indication of the total effects induced by these heteroarotinoids. Therefore, the reduced activities of the heteroarotinoids in comparison to that of the natural retinoids is not necessarily undesirable if the end result is increased specificity. The specificity is under investigation.

Although there is a linear correlation between RARE transactivation and growth inhibition in the cell line described herein, other activities of these retinoids, such as the anti-AP1 effects, may well contribute to growth. Agents **8**, **10**, and **13** displayed higher growth inhibitory activity then expected from the corresponding transactiviation activity (Figure 2, panel A).

The other anticancer activity of the heteroarotinoids screened in this study involved induction of TGase activity in HEL cells.[10,32-34,61,62] All of the agents evaluated induced TGase activity in the range of 16–52% relative to that of *t*-RA. A linear correlation ($R = 0.96$) between the degree of TGase inducation in HEL cells and the degree of RARE transactivation in CC-B was observed (Figure 1, panel B).

In summary, this study demonstrates for the first time that certain heteroarotinoids transactivate the RARE from the RARβ gene through endogenous receptors. This transactivation correlates in a statistically signficant manner with TGase induction in human erythroleukemia cells and with growth inhibition in a cervical carcinoma cell line. These assays clearly show strong correlations between the profiles of heteroarotinoid activities. This is the first systematic study involving only heteroarotinoids in such assays and demonstrates that potentially useful chemotherapeutic properties are present in such retinoids.

The uses of retinoids as preventive and therapeutic agents for the treatment of various forms of cancer have been delineated.[10,48,58-68] However, toxicity remains a serious problems with many retinoids, including synthetics. In view of the above activity as well as the ODC and TOC activity of **8**,[49] **10**,[49] and **13**,[20] a second major objective was to utilize rats to determine the maximum tolerated doses (MTDs), to identify target organs for

toxicity, and to define toxicity relative to *t*-RA (**1**) and TTNPB (**5**). In addition, an examination of agents **8**, **10**, and **13** allows an assessment of both the influence of structural variation and the nature of the heteroatom on toxicity in such heteroarotinoids. Such information could be quite instructive in terms of improved agent design and potential anticarcinogenic efficacy. Table 2 summarizes the toxicological effects observed for each retinoid in this study. Overt toxicity, as evidenced by rapid onset of clinical signs of hypervitaminosis A syndrome (Table 3) with high incidence of mortality, was observed in the 0.1, 0.2, 0.4, and 0.8 mg/kg TTNPB groups. Incidence and latency to mortality was related to dose. Fracture incidence and average number of fractures per mouse decreased with increasing dose. This was attributed to death from overt toxicity prior to development of fractures. Three mortalities occurred in the 0.05 mg/kg TTNPB group, with deaths from days 14 to 22. Fracture incidence was 7/8 in this group, with clinical signs of factures (avoiding pressure on limbs) beginning day 11 (Table 3). Fracture incidence was 1/7 for the 0.01 mg/kg TTNPB group. Clinical signs of vitamin A skin toxicity (alopecia and/or skin scaling; Table 3) were present at about day 7 in the 0.05 mg/kg group and day 14 in the 0.01 mg/kg group. Final body weights in the 0.01 and 0.05 mg/kg groups were significantly reduced from vehicle 2 values at termination on day 25. Body weight reductions were dose-related with 10% body weight reduction form vehicle 2 values at approximately day 8 in the 0.05 mg/kg TTNPB group.

Significant toxicity in the **13** groups did *not* occur until the increases in dosages on day 30. Mortalities occurred in the mid-dose 2 (4, days 57 to 63) and high-dose (8, days 43 to 62) groups. Except for the high-dose group, in which high mortality may have precluded the development of fractures, fracture incidence, average number of fractures per rat, and onset of fractures, as evidenced by the clinical sign of avoiding pressure on limbs, was related to dose. Clinical signs of skin toxicity were present at high incidence in all groups. Final body weights were significantly reduced from vehicle 1 values of 8.8, 16.5, and 27.9%, respectively, in the low-dose, mid-dose 1, and mid-dose 2 groups. Onset of 10% body weight reduction from vehicle 1 values was dose-related, with days of onset of 51, 41, and 36 in the mid-dose 1, mid-dose 2, and high-dose groups, respectively. There was no 10% body weight reduction from vehicle 1 values in the low-dose group.

Significant toxicity was not observable in the **8** and **10** groups until the increases in dosage on day 51. The toxicity was limited to the mid-dose 2 of **10** and high-dose of **8** and **10** groups. There was only one mortality, which was on day 57, in the mid-dose 1 of the **10** group. A high incidence of fractures was noted in the mid-dose 2 and high-dose of **10** and high-dose of **8** groups, with onset of fractures on day 62 in the high-dose of the **10** group. Since there were no clinical signs of fractures in the mid-dose 2 of the **10** and high-dose of the **8** groups, one can conclude that onset of bone toxicity occurred relatively near study termination on day 65. Clinical signs of skin toxicity were present predominately in the high dose of the **10** group. Final body weights were significantly reduced from vehicle 1 values of 9.6, 17.4, and 9.6%, respectively, in the mid-dose 2 and high-dose of **10**, and high-dose of **8** groups. Onset of 10% body weight reduction from vehicle 1 values were days 61, 53, and 54 in the mid-dose 2, the high-dose of **10**, and the high-dose of **8** groups, respectively. No body weight reductions of ≤10% from vehicle 1 values occurred in the remaining **10** and **8** groups.

Table 4 summarizes the significant ($p \leq 0.05$ relative to vehicle) effects of the three compounds, as revealed by hematology and clinical chemistry. On day 8 in the 0.1, 0.2, 0.4, and 0.8 mg/kg TTNPB groups, large increases in numbers of circulating white and red blood cell counts, hematocrit, and hemoglobin and large decreases in serum triglycerides and cholesterol were observed. These changes reflected the overt toxicity in these groups. The only significant clinical pathologic effect in the 0.01 mg/kg TTNPB group was a 3-fold increase in circulating white blood cells at study termination (day 25–6.58 ± 4.24 10^3/mm versus 2.10 ± 0.47 for vehicle 2). Large decreases in serum triglycerides and large increases in circulating white blood cells in the 0.05 mg/kg TTNPB group at study termination were attributed to overt toxicity.

With the **8** and **10** compounds, elevations in serum triglyceride were the primary alterations as detected by clinical pathology. Effects were evident as early as day 8 and were seen in the low-dose and mid-dose groups. Effects tended to be restricted to the first portion of the study. Elevations, relative to vehicle 1, ranged from 35 to 116%. For **13**, significant effects on serum triglycerides were limited to day 29 in the mid-dose 1 and high dose groups. Elevations were 82 and 100% higher relative to vehicle 1, respectively.

For all three compounds, elevations in serum alkaline phosphatase occurred at day 29 and/or later in all dose groups and in a dose-related manner. Elevations ranged from 14 to 143% above vehicle 1 values.

Elevations in circulating white blood cell counts occurred on day 8 in the low-dose 2 and high-dose of **10** and **8** groups (196, 45, 143, and 175% higher, respectively). For **13**, increased circulating white blood cells occurred on day 8 in the mid-dose 2 group (76% higher relative to vehicle 1) and in a dose-related fashion at study termination (day 65, no significant elevation in the high-dose group). Elevations were 180, 259, and 330% in the low- and mid-dose 1 and 2 groups, respectively.

Organ weight effects of each retinoid have been presented in Table 5. Testes-to-body weight ratios were reduced, 24–55% from vehicle 1 values, in all dose levels of the three compounds. Significant changes in organ-to-body weight ratios relative to vehicle were also observed for thymus in the low-dose of **8** group (+28.1%), the mid-dose 2 of **13** group (−30.2%), and the 0.05 mg/kg TTNPB group (−41.3%), adrenal in the 0.05 mg/kg TTNPB group (+56.8%) and spleen in the mid-dose 2 and high-dose of **10** groups (+36.4 and 48.6%, respectively), low and mid-dose 1 and 2 of **8** groups (+82.4, +151.1, and +256.5%, respectively), and 0.01 and 0.05 mg/kg TTNPB groups (+124.6 and +335.7%, respectively).

Table 6 summarizes the gross observations of pathologic efforts at necropsy for each retinoid. Enlarged lymph nodes and spleens were observed in high incidence in all **13** and TTNPB groups. (Enlarged spleens were not observed in the 0.2, 0.4 and 0.8 mg/kg TTNPB

Table 2. Summary of Toxicological Parameters in the Toxicity Study on **8**, **10**, and **13** in Male B6D2F1 Mice

dose group	total dose (mg/kg) to termination[a]	mortalities[b] (day of death)	final body weights, g, mean ± SD $N = 8$ (% control)	fracture incidence average no. fractures/rat	approximate day to 10% weight loss	total dose of 10% weight loss (mg·kg)	calculated 30-day maximally tolerated dose (mg/kg) based on 10% weight loss[c]	target organs[d]
vehicle 1		0 (−)	25.97 ± 0.94 (−)	0/8, 0				
TTNPB (**5**)								
0.1 mg/kg/day	*e*	7 (12−24)	*e*	5.7, 2.0	*f*	*f*	*f*	skin, bone, spleen, lymph nodes
0.2 mg/kg/day	*e*	6 (8−19)	*e*	3/8, 1/3	*f*	*f*	*f*	skin, bone, lymph nodes
0.4 mg/kg/day	*e*	14 (8−19)	*e*	3/12, 0.4	*f*		*f*	skin, bone, lymph nodes
0.8 mg/kg/day	*e*	16 (7−8)	*e*	0/16, 0	*f*	*f*	*f*	skin, bone lymph nodes
8								
low-dose	207.1	0 (−)	25.49 ± 2.42[g] (98.2)	0/8, 0	>65	>207.1	>6.9	skin, lymph nodes, thymus, testes
mid-dose 1	414.2	1 (57)	24.94 ± 1.39[g] (96.0)	0/8, 0	>65	>414.2	>13.8	skin, lymph nodes, testes
mid-dose 2	828.4	0 (−)	25.66 ± 1.40[g] (98.9)	0/8, 0	>65	>828.4	>27.6	skin, lymph nodes, testes
high-dose	3106.5	0 (−)	23.47 ± 1.26[g] (90.4)	2/8, 0.3	54	1016.8	33.9	skin, bone, lymph nodes, testes
10								
low-dose	207.1	0 (−)	24.47 ± 1.66[g] (94.2)	0/8, 0	>65	>207.1	>6.9	lymph nodes, testes
mid-dose 1	414.2	0 (−)	24.53 ± 2.21[g] (94.5)	0/8, 0	>65	>414.2	>13.8	skin, lymph nodes, testes
mid-dose 2	828.4	0 (−)	23.49 ± 1.04[g] 90.4)	4/8, 0.6	61	732.4	24.4	skin, bone, spleen, lymph nodes, testes
high-dose	3106.5	0 (−)	21.46 ± 2.51[g] (82.6)	3/8, 1.0	53	952.8	31.8	skin, bone, spleen, lymph nodes, testes
13								
low-dose	147.1	0 (−)	23.69 ± 1.94[g] (91.2)	2/8, 0.4	>65	147.1	>4.9	skin, bone, spleen, lymph nodes, testes
mid-dose 1	294.2	0 (−)	21.68 + 3.01[g] (83.5)	5/9, 1.9	51	190.2	6.3	skin, bone, spleen, lymph nodes, testes
mid-dose 2	588.4	4 (37−63)	18.73 + 1.64[g] (72.1)	6/7, 3.4	41	220.6	7.4	skin, bone, spleen, lymph nodes, testes
high-dose	1176.8	8 (43−62)	*b*	4/7, 1.3	36	280.8	9.4	skin, bone, spleen, lymph nodes
vehicle 2		0 (−)	24.5 ± 1.80 (100)	0.8, 0				
TTNPB (**5**)								
0.01 mg/kg/day	0.25	0 (−)	22.13 ± 2.04[g] (92.0)	1/7, 0.1	>25	>0.25	>0.008	skin, bone, spleen, lymph nodes
0.05 mg/kg/day	1.25	3 (14−22)	15.96 ± 0.53[g] (66.4)	7/8, 3.3	8	0.4	0.001	skin, bone spleen, lymph nodes thymus, adrenal

[a] Day 65 or day 25. [b] Animals found dead or sacrificed moribund. [c] Total dose to 10% weight loss divided by 30 days. [d] Based on clinical signs, X-rays, gross observation at necropsy, and organ weight determinations. [e] No survivors at termination. [f] Unable to determine due to overt toxicity. [g] $p \leq 0.5$ relative appropriate vehicle control.

Table 3. Clinical Signs of Hypervitaminosis in the Toxicity Study of **8**, **10**, and **13** in Male B6D2F1 Mice

group	dose	skin scaling[a]	alopecia[b]	avoiding pressure on limbs
vehicle 1		0/16 (−)[c]	0/16 (−)	0/16 (−)
TTNPB (**5**)				
	0.1 mg/kg/day	14/16 (8)	10/16 (8)	0/16 (−)
	0.2 mg/kg/day	14/16 (6)	10/16 (8)	0/16 (−)
	0.4 mg/kg/day	15/16 (6)	8/16 (6)	0/16 (−)
	0.8 mg/kg/day	12/16 (6)	3/16 (6)	0/16 (−)
8	low-dose	0/16 (−)	1/16 (38)	0/16 (−)
	mid-dose 1	1/16 (41)	1/16 (10)	0/16 (−)
	mid-dose 2	0/16 (−)	1/16 (19)	0/16 (−)
	High Dose	0/16 (−)	1/16 (45)	0/16 (−)
10	low-dose	0/16 (−)	0/16 (−)	0/16 (−)
	mid-dose 1	0/16 (−)	2/16 (38)	0/16 (−)
	mid-dose 2	0/16 (−)	3/16 (41)	0/16 (−)
	high-dose	2/16 (57)	4/16 (36)	4/16 (62)
13	low-dose	7/16 (61)	6/16 (26)	1/16 (65)
	mid-dose 1	6/16 (57)	6/16 (43)	2/16 (62)
	mid-dose 2	7/16 (38)	7/16 (45)	6/16 (45)
	high-dose	6/16 (36)	4/16 (41)	4/16 (36)
vehicle 2				
TTNPB (**5**)	0.01 mg/kg/day	4/8 (14)	6/8 (16)	0/8 (−)
	0.05 mg/kg/day	8/8 (9)	8/8 (7)	7/8 (11)

[a] Sites of skin scaling were ears, mouth, nose, eyelids, feet, tail, and/or ventral body. [b] Sites of alopecia were face, ventral body, and/or forlimbs. [c] Number of animals in group with clinical sign/total animals in group (day of first observation).

groups.) No enlarged spleens were observed in the **8** group. There were only low incidences of enlarged lymph nodes.

Calculations of 30-day maximally tolerated dose, based upon total dose to 10% weight loss, show that the three compounds have a favorable toxicologic profile relative to the reference retinoids. Maximally tolerated doses (Table 2) were approximately 34 mg/kg for **8**, 24−32 mg/kg for **10**, 6−9 mg/kg for **13**, and 0.01 mg/kg for TTNPB. In male B6D2F1 mice, the 30-day maximally tolerated dose of 10% weight loss for *t*-RA is approximately 10 mg/kg.[69−71] With this relative measure of toxicity, **10** and **8** are approximately *3-fold less toxic*, on a mg/kg basis, than *t*-RA (**1**) and *3000-fold less toxic than TTNPB (5)*. In contrast, **13** is approximately equal in toxicity to *t*-RA (**1**) and 630−940-fold less toxic than TTNPB (**5**). This reduced toxicity of **13** is significant compared to other arotinoids[59,69] and suggests that insertion of a heteroatom into the cyclohexenyl ring of arotinoids will result in significantly reduced toxicity. Thus, removal of the dimethyl group at the 4-position from the cyclohexenyl ring in **5** results in reduced toxicity of **13** relative to TTNPB (**5**).[69] This lowered toxicity does not appear to be due solely to reduced absorption *in vivo* since elevated triglycerides were observed as early as day 8 to 0.1 mg/kg for the **8** and **10** agents, and increased circulating white blood cell counts were observed at day 8 at 0.4 mg/kg for **13**. Within the three compounds, **13** can be ranked as 3−4-fold more toxic than **8** and **10**, with **10** being more toxic than **8** based upon the observance of fractures in the mid-dose 2 group and skin scaling in the high-dose group.

These data suggest that target organs for the three compounds are those typically found for retinoids.[10,62] In the absence of the hypervitaminosis A syndrome, target organ effects of retinoids are in skin (epithelial hyperplasia, hyperkeratoses and subacute inflammation), spleen (lymphoid hyperplasia, hematopoietic cell

Table 4. Summary of Selected[a] Clinical Pathology Effects in the Toxicity Study of **8**, **10**, and **13** in Male B6D2F1 Mice

parameter	low-dose	mid-dose 1	mid-dose 2	high-dose
8				
triglycerides	8, 70.9 ± 10.9, 97.5 ± 20.1 (+38) 29, 96.5 ± 27.5, 176.4 ± 67.6 (+38) 36, 138.8 ± 36.8, 194.9 ± 40.0 (+40)	8, 70.9 ± 10.9, 97.6 ± 31.8 (+38) 29, 96.5 ± 27.5, 174.1 ± 53.3 (+80) 36, 138.8 ± 36.8, 212.7 ± 49.2 (+53)	29, 96.5 ± 27.5, 153.4 ± 49.6 (+59)	29, 96.5 ± 27.5, 164.2 ± 47.5 (+70)
alkaline phosphatase	29, 82.9 ± 7.4, 94.9 ± 7.6 (+14) 65, 94.2 ± 8.0, 127.5 ± 10.4 (+35)	65, 94.2 ± 8.0, 135.8 ± 13.2 (+44)	36, 63.1 ± 6.3, 75.6 ± 16.4 (+20) 65, 94.2 ± 8.0, 136.8 ± 12.0 (+45)	29, 82.9 ± 7.4, 94.6 ± 11.9 (+14) 36, 63.1 ± 6.3, 84.6 ± 11.5 (+34) 65, 94.2 ± 8.0, 159.0 ± 15.4 (+69)
white blood cells				65, 1.52 ± 0.49, 4.18 ± 2.19 (+175)
10				
triglycerides	8, 70.9 ± 10.9, 96.8 ±40.0 (+36)[b] 29, 96.5 ± 27.5, 179.4 ± 40.8 (+86) 36, 138.8 ± 36.8, 187.1 ± 46.4 (+35)	8,70.9 ± 10.9, 97.2 ± 16.8 (+37) 29, 96.5 ± .5, 167.3 ± 34.5 (+73) 36, 138.8 ± 36.8, 202.8 ± 51.9 (+46)	8, 70.9 ± 10.9, 112.3 ± 34.2 (+58) 29, 96.5 ± 27.5, 159.3 ± 33.9 (+65) 36, 138.8 ± 36.8, 195.9 + 32.8 (+41)	29, 96.5 ± 27.5, 208.2 ± 85.8 (+116)
alkaline phosphatase	29, 82.9 ± 7.4, 96.8 ± 24.4 (+17) 65, 94.2 ± 8.0, 115.7 ± 9.5 (+23)	65, 94.2 ± 8.0, 127.1 ± 8.3 (+35)	65, 94.2 ± 8.0, 136.8 ± 16.5 (+45)	36, 63.1 ± 6.3, 91.9 ± 17.7 (+46) 65, 94.2 ± 8.0, 156.8 ± 15.8 (+66)
white blood cells	8, 2.99 ± 1.25, 4.84 ± 1.29 (+62)		65, 1.52 ± 0.49, 4.51 ± 2.72 (+196)	65, 1.52 ± 0.49, 3.70 ± 1.47 (+143)
13				
triglycerides		29, 96.5 ± 27.5, 175.9 ± 77.4 (+82)		29, 96.5 ± 27.5, 192.0 ± 88.3 (+100)
alkaline phosphatase	36, 63.1 ± 6.3, 77.4 ± 12.5 (+23) 65, 94.2 ± 8.0, 115.0 ± 16.5 (+22)	36, 63.1 ± 6.3, 81.5 ± 5.9 (+29) 65, 94.2 ± 8.0, 123.1 ± 17.5 (+31)	29, 82.9 ± 7.4, 106.3 ± 7.6 (+28) 36, 63.1 ± 6.3, 93.3 ± 18.0 (+48) 65, 94.2 ± 8.0, 134.8 ± 20.0 (+43)	29, 82.9 ± 7.4, 103.1 ± 17.2 (+24) 36, 63.1 ± 6.3, 86.3 ± 12.6 (+38)
white blood cells	65, 1.52 ± 0.49, 4.27 ± 1.31 (+180)	65, 1.52 ± 0.49, 5.46 ± 2.10 (+259)	8, 2.99 ± 1.25, 5.26 ± 2.68 65, 1.52 ± 0.49, 6.55 ± 1.77 (+330)	

[a] $p \leq 0.05$ relative to corn oil vehicle. [b] Day of effect, vehicle mean ± SD, group mean ± SD (% relative to vehicle).

Table 5. Effects of Retinoids on Organ-to-Body Weight Ratios in the Toxicity Study on **8**, **10**, and **13** in Male b6d2fl Mice

group	dose	thymus (ratio × 1000)	right testicle (ratio × 1000)	spleen (ratio × 1000)	adrenal (ratio × 1000)
		day 65	day 65	day 65	day 65
vehicle 1		1.3 ± 0.1 (−)[a]	4.2 ± 0.3 (−)	2.0 ± 0.3 (−)	0.4 ± 0.2 (−)
TTNPB (**5**)	0.1 mg/kg/day	*b*	*b*	*b*	*b*
	0.2 mg/kg/day	*b*	*b*	*b*	*b*
	0.4 mg/kg/day	*b*	*b*	*b*	*b*
	0.8 mg/kg/day	*b*	*b*	*b*	*b*
8	low-dose	1.7 ± 0.2 (+28.1)	2.6 ± 0.8 (−37.8)[c]	2.1 ± 0.6 (+3.3)	0.3 ± 0.1 (−34.5)
	mid-dose 1	1.5 ± 0.3 (+14.9)	3.1 ± 0.3 (−26.5)[c]	2.0 ± 0.3 (+1.0)	0.3 ± 0.1 (−13.1)
	mid-dose 2	1.6 ± 0.2 (+16.7)	2.8 ± 0.2 (−32.7)[c]	2.2 ± 0.5 (+8.0)	0.3 ± 0.2 (−23.6)
	high-dose	1.5 ± 0.3 (+13.1)	2.7 ± 0.3 (−37.1)[c]	2.6 ± 0.3 (−37.1)[c]	2.6 ± 0.4 (−28.0)
10	low-dose	1.6 ± 0.3 (+17.1)	3.2 ± 0.6 (−24.4)[c]	2.3 ± 0.5 (+14.2)	0.3 ± 0.1 (−34.8)
	mid-dose 1	1.6 ± 0.3 (+20.8)	2.9 ± 0.6 (−32.5)[c]	2.5 ± 0.6 (+26.5)	0.3 ± 0.2 (−31.4)
	mid-dose 2	1.5 ± 0.5 (+13.7)	3.3 ± 0.3 (−22.3)[c]	2.7 ± 0.2 (+36.4)[c]	0.3 ± 0.1 (−15.1)
	high-dose	1.3 ± 0.4 (−0.1)	3.0 ± 0.5 (−28.9)[c]	3.0 ± 0.4 (+48.6)[c]	0.4 ± 0.2 (−7.6)
13	low-dose	1.5 ± 0.3 (14.8)	1.9 ± 0.1 (−55.1)[c]	3.6 ± 0.9 (+82.4)[c]	0.3 ± 0.1 (−14.9)
	mid-dose 1	1.3 ± 0.4 (−6.7)	2.0 ± 0.2 (−53.5)[c]	5.0 ± 1.4 (+151.1)[c]	0.4 ± 0.2 (−3.1)
	mid-dose 2	0.9 ± 0.5 (−30.2)[c]	1.9 ± 0.1 (−54.8)[c]	7.1 ± 0.7 (+256.5)[c]	0.3 ± 0.1 (−30.5)
	high-dose	*b*	*b*	*b*	*b*
		day 25	day 25	day 25	day 25
vehicle 2		1.7 ± 0.2 (−)	4.0 ± 0.7 (−)	2.2 ± 0.5 (−)	0.3 ± 0.1 (−)
TTNPB (**5**)					
	0.01 mg/kg/day	1.9 ± 0.3 (+11.8)	3.9 ± 0.5 (−1.2)	4.9 ± 1.2 (+124.6)[c]	0.3 ± 0.0 (−9.6)
	0.05 mg/kg/day	1.0 ± 0.4 (−41.3)[c]	4.0 ± 0.3 (+1.5)	9.5 ± 1.7 (+335.7)[c]	0.4 ± 0.1 (+56.8)[c]

[a] Mean ± SD for 4 to 8 animals (% difference from vehicle). [b] All animals dead by day 65. [c] *P* less than 0.05 relative to appropriate vehicle group.

Table 6. Gross Pathological Observations Consistent with Hypervitaminosis A in the Toxicity Study of **8**, **10**, and **13** in the Male B6D2F1 Mice

group	dose	enlarged spleen	lymph nodes enlarged[b]
vehicle 1		0/16[a]	0/16
TTNPB (**5**)	0.1 mg/kg/day	4/16	16/16
	0.2 mg/kg/day	0/16	16/16
	0.4 mg/kg/day	0/16	12/16
	0.8 mg/kg/day	0/16	8/16
8	low-dose	0/16	1/16
	mid-dose 1	0/16	0/16
	mid-dose 2	0/16	2/16
	high-dose	0/16	1/16
10	low-dose	0/16	1/16
	mid-dose 1	1/16	2/16
	mid-dose 2	0/16	1/16
	high-dose	0/16	1/16
13	low-dose	3/16	6/16
	mid-dose 1	5/16	6/16
	mid-dose 2	6/16	8/16
	high-dose	4/16	8/16
vehicle 2			
TTNPB (**5**)	0.01 mg/kg/day	5/8	6/8
	0.05 mg/kg/day	5/8	8/8

[a] Number of animals in group with gross observations/total number of animals in group. [b] Mesenteric, mandibular, inguinal, iliac, renal, and/or axillary.

proliferation), thymus (cortical epithelial necrosis), adrenal (hypertrophy of zona fasciculata), liver (periportal cytoplasmic vacuolization, hematopoietic cell proliferation), forestomach (squamous epithelial hyperplasia, subacute inflammation), bone (osteodystrophy), and lymph nodes (lymphoid hyperplasia).[62,69]

Some structure–activity relationships among retinoids are suggested from these studies. Agents **8** and **10** appear to have high selectivity for elevation of serum triglycerides, and **13** has selectivity for elevation of serum alkaline phosphatase. Organ weight data suggest higher activity for adrenal effects for TTNPB (**5**) and testicular effects for all three compounds. The high incidence of enlarged lymph nodes and spleens, higher spleen weights, and increases in circulating white blood cell count suggest higher selectivity for retinoid immunologic effects with TTNPB (**5**) and **13**. It should be stated, however, that organ weight determinations and gross observations of pathologic effects require histopathologic confirmation.

In summary, the heteroarotinoids cited have very favorable toxicologic profiles relative to the reference retinoids, *t*-RA (**1**) and TTNPB (**5**). The MTD of **13** was 9.4 mg/kg/day which is equal in toxicity to *t*-RA (**1**)[70,71] and 1000-fold less than TTNPB (**5**) (0.001 mg/kg/day). The MTD values for **8** and **10** are 34 and 32 mg/kg/day, respectively, which is 3-fold less toxic than *t*-RA (**1**) and 3000-fold less toxic than TTNPB (**5**), suggesting the presence of the polyene side chain may be more favorable than the aryl group. This 3000-fold reduced toxicity, compared with only 27% reduction in biological activity of **8** and **10** in relation to that of TTNPB observed in our assays, indicates an improved therapeutic ration of the heteroarotinoids over the parent. In view of the important receptor binding capabilities and growth-promoting characteristics of heteroarotinoids **10** and **14**,[48] these systems deserve additional study. The therapeutic ultility of these compounds awaits data from *in vivo* efficacy and pharmacokinetic studies.

Experimental Section

Chemical Methods. General. IR spectra were recorded on a Perkin-Elmer 2000 FT-IR as films or as KBr pellets. Mass spectral data (EI and FAB) were obtained from an HP GC-MS ENGINE, Model 5989B, using 3-nitrobenzyl alcohol as the matrix. All ^{1}H and ^{13}C NMR spectra were taken on a Varian XL-400 BB NMR spectrometer operating at 399.99 and 100 Hz, respectively, and signals were referenced to TMS. Melting points were determined with a Thomas-Hoover melting point apparatus and were uncorrected. Syntheses were executed, unless otherwise indicated, under N_2 and with the aid of a magnetic stir bar. Acids **8**,[49] **9**,[49] **10**,[49] **11**,[51] and **13**[20] as well as esters **14**,[20] **15**,[20] and **22**[72] were prepared as reported. Intermediates were used at once, but intermediates and products could be safely stored in a dark freezer for several weeks without appreciable decay.

(2*E*,4*E*,6*E*)-7-(2,3-Dihydro-3,3-dimethylbenzo[*b*]thien-5-yl)-3-methyl-2,4,6-octatrienoic Acid (12) with Intermediate 29. A solution of *n*-butyllithium (1.6 M, 3.4 mL, 5.4 mmol) in hexane was added to a stirred solution of **28** (3.00 g, 5.35 mmol) in dry ether (35 mL). After stirring (dark) at room temperature (15 min), the Wittig reagent was cooled to −78 °C, and a solution of ethyl (*E*)-OHCC(CH_3)=CHCO$_2$Et (0.76 g, 5.35 mmol) in dry ether (10 mL) was added dropwise. The mixture was allowed to warm to room temperature and then was stirred (45 h). Hexane was added, and the mixture was filtered. The filtered solid was rinsed with ether which was added to the hexane solution. Chromatography on silica gel (hexanes:ether, 20:1) yielded slightly crude ester **29** as a yellow oil (0.67 g, 36.3%). This light-sensitive ester **29** (0.665 g, 1.94 mmol) was treated (dark) with 35% aqueous KOH (2.0 mL) in absolute ethanol (8 mL) with stirring and at reflux (1 h). After being cooled to room temperature, the solution was diluted with ethyl acetate and water, followed by quenching with 50% aqueous acetic acid. Extracts (EtOAc) of the solution were dried (Na_2SO_4), filtered, and evaporated to a yellow solid. Recrystallization (absolute ethanol), followed by filtration, washing (chilled ethanol, then hexanes), and drying gave free acid **12** as yellow flakes (0.424 g, 25% from **28**): mp 209.5–210.7 °C; IR (KBr) 1683 (C=O of CO_2H) cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.40 [s, 6 H, C(CH_3)$_2$], 2.24 [s, 3 H, H(11)], 2.39 [s, 3 H, H(16)], 3.20 [s, 2 H, H(2)], 5.85 [bs, 1 H, H(17)], 6.41 [d, J = 14.9 Hz, 1 H, H(14)], 6.56 [d, J = 11.3 Hz, 1 H, H(12)], 7.06 [dd, J = 14.9 Hz, J = 11.2 Hz, 1 H, H(13)], 7.13–7.28 [m, 3 H, H(4,6,7)]; ^{13}C NMR ($DCCl_3$) δ 14.1 [C(16)], 16.5 [C(11)], 27.4 [C(8,9)], 47.2 [C(3)], 47.5 [C(2)], 117.5 [C(17)], 119.9 [C(4)], 122.2 [C(7)], 125.2 [C(6)], 125.6 [C(12)], 132.1 [C(7a) and C(13)], 135.4 [C(14)], 139.0 [C(5)], 140.7 [C(10)], 148.2 [C(3a)], 155.2 [C(15)], 171.2 [C(18)]. Anal. ($C_{19}H_{22}O_2S$) C, H.

Ethyl (*E*)-4-[2-(3,4-Dihydro-2-*n*-octyl-2*H*-1-benzothiopyran-6-yl)-1-propenyl]benzoate (16, R = *n*-octyl). To a boiling mixture of phosphonium salt **36b** (2.0 g, 3 mmol), K_2CO_3 (0.4 g, 3 mmol), and 18-C-6 (30 mg) in H_2CCl_2 (15 mL) was added ethyl 4-formylbenzoate (0.5 g, 3 mmol) in H_2CCl_2 (10 mL) in a single portion. The mixture was boiled (12 h) and was then concentrated to an orange oil which was treated with hexane (150 mL). A suspension formed and was filtered. The filtrate was washed with brine, dried (Na_2SO_4), and concentrated to a yellow oil which was separated on a silica gel column (eluent, hexane:ethyl acetate, 1:1) to give sulfide **16** (or **37b**) (1.1 g, 79%) as a white solid: mp 72–73 °C; IR (neat) 1750 cm^{-1} (C=O); ^{1}H NMR ($DCCl_3$) δ 0.95 [t, J = 7.5 Hz, 3 H, (CH_2)$_7$CH_3], 1.1–1.7 [m, 17 H, (CH_2)$_7$CH$_3$ and OCH$_2$CH_3], 2.8–2.95 [m, 1 H, H(3)], 2.15–2.3 [m, 4 H, H(3) and H(10)], 2.8–2.9 [m, 2 H, H(4)], 3.1–3.2 [m, 1 H, H(2)], 4.3 [q, J = 7.5 Hz, 2 H, OCH_2CH$_3$], 6.8 [s, 1 H, H(11)], 7.0–8.1 [m, 7 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 14.05 [(CH_2)$_3$CH$_3$]; aliphatic-C: 14.31, 17.46, 22.61, 25.55, 27.08, 29.26, 29.54, 29.63, 31.87, 36.83, 37.83, 42.31; 60.79 [C(19)]; ArC and vinylic C: 124.03, 125.63, 126.34, 127.39, 128.93, 129.36, 132.08, 148.01, 149.10, 149.32, 149.8; 168.82 [C(18)]; mass spectral (EI) data calcd for $C_{29}H_{38}O_2S$ m/z ($M^{\bullet+}$) 450.2593, found 450.2595. Anal. ($C_{29}H_{38}O_2S$) C, H.

Ethyl (*E*)-4-[2-(3,4-Dihydro-2-ethyl-2*H*-1-benzothiopyran-6-yl)-1-propenyl]benzoate (17). To a stirred mixture of Ti(O-*i*-Pr)$_4$ (1.44 g, 5 mmol) and (+)-diethyl L-tartrate (2.0 g, 10 mmol) in H_2CCl_2 (50 mL) was introduced water (88 μL syringe) in a single portion. The mixture was stirred to a homogeneous solution. To this solution was added sulfide **37a** (1.78 g, 5 mmol, R = ethyl) in H_2CCl_2 (15 mL). To this cooled (−20 °C) mixture was added *tert*-butyl hydroperoxide (TBHP, 0.5 g, 5.5 mmol) in H_2CCl_2 (1.6 mL). Stirring was continued (4 h) at −20 °C, and water (50 mL) was then added over 10 min. Stirring was continued at −20 °C (1 h) and at room temperature (1 h). A white gel formed and was filtered off, and the filtrate was dried (Na_2SO_4) and evaporated to yield sulfoxide **17** as a light yellow solid. Recrystallization ($HCCl_3$ and then ethanol) gave **17** as a white solid (1.2 g, 64%): mp 88–89 °C; ^{1}H NMR ($DCCl_3$) δ 0.85 [t, J = 7.3 Hz, 3 H, CH$_2$CH_3], 1.05 [t, J = 7.4 Hz, 3 H, H(20)], 1.2–1.4 [m, 1 H, H(3)], 1.5–1.7 [m, 2 H, CH_2CH$_3$], 1.8–1.9 [s, 3 H, H(10)], 2.2–2.3 [m, 1 H, H(3)], 2.5–2.8 [m, 4 H, H(2) and H(4)], 4.1 [q, J = 7.4 Hz, 2 H, H(19)], 6.8 [s, 1 H, H(11)], 7.25–8.05 [m, 7 H, Ar-H]; ^{13}C NMR ($DCCl_3$) δ 10.8 [CH$_2$CH$_3$]; aliphatic-C: 14.14, 17.39, 20.51, 21.48, 26.57, 59.53, 60.70; Ar-C and vinylic-C: 124.76, 126.89, 128.01, 128.40, 128.80, 129.06, 129.25, 135.43, 138.08, 138.35, 142.09, 145.83, 166.11 [C=O]; at 26 °C $[\alpha]_D$ = +39.60° (acetone); mass spectral (EI) data calcd for $C_{23}H_{26}O_3S$ m/z (M^+) 382.1602, found 382.1600. Anal. ($C_{23}H_{26}O_3S$) C, H, S.

Ethyl 4-[[(4,4-Dimethyl-3,4-dihydro-2*H*-benzo[*b*]pyran-6-yl)carbonyl]oxy]benzoate (18). Carboxylic acid **39** (0.500 g, 2.45 mmol) and ethyl 4-hydroxybenzoate (0.610 g, 3.67 mmol) in CH_2Cl_2 (30 mL) were mixed. To this mixture was added dicyclohexylcarbodiimide (~1.52 g, 7.35 mmol) and a catalytic amount of DMAP (20 mg). The resulting cloudy solution was stirred at room temperature (24 h) and then filtered. The solvent was evaporated *in vacuo*, and the heavy oil was subjected to chromatography (CH_2Cl_2:MeOH, 200:1). To the resulting oil was added 2 mL of a solution of hexane: EtOAc (4:1). The solution was cooled at 0–4 °C for 15 h, during which time crystallization took place. The solid **18** was filtered (mp 108–110 °C): 0.721 g (83%); IR (KBr) 1730 (C=O) cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.39 [s, 6 H, C(CH_3)$_2$], 1.43 [t, 3 H, J = 7.2 Hz, CH$_3$], 1.88 [t, 2 H, J = 6 Hz, CCH_2], 4.29 [t, 2 H, J = 6 Hz, CH_2O], 4.39 [q, 2 H, J = 7.2, OCH$_2$], 6.87 [d, 1 H, Ar-H], 7.30 [m, 2 H, Ar-H], 7.90 [dd, 2 H, Ar-H], 8.10 [d, 1 H, Ar-H]; ^{13}C NMR ($DCCl_3$) δ 14.29 [CH$_3$], 30.60 [C(CH_3)$_2$], 36.89 [ArCCH_2], 61.02 [OCH$_2$], 63.51 [ArOCH_2], 117.32–158.66 [Ar-C], 164.64 [C=O], 165.98 [C=O]. Anal. ($C_{21}H_{22}O_5$) C, H.

Ethyl 4-(*N*,4,4-Trimethyl-1,2,3,4-tetrahydroquinolinyl)benzoate (19). To a mixture of the carboxylic acid **45** (0.174 g, 0.794 mmol) and ethyl 4-hydroxybenzoate (0.198 g, 1.08 mmol) in CH_2Cl_2 (6 mL) was added dicyclohexylcarbodiimide (~0.025 g, 7.9 mmol) and a catalytic amount of DMAP (7 mg). The resulting cloudy solution was stirred at room temperature (48 h) and then filtered. The solvent was evaporated *in vacuo*, and the product was chromatographed ($HCCl_3$:MeOH, 50:1) to give the solid **19** (0.105 g, 45%): mp 116–118 °C; IR (KBr) 1720 (C=O) cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.32 [s, 6 H, C(CH_3)$_2$], 1.40 [t, 3 H, J = 7.2 Hz, CH_3], 1.76 [t, 2 H, J = 6 Hz, CCH_2], 3.03 [s, 3 H, NCH$_3$], 3.39 [t, 2 H, J = 6 Hz, CH$_2$N), 4.38 [q, 2 H, J = 7.2 Hz, NCH$_2$], 6.56 [d, 1 H, Ar-H], 7.27 [d, 2 H, Ar-H], 7.90 [dd, 2 H, Ar-H], 7.98 [d, 1 H, Ar-H], 8.10 [dd, 1 H, Ar-H]; ^{13}C NMR ($DCCl_3$) δ 14.62 [CH$_3$], 30.28 [C(CH$_3$)$_2$], 36.42 [ArCCH$_2$], 39.31 [NCH$_3$], 47.79 [NCH$_2$], 61.27 [ArNCH$_2$], 109.87–155.60 [Ar-C], 165.53 [C=O], 166.43 [C=O]. Anal. ($C_{22}H_{25}NO_4$) C, H, N.

4-[[(4,4-Dimethyl-3,4-dihydro-2*H*-benzo[*b*]pyran-6-yl)-carbonyl]oxy]benzaldehyde Thiosemicarbazone (20). Thiosemicarbazide (0.017 g, 0.184 mmol) was dissolved in H_2O (1 mL) and AcOH (1 drop). Aldehyde **46** (0.050 g, 0.161 mmol) dissolved in hot EtOH (3 mL) was added hot to the thiosemicarbazide solution. Crystals formed after 10 s, and the mixture was allowed to stand at room temperature (8 h) and then was cooled (12 h). The crystals were filtered and dried to give **20** (0.50 g, 81%): mp 185–186 °C; IR (KBr) 3436, 3403, 3266, 3164 (N-H) cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.33 [s, 6 H, C(CH_3)$_2$], 1.82 [t, 2 H, J = 5.6 Hz, CCH$_2$], 4.26 [t, 2 H, J = 5.6 Hz, CH$_2$O], 6.90 [d, 1 H, Ar-H], 7.31 [d, 2 H, Ar-H], 7.82 [dd, 1 H, Ar-H], 7.84 [d, 2 H, Ar-H], 8.06 [d, 1 H, Ar-H], 8.21 [s, 2 H, NH$_2$], 11.46 [s, 1 H, C=NH]. ^{13}C NMR ($DCCl_3$) δ 30.26 [C(CH$_3$)$_2$], 36.13 [ArCCH$_2$], 38.87 [CH$_2$C], 63.17 [ArOCH$_2$], 117.25–158.30 [Ar-C], 164.23 [C=O], 178.04 [C=N]. Anal. ($C_{20}H_{21}N_3O_4S$) C, H, N.

Methyl 4-(4,4,5,7-Tetramethyl-6-coumaryl)benzoate (21). Phenol **47**[56] (0.125 g, 0.568 mmol) and monomethylterephthalate (0.122 g, 0.682 mmol) were added to CH_2Cl_2 (10 mL). To this cloudy mixture were added dicyclohexylcarbodiimide (~0.352 g, 1.7 mmol) and a catalytic amount of DMAP (10 mg). The resulting cloudy solution was stirred at room temperature (36 h) and then filtered. The solvent was evaporated *in vacuo*, and the heavy oil was subjected to chromatography (0.67:1.33:15, hexane:EtOAc:CH_2Cl_2). The white solid ester **21** was obtained in a yield of 51% (0.109 g): mp 174–175 °C; IR (KBr) 1788 (C=O), 1737 cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.48 [s, 6 H, C(CH_3)$_2$], 2.16 [s, 3 H, Ar-CH$_3$], 2.29 [s, 3 H, Ar-CH$_3$], 2.62 [s, 2 H, C(O)CH$_2$], 3.99 [s, 3 H, OCH$_3$], 6.87 [s, 1 H, Ar-H], 8.20

[d, 2 H, Ar-H], 8.30 [d, 2 H, Ar-H]; ^{13}C NMR ($DCCl_3$) δ 15.10 [CH_3], 35.50 [$C(CH_3)_2$], 45.64 [Ar*C*CH_2], 52.56 [CH_2], 117.53–148.98 [Ar-C], 163.76 [C=O], 166.12 [C=O], 168.07 [C=O]; MS (EI) calcd for 382.1416, found 382.1411. Anal. ($C_{22}H_{22}O_6$) C, H.

An NMR variable-temperature study was made with **21** in $DCCl_3$ over a range of 0–30 °C in a 5 mm tube. The equation utilized to calculate the ΔG^* was $\Delta G^* = 4.58T_c[9.97 + \log(T_c/\Delta\nu]$.[73] Two peaks for the *gem*-dimethyl groups appeared at δ 1.46 and δ 1.49 at 26 °C and coalesced at 27 °C. The value for ΔG^* was determined to be 15.72 kcal/mol ($\Delta\nu$ = 9.3 Hz) at 27 °C.

2-Methyl-1-phenylpropanol (23). To a cooled (0 °C) solution of methylmagnesium iodide (0.17 mol) in 100 mL of dry ether was added slowly a solution of ester **22** (10 g, 54.9 mol) in dry ether (15 mL). The resulting mixture was stirred at 0–25 °C for 1 h and then at reflux (18 h). The final mixture was diluted with ether, cooled (0 °C), and quenched with water, 20% NH_4Cl, and finally with 10% H_2SO_4 in that order. Ether extracts were washed with 5% $NaHCO_3$ and dried (Na_2SO_4). Evaporation of the solvent gave an oil which contained an impurity that was detrimental to the next cyclization step. Removal of the impurity was effected by adding a solution of I_2 and NaI in water to the oil which was suspended in a 6% solution of KOH in H_3COH. The resulting mixture was stirred for 5 min with gentle warming and 5 min at room temperature. Ether extracts of the resulting mixture were dried (Na_2SO_4) and evaporated to an oil which was vacuum distilled with the main fraction distilling at 80–85 °C/0.12 mm [lit.[51,74] 136–137 °C/12 mm]. Chromatography of the oil over silica gel (3:1 hexanes:ether) gave **22** as a yellow oil (5.6 g, 56%). Alcohol **23** has not been well characterized to date. Thus, we obtained the following data to substantiate the structure; $n^{26.8}$ 1.5582 [lit.[74] n^{23} 1.5609]; IR (neat) 3650–3150 cm^{-1} (O–H); ^{1}H NMR ($DCCl_3$) δ 1.29 [s, 6 H, $C(CH_3)_2$], 2.31 [bs, 1 H, O-*H*], 3.11 [s, 2 H, CH_2], 7.13–7.21 [m, 1 H, Ar-H], 7.22–7.32 [m, 2 H, Ar-H], 7.41 [m, 2 H, Ar-H]; ^{13}C NMR ($DCCl_3$) δ 28.6 [$C(CH_3)_2$], 48.2 [CH_2], 70.6 [$C(CH_3)_2$], Ar-C [125.9, 128.7, 129.2, 136.8].

2,3-Dihydro-3,3-dimethylbenzo[*b*]thiophene (24). Alcohol **23** (9.60 g, 52.7 mmol) in CS_2 (55 mL) was added dropwise to a stirred suspension of $AlCl_3$ (25.0 g, 0.18 mol) in CS_2 (50 mL). The resulting orange-red suspension was stirred at reflux (3 h). The cooled mixture was diluted (H_2CCl_2) and quenched with 5% HCl. Extracts (H_2CCl_2) were washed (saturated $NaHCO_3$, brine), dried ($MgSO_4$), and evaporated to an oil. Vacuum distillation (56–58 °C/0.37 mm) gave a pale yellow oil (6.97 g, 80.5%) which was further purified over silica gel (hexanes); $n^{25.6}$ 1.5757. The compound **24** has not been well characterized, but certain derivatives have been reported.[74] Our spectral data are as follows: ^{1}H NMR ($DCCl_3$) δ 1.35 [s, 6 H, $C(CH_3)_2$], 3.16 [s, 2 H, SCH_2], 7.02–722 [m, 4 H, Ar-H]; ^{13}C NMR ($DCCl_3$) δ 27.2 [$C(CH_3)_2$], 46.9 [SCH_2], 46.9 [$C(CH_3)_2$], Ar-C [122.0, 122.3, 124.1, 127.0, 140.1, 147.5]. Anal. ($C_{10}H_{12}S$) C, H.

1-(2,3-Dihydro-3,3-dimethylbenzo[*b*]thien-5-yl)ethanone (25). A solution of thioether **24** (8.00 g, 48.7 mmol) and freshly distilled acetyl chloride (4.40 g, 56.1 mmol) in CS_2 (65 mL) was added dropwise to a stirred suspension of $AlCl_3$ (9.8 g, 73 mmol) in CS_2 (70 mL). Stirring was continued at room temperature (2 h) after which the mixture was cooled (0 °C) and quenched (H_2O). Ether extracts of the resulting mixture were washed with 5% $NaHCO_3$ and then brine. After drying (Na_2SO_4), the solution was evaporated to an oil which was chromatographed over silica gel (hexanes:ether, 4:1). The oil dissolved in a minimum of hexane and was cooled to −78 °C which resulted in an initial precipitation of a light yellow solid. Intermittent cooling and warming from −78 to −60 °C produced a crystalline, very lightly colored solid. Immediate removal of the crystals, followed by vacuum drying (room temperature/0.3 mm), produced off-white crystals of **25** (8.28 g, 82.4%), mp 20.1–21.4 °C. Concentration of the mother liquors gave an additional 0.65 g of the same crystals for a total yield of 8.93 g (88.9%). Other properties of **25** were n^{26} 1.6048; IR (neat) 1683 cm^{-1} (C=O); ^{1}H NMR ($DCCl_3$) δ 1.39 [s, 6 H, $C(CH_3)_2$], 2.57 [s, 3 H, CH_3], 3.24 [s, 2 H, SCH_2], 7.25 [d, 1 H, Ar-H], 7.67 [d, 1 H, Ar-H], 7.73 [dd, 1 H, Ar-H]; ^{13}C NMR ($DCCl_3$) δ 26.4 [CH_3], 27.6 [$C(CH_3)_2$], 47.3 [$C(CH_3)_2$], 47.7 [SCH_2], Ar-C [122.7, 122.9, 129.2, 134.9, 149.0, 149.5], 198.3 [*C*=O]. Anal. ($C_{12}H_{14}OS$) C, H.

2-(2,3-Dihydro-3,3-dimethylbenzo[*b*]thien-5-yl)-3-buten-2-ol (27). To a fresh solution of H_2C=CHMgBr (**26**, 33 mmol) in dry THF (25 mL) was slowly added a solution of ketone **25** (2.00 g, 9.69 mmol) in dry THF (20 mL). After being stirred at room temperature (3 h) and at reflux (1 h), the mixture was cooled and quenched by the dropwise addition of saturated aqueous NH_4Cl solution. Ether extracts of the mixture were washed with 5% $NaHCO_3$ and brine and then dried (Na_2SO_4), filtered, and evaporated to a yellow oil (2.35 g, qt). The alcohol **27** appeared to become colored quickly and was used immediately to make **28**: IR (neat) 3600–3150 cm^{-1} (O–H); ^{1}H NMR ($DCCl_3$) δ 1.35 [s, 6 H, $C(CH_3)_2$], 1.60 [s, 3 H, CH_3], 2.32 [bs, 1 H, OH], 3.15 [s, 2 H, SCH_2], 5.11 [dd, J_{cis} = 10.6 Hz, J_{gem} = 1.1 Hz, CH=C(*H*)H], 5.26 [dd, J_{trans} = 17.2 Hz, J_{gem} = 1.1 Hz, CH=C(H)*H*], 6.12 [dd, J_{trans} = 17.2 Hz, J_{cis} = 10.6 Hz, 1 H, C*H*=CH_2], 7.08–7.22 [m, 3 H, Ar-H].

[3-(2,3-Dihydro-3,3-dimethylbenzo[*b*]thien-5-yl)-2-buten-1-yl]triphenylphosphonium Bromide (28). A solution of alcohol **27** (2.00 g, 8.53 mmol) was added slowly to a stirred and cooled (0 °C) mixture of $Ph_3PH^+Br^-$ (2.90 g, 8.45 mmol) in H_3COH (10 mL). An immediate blue-green-colored mixture appeared which turned to bright yellow after 14 h of stirring. The mixture was evaporated to about 7 mL, and dry ether was added. Trituration caused the salt **28** to precipitate which was recrystallized (H_3COH). An analytical sample was obtained by allowing a methanol solution of the salt to stand in a diffusion chamber with an adjacent container of ether whose vapors induced precipitation of white, crystalline **28** (3.53 g, 74.7%): mp 236–238 °C; ^{1}H NMR ($DCCl_3$) δ 1.32 [s, 6 H, $C(CH_3)_2$], 1.61 [dd, $^5J_{PH}$ = 4.4 Hz, J = 1 Hz, 3 H, H_3-CC=CHCH_2P], 3.14 [s, 2 H, SCH_2], 4.91 [dd, $^3J_{PH}$ = 15.2 Hz, J = 8.0 Hz, 2 H, CH_2P], 5.55–5.65 [m, 1 H, H_3CC=C*H*CH_2P], 6.85 [d, J = 8.0 Hz, 1 H, H(4)], 6.90 [dd, J = 8.0 Hz, J = 1.8 Hz, 1 H, H(6)], 7.06 [d, J = 8.0 Hz, 1 H, H(7)], 7.6–8.0 [m, 15 H, $P(C_6H_5)_3$]. Anal. ($C_{32}H_{32}BrPS$) C, H.

3,4-Dihydro-2*H*-1-benzothiopyran (30). Thiochroman-4-one (3 g, 18 mmol), toluene (75 mL), water (120 mL), concentrated HCl (60 mL), and the Clemmenson–Martin[75] amalgam [50 g, prepared by shaking for 5 min a mixture of mossy Zn (50 g, 765 g atom), mercuric chloride (5 g, 18 mmol), concentrated HCl (2.5 mL), and water (75 mL)] were mixed. The heterogeneous mixture was boiled and stirred for 72 h, adding 20-mL portions of concentrated HCl at intervals of about 6 h to maintain a total volume of 500 mL. The mixture was allowed to cool to room temperature (1 h) and was then gravity filtered. Toluene extracts of the aqueous layer and the original organic layer were combined and washed with saturated $NaHCO_3$, water, and brine. The solution was dried ($MgSO_4$) and evaporated to give **30** as a yellow oil (2.7 g, 98%), which was used directly to prepare sulfoxide **31**: IR (neat) 1680 (C=O, weak) cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 2.1 [quintet, $^3J_{HCCH}$ = 6.1 Hz, 2 H, H(3)], 2.8 [t, $^3J_{HCCH}$ = 6.2 Hz, 2 H, H(4)], 3.0 [t, $^3J_{HCCH}$ = 6.0 Hz, 2 H, H(2)], 7.05–7.2 [m, 4 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 22.75 [C(3)], 27.46 [C(4)], 29.56 [C(2)]; ArC: 123.79, 126.28, 126.45, 129.87, 132.77, 133.73. Reported properties: [53] bp 81.5–82.5 °C/1.2 mm; ^{1}H NMR ($DCCl_3$) δ 1.90 [m, 2 H, H(3)], 2.92 [quintet, 4 H, H(2) and H(4)], 6.90 [s, 4 H, ArH].

3,4-Dihydro-2*H*-1-benzothiopyran 1-Oxide (31).[54] To a solution of Ti(*i*-OPr)$_4$ (43 mL, 40.6 g, 143 mmol) and (+)-diethyl L-tartrate (49 mL, 58.9 g, 286 mmol) in H_2CCl_2 (250 mL) was added water (2.6 mL) via a syringe. The resulting mixture was stirred (20 min) to a homogeneous solution. To this solution was added sulfide **30** (21.45 g, 143 mmol). The mixture was cooled to −20 °C, and a 3.1 M solution of TBHP (180 mmol) in H_2CCl_2 (51 mL) was introduced dropwise. Stirring was continued at −20 °C (4 h), and 25 mL of water was then added dropwise. Stirring was continued at −20 °C (1 h) and then at room temperature (1 h). A white gel formed and was filtered off, and the filtrate was evaporated and dried (Na_2SO_4). The resulting mixture was separated on silica gel (column chromatography; ethyl acetate:hexane = 85:15, 100% MeOH). The fraction from methanol was evaporated to yield **31** (19 g, 82%) as a yellow oil. The system has not been well

characterized previously, and the following data were thus taken: ^{1}H NMR ($DCCl_3$) δ 2.0–2.2 [m, 1 H, H(3)], 2.4–2.7 [m, 1 H, H(3)], 2.8–3.3 [m, 4 H, H(2) and H(4)], 7.2–7.9 [m, 4 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 14.12 [C(3)], 28.43 [C(4)], 46.37 [C(2)]; ArC: 127.42, 130.51, 130.81, 131.65, 136.01, 138.04. The optical rotation of **31** was taken in cells (l cm × 10 cm) on a Perkin-Elmer 241 polarimeter. At 26 °C, $[\alpha]_D$ = −114.15° (acetone). Reported[54] properties: bp 117–120 °C/0.04 mm; ^{1}H NMR δ 1.09–3.50 [m, 6 H], 7.20–7.90 [m, 4 H]. The reported[52] specific rotation was $[\alpha]_D$ = −21.8° (acetone) at 25 °C.

3,4-Dihydro-2-ethyl-2*H*-1-benzothiopyran 1-Oxide (32a, R = ethyl).[54] To a cooled (−78 °C) solution of diisopropylamine (6.0 mL, 43 mmol) in THF (50 mL) was added a solution of *n*-butyllithium (26 mL, 1.6 M) in hexanes. The reaction mixture was allowed to warm to room temperature and was stirred for 1 h and then was again cooled to −78 °C. The sulfoxide **31** (6.5 g, 39 mmol) in THF (50 mL) was added to this solution. The reaction mixture was allowed to warm to −30 °C (1 h) and was again cooled to −78 °C, after which ethyl iodide (3.1 mL, 43 mmol) was added. Stirring continued for 12 h, after which time 5% hydrochloric acid (50 mL) was added, and the resulting solution was then extracted ($HCCl_3$). Combined extracts were washed with water, 5% $NaHCO_3$, water, and brine. When dried (Na_2SO_4), the solution was concentrated to a brown oil which was separated on a silica gel column (hexane:$HCCl_3$:ethyl acetate = 4:1:1). The second fraction gave the alkylated product **32a** (4.2 g, 80%) as a light yellow oil which was used immediately: ^{1}H NMR ($DCCl_3$) δ 1.13 [t, $^3J_{HCCH}$ = 7.5 Hz, 3 H, CH_3], 1.47 [m, 1 H, H(3)], 1.94 [m, 2 H, CH_2CH_3], 2.5 [m, 1 H, H(3)], 2.92–3.25 [m, 3 H, H(2) and H(4)], 7.21–7.85 [m, 4 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 10.97 [C(3)], 20.67 [CH_2CH_3], 21.27 [CH_2CH_3], 26.6 [C(4)], 59.7 [C(2)]; ArC: 127.2, 129.1, 129.6, 130.9, 136.6, 138.1. Recorded[52] properties: ^{1}H NMR ($DCCl_3$) δ 1.09 [t, 3 H, CH_3], 1.40–3.45 [m, 7 H], 6.83–7.08 [m, 4 H]. No other properties of **32a** were revealed in a search of the literature.

3,4-Dihydro-2-n-octyl-2*H*-1-benzothiopyran 1-Oxide (32b, R = *n*-octyl).[53,54] To a cooled (−78 °C) solution of diisopropylamine (4.8 mL, 34 mmol) in THF (50 mL) was added dropwise *n*-butyllithium (22 mL, 1.6 M in hexanes) over a period of 1 h. The resulting solution was stirred at room temperature for 1 h. After the solution was again cooled to −78 °C, the sulfoxide **31** (5.72 g, 30 mmol) in THF (50 mL) was added (15 min). The solution was then allowed to warm to −30 °C (1 h) and again cooled to −78 °C. Then *n*-octyl bromide (5.4 mL, 34 mmol) was added via syringe in a single portion. Stirring was continued for another 12 h, after which 5% hydrochloric acid (50 mL) was added, and the solution was extracted ($HCCl_3$). Combined extracts were washed with water, 5% $NaHCO_3$, water, and brine. When dried (Na_2SO_4), the solution was concentrated to a brown oil which was separated on a silica gel column (hexane:$HCCl_3$:ethyl acetate, 4:1:1). The second fraction gave the alkylated product **32b** (3.2 g, 40%) as a light yellow oil which was used at once: ^{1}H NMR ($DCCl_3$) δ 0.9 [t, $^3J_{HCCH}$ = 0.7 Hz, 3 H, CH_3], 1.2–1.4 [bs, 12 H, $(CH_2)_6CH_3$], 1.4–1.7 [m, 2 H, $CH_2(CH_2)_6$], 1.85 [m, 1 H, H(3)], 2.45 [m, 1 H, H(3)], 2.8–3.1 [m, 3 H, H(2) and H(4)], 7.1–7.8 [m, 4 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 14.06 [C(3)]; aliphatic-C: 20.75, 22.54, 26.32, 26.53, 28.31, 29.08, 29.25, 29.40, 31.71; 57.99 [C(2)]; ArC: 127.2, 129.18, 129.46, 130.67, 135.47, 140.0. Recorded[53] properties: ^{1}H NMR ($DCCl_3$) δ 0.88 [t, 3 H], 1.1–3.10 [m, 22 H], 7.24–8.10 [m, 4 H]. No other properties of **32b** have been reported.

3,4-Dihydro-2-ethyl-2*H*-1-benzothiopyran (33a, R = ethyl).[53] To a stirred mixture of sulfoxide **32a** (2.0 g, 10 mmol) and NaI (3.7 g, 25 mmol) in acetone at 0 °C was added slowly trifluoroacetic anhydride (4.2 mL, 30 mmol) in acetone (25 mL). The reaction mixture was stirred at 0 °C (1 h) after which time acetone was evaporated. Water was added to the resulting mixture which was then extracted with ether. The ether extracts were washed (water, saturated $Na_2S_2O_3$, water, and brine). After drying ($MgSO_4$), the solvent was evaporated, and the dark red oil obtained was subjected to flash chromatography (silica gel, hexane). Hexane was evaporated to give the sulfide **33a** (1.8 g, 98%) as a light yellow oil: ^{1}H NMR ($DCCl_3$) δ 1.07 [t, $^3J_{HCCH}$ = 7.3 Hz, 3 H, CH_3], 1.65–1.85 [m, 3 H, CH_2-CH_3 and H(3)], 2.2–2.3 [m, 1 H, H(3)], 2.75–2.95 [m, 2 H, H(4)], 3.2–3.3 [m, 1 H, H(2)], 6.9–7.1 [m, 4 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 11.4 [CH_3]; aliphatic-C: 29.36, 29.41, 29.43, 43.79; ArC: 123.74, 126.32, 126.38, 129.51, 133.58, 133.83. Recorded[53] properties: ^{1}H NMR ($DCCl_3$) δ 1.04 [t, 3 H], 1.40–3.45 [m, 7 H], 6.83–7.60 [m, 4 H, ArH]. No other properties of **33a** have been reported. Sulfide **33a** was used immediately to prepare ketone **34a**.

3,4-Dihydro-2-*n*-octyl-2*H*-1-benzothiopyran (33b, R = *n*-octyl).[76] To a stirred mixture of the sulfoxide **32b** (1.7 g, 6 mmol) and NaI (2.2 g, 15 mmol) in acetone at 0 °C (ice–water bath) was added slowly trifluoroacetic anhydride (2.5 mL, 18 mmol) in acetone. The reaction mixture was stirred (1 h) at 0 °C. Acetone was evaporated, water was added, and the mixture was extracted (ether). The ether extracts were washed (water, saturated $Na_2S_2O_3$, water, and brine). After drying ($MgSO_4$), the solvent was evaporated, and the red oil obtained was passed through a short silica gel column (hexane). Solvent evaporation gave sulfide **33b** (1.5 g, 95%) as a light yellow oil which was used directly to make ketone **34b**: ^{1}H NMR ($DCCl_3$) δ 0.88 [t, $^3J_{HCCH}$ = 0.7 Hz, 3 H, CH_3], 1.2–1.5 [bs, 12 H, $(CH_2)_6CH_3$], 1.6–1.8 [m, 3 H, $CH_2(CH_2)_6$ and H(3)], 2.2–2.3 [m, 1 H, H(3)], 2.75–2.9 [m, 2 H, H(4)], 3.25–3.57 [m, 1 H, H(2)], 6.9–7.1 [m, 4 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 13.67 [CH_3]; aliphatic-C: 22.22, 22.37, 25.40, 26.70, 28.88, 29.13, 29.27, 31.43, 33.92, 37.21; ArC: 123.14, 123,31, 125.89, 125.92, 126.05, 129.44. Recorded[53] properties: ^{1}H NMR ($DCCl_3$) δ 1.03 [t, 3 H, CH_3], 1.43 [m, 12 H, $(CH_2)_6CH_3$], 1.8–2.62 [m, 4 H, $CH_2(CH_2)_6$ and H(4)], 2.80–3.10 [dd, 2 H, H(3)], 3.15–3.57 [bs, 1 H, H(2)], 6.97–7.31 [m, 4 H, ArH]. No other properties of **33b** have been reported.

6-Acetyl-2-ethylthiochroman (34a, R = ethyl). To a stirred suspension of $AlCl_3$ (2.9 g, 22 mmol) in nitromethane (50 mL) at 0 °C was added acetic anhydride (0.9 mL, 10 mmol). To the mixture was added a solution of thiochroman **33a** (1.8 g, 10 mmol) in CS_2:nitromethane (1:5, 25 mL). The solution was stirred at 0 °C (1 h) and then allowed to warm to room temperature. Stirring was continued (48 h), and then the solution was cooled (0 °C) and slowly quenched (H_2O). The aqueous layer was extracted ($HCCl_3$), and the combined organic extracts were washed (saturated $NaHCO_3$, water, and brine). After drying (Na_2SO_4), the solution was evaporated to a red oil which was separated on a silica gel column (hexane: ether, 20:80) to give the ketone **34a** (1.5 g, 72%) as an orange oil. Ketone **34a** was used at once to prepare alcohol **35a**: IR (neat) 1680 (C=O) cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.05 [t, $^3J_{HCCH}$ = 7.4 Hz, 3 H, CH_3], 1.6–1.8 [m, 3 H, H(3) and CH_2CH_3], 2.2–2.3 [m, 1 H, H(3)], 2.3 [m, 1 H, H(3)], 2.5–2.6 [s, 3 H, CH_3C(O)], 2.65–3.0 [m, 2 H, H(4)], 3.2–3.3 [m, 1 H, H(2)], 7.2–7.7 [m, 3 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 11.25 [CH_3]; aliphatic-C: 26.26, 28.75, 29.24, 29.82, 44.0; ArC: 126.01, 126.22, 129.23, 132.70, 133.41, 141.27; 197.25 [C(O)].

6-Acetyl-2-*n*-octylthiochroman (34b, R = *n*-octyl). Acetic anhydride (0.5 mL, 6 mmol) was added to a stirred suspension of $AlCl_3$ (1.4 g, 9 mmol) in nitromethane (50 mL, 0 °C). To the stirred mixture was added a solution of 2-*n*-octylthiochroman **33b** (1.5 g, 6 mmol) in CS_2:nitromethane (1: 5, 25 mL). The solution was stirred at 0 °C (1 h) and then allowed to warm to room temperature; stirring was continued (48 h). The solution was cooled (0 °C) and then was quenched (H_2O). The aqueous layer was extracted ($HCCl_3$). The extracts and the original organic layer were combined and washed (saturated $NaHCO_3$, water, and brine). After drying (Na_2SO_4), the solution was evaporated to a red oil which was separated on a silica gel column (hexane:ether, 20:80) to give the ketone **34b** (R = *n*-octyl, quantitative yield) as an orange oil. Ketone **34b** was used directly to make alcohol **35b**. IR (neat) 1680 (C=O) cm^{-1}; ^{1}H NMR ($DCCl_3$,) δ 0.9 [t, $^3J_{HCCH}$ = 0.7 Hz, 3 H, CH_3], 1.35–1.45 [m, 14 H, $(CH_2)_7CH_3$], 2.0 [m, 1 H, H(3)], 2.3 [m, 1 H, H(3)], 2.7 [s, 3 H, CH_3C(O)], 2.9–3.3 [m, 3 H, H(2) and H(4)], 7.2–7.7 [m, 3 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 14.07 [CH_3]; aliphatic-C: 22.62, 22.90, 25.11, 27.99, 29.37, 29.42, 29.51, 29.62, 31.82, 34.19, 37.67; ArC: 126.29, 126.34, 129.56, 132.67, 137.75, 139.89; 197.42 [C(O)].

2-Ethyl-α-methylthiochroman-6-methanol (35a, R = ethyl). To a stirred suspension of $LiAlH_4$ (0.39 g, 10 mmol)

in dry ether (10 mL) was added a solution of ketone **34a** (1.5 g, 7 mmol) in dry ether (15 mL). The resulting mixture was boiled (6 h). It was then cooled (0 °C), and ethyl acetate (25 mL) was slowly added followed by 5% HCl (10 mL). The new mixture was stirred (5 min). The aqueous layer was extracted with ether (3 × 25 mL). Combined organic layers were washed with saturated $NaHCO_3$, water, and brine. After drying ($MgSO_4$), the solvent was evaporated to an orange oil which was separated on a silica gel column (hexane:ether, 1:1) to give alcohol **35a** (1.3 g, 86%) as a yellow oil. Alcohol **35a** was used directly to prepare salt **36a**: IR (neat) 3350 (O−H) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.05 [t, $^3J_{HCCH}$ = 7.4 Hz, 3 H, CH_3], 1.4−1.5 [d, $^3J_{HCCH}$ = 6.4 Hz, 3 H, $CH_3C(OH)$], 1.6−1.8 [m, 3 H, H(3) and CH_3CH_2], 1.9−2.05 [s, 1 H, OH], 2.2−2.3 [m, 1 H, H(3)], 2.8−2.9 [m, 2 H, H(4)], 3.15−3.25 [m, 1 H, H(2)], 4.75−4.85 [m, $^3J_{HCCH}$ = 6.4 Hz, 1 H, $CH_3CH(OH)$], 6.9−7.1 [m, 3 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 11.39 [CH_3]; aliphatic-C: 24.92, 29.32, 29.36, 29.46, 43.76; 69.97 [$CH_3C(OH)$]; ArC: 123.51, 123.59, 126.42, 126.61, 133.82, 141.72.

2-*n*-Octyl-α-methylthiochroman-6-methanol (35b, R = *n*-octyl). A solution of the ketone **34b** (1.5 g, 6 mmol) in dry ether (15 mL) was added to a stirred suspension of $LiAlH_4$ (0.38 g, 9 mmol) in dry ether (10 mL). This mixture was heated at reflux (6 h). It was then cooled to 0 °C, and ethyl acetate (25 mL) was added slowly followed by 5% HCl (10 mL). The mixture was stirred (5 min), and the aqueous layer was extracted (ether). The organic layer and extracts were combined and washed (saturated $NaHCO_3$, water, and brine). After drying ($MgSO_4$), the solvent was evaporated to an orange oil which was separated on a silica gel column (hexane:ether, 1:1) to give alcohol **35b** (1.2 g, 80%) as a yellow oil and which was converted immediately to salt **36b**: IR (neat) 3350 (O−H) cm^{-1}; ^1H NMR ($DCCl_3$) δ 0.9 [t, $^3J_{HCCH}$ = 0.7 Hz, 3 H, CH_3], 1.1−1.6 [m, 17 H, $(CH_2)_7CH_3$ and $CH_3C(OH)$], 1.75−1.95 [m, 2 H, OH and H(3)], 2.0−2.2 [m, 1 H, H(3)], 2.8 [m, 2 H, H(4)], 3.1−3.15 [m, 1 H, H(2)], 4.65−4.8 [m, 1 H, $CH_3CH(OH$], 6.9−7.1 [m, 3 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 14.07 [CH_3]; aliphatic-C: 22.62, 22.70, 25.68, 25.70, 27.11, 29.27, 29.55, 29.64, 31.83, 34.34, 37.75; 70.05 [$CH_3C(OH)$]; ArC: 123.540, 123.62, 126.50, 126.92, 126.98, 139.08, 141.01.

1-[(2-Ethylthiochroman-6-yl)ethyl]triphenylphosphonium Bromide (36a, R = ethyl). A solution of alcohol **35a** (1.3 g, 6 mmol) and triphenylphosphine hydrobromide (2.0 g, 6 mmol) in H_2CCl_2 (50 mL) was stirred (room temperature, 24 h). After concentration, the resulting orange oil was triturated (dry ether) to give phosphonium salt **36a** (3.0 g, 96%) as a slightly hygroscopic, white solid which was used at once to prepare **37a**.

1-[(2-*n*-Octylthiochroman-6-yl)ethyl]triphenylphosphonium Bromide (36b, R = *n*-octyl). A solution of alcohol **35b** (4.4 g, 14 mmol) and triphenylphosphonium hydrobromide (5.0 g, 14 mmol) in H_2CCl_2 (50 mL) was stirred (room temperature, 24 h). The solvent was evaporated, and the oil obtained was triturated (room temperature, dry ether) to obtain **36b** as a white solid (9.0 g, 98%), mp 67−68 °C. Salt **36b** was used directly to prepare heteroarotinoid **37b**: ^1H NMR ($DCCl_3$) δ 0.9 [t, $^3J_{HCCH}$ = 6.6 Hz, 3 H, CH_3], 1.1−1.6 [m, 14 H, $(CH_2)_7CH_3$], 1.7−1.95 [m, 4 H, CH_3CH and H(3)], 2.0−2.2 [m, 1 H, H(3)], 2.5−2.7 [m, 1 H, H(4)], 2.8 [m, 1 H, H(4)], 3.0−3.1 [m, 1 H, H(2)], 6.55 [m, 1 H, CH_3CH], 6.9−7.1 [m, 3 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 14.15 [CH_3]; aliphatic-C: 16.89, 17.29, 22.68, 26.74, 26.95, 29.36, 29.44, 29.50, 29.74, 31.9, 34.12, 37.55 [$CH_3C(H)$]; ArC: 117.42, 117.52, 118.24, 118.33, 126.88, 128.55, 128.67, 129.26, 129.36, 130.04, 130.08, 130.12, 130.16, 130.21, 130.24, 132.11, 132.21, 133.82, 133.97, 134.64, 134.67, 134.73, 134.76, 134.82.

Ethyl (*E*)-4-[2-(3,4-Dihydro-2-ethyl-1-oxy-2*H*-1-benzothiopyran-6-yl)-1-propenyl]benzoate (37a). A mixture of **36a** (3.9 g, 11 mmol), K_2CO_3 (1.5 g, 11 mmol), and 18-C-6 (30 mg) in H_2CCl_2 (25 mL) was boiled (2 h). Ethyl 4-formylbenzoate (1.7 g, 10 mmol) was added rapidly to the mixture. After boiling (12 h), the resulting mixture was concentrated to an orange oil which was diluted with hexane. A suspension formed and was filtered, and the filtrate was washed (brine) and dried (Na_2SO_4). Concentration left a yellow oil which was chromatographed on silica gel (hexane:ethyl acetate, 1:1) to give sulfide **37a** as a yellow solid. Recrystallization (ether) produced **37a** as a white solid (2.3 g, 91%): mp 63−64 °C; IR (neat) 1750 cm^{-1} (C=O); ^1H NMR ($DCCl_3$) δ 1.05 [t, J = 7.4 Hz, 3 H, CH_2CH_3], 1.4 [t, J = 7.3 Hz, 3 H, OCH_2CH_3], 1.6−1.85 [m, 3 H, CH_2CH_3 and H(3)], 2.25−2.35 [m, 4 H, H(3) and H(10)], 2.8−2.9 [m, 2 H, H(4)], 3.2−3.3 [m, 1 H, H(2)], 4.4 [q, J = 7.3 Hz, 2 H, OCH_2CH_3], 6.8 [s, 1 H, H(11)], 7.05−8.15[m, 7 H, ArH]; ^{13}C NMR ($DCCl_3$) δ 11.49 [CH_2CH_3]; aliphatic-C: 14.39,17.54, 29.43, 29.47, 29.66, 43.98, 60.90 [C(19)]; ArC and vinylic C: 124.11, 125.80, 126.38, 127.16, 127.33, 127.72, 129.09, 129.46, 133.18, 133.65, 139.02, 143.10, 166.14 [C(18)]; mass spectral (EI) data calcd for $C_{23}H_{26}O_2S$ m/z 366.1653, found 366.1650. Anal. Calcd for $C_{23}H_{26}O_2S$: C, 75.38; H, 7.16. Anal. ($C_{23}H_{26}O_2S{\cdot}0.2H_2O$) C, H.

4,4-Dimethyl-3,4-dihydro-2*H*-1-benzopyran-6-carboxylic Acid (39). Ketone **38**[20,55] (2.0 g, 0.01 mol), ethanol (35 mL), and Clorox (100 mL) were mixed, and the resulting mixture was boiled for 24 h. The solution was cooled (ice bath), and then $Na_2S_2O_5$ (75 mL) was added cautiously, followed by concentrated HCl (20 mL). The resulting mixture was vacuum filtered and the white solid was recrystallized (95% ethanol) to give **39** (1.31 g, 65%): mp 227.5−228.5 °C [lit.[55] mp 227−229 °C]; IR (KBr) 3600−2595 (CO_2H), 1685 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.38 [s, 6 H, $C(CH_3)_2$], 1.86 [t, 2 H, J = 6 Hz, CCH_2], 4.27 [t, 2 H, J = 6 Hz, CH_2O], 6.83 [d, 1 H, Ar-H], 7.82 [dd, 1 H, Ar-H], 8.07 [d, 1 H, Ar-H]. ^{13}C NMR ($DCCl_3$) δ 30.59 [$(CH_3)_2$], 30.75 [$ArCCH_2$], 36.49 [CCH_2], 63.49 [CH_2O], 117.1−158.5 [Ar-C], 171.8 [C=O].

***N*-(4-Bromophenyl)-3-methyl-2-butenamide (41).** To 4-bromoaniline (**40**) (6.13 g, 0.035 mol) in 225 mL of $HCCl_3$ was added slowly 3,3-dimethylacryloyl chloride (2.06 g, 0.017 mol) in $HCCl_3$ (10 mL). The resulting cloudy, tan mixture was then boiled for 5 h. After the mixture was cooled to room temperature, the precipitate that formed was removed (vacuum filtration). The solution was then washed (2 M HCl, saturated $NaHCO_3$, and brine). After drying ($MgSO_4$), the solution was concentrated *in vacuo*. Recrystalliztion (95% ethanol) of the product gave off-white, flaky crystals of **41** (7.7 g, 87%): mp 118−119 °C; IR (KBr) 3320 (N−H), 1680 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.88 [s, 3 H, CH_3 (*cis*)], 2.21 [s, 3 H, CH_3 (*trans*)], 5.69 [t, 1 H, J = 1.2 Hz, C=CH], 7.26−7.45 [m, 4 H, Ar-H]; ^{13}C NMR ($DCCl_3$) δ 19.99 [CH_3 (*cis*)], 27.41 [CH_3 (*trans*)], 116.40−137.30 [Ar-C, 118.29 [C=CH], 154.22 [(CH_2C=CH)], 165.00 [C=O]; MS (FAB) calcd for $C_{11}H_{13}BrNO$ 254, found 255 (MH^+). Anilide **41** was used at once to make lactam **42**.

6-Bromo-4,4-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoline (42). Anilide **41** (2.01 g, 7.94 mmol) was placed in a flask which was then heated to 130−140 °C. Then $AlCl_3$ (1.57 g 0.012 mol) was added portionwise over 1 h. The flask was allowed to cool to 80 °C, and one final portion of $AlCl_3$ (0.2 g) was slowly added. The mixture was then stirred at that temperature (0.5 h). After the mixture was cooled to room temperature, 20 mL of ice water was cautiously added to the dark brown solid. Chloroform (50 mL) was then added to dissolve the solid. The solution was then stirred (15 min), and 10 mL of 2 M HCl was added. The aqueous layer was extracted with $HCCl_3$. The combined organic layers were washed (saturated $NaHCO_3$ and brine). After drying ($MgSO_4$), the solution was concentrated *in vacuo* to give **42** (1.57 g, 78%) as a crude orange solid. Recrystallization (95% ethanol) gave white crystals of **42** (1.31 g, 65%): mp 174−175 °C; IR (KBr) 3240 (N−H), 1700 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.6 [s, 6 H, $(CH_3)_2$], 2.48 [s, 2 H, CH_2C=O], 6.7−7.40 [m, 3 H, Ar-H]. ^{13}C NMR ($DCCl_3$) δ 27.47 [CH_3], 27.50 [CH_3], 34.11 [$ArCCH_2$], 44.86 [CH_2], 116.15−134.99 [Ar-C], 171.15 [C=O]; MS (FAB) calcd for $C_{11}H_{13}BrNO$ 254, found 255 (MH^+). Lactam **42** was reduced at once to **43**.

6-Bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline (43). Lactam **42** (1.0 g, 3.95 mmol) in 10 mL of dry, distilled toluene was cooled (ice bath; 0−4 °C). The borane−dimethyl sulfide (0.31 g, 4.10 mmol, 5% excess) complex was added dropwise. The reaction mixture was stirred at 0 °C (15 min) and then was boiled (5 h). After being cooled to room temperature, the solution was treated with 10% Na_2CO_3 (15 mL). The solution was then stirred at room temperature (30 min). The organic layer was separated, dried ($MgSO_4$), and concentrated *in vacuo*

to give **43** (0.85 g, 90%, light yellow oil) which was used immediately to make **44**: IR (neat) 3420 (N–H) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.27 [s, 6 H, $(CH_3)_2$], 1.70 [t, 2 H, J = 1.2 Hz, CCH_2], 3.29 [t, 2 H, J = 1.2 Hz, CH_2N], 6.33 [d, 1 H, Ar-H], 7.01 [dd, 1 H, Ar-H], 7.24 [d, 1 H, Ar-H]; ^{13}C NMR ($DCCl_3$) δ 30.71 [CH_3], 30.75 [CH_3], 31.92 [$ArCCH_2$], 36.65 [CCH_2], 38.24 [CH_2N], 108.39–142.53 [Ar-C]; MS (FAB) calcd for $C_{11}H_{14}BrN$ 239, found 239.

6-Bromo-*N*,4,4-trimethyl-1,2,3,4-tetrahydroquinoline (44). The quinoline **43** (2.5 g 0.01 mol), $NaHCO_3$ (1.51 g 0.018 mol), and H_2O (2 mL) were mixed in a flask and cooled (15–18 °C). Dimethyl sulfate (1.64 g 0.013 mol) was added dropwise. The cloudy light yellow mixture was stirred at room temperature until the evolution of CO_2 ceased (~1 h). The solution was then heated to and maintained at 50 °C until all CO_2 ceased to be released (~1 h). After being cooled to room temperature, the solution was diluted with chloroform (25 mL), and the layers were separated. Chloroform extracts of the aqueous layer and the organic layer were combined and then washed (H_2O and brine). After drying ($MgSO_4$), the solution was concentrated *in vacuo*. The product was then cooled to 0 °C overnight during which time solid impurities formed. The mixture was vacuum filtered and washed with hexane. Concentration *in vacuo* gave the *N*-methylated product **44** as a yellow oil (2.18 g, 86%), which was used directly to make **45**: ^1H NMR ($DCCl_3$) δ 1.25 [s, 6 H, $C(CH_3)_2$], 1.73 [t, 2 H, J = 6 Hz, CCH_2], 2.86 [s, 3 H, NCH_3], 3.20 [t, 2 H, J = 6 Hz, CH_2N], 6.42 [d, 1 H, Ar-H], 7.13 [dd, 1 H, Ar-H], 7.23 [d, 1 H, Ar-H]; ^{13}C NMR ($DCCl_3$) δ 30.70 [$(CH_3)_2$], 32.30 [$ArCCH_2$], 36.88 [CCH_2], 39.23 [CH_2N], 47.48 [NCH_3], 107.94–144.29 [Ar-C]; MS (FAB) calcd for $C_{12}H_{16}BrN$ 253, found 253.

***N*,4,4-Trimethyl-1,2,3,4-tetrahydroquinoline-6-carboxylic Acid (45).** To 1.6 M *n*-BuLi (4.90 mL, 784 mmol) in THF was added dropwise the tetrahydroquinoline **44** (1.0 g, 4.11 mmol) in ether (5 mL). The solution was stirred at room temperature (24 h). The cloudy orange mixture was then poured over solid CO_2 in excess ether. The mixture was stirred until it warmed to room temperature (~2 h), and then H_2O (20 mL) was added. The layers were separated, and the aqueous layer was acidified (2 M HCl, pH ~3). The resulting mixture was extracted (CH_2Cl_2), and the extracts and organic layer were combined and dried ($MgSO_4$). The solution was concentrated *in vacuo*, and the resulting solid was recrystallized (95% ethanol). White flaky crystals (0.270 g, 30%) of the carboxylic acid **45** were obtained: mp 223–225 °C; IR (KBr) 3500–2400 (CO_2H) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.30 [s, 6 H, $C(CH_3)_2$], 1.75 [t, 2 H, J = 6 Hz, CCH_2], 3.00 [s, 3 H, NCH_3], 3.70 [t, 2 H, J = 6 Hz, CH_2N], 6.53 [d, 1 H, Ar-H], 7.82 [dd, 1 H, Ar-H], 7.92 [d, 1 H Ar-H]; ^{13}C NMR ($DCCl_3$) δ 30.03 [$C(CH_3)_2$], 31.88 [$ArCCH_2$], 36.22 [CCH_2], 38.99 [CH_2N], 47.49 [NCH_3], 109.44–149.25 [Ar-C], 171.95 [C=O]; MS (FAB) calcd for $C_{13}H_{17}NO_2$ 219, found 219. Acid **45** was converted at once to ester **19**.

4-[[(4,4-Dimethyl-3,4-dihydro-2*H*-benzo[*b*]pyran-6-yl)carbonyl]oxy]benzaldehyde (46). To a mixture of the carboxylic acid **39** (0.300 g, 1.45 mmol) and 4-formylphenol (0.265 g, 2.17 mmol) in CH_2Cl_2 (20 mL) was added dicyclohexylcarbodiimide (~0.895 g, 4.33 mmol) and a catalytic amount of DMAP (10 mg). The resulting cloudy solution was stirred at room temperature (48 h) and then filtered. The filtrate was cooled to 0 °C (24 h) and filtered again. Evaporation of the solvent *in vacuo* gave an oil which was allowed to stand at room temperature (24 h), during which time crystallization took place. Recrystallization (hexane:EtOAc, 3:1) gave solid **46** (0.195 g, 43%): mp 132–133.5 °C; IR (KBr) 2850 ((O)C–H), 2724, 1700, 1730 (C=O) cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.40 [s, 6 H, $C(CH_3)_2$], 1.87 [t, 2 H, J = 5.6 Hz, CCH_2], 4.30 [t, 2 H, J = 5.6 Hz, CH_2O], 6.88 [d, 1 H, Ar-H], 7.39 [d, 2 H, Ar-H], 7.91 [d, 1 H, Ar-H], 7.97 [d, 2 H, Ar-H], 8.13 [d, 1 H, Ar-H], 10.02 [s, 1 H, C(O)H]; ^{13}C NMR ($DCCl_3$) δ 30.61 [$(CH_3)_2$], 36.86 [$ArCCH_2$], 63.54 [$ArOCH_2$], 117.39–158.81 [Ar-C], 164.47 [C=O], 191.07 [C=O]; MS (FAB) calcd for $C_{15}H_{19}O_4$ 310, found 311 (MH^+). The aldehyde **46** was converted immediately to the corresponding thiosemicarbazone **20**.

Transactivation Assay. The CC-B cervical tumor cell line,[57] which contains stabilized integrated copies of RARβ-RARE-tk-CAT reporter plasmid, was used to evaluate activation of the heteroarotinoids via endogenous nuclear receptors. A concentration of 4×10^5 cells per well was used to inoculate 6-well culture dishes containing Eagles Minimal Essential Media (contains Earle's Salts and L-glutamine via Cellgro Mediatech, Herndon, VA) supplemented with nonessential amino acids, sodium pyruvate, and 10% fetal bovine serum (FBS). Only lots of FBS that contained negligible quantities of *t*-RA (**1**), as determined by HPLC analysis, were used. Triplicate cultures were treated with 1000× stocks (DMSO) of the agents. The final concentration in DMSO in control and treated cultures was 0.1%. After 48 h, cell extracts were prepared and CAT activity was assayed as previously described[57] with the exception that [^3H]acetyl-CoA was used instead of [^{14}C]acetyl-CoA. Protein concentrations of the extracts were determined using the Bio-Rad protein assay.[77] Transactivation activities were derived by dividing the average of the CAT activity per milligram of protein in the drug-treated culture by that in the control culture. Relative transactivation activity was derived by dividing the activities observed in the heteroarotinoid-treated cultures by that in cultures treated with *t*-RA (**1**) (Table 1). The results, presented as percentages in Table 1, are the average of three experiments.

Growth Rate. Six-well tissue culture dishes were inoculated with a concentration of 4×10^4 CC-B cells per well. Twenty-four hours after plating, the media was replenished, and triplicate cultures were treated with retinoids and heteroarotinoids as described above. The media and agents were replenished every 2 days. After 7 days, the number of cells per well in the treated and control cultures were determined using a Particle Counter (Coulter ZM). Percent growth rate was determined by dividing the average of the number of cells in the treated culture by that in the control culture and multiplying by 100. The results presented in Figure 1 (panel A) are the average of three separate experiments.

General Method for Assaying Transglutaminase Activity. Human erythroleukemia cells (GMO6141A, Human Genetic Mutant Cell Respository, Camden, NJ) were inoculated into T-25 flasks (Corning, Corning, NY) containing McCoy's Medium 5a (GIBCO/BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (Intergen, Purchase, NY) at a density of 2×10^5 cells/mL. The heteroarotinoids were dissolved either in ethanol or DMSO. Twenty-four hours after subculture, the cells were treated with a 10 μM solution of either *t*-RA (**1**), the vehicle alone (control), or the heteroarotinoid. The *t*-RA (**1**; Sigma, St. Louis, MO) was added to one flask in all experiments to ensure that transglutaminase induction occurred, and it was also used as the standard to which the heteroarotinoid induction was compared. Cultures were covered with aluminum foil to protect them from light and then incubated at 37 °C (48 h). Cells ((10–40) $\times 10^6$) were collected by centrifugation at 2000 rpm (5 min). The resulting cell pellet was resuspended in 10 mL of Ca,Mg-free Earles solution, and the cell suspension was centrifuged at 2000 rpm (3 min). These cells were twice washed again. The final washed cell pellet was resuspended in 1 mL of Tris-buffered saline (150 mM NaCl-1 mM EDTA-20 mM Tris-HCl, pH 7.4), and the cell suspension was disrupted by a 10-s sonication. Cell lysates were quantitated for trans-glutaminase activity by measuring the covalent incorporation of ^{14}C-labeled putrescine into dimethylcasein in a calcium-dependent manner as previously described.[78] Protein was estimated using the Bio-Rad procedure.[77] The values for the TGase activity were determined as the activity/mg of protein in the treated cultures divided by that in solvent control cultures and represent the mean of duplicate assays performed on replicate samples. Figure 1 plots such activity versus RARE transactivation (panel B).

Materials and Methods for the Toxicity Studies. The syntheses of **8**, **10**, and **13** were recorded previously.[20,49] A sample of TTNPB (**5**) was kindly provided by Dr. Peter F. Sorter, Hoffmann-La Roche, Inc. Qualitative checks for purity using high-pressure liquid chromatography (column, Spherisorb, ODS, 5 μm; Solvents, acetonitrile; 1% ammonium acetate buffer, 85:15; wavelength, 254 and 340 nm; flow, 1 mL/min; concentration, 1 mg/mL H_3CCN) and melting point determina-

tions indicated **10** to be 99.8–100% pure and **8** and **13** to be 100% pure. When not in use, the compounds were stored in sealed amber vials under a nitrogen atmosphere at −70 °C. All compounds were weighed, and dosage formulations were prepared in subdued light. After the compounds were formulated in corn oil for dosing, the solutions were stored at room temperature in sealed amber vials under a nitrogen atmosphere.

Male B6D2F1 mice, 4 weeks of age, were obtained from Charles River Laboratories (Raleigh, NC). Mice were quarantined for a minimum of 7 days prior to initiation of dosing. Mice were also housed individually in suspended $7^3/_4$ in. × $10^5/_8$ in. × $5^1/_{16}$ in. solid-bottom polycarbonate cages fitted with spun-bonded polyester fiber filter. Heat-treated hardwood ships were used as bedding. Feed (NIH-07 open formula) and city tap water (Edstrom automatic watering system) were administered *ad libitum*. A light/dark cycle of 12 h light and 12 h dark was used. The optimal temperature and relative humidity ranges for the animal room were 69–75 °F and 35–65%, respectively. All animal care procedures conformed to the guidelines found in *Guide for the Care and Use of Laboratory Animals*, DHEW Publication No. (NIH)85-23 (Revised 1985).

Groups of 16 animals each were dosed daily by gavage (10 mL/kg) with either corn oil (vehicle 1) or 0.1, 0.2, 0.4 or 0.8 mg/kg of each retinoid. Due to overt toxicity at these dose levels in the TTNPB groups, groups of eight animals each were added to the study and dosed with either corn oil (vehicle 2), 0.01 or 0.05 mg/kg of TTNPB (**5**). *Due to the lack of toxicity*, dose levels in the **8**, **10**, and **13** groups were increased 4-fold on day 15, 10-fold on day 29, and 2-fold (**8** and **10** only) on day 51. The study was terminated on day 65 for all three compounds and on day 25 for the vehicle 2, 0.01 and 0.05 mg/kg TTNPB groups. The toxicity data and effects induced by *t*-RA (**1**) have been recorded independently on many occasions, are well-known[69–71] to be approximately 10 mg/kg/day for many organs, are entirely reproducible, and are thus not included herein.

Body weights and clinical signs were measured three times weekly. On study days 8, 15, 29, 36, and at terminal sacrifice (day 8 and at terminal sacrifice for the vehicle 2, 0.01 and 0.05 mg/kg groups), blood was collected from each animal from the retro-orbital sinus under CO_2 anesthesia. Portions were placed in tubes containing potassium EDTA for hematology analyses or in tubes without anticoagulant for alkaline phosphatase, cholesterol, and triglyceride analyses. Serum was collected after centrifugation at 2000g for 2 min. Hematology analyses were performed with an Ortho ELT-8 automated hematology analyzer, which uses a laser light source to detect RBC, WBC, and platelets. Hematocrit values were based upon erythrocyte counts, and hemoglobin was measured by the colorimetric determination of cyanomethemoglobin. Serum chemistries were performed with a Centrifichem 500 automated centrifugal analyzer, which used test-specific reagent kits obtained from the manufacturer and provided quantitative, spectrophotometric analysis.

All animals were necropsied, with 43 tissues from each being animal examined for gross observations of pathologic effects. Each carcass was X-rayed (40 kV, 3 mA, 2 min). At study termination, the organs weighed included brain, liver, right kidney, thymus, heart, lungs, right testicle, and spleen. The method of euthansia was CO_2 asphyxiation. Analysis of variance, with comparisons of means by Students' *t*-test, was performed. Differences were considered significant if the value for p was ≤ 0.05.

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