Novel Heteroarotinoids as Potential Antagonists of *Mycobacterium bovis* BCG

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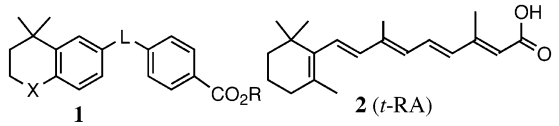
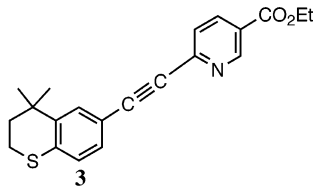
A series of 15 heteroarotinoids has been prepared and evaluated for activity against *Mycobacterium bovis* BCG with the thiourea-containing isoxyl (**7**) (0.5 μg/mL) as the standard. 2,2,4-Trimethyl-2*H*-chromen-7-yl 4-(methoxycarbonyl)benzoate (**8**) displayed the most significant activity (2.0−4.0 μg/mL) in terms of the lowest concentration (μg/mL) (MIC, minimum inhibitory concentration) required to produce a 99% reduction in the number of colonies on a plate as compared to that system free of the agent at the same dilution of the culture suspension. Ethyl 4-{[*N*-(2,2,4,4-tetramethylchroman-6-yl)thiocarbamoyl]amino}benzoate (**9**) and {[(1*E*,3*Z*,5*E*)-1-aza-4-methyl-6-(1,2,2,4-tetramethyl(1,2-dihydroquinolyl))hexa-1,3,5-trienyl]-amino}aminomethane-1-thione (**10**) exhibited activity at 5.0−10.0 and 10.0−20.0 μg/mL, respectively, while the other examples had MIC values of 20 μg/mL or greater. The inhibitory ability of **8** may occur via the inhibition of mycolic acid synthesis in a like manner as found with **7**, but this requires further study. The heteroarotinoids are the first examples to exhibit inhibitory ability against the growth of *Mycobacterium bovis* BCB.

Introduction

The biology−chemistry of retinoids is an extremely active area, especially with new synthetic examples for the treatment of malignancies[1] and dermatological disorders.[2] Heteroarotinoids (**1**) are structurally related



1 **2** (*t*-RA)



3

to *trans*-retinoic acid (*t*-RA, **2**), but the former are generally much less toxic.[3] Tazarotene (**3**) is an example of a heteroarotinoid that is currently in clinical trials for the treatment of psoriasis.[2] To the best of our knowledge, there are no reports of the utility of heteroarotinoids as chemotherapeutic agents to treat any strains of mycobacteria.

Mycobacterium tuberculosis (*M. tuberculosis*, or Mtb), a human pathogen causing tuberculosis (TB), is responsible for the death of millions of people each year and continues to claim more lives than any other single infectious agent.[4] Its pathogenicity is believed to arise from an ability to survive in host cells by colonizing macrophages and remaining quiescent for long periods of time, only to become active decades later.[5] Approximately one-third of the world's population is infected with Mtb, 10% of which are predicted to develop the disease over the course of their lives.[6] One current treatment for active TB is a four-drug regimen comprised of isoniazid, rifampin, pyrazinamide, and ethambutol for a period of at least 6 months.[7] A failure of patients to complete the therapy has led to the emergence of multidrug-resistant (MDR) tuberculosis. The increasing number of cases of MDR tuberculosis has become such a public health threat that the World Health Organization (WHO) has declared TB a global public health emergency.[8] Consequently, there is an urgent need to create high efficacy or rapid-acting agents to treat Mtb.

One approach is the development of agents that will successfully inhibit the actions of the enzyme dihydrofolate reductase (DHFR). DHFR is essential for folate metabolism in both eukaryotic and prokaryotic cells.[9] It is important in the treatment of many types of infections[10] and in the treatment of patients with HIV and Mtb infections.[11] Agents such as methotrexate [MTX (**4**), an antiinflammatory and immunosuppressive agent],[12] trimethoprim (TMP, **5**),[5] and Br-WR99210 [**6**,[5] a bromine analogue of triazine (WR99210)] are important as DHFR inhibitors. A structural comparison study of DHFRs from human tissue and *M. tuberculosis* performed by Hol and co-workers[5] provided several useful observations. Through a sequence alignments of the amino acids in the DHFRs of human and Mtb, Hol

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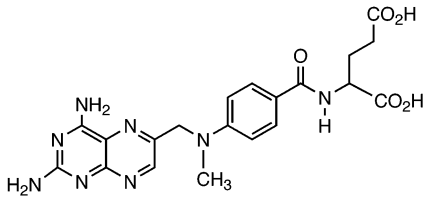
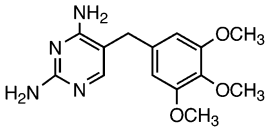
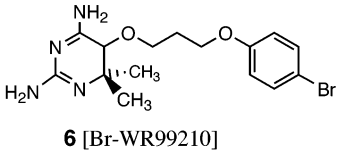


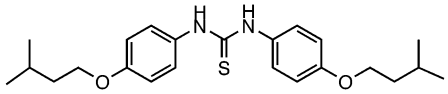
4 [Methotrexate, MTX]



5 [Trimethoprim, TMP]



6 [Br-WR99210]

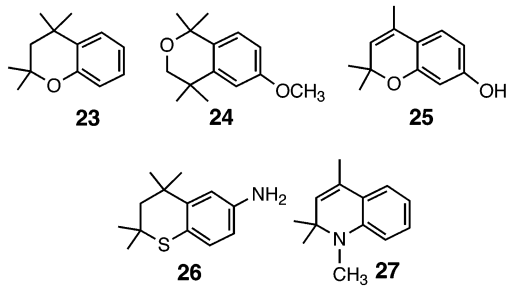


7 [Isoxyl]

noted only a ~26% sequence identity and indicated key differences that might be considered in the future development of selective inhibitors of the DHFR of Mtb. Isoxyl (**7**) has demonstrated potent activity against various *Mycobacterium* strains, including *M. tuberculosis* (Mtb).[13] Isoxyl is also known to inhibit mycolic acid synthesis and fatty acid synthesis in the same bacteria.[13] As can be noted, **7** contains a flexible thiourea group connected to two aryl rings, which are also in **4**−**6**. Similar H-bonding NH groups are in **4**−**7**, possibly important for inhibition. We discovered that certain heteroarotinoids with thiourea linkages exhibit strong anticancer activity.[14,15] We now report the antimycobacterial activity of some heteroarotinoids with H-bonding groups and structurally related to **7** but with saturated rings fused to one aryl ring.

Chemistry

None of the heteroarotinoids reported herein are commercially available. The preparation of members of **8**−**22** (Table 1) required the use of key starting materials **23**−**27**. Direct methods to obtain such intermediates



have been generally laborious. Heterocycles **24**[15] and **26**[15] are known, while **23**[16] and **25**[17] have been partially characterized and **27** is unknown. Structures of the latter three materials have now been firmly established.

Direct nitration of **23** could only be accomplished in reasonable yield at −5 °C by using a combination of nitric acid and freshly distilled acetic anhydride (Scheme 1). A mixture of isomers **28a** and **28b** was isolated in pure form as creamy white solids in yields of 43% and 26%, respectively, after rigorous chromatography. Reduction of the nitro groups in a mixture of these isomers was achieved in highest conversions with iron in acetic acid and absolute ethanol and provided **29a** and **29b**, which were easily separated by chromatography. In fact, later experiments revealed that the best returns of **29a** and **29b** were realized by subjecting the crude mixture of **28a** and **28b** to reducing conditions and then separating **29a** and **29b** via immediate chromatography.

Treatment of **29a** and/or **29b** with the correspondingly required isocyanate or isothiocyanate as illustrated in Scheme 2 led to **9**, **11**, and **13** as well as to **19**, **22**, and **21**, respectively. Yields of the ureas and thioureas were good to excellent with all of the solids being purified by recrystallization to sharp melting points. Obviously, the urea or thiourea groups in the 6- or 8-positions in the systems could elicit a different response in the assay.

To assess the impact of the sulfur heteroatom on activity, as compared to that observed with the oxygen-containing cyclohexyl ring systems **9**, **11**, and **13**, esters **15** and **17** were examined. Although reported earlier,[14] no preparations were disclosed for these latter derivatives. Starting with **26**,[15] the condensation was straightforward and gave **15** and **17** under conditions similar to those reported for **9**, **11**, **13**, **19**, **20**, and **21**.

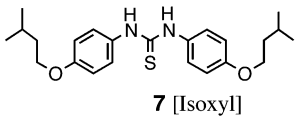
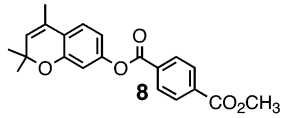
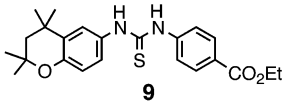
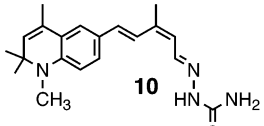
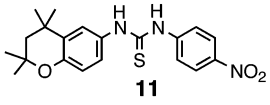
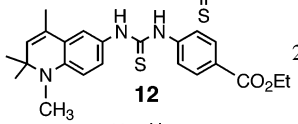
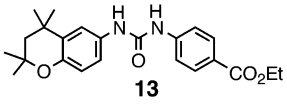
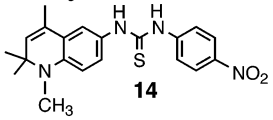
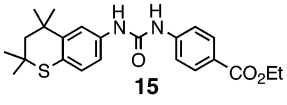
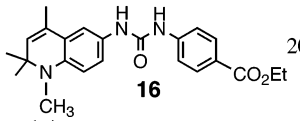
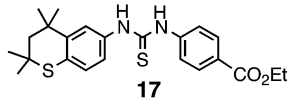
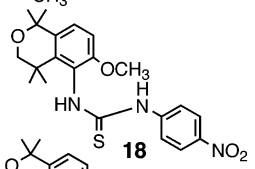
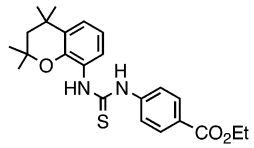
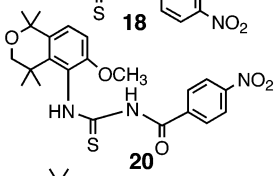
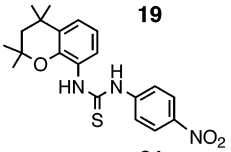
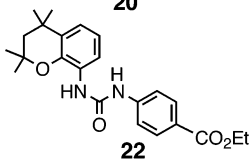
A series of nitrogen-possessing analogues was investigated to ascertain the influence of this heteroatom and also the substituent on the cyclohexyl ring unit in the form of one less methyl group at C-4. The condensation of aniline with acetone and a trace of iodine in an acidic medium afforded **30**, which was an oil and was used at once to prepare the *N*-methyl derivative **27**, also an oil (Scheme 3). Likewise, **27** was immediately treated with $LiClO_4$ and freshly prepared bis(2,2,2-trichloroethyl)azodicarboxylate (**31**) in ether. Decomposition of the intermediate azide with zinc/concentrated acetic acid afforded **32** as a solid (51%). To avoid degradation, **32** was treated immediately with the appropriate thioisocyanates/isocyanate and gave **12**, **14**, and **16**.

To determine if an extended side chain would induce stronger activity in this nitrogen series, **27** was subjected to formylation with $POCl_3$/DMF and yielded **33** (Scheme 4). Lactonization of **33** in a base-catalyzed experiment with ethyl 3,3-dimethylacrylate generated **34**, which was immediately reduced with DIBAL-H in THF at −78 °C and gave **35**. Ring opening of **35** under dilute acid conditions in 1,2-dichloroethane led to aldehyde **36**. Treatment of **36** with thiosemicarbazone in acetic acid−ethanol at 60 °C produced the thiocarbazone **10**. Another reason for assessing **10** in the current assay was based upon previous work with two heteroarotinoids that gave thiocarbazones that exhibited retinoid receptor activation and/or growth inhibition of ovarian cancer cells.[3,14]

For comparison purposes, **8** was targeted for synthesis, since it had oxygen rather than nitrogen in the cyclohexyl ring, was void of one methyl group at C-4, contained a shorter linking ester group, and had the linking group at C-7. Lactone **37**[17] reacted with excess methylmagnesium bromide in THF at room temperature for 3 days to give **25** in modest yield (40%) (Scheme 5). The anion of **25** condensed smoothly with the required acid chloride shown and created ester **8**.

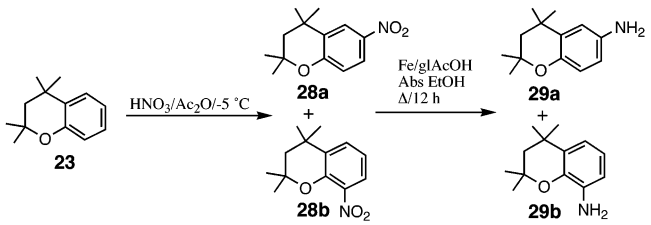
Changing the position of the heteroatom in the ring was the impetus for preparing **18** and **20**. In addition,

Table 1. Antibacterial Activity of Selected Heteroarotinoids against *M. bovis*[a]

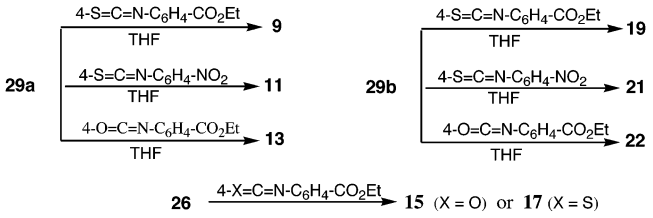
Compound	MIC (μg/mL)[b]	Compound	MIC (μg/mL)[b]
 **7** [Isoxyl]	0.5-1.0	 **8**	2.0-4.0
 **9**	5.0-10.0	 **10**	10.0-20.0
 **11**	20.0-40.0	 **12**	20.0-40.0
 **13**	20.0-40.0	 **14**	20.0-40.0
 **15**	20.0-40.0	 **16**	20.0-40.0
 **17**	20.0-40.0	 **18**	20.0-40.0
 **19**	20.0-40.0	 **20**	20.0-40.0
 **21**	20.0-40.0	 **22**	20.0-40.0

[a] The results are from the treatment of M. bovis (BCG) with the agents listed. See the Experimental Section for details of the assay. [b] MIC is the lowest concentration (μg/mL) of an agent resulting in a 99% reduction in the number of colonies on a plate as compared to those on a plate free of the agent at the same dilution of the culture suspension.

Scheme 1

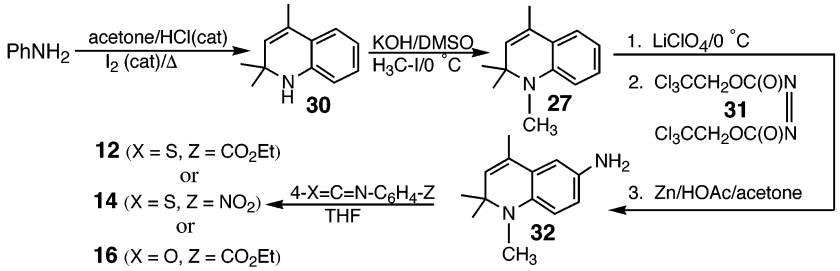


Scheme 2

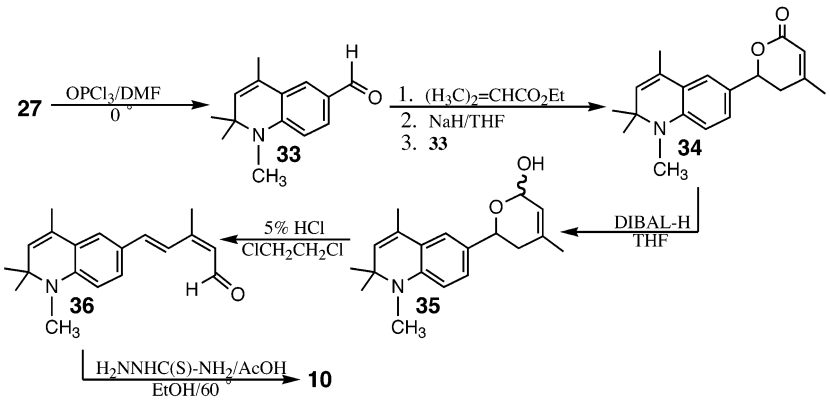


inserting a group at C-7 would alter the degree of rotation around the thiourea-type linking group and might well affect the biological activity of the compounds. Nitration of **24**[15] under conditions as illustrated in Scheme 6 gave a mixture of the 5-nitro isomer, the 7-nitro isomer, and some dinitro-substituted compound. Proton NMR analysis revealed a ratio of approximately 3:5:1, respectively, of three products. Repeated and very careful flash chromatography allowed the isomer **38** (32%) to be obtained, but it was best reduced at once to amine **39**. The optimum conditions for reduction of **38** to **39** (89%) proved to be the use of $TiCl_3$ in an acidic medium. Treatment of **39** with 4-nitrophenyl thioisocyanate in THF afforded **18** under the usual conditions. In the reaction of **39** with 4-nitrophenylbenzoyl isothiocyanate, all reagents and equipment had to be exceptionally dry to avoid severe mixtures in the product. Although **20** was a solid and could be recrystallized, it retained a small amount of water in the solid state, even after extensive drying. The rationale for the additional atom in the linking group of **20**, compared to that in **18**, was to give a measure of activity with a more flexible linker.

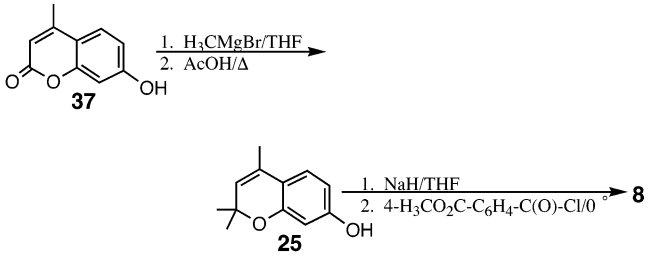
Scheme 3



Scheme 4



Scheme 5



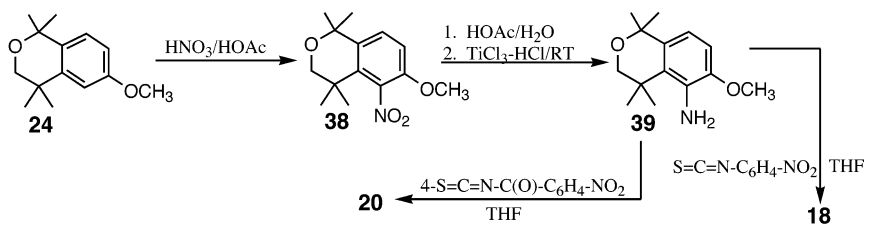
Biological Activity

The 15 heteroarotinoids listed in Table 1 were tested for antibacterial activity against *Mycobacterium bovis* (BCG) and compared to that of Isoxyl (**7**) by the technique previously reported from our group.[13] The results are listed as MIC values in Table 1. The MIC value has been defined as the lowest concentration (in μg/mL) of drug resulting in 99% reduction in the number of bacterial colonies on a plate compared to those on a plate free of the drug at the same dilution of the bacterial culture suspension.[13] Isoxyl (**7**) demonstrated a MIC value of 0.5 μg/mL against BCG (Table 1), indicating potent antibacterial activity. Several of the heteroarotinoids exhibited modest antibacterial activity, with MIC values of 20.0−40.0 μg/mL. Compounds **9** and **10** showed promising activity with MIC values of 5.0−10.0 and 10.0−20.0 μg/mL, respectively. Although heteroarotinoids **9**, **11**, and **12** are structurally similar to **7**, the activities of the former are reduced. In contrast, agent **8**, which is less structurally similar to **7**, demonstrated the best activity against BCG with a MIC value of 2.0−4.0 μg/mL.

Results and Discussion

In summary, 15 heteroarotinoids were evaluated for antibacterial activity against *M. bovis* with isoxyl (**7**) as the reference. Compound **8** displayed the best activity with a MIC of 2.0−4.0 μg/mL. Both **9** and **10** exhibited MIC values of 5.0−10 and 10.0−20.0 μg/mL, respectively, while all other examples had values >20.0 μg/mL. It is speculated that the antibacterial activity may be the result of the inhibition of mycolic acid synthesis and/or the inhibition of DHFR. The latter might result from the cleavage of the ester group in **8**, for example, to a carboxyl anion, as certain studies conclude from the interactions of some endogenous ligands with receptor proteins,[15,18,19] and a "salt bridge" could be created via interactions with Arg32 and Arg60, as in **4**.[5] Another observation[5] made on the DHFR enzyme was that inhibitors **4**−**6** adopted a "curved conformation" with the nitrogen-containing rings oriented toward the binding site in the interior of the enzyme, while the aromatic ring was closer to the surface. The structure of **8** is slightly shorter than that of **4** or **6** and can assume a near linear conformation and/or a curved conformation

Scheme 6



as judged by molecular models. Consequently, **8** may be able to locate between the interior, hydrophobic amino acid residues as well as extending toward the exterior Arg32 and Arg60 residues of the ligand binding site within the enzyme. Such a position could endow **8** with antibacterial properties. Further work is needed to delineate a more exact role of heteroarotinoids in terms of antibacterial chemotherapy. The compounds herein are the first examples of heteroarotinoids to display inhibitory ability against *M. bovis*.

Experimental Section

General. Commercial reagents and solvents were used as received unless otherwise noted. Anhydrous THF was obtained by distillation from a purple solution of sodium and benzophenone. All of the isocyanates and isothiocyanates were commercially available and were obtained from Lancaster Synthesis, Inc., Windham, NH. Unless otherwise indicated, all reactions were run under dry N_2, and all purification beyond recrystallization was done by flash chromatography (J. T. Baker flash chromatography silica gel packing, 40 μm particle size). Melting points were determined on a Thomas-Hoover melting point apparatus and were uncorrected. IR spectra were obtained using a Perkin-Elmer 2000 FT-IR spectrometer as films or KBr pellets. Unless stated otherwise, all ^{1}H and ^{13}C NMR spectra were performed using $DCCl_3$ (unless otherwise noted) as the solvent and obtained on a Varian ^{UNITY}INOVA 400 BB NMR spectrometer operating at 399.99 and 100.01 MHz, respectively. Signals were referenced to TMS. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Although the intermediates and final products appeared relatively stable in light, precautions were taken to minimize exposure to any light source and to the atmosphere. In the purified state, all products were stored in the cold and dark without significant decomposition. Compounds **24** and **26** were prepared by known methods.[15]

2,2,4-Trimethyl-2*H*-chromen-7-yl 4-(Methoxycarbonyl)benzoate (8). A suspension of NaH (0.054 g, 2.14 mmol, 1.02 equiv) in dry THF (1 mL) was chilled to 0 °C, and then **25** (0.400 g, 2.10 mmol) in dry THF (3 mL) was added dropwise. When the addition was complete (~10 min), monomethyl terephthaloyl chloride (04.64 g, 2.33 mmol, 1.1 equiv) was added dropwise. The resulting mixture was allowed to warm to room temperature slowly and then was stirred for 12 h. The final mixture was poured into water (15 mL) containing 3 drops of glacial acetic acid. Two layers separated, and the water layer was extracted (EtOAc, 4 × 15 mL). The combined organic layer and extracts was washed with 10% NaOH (2 × 15 mL), water (15 mL), and brine (15 mL). After drying (Na_2SO_4), the solution was evaporated to a solid which was recrystallized ($HCCl_3$:pentane, 1:1) to afford **8** (0.270 g, 36%) as a shiny, white solid: mp 94−95 °C; IR 1740, 1731 cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.41 (s, 6 H), 2.01 (d, 3 H, J = 1.89 Hz), 3.96 (s, 3 H), 5.41 (m, 1 H), 6.68 (d, 1 H, J = 2.0 Hz), 6.74 (dd, 1 H, J = 2.01, 7.97 Hz), 7.17 (d, 1 H, 7.98 Hz) 8.16 (d, 2 H, 8.01 Hz), 8.24 (d, 2 H, J = 8.02 Hz); ^{13}C NMR ($DCCl_3$) ppm 17.94, 28.15, 52.46, 76.48, 109.76, 113.25, 121.08−153.94, 164.17, 166.14. Anal. ($C_{21}H_{20}O_5$) C, H.

General Procedure for the Preparation of Heteroarotinoids 9, 11, 13, 19, 21, and 22 from Corresponding Amine 29a or 29b. To a solution of amine **29a** (205 mg, 1.0 mmol) or **29b** (205 mg, 1.0 mmol) in dry THF (4.5 mL) at 0 °C was added dropwise the desired isocyanate or isothiocyanate (1.04 mmol) in dry THF (5 mL) over 3 min. After the addition, the reaction mixture was allowed to warm to room temperature and was stirred for 24 h. The solvent was evaporated, and the resulting residue was purified to give an analytically pure sample of the corresponding heteroarotinoid.

Ethyl 4-{[*N*-(2,2,4,4-Tetramethylchroman-6-yl)thiocarbamoyl]amino}benzoate (9). The compound was prepared from amine **29a** and 4-ethoxycarbonylphenyl isothiocyanate (215 mg, 1.04 mmol). Evaporation of the solvent gave a tan, viscous oil, which was subjected to flash chromatography [Et_2O:hexanes (1:1)] to afford **9** (284 mg, 69%) as a flaky, white solid: mp 102−104 °C; IR (KBr) 3351, 3289, 1714 cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.35 (m, 15 H), 1.85 (s, 2 H), 4.35 (q, J = 7.16 Hz, 2 H), 6.85 (d, J = 8.07 Hz, 1 H), 7.04 (dd, J = 7.98, 2.07 Hz, 1 H), 7.24 (d, J = 2.04 Hz, 1 H), 7.57 (d, J = 8.12 Hz, 2 H), 7.81 (bs, 1 H), 8.01 (d, J = 8.05 Hz, 2 H), 8.09 (bs, 1 H); ^{13}C NMR ($DCCl_3$) ppm 14.28, 28.45, 31.06, 32.70, 48.47, 60.96, 75.09, 119.39, 123.01, 124.85, 124.92, 127.47, 128.25, 130.42, 133.32, 141.99, 152.35, 152.35, 165.85, 179.47. Anal. ($C_{23}H_{28}N_2O_3S$) C, H, N, S.

{[(1*E*,3*Z*,5*E*)-1-Aza-4-methyl-6-(1,2,2,4-tetramethyl-(1,2-dihydroquinolyl))hexa-1,3,5-trienyl]amino}aminomethane-1-thione (10). To thiosemicarbazide (71.31 mg, 0.76 mmol), dissolved in 4 mL of water and AcOH (1 drop), was added dropwise a warmed (60 °C) solution of aldehyde **36** (200 mg, 0.71 mmol) dissolved in EtOH (95%, 5 mL). A precipitate formed immediately. The reaction mixture stood for 24 h (0 °C), and then the solid was filtered off. Recrystallization (EtOAc:diethyl ether, 1:1) of the solid afforded **10** (123 mg, 41%) as a light orange solid: mp 177−179 °C; IR (neat) 3428, 3254, 3156 cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.32 (s, 6 H), 2.01 (d, 3 H), 2.06 (d, 3 H), 2.84 (s, 3 H), 5.32 (d, 1 H), 6.17 (d, 1 H), 6.47 (d, 1 H), 6.72 (s, 1 H), 7.14−7.25 (m, 3 H); 7.88 (s, 1 H), 7.91 (s, 1H), 9.28 (s, 1 H), ^{13}C NMR ($DCCl_3$) ppm, 18.57, 27.64, 30.79, 56.63, 110.55, 121.81−155.04, 177.75. Anal. ($C_{20}H_{26}N_4S$) C, H, N.

[(4-Nitrophenyl)amino][(2,2,4,4-tetramethylchroman-6-yl)amino]methane-1-thione (11). The compound was prepared from amine **29a** and 4-nitrophenyl isothiocyanate (187 mg, 1.04 mmol). Evaporation of the solvent gave a solid, which was subjected to flash chromatography (Et_2O:hexanes, 2:1) to afford **11** (327 mg, 85%) as a light yellow solid: mp 166−168 °C; IR (KBr) 3346, 3215 cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.35 (s, 6 H), 1.38 (s, 6 H), 1.86 (s, 2 H), 6.87 (d, J = 7.98 Hz, 1 H), 7.04 (dd, J = 8.01, 2.06 Hz, 1 H), 7.23 (d, J = 2.01 Hz, 1 H), 7.74 (d, J = 8.03 Hz, 2 H), 7.87 (bs, 1 H), 8.18 (d, J = 8.0 Hz, 2 H) 8.35 (s, 1 H); ^{13}C NMR ($DCCl_3$) ppm 28.45, 31.09, 32.72, 48.38, 75.21, 119.66, 122.81, 124.42, 124.89, 127.69, 133.65, 144.03, 144.32, 152.71, 179.15. Anal. ($C_{20}H_{23}N_3O_3S$) C, H, N, S.

Ethyl 4-({[(1,2,2,4-Tetramethyl-1,2-dihydroquinol-6-yl)amino]thioxomethyl}amino)benzoate (12). To a solution of (1,2,2,4-tetramethyl-1,2-dihydroquinol-6-yl)amine (**32**, 150 mg, 0.74 mmol) in 4 mL of dry THF in oven-dried equipment and cooled to −5 °C was added dropwise ethyl 4-isothiocyanatobenzoate (161 mg, 7.78 mmol, 1.05 equiv) dissolved in dry THF (5 mL, 30 min). After the addition, the reaction mixture was allowed to warm to room temperature and was then stirred (24 h). The solvent was evaporated to a solid which was recrystallized (H_2CCl_2:pentane, 1:2) to afford **12** (275 mg, 91%) as a pale yellow solid: mp 161−162 °C; IR (KBr pellet) 3344, 3289, 1712 cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.34 (s, 6 H), 1.37 (t, 3 H), 1.60 (bs, 1 H), 1.95 (s, 3 H), 2,83 (s, 3 H), 4.35 (q, 2), 5.36 (s, 1 H), 6.51 (d, 1 H, J = 8.7 Hz), 6.94 (d, 1 H, J = 2.4 Hz), 7.13 (q, 1 H, J = 2.4 Hz, J = 8.7 Hz), 7.60 (d, 2 H, J = 8.7 Hz), 7.75 (bs, 1 H), 8.01 (d, 2 H, J = 8.7 Hz); ^{13}C NMR ($DCCl_3$) ppm 14.30, 18.53, 27.77, 30.86, 56.75, 60.92, 111.04, 121.66, 123.06−143.5, 165.97, 179.92. Anal. ($C_{23}H_{27}N_3O_2S$) C, H, N.

Ethyl 4-{[*N*-(2,2,4,4-Tetramethylchroman-6-yl)carbamoyl]amino}benzoate (13). The ester was prepared from amine **18a** and 4-ethoxycarbonylphenyl isocyanate (199 mg, 1.04 mmol). Evaporation of the solvent gave a white solid, which was recrystallized (EtOAc) to afford **13** (261 mg, 66%) as a white solid: mp 234−235 °C; IR (KBr) 3346, 3195, 1713, 1655 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.28 (m, 15 H), 1.80 (s, 2 H), 4.29 (q, J = 7.09 Hz, 2 H), 6.65 (d, J = 7.99 Hz, 1 H), 7.09 (dd, J = 7.97, 1.98 Hz, 1 H), 7.44 (d, J = 2.01 Hz, 1 H), 7.58 (d, J = 8.08 Hz, 2 H), 7.88 (d, J = 8.06 Hz, 2 H), 8.52 (s, 1 H), 8.99 (s, 1 H); ^{13}C NMR (DMSO-d_6) ppm 14.23, 28.10, 30.61, 32.50, 48.35, 60.20, 73.84, 117.10, 117.41, 117.51, 118.57, 122.41, 130.33, 131.29, 132.10, 144.56, 147.48, 152.33, 165.43. Anal. ($C_{23}H_{28}N_2O_4$) C, H, N.

[(4-Nitrophenyl)amino][(1,2,2,4-tetramethyl(1,2-dihydroquinol-6-yl))amino]methane-1-thione (14). To a chilled (−5 °C) solution of (1,2,2,4-tetramethyl-1,2-dihydroquinol-6-yl)amine (**32**, 150 mg, 0.74 mmol) dissolved in dry THF (5 mL) was added dropwise 4-nitrophenyl isothiocyanate (141 mg, 7.78 mmol, 1.05 equiv) dissolved in dry THF (5 mL, 30 min). After the addition, the reaction mixture was allowed to warm to room temperature and was then stirred for 24 h. The solvent was evaporated, and the resulting solid was recrystallized (EtOAc:hexane, 1:1) to afford **14 (**184 mg, 65%) as an orange-yellow solid: mp 172−173.5; IR (KBr pellet) 3338, 3181 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.39 (s, 6 H), 1.93 (s, 3 H), 2,82 (s, 3 H), 5.30 (s, 1 H), 6.52 (d, 1 H, J = 8.7 Hz), 6.92 (d, 1 H, J = 2.4 Hz), 7.02 (q, 1 H, J = 2.4 Hz, J = 8.7 Hz), 7.67 (bs, 1 H), 7.77 (d, 2 H, J = 9.0 Hz), 7.87 (bs, 1 H), 8.19 (d, 2 H, J = 9.0 Hz); ^{13}C NMR ($DCCl_3$) ppm 18.52, 27.89, 30.91, 56.85, 111.08, 121.61, 122.81−145.44, 179.65. TLC analysis for $C_{20}H_{22}N_4O_2S$ showed one spot in the following solvent systems: hexane: diethyl ether:H_2CCl_2, (1:1:1), R_f 0.40; chloroform:pentane, (2:1), R_f 0.19; hexane:EtOAc, (2:1), R_f 0.14. Anal. ($C_{20}H_{22}N_4O_2S\cdot 1.37\ H_2O$) C, H, N.

Ethyl 4-{[*N*-(2,2,4,4-Tetramethylthiochroman-6-yl)carbamoyl]amino}benzoate (15). A solution of **26** (400 mg, 1.81 mmol) in dry THF (8 mL) was treated dropwise with 4-ethoxycarbonylphenyl isocyanate (380 mg, 1.99 mmol) in dry THF (8 mL) at 0−5 °C. The reaction was warmed to room temperature and was stirred overnight. Evaporation of the solvent gave a solid which was recrystallized (pentane:H_2CCl_2, 1:2) to give **15** (480 mg, 63%) as a white solid: mp 200−201 °C; IR (KBr) 1688 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.33 (s, 6 H), 1.37 (t, 3 H,), 1.38 (s, 6 H), 1.89 (s, 2 H), 4.35 (q, 2 H), 6.92 (dd, 1 H), 7.06 (s, 1 H), 7.26 (d, 1 H), 7.36 (d, 2 H), 7.43 (d, 2 H), 7.52 (s, 1 H), 7.92 (s, 2 H); ^{13}C NMR ($DCCl_3$) ppm 14.20, 31.46, 32.25, 35.66, 42.07, 54.10, 60.91, 118.49, 120.01, 120.66, 124.80, 128.93, 129.02, 130.97, 134.49, 142.95, 144.23, 153.35, 166.73. Anal. ($C_{23}H_{28}N_2O_3S$) C, H, N, S.

Ethyl 4-{[(1,2,2,4-Tetramethyl(1,2-dihydroquinol-6-yl)-carbamoyl]amino]benzoate (16). A solution of (1,2,2,4-tetramethyl-1,2-dihydroquinol-6-yl)amine (**27**, 150 mg, 0.74 mmol) dissolved in dry THF (4 mL) in oven-dried equipment was cooled to −5 °C (ice and NaCl), and ethyl 4-isocyanatobenzoate (148.5 mg, 7.78 mmol, 1.05 equiv) dissolved in dry THF (5 mL) was then added dropwise (30 min). After the addition, the reaction mixture was allowed to warm to room temperature and was then stirred for 24 h. The solvent was evaporated, and the solid was recrystallized (pentane:$HCCl_3$, 1:1) to afford **16** (206 mg, 71%) as a white, flaky solid: mp 211−212 °C; IR (KBr pellet) 3352, 3262, 1709 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.29 (s, 6 H), 1.36 (t, 3 H), 1.63 (bs, 1 H), 1.94 (s, 3 H), 2,87 (s, 3 H), 4.37 (q, 2 H), 5.33 (s, 1 H), 6.47 (d, 1 H, J = 8.3 Hz), 6.67 (bs, 1 H), 6.97 (d, 1 H, J = 1.9 Hz), 7.13 (q, 1 H, J = 1.9 Hz, J = 8.3 Hz), 7.4 (d, 2 H, J = 9.0 Hz), 7.92 (d, 2 H, J = 9.0 Hz); ^{13}C NMR ($DCCl_3$) ppm 14.33, 18.50, 27.21, 30.75, 56.39, 60.70, 111.11, 118.14, 120.64−143.96, 154.23, 180.45. TLC analysis for $C_{23}H_{27}N_3O_3$ showed one spot in the following solvent systems: hexane:diethyl ether:H_2CCl_2, (1:1:1), R_f 0.46; chloroform:pentane, (2:1), R_f 0.40; hexane:EtOAc, (2:1), R_f 0.18. Anal. ($C_{23}H_{27}N_3O_3\cdot 0.3H_2O$) C, H, N.

N*-4-[2,3-Dihydro-2,2,4,4-tetramethyl-2H-1-benzothiopyran-6-yl)-N-(4-ethoxycarbonylphenyl)*urea (17). A solution of crude amine **26** (400 mg, 1.70 mmol) in THF (10 mL) was treated dropwise with 4-ethoxycarbonylphenyl isocyanate (412 mg, 1.99 mmol) in THF (10 mL) at 0−5 °C. The reaction mixture was allowed to warm to room temperature and was stirred overnight. Evaporation of the solvent gave a solid. Recrystallization (pentane:CH_2Cl_2, 6:1) gave **17** (710 mg, 92%) as a white solid: mp 134−135 °C; IR (KBr) 1688 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.38 (s, 6 H), 1.40 (t, 3 H, J = 7.2 Hz), 1.43 (s, 6 H), 1.95 (s, 2 H), 4.35 (q, 2 H, J = 7 Hz), 7.04 [dd, 1 H, Ar−H], 7.16 [s, 1 H, Ar−H], 7.36 [d, 1 H, Ar−H], 7.55 [d, 2 H, Ar−H], 8.00 [s, 1 H, NH], 8.02 [d, 2 H, Ar−H], 8.35 [s, 1 H, NH]; ^{13}C NMR ($DCCl_3$) ppm 14.16, 31.46, 32.35, 35.67, 42.25, 53.72, 60.97, 122.95, 123.17, 124.05, 127.72, 129.27, 130.65, 132.97, 133.08, 141.86, 144.70, 165.96, 179.21. Anal. ($C_{23}H_{28}N_2O_2S_2$) C, H, N, S.

[(6-Methoxy-1,1,4,4-tetramethylisochroman-5-yl)amino]-[(4-nitrophenyl)amino]methane-1-thione (18). To a cooled (−5 °C) solution of (6-methoxy-1,1,4,4-tetramethylisochroman-5-yl)amine [**39**, 200 mg, 0.85 mmol] dissolved in dry THF (5 mL) in oven-dried equipment was added dropwise (1 h) 4-nitrophenyl isothiocyanate (160.7 mg, 8.92 mmol, 1.05 equiv). After the addition, the reaction mixture was allowed to warm to room temperature and was then stirred (24 h). The solvent was evaporated, and resulting solid was recrystallized (pentane:H_2CCl_2, 1:1) to afford **18** (251 mg, 71%) as a light yellow solid: mp 181−182 °C; IR (KBr pellet) 3368, 3214 cm^{-1}; ^1H NMR [$D_3C(O)CD_3$] δ 1.24 (s, 6 H), 1.46 (s, 6 H), 3.56 (s, 2 H), 3.87 (s, 3 H), 7.01 (d, 1 H, J = 8.7 Hz), 7.25 (d, 1 H, J = 8.7 Hz), 7.60 (bs, 1 H), 8.01 (d, 2 H, J = 8.5 Hz), 8.15 (d, 2 H, J = 8.5 Hz), 8.48 (bs, 1 H); ^{13}C NMR [$D_3C(CO)CD_3$] ppm 18.06, 26.72, 28.91, 34.54, 56.10, 75.55, 108.65, 122.11−145.87, 181.15. Anal. ($C_{21}H_{25}O_4N_3S$) C, H, N, S.

Ethyl 4-{[*N*-(2,2,4,4-Tetramethylchroman-8-yl)thiocarbamoyl]amino}benzoate (19). The ester was prepared from amine **29b** and 4-ethoxycarbonylphenyl isothiocyanate (215 mg, 1.04 mmol). Evaporation of the solvent gave a tan, viscous oil, which was subjected to flash chromatography (Et_2O: hexanes, 1:1) to afford **19** (412 mg, 88%) as a white solid: mp 56−58 °C; IR (KBr) 3325, 3199, 1715 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.29 (m, 15 H), 1.83 (s, 2 H), 4.38 (q, J = 7.12 Hz, 2 H), 6.94 (m, 1 H), 7.15 (dd, J = 7.91, 1.98 Hz, 1 H), 7.51 (d, J = 7.99 Hz, 2 H), 7.98 (m, 1 H), 8.07 (d, J = 8.02 Hz, 2 H), 8.30 (bs, 1 H), 8.40 (bs, 1 H); ^{13}C NMR ($DCCl_3$) ppm 14.26, 28.47, 31.02, 32.57, 48.71, 61.02, 75.98, 120.14, 120.49, 123.01, 124.06, 126.38, 127.64, 130.93, 132.27, 141.45, 143.91, 165.73, 177.83. Anal. ($C_{23}H_{28}N_2O_3S$) C, H, N, S.

***N*-{[(6-Methoxy-1,1,4,4-tetramethylisochroman-5-yl)-amino]thioxomethyl}(4-nitrophenyl)carboxamide (20)**. To a cooled (−5 °C) solution of (6-methoxy-1,1,4,4-tetramethyl-isochroman-5-yl)amine [**39**, 200 mg, 0.85 mmol] dissolved in dry THF (5 mL) in oven-dried equipment was added dropwise (1 h) ethyl (4-nitrophenyl)oxomethane isocyanate (186 mg, 8.92 mmol, 1.05 equiv) dissolved in dry THF (5 mL). After the addition, the reaction mixture was allowed to warm to room temperature and was then stirred (24 h). The solvent was evaporated, and the resulting solid was recrystallized (pentane: $HCCl_3$, 1:2) to afford **20** (305 mg, 81%) as a yellow solid: mp 179 °C (dec); IR (KBr pellet) 3229, 3171, 1686 cm^{-1}; ^1H NMR [$D_3C(O)CD_3$] δ 1.33 (s, 6 H), 1.56 (s, 6 H), 3.57 (s, 2 H), 3.80 (s, 3 H), 6.85 (d, 1 H, J = 8.3 Hz), 7.10 (d, 1 H, J = 8.3 Hz), 8.10 (d, 1 H, J = 7.8 Hz), 8.39 (dd, 1 H, J = 7.8 Hz), 10.85 (s, 1 H), 11.82 (s, 1 H); ^{13}C NMR [$D_3C(O)CD_3$] ppm 24.10, 26.67, 34.55, 56.05, 71.34, 111.19, 124.24−135.77, 168.77, 182.56. Anal. ($C_{22}H_{25}N_3O_5S\cdot 0.6H_2O$) C, H, N, S.

[(4-Nitrophenyl)amino][(2,2,4,4-tetramethylchroman-8-yl)amino]methane-1-thione (21). Ether **21** was prepared from amine **29b** and 4-nitrophenyl isothiocyanate (187 mg, 1.04 mmol). Evaporation of the solvent gave a yellow oil, which was subjected to flash chromatography (Et_2O:hexanes, (2:1) to afford **21** (316 mg, 82%) as a yellow solid: mp 160−161 °C; IR (KBr) 3327, 3232 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.35 (s, 6 H), 1.36 (s, 6 H), 1.87 (s, 2 H), 6.96 (m, 1 H), 7.22 (dd, J = 8.02, 2.04 Hz, 1 H), 7.62 (m, 1 H), 7.68 (d, J = 8.07 Hz, 2 H), 8.21 (d, J = 8.07 Hz, 2 H), 8.30 (bs, 1 H), 8.34 (bs, 1 H); ^{13}C NMR ($DCCl_3$) ppm 28.51, 31.09, 32.61, 48.67, 76.26, 120.48, 12.37, 122.53, 124.79, 125.24, 125.47, 133.10, 143.91, 144.21, 144.80, 178.21. Anal. ($C_{20}H_{23}N_3O_3S$) C, H, N, S.

Ethyl 4-{[*N*-(2,2,4,4-Tetramethylchroman-8-yl)carbamoyl]amino}benzoate (22). Ether **22** was prepared from amine **29b** (0.130 g, 0.63 mmol) and 4-ethoxycarbonylphenyl isocyanate (199 mg, 1.04 mmol). Evaporation of the solvent gave a tan residue, which was flash chromatographed [Et_2O: hexanes (8:1)] to afford **22** (332 mg, 84%) as a white solid: mp 174−176 °C; IR (KBr) 3348, 3201, 1715, 1675 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.27 (m, 15 H), 1.78 (s, 2 H), 4.34 (q, J = 7.21 Hz, 2 H), 6.87 (m, 1 H), 6.97 (dd, J = 8.03, 1.98 Hz, 1 H), 7.44 (d, J = 8.07 Hz, 2 H), 7.74 (s, 1 H), 7.91 (d, J = 7.98 Hz, 2 H), 7.95

(d, J = 1.99 Hz, 1 H), 8.03 (s, 1 H); ^{13}C NMR ($DCCl_3$) ppm 14.28, 28.41, 30.97, 32.53, 49.02, 60.28, 75.45, 117.31, 118.26, 120.49, 120.89, 124.21, 127.61, 130.80, 131.36, 141.45, 143.39, 152.80, 166.66. Anal. ($C_{23}H_{28}N_2O_4$) C, H, N.

2,2,4,4-Tetramethylchroman (23). To 3.0 M methylmagnesium bromide in diethyl ether (121 mL, 0.36 mol), along with dry THF (240 mL), was added dropwise (room temperature) 4,4-dimethylcoumarin **14**[20] (16.00 g, 90.8 mmol) dissolved in THF (10 mL), and the resulting mixture was stirred at reflux for 4 days. The mixture was allowed to cool to room temperature and then was cooled to 0 °C (ice bath). Saturated, aqueous NH_4Cl solution (820 mL) was added, the resulting aqueous layer was extracted (Et_2O, 4 × 150 mL), and the combined organic extracts were washed with H_2O (150 mL) and brine (150 mL) and then were dried (Na_2SO_4). Evaporation of the solvent gave a pale yellow oil, which crystallized on standing. Recrystallization (petroleum ether) of the yellow solid gave 4-(2-hydroxyphenyl)-2,4-dimethyl-2-pentanol (16.00 g, 84%) as clear, needlelike crystals: mp 88−90 °C (lit.[16] mp 91 °C); IR (KBr) 3406, 1731 cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.17 (s, 6 H), 1.41 (s, 6 H), 2.24 (s, 2 H), 6.58 (dd, J = 7.21, 1.56 Hz, 1 H), 6.87 (m, 1 H), 7.04 (m, 1H), 7.35 (dd, J = 7.18, 1.59 Hz, 1 H); ^{13}C NMR ($DCCl_3$) ppm 30.89, 30.94, 37.50, 52.31, 73.43, 117.15, 120.89, 127.50, 127.71, 133.98, 155.05. Previous data on this diol were an elemental analysis[16] and some spectral information on a crude sample.[20]

A mixture of the above diol[18] (45.02 g, 0.22 mol), 85% H_3PO_4 (21.21 mL, 0.18 mol), and benzene (225 mL) was stirred and heated (80 °C). After ∼5 min, the first of 3 portions of P_2O_5 (25.42 g, 0.54 mol) was added. The other 2 portions of P_2O_5 were added at ∼7 h intervals for a total of 76.26 g (3 × 25.42 g), and the reaction mixture was allowed to reflux (24 h). The reaction mixture was allowed to cool to room temperature, and the benzene solution was decanted from a dark, purple residue in the bottom of the reaction flask. The residue was simply rinsed (ether, 3× 150 mL), and the ether extracts were combined with the benzene solution. The combined organic extracts were washed with 5% aqueous $NaHCO_3$ (3 × 100 mL) and brine (3 × 100 mL) and were then dried (Na_2SO_4). The solvent was evaporated to a liquid which was subsequently vacuum distilled to afford 2,2,4,4-tetramethylchroman (**23**) (21.5 g, 54%) as a colorless oil: bp 69−71 °C/1.5 mmHg (lit.[16] bp 102−104 °C); ^{1}H NMR ($DCCl_3$) δ 1.34 (s, 6 H), 1.35 (s, 6 H), 1.84 (s, 2 H), 6.79 (dd, J = 7.68, 1.79 Hz, 1 H), 6.85 (m, 1 H), 7.08 (m, 1 H), 7.31 (dd, J = 7.76, 1.83 Hz, 1 H); ^{13}C NMR ($DCCl_3$) ppm 28.45, 30.74, 32.70, 49.20, 74.30, 117.97, 120.66, 126.82, 127.05, 131.63, 152.62. The only prior information on chroman **23** was an elemental analysis.[16]

2,2,4-Trimethyl-2*H*-1-benzopyran-7-ol (25). To 3.0 M methylmagnesium bromide in diethyl ether (30 mL, 90.8 mmol), along with dry THF (60 mL), was added dropwise (room temperature) coumarin **37**[17] (4.00 g, 22.7 mmol) dissolved in THF (25 mL), and the resulting mixture was stirred at room temperature (2 days). During the addition, some heat evolved, and a yellow precipitate formed. The reaction was quenched with saturated, aqueous NH_4Cl (250 mL), and the resulting mixture was separated into two layers. The aqueous layer was extracted (Et_2O, 5 × 50 mL), and the combined organic extracts were dried (Na_2SO_4). Evaporation of the solvent afforded a dark orange oil which was dissolved in 20 mL of glacial acetic acid, and the resulting solution was stirred and heated gently (slightly below the boiling point of acetic acid) for 2 h. The reaction mixture was allowed to cool to room temperature and was poured into H_2O (100 mL). The resulting emulsion was extracted (Et_2O, 5 × 50 mL), and the combined organic extracts were washed with saturated, aqueous $NaHCO_3$ (3 × 50 mL) and brine (2 × 50 mL) and were dried (Na_2SO_4). Evaporation of the solvent gave a dark brown, viscous oil, which was subjected to flash chromatography (ether:hexanes, 5:1) that provided **25** (1.51 g, 40%) as a white solid: mp 128−130 °C; IR (KBr) 3330 cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.38 (s, 6 H), 1.96 (d, J = 2.09 Hz, 3 H), 5.17 (bs, 1 H), 5.28 (m, 1 H), 6.32 (d, J = 2.01 Hz, 1 H), 6.35 (dd, J = 8.01, 2.0 Hz, 1 H), 6.99 (d, J = 8.03 Hz, 1 H); ^{13}C NMR ($DCCl_3$) ppm 17.92, 28.03, 76.38, 103.67, 107.42, 116.62, 124.29, 124.87, 127.41, 154.22, 156.34. Anal. ($C_{12}H_{14}O_2$) C, H.

1,2,2,4-Tetramethyl-1,2-dihydroquinoline (27). A mixture of powdered KOH (299 mg, 5.7 mmol) dissolved in DMSO (10 mL) was stirred at room temperature until all KOH dissolved, and then the temperature was adjusted to 10 °C. 2,2,4-Trimethyl-1,2-dihydroquinoline (**30**, 1.00 g, 5.77 mmol) dissolved in DMSO (5 mL) was added dropwise, followed immediately by the addition of CH_3I (1.09 g, 7.7 mmol). The reaction mixture was stirred (0.5 h) and then was poured into ice-cold water (10 mL). Extracts with H_2CCl_2 (3 × 5 mL) were combined and washed with water (1 × 10 mL) and brine (1 × 10 mL) and then dried (Na_2SO_4). Evaporation of the solvent and flash chromatography (silica gel, hexanes) of the residue afforded **27** as a yellow oil (0.784 g, 71%): IR (neat) 1048 cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.37 (s, 6 H), 2.23 (s, 3 H), 2,96 (s, 3 H), 5.36 (s, 1 H), 6.87−7.24 (m, 4 H); ^{13}C NMR ($DCCl_3$) ppm 18.64, 25.74, 30.60, 54.53, 111.43, 112.5, 118.12−144.56. The compound was used immediately to prepare **32** and/or **33**.

2,2,4,4-Tetramethyl-6-nitrochroman (28a) and 2,2,4,4-Tetramethyl-8-nitrochroman (28b). To a solution of the chroman **23**[17] (5.00 g, 26.3 mmol) in freshly distilled Ac_2O (5 mL) at −5 °C (ice/salt) was added dropwise a mixture of cold, concentrated HNO_3 (1.89 mL) and Ac_2O (4.42 mL) over 10 min. After being stirred at −5 °C (90 min), the reaction mixture was then poured into saturated, aqueous $NaHCO_3$ (50 mL). The resulting emulsion was extracted (H_2CCl_2, 3 × 30 mL), and the combined extracts were washed with H_2O (40 mL) and brine (40 mL) and were then dried (Na_2SO_4). Evaporation of the solvent gave a dark brown liquid, which was subjected to flash chromatography (hexanes:Et_2O, 20:1) to provide isomers **28a** and **28b** (isomer **28a** was collected in fractions 5−12, and isomer **28b** was collected in fractions 15−20). The 6-isomer **28a** (2.67 g, 43%) was isolated as a pale yellow solid: mp 56−58 °C; IR (KBr) 1581, 1341 cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.39 (s, 6 H), 1.41 (s, 6 H), 1.89 (s, 2 H), 6.85 (d, J = 7.93 Hz, 1 H), 7.98 (dd, J = 7.01, 1.98 Hz, 1 H), 8.22 (d, J = 1.97 Hz, 1 H); ^{13}C NMR ppm 28.48, 31.12, 32.69, 48.07, 76.39, 118.45, 123.18, 123.54, 131.93, 141.38, 158.46. The 8-isomer **28b** (1.61 g, 26%) was obtained as a creamy white solid: mp 52−54 °C; IR (KBr) 1582, 1341 cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.37 (s, 6 H), 1.39 (s, 6 H), 1.91 (s, 2 H), 6.94 (m, 1 H), 7.48 (dd, J = 7.63, 1.87 Hz, 1 H), 7.57 (dd, J = 7.59, 1.83 Hz, 1 H); ^{13}C NMR ($DCCl_3$) ppm 28.35, 31.30, 32.55, 48.33, 76.86, 118.45, 119.52, 122.68, 130.86, 135.12, 146.89, 146.89. Both isomers were immediately used to prepare **29a** and **29b**, respectively.

2,2,4,4-Tetramethyl-6-aminochroman (29a) and 2,2,4,4-Tetramethyl-8-aminochroman (29b). A mixture of nitro compound **28a** (1.60 g, 6.8 mmol) or **28b**, iron powder (1.36 g, 24.3 mmol, Sigma-Aldrich Chemical Co.), glacial acetic acid (2.86 g, 47.6 mmol), and absolute EtOH (17 mL) was stirred at reflux (12 h). The reaction was allowed to cool to room temperature and poured into H_2O (68 mL), and the resulting emulsion was extracted with Et_2O (2 × 65 mL) and $HCCl_3$ (3 × 65 mL). The combined organic extracts were washed with H_2O (3 × 65 mL), dried (Na_2SO_4), and concentrated to a dark oil. This crude mixture was then dissolved in Et_2O (45 mL), and the resulting solution was extracted (2 N HCl, 2 × 55 mL). The acid solution was neutralized with 40% Na_2CO_3 (pH ∼ 8), and the resulting solution was extracted (Et_2O, 2 × 55 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was evaporated to amine **29a** (or **29b**) as an oil which crystallized upon standing at room temperature. The 6-isomer **29a** (586 mg, 42%) was obtained as an off-white solid: mp 40−42 °C; IR (KBr) 3433, 3357 cm^{-1}; ^{1}H NMR ($DCCl_3$) δ 1.30 (s, 6 H), 1.31 (s, 6 H), 1.78 (s, 2 H), 3.29 (bs, 2 H), 6.46 (dd, J = 8.03, 1.99 Hz, 1 H), 6.61 (d, J = 1.80 Hz, 1 H), 6.63 (d, J = 7.89 Hz, 1 H); ^{13}C NMR ($DCCl_3$) ppm 28.35, 30.96, 32.57, 49.32, 73.81, 113.41, 114.88, 118.29, 132.28, 139.51, 145.35.

It was discovered that a crude mixture of the isomeric nitro compounds **29a** and **29b** could be coreduced by the above method. The final mixture of amines **29a** and **29b** was more easily separated by flash column chromatography (hexanes: Et_2O, 1:1) than the corresponding mixture of nitro compounds

(fractions 4−10 yielded **29b** and fractions 17−25 yielded **29a**). The 8-isomer **29b** (430 mg, 31%) was obtained as a very light pink solid: mp 43−45 °C; IR (KBr) 3455, 3360 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.32 (s, 6 H), 1.36 (s, 6 H), 1.83 (s, 2 H), 3.54 (bs, 2 H), 6.54 (dd, J = 7.94, 2.02 Hz, 1 H), 6.70 (m, 2 H); ^{13}C NMR ($DCCl_3$) ppm 28.63, 30.96, 32.58, 49.34, 74.48, 112.37, 116.03, 120.24, 131.33, 136.11, 140.07. Amines **29a** and **29b** were used at once to prepare **9**, **11**, and **13** as well as **19**, **21**, and **22**, respectively.

2,2,4-Trimethyl-1,2-dihydroquinoline (30). A mixture of freshly distilled aniline (20.0 g, 0.21 mol) together with a catalytic amount of iodine (0.3 g) and concentrated HCl (0.2 mL) were heated to 155 °C, and then acetone (∼ 400 mL) was added slowly at such a rate so that the temperature of the mixture did not fall below 140 °C. Unreacted acetone and H_2O (a reaction byproduct) distilled off during the addition process. After another addition of acetone (250 mL, ∼3.5 h), the reaction mixture was stirred (1 h) and was then allowed to cool to room temperature. Extraction with hexane (3 × 100 mL) was followed by washing the combined organic extracts with H_2O (1 × 100 mL) and brine (1 × 100 mL) and then drying ($MgSO_4$). The hexane was evaporated, and the resulting product was purified by distillation (bp 109−111 °C/0.75 mmHg) to yield **30** as a pale yellow oil (27.9 g, 76%): IR (neat) 3301 [N−H] cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.41 (s, 6 H), 2.18 (s, 3 H), 3.79 (bs, 1 H), 5.45 (s, 1 H), 6.55−7.15 (m, 4 H); ^{13}C NMR ($DCCl_3$) ppm 18.64, 23.44, 54.34, 110.53, 111.91, 116.12−145.07. The compound **30** was used immediately to prepare **27**.

Bis(2,2,2-trichloroethyl) Azodicarboxylate (31). It was discovered that fresh and highly purified **31** was required in the preparation of **32**. Slightly crude commercial material and the long preparation in *Organic Syntheses*[21] for **31** prompted us to modify the latter approach. A solution of 1.0 g (0.023 mol) of 85% hydrazine hydrate in 6 mL of 95% ethanol was cooled in an ice bath, and 9.6 g (0.046 mol) of 2,2,2-trichloroethyl chloroformate was added dropwise so that the temperature was kept below 20 °C. During the addition of 1 equiv of the chloroformate, a white precipitate formed. After one-half of the chloroformate had been added, a solution of sodium carbonate (2.5 g, 0.024 mol) in water (10.0 mL) was added dropwise (2 h) along with the remaining chloroformate. The rate of addition of these two reagents was such that the flow of the chloroformate was faster than that of the sodium carbonate so that an excess of chloroformate was present at all times. The temperature was kept below 20 °C during the addition. As the second equivalent of chloroformate was added, the white precipitate dissolved. After the addition of the reactants was complete (4 h), the reaction was allowed to stir for an additional 30 min while the solution warmed to room temperature. The reaction mixture was then transferred to a separatory funnel, and the viscous, organic layer (bottom) was separated from the aqueous layer. The organic layer was dissolved in ether (20 mL), and the reaction vessel was washed with ether (10 mL). This ether washing was used to extract the aqueous layer again. The ether extracts were combined, dried ($MgSO_4$), and filtered, which was followed by evaporation of the solvent.

Bromine (1.6 g, 20.0 mmol) in dichloromethane (150 mL) was added dropwise (1 h) to a solution of dichloromethane (500 mL), hydrazide (7.0 g, 18.2 mmol, 85%), and pyridine (1.50 g, 20.0 mmol), and solution was cooled to 0 °C (ice bath) under argon. The reaction mixture turned from colorless to yellow upon the addition. The reaction was complete after 30−60 min at room temperature, as determined by TLC (silica gel, EtOAc: hexane, 1:1). The reaction mixture was then diluted to 1000 mL with dichloromethane and washed with 5% HCl (2 × 300 mL), saturated sodium bicarbonate (300 mL), water (3 × 300 mL), and saturated NaCl (1 × 300 mL) to give diazo compound **31** as light yellow solid [4.26 g, 56%]: mp 115−116 °C (lit.[21] mp 109−110.5 °C); IR (KBr pellet) 1786, 1723 cm^{-1}; ^1H NMR ($DCCl_3$) δ 5.08 (s, 4 H); ^{13}C NMR ($DCCl_3$) ppm 77.45, 93.09, 158.42. Diazo compound **31** was used immediately to prepare **32**.

[1,2,2,4-Tetramethyl-1,2-dihydroquinol-6-yl]amine (32). To 1,2,2,4-tetramethyl-1,2-dihydroquinoline (**27**, 1.00 g, 5.3 mmol) dissolved in a 3 M solution of $LiClO_4$ in diethyl ether (5 mL) was added dropwise freshly prepared bis(2,2,2-trichloroethyl) azodicarboxylate (**31**, 4.5 g, 11.8 mmol) dissolved in diethyl ether (5 mL) at 0 °C. The solution was then carefully warmed to 55 °C and stirred at this temperature (3 h). The new reaction mixture was cooled to 0 °C, and ice water (5 mL) was added. Extraction with H_2CCl_2 (3 × 10 mL), followed by washing the extracts with brine (1 × 5 mL) and drying (Na_2SO_4), yielded an aryl azide (2.58 g, 85%). This aryl azide was reduced, without purification, by dissolving it in concentrated acetic acid (5 mL). To this solution was added approximately 1 equiv by weight of Zn dust (2.6 g). The reaction mixture was stirred for 15 min, and 9 μL of acetone was added. After stirring the reaction mixture (3 h) at room temperature, the new mixture was filtered through Celite. Then a saturated, aqueous solution of $NaHCO_3$ (5 mL) was added, and the mixture was extracted (H_2CCl_2, 3 × 10 mL). Flash chromatography of the concentrated extracts with silica gel (hexane: EtOAc, 1:1) gave solid **32** (546 mg, 51% from **27**): mp 87−89 °C; IR (neat) 3338, 3224 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.30 (s, 6 H), 1.97 (s, 3 H), 2,78 (s, 3 H), 5.38 (s, 1 H), 6.42 (d, 1 H, J = 9.3 H), 6.48 (d, 1 H, J = 2.3 Hz), 6.55 (q, 1 H, J = 2.1 Hz, J = 9.3 Hz), 6.56 (s, 1 H), 6.8 (bs, 1 H); ^{13}C NMR ($DCCl_3$) ppm 18.53, 25.70, 30.68, 55.60, 111.91, 112.14, 115.72−138.78. Amine **32** was used at once to prepare **12**, **14**, and **16**.

1,2,2,4-Tetramethyl-1,2-dihydroquinoline-6-carbaldehyde (33). Phosphorus oxychloride (4.1 g, 0.026 mol) was added dropwise to DMF (12 mL) at 0 °C. After the reaction of $OPCl_3$ with DMF had subsided, 1,2,2,4-tetramethyl-1,2-dihydroquinoline [(**31**), 5 g, 0.026 mol] dissolved in DMF (30 mL) was slowly added at 0 °C. The reaction mixture was allowed to stir (24 h) at room temperature and was then cooled to 0 °C, after which cold water (5 mL) was carefully added. The reaction mixture was extracted with methylene chloride (3 × 30 mL), and the combined organic extracts were washed with water (1 × 10 mL) and brine (1 × 10 mL) and dried ($MgSO_4$). After evaporation of the solvents and refrigeration of the oil (24 h), aldehyde **33** crystallized as a pale yellow solid (no purification required, 3.57 g, 64%): mp 39−41 °C; IR (pellet) 2733, 1669 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.38 (s, 6 H), 2.03 (s, 3 H), 2.91 (s, 3 H), 5.30 (s, 1 H), 6.50 [d, 1 H, J = 8.4 Hz, Ar-*H*], 7.52 [d, 1 H, J = 2.4 Hz, Ar-*H*], 7.52 [q, 1 H, J = 8.4 Hz, J = 2.4 Hz, Ar-*H*], 9.67 (s, 1 H); ^{13}C NMR ($DCCl_3$) ppm 18.57, 28.75, 31.21, 57.60, 109.14, 121.76−150.04, 190.19. Aldehyde **33** was used at once to prepare **34**.

4-Methyl-6-(1,2,2,4-tetramethyl(1,2-dihydroquinolin-6-yl))-5,6-dihydropyran-2-one (34). To a solution of ethyl 3,3-dimethylacrylate (205 mg, 1.5 mmol) and dry THF (3 mL) was added dropwise LDA (0.51 mL, 1.53 mmol, 3 M solution in toluene) at −78 °C. Following the addition, the reaction mixture was stirred (1 h), after which **33** (0.3 g, 1.46 mmol) dissolved in dry THF (2 mL) was added. After stirring the reaction mixture for 1 h at −78 °C, the reaction was quenched with a saturated, aqueous solution of ammonium chloride (1.5 mL) The resulting mixture was allowed to warm to room temperature and was then stirred for an additional 1 h. Extraction of the mixture with EtOAc (3 × 3 mL) was followed by washing the combined organic extracts with water (1 × 1 mL) and brine (1 × 1 mL). After drying ($MgSO_4$), the solvent was removed, and a thick, dark-red oil was recovered as **34** (171 mg, 43%): IR (neat) 1725 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.28 (s, 6 H), 2.03 (s, 3 H), 2.05 (s, 3 H), 2.91 (s, 3 H), 2.38 (dd, 2 H), 2.75, (d, 2 H), 5.25 (s, 1 H), 5.91 (s, 1 H), 6.50 (d, 1 H), 7.45 (m, 1 H); ^{13}C NMR ($DCCl_3$) ppm 18.57, 28.75, 31.21, 56.60, 109.14, 121.76−150.04, 168.35. Amine **34** was used immediately to prepare **35**.

4-Methyl-6-(1,2,2,4-tetramethyl(1,2-dihydroquinolin-6-yl))-5,6-dihydropyran-2-ol (35). To a chilled solution (−78 °C) of DIBAL-H in hexane (0.37 mL. 0.59 mmol, 1.6 M) was added **34** (107 mg, 0.36 mmol) in dry THF (2 mL). The mixture was stirred (20 min) and then quenched with 0.75 mL of a saturated, aqueous solution of Rochelle salt (saturated solution

of sodium and potassium tartrate, 1:1). After allowing the reaction mixture to warm to room temperature, the mixture was extracted with EtOAc (2 × 2 mL), and the extracts were washed with water (1 × 1 mL) and brine (1 × 1 mL). After drying (Na_2SO_4), the solvent was evaporated to give a thick, red oil (**35**) [one product via TLC (hexane:EtOAc, 2:1) (92 mg, 85%)]. IR (neat) for **35** showed 3452 [O−H] cm^{-1}. Compound **35** was used at once and without further purification to generate **36**.

(2*Z*,4*E*)-3-Methyl-5-(1,2,2,4-tetramethyl(1,2-dihydroquinolin-6-yl))penta-2,4-dienal (36). To lactol **35** (150 mg, 0.5 mmol) dissolved in $ClCH_2CH_2Cl$ (2 mL) was slowly added 5% HCl (2 mL). The reaction mixture was warmed to 55 °C (3 h) and monitored by TLC (hexane:EtOAc, 4:1) until completion. The mixture was then cooled to room temperature and carefully neutralized with a saturated, aqueous solution of $NaHCO_3$. The aqueous layer was extracted (H_2CCl_2; 2 × 3 mL), and the combined extracts were washed with water (1 × 2 mL) and brine (1 × 2 mL). After drying ($MgSO_4$) and evaporation of the solvent, the residue was purified over silica gel (hexane: EtOAc, 1:1) to yield a bright red oil as **36** (87 mg, 62%): IR (neat) 2785, 1655 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.28 (s, 6 H), 2.03 (s, 3 H), 2.35 (s, 3 H, J = 0.3 Hz), 2.91 (s, 3 H), 5.25 (s, 1 H), 5.91 (d, 1 H), 6.25 (d, 1 H), 6.50 (d, 1 H), 7.45 (m, 2 H), 10.15 (d, 1 H); ^{13}C NMR ($DCCl_3$) ppm 13.06, 18.57, 27.90, 30.21, 56.86, 110.14, 122.76−136.04, 191.04. Aldehyde **36** was used at once to prepare **10**.

6-Methoxy-1,1,4,4-tetramethyl-5-nitroisochromane (38). To a solution of 6-methoxy-1,1,4,4-tetramethylisochromane (**24**, 18.0 g, 81.70 mmol) dissolved in Ac_2O (36 mL) at −5 °C was added dropwise a mixture of ice-cold concentrated HNO_3 (18 mL) and Ac_2O (36 mL), and the resulting mixture was then stirred (1 h). The final mixture was poured into a solution of saturated $NaHCO_3$ (300 mL), and the mixture was extracted (H_2CCl_2; 3 × 120 mL). The organic layer was washed with water (150 mL) and brine (150 mL) and then dried (Na_2SO_4). The solvent was evaporated to give a thick yellow oil which was triturated with pentane to give a light yellow solid. Recrystallization (95% EtOH) gave **38** (6.91 g, 32%) as a white solid: mp 82−83 °C; IR (KBr) 1241 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.28 (s, 6 H), 1.51 (s, 6 H), 3.48 (s, 2 H), 3.83 (s, 3 H), 6.88 (d, 1 H, J = 2.5 Hz), 7.12 (d, 1 H, J = 2.5 Hz); ^{13}C NMR ($DCCl_3$) ppm 24.09, 30.05, 56.35, 71.68, 110.65, 128.18−159.34; MS (EI) calcd m/z (M^+) for $C_{14}H_{19}NO_4$ 265, found 265. Ether **38** was used directly to prepare **39**.

(6-Methoxy-1,1,4,4-tetramethylisochromane-5-yl)-amine (39). To a solution of 6-methoxy-1,1,4,4-tetramethyl-5-nitroisochromane (**38** 5.7 g, 17.71 mmol) dissolved in acetic acid (206 mL) and water (42 mL) was added dropwise a solution of a $TiCl_3$/HCl complex (30% solution, 120 g, 177.1 mmol). The resulting purple reaction mixture was stirred (13 h, room temperature). The new mixture was cooled (0 °C), and NaOH (30%, 500 mL) was added dropwise (4 h). The combined organic layer and extracts (EtOAc; 8 × 50 mL) of the aqueous layer were washed with water (2 × 50 mL) and saturated $NaHCO_3$ (2 × 100 mL) and then dried ($MgSO_4$). Evaporation of the solvent gave a solid which was recrystallized (95% EtOH) to yield amine **39** (4.6 g, 89%) as a white solid: mp 110−112 °C; IR (KBr) 3449, 3338 cm^{-1}; ^1H NMR ($DCCl_3$) δ 1.37 (s, 6 H), 1.49 (s, 6 H), 3.53 (s, 2H), 3.83 (s, 3 H), 3.98 (s, 2 H), 6.50 (d, 1 H, J = 8.5 Hz), 7.69 (d, 1 H, J = 8.5 Hz); ^{13}C NMR ($DCCl_3$) ppm 27.02, 29.73, 55.41, 71.16, 74.83, 111.79, 122.73−146.24; MS (EI) calcd m/z (M^+) for $C_{14}H_{19}NO_4$ 235, found 235. Ether **39** was promptly used to prepare **18** and **20**.

Biological Assay. Determination of the MICs. In this work, the MICs of the agents on solid medium were determined by the microdrop agar proportion test of McClatchy[22] as modified by Brennan and co-workers.[13] Briefly, a series of 10-fold dilutions of cultures of *M. bovis* BCG were prepared by using phosphate-buffered saline as a diluent. An aliquot (5 μL) of each dilution was spotted on plates of 7H11 agar (Difco) containing oleic acid−albumin−dextrose−citric acid (OADC) as a supplement and 0.1, 0.5, 1.0, 2.0, 2.5, 5.0, 10.0, and 20.0 mg of each agent per milliliter. The plates were incubated at 37 °C for 14 days, and the number of viable bacteria was scored by counting colonies. The experments were run separately and in duplicate. The MIC value was defined as the lowest concentration of drug resulting in 99% reduction in the number of colonies on each plate compared to those on a plate free of the agent at the same dilution of the culture suspension. The technique had previously been utilized to demonstrate the prowess of similar compounds in terms of inhibition of mycolic acid synthesis.[13]

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