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(54) Title: PYRANOCOUMARIN COMPOUNDS AS A NOVEL PHARMACOPHORE WITH ANTI-TB ACTIVITY

(57) Abstract: The present invention relates to compounds and compositions useful in treating or preventing conditions and diseases related to*Mycobacterium* infection, and methods of use directed thereto.

PYRANOCOUMARIN COMPOUNDS AS A NOVEL PHARMACOPHORE WITH ANTI-TB ACTIVITY

Priority Data and Governmental Rights

This application claims priority to U.S. Provisional application serial number 60/276,531, filed March 16, 2001. This work was supported in part by the National Institutes of Health (NIH) through SBIR Grant (# 1 R43 AI49053-01). Accordingly, the United States government may have certain rights to the invention.

Field of the Invention

The present invention relates to methods for the treatment of conditions related to *Mycobacterium tuberculosis* infection. The invention also relates to compounds and compositions useful in the treatment of *Mycobacterium tuberculosis* infection. The invention further relates to a method useful in the stepwise reductive amination of ketones.

Background of the Invention

Infectious diseases remain the largest cause of death in the world today, greater than cardiovascular disease or cancer[1]. Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, a facultative intracellular bacillus, is the world's number one killer among the infectious diseases and the leading cause of death among women of reproductive age[2]. Even though the improved methods of prevention, detection, diagnosis, and treatment have greatly reduced the number of people who contract the disease and die from it, the emergence of multidrug-resistant (MDR) strains[3] and the global human immunodeficiency virus (HIV) pandemic have amplified the incidence of TB.

It has been estimated one-third (about 2 billion) of the world's population, including 15 million Americans, is infected with *M. tuberculosis*[4]. The lifetime risk of developing TB is approximately 10% of infected persons, while the remaining 90% have latent infection with viable bacilli. This 10% rate of TB accounts for the 8 million cases of each year, resulting in 3 million deaths. The gravity of the situation led the World Health Organization (WHO) in 1993 to declare TB a global emergency in an attempt to heighten public and political awareness.

HIV is the most powerful factor known to increase the risk of TB. At first, HIV increases a person's susceptibility to infection with *M. tuberculosis*. In 1995, about one third of the 17 million HIV-infected people worldwide were also co-infected with *M. tuberculosis*[5]. As HIV infection progresses, CD4+ lymphocytes decline in number and function and, therefore, the immune system is less able to prevent the growth and local spread of *M. tuberculosis*, rendering a rapid progression of TB infection to disease. An individual co-infected with HIV and *M. tuberculosis* has a 10 times greater risk of developing TB, compared to an individual who is not infected with HIV. On the other hand, TB infection in an HIV-infected person may allow HIV to multiply more quickly and lead to a more rapid disease progression of AIDS[5].

The recommended treatment of TB is Directly Observed Therapy Short-course (DOTS), which uses a combination of drugs with isoniazid and rifampin taken over 6 months, supplemented with pyrazinamide for the first 2 months, and addition of ethambutol when isoniazid resistance is suspected. DOTS is generally successful, even though the treatment may need to be extended, sometimes to as long as 2 years, in order to fully cure the patient of infectious bacteria. However, poor compliance with such a long, complex and unpleasant combination of drugs results in a significant treatment failure rate. Worse still, resistance may emerge to these first-line agents, and thereafter to a wide range of second-line anti-mycobacterials. Not only are multi-drug resistant-TB (MDR-TB) strains difficult to treat but these strains are also life threatening, sometimes resulting in a high mortality rate (*e.g.*, 72 to 89%) in a short period of time (e.g., 4 to 6 weeks)[6]. In general, treating individuals infected with MDR-TB is expensive, intolerable in toxicity, and frequently unsuccessful. Treatment of drug susceptible TB costs about $2,000 per patient, whereas the cost increases to as much as $250,000 per case for MDR-TB[7]. In late 1998, FDA approved a new drug rifapentine, a derivative of rifampin, the first anti-TB drug to be approved in 25 years[8]. Although TB relapse rate for rifapentine is slightly higher (10%) than that for rifampin (5%), FDA approved the new medication because it only has to be taken once weekly during the last four months of treatment, as opposed to twice weekly for rifampin.

Currently, there is no standard optimal antimicrobial therapy in AIDS patients and no single agent that is active against both infections. Challenges of management of TB in patients with AIDS are significantly higher than that in patients without AIDS. The first challenge is the pill burden. DOTS program for TB requires a patient to take 10 to 12 pills a day and the recommended highly active antiretroviral therapy (HAART) for HIV infection normally adds no less than another 20 pills. All the medications have to be taken daily, around the clock, with or without food restrictions, creating a tremendously difficult drug regimen for the patient. The second challenge is the interactions between the drugs for TB and HIV infections, which may lead to regimen intolerance and/or contraindication and add more difficulties in the treatment design. For example, rifampin is not recommended for concurrent use with almost all the anti-HIV NNRTIs and protease inhibitors, due to their contraindicated interactions.

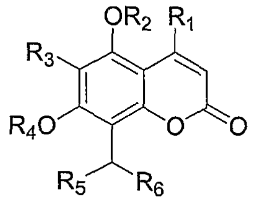
It is clear that there is an urgent need for anti-TB drugs with improved properties such as enhanced activity against MDR strains, reduced toxicity, shortened duration of therapy, rapid mycobactericidal mechanism of action, ability to penetrate host cells and exert antimycobacterial effect in the intracellular environment.

It is an object of this invention to provide for the design, synthesis and evaluation of a library of pyranocoumarin analogues, with an ultimate goal of developing a novel anti-TB drug which should maintain the same unique resistance profile and unique mechanism of action as demonstrated by (+)-calanolide A but have improved potency.

It is another objective of this invention to further understand the structural features of pyranocoumarin necessary for the unique anti-TB activity. The compounds of the present invention are useful tool to study a structure-activity relationship (SAR), to select and/or design other molecules to inhibit and/or kill *M. tuberculosis*. In addition, the instant compounds of the present invention are useful tools and/or reagents to identify and validate novel targets in the life cycle of *M. tuberculosis* for anti-TB drug development. Furthermore, the instant compounds of the present invention can be used to probe the mechanism of actions for anti-TB agents.

Summary of the Invention

The present invention provides for compounds according to formula I, compositions comprising the compounds of formula I, and methods for treating a patient who has a condition or disease associated with *Mycobacterium* infection and who is in need of such treatment which comprises administration of a therapeutically effective amount of at least one compound of formula I:



(I)

wherein R_1 is alkyl, alkenyl, alkynyl, aryl, OH, or NH_2;

R_2 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_3;

R_3 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_2;

R_4 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_5;

R_5 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_4; and

R_6 is selected from the group consisting of =O, OH, =NH, NH_2, SH, $P(O)_nH_m$ substituted imines, and substituted amines, wherein n is 2-4 and m is 1-3;

The invention also provides a method for the reductive amination of a ketone comprising contacting the ketone with a compound of the formula R'NH_2, wherein R' is selected from the group consisting of H, alkyl, alkenyl, alkynyl, and aryl.

Detailed Description of the Preferred Embodiments

Brief Description of the Figures:

Figure 1 discloses the structures of several pyranocoumarin compounds. The ring nomenclature (i.e. rings A, B, and C) as well as the numbering scheme are provided for (+)-calanolide-A (**1**).

Figure 2 discloses several of the structural features in the pyranocoumarins thought to be important in modulating anti-TB activity, and also provides the generic structures of four general types of compounds (I-IV) of the invention.

Figure 3 discloses several pyranocoumarin compound analogues.

Definitions

As used herein the terms "*Mycobacterium*" is taken to mean any strain of bacteria classified as *Mycobacterium*. In certain contexts, particular strain(s) of *Mycobacterium* include, but are not limited to, *Mycobacterium avium* complex (MAC), *Mycobacterium kansaii, Mycobacterium marinum, Mycobacterium phlei, Mycobacterium ulcerans,* *Mycobacterium xenopi, Mycobacterium gordonae, Mycobacterium terrae complex, Mycobacterium haemophilum, Mycobacterium fortuitum, Mycobacterium tuberculosis, Mycobacterium laprae, Mycobacterium scrofulaceum and Mycobacterium smegmatis.*

As used herein, the terms "conditions related to infection by *Mycobacterium*" or "diseases related to infection by *Mycobacterium*" are taken to mean any disease or condition recognized as being caused by, or further exacerbated by, an infection by *Mycobacterium*, as defined above.

As used herein, "alkyl", "lower alkyl", or "C_{1-6} alkyl" is meant to include a straight or branched hydrocarbon having from 1 to 12 carbon atoms and includes, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, n-hexyl, and the like.

As used herein, "alkenyl" means an alkyl group of 2 to 12 carbon atoms, wherein at least one carbon-carbon single bond is replaced by a carbon-carbon double bond. Examples of such groups include ethylene, 1-propene, 2-propene, 1-butene, 2-butene, isobutene, and the isomers of pentene and hexene and the like. All cis- and trans- isomers are included in the scope of the definition.

As used herein, "alkynyl" means an alkyl group of 2 to 12 carbon atoms, wherein at least one carbon-carbon single bond is replaced by a carbon-carbon triple bond. Examples of such groups include acetylene, 1-propyne, 2-propyne, 1-butyne, 2-butyne, 3-butyne, and the isomers of pentyne and hexyne and the like.

All of alkyl, alkenyl, and alkynyl groups can be optionally substituted with one or more substituent listed below for aryl, on any carbon atom that results in a stable structure that is available by conventional synthetic methods.

The term "aryl" means an aromatic carbocyclic or heterocyclic group having a single ring (e.g., phenyl, pyridine, or thiophene), multiple rings (e.g., biphenyl, indole, or benzoimidazole), or multiple condensed rings in which at least one is aromatic (e.g., 1,2,3,4-tetrahydronaphthyl, naphthyl, anthracyl, phenanthryl, or 1,2-dihydroindole), unsubstituted or substituted by 1 to 4 substituents selected from alkyl, O-alkyl (alkoxy) and S-alkyl, OH, SH, -CN, halogen, 1,3-dioxolanyl, CF_3, NO_2, NH_2, $NHCH_3$, $N(CH_3)_2$, NHCO-alkyl, $-(CH_2)_mCO_2H$, $-(CH_2)_mCO_2$-alkyl, $-(CH_2)_mSO_3H$, –NH alkyl, $-N(alkyl)_2$, $-(CH_2)_mPO_3H_2$, $-(CH_2)_mPO_3(alkyl)_2$, $-(CH_2)_mSO_2NH_2$, and $-(CH_2)_mSO_2NH$-alkyl wherein alkyl is defined as above and m is 0, 1, 2, or 3.

By "alkoxy", "lower alkoxy" or "C_{1-6} alkoxy" in the present invention is meant straight or branched chain alkoxy groups having 1-6 carbon atoms, such as, for example, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, sec-butoxy, tert-butoxy, pentoxy, 2-pentyl, isopentoxy, neopentoxy, hexoxy, 2-hexoxy, 3-hexoxy, and 3-methylpentoxy and the like.

As used herein, "4 to 7-membered ring" is meant to include an aromatic or non-aromatic ring having, zero, one, or two carbon-carbon double bonds, and optionally containing one to two heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur, which are stable and available by conventional chemical synthesis. Preferred rings comprise heterocyclic 6-membered rings, including those that contain a single oxygen atom, and optionally comprise no carbon-carbon double bonds, or a single carbon-carbon double bond.

Recently, through the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) screening program sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), (+)-calanolide A (**1** in **Fig. 1**) was found to be active against TB. (+)-Calanolide A, a natural product originally isolated from the rain forest tree *Calophyllum lanigerum*, has been previously identified to be an anti-HIV agent and it is currently undergoing clinical trials for HIV infection.

Against H37Rv strain of *M. tuberculosis* in BACTEC 12B medium using the BACTEC 460 radiometric system, (+)-calanolide A demonstrated greater than 96% inhibition at a concentration of 12.5 μg/mL. The minimum inhibitory concentration (MIC) for (+)-calanolide A, defined as the lowest concentration inhibiting 99% of the inoculum, was then determined to be 3.13 μg/mL or 8 μM. The MIC value suggests that activity of (+)-calanolide A against TB might be marginal. For comparison, MICs for the first line anti-TB drugs are 0.025-0.05 μg/mL for isoniazid, 0.005-0.2 μg/mL for rifampin, and 0.4-10 μg/mL for ethambutol. However, in a bone marrow-derived murine macrophage model, the BC 99 value of (+)-calanolide A, a concentration at which 99% of the inoculum of *M. tuberculosis* is destroyed, was 1.1 μg/mL (3 μM) against strain Erdman (TMCC 107/ATCC 35801) and 1.8 μg/mL (5 μM) against strain CSU 93 (CDC-95-031551), compared to 0.7 - 0.8 μg/mL (5-6 μM) for the positive control drug isoniazide. The drug inhibition patterns observed in this study led to the belief that (+)-calanolide A was a bactericidal agent. In addition, (+)-

calanolide A was weakly active against *M. avium*, exhibiting 81% inhibition at a concentration of 12.5 μg/mL.

More importantly, (+)-calanolide A was active against all the first-line anti-TB drug resistant strains. As indicated in **Table 1**, (+)-calanolide A did not lose activity toward strains of *M. tuberculosis*, which are resistant to isoniazid, rifampin, streptomycin, and ethambutol, respectively.

Table 1. Activity of (+)-Calanolide A against anti-TB Drug Resistant Strains

	MIC (μg/mL) (fold resistance)				
Strains	**(+)-Calanolide A**	**Isoniazid**	**Rifampin**	**Streptomycin**	**Ethambutol**
H37Ra	16	0.063	ND	ND	4
H37Rv	8	0.031	0.016	0.25	2
1369	8 (1)	0.031 (1)	64 (4,000)	0.25 (1)	2 (1)
1371	8 (1)	0.031 (1)	0.016 (1)	> 128 (>512)	2 (1)
1348	8 (1)	> 128 (>4100)	0.031 (2)	0.25 (1)	8 (4)
1356	8 (1)	0.25 (8)	0.031 (2)	0.25 (1)	64 (32)

In the preliminary mechanistic studies, the effect of (+)-calanolide A on the *M. tuberculosis*' synthesis of protein, RNA, DNA and lipid was assessed by measuring incorporation of appropriate radiolabeled precursors into mycobacterial macromolecular components. It was apparent that (+)-calanolide A inhibited all these biological events at a concentration of 8x MIC (64 μg/mL). However, the pattern of its inhibition was somewhat different from the respective positive control drug. For instance, the effect of (+)-calanolide A on the protein synthesis of *M. tuberculosis* occurred earlier than that observed with the positive control streptomycin (32% inhibition at 2 hours vs 43% at 5 hours). Streptomycin is a known inhibitor of protein synthesis by binding to the 30S ribosomal subunit and subsequently "freezing" the initiation complex. The fact that the inhibition pattern observed for (+)-calanolide A was different from streptomycin suggests that (+)-calanolide A may affect a target that ultimately, but not directly, affects protein synthesis. (+)-Calanolide A inhibited RNA synthesis to the extent and in the time frame very similar to the positive control rifampin, a known inhibitor of DNA dependent RNA polymerase. Also, (+)-calanolide A appears to inhibit both DNA and lipid syntheses. Approximately 77% of DNA synthesis was inhibited in a period of 3 hours while 99% of lipid synthesis was inhibited when measured at 12-hour time point. However, these inhibitions should be viewed with some reservation as DMSO at a concentration equivalent to that used to dissolve (+)-calanolide A showed 24% and 51% inhibition, respectively, of DNA synthesis and lipid synthesis during the same sampling time point.

Based on the data presented above, it appears that the effect of (+)-calanolide A on TB was more similar to rifampin than other agents. However, (+)-calanolide A is active against rifampin-resistant strain, suggesting that both compounds may affect the same biological event (RNA synthesis) but involve different targets. The inhibitory effect of (+)-calanolide A on protein and lipid syntheses is probably secondary since the target affected proceeds these biological events. It is therefore not surprising that both isoniazid- and streptomycin-resistant strains are susceptible to (+)-calanolide A (**Table 1**). Clearly, (+)-calanolide A represents a novel pharmacophore for anti-TB activity.

Encouraged by the (+)-calanolide A results, seven (7) other anti-HIV-1 active pyranocoumarin compounds (see **Fig. 1** for structures) were also screened for anti-TB activity. Interestingly, compounds **2** - **5** were all determined to be active against H37Rv strain of *M. tuberculosis*, with MIC values at the same level of (+)-calanolide A, as shown in **Table 2**. However, compounds **6** - **8** were weakly active, since they only exhibited 43 to 78% inhibition at a concentration of 12.5 μg/mL.

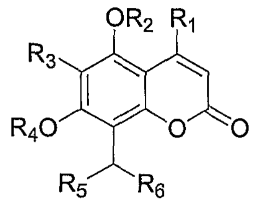
Table 2. Activity of Pyranocoumarin Compounds against Strain H37Rv of *M. tuberculosis*

Compound	% Inhibition	MIC (μg/mL)	IC_{50} (μg/mL)	SI (IC_{50}/MIC)
(+)-Calanolide A (**1**)	96	3.13	7.60	2.43
(-)-Calanolide A (**2**)	98	6.25	> 10	> 1.6

(-)-Calanolide B (**3**)	99	6.25	-----	-----
Soulattrolide (**4**)	99	3.13	-----	-----
7,8-Dihydrosoulattrolide (**5**)	99	6.25	-----	-----
(+)-12-Oxocalanolide A (**6**)	78	> 12.5	-----	-----
(±)-**6**	59	> 12.5	-----	-----
(±)-7,8-Dihydro-12-Oxocalanolide A (**7**)	43	> 12.5	-----	-----
(±)-Calanolide D (**8**)	57	> 12.5	-----	-----

The results reported above reveal a few key features of structural requirements for these pyranocoumarins to exert their anti-TB activity. First of all, the 12-OH group appears to be the single most important structural element, since compounds with such a group as in **2** - **5** are active while those without, as in **6** - **8**, which all possess a carbonyl group at the 12-position, are inactive. In contrast to the anti-HIV activity,[i] however, the stereochemistry of the 12-OH group in these pyranocoumarins may not be critical for anti-TB activity because (-)-calanolide A (**2**), possessing the 12-OH group in the β position, was as active against TB as (+)-calanolide A (**1**) and compounds **3** - **5**, which all have a 12-OH group in the α position. Furthermore, it is apparent that the coumarin (Ring A, see the ring designation in **Fig. 1**) and chromene (Ring B) rings are flexible to modifications. For example, either replacement of the *n*-propyl group in **3** with a phenyl, resulting in **4**, or saturation of the double bond in Ring B of **4**, leading to **5**, still maintains the anti-TB activity. However, the necessity of methyl groups in the chroman ring (Ring C) is not clear. Therefore, the structural features in the pyranocoumarins required for anti-TB activity can be summarized by structure **I** as shown in **Fig. 2**.

In one embodiment, the invention provides for compounds of formula I or pharmaceutically acceptable salts thereof:



(I)

wherein R_1 is alkyl, alkenyl, alkynyl, aryl, OH, or NH_2;

R_2 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_3;

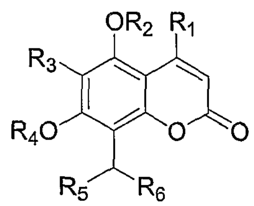
R_3 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_2;

R_4 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_5;

R_5 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_4; and

R_6 is selected from =O, =NH, OH, NH_2, NRH, NR, SH, and $P(O)_nH_m$, wherein n is 2-4 and m is 1-3.

In one embodiment, this invention provides for methods for treating a patient who has a condition or disease related to *Mycobacterium* infection and who is in need of such treatment which comprises administration of a therapeutically effective amount of at least one compound of formula I or a pharmaceutically acceptable salt thereof:



(I)

wherein R_1 is alkyl, alkenyl, alkynyl, aryl, OH, or NH_2;

R_2 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_3;

R_3 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_2;

R_4 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_5;

R_5 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_4; and

R_6 is selected from the group consisting of =O, OH, =NH, NH_2, SH, $P(O)_nH_m$ substituted imines, and substituted amines, wherein n is 2-4 and m is 1-3;

In an embodiment, this method of treatment can be used where the disease is tuberculosis.

In an embodiment, this method of treatment can be used where the disease is tuberculosis associated with an immunodeficiency including, but not limited to, human immunodeficiency virus (HIV) infection, acquired immune deficiency syndrome (AIDS), or AIDS related complex (ARC).

In an embodiment, this method of treatment can be used to treat a condition or disease related to an existing *Mycobacterium* infection.

In an embodiment, this method of treatment can be used to prevent *Mycobacterium* infection, for example, in a patient with a compromised immune system.

In preferred embodiments, this method of treatment can be used to treat or prevent *Mycobacterium* infection, especially when the *Mycobacterium* is *Mycobacterium tuberculosis.*

The compounds of the invention may be formulated as a solution of lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. The liquid formulation is generally a buffered, isotonic, aqueous solution. Examples of suitable diluents are normal isotonic saline solution, standard 5% dextrose in water or in buffered sodium or ammonium acetate solution. Such formulation is especially suitable for parenteral administration, but may also be used for oral administration. It may be desirable to add excipients such as polyvinylpyrrolidone, gelatin, hydroxy cellulose, acacia, polyethylene glycol, mannitol, sodium chloride or sodium citrate.

Alternatively, the compounds of the present invention may be encapsulated, tableted or prepared in an emulsion (oil-in-water or water-in-oil) syrup for oral administration. Pharmaceutically acceptable solids or liquid carriers, which are generally known in the pharmaceutical formulary arts, may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Solid carriers include starch (corn or potato), lactose, calcium sulfate dihydrate, terra alba, croscarmellose sodium, magnesium stearate or stearic acid, talc, pectin, acacia, agar, gelatin, maltodextrins and microcrystalline cellulose, or colloidal silicon dioxide. Liquid carriers include syrup, peanut oil, olive oil, corn oil, sesame oil, saline and water. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax. The amount of solid carrier varies but, preferably, will be between about 10 mg to about 1 g per dosage unit.

The dosage ranges for administration of the compounds of the invention are those to produce the desired affect whereby symptoms of infection are ameliorated. For example, as used herein, a pharmaceutically effective amount for a mycobacterium infection refers to the amount administered so as to maintain an amount which suppresses or inhibits mycobacterium infection as evidenced by standard assay(s). The dosage will also be determined by the existence of any adverse side effects that may accompany the compounds. It is always desirable, whenever possible, to keep adverse side effects to a minimum.

One skilled in the art can easily determine the appropriate dosage, schedule, and method of administration for the exact formulation of the composition being used in order to achieve the desired effective concentration in the individual patient. However, the dosage can vary from between about 0.001 mg/kg/day to about 50 mg/kg/day, but preferably between about 0.01 to about 20 mg/kg/day.

The pharmaceutical composition may contain other pharmaceuticals in conjunction with the compounds of the invention for use in combination therapy. For example, other pharmaceuticals may include, but are not limited to, other antiviral compounds (e.g., AZT, ddC, ddI, D4T, 3TC, acyclovir, gancyclovir, fluorinated nucleosides and non-nucleoside analog compounds such as TIBO derivatives and nevirapine, α-interfon and recombinant CD4), protease inhibitors (e.g., indinavir, saquinavir, ritonavir, and nelfinavir), immunomodulators such as, for example, immunostimulants (e.g., various interleukins and cytokines), antibiotics (e.g., antimicrobials such as the anti-TB agents isoniazid, rifampin, rifabutin, rifapentine, pyrazinamide, and ethambutol, as well as antibacterial, antifungal, anti-pneumocysitis agents), and chemokine inhibitors. Administration of the inhibitory compounds with anti-retroviral agents that act against other HIV proteins such as protease, intergrase and TAT will generally inhibit most or all replicative stages of the viral life cycle.

The compounds described herein can be used either alone or in conjunction with other pharmaceutical compounds to combat multiple infections. For example, the compounds of the invention can be used either alone or combined with acyclovir in a combination therapy to treat HSV-1; with one or more anti-mycobacterial agents such as anti-TB agents such as Isoniazid, rifamycins (e.g., rifampin, rifabutin and rifapentine), pyrazinamide, and ethambutol as a prophylatic or therapeutic treatment; in combination with Intron A and/or a biflavanoid for treating Hepatitis B; with gancyclovir, progancyclovir, famcyclovir, foscarnet, vidarabine, cidovir, and/or acyclovir for treating herpes viruses; and with ribavarin, amantidine, and/or rimantidine for treating respiratory viruses.

In another embodiment the present invention relates to a method of stepwise reductive amination of a ketone comprising the steps of:

(a) contacting the ketone with a solution comprising an amount of a compound of the formula $R'NH_2$,

wherein R' is selected from the group consisting of H, alkyl, alkenyl, alkynyl, and aryl under conditions that allow formation of an imine intermediate compound; and

(b) contacting the imine intermediate compound generated in step (a) with an amount of a reducing agent sufficient to reduce the imine intermediate compound to its amino analog, under conditions that allow reduction of the imine intermediate compound to its amino analog.

In one embodiment, the amount of $R'NH_2$ ranges between 1 to 500 molar equivalents relative to the amount of ketone to be reduced. Use of excessive amount of $R'NH_2$ tends to shorten the reaction time for the intermediate imine formation as well as drive the reaction to completion. In preferred embodiments, the method comprises 30 to 70 molar equivalents of ammonia (R' is H) or 10 to 40 molar equivalents of amine compound (i.e. R' is not H).

In preferred embodiments the solvents used in the method are alcohols and ethers including, but not limited to, methanol, ethanol, propanol, isopropanol, butanol, ethyl ether, methyl t-butyl ether, dioxane, tetrahydrofuran, diglyme, tetraglyme, and the like. Compounds of formula $R'\text{-}NH_2$ can either be added to the solution comprising the ketone, or can be present in the solution to which the ketone is added. In preferred embodiments, the ketone compound is added to the solution comprising the compound $R'NH_2$.

The compound of formula $R'NH_2$ can be added to the solvent by any method known in the art including, but not limited to, bubbling of gas phase $R'NH_2$ through the solvent, or by addition of liquid $R'NH_2$ to the solvent.

In one embodiment, this step of the method can be carried out at –78 to 120 °C. In preferred embodiments, the reaction temperature is about room temperature.

In one embodiment, the reducing agents comprise common reducing agents capable of reducing an imine to an amine, and are known to those of skill in the art. Examples of such reducing agents include, but are not limited to, $LiAlH_4$, $(i\text{-}Bu)_2AlH$, $(n\text{-}Bu)_3SnH$, $NaBH_3CN$, $NaB(OAc)_3H$, $LiBH_4$, $NaBH_4$, $Zn(BH_4)_2$, BH_3, or a borane compound such as 9-BBN and DIP-chloride. The imine intermediate can be isolated and purified before it is reduced, or the imine intermediate can be reduced without substantial purification. In preferred embodiments, the reduction is performed on the crude product, without substantial purification. The reaction can be carried out at –78 to 120 °C. In preferred embodiments, the reaction temperature is about room temperature.

In one embodiment, the method further comprises a metal additive, added at the step including the reducing agent. Examples of metal additives include, but are not limited to, $CeCl_3$, $ZnCl_2$, $AlCl_3$, $TiCl_4$, $SnCl_3$, and $LnCl_3$.

In one embodiment the amount of reducing agent used ranges from 0.1 to 50 molar equivalents.

In a preferred embodiment, the reducing agent is added to the crude imine intermediate, and comprises $NaBH_4$. In an especially preferred embodiment, the added amount of $NaBH_4$ is 3 to 6 molar equivalents, relative to the amount of imine intermediate, and is carried out at about room temperature.

In another embodiment the present invention relates to a method of making compounds of formula I (R_6 is =NH, or substituted imines) comprising the step of: (a) contacting the ketone with a solution comprising an amount of a compound of the formula $R'NH_2$, wherein R' is selected from the group consisting of H, alkyl, alkenyl, alkynyl, and aryl, under conditions that allow formation of an imine intermediate compound.

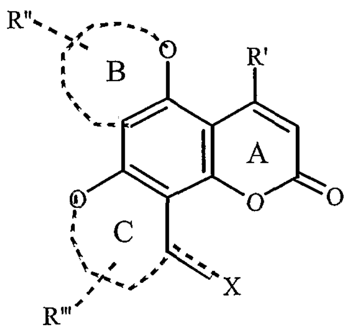
In a preferred embodiment, the method of stepwise reductive amination is used to generate a compound of formula (I) wherein R_6 is NH_2 or RNH comprising: (a) contacting a ketone compound of formula (I) (wherein R_6 is =O) with an excess of a compound of the formula $R'NH_2$, under conditions that allow formation of an imine intermediate compound of formula (I) (R_6 is =NH or =NR); and (b) contacting the imine intermediate compound of formula (I) with an amount of a reducing agent sufficient to reduce the imine moiety of the imine intermediate compound of formula (I) to its amino analog, under conditions that allow for said reduction.

SYNTHESIS OF COMPOUNDS

One of skill in the art can identify alternative synthetic pathways that can be used to make the compounds of the instant invention. The following represents an example of one synthetic approach and should not be viewed as limiting the spirit or scope of the invention.

One synthetic approach useful in generating the compounds of the invention is to systematically modify the chromene and chroman rings (B and C of formula (II), *vide infra*). A number of characteristics of analogs of interest have been identified and listed in **Fig. 3**. Initially, a small number of compound library was designed, synthesized and tested for anti-TB activity (**Fig. 3**). The first three categories of compounds listed in **Fig. 3** are set to further clarify the importance of Ring B and C, while the fourth category represents the corresponding amino derivatives. The amino compounds may be of particular interest because they can participate in hydrogen bonding, and is less prone to elimination under acidic conditions, relative to an OH group. Further, an amino compound tends to have an increased water-solubility, especially by forming a salt with an acid, which offers an enhanced bioavailability.

Based on the initial biological screening results, the compound library was expanded to encompass compounds selected from the structure shown below as formula (II). B and C can be open structure, or 4 to 7-membered ring, preferably a 6-membered ring, with or without substituents; X can be O, OH NH, NH_2, NRH, NR, SH, and $P(O)_nH_m$, wherein n is 2-4 and m is 1-3.

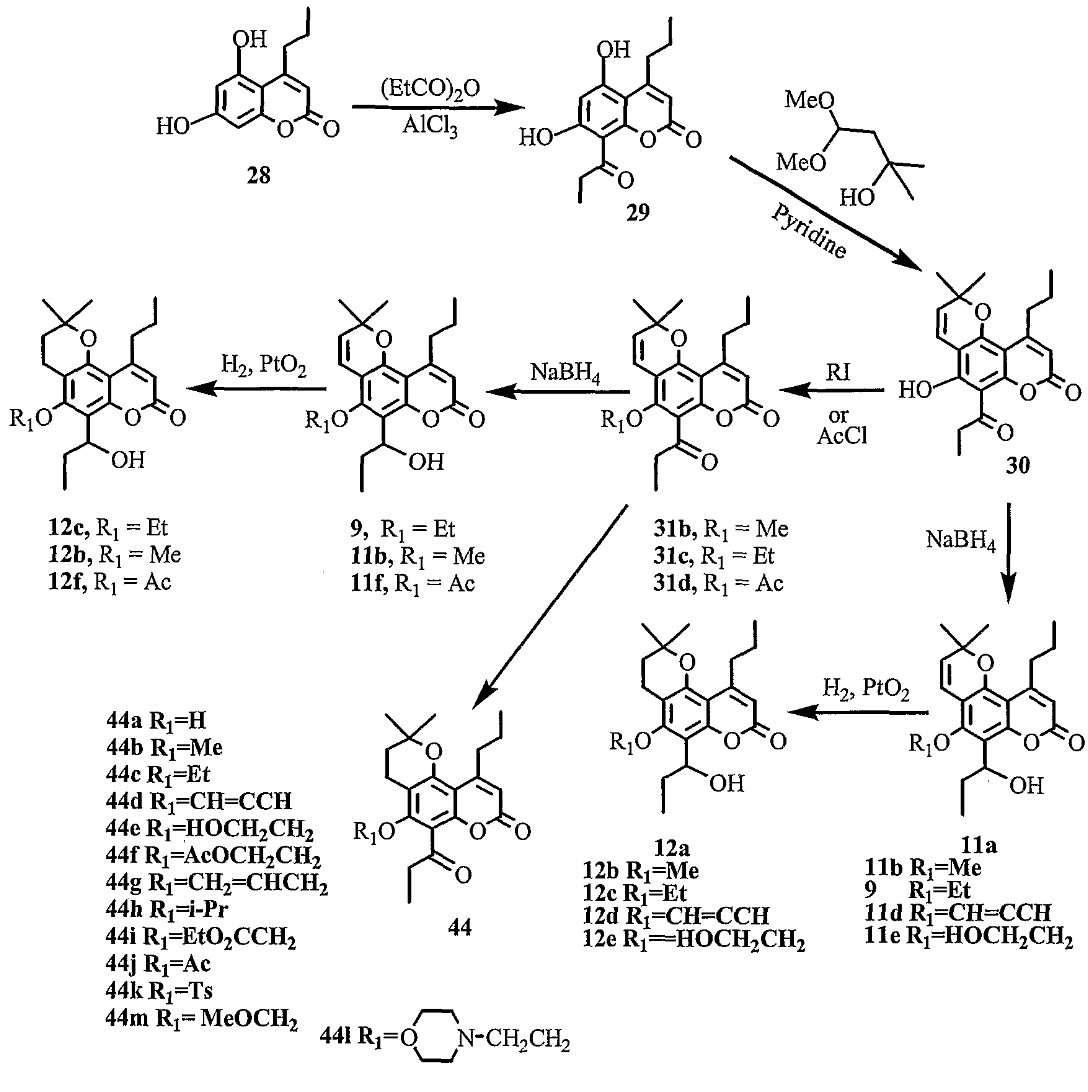


(II)

Synthesis of Type I Compounds

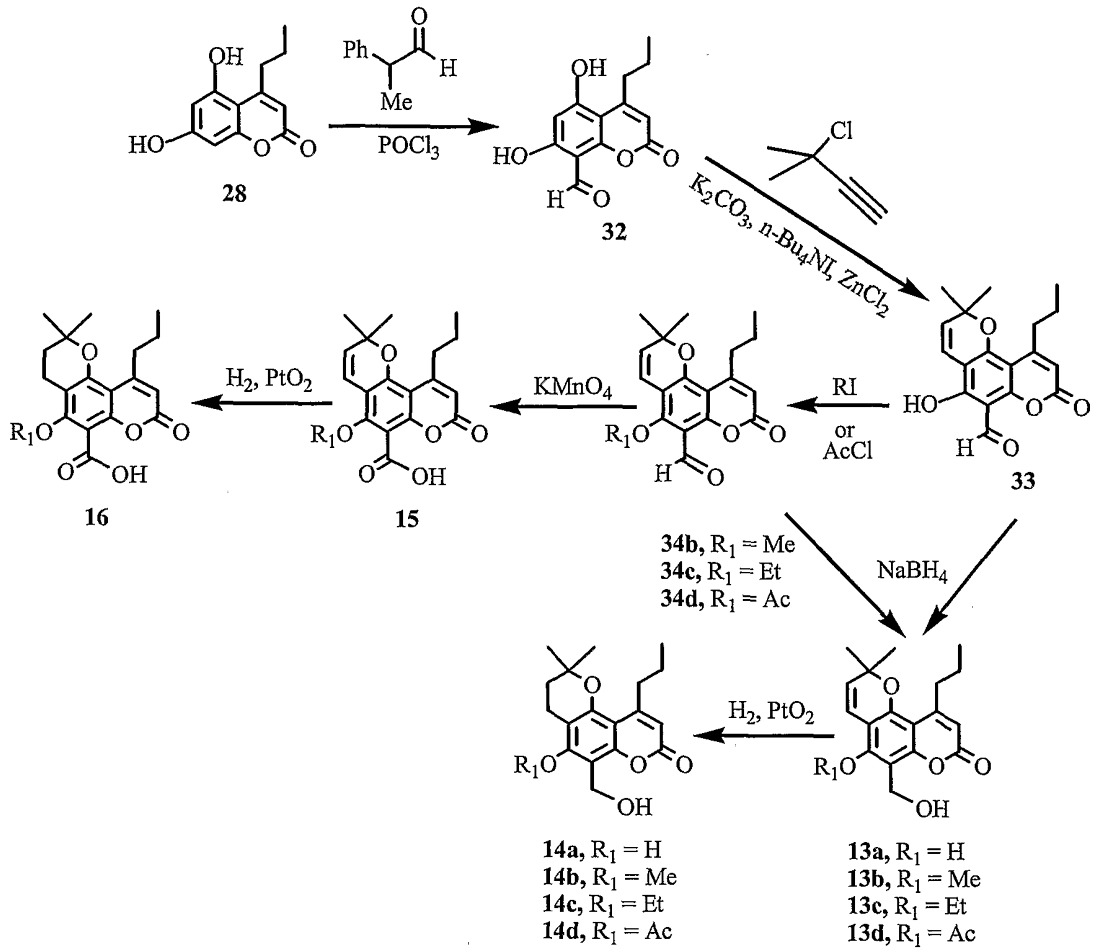
Type I compounds resulted from the modification on Ring C to determine if the rigidity of the ring system is required. Compound **9** can be viewed as an acyclic form of calanolide A or B, with the same number of carbon atoms as the latter molecules but with two fewer chiral centers, allowing for easy manufacture. This compound has been synthesized previously[10] and, therefore, the reported procedures, as described in **Scheme 1**, are followed, starting from coumarin **28**. Briefly, selective Friedel-Crafts acylation of **28** with propionic anhydride in the presence of $AlCl_3$ affords 8-acylated coumarin **29**, which is then treated with 4,4-dimethoxy-2-methylbutan-2-ol to furnish **30**. Alkylation of **30** with iodoethane, followed by reduction with sodium borohydride, provides **9**. Hydrogenation of **9** with NH_4OH-poisoned PtO_2 as catalyst[11] affords the corresponding dihydro compound **10**. Likewise, treatment of **30** with iodomethane or acetyl chrolide, followed by reduction with $NaBH_4$, provides **11b** and **11d** (**Scheme 1**). Direct reduction of **30** leads to the formation of **11a**. Also, hydrogenation of **11a**, **11b** and **11d** with NH_4OH-poisoned PtO_2 as catalyst furnishes the corresponding dihydro compound **12a**, **12b** and **12d**, respectively. Hydrogenation of **31** yields the corresponding saturated ketone **44**.

Scheme 1



Since formylated chromeno-coumarin **33** has been reported in the literature[12], compounds **13** – **16** are easily prepared from this intermediate (**Scheme 2**). Thus, Vilsmeier reaction on coumarin **28** by treating with N-methylformanilide in the presence of $POCl_3$ gives 8-formylated coumarin **32**.

Scheme 2



Subsequent treatment of **32** with 3-chloro-3-methyl-1-butyne in the presence of K_2CO_3 and n-Bu_4NI in DMF and 2-butenone, followed by addition of anhydrous $ZnCl_2$, produces **33**. Alkylation or acylation of **33** following the similar procedures as described above affords **34**. Reduction of **33** and **34** with $NaBH_4$ provides the designed compound **13a-d (Scheme 2)**. Oxidation of **34** with CrO_3 or $KMnO_4$ delivers the acid derivative **15.** Once again, hydrogenation of **13a-d** and **15** catalyzed by Pd/C or PtO_2 furnishes the corresponding dihydro compound **14a-d** and **16**, respectively.

Following the same chemistry described above, a number of library compounds were made by varying the substituents R_1, R_2, and R_3.

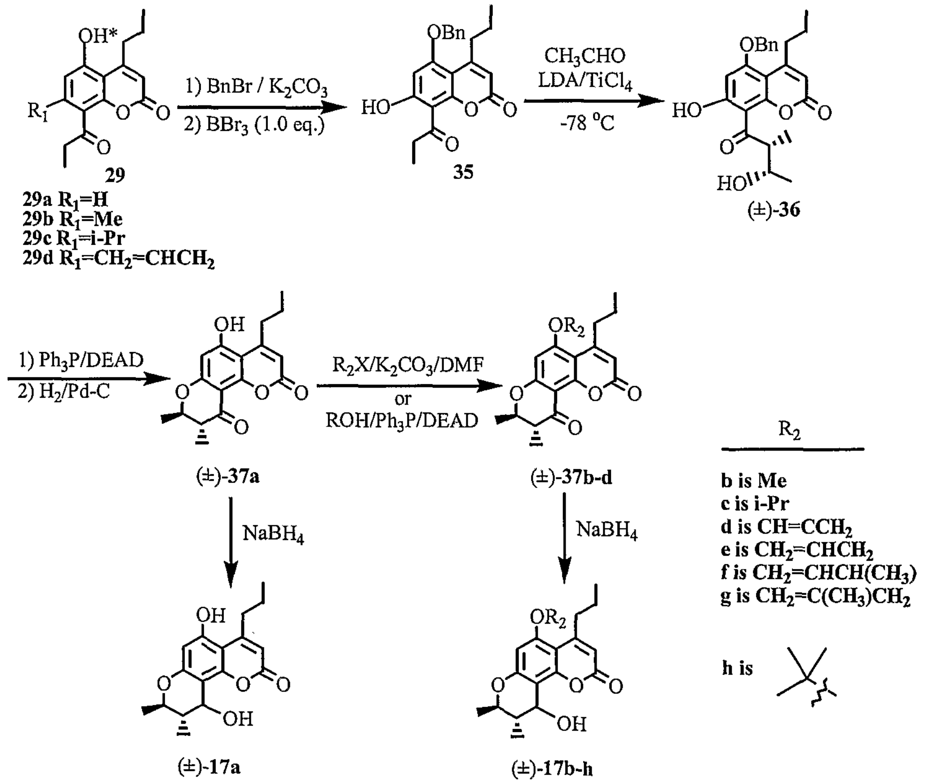
Synthesis of Type II Compounds

Type II compounds address the importance of Ring B by maintaining Ring C (formula II). We have previously developed a method for the synthesis of coumarino-chromanone **37a**[13] and demonstrated its ready alkylation of the phenol group (**Scheme 3**).

Therefore, **29** is readily protected by benzyl groups. The 7-benzyl group adjacent to the carbonyl function is then selectively deprotected using BBr_3 (1.0 equiv.), providing phenol **35**. Aldol reaction with CH_3CHO in the presence of $TiCl_4$ affords *syn* diastereomer **36** as a crystalline compound. Mitsunobu cyclization of **36**, followed by debenzylation, furnishes the *trans*-chromanone **37a**.

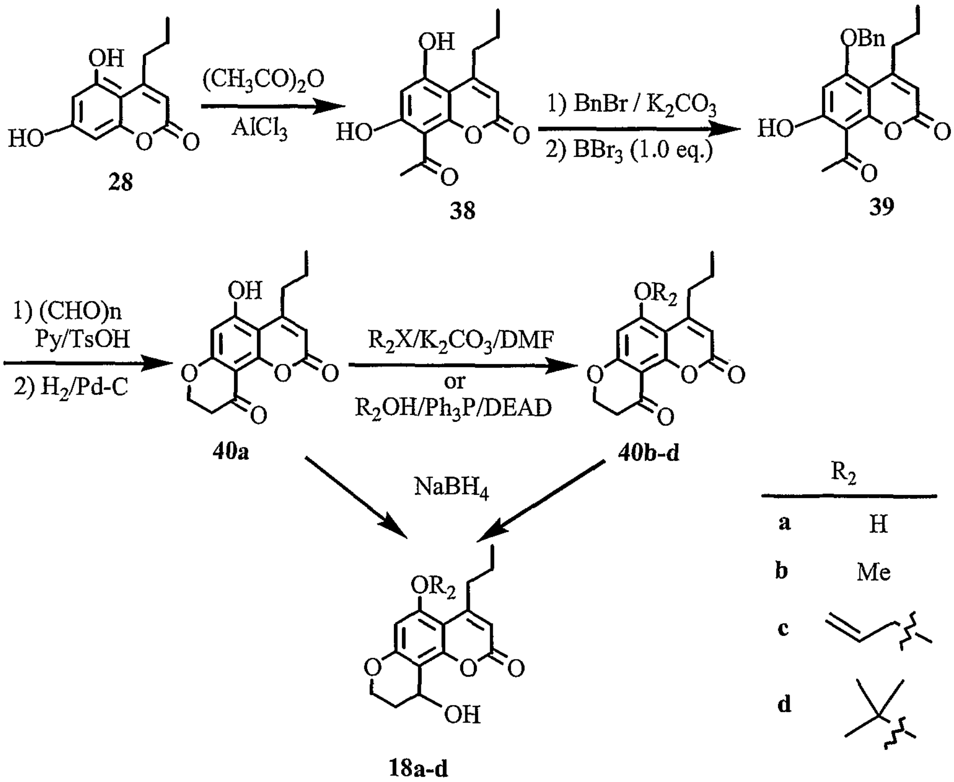
Alkylation of **37a** using allyl bromide in DMF in the presence of K_2CO_3 is generally completed in a couple of hours, leading to the formation of **37c**, with only trace amount of the *cis* isomer.[13] The alkylation of **37a** is extended to other alkylating agents (RX) as specified in **Scheme 3**, yielding phenyl ethers **37b** and **37d**. Alternatively, Mitsunobu coupling of **37a** with ROH forms a phenol ether bond. Reduction of **37a-d** with $NaBH_4$ provides the designed compound **17a-d** (**Scheme 3**). It is realized that both α and β isomers are formed during the reduction. However, in the presence of $CeCl_3$[14], the reduction using $NaBH_4$ results in an β form predominately.

Scheme 3



Compound **18** determined if the methyl groups in Ring C are needed. Relative to calanolide compounds, compound **18** has two less chiral centers and should be relatively easier to manufacture even if the Ring C system needs to be maintained for anti-TB activity. The synthesis of **18** is described in **Scheme 4**. Analogous to coumarin **29**, 8-acetyl coumarin **38** is synthesized via the selective Friedel-Crafts acylation[14] of coumarin **28** with acetic anhydride in the presence of $AlCl_3$. Benzylation of **38**, followed by selective debenzylation using 1.0 equiv. of BBr_3,[13] yields phenol **39**. Treatment of **39** with paraformaldehyde in the presence of pyridinium *p*-totuenesulfonate (PPTS),[ii] followed by debenzylation, furnished the ketone derivative **40a**. Alkylation of **40a** with alkylating agents in DMF in the presence of K_2CO_3, or Mitsunobu coupling with the corresponding alcoholic compounds, leads to the formation of phenyl ethers **40b-d**. Reduction of **40a-d** with $NaBH_4$ provides the designed compound **18a-d (Scheme 4)**. Once again, the α and β isomers of **18a-d** are not separated for the biological screening.

Scheme 4



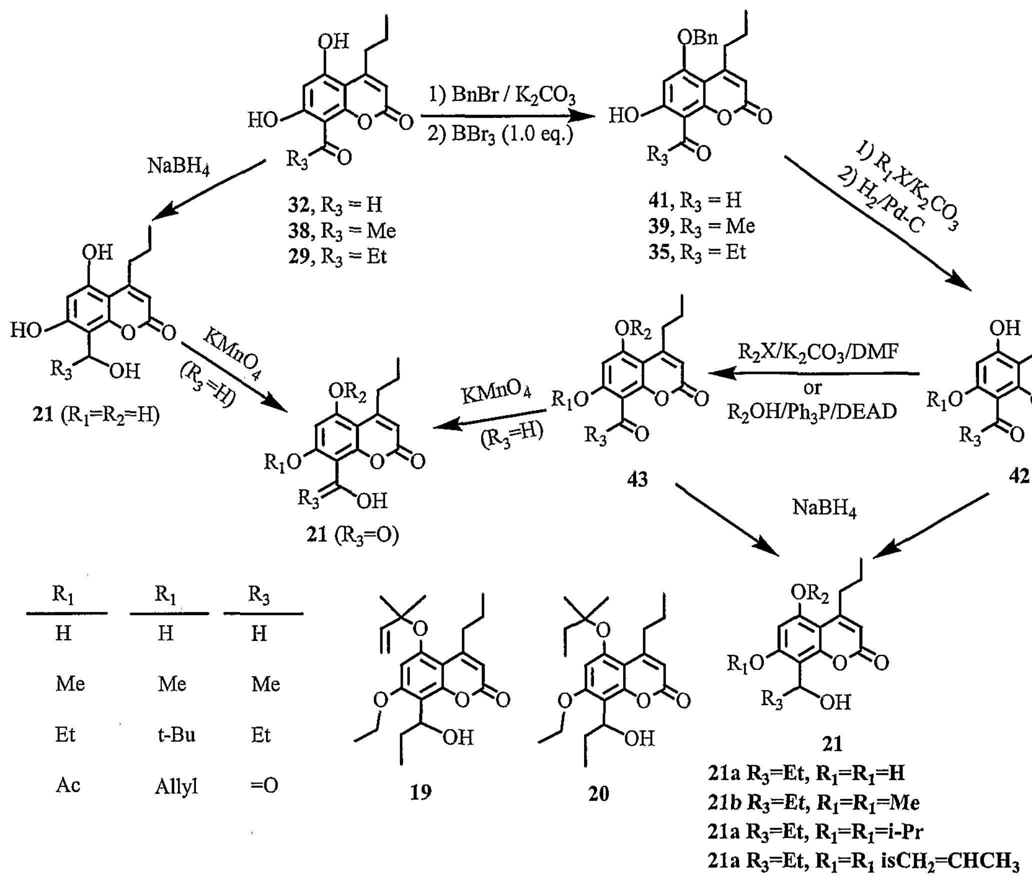
Following the same chemistry described above, a good number of library compounds are built up by varying the substituents R_1, R_2, R_3, and R_4.

Synthesis of Type III Compounds

The representative Type III compounds as identified in **Fig. 3** can be synthesized via conventional medicinal chemistry approach. The first representative compound of this series is compound **19**, which is a dual-acyclic form of calanolide A or B, with the same number of carbon atoms as the latter molecules. A more generalized structure, with elimination of both Ring B and Ring C, is illustrated in **21**. The synthesis of these compounds is very straightforward.

Therefore, as shown in the previous schemes, 8-acylated coumarins **29**, **32**, and **38** are obtained by following the literature procedures. Reduction of these compounds with $NaBH_4$ affords the desired **21** (R_1=R_2=H, R_3=H, Me or Et, in **Scheme 5**). Benzylation of **29**, **32**, and **38**, followed by selective debenzylation using 1.0 equiv. of BBr_3,[13] yields monobenzylated phenols **32**, **39**, and **41**. Alkylation of the latter compounds with alkylating agents (R_1X) in DMF in the presence of K_2CO_3 leads to the formation of phenyl ethers **42** after debenzylation. A similar alkylation process on **42** yields **43**. Reduction of **42** and **43** with $NaBH_4$ affords the designed compounds **21** (**Scheme 5**). Compound **19** is a special case of **21** and is prepared following the same sequence. Hydrogenation of **19** catalyzed by poisoned PtO_2 furnishes **20**. Furthermore, oxidation of **43** (R_3=H) and **21** (R_1=R_2=R_3=H) with CrO_3 or $KMnO_4$ delivers the acid derivative **21** (R_3=carbonyl) (**Scheme 5**).

Scheme 5



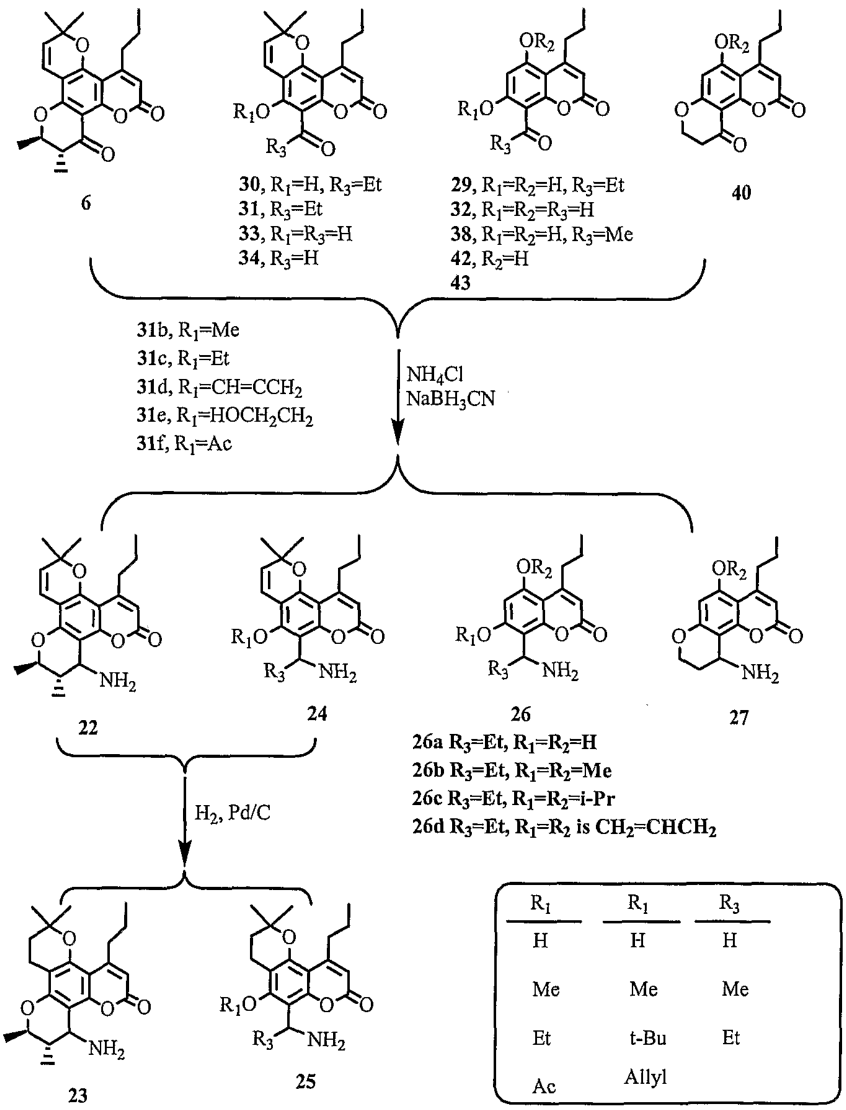
Following the same chemistry described above, a larger library of Type III compounds were investigated using either medium- or high-throughput combinatorial approaches.

Synthesis of Type IV Compounds

Type IV compounds are the amino derivatives of formula (I). As stated above, the amino compounds may be of particular interest for a number of reasons, including the ability to form hydrogen bonds and an increased water-solubility, especially by salt formation with an acid. These compounds of the invention offer an enhanced bioavailability and facilitate the future *in vivo* and clinical studies.

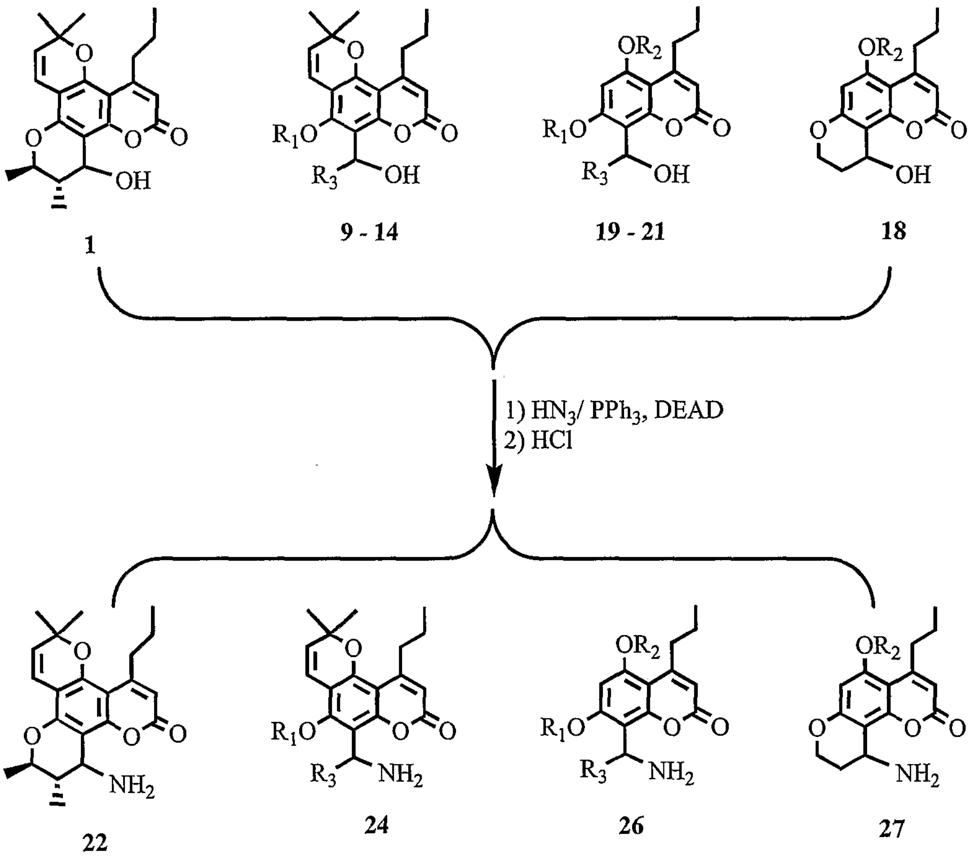
In general, amino compounds can be derived either from reductive amination from the corresponding carbonyl derivatives[16] or from direct replacement of hydroxyl group with amines[17]. Amino compound **23** is known and has been synthesized by nucleophilic displacement of the calanolide triflate ester with NaN_3, followed by hydrogenolysis with the poisoned PtO_2 catalyst. Initially, reductive amination approach is investigated. The precursor carbonyl compounds such as 12-oxocalanolide A (**6**), **29**, **30** and **31** (**Scheme 1**), **32**, **33** and **34** (**Scheme 2**), **38** and **40** (**Scheme 4**), as well as **42** and **43** (**Scheme 5**), are obtained by following the procedures described above. Reductive amination of these carbonyl compounds with NH_4Cl and $NaBH_3CN$[16] affords the corresponding amino derivatives **22**, **24**, **26**, **27** (**Scheme 6**). Catalytic hydrogenation of **22** and **24** provides the dihydro amino compound **23** and **25**.

Scheme 6



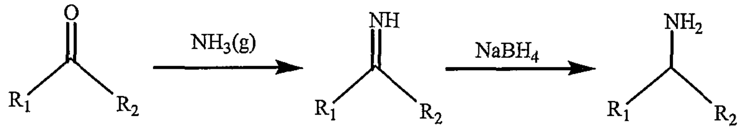
Alternatively, amino compounds **22**, **24**, **26**, **27** are obtained by treating the corresponding alcoholic precursors with HN_3 in the presence of PPh_3 and DEAD followed by the hydrolysis[17] (**Scheme 7**).

Scheme 7

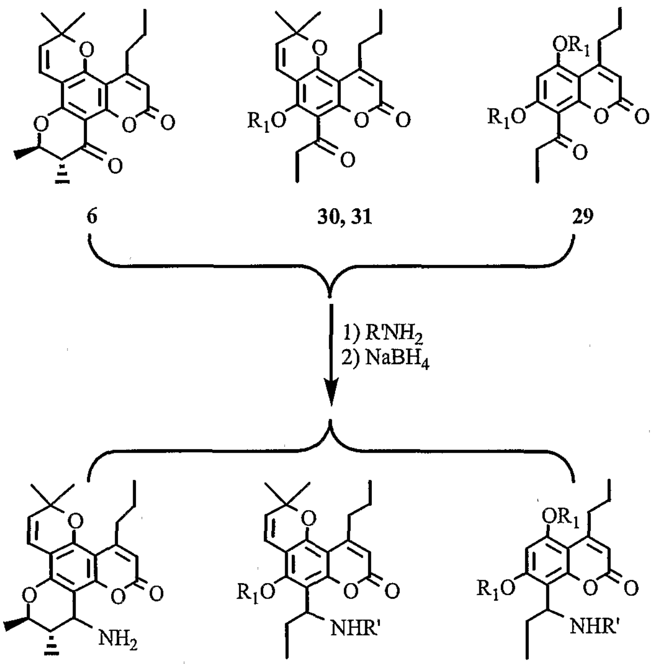


Applicants have successfully developed a one-pot stepwise reductive amination which would have a very general application. Applicants have found that the treatment of a ketone with an excessive amount of NH_3(g) in organic solvent led to the formation of a polar spot as shown on TLC, presumably to be the corresponding imine **A** (**Scheme 8**). ^{1}H NMR on the isolated product confirmed the structure to be imine **A**. Reduction of imine **A** with $NaBH_4$ furnished the corresponding amine **B**. To Applicants' knowledge, this is the first time that NH_3(g) is utilized in the stepwise reductive amination. Furthermore, when NH_3(g) is replaced with amine derivatives (R'NH_2), the corresponding substituted amino analogues can be prepared. Thus, ketone compounds **6, 29, 30**, and **31** were treated with NH_3(g) or amine derivatives (R'NH_2), followed by $NaBH_4$ reduction, affording the corresponding amino compounds **22, 45**, and **46** (**Scheme 8**).

Scheme 8



A B

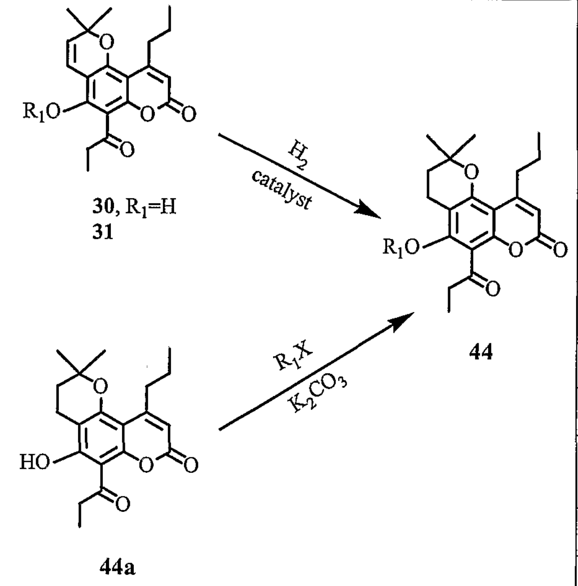


22 24 26

R_1 / R'	24 Yield%	26 Yield%
a, H / H		
b, Me / H	98	60
c, Et / H		
d, CH≡CCH$_2$ / H		
e, HOCH$_2$CH$_2$ / H		
f, Me / Me		
g, Me / Et	27	
h, Me / i-Pr		
i, Me / HOCH$_2$CH$_2$	22	
j, Me / Bn		
k, i-Pr / H		23
l, CH$_2$=CHCH$_2$ / H		46

In order to further evaluate the flexibility of the OH group, identified to be the key structural elements required for anti-TB activity in the lead pyranocoumarin compounds, 7,8-dihydroketones **44** were synthesized (**Scheme 9**). Two synthetic routes have been utilized. One is the hydrogenation of **30** and **31**, and the other is the direct alkylation of **44a**.

Scheme 9

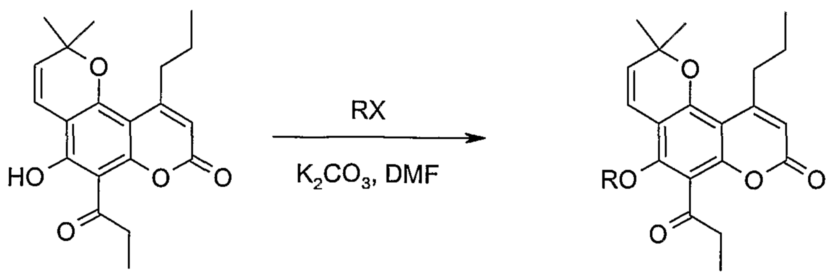


R1	Catalyst or Reagents	Yield%
a, H	PtO_2 (NH_3) in toluene	
b, Me	5% PtS_x on C in EtOH	68
c, Et	PtO_2 (NH_3) in EtOH	61
d, $CH\equiv CCH_2$	$CH\equiv CCH_2Cl$	72
g, CH_2=$CHCH_2$	CH_2=$CHCH_2Br$	93
n, CH_2=$CHCH(CH_3)$	CH_2=$CHCH(CH_3)$	
o, CH_2=$C(CH_3)CH_2$	CH_2=$C(CH_3)CH_2$	
h, i-Pr	i-PrBr	89
f, $AcOCH_2CH_2$	$AcOCH_2CH_2Br$	82
e, $HOCH_2CH_2$		33
m, $MeOCH_2$	$MeOCH_2Br$	92
l, 2-(4-morpholin)-ethyl	2-(4-morpholin)ethyl Bromide	73
i, EtO_2CCH_2	EtO_2CCH_2Br	81

EXAMPLES

The following examples are presented merely for illustrative purposes and are not intended to limit the spirit or scope of the instant invention.

Example 1: *5-Alkyloxy-2,2-dimethyl-6-propionyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one*(**31**)

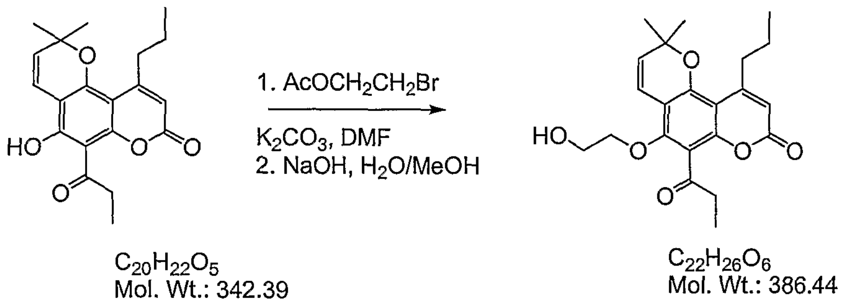


(30) **(31)**

To a mixture of 5-hydroxy-2,2-dimethyl-6-propionyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**30**) (1.0 eq) and potassium carbonate (5-10 eq) in DMF (10-30 mL/mmol) was added an alkylating reagent. The mixture was stirred at ambient temperature until the reaction was completed (16 to 24 h). The mixture was partitioned between water (10 volumes of DMF) and ethyl acetate (50 mL/mmol). The organic layer was separated, washed successively with water and brine, dried over anhydrous sodium sulfate and concentrated. The desired product was purified on a silica gel column chromatography eluted with 10-30% ethyl acetate in hexanes. Compound **31b** is the representative and its analytical data is shown below.

5-Methoxy-2,2-dimethyl-6-propionyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**31b**): 73% yield. ^{1}H NMR ($CDCl_3$): 1.04 (t, J=7.5 Hz, 3H), 1.20 (t, J=7.5 Hz, 3H), 1.52 (s, 6H), 1.65 (m, 2H), 2.91 (m, 4H), 3.79 (s, 3H), 5.66 (d, J=10.0 Hz, 1H), 6.01 (s, 1H), 6.54 (J=10.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): 7.86, 13.78, 23.03, 27.76, 38.41, 38.48, 63.64, 78.15, 106.33, 111.12, 112.70, 116.39, 117.66, 128.70, 151.90, 152.59, 155.19, 157.32, 159.50, 202.88; IR: 1728, 1701 cm^{-1}; MS (ACPI +): 357 (M+1); Anal. Calcd. for $C_{21}H_{24}O_5$: C 70.77, H 6.79; Found: C 70.41, H 6.75.

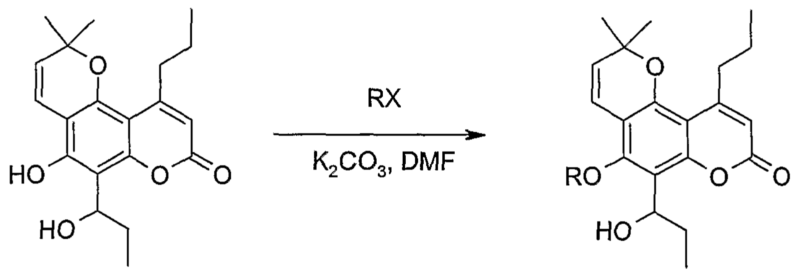
Example 2: *5-(2-Hydroxy-ethoxy)-2,2-dimethyl-6-propionyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one* (**31e**)



(**30**) (**31e**)

To a solution of 5-hydroxy-2,2-dimethyl-6-propionyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**30**) (3.40g, 10 mmol, 1.0 eq) in DMF (60 mL) was added potassium carbonate (13.8g 100mmol, 10.0 eq) followed by 2-bromoethyl acetate (5g, 30mmol, 3.0 eq). The reaction mixture was stirred for 2 days at ambient temperature, which was partitioned between water (600mL) and ethyl acetate (300ml). The organic layer was washed with an additional portion of water (600mL), dried and concentrated. The solid residue was re-dissolved in methanol (200 mL) followed by addition of 2 N aqueous sodium hydroxide (20 mL). The mixture was stirred for 2 h at room temperature, pH adjusted to 6.0 with concentrated hydrochloric acid and concentrated under vacuum. The residue was partitioned between water (200 mL) and ethyl acetate (200 mL). The organic layer was separated, washed successively with water (100 mL) and brine (100 mL), dried over anhydrous sodium sulfate and concentrated under vacuum. The solid residue was crystallized from cyclohexane/ethanol 80/20 and re-crystallized from methanol/water 95/5, affording 1.23 g of **31e** (32% yield). ^{1}H NMR ($CDCl_3$): 1.05 (t, J=7.5 Hz, 3H), 1.21 (t, J=7.5 Hz, 3H), 1.52 (s, 6H), 1.67 (m, 2H), 2.92 (m, 4H), 3.29 (t, J=6.5 Hz, 1H), 3.81 (m, 2H), 4.13 (m, 2H), 5.66 (d, J=10.0 Hz, 1H), 6.03 (s, 1H), 6.54 (J=10.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): 7.99, 13.89, 23.04, 27.60, 38.50, 38.54, 62.01, 78.08, 78.41, 106.17, 110.77, 112.63, 116.92, 117.04, 128.47, 152.27, 153.12, 154.78, 157.39, 159.38, 203.60; IR: 1708 cm^{-1}; MS (ACPI +): 387 (M+1); Anal. Calcd. for $C_{22}H_{26}O_6$: C 68.38, H 6.78; Found: C 68.21, H 6.90.

Example 3: *5-Alkyloxy-6-(1-hydroxypropyl)-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one* (**11**)

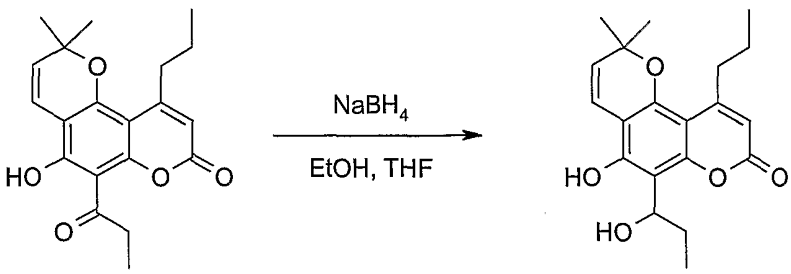


(**11a**) (**11**)

To a mixture of 5-hydroxy-6-(1-hydroxy-propyl)-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**11a**) (1.0 eq) and potassium carbonate (10 eq) in anhydrous DMF (10-15 mL/mmol of starting material) was added alkylating reagent (2.0-2.5 eq). The reaction mixture was stirred overnight at ambient temperature. The mixture was poured into water (10 volumes of DMF) and extracted with ethyl acetate (50 mL/mmol of starting material). The organic layer was separated, washed with brine and dried over anhydrous sodium sulfate. Solvent was removed under vacuum and the product purified on a silica gel column eluted with 10-30% ethyl acetate in hexanes. Compound **11b** is the representative and its analytical data is shown below.

6-(1-Hydroxypropyl)-5-methoxy-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**11b**): 81% yield. ^{1}H NMR ($CDCl_3$): 1.02 (m, 6H), 1.49 (s, 3H), 1.52 (s, 3H), 1.66 (m, 2H), 1.88 (m, 2H), 2.08 (m, 1H), 2.91 (m, 2H), 3.11 (d, J=10.0 Hz, 1H), 3.84 (s, 3H), 5.04 (m, 1H), 5.65 (d, J=10.0 Hz, 1H), 6.03 (s, 1H), 6.54 (d, J=10.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): 10.93, 13.94, 23.12, 27.32, 27.81, 30.61, 38.69, 63.05, 69.37, 77.47, 106.93, 111.01, 112.24, 117.07, 117.19, 128.49, 151.20, 153.37, 156.65, 158.14, 159.94; IR: 1729 cm^{-1}; MS (ACPI +): 359 (M+1), 341 (M+1-H2O); Anal. Calcd. for $C_{21}H_{26}O_5$: C 70.37, H 7.31; Found: C 70.15, H 7.35.

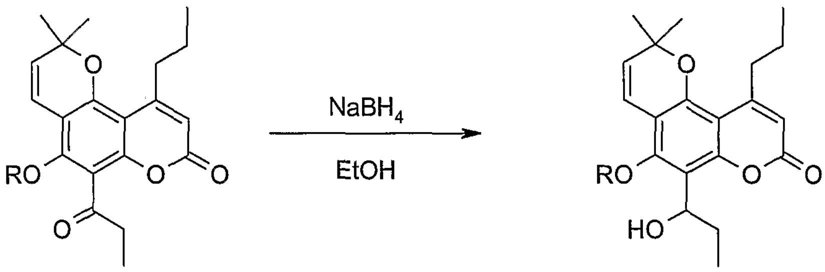
Example 4: *5-Hydroxy-6-(1-hydroxypropyl)-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one* (**11a**)



(30) **(11a)**

A solution of 5-hydroxy-2,2-dimethyl-6-propionyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**30**) (10.0 g, 29.2 mmol, 1.0 eq) in a mixture of ethanol (500 mL) and THF (200 mL) was cooled to 0 °C and sodium borohydride (1.7 g, 45.0 mmol, 1.54 eq) was add portionwise within 1h. The reaction mixture was allowed to warm up room temperature and stirred for 3.5 h. The reaction was quenched with saturated aqueous ammonium chloride (20 mL). The solvents were removed under vacuum and the residue was partitioned between ice-cold 1N hydrochloric acid (200 mL) and ethyl acetate (500 mL). The organic layer was separated and washed successively with saturated sodium bicarbonate (200 mL) and brine (200 mL). The organic solution was dried over anhydrous sodium sulfate and concentrated under vacuum. The product was purified on a silica column eluted with 20-50% ethyl acetate in hexanes, affording 7.1 g of **11a** (71% yield). ^{1}H NMR ($CDCl_3$): 1.01 (q, J=7.5 Hz, 6H), 1.46 (s, 3H), 1.50 (s,3H), 1.61 (m, 2H), 1.88 (m, 2H), 2.78 (m, 1H), 2.89 (m, 1H), 4.96 (d, J=3.5 Hz, 1H), 5.54 (d, J=10.0 Hz, m, 2H), 5.78 (s, 1H), 6.69 (d, J=10.0 Hz, 1H), 10.18 (s, 1H); ^{13}C NMR ($CDCl_3$): 9.57, 14.04, 23.10, 27.47, 28.08, 29.78, 38.59, 70.73, 77.58, 102.85, 107.15, 107.62, 109.12, 116.54, 126.76, 150.89, 151.28, 156.08, 159.70, 162.01; IR: 1683 cm^{-1}; MS (ACPI +): 345 (M+1); Anal. Calcd. for $C_{20}H_{24}O_5$: C 69.75, H 7.02; Found: C 69.90, H 7.05.

Example 5: 5-Alkyloxy-6-(1-hydroxy-propyl)-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**11**)

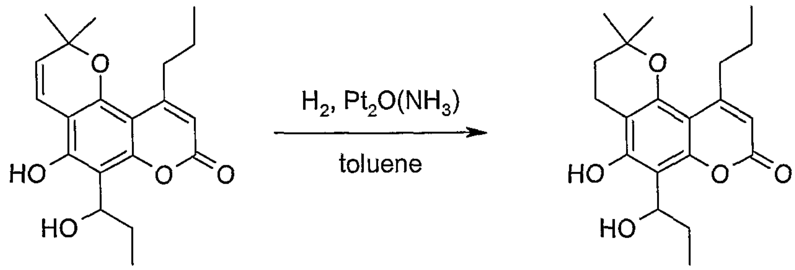


(31) **(11)**

To a solution of 5-alkyloxy-2,2-dimethyl-6-propionyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**31**) in ethanol (100 mL/mmol) was added portionwise sodium borohydride (3-5 eq) at 0 °C. The reaction mixture was allowed to reach room temperature and was stirred until all starting material was consumed (3-4 h). Excess of sodium borohydride was quenched with saturated aqueous ammonium chloride and the mixture was concentrated under vacuum. The residue was partitioned between water and ethyl acetate. The organic layer was separated, washed successively with water and brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was chromatographed on silica gel to provide the desired compound **11**. Compound **11e** is the representative and its analytical data is shown below.

5-(2-Hydroxyethoxy)-6-(1-hydroxypropyl)-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**11e**): 64% yield. ^{1}H NMR ($CDCl_3$): 0.96 (t, J=7.5 Hz, 3H), 1.04 (t, J=7.5 Hz, 3H), 1.49 (s, 3H), 1.52 (s, 3H), 1.66 (m, 2H), 1.93 (m, 1H), 2.14 (m, 1H), 2.91 (m, 2H), 3.78 (br. s, 2H), 3.94 (m, 2H), 4.10 (m, 2H), 5.63 (d, J=10.0 Hz, 1H), 6.03 (s, 1H), 6.56 (d, J=10.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): 10.88, 13.93, 23.13, 27.21, 27.68, 29.82, 38.68, 61.66, 68.70, 77.00, 77.25, 106.65, 111.20, 112.134, 116.96, 117.47, 128.25, 151.31, 153.17, 156.36, 158.27, 160.36; IR: 1730 cm^{-1}; MS (ACPI +): 371 (M-H_2O+1); Anal. Calcd. for $C_{22}H_{28}O_6$: C 68.02, H 7.27; Found: C 68.04, H 7.21.

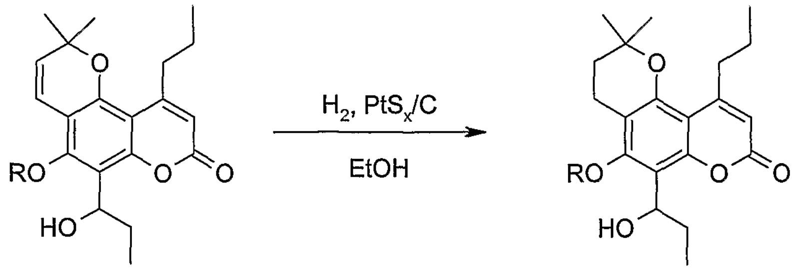
Example 6: 5-Hydroxy-6-(1-hydroxypropyl)-2,2-dimethyl-10-propyl-3,4-dihydro-2H-pyrano[2,3-f]chromen-8-one (**12a**)



(11a) (12a)

A mixture of 5-hydroxy-6-(1-hydroxypropyl)-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**11a**) (400 mg, 1.16 mmol) and platinum oxide poisoned with ammonia (100 mg) in toluene (50 mL) was hydrogenated under atmospheric pressure of hydrogen at ambient temperature for 2 hours. Column chromatography on silica gel eluted with 30% ethyl acetate in hexanes and subsequent crystallization from 20% acetone in hexanes provided 252 mg of **12a** (63% yield). ^{1}H NMR ($CDCl_3$): 1.02 (t, J=7.5 Hz, 3H), 1.22 (t, J=7.5 Hz, 3H), 1.41 (s, 6H), 1.62 (m, 2H), 1.82 (t, J=6.5 Hz, 2H), 2.75 (t, J=6.5 Hz, 2H), 2.91 (m, 4H), 3.78 (s, 3H), 6.01 (s, 1H); ^{13}C NMR ($CDCl_3$): 7.97, 13.84, 17.20, 23.19, 26.66, 31.33, 38.48, 38.99, 62.14, 76.35, 106.14, 110.66, 112.48, 116.55, 151.24, 153.50, 157.51, 157.92, 159.92, 203.40; IR: 1724, 1704 cm^{-1}; MS (ACPI +): 359 (M+1); Anal. Calcd. for $C_{21}H_{26}O_5$: C 70.37, H 7.31; Found: C 70.15, H 7.28.

Example 7: *5-Alkyloxy-6-(1-hydroxy-propyl)-2,2-dimethyl-10-propyl-3,4-dihydro-2H-pyrano[2,3-f]chromen-8-one* (**12**)



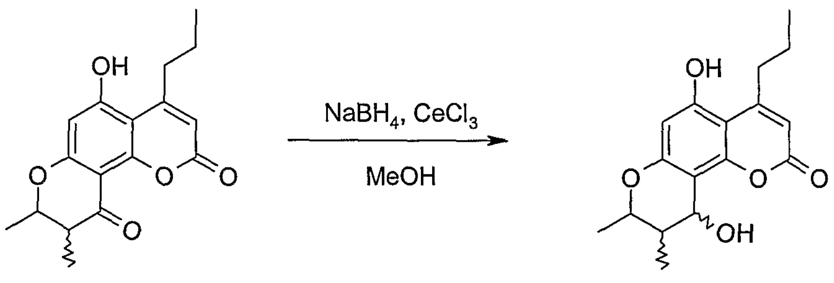
(11) (12)

A solution of 5-alkyloxy-6-(1-hydroxy-propyl)-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**11**) in ethanol (50 mL/mmol) was flushed with nitrogen for 15 min. Sulfided platinum on carbon (Aldrich, 100-150 mg per 1 mmol) was added to the solution and the mixture stirred overnight at ambient temperature under atmospheric pressure of hydrogen. The mixture was filtered through a pad of Celite and the filtrate was concentrated under vacuum. The product was purified on a silica column eluted with 15-30% ethyl acetate in hexanes. The representative compounds are shown below.

6-(1-Hydroxypropyl)-5-methoxy-2,2-dimethyl-10-propyl-3,4-dihydro-2H-pyrano[2,3-f]chromen-8-one (**12b**): 73% yield. ^{1}H NMR ($CDCl_3$): 1.02 (m, 6H), 1.38 (s, 3H), 1.42 (s, 3H), 1.62 (m, 2H), 1.85 (m, 4H), 2.13 (m, 1H), 2.83 (m, 4H), 3.08 (d, J=11.0 Hz, 1H), 3.83 (s, 3H), 5.01 (m, 1H), 6.01 (s, 1H); ^{13}C NMR ($CDCl_3$): 11.06, 13.90, 17.50, 23.27, 26.11, 27.28, 30.64, 31.52, 39.23, 61.48, 69.63, 75.79, 106.98, 110.57, 112.11, 116.38, 152.08, 152.81, 156.71, 158.81, 160.25; IR: 1711 cm^{-1}; MS (ACPI +): 361 (M+1), 343 (M+1-H2O); Anal calc for $C_{21}H_{28}O_5 \cdot 1/3H_2O$: C 68.83, H 7.88; Found: C 68.50, H 7.89.

5-(2-Hydroxyethoxy)-6-(1-hydroxypropyl)-2,2-dimethyl-10-propyl-3,4-dihydro-2H-pyrano[2,3-f]chromen-8-one (**12e**): 365 yield. ^{1}H NMR ($CDCl_3$): 0.94 (t, J=7.0 Hz, 3H), 1.03 (t, J=7.0 Hz, 3H), 1.40 (s, 3H), 1.41 (s, 3H), 1.64 (m, 2H), 1.86 (m, 3H), 2.17 (m, 1H), 2.80 (t, J=6.5 Hz, 2H), 2.90 (m, 2H), 3.92 (m, 1H), 4.01 (m, 1H), 4.14 (m, 1H), 5.17 (t, J=7.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): 11.02, 13.90, 17.98, 23.27, 26.40, 27.01, 29.89, 31.54, 39.24, 61.94, 69.07, 75.11, 75.78, 106.76, 110.68, 112.04, 116.18, 152.13, 152.58, 158.18, 158.79, 160.56; IR: 1721, 1685 cm^{-1}; MS (ACPI +): 373 (M+1-H2O); Anal calc for $C_{22}H_{30}O_6$: C 67.67, H7.74; Found C 67.31, H 7.69.

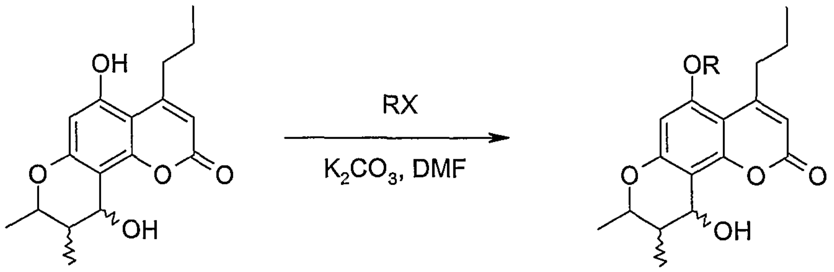
Example 8: *5,10-Dihydroxy-8,9-dimethyl-4-propyl-9,10-dihydro-8H-pyrano[2,3-f]chromen-2-one* (cis- and trans-**17a**)



(37a) **(17a)**

A solution of 5-hydroxy-8,9-dimethyl-4-propyl-8,9-dihydro-pyrano[2,3-f]chromene-2,10-dione (a mixture of cis- and trans-**37a**) (2 g, 6.61 mmol, 1.00 eq) and cerium(III) chloride septahydrate (2.5 g, 6.71 mol, 1.02 eq) in methanol (300 mL) was cooled to 0 °C. Sodium borohydride (0.62 g, 16.39 mmol, 2.48 eq) was added portionwise to the stirred solution within 1 h while the temp was maintained at 0-5 °C. The reaction mixture was allowed to warm up to room temperature and stirring continued for 2 h. Solvent was removed under vacuum and the residue partitioned between dichloromethane (200 mL) and ice-cold 1N hydrochloric acid (100 mL). The organic layer was separated, washed successively with saturated solution of sodium bicarbonate (200 mL) and brine (200 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum yielding 1.97 g (98%) of a mixture of cis- and trans-**17a**. The crude product was used for the next step without further purification.

Example 9: *5-Alkyloxy-10-hydroxy-8,9-dimethyl-4-propyl-9,10-dihydro-8H-pyrano[2,3-f]chromen-2-one* (cis- and trans-**17**)



(±**17a**) (±**17**)

To a stirred mixture of a crude 10-hydroxy-5-methoxy-8,9-dimethyl-4-propyl-9,10-dihydro-8H-pyrano[2,3-f]chromen-2-one (a mixture of cis- and trans-**17a**) (0.5 g, 1.64 mmol, 1.00 eq) and potassium carbonate (4.18 g, 30.24 mmol, 18.44 eq) in anhydrous DMF (50 mL) was added alkylating reagent (4.00-5.00 eq). The reaction mixture was stirred overnight at room temperature. The mixture was then poured into water (200 mL) and extracted with ethyl acetate (100 mL). The organic layer was separated and washed successively with water (2x 100 mL) and brine. The organic solution was dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified on short silica column (15-30% ethyl acetate in hexanes) to provide a mixture of isomers. Two major isomers were separated on preparative HPLC (Alltech Econosil 10 um, 250x22 mm column, 15-30% ethyl acetate in hexanes) in a ratio of 65:45. The representative compounds are shown below.

5-Methoxy-10-hydroxy-8,9-dimethyl-4-propyl-9,10-dihydro-8H-pyrano[2,3-f]chromen-2-one (cis- and trans-**17b**): 69% combined yield. 8,9-trans-**17b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.01 (3H, t, J = 7.5 Hz), 1.15 (3H, d, J = 7.0 Hz), 1.42 (3H, d, J = 6.5 Hz), 1.61 (2H, sextet, J = 7.0 Hz), 2.29 (1H, ddq, J = 3.5, 4.5, 7.5 Hz), 2.80-2.92 (2H, m), 3.17 (1H, d, J = 3.5 Hz), 3.86 (3H, s), 4.37 (1H, dq, J = 3.3, 6.6 Hz), 5.10 (1H, dd, J = 3.5, 5.0 Hz), 5.96 (1H, s), 6.25 (1H, s); ^{13}C NMR (125.65 Hz, $CDCl_3$) δ 9.5, 14.0, 16.1, 22.8, 35.7, 38.7, 55.7, 75.7, 96.0, 104.1, 106.0, 109.9, 155.6, 156.9, 158.1, 159.0, 160.7; IR (film) 3471, 2998, 2963, 2937, 2891, 2873, 1702, 1614, 1584, 1486, 1455, 1378, 1346, 1300, 1280, 1204, 1158, 1115, 1063, 1031, 965, 820 cm^{-1}; MS (APCI) m/e 319 (M+1), 301 (M-OH); Anal. Calcd. for $C_{18}H_{22}O_5$: C, 67.91; H, 6.97. Found: C, 67.65; H, 7.08. 8,9-cis-**17b:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.01 (3H, t, J = 7.5 Hz), 1.14 (3H, d, J = 7.0 Hz), 1.45 (3H, d, J = 6.0 Hz), 1.61 (2H, sextet, J = 7.3 Hz), 1.94 (1H, sextet, J = 7.2 Hz), 2.80-2.92 (2H, m), 3.60 (1H, d, J = 2.5 Hz), 3.85 (3H, s), 3.95 (1H, dq, J = 8.7, 6.2 Hz), 4.73 (1H, dd, J = 2.5, 7.5 Hz), 5.96 (1H, s), 6.26 (1H, s); ^{13}C NMR (125.65 Hz, $CDCl_3$) δ 14.0, 15.1, 18.9, 22.9, 38.7, 40.4, 55.8, 66.9, 77.1, 95.9, 104.4, 106.5, 109.9, 155.5, 157.9, 158.0, 159.0, 160.5; IR (film) 3480, 3403, 2965, 2934, 2898, 2872, 1695, 1615, 1585, 1465, 1374, 1346, 1327, 1297, 1233, 1200, 1166, 1129, 1116, 1109, 1060, 1031, 819 cm^{-1}; MS (APCI) m/e 319 (M+1), 301 (M-OH); Anal. Calcd. for $C_{18}H_{22}O_5 \cdot 1/4H_2O$: C, 66.96; H, 7.02. Found: C, 66.92; H, 7.13.

5-(i-Propyloxy)-10-hydroxy-8,9-dimethyl-4-propyl-9,10-dihydro-8H-pyrano[2,3-f]chromen-2-one (cis- and trans-**17e**): 74% combined yield. 8,9-trans-**17e**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.01 (3H, t, J = 7.2 Hz), 1.14 (3H, d, J = 7.5 Hz), 1.40 (6H, d, J = 6.0 Hz), 1.41 (3H, d, J = 6.5 Hz), 1.63 (2H, d-sextet, J = 2.2, 7.9 Hz), 2.24-2.32 (1H, m), 2.82-2.95 (2H, m), 3.31 (1H, br. s), 4.35 (1H, dq, J = 3.1, 6.7 Hz), 4.64 (1H, septet, J = 6.0 Hz), 5.09 (1H, d, J = 5.0 Hz), 5.94 (1H, s), 6.22 (1H, s); ^{13}C NMR (125.65 Hz, $CDCl_3$) δ 9.5, 13.9, 16.1, 21.7, 23.2, 35.8, 39.0, 62.9, 70.7, 75.7, 97.0, 104.5, 105.5, 109.9, 155.8, 156.2, 156.9, 159.3, 160.8; IR (film) 3433, 2975, 2934, 2874, 1723, 1701, 1615, 1584, 1480, 1451, 1381, 1349, 1331, 1298, 1231, 1202, 1161, 1130, 1108, 1062, 1034, 967, 830 cm^{-1}; MS (APCI) m/e 347 (M+1); Anal. Calcd. for $C_{20}H_{26}O_5 \cdot 2/3\ H_2O$: C, 67.02; H, 7.69. Found: C, 67.08; H, 7.68. 8,9-cis-**17e:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.01 (3H, t, J = 7.5 Hz), 1.14 (3H, d, J = 7.0 Hz), 1.39 (3H, d, J = 6.5 Hz), 1.41 (3H, d, J = 6.0 Hz), 1.44 (3H, d, J = 6.5 Hz), 1.62 (2H, sextet, J = 7.3 Hz), 1.91-1.97 (1H, m), 2.83-2.94 (2H, m), 3.61 (1H, br. s), 3.90-3.98 (1H, m), 4.63 (1H, septet, J = 6.0 Hz), 4.72 (1H, d, J = 8.0 Hz), 5.94 (1H, s), 6.23 (1H, s); ^{13}C NMR (125.65 Hz, $CDCl_3$) δ 13.9, 15.1, 18.9, 21.6, 21.8, 23.2, 39.0, 40.5, 67.0, 70.7, 76.7, 96.9, 104.8, 106.1, 109.9, 155.7, 156.1, 157.9, 159.3, 160.6; IR (film) 3357, 2969, 2934, 2873, 1722, 1696, 1615, 1585, 1480, 1453, 1377, 1350, 1295, 1232, 1199, 1169, 1110, 1063, 1029 cm^{-1}; MS (APCI) m/e 347 (M+1), 329 (M-OH); Anal. Calcd. for $C_{20}H_{26}O_5 \cdot H_2O$: C, 65.92; H, 7.74. Found: C, 65.46; H, 7.48.

5-(Prop-2-ynyloxy)-10-hydroxy-8,9-dimethyl-4-propyl-9,10-dihydro-8H-pyrano[2,3-f]chromen-2-one (cis- and trans-**17f**): 71% combined yield. 8,9-trans-**17f**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.03 (3H, t, J = 7.2 Hz), 1.15 (3H, d, J = 7.0 Hz), 1.42 (3H, d, J = 7.0 Hz), 1.63 (2H, sextet, J = 7.1 Hz), 2.28 (1H, ddq, J = 3.0, 5.0, 7.0 Hz), 2.58 (1H, t, J = 2.5 Hz), 2.82-2.94 (2H, m), 3.21 (1H, d, J = 3.0 Hz), 4.37 (1H, dq, J = 3.0, 6.7 Hz), 4.72 and 4.73 (2H, d-AB type, J_d = 2.5 Hz, J_{AB} = 15.4 Hz), 5.10 (1H, dd, J = 2.0, 5.0 Hz), 5.98 (1H, s), 6.32 (1H, s); ^{13}C NMR (125.65 Hz, $CDCl_3$) δ 9.5, 13.9, 16.1, 23.0, 35.7, 38.8, 56.4, 62.9, 75.8, 76.4, 97.2, 104.3, 106.7, 107.9, 110.4, 155.6, 155.9, 156.7, 158.7, 160.5; IR (film) 3431, 3288, 2962, 2936, 2874, 2125, 1706, 1616, 1588, 1483, 1454, 1384, 1349, 1297, 1231, 1202, 1161, 1130, 1107, 1064, 1035, 1000, 968 cm^{-1}; MS (APCI) m/e 343 (M+1), 325 (M-OH); Anal. Calcd. for $C_{20}H_{22}O_5\cdot 1/2H_2O$: C, 68.36; H, 6.60. Found: C, 68.26; H, 6.57. 8,9-cis-**17f**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.03 (3H, t, J = 7.2 Hz), 1.15 (3H, d, J = 7.0 Hz), 1.45 (3H, d, J = 6.5 Hz), 1.63 (2H, sextet, J = 7.2 Hz), 1.94 (1H, sextet, J = 7.8 Hz), 2.58 (1H, t, J = 2.5 Hz), 2.82-2.94 (2H, m), 3.70 (1H, d, J = 3.0 Hz), 3.95 (1H, dq, J = 8.7, 6.7 Hz), 4.72 (3H, m), 5.99 (1H, s), 6.32 (1H, s); ^{13}C NMR (125.65 Hz, $CDCl_3$) δ 13.9, 15.1, 18.9, 23.0, 38.8, 40.4, 56.4, 66.9, 76.4, 76.8, 97.0, 104.6, 107.4, 107.9, 110.3, 155.5, 155.7, 157.8, 158.9, 160.5; IR (film) 3467, 3380, 3212, 2964, 2933, 2899, 2872, 2117, 1699, 1613, 1589, 1458, 1384, 1344, 1326, 1292, 1259, 1235, 1170, 1127, 1110, 1065, 1034, 997, 813 cm^{-1}; MS (APCI) m/e 343 (M+1), 325 (M-OH); Anal. Calcd. for $C_{20}H_{22}O_5\cdot 1/2H_2O$: C, 68.36; H, 6.60. Found: C, 68.37; H, 6.91.

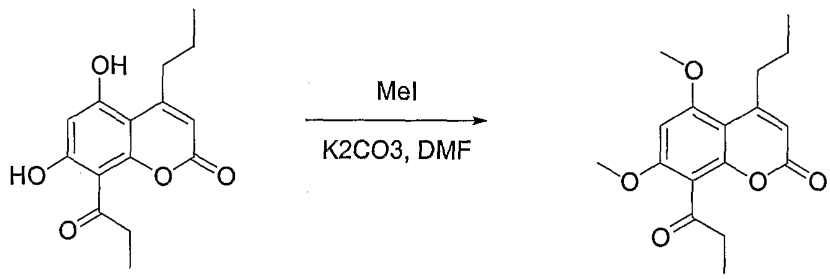
5-(Allyloxy)-10-hydroxy-8,9-dimethyl-4-propyl-9,10-dihydro-8H-pyrano[2,3-f]chromen-2-one (cis- and trans-**17c**): 69% combined yield. 8,9-trans-**17c**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99 (3H, t, J = 7.2 Hz), 1.14 (3H, d, J = 7.0 Hz), 1.41 (3H, d, J = 6.5 Hz), 1.61 (2H, sextet, J = 7.5 Hz), 2.24-2.31 (1H, m), 2.82-2.94 (2H, m), 3.29 (1H, br. s), 4.36 (1H, dq, J = 3.5, 6.7 Hz), 4.56 (2H, d, J = 6.0 Hz), 5.09 (1H, d, J = 5.0 Hz), 5.36 (1H, dd, J = 2.0, 9.5 Hz), 5.43 (1H, dd, J = 1.5, 17.0 Hz), 5.96 (1H, s), 6.03-6.11 (1H, m), 6.24 (1H, s); ^{13}C NMR (125.65 Hz, $CDCl_3$) δ 9.7, 13.9, 16.1, 23.0, 35.7, 38.8, 62.9, 70.0, 75.7, 96.9, 104.2, 106.1, 110.0, 119.2, 132.0, 155.6, 156.8, 157.1, 159.0, 160.7; IR (film) 3436, 3083, 2964, 2934, 2874, 1723, 1617, 1587, 1483, 1456, 1423, 1382, 1350, 1299, 1231, 1195, 1162, 1108, 1064, 1034, 991, 967, 924, 819 cm^{-1}; MS (APCI) m/e 345 (M+1); Anal. Calcd. for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 69.17; H, 7.09. 8,9-cis-**17c**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99 (3H, t, J = 7.2 Hz), 1.14 (3H, d, J = 7.0 Hz), 1.45 (3H, d, J = 6.0 Hz), 1.61

(2H, sextet, J = 7.3 Hz), 1.94 (1H, sextet, J = 7.0 Hz), 2.82-2.94 (2H, m), 3.67 (1H, d J = 3.0 Hz), 3.94 (1H, dq, J = 8.5, 6.2 Hz), 4.56 (2H, d, J = 5.5 Hz), 4.72 (1H, dd, J = 2.5, 8.0 Hz), 5.36 (1H, dd, J = 1.0, 10.5 Hz), 5.42 (1H, dd, J = 1.0, 17.0 Hz), 5.96 (1H, s), 6.03-6.11 (1H, m), 6.25 (1H, s); ^{13}C NMR (125.65 Hz, $CDCl_3$) δ 13.9, 15.1, 18.9, 23.0, 38.9, 40.4, 66.9, 70.0, 76.7, 96.8, 104.5, 106.7, 110.0, 119.2, 132.0, 155.5, 156.9, 157.8, 159.1, 160.5; IR (film) 3448, 2970, 2936, 2891, 2870, 1690, 1615, 1583, 1485, 1455, 1377, 1359, 1347, 1289, 1229, 1200, 1175, 1106, 1065, 1054, 1036, 819 cm^{-1}; MS (APCI) m/e 345 (M+1), 327 (M-OH); Anal. Calcd. for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 68.94; H, 7.16

5-(1-Methylallyloxy)-10-hydroxy-8,9-dimethyl-4-propyl-9,10-dihydro-8H-pyrano[2,3-f]chromen-2-one (cis- and trans-**17g**): 75% combined yield. 8,9-trans-**17g**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.01 (3H, t, J = 7.2 Hz) [0.98 (t, J = 7.2 Hz)], 1.14 (3H, d, J = 7.0 Hz) [1.13 (d, J = 7.0 Hz)], 1.40 (3H, d, J = 6.5 Hz), 1.50 (3H, d, J = 6.5 Hz) [1.51 (d, J = 6.0 Hz)], 1.64 (2H, sextet, J = 7.2 Hz) [1.61 (sextet, J = 7.1 Hz)], 2.27 (1H, ddq, J = 1.0, 4.5, 7.0 Hz), 2.82-2.96 (2H, m), 3.18 (1H, br. s), 4.34 (1H, dq, J = 3.0, 7.0 Hz), 4.89 (1H, sextet, J = 4.0 Hz), 5.10 (1H, dd, J = 2.5, 4.5 Hz), 5.25 (1H, d, J = 10.5 Hz), 5.30 (1H, dd, J = 1.0, 16.5 Hz), 5.86-5.95 (1H, m), 5.95 (1H, s), 6.23 (1H, s) [6.24 (1H, s)]; ^{13}C NMR (125.65 Hz, $CDCl_3$) δ 9.5, 13.9, 16.1, 21.0 (21.2), 23.2 (23.1), 35.7, 39.0 (38.9), 63.0, 69.7, 75.7 (75.6), 98.0 (96.8), 104.4 (104.2), 105.8 (105.7), 110.1 (110.0), 116.7 (116.6), 137.8, 155.7, 156.3, 156.7 (157.2), 159.1, 160.7; IR (film) 3438, 3086, 2963, 2934, 2874, 1721, 1616, 1586, 1480, 1448, 1380, 1349, 1296, 1231, 1199, 1162, 1130, 1104, 1064, 1035, 967 cm^{-1}; MS (APCI) m/e 359 (M+1), 341 (M-OH), 287 (M-OH-C_4H_6); Anal. Calcd. for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 69.99; H, 7.21. 8,9-cis-**17g**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.01 (3H, t, J = 7.2 Hz) [0.98 (t, J = 7.2 Hz)], 1.14 (3H, d, J = 6.5 Hz) [1.13 (d, J = 7.0 Hz)], 1.43 (3H, d, J = 6.5 Hz), 1.50 (3H, d, J = 6.5 Hz) [1.51 (d, J = 6.0 Hz)], 1.64 (2H, sextet, J = 7.5 Hz) [1.61 (sextet, J = 7.2 Hz)], 1.92 (1H, sextet, J = 7.2 Hz), 2.82-2.96 (2H, m), 3.58 (1H, br. s), 3.92 (1H, m), 4.71 (1H, dd, J = 2.0, 7.5 Hz), 4.88 (1H, sextet, J = 6.0 Hz), 5.23-5.32 (2H, m), 5.85-5.95 (1H, m), 5.96 (1H, s), 6.24 (1H, s) [6.23 (1H, s)]; ^{13}C NMR (125.65 Hz, $CDCl_3$) δ 13.8, 15.1, 18.9, 21.1, 23.2 (23.1), 39.0 (38.9), 40.4, 67.0, 69.7, 75.6 (75.5), 97.8 (96.6), 104.6 (104.5), 106.4, 110.0, 116.7 (116.6), 137.8 (137.6), 155.5, 156.1, 157.8 (157.1), 159.1, 160.5; IR (film) 3427, 2966, 2933, 2873, 1723, 1617, 1587, 1480, 1448, 1376, 1293, 1232, 1195, 1172, 1104, 1064, 1028, 992, 895, 830 cm^{-1}; MS (APCI) m/e 359 (M+1), 341 (M-OH), 287 (M-OH-C_4H_6); Anal. Calcd. for $C_{21}H_{26}O_5 \cdot 1/2H_2O$: C, 68.65; H, 7.27. Found: C, 68.89; H, 7.39.

5-(2-Methylallyloxy)-10-hydroxy-8,9-dimethyl-4-propyl-9,10-dihydro-8H-pyrano[2,3-f]chromen-2-one (cis- and trans-**17h**): 75% combined yield. 8,9-trans-**17h**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (3H, t, *J* = 7.2 Hz), 1.14 (3H, d, *J* = 7.0 Hz), 1.41 (3H, d, *J* = 7.0 Hz), 1.62 (2H, sextet, *J* = 7.2 Hz), 1.87 (3H, s), 2.24-2.31 (1H, m), 2.82-2.94 (2H, m), 3.23 (1H, d, *J* = 3.0 Hz), 4.36 (1H, dq, *J* = 3.2, 6.7 Hz), 4.47 (2H, s), 5.06 (1H, s), 5.09 (2H, s), 5.97 (1H, s), 6.25 (1H, s); ^{13}C NMR (125.65 Hz, $CDCl_3$) δ 9.5, 13.8, 16.1, 22.8, 35.7, 38.7, 62.9, 73.2, 75.7, 97.0, 104.2, 106.1, 109.9, 114.5, 139.6, 155.6, 155.8, 157.4, 159.0, 160.6; IR (film) 3513, 3083, 2957, 2932, 2911, 2874, 1707, 1616, 1587, 1487, 1460, 1438, 1382, 1368, 1261, 1202, 1159, 1132, 1110, 1063, 1033, 970, 923, 843, 823 cm^{-1}; MS (APCI) m/e 359 (M+1), 341 (M-OH); Anal. Calcd. for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.08; H, 7.32. 8,9-cis-**17h**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (3H, t, *J* = 7.2 Hz), 1.14 (3H, d, *J* = 6.5 Hz), 1.44 (3H, d, *J* = 6.0 Hz), 1.62 (2H, sextet, *J* = 7.3 Hz), 1.86 (3H, s), 1.94 (1H, sextet, *J* = 7.2 Hz), 2.82-2.94 (2H, m), 3.71 (1H, br. s), 3.94 (1H, dq, *J* = 8.7, 6.2 Hz), 4.47 (2H, s), 4.72 (1H, d, *J* = 7.5 Hz), 5.06 (1H, s), 5.09 (1H, s), 5.97 (1H, s), 6.25 (1H, s); ^{13}C NMR (125.65 Hz, $CDCl_3$) δ 13.8, 15.1, 18.9, 19.7, 22.8, 38.8, 40.4, 66.9, 73.2, 76.7, 96.9, 104.5, 106.7, 109.8, 114.5, 139.6, 155.5, 157.2, 157.8, 159.1, 160.6; IR (film) 3441, 3203, 2938, 2900, 2874, 1698, 1618, 1586, 1486, 1384, 1355, 1325, 1297, 1275, 1229, 1210, 1174, 1127, 1109, 1068, 1029, 882 cm^{-1}; MS (APCI) m/e 359 (M+1), 341 (M-OH); Anal. Calcd. for $C_{21}H_{26}O_5 \cdot 1/4H_2O$: C, 69.50; H, 7.36. Found: C, 69.47; H, 7.38.

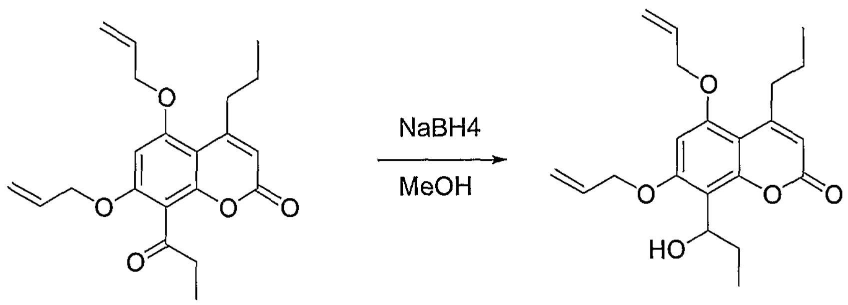
Example 10: *5,7-Dimethoxy-8-propionyl-4-propyl-chromen-2-one* (**43b**)



(38) **(43b)**

The same procedure as used in the preparation of **31a**. Compound **43b**: ^{1}H NMR ($CDCl_3$): 1.01 (t, 3H), 1.19 (t, 3H), 1.59 (m, 2H), 2.85 (m, 4H), 3.89 (s, 3H), 3.94 (s, 3H), 5.96 (s, 1H), 6.32 (s, 1H); Calcd. for $C_{17}H_{20}O_5$: C 67.09, H 6.62; Found C 67.21, H 6.66.

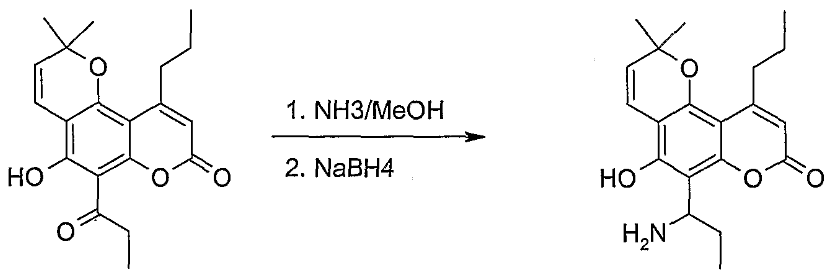
Example 11: *5,7-Bis-allyloxy-8-(1-hydroxy-propyl)-4-propyl-chromen-2-one* (**21d**)



(21d)

The same procedure as used in the preparation of **11e**. Compound **21d**: ^{1}H NMR ($CDCl_3$): 0.89 (t, J=7.5 Hz, 3H), 0.90 (t, J=7.5 Hz, 3H), 1.61 (m, 2H), 1.86 (m, 1H), 2.04 (m, 1H), 2.89 (t, J=7.5 Hz, 2H), 3.28 (d, 1H), 4.62 (d, 2H), 4.66 (d, 2H), 5.40 (m, 2H), 5.99 (s, 1H), 6.06 (m, 2H), 6.37 (s, 1H); ^{13}C NMR ($CDCl_3$): 10.63, 13.86, 22.89, 30.23, 38.82, 68.76, 69.48, 70.10, 93.62, 104.42, 111.06, 112.79, 118.56, 119.20, 132.05, 153.79, 156.52, 158.24, 158.73, 160.32; IR: 1708 cm^{-1}; MS (APCI+): 359 (M+1), 341 ($M-H_2O+1$).

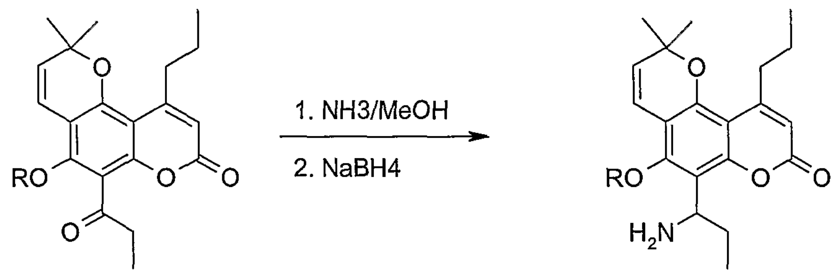
Example 12: *6-(1-Aminopropyl)-5-hydroxy-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one* (**24a**)



(30) **(24a)**

5-Hydroxy-2,2-dimethyl-6-propionyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**30**) (1.0 g, 2.9 mmol, 1.0 eq) was dissolved in 7 N ammonia in methanol (100 mL, 0.7 mol, 241 eq). The mixture was stirred overnight at ambient temperature. Solvent and excess of ammonia were removed under vacuum and the residue re-dissolved in methanol (100 mL). The solution was cooled to 0 °C and sodium borohydride (550 mg, 14.6 mmol, 5.0 eq) added portionwise. The mixture was allowed to warm up room temperature and stirred for 3h. The mixture was then poured into ice-water and pH adjusted to 8.5 with concentrated hydrochloric acid. The product was extracted into ethyl acetate (3 x 50 mL). The extracts were combined and washed with brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was chromatographed on silica gel column eluted with 5-10% methanol in dichloromethane to yield 612 mg of **24a** (61%yield). ^{1}H NMR ($CDCl_3$): 0.97 (t, J=7.5 Hz, 3H), 1.03 (t, J=7.5 Hz, 3H), 1.47 (s, 3H), 1.49 (s, 3H), 1.67 (m, 2H), 1.78 (m, 2H), 2.89 (m, 2H), 4.85 (t, J=7.0 Hz, 1H), 5.52 (d, J=10.0 Hz, 1H), 5.88 (s, 1H), 6.67 (d, J=10.0 Hz, 1H); ^{13}C NMR (DMSO-d_6): 10.17, 13.99, 23.54, 26.24, 27.23, 27.31, 37.99, 49.84, 76.35, 96.60, 102.88, 103.95, 107.80, 118.29, 123.55, 150.87, 153.82, 158.66, 160.26, 167.83; IR: 1683 cm^{-1}; MS (ACPI +): 344 (M+1), 327 (M-NH_3+1); Anal. Calcd. for $C_{20}H_{25}NO_4$: C 69.95, H 7.34, N 4.08; Found C 69.97, H 7.30, N 4.08.

Example 13: *5-Alkyloxy-6-(1-amino-propyl)-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one* (**24**)

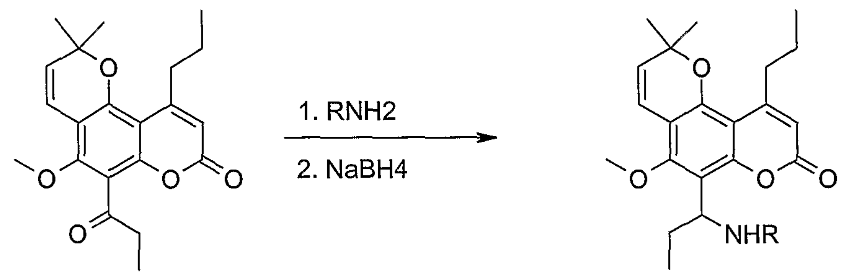


(31) **(24)**

5-Alkyloxy-2,2-dimethyl-6-propionyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**31**) was dissolved in 7N methanolic ammonia (5-10 mL/mmol). The reaction mixture was stirred at ambient temperature overnight or until the starting material was completely consumed. Volatiles were removed on Rotavapor and the residue was re-dissolved in methanol (5-10 ml/mmol) and the solution was cooled to 0 deg C. Sodium borohydride (5 eq) was added portionwise and the mixture was allowed to reach room temperature. The mixture was stirred for 2 h, quenched with water, diluted with ethyl acetate (50 mL/mmol). The mixture was washed successively with water and brine, dried over anhydrous sodium sulfate and the solution was concentrated on Rotavapor. The desired product was separated on silica gel column eluted with 2-10% methanol in dichloromethane. Compound **24e** is the representative and its analytical data is shown below.

6-(1-Aminopropyl)-5-(2-hydroxyethoxy)-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**24e**): 47% yield. ^{1}H NMR ($CDCl_3$): 0.86 (t, J=7.5 Hz, 3H), 1.03 (t, J=7.5 Hz, 3H), 1.50 (s, 3H), 1.52 (s, 3H), 1.66 (m, 2H), 2.01 (m, 1H), 2.11 (m, 1H), 2.91 (m, 4H), 3.80 (m, 2H), 4.13 (m, 1H), 4.21 (m,1H), 4.63 (t, J=7.5 Hz, 1H), 5.63 (d, J=10.0 Hz, 1H), 6.02 (s, 1H), 6.50 (J=10.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): 11.51, 13.97, 23.15, 27.27, 27.58, 29.25, 38.71, 49.23, 61.65, 78.58, 106.36, 110.64, 111.98, 118.02, 127.82, 151.41, 153.77, 158.25, 160.36; IR: 1721 cm^{-1}; MS (ACPI +): 388 (M+1), 371 (M+1-NH3); Anal calc for $C_{22}H_{29}O_5 \cdot 1/2H_2O$: C 66.65, H 7.63, N 3.53. Found C 66.61, H 7.31, N 3.29.

Example 14: *N-Substituted 6-(1-aminopropyl)-5-methoxy-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one* (**24**)

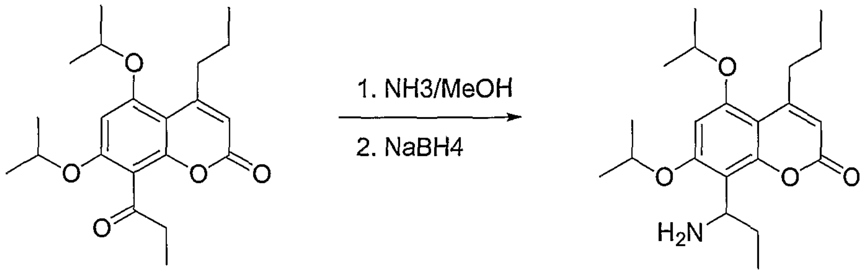


(**31b**) (**24**)

A solution of 5-methoxy-2,2-dimethyl-6-propionyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**31b**) and an amine (10-40 eq) in methanol or methanol/tetrahydrofurane (50-100 mL per mmol) was stirred until all starting material was consumed (16-36 h). Sodium borohydride (5 eq) was then added portionwise to the reaction mixture and stirring was continued until the reaction was completed (2-4 h). Excess of sodium borohydride was quenched with water and the mixture concentrated under vacuum. The residue was partitioned between water (50 mL/mmol) and ethyl acetate (100 mL/mmol). The organic layer was separated and washed consecutively with water (2 x 50 mL) and brine. The organic solution was dried over anhydrous sodium sulfate and concentrated. The residue was chromatographed on silica gel column eluted with 2-10% methanol in dichloromethane. Compound **24g** is the representative and its analytical data is shown below.

6-(1-Ethylaminopropyl)-5-methoxy-2,2-dimethyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one, hydrochloride (**24g**): 27% yield. ^{1}H NMR (DMSO-d_6): 0.85 (t, J=7.0 Hz, 3H), 1.05 (t, J=7.5 Hz, 3H), 1.10 (t, J=7.5 Hz, 3H), 1.50 (s, 3H), 1.52 (s, 3H), 1.66 (m, 2H), 2.09 (m, 1H), 2.52 (m, 4H), 2.91 (m, 2H), 3.80 (s, 3H), 5.64 (d, J=10.0 Hz, 1H), 6.03 (s, 1H), 6.54 (d, J=10.0 Hz, 1H); ^{13}C NMR (DMSO-d_6): 10.76, 13.74, 22.92, 27.23, 37.68, 63.46, 66.31, 77.94, 110.33, 112.33, 116.48, 152.28, 157.23, 157.78, 158.32; IR:1733 cm^{-1}; MS (ACPI +): 386 (M+1), 341 (M-$C_2H_5NH_2$+1); Anal calcd. for $C_{23}H_{32}ClNO_4$: C 65.47, H 7.64, N 3.32; Found C 65.15, H 7.76, N 3.32.

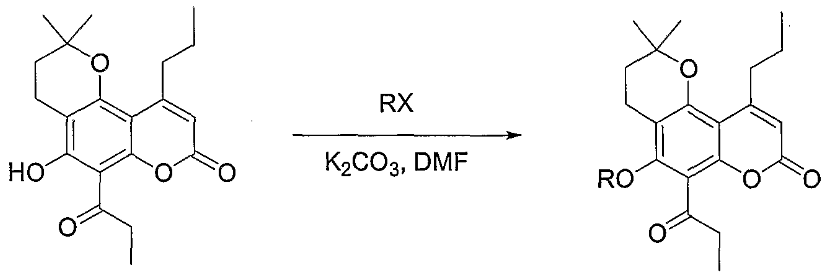
Example 15: *8-(1-Aminopropyl)-5,7-diisopropoxy-4-propyl-chromen-2-one, hydrochloride* **(26k)**



(26k)

The same procedure as used in the preparation of **24e**. Compound **26k**: 1H NMR (DMSO-d_6): 0.77 (t, 3H), 0.98 (t, 3H), 1.36 (d, 12H), 1.57 (m, 2H), 2.05 (m, 2H), 2.87 (m, 2H), 4.62 (bs, 1H), 4.99 (m, 2H), 6.04 (s, 1H), 6.67 (s, 1H), 8.22 (bs, 3H); 13C NMR (DMSO-d_6): 10.39, 13.67, 21.32, 22.94, 38.09, 46.67, 70.65, 71.17, 94.70, 103.05, 104.07, 110.27, 154.32, 156.67, 158.19, 158.78, 158.91; IR: 1720 cm^{-1}; MS (APCI+): 362 (M+1), 345 (M-NH_3+1); Anal. Calcd. for $C_{21}H_{32}ClNO_4 \cdot 1/2H_2O$: C 61.98, H 8.17, N 3.44; Found C 61.85, H 8.04, N 3.56.

Example 16: *5-Alkyloxy-2,2-dimethyl-6-propionyl-10-propyl-3,4-dihydro-2H-pyrano[2,3-f]chromen-8-one* **(44)**



(44a) **(44)**

To a mixture of 5-hydroxy-2,2-dimethyl-6-propionyl-10-propyl-3,4-dihydro-2H-pyrano[2,3-f]chromen-8-one (**44a**) and potassium carbonate (10 eq) in DMF (15 mL/mmol) was added alkyl halide (2-5 eq) and the reaction was carried out 16h at ambient temperature. The mixture was partitioned between water (10 volumes of DMF) and ethyl acetate (50 mL/mmol). The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate and concentrated in vacuum. The product was purified by column chromatography. The representative compounds are shown below.

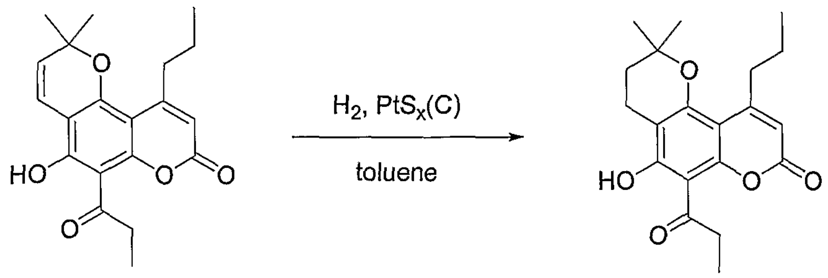
5-Isopropoxy-2,2-dimethyl-6-propionyl-10-propyl-3,4-dihydro-2H-pyrano[2,3-f]chromen-8-one (**44i**): 89% yield. ^{1}H NMR ($CDCl_3$): 1.02 (t, J=7.5 Hz, 3H), 1.24 (d, J=6.0 Hz, 6H), 1.40 (s, 6H), 1.64 (m, 2H), 1.80 (t, J=7,0 Hz, 2H), 2.74 (t, J=6.5 Hz, 2H), 2.90 (m, 4H), 4.26 (m, 1H), 5.99 (s, 1H); ^{13}C NMR ($CDCl_3$): 7.92, 13.87, 18.20, 22.41, 23.21, 26.68, 31.58, 38.36, 39.02, 76.18, 76.75, 105.87, 111.38, 112.35, 116.90, 151.13, 153.26, 155.54, 157.89, 160.05, 203.53; IR: 1711 cm^{-1}; MS (ACPI +): 387 (M+1); Anal. Calcd. for $C_{23}H_{30}O_5$: C 71.48, H 7.82. Found: C 71.61, H 7.89.

5-(2-Hydroxyethoxy)-2,2-dimethyl-6-propionyl-10-propyl-3,4-dihydro-2H-pyrano[2,3-f]chromen-8-one (**44k**): 33% yield. ^{1}H NMR ($CDCl_3$): 1.03 (t, J=7.5 Hz, 3H), 1.21 (t, J=7.5 Hz, 3H), 1.42 (s, 6H), 1.64 (m, 2H), 1.82 (m, 2H), 2.78 (t, J=6.5 Hz, 2H), 2.91 (m, 4H), 3.05 (t, J=6.5 Hz, 1H), 3.85 (m, 2H), 4.07 (m, 2H), 6.19 (s, 1H); ^{13}C NMR ($CDCl_3$): 8.05, 13.86, 17.87, 23.18, 26.68, 31.35, 38.59, 39.01, 62.10, 76.47, 76.52, 106.21, 110.46, 112.56, 116.36, 151.49, 153.92, 156.70, 157.93, 159.78, 204.71; IR: 1720, 1694 cm^{-1}; MS (ACPI +): 388 (M+1); Anal. Calcd. for $C_{22}H_{28}O_6$: C 68.02, H 7.27; Found: C 68.03, H 7.30.

2,2-Dimethyl-5-(2-morpholin-4-yl-ethoxy)-6-propionyl-10-propyl-3,4-dihydro-2H-pyrano[2,3-f]-chromen-8-one (**44m**): 73% yield. ^{1}H NMR ($CDCl_3$): 1.02 (t, J=7.5 Hz, 3H), 1.20 (t, J=7.5 Hz, 3H), 1.41 (s, 6H), 1.81 (t, J=7.0 Hz, 2H), 2.53 (m, 4H), 2.70 (t, J=5.0 Hz, 2H), 2.78 (t, J=7.0 Hz, 2H), 2.91 (m, 4H), 3.73 (t, J=4.5 Hz, 4H), 4.00 (t, J=5.0 Hz, 2H), 6.01 (s, 1H); ^{13}C NMR ($CDCl_3$): 7.92, 13.85, 17.34, 23.18, 26.67, 31.37, 38.45, 38.97, 53.87, 58.32, 66.85, 71.76, 76.33, 106.33, 110.86, 112.59, 116.97, 151.15, 153.45, 156.40, 157.87, 159.87, 203.50; IR: 1713 cm^{-1}; MS (ACPI +): 458 (M+1); Anal. Calcd. for $C_{26}H_{35}NO_6$: C 68.25, H 7.71, N 3.06. Found: C 68.13, H 7.76, N 3.04.

5-Ethoxycarbonylmethyl-2,2-dimethyl-6-propionyl-10-propyl-3,4-dihydro-2H,8H-pyrano[2,3-f]chromen-8-one (**44n**): 81% yield. ^{1}H NMR ($CDCl_3$): 1.03 (t, J=7.5 Hz, 3H), 1.21 (t, J=7.5 Hz, 3H), 1.41 (s, 6H), 1.63 (m, 2H), 1.84 (t, J=6.5 Hz, 2H), 2.70 (t, J=7.0 Hz, 2H), 2.91 (m, 4H), 4.09 (t, J=4.5 Hz, 2H), 4.34 (t, J=4.5 Hz, 2H), 6.02 (s, 1H); ^{13}C NMR ($CDCl_3$): 7.92, 13.83, 17.20, 20.79, 23.16, 26.64, 31.30, 38.46, 38.95, 63.13, 72.46, 77.39, 106.48, 110.77, 112.71, 116.87, 151.20, 153.51, 155.78, 157.79, 159.75, 170.74, 203.26; IR: 1727 cm^{-1}; MS (ACPI +): 331 (M+1); Anal. Calcd. for $C_{24}H_{30}O_7$: C 66.96, H 7.02; Found: C 69.76, H 7.02.

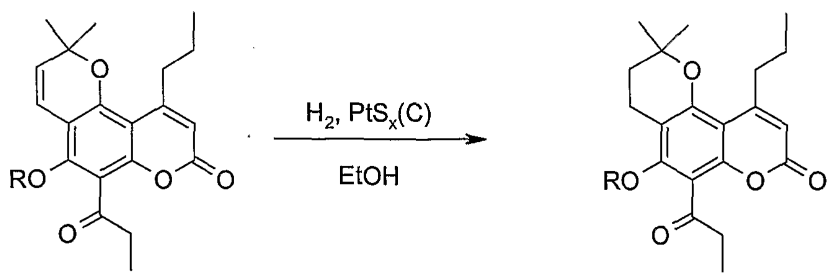
Example 17: *5-Hydroxy-2,2-dimethyl-6-propionyl-10-propyl-3,4-dihydro-2H-pyrano[2,3-f]chromen-8-one* (**44a**)



(30) (44a)

A mixture of 5-hydroxy-2,2-dimethyl-6-propionyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**30**) (5 g, 14.60 mmol) and sulfided platinum (5% on carbon, 1 g) in toluene/isopropyl alcohol (200/50 mL) was hydrogenated 16 h at room temperature under atmospheric pressure of hydrogen. The catalyst was filtered off and the filtrate concentrated under vacuum. The solid residue was crystallized from ethanol and re-crystallized from acetone to provide 2.66 g of **44a** (53% yield). ^{1}H NMR ($CDCl_3$): 1.03 (t, J=7.5 Hz, 3H), 1.23 (t, J=7.5 Hz, 3H), 1.42 (s, 6H), 1.64 (m, 2H), 1.84 (t, J=6.5 Hz, 2H), 2.70 (t, J=6.5 Hz, 2H), 2.90 (m, 2H), 3.34 (q, J=7.0 Hz, 2H), 5.98 (s, 1H), 14.61 (s, 1H); ^{13}C NMR ($CDCl_3$): 8.45, 13.89, 16.42, 23.37, 26.62, 31.20, 38.13, 39.38, 77.60, 102.77, 103.43, 105.30, 110.22, 156.41, 157.29, 158.94, 159.76, 165.77, 206.67; IR: 1730 cm^{-1}; MS (ACPI +): 345 (M+1); Anal. Calcd. for $C_{20}H_{24}O_5$: C 69.75, H 7.02; Found C 70.03, H 7.18.

Example 18: *5-Alkyloxy-2,2-dimethyl-6-propionyl-10-propyl-3,4-dihydro-2H-pyrano[2,3-f]chromen-8-one* (**44**)



(30) or (31) (44)

A solution of 5-alkyloxy-2,2-dimethyl-6-propionyl-10-propyl-2H-pyrano[2,3-f]chromen-8-one (**30** or **31**) in ethanol (30-100mL/mmol) was hydrogenated under atmospheric pressure of hydrogen in the presence of sulfided platinum (Aldrich, 5% on carbon, 100 mg/mmol). The reaction was carried out overnight at ambient temperature. The mixture was filtered through a pad of Celite and the filtrate concentrated under vacuum. The residue was chromatographed on silica gel column eluted with 15-30% ethyl acetate in hexanes. Compound **44b** is the representative and its analytical data is shown below.

5-Methoxy-2,2-dimethyl-6-propionyl-10-propyl-3,4-dihydro-2H-pyrano[2,3-f]chromen-8-one (**44b**): 68% yield. ^{1}H NMR ($CDCl_3$): 1.02 (t, J=7.5 Hz, 3H), 1.22 (t, J=7.5 Hz, 3H), 1.41 (s, 6H), 1.62 (m, 2H), 1.82 (t, J=6.5 Hz, 2H), 2.75 (t, J=6.5 Hz, 2H), 2.91 (m, 4H), 3.78 (s, 3H), 6.01 (s, 1H); ^{13}C NMR ($CDCl_3$): 7.97, 13.84, 17.20, 23.19, 26.66, 31.33, 38.48, 38.99, 62.14, 76.35, 106.14, 110.66, 112.48, 116.55, 151.24, 153.50, 157.51, 157.92, 159.92, 203.40; IR: 1724, 1704 cm^{-1}; MS (ACPI +): 359 (M+1); Anal. Calcd. for $C_{21}H_{26}O_5$: C 70.37, H 7.31; Found: C 70.15, H 7.28.

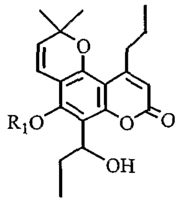
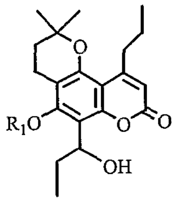
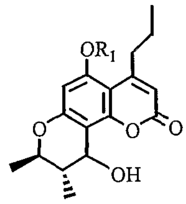
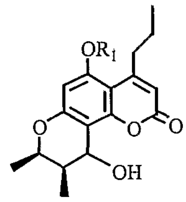
Example 20: Evaluation of Compounds Synthesized for Activity against *M. tuberculosis*

The compounds synthesized above were evaluated for activity against *M. tuberculosis* H37Ra using a colorimetric microdilution broth assay that incorporated the REDOX indicator alamar Blue, which indicates the growth and viability by the metabolic reduction of the dye from blue to red[18].

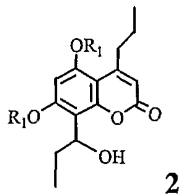
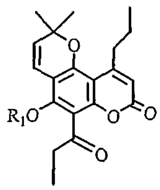
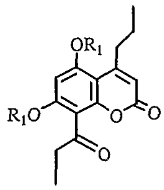
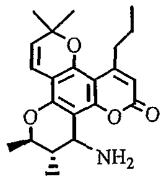
In an initial screen, the compounds were assayed in duplicate at four $\log_{10}$ dilutions, with the highest concentration being 128 μg/mL (therefore, tested concentrations were 0.128, 1.28, 12.8, and 128 μg/mL). Each solubilized test compound was diluted in broth medium (Middlebrook 7H9 + ADC enrichment + 0.2% glycerol) at twice the desired concentration and 0.05 mL of each dilution then added to appropriate wells in duplicate in a 96 well (U-shaped) microtiter plates. A plate format was designed for up to seven (7) compounds per plate. Each plate also included uninoculated drug controls, viability controls, uninoculated medium controls, and positive drug controls. The inoculum for each well consisted of 0.05 mL of culture, standardized as described above to provide an inoculum in each microtiter well of approximately 10^5 CFU/mL. The plates were covered with a lid, placed in polyethylene bags and incubated for at 37 °C for 6 days. At that time, alamar Blue, diluted in Tween 80, was added to each well followed by further incubation for about 20 hours. The plates were read in an optical microplate reader programmed to subtract absorbance at 600 nm from absorbance at 570 nm. This effectively blanked out turbidity and absorbance due to oxidized dye. The amount of dye reduced, resulting in a change from blue to red, is indicative of drug yielding a differential absorption of zero or less. The initial screening results are shown in **Table 3**.

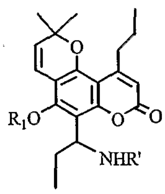
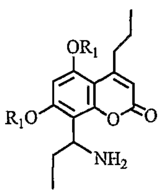
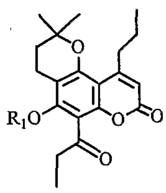
The method is easily modified to medium or high throughput screening in order to evaluate more compounds. For example, instead of 4 concentrations in duplicate, the compounds are tested singly at one cut-off concentration (e.g., 12.8 μg/mL).

Table 3. Activity of Compounds against *M. tuberculosis* H37Ra from the Initial Screening

Type I Compounds				Type II Compounds		
 **11**		 **12**		 *trans*-**17**;  *cis*-**17**		
R_1	**MIC**	**R_1**	**MIC**	**R_1**	**MIC of *trans*- 17**	**MIC of *cis*-17**
a. H	>12.8 <128	**a.** H	>12.8 <128	**a.** H		
b. Me (9)	>12.8 <128	**b.** Me	>12.8 <128	**b.** Me	>12.8 <128	>128
c. Et	>12.8 <128	**c.** Et	>128	**e. *i-Pr***	>12.8 <128	***>1.28 <12.8***
d. $CH \equiv CCH_2$	>12.8 <128	**d.** $CH \equiv CCH_2$	>12.8 <128	**d.** $CH \equiv CCH_2$	>128	>12.8 <128
e. $HOCH_2CH_2$	>12.8 <128	**e.** $HOCH_2CH_2$	>12.8 <128	**c.** CH_2=$CHCH_2$	>128	>12.8 <128
				f, *CH_2=$CHCH(CH_3)$*	>12.8 <128	***>1.28 <12.8***
				g, CH_2=$C(CH_3)CH_2$	>128	>12.8 <128

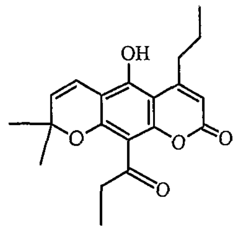
Type III	Type IV Compounds

 **21**		 **31**		 **29**		 **22**
R_1	**MIC**	**R_1**	**MIC**	**R_1**	**MIC**	**MIC**
a, H	N/P	**30**. H	>128	**a**, H	>12.8 <128	***>1.28 <12.8***
b, Me	>12.8 <128	**31b**. Me	>12.8 <128	**b**, Me	>128	
c, i-Pr	>128	**31c**. Et	>128	**c**, i-Pr	>128	
d, CH_2=CHCH$_2$	***>1.28 <12.8***	**31d**. CH≡CCH$_2$	>128	**d**, CH_2=CHCH$_2$	>128	
		31e. HOCH$_2$CH$_2$	>12.8 <128			
		31f. Ac	>12.8 <128			

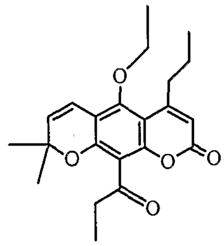
Type IV Compounds						**Others**	
 **24**		 **26**		 **44**			
R_1 / R'	**MIC**	**R_1**	**MIC**	**R_1**	**MIC**	**Cmpd**	**MIC**
a, H / H	>12.8 <128	**a**, H	N/P	**a**. H	>128	**47**	>12.8 <128
b, Me / H	>12.8 <128	**b**, Me	>12.8 <128	***b. Me***	***>1.28 <12.8***	**48**	>12.8 <128
c, Et / H		***c, i-Pr***	***>1.28 <12.8***	**c**. Et	>128		

d, $CH\equiv CCH_2$ / H		**d**, CH_2=CHCH2	>12.8 <128	**d**. $CH\equiv CCH_2$	>128		
e, ***$HOCH_2CH_2$ /H***	***>1.28 <12.8***			**e**. $HOCH_2CH_2$	>12.8 <128		
f, Me / Me				**f**. $AcOCH_2CH_2$	>12.8 <128		
g, Me / Et	>12.8 <128			**g**, CH_2=CHCH2	>128		
h, Me / i-Pr				**h**, i-Pr	>128		
i, Me/$HOCH_2CH_2$	>12.8 <128			**i**, EtO_2CCH_2	>128		
j, Me / Bn				**j**, Ac	>128		
k, i-Pr / H				**k**, Ts	>128		
l, Allyl / H				**l**, O N—CH_2CH_2	>128		
				m. $MeOCH_2$	>128		

MIC: µg/mL



47



48

Compounds exhibiting activity, with MIC range less than 12.8 µg/mL, in the initial screening were further assayed again within an appropriate concentration range using 2-fold dilutions to determine the minimum inhibitory concentration (MIC), which is defined as the lowest concentration of drug that completely inhibits growth of *M. tuberculosis*, as well as the MBC (minimum bactericidal concentration). The results are shown in **Table 4**. Four of the compounds (***cis*-17c**, **22**, **45e**, and **26c**) were bactericidal in their effect on *M. tuberculosis* since their MIC/MBC were 4 or less. All of these compounds had an MIC value of 16 µg/mL.

Table 4. MIC and MBC Values of Compounds against *M. tuberculosis* H37Ra

Compound	MIC	MBC	MIC / MBC
***cis*-17c**	16	64	4
***cis*-17f**	16	>64	>4
21d	16	>64	>4
22	16	32	2
45e	16	32	2
26c	16	64	4
44b	16	>64	>4

MIC and MBC: μg/mL

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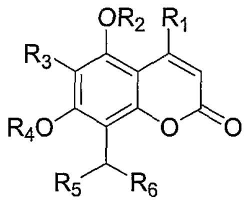
CLAIMS

We claim:

1. A compound according to formula (I) or a pharmaceutically acceptable salt thereof:



(I)

wherein R_1 is alkyl , alkenyl, alkynyl, aryl, OH, or NH_2;

R_2 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_3;

R_3 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_2;

R_4 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_5;

R_5 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_4; and

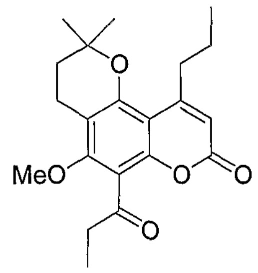
R_6 is selected from the group consisting of =O, OH, =NH, NH_2, SH, $P(O)_nH_m$ substituted imines, and substituted amines, wherein n is 2-4 and m is 1-3;

with the proviso that only one of either R_2 and R_3 or R_4 and R_5 optionally form a 6-membered ring, resulting in a compound of formula (I) comprising three fused 6-membered rings.

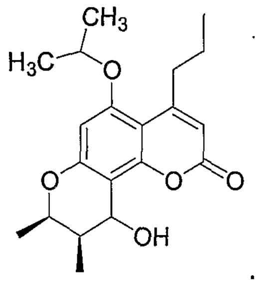
2. The compound of claim 1, wherein the optionally formed 4 to 7-membered ring between R_2 and R_3 is a 6-membered ring.

3. The compound of claim 1, wherein the optionally formed 4 to 7-membered ring between R_4 and R_5 is a 6-membered ring.

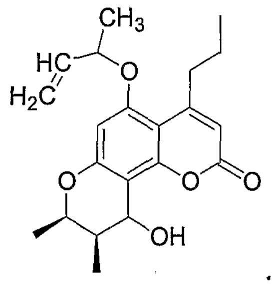
4. The compound of claim 1, wherein the compound is:



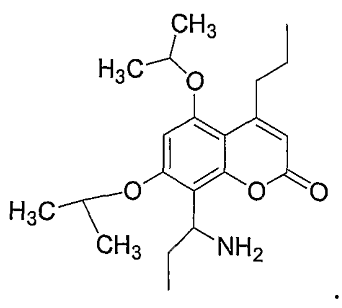
5. The compound of claim 1, wherein the compound is:



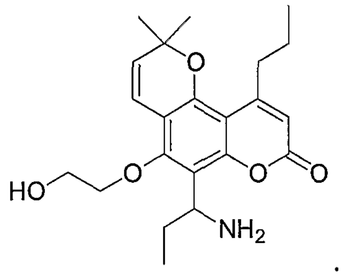
6. The compound of claim 1, wherein the compound is:



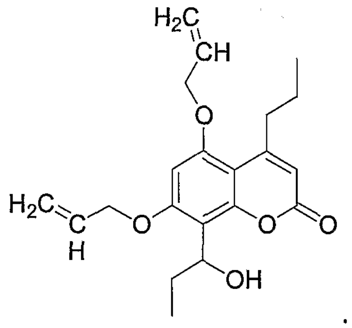
7. The compound of claim 1, wherein the compound is:



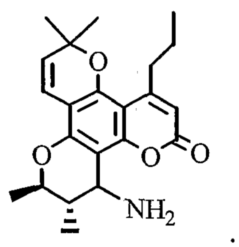
8. The compound of claim 1, wherein the compound is:



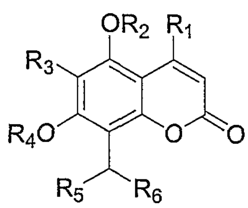
9. The compound of claim 1, wherein the compound is:



10. A compound of the formula:



11. A composition comprising a compound according to formula I, and a pharmaceutically acceptable carrier:



(I)

wherein R_1 is alkyl , alkenyl, alkynyl, aryl, OH, or NH_2;

R_2 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_3;

R_3 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_2;

R_4 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_5;

R_5 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_4; and

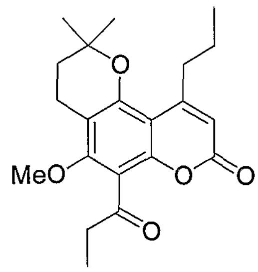
R_6 is selected from the group consisting of =O, OH, =NH, NH_2, SH, $P(O)_nH_m$ substituted imines, and substituted amines, wherein n is 2-4 and m is 1-3;

with the proviso that only one of either R_2 and R_3 or R_4 and R_5 optionally form a 6-membered ring, resulting in a compound of formula (I) comprising three fused 6-membered rings.

12. The composition of claim 11, wherein the optionally formed 4 to 7-membered ring between R_2 and R_3 is a 6-membered ring.

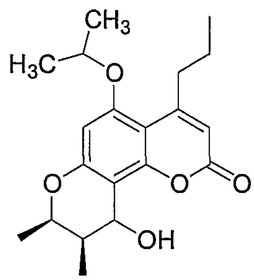
13. The composition of claim 11, wherein the optionally formed 4 to 7-membered ring between R_4 and R_5 is a 6-membered ring.

14. A composition comprising the compound



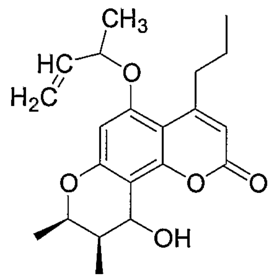
and a pharmaceutically acceptable carrier.

15. A composition comprising the compound



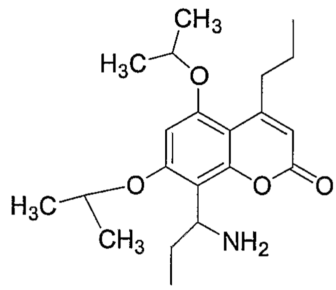
and a pharmaceutically acceptable carrier.

16. A composition comprising the compound



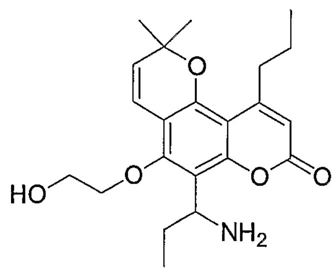
and a pharmaceutically acceptable carrier.

17. A composition comprising the compound



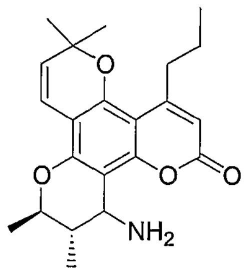
and a pharmaceutically acceptable carrier.

18. A composition comprising the compound



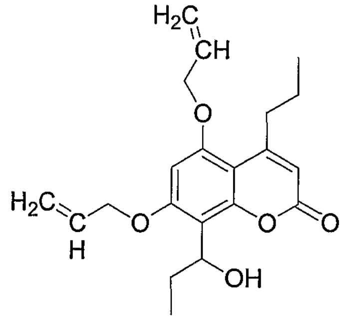
and a pharmaceutically acceptable carrier.

19. A composition comprising the compound



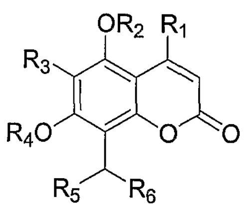
and a pharmaceutically acceptable carrier.

20. A composition comprising the compound



and a pharmaceutically acceptable carrier.

21. A method of treating infection or conditions related to infection by *Mycobacterium* in a mammal in need of such treatment comprising administering a therapeutically effective amount of a composition comprising one or more non-toxic pharmaceutically acceptable carriers and a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



(I)

wherein R_1 is alkyl , alkenyl, alkynyl, aryl, OH, or NH_2;

R_2 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_3;

R_3 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_2;

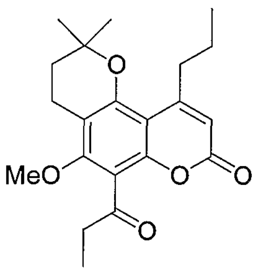
R_4 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_5;

R_5 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_4; and

R_6 is selected from the group consisting of =O, OH, =NH, NH_2, SH, $P(O)_nH_m$ substituted imines, and substituted amines, wherein n is 2-4 and m is 1-3;

with the proviso that only one of either R_2 and R_3 or R_4 and R_5 optionally form a 6-membered ring, resulting in a compound of formula (I) comprising three fused 6-membered rings.

22. The method of claim 21, wherein the compound of formula (I) is selected from the group consisting of:



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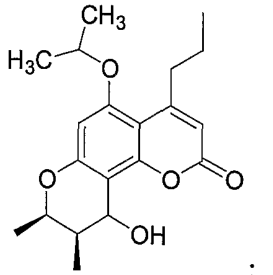

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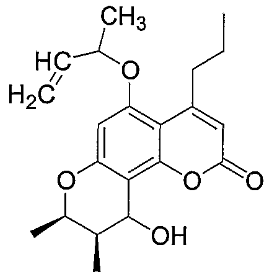

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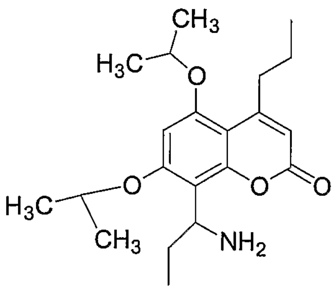

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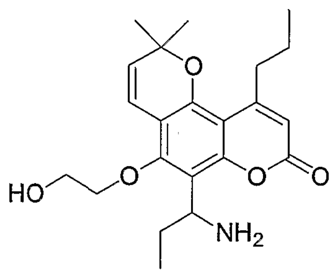
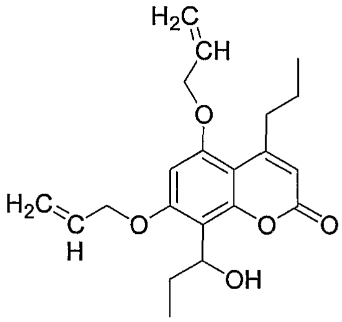


; and



23. The method of claim 21, wherein the *Mycobacterium* is selected from the group consisting of *Mycobacterium avium* complex (MAC), *Mycobacterium kansaii, Mycobacterium marinum, Mycobacterium phlei, Mycobacterium ulcerans, Mycobacterium xenopi, Mycobacterium gordonae, Mycobacterium terrae complex, Mycobacterium haemophilum, Mycobacterium fortuitum, Mycobacterium tuberculosis, Mycobacterium laprae, Mycobacterium scrofulaceum and Mycobacterium smegmatis.*

24. The method of claim 21, wherein the *Mycobacterium* is *Mycobacterium tuberculosis.*

25. A method of treating infection or conditions related to infection by *Mycobacterium* in a mammal in need of such treatment wherein the *Mycobacterium* is selected from the group consisting of *Mycobacterium avium* complex (MAC), *Mycobacterium kansaii, Mycobacterium marinum, Mycobacterium phlei, Mycobacterium ulcerans, Mycobacterium xenopi, Mycobacterium gordonae, Mycobacterium terrae complex, Mycobacterium haemophilum, Mycobacterium fortuitum, Mycobacterium tuberculosis, Mycobacterium laprae, Mycobacterium scrofulaceum and Mycobacterium smegmatis,* comprising administering to the mammal a compound according to any of claims 2-10, or a pharmaceutically acceptable salt thereof.

26. A method of treating infection or conditions related to infection by *Mycobacterium* in a mammal in need of such treatment wherein the *Mycobacterium* is *Mycobacterium tuberculosis*, comprising administering to the mammal a compound according to any of claims 2-10, or a pharmaceutically acceptable salt thereof.

27. The method according to claim 21, further comprising the administration of anti-microbial agent, an antiviral compound, an immunostimulant, an immunomodulator, an antibiotic, a chemokine inhibitor, or a pharmaceutically acceptable salt thereof.

28. The method of claim 27, wherein the anti-microbial agent is an anti-mycobacterial agent.

29. The method of claim 28, wherein the anti-mycobacterial agent is an anti-TB agent.

30. The method of claim 29, wherein the anti-TB agent comprises isoniazid, rifampin, rifabutin, rifapentine, pyrazinamide or ethambutol.

31. The method of claim 27, wherein the antiviral compound is a protease inhibitor.

32. The method of claim 31, wherein the protease inhibitor is selected from the group consisting of indinavir, saquinavir, ritonavir, and nelfinavir.

33. The method of claim 27, wherein the antiviral compound is a biflavanoid.

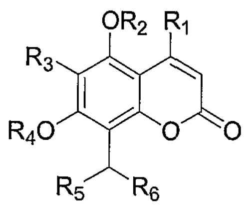
34. The method of claim 33, wherein the biflavanoid is selected from the group consisting of robustaflavone, amentoflavone, and a derivative or salt thereof.

35. The method of claim 27, wherein the antiviral compound is selected from the group consisting of AZT, ddC, ddI, D4T, 3TC, acyclovir, gancyclovir, fluorinated nucleosides and nonnucleoside analog compounds such as delavirdine and nevirapine, and efavirenz, α-interfon, recombinant CD4, amantadine, rimantadine, ribavirin, and vidarabine.

36. The method of claim 27, wherein the immunostimulant is an interleukin or cytokine.

37. The method of claim 27, wherein the antibiotic is an antibacterial agent, antifungal agent, or anti-pneumocysitis agent.

38. A method of treating a patient who has a disease or condition selected from the group consisting of tuberculosis, tuberculosis associated with immunosuppression, tuberculosis associated with an immunodeficiency, tuberculosis associated with infection by human immunodeficiency virus (HIV), and tuberculosis associated with acquired immune deficiency syndrome (AIDS) and who is in need of such treatment which includes administration of a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt thereof:



(I)

wherein R_1 is alkyl , alkenyl, alkynyl, aryl, OH, or NH_2;

R_2 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_3;

R_3 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_2;

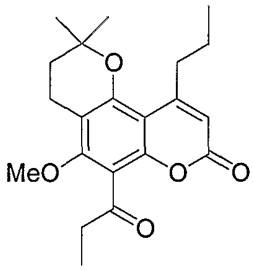
R_4 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_5;

R_5 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_4; and

R_6 is selected from the group consisting of =O, OH, =NH, NH_2, SH, $P(O)_nH_m$ substituted imines, and substituted amines, wherein n is 2-4 and m is 1-3;

with the proviso that only one of either R_2 and R_3 or R_4 and R_5 optionally form a 6-membered ring, resulting in a compound of formula (I) comprising three fused 6-membered rings.

39. The method of claim 38, wherein the compound of formula (I) is selected from the group consisting of:



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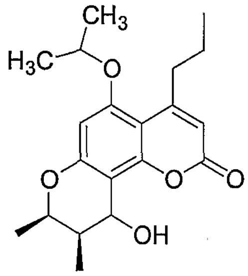

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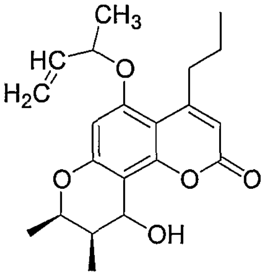

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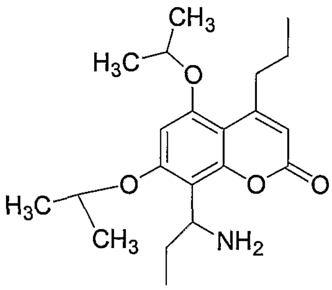

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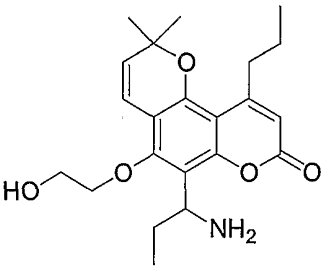

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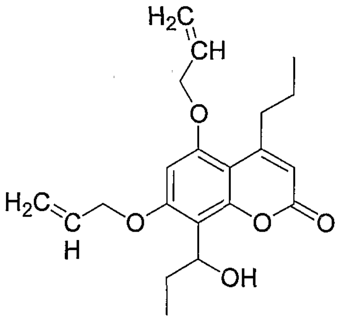


40. A method of stepwise reductive amination comprising:

(a) contacting a compound comprising ketone moiety with an excess of a compound of the formula R'NH_2, wherein R' is selected from the group consisting of H, alkyl, alkenyl, alkynyl, and aryl under conditions that allow the ketone moiety to form an imine intermediate compound; and

(b) contacting the intermediate compound of step (a) with an amount of a reducing agent sufficient to reduce the imine moiety of the intermediate compound to its amino analog, under conditions that allow reduction of the imine intermediate compound to its amino analog.

41. A method of stepwise reductive amination to prepare a compound of formula (I) wherein R_6 is NH_2 comprising:

(a) contacting a compound of formula (I), wherein R_6 is =O, with an excess of a compound of the formula $R'NH_2$, wherein R' is selected from the group consisting of H, alkyl, alkenyl, and alkynyl, under conditions that allow formation of an imine intermediate compound of formula (I), wherein R_6 is =NH; and

(b) contacting the intermediate compound of step (a) with an amount of a reducing agent sufficient to reduce the imine moiety of the intermediate compound to its amino analog, under conditions that allow reduction of the imine intermediate compound to its amino analog.

42. The method of claim 41, wherein the compound of formula $R'NH_2$ is NH_3.

43. The method of claim 41, wherein the reducing agent is $NaBH_4$.

44. The method of claim 41, wherein the compound of formula (I), wherein R_6 is =O, is added to a solution comprising the compound of formula $R'NH_2$.

45. The method of claim 41, wherein the amount of reducing agent is from 0.1 to 50 molar equivalents with respect to the amount of imine intermediate.

46. The method of claim 41, wherein the amount of compound of formula $R'NH_2$ is from 1 to 500 molar equivalents with respect to the amount of compound of formula (I), wherein R_6 is =O.

47. The method of claim 41, further comprising a metal additive in the step comprising reduction of the imine intermediate.

48. The method of claim 47, wherein the metal additive is selected from the group consisting of $CeCl_3$, $ZnCl_2$, $AlCl_3$, $TiCl_4$, $SnCl_3$, and $LnCl_3$.

49. The method of claim 41, wherein the method is performed at a temperature from about –78 to 120 °C.

50. The method of claim 41, wherein the method is performed at a temperature of about room temperature.

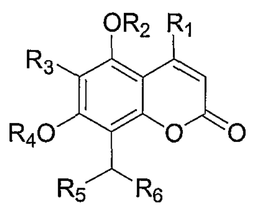
51. A method of preparing a compound of formula (I) wherein R_6 is =NH or =NR' comprising:

(a) contacting a compound of formula (I), wherein R_6 is =O, with an excess of a compound of the formula $R'NH_2$,

wherein R' is selected from the group consisting of H, alkyl, alkenyl, and alkynyl,

under conditions that allow formation of an imine or substitututed imine compound of formula (I), wherein R_6 is =NH or =NR'.

52. A compound according to formula (I) or a pharmaceutically acceptable salt thereof:



(I)

wherein R_1 is alkyl , alkenyl, alkynyl, aryl, OH, or NH_2;

R_2 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_3;

R_3 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_2;

R_4 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_5;

R_5 is selected from H, alkyl, alkenyl, alkynyl, aryl, and can optionally form a 4 to 7-membered ring with R_4; and

R_6 is selected from the group consisting of =O, OH, =NH, NH_2, SH, $P(O)_nH_m$ substituted imines, and substituted amines, wherein n is 2-4 and m is 1-3;

with the proviso that if both R_2 and R_3, and R_4 and R_5 form a six-membered ring, then R_6 cannot be =O or OH.

53. A composition comprising the compound of claim 52 and a pharmaceutically acceptable carrier.

54. A method of treating infection or conditions related to infection by *Mycobacterium* in a mammal in need of such treatment comprising administering a therapeutically effective amount a compound of claim 52 or a pharmaceutically acceptable salt thereof.

55. A method of treating infection or conditions related to infection by *Mycobacterium* in a mammal in need of such treatment comprising administering a therapeutically effective amount of a composition comprising one or more non-toxic pharmaceutically acceptable carriers and a compound of claim 52 or a pharmaceutically acceptable salt thereof.

56. The method of claim 55, further comprising the administration of anti-microbial agent, an antiviral compound, an immunostimulant, an immunomodulator, an antibiotic, a chemokine inhibitor, or a pharmaceutically acceptable salt thereof.

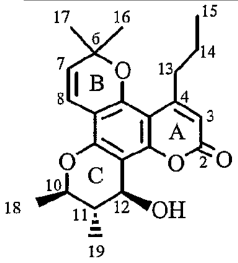
57. A method of treating infection or conditions related to infection by *Mycobacterium* in a mammal in need of such treatment comprising administering a therapeutically effective amount of the compound of claim 10 or a pharmaceutically acceptable salt thereof.

58. A method of treating infection or conditions related to infection by *Mycobacterium* in a mammal in need of such treatment comprising administering a therapeutically effective amount of a composition comprising one or more non-toxic pharmaceutically acceptable carriers and the compound of claim 10 or a pharmaceutically acceptable salt thereof.

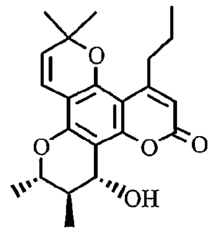
59. The method of claim 58, further comprising the administration of anti-microbial agent, an antiviral compound, an immunostimulant, an immunomodulator, an antibiotic, a chemokine inhibitor, or a pharmaceutically acceptable salt thereof.

60. A method of treating a patient who has a disease or condition selected from the group consisting of tuberculosis, tuberculosis associated with immunosuppression, tuberculosis associated with an immunodeficiency, tuberculosis associated with infection by human immunodeficiency virus (HIV), and tuberculosis associated with acquired immune deficiency syndrome (AIDS) and who is in need of such treatment which includes administration of a therapeutically effective amount of a compound of claim 10 or a pharmaceutically acceptable salt thereof.

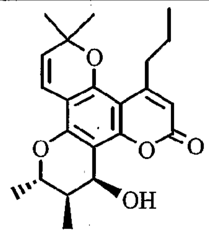
Figure 1: Structures of Pyranocoumarin Compounds



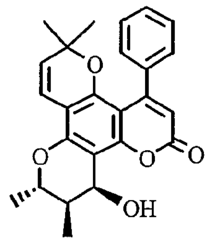
(+)-Calanolide A (**1**)



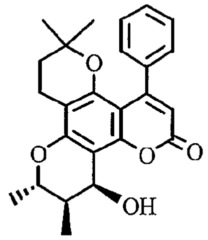
(-)-Calanolide A (**2**)



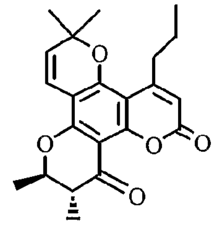
(-)-Calanolide B (**3**)



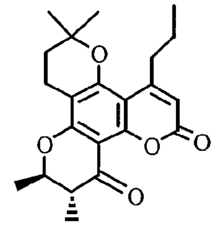
Soulattrolide (**4**)



7,8-Dihydrosoulattrolide (**5**)

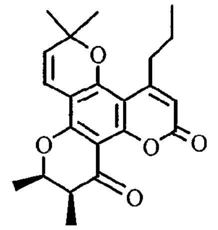


(+)-12-Oxocalanolide A (**6**)



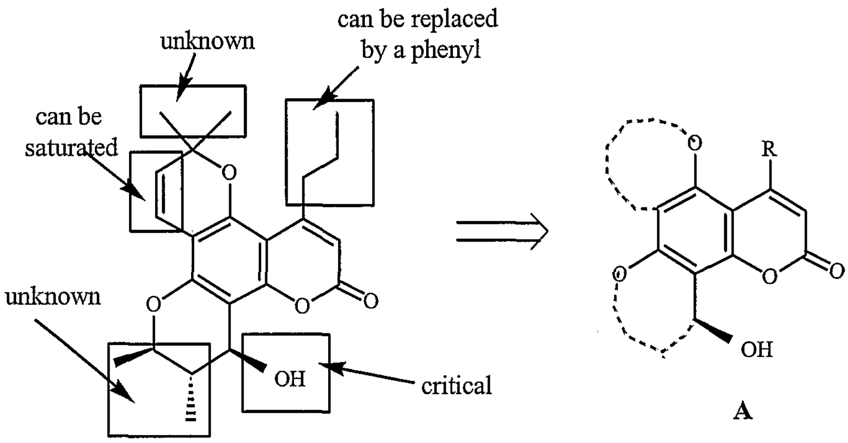
(±)-7,8-Dihydro-12-oxocalanolide A

(**7**)



(±)-Calanolide D (**8**)

Figure 2: Structural Features in Pyranocoumarins Critical for Anti-TB Activity



Selected General Structures Types I-IV

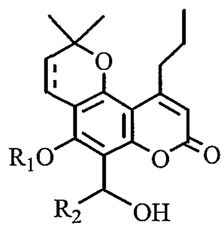
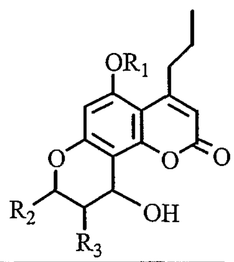
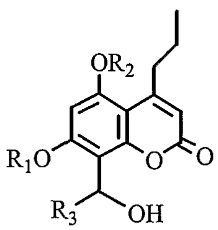
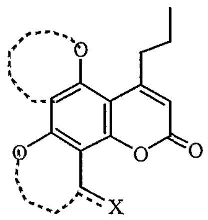
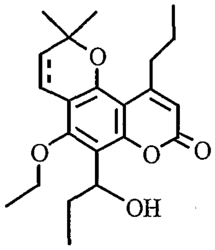
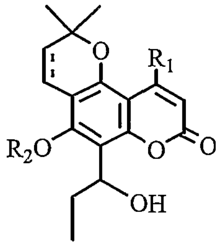
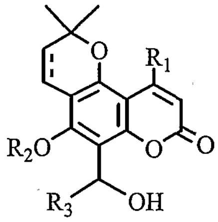
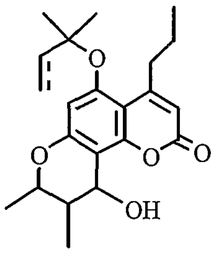
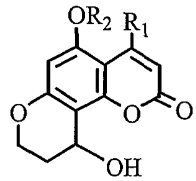
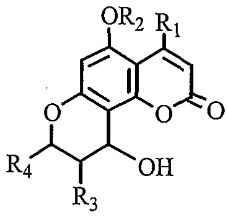
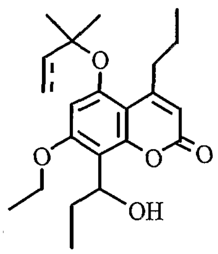
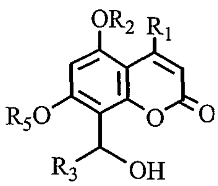
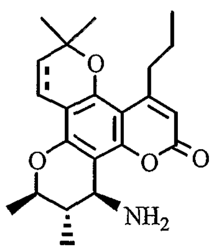
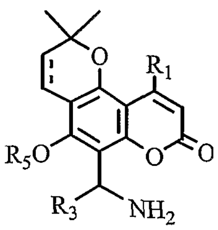
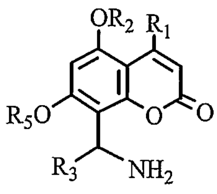
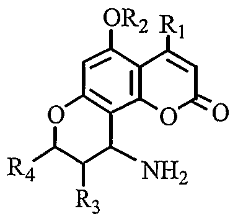
Type I	Type II	Type III	Type IV
			

Figure 3: Design, Synthesis and Evaluation of Pyranocoumarin Analogues

Category	Compound Examples
Type I	 **9, 10**  **11, 12**  **13-16**
Type II	 **17a, 17b**  **18**  **18a**
Type III	 **19, 20**  **21** R_1 = H, Me, n-Pr, c-PrCH$_2$, Ph R_2 = H, Me, Et, allyl, t-Bu, Ac R_3 = H, Me, Et, allyl, t-Bu, O, OH R_4 = H, Me, Et, allyl, t-Bu, OH R_5 = H, Me, Et, allyl, t-Bu, Ac
Type IV	 **22, 23**  **24, 25**  **26**  **27**