Synthesis Of New Psoralens And Coumarins As Epidermal Growth Factor Antagonists.

Order Number 9513122

Synthesis of new psoralens and coumarins as epidermal growth factor antagonists

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Lehigh University, 1994

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Synthesis of New Psoralens and Coumarins as Epidermal Growth Factor Antagonists

by

Keith W. Kardos

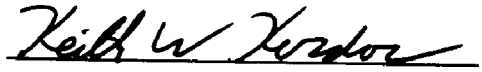
A Dissertation
Presented to the Graduate Committee
of Lehigh University
in Candidacy for the Degree of
Doctor of Philosophy
in
Chemistry

Lehigh University

July, 1994

CERTIFICATE OF PRESENTATION

This dissertation is respectfully submitted to the Graduate Faculty of Lehigh University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.



Keith W. Kardos

A CERTIFICATE OF APPROVAL

Approved and recommended for acceptance as a dissertation in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

8-1-94
Date

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ACKNOWLEDGEMENTS

The author would like to extend his gratitiude to Dr. Ned D. Heindel for his guidance, support and his occasional redirection of my focus. I would also like to thank Dr. Daniel Lima, Mr. William Anderson and everyone who helped me throughout my time at Lehigh.

DEDICATION

This Dissertation is dedicated to my wife, Stephanie, whose time and patience brought this work to a timely conclusion.

This research is also dedicated to the members of the Heindel group, whose choice of a summer student made the previous statement possible.

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Abstract

Coumarins and psoralens (furocoumarins) are naturally occuring compounds which have been used for medicinal purposes since 2000 B.C. The systematic investigation of the biological effects of these agents began in 1938 and continues to this day. Psoralen photochemotherapy (psoralens + UVA light) has been shown to stimulate melanin secretion in vitiligo patients and inhibit cell division in the treatment of psoriasis.

Psoralens were once believed to exert their biological activity exclusively by intercalating and cross-linking nuclear DNA. Evidence of an alternative binding site for psoralens was presented by Laskin, who identified specific psoralen receptors in the cytoplasmic membrane of epidermal cells. The binding of a psoralen or coumarin to its receptor inhibits the binding of ^{125}I-EGF to its receptor. This molecular event appeared causual for the beneficial photopharmacology of these agents.

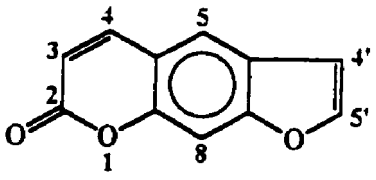
A series of psoralens and coumarins were synthesized to further evaluate the ability of these agents to inhibit the binding of ^{125}I-EGF to its receptor. The compounds described in this thesis along with compounds previously synthesized in our laboratory will allow further comment on the structure activity relationship of these compounds with respect to the inhibition of EGF binding to its receptor.

The compounds described in this thesis were synthesized

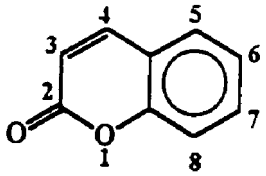
using improvements of previously described methods as well as by using new synthetic procedures for the synthesis of psoralens and coumarins. This research has provided four new series of candidate therapeutics that have not been previously studied. The series of compounds synthesized includes: 1) 4-trifluoromethyl coumarins and psoralens; 2) C-5' substituted derivatives of trioxsalen; 3) 7-alkoxy-3-bromo coumarins; and 4) Iodinated coumarins and psoralens.

Historical

Coumarins and psoralens (furocoumarins) are naturally occurring compounds which have been used for medicinal purposes since ancient times.[1-4] The name coumarin derives from the Caribbean word coumarou for tonka tree, from which the original coumarin was isolated. The name psoralen derives from the *Psoralea corylifolia*, from which the original psoralen was isolated.[5] These common names are now accepted when referring to these two classes of compounds. The variety of other common names that exist for coumarins and psoralens are conveniently indexed in *The Natural Coumarins*, by Robert Murray.[5] The common base structures and the currently accepted numbering system are shown in Figure 1.



Psoralen



Coumarin

Figure 1

The use of psoralens for medicinal purposes can be traced back to approximately 2000 B.C.[1-6] Ancient writings from the Egyptians and the Hindus of India mentioned the use of extracts from umbelliferous plants to treat various skin disorders and irregular pigmentation of the skin. The patients would either ingest the plant extract or apply it topically

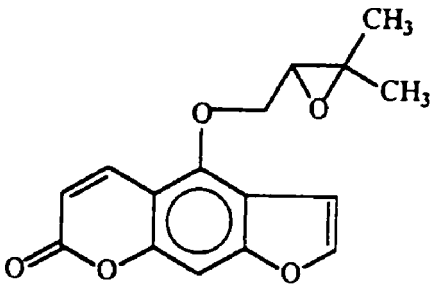
and expose themselves to sunlight. The use of psoralen containing plants is also mentioned throughout writings of many other cultures.

The most common plants associated with psoralens and coumarins include: Rutacea (lime), Umbelliferone (*Ammi Majus*), Leguminosae (*Psoralea Corylifolia*), and Moraceae (figs). The presence of psoralens and coumarins in a variety of other botanical sources has also been well documented. Single plants have been reported to yield many different compounds.[1,2,5,6]

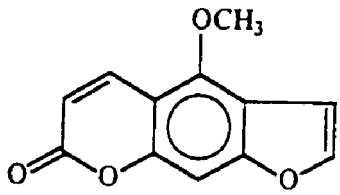
Although the biological activity of these plants was well established, it was not until 1938 that the Swiss dermatologist Kuske reported that psoralens were the active species which induced the photodermatitis caused by these plants.[7] The two psoralens that Kuske investigated were 5-methoxypsoralen (bergapten) from the oil of bergamot, and oxypeucedanin from the *Peucedenum ostruthium* (Figure 2).

Psoralen research was continued in 1941 when Fahmy started research with the Egyptian powder Atrillal, used by Egyptian herb doctors for treatment of vitiligo. Fahmy discovered that the active ingredients in this powder were 8-methoxypsoralen, 5-methoxypsoralen, and 8-isoamylenoxy-psoralen. The repigmentation of vitiligo patients with the compounds was then studied. Many other more sophisticated clinical trials were performed after Fahmy's original study and the use of psoralens for the treatment of vitiligo became

an important area of study.[1,2,8]



oxypeucedanin



5-methoxypsoralen

Figure 2

The use of psoralens for the treatment of psoriasis was first suggested in 1953 by Lerner who was familiar with the use of photosentizers for the treatment of this disorder.[1,2,8] Psoriasis is a common heritable disease characterized by deep red scaling lesions caused by the hyperproliferation of cells. In psoratic skin, the basal keratinocytes are not given the required time to differentiate and, as a result, undergo incomplete keratinization. This causes the skin to lose its resiliant properties and induces the red patches associated with psoriasis. Psoriatic lesions have responded to phototherapy in a number of cases and in some cases exposure to UV light alone has sent the psoriasis into remission.[9]

In 1962, the first successful treatment of psoriasis with psoralens was reported by Allyn. Many clinical trials followed this initial report and in 1982 the FDA approved the

use of psoralens for the treatment of severe psoriasis. The common acronym associated with psoralen photochemotherapy is PUVA (psoralens + UVA light).[1,2,10-12]

Mechanism of Action

The focus of psoralen research has been to determine the mechanisms by which these compounds exert their biological effects and to develop new agents which have increased therapeutic benefit and decreased toxicity. This effort has led to the synthesis of a number of new compounds and reports of many modes of action for the psoralens. To date there is no one mechanism of action universally accepted for psoralen activity. Several mechanisms have been suggested and are described below.

The complete biological activity of the psoralens is difficult to explain since a single drug can both stimulate melanocytes and inhibit keratinocytes.[2] Psoralens have been shown to intercalate and photobind to DNA,[13-17] to photobind to proteins [18-26] and lipids [27,28] and also to generate singlet oxygen [3,4]. Various workers have proposed each of these theories as the explanation for the biological effects observed when psoralen therapy is used. No one theory is universally accepted which explains all of the biological effects observed during psoralen photochemotherapy.

The ability of psoralens to intercalate and photobind to DNA has been extensively studied and is one of the dominant explanations for the activity of these compounds. It has been demonstrated that psoralens form a reversible intercalation complex with DNA which becomes irreversibly bound after irradiation with UVA light.[13-17] The psoralens have been shown

to form a 2+2 cycloaddition adduct between the 4',5' double bond of the pyrimidine base and the 3,4 or 4',5' double bonds of the psoralen. The 4',5' monoadducts can further react to form DNA crosslinks whereas the 3,4 adducts cannot absorb another photon of light required to form the crosslink (Figure 3). The structures of these photoproducts have been the subject of intense study and are well defined.[13-17]

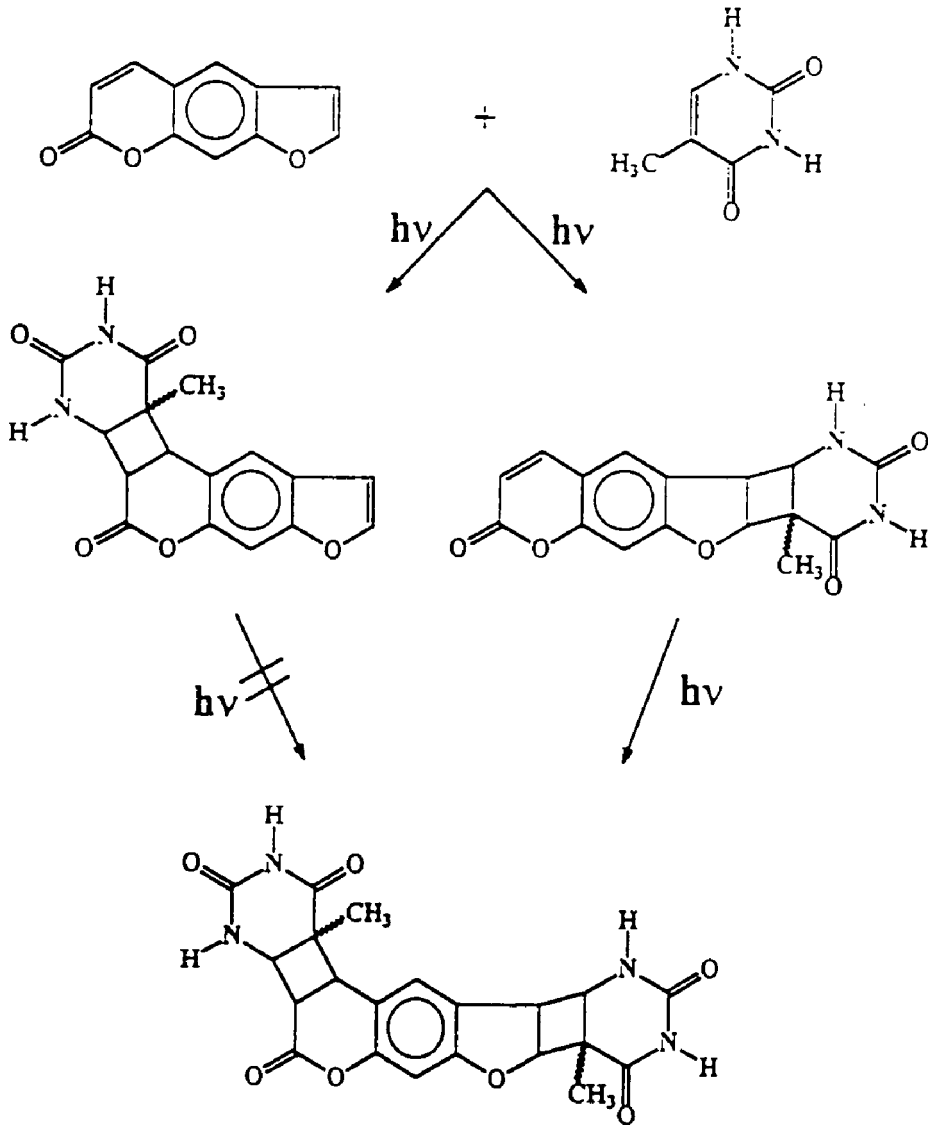


Figure 3

Many researchers believe that the formation of covalent bonds with DNA under UV light is the dominant cause of the cytotoxicity of psoralens.[13-17] Importantly, the DNA adducts are repaired by a random process involving DNA-polymerase-a. Monoadducts have been shown to be repaired at a faster rate than the diadducts which require a more complex repair process.[3,4] The mutagenicity of psoralens has been attributed to failures in the DNA repair process which can sometimes induce the onset of various cancers associated with psoralen therapy.

That psoralens generate singlet oxygen in the presence of UVA light has also been the subject of investigations.[3,4] It has been well documented that oxygen can be promoted to the singlet state by the energy transfer from the excited psoralen triplet state. The singlet oxygen has the potential to oxidize lipids or proteins and render them inactive for their desired purpose, thus causing the observed biological effects. Although there has been some correlation between the ability to generate singlet oxygen and photosensitization, photosensitizers such as 8-MOP, a very active photosensitizer, are very poor singlet oxygen generators. These conflicting findings indicate that singlet oxygen may play some role in photosensitization but it is not the primary cause.[3,4]

The formation of covalently bound adducts between psoralens and unsaturated fatty acids has also been reported. For example, several photoproducts resulting from the

irradiation of psoralens in the presence of linolenic acid have been isolated. These photoproducts derive from the 2 + 2 addition between the 4',5', or 3,4 double bond of the psoralen and the 12, 13 double bond of the fatty acid.[27,28] The significance of lipid binding was also shown in the photoreaction between rat epidermis and methoxalen, where 60% of the methoxalen was bound to lipid, 20% to nucleic acid and 20% to protein.[4] The photoreaction between psoralens and lipids could lead to the inhibition of cell proliferation due to inhibition of lipid hydrolysis reactions needed for cell growth.

Laskin and coworkers reported that psoralens photobind to specific high affinity cellular receptors.[18-20] The binding of psoralens to their receptors effectively inhibits the binding of epidermal growth factor (EGF) to its receptor. EGF is important for the production of intracellular growth regulatory signals and is known to be over expressed in many cancerous cells. The psoralens do not directly compete for the EGF binding site. Rather, they bind to a receptor so close to the EGF receptor that the affinity of EGF for its receptor diminishes. The psoralens were shown to bind to both high and low affinity receptors on the surface of the cell through a Scatchard analysis. Laskin was the first to isolate a high affinity psoralen receptor, which has a molecular weight of 22 kD (compared with 170 kD EGF receptor).[18-20]

The interaction of psoralens with proteins is well

documented, but the mechanism by which the psoralens bind to proteins is not well understood. There have been many reports of psoralen-protein interaction under a variety of conditions, suggesting that there may be a number of competing pathways for this interaction. Psoralens have been shown to bind to proteins by light-dependent and light-independent pathways, and also with or without oxygen.[18-26]

Artuc and coworkers have shown that 8-MOP and 5-MOP will reversibly bind to albumin in the absence of light.[25] The two compounds are assumed to bind to two specific receptors since 5-MOP does not displace bound ^{3}H-8-MOP from the albumin. Curently, albumin is the only protein known to interact with psoralens in the absence of light.

There have been a number of reports of the photoaddition of psoralens to a variety of proteins *in vitro*. The psoralens have been shown to bind to proteins in the presence or absence of oxygen along with UVA light. Since the photobinding has been observed with or without oxygen, the possibility exists that there could be two or more mechanisms for the photobinding. Psoralens or the psoralen photodecomposition products may bind to the protein.

There has been evidence that the psoralens can bind directly to proteins through a free radical intermediate.[22] The photoreactions of a variety of psoralens with aromatic amino acids were studied by a photo-CIDNP technique.[22] Solutions of psoralens and amino acids were irradiated and the

nuclear spin polarization observations were made. The most intense reaction was observed when psoralens were irradiated in the presence of N-acetyltyrosine. The first step in the mechanism was reported to be a reversible electron transfer from the tyrosine to the psoralen, accounting for the CIDNP effect. The pathway by which these molecules would then couple was not discussed and is still not understood.

Tyrosine has been shown to interact with psoralens *in vitro*. Yoshikawa and coworkers reported that psoralens bind to bovine serum albumin in the presence of oxygen and UVA light.[23] This photo-addition is dramatically decreased if the tyrosine residues of the protein are acetylated, thus indicating that tyrosine is the major site of psoralen binding. That tyrosine is the major site of photo-addition is also supported by the work reported by Marko since tyrosine showed the greatest CIDNP effect in the presence of psoralens.[22]

The first attempt to isolate an adduct of tyrosine and 8-methoxypsoralen was attempted by Akyea.[26] Solutions of 8-MOP and N-acetyl-L-tyrosine ethyl ester in acetonitrile were irriadiated at 350 nm for 88 hours. A psoralen-tyrosine adduct, which was analyzed by HPLC-MS, was isolated. The MS of the photoreaction showed peaks with mass units of 352, 399, and 438. In combination with NMR spectra, structures were proposed which fit the data. It is certain that there were adducts formed between tyrosine and 8-methoxypsoralen although

the actual structures were not unequivocally determined. The significance in this work was that adducts of psoralen and tyrosine were obtained.

Introduction

The research described in this dissertation was focused on the preparation of new psoralens and coumarins to further study the reactivity of psoralens with respect to the inhibition of EGF binding to its receptor. The specific aims of this research were to synthesize and evaluate:

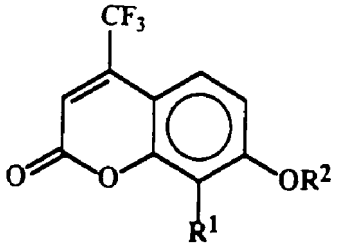
1. 4-trifluoromethyl coumarins and psoralens.
2. New analogs of trioxsalen, which are functionalized at the C-5' methyl.
3. 7-alkoxy-3-bromo coumarins.
4. Iodinated coumarins and psoralens to be used as potential radiolabeled probes.

Over the course of this research, new coumarin and psoralen analogs having structural features in common with compounds of known activity have been synthesized. These compounds have been fully characterized and their ability to inhibit EGF binding has been determined. The primary focus of our laboratory has been to develop new antipsoratic drugs which maximize the inhibition of cell growth and also limit the toxic side effects associated with the currently used compounds. The general structures of the compounds described in this thesis are shown in Figure 4.

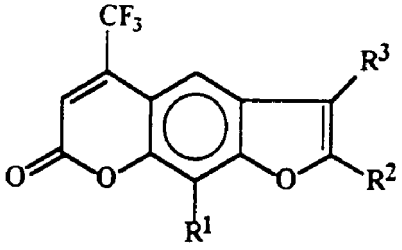
It is well established that psoralens intercalate and photobind to DNA, factors which could explain their photoreactivity. The biological evaluation used over the

course of this research was the EGF binding assay designed and perfected by Laskin.[19] This assay has become the standard for evaluating new compounds in this laboratory.

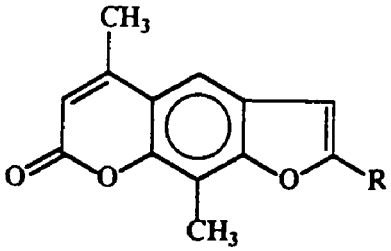
The object of this work was to synthesize compounds which would avidly bind to the psoralen receptor and inhibit the binding of EGF to its receptor.



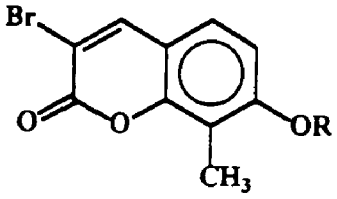
4-trifluoromethylcoumarins



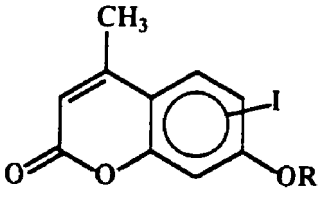
4-trifluoromethylpsoralens



5'-substituted trioxsalens



7-alkoxy-3-bromocoumarins



iodocoumarins

Figure 4

Results and Discussion

This dissertation reports the synthesis and evaluation of a series of new psoralens and coumarins as potential inhibitors of epidermal growth factor. The results and discussion will be divided into four sections on the synthesis and evaluation of: 1) 4-trifluoromethyl psoralens and coumarins, 2) 5' substituted trioxsalen derivatives 3) 7-alkoxy-3-bromo-8-methylcoumarins and 4) iodinated coumarins and psoralens.

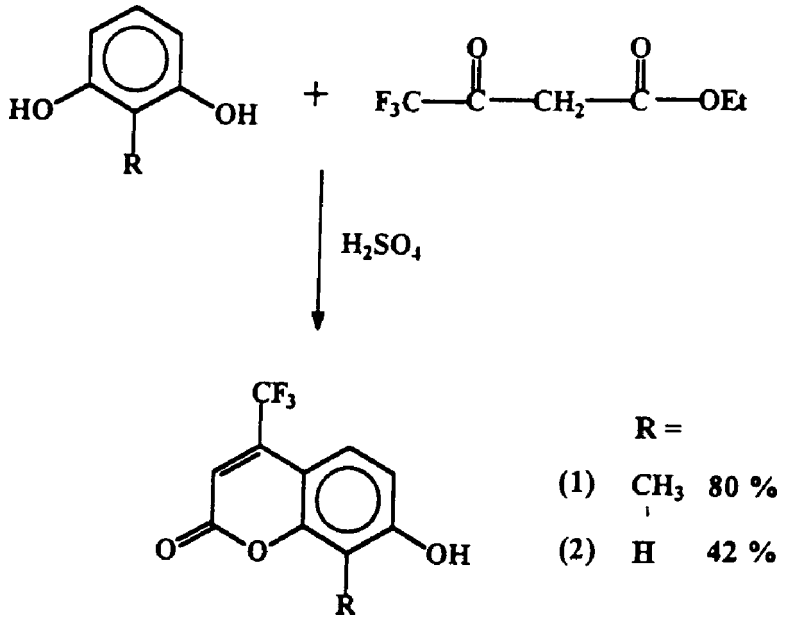
Synthesis of 4-trifluoromethyl psoralens and coumarins

The suggestion by Marko[22] that the charge transfer complex formation between tyrosine (electron donor) and psoralens (electron acceptors) was an important process in the psoralen photocoupling reaction encouraged us to synthesize 4-trifluoromethyl psoralens and coumarins. The trifluoromethyl group should make the pyrone ring electron deficient with respect to the parent methyl compound and thus facilitate the electron transfer reaction.

Further indications that electron acceptor behavior in the pyrone ring might contribute to the desired photobiology are the results obtained with nitro and carboethoxy withdrawing groups positioned in that ring. 3-Nitro-4',5'-dihydropsoralens developed in this laboratory by Sachais possessed impressive IC_{50}'s. Similarly, published work from other groups has claimed the 3-carboethoxypsoralens as

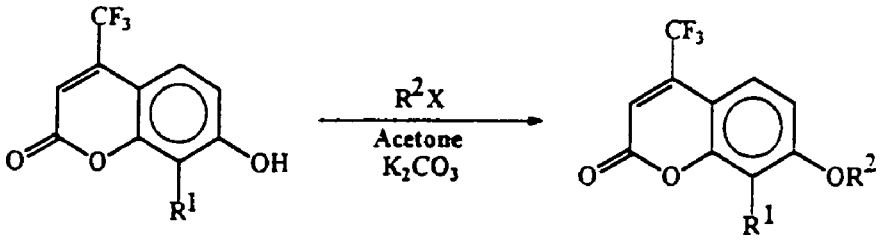
promising dermatological phototherapeutics.[3,4] While pyrone-substituted nitro and carboethoxy derivatives are known, the introduction of an inductive electron withdrawer like trifluoromethyl has not been reported.

The substitution of a trifluoromethyl group for a methyl group is precedented in a variety of pharmaceutical agents.[29-32] The trifluoromethyl group enhances the lipophilicity in addition to altering the electronic properties compared to the methyl group. The Van der Waals volume (hemisphere) of the trifluoromethyl group (42.5 Å^3) is approximately 2.5 times greater than that of the methyl group (16.8 Å^3). A trifluoromethyl substituted compound is often found to have similar activity to the parent compound. This substitution can be effective if the trifluoromethyl group does not disrupt a critical event needed for activity.[29-32]



Scheme 1

The coumarins selected as promising starting materials for the synthesis of all the 4-trifluoromethyl alkoxy coumarins and psoralens were synthesized by a Pechmann condensation between the corresponding resorcinol and 4,4,4-trifluoroacetoacetate[33] (Scheme 1). The 7-alkoxy coumarins were synthesized by alkylating the coumarin with the corresponding alkyl halide in an acetone/K_2CO_3 system. The general reaction and compounds synthesized are shown in Scheme 2.



Compound	R^1	R^2
3	CH_3	CH_3
5	CH_3	$CH_2CH_2CH_3$
7	CH_3	$CH_2CH{=}CH_2$
9	CH_3	$CH_2C{\equiv}CH$
11	CH_3	$CH_2CH{=}C(CH_3)_2$
4	H	CH_3
6	H	$CH_2CH_2CH_3$
8	H	$CH_2CH{=}CH_2$
10	H	$CH_2C{\equiv}CH$
12	H	$CH_2CH{=}C(CH_3)_2$

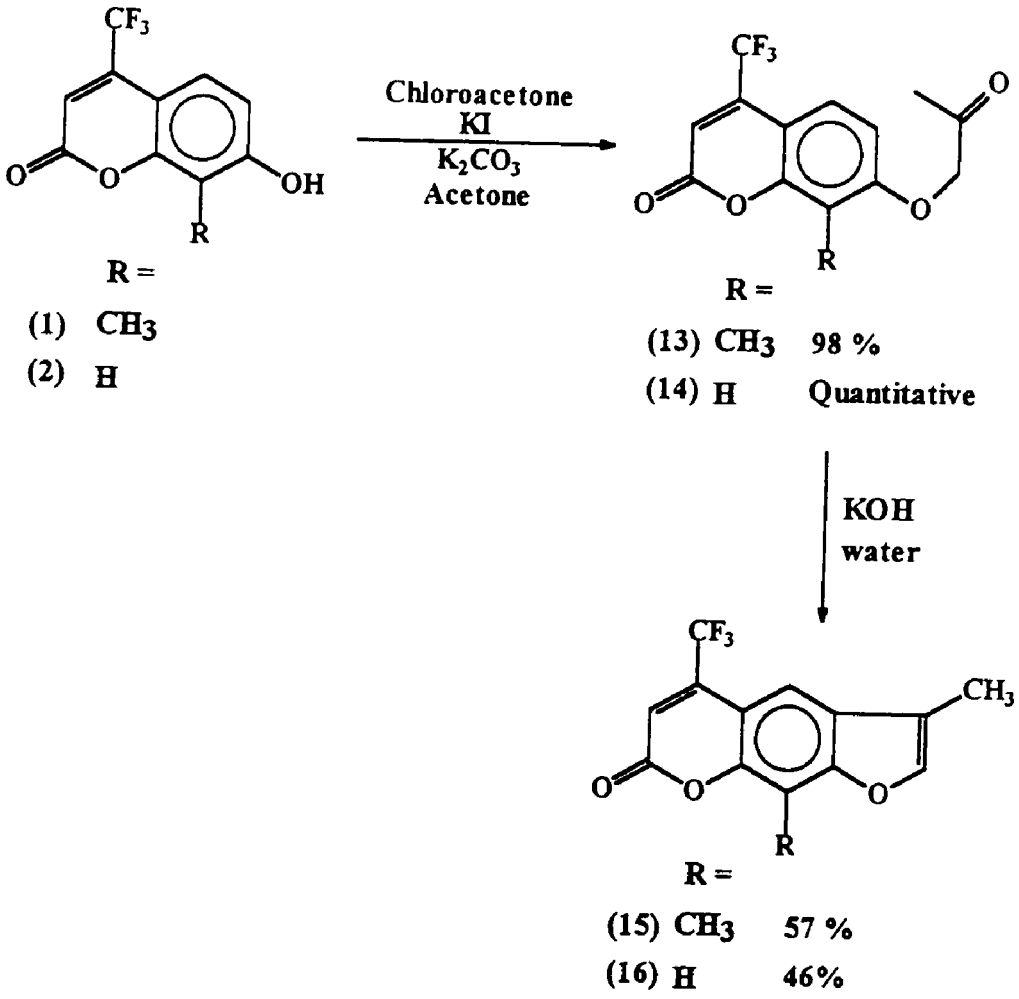
Scheme 2

Note that five of the compounds (**3, 5, 7, 9, 11**) shown above as the products of reaction Scheme 2 were selected to be partial mimics of trioxsalen (i.e., the 4-trifluoromethyl-8-methyl analogs). The others in the set (**4, 6, 8, 10, 12**) allow for evaluation of the role of the 8-methyl in biological activity. Structure-activity correlations of other coumarins studied in the work of Jetter[42] indicate that the additional methyl at C-8 (which is R^1 in the structure of Scheme 2) enhances reactivity.

The specific side chains selected for incorporation into these coumarins allow for a marked variation in lipophilicity from a single methyl to the five-carbon assembly of the 3-methyl-2-buten-1-yl- as attachments at the 7-oxy site (R^2 in the product structure of Scheme 2). Also, the array of side chains chosen for synthetic incorporation displayed measurable, but widely variant, activity in Jetter's studies with simple 4-methyl coumarins.

The 4-trifluoromethyl-4',8-dimethylpsoralen and 4-trifluoromethyl-4'-methylpsoralen derivatives were made by heating the corresponding 7-acetonyloxy compounds (**13, 14**), obtained by alkylating the corresponding coumarin with chloroacetone in an aqueous KOH solution. This technique constituted a modification of the method reported by Kauffman.[34] The reaction proceeds by hydrolysis of the pyrone ring, forming the cis-cinnamic acid, and alkylation of the aromatic ring occurs followed by dehydration. Upon acidification the

pyrone ring readily closes to give the desired compound. The reaction is shown in Scheme 3. The hitherto unknown 4-trifluoromethyl-4',8-dimethylpsoralen (15) and 4-trifluoromethyl-4'-methylpsoralen (16) were obtained in 57% and 46% yields, respectively.



R =

(1) CH_3

(2) H

R =

(13) CH_3 98 %

(14) H Quantitative

R =

(15) CH_3 57 %

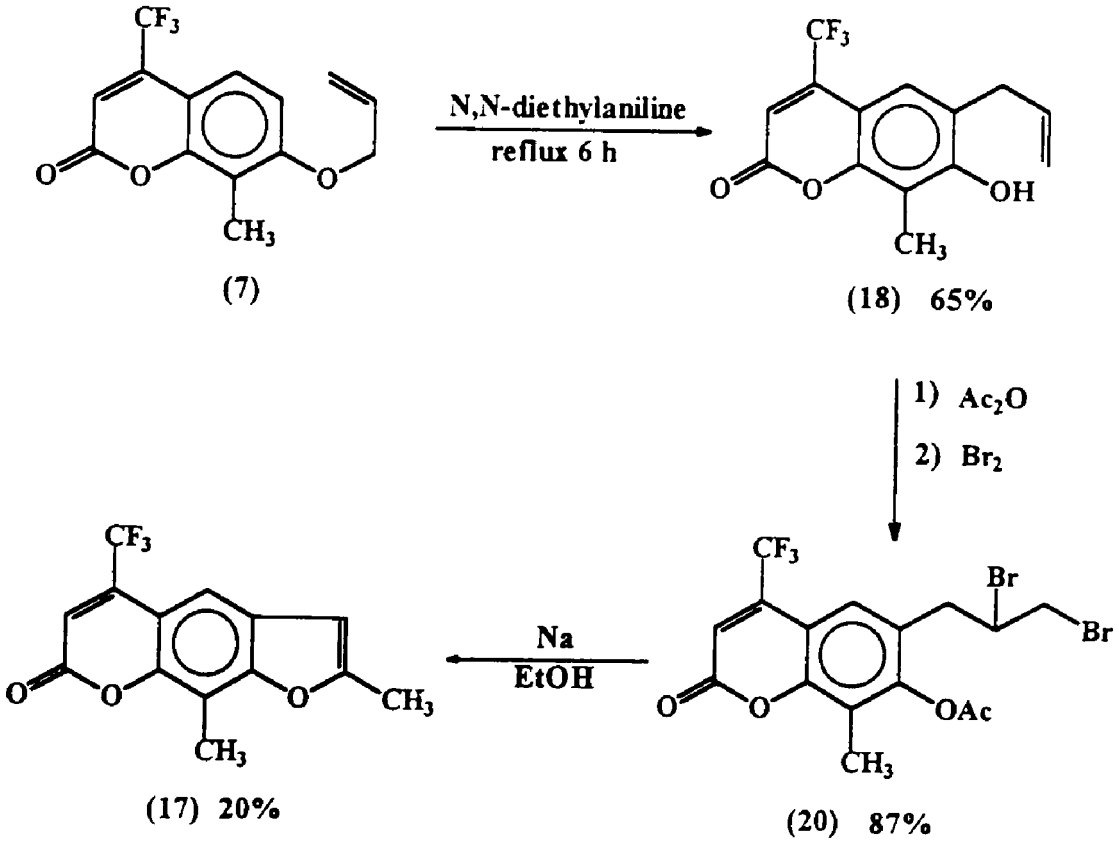
(16) H 46%

Scheme 3

Because, in the original parent trimethylpsoralen family, both isomers with the furan-ring methyl at C-4' (often called by the common name "isotrioxsalen") and with the methyl at C-5' (also known as trioxsalen) are biologically active, we sought to obtain the corresponding 4-trifluoromethyl counterparts. As noted above, the syntheses of the two different 4-trifluoromethyl-4'-methyl compounds (**15**, **16**) was rather uncomplicated. Preparation of the 5'-methyl target presented more difficulties due to the greater number of individual synthetic steps and the inherently lower yields. Several alternative routes were developed for comparison.

The synthesis of 4-trifluoromethyl-5',8-dimethylpsoralen was attempted by a variety of literature methods reported for the synthesis of trioxsalen.[34-36] The first attempted synthesis utilized the methodology for the original synthesis of trioxsalen (Scheme 4).[34] The published procedure needed some modifications, however, due to inherent differences in the reactivities and solubilities of the various substrates used in the reaction sequences. 4-Trifluoromethyl-7-hydroxy-4,8-dimethylcoumarin was alkylated with allyl bromide to form 7-allyloxy-4-trifluoromethyl-8-methylcoumarin (**7**) which was then subjected to the Claisen rearrangement in refluxing N,N-diethylaniline to yield 6-allyl-4-trifluoromethyl-7-hydroxy-8-methylcoumarin (**18**). This compound was then acylated to yield compound **19** and subsequently brominated to yield compound **20**. The yield for the two steps was 87% overall. The psoralen was

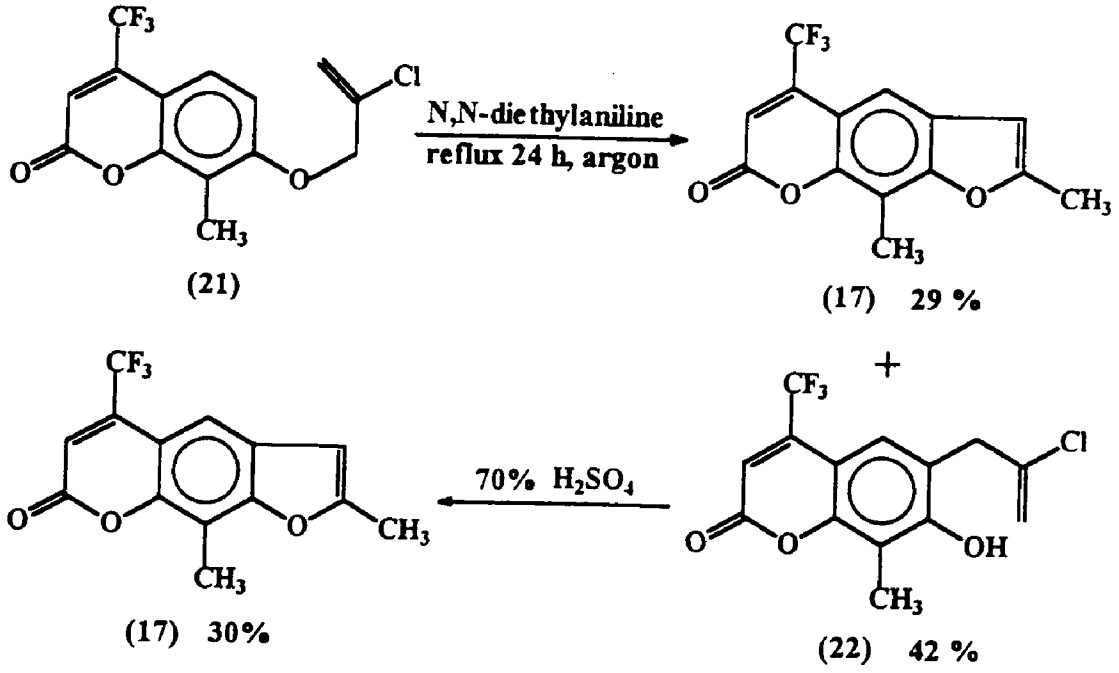
obtained by heating **20** in an ethanolic solution of sodium ethoxide. This reaction takes advantage of the fact that once the acetate is hydrolyzed, the five membered ring will form faster than the six membered ring yielding the desired compound. The series of reactions gave an 11% overall yield.



Scheme 4

A second method was attempted to directly synthesize the desired compound using a modification of a method reported by

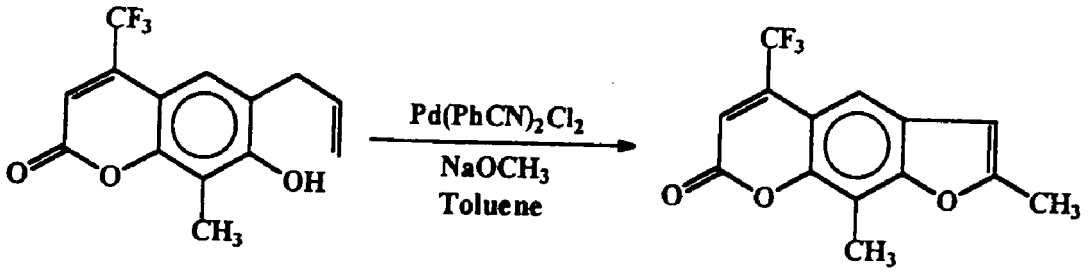
Bender.[35] The reaction is diagramed in Scheme 5. 4-Trifluoromethyl-7-hydroxy-4,8-dimethylcoumarin was alkylated with 2,3-dichloropropene to form 7-(2-chloroallyloxy)-4-trifluoromethyl-8-methylcoumarin (21). The ether was heated under reflux with N,N-diethylaniline for 24 hours giving a 42% yield of the expected phenolic compound (22) and 29% of the 4-trifluoro-methyl-5,8-dimethylpsoralen (17). Attempts were made to improve the yield by increasing the reaction time, but this led to an intractable tar resulting from decomposition of both the solvent and reactants. The phenolic product (22) was converted to the desired psoralen (17) in a 30% yield from 22 by stirring in 70% sulfuric acid.



Scheme 5

The progress of this cyclization pathway and the characterization of the Claisen-derived intermediate (**22**) can be monitored by both TLC and NMR. Chromatographically, the compounds differed sufficiently in polarity to provide for easy separation on silica using chloroform as the eluent. The more polar phenolic compounds remained at the origin and the non-phenolic compounds migrated up the plate. Spectrally, in ^{1}H-NMR one observes that the Claisen derived intermediate (**22**) has a singlet for the C-5 proton. A doublet is observed for the C-5 proton in the initial starting material (**21**) due to coupling to the C-6 proton.

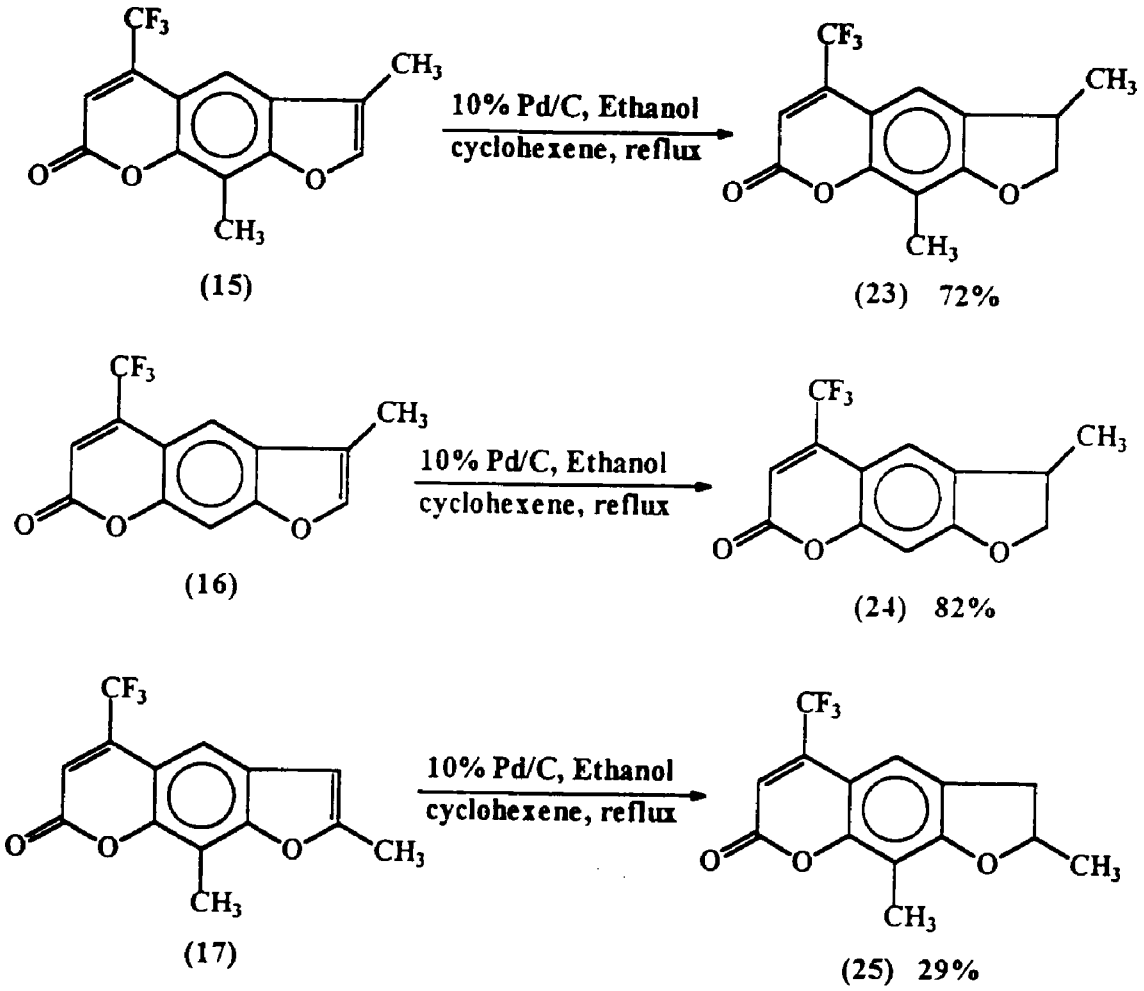
A third method was attempted for the synthesis of **17** using a palladium promoted cyclization of **18** to yield the desired psoralen.[36] Molar equivalents of palladium bis benzonitrile dichloride and sodium methoxide were combined with **18** in toluene under an argon atmosphere and heated under reflux for three hours to yield **17** in 14% yield (Scheme 6). Attempts to synthesize **17** using less than the molar equivalent of palladium acetate with copper acetate as the oxidant were unsuccessful. Only starting materials were recovered.



(**18**) (**17**) 14%

Scheme 6

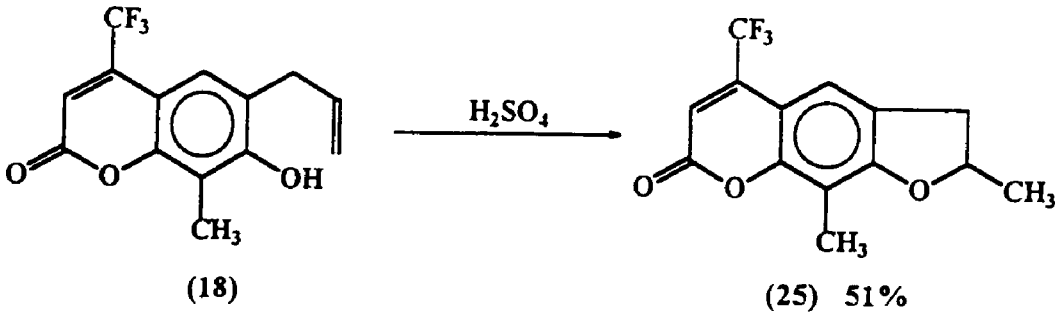
It has been well established that psoralen derivatives in which the 4',5' double bond is reduced retain activity and in some cases have enhanced activity in the EGF assay.[21,37] The 4',5' furan double bonds of the three reported psoralens were reduced by a transfer hydrogenation with cyclohexene over 10% palladium on carbon (Scheme 7). The two 4'-methyl psoralen derivatives (**23, 24**) reduced with good efficiency, but the 5'-methyl derivative was strangely resistant to reduction. Heindel has reported that in trioxsalen itself, the reduction is invariably incomplete and heroic chromatographic methods are needed to obtain the 4',5'-dihydro product in pure form.[21] As a result the 5'-methyl dihydro derivative was synthesized by an alternate route. The dihydro furan ring (**25**) was formed directly via a sulfuric acid promoted ring closure of the 6-allyl phenol (**18**) as shown in Scheme 8. This type of ring closure has previously been reported in the synthesis of trioxsalen analogs.[35]



Scheme 7

Because both compound **18** and compound **25** possessed activity in the bioassay for inhibition of EGF binding, and because the pharmacological tester observed a transient fluorescence when a sample of **18** was irradiated with HeLa cells in the assay, the possibility that the former was ring closing to the latter under light activation was considered. The possible conversion of **18** to compound **25** was explored preparatively under photolytic conditions by Dr. Rapp of Albright College, since precedent exists for ring closures of this type in structurally related systems.[39]

The ultraviolet spectra of **18** and **25** were first obtained and these spectra were sufficiently different to detect if **18** was converted to **25** under photolytic conditions. A 2.0×10^{-3} M solution of **18** in methanol was irradiated for 4 hours with a Sylvania GTE ST122480 fluorescent lamp after which the ultraviolet spectrum was examined. While the characteristic maxima for the 7-hydroxy compound (**18**) at 215, 235 and 339 nm did fall during the 4 hours of exposure, the spectrum did not transform into that of the 4',5'-dihydropsoralen (**25**). The photoinstability of **18** may be the result of dimerization of the pyrone double bond, since this reaction has strong literature precedence.[3-5]



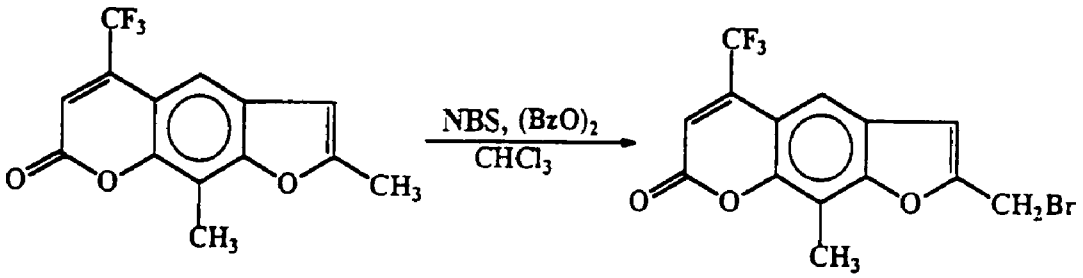
(18) (25) 51%

Scheme 8

The bromomethyl derivatives of both 4-trifluoro-4',8-dimethylpsoralen (**15**) and 4-trifluoro-5',8-dimethylpsoralen (**17**) were made because the bromomethyl substituent has been previously shown to increase the activity of trioxsalen.[26] The activity may be due to: 1) the bromine being displaced by

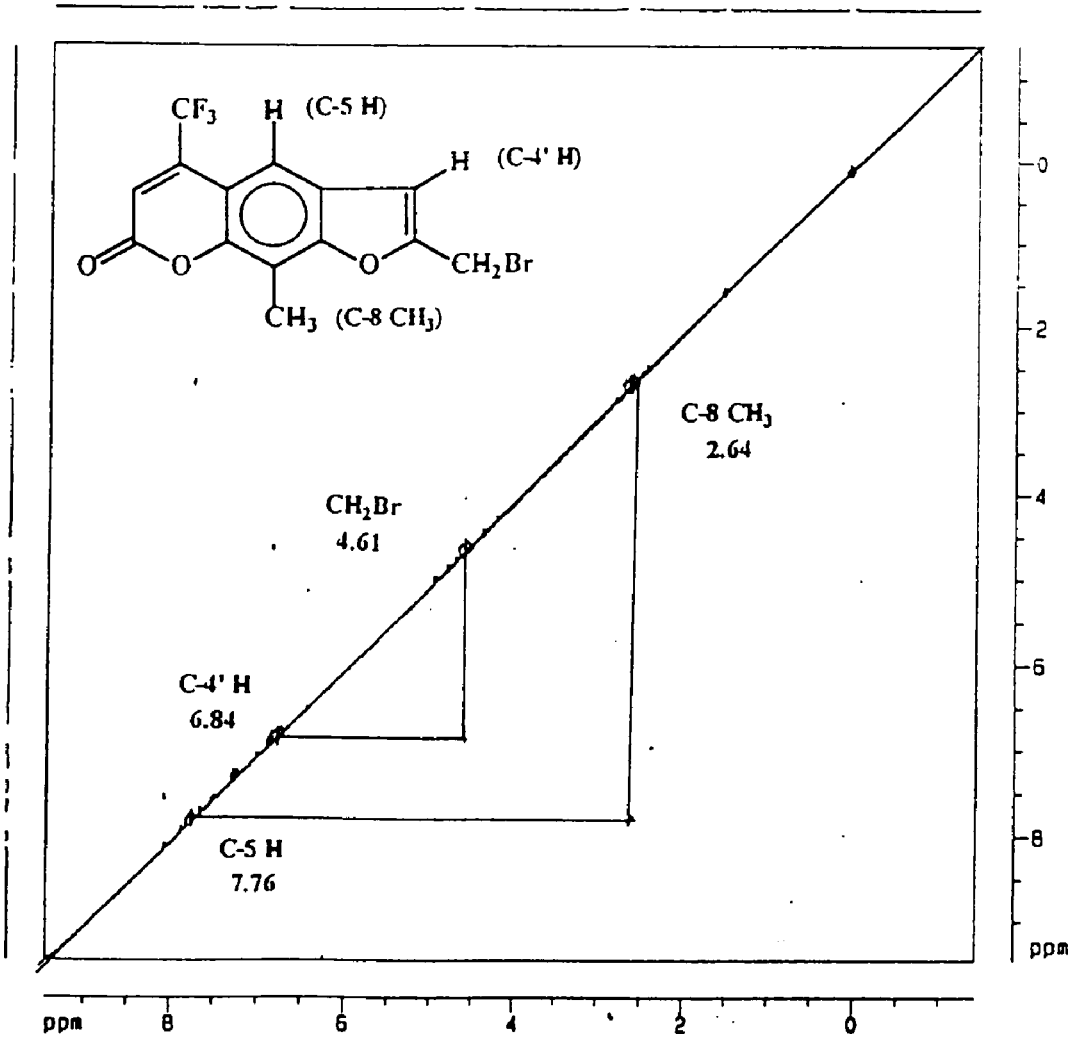
amino groups in the binding site; or 2) the carbon-bromine bond being photolytically cleaved to yield an active alkylating agent which can then bind to the receptor.

5'-Bromomethyl-4-trifluoromethyl-8-methylpsoralen (26) was synthesized utilizing a selective free radical bromination as shown in Scheme 9. The ability to exclusively brominate the 5' methyl group of psoralen derivatives has been reported using both thermal [26] and photochemical [40] processes. The psoralen 17 is heated with a molar equivalent of N-bromo-succinimide using benzoyl peroxide as the radical initiator. The reaction yielded the desired product 26 in a 62% yield. The structure of the product was confirmed by a long-range COSY shown in Figure 5. Cross-peaks were observed that indicate that the C-5' methylene and the C-4' proton were long range coupled and that the C-8 methyl and the C-5 proton were coupled.



(17) (26) 62%

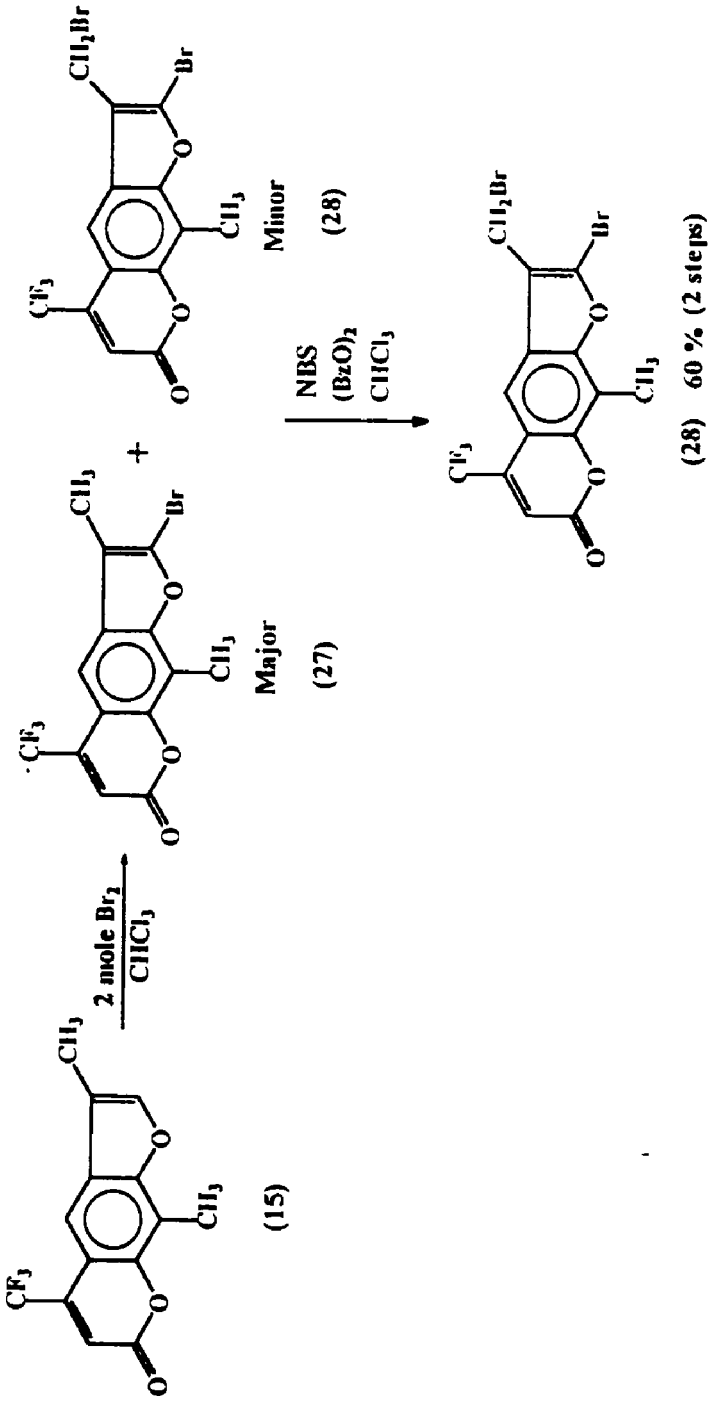
Scheme 9



^{1}H-^{1}H COSY for 5'-bromomethyl-4-trifluoromethyl-8-methylpsoralen (26)

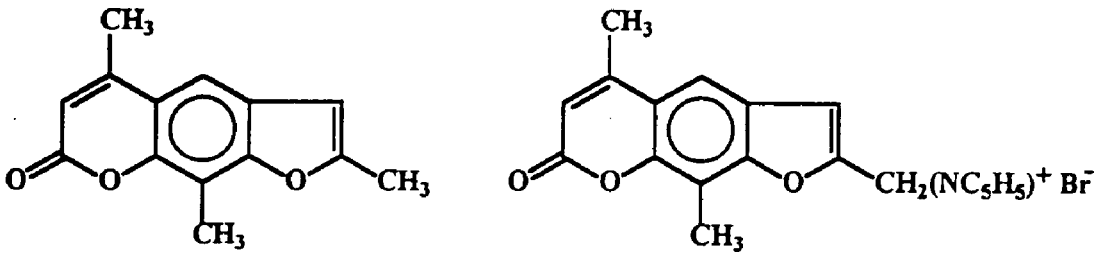
Figure 5

The bromination of the 4-trifluoromethyl-4',8-dimethylpsoralen (**15**) was accomplished by a two step procedure (shown in Scheme 10) using molecular bromine and yielding a mixture of compounds **27** and **28**. The product distribution did not parallel that reported by Akyea in his bromination studies on trioxsalen. Akyea reported that the major product was the di-bromo compound, but in this work the mono-bromo compound **27** was the major product (85%) whereas the di-bromo product (**28**) amounted to 15%. The product distribution was determined by NMR analysis. Since the desired product was the dibromo, the crude product was subjected to a free radical initiated bromination to enhance the overall yield of the dibromo product (**28**). Attempts to brominate the parent compound (**15**) under similar conditions yielded only the the 5'-bromo-4-trifluoromethyl-4',8-dimethylpsoralen (**27**). This product distribution is consistent with previously reported attempts at brominations of 4,4',8-trimethylpsoralen with N-bromosuccinimide.[26]


2 mole Br2
CHCl3
(15)
Major
(27)
+
Minor
(28)
NBS
(BzO)2
CHCl3
(28) 60 % (2 steps)

Scheme 10

One of the persistent problems with the fluoro psoralens is that they are much less water soluble than their non-fluorinated analogs. To increase the water solubility of these compounds, quaternary pyridinium groups were added to the furan methyl of the psoralens via a bromomethyl intermediate. The quaternary compounds have been previously shown to have comparable IC_{50} values to trioxsalen in the EGF assay (Figure 6). These charged molecules cannot cross the cell membrane and thus cannot bind to the DNA. This provides more evidence to support the contention that psoralens exert their biological activity at a membrane receptor.

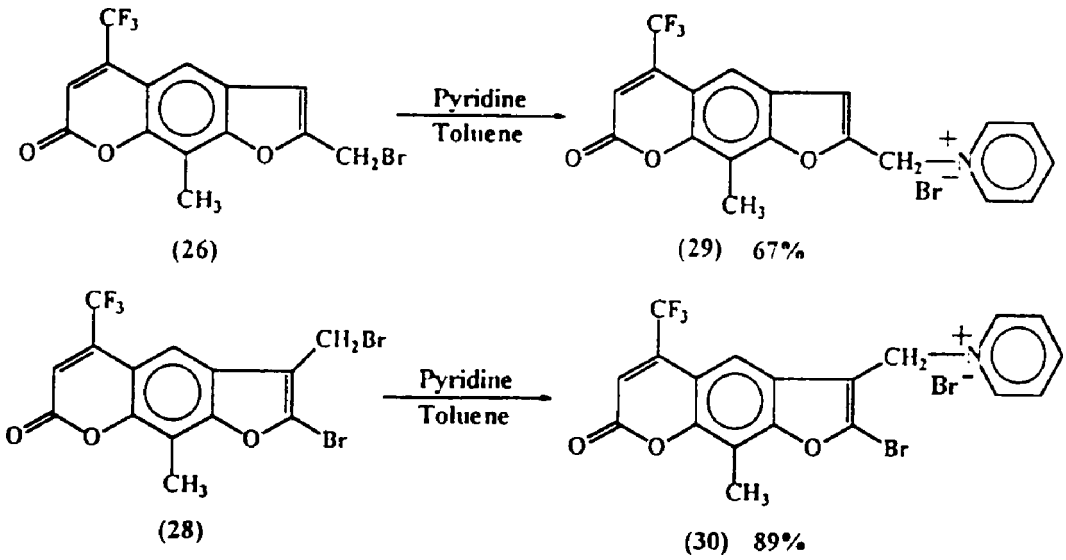


IC_{50} = 6.6 μM IC_{50} = 7.5 μM

Figure 6

The quaternary compounds were synthesized by heating a toluene solution of the bromomethyl compound and a 10 molar excess of pyridine. The quaternary compounds precipitated from the reaction medium and were isolated by filtration. The two quaternary psoralens, 4-trifluoromethyl-5'[(N-pyridinium)-methyl]-8-methylpsoralen bromide (**29**) and 5'-bromo-4-trifluoromethyl-4'[(N-pyridinium)methyl]-8-methylpsoralen bromide (**30**) were isolated in 67% and 89% yields respectively.

The two reaction reactions are diagramed in Scheme 11.



Scheme 11

Evaluation of the electron localization in the pyrone ring of the 4-trifluoromethyl psoralens and coumarins in comparison to the parent compounds.

Replacing the 4-methyl with the 4-trifluoromethyl group should alter the electronic localization of the pyrone ring and thus enhance its ability to accept an electron in an electron transfer reaction. Evidence to support this contention was recently reported by Nagasawa who has shown that a series of 7-amino-4-trifluoromethylcoumarins were better electron acceptors than their corresponding methyl analogs.[41] The electron density was further investigated by ^{1}H and ^{13}C NMR studies and by theoretical calculations.

Theoretical semi-empirical calculations were performed using the Spartan software (Figure 7). The calculations indicated that the pyrone ring of the 4-trifluoromethylpsoralens and coumarins was indeed electronically altered in comparison to their methyl counterparts. The electron density on the C-3 carbon was decreased but the electron density on the C-4 carbon was increased.

NMR studies appear to support the semi-empirical molecular orbital calculations. The C-3 proton of the 4-trifluoromethyl psoralens and coumarins in the ^{1}H NMR was shifted downfield with respect to the 4-methyl analogs. The chemical shift of the C-3 proton of 4-trifluoromethyl coumarins and 4-trifluoromethyl-4',5'-dihydropsoralens ranged

Compound	Charge				
	O-1	O-2	C-2	C-3	C-4
4-trifluoromethyl-7-hydroxy-8-methylcoumarin (1)	-0.1860	-0.2696	0.3355	-0.1859	-0.0307
4,8-dimethyl-7-hydroxycoumarin	-0.1951	-0.2936	0.3406	-0.2553	0.0515
4-trifluoromethyl-7-methoxy-8-methylcoumarin (3)	-0.1876	-0.2726	0.3357	-0.1878	-0.0293
4,8-dimethyl-7-methoxycoumarin	-0.1967	-0.2964	0.3408	-0.2567	0.0525
4-trifluoromethyl-5',8-dimethylpsoralen (17)	-0.1819	-0.2698	0.3339	-0.1781	-0.0388
4,5',8-trimethylpsoralen	-0.1916	-0.2940	0.3405	-0.2577	0.0486
4-trifluoromethyl-4',8-dimethylpsoralen (15)	-0.1818	-0.2698	0.3338	-0.1779	-0.0391
4,4',8-trimethylpsoralen	-0.1912	-0.2919	0.3391	-0.2475	0.0431

The more negative the number the greater the electron density.

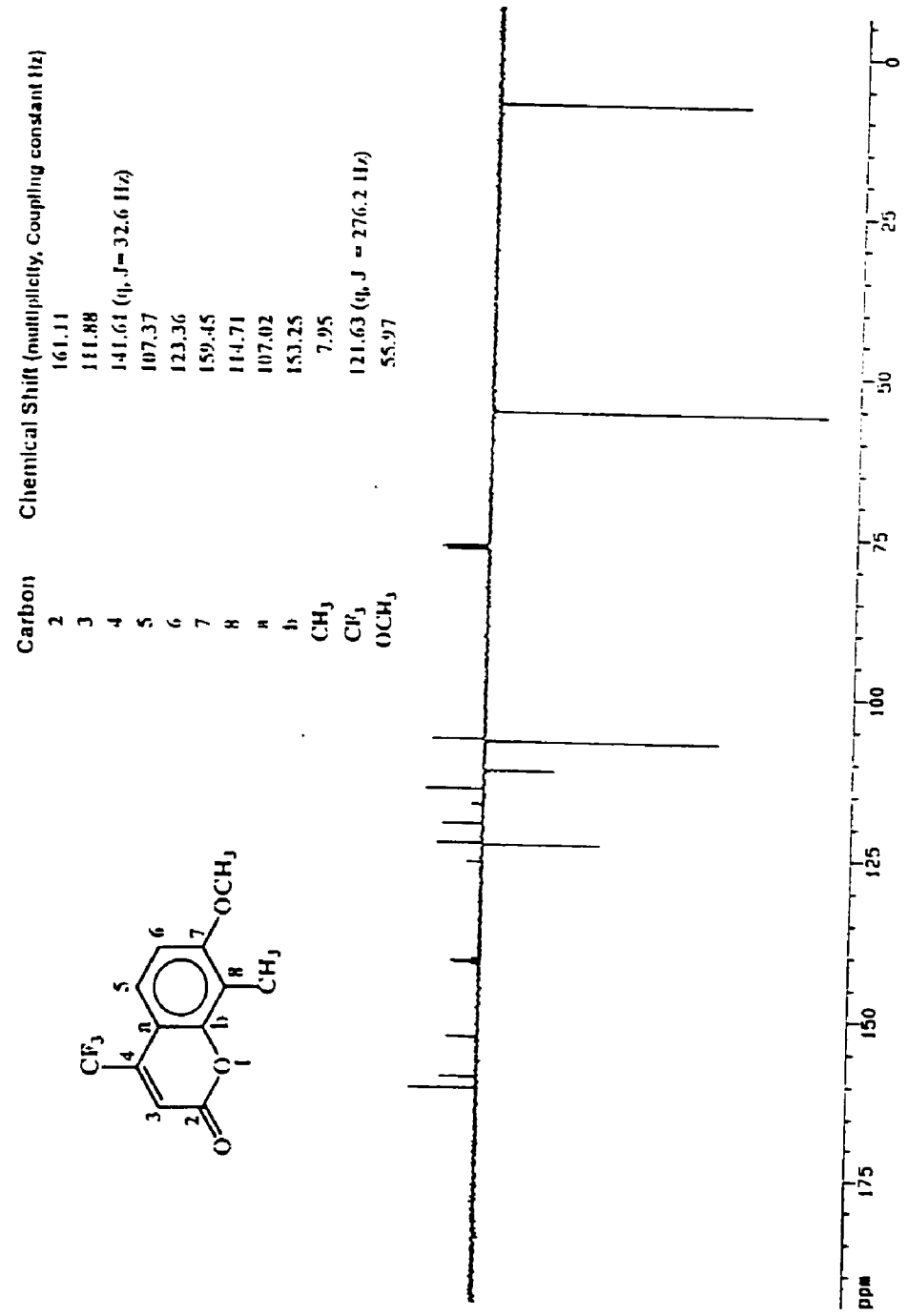
Summary of molecular orbital calculations for selected psoralens and coumarins

Figure 7

from 6.55-6.60 ppm compared to 6.09-6.13 ppm for the 4-methyl derivatives.[42] The chemical shift of the C-3 proton of the 4-trifluoromethylpsoralens ranged from 6.76-6.87 ppm compared to 6.56-6.69 ppm for the 4-methyl derivatives. These data are consistent with the fact that the electron density on the C-3 carbon is reduced in the 4-trifluoromethyl derivatives.

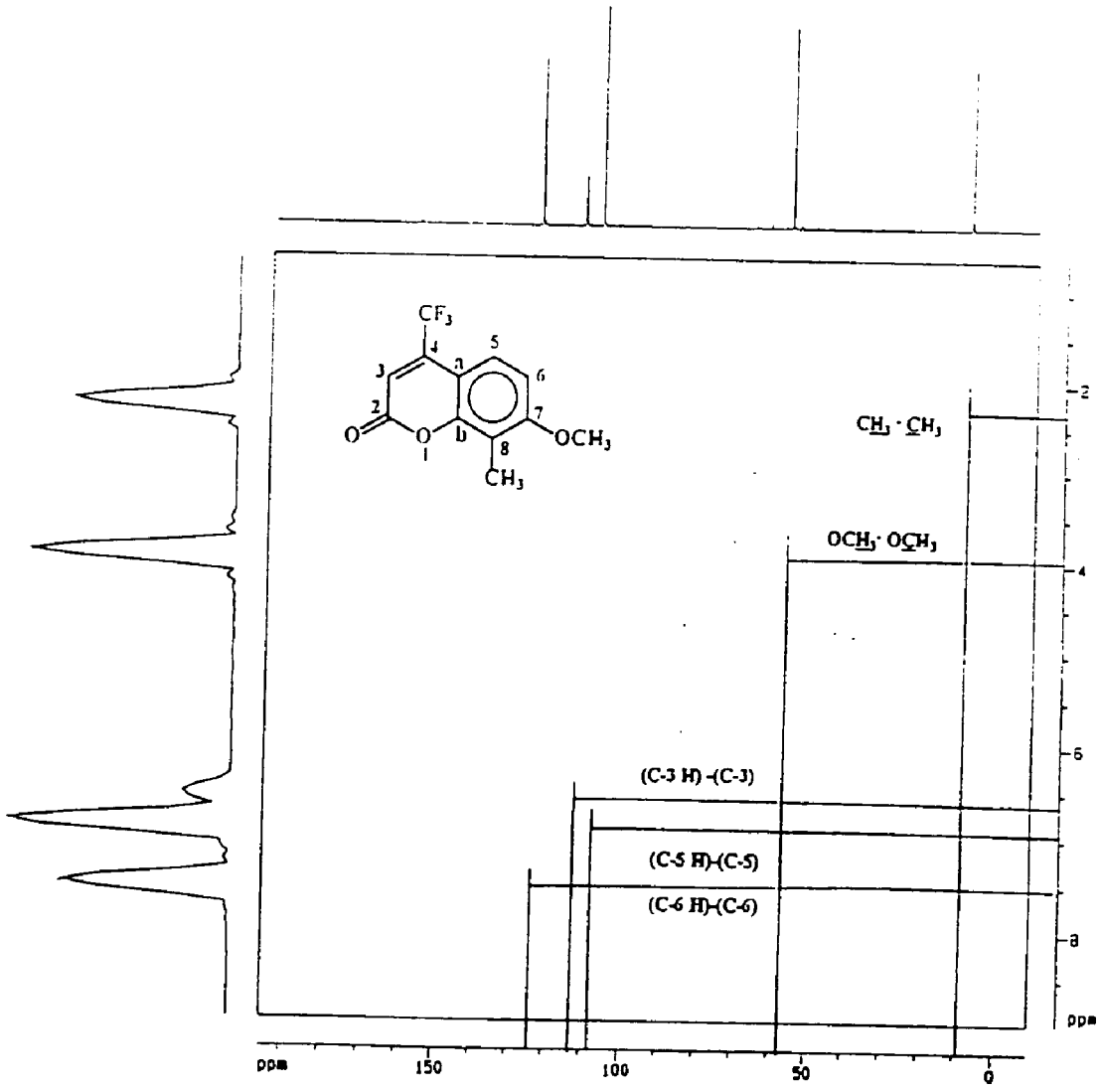
To compare the ^{13}C chemical shifts of the C-3 and C-4 carbons of the pyrone ring of the 4-trifluoro verses the 4-methyl compounds, 4-trifluoromethyl-7-methoxy-8-methylcoumarin (**3**) and 7-methoxy-4,8-dimethylcoumarin were used as representative compounds. The chemical shifts of the respective carbons were determined using both APT and HETCOR. The APT and HETCOR spectra for 4-trifluoromethyl-7-methoxy-8-methylcoumarin are shown in Figures 8 and 9.

The chemical shifts of C-3 and C-4 for the 4-trifluoromethyl-7-methoxy-8-methyl coumarin were 111.88 and 141.61 ppm respectively. The chemical shifts of C-3 and C-4 for the 7-methoxy-4,8-dimethylcoumarin were 111.53 and 152.37 ppm respectively. The downfield shift of C-3 (+ 0.35 ppm) and the upfield shift of the C-4 (- 10.76 ppm) proton also agree with what would be expected from the previously mentioned calculations and observations since the C-3 carbon is deshielded and the C-4 carbon is shielded in the trifluoromethyl derivatives.



^{13}C-APT of 4-trifluoromethyl-7-methoxy-8-methylcoumarin (3)

Figure 8



^{1}H-^{13}C HETCOR of 4-trifluoromethyl-7-methoxy-8-methylcoumarin (3)

Figure 9

BIOLOGICAL ACTIVITY OF THE 4-TRIFLUOROMETHYL DERIVATIVES

Since all psoralens and psoralen-mimics are clinically used in conjunction with UV light (PUVA therapy), any bioassay must control for cell type, cell population, cell viability, concentration of reference agent (e.g., ^{125}I EGF), temperature, light exposure intensity, wavelength of exposure, and duration of illumination. Because so many variables are involved in the molecular-cellular process being probed, a wide variability of the data (expressed as IC_{50} or the concentration of candidate agent to inhibit by 50% the degree of ^{125}I EGF binding) is to be expected.

The EGF binding assay is performed by producing a dose response curve for a given compound using duplicate samples for each concentration (Figure 10). The IC_{50} for the compound is then determined from the curve. Previous work has resulted in IC_{50} values for a given compound which differ +/- 40% or less. In comparing the IC_{50} values obtained for various compounds, a number which differs by +/- 80% (2 standard deviations) is considered to be significantly different.

All compounds were evaluated by our collaborators at the University of Medicine and Dentistry of New Jersey in an EGF binding assay developed and previously published by Laskin.[18-20] The biological activity data for the 4-trifluoromethyl compounds are summarized in Tables 1-5.

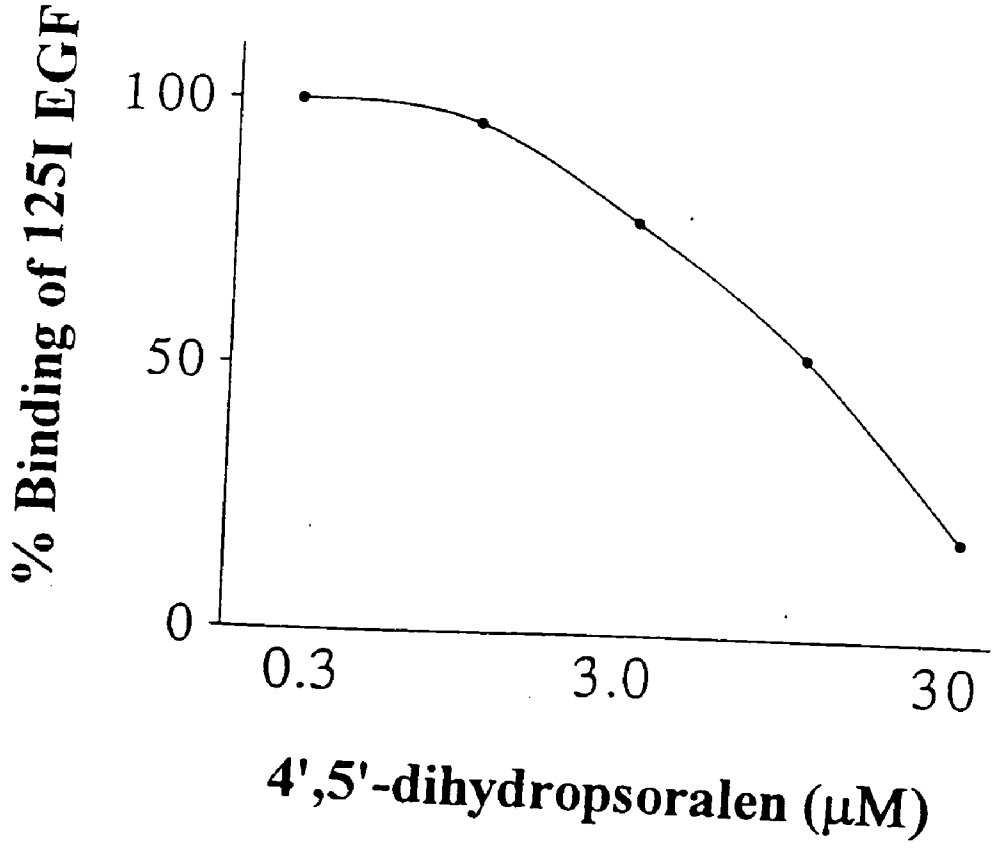
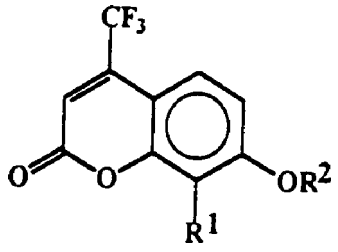


Figure 10

Table 1

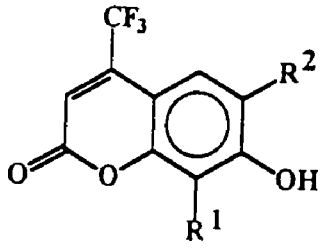
IC_{50} values for 7-alkoxy-4-trifluoromethylcoumarins



Compound	R^1	R^2	IC_{50} (μM)
3	CH_3	CH_3	> 106
5	CH_3	$CH_2CH_2CH_3$	> 105
7	CH_3	$CH_2CH{=}CH_2$	107
9	CH_3	$CH_2C{\equiv}CH$	> 106
11	CH_3	$CH_2CH{=}C(CH_3)_2$	20
4	H	CH_3	> 130
6	H	$CH_2CH_2CH_3$	> 122
8	H	$CH_2CH{=}CH_2$	> 110
10	H	$CH_2C{\equiv}CH$	> 112
12	H	$CH_2CH{=}C(CH_3)_2$	> 100

Table 2

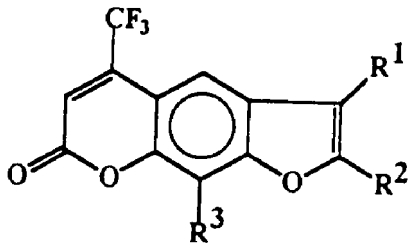
IC_{50} values 7-hydroxycoumarins



Compound	R^1	R^2	IC_{50} (μM)
1	CH_3	H	23
2	H	H	> 130
18	CH_3	$CH_2CH{=}CH_2$	7
22	CH_3	$CH_2C(Cl){=}CH_2$	> 94

Table 3

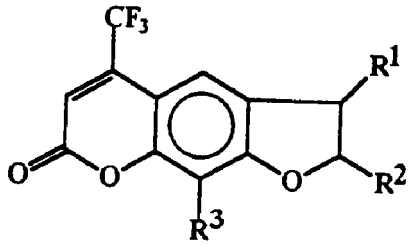
IC_{50} values for 4-trifluoromethylpsoralens



Compound	R^1	R^2	R^3	IC_{50} (μM)
15	CH_3	H	CH_3	> 106
16	CH_3	H	H	> 112
17	H	CH_3	CH_3	> 106

Table 4

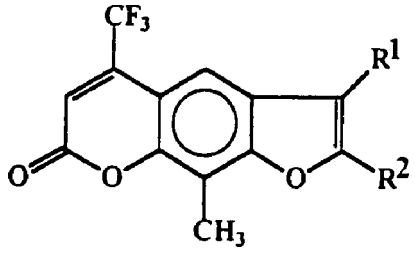
IC_{50} values for 4-trifluoromethyl-4',5'-dihydropsoralens



Compound	R^1	R^2	R^3	IC_{50} (μM)
23	CH_3	H	CH_3	70
24	CH_3	H	H	> 111
25	H	CH_3	CH_3	32

Table 5

IC_{50} values for brominated and quaternary 4-trifluoromethylpsoralens



Compound	R^1	R^2	IC_{50}
26	H	CH_2Br	11
29	H	$CH_2(NC_5H_5)^+Br^-$	> 68
27	CH_3	Br	> 83
28	CH_2Br	Br	> 68
30	$CH_2(NC_5H_5)^+Br^-$	Br	> 56

4-TRIFLUOROMETHYLCOUMARINS

Four compounds in the trifluoromethylcoumarin series were active in the standard bioassay. The remaining compounds were markedly limited in water solubility because of the increased hydrophobicity imparted by the fluorines. These compounds could not be dissolved in the test media at high enough concentration to observe activity. We characterize this subset as having IC_{50} values greater than the highest concentration tested in the assay.

Biologically active compounds in the trifluoromethyl series included two 7-alkoxy derivatives (**7**, **11**) and two 7-hydroxy compounds (**1**, **18**). The inability of the remaining compounds in this series to inhibit EGF binding prompted the investigation of both the molar absorptivities and the lipophilicities of a subset of these compounds to determine if there was any inherent property of these agents which would correlate with their inactivity and the activity observed in the subset of four compounds.

The choice of these two physico-chemical parameters (molar absorptivities and lipophilicities) for testing as correlating quantities in the structure-activity evaluation is not an arbitrary one. First, since all beneficial pharmacology of the psoralens requires a photoactivation, the possibility of substantial differences in light-absorbing properties contributing to biological variations cannot be discounted. Second, since the cellular target for biological

activity is believed to be a membrane receptor - normally a highly lipophilic environment - transport and uptake into this structure could be a prime determinant of activity.

The molar absorptivities for a subset of compounds are shown in Table 6. The values were determined at the maximum of the peak between 320 and 400 nm since this is the wavelength range over which these agents are irradiated in the biological assay. There are no significant differences between the molar absorptivities of active compounds compared to inactive compounds.

Table 6

Compound	λ	Molar absorptivity (10^4)
4-trifluoromethyl-7-hydroxy-8-methylcoumarin (**1**)	332	1.29
4-trifluoromethyl-7-hydroxy-coumarin (**2**)	332	1.21
6-allyl-4-trifluoromethyl-7-hydroxycoumarin (**18**)	339	1.26
4-trifluoromethyl-7-methoxy-8-methylcoumarin (**3**)	332	1.43
7-allyloxy-4-trifluoromethyl-8-methylcoumarin (**7**)	331	1.44
4-trifluoromethyl-8-methyl-7-[(3-methyl-2-butene-1-yl)oxy] coumarin (**11**)	332	1.29

The lipophilicities of the 4-trifluoromethylcoumarins were determined by the HPLC method developed by Akyea.[44,26] The lipophilicities are calculated from the capacity factors of the given agents. The system is calibrated using a series of

compounds with known partition coefficients and plotting log K' versus log P.[26] The slope and Y intercept of the resultant straight line are then used to convert the capacity factor to log P. The general equation is shown in Figure 11. A more complete description of this technique is given in the experimental section of this thesis.

$$\log P = A \log K' + \log K$$

Figure 11

Akyea reported that a series of 7-alkoxy-4,8-dimethylcoumarins exhibited a parabolic relationship between the log of the partition coefficient and the log of the inverse of the IC_{50}. The greatest activity was observed for agents which had log P values between 2.5 and 4.0. The log P values of six of the coumarins were determined and are listed in Table 7. The trifluoromethyl group increased the value of log P for the coumarins by an average value of 0.89 in comparison to their methyl counterparts.

The influences of lipophilicity on the activity of these agents are not apparent. The most lipophilic compound **11** has activity while those agents (such as **3**) with lipophilicities within the same range as active compounds are completely inactive. These data indicate that lipophilicity by itself may not be a primary predictive property with respect to biological activity for coumarins as a general class. This is despite the fact that it has previously shown a correlation in

a select series of compounds in which all structural features were fixed except for attachment on the C-7 oxygen of the coumarin.

Table 7

Compound	Log P	Log $1/IC_{50}$
4-trifluoromethyl-7-hydroxy-coumarin (**2**)	2.12	-----
4-trifluoromethyl-7-hydroxy-8-methylcoumarin (**1**)	2.45	-1.36
6-allyl-4-trifluoromethyl-7-hydroxycoumarin (**18**)	3.40	-0.845
4-trifluoromethyl-7-methoxy-8-methylcoumarin (**3**)	3.22	-----
7-allyloxy-4-trifluoromethyl-8-methylcoumarin (**7**)	3.84	-2.03
4-trifluoromethyl-8-methyl-7-[(3-methyl-2-butene-1-yl)oxy] coumarin (**11**)	4.75	-1.30

4-TRIFLUOROMETHYLPSORALENS

The parent 4-trifluoromethylpsoralens also did not display activity over the concentration range tested. The active compounds found in this series were **23**, **25**, and **26** (Tables 4 and 5). Compounds **23** and **25** are the reduced analogs of **15** and **17** which did not show activity. The molar absorptivities of the four compounds are shown in Table 8. The molar absorptivities of the reduced analogs are approximately 4.5 times greater than those for the unsaturated analogs. This difference is probably not significant enough to account for the vast increase in biological activity

observed since a similar difference in molar absorptivity is present between the parent trioxsalen (0.677×10^{-4}) and the 4',5'-dihydrotrioxsalen analog (1.53×10^{-4}) which have similar IC_{50} values (6.6 and 7.0 μM, respectively).

The 5'-bromomethyl-4-trifluoromethyl-8-methylpsoralen (**26**) possessed very significant biological activity (IC_{50} =11 μM), but it should be remembered that this compound has the capability of binding to the receptor via the bromomethyl group and thus cannot be compared with the rest of the series. The 5'-bromo-4'-bromomethyl-4-trifluoromethyl-8-methylpsoralen (**28**) did not show evidence of activity, but this parallels the results obtained for the 5'-bromo-4'-bromomethyl-8-methylpsoralen which was also inactive. The explanation for why the 4' bromomethyl derivative does not bind to the receptor is not known, but may be due to hinderance from the 5' bromo substituent.

Table 8

Compound	λ	Molar absorptivity (10^4)
4-trifluoromethyl-4',8-dimethylpsoralen (**15**)	353	0.389
4-trifluoromethyl-5',8-psoralen (**17**)	348	0.349
4-trifluoromethyl-5',4' dihydro-4',8-dimethyl psoralen (**23**)	348	1.77
4-trifluoromethyl-4',5' dihydro-5',8-methyl psoralen (**25**)	350	1.58

General Conclusions

The series of trifluoromethyl coumarins and psoralens developed in this project provides new compounds with measured activities below those of their methyl counterparts. The trifluoromethyl derivatives were initially synthesized in an attempt to enhance an electron transfer reaction between tyrosine, a presumed binding component in the target receptor, and the psoralen or coumarin. However, the trifluoromethyl compounds were less effective than their methyl counterparts.

Experimental and theoretical data have clearly shown that the trifluoromethyl group decreases the electron density of the pyrone ring. The overall effect of the trifluoromethyl group on the photoreactivity of the pyrone ring of a psoralen or coumarin is unclear. It has been shown that the trifluoromethyl coumarins and psoralens (with respect to their methyl counterparts) are generally less able to inhibit the binding of EGF to its receptor. The inability to efficiently inhibit the binding of EGF could be attributed to one of the following explanations:

1. The difference in size between the trifluoromethyl and the methyl groups could be significant enough to hinder the initial binding of these agents to their receptor.

2. The increased hydrophobicity induced by the trifluoromethyl group could inhibit the initial binding of the agent to its receptor.

The substitution of fluorine for hydrogen has been shown

to produce increased beneficial effects in various other compounds.[29b] For example, the substitution of a fluorine to the 9 α position of corticosteroids has been reported to increase the therapeutic benefit of these compounds.[29b] The substitution of a fluorine does not cause any steric effects since it is remote from the binding site and is similar in size to hydrogen. Also, the fluorine cannot affect the electronic properties at the binding site of the molecule since it is not attached to a pi system. In these cases the fluorine substitution serves only to alter the lipophilicity of the molecule thereby enhancing binding to its appropriate target.

The trifluoromethyl substitution for a methyl group in the psoralen and coumarin series does not impart any beneficial effect with respect to the inhibition of EGF binding. The trifluoromethyl group is larger than a methyl group (42.5 $Å^3$ vs. 16.8 $Å^3$) and thus substantially alters steric interactions of these compounds at the receptor site. The trifluoromethyl group was also shown to signifigantly alter the electron localization of the pyrone ring which is important in the binding of these compounds to their receptor. Finally, the lipophilicity of these compounds was increased with respect to their methyl counterparts. The simultaneous alteration of three physical properties makes the determination of the individual effects imparted by each difficult to determine.

Trioxsalen Analogs

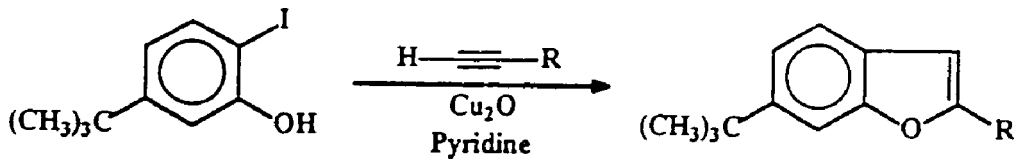
Trioxsalen (4,5',8-trimethylpsoralen) is one of the most active agents in the EGF assay and is commonly used in the treatment of psoriasis. Frequently, the furan ring of the psoralen is formed through the 6-allyl phenolic compound. The furan ring is formed by an oxidative cyclization process discussed previously in the synthesis of 4-trifluoromethyl-5',8-dimethylpsoralen. This sequence of reactions (some of which employ harsh conditions) limits the substituents that can be directly appended to the furan ring of the psoralen. Post-cyclization modification has proven to be a valuable synthetic method.

Only a limited number of 5' substituted trioxsalen derivatives have been reported in the literature because there are few available methods to synthesize them starting from the parent molecule. The current procedures provide for the incorporation an aminomethyl or halomethyl substituent on the furan ring of a fully formed psoralen.[46,47,48] These derivatives have been used as potential phototherapeutics as well as photoactivated anchors to oligonucleotides in antisense therapy applications.[49,50]

The 5' substituted derivatives of trioxsalen currently available derive from 5'-bromomethyl-4,8-dimethyl psoralen.[26] Our goal was to directly synthesize trioxsalen derivatives substituted at the C-5' position under closure conditions sufficiently mild that simple polar functions (amino and

hydroxyl) might survive. These derivatives along with those previously synthesized should enable us to further understand the effects of specific substitutions on the activity of the trioxsalen derivatives.

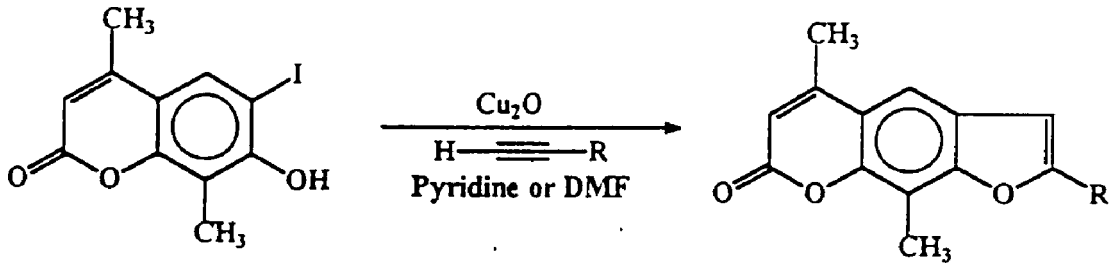
The trioxsalen derivatives were synthesized using a new modification of the Castro reaction. In the typical Castro reaction, aryl halides with nucleophilic ortho substituents react with copper(I) acetylides to form an acetylene compound which subsequently undergoes a cyclization reaction giving the corresponding heterocycle (Scheme 12).[52-54] This new modification of the Castro reaction provides a more convenient method to synthesize C-5' substituted trioxsalen derivatives.



Scheme 12

The initial starting material for the synthesis of the trioxsalen derivatives was 7-hydroxy-4,8-dimethylcoumarin (**31**). 7-Hydroxy-4,8-dimethylcoumarin was iodinated with an iodine/potassium iodide mixture in a dioxane/ammonia/water solvent system to yield 4,8-dimethyl-7-hydroxy-6-iodocoumarin (**32**) in a 95% yield. The active iodinating species is presumed to be the tri-iodide known to form in the presence of iodine and ammonia.[55]

The trioxsalen analogs were synthesized by heating the 7-hydroxy-6-iodo-4,8-dimethylcoumarin (32) with an excess of copper oxide and the corresponding alkyne using either pyridine or DMF as the solvent. The active species in this reaction is the copper acetylide which is generated *in situ*.[51,52] The copper acetylide reacts with the iodocoumarin to form the corresponding psoralen compound. This reaction has provided four new trioxsalen derivatives. The general reaction and the corresponding yields are shown in Scheme 13.



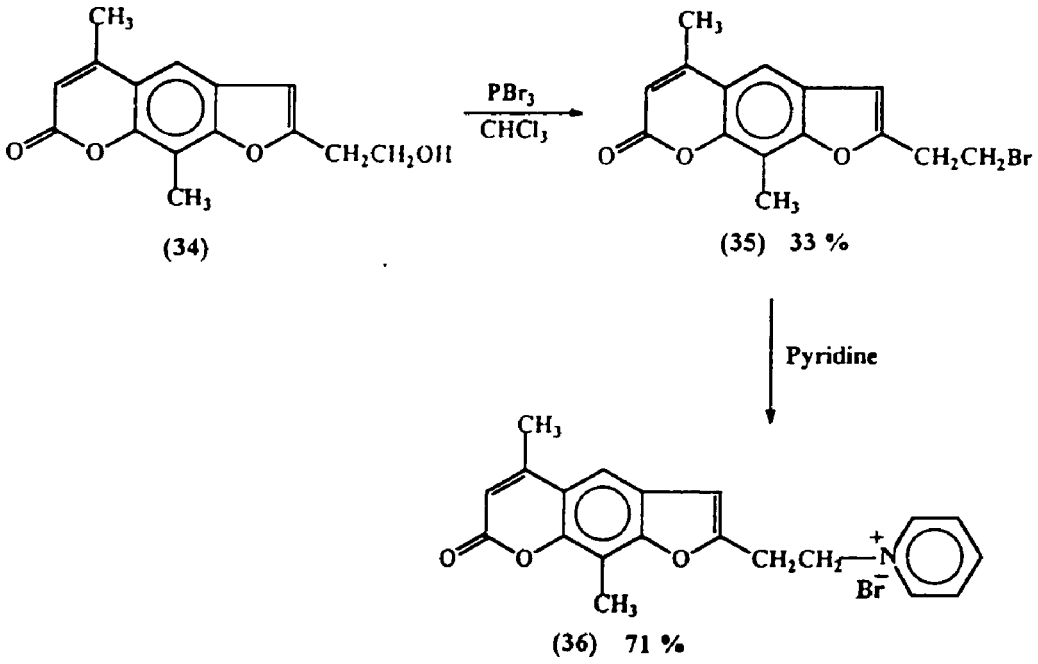
Compound	R	Yield (%)
33	CH_2OH	64
34	CH_2CH_2OH	56
37	$CH_2N(CH_3)_2$	70
39	$CO_2CH_2CH_3$	66

Scheme 13

The synthesis of the 5'-hydroxymethyl-4,8-dimethylpsoralen provided further evidence to confirm the structure of the 5'-bromomethyl-4,8-dimethylpsoralen synthesized by Akyea. Akyea reported the synthesis of the 5'-hydroxymethyl-4,8-dimethylpsoralen from the bromomethyl

compound.[26] The two hydroxymethyl compounds had identical physical and spectroscopic properties which confirms again that the initial structural assignments were correct.

The reported trioxsalen derivatives were treated further to yield four additional compounds. Compound **34** was treated with phosphorous tribromide to yield 4,8-dimethyl-5-(2-bromoethyl)psoralen (**35**). This product was subsequently treated with pyridine to form the corresponding quaternary compound (**36**). This series of reactions is diagramed in Scheme 14.

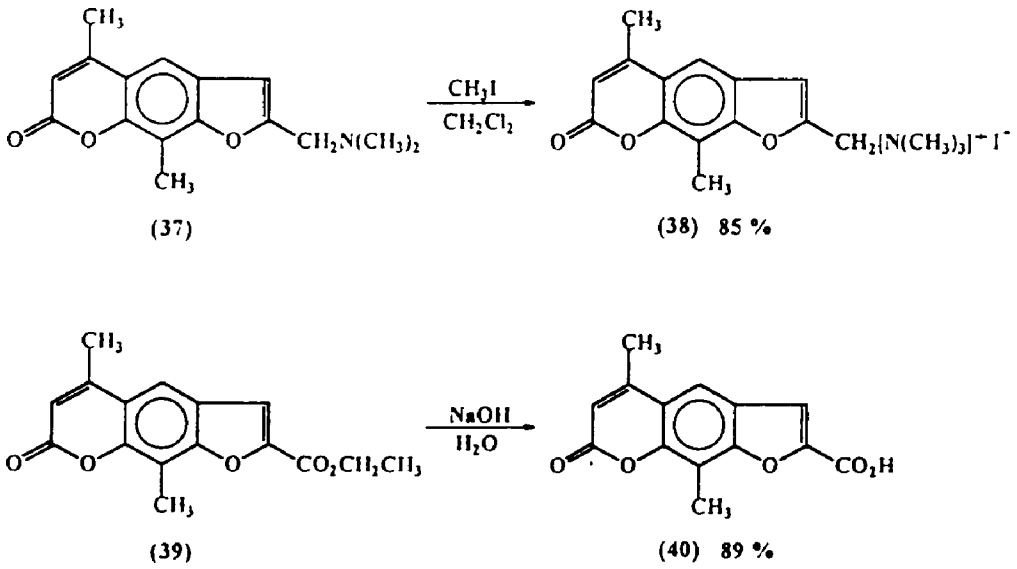


(34) (35) 33 % (36) 71 %

Scheme 14

The 4,8-dimethyl-5'-(N,N-dimethylaminomethyl)psoralen was converted to the corresponding quaternary compound (**38**) by

reaction with methyl iodide. The 5'-ethoxycarbonyl-4,8-dimethylpsoralen (**39**) was saponified to the corresponding acid (**40**) by reaction with aqueous potassium hydroxide followed by acidification. The two reactions are shown in Scheme 15.



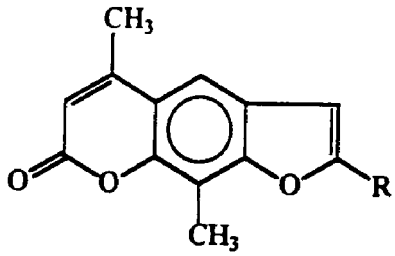
Scheme 15

BIOLOGICAL ACTIVITY OF THE TRIOXSALEN DERIVATIVES

The IC_{50} values of the trioxsalen derivatives are shown in Table 9. The molar absorptivities of selected compounds synthesized are shown in Table 10.

Table 9

IC_{50} values for trioxsalen derivatives



Compound	R	IC_{50} (μM)
34	CH_2CH_2OH	27
35	CH_2CH_2Br	22
37	$CH_2N(CH_3)_2$	26
39	$CO_2CH_2CH_3$	35

Table 10

Compound	λ	Molar Absorptivity (10^4)
4,5',8-trimethyl psoralen (trioxsalen)	333	6.77
4,8-dimethyl-5'-hydroxymethylpsoralen (**33**)	331	9.40
4,8-dimethyl-5'-(2-hydroxyethyl) psoralen (**34**)	333	5.98
4,8-dimethyl-5'-(dimethylamino)methyl psoralen (**37**)	331	9.41
4,8-dimethyl-5'-(trimethylamino)methyl psoralen iodide (**38**)	327	8.56
5'-ethoxycarbonyl-4,8-dimethylpsoralen (**39**)	333	17.7

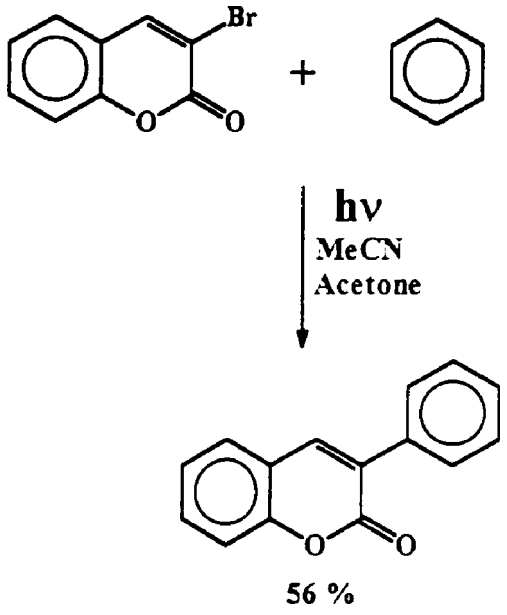
Conclusions

Our biological activity measurements in the C-5' class indicate that the substitution at the C-5' position does not affect the ability of psoralen to bind to its receptor. The measured activity of the trioxsalen derivatives synthesized herein does not seem to be influenced by varying substitution at the C-5' position. Aside from their potential as photosensitizers, some of these compounds (**33**, **34**, **35**, **40**) provide additional compounds which could be used in situations where psoralens need to be tethered to various substrates.

7-Alkoxy-3-bromo-8-methylcoumarins

Recently, a report by Meng and coworkers described the efficient synthesis of 3-arylcoumarins by the photochemically promoted reaction between 3-bromocoumarin and a variety of aromatic compounds.[56] This discovery has potential application in the design of new coumarin-like phototherapeutics. As noted earlier herein, the photoanchoring of psoralens to their protein receptor appears to involve an aryl-to-aryl coupling process. Marko's results, in fact, imply that this coupling involves the pyrone ring of the psoralen with an aryl ring of the protein (probably a tyrosine).[26] Also as noted before herein, Akyea has shown that with the model "protein" [ethyl N-acetyltyrosine] such 1:1 photo adducts with 8-methoxypsoralen are readily generated.[26] Having available a series of 3-bromocoumarins - for which photocoupling to aromatic residues is already established *in vitro* - might generate a promising set of candidate phototherapeutics.

An example of the synthesis of a 3-arylcoumarin utilizing this reaction is shown in in Figure 12.



56 %

Figure 12

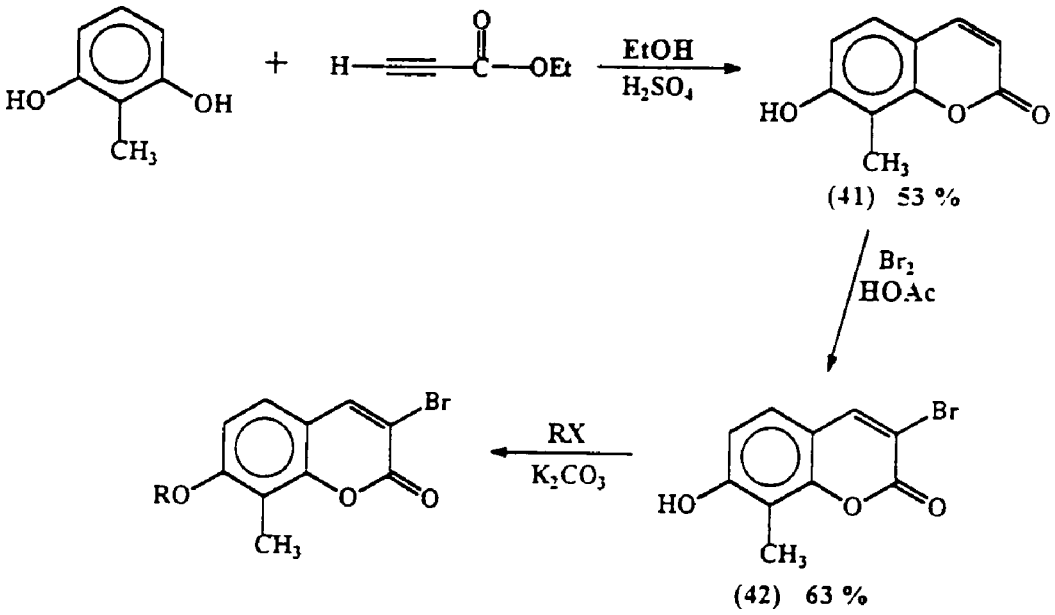
Based on the reported photocoupling reaction, we synthesized a variety of 3-bromocoumarins as potential photosensitizers. As previously mentioned, the proposed mode of EGF binding inhibition involves the photocoupling of a psoralen to an aromatic amino acid, especially tyrosine. The 7-alkoxy-3-bromo-8-methylcoumarins should bind efficiently to one of the aromatic amino acids in the psoralen binding site since compounds of similar structure are known to photocouple to aromatic substrates.

The starting material for the synthesis of these analogs was 7-hydroxy-8-methylcoumarin (**41**). This was synthesized by a method reported by Kauffman which combines 2-methyl-resorcinol with ethyl propiolate in an ethanol/sulfuric acid solvent system (5.7/1) to yield the desired compound (**41**).[57]

This reaction is one of the few known that produces unsubstituted coumarins in high yields.

Compound **41** was selectively brominated using a procedure based on that recently reported by Reish.[58] One equivalent of bromine was added to a dilute solution of **41** in acetic acid to yield 3-bromo-7-hydroxy-8-methylcoumarin (**42**) in 63% yield. The selectivity of this reaction derives from protonation of the phenolic hydroxyl group which deactivates the aromatic ring with respect to the coumarin ring and thus bromination first occurs on the coumarin ring. The amount of bromine still must be carefully controlled to avoid multiple bromination products.

A series of five 7-alkoxy derivatives was synthesized by the Williamson ether synthesis previously mentioned. The alkoxy groups chosen offer a wide range of lipophilicities for this series of compounds. The entire series of compounds is shown in Scheme 16.



Compound	R	Yield (%)
43	CH_3	80
44	$CH_2CH_2CH_3$	70
45	$CH_2CH{=}CH_2$	83
46	$CH_2C{\equiv}CH$	79
47	$CH_2CH{=}C(CH_3)_2$	83

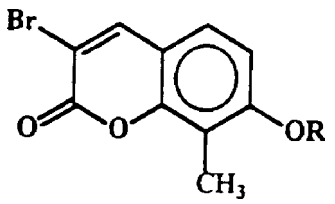
Scheme 16

Biological Activity of the 7-Alkoxy-3-bromo-8-methylcoumarins

The 7-alkoxy-3-bromo-8-methylcoumarin derivatives are among the most active agents tested in the EGF binding assay. The complete list of compounds along with their biological activity are shown in Table 11. The molar absorptivities and the lipophilicities of the various compounds are shown in Tables 12 and 13 respectively.

Table 11

IC_{50} values for 7-alkoxy-3-bromo-8-methylcoumarins



Compound	R	$IC_{50}(\mu M)$
42	H	----
43	CH_3	2.6
44	$CH_2CH_2CH_3$	2.4
45	$CH_2CH{=}CH_2$	3.4
46	$CH_2C{\equiv}CH$	17
47	$CH_2CH{=}C(CH_3)_2$	2.2

Table 12

Compound	λ	Molar Absorptivity (10^4)
3-bromo-7-hydroxy-8-methylcoumarin (**42**)	332	1.76
3-bromo-7-methoxy-8-methylcoumarin (**43**)	332	1.92
3-bromo-8-methyl 7-propoxycoumarin (**44**)	332	1.86
7-allyloxy-3-bromo 8-methylcoumarin (**45**)	332	1.92
3-bromo-8-methyl 7-propargyloxycoumarin (**46**)	330	1.87
3-bromo-8-methyl-7-[(3-methyl-2-butene-1-yl)oxy] coumarin (**47**)	332	1.94

Table 13

Compound	Log P	Log $1/IC_{50}$
3-bromo-7-hydroxy-8-methylcoumarin (**42**)	1.81	------
3-bromo-8-methyl 7-propargyloxycoumarin (**46**)	2.51	-1.23
3-bromo-7-methoxy-8-methylcoumarin (**43**)	2.59	-0.415
7-allyloxy-3-bromo 8-methylcoumarin (**45**)	3.22	-0.531
3-bromo-8-methyl 7-propoxycoumarin (**44**)	3.62	-0.380
3-bromo-8-methyl-7-[(3-methyl-2-butene-1-yl)oxy] coumarin (**47**)	4.06	-0.342

Conclusions

The substitution of a bromine on the C-3 position of the coumarin increases the biological activity of these compounds with respect to their non-brominated counterparts. It is concluded that this substitution increases the photoreactivity of these compounds thus providing for a more efficient coupling to their target receptor. The lipophilicity of these compounds is not critical since no relationship exists between lipophilicity and the ability to inhibit the binding of EGF to its receptor for these compounds. This series provides a promising new class of photosensitizers which can be explored further by future investigators.

Iodinated Psoralens and Coumarins

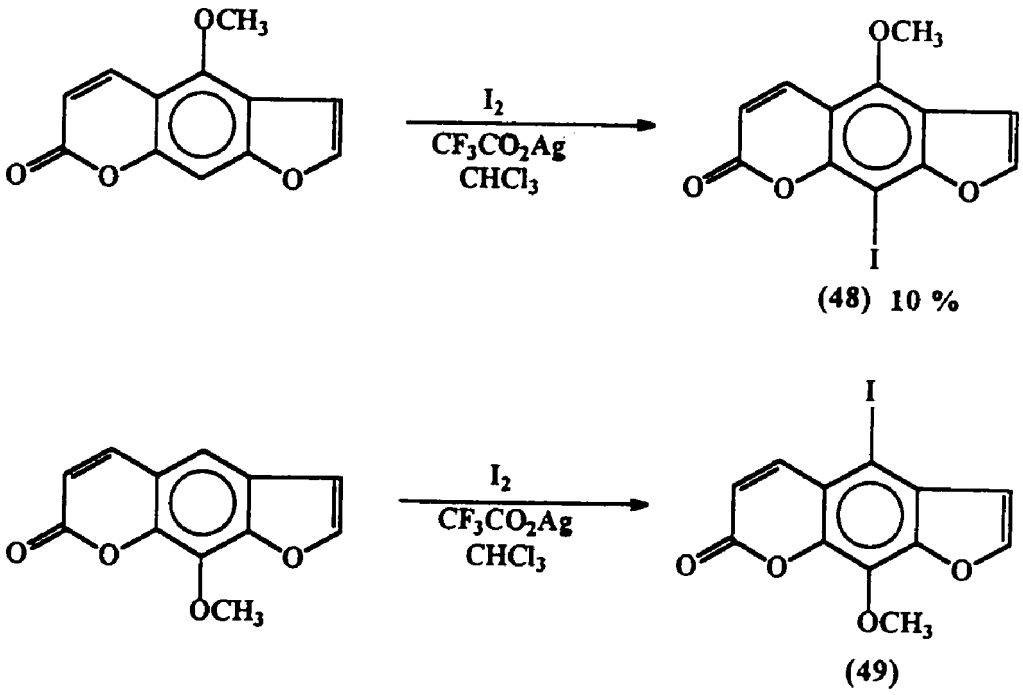
The study of the interaction and localization of psoralens within cells and organs is a continuing interest. Radiochemical techniques are a common tool used in such studies. Currently, the studies of psoralen distribution in cells are performed with tritiated psoralens. It was desired to produce a psoralen or coumarin with a higher radiochemical specific activity to make biotransport events easier to study, because the tritiated psoralens gave high background readings and made such studies difficult. The focus of this part of the project was to synthesize iodinated psoralen and coumarin derivatives as target compounds for an ^{125}I derivative to be used in cell localization studies.

In addition, the developing field of *in situ* radiotherapy of cancer is being advanced by use of ^{125}I-labeled materials with high affinity for specific types of malignant tissue. The possibility exists that a radioiodinated coumarin/psoralen may display sufficiently specific uptake in an epidermal cancer to serve as an *in situ* radiotherapeutic.

Any targets for a radiolabeled derivative must first be synthesized and tested for activity since an agent must show activity to warrant the subsequent synthesis of the radio-labeled analog. The synthesis of the radiolabeled analog was not a part of this project although preparation of these compounds has strong literature precedent. The corresponding tri-alkyl tin derivatives of the respective iodo compounds

could then be synthesized and subsequently radioiodinated.[59]

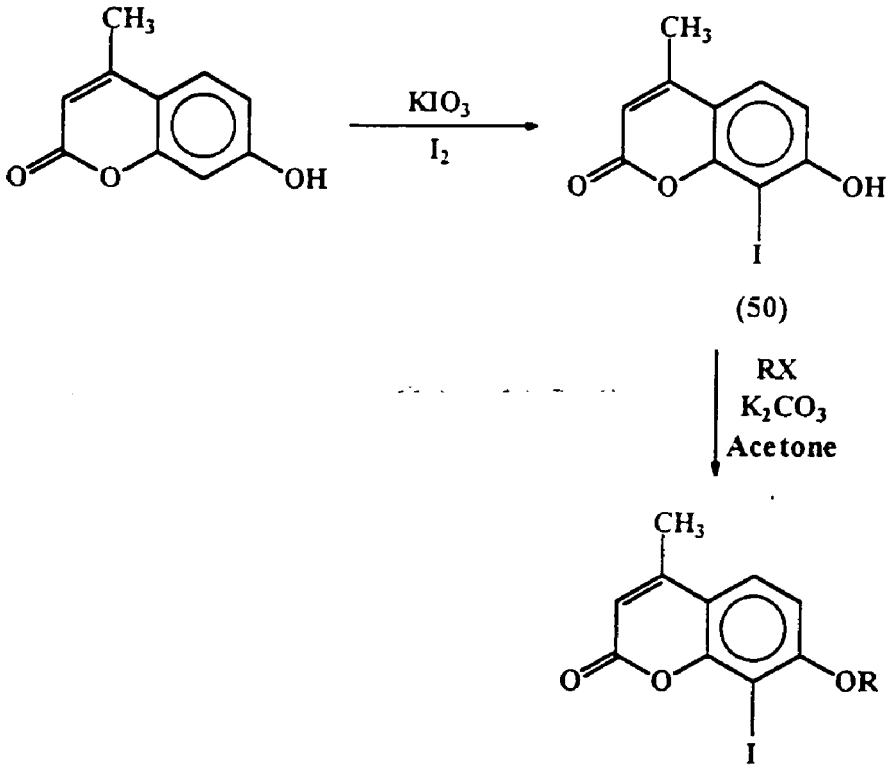
The initial candidate was an iodinated 5-methoxypsoralen derivative. The 5-methoxypsoralen was iodinated in an iodine/silver trifluoroacetate system, common for compounds resistant to iodination, and using chloroform as the solvent to yield 8-iodo-5-methoxypsoralen (**48**) in 10% yield (Scheme 17). This derivative is a structural isomer of the 5-iodo-8-methoxypsoralen (**49**) synthesized by Mack using the same system.[60]



Scheme 17

Two 7-alkoxy-8-iodo-4-methylcoumarin derivatives were synthesized by alkylation of 7-hydroxy-8-iodo-4-methyl coumarin (Scheme 18). This iodocoumarin was obtained from a

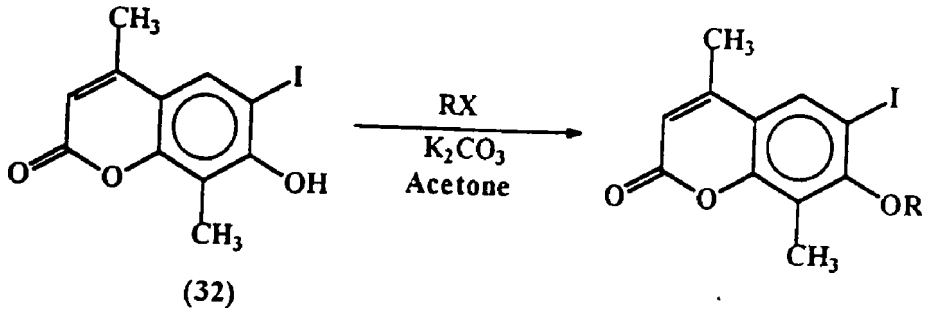
selective iodination of 7-hydroxy-4-methylcoumarin.[42] We were encouraged to synthesize this series of iodinated coumarins since Jetter reported that 8-iodo-4-methyl-7-(3-methyl-2-buten-1-yl)oxycoumarin was highly active in the EGF assay.[42]



Compound	R =	Yield (%)
51	CH_3	44
52	$CH_2CH_2CH_3$	49
53	$CH_2CH{=}C(CH_3)_2$	55

Scheme 18

Three 7-alkoxy-6-iodo-4,8-dimethylcoumarins were synthesized by alkylation of the 7-hydroxy-6-iodo-4,8-dimethylcoumarin which was obtained by the previously mentioned method (Scheme 19). This series of derivatives was synthesized since many of the previous studies on the inhibition of EGF binding were performed on 7-alkoxy-4,8-dimethylcoumarins. Thus, an active radioiodinated derivative of this type could be very useful in cell binding studies.



(32)

Compound	R =	Yield (%)
54	CH_3	61
55	$CH_2CH_2CH_3$	31
56	$CH_2CH{=}C(CH_3)_2$	76

Scheme 19

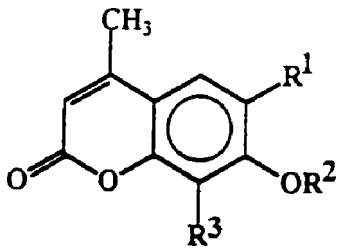
Biological Activity of the

Iodinated Psoralens and Coumarins

The biological activities of the iodinated coumarins are shown in Table 14. The 8-iodo-5-methoxypsoralen did not show activity at the highest concentration (92 μM) tested.

Table 14

IC_{50} values for iodinated coumarins



Compound	R^1	R^2	R^3	$IC_{50}(\mu M)$
50	H	H	I	----
51	H	CH_3	I	> 95
52	H	$CH_2CH_2CH_3$	I	> 87
53	H	$CH_2CH{=}C(CH_3)_2$	I	1.5
32	I	H	CH_3	> 95
54	I	CH_3	CH_3	> 91
55	I	$CH_2CH_2CH_3$	CH_3	> 84
56	I	$CH_2CH{=}C(CH_3)_2$	CH_3	78

Conclusions

It is concluded that the iodinated compounds synthesized in this work are not suitable targets for radiolabeling. The most critical property of a radiolabeled probe is that it must have similar behavior to the compound of interest. The compounds synthesized did not have the ability to effectively inhibit the binding of EGF to its receptor thus making them poor targets for radiolabeling.

Summary of Conclusions

As the reader has noted, each of the four individual sections of this thesis has had an independent conclusion. In faithfulness to the traditional organization of doctoral dissertations, an overall assessment of accomplishment is required. Based on the information presented, the following conclusions can be made:

1. The addition of the CF_3 functionality does not enhance biological activity.
2. The new modification of the Castro reaction provides for a one step synthesis of 5' substituted trioxsalen derivatives from a common intermediate.
3. The substitution of a bromine on the C-3 position of a coumarin enhances the ability of the coumarin to inhibit the binding of EGF to its receptor.
4. The relationship between biological activity and lipophilicity does not apply to all sets of coumarins.

Experimental

General

Chemicals and solvents were obtained from commercial sources and used without further purification. Infrared spectra were obtained on a Perkin-Elmer Model 283 infrared spectrophotometer as 2.5-5% KBr pellets. Ultraviolet absorption spectra were recorded on a Perkin-Elmer Lambda 5 UV/VIS spectrophotometer using quartz cuvettes with a path length of 1 cm. HPLC analyses were performed on a Waters HPLC equipped with binary pump model LC 501, 486 tunable absorbance detector, and a C-18 3.9 x 15 mM column with a 4 μM particle size. Melting points were determined on a Melt-Temp apparatus and are reported uncorrected. Elemental analyses were performed by Quantitative Technologies, Inc., White House, New Jersey.

^{1}H-NMR were recorded on Bruker 500 or 360 MHz or JOEL FX-90Q 90 MHz NMR using deuterated solvents. Chemical shifts are reported in parts per million downfield from tetramethylsilane (TMS). Peak assignments are given in the following order: (multiplicity, number of protons, coupling constants in Hz, assignment). Multiplicity is designated as follows: s= singlet, d= doublet, t= triplet, q= quartet, m= multiplet, c= complex, b= broad. The decoupled ^{13}C NMR, HETCOR, APT and COSY spectra were recorded on a Brucker 360 MHz NMR. The deuterated solvent, DMSO-d_6 or $CDCl_3$, was used as the internal reference for these spectra.

4-Trifluoromethyl-7-hydroxy-8-methylcoumarin (1)

a) A slurry of 2-methylresorcinol (12.4 g, 100 mmole) and ethyl 4,4,4-trifluoroacetoacetate (18.4 g, 100 mmole) was added slowly to 100 mL of ice cold sulfuric acid with vigorous stirring. After completion of addition, the solution was allowed to warm to room temperature and was stirred for 24 hours. The reaction mixture was then slowly added to 500 mL of ice water (300 g ice and 200 mL H_2O) with vigorous stirring. The desired product precipitated from the solution as a light pink solid. The solid was then isolated by vacuum filtration, transferred to a 500 mL beaker, 200 mL of water was added to achieve a slurry and the pH was adjusted to 6 with sodium bicarbonate. The solid was then suction filtered and recrystallized from ethanol to yield 46-80% of **1**.

b) To a solution of 2-methylresorcinol (3.1 g, 25 mmole) and ethyl 4,4,4-trifluoroacetoacetate (4.6 g, 25 mmole) was added 6.3 mL of trifluoroacetic acid. The reaction was refluxed for 30 minutes and then cooled to room temperature. The reaction mixture was then poured into 75 mL of ice water and suction filtered to yield the crude product which ranged in color from pink to purple. The crude product could be recrystallized from ethanol to yield 4.3 g (73 %) of **1** as white crystals. ^1H NMR (D_3COD): δ 2.2 (s, 3H, CH_3), 6.5 (s, 1H, C3 -H), 6.7 (d, 1H, J = 8 Hz, C6 -H), 7.35 (d, 1H, J = 8 Hz, C5 -H). IR (KBr) 3414 cm^{-1} (OH), 1713 cm^{-1} (C=O). The melting point of the product is 197-198 °C.

Elemental analysis:

Anal. calcd. for $C_{11}H_7F_3O_3$: C 54.10 H 2.90 F 23.40

Found: C 54.01 H 2.83 F 23.73

4-Trifluoromethyl-7-hydroxycoumarin (2)

A slurry of resorcinol (11.0 g, 100 mmole) and ethyl 4,4,4-trifluoroacetoacetate (18.4 g, 100 mmole) was added slowly to 100 mL of ice cold sulfuric acid while vigorously stirring. After completion of addition, the solution was allowed to warm to room temperature and was stirred for 24 hours. The reaction mixture was then slowly added to 500 mL of ice water (300 g ice and 200 mL H_20) with vigorous stirring. The product precipitated from the solution as a light pink solid. The solid was isolated by vacuum filtration, transferred to a 500 mL beaker, slurried with 200 mL of water and the pH was adjusted to 6 with sodium bicarbonate. The isolated solid was recrystallized from an ethanol/water mixture to yield 9.56 g (42%) of pink crystals, mp 183-85 °C, 187 °C (lit).[61] ^{1}H NMR (D_3COD): δ 6.58 (s, 1H, C3 -H), 6.74 - 6.91 (c, 2H, C6 + C8 -H), 7.64 (b d, 1H, C5 -H).

4-Trifluoromethyl-7-methoxy-8-methylcoumarin (3)

To a solution of **1** (0.50 g, 2.0 mmole) in 10 mL of dry acetone was added 2.5 g of K_2CO_3. Methyl iodide (0.62 mL, 1.4 g, 10 mmole) was added to the solution and stirred at room temperature for 4 hours. The solution was filtered and

evaporated to dryness. The solid was then dissolved in 50 mL of chloroform and washed with 3 x 25 mL of water. The chloroform was evaporated under reduced pressure and the solid was recrystallized from ethanol yielding a fluffy white solid, 0.50 g (96%), m.p. 109.5-111°C. ^{1}H NMR ($CDCl_3$): δ 2.28 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 6.59 (s, 1H, C3 -H), 6.89 (d, 1H, J = 8.3 Hz, C6 -H), 7.60 (d, 1H, J = 8.3 Hz, C5 -H). IR (KBr): 1738 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{12}H_9F_3O_3$: C 55.81 H 3.49 F 22.09

Found: C 55.70 H 3.41 F 22.09

4-Trifluoromethyl-7-methoxycoumarin (4)

To a solution of **2** (0.46 g, 2.0 mmole) in 10 mL of dry acetone was added 2.5 g of K_2CO_3. Methyl iodide (0.62 mL, 1.4 g, 10 mmole) was added to the solution and stirred at room temperature for 5 hours. The solution was filtered and evaporated to dryness. The solid was then dissolved in 50 mL of chloroform and washed with 3 x 25 mL of water. The chloroform was evaporated under reduced pressure to yield 0.40 g (81%), of white crystals, m.p. 112.5-113.5 °C. ^{1}H NMR ($CDCl_3$): δ 3.89 (s, 3H, CH_3), 6.62 (s, 1H, C3 -H), 6.87-6.98 (c, 2H, C8 + C6 -H), 7.64 (d, 1H, C5 -H). IR (KBr): 1734 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{11}H_7F_3O_3$: C 54.10 H 2.87 F 23.36

Found: C 54.02 H 2.70 F 23.33

4-Trifluoromethyl-8-methyl-7-propoxycoumarin (5)

To a solution of **1** (0.50 g, 2.0 mmole) in 10 mL of dry acetone was added 2.5 g of K_2CO_3. Bromopropane (1.2 g, 10 mmole) was added to the solution and the reaction was refluxed for 6 hours. The solution was filtered and evaporated to dryness. The solid was then dissolved in 50 mL of chloroform and washed with 3 x 50 mL of water. The chloroform was evaporated and the solid was recrystallized from ethanol yielding light tan crystals, 0.47 g (80%), mp 123.5-124.5 °C ^1H NMR ($CDCl_3$): δ 1.1 (t, 3H, J = 7.33 Hz, CH_3), 1.90 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 4.06 (t, 2H, J = 6.59 Hz, OCH_2), 6.60 (s, 1H, C3 -H), 6.88 (d, 1H, J = 9.28 Hz, C6 -H), 7.55 (d, 1H, J = 9.28 Hz, C5 -H). IR (KBr): 1733 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{14}H_{13}F_3O_3$: C 58.74 H 4.55 F 19.93

Found: C 58.35 H 4.42 F 19.92

4-Trifluoromethyl-7-propoxycoumarin (6)

To a solution of **2** (0.46 g, 2.0 mmole) in 10 mL of dry acetone was added 2.5 g of K_2CO_3. Bromopropane (1.2 g, 10 mmole) was added to the solution and the reaction was refluxed for 6 hours. The solution was filtered and evaporated to dryness. The solid was then dissolved in 50 mL of chloroform and washed with 3 x 50 mL of water. The solvent was removed under reduced pressure and the resulting solid was recrystallized from ethanol to yield 0.54 g (77%) of white

crystals, m.p. 97-98 °C. ^{1}H NMR ($CDCl_3$): δ 1.07 (t, 3H, J = 7.3 Hz, CH_3), 1.88 (m, 2H, J = 7.3 Hz, CH_2), 4.02 (t, 2H, J = 6.6 Hz, CH_2), 6.62 (s, 1H, C3 -H), 6.87-6.99 (c, 2H, C6 + C8 -H), 7.63 (d, 1H, C5 -H). IR (KBr): 1730 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{13}H_{11}F_3O_3$: C 57.35 H 4.04 F 20.96

Found: C 57.25 H 3.90 F 20.95

7-Allyloxy-4-trifluoromethyl-8-methylcoumarin (7)

To a solution of **1** (5.00 g, 20.0 mmole) in 100 mL of dry acetone was added 25 g of K_2CO_3. Allyl bromide (12.1 g, 100 mmole) was added to the solution and refluxed for 1.5 hours. The reaction was then cooled, filtered and evaporated to dryness. The solid was taken up in 200 mL of chloroform and washed with 3 x 50 mL of water. The chloroform was removed under reduced pressure and the solid was recrystallized from ethanol to yield **7** as white needles: 5.40 g (92%), m.p. 114.5-115.5°C. ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 3H, CH_3), 4.70 (d, 2H, $-OCH_2$, J = 4.88 Hz), 5.30 - 5.56 (c, 2H, $=CH_2$), 5.90-6.37 (m, 1H, -CH=), 6.63 (s, 1H, C3 -H), 6.90 (d, 1H, C6 -H, J = 8.8 Hz), 7.55 (d, 1H, C5 -H, J = 8.8 Hz). IR (KBr): 1734 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{14}H_{11}F_3O_3$: C 59.15 H 3.87 F 20.07

Found: C 58.92 H 3.72 F 20.11

7-Allyloxy-4-trifluoromethylcoumarin (8)

To a solution of **2** (0.46 g, 2.0 mmole) in 100 mL of dry acetone was added 2.5 g of K_2CO_3. Allyl bromide (1.2 g, 10 mmole) was added to the solution and refluxed for 1.5 hours. The reaction was then cooled, filtered and evaporated to dryness. The solid was taken up in 200 mL of chloroform and washed with 3 x 50 mL of water. The chloroform was removed under reduced pressure and the residue was recrystallized from ethanol to yield 0.41 g (76%) of white crystals, m.p. 69-71°C. ^1H NMR ($CDCl_3$): δ 4.64 (d, 2H, J = 4.9 Hz, $-OCH_2$), 5.30 - 5.55 (c, 2H, $=CH_2$), 5.87-6.35 (m, 1H, -CH=), 6.63 (s, 1H, C3 - H), 6.89-7.01 (c, 2H, C6 + C8 -H), 7.63 (d, 1H, C5 -H). IR (KBr): 1729 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{13}H_9F_3O_3$: C 57.78 H 3.33 F 21.11

Found: C 57.44 H 3.22 F 21.02

4-Trifluoromethyl-8-methyl-7-propargyloxycoumarin (9)

To a solution of **1** (0.50 g, 2.0 mmole) in 10 mL of dry acetone was added 2.5 g of K_2CO_3 and 50 mg of potassium iodide. Propargyl chloride (0.74 g, 0.72 mL, 10 mmole), was added and the reaction was refluxed for 4 hours. The reaction was cooled, filtered and evaporated to dryness. The solid was dissolved in 50 mL of chloroform and washed with 3 x 25 mL of water. The chloroform layer was evaporated to dryness and the

solid was recrystallized from ethanol to yield light tan crystals: 0.45 g (80%), m.p. 160-161.5. ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 2.60 (b s, 1H, $\equiv$CH), 4.85 (d, 2H, J = 2.0 Hz, CH_2), 6.69 (s, 1H, C3 -H), 7.04 (d, 1H, J = 8.79 Hz, C6 -H), 7.60 (d, 1H, J = 8.79 Hz, C5). IR (KBr): 1724 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{14}H_9F_3O_3$: C 59.57 H 3.19 F 20.21

Found: C 59.33 H 3.07 F 19.99

4-Trifluoromethyl-7-propargyloxycoumarin (10)

To a solution of **2** (0.46 g, 2.0 mole) in 10 mL of dry acetone was added 2.5 g of K_2CO_3 and 50 mg of potassium iodide. Propargyl chloride (0.75 g, 0.72 mL, 10 mmole), was added and the reaction was refluxed for 4 hours. The reaction was cooled, filtered and evaporated to dryness. The solid was dissolved in 50 mL of chloroform and washed with 3 x 25 mL of water. The chloroform layer was evaporated to dryness and the solid was recrystallized from ethanol to yield light tan crystals: 0.40 g (75%), m.p. 110.5-112.0°C . ^{1}H NMR ($CDCl_3$): δ 2.60 (t, 1H, J = 2.5 Hz, $\equiv$C-H), 4.79 (d, 2H, J = 2.5 Hz, -OCH_2-), 6.65 (s, 1H, C3 -H), 6.92-7.06 (c, 2H, C6 + C8 -H), 7.66 (b d, 1H, C5 -H). IR (KBr): 1744 cm^{-1}

Elemental analysis:

Anal. calcd. for $C_{13}H_7F_3O_3$: C 58.21 H 2.61 F 21.27

Found: C 57.82 H 2.48 F 21.05

4-Trifluoromethyl-8-methyl-7-[(3-methyl-2-buten-1-yl)oxy]coumarin (11)

To a solution of **1** (0.50 g, 2.0 mmole) in 10 mL of dry acetone was added 2.5 g of K_2CO_3. 4-bromo-2-methyl-2-butene (1.5 g, 10 mmole) was added and the solution was stirred for 6 hours. The reaction was filtered and evaporated to dryness. The solid was dissolved in 50 mL of chloroform and washed with 3 x 25 mL of water. The chloroform was evaporated to dryness and the solid was recrystallized from ethanol to yield white crystals: 0.55 g (86%), m.p. 112-113 °C ^{1}H NMR ($CDCl_3$): δ 1.79 (s, 3H, CH_3), 1.83 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 4.68 (d, 2H, J = 6.84, OCH_2), 5.5 (b t, 1H, -CH=), 6.62 (s, 1H, C3 -H), 6.91 (d, 1H, J = 9.04 Hz, C6 -H), 7.55 (d, 1H, J = 9.04 Hz, C5 -H). IR (KBr): 1736 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{16}H_{15}F_3O_3$: C 61.54 H 4.81 F 18.27

Found: C 61.37 H 4.74 F 18.48

4-Trifluoromethyl-7-[(3-methyl-2-buten-1-yl)oxy]coumarin(12)

To a solution of **2** (0.46 g, 2.0 mmole) in 10 mL of dry acetone was added 2.5 g of K_2CO_3. 4-bromo-2-methyl-2-butene (1.5 g, 10 mmole) was added and the solution was stirred for 6 hours. The reaction was filtered and evaporated to dryness. The solid was dissolved in 50 mL of chloroform and washed with 3 x 25 mL of water. The chloroform was evaporated to dryness and the solid was recrystallized from ethanol to yield white

crystals: 0.40 g (67%), m.p. 76-77 °C ^{1}H NMR ($CDCl_3$): δ 1.79 (s, 3H, CH_3), 1.83 (s, 3H, CH_3), 4.68 (d, 2H, J = 6.35 Hz, OCH_2), 5.49 (b t, 1H, =CH), 6.62 (s, 1H, C3 -H), 6.89-7.00 (c, 2H, C6 + C8 -H), 7.63 (b d, 1H, C5 -H). IR (KBr): 1737 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{15}H_{13}F_3O_3$: C 60.40 H 4.36 F 19.13

Found: C 60.16 H 4.21 F 19.27

Synthesis of trifluoromethylpsoralens

7-Acetonyloxy-4-trifluoromethyl-8-methyl-coumarin (13)

To a solution of 10 g of potassium carbonate in 20 mL of dry acetone was added 1.0 g of **1** (4.0 mmoles). To this reaction mixture was then added 1.0 mL of chloroacetone (13 mmoles) and 0.10 g of potassium iodide. The reaction was refluxed for 4 hours and then evaporated to dryness under reduced pressure. The crude product was taken up in chloroform and washed with 3 x 50 mL of water. The solution was evaporated to dryness and recrystallized from toluene to yield 1.2 g of white crystals (98%): mp. 143-144.5 °C. ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 4.71 (s, 2H, OCH_2), 6.72 (d, 2H, J = 9.1 Hz, C5 and overlapping C3), 7.60 (d, 1H, J = 9.1 Hz, C6). IR (KBr): 1743 cm^{-1} (O-C=O), 1716 cm^{-1} (C=O).

Elemental analysis:

Anal. calcd. for $C_{14}H_{11}F_3O_3$: C 56.00 H 3.67 F 19.00

Found: C 56.00 H 3.62 F 19.00

7-Acetonyloxy-4-trifluoromethylcoumarin (14)

To a solution of 5.0 g of potassium carbonate in 20 mL of dry acetone was added 0.92 g of **2** (4.0 mmoles). To this reaction mixture was then added 1.6 mL of chloroacetone (20 mmoles) and 0.10 g of potassium iodide. The reaction was refluxed for 4 hours and then evaporated to dryness under

reduced pressure. The crude product was taken up in chloroform and washed with 3 x 50 mL of water. The solution was evaporated to dryness to yield 1.2 g (100%) of the desired product m.p. 158-159°C. ^{1}H NMR ($CDCl_3$): δ 2.33 (s, 3H, CH_3), 4.69 (s, 2H, OCH_2), 6.67 (s, 1H, C3 -H), 6.80 (d, 1H, 2.4 Hz, C8 -H), 7.05 (d d, 1H, J = 8.8, 2.4 Hz, C6 -H), 7.70 (d, 1H, J = 8.8 Hz, C5 -H). IR (KBr): 1737 cm^{-1} (O-C=O), 1725 cm^{-1} (C=O).

Elemental analysis:

Anal. calcd. for $C_{13}H_9F_3O_4$: C 54.55 H 3.15 F 19.93

Found: C 54.43 H 3.09 F 19.75

4-Trifluoromethyl-4',8-dimethylpsoralen (15)

A heterogeneous mixture of 3.0 g **13** (10 mmole), 1.4 g of potassium hydroxide, and 170 mL of water was refluxed for 4 hours. The resulting homogeneous solution was cooled to 0°C degrees and acidified with 1 N HCl , accompanied by vigorous stirring, to precipitate product. The product was separated by filtration, dried, and recrystallized from ethanol to yield 1.6 g (57%) of the desired product, mp. 185-186 °C. ^{1}H NMR (CD_3COCD_3): δ 2.30 (s, 3H, C-8 CH_3), 2.55 (s, 3H, C-4' CH_3), 6.87 (s, 1H, C3 -H), 7.80 (c, 2H, overlapping C5 + C5' -H). IR (KBr): 1735 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{14}H_9F_3O_3$: C 59.57 H 3.19 F 20.21

Found: C 59.36 H 3.05 F 19.93

4-Trifluoromethyl-4'-methylpsoralen (16)

A heterogeneous mixture of 0.50 g **14** (1.9 mmole), 0.25 g of potassium hydroxide, and 170 mL of water was refluxed for 4 hours. The resulting homogeneous solution was cooled to 0°C and acidified with 1 N HCl to precipitate product. The solid was isolated by filtration, dried, and recrystallized from ethanol to yield 0.22 g (46%) of **16** as a yellow solid, m.p. 170-173°C. ^{1}H NMR ($CDCl_3$): δ 2.24 (s, 3H, CH_3), 6.80 (s, 1H, C3 -H), 7.50 (s, 1H, aromatic -H), 7.54 (s, 1H, aromatic -H), 7.83 (s, 1H, C5' -H). IR (KBr): 1738 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{13}H_7F_3O_3$: C 58.21 H 2.61 F 21.27

Found: C 58.05 H 2.47 F 20.99

4-Trifluoromethyl-5',8-dimethylpsoralen (17)

a) A mixture of **21** (5.0 g, 16 mmole) and N,N-diethylaniline (50 mL) was refluxed for 24 hours under argon while stirring. The solution was cooled to room temperature and then dissolved in 200 mL of chloroform. The chloroform was extracted with 5 x 50 mL portions of 5% HCl and 2 x 50 protions of water. The chloroform layer was then extracted with 25 mL portions of 5% NaOH until the base washings were colorless. The sodium hydroxide solution was slowly acidified with concentrated HCl to precipitate the phenolic product. The solid was digested for approximatley 1 hour, filtered and washed with water. The solid was dried under reduced pressure

to yield 2.1 grams (42%) of 6-(2-Chloroallyl)-4-trifluoromethyl-7-hydroxy-8-methylcoumarin (**22**), m.p. 182-83 °C. The chloroform layer was evaporated under reduced pressure and the residue was recrystallized from an ethanol-water system. The resulting crystals were filtered and washed with cold ethanol to yield 0.86 g of **17**. The filtrate was evaporated to dryness and purified by flash chromatography ($CHCl_3$) to yield 0.42 grams of **17**. The total yield of desired product from this reaction was 1.3 g (29%). The phenolic product could be recrystallized from ethanol to yield an off white solid m.p. 182-183°C. ^1H NMR of **22** (CD_3OD): 2.33 (s, 3H, CH_3), 3.76 (s, 2H, CH_2), 5.19 (d, 1H, J = 1.5 Hz, =CH), 5.28 (d, 1H, J = 1.5 Hz, =CH), 6.67 (s, 1H, C3 -H), 7.45 (s, 1H, C5 -H). IR (KBr): 1710 cm^{-1}. ^1H NMR of **17** (CD_3COCD_3): δ 2.55 (s, 6H, 2 x CH_3), 6.67 (s, 1H, C3 -H), 6.80 (s, 1H, C4' -H), 7.70 (s, 1H, C5 -H). IR (KBr): 1736 cm^{-1}. The melting point of the product is 161-162.5 °C.

Elemental analysis:

Anal. calcd. for $C_{14}H_9F_3O_3$ (**17**): C 59.57 H 3.19 F 20.21

Found: C 59.47 H 3.15 F 20.04

Elemental Analysis

Anal. Calcd. for $C_{14}H_{10}F_3ClO_3$ (**22**): C 52.75 H 3.14 F 17.90

Cl 11.15

Found: C 52.38 H 3.10 F 17.51

Cl 11.31

b) The crude **22** (1.0 g, 3.0 mmoles) from procedure A was

added to 50 mL of 70% sulfuric acid and stirred vigorously at room temperature for 1 hour. The solution was slowly added to 250 mL of cold water in a 500 mL separatory funnel and the flask was rinsed with 2 x 50 mL portions of water, which were added to the seperatory funnel. A precipitate formed in the seperatory funnel. The aqueous layer was extracted with 4 x 50 mL portions of methylene chloride. The methylene chloride layer was washed with 4 x 50 mL portions of 5% NaOH and 3 x 50 mL portions of water. The methylene chloride was evaporated to yield 0.51 g of crude **17**. The product was recrystallized twice from ethanol-water to yield 0.27 g (30%) of analytically pure **17**. The base wash layers were acidified to yield 0.21 g of the initial starting material.

c) Into a 100 mL round bottom flask was added 1.0 g (3.5 mmole) of **18** dissolved in 50 mL of anhydrous toluene under an argon atmosphere. To the solution were added 0.20 mg (3.5 mmole) of 95% sodium methoxide and 1.4 g (3.5 mmole) of bis(benzonitrile)palladium (II) chloride and the reaction was heated under reflux for 3 hours. TLC ($CHCl_3$) of the reaction mixture showed spots corresponding to product, benzonitrile, and starting material. The reaction mixture was filtered through a small silica column, using chloroform as the eluent, to remove the palladium. The resulting solution was evaporated to dryness. The resulting solid was purified by flash chromatography, using chloroform as the eluent, to yield 0.14 g of yellow crystals (14%).

d) The crude **20** (0.25 g, 0.50 mmole) was added to a solution of 0.06 g of sodium (2.8 mmole) in 5 mL of absolute ethanol and the solution was heated at reflux under argon for 2.5 hours. The reaction was cooled to room temperature and acidified dropwise with 6 N hydrochloric acid to precipitate the desired product. The heterogeneous solution was extracted with chloroform and evaporated to dryness under reduced pressure. The resulting tarry brown solid was dissolved in 50 mL chloroform and washed with 1 x 25 mL of water, 3 x 25 mL of 5% NaOH and 3 x 25 mL of water. The solvent was dried over magnesium sulfate, filtered and removed under reduced pressure to yield a yellow-brown solid. The solid was recrystallized from an ethanol/water mixture to yield 32 mg (20%) of a bright yellow solid.

e) A solution of **18** (0.50 g, 1.8 moles) in 25 mL of methanol was added to a 100 mL round bottom flask equipped with a reflux condenser. To the solution was added 180 mg (0.90 mmoles) of copper acetate monohydrate and 10 mg (0.045 mmoles) of palladium acetate. The reaction was heated to approximately 55°C and a slow stream of air was bubbled through the reaction via a gas dispersion tube for 24 hours. The reaction was checked by TLC and there was no sign of the desired product.

6-Allyl-4-trifluoromethyl-7-hydroxy-8-methylcoumarin (18)

A heterogeneous mixture of **7** (2.0 g) and N,N-

diethylaniline (4 mL) was refluxed for 6 hours under argon while stirring. The solution was cooled to room temperature and dissolved in 150 mL of chloroform. The chloroform was extracted with 4 x 50 mL portions of 5% sodium hydroxide. The sodium hydroxide solution was slowly acidified with concentrated HCl to precipitate the desired product. The solid was suction filtered and washed with water. The solid was dissolved in chloroform and washed with 3 x 50 mL of water to remove any inorganic salts. The chloroform was evaporated under reduced pressure to yield 1.3 g (65%) of **18** as a light brown solid, m.p. 172-173 °C. The original chloroform layer was washed with 3 x 50 mL of 5% HCL and evaporated to yield 950 mg of crude solid that had a major spot on TLC corresponding to starting material. ^{1}H NMR ($CDCl_3$): δ 2.32 (s, 3H, CH_3), 3.47 (d, 2H,J = 6.3 Hz, $-CH_2$), 5.25 (c, 2H, $=CH_2$), 5.81 (s, 1H, OH), 6.00 (m, 1H, =CH), 6.59 (s, 1H, C3 -H), 7.31 (s, 1H, C5 -H). IR (KBr): 1700 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{14}H_{11}F_3O_3$: C 59.15 H 3.87 F 20.07

Found: C 58.76 H 3.85 F 19.80

7-Acetoxy-6-allyl-4-trifluoromethyl-8-methylcoumarin (19)

A mixture of the crude **18** (3.0 g), 25 mL of acetic anhydride, and a few crystals of fused sodium acetate were refluxed for 6 hours. The reaction was cooled to room temperature and 25 mL of water was added and the reaction was

stirred. The product precipitated and was suction filtered, washed with water, and dried to yield 3.2 g (92%) of **19** as white solid, m.p. 151-52 °C. ^{1}H NMR ($CDCl_3$): 2.28 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 3.36 (d, 2H, CH_2, J = 6.3 Hz), 5.07 (d d, 1H, trans H, J = 2.9 Hz, J = 1.3 Hz), 5.22 (d, 1H, cis H, J = 1.3 Hz), 5.68-6.05 (m, 2H, $=CH_2$), 6.79 (s, 1H, C-3 -H), 7.47 (s, 1H, C-5 -H). IR (KBr): 1760 cm^{-1}, 1740 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{16}H_{13}F_3O_4$: C 58.90 H 3.99 F 17.48

Found: C 58.73 H 3.94 F 17.41

7-Acetoxy-6-(2,3-dibromopropyl)-4-trifluoromethyl-8-methylcoumarin (20)

A solution of bromine (1.0 g, 6.5 mmoles) in 3 mL of chloroform was added dropwise to a stirred solution of **19** (2.1 g, 6.5 mmoles) in 15 mL of chloroform over a 5 minutes. The reaction was then stirred an additional 2 hours. The reaction was then transferred to a separatory funnel and washed with 3 x 10 mL portions of water. The chloroform layer was then evaporated to yield 3.0 g (94%) of crude **20** , m.p. 120-122 ^{0}C, which was subsequently used as such. ^{1}H NMR ($CDCl_3$): δ 2.25 (s, 3H, CH_3), 2.43 (s, 3H, $COCH_3$), 2.80 (m, 1H, $-CH_2$), 3.66 (m, 1H, $-CH_2$), 3.71 (m, 1H, CH_2Br), 3.93 (m, 1H, CH_2Br), 4.24 (m, 1H, CHBr), 6.78 (s, 1H, C3 -H), 7.52 (s, 1H, C5 -H). IR (KBr): 1772 cm^{-1}, 1740 cm^{-1}.

7-2-Chloroallyloxy-4-trifluoromethyl-8-methylcoumarin (21)

To a solution of **1** (3.0 g, 12 mmole) in 60 mL of dry acetone was added 15 g of K_2CO_3, 1.2 g (6.0 mmoles) potassium iodide, 2,3-dichloropropene (5.6 mL, 60 mmoles). The mixture was refluxed for 5 hours, cooled, filtered and evaporated to dryness. The solid was dissolved in 200 mL of chloroform and washed with 2 x 100 mL of water, 2 x 100 mL of 5% NaOH, and 2 x 100 mL of water. The chloroform was dried over magnesium sulfate and removed under reduced pressure. The solid residue was recrystallized from ethanol-water to yield 2.2 g (57%) of **21** as a white solid m.p. 114.5-116.0 °C. ^{1}H NMR ($CDCl_3$): 2.37 (s, 3H, CH_3), 4.71 (s, 2H, CH_2), 5.51 (s, 1H, =CH), 5.58 (s, 1H, =CH), 6.64 (s, 1H, C3 -H), 6.85 (d, 1H, C6 -H, J= 8.8 Hz), 7.55 (d, 1H, C5 -H, J= 8.8 Hz). IR (KBr): 1735 cm^{-1}.

Elemental Analysis:

Anal. Calcd. for $C_{14}H_{10}F_3ClO_3$: C 52.75 H 3.14 F 17.90
Cl 11.15

Found: C 52.69 H 2.96 F 17.90
Cl 11.28

4-Trifluoromethyl-4',5'-dihydro-4',8-dimethylpsoralen (23)

To a 100 mL round bottom flask was added **15** (0.50 g, 1.8 mmoles), 10% palladium on carbon (1.2 g), 95% ethanol (50 mL), and cyclohexene (1.3 mL). The reaction was heated under reflux for 2 hours and then an additional 1.3 mL of cyclohexene was added followed by an additional hour of

reflux. The reaction was cooled to room temperature and the ethanol was removed under reduced pressure. The palladium was washed repetitively with chloroform (75 mL) and the subsequent solution was filtered to remove suspended palladium. The chloroform was removed under reduced pressure to yield 0.46 g of a yellow solid. The mixture was analyzed by NMR which showed that the solid was 72% of the desired product and 28% starting material. The product was obtained by flash chromatography using chloroform as the mobile phase (rf starting material 0.65, rf product 0.53) to yield **23** as a yellow solid, m.p. 121-122°C. ^{1}H NMR ($CDCl_3$): δ 1.36 (d, 3H, CH_3), 2.27 (s, 3H, CH_3), 3.61 (m, 1H, C4' -H), 4.21 (m, 1H, C5' -H), 4.82 (m, 1H, C5' -H), 6.56 (s, 1H, C3 -H), 7.28 (s, 1H, C5 -H). IR (KBr): 1730 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{14}H_{11}F_3O_3$: C 59.15 H 3.87 F 20.07

Found: C 59.29 H 3.84 F 20.27

4-Trifluoromethyl-4',5'-dihydro-4'-methylpsoralen (24)

To a 100 mL round bottom flask was added **16** (0.50 g, 1.9 mmoles), 10% palladium on carbon (1.2 g), 95% ethanol (50 mL), and cyclohexene (1.3 mL). The reaction was heated under reflux for 2 hours and then an additional 1.3 mL of cyclohexene was added followed by an additional hour of reflux. The reaction was cooled to room temperature and the ethanol was removed under reduced pressure. The palladium was

washed repetitively with chloroform (75 mL) and the subsequent solution was filtered to remove suspended palladium. The chloroform was removed under reduced pressure to yield 0.41 g of an off white solid. The mixture, analyzed by NMR, contained 82% of the desired product and 18% starting material. The product was isolated by flash chromatography using chloroform as the mobile phase (rf starting material 0.61, rf product 0.47) to yield **24** as a white solid m.p. 168-169.5°C. ^{1}H NMR ($CDCl_3$): δ 1.36 (d, 3H, CH_3), 3.60 (m, 1H, C4' -H), 4.22 (m, 1H, C5' -H), 4.81 (m, 1H, C5' -H), 6.55 (s, 1H, C3 -H), 6.74 (s, 1H, C8 -H), 7.39 (s, 1H, C5 -H). IR (KBr): 1731 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{13}H_9F_3O_3$: C 57.78 H 3.33 F 21.11

Found: C 57.46 H 3.17 F 20.87

4',5'Dihydro-4-trifluoromethyl-5',8-dimethylpsoralen (25)

a) A solution of **18** (0.50 g) in 20 mL of concentrated sulfuric acid was stirred vigorously for four hours and then poured into 200 mL of ice cold water. The slurry was poured into a separatory funnel and extracted with four 25 mL portions of chloroform. The chloroform layer was extracted with 1 x 50 mL H_2O, 3 x 50 mL 5% NaOH, and 3 x 50 mL H_2O. The chloroform layer was removed under reduced pressure and the solid residue was recrystallized from ethanol/water to yield 0.25 g (51%) of the desired product. m.p. 155-56 °C.

b) To a 50 mL round bottom flask was added 4-trifluoromethyl-5',8-dimethylpsoralen (0.25 g, 0.89 mmoles), 10% palladium on carbon (0.61 g), 95% ethanol (25 mL), and cyclohexene (0.63 mL). The reaction was heated under reflux for 2 hours and then an additional 0.63 mL of cyclohexene was added followed by an additional hour of reflux. The reaction was cooled to room temperature and the ethanol was removed under reduced pressure. The palladium was washed repetitively with chloroform (50 mL) and the solution subsequently was filtered to remove suspended palladium. The chloroform was removed under reduced pressure to yield 0.22 g of a yellow solid. The mixture, analyzed by NMR, contained 29% of **25** and 71% starting material. ^1H NMR ($CDCl_3$): δ 1.52 (d, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.90 (m, 1H, C4' -H), 3.42 (m, 1H, C4' -H), 5.08 (m, 1H, C5' -H), 6.56 (s, 1H, C3 -H), 7.33 (s, 1H, C5 -H). IR (KBr): 1732 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{14}H_{11}F_3O_3$: C 59.15 H 3.87 F 20.07

Found: C 59.07 H 3.88 F 19.71

5'Bromomethyl-4-trifluoromethyl-8-methylpsoralen (26)

Into an argon purged and oven dried 10 mL round bottom flask equipped with a reflux condenser, Claisen head, and septum were added **17** (0.13 g, 0.44 mmoles), N-bromosuccinimide (0.08 g, 0.50 mmoles), and 5 mL of anhydrous chloroform. The flask was wrapped with aluminum foil to exclude light and

brought to reflux. A solution of benzoyl peroxide (0.06 g, 0.25 mmoles) in 1 mL of anhydrous chloroform was added in 0.2 mL portions every 15 minutes for 1.25 h. After completion of addition, reflux was continued for 1.75 h after which the solution was cooled to room temperature. The solution was transferred to a separatory funnel and the reaction flask was rinsed with 45 mL of chloroform. The chloroform was washed with 3 x 10 mL of saturated sodium bicarbonate and 3 x 10 mL of water, dried over magnesium sulfate, and evaporated to dryness under reduced pressure. The yellow solid was washed repetitively with methanol and dried to yield 0.10 g (62%) of **29**, m.p. 202-204 °C. ^{1}H NMR ($CDCl_3$): δ 2.64 (s, 3H, CH_3), 4.61 (s, 2H, CH_2), 6.78 (s, 1H, C3 -H), 6.84 (s, 1H, C4' -H), 7.76 (s, 1H, C5 -H). IR (KBr): 1739 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{14}H_8F_3BrO_3$: C 46.54 H 2.22 F 15.79 Br 22.16

Found: C 46.15 H 2.27 F 15.70 Br 22.38

5'-Bromo-4-trifluoromethyl-4',8-dimethylpsoralen (27)

To a solution of **15** (0.28 g, 1.0 mmole) in 20 mL of chloroform was added bromine (0.32 g, 2.0 mmole) and the solution was stirred for 48 hours. The solvent was removed under reduced pressure and the resulting solid was recrystallized from methanol to yield 0.35 g of a bright yellow solid. The solid was determined to be 85% of the desired product and 15% of 5'-bromo-4'-bromomethyl-4-

8-methylpsoralen (**28**) by NMR analysis. The two products were virtually inseparable on TLC. The pure product could be isolated by heating the mixture in pyridine to convert the di-bromo product to the corresponding quaternary salt (**30**) which precipitated from the solution. The solution was then filtered and the filtrate was evaporated to yield an off yellow solid which when washed with methanol gave **27** as a bright yellow solid, m.p. 226-228.5 °C. ^{1}H NMR ($CDCl_3$): δ 2.22 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 6.76 (s, 1H, C3 -H), 7.72 (s, 1H, C5 -H). IR (KBr): 1734 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{14}H_8F_3BrO_3$: C 46.54 H 2.22 F 15.97
Br 22.16

Found : C 46.56 H 2.31 F 15.68
Br 21.91

5'-Bromo-4'-bromomethyl-4-trifluoromethyl-8-methylpsoralen (28)

Into an argon purged and oven dried 25 mL round bottom flask equipped with a reflux condenser, Claisen head, and septum were added **27** (0.35 g, 0.97 mmoles), N-bromosuccinimide (0.16 g, 0.60 mmoles), and 10 mL of anhydrous chloroform. The flask was wrapped with aluminum foil to exclude light and brought to reflux. A solution of benzoyl peroxide (0.08 g, 0.33 mmoles) in 1.5 mL of anhydrous chloroform was added in 0.30 mL portions every 15 minutes for 1.25 h. After

completion of addition, reflux was continued for 1.75 h whereafter the solution was cooled to room temperature. The solution was transferred to a separatory funnel and the reaction flask was rinsed with 40 mL of chloroform. The chloroform was washed with 3 x 10 mL of saturated sodium bicarbonate and 3 x 10 mL of water, dried over magnesium sulfate, and evaporated to dryness under reduced pressure. The yellow solid was washed repetitively with methanol and dried to yield 0.26 g (60%) of **28** as a light yellow solid, m.p. 248-251 °C. ^{1}H NMR ($CDCl_3$): δ 2.59 (s, 3H, CH_3), 4.54 (s, 2H, CH_2), 6.80 (s, 1H, C3 -H), 7.78 (s, 1H, C5 -H). IR (KBr): 1745 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{14}H_7F_3Br_2O_3$: C 38.18 H 1.59 F 12.95
Br 36.36

Found : C 37.95 H 1.44 F 12.65
Br 36.45

4-Trifluoromethyl-5'[(N-pyridinium)methyl]-8-methylpsoralen bromide (29)

Into an argon purged flask were added **26** (80 mg, 0.22 mmoles), pyridine (0.18 mL, 0.18 g, 2.2 mmoles), and 15 mL of anhydrous toluene. The mixture was brought to reflux for 8 hours after which the heterogeneous solution was cooled to room temperature. The precipitate was filtered and washed with anhydrous toluene and anhydrous chloroform, until no

color was observed in either of the wash layers, to yield 65 mg (67%) of **29** as a cream colored solid, m.p. 225-27°C. ^{1}H NMR (CD_3OD): δ 2.56 (s, 3H, CH_3), 6.17 (s, 2H, CH_2), 6.89 (s, 1H, C3 -H), 7.41 (s, 1H, C4' -H), 7.96 (s, 1H, C5 -H), 8.22 (m, 2H, C3 pyridine -H), 8.69 (m, 1H, C4 pyridine -H), 9.22 (m, 2H, C2 pyridine -H). IR (KBr): 1744 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{19}H_{13}F_3BrNO_3$ .0.25 H_2O: C 50.80 H 3.12
N 3.11

Found: C 51.00 H 3.03
N 3.13

5'Bromo-4-trifluoromethyl-4'[(N-pyridinium)methyl]-8-methyl-psoralen bromide (30)

Into and argon purged flask were added **29** (0.10 g, 0.22 mmoles), pyridine (0.18 mL, 0.18 g, 2.2 mmoles), and 15 mL of anhydrous toluene. The heterogeneous mixture was brought to reflux and the solution became homogeneous. Reflux was continued for 8 hours after which the heterogeneous mixture was cooled to room temperature. The precipitate was filtered and washed with anhydrous toluene and anhydrous chloroform until no color was observed in either of the wash layers to yield 0.11 g (89%) of **30** as a cream colored solid, m.p. 255-57 °C. ^{1}H NMR (CD_3OD): δ 2.56 (s, 3H, CH_3), 6.07 (s, 2H, CH_2), 6.87 (s, 1H, C3 -H), 7.82 (s, 1H, C5 -H), 8.12 (m, 2H, C3 pyridine -H), 8.61 (m, 1H, C4 pyridine -H), 9.03 (m, 2H, C2

pyridine -H). IR (KBr): 1745 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{19}H_{12}F_3Br_2NO_3$:

C 43.93 H 2.31 N 2.70 Br 30.83 F 10.98

Found: C 43.79 H 2.29 N 2.69 Br 30.83 F 10.77

5' Substituted Trioxsalen Derivatives

4,8-Dimethyl-7-hydroxycoumarin (31)

This compound was obtained from the Regis Chemical Company.

4,8-Dimethyl-7-hydroxy-6-iodocoumarin (32)

7-hydroxy-4,8-dimethylcoumarin (10.0 g, 52.0 mmole) was dissolved in 80 mL of dioxane and then 200 mL of concentrated ammonia was added. A solution of iodine (13.6 g, 53.5 mmoles) in 500 mL of 5% potassium iodide was added over a two hour period and the solution was stirred until the greenish color disappeared. A yellow precipitate formed over the course of the reaction. The solution was neutralized to pH 3 and the product was filtered. The product was dissolved in ethyl acetate and washed with 3 x 50 mL of water. The solvent was then evaporated to yield 15.7 g (95%) **32**, m.p. 219-220 °C. ^1H NMR (DMSO-d_6): 2.25 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 6.13 (s, 1H, C3 -H), 7.84 (s, C5, -H), 10.11 (s, 1H, OH). IR (KBr): 1682 cm^{-1}, 3400 cm^{-1}.

4,8-Dimethyl-5'-hydroxymethylpsoralen (33)

A solution of **32** (1.0 g, 3.2 mmole) in 13 mL of pyridine was added to a suspension of Cu_2O (0.60 g, 4.3 mmole) and propargyl alcohol (0.36 g, 6.4 mmole) in 6 mL of anhydrous pyridine. The mixture was refluxed under argon for 3 hours.

The reaction was filtered through a small silica column using ethyl acetate as the eluent. The solution was washed with 3 x 50 mL of 5% HCl, 2 x 50 mL water, 2 x 50 mL 5% sodium bicarbonate, and 100 mL of water. The ethyl acetate was removed under reduced pressure and the residue was passed through another silica column using ethyl acetate. The ethyl acetate was evaporated to yield 0.50 g (64%) **33**, m.p. 211-213°C. ^{1}H NMR (DMSO-d_6): δ 2.47 (s, 6H, 2 x CH_3), 4.62 (d, 2H, J = 6.0 Hz, CH_2), 5.59 (t, 1H, J = 6.0 Hz, OH), 6.30 (s, 1H, C3 -H), 6.82 (s, 1H, C4' -H), 7.74 (s, 1H, C5 -H). IR (KBr): 1680 cm^{-1} (C=O), 3395 cm^{-1} (-OH).

Elemental analysis:

Anal. calcd. for $C_{14}H_{12}O_4$: C 68.85 H 4.92

\+ 0.50 H_2O: C 66.39 H 5.17

Found: C 66.05 H 4.86

4,8-Dimethyl-5'-(2-hydroxyethyl)psoralen (34)

Into an argon purged 100 mL flask was added **32** (2.0 g, 6.4 mmoles), Cu_2O (0.65 g, 4.5 moles) 30 mL of anhydrous pyridine and 3-butyn-1-ol (0.83 g, 0.89 mL, 12 mmoles). The mixture was refluxed under argon for 2 hours. The pyridine was removed under reduced pressure and the residue was rinsed from the flask with methylene chloride and eluted over a silica column, which was pre-equilibrated with methylene chloride, using ethyl acetate as the eluent. The fractions containing product were combined and evaporated to dryness.

The residue was dissolved in 250 mL of methylene chloride and the solution was washed with 3 x 50 mL of 5% NaOH, 3 x 50 mL water, 3 x 50 mL 5% HCl, and 3 x 50 mL of water. The solvent was removed under reduced pressure and the resulting solid was recrystallized from ethanol/water to yield 0.92 g (56%) of **34**, m.p. 141-142.5 °C. ^{1}H NMR (CD_3OD): 2.44 (s, 6H, 2 x CH_3), 3.03 (t, 2H, J = 6.5 Hz, CH_2), 3.96 (t, 2H, J = 6.5 Hz, OCH_2), 4.92 (s, 1H, OH), 6.16 (s, 1H, C3 -H), 6.61 (s, 1H, C4' -H), 7.60 (s, 1H, C8 -H). IR (KBr): 3423 cm^{-1} (-OH), 1688 cm^{-1} (C=O). Elemental analysis:

Anal. calcd. for $C_{15}H_{14}O_4$: C 69.77 H 5.43

\+ 0.25 H_2O: C 68.57 H 5.56

Found: C 68.78 H 5.37

4,8-Dimethyl-5'-(2-bromoethyl)psoralen (35)

Into a dry 100 mL three necked flask containing a magnetic stir bar and fitted with a drying tube and rubber septum was added **34** (0.50 g, 1.9 mmoles) and 20 mL of chloroform. Phosphorous tribromide (5.0 mL, 14 g, 53 mmoles) was added dropwise to the solution and the reaction was stirred at room temperature for 5 days. The reaction was cooled in an ice bath while 40 mL of water was slowly added with vigorous stirring. The reaction was stirred until the evolution of HBr ceased. The mixture was transferred to a separatory funnel and the organic layer was removed. The aqueous layer was extracted with 2 X 10 mL of chloroform. The

combined organic layers were washed with 2 x 20 mL of saturated sodium bicarbonate and 2 x 20 mL of water. The chloroform was dried over magnesium sulfate and evaporated under reduced pressure to yield 0.54 g of crude product. The crude product was purified by column chromatography (CH_2Cl_2) to yield 0.22 g of **35**, m.p. 173-173.5 °C, and 0.11 g of the starting material. ^1H NMR ($CDCl_3$): δ 2.45 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 3.34 (t, 2H, J = 6.35 Hz, $-CH_2$), 3.70 (t, 2H, J = 6.35, OCH_2), 6.21 (s, 1H, C3 -H), 6.57 (s, 1H, C4' -H), 7.53 (s, 1H, C5 -H). IR (KBr): 1709 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{15}H_{13}O_3Br$: C 56.10 H 4.08 Br 24.88

Found: C 55.72 H 4.13 Br 24.89

4,8-Dimethyl-5'-(2-pyridinium-ethyl)psoralen bromide (36)

Into a 10 mL reaction flask containing a magnetic stir bar was added **35** (0.20 g, 0.62 mmoles) and 4.0 mL of anhydrous pyridine. The reaction, initially homogeneous, started to form a precipitate after two hours of stirring. The reaction was stirred for a total of 36 hours followed by the evaporation of the excess pyridine under reduced pressure. The residue was recrystallized from methanol to yield 0.19 g (71%) **37**, m.p. 273-274°C. ^1H NMR (CD_3OD): δ 2.40 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 3.59 (t, 2H, CH_2 J = 6.7 Hz), 5.04 (t, 2H, CH_2 J = 6.7 Hz), 6.20 (s, 1H, C3 -H), 6.66 (s, 1H, C4' -H), 7.69 (s, 1H, C5 -H), 8.05 (m, 2H, C3 pyridine -H), 8.56 (m, 1H, C4

pyridine -H), 8.94 (m, 2H, C2 pyridine -H). IR (KBr): 1706 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{20}H_{18}NO_3Br$: C 60.01 H 4.53 Br 19.96 N 3.50

Found: C 59.67 H 4.51 Br 19.85 N 3.38

4,8-Dimethyl-5'-(dimethylamino)methylpsoralen (37)

Into an argon purged 100 mL flask was added **32** (2.0 g, 6.4 mmoles), Cu_2O (0.65 g, 4.5 mmoles) 30 mL of anhydrous pyridine and 1-dimethylamino-2-propyne (0.98 g, 1.3 mL, 12 mmoles). The mixture was refluxed under argon for 2 hours. The pyridine was removed under reduced pressure and the residue was rinsed from the flask using methylene chloride and eluted over a silica column, which was pre-equilibrated with methylene chloride, using methylene chloride (50%) ethyl acetate (40%) and methanol (10%) as the eluent. The fractions containing product were combined and evaporated to dryness. The residue was dissolved in 250 mL of methylene chloride and the solution was washed with 3 x 50 mL of 5% NaOH, 3 x 50 mL water. The solvent was removed under reduced pressure and the resulting solid was eluted over another silica column using the previously stated conditions to yield 1.2 g (70%) of **37**, m.p. 132-134 °C. ^1H NMR (CD_3OD): 2.37 (s, 6H, $N(CH_3)_2$), 2.51 (d, 3H, J = 1 Hz, C4 CH_3), 2.54 (s, 3H, CH_3), 3.73 (s, 2H, CH_2), 6.25 (d, 1H, J = 1 Hz, C3 -H), 6.84 (s, 1H, C4' -H), 7.79 (s, 1H, C5 -H). IR (KBr): 1709 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{16}H_{17}NO_3$: C 70.85 H 6.27 N 5.17

Found: C 70.53 H 6.31 N 4.73

4,8-Dimethyl-5'-(trimethylammonium)methylpsoralen iodide (38)

To a solution of **37** (0.15 g, 0.55 mmoles) in 10 mL of methylene chloride was added methyl iodide (0.18 mL, 2.8 mmoles). The solution was stirred at room temperature for one hour. The precipitate was filtered and washed with methylene chloride to yield 0.20 g (85%) of **38**, m.p. 240-41 °C. ^{1}H NMR (DMSO-d_6): 2.52 (s, 6H, CH_3), 3.19 (s, 9H, $N(CH_3)_3I$), 4.89 (s, 2H, CH_2), 6.40 (s, 1H, C3 -H), 7.42 (s, 1H, C4' -H), 8.05 (s, 1H, C5 -H). IR (KBr): 1710 cm^{-1}.

Elemental Analysis:

Anal. calcd. for $C_{17}H_{20}NO_3$: C 49.39 H 4.84 N 3.39

+ 0.25 H_2O: C 48.86 H 4.91 N 3.35

Found : C 48.88 H 4.88 N 3.22

5'-Ethoxycarbonyl-4,8-dimethylpsoralen (39)

A solution of **32** (5.0 g, 16 mmole) in 75 mL of anhydrous dimethyl formamide was added to a suspension of copper 1 oxide (1.5 g, 10 mmole) and ethyl propiolate (2.2 g, 22 mmole) in 25 mL of anhydrous dimethyl formamide. The reaction was heated at 115 °C for 24 hours. The reaction was cooled and filtered through a small silica column using ethyl acetate. The solvent was evaporated under reduced pressure to

yield a tarry material (due to residual DMF). The tarry material was extacted repetitively with diethyl ether to yield 2.2 g of **39** as a light yellow solid. The wash layers were evaporated to dryness to yield a tarry material that was subsequently dissolved in 50/50 chloroform-ethyl acetate and washed with 4 x 25 mL portions of 5% NaOH, 2 x 25 mL water, 1 x 25 mL 5% HCl, and 2 x 25 mL water. The solvent was evaporated and the resulting material was recrystallized from chloroform-cyclohexane to yield 0.82 g of additional product. The total product isolated was 3.0 g (66%). The melting point of this compound is 198-199°C. ^{1}H NMR ($CDCl_3$): δ 1.40 (t, 3H, J = 7.1 Hz, CH_3), 2.47 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 4.47 (q, 2H, J = 7.1 Hz, CH_2), 6.30 (s, 1H, C3 -H), 7.55 (s, 1H), 7.75 (s, 1H). IR (KBr): 1730 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{16}H_{14}O_5$: C 67.13 H 4.90

Found: C 67.02 H 5.02

5'-Carboxy-4,8-dimethylpsoralen (40)

A heterogeneous mixture of **39** (0.60 g, 2.0 mmoles) and 60 mL of 20% NaOH was refluxed for 2 hours and then cooled to room temperature. The homogeneous solution was diluted to 200 mL with water and acidified to a pH of 1 with concentrated HCl to precipitate the desired product. The solution was allowed to digest for one hour and then the aqueous layer was decanted and the precipitate was washed with 150 mL of water and then

filtered to yield 0.48 g (89%) of **40**, m.p. 345°C with dec. ^1H NMR ($CDCl_3$): δ 2.48 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 6.26 (s, 1H, C3 -H), 7.62 (s, 1H, C4' -H), 7.96 (s, 1H, C5 -H). IR (KBr): 1721 cm^{-1} (C=O), 1671 cm^{-1} (O=C-OH).

Elemental analysis:

Anal. calcd. for $C_{14}H_{10}O_5$:	C 65.11	H 3.90
0.1 M water:	C 64.67	H 3.93
Found:	C 64.42	H 3.92

7-Alkoxy-3-bromo-8-methylcoumarins

8-Methylumbelliferone (41)

Prepared according to the method of Kauffman.[57] The m.p. of **41** was 257-258.5°C, lit. m.p. 259.5-260.5°C.

3-Bromo-7-hydroxy-8-methyl coumarin (42)

To a solution of **41** (3.0 g, 17 mmol) in 110 mL of glacial acetic acid was added dropwise a solution of bromine (0.88 mL, 17 mmol) in 450 mL of glacial acetic acid. The solution was stirred at room temperature for 48 hours, after which it was filtered and evaporated to dryness under reduced pressure. The solid was recrystallized from ethanol yielding 2.8 g (63.4%) of pure **42**, m.p. 209-209.5 °C. This compound was converted to **43** and combustion analysis was performed. ^1H NMR (CD_3OD): δ 2.09 (s, 3H, CH_3), 6.65 (d, 1H, J = 8.6 Hz, C6 -H), 7.11 (d, 1H, J = 8.6 Hz, C5 -H), 8.05 (s, 1H, C4 -H). IR (KBr): 3244 cm^{-1} (-OH), 1690 cm^{-1} (C=O).

3-Bromo-7-methoxy-8-methyl coumarin (43)

To a solution of **41** (0.25 g, 0.98 mmol) in 5.0 mL of dry acetone were added 1.0 g of K_2CO_3 and methyl iodide (0.30 mL, 4.9 mmol). The solution was stirred at room temperature for 2.5 hours, filtered and the filtrate evaporated to dryness. The solid was dissolved in 50 mL of chloroform, washed with 2 x 25 mL of water, 3 x 25 mL 5% NaOH solution and finally with

2 x 25 mL of water. The chloroform was then evaporated under reduced pressure and the solid was recrystallized from ethanol yielding 0.19 g (70%) of pure **43**, m.p. 154-155°C. ^{1}H NMR ($CDCl_3$): δ 2.26 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 6.82 (d, 1H, J = 8.6 Hz, C6 -H), 7.24 (d, 1H, J = 8.6 Hz, C5 -H), 7.97 (s, 1H, C4 -H). IR (KBr): 1716 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{11}H_9BrO_3$: C 49.07 H 3.35 Br 29.74

Found: C 49.04 H 3.34 Br 29.88

3-Bromo-7-propoxy-8-methyl coumarin (44)

To a solution of **41** (0.25 g, 0.98 mmol) in 5.0 mL of dry acetone were added 1.0 g of K_2CO_3 and n-propyl bromide (0.44 mL, 4.9 mmol). The mixture was heated under reflux while stirring for 5 hours. The solution was filtered and evaporated to dryness. The solid was dissolved in 50 mL of chloroform and was washed with 2 x 25 mL of water, 3 x 25 mL of 5% NaOH solution and finally with 2 x 25 mL of water. The chloroform was then evaporated under reduced pressure and the solid was recrystallized from ethanol yielding 0.20 g (70%) of pure **44**, m.p. 139.5-140°C. ^{1}H NMR ($CDCl_3$): δ 1.05 (t, 3H, CH_3, J = 7.4 Hz), 1.85 (m, 2H, CH_2), 2.28 (s, 3H, CH_3), 4.01 (t, 2H, OCH_2, J = 12.8 Hz), 6.80 (d, 1H, C6 -H, J = 8.6 Hz), 7.21 (d, 1H, C5 -H, J = 8.6 Hz), 7.97 (s, 1H, C8 -H). IR (KBr): 1714 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{13}H_{13}BrO_3$: C 52.53 H 4.38 Br 26.78

Found: C 52.34 H 4.33 Br 26.71

3-Bromo-7-allyloxy-8-methyl coumarin (45)

To a solution of **41** (0.25 g, 0.98 mmol) in 5 mL of dry acetone were added 1.0 g of K_2CO_3 and allyl bromide (0.42 ml, 4.9 mmol). The solution was heated under reflux while stirring for 2 hours. The solution was then filtered and evaporated to dryness. The solid was dissolved in 50 mL of chloroform and was washed with 2 x 25 mL of water, 3 x 25 mL 5% NaOH solution and finally with 2 x 25 mL of water. The chloroform was evaporated under reduced pressure and the solid was recrystallized from ethanol yielding 0.24 g (83%) of **45**, m.p. 143-143.5°C. ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 4.62 (m, 2H, CH_2), 5.36 (m, 2H, $=CH_2$), 6.00 - 6.09 (m, 1H, CH=), 6.80 (d, 1H, J = 8.6 Hz, C6 -H), 7.22 (d, 1H, J = 8.6 Hz, C5 -H), 7.97 (s, 1H, C4 -H). IR (KBr): 1714 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{13}H_{11}BrO_3$: C 52.88 H 3.73 Br 27.12

Found: C 52.77 H 3.72 Br 26.89

3-Bromo-7-propargyloxy-8-methyl coumarin (46)

To a solution of **41** (0.25 g, 0.98 mmol) in 5.0 mL of dry acetone were added 1.0 g of K_2CO_3 and propargyl chloride (0.35 mL, 4.9 mmol). The solution was heated under reflux while

stirring for 5 hours. The solution was filtered and evaporated to dryness. The solid was dissolved in 50 mL of chloroform and washed with 2 x 25 mL of water, 3 x 25 mL of 5% NaOH solution, and finally with 2 x 25 mL of water. The chloroform was then evaporated under reduced pressure and the solid was recrystallized from ethanol yielding 0.23 g (79%) of **46**, m.p. 181-182°C. ^{1}H NMR ($CDCl_3$): δ 2.30 (s, 3H, CH_3), 2.53 (t, 1H, J = 2.4 Hz, $\equiv$CH), 4.79 (d, 2H, J = 2.4 Hz, CH_2), 6.96 (d, 1H, J = 8.7 Hz, C6 -H), 7.26 (d, 1H, J = 8.7 Hz, C5 -H), 7.99 (s, 1H, C4 -H). IR (KBr): 1714 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{13}H_{11}BrO_3$: C 53.24 H 3.07 Br 27.49

Found: C 53.40 H 3.09 Br 27.49

3-Bromo-7-[(3-methyl-2-buten-1-yl)oxy]-8-methyl coumarin (47)

To a solution of **41** (0.25 g, 0.98 mmol) in 5 mL of dry acetone were added 1.0 g of K_2CO_3 and 3,3-dimethylallyl bromide (0.56 mL, 4.9 mmol). The mixture was stirred at room temperature for 12 hours, filtered and evaporated to dryness. The solid was dissolved in 50 mL of chloroform and washed with 2 x 25 mL of water, 3 x 25 mL 5% NaOH solution and finally with 2 x 25 mL of water. The chloroform was then evaporated under reduced pressure and the solid was recrystallized from ethanol yielding 0.26 g (83%) of pure **47**, m.p. 131.5-133°C. ^{1}H NMR ($CDCl_3$): δ 1.73 (s, 3H, CH_3), 1.78 (d, 3H, J = 0.8 Hz, trans CH_3), 2.27 (s, 3H, CH_3), 4.60 (d, 2H, J = 6.6 Hz, OCH_2),

5.45 (m, 1H, CH=), 6.82 (d, 1H, J = 8.5 Hz, C6 -H), 7.21 (d, 1H, J = 8.5 Hz, C5 -H), 7.97 (s, 1H, C4 -H). IR (KBr): 1715 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{15}H_{15}BrO_3$: C 55.73 H 4.64 Br 24.93

Found: C 55.80 H 4.61 Br 24.93

Iodinated Psoralens and Coumarins

8-Iodo-5-methoxypsoralen (48)

A solution of 5-methoxypsoralen (0.44 g, 2.0 mmoles) in 20 mL of chloroform was added to a 100 mL flask containing 0.44 g of silver trifluoroacetate over 15 minutes. To the flask was added a solution of iodine (0.55 g, 2.2 mmoles) in 40 mL chloroform over a period of one hour. An additional 0.10 g of silver trifluoroacetate was added and the reaction was stirred for 3 hours. The mixture was washed with 2 x 25 mL portions of water, 2 x 25 mL of 5% sodium bisulfite, and 2 x 25 mL of water and was evaporated to dryness. TLC of the crude material showed two spots using methylene chloride as the mobile phase (R_f 0.42 product, 0.31 starting material). The crude material was purified by flash chromatography using methylene chloride as the mobile phase. The pure fractions were combined and evaporated to dryness to yield 67 mg (10%) of **48**, m.p. 226-28 °C. ^{1}H NMR ($CDCl_3$): δ 4.30 (s, 3H, OCH_3), 6.29 (d, 1H, J= 10 Hz, C3 -H), 7.19 (d, 1H J= 2.4 Hz, C4' -H), 7.70 (d, 1H, J= 2.4 Hz, C5' -H), 8.11 (d, 1H, J= 10 Hz). IR (KBr): 1715 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{12}H_7IO_4$: C 42.10 H 2.05 I 37.13

Found: C 42.20 H 1.92 I 37.28

8-Iodo-7-methoxy-4-methylcoumarin (51)

To a solution of **8-iodo-7-hydroxy-4-methylcoumarin** (0.30 g, 1.0 mmole) in 5.0 mL of dry acetone were added 1.3 g of K_2CO_3 and methyl iodide (0.31 mL, 0.71 g, 5.0 mmoles). The solution was stirred at room temperature for 6 hours, filtered and evaporated to dryness. The solid was then dissolved in 50 mL of chloroform and washed with 3 x 25 mL of water. The chloroform was then evaporated under reduced pressure and the solid was recrystallized from ethanol yielding 0.14 g (44%) of **51** as an off white solid m.p. 194-195°C. ^1H NMR ($CDCl_3$): δ 2.42 (s, 3H, C4 CH_3), 4.00 (s, 3H, OCH_3), 6.15 (s, 1H, C3 -H), 6.82 (d, 1H, J = 8.8 Hz, C6 -H), 7.57 (d, 1H, J = 8.8 Hz, C5 -H). IR (KBr): 1719 cm^{-1}.

Elemental Analysis:

Anal. calcd. for $C_{11}H_9IO_3$: C 41.77 H 2.85 I 40.19

Found: C 41.81 H 2.78 I 40.04

8-Iodo-7-propoxy-4-methylcoumarin (52)

To a solution of **8-iodo-7-hydroxy-4-methylcoumarin** (0.30 g, 1.0 mmole) in 5.0 mL of dry acetone were added 1.3 g of K_2CO_3 and bromopropane (0.44 mL, 0.60 g, 4.9 mmoles). The solution was heated under reflux for 6 hours, filtered and evaporated to dryness. The solid was then dissolved in 50 mL of chloroform and washed with 3 x 25 mL of water. The chloroform was evaporated under reduced pressure and the solid was recrystallized from ethanol yielding 0.17 g (49%) of **52** as

an off white solid m.p. 129-130 °C. ^{1}H NMR ($CDCl_3$): δ 1.13 (t, 3H, J= 7.33 Hz, CH_3), 1.85 (m, 2H, CH_2), 2.41 (s, 3H, CH_3), 4.10 (t, 2H, J = 6.36 Hz, OCH_2), 6.14 (s, 1H, C3 -H), 6.77 (d, 1H, J = 8.8 Hz, C6 -H), 7.54 (d, 1H, J = 8.8 Hz, C5 -H). IR (KBr): 1709 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{13}H_{13}IO_3$: C 45.35 H 3.78 I 36.92

Found: C 45.34 H 3.82 I 37.05

6-Iodo-7-methoxy-4,8-dimethylcoumarin (54)

To a solution of **32** (0.50 g, 1.6 mmole) in 10 mL of dry acetone were added 2.5 g of K_2CO_3 and methyl iodide (0.49 mL, 1.1 g, 8.0 mmoles). The reaction was stirred at room temperature for 6 hours, filtered and evaporated to dryness. The solid was then dissolved in 50 mL of chloroform and washed with 3 X 25 mL of 5% NaOH and 3 x 25 mL of water. The chloroform was then evaporated under reduced pressure and the solid was recrystallized from ethanol yielding 0.32 g (61%) of **54** as a light yellow solid. The solid was further purified by flash chromatography (CH_2Cl_2) on a column which was preequilibrated with hexanes. The product fractions were combined and evaporated to dryness to yield 0.27 g of the desired product, m.p. 158.5-60°C. ^{1}H NMR ($CDCl_3$): 2.40 (d, 3H, J = 1.2 Hz, C4 -CH_3), 2.43 (s, 3H, C8 -CH_3), 3.83 (s, 3H, OCH_3), 6.21 (d, 1H, J = 1.2 Hz, C3 -H), 7.86 (s, 1H, C5 -H). IR (KBr): 1711 cm^{-1}.

Elemental Analysis:

Anal. calcd. for $C_{12}H_{11}IO_3$: C 43.64 H 3.33 I 38.48

Found: C 43.64 H 3.33 I 38.28

6-Iodo-7-propoxy-4,8-dimethylcoumarin (55)

To a solution of **32** (1.0, 3.2 mmole) in 20 mL of dry acetone were added 5.0 g of K_2CO_3 and bromopropane (1.5 mL, 2.0 g, 16 mmoles). The solution was heated under reflux for 6 hours, filtered and evaporated to dryness. The solid was then dissolved in 50 mL of chloroform and washed with 3 X 25 mL of 5% NaOH and 3 x 25 mL of water. The chloroform was evaporated under reduced pressure. The solid was purified by flash chromatography (CH_2Cl_2) on a column which was preequilibrated with hexanes. The product fractions were combined and evaporated to dryness to yield 0.35 mg (31%) of **32** m.p. 124.5-6°C. ^1H NMR ($CDCl_3$): 1.13 (t, 3H, J = 7.33 Hz, CH_3), 1.98 (m, 2H, CH_2), 2.41 (s, 6H, CH_3), 3.86 (t, 2H, J = 6.59 Hz, CH_2), 6.21 (s, 1H, C3 -H), 7.87 (s, 1H, C5 -H). IR (KBr): 1745 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{14}H_{15}IO_3$: C 46.93 H 4.19 I 35.47

Found: C 46.79 H 4.07 I 35.27

6-Iodo-4,8-dimethyl-7-[(3-methyl-2-buten-1-yl)oxy]coumarin (56)

To a solution of **32** (1.0, 3.2 mmole) in 20 mL of dry acetone were added 5.0 g of K_2CO_3 and 4-bromo-2-methyl-2-butene

(1.8 ml, 2.4g, 16 mmole). The solution was stirred for 6 hours, filtered and evaporated to dryness. The solid was then dissolved in 100 mL of chloroform and washed with 3 X 50 mL of 5% NaOH and 3 x 50 mL of water. The chloroform was evaporated under reduced pressure. The solid was purified by flash chromatography (CH_2Cl_2) on a column which was preequilibrated with hexanes. The product fractions were combined and evaporated to dryness to yield 0.94 g (76%) of **56**, m.p. 146-147°C. ^1H NMR ($CDCl_3$): δ 1.73 (d, 3H, J = 1 Hz, trans CH_3), 1.80 (s, 3H, cis CH_3), 2.37 (d, 3H, J = 1.2 Hz, C4 CH_3), 2.40 (s, 3H, C8 CH_3), 4.42 (d, 2H, J = 7.3 Hz, OCH_2), 5.62 (m, 1H, -CH=), 6.18 (d, 1H, J = 1.2 Hz, C3 -H), 7.85 (s, 1H, C5 -H). IR (KBr): 1719 cm^{-1}.

Elemental analysis:

Anal. calcd. for $C_{16}H_{17}IO_3$: C 50.00 H 4.43 I 33.07

Found: C 50.20 H 4.41 I 32.90

General Procedure for Determination of Lipophilicities

The lipophilicities of the compounds were determined using a technique which relates the capacity factor measurements to the partition coefficients. All of the capacity factors were determined using 60% aqueous methanol at a flow rate of 0.5 mL/min under isocratic conditions.

Samples (15 μL) of 10^{-4}M solutions of the test compounds were injected and their retention times were determined. The injections were repeated three times and the average was used as the retention time of the compounds. The retention time of an unretained compound was determined using potassium acid phthalate. The capacity factor K' for each compound was calculated using the following equation:

$$K' = t_r - t_o / t_o$$

t_r= the retention time of the test compound

t_o= the retention time of the reference compound

The conversion of the capacity factors to the log of the partition coefficients (log P) was accomplished using the following equation:

$$\log P = A \log K' + \log K$$

log K- the correction factor for the system

A- the linearity constant

The values for log K and A were determined using compounds with known lipophilicities and plotting log P vs log K' according to the procedure described by Akyea.[26]

General procedure for the EGF assay

To assay compounds for inhibition of EGF binding, human cells (HeLa) grown *in vitro* were used. A constant concentration of cells was inoculated into 5 cm culture dishes in growth medium consisting of Dulbecco's modified Eagle's medium supplemented with 10% newborn calf serum. After 4-5 days at 37° in a humidified CO_2 incubator, the cells were washed three times with 2 mL of phosphate-buffered saline and then incubated with the test compounds in 2 mL of Eagle's salt solution supplemented with 5.2 mM D-glucose/25 nM Hepes buffer, pH 7.2. In some cases DMSO was added to increase the solubility of various agents in the culture medium. Control cultures were incubated in 2 mL of Eagle's salt solution in the absence of the test compounds. An appropriate amount of DMSO was added to the control cultures if DMSO was used with the test compounds.

The cells were incubated for 30 minutes and then exposed to ultraviolet light (UVA, 320-400 nM) emitted from a bank of four BLB fluorescent light tubes (F40BL/Sylvania) placed approximately 10 cM above the cell culture plates. After this light exposure, the cells were rinsed with phosphate-buffered saline and submitted for assay of epidermal growth factor binding. The cells were then treated with a constant ammount of ^{125}I epidermal growth factor and incubated for 30 minutes at 37°C. The cells were then solublized with a 0.2 M solution of sodium hydroxide and then analyzed with an ^{125}I counter to

evaluate the ^{125}I-EGF binding. A plot of percent activity bound to cells versus concentration was utilized to determine th IC_{50} of the agent.

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Vita

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