COMBINATORIAL LIBRARIES OF STILBENE FUSED CHALCONE AND FLAVANONE DERIVATIVES: SYNTHESIS AND ANTI-PROLIFERATIVE PROPERTIES

A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in Chemistry

by

İsmail AKÇOK

July 2009
İZMİR

We approve the thesis of **İsmail AKÇOK**

Assist. Prof. Dr. Ali ÇAĞIR
Supervisor

Prof. Dr. Serdar ÖZÇELİK
Committee Member

Assoc. Prof. Dr. Oğuz BAYRAKTAR
Committee Member

09 July 2009

Prof. Dr. Levent ARTOK
Head of the Chemistry Department

Prof. Dr. Hasan BÖKE
Dean of the Graduate School of
Engineering and Science

ACKNOWLEDGEMENTS

First of all, I would like to thank to my advisor Assistant Prof. Dr. Ali ÇAĞIR who always gave encourage, made me more systematic person and guided me whenever I need a pathfinder. It is a big honour to work such a wonderful person. I grateful him to provide me opportunity to work his research group and to complete my master studies.

I would also like to thank to State Planning Organization for financial support for this project (DPT-2003K120690 (DPT10)). Next, I thank Specialist Özgür Yılmazer and Biotechnology and Bioengineering Central Research Laboratories for their help and support during the biological activity tests.

I would like to thank my labmates for their invaluable help and the kind in the laboratory. Also I would like to thank all of my friends in IYTE.

I would like to special thanks to "*My Angel*" for her unfailing encouragement, and never ending friendship.

My parents... They are my all. They were always with me even I failed sometimes. They are the ones who always beside me with their pray however far from me. I want to biggest thanks to my father Mehmet and my mother Kamuran. This job is yours!

To my family...

ABSTRACT

COMBINATORIAL LIBRARIES OF STILBENE FUSED CHALCONE AND FLAVANONE DERIVATIVES: SYNTHESIS AND ANTI-PROLIFERATIVE PROPERTIES

Flavonoids and stilbenes have attracted great attention as potential pharmaceuticals. Up to date more than 5000 natural flavonoid derivatives have been isolated from natural resources. Flavonoids are most commonly known for their anti-oxidant activity. Additionally they might show various biological activities such as antibacterial, antiviral, anti-fungal, topoisomerase I and telomerase inhibitors, antiangiogenesis etc. Stilbenes are another class of compounds, which are also isolated from natural sources having anti-cancer, anti-inflammatory, blood sugar lowering and beneficial cardiovascular activities.

In this study, synthesis of stilbene fused chalcone and stilbene fused flavanone systems were aimed. By means of that, it might be possible to produce a compound which can be used for at least two different biological activity or they might show a single activity with a great enhancement.

For this purpose, a series of chalcone, flavanone, stilbene, stilbene fused chalcone, and stilbene fused flavanone derivatives were synthesized. Synthesis of stilbene fused chalcones and stilbene fused flavanones were accomplished by two different pathways. Two small combinatorial libraries of stilbene fused chalcones and stilbene fused flavanones have been built starting from four different acetophenones and four different styrenes. Preliminary anti-tumor activities of selected examples against human mammary adenocarcinoma cells (MCF-7) and human prostate cancer cell lines (PC3) were also evaluated.

ÖZET

STİLBEN KAYNAŞTIRILMIŞ ÇALKON VE FLAVANON TÜREVLERİNİN TÜMLEŞİK KÜTÜPHANELERİ: SENTEZ VE ANTİ-PROLİFERATİF ÖZELLİKLER

Flavonoidler ve stilbenler potansiyel ilaç olma özelliklerinden dolayı büyük ilgi çekmişlerdir. Günümüze kadar doğal kaynaklardan izole edilmiş beş binden fazla flavonoid tanımlanmıştır. Flavonoidler genellikle antioksidan özellikleriyle bilinmektedirler. Bunun yanında antibakteriyel, antiviral, mantarları yok eden, topoizomeraz I ve telomeraz inhibitörleri olarak ve kanser çevresindeki yeni damar oluşumlarının engellenmesi gibi çeşitli biyolojik aktiviteler gösterebilmektedirler. Stilbenler de doğadan izole edilen ve antikanser, kan şekerini düşürücü, iltihap giderici ve kalp ve damar hastalıklarına karşı aktiviteleri olan diğer bir grup bileşiklerdir.

Bu çalışmada stilben kaynaştırılmış çalkon ve stilben kaynaştırılmış flavanon sistemlerinin sentezleri amaçlanıştır. Bu sayede en az iki farklı biyolojik aktiviteye sahip ya da tek bir biyolojik aktiviteyi büyük bir potansiyel ile gösterebilen maddelerin üretilmesi beklenmektedir.

Bu amaçla, bir seri çalkon, flavanon, stilben ve stilben kaynaştırılmış çalkon ve flavanonların sentezi tamamlandı. Bu maddeler arasından, stilben kaynaştırılmış çalkonlar ile stilben kaynaştırılmış flavanonlar iki farklı yöntemle sentezlendi. Dört farklı asetofenon ve dört farklı stirenden başlanarak, stilben kaynaştırılmış çalkonlar ve stilben kaynaştırılmış flavanonlardan oluşan iki küçük kombinatoryal kütüphane oluşturuldu. Bu kütüphanelerden seçilen bazı örnek bileşiklerin meme kanseri hücre hattı (MCF-7) ve prostat kanseri hücre hattına (PC3) karşı antitümör aktiviteleri değerlendirildi.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Cancer is a group of diseases which is characterized by unregulated cell growth, invasion or spread of cells from the site of origin (or primary site) to other sites in the body. Cancer cells are characterized by six hallmarks; growth signal autonomy, evasion of growth inhibitory signal, evasion of apoptosis, unlimited replicative potential, angiogenesis, invasion and metastasis. (Hanahan and Weinberg, 2000) Each hallmark distinguishes cancer cells from normal cells by the following statements.

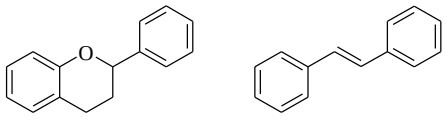
- Growth signal autonomy: Normal cells acquire external growth factors to divide, whereas cancer cells are not dependent on this external stimulus.
- Evasion of growth inhibitory signals: Normal cells response to inhibitory signals to provide their growth blockage. However cancer cells do not respond to inhibitory signal, so cancer cells become immortal.
- Evasion of apoptosis: Apoptosis is defined as programmed cell death. A normal cell can end its life by apoptosis if there is irreversible damage, but cancer cells evade apoptotic signals.
- Unlimited replicative potential: Normal cells have a device which defines a finite number of cell doublings. This cellular counting device is shortening the chromosomal ends, telomeres that occur during every round of DNA replication. However cancer cell maintain the length of telomeres. Unlimited replicative potential caused by the altered regulation of telomere maintenance.
- Angiogenesis: Normal cells depend on blood vessels to supply oxygen, and the vascular architecture is more or less constant in adult's body. However, cancer cells induce angiogenesis which is the formation of new blood vessels to help carrying more foods to survive and expand.
- Invasion and metastasis: Normal cells maintain their location in body, and they do not change their place. Although the mechanism is not well understood, cancer cells can migrate to other parts of the body and this circumstance can be the major cause of death of cancer patients. (Pecorino 2005)

There can be many factors which can be blamed for the formation of cancers such as tobacco, radiation, chemicals or infectious organisms which are external factors. Also there are some internal factors like inherited mutations, hormones, and immune conditions. These factors may act together or in sequence, can initiate or promote carcinogenesis. Today there are many different cancer treatment strategies. For example cancer is treated by surgery, chemotherapy, biological therapy, hormone therapy, radiation and targeted therapy. (American Cancer Society 2009)

Chemotherapy is the treatment of cancer by pills, containing chemical ingredients. These drugs might be taken before or after surgery. Drugs might be taken with other type of therapy (e.g. radiotherapy) or alone. Patient may get chemotherapy once a day, once a week, or even once a month, depending on the type of cancer the patient has and the medicine the patient is taking. The length of the chemotherapy depends on the type of cancer, and how she/he responds to the drugs. (American Cancer Society)

Many useful drugs have been discovered from various plants and they are excellent sources for the treatment of many diseases, and cancer is one of them. There are many therapeutic agents to treat cancer, which are synthetic or semi-synthetic. Beside, many naturally occurring biologically active compounds were also identified and clinically used against cancer. Natural products or their semi-synthetic analogues constitute approximately 74% of all new chemical entities marketed as anticancer drugs between 1981 and 2006. (Newman and Cragg 2007) For this reason, in the war with cancer, isolation, synthesis and evaluation of the new compounds against cancer is one of the important tools.

Flavonoids **(1)** and stilbenes **(2)** are among the best known natural products which may possess a wide range of biological activity. Because of their valuable role in biological systems more than 5000 flavonoid derivatives either were isolated from natural products or synthesized in laboratories.



1 **2**

Figure 1.1. Basic flavonoid **(1)** and stilbene **(2)** structures.

Flavonoids **(1)** are class of compounds which are secondary metabolites of plants. (Figure 1.1) They are best known for their antioxidant activity. Beside their antioxidant activity they have other health benefits, and they can help to reduce the risk of having diseases like cancer and heart disease.

Flavonoids are derived from the two basic metabolites malonyl-CoA and p-coumaroyl-CoB. Three molecules of malonyl-CoA and one molecule p-coumaryl-CoB produce secondary metabolite chalcone **(3)** intermediate which can be classified as a member of flavonoids. Chalcones have 2 phenolic rings and open three carbon chains. Intramolecular cyclization of the chalcone **(3)** gives another member of flavonoid class of substance named as flavanone **(4)**. Then a series of enzymatic modifications occur to yield dihydro-flavonols **(5)**, leuco-anthocyanins **(6)**, antho-cyanidins **(7)**, antho-cyanins **(8)**, flavones **(9)**, flavonols **(10)**, flavan-3-ols **(11,12)** and some other phenolics. (Figure1.2)

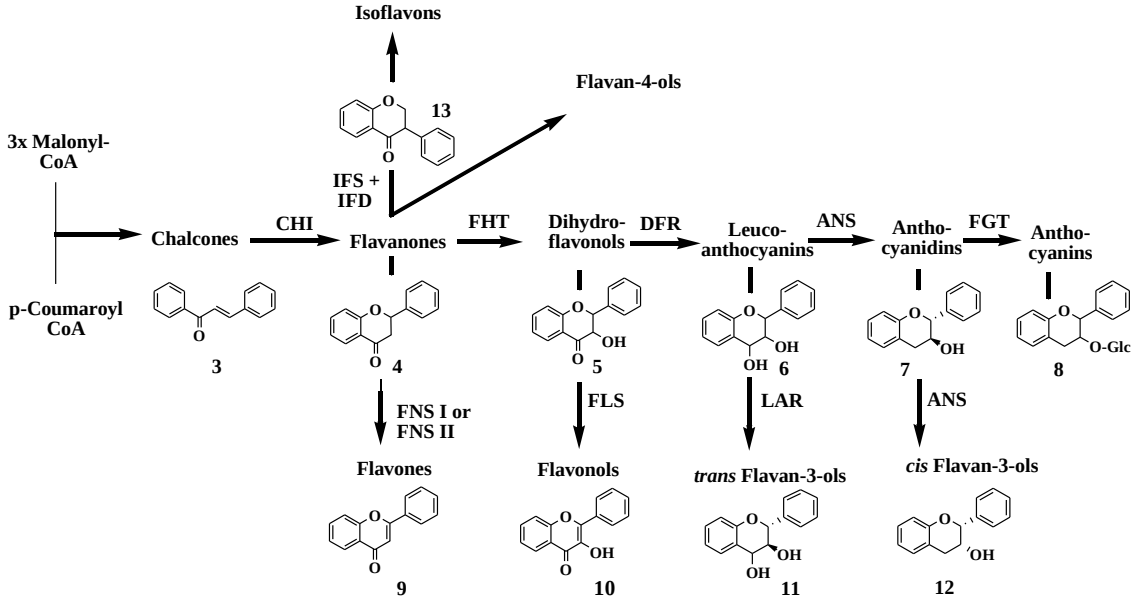


Figure 1.2. Biological pathways for the biosynthesis of flavonoids.

In 1995, Linuma and co-workers isolated six interesting compounds, alopecurones A-F **(14-19)**, from the extracts of the roots of *Sophora alopecuroides.* These compounds were classified as flavanostilbenes because they are composed of flavanones condensed with stilbene. (Linuma, et al. 1995)

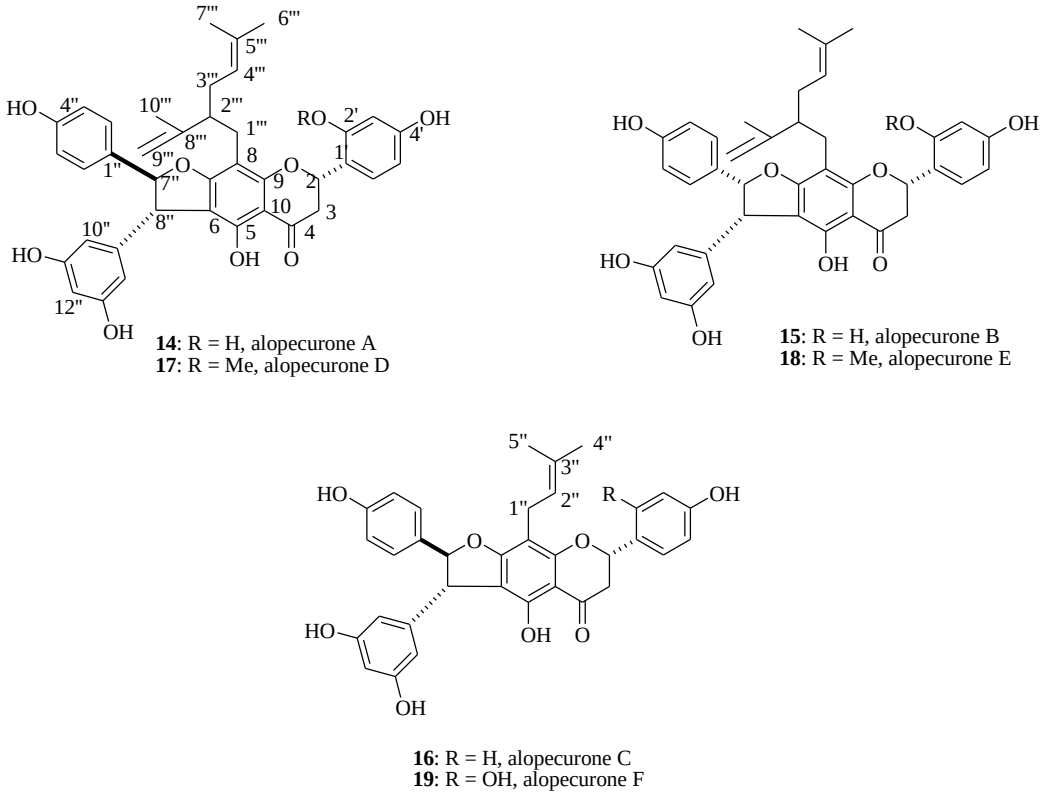


Figure 1.3. Six flavanostilbenes isolated from the *Sophora alopecuroides* **(14-19)**.

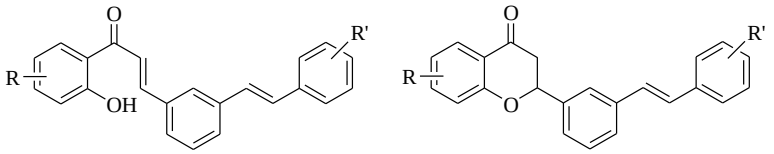
Antibacterial activity of three isolated flavanostilbenes (alopecurones A-C, **14, 15, 16**) evaluated on the strains of *Stphylococcus aureus* which are known for their methicillin-resistant property. Results showed that flavanostilbenes uniformly inhibited the growth of 21 strains of *Stphylococcus aureus* with minimum inhibitory concentrations in the range of 3.13-6.25 μg/ml. (Sato, et al. 1995)

In pharmaceutical industry, design and synthesis of dual acting drugs, dual acting enzyme inhibitors are very important. There are several advantages to define such compound which inhibits two different enzyme rather than two compounds instead, one compound for each enzyme.

First of all, with two drugs, two separate synthesis, two formulations and two separate metabolism studies will be necessary. Second, two drugs may have different pharmacokinetics rates and metabolic profiles so it might be difficult to adjust the concentrations to optimum in the same time intervals. Third, the changes that both drugs would progress to the clinic at the same rate are small. Fourth, number of clinical trials and safety studies for both compounds will be expensive. Lastly, the probability for a single drug just starting clinical trials to be afforded for the drug market is 1:10, probability for two compound is 1:100. (Silverman 2004)

As it is mentioned above, polyphenols such as flavonoids and stilbenes have variety of biological activities. Somehow, *sophora alopecuroides* combined these two functional groups in the same structure, which possess antibacterial property. In these structures the double bond and the planarity of the stilbene get lost, and it might be highly possible that biological systems might not recognize this part of molecule as stilbene anymore.

In this work, it is aimed to combine (to fuse) the stilbene structure into either to a chalcone or a flavanone without any lost in their structural features. By means of this, target molecules can carry the biological properties of stilbene-flavanones, or stilbene-chalcones at the same time. (Figure 1.4)



20 **21**

Figure 1.4. Proposed structures of stilbene fused chalcones **(20)** and flavanones **(21)**.

To better understand the importance of the analogues, it is necessary to look at the biological activities of chalcones, stilbenes and flavanones separately. Rest of the introduction will discuss the biological activities and synthesis methodologies of these structures. Although it is not possible to list all of these compounds and their biological activities, few of them will be represented as a short summary.

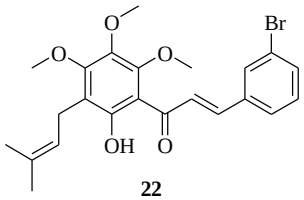
1.1 Chalcones

Chalcone **(3)** is an aryl styryl ketone that forms the central core for a variety of important biological compounds. Chalcones are secondary metabolites of terrestrial plants, and precursor for the synthesis of flavonoids. They can be isolated from many different plants. In literature plenty of studies exist showing the isolation of the chalcones from natural sources. (Adesanwo, et al. 2009, Van Puyvelde, et al. 1989).

1.1.1. Biological Activities of Chalcones

1.1.1.1. Anti-invasive Activity

Invasion is one of the hallmarks of malignant tumours and generally leads to metastasis, which is the major cause of cancer death. Anti-invasive property of chalcone 1-[2-hydroxy-3-(3-methyl-2-butenyl)-4,5,6,-trimethoxyphenyl]-3-(3-bromophenyl) propenone **(22)**, was investigated against human MCF-7/6 mammary carcinoma cells. (Figure 1.5) It is found to be effective in inhibiting the invasion of cancer cells onto normal cells at 10 μM concentration, which makes it as a lead compound for further studies to design new analogues showing anti-invasive activity. (Mukherjee, et al. 2001)



22

Figure 1.5. Structure of 1-[2-hydroxy-3-(3-methyl-2-butenyl)-4,5,6,-trimethoxyphenyl]-3-(3-bromophenyl)propenone **(22)**.

1.1.1.2. Antimalarial Activity

Nowadays malaria is again becoming a health problem to deal with because of the development of resistance by lethal causative parasitic species, *plasmodium falciparum* to the drugs. Boyom et al. isolated bartericin A **(23)**, stipulin **(24)**, 4-hydroxylonchocarpin **(25)** from *Dorstenia barteri var. subtriangularis,* and compound **23**, **24** and **25** were evaluated for their antimalarial activity in vitro against *P. Falciparum* and it is found that they are all effective against malaria with IC_{50} values of 2.15, 5.13 and 3.36 μM, respectively. (Boyom, et al. 2007)

A similar study was done by Mishra et al. A series of chalcone derivatives were synthesized and tested against human malarian parasite and *plasmodium falciparum* in vitro. Among the tested compounds, chalcones **26-28** were the most active compounds with the IC_{50} values 2.93, 2.5 and 1.50 μM respectively. (Mishra, et al. 2008)

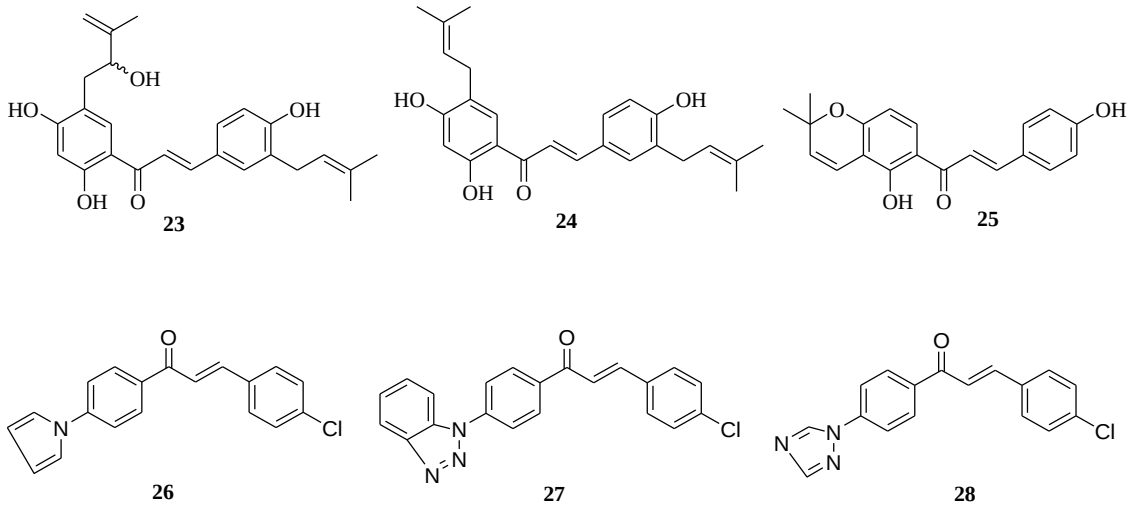
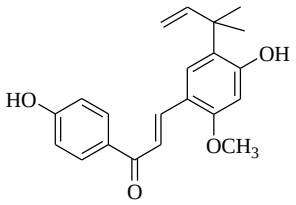


Figure 1.6. Anti-malarian active chalcone derivatives, bartericin A **(23)**, stipulin **(24)**, 4-hydroxylonchocarpin (**25)** and other chalcones **(26-28)**.

1.1.1.3. Antibacterial Activity

Tuberculosis is a disease caused by *Mycobacterium tuberculosis* which is the main fatal infectious diseases that mainly infect women of reproductive age. Licochalcone A is one of the naturally occurring chalcone that inhibits the *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium kansasii, Mycobacterium marinum and Mycobacterium xenophii* with MIC < 20 mg/L. (Nowakowska 2007)



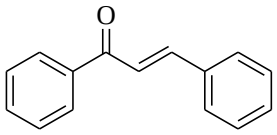
Licochalcone A

29

Figure 1.7. Structure of licochalcone A showing antibacterial activity.

1.1.1.4. Trypanocidal Activity

Trypanosoma cruzi is a parazite which causes sleeping sickness. Many derivatives of chalcones were synthesized by Lunardi et al. and tested against anti-trypasomes activity. Unsubstituted chalcone **(3)** was found to be the most active compound with IC_{50} at 24.8 μM. (Lunardi, et al. 2003)

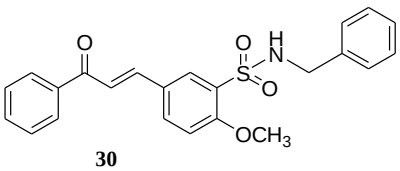


3

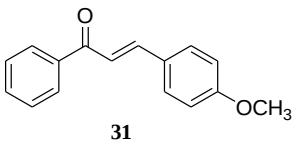
Figure 1.8. Structure of chalcone **(3)** showing trypanocidal activity.

1.1.1.5. Antileishmanial Activity

Leishmanial is an infectious disease that affects millions of people. Fröhner and co-workers synthesized sulphonamide derivatives of 4-methoxychalcone and tested for their antileishmanial activities to overcome unsatisfactory treatment protocols.(Figure 1.9) Results showed that compound **30** is the most active sulphonamide chalcone derivative, and all tested sulphonamide chalcones show higher activity compared to their chalcone equivalent 4-methoxychalcone **(31)**. IC_{50} values for compounds **30** and **31** are 3.5 μM and 16.6 μM respectively. (Fröhner, et al. 2009)



30



31

Figure 1.9. Structures of 4-methoxy-sulfonamidechalcone **(30)** and 4-methoxychalcone **(31)**.

1.1.1.6. Antiviral Activity

Virus infectious can cause many diseases. Antiviral acting compounds can be useful in the treatment of these diseases. AIDS is one of the fatal diseases caused by HIV (human immunodeficiency virus). Wu et al. demonstrated that compound **32** showed potent anti-HIV activity. (EC_{50} = 0.022 μg/ml) Beside this, Uchiumi et al. found that licochalcone A **(29)**, licochalcone B **(33)** and tetrahydroxymethoxychalcone **(34)** have transcription suppression effect on HIV promoter region. (Uchiumi, et al. 2002)

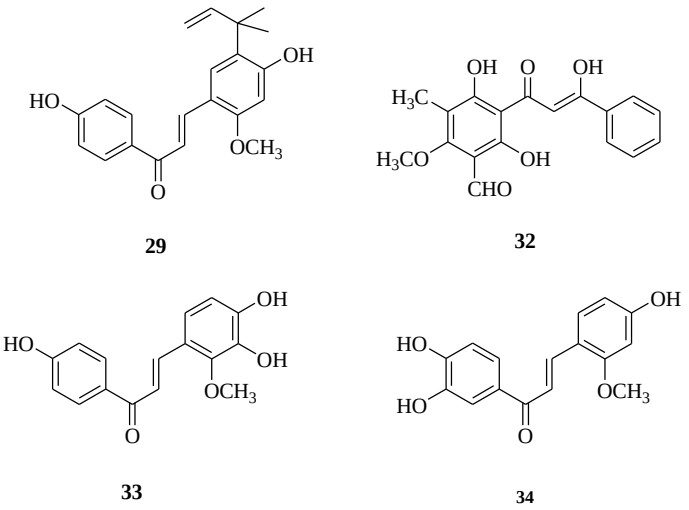


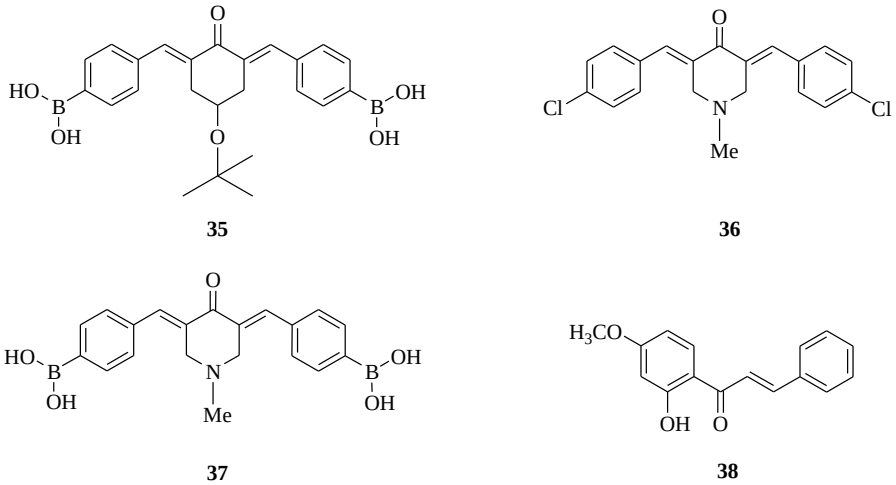
Figure 1.10. Structures of antiviral chalcones: **(29** and **32-34)**.

1.1.1.7. Anticancer Activity

In literature large number of chalcones were synthesized and evaluated for their possible anticancer activity. As an example, Modzelewska and colleagues synthesized several chalcone and bis-chalcone derivatives. (Figure 1.11) Antiproliferative properties of three bis-chalcones **35-37** were evaluated on the wild type of MCF-7 breast cancer cell lines at nanomolar concentrations. Especially compound **37** has IC_{50} value at 350 nM. (Modzelewska, et al. 2006)

Another similar study was carried out by Lee and colleagues. They evaluated the antitumor activity of 2'-hydroxy-4'-methoxychalcone **(38)** *in vivo* and *in vitro.* It is

found that, compound **38** shows antiproliferative activity on cultured pulmonary artery endothelial cell lines and its IC_{50} concentration was found as 12.7 µM. (Lee, et al. 2006)

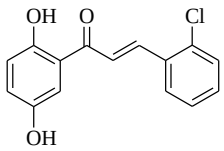


35 **36** **37** **38**

Figure 1.11. Structures of chalcones **(35-38)** having antiproliferative properties.

1.1.1.8. Antiangiogenic Activity

Angiogenesis is formation of new blood vessels and it is essential for a cancer cell to survive. The inhibition of angiogenesis is important to prevent proliferation of cancer cells. Nam et al. synthesized 2',5'-dihydroxychalcones and evaluated its cytotoxicity on different tumor cell lines. Among the tested compounds the most effective one was (E)-3-(2-chlorophenyl)-1-(2,5-dihydroxyphenyl)prop-2-en-1-one **(39)** on A431 human epidermoid carcinoma cell lines, with IC_{50} concentration at 0.03 µM.(Figure 1.12) This compound also exhibited strong inhibitory effects on the HUVEC tube formation in an in vitro model. When administered into BDF1 mice bearing Lewis lung carcinoma cells at 50 mg/kg.day, **39** was found to inhibit the growth of tumor mass by 60.5%. (Nam, et al. 2003)

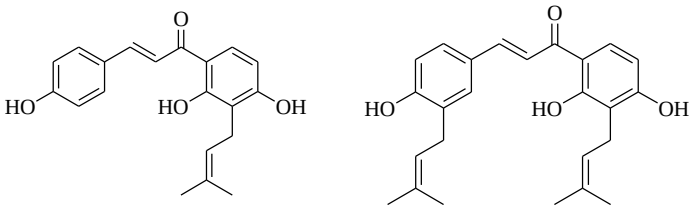


39

Figure 1.12. Structure of 2-chloro-2',5'-dihydroxychalcone **(39)**.

1.1.1.9. Antimicrobial Activity

Mbaveng et al. evaluated the antimicrobial activity of crude extracts of *Dorstenia barteri*. Isobavachalcone **(40)** and kanzonol C **(41)**, isolated from crude extract, prevented the growth of all tested microorganisms effectively. (Figure 1.13) (MIC value of 0.3 μg/ml for compound **40** on the six bacteria of tested twenty-two.) (Mbaveng, et al. 2008)



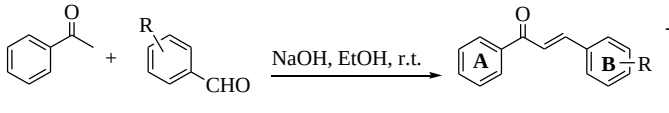
40 **41**

Figure 1.13. Structures of isobavachalcone **(40)** and kanzonol C **(41)**.

1.1.2. Synthesis of Chalcones

There is a huge library of chalcones in literature either isolated from nature or synthesized by a chemist in a laboratory. There are variety of methods to synthesize chalcones in literature. Base catalyzed Claisen-Schmidt condensation is the most widely used method and mostly carried out in the presence of strong bases such as NaOH, $Ba(OH)_2$ and KOH.

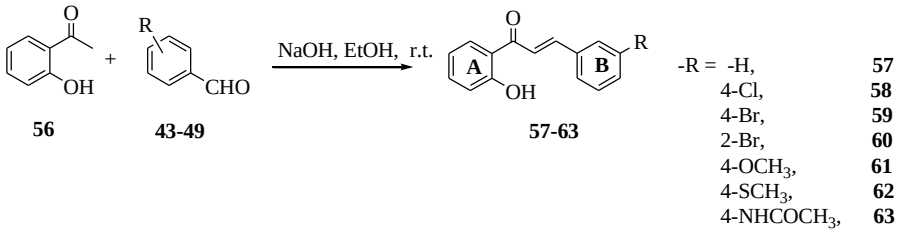
A series of chalcones **(3, 50-55)** and 2-hydroxychalcones **(57-63)** have been synthesized by Cabrera et al., starting from acetophenone and substituted benzaldehyde via base catalyzed aldol condensation. In the process, acetophenone or 2-hydroxyacetophenone was reacted with corresponding aldehydes. Chalcone derivatives (Figure 1.14.) and 2-hydroxychalcone derivatives (Figure 1.15.) having substitution on ring B, were prepared starting from substituted benzaldehyde in high yields. (Cabrera, et al. 2007) This method can tolerate wide range of substituents on the aryl rings.



42 **43-49** **3, 50-55**

-R = -H, **3**
4-Cl, **50**
4-Br, **51**
2-Br, **52**
4-OCH_3, **53**
4-SCH_3, **54**
4-$NHCOCH_3$, **55**

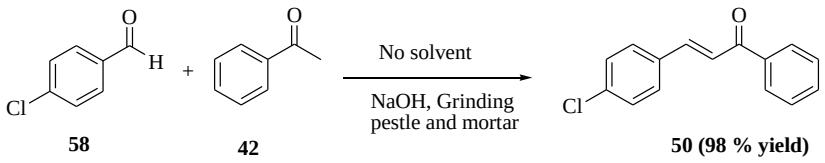
Figure 1.14. Synthesis of chalcone derivatives **(3, 50-55)** under basic conditions.



56 43-49 57-63

Figure 1.15. Synthesis of 2-hydroxychalcone derivatives **(57-63)** under basic condition.

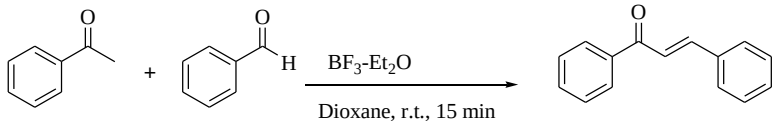
In 1990, Toda et al. has worked on the aldol condensation in the absence of solvent. They observed that in solvent free condition some aldol reactions proceeded more efficiently. Acetophenone **(42)** and 4-chlorobenzaldehyde **(58)** were grinding in the presence of NaOH by pestle and mortar to give chalcone **50** with 98% yield. (Toda, et al. 1990)



58 42 50 (98 % yield)

Figure 1.16. Synthesis of chalcone derivative **(50)** in the absence of solvent under basic conditions.

Acid catalyzed Claisen-Schmidt condensations are also useful reactions to prepare chalcones. $AlCl_3$, HCl, $TiCl_4$ are well known catalysts in these reactions. A similar acid catalyzed reaction was developed by Narender and Reddy. (Figure 1.17) Borontrifluoride-etherate complex was used as Lewis acid. It is known that, reaction time is long (2-3 days) and side reaction probability is high in hydroxide catalyzed reactions. Contrarily very short reaction times and very high reaction yields were reported for BF_3-Et_2O catalyzed Claisen-Schmidt reactions. No side reaction was observed. Also there are other advantages of this method, such as solvent–free reaction can occur in liquid reactants. BF_3-Et_2O can tolerate the presence of amine and ester functionalities, and chalcones can be synthesized in high yields. In summary this method is also very efficient for chalcone synthesis. (Narender and Reddy 2007)



42 43 3

Figure 1.17. Synthesis of chalcone **(3)** catalyzed by BF_3-Et_2O.

In another work, L-proline was utilized as an organocatalyst for the synthesis of substituted chalcones and flavanones. Chalcones and flavanones were formed simultaneously in this method. Procedure is very simple, 2-hydroxyacetophenone derivatives **(56, 64, 65)** and benzaldehyde **(43)** were stirred in the presence of L-proline (30 mol %) in DMF (0.02 M) at 80 ^{o}C for 18 h. After the usual work up and chromatography, chalcones **(57, 66, 67)** were furnished. (Figure 1.18) (Chandrasekhar, et al. 2005)

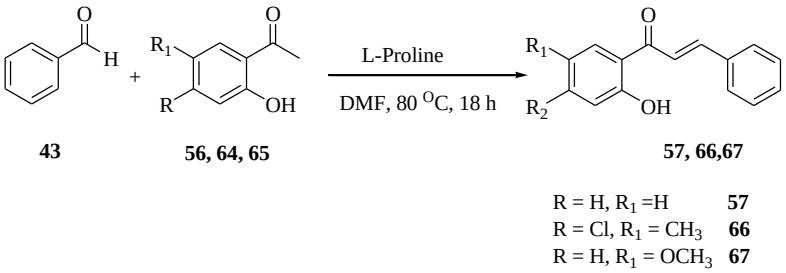


Figure 1.18. L-proline catalyzed synthesis of chalcones **(57, 66, 67)**.

As an another interesting study, Comisar and Savage studied on crossed aldol condensation in high-temperature water (HTW). High-temperature water (HTW) is defined as liquid water above 200 ^{o}C. Benzaldehyde **(43)** and acetophenone **(42)** was reacted at 200 ^{o}C, 250 ^{o}C, and 300 ^{o}C and the maximum yield of chalcone **(3)** was reported as 21% at 250 ^{o}C after 15 hours of reaction time. (Figure 1.19) (Comisar and Savage 2004)

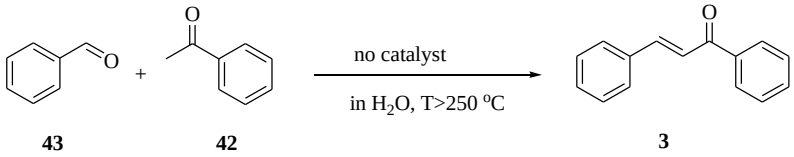


Figure 1.19. High-temperature of water (HTW) catalyzed synthesis of chalcone **(3)**.

Alternatively, bamboo char sulfonic acid, a novel solid catalyst, was prepared and used in the synthesis of chalcones under solvent free conditions. (Figure 1.20) Yields of reactions were relatively high. (60-82%) (Xu, et al. 2008)

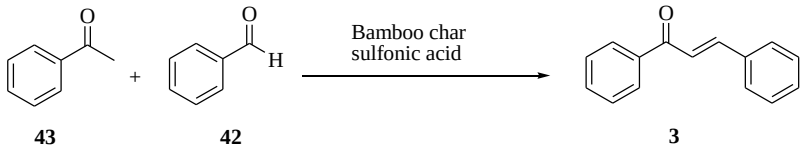


Figure 1.20. Synthesis of chalcone **(3)** in the presence of bamboo char sulfonic acid.

1.2. Flavanones

Flavanones **(4)** are one of the subclasses of flavonoids, having strong antioxidant activity and generally formed by intramolecular Michael addition type cyclization of 2-hydroxychalcones. (Figure 1.21)

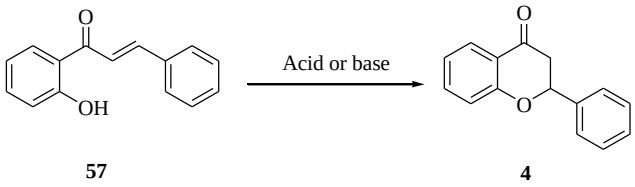


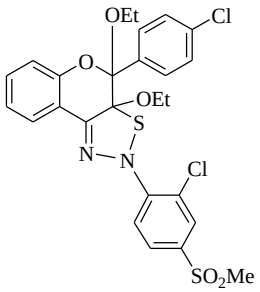
Figure 1.21. 2-hydroxychalcone **(57)** as precursor of flavanone **(4)**.

Like chalcones, flavanones also have variety of biological activities, such as antileukemia, antibacterial, aromatase inhibitor activity. These biological activities will be discussed in the following sections.

1.2.1. Biological Activities of Flavanones

1.2.1.1. Antileukemia Activity

Qiaojun He et al. demonstrated that newly synthesized flavanone derivative 2-(2-chloro-4-(methylsulfonyl)phenyl)-4-(4-chlorophenyl)-3,4-diethoxy-3,4-dihydro-2H-chromeno[4,3][1,2,3]thiadiazole (MSFTZ, figure 1.22) **(68)** inhibits the proliferation of leukemia cells. It is also shown that MSFTZ induces apoptosis in HL-60 leukemia cells with IC_{50} value of 1.60 μM. (He, et al. 2006)

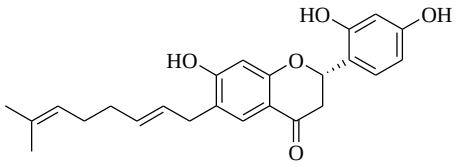


68

Figure 1.22. Structure of MSFTZ **(68)**.

1.2.1.2. Antibacterial Activity

Sepicanin A (Figure 1.23) is a flavanone which is isolated from *Artocarpus sepicanus*. Sepicanin A showed significant selectivity against methillicin–resistant *Staphylococcus aureus* (MRSA) with IC_{50} concentration at 1.4 μM and MIC at 2.9 μM. (Radwan, et al. 2009)

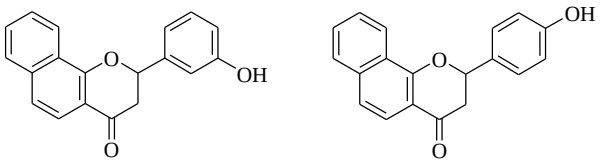


69

Figure 1.23. Structure of sepicenin A **(69)**.

1.2.1.3. Aromatase Inhibitory Activity

Aromatase is an enzyme and plays an important role in development of breast cancer. For that reason it is important to develop aromatase inhibitors to treat the breast cancer. Structures of two new benzophenone derivatives of flavanone (**70**,**71**) are shown in figure 1.24. Activities of these two compounds were reported as nine times more potent than aminogluthetimide which was the first clinically used aromatase inhibitor. (IC_{50} = 0.61 μM for compound **70** and IC_{50} = 0.63 μM for compound **71**). (Yahiaoui, et al. 2007)

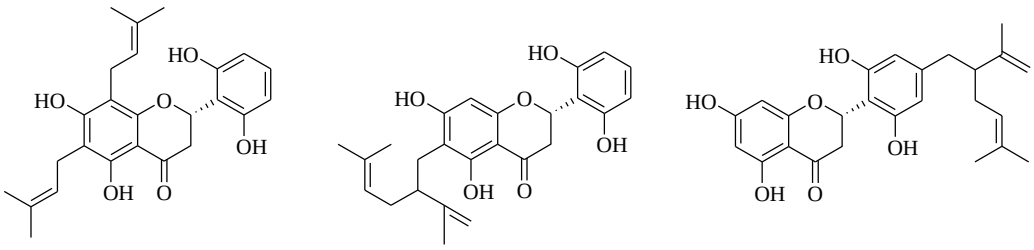


70 **71**

Figure 1.24. Derivatives of 7,8-benzoflavanones (**70**,**71**) as potent aromatase inhibitors.

1.2.1.4. Anticancer Activity

One of the well known biological activities of flavanones is anticancer activity. There are known isolated and synthetic flavanones which show potent anticancer activity. Peng et al. isolated six flavanones from the leaf of *Patrinia villosa* Juss, and evaluated their anticancer activities in vitro. Three of them (**72, 73, 74**) exhibited strong anticancer activity to K562 (human erythromyeloblastoid leukemia), HT29 (human colon adenocarcinoma) and MCF7 (human breast adenocarcinoma) cell lines. (Figure 1.25) Reported IC_{50} values of compound **72** against tested cancer cell lines were 1.64, 2.42 and 3.04 μM respectively. Those values for compound **73** were 4.08, 5.28 and 4.26 μM, and for compound **74** were 5.72, 7.94 and 7.62 μM respectively. (Peng, et al. 2006)



72 **73** **74**

Figure 1.25. Structures of flavanones isolated from *Patrinia villosa* Juss.

1.3.1.5. Metastasis Inhibition Activity

Beside anticancer activity metastasis inhibition effects of flavanones were also reported in literature. (Hsiao, et al. 2007) Antiproliferative activity and metastasis inhibition potential of flavanone **(4)** and 2'-hydroxyflavanone **(75)** were

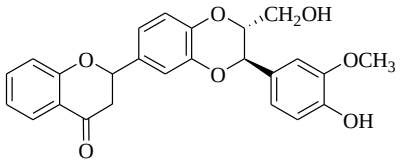
evaluated.(Figure 1.26) Tested compounds show little influence on cell viability, but inhibit the invasion and metastasis of A549 cells.

4 **75**

Figure 1.26. Structures of flavanone **(4)** and 2'-hydroxyflavanone **(75)**.

1.3.1.6. Chemoprevention Activity

Silibinin **(76)** is a flavanone which is isolated from the fruits of milk thistle. (Figure 1.27) It has strong anticancer efficacy against human androgen-dependent and independent human prostate carcinoma cell cultures. (Zi, et al. 1998, Zi, et al. 1999) Silibinin strongly inhibits cell growth and DNA synthesis, and cause apoptotic cell death in rat prostate cancer cell. (Tyagi, et al. 2002)



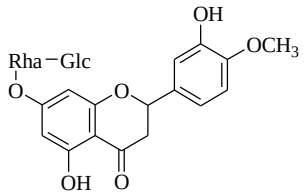
76

Figure 1.27. Structure of silibinin **(76)**.

1.3.1.7. Antioxidant Activity

Antioxidants play important roles to protect the cells being damaged from radicals. Chronic disease (cancer or heart disease) risks can be reduced by taking antioxidants. (Stanner, et al. 2003)

Hesperidin **(77)** has a disaccharide attached flavanone structure, and it shows strong antioxidant activity with IC_{50} concentration at 11 µM.(Figure 1.28) (Rao, et al. 2003)



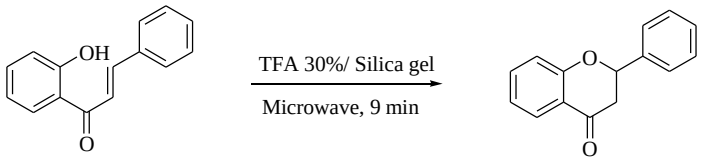
77

Figure 1.28. Structure of hesperidin **(77)** as an example of antioxidant flavanone.

1.3.2. Synthesis of Flavanones

It was mentioned that flavanones can be prepared from chalcones by Michael addition type cyclization reaction. Cyclization can be done under either acidic or basic catalyst. In literature, biosynthetic pathways are also reported for this transformation. Different approaches toward the asymmetric syntheses of flavanones are also present. Reactions, giving flavanone structures, will be discussed briefly in this section.

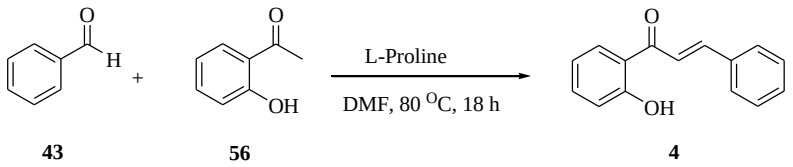
One of the simplest transformations of 2-hydroxychalcones to flavanone is refluxing in glacial acetic acid as remarked by Cabrera and colleagues. (Cabrera, et al. 2007)(Figure 1.29)



57 4

Figure 1.29. Acid catalyzed synthesis of flavanone **(4)** starting from 2-hydroxychalcone **(56)**.

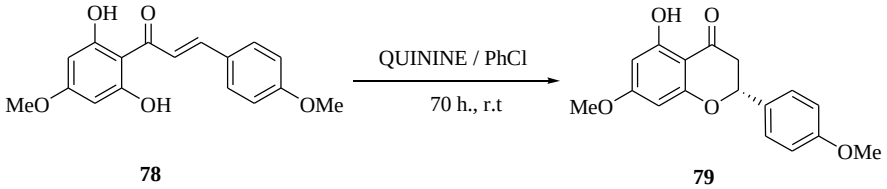
As discussed in chalcone synthesis L-proline is an effective catalyst for the Claisen-Schmidt reaction. In this reaction flavanones were reported as major products.(Figure 1.30) The ratios of flavanones to chalcones changes by the reactants but roughly it gives 7:3 mixtures of flavanone and chalcone. (Chandrasekhar, et al. 2005)



43 **56** **4**

Figure 1.30. L-proline catalyzed synthesis of flavanone **(4)**.

Asymmetric cyclization of 2'-hydroxychalcone to flavanone is enzyme-catalyzed step in biosynthesis of flavonoids. This reaction occurs easily by enzyme catalyst, but it is quite difficult to perform same reaction with a non-enzyme catalyst. There are studies to develope such catalysts in the literature. For example cinchona alkaloids used as a chiral Bronsted base catalyst for such asymmetric cyclization reaction. (Figure 1.31) Cinchonine (CN), cinchonidine (CD), quinine (QN), and quinidine (QD) were also used as asymmetric catalyst for cyclization of 2-hydroxychalcones, and up to 64% enantiomeric excess was observed in these trials. (Dittmer, et al. 2007)



78 **79**

Figure 1.31. Example for asymmetric synthesis of flavanone **(79)**.

Katsuyama et al. synthesized flavanones in *Esherichia coli.* (Figure 1.32) Their artificial biosynthetic pathway includes: p-coumaroyl **(80)** synthesis from p-coumaric acid **(81)** by coenzyme A ligase (4CL), and chalcone **(82)** synthesis from p-coumaroyl by chalcone synthase enzyme (CHS), then synthesis of asymmetric (2S) flavanones **(83)** from chalcones by chalcone isomerise (CHI). (Katsuyama, et al. 2007)

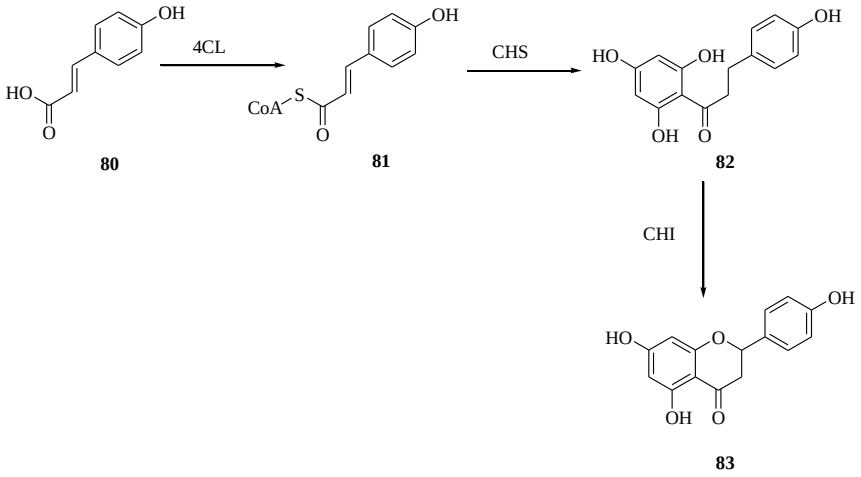


Figure 1.32. Artificial biosynthetic pathway for the synthesis of flavanone **(83)**, reported by Katsuyama.

Syntheses of flavanones under solvent-free conditions were performed by Sagrera et al. by using microwave irradiation of chalcones. Reactions were carried out in unmodified household microwave oven. Irradiation of chalcones with 30% trifluoroacetic acid over silica gel furnished flavanones in high yields. (Sagrera, et al. 2005)

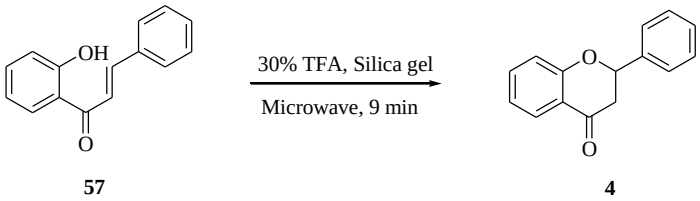
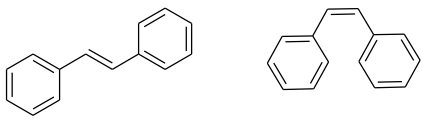


Figure 1.33. Microwave accelerated synthesis of flavanones **(4)**.

1.3. Stilbenes

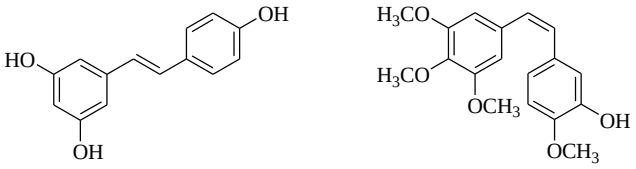
Stilbenes (diarylethenes) are a class of biologically active components, isolated from nature that possesses various medicinal properties. Two isomers of stilbene exist, which are (E)-stilbene **(84)** and Z-stilbene **(85)**. (Figure 1.34)



84 **85**

Figure 1.34. Structures of (E)-stilbene **(84)** and (Z)-stilbene **(85)**.

The most famous stilbenes are resveratrol **(86)** and combretastatin A-4 **(87)**. Both molecules are naturally occurring, and biologically active compounds. (Nam 2003)



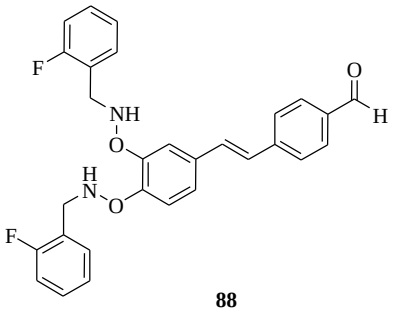
86 **87**

Figure 1.35. Structures of resveratrol **(86)** and combretastatin A-4 **(87)**.

1.3.1. Biological Activities of Stilbenes

1.3.1.1. Antioxidant Activity

Stilbenes, well known for their antioxidant activity, are found in grapes, red wine, berries, and peanuts. Many studies were done on this subject, for example Jun and co-workers recently synthesized trans-stilbene derivatives and evaluated their anti-oxidant activities. Among the synthesized molecules, an amine derivative of stilbene **(88)** shows three times more potent antioxidant activity than resveratrol does. (Jun, et al. 2009, figure 1.36)

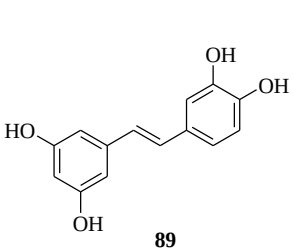


88

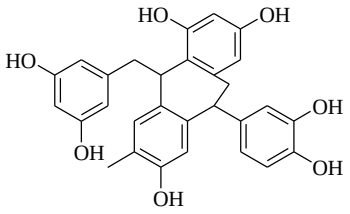
Figure 1.36. Structure of (E)-4-(3,4-bis(2-fluorobenzylaminooxy)styryl)benzaldehyde **(88)**.

1.3.1.2 Antitumor Activity

Three stilbene derivatives, (resveratrol **(86)**, piceatannol **(89)**, and cassigarol A (piceatannol dimer) **(90)**) were isolated from the medicinal plants and inhibited tumor growth and metastasis through the inhibition of tumor-induced angiogenesis. (Kimura, et al. 2000, 2000, 2001)



89



90

Figure 1.37. Stilbenes showing antitumor properties: piceatannol **(89)**, cassigarol A **(90)**.

1.3.2 Synthesis of Stilbenes

There are many reactions to form double bond functionality, and Wittig reaction is one of them.(Figure 1.38) Wittig reaction of phosphonium bromide **(91)** with benzaldehyde in THF in the presence of sodium hydride yields *cis* and *trans*-stilbene together, and *cis*-stilbene is the major product (66%) for this reaction. (Cushman, et al. 1991)

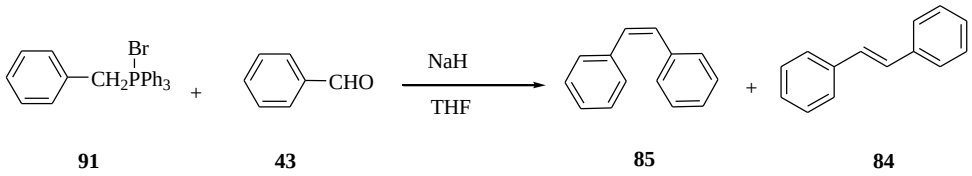


Figure 1.38. Synthesis of *trans* **(84)** and *cis* **(85)** stilbene by Wittig reaction.

Similarly, trans stilbene can be synthesized by Wittig-Horner reaction of phosphonate ester **(92)** with benazaldehyde in the presence of sodium methoxide as base and DMF as solvent with 87% yield.(Cushman, et al. 1991)

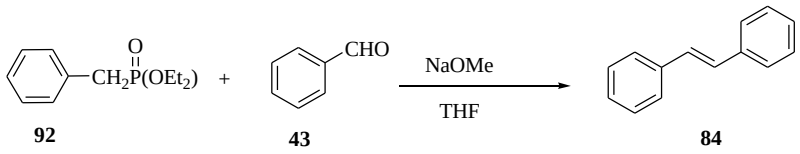


Figure 1.39. Synthesis of *trans*-stilbene **(84)** by Wittig-Horner reaction.

Stilbenes can also be synthesized by biosynthesis. As shown Figure 1.40. Hatsuyama and colleagues synthesized stilbenes from p-coumaroyl-CoA by stilbene synthase enzyme (STS). (Katsuyama, et al. 2007)

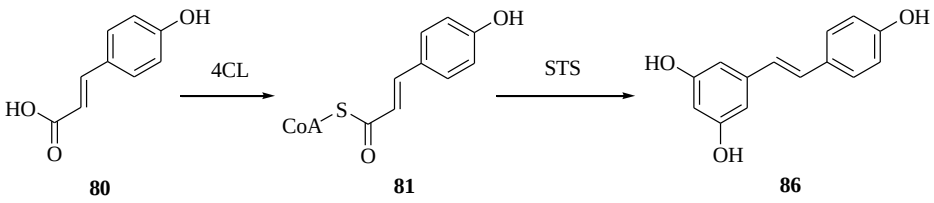
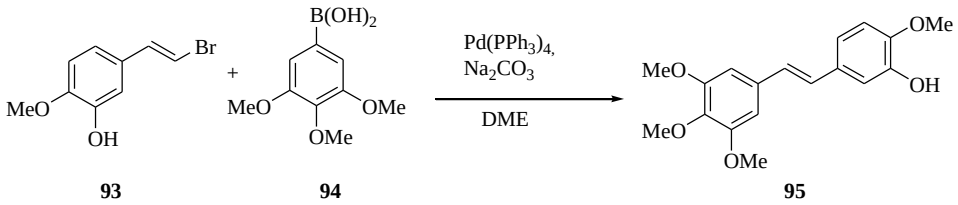


Figure 1.40. Synthesis of resveratrol **(86)** via biosynthetic pathway.

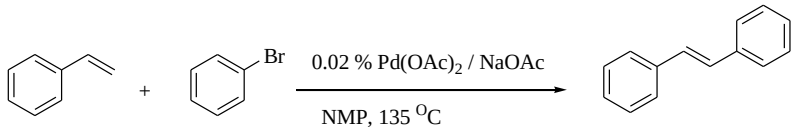
As an alternative, it is shown that *trans* isomer of combretastatin A-4 can be synthesized by using palladium catalyzed Suzuki cross coupling reaction of (E)-5-(2'Bromoethenyl)-2-methoxyphenol and 3,4,5-trimethoxybenzeneboronic acid. (Gaukrager, et al. 2001)



93 **94** **95**

Figure 1.41. Synthesis of trans-combretastatin A-4 **(95)** by Suzuki cross coupling reaction.

The last example for the synthesis of *trans*-stilbene derivatives is ligand-free palladium catalyzed Heck reaction of bromobenzene with styrene (Figure 1.42). In this method, Vries and colleagues used very low amount of palladium acetate in N-methyl pyrollidone, and synthesized *trans*-stilbene at high yields. (Vries, et al. 2003)



96 **97** **84**

Figure 1.42. Synthesis of trans-stilbene **(84)** by Heck reaction.

CHAPTER 2

RESULTS AND DISCUSSIONS

2.1. Toward the Synthesis of Stilbene Fused Chalcones and Flavanones

Pharmaceutical sciences need to develop compounds which show strong biological activity. The other important parameter is the availability of that compound. Biologically active compounds having complex structures, whose synthesis require large numbers of steps, consumes huge amount of time and resources.

On the other hand to develop biologically active compounds, synthesized by few step, is very important. By means of this, amount of time and resources spent to carry biologically active compound to the market could be much lower.

Flavanones, chalcones and stilbenes are relatively simple molecules and can be synthesized in one or maximum two steps, so preparing them in large quantities are not a big deal. As it is noted in introduction part, main aim of this thesis is to prepare the stilbene fused chalcones and flavanones, which are relatively simple molecules.

Retrosynthetic analyses of target molecules are shown in figure 2.1. Synthesis of either stilbene fused chalcone or stilbene fused flavanone can simply be enough, because one of them can be converted to other one in the presence of acids or bases.

It might be possible to synthesize stilbene fused chalcones and flavanones by two different routes. Both routes require Claisen-Schmidt reaction, Michael addition and Heck reaction steps. Only difference is the order of the reactions. In route A, chalcone synthesis is planned at the beginning then it will be converted to flavanone and finally stilbene will be fused to flavanones by Heck reaction.

Contrarily in route B Heck reaction is the first reaction, then formed stilbene will be transformed into chalcones and flavanones in the next steps.

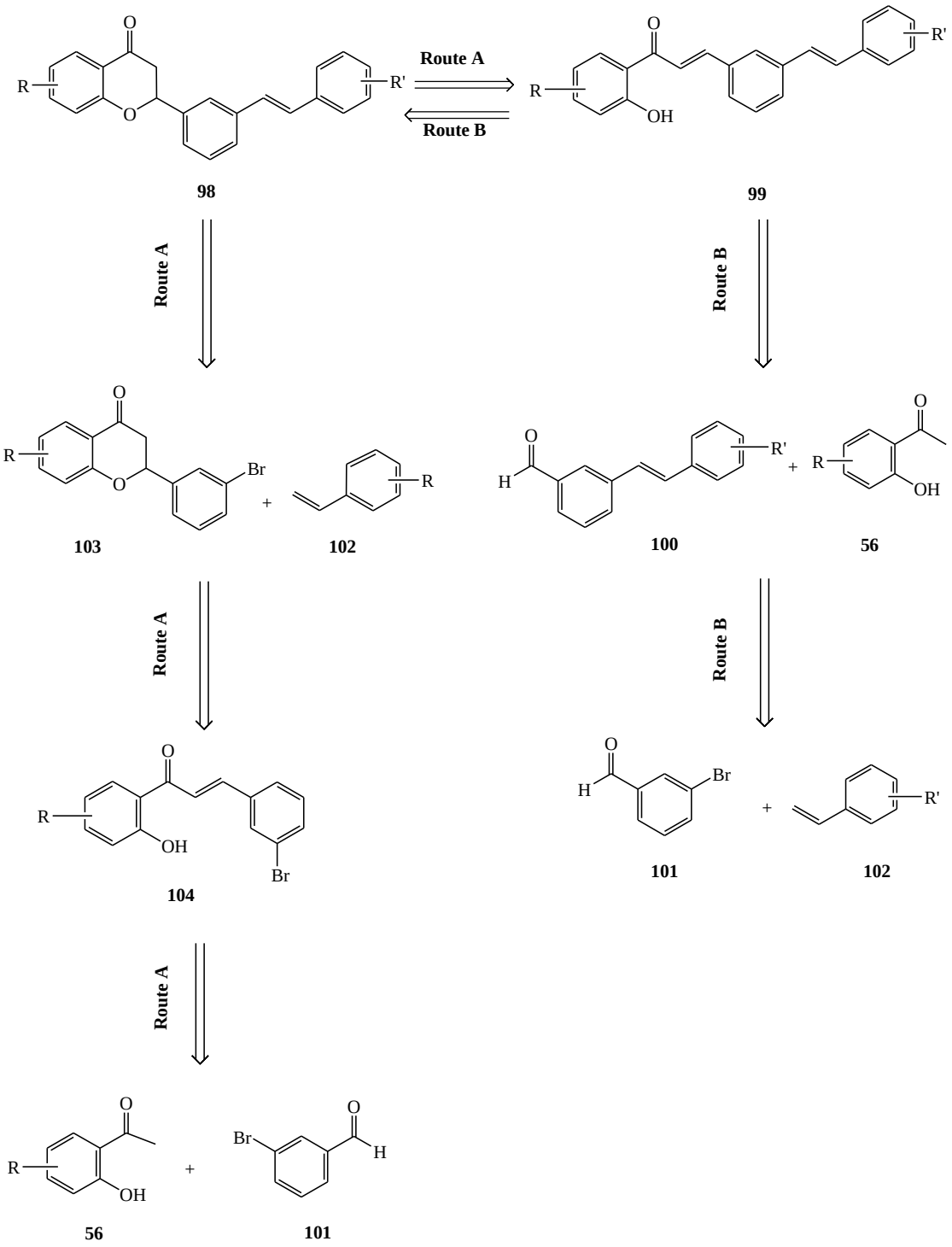


Figure 2.1 Retrosynthetic analysis of stilbene fused chalcone and flavanone system.

The main difference between the routes is limitation of the possible conditions for cyclization reaction of route B. Double bonds are kind of sensitive towards acids so using an acid catalyst might not be good in cyclization step of route B. On the other hand at Route A Heck reaction may cause a problem because it is well known that α,β-

unsaturated ketones are good substrates for Heck reactions. It is well known that Heck reaction should be carried out under basic condition which will cause an equilibrium reaction between flavanone and chalcone. Later chalcone can be a substrate for a self Heck reaction under basic conditions. By keeping these in mind, trials for the synthesis of target molecules started by route A.

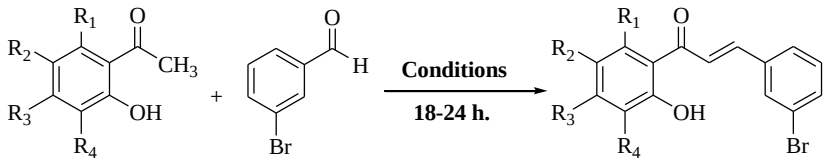
2.2 Route A: Heck Reactions of Flavanone Derivatives

Commercial available 3-bromobenzaldehyde has been chosen as a cornerstone in this work. It has the functionalities for both Heck and aldol condensation reactions. By keeping it still, different 2-hydroxyacetophenones are tried for aldol condensation reactions in sodium hydroxide containing ethanol at room temperature.

As it is listed in table 2.1 reactions gave the chalcone products in high yields (90-93%). All acetophenones, used in these trials, have electron donating substituents except entry 2.

A similar aldol condensation reaction was also tried in the presence of L-proline as catalyst (entry 6). This reaction gave 15% chalcone with 21% flavanone products. Although acetophenone has electron withdrawing group, yield was lower than expected in this trial. Hence L-proline catalyst was abandoned.

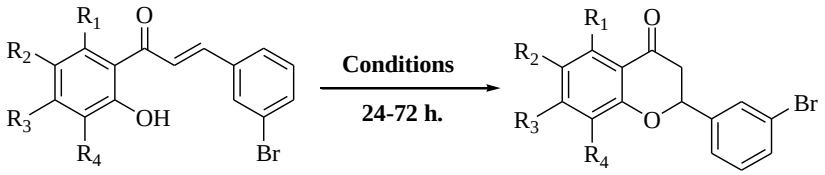
Table 2.1. Synthesis of chalcone derivatives **(110-115)**.



Entry	Acetophenone				Conditions	Product	Yield (%)
	R_1	R_2	R_3	R_4			
1	H	OCH_3	H	H	NaOH, EtOH, RT	**110**	90
2	H	Cl	H	H	NaOH, EtOH, RT	**111**	93
3	OCH_3	H	OCH_3	H	NaOH, EtOH, RT	**112**	92
4	OCH_3	H	H	H	NaOH, EtOH, RT	**113**	91
5	H	H	OH	CH_3	NaOH, EtOH, RT	**114**	90
6	H	CH_3	H	NO_2	L-Proline, DMF, 80^0C	**115**	15

In the second step, cyclizations of 2-hydroxy chalcones with intramolecular Michael reactions were studied under acidic and basic conditions. In these attempts, first 25 ml of glacial acetic acid were used for each mmol of 2-hydroxychalcone under reflux (Table 2.2). Except entry 2, all 2-hydroxychalcones are substituted by electron donating groups. All reactions gave the products with very good yields except the dimethoxy substituted chalcone case. Although there was not any reasonable explanation for this we continued the trials by base catalyzed cyclization reactions. Sodium acetate, anhydrous or trihydrate was used as catalyst for cyclization reactions in refluxing ethanol. Contrarily to trihyrated one, anhydrous sodium acetate gave product with 45% yield. This method was found much more useful compared to acetic acid method. Because of easier workup, requirement of small amount of catalyst and cleaner TLC results. Anhydrous sodium acetate was chosen as the best suited catalyst for this reaction and will be used in the rest of cyclization reactions in this thesis.

Table 2.2. Synthesis of flavanone derivatives **(116-120)**.

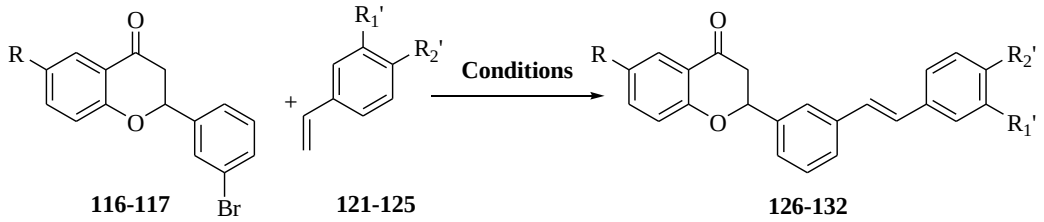


110-114 **116-120**

Entry	2-Hydroxychalcone				Conditions	Product	Yield (%)
	R_1	R_2	R_3	R_4			
1	H	OCH_3	H	H	Acetic acid, Reflux	**116**	79
2	H	Cl	H	H	Acetic acid, Reflux	**117**	85
3	OCH_3	H	OCH_3	H	Acetic acid, Reflux	**118**	No Rxn
4	OCH_3	H	H	H	Acetic acid, Reflux	**119**	90
5	OCH_3	H	H	H	NaOAc, EtOH Reflux	**119**	45
6	H	H	OH	CH_3	NaOAc.$3H_2O$, EtOH Reflux	**120**	No Rxn
7	OCH_3	H	OCH_3	H	NaOAc.$3H_2O$, EtOH Reflux	**118**	No Rxn

In the last step of route A, Heck reactions of synthesized flavanones with selected styrenes were performed in the presence of $Pd(OAc)_2$ with or without ligands. Previously Li and Wang showed that Heck reaction can successfully produces stilbene in a mixture of 1% $Pd(OAc)_2$ in triethanolamine at 100 ^oC. (Li and Wang 2006) Same procedure was applied to the synthesized flavanones in varying amount of palladium (II) acetate (entry 1-6). All reactions were monitored by TLC and formations of many new spots have been observed in all cases. Among these trials, purification of the product was successful for only first reaction after many attempts. (Table 2.3)

Table 2.3. Synthesis of stilbene fused flavanones **(126-132)**.



116-117 **121-125** **126-132**

Entry	R	R_1'	R_2'	Conditions	Product	Yield(%)
1	OCH_3	H	OCH_3	1% $Pd(OAc)_2$, $N(C_2H_4OH)_3$, 10 h.	**126**	44
2	Cl	H	OCH_3	0,1% $Pd(OAc)_2$, $N(C_2H_4OH)_3$, 15 h.	**127**	(Inseparable mixture)
3	OCH_3	CH_3	H	1% $Pd(OAc)_2$ / $N(C_2H_4OH)_3$, 14 h.	**128**	(Inseparable mixture)
4	Cl	H	CH_3	1% $Pd(OAc)_2$, $N(C_2H_4OH)_3$, 15 h.	**129**	(Inseparable mixture)
5	OCH_3	H	F	20% $Pd(OAc)_2$, $N(C_2H_4OH)_3$, 48 h.	**130**	(Inseparable mixture)
6	OCH_3	H	NH_2	4% $Pd(OAc)_2$, $N(C_2H_4OH)_3$, 48 h	**131**	(Inseparable mixture)
7	OCH_3	H	OCH_3	1% $Pd(OAc)_2$, 2%$P(Ph)_3$, Et_3N, CH_3CN 24 h.	**126**	(Inseparable mixture)
8	OCH_3	H	OCH_3	1% $Pd(OAc)_2$, 2%$P(Ph)_3$, Na_2CO_3, Toluene, 24 h.	**126**	(Inseparable mixture)
9	OCH_3	H	CH_3	1% $Pd(OAc)_2$, 2%$P(Ph)_3$, Na_2CO_3, Toluene, 24 h.	**132**	(Inseparable mixture)

Heck reactions of flavanones with styrene were also tried in presence of a ligand and a base (entry 7-9). Again all of them produced inseparable mixtures of products. For this reason this route was abandoned and further studies were concentrated on route B.

In these inseparable mixtures no product could be isolated and characterized to better understand the reason for the fail of Heck reaction with flavanone. We can only speculate that flavanones and chalcones can exist in equilibrium under basic conditions and chalcones can be a good substrate for Heck reaction. In such equilibrium α,β-unsaturated ketones are more active toward Heck reactions than the styrenes. So it is

possible to form a self Heck reaction of bromine substituted chalcones (Figure 2.2). Further similar reactions may produce many additional products.

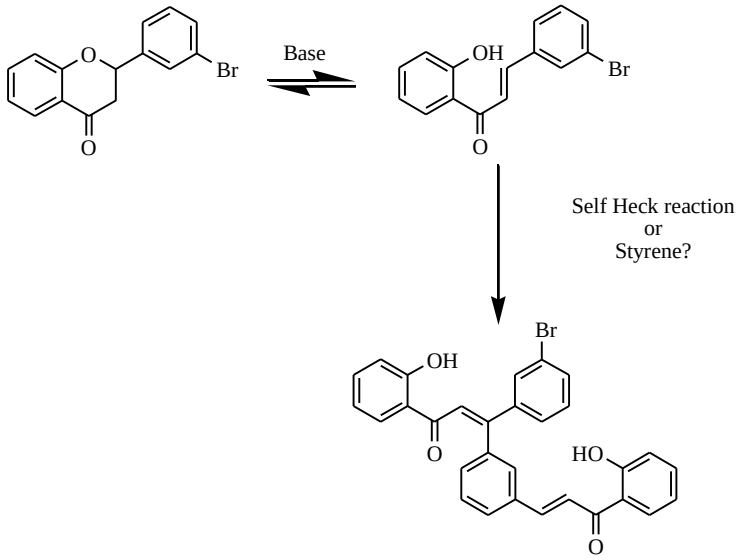


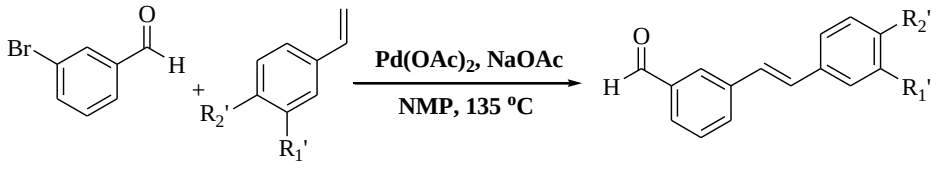
Figure 2.2. Possible self Heck reaction of chalcone.

2.3 Route B: Stilbene Formation as the First Step to Prepare Stilbene Fused Chalcone and Flavanone Libraries

Reaction conditions were almost optimized for aldol reaction and Michael Addition in route A experiments. Alkaline ethanol solution was quite suitable for aldol reaction, and sodium acetate solution in ethanol gave clear product formation in cyclization reaction. Because both conditions are basic, there is no harm to try the complete the synthesis starting from a stilbene.

On the other hand, it is necessary to optimize the Heck reaction conditions to complete the synthesis efficiently. For this purpose, modified Heck reactions, developed by Vries and co-workers, were used. This procedure requires only 0.02% palladium (II) acetate as catalyst and sodium acetate was used as base. Beside, no ligand was necessary. Reactions were carried out for the synthesis of five different stilbenes **(133-137)** in N-methyl pyrolidone at 135 ^{o}C. Except 4-aminostyrene **(125)**, rest of the reactions yielded target stilbenes in high yields (75-93%).

Table 2.4. Synthesis of stilbene derivatives **(133-137)** under ligand free conditions.



101 **121-125** **133-137**

Entry	Styrene	R_1'	R_2'	Product	Yield (%)
1	**121**	H	OCH_3	**133**	93
2	**122**	H	CH_3	**134**	80
3	**123**	CH_3	H	**135**	90
4	**124**	F	H	**136**	75
5	**125**	H	NH_2	**137**	No Rxn

After optimization the Heck reaction it is aimed to produce libraries of stilbene fused chalcones and flavanones. Stilbenes, formed from the Heck reactions, were used for this purpose. To complete the libraries four different acetophenones (2-hydroxy-5-methoxy acetophenone **(65)**, 5-chloro-2-hydroxy acetophenone **(105)**, 2-hydroxy-6-methoxy acetophenone **(107)** and 2-hydroxy-4-methoxy acetophenone **(138)**) were chosen. Starting materials for these combinatorial libraries were pictured in figure 2.3.

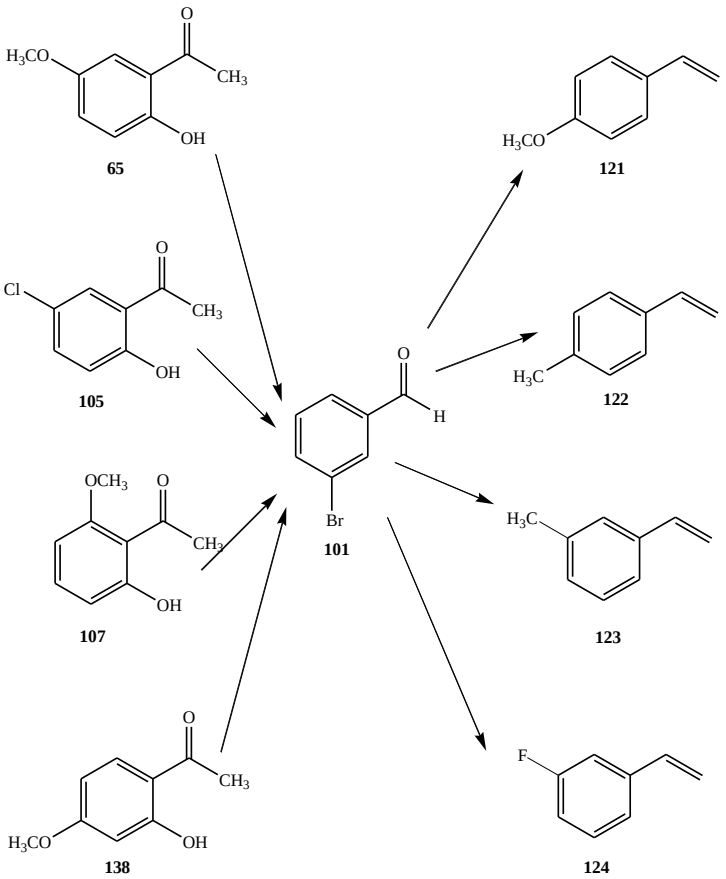


Figure 2.3. Selected ketones and styrenes for combinatorial libraries.

To complete the libraries, formed stilbenes were reacted with selected acetophenones **(65, 105, 107, 138)** in alkaline ethanol at room temperature. After 24 hours, reactions were quenched and products were purified in silica gel column chromatography. Isolated yields for these reactions were listed in table 2.5. It seems like, some of the yields are relatively low. Difficulty in purification step of formed chalcone from the starting materials, especially the acetophenones, was the main reason for these lower yields. Products of 2-hydroxy-4-methoxy acetophenone could not be

purified completely from acetophenone during silica gel column. Small amount of impurity (less than 5 %) were present in NMR spectrum. (Table 2.5)

In the last step, stilbene fused chalcones were divided into two parts. One portion of each stilbene fused chalcone treated with sodium acetate in ethanol to complete the cyclization reactions. Reactions were quenched after 48 hours and products were isolated by silica gel column in good yields. For these reactions isolated yields were given in table 2.6. Lower yields are resulted from the difficulties during the purification in flash chromatography. (Table 2.6)

Addition to combinatorial libraries, one more stilbene fused chalcone **(139)** and flavanone **(140)** were synthesized as shown in figure 2.4.

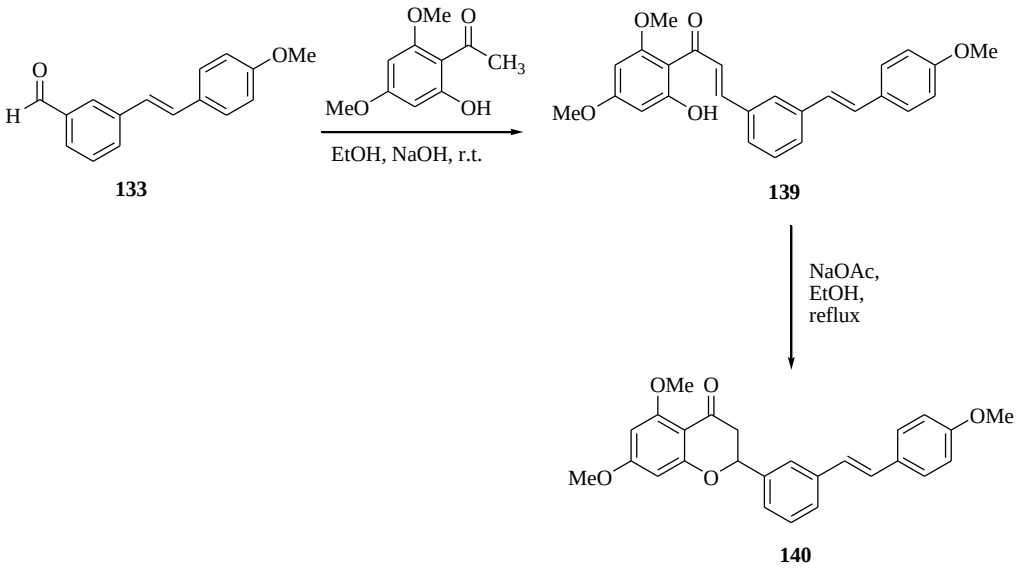
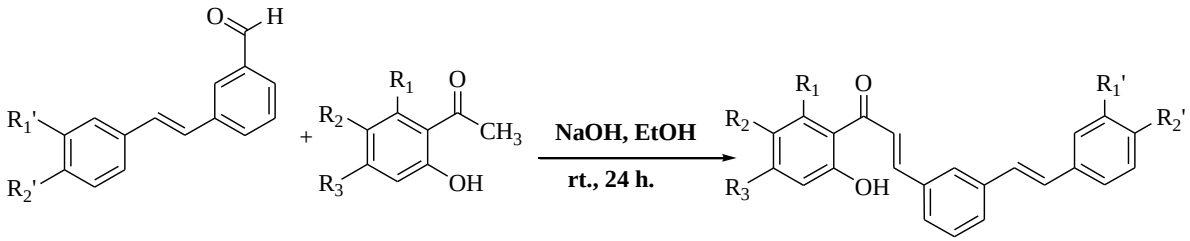


Figure 2.4. Synthesis of stilbene fused chalcone **(139)** and flavanone **(140)** substituted by two methoxy on ring A.

Aldol reaction of stilbene **(133)** with 2-hydroxy-4,6-dimethoxy acetophenone gave the stilbene fused chalcone **(139)** with 44% yield. Then it was transformed to stilbene fused flavanone **(140)** with 65% yield. Overall summaries for the libraries of stilbene fused chalcones and stilbene fused flavanones were reviewed in table 2.7 and table 2.8.

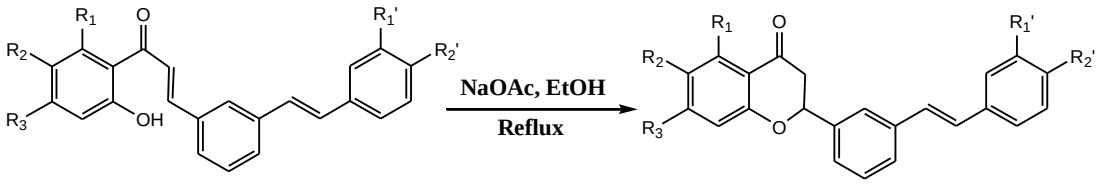
Table 2.5. Synthesis of stilbene fused chalcones **(141-156)**.



133-137 **65, 105, 107, 138** **141-156**

Entry	Stilbene		Acetophenone			Product	Yield (%)
	R_1'	R_2'	R_1	R_2	R_3		
1	H	OCH_3	H	OCH_3	H	**141**	80
2	H	CH_3	H	OCH_3	H	**142**	72
3	CH_3	H	H	OCH_3	H	**143**	10
4	F	H	H	OCH_3	H	**144**	57
5	H	OCH_3	H	Cl	H	**145**	62
6	H	CH_3	H	Cl	H	**146**	54
7	CH_3	H	H	Cl	H	**147**	41
8	F	H	H	Cl	H	**148**	23
9	H	OCH_3	OCH_3	H	H	**149**	55
10	H	CH_3	OCH_3	H	H	**150**	44
11	CH_3	H	OCH_3	H	H	**151**	78
12	F	H	OCH_3	H	H	**152**	77
13	H	OCH_3	H	H	OCH_3	**153**	20
14	H	CH_3	H	H	OCH_3	**154**	45
15	CH_3	H	H	H	OCH_3	**155**	56
16	F	H	H	H	OCH_3	**156**	27

Table 2.6. Synthesis of stilbene fused flavanones **(157-171)**.

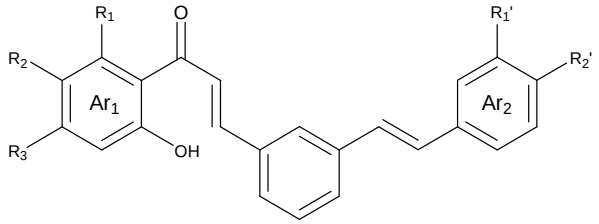


142-156 **157-171**

Entry	Compound	R_1	R_2	R_3	R_1'	R_2'	Product	Yield (%)
1	**142**	H	OCH_3	H	H	CH_3	**157**	73
2	**143**	H	OCH_3	H	CH_3	H	**158**	72
3	**144**	H	OCH_3	H	F	H	**159**	76
4[*i*]	**145**	H	Cl	H	H	OCH_3	**160**	63
5	**146**	H	Cl	H	H	CH_3	**161**	26
6	**147**	H	Cl	H	CH_3	H	**162**	76
7	**148**	H	Cl	H	F	H	**163**	77
8	**149**	OCH_3	H	H	H	OCH_3	**164**	79
9	**150**	OCH_3	H	H	H	CH_3	**165**	64
10	**151**	OCH_3	H	H	CH_3	H	**166**	61
11	**152**	OCH_3	H	H	F	H	**167**	56
12	**153**	H	H	OCH_3	H	OCH_3	**168**	68
13	**154**	H	H	OCH_3	H	CH_3	**169**	32
14	**155**	H	H	OCH_3	CH_3	H	**170**	73
15	**156**	H	H	OCH_3	F	H	**171**	49

i: Conditions of entry 4: Potassium tert-butoxide / EtOH, r.t. 24 h.

Table 2.7. Yields for library of stilbene fused chalcone systems **(139,141-156)**.



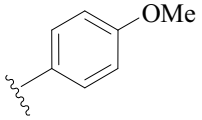
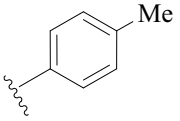
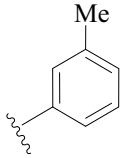
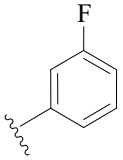
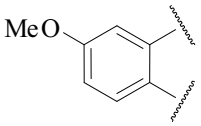
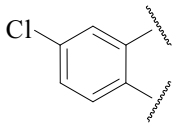
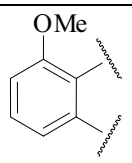
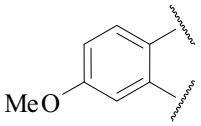
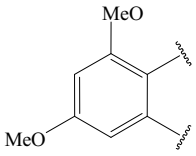
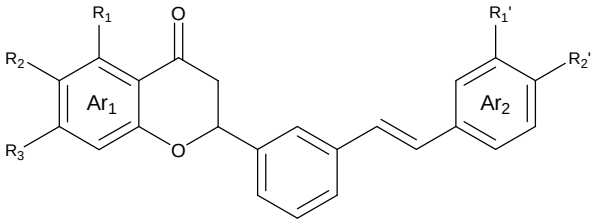
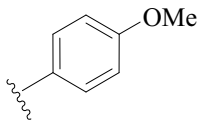
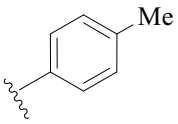
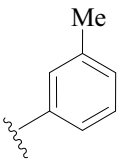
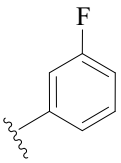
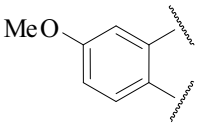
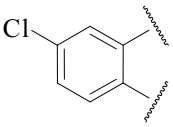
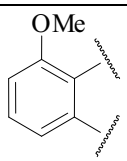
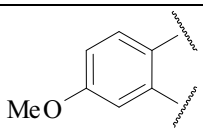
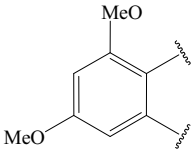
Ar_1 \ Ar_2				
	**141** (80%)	**142** (72%)	**143** (10%)	**144** (57%)
	**145** (62%)	**146** (54%)	**147** (41%)	**148** (23%)
	**149** (55%)	**150** (44%)	**151** (78%)	**152** (77%)
	**153** (20%)	**154** (45%)	**155** (56%)	**156** (27%)
	**139** (44%)			

Table 2.8. Yields for library of stilbene fused flavanone systems **(126,140, 157-171)**.



Ar1 \ Ar2				
	**126** (44%)	**157** (73%)	**158** (72%)	**159** (76%)
	**160** (63%)	**161** (26%)	**162** (76%)	**163** (77%)
	**164** (79%)	**165** (64%)	**166** (61%)	**167** (56%)
	**168** (68%)	**169** (32%)	**170** (73%)	**171** (49%)
	**140** (65%)			

2.5 Preliminary Cytotoxic Properties of Selected Stilbene Fused Chalcone and Flavanone Derivatives

Ultimate goal of this work is to combine two biologically active compounds in a way to produce a new one which has either one type of biological activity with more potency or multi-bioactivity. For this purpose synthesis of stilbene fused chalcones and

flavanones were completed. To show the multi-biological activity of synthesized compounds, it is necessary to test synthesized compounds in more than one possible target.

Two different set of biological activity tests for each library will require large amount of time. Instead it might be better to evaluate the efficiency of compounds in one type of biological activity as a starting point.

For this purpose one stilbene fused flavanone (**125)** was chosen and tested against MCF-7 and PC3 cancer cells. To compare the potency of selected compound, a chalcone **(110)**, a flavanone **(116)** and a stilbene **(133)** (Figure 2.5) were also prepared and tested against same cancer cell lines. As it is discussed earlier it was expected that fused product should have more potency than separate flavanone and stilbene. It might also have more potency compared the synergetic effect of selected stilbene **(133)** and flavanone **(116)**. Cytotoxic effects of the mixtures of these compounds were also evaluated against cancer cell lines at different concentrations. Test results, showing 24 hours incubation for PC3 and 48 hours incubation of PC3 and MCF-7 were given in figures 2.6.-2.8.

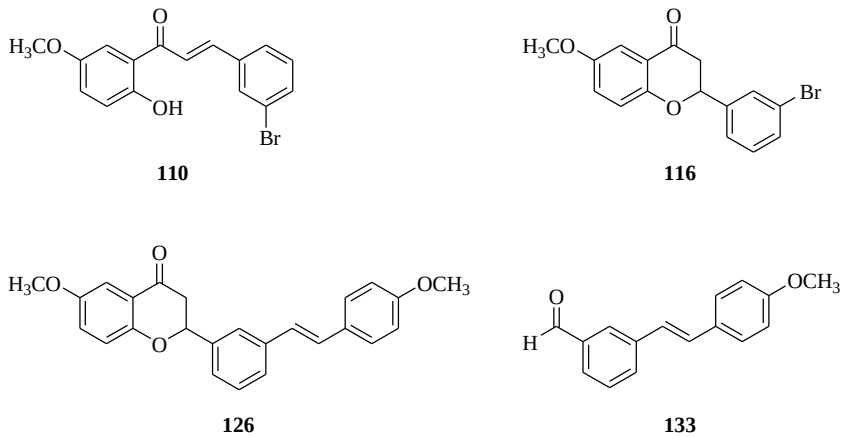


Figure 2.5. Selected chalcone **(110)**, flavanone **(116)**, stilbene fused flavanone **(126)** and stilbene **(133)** structures for cytotoxicity tests on PC3 and MCF-7 cancer cell lines.

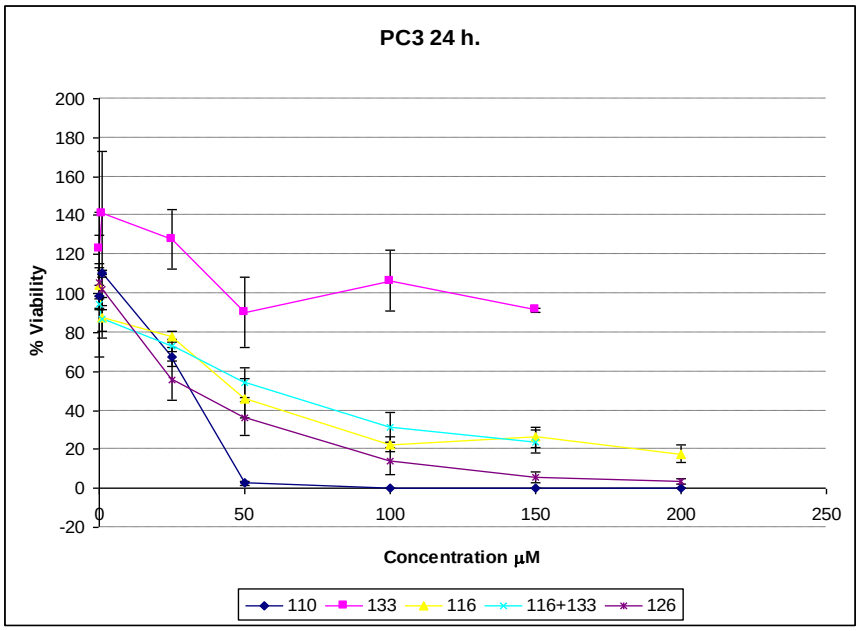


Figure 2.6. Dose dependent cytotoxicities of compounds **110**, **133**, **116**, **116**+**133** and **126** on PC3 cancer cell lines after 24 hours incubation.

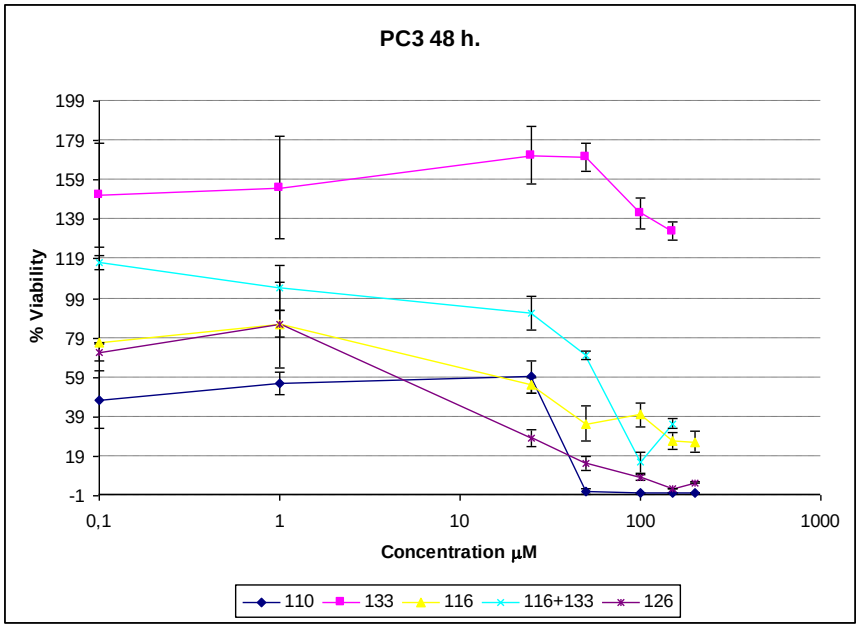


Figure 2.7. Dose dependent cytotoxicities of compounds **110**, **133**, **116**, **116**+**133** and **126** on PC3 cancer cell lines after 48 hours incubation.

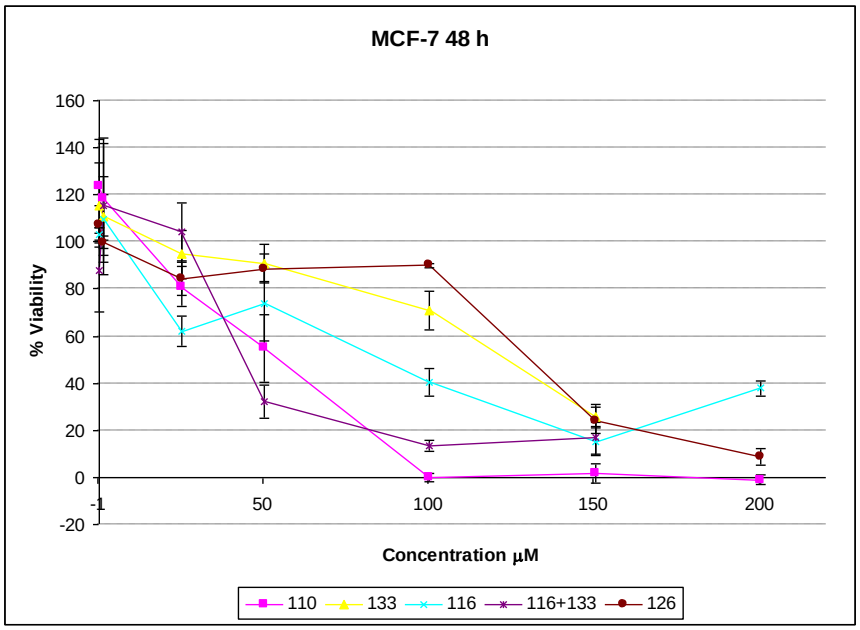


Figure 2.8. Dose dependent cytotoxicities of compounds **110**, **133**, **116**, **116**+**133** and **126** on MCF-7 cancer cell lines after 48 hours incubation.

It is clear from graphs that standard deviations are getting larger when the concentrations of the compounds are far below the cytotoxic levels.

Response of PC3 cells to the tested compounds after 24 hours shows that the most cytotoxic compound is chalcone **(110)**. Stilbene **(133)** produces no cytotoxicity at all tested concentrations. Additionally cytotoxicity levels of flavanone **(116)**, mixture of stilbene flavanone and stilbene fused flavanone **(126)** have similar trend, and levels of cytotoxicities were very close to that of chalcone **(110)**. After 48 hours potency of chalcone **(110)** is still the highest. On the other hand fused system clearly works better compared to flavanone and stilbene mixture at all tested concentrations. At 1 µM fused system **(126)** is still cytotoxic (85% cell viability), but mixture has no effect at all in these concentrations.

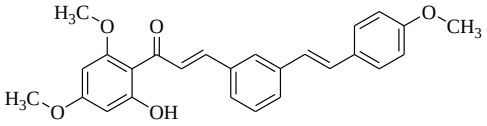
For MCF-7, stilbene **(133)** is quite cytotoxic at high concentrations (100-200 µM) but its fused form **(126)** only active at concentrations greater than 150 µM. Remaining tested compounds have similar trend with PC3. These early results imply that activity of stilbene fused flavanones have opposite trend compare to that of stilbene only.

After this short comparison of biological activity of the fused product with simple chalcone, flavanone and stilbene, more stilbene fused chalcones and flavanones were tested on PC3 and MCF-7 cell lines. Structures of tested compounds are given in figure 2.9 and 2.10.

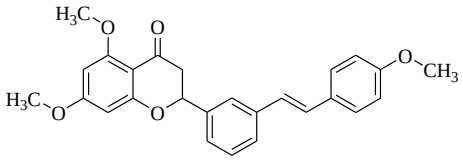
Among the tested samples, stilbene fused chalcones **139** and **156** and stilbene fused flavanones **140**, **158**, **160**, **167** and **171** have no cytotoxic effects on MCF-7 and PC3 cancer cell lines at tested concentrations below 50 μM.

On the other hand chalcones **145** and **141**, **143**, **149** and **152** are quite effective on PC3 at concentrations higher than 10 μM and on MCF-7 at concentrations higher than 25 μM. Especially chalcone **143** is highly cytotoxic at submicromolar concentrations for both cancer cell lines.

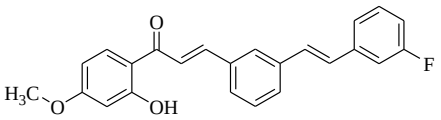
Although it is too early to talk about the complete structure activity relationship for the libraries, these preliminary cytotoxicity test results imply that stilbene fused chalcones are much more cytotoxic compared to their flavanone isomers. Activities of chalcones depend on substituents originated from styrenes and acetophenones.



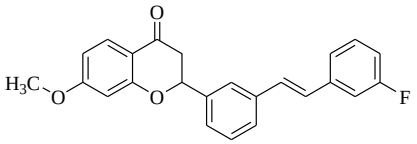
139



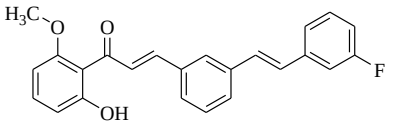
140



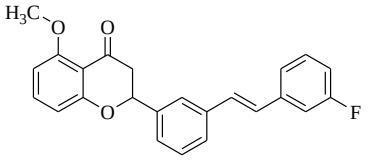
156



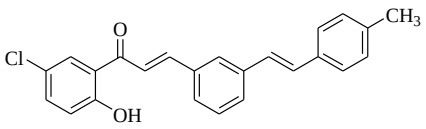
171



152

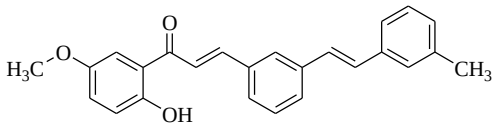


167

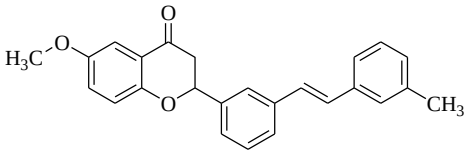


145

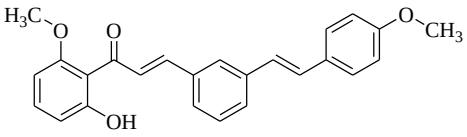
Figure 2.9. Structures of selected stilbene fused chalcones **(139,145,152** and **156)** and stilbene fused flavanones **(140,167** and **171)**.



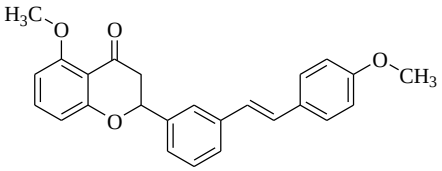
143



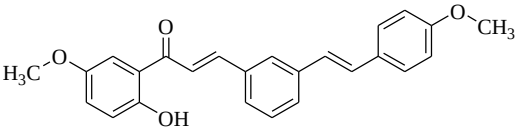
158



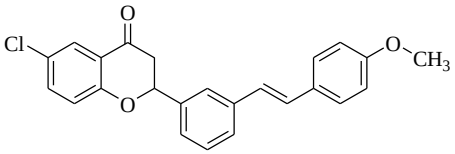
149



164



141



160

Figure 2.10. Structures of selected stilbene fused chalcones **(141,143** and **149)** and stilbene fused flavanones **(158,160** and **164)**.

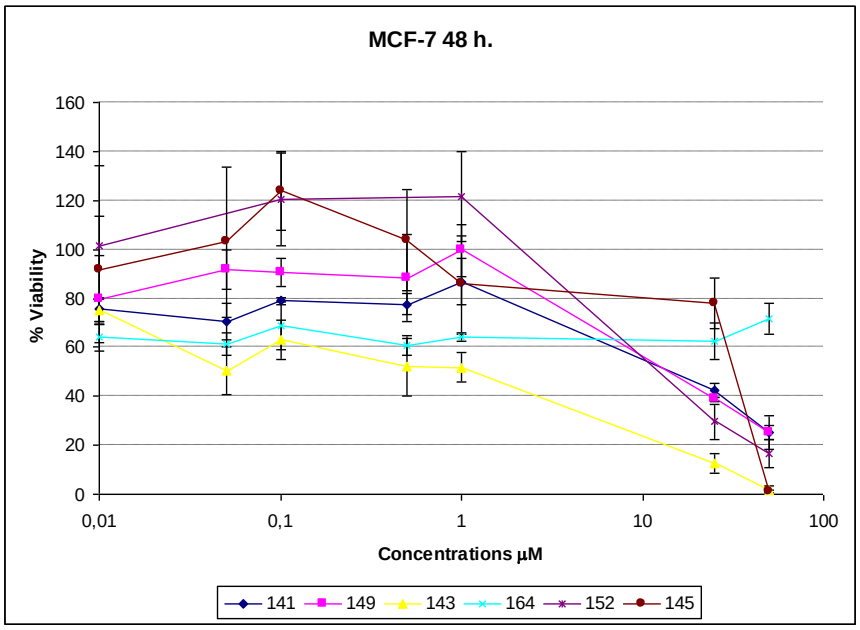


Figure 2.11. Dose dependent cytotoxicities of compounds **141**, **143**, **145**, **149**, **152** and **164** on MCF-7 cancer cell lines after 48 hours incubation.

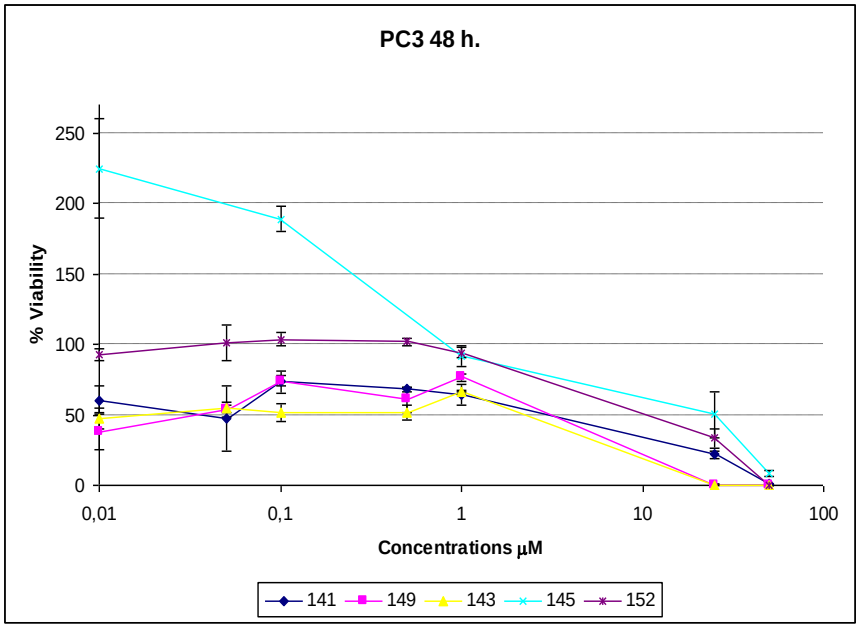


Figure 2.12. Dose dependent cytotoxicities of compounds **141**, **143**, **145**, **149** and **152** on PC3 cancer cell lines after 48 hours incubation

CHAPTER 3

EXPERIMENTAL

3.1. Chemistry Part

3.1.1. General Methods

Reagents were commercial grade and were used as supplied. Reactions were monitored by thin layer chromatography by using Merck TLC plates (Silica gel 60 F254). Chromatographic separations and isolations of compounds were performed by column chromatography. 70-230 mesh silica gel was used for column chromatography. Solvents were also commercial grade and were used as supplied. ^{1}H NMR and ^{13}C NMR data were recorded on Varian 400-MR (400 MHz) spectrometer. Chemical shifts for ^{1}H-NMR and ^{13}C-NMR are reported in δ (ppm). $CDCl_3$ peaks were used as reference in ^{1}H-NMR (7.26 ppm), and ^{13}C-NMR (77.36 ppm) respectively.

3.1.2. Synthesis of Chalcone Derivatives (110-115)

3.1.2.1. (E)-3-(3-bromophenyl)-1-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one (110)

A mixture of 950.0 mg (5.72 mmol) of 2-hydroxy-5-methoxyacetophenone and 1.05 g (5.68 mmol) of 3-bromobenzaldehyde in anhydrous ethanol was prepared, and stirred for 5 minutes. Then 700.0 mg (18.00 mmol) of NaOH (3.15 eq.) was added to this solution. The reaction mixture was stirred 18 h. at room temperature. Then 10% HCl was added to neutralize this solution. Resulting solution was extracted with ethyl acetate (2x40 ml) and combined organic phase was washed with brine solution (2x25 ml). Resulting organic phase was dried over $MgSO_4$. After the removal of the solvent

under vacuum, crude product was purified by column chromatography on silica gel (1:12 EtOAc/Hexanes) to furnish 909.4 mg of (E)-3-(3-bromophenyl)-1-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one as orange solid with 46% yield. Rf = 0.50 (ethyl acetate/hexanes, 1:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.26 (s, 1H), 7.87–7.78 (m, 2H), 7.62–7.52 (m, 3H), 7.36–7.27 (m, 2H), 7.16 (dd, *J* = 9.1, 3.0 Hz, 1H), 6.99 (d, *J* = 9.1 Hz, 1H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 193.35, 158.37, 152.14, 144.07, 137.04, 133.98, 131.32, 130.90, 127.84, 124.52, 123.53, 121.85, 119.85, 119.81, 113.28, 56.57.

3.1.2.2. (E)-3-(3-bromophenyl)-1-(5-chloro-2-hydroxyphenyl)prop-2-en-1-one (111)

A mixture of 1.05 g (6.18 mmol) of 2-hydroxy-5-chloroacetophenone and 1.1 g (5.95 mmol) of 3-bromobenzaldehyde in anhydrous ethanol was prepared, and stirred for 5 minutes. Then 1.1 g (27.5 mmol) of NaOH (4.62 eq.) was added to this solution. The reaction mixture was stirred 24 h. at room temperature. Then 10% HCl was added to neutralize this solution. Resulting solution was extracted with ethyl acetate (3x30 ml) and combined organic phase was washed with brine solution (2x25 ml). Resulting organic phase was dried over $MgSO_4$. After the removal of the solvent under vacuum, crude product was purified by column chromatography on silica gel (1:12 EtOAc/Hexanes) to furnish 1.9 mg of (E)-3-(3-bromophenyl)-1-(5-chloro-2-hydroxyphenyl)prop-2-en-1-one as orange solid with 93% yield. Rf = 0.40 (ethyl acetate/hexanes, 1:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.61 (s, 1H), 7.86–7.79 (m, 3H), 7.60–7.50 (m, 3H), 7.47–7.42 (m, 1H), 7.32 (dd, *J* = 7.9, 7.9 Hz, 1H), 7.01–6.96 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 192.75, 162.42, 144.92, 136.77, 136.68, 134.25, 131.50, 130.92, 129.12, 127.91, 123.98, 123.57, 121.04, 120.75, 120.64.

3.1.2.3.(E)-3-(3-bromophenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one (112)

A mixture of 470.0 mg (2.40 mmol) of 2-hydroxy-4,6-dimethoxyacetophenone and 278.0 mg (2.43 mmol) of 3-bromobenzaldehyde in anhydrous ethanol was prepared, and stirred for 5 minutes. Then 600.0 mg (15.00 mmol) of NaOH (6.25 eq.)

was added to this solution. The reaction mixture was stirred 24 h. at room temperature. Then 10% HCl was added to neutralize this solution. Resulting solution was extracted with ethyl acetate (2x30 ml) and combined organic phase was washed with brine solution (2x25 ml). Resulting organic phase was dried over $MgSO_4$. After the removal of the solvent under vacuum, crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to furnish 333.0 mg of (E)-3-(3-bromophenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl)prop-2-en-1-one as orange solid with 92% yield. Rf = 0.25 (ethyl acetate/hexanes, 1:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.84 (d, *J* = 15.6 Hz, 1H), 7.72 (dd, *J* = 1.8 Hz, 1H), 7.65 (d, *J* = 15.6 Hz, 1H), 7.53–7.46 (m, 2H), 7.27 (dd, *J* = 7.9, 7.9 Hz, 1H), 6.11 (d, *J* = 2.4 Hz, 1H), 5.96 (d, *J* = 2.4 Hz, 1H), 3.92 (s, 3H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 192.49, 168.77, 166.76, 162.79, 140.60, 138.07, 133.01, 131.17, 130.66, 129.21, 127.28, 123.30, 106.57, 94.12, 91.63, 56.24, 55.92.

3.1.2.4. (E)-3-(3-bromophenyl)-1-(2-hydroxy-6-methoxyphenyl)prop-2-en-1-one (113)

A mixture of 250.0 mg (1.51 mmol) of 2-hydroxy-6-methoxyacetophenone and 278.0 mg (1.50 mmol) of 3-bromobenzaldehyde in anhydrous ethanol was prepared, and stirred for 5 minutes. Then 200.0 mg (5.00 mmol) of NaOH (3.33 eq.) was added to this solution. The reaction mixture was stirred 24 h. at room temperature. Then 10% HCl was added to neutralize this solution. Resulting solution was extracted with ethyl acetate (2x30 ml) and combined organic phase was washed with brine solution (2x25 ml). Resulting organic phase was dried over $MgSO_4$. After the removal of the solvent under vacuum, crude product was purified by column chromatography on silica gel (1:12 EtOAc/Hexanes) to furnish 454.0 mg of (E)-3-(3-bromophenyl)-1-(2-hydroxy-6-methoxyphenyl)prop-2-en-1-one as orange solid with 91% yield. Rf = 0.40 (ethyl acetate/hexanes, 1:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.95 (s, 1H), 7.73 (d, *J* = 15.6 Hz, 1H), 7.65 (dd, *J* = 1.8, 1.8 Hz, 1H), 7.60 (d, *J* = 15.6 Hz, 1H), 7.45–7.40 (m, 2H), 7.32–7.25 (m, 1H), 7.22–7.16 (m, 1H), 6.53 (dd, *J* = 8.4, 1.0 Hz, 1H), 6.35 (dd, *J* = 8.3, 0.8 Hz., 1H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 194.44, 165.21, 161.29, 141.20, 137.80, 136.52, 133.27, 131.27, 130.72, 129.23, 127.40, 123.34, 112.17, 111.30, 101.88, 56.34.

3.1.2.5. (E)-3-(3-bromophenyl)-1-(2-4-dihydroxy-3-methylphenyl)prop-2-en-1-one (114)

A mixture of 997.0 mg (6.00 mmol) of 2,4-dihydroxy-3-methylacetophenone and 1.1 g (5.95 mmol) of 3-bromobenzaldehyde in anhydrous ethanol was prepared, and stirred for 5 minutes. Then 1.1 g (27.5 mmol) of NaOH (4.62 eq.) was added to this solution. The reaction mixture was stirred 24 h. at room temperature. Then 10% HCl was added to neutralize this solution. Resulting solution was extracted with ethyl acetate (3x40 ml) and combined organic phase was washed with brine solution (2x25 ml). Resulting organic phase was dried over $MgSO_4$. After the removal of the solvent under vacuum, crude product was purified by column chromatography on silica gel (1:12 EtOAc/Hexanes) to furnish 1.8 g of (E)-3-(3-bromophenyl)-1-(2,4-dihydroxy-3-methylphenyl)prop-2-en-1-one as orange solid with 90% yield. Rf = 0.20 (ethyl acetate/hexanes, 1:4); formation of the product was observed from NMR spectrum of crude product, and this crude product was used in next step (Formation of product 120).

3.1.2.6.(E)-3-(3-bromophenyl)-1-(2-hydroxy-5-methyl-3-nitrophenyl) prop-2-en-1-one (115)

A mixture of 390.0 mg (2.00 mmol) of 2-hydroxy-5-methyl-3-nitroacetophenone and 370.0 mg (2.00 mmol) of 3-bromobenzaldehyde was stirred together in the presence of 30% L-Proline in DMF (0.02 M) at 80 ^{o}C for 18 hours. Then solution was extracted with ethyl acetate (2x30 ml) and combined organic phase was washed with brine solution (2x20 ml). Resulting organic phase was dried over $MgSO_4$. After the removal of the solvent under vacuum, crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to furnish 41.0 mg of (E)-3-(3-bromophenyl)-1-(2-hydroxy-5-methyl-3-nitrophenyl)prop-2-en-1-one as orange solid with 15% yield. Rf = 0.25 (ethyl acetate/hexanes, 1:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.09–8.05 (m, 1H), 7.94 (dd, *J* = 2.3, 0.6 Hz, 1H), 7.84–7.76 (m, 2H), 7.60–7.49 (m, 4H), 7.32 (dd, *J* = 7.9, 7.9 Hz, 1H).

3.1.3. Synthesis of Flavanone Derivatives (116,117,119)

3.1.3.1. 2-(3-bromophenyl)-6-methoxychroman-4-one (116)

A solution of 1.60 g (4.80 mmol) of (E)-3-(3-bromophenyl)-1-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one in 125.0 ml of glacial acetic acid was heated at reflux for 72 h. At the end of this period, reaction mixture was cooled to room temperature, and solution was poured into 40.0 ml of water and extracted with ethyl acetate (3x40ml). Combined organic phase was washed with brine (2x50 ml) and dried over $MgSO_4$. After removal of the solvent under vacuum, crude product was purified by column chromatography on silica gel (1:12 EtOAc/Hexanes) to furnish 1.30 g of 2-(3-bromophenyl)-6-methoxychroman-4-one as yellow solid with 79% yield. Rf = 0.16 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.67 (dd, *J* = 1.6, 1.6 Hz, 1H), 7.53–7.48 (m, 1H), 7.40–7.25 (m, 3H), 7.13 (dd, *J* = 9.0, 3.1 Hz, 1H), 7.00 (d, *J* = 9.0 Hz, 1H), 5.40 (dd, *J* = 13.2, 3.1 Hz, 1H), 3.82 (s, 3H), 3.00 (dd, *J* = 16.9, 13.2 Hz, 1H), 2.87 (dd, *J* = 16.9, 3.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 191.75, 156.26, 154.76, 141.48, 132.09, 130.72, 129.58, 125.80, 124.95, 123.27, 121.10, 119.73, 107.79, 79.14, 56.17, 44.91.

3.1.3.2. 2-(3-bromophenyl)-6-chlorochroman-4-one (117)

A solution of 1.85 g (5.49 mmol) of (E)-3-(3-bromophenyl)-1-(5-chloro-2-hydroxyphenyl)prop-2-en-1-one in 150.0 ml of glacial acetic acid was heated at reflux during 72 hours. At the end of this period, reaction mixture was cooled to room temperature, and solution was poured into 40 ml of water and extracted with ethyl acetate (3x40ml). Combined organic phase was washed with brine (2x50 ml) and dried over $MgSO_4$. After removal of the solvent under vacuum, crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to furnish 1.30 g of 2-(3-bromophenyl)-6-chlorochroman-4-one as white solid with 90% yield. Rf = 0.18 (ethyl acetate/hexanes, 1:8); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.93–7.82 (m, 1H), 7.71–7.62 (m, 1H), 7.58–7.22 (m, 4H), 7.10–6.97 (m, 1H), 5.44 (dd, *J* = 12.8, 2.8 Hz, 1H), 3.14–2.79 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 190.11, 159.60, 140.52, 136.10, 131.96, 130.45, 129.21, 127.44, 126.41, 124.60, 122.98, 121.64, 119.82, 78.87, 44.23.

3.1.3.3. 2-(3-bromophenyl)-5-methoxychroman-4-one (119)

A solution of 533.0 mg (1.60 mmol) of (E)-3-(3-bromophenyl)-1-(2-hydroxy-6-methoxyphenyl)prop-2-en-1-one in ethanol was prepared. 1.3 g of anhydrous sodium acetate was added to this solution. Final solution was heated at reflux for 24 h. At the end of this period, reaction mixture was cooled to room temperature, and solution was poured into 40 ml of water and extracted with ethyl acetate (3x40ml). Combined organic phase was washed with brine (2x50 ml) and dried over $MgSO_4$. After removal of the solvent under vacuum, crude product was purified by column chromatography on silica gel (1:12 EtOAc/Hexanes) to furnish 240.0 mg of 2-(3-bromophenyl)-5-methoxychroman-4-one as yellow solid with 45% yield. Rf = 0.16 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.66 (s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.47–7.35 (m, 2H), 7.34–7.25 (m, 1H), 6.68 (d, *J* = 8.3 Hz, 1H), 6.58 (d, *J* = 8.4 Hz, 1H), 5.41 (d, *J* = 13.1 Hz, 1H), 3.94 (s, 3H), 3.08–2.95 (m, 1H), 2.86 (d, *J* = 16.4 Hz, 1H).

3.1.4. Synthesis of Stilbene Derivatives (133-136)

3.1.4.1. Preparation of Catalyst for Heck Reaction

In a 100 ml flask, 6.3 mg ($2.8x10^{-4}$ mmol) of $Pd(OAc)_2$ was dissolved in 100 ml of NMP and $2.8x10^{-3}$ M stock solution was prepared. This solution was stored in cold medium.

3.1.4.2. (E)-3-(4-methoxystyryl)benzaldehyde (133)

A double neck round bottom flask was filled with 451.0 mg (5.50 mmol) of NaOAc, 925.1 mg of (5.00 mmol, 583.0 µl) 3-bromobenzaldehyde and 10 ml NMP. 10.7 ml of stock solution of catalyst (containing 0.06 mol% $Pd(OAc)_2$ compared to 3-bromobenzaldehyde) was added to reaction vessel by a syringe under nitrogen atmosphere. The reaction mixture was stirred and heated to 120 ^oC, and then 670.9 mg (5.00 mmol, 672.0 µl) of 4-methoxystyrene was added to reaction mixture. Then reaction mixture was heated to 135 ^oC, and stirred at this temperature for 18 hours. The

resulting mixture was cooled to room temperature and poured into the 50.0 ml of water. Then mixture extracted with ethyl acetate (3x40 ml). The combined organic phase washed with brine (3x40 ml) and dried over $MgSO_4$. After removal of solvent under vacuum, purification of the crude product was performed by column chromatography on silica gel (1:10 EtOAc/Hexanes) gave 1.1 g of (E)-3-(4-methoxylstyryl)benzaldehyde as white powder with 93% yield. Rf = 0.55 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.00 (d, *J* = 5.0 Hz, 1H), 7.95 (s, 1H), 7.75–7.63 (m, 2H), 7.52–7.39 (m, 3H), 7.12 (d, *J* = 16.3 Hz, 1H), 7.02–6.85 (m, 3H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 192.53, 159.85, 138.80, 136.94, 132.19, 130.14, 129.63, 129.45, 128.53, 128.16, 127.11, 125.04, 114.40, 55.45.

3.1.4.3. (E)-3-(4-methylstyryl)benzaldehyde (134)

A double neck round bottom flask was filled with 451.0 mg (5.50 mmol) of NaOAc, 925.1 mg of (5.00 mmol, 583.0 µl) 3-bromobenzaldehyde and 10 ml NMP. 10.7 ml of stock solution of catalyst (containing 0.06 mol% $Pd(OAc)_2$ compared to 3-bromobenzaldehyde) was added to reaction vessel by a syringe under nitrogen atmosphere. The reaction mixture was stirred and heated to 120 ^{o}C, and then 590.9 mg (5.00 mmol, 659.0 µl) of 4-methylstyrene was added to reaction mixture. Then reaction mixture was heated to 135 ^{o}C, and stirred at this temperature for 18 hours. The resulting mixture was cooled to room temperature and poured into the 50.0 ml of water. Then mixture extracted with ethyl acetate (3x40 ml). The combined organic phase washed with brine (3x40 ml) and dried over $MgSO_4$. After removal of solvent under vacuum, purification of the crude product was performed by column chromatography on silica gel (1:10 EtOAc/Hexanes) gave 889.0 mg of (E)-3-(4-methylstyryl)benzaldehyde as white powder with 80% yield. Rf = 0.60 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.04 (s, 1H), 8.00 (dd, *J* = 1.6, 1.6 Hz, 1H), 7.78–7.70 (m, 2H), 7.56–7.47 (m, 1H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.29–7.02 (m, 4H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 192.62, 138.80, 138.41, 137.06, 134.20, 132.46, 130.69, 129.78, 129.58, 128.86, 127.39, 126.90, 126.33, 21.58.

3.1.4.4. (E)-3-(3-methylstyryl)benzaldehyde (135)

A double neck round bottom flask was filled with 451.0 mg (5.50 mmol) of NaOAc, 925.1 mg of (5.00 mmol, 583.0 µl) 3-bromobenzaldehyde and 10 ml NMP. 10.7 ml of stock solution of catalyst (containing 0.06 mol% $Pd(OAc)_2$ compared to 3-bromobenzaldehyde) was added to reaction vessel by a syringe under nitrogen atmosphere. The reaction mixture was stirred and heated to 120 ^oC, and then 591.0 mg (5.00 mmol, 664.0 µl) of 3-methylstyrene was added to reaction mixture. Then reaction mixture was heated to 135 ^oC, and stirred at this temperature for 18 hours. The resulting mixture was cooled to room temperature and poured into the 50.0 ml of water. Then mixture extracted with ethyl acetate (3x40 ml). The combined organic phase washed with brine (2x40 ml) and dried over $MgSO_4$. After removal of solvent under vacuum, purification of the crude product was performed by column chromatography on silica gel (1:10 EtOAc/Hexanes) gave 1.0 g of (E)-3-(3-methylstyryl)benzaldehyde as white powder with 90% yield. Rf = 0.60 (ethyl acetate/hexanes, 1:6); ^1H NMR (400 MHz, $CDCl_3$) δ = 10.05 (s, 1H), 8.02 (s, 1H), 7.79–7.71 (m, 2H), 7.56–7.48 (m, 1H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.28 (dd, *J* = 11.6, 4.2 Hz, 1H), 7.23–7.08 (m, 3H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 192.58, 138.70, 138.62, 137.08, 136.92, 132.54, 130.88, 129.61, 129.27, 128.99, 128.96, 127.67, 127.47, 127.13, 124.18, 21.73.

3.1.4.5. (E)-3-(3-fluorostyryl)benzaldehyde (136)

A double neck round bottom flask was filled with 451.0 mg (5.50 mmol) of NaOAc, 925.1 mg of (5.00 mmol, 583.0 µl) 3-bromobenzaldehyde and 10 ml NMP. 10.7 ml of stock solution of catalyst (containing 0.06 mol% $Pd(OAc)_2$ compared to 3-bromobenzaldehyde) was added to reaction vessel by a syringe under nitrogen atmosphere. The reaction mixture was stirred and heated to 120 ^oC, and then 610.7 mg (5.00 mmol, 596.0 µl) of 3-fluorostyrene was added to reaction mixture. Then reaction mixture was heated to 135 ^oC, and stirred at this temperature for 20 hours. The resulting mixture was cooled to room temperature and poured into the 50.0 ml of water. Then mixture extracted with ethyl acetate (3x40 ml). The combined organic phase washed with brine (2x40 ml) and dried over $MgSO_4$. After removal of solvent under vacuum, purification of the crude product was performed by column chromatography on silica

gel (1:10 EtOAc/Hexanes) gave 850.0 mg of (E)-3-(3-fluorostyryl)benzaldehyde as white powder with 75% yield. Rf = 0.50 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.03 (s, 1H), 7.99 (dd, *J* = 1.7, 1.7 Hz, 1H), 7.79–7.74 (m, 1H), 7.71 (ddd, *J* = 5.0, 3.2, 1.8 Hz, 1H), 7.51 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.36–7.24 (m, 2H), 7.23–7.17 (m, 1H), 7.11 (d, *J* = 2.5 Hz, 2H), 6.98 (tdd, *J* = 8.4, 2.6, 1.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 192.41, 164.62, 162.17, 139.33, 139.26, 138.05, 137.08, 132.65, 130.53, 130.44, 129.66, 129.47, 129.44, 128.63, 127.48, 122.95, 122.92, 115.27, 115.06, 113.30, 113.08.

3.1.5. Synthesis of Stilbene Fused Chalcone Derivatives (139,141-156)

3.1.5.1. (E)-1-(2-hydroxy-5-methoxyphenyl)-3-(3-((E)-4-methoxystyryl) phenyl)prop-2-en-1-one (141)

A mixture of 238.0 mg (1.00 mmol) of (E)-3-(4-methoxystyryl)benzaldehyde and 166.0 mg (1.00 mmol) of 2-hydroxy-5-methoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 2x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 309.0 mg of (E)-1-(2-hydroxy-5-methoxyphenyl)-3-(3-((E)-4-methoxystyryl) phenyl)prop-2-en-1-one as orange solid with 80% yield. Rf = 0.29 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.39 (s, 1H), 7.93 (d, *J* = 15.5 Hz, 1H), 7.72 (s, 1H), 7.62 (d, *J* = 15.5 Hz, 1H), 7.59–7.45 (m, 4H), 7.44–7.37 (m, 2H), 7.19–7.09 (m, 2H), 7.04–6.88 (m, 4H), 3.85 (s, 3H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 193.67, 159.90, 158.26, 152.03, 145.95, 138.88, 135.26, 130.00, 129.77, 129.64, 128.85, 128.23, 127.57, 126.96, 125.82, 124.11, 120.61, 119.99, 119.68, 114.53, 113.45, 56.53, 55.68.

3.1.5.2. (E)-1-(2-hydroxy-5-methoxyphenyl)-3-(3-((E)-4-methystyryl) phenyl)prop-2-en-1-one (142)

A mixture of 222.0 mg (1.0 mmol) of (E)-3-(4-methylstyryl)benzaldehyde and 166.0 mg (1.0 mmol) of 2-hydroxy-5-methoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 267.0 mg of (E)-1-(2-hydroxy-5-methoxyphenyl)-3-(3-((E)-4-methylstyryl) phenyl)prop-2-en-1-one as orange solid with 72% yield. Rf = 0.43 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.43 (d, *J* = 1.4 Hz, 1H), 7.92 (d, *J* = 15.5 Hz, 1H), 7.71 (s, 1H), 7.64–7.50 (m, 3H), 7.47–7.35 (m, 4H), 7.22–7.03 (m, 5H), 6.99 (d, *J* = 9.1 Hz, 1H), 3.85 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 193.61, 158.24, 152.01, 145.83, 138.69, 138.28, 135.24, 134.41, 130.13, 129.79, 129.61, 128.93, 127.73, 127.09, 126.91, 126.88, 124.07, 120.58, 119.96, 119.64, 113.43, 56.48, 21.61.

3.1.5.3. (E)-1-(2-hydroxy-5-methoxyphenyl)-3-(3-((E)-3-methylstyryl) phenyl)prop-2-en-1-one (143)

A mixture of 222.0 mg (1.0 mmol) of (E)-3-(3-methylstyryl)benzaldehyde and 166.0 mg (1.0 mmol) of 2-hydroxy-5-methoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 37.0 mg of (E)-1-(2-hydroxy-5-methoxyphenyl)-3-(3-((E)-3-methylstyryl) phenyl)prop-2-en-1-one as orange solid with 10% yield. Rf = 0.43 (ethyl

acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.37 (s, 1H), 7.94 (d, *J* = 15.4 Hz, 1H), 7.75 (s, 1H), 7.66–7.53 (m, 3H), 7.47–7.33 (m, 4H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.20–7.08 (m, 4H), 7.00 (d, *J* = 9.1 Hz, 1H), 3.86 (s, 3H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 193.67, 158.28, 152.06, 145.85, 138.68, 138.66, 137.16, 135.33, 130.38, 129.69, 129.19, 129.05, 129.00, 127.93, 127.76, 127.67, 127.16, 124.19, 124.11, 120.72, 120.01, 119.69, 113.52, 56.56, 21.79.

3.1.5.4. (E)-1-(2-hydroxy-5-methoxyphenyl)-3-(3-((E)-3-fluorostyryl) phenyl)prop-2-en-1-one (144)

A mixture of 226.0 mg (1.0 mmol) of (E)-3-(4-fluorostyryl)benzaldehyde and 166.0 mg (1.0 mmol) of 2-hydroxy-5-methoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 214.0 mg of (E)-1-(2-hydroxy-5-methoxyphenyl)-3-(3-((E)-4-fluorostyryl) phenyl)prop-2-en-1-one as orange solid with 57% yield. Rf = 0.39 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.33 (d, *J* = 1.8 Hz, 1H), 7.93 (d, *J* = 15.5 Hz, 1H), 7.74 (dd, *J* = 1.6, 1.6 Hz, 1H), 7.65–7.55 (m, 3H), 7.44 (dd, *J* = 7.7 Hz, 1H), 7.39 (d, *J* = 3.0 Hz, 1H), 7.37–7.22 (m, 3H), 7.16 (dd, *J* = 9.1, 3.0 Hz, 1H), 7.13 (s, 2H), 7.03–6.95 (m, 2H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 193.62, 164.78, 162.34, 158.32, 152.11, 145.61, 139.68, 139.60, 138.11, 135.47, 130.58, 130.49, 129.76, 129.36, 129.15, 129.09, 129.06, 128.29, 127.32, 124.14, 122.98, 122.95, 120.94, 120.02, 119.71, 115.23, 115.01, 113.60, 113.38, 113.16, 56.57.

3.1.5.5. (E)-1-(2-hydroxy-5-chlorophenyl)-3-(3-((E)-4-methoxystyryl) phenyl)prop-2-en-1-one (145)

A mixture of 80.0 mg (0.34 mmol) of (E)-3-(4-methoxylstyryl)benzaldehyde and 58.0 mg (0.34 mmol) of 5-chloro-2-hydroxyacetophenone in anhydrous ethanol was

stirred for 5 min. Then 40 mg (1.0 mmol) of NaOH (3.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 80.0 mg of (E)-1-(2-hydroxy-5-chlorophenyl)-3-(3-((E)-4-methoxylstyryl) phenyl)prop-2-en-1-one as yellow solid with 62% yield. Rf = 0.48 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.61 (s, 1H), 7.81 (d, *J* = 15.4 Hz, 1H), 7.74 (s, 1H), 7.58 (s, 1H), 7.47–7.37 (m, 3H), 7.37–7.24 (m, 4H), 7.03–6.94 (m, 1H), 6.90–6.82 (m, 2H), 6.78 (d, *J* = 7.8 Hz, 2H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 193.07, 162.41, 159.92, 146.84, 138.94, 136.52, 134.98, 129.97, 129.85, 129.70, 129.68, 129.15, 128.25, 127.77, 127.05, 125.72, 123.85, 120.95, 120.91, 120.57, 119.83, 119.82, 114.53, 55.66.

3.1.5.6. (E)-1-(2-hydroxy-5-chlorophenyl)-3-(3-((E)-4-methylstyryl) phenyl)prop-2-en-1-one (146)

A mixture of 175.0 mg (0.8 mmol) of (E)-3-(4-methylstyryl)benzaldehyde and 135.0 mg (0.8 mmol) of 5-chloro-2-hydroxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 320 mg (8.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 160.0 mg of (E)-1-(2-hydroxy-5-chlorophenyl)-3-(3-((E)-4-methylstyryl) phenyl)prop-2-en-1-one as yellow solid with 54% yield. Rf = 0.65 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.74 (s, 1H), 7.97 (d, *J* = 15.4 Hz, 1H), 7.90 (d, *J* = 2.5 Hz, 1H), 7.76 (s, 1H), 7.64–7.53 (m, 3H), 7.48–7.40 (m, 4H), 7.23–7.06 (m, 4H), 7.00 (d, *J* = 8.9 Hz, 1H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 193.10, 162.42, 146.84, 138.82, 138.37, 136.57, 135.01, 134.41, 130.29, 129.83, 129.73, 129.27, 129.18, 128.00, 127.20, 126.92, 126.88, 123.88, 120.93, 120.60, 119.92, 21.66.

3.1.5.7. (E)-1-(2-hydroxy-5-chlorophenyl)-3-(3-((E)-3-methylstyryl) phenyl)prop-2-en-1-one (147)

A mixture of 222.0 mg (1.0 mmol) of (E)-3-(3-methylstyryl)benzaldehyde and 170.0 mg (1.0 mmol) of 5-chloro-2-hydroxyoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 155.0 mg of (E)-1-(2-hydroxy-5-chlorophenyl)-3-(3-((E)-3-methylstyryl) phenyl)prop-2-en-1-one as yellow solid with 41% yield. Rf = 0.69 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.76 (s, 1H), 7.95 (d, *J* = 15.4 Hz, 1H), 7.88 (d, *J* = 2.5 Hz, 1H), 7.74 (s, 1H), 7.63–7.52 (m, 3H), 7.48–7.40 (m, 2H), 7.39–7.33 (m, 2H), 7.31–7.25 (m, 1H), 7.16–7.08 (m, 3H), 6.99 (d, *J* = 8.9 Hz, 1H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 193.01, 162.40, 146.72, 138.66, 138.64, 137.09, 136.52, 134.98, 130.40, 129.70, 129.29, 129.20, 129.13, 128.97, 128.12, 127.65, 127.61, 127.25, 124.20, 123.83, 120.88, 120.57, 119.84, 21.79.

3.1.5.8. (E)-1-(2-hydroxy-5-chlorophenyl)-3-(3-((E)-3-fluorostyryl) phenyl)prop-2-en-1-one (148)

A mixture of 226.0 mg (1.0 mmol) of (E)-3-(3-fluorostyryl)benzaldehyde and 170.0 mg (1.0 mmol) of 5-chloro-2-hydroxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 86.2 mg of (E)-1-(2-hydroxy-5-chlorophenyl)-3-(3-((E)-3-fluorostyryl) phenyl)prop-2-en-1-one as yellow solid with 23% yield. Rf = 0.58 (ethyl

acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.72 (s, 1H), 7.96 (d, *J* = 15.4 Hz, 1H), 7.90 (d, *J* = 2.5 Hz, 1H), 7.77 (d, *J* = 1.6 Hz, 1H), 7.63–7.56 (m, 3H), 7.49–7.42 (m, 2H), 7.38–7.24 (m, 3H), 7.14 (s, 2H), 7.00 (d, *J* = 8.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 193.05, 164.79, 162.46, 162.34, 146.55, 139.64, 139.57, 138.19, 136.60, 135.19, 130.59, 130.51, 129.83, 129.44, 129.27, 129.20, 129.17, 128.50, 127.43, 123.92, 123.00, 122.97, 120.95, 120.64, 120.20, 115.27, 115.06, 113.40, 113.19.

3.1.5.9. (E)-1-(2-hydroxy-6-methoxyphenyl)-3-(3-((E)-4-methoxystyryl) phenyl)prop-2-en-1-one (149)

A mixture of 238.0 mg (1.0 mmol) of (E)-3-(4-methoxylstyryl)benzaldehyde and 166.0 mg (1.0 mmol) of 2-hydroxy-6-methoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 211.0 mg of (E)-1-(2-hydroxy-6-methoxyphenyl)-3-(3-((E)-4-methoxylstyryl) phenyl)prop-2-en-1-one as yellow solid with 55% yield. Rf = 0.24 (ethyl acetate/hexanes, 1:6; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 13.15 (s, 1H), 7.92–7.78 (m, 2H), 7.67 (s, 1H), 7.57–7.44 (m, 4H), 7.42–7.33 (m, 2H), 7.11 (d, *J* = 16.3 Hz, 1H), 6.99 (d, *J* = 16.3 Hz, 1H), 6.95–6.88 (m, 2H), 6.64 (dd, *J* = 8.4, 0.8 Hz, 1H), 6.44 (dd, *J* = 8.3, 0.7 Hz, 1H), 3.96 (s, 3H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 194.76, 165.18, 161.31, 159.85, 143.21, 138.68, 136.28, 136.02, 130.10, 129.50, 128.23, 128.18, 128.13, 127.13, 127.08, 126.08, 114.52, 112.33, 111.29, 101.90, 56.33, 55.66.

3.1.5.10. (E)-1-(2-hydroxy-6-methoxyphenyl)-3-(3-((E)-4-methylstyryl) phenyl)prop-2-en-1-one (150)

A mixture of 222.0 mg (1.0 mmol) of (E)-3-(4-methylstyryl)benzaldehyde and 166.0 mg (1.0 mmol) of 2-hydroxy-6-methoxyacetophenone in anhydrous ethanol was

stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 163.0 mg of (E)-1-(2-hydroxy-6-methoxyphenyl)-3-(3-((E)-4-methylstyryl) phenyl)prop-2-en-1-one as yellow solid with 44% yield. Rf = 0.35 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 13.12 (s, 1H), 7.93–7.80 (m, 2H), 7.70 (s, 1H), 7.58–7.48 (m, 2H), 7.46–7.34 (m, 4H), 7.22–7.03 (m, 4H), 6.64 (dd, *J* = 8.4, 0.8 Hz, 1H), 6.45 (d, *J* = 8.3 Hz, 1H), 3.97 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 194.66, 165.17, 161.25, 143.07, 138.45, 138.12, 136.22, 135.95, 134.45, 129.80, 129.73, 129.43, 128.30, 128.06, 127.23, 127.15, 127.11, 126.81, 112.23, 111.20, 101.84, 56.22, 21.56.

3.1.5.11. (E)-1-(2-hydroxy-6-methoxyphenyl)-3-(3-((E)-3-methylstyryl) phenyl)prop-2-en-1-one (151)

A mixture of 222.0 mg (1.0 mmol) of (E)-3-(3-methylstyryl)benzaldehyde and 166.0 mg (1.0 mmol) of 2-hydroxy-6-methoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 286.0 mg of (E)-1-(2-hydroxy-6-methoxyphenyl)-3-(3-((E)-3-methylstyryl) phenyl)prop-2-en-1-one as yellow solid with 78% yield. Rf = 0.38 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.16 (d, *J* = 3.6 Hz, 1H), 7.93–7.79 (m, 2H), 7.70 (s, 1H), 7.59–7.50 (m, 2H), 7.45–7.32 (m, 4H), 7.31–7.23 (m, 1H), 7.16–7.08 (m, 3H), 6.64 (dd, *J* = 8.4, 0.9 Hz, 1H), 6.45 (d, *J* = 8.2 Hz, 1H), 3.97 (s, 3H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 194.74, 165.21, 161.34, 143.06, 138.61,

138.49, 137.28, 136.25, 136.11, 130.12, 129.52, 129.09, 128.96, 128.42, 128.25, 128.03, 127.63, 127.44, 127.24, 124.14, 112.38, 111.33, 101.93, 56.33, 21.75.

3.1.5.12. (E)-1-(2-hydroxy-6-methoxyphenyl)-3-(3-((E)-3-fluorostyryl) phenyl)prop-2-en-1-one (152)

A mixture of 226.0 mg (1.0 mmol) of (E)-3-(3-fluorostyryl)benzaldehyde and 166.0 mg (1.0 mmol) of 2-hydroxy-6-methoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 288.0 mg of (E)-1-(2-hydroxy-6-methoxyphenyl)-3-(3-((E)-3-fluorostyryl) phenyl)prop-2-en-1-one as yellow solid with 77% yield. Rf = 0.31 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 13.13 (s, 1H), 7.89 (d, *J* = 15.6 Hz, 1H), 7.82 (d, *J* = 15.6 Hz, 1H), 7.69 (s, 1H), 7.58–7.51 (m, 2H), 7.45–7.20 (m, 5H), 7.11 (s, 2H), 7.02–6.94 (m, 1H), 6.63 (dd, *J* = 8.4, 1.0 Hz, 1H), 6.44 (dd, *J* = 8.3, 0.7 Hz, 1H), 3.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 194.72, 165.22, 164.77, 162.32, 161.34, 142.87, 139.76, 139.69, 137.91, 136.32, 136.22, 130.56, 130.47, 129.62, 129.61, 128.79, 128.77, 128.53, 128.41, 127.87, 127.42, 122.94, 122.91, 115.13, 114.92, 113.32, 113.10, 112.37, 111.36, 101.94, 56.35.

3.1.5.13. (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-4-methoxystyryl)phenyl)prop-2-en-1-one (153)

A mixture of 238.0 mg (1.0 mmol) of (E)-3-(4-methoxystyryl)benzaldehyde and 166.0 mg (1.0 mmol) of 2-hydroxy-4-methoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml)

and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 78.5 mg of (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-4-methoxystyryl) phenyl)prop-2-en-1-one as yellow solid with 20% yield. Rf = 0.40 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 13.45 (s, 1H), 7.93–7.83 (m, 2H), 7.72 (s, 1H), 7.60 (d, *J* = 15.5 Hz, 1H), 7.57–7.45 (m, 4H), 7.40 (dd, *J* = 7.7, 7.7 Hz, 1H), 7.12 (d, *J* = 16.3 Hz, 1H), 7.04–6.88 (m, 3H), 6.54–6.46 (m, 2H), 3.86 (s, 3H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 192.14, 167.09, 166.60, 159.91, 144.71, 138.84, 135.51, 131.62, 130.09, 129.69, 129.59, 128.61, 128.24, 128.22, 127.50, 126.75, 125.96, 120.85, 114.55, 114.46, 108.13, 101.42, 55.95, 55.68.

3.1.5.14. (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-4-methylstyryl) phenyl)prop-2-en-1-one (154)

A mixture of 222.0 mg (1.0 mmol) of (E)-3-(4-methylstyryl)benzaldehyde and 166.0 mg (1.0 mmol) of 2-hydroxy-4-methoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 167.5 mg of (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-4-methylstyryl) phenyl)prop-2-en-1-one as yellow solid with 45% yield. Rf = 0.39 (ethyl acetate/hexanes, 1:6); product could not be purified from 2-hydroxy-4-methoxyacetophenone completely. However it was used in synthesis of product 169.

3.1.5.15. (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-3-methylstyryl) phenyl)prop-2-en-1-one (155)

A mixture of 222.0 mg (1.0 mmol) of (E)-3-(3-methylstyryl)benzaldehyde and 166.0 mg (1.0 mmol) of 2-hydroxy-4-methoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this

solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 163.0 mg of (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-3-methylstyryl) phenyl)prop-2-en-1-one as yellow solid with 56% yield. Rf = 0.40 (ethyl acetate/hexanes, 1:6); product could not be purified from 2-hydroxy-4-methoxyacetophenone completely. However it was used in synthesis of product 170.

3.1.5.16. (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-3-fluorostyryl) phenyl)prop-2-en-1-one (156)

A mixture of 226.0 mg (1.0 mmol) of (E)-3-(3-fluorostyryl)benzaldehyde and 166.0 mg (1.0 mmol) of 2-hydroxy-4-methoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 400 mg (10.0 mmol) of NaOH (10.0 eq.) was added to this solution. The reaction mixture was stirred for 24 h at room temperature. Resulting solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 101.0 mg of (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-3-fluorostyryl) phenyl)prop-2-en-1-one as yellow solid with 27% yield. Rf = 0.40 (ethyl acetate/hexanes, 1:6); product could not be purified from 2-hydroxy-4-methoxyacetophenone completely. However it was used in synthesis of product 171.

3.1.5.17. (E)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(3-((E)-4-methoxystyryl)phenyl)prop-2-en-1-one (139)

A mixture of 200.0 mg (0.8 mmol) of (E)-3-(4-methoxylstyryl)benzaldehyde and 170.0 mg (0.8 mmol) of 2-hydroxy-4,6-dimethoxyacetophenone in anhydrous ethanol was stirred for 5 min. Then 280 mg (7.0 mmol) of NaOH (9.0 eq.) was added to this solution. The reaction mixture was stirred for 18 h at room temperature. Resulting

solution was neutralized by 10% HCl. Obtained mixture was extracted with 3x30 ml ethyl acetate. The combined organic phase was washed with brine solution (2x25 ml) and dried over $MgSO_4$. After the removal of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (1:10 EtOAc/Hexanes) to give 153.0 mg of (E)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(3-((E)-4-methoxylstyryl)phenyl)prop-2-en-1-one as yellow solid with 44% yield. Rf = 0.20 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.95–7.88 (m, 1H), 7.79 (d, *J* = 15.6 Hz, 1H), 7.65 (s, 1H), 7.54–7.44 (m, 4H), 7.37 (dd, *J* = 7.7 Hz, 1H), 7.10 (d, *J* = 16.3 Hz, 1H), 6.98 (d, *J* = 16.3 Hz, 1H), 6.94–6.88 (m, 2H), 6.11 (d, *J* = 2.4 Hz, 1H), 5.95 (dd, *J* = 9.9, 2.4 Hz, 1H), 3.92 (s, 3H), 3.83 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 192.88, 168.73, 166.55, 162.81, 159.81, 142.56, 138.60, 136.24, 130.12, 129.42, 129.37, 128.15, 128.05, 127.97, 127.00, 126.97, 126.14, 114.49, 106.65, 94.12, 91.58, 56.19, 55.89, 55.64.

3.1.6. Synthesis of Stilbene Fused Flavanone Derivatives (126,140,157-171)

3.1.6.1. (E)-6-methoxy-2-(3-(4-methoxystyryl)phenyl)chroman-4-one (126)

185.0 mg (0.6 mmol) of 2-(3-bromophenyl)-6-methoxychroman-4-one and 80.0 mg (0.6 mmol) of 4-methoxystyrene were placed in two necked flask with magnetic bar under nitrogen atmosphere. To this solution 15.0 mg (0.07 mmol) of palladium (II) acetate and 2.0 ml of triethanolamine were added. The mixture was stirred and heated at 100 ^{o}C for 10 h. Then solution was cooled to room temperature and extracted with diethyl ether (3x30 ml). The combined organic phase was washed with water (40 ml) and brine (2x40 ml) and dried over$MgSO_4$. After removal of the solvent under vacuum, purification of the crude product was performed by column chromatography on silica gel (1:8 EtOAc/Hexanes) to furnish 93.0 mg of (E)-6-methoxy-2-(3-(4-methoxystyryl)phenylchroman-4-one as white powder with 43% yield. Rf = 0.24 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.60 (s, 1H), 7.53–7.44 (m, 3H), 7.44–7.31 (m, 3H), 7.18–7.07 (m, 2H), 7.06–6.96 (m, 2H), 6.95–6.87 (m, 2H), 5.45 (dd, *J* = 13.4, 2.9 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.10 (dd, *J*=17.0, 13.5 Hz, 1H), 2.90

(dd, *J* = 17.0, 2.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 192.32, 159.79, 156.57, 154.57, 139.59, 138.64, 130.16, 129.44, 129.35, 128.13, 126.82, 126.29, 125.68, 125.14, 124.26, 121.10, 119.77, 114.49, 107.71, 80.04, 56.12, 55.63, 44.96.

3.1.6.2. (E)-6-methoxy-2-(3-(4-methylstyryl)phenyl)chroman-4-one (157)

89 mg (0.240 mmol) of (E)-1-(2-hydroxy-5-methoxyphenyl)-3-(3-((E)-4-methylstyryl)phenyl)prop-2-en-1-one and 196.8 mg (2.4 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 65.4 mg of (E)-6-methoxy-2-(3-(4-methylstyryl)phenylchroman-4-one as white solid with 73.4% yield. Rf = 0.33 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.62 (s, 1H), 7.52 (d, *J* = 7.6 Hz, 1H), 7.47–7.32 (m, 5H), 7.22–7.05 (m, 5H), 7.03 (d, *J* = 9.0 Hz, 1H), 5.45 (dd, *J* = 13.4, 2.4 Hz, 1H), 3.83 (s, 3H), 3.10 (dd, *J* = 16.9, 13.5 Hz, 1H), 2.91 (dd, *J* = 17.0, 2.8 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 192.35, 156.58, 154.58, 139.61, 138.50, 138.12, 134.58, 129.76, 129.47, 127.40, 126.98, 126.83, 125.73, 125.35, 124.42, 121.09, 119.79, 107.69, 80.04, 56.13, 44.97, 21.60.

3.1.6.3. (E)-6-methoxy-2-(3-(3-methylstyryl)phenyl)chroman-4-one (158)

125 mg (0.337 mmol) of (E)-1-(2-hydroxy-5-methoxyphenyl)-3-(3-((E)-3-methylstyryl)phenyl)prop-2-en-1-one and 277 mg (3.37 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes)

to afford 90 mg of (E)-6-methoxy-2-(3-(3-methylstyryl)phenylchroman-4-one as white solid with 72.0% yield. Rf = 0.33 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.64 (s, 1H), 7.53 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.46–7.32 (m, 5H), 7.30–7.24 (m, 1H), 7.17–7.08 (m, 4H), 7.03 (d, *J* = 9.0 Hz, 1H), 5.45 (dd, *J* = 13.4, 2.9 Hz, 1H), 3.83 (s, 3H), 3.10 (dd, *J* = 16.9, 13.5 Hz, 1H), 2.97–2.84 (m, 1H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 192.23, 156.49, 154.52, 139.60, 138.52, 138.33, 137.24, 129.87, 129.42, 128.97, 128.89, 128.13, 127.56, 126.98, 125.64, 125.43, 124.47, 124.07, 121.05, 119.72, 107.64, 79.94, 56.05, 44.89, 21.72.

3.1.6.4. (E)-6-methoxy-2-(3-(3-fluorostyryl)phenyl)chroman-4-one (159)

75 mg (0.240 mmol) of (E)-1-(2-hydroxy-5-methoxyphenyl)-3-(3-((E)-3-fluorostyryl)phenyl)prop-2-en-1-one and 196.8 mg (2.40 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 57.3 mg of (E)-6-methoxy-2-(3-(3-fluorostyryl)phenylchroman-4-one as white solid with 76.4% yield. Rf = 0.30 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.62 (s, 1H), 7.52 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.42 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.39–7.35 (m, 2H), 7.35–7.24 (m, 2H), 7.24–7.19 (m, 1H), 7.16–7.09 (m, 3H), 7.01 (d, *J* = 9.0 Hz, 1H), 6.96 (dddd, *J* = 9.6, 8.6, 2.6, 1.3 Hz, 1H), 5.45 (dd, *J* = 13.4, 2.9 Hz, 1H), 3.82 (s, 3H), 3.08 (dd, *J* = 17.0, 13.4 Hz, 1H), 2.90 (dd, *J* = 17.0, 2.9 Hz, 1H).
^{13}C NMR (100 MHz, $CDCl_3$) δ = 192.20, 164.69, 162.25, 156.49, 154.59, 139.75, 139.68, 137.79, 130.50, 130.41, 129.71, 129.54, 128.60, 128.57, 127.19, 125.92, 125.71, 124.64, 122.87, 122.84, 121.08, 119.74, 115.03, 114.82, 113.26, 113.04, 107.69, 79.90, 56.10, 44.94.

3.1.6.5. (E)-6-chloro-2-(3-(4-methoxystyryl)phenyl)chroman-4-one (160)

A solution of 39 mg (0.100 mmol) of (E)-1-(2-hydroxy-5-chlorophenyl)-3-(3-((E)-4-methoxylstyryl)phenyl)prop-2-en-1-one and 13.46 mg (1.20 mmol) of potassium tert-butoxide were heated in refluxing ethanol (8 ml) for 48 hours nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (1:14 EtOAc/Hexanes) to afford 24 mg of (E)-6-chloro-2-(3-(4-methoxystyryl)phenylchroman-4-one as white solid with 62% yield. Rf = 0.35 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.90 (d, *J* = 2.6 Hz, 1H), 7.58 (s, 1H), 7.55–7.38 (m, 5H), 7.32 (d, *J* = 7.6 Hz, 1H), 7.18–6.95 (m, 3H), 6.95–6.88 (m, 2H), 5.48 (dd, *J* = 13.3, 2.9 Hz, 1H), 3.84 (s, 3H), 3.11 (dd, *J* = 17.0, 13.3 Hz, 1H), 2.93 (dd, *J* = 17.0, 3.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 191.15, 160.29, 159.85, 139.01, 138.76, 136.38, 130.10, 129.55, 129.52, 128.17, 127.56, 127.05, 126.75, 126.16, 125.12, 124.26, 122.04, 120.24, 114.52, 80.19, 55.67, 44.69.

3.1.6.6. (E)-6-chloro-2-(3-(4-methylstyryl)phenyl)chroman-4-one (161)

96.5 mg (0.256 mmol) of (E)-1-(2-hydroxy-5-chlorophenyl)-3-(3-((E)-4-methylstyryl)phenyl)prop-2-en-1-one and 210.0 mg (2.56 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 15 mg of (E)-6-chloro-2-(3-(4-methylstyryl)phenylchroman-4-one as white solid with 26.4% yield. Rf = 0.50 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.91 (d, *J* = 2.5 Hz, 1H), 7.60 (s, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.50–7.39 (m, 4H), 7.33 (d, *J* = 7.6 Hz, 1H), 7.22–7.01 (m, 5H), 5.49 (dd, *J* = 13.3, 2.5 Hz, 1H), 3.11 (dd, *J* = 17.0, 13.3 Hz, 1H), 2.93 (dd, *J* = 17.0, 2.9 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR

(100 MHz, $CDCl_3$) δ = 191.13, 160.29, 139.03, 138.63, 138.23, 136.38, 134.52, 129.93, 129.80, 129.57, 127.58, 127.28, 127.19, 126.85, 126.76, 125.32, 124.42, 122.05, 120.24, 80.17, 44.70, 21.62.

3.1.6.7. (E)-6-chloro-2-(3-(3-methylstyryl)phenyl)chroman-4-one (162)

93 mg (0.248 mmol) of (E)-1-(2-hydroxy-5-chlorophenyl)-3-(3-((E)-3-methylstyryl)phenyl)prop-2-en-1-one and 203 mg (2.48 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 71 mg of (E)-6-chloro-2-(3-(3-methylstyryl)phenylchroman-4-one as white solid with 76.3% yield. Rf = 0.50 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.81 (d, *J* = 2.3Hz, 1H), 7.50 (s, 1H), 7.44 (d, *J* = 7.7 Hz, 1H), 7.39–7.29 (m, 2H), 7.28–7.12 (m, 4H), 7.05–6.91 (m, 4H), 5.37 (d, *J* = 13.2 Hz, 1H), 3.07–2.92 (m, 1H), 2.82 (d, *J* = 17.0 Hz, 1H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $cdcl_3$) δ = 191.03, 160.23, 139.04, 138.59, 138.48, 137.21, 136.33, 130.06, 129.54, 129.07, 128.94, 128.03, 127.60, 127.53, 127.21, 126.72, 125.42, 124.48, 124.10, 122.01, 120.20, 80.10, 44.63, 21.75.

3.1.6.8. (E)-6-chloro-2-(3-(3-fluorostyryl)phenyl)chroman-4-one (163)

77 mg (0.203 mmol) of (E)-1-(2-hydroxy-5-chlorophenyl)-3-(3-((E)-3-fluorostyryl)phenyl)prop-2-en-1-one and 166 mg (2.03 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 58 mg of (E)-6-chloro-2-(3-(3-fluorostyryl)phenylchroman-4-one as white solid with 77.3% yield. Rf = 0.45 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz,

$CDCl_3$) δ = 7.90 (s, 1H), 7.64–7.40 (m, 4H), 7.40–7.19 (m, 4H), 7.12 (s, 2H), 7.05 (dd, *J* = 8.8, 1.9 Hz, 1H), 6.98 (dd, *J* = 8.4, 8.4 Hz, 1H), 5.49 (d, *J* = 13.2 Hz, 1H), 3.18–3.03 (m, 1H), 2.93 (d, *J* = 16.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 207.10, 190.82, 164.58, 162.14, 160.08, 139.58, 139.50, 139.06, 137.78, 136.23, 136.22, 130.41, 130.32, 130.31, 129.48, 128.62, 128.60, 127.46, 127.26, 126.61, 126.60, 126.56, 125.76, 124.53, 124.51, 122.77, 122.75, 121.90, 120.07, 114.98, 114.76, 113.16, 113.15, 112.94, 79.89, 44.51.

3.1.6.9. (E)-5-methoxy-2-(3-(4-methoxystyryl)phenyl)chroman-4-one (164)

75 mg (0.194 mmol) of (E)-1-(2-hydroxy-6-methoxyphenyl)-3-(3-((E)-4-methoxystyryl)phenyl)prop-2-en-1-one and 159 mg (1.94 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 59.1 mg of (E)-5-methoxy-2-(3-(4-methoxystyryl)phenylchroman-4-one as white solid with 78.8% yield. Rf = 0.25 (ethyl acetate/hexanes, 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.57 (s, 1H), 7.50–7.35 (m, 5H), 7.30 (d, *J* = 7.7 Hz, 1H), 7.09 (d, *J* = 16.3 Hz, 1H), 6.98 (d, *J* = 16.3 Hz, 1H), 6.92–6.86 (m, 2H), 6.68 (dd, *J* = 8.3, 0.6 Hz, 1H), 6.54 (d, *J* = 8.2 Hz, 1H), 5.42 (dd, *J* = 13.2, 2.8 Hz, 1H), 3.92 (s, 3H), 3.81 (s, 3H), 3.08 (dd, *J* = 16.4, 13.3 Hz, 1H), 2.86 (dd, *J* = 16.4, 2.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 190.95, 163.44, 161.05, 159.72, 139.42, 138.55, 136.35, 130.14, 129.38, 129.26, 128.10, 126.75, 126.27, 125.09, 124.18, 114.45, 111.65, 110.51, 104.38, 79.24, 56.51, 55.60, 46.30.

3.1.6.10. (E)-5-methoxy-2-(3-(4-methylstyryl)phenyl)chroman-4-one (165)

85 mg (0.23 mmol) of (E)-1-(2-hydroxy-6-methoxyphenyl)-3-(3-((E)-4-methylstyryl)phenyl)prop-2-en-1-one and 189 mg (2.3 mmol) of sodium acetate were

heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 54 mg of (E)-5-methoxy-2-(3-(4-methylstyryl)phenylchroman-4-one as white solid with 63.5% yield. Rf = 0.35 (ethyl acetate/hexanes, 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.60 (s, 1H), 7.53–7.49 (m, 1H), 7.46–7.38 (m, 4H), 7.34 (d, *J* = 7.7 Hz, 1H), 7.20–7.15 (m, 2H), 7.11 (d, *J* = 7.1 Hz, 2H), 6.70 (dd, *J* = 8.4, 0.9 Hz, 1H), 6.56 (dd, *J* = 8.4, 0.7 Hz, 1H), 5.45 (dd, *J* = 13.2, 2.9 Hz, 1H), 3.94 (s, 3H), 3.10 (dd, *J* = 16.4, 13.2 Hz, 1H), 2.89 (dd, *J* = 16.5, 2.9 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 190.93, 163.44, 161.06, 139.45, 138.41, 138.04, 136.36, 134.56, 129.72, 129.67, 129.39, 127.39, 126.89, 126.79, 125.29, 124.33, 111.65, 110.51, 104.39, 79.22, 56.52, 46.31, 21.57.

3.1.6.11. (E)-5-methoxy-2-(3-(3-methylstyryl)phenyl)chroman-4-one (166)

99 mg (0.267 mmol) of (E)-1-(2-hydroxy-6-methoxyphenyl)-3-(3-((E)-3-methylstyryl)phenyl)prop-2-en-1-one and 219 mg (2.67 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 60 mg of (E)-5-methoxy-2-(3-(3-methylstyryl)phenylchroman-4-one as white solid with 60.6% yield. Rf = 0.35 (ethyl acetate/hexanes, 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.61 (s, 1H), 7.52 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.45–7.38 (m, 2H), 7.37–7.31 (m, 3H), 7.30–7.23 (m, 1H), 7.13 (s, 2H), 7.10 (d, *J* = 7.4 Hz, 1H), 6.70 (dd, *J* = 8.3, 0.9 Hz, 1H), 6.56 (dd, *J* = 8.4, 0.8 Hz, 1H), 5.45 (dd, *J* = 13.2, 2.9 Hz, 1H), 3.93 (s, 3H), 3.10 (dd, *J* = 16.5, 13.2 Hz, 1H), 2.89 (dd, *J* = 16.5, 2.9 Hz, 1H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 190.85, 163.40, 161.03, 139.46, 138.50, 138.28, 137.25, 136.33,

129.81, 129.38, 128.93, 128.87, 128.15, 127.54, 126.92, 125.39, 124.40, 124.05, 111.63, 110.48, 104.37, 79.17, 56.48, 46.27, 21.71.

3.1.6.12. (E)-5-methoxy-2-(3-(3-fluorostyryl)phenyl)chroman-4-one (167)

110 mg (0.294 mmol) of (E)-1-(2-hydroxy-6-methoxyphenyl)-3-(3-((E)-3-fluorostyryl)phenyl)prop-2-en-1-one and 241 mg (2.94 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 62 mg of (E)-5-methoxy-2-(3-(3-fluorostyryl)phenylchroman-4-one as white solid with 56.4% yield. Rf = 0.30 (ethyl acetate/hexanes, 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.59 (s, 1H), 7.49 (dt, *J* = 7.6, 1.4 Hz, 1H), 7.43–7.23 (m, 5H), 7.23–7.17 (m, 1H), 7.11–7.07 (m, 2H), 6.97–6.90 (m, 1H), 6.67 (dd, *J* = 8.3, 0.8 Hz, 1H), 6.54 (d, *J* = 8.4 Hz, 1H), 5.43 (dd, *J* = 13.2, 2.9 Hz, 1H), 3.92 (s, 3H), 3.07 (dd, *J* = 16.4, 13.2 Hz, 1H), 2.86 (dd, *J* = 16.4, 3.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 190.73, 164.65, 163.34, 162.20, 161.04, 139.74, 139.67, 139.60, 137.70, 136.36, 130.45, 130.36, 129.71, 129.46, 128.50, 128.48, 127.09, 125.84, 124.56, 124.54, 122.82, 122.79, 114.95, 114.74, 113.22, 112.99, 111.63, 110.45, 104.43, 104.42, 79.09, 79.07, 56.50, 56.46, 46.27.

3.1.6.13. (E)-7-methoxy-2-(3-(4-methoxystyryl)phenyl)chroman-4-one (168)

78.5 mg (0.203 mmol) of (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-4-methoxystyryl)phenyl)prop-2-en-1-one and 166 mg (2.03 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude

product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 53.3 mg of (E)-7-methoxy-2-(3-(4-methoxystyryl)phenylchroman-4-one as white solid with 67.9% yield. Rf = 0.16 (ethyl acetate/hexanes, 1:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.89 (dd, *J* = 8.2, 3.8 Hz, 1H), 7.60 (s, 1H), 7.53–7.44 (m, 3H), 7.41 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.33 (d, *J* = 7.7 Hz, 1H), 7.11 (d, *J* = 16.3 Hz, 1H), 7.00 (d, *J* = 16.3 Hz, 1H), 6.94–6.87 (m, 2H), 6.63 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.53 (d, *J* = 2.4 Hz, 1H), 5.47 (dd, *J* = 13.3, 2.9 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.06 (dd, *J* = 16.9, 13.3 Hz, 1H), 2.85 (dd, *J* = 16.9, 2.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 190.80, 166.47, 163.80, 159.77, 139.53, 138.62, 130.12, 129.43, 129.33, 129.06, 128.12, 126.81, 126.24, 125.13, 124.25, 115.13, 114.47, 110.57, 101.25, 101.22, 80.31, 80.29, 55.96, 55.92, 55.62, 55.58, 44.68.

3.1.6.14. (E)-7-methoxy-2-(3-(4-methylstyryl)phenyl)chroman-4-one (169)

82 mg (0.223 mmol) of (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-4-methylstyryl)phenyl)prop-2-en-1-one and 183 mg (2.23 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 26.5 mg of (E)-7-methoxy-2-(3-(4-methylstyryl)phenylchroman-4-one as white solid with 32.3% yield. Rf = 0.68 (ethyl acetate/hexanes, 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.90 (d, *J* = 8.8 Hz, 1H), 7.62 (s, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.46–7.38 (m, 3H), 7.35 (d, *J* = 7.7 Hz, 1H), 7.22–7.15 (m, 2H), 7.12 (d, *J* = 7.1 Hz, 2H), 6.64 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.54 (d, *J* = 2.4 Hz, 1H), 5.49 (dd, *J* = 13.3, 2.9 Hz, 1H), 3.85 (s, 3H), 3.07 (dd, *J* = 16.9, 13.3 Hz, 1H), 2.86 (dd, *J* = 16.9, 3.0 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 190.84, 166.52, 163.84, 139.58, 138.53, 138.13, 134.58, 129.77, 129.48, 129.10, 127.39, 127.00, 126.83, 125.36, 124.43, 115.16, 110.65, 110.63, 101.28, 101.26, 101.25, 80.33, 80.32, 56.00, 55.97, 44.72, 21.61, 21.59.

3.1.6.15. (E)-7-methoxy-2-(3-(3-methylstyryl)phenyl)chroman-4-one (170)

75 mg (0.202 mmol) of (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-3-methylstyryl)phenyl)prop-2-en-1-one and 165.6 mg (2.02 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 54.8 mg of (E)-7-methoxy-2-(3-(3-methylstyryl)phenylchroman-4-one as white solid with 73% yield. Rf = 0.70 (ethyl acetate/hexanes, 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.93–7.86 (m, 1H), 7.63 (s, 1H), 7.56–7.50 (m, 1H), 7.43 (dd, *J* = 9.5, 5.7 Hz, 1H), 7.38–7.31 (m, 3H), 7.26 (dd, *J* = 9.4, 5.7 Hz, 1H), 7.13 (s, 2H), 7.10 (d, *J* = 7.4 Hz, 1H), 6.64 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.54 (d, *J* = 2.4 Hz, 1H), 5.48 (dd, *J* = 13.3, 2.9 Hz, 1H), 3.84 (s, 3H), 3.07 (dd, *J* = 16.9, 13.3 Hz, 1H), 2.86 (dd, *J* = 16.9, 3.0 Hz, 1H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 190.81, 166.51, 163.81, 139.59, 138.57, 138.41, 137.28, 129.94, 129.48, 129.09, 129.01, 128.93, 128.16, 127.59, 127.05, 125.48, 124.51, 124.10, 115.14, 110.63, 101.26, 80.28, 55.97, 44.70, 21.75.

3.1.6.16. (E)-7-methoxy-2-(3-(3-fluoroystyryl)phenyl)chroman-4-one (171)

58 mg (0.155 mmol) of (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-3-fluorostyryl)phenyl)prop-2-en-1-one and 127 mg (1.55 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 28.6 mg of (E)-7-methoxy-2-(3-(3-fluorostyryl)phenylchroman-4-one as white solid with 49.3% yield. Rf = 0.70 (ethyl acetate/hexanes, 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.81 (d, *J* = 8.8 Hz, 1H), 7.55 (s, 1H), 7.45 (d, *J* = 7.6 Hz, 1H), 7.35 (dd, *J* =

7.6, 7.6 Hz, 1H), 7.32–7.12 (m, 4H), 7.04 (d, *J* = 2.3 Hz, 2H), 6.93–6.85 (m, 1H), 6.55 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.45 (d, *J* = 2.4 Hz, 1H), 5.41 (dd, *J* = 13.3, 2.9 Hz, 1H), 3.76 (s, 3H), 2.98 (dd, *J* = 16.9, 13.3 Hz, 1H), 2.78 (dd, *J* = 16.9, 3.0, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 190.73, 166.56, 164.73, 163.79, 162.29, 139.79, 139.75, 139.71, 137.85, 130.53, 130.44, 129.74, 129.60, 129.14, 128.66, 128.63, 127.25, 125.96, 124.68, 124.67, 122.89, 122.86, 115.17, 115.07, 114.86, 113.29, 113.07, 110.69, 110.66, 101.31, 101.29, 101.27, 80.24, 80.23, 56.03, 55.98, 44.74.

3.1.6.17. (E)-5,7-dimethoxy-2-(3-(4-methoxystyryl)phenyl)chroman-4-one (140)

102 mg (0.245 mmol) of (E)-1-(2-hydroxy-4-methoxyphenyl)-3-(3-((E)-3-fluorostyryl)phenyl)prop-2-en-1-one and 201 mg (2.45 mmol) of sodium acetate were heated in refluxing ethanol (8 ml) for 48 hours under nitrogen atmosphere. The mixture then allowed to cool to room temperature and poured into cold water (20 ml) and then extracted with ethyl acetate (2x25 ml). The combined organic phase was washed with brine and dried over $MgSO_4$. After concentrated under vacuum, crude product was purified by column chromatography on silica gel (12.5-25 % ethyl acetate in hexanes) to afford 66.3 mg of (E)-5,7-methoxy-2-(3-(4-methoxystyryl)phenylchroman-4-one as white solid with 65% yield. Rf = 0.13 (ethyl acetate/hexanes, 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.57 (s, 1H), 7.52–7.43 (m, 3H), 7.39 (dd, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 7.7 Hz, 1H), 7.10 (d, *J* = 16.3 Hz, 1H), 6.99 (d, *J* = 16.3 Hz, 1H), 6.94–6.86 (m, 2H), 6.19 (d, *J* = 2.3 Hz, 1H), 6.10 (d, *J* = 2.3 Hz, 1H), 5.42 (dd, *J* = 13.2, 2.9 Hz, 1H), 3.90 (s, 3H), 3.82 (s, 6H), 3.05 (dd, *J* = 16.5, 13.3 Hz, 1H), 2.82 (dd, *J* = 16.6, 2.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 189.48, 166.27, 165.26, 162.57, 159.73, 139.47, 138.54, 130.14, 129.37, 129.25, 128.09, 126.74, 126.28, 125.10, 124.20, 114.45, 106.27, 93.86, 93.49, 79.50, 56.45, 55.91, 55.60, 45.93.

3.2. Measuring Cell Viability Test

3.2.1. General Methods

PC3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum (FBS), 100 µg/ml streptomycin/100IU/ml penicillin, MCF7 cell line was maintained in Roswell Park Memorial Institude-1640 (RPMI-1640) containing 15% FBS (BIO-IND), 100 µg/ml streptomycin/100IU/ml penicillin incubated at 37 ºC in the dark with 5% CO_2 humidified incubator and passaged when they reached 80-85% confluency. Cells used in experiments were maintained from 10-20^{th} passages.

3.2.2. MTT Tests for Compounds

To investigate the cytotoxic activity of the compounds, 95 µl of cell suspension was inoculated into 96-well microculture plates at $1x10^4$ cells density per well in culture media containing FBS, penicillin/streptomycin. Compounds were dissolved in dimethylsulfoxide (DMSO) (Sigma Chemical Co.), filter sterilized, diluted at the appropriate concentrations with the culture medium. In all well, 1% DMSO concentration was fixed. Dilutions of compounds were freshly prepared before each experiment. After 24h cultivation for cell attachment, compounds were added at final concentration 50, 25, 1, 0.5, 0.1, 0.05, and 0.01 µM for triplicate assay. Cells were treated with the compounds for 48 hours and cytotoxic effects were determined by tetrazolium (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma Chemical Co.) based colorimetric assay. This method depends on the cleavage of tetrazolium salt to purple formazan crystals by mitochondrial enzymes of metabolically active cells (Ciapetti, et al. 1993). Briefly; 4 hours before end of incubation period, medium of the cells was removed and wells were washed by pre-warmed phosphate-buffered saline (PBS) to remove any trace of compounds and to prevent colour interference while optical density determination. MTT stock solution (5mg/ml) was diluted at 1:10 ratio into complete culture media, 100 µl of MTT dilution was added into each well and incubated. After 3.5 hours plates were centrifuged at 1800 rpm for 10 minute at room temperatures to avoid accidental removal of formazan crystals. Crystals

were dissolved with 100µl DMSO. The absorbance was determined at 540nm. Results were represented as percentage viability and calculated by the following formula (Equation 3.1).

$$\% \text{ viability}=[(OD_s-OD_b/OD_c-OD_b)x100] \quad (3.1)$$

CHAPTER 4

CONCLUSION

Stilbenes, flavanones and chalcones are valuable, biologically active compounds. In this thesis, stilbene fused chalcones and stilbene fused flavanones were designed as potential biologically active compounds.

Syntheses of target compounds were accomplished by two alternative routes. Because of the problematic Heck reactions of halogenated flavanones at the first route, stilbene formations were done in first step at second route. Then formed stilbenes were subjected to aldol reactions in basic ethanol solution. Finally cyclization reactions were carried out with sodium acetate in ethanol. Synthesis of two small libraries of stilbene fused chalcones and stilbene fused flavanones were successfully prepared.

Selected examples from these libraries were evaluated their cytotoxic activities over PC3 and MCF-7 cancer cell lines. One of the stilbene fused flavanones' cytotoxicity compared with the cytotoxicities of corresponding simple flavanone and stilbene structure. These preliminary cytotoxicity tests show that selected stilbene fused flavanone shows higher cytotoxicity than simple flavanone and stilbene structure over PC3 cell lines. Addition to that, stilbene fused chalcones are much more toxic than stilbene fused flavanones over both cancer cell lines. Finally cytotoxicities of stilbene fused chalcones can be altered by changing the substituents coming from styrene and acetophenones started with. Stilbene fused chalcones, having methyl substituent on styryl part, has found the most active compound so far. To tell more about the structure activity relationship of libraries more cytotoxicity test is on the way.

REFERENCES

American Cancer Society 2007. Cancer Facts & Figures 2007.

Cabrera, M., Simoens, M., Falchi, G., Lavaggi, M. L., Piro, O. E., Castellano, E. E., Vidal, A., Azqueta, A., Monge, A., de Cerain, A. L., Sagrera, G., Seoane, G., Cerecetto, H. and Gonzalez, M. 2007. Synthetic chalcones, flavanones, and flavones as antitumoral agents: Biological evaluation and structure–activity relationships. *Bioorganic & Medicinal Chemistry* 15:3356–3367.

Chandrasekhar, S., Vijeender, K. and Reddy, K: V. 2005. New synthesis of flavanones catalyzed by L-proline. *Tetrahedron Letters* 46:6991–6993.

Ciapetti, G.: Cenni, E., Pratelli, L., Pizzoferrato, A. 1993**.** In vitro evaluation of cell/biomaterial interaction by MTT assay. *Biomaterials*14:359-364.

Comisar, C. M. and Savage, P. E. 2004. Kinetics of crossed aldol condensations in high-temperature water. *Green Chem* . 6:227–231.

Cushman, M., Nagarathnam, D., Gopal, D., Chakraborti, A. K., Lin, C. M., Hamel, E. 1991. Synthesis and Evaluation of Stilbene and Dihydrostilbene Derivatives as Potential Anticancer Agents That Inhibit Tubulin Polymerization. *J. Med. Chem.* 34, 2579-2588.

Dittmer, C., Raabe, G. and Hintermann, L. 2007. Asymmetric Cyclization of 2_-Hydroxychalcones to Flavanones: Catalysis by Chiral Brønsted Acids and Bases. *Eur. J. Org. Chem* 5886–5898.

Fröhner, C. R. A., de Oliveira, K. N., Silva, D. G., Pacheco, L. K., Joussef, A. C., Steindel, M., Claudia M.O., de Souza, Simoes Alessandra M.T., Magalhaes, U. O., Afonso, I. F., Rodrigues, C. R., Nunes, R. J., Castro, H. C., 2009. Synthesis, biological evaluation and SAR of sulphonamide 4-methoxychalcone derivatives with potential antileishmanial activity *European Journal of Medicinal Chemistry* 44:755-763.

Gaukroger, K., Hadfield, J. A., Hepworth, L. A., Lawrence, N. J. and McGown, A. T. 2001. Novel Syntheses of Cis and Trans Isomers of Combretastatin A-4. *J. Org. Chem. 66:* 8135-8138.

Goldberg, D. M., Eric, N.G., Karumanchiri, A., Diamandis, E. P., Soleas, G. J. 1996. Resveratrol glucosides are important components of commercial wines. *Am. J. Enol. Vitic.* 47:415-420.

Hanahan, D. and Weinberg R.A. 2000. The hallmarks of cancer. *Cell* 100:57-70.

Hea, Q., Li, R., Fang, L., Ying, H., Hu Y.and Yang, B. 2006. Antileukemia activity of MSFTZ–a novel flavanone analog. *Anti-Cancer Drugs* 17: 641-647.

Heynekamp, J. J., Weber, W. M., Hunsaker, L. A., Gonzales, A. M., Orlando, R. A., Deck, L. M. and Jagt, D. L. V. 2006. Substituted *trans*-Stilbenes, Including Analogues of the Natural Product Resveratrol, Inhibit the Human Tumor Necrosis Factor Alpha-Induced Activation of Transcription Factor Nuclear Factor KappaB. *J. Med. Chem.* 49:7182-7189.

Hsiao, Y. C., Kuo, W. H., Chen, P. N., Change, H. R., Lin, T. H., Yang, W. E., Hsieh, Y. S., Chu, S. C. 2007. Flavanone and 2'-OH flavanone inhibit metastasis of lungcancer cells via down-regulation of proteinases activities and MAPK pathway. *Chemico-Biological Interactions* 167:193–206.

Katsuyama, Y., Funa, N., Miyahisa, I. and Horinouchi, S. 2007. Synthesis of Unnatural Flavonoids and Stilbenes by Exploiting the Plant Biosynthetic Pathway in *Escherichia coli*. *Chemistry & Biology* 14:613–621.

Kaur, K., Jain, M., Kaur, T., Jain, R. 2009. Antimalarials from nature. *Bioorganic & Medicinal Chemistry* 17:3229–3256.

Kimura, Y. and Okuda H. 2001. Resveratrol isolated from *Polygonum cuspidatum* roootprevents tumor growth and metastasis to lung and tumor-induced neovascularizatio in Lewis lung carcinoma-bearing mice. *J Nutr*, 131:1844–1849.

Kimura, Y. and Okuda T. 2000. Effects of naturally occurring stilbene glucosides from medicinal plants and wine, on tumor growth and lung metastasis in Lewis lung carcinoma-bearing mice. *J Pharm Pharmacol* 52:1287–1295.

Kimura, Y., Baba, K., Okuda, T. 2000. Inhibitory effects of active substances isolated from *Cassia garretiana* heartwood on tumor growth and lung metastasis in Lewis lung carcinoma-bearing mice (Part 2). *Anticancer Res* 20:2923–2930.

Kimura, Y., Sumiyoshi, M. and Baba, K. 2008. Antitumor activities of synthetic and natural stilbenes through antiangiogenic action. Japanese Cancer Association.

Lauren Pecorino 2005. *Molecular Biology of Cancer: Mechanism, Targets and Therapeutics.* New York: Oxford University Press.

Lee, Y. S., Lim, S. S., Shin, K. H., Kim, Y. S., Ohuchi, K. and Jung, S. H. 2006. Anti-angiogenic and Anti-tumor Activities of 2'-Hydroxy-4'methoxychalcone. *Biol. Pharm. Bull.* 29:1028.

Linuma, M., Ohyama, M. and Tanaka, T. 1995. Six Flavonostilbenes and a flavanone in roots of *Sophora Alopecuroides. Phytochemistry*. 32:519-525.

Lunardi, F., Guzela, M., Rodrigues, A. T., Correˆa, R., Mangrich, I. E., Steindel, M., Grisard, E. C., Assreuy, J., Calixto, J. B. and Santos, Adair R. S. 2003. Trypanocidal and Leishmanicidal Properties of Substitution-Containing Chalcones. *Antimicrobial Agents and Chemotherapy* 1449–1451.

Mbaveng, A. T., Ngameni, B., Kuete, V., Simo, I. K., Ambassa, P., Roy, R., Bezabih, M., Etoa,F. X., Ngadjui, B. T., Abegaz, B. M., Meyer, J. J. M., Lall, N., Beng, V. P. 2008. Antimicrobial activity of the crude extracts and five flavonoids from the twigs of *Dorstenia barteri* (Moraceae) *Journal of Ethnopharmacology* 116:483–489.

Mishra, N., Arora, P., Kumar, B., Mishra, L: C., Bhattacharya, A., Awasthi, S. K., Bhasin, V. K. 2008. Synthesis of novel substituted 1,3-diaryl propenone derivatives and their antimalarial activity in vitro. *European Journal of Medicinal Chemistry* 43:1530-1535.

Modzelewska, A., Pettit, C., Achanta, G., Davidson, N. E., Huang, P. and Khan, S. R. 2006. Anticancer activities of novel chalcone and bis-chalcone derivatives. *Bioorganic & Medicinal Chemistry* 14:3491–3495.

Mukherjee, S., Kumar, V., Prasad, A. K., Raj, H. G., Bracke, M. E., Olsen, C. E., Jain, S. C. and Parmar, V. S. 2001. Synthetic and Biological activity evaluation Studies on Novel 1,3-Diarylpropenones. *Bioorganic & Medicinal Chemistry* 9:337-345.

Nam, H. N., Kim, Y., You, Y. J., Hong, D. H., Kim, H. M., Ahn, B. Z. 2003. Cytotoxic 2',5'-dihydroxychalcones with unexpected antiangiogenic activity. *European Journal of Medicinal Chemistry* 38:179-187.

Nam, N. 2003. Combretastatin A-4 Analogues as Antimitotic Antitumor Agents. *Current Medicinal Chemistry* 10:1697-1722.

Narender, T. and Reddy, K. P. 2007. A simple and highly efficient method for the synthesis of chalcones by using borontrifluoride-etherate. *Tetrahedron Letters* 48:3177–3180.

Newman, D.J., Cragg, G.M. 2007. The influence of natural products upon drug discovery. *Natural Product Reports* 17:215-234.

Ngameni, B., Watchueng, J., Boyom,F. K., Keumedjio, F., Ngadjui, B. T., Gut, J., Abegaz, B. M. and Rosenthal, P. J. 2007. Antimalarial prenylated chalcones from the twigs of *Dorstenia barteri var. Subtriangularis*. *Arkivoc* 116-123.

Nowakowska, Z. 2007. A review of anti-infective and anti-inflammatory chalcones. *European Journal of Medicinal Chemistry* 42:125-137.

Pan, M. H., Gao, J. H., Lai, C. S., Wang, Y. J., Chen, W. M., Lo, C. H., Wang, M., Dushenkov, S. and Ho, C.T. 2008. Antitumor Activity of 3,5,4'-Trimethoxystilbene in COLO 205 Cells and Xenografts in SCID Mice. *Molecular Carcinogenesis* 47:184–196.

Peng, J., Fan, G., Wu, Y. 2006.Preparative isolation of four new and two known flavonoids from the leaf of *Patrinia villosa* Juss. by counter-current chromatography and evaluation of their anticancer activities in vitro. *Journal of Chromatography A* 1115:103–111.

Radwan, M. M., Guzman, R. R., Manly, S. P., Jacob, M., Ross, S. A. 2009. Sepicanin A—A new geranyl flavanone from *Artocarpus sepicanus* with activity against methicillin-resistant *Staphylococcus aureus* (MRSA). *Phytochemistry Letters* (in press).

Rao, Y. K., Geethangili, M., Fang, S. H., Tzeng, Y. M. 2007. Antioxidant and cytotoxic activities of naturally occurring phenolic and related compounds: A comparative study. *Food and Chemical Toxicology* 45:1770–1776.

Richard B. Silverman 2004. The Organic Chemistry of Drug Design and Drug Action, second edition, Elseiver Inc, USA.

Sagrera, G. J. and Seoane, G. A. 2005. Microwave Accelerated Solvent-Free Synthesis of Flavanones. *J. Braz. Chem*. Soc.16: 851-856.

Sato, M., Tsuchiya, H., Miyazaki, T., Ohyama, M., Tanaka, T. and Linuma, M. 1995. Antibacterial activity of flavanostilbenes against methicillin-resistant *Staphylococcus aureus. Letters in Applied Microbiology* 21:219-222.

Stanner, S. A., Hughes, J., Kelly, C. N. M. and Buttriss J. 2004. A review of the epidemiological evidence for the 'antioxidant hypothesis'. *Public Health Nutrition* 7:407-422.

Toda, F., Tanaka, K. and Hamai, K. 1990. Aldol Condensations in the Absence of Solvent: Acceleration of the Reaction and Enhancement of the Stereoselectivity. *J. Chem. Soc. Perkin Trans. 1* 3207-3209.

Tyagi, A., Bhatia, N., Condon, M.S., Bosland, M.C., Agarwal, C., Agarwal, R. 2002 Antiproliferative and antiapoptotic effect of silibinin in rat prostate cancer cells. *Prostate* 53:211–217.

Uchiumi, F., Hatano, T., Ito, H., Yoshida, T., Tanuma, T. 2003. Transcriptional suppression of the HIV promoter by natural compounds. *Antiviral Research* 58:89–98.

Vries, Andre´ H. M., Mulders, Jan M. C. A., Mommers, John H. M., Henderickx, Huub J. W. and Vries, Johannes G. 2003. Homeopathic Ligand-Free Palladium as a Catalyst in the Heck Reaction. AComparison with a Palladacycle. *Org. Lett.* 5:3285-3288.

Wu, J. H., Wang, X. H., Yi, Y. H., Lee, K. H. 2003. Anti-AIDS agents 54. A potent anti-HIV chalcone and flavonoids from genus *Desmos. Bioorg. Med. Chem. Lett*. 13:1813-1815.

Xu, Q., Yang, Z., Yin, D., Zhang, F. 2008. Synthesis of chalcones catalyzed by a novel solid sulfonic acid from bamboo. *Catalysis Communications*, 9:1579–1582.

Yahiaoui, S., Fagnere, C., Pouget, C., Buxeraud, J. and Chulia, A. J. 2007. New 7,8 benzoflavanones as potent aromatase inhibitors: Synthesis and biological evaluation. *Bioorganic & Medicinal Chemistry* 16:1474–1480.

Zi, X. and Agarwal, R. 1999. Silibinin decreases prostate-specific antigen with cell growth inhibition via G1 arrest, leading to differentiation of prostate carcinoma cells: implications for prostate cancer intervention. *Proc. Natl. Acad. Sci.* U.S.A. 96.

Zi, X., Grasso, A. W., Kung, H., Agarwal, R. 1998. A flavonoid antioxidant, silymarin, inhibits activation of erbB1 signaling and induces cyclin-dependent kinase inhibitors, G1 arrest, and anticarcinogenic effects in human prostate carcinoma DU145 cells. *Cancer Res*. 58:1920–1929.

Zi, X., Zhang, J., Agarwal, R., Pollak, M. 2000. Silibinin up-regulates insulin-like growth factor-binding protein 3 expression and inhibits proliferation of androgen-independent prostate cancer cells. *Cancer Res.* 60:5617-5620.

APPENDIX

^{1}H and ^{13}C NMR SPECTRUM OF COMPOUNDS 110,116,126,134,139 and 140

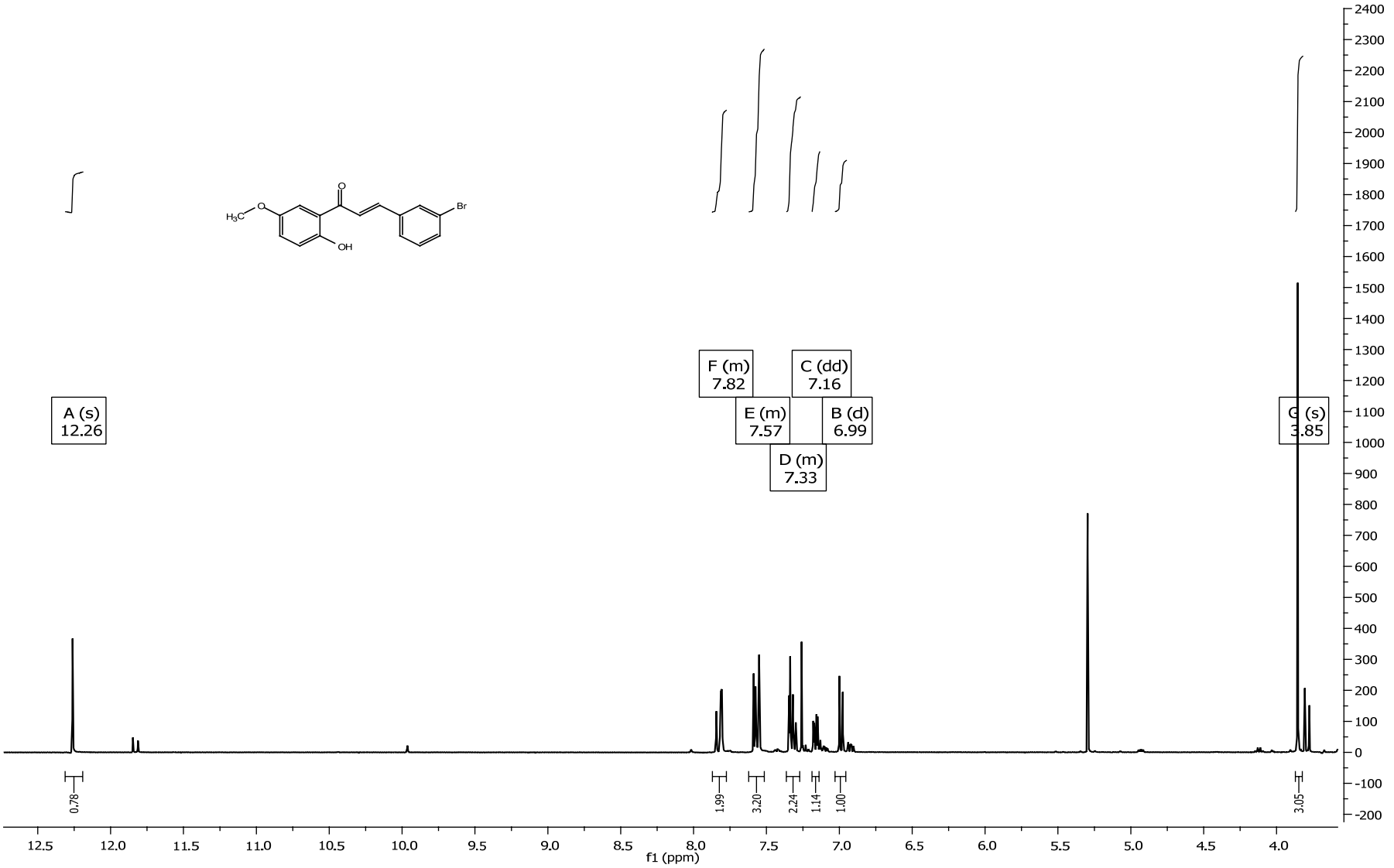


Figure A. 1. ^{1}H Spectra of (E)-3-(3-bromophenyl)-1-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one (110)

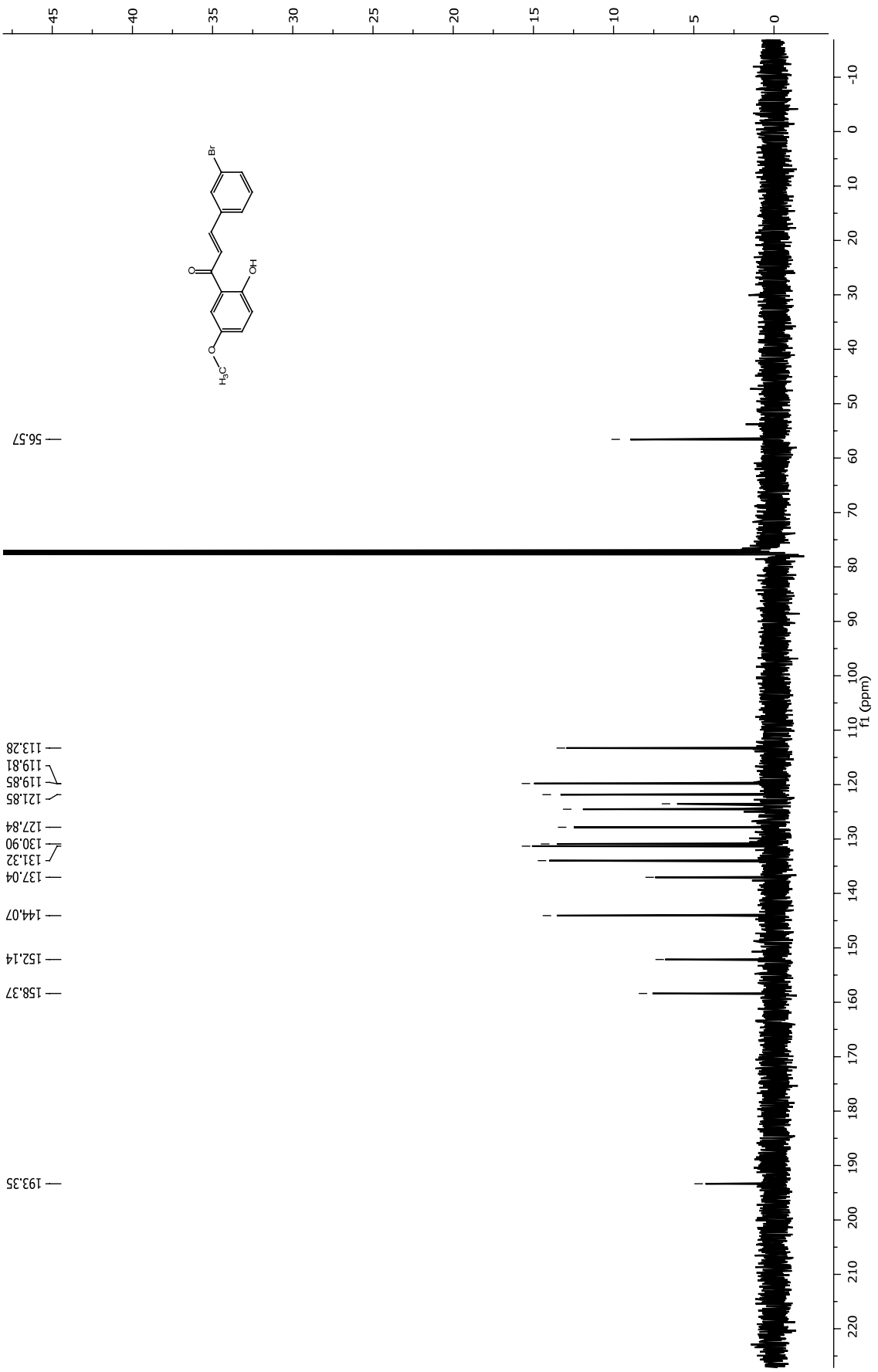


Figure A. 2. ^{13}C Spectra of (E)-3-(3-bromophenyl)-1-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one (110)

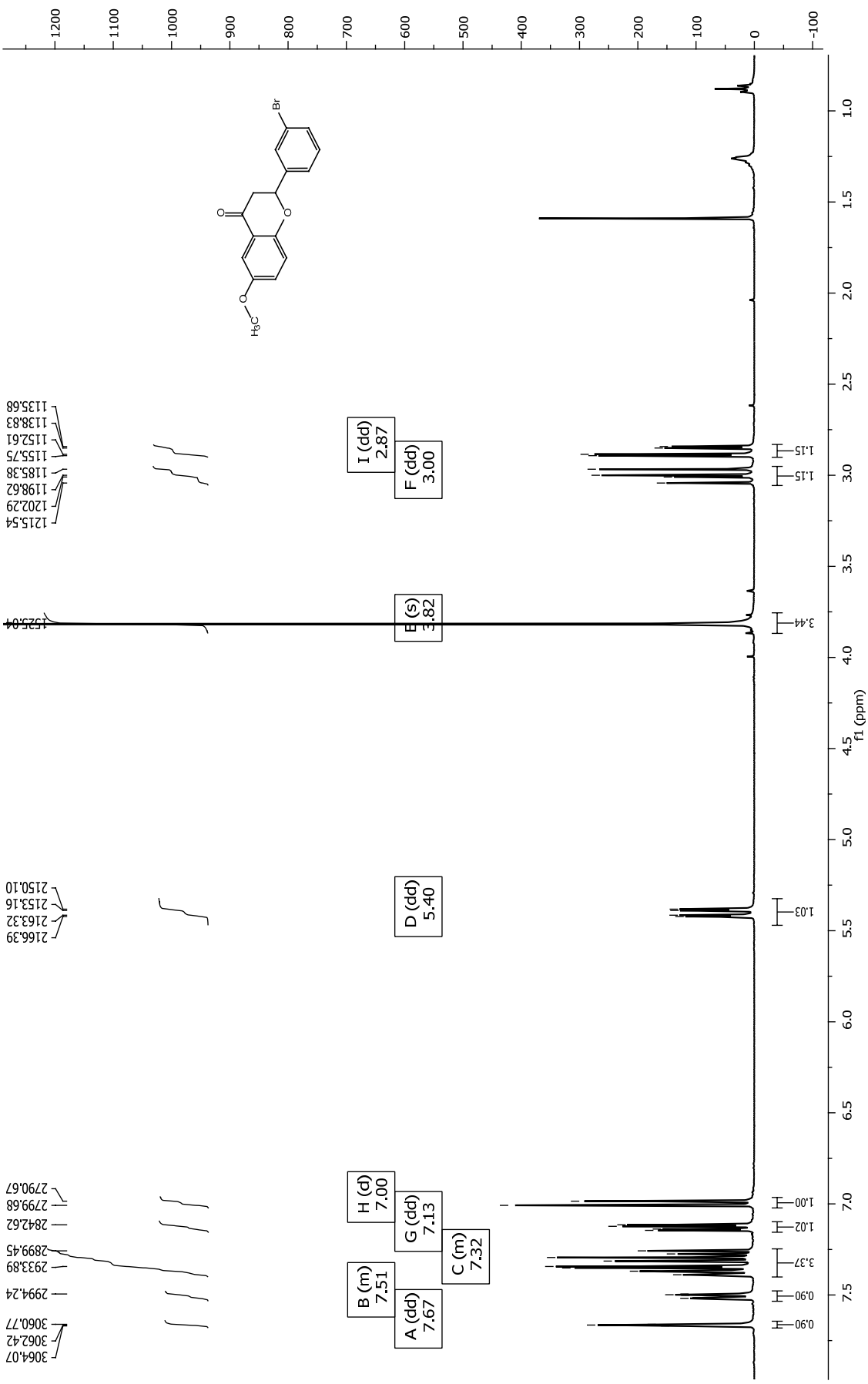


Figure A. 3. ^{1}H Spectra of 2-(3-bromophenyl)-6-methoxychroman-4-one (116)

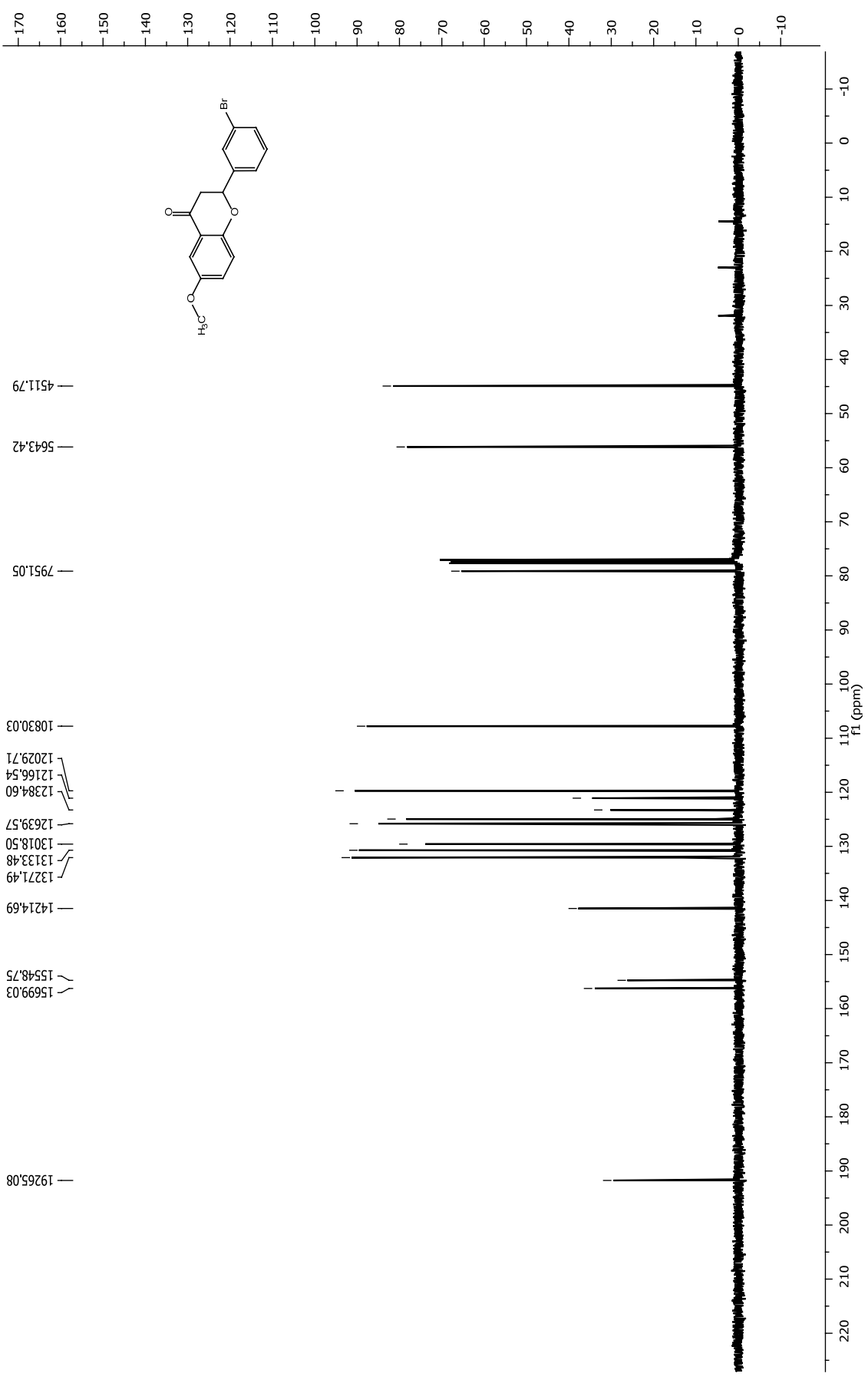


Figure A. 4. ^{13}C Spectra of 2-(3-bromophenyl)-6-methoxychroman-4-one (116)

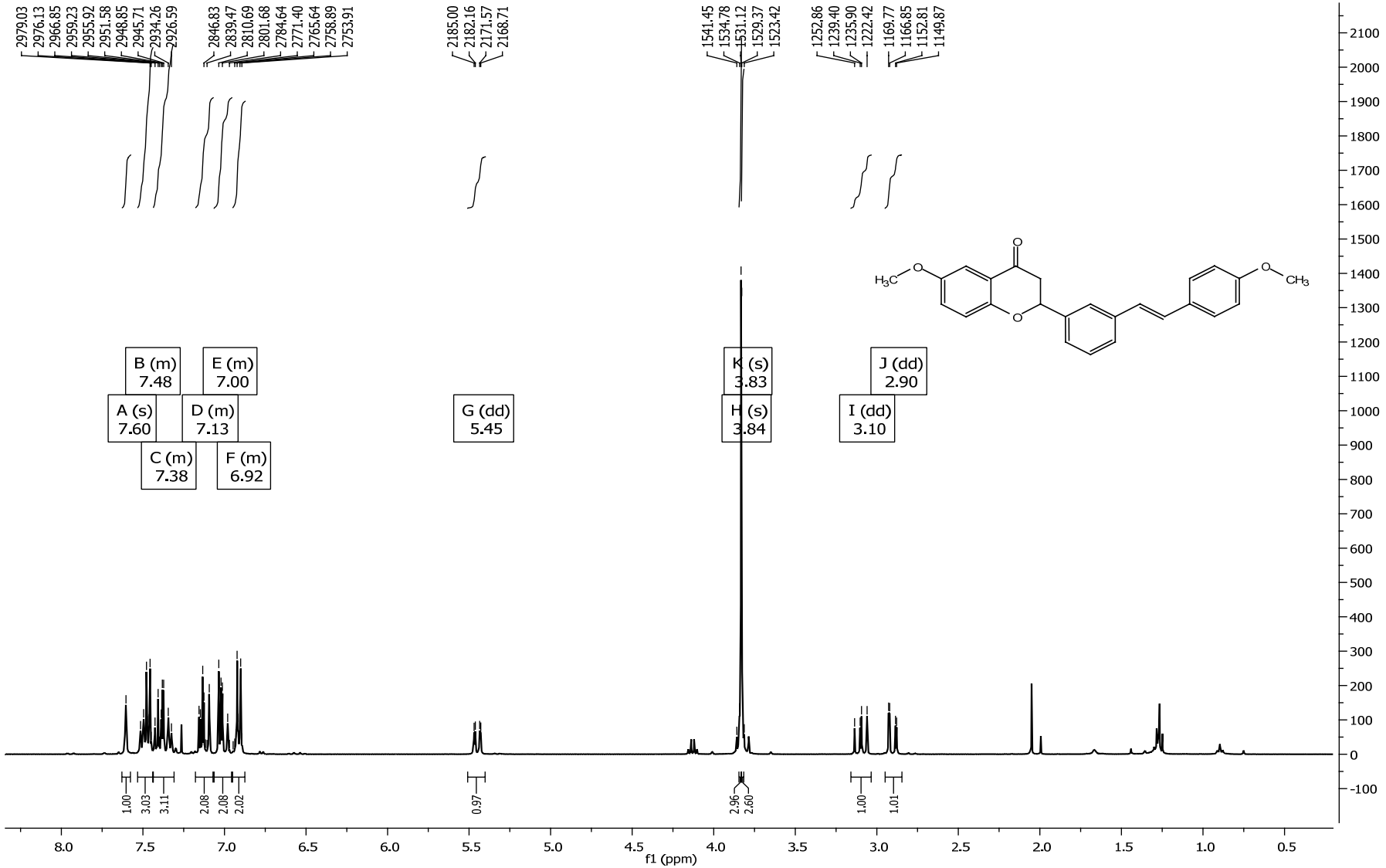


Figure A. 5. ^{1}H Spectra of (E)-6-methoxy-2-(3-(4-methoxystyryl)phenyl)chroman-4-one (126)

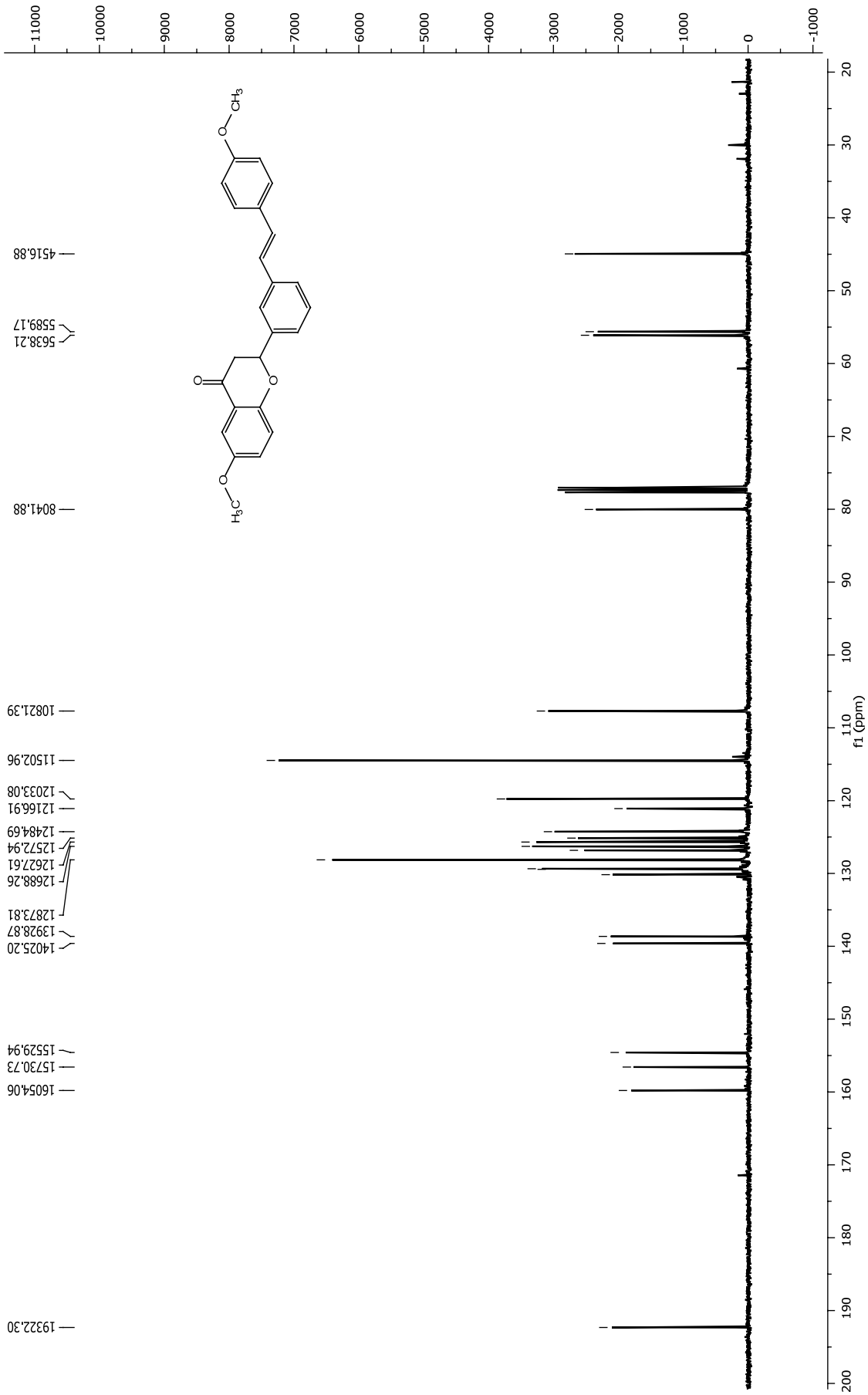


Figure A. 6. ^{13}C Spectra of (E)-6-methoxy-2-(3-(4-methoxystyryl)phenyl)chroman-4-one (126)

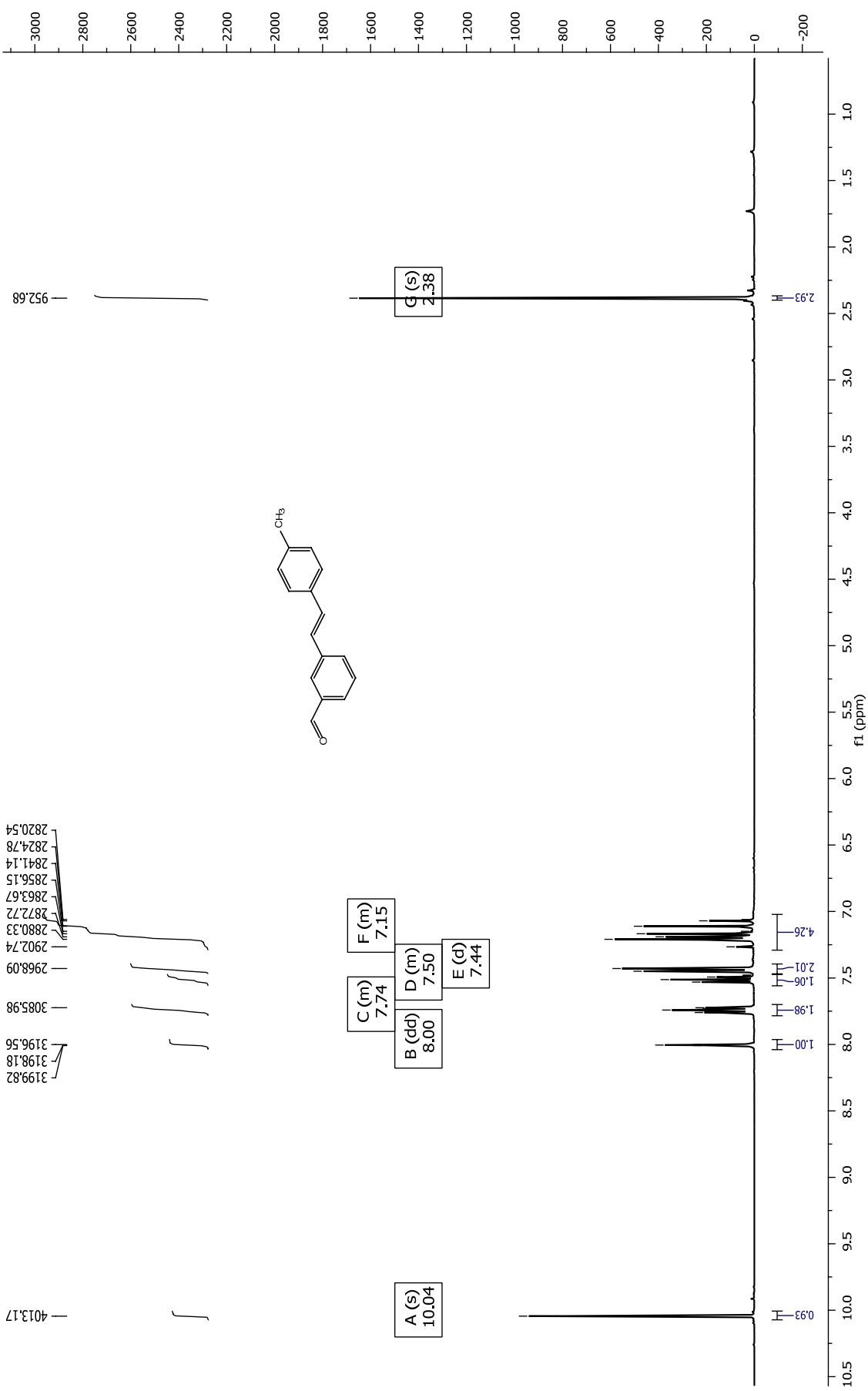


Figure A. 7. ^{1}H Spectra of (E)-3-(4-methylstyryl)benzaldehyde (134)

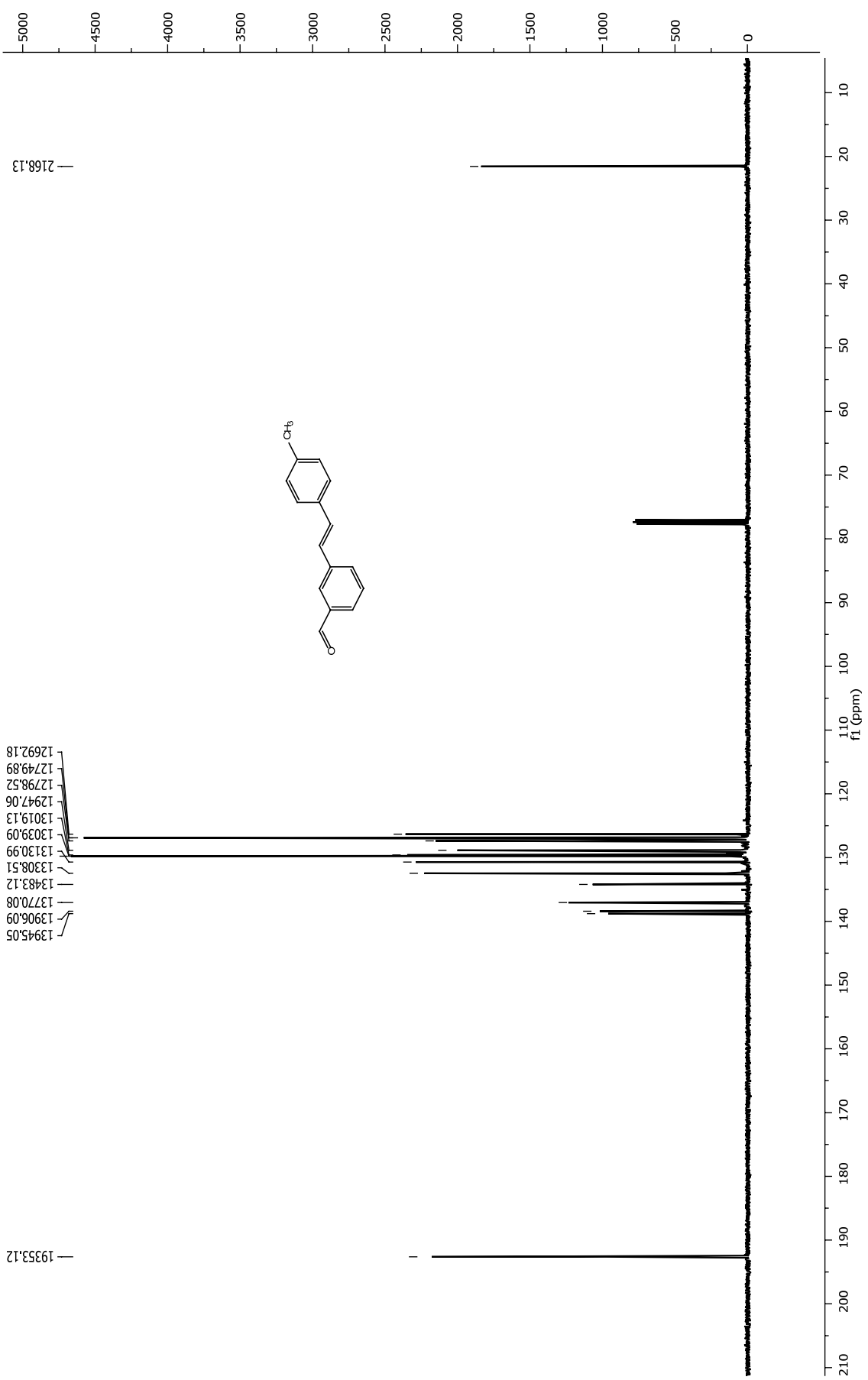


Figure A. 8. ^{13}C Spectra of (E)-3-(4-methylstyryl)benzaldehyde (134)

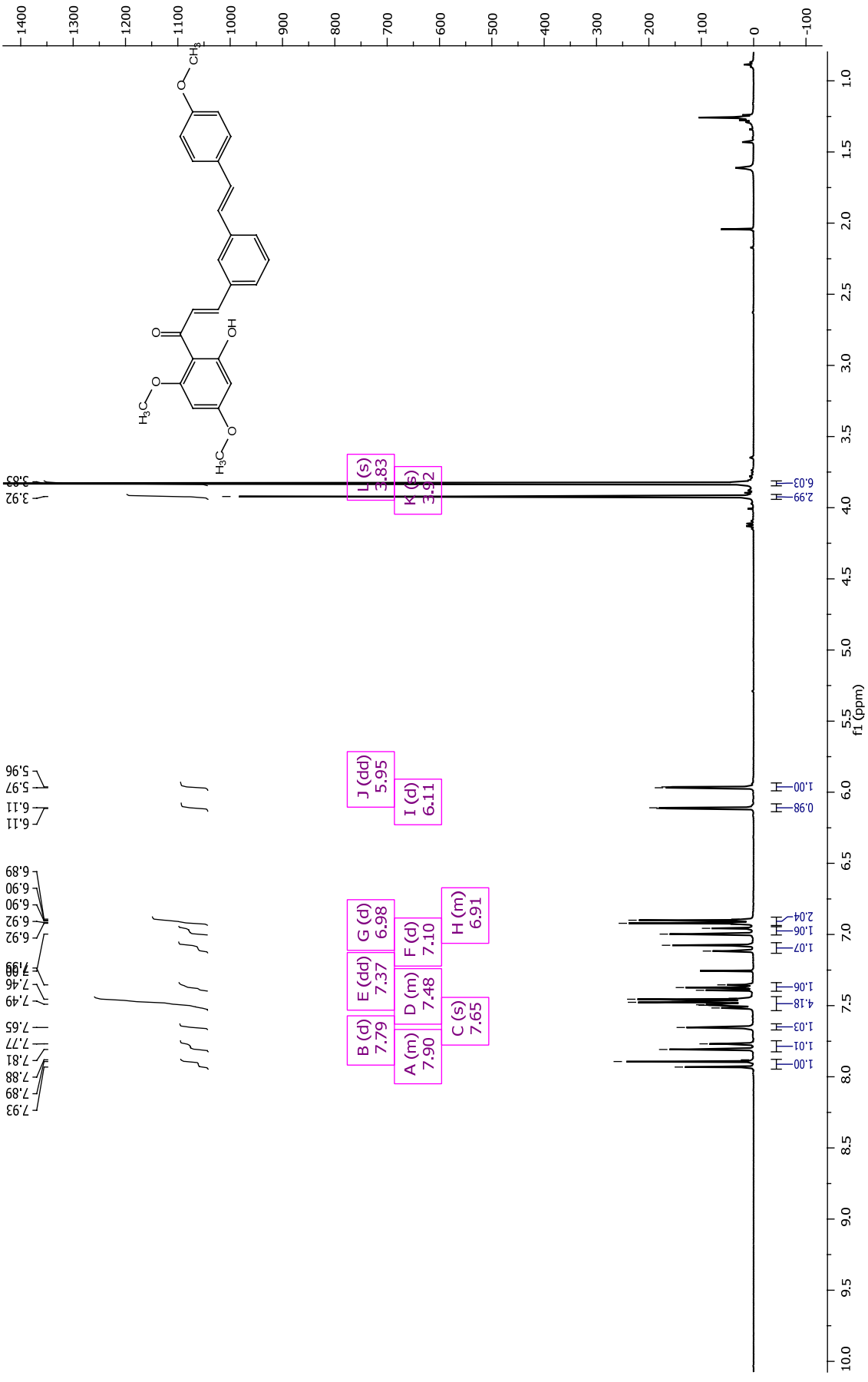


Figure A. 9. ^{1}H Spectra of (E)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(3-((E)-4-methoxystyryl)phenyl)prop-2-en-1-one (139)

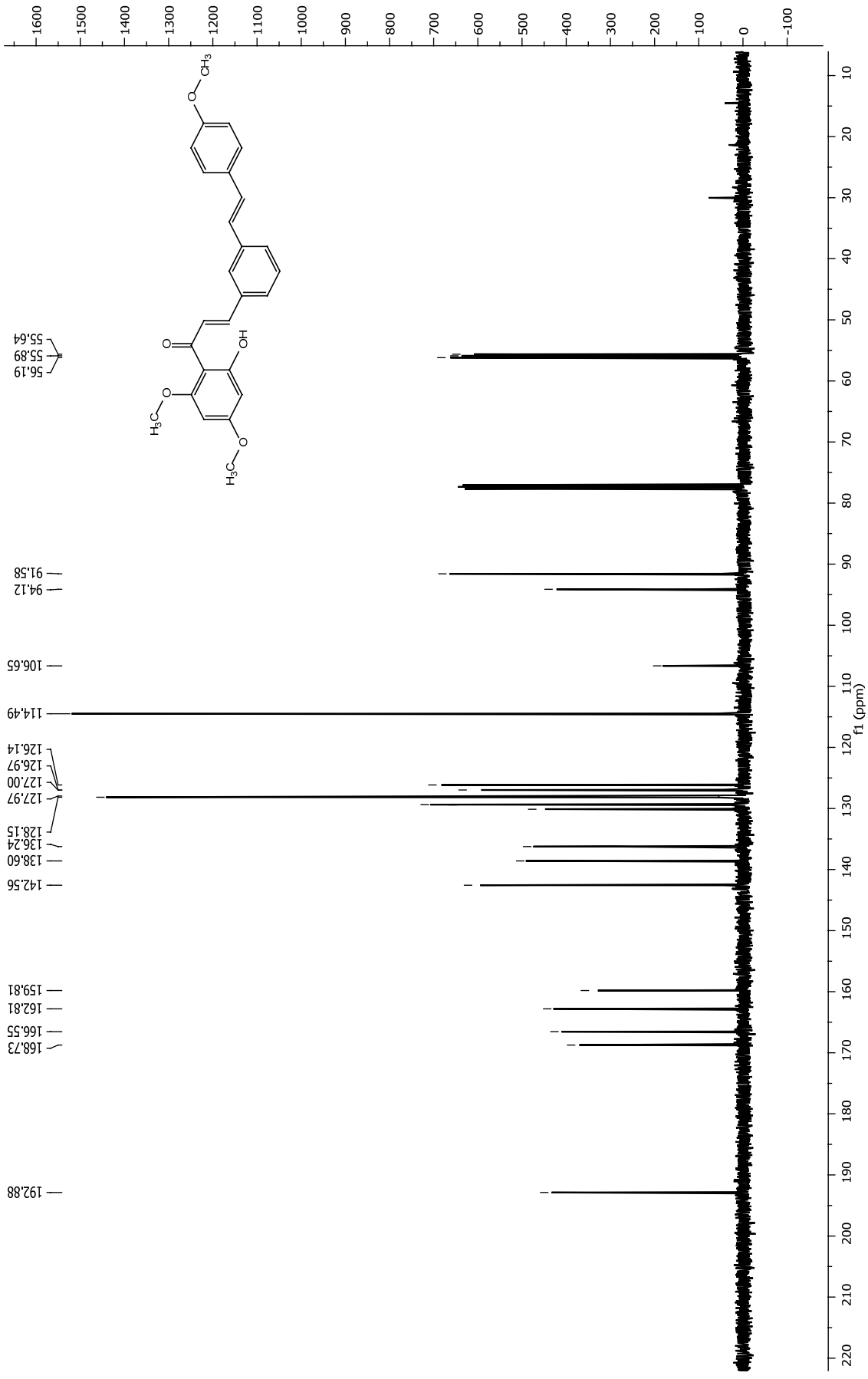


Figure A. 10. ^{13}C Spectra of (E)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(3-((E)-4-methoxystyryl)phenyl)prop-2-en-1-one (139)

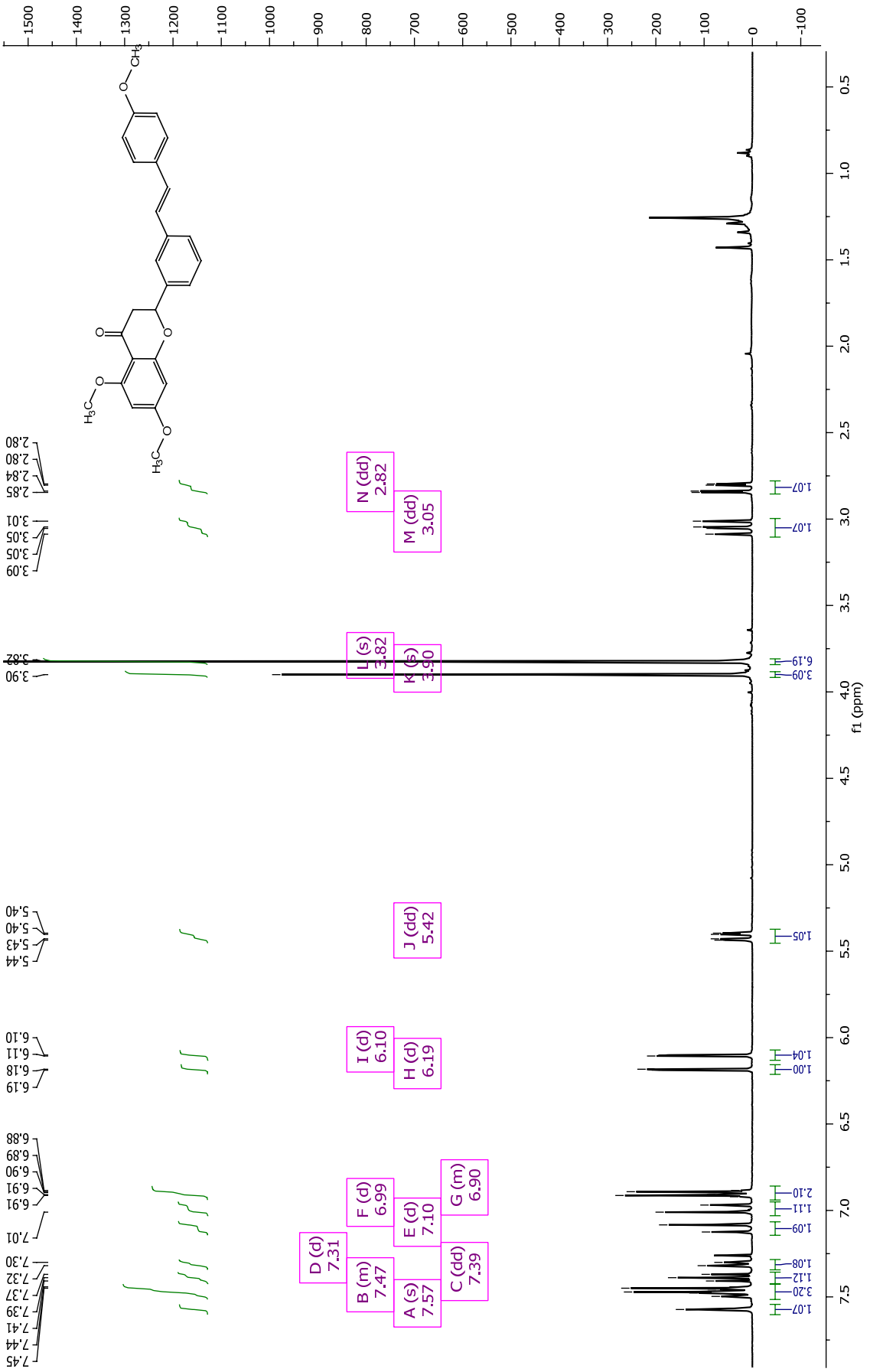


Figure A. 11. ^{1}H Spectra of (E)-5,7-dimethoxy-2-(3-(4-methoxystyryl)phenyl)chroman-4-one (140)

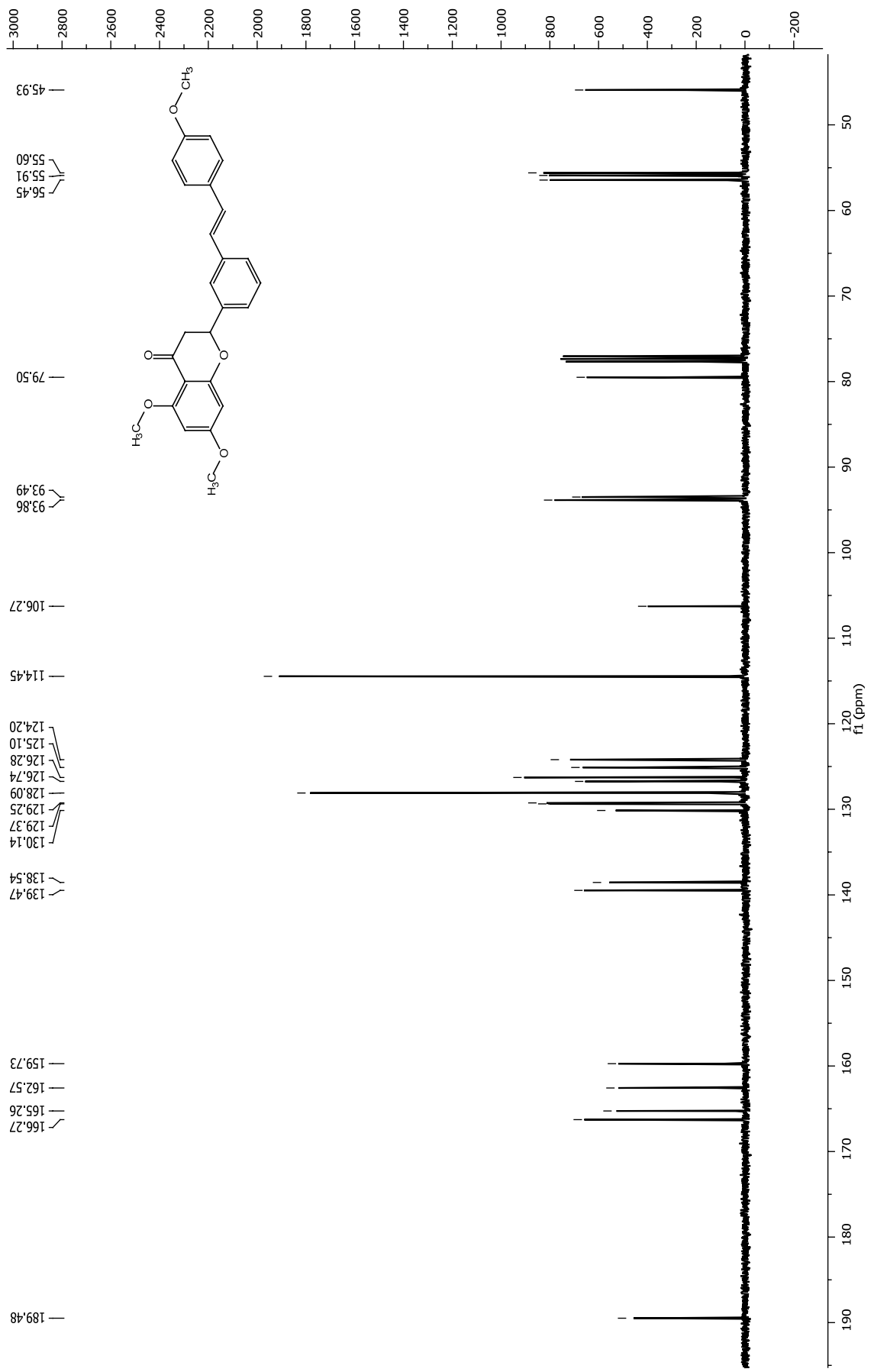


Figure A. 12. ^{13}C Spectra of (E)-5,7-dimethoxy-2-(3-(4-methoxystyryl)phenyl)chroman-4-one (140)