SYNTHESIS AND BIOLOGICAL ACTIVITIES OF NATURAL HOMOISOFLAVANONES

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M.Sc. (Organic Chemistry)

Dissertation submitted for the degree

DOCTOR OF PHILOSOPHY

(Pharmaceutical Chemistry)

in the

School of Pharmacy and Pharmacology

at the

University of KwaZulu-Natal (Westville Campus)

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Westville, Durban

March 2011

ABSTRACT

Plants have formed the foundation of traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies for various ailments. A large portion of the black South African population still depends on medicinal plants as primary health care due to its affordability, accessibility and cultural importance. These medicinal plants need to be investigated since new lead compounds are often found in nature.

Homoisoflavanones isolated from South African and Indian plants were found to exhibit anti-inflammatory activities although the mechanism of action has not yet been determined. A few reports on the antifungal activities of these compounds were also found.

Four new and three known homoisoflavanones of the 3-benzylidene-4-chromanone type were synthesized and tested for anti-inflammatory and antifungal activities. Two novel intermediates were also synthesised. Enantiomers of a homoisoflavanone of the 3-benzyl-4-chromanone types were also synthesized from the corresponding 3,5-dimethoxy phenol via 4-chromanone in six steps. This is the first report of the synthesis of an enantiomerically pure homoisoflavanone compound together with its opposite isomer. The enantiomers and racemate were tested for anti-inflammatory activity. All the synthesized homoisoflavanones were screened for cytotoxicity. The structures of these homoisoflavanones were elucidated by NMR spectroscopy along with HRMS data. The crystal structure of a homoisoflavanone with anti-inflammatory and antifungal activity is reported.

The anti-inflammatory activity of the homoisoflavanones was determined in an acute croton oil-induced auricular dermatitis mouse model. The antifungal activity was performed in *vitro* against a *Candida albicans* strain. Compounds were tested for cytotoxicity against a Chinese Hamster Ovarian (CHO) cell line using the 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazoliumbromide (MTT) assay.

In conclusion, the synthetic homoisoflavanones showed anti-inflammatory as well as antifungal activity. Some of the compounds showed anti-inflammatory activity comparable to that of the commercially available diclofenac.

DECLARATIONS

Declaration 1 - Plagiarism

I, …….declare that

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2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Declaration 2 - Publications

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

Publication 1
Mahidansha M. Shaikh, Hendrik G. Kruger, Johannes Bodenstein, Peter Smith and Karen du Toit. Anti-inflammatory activities of selected homoisoflavanones, *Natural Product Research*, Accepted for publication, **2010**.
Contributions: All compounds were synthesized and all bioassays were conducted by the student. Cytotoxicity tests were carried out by Peter Smith. Johannes Bodenstein assisted in the statistical evaluation of the bioassay. The other authors supervised this project.

Publication 2
Mahidansha M. Shaikh, Hendrik G. Kruger, Peter Smith, Orde Q. Munro, Johannes Bodenstein and Karen du Toit. Synthesis and antifungal activity of homoisoflavanone analogues, *Journal of Natural Products*, Submitted for publication **2010**.
Contributions: All compounds were synthesized and all bioassays were conducted by the student. Cytotoxicity tests were carried out by Peter Smith. Johannes Bodenstein assisted in the statistical evaluation of the bioassay. Orde Q. Munro advised on X-ray data. The other authors supervised this project.

Publication 3
Mahidansha M. Shaikh, Hendrik G. Kruger, Katja Petzold and Karen du Toit. Synthesis and NMR elucidation of homoisoflavanone analogues, *Structural Chemistry*, Accepted for publication **2010**.
Contributions: All compounds were synthesized by the student. Katja Petzold assisted in the interpretation of data and the other authors were supervisors.

Publication 4
Mahidansha M. Shaikh, Hendrik G. Kruger, Johannes Bodenstein, Peter Smith and Karen du Toit. Does Nature Provide the Best Therapeutic Options? Synthesis and Anti-inflammatory activity of a naturally occurring homoisoflavanone and its enantiomer, *Journal of Natural Products,* Submitted for publication, **2010**.
Contributions: All compounds were synthesized and all bioassays were conducted by the student. Cytotoxicity tests were carried out by Peter Smith. Johannes Bodenstein assisted in the statistical evaluation of the bioassay. The other authors supervised this project.

Publication 5
Mahidansha M. Shaikh, Glenn E.M. Maguire, Hendrik G. Kruger and Karen du Toit. 5,7-dimethoxy-3-benzyl-4-chroman-ol, *Acta Crystallography*, Accepted for publication, **2010**.
Contributions: The compound was synthesized by the student and the paper was written with assistance of Glenn E.M. Maguire. All other authors were supervisors.

Signed:

Declaration 3 - Research

I hereby declare that the synthesis of homoisoflavanones described in this thesis was carried out in the School of Chemistry, University of Kwazulu-Natal, Westville Campus, Durban. The anti-inflammatory assays were conducted at the Biomedical Research Unit (BRU) and the antifungal assays were conducted in the School of Pharmacy and Pharmacology, University of Kwazulu-Natal, Westville Campus, Durban. The cytotoxicity assays were conducted in the Division of Pharmacology, University of Cape Town, Rondebosch, South Africa. This study was supervised by Dr. Karen du Toit and Prof. Hendrik G. Kruger.

The research contained in this thesis is original work by author, except when otherwise acknowledged in the customary manner, and has not been submitted previously for a degree at any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made.

Signed:

…….

Mahidansha M. Shaikh

I hereby certify that the above statement is correct.

	Signature	**Date**
Supervisor (K. Du Toit)		
Co-Supervisor (H. G. Kruger)		

DEDICATION

To my father, for direct my interest in science…

To my mother, for support and encouragement…

And to my wife, for unfailing love and understanding…

ACKNOWLEDMENTS

The contribution of many people has made the completions of the study a success. I would like to sincerely thank the following people:

- First, I would like to special thanks to my supervisor, Dr. Karen du Toit for her unwavering support, enthusiasm, independent throughout my study and high expectations that will ultimately make me a better and more successful scientist.
- I would like to sincere thanks to my co-supervisor, Prof. Hendrik G. Kruger for his valuable guidance, encouragement during my study.
- I would also like to thanks to Dr. Johannes Bodenstein for his kind support in a number of ways.
- I would thanks to Ms Sithabile Buthelezi and Mr Dennis Ndwandwe for bioassay experimental assistance.
- I would like to special thanks to Mr. Rajshekar Karpoormath, Mr. Saikumar Chakka, Mr. Peters Byron and Dr. Venugopala K.N. for needed help throughout my study.
- I would also like to sincere thanks to my lab mates, instrumentation Staff and other friends.
- I am pleased to express my gratitude to all faculty and staff member of school of chemistry and school of pharmacy and pharmacology for their valuable co-operation.
- I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.
- Finally, I would not be who or where I am today without my family. To my father and mother, thank you for allowing me to choose my own path as you provided support and advice that continues to this day. To my brothers, thanks for their continue encouragement and thanks to my wife always being so supportive, especially when I was away for weeks, months and year at a time. This achievement is as much yours as it is mine.

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LIST OF PUBLICATIONS

1. Anti-inflammatory activities of selected homoisoflavanones.
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   Accepted for publication in *Natural Product Research*.
2. Synthesis and antifungal activity of homoisoflavanone analogues.
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5. 5,7-dimethoxy-3-benzyl-4-chroman-ol.
   Mahidansha M. Shaikh, Glenn E.M. Maguire, Hendrik G. Kruger and Karen du Toit.
   Accepted to *Acta-E Crystallography*.

CHAPTER-1

INTRODUCTION

1.1 BACKGROUND

Plants have been used for medicinal purposes throughout human history, and the first pharmaceutical agents were derived from medicinal plants.[1] Approximately three quarters of the world's population and 70-80% of the African population still depend on traditional medicines as a primary source of healthcare.[2] The African continent provides a wide diversity of plants useful for treatment of a variety of ailments.[3] Medicinal plants of the sub-Saharan region are readily available, relatively cheap and therefore more attractive as therapeutic agents than modern medicines.[4, 5] South Africa hosts around 30,000 different plant species and medicinal plants still play an important role in the lives of most black South Africans.[6]

The World Health Organization (WHO) recommends that proven traditional remedies should be incorporated within national drug policies.[2] Therefore it becomes important to investigate the scientific rationale behind the medicinal uses of South African plants. The destruction of the natural habitats of medicinal plants as well as urbanization through which traditional knowledge is lost increase the urgency to investigate compounds isolated from plants.[7]

The potential of synthesized compounds with known modes of action is starting to decrease. Attention is therefore being focused on natural sources of lead compounds.[8] Many drugs were discovered through isolation of active compounds from medicinal plants.[7,8] The science of ethnobotany and ethnopharmacognosy are being used to established new sources and classes of compounds with novel modes of action.[7] These are useful to provide lead compounds for different ailments.

1.2 HOMOISOFLAVANONES

1.2.1 BACKGROUND

Böhler and Tamm isolated the first homoisoflavanones, eucomin and eucomol from *Eucomis bicolor* (Hyacinthaceae).[9] Subsequently, various homoisoflavanones were also isolated from

other plant families. Additional plant families from which the homoisoflavanones have been isolated are Agavaceae,[10] Fabaceae,[11] Liliaceae,[12] Polygonaceae.[13] A few reports of synthetic homoisoflavanones exist.[14] Homoisoflavanone-containing plants are mostly used for pain, inflammation, microbial infection, digestive disorder and endocrine dysfuctions.[15]

Isolated homoisoflavanones were found to exhibit a wide range of activities[15] and may be important lead compounds for anti-inflammatory and antifungal therapies.[16]

1.2.2 BASIC STRUCTURE

Homoisoflavanones form a small class of naturally occurring oxygen heterocycles and their structures are similar to that of the more familiar isoflavonoids.[17] Homoisoflavanones consist of a sixteen-carbon skeleton which include either a chromanone, chromone or chromane system with a benzyl or benzylidene group at position-3.[18] Homoisoflavanones consists of four basic types, namely 3-benzylidene-4-chromanones **1**, 3-benzyl-4-chromanones **2**, 3-benzyl-3-hydroxy-4-chromanones **3** and scillascillins **4**.[15] In this study, the 3-benzylidene-4-chromanones **1** and 3-benzyl-4-chromanones **2** types were investigated as lead compounds.

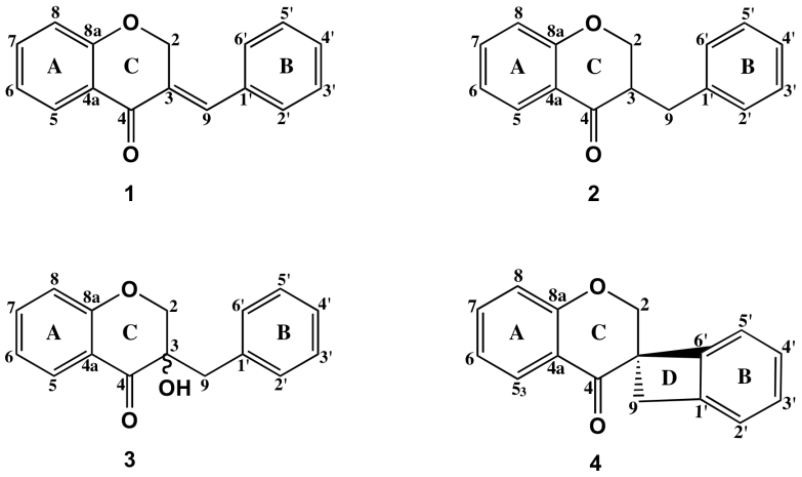


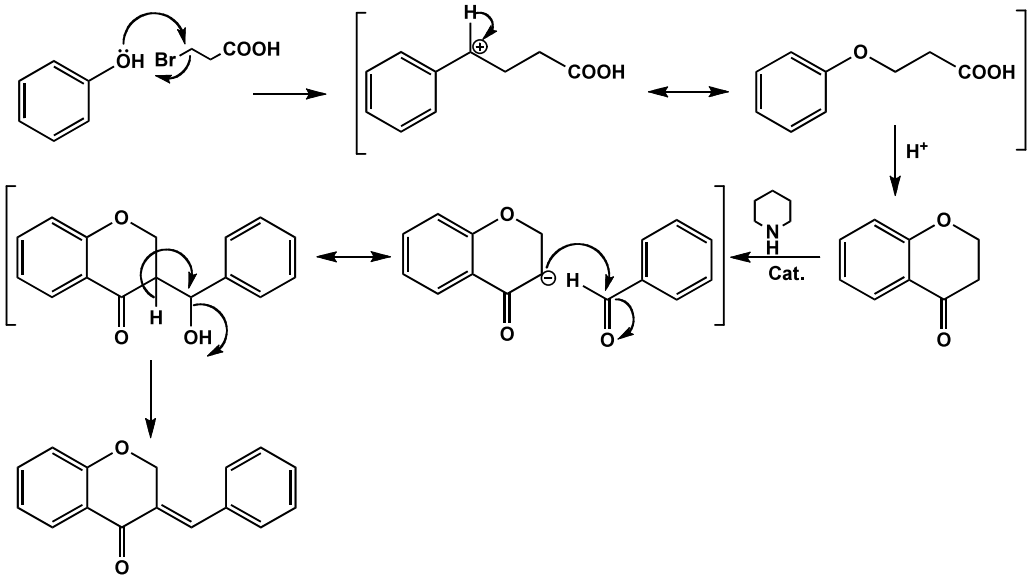
Figure 1. The four different types of homoisoflavanones

The 3-benzylidene-4-chromanones type **1** consists of (*E*)- and (*Z*)-isomers which can undergo chemical interconversion.[17] The homoisoflavanones **2** and **3** contains asymmetric centres at C-3 and, therefore consists of (*R*)- and (*S*)-enantiomers. The absolute configuration at C-3 was determined for a series of natural homoisoflavanones using circular dichroism and the

absolute (*R*)-configuration at C-3 was established for naturally occurring homoisoflavanones.[19]

In the ^{1}H-NMR spectra of 3-benzyl-4-chromanones the H-2 and H-9 protons appear as two ABX systems with geminal as well as vicinal coupling in the presence of H-3. The H-2 protons of 3-benzylidene-4-chromanones appear as one AB system in the ^{1}H-NMR spectra and the alkene proton is deshielded in the aromatic region due to conjugation with the carbonyl group at C-4 as well as with the aromatic ring-B.[17]

1.2.3 MECHANISM OF SYNTHESIS



General synthetic mechanism of the homoisoflavanone.

1.3 BIOLOGICAL ACTIVITIES OF HOMOISOFLAVANONES

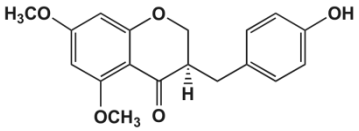
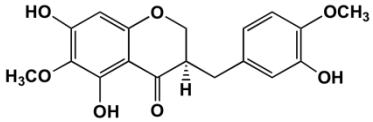
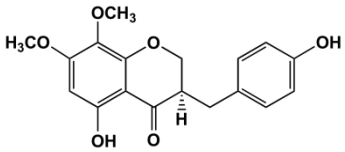
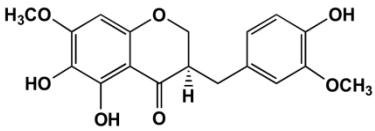
1.3.1 ANTI-INFLAMMATORY ACTIVITY

A wide range of homoisoflavanones have been isolated from different plants around the world but mostly from the Hyacinthaceae family. Only few reports on the anti-inflammatory activity of homoisoflavanones have been found.[15]

The anti-inflammatory activity of homoisoflavanones of 3-benzylidene-4-chromanone type, **5-8** was investigated using microsomal cell fractions as well as COX enzymes by Du Toit and co-workers. Activity was measured as % inhibition of prostaglandin synthesis and of COX-1 and -2 enzymes at test concentrations of 250 μg/ml.[20] The results revealed

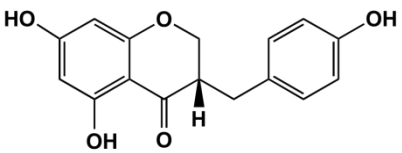
significant inhibition of prostaglandin synthesis but weak activity against COX enzymes (Table 1).

Table 1. The structure and anti-inflammatory activity of homoisoflavanones isolated from different plant sources.[20]

Comp	Structure	Plant source	% Inhibition		
			PG synthesis	COX-1	COX-2
5		*Drimiopsis burkei Bak.*	81	43	NA
6		*Merwilla plumbea Lindl*	70	21	12
7		*Drimiopsis maculata Lindl*	83	26	NA
8		*Scilla zebrine Baker*	70	24	NA

Note: NA = No Activity

Homoisoflavanones of the 3-benzyl-4-chromanone **9** and 3-benzyl-3-hydroxy-4-chromanone **10** types were isolated from *Dracaena loureiroi* Gagnep. showed weak anti-inflammatory activity respectively against COX-1 and COX-2 enzymes at test concentrations of 10 μg/ml.[10] These results correlate with that of Toit and co-workers.



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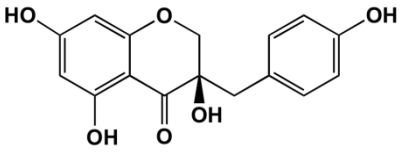

10

Figure 2. Two homoisoflavanones isolated from *Dracaena loureiroi* Gagnep.

No significant COX enzyme activity was reported although good activity was reported in microsomal cell fractions. Therefore the mechanism of action is still unknown.[20]

The anti-inflammatory effects of (*E*)- and (*Z*)-isomers **11** and **12** were established in a homoisoflavanone isolated from *Caesalpinia pulcherrima* (L.) Sw. (Fabaceae). The nitric oxide (NO) production was determined by Rao et. al. using lipopolysaccharide (LPS) and interferon (IFN)-γ-induced systems in cultured macrophage cells.[21]

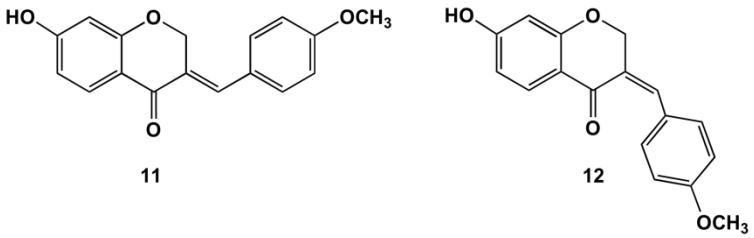

Figure 3. Two (*E*)- and (*Z*)-homoisoflavanones isolated from *Caesalpinia pulcherrima*

Compounds **11** and **12** at test concentrations of 40 μM inhibited the production of NO respectively with 75 and 92%. This report gave insight into a possible mechanism of action.[21]

1.3.2 ANTIFUNGAL ACTIVITY

The antifungal activity of **12** isolated from *Caesalpinia pulcherrima* showed moderate antifungal activity against *Aspergillus niger* and *Candida albicans* however, it was inactive against *Rhizopus oryzae*.[16]

Antifungal activity of synthetic homoisoflavanones of the 3-benzyl-4-chromanone and 3-benzylidene-4-chromanone types were reported using the agar cup method. Moderate antifungal activity was found against *Aspergillus niger* and *Candida albicans* in 3-benzylidene-4-chromanones but not in 3-benzyl-4-chromanones.[22] This method of testing is not always decisive because solubility differences of compounds are not taken into account.

1.3.3 CYTOTOXICITY

Rao and co-workers reported that compounds **11** and **12** were not toxic against macrophages as measured by MTT assays.[22] However, four 3-benzyl-4-chromanones isolated from

Disporopsis aspera Engl. (Liliaceae) were reported to exhibit cytotoxic effects against six human cancer cell lines with IC_{50} values ranging from 15 to 200 uM.[12]

1.4 CONCLUSION

Homoisoflavanones were reported to exhibit varying degrees of anti-inflammatory and antifungal activities. However, only a few reports exist and it is important to expand the literature studies reporting these activities. Bioactive natural compounds can be considered a very promising starting point for the development of new therapeutic agents with less side-effects and new modes of action.

1.5 AIM OF THIS STUDY

- To synthesize novel homoisoflavanones of the 3-benzylidene-4-chromanone and 3-benzyl-4-chromanone types. The structures of these compounds will also be elucidated.
- To screen the synthesized homoisoflavanones for anti-inflammatory and antifungal activity.
- To determine the cytotoxicity of synthetic homoisoflavanones.

1.6 RESEARCH REPORTED IN THIS THESIS

The homoisoflavanone analogues of the 3-benzylidene-4-chromanone types were synthesized as well as enantiomers of a homoisoflavanone of the 3-benzyl-4-chromanone type. ^{1}H and ^{13}C NMR spectroscopy along with HRMS studies supported the elucidation of structures of synthetic homoisoflavanones. The homoisoflavanone analogues of the 3-benzylidene-4-chromanone type were screened for anti-inflammatory and antifungal activity. The enantiomerically pure homoisoflavanones were screened for anti-inflammatory activity. All of these homoisoflavanones were screened for cytotoxicity.

These results are presented as a series of five publications dealing with the synthesis, structural elucidation and biological activities of different homoisoflavanone analogues. The format (numbering, experimental format etcetera) of each results chapter is different since the work was published (or submitted for publication) in different journals.

In paper 1 (Chapter 2), a series of four homoisoflavanone compounds have been synthesized from corresponding substituted phenols via 4-chromanone in three steps. The synthesized

compounds were screened for anti-inflammatory activity and cytotoxicity. The paper was accepted for publication in *Natural Product Research*.

In paper 2 (Chapter 3), a series of seven homoisoflavanones compounds have been synthesized from different substituted phenols. These compounds were screened for antifungal activity and cytotoxicity. A crystal structure of the compound exhibiting the highest antifungal activity was reported. The paper was submitted to *Journal of Natural Products*.

In paper 3 (Chapter 4), the NMR and mass spectroscopy of five synthetic homoisoflavanones were reported. The paper was accepted for publication in *Structural Chemistry*.

In the final paper 4 (Chapter 5), the synthesis of the pure enantiomers of a naturally occurring homoisoflavanone was conducted. The synthesized enantiomers along with the racemic mixture were screened for anti-inflammatory activity. The paper was submitted to *Journal of Natural Products*.

In the final paper 5 (Chapter 6), the synthesis and crystal data of intermediate of the homoisoflavanone was reported. The paper was accepted to *Acta Crystallography*.

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CHAPTER-2

ANTI-INFLAMMATORY ACTIVITIES OF SELECTED SYNTHETIC HOMOISOFLAVANONES

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ABSTRACT

Aims of study: The aims of this study were to synthesize four homoisoflavanones (compounds **4a-d**) of the 3-benzylidene-4-chromanone type, some of which were previously isolated from *Caesalpinia pulcherrima*, and to determine their anti-inflammatory activity and cytotoxicity.

Materials and methods: A range of four different homoisoflavanones (compounds **4a-d**) were synthesized from the corresponding substituted phenols. ^{1}H and ^{13}C-NMR data together with high-resolution mass spectroscopy (HRMS) data were employed to elucidate the structures. Anti-inflammatory activity was determined in an acute croton oil-induced auricular dermatitis in a mouse model. *In vitro* cytotoxicity was tested against a Chinese Hamster Ovarian (CHO) cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay.

Results: Compound **4a** exhibited a tendency to inhibit oedema in a dose dependent manner after 3 and 6 hours treatment. Compounds **4b-d** also inhibited oedema although a clear dose-response relationship was not observed. Compounds **4a-c** were found not to be cytotoxic whilst compound **4d** showed some evidence of cytotoxicity.

Conclusion: Compounds **4a-d** exhibited anti-inflammatory activity and did not display significant levels of cytotoxicity.

Keywords

Homoisoflavanones, synthesis, anti-inflammatory, cytotoxicity

Abbreviations

NMR, nuclear magnetic resonance; Hz, hertz; MHz, megahertz; HRMS, high resolution mass spectroscopy; TLC, thin-layer chromatography; DMF, *N,N*-dimethyl formamide; DMSO, dimethyl sulphoxide; NaH, sodium hydride; NaOH, sodium hydroxide; HCl, hydrochloric acid; CHO, chinese hamster ovarian, MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide; MIC, minimum inhibition concentration; mp, melting point, PPA; phosphoric acid.

INTRODUCTION

Inflammation represents a series of homeostatic events that form part of a mechanism for human survival against pathogens and tissue injury. Non-steroidal anti-inflammatory drugs in general inhibit cyclooxygenase enzymes and are useful for the treatment of acute inflammation. However, their ability to inhibit the progression of inflammation in chronic cases is controversial.[1] These compounds also possess several unwanted side effects including intestinal ulceration, inhibition of platelet function and alterations in renal functions.[1] Therefore, a need for effective and safe anti-inflammatory drugs exists and new lead compounds from natural sources are investigated. Plants are an established natural source of lead compounds.[2]

Many homoisoflavanones have been isolated from different plants across the world, of which many are used for medicinal purposes.[3, 4] These homoisoflavanone-containing plants are mainly used for conditions that can be ascribed to pain, inflammation, microbial infections, digestive disorders and endocrine dysfunctions.[4] Phytochemical investigations of *Caesalpinia pulcherrima* (L.) Sw. (Fabaceae), an Indian medicinal plant have resulted in the isolation of homoisoflavanones of the 3-benzylidene-4-chromanone type with anti-inflammatory activity.[5] This plant has been reported to be useful in treating tridosha, fever, ulcers, asthma, tumors, vata and skin diseases and is also used as an abortifacient and emmengogue.[5, 6]

Anti-inflammatory activities of the (*E*)- and (*Z*)-isomers of 7-hydroxy-3-(4'-methoxybenzylidene)chroman-4-one (bonducellin) previously isolated from *C. pulcherrima* were reported by Rao and co-workers.[2] Both isomers inhibited LPS/IFN-γ-induced NO production in mouse peritoneal macrophages with IC_{50} values of 20 μM and 30 μM for the (*Z*)- and (*E*)-isomer respectively.[2] Synthetic bonducellin and (*E*)-7-methoxy-3-(4'-methoxybenzylidene) chroman-4-one both previously isolated from *C. pulcherrima* were also found to inhibit 5-lipoxygenase enzymes. Inhibitors of these enzymes could be useful in treating asthma and other inflammatory disorders.[7]

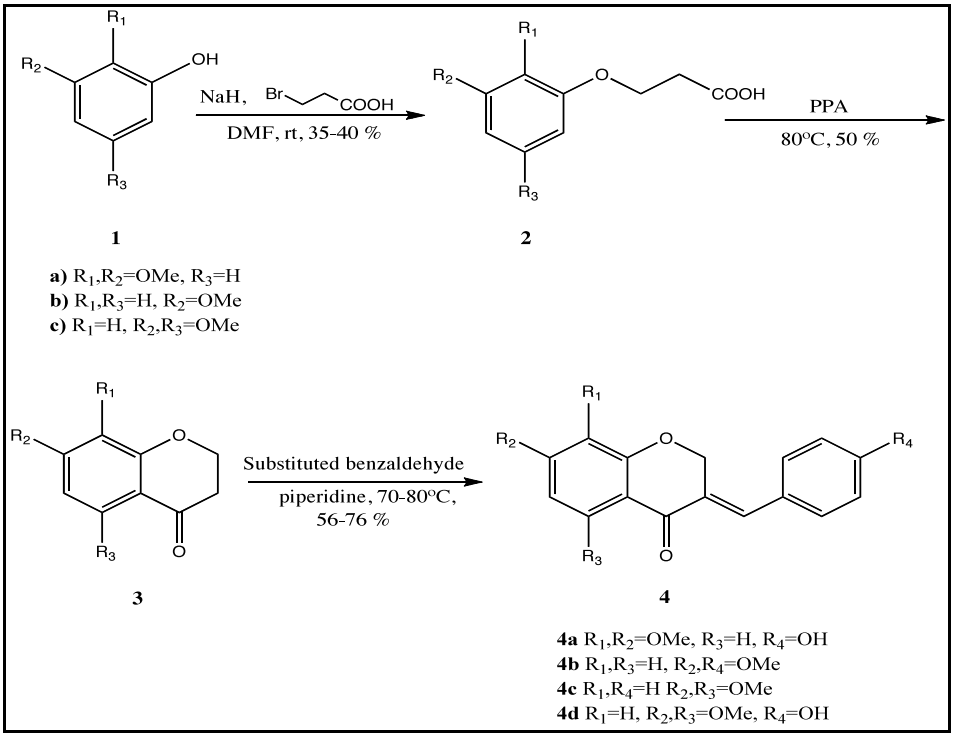
In view of the anti-inflammatory properties displayed by the benzylidene-4-chromanone compounds isolated from *C. pulcherrima,* this study aims to synthesize two compounds previously isolated from this plant including (*E*)-7-methoxy-3-(4'-methoxybenzylidene) chroman-4-one together with two structurally similar compounds of which one is novel.[7] Furthermore the anti-inflammatory activity in an acute croton oil-induced auricular dermatitis mouse model as well as the cytotoxicity of these compounds were determined.

MATERIALS AND METHODS

Chemistry

A range of four different homoisoflavanones were synthesized from the corresponding substituted phenols. The synthesis of homoisoflavanones and their derivatives from commercially available reagents was carried out using the general synthetic approach shown in Scheme 1.

The reaction of the substituted phenols (**1a-c)** with 3-bromopropanoic acid using sodium hydroxide as base furnished substituted 3-phenyloxypropanoic acids (**2a-c**).[8] The propanoic acids were cyclised using polyphosphoric acid to give the substituted chroman-4-ones (**3a-c**).[9] Base catalyzed condensation of substituted chroman-4-one with different substituted benzaldehydes afforded the homoisoflavanones (**4a-d**). The method was adapted from literature.[7-11] The homoisoflavanone (**4d**) is novel and the other three homoisoflavanones (**4a-c**) were previously reported. The ^{1}H NMR data for homoisoflavanone (**4a**), (**4b**) and (**4c**) coincide with the literature values.[7, 12]



Scheme 1. Reagents and conditions for the chemical synthesis of substituted homoisoflavanones.

EXPERIMENTAL

All reaction mixtures were magnetically stirred and monitored by TLC using Kieselgel 60 F254 from Merck (Darmstadt, Germany). ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker AVANCE III at 400 MHz with $CDCl_3$ as internal reference. The value for the chemical shift (δ) is given in ppm and coupling contants (*J*) are reported in Hertz (Hz). Melting points were recorded with a Mel-Temp melting point apparatus in open capillaries and are uncorrected. The high-resolution mass spectroscopy (HRMS) data was recorded on a Waters Micromass Q-Tof Micro mass spectrometer with a lock spray source.

Preparation of substituted 3-phenoxypropanoic acid derivatives (2a-c)

To a DMF solution (10 ml), NaH (32.46 mmol) was added at a temperature of 10–15 °C. Each substituted phenol (32.46 mmol) in DMF (15 ml) was added to the reaction mixture and stirred at room temperature for 1 hour. A solution of 3-bromopropionic acid (38.96 mmol) in DMF (15 ml) was then added dropwise at 0 °C and the reaction mixture was stirred for 12 hours at room temperature. It was then diluted with methanol (20 ml) and acidified with 10%

HCl. The product was extracted with ethyl acetate (3× 50 ml). The combined ethyl acetate layer was washed with water (1×50 ml), brine (1×50 ml) and then dried over magnesium sulphate. The residue obtained after evaporation of the solvent was chromatographed over a silica gel column, using a mixture of ethyl acetate/hexane (30:70) as eluent to obtain the title products **2a-c** (yield, 35-40%).

3-(2,3-dimethoxyphenoxy) propanoic acid (**2a**)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.88 (t, *J*=6.3 Hz, 2H, H-2), 3.81 (s, 3H, Ar-OMe-2'), 3.84 (s, 3H, Ar- OMe-3'), 4.29 (t, *J*=6.3 Hz, 2H, H-1), 6.59 (d, *J*=8.4 Hz, 2H, H-4' & H-6'), 6.96 (dd, *J*=8.3, 8.3 Hz, 1H, H-5'); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 34.4 (C-2), 56.1 (OMe-3'), 60.8 (OMe-2'), 64.4 (C-1), 105.9 (C-4'), 107.2 (C-6'), 123.6 (C-5'), 138.7 (C-2'), 152.2 (C-1'), 153.6 (C-3'), 176.8 (C-3); Mass *m/z* = 227 $[M+1]^+$; Rf = 0.23 on silicagel with ethyl acetate:hexane (30:70).

3-(3-methoxyphenoxy) propanoic acid (**2b**)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.84 (t, *J*=6.3 Hz, 2H, H-2), 3.78 (s, 3H, Ar- OMe-3'), 4.23 (t, *J*=6.2 Hz, 2H, H-1), 6.46-6.53 (m, 3H, H-2', H-4', H-6'), 7.17 (t, 1H, H-5'); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 34.3 (C-2), 55.2 (OMe-3'), 63.0 (C-1), 101.1 (C-2'), 106.6 (C-4'), 106.8 (C-6'), 129.9 (C-5'), 159.6 (C-1'), 160.8 (C-3'), 177.1 (C-3); Mass *m/z* = 197 $[M+1]^+$; Rf = 0.25 on silicagel with ethyl acetate:hexane (30:70).

3-(3,5-dimethoxyphenoxy) propanoic acid (**2c**)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.83 (t, *J*=6.2 Hz, 2H, H-2), 3.76 (s, 6H, Ar- OMe-3',5'), 4.20 (t, *J*=6.2 Hz, 2H, H-1), 6.09 (s, 3H, H-2', H-4', H-6'); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 34.2 (C-2), 55.3 (OMe-3',5') 63.0 (C-1), 93.4 (C-4'), 93.5 (C-2',6'), 160.2 (C-1'), 161.5 (OMe-3',5'), 176.8 (C-3); Mass *m/z* = 227 $[M+1]^+$; Rf = 0.24 on silicagel with ethyl acetate:hexane (30:70).

Preparation of substituted chroman-4-ones (3a-c)

A mixture of substituted 3-phenoxypropanoic acid (10 mmol) and polyphosphoric acid (10 g) was stirred whilst being heated to 85-90 °C for 2 h. The red coloured syrup thus obtained was poured into crushed ice and extracted with diethyl ether (2×50 ml). The extract was washed with 3M NaOH (30 ml), water (50 ml) and dried over magnesium sulphate. The residue obtained after evaporation of the solvent was chromatographed through a silica gel

column using an appropriate mixture of ethyl acetate/hexane as eluent to obtain the title product (**3a-c**) (yield, 50-55%).

7,8-dimethoxychroman-4-one (**3a**)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.74 (t, *J*=6.3 Hz, 2H, H-3), 3.83 (s, 3H, Ar-OMe-7), 3.88 (s, 3H, Ar- OMe-8), 4.54 (t, *J*=6.4 Hz, 2H, H-2), 6.60 (d, *J*=8.9, 1H, H-6), 7.64 (d, *J*=8.9 Hz, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 37.5 (C-3), 56.1 (OMe-7), 61.0 (OMe-8), 67.6 (C-2), 105.5 (C-6), 116.5 (C-4a), 123.1 (C-5), 136.7 (C-8), 155.7 (C-8a), 158.5 (C-7), 190.7 (C-4); Mass *m/z* = 209 $[M+1]^+$; Rf = 0.55 on silicagel with ethyl acetate:hexane (30:70).

7-methoxychroman-4-one (**3b**)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.74 (t, *J*=6.4 Hz, 2H, H-3), 3.83 (s, 3H, Ar- OMe-7), 4.51 (t, *J*=6.4 Hz, 2H, H-2), 6.40 (d, *J*=2.4 Hz, 1H, H-8), 6.57 (dd, *J*=2.4, 8.9 Hz, 1H, H-6), 7.82 (d, *J*=8.8 Hz, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 37.4 (C-3), 55.6 (OMe-7), 67.3 (C-2), 100.7 (C-8), 109.8 (C-6), 115.2 (C-4a), 128.8 (C-5), 163.7 (C-8a), 165.9 (C-7), 190.5 (C-4); Mass *m/z* = 179 $[M+1]^+$; Rf = 0.57 on silicagel with ethyl acetate:hexane (30:70).

5,7-dimethoxychroman-4-one (**3c**)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.71 (t, *J*=6.4 Hz, 2H, H-3), 3.81 (s, 3H, Ar- OMe-7), 3.87 (s, 3H, Ar- OMe-5), 4.43 (t, *J*=6.4 Hz, 2H, H-2), 6.04 (s, 2H, H-6 & H-8); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 38.7 (C-3), 55.5 (OMe-7), 56.0 (OMe-5), 66.7 (C-2), 92.8 (C-8), 93.3 (C-6), 106.3 (C-4a), 162.2 (C-5), 165.2 (C-8a), 165.7 (C-7), 189.1 (C-4); Mass *m/z* = 209 $[M+1]^+$; Rf = 0.54 on silicagel with ethyl acetate:hexane (30:70).

Preparation of substituted homoisoflavanones

A mixture of substituted chroman-4-one (2.4 mmol), substituted benzaldehyde (3.6 mmol) and piperidine (7-10 drops) was heated at 80 °C for 2-48 h. The reaction was monitored by TLC, using 3:7 ethyl acetate/hexane as solvent system. The reaction mixture was cooled, diluted with water (15 ml), acidified with 10% HCl and then extracted with ethyl acetate (3×30 ml). The combined ethyl acetate layer was washed with water (30 ml), brine (30 ml) and dried over magnesium sulphate. The residue obtained after evaporation of the solvent was chromatographed with a silica gel column using an appropriate mixture of ethyl acetate/hexane as eluent to obtain homoisoflavanones (yield, 58-86%).

(*E*)-7,8-dimethoxy-3-(4-hydroxybenzylidene)-4-chromanone (**4a**)

Yield 58 %; mp 172-175 ºC; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.87 (s, 3H, Ar-OMe-7), 3.94 (s, 3H, Ar-OMe-8), 5.41 (d, *J*=1.52 Hz, 2H, H-2), 5.90 (broad s, 1H, OH), 6.70 (d, *J*=8.8 Hz, 1H, H-6), 6.93 (d, *J*=8.8 Hz, 2H, H-3',5'), 7.22 (d, *J*=8.5 Hz, 2H, H-2',6'), 7.80 (s, 1H, H-9), 7.81 (d, *J*=8.8 Hz, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 54.3 (OMe-7), 59.3 (OMe-8), 66.3 (C-2), 104.2 (C-6), 114.0 (C-4a), 115.3 (C-3',5'), 122.2 (C-5), 125.2 (C-1'), 126.8 (C-2',6'), 130.3 (C-3), 134.8 (C-9), 135.4 (C-8), 153.0 (C-8a), 155.2 (C-4), 156.7 (C-7), 179.7 (C-4); HRMS calculated for $C_{18}H_{17}O_5$ $[M + H]^+$ 313.0998, found 313.1071; Rf = 0.51 on silicagel with ethyl acetate:hexane (30:70).

(*E*)-7-methoxy-3-(4-methoxybenzylidene)-4-chromanone (**4b**)

Yield 70 %; mp 126-128 ºC; pale yellow needles; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.83 (s, 3H, Ar-OMe-7), 3.85 (s, 3H, Ar-OMe-4'), 5.35 (d, *J*=1.8 Hz, 2H, H-2), 6.39 (d, *J*=2.4 Hz, 1H, H-8), 6.62 (dd, *J*=2.4, 8.8 Hz, 1H, H-6), 6.95 (d, *J*=8.8 Hz, 2H, H-3',5'), 7.25 (d, *J*=8.8 Hz, 2H, H-2',6'), 7.80 (s, 1H, H-9), 7.95 (d, *J*=8.8 Hz, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.3 (C-4'-OMe), 55.6 (C-7-OMe), 68.0 (C-2), 100.7 (C-8), 110.3 (C-6), 114.2 (C-4a), 115.8 (C-3',5'), 127.1 (C-1'), 128.8 (C-5), 129.6 (C-2',6'), 131.9 (C-3), 136.5 (C-9), 160.5 (C-4'), 162.9 (C-8a), 165.8 (C-7), 180.9 (C-4); HRMS calculated for $C_{18}H_{17}O_4$ $[M + H]^+$ 297.1049, found 297.1121; Rf = 0.62 on silicagel with ethyl acetate:hexane (30:70).

(*E*)-5,7-dimethoxy-(3-benzylidene)-4-chromanone (**4c**)

Yield 76 %; mp 100-103 ºC; dark yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.82 (s, 3H, Ar-OMe-5), 3.91 (s, 3H, Ar-OMe-7), 5.20 (d, *J*=1.7 Hz, 2H, H-2), 6.12 (d, *J*=2.2 Hz, 1H, H-6), 6.06 (d, *J*=2.2 Hz, 1H H-8), 7.27 (d, *J*=8.0 Hz, 2H, H-2',6'), 7.27-7.37 (m, 3H, H-3', H-4', H-5'), 7.82 (s, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.1 (OMe-7), 55.6 (OMe-5), 67.4 (C-2), 93.6 (C-6,8), 107.2 (C-4a), 128.6 (C-4'), 129.9 (C-2',6'), 131.9 (C-3), 134.8 (C-1'), 135.8 (C-9), 162.0 (C-8a), 164.7 (C-5), 165.8 (C-7), 179.5 (C-4); HRMS calculated for $C_{18}H_{17}O_4$ $[M + H]^+$ 297.1049, found 297.1121; Rf = 0.58 on silicagel with ethyl acetate:hexane (30:70).

Novel (*E*)-5,7-dimethoxy-3-(4-hydroxybenzylidene)-4-chromanone (**4d**)

Yield 68 %; mp 197-200 ºC; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.76 (s, 3H, Ar-OMe-7), 3.82 (s, 3H, Ar-OMe-5), 5.17 (d, *J*=1.4 Hz, 2H, H-2), 6.00 (d, *J*=2.2 Hz, 1H, H-6),

6.05 (d, *J*=2.2 Hz, 1H, H-8), 7.11 (d, *J*=8.5 Hz, 2H, H-2',6'), 6.85 (d, *J*=8.5 Hz, 2H, H-3',5'), 7.70 (s, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 54.5 (OMe-7), 55.0 (OMe-5), 66.5 (C-2), 92.5 (C-6), 92.5 (C-8), 106.1 (C-4a), 114.7 (C-3',5'), 126.0 (C-1'), 128.0 (C-3), 130.9 (C-2',6'), 135.0 (C-9), 155.9 (C-4'), 163.6 (C-8a), 164.5 (C-5), 164.7 (C-7), 178.8 (C-4); HRMS calculated for $C_{18}H_{17}O_5$ $[M + H]^+$ 313.0998, found 313.1071; Rf = 0.50 on silicagel with ethyl acetate:hexane (30:70).

Assessment of croton oil-induced oedema

The synthesized homoisoflavanones were assessed for their potential anti-inflammatory activity. Ethical approval (003/09/Animal) from the University of KwaZulu-Natal Animal Ethics subcommittee was obtained prior to the investigation of acute croton oil-induced auricular dermatitis in a mouse model. Guidelines by the University of KwaZulu-Natal Animal Ethics Subcommittee and Biomedical Resources Unit for the maintenance and treatment of laboratory animals were followed.

Eight-week old male Balb/c mice of approximately 30 g each were used. Equal volumes of croton oil (25 μl) were mixed with acetone (25 μl) as vehicle and applied (50 μl total volume; 1 hour) onto the inner surface of the right auricle of each mouse to induce oedema.[13] Acetone has not been documented to have an anti-inflammatory effect by itself.[14] Thereafter, the homoisoflavanones were dissolved in acetone and applied (0.05, 0.1 and 0.5 mg in 50 μl; 3 or 6 hours treatment) onto the right auricle to assess the reduction in oedema. The non-steroidal anti-inflammatory drug diclofenac (0.05 mg; 6 hours treatment) was included as a positive control.

Mice were euthanized after treatment for 3 and 6 hours. From each mouse, left and right auricle biopsy specimens were obtained with a 6 mm biopsy punch and then weighed.

Cytotoxicity assay

In vitro cytotoxicity of the synthesized homoisoflavanones was tested against a Chinese Hamster Ovarian (CHO) cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay.

The MTT assay is a colorimetric assay to determine cellular growth and survival, and compares well with other available assays.[15, 16] The tetrazolium salt MTT was used to measure cell viability.

The homoisoflavanones were prepared in a 2 mg/ml stock solution containing 10% v/v DMSO. Emetine was used as the reference drug at an initial concentration of 100 μg/ml serially diluted in 10-fold to obtain 6 concentrations, the lowest being 0.001 μg/ml. Homoisoflavanones were diluted similarly. The DMSO solvent system had no measurable effect on cell viability (data not shown).

Data analysis

Data are reported as the mean ± standard error of the mean of 3 (cytotoxicity assays) and at least 4 (anti-inflammatory assays) independent experiments with triplicate and duplicate measurements respectively. For the anti-inflammatory assays, oedema was quantified by calculating the difference in weights of the right and left auricular biopsy specimens. The value is expressed as a percentage of the croton oil control. For the cytotoxicity assays, the 50% inhibitory concentration (IC_{50}) values were obtained from full dose-response curves using a non-linear dose-response curve fitting analysis. GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA) was used to analyse and present the data. Statistical comparisons were made by one-way ANOVA followed by Bonferroni's post-test for multiple comparisons, or by Student's two-tailed paired t test to determine *P* values. A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Reduction of croton oil-induced oedema

Four homoisoflavanones with different substitution patterns were synthesized and tested for anti-inflammatory activities. The ability of the homoisoflavanones to inhibit acute croton oil-induced auricular contact dermatitis was assessed (Figure-1).

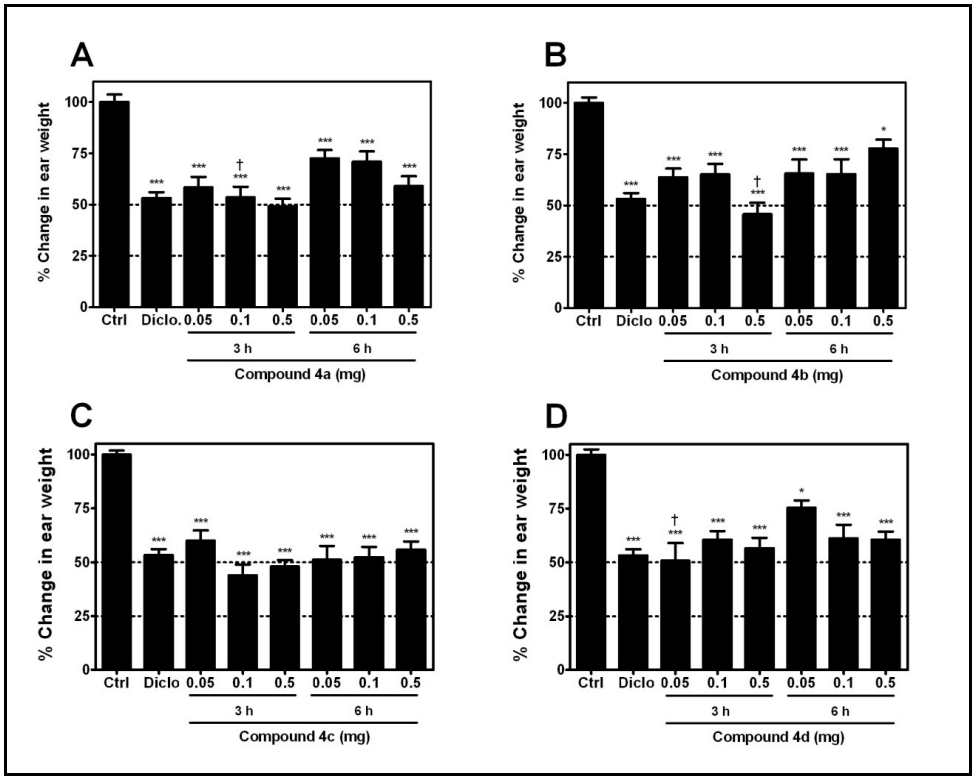


Figure 1. The anti-inflammatory activity of synthetic homoisoflavanones.

Compounds **4a-d** (represented by A-D) were tested in a mouse model of acute croton oil-induced auricular contact dermatitis. Oedema was measured after treatment with 0.05, 0.1 and 0.5 mg homoisoflavanone in acetone for 3 and 6 hours. Treatment with 0.05 mg diclofenac was included as a positive control (indicated as "Diclo"). Data are the mean ± standard error of the mean of duplicate measurements from 4 independent experiments. Statistical comparisons of activity were by one-way ANOVA followed by Bonferroni's post-test for multiple comparisons; *, $P < 0.05$; ***, $P < 0.001$ versus croton oil only control; †, $P < 0.05$ versus 6 hours treatment.

Compound **4a** exhibited a tendency to inhibit oedema in a dose dependent manner after 3 and 6 hours treatment. Activity increased with increasing doses and optimal activity was observed at 0.5 mg. After 6 hours treatment, activity decreased for all doses and was significant for 0.1 mg (3 h: 46.4 ± 5.1%; 6 h: 29.0 ± 5.0%). Compounds **4b-d** also inhibited oedema although a clear dose-response relationship was not observed. For compounds **4b-d**, optimal activity was observed with 0.5 mg, 0.1 mg and 0.05 mg respectively after 3 hours treatment. After 6 hours treatment, the activity of compounds **4b** and **4d** respectively was significantly decreased at 0.5 mg (3 h: 54.2 ± 5.6%; 6 h: 22.2 ± 4.4%) and 0.05 mg (3 h: 49.2

± 8.3%; 6 h: 39.4 ± 3.8%). However, compound **4c** did not exhibit a significant decrease in activity after 6 hours at the doses tested.

Compound **4a** and **4d** have similar substitution patterns. The presence of the methoxy group at C-8 (**4a**) or at C-5 (**4d**) in ring A apparently has an insignificant influence on the biological activity of these compounds. Compounds **4c** and **4d** have the same substitution pattern in ring A, but **4c** lacks the hydroxyl group at C-4'of ring B. This compound exhibited a tendency of higher activity that was maintained after 6 hours. Overall, the compound with an unsubstituted B ring (**4c**) seemed to show higher activity than compounds with substituted B rings (**4a**, **4b**, **4d**).

Cytotoxicity

Cytotoxicity is an important factor to consider when testing for any biological activity. A relatively high value may reduce the therapeutic potential of the compounds tested. The *in vitro* cytotoxicity of compounds **4a-d** was investigated and the IC_{50} values are represented in Table 1. Compounds **4a-c** were found not to be cytotoxic whilst compound **4d** showed some evidence of cytotoxicity.

CONCLUSION

In conclusion we were able to synthesize four structurally similar homoisoflavanones. Structures were assigned based on NMR and Mass spectrometric data. These compounds were tested for the first time in an animal model and all of them were found to be active. It appeared as if the substitution pattern in ring A did not have a significant effect on the activity but an unsubstituted B ring increased activity. Compounds **4a-c** were not cytotoxic. Only compound **4d** showed some degree of cytotoxicity but this was 40 times lower than the cytotoxic control, emetine.

Table 1. The in vitro cytotoxicity of synthetic homoisoflavanones against Chinese Hamster Ovarian (CHO) cells using the MTT assay.

Compound	IC_{50} (µg/ml)
4a	67.1 ± 16.2
4b	>100
4c	45.3 ± 8.7
4d	2.6 ± 0.5
emetine	0.06 ± ND

ACKNOWLEDGEMENT

We acknowledge the University of KwaZulu-Natal Competitive Research Fund and Rolexsi (Pty) Ltd for financial support, and Ms Sithabile Buthelezi and Mr Dennis Ndwandwe for experimental assistance.

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CHAPTER-3

SYNTHESIS AND ANTIFUNGAL ACTIVITY OF HOMOISOFLAVANONE ANALOGUES

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ABSTRACT

A series of seven homoisoflavanone analogues **4a-g** were synthesized from the corresponding substituted phenols via 4-chromanone in three steps. The structures were elucidated with ^{1}H and ^{13}C-NMR together with High-Resolution Mass Spectroscopy (HRMS) data. The X-ray crystal structure of compound **4c** (*E*)-5,7-dimethoxy-3-benzylidene-4-chromanone, is reported. Analogues were evaluated for their antifungal activity against *Candida albicans* and compound **4c** showed the highest activity. The cytotoxicity of the compounds were investigated against Chinese Hamster Ovarian (CHO) cells in a MTT assay.

Keywords

Homoisoflavanones, synthesis, anti-fungal, cytotoxicity, crystal structure.

Abbreviations

CHO, Chinese Hamster Ovarian; DMF, *N,N*-dimethyl formamide; DMSO, dimethyl sulphoxide; HCl, hydrochloric acid; HRMS, high resolution mass spectroscopy; Hz, hertz; IC_{50}, inhibition concentration 50 %; MHz, megahertz; MIC, minimum inhibition concentration; mp, melting point; MTT, 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl tetrazoliumbromide; NaH, sodium hydride; NaOH, sodium hydroxide; NMR, nuclear magnetic resonance; PPA, polyphosphoric acid; TLC, thin-layer chromatography

INTRODUCTION

There has been a growing concern regarding the increase in severe opportunistic fungal infections that threaten public health.[1] This is associated with the wide-spread use of broad-spectrum antibiotics as well as immunosuppressive, anticancer, and antiretroviral drugs.[2-4] *Candida albicans* is present in the gut of about 80% of the human population and a major opportunistic pathogen.[5] The high incidence of acquired immune deficiency syndrome (AIDS) in sub-Saharan Africa allowed this fungus to become a source of major health problems in these developing countries.[6]

The successful treatment of fungal infections has remained problematic due to the emergence of wide-spread resistance against current antifungal drugs. The deficiency of health care clinics adequately equipped to treat patients in Southern Africa further contribute towards the problem. Many of these patients revert to traditional healers who use medicinal plants to treat *Candida* infections.[6] Medicinal plants are good sources of potential antifungal drugs.

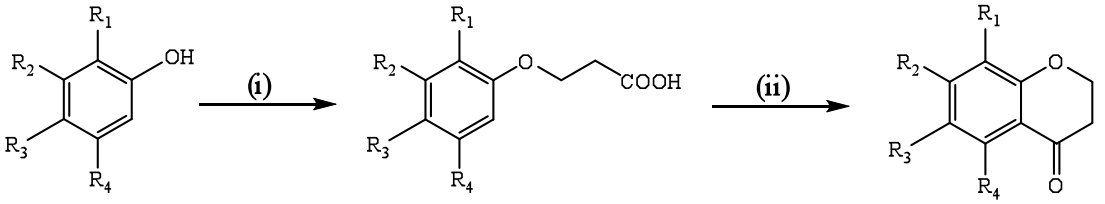
Homoisoflavanone-containing plants have been used medicinally to treat fungal and other skin infections.[7-10] Homoisoflavanones have also been reported to possess antifungal activity.[11-13] These compounds are structurally related to isoflavonoids, but consist of a sixteen-carbon atom skeleton as opposed to the fifteen-carbon atom skeleton of isoflovanoids. Four types of homoisoflavanones can be distinguished, namely 3-benzyl-4-chromanones, 3-benzylidene-4-chromanones, 3-benzyl-3-hydroxy-4-chromanones and scillascilins.[14] The 3-benzylidene-4-chromanone type was reported to exhibit antifungal activity.[11-13]

In this study, we report (1) the synthesis of 3-benzylidene-4-chromanone analogues with varying substitution patterns, (2) the antifungal activities and cytotoxicity of the analogues, and (4) the crystal structure of the most active antifungal compound, **4c**. The chemical structure of compounds **4c**, **4d** and **4f-g** were not previously reported, and the antifungal activities of all the synthesized compounds are unknown.

Chemistry

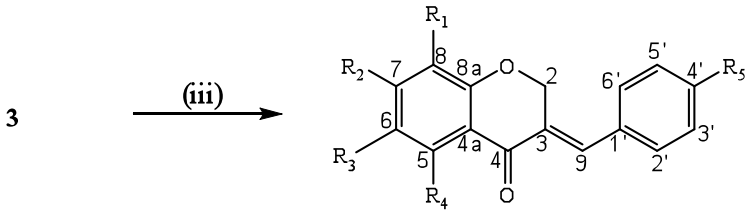
The synthesis of homoisoflavanones and its derivatives from commercially available reagents was carried out using the general synthetic approach shown in Scheme 1. The reaction of substituted phenols **1a-c** with 3-bromopropanoic acid using sodium hydride as base furnished

substituted 3-phenyloxypropanoic acids **2a-c**,[15] which were cyclised using polyphosphoric acid to give substituted chroman-4-ones **3a-c**.[16] Base catalyzed condensation of the substituted chroman-4-ones with a series of substituted benzaldehydes afforded homoisoflavanones **4a-g**. The method was adapted from literature.[17-19]



1 **2** **3**

a) R_1,R_2=OMe, R_3,R_4=H
b) R_1,R_3,R_4=H, R_2=OMe
c) R_1,R_3=H, R_2,R_4=OMe



4

4a R_1,R_2=OMe, R_3,R_4=H, R_5=OH
4b R_1,R_3,R_4=H, R_2,R_5=OMe
4c R_1,R_3,R_5=H R_2,R_4=OMe
4d R_1,R_3=H, R_2,R_4=OMe, R_5=OH
4e R_1,R_3=H, R_2,R_4=OMe, R_5=OMe
4f R_1,R_3=H, R_2,R_4=OMe, R_5=Cl
4g R_1,R_3= H, R_2,R_4=OMe, R_5=F

Reagents and conditions: (i) 3-Bromopropionic acid, NaH, DMF, rt, 12h, 35-40 %;
(ii) PPA, 80 °C, 4h, 50-55 %; (iii) substituted benzaldehyde, piperidine, 70-80 °C, 2-48 h, 52-86 %

Scheme 1. Chemical synthesis of substituted homoisoflavanones.

The X-ray crystal structure of compound **4c** was determined at low temperature from a suitable single crystal of the compound (Table 1, Figure 1).

Table 1. Crystallographic Parameters and Refinement Statistics for Compound **4c**.

Crystal data	
Chemical formula	$C_{18}H_{16}O_4$
M_r	296.31
Crystal system, space group	Monoclinic, $P2_1/c$
Temperature (K)	173
a, b, c (Å)	14.8454 (10), 5.4119 (4), 17.3821 (12)
b (°)	93.738 (1)
V (Å^3)	1393.54 (17)
Z	4
Radiation type	Mo Ka
m (mm^{-1})	0.10
Crystal size (mm)	0.19 × 0.14 × 0.08
Data collection	
Diffractometer	Bruker Kappa Duo Apex II Diffractometer
Absorption correction	Multi-scan *SADABS* (Sheldrick, 1997)
T_{min}, T_{max}	0.666, 0.746
No. of measured, independent and	14158, 3464, 2833
R_{int}	0.040
Refinement	
$R[F^2 > 2s(F^2)]$, $wR(F^2)$, S	0.040, 0.109, 1.04
No. of reflections	3464
No. of parameters	199
No. of restraints	0
H-atom treatment	H-atom parameters constrained
$D\rho_{max}$, $D\rho_{min}$ (e Å^{-3})	0.32, –0.21

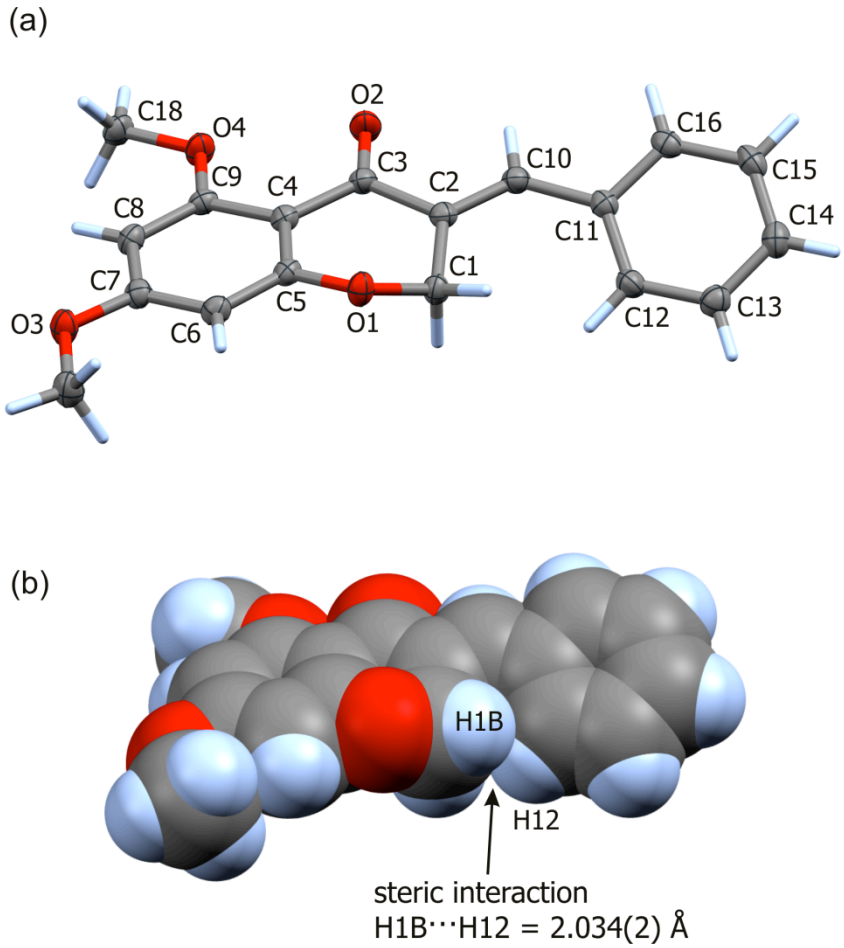


Figure 1. (a) X-ray crystal structure of compound **4c** showing the molecular conformation and numbering scheme. All non-hydrogen atoms are indicated as thermal ellipsoids with a probability level of 50%. Hydrogen atoms are rendered as capped cylinders. (b) Space-filling plot (van der Waals radii) of the X-ray structure of **4c** highlighting the intramolecular H···H steric interaction that leads to the nonplanar conformation of the molecule.

The structure of **4c** exhibits a conspicuously nonplanar conformation characteristic of all 5,7-dimethoxy-3-(benzylidene)-4-chromanone derivatives (Figure 1). The C3–C2–C10–C11 and C2–C10–C11–12 torsion angles measure 173.1(1)^{o} and 19.3(2)^{o}, respectively. The dihedral angle between the 4-chromanone ring and the phenyl ring containing C11 (Ring B in Scheme 1) is 31.6(3)^{o}, consistent with a substantial out-of-plane tilt of this substituent ring. The 4-chromanone ring is essentially planar as whole, but with localized nonplanarity confined to the region encompassing the ethereal oxygen of Ring C (Scheme 1, Figure 1). The deviations of the ether oxygen atom O1 and methylene carbon atom C1 from the mean plane of the 4-chromanone ring system measure 0.24(1) Å and 0.33(1) Å, respectively. One important conformation-defining *intramolecular* short contact exists for **4c**, specifically the

hydrogen···hydrogen interaction H12···H1B (2.034(2) Å). This is shown in the van der Waals plot of Figure 1b and is considerably shorter than the sum of the van der Waals radii of two hydrogen atoms (2.4 Å). Analysis of the unit cell packing of **4c** indicates that there are symmetric (aromatic)C–H···O type hydrogen bonds between neighbouring molecules in the solid state (Figure 2) such that **4c** crystallizes as an inversion pair or dimer with crystallographically-imposed inversion symmetry. One short H···O contact (shorter than the limit ∑(van der Waals radii) – 0.2 Å) exists between the carbonyl oxygen O_2 and a neighbouring methoxy group's hydrogen atom (H18C···O2, 2.49(1) Å). This interaction is inconsequential to the molecular conformation of **4c**.

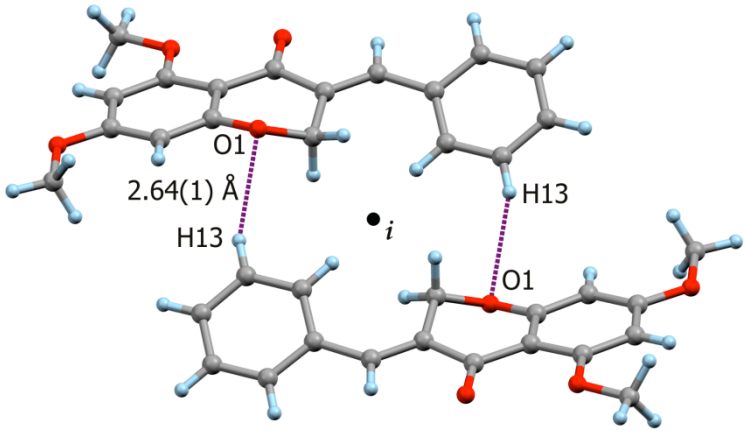


Figure 2. Ball and cylinder model of the hydrogen-bonded dimer involving symmetric (aromatic)C–H···O interactions between neighbouring molecules of **4c**. The dimer has crystallographic inversion symmetry (centre of inversion, ***i***, indicated).

RESULTS AND DISCUSSION

In this study, seven homoisoflavanones were synthesized of which four are novel. The antifungal activities were unknown.

Chemistry

Five of the synthesized homoisoflavanones have identical substitution patterns in ring A **4d-g**. Ring B contains either no substituent or substituents varying in hydrophobicity, electronic properties or size. The influence of substituents on ring A was investigated by adding two homoisoflavanones **4a-b** to the series.

X-ray structure of 4c

The X-ray structures of eleven homoisoflavanones have been reported in the literature[20]; the present structure of **4c** is, however, novel. Inspection of the available crystallographic data suggests that the 4-chromanone ring is conformationally flexible in all of these compounds with the 2,3-dihydro-4*H*-pyran4-one moiety capable of adopting half-chair conformations in which the methylene carbon (C2, Scheme 1) is either displaced above or below the mean plane of the bicyclic 4-chromanone ring system. Thus, for example, the parent compound, (3*E*)-5,7-dimethoxy-3-(benzylidene)-4-chromanone, crystallizes in the triclinic space group *P*-1 with the unit cell containing the inversion-related pair of conformers with the methylene carbon above and below the mean plane of the 4-chromanone ring system.[21] The present compound crystallizes in the space group $P2_1/c$ and, because of the inversion centre shown in Figure 2, both conformers of the 2,3-dihydro-4*H*-pyran-4-one moiety are simultaneously present in the solid state.

As noted above, the salient feature of the molecular structure of **4c** is its nonplanar conformation, this despite a conjugated p-electron system involving Ring A, the carbonyl group of Ring C, and the benzylidene substituent. The key determinant of the nonplanar conformational architecture is the van der Waals repulsion that occurs between the hydrogen atoms of the methylene group of Ring C and the benzylidine *ortho*-H atom (6' position in Scheme 1). This is readily seen in the space-filling plot of Figure 1b; the H1B···H12 contact distance is 0.37-Å shorter than the sum of the van der Waals radii of the two hydrogen atoms, consistent with a considerable degree of steric strain brought about by the electronic requirement of a planar conjugated p-electron system and the steric clash engendered by the juxtaposition of the *ortho*-H atom of the benzylidine group and the methylene hydrogen atoms. The solution to these opposing intramolecular forces is evidently to have partial rotation about the benzylidene double bond (i.e., an out-of-plane tilt of 7° for the C3–C2–C10–C11 torsion angle) and a canted phenyl group orientation (*ca.* 32°) relative to the mean plane of the 4-chromanone ring system. The latter distortion is naturally achieved by a *ca.* 19° rotation about the C10–C11 bond in the molecule (Figure 1). Finally, it is worth noting that the overall conformational architecture observed for **4c** matches the conformations of other substituted 4-chromanones (torsion angles within 13°)[22]; the methoxy groups of **4c** do

not, as might be expected, have any significant impact on the overall conformation of rings A–C of the compound.

Assessment of antifungal activity

The susceptibility of *Candida albicans* to compounds **4a-g** was determined and depicted in Figure 3. The MIC_{50} values depicted in Table 2 represents the potency of the synthesized compounds, whilst the E_{max} values are indicative of their efficacies. A relatively low potency suggests that higher concentrations are needed to achieve 50% activity. Efficacy is indicative of the maximum response obtainable. The MIC_{50} and E_{max} values are summarised in Table 2.

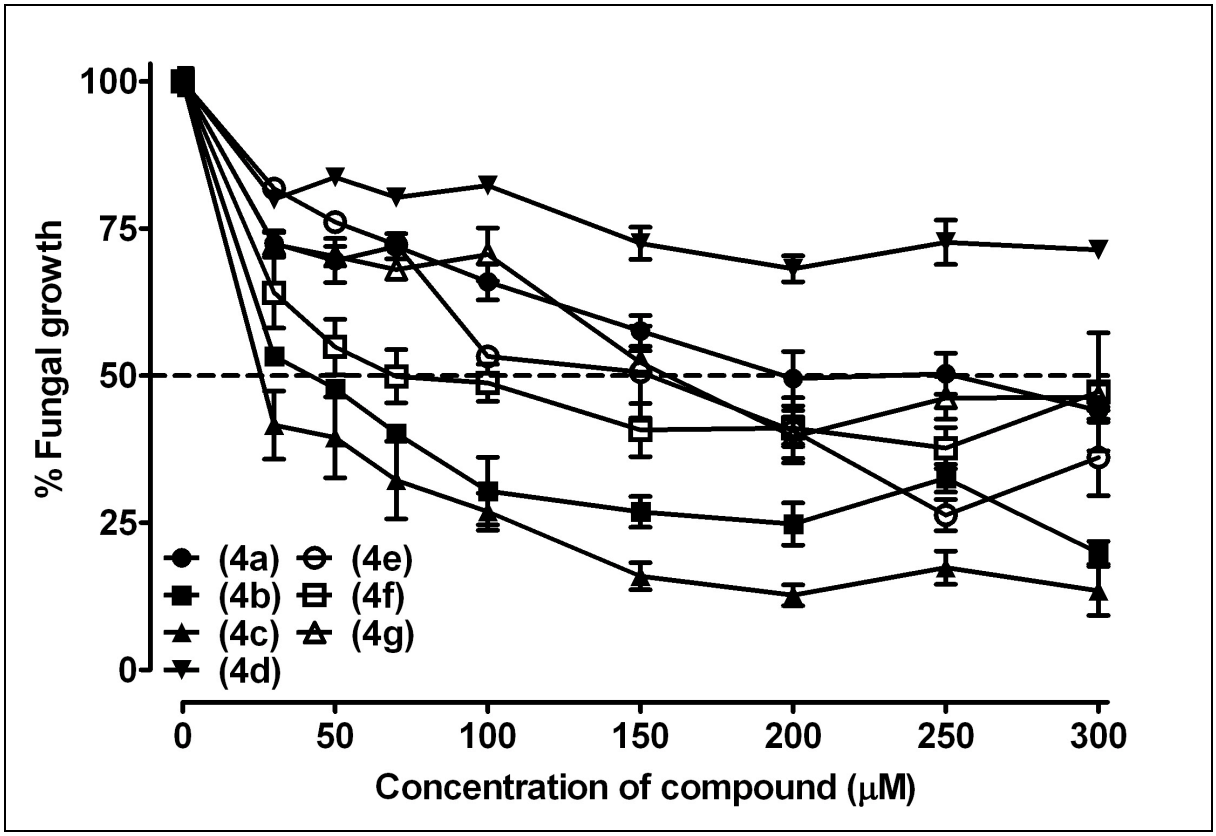


Figure 3. Fungal susceptibility to synthetic homoisoflavanones. Growth of *Candida albicans* was measured against increasing concentrations (0, 30, 50, 70, 100, 150, 200, 250 and 300 μM) of compounds **4a-g**. Data are the mean ± standard error of the mean of duplicate measurements from four independent experiments. For each compound, growth was significantly inhibited ($P < 0.05$ or less) at each concentration tested.

Table 2. The MIC_{50} and E_{max} values of compounds **4a-g** against *Candida albicans*.

Compound	MIC_{50} (μM)	E_{max} (% inhibition)
(4a)	200	55.9 ± 2.0
(4b)	42	80.1 ± 2.0
(4c)	25	87.3 ± 1.8
(4d)	>300	31.8 ± 2.2
(4e)	150	73.7 ± 2.7
(4f)	70	62.3 ± 3.5
(4g)	160	60.4 ± 4.5

Compound **4c** exhibited the highest potency and efficacy. The B ring of this compound was unsubstituted. Compounds **4d-g** were substituted respectively with hydroxy, methoxy, chloride and fluoride in the 4' position. Of these compounds, **4e** exhibited the highest efficacy followed by **4f-g** which exhibited slightly lower efficacies. Compound **4d** exhibited the lowest efficacy. These results suggest that the size and hydrophobicity of the substituents may play a role in the activity. Compounds **4a** and **4d** both contain a 4'-hydroxy group in ring B but respectively 7,8-dimethoxy or 5,7-dimethoxy substituents in ring A. Results suggest that the 7,8-dimethoxy substitution pattern leads to reduced activity. The activity of compound **4e** was increased by removing the 5-methoxy group in ring A, resulting in compound **4b** with a monosubstituted ring A. It is suggested that the substituents in the B ring increases the activity in the following order: hydroxy < halogens < methoxy < unsubstitued.

These results are encouraging and provide novel lead compounds in the search for antifungal drugs with favourable toxicity profiles.

Cytotoxicity

The *in vitro* cytotoxicity of compounds **4a-g** was investigated and the IC_{50} values are represented in Table 3.

Assessment of cytotoxicity in mammalian cells is important in the development of new drugs to ensure selectivity between species. Even if the cytotoxicity profile of a compound is not favourable, it does not prohibit its future development. Many fungal infections are superficial and topical application of drugs may reduce systemic toxicity. Compounds **4d**, **4f** and **4g** were the most cytotoxic compounds with IC_{50} values below 5 μg/ml. Compounds **4a**

and **4e** showed slight cytotoxicity and compound **4b** was not cytotoxic at the concentrations tested. Considering activity and cytotoxicity profiles, it is suggested that compounds **4b** and **4e** are most favorable, although compound **4c** exhibited the highest antifungal potential. Compound **4c** was significantly more potent than all the other compounds tested, suggesting that a relatively lower dose may be needed to reach optimum activity.

Table 3. The in vitro cytotoxicity of synthetic homoisoflavanones against Chinese Hamster Ovarian (CHO) cells using the MTT assay.

Compound	**IC_{50} (µg/ml)**
4a	67.1 ± 16.2
4b	>100 ± ND
4c	2.6 ± 0.5
4d	45.3 ± 8.7
4e	59.4 ± 18.9
4f	4.9 ± 1.9
4g	3.8 ± 0.3
emetine	0.06 ± ND

EXPERIMENTAL

Chemistry

General

^{1}H and ^{13}C-NMR spectra were recorded at ambient temperature at 400 and 100 MHz, respectively, using a Bruker AVANCE III spectrophotometer. All the spectra were acquired in $CDCl_3$ with chemical shifts reported as δ values in part per million (ppm) and were calibrated according to the internal $CDCl_3$ (7.24 ppm) solvent residual peak. The data are reported as follows: multiplicity (br, broad; s, singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet), coupling constants (*J*) are reported in Hertz (Hz). Melting points were

recorded with a Melt-Temp melting point apparatus in open capillaries and are uncorrected. The high-resolution mass spectroscopy (HRMS) was recorded on a Waters Micromass Q-Tof-II Micro mass spectrometer with a lock spray source.

Preparation of substituted 3-phenoxypropanoic acid 2a-c

To a DMF solution (10 ml), NaH (32.46 mmol) was added at a temperature of 10–15°C. Each substituted phenol (32.46 mmol) in DMF (15 ml) was added to the reaction mixture and stirred at room temperature for 1 hour. A solution of 3-bromopropionic acid (38.96 mmol) in DMF (15 ml) was then added dropwise at 0°C and the reaction mixture was stirred for 12 hours at room temperature. It was then diluted with methanol (20 ml) and acidified with 10% HCl. The product was extracted with ethyl acetate (3× 50 ml). The combined ethyl acetate layer was washed with water (1×50 ml), brine (1×50 ml) and then dried over magnesium sulphate. The residue obtained after evaporation of the solvent was chromatographed over a silica gel column, using a mixture of ethyl acetate/hexane (30:70) as eluent to obtain the title products **2a-c** (yield, 35-40%).

3-(2,3-dimethoxyphenoxy) propanoic acid 2a

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.88 (t, *J*=6.3 Hz, 2H, H-2), 3.81 (s, 3H, Ar-OMe-2'), 3.84 (s, 3H, Ar-OMe-3'), 4.29 (t, *J*=6.3 Hz, 2H, H-1), 6.59 (d, *J*=8.4 Hz, 2H, H-4' & H-6'), 6.96 (dd, *J*=8.3, 8.3 Hz, 1H, H-5'); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 34.4, 56.1, 60.8, 64.4, 105.9, 107.2, 123.6, 138.7, 152.2, 153.6, 176.8; MS (ESI): *m/z* 227 $[M+1]^+$.

3-(3-methoxyphenoxy) propanoic acid 2b

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.84 (t, *J*=6.3 Hz, 2H, H-2), 3.78 (s, 3H, Ar-OMe-3'), 4.23 (t, *J*=6.2 Hz, 2H, H-1), 6.46-6.53 (m, 3H, H-2', H-4', H-6'), 7.17 (t, 1H, H-5'); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 34.3, 55.2, 63.0, 101.1, 106.6, 106.8, 129.9, 159.6, 160.8, 177.1; MS (ESI): *m/z* 197 $[M+1]^+$.

3-(3,5-dimethoxyphenoxy) propanoic acid 2c

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.83 (t, *J*=6.2 Hz, 2H, H-2), 3.76 (s, 6H, Ar-OMe-3',5'), 4.20 (t, *J*=6.2 Hz, 2H, H-1), 6.09 (s, 3H, H-2', H-4', H-6'); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 34.2, 55.3, 63.0, 93.4, 93.5, 160.2, 161.5, 176.8; MS (ESI): *m/z* 227 $[M+1]^+$.

Preparation of substituted 4-chromanones 3a-c

A mixture of substituted 3-phenoxypropanoic acid (10 mmol) and polyphosphoric acid (10 g) was stirred whilst being heated to 85-90°C for 2 h. The red coloured syrup thus obtained was poured into crushed ice and extracted with diethyl ether (2×50 ml). The extract was washed with 3N NaOH (30 ml), water (50 ml) and dried over magnesium sulphate. The residue obtained after evaporation of the solvent was chromatographed through a silica gel column using an appropriate mixture of ethyl acetate-hexane as eluent to obtain the title product **3a-c** (yield, 50-55%).

7,8-dimethoxy-4-chromanone 3a

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.74 (t, *J*=6.3 Hz, 2H, H-3), 3.83 (s, 3H, Ar-OMe-7), 3.88 (s, 3H, Ar- OMe-8), 4.54 (t, *J*=6.4 Hz, 2H, H-2), 6.60 (d, *J*=8.9, 1H, H-6), 7.64 (d, *J*=8.9 Hz, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 37.5, 56.1, 61.0, 67.6, 105.5, 116.5, 123.1, 136.7, 155.7, 158.5, 190.7; Mass *m/z* = 209 $[M+1]^+$; Rf = 0.55 on silicagel with ethyl acetate:hexane (30:70).

7-methoxy-4-chromanone 3b

^{1}H NMR data were in agreement with those previously reported[23]; MS (ESI): *m/z* 179 $[M+1]^+$.

5,7-dimethoxy-4-chromanone 3c

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.71 (t, *J*=6.4 Hz, 2H, H-3), 3.81 (s, 3H, Ar- OMe-7), 3.87 (s, 3H, Ar- OMe-5), 4.43 (t, *J*=6.4 Hz, 2H, H-2), 6.04 (s, 2H, H-6 & H-8); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 38.7, 55.5, 56.0, 66.7, 92.8, 93.3, 106.3, 162.2, 165.2, 165.7, 189.1; Mass m/z = 209 $[M+1]^+$; Rf = 0.54 on silicagel with ethyl acetate:hexane (30:70).

Preparation of substituted homoisoflavanones

A mixture of substituted chroman-4-one (2.4 mmol), substituted benzaldehyde (3.6 mmol) and piperidine (7-10 drops) was heated at 80°C for 2-48 h. The reaction was monitored by TLC, using 4:6 ethyl acetate/hexane as solvent system. The reaction mixture was cooled, diluted with water (15 ml), acidified with 10% HCl and then extracted with ethyl acetate (3×30 ml). The combined ethyl acetate layer was washed with water (30 ml), brine (30 ml) and dried over magnesium sulphate. The residue obtained after evaporation of the solvent

was chromatographed with a silica gel column using an appropriate mixture of ethyl acetate-hexane as eluent to obtain homoisoflavanones (yield, 52-86%).

(*E*)-7,8-dimethoxy-3-(4-hydroxybenzylidene)-4-chromanone 4a

Yield 58%; mp 172-175°C; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.87 (s, 3H, Ar-OMe-7), 3.94 (s, 3H, Ar-OMe-8), 5.41 (d, *J*=1.52 Hz, 2H, H-2), 5.90 (broad s, 1H, OH), 6.70 (d, *J*=8.8 Hz, 1H, H-6), 6.93 (d, *J*=8.8 Hz, 2H, H-3',5'), 7.22 (d, *J*=8.5 Hz, 2H, H-2',6'), 7.80 (s, 1H, H-9), 7.81 (d, *J*=8.8 Hz, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 54.3, 59.3, 66.3, 104.2, 114.0, 115.3, 122.2, 125.2, 126.8, 130.3, 134.8, 135.4, 153.0, 155.2, 156.7, 179.7; HRMS calculated for $C_{18}H_{17}O_5$ $[M + H]^+$ 313.0998, found 313.1071.

(E)-7-methoxy-3-(4-methoxybenzylidene)-4-chromanone 4b

Yield 70%; mp 126-128°C; pale yellow needles; ^{1}H NMR data were in agreement with those previously reported[19]; HRMS calcd for $C_{18}H_{17}O_4$ $[M+1]^+$ 297.1049, found 297.1121.

(E)-5,7-dimethoxy-3-(4-benzylidene)-4-chromanone 4c

Yield 76%; mp 100-103°C; dark yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.82 (s, 3H, Ar-OMe-7), 3.91 (s, 3H, Ar-OMe-5), 5.2 (d, *J*=1.7 Hz, 2H, H-2), 6.06 (d, *J*=2.2 Hz, 1H, H-6), 6.12 (d, *J*=2.2 Hz, 1H, H-8), 7.27 (d, *J*=8.0 Hz, 2H, H-2',6'), 7.27-7.37 (m, 3H, H-3', H-4', H-5'), 7.82 (s, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.1, 55.6, 67.4, 93.6, 107.2, 128.6, 128.9, 129.9, 131.9, 134.8, 135.8, 162.8, 164.7, 165.8, 179.5; HRMS calculated for $C_{18}H_{17}O_4$ $[M + H]^+$ 297.1049, found 297.1121.

Novel (E)-5,7-dimethoxy-3(4-hydroxybenzylidene)-4-chromanone 4d

Yield 68%; mp 197-200°C; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.76 (s, 3H, Ar-OMe-7), 3.82 (s, 3H, Ar-OMe-5), 5.17 (d, *J*=1.4 Hz, 2H, H-2), 6.00 (d, *J*=2.2 Hz, 1H, H-8), 6.05 (d, *J*=2.2 Hz, 1H, H-6), 6.85 (d, *J*=8.5 Hz, 2H H-3',5'), 7.11 (d, *J*=8.5 Hz, 2H, H-2',6'), 7.70 (s, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 54.5, 55.0, 66.5, 92.5, 106.1, 114.7, 126.0, 128.0, 130.9, 135.0, 155.9, 163.6, 164.7, 164.7, 178.8; HRMS calcd for $C_{18}H_{17}O_5$ $[M+1]^+$ 313.0998, found 313.1071.

(E)-5,7-dimethoxy-3-(4-methoxybenzylidene)-4-chromanone 4e

Yield 71%; mp 171-174°C; pale yellow powder; ^{1}H NMR data were in agreement with those previously reported[23]; HRMS calcd for $C_{19}H_{19}O_5$ $[M+1]^+$ 327.1154, found 327.1227

Novel (E)-5,7-dimethoxy-3-(4-chlorobenzylidene)-4-chromanone 4f

Yield 86%; mp 126-129°C; pale yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.74 (s, 3H, Ar-OMe-7), 3.82 (s, 3H, Ar-OMe-5), 5.08 (d, *J*=1.8 Hz, 2H, H-2), 5.97 (d, *J*=2.2 Hz, 1H, H-8), 6.03 (d, *J*=2.2 Hz, 1H, H-6), 7.11 (d, *J*=8.4 Hz, 2H, H-2', 6'), 7.30 (d, *J*=2.2 Hz, 2H, H-3', 5'), 7.65 (s, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 54.5, 55.1, 66.2, 92.5, 92.6, 106.2, 127.0, 129.4, 131.0, 131.5, 133.5, 133.9, 161.2, 163.5, 164.8, 178.0; HRMS calcd for $C_{18}H_{16}ClO_4$ $[M+1]^+$ 331.0659, found 331.0732.

Novel (E)-5,7-dimethoxy -3-(4-fluorobenzylidene)-4-chromanone 4g

Yield 63%; mp 102-105°C; pale yellow needles; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.83 (s, 3H, OMe-7), 3.92 (s, 3H, OMe-5), 5.19 (d, *J*=1.6 Hz, 2H, H-2), 6.07 (d, *J*=2.3 Hz, 1H, H-8), 6.13 (d, *J*=2.3 Hz, 1H, H-6), 7.12 (t, 2H, H-3',5'), 7.25-7.28 (m, 2H, H-2', 6'), 7.77 (s, 1H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.6, 56.2, 67.3, 93.6, 93.6, 107.1, 115.8, 130.9, 131.6, 134.6, 137.7, 161.6, 162.8, 164.6, 165.8, 179.3; HRMS calcd for $C_{18}H_{16}FO_4$ $[M+1]^+$ 315.0954, found 315.1027.

Crystallography

Single-crystal X-ray diffraction data were collected on a Bruker KAPPA APEX II DUO diffractometer using graphite-monochromated Mo-Kα radiation (χ = 0.71073 Å). Data collection was carried out at 173(2) K to minimise thermal motion effects. Temperature was controlled by an Oxford Cryostream cooling system (Oxford Cryostat). Cell refinement and data reduction were performed using the program SAINT.[24] The data were scaled and empirical absorption corrections were performed using SADABS.[25] The structure was solved by direct methods using SHELXS-97[25] and refined by full-matrix least-squares methods based on F^2 using SHELXL-97[25] and using the graphic interface program X-Seed.[26, 27] The program Mercury 2.3 was used to prepare molecular graphic images.

Assessment of fungal susceptibility

Sabouraud dextrose broth was inoculated with *Candida albicans* and grown in an incubator (37°C; optical density of 0.5 at 600 nm). *C. albicans* (ATCC strain 10231) culture was obtained from American Type Culture Collection (Manassas, VA, USA). The broth was prepared according to the manufacturer's protocol. The fungal susceptibility assay was based on a microplate method but with modifications. Compounds **4a-g** were prepared in pure DMSO at stock concentrations of 1.5, 2.5, 3.5, 5, 7.5, 10, 12.5 and 15 mM. Firstly, 100 μl/well of sterile broth was added into a clear, sterile 96-well microtitre plate (Corning Life Sciences, Acton, MA, USA). Secondly, 6 μl/well of the compound at the appropriate concentration above was added and the plate tapped to mix the contents. Thirdly, 94 μl/well of sterile water was added and the plate tapped. Finally, 100 μl/well of the culture was added and the plate tapped and incubated (37°C; 18 hours). Therefore, with a final volume/well of 300 μl and a dilution factor of 50×, the final concentration of DMSO/well was 2% v/v and the final concentrations of each compound/well were 30, 50, 70, 100, 150, 200, 250 and 300 μM. Fungal growth was not significantly inhibited by the 2% v/v DMSO (data not shown). The positive control was amphotericin B (100 μM final concentration) against *C. albicans* (inhibition of growth 98.9 ± 0.7%). Fungal growth was quantified by optical density (600 nm) in a microplate reader (BioTek ELx800, Winooski, VT, USA).

Cytotoxicity assay

In vitro cytotoxicity of the synthesised homoisoflavanones was tested against a Chinese Hamster Ovarian (CHO) cell line using the 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazoliumbromide (MTT) assay.

The MTT assay is a colourimetric assay to determine cellular growth and survival, and compares well with other available assays.[28, 29] The tetrazolium salt MTT was used to measure cell viability.

The homoisoflavanones were prepared in a 2 mg/ml stock solution containing 10% v/v DMSO. Emetine was used as the reference drug at an initial concentration of 100 μg/ml serially diluted in 10-fold to obtain 6 concentrations, the lowest being 0.001 μg/ml. Homoisoflavanones were diluted similarly. The DMSO solvent system had no measurable effect on cell viability (data not shown).

Data analysis

Data are reported as the mean ± standard error of the mean of four independent experiments with duplicate measurements. Fungal growth was quantified as a percentage of the control without the test compound. GraphPad Prism (version 5.02; GraphPad Software, San Diego, CA, USA) was used to present and analyze the data. MIC_{50} values were deduced from the graphs. Statistical comparisons between 0 and each concentration for each compound were made by one-way ANOVA followed by Bonferroni's post-test to determine *P* values. A value of $P < 0.05$ was considered significant.

ACKNOWLEDEMENT

The authors would like to thank the University of KwaZulu-Natal Competitive Research Fund and Rolexsi (Pty) Ltd for financial support, and Ms Sithabile Buthelezi for experimental assistance.

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CHAPTER-4

SYNTHESIS AND NMR ELUCIDATION OF HOMOISOFLAVANONE ANALOGUES

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ABSTRACT

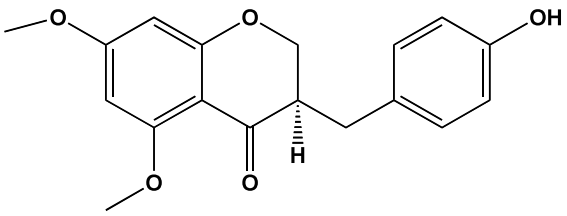
A series of five homoisoflavanone analogues have been synthesized from the corresponding 3,5-methoxy phenols *via* 4-chromanone in three steps. The complete NMR elucidation of these homoisoflavanone analogues is reported. The use of 2D NMR techniques (COSY, NOESY, HSQC and HMBC) proved to be very useful tools in the elucidation of homoisoflavanone analogues. The homoisoflavanone analogues exhibit an AA'BB' spin pattern in the ring B of the homoisoflavanone. These homoisoflavanone analogues are potential antifungal and anti-inflammatory agents.

Keywords

Homoisoflavanone, Synthesis, Anti-inflammatory, Antifungal, 2D NMR.

INTRODUCTION

Homoisoflavanones belong to a small homogeneous group of naturally occurring oxygen heterocycles.[1] The basic homoisoflavanone structure consists of a 16-carbon skeleton, which includes a chromane system with a benzyl or benzylidine group at position 3.[2] Natural homoisoflavanone (3-benzylidine-4-chromanones) such as Bonducellin, Isobonducellin, 2'-Methoxybonducellin and Sappanone *A* have been isolated from different plants.[3-8] Few reports on the biological activity of homoisoflavanones have been found. However according to previous studies, natural homoisoflavanones exhibit anti-inflammatory, antibacterial, anti-mutagenic, hypo chloesterolemic,[9-13] antioxidant and anti-viral activities.[14-16]

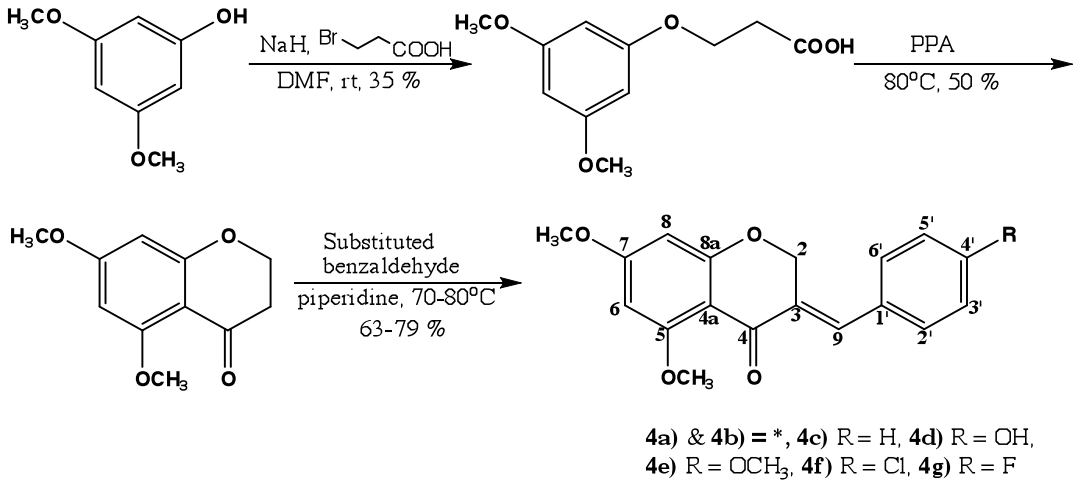


7a

The synthesis of homoisoflavanones (3-benzylidine-4-chromanones) is well established[17] and although a variety of homoisoflavanones have been isolated from many different plants, it appears that more is known about the biosynthesis and chemistry of these compounds than about their biological activities and its relation to their structures. Previously, a NMR study of a similar type was reported on natural homoisoflavanones.[18-20] In this study, we report the synthesis of 3-benzylidene-4-chromanone analogues with varying substitution patterns at the 4'-position of the B-ring. The lead compound (3,5-dimethoxy-3-benzyl-4-chromanone **7a,** chapter-5), a type of homoisoflavanone, was isolated from the Southern African Hyacinthaceae family and screened for anti-inflammatory activity.[9] Based on the lead compound we synthesized compound **4d** and its analogues with different substitution patterns at the 4'-position of the B-ring The products showed good anti-inflammatory[17] and anti-fungal activity.[21] The structures of compounds **4c**[22] and **4e**[23, 24] have been previously reported, respectively.

RESULTS AND DISCUSSION

This study describes the synthesis and structural elucidation of homoisoflavanones **4c-g**. The synthesis of homoisoflavanone analogues from commercially available reagents was carried out using the general synthetic approach shown in Scheme 1. The reaction of 3,5 dimethoxy phenol with 3-bromopropanoic acid using sodium hydride as base furnished 3-(3,5-dimethoxy phenoxy)propanoic acid,[25] which was cyclised using polyphosphoric acid to give 7,8 dimethoxy chroman-4-one.[26] Base catalyzed condensation of 7,8-dimethoxy-4-chromanone with different substituted benzaldehyde results in the synthesis of homoisoflavanones[14] **(Scheme 1: 1-5)**.



4a) & **4b) = ***, **4c)** R = H, **4d)** R = OH,
4e) R = OCH_3, **4f)** R = Cl, **4g)** R = F

Note: * = not studied 2D NMR spectroscopy

Scheme 1: General synthesis of homoisoflavanone analogues **4c-g**.

Infrared spectroscopy (IR) is regularly used to confirm the functional groups of organic compounds and was therefore applied to our modified compounds **4c-g**. The structure of compound **4c**, with no substitution at C-4' in the ring-B, is a good starting point for the elucidation. The basic IR peaks for compound **4c** resulted in a sharp single peak at 2940 cm^{-1} for the C-H stretching mode in the aromatic rings A and B. The peak at 1664 cm^{-1} and 1567 cm^{-1} indicated the presence of a carbonyl group (C=O, C-4) with symmetric and asymmetric conjugation with the double bond (C=C, C-3 and C-9). The peaks at 1465 cm^{-1} and 1421 cm^{-1} represents the symmetrical stretching of alkene (C=C) group of aromatic ring and 1339 cm^{-1} and 1317 cm^{-1} indicated asymmetrical stretching of the aromatic ring. Furthermore the peak at 1107 cm^{-1} indicated the C-O-C (C-8a and C-2) ether stretching in compound **4c**, confirming the most prominent functional groups in this molecule. In compound **4d**, the hydroxy-group (OH) at C-4' in ring-B causes a broad single peak at 3377 cm^{-1}. As expected, no significant differences in the IR spectra for the remaining compounds were observed.

Nuclear magnetic resonance (NMR) spectroscopy has become an essential tool for studying the structure of a broad variety of systems, and was here applied to elucidate the structures of the substituted compounds **4c** to **4g**. All 1D ^{1}H and ^{13}C chemical shifts and couplings of the homoisoflavanone analogues are reported in Table 1. The only methylene protons (H-2) and the well-separated H-9 alkene proton are two convenient points of entry for the elucidation of compound **4c**. The 1D NMR spectrum of compound **4c** shows two methylene protons, which exhibit a geminal coupling constant ($^{2}J_{HH}$) of 1.7 Hz. The well separated alkene proton H-9

appears as a singlet and is deshielded in the aromatic region due to conjugation with the carbonyl group at C-4, as well as with the aromatic ring-B. The ^{1}H-NMR spectrum showed two *meta*-coupled protons doublet, which is therefore assigned to H-6 and H-8 in the aromatic ring B, respectively, indicative of a 5,7 disubstituted aromatic ring. Furthermore the ^{1}H-NMR spectrum shows a doublet for two protons (by integration), which were coupled with 3 adjacent protons, registering as overlaying multiplets. These remaining protons were assigned to H-2' and H-6' of the aromatic ring B.

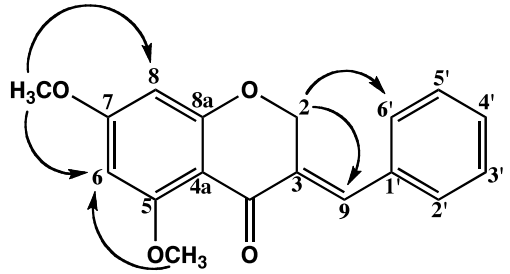


Figure 1: Long range interactions of homoisoflavanone found by NMR measurements.

The COSY and NOESY spectra enabled us to confirm the prior assignment. The COSY spectrum shows a correlation between H-2 and the methine bridge proton H-9. COSY correlations are most intense for vicinal H-C-C-H protons ($^{3}J_{HH}$), the COSY correlation between H-2 and H-9 is transfered via an additional carbon atom and is therefore accounted as long-range couplings ($^{4}J_{HH}$). These are only observed for specific conformations.[27] We also observed a NOESY correlation between H-2 and H-2'/H-6' (overlapping) and between H-2 and H-9, though with less intensity, indicating that ring B is positioned as the *E*-isomer (the *cis*-isomer). The NOESY spectrum shows correlation of H-6 with the protons of the methoxy group $OC(H\text{-}5)_3$ and H-8 with the methoxy group $OC(H\text{-}7)_3$. These observations further indicated a disubstituted methoxy group at position C-5 and C-7 in ring A. Confirmation of the assignments for $OC(H\text{-}5)_3$ and $OC(H\text{-}7)_3$ is possible by comparing the chemical shifts of the methyl protons. Since $OC(H\text{-}5)_3$ is close to the carbonyl oxygen at C-4, it should experience a through space deshielding effect (H-bonding between the $OC(H\text{-}5)_3$ methyl protons and the carbonyl oxygen group at C-4 can also contribute to the deshielding effect), moving the $OC(H\text{-}5)_3$ protons at higher δ value. The COSY spectrum also shows correlation between the protons H-2'/H-6' and H-3'/H-5' and a complex correlation between H-3'/H-5' and H-4'.

After the unambiguous assignment of the existing protons, the corresponding carbon atoms were identified using the HSQC and HMBC spectra. In the HMBC spectrum a correlation of H-2, our preferred starting point, with a carbonyl carbon was observed. The latter was therefore assigned to C-4 in ring C. Correlations of H-2 to a quaternary alkene carbon led to identification of C-3 and the alkene bridge carbon C-9. These assignments were also confirmed from the HSQC data, indicating that compound **4c** is a homoisoflavanone of the 3-benzylidene-4-chromanone type. In addition, the HMBC spectrum shows correlations of H-6 with a quaternary carbon C-4a, a tertiary carbon C-8, the latter confirmed by HSQC, and two quaternary carbons bound to oxygens which were assigned to C-5 and C-7, respectively. It was possible to distinguish between the C-5 and C-7 methoxy groups, because the HMBC spectrum shows correlation of H-8 with carbon atom C-7, the carbon of the methoxy group attached to C-7, and to a quaternary carbon, assigned to C-8a. The H-6 proton shows correlations to carbon atom C-5, C-7, C-4a and methoxy carbon at C-5 but not to methoxy group at C-7. The HMBC spectrum also allowed for the assignment of the carbon atoms in ring B by first correlating H-9 to the quaternary carbon C-1' and the two equivalent tertiary carbons C-2'/C-6'. HSQC correlations of these carbons with H-2'/H-6' confirm these assignments. H-3'/H-5' show HMBC correlations with the previously assigned C-2'/C-6' carbon atoms and also with three aromatic/tertiary carbons, assigned to the chemical equivalent C-3'/C-5' and to C-4'. It was possible to distinguish between C-3'/C-5' and C-4' by utilizing the HMBC spectrum. The details of proton and carbon chemical shifts values for compound **4c** are reported in table 1.

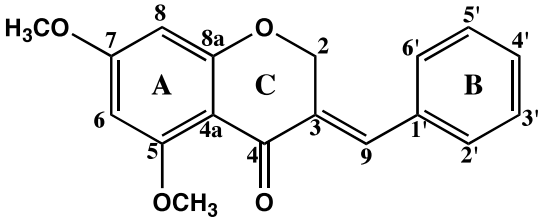


Figure 2: Compound **4c** and labeling of atoms as used in this paper

Table 1: ^{1}H and ^{13}C chemical shift (δ) and J_{HH}-couplings data[a] of compound **4c**.

Atom	δ $^{1}H^{b}$ in ppm	*J*(Hz)	δ $^{13}C^{b}$ in ppm
2	5.21	1.7	67.4
3	--	--	131.9
4	--	--	179.5
4a	--	--	107.2
5	--	--	164.7
6	6.12	2.2	93.6
7	--	--	165.8
8	6.06	2.2	93.6
8a	--	--	162.8
1’	--	--	134.8
2’/6’	7.27	8.0	129.9
3’/5’	7.27-7.34	--	128.6
4’	7.27-7.34	--	128.9
9	7.82	--	135.8
$OC(H\text{-}5)_3$	3.91	--	56.1
$OC(H\text{-}7)_3$	3.82	--	55.6

[a] 9.4T Bruker Avance III (400 MHz ^{1}H and 100 MHz ^{13}C)

[b] Referring to the solvent $CDCl_3$.

The ^{1}H and ^{13}C NMR spectra of compounds **4d-g** were also completely assigned based on the same methodology described for compound **4c**. The change in NMR data of these compounds are reported in tables 2 and 3.

Table-2: ^{1}H chemical shift differences ($\Delta\delta$) in ppm data[a] between compound **4d** to **4g** relative to compound **4c**.

Atom	Compound 4d ($\Delta\delta$ $^{1}H^{b}$) ppm	Compound 4e ($\Delta\delta$ $^{1}H^{b}$) ppm	Compound 4f ($\Delta\delta$ $^{1}H^{b}$) ppm	Compound 4g ($\Delta\delta$ $^{1}H^{b}$) ppm
2	nc	0.02	0.05	0.04
3	--	--	--	--
4	--	--	--	--
4a	--	--	--	--
5	--	--	--	--
6	nc	nc	nc	nc
7	--	--	--	--
8	nc	nc	nc	nc
8a	--	--	--	--
1’	--	--	--	--
2’/6’	0.16	0.03	0.16	nc
3’/5’	0.45	0.36	0.19	0.12
4’	--	--	--	--
9	0.06	0.05	nc	0.18
$OC(H\text{-}5)_3$	nc	nc	nc	nc
$OC(H\text{-}7)_3$	0.03	nc	0.17	nc

[a] 9.4T Bruker Avance III (400 MHz ^{1}H and 100 MHz ^{13}C)

[b] Referring to the solvent $CDCl_3$.

‘nc’ = no change was detectable.

Comparing the chemical shifts of the molecules due to substitution at position C-4’, no or only insignificantly small changes where observed for the ^{1}H and ^{13}C resonances on ring A and B, with the exception of the bridge atoms C-9 and its surroundings, such as C-2 and C-3 (see Table 2 and 3). The strongest changes in general, as expected are found in the change of chemical shift for atom C-4’, followed by C-3’/C-5’, C1’ and C-2’/C-6’, with exception of compound **4f**, where carbon chemical shift changes of C-1’ and C-2’/C-6’ are larger than for C-3’/C-5’.

One can hypothesize that in the ring-B of compound **3,** the electron pair of the hydroxyl group substitution at C-4’ generates an electron donating effect in the aromatic ring with the result of a larger shielding effect for C-3’/C-5’ than for C-2’/C-6’. Similarly the methoxy-group of compound **4f** at C-4’ causes an electron donating effect in the aromatic ring which

results in a larger shielding effect for C-3'/C-5' than for C-2'/C-6'. In compound **4f**, the protons of C-3'/C-5' experience a larger de-shielding effect than H-2'/H-6' due to two opposing effects exerted by the (C-4')-Cl group: an electron donation effect due to conjugation and an electron withdrawal effect due to induction. However chlorine is a large atom and has a smaller electronegative effect therefore, for compound **4g**, the substitution of fluorine at the C-4' position of the aromatic ring-B influences the chemical shift in the opposite way than that of compound **4f** most possibly due to the smaller fluorine atom with a stronger electron withdrawing inductive effect on ring-B. In compound **4g**, the protons H-2'/H-6' couple with protons H-3'/H-5' as well as with the fluorine atom. One therefore expects to observe a multiplet but the splitting pattern merged into a triplet (*J*=5,8.6 Hz).[28] The splitting pattern for protons H-3'/H-5' clearly shows a multiplet. Interestingly, the protons on the methoxy-group at C7 [$OC(H\text{-}7)_3$] in compound **4f** shows slight shielding ($\Delta\delta$ ^1H of 0.17 ppm) compared to compound **4c**. Furthermore, the bridge proton H9 of compounds **4d-g** shows significant shielding in comparison to compound **4c**, ranging from $\Delta\delta$ ^1H = 0.05 to 0.09 ppm. The ^{13}C chemical shift values for compound **4f** and **4g** differs due to substitution with halogens as well as in comparison to the unsubstituted compound **4c**.

Table-3: ^{13}C chemical shift differences (Δδ) in ppm data[a] of compounds **4d** to **4g**

Atom	Compound 4d ($\Delta\delta$ $^{13}C^{b}$)	Compound 4e ($\Delta\delta$ $^{13}C^{b}$)	Compound 4f ($\Delta\delta$ $^{13}C^{b}$)	Compound 4g ($\Delta\delta$ $^{13}C^{b}$)
2	nc	nc	1.2	nc
3	3.1	1.8	nc	nc
4	nc	nc	1.5	nc
4a	1.1	nc	1.2	nc
5	nc	2.0	2.9	1.9
6	nc	nc	nc	nc
7	1.1	nc	1.0	nc
8	1.3	nc	nc	nc
8a	nc	1.7	nc	1.8
1’	8.6	7.4	2.6	3.8
2’/6’	2.0	-1.7	2.2	-1.7
3’/5’	13.9	14.5	-1.3	13.0
4’	27.0	31.4	5.0	32.7
9	nc	nc	2.5	1.2
$OC(H\text{-}5)_3$	1.1	nc	1.1	nc
$OC(H\text{-}7)_3$	1.1	nc	1.1	nc

[a] 9.4T Bruker Avance III (400 MHz ^{1}H and 100 MHz ^{13}C)

[b] referring to the solvent $CDCl_3$.

‘nc’ = no change was detectable

EXPERIMENTAL

All reagents and solvents were purchased from Aldrich, Merck and Fluka. TLC used Kieselgel 60 F254 from Merck (Darmstadt, Germany). All NMR spectra were recorded on a 9.4T Bruker AVANCE III 400 MHz instrument at room (298K) temperature with $CDCl_3$ as an internal standard using a sample concentration in the range of 10 (mg/µl). The spectrometer was equipped with a BBO probe with 400 MHz S1 and with a z gradient. The data was recorded and analyzed with Topspin 2.1 (Bruker, Karlsruhe, Germany). Standard Bruker pulse sequences were used: 1D ^{1}H (32 scans) and ^{13}C with ^{1}H decoupling and 1024 scans, ^{1}H and ^{13}C hard pulse width for all experiments was 10 µs at -3 db and 8.4 µs at -2 db, respectively. ^{1}H and ^{13}C spectral width was 20.54 and 238 ppm, respectively and therefore

acquisition time was 1.9923 and 1.38s. Relaxation delays were set to 1,5 and 2 s for ^{1}H and ^{13}C, respectively and carrier frequency was set to 4 ppm for ^{1}H or 100 ppm for ^{13}C.

2D ^{1}H-^{13}C HSQC and HMBC were acquired with the same parameters, 256 and 128 complex t1 points were recorded, respectively. The number of scans was adapted to the sample concentration to assure a proper signal to noise ratio. The HSQC experiment was recorded using Echo/Antiecho-TPPI gradient selection with decoupling during acquisition and using trim pulses for transfer of magnetization. The HMBC experiment was optimized for long-range couplings and uses gradient pulses to select zero and double quantum coherence. 2D ^{1}H-^{1}H NOESY and COSY experiments were recorded with 256 and 128 complex t1 points respectively. For ^{1}H-^{1}H NOESY experiment the mixing time was recorded with 300 ms. For the COSY experiment gradient pulses were used for coherence selection. The values for chemical shift (δ) is given in ppm and coupling contants (*J*) in Hertz (Hz), presented in table 1-3 for compounds **4c-g**.

Melting points were recorded with a Mel-Temp melting point apparatus in open capillaries and are uncorrected. The high-resolution mass spectroscopy (HRMS) were recorded on a Waters Micromass Q-Tof Micro mass spectrometer with a lock spray source. The mass spectroscopy (MS) were recorded ona Waters Acquity Ultra Performance LC with ZQ detector in ESI mode.

General procedure for the synthesis of substituted homoisoflavanones

A mixture of substituted chroman-4-one (2.4 mmol, 500 mg), substituted benzaldehyde (3.6 mmol) and piperidine (7-10 drops) was heated at 80^{O}C for 2 h - 36 h (reaction was monitored by TLC – detail follow for each compound). The reaction mixture was cooled, diluted with water (15 ml) and acidified with 10 % HCl. The ethyl acetate layer was extracted (3 × 30 ml), the combined ethyl acetate layers were washed with water (30 ml), brine (30 ml) and dried over magnesium sulphate. The residue obtained after evaporation of the solvent was chromatographed using a silica gel column with a mixture of ethyl acetate-hexane (20:80) as eluent to give homoisoflavanones **4c-g** (63-79 %) yield. The NMR data for all compounds are included in Tables 1 – 3.

(*E*)-5,7-dimethoxy-3-benzylidene-4-chromanone (4c)

Yield 76 %; mp 100-103 ºC; dark yellow powder (Rf = 0.61 in 30 % ethyl acetate in hexane). IR: 2940 cm^{-1}, 1664 cm^{-1}, 1602 cm^{-1}, 1466 cm^{-1}, 1421 cm^{-1}, 1250 cm^{-1}, 1209 cm^{-1}, 1109 cm^{-1},

940 cm^{-1}, 755 cm^{-1}. HRMS calculated for $C_{18}H_{17}O_4$ ($M + H^+$) 297.1049, found 297.1131.

(*E*)-5,7-dimethoxy-3-(4-hydroxybenzylidene)-4-chromanone (4d)

Yield 68 %; mp 197-200 ºC; yellow powder (Rf = 0.43 in 30 % ethyl acetate in hexane).
IR 3176 cm^{-1}, 2923 cm^{-1}, 1644 cm^{-1}, 1601 cm^{-1}, 1570 cm^{-1}, 1452 cm^{-1}, 1420 cm^{-1}, 1265 cm^{-1}, 1210 cm^{-1}, 1157 cm^{-1}, 969 cm^{-1}, 809 cm^{-1}. HRMS calculated for $C_{18}H_{17}O_5$ ($M + H^+$) 313.0998, found 313.1070.

(*E*)-5,7-dimethoxy-3-(4-methoxybenzylidene)-4-chromanone (4e)

Yield 71 %; mp 171-174 ºC; pale yellow powder (Rf = 0.58 in 30 % ethyl acetate in hexane).
IR: 2935 cm^{-1}, 1664 cm^{-1}, 1602 cm^{-1}, 1456 cm^{-1}, 1421 cm^{-1}, 1247 cm^{-1}, 1208 cm^{-1}, 1109 cm^{-1}, 945 cm^{-1}, 798 cm^{-1}. HRMS calculated for $C_{19}H_{19}O_5$ ($M + H^+$) 327.1154, found 327.1225.

(*E*)- 5,7-dimethoxy-3-(4-chlorobenzylidene)-4-chromanone (4f)

Yield 79 %; mp 126-129 ºC; pale yellow powder (Rf = 0.54 in 30 % ethyl acetate in hexane).
IR: 2938 cm^{-1}, 1664 cm^{-1}, 1602 cm^{-1}, 1456 cm^{-1}, 1249 cm^{-1}, 1208 cm^{-1}, 1109 cm^{-1}, 798 cm^{-1}.
HRMS calculated for $C_{18}H_{16}ClO_4$ ($M + H^+$) 331.0659, found 331.0733.

(*E*)-5,7-dimethoxy-3-(4-fluorobenzylidene)-4-chromanone (4g)

Yield 63 %; mp 102-105ºC; pale yellow needles (Rf = 0.52 in 30 % ethyl acetate in hexane).
IR: 2938 cm^{-1}, 1646 cm^{-1}, 1601 cm^{-1}, 1586 cm^{-1}, 1421 cm^{-1}, 1290 cm^{-1}, 1205 cm^{-1}, 1154 cm^{-1}, 1078 cm^{-1}, 1109 cm^{-1}, 821 cm^{-1}. HRMS calculated for $C_{18}H_{16}FO_4$ ($M + H^+$) 315.0954, found 315.1047.

CONCLUSION

The synthesis and 2D-NMR elucidation of five homoisoflavanone (3-benzylidene-4-chromanone) analogues was successfully carried out. The synthesis of these homoisoflavanone analogues were based on a natural lead compound (3-benzyl-4-chromanone) with different substitution patterns at the 4'-position of the B-ring. We could confirm the influence of the R-group (R = H, OH, OMe, Cl and F) on the chemical shift of the ring B.

SUPPLEMENTARY MATERIAL

The NMR spectra of all products are available as supplementary material.

ACKNOWLEGMENT

The authors thank the University of KwaZulu-Natal for a Competitive Research Fund, NRF (Gun RH-6030732) and Rolexsi (Pty) Ltd for financial support. The author also thanks the NRF (Gun 48505) for postdoctoral fellowship to Dr. Katja Petzold. The authors also thank Mr. Dilip Jagjivan for valuable discussions on acquiring the NMR data

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CHAPTER-5

DOES NATURE PROVIDE THE BEST THERAPEUTIC OPTIONS? SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF A NATURALLY OCCURRING HOMOISOFLAVANONE AND ITS ENANTIOMER

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ABSTRACT

The Hyacinthaceae family is one of the most important plant families across the eastern seaboard. The *(R)*-enantiomer of a homoisoflavanone with anti-inflammatory activity was previously isolated from members of this family, namely *Drimiopsis burkei* Bak. and *Scilla nervosa* (Burch.) Jessop. However the activity of the *(S)*-enantiomer is unknown. In this paper, we report the synthesis and structural elucidation, *in vivo* anti-inflammatory activity and *in vitro* cytotoxic properties of both the *(R)*- and *(S)*-enantiomers and the racemate. The enantiomers and racemate exhibited a relatively short duration of action and activity similar to that of the known non-steroidal anti-inflammatory drug diclofenac. The naturally occurring enantiomer exhibited the least cytotoxicity.

INTRODUCTION

A homoisoflavanone, *(3R)*-5,7-dimethoxy-(4'-hydroxybenzyl)-4-chromanone, was previously isolated from *Scilla nervosa* (Burch.) Jessop[1] as well as from *Drimiopsis burkei* Bak.[2] The traditional use of *S. nervosa* for rheumatic fever indicates possible anti-inflammatory properties of its constituents.[3] Subsequent studies showed strong inhibition of

prostaglandin synthesis in microsomal cells by the isolated homoisoflavanone, supporting the traditional use of *S. nervosa*. This compound was found to exhibit weak activity against COX-1 enzymes and to be inactive against COX-2 enzymes. The anti-inflammatory activity can therefore not be attributed exclusively to COX enzyme inhibition, and an unknown mechanism of action was proposed.[4]

Studies indicate that stereoselectivity plays an important role in the anti-inflammatory activities of non-steroidal anti-inflammatory drugs.[5] The decision to employ either a racemate or a pure enantiomer for therapeutic purposes is usually based on the diverse mechanisms of actions of the enantiomers.[6] The absolute configuration at C-3 of a series of naturally occurring homoisoflavanones was investigated using circular dichroism.[7] The *R*-configuration was established for all of these compounds.[2, 7] Therefore, the anti-inflammatory activity of the naturally occurring (*R*)-enantiomer is known, but the activity of the (*S*)-enantiomer and racemate is unknown. A study of the anti-inflammatory activity of both the enantiomers could provide an answer to the question whether nature truly provides the best therapeutic options.

RESULTS AND DISCUSSION

The synthesis of the enantiomers of the homoisoflavanone from commercially available reagents was carried out using the general synthetic approach shown in the synthetic scheme (Scheme 1). The reaction of 3,5-dimethoxyphenol **1** with 3-bromopropanoic acid using sodium hydride as base furnished 3-(3,5-dimethoxyphenoxy)-propanoic acid **2**,[8] which was cyclised using polyphosphoric acid to give rise to a substituted chroman-4-one **3**.[9] Base catalyzed condensation of **3** with *p*-hydroxybenzaldehyde resulted in **4**.[10] Subsequent reduction of the olefinic double bond of **4** in MeOH-THF (1:1) was achieved by passing hydrogen gas in the presence of palladium on charcoal through the solution to give rise to **5**.[11] The reduction of the carbonyl group in **5**, using sodium borohydrate as a reducing agent in MeOH, afforded a diastereomeric mixture of **6a** and **6b** in a ratio of 2:1 with an 88 % yield.[12] An appreciable difference in R_f values between these compounds allowed separation of the two diastereomers by column chromatography. Finally, **6a** and **6b** were oxidized by using CrO_3 and acetic acid which afforded pure enantiomers **7a** and **7b** of an approximate yield of 40 %.[13]

The optical rotation of both the enantiomers was measured and correlated with literature values of the natural homoisoflavanone to establish the absolute stereochemistry.[2] It was found that (3*R*)-5,7-dimethoxy-(-4'-hydroxybenzyl)-4-chromanone correlated with **7a** and (3*S*)-5,7-dimethoxy-(-4'-hydroxybenzyl)-4-chromanone with **7b.** The spectral data of **7a** coincided with that of the natural homoisoflavanone.[2]

The anti-inflammatory activities of **7a** and **7b** and the racemate were assessed in a mouse model of acute contact dermatitis. Eight-week old male Balb/c mice of approximately 30 g each were used. Equal volumes of croton oil (25 μl) were mixed with acetone (25 μl) as vehicle and applied (50 μl total volume; 1 hour) onto the inner surface of the right auricle of each mouse to induce oedema.[14] The left auricle was untreated and used a negative control. Acetone has not been documented to have an anti-inflammatory effect by itself.[15] A known non-steroidal anti-inflammatory drug, diclofenac, was used as a positive control. Treatment of inflammation was initiated an hour after induction with croton oil and the reduction in oedema was measured after 3 (Fig. 1, left panel) and 6 h (Fig. 1, right panel) with **7a** and **7b**. Mice were euthanized after treatment. From each mouse, left and right auricle biopsy specimens were obtained with a 6 mm biopsy punch and weighed. Each compound at a dose of 0.1 mg significantly inhibited oedema after 3 and 6 h treatment ($P < 0.001$). It was established that a dose of 0.1 mg of **7a**, **7b** and diclofenac exhibited optimal activity (data not shown). Thus, the optimal anti-inflammatory activities of **7a**, **7b** and the racemate were comparable to that of diclofenac.

After 3 h treatment, diclofenac inhibited oedema by 55.7 ± 8.4 %. Compound **7a** was the least active (50.1 ± 4.2 %), whilst compound **7b** and the racemate exhibited slightly higher activities (58.9 ± 4.0 % and 60.0 ± 2.5 % respectively). The difference in activity between **7a** and the racemate was significant ($P < 0.05$). After 6 h treatment, the activity of diclofenac, **7b** and the racemate decreased significantly, suggesting a relatively short duration of action. The difference in activity of **7a** between 3 and 6 h was the least significant ($P > 0.05$). After 6 h treatment, diclofenac was the least active (34.7 ± 7.2 %; $P < 0.001$), followed by **7b** (39.0 ± 4.6 %; $P < 0.05$), **7a** (40.1 ± 8.4 %) and the racemate (42.4 ± 4.0 %; $P < 0.01$).

Compound **7a**, previously isolated from plants, was the least active at 3 h but its duration of action was not statistically significantly different between 3 and 6 h treatment, suggesting a relatively longer duration of action.

Cytotoxicity is an important factor to consider when testing for any biological activity. The MTT assay is a colourimetric assay to determine cellular growth and survival, and compares well with other available assays.[16, 17] The tetrazolium salt MTT was used to measure cell viability. A low IC_{50} value indicates increased cytotoxicity, thereby decreasing the therapeutic potential of the compounds tested. The *in vitro* cytotoxicity of the compounds were tested in mammalian cells and compared to diclofenac and the known cytotoxic drug emetine. IC_{50} values are represented in Table 1. Diclofenac was the least toxic, followed by **7a**, **7b** and the racemate. The racemate was approximately 10-fold more toxic than **7b**, and approximately 20-fold more toxic than **7a**. This difference in cytotoxicity profiles may indicate interactions with different receptor systems.

Although **7a** was previously isolated from plants and tested *in vitro* for anti-inflammatory activity, no reports on the synthesis or biological activity of **7b** or the racemate exist. *In vitro* tests indicated that mechanisms other than inhibition of COX enzymes play a role in the anti-inflammatory activity.[4] The decision to employ either a racemate or a pure enantiomer therapeutically is usually based on the diverse mechanisms of action.[6] The *in vivo* studies reported here consider all mechanisms of action and provide important insight regarding the activity as well as the toxicity of the enantiomers and racemate.

Anti-inflammatory activity was found for both enantiomers and the racemate. Compound **7b** and the racemate were more active than **7a** after 3 h treatment, however, cytotoxicity profiles indicated that **7a** is less cytotoxic against mammalian cells. Furthermore, **7a** had a more sustained duration of action.

In conclusion, **7a** which is naturally found, does provide the best therapeutic option in terms of a favourable cytotoxicity profile. The varying anti-inflammatory activities and cytotoxicity profiles seem to suggest that **7a** and **7b** does not share the same mechanism of action.

ACKNOWLEDGMENT

We acknowledge the University of KwaZulu-Natal Competitive Research Fund, NRF (Gun RH-6030732) and Rolexsi (Pty) Ltd for financial support. We also thank Ms Sithabile Buthelezi and Mr Dennis Ndwandwe for experimental assistance.

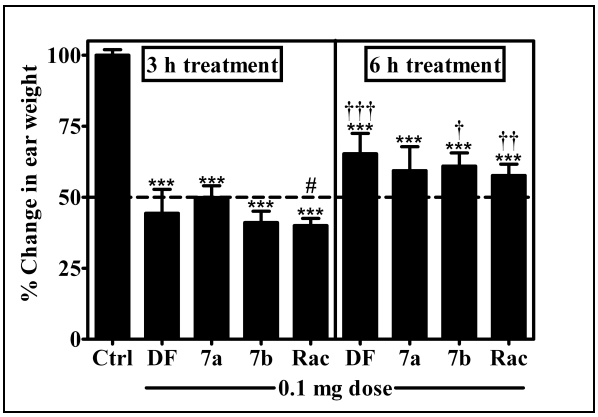
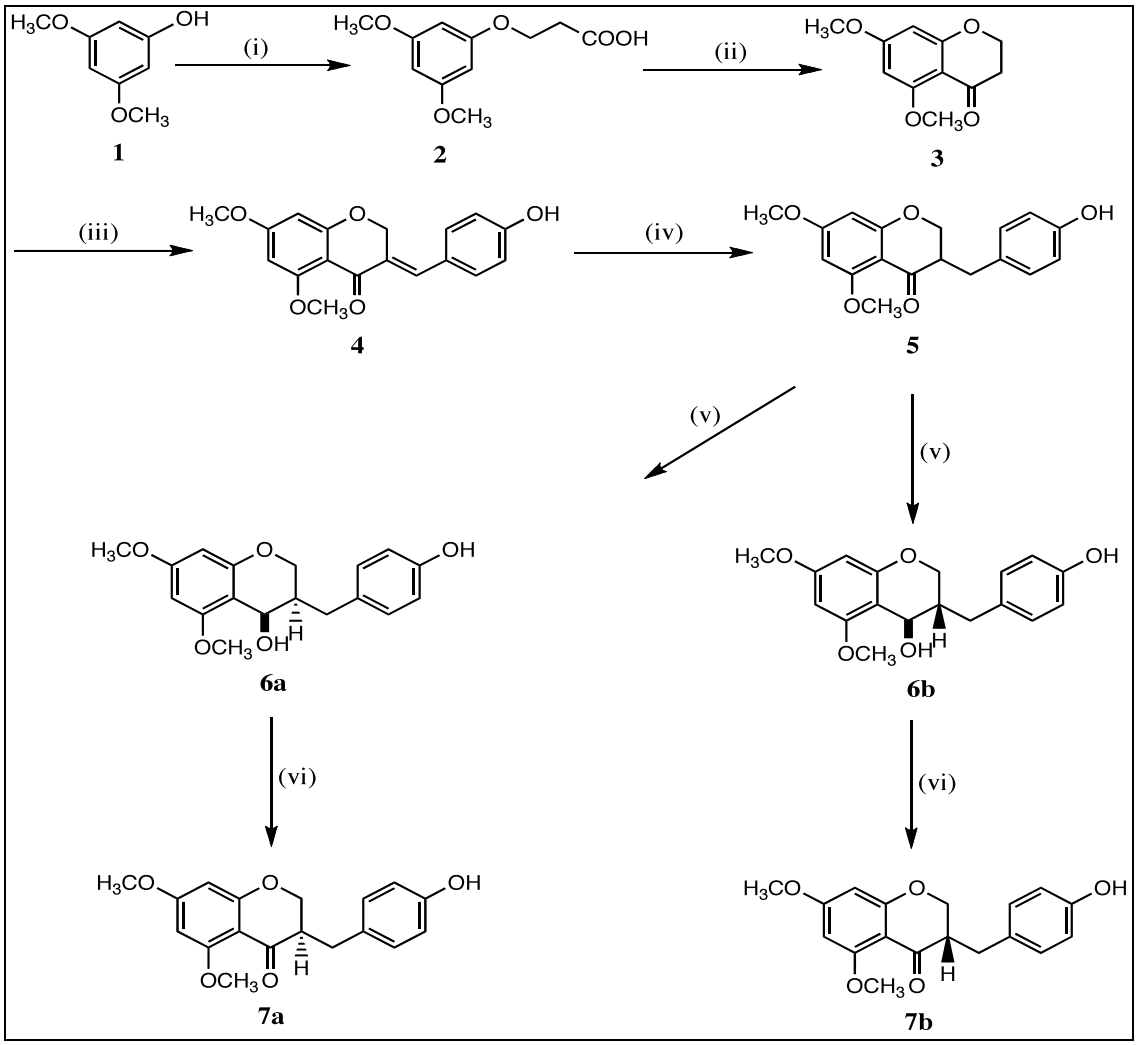


Figure 1. The anti-inflammatory activity of synthetic homoisoflavanones. Diclofenac (DF), **7a**, **7b** and the racemate (Rac) at 0.1 mg each were tested in a mouse model of acute croton oil-induced auricular contact dermatitis. Oedema was measured after treatment for 3 and 6 h. ***, $P < 0.001$ of compound versus croton oil only control (no treatment); †, $P < 0.05$; ††, $P < 0.01$; †††, $P < 0.001$ of compound at 6 h versus 3 hour treatment; #, $P < 0.05$ of Rac versus **7a**.



Scheme 1. Reagents and conditions: (i) 3-bromopropionic acid, NaH, DMF, rt, 12h, 38 %; (ii) PPA, 80 °C, 4h, 45 %; (iii) *para*-hydroxy benzaldehyde, piperidine, 70-80 °C, 6h, 86 %; (iv) H_2, Pd/c, MeOH-THF, rt, 0.5h, 68 %; (v) $NaBH_4$, MeOH, rt, 1h, 88 %; (vi) CrO_3, CH_3COOH, rt, 0.5h, 40 %.

Table 1. The *in vitro* cytotoxicity of synthetic homoisoflavanones **7a**, **7b**, Rac and DF against Chinese Hamster Ovarian (CHO-K1) cells using the MTT assay.

Compound	IC_{50} (µg/ml)
DF	>100
7a	76.38 ± 7.87
7b	36.48 ± 6.30
Rac	3.68 ± 1.77
emetine	0.06 ± 0.02

EXPERIMENTAL SECTION

General Experimental Procedures

All reagents were obtained from Aldrich chemicals suppliers and solvents were obtained from a commercial supplier and used without further purification. All reaction mixtures were magnetically stirred and monitored by TLC using Kieselgel 60 F254 obtained from Merck (Darmstadt, Germany). ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker AVANCE III at 400 MHz with $CDCl_3$ as internal reference. The value for chemical shift (δ) is given in ppm and coupling constants (*J*) in Hertz (Hz). Melting points were recorded with a Mel-Temp melting point apparatus in open capillaries and are uncorrected. Optical rotations were measured at room temperature in chloroform using a Perkin Elmer Polarimeter-Model 341. High-resolution mass spectroscopy (HRMS) data was recorded on a Waters Micromass Q-Tof Micro mass spectrometer with a lock spray source.

Procedure for the synthesis of 3-(3,5-dimethoxyphenoxy) propanoic acid 2

To a mixture of 3,5 dimethoxy phenol (5.0 g, 32.46 mmol) in DMF (15 ml), NaH (0.7 g, 32.46 mmol) was added at a temperature of 10-15 °C. The reaction mixture was stirred at room temperature for 1 h. A solution of 3-bromopropionic acid (5.9 g, 38.96 mmol) in DMF (15 ml) was then added dropwise at a temperature of 0 °C and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with methanol (20 ml) and acidified with 10 % HCl and extracted with ethyl acetate (3× 50 ml). The combined

ethyl acetate layers were washed with water (1× 50 ml), brine (1× 50 ml) and dried over magnesium sulphate. The residue obtained after evaporation of the solvent was chromatographed over a silica gel column, using a mixture of ethyl acetate/hexane (30:70) as eluent to produce the title product **2**. Yield 38 %; R_f = 0.25 (30:70 ethyl acetate/hexane); mp 124-126 °C; light yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.83 (2H, t, *J*=6.2 Hz, H-2), 3.76 (6H, s, Ar-OCH_3-3', 5'), 4.20 (2H, t, *J*=6.2 Hz, H-3), 6.09 (3H, s, H-2', H-4', H-6'); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 34.2 (CH_2, C-1), 55.3 (OCH_3, C-3' & C-5'), 63.0 (C-2), 93.4 (C-4'), 93.5 (C, C-2' & C-6'), 160.2 (C-3'), 161.5 (C-5'), 176.8 (C-3); Mass *m/z* = 227 $(M+1)^+$.

Procedure for the synthesis of 5,7-dimethoxy-4-chromanone 3

A mixture of 3-(3,5-dimethoxyphenoxy)-propanoic acid (2.0 g, 10 mmol) and polyphosphoric acid (10 g) was stirred at 85-90 °C for 2 h. The red coloured syrup thus obtained was poured onto crushed ice and extracted with diethyl ether (2× 50 ml). The combined ether solution was washed with 3N NaOH (1× 30 ml), water (1× 50 ml) and dried over magnesium sulphate. The residue obtained after evaporation of the solvent was chromatographed over a silica gel column using a mixture of ethyl acetate/hexane (20:80) as eluent to produce the title product **3**. Yield 45 %; R_f = 0.54 (20:80 ethyl acetate/hexane); mp 101-103 °C; white powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.71 (2H, t, *J*=6.4 Hz, H-3), 3.81 (3H, s, Ar-OCH_3-7), 3.87 (3H, s, Ar-OCH_3-5), 4.43 (2H, t, *J*=6.4 Hz, H-2), 6.04 (2H, s, H-6 & H-8); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 38.7 (CH_2, C-3), 55.5 (OCH_3, C-7), 56.0 (OCH_3, C-5), 66.7 (CH_2, C-2), 92.8 (CH, C-8), 93.3 (CH, C-6), 106.3 (C, C-4a), 162.2 (C, C-5), 165.2 (C, C-8a), 165.7 (C, C-7), 189.1 (C, C-4); Mass *m/z* = 209 $(M+1)^+$.

Procedure for the synthesis of (E)-5,7-dimethoxy-3-(4-hydroxybenzylidene)-4-chromanone 4

A mixture of 5,7-dimethoxychroman-4-one (0.5 g, 2.4 mmol), *p*-hydroxybenzaldehyde (0.4 g, 3.6 mmol) and piperidine (7-10 drops) was heated at a temperature of 80 °C for 6 h (reaction was monitored by TLC). The reaction mixture was cooled, diluted with water (15 ml) and acidified with 10 % HCl. The mixture was then extracted with ethyl acetate (3× 30), and the combined ethyl acetate layer was washed with water (1× 30 ml), brine (1× 30 ml) and dried over magnesium sulphate. The residue obtained after evaporation of the solvent was chromatographed over a silica gel column using a mixture of ethyl acetate/hexane (20:80) as

eluent to produce **4**. Yield 86 %; R_f = 0.41 (20:80 ethyl acetate/hexane); mp 197-200 °C; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.76 (3H, s, Ar-OCH_3-7), 3.82 (3H, s, Ar-OCH_3-5), 5.17 (2H, d, *J*=1.4 Hz, H-2), 6.00 (1H, d, *J*=2.2 Hz, H-8), 6.05 (1H, d, *J*=2.2 Hz, H-6), 6.85 (2H, d, *J*=8.5 Hz, H-3',5'), 7.11 (2H, d, *J*=8.5 Hz, H-2',6'), 7.70 (1H, s, H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 54.5 (OCH_3, C-7), 55.0 (OCH_3, C-5), 66.5 (CH_2, C-2), 92.5 (CH, C-6), 92.5 (CH, C-8), 106.1 (C, C-4a), 114.7 (CH, C-3',5'), 126.0 (C, C-1'), 128.0 (C, C-3), 130.9 (CH, C-2',6'), 135.0 (CH, C-9), 155.9 (C, C-4'), 163.6 (C, C-8a), 164.5 (C, C-5), 164.7 (C, C-7), 178.8 (C, C-4); HRMS (EI) calcd for $C_{18}H_{17}O_5$ 313.0998, found 313.1071.

Procedure for the synthesis of 5,7-dimethoxy-3-(4-hydroxybenzyl) -4-chromanone 5

To a solution of 5,7-dimethoxy-3-(4-hydroxybenzylidene)-4-chromanone (1.0 g, 3.2 mmol) in a mixture of anhydrous MeOH/THF (1:1, 20 ml) at a temperature of 0 °C, Pd/c (0.4 g, 3.8 mmol) was added portion wise. H_2 gas was passed through the stirred mixture at room temperature for 0.5 h after which it was filtered through celite and concentrated under reduced pressure. The residue obtained after evaporation of the solvent was chromatographed over a silica gel column using mixture of ethyl acetate/hexane (20:80) as eluent to produce the homoisoflavanone **5**. Yield 68 %; R_f = 0.43 (20:80 ethyl acetate/hexane); mp 174-176 °C; light yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.65 (1H, dd, *J*=10.4, 13.5 Hz, H-9a), 2.68-2.70 (1H, m, H-3), 3.15 (1H, dd, *J*=4.1, 13.4 Hz, H-9b), 3.81 (3H, s, Ar-OCH_3-7), 3.86 (3H, s, Ar-OCH_3-5), 4.12 (1H, dd, *J*=4.2, 7.0 Hz, H-2a), 4.27 (1H, dd, *J*=3.9, 11.2 Hz, H-2b), 6.06 (1H, s, H-8), 6.07 (1H, s, H-6), 6.80 (2H, d, *J*=8.4 Hz, H-2',6'), 7.07 (2H, d, *J*=8.4 Hz, H-3',5'); ^{13}C NMR (100 MHz, $CDCl_3$) 32.1 (CH_2, C-9), 48.6 (CH, C-3), 55.0 (OCH_3, C-7), 55.8 (OCH_3, C-5), 68.8 (CH_2, C-2), 92.8 (CH, C-8), 93.2 (CH, C-6), 130.2 (CH, C-2',6'), 105.4 (C, C-4a), 115.5 (CH, C-3',5'), 130.4 (C, C-1'), 154.7 (C, C-4'), 162.8 (C, C-7), 165.0 (C, C-8a), 165.7 (C, C-5), 191.9 (C, C-4); HRMS (EI) calcd for $C_{18}H_{19}O_5$ 315.1154, found 315.1224.

Procedure for synthesis of 5,7-dimethoxy-3-(4-hydroxybenzyl) -4-chromanone 6a/6b

$NaBH_4$ (0.3 g, 9.5 mmol) was added portionwise to a solution of 5,7-dimethoxy-3-(4-hydroxybenzyl)-4-chromanone (1.0 g, 3.1 mmol) in anhydrous MeOH (15 ml) at a temperature of 0 °C under nitrogen athmosphere. The mixture was then allowed to reach room temperature and stirred for 1 h. The reaction mixture was quenched with water and extracted with ethyl acetate (3×30). The organic layer was washed with brine, dried over

magnesium sulphate, and concentrated under reduced pressure to produce a viscous oil mixture of **6a** and **6b**. The residue obtained after evaporation of the solvent was chromatographed over a silica gel column using mixture of ethyl acetate/hexane (30:70) as eluent to produce an oily syrup at a yield of 88 %. Compound **6a**; R_f = 0.48 (30:70 ethyl acetate/hexane); oily syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.08-2.15 (1H, m, H-3), 2.58 (1H, dd, *J*=2.6, 7.2 Hz, H-9a), 2.85 (1H, dd, *J*=2.6, 7.2 Hz, H-9b), 3.78 (3H, s, Ar-OCH_3-5), 3.83 (3H, s, Ar-OCH_3-7), 3.99 (2H, d, *J*=8.2 Hz, H-2a & 2b), 4.66 (1H, d, *J*=2.5 Hz, H-4), 5.99 (1H, d, *J*=7.1 Hz, H-8), 6.01 (1H, d, *J*=7.1 Hz, H-6), 6.76 (2H, d, *J*=8.2 Hz, H-3',5'), 7.12 (2H, d, *J*=8.0 Hz, H-2',6'); ^{13}C NMR (100 MHz, $CDCl_3$) 31.9 (CH_2, C-9), 40.1 (CH, C-3), 55.3 (OCH_3, C-7), 55.4 (OCH_3, C-5), 59.6 (CH, C-4), 65.2 (CH_2, C-2), 91.3 (CH, C-6), 93.0 (CH, C-8), 106.6 (C, C-4a), 115.2 (CH, C-3',5'), 130.2 (C, C-1'), 131.6 (CH, C-2',6'), 153.8 (C, C-4'), 155.9 (C, C-5), 159.2 (C, C-8a), 161.1 (C, C-7); Mass *m/z* = 317 $(M+1)^+$. Compound **6b**; R_f = 0.45 (30:70 ethyl acetate/hexane); oily syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.12-2.18 (1H, m, H-3), 2.40 (1H, dd, *J*=2.9, 7.9 Hz, H-9a), 2.55 (1H, dd, *J*=2.9, 7.9 Hz, H-9b), 3.76 (3H, s, Ar-OCH_3-5), 3.81 (3H, s, Ar-OCH_3-7), 3.90 (1H, dd, *J*=1.8, 1.8 Hz, H-2a), 4.07 (1H, dd, *J*=1.9, 2.0 Hz, H-2b), 4.62 (1H, s, H-4), 6.06 (1H, d, *J*=3.9 Hz, H-6), 6.07 (1H, d, *J*=3.9 Hz, H-8), 6.74 (2H, d, *J*=8.3 Hz, H-3',5'), 7.04 (2H, d, *J*=8.3 Hz, H-2',6'); ^{13}C NMR (100 MHz, $CDCl_3$) 33.6 (CH_2, C-9), 40.5 (CH, C-3), 55.3 (OCH_3, C-7), 55.5 (OCH_3, C-5), 62.9 (CH, C-4), 64.3 (CH_2, C-2), 91.8 (CH, C-6), 93.2 (CH, C-8), 104.9 (C, C-4a), 115.3 (CH, C-3',5'), 130.2 (C, C-1'), 131.2 (CH, C-2',6'), 154.2 (C, C-4'), 155.8 (C, C-5), 159.8 (C, C-8a), 161.0 (C, C-7); Mass *m/z* = 317 $(M+1)^+$.

Procedure for synthesis of optically pure 5,7-dimethoxy-3-(4-hydroxybenzyl)-4-chromanone 7a/7b

To a mixture of either **6a** or **6b** respectively (0.1 g, 1.0 mmol) in acetic acid (4 ml) was added CrO_3 (0.16 g, 5.0 mmol). The reaction mixture was stirred at room temperature and allowed to stand for 0.5h. The solvent was evaporated and extracted with ethyl acetate (2×15 ml). The organic layer was washed with brine (2×15 ml) and dried over magnesium sulphate. The residue obtained after evaporation of the solvent was chromatographed over a silica gel column using a mixture of ethyl acetate/hexane (20:80) as eluent to produce the respective title products **7a** or **7b** as a semi-solid overall yield 40 %. Compound **7a**; R_f = 0.44 (20:80 ethyl acetate/hexane); off white semi-solid; $[\alpha]^{25}_D$ = -25.33 (c = 0.03 g/100 mL); ^{1}H NMR (400 MHz, MeOD) δ: 2.63 (1H, dd, *J*=10.7, 13.3 Hz, H-9a), 2.70-2.72 (1H, m, H-3), 3.15

(1H, dd, *J*=4.0, 13.5 Hz, H-9b), 3.82 (3H, s, Ar-OCH_3-7), 3.87 (3H, s, Ar-OCH_3-5), 4.10 (1H, dd, *J*=6.9, 11.2 Hz, H-2b), 4.27 (1H, dd, *J*=3.9, 11.2 Hz, H-2a), 6.06 (1H, s, H-6), 6.07 (1H, s, H-8), 6.80 (2H, d, *J*=8.4 Hz, H-2',6'), 7.07 (2H, d, *J*=8.4 Hz, H-3',5'); ^{13}C NMR (100 MHz, MeOD) 32.1 (CH_2, C-9), 48.5 (CH, C-3), 55.0 (OCH_3, C-7), 55.9 (OCH_3, C-5), 68.9 (CH_2, C-2), 92.9 (CH, C-8), 93.2 (CH, C-6), 105.3 (C, C-4a), 115.5 (CH, C-3',5'), 130.2 (C, C-1'), 130.3 (CH, C-2',6'), 154.5 (C, C-4'), 162.6 (C, C-7), 165.0 (C, C-8a), 165.9 (C, C-5), 191.7 (C, C-4); HRMS (EI) calcd for $C_{18}H_{19}O_5$ 315.1154, found 315.1226. Compound **7b**; R_f = 0.44 (20:80 ethyl acetate/hexane); off white semi-solid; $[\alpha]^{25}_D$ = +25.66 (c = 0.03 g/100 mL); ^{1}H NMR (400 MHz, MeOD) δ: 2.64 (1H, dd, *J*=10.4, 13.5, H-9a), 2.69-2.70 (1H, m, H-3), 3.14 (1H, dd, *J*=4.1, 13.4 Hz, H-9b), 3.82 (3H, s, Ar-OMe-7), 3.86 (3H, s, Ar-OMe-5), 4.11 (1H, dd, *J*=4.2, 7.0 Hz, H-2b), 4.27 (1H, dd, *J*=3.9, 11.2 Hz, H-2a), 6.06 (1H, s, H-6), 6.07 (1H, s, H-8), 6.80 (2H, d, *J*=8.4 Hz, H-2',6'), 7.07 (2H, d, *J*=8.4 Hz, H-3',5'); ^{13}C NMR (100 MHz, MeOD) 32.1 (CH_2, C-9), 48.5 (CH, C-3), 55.0 (OCH_3, C-7), 55.9 (OCH_3, C-5), 68.9 (CH_2, C-2), 92.8 (CH, C-8), 93.2 (CH, C-6), 105.3 (C, C-4a), 115.5 (CH, C-3',5'), 130.1 (C, C-1'), 130.2 (CH, C-2',6'), 154.7 (C, C-4'), 162.6 (C, C-7), 165.0 (C, C-8a), 165.9 (C, C-5), 191.9 (C, C-4); HRMS (EI) calcd for $C_{18}H_{19}O_5$ 315.1154, found 315.1220.

Assessment of croton oil-induced oedema

Compound **7a**, **7b** and the racemate were assessed for their potential anti-inflammatory activity. Ethical approval (003/09/Animal) from the University of KwaZulu-Natal Animal Ethics subcommittee was obtained prior to the investigation of acute croton oil-induced auricular dermatitis in a mouse model. Guidelines by the University of KwaZulu-Natal Animal Ethics Subcommittee and Biomedical Resources Unit for the maintenance and treatment of laboratory animals were followed. Eight-week old male Balb/c mice of approximately 30 g each were used. Equal volumes of croton oil (25 μl) were mixed with acetone (25 μl) as vehicle and applied (50 μl total volume; 1 h) onto the inner surface of the right auricle of each mouse to induce oedema.[14] Acetone has not been documented to have an anti-inflammatory effect by itself.[18] Thereafter, **7a**, **7b** or the racemate were dissolved in acetone and 0.1 mg applied for 3 or 6 h treatment onto the right auricle to assess the reduction in oedema. The non-steroidal anti-inflammatory drug diclofenac was included as a positive control. Mice were euthanized after treatment for 3 and 6 h. From each mouse, left and right auricle biopsy specimens were obtained with a 6 mm biopsy punch and weighed.

Cytotoxicity Assay

In vitro cytotoxicity of **7a**, **7b** and the racemate was tested against a Chinese Hamster Ovarian (CHO-K1) cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. This cell line was obtained from American Type Culture Collection (ATCC, CCL-61). The MTT assay is a colourimetric assay to determine cellular growth and survival, and compares well with other available assays. The tetrazolium salt MTT was used to measure cell viability. The test compounds were prepared in a 2 mg/ml stock solution containing 10 % v/v DMSO. Emetine was used as the reference drug at an initial concentration of 100 μg/ml and serially diluted in 10-fold to obtain 6 concentrations, the lowest being 0.001 μg/ml. Compounds **7a**, **7b** and the racemate were diluted similarly. The DMSO solvent system had no measurable effect on cell viability (data not shown).

Data analysis

Data are reported as the mean ± standard error of the mean of at least three independent experiments with duplicate measurements. Oedema was quantified by calculating the difference in weights of the right and left auricular biopsy specimens. The value is expressed as a percentage of the croton oil control. The 50 % inhibitory concentration (IC_{50}) values of the cytotoxicity assays were obtained from full dose-response curves using a non-linear dose-response curve fitting analysis. GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA) was used to analyse and present the data. Statistical comparisons were made by one-way ANOVA followed by Bonferroni's post-test for multiple comparisons, or by Student's two-tailed paired *t* test for individual comparisons to determine *P* values. A value of $P < 0.05$ was considered significant.

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CHAPTER-6

5,7-DIMETHOXY-3-BENZYL-4-CHROMAN-OL

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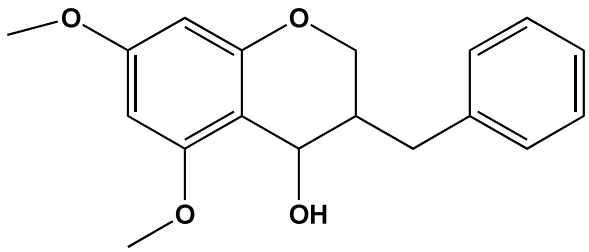
ABSTRACT

The title compound $C_{18}H_{20}O_4$, at 100 K has monoclinic ($P2_1/c$) symmetry. The structure displays O2—H···O1 hydrogen bonding. This hydrogen-bonding system connects the molecules in two dimensional plains. There is no π -stacking found in the structure.

RELATED LITRATURE

For examples of structural analogues see.[1, 2] For synthesis see.[3, 4]



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EXPERIMENTAL

To a solution of 5,7-dimethoxy-3-(benzyl)-4-chromanone (1.0 g, 3.3 mmol) in anhydrous MeOH (15 ml), $NaBH_4$ (0.38 g, 10.0 mmol) was added portionwise at a temperature of 0 °C under a nitrogen atmosphere. The mixture was then allowed to reach room temperature and stirred for 1 h. The reaction mixture was quenched with water and extracted with ethyl acetate (3x30 mL). The organic layer was washed with brine, dried over magnesium sulphate, and concentrated under reduced pressure to produce a viscous oil mixture. The residue obtained after evaporation of the solvent was chromatographed over a silica gel column using a mixture of ethyl acetate/hexane (30:70) as eluent to yield a product of 88 % (0.88 g).

Off-white solid; m.p. 118-121 °C. The title compound was recrystalised from a solution of ethyl acetate/hexane (30:70) at room temperature.

^{1}H NMR (400 MHz, $CDCl_3$, δ, p.p.m.): 7.33-7.26 (m, 5H), 6.02 (d, *J*=2.20 Hz, 1H), 6.00 (d, *J*=2.20 Hz, 1H), 4.70 (d, *J*=2.40 Hz, 1H), 4.02 (dd, *J*=3.68, 6.20 Hz, 2H), 3.77 (s, 3H), 3.73 (s, 3H), 2.95 (dd, *J*=2.5, 7.4 Hz, 1H), 2.66 (dd, *J*=2.5, 7.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, p.p.m.): 161.1, 159.2, 155.9, 139.6, 129.1, 128.4, 126.1, 106.7, 93.0, 91.4, 65.2, 59.6, 55.4, 55.3, 40.0, 32.9.IR: 3501, 2946, 1592, 1453, 1304, 1200, 1052; HRMS (EI): Calcd for $C_{18}H_{20}O_4Na$ 323.1254, found 323.1271.

Crystal data

$C_{18}H_{20}O_4$	V = 1544.6 (13) Å^3
M_r = 300.34	Z = 4
Monoclinic, $P2_1/c$	Mo $K\alpha$
a = 9.870 (5) Å	μ = 0.09 mm^{-1}
b = 11.211 (6) Å	T = 100 (2) K
c = 14.603 (7) Å	0.37 × 0.24 × 0.20 mm
β = 107.072 (7)°	

Data collection

Bruker Kappa Duo Apex II Diffractometer	3369 reflections with $I > 2\sigma(I)$
Absorption correction: none	R_{int} = 0.021
12055 measured reflections	Standard reflections: n/a
3882 independent reflections	

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.037	1 restraint
$wR(F^2)$ = 0.100	H atoms treated by a mixture of independent and constrained refinement
S = 1.04	$\Delta\rho_{max}$ = 0.38 e Å^{-3}
3882 reflections	$\Delta\rho_{min}$ = −0.21 e Å^{-3}
203 parameters	

Table 1 Hydrogen-bonding geometry (Å, °)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O2—H2O···O1^{i}	0.952 (9)	1.931 (11)	2.8366	158.2 (15)

Symmetry codes: (i) −*x*, *y*+1/2, −*z*+1/2

COMPUTING DETIALS

Data collection: *APEX* [5]; cell refinement: *SAINT* **[5]**; data reduction: *SAINT* **[5]**; program(s) used to solve structure: *SHELXS97* **[6]**; program(s) used to refine structure: *SHELXL97* **[6]**; molecular graphics: OLEX2**[7]**; software used to prepare material for publication: *SHELXL97*.**[8]**

SUPPORTING INFORMATION

Crystallographic information file (CIF) is avialable in CD.

ACKNOWLEDGEMENT

The authors would like to thank Dr Hong Su (University of Capetown) for the data collection and structure refinement.

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CHAPTER-7

SUMMARY AND CONCLUSION

Four homoisoflavanones of the 3-benzylidene-4-chromanone type, of which one compound was novel, were synthesized and screened for anti-inflammatory activity. None of these compounds were reported to exhibit anti-inflammatory activity before.

The synthesis of two enantiomers of the 3-benzyl-4-chromanone type (one of which is naturally found in the plant kingdom) along with the racemate is reported. This is the first report of the synthesis of a homoisoflavanone enantiomer with its opposite isomer. The compounds were screened for anti-inflammatory activity. Optimal activity was generally observed after 3 hours but activity was significantly reduced after 6 hours indicating a short duration of action. However, the compound found in nature showed a sustained activity. The other enantiomer and the racemate showed potent activity comparable to that of the commercial drug, diclofenac but showed more pronounced cytotoxicity in comparison to the natural enantiomer (Chapters 2 and 5).

Seven homoisoflavanones of the 3-benzylidene-4-chromanone type of which four were novel were screened for antifungal activity. The B-ring of each compound had a different substitution pattern. Antifungal activity was exhibited by all of these compounds but the compound containing an unsubstituted B-ring showed exceptional activity. This suggested that the size and hydrophobicity of the substituent may play a important role in the activity (Chapter 3). The homoisoflavanones exhibited varying degrees of cytotoxicity (Chapters 2-3 and 5).

The structural elucidation of all the synthesized homoisoflavanones of the 3-benzylidene-4-chromanone and 3-benzyl-4-chromanone types is reported (Chapter-4).

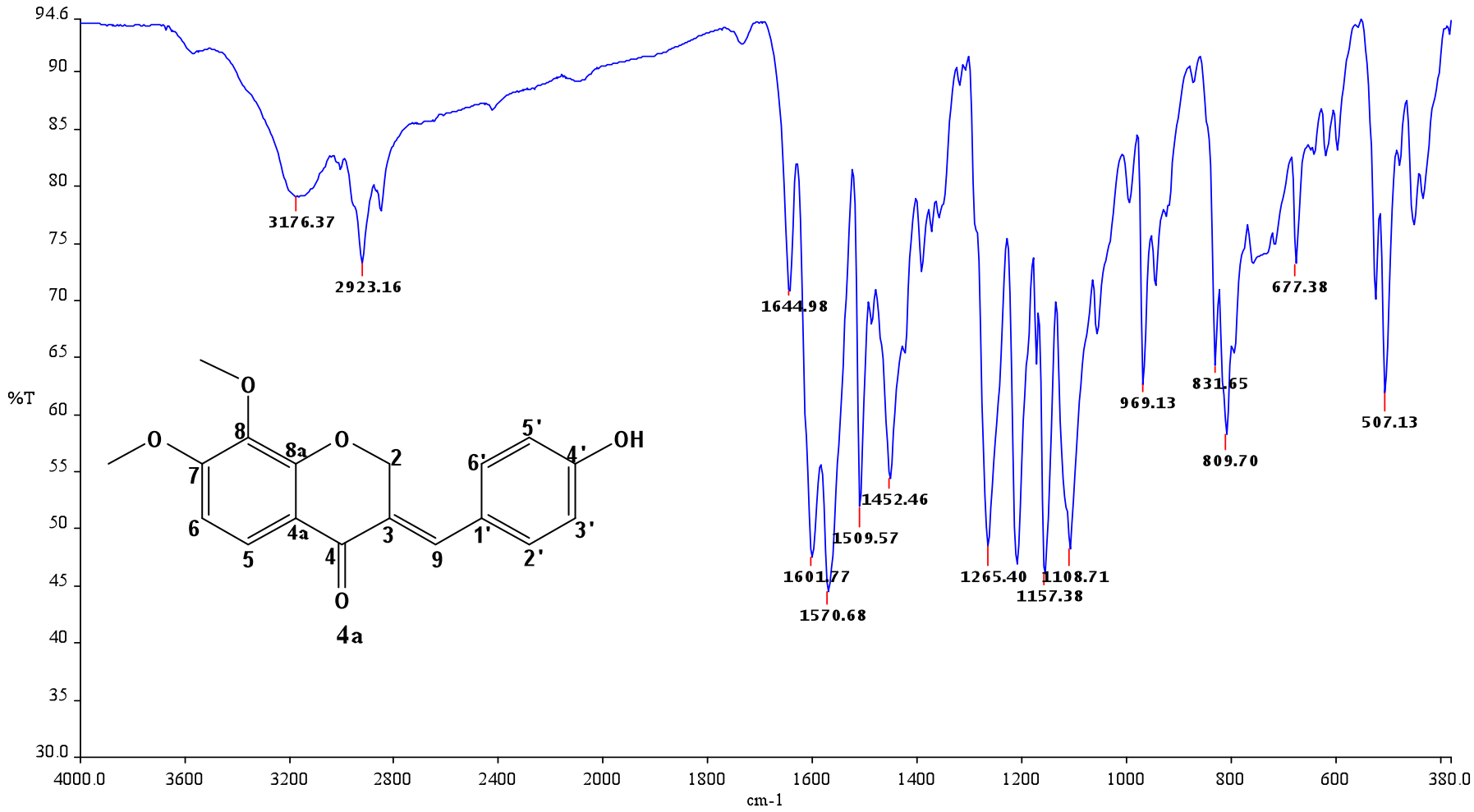
Some of the homoisoflavanones reported in this thesis exhibited significant anti-inflammatory and antifungal activity and can be useful in the development of drugs from natural sources. Some limitations were however experienced.

1) The yields for the first two steps of the homoisoflavanone synthesis were comparatively low and will need to be improved.

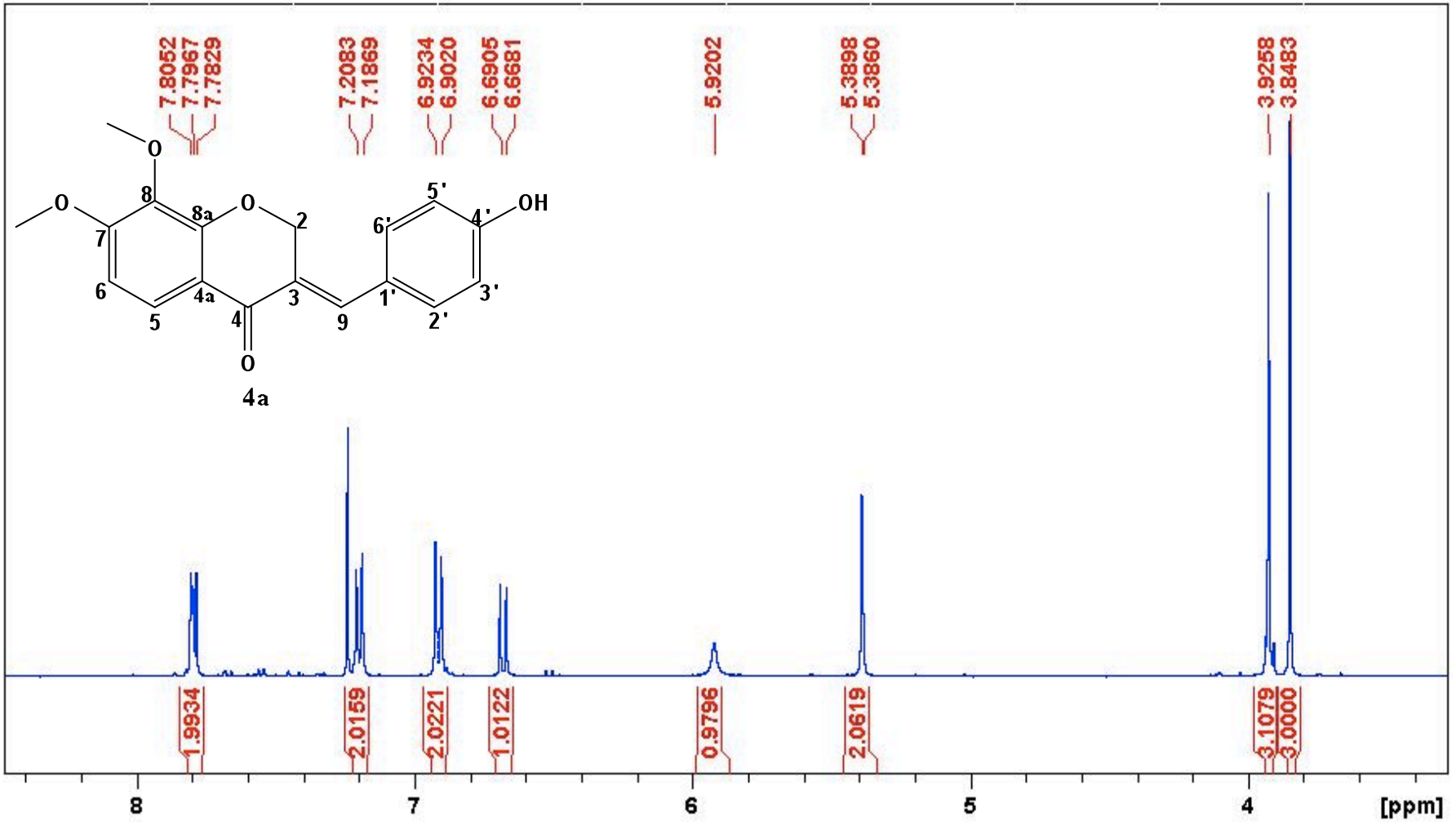
2) More homoisoflavanones analogues also need to be synthesized followed by QSAR study to enable further modification and drug design.
3) Only one fungal strain was used to determine the antifungal activity. A study including a wider variety of strains will have to be conducted.
4) Different types of activities could be investigated for example anticancer activity.
5) The anti-inflammatory mode of action still needs to be determined.

APPENDICES

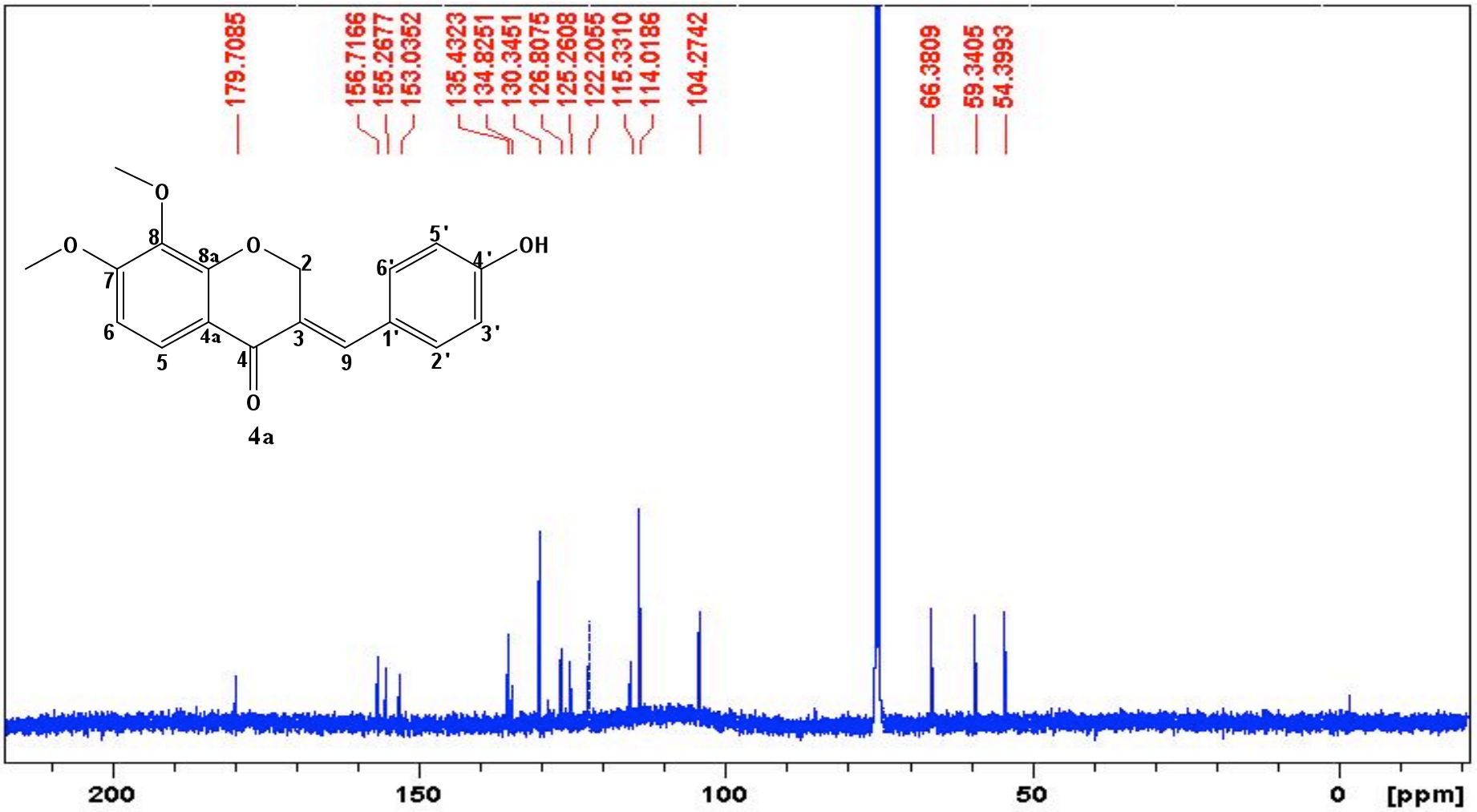
Table of content



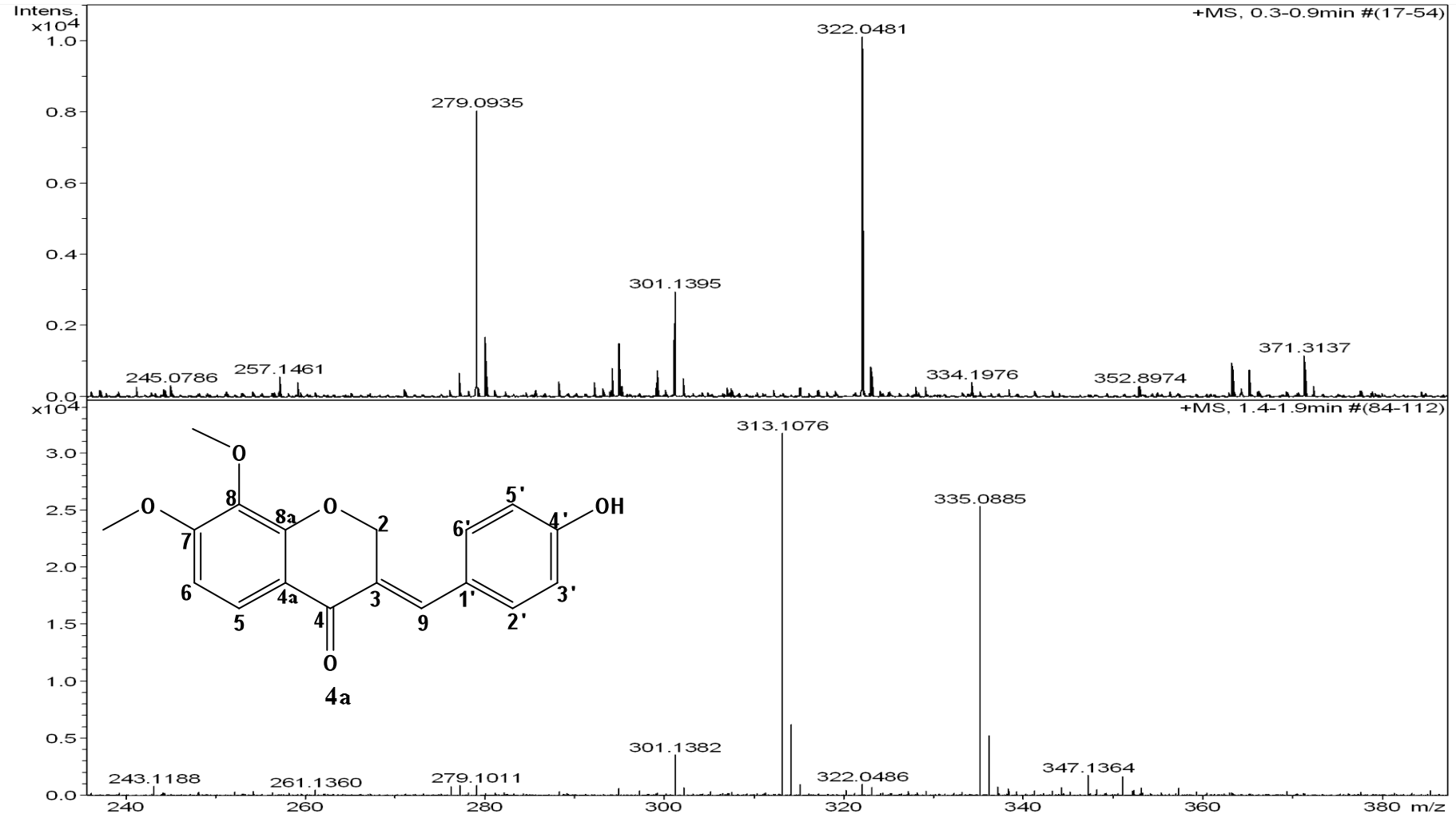
IR spectrum of compound **4a**



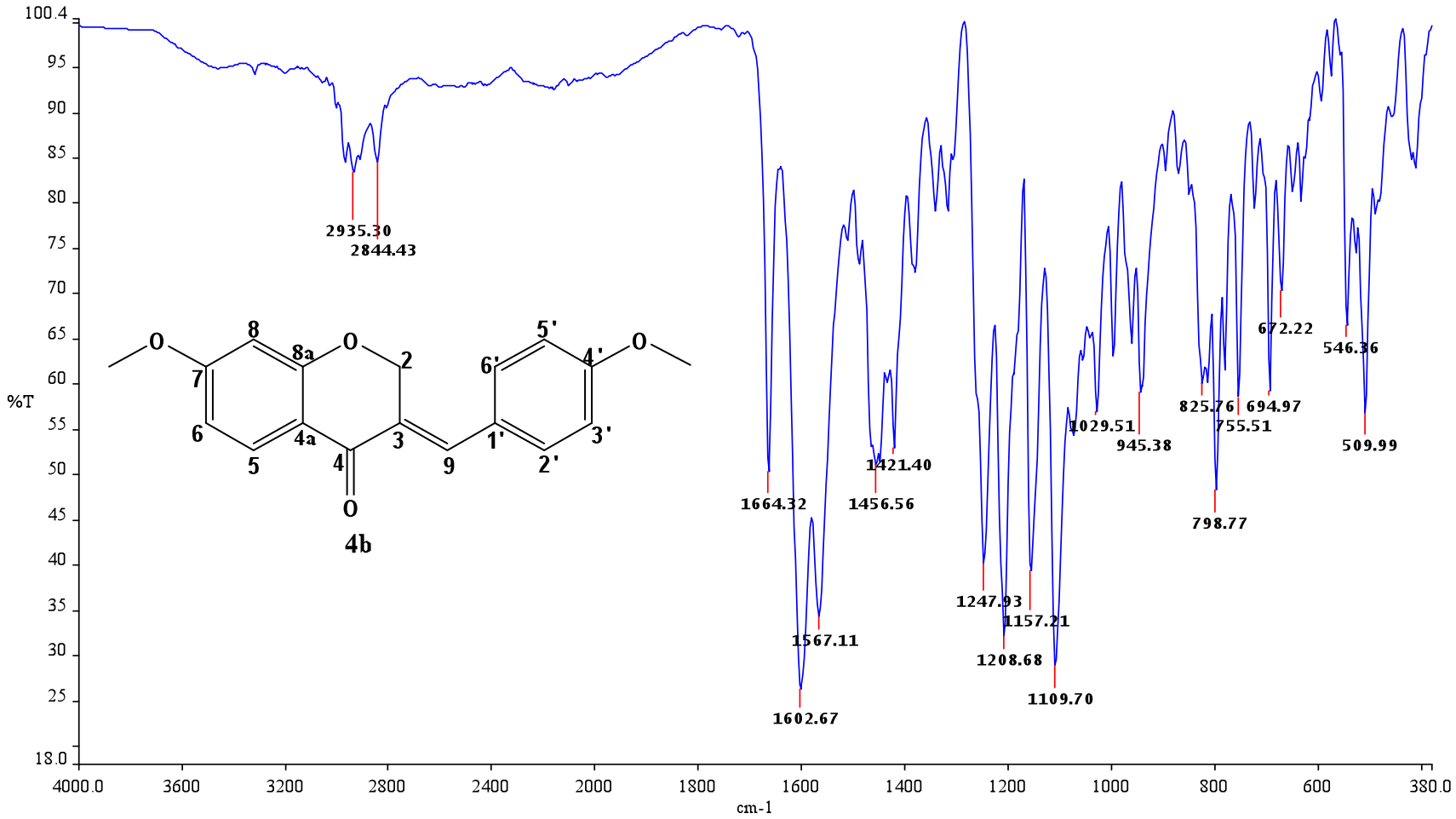
^{1}H-NMR spectrum of compound **4a** in $CDCl_3$ (400 MHz)



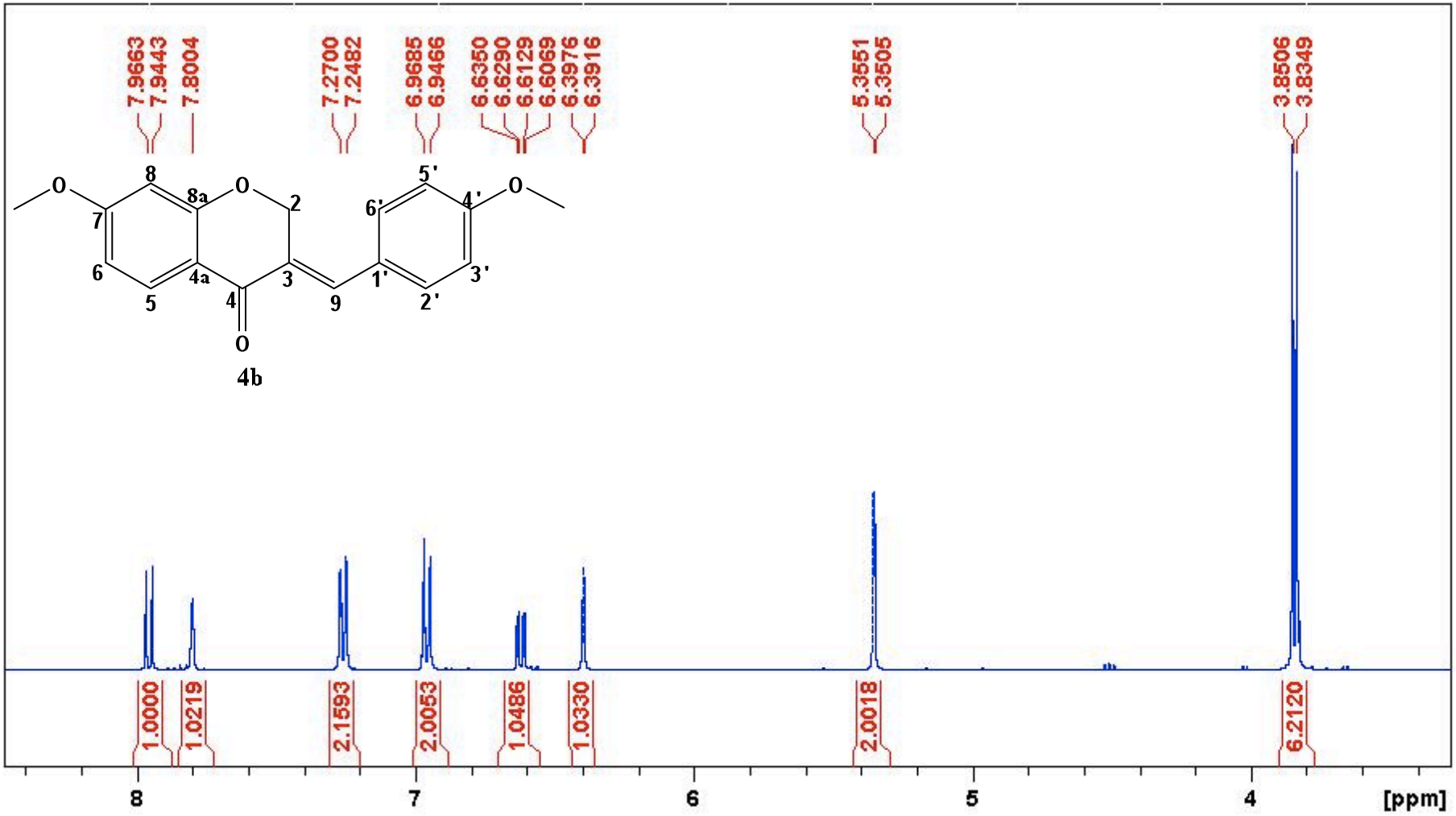
^{13}C-NMR spectrum of compound **4a** in $CDCl_3$ (400 MHz)



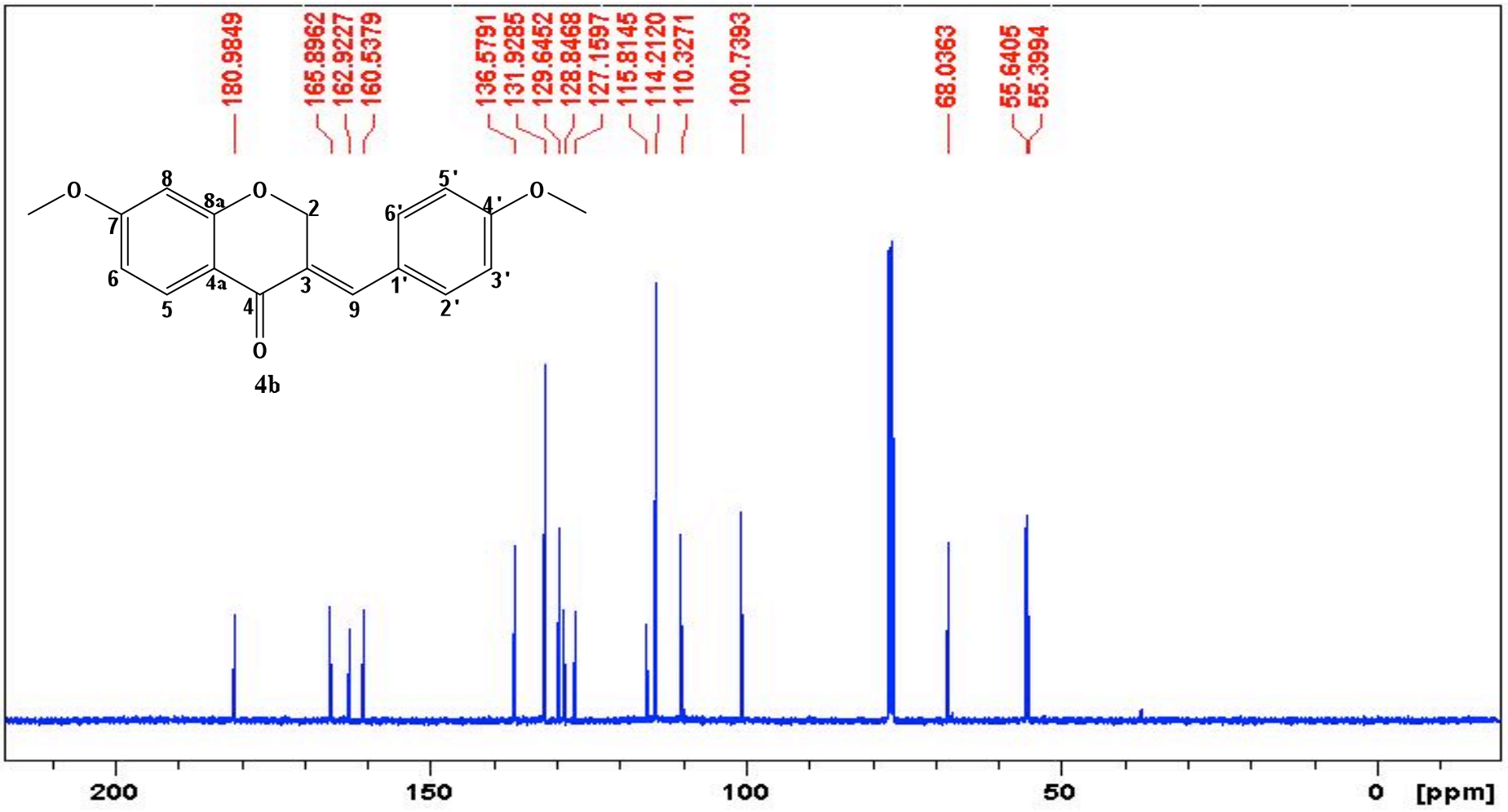
HRMS spectrum of compound **4a**



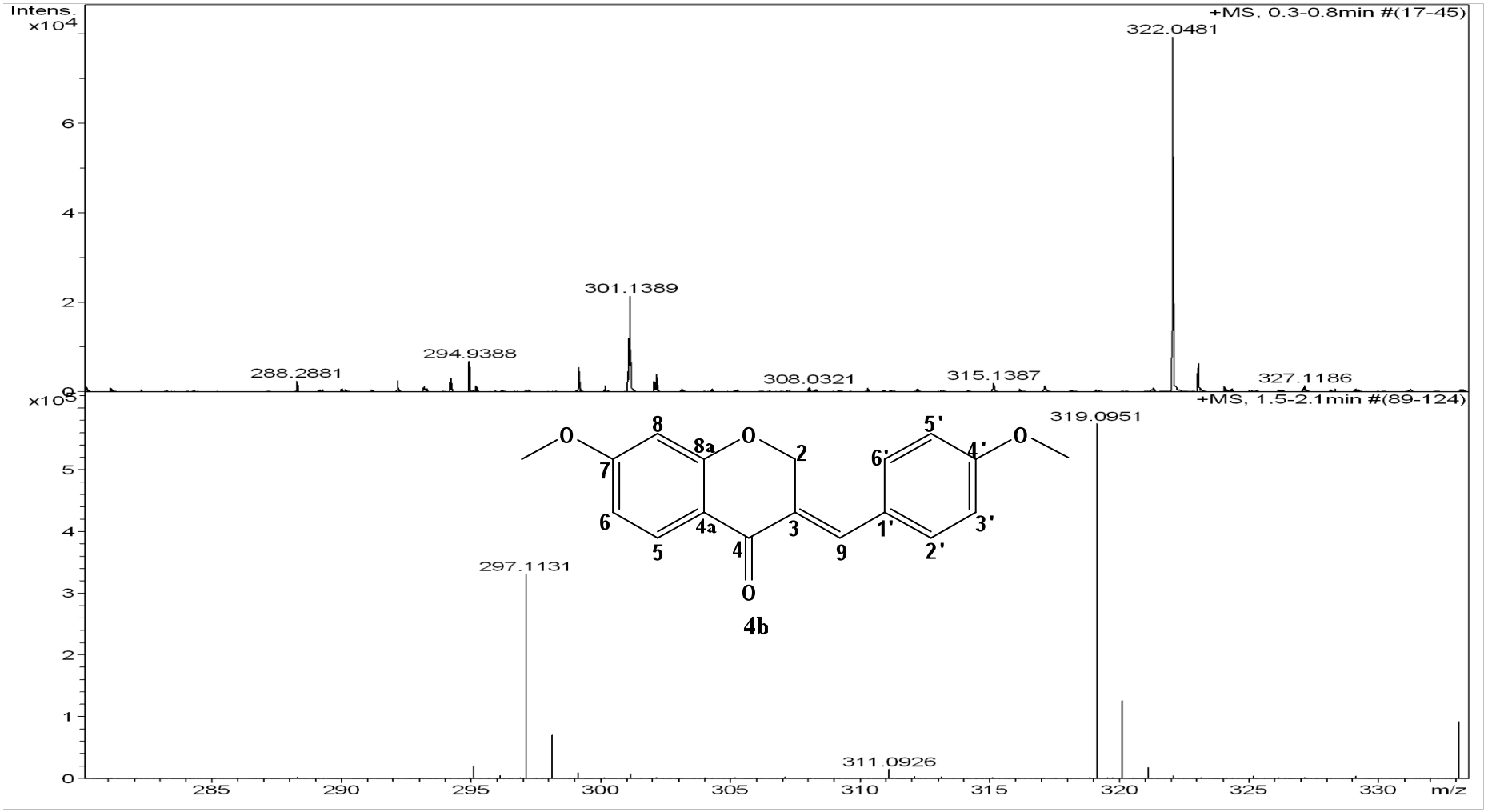
IR spectrum of compound **4b**



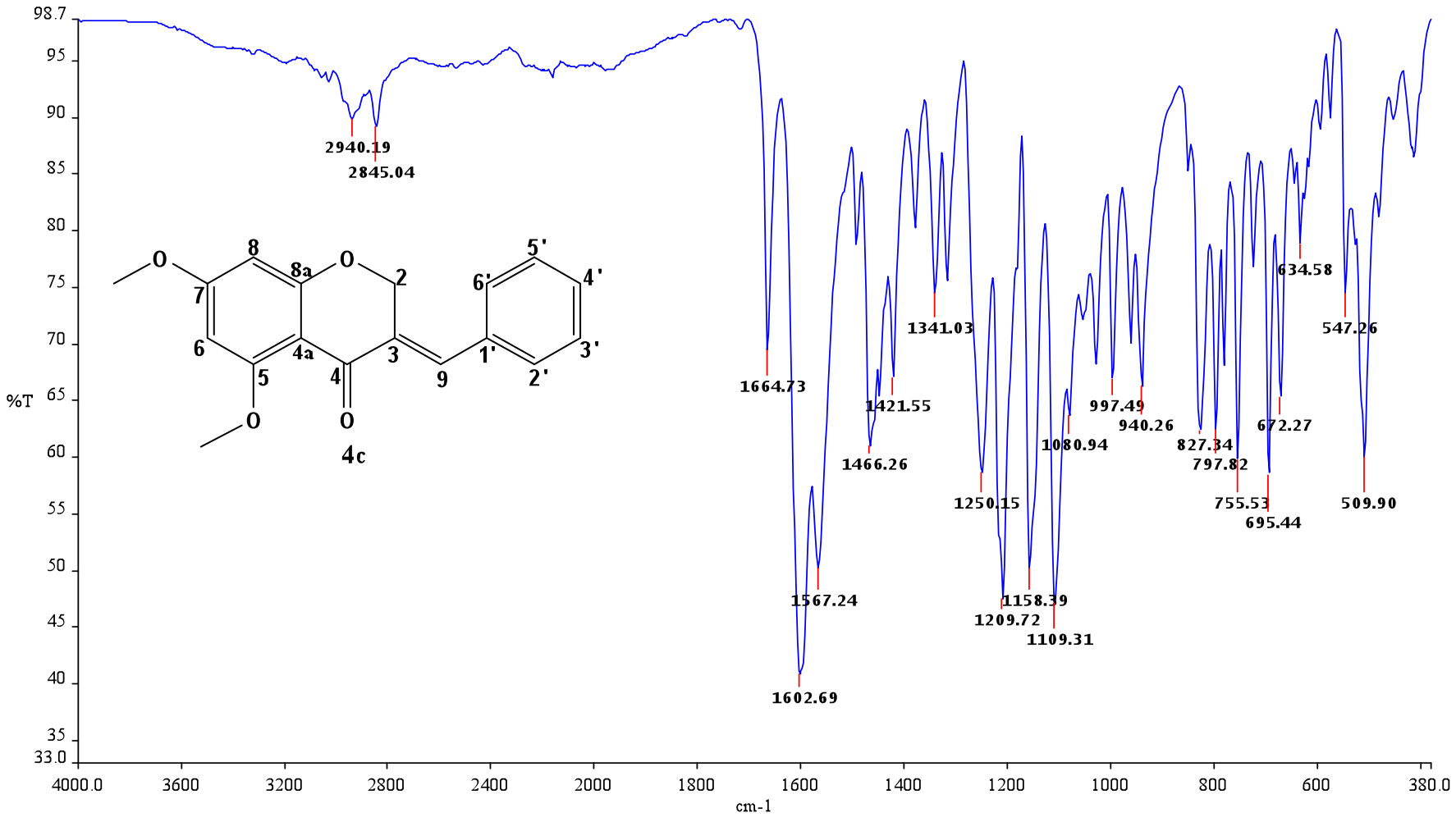
^{1}H-NMR spectrum of compound **4b** in $CDCl_3$ (400 MHz)



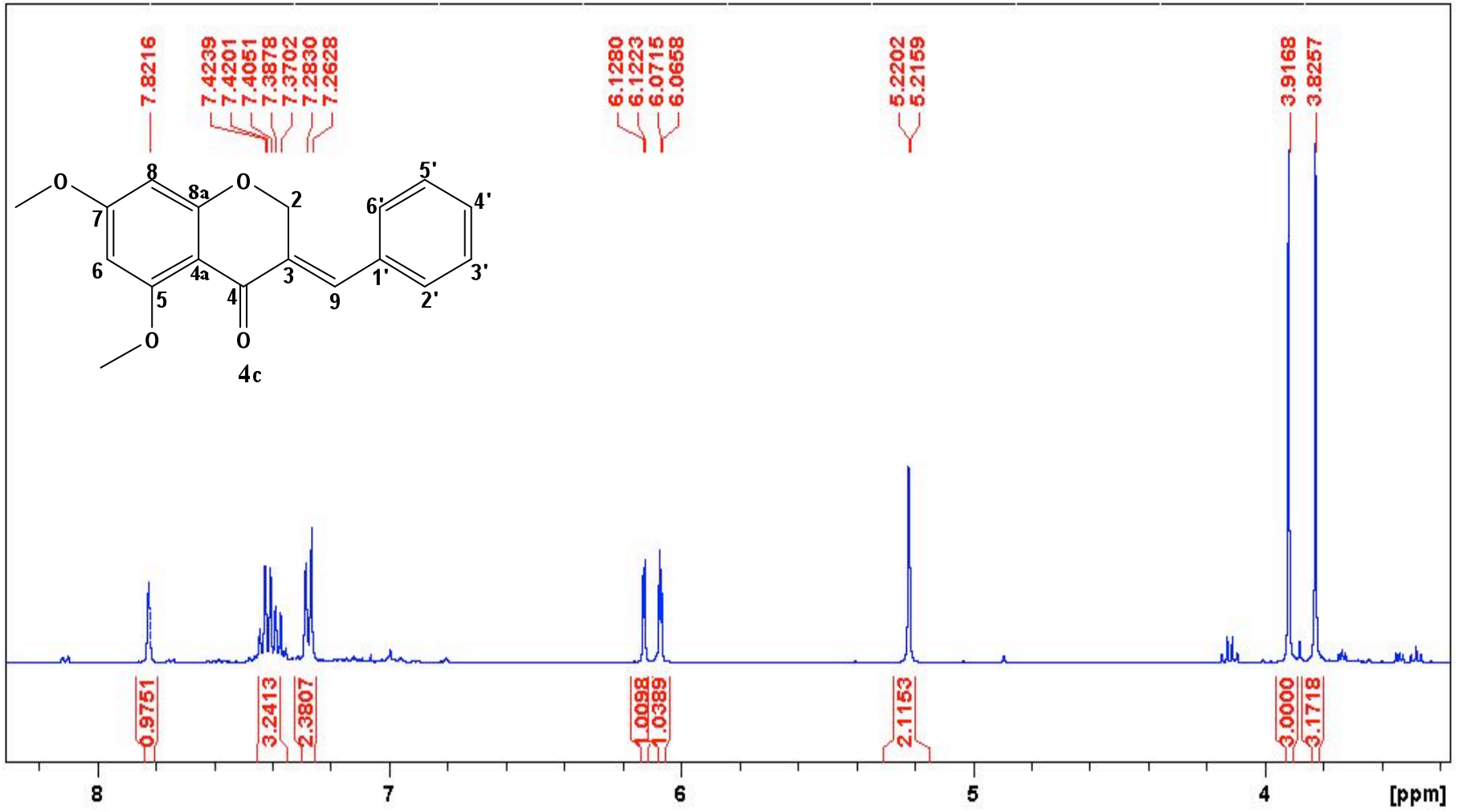
^{13}C-NMR spectrum of compound **4b** in $CDCl_3$ (400 MHz)



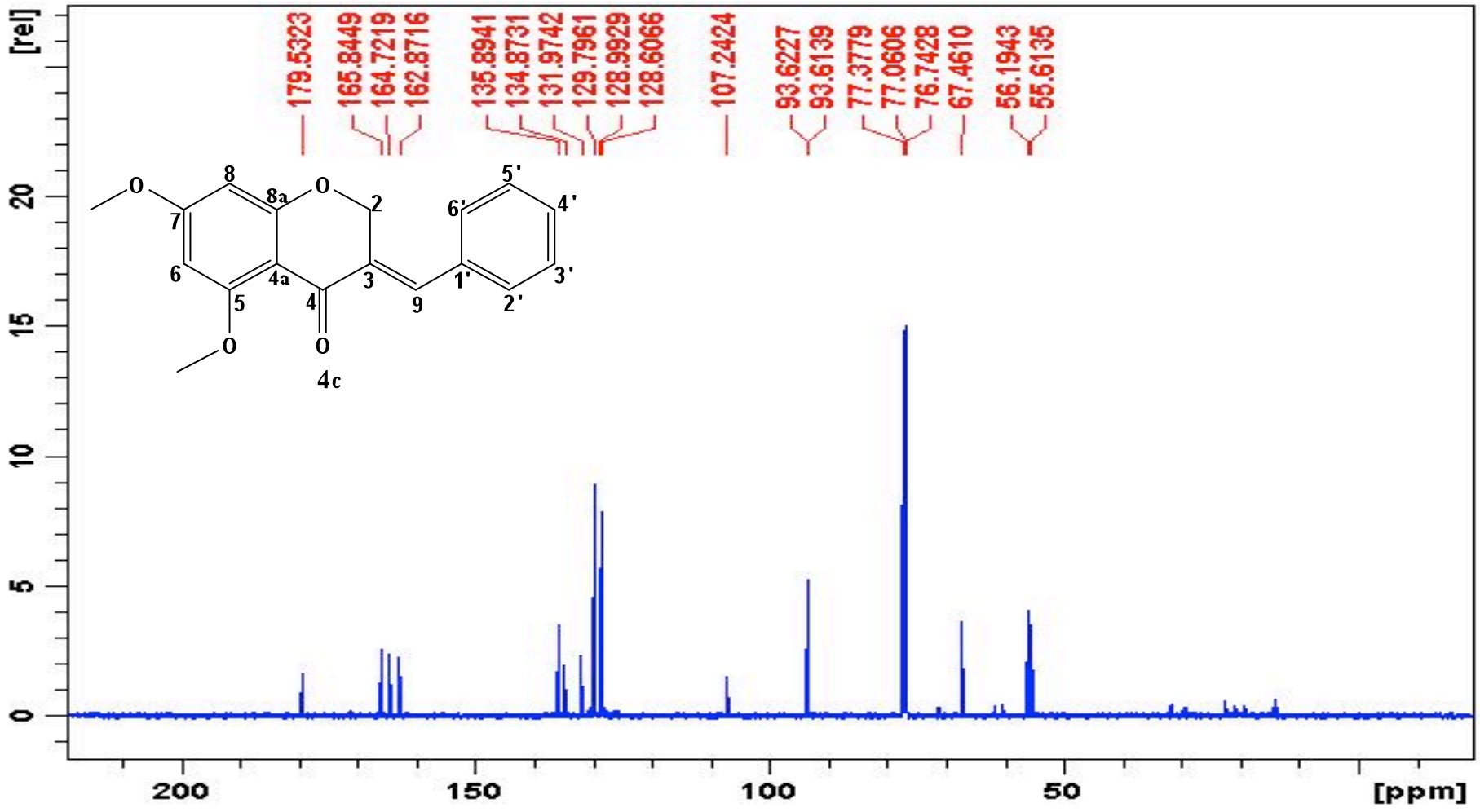
HRMS spectrum of compound **4b**



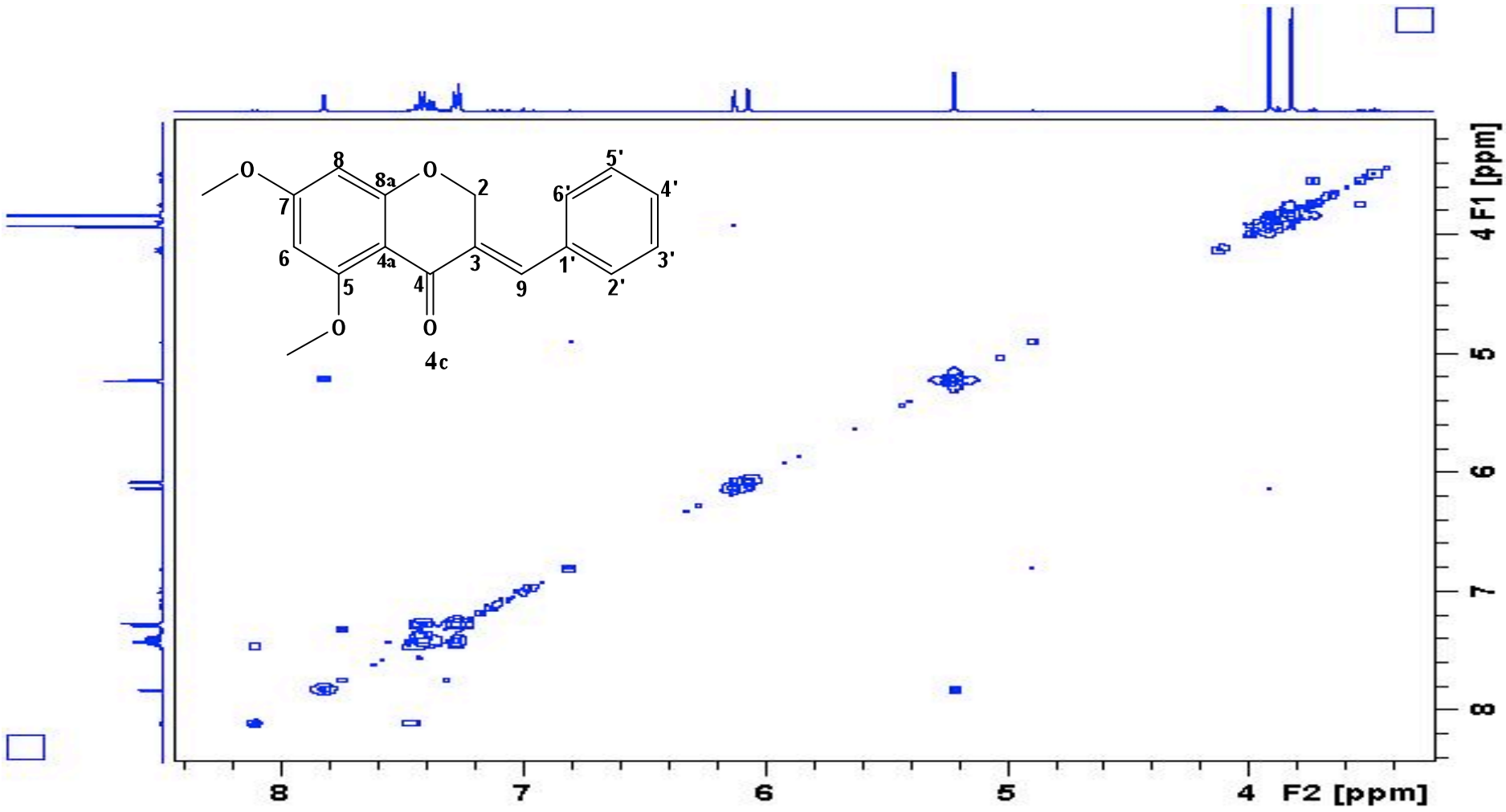
IR spectrum of compound **4c**



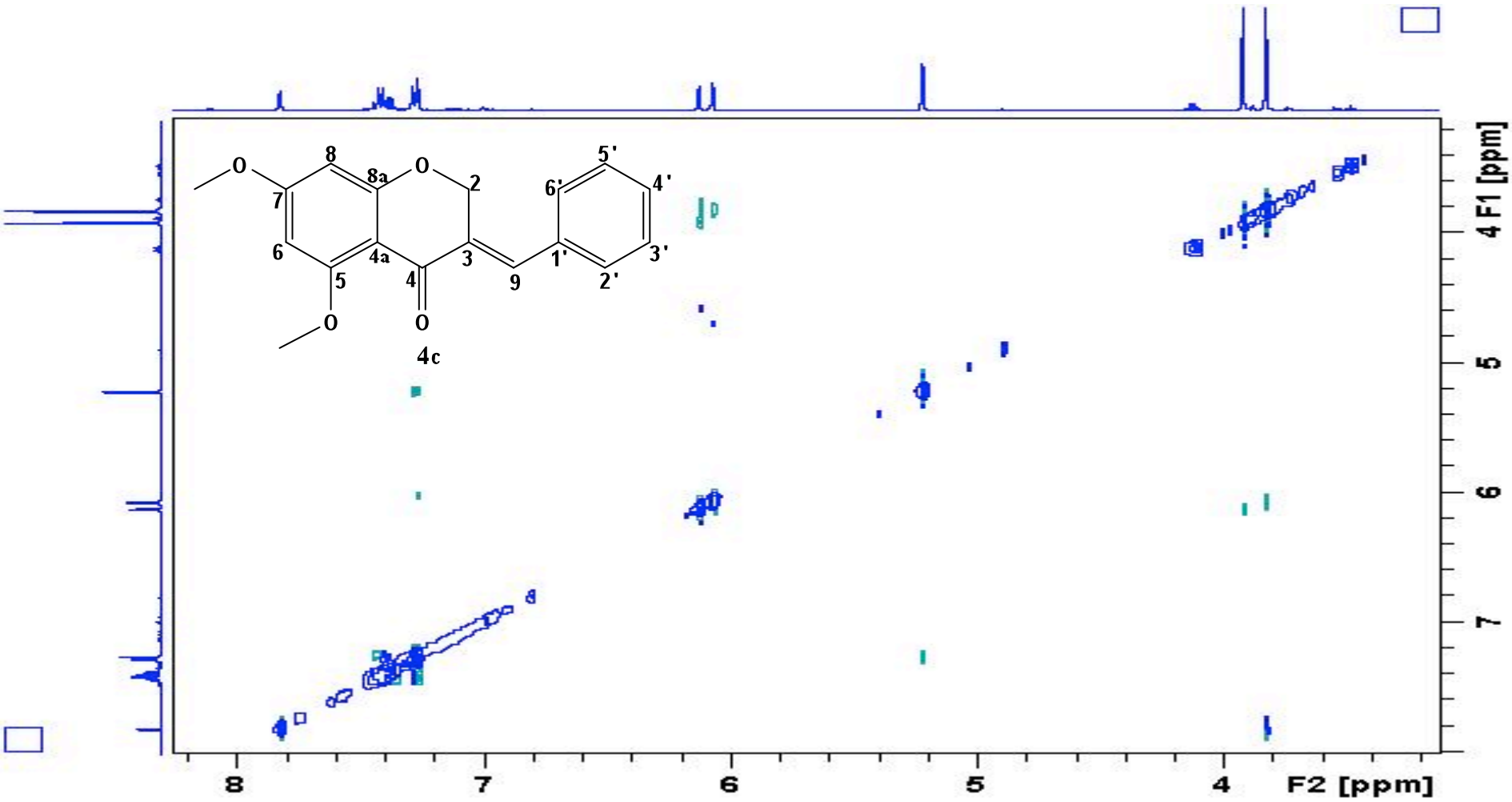
^{1}H-NMR spectrum of compound **4c** in $CDCl_3$ (400 MHz)



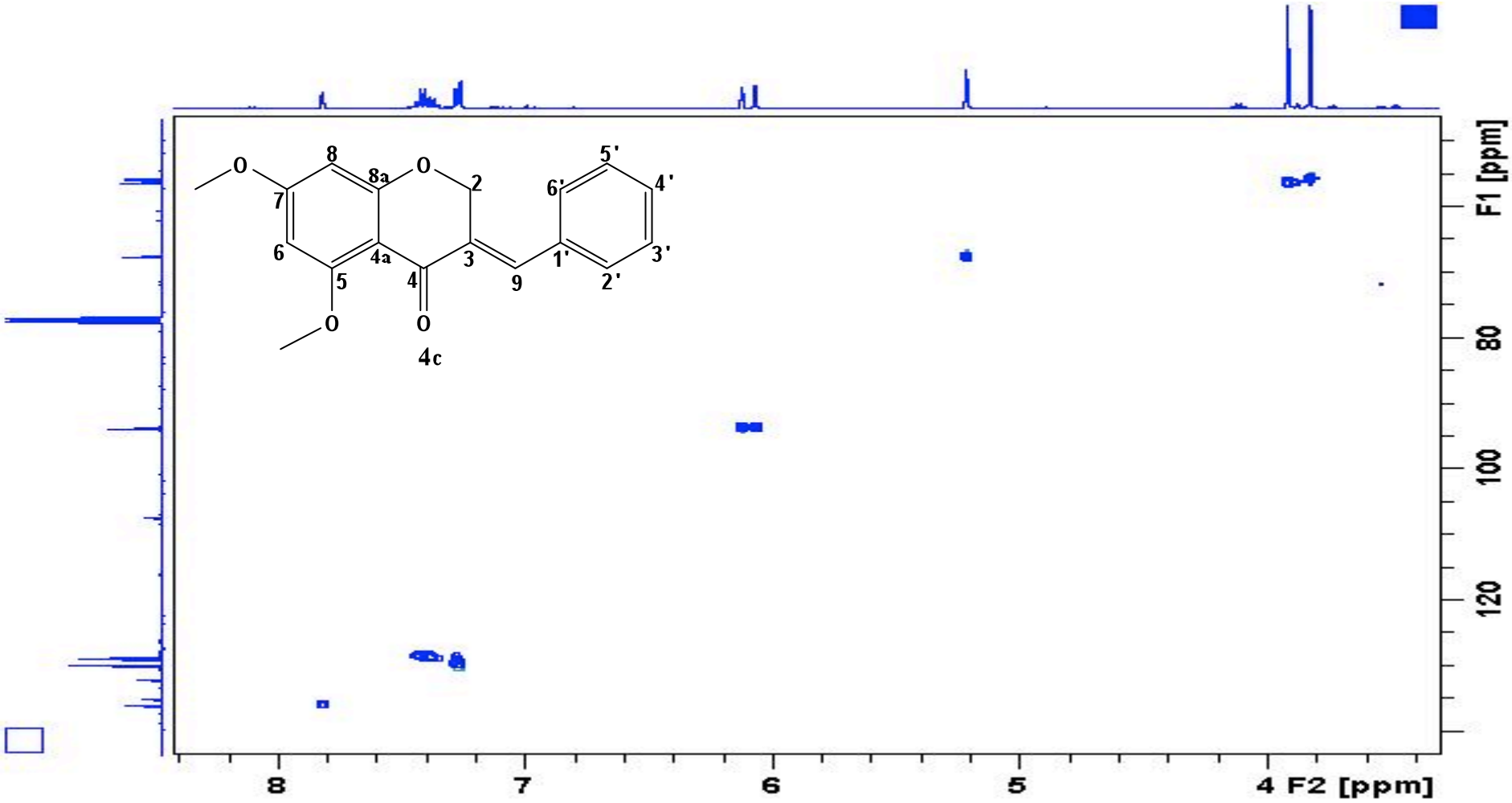
^{13}C-NMR spectrum of compound **4c** in $CDCl_3$ (400 MHz)



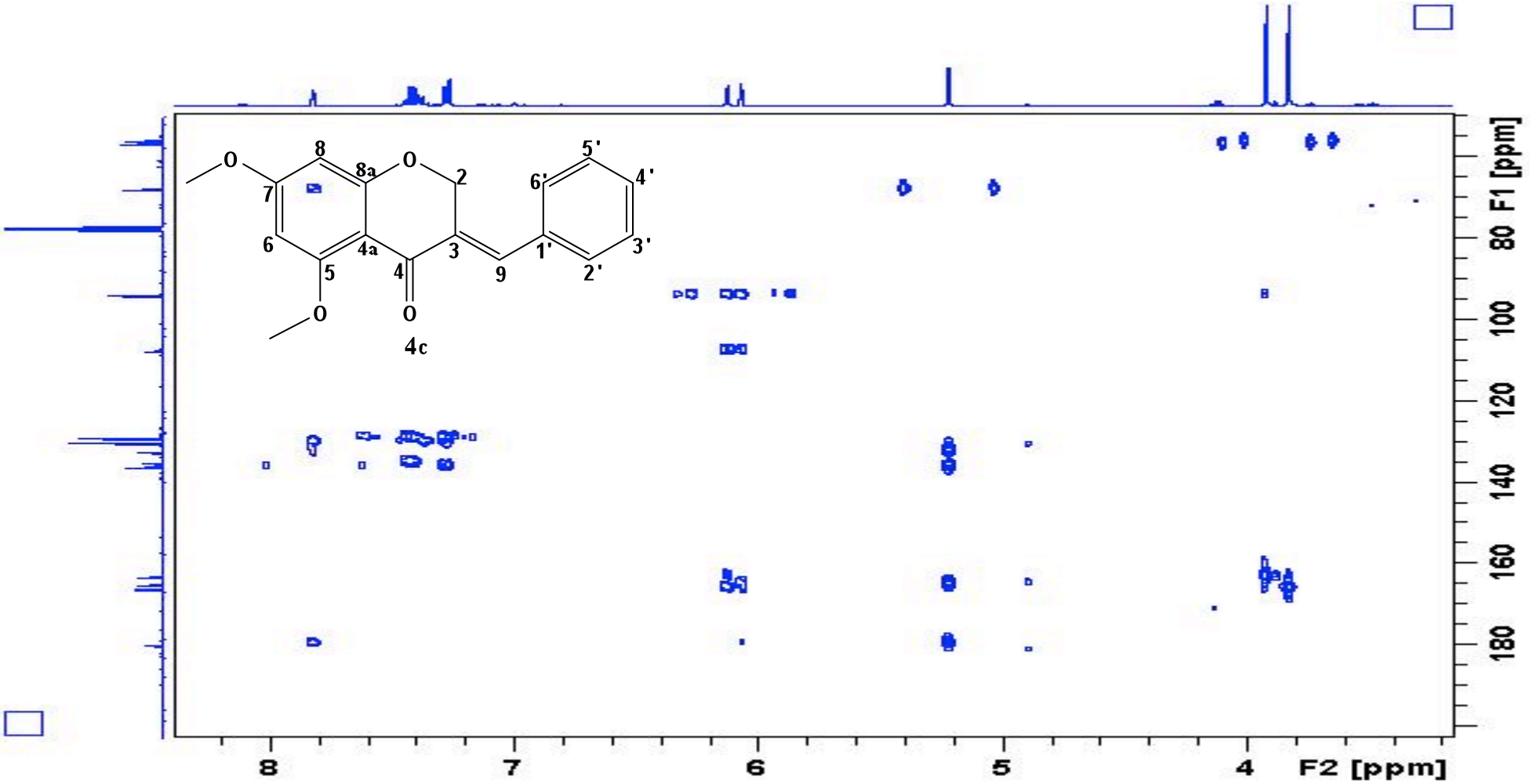
COSY spectrum of compound **4c** in $CDCl_3$ (400 MHz)



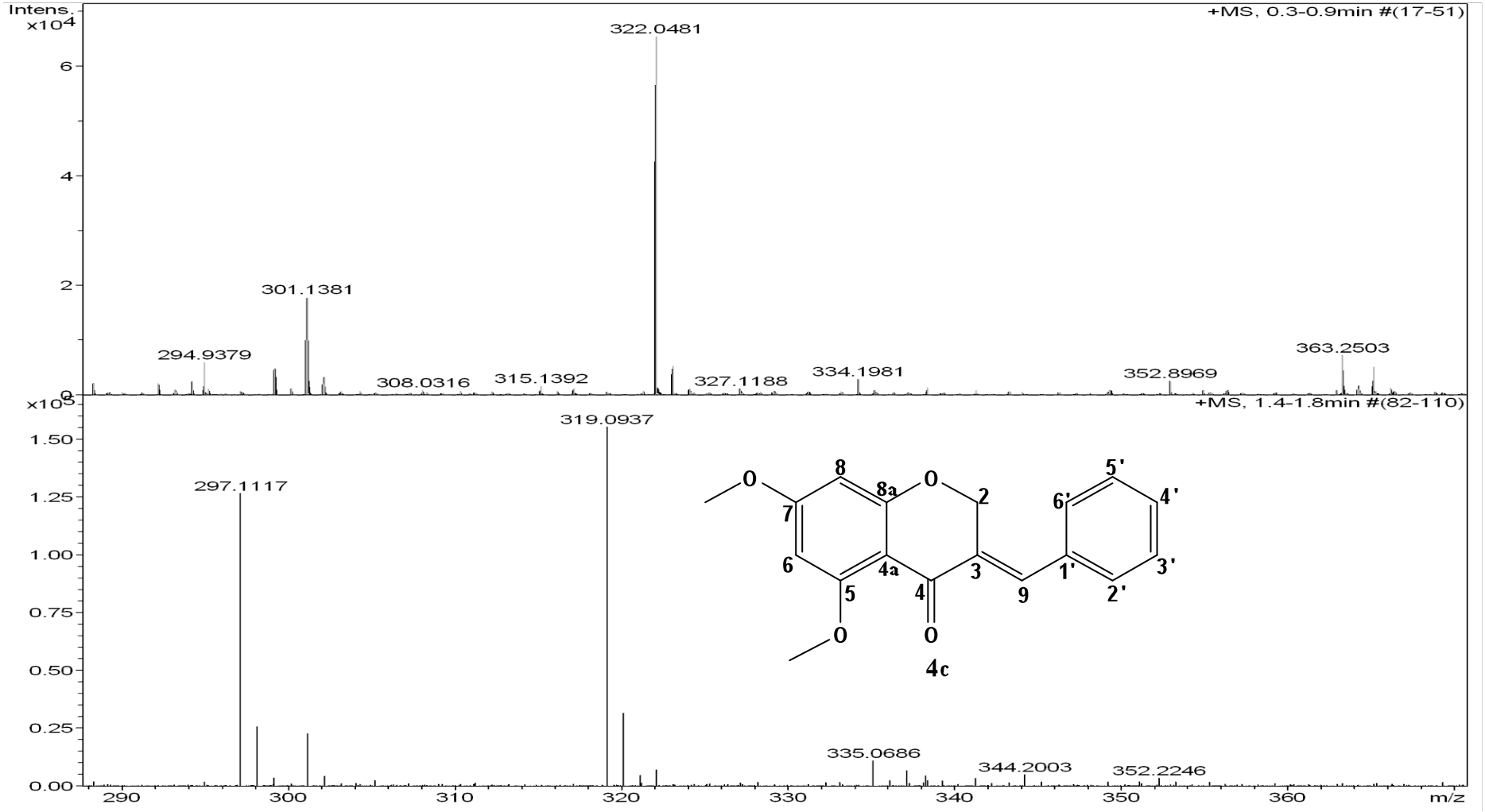
NOESY spectrum of compound **4c** in $CDCl_3$ (400 MHz)



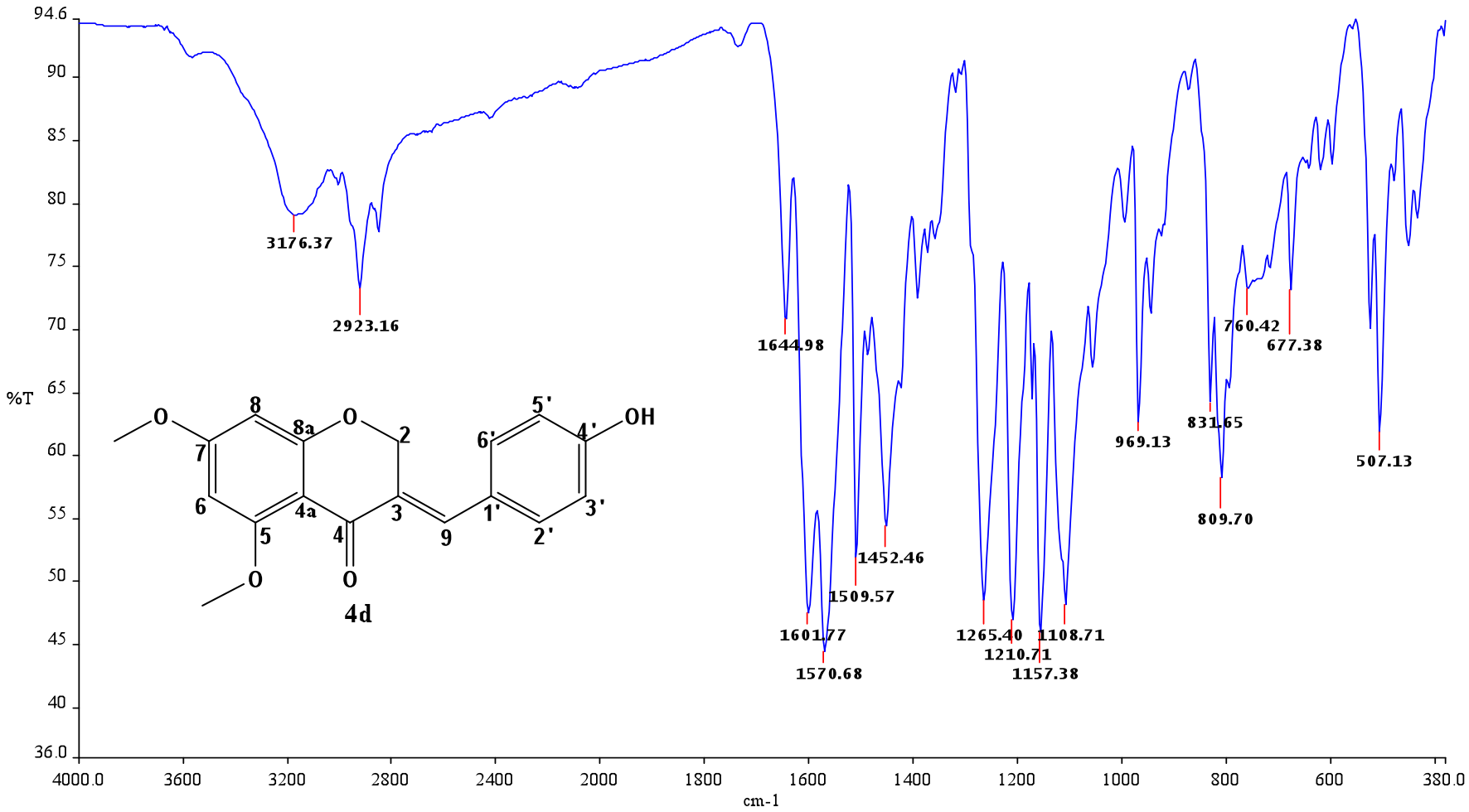
HSQC spectrum of compound **4c** in $CDCl_3$ (400 MHz)



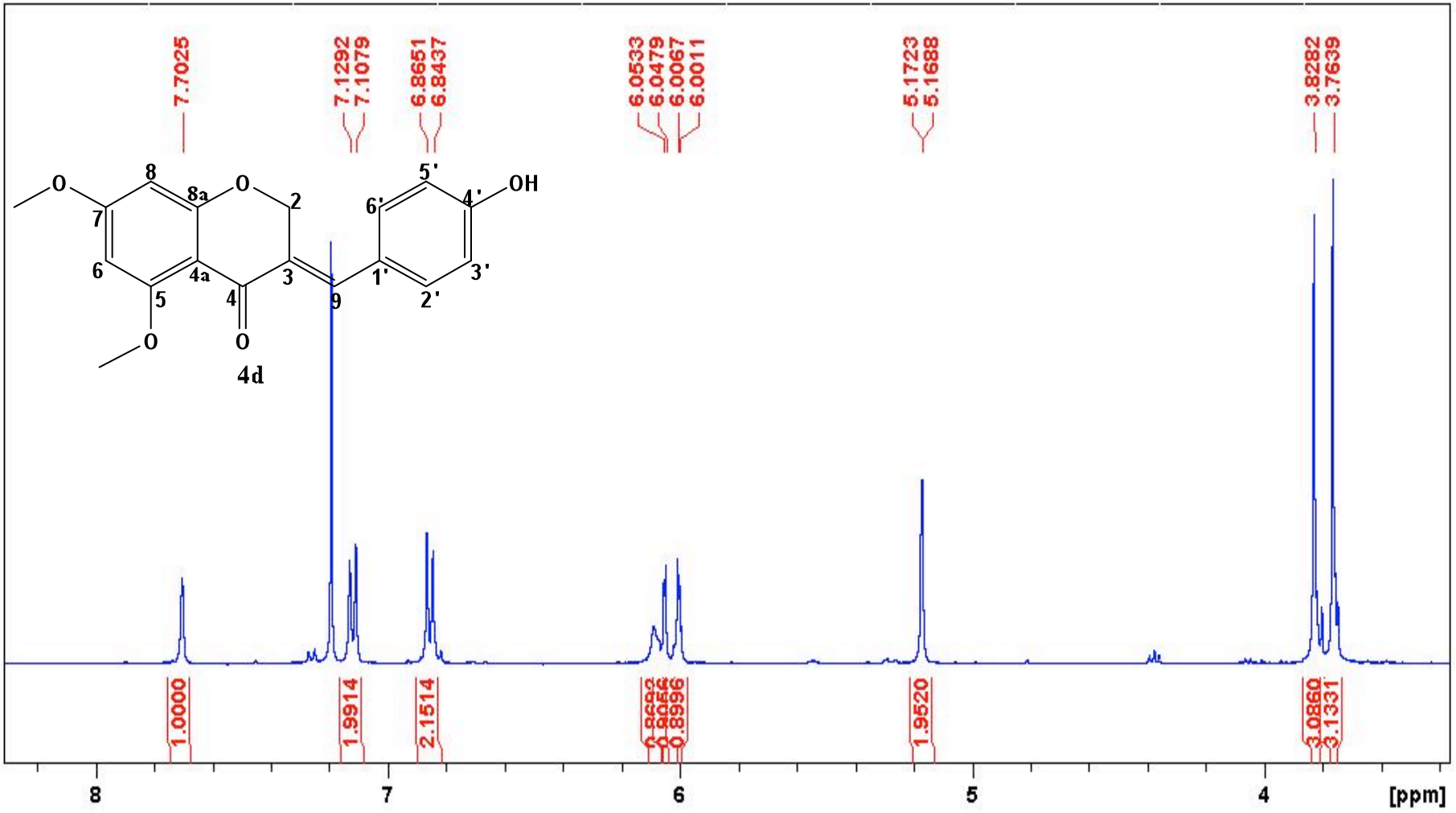
HMBC spectrum of compound **4c** in $CDCl_3$ (400 MHz)



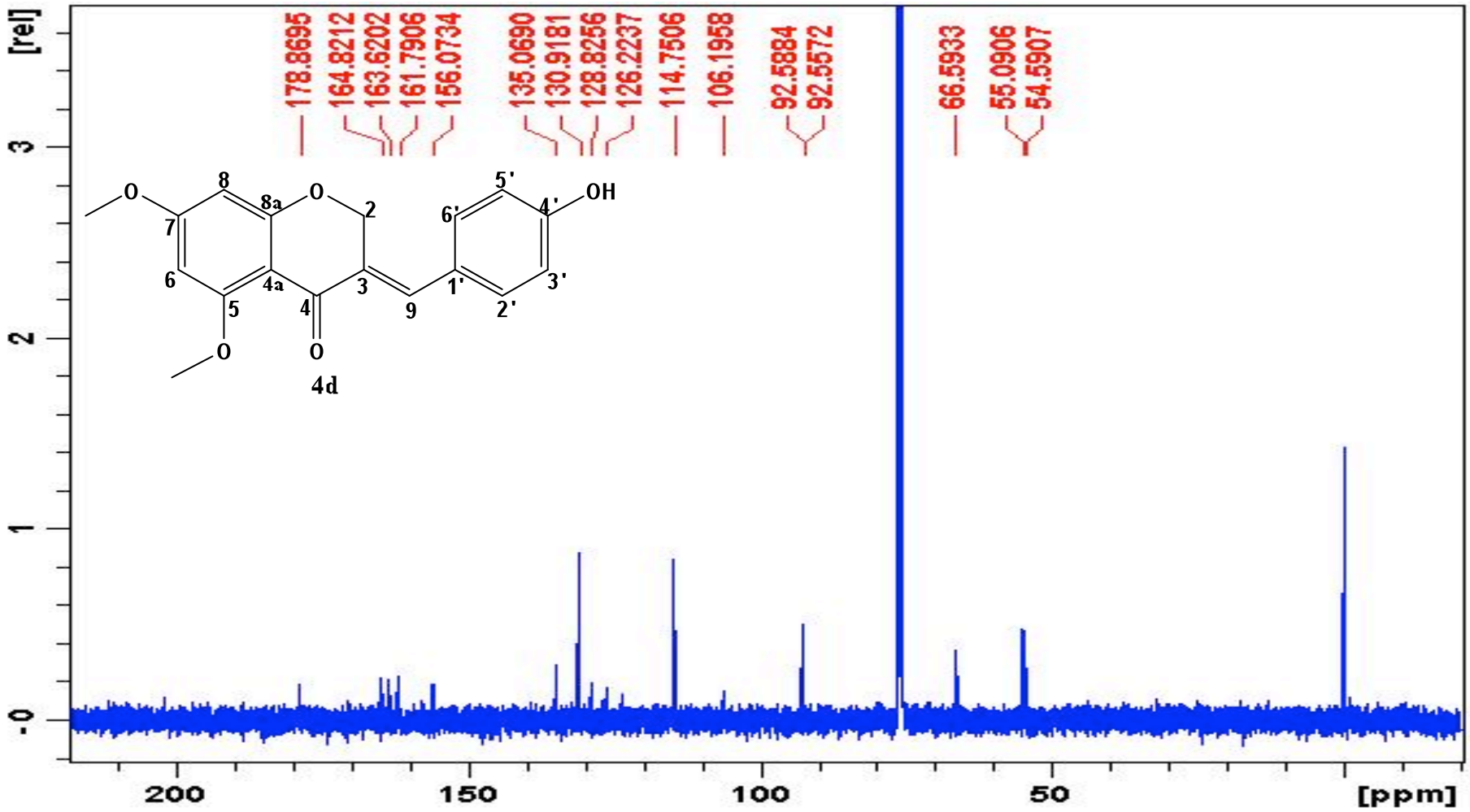
HRMS spectrum of compound **4c**



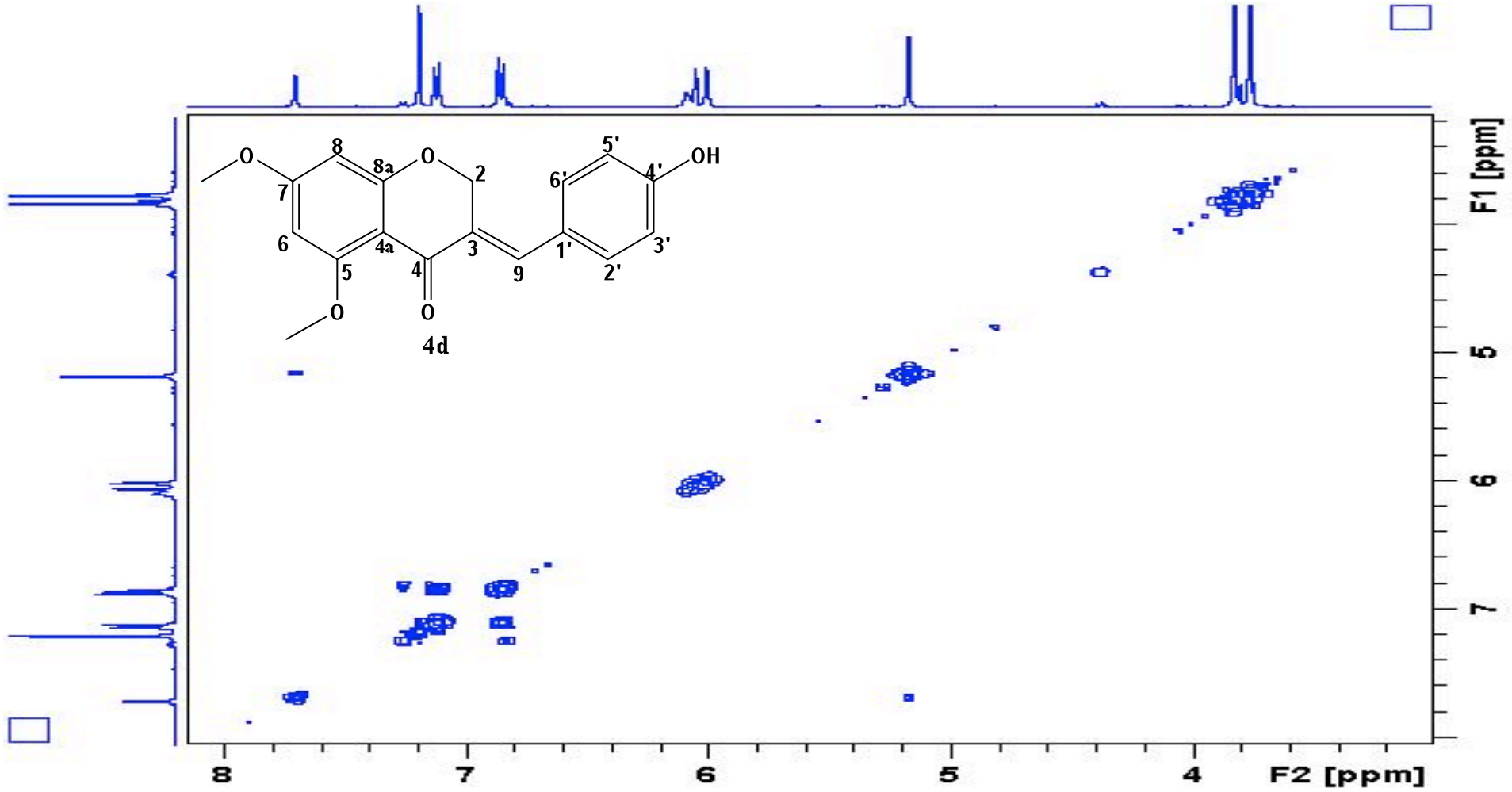
IR spectrum of compound **4d**



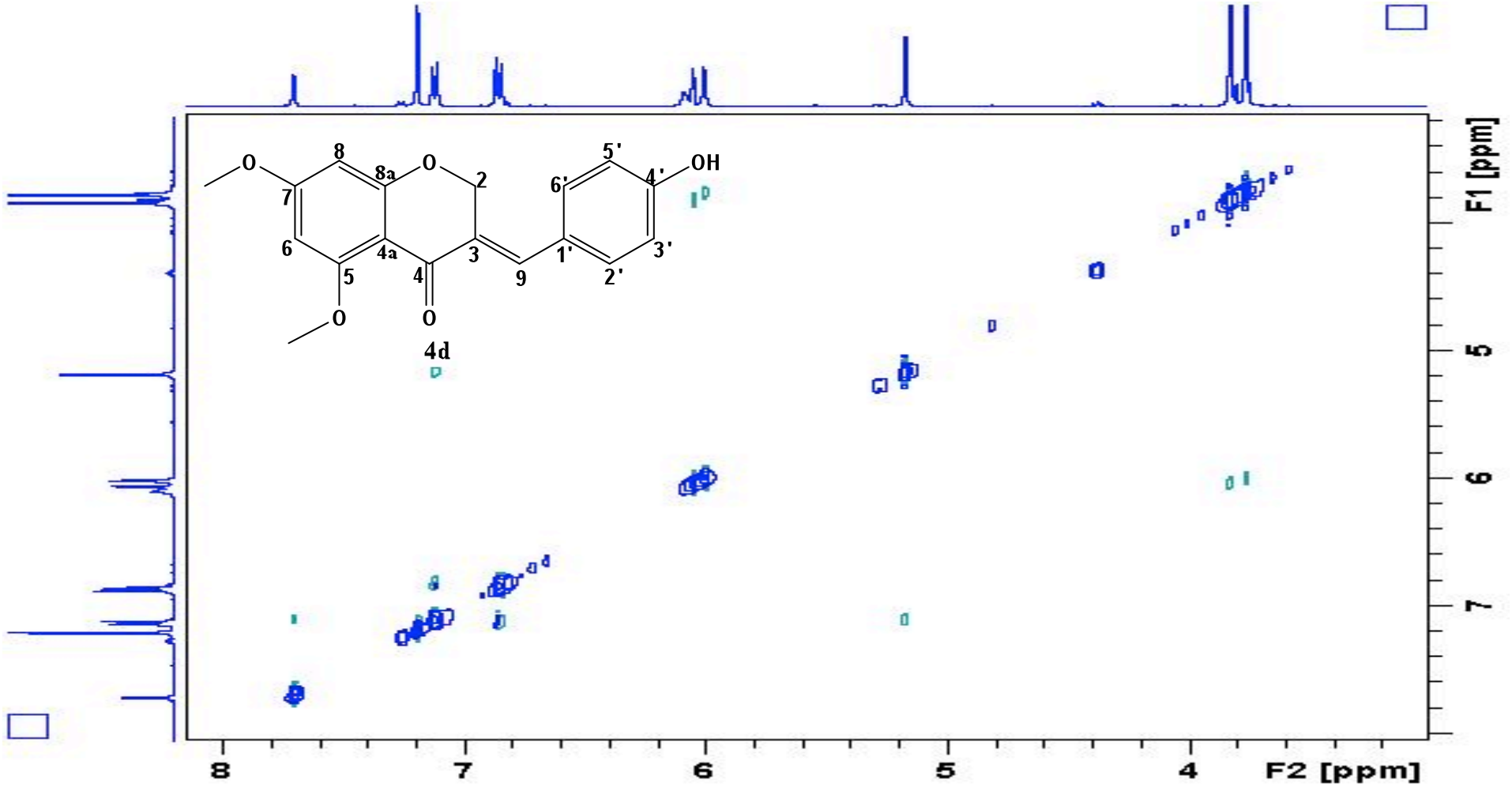
^{1}H-NMR spectrum of compound **4d** in $CDCl_3$ (400 MHz)



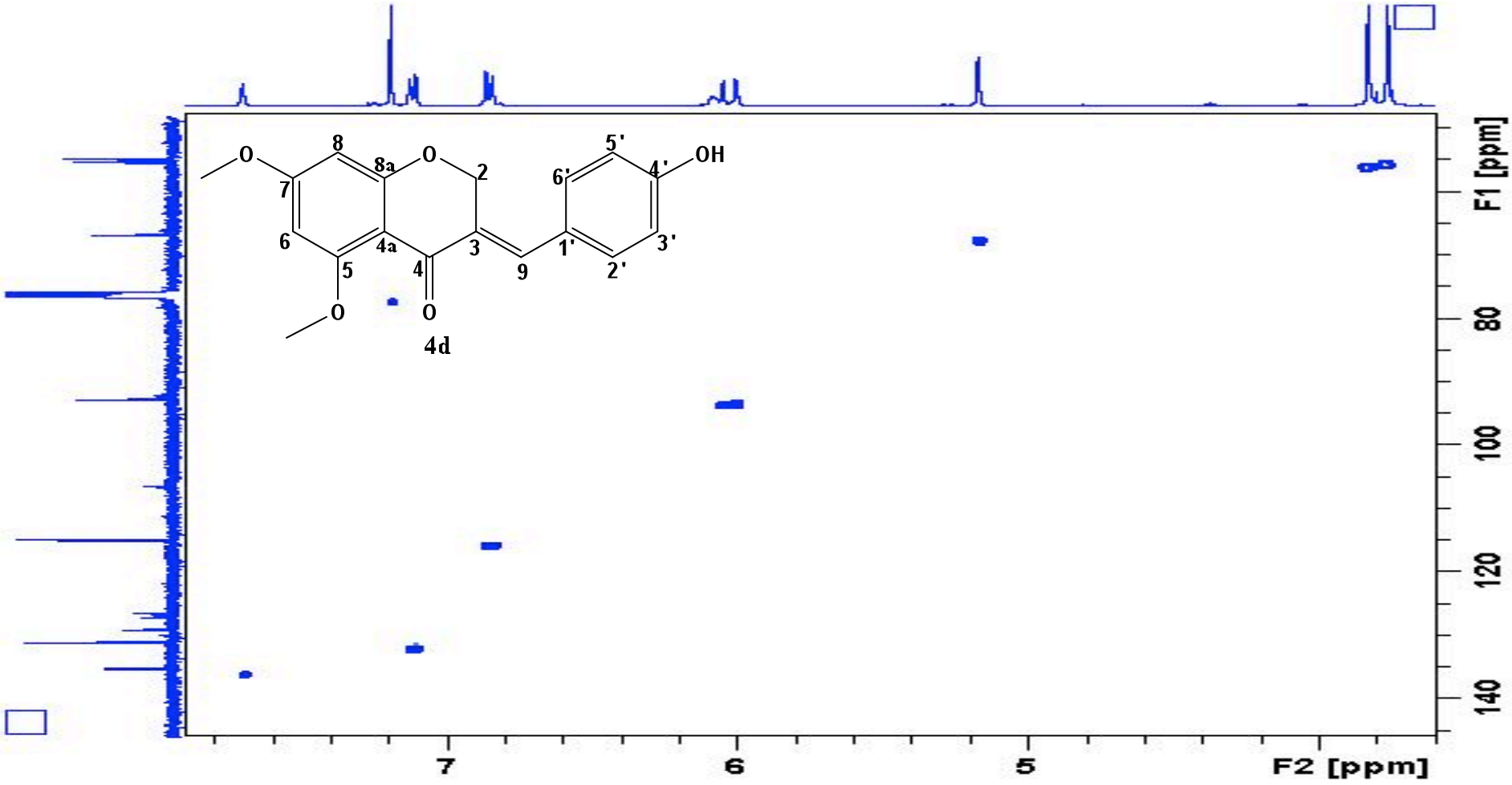
^{13}C-NMR spectrum of compound **4d** in $CDCl_3$ (400 MHz)



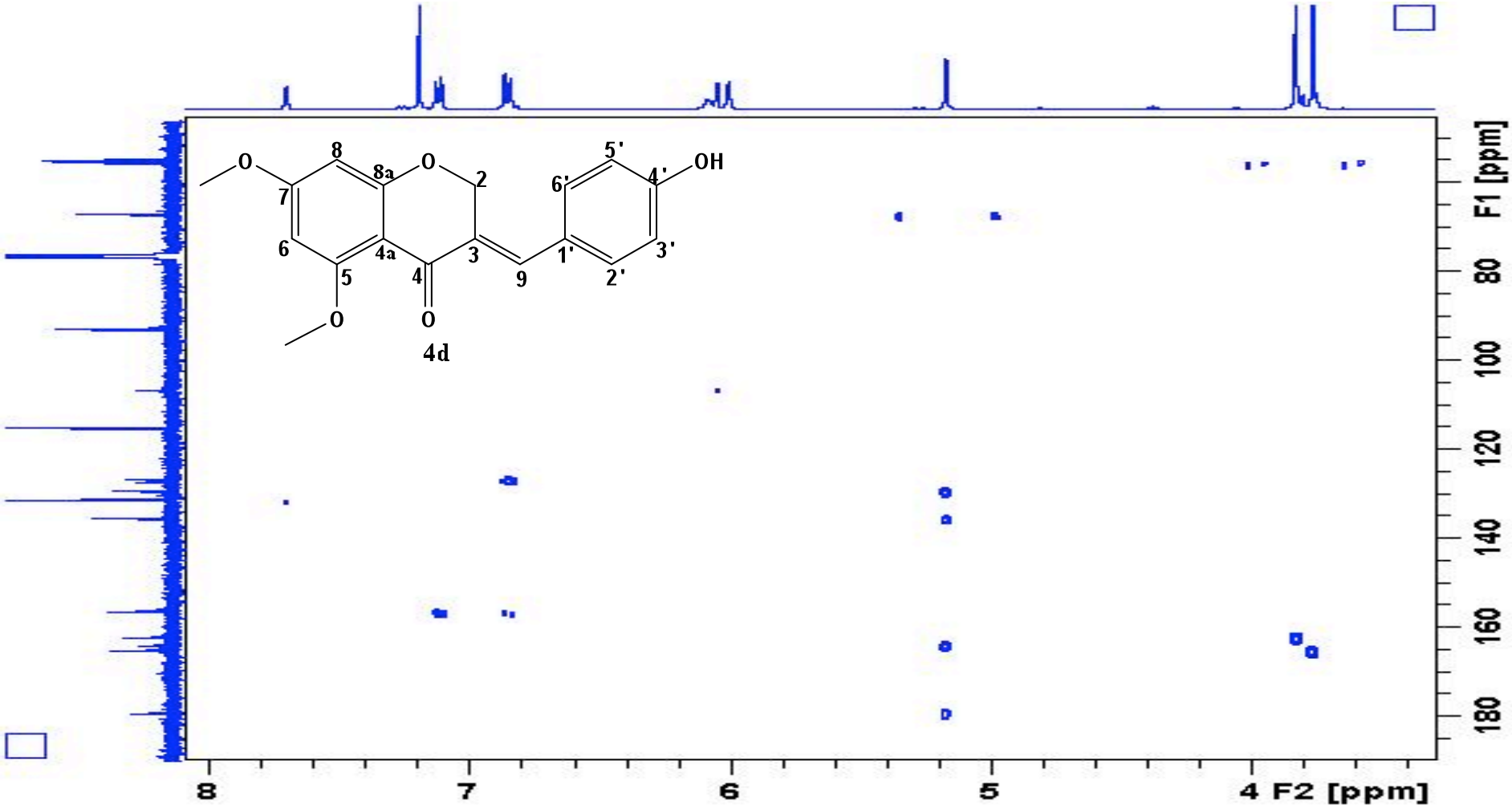
COSY spectrum of compound **4d** in $CDCl_3$ (400 MHz)



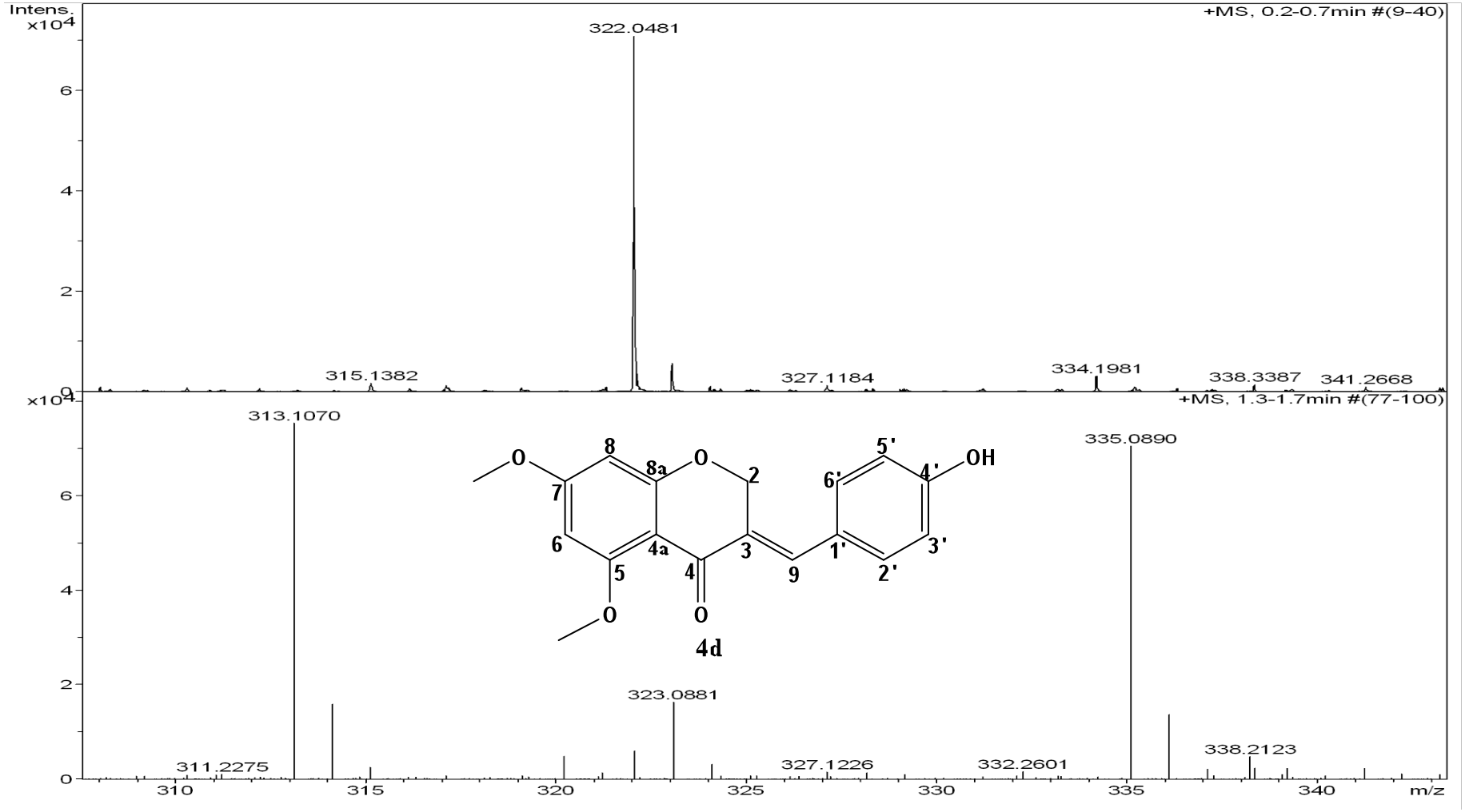
NOESY spectrum of compound **4d** in $CDCl_3$ (400 MHz)



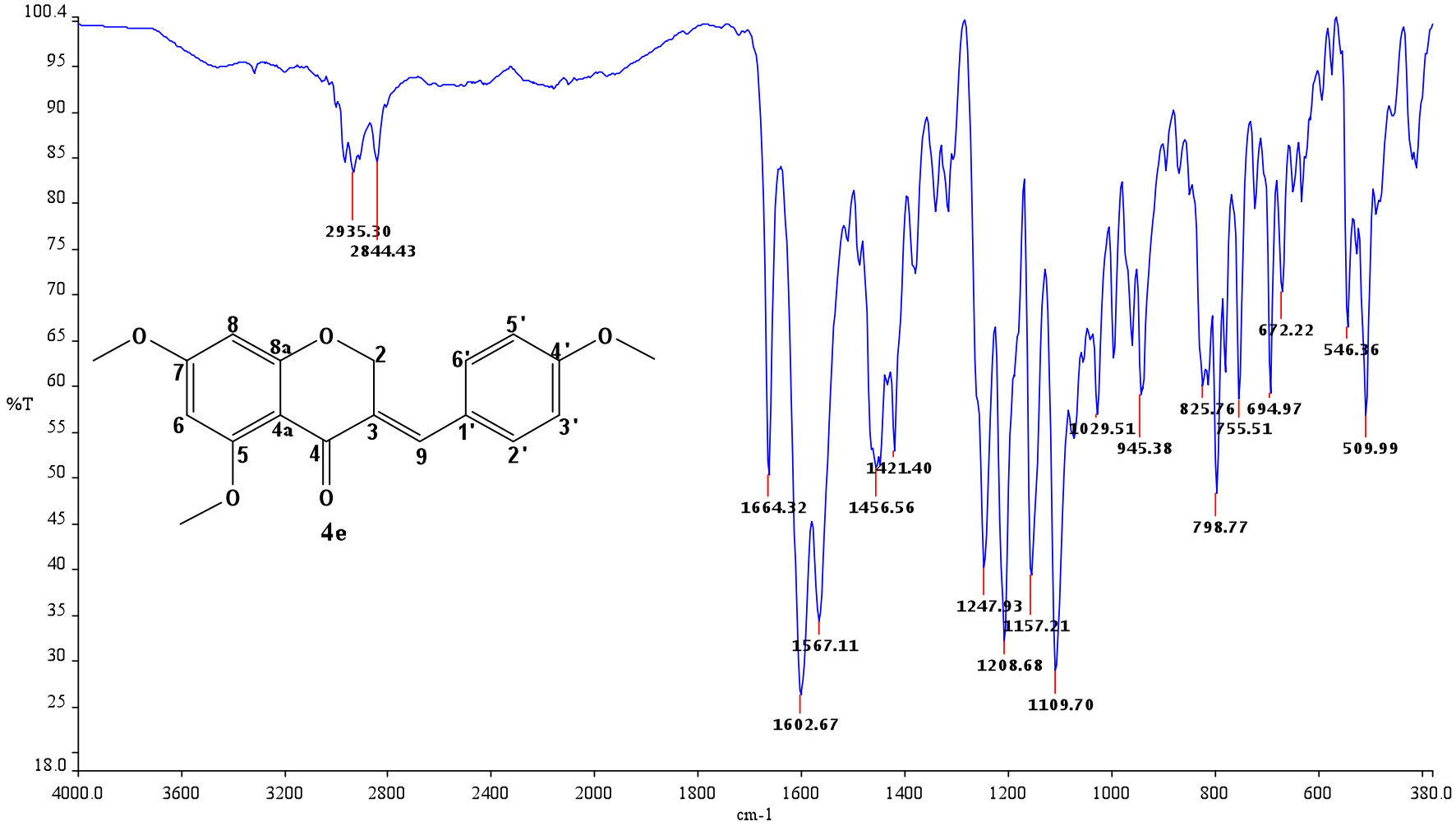
HSQC spectrum of compound **4d** in $CDCl_3$ (400 MHz)



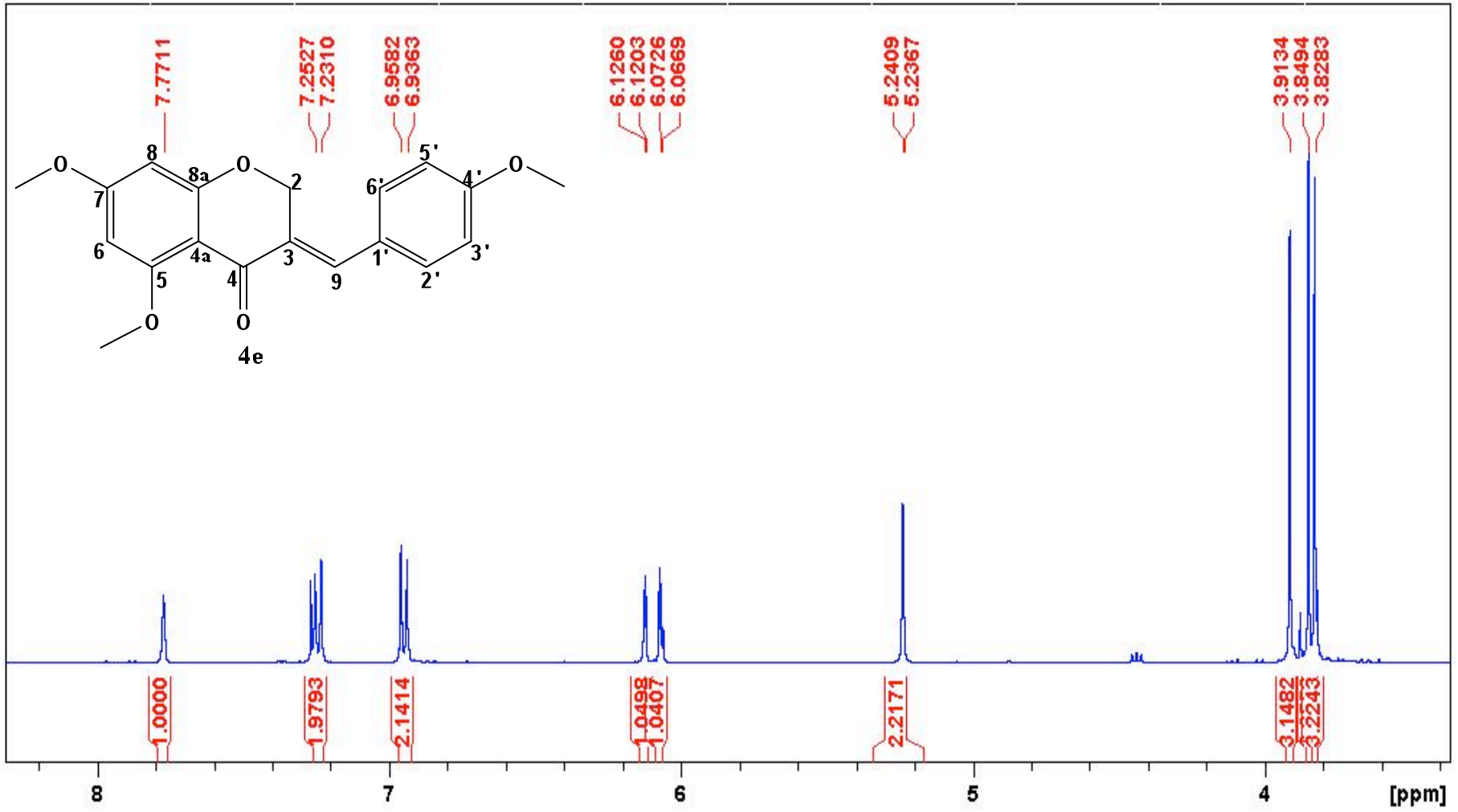
HMBC spectrum of compound **4d** in $CDCl_3$ (400 MHz)



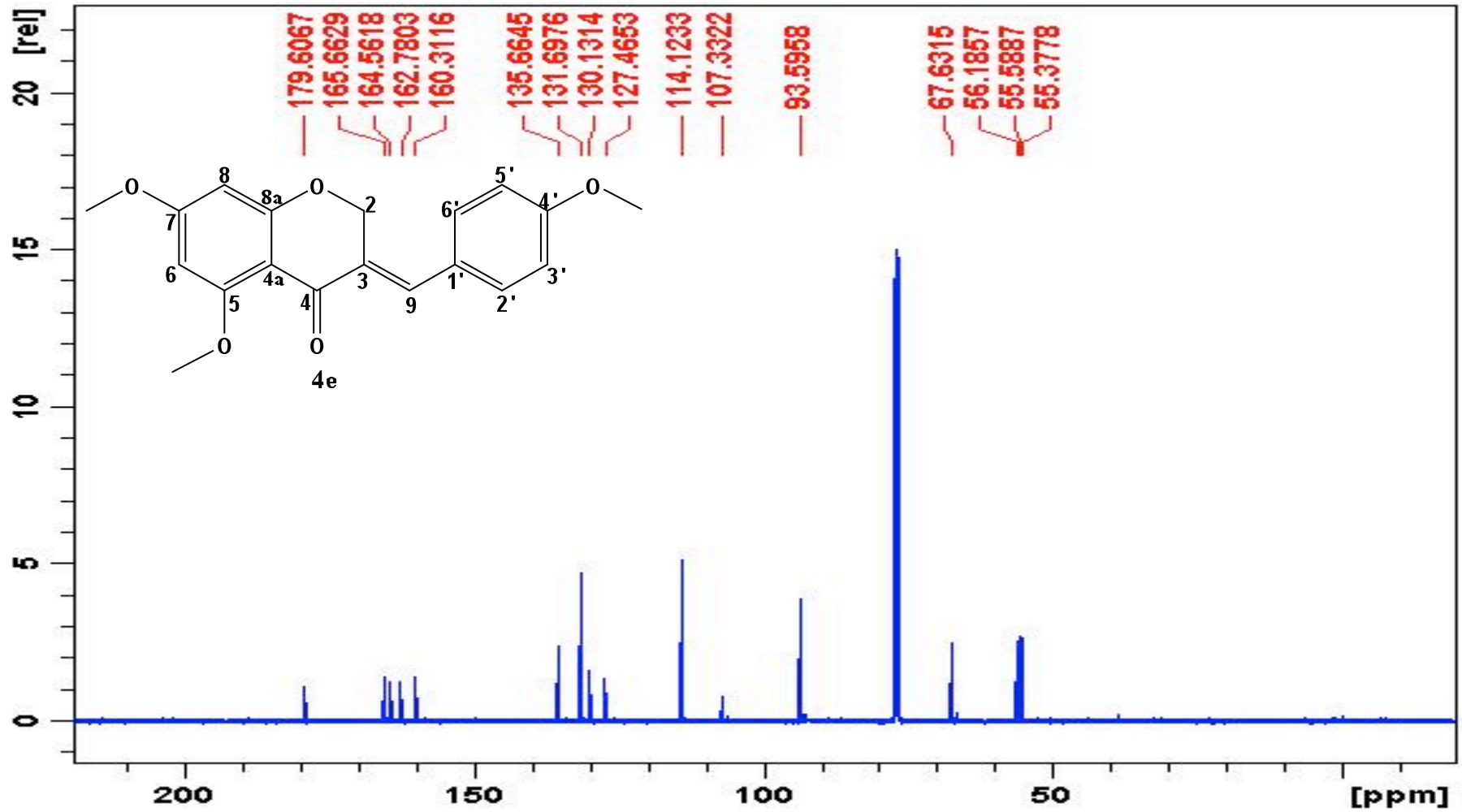
HRMS spectrum of compound **4d**



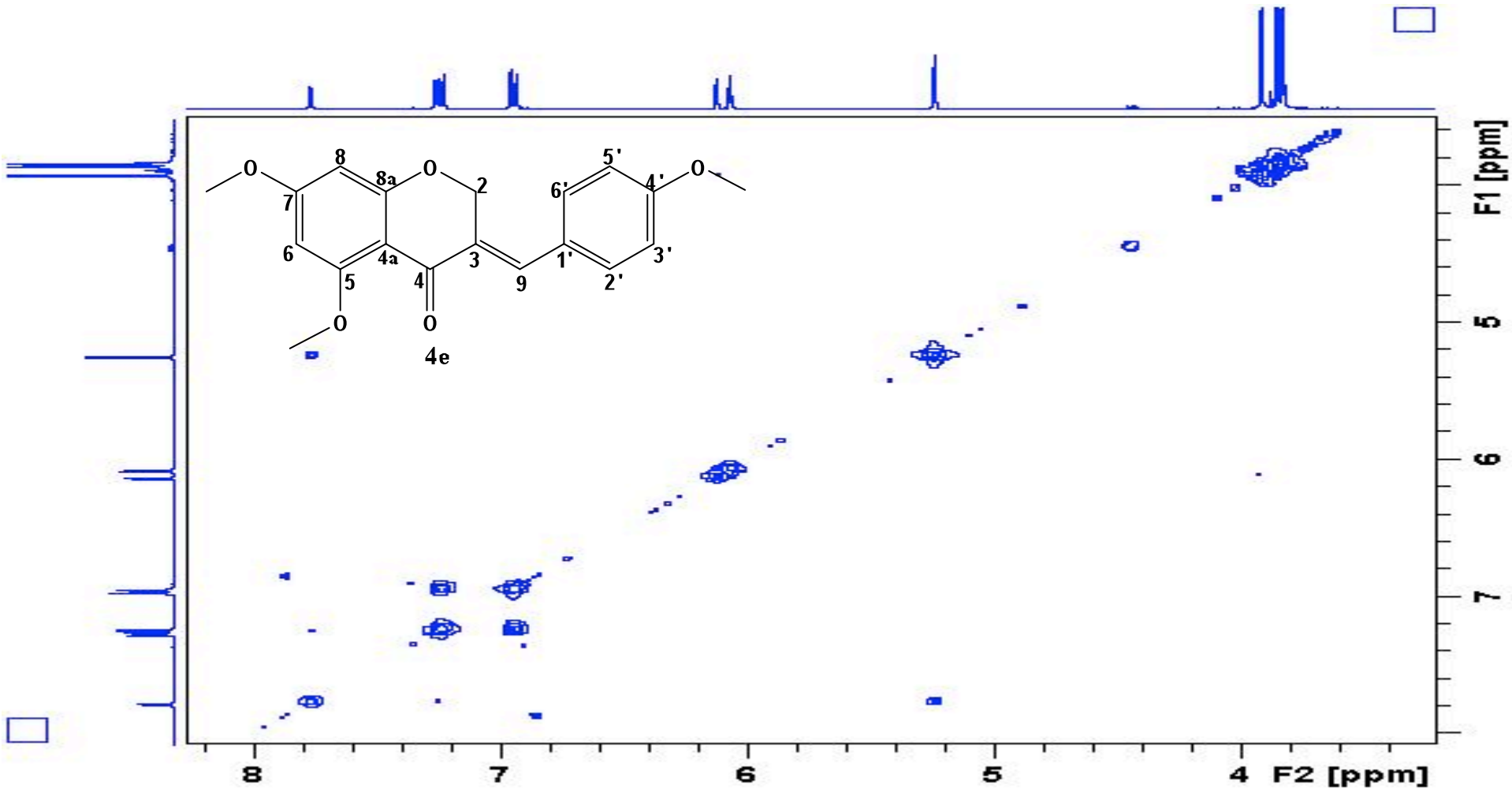
IR spectrum of compound **4e**



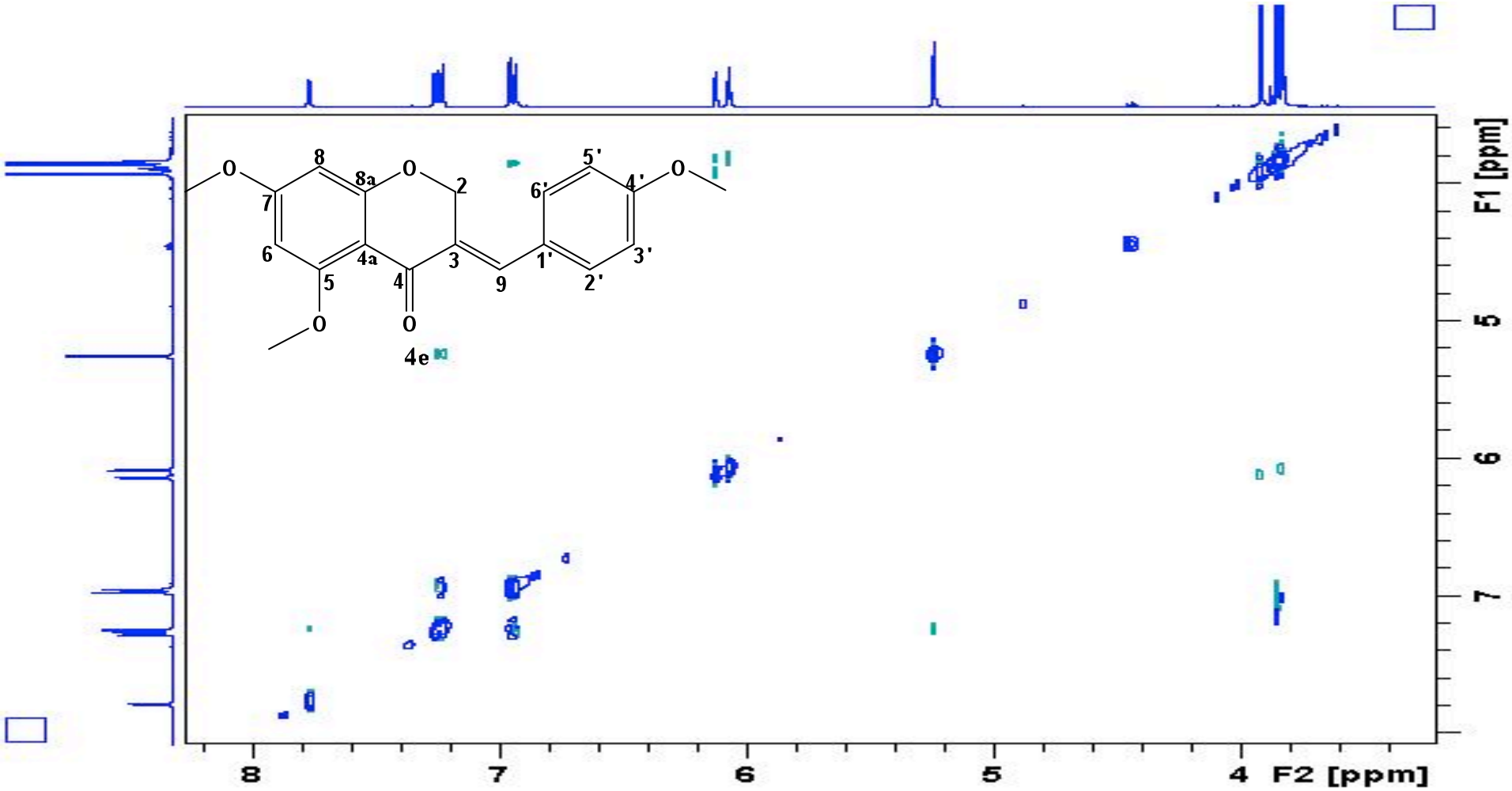
^{1}H-NMR spectrum of compound **4e** in $CDCl_3$ (400 MHz)



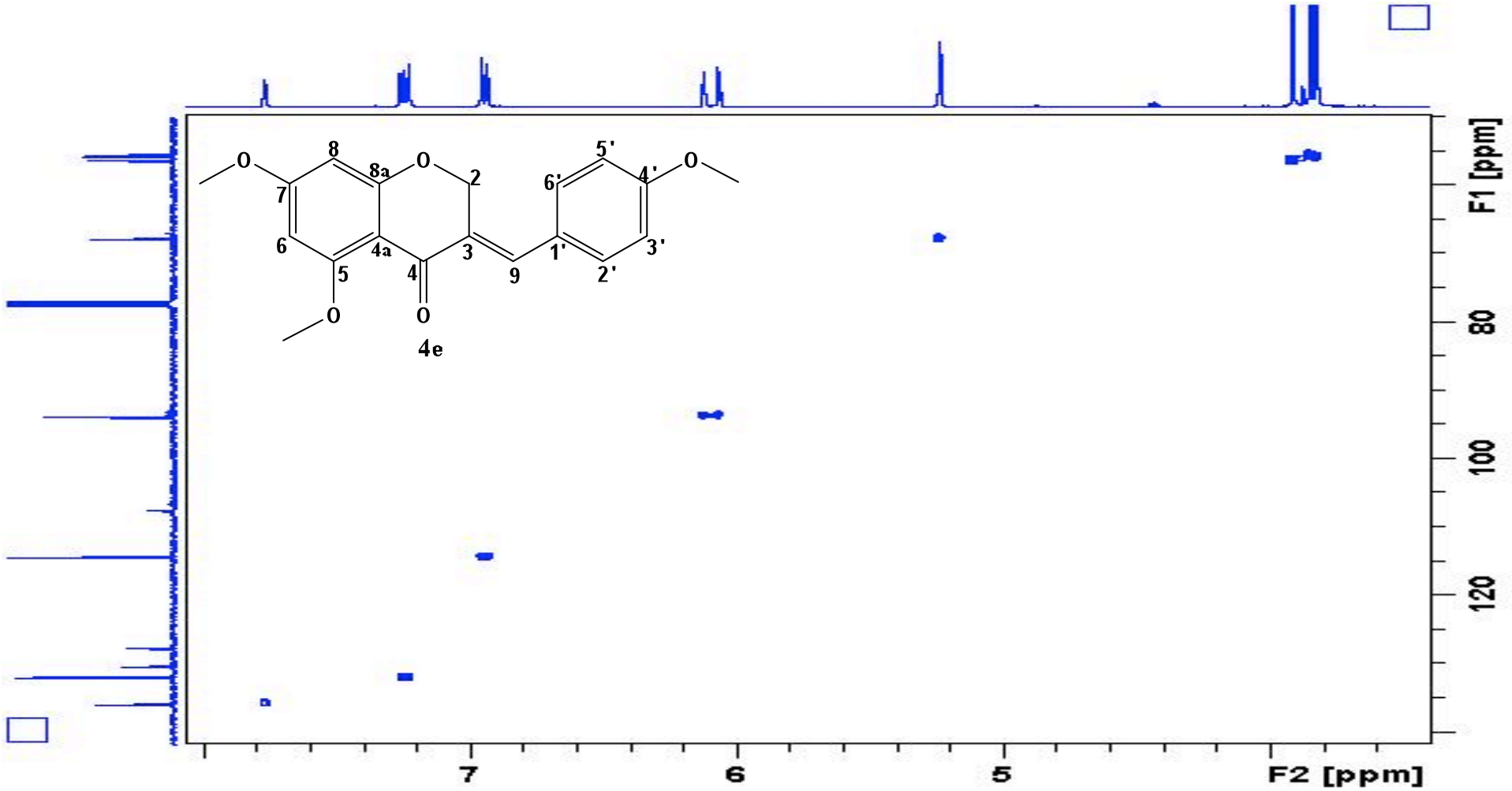
^{13}C-NMR spectrum of compound **4e** in $CDCl_3$ (400 MHz)



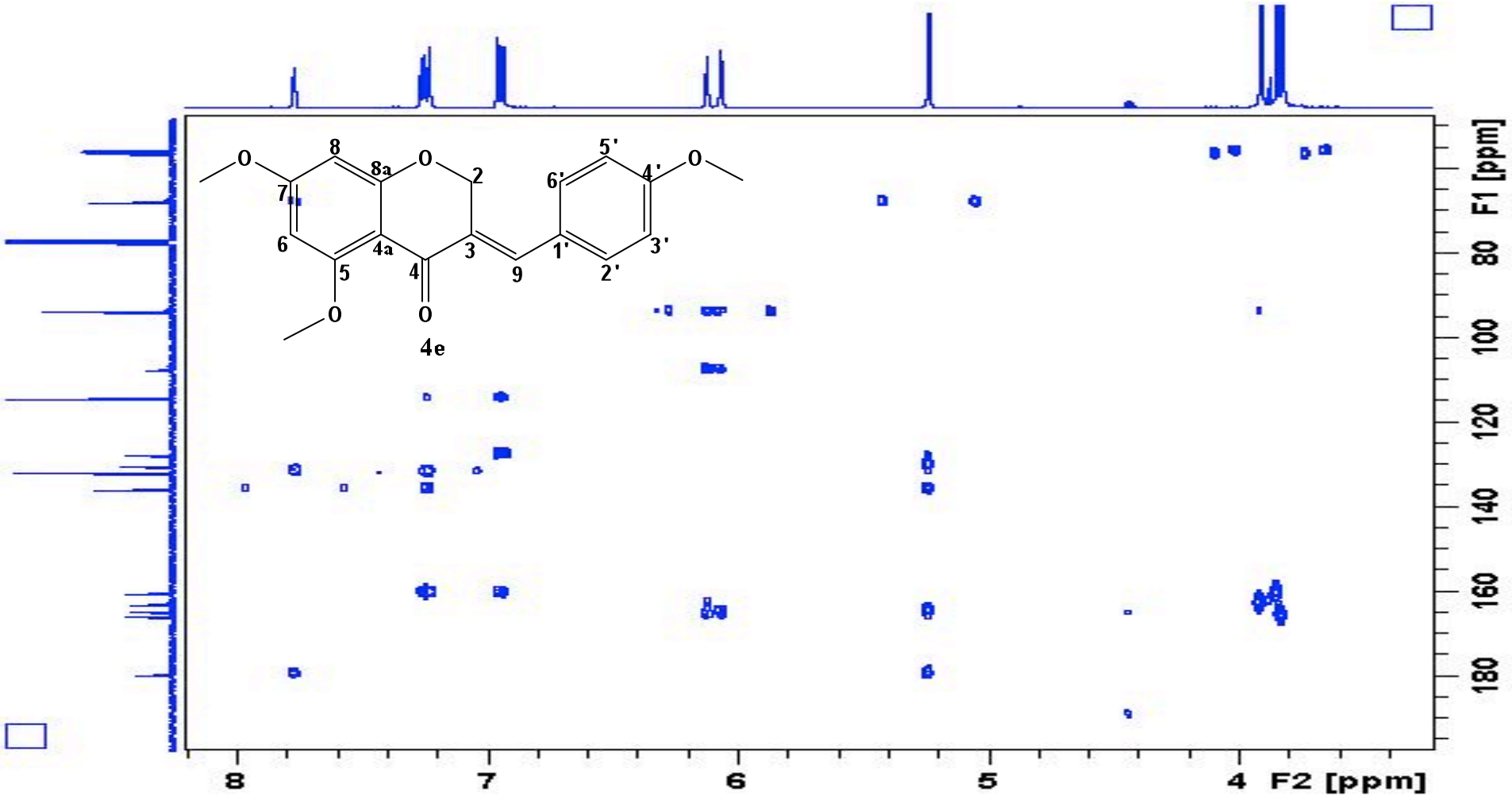
COSY spectrum of compound **4e** in $CDCl_3$ (400 MHz)



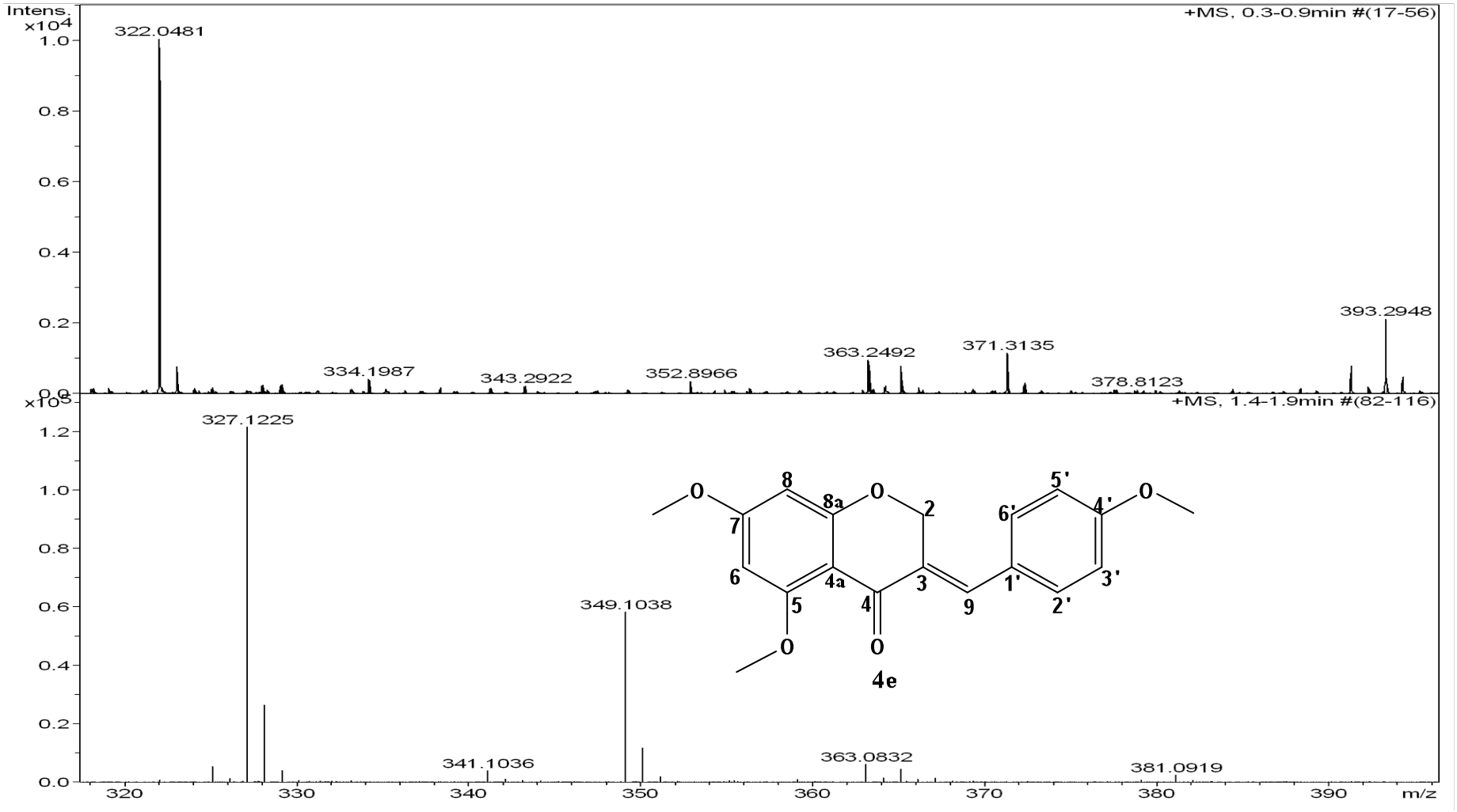
NOESY spectrum of compound **4e** in $CDCl_3$ (400 MHz)



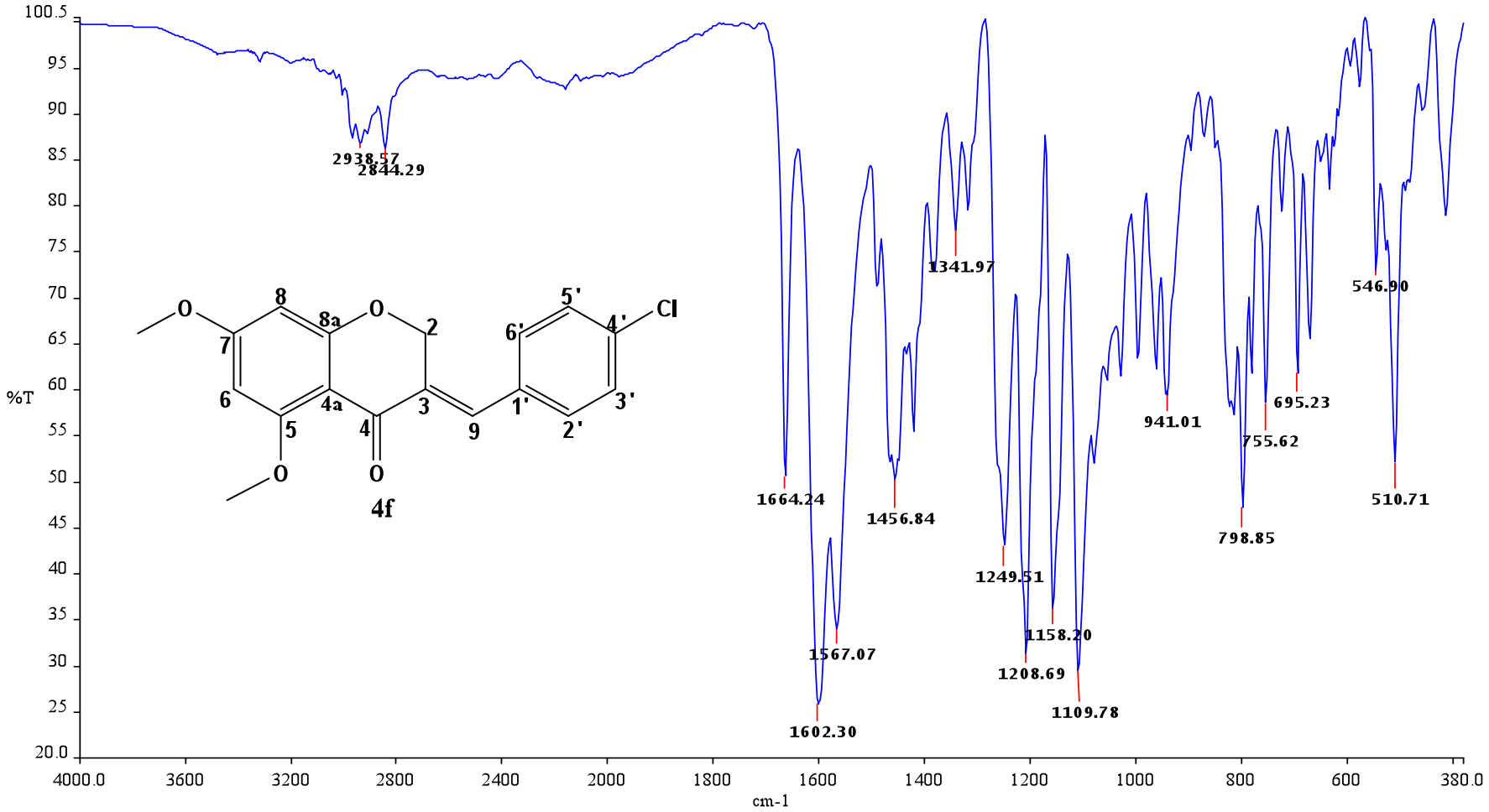
HSQC spectrum of compound **4e** in $CDCl_3$ (400 MHz)



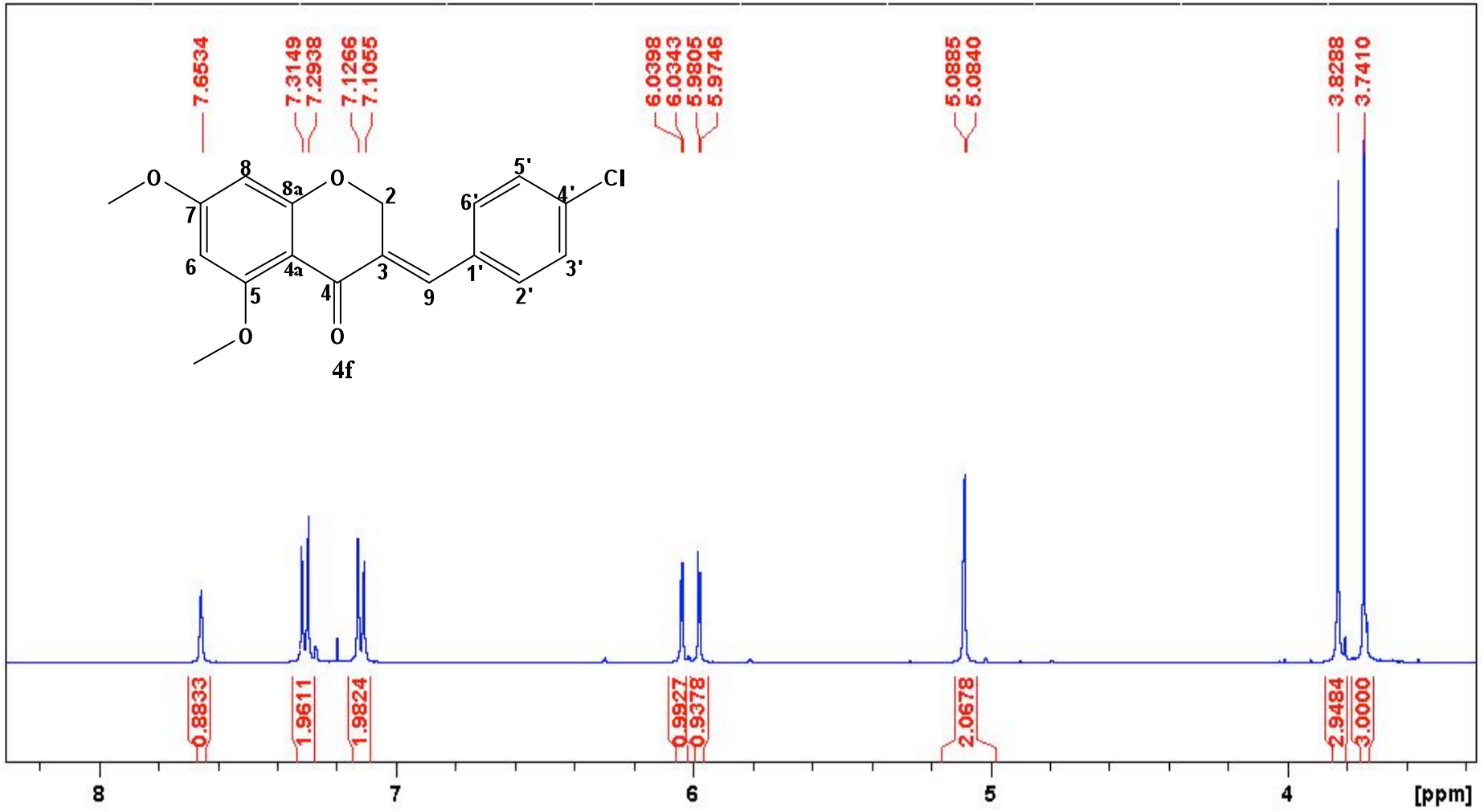
HMBC spectrum of compound **4e** in $CDCl_3$ (400 MHz)



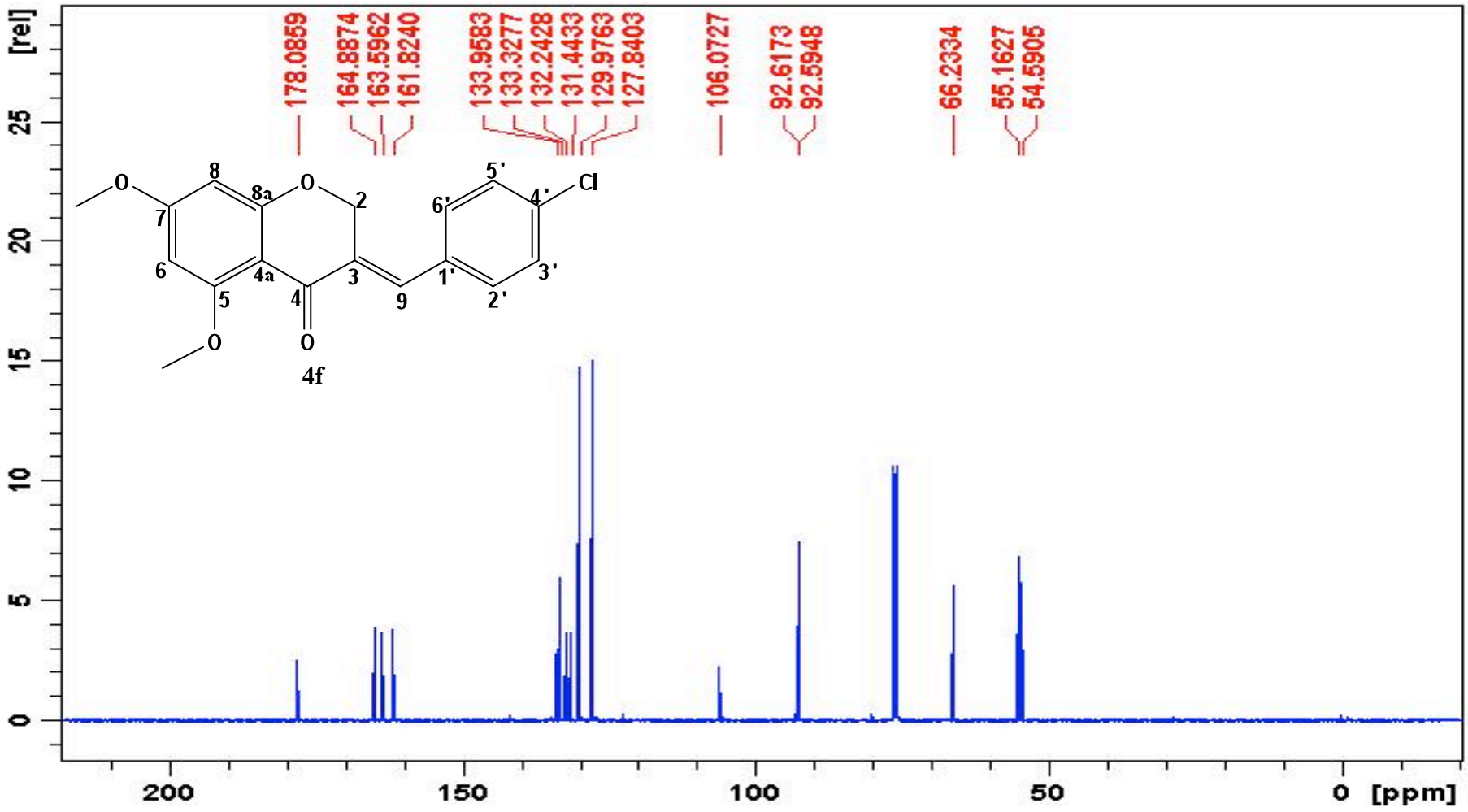
HRMS spectrum of compound **4e**



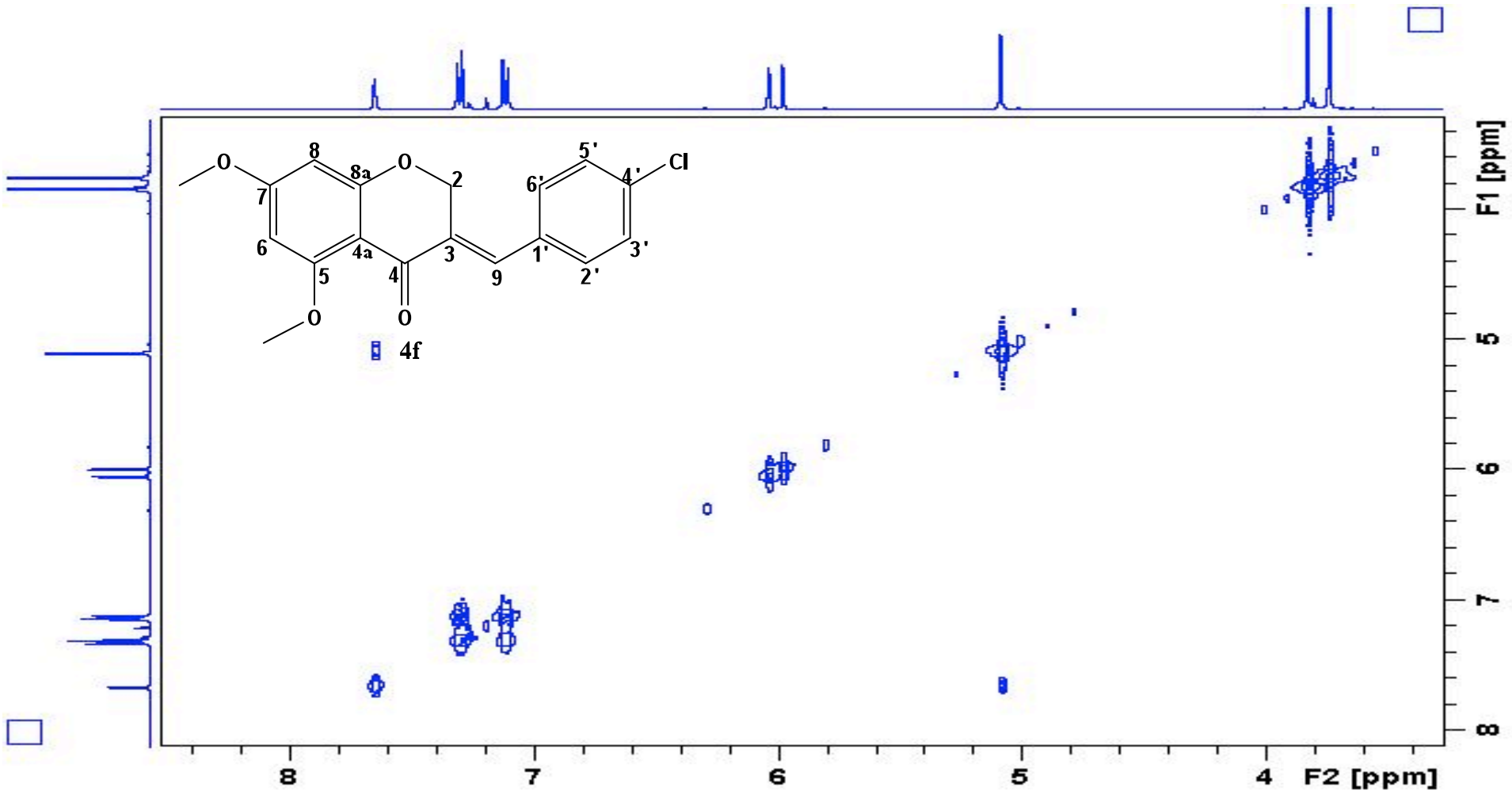
IR spectrum of compound **4f**



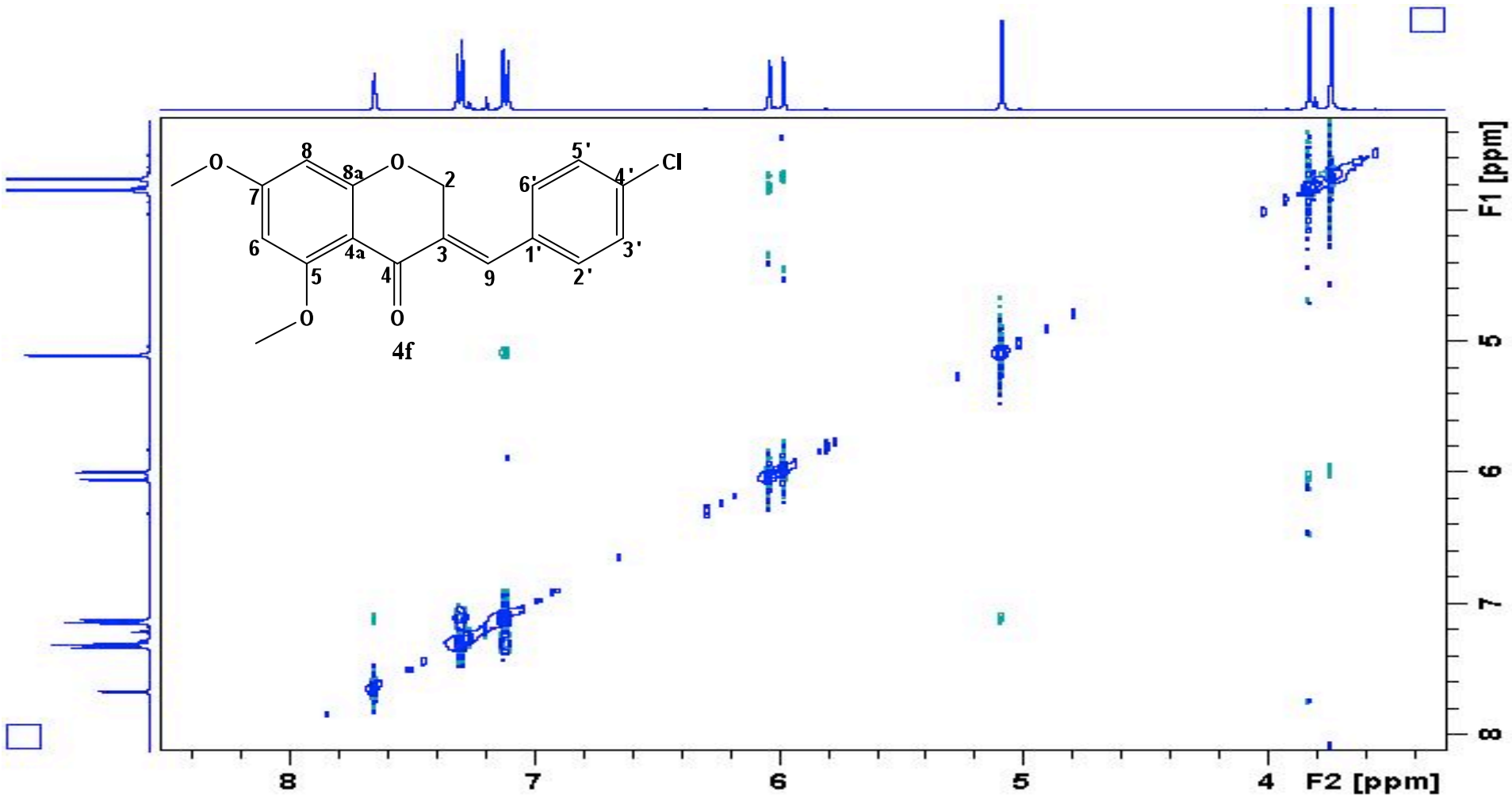
^{1}H-NMR spectrum of compound **4f** in $CDCl_3$ (400 MHz)



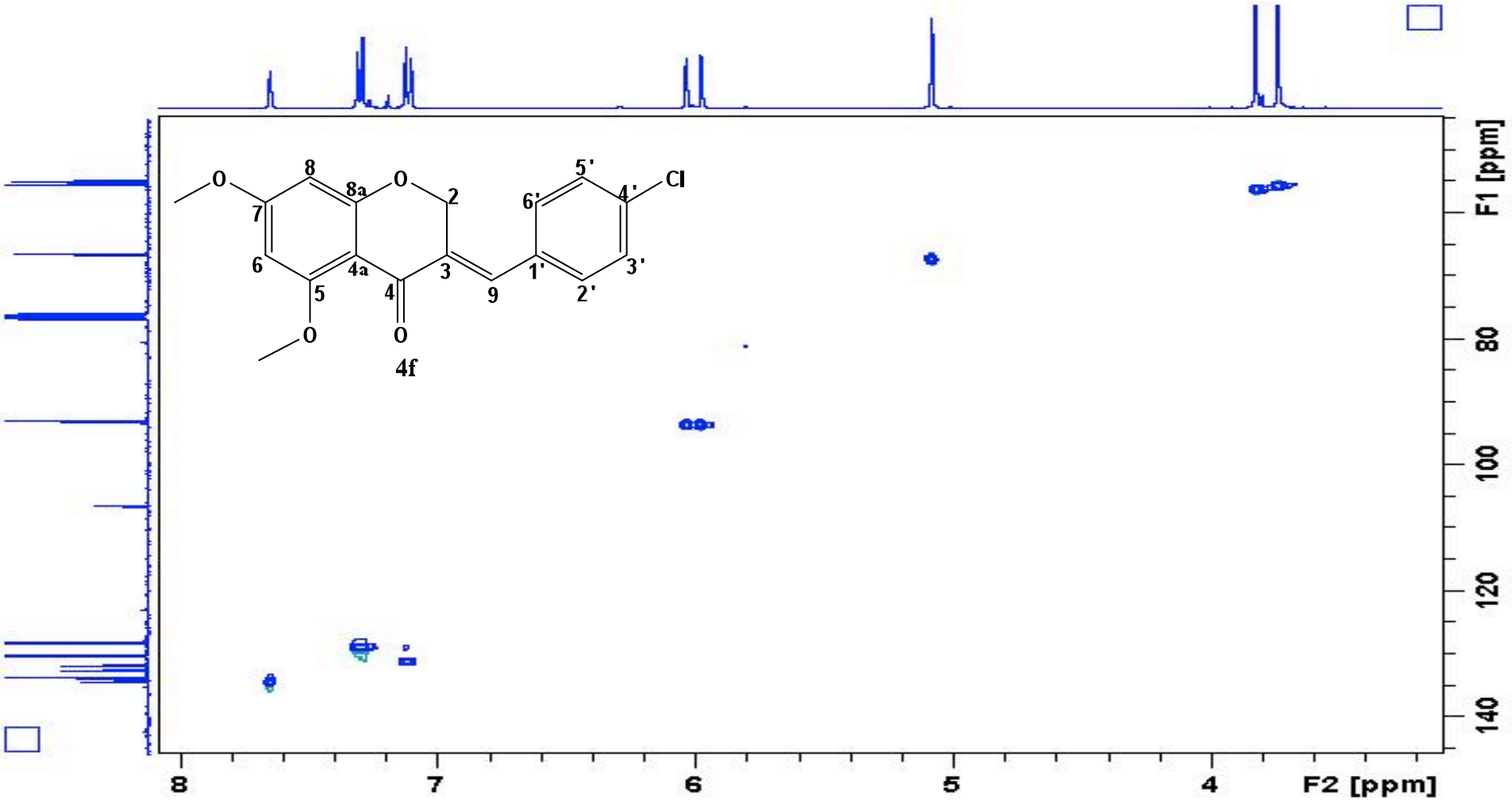
^{13}C-NMR spectrum of compound **4f** in $CDCl_3$ (400 MHz)



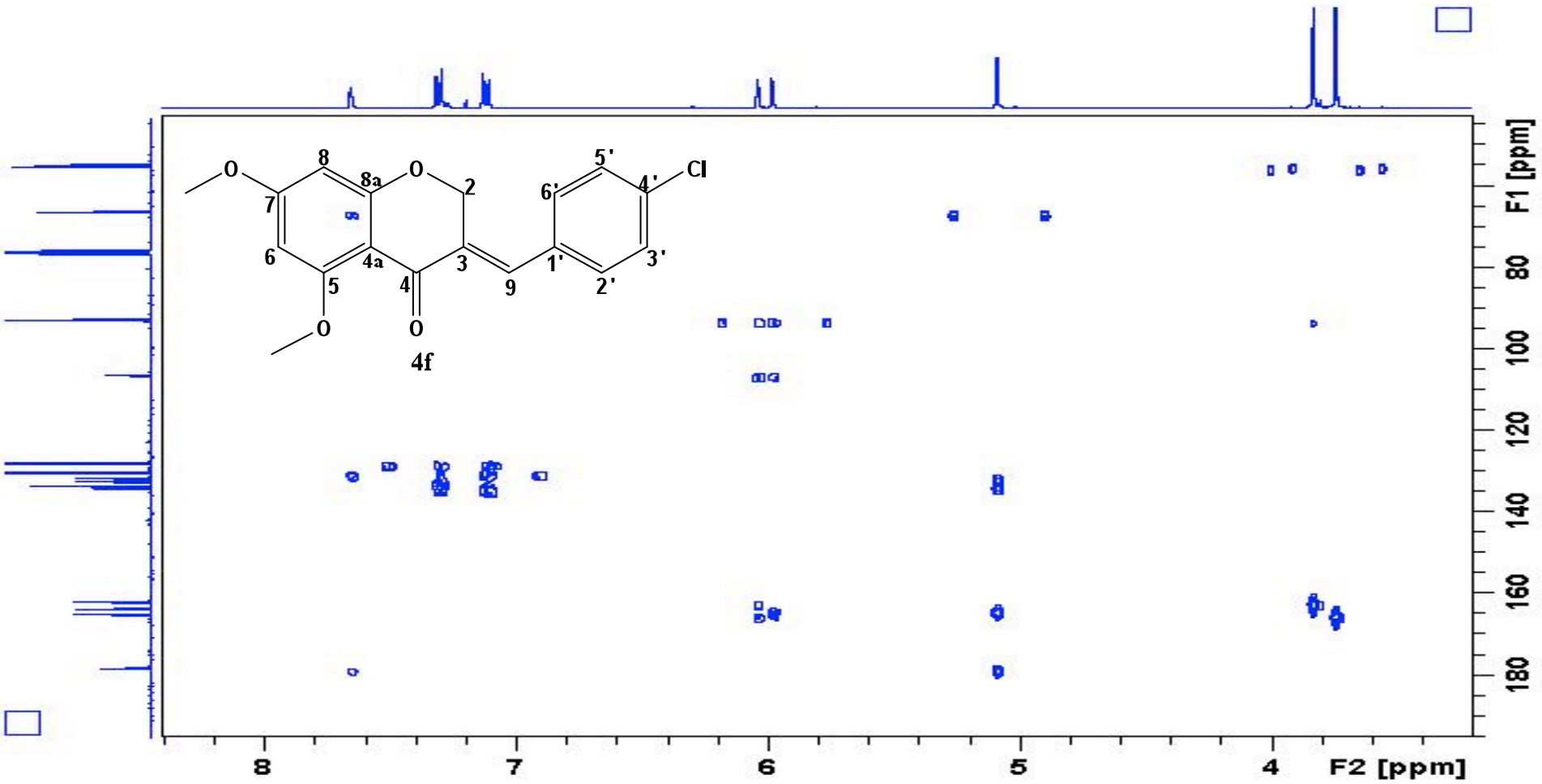
COSY spectrum of compound **4f** in $CDCl_3$ (400 MHz)



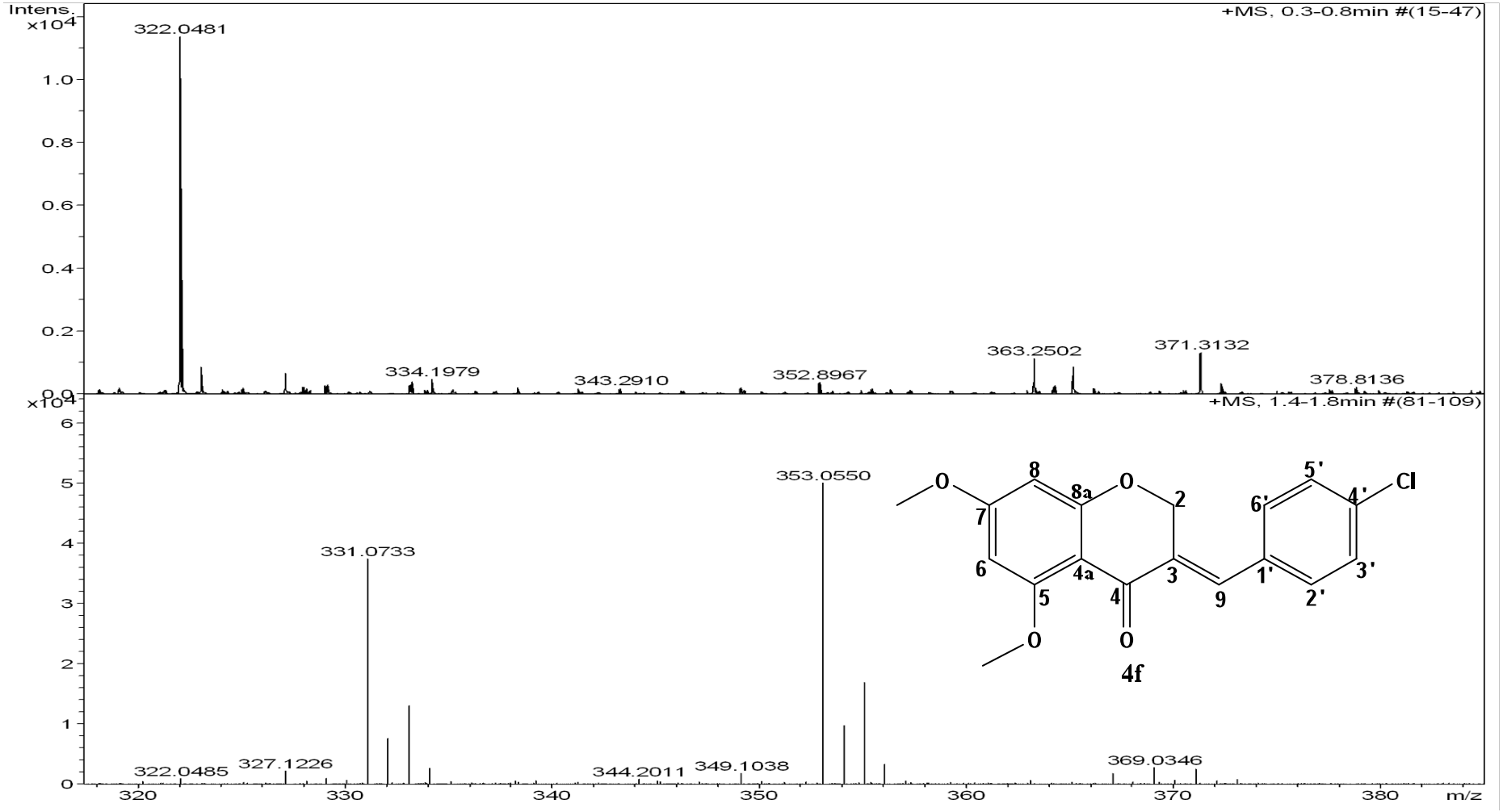
NOESY spectrum of compound **4f** in $CDCl_3$ (400 MHz)



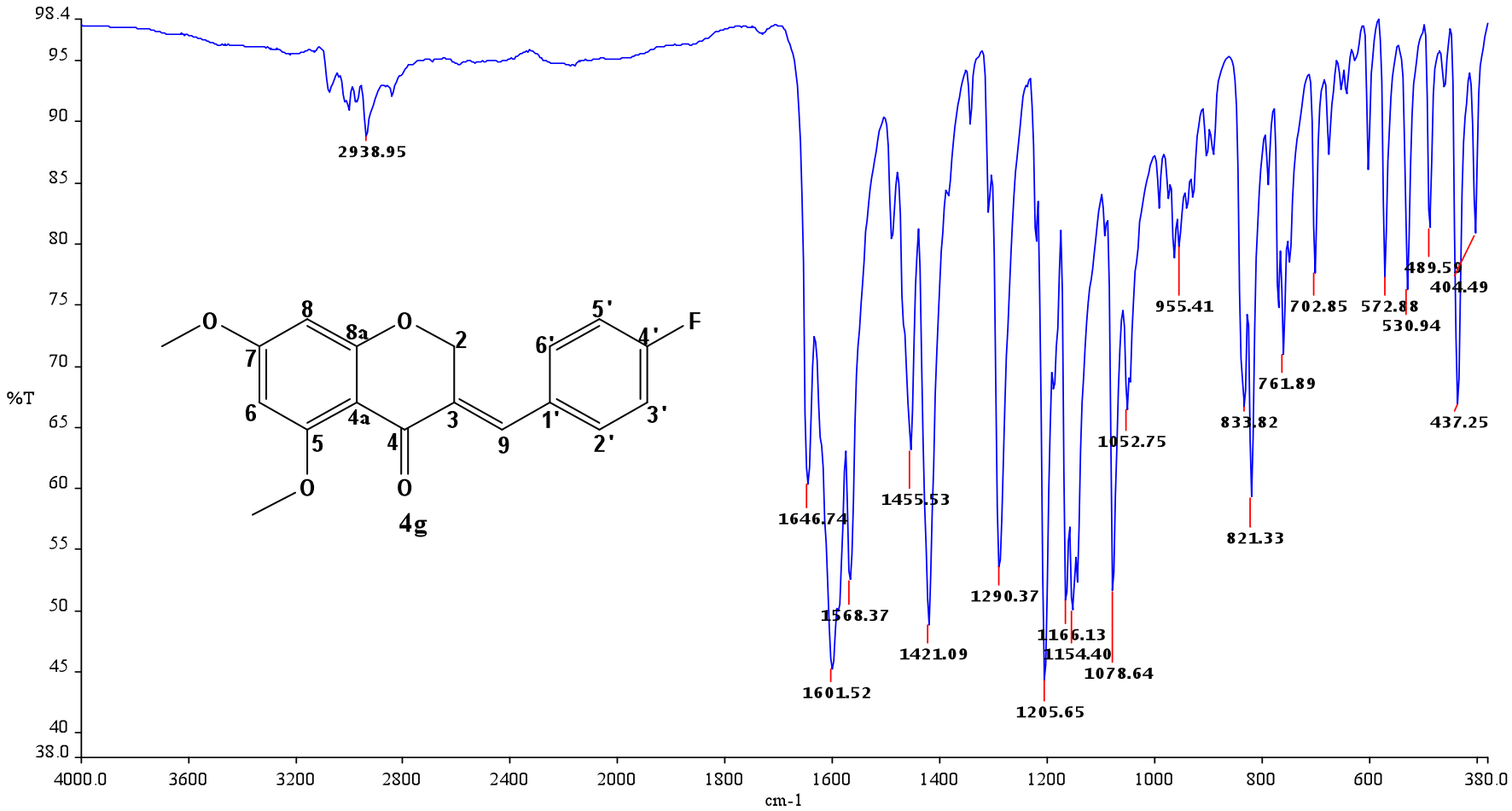
HSQC spectrum of compound **4f** in $CDCl_3$ (400 MHz)



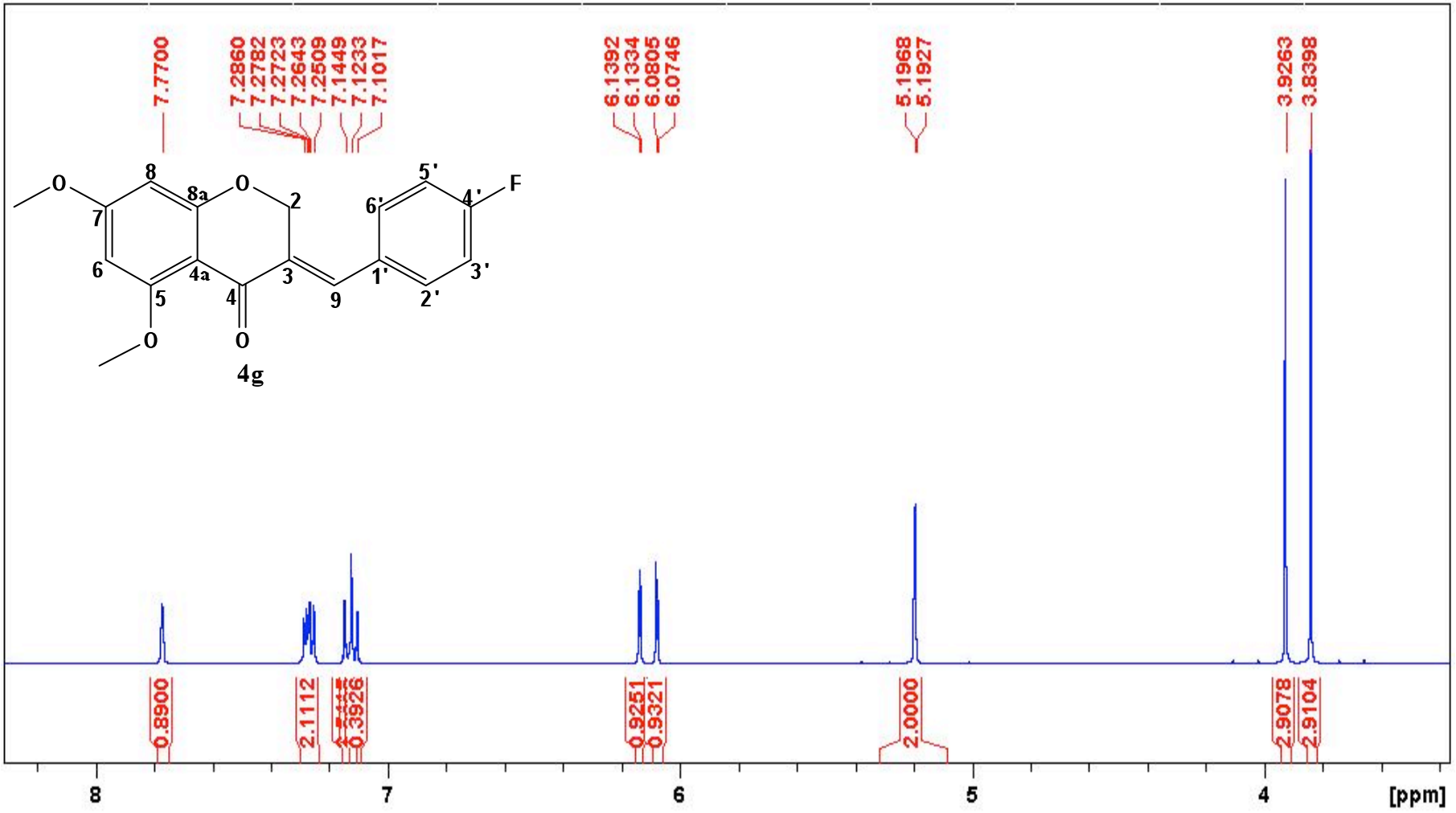
HMBC spectrum of compound **4f** in $CDCl_3$ (400 MHz)



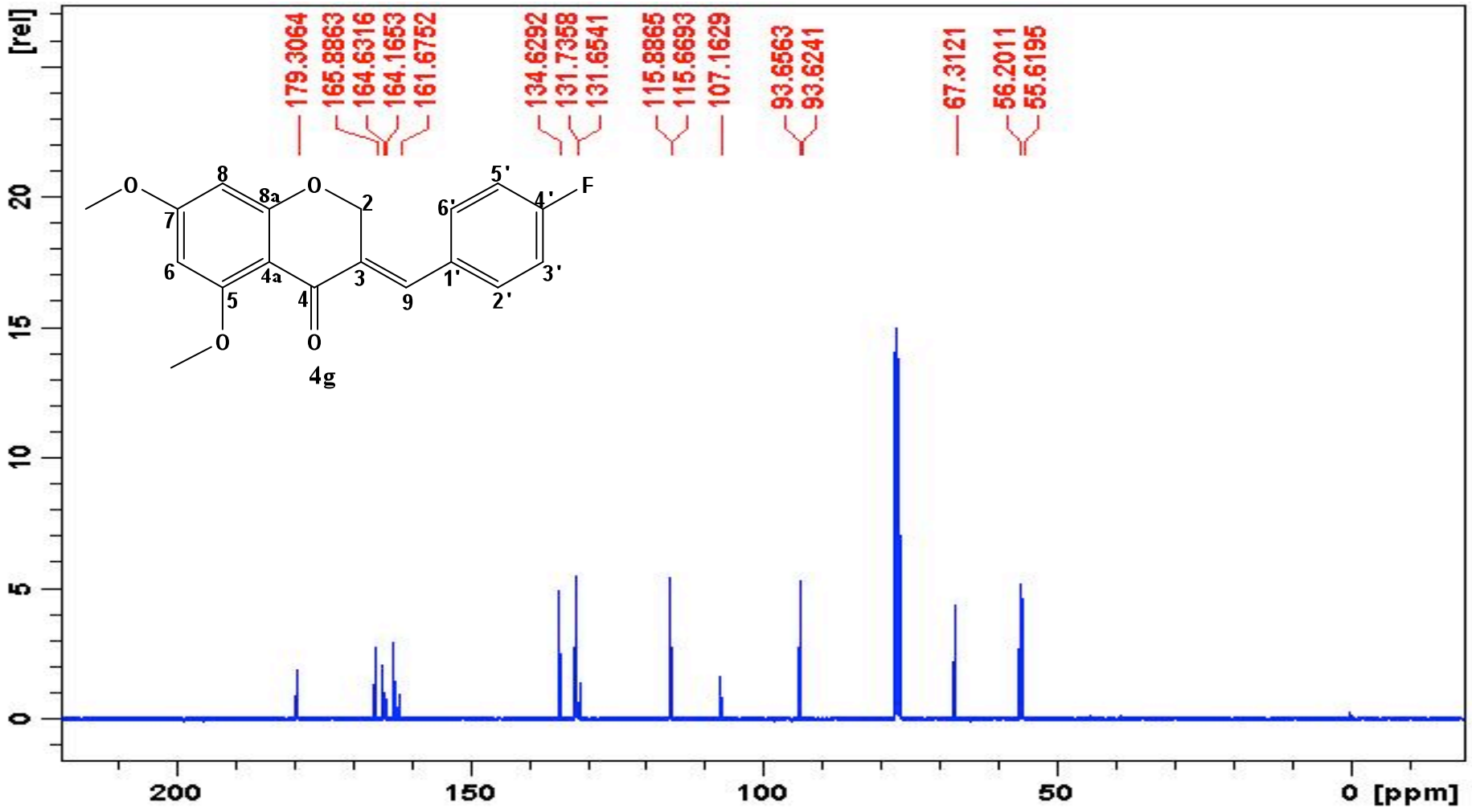
HRMS spectrum of compound **4f**



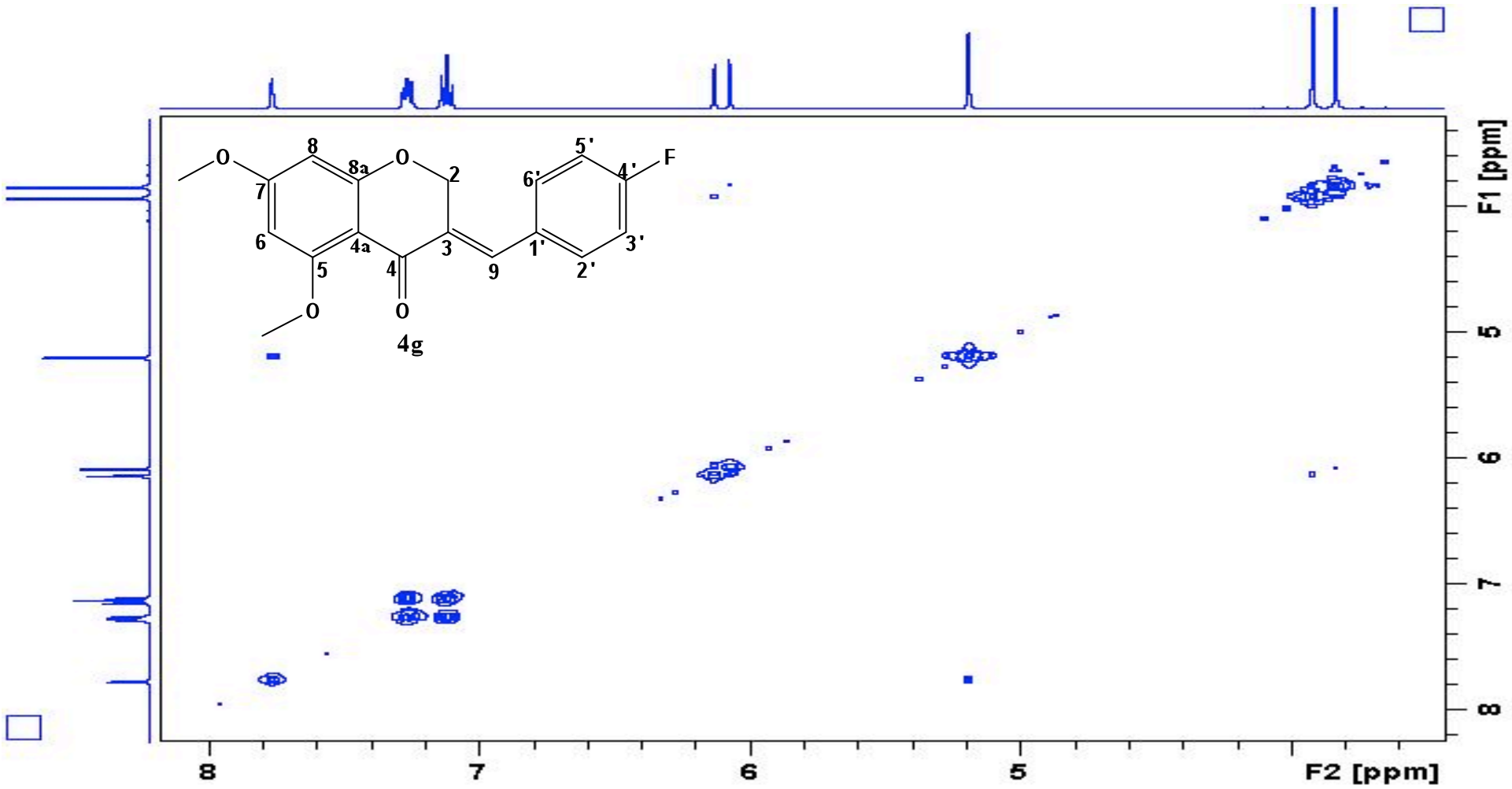
IR spectrum of compound **4g**



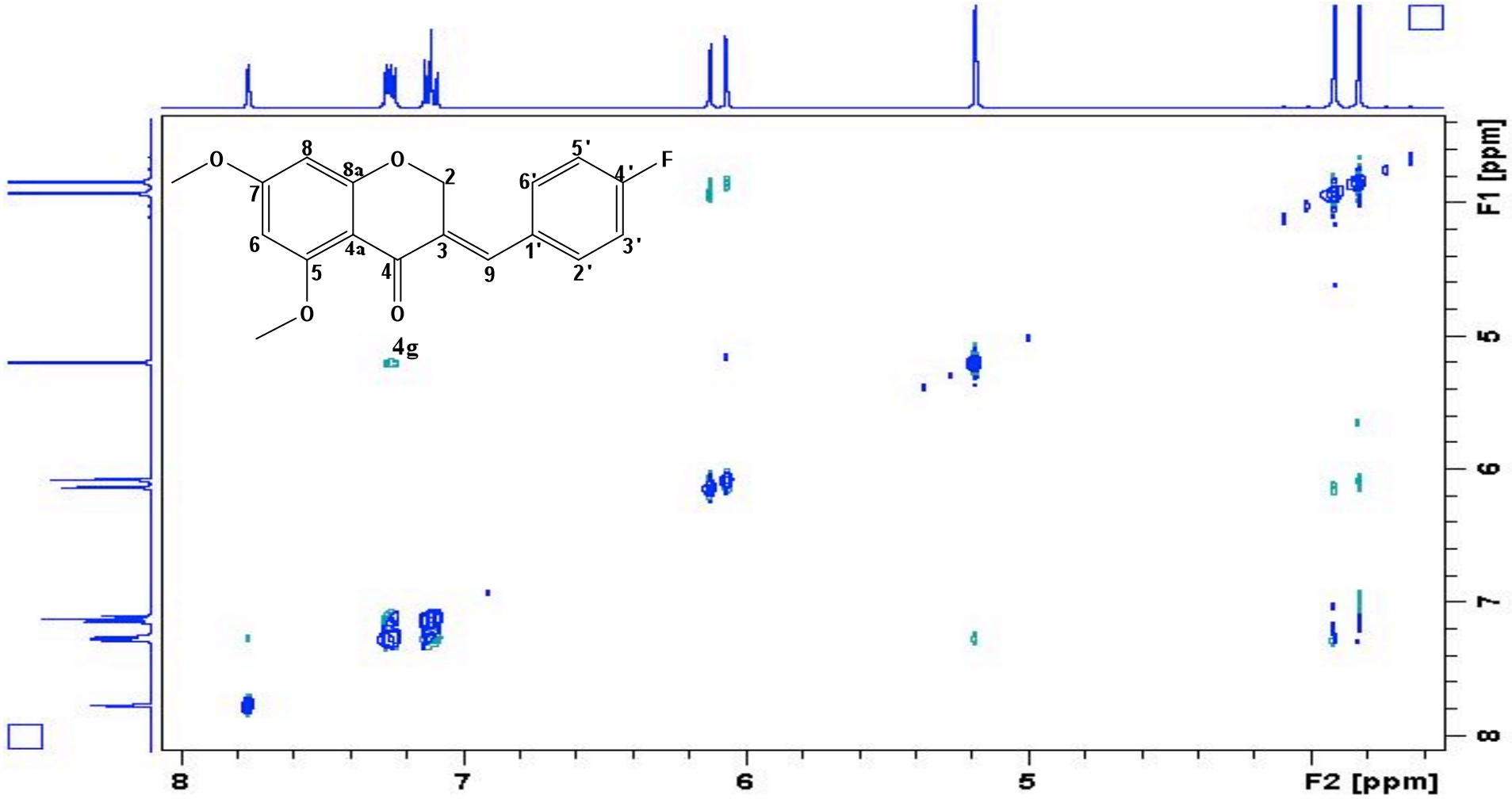
^{1}H-NMR spectrum of compound **4g** in $CDCl_3$ (400 MHz)



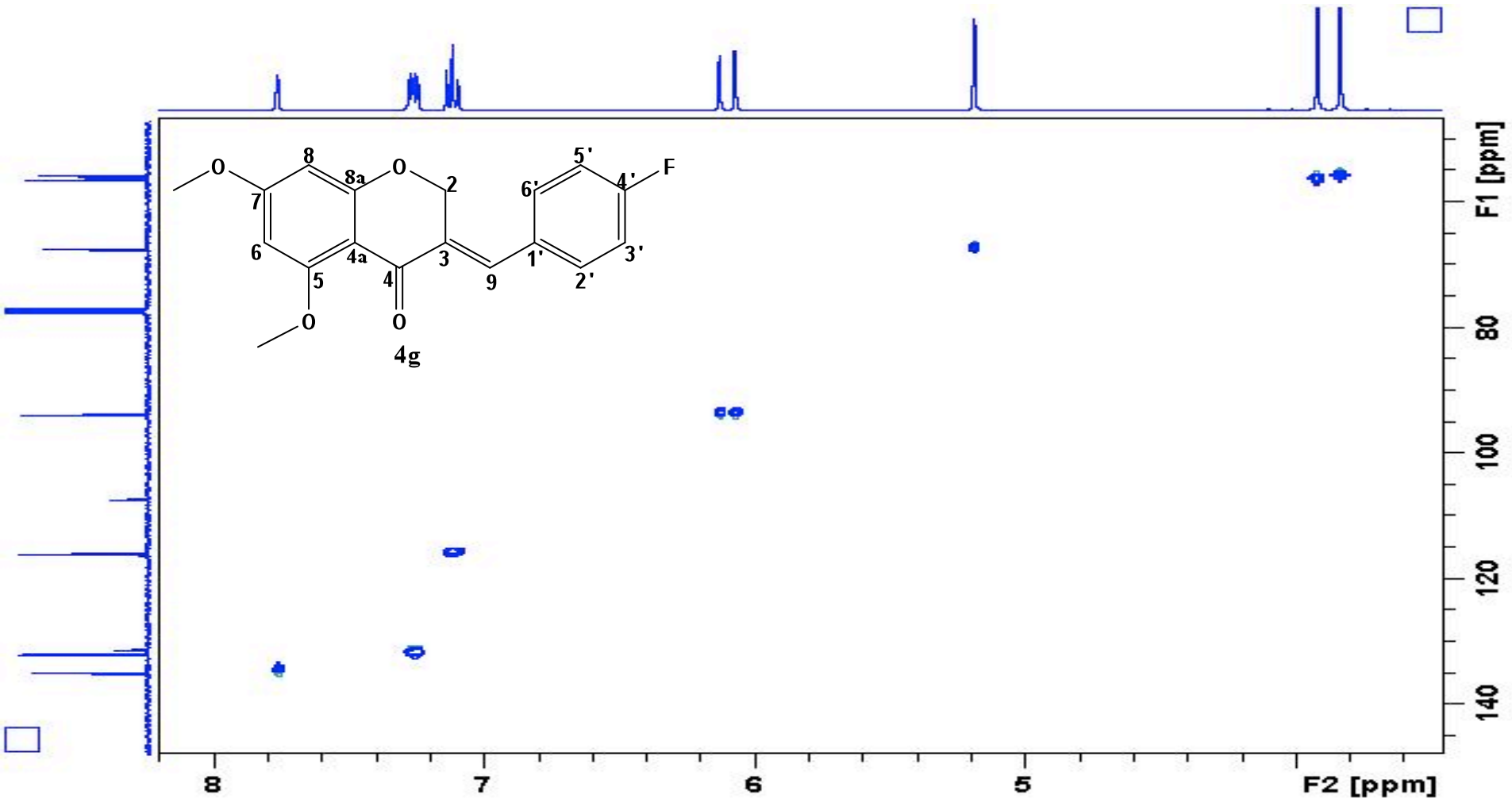
^{13}C-NMR spectrum of compound **4g** in $CDCl_3$ (400 MHz)



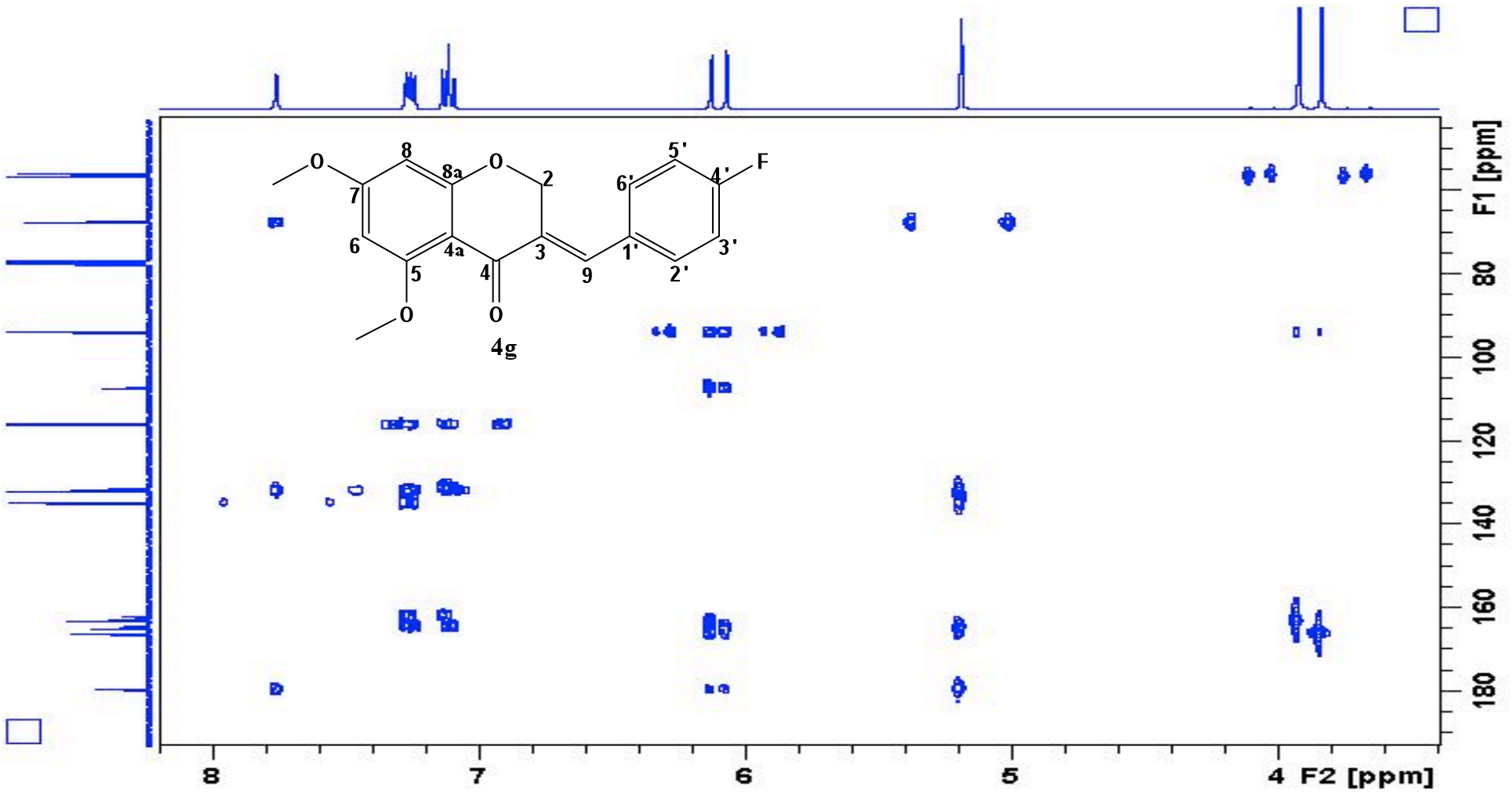
COSY spectrum of compound **4g** in $CDCl_3$ (400 MHz)



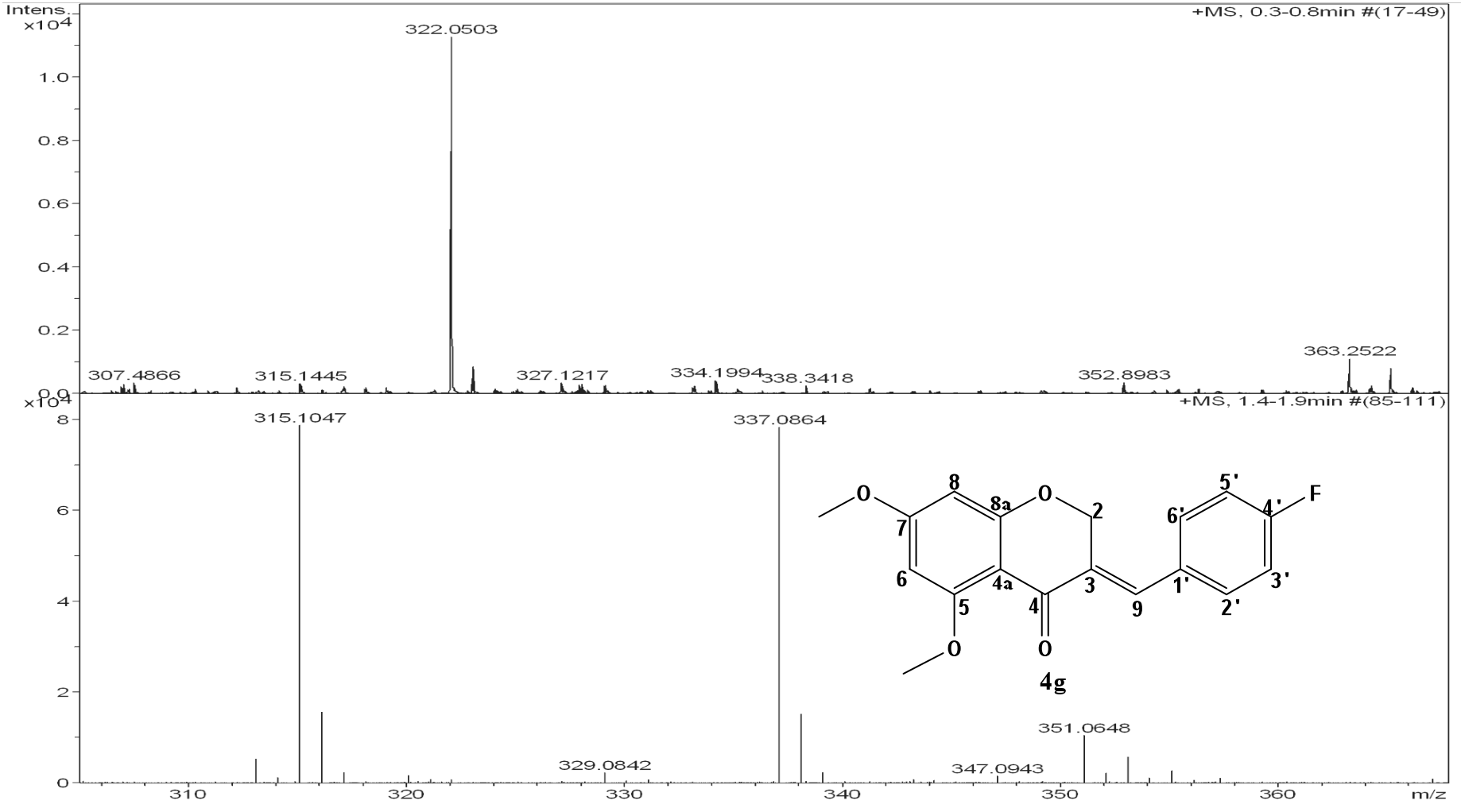
NOESY spectrum of compound **4g** in $CDCl_3$ (400 MHz)



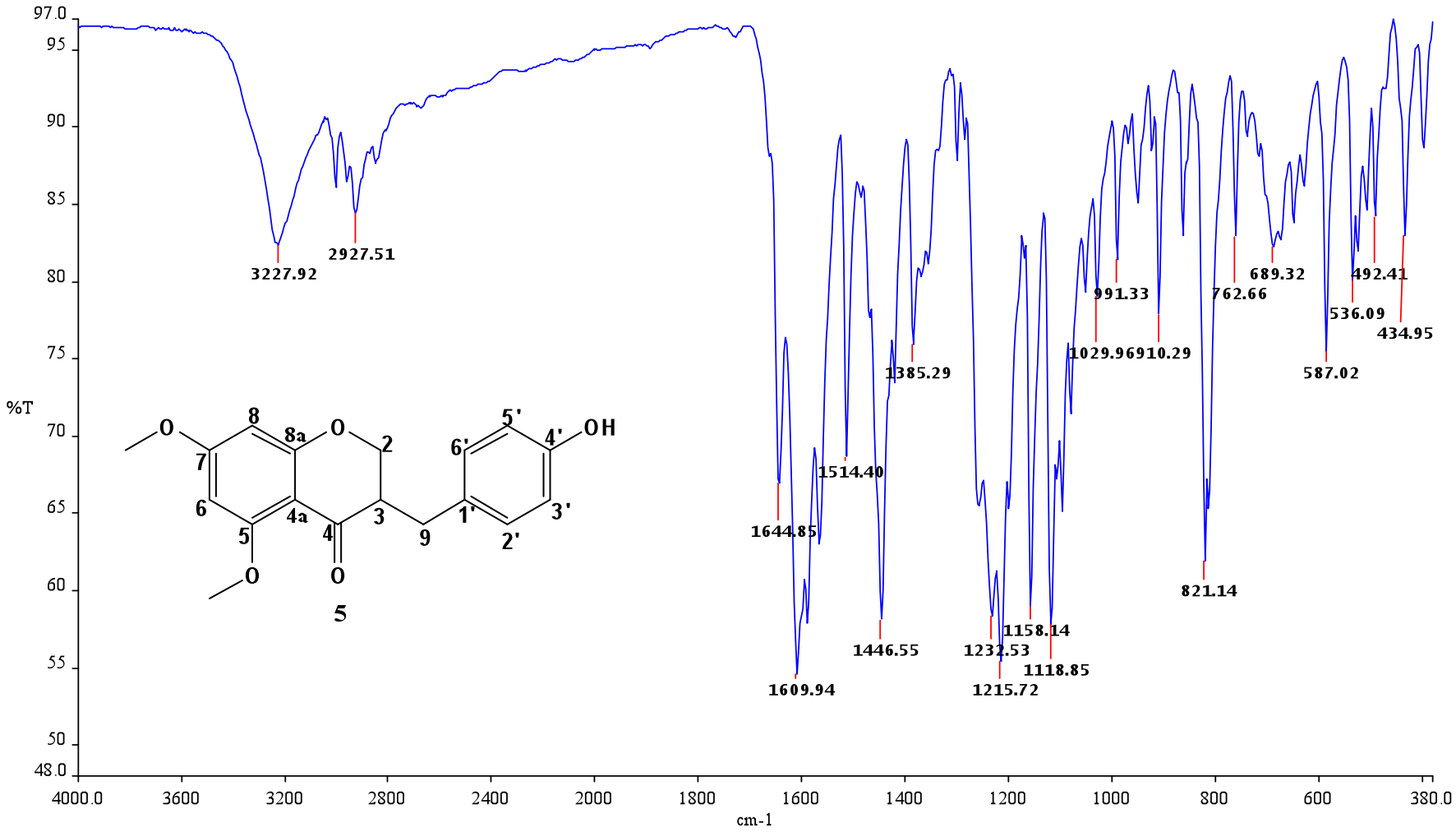
HSQC spectrum of compound **4g** in $CDCl_3$ (400 MHz)



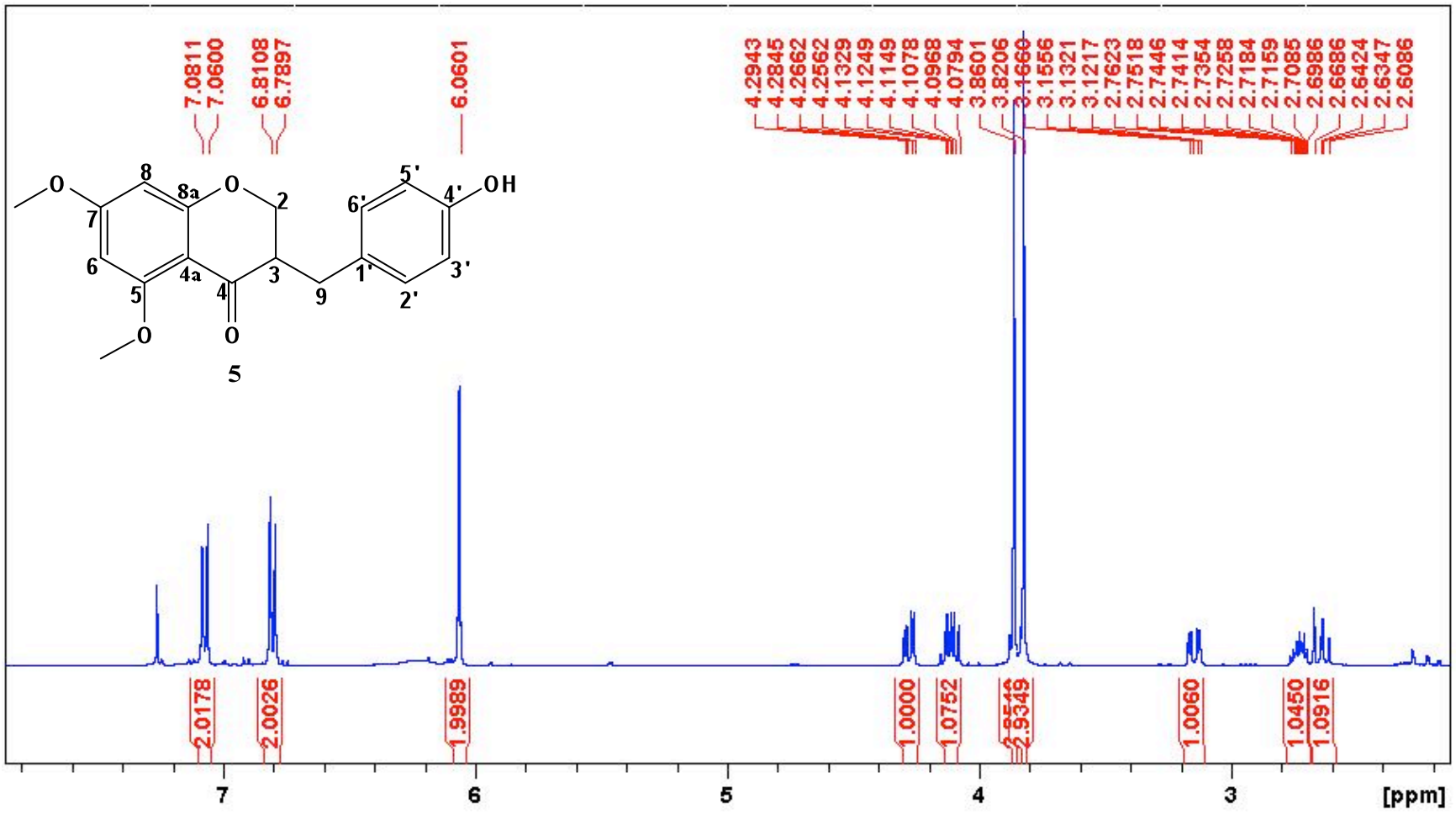
HMBC spectrum of compound **4g** in $CDCl_3$ (400 MHz)



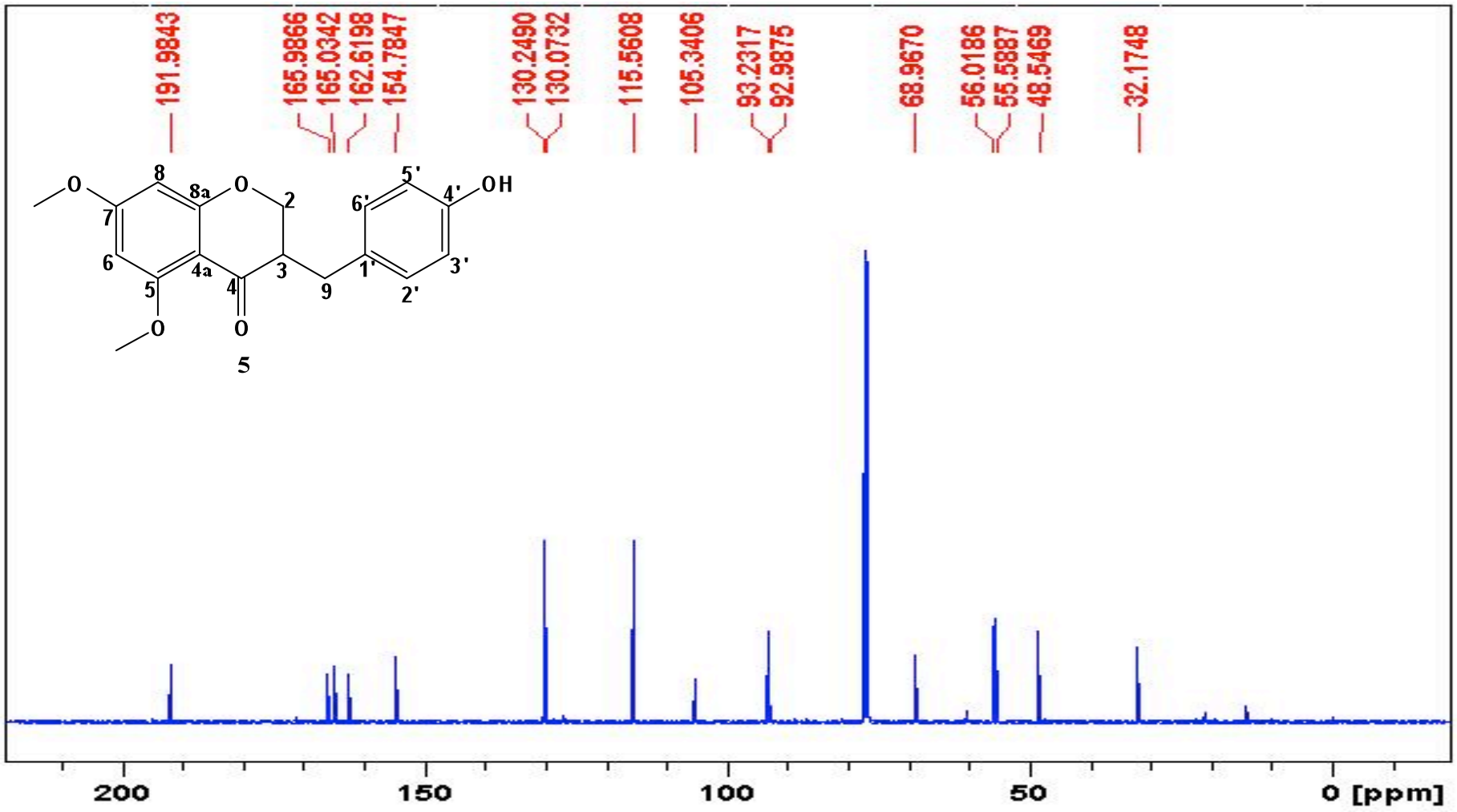
HRMS spectrum of compound **4g**



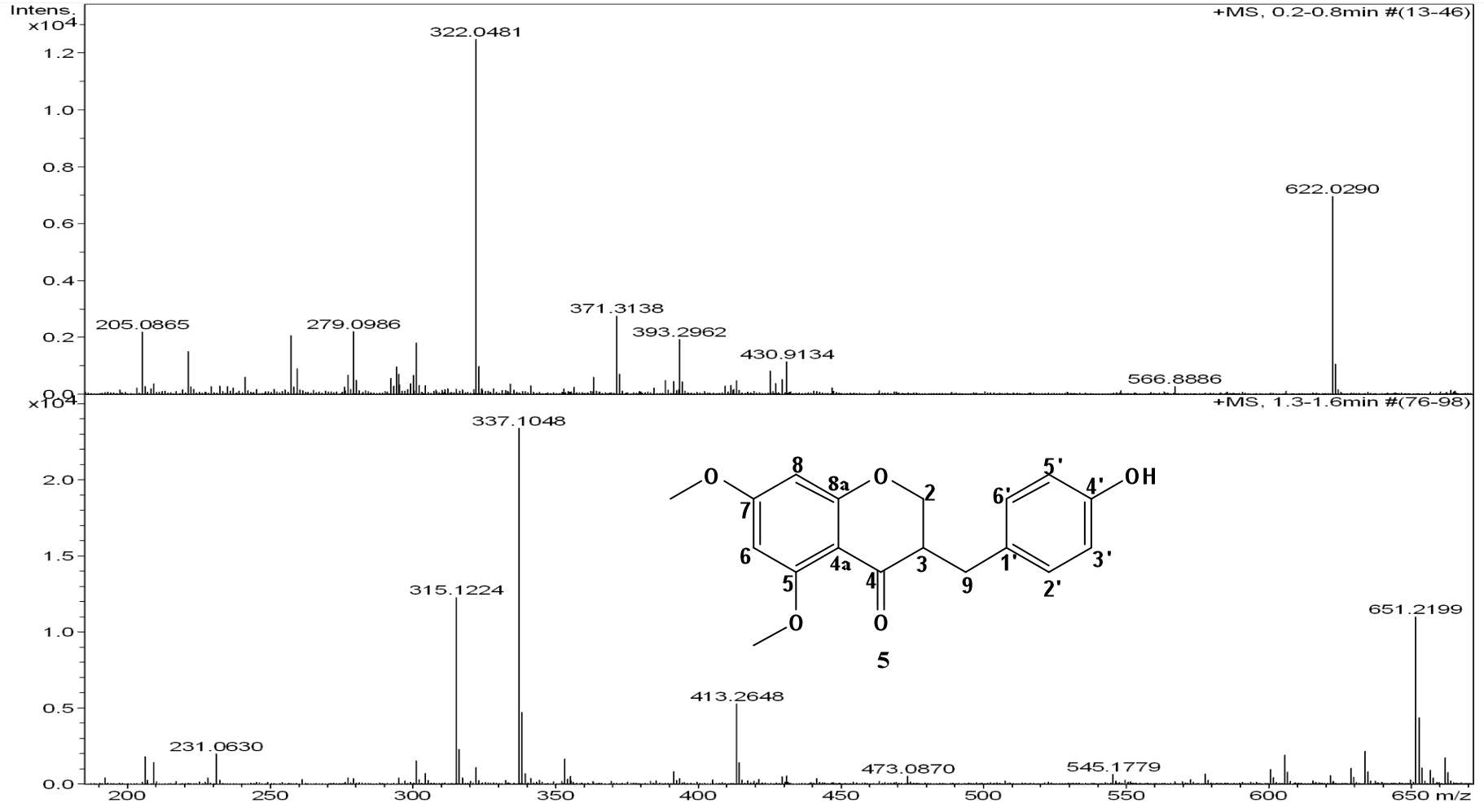
IR spectrum of compound **5**



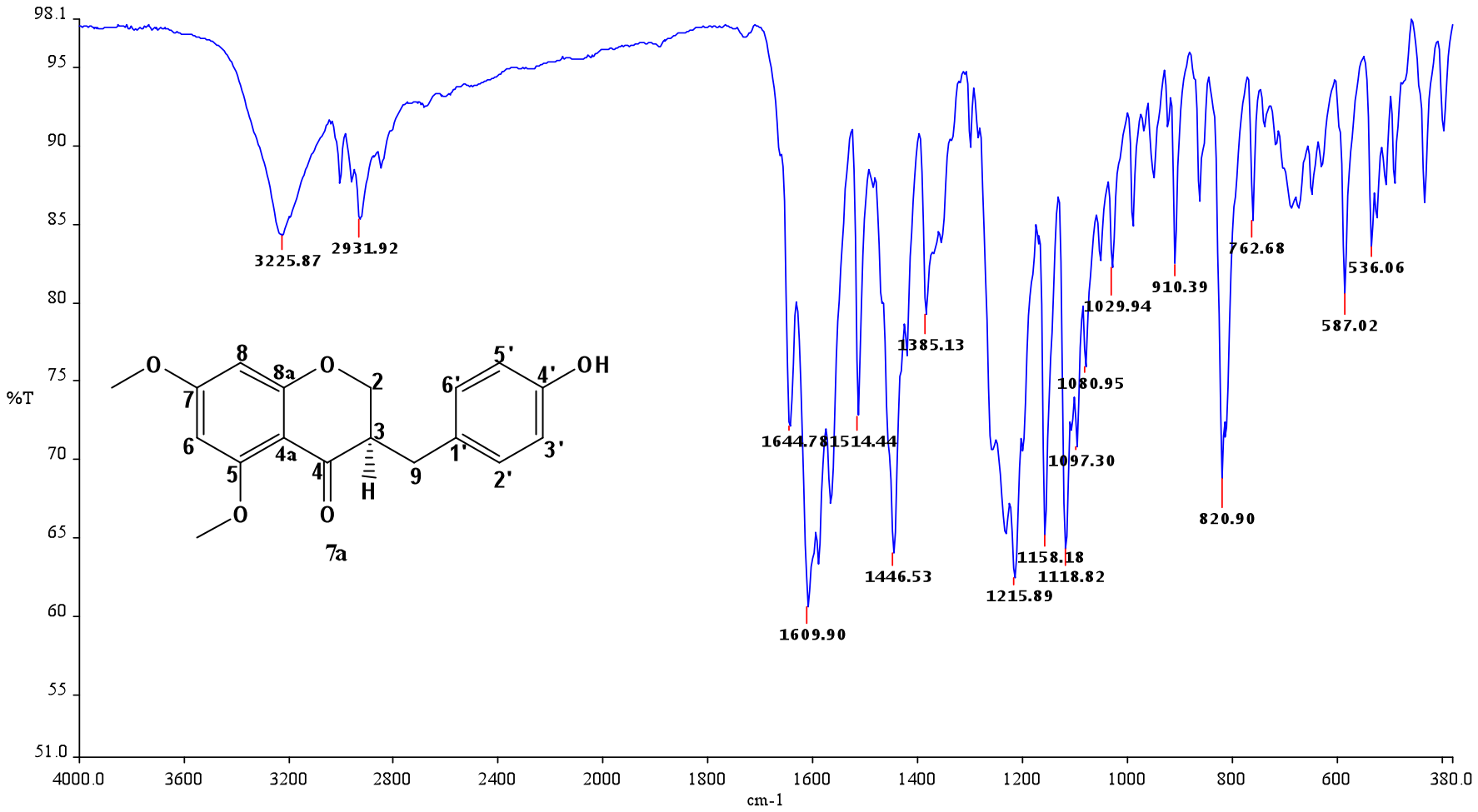
^{1}H-NMR spectrum of compound **5** in $CDCl_3$ (400 MHz)



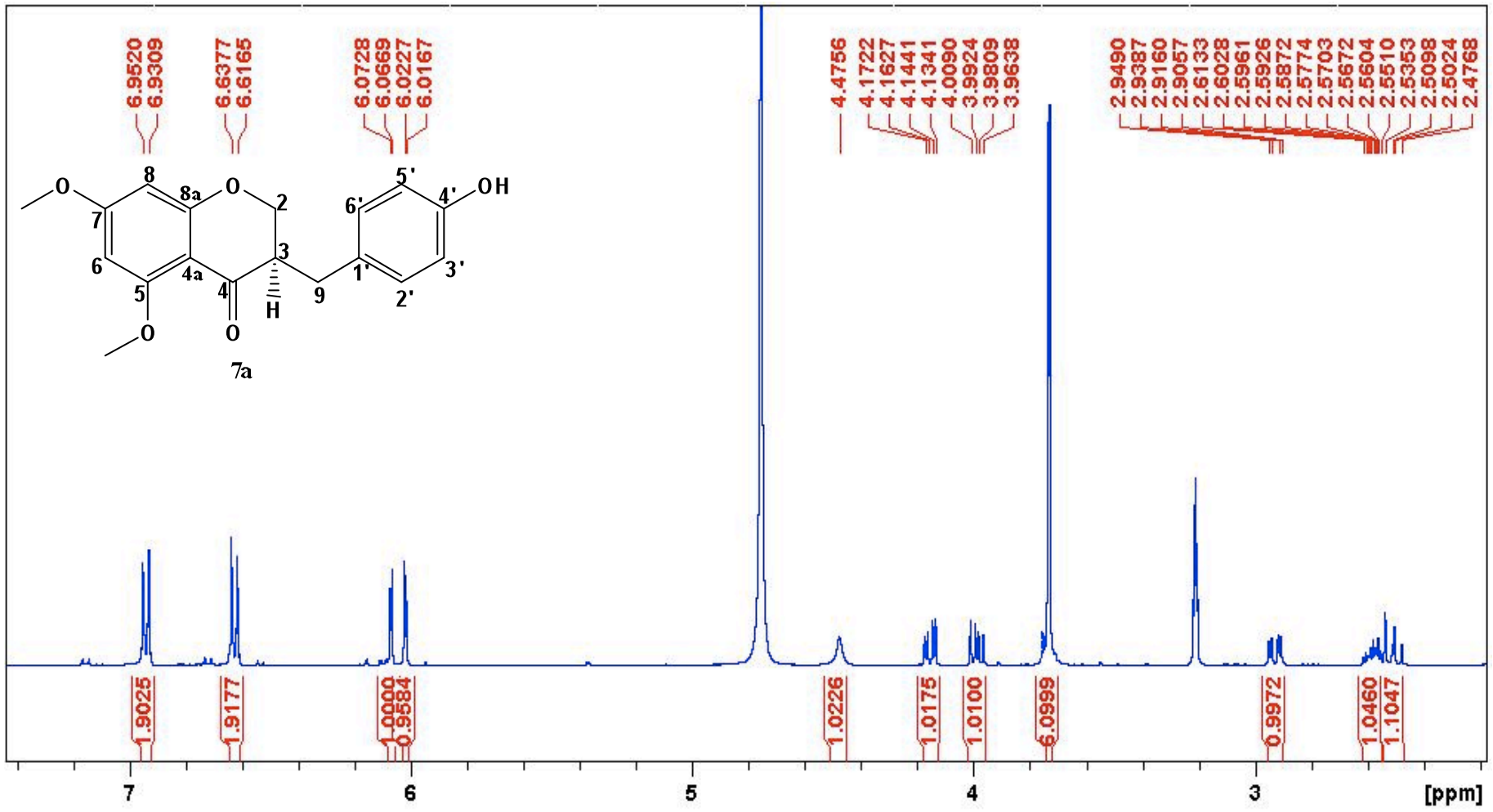
^{13}C-NMR spectrum of compound **5** in $CDCl_3$ (400 MHz)



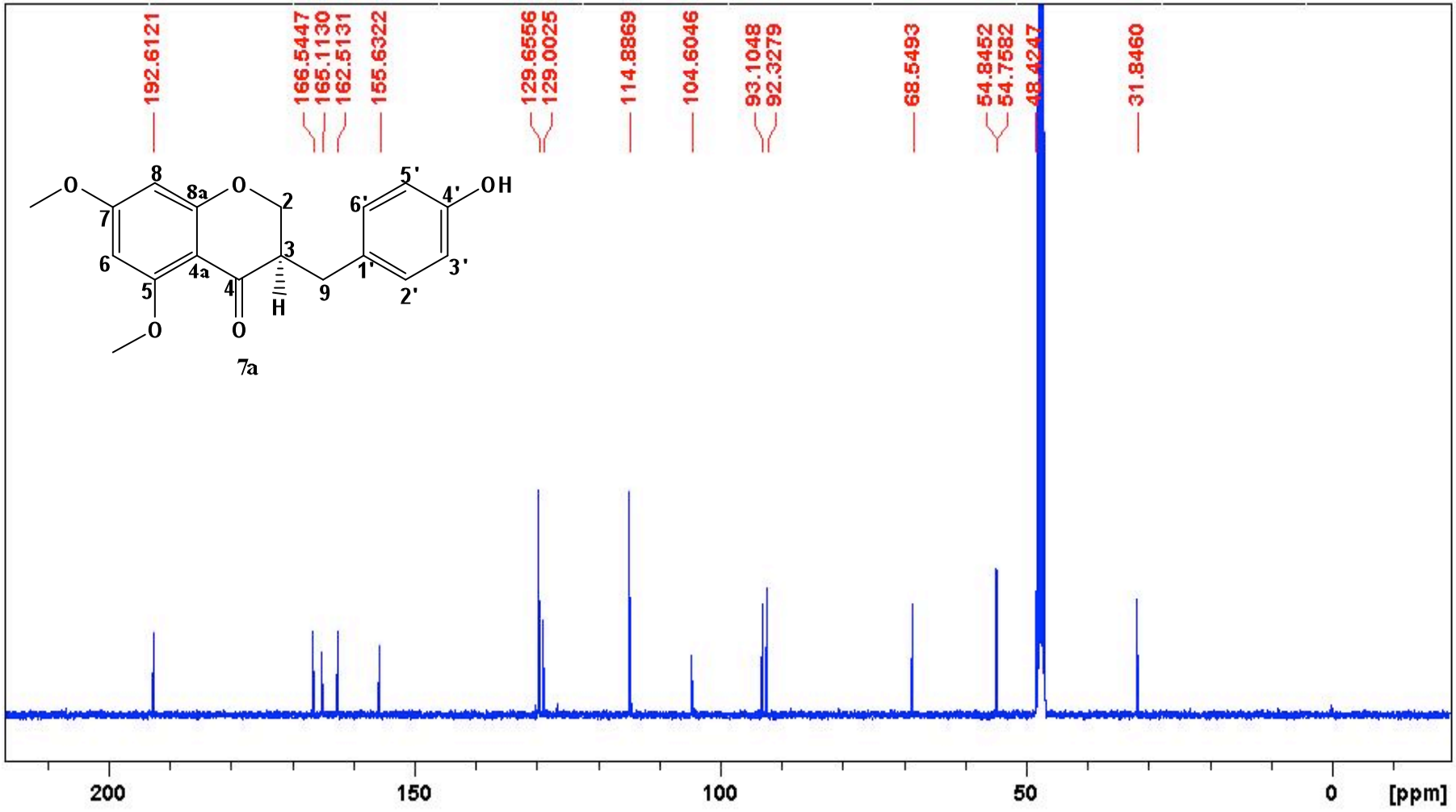
HRMS spectrum of compound **5**



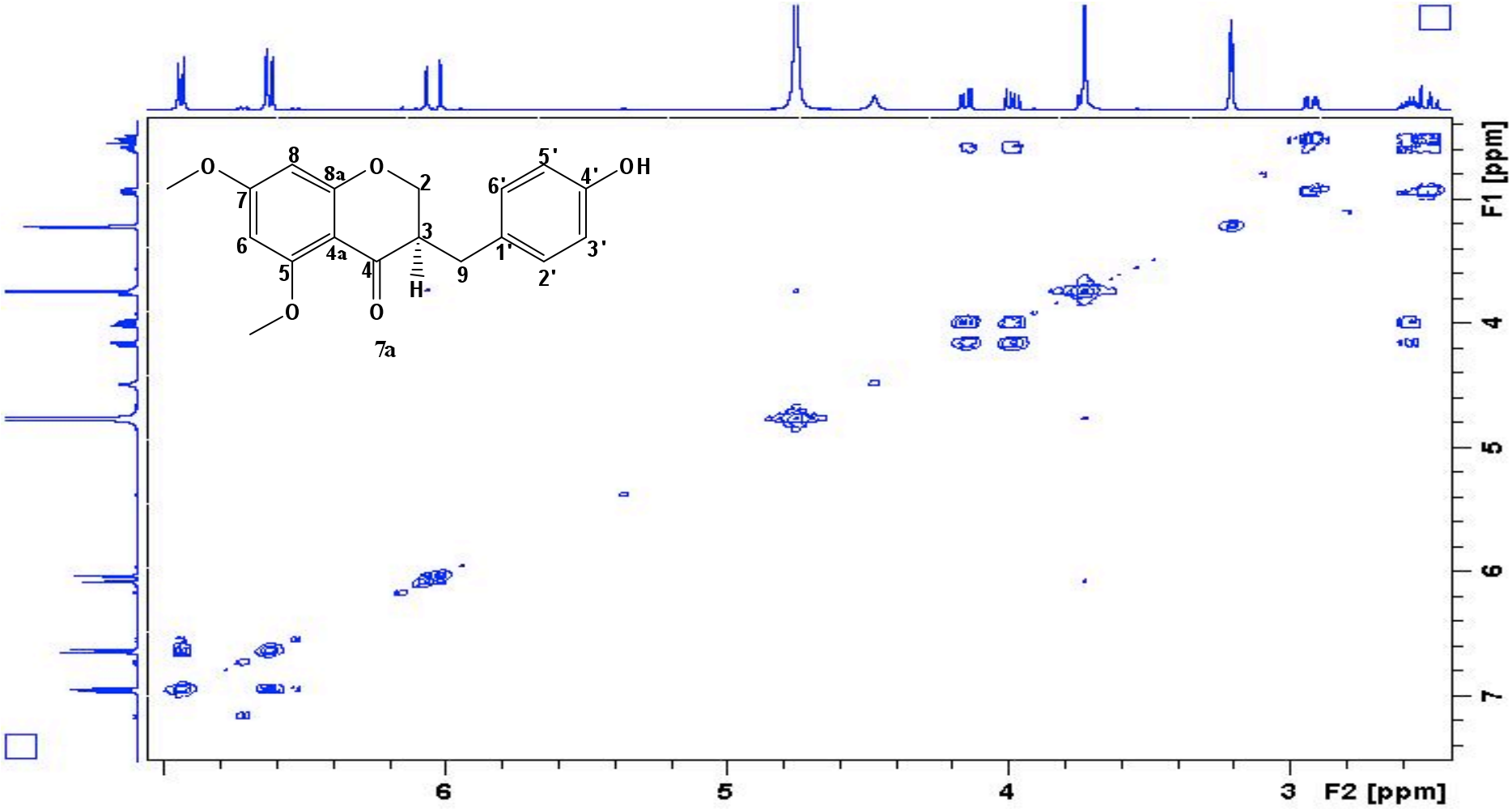
IR spectrum of compound **7a**



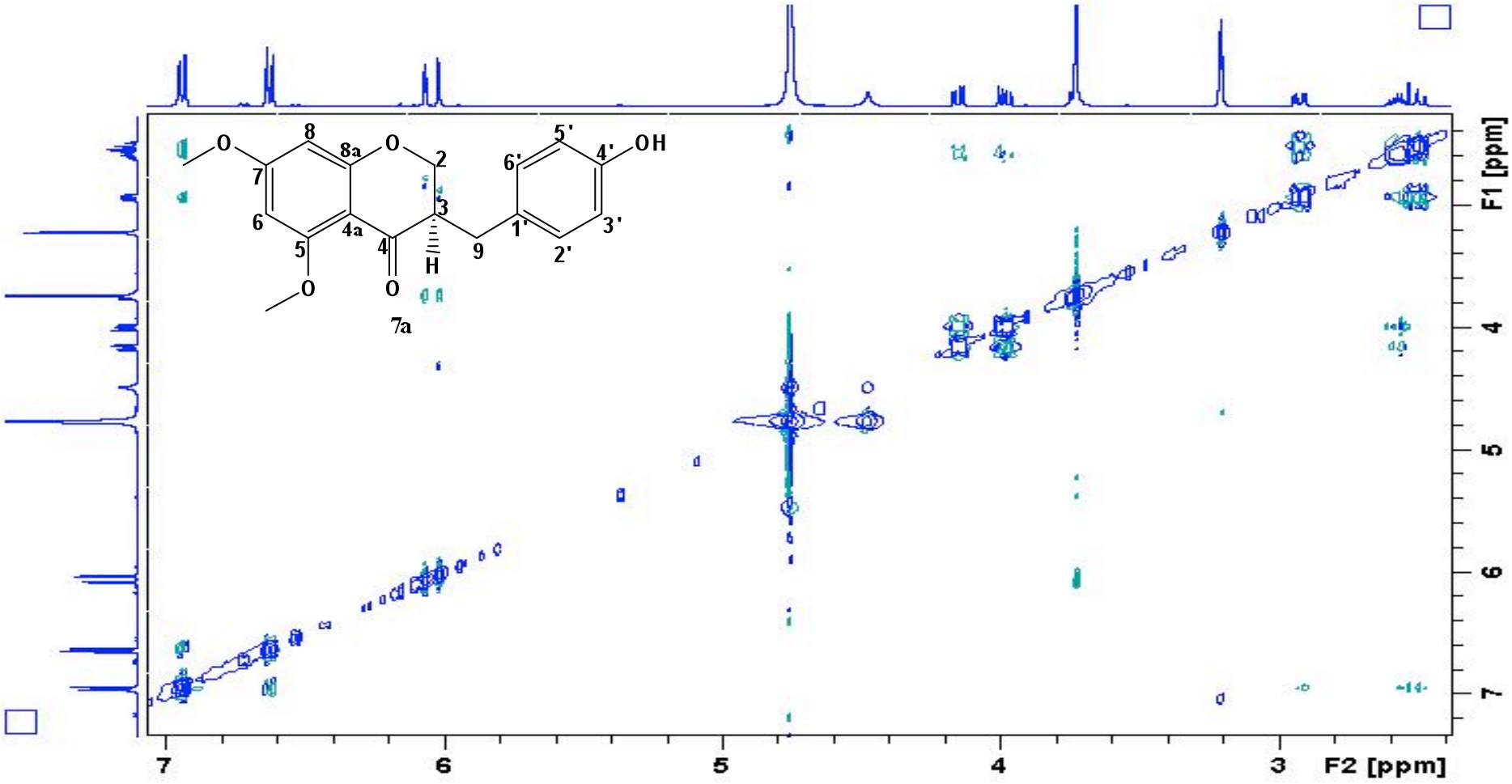
^{1}H-NMR spectrum of compound **7a** in MeOH (400 MHz)



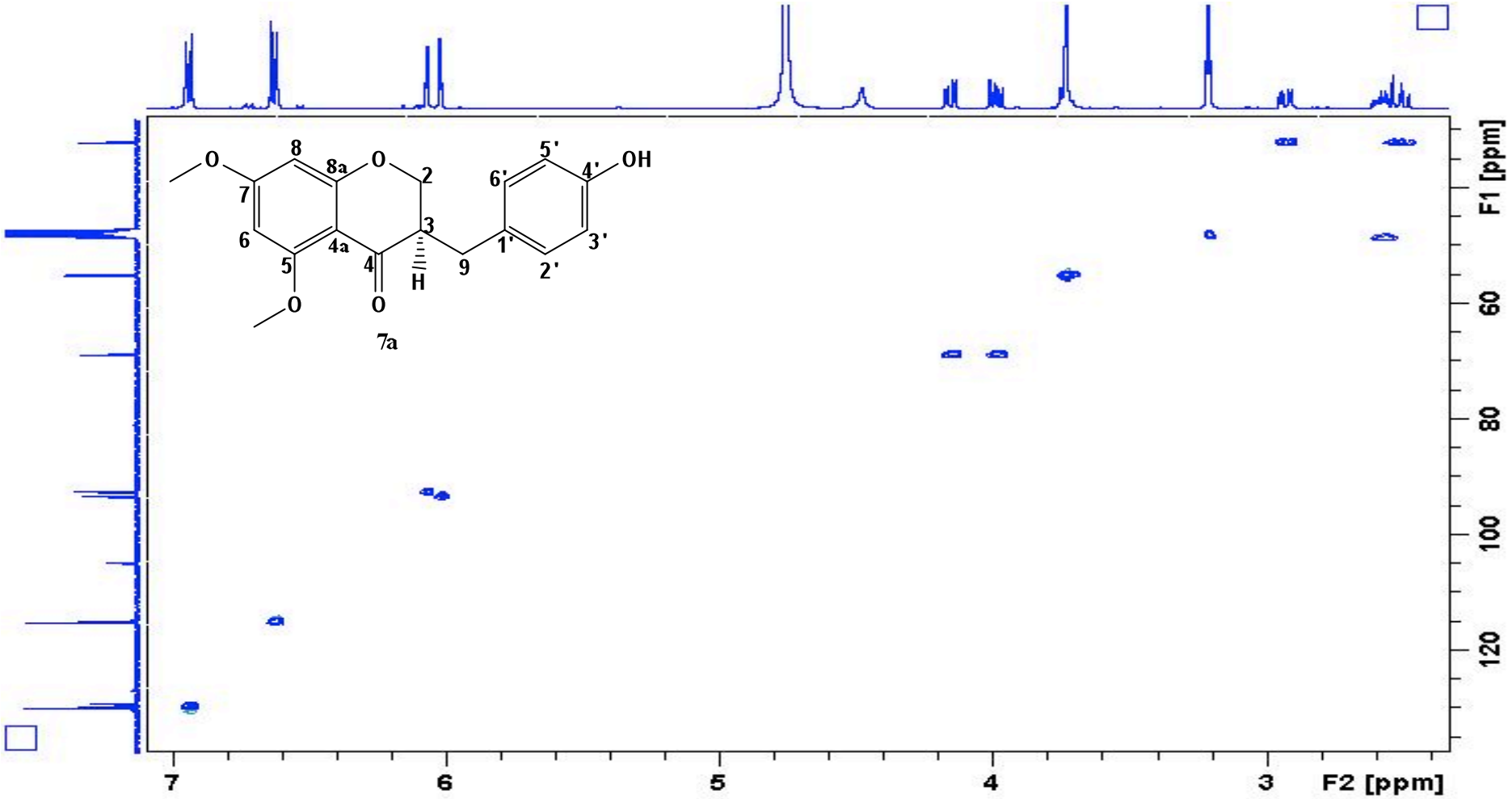
^{13}C-NMR spectrum of compound **7a** in MeOH (400 MHz)



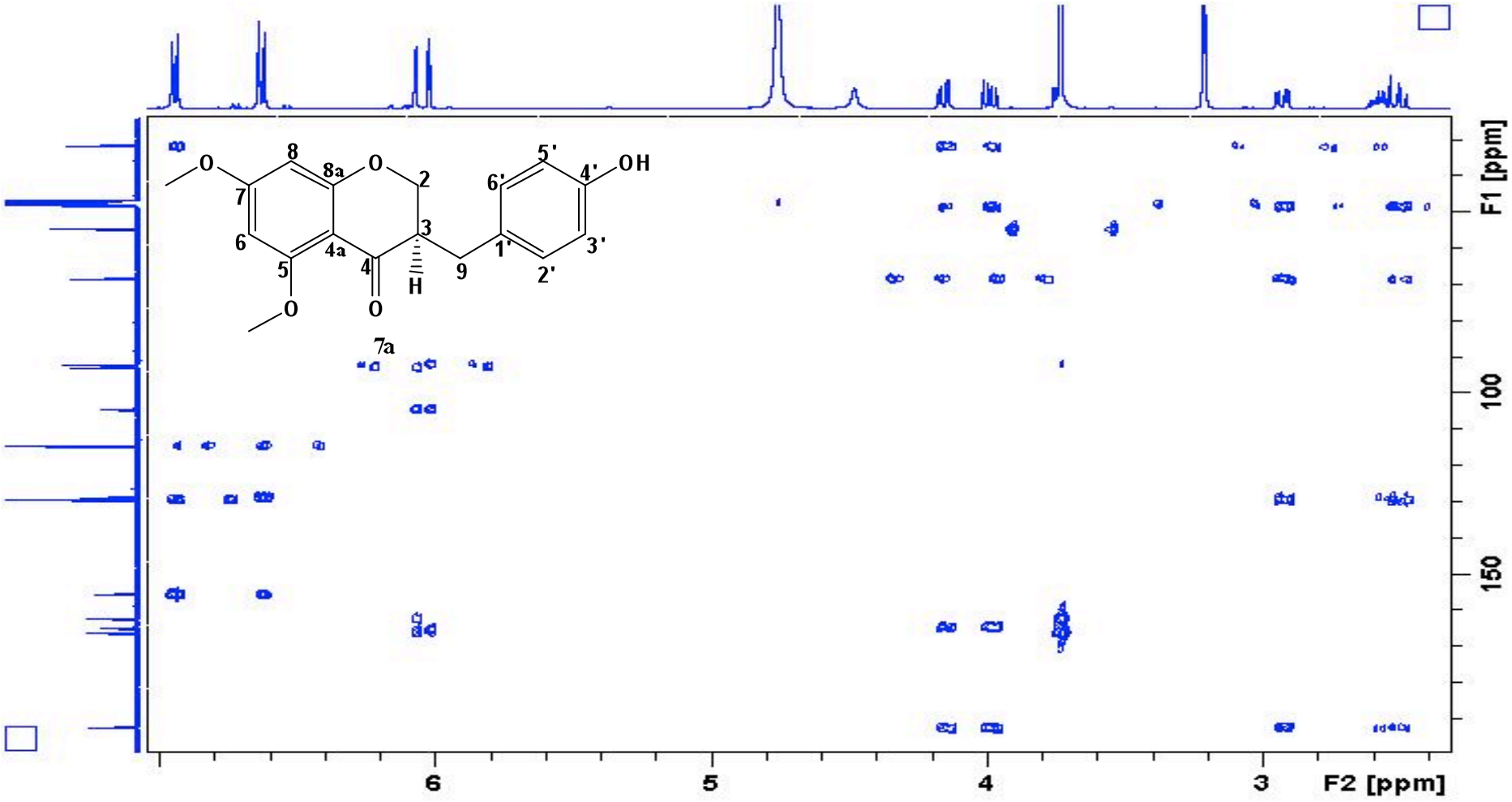
COSY spectrum of compound **7a** in MeOH (400 MHz)



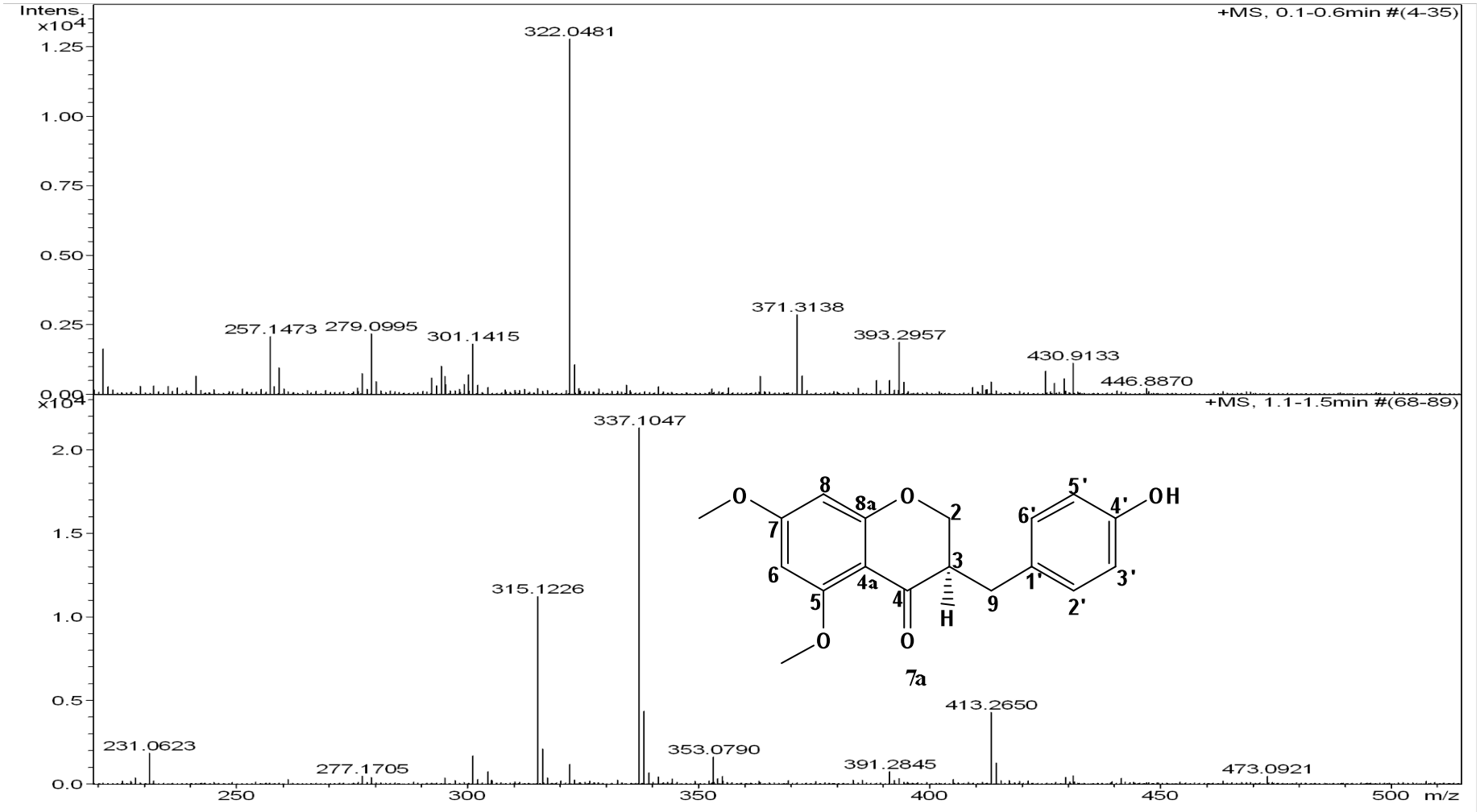
NOESY spectrum of compound **7a** in MeOD (400 MHz)



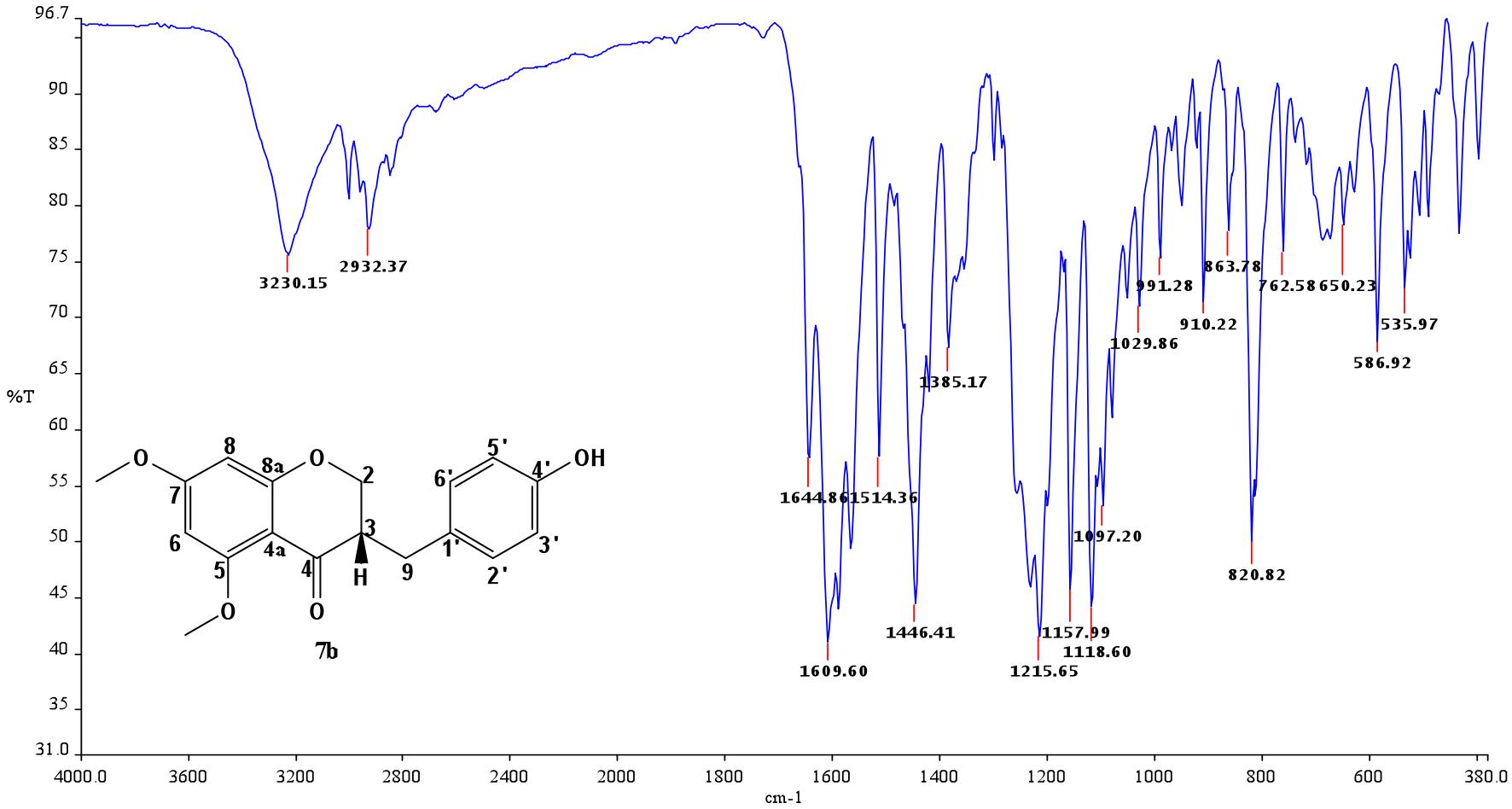
HSQC spectrum of compound **7a** in MeOD (400 MHz)



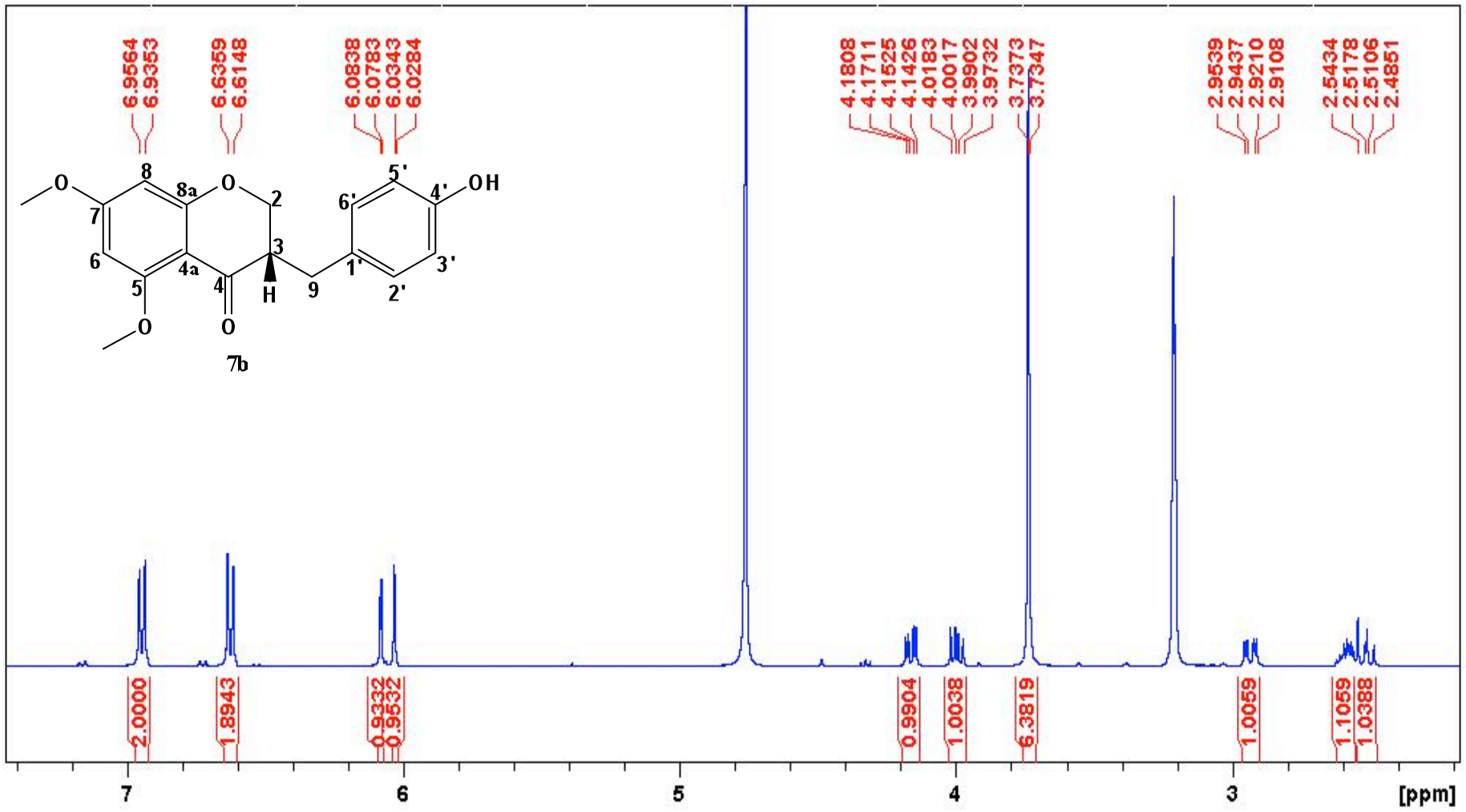
HMBC spectrum of compound **7a** in MeOD (400 MHz)



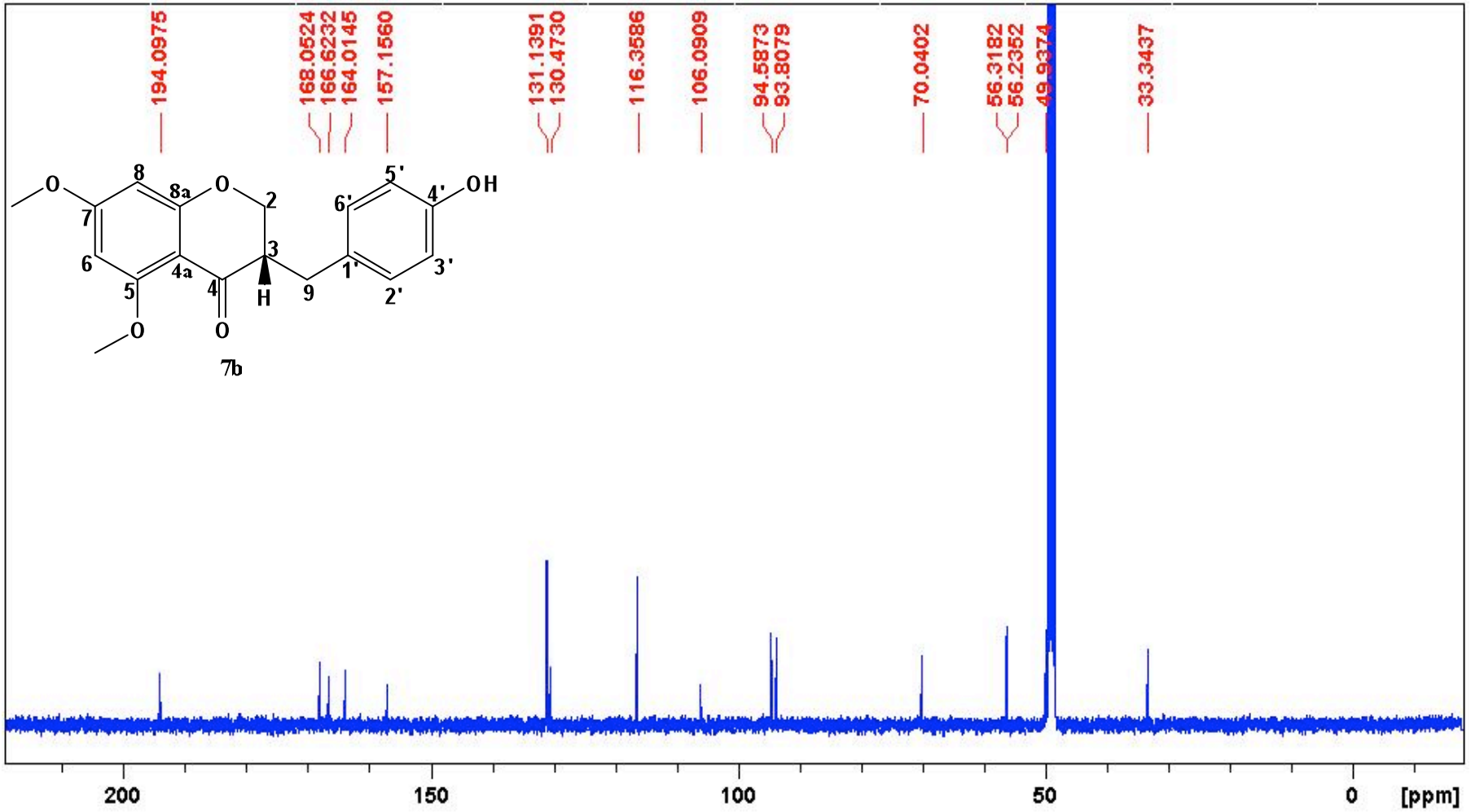
HRMS spectrum of compound **7a**



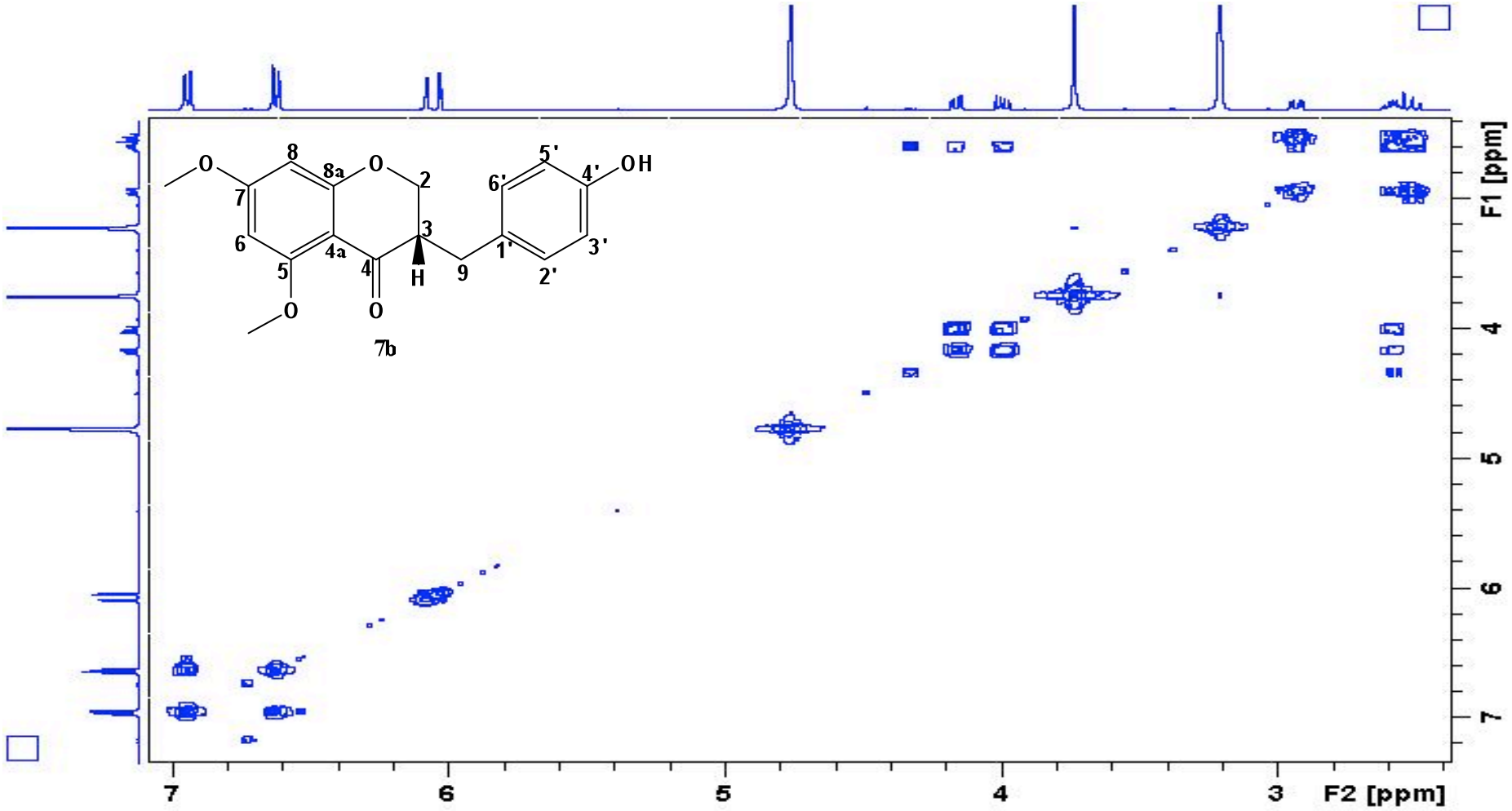
IR spectrum of compound **7b**



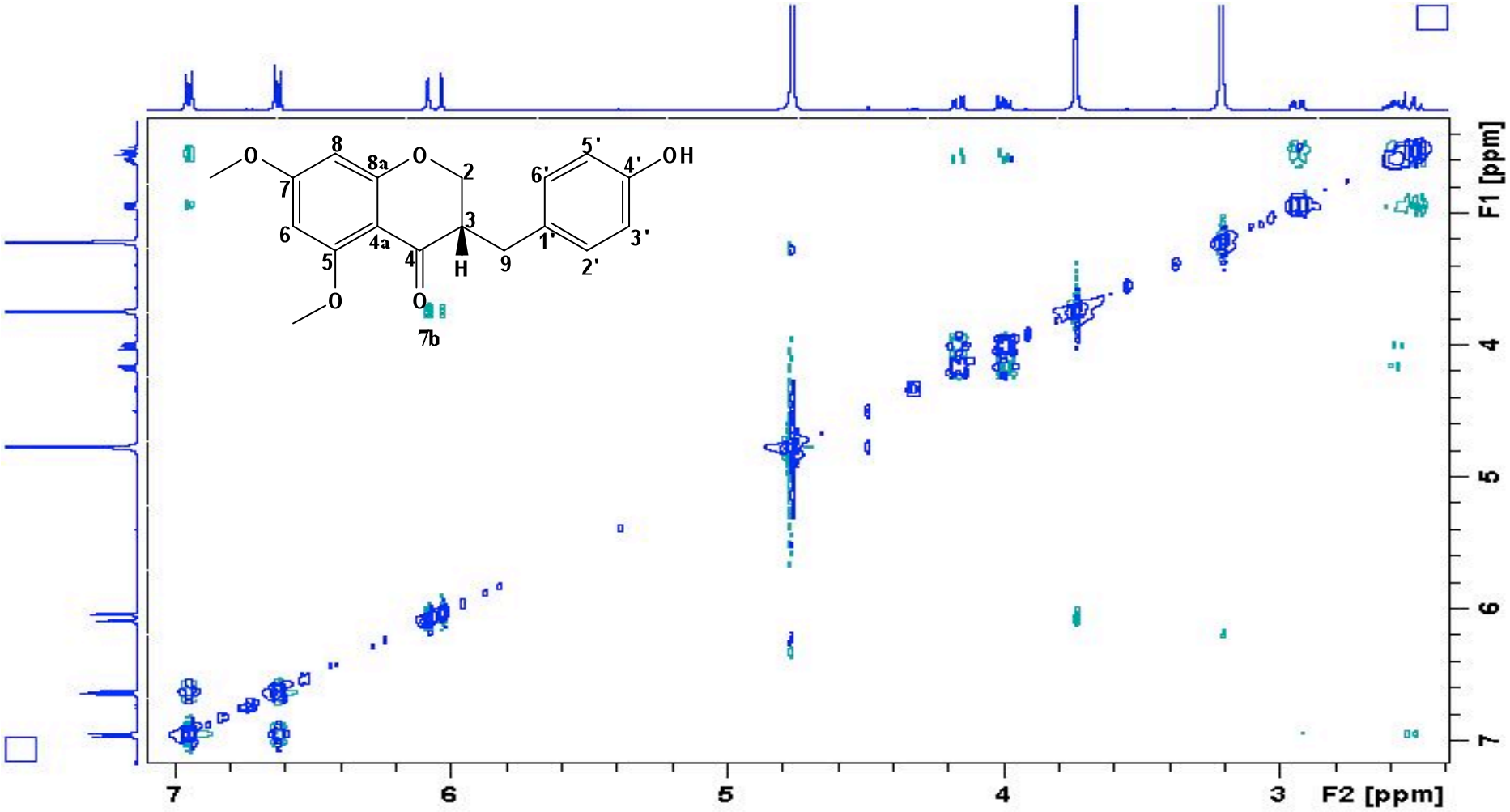
^{13}C-NMR spectrum of compound **7b** in $CDCl_3$ (400 MHz)



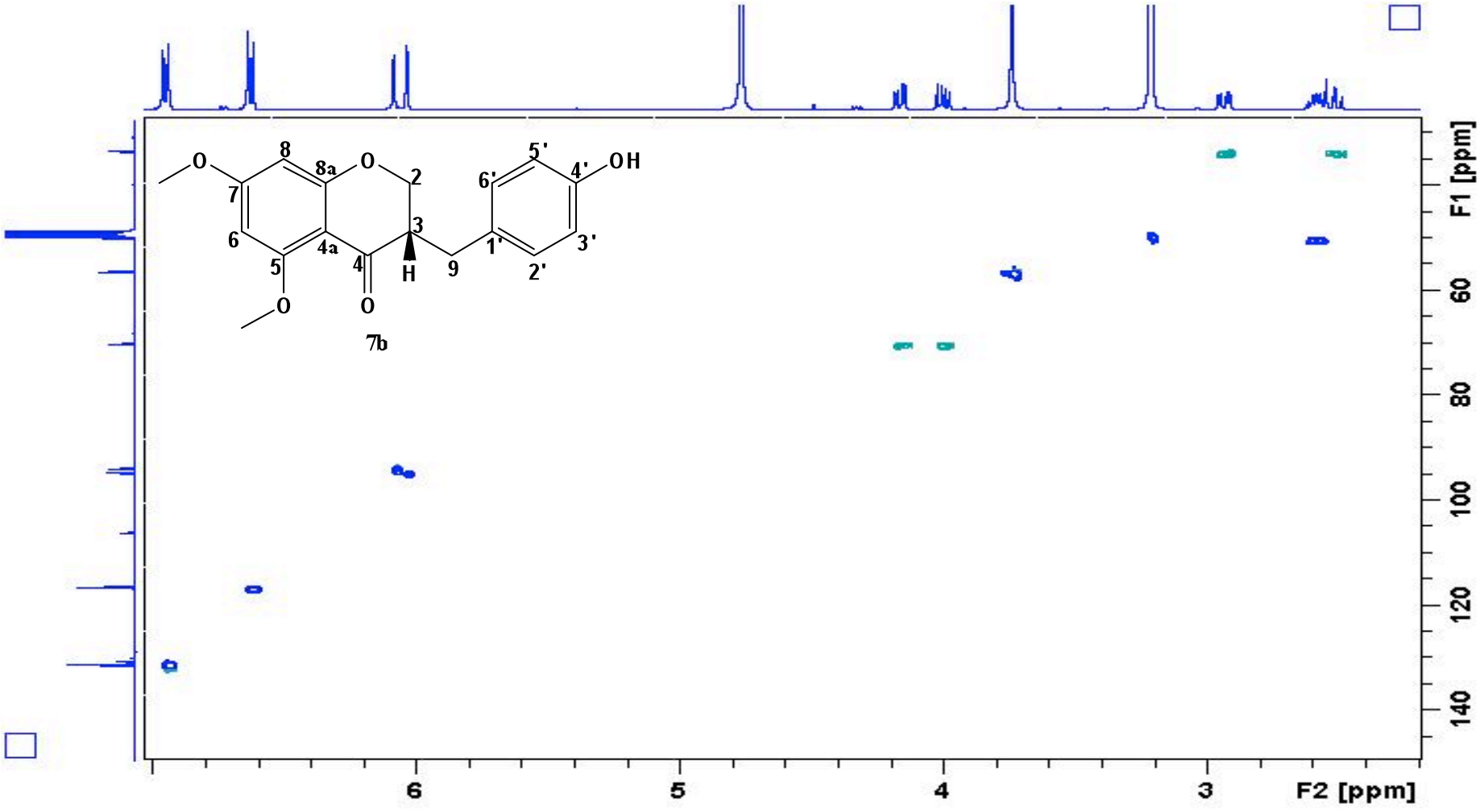
^{13}C-NMR spectrum of compound **7b** in MeOH (400 MHz)



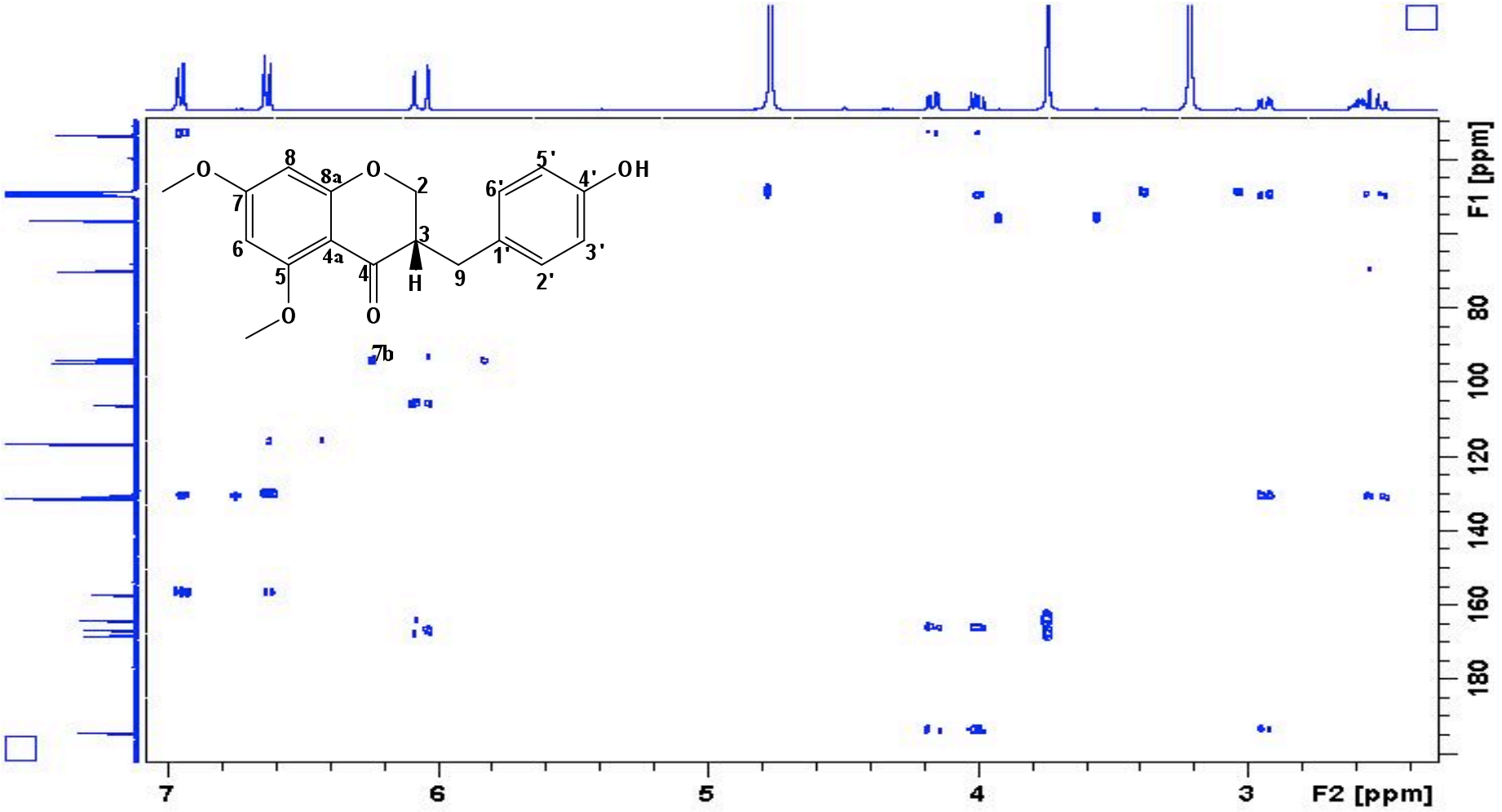
COSY spectrum of compound **7b** in MeOD (400 MHz)



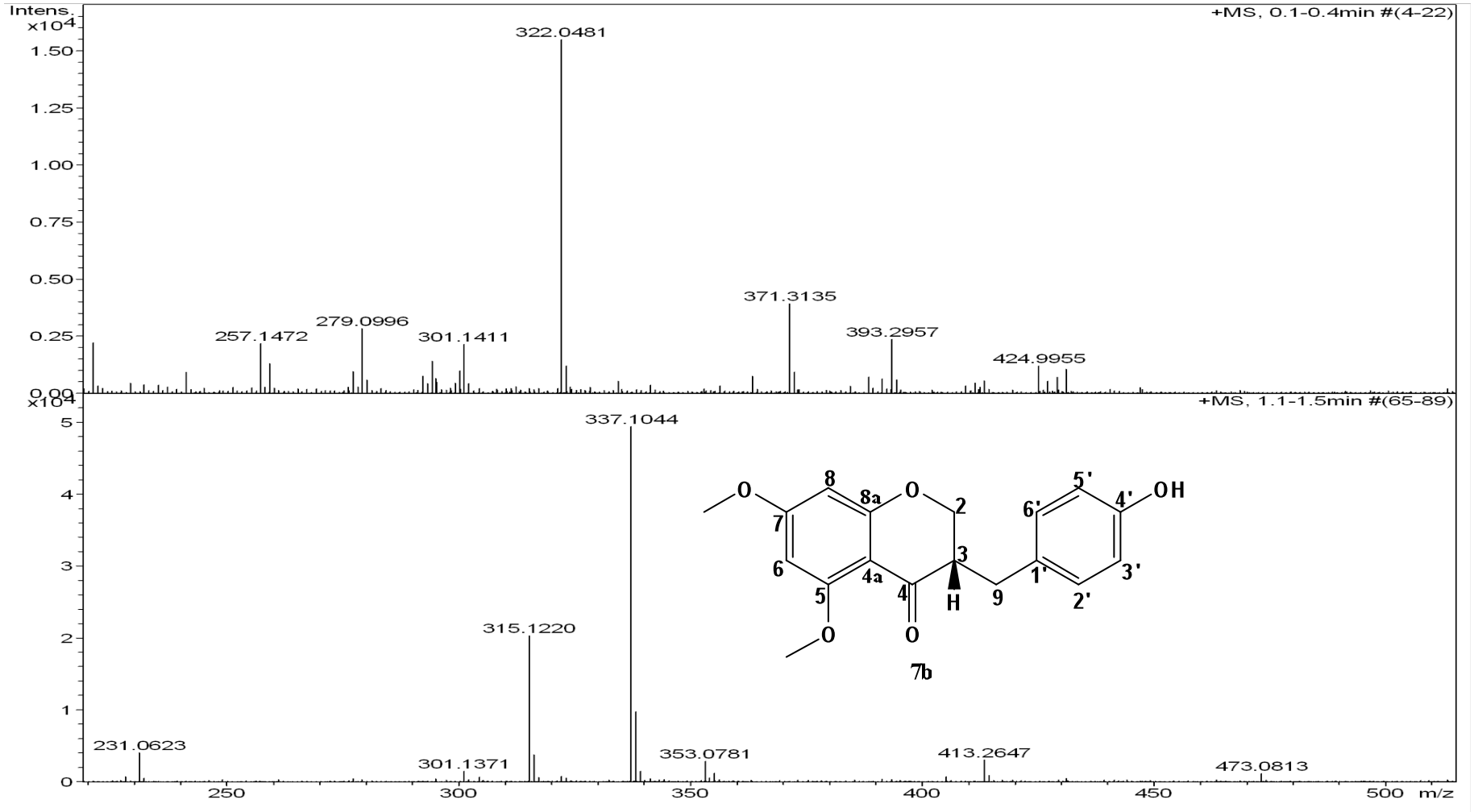
COSY spectrum of compound **7b** in MeOH (400 MHz)



HSQC spectrum of compound **7b** in MeOH (400 MHz)



HMBC spectrum of compound **7b** in MeOH (400 MHz)



HRMS spectrum of compound **7b**