

SYNTHESIS AND CHARACTERIZATION OF 1, 1-DIARYL VINYL SULFONES AS POTENTIAL ANTICANCER AGENTS

BY

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**Ph.D. Thesis submitted to the Department of Chemistry in partial fulfillment for the
Award of Doctor of Philosophy in Chemistry,
University of Ilorin, Ilorin, Nigeria**

MARCH, 2018.

CERTIFICATION

This is to certify that the thesis **SYNTHESIS AND CHARACTERIZATION OF 1, 1-DIARYL VINYL SULFONES AS POTENTIAL ANTICANCER AGENTS** is an original work carried out by **EGHAREVBA God'shelp Osas** with Matriculation Number **13/68EY002**. The report has been read, approved, and partially accepted on meeting the requirements for the award of the degree of Doctor of Philosophy in Chemistry of the University of Ilorin, Ilorin, Nigeria.

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DEDICATION

This project is dedicated to my Lord and Saviour, Jesus Christ.

ACKNOWLEDGEMENT

My avowed adoration unto my Lord, my King, and the Great Father of glory, pure Father of Light, who dwells in the splendour of unsearchable Light, the immortal, the invincible, the invisible, the only wise God. The one who stretches out the heavens as a curtain, spreads it out as a tent to dwell in, the God who sits upon the circle of the earth. The Most blessed, most glorious, most merciful, most compassionate, the Ancient of Days, the Almighty, the Victorious, and thy great name I praise.

Oh! Precious and beloved Jesus, my Saviour, with the greatness of thy arm you have brought this to pass. By your stretched out arm, you carried me over many waters, delivered me from great terrors of men and diverse temptations, and performed mighty signs and miracles. Indeed, you have helped me that was without strength and power; you saved me that had no saviour. Hmmm! You broke the arms of the wicked and the evil men. For I can boldly say that, “I got not the land of my possession by my own strength, neither did my own arm save me: but thy right hand and thy strong arm, Almighty God, and the light of the countenance of thy only begotten Son Jesus, has brought this great favour upon me and my household. Oh! I sing unto you my Lord a new song, for you have done marvellous things, your right hand and your holy arm, has got me this victory.

Now, I can boldly worship the King of kings, the Lord of lords. He is my shield and my defender, the giver of life. I bask in your bountiful care oh God, and tell of your measureless might and ineffable love. This poor boy, God’shelp, has enjoyed your mercies and grace despite how feeble, frail and unworthy I am thank you Jesus, my Redeemer, and best Friend.

As I thought and pondered on the need to go for further studies, I met a dear brother, Mr. Olowe Kehinde, a technologist at the department of Chemistry, University of Ilorin, Ilorin, Kwara State, and shared my desire with him. He opted to take me to someone he had in mind; a person he believed will guide me well. Little did I know that God was leading me on,

through the kindness of this beloved servant of God. Thanks so much Bro Olowe for guidance, I did not regret the choice of supervisor God led you to lead me to. God bless you Sir. May your generation continue to enjoy the providence of divine guidance in Jesus name; Amen.

My warm admiration goes to my supervisor, Prof. Omotayo O. Dosumu. I am deeply thankful for your supervision, love, care, and many other wonderful things you have done for me. Thanks so much for providing me with support when I needed it the most. You are more than a supervisor; you are a father, a mentor, a guide, above all a brother in Christ. You prayed for me and with me, counselled me to be patient when I felt anxious, you invited me to eat with you on several occasions, and you gave me your office and lab to use for my study and research respectively. You never yelled at me for once in five years of being under your tutelage, even when I made several mistake at the outset of my study. Consistently, you reached out to me when I travelled abroad for two whole years. Whenever I called you with my air time, you terminated the call and called me back again not minding the cost. Really, I am grateful Sir. Thanks for being such an example of a patient, godly, Christ like, and never angry supervisor. I celebrate you Sir. Words will fail me to tell of all the wonderful experiences I have had under your auspicious guidance. May the Lord continue to bless you and all yours in ways beyond human comprehension in Jesus name; Amen.

To my co-supervisor, Dr. S. O. Oguntoye, I am so grateful Sir. The quality of your supervisor-ship style has greatly inspired me. Thanks for being so approachable and ever supportive. You were always there for me, offering constructive productive positive correction to my research. As little as I am to you Sir, you always referred to me as your brother; this humbled me so many a times. When you had a lot to do and could not readily assist you were so quick to apologize for the delay and making up for it immediately. I cannot thank you enough Sir. You read through my thesis with such dexterity within a short while. I

am very grateful Sir. The good Lord will reward you greatly. You and your household shall never lack the supportive hands of the Almighty God. Amen.

Cheerful I am as I reminiscence over the support got from the department of Chemistry and the department of Industrial Chemistry, University of Ilorin, Ilorin. My appreciation goes to all the academic and non academic staff of this twin department. Thank you so much for your individual and collective assistance. Truly I am grateful. God bless you all. Time would not permit me to acknowledge the names of everyone one who has contributed immensely to the success of this research one after the other, nonetheless, I will not fail to appreciate the HOD department of Chemistry, Dr. L. A. Usman, the Post Graduate coordinator, Dr. A. C. Tella, the Academic Planning Committee chairman, Prof. J. A. Obaleye, Dr. Baba Alafara, the HOD Industrial Chemistry, Dr. O. O. Oluwaniyi, and a host of many VVIP's. God bless you all.

The World Academic of Science (TWAS) Trieste, Italy and Council of Scientific and Industrial Research (CSIR), Hyderabad, India is delightedly appreciated for their tremendous support in providing me the TWAS-CSIR fellowship. A huge thank you to CSIR-Indian Institute of Chemical Technology, Hyderabad, India for the bench space and the conducive working atmosphere offered to me to carry out my research. My ecstatic gratitude goes to my host supervisor at CSIR-IICT, Hyderabad, Dr. Ahmed Kamal, the then only Outstanding Scientist in the institute. Thanks so much Sir for your great support. God bless you Sir. I call to remembrance the unforgettable support of the scientist, technologist, research scholars, administrative staff and casual workers as well as the entire CSIR-IICT community for their love and care. Thank you for making a home for me in the institute. May the good Lord reward you abundantly. Amen.

Christian Fellowship Centre (CFC), Hyderabad, India blessed art thou. Thanks so much for the Christ like love and care showered over me while I was with you. Thank you for

unveiling Jesus to me in ways I never knew. I am grateful to my beloved brother and discipler, Bro. Zac Poone and his helpmeet Dr. Annie Poone. To my brethren Bro. Marredy and family, Bro. Balulu as I fondly call him, Bro. Rejo and family, Bro. Kishore and family, Bro. Ravie and family, Bro. Rohan and family, and the entire family of CFC in diaspora. Thank you for giving to the Lord. The impact of your love, fellowship, simplicity of worship and life, and christlikeness has impacted me more than you can ever imagine. Thank you all. God bless you.

God brings you across certain person(s) who become pillars in your life. One of such a rear gem is Pastor M. R. Popoola, a transparent addict follower of Jesus Christ, the general overseer of the New Testament Christian Mission (NTCM) Int'l and his beloved wife, Mummy F. M. Popoola. Thanks so much Daddy and Mummy for all your support in cash and in kind, prayers, encouragement and for the spiritual nourishment of my soul. I am very grateful. How can I thank God enough for this very great and loving family of God in NTCM. You have been so wonderful. Thank you for your love, visits, prayers, counsels, contributions of all sorts, and follow up at various stages of the research. I am truly grateful. God bless you all richly. To my very understanding and ever supportive brethren, amongst whom are: Pastor C.O. Bukoye and wife, Pastor J.O. Adeoti and wife, Daddy and Mummy T. J. Olanrewaju, Daddy J. K. Ogundele and wife, Pastor J. J. Buoye and wife, Pastor J.O. Afolayan and wife, Pastor Wale Jimoh and wife, all the Pastors in this great family of God, the dynamic fellowship of the young adult, the NTCM churches within Ilorin and outside Ilorin, the list is so long, no matter how hard I try, I will not be able to mention them all one after the other. You have all done wonderfully well. The Lord whom you have served will reward you greatly individually. God bless you all.

I am warmly thankful to Mummy Oni, of KSTSC for her splendid support. Thanks so much Ma, for the love and priceless assistance rendered to me and my family during my course of

study. The eternal God will reward you abundantly. You and your household shall not lack the help of God in all your ways, God bless you richly Ma. Many thanks to the incumbent Principal, A. S. Raman, the former Principal, Alhaji Jimoh, the entire staff and students of Government Day Secondary School, Kulende, Ilorin. Thanks so much to you all. I cannot but say a very big thank you to the HOD Science, Mummy Adeniyi, T. V., for your ever supportive counsels and ceaseless encouragement to me and my family. God bless you Ma. Very plenty thank you to Dr. Amao, J. O., Mr. Abubarkar, Mrs. Hamzat, Mrs. Balogun, Mrs. Akojede B., and a host of many others. Thank you so much for your support. God bless you tremendously.

I am eternally grateful to all my teachers who laboured tirelessly over me to build a solid foundation upon which my degree studies were founded. Sincere thanks to: Mr. Sanusi, Mrs. Asene, Mr. Oyinlo, Mr. Adebayo, Prof. O.T. Asekun, Prof. O. B. FAMILONI, Dr. C. Isanbor, Dr. Mrs. Babatunde, Dr. O.T. Olaniyi, Prof. Jide Ige, Dr. Adeniyi, A.A., Dr. Owoyomi, just to mention a few. Many thanks to you all, God bless you.

And finally, my sincere unrelenting appreciation goes to my family members. You have done more than my words can possible express. You have spent money; time, energy, and everything spendable just to see to it that this day come to be. How can I forget your endless continual prayers for me, your sweet words of constant reassurances and motivation, those doses of kind gestures, and tireless efforts to provide a consistent conducive atmosphere love, peace, smiles, grace, and tranquillity, the tons of unmentioned hidden sacrifices you put in, just to see to it that I was not distracted from achieving our set goals. God helped you all to keep uplifting my spirit, thus I was never depressed. While away for study abroad, I had no reason to run helter skelter, my mind was steadfast and sure because of God's abundant life in you all and your graceful comportment. Thanks so so much for all you have done for me my dearly beloved family. I love you all. Frankly speaking, words cannot suffice to convey

my depth of gratitude to each and every one of you. But the good Lord, the great God of heaven whom you have served in this regard will reward you greatly.

A standing ovation to my one and only best friend for life, Mrs. Egharevba Peace Boluwatife, the wife of my youth, the mother of my children, Jedidiah and John, the one who felt and shared and bore the very heat of the journey. My DEAR, you literally partook in the ups and downs that came with this work, but you lovingly and caringly endured all of it, prayed passionately for God's help and timely intervention. You starved yourself of the luxuries life, and many times denied yourself of some necessary needs of life and comfort just to make sure we achieve this goal together, you have done excellently well. Thanks so much my DEAR, thank you my beloved GRACIOUS VINE. God bless you abundantly. Further appreciation goes to my wonderful amiable loving ever caring ever praying Christ centred Parents: Daddy Egharevba Alexander, Daddy Ajileye S. S., Mummy Egharevba Sarah, and Mummy, Dr. Mrs. Ajileye, M. K, you have all laboured tirelessly over me my beloved parents and stood firm with us to see the accomplishment of this laudable goal. God bless you richly, you shall live long to enjoy the fruit thereof. In the same breath, I am saying thank you to my wonderful siblings: Sis. Gloria Osaga and her hubby Pastor Sage Osaga, Sis. Blessing, Sis. Charity, Sis. Joy and her hubby, Barr. Kelly Agbonzie, Bro. Femi, Bro. Segun, Sis. Esther, and Bro Benjamin. I cannot appreciate you all enough. God bless you and reward you marvellously.

Appreciating men is a tedious and also a laborious task, doing it appropriately is more complicated. So, if I have omitted any, it's not on purpose, please pardon me. To everybody mentioned or unmentioned who have contributed in one way or the other, God bless you abundantly in Jesus mighty name. Amen.

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LIST OF ABBREVIATIONS

¹³C-NMR: Carbon-13 Nuclear Magnetic Resonance

¹H-NMR: Proton Nuclear Magnetic Resonance

[AKL-CA-1: 5-\(2,2-dibromovinyl\)-1,2,3-trimethoxybenzene.](#)

[AKL-CA-2: C₁₁H₁₂O₃, 5-ethynyl-1,2,3-trimethoxybenzene](#)

[AKL-CA-3: C₁₈H₁₉IO₅S, \(E\)-5-\(1-iodo-2-tosylvinyl\)-1,2,3-trimethoxybenzene](#)

[AKL-CA-4A: C₂₄H₂₄O₅S, \(Z\)-1,2,3-trimethoxy-5-\(1-phenyl-2-tosylvinyl\)benzene](#)

[AKL-CA-4B: C₂₅H₂₆O₆S, \(Z\)-1,2,3-trimethoxy-5-\(1-\(4-methoxyphenyl\)-2-tosylvinyl\)benzene](#)

[AKL-CA-4C: C₂₆H₂₈O₇S, \(E\)-5-\(1-\(2,4-dimethoxyphenyl\)-2-tosylvinyl\)-1,2,3-trimethoxybenzene](#)

[AKL-CA-4D: C₂₆H₂₈O₇S, \(Z\)-5-\(1-\(3,4-dimethoxyphenyl\)-2-tosylvinyl\)-1,2,3-trimethoxybenzene](#)

[AKL-CA-4E: C₂₆H₂₈O₇S, \(Z\)-5-\(1-\(3,5-dimethoxyphenyl\)-2-tosylvinyl\)-1,2,3-trimethoxybenzene](#)

[AKL-CA-4F: C₁₈H₁₈O₅S, 4-methylphenyl \(3,4,5-trimethoxyphenyl\)ethynyl sulfone](#)

[AKL-CA-4G: C₂₄H₂₃FO₅S, \(Z\)-5-\(1-\(4-fluorophenyl\)-2-tosylvinyl\)-1,2,3-trimethoxybenzene](#)

[AKL-CA-4H: C₂₅H₂₃F₃O₅S, \(Z\)-1,2,3-trimethoxy-5-\(2-tosyl-1-\(4-\(trifluoromethyl\)phenyl\)vinyl\)benzene](#)

[AKL-CA-4I: C₂₄H₂₂F₂O₅S, \(E\)-5-\(1-\(2,4-difluorophenyl\)-2-tosylvinyl\)-1,2,3-trimethoxybenzene](#)

[AKL-CA-4J: C₂₄H₂₂F₂O₅S, \(E\)-5-\(1-\(3,4-difluorophenyl\)-2-tosylvinyl\)-1,2,3-trimethoxybenzene](#)

AKL-CA-4K: $C_{26}H_{27}FO_6S$, (E)-5-(1-(5-ethoxy-2-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

AKL-CA-4L: $C_{24}H_{22}ClFO_5S$, (E)-5-(1-(3-chloro-4-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

AKL-CA-4M: $C_{24}H_{23}ClO_5S$, (Z)-5-(1-(4-chlorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

AKL-CA-4N: $C_{24}H_{22}Cl_2O_5S$, (E)-5-(1-(3,4-dichlorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

AKL-CA-4O: $C_{25}H_{23}NO_5S$, (Z)-4-(2-tosyl-1-(3,4,5-trimethoxyphenyl)vinyl)benzonitrile

AKL-CA-4Q: $C_{25}H_{22}F_4O_5S$, (E)-5-(1-(2-fluoro-3-(trifluoromethyl)phenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

AKL-CA-4R: $C_{25}H_{25}FO_5S$, (E)-5-(1-(2-fluoro-5-methylphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

AKL-CA-4T: $C_{25}H_{25}FO_5S$, (E)-5-(1-(3-fluoro-4-methylphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

AKL-CA-4U: $C_{30}H_{28}O_5S$, (Z)-4-(2-tosyl-1-(3,4,5-trimethoxyphenyl)vinyl)-1,1'-biphenyl

AKL-CA-4V: $C_{18}H_{18}O_5S$, 4-methylphenyl (3,4,5-trimethoxyphenyl)ethynyl sulfone

AZT: 3'-azido-3'-deoxythymidine (zidovudine)

BC: Before Christ

BRCA1: Breast cancer 1

CA 1: Combretastatin A1

CA 4: Combretastatin A4

CA-4P: Combretastatin-4,3-O-phosphate

CFC: Chlorofluorocarbons

DACH: Diaminocyclohexane

DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene

DDC: 2'-3'-dideoxycytidine (zalcitabine)

DDT: Dichlorodiphenyltrichloroethane

DIB: Diacetoxiodo benzene

DNA: Deoxyribonucleic acid

EBV: Epstein-barr virus

EMR: Electromagnetic radiation

EPIC: European Prospective Investigation into Cancer

ESI: Electro spray ionization

EW: Electron withdrawing

HBV: Hepatitis B virus

HCV: Hepatitis C virus Helicobacter pylori (H.pylori)
HHV-8: Human herpesvirus
HIV: Human Immunodeficiency Virus
HPV: Human papilloma viruses
HRMS: High resolution mass spectroscopy
HTLV-1: Human T-cell leukemia virus type 1
IARC: International Agency for Research on Cancer
IR: Infra red
MRI: Magnetic resonance imaging
MS: Mass spectroscopy
MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
NCI: National Cancer Institute
NMR: Nuclear Magnetic Resonance
NMSC: Non Melanoma Skin Cancer
PDE4: Phosphodiesterase 4
RNA: Ribonucleic acid
SAR: Structural Activity Relationship
USDA: United State Department of Agriculture
UV: Ultraviolet
WHO: World Health Organization

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ABSTRACT

Cancer has become a major health concern globally. Interestingly, anticancer agents are being synthesized from small molecules derived from either plants or aquatic organisms. For example, Combretastatin A-4 (CA-4) isolated from the bark of the South African tree *Combretum caffrum*. CA-4 is one of such important anticancer agents which have gained the attention of a vast majority of investigators. However, it has problems of bioavailability and isomerisation of the biologically active Z-configured double bond into the inactive E-configuration. There is therefore the need to synthesize a new library of CA-4 analogues. The objectives were to: (i) synthesize 1,1-diarylethene derivatives having a sulfonyl (SO₂R) group at the 2 - carbon position; (ii) characterize all synthesized compounds; and (iii) determine the cytotoxicity of the synthesized compounds using various human cancer cell lines.

The desired target molecules were obtained from 3,4,5-trimethoxy benzaldehyde prepared by Corey-Fuchs olefination to yield 5-(2,2-dibromovinyl)-1,2,3-trimethoxybenzene. This was reacted with 1, 8- diazabicyclo[5.4.0]undec-7-ene (DBU) in acetonitrile to give 5-ethynyl-1,2,3- trimethoxybenzene. Further treatment of the product with diacetoiodobenzene (DIB), sodium-p-toluenesulfonate and potassium iodide in acetonitrile yielded (E)-5-(1-iodo-2-tosylvinyl)-1,2,3-trimethoxybenzene). This was made to undergo Suzuki-Miyaura coupling with different substituted arylboronic acids in tetrahydrofuran (THF) to give different combretastatin sulfonyl compounds. The compounds were characterized using proton (¹H) and carbon-13 (¹³C) Nuclear Magnetic Resonance (NMR) Spectroscopy, Fourier Transform Infra Red Spectroscopy (FTIR), Electro Spray Ionization Mass Spectrometry (ESI-MS) and High Resolution Mass Spectrometry (HRMS). The *in vitro* cytotoxicity assay was carried out against four human cancer cell lines MDA-MB 231(breast cancer), HeLa (cervical cancer), A549 (lung cancer), and IMR-32 (neuroblast cancer), along with normal cell line HEK-293 (human embryonic kidney cell) by employing 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.

The following results were obtained:

- (i) twenty novel 1,1, diaryl sulfones analogues of combretastatin CA-4 were successfully synthesized;
- (ii) the sulfonyl group was fully characterized with the following signals: ¹H and ¹³C NMR at δ2.36 ppm and δ21.50 ppm respectively, which confirmed the presence of the methyl group attached to the aryl sulfonyl group. Proton resonating around δ7.14 ppm - 7.46 ppm also established the presence of the aromatic protons attached to the aryl sulfonyl group. Infra red (IR) band around 1361cm⁻¹ and 1150 cm⁻¹ signified the presence of SO₂ in the compound. HRMS molecular formula C₂₆H₂₈O₇NaS also supported the presence of SO₂ in the compound;

- (iii) the compounds showed significant cytotoxicity with IC_{50} values ranging from 4.10 - 23.94 μ M when compared to the standard, doxorubicin (IC_{50} values ranging from 0.23 – 2.06 μ M);
- (iv) conjugate (Z)-1,2,3-trimethoxy-5-(1-(4-methoxyphenyl)-2-tosylvinyl)benzene displayed potent antiproliferative activity against A549 and IMR-32 cancer cell lines; and
- (v) all the synthesized compounds were non toxic to normal cell line HEK-293. with the exception of 4-methylphenyl(3,4,5-trimethoxyphenyl)ethynyl sulfone.

In conclusion, the synthesized CA-4 analogues were found to be effective against A549 cancer cell lines especially compound (Z)-1,2,3-trimethoxy-5-(1-(4-methoxyphenyl)-2-tosylvinyl)benzene. It is therefore recommended for consideration as a possible drug for lung cancer treatment.

CHAPTER ONE

1.0

INTRODUCTION

1.1 Cancer

Cancer is a disease which occurs when changes in a group of normal cells within the body lead to uncontrolled growth causing a lump called tumour (Figure 1.1); this is true of all cancers except leukaemia (cancer of the blood). If left untreated, tumours can grow and spread into the surrounding normal tissue, or to other parts of the body via the bloodstream and lymphatic systems, and can affect the digestive, nervous and circulatory systems [1, 8]. There are many kind of cancer, but they all start because of out-of-control growth of abnormal cells (Figure 1.1).

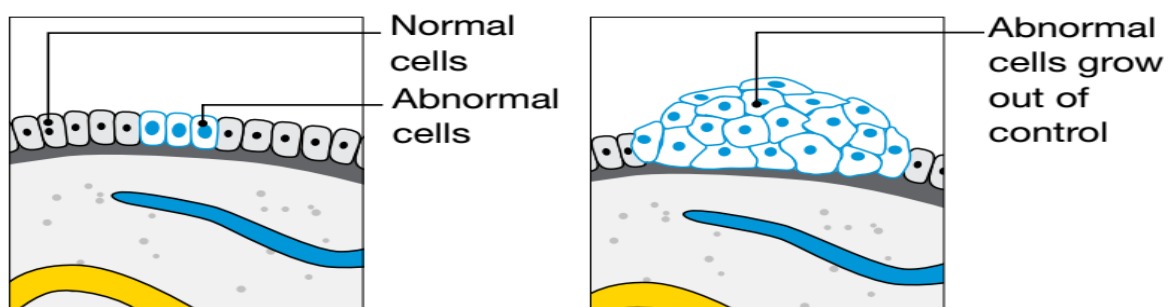


Figure 1.1: Normal cells Versus Abnormal Cell [9]

Our oldest description of cancer (although the word cancer was not used) was discovered in Egypt and dated back to about 3000 BC. It is called the Edwin Smith Papyrus and is a copy of part of an ancient Egyptian textbook on trauma surgery [2]. It described 8 cases of tumors or ulcers of the breast that were removed by cauterization with a tool called the fire drill [2]. The writing says about the disease, “There is no treatment.”[2]. The spread has continued up till now in an alarming rate.

Cancer has become a major source of health challenges and death globally [3]. In 2008, there were 12.7 million new cases and 7.6 million cancer-related deaths [4]. Majorly, 56 % of these

newly reported cancer cases occurred in low-income countries and it is projected that by 2030, 70 % of all new cases of cancer will be found in low-income countries [4].

In Nigeria, members of the public still see cancer as a disease of the wealthy, the elderly and developed countries. Many sufferers of the disease in the country still regard it as their fate and, as such, a death sentence. Cancer is not just a health issue but has far-reaching social and economic implications. It is a global epidemic that affects all ages with low and middle income countries bearing a disproportionate burden of it. There are many types of cancer: skin, lung, cervical, breast, leukaemia, nasal cavity, bladder, prostate etc. Breast and cervical cancers are commonest among women while prostate cancer is more prevalent in men in Nigeria. Since there is low awareness of the disease in the country as at now, more efforts should be directed at the area of massive awareness for the general populace (Table 1.0).

Table 1.1: **Cancer Statistics in Nigeria**

Cancer statistics in Nigeria	Estimate
Population in 2012	166.6 m
People newly diagnosed with cancer (excluding NMSC) / yr	102,100
Age-standardised rate, incidence per 100,000 people/yr	100.1
Risk of getting cancer before age 75	10.4 %
People dying from cancer /yr	71,600 [5-7]

The study of cancer, called *oncology*, is the work of countless doctors and scientists around the world whose discoveries in Anatomy, Physiology, Chemistry, Epidemiology, and other related fields made oncology what it is today. Technological advances and the ever-increasing understanding of cancer make this field, one of the most rapidly evolving areas of modern medicine [1, 8].

1.2 Causes of Cancer

Information on causation of cancer came from investigation of the research on cancer in human populations and the induction of tumours in experimental animals following treatment with cancer-causing agents. The most important human carcinogens include tobacco, asbestos, aflatoxins and ultraviolet light [10]. Almost 20 % of cancers are associated with viral and bacterial infections, the most significant ones being hepatitis B and hepatitis C viruses, respectively (HBV, HCV), human papilloma virus (HPV) and *Helicobacter pylori* (*H.pylori*) [11]. There is increasing recognition of the causative role of lifestyle factors, including diet, physical activity, and alcohol consumption [12]. Genetic susceptibility may significantly alter the risk from environmental exposures [13]. As with most illnesses, causes of cancer are multi-factorial, meaning there is no one single cause for it [14].

1.3 Cancer-causing substances (carcinogens) - Genes are coded messages inside a cell that instructs it how to behave (that is, which proteins to make). Mutation or changes to the gene, such as damage or loss, can alter how that cell behaves. For example, a mutation may mean that too much protein is made, or too little protein is made or that protein is not made at all [15]. Significantly, there is need for a number of genetic mutations within a cell before it becomes cancerous. Something that damages a cell, changes its behaviour and making it more likely to be susceptible to cancerous cells is called a 'carcinogen'[16].

1.3.1 Carcinogenic agents in tobacco

The use of tobacco has been identified by World Health Organisation (WHO) as the major preventable cause of cancer in humankind [17]. Cigarettes represent the most important tobacco product worldwide [18]. Tobacco is also chewed, alone or with lime, betel nut or other plants [19]. Tobacco snuff is taken in many countries, notably in Scandinavia, India and its neighbouring countries, the Mediterranean and Southern Africa [20]. The composition of chewing tobacco and snuff greatly varies geographically [21]. In India and its neighbouring

countries, smokeless tobacco is widely used and “bidi” smoking is also common, this being the cheapest form of tobacco consumption available [22]. Non-smokers are exposed to environmental tobacco smoke, the extent of exposure being determined primarily by whether family members smoke and by workplace conditions. The amount of tobacco smoke inhaled as a consequence of atmospheric pollution is much less than that inhaled by active smokers [23].

Tobacco smoking is one of the main known causes of human cancer-related death worldwide [24]. Smoking is the most commonly causes of lung cancer (Figure 1.2) [25]. For a smoker, lung cancer risk is related to the parameters of tobacco smoking in accordance with the basic principles of chemical carcinogenesis: risk is determined by the dose of carcinogen, the duration of administration and the intensity of exposure [25, 26]. In respect of these determinants of lung cancer risk, women are less as susceptible as men. Tobacco smoking has been estimated to cause approximately 25 % of all cancers in men and 4 % in women, and, in both genders, approximately 16 % of cancer in more developed countries and 10% in less developed countries [27], although some estimates are as high as 30 % [28].

Lung cancer risk is determined by the amount of daily consumption of tobacco, duration of smoking and the depth of inhalation. For regular smokers, the relative risk for the development of lung cancer is more than 20 times higher than for non-smokers [29]. Mainstream smoke (the material inhaled by smokers) is an aerosol including approximately 4,000 specific chemicals and containing 1,010 particles per ml [30]. The particulate matter (tar) is made up of some 3,500 compounds, the most abundant being nicotine (0.1-2.0 mg per cigarette) and also including most of the polycyclic aromatic hydrocarbons occurring in the smoke [31].

Another class of carcinogens present in tobacco smoke is N-nitroso compounds, particularly including the nitroso derivatives of nicotine and nor nicotine [32]. Chemicals such as

aromatic amines, benzene and heavy metals, independently established as carcinogenic for human, are present in tobacco smoke (Table 1.2). Use of smokeless tobacco results in exposure to tobacco related nitroso compounds, but not to polycyclic aromatic hydrocarbons, which are products of combustion [33]. Cancer causation by tobacco smoke is not attributable to any one chemical component, or any one class of chemicals present, but to an overall effect of the complex mixture of chemicals in smoke (Table 1.2).

Most chemical carcinogens in tobacco smoke require metabolic activation in order to exert a carcinogenic effect [34]. The requisite enzymes are present in lung and other “target” organs [35]. Individual risk may be affected by the activity and levels of enzymes such as glutathione-S-transferase, cytochrome P450 and N-acetyl transferases [36]. In the course of metabolism reactive forms of polycyclic aromatic hydrocarbons, nitrosamines and aromatic amines are generated and become covalently bound to DNA in relevant tissues. Such DNA adducts have been detected in tissues, bodily fluids and urine from smokers and from persons exposed to environmental tobacco smoke [37, 38, 39].

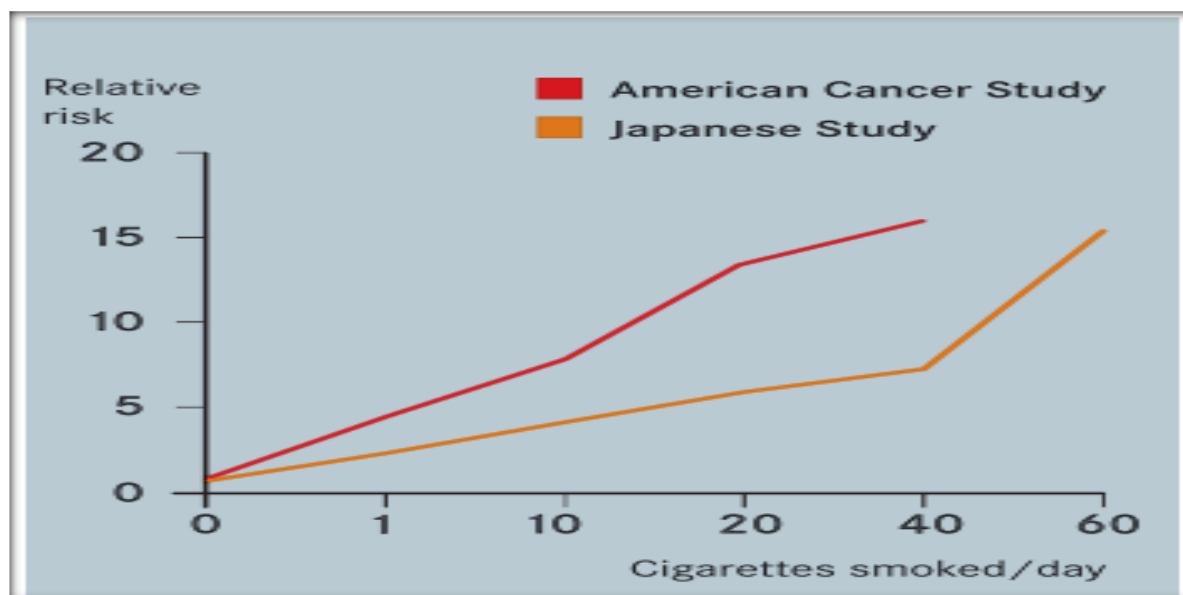


Figure 1.2: Risk of lung cancer determined by number of cigarettes smoked [25].

Table 1.2: Carcinogenic agents in tobacco smoke and smokeless tobacco.

S/N	Substance	Tobacco smoke (per cigarette)	Smokeless tobacco (ng/g)
1	Volatile aldehydes		
	Formaldehyde	20-105 µg	2,200-7,400
	Acetaldehyde	18-1400 µg	1,400-27,400
	Crotonaldehyde	10-20 µg	200-2,400
2	N-Nitrosoamines		
	N-Nitrosodimethylamine	0.1-180 ng	0-220
	N-Nitrosodiethylamine	0-36 ng	40-6,800
	N-Nitrosopyrrolidine	1.5-110 ng	0-337
3	Tobacco-specific nitrosoamines		
	N'-Nitrosonornicotine (NNN)	3-3,700 ng	400-154,000
	4-(Methylnitrosoamino)-1-(3-pyridyl)-1 butanone(NNK)	0-770 ng	0-13,600
	4-(Methylnitrosoamino)-1-(3-pyridyl)-1 butanol (NNAL)	+	+
	N'-Nitrosoanabasine (NAB)	14-46 ng	0-560
4	Metals		
	Nickel	0-600 ng	180-2,700
	Cadmium	41-62 ng	700-790
	Polonium 210	1-10 mBq	0.3-0.64 pci/g
	Uranium 235 and 238	-	2.4-19.1 pci/g
	Arsenic	40-120 ng	
5	Polycyclic aromatic hydrocarbons		
	Benzo[a]pyrene	20-40 ng	>0.1-90
	Benzo[a]anthracene	20-70 ng	-
	Benzo[b]fluoranthene	4-22 ng	-
	Chrysene	40-60 ng	-
	Dibenzo[a,l]pyrene	1.7-3. 2ng	-
	Dibenzo[a,h]anthracene	+	-

Key: + = *present*, - = *absent*

[32]

1.3.2 Alcohol consumption

Heavy alcohol drinking causes cancer of the oral cavity, pharynx, larynx, oesophagus, inflammation, cirrhosis of the liver, liver cancer, and may increase the risk of breast and colorectal cancer [40, 41]. Most alcoholic beverages can be grouped as either beers (brewed by fermenting malted barley and typically containing 5 % volume of alcohol), wines (made by fermenting grape juice or crushed grapes, containing 12 % alcohol) or spirits (made by distilling fermented products of a variety of cereals, vegetables and fruits, containing 40 % alcohol) [42]. On a global scale, the consumption of alcoholic beverages by adults as calculated from official figures is equivalent to 4 L of alcohol per year (or 9 g/day), corresponding to approximately 3 % of the average total intake of calories while official consumption, however, is estimated to account for an additional amount corresponding to 20-100 % of the official figures, depending on the country [43].

Studies of cancer risk in brewery workers and in alcoholic patients have provided important evidence on the carcinogenic role of alcohol. A causal association is also established in the case of breast cancer and is probable for colon and rectal cancer [44, 45]. For all cancers caused by drinking alcohol, the risk of cancer is a linear function of the level of consumption, up to an intake of about 80 g/day (one litre of wine, a quarter of a litre of spirits), above which level the dose-response relationship is less clearly defined (Table 1.3). Alcohol drinking and tobacco smoking show a synergistic interaction in the etiology of cancers of the oral cavity, pharynx, larynx and oesophagus; the risk of cancer for heavy consumers of both products relative to that for subjects who neither smoke nor drink is higher than the product of the risks attributable respectively to heavy drinking and heavy smoking separately (Figure 1.3) [46].

Table 1.3: Consumption of alcohol and relative risk of cancer of the upper gastrointestinal tract.

Mean alcohol consumption	Relative risk (95 % confidence)
No alcohol	1
>0 to 30 g/day	1.2 (0.4 – 3.4)
> 30 to 60 g/day	3.2 (1.0 – 10.1)
>60 g/day	9.2 (2.8 -31.0) [4]

*adjusted for follow-up time, sex, education, body mass index(BMI), vegetable and fruit consumption, tobacco smoking and energy intake

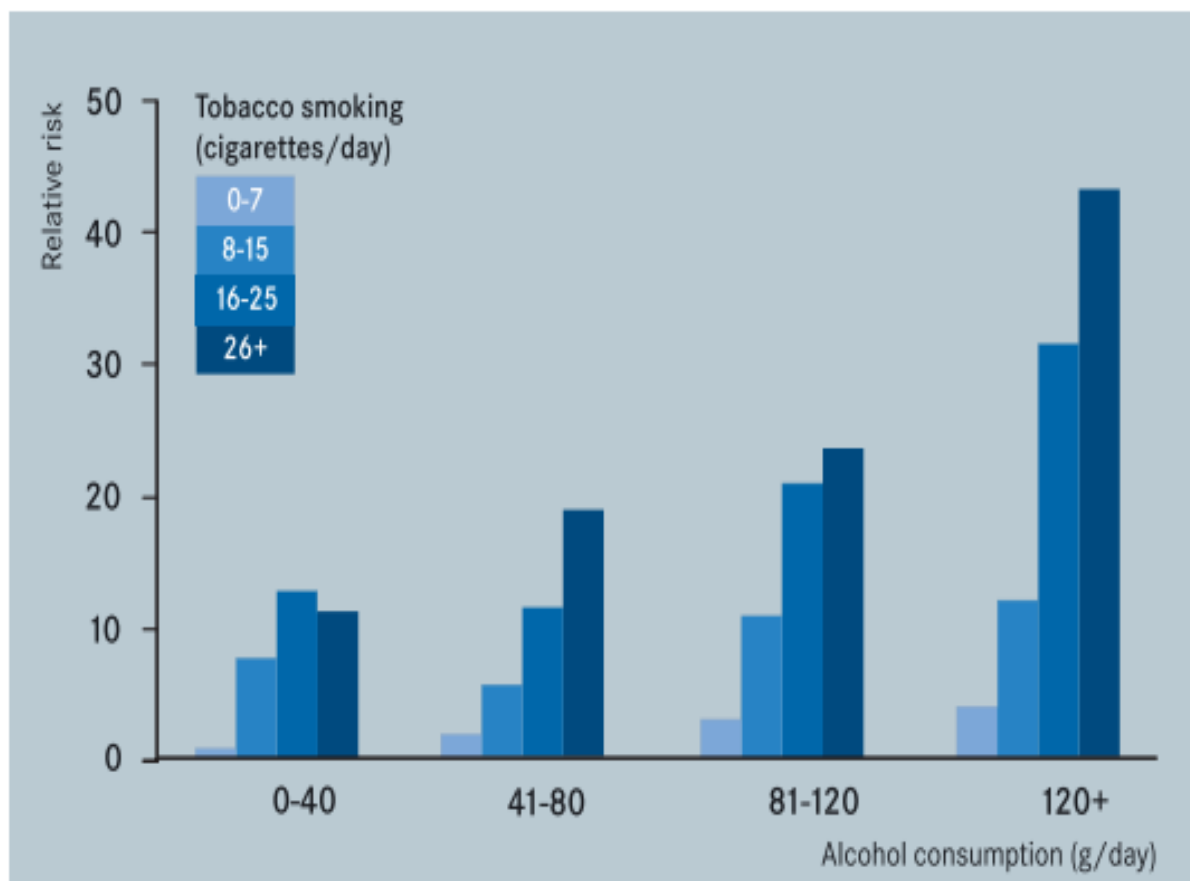


Figure 1.3: Multiplicative increase in relative risk of laryngeal cancer as a consequence of both alcohol drinking and active smoking (colour coding approximates progressive doubling of risk as exposure increases).

[46]

1.3.3 Occupational Exposure

Many occupations and some specific chemicals encountered at work are associated with increased risk of cancer. The first reports of associations between risk of cancer and employment in particular occupations appeared during the 18th century, scrotal cancer among chimney sweeps [47] and 19th century, bladder cancer in workers exposed to dyes [32].

However, majority of studies establishing a link between an increased risk of cancer and a particular working environment were published between 1950 and 1975 [48]. The International Agency for Research on Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Human evaluate data relevant to the carcinogenic hazard to human as a consequence of exposure to particular chemical, physical and biological agents and mixtures [49]. At present, 25 chemicals, groups of chemicals or mixtures for which exposures are mostly occupational, have been established as human carcinogens (Table 1.4). While some of these agents, such as asbestos, crystalline silica and heavy metals, are currently encountered in the work places of many countries, other agents have been phased out and are mainly of historical interest (e.g. mustard gas and 2-naphthylamine). An additional 25 agents presenting a hazard on the basis of workplace exposure are classified as probably carcinogenic to human. Most of these agents have been shown to be carcinogenic in experimental animals, and less than conclusive evidence of carcinogenicity in human from epidemiological studies (Table 1.5). In many instances, it is possible to associate increased risk of cancer with a group of agents or a particular work environment rather than with a single compound.

Table 1.4: Chemicals classified as human carcinogens (IARC Group 1) for which exposures are mostly occupational

S/N	Agent	Cancer site/cancer	Main industry/use
1	4-Aminobiphenyl	Bladder	Rubber manufacture
2	Arsenic and arsenic compounds*	Lung, skin	Glass, metals, pesticides
3	Asbestos	Lung, pleura, peritoneum	Insulation, filter material, textiles
4	Benzene	Leukaemia	Solvent, fuel
5	Benzidine	Bladder	Dye/pigment manufacture
6	Beryllium and beryllium compounds	Lung	Aerospace industry/metals
7	Bis(chloromethyl) ether	Lung	Chemical intermediate/by-product
8	Cadmium and cadmium compounds	Lung	Dye/pigment manufacture
9	Chloromethyl methyl ether	Lung	Chemical intermediate/by-product
10	Chromium[VI] compounds	Nasal cavity, lung	Metal plating, dye/pigment manufacture
11	Coal-tar pitches	Skin, lung, bladder	Building material, electrodes
12	Coal-tars	Skin, lung	Fuel
13	Ethylene oxide	Leukaemia	Chemical intermediate, sterilising agent
14	Mineral oils, untreated and mildly-treated	Skin	Lubricants
15	Mustard gas (sulfur mustard)	Pharynx, lung	War gas
16	2-Naphthylamine	Bladder	Dye/pigment manufacture
17	Nickel compounds	Nasal cavity, lung	Metallurgy, alloys, catalyst
18	Shale-oils	Skin	Lubricants, fuels
19	Silica, crystalline	Lung	Stone cutting, mining, foundries
20	Soots	Skin, lung	Pigments
21	Strong-inorganic-acid mists containing sulfuric acid	Larynx, lung	Metal, batteries
22	Talc containing asbestiform fibres	Lung	Paper, paints
23	2,3,7,8-Tetrachlorodibenzo-para-dioxin (TCDD)	Several organs	Contaminant
24	Vinyl chloride	Liver	Plastics monomer
25	Wood dust	Nasal cavity	Wood industry

* This evaluation applies to the group of chemicals as a whole and not necessarily to all individual chemicals within the group.

[50]

Table 1.5: Chemicals classified as probably carcinogenic to human (IARC Group 2A) for which exposures are mostly occupational

S/N	Agent	Cancer site/cancer	Main industry/use
1	Acrylonitrile	Lung, prostate, lymphoma	Plastics, rubber, textiles, monomer
2	Benz[a]anthracene	Lung, skin	Combustion fumes
3	Benzidine-based dyes	Bladder	Paper, leather, textile dyes
4	Benzo[a]pyrene	Lung, skin	Combustion fumes
5	1,3-Butadiene	Leukaemia, lymphoma	Plastics, rubber, monomer
6	Captafol -	-	Pesticide
7	Chlorinated toluenes (trichlorobenzene, benzal chloride, benzyl chloride, benzoyl chloride)	Lung	Chemical intermediates
8	para-Chloro-ortho-toluidine(and its strong acid salts)	Bladder	Dye/pigment manufacture, textiles
9	4-Chloro-ortho-toluidine	Bladder	Dye/pigment manufacture, insecticide
10	Creosotes	Skin	Wood preservation
11	Dibenz[a,h]anthracene	Lung, skin	Combustion fumes
12	Diethyl sulfate	-	Chemical intermediate
13	Dimethylcarbonyl chloride	-	Chemical intermediate
14	Dimethyl sulfate	-	Chemical intermediate
15	Epichlorohydrin	-	Plastics/resins monomer
16	Ethylene dibromide	-	Chemical intermediate, fumigant, fuels
17	Formaldehyde	Nasopharynx	Plastics, textiles, laboratory agent
18	Glycidol	-	Chemical intermediate, sterilising agent
19	4,4'-Methylenebis(2-chloroaniline) (MOCA)	Bladder	Rubber manufacture
20	Methyl methanesulfonate	-	Laboratory research
21	ortho-Toluidine	Bladder	Dye/pigment manufacture
22	Polychlorinated biphenyls	Liver, bile ducts, leukaemia, lymphoma	Electrical components
23	Styrene oxide	-	Plastics, chemical intermediate
24	Tetrachloroethylene	Oesophagus, lymphoma	Solvent, dry cleaning
25	Trichloroethylene	Liver, lymphoma	Solvent, dry cleaning, metal
26	Tris(2,3-dibromopropyl) phosphate	-	Plastics, textiles, flame retardant
27	Vinyl bromide	-	Plastics, textiles, monomer

[50]

1.3.4 Environmental Pollution

In a broad sense, “environmental factors” are implicated in the causa of the majority of human cancers [51]. In respect of many such environmental factors, such as active smoking, alcohol intake, sun exposure and dietary make-up, individual exercise a degree of control over their levels of exposure. However in the present context, “environmental pollution” refers to a specific subset of cancer-causing environmental factors; namely, contaminants of air, water and soil. One characteristic of environmental pollutants is that individuals lack control over their level of exposure. The carcinogenic pollutants for which most information is available include asbestos, toxic agents in urban air, indoor air pollutants and chlorination by-products and other contaminants of drinking water (Figure 1.4).

Relevant risk factors include place of residence: whether rural or urban, and the relationship to major industrial emission sources. Various determinations suggest that environmental pollution accounts for 1-4 % of the total burden of cancer in developed countries [52, 53]. A small proportion of lung cancer (<5 %) is attributable to outdoor air pollution by industrial effluent, engine exhaust products and other toxins e.g hydrocarbons, nitrogen oxides, carbon monoxide, carbon dioxide, sulphur dioxide, particulate matter (mostly , soot), benzene, acetaldehyde, 1,3-butadiene, etc... [54].

Carcinogenic indoor air pollutants is another great source of environmental pollution, these include tobacco smoke, heating fuels, fumes from frying oils and cooking fumes in many regions of the world. In circumstances of high exposure, more than 50 % of cases of lung cancer among women can be attributed to indoor air pollution [55, 56, 57].

Chlorofluorocarbons (CFCs), a major source of air pollution is believed to cause destruction of the ozone layer and enhance the risk of skin cancer through increased ultraviolet radiation. These chemicals, including halons, carbon tetrachloride, and methyl chloroform are emitted from home air conditioners, foam cushions, and many other products. CFC’s are carried by

winds into the stratosphere, where the action of strong solar radiation releases chlorine and bromine atoms which react with, and thereby eliminate molecules of ozone. Depletion of the ozone layer is believed to be responsible for global increases in ultraviolet B (UVB) radiation [58].

Tobacco smoke is an important source of indoor air pollution. In non adult smokers, chronic exposure to environmental tobacco smoke increases mortality from lung cancer by between 20 % and 30 % [59]. Among adults, exposure to environmental tobacco smoke has been linked to lung cancer and heart disease, while environmental exposure to tobacco smoke in children has been identified as a cause of respiratory disease, middle ear disease, asthma attacks and sudden infant death syndrome [23].

Carcinogenic agents can be introduced into drinking water via water and soil pollution. These include a variety of potentially carcinogenic agents such as, chlorination by-products (trihalomethanes and chloroform), and arsenic [60]. Studies on bladder cancer have suggested an increased risk associated with consumption of chlorinated drinking water [61].

Arsenic causes cancer in the skin, lung and other organs [62]. The main source of environmental exposure to arsenic for the general population is through ingestion of contaminated water. High exposure to arsenic from drinking water is found in several areas of Alaska, Argentina, Bangladesh, Chile, India, Mexico, Mongolia, Taiwan and the USA. There is strong evidence of an increased risk of bladder, skin and lung cancers following consumption of water with high arsenic contamination [63].

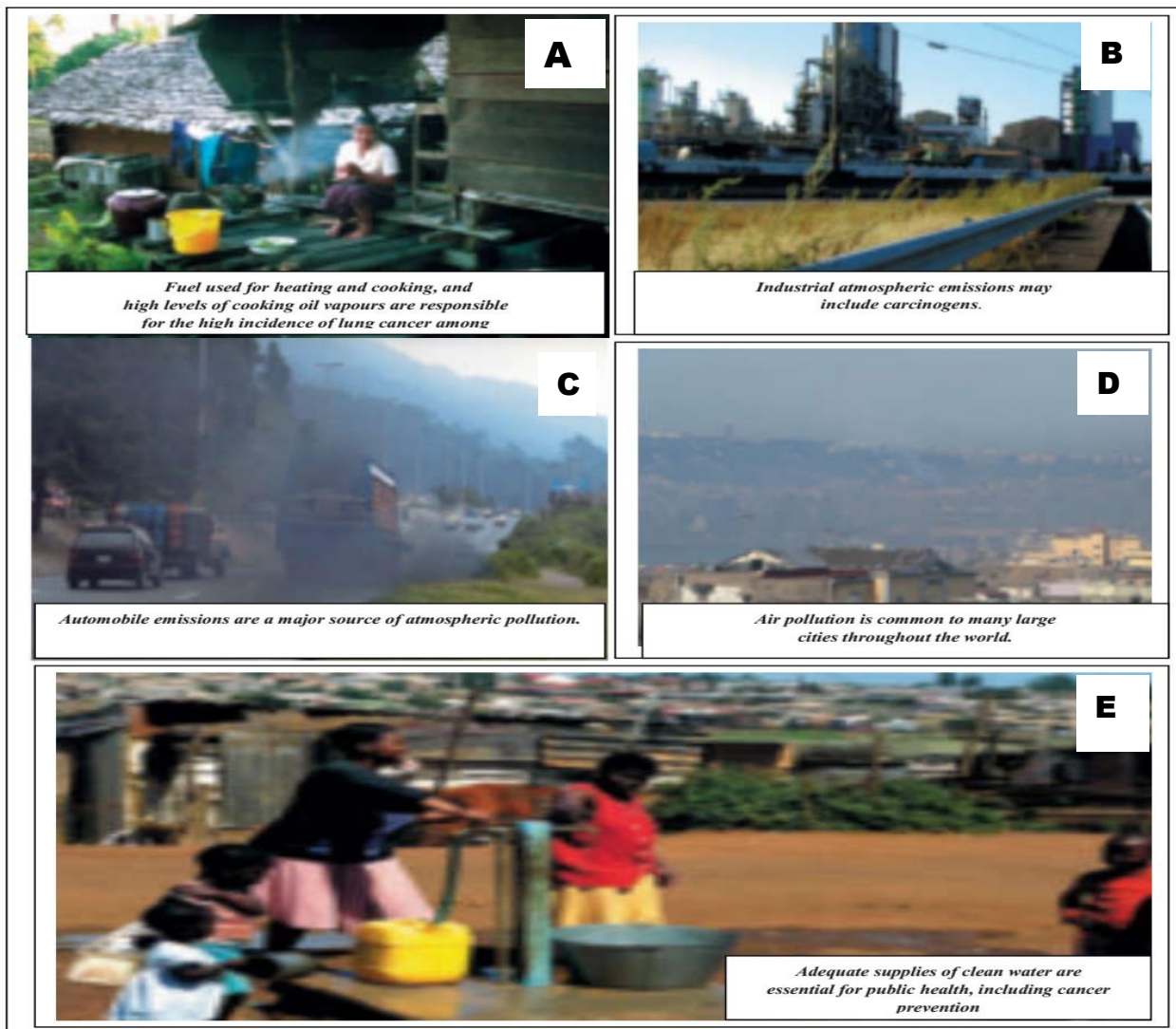


Figure 1.4: Some Environmental pollutants that cause Cancer

- A: Fuel used for heating and cooking, and high levels of cooking oil vapours is responsible for the high incidence of lung cancer among women in some parts of the world [56].
- B: Industrial atmospheric emissions may include carcinogens [54].
- C: Automobile emissions are a major source of atmospheric pollution [59].
- D: Air pollution is common to many large cities throughout the world [56]
- E: Adequate supplies of clean water are essential for public health, including cancer prevention [60].

1.3.5 Food Contamination

Contamination of food may occur directly during its production, storage and preparation. For example, grains and cereals are subjected to fungal growth and contamination by mycotoxins during storage. Indirect contamination of food can occur when animals have been given contaminated feed or been otherwise treated with various products. The most contentious residues occurring in meat, milk and eggs are antibacterial drugs, hormonal growth promoters and certain pesticides, heavy metals and industrial chemicals [64].

An additional category of contaminants comprises those generated in the course of food preparation. Aflatoxins, a family of related compounds (designated B1, B2, G1, G2 and M) which occur as food contaminants in hot, humid parts of the world, with particularly high levels in traditional diets based upon maize and groundnuts (peanuts) of subSaharan Africa, South-east Asia and South America have been categorized as carcinogenic hazards. Aflatoxins are products of the *Aspergillus* fungi and particularly accumulate during storage of grains [65]. Aflatoxin B1 (the most common aflatoxin) causes liver cancer in experimental animals [66]. In liver cells, aflatoxin B1 is metabolized to form an epoxide which binds to the N7 position of specific guanines, leading to the formation of G to T transversions (carcinogen activation and DNA repair) [67]. Mutations induced by aflatoxin B1 are found in several genes involved in hepatocellular carcinogenesis [68]. Naturally occurring aflatoxins are categorized by IARC as Group 1 carcinogens (causing cancer in human)[69].

Fusarium sporotrichioides produces T-2 toxin, which may have played a significant role in large-scale human poisonings in Siberia in the last century and may be carcinogenic [69]. *Ochratoxin A*, also a fungal metabolite (Figure 1.5), has been classed as a possible human carcinogen. This mycotoxin may contaminate grain and pork products and has been detected in human blood and milk [69, 70].

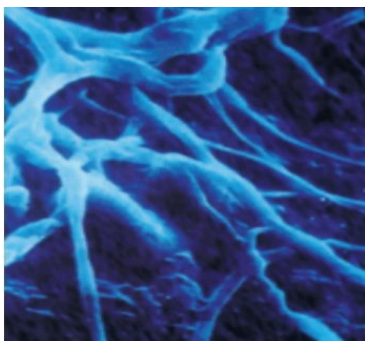


Figure 1.5: The fungi *Aspergillus* [70]

Pyrrolizidine alkaloids (including lasiocarpine and monocrotaline) are naturally occurring plant toxins which may be ingested by animals, and by human eating some medicinal plants (e.g. comfrey) or honey, in some areas [71]. Several pyrrolizidine alkaloids have been found to cause DNA damage and show mutagenic properties *in vitro* [72]. Chronic consumption of some pyrrolizidine alkaloids may cause liver tumours in rodents, but has not been associated with cancer in human [73].

Certain organochlorines, including dichlorodiphenyltrichloroethane (DDT) and other pesticides, are resistant to degradation, are very lipid-soluble and hence persist in the environment and are bioconcentrated up the human food chain. DDT and a number of other organochlorine pesticides cause liver cancer in rats. DDT in particular has been associated with increased risk of pancreatic cancer, breast cancer, lymphoma and leukaemia in human [74].

Some chemicals formed during food preparation may present a carcinogenic hazard [75]. Some examples include certain heterocyclic amines that are formed by pyrolysis of two amino acids, creatine and creatinine, during cooking of meat and fish at high temperature [76]. Heterocyclic amines are carcinogenic in various organs of mice, rats and non-human primates, although, their carcinogenic potential in human has not yet been established [77].

Polycyclic aromatic hydrocarbons can be generated in meat when it is fried, roasted or cooked over an open flame, and many members of this chemical class are carcinogenic, such

as benzo[a]pyrene and benzanthracene, which are present in smoke from the burning of fuels, tobacco and weeds [77].

N-Nitroso compounds are a wide class of chemicals, many of which (particularly N-nitrosamines) are potent carcinogens in several species of experimental animals, and probably in human [78]. Nitrosamines may be formed by chemical reactions in foods containing added nitrates and nitrites, such as salt-preserved fish and meat, and in foods processed by smoking or direct fire drying (Figure 1.6). The use of fertilizers may influence the level of nitrites in food, which may be a factor in determining generation of nitrosamines during food preparation and storage [78].



Figure 1.6: Smoked fish consumed in many parts of the world [78].

1.3.6 Synthetic Drugs

Modern medicine has at its disposal hundreds of synthetic drugs, many of which are essential for the effective treatment of an enormous range of human diseases. A small fraction of such drugs has been found to be carcinogenous to human as a side-effect (Table 1.6). This is most

likely for some drugs that must be given at high doses or for prolonged periods [79]. Synthetic drugs that have been found to be carcinogenic to human include some antineoplastic drugs and drug combinations [79], certain hormones and hormone antagonists [80, 81], some immune suppressants and a small number of miscellaneous agents [79, 82]. Hormones are potent regulators of bodily functions therefore, hormonal imbalances can cause increased risk of certain cancers. Drugs with hormonal activity or which block hormonal effects may increase risk of some hormonally-responsive cancers, while reducing the risk of others [83].

Some examples include, Tamoxifen, which is an antiestrogen that may be given to women with estrogen receptor-positive breast tumours to block estrogen from entering the breast tissues. It is an effective drug for prevention of contralateral breast cancer in breast cancer patients, but it also increases the risk of cancer of the endometrium [84]. Diethylstilbestrol, a synthetic estrogen, originally prescribed to prevent miscarriage caused by malformations of the reproductive organs, is associated with increased risk of vaginal adenocarcinoma [85].

Some drugs that were introduced into human medicine, including the antiretroviral drugs zidovudine [3'-azido-3'-deoxythymidine] (AZT) and zalcitabine [2'-3'-dideoxycytidine] (DDC), are carcinogenic in experimental animals and may possibly be carcinogenic to human (Table 1.7), although, there is yet no direct evidence of increased cancer risk in treated patients [86]. Surgical implants of various kinds are widely used for both therapeutic and cosmetic purposes [87]. Foreign bodies of many kinds cause development of malignant tumours of connective tissue (sarcomas) when implanted in tissues or body cavities of experimental rodents and left in place for long periods. Foreign bodies include both metallic and non-metallic solid objects, and non-absorbable or very slowly absorbable liquid suspensions. Sarcomas develop in rodents immediately adjacent to the foreign body, in the soft connective tissues or in bone and/or cartilage [87]. There have been more than 60

published case reports of sarcomas and other kinds of cancers that have developed in human at the sites of surgical implants or other foreign bodies. However, there are no controlled studies that would allow a conclusion that these cancers were indeed caused by the pre-existing foreign body [88].

Table 1.6: Synthetic drugs classified as being carcinogenic to human (IARC Group 1)

S/N	Drug or drug combination [IARC Group 1]	Cancer site/cancer
1	Analgesic mixtures containing phenacetin	Kidney, bladder
2	Azathioprine	Lymphoma, skin, liver and bile ducts, soft connective tissues
3	N,N-bis(2-chloroethyl)-2-naphthylamine (Chlornaphazine)	Bladder
4	1,4-Butanediol dimethane-sulfonate (Myleran; Busulfan)	Leukaemia
5	Chlorambucil	Leukaemia
6	1-(2-Chloroethyl)-3-(4-methyl-cyclohexyl)-1-nitrosourea (Methyl-CCNU)	Leukaemia
7	Ciclosporin	Lymphoma, Kaposi sarcoma
8	Cyclophosphamide	Leukaemia, bladder
9	Diethylstilbestrol	Cervix, vagina
10	Etoposide in combination with cisplatin and bleomycin	Leukaemia
11	Fowler's solution (inorganic arsenic)	Skin
12	Melphalan	Leukaemia
13	8-Methoxypsoralen (Methoxsalen) plus ultraviolet radiation	Skin
14	MOPP and other combined [anticancer] chemotherapy including alkylating agents	Leukaemia
15	Estrogen therapy, postmenopausal	Breast, uterus
16	Estrogens, non-steroidal	Cervix/vagina
17	Estrogens, steroidal	Uterus, breast
18	Oral contraceptives, combined ^a	Liver
19	Oral contraceptives, sequential	Uterus
20	Tamoxifen ^b Uterus	Uterus
21	Thiotep ^a	Leukaemia
22	Treosulfan	Leukaemia [82, 84]

^a There is also conclusive evidence that these agents have a protective effect against cancers of the ovary and endometrium.

^b There is conclusive evidence that tamoxifen has a protective effect against second breast tumours in patients with breast cancer.

Table 1.7: Synthetic drugs that are probably carcinogenic to human (IARC Group 2A)

S/N	Drug or drug combination [IARC Group 2]	Cancer
1	Androgenic (anabolic) steroids	Liver cancer
2	Bis(chloroethyl) nitrosourea (BCNU)	Leukaemia
3	Chloramphenicol	Leukaemia
4	1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)	Leukaemia
5	Etoposide	Leukaemia
6	5-Methoxypsoralen	Skin cancer
7	Nitrogen mustard	Skin cancer
8	Procarbazine hydrochloride	Leukaemia
9	Teniposide	Leukaemia

[82, 84]

1.3.7 Radiation

Exposure to ionizing radiation from industrial, medical and other sources can cause a variety of neoplasms, including leukaemia, breast cancer and thyroid cancer [89]. Human are exposed to both X-rays and γ -rays from natural sources (including cosmic radiation and radioactivity present in rocks and soil) and to a much lower extent from man-made sources (Figure 1.7). On the average, for members of the general public, the greatest contribution comes from medical X-rays and the use of radiopharmaceuticals, with lower doses from fallout from weapons testing, nuclear accidents (such as Chernobyl) and routine releases from nuclear installations [90]. Medical exposures occur both in the diagnosis (e.g. radiography) of diseases and injuries and in the treatment (e.g. radiotherapy) of cancer and of some benign diseases [91]. Occupational exposure to ionizing radiation occurs in a number of jobs including the nuclear industry and medicine. Airline pilots and crew are exposed to cosmic radiation. Therefore, ionizing radiation is one of the most intensely studied carcinogens [92, 93, 94].

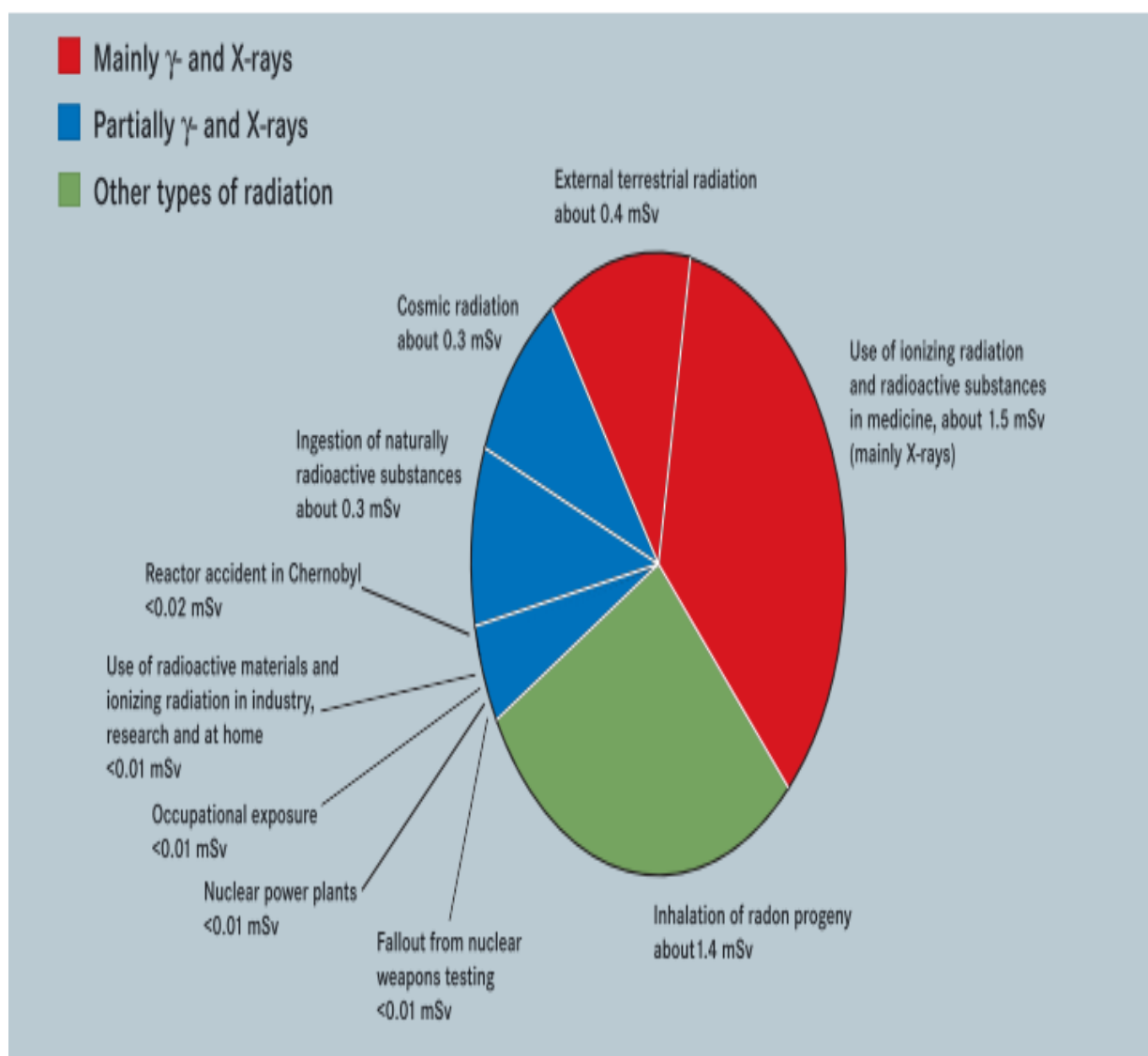


Figure 1.7: Estimated annual dose of ionizing radiation received by a member of the general public [89].

Solar radiation, specifically the ultraviolet component, causes cutaneous malignant melanoma and non-melanocytic skin cancer [95]. Exposure of skin to ultraviolet radiation causes DNA damage (Carcinogen activation and DNA repair) and also the conversion of trans-urocanic acid to cis-urocanic acid, which leads to cell injury and ultimately to cancer [96]. Incidence of skin cancer is increasing rapidly among fair-skinned populations (Figure 1.8) [97].

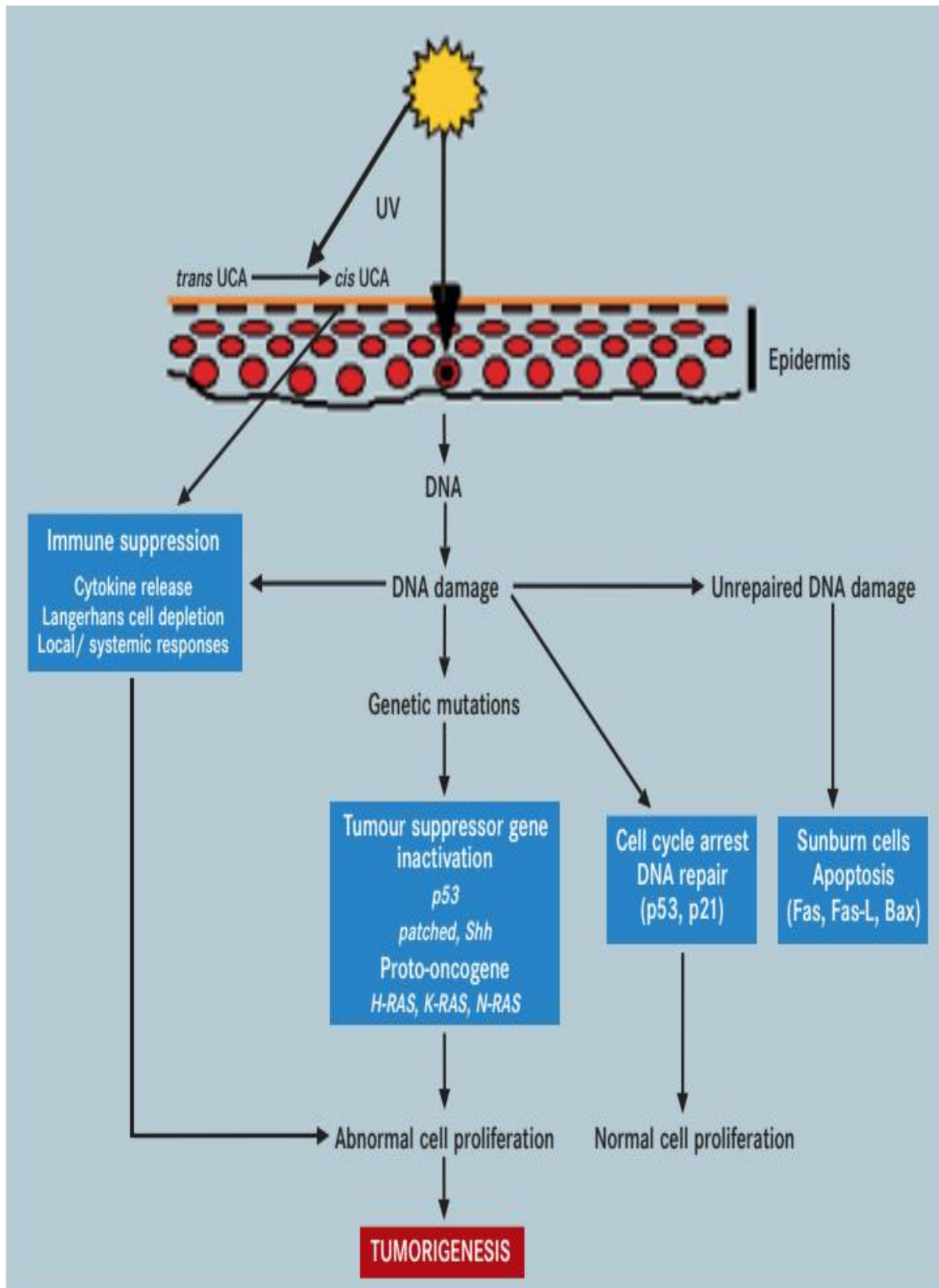


Figure 1.8: Pathways implicated in the induction of non-melanoma skin cancer by ultraviolet radiation (UCA = urocanic acid) [97].

Radiations are from various sources and Table 1.8 shows some sources of radiation and the kind of cancer they cause.

Table 1.8: Various forms of radiation that are carcinogenic to human (IARC Group 1) or probably carcinogenic to human (IARC Group 2A)

S/N	Forms of radiation	Cancer site/cancer
IARC Group 1: Carcinogenic		
1	X-rays and gamma-radiation	Various – all sites
2	Solar radiation	Skin
3	Radon-222 and its decay products	Lung
4	Radium-224, -226, -228 and their decay products	Bone
5	Thorium-232 and its decay products	Liver, including haemangiosarcoma; leukaemia
6	Radioiodines (including iodine-131)	Thyroid
7	Plutonium-239 and its decay products (aerosols)	Lung, liver, bone
8	Phosphorus-32	Leukaemia
9	Neutrons	Various
	Alpha (α) particle-emitting radionuclides	Various
	Beta (β) particle-emitting radionuclides	Various
IARC Group 2A: Probably carcinogenic to human		
1	Sunlamps and sun beds	Skin
2	Ultraviolet radiation	Skin

[97]

1.3.8 Chronic Infection

Infectious agents are one of the main causes of cancer, accounting for 18 % of cases worldwide with majority occurring in developing countries [98]. Today, experimental and epidemiological evidence revealed that a variety of infectious agents constitute one of the main causes of cancer worldwide [99]. Viruses are the principal ones, with at least eight different viruses associated with particular tumour types, with varying degrees of certainty. Other infectious agents involved in carcinogenesis are four parasites and one bacterium [100-104] (Table 1.9).

Table 1.9: The Burden of Cancer caused by Infectious Agents Worldwide

S/N	Infectious agent	IARC classification ¹	Cancer site/cancer	Number of cancer cases	% of cancer cases Worldwide
1	<i>H. pylori</i>	1	Stomach	490,000	5.4
2	HPV	1 , 2A	Cervix and other sites	550,000	6.1
3	HBV, HCV	1	Liver	390,000	4.3
4	EBV	1	Lymphomas and nasopharyngeal carcinoma	99,000	1.1
5	HHV-8	2A	Kaposi sarcoma	54,000	0.6
6	<i>Schistosoma haematobium</i>	1	Bladder	9,000	0.1
7	HTLV-1	1	Leukaemia	2,700	0.1
8	Liver flukes <i>Opisthorchis viverrini</i> <i>Clonorchis sinensis</i>	1 2A	Cholangiocarcinoma (biliary system)	800	
			Total infection related cancers	1,600,000	17.7
[100-104]					

¹Group 1= carcinogenic to human,

Group 2A= probably carcinogenic to human. ²Applies only to cervical cancer.

It has been estimated that 60 % of cases of primary liver cancer worldwide and 67 % of cases in developing countries can be attributed to chronic persistent infection with hepatitis B virus (HBV) [99]. About 25 % of cases of liver cancer in the world are attributable to hepatitis C virus HCV [100]. Human papillomavirus (HPV) DNA is found in virtually all invasive cervical cancers, indicating that HPV is a necessary cause. Moreover, about 80 % of anal cancers and 30% of cancers of the vulva, vagina, penis and oro-pharynx can be attributed to HPV [105]. Epstein-Barr virus (EBV) infection is ubiquitous [106]. In developing countries, infection is acquired in childhood, while in developed countries infection is delayed until adolescence [104].

Individuals with high titres of antibodies to various early and late EBV antigens have a higher risk of developing Burkitt lymphoma and Hodgkin disease [107]. Molecular evidence showed that EBV DNA and viral products are regularly detected (monoclonally) in cancer cells, but

not in normal cells thus providing a strong indication of a causal role for EBV in nasopharyngeal carcinoma and sinonasal angiocentric T-cell lymphoma [108]. The prevalence of Human Immunodeficiency Virus (HIV) infection is highest in sub-Saharan Africa (15-20 %) [109]]. HIV infection enhances the risk of Kaposi sarcoma by approximately 1,000-folds, of non-Hodgkin lymphoma by 100-folds, and of Hodgkin disease by 10-folds [103]. Increased risks of cancer of the anus, cervix and conjunctiva have also been observed. In all these cases, the role of HIV is probably as an immunosuppressive agent and hence indirect, the direct etiological agents being other cancer viruses (i.e. human herpesvirus 8 (HHV-8), EBV and HPV) [102-104].

Human T-cell lymphotropic virus type 1 (HTLV-1) infection occurs in clusters in Japan, Africa, the Caribbean, Colombia and Melanesia. A strong geographical correlation suggested that HTLV-1 is the main etiological factor in adult T-cell leukaemia/lymphoma [103]. HHV-8 infection appears to be common in Africa and in some Mediterranean countries but rare elsewhere [110]. HHV-8 DNA has been detected in over 90 % of Kaposi sarcomas and rarely in control patients [111]. Seropositivity rates are also higher in cases than controls, with relative risks over 10 in most studies [112]. Accordingly, the evidence linking HHV-8 to Kaposi sarcoma is strong [113]. Certain lymphoproliferative diseases such as primary effusion lymphoma and Castleman disease have also been linked to HHV-8, but the evidence is very limited [104].

Infection with *Helicobacter pylori* is one of the most common bacterial infections worldwide. In developing countries, the prevalence of *H. pylori* among adults ranges from 80 to 90% whilst in developed areas it is around 50 %. *H. pylori* is the main cause of gastritis and peptic ulcer; the infection may be lifelong if not treated with antibiotics [114]. The relationship between gastric cancer and *H. pylori* has been difficult to determine due to the very high prevalence of *H. pylori* in most populations where the cancer is endemic and the very low

bacterial load usually found in gastric cancer patients. It is clear that *H. pylori* plays a role in gastric cancer, but other cofactors (e.g. diet) are also contributory [114].

Two liver flukes, *Opisthorchis viverrini* and *Clonorchis sinensis*, have been identified to be associated with cholangio carcinoma in parts of Asia (Liver cancer) [115]. Infection by these flukes is acquired by eating raw or undercooked freshwater fish containing the infective stage of the fluke; the fluke matures and produces eggs in the small intrahepatic ducts [101]. The evidence for cancer causation by *O. viverrini*, a parasite mainly prevalent in Thailand, is stronger than for *C. sinensis* due to its multiple pathways to cancer [115]. The incidence of cholangiocarcinoma in areas where these liver flukes are non-endemic is very low [116]. Schistosomes are trematode worms. The cercarial stage infects human by skin penetration. The worms mature and lay eggs in the bladder or intestine of the host, provoking symptoms of a disease known as bilharzia. *Schistosoma haematobium* infection is prevalent in Africa and the Middle East and has been identified as a cause of bladder cancer. *Schistosoma japonicum* infection is prevalent in Japan and China and has been associated with cancers of the liver, stomach and colorectum, but the evidence is weak and inconsistent [101].

1.3.9 Diet and Nutrition

Up to 30 % of human cancers are probably related to diet and nutrition [117]. These observations, which were made more than 30 years ago with the publication of the first reliable data on cancer incidence from population-based cancer registries [118, 119] are still substantially valid. Consumption of vegetables and fruits is associated with reduced risk of cancers of the pharynx, larynx, lung, oesophagus, stomach and cervix uteri, while only vegetables, but not fruit, seem to protect against cancers of the colon and rectum [120] (Figure 1.9).

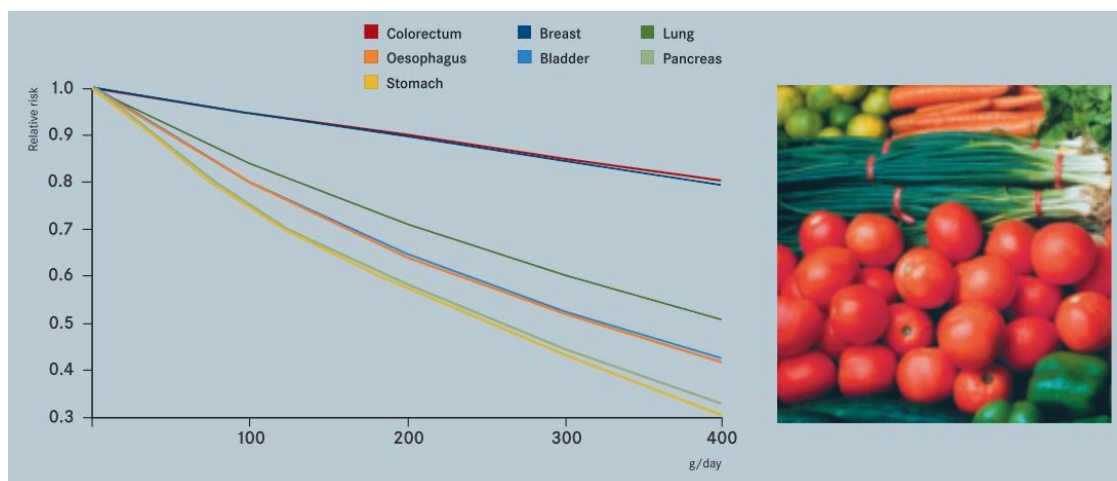


Figure 1.9: Fruit consumption associated with reduced risk of cancer (according to results of meta-analyses). Findings are essentially similar for vegetable consumption [120].

Preliminary results from the large European Prospective Investigation into Cancer and Nutrition (EPIC) study confirmed these results, for example, a daily consumption of 500 g of fruit and vegetables can decrease incidence of cancers of the digestive tract by as much as 25 % [121]. Fruit and vegetables do not represent a major source of protein, fat, carbohydrates and therefore energy, but they can be major contributors of fibre, several vitamins, minerals and other biologically active compounds. Current hypotheses on mechanisms through which fruit and vegetables may protect against cancer invoke the interaction of micro-constituents with the processes of carcinogen metabolism, protection of DNA integrity and intercellular communication.

Consumption of salt added to food and salt-preserved foods has been investigated mainly in relation to cancers of the stomach, colorectum and nasopharynx. Several studies conducted in Europe, South America and Eastern Asia have reported increased relative risks of stomach cancer in relation to the consumption of salt and salt-preserved foods, particularly in populations with high stomach cancer incidence and high salt intake [122] (Figure. 1.10).

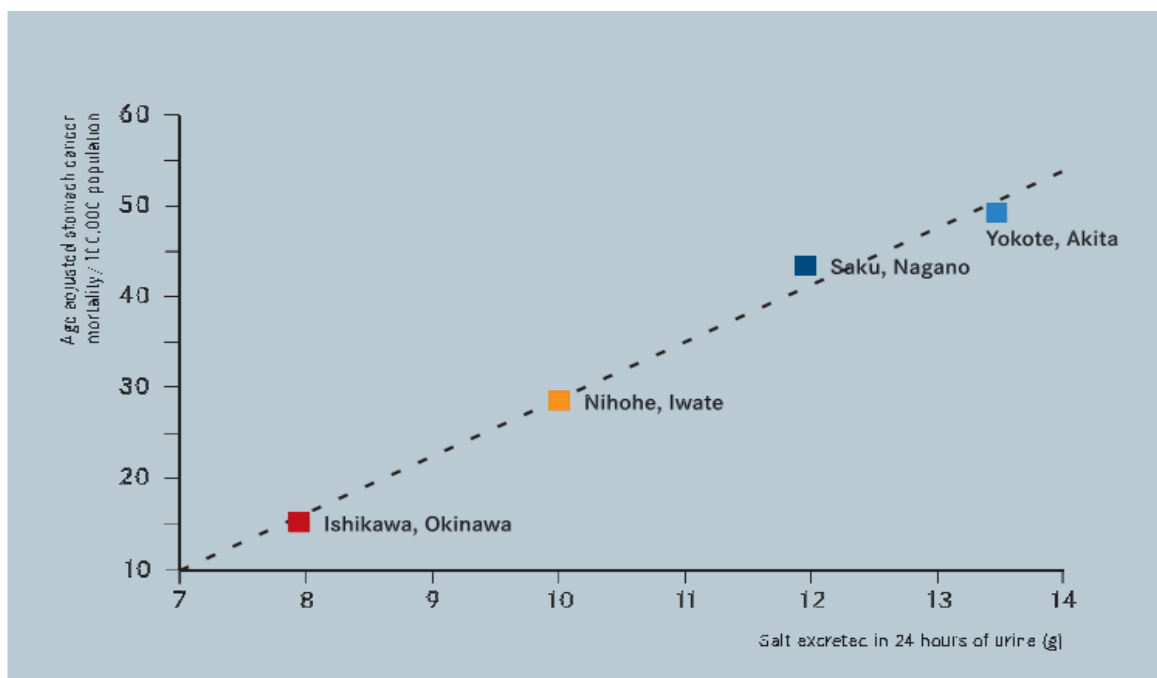


Figure 1.10. The age-adjusted mortality rate for gastric cancer increases with increasing salt consumption, measured by 24-hour urine sodium excretion, in selected regions of Japan [122].

Salted, smoked, pickled and preserved food (rich in salt, nitrite and preformed N-nitroso compounds) are associated with increased risk of gastric cancer. Such high salt intake, together with *Helicobacter pylori* infection, may contribute to the development of atrophic gastritis, and hence gastric cancer [123]. Domestic refrigeration and reduced salt consumption are likely to have contributed to the observed decreased stomach cancer incidence in developed countries during the 20th century [122, 124]. Epidemiological studies on meat consumption and cancer risk support the existence of a specific association with colorectal cancer risk [125]. This association, however, seems to have been found more consistently for consumption of red meat (beef, lamb and pork) and processed meat (ham, salami, bacon and other charcuterie) for which consumption of 80 g per day may increase colorectal cancer risk by 25 and 67 %, respectively [126]. Several biological mechanisms

have been investigated which could explain the possible effect of meat consumption on colorectal carcinogenesis. These include the influence of meat and/or fat consumption on the production and metabolism of bile salts and bile acids by gut flora [127].

Other hypotheses concerning the potential carcinogenic effect of certain compounds that can be formed in meat during cooking, such as heterocyclic amines and polycyclic aromatic hydrocarbons or as a consequence of preserved meat processing (nitrates and nitrites) or endointestinal metabolism (various N-nitroso compounds) [128]. Food additives are chemicals added to food for the purpose of preservation or to enhance flavour, texture or colour. Less than comprehensive toxicological data are available for most additives, although, some have been tested for mutagenic or carcinogenic activity [129]. In *in vitro* assay systems, some additives, such as dietary phenolics, have both mutagenic and antimutagenic effects [130]. In the past, some chemicals were employed as food additives before their carcinogenicity in animals was discovered, e.g. the colouring agent “butter yellow” (dimethylaminoazobenzene) and, in Japan, the preservative AF2 (2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide). Saccharin and its salts have been used as sweeteners for nearly a century. Although, some animal bioassays have revealed an increased incidence of urinary bladder cancer, there is inadequate evidence for carcinogenicity of saccharin in human [131].

Physical activity, avoidance of obesity, and frequent daily intake of fresh fruits and vegetables reduce the risk of oral cavity, lung, cervix uteri and other cancers [132, 133, 134]. Olive oil in the context of the Mediterranean dietary tradition is associated with a reduced risk of cancer [129]. A Western diet (highly caloric food rich in animal fat and protein), often combined with a sedentary lifestyle and hence energy imbalance, increases the risk of colon, breast, prostate, endometrial and other cancers [135].

1.3.10 Immunosuppression

Immunosuppression is a reduction in the capacity of the immune system to respond effectively to foreign antigens, and can be either transient or permanent. Persistent suppression of the immune system results in an increased cancer risk [136]. Apart from deliberate suppression of the immune response in the context of organ transplantation, immunosuppression may arise as a side-effect of some drugs [137], and specifically many cytotoxic agents widely used in cancer chemotherapy (Table 1.10) [138]. This action may contribute to the development of “second cancers”, particularly in children. More generally, patients receiving cancer chemotherapy are vulnerable to infectious disease as a result of their immune system being compromised [138].

Table 1.10: Immunosuppressive agents associated with development of cancer.

S/N	Drug or infectious agent	Cancer site/cancer
1	Azathioprine	Non-Hodgkin lymphoma, Kaposi sarcoma, squamous cell carcinoma of the skin, hepatobiliary cancers, mesenchymal tumours.
2	Cyclophosphamide	Bladder cancer
3	Ciclosporin	Non-Hodgkin lymphoma, Kaposi sarcoma
4	Human immunodeficiency virus-1 (HIV-1)	Non-Hodgkin (B-cell) lymphoma, Kaposi sarcoma (increased risk by coinfection with herpesvirus 8)
5	Epstein-Barr virus	Burkitt lymphoma (in conjunction with malaria infection), non-Hodgkin (B-cell) lymphoma in immunosuppressed patients, Hodgkin disease, smooth muscle tumours in immunosuppressed individuals
6	Human herpesvirus 8	Kaposi sarcoma
7	Human papillomaviruses	Cancers of cervix, vulva and anus

[138]

An increased incidence of malignant lymphomas, of which the majority contains the Epstein-Barr virus, is caused by immunosuppressive drugs used to prevent the rejection of organ transplants [1391- 142]. Infectious agents that cause severe immune suppression, such as the HIV, are associated with an increased incidence of several tumours, including non-Hodgkin lymphoma and Kaposi sarcoma [143, 144].

1.3.11 Hereditary Susceptibility

Inherited factors clearly contributed to some percentages of occurrence of cancer, particularly childhood cancer and cancer in early adulthood. Generally, cancer increases exponentially with age except for a blip on the curve for childhood cancer, which is thought to be mainly due to inheritance of a mutant cancer gene [145, 146]. Heredity is likely to affect susceptibility to all cancers. Inherited cancer syndromes, usually involving germline mutations in tumour suppressor or DNA repair genes, may account for up to 4 % of all cancers [147]. Inherited mutations of the breast cancer 1 (*BRCA1*) gene account for a small proportion of all breast cancers, but family members that have been affected stand the chance of a greater than 70 % lifetime risk of developing breast or ovarian cancer [148, 149]. The lifetime risk of cancer is high and there are usually recognizable phenotypic features that make the syndromes easy to identify clinically as shown in Table 1.11.

Table 1.11: Inherited cancer syndromes caused by a single genetic defect

S/N	Syndrome	Gene	Location	Cancer site/cancer
1	Familial retinoblastoma	RB1	13q14	Retinoblastoma, osteosarcoma
2	Multiple endocrine neoplasia II	RET	10q11	Medullary thyroid carcinoma, pheochromocytoma
3	Multiple endocrine neoplasia I	MEN1	11q13	Adrenal, pancreatic islet cells
4	Neurofibromatosis type I	NF1	17q11	Neurofibromas, optic gliomas, pheochromocytoma
5	Neurofibromatosis type II	NF2	22q2	Bilateral acoustic neuromas, meningiomas, cerebral astrocytomas
6	Bloom syndrome	BLM	15q26	Leukaemia, lymphoma
7	Familial adenomatous polyposis	APC	5q21	Colorectal, thyroid
8	Von Hippel-Lindau	VHL	3p25	Renal cell carcinoma, pheochromocytoma
9	Familial Wilms tumour	WT1	11q	Wilms tumour (kidney)
10	Xeroderma pigmentosum	XP(A-D)	9q, 3p, 19q, 15p	Basal cell carcinoma, squamous cell carcinoma, melanoma (skin)
11	Fanconi anaemia	FAC	16q, 9q, 3p	Acute leukaemia
12	Li-Fraumeni syndrome	p53	17p13	Breast and adrenocortical carcinomas, bone and soft tissue sarcomas, brain tumours, leukaemia
13	Cowden syndrome	PTEN	10q22	Breast, thyroid
14	Gorlin syndrome	PTCH	9q31	Basal cell carcinoma
15	X-linked proliferative disorder	XLP	Xq25	Lymphoma
16	Peutz-Jeghers syndrome	LKB1	19p	Breast, colon
17	Ataxia telangiectasia	ATM	11q22	Leukaemia, lymphoma

[147]

1.4 Classification of Cancer

There are more than 100 types of cancer. They can be classified in terms of their behaviour as either ‘benign’ or ‘malignant’, as well as by the type of tissue in which the cancer originates (histological type) and by primary site, or the location in the body where the cancer first developed [9].

1.4.1 Benign tumours are not cancerous and rarely threaten life [150 -151]. They tend to grow quite slowly (Figure 1.11), do not spread to other parts of the body by invasion or metastasis hence, they grow locally and are usually made up of cells quite similar to normal / healthy cells [152]. They will only cause a problem if they grow very large, becoming uncomfortable or press on other organs - for example a brain tumour inside the skull.

1.4.2 Malignant tumours are faster growing than benign tumours and have the ability to spread by invasion and metastasis and destroy neighbouring tissue (Figure 1.11). Cells of malignant tumours can break off from the main (primary) tumour and spread to other parts of the body through a process known as metastasis. Upon invading healthy tissue at the new site, they continue to divide and grow. These secondary sites are known as metastases and the condition is referred to as metastatic cancer [150. 152].

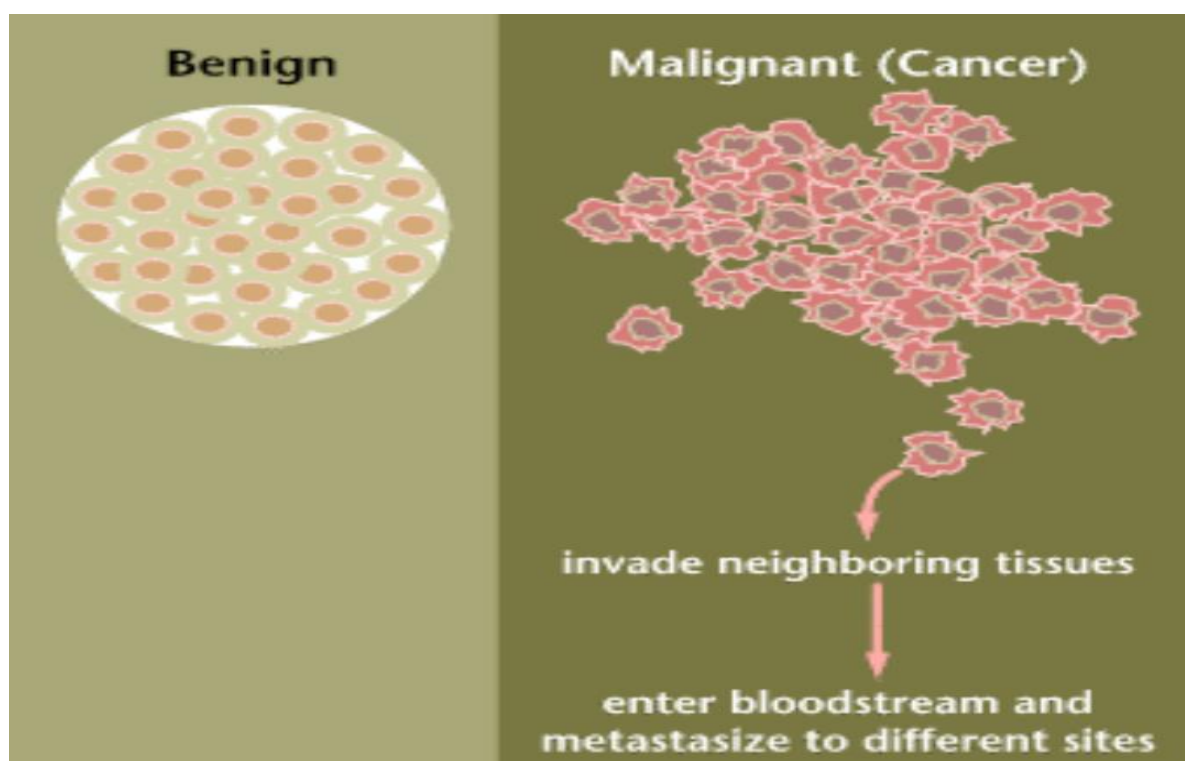


Figure 1.11: Benign Vs. Malignant Tumors [152]

1.4.3 Cancer type according to histological classification [153]:

From a histological standpoint, there are hundreds of different cancers, which are grouped into six major categories namely, carcinoma, sarcoma, myeloma, leukaemia, lymphoma, and mixed types [9].

1.4.3.1 Carcinoma– A cancer that arises from the epithelial cells (the lining of cells that protect or enclose organs). Carcinomas may invade the surrounding tissues and organs and metastasise to the lymph nodes and other areas of the body [154]. The most common forms of cancer in this group are breast, prostate, lung and colon cancer.

1.4.3.2 Myeloma- Myeloma is cancer that originates in the plasma cells of bone marrow [155]. The plasma cells produce some of the proteins found in blood.

1.4.3.3 Sarcoma – A type of malignant tumour of the bone or soft tissue (fat, muscle, blood vessels, nerves and other connective tissues that support and surround organs). The most common forms of sarcoma are leiomyosarcoma, liposarcoma and osteosarcoma.

1.4.3.4 Lymphoma– Lymphoma is a cancer of the lymphatic system, which runs all through the body, and can therefore occur anywhere. The two main forms are non-Hodgkin's (which begins with uncontrolled growth of the lymphocytes of the immune system) and Hodgkin's lymphoma in which cells of the lymph nodes become cancerous.

1.4.3.5 Leukaemia- Leukaemia is a cancer of the white blood cells and bone marrow (the tissue that forms blood cells). There are several subtypes but the most common are lymphocytic leukaemia and chronic lymphocytic leukaemia.

1.4.3.6 Mixed type- The type components may be within one category or from different categories. Some examples are: adenosquamous carcinoma, mixed mesodermal tumor, carcinosarcoma, teratocarcinoma [154].

1.4.4 Cancer types by primary site

Medical professionals frequently refer to cancers based on their histological type. However, the general public is more familiar with cancer names based on their primary sites (Figure 1.12). The most common sites in which cancer develops include: skin, lungs, female breasts, prostate, colon and rectum, cervix and uterus [156].

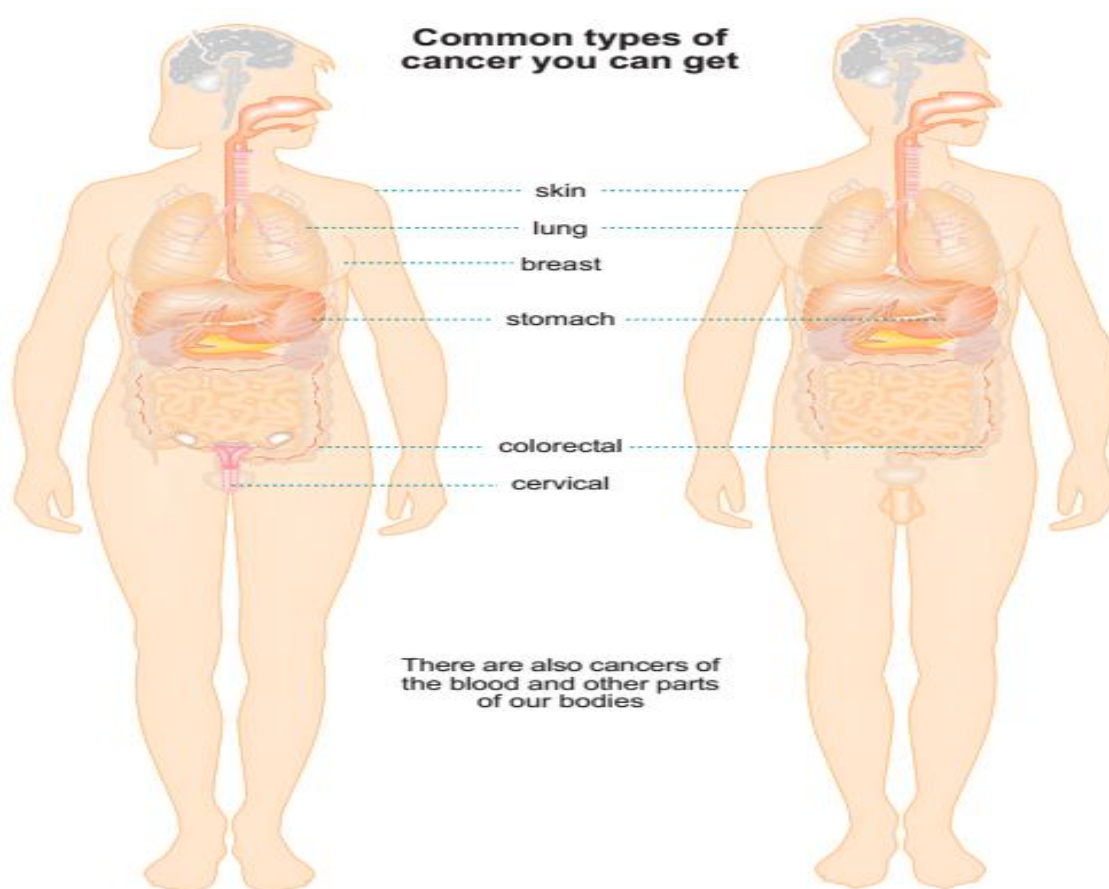


Figure 1.12: Cancer names based on their primary sites [156]

1.5 Anticancer agents from medicinal plants

Cancer is a major public health burden in both developed and developing countries. Medicinal plant derived agents are being used for the treatment of cancer. Several anticancer agents including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan, irinotecan, and etoposide derived from epipodophyllotoxin are in clinical use all over the

world. A number of promising anticancer agents such as flavopiridol, roscovitine, combretastatin A-4, betulinic acid and silvestrol are in clinical or preclinical development [157].

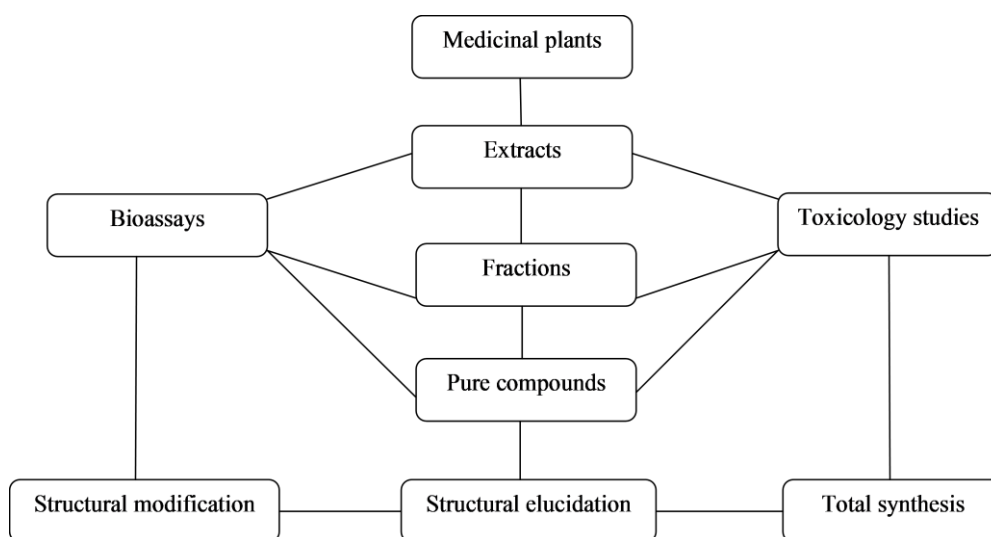
Medicinal plants have been utilized as medicines for thousands of years [158]. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations [158,159]. The specific plants to be used and the methods of application for particular ailments were passed down through oral history. Eventually, information regarding medicinal plants was recorded in herbals. In more recent history, the use of plants as medicines has involved the isolation of active compounds, beginning with the isolation of morphine from opium in the early 19th century [158,160]. Drug discovery from medicinal plants led to the isolation of early drugs such as cocaine, codeine, digitoxin, and quinine, in addition to morphine, of which some are still in use [158,161,162]. Isolation and characterization of pharmacologically active compounds from medicinal plants continue today. More recently, drug discovery techniques have been applied to the standardization of herbal medicines, to elucidate analytical marker compounds [162].

The process typically begins with a botanist, ethnobotanist, ethnopharmacologist, or plant ecologist who collects and identifies the plant(s) of interest. Collection may involve species with known biological activity for which active compound(s) have not been isolated (e.g., traditionally used herbal remedies) or may involve taxa collected randomly for a large screening program. Phytochemists (natural product chemists) prepare different extracts from the plant materials, subject these extracts to biological screening using pharmacologically relevant assays, and commence the process of isolation and characterization of the active compound(s) through bioassay-guided fractionation (Figure 1.13). Molecular biology has become essential to medicinal plant drug discovery through the determination and implementation of appropriate screening assays directed towards physiologically relevant

molecular targets [157,163] as well as pharmacognosy which involves the physical, chemical, biochemical and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources [164, 165].

As for cancer protection, it has been estimated that diets rich in phytochemicals can reduce cancer risk by 20 % [166]. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites [167, 168]. Plant natural product chemistry has played an active role in generating a significant number of drug candidate compounds in a drug discovery program [169]. Recently, it has been reported in literature that approximately 49 % of 877 small molecules that were introduced as new pharmaceuticals between 1981 and 2002 by New Chemicals Entities were either natural products or semi-synthetic analogs or synthetic products based on natural product models [170].

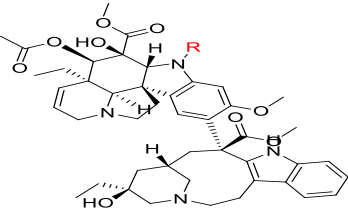
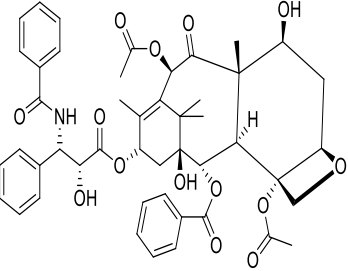
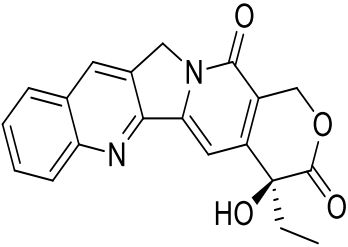
A summary of the processes involved in obtaining active biological substances is summarized below (Scheme 1.0).

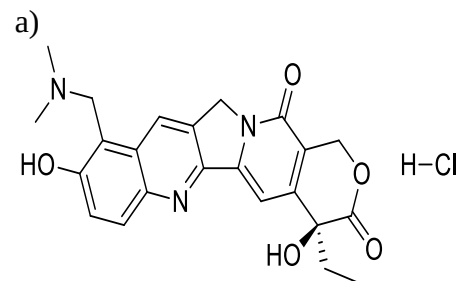


Scheme 1.0: Routes for obtaining active substances from plants [168, 170].

1.5.1 Plant-derived anticancer agents in clinical use: A few examples as illustrated in Table 1.12

Table 1.12: Plants-derived anticancer agents in clinical use

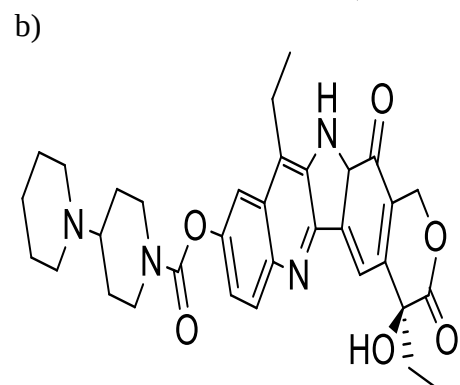
S/N	NAME OF PLANT	CLASS OF SECONDARY METABOLITE	ISOLATED COMPOUND	NAME	USED AGAINST CANCER TYPE(S)	MODE OF ACTION
1	<i>Catharanthus roseus</i> . G. Don. (Madagascar periwinkle) [166,171]	Alkaloids (vinka alkaloids)		Vinblastine (VLB)[R=-CH ₃] vincristine (VCR)[R= -CHO]	Leukemias, lymphomas, advanced testicular cancer, breast and lung cancers, and Kaposi's sarcoma	Anti-mitotic and anti-microtubule
2	<i>Taxus brevifolia</i> (Pacific yew) [172]	Alkaloids		P aclitaxel	Ovarian, breast, lung, pancreatic and other cancers	Inducing apoptosis (interference with the normal breakdown of microtubules during cell division.)
3	<i>Camptotheca acuminata</i> [173,174]	Alkaloids		Camptothecin (CPT) (stoppage of usage due to bladder toxicity) modified to give 2 semi-synthetic derivatives		



a) Topotecan [173]

a) Ovarian cancer, lung cancer and other cancer types.

a) Intercalates between DNA bases in the topoisomerase-I cleavage complex

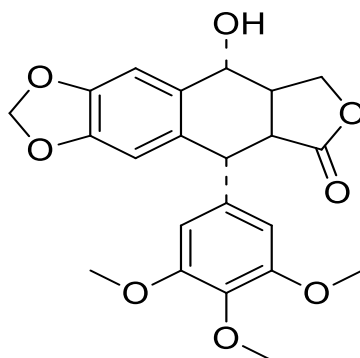


b) Irinotecan [174]

b) Colon cancer

b) Prevents DNA from unwinding by inhibition of topoisomerase-1

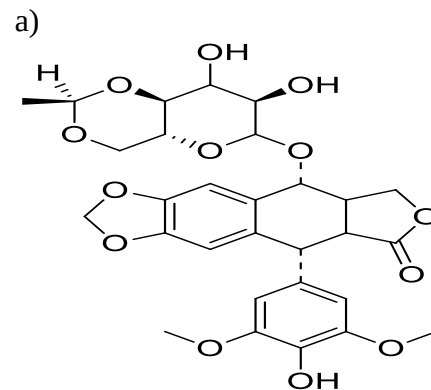
4 *Podophyllum peltatum* [175, 176] Non-alkaloids



Epipodophyllotoxin modified to give 2 semi-synthetic derivatives

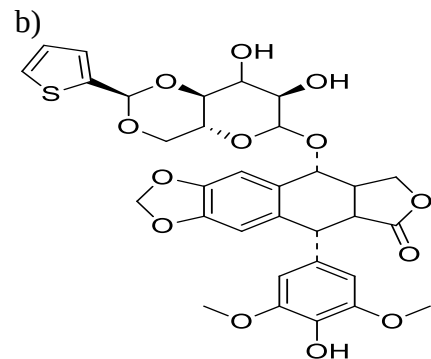
Treatment of cancer

Inhibition of topoisomerase II



a) Etoposide

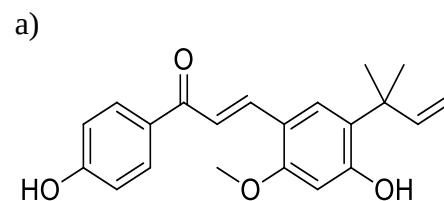
a) Kaposi's sarcoma, Ewing's sarcoma, lung cancer, testicular cancer, lymphoma, nonlymphocytic leukemia, and glioblastoma multiforme.



b) Teniposide.

b) Childhood acute lymphocytic leukemia (ALL), Hodgkin's lymphoma, certain brain tumours, and other types of cancer.

5 *Glycyrrhiza glabra* Flavonoids



a) Licochalcone A [177]

a) Gastric cancer

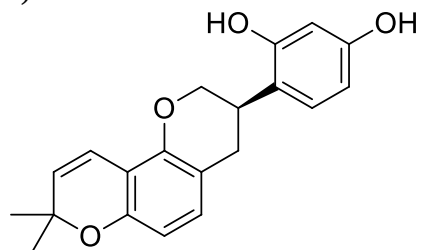
a) Inhibits cell cycle and induces cell apoptosis

6

*Scutellaria
lateriflora*
[179, 180]

Flavone

b)

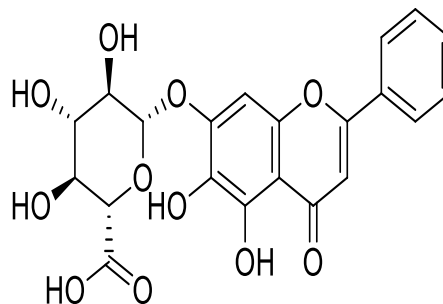


b) Glabridin[178]

b) Breast cancer

b) Inhibits cell
metastasis,
decrease tumor
angiogenesis

a)

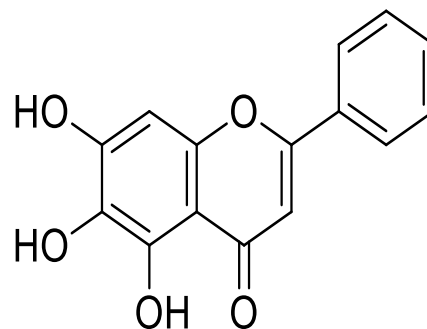


a) Baicalin

a) Protects the
skin. It also
reduces UV-
related apoptosis
(cell death)

a) Protects the
skin with
antioxidant
effects. It also
reduces UV-
related apoptosis
(cell death)

b)

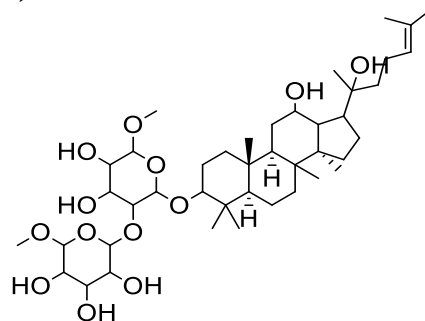


b) Baicalein

b) Breast cancer,
human colorectal
cancer

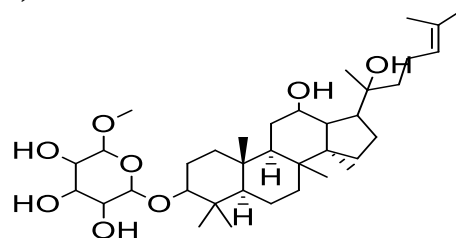
b) Suppressed
proliferation,
migration, and
invasion of breast
cancer cells

a)

a) Ginsenoside
RG3[181, 182]a) Leukaemia,
prostate cancer

a) Rg3 can promote TRAIL (Tnf Related Apoptosis Inducing Ligand) by inducing apoptosis of hepatocellular carcinoma cell line.

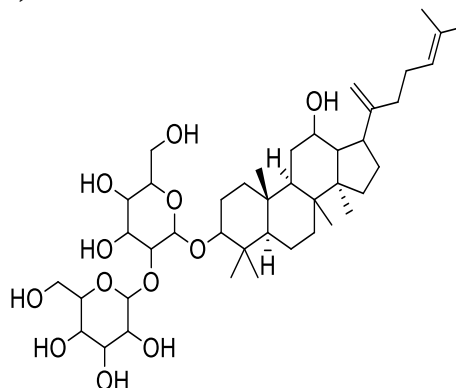
b)

b) Ginsenoside RH2
[181,183]

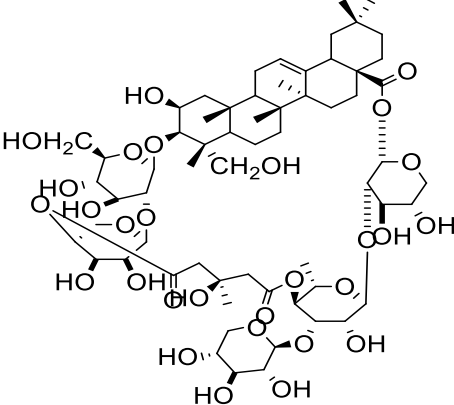
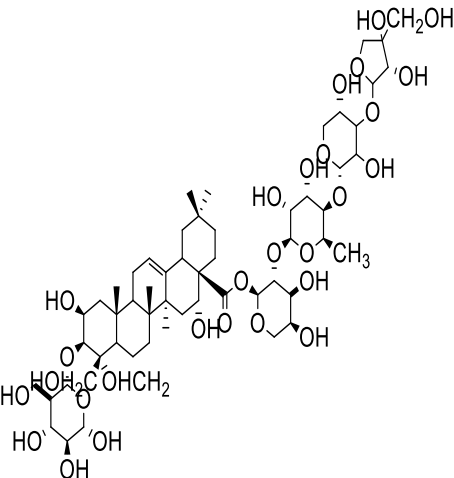
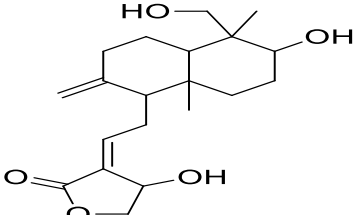
b) Leukaemia

b) Rh2 can treat leukemia by blocking the cell cycle; treat pancreatic cancer by inhibiting proliferation, migration, invasion and inducing apoptosis of cancer cells.

c)

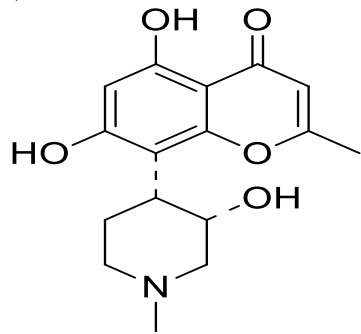
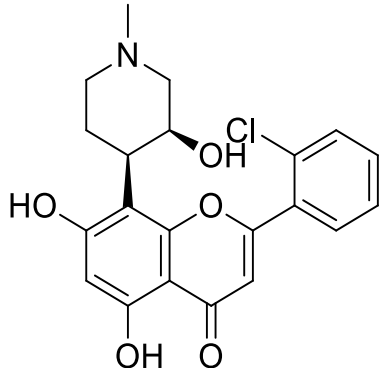
c) Ginsenoside Rk1
[184]c) Colon cancer, a
variety of cancer
cells growth

c) Inhibited telomerase activity and cell growth along with significant morphological change and induced apoptosis

8	<i>Bolbostemma paniculatum</i> [185]	Saponins		Tubeimoside-1 (TBMS1)	Esophageal cancer	Cell proliferation
9	<i>Platycodon grandiflorum</i> [186]	Saponins		Platycodin	Prostate cancer cells	Induce apoptosis
10	<i>Andrographis paniculata</i> [187]	Terpenoid		Andrographolide	Breast cancer	inhibit cancer cell proliferation, migration and arrest cell cycle at G2/M phase

1.5.2 Plant-derived anticancer agents in clinical development: A few examples as illustrated in Table 1.13

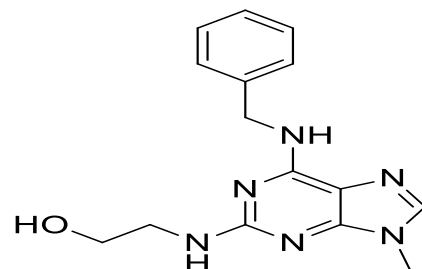
Table 1.13: Plants derived anticancer agents in clinical development

S/N	NAME OF PLANT	CLASS OF SECONDARY METABOLITE	ISOLATED COMPOUND	NAME	USED AGAINST CANCER TYPE(S)	MODE OF ACTION
1	<i>Dysoxylum binectariferum</i> Hook. f. [188, 189]	Flavonoid alkaloid	a) 	a) Rohitukine modified to	Acute myeloid leukemia, breast and lung carcinoma	Inhibition of P-TEFb and the loss of mRNA production
			b) 	b) Flavopiridol		

2	<i>Combretum caffrum</i> (bush willow) [190,191]	Stibenos	a) Chemical structure of Combretastatin A1 (a): A stilbenoid molecule consisting of two phenyl rings connected by a 1-hydroxyethyl chain. The left ring is a 3,4,5-trimethoxyphenyl group. The right ring is a 3,4-dimethoxyphenyl group with a hydroxyl group at the 1-position.	a) Combretastatins	Tumors	Act as anti-angiogenic agents, causing vascular shutdown in tumors and resulting in tumor necrosis
			b) Chemical structure of Combretastatin A4 (b): A stilbenoid molecule consisting of two phenyl rings connected by a trans-vinyl chain. The left ring is a 3,4,5-trimethoxyphenyl group. The right ring is a 3,4-dimethoxyphenyl group with a hydroxyl group at the 1-position.	b) Combretastatins A4		

3 *Raphanus sativus*
L. [192, 193]

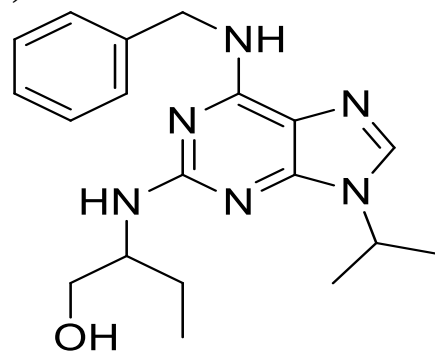
a)



a) Olomucine

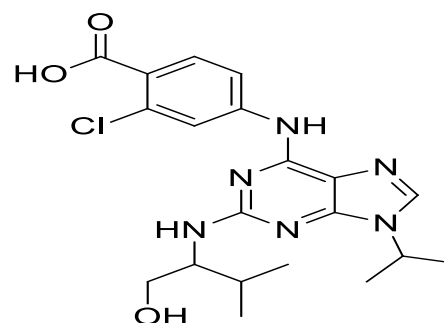
Inhibit cyclin-
dependent kinases
(Cdk)

b)



b) Roscovitine

c) purvalanols



c) Purvalanol
(preclinical
development)

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Combretastatins

Combretastatins are compounds isolated from the South African “bush willow”, *Combretum caffrum* (Eckl. & Zeyh.) Kuntze (Combretaceae), collected in southern Africa in the 1970s as part of a random collection program for the National Cancer Institute (NCI) by the United State Department of Agriculture (USDA), working in collaboration with the Botanical Research Institute of South Africa [194]. Species of the *Combretum* and *Terminalia*, both of which belong to the Combretaceae family, are used in African and Indian traditional medicine for the treatment of a variety of diseases, including hepatitis and malaria, with several *Terminalia* species reportedly used in the treatment of “cancer” [195].

The combretastatins are a family of stilbenes which act as anti-angiogenic agents, causing vascular shutdown in tumors and resulting in tumor necrosis. A water-soluble analog, combretastatin A4 phosphate (CA4), has shown promise in early clinical trials, and a number of combretastatin (CA4) mimics are being developed. Three are in clinical trials, while 11 are in preclinical development [196]. This chemical class has served as a model for the synthesis of a host of analogs containing the essential trimethoxy aryl moiety linked to substituted aromatic moieties through a variety of two or three atom bridges including heterocyclic rings and sulfonamides, thus providing an impressive display of the power of a relatively simple natural product structure to spawn a prolific output of medicinal and combinatorial chemistry [190]. Combretastatin is active against colon, lung and leukaemia cancers and it is expected that this molecule is the most cytotoxic phytomolecule [196,197].

2.2 Isolates from *Combretum caffrum*

In 1982, Pettit *et al.* [198, 200, 201] reported the isolation and structure of Combretastatin, the first member of a series of biologically active bibenzyls, stilbenes and phenantherenes

from the bark of *C. caffrum* at Arizona State University, USA. Combretastatins A-1 and A-4 were isolated by the same group in 1987 and 1989 respectively [201,202]. Chemically, they are stilbene derivatives having two phenyl rings separated by a C-C double bond. Ring-A has three methoxy groups in 3,4,5-positions while in ring B one hydroxy group is at the C-3 position and one methoxy group at the C-4 position. (Figure 1.16)

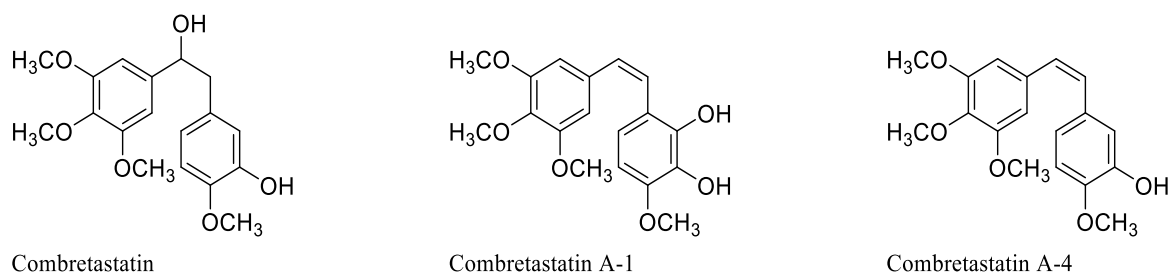


Figure 2.0: Structures of Combretastatin and Combretastatin A-1 and A-4

2.3 Structure Activity Relationship (SAR) of Combretastatin Analogues

A number of studies have been reported on the structure activity relationship of combretastatins [203,204]. For a minimal cytotoxic activity of such compounds, a diaryl system should be separated through a double bond along with a trimethoxy system in one of the rings (Figure 2.1).

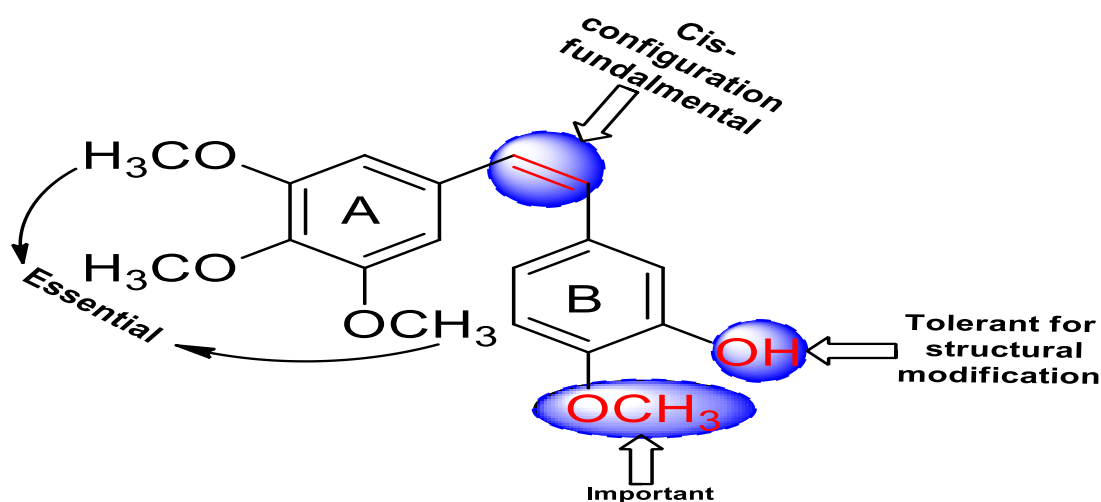


Figure 2.1: SAR of Combretastatin Analogues

The benzene ring 'A' contain the trimethoxy benzene moiety essential for activity [205], and the two aryl groups 'A' and 'B' separated through a double bond which is the cis (Z) isomer is preferred over the trans (E) as cis is more active than trans [206].

Cushman *et al.* [206] reported that the 3-hydroxyl group on ring B of CA-4 is not necessary for potent activity. However, a 4-methyl or 4-methoxy group is required in ring B for strong cytotoxicity activity [207]. An additional hydroxyl group at C-2 on ring B of CA-4 is less preferred as it decreases its activity as in CA-1 [208]. Introduction of an amino group in ring B significantly decreased antimitotic activity but showed strong cytotoxicity, more potent than that of CA-4 [200].

2.4 Biological Activity and Mode of Action of CA-4

CA-4 is an investigation drug of the National Cancer Institute and the University of Arizona, USA. The compound is active against colon, lung and leukaemia cancers. CA-4 exhibited an LD₅₀ value of 7 nm (0.007 lM) against murine L1210 leukaemia cell lines [209, 210].

In vitro studies showed that CA-4 competes with colchicine for binding sites on tubulin hence, it is a member of the colchicine-like inhibitors of microtubulin assembly rather than the vinca alkaloid type compound (i.e., tubulin polymerization inhibitors) [211]. McGown *et al.* [212] suggested that the trimethoxy benzene moiety in all these compounds (colchicine, CA-4 and podophyllotoxin) probably provides a favourable binding site for tubulin [197, 212]. Several *in vivo* experiments have been done in rats. Magnetic Resonance Imaging (MRI) experiments showed [213] that combretastatin-4,3-O-phosphate (CA-4P) significantly reduced blood flow to the tumour cells in a dose-dependent manner. Thus, it acted as an antivasular targeting agent [214] which blocks tumour blood supply. In animal tumours, it can cause the shutdown of blood flow [215] leading to extensive tumour necrosis. A sodium phosphate derivative of CA-4 induced a complete vascular shutdown within metastatic

tumours at doses one-tenth of the maximum tolerated dose [216] while the reduction in blood flow by CA-4 is up to 70 %.

2.5 Synthetic Analogues of CA-4

Varied modifications have been reported in the CA-4 molecule. In some analogues, only the functional groups have been modified but in several analogues the total aryl ring is either replaced or totally modified by some other groups. However, in almost all cases 3,4,5-trimethoxy aryl or ring A was kept intact since it is considered indispensable for cytotoxicity of the molecule [205].

2.6 Modifications in Linker Alkene

From the SAR studies, it was concluded that the presence of an alkene is not necessary for activity. However, the restricted rotation of rings A and B of CA-4 can also be maintained by introducing suitable conformational restricted arrangements. Sun *et al.* [217] reported 1,4-disubstituted azetidinone ring system having good cytotoxicity against MCF-7, CHO-K, and NCI-H69 cancer cell lines. Thiophene-based analogues of CA-4 have shown tubulin polymerization inhibition activity comparable to CA-4. Gwaltney *et al.* [218] used a sulfonate group between the aryl groups for restricted rotation and compounds 22 and 23 showed cytotoxicity comparable to CA-4 (Table 2.0 and Figure 1.18).

Table 2.0: Cytotoxicities of sulfonate derivatives of CA-4

S/N	COMPOUND	HCT-15 human colonm Carcinoma IC ₅₀ (nM)	NCI-H460 human lung Carcinoma IC ₅₀ (nM)
1	CA-4	1.7	3
2	17- Paclitaxel	450	15
3	22	3.3	3.1
4	23	4.1	2.7

[218]

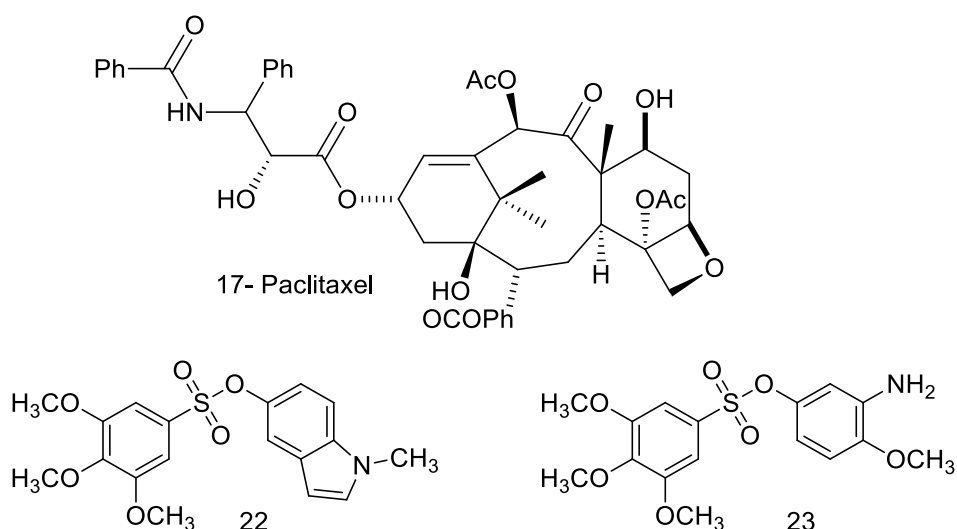


Figure 2.2: Novel sulfonate analogues of CA-4

2.7 Review of Combrestatin CA-4 Analogues

Aziz *et al.* [219] reported the synthesis and structure activity relationships associated with a series of 1,1-diarylethylene (Figure 2.3) tubulin polymerization inhibitors 3 and 4. The key step for their preparation involved a palladium catalyzed coupling of N-arylsulfonylhydrazones with aryl halides, thus providing flexible and convergent access to tri- and tetra-substituted 1,1-diarylethylens 3 and 4 related to isocombretastatin A-4 (iso CA-4). These compounds have been evaluated for tubulin polymerization inhibitory activity as well as for cytotoxic activity.

The most potent compounds are 1,1-diaryl-2-methoxyethylenes (**4b**, **4d** and **4e**) having a trisubstituted double bond. They exhibited good antiproliferative activity against various human cancer cell lines ($GI_{50} = 8^{-80}$ nM). Compounds **4b** and **4e** strongly inhibited tubulin polymerization with IC_{50} values of 2 and 3 μ M, respectively, and induced cell cycle arrest in the G2/M phase in the K562 cell line. Docking studies in the colchicine binding site of tubulin allowed identification of residues most likely to interact with these inhibitors and explain their potent anti-tubulin activity [220].

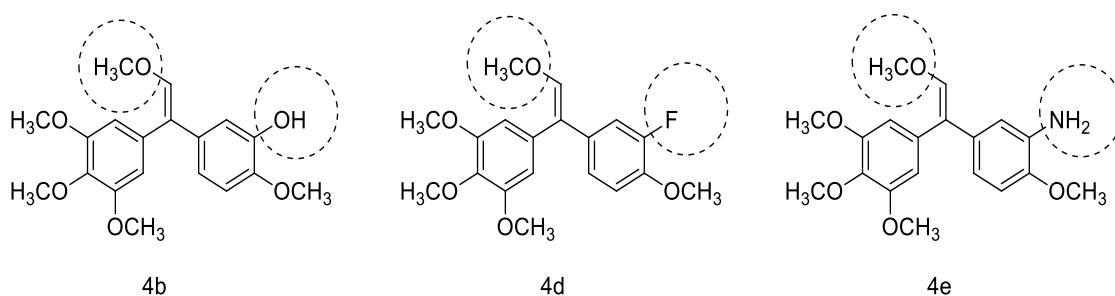


Figure 2.3: 1,1-diarylethylene

Sanghai *et al.* [220] based on the pharmacophoric features of the natural product combretastatin A-4 (CA-4) and its synthetic analogues that inhibited tubulin polymerization, designed a series of novel 2-aryl-3-arylamino-imidazo-pyridines/ pyrazines (Figure 2.4) as potential antitubulin anticancer agents. They were synthesized by a one-pot method involving preparation of isocyanides from the anilines via formylation and subsequent dehydration followed by their reactions with heterocyclic-2-amidines and aldehydes.

Compounds **1**, **2**, **14**, and **15** (Figure 2.4), were found to exhibit significant tubulin polymerization inhibition and disruption of tubulin– microtubule dynamics similar to that of CA-4. They showed potent anticancer activity in kidney, breast and cervical cancer cell lines and relatively low toxicity to normal cells compared to CA-4. The compounds induced DNA damage, chromosomal damage and apoptosis via cell cycle arrest in the G2/M phase. The molecular docking and molecular dynamics (MD) simulation studies revealed that disruption of microtubule dynamics might occur by interaction of the compounds at the colchicine binding site of the α,β -tubulin heterodimer interface, similar to that of CA-4.

Molecular modeling analysis showed that two of the three methoxy groups at ring A of all four potent compounds **1**, **2**, **14**, and **15** (Figure 2.4) were involved in bifurcated hydrogen bonding with Cys β 241 [220], an important molecular recognition interaction to show tubulin inhibitory activity. In comparison to CA-4, the bridging NH and the imidazo-pyridine/pyrazine moieties in the compounds provide flexibility for attaining the required

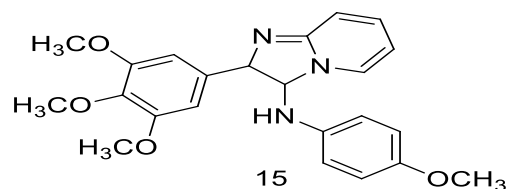
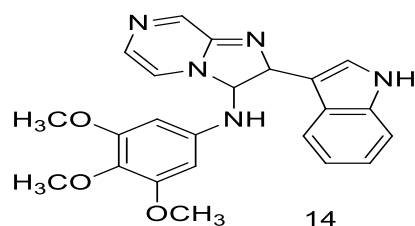
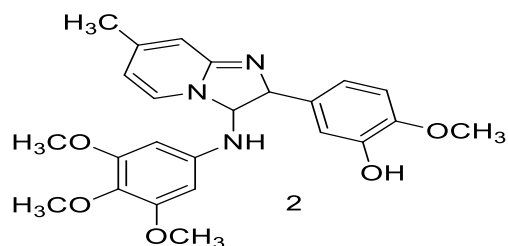
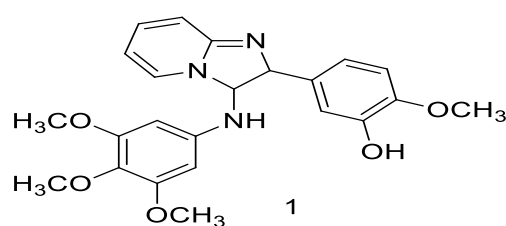
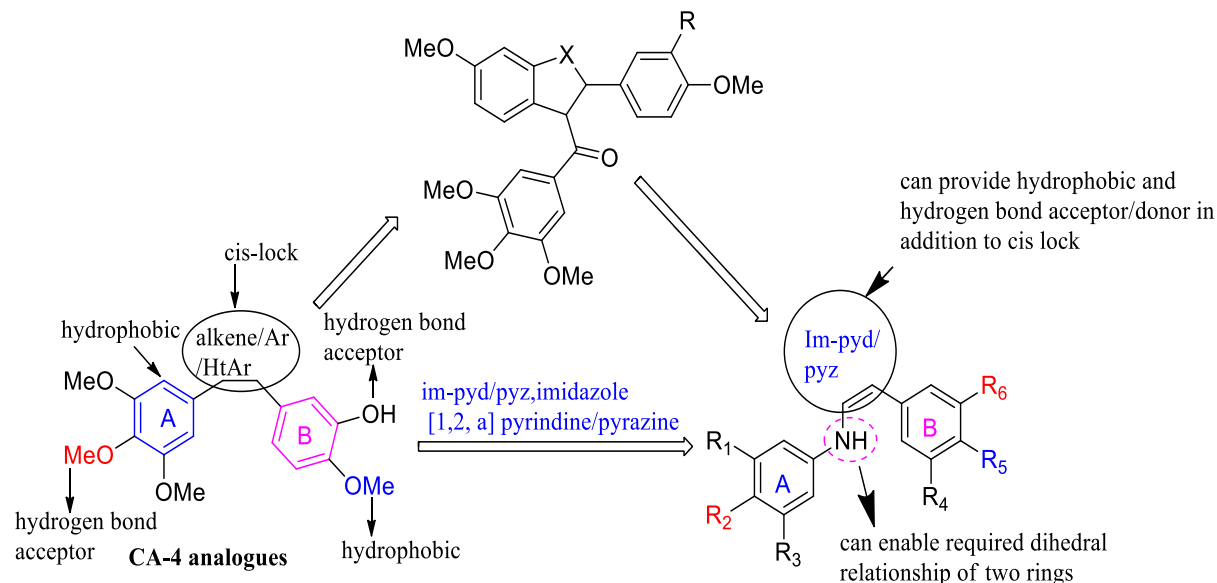


Figure 2.4: Design of novel 2-aryl-3-aryl-amino-imidazo-pyridines/pyrazines as potential antitubulin anticancer agent.

Jiang *et al.* [221] investigated and reported a potent combretastatin A-4 (CA-4) like tubulin polymerization inhibitor **22b** (Figure 2.5) which was previously found with strong antitumor activity. However, it easily undergoes cis – trans isomerization under natural light and the resulting decrease in activity limits its further applications. In the study, the quantum

chemistry calculations were used to explore the molecular basis of its instability. The calculations were aided by, two rounds of structural optimization of **22b** (Figure 2.5).

Accelerated quantitative light stability testing confirmed that the stability of these designed compounds was significantly improved as predicted. Among them, compounds **1** and **3b** (Figure 2.5) displayed more potent inhibitory activity on tumor cell growth than **22b** (Figure 2.5). In addition, the potent *in vivo* antitumor activity of compound **1** was confirmed. Quantum chemistry calculations were used in the optimization of stilbene-like molecules, providing new insight into stilbenoid optimization and important implications for the future development of novel CA-4-like tubulin polymerization inhibitors [222].

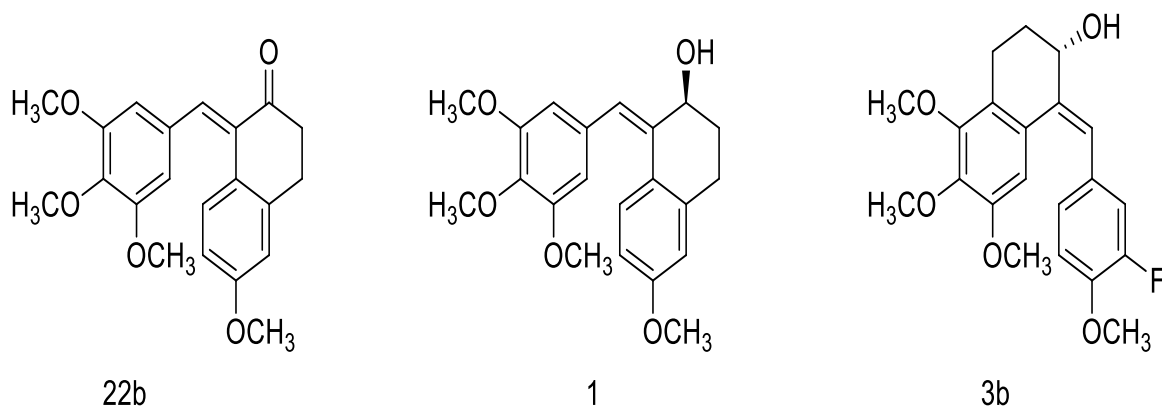


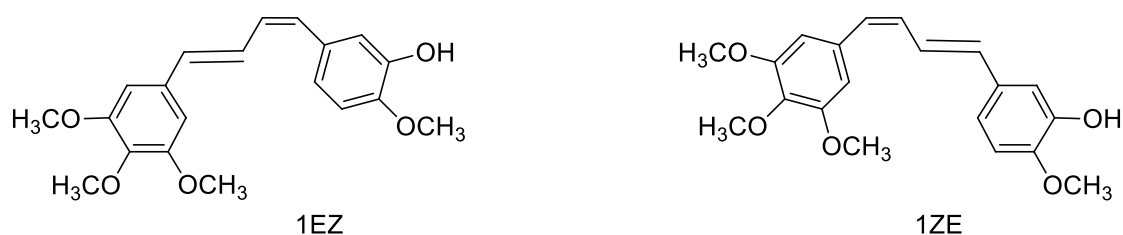
Figure 2.5: Combretastatin A-4 (CA-4) like tubulin polymerization inhibitor

A series of combretastatins possessing both a trimethoxy unit and other substituents on ring A has been synthesized [222] and tested for cytotoxicity and their ability to interact with the protein tubulin. All previous studies have indicated that the trimethoxy unit is essential for interaction with tubulin. The studies herein showed that molecules possessing functionalities other than trimethoxy can also interact with tubulin.

Importantly, a trimethyl substituted agent **52a** (Figure 2.6) has shown reduced cytotoxicity, but increased potency in its ability to inhibit the assembly of tubulin. Of the non-trimethoxy

52a

Stereospecific syntheses of the Z-E (**1ZE**) and E-Z (**1EZ**) vinylogues of CA-4 (Figure 2.7), and two B-ring related analogues were synthesized [223] via a Suzuki–Miyaura coupling. As compared to CA-4, the derivative with a phenyl moiety showed increased potency in its ability to inhibit tubulin polymerisation. It was demonstrated that the replacement of the cis double bond between the two aromatic rings of CA-4 by an (E, Z) butadiene moiety, gave compounds with equal or greater depolymerising microtubule activity than CA-4. Also, the replacement of the cis double bond between the two aromatic rings of CA-4 by an (E, Z) butadiene moiety, gave compounds with equal or greater depolymerising microtubule activity than CA-4 [223].



Wang *et al.* [224] designed a series of novel 2,3-diarylthiophene analogues of CA-4 with a rigid thiophene moiety (Figure 2.8) to retain the cis-olefin configuration of CA-4 and were subsequently Synthesized. These compounds were evaluated for their *in vitro* anti-proliferative activities. Among these compounds, compounds **5f** and **8d** (Figure 2.8) exhibited superior potency against different tumour cell lines with IC₅₀ values at

submicromolar levels [224]. Moreover, compound **8d** significantly inhibited tubulin polymerisation to microtubules and caused microtubule destabilization [224]. In addition, a molecular modelling study of compound **8d** was performed to clarify its binding mode at the colchicine site in the tubulin dimer and to provide a basis for further structure-guided design of novel CA-4 analogues.



Figure 2.8: 2,3-diarylthiophene analogues of CA-4

Chaudhary *et al.* [225] had a great interest in discovering CA-4 inspired new chemical entities, hence a novel series of 4,5- diaryl-2-aminoimidazole analogues (Figure 2.9) of the compound was designed and Synthesized by an efficient and diversity-feasible route involving atom economical arene C-H bond arylation. Interestingly, four compounds showed potent cell-based antiproliferative activity in nanomolar concentrations [225]. Among the compounds, compound **12** inhibited the proliferation of several types of cancer cells much more efficiently than CA-4 [225]. It depolymerized microtubules, induced spindle defects and stalled mitosis in cells. Compound **12** bound to tubulin and inhibited the polymerization of tubulin *in vitro*.

In addition, podophyllotoxin and CA-4 inhibited the binding of compound 12 to tubulin. The distinctive pharmacophoric features of the bridging motif as well as quinoline nucleus were explored.

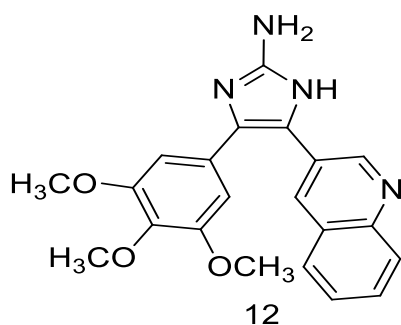


Figure 2.9: A novel series of 4,5- diaryl-2-aminoimidazole analogues

A series of novel pyridine-bridged analogues (Figure 2.10) of CA-4 were designed and Synthesized by Zheng *et al.* [226]. Activities of the analogues with 3-atom linker varied widely depending on the phenyl ring substitutions, and the 3-atom linker containing nitrogen represents the more favourable linker structure. Among them, three analogues, compounds (**4h**, **4s**, and **4t**) (Figure 2.10) potently inhibited cell survival, cell growth, arrested cell cycle, and blocked angiogenesis and vasculature formation *in vivo* in ways comparable to CA-4 [226]. The superposition of compounds **4h** and **4s** on the colchicine-binding pocket of tubulin shows the binding posture of CA-4, **4h**, and **4s** are similar, as confirmed by the competitive binding assay where the ability of the ligands to replace tubulin-bound colchicine was measured. The binding data were consistent with the observed biological activity in antiproliferation and suppression of angiogenesis but are not predictive of their antitubulin polymerization activity.

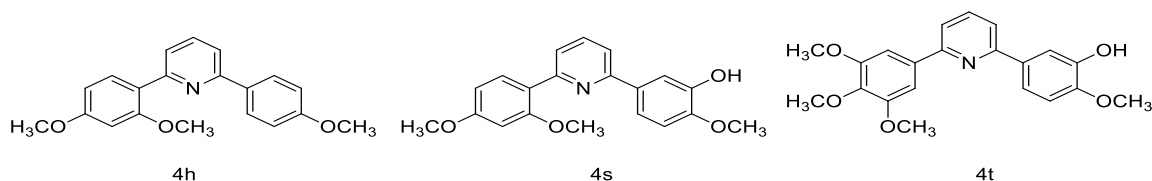


Figure 2.10: Novel pyridine-bridged analogues of CA-4

Wang *et al.* [227] investigated the synthesis and structure activity relationship study of a series of compounds with heterocycles in place of the cis double bond in CA-4. Substituted tosylmethyl isocyanides were found to be the key intermediates in construction of the

heterocycles. Cytotoxicities of the heterocycle-based CA-4 analogues (Figure 2.11) were evaluated against NCI-H460 and HCT-15 cancer cell lines. 3-Amino-4-methoxyphenyl and N-methyl-indol-5-yl were the best replacements for the 3-hydroxy-4-methoxyphenyl in CA-4. 4,5-disubstituted imidazole was found to be the best for the replacement of the cis double bond in CA-4. Medicinal chemistry efforts led to the discovery of compounds **24h** and **25f** that were found to be 32 and 82 % bioavailable respectively in rat. Evaluation of compounds **24h** and **25f** (Figure 2.11) against murine M5076 reticulum sarcoma in mice revealed that both compounds were orally efficacious with an increase in life span of 38.5 and 40.5 %, respectively.

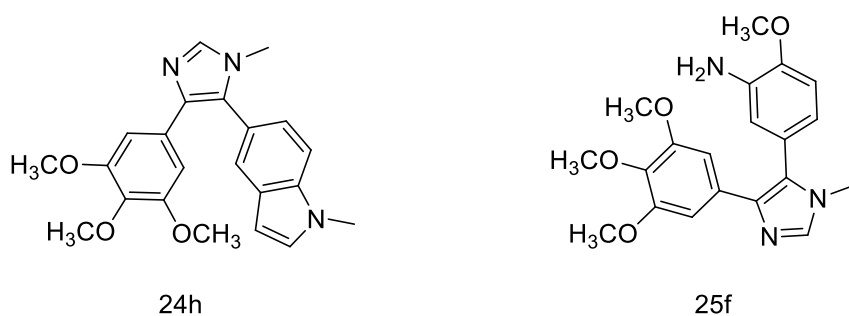


Figure 2.11: Heterocycle-based CA-4 analogues

A new series of 2,3-diaryl-4/5-hydroxy-cyclopent-2-en-1-one analogues (Figure 2.12) replacing the cis double bond of CA-4 by 4/5-hydroxy cyclopentenone moieties was designed and synthesized [228]. The analogues displayed potent cytotoxic activity ($IC_{50} < 1 \mu\text{g/ml}$) against a panel of human cancer cell lines and endothelial cells. The most potent analogues, **11** and **42** (Figure 2.12) belonging to the 5-hydroxy cyclopentenone class were further evaluated for their mechanism of action. Both analogues led to cell cycle arrest at G2/M phase and induced apoptosis in endothelial cells. Antitubulin property of compound **42** was superior to compound **11** and comparable to CA-4. The compound **42** had better aqueous solubility, metabolic stability and pharmacokinetic profile than CA-4 and also demonstrated significant tumor regression in the human colon xenograft model [228]. Their data suggested

that cis-restricted analogues of CA-4 are a new class of molecules that have the potential to be developed as novel agents for the treatment of cancer.

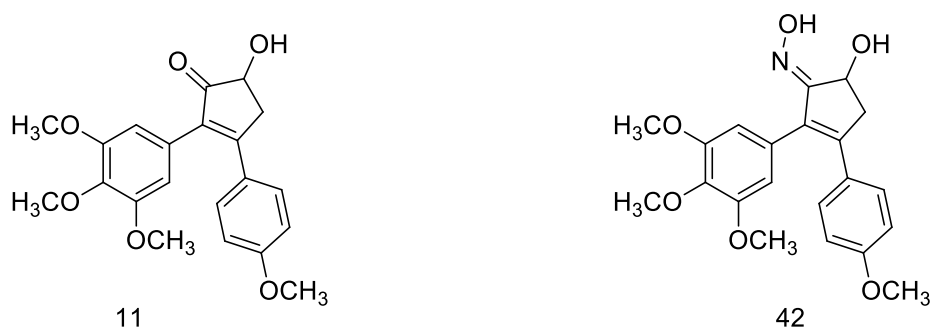


Figure 2.12: 2,3-diaryl-4/5-hydroxy-cyclopent-2-en-1-one analogues

Two new series of compounds, E-2,3,4-trimethoxy-6-styrylbiphenyls and 2,3,4-trimethoxy-6-(1-phenylvinyl)biphenyls were synthesized and evaluated for antitubulin activity [229]. A common intermediate 4,5,6-trimethoxybiphenyl-2-carbaldehydes was employed to generate the two scaffolds. Majority of the analogs inhibited cell proliferation and those functionalized with 3,4-(1,3-dioxolane) and 3,4-difluoro groups were identified as effective inhibitors in both series. Treatments with compounds **19b**, **19c**, **22b** and **22c** (Figure 2.13) arrested cells at G2/M phase, disrupted microtubule network, accumulated tubulin in the soluble fraction and manifested as an increased expression of the G2/M marker Cyclin B1 [229]. Molecular docking analysis demonstrated the interaction of these compounds at the colchicine binding site of tubulin.

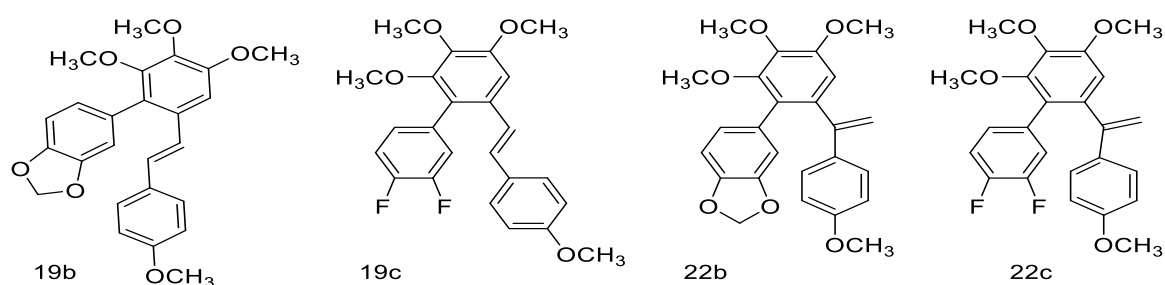


Figure 2.13: E-2,3,4-trimethoxy-6-styrylbiphenyls and 2,3,4-trimethoxy-6-(1-phenylvinyl)biphenyls

Isocombretastatins A are 1,1-diarylethene isomers of combretastatins A. Álvarez *et al* [230] synthesized the isomers of combretastatin A-4, deoxycombretastatin A-4, 3-amino-deoxycombretastatin A-4 (AVE-8063), naphthylcombretastatin and the N-methyl- and N-ethyl-5-indolyl analogues of combretastatin A-4. They prepared analogues with a 2,3,4-trimethoxyphenyl ring instead of the 3,4,5-trimethoxyphenyl ring (Figure 2.14). They reported that isocombretastatins A strongly inhibited tubulin polymerization and were potent cytotoxic compounds, some of them with IC₅₀ in the nanomolar range (compound **5d**, **5n**, and **5p**) (Figure 2.14). This new family of tubulin inhibitors showed higher or comparable potency when compared to phenstatin or combretastatin analogues. These results suggested that one carbon bridges with a geminal diaryl substitution can successfully replace the two carbon bridge of combretastatins and that the carbonyl group of phenstatins is not essential for high potency [230].

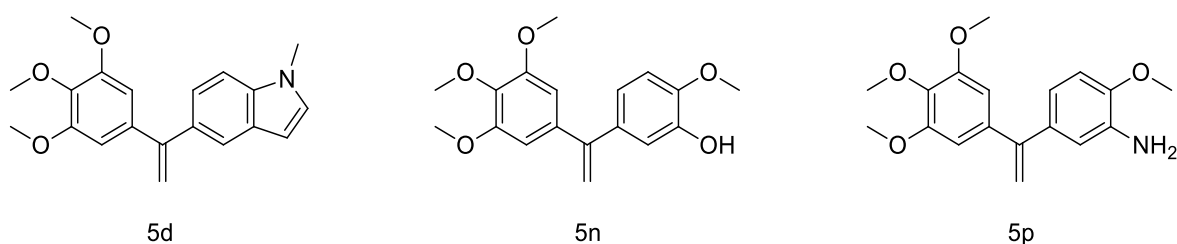


Figure 2.14: Analogues with a 2,3,4-trimethoxyphenyl ring

A series of 1,1-diarylethene derivatives were prepared by Ruchelman *et al* [231] to optimize the properties of CC-5079 (**1**), a dual inhibitor of tubulin polymerization and phosphodiesterase 4 (PDE4). By using the 3-ethoxy-4-methoxyphenyl PDE4 pharmacophore as one of the aromatic rings, a significant improvement in PDE4 inhibition was achieved. Compound **28** (Figure 2.15) was identified as a dual inhibitor with potent PDE4 (IC₅₀ = 54 nM) and antitubulin activity (HCT-116 IC₅₀ = 34 nM) and tubulin polymerization (IC₅₀ of 11 nM). While the nitrile group at the alkene terminus was generally required for potent antiproliferative activity, its replacement was tolerated if there was a hydroxyl or amino

group on one of the aryl rings [231]. It was reported that this group conveniently could also serve as a handle for amino acid derivatization to improve the compounds' solubility. The glycineamide analog compound **45** showed significant efficacy in the HCT-116/xenograft model with 64 % inhibition of tumor growth upon dosing at 20 mg/kg qd [231].

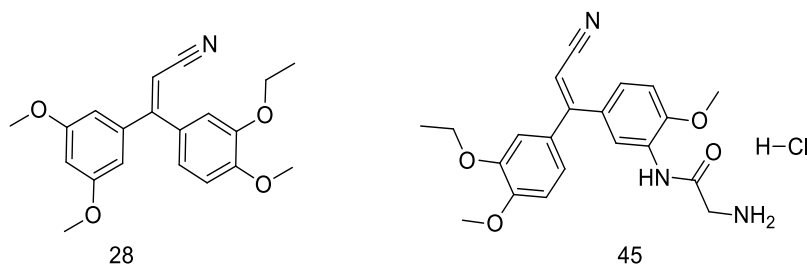


Figure 2.15: A dual inhibitor with potent Phosphodiesterase 4 (PDE4)

Zhang *et al.* [232] found that the synthetic compound **CC-5079** (Figure 2.16) potently inhibited cancer cell growth *in vitro* and *in vivo* by a novel combination of molecular mechanisms. Compound **CC-5079** inhibited proliferation of cancer cell lines from various organs and tissues at nanomolar concentrations [232]. Its IC_{50} value ranged from 4.1 to 50 nmol/L. They stated that the effect of compound **CC-5079** on cell growth associated with cell cycle arrest in G2-M phase, increased phosphorylation of G2-M checkpoint proteins and apoptosis. Compound **CC-5079** prevented polymerization of purified tubulin in concentration-dependent manner *in vitro* and depolymerized microtubules in cultured cancer cells. In competitive binding assays, compound **CC-5079** competed with [3H] colchicine for binding to tubulin; however, it did not compete with [3H] paclitaxel (Taxol) or [3H] vinblastine [233].

Their data indicated that compound **CC-5079** inhibited cancer cell growth with a mechanism of action similar to that of other tubulin inhibitors. **CC-5079** remains active against multidrug-resistant cancer cells unlike other tubulin-interacting drugs such as Taxol and colchicine. Interestingly, compound **CC-5079** also inhibits tumor necrosis factor-A (TNF-A) secretion from lipopolysaccharide-stimulated human peripheral blood mononuclear cells

(IC₅₀ = 270 nmol/L). These reported data by Zhang *et al.* [232] indicated that compound **CC-5079** represents a new compound with novel mechanisms of action and that it has the potential to be developed for neoplastic and inflammatory disease therapy.

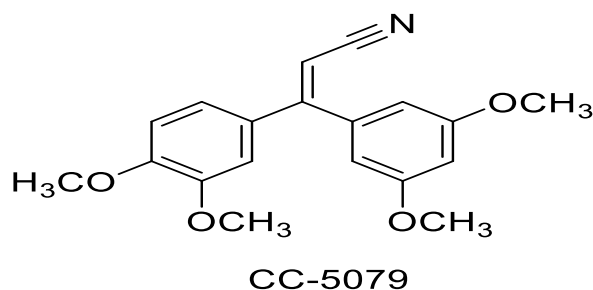


Figure 2.16: The synthetized compound CC-5079

Messaoudi *et al.* [233] reported a convergent synthesis of isocombretastatins A, Compounds **12** and **14** (Figure 2.17) a novel class of potent antitubulin agents. These compounds having a 1,1-diarylethylene scaffold which constitute the simplest isomers of natural Z-combretastatins A that are easy to synthesize without the necessary to control the Z-olefin geometry. Their discovery of iso CA-4 with different biological activities comparable to that of CA-4 represents a major progress in this field.

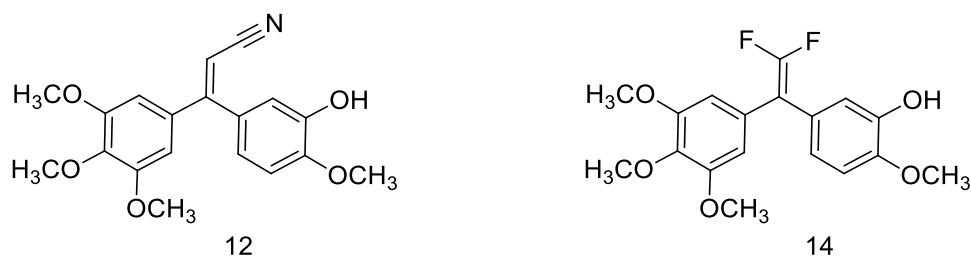


Figure 2.17: Isocombretastatins A, compounds 12 and 14

A novel class of isocombretastatin A-4 (isoCA-4) analogues with modifications at the 3'-position of the B-ring by replacement with C-linked substituents was studied by Hamze *et al.* [234]. Exploration of the structure activity relationships of these analogues led to the identification of several compounds that exhibited excellent anti-proliferative activity in the nanomolar concentration range against H1299, MDA-MB231, HCT116 and K562 cancer cell lines. They also inhibited tubulin polymerization with potency similar to that of isoCA-4. 1,1-

Diarylethylenes, compounds **8** and **17**, respectively with (E)-propen-3-ol and propyn-3-ol substituents at the 3'-position of the B-ring, proved to be the most active in this series. Both compounds led to the arrest of various cancer cell lines at the G2/M phase of the cell cycle and strongly induced apoptosis. Docking of compounds **8** and **17** in the colchicine binding site indicated that their C3' substituents guide the positioning of the B-ring in a manner different from that observed for isoCA-4.

Mousset *et al.* [235] synthesized a series of benzil derivatives (Figure 2.18) related to CA-4 by oxidation of diarylalkynes promoted by PdI₂ in DMSO. Using this new protocol, 14 benzils were prepared with their biological activity studied. Several benzils exhibited excellent antiproliferative activity: for example, compounds **4j** and **4k** (Figure 2.18) bearing the greatest resemblance to CA-4 and AVE-8062 respectively were found to inhibit cell growth at the nanomolar level (20–50 nM) on four human tumor cell lines. Flow cytometric analysis indicated that these compounds act as antimitotics and arrest the cell cycle in G2/M phase. A cell-based assay indicated that compounds **4j** and **4k** displayed a similar inhibition of tubulin assembly with an IC₅₀ value similar to CA-4. These results clearly demonstrated that the Z-double bond of CA-4 can be replaced by a 1,2-diketone unit without significant loss of cytotoxicity and inhibition of tubulin assembly potency [236].



Figure 2.18: Benzil derivatives related to CA-4

Hall *et al.* [236] reported that trifluorinated amino-combretastatin analogue (Figure 2.19), (Z)-2-(4'-methoxy-3'-aminophenyl)-1-(3,4,5-trifluorophenyl)ethene (**8**), prepared by chemical synthesis, was found to be a potent inhibitor of tubulin assembly (IC₅₀ = 2.9 μM)

and cytotoxic against selected human cancer cell lines. This new lead compound was among the most active chemotherapeutic agent from a group of related structural modifications.

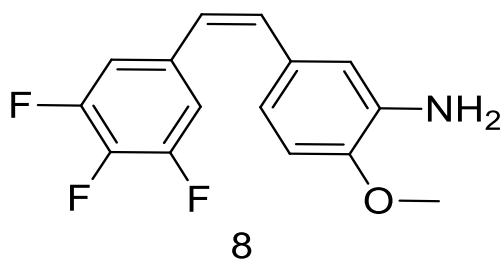


Figure 2.19: Trifluorinated amino-combretastatin analogue

A series of 3,4-diaryl squaric acid analogs (figure 2.20) related to CA-4 using squaric acid as the cis-restricted linker were prepared and studied for their anticancer activity against selected human cancer cell lines [237]. New compounds **4g**, **4k**, **4m**, **4n**, **4p**, **4q** and **4r** exhibited strong activities against human leukemia cells with IC_{50} values of ≤ 20 nM and compounds **4k**, **4n**, **4p**, **4q** and **4r** showed higher potency against a panel of human tumor cell lines [237]. Compounds **4n** and **4p** arrested tumor cell cycle in G2-M phase. Computational modeling analysis suggested that the binding mechanism of compound **4n** to the colchicine binding site on the microtubules is similar to that of CA4

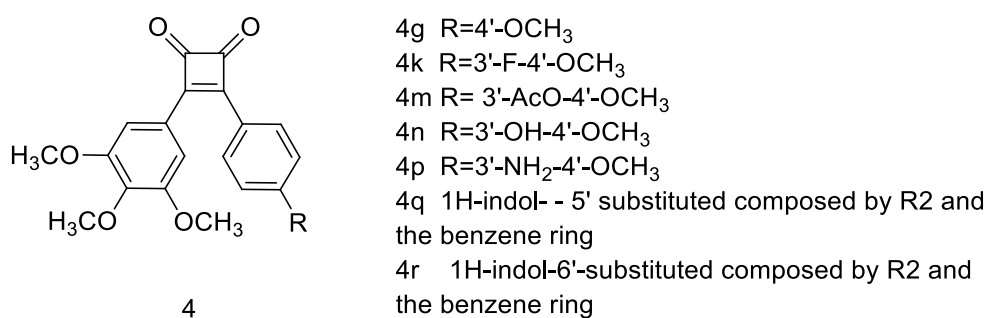


Figure 2.20: 3,4-diaryl squaric acid analogs

The *in vitro* antitumor activity of combretastatin-like 1,5- and 1,2-diaryl-1H-imidazoles was evaluated against the NCI 60 human tumor cell lines [238] and compounds **2d** and **2g**

(Figure 2.21) proved to be more cytotoxic than CA-4 in the tests involving their evaluation over a 10^{-4} – 10^{-8} range.

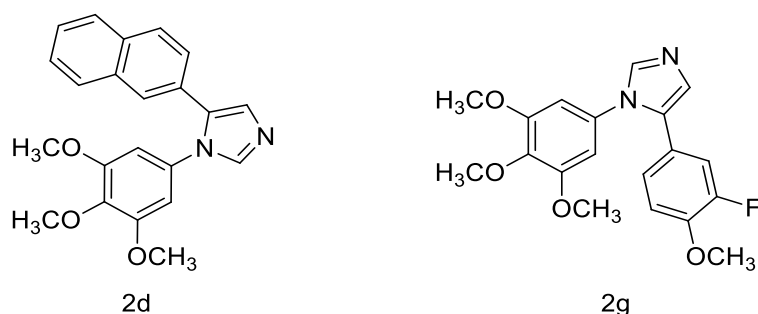


Figure 2.21: Combretastatin-like 1,5- and 1,2-diaryl-1H-imidazoles

A series of 4,5-disubstitute-1,2,3-thiadiazole compounds (Figure 2.22) were designed and synthesized as potent anticancer agents by Wu *et al.* [239] and some of them exhibited excellent *in vitro* and *in vivo* inhibitory activity. From the series of compounds synthesized, compounds **4b**, **4e**, **4i** and **4r** showed significant antitumor activity.

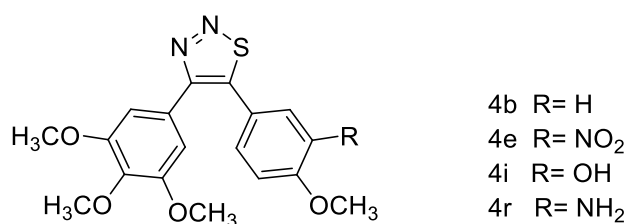


Figure 2.22: 4,5-disubstitute-1,2,3-thiadiazole compounds

Eleven 1,2,3,4-tetrahydro-2-thioxopyrimidine analogs of CA-4 were synthesized [240] and their cytotoxicity against the growth of two murine cancer cell lines (B16 melanoma and L1210 leukemia) in culture were determined using an MTT assay. Two 2-thioxopyrimidine analogs **8f** and **9a** (Figure 2.23) exhibited prominent activity ($IC_{50} < 1 \mu M$ for L1210 and $< 10 \mu M$ for B16 cells). Exposure of A-10 cells to compound **8f** and **9a** produced a remarkable reduction in cellular microtubules in interphase cells, with an EC_{50} value of 4.4 and 2.9 μM , respectively, for microtubule loss [240]. Molecular modeling studies using MacSpartan indicated that the two active 2-thioxopyrimidine analogs preferably adopt a twisted

conformation, similar to CA-4, affirming that conformation and structure are connected to activity [241].

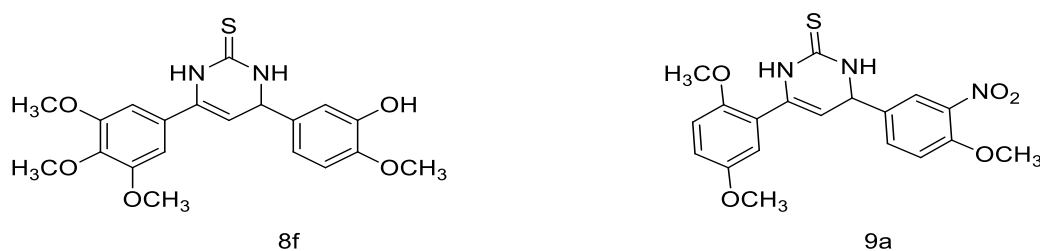


Figure 2.23: 1,2,3,4-tetrahydro-2-thioxopyrimidine analogs of CA-4

Monk *et al.* [241] synthesized a series of analogs with nitro or serinamide substituents at the C-2'-, C-5'-, or C-6'-position of CA-4 B-ring and evaluated for cytotoxic effects against heart endothelioma cells, blood flow reduction to tumors in SCID mice, and as inhibitors of tubulin polymerization. The synthesis of these analogs typically featured a Wittig reaction between a suitably functionalized arylaldehyde and an arylphosphonium salt followed by separation of the resultant E- and Z-isomers. They observed that many of these nitrogen-modified CA-4 derivatives (both amino and nitro) demonstrated remarkable inhibition of tubulin assembly as well as cytotoxicity and *in vivo* blood flow reduction [241]. 2'-Aminostilbenoid compound **7** and 2'-amino-3-hydroxystilbenoid compound **29** proved to be the most active in this series. Both compounds **7** and **29** (Figure 2.24), were reported to have the potential for further pro-drug modification and development as vascular disrupting agents for the treatment of solid tumor cancers and certain ophthalmological diseases.

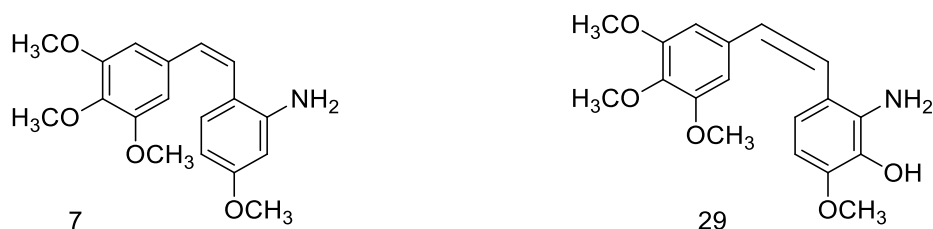


Figure 2.24: 2'-Aminostilbenoid (7) and 2'-amino-3-hydroxystilbenoid (29)

2.8 Boronic acids in medicinal chemistry application

A boronic acid is an alkyl or aryl substituted boric acid (Figure 2.25) containing a carbon-boron bond belonging to the larger class of organoboranes. Boronic acids act as Lewis acids. Their unique feature is in their ability to form reversible covalent complexes with sugars, amino acids, hydroxamic acids, and molecules with vicinal bonds [242a].

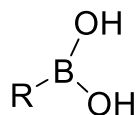


Figure 2.25: The general structure of a boronic acid, where **R** (alkyl or aryl) is a substituent.

Boronic acids have been known in literature since 1860 [242b], developing over the years into a widely studied area of chemistry. The ease of synthesis and stability of the group has led to its use in a variety of important synthetic reactions, from hydroboration [243] (C–H, C–OH, C–S bonds), Suzuki–Miyaura coupling [244] (C–C bonds) and the Chan–Lam coupling [245] (C–N and C–O bonds). Initially, the use of boronic acids in medicinal chemistry was limited to formation of intermediates for synthesis towards, or further elaboration of a lead compound. Some examples of boronic acids used in this study as shown in Figure 2.26.

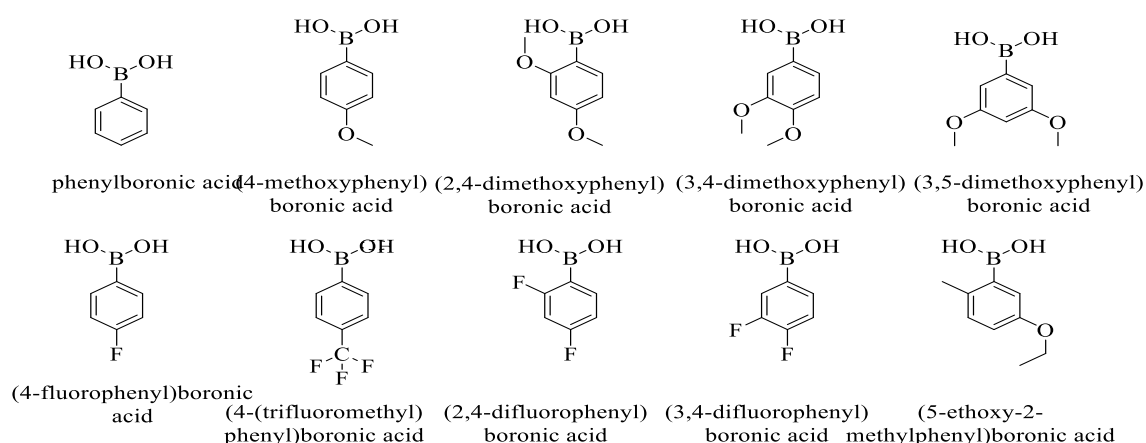


Figure 2.26: Some examples of Boronic acids

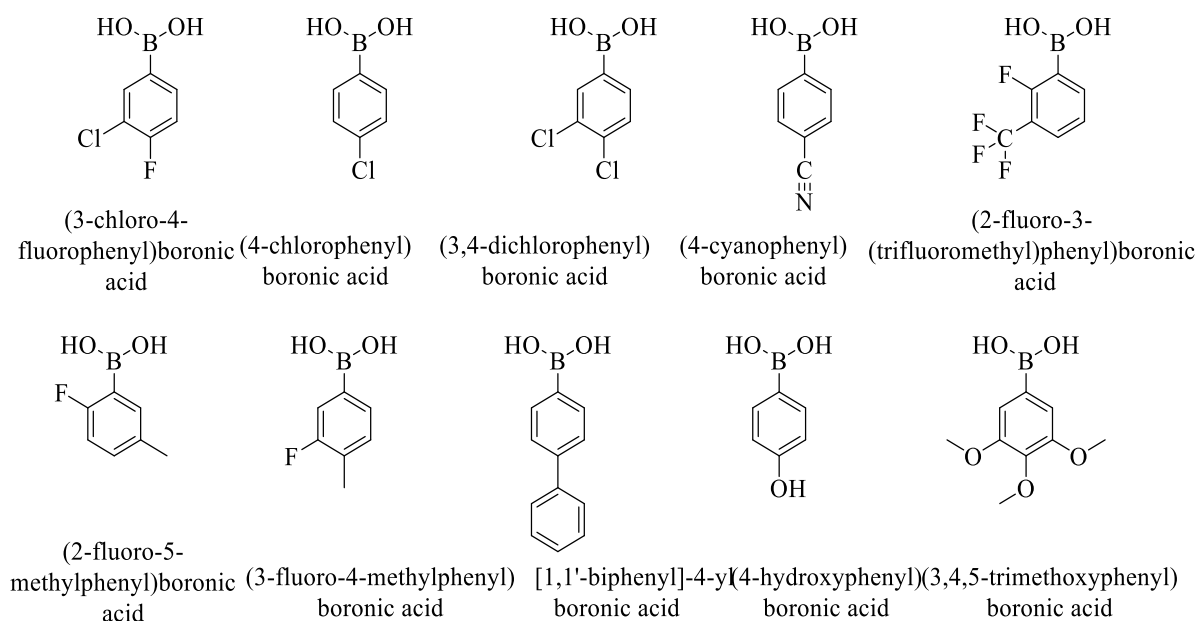
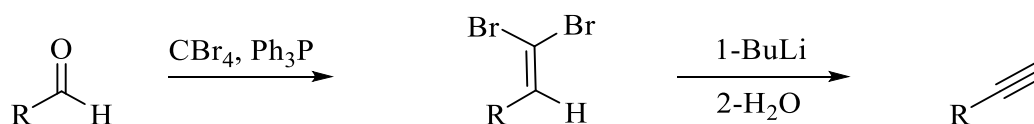


Figure 2.26: Some examples of Boronic acids continue

2.9 Corey–Fuchs reaction

The Corey-Fuchs reaction [246-248] also known as the Ramirez-Corey-Fuchs reaction (Scheme 2.1) is a series of chemical reactions designed to transform an aldehyde into an alkene or alkyne. The formation of the 1,1-dibromoolefins via phosphinedibromomethylenes was originally discovered by Desai and McKelvie [249]. This reaction is a two-step process to prepare a terminal alkyne with one-carbon homologation of an aldehyde or to prepare an alkyne derivative by the treatment of the alkynide intermediate with an electrophile before an aqueous workup and is generally known as the Corey–Fuchs reaction. Because dihaloalkene is formed as a reaction intermediate that can be obtained before the treatment with a strong base, this reaction is also referred to as the Corey-Fuchs olefination [249].



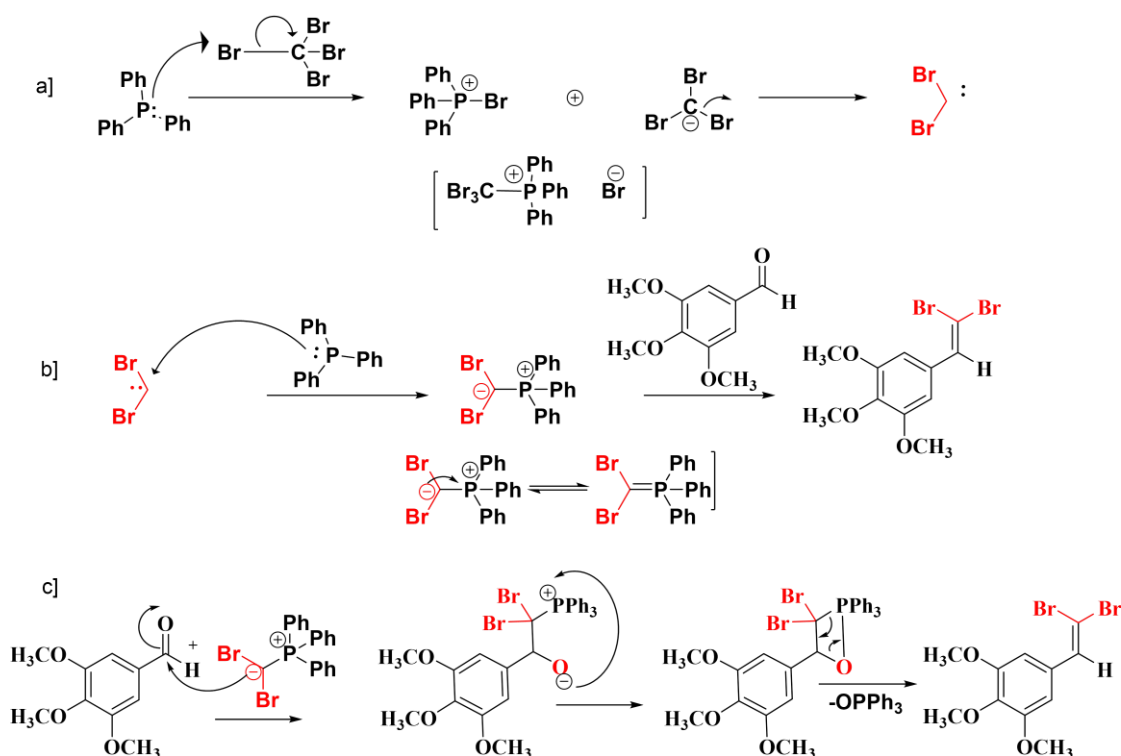
Where R can be an aromatic ring or an alkyl substituent

Scheme 2.1: Ramirez-Corey-Fuchs reaction

The first step is comparable to a Wittig Reaction, and leads to a dibromoalkene. Treatment with a lithium base (BuLi, LDA) generates a bromoalkyne intermediate via dehydrohalogenation, which undergoes metal-halogen exchange under the reaction conditions and yields the terminal alkyne upon work-up.

2.10 Reaction Mechanism

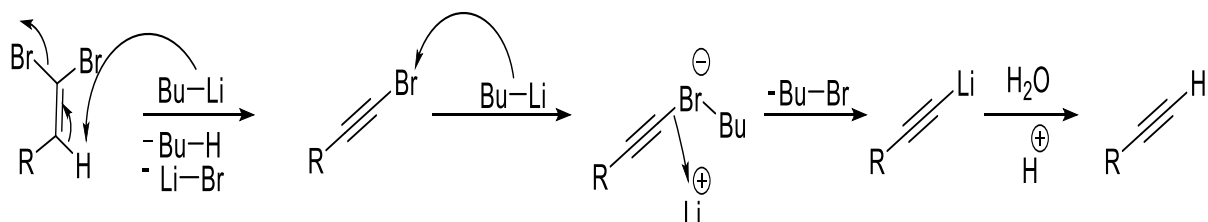
The Corey-Fuchs reaction is based on a special case of the Wittig Reaction, where the phosphorus ylide is formed from dibromocarbene (Scheme (2.2a)). This carbene is generated *in situ* from the reaction of Triphenylphosphine (TPP) and carbon tetrabromide (CBr₄) (Scheme 2.2a).



Scheme 2.2: Reaction Mechanism

- Formation of phosphorus ylide
- Reaction of carbene with TPP
- Addition of the ylide to the aldehyde

Triphenylphosphine then attacks the nascent carbene to form the reactive ylide (Scheme (2.2b)). This ylide undergoes a Wittig Reaction when exposed to an aldehyde. The addition of the ylide to the aldehyde (Scheme (2.2c)) yields the desired bromoalkyne. Reaction of the dibromoalkene with ButylLithium (BuLi) (Scheme 2.3):

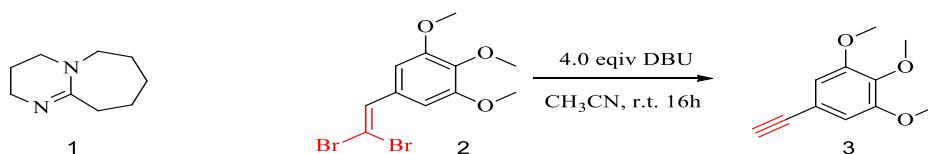


Scheme 2.3: Geminal dibromoalkenes with a strong base

Traditionally, terminal alkynes are prepared by a Corey–Fuchs reaction using geminal dibromoalkenes in the presence of a strong base as shown in Scheme 2.3 above. Because of the importance of alkynes as versatile building blocks in organic synthesis, a mild and practical protocol for their preparation was developed by Morri *et al.* [250].

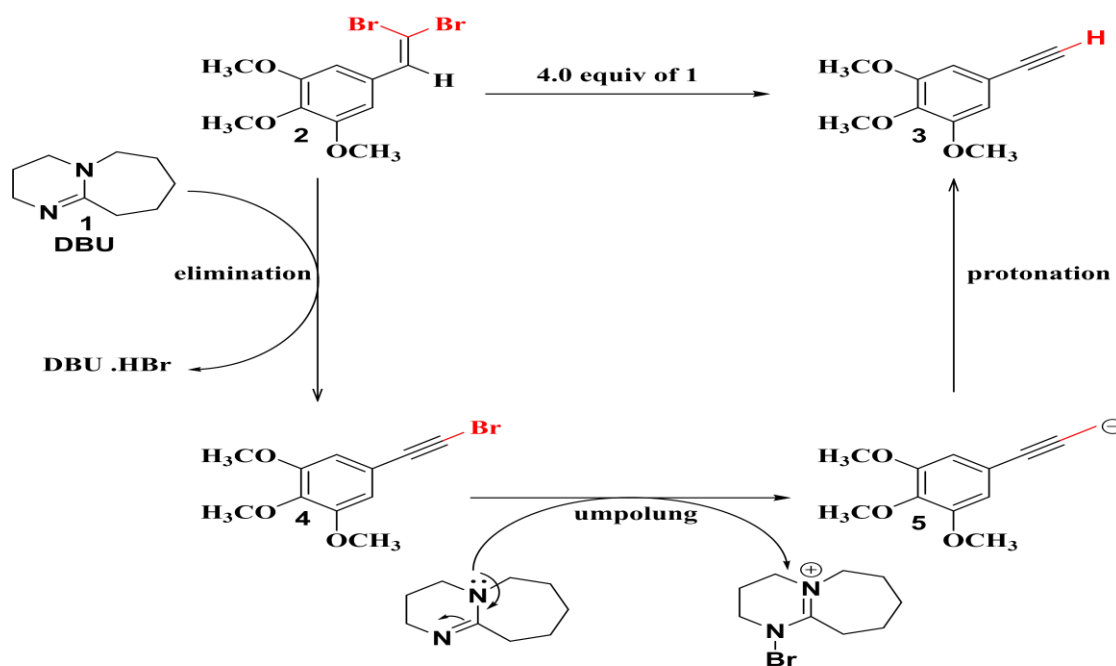
The dual role of the bicyclic amidine base 1,8- diazabicyclo[5.4.0]undec-7-ene (DBU) was demonstrated in a synthesis of terminal aryl- and styryl-acetylenes. Mechanistically, a tandem process involving elimination/Umpolung/ protonation occurs in a single step to generate terminal aryl and styryl-acetylenes from geminal dibromoalkenes. The key to the success of this transformation lies in the organobase mediated generation of the acetylide from the 1-bromoalkynes at room temperature (Scheme 2.3).

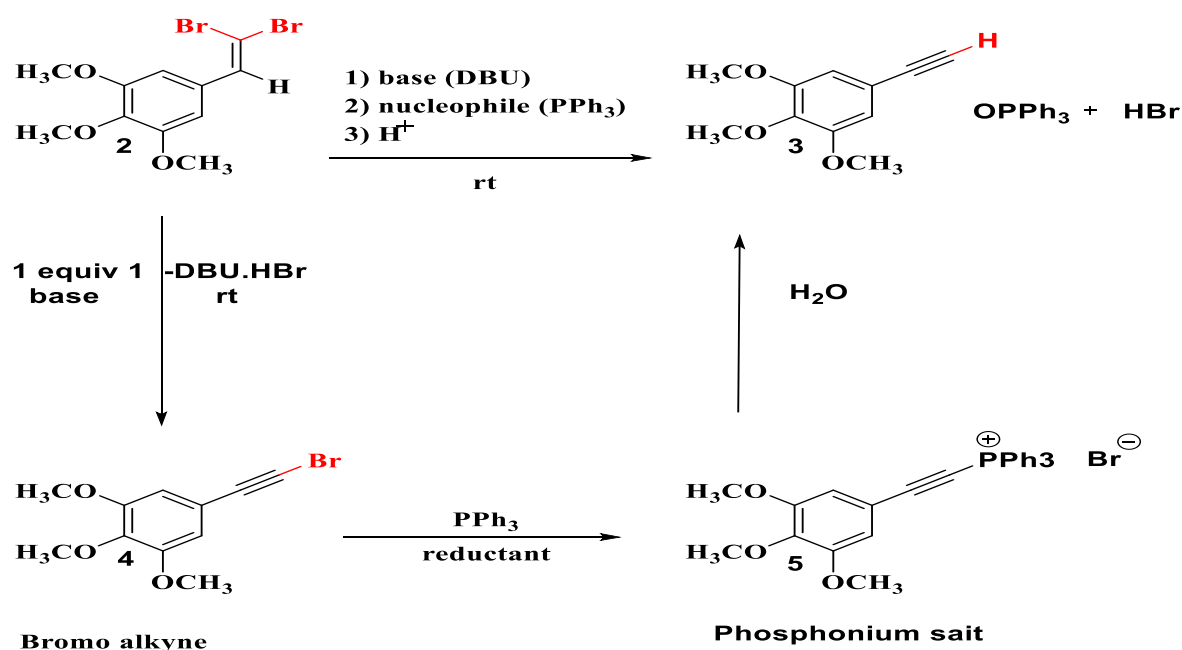
The unique characteristics of DBU as an inherently safer reagent make it an attractive alternative to previous systems wherein required pyrophoric reagents and non ambient temperatures remain unsolved issues [250]. Hence, this synthetic route was employed in the study of synthesized acetylenes (Scheme 2.4).



Scheme 2.4: Acetylide from the 1-bromoalkynes at room temperature

On the basis of the basic reactivity of DBU at ambient temperature, a sequential elimination reaction of **2** with **1** (1 equiv) (Scheme 2.4), followed by a nucleophilic attack on the resulting 1-bromoalkyne intermediate **4** by 1 mol of phosphine to generate an alkynyl phosphonium salt via addition/ elimination was envisioned. A subsequent workup furnished the desired alkyne **3** and triphenylphosphine oxide in the presence of water (Scheme 2.5a. 2,5b) [250]. The presented method features numerous advantages: (i) it is simple; (ii) it avoids the use of pyrophoric reagents or other reagents, such as phosphines, that complicate the purification process; (iii) it obviates column chromatography; and (iv) it is safer and greener than previous protocols. In general, the protocol is sufficiently mild to tolerate a wide range of functional groups [250].

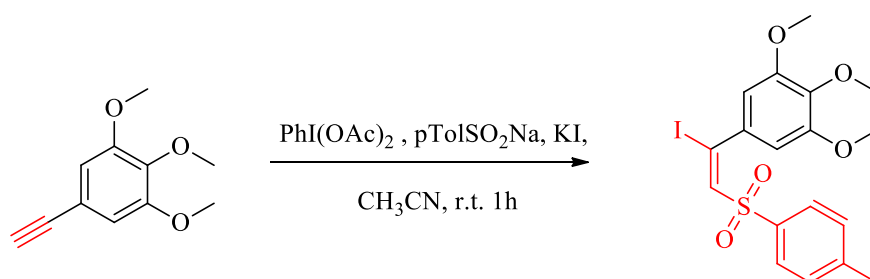




Scheme 2.5: Design pathway and proposed mechanism for the synthesis of terminal Alkynes [251]

2.11 Synthesis of β -Iodovinyl Sulfones

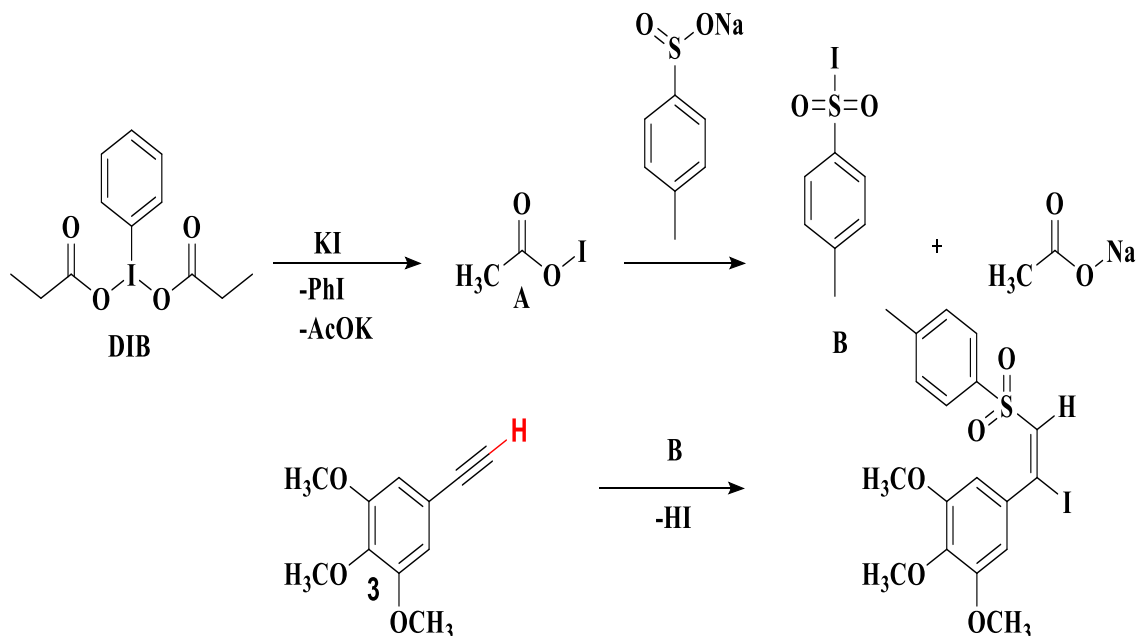
(Diacetoxyiodo)benzene [PhI(OAc)₂, DIB] promoted the reaction of sodium aryl sulfonate and potassium iodide (KI) with alkynes to afford the corresponding β -iodovinyl sulfones (Scheme 2.6), in good yields. The salient features of this reaction are: it employs a commercially available and environmentally benign hypervalent iodine (III) reagent; a one-step reaction; a short reaction time; and mild reaction conditions [251].



Scheme 2.6: Synthesis of β -Iodovinyl Sulfones

A probable mechanism for the formation of vinyl sulfones is outlined in Scheme 2.6. DIB promotes the oxidation of the iodide anion, yielding acetylhypoiodite **A** as a putative

intermediate. Subsequent reaction of **A** with aryl sulfonate gives arylsulfonyl iodide **B**, which readily mediates iododisplacement of the alkyne [252]. Spontaneous elimination of HI then takes place when the R group is an aryl or conjugated functional group.

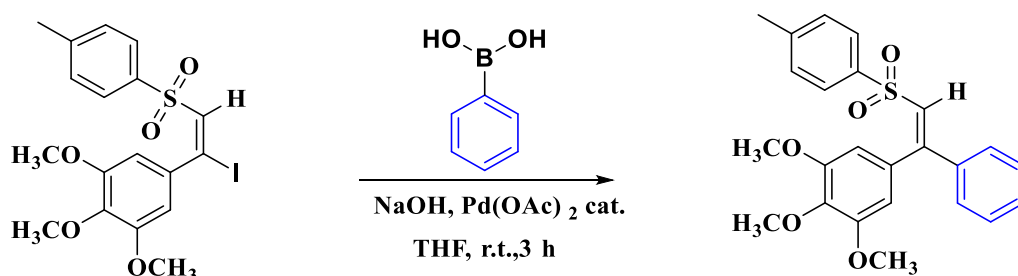


Scheme 2.7: Reaction mechanism of the Synthesis of β -Iodovinyl Sulfones

2.13 Synthesis of Suzuki-Miyaura cross-coupled products

The Suzuki reaction is an exceptionally useful cross-coupling process that has been widely applied in synthetic chemistry, and boronic acids and by far, the most commonly employed coupling partner (Scheme 2.8). To date, however, no versatile method has been developed for cross-coupling boronic acids with unactivated alkyl (as opposed to aryl or vinyl) electrophiles [253]. The availability of the reagents and the mild reaction conditions all contribute to the versatility of this reaction. The coupling reaction offers several additional advantages, such as being largely unaffected by the presence of water, tolerating a broad range of functional groups, and proceeding generally regio- and stereoselectively. Moreover, the inorganic by-product of the reaction is non-toxic and easily removed from the reaction mixture thereby

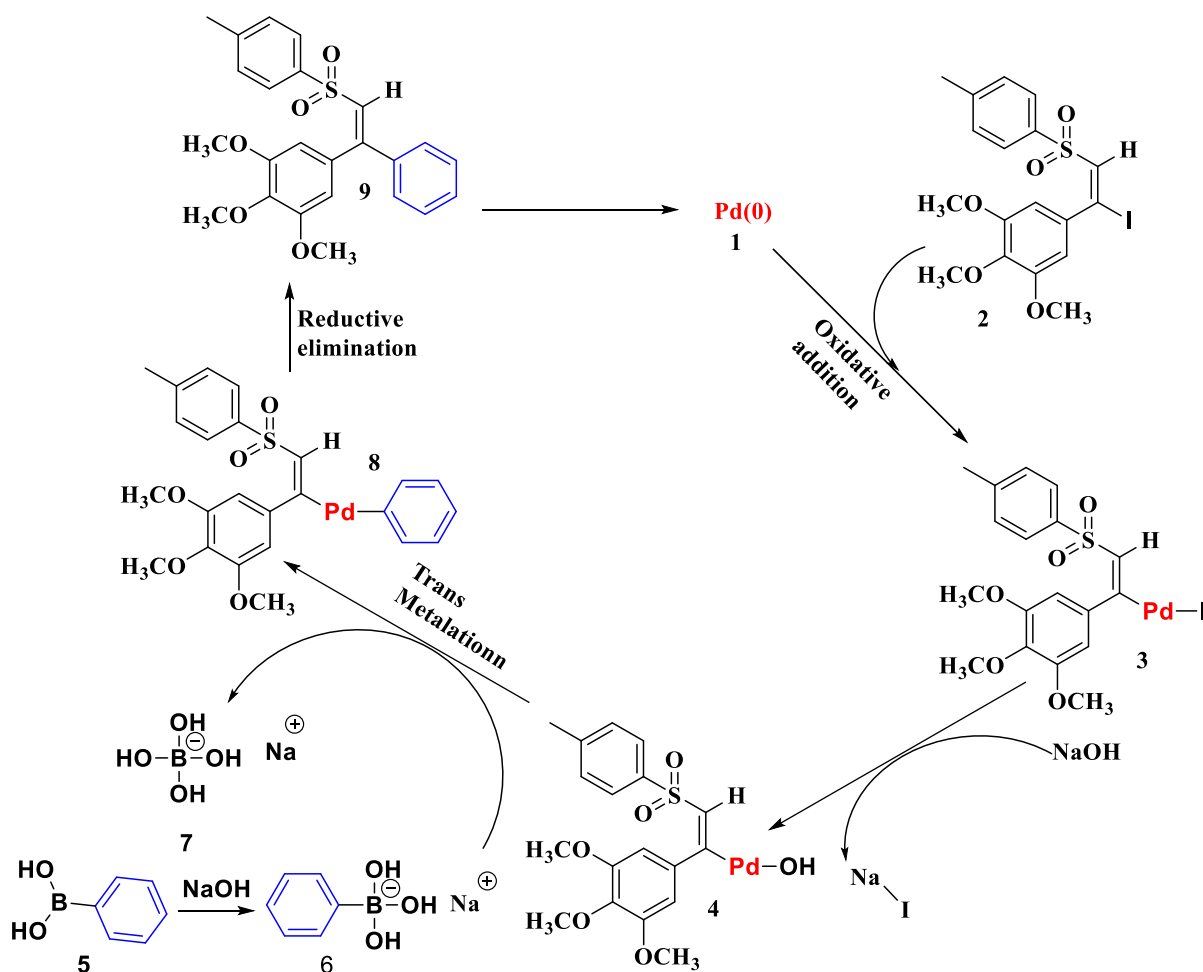
making the Suzuki coupling suitable not only for laboratories but also for industrial processes [254].



Scheme 2.8: Suzuki Miyaura Coupling

The mechanism of the Suzuki reaction is best viewed from the perspective of the palladium catalyst. The first step is the oxidative addition of palladium to the halide **2** to form the organopalladium species **3**. Reaction with base gives intermediate **4**, which via transmetalation with the boron-ate complex **6** (produced by reaction of the boronic acid **5** with base) forms the organopalladium species **8**. Reductive elimination of the desired product **9** restores the original palladium catalyst **1** which completes the catalytic cycle as shown in Scheme 2.9 [255].

2.13.1 Oxidative Addition: In most cases, the oxidative addition is the rate determining step of the catalytic cycle [170]. During this step, the palladium catalyst is oxidized from palladium (0) to palladium (II). The palladium catalyst **1** is coupled with the alkyl halide **2** to yield an organopalladium complex **3**. As shown in the Scheme 2.9 below, the oxidative addition step breaks the carbon-halogen bond where the palladium is now bound to both the halogen and the **Ar** group.



Scheme 2.9: Mechanism of Suzuki Miyaura Coupling

2.13.2 Transmetalation is an organometallic reaction where ligands are transferred from one species to another [256]. In the case of the Suzuki coupling the ligands are transferred from the organoboron species **6** to the palladium (II) complex **4** where the base that was added in the prior step is exchanged with the Ar_2 substituent on the organoboron species to give the new palladium (II) complex **8** (Scheme 2.9). The exact mechanism of transmetalation for the Suzuki coupling remains to be discovered. The organoboron compounds do not undergo transmetalation in the absence of base and it is therefore widely believed that the role of the base is to activate the organoboron compound as well as facilitate the formation of $\text{Ar}_2\text{-Pd(II)-OH}$ from $\text{Ar}_2\text{-Pd(II)-X}$ [257].

2.13.3 Reductive elimination is the final step where the palladium(II) complex **8** eliminates the product **9** and regenerates the palladium (0) catalyst (1) (Scheme 2.9) [257].

2.14 Structure Elucidation and Derivatization of the Synthesized Compounds

The synthesized compounds were characterized by Spectroscopic methods. The four basic types of Spectroscopic methods that were utilized in the characterization of the purified synthesized compounds include Infra red (IR), Mass spectrometry (MS) and Nuclear Magnetic Resonance (NMR) techniques. MS is an instrumental technique, while the other three utilizes different parts of the broad electromagnetic radiation (EMR) spectrum. The infra red (IR) region of EMR was utilized to detect different vibration frequencies of different chemical bonds present in the molecule. IR gave information about the kind of functional groups present in the molecule while the MS analytical technique provided information on the molecular ion useful for structural characterizations and fragmentation pattern of the molecule. The NMR spectroscopy revealed the connectivity of nuclei (^1H and ^{13}C -NMR) in the synthesized molecule.

2.15 Aim and Objectives

To synthesize and characterize 1, 1-diaryl vinyl sulfones as potential anticancer agents.

The objectives of this work were to:

- (i) synthesize 1,1-diarylethene derivatives having a sulfonyl (SO_2R) group at the 2 - carbon position;
- (ii) characterize all synthesized compounds; and
- (iii) determine the cytotoxicity of the synthesized compounds using various human cancer cell lines.

2.16 Synthetic Work Plan

From the literature survey data, it was obvious that 1,1-diarylethenes exhibit anticancer activity by strongly binding to colchicine site of tubulin and thus inhibiting tubulin polymerization. One of the advantages of the 1,1-diarylethene units is its structural simplicity and the consequent ease of synthesis. This study was designed to exploit these virtues by designing and synthesizing new 1,1-diarylethene derivatives modified with substituent on the carbon 2-position of the alkene. As described earlier [232], the synthetic compound CC-5079, that incorporates a 1,1-diarylethene unit has proven to be a potent tubulin binder. It is noteworthy that the carbon 2-position of the 1,1-diarylethene unit in CC-5079 (Figure 2.7) is substituted with an electron withdrawing (**EW**) cyano group.

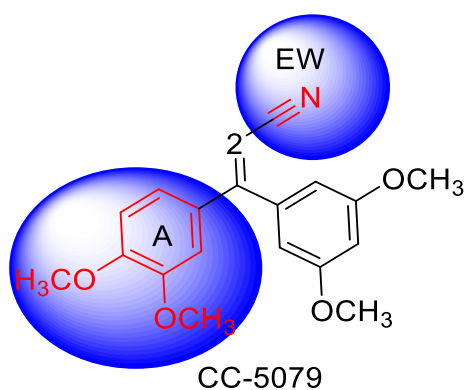
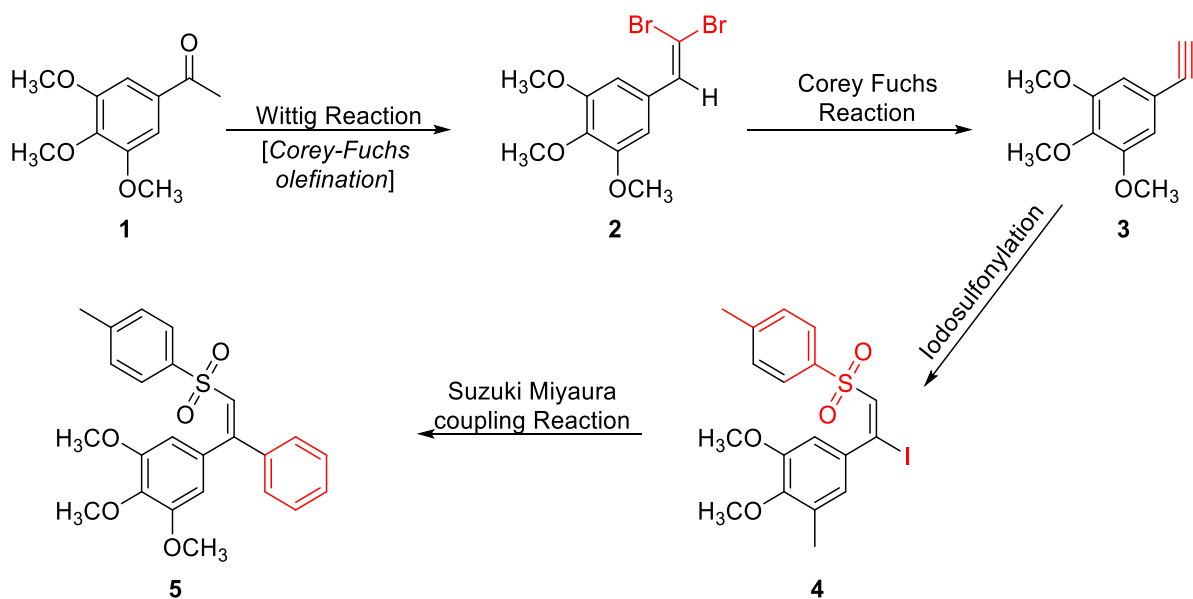


Figure 2.27: CC-5079

The properties of this structural scaffold could be fine-tuned by replacing the carbon 2-substituent with other electron withdrawing substituents that have favourable pharmacological profile.



Scheme 2.10: Synthesis of 1,1-diarylsulfonyl compounds

Also, this study fine-tuned the ring **A** by replacing the 3,4-dimethoxy phenyl ring with a trimethoxy phenyl ring (Scheme 2.10). The trimethoxy benzaldehyde **1** undergoes Corey-Fuchs reaction to generate aryl alkynes **3**, which in turn undergo an iodosulfonylation reaction to give an aryl iodosulfonyl compound **4**, which provide Suzuki Miyaura reaction with different aryl boronic acids to afford 1,1-diarylethene carrying a sulfonyl group **5** (Scheme 2.10).

CHAPTER THREE

3.0

MATERIALS AND METHODS

3.1 Materials

All reactions were performed in flame-dried round-bottom flasks, fitted with rubber septa under nitrogen gas. Removal of solvent was done using a Heidolph Hei-VAP Value Digital G3: 560-01302-00 rotary evaporator attached to a vacuum pump (~3 mmHg). Analytical thin-layer chromatography (TLC) was performed using aluminum foil UVF₂₅₄ precoated silica gel flexible plates. Column chromatography was conducted using silica gel (60 –120 mesh and 100 –200 mesh) packed in glass columns. This was carried out by serial dilution method for the purification of all the synthesized compounds.

Melting points were obtained in open capillary tubes using a 1101D Mel-Temp® Digital Melting Point Apparatus from Cole-Palmer Limited. Technical grade solvents which include: n-hexane, methanol, ethyl acetate, acetone, chloroform, dichloromethane, and ethanol were distilled before use. All chemicals used were supplied by Sigma-Aldrich Company, USA. These include: Phenylacetylene, Iodosobenzene diacetate, p-Toluenesulfonic acid sodium salt, Boronic Acids, 1,2,3-trimethoxy benzaldehyde, Sodium thiosulphate, Potassium iodide, etc.

A panel of four (4) cancer cell lines used for testing the *in vitro* cytotoxicity include: MDA-MB-231 derived from human breast adenocarcinoma cells (ATCC No HTB-26), HeLa derived from human cervical cancer cells (ATCC No. CCL-2), A549 derived from human alveolar adenocarcinoma epithelial cells (ATCC No. CCL-185) and IMR-32 derived from human neuroblastoma cell line (ATCC No CCL-127), with a normal cell line HEK-293 (human embryonic kidney cell). These cancer cell lines were obtained from the American Type Culture Collection (ATCC), Manassas, VA, USA, and stored before use.

3.2 Spectroscopic Measurement and Structure Elucidation

The synthesized compounds were characterized by spectroscopic methods using NMR (^1H NMR, and ^{13}C NMR), IR, and MS (ESI-MS and HRMS). The operating condition of each instrumentation technique is as stated below.

3.2.1 Infrared Spectroscopy (IR)

The infrared spectra were recorded on Thermo Nicolet Nexus 670 Spectrometer with 4 cm^{-1} resolution using KBr beam splitter and wave numbers of maximal absorption peaks were presented in cm^{-1} .

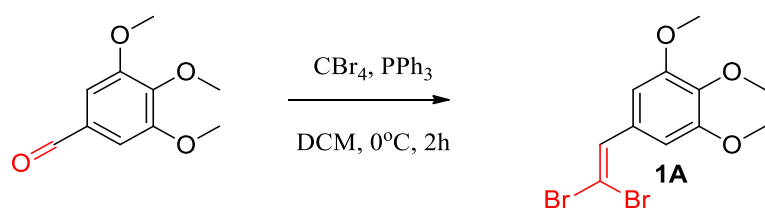
3.2.2 NMR Spectroscopy (NMR)

NMR spectra were recorded on Bruker 300, 400, 500 and 600 MHz NMR Spectrometers, ^{13}C -NMR spectra were recorded at 75, 100, 125, 150 MHz. Multiplicity of the signals were determined by DEPT 135 experiments. The proton resonances are annotated as chemical shifts δ parts per million (ppm) relative to tetramethylsilane (δ 0.0) using the residual solvent signal as an internal standard or tetramethylsilane itself: chloroform- d (δ 7.26, singlet), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), coupling constant J , in hertz (Hz), and the number of protons for a given resonance indicated by $n\text{H}$. The chemical shifts of ^{13}C NMR are reported in ppm, relative to the central line of the triplet at δ 77.0 ppm for CDCl_3 .

3.2.3 Mass Spectrometry (MS)

ESI spectra were recorded on Micro mass Quattro LC using ESI+ software with a capillary voltage of 3.98 kV and an ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on a QSTAR XL hybrid MS-MS mass spectrometer.

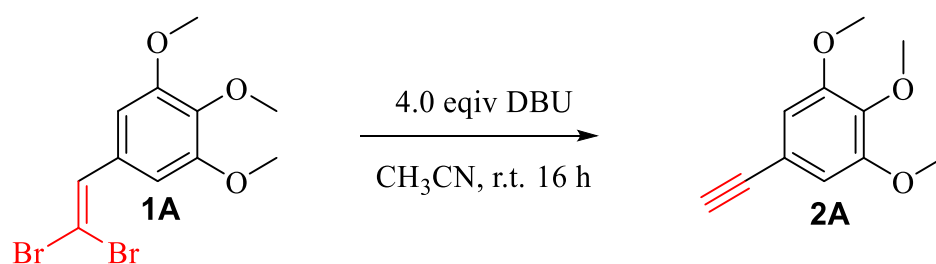
3.3 Synthesis of 1, 1-dibromoalkenes



Scheme 3.0: 5-(2,2-dibromovinyl)-1,2,3-trimethoxybenzene (**1A**) [249]

To a stirred solution of 3,4,5-trimethoxybenzaldehyde (686 mg, 3.5 mmol) and carbon tetrabromide (2.317 g, 7 mmol) in anhydrous dichloromethane (10 ml) was added triphenylphosphine (3.654 g, 14 mmol, 4 equiv) in portions over a period of 20 mins at 0°C , under inert atmosphere and stirred at 0°C temperature for 2 h. After completion of reaction (monitored by TLC), reaction mixture was quenched with water (15 ml). The reaction mixture was extracted with dichloromethane (2 x 20 ml); organic layers were washed with brine (10 ml). The organic layers were dried over anhydrous Na_2SO_4 ; solvent was evaporated under reduced pressure to afford a crude residue. The crude product was purified on silica gel (SiO_2) (60-120 mesh) packed in a glass column using Hexane/EtOAc (95:5) as eluents to afford pure compound **1A** (Scheme 3.0).

3.4 Preparation of Terminal Alkynes.



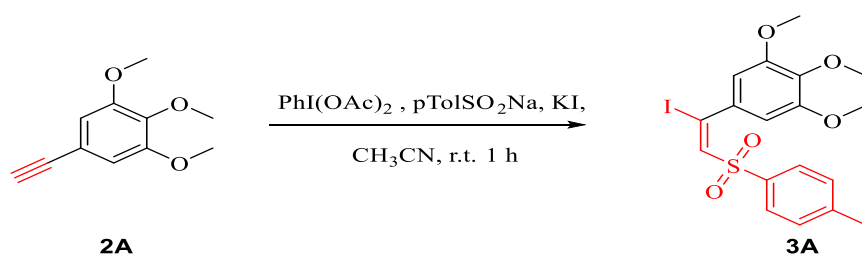
Scheme 3.1: 5-ethynyl-1,2,3-trimethoxybenzene (**2A**) [250]

To a stirred solution of dibromoalkene **1A** (352 mg, 1 mmol) in anhydrous CH_3CN (2 ml) was added DBU (0.59 mL, 4.0 mmol, 4 equiv) drop wise over a period of 10 minutes at ambient temperature ($25\text{--}30^\circ\text{C}$). The reaction mixture was allowed to stir at ambient

temperature for 16 h. After completion of reaction (monitored by TLC), reaction mixture was cooled at 15 °C and quenched by drop wise addition of 5N aqueous HCl (10 ml) over a period of 15 mins then continued stirring for 5 mins. The reaction mixture was extracted with EtOAc/hexane (1:1, 2 x 10 ml); organic layers were washed with water (10 ml). The organic layers were dried over anhydrous K₂CO₃, solvent was evaporated under reduced pressure, and resulting residues were dried in high vacuum to afford the pure compound **2A** (Scheme 3.1).

3.5 One-Step Synthesis of Vinyl Sulfones

PhI(OAc)₂ (241.6 mg, 0.75 mmol) was added to a suspension of the alkyne **2A** (0.5 mmol), sodium arenesulfonate (2.0 mmol), and KI (83.0 mg, 0.5 mmol) in CH₃CN (2 ml), and the reaction mixture was vigorously stirred at room temperature for 1 h. Upon completion of the reaction, the reaction mixture was quenched by the addition of a saturated aqueous solution of Na₂S₂O₃ (5 ml) and basified with a saturated aqueous solution of NaHCO₃ (5 ml). Further stirring was followed by extraction with EtOAc (3 x 15 ml). The combined organic extract was washed with H₂O (15 ml) and brine (15 ml), dried (MgSO₄), filtered, and concentrated (aspirator). The residue was purified by column chromatography using Hexane/EtOAc (95:5) as eluents to afford compound **3A** (Scheme 3.2).

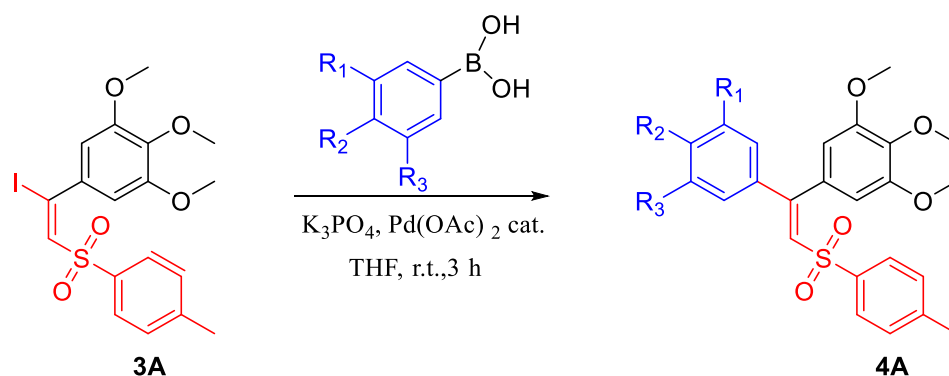


Scheme 3.2: (E)-5-(1-iodo-2-tosylvinyl)-1,2,3-trimethoxybenzene (3A)[251]

3.6 Synthesis of Suzuki-Miyaura Cross-Coupled Products

To a stirred mixture of (E)-5-(1-iodo-2-tosylvinyl)-1,2,3-trimethoxybenzene **3A** (50 mg, 0.13 mmol, 1 eq), aryl boronic acid (0.156 mmol, 1.2 eq) and K₃PO₄ (2 M in H₂O, 1 ml) in THF,

$\text{Pd}(\text{OAc})_2$ catalyst (0.0006mmol, 0.005 eq) was added and reaction was stirred at room temperature under argon/nitrogen for 3 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The combined organic fraction was dried and concentrated. The residue was purified by column chromatography with a mixture of Hexane/EtOAc (95:5) as eluents to afford the corresponding coupled product compound **4A** (Scheme 3.3).



Scheme 3.3: (Z) -1,2,3-trimethoxy-5-(1-phenyl-2-tosylvinyl)benzene [253]

Compounds AKL-CA-4A to AKL-CA-4V, were prepared by the same methods described above (Scheme 3.3).

3.7 Biological Activity

3.7.1 Anti-proliferative assay

Cell proliferation and viability of compounds AKL-CA-3 – 4V were determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay [258]. The pale yellow coloured tetrazolium salt (MTT) reduced to a dark blue water-insoluble formazon by metabolically active cells and the product was measured quantitatively after solubility in DMSO. The absorbance of the soluble formazon is directly proportional to the number of viable cells. A panel of four cancer cell lines were used for testing the *in vitro* cytotoxicity are stated in Section 3.1.7.

These cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) containing non-essential amino acids and 10 % FBS. All the cells were maintained under humidified conditions of 5 % CO_2 atmosphere at 37 °C in a CO_2 incubator (Model Galaxy 170S,

Eppendorf, USA). The 96-well micro titer plates were incubated for 24 h prior to addition of the experimental compounds. Cells were treated with vehicle alone (DMSO) or compounds (drugs were dissolved in DMSO previously) at different concentrations (0.1, 1, 10 and 25 μ M) of test compounds for 48 hours. The assay was completed with the addition of MTT (5 %, 10 μ L) and incubated for 60 mins at 37 $^{\circ}$ C. The supernatant was aspirated and plates were air dried and the MTT-formazon crystals were dissolved in 100 μ L of DMSO.

The optical density (O.D) was measured at 560 nm using TECAN multimode reader ((Infinite[®] M200Pro, Tecan, Switzerland)). The growth percentage of each treated well of 96 well plate were calculated based on test wells relative to control wells. Doxorubicin and Nocodazole were used as positive controls for comparison purpose and 1 % DMSO as a vehicle control. Antiproliferative activity of the cancer cells to the test compounds was expressed in terms of IC₅₀ value, which is defined as a concentration of compound that produced 50 % reduction absorbance relative to control, and the absorbance at 570 nm wavelength was recorded [258].

CHAPTER FOUR

4.0

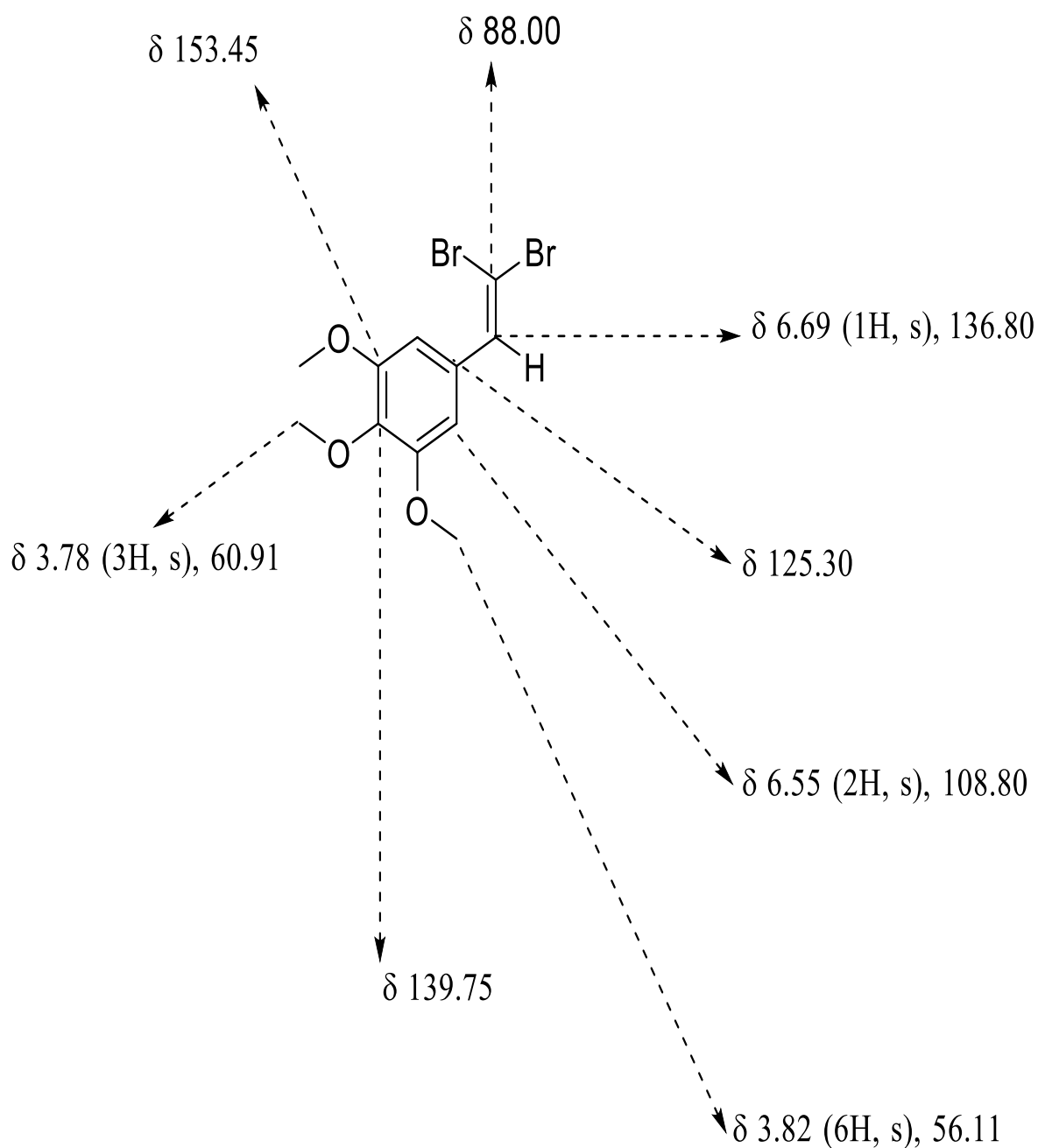
RESULTS AND DISCUSSION

4.1 Results

Twenty-three compounds (AKL-CA-1 to AKL-CA-4V) were synthesized. Two of these compounds, AKL-CA-1 and AKL-CA-2 have been reported previously by Corey-Fuchs [247] and Dasai *et al.* [249]. Twenty-one (20) compounds (AKL-CA-3 to AKL-CA-4V) are novel compounds. The novelties of these twenty synthetic compounds were confirmed by searching the Scifinder, and Reaxys library.

The desired target molecules (AKL-CA-3 to AKL-CA-4V) were obtained from the starting material of 3,4,5-trimethoxy benzaldehyde (**1**) prepared by Corey Fuchs olefination in the presence of carbontetrabromide and triphenylphosphine in dichloromethane to afford the 5-(2,2-dibromovinyl)-1,2,3-trimethoxybenzene (**2**) which in reacted with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in acetonitrile to give 5-ethynyl-1,2,3-trimethoxybenzene (**3**). Further treatment of molecule (**3**) with diacetoiodobenzene (DIB), sodium-p-toluenesulfonate and potassium iodide in acetonitrile yields ((E)-5-(1-iodo-2-tosylvinyl)-1,2,3-trimethoxybenzene) (**4**). ((E)-5-(1-iodo-2-tosylvinyl)-1,2,3-trimethoxybenzene) (**4**) undergoes Suzuki Miyaura coupling with different substituted arylboronic acids in tetrahydrofuran (THF), in the presence of a base, potassium phosphate with palladium acetate as catalyst to give different combretastatin sulfonyl compounds (AKL-CA-4A – 4V).

4.1.1. AKL-CA-1



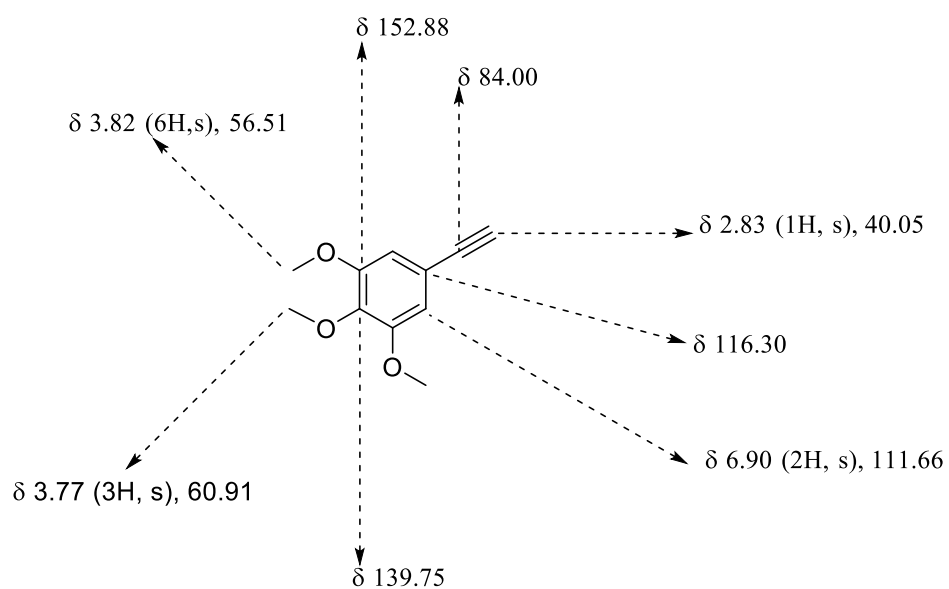
(Light brown liquid, 3.786 g, 80 % yield).

Melting Point: 51-53 °C

^1H NMR (400 MHz, CDCl_3) δ 6.69 (1H, s), 6.55 (2H, s), 3.82 (6H, s), 3.78 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 153.45, 139.75, 136.80, 125.30, 108.70, 88.00, 60.91, 56.15

4.1.2. AKL-CA-2



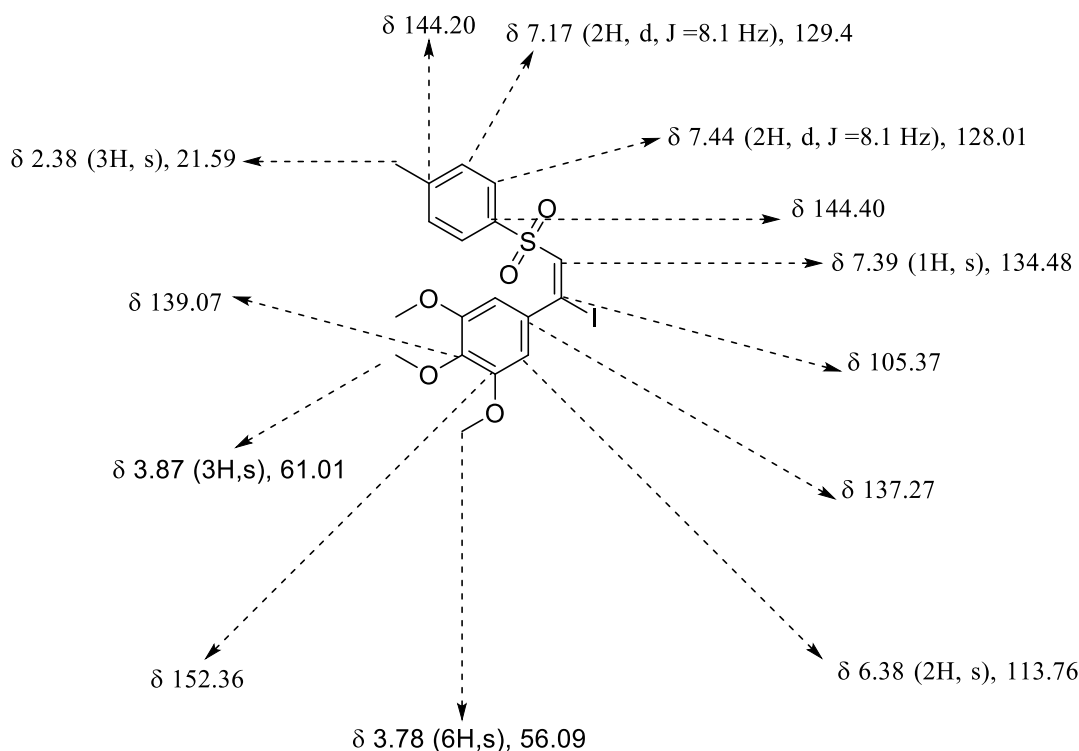
(Yellow oil, 2.560g, 92 % yield).

Melting Point: 68-70°C

^1H NMR (400 MHz, CDCl_3) δ 6.90 (2H, s), 3.82 (6H, s), 3.77 (3H, s), 2.83 (1H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 152.88, 139.75, 116.30, 111.66, 84.00, 60.91, 56.15, 40.05

4.1.3. AKL-CA-3



(Brown solid 240mg, 92 % yield).

Melting Point: 169-171 °C

IR (KBr) ν_{max} : 3039 (C-H Aromatic stretching), 2928 (C-H Olefinic stretching), 2835 (C-H Aliphatic stretching), 1582 (C=C Aromatic), 1270 (S=O Symmetrical), 1130 (C-O Alkoxy stretching), 994 (C-H Aromatic out of plane), 802 (C-I) cm^{-1}

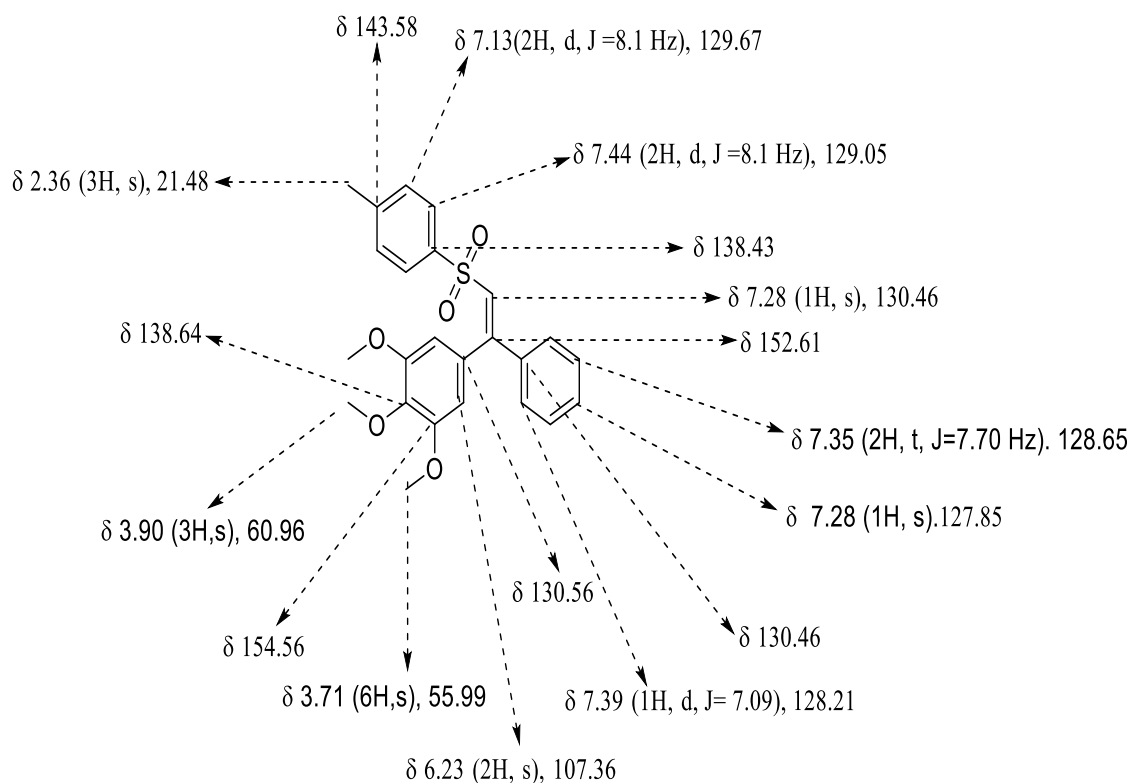
^1H NMR (500 MHz, CDCl_3) δ 7.44 (2H, d, $J=8.4$), 7.39 (1H, s), 7.17 (2H, d, $J=8.1$), 6.38 (2H, s), 3.87 (3H, s), 3.78 (6H, s), 2.38 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 152.36, 144.40, 142.08, 139.07, 137.27, 134.48, 129.41, 128.01, 113.76, 105.37, 61.01, 56.09, 21.59

MS (ESI): m/z 475 $[\text{M}+1]^+$

HRMS (ESI): calcd for $\text{C}_{18}\text{H}_{20}\text{IO}_5\text{S}$ $[\text{M}+1]^+$ 475.00706, found 475.00934

4.1.4. AKL-CA-4A



(White solid, 52 mg, 85 % yield).

Melting Point: 139-141 °C

IR (KBr) ν_{max} : 3035 (C-H Aromatic stretching), 2934 (C-H Olefinic stretching), 2837 (C-H Aliphatic stretching), 1583 (C=C Aromatic), 1309 (S=O Anti symmetrical stretching), 1140 (C-O Alkoxy stretching), 1083 (S=O Symmetrical stretching), 813 (C-H bend trisubstituted), 760 (C-H Aromatic out of plane) cm^{-1}

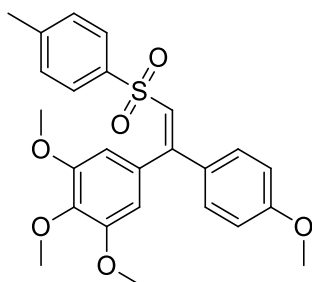
^1H NMR (500 MHz, CDCl_3) δ 7.44 (2H, d, $J=8.192$), 7.39 (1H, d, $J=7.091$), 7.35 (2H, t, $J=7.703$), 7.28 (1H, s), 7.13 (2H, d, $J=8.069$), 7.05 (1H, s), 6.23 (1H, s), 3.90 (3H, s), 3.71 (6H, s), 2.36 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 154.56, 152.61, 143.53, 138.64, 138.43, 138.36, 130.58, 130.46, 129.67, 129.05, 128.65, 128.21, 127.85, 107.36, 60.96, 55.99, 21.48

MS (ESI): m/z 425 $[\text{M}+1]^+$

HRMS (ESI) calcd for $\text{C}_{24}\text{H}_{25}\text{O}_5\text{S}$ $[\text{M}+1]^+$ 425.14172, found 425.14311

4.1.5. AKL-CA-4B



(White solid, 87 mg, 80 % yield).

Melting Point: 124-126 °C

IR (KBr) vmax: 3056, 2939, 2839, 1583, 1305, 1246, 1127, 838 cm⁻¹

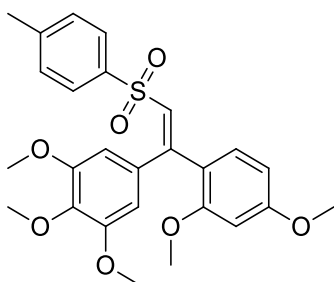
¹H NMR (500 MHz, CDCl₃) δ 7.42 (2H, d, 7.947), 7.22 (2H, d, J=8.861), 7.12 (2H, d, J=7.947), 6.98 (1H s), 6.85 (2H d, J=8.681), 6.21 (2H, s), 3.90 (3H, s), 3.81 (3H, s), 3.70 (6H, s), 2.35 (3H, s)

¹³C NMR (100 MHz, CDCl₃) δ 161.59, 154.21, 152.56, 143.33, 138.72, 138.22, 130.83, 129.78, 128.99, 127.79, 127.39, 114.03, 107.33, 60.96, 55.95, 55.45, 21.46

MS (ESI): m/z 455 [M+1]⁺

HRMS (ESI) calcd for C₂₅H₂₇O₆S [M+1]⁺ 455.15229, found 455.15419

4.1.6. AKL-CA-4C



(Yellow solid, 53 mg, 75 % yield).

Melting Point: 107-109 °C

IR (KBr) vmax: 3261, 2933, 2842, 1584, 1414, 1297, 1130, 839 cm⁻¹

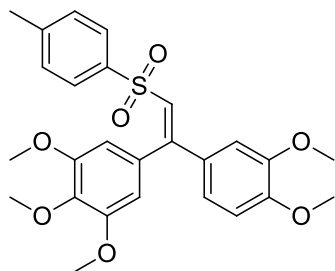
¹H NMR (400 MHz, CDCl₃) δ 7.44 (2H, d, J=8.069), 7.29 (1H, s), 7.12 (2H, d, J=7.947), 6.83 (1H, d, J=8.681), 6.47 (1H, s), 6.37-6.35 (1H, dd, J=8.681), 6.22 (1H, s), 3.88 (3H, s), 3.80 (6H, s), 3.70 (6H, s), 2.35 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 162.14, 159.26, 152.29, 151.10, 143.01, 139.16, 137.91, 133.23, 131.22, 130.90, 129.70, 128.89, 128.62, 127.68, 126.46, 120.27, 107.33, 104.51, 99.02, 60.92, 55.93, 55.69, 55.46, 30.95, 21.45

MS (ESI): m/z 485 $[\text{M}+1]^+$

HRMS (ESI): calcd for $\text{C}_{26}\text{H}_{29}\text{O}_7\text{S}$ $[\text{M}+1]^+$ 485.16285, found 485.16490

4.1.7. AKL-CA-4D



(Yellow solid, 70 mg, 92 % yield).

Melting Point: 173-175°C

IR (KBr) ν_{max} : 3026, 2938, 2844, 1583, 1278, 1134, 1079, 839 cm^{-1}

^1H NMR (400 MHz, CDCl_3) δ 7.42 (2H,

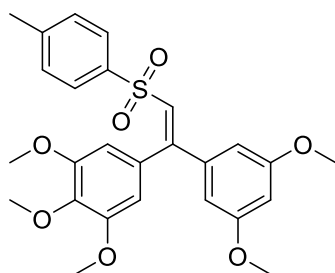
d, $J=8.314$), 7.12 (2H, d, $J=8.069$), 7.01 (1H, s), 6.84 (1H, s), 6.77 (2H, s), 6.20 (2H, s), 3.91 (3H, s), 3.88 (3H, s), 3.64 (3H, s), 3.70 (6H, s), 2.36 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 154.35, 152.52, 151.31, 148.97, 143.36, 138.64, 138.24, 131.01, 128.99, 127.81, 127.67, 122.34, 110.72, 110.37, 107.38, 60.97, 56.13, 55.96, 21.46

MS (ESI): m/z 485 $[\text{M}+1]^+$

HRMS (ESI) calcd for $\text{C}_{26}\text{H}_{29}\text{O}_7\text{S}$ $[\text{M}+1]^+$ 485.16285, found 485.16490

4.1.8. AKL-CA-4E



(White solid, 93 mg, 77 % yield).

Melting Point: 163-165°C

IR (KBr) ν_{max} : 3028, 2940, 2838, 1588, 1284, 1080, 841, 571 cm^{-1}

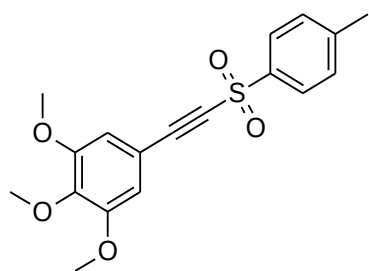
^1H NMR (500 MHz, CDCl_3) δ 7.42 (2H, d, $J=8.240$), 7.12 (2H, d, $J=7.934$), 7.03 (1H, s), 6.48 (1H, t, $J=2.289$), 6.38 (2H, d, $J=2.289$), 6.21 (2H, s), 3.89 (3H, s), 3.75 (6H, s), 3.71 (6H, s), 2.36 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 160.77, 154.44, 152.54, 143.55, 140.72, 138.31, 130.37, 130.09, 129.04, 127.84, 107.29, 106.66, 101.98, 60.95, 55.97, 55.55, 21.48

MS (ESI): m/z 485 $[\text{M}+1]^+$

HRMS (ESI) calcd for $\text{C}_{26}\text{H}_{29}\text{O}_7\text{S}$ $[\text{M}+1]^+$ 485.16285, found 485.16490

4.1.9. AKL-CA-4F



(Yellow solid, 60 mg, 37 % yield).

Melting Point: 121-123°C

IR (KBr) ν_{max} : 3090, 2937, 2841, 1378, 1127, 828, 757 cm^{-1}

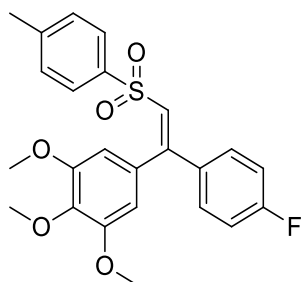
^1H NMR (400 MHz, CDCl_3) δ 7.97 (2H, d, $J=8.3$), 7.41 (2H, d, $J=8.2$), 6.75 (2H, s), 3.87-3.83 (9H, s), 2.47 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 153.22, 145.39, 141.58, 138.96, 130.03, 127.52, 112.49, 110.07, 93.43, 84.78, 61.07, 56.33, 21.78

MS (ESI): m/z 347 $[\text{M}+1]^+$

HRMS (ESI) calcd for $\text{C}_{18}\text{H}_{19}\text{O}_5\text{S}$ $[\text{M}+1]^+$ 347.09477, found 347.09634

4.1.10. AKL-CA-4G



(White solid, 43 mg, 63 % yield).

Melting Point: 141-143°C

IR (KBr) vmax: 3007, 2925, 2853, 1582, 1298, 1126, 875 cm⁻¹

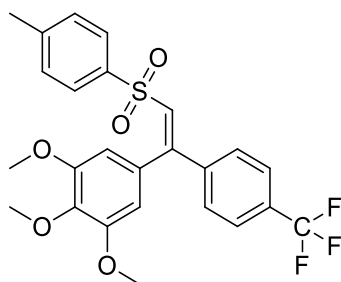
¹H NMR (500 MHz, CDCl₃) δ 7.44 (2H, s, J=8.1), 7.27-7.24 (2H, m), 7.13 (2H, d, J=8.1), 7.04 - 6.99 (3H, m), 6.22 (2H, s), 3.90 (3H, s), 3.71 (6H, s), 2.36 (3H, s)

¹³C NMR (100 MHz, CDCl₃) δ 164.10 (d, J=252.49 Hz) 153.39, 152.68, 143.61, 138.39 (d, J=19.98 Hz), 134.80, 132.46, 130.90, 130.39, 130.22 (d, J=9.08 Hz), 129.09, 127.83, 115.76 (d, J= 21.80 Hz), 107.29, 60.95, 55.99, 21.47

MS (ESI): m/z 443[M+1]⁺

HRMS (ESI) calcd for C₂₄H₂₄FO₅S [M+1]⁺ 443.13230, found 443.13405

4.1.11. AKL-CA-4H



(White solid, 82 mg, 66 % yield).

Melting Point: 125-127°C

IR (KBr) vmax: 3052, 2927, 2849, 1581, 1313, 1124, 1067, 834cm⁻¹

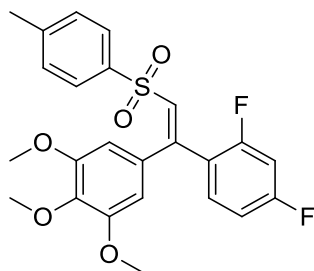
¹H NMR (500 MHz, CDCl₃) δ 7.60 (2H, d, J=8.2), 7.46 (2H, d, J= 8.1), 7.39 (2H, d, J= 8.1), 7.15 (2H, d, J= 7.9), 7.07 (1H, s), 6.23 (2H, s), 3.91 (3H, s), 3.72 (3H, s), 2.37 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 152.90, 152.81, 143.91, 138.60, 137.94, 131.65, 128.89 (q), 125.65, 107.21, 60.99, 56.03, 21.52

MS (ESI): m/z 493 $[\text{M}+1]^+$

HRMS (ESI) calcd for $\text{C}_{25}\text{H}_{24}\text{F}_3\text{O}_5\text{S}$ $[\text{M}+1]^+$ 493.12911, found 493.13163

4.1.12. AKL-CA-4I



(White solid, 88 mg, 60 % yield).

Melting Point: 113-115 $^{\circ}\text{C}$

IR (KBr) ν_{max} : 3049, 2993, 2934, 1594, 1128, 845 cm^{-1}

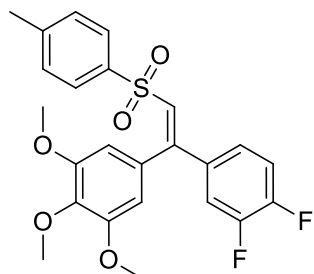
^1H NMR (500 MHz, CDCl_3) δ 7.48 (2H, d, $J = 8.2$), 7.15 (2H, d, $J = 7.9$), 7.03-6.97 (2H, m), 6.88-6.80 (2H, m), 6.27 (2H, s), 3.89 (3H, s), 3.73 (6H, s), 2.37 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 152.66, 147.74, 143.74, 138.61, 138.16, 133.72, 133.66, 130.57, 129.16, 127.81, 123.23, 111.85, 111.68, 107.17, 105.10, 104.89, 104.68, 60.96, 56.04, 21.51

MS (ESI): m/z 461 $[\text{M}+1]^+$

HRMS (ESI) calcd for $\text{C}_{24}\text{H}_{23}\text{F}_2\text{O}_5\text{S}$ $[\text{M}+1]^+$ 461.12288, found 461.12483

4.1.13. AKL-CA-4J



(White solid, 76 mg, 63 % yield).

Melting Point: 140-142 $^{\circ}\text{C}$

IR (KBr) ν_{max} : 3031, 2924, 2847, 1516, 1288, 1128, 836 cm^{-1}

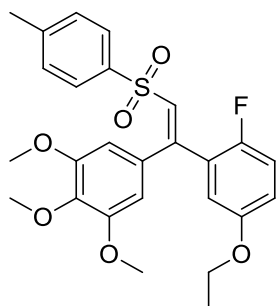
^1H NMR (500 MHz, CDCl_3) δ 7.44 (2H, d, $J=8.1$), 7.15 (3H, d, $J=8.1$), 7.10 (2H, m), 6.98 (1H, s), 6.21 (2H, s), 3.91 (3H, s), 3.72 (6H, s), 2.37 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 152.80, 152.73, 152.22, 143.84, 138.62, 138.01, 135.76, 130.56, 129.76, 129.17, 127.89, 124.56, 117.66, 117.52, 117.45, 117.31, 107.19, 60.99, 56.01, 21.51

MS (ESI): m/z 461 $[\text{M}+1]^+$

HRMS (ESI) calcd for $\text{C}_{24}\text{H}_{23}\text{F}_2\text{O}_5\text{S}$ $[\text{M}+1]^+$ 461.12288, found 461.12513

4.1.14. AKL-CA-4K



(Yellow solid, 108 mg, 70 % yield).

Melting Point: 125-127 $^{\circ}\text{C}$

IR (KBr) ν_{max} : 3074, 2939, 2826, 1584, 1255, 1126, 831 cm^{-1}

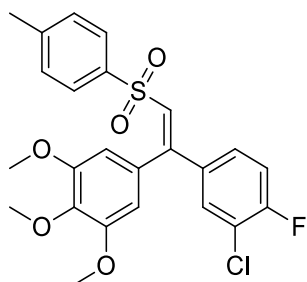
^1H NMR (500 MHz, CDCl_3) δ 7.47 (2H, d, $J=8.2$), 7.14 (2H, d, $J=8.1$), 7.04-6.98 (2H, m), 6.84 - 6.81 (1H, dt, $J=8.96$), 6.50 (1H, dd, $J=6.2$), 6.29 (2H s), 3.88 (5H, m), 3.73(6H, s), 2.36 (3H, s), 1.34 (3H, t, $J=6.9$)

^{13}C NMR (100 MHz, CDCl_3) δ 155.58, 154.79, 152.56, 148.70, 143.63, 138.54, 138.25, 133.91, 133.84, 130.66, 129.10, 127.80, 117.24, 116.53, 107.20, 64.18, 60.93, 56.04, 21.50, 14.70

MS (ESI): m/z 487 $[\text{M}+1]^+$

HRMS (ESI) calcd for $\text{C}_{26}\text{H}_{28}\text{FO}_6\text{S}$ $[\text{M}+1]^+$ 487.15851, found 487.16028

4.1.15. AKL-CA-4L



(Yellow solid, 66 mg, 44 % yield).

Melting Point: 123-125°C

IR (KBr) ν_{max} : 3038, 2958, 2837, 1583, 1284, 1123, 815 cm^{-1}

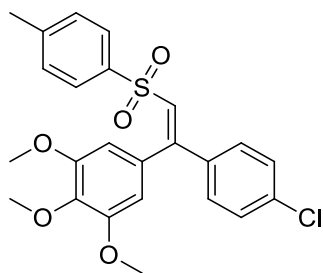
^1H NMR (400 MHz, CDCl_3) δ 7.44 (2H, d, J = 8.3), 7.30 (1H, dd, J = 6.9), 7.15 - 7.12 (4H, m), 6.97 (1H, s), 6.21 (2H, s), 3.91 (3H, s), 3.72 (6H, s), 2.37 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 152.81, 152.14, 143.84, 138.02, 130.61, 130.41, 129.77, 129.17, 128.21, 127.88, 116.77, 107.25, 60.97, 56.03, 21.50

MS (ESI): m/z 477 [$\text{M}+1$] $^+$

HRMS (ESI) calcd for $\text{C}_{24}\text{H}_{23}\text{ClFO}_5\text{S}$ [$\text{M}+1$] $^+$ 477.09333, found 477.09542

4.1.16. AKL-CA-4M



(White solid, 61 mg, 42 % yield).

Melting Point: 96-98°C

IR (KBr) ν_{max} : 3035, 2924, 2849, 1584, 1287, 1141, 1127, 852 cm^{-1}

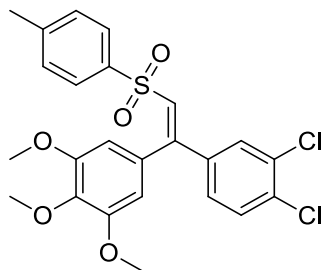
^1H NMR (500 MHz, CDCl_3) δ 7.44 (2H, d, J = 7.93), 7.31 (2H, d, J =8.24), 7.20 (2H, d, J = 8.4), 7.14 (2H, d, J = 7.78), 7.01 (1H, s), 6.21 (2H, s), 3.90 (3H, s), 3.72 (6H, s), 2.36 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 153.25, 152.72, 143.70, 138.52, 138.22, 137.16, 130.15, 129.99, 129.48, 129.12, 128.91, 127.86, 107.29, 60.97, 56.01, 21.49

MS (ESI): m/z 459 [M+1]⁺

HRMS (ESI) calcd for C₂₄H₂₄ClO₅S [M+1]⁺ 459.10275, found 459.10496

4.1.17. AKL-CA-4N



(Yellow solid, 65 mg, 42 % yield).

Melting Point: 151-153°C

IR (KBr) vmax: 3064, 2940, 2837, 2360, 1579, 1127, 1028, 829 cm⁻¹

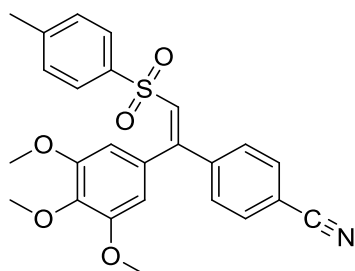
¹H NMR (400 MHz, CDCl₃) δ 7.44 (2H, m), 7.41 (1H, s), 7.34 (1H, d, J=2.08), 7.15 (2H, d, J= 8.07), 7.00 (1H, dd, J=2.08), 6.21 (2H, s), 3.91 (3H, s), 3.73 (6H, s), 2.37 (3H, s)

¹³C NMR (126 MHz, CDCl₃) δ 152.83, 152.03, 143.89, 138.76, 137.94, 134.75, 133.13, 131.04, 130.62, 129.64, 129.19, 127.89, 127.37, 107.23, 60.97, 56.04, 21.51

MS (ESI): m/z 493[M+1]⁺

HRMS (ESI) calcd for C₂₄H₂₃Cl₂O₅S [M+1]⁺ 493.06378, found 493.06600

4.1.18. AKL-CA-4O



(Yellow solid, 72 mg, 51 % yield).

Melting Point: 165-167°C

IR (KBr) vmax: 3050, 2942, 2839, 2227, 1580, 1240, 842 cm⁻¹

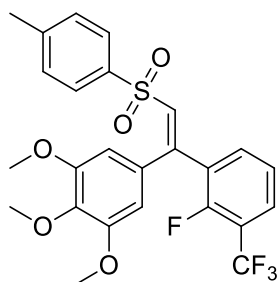
¹H NMR (400 MHz, CDCl₃) δ 7.63 (2H, d, J =8.31), 7.46 (2H, d, J= 8.07), 7.38(2H, d, J= 8.31), 7.16 (2H, d, J= 7.95), 7.06 (1H, s), 6.22 (2H, s), 3.91 (3H, s), 3.72 (6H, s), 2.38 (3H, s)

^{13}C NMR (126 MHz, CDCl_3) δ 152.90, 152.26, 144.06, 143.08, 138.77, 137.79, 132.38, 129.43, 129.24, 128.77, 127.93, 118.09, 113.83, 107.22, 60.97, 56.04, 21.51

MS (ESI): m/z 450 $[\text{M}+1]^+$

HRMS (ESI) calcd for $\text{C}_{25}\text{H}_{24}\text{NO}_5\text{S}$ $[\text{M}+1]^+$ 450.13697, found 450.13898

4.1.19. AKL-CA-4Q



(White solid, 71 mg, 44 % yield).

Melting Point: 128-130 °C

IR (KBr) ν_{max} : 3042, 2950, 2837, 1584, 1332, 1133, 851 cm^{-1}

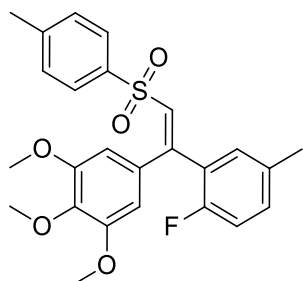
^1H NMR (500 MHz, CDCl_3) δ 7.63 (1H, t, J = 6.41), 7.61 (2H, d, J = 8.24), 7.24- 7.16 (4H, m), 7.04 (1H, s), 6.31 (2H, s), 3.89(3H, s), 3.74 (6H, s), 2.38 (3H, s)

^{13}C NMR (100 MHz, CDCl_3) δ 152.79, 146.77, 143.99, 143.99, 137.91, 135.33, 130.16, 129.26, 127.86, 124.15, 107.17, 60.95, 56.11, 21.53

MS (ESI): m/z 511 $[\text{M}+1]^+$

HRMS (ESI) calcd for $\text{C}_{25}\text{H}_{23}\text{F}_4\text{O}_5\text{S}$ $[\text{M}+1]^+$ 511.11968, found 511.12183

4.1.20. AKL-CA-4R



(White solid, 55 mg, 38 % yield).

Melting Point: 118-120 °C

IR (KBr) ν_{max} : 3025, 2940, 1583, 1503, 1415, 1216, 1128, 822 cm^{-1}

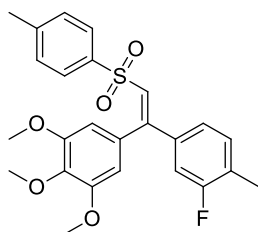
¹H NMR (400 MHz, CDCl₃) δ 7.48 (2H, d, J= 7.95), 7.14 (3H, d, J= 7.82), 7.01-6.94 (2H, m), 6.79 (1H, d), 6.29 (2H, s), 3.89 (3H, s), 3.73(6H, s), 2.36 (3H, s), 2.24 (3H, s)

¹³C NMR (100 MHz, CDCl₃) δ 152.54, 149.03, 143.57, 138.34, 133.81, 133.61, 132.03, 131.63, 129.09, 127.80, 116.21, 115.98, 107.20, 60.94, 56.05, 21.50, 20.60

MS (ESI): m/z 457 [M+1]⁺

HRMS (ESI) calcd for C₂₅H₂₆FO₅S [M+1]⁺ 457.14795, found 457.14966

4.1.21. AKL-CA-4T



(Light yellow, 76 mg, 53 % yield).

Melting Point: 124-126 °C

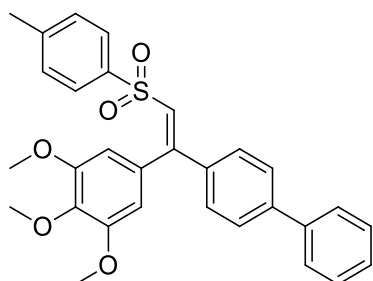
¹H NMR (400 MHz, CDCl₃) δ 7.43 (2H, d, J= 8.31), 7.13 (3H, m), 7.01 (1H, s), 6.98 (1H, dd, J= 1.71), 6.89 (1H, dd, J= 1.71), 6.21 (2H, s), 3.90 (3H, s), 3.71 (6H, s), 2.36 (3H, s), 2.28 (3H, s)

¹³C NMR (100 MHz, CDCl₃) δ 153.28, 152.67, 143.62, 138.45, 138.29, 131.57, 130.15, 129.74, 129.09, 127.86, 123.51, 114.92, 114.68, 107.27, 60.97, 55.99, 21.48, 14.54

MS (ESI): m/z 457 [M+1]⁺

HRMS (ESI) calcd for C₂₅H₂₆FO₅S [M+1]⁺ 457.14795, found 457.15009

4.1.22. AKL-CA-4U



(White solid, 94 mg, 59 % yield).

Melting Point: 132-134 °C

¹H NMR (400 MHz, CDCl₃) δ 7.57 (4H, s), 7.44 (4H, s), 7.35(3H, s), 7.13 (3H, m), 6.26 (2H, s), 3.92 (3H, s), 3.73 (6H, s), 2.37 (3H, s)

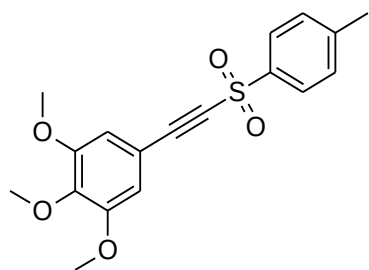
¹³CNMR (101 MHz, CDCl₃) δ 154.10, 152.66, 143.53, 143.29, 139.83, 138.49, 137.38, 130.51, 129.38, 129.07, 128.96, 128.68, 128.03, 127.87, 127.27, 127.07, 107.37, 77.36, 77.04, 76.73, 60.98, 56.01, 21.49.

MS (ESI): m/z 501 [M+1]⁺

HRMS (ESI) calcd for C₃₀H₂₉O₅S [M+1]⁺ 501.17302, found 501.17500

IR (KBr) v_{max}: 3058, 2925, 1580, 1305, 1130, 823 cm⁻¹

4.1.23. AKL-CA-4V



(Yellow solid, 84 mg, 67 % yield).

Melting Point: 119-121 °C

IR (KBr) v_{max}: 3090, 2937, 2841, 1573, 1298, 803 cm⁻¹

¹H NMR (400 MHz, CDCl₃) δ 7.97 (2H, d, J=8.3), 7.41 (2H, d, J=8.2), 6.75 (2H, s), 3.87-3.83 (9H, s), 2.47(3H, s)

¹³CNMR (100 MHz, CDCl₃) δ 153.22, 145.38, 141.59, 138.97, 130.02, 127.53, 112.50, 110.07, 93.43, 84.78, 61.08, 56.33, 21.79

MS (ESI): m/z 347 [M+1]⁺

HRMS (ESI) calcd for C₁₈H₁₉O₅S [M+1]⁺ 347.09477, found 347.09608

4.2 Discussions

A close look at the percentage yield of the synthesized compounds (AKL-CA-4A – 4V) reveals interesting information. Compounds possessing electron donating groups gave a higher percentage yield while those possessing electron withdrawing groups had a lower percentage yield. This might be as a result of their respective electronic induction, which affect their yield.

4.2.1 Important Characteristic Spectroscopic Signals

The spectroscopic data obtained for the synthesized analogues possess certain common signals which reveal the presence of some functional groups that characterised the series of the synthesized analogues. Annotated Compound AKL-CA-4A is used for this illustration.

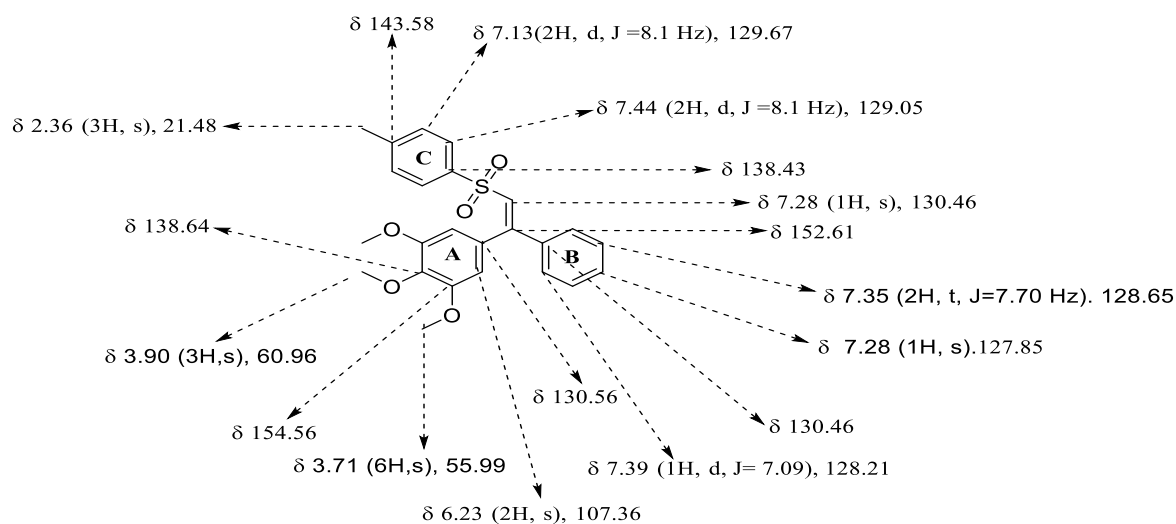


Figure 4.1: Annotated diagram of Compound AKL-CA-4A

The IR band at 1309 cm^{-1} (S=O Anti symmetrical stretching), and 1083 cm^{-1} (S=O Symmetrical stretching) are characteristic bands of sulfonyl group observed in the IR spectra of the compound AKL-CA-4A. Chang [259] confirms this in his report on observing the infra red band at 1326 cm^{-1} and 1060 cm^{-1} study of SO_2 on gamma alumina. Praewpan *et al.*, [260] and and Kerale *et al.*, [261] reinforced our findings with similar IR band for the sulfonyl group attached to the aromatic ring C to be at 1315 cm^{-1} (S=O Anti symmetrical stretching), and 1086 cm^{-1} (S=O Symmetrical stretching) respectively for the compound (E)-1-Methyl-4-(styrylsulfonyl)benzene.

The IR band at 1140 cm^{-1} (C-O Alkyl Ethers stretch) is characteristic of an ether group, thus confirming the presence of C-O functional group arising from the methoxy substituents on aromatic ring A. Robert *et al.*, [262] gave the IR band for alkyl ethers (C-O stretching) to be in the range of $1060 - 1150\text{ cm}^{-1}$.

The IR bands at 3035 cm^{-1} is characteristic of a C-H Aromatic stretching, 2934 cm^{-1} is characteristic of C-H Olefinic stretching, while the band at 2837 cm^{-1} signifies the presence of a C-H Aliphatic stretch, likewise IR bands at 1583 cm^{-1} also confirms the presence of a C=C Aromatic, and the IR band at 813 cm^{-1} gives credence to the presence of C-H bend trisubstituted alkene. Praewpan *et al.*, [260] quoted IR bands for C-H Aromatic stretch (3046 cm^{-1}), C-H Olefinic stretch (2924 cm^{-1}), C=C Aromatic (1595 cm^{-1}), C-H Aliphatic stretch (2840 cm^{-1}), for the compound (E)-1Methyl-4-(styrylsulfonyl)benzene. Robert *et al.*, [262] further stated that the IR band for C-H Aromatic stretch to be 3050 cm^{-1} , C-H Olefinic stretch to be 2930 cm^{-1} , C-H Aliphatic stretch 2843 cm^{-1} , C=C Aromatic 1560 cm^{-1} , and C-H bend trisubstituted alkene 810 cm^{-1} .

^1H NMR signals at $\delta\ 3.71\text{ ppm}$ (6H, s, $-\text{OCH}_3$), $\delta\ 3.90\text{ ppm}$ (6, s, $-\text{OCH}_3$) and ^{13}C NMR signals at $\delta\ 60.96\text{ ppm}$ and $\delta\ 55.99\text{ ppm}$ respectively confirms the presence of the methoxy (OCH_3) proton and a carbon next to oxygen on the aromatic ring A. Monk *et al.*, [241] in like manner reported the ^1H signals for methoxy proton to be around $\delta\ 3.83 - 3.89\text{ ppm}$ for (Z)-2-(2-Nitro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethane, while Lee *et al.*, [240] emphasized the ^1H and ^{13}C NMR signals for methoxy proton and carbon next to oxygen to be around $\delta\ 3.88 - 3.91\text{ ppm}$ and $\delta\ 57.1 - 60.7\text{ ppm}$ respectively for the compound 2-(2'-Methoxyphenyl)-5-(3'',4'',5''-trimethoxyphenyl)-2-acetyl-2,3-dihydro-1,3,4-oxadiazoline.

Mousset *et al.*, [235] concluded that ^1H and ^{13}C NMR signals for methoxy protons and carbon next to oxygen to be around $\delta\ 3.87, 3.93\text{ ppm}$ and $\delta\ 56.0, 61.0\text{ ppm}$ respectively for the compound 1-(4-hydroxy-3-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethane-1,2-dione.

The ^1H NMR signal resonating at $\delta\ 7.28\text{ ppm}$ (1H, s, -vinyl-H) is characteristic of the presence of an olefinic proton bonded to an electron withdrawing group. Pui-Kai *et al.*, [263] in the same vein indicated ^1H signal for the compound (E)-1,3-Dihydro-6-methoxy-3-(2,4,6-trimethoxybenzylidene)-1H-indol-2-one to range from $\delta\ 7.23\text{ ppm}$ to $\delta\ 7.45$, depending on the type of electron withdrawing substituents bonded to the vinyl carbon. Ruchelman *et al.*, [231] corroborated the fact that the ^1H NMR signal of vinyl-H resonate at $\delta\ 7.28\text{ ppm}$.

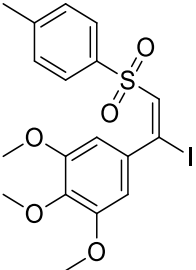
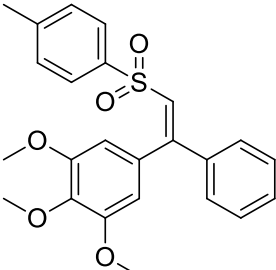
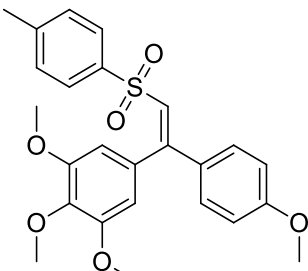
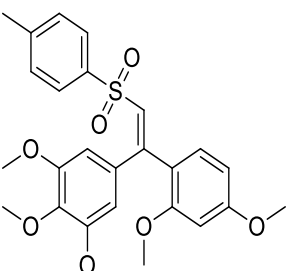
^1H NMR signals at δ 2.36 ppm (3H, s, $-\text{CH}_3$), and ^{13}C NMR signals at δ 21.48 ppm respectively confirms the presence of the methyl proton (CH_3) proton and a carbon attached to the aromatic ring C. Aziz *et al.*, [219] augmented the fact that the ^1H signals for methyl proton to be around δ 2.41 ppm (3H, s) and ^{13}C NMR signal at δ 21.7 ppm for compound (Z/E)-1-(2-Methoxy-1-(3,4,5-trimethoxyphenyl)propylidene)-2-tosylhydrazine. Praewpan *et al.*, [260] in addition reinforced our findings with similar chemical shift for methyl protons attached to an aromatic ring to be at δ 2.46 ppm (3H, s, CH_3) and ^{13}C NMR signals at δ 21.6 ppm respectively for the compound (E)-1-Methyl-4-(styrylsulfonyl)benzene.

4.2.2 Anticancer Activity

All the newly synthesized compounds (AKL CA-3 to AKL-CA-4V) were evaluated for their anticancer activity on a panel of four human cancer cell lines namely MDA-MB 231 (breast cancer), HeLa (cervical cancer), A549 (lung cancer), and IMR-32 (neuroblast cancer), along with normal cell line HEK-293 (human embryonic kidney cell) by employing MTT assay [244]. The results were summarized in Table 4.1 and expressed as IC_{50} (μM) values. The *in vitro* cytotoxicity assay reveals that all the synthesized compounds (AKL CA-3 to AKL-CA-4V) showed potent activity against A549 and IMR-32 cancer cell lines with IC_{50} values in the range of $4.10 \pm 0.96 - 12.03 \pm 0.02 \mu\text{M}$.

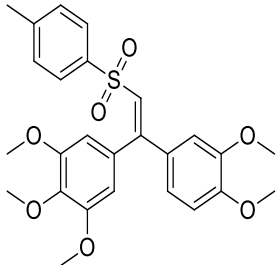
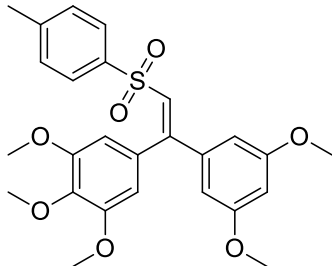
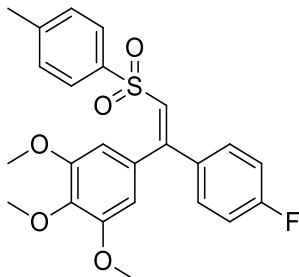
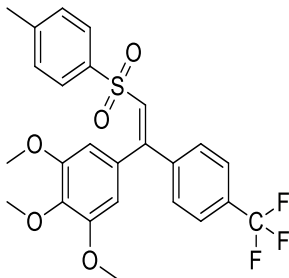
Among some of the compounds; AKL-CA-3 and AKL-CA-4B showed more potent remarkable cytotoxicity against A549 and IMR-32 cancer cell lines with IC_{50} values in the range of $4.10 \pm 0.96 - 4.74 \pm 0.56 \mu\text{M}$ when compared to the standard, Doxorubicin with IC_{50} values from $0.23 \pm 0.76 - 2.06 \pm 0.09 \mu\text{M}$. The synthesized compounds (AKL CA-3 to AKL-CA-4V) also showed significant activity on MDA-MB-231 cancer cell line with IC_{50} values in the range of $9.85 \pm 0.72 - 23.94 \pm 0.156 \mu\text{M}$, and on HeLa cancer cell line with IC_{50} values in the range of $8.39 \pm 0.99 - 11.70 \pm 0.81 \mu\text{M}$. Furthermore, all these compounds were evaluated for their cytotoxicity towards normal cell line, HEK-293. Results revealed that all the compounds did not affect the growth of normal cells, HEK-293 except compounds AKL-CA-3 which was toxic with IC_{50} $43.25 \mu\text{M}$.

Table 4.1: IC₅₀ values (in μ M) for synthesized compounds in selected human cancer cell lines

S/N	Compound	Structure	^a MDA-MB 231	^b HeLa	^c A549	^d IMR32	^e HEK-293
1	AKL-CA-3		18.03 $\pm$ 1.70	11.03 $\pm$ 0.70	4.69 $\pm$ 0.13	4.74 $\pm$ 0.56	NA
2	AKL-CA-4A		19.40 $\pm$ 1.72	9.40 $\pm$ 0.72	6.39 $\pm$ 0.68	6.40 $\pm$ 0.82	NA
3	AKL-CA-4B		14.59 $\pm$ 0.85	8.59 $\pm$ 0.58	4.68 $\pm$ 0.46	4.10 $\pm$ 0.96	NA
4			13.56 $\pm$ 1.56	10.56 $\pm$ 0.56	7.04 $\pm$ 0.08	7.04 $\pm$ 0.83	NA
	Doxorubicin		0.89 $\pm$ 0.02	1.68 $\pm$ 0.24	2.06 $\pm$ 0.09	0.23 $\pm$ 0.76	NT

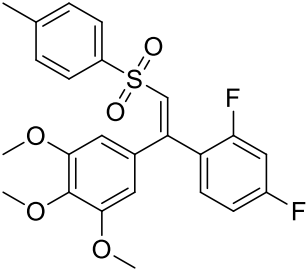
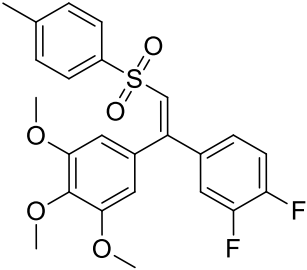
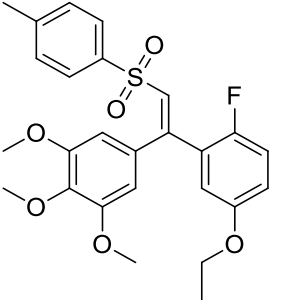
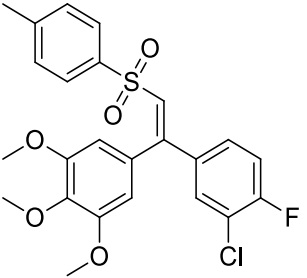
Key: ^aMDA-MB 231(Breast cancer), ^bHeLa (Cervical cancer), ^cA549 (Lung cancer), ^dIMR-32 (neuroblast), ^eHEK-293 (Human embryonic kidney Normal cell line), NA (Not active), NT (Not tested). Data are the means of three experiments and are reported as means $\pm$ standard error of the mean (SEM).

Table 4.1 Contd.: IC₅₀ values (in μ M) for synthesized compounds in selected human cancer cell lines

S/N	Compound	Structure	^a MDA-MB 231	^b HeLa	^c A549	^d IMR32	^e HEK-293
5	AKL-CA-4D		14.87 $\pm$ 1.54	10.87 $\pm$ 0.54	5.52 $\pm$ 0.06	6.25 $\pm$ 0.03	NA
6	AKL-CA-4E		14.12 $\pm$ 1.51	11.12 $\pm$ 0.51	5.32 $\pm$ 0.92	6.23 $\pm$ 0.09	NA
7	AKL-CA-4G		13.25 $\pm$ 1.49	8.73 $\pm$ 0.49	6.92 $\pm$ 0.65	7.29 $\pm$ 0.46	NA
8	AKL-CA-4H		15.92 $\pm$ 1.44	9.62 $\pm$ 0.44	6.84 $\pm$ 0.70	7.48 $\pm$ 0.13	NA
	Doxorubicin		0.89 $\pm$ 0.02	1.68 $\pm$ 0.24	2.06 $\pm$ 0.09	0.23 $\pm$ 0.76	NT

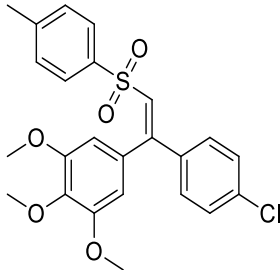
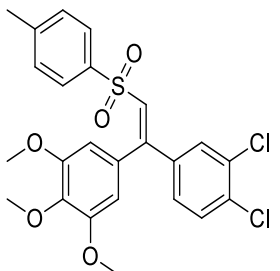
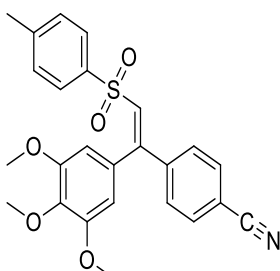
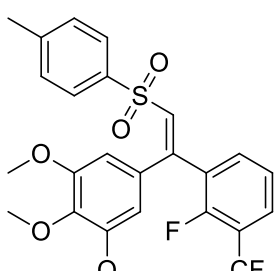
Key: ^aMDA-MB 231(Breast cancer), ^bHeLa (Cervical cancer), ^cA549 (Lung cancer), ^dIMR-32 (neuroblast), ^eHEK-293 (Human embryonic kidney Normal cell line), NA (Not active), NT (Not tested). Data are the means of three experiments and are reported as means $\pm$ standard error of the mean (SEM).

Table 4.1 Contd.: IC₅₀ values (in μ M) for synthesized compounds in selected human cancer cell lines

S/N	Compound	Structure	^a MDA-MB 231	^b HeLa	^c A549	^d IMR32	^e HEK-293
9	AKL-CA-4I		12.72 $\pm$ 0.40	9.46 $\pm$ 0.40	13.08 $\pm$ 0.86	15.10 $\pm$ 0.16	NA
10	AKL-CA-4J		13.14 $\pm$ 0.92	9.98 $\pm$ 0.42	11.30 $\pm$ 0.83	12.03 $\pm$ 0.02	NA
11	AKL-CA-4K		13.56 $\pm$ 0.89	9.50 $\pm$ 0.49	7.02 $\pm$ 0.84	8.06 $\pm$ 0.09	NA
12	AKL-CA-4L		13.38 $\pm$ 1.49	8.74 $\pm$ 0.49	8.54 $\pm$ 0.85	5.12 $\pm$ 0.36	NA
	Doxorubicin		0.89 $\pm$ 0.02	1.68 $\pm$ 0.24	2.06 $\pm$ 0.09	0.23 $\pm$ 0.76	NT

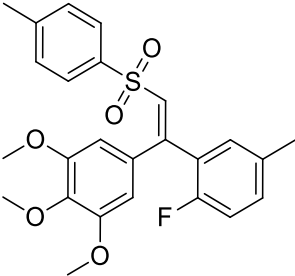
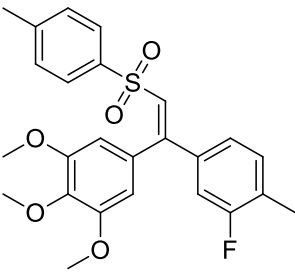
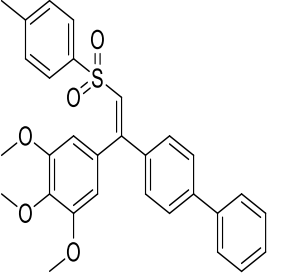
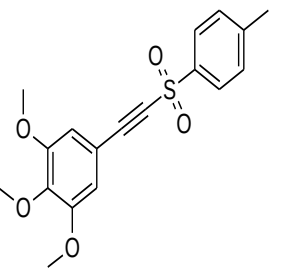
Key: ^aMDA-MB 231(Breast cancer), ^bHeLa (Cervical cancer), ^cA549 (Lung cancer), ^dIMR-32 (neuroblast), ^eHEK-293 (Human embryonic kidney Normal cell line), NA (Not active), NT (Not tested). Data are the means of three experiments and are reported as means $\pm$ standard error of the mean (SEM).

Table 4.1 Contd.: IC₅₀ values (in μ M) for synthesized compounds in selected human cancer cell lines

S/N	Compound	Structure	^a MDA-MB 231	^b HeLa	^c A549	^d IMR32	^e HEK-293
13	AKL-CA-4M		11.14 $\pm$ 1.50	10.20 $\pm$ 1.80	5.12 $\pm$ 0.08	6.79 $\pm$ 0.44	NA
14	AKL-CA-4N		9.85 $\pm$ 0.72	9.64 $\pm$ 0.96	6.79 $\pm$ 0.19	9.14 $\pm$ 0.84	NA
15	AKL-CA-4O		12.38 $\pm$ 1.67	9.83 $\pm$ 0.76	9.14 $\pm$ 0.91	9.08 $\pm$ 0.07	NA
16	AKL-CA-4Q		12.25 $\pm$ 1.61	10.69 $\pm$ 0.74	9.08 $\pm$ 0.04	7.46 $\pm$ 0.61	NA
	Doxorubicin		0.89 $\pm$ 0.02	1.68 $\pm$ 0.24	2.06 $\pm$ 0.09	0.23 $\pm$ 0.76	NT

Key: ^aMDA-MB 231(Breast cancer), ^bHeLa (Cervical cancer), ^cA549 (Lung cancer), ^dIMR-32 (neuroblast), ^eHEK-293 (Human embryonic kidney Normal cell line), NA (Not active), NT (Not tested). Data are the means of three experiments and are reported as means $\pm$ standard error of the mean (SEM).

Table 4.1 Contd.: IC₅₀ values (in μ M) for synthesized compounds in selected human cancer cell lines

S/N	Compound	Structure	^a MDA-MB 231	^b HeLa	^c A549	^d IMR32	^e HEK-293
17	AKL-CA-4R		12.22 $\pm$ 0.54	10.46 $\pm$ 1.03	7.46 $\pm$ 0.07	6.14 $\pm$ 0.03	NA
18	AKL-CA-4T		10.44 $\pm$ 1.55	8.39 $\pm$ 0.99	7.22 $\pm$ 0.81	6.29 $\pm$ 0.07	NA
19	AKL-CA-4U		23.94 $\pm$ 1.56	11.70 $\pm$ 0.81	11.14 $\pm$ 0.78	11.77 $\pm$ 0.13	NA
20	AKL-CA-4V		10.87 $\pm$ 1.49	8.53 $\pm$ 0.76	6.29 $\pm$ 0.79	9.60 $\pm$ 0.34	43.25
	Doxorubicin		0.89 $\pm$ 0.02	1.68 $\pm$ 0.24	2.06 $\pm$ 0.09	0.23 $\pm$ 0.76	NT

Key: ^aMDA-MB 231(Breast cancer), ^bHeLa (Cervical cancer), ^cA549 (Lung cancer), ^dIMR-32 (neuroblast), ^eHEK-293 (Human embryonic kidney Normal cell line), NA (Not active), NT (Not tested). Data are the means of three experiments and are reported as means $\pm$ standard error of the mean (SEM).

Huan *et al.*, [264] reported the *in vitro* antiproliferative activities of the cyclopropylamide analogues of CA-4 to be in the range of IC_{50} (μM) in the range of $24.1 \pm 2.1 - >100 \mu M$ for Lung cancer cell line (A549) and IC_{50} (μM) in the range of $8.1 \pm 1.3 - >100 \mu M$ for Cervical cancer cell line (HeLa). The results of our synthesized AKL-CA-4 analogues containing sulfonyl (SO_2) group showed more potent antiproliferative activity on A549 cancer cell line with IC_{50} (μM) in the range of $4.68 \pm 0.13 - 13.08 \pm 0.86 \mu M$, as well as cervical cancer cell line (HeLa) with IC_{50} (μM) in the range of $8.53 \pm 0.76 - 11.70 \pm 0.81 \mu M$. This underscores the fact sulfonyl (SO_2) CA-4 analogues showed more remarkable cytotoxicity on A549 cancer cell line and HeLa cancer cell lines than the cyclopropylamide CA-4 analogues.

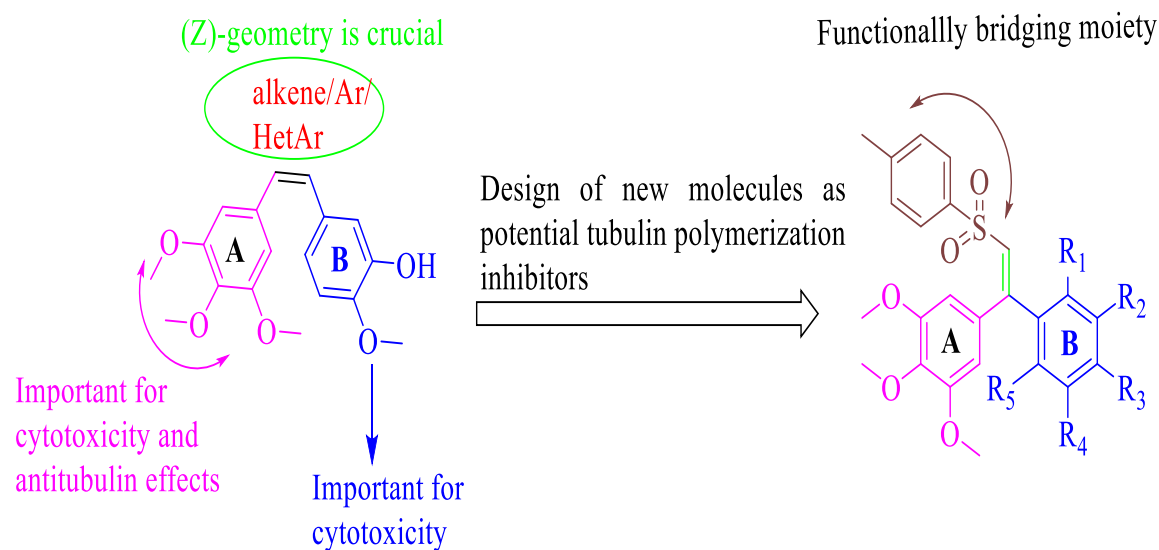
Zheng *et al.*, [226] synthesized novel pyridine-bridge analogues of CA-4. They evaluated their synthesized compounds against three (3) panel of cancer cell lines namely: breast cancer (MDA-MB-231), lung cancer (A549), and cervical cancer (HeLa). The *in vitro* cytotoxicity assay of these compounds showed varied antiproliferative activities. In their report they stated that their pyridine-bridge analogues of CA-4 showed activity on MDA-MB-231 with IC_{50} in the range of $2.63 \pm 0.18 - >100 \mu M$, and A549 with IC_{50} (μM) in the range of $0.086 \pm 0.18 - >100 \mu M$. Comparing the results of the cytotoxicity assay of the pyridine-bridge analogues of CA-4 with that of our newly synthesized sulfonyl bridge CA-4 analogues, an interesting trend is seen.

The pyridine-bridge analogues [227] of CA-4 showed IC_{50} values in the range of $2.63 \pm 0.18 - >100 \mu M$ for MDA-MB-231 cancer cell line, $0.089 \pm 0.008 - >100 \mu M$ for A549 cancer cell line and $0.0038 \pm 0.0001 - >50 \mu M$ for HeLa cancer cell line while the AKL-CA-4 sulfonyl bridge analogues of CA-4 showed IC_{50} in the range of $9.85 \pm 0.72 - 23.94 \pm 1.56 \mu M$ for MDA-MB-231 cancer cell line, $4.69 \pm 0.13 - 13.08 \pm 0.86 \mu M$ for A549 cancer cell line and $8.39 \pm 0.99 - 11.70 \pm 0.81 \mu M$ for HeLa cancer cell line respectively. From this reported results, it can be inferred that the pyridine-bridge analogues of CA-4 showed more potent

antiproliferative activity on MDA-MB-231, A549, and HeLa cancer cell lines than AKL-CA-4 sulfonyl bridge analogues of CA-4.

Kumar *et al.*, [229] reported that biaryl aryl stilbenes CA-4 analogues showed antiproliferative activity against A549 and HeLa cancer cell lines with IC_{50} in the range of $6.3 \pm 0.5 - 79.00 \pm 8.4 \mu M$ and $9.2 \pm 0.1 - 89 \pm 5.8 \mu M$ respectively, where as the sulfonyl bridge analogues of AKL- CA-4 showed IC_{50} in the range of $4.69 \pm 0.13 - 13.08 \pm 0.86 \mu M$ for A549 cancer cell line and $8.39 \pm 0.99 - 11.70 \pm 0.81 \mu M$ for HeLa cancer cell line. The results of these *in vitro* cytotoxicity showed that the SO_2 based CA-4 analogues posses more potent cytotoxicity activities than the biaryl aryl stilbenes CA-4 analogues.

4.3 Structure Activity Relationship (SAR)



Scheme 4.1: SAR of CA-4 and its analogues

In addition, it was envisioned that variation of the substituents on ring-B would provide remarkable SAR information about these compound AKL-CA-4 congeners (Scheme 4.1). The introduction of substituent's on ring B were exemplified by preparing analogs possessing electron donating groups (AKL-CA-4B – 4E), electron withdrawing groups (AKL-CA-4G – 4J and AKL-CA-4I – 4Q) as well as both electron donating and withdrawing groups (AKL-CA-4K, AKL-CA-4R, and AKL-CA-4K). However, compound possessing

biphenyl group (AKL-CA-4U) on ring **B** shows poor cytotoxic activity compared to the simple phenyl group (AKL-CA-4A) thereby increase in the number of phenyl moieties, activity was declined. Compound AKL-CA-4B displayed prominent potent antiproliferative activity against lung cancer (A549), human neuroblastoma (IMR32), and cervical cancer (HeLa). So the presence of 4-methoxy phenyl on ring B is essential for cytotoxic activity. In contrast, order of reactivity of electron donating substituents on ring B was 4- methoxy (AKL-CA-4B) > 3,5-dimethoxy (AKL-CA-4E) > 3,4- dimethoxy (AKL-CA-4D) > 4,6-dimethoxy (AKL-CA-4C). It was observed that compounds with electron withdrawing substituents like fluoro, difluoro, chloro, dichloro, cyano and trifluoro methyl on ring B exhibit moderate cytotoxic activity. The compounds possessing both electron donating and electron withdrawing groups showed moderate activity. Therefore, it was concluded that electron donating substituent on ring B enhances the cytotoxic activity of the synthesized compounds.

4.4 Conclusion

A series of 20 compounds of the analogues of CA-4 using Suzuki Miyaura coupling method were successfully synthesized and fully characterized using different spectroscopic techniques. The compounds possessing electron donating substituents gave higher percentage yields than those possessing electron withdrawing substituent. This is due to their positive electronic induction effect. All the synthesized compounds evaluated for their anticancer activity against four cancer cell lines, MDA-MB 231 (breast cancer), HeLa (cervical cancer), A549 (lung cancer), and IMR-32 (neuroblast cancer), along with normal cell line HEK-293 (human embryonic kidney cell) by employing MTT assay. Among all the compounds, compound AKL-CA-4B showed remarkable cytotoxic activity against human lung cancer cell line (A549).

4.5 Recommendation

1. The synthesized sulfonyl CA-4 analogues were found to be effective against A549 cancer cell lines especially compound (Z)-1,2,3-trimethoxy-5-(1-(4-methoxyphenyl)-2-tosylvinyl)benzene. It is therefore recommended for consideration as a possible drug for lung cancer treatment.
2. Further biological evaluation should be carried out on compound AKL-CA-4B in order to determine its cell cycle analysis, and tubulin polymerization activity. Also, other cytotoxicities studies should be considered and evaluated on the other molecules and more variation of electron donating groups on the ring B should also be analysed to enhance the biological activity of the molecule.

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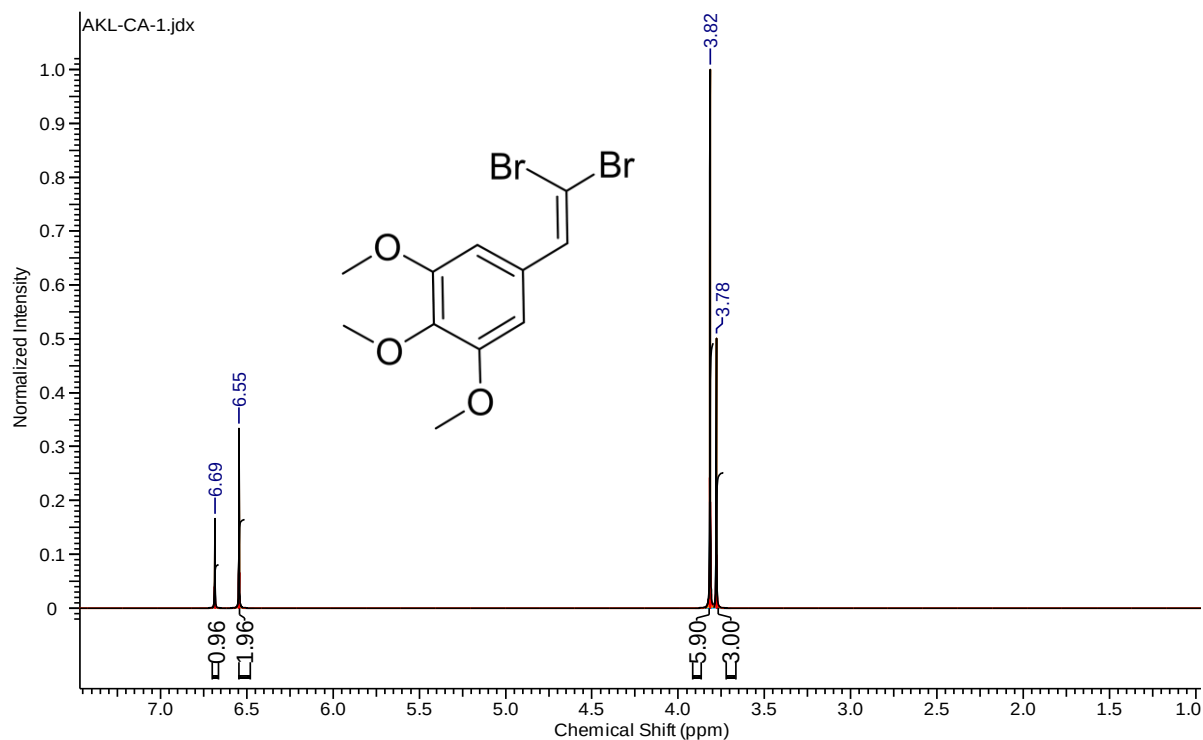
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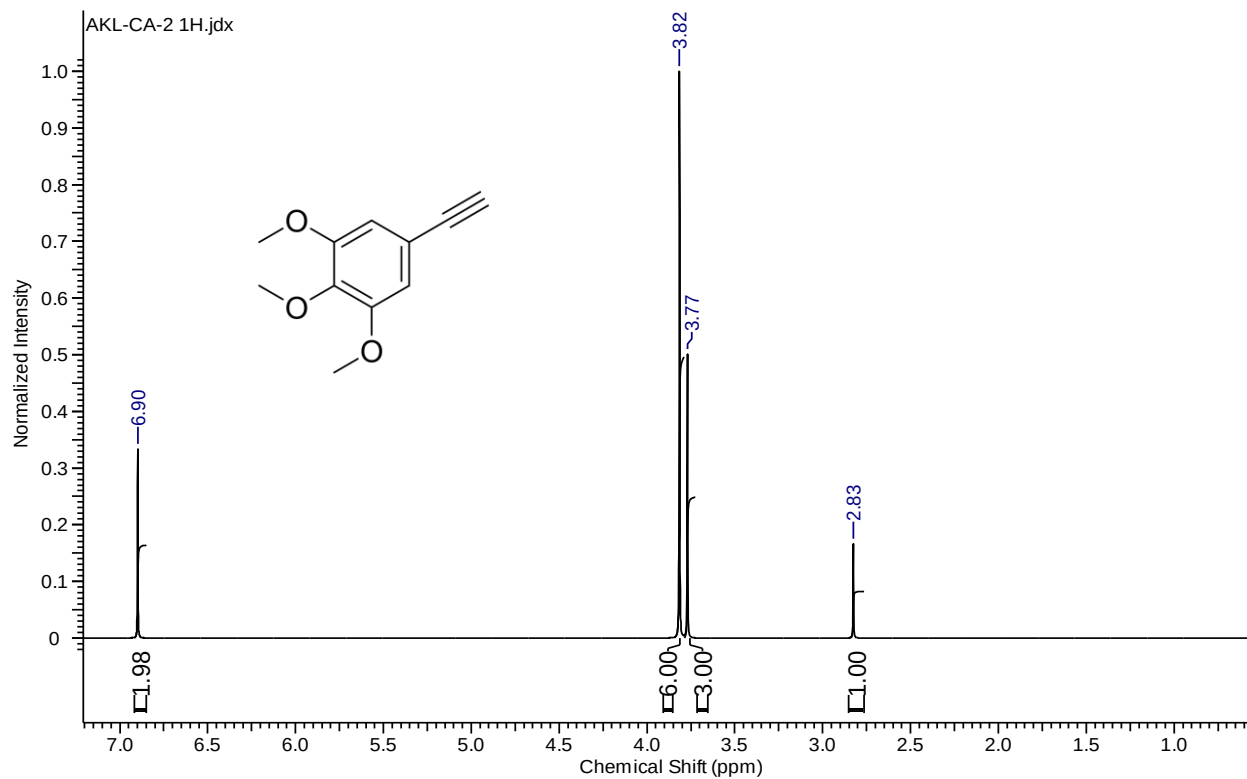
Appendices

APPENDICES I: ^1H NMR SPECTRA OF SYNTHESIZED COMPOUNDS

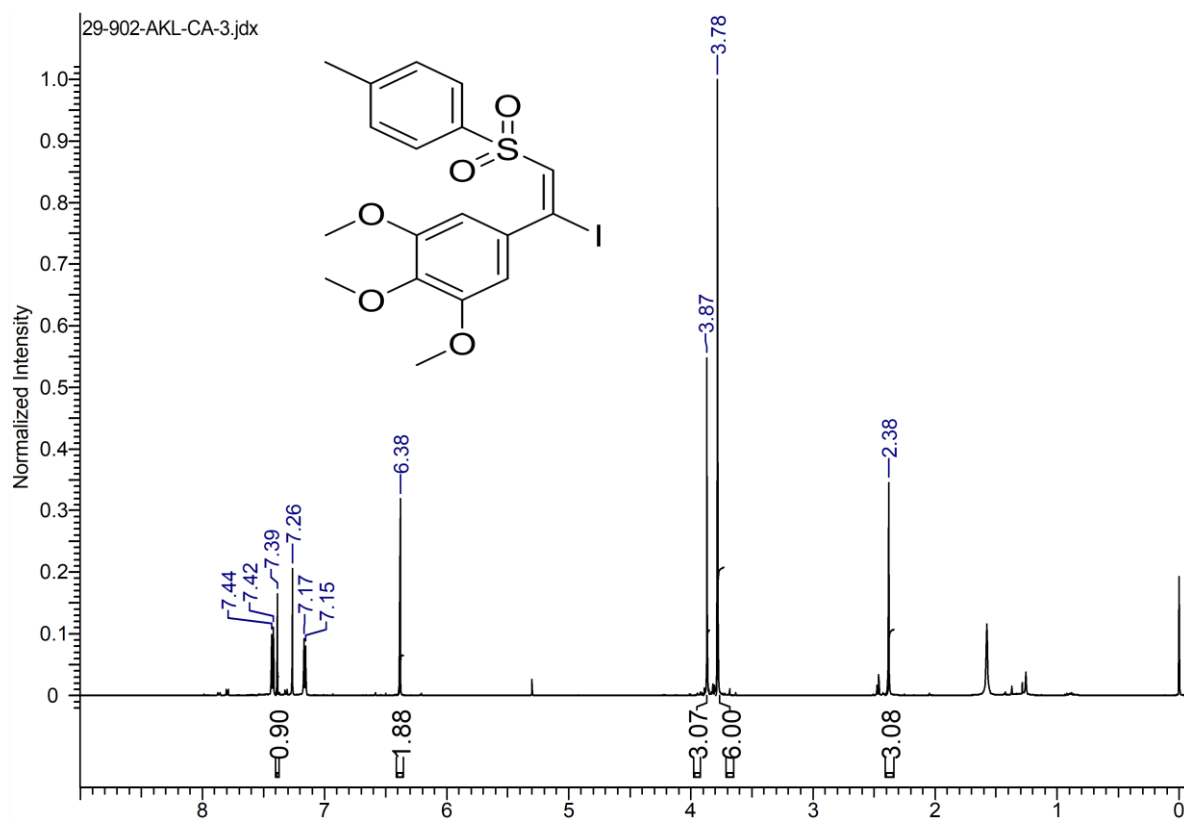
1. AKL-CA-1, $\text{C}_{11}\text{H}_{12}\text{Br}_2\text{O}_3$; 5-(2,2-dibromovinyl)-1,2,3-trimethoxybenzene



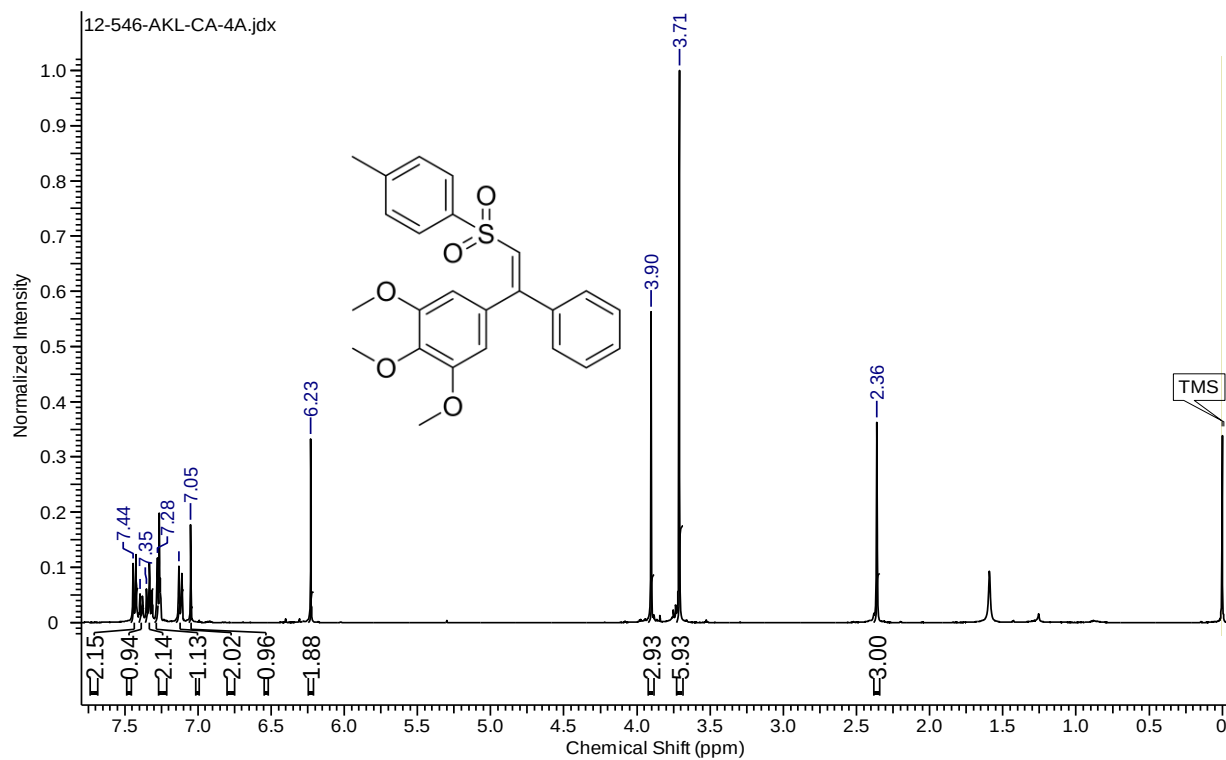
2. AKL-CA-2, $\text{C}_{11}\text{H}_{12}\text{O}_3$, 5-ethynyl-1,2,3-trimethoxybenzene



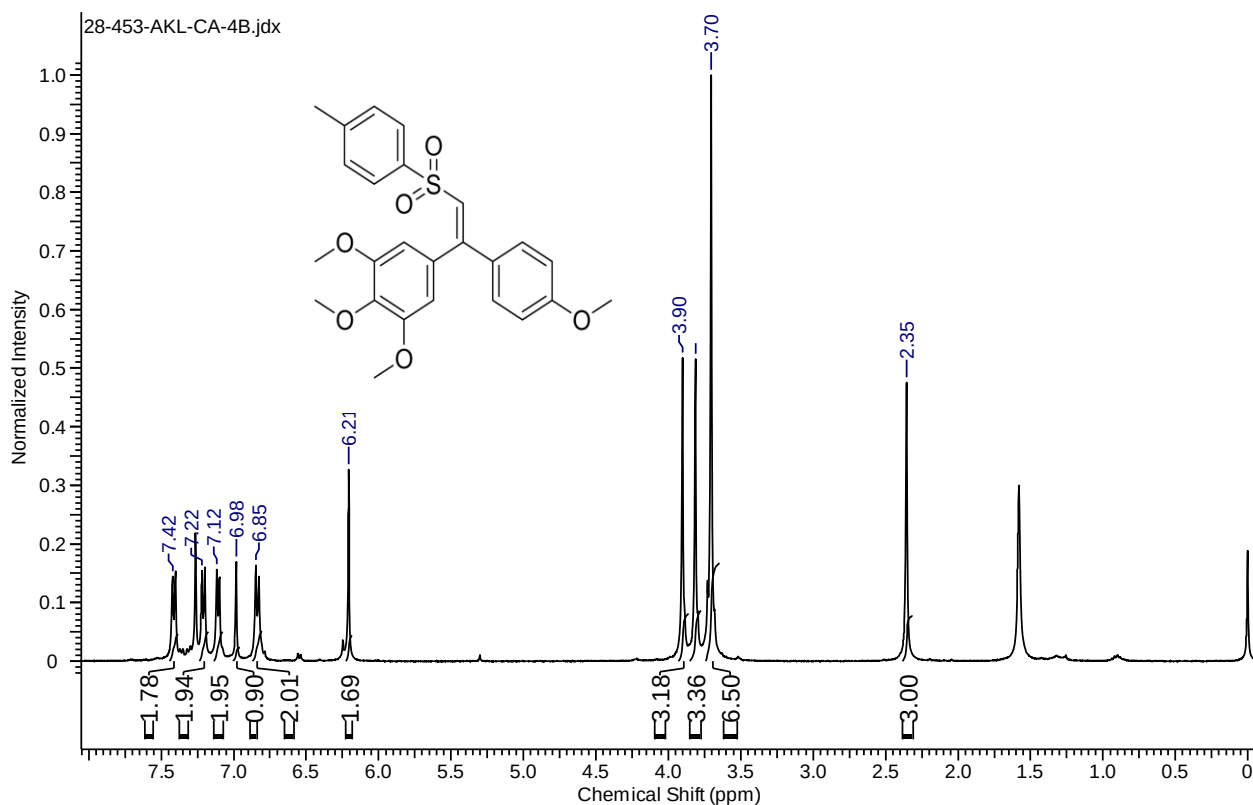
3. AKL-CA-3, C₁₈H₁₉IO₅S, (E)-5-(1-iodo-2-tosylvinyl)-1,2,3-trimethoxybenzene



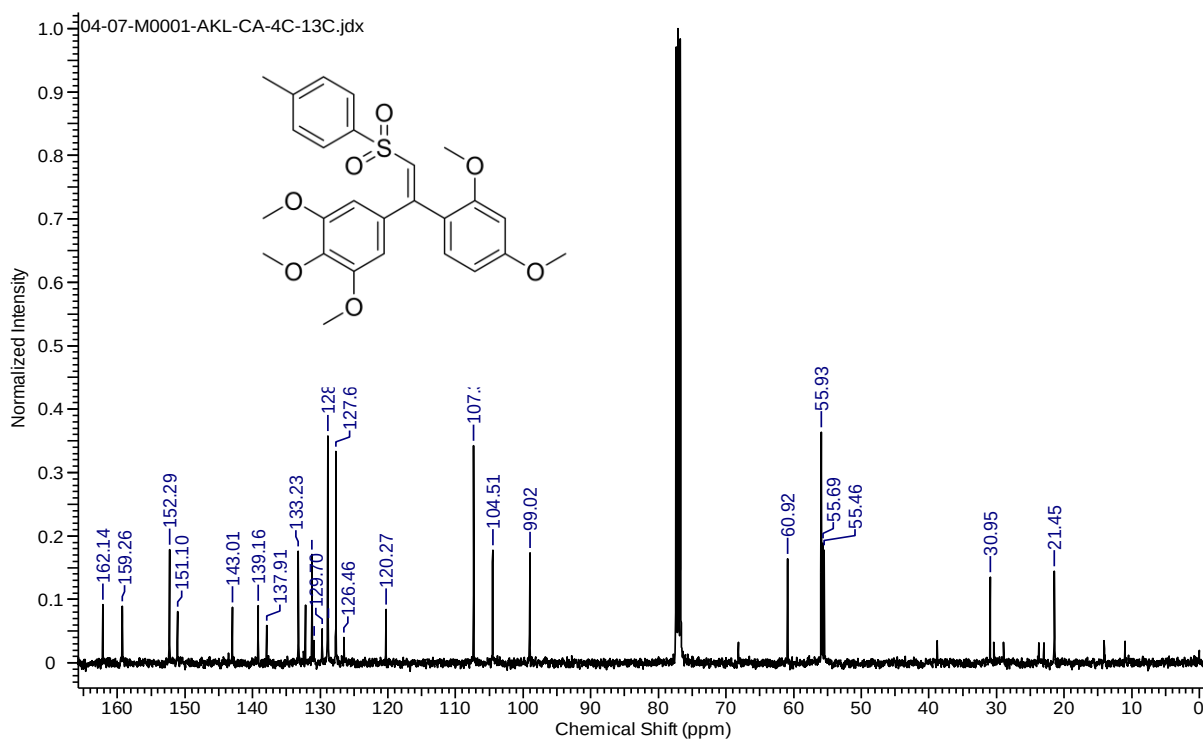
4. AKL-CA-4A, C₂₄H₂₄O₅S, (Z)-1,2,3-trimethoxy-5-(1-phenyl-2-tosylvinyl)benzene



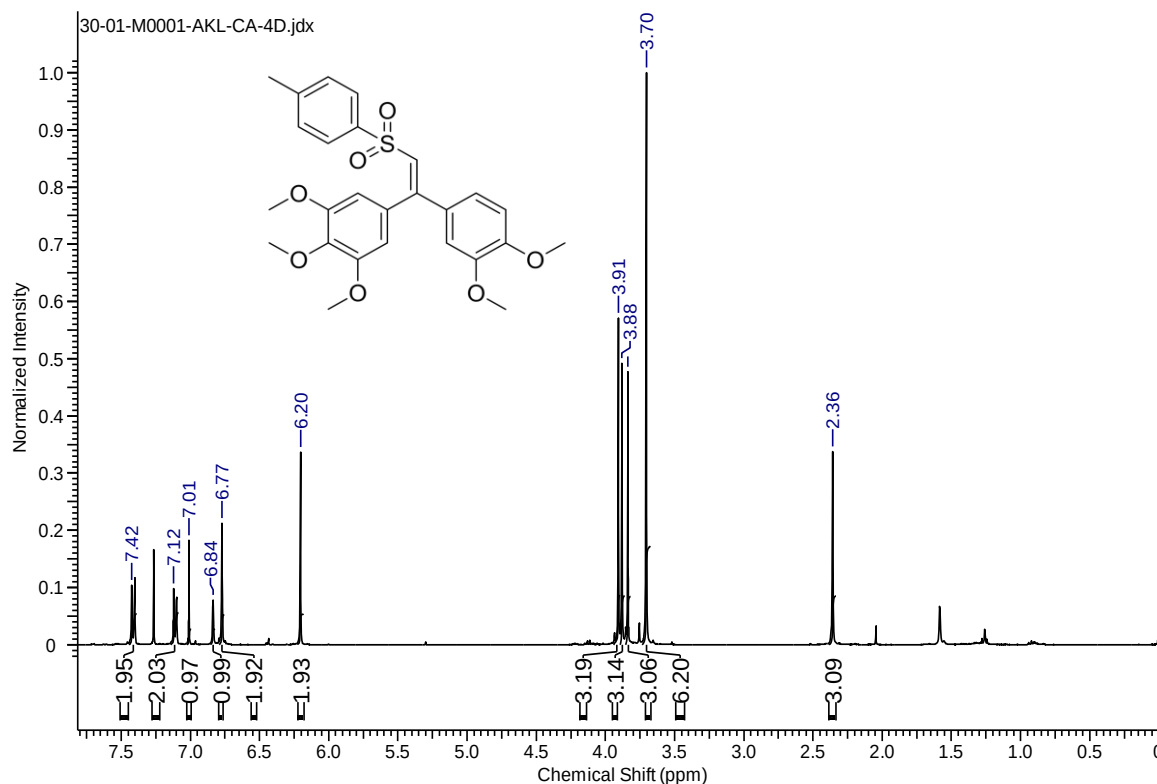
5. AKL-CA-4B, (Z)-1,2,3-trimethoxy-5-(1-(4-methoxyphenyl)-2-tosylvinyl)benzene



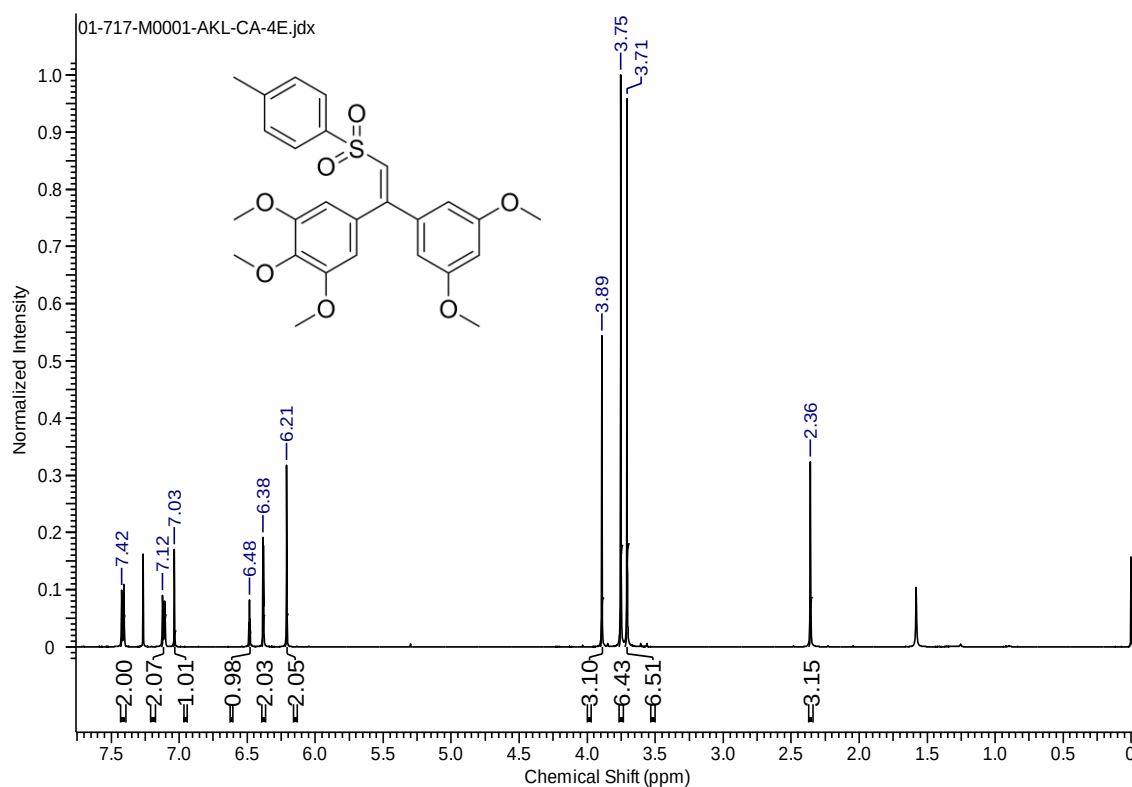
6. AKL-CA-4C, C₂₆H₂₈O₇S, (E)-5-(1-(2,4-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



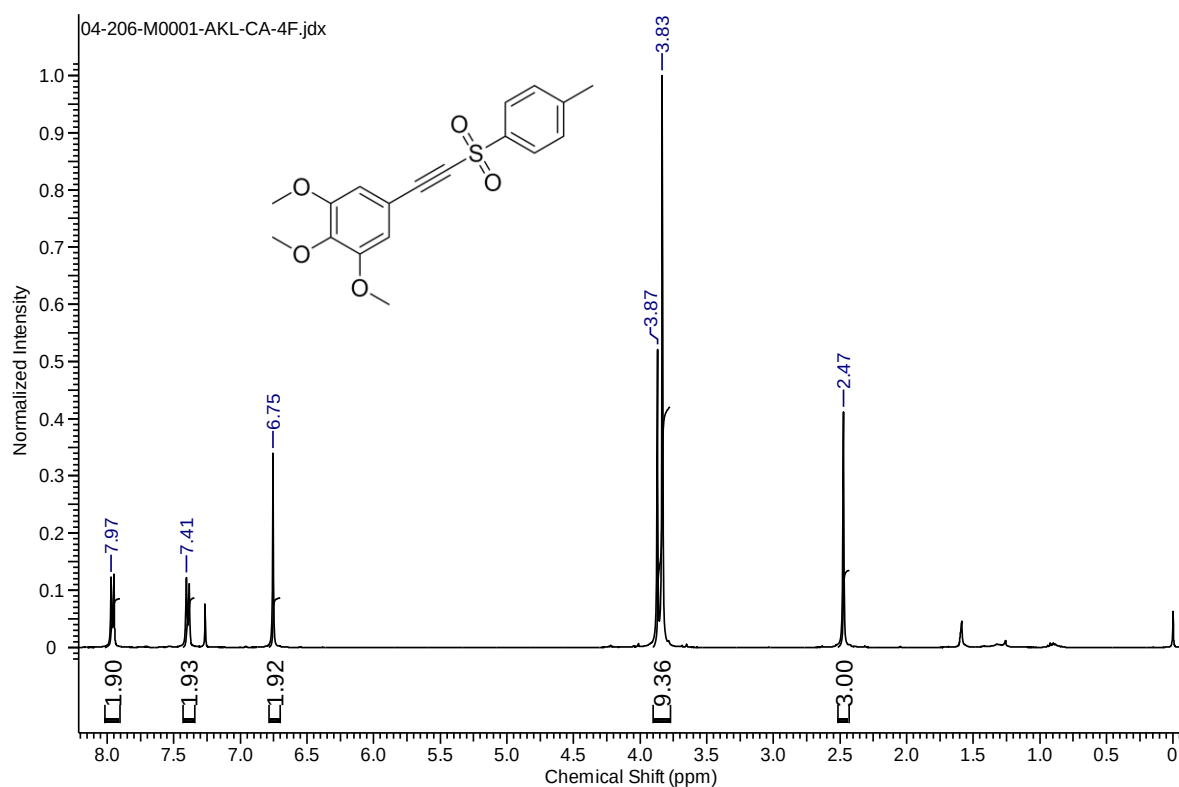
7. AKL-CA-4D, C₂₆H₂₈O₇S, (Z)-5-(1-(3,4-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



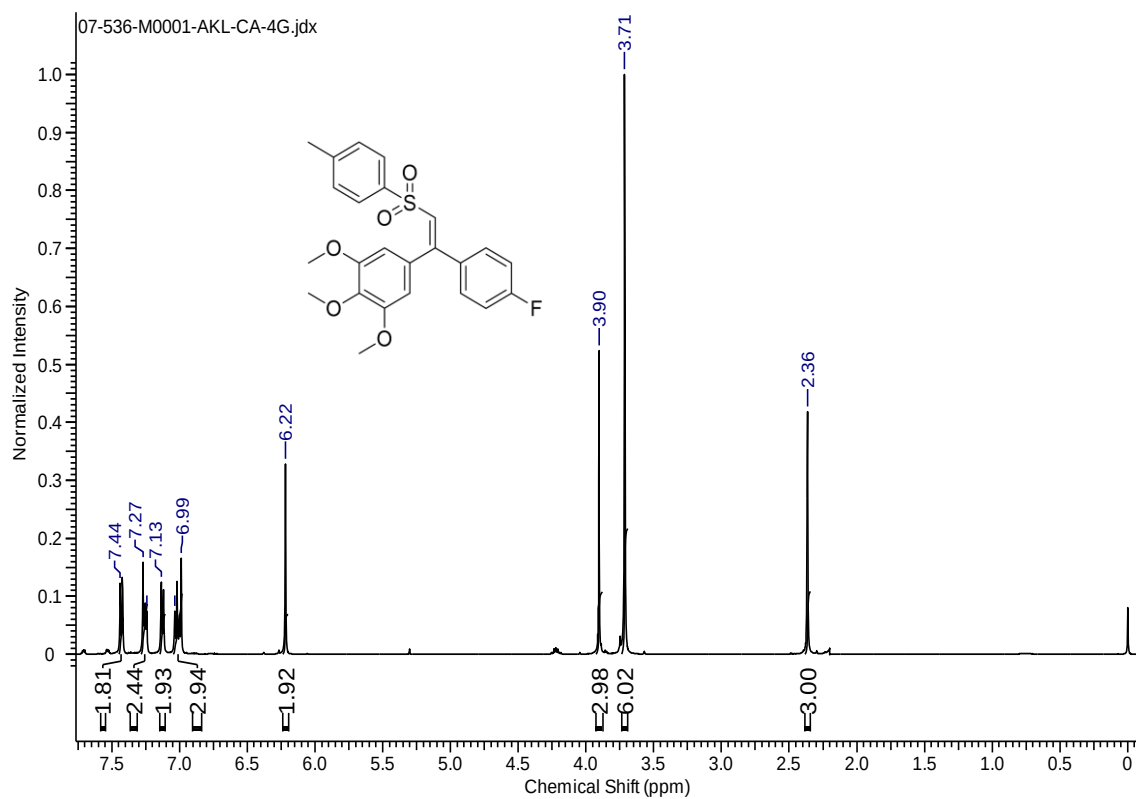
8. AKL-CA-4E, C₂₆H₂₈O₇S, (Z)-5-(1-(3,5-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



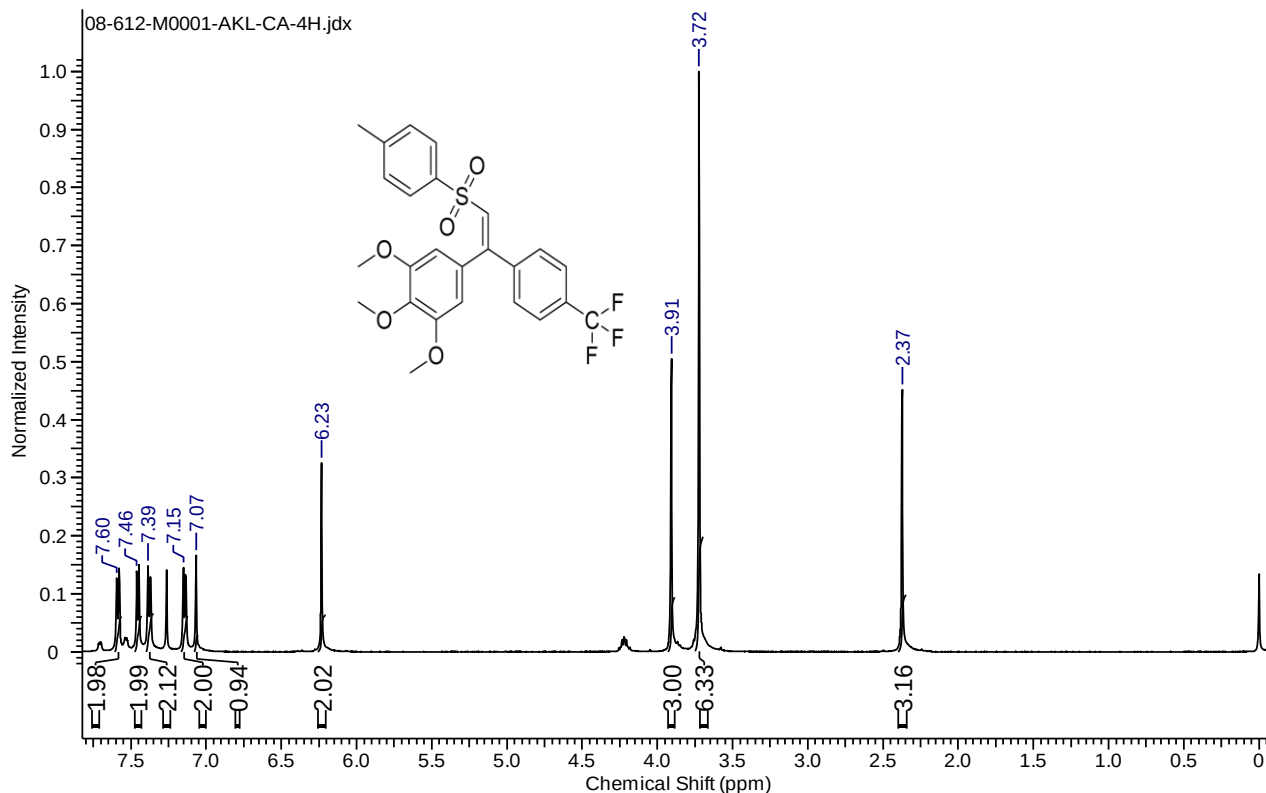
9. AKL-CA-4F, C₁₈H₁₈O₅S, 4-methylphenyl (3,4,5-trimethoxyphenyl)ethynyl sulfone



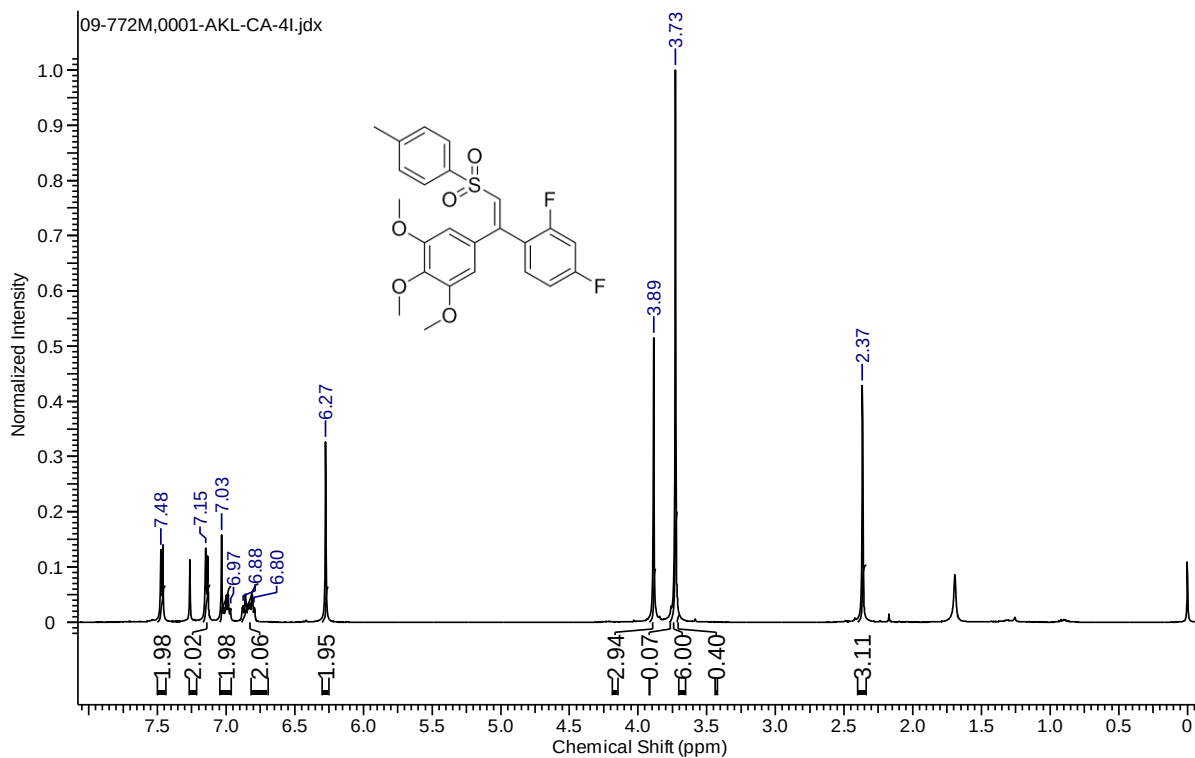
10. AKL-CA-4G, C₂₄H₂₃FO₅S, (Z)-5-(1-(4-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



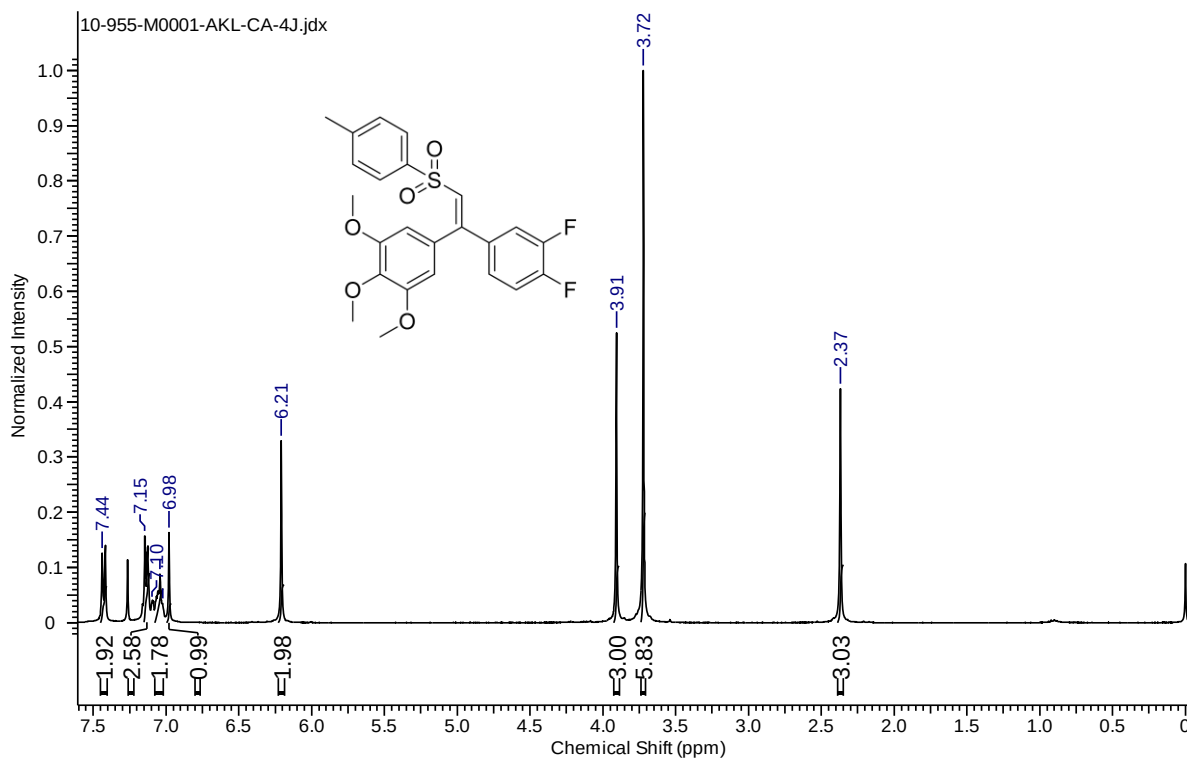
11. AKL-CA-4H, C₂₅H₂₃F₃O₅S, (Z)-1,2,3-trimethoxy-5-(2-tosyl-1-(4-(trifluoromethyl)phenyl)vinyl)benzene



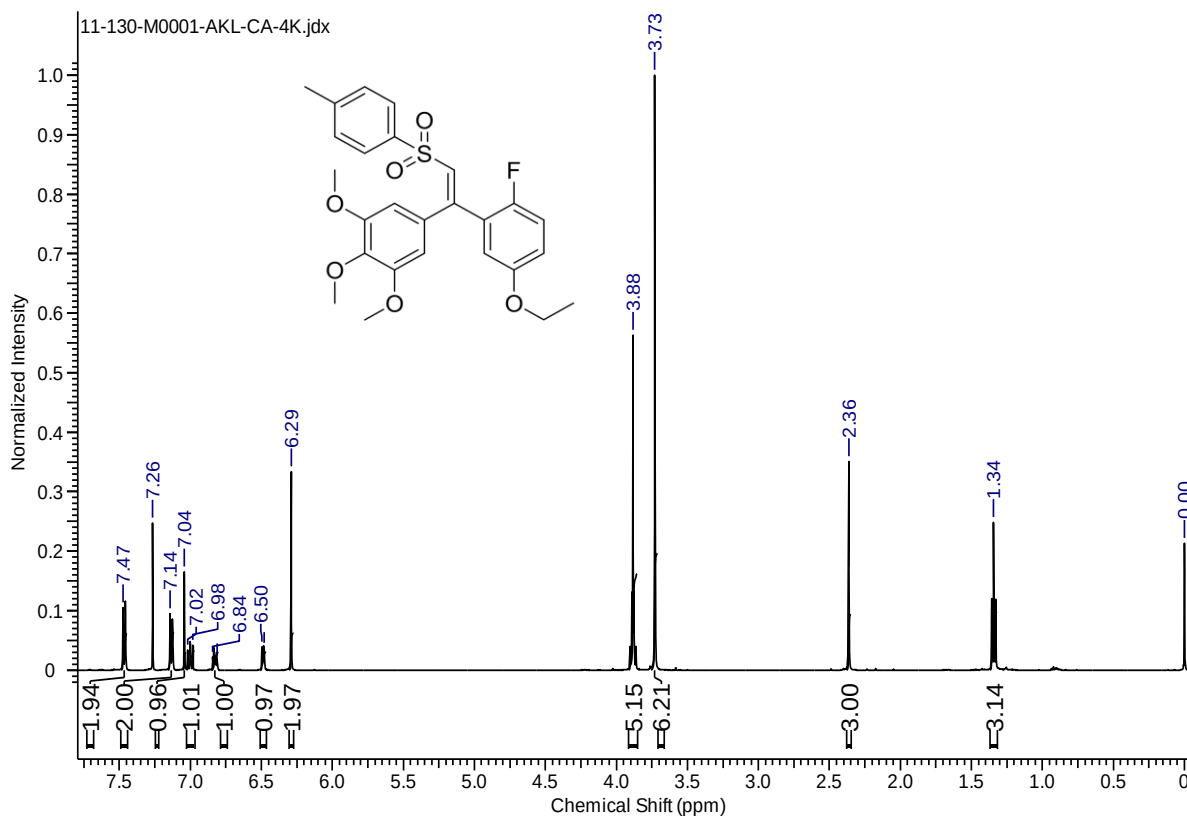
12. AKL-CA-4I, C₂₄H₂₂F₂O₅S, (E)-5-(1-(2,4-difluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



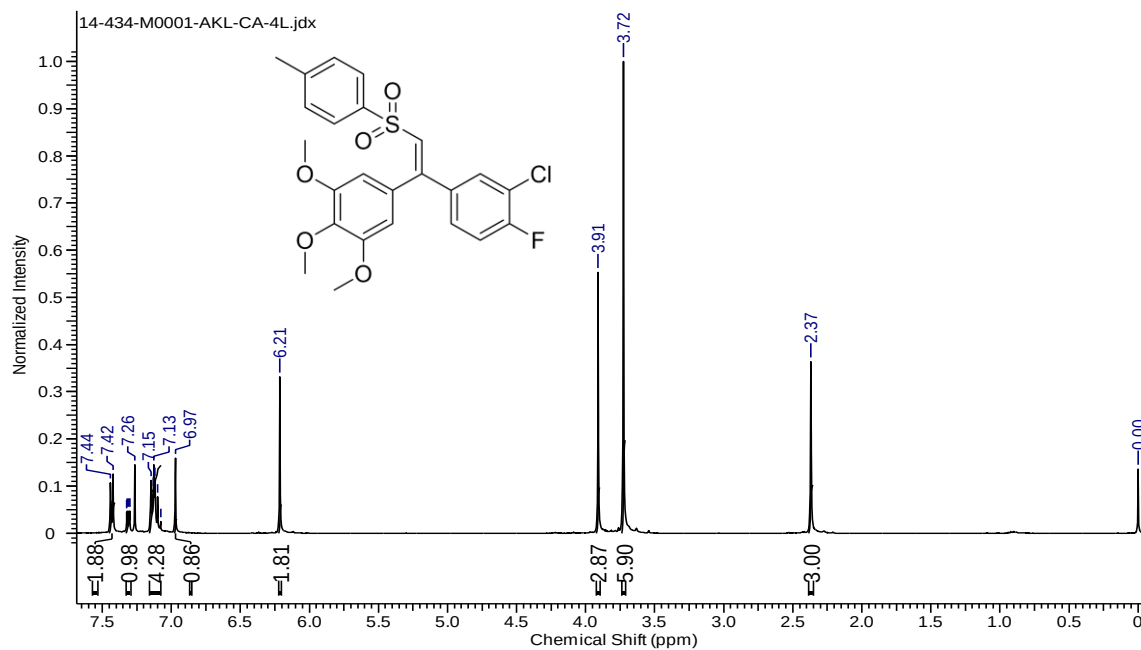
13. AKL-CA-4J, C₂₄H₂₂F₂O₅S, (E)-5-(1-(3,4-difluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



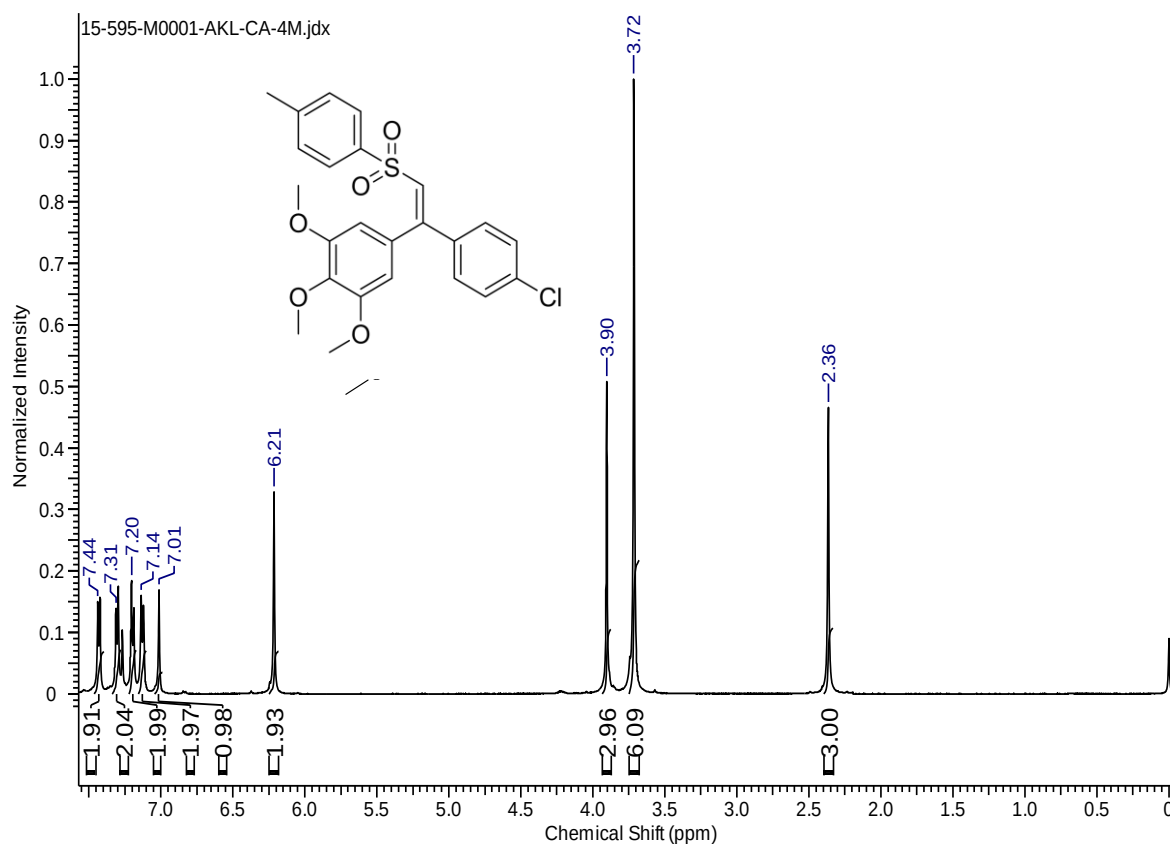
14. AKL-CA-4K, C₂₆H₂₇FO₆S, (E)-5-(1-(5-ethoxy-2-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



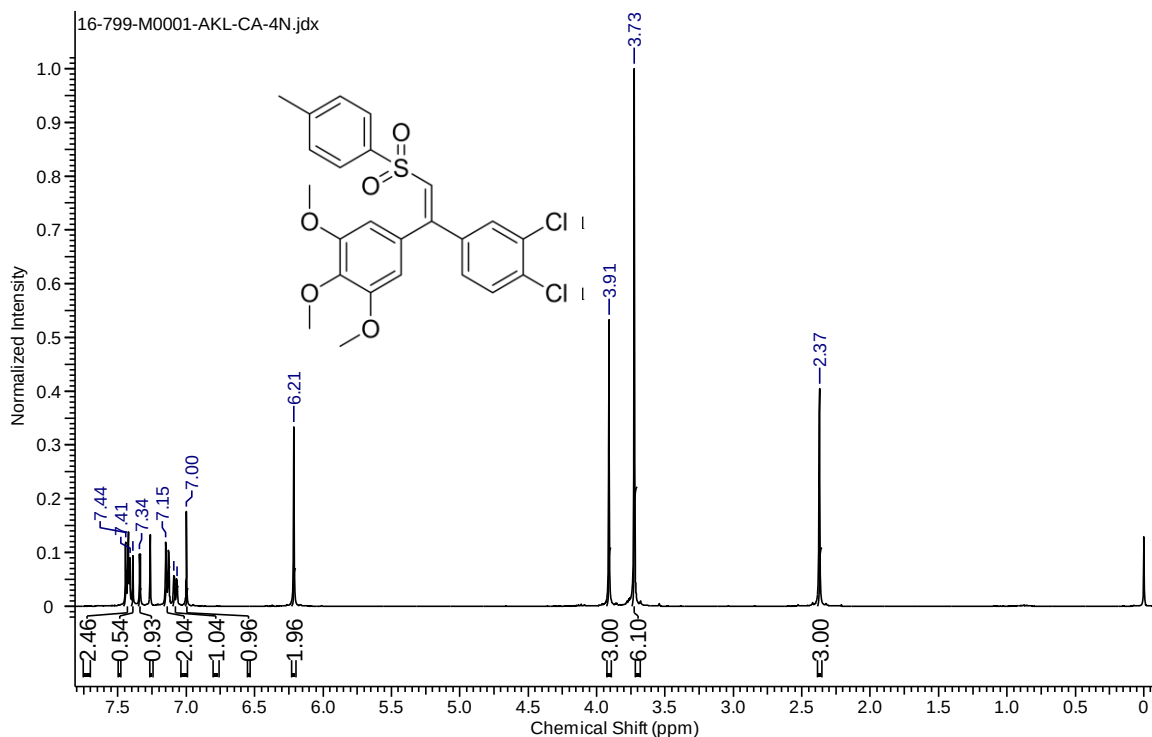
15. AKL-CA-4L, C₂₄H₂₂ClFO₅S, (E)-5-(1-(3-chloro-4-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



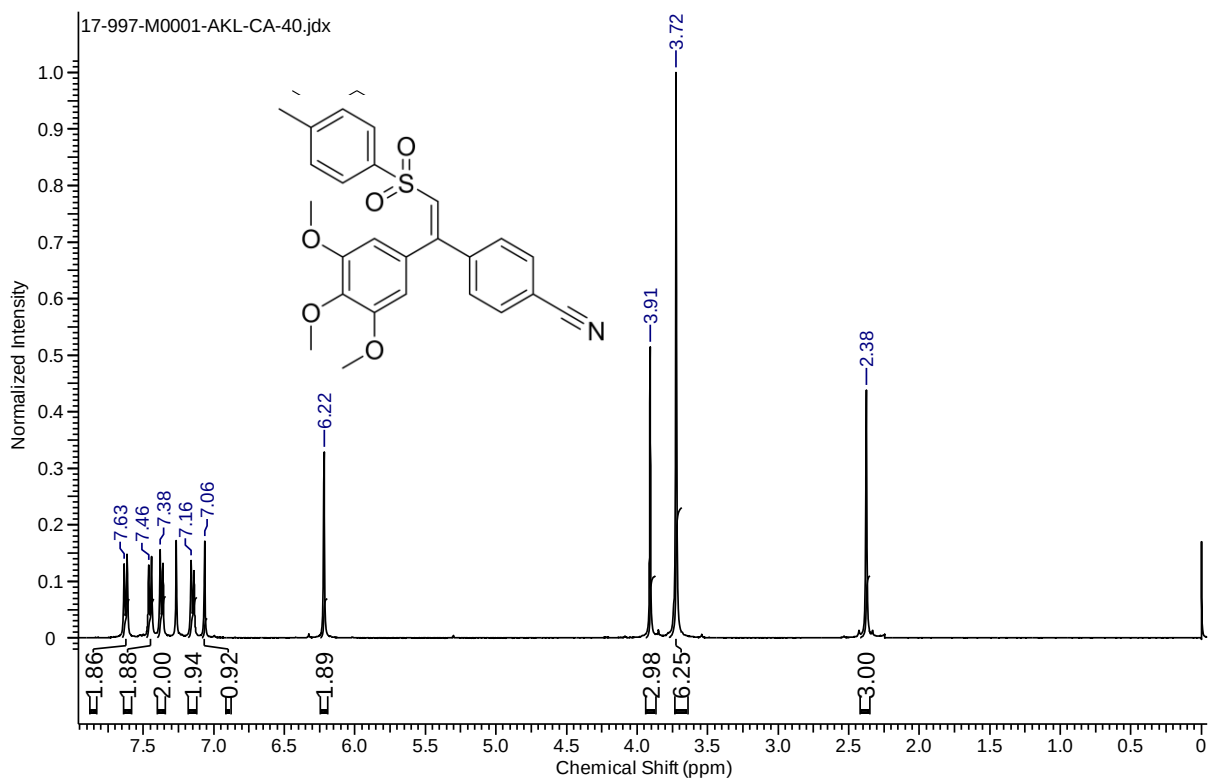
16. AKL-CA-4M, C₂₄H₂₃ClO₅S, (Z)-5-(1-(4-chlorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



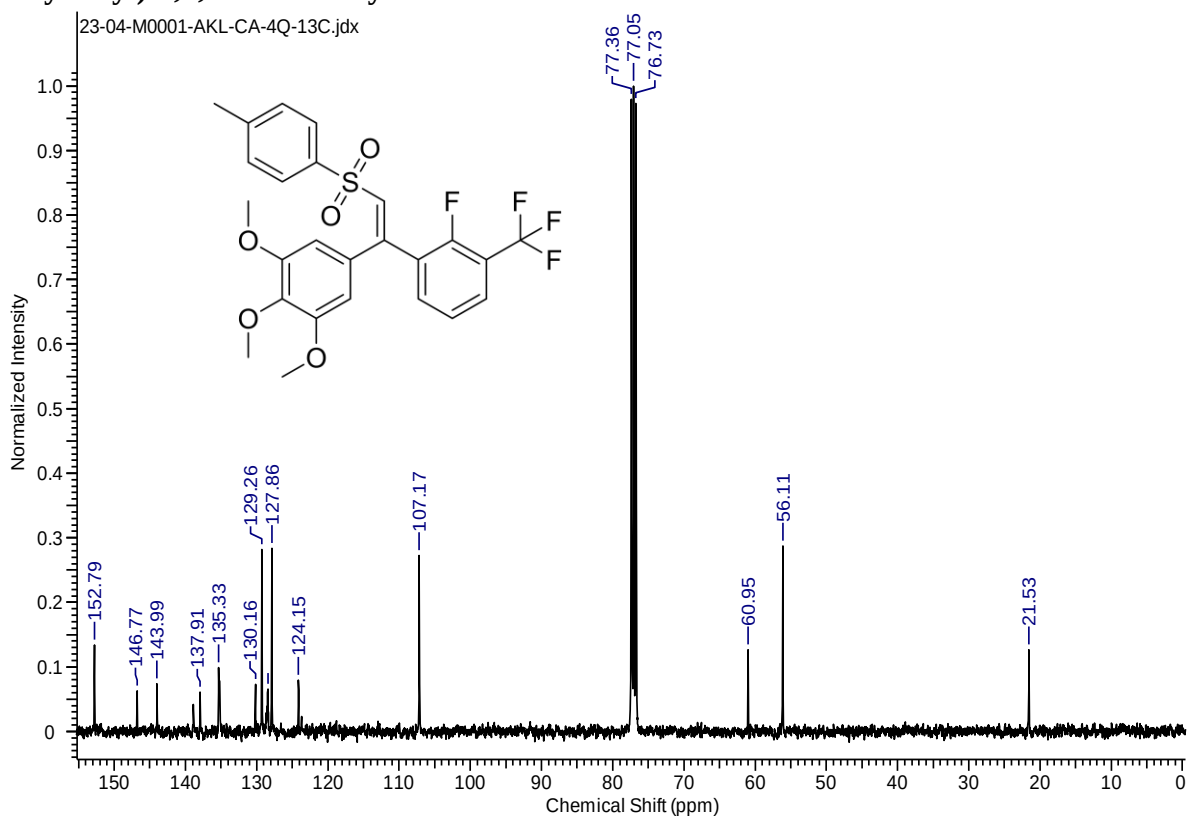
17. AKL-CA-4N, C₂₄H₂₂Cl₂O₅S, (E)-5-(1-(3,4-dichlorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



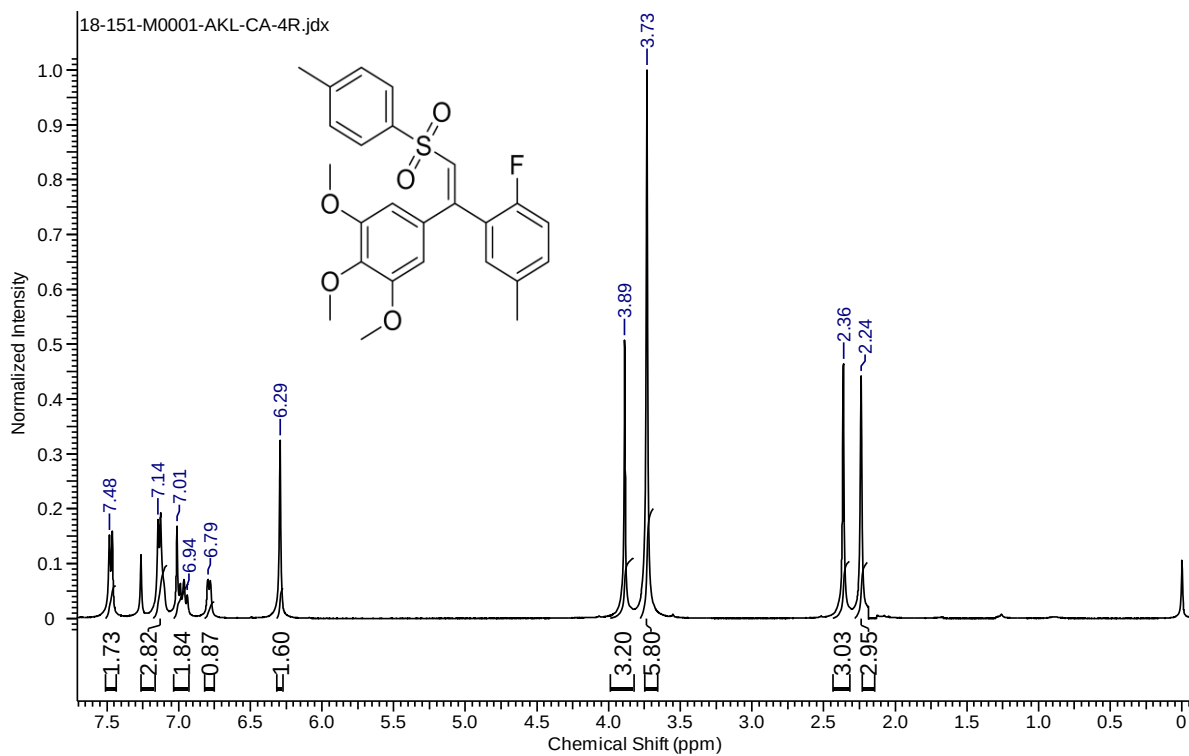
18. AKL-CA-4O, C₂₅H₂₃NO₅S, (Z)-4-(2-tosyl-1-(3,4,5-trimethoxyphenyl)vinyl)benzonitrile



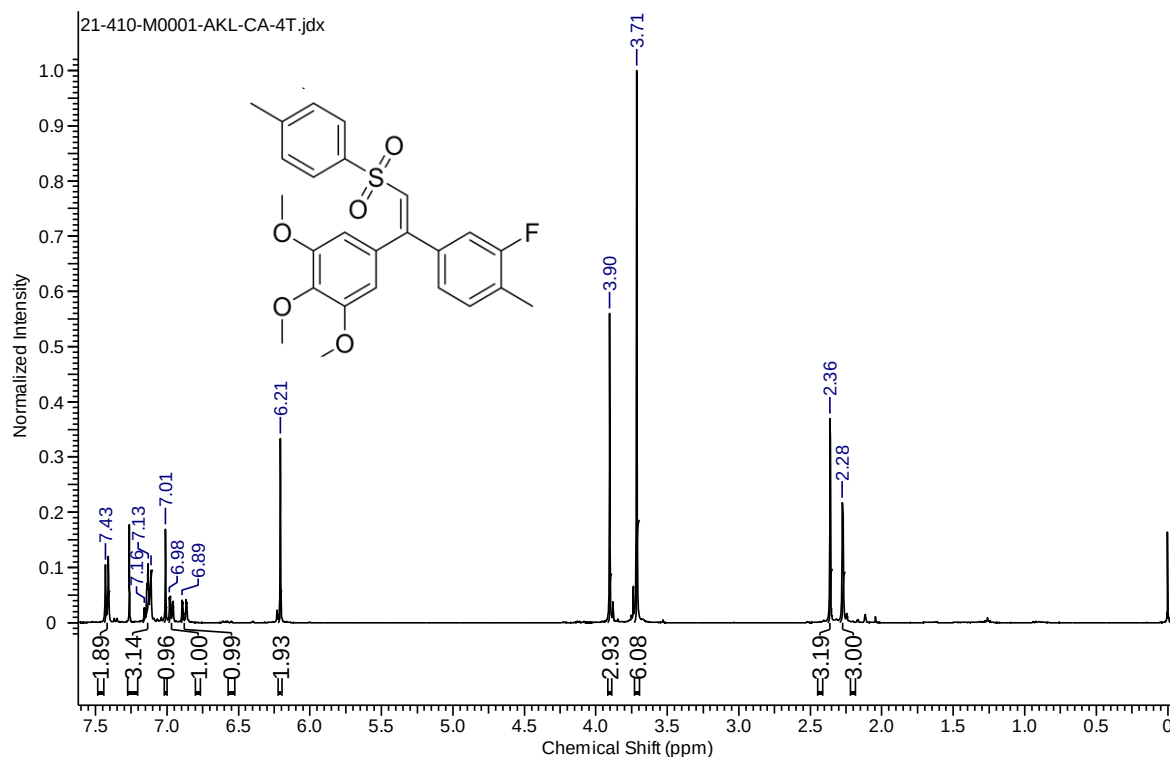
19. AKL-CA-4Q, C₂₅H₂₂F₄O₅S, (E)-5-(1-(2-fluoro-3-(trifluoromethyl)phenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



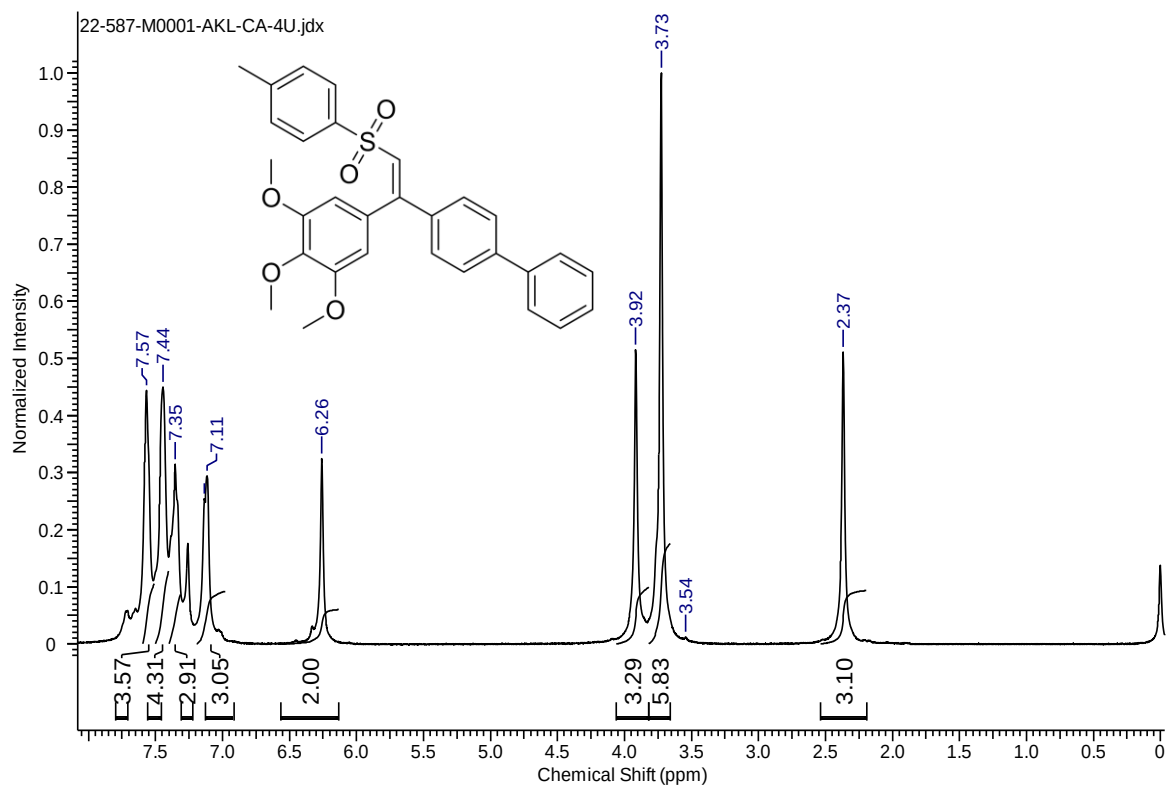
20. AKL-CA-4R, C₂₅H₂₅FO₅S, (E)-5-(1-(2-fluoro-5-methylphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



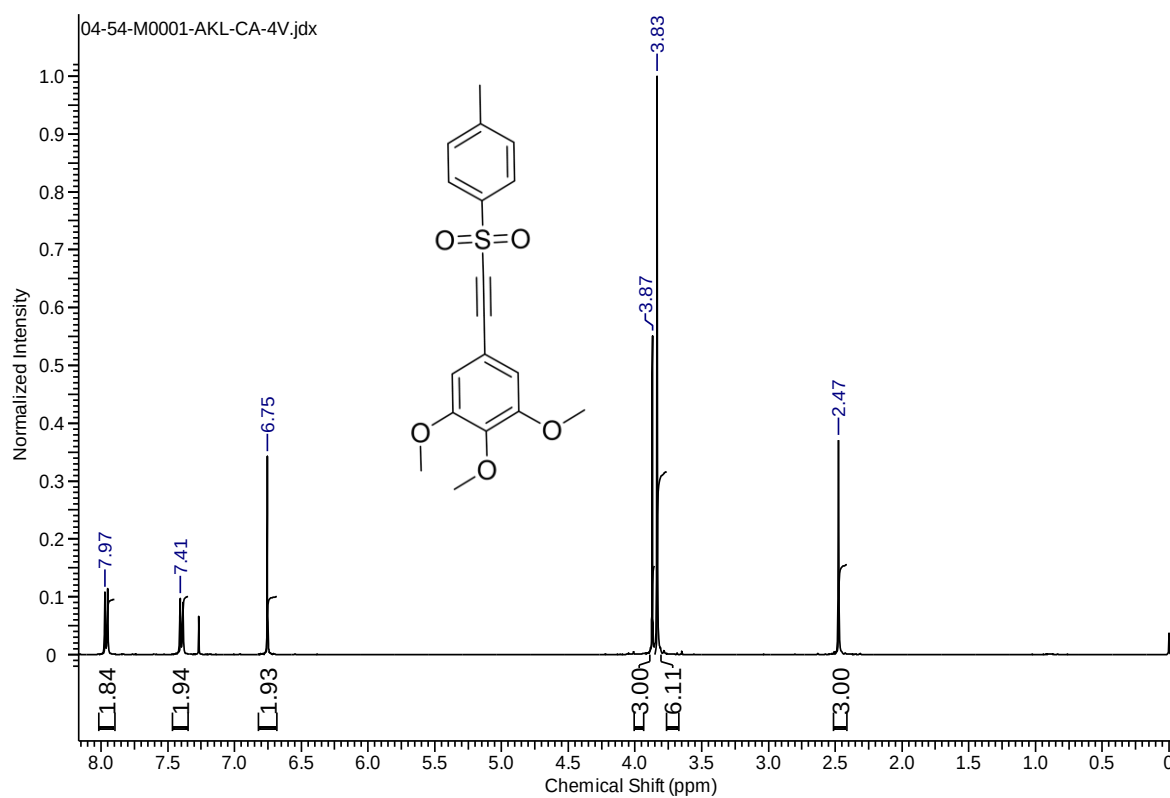
21. AKL-CA-4T, C₂₅H₂₅FO₅S, (E)-5-(1-(3-fluoro-4-methylphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



22. AKL-CA-4U, C₃₀H₂₈O₅S, (Z)-4-(2-tosyl-1-(3,4,5-trimethoxyphenyl)vinyl)-1,1'-biphenyl

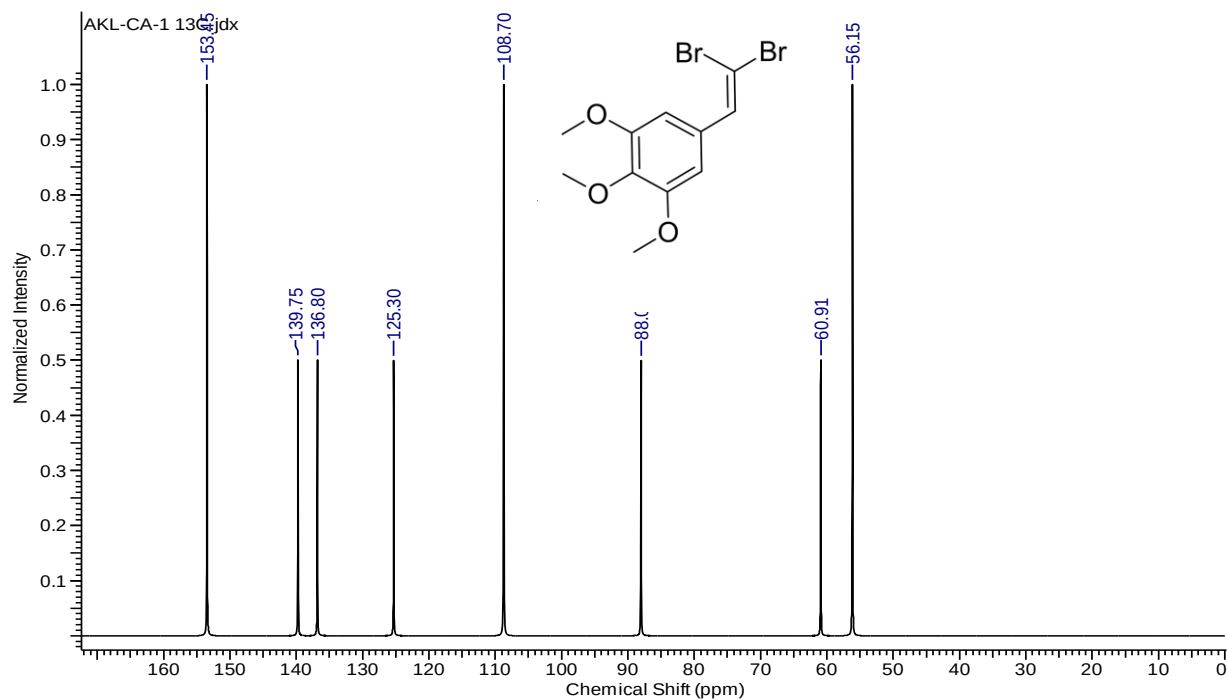


23. AKL-CA-4V, C₁₈H₁₈O₅S, 4-methylphenyl (3,4,5-trimethoxyphenyl)ethynyl sulfone

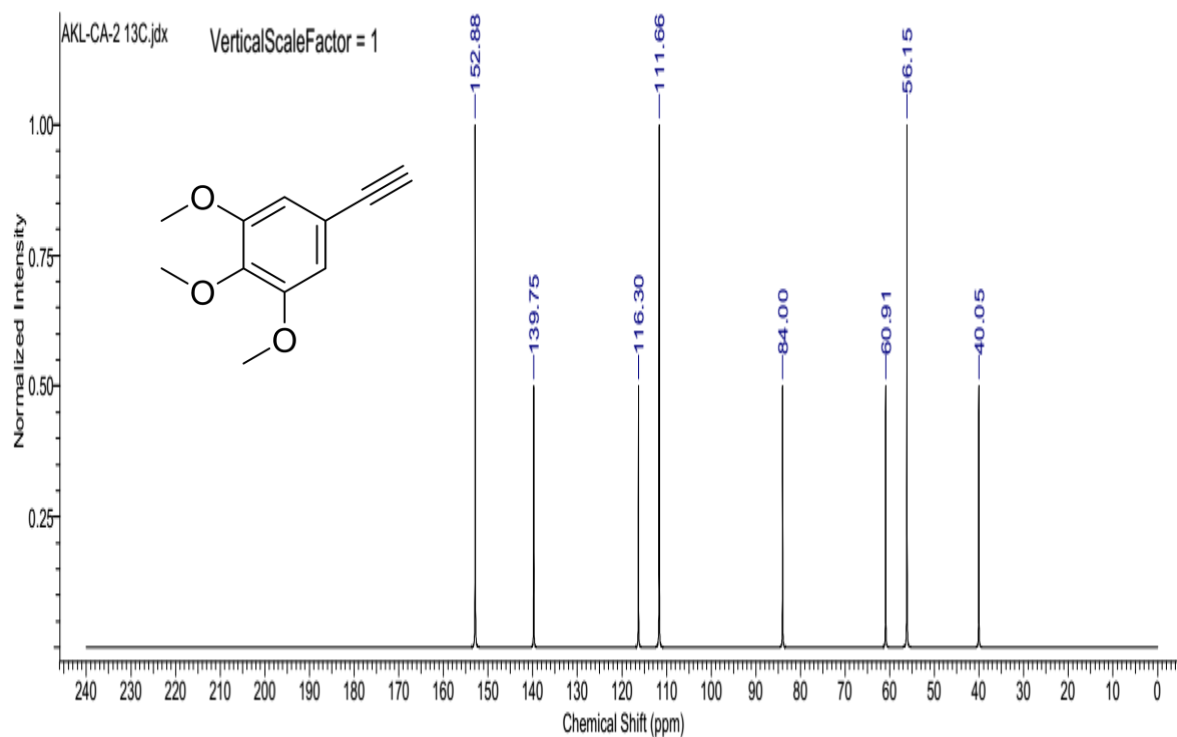


APPENDICES 2: ¹³C NMR SPECTRA OF SYNTHESIZED COMPOUNDS

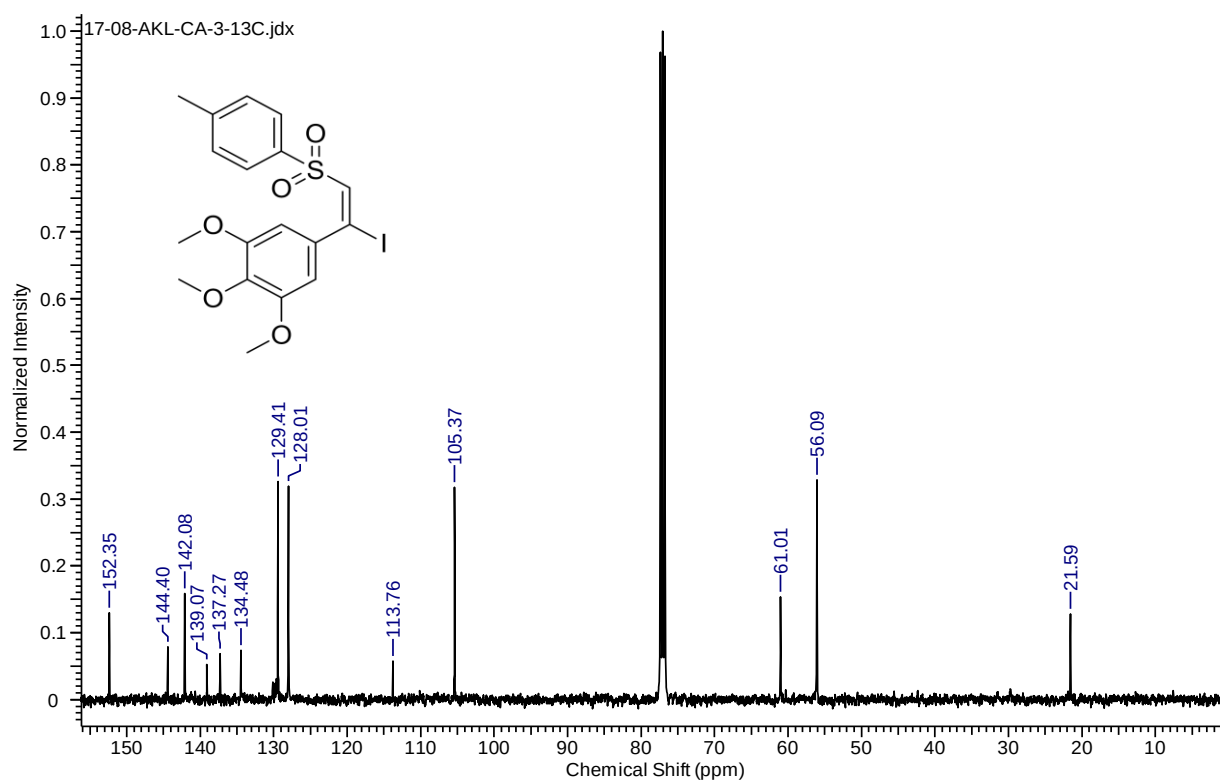
1. AKL-CA-1, C₁₁H₁₂Br₂O₃; 5-(2,2-dibromovinyl)-1,2,3-trimethoxybenzene



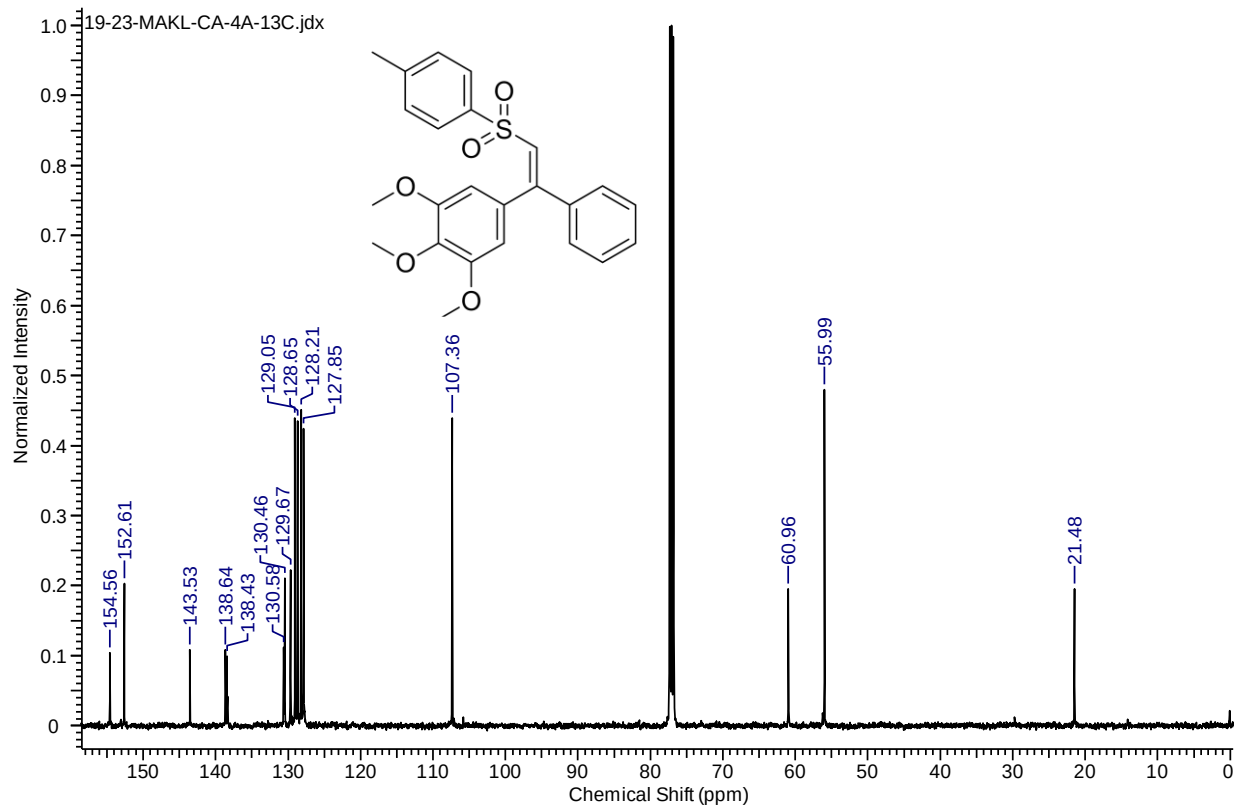
2. AKL-CA-2, C₁₁H₁₂O₃, 5-ethynyl-1,2,3-trimethoxybenzene



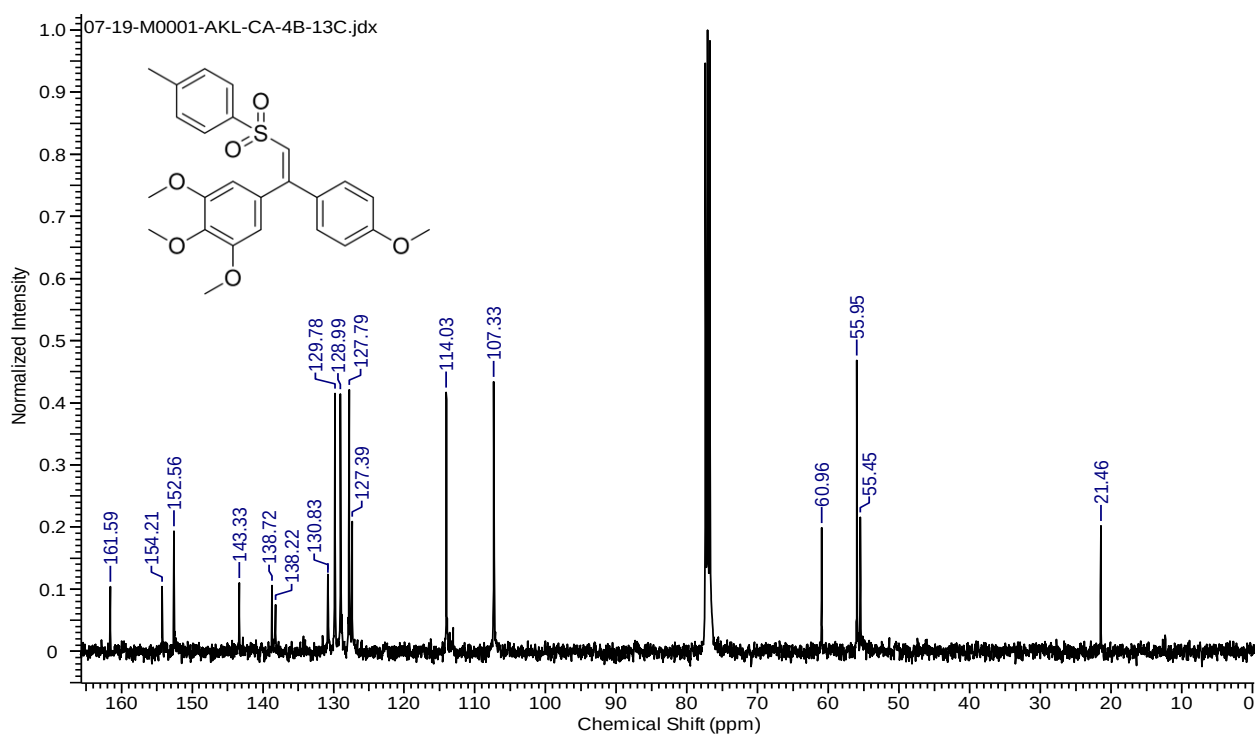
3. AKL-CA-3, C₁₈H₁₉IO₅S, (E)-5-(1-iodo-2-tosylvinyl)-1,2,3-trimethoxybenzene



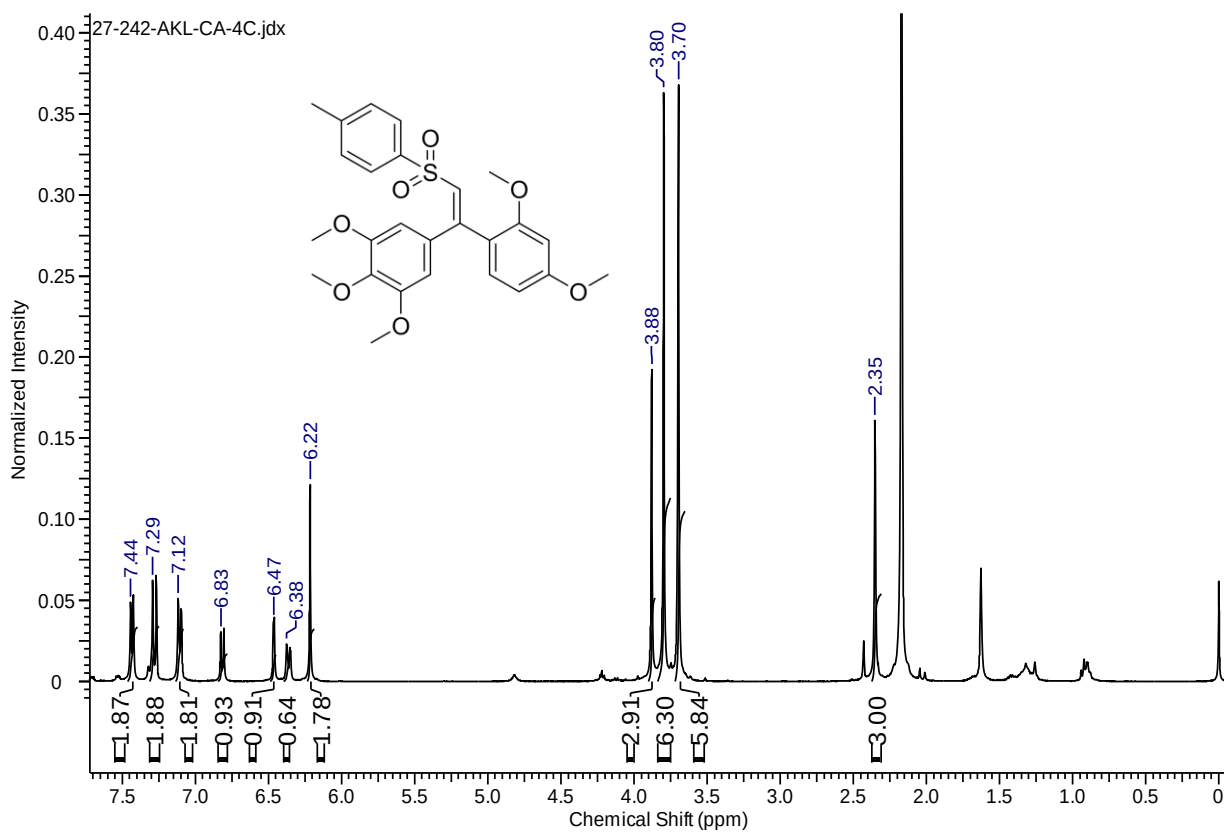
4. AKL-CA-4A, C₂₄H₂₄O₅S, (Z)-1,2,3-trimethoxy-5-(1-phenyl-2-tosylvinyl)benzene



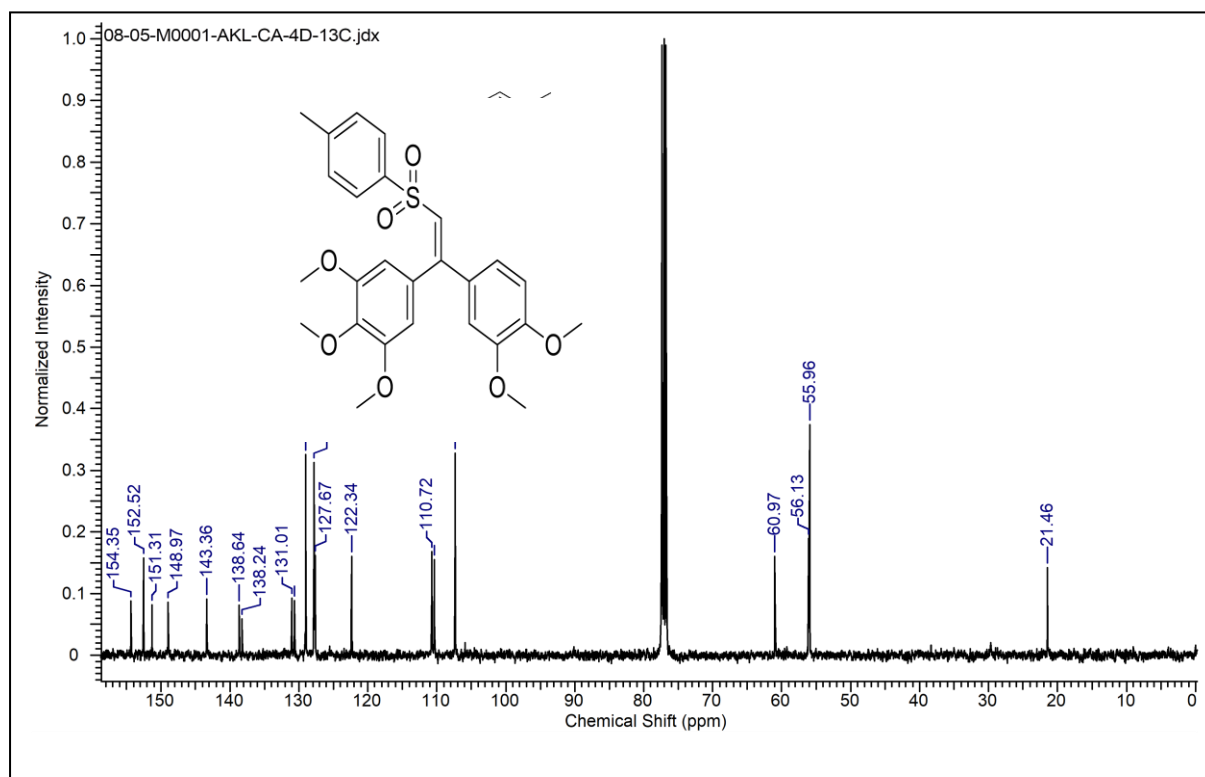
5. AKL-CA-4B, (Z)-1,2,3-trimethoxy-5-(1-(4-methoxyphenyl)-2-tosylvinyl)benzene



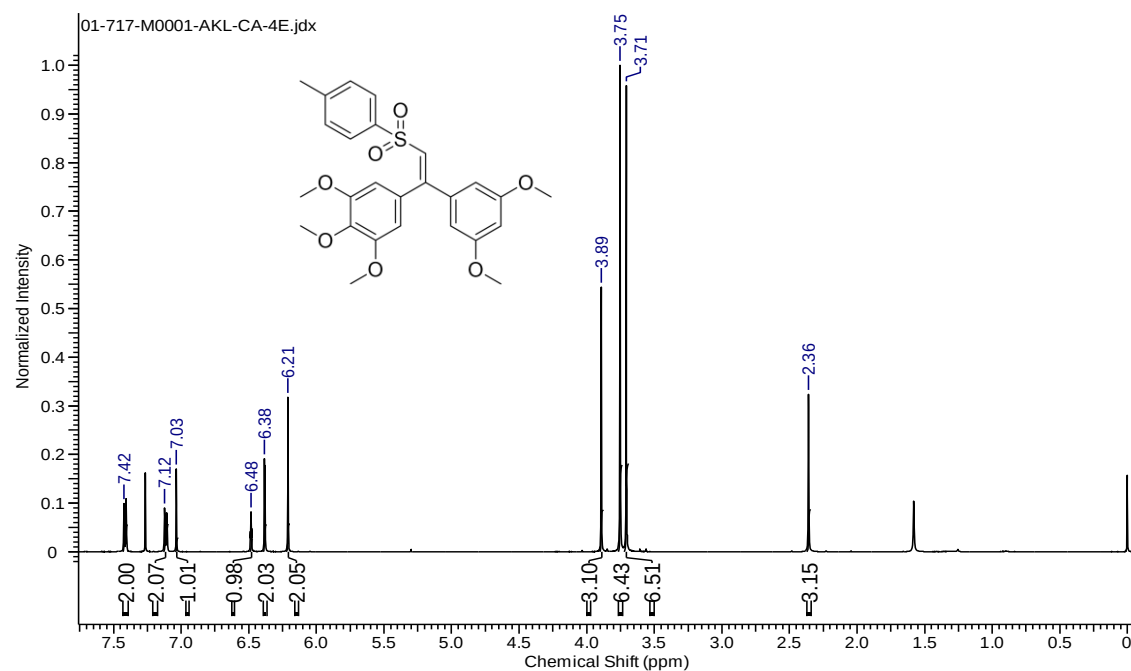
6. AKL-CA-4C, C₂₆H₂₈O₇S, (E)-5-(1-(2,4-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



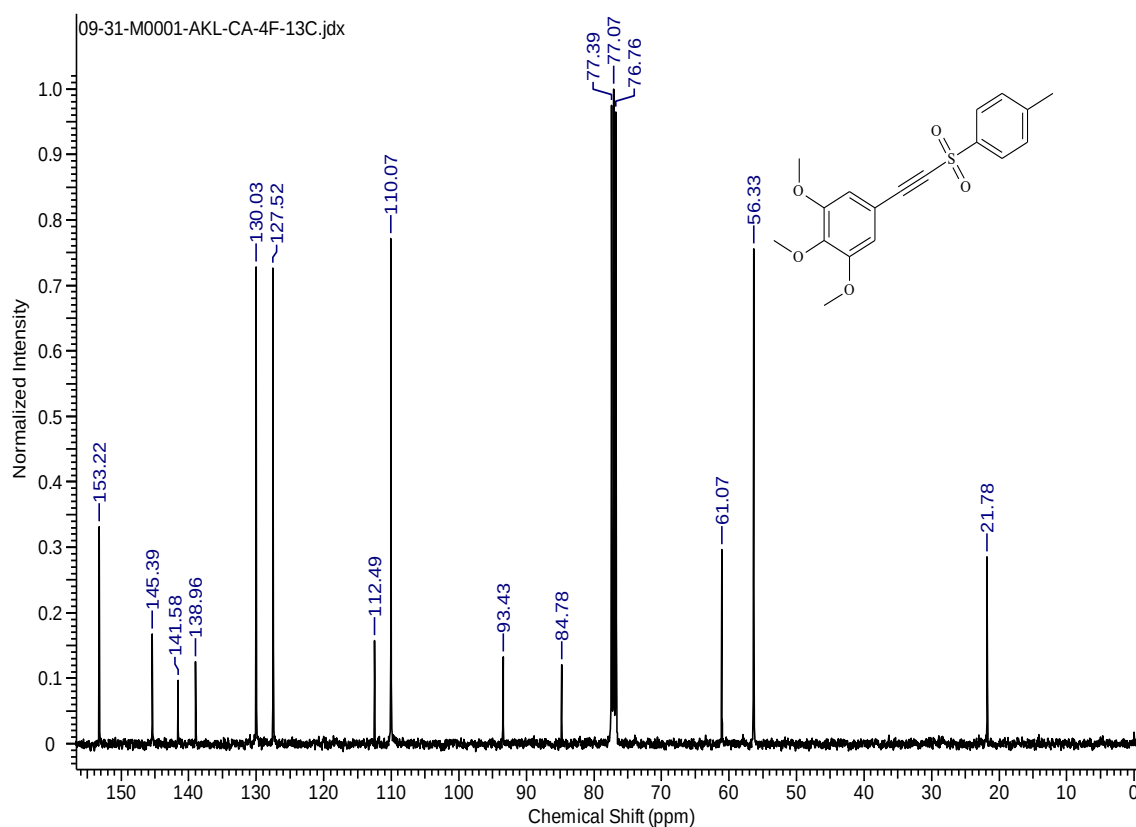
7. AKL-CA-4D, C₂₆H₂₈O₇S, (Z)-5-(1-(3,4-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



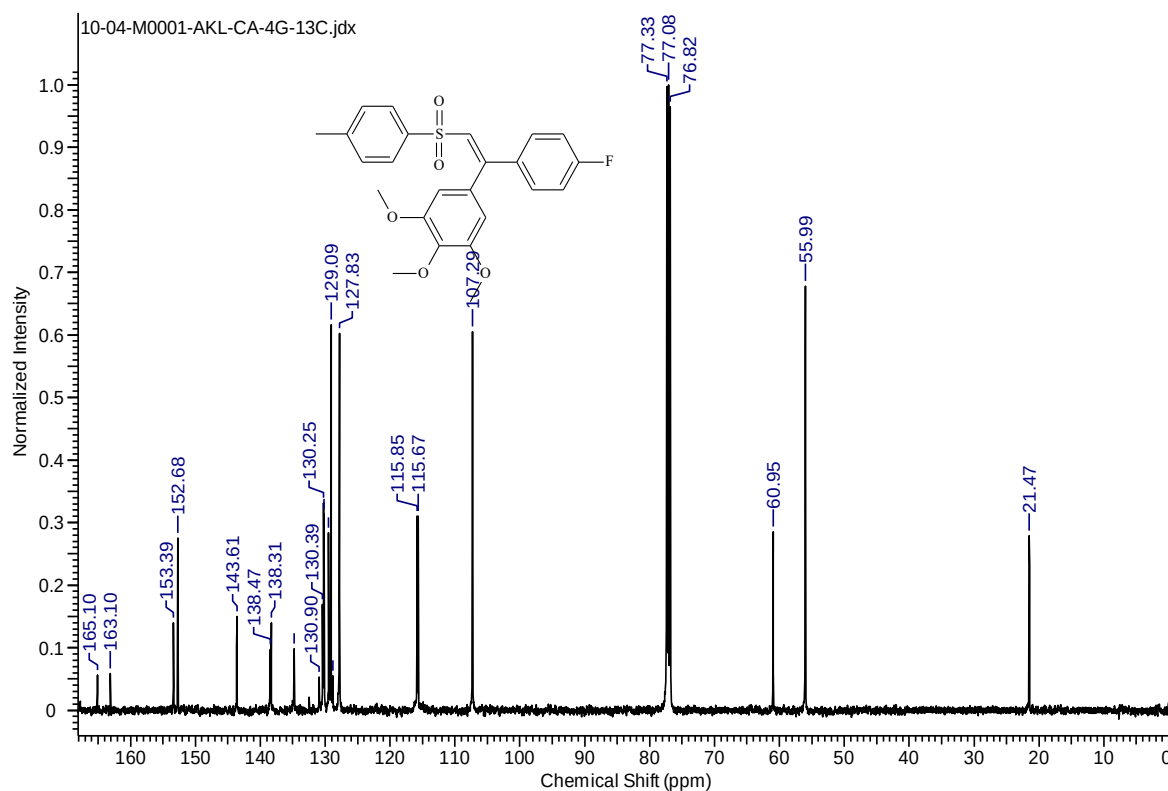
8. AKL-CA-4E, C₂₆H₂₈O₇S, (Z)-5-(1-(3,5-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



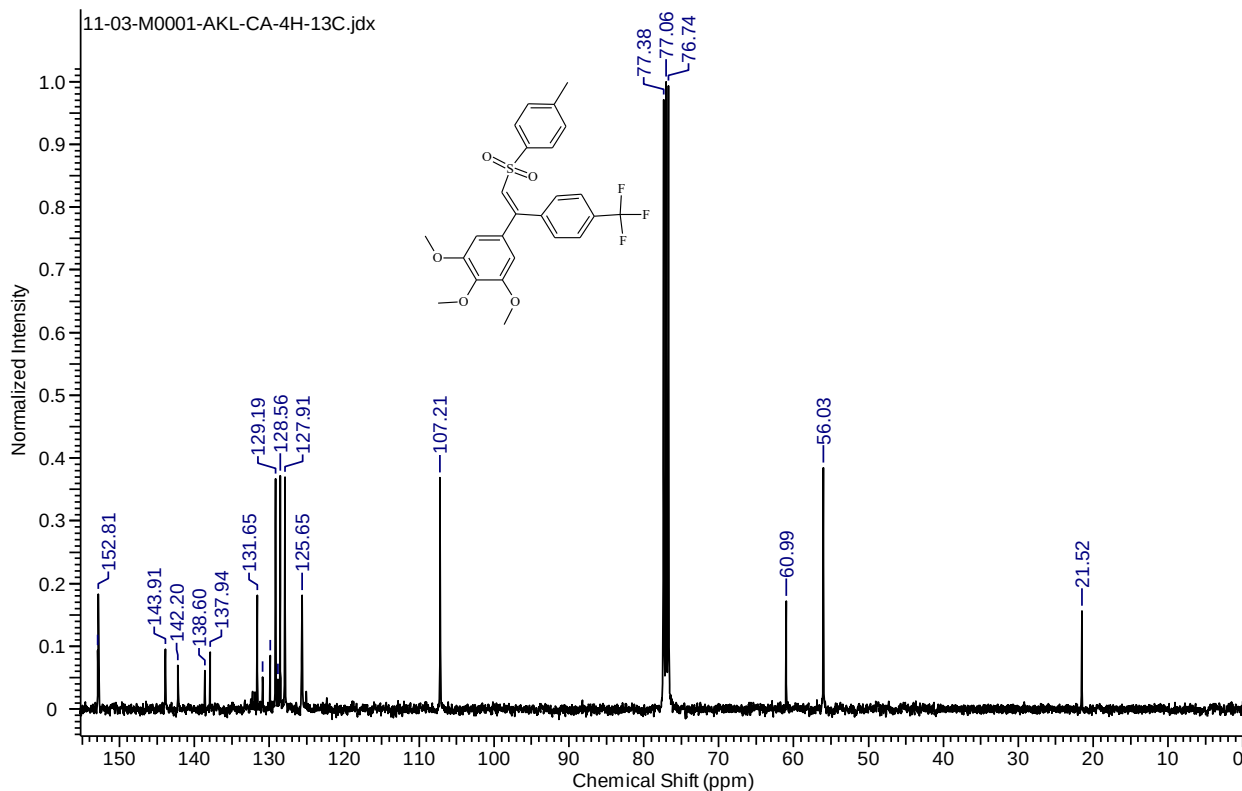
9. AKL-CA-4F, C₁₈H₁₈O₅S, 4-methylphenyl (3,4,5-trimethoxyphenyl)ethynyl sulfone



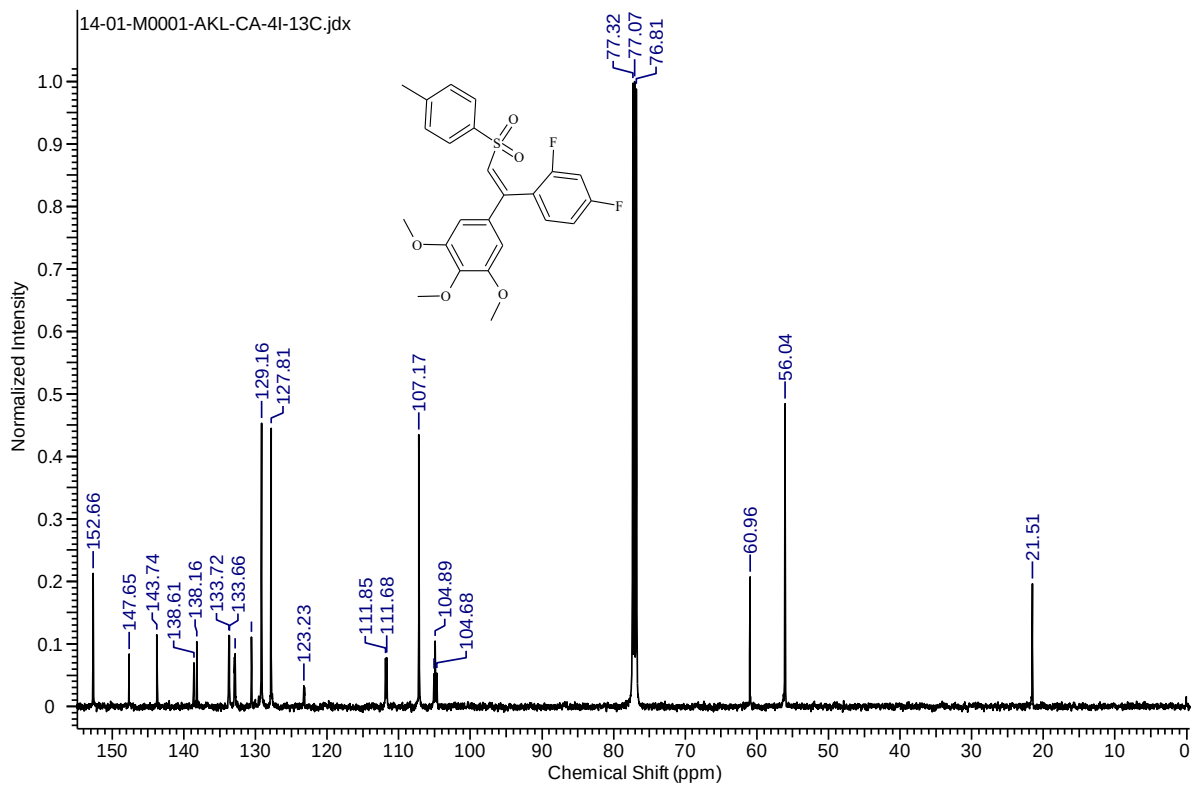
10. AKL-CA-4G, C₂₄H₂₃FO₅S, (Z)-5-(1-(4-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



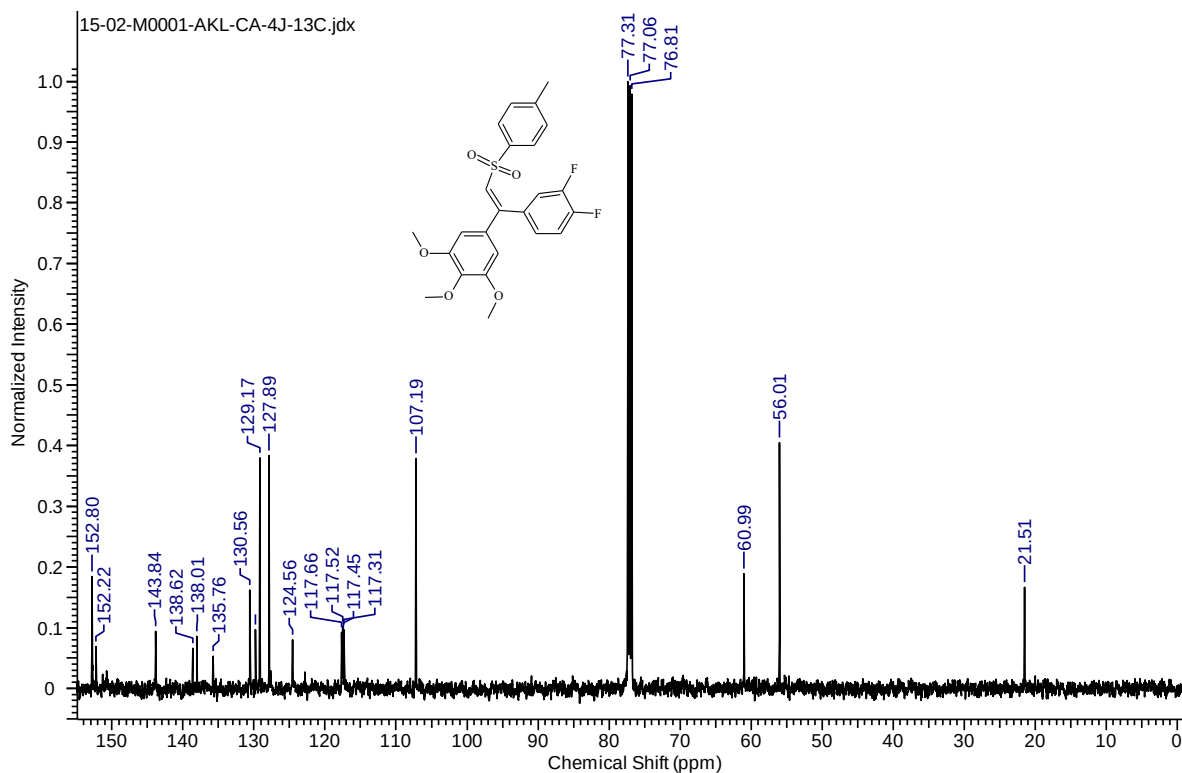
11. AKL-CA-4H, C₂₅H₂₃F₃O₅S, (Z)-1,2,3-trimethoxy-5-(2-tosyl-1-(4-(trifluoromethyl)phenyl)vinyl)benzene



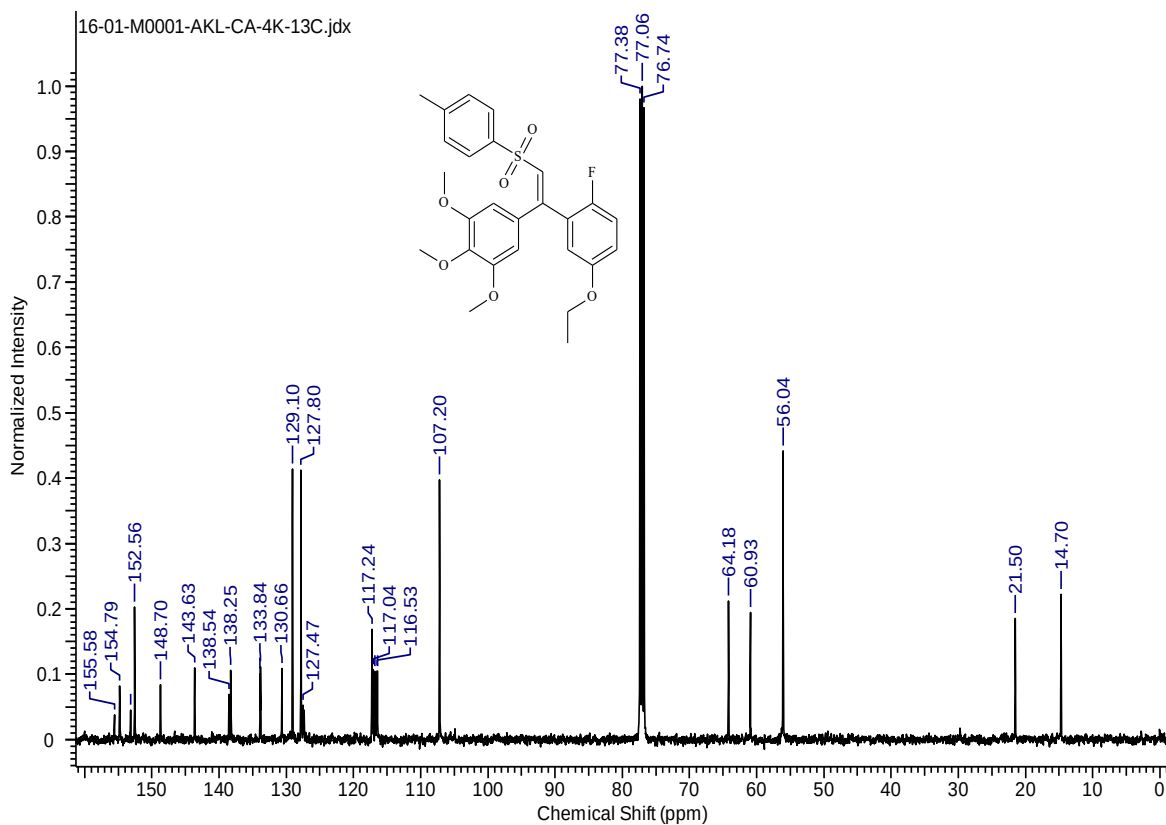
12. AKL-CA-4I, C₂₄H₂₂F₂O₅S, (E)-5-(1-(2,4-difluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



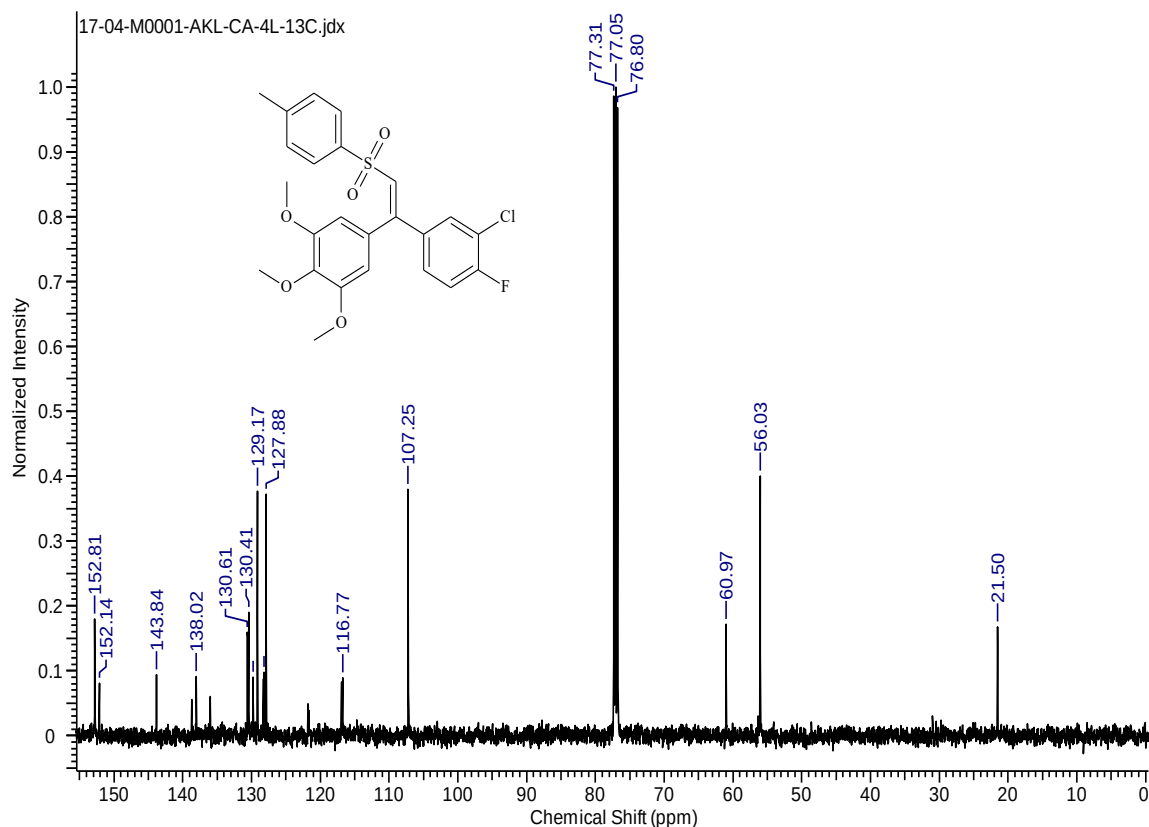
13. AKL-CA-4J, C₂₄H₂₂F₂O₅S, (E)-5-(1-(3,4-difluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



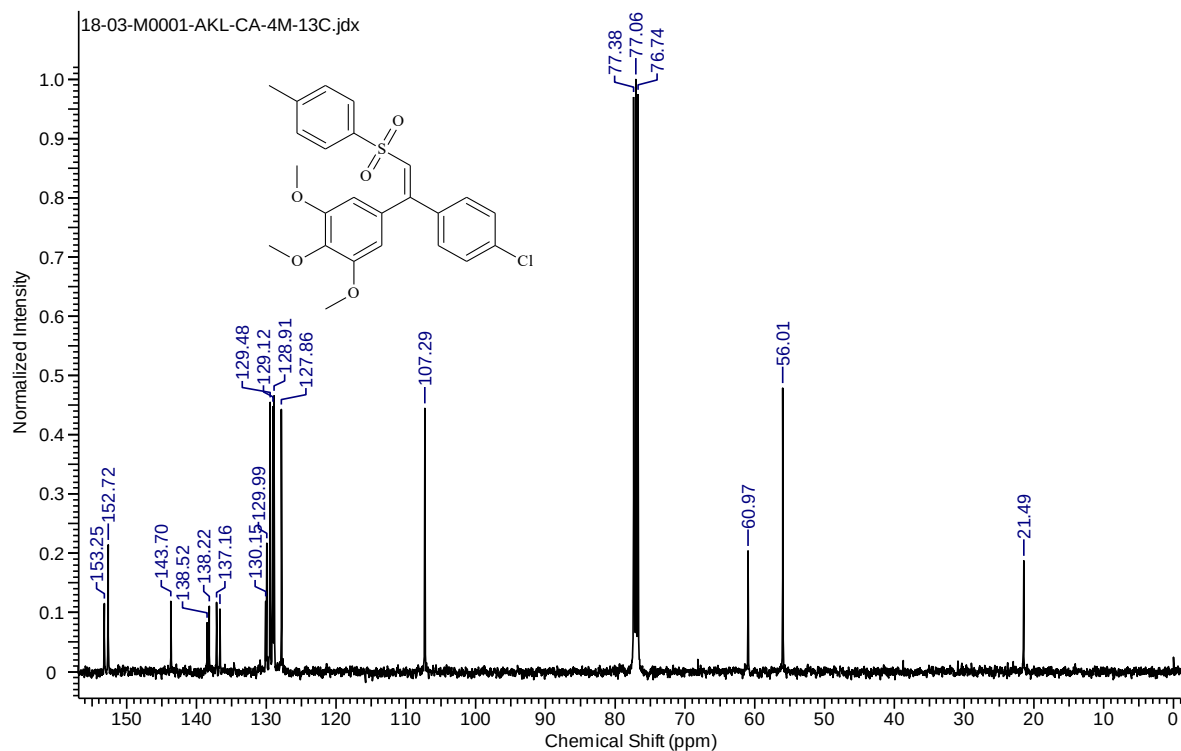
14. AKL-CA-4K, C₂₆H₂₇FO₆S, (E)-5-(1-(5-ethoxy-2-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



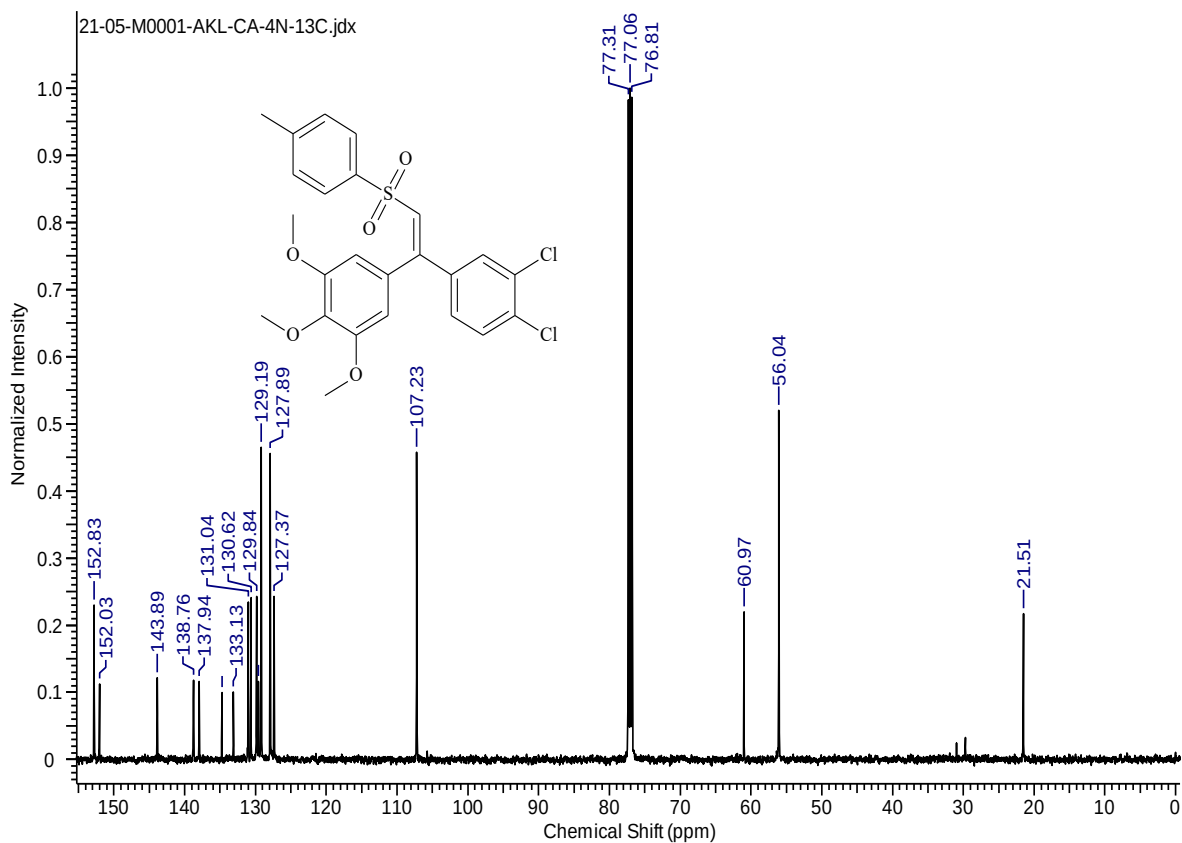
15. AKL-CA-4L, C₂₄H₂₂ClFO₅S, (E)-5-(1-(3-chloro-4-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



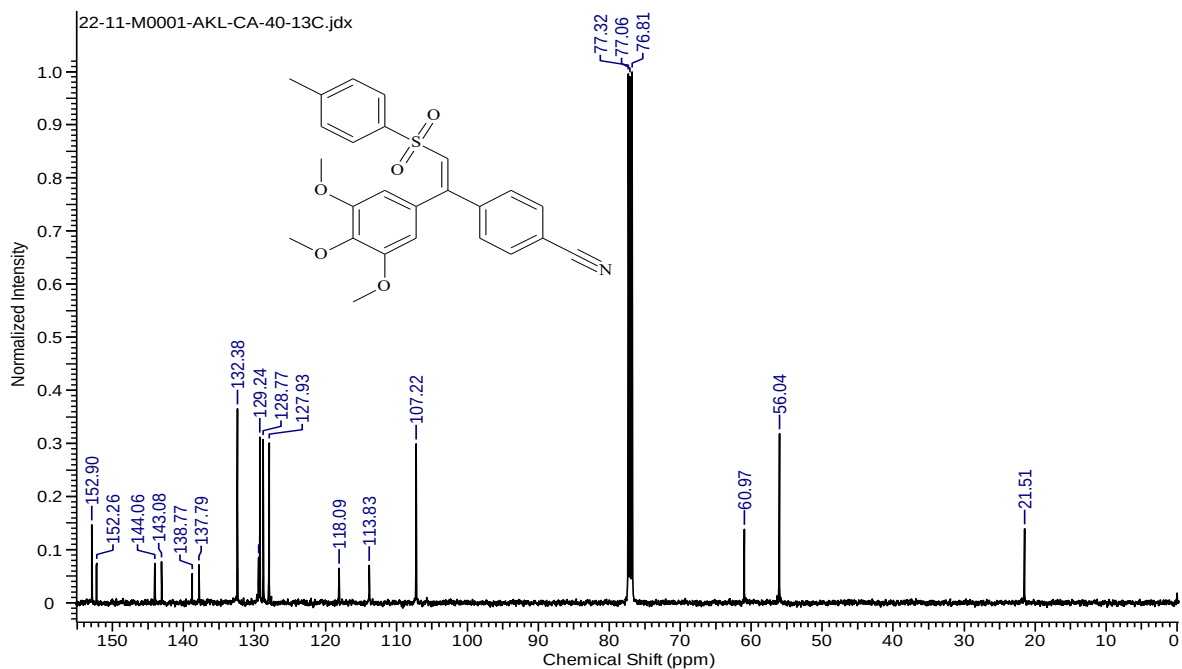
16. AKL-CA-4M, C₂₄H₂₃ClO₅S, (Z)-5-(1-(4-chlorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



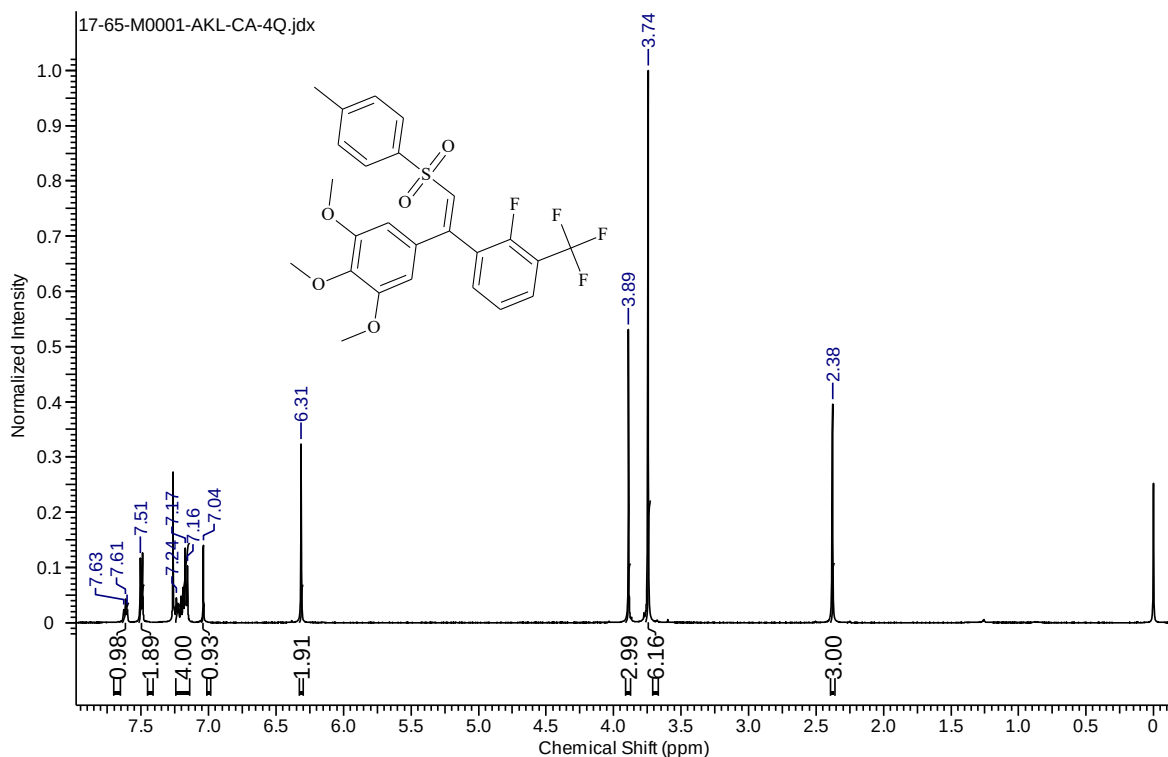
17. AKL-CA-4N, C₂₄H₂₂Cl₂O₅S, (E)-5-(1-(3,4-dichlorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



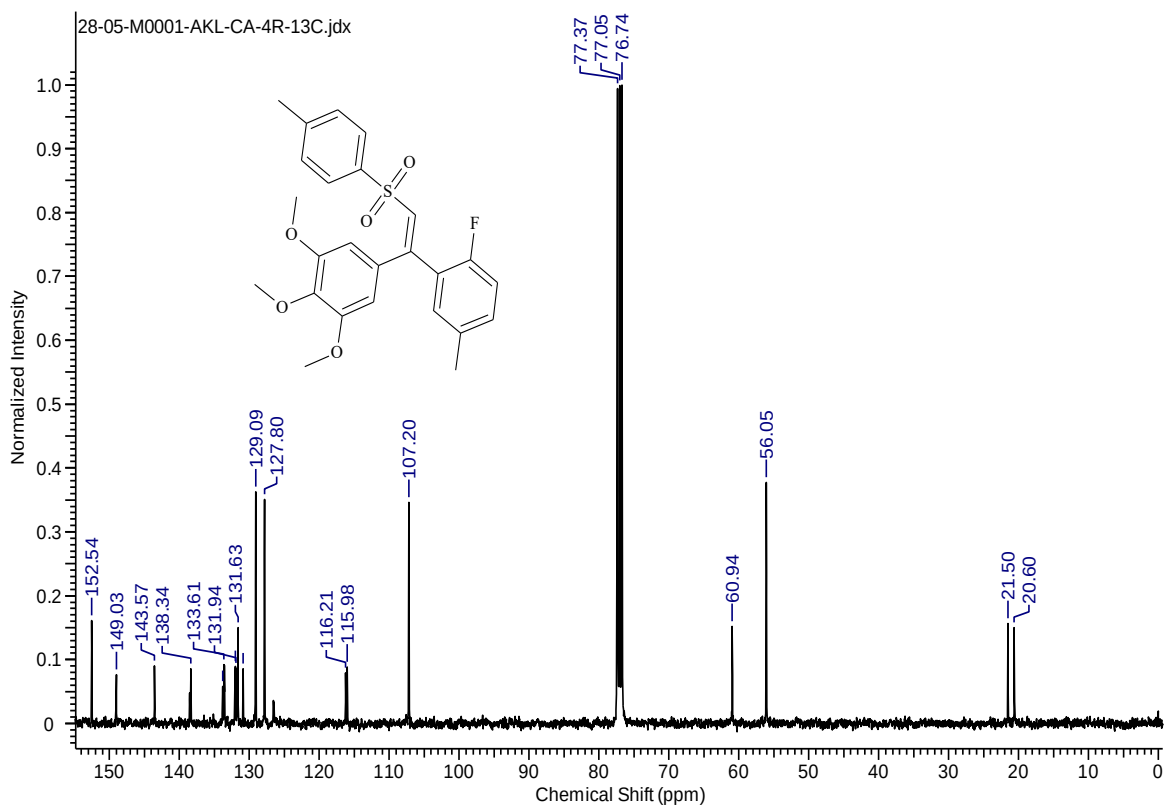
18. AKL-CA-40, C₂₅H₂₃NO₅S, (Z)-4-(2-tosyl-1-(3,4,5-trimethoxyphenyl)vinyl)benzonitrile



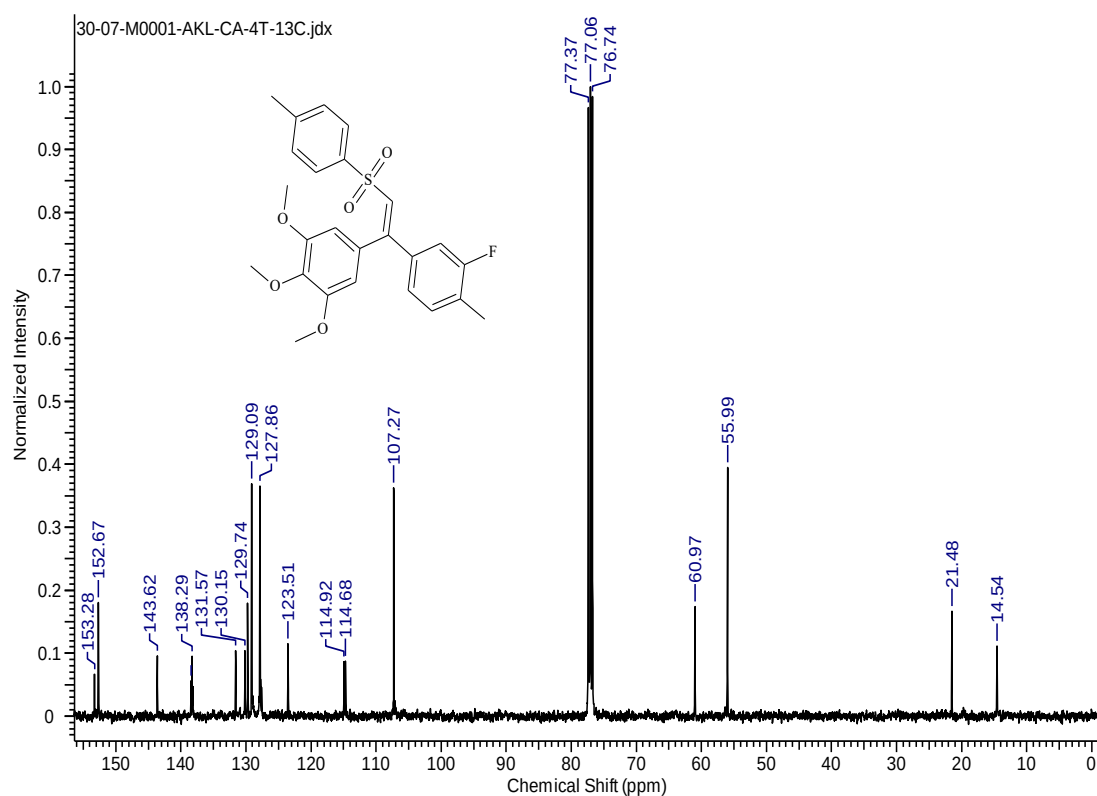
19. AKL-CA-4Q, C₂₅H₂₂F₄O₅S, (E)-5-(1-(2-fluoro-3-(trifluoromethyl)phenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



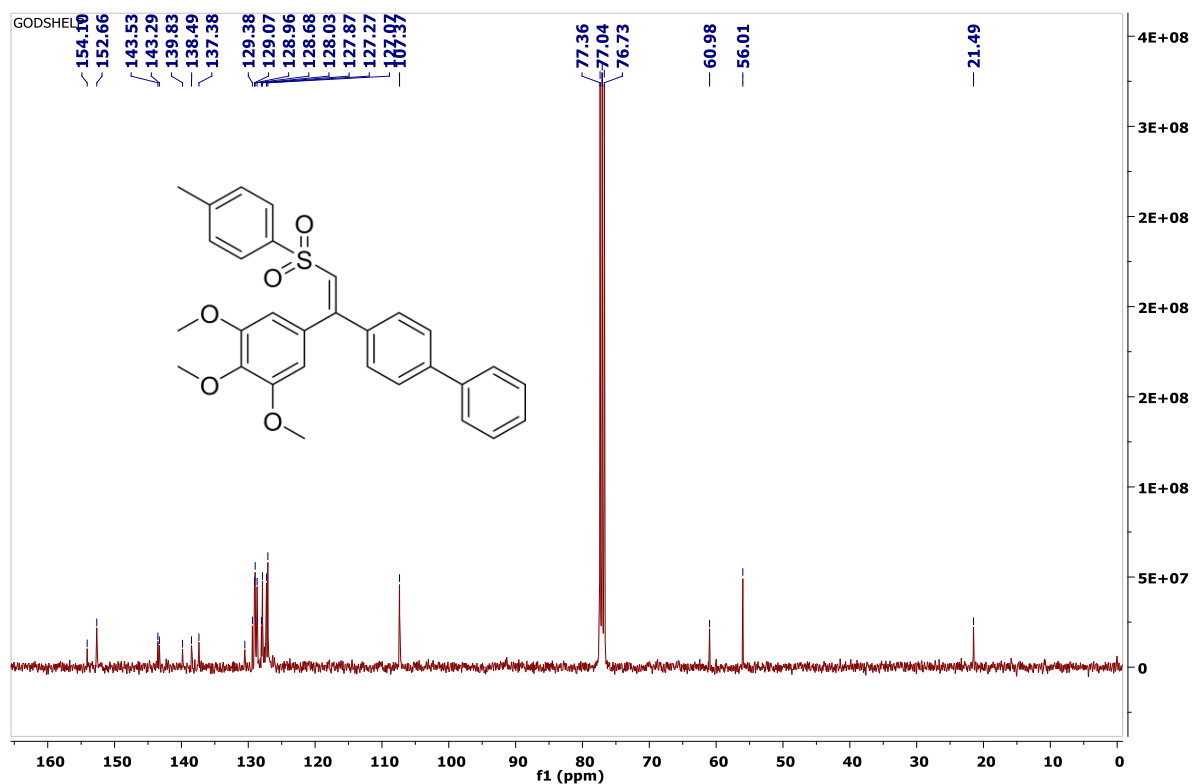
20. AKL-CA-4R, C₂₅H₂₅FO₅S, (E)-5-(1-(2-fluoro-5-methylphenyl)-2-tosylvinyl)-1,2,3-Trimethoxybenzene



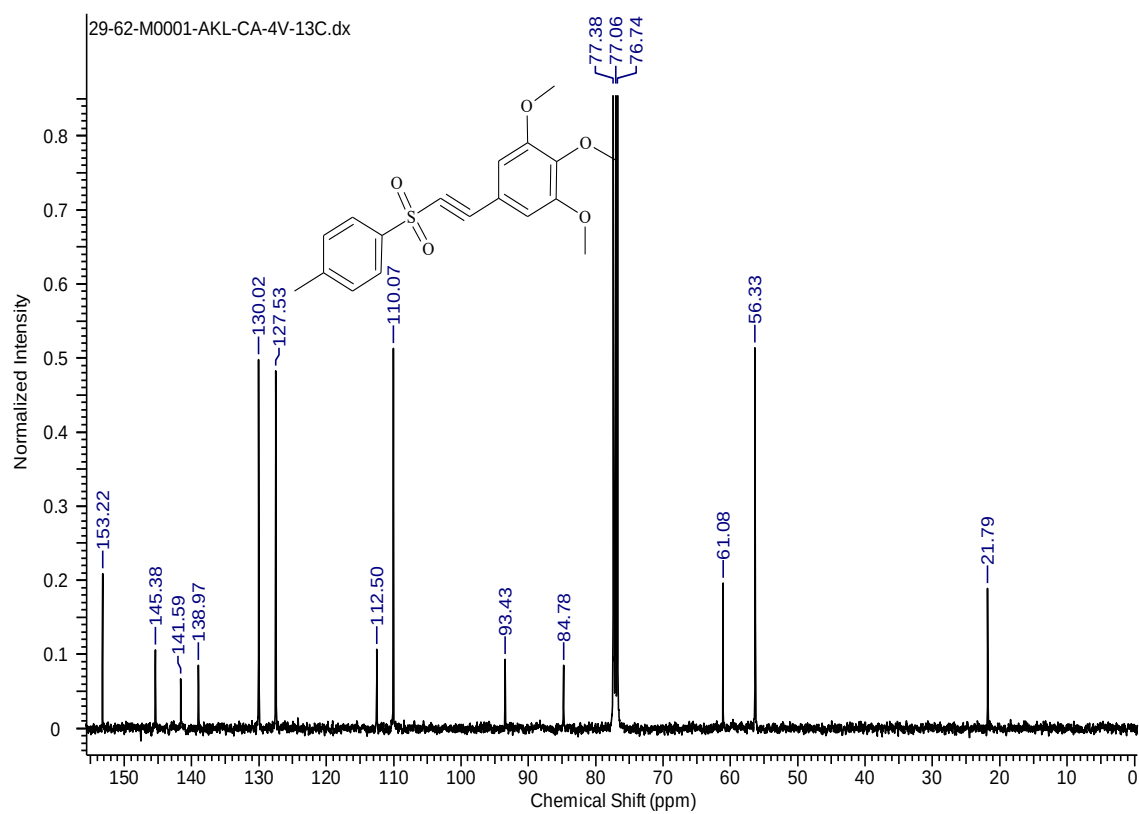
21. AKL-CA-4T, C₂₅H₂₅FO₅S, (E)-5-(1-(3-fluoro-4-methylphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



22. AKL-CA-4U, C₃₀H₂₈O₅S, (Z)-4-(2-tosyl-1-(3,4,5-trimethoxyphenyl)vinyl)-1,1'-biphenyl



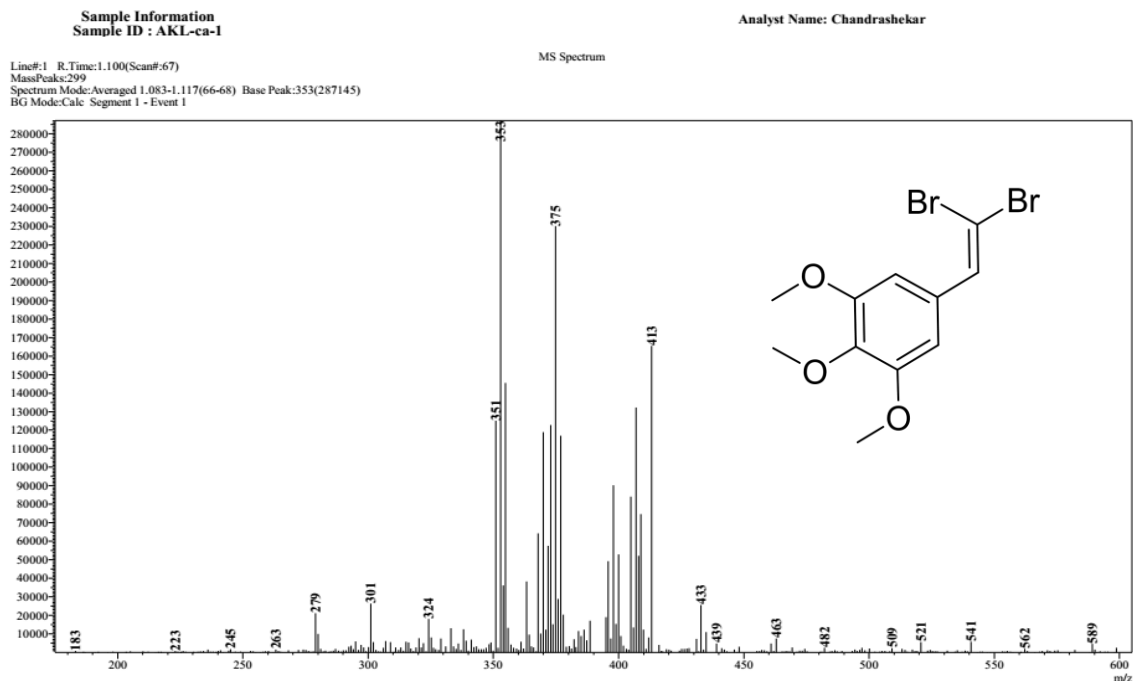
23. AKL-CA-4V, C₁₈H₁₈O₅S, 4-methylphenyl (3,4,5-trimethoxyphenyl)ethynyl sulfone



APPENDICES 3: MS SPECTRA OF THE SYNTHESIZED COMPOUNDS

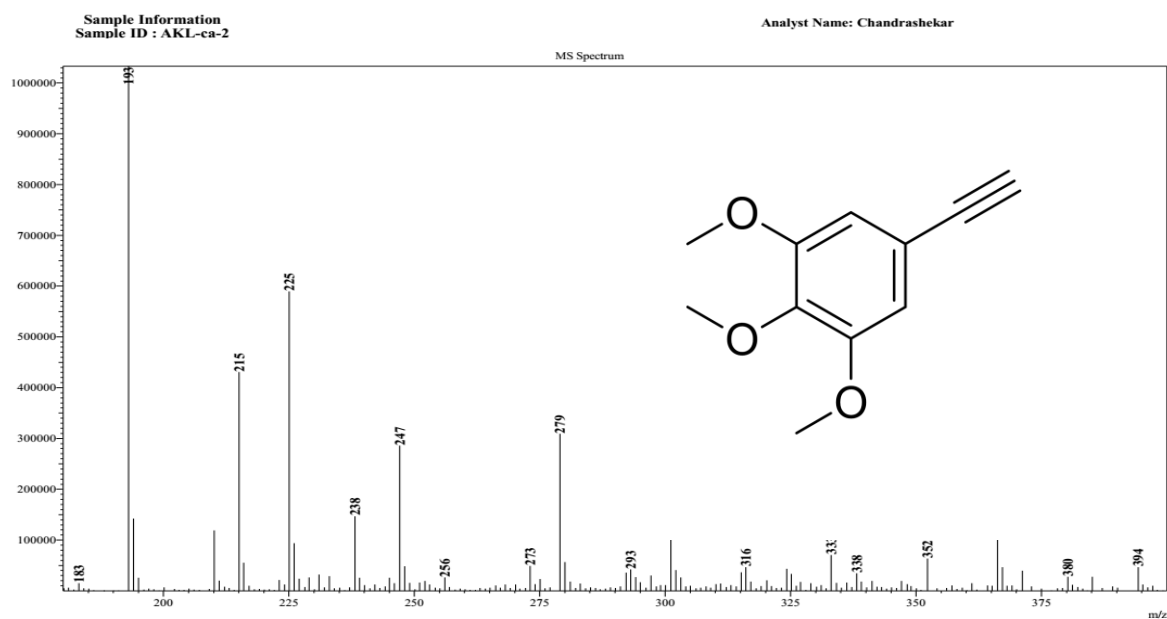
1. AKL-CA-1, C₁₁H₁₂Br₂O₃; 5-(2,2-dibromovinyl)-1,2,3-trimethoxybenzene

Biotransformation Lab



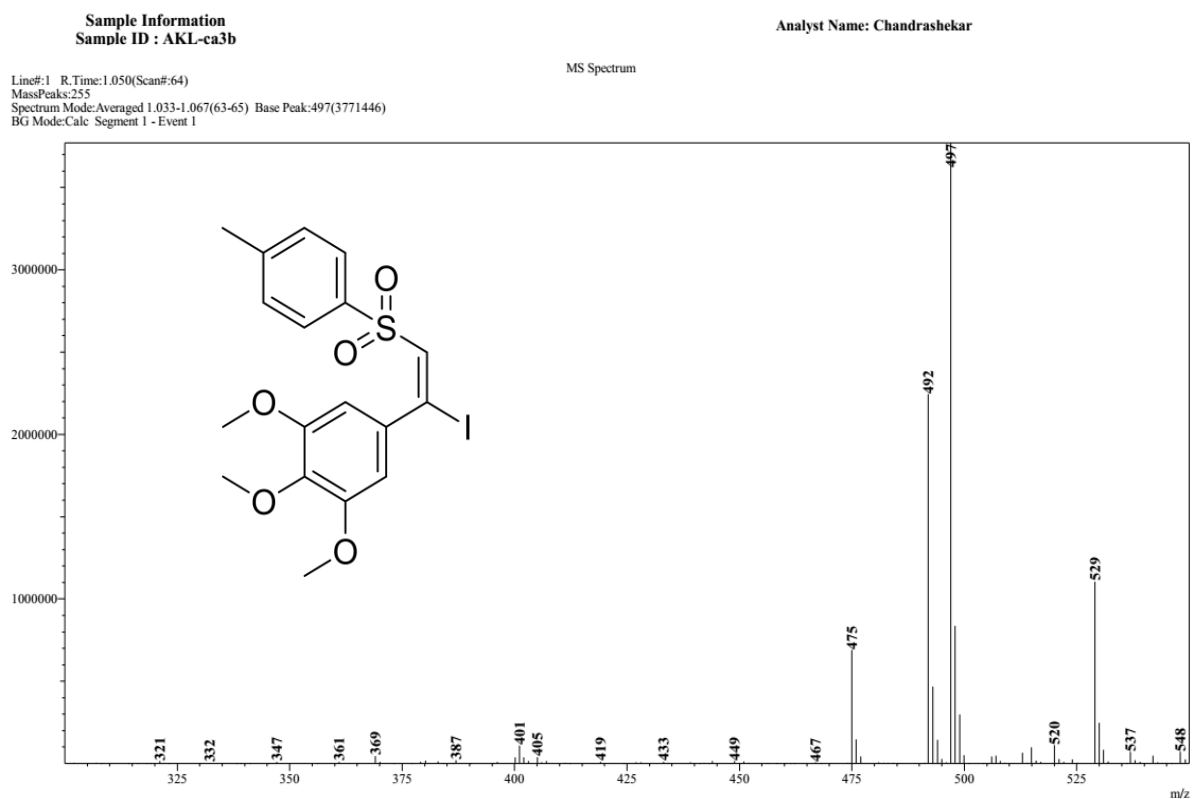
2. AKL-CA-2, C₁₁H₁₂O₃, 5-ethynyl-1,2,3-trimethoxybenzene

Biotransformation Lab



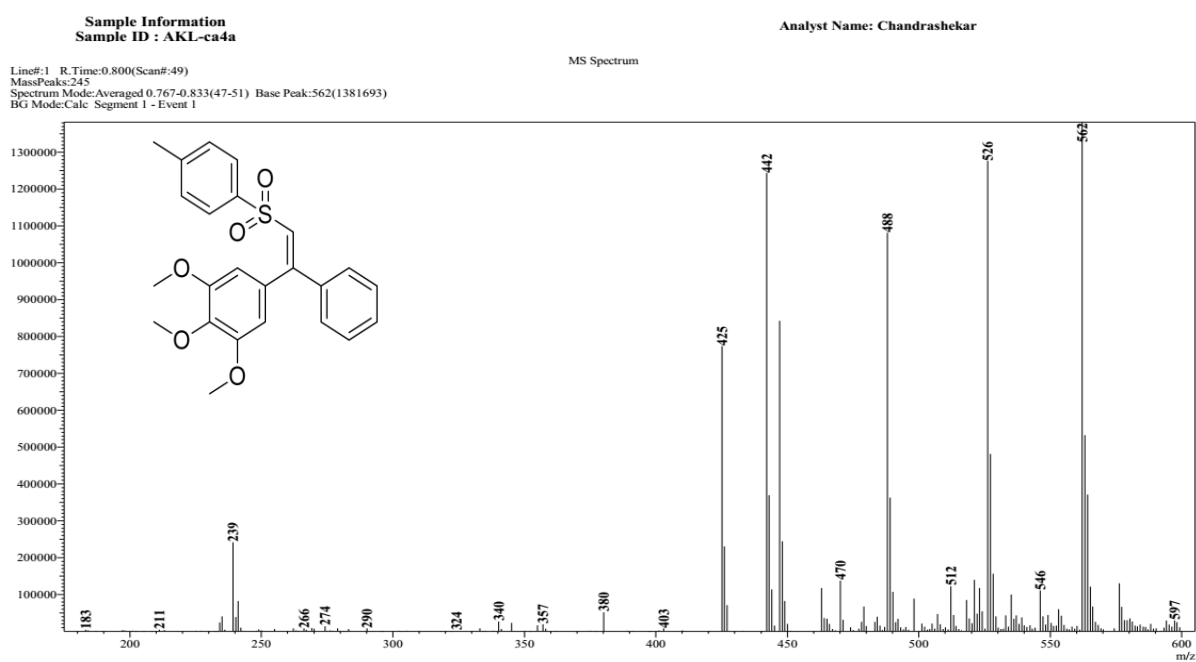
3. AKL-CA-3, C₁₈H₁₉IO₅S, (E)-5-(1-iodo-2-tosylvinyl)-1,2,3-trimethoxybenzene

Biotransformation Lab

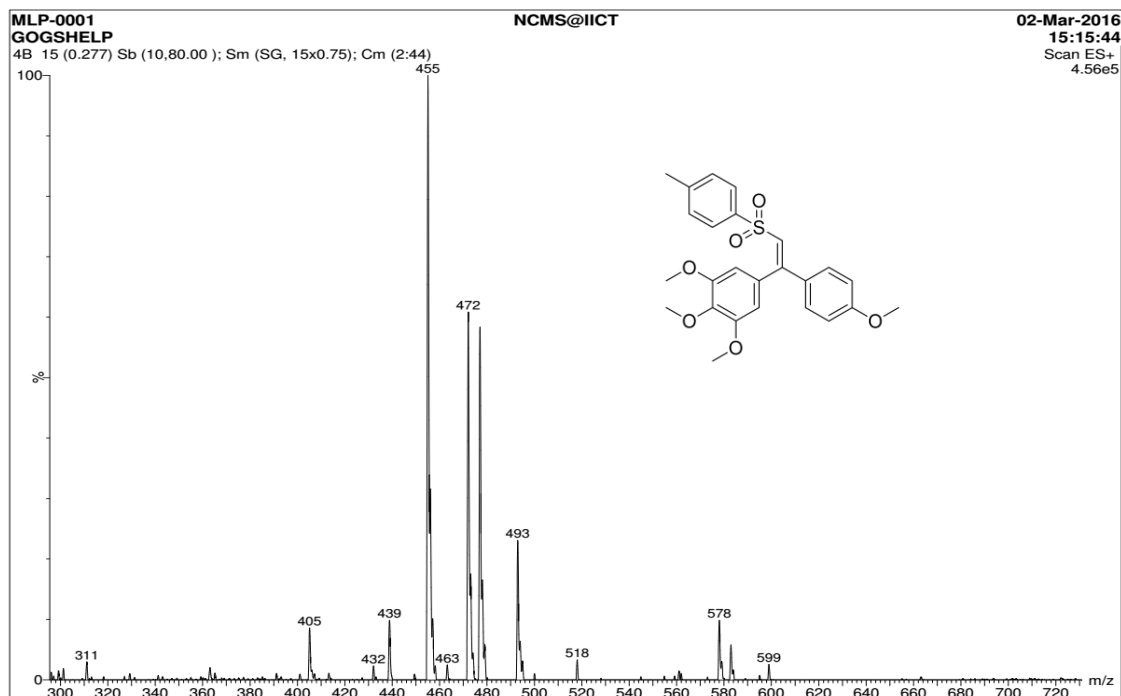


4. AKL-CA-4A, C₂₄H₂₄O₅S, (Z)-1,2,3-trimethoxy-5-(1-phenyl-2-tosylvinyl)benzene

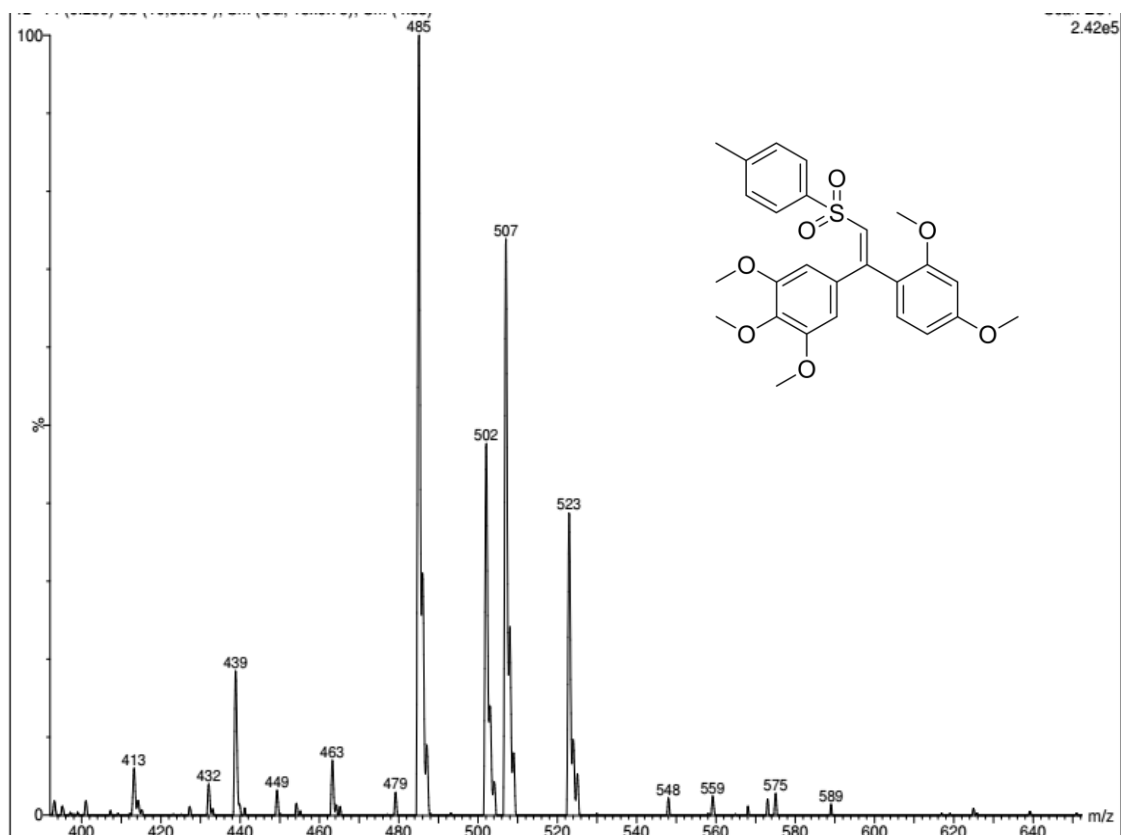
Biotransformation Lab



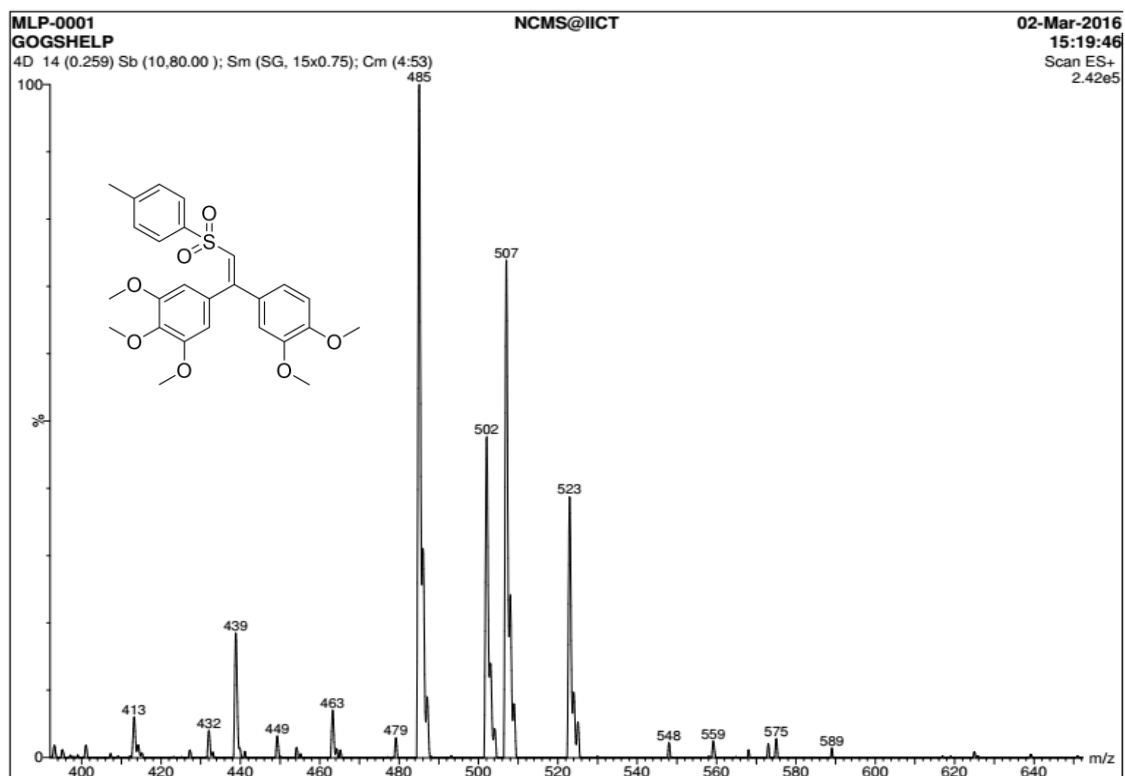
5. AKL-CA-4B, (Z)-1,2,3-trimethoxy-5-(1-(4-methoxyphenyl)-2-tosylvinyl)benzene



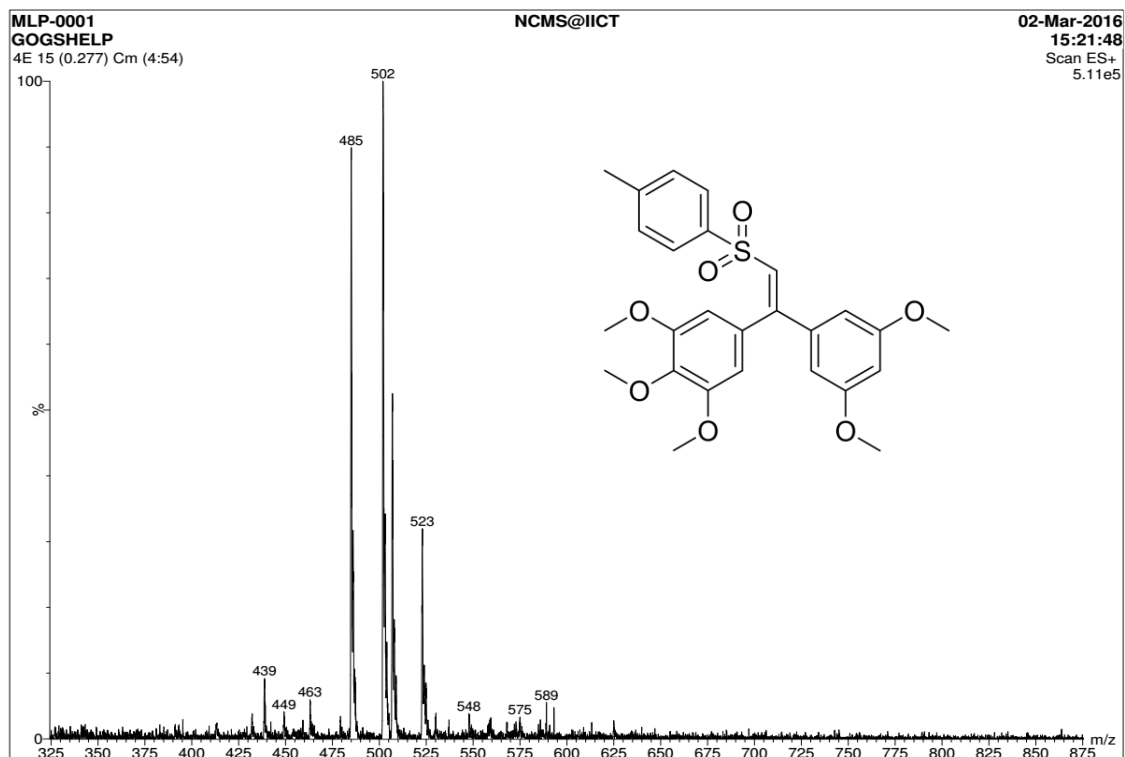
6. AKL-CA-4C, C₂₆H₂₈O₇S, (E)-5-(1-(2,4-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



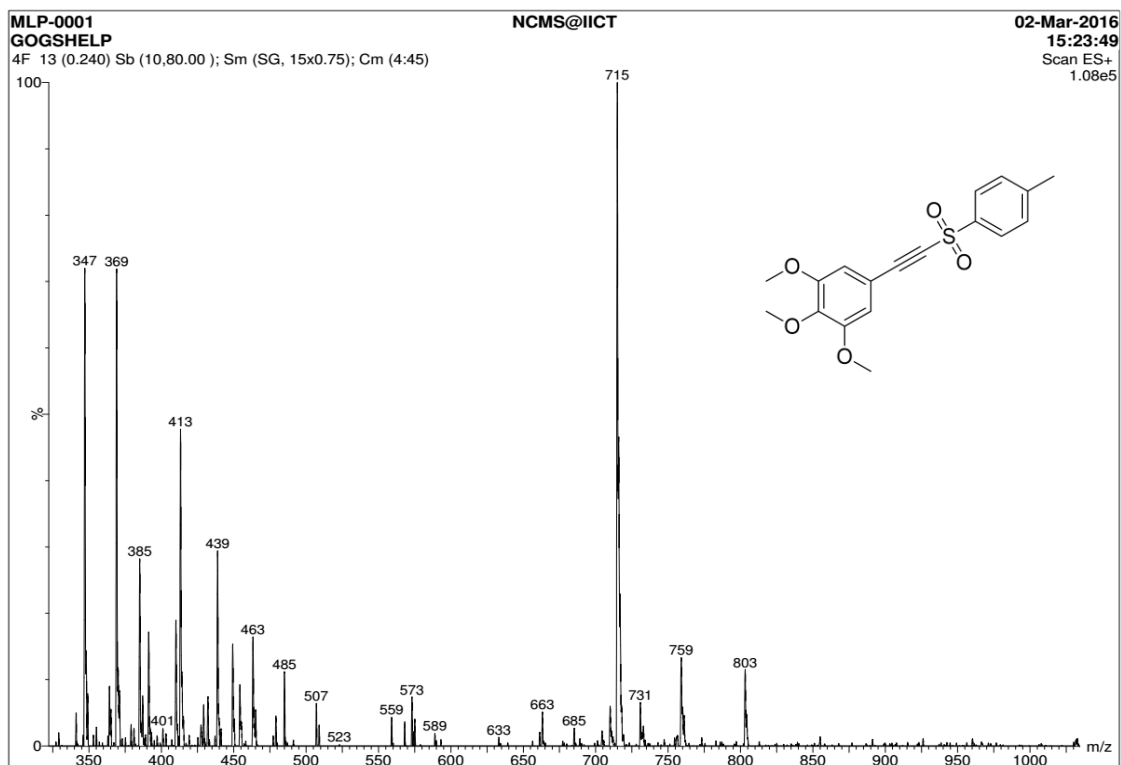
7. AKL-CA-4D, C₂₆H₂₈O₇S, (Z)-5-(1-(3,4-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



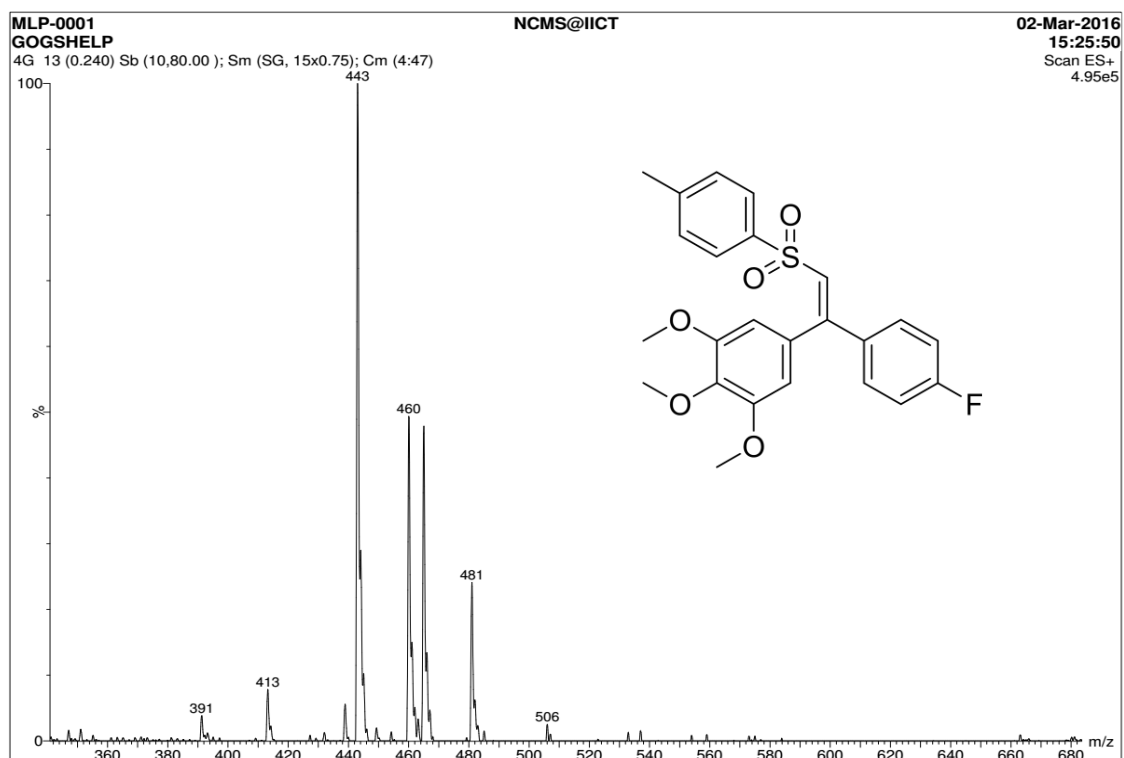
8. AKL-CA-4E, C₂₆H₂₈O₇S, (Z)-5-(1-(3,5-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



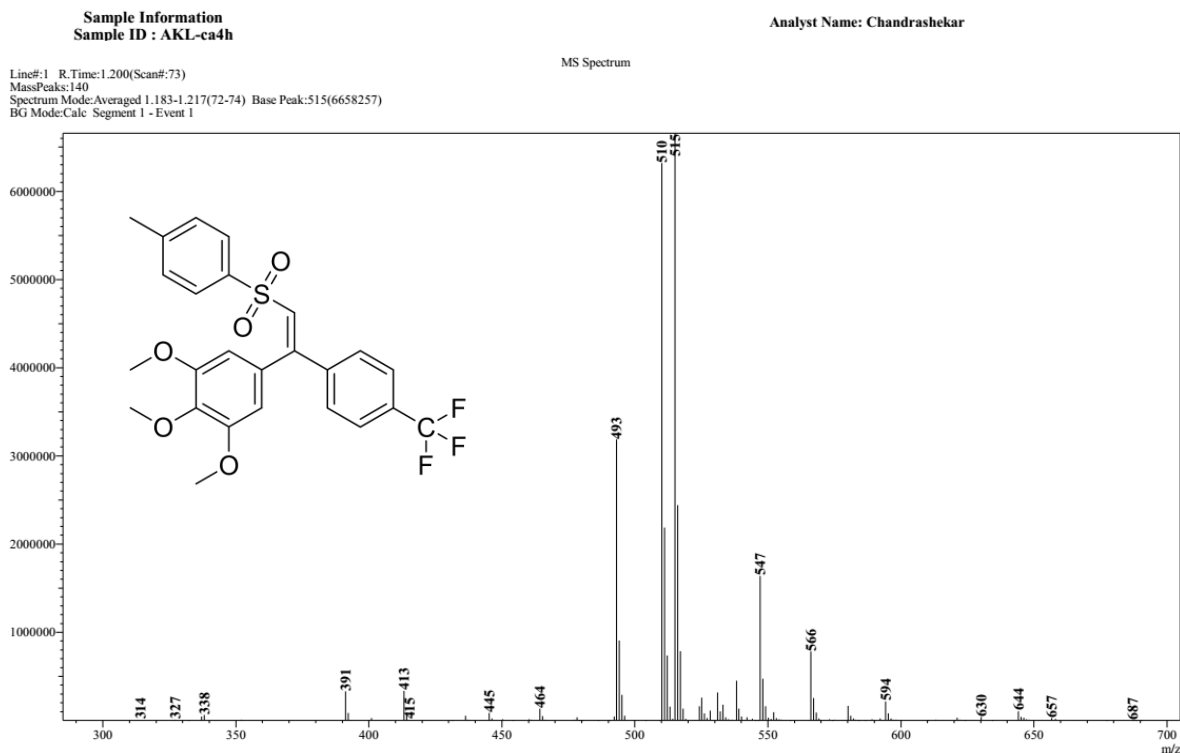
9. AKL-CA-4F, C₁₈H₁₈O₅S, 4-methylphenyl (3,4,5-trimethoxyphenyl)ethynyl sulfone



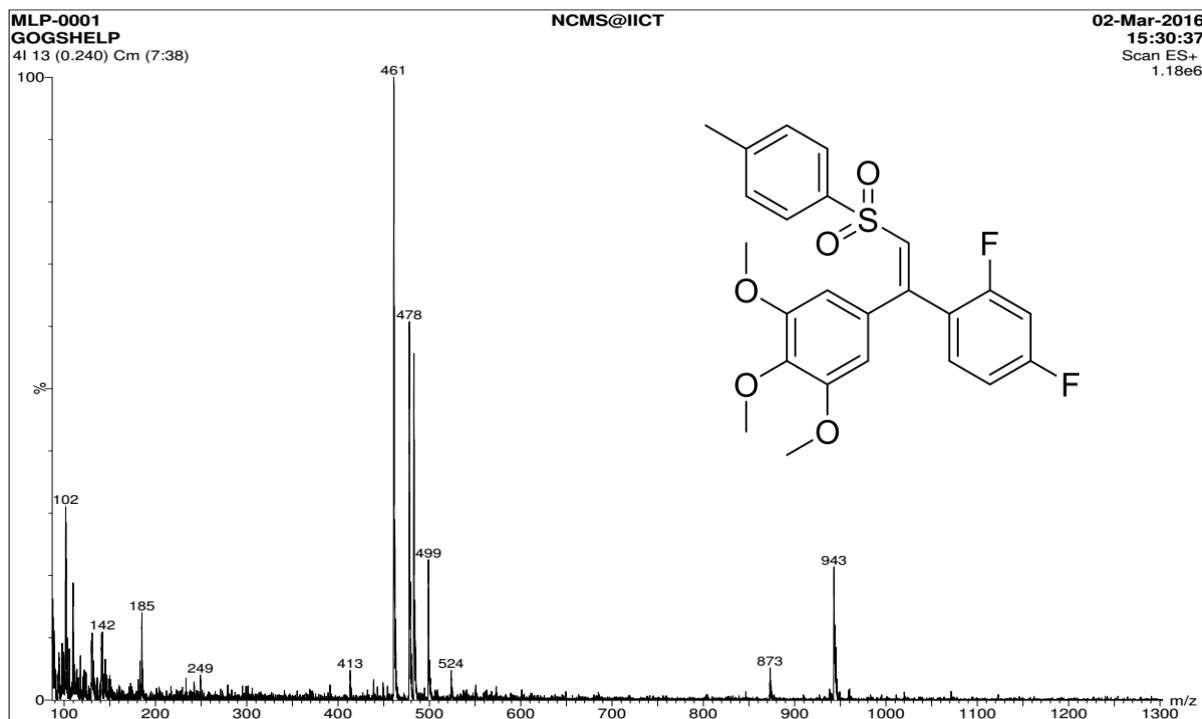
10. AKL-CA-4G, C₂₄H₂₃FO₅S, (Z)-5-(1-(4-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



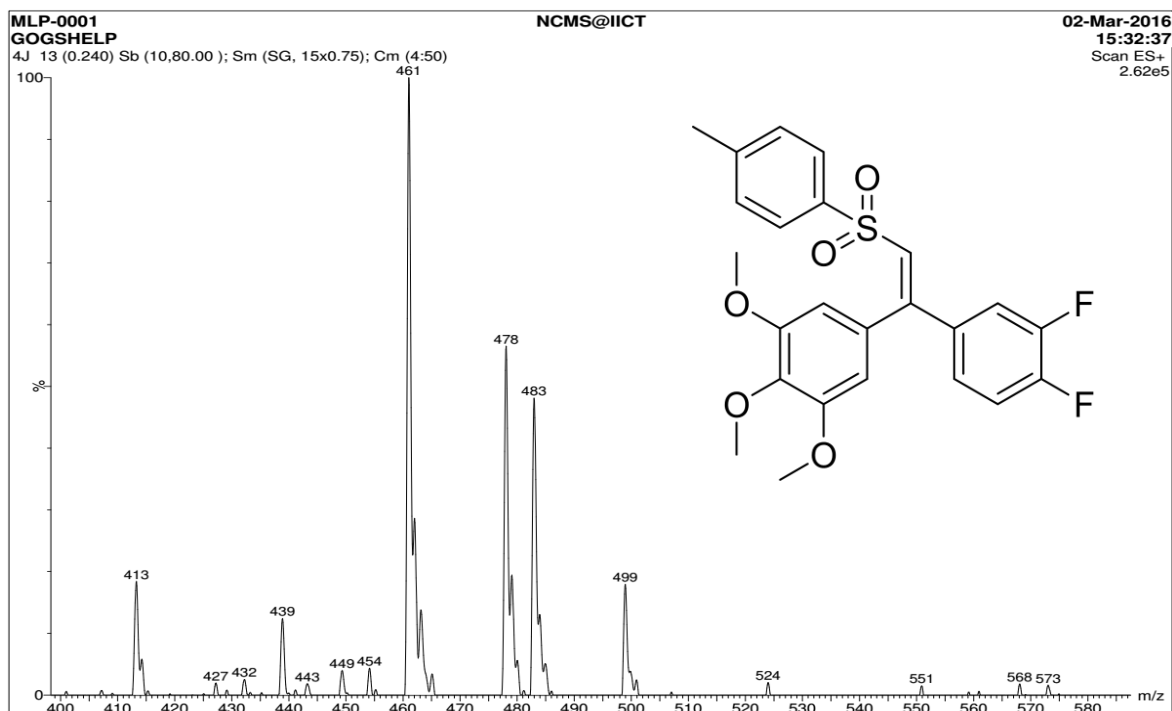
11. AKL-CA-4H, $C_{25}H_{23}F_3O_5S$, (Z)-1,2,3-trimethoxy-5-(2-tosyl-1-(4-(trifluoromethyl)phenyl)vinyl)benzene



12. AKL-CA-4I, $C_{24}H_{22}F_2O_5S$, (E)-5-(1-(2,4-difluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

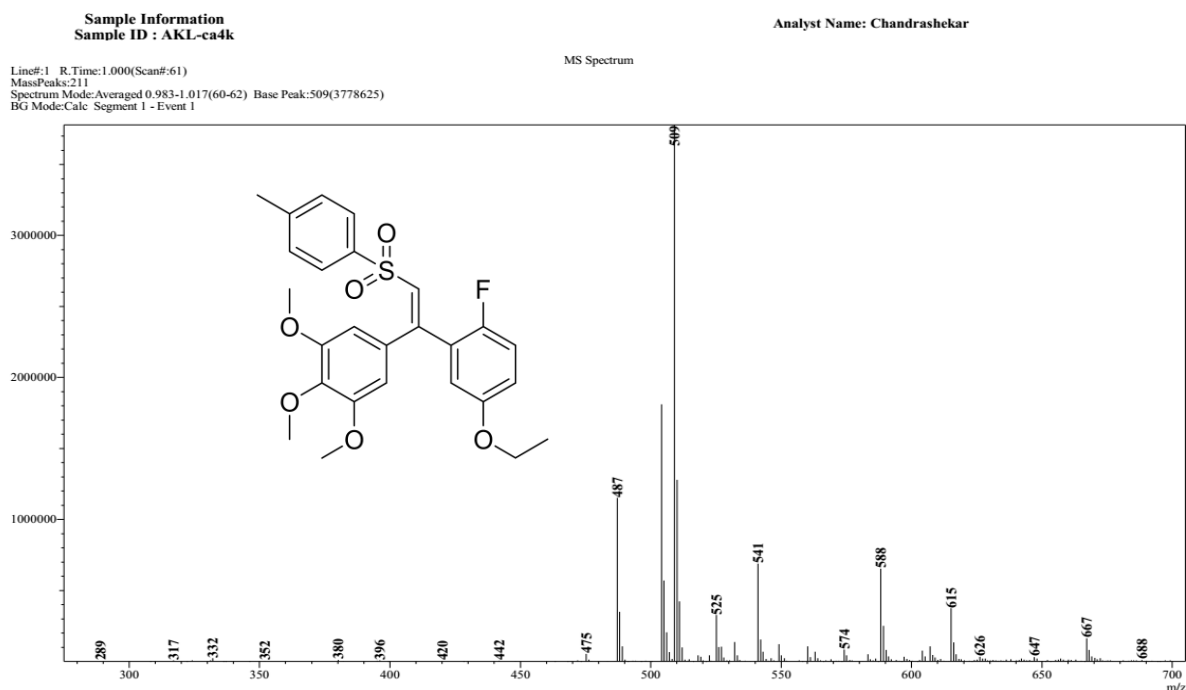


13. AKL-CA-4J, C₂₄H₂₂F₂O₅S, (E)-5-(1-(3,4-difluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



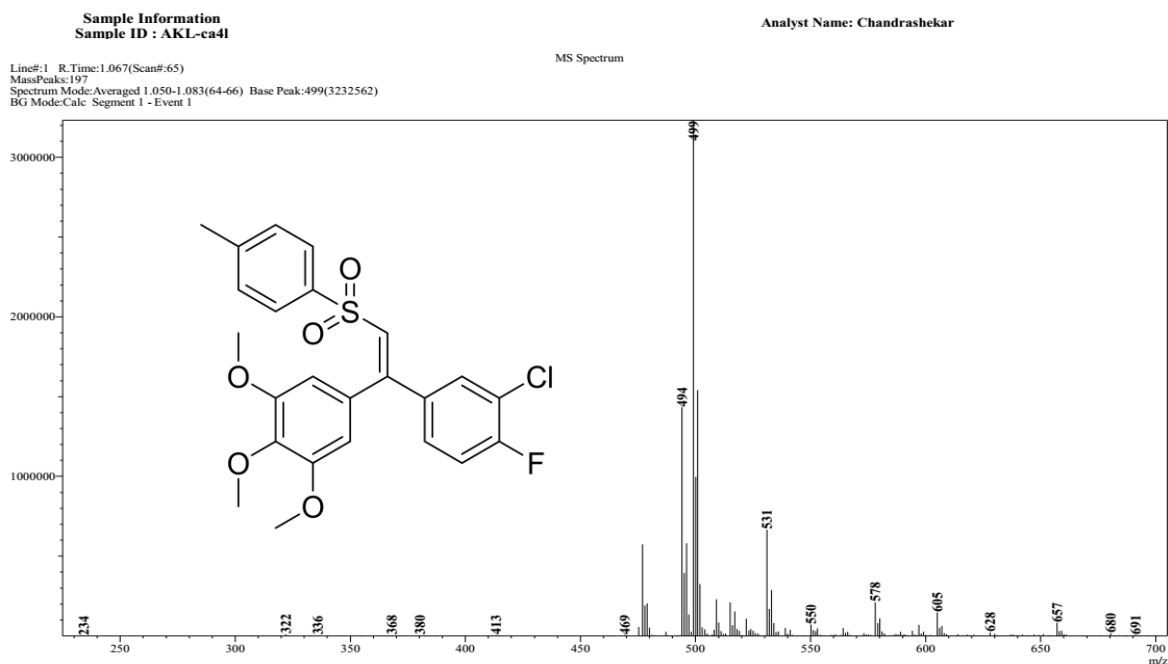
14. AKL-CA-4K, C₂₆H₂₇FO₆S, (E)-5-(1-(5-ethoxy-2-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

Biotransformation Lab



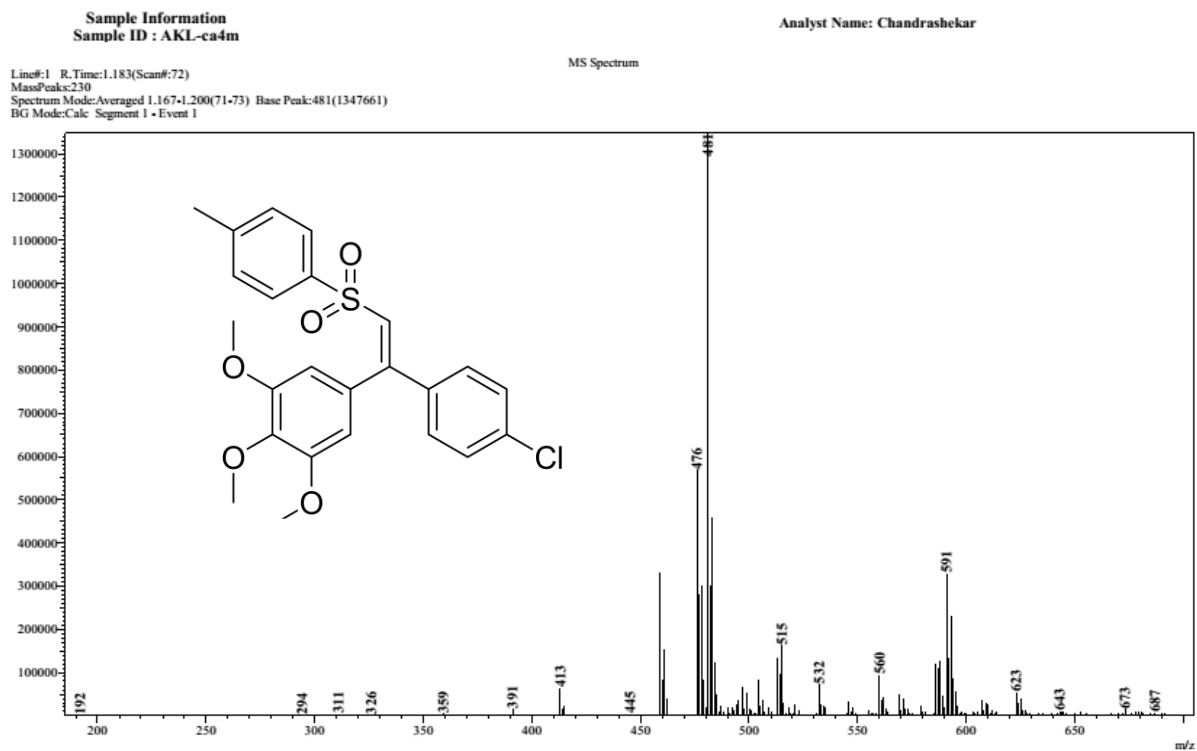
15. AKL-CA-4L, C₂₄H₂₂ClFO₅S, (E)-5-(1-(3-chloro-4-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

Biotransformation Lab

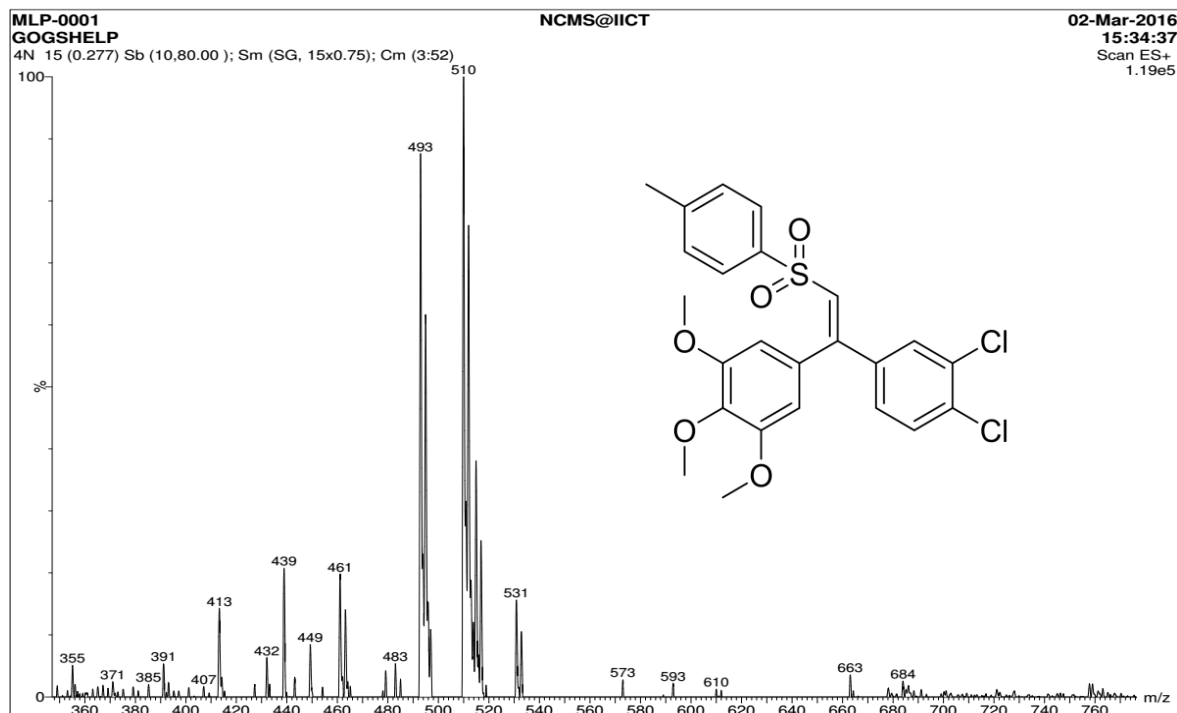


16. AKL-CA-4M, C₂₄H₂₃ClO₅S, (Z)-5-(1-(4-chlorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

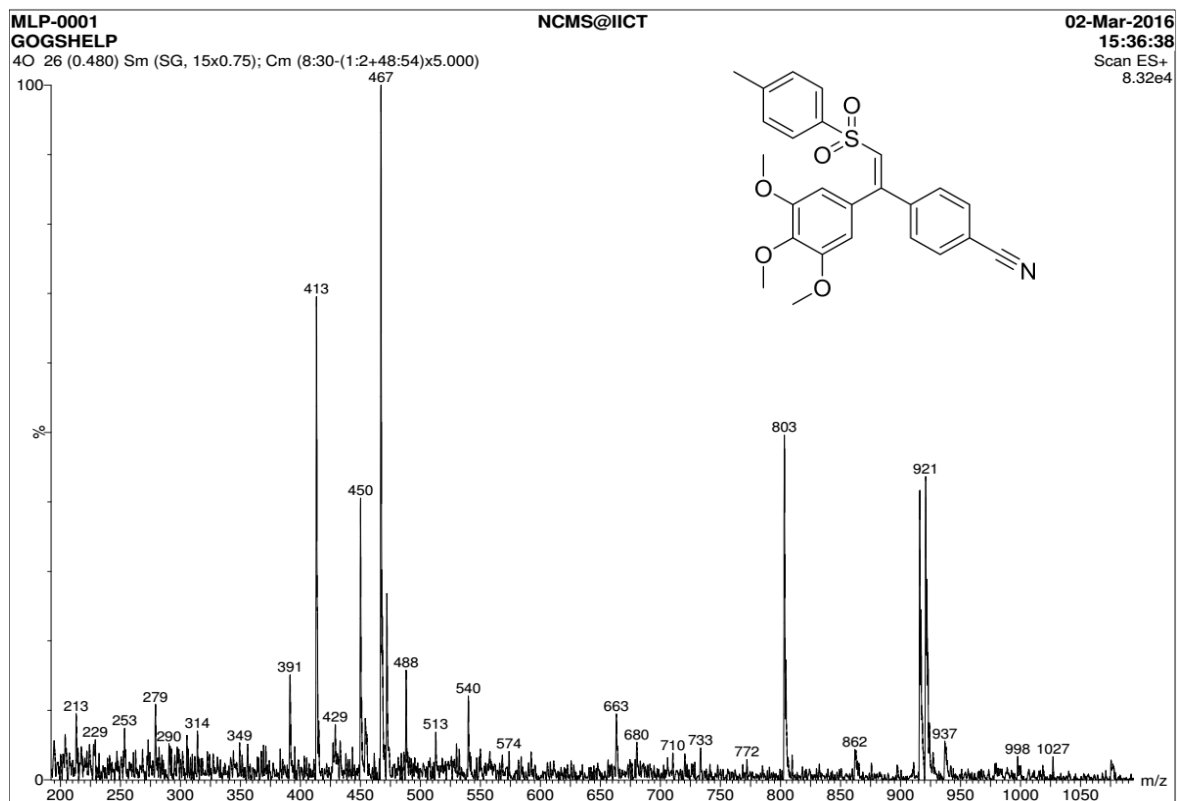
Biotransformation Lab



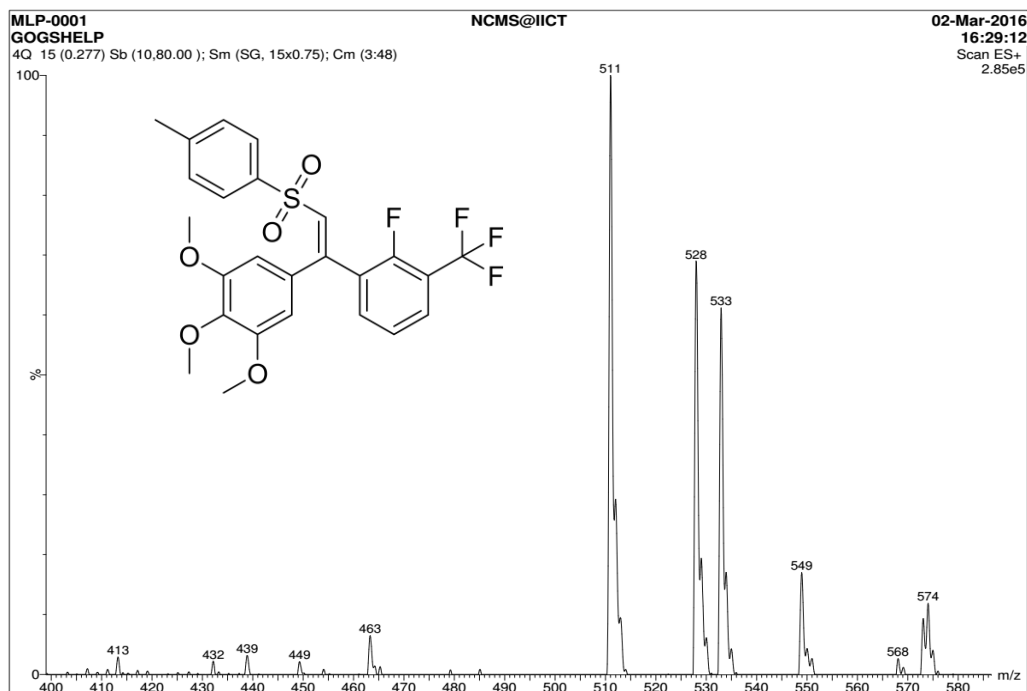
17. AKL-CA-4N, C₂₄H₂₂Cl₂O₅S, (E)-5-(1-(3,4-dichlorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



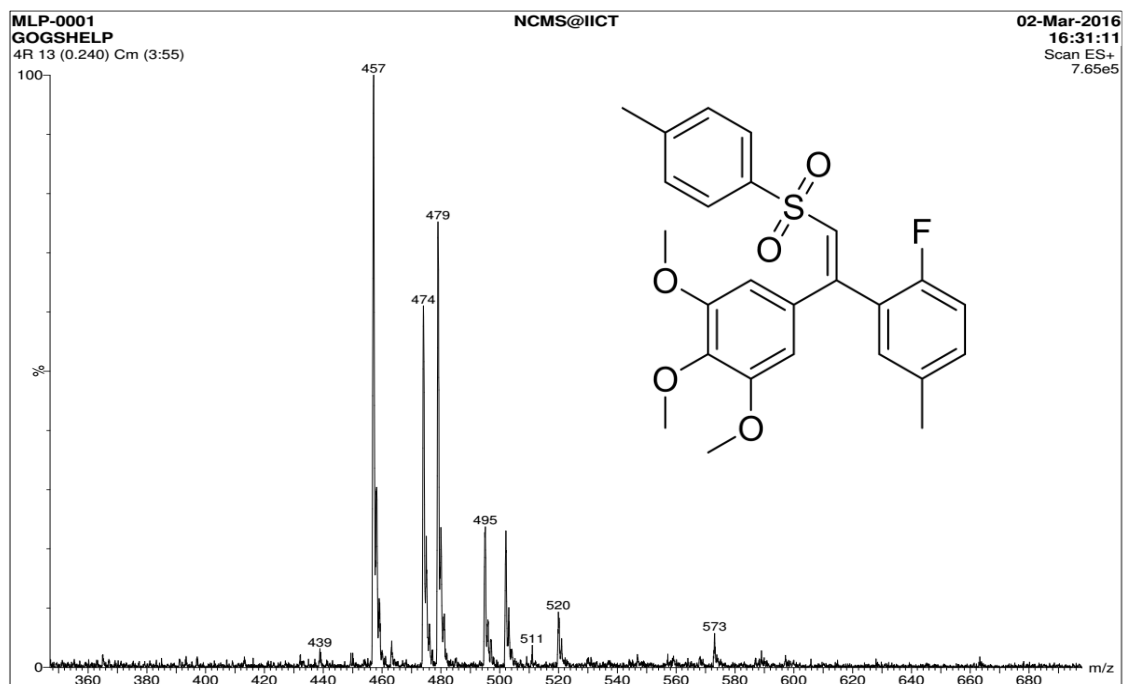
18. AKL-CA-4O, C₂₅H₂₃NO₅S, (Z)-4-(2-tosyl-1-(3,4,5-trimethoxyphenyl)vinyl) benzonitrile



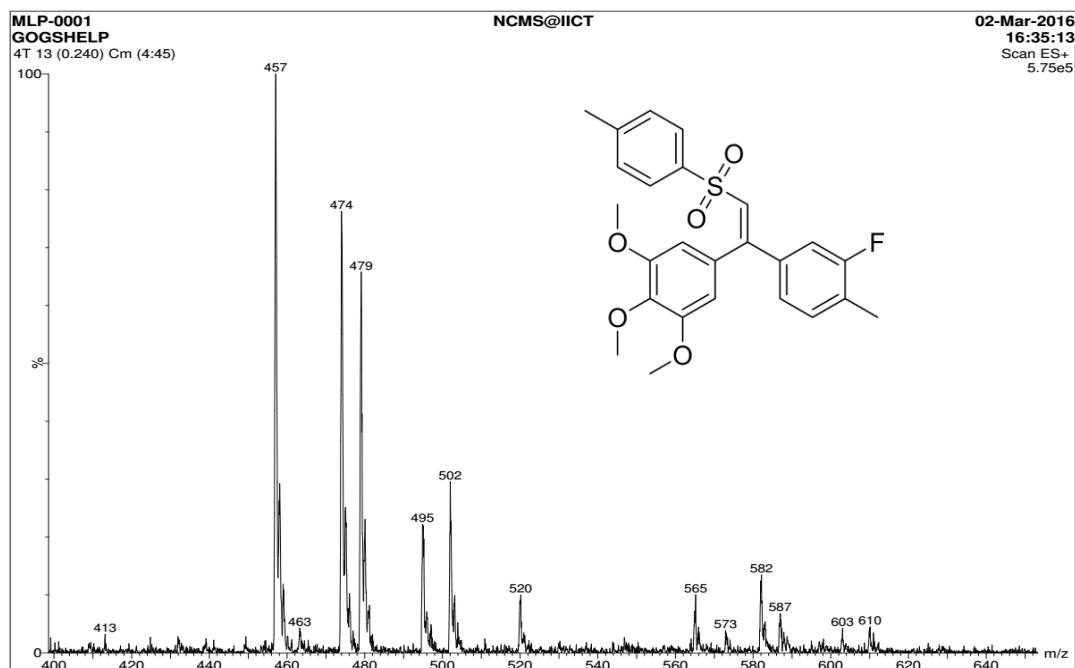
19. AKL-CA-4Q, C₂₅H₂₂F₄O₅S, (E)-5-(1-(2-fluoro-3-(trifluoromethyl)phenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



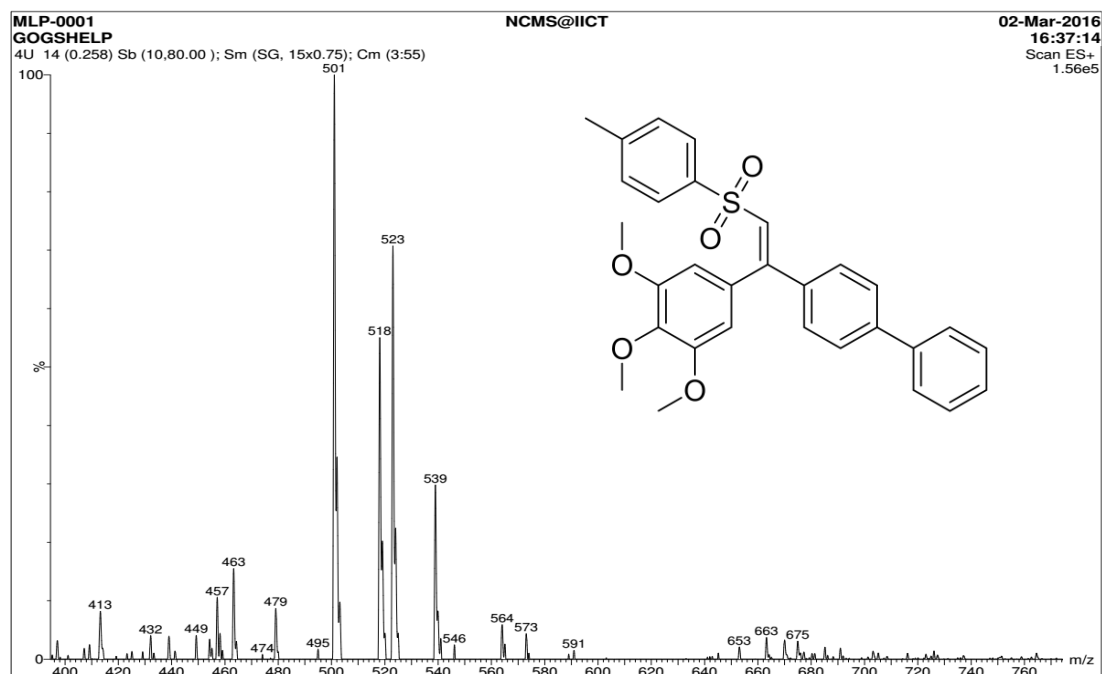
20. AKL-CA-4R, C₂₅H₂₅FO₅S, (E)-5-(1-(2-fluoro-5-methylphenyl)-2-tosylvinyl)-1,2,3-Trimethoxybenzene



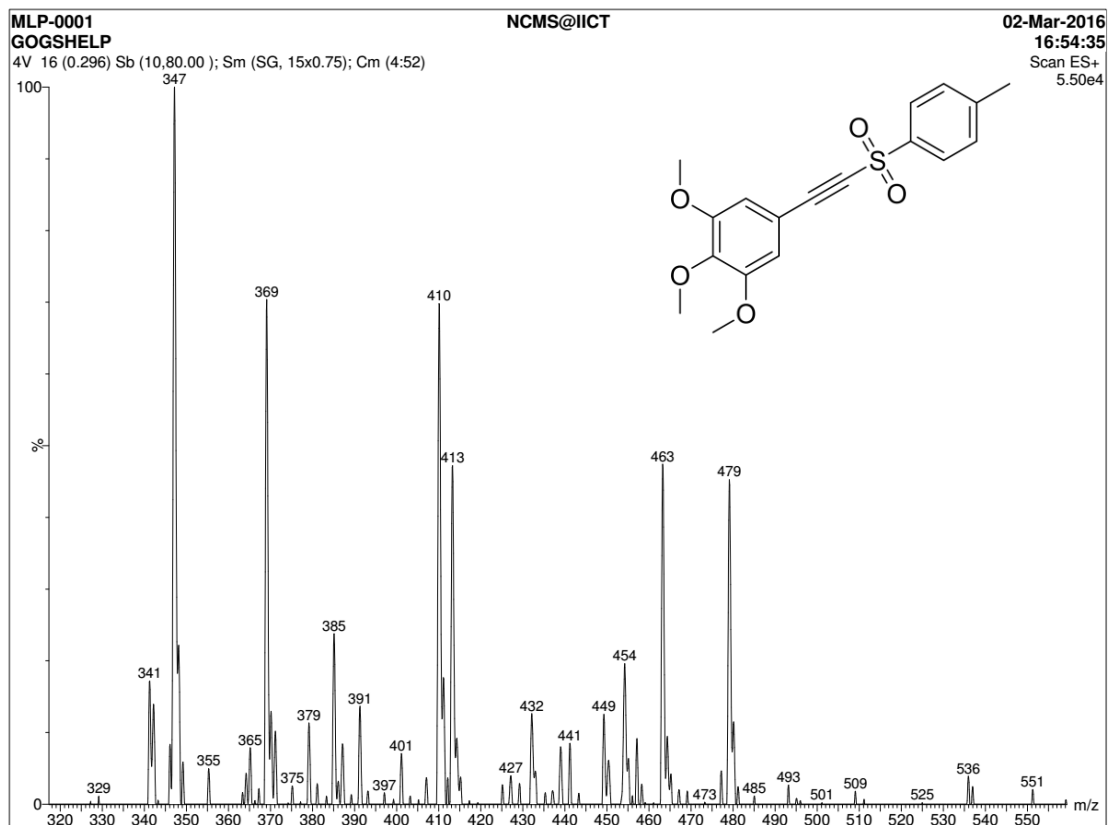
21. AKL-CA-4T, C₂₅H₂₅FO₅S, (E)-5-(1-(3-fluoro-4-methylphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



22. AKL-CA-4U, C₃₀H₂₈O₅S, (Z)-4-(2-tosyl-1-(3,4,5-trimethoxyphenyl)vinyl)-1,1'-biphenyl



23. AKL-CA-4V, C₁₈H₁₈O₅S, 4-methylphenyl (3,4,5-trimethoxyphenyl)ethynyl sulfone



APPENDICES 4: HRMS SPECTRA OF SYNTHESIZED COMPOUNDS

1. AKL-CA-3, C₁₈H₁₉IO₅S, (E)-5-(1-iodo-2-tosylvinyl)-1,2,3-trimethoxybenzene

C:\NICT HRMS-03.09.2014\...AKL-CA3

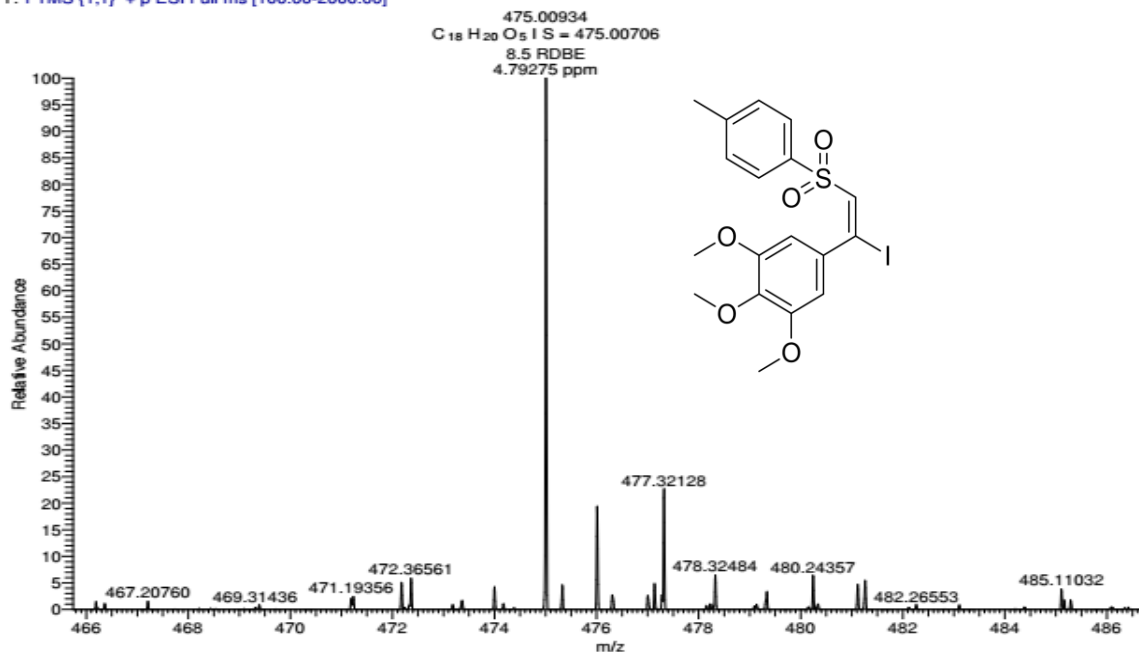
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 02:18:06

Analysed By G SaiKrishna

AKL-CA3 #5-43 RT: 0.02-0.15 AV: 39 NL: 4.36E5

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



2. AKL-CA-4A, C₂₄H₂₄O₅S, (Z)-1,2,3-trimethoxy-5-(1-phenyl-2-tosylvinyl)benzene

C:\NICT HRMS-03.09.2014\...AKL-CA-4A

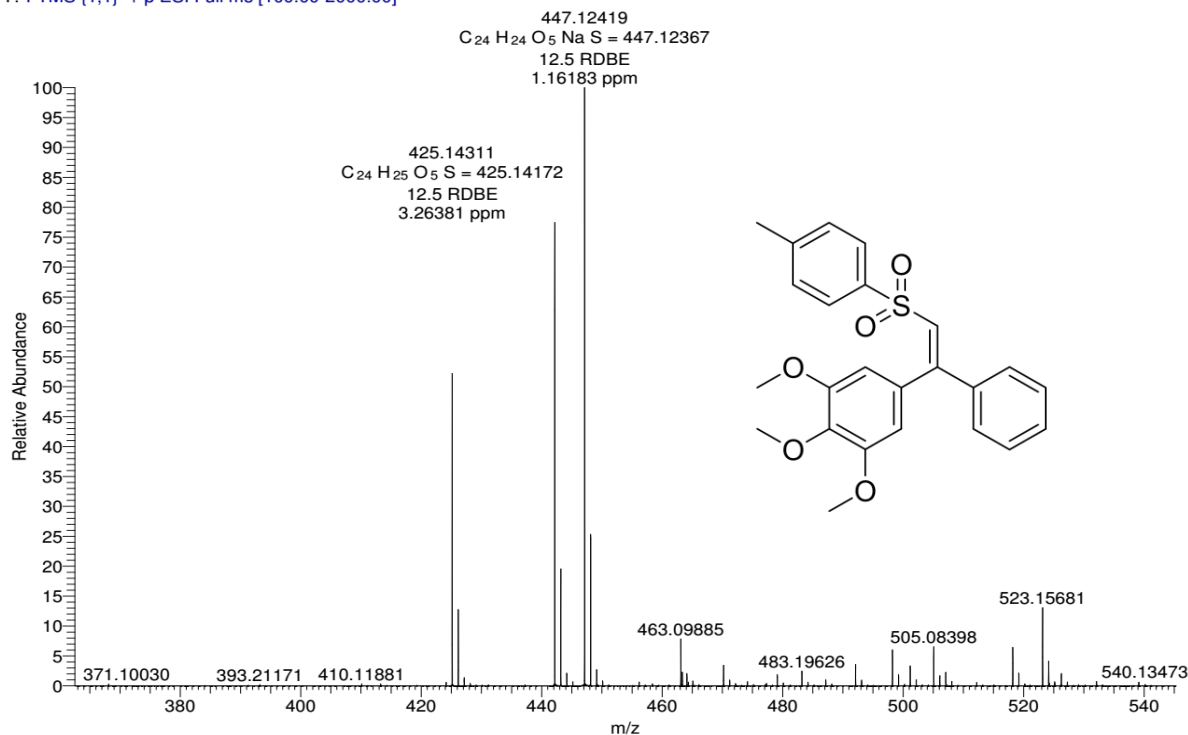
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 02:15:23

Analysed By G SaiKrishna

AKL-CA-4A #1-29 RT: 0.01-0.10 AV: 29 NL: 1.78E7

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



3. AKL-CA-4B, (Z)-1,2,3-trimethoxy-5-(1-(4-methoxyphenyl)-2-tosylvinyl)benzene

C:\NICT HRMS-03.09.2014\...AKL-CA-4B

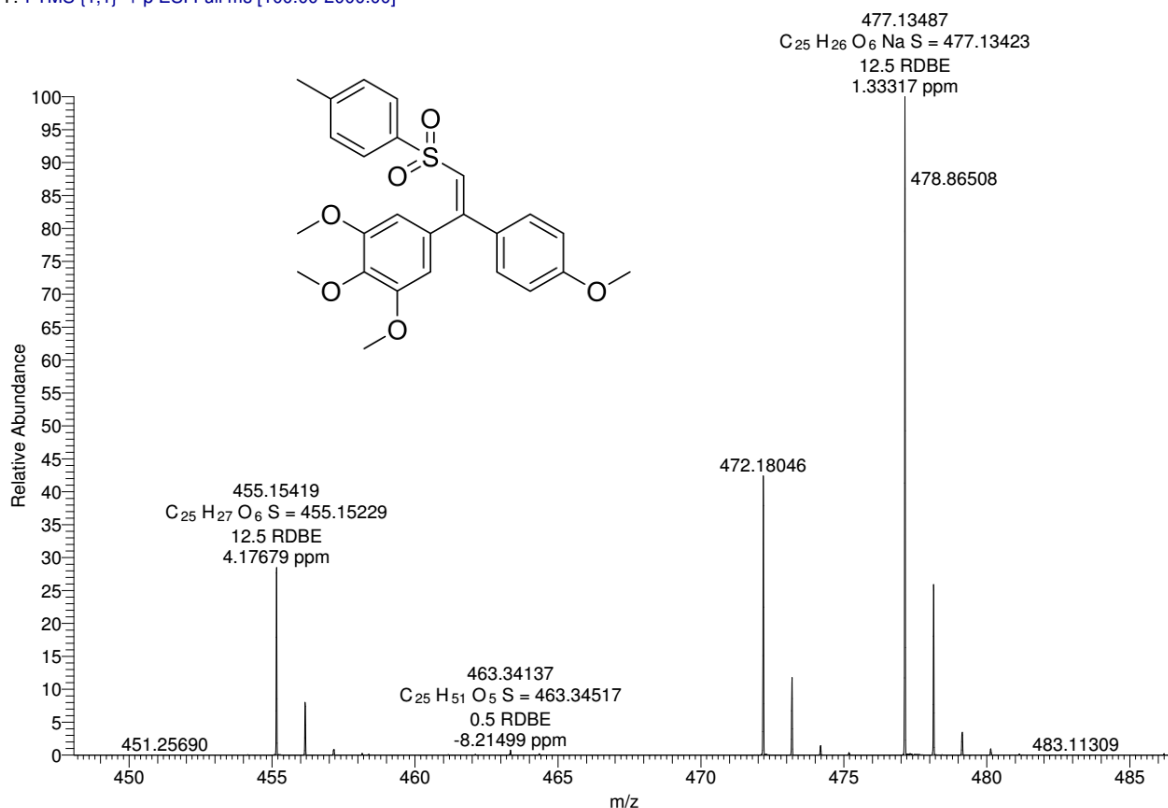
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 02:12:40

Analysed By G SaiKrishna

AKL-CA-4B #4-29 RT: 0.02-0.10 AV: 26 NL: 3.07E7

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



4. AKL-CA-4C, C₂₆H₂₈O₇S, (E)-5-(1-(2,4-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

C:\NICT HRMS-03.09.2014\...AKL-CA 4C

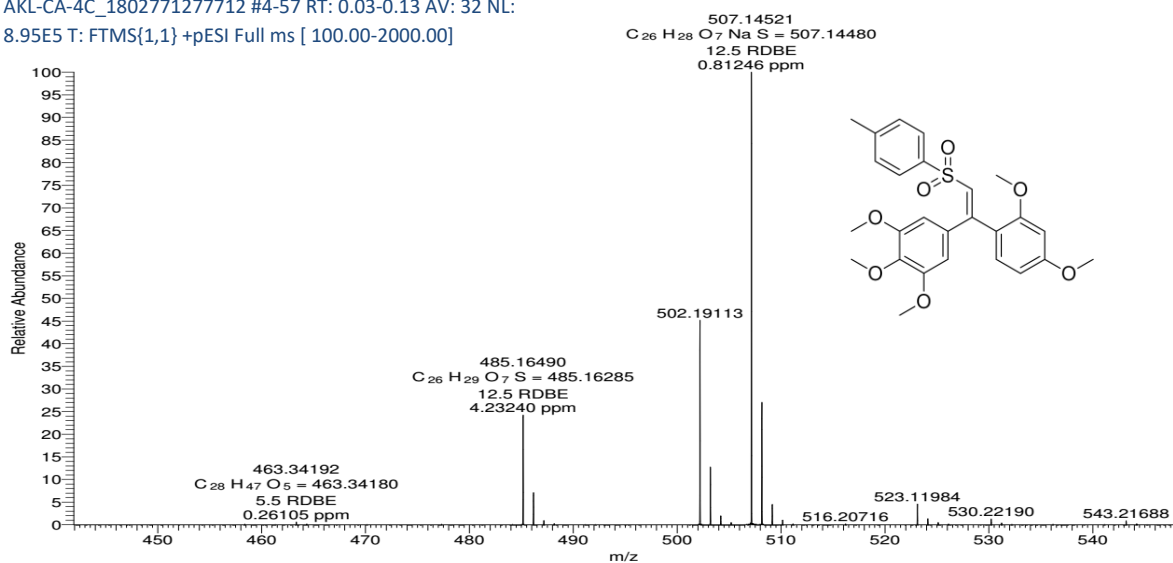
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 02:07:16

Analysed By G SaiKrishna

AKL-CA-4C_1802771277712 #4-57 RT: 0.03-0.13 AV: 32 NL:

8.95E5 T: FTMS{1,1} +pESI Full ms [100.00-2000.00]



5. AKL-CA-4D, C₂₆H₂₈O₇S, (Z)-5-(1-(3,4-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

C:\ICT HRMS-03.09.2014\...AKL-CA-4D

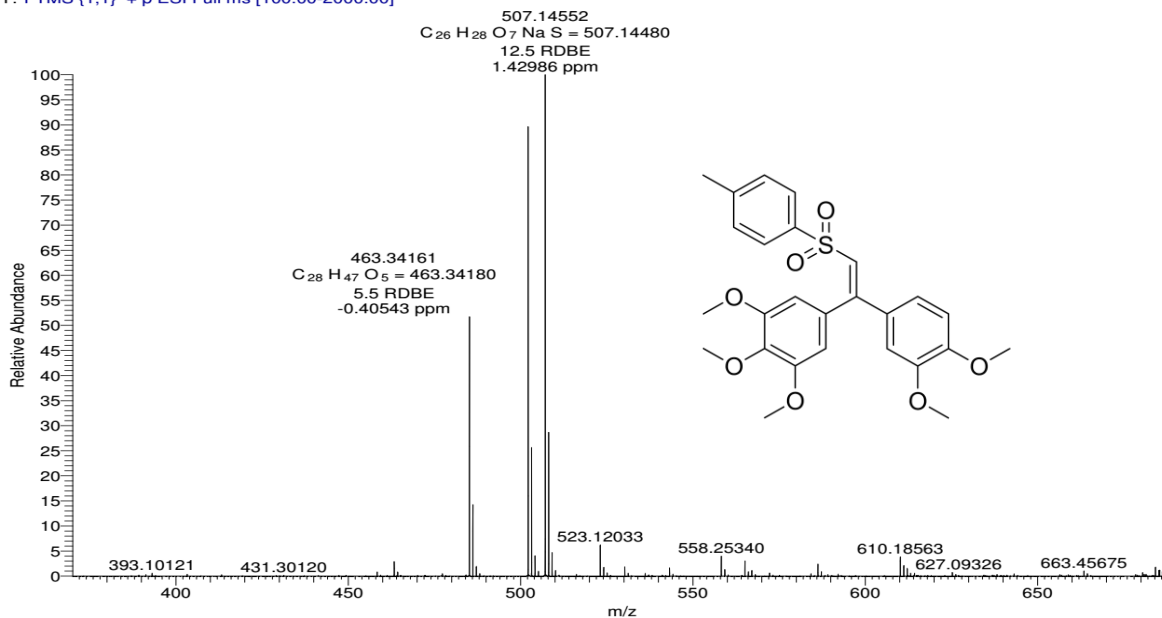
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 02:09:57

Analysed By G SaiKrishna

AKL-CA-4D #2-44 RT: 0.01-0.15 AV: 43 NL: 1.44E7

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



6. AKL-CA-4E, C₂₆H₂₈O₇S, (Z)-5-(1-(3,5-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

C:\ICT HRMS-03.09.2014\...AKL-CA-4E

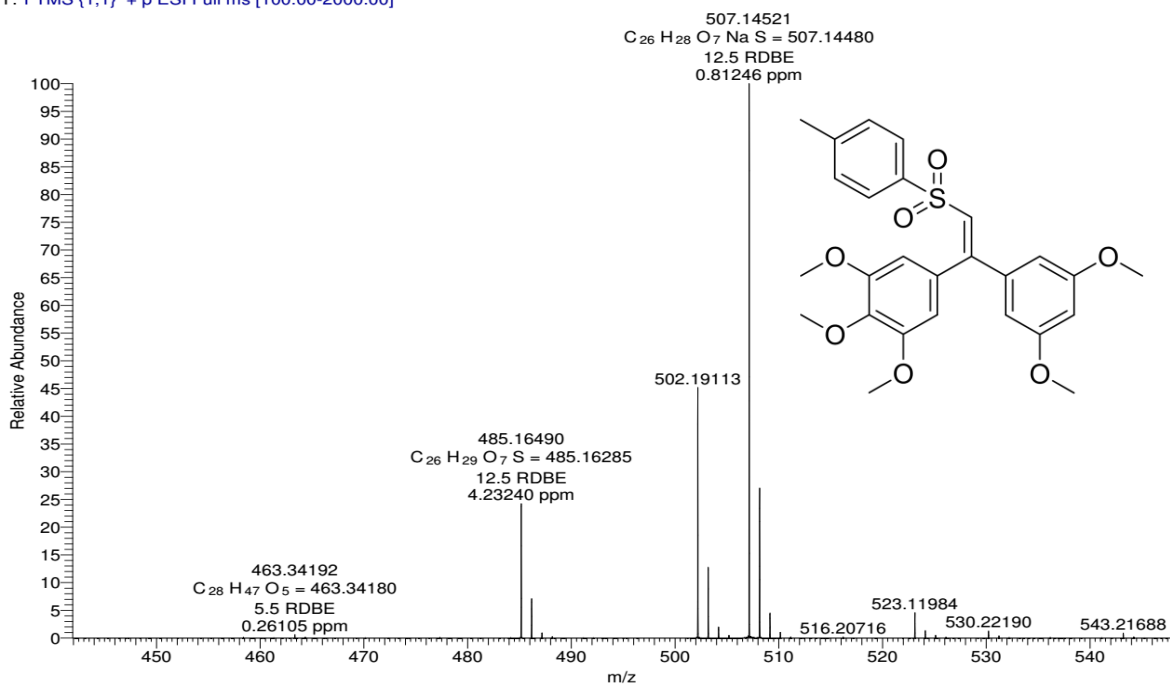
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 02:07:16

Analysed By G SaiKrishna

AKL-CA-4E #9-33 RT: 0.03-0.12 AV: 25 NL: 3.83E7

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



7. AKL-CA-4F, C₁₈H₁₈O₅S, 4-methylphenyl (3,4,5-trimethoxyphenyl)ethynyl sulfone

C:\NICT HRMS-03.09.2014\...AKL-CA-4F

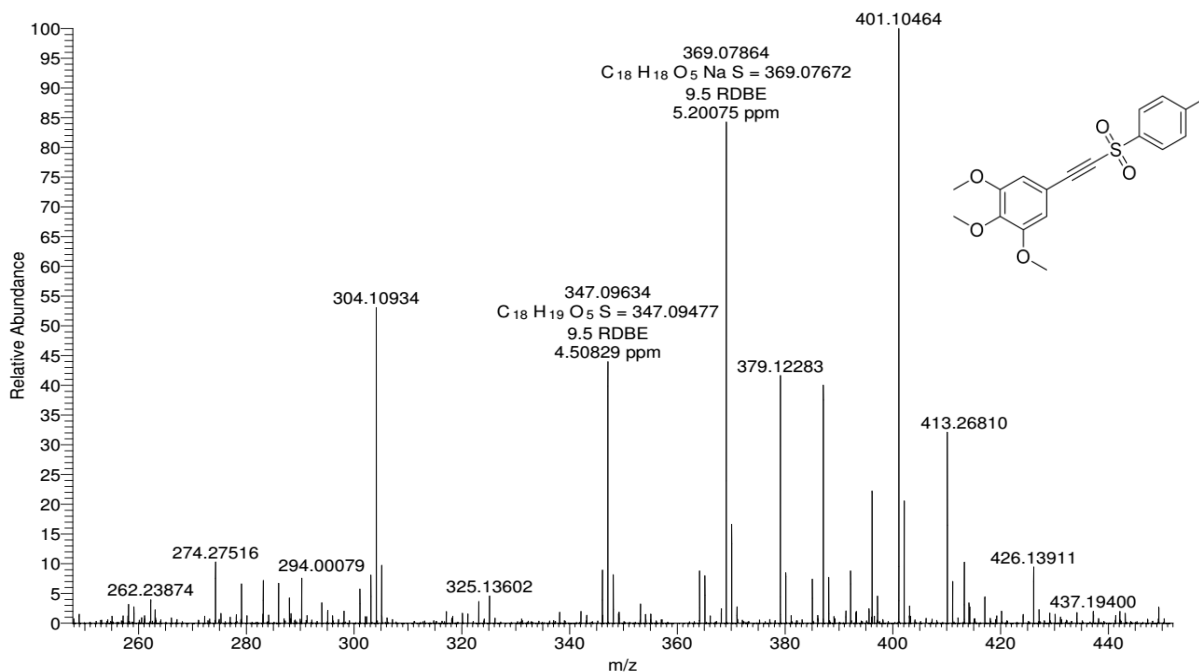
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 02:04:35

Analysed By G SaiKrishna

AKL-CA-4F #4-38 RT: 0.02-0.13 AV: 35 NL: 1.32E6

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



8. AKL-CA-4G, C₂₄H₂₃FO₅S, (Z)-5-(1-(4-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

C:\NICT HRMS-03.09.2014\...AKL-CA-4G

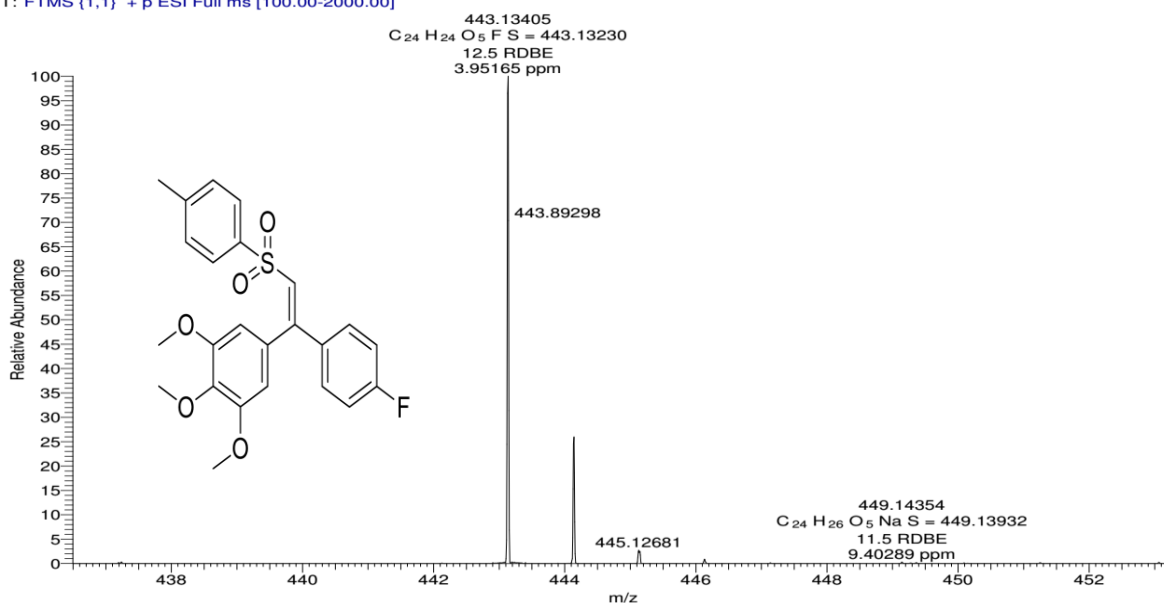
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 02:01:54

Analysed By G SaiKrishna

AKL-CA-4G #3-23 RT: 0.01-0.08 AV: 21 NL: 1.10E7

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



9. AKL-CA-4H, $C_{25}H_{23}F_3O_5S$, (Z)-1,2,3-trimethoxy-5-(2-tosyl-1-(4-(trifluoromethyl)phenyl)vinyl)benzene

AKL-CA-4H_160324015911

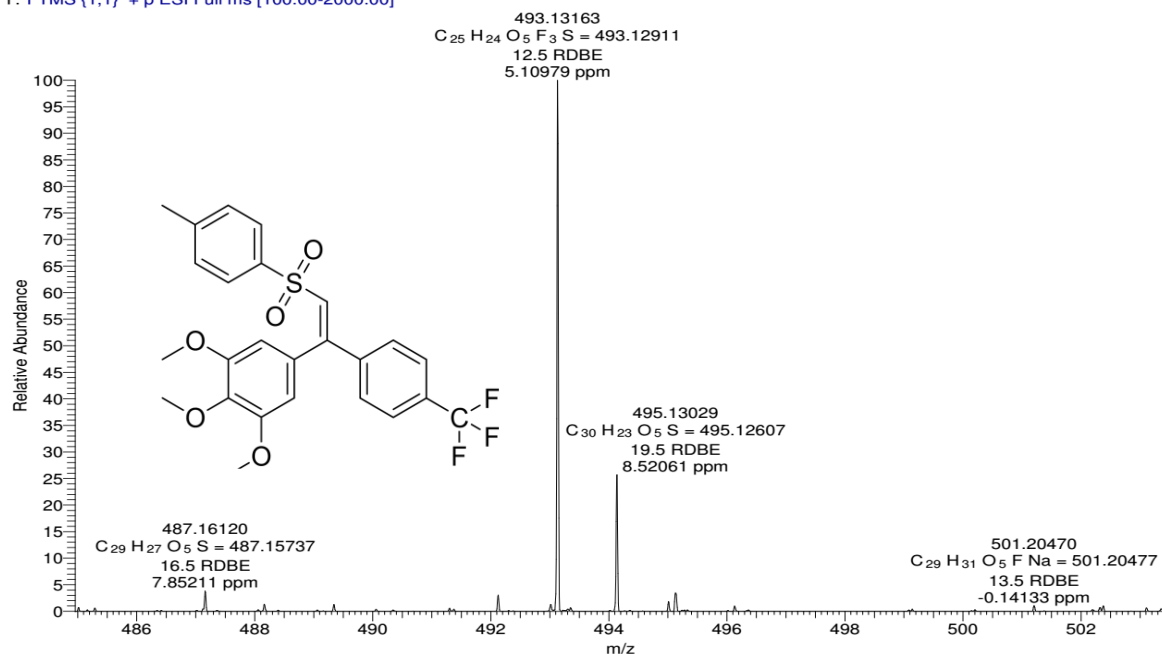
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 01:59:11

Analysed By G SaiKrishna

AKL-CA-4H_160324015911 #7-38 RT: 0.03-0.13 AV: 32 NL: 8.95E5

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



10. AKL-CA-4I, $C_{24}H_{22}F_2O_5S$, (E)-5-(1-(2,4-difluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

C:\ICT HRMS-03.09.2014\...AKL-CA-4I

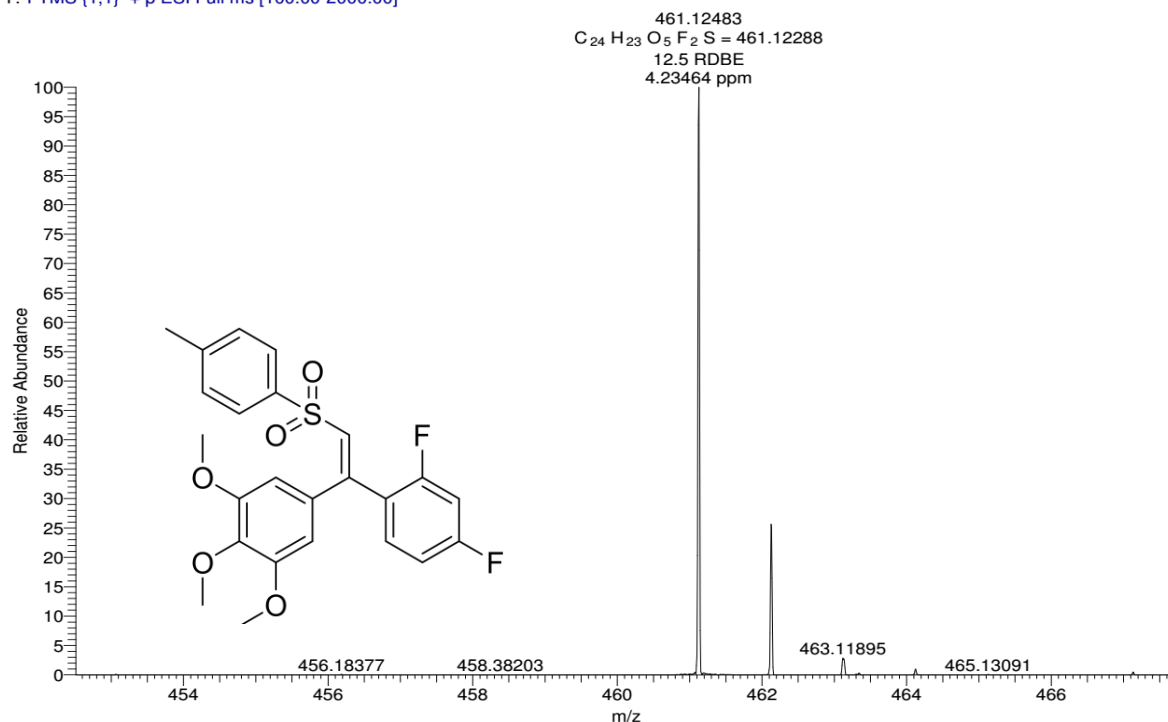
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 01:56:30

Analysed By G SaiKrishna

AKL-CA-4I #9-19 RT: 0.03-0.07 AV: 11 NL: 1.48E7

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



11. AKL-CA-4J, C₂₄H₂₂F₂O₅S, (E)-5-(1-(3,4-difluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

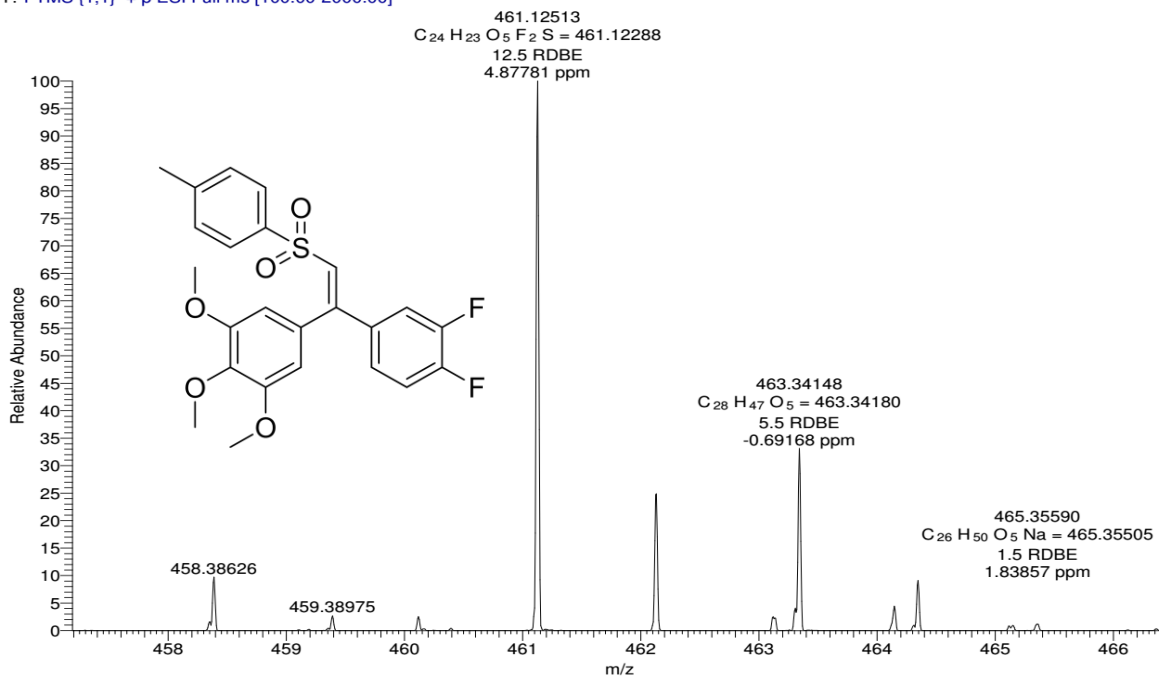
C:\ICT HRMS-03.09.2014\...AKL-CA-4J

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24-03-16 01:53:49

Analysed By G SaiKrishna

AKL-CA-4J #4-49 RT: 0.02-0.17 AV: 46 NL: 1.14E6
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



12. AKL-CA-4K, C₂₆H₂₇FO₆S, (E)-5-(1-(5-ethoxy-2-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

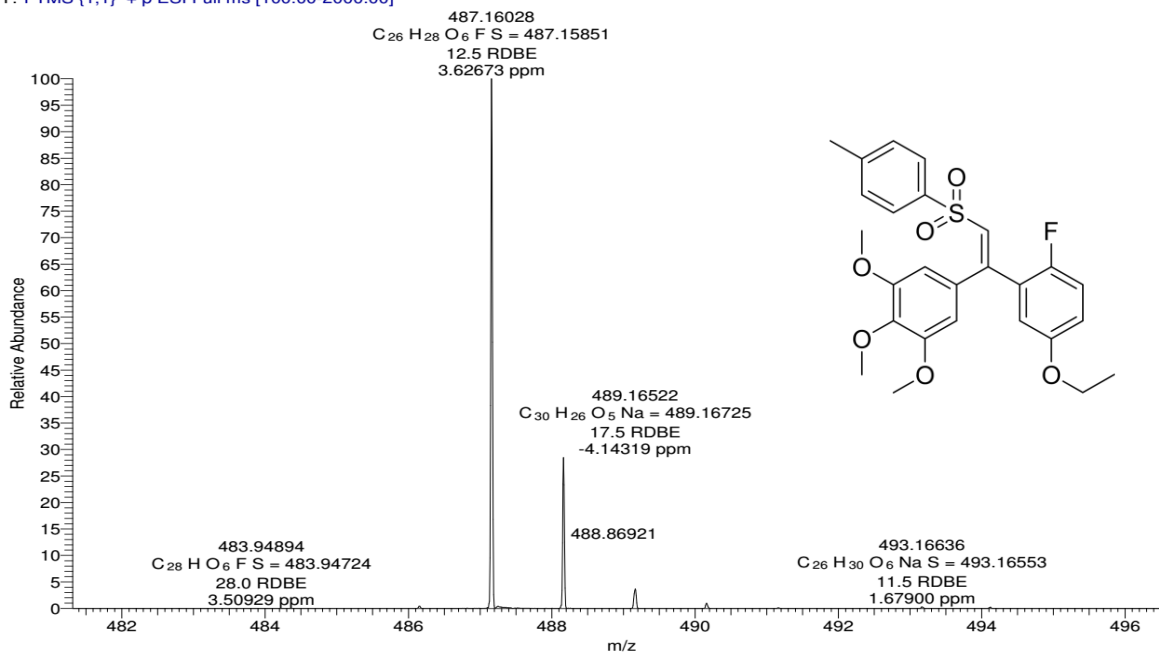
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24-03-16 01:51:08

Analysed By G SaiKrishna

AKL-CA-4K #7-34 RT: 0.03-0.12 AV: 28 NL: 1.30E7
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



13. AKL-CA-4L, C₂₄H₂₂ClFO₅S, (E)-5-(1-(3-chloro-4-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

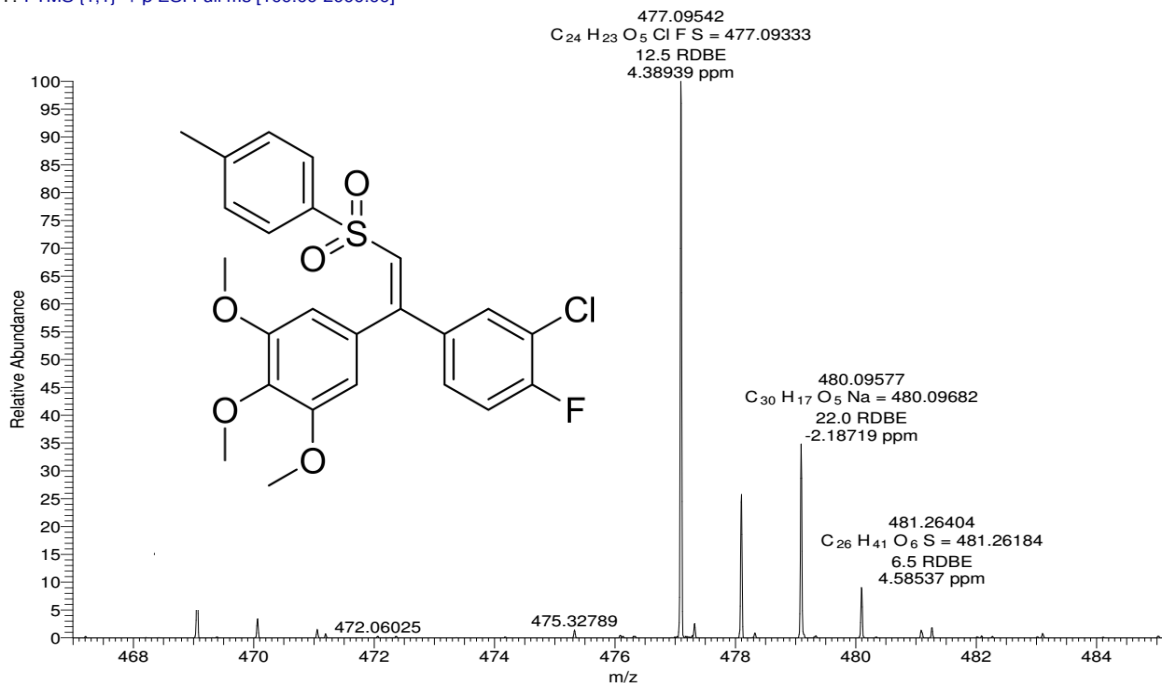
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24-03-16 01:48:25

Analysed By G SaiKrishna

AKL-CA-4L #5-20 RT: 0.02-0.07 AV: 16 NL: 1.56E6
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



14. AKL-CA-4M, C₂₄H₂₃ClO₅S, (Z)-5-(1-(4-chlorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

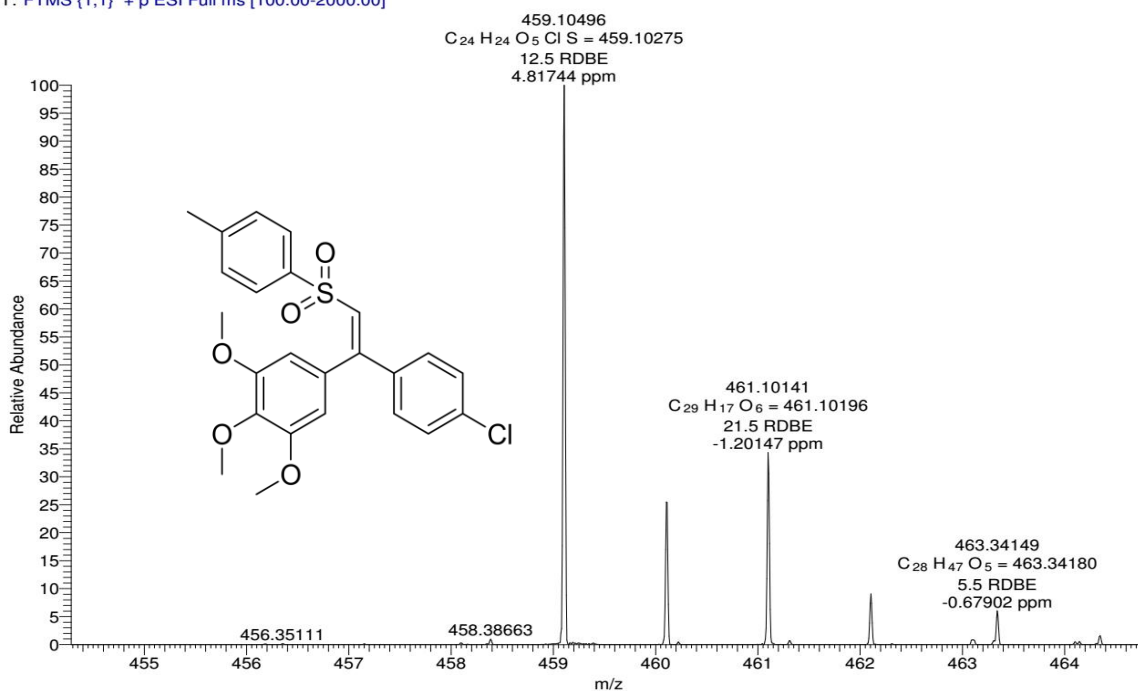
C:\ICT HRMS-03.09.2014\...AKL-CA-4M

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24-03-16 01:45:44

Analysed By G SaiKrishna

AKL-CA-4M #4-29 RT: 0.02-0.10 AV: 26 NL: 4.26E6
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



15. AKL-CA-4N, C₂₄H₂₂Cl₂O₅S, (E)-5-(1-(3,4-dichlorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

C:\ICT HRMS-03.09.2014\...AKL-CA-4H

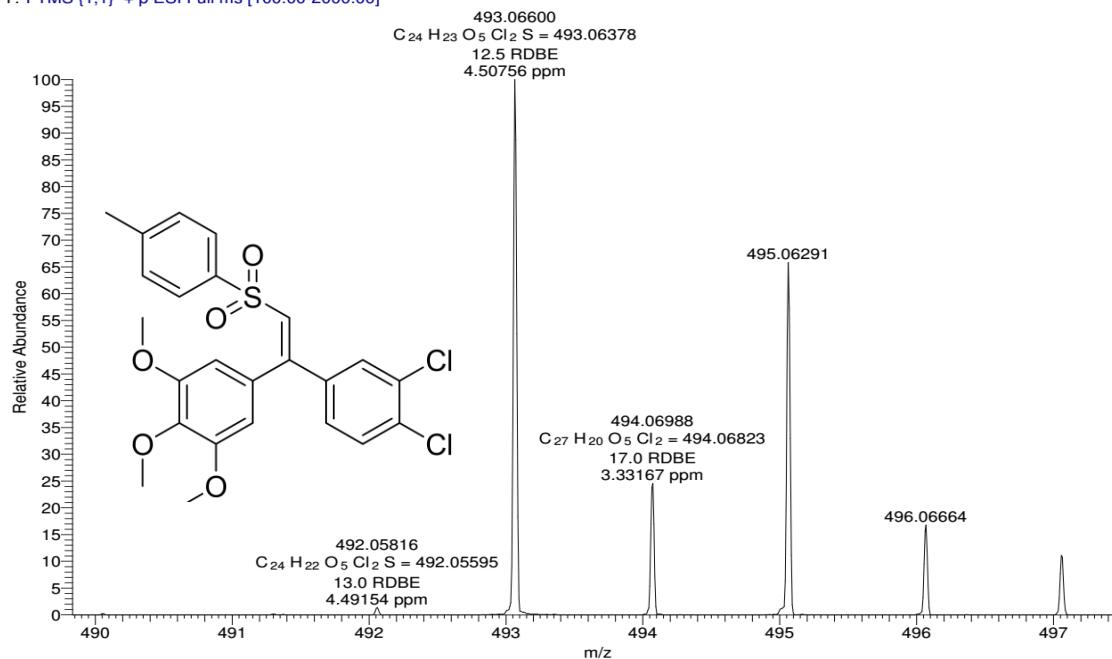
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 01:43:03

Analysed By G SaiKrishna

AKL-CA-4H #7-27 RT: 0.03-0.10 AV: 21 NL: 1.19E6

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



16. AKL-CA-4O, C₂₅H₂₃NO₅S, (Z)-4-(2-tosyl-1-(3,4,trimethoxyphenyl)vinyl)benzonitrile

C:\ICT HRMS-03.09.2014\...AKL-CA-4O

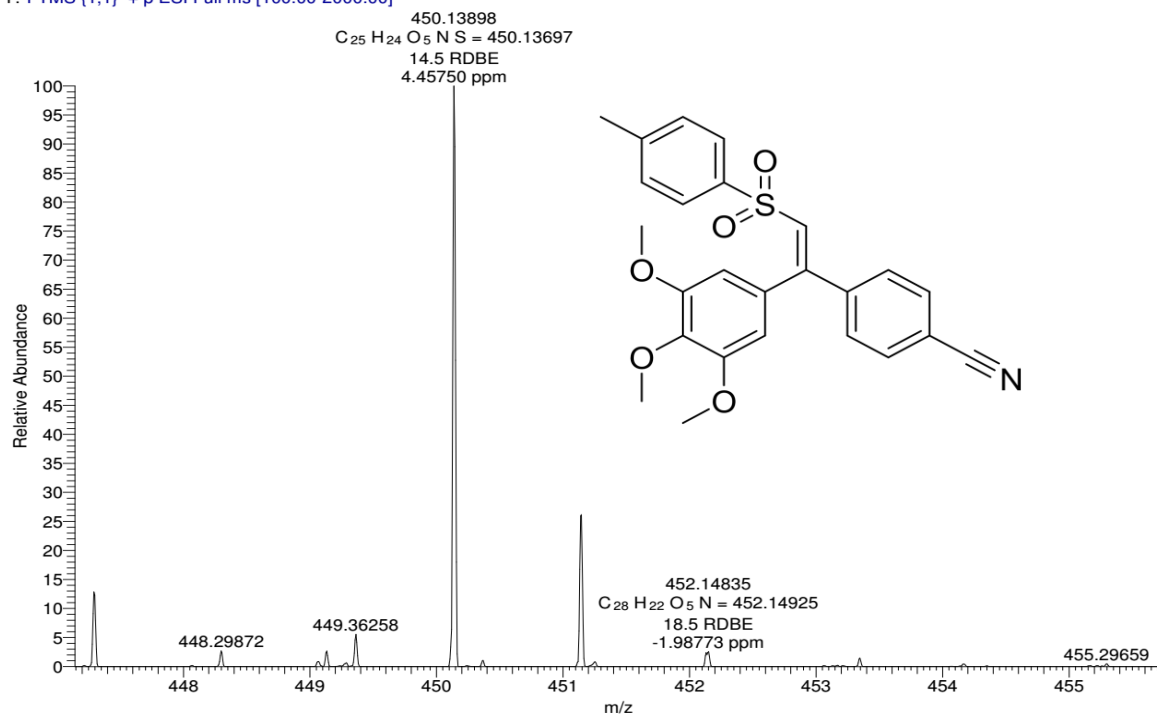
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 01:40:22

Analysed By G SaiKrishna

AKL-CA-4O #11-21 RT: 0.04-0.08 AV: 11 NL: 2.37E5

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



17. AKL-CA-4Q, C₂₅H₂₂F₄O₅S, (E)-5-(1-(2-fluoro-3-(trifluoromethyl)phenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

C:\NICT HRMS-03.09.2014\...AKL-CA-4Q

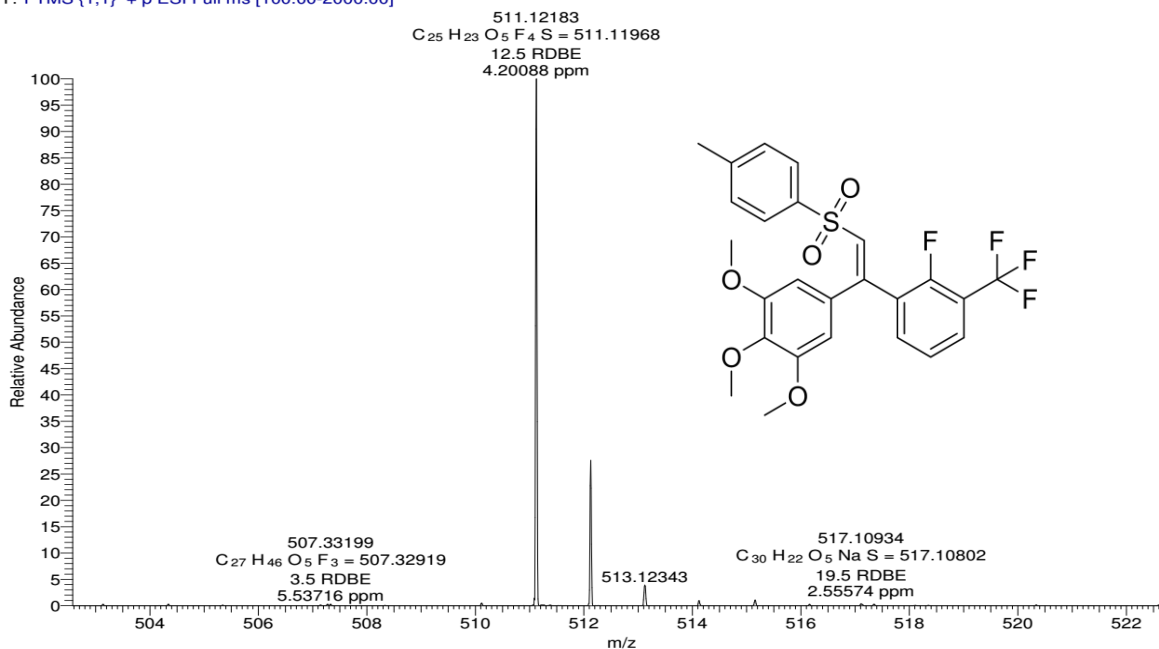
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 01:34:56

Analysed By G SaiKrishna

AKL-CA-4Q #11-21 RT: 0.04-0.08 AV: 11 NL: 6.12E6

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



18. AKL-CA-4R, C₂₅H₂₅FO₅S, (E)-5-(1-(2-fluoro-5-methylphenyl)-2-tosylvinyl)-1,2,3-Trimethoxybenzene

C:\NICT HRMS-03.09.2014\...AKL-CA-4R

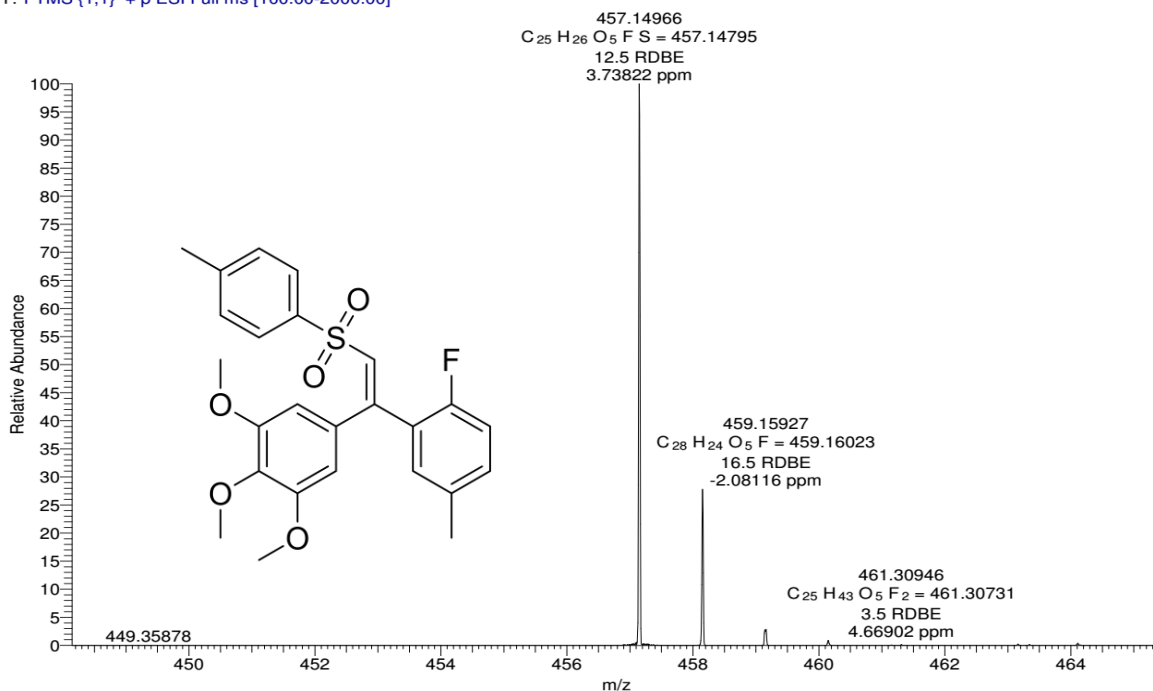
CSIR-INDIAN INSTITUTE OF CHEMICAL TECHNOLOGY
NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 01:32:11

Analysed By G SaiKrishna

AKL-CA-4R #10-20 RT: 0.04-0.07 AV: 11 NL: 1.98E7

T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



19. AKL-CA-4T, C₂₅H₂₅FO₅S, (E)-5-(1-(3-fluoro-4-methylphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene

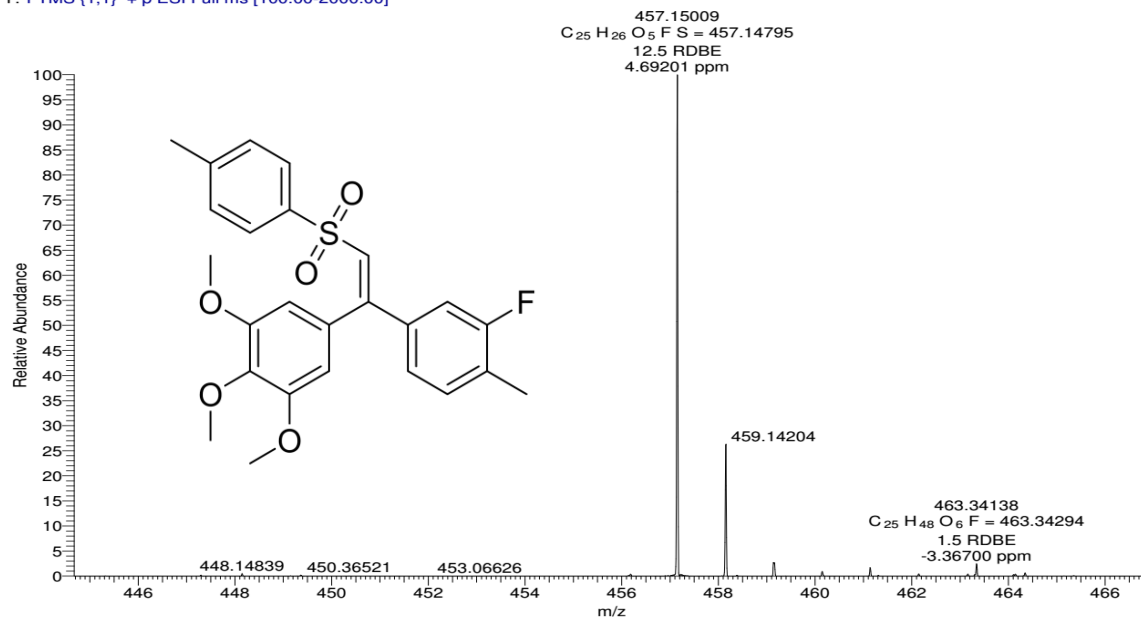
C:\ICT HRMS-03.09.2014\...AKL-CA-4T

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NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 01:26:41

Analysed By G SaiKrishna

AKL-CA-4T #5-19 RT: 0.02-0.07 AV: 15 NL: 7.71E6
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



20. AKL-CA-4U, C₃₀H₂₈O₅S, (Z)-4-(2-tosyl-1-(3,4,5-trimethoxyphenyl)vinyl)-1,1'-biphenyl

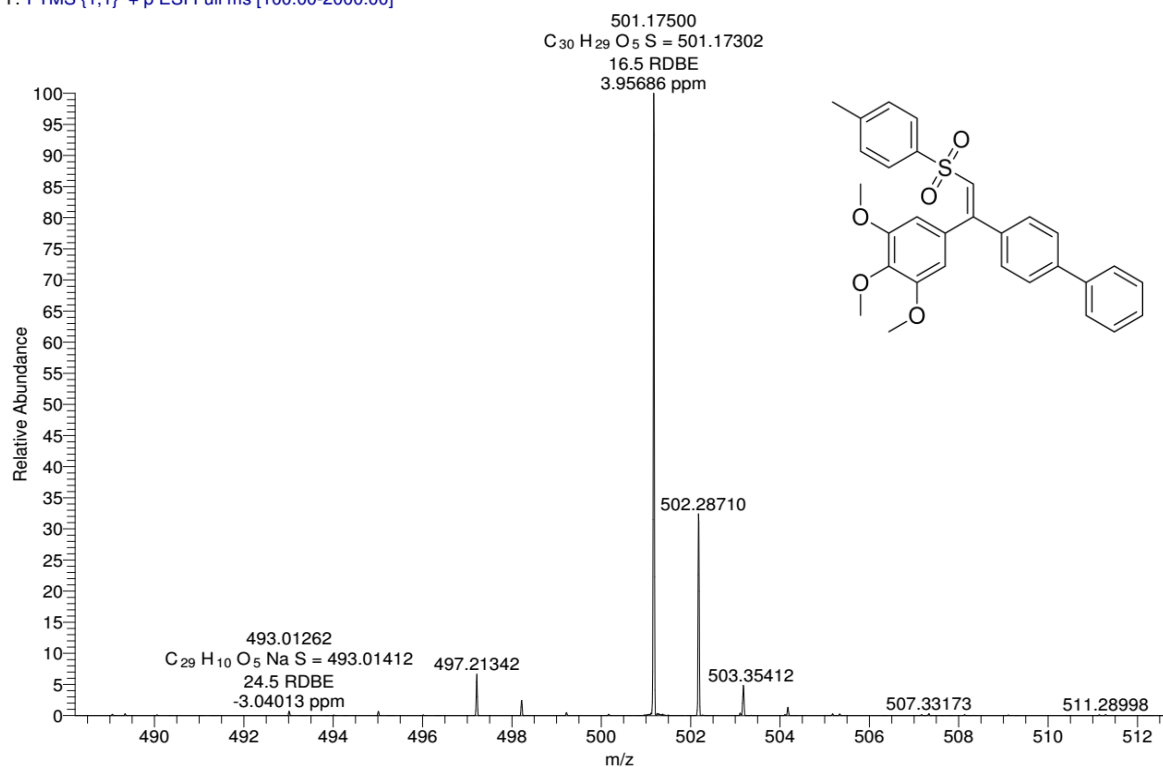
C:\ICT HRMS-03.09.2014\...AKL-CA-4U

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24-03-16 01:23:58

Analysed By G SaiKrishna

AKL-CA-4U #7-22 RT: 0.03-0.08 AV: 16 NL: 4.89E6
T: FTMS {1,1} + p ESI Full ms [100.00-2000.00]



21. AKL-CA-4V, C₁₈H₁₈O₅S, 4-methylphenyl (3,4,5-trimethoxyphenyl)ethynyl sulfone

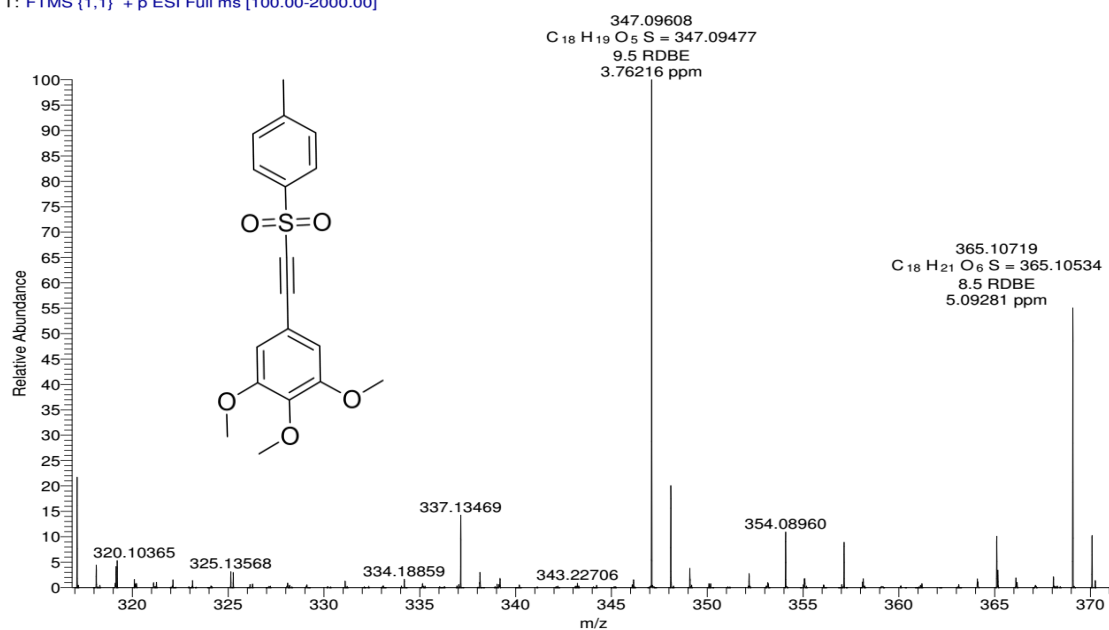
CAICT HRMS-03.09.2014\...AKL-CA-4V

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NATIONAL CENTRE FOR MASS SPECTROMETRY

24-03-16 01:21:17

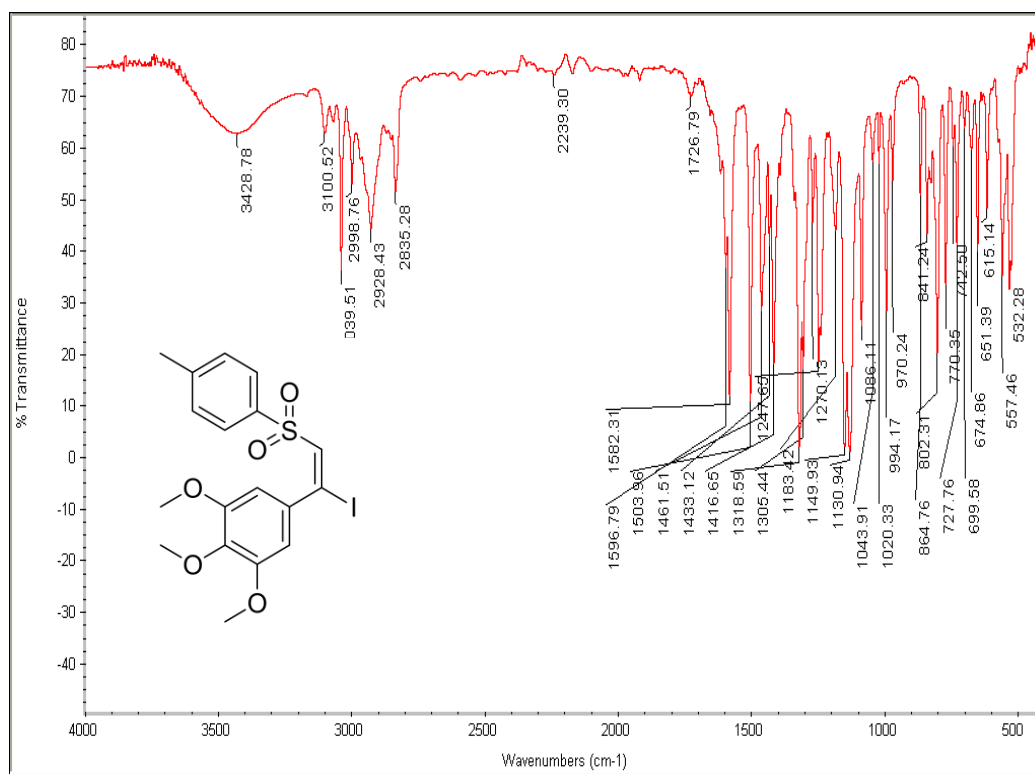
Analysed By G SaiKrishna

AKL-CA-4V #10-19 RT: 0.04-0.07 AV: 10 NL: 2.25E6
T: FTMS (1,1) + p ESI Full ms [100.00-2000.00]

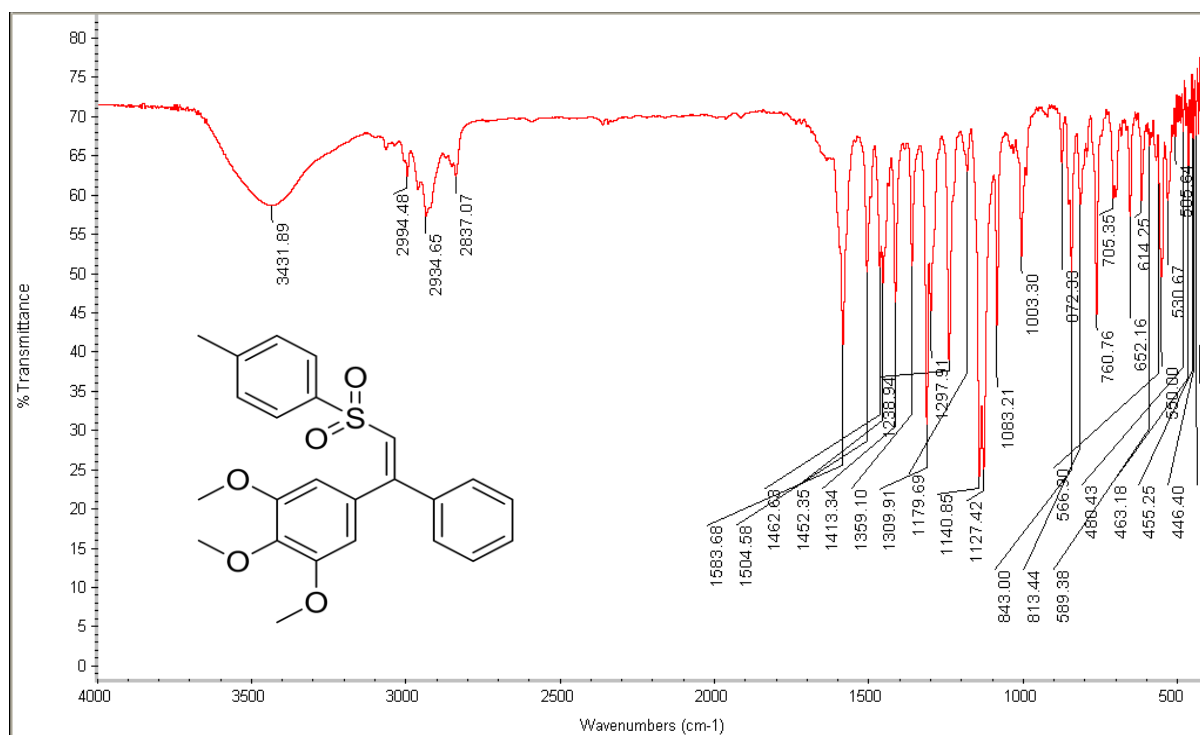


APPENDICES 5: IR SPECTRA OF SYNTHESIZED COMPOUNDS

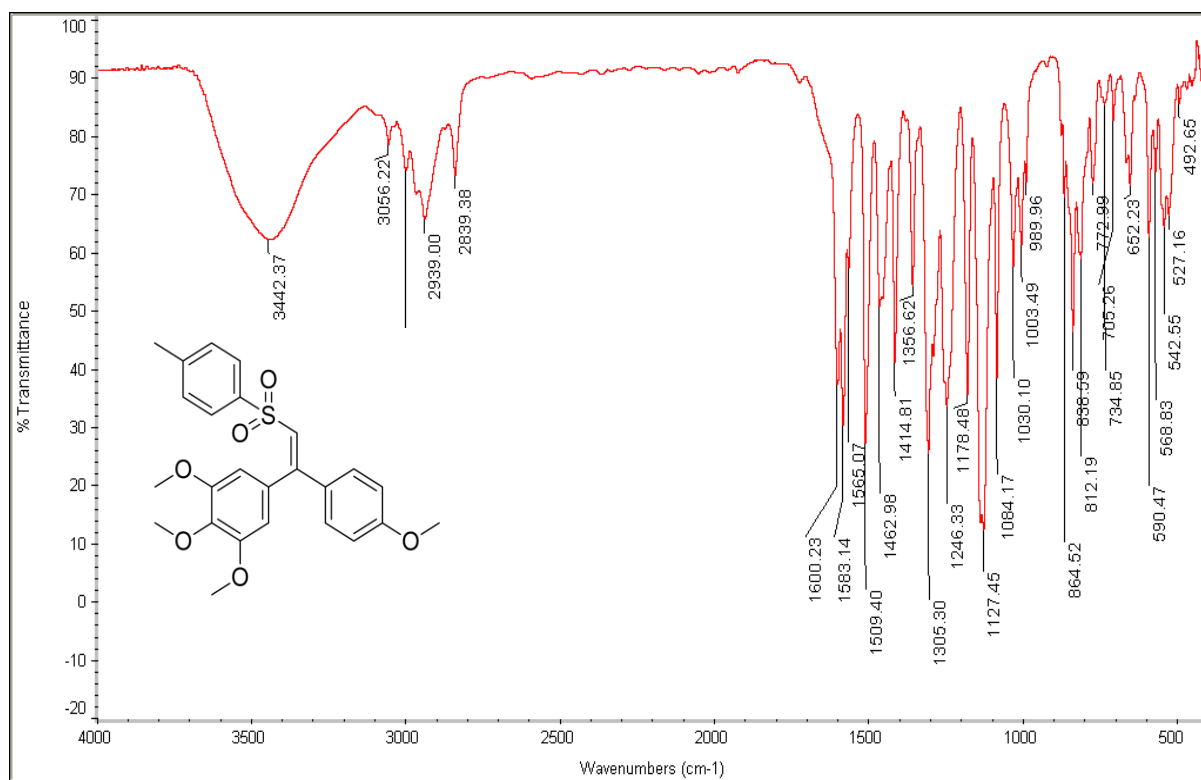
1. AKL-CA-3, $C_{18}H_{19}IO_5S$, (E)-5-(1-iodo-2-tosylvinyl)-1,2,3-trimethoxybenzene



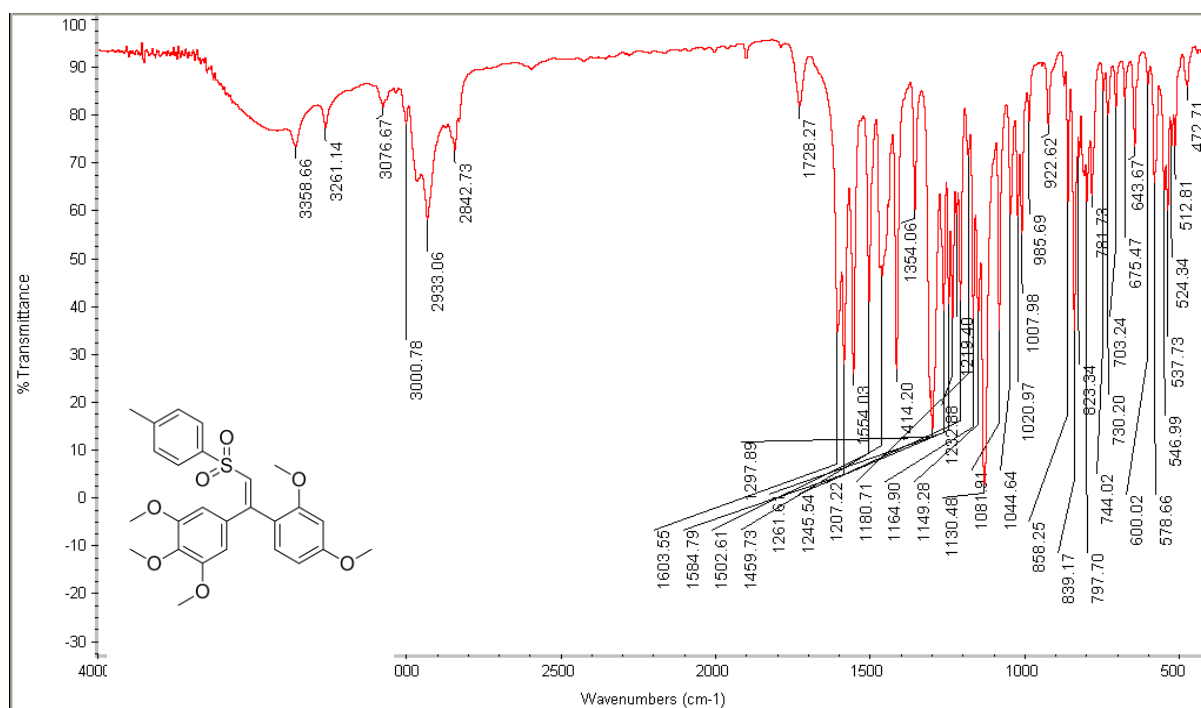
2. AKL-CA-4A, $C_{24}H_{24}O_5S$, (Z)-1,2,3-trimethoxy-5-(1-phenyl-2-tosylvinyl)benzene



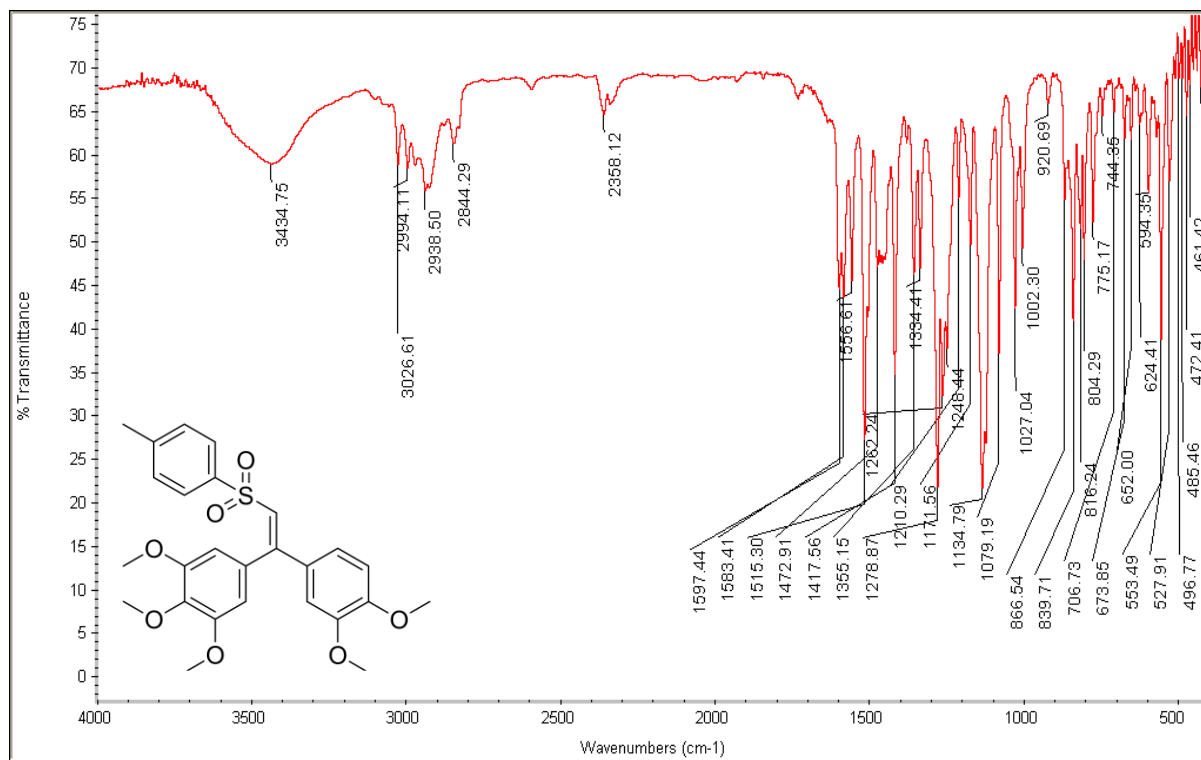
3. AKL-CA-4B, (Z)-1,2,3-trimethoxy-5-(1-(4-methoxyphenyl)-2-tosylvinyl)benzene



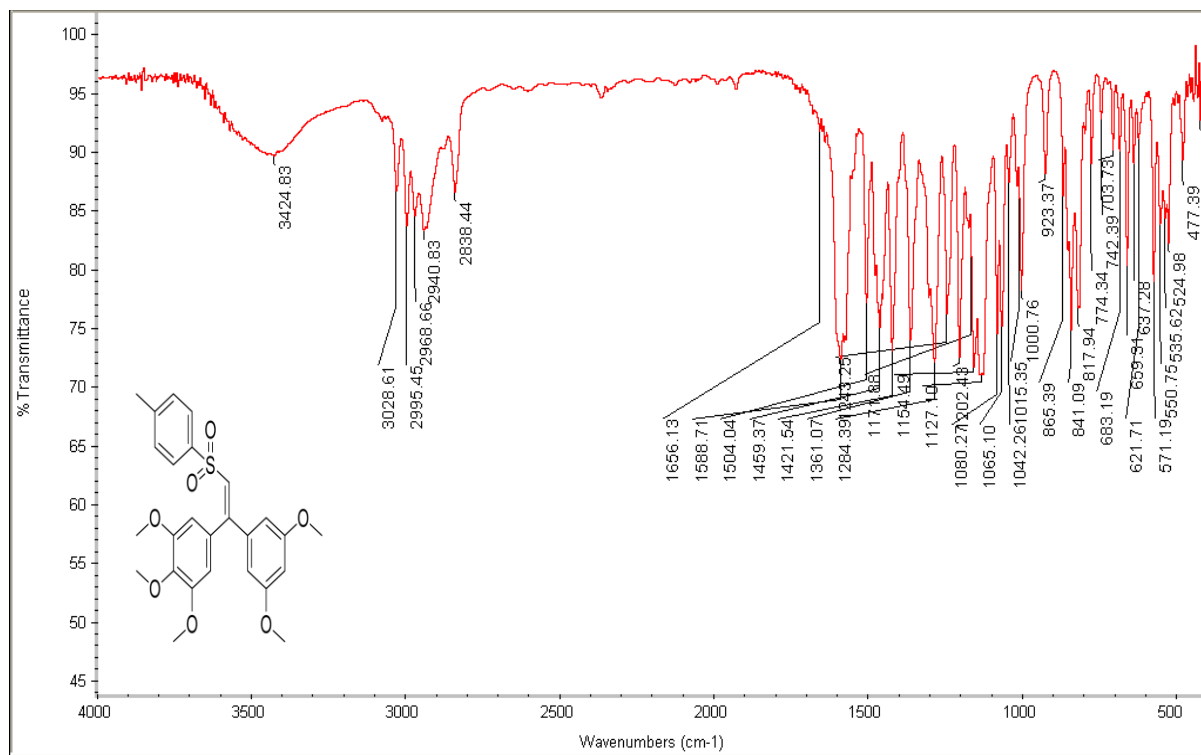
4. AKL-CA-4C, C₂₆H₂₈O₇S, (E)-5-(1-(2,4-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



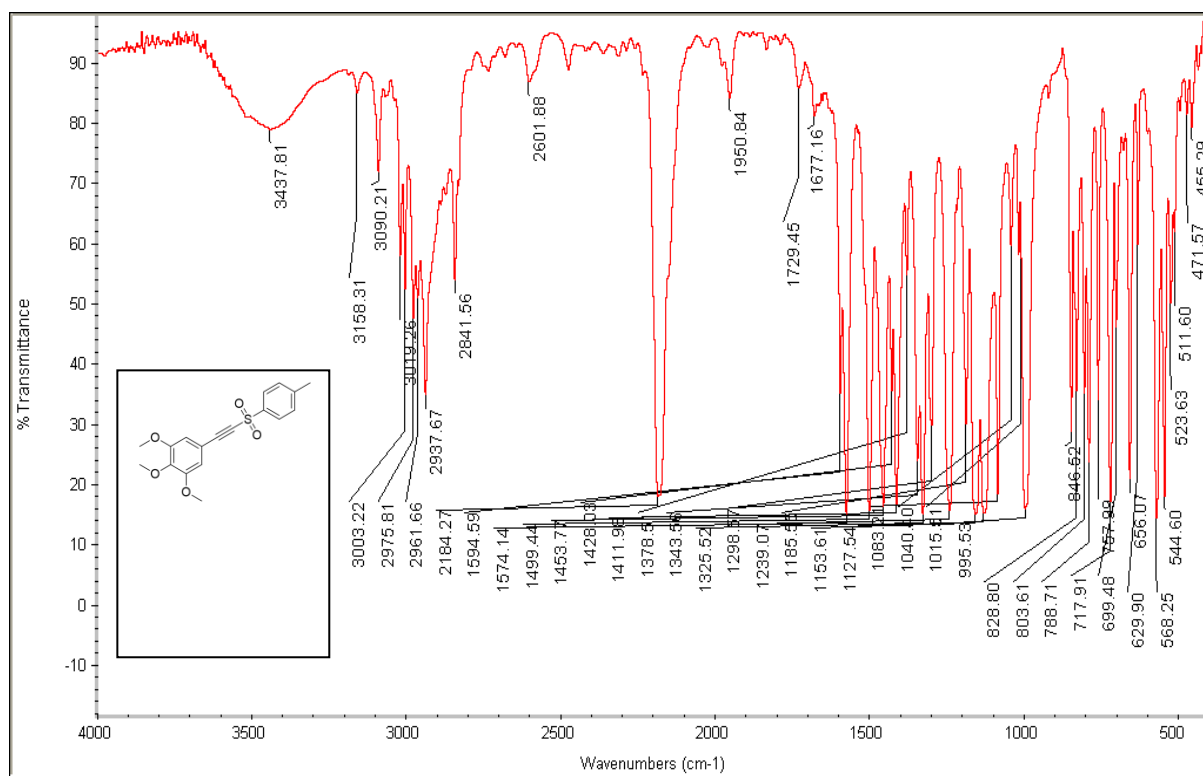
5. AKL-CA-4D, C₂₆H₂₈O₇S, (Z)-5-(1-(3,4-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



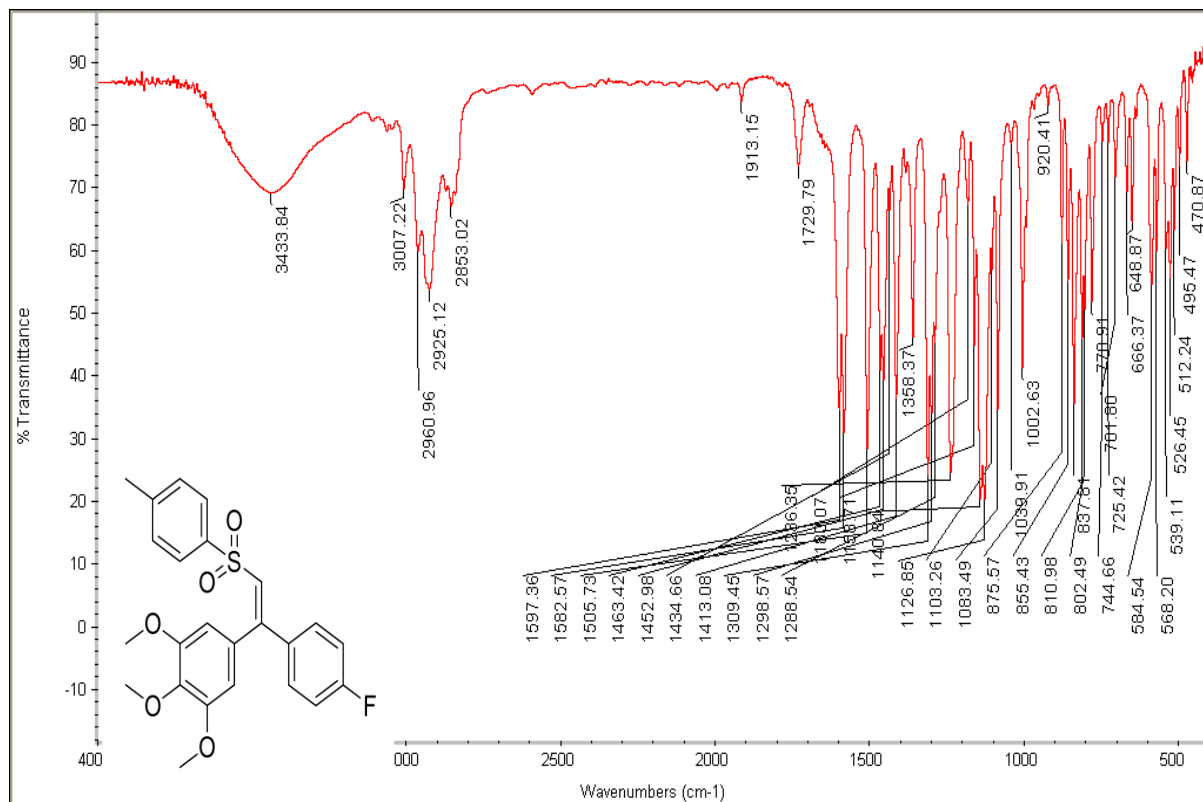
6. AKL-CA-4E, C₂₆H₂₈O₇S, (Z)-5-(1-(3,5-dimethoxyphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



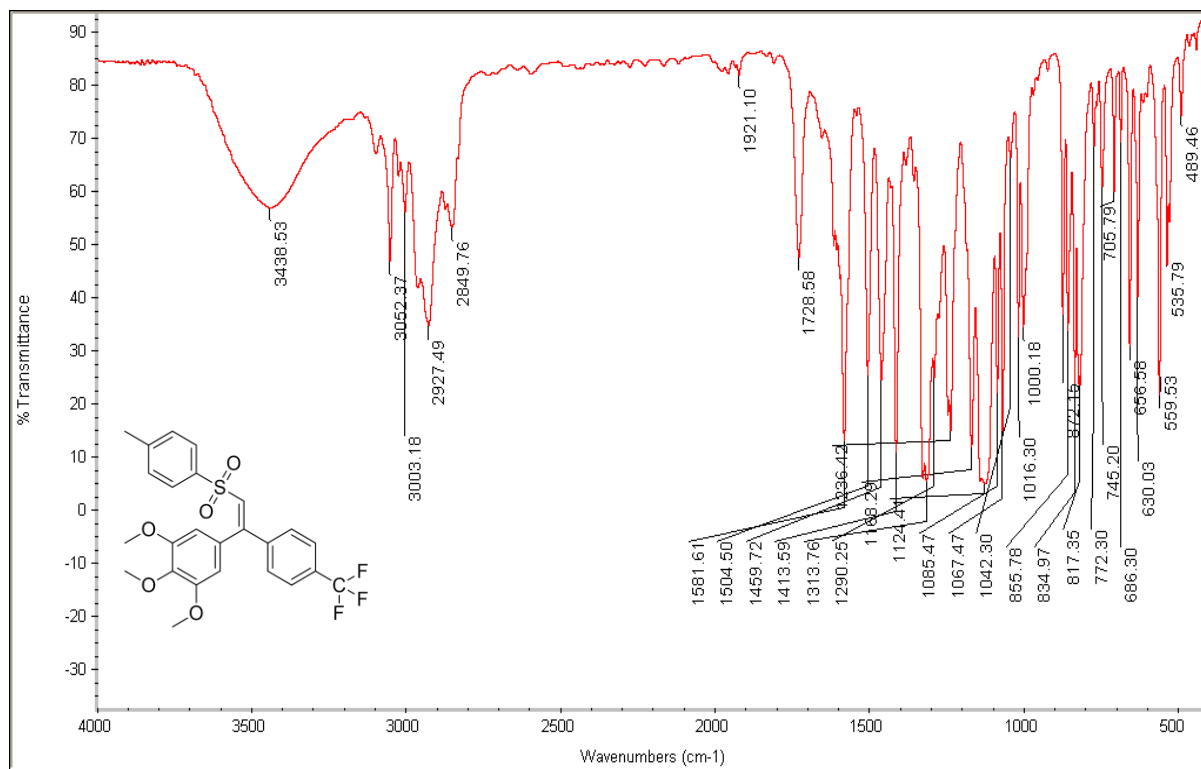
7. AKL-CA-4F, $C_{18}H_{18}O_5S$, 4-methylphenyl (3,4,5-trimethoxyphenyl)ethynyl sulfone



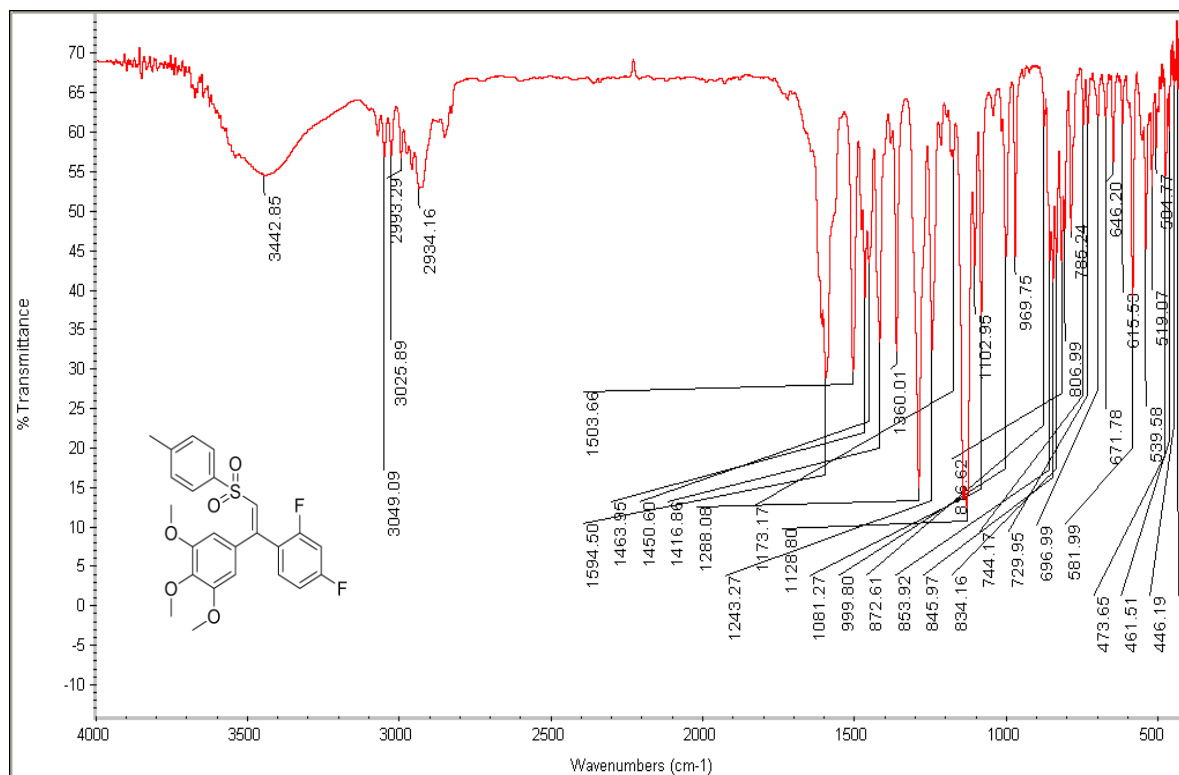
8. AKL-CA-4G, $C_{24}H_{23}FO_5S$, (Z)-5-(1-(4-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



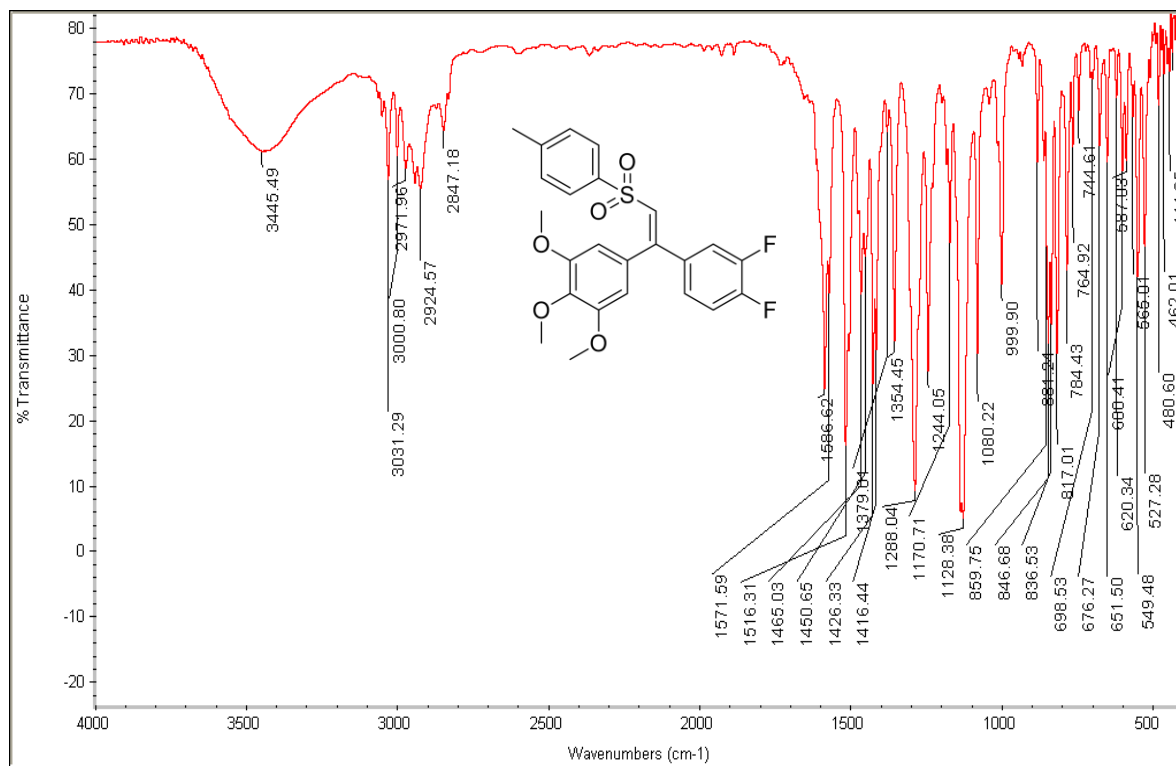
9. AKL-CA-4H, $C_{25}H_{23}F_3O_5S$, (Z)-1,2,3-trimethoxy-5-(2-tosyl-1-(4-(trifluoromethyl)phenyl)vinyl)benzene



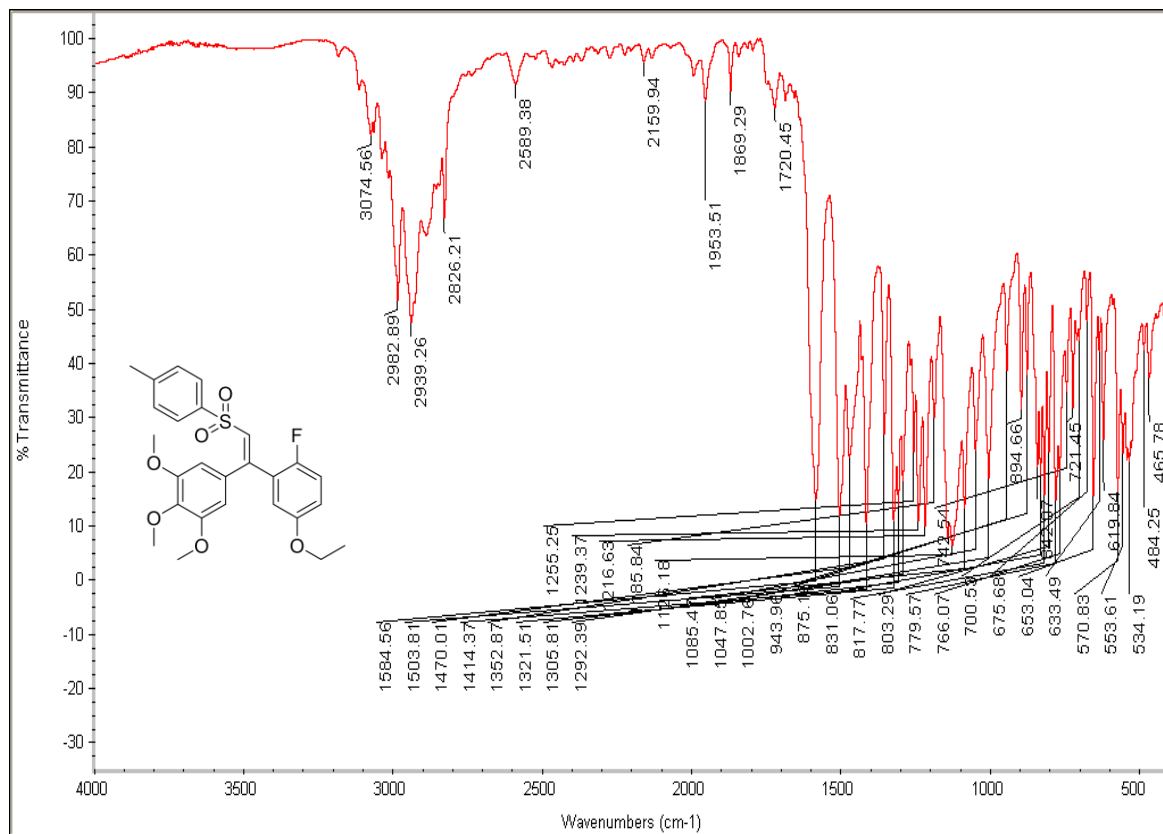
10. AKL-CA-4I, $C_{24}H_{22}F_2O_5S$, (E)-5-(1-(2,4-difluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



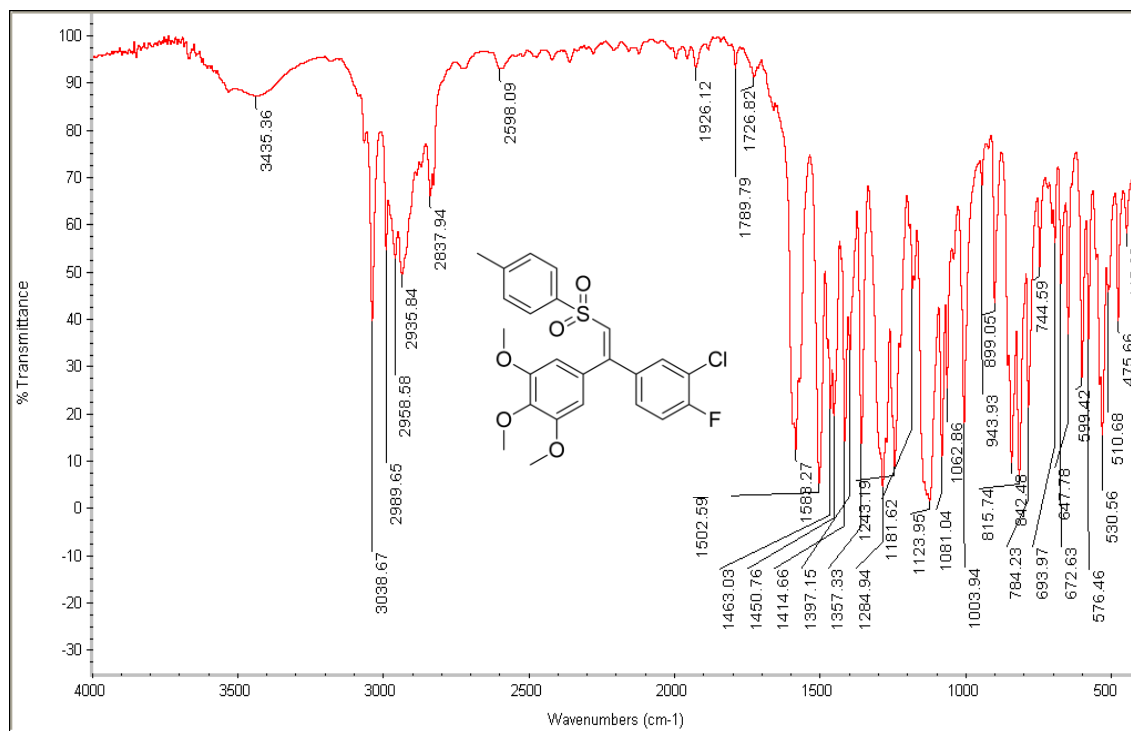
11. AKL-CA-4J, C₂₄H₂₂F₂O₅S, (E)-5-(1-(3,4-difluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



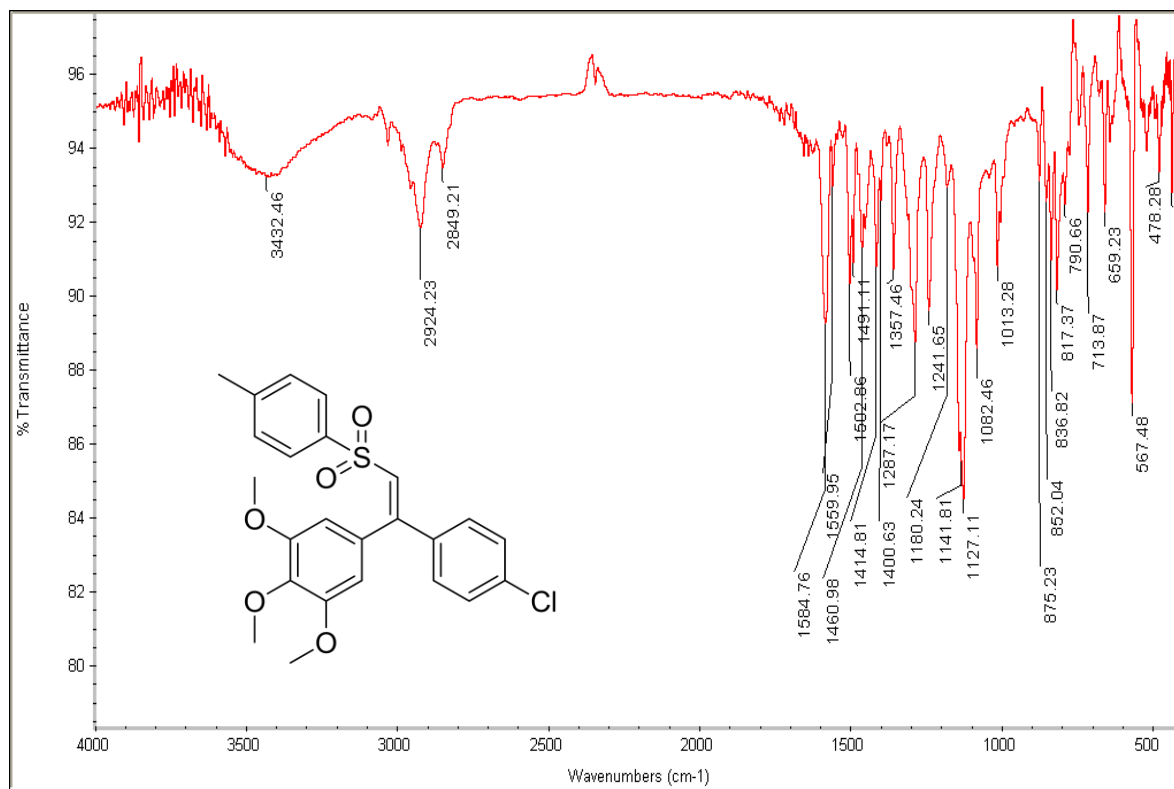
12. AKL-CA-4K, C₂₆H₂₇FO₆S, (E)-5-(1-(5-ethoxy-2-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



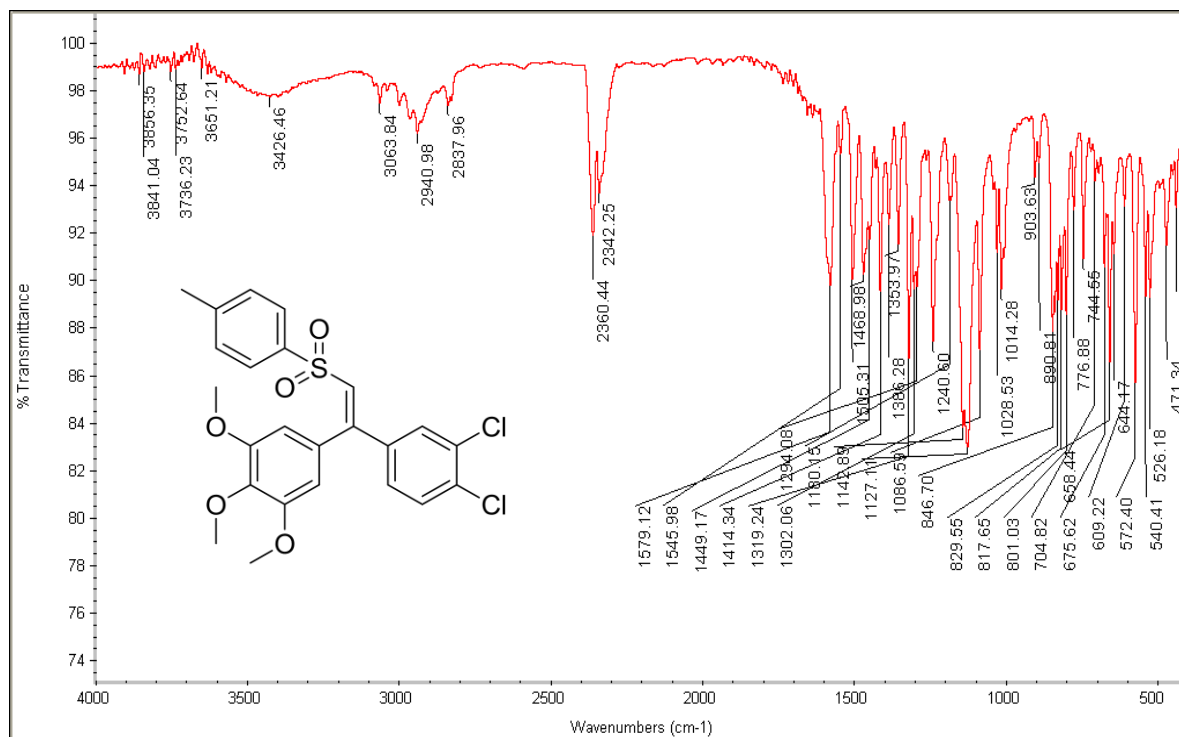
13. AKL-CA-4L, C₂₄H₂₂ClFO₅S, (E)-5-(1-(3-chloro-4-fluorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



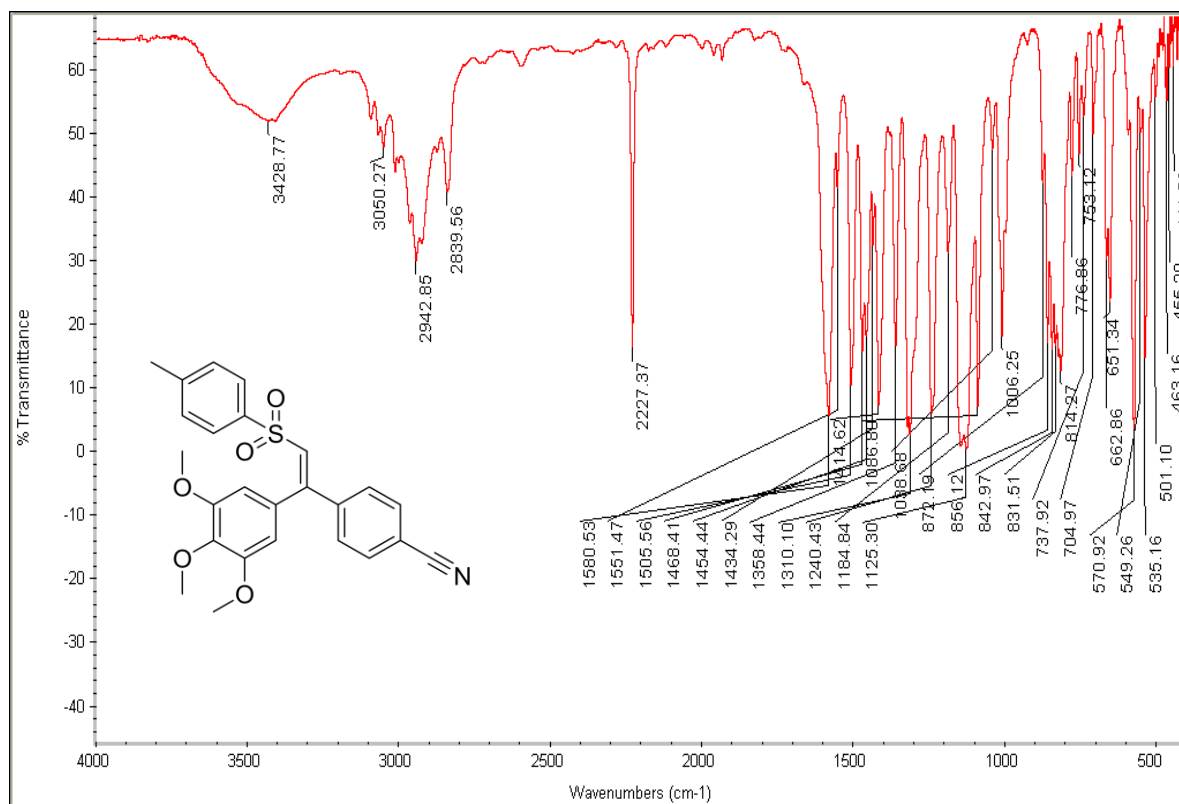
14. AKL-CA-4M, C₂₄H₂₃ClO₅S, (Z)-5-(1-(4-chlorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



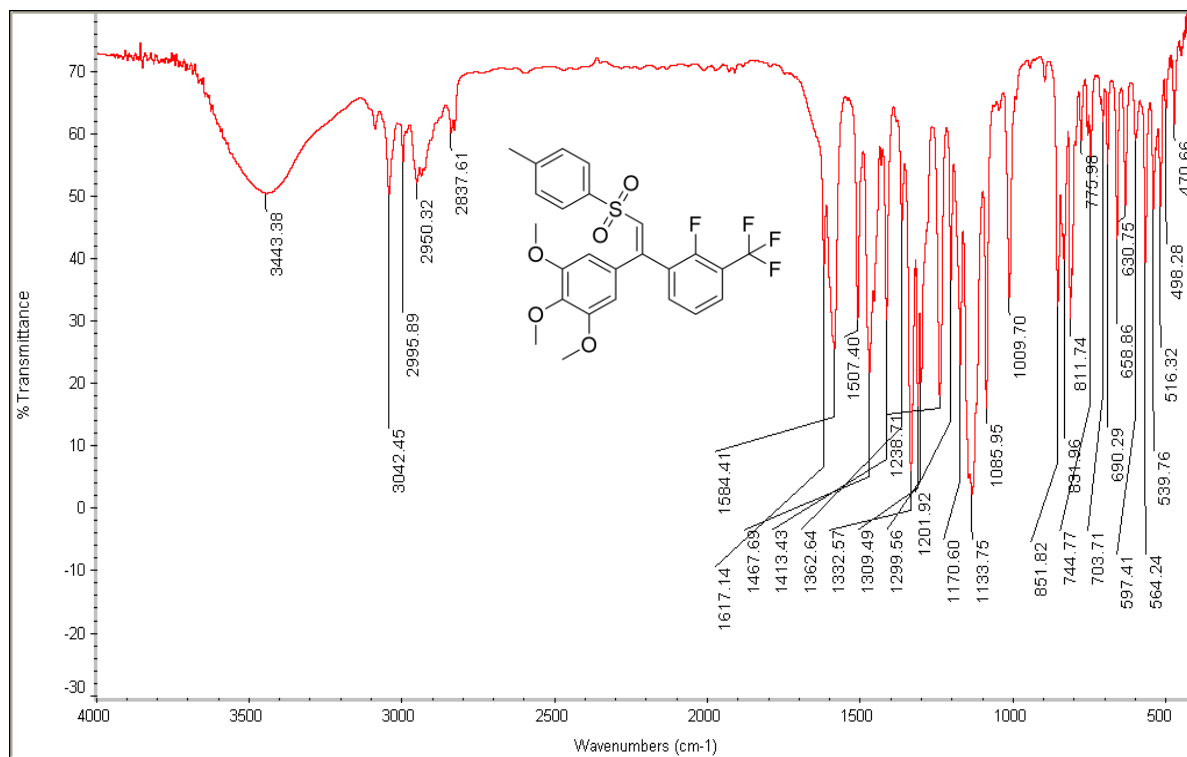
15. AKL-CA-4N, C₂₄H₂₂Cl₂O₅S, (E)-5-(1-(3,4-dichlorophenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



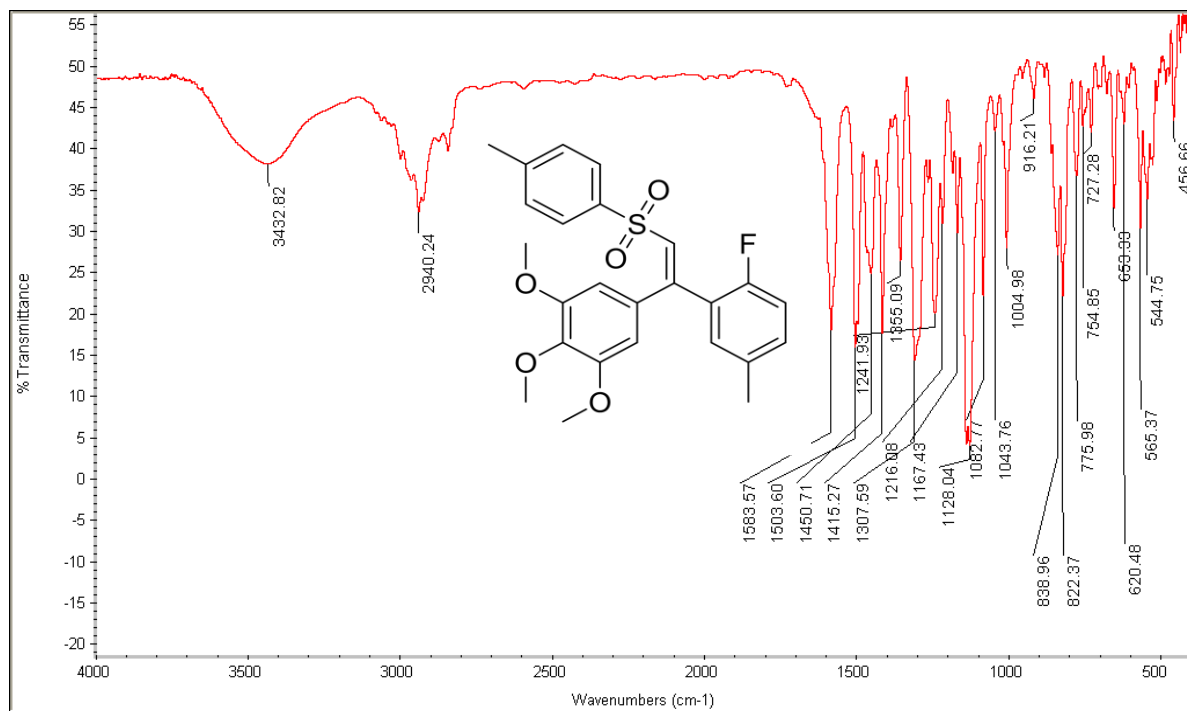
16. AKL-CA-4O, C₂₅H₂₃NO₅S, (Z)-4-(2-tosyl-1-(3,4,trimethoxyphenyl)vinyl)benzonitrile



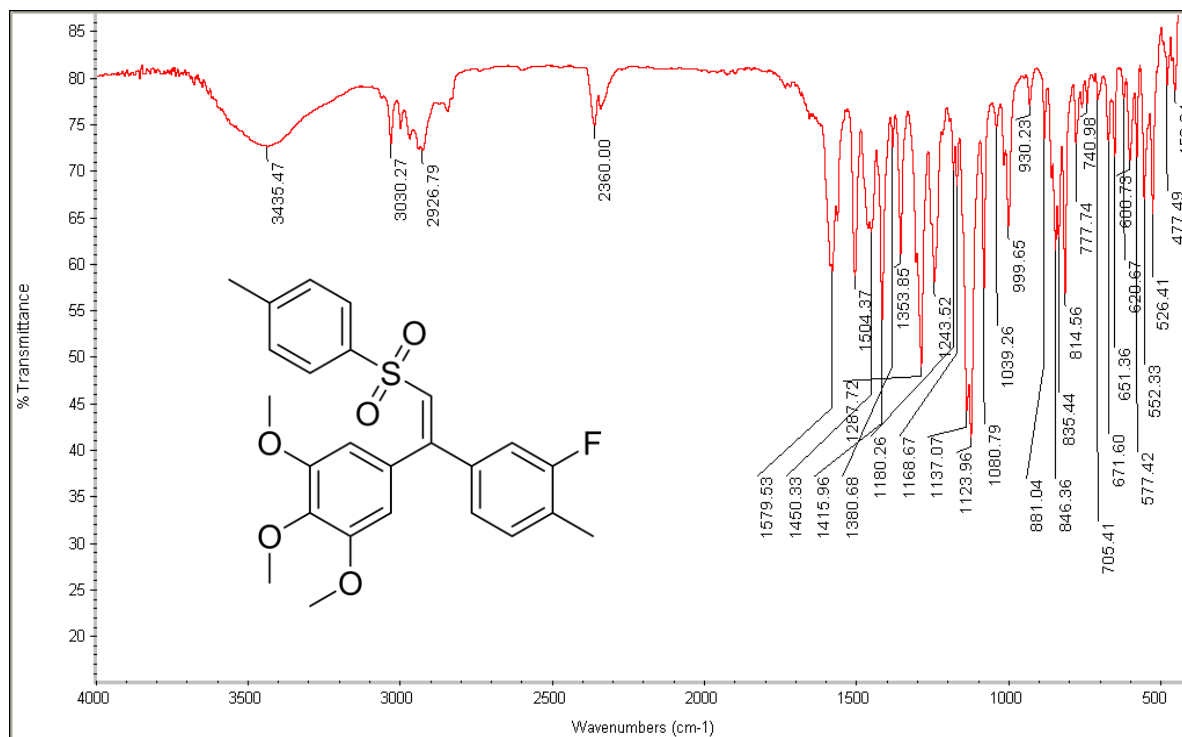
17. AKL-CA-4Q, C₂₅H₂₂F₄O₅S, (E)-5-(1-(2-fluoro-3-(trifluoromethyl)phenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



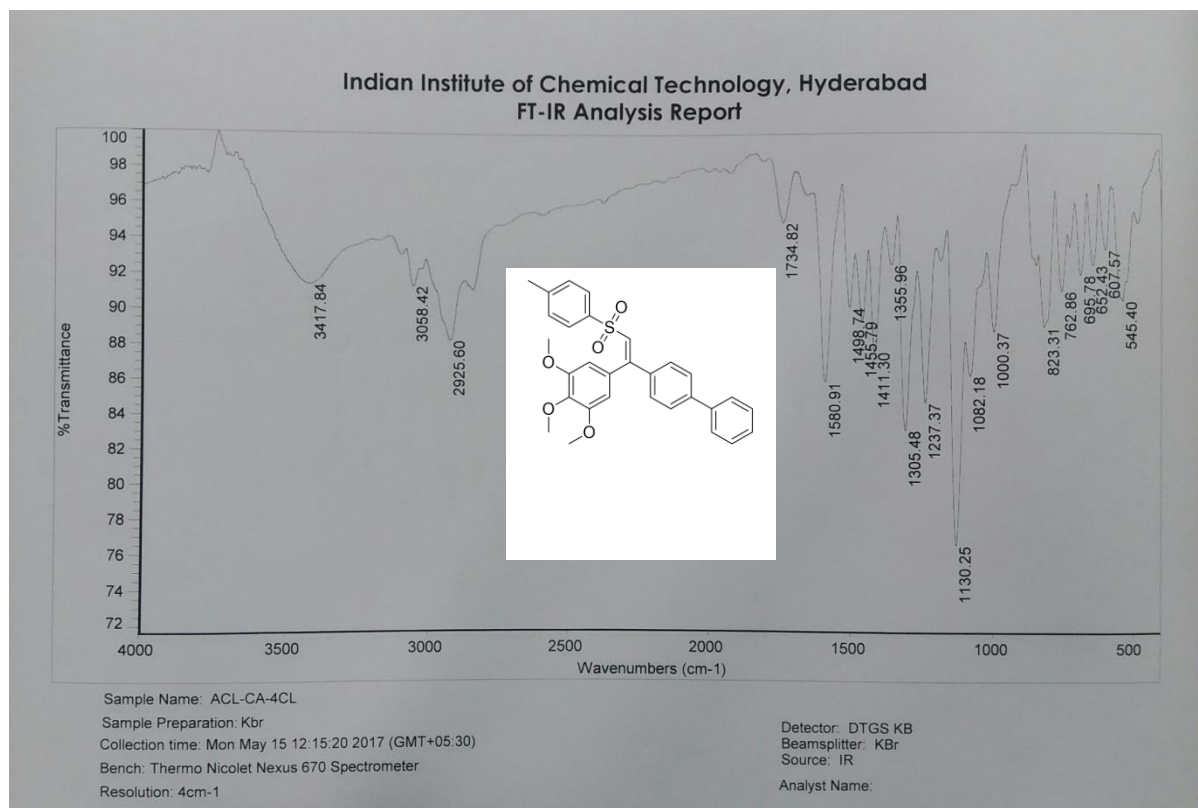
18. AKL-CA-4R, C₂₅H₂₅FO₅S, (E)-5-(1-(2-fluoro-5-methylphenyl)-2-tosylvinyl)-1,2,3-Trimethoxybenzene



19. AKL-CA-4T, C₂₅H₂₅FO₅S, (E)-5-(1-(3-fluoro-4-methylphenyl)-2-tosylvinyl)-1,2,3-trimethoxybenzene



20. AKL-CA-4U, C₃₀H₂₈O₅S, (Z)-4-(2-tosyl-1-(3,4,5-trimethoxyphenyl)vinyl)-1,1'-biphenyl



21. AKL-CA-4V, C₁₈H₁₈O₅S, 4-methylphenyl (3,4,5-trimethoxyphenyl)ethynyl sulfone

