SYNTHESIS OF BAICALEIN DERIVATIVES AS ANTI-DENGUE AGENTS

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จุฬาลงกรณ์มหาวิทยาลัย
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การสังเคราะห์อนุพันธ์ไบคาไลน์เพื่อเป็นสารต้านไวรัสไข้เลือดออก

น.ส.อภิญญา ปาฏิโก

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อภิญญา ปาฏิโก : การสังเคราะห์อนุพันธ์ไบคาไลน์เพื่อเป็นสารต้านไวรัสไข้เลือดออก. (SYNTHESIS OF BAICALEIN DERIVATIVES AS ANTI-DENGUE AGENTS) อ.ที่ปรึกษาหลัก : ผศ.ธนธรณ์ ขอทวีวัฒนา

ไข้เลือดออกเป็นโรคที่เกิดจากเชื้อไวรัสที่มียุงเป็นพาหะ ในปัจจุบันถือเป็นปัญหาด้านสุขภาพที่สำคัญระดับนานาชาติ ด้วยเหตุนี้การพัฒนายาต้านไข้เลือดออกจึงเป็นที่ต้องการอย่างมาก ไบคาไลน์เป็นสารประกอบฟลาโวนที่แยกได้จากรากของ *Scutellaria baicalensis* และ *Scutellaria lateriflora* ก่อนหน้านี้ได้มีการรายงานว่าอนุพันธ์ของไบคาไลน์มีแนวโน้มแสดงการออกฤทธิ์ต้านเชื้อไวรัสไข้เลือดออกที่ดี โดยในงานวิจัยนี้อนุพันธ์ของไบคาไลน์ใหม่ 5 ตัวและที่มีอยู่แล้ว 13 ตัว ถูกสังเคราะห์ผ่านการสังเคราะห์แบบ semi synthesis จากไบคาไลน์ด้วยการดัดแปลงกลุ่มไฮดรอกซี่ตรงคาร์บอนตำแหน่งที่ 5, 6, 7 และการแทนที่ไฮโดรเจนอะตอมตรงคาร์บอนตำแหน่งที่ 8 บนวงแหวน A และการสังเคราะห์แบบ total synthesis ซึ่งมีหกขั้นตอน โดยมีการดัดแปลงโดยการแทนที่ไฮโดรเจนอะตอมของคาร์บอนที่ตำแหน่งพาราบนวงแหวน B ซึ่งมีทั้งหมู่ดึงอิเล็กตรอน (electron withdrawing group) และหมู่ให้อิเล็กตรอน (electron donating group) โดยโครงสร้างของอนุพันธ์ไบคาไลน์ที่สังเคราะห์ทั้งหมดได้รับการยืนยันโครงสร้างโดย ^{1}H และ ^{13}C NMR สารประกอบของอนุพันธ์ไบคาไลน์ส่วนใหญ่แสดงฤทธิ์ต้านไข้เลือดออกชนิดที่ 2 (DENV2) ในเซลล์ LLC/MK2 ที่ความเข้มข้น 10 ไมโครโมลาร์ ยกเว้นอนุพันธ์ของ 8-โบรโม-5,6,7-ไตรโพรไพโอนิล (1h) และ 4'-อะมิโน (6d) การมีอยู่ของอนุพันธ์โพรพิโอนิล (1d, EC_{50} = 0.070 ± 0.015 μM และ 1e, EC_{50} = 0.068 ± 0.040 μM) แสดงการยับยั้งการมีฤทธิ์ของ DENV2 อย่างมีประสิทธิภาพ และไม่มีพิษต่อเซลล์ปกติ (1d, %viability = 83.79±2.61 และ 1e, %viability = 89.62±5.95) ความสัมพันธ์ระหว่างโครงสร้างกับฤทธิ์การยับยั้งเชื้อไวรัสไข้เลือกออก (Structure-Activity Relationship, SAR) ของสารประกอบเหล่านี้แสดงให้เห็นว่าหมู่แทนที่ที่เป็นหมู่ดึงอิเล็กตรอน สามารถเพิ่มฤทธิ์ต้านไข้เลือดออกได้ โดยเฉพาะที่คาร์บอนตำแหน่ง 6, 7 และ 8 บนวงแหวน A และตำแหน่งพาราบนวงแหวน B นอกจากนี้สารประกอบไบคาไลน์ที่สังเคราะห์ได้ส่วนใหญ่มีสมบัติทางเคมีกายภาพและคุณสมบัติการเป็นยาที่ดี ตามกฎของ Lipinski's rule of five และ Verber parameters ดังนั้นจึงสรุปได้ว่าการปรับเปลี่ยนไบคาไลน์ทั้งวงแหวน A และ B อาจช่วยเพิ่มประสิทธิภาพในการยับยั้งไข้เลือดออก และอนุพันธ์ไบคาไลน์ที่มีการแทนที่ด้วยหมู่ดึงอิเล็กตรอน อาจกลายเป็นตัวเลือกที่น่าสนใจสำหรับยาต้านไวรัสไข้เลือดออกในอนาคต

สาขาวิชา เคมี ลายมือชื่อนิสิต .....

ปีการศึกษา 2564 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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Dengue, a mosquito-borne viral disease, is now considered a major international health concern, and hence the development of a new anti-dengue drug is highly desirable. Baicalein is a flavone isolated from the roots of *Scutellaria baicalensis* and *Scutellaria lateriflora*. Baicalein derivatives have been shown previously to exhibit a promising anti-dengue activity. In this study, five novel and thirteen known baicalein derivatives were synthesized via semi synthesis from baicalein with modifications at C-5, -6, -7, and -8 positions on ring A and a six-step total synthesis with modifications at the para position on the ring B with a range of electron-withdrawing or electron-donating groups. The structures of the synthesized derivatives were confirmed by ^{1}H and ^{13}C NMR. Most of the compounds exhibited the anti-dengue activity (DENV2) in LLC/MK2 cell at the concentration of 10 μM, except for the 8-bromo-5,6,7-tripropionyl (1h) and 4'-amino (6d) derivatives. Strikingly, the presence of propionyl derivatives (1d, EC_{50} = 0.070 ± 0.015 μM and 1e, EC_{50} = 0.068 ± 0.040 μM) showed efficiently inhibited DENV2 activity with great viability (1d, viability = 83.79±2.61 and 1e, viability = 89.62±5.95). The structure-activity relationship (SAR) of these compounds demonstrated that electron-withdrawing substituents could Improve anti-dengue activity, especially at the C-6, -7, and -8 positions on ring A and para position on ring B. Moreover, most of the synthetic baicalein compounds have good physicochemical properties and drug-likeness according to Lipinski's rule of five and Verber parameters. In conclusion, modification on both ring A and B of baicalein can potentially enhance the efficiency of dengue inhibition and the derivatives with electron-withdrawing substituents could become interesting candidates for anti-dengue agents in the future.

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Chapter I

Introduction

1. Background research

Dengue is a mosquito-borne viral disease which is now considered a major international health concern with over 390 million infections per year according to the World Health Organization (WHO).[24] In the case of Thailand, Dr Opas Kankawinpong, Director-General of the DDC, said that there had been three deaths from the dengue virus during the first three months of this year compared to six fatalities in the whole of last year. Dengue fever derived from dengue virus type 2 (DENV-2) can induce severe symptoms and a high mortality rate.[20] Currently, there is no specific antiviral drug available for treating dengue infection. However, the only available vaccine, Dengvaxia® (CYD-TDV), has suffered from various limitations.[11] Therefore, the development of a new anti-dengue drug is highly desirable.

In the last few decades, several nature flavones demonstrated significant anti-dengue activity.[2] Recently, the study of synthetic flavones with anti-dengue activity have gained significant attention.[29] Among them, 8-bromobaicalein was found to be the most potent derivative with almost 5-fold greater antiviral activity against DENV-2 than the original baicalein. However, the scope of the derivatives in this work is mostly limited to the halogenated species, thus a broad range of possible structural modifications remains unexplored.

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Therefore, in this work, we aim to expand the scope of the baicalein analogs even further, including several substitution patterns on both rings A and B using both semi- and total synthesis approaches in order to construct the relationship between chemical structure and the anti-dengue activity of the synthesized flavone analogs. The knowledge of both the structure-activity relationship (SAR) will be tremendously crucial for the further developing of the flavone-based anti-dengue agents in the future.

2. Literature review

2.1 Dengue virus

Dengue virus is a mosquito-borne viral infection transmitted by the *Aedes mosquito* (*Ae. aegypti* or *Ae. albopictus*). Dengue virus is transmitted by mosquito-to-human and human-to-mosquito by the bites of infected female mosquitoes.

Dengue virus spreads throughout the tropics. The local risk varies with rainfall, temperature, and relative humidity. and the unplanned rapid urbanization. Dengue fever can cause a wide range of diseases ranging from asymptomatic disease to flu-like symptoms in those who are infected. Severe dengue has an increased risk of death if not treated properly which mortal rate can be reach to 20%. Severe dengue was first recognized in 1950 during dengue outbreaks in the Philippines and Thailand.[22]

Severe dengue also affects most Asian and Latin American countries. Severe dengue has become the leading cause of hospitalizations and deaths among children and adults in these regions. According to the World Health Organization (WHO), dengue fever is now a major international health problem which indicates 390 million cases of dengue virus annually. Dengue infection has increased 30-fold over the past 50 years, and more than 50% of the world's population living in risk areas.[22]

2.3 Symptoms of dengue fever

The symptoms of dengue fever can be confused with other diseases that can cause fever, aches, or a rash. The most common symptom of dengue fever is high fever (40°C), followed by two symptoms such as nausea, vomiting, rash, and aches (eye pain, often pain behind the eyes, muscle, joint, or bone pain). Dengue fever usually lasts 2-7 days. Most people recover after about a week. Severe cases of dengue fever are called the critical phase about 3-7 days after the onset of illness. Severe dengue is a potentially fatal complication due to plasma leakage fluid accumulation respiratory distress, severe bleeding or organ impairment, patient's fever decreased (below 38°C) and warning signs such as severe abdominal pain, vomiting, rapid breathing, scurvy restless and vomiting blood. If the patient shows these symptoms during a crisis. Close monitoring during the next 24-48 hours is essential to be able to provide appropriate medical care to avoid complications and the risk of death.[4]

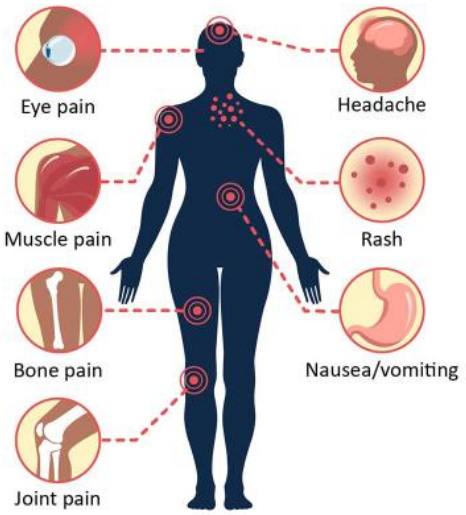

Figure 1 Main dengue fever symptoms

2.4 Currently available medication and vaccination

There is no specific treatment for dengue fever. The symptoms of muscle aches, pains and fever were reduced by acetaminophen or paracetamol. Furthermore, NSAIDs (non-steroidal anti-inflammatory drugs), such as ibuprofen and aspirin should be avoided because these drugs affect thinning the blood, and disease with risk of hemorrhage, blood thinners may exacerbate the prognosis.[4]

Dengvaxia® (CYD-TDV) is the first dengue vaccine developed by Sanofi Pasteur in December 2015 and approved by regulatory authorities in ~20 countries. However, the analysis showed that the subset of trial participants at the time of first vaccination had a higher risk of more severe dengue. Therefore, use of the vaccine is targeted for persons living in endemic areas, ranging from 9-45 years of age, who have had at least 1 documented dengue virus infection previously.[11]

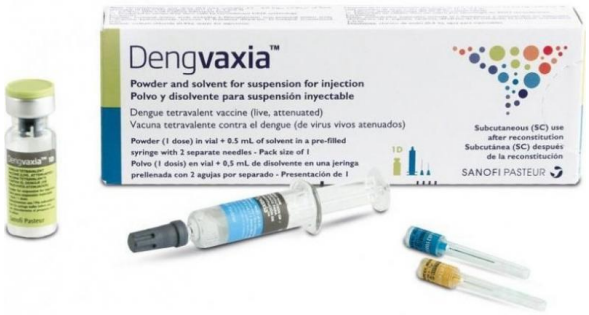

Figure 2 The first vaccine Dengvaxia® (CYD-TDV)

2.5 Dengue virus structure and their functions

The RNA genome encodes three structural proteins (core protein, membrane associated protein, and envelope protein) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). There is a polyprotein precursor in the order NH_2-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-COOH[21] as shown in figure 3.

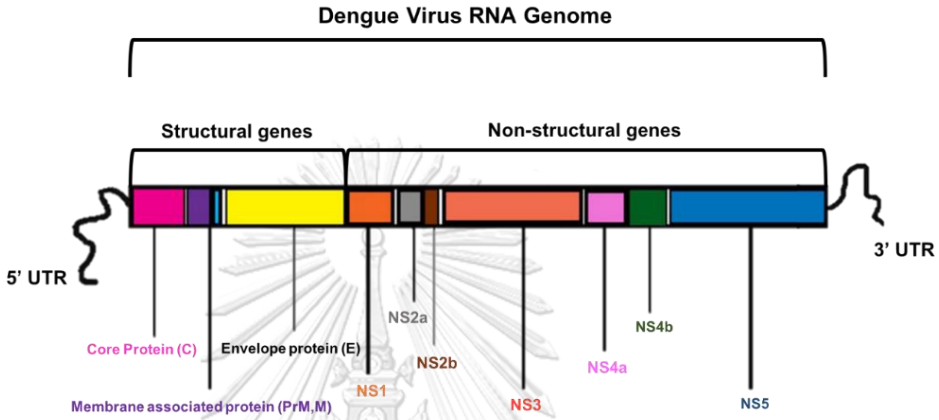

Figure 3 The dengue virus RNA genome[21]

2.5.1 The structural proteins

The structural capsid protein (C protein) has several residues of basic amino acids such as lysine and arginine, which allow these to interact with the viral genome for construction of the nucleocapsid. The membrane associated protein (prM protein) is a glycoprotein which cleavages with the N-terminal region, originating the M protein. The cleavage process is associated with the virus maturation. The M protein is one of the two proteins that form the viral envelope, and it is involved in the penetration of the virus of the host cell. The envelope protein (E protein) plays an important role in the virus and the host cells causing cell tropism, a membrane fusion cell, resulting in the neutralization of antibodies and hemagglutination. This glycoprotein has three distinct domains bound by sulphur-sulphur interaction: I, II, and III domains have 495 amino acids.[21]

2.5.2 The nonstructural proteins

The NS1 protein has a N-linked glycoprotein involved in the RNA replication. The NS1 protein exists in multiple forms in different compartments

of insect cells and dengue virus-infected mammals such as synthesized as a monomer and, after processing in endoplasmic reticulum and the trans-Golgi network secreted as a hexameric lipoprotein particle into the extracellular space and blood. The NS1 protein can be found in cell culture mediums or serums from infected patients. The NS2A is a hydrophobic protein that was previously shown to be important for viral replication and pathogenesis. NS2A participates in viral RNA synthesis, viral assembly, virus-induced membrane formation, contributes to the production of NS1, and inhibits interferon. The NS2B is a co-factor of the NS3 protein. NS2B cofactor is activated before the catalytic activity of the NS3 protein. NS3 acts as a chymotrypsin-like serine protease for polyprotein processing, RNA triphosphatase (RTP/NTPase) for capping nascent viral RNA and a helicase for unwinding the double-stranded replicative form of RNA. The flavivirus proteases, including NS2B-NS3 protease, are essential for cleaving the DENV polyprotein as well as RNA replication and infectivity. Rothan and collaborators demonstrate that dengue virus infection is reduced by 80% which treats the cells with a peptide that inhibits NS2B-NS3 protease. The NS4A protein has been implicated in substantial rearrangements of internal membranes, permitting facile virus RNA synthesis and assembly. However, the mechanism of this protein is unknown. NS4B protein serves the RNA replication though its direct interaction with NS3 and blocks interferon-induced signal transduction. NS5 is the most conserved protein in DENV. It is also a bifunctional enzyme with a methyltransferase domain (MTase; residues 1-296) at its N-terminal end and an RNA-dependent RNA polymerase (RdRp; residues 320-900) at its C-terminal end. Specifically, residues 320-368 are strictly conserved among the flaviviruses.[21]

2.6 Dengue virus type

Dengue is caused by a virus of the Flaviviridae family, which also includes West Nile virus (WNV), yellow fever virus (YFV), and Japanese encephalitis virus (JEV). Flaviviruses are small-enveloped viruses containing a single molecule of positive-strand RNA. The RNA genome is approximately 11 kb in length and encodes for three structural proteins (core protein [C], premembrane [PrM], and envelope [E] proteins) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The

dengue virus has four serotypes, but approximately related about 65% amino acid sequence, serotypes of the virus that cause dengue (DENV1, DENV2, DENV3 and DENV4). The first infection is believed to provide lifelong immunity against that serotype. However, secondary infection by other serotypes increases the risk of developing severe dengue.[28]

2.7 Baicalein and other flavonoids

Flavonoids are a group of low-molecular-weight substances which have the general structure of a 15-carbon skeleton and consisting of two phenyl rings and a heterocyclic ring. In general, flavonoids are classified by oxidation degree, annularity of ring C and connection position of ring B. The classification of flavonoids has shown in Figure 4. Baicalein (5,6,7-trihydroxyflavone) is a flavone isolated from the roots of *Scutellaria baicalensis* and *Scutellaria lateriflora*.[2]

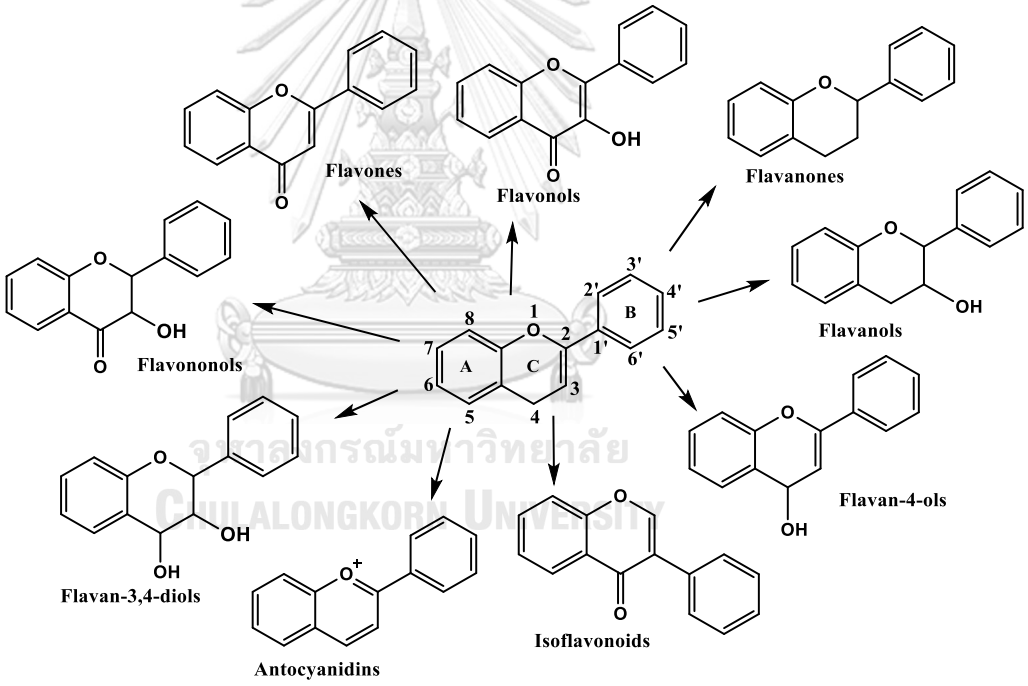

Figure 4 Basic structure of flavonoids and different classes[2]

2.8 Anti-dengue activity in baicalein and the other flavonoids

Over the past few decades, several studies revealed that a broad range of plant flavonoids exhibited a wide spectrum of anti-dengue activity, both in vitro, in vivo, and in silico. For example, biflavonoid (Figure. 5a), from L. R. F. de Sousa et. al. work in 2015 exhibit inhibitory activity against DENV2 and DENV3 protease with an IC_{50} of 15.1 ± 2.2 and 17.5 ± 1.4 μM, respectively. Additionally, they showed that

quercetin (Figure. 5b) greatly inhibited the replication of DENV2 and DENV3 with an IC_{50} of 35.2 ± 2.3 and 22.7 ± 2.3 μM, respectively. Moreover, this work confirmed the interaction between the inhibitors and the NS2B/NS3 protease. Hydroxy groups on the left-hand side of quercetin form hydrogen bonds with Gln88, Gln167, and Gly124. The para-hydroxy group on the right-hand side forms hydrogen-bonding interactions with the side chain of Asn152 and the backbone of Lys73. All interactions show in Figure 6. Additionally, hydrophobic interactions of compound with Lys74, Ile123, and Gln167 were proposed.[7]

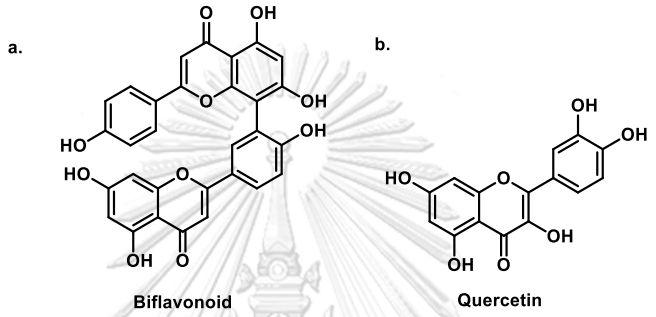

Figure 5 Structure of biflavonoid and quercetin

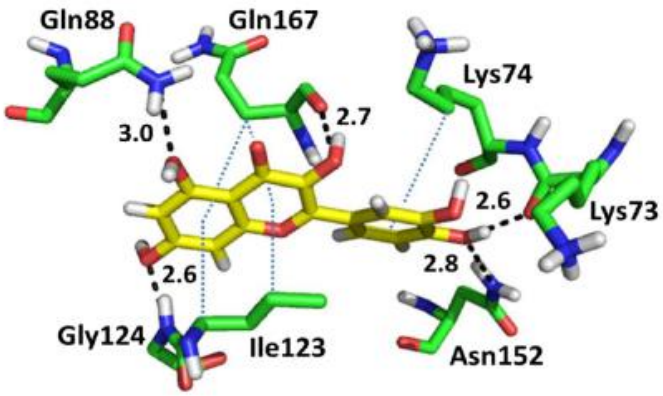

Figure 6 The interaction between quercetin and the NS2B-NS3 protease

In 2017, Peng and co-workers demonstrated that luteolin (Figure. 7a) exhibited significant anti-dengue activity against DENV type 1, 3, and 4 with EC_{50} = 4.36, 5.69 and 8.36 μM, respectively with high SI values. Moreover, the in vivo study showed that there was a 10-fold reduction of the viral load in mice administered with luteolin.[33] Later on, the inhibitory activity of five flavone analogs namely agathisflavone (Figure. 7b), quercitrin (Figure. 7c), isoquercitrin (Figure. 7d), myricetin

(Figure. 7e), and kaempferol (Figure. 7f) against DENV2 NS2B/NS3 and DENV3 NS2B/NS3 was reported with the IC_{50} values in the range of 15 to 44 μM.[7]

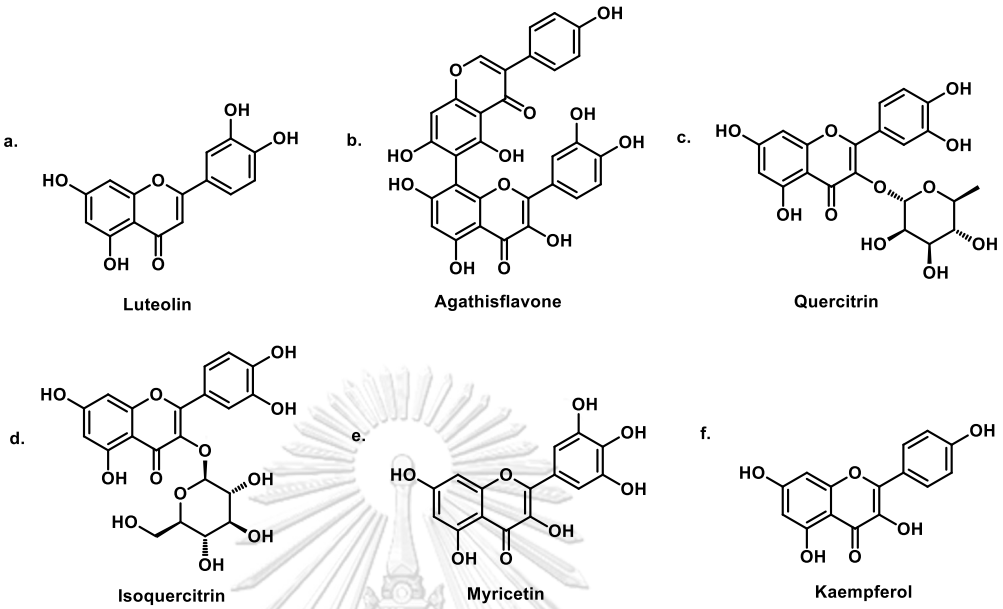

Figure 7 Structure of Luteolin, Agathisflavone, Quercitrin, Isoquercitrin, Myricetin and Kaempferol

Zandi and co-workers showed that baicalein (Figure. 8a) could inhibit the DENV2 replication in Vero cells with IC_{50} = 23.9 μM as well as exhibit the direct virucidal activity with IC_{50} = 5.73 μM (SI = 74.3).[33] Moreover, the glucuronide metabolite of baicalein, baicalin (Figure. 8b), could also inhibit the DENV2 replication with the IC_{50} value of 19.58 μM.[19] Hassandarvish et al. in 2016 reported that baicalein and baicalin showed hydrogen bond, pi-pi interaction, pi-sigma interaction, pi-cation interaction and close interaction with NS3/NS2B, NS5 and envelop protein (Figure. 9). The synthetic flavone started from halogenated flavonoid by Thanh, T. H. N. work in 2019. 8-bromobaicalein (Figure. 8c), showed the greatest DENV2 inhibition with an EC_{50} value of 0.88 ± 0.14 μM.[29]

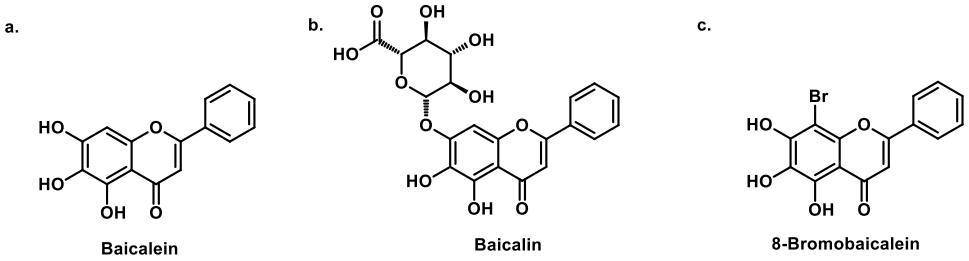

Figure 8 Structure of baicalein, baicalin and 8-bromobaicalein

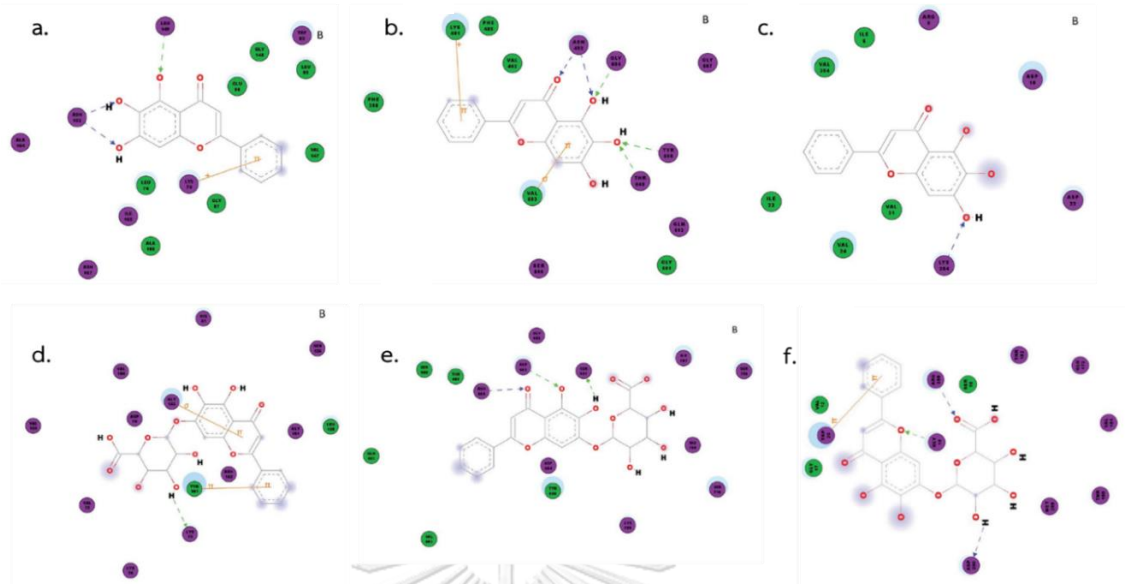

Figure 9 The interaction of baicalein and baicalin with DENV protein a). The interaction of baicalein with NS3/NS2B (2FOM) show H-bonding, pi–cation interaction and close contact, b). The interaction of baicalin with NS3/NS2B (2FOM) show H-bonding, pi–sigma interaction, pi–pi interaction and close contact, c). The interaction of baicalein with NS5 (2J7U) show H-bonding, pi–pi interaction and close contact, d). The interaction of baicalin with NS5 (2J7U) show H-bonding and close contact, e). The interaction of baicalein with envelop protein(1OKE) show H-bonding, pi–pi interaction and close contact, and f). The interaction of baicalin with envelop protein (1OKE) show H-bonding, pi–pi interaction and close contact[12]

2.9. Physicochemical properties prediction via SwissADME

ADME parameters (absorption, distribution, metabolism and excretion) can be used to predict whether compounds demonstrated good drug-like properties. Several computational programs can be used to predict these properties, such as SwissADME which is a free web tool to determine physicochemical properties and drug-likeness properties. Researchers can access the SwissADME tool by following the website http://www.swissadme.ch. Physicochemical parameters were predicted in this tool based on the Lipinski's rule of five[6] such as molecular weight (MW, ≤ 500 Da), hydrogen bond donors (HBD, ≤ 5), hydrogen bond acceptors (HAD, ≤ 10), molecular refractivity (MR), lipophilicity (log P, ≤ 5) and Verber's rule[9] such as rotatable bonds (No. of RB, ≤ 10) and total polar surface area (TPSA, ≤ 140 $Å^2$) including solubility (log S, between -6 to 0) or water solubility which is high-value effect to the effective concentration of drug molecules at the intestinal membrane for absorption. In contrast, high lipophilicity (log P)

induces low solubility and poor oral absorption. Moreover, extremely lipophilic compounds prefer to bind hydrophobic targets than require targets increasing the risk of toxicity. Cell permeability can be determined from the total polar surface area (TPSA) parameter where the lower value (less than 140 $Å^2$) is more preferred, which can be achieved by decreasing the sum of the hydrogen-bond accepting and donating moieties in the compound (corresponding to HBD and HAD in Lipinski's rule of five) including molar refractivity (MR) which is a measure of the volume of the drug molecules. Moreover, MR indicates London dispersion forces of the drug-receptor interaction.

3. Objectives

3.1. To design, synthesize and characterize baicalein derivatives

3.2. To evaluate the anti-dengue activity of the baicalein derivatives

3.3. To establish the structure-activity relationship (SAR) of the baicalein derivatives

4. Scope of the research

1.1. Synthesis of baicalein derivatives

1.2. Evaluate the anti-dengue activity of the synthesized baicalein derivatives

1.3. Establish the structure-activity relationship (SAR)

5. Beneficial outcome

The structure-activity relationship (SAR) of modified baicalein derivatives for improving anti-dengue activity

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Chapter II

Experimental

The methodology in this research divide into 2 steps. First, the chemical structure is modified on rings A and B of baicalein derivative via semi- and total synthesis. Next, all synthetic compounds have been evaluated for *in vitro* anti-dengue activity and cell viability in LLC/MK2 cells including EC_{50} values of some compounds in collaboration with Assoc. Prof. Dr Siwaporn Boonyasuppayakorn, Department of Microbiology, Faculty of Medicine, Chulalongkorn University.

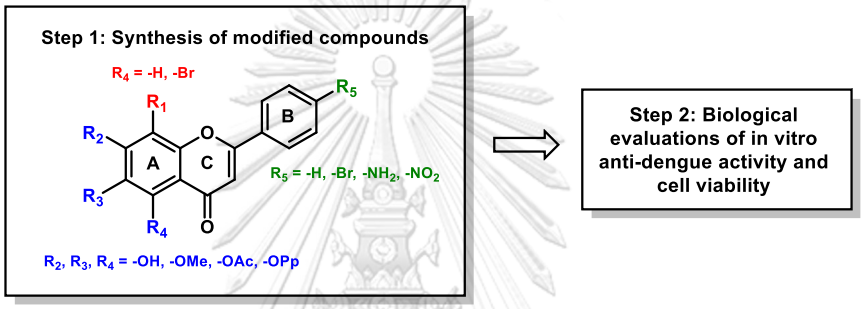
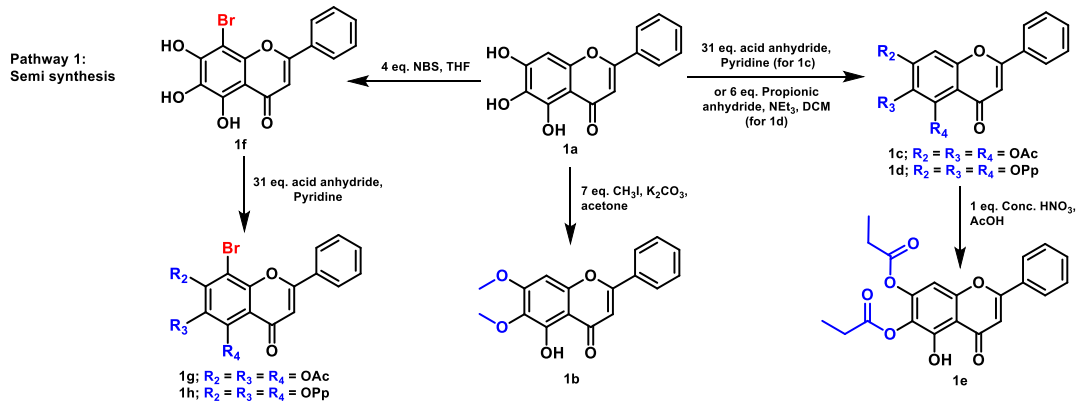


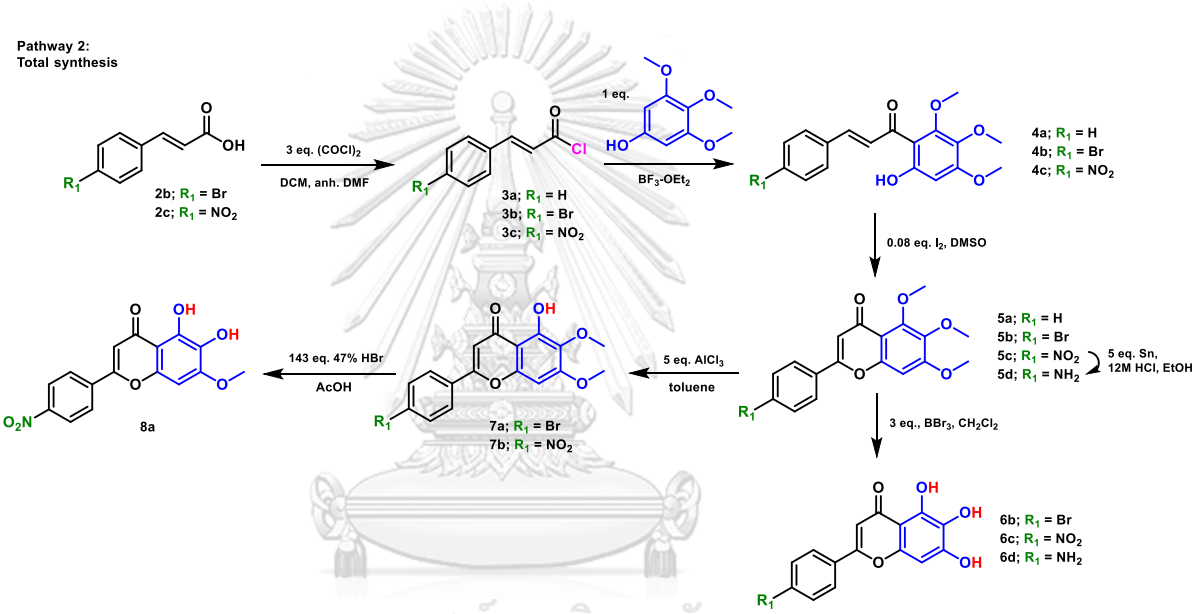
Figure 10 The methodology for this research project

1. Synthesis of modified baicalein derivatives

The modified baicalein derivatives have been designed and synthesized. The synthetic procedures are mainly divided into 2 pathways. For the semi synthesis starts with the commercially available baicalein compound, then modified by methylation[1], acylation[30] and bromination[23]. For the total synthesis starts with the commercially available cinnamic acid and acid chloride, then continue with acylation[5], cyclization[25] and demethylation[13]. The procedures are appreciatively adjusted to the previously mentioned reports.



Scheme 1 Synthesis of baicalein derivatives by semi synthesis



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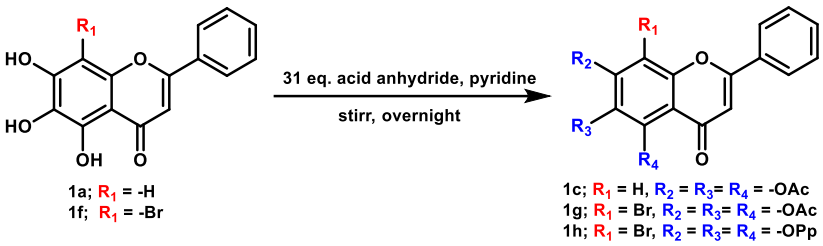
Scheme 2 Synthesis of baicalein derivatives by total synthesis

1.1. Material and methods

All reagents and solvents were obtained from Sigma–Aldrich (St. Louis, MO, USA), TCI chemicals (Tokyo, Japan) and Merck (Darmstadt, Germany). All solvents for column chromatography from RCI Labscan (Samutsakorn, Thailand) were ditilled before use. Reactions were monitored by thin–layer chromatography (TLC) using aluminium Merck TLC plates coated with silica gel 60 F254. Normal phase column chromatography was performed using silica gel 60 (0.063–0.200 mm, 70–230 mesh ASTM, Merck, Darmstadt, Germany). Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a Jeol JNM–ECZ500/S1 (500 MHz). Chemical shifts were expressed in parts per million (ppm), J values were in Hertz (Hz).

1.2. General procedure

1.2.1. General procedure A

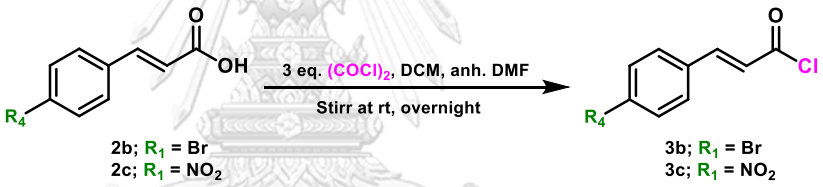


1a; R_1 = -H
1f; R_1 = -Br

1c; R_1 = H, R_2 = R_3= R_4 = -OAc
1g; R_1 = Br, R_2 = R_3= R_4 = -OAc
1h; R_1 = Br, R_2 = R_3= R_4 = -OPp

Compounds **1c**, **1g** and **1h** were synthesized by modified previous report[30]. A mixture of baicalein analogs, acid anhydride (31 equiv.) and pyridine was stirred at room temperature overnight. The reaction monitored by TLC. The reaction was quenched with DI water and extracted with CH_2Cl_2. The combined organic layers were washed with 10% NaOH, 1M HCl and brine. The crude mixture was concentrated *in vacuo* to give the product.

1.2.2. General procedure B



2b; R_1 = Br
2c; R_1 = NO_2

3b; R_1 = Br
3c; R_1 = NO_2

Compounds **3b** and **3c** were synthesized by modified previous report[5]. The $(COCl)_2$ (3 equiv.) was added into the solution of cinnamic acid in DCM. Anh. DMF was added into the mixture under N_2 gas. The reaction was stirred at room temperature overnight. The reaction monitored by TLC. The crude mixture was concentrated *in vacuo* to give the product.

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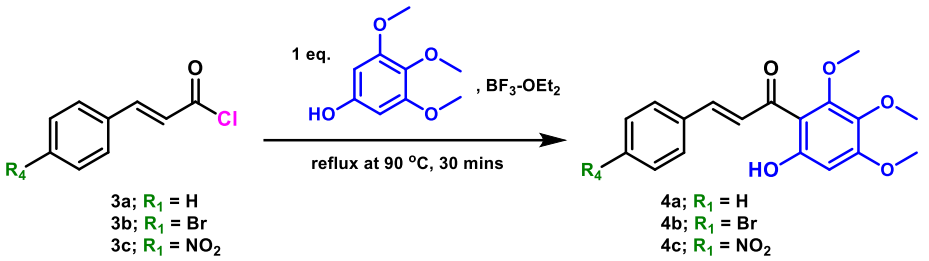
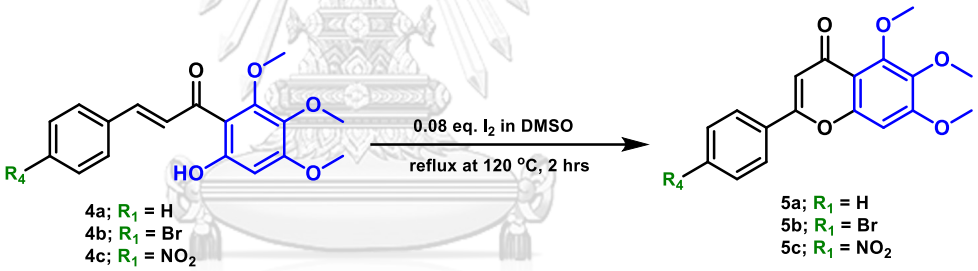
1.2.3. General procedure C



Compounds **4a** - **4c** were synthesized by modified previous report[5]. A BF_3-OEt_2 (1 equiv.) was slowly added into the acid chloride in cold bath, then cooling down to room temperature. The reaction was refluxed at 90 ºC for 0.5-1 hours and monitored by TLC. The reaction was quenched with DI water and extracted with EtOAc (3 times). The combined organic layers were dried with anh. Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by column chromatography using a mixture of EtOAc and hexane as an eluent to give the product.

1.2.4. General procedure D



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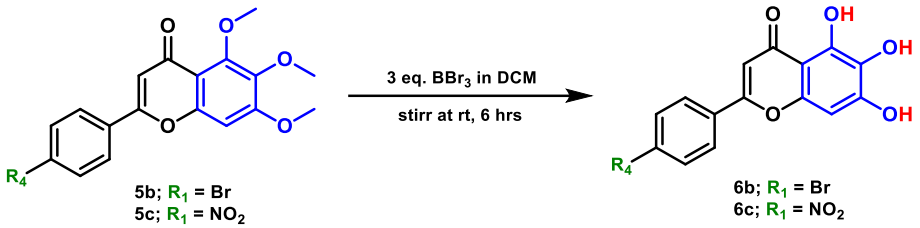
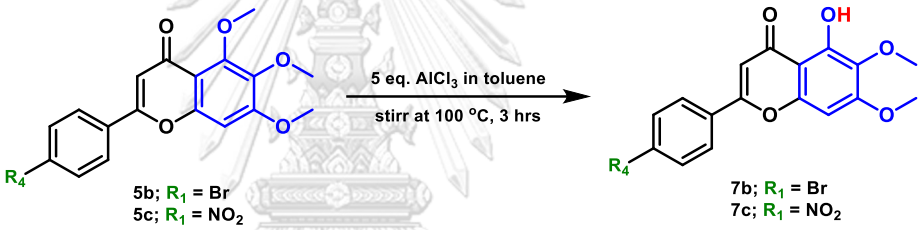
Compounds **5a** - **5c** were synthesized by modified previous report[5]. The I_2 (0.08 equiv.) was added into the solution of 6-hydroxy-2,3,4-trimethoxychaclone in DMSO. The reaction was refluxed at 120 ºC for 2 hrs. and monitored by TLC. The reaction was quenched with DI water and extracted with EtOAc (3 times). The combined organic layers were dried with anh. Na_2SO_4 and concentrated *in vacuo* to give the product.

1.2.5. General procedure E



Compounds **6b** and **6c** were synthesized by modified previous report[13]. The BBr_3 (3 equiv.) was added into the solution of 5,6,7-trimethoxybaicalein in DCM at 0 °C. The reaction was stirred at room temperature for 6 hrs. and monitored by TLC. The reaction was quenched with 1M HCl and extracted with EtOAc (3 times). The combined organic layers were dried with anh. Na_2SO_4 and concentrated *in vacuo* to give the product.

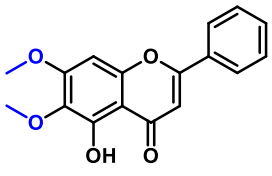
1.2.6. General procedure F



Compounds **7b** and **7c** were synthesized by modified previous report[15]. The $AlCl_3$ (5 equiv.) was added into the solution of 5,6,7-trimethoxybaicalein in toluene. The reaction was stirred at 100 °C for 3 hrs. and monitored by TLC. The reaction was quenched with 1M HCl and left stirring for an hour. The mixture was extracted with EtOAc (3 times). The combined organic layers were dried with anh. Na_2SO_4 and concentrated *in vacuo* to give the product.

1.3. Synthesis of baicalein derivatives

1.3.1. 5-hydroxy-6,7-dimethoxy-2-phenyl-4H-chromen-4-one (1b)



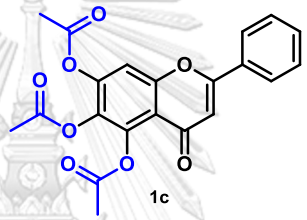
1b

The title compound was synthesized using the previously described method[1]. Methyl iodide (43 µL, 0.7 mmol) was added in the solution of baicalein (28 mg, 0.1 mmol) in dry acetone (4 mL). Potassium carbonates (94 mg, 0.7 mmol) was added into

the mixture. The reaction was refluxed at 65 °C for 6 hours and monitored by TLC. The mixture was filtered. The filtrate was concentrated *in vacuo* to give the crude mixture. The crude mixture was purified by column chromatography using a mixture of EtOAc and hexane as an eluent to give the title compound (28 mg, 19% yield) as a light-yellow solid.

^{1}H NMR (500 MHz, acetone-d_6): δ 12.80 (br s, 1H), 8.08–8.06 (m, 2H), 7.60–7.57 (m, 3H), 6.88 (s, 1H), 6.81 (s, 1H), 3.97 (s, 3H), 3.77 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 182.9, 164.1, 159.0, 153.4, 153.1, 132.8, 132.0, 131.4, 129.2, 126.4, 106.4, 105.7, 90.7, 61.0, 56.4. ^{1}H and ^{13}C NMR data are consistent with literature values[1].

1.3.2. 4-oxo-2-phenyl-4H-chromene-5,6,7-triyl triacetate (1c)



1c

The title compound was synthesized following the General Procedure A using baicalein (20 mg, 0.07 mmol), pyridine (60 µL), and acetic anhydride (2.2 mL, 2.2 mmol). The reaction was stirred at room temperature for 4 hours. The reaction was quenched with DI water and extracted with EtOAc. The crude mixture was concentrated *in vacuo* to give the title compound (20 mg, 100% yield) as a pale-yellow solid.

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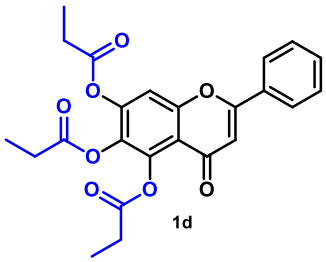
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.84 (d, 2H), 7.54–7.50 (m, 3H), 7.49 (s, 1H), 6.64 (s, 1H), 2.43 (s, 3H), 2.34 (s, 3H), 2.33 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 176.3, 168.4, 167.3, 167.1, 162.9, 154.3, 147.0, 142.2, 131.9, 131.1, 129.3, 129.2, 126.5, 126.4, 126.3, 110.4, 108.3, 105.9, 20.9, 20.8, 20.2. ^{1}H and ^{13}C NMR data are consistent with literature values[10].

1.3.3. 4-oxo-2-phenyl-4H-chromene-5,6,7-triyl tripropionate (1d)



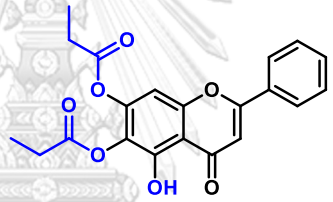
1d

The synthesis of the title compound was modified by the previously described method. Propionic anhydride (2.4 mL, 15 mmol) was added into the solution of baicalein

(1 g, 3.7 mmol) in DCM (50 mL). NEt_3 (2 mL, 3.7 mmol) was added into the mixture. The reaction was stirred at room temperature for 2 hours and monitored by TLC. The reaction was quenched by DI water and extracted with DCM. The combined organic layers were washed with 10% NaOH solution, 1M HCl and brine. The crude mixture was concentrated *in vacuo* to give the title compound (1.3 g, 80% yield) as a cottage white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.84 (dd, *J* = 8.1, 1.5 Hz, 2H), 7.54–7.47 (m, 4H), 6.63 (s, 1H), 2.75 (q, *J* = 7.5 Hz, 2H), 2.60 (q, *J* = 7.6, 2.4 Hz, 4H), 1.32–1.25 (m, 9H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 176.4, 171.8, 170.9, 170.7, 162.7, 154.2, 147.1, 142.3, 132.8, 131.9, 131.1, 129.2, 126.3, 115.7, 110.3, 108.3, 27.7, 27.6, 27.2 9.4, 9.0, 8.9. ^{1}H and ^{13}C NMR data are consistent with literature values[30].

1.3.4. 5-hydroxy-4-oxo-2-phenyl-4H-chromene-6,7-diyl dipropionate (1e)



1e

5,6,7-tripropionylbaicalein (200 mg, 0.46 mmol) was dissolved in acetic acid (2.76 mL, 0.46 mmol). Nitric acid concentrate was dropwise added into the mixture in cold bath. After warming to room temperature, the reaction was stirred at 65 °C for 2 hours and monitored by TLC. The reaction was quench by DI water and filtered. The residue was recrystallized by ethanol and filtered to give the title compound (105 mg, 60% yield) as a pale-yellow solid.

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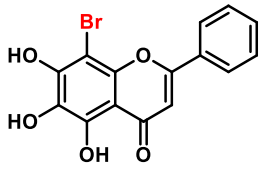
^{1}H NMR (500 MHz, $CDCl_3$): δ 12.89 (s, 1H), 7.87 (d, *J* = 7.1 Hz, 2H), 7.56–7.51 (m, 3H), 6.96 (s, 1H), 6.72 (s, 1H), 2.66–2.60 (m, 4H), 1.31–1.27 (m, 6H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 178.8, 171.3, 171.1, 165.1, 158.11, 153.5, 148.6, 132.4, 131.0, 129.3, 129.3, 126.6, 126.5, 126.5, 111.5, 105.9, 101.7, 27.7, 27.2, 9.3, 9.1; **HRMS** (m/z): $[M + H]^+$ calcd. for $C_{21}H_{18}O_7$, 383.1131; found, 383.1135; **m.p.** 132-134 °C.

1.3.5. 8-bromo-5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one (1f)

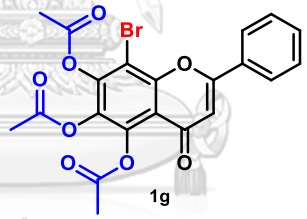


1f

Baicalein (54 mg, 0.2 mmol) was dissolved in THF (6.2 mL). NBS (54 mg, 0.3 mmol) was added into the mixture. The reaction was stirred at room temperature for 3 hours and monitored by TLC. The reaction was quenched by DI water and extracted with EtOAc. The combined organic layers were concentrated *in vacuo* to give the title compound (65 mg, 93% yield) as a light-yellow solid.

^{1}H NMR (500 MHz, acetone-d_6): δ 12.82 (br s, 1H), 8.18 (dd, *J* = 7.6, 2.1 Hz, 2H), 7.67–7.62 (m, 3H), 6.90 (s, 1H); **^{13}C NMR** (126 MHz, acetone-d_6): δ 183.0, 163.8, 150.8, 147.4, 146.6, 132.1, 131.3, 130.4, 129.4, 129.3, 126.50, 126.4, 105.3, 104.6, 86.6. ^{1}H and ^{13}C NMR data are consistent with literature values[8].

1.3.6. 8-bromo-4-oxo-2-phenyl-4H-chromene-5,6,7-triyl triacetate (1g)

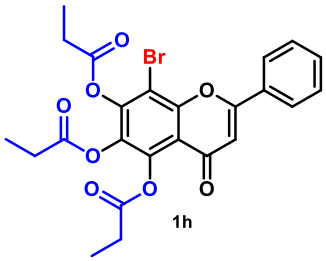


1g

The title compound was synthesized following the General Procedure A using 8-bromobaicalein (17.5 mg, 0.05 mmol), pyridine (40.5 µL), and acetic anhydride (1.5 mL, 1.5 mmol). The reaction was stirred at room temperature for an hour and monitored by TLC. The reaction was quenched by DI water and extracted with EtOAc. The crude mixture was concentrated *in vacuo* to give the title compound (15 mg, 63% yield) as a pale white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95 (d, *J* = 6.9 Hz, 2H), 7.57–7.51 (m, 3H), 6.70 (s, 1H), 2.42 (s, 3H), 2.41 (s, 3H), 2.34 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 181.1, 169.5, 165.5, 169.2, 162.8, 151.7, 151.0, 141.5, 133.2, 132.3, 129.4, 129.3, 126.5, 126.5, 126.5, 112.4, 105.6, 104.6, 20.9, 20.4, 20.1. ^{1}H and ^{13}C NMR data are consistent with literature values[32].

1.3.7. 8-bromo-4-oxo-2-phenyl-4H-chromene-5,6,7-triyl tripropionate (1h)



1h

The title compound was synthesized following the General Procedure A using 8-bromobaicalein (94 mg., 0.269 mmol), pyridine (22 µL), and acetic anhydride (1.1 mL, 8.3 mmol). The reaction was stirred at room temperature overnight and monitored by TLC. The reaction was quenched by DI water and extracted with EtOAc. The combined organic layers were washed by sat. NH_4Cl, 10% NaOH and brine. The crude mixture was concentrated *in vacuo* to give the title compound (43 mg, 59% yield) as a pale white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.97 (d, *J* = 7.9 Hz, 2H), 7.57–7.51 (m, 3H), 6.92 (s, 1H), 2.71 (q, *J* = 7.6 Hz, 4H), 2.38 (q, *J* = 7.5 Hz, 2H), 1.31 (t, *J* = 7.6 Hz, 9H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 183.2, 176.0, 174.6, 172.9, 163.8, 150.6, 149.1, 141.7, 137.3, 132.5, 129.9, 129.4, 126.9, 126.3, 126.2, 120.5, 112.2, 105.1, 27.4, 27.2, 27.0, 9.6, 9.5, 9.4; **HRMS** (m/z): $[M + Na]^+$ calcd. for $C_{24}H_{21}BrO_8$, 460.1134; found, 463.0212; m.p. 113-115 °C.

1.3.8. 5,6,7-trimethoxy-2-phenyl-4H-chromen-4-one (5a)

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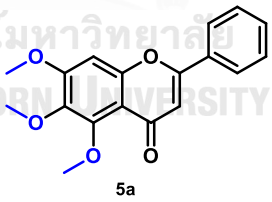


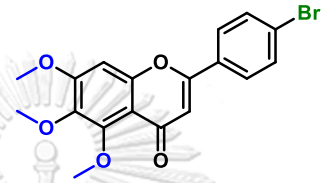
5a

The title compound started with following the General Procedure C using BF_3-OEt_2 (5 mL, 41 mmol), cinnamoyl chloride (866 mg, 5 mmol), and 3,4,5-trimethoxyphenol (958 mg, 5 mmol). The reaction was refluxed at 90 °C for an hour and monitored by TLC. The reaction was quenched by DI water and extracted with EtOAc. The crude mixture was concentrated *in vacuo* to give the 6-hydroxy-2,3,4-trimethoxychaclone **4a** (2.7 g, 100% yield) as a pale white solid. Then, the title compound was synthesized following the General Procedure D using **4a** (314 mg, 1 mmol), I_2 (20 mg, 0.08 mmol) and DMSO (12 mL). The reaction was refluxed at 90 °C for 3 hours and monitored by TLC. The reaction was quenched by DI water and extracted with DCM. The crude mixture was

concentrated *in vacuo* to give the title compound (126 mg, 40% yield) as a dark-yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.86 (d, *J* = 7.9 Hz, 2H), 7.51–7.47 (m, 3H), 6.80 (s, 1H), 6.66 (s, 1H), 3.97 (s, 6H), 3.90 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 177.1, 160.1, 158.0, 154.5, 152.7, 140.6, 132.4, 130.6, 127.5, 126.0, 113.0, 108.6, 96.3, 62.3, 61.7, 56.4. ^{1}H and ^{13}C NMR data are consistent with literature values[17].

1.3.9. 2-(4-bromophenyl)-5,6,7-trimethoxy-4H-chromen-4-one (5b)



5b

The title compound started with following the General Procedure B using 4'-bromocinnamic acid (1.14 g, 5 mmol), $(COCl)_2$ (1.3 mL, 15 mmol), DCM (4.5 mL), and anh. DMF (231 µL, 3 mmol). The reaction was stirred overnight. The crude mixture was concentrated *in vacuo* to give a of 4'-bromocinnamoyl chloride **3b** (1.23 g, 100% yield) as a light-yellow solid. The title compound was continuously synthesized following the General Procedure C using BF_3-OEt_2 (8.2 mL, 67 mmol), **3b** (1.23 g, 5 mmol), and 3,4,5-trimethoxyphenol (921 mg, 5 mmol). The reaction was refluxed at 90 °C for 30 mins and monitored by TLC. The reaction was quenched by DI water and extracted with EtOAc. The crude mixture was concentrated *in vacuo* to give the 4'-bromo-6-hydroxy-2,3,4-trimethoxychaclone **4b** (702 mg, 36% yield) as a dark orange solid. Then, the title compound was synthesized following General Procedure D using **4b** (650 mg, 1.7 mmol), I_2 (34 mg, 0.14 mmol) and DMSO (18 mL). The reaction was refluxed at 120 °C for an hour and monitored by TLC. The reaction was quenched by DI water and extracted with DCM. The crude mixture was purified by column chromatography, eluting with ethyl acetate/hexane (1:4) and concentrated *in vacuo* to give the title compound (419 mg, 65% yield) as a dark-yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.72 (d, *J* = 8.8 Hz, 2H), 7.62 (d, *J* = 8.8 Hz, 2H), 6.79 (s, 1H), 6.63 (s, 1H), 3.97 (s, 6H), 3.90 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 177.1, 160.1, 158.0, 154.5, 152.7, 140.6, 132.4, 130.6, 127.5, 126.0, 113.0, 108.6, 96.3, 62.3, 61.7, 56.4. ^{1}H and ^{13}C NMR data are consistent with literature values[25].

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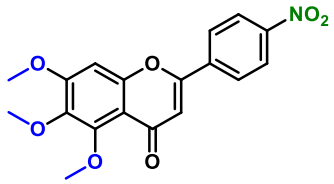
1.3.10. 5,6,7-trimethoxy-2-(4-nitrophenyl)-4H-chromen-4-one (5c)



5c

The title compound started with following the General Procedure B using 4'-nitrocinnamic acid (965 mg, 5 mmol), $(COCl)_2$ (1.3 mL, 15 mmol), DCM (5 mL), and anh. DMF (230 µL, 3 mmol). The reaction was stirred overnight. The crude mixture was concentrated *in vacuo* to give a of 4'-nitrocinnamoyl chloride **3c** (1.31 g, 100% yield) as a light-yellow solid. The title compound was continuously synthesized following General Procedure C using BF_3-OEt_2 (7.38 mL, 59 mmol), **3c** (1.3 g, 6.2 mmol), and 3,4,5-trimethoxyphenol (1.14 g, 6.2 mmol). The reaction was refluxed at 90 °C for 45 mins and monitored by TLC. The reaction was quenched by DI water and extracted with EtOAc. The crude mixture was purified by column chromatography, eluting with ethyl acetate/hexane (1:5) and concentrated *in vacuo* to give the 6-hydroxy-2,3,4-trimethoxy-4'-nitrochaclone **4c** (675 mg, 31% yield) as an orange-brown solid. Then, the title compound was synthesized following General Procedure D using **4c** (620 mg, 1.7 mmol), I_2 (35 mg, 0.14 mmol) and DMSO (20 mL). The reaction was refluxed at 120 °C for an hour and monitored by TLC. The reaction was quenched by DI water and extracted with DCM. The crude mixture was concentrated *in vacuo* to give the title compound (366 mg, 60% yield) as a dark-yellow solid.

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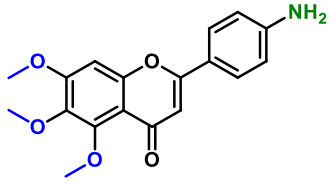
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.35 (d, *J* = 8.6 Hz, 2H), 8.04 (d, *J* = 8.7 Hz, 2H), 6.82 (s, 1H), 6.75 (s, 1H), 3.99 (s, 3H), 3.98 (s, 3H), 3.92 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 176.8, 162.9, 158.5, 154.5, 152.7, 149.3, 140.9, 137.6, 128.9, 123.5, 113.1, 110.6, 96.3, 62.3, 61.7, 56.5. ^{1}H and ^{13}C NMR data are consistent with literature values[25].

1.3.11. 2-(4-aminophenyl)-5,6,7-trimethoxy-4H-chromen-4-one (5d)



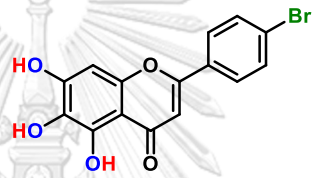
5d

The synthesis of the title compound was modified by the previously described method. **5c** (60 mg, 0.17 mmol) was dissolved in ethanol (2.7 mL) in cold bath. 12M HCl

(2.7 mL) was slowly added into the solution followed by Sn (100 mg, 0.84 mmol). The reaction was stirred at room temperature for 1 hour 30 mins and monitored by TLC. The reaction was quenched by $NaHCO_3$ and extracted with EtOAc. The crude mixture was concentrated *in vacuo* to give the title compound (54 mg, 100% yield) as a yellow-orange solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (d, *J* = 8.6 Hz, 2H), 6.76 (s, 1H), 6.73 (d, *J* = 8.6 Hz, 2H), 6.52 (s, 1H), 3.97 (s, 1H), 3.96 (s, 1H), 3.90 (s, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 177.4, 161.8, 157.5, 154.5, 152.6, 149.6, 140.3, 127.7, 121.1, 114.8, 112.9, 106.0, 96.3, 62.3, 61.6, 56.3. ^{1}H and ^{13}C NMR data are consistent with literature values[25].

1.3.12. 2-(4-bromophenyl)-5,6,7-trihydroxy-4H-chromen-4-one (6b)

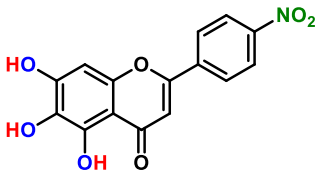


6b

The title compound was synthesized following the General Procedure E using **5b** (50 mg, 0.128 mmol), DCM (1 mL), and BBr_3 (36 µL, 0.384 mmol). The reaction was refluxed at room temperature for 6 hours and monitored by TLC. The reaction was quenched by 1M HCl and diluted with EtOAc. The crude mixture was concentrated *in vacuo* to give the title compound (42 mg, 94% yield) as a dark green solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.01 (d, *J* = 8.6 Hz, 2H), 7.77 (d, *J* = 8.6 Hz, 2H), 6.98 (s, 1H), 6.62 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6): δ 182.6, 162.3, 154.2, 150.3, 147.4, 132.7, 130.7, 129.9, 128.8, 126.1, 105.3, 104.8, 94.6; ^{1}H and ^{13}C NMR data are consistent with literature value[5].

1.3.13. 5,6,7-trihydroxy-2-(4-nitrophenyl)-4H-chromen-4-one (6c)



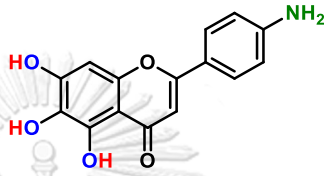
6c

The title compound was synthesized following the General Procedure E using **5c** (20 mg, 0.056 mmol), DCM (1 mL), and BBr_3 (50 µL, 0.527 mmol). The reaction was refluxed at room temperature for 6 hours and monitored by TLC. The reaction was

quenched by 1M HCl and diluted with EtOAc. The crude mixture was concentrated *in vacuo* to give the title compound (13 mg, 74% yield) as a dark green solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ 12.45 (s, 1H), 10.69 (s, 1H), 8.88 (s, 1H), 8.35 (d, *J* = 7.1 Hz, 2H), 8.30 (d, *J* = 6.9 Hz, 2H), 7.11 (s, 1H), 6.62 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6): δ 184.1, 162.6, 158.7, 154.3, 151.3, 147.5, 145.2, 136.5, 128.0, 124.6, 107.5, 104.9, 94.7. ^{1}H and ^{13}C NMR data are consistent with literature value[14].

1.3.14. 2-(4-aminophenyl)-5,6,7-trihydroxy-4H-chromen-4-one (6d)

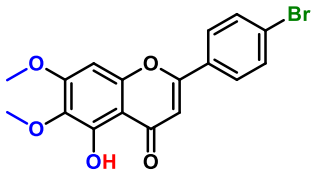


6d

The title compound was synthesized by Thamonwan Chokmahasarn following the General Procedure E using **6c** (40 mg, 0.12 mmol), DCM (1 mL), and BBr_3 (50 µL, 0.527 mmol). The reaction was refluxed at room temperature for 2 hours and monitored by TLC. The reaction was quenched by 1M HCl and diluted with EtOAc. The crude mixture was concentrated *in vacuo* to give the title compound (30 mg, 86% yield) as a as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.70 (d, *J* = 9.0 Hz, 2H), 6.72 (d, *J* = 8.9 Hz, 2H), 6.53 (s, 1H), 6.48 (s, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 182.3, 167.5, 158.9, 154.0, 150.0, 147.3, 143.2, 128.5, 123.4, 114.0, 106.2, 104.3, 94.2; **HRMS** (m/z): $[M + H]^+$ calcd. for $C_{15}H_{11}NO_5$, 286.0715; found, 286.0719; m.p. >250 ºC (decompose).

1.3.15. 2-(4-bromophenyl)-5-hydroxy-6,7-dimethoxy-4H-chromen-4-one (7a)



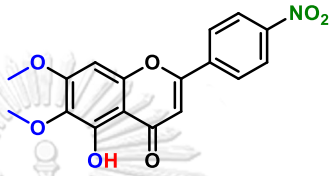
7a

The title compound was synthesized following the General Procedure F using **5b** (30 mg, 0.077 mmol), toluene (5.6 mL, 52.5 mmol), and $AlCl_3$ (52 mg, 0.385 mmol). The mixture was stirred at 100 ºC for 3 hours and monitored by TLC. The reaction was quenched by 1M HCl, then stirring an hour. The mixture was extracted with EtOAc. The

crude mixture was concentrated *in vacuo* to give the title compound (63 mg, 100% yield) as a brown solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75 (d, *J* = 8.7 Hz, 2H), 7.65 (d, *J* = 8.7 Hz, 2H), 6.65 (s, 1H), 6.55 (s, 1H), 3.96 (s, 3H), 3.92 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 182.7, 162.9, 159.1, 153.3, 153.1, 132.9, 132.5, 130.3, 127.8, 126.7, 106.4, 105.9, 90.8, 61.0, 56.5. ^{1}H and ^{13}C NMR data are consistent with literature value[(5)].

1.3.16. 5-hydroxy-6,7-dimethoxy-2-(4-nitrophenyl)-4H-chromen-4-one (7b)

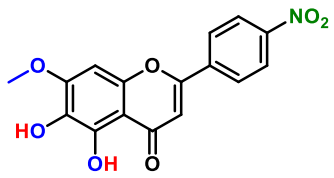


7b

The title compound was synthesized following the General Procedure F using **5c** (30 mg, 0.092 mmol), toluene (6.7 mL, 62.74 mmol), and $AlCl_3$ (61 mg, 0.46 mmol). The mixture was stirred at 100 ºC for 3 hours and monitored by TLC. The reaction was quenched by 1M HCl, then stirring an hour. The mixture was extracted with EtOAc. The crude mixture was concentrated *in vacuo* to give the title compound (59 mg, 81% yield) as a brown solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.38 (d, *J* = 9.0 Hz, 2H), 8.07 (d, *J* = 8.9 Hz, 2H), 6.77 (s, 1H), 6.59 (s, 1H), 3.98 (s, 3H), 3.93 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 182.4, 161.1, 159.5, 157.6, 155.7, 153.1, 137.2, 133.1, 127.3, 124.4, 107.9, 104.1, 90.9, 61.0, 56.6; **HRMS** (m/z): $[M + H]^+$ calcd. for $C_{17}H_{13}NO_7$, 344.0770; found, 344.0779; m.p. 145–150 ºC.

1.3.17. 5,6-dihydroxy-7-methoxy-2-(4-nitrophenyl)-4H-chromen-4-one (8a)



8a

The synthesis of the title compound was modified by the previously described method. **7b** (30 mg, 0.087 mmol) was dissolved in Acetic acid (1.36 mL, 23.8 mmol) in cold bath. 47% HBr (680 µL, 12.5 mmol) was added into the solution. The reaction was refluxed at 120 ºC for 3 hours and monitored by TLC. The reaction was quenched by

$NaHCO_3$ and extracted with EtOAc. The crude mixture was concentrated *in vacuo* to give the title compound (25 mg, 89% yield) as a dark brown solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (d, *J* = 8.9 Hz, 2H), 8.06 (d, *J* = 8.9 Hz, 2H), 6.77 (s, 1H), 6.65 (s, 1H), 4.02 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$): δ 181.7, 162.3, 160.2, 152.6, 151.6, 147.4, 135.3, 129.1, 127.3, 123.4, 107.1, 103.6, 95.4, 64.0; **HRMS** (m/z): $[M + H]^+$ calcd. for $C_{16}H_{11}NO_7$, 330.0614; found, 330.0610; m.p. 74–78 °C.

2. Anti-dengue activity evaluation

Baicalein derivatives were evaluated for *in vitro* anti-dengue efficacy and cell toxicity in LLC/MK2 cells including EC_{50} values of some compounds in collaboration with Assoc. Prof. Dr Siwaporn Boonyasuppayakorn, Department of Microbiology, Faculty of Medicine, Chulalongkorn University.

2.1. Material for anti-dengue activity evaluation

The cell lines of LLC/MK2 (ATCC®CCL-7) and C6/36 (ATCC®CRL-1660) were maintained in minimal essential medium (MEM) (Gibco®, Langley, USA) supplemented with 10% fetal bovine serum (Gibco®, Langley, USA); 100 I.U./ml penicillin, and 100 µg/ml streptomycin (Bio Basic Canada, Ontario, Canada); 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazine-ethane-sulfonic acid) (Sigma Aldrich, St. Louis, USA) at 37 °C under condition of 5% CO_2 and 28°C, respectively[26]. Reference strain of DENV2 (New Guinea C strain) was propagated in C6/36 and LLC/MK2 cell line with MEM medium added with 1% FBS, 100 I.U./ml penicillin, 100 µg/ml streptomycin, and 10 mM HEPES at 37 °C in 5% CO_2 incubator[1].

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2.2. Anti-dengue efficacy

LLC/MK2 (ATCC® CCL-7), and C6/36 (ATCC® CRL-1660) cell lines were propagated and maintained as previously described[27]-[3]. Effective concentration (EC_{50}) of the compounds against the DENV2 were tested using LLC/MK2 cells[27],[26]. Briefly, cells were seeded overnight and infected with each virus at the multiplicity of infection (M.O.I.) of 0.1 for 1 h. The compound was added during and after infection, and cells were incubated for 72 h. Supernatants were collected for plaque titration[14]. EC_{50} results were means and standard errors of three independent experiments

2.3. Cell toxicity

Cytotoxicity of the compounds was also accessed at the concentration of 10 μM in parallel with the viral inhibition screening. LLC/MK2 cells ($1x10^4$) were seeded in 96-well plate and incubated at 37°C under 5% CO_2 overnight; compounds were added after 24 hrs, and then incubated for 2 days. DMSO at 1% was used as mock treatment. Cytotoxicity was measured using CellTiter 96® Aqueous One Solution Cell Proliferation Assay (MTS) kit (Promega, Wisconsin-Madison, USA) according to the manufacturer's instruction and analyzed by EnSight Multimode Plate Reader spectrophotometry at A_{450nm}. (Perkin Elmer, Waltham, MA, USA).

2.4. Physicochemical properties prediction

All baicalein derivatives were analyzed in terms of physicochemical descriptors, drug-likeness, and the ADME properties using the SwissADME webserver[6]. The Lipinski and Verber parameters are commonly used to estimate the drug-likeness properties by considering the following criteria; molecular weight ≤500, hydrogen bond donors (HBDs) ≤5, hydrogen bond acceptors (HBAs) ≤10, log of octanol to water partition coefficient (Log P) ≤5, the number of rotational bonds ≤10, total polar surface area (TPSA, $Å^2$) ≤140, Verber Violations (Ver. Vio), log of aqueous solubility (Log S, mol/L) −6 to 0 and molecular refractivity (MR, cm^3/mol) 40 to 130[18].

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Chapter III

Results and discussion

All the baicalein derivatives have been synthesized via semi and total synthesis except for the commercially available baicalein. Semi synthesis is a slight adjustment on ring A of baicalein such as methylation, acylation and bromination. The total synthesis consists of up to 6 step process, with the key steps being the Friedel craft acylation and oxidative cyclization. The evaluated anti-dengue activity can be used to establish the relationship between structural modification of baicalein derivatives and the efficacy, called the structure–activity relationship (SAR), for improving anti-dengue activity and toxicity on LLC/MK2 cell.

1. Semi synthesis of baicalein derivatives

All 7 semi synthesized compounds (**1b**-**1h**) were prepared starting from commercially available baicalein (**1a**, CAS Number 491-67-8) as shown in Scheme 3. Dimethylated baicalein derivative (**1b**) was synthesized following the previously described alkylation method. However, the product was obtained in low yield (19%) as the crude mixture contained another product, possibly the mono substitution which struck on the silica gel during column chromatography. Tri-substitution of acetyl (**1c**) and propionyl (**1d**) group were achieved by using acetic anhydride with pyridine for acetylation, and propionic anhydride with NEt_3 in DCM for propionylation at room temperature to obtain the corresponding products in excellent yields (100% and 80%, respectively). For compound **1d**, the yield was slightly lower than expected because it was quite difficult to remove the left over pyridine in the crude by either extraction or column chromatography. Tri propionyl derivative **1d** was hydrolyzed by acid-catalyzed ester hydrolysis to give di substitution of propionyl group (**1e**) in moderated yield (60%). Selectivity on C-8 position as a result of the possibility to form intramolecular hydrogen bonding with the nearest carbonyl group at C-4 position on ring C. Therefore, carbon atom of carbonyl group is more electrophile for hydrolysis. Bromination of baicalein (**1f**) was obtained through various methods as shown in table 1. The best way to synthesize the brominated baicalein analogue is method III. Although, method I gives product in excellent yield but NMR result of the product found many the H grease and ethyl acetate impurities. Tri substitution of brominated derivative **1f** was produced tri acetyl (**1g**) and propionyl (**1h**) via acetylation and propionylation, respectively in moderated yields (63% and 59%, respectively). Di propionyl derivative **1e** and brominated tri propionyl derivative **1h** are the novel compounds.

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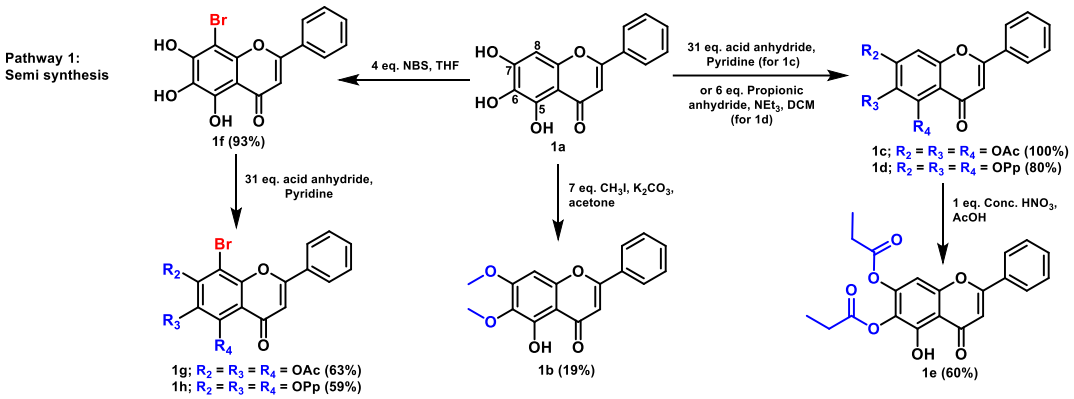
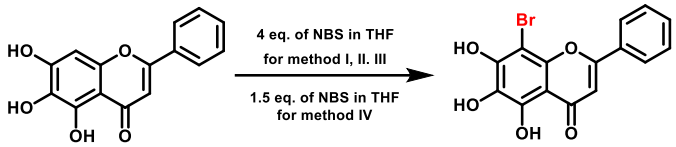


Scheme 3 Synthesis of baicalein derivatives by semi synthesis

Table 1 The various methods for bromination of baicalein derivative



Procedure	Condition	Time	%Yield
Method I	Conc. H_2SO_4, Refluxed at 60 °C	1 hour	100[a]
Method II	Conc. H_2SO_4, Room temperature	40 mins	63
Method III	Room temperature	3 hours	93
Method IV[b]	Conc. H_2SO_4, Room temperature	12 hours	35

[a] found many the H grease and ethyl acetate impurities in the crude [b] from previous work[5]

The ^{1}H NMR spectra of compound **1a**-**1b** and **1d**-**1f** are shown Figure 11. The chemical shift and integration were determined for all protons according to the compound structures. The results demonstrated that the crucial peaks of methyl groups (-CH_3, green line) in compound **1b** (Figure 11B) are similar to ethyl groups (-CH_2CH_3, orange lines) in compound **1c**-**1d** and **1g**-**1h** in upfield region (low chemical shift). Compound **1e** (Figure 11D) showed the disappearance of proton integration of ethyl groups (-CH_2CH_3, orange lines) from 15 protons to 10 protons. This suggests that compound **1d** (Figure 11C) was hydrolyzed at the C5 position to obtain compound **1e**. Additionally, the hydrolyzed compound **1e** was confirmed by 2D NMR (HMBC, shown in Figure 12) which showed only a correlation between carbon atom at C5 position and hydrogen atom at C8 position as shown in Figure 12. Substitution of bromo group in compound **1f** (Figure 11E) led to the absence of proton at the C8 position. Moreover, the chemical shift of brominated compound **1f** was slightly shifted towards downfield due to the shielding electron of the bromo group.

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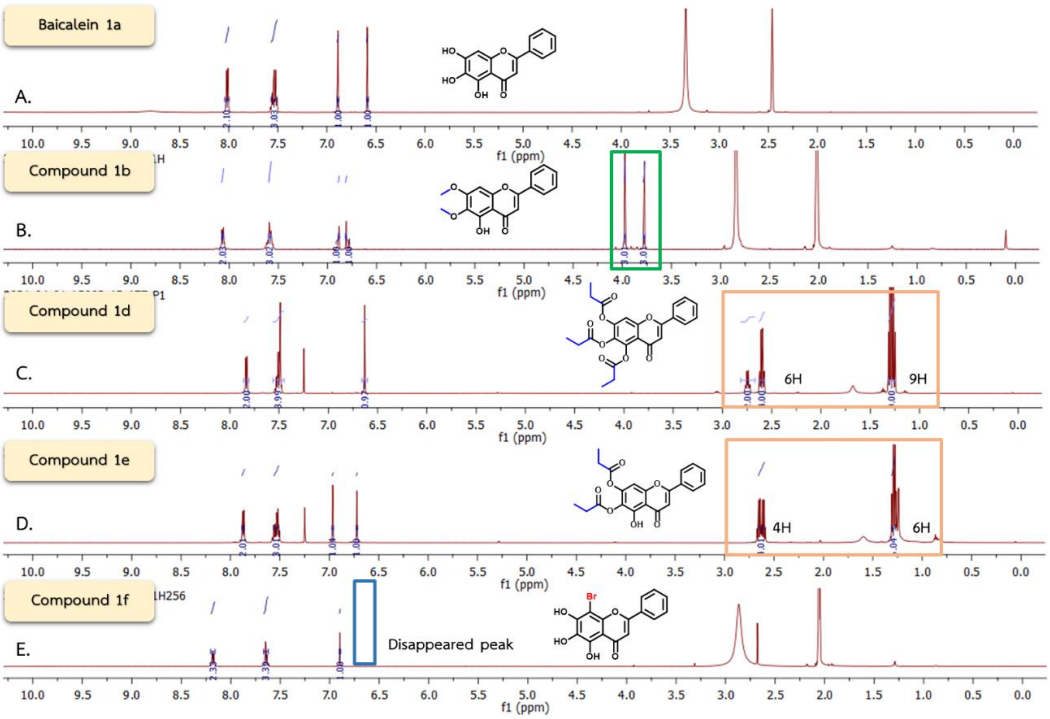


Figure 11 ^{1}H NMR examples of compound **1a**-**1c**, **1e** and **1f**

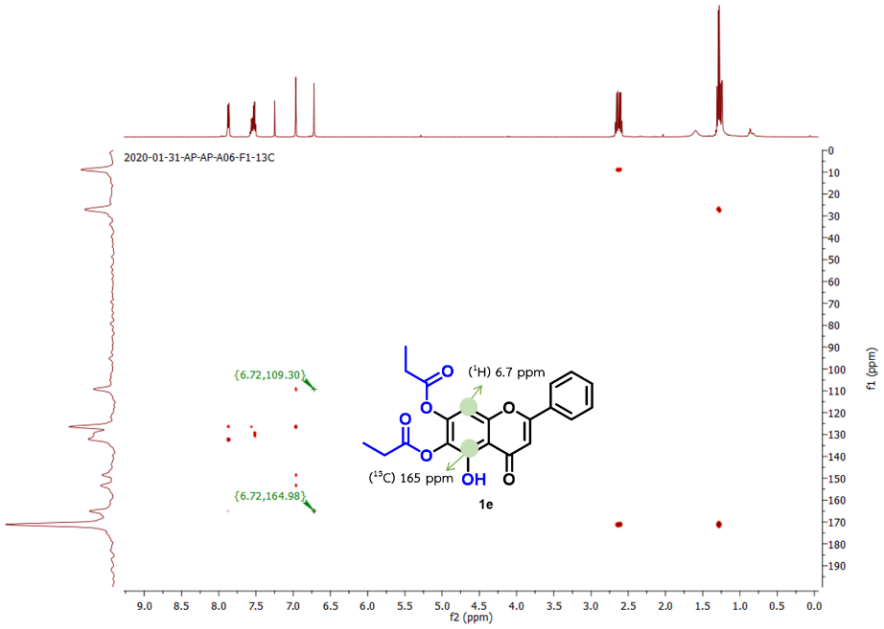
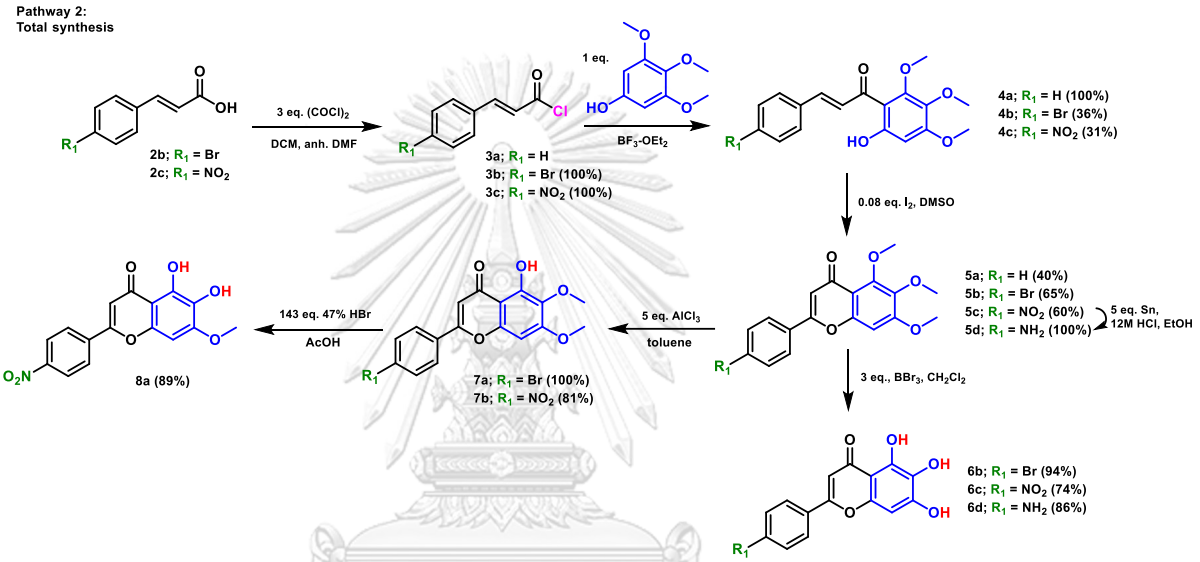


Figure 12 HMBC of compound **1e**

2. Total synthesis of baicalein derivatives

The total synthetic route for baicalein derivatives is outlined in Scheme 4. Unlike the unsubstituted cinnamoyl chloride **3a** which is commercially available, the substituted cinnamoyl chlorides **3b** and **3c** were synthesized from the corresponding

cinnamic acids **2b**-**2c** in excellent yields using oxalyl chloride. The cinnamoyl chlorides **3a**-**3c** were immediately reacted with 3,4,5-trimethoxyphenol under Lewis acid BF_3-OEt_2 as a mediator *via* Friedel–Crafts acylation to generate chalcones **4a**-**c** by the attack of electron-rich aromatic ring at the carbonyl carbon as shown in Figure 13, mechanism I in moderated to excellent yields. For the formation of **4b** and **4c** with relatively low %yields (36% and 31%, respectively), we observed by-products which might be resulted from the direct esterification between the hydroxy group on the 3,4,5-trimethoxyphenol group and the corresponding acid chloride as shown in Figure 13, mechanism II.



Scheme 4 Synthesis of baicalein derivatives by total synthesis

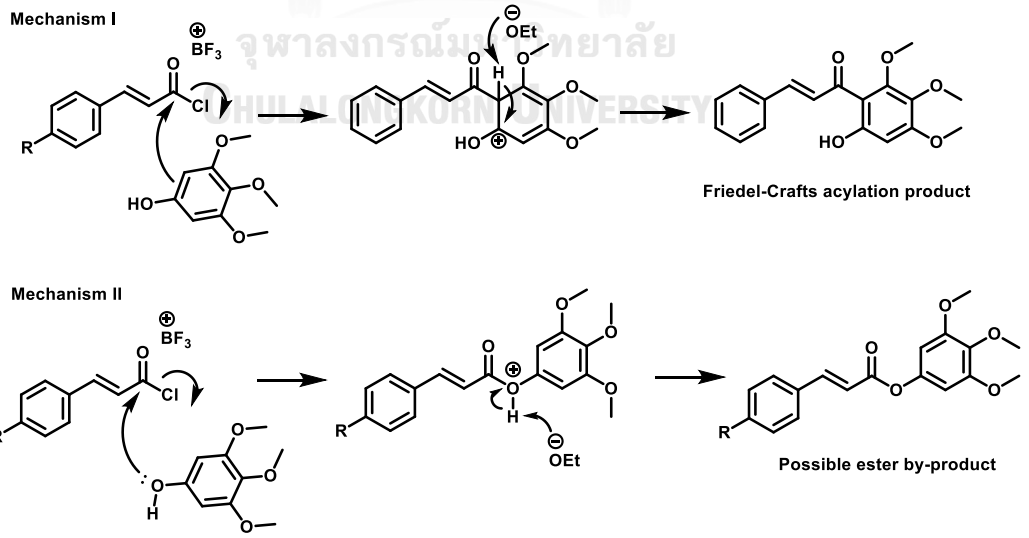


Figure 13 Mechanism of Friedel–Crafts acylation product and possible ester by-product

Subsequently, the iodine-mediated cyclization of chalcone **4a**-**c** using iodine in DMSO gave the 5,6,7-trimethylbaicalein derivatives **5a**-**c** via oxidative cyclization in moderated yields (40% to 65%). The mechanism of cyclization starts from generating the reactive species in which iodine forms a bond with DMSO solvent to activate the conjugated carbonyl system and leave iodide ion to attack the alkene at the beta position. Then, a lone pair electron of the hydroxy group attacks the carbon atom, replacing the iodide leading to cyclization as shown in Figure 14 which is adapted from the previous report[16]. The nitro group of the derivative **5c** was then reduced using tin as a reducing agent in acidic condition to provide the 4'-amino-5,6,7-trimethylbaicalein derivative **5d** in excellent yield. In the final step, the 5,6,7-trimethoxyl derivatives **6b**-**d** were demethylated using Lewis acidic boron (BBr_3) in DCM at 0 ºC leading to baicalein derivatives **6b**-**d** in good yields. Prior attempts with HBr were unsuccessful, due to starting material being used up but the product decomposed. Additionally, **5b** and **5c** was demethylated on one position using Lewis acid of $AlCl_3$ gave bromo-5,6-dimethyl baicalein derivative **7a** and nitro-5,6-dimethyl baicalein derivative **7b** in excellent yield. Demethylation of nitro-5,6-dimethyl baicalein derivative **7b** using HBr in acetic acid gave a novel compound of nitro-5-methyl baicalein derivative **8a** in excellent yield. Trimethyl compounds were demethylated all position by BBr_3 because BBr_3 is strongly Lewis acid. Di methyl compounds were demethylation by $AlCl_3$ only one position which is proposed on C5 position due to a result of nearest carbonyl group at C4 position on ring C. Therefore, methoxy group on C5 position might be crowded electron for chelating covalent with $AlCl_3$. Dimethyl compound was demethylated by HBr which is strong protic acid, but it is not enough to demethylated all trimethyl compound so dimethyl compound can be demethylated to obtain compound **8a** by HBr. Moreover, nitro baicalein derivatives **7b**, **8a** and amino baicalein derivative **6d** are also a novel compound.

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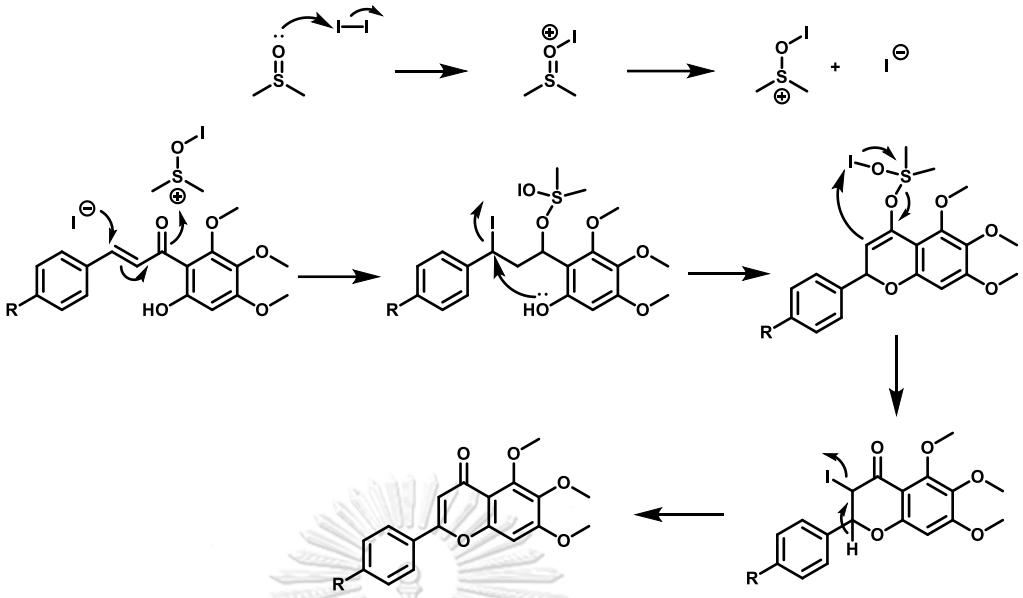

Figure 14 Possible mechanism of iodine-mediated cyclization[30]

The ^{1}H NMR spectra of compound **4a**, **5a**, **5c**, **5d** and **6d** are shown in Figure 15. The chemical shift and integration were determined for all protons according to the compound structures. The results demonstrated that the essential peaks of alkene group in compounds **4a**-**4c** (example of compound **4a** shown in Figure 15A, orange lines) disappeared upon cyclization to obtain compounds **5a**-**5c** (example of compound **5a** shown in Figure 15B, blue lines). Compound **5d** (Figure 15D) was reduced from compound **5c** (Figure 15C) which chemical shifts of aromatic proton at C3' and C5' position was induced to downfield (green lines) due to the electron-donating amino group (-NH_2). Compounds **6b**-**6d** (example of compound **6d** shown in Figure 15E) were completely demethylated by observing the disappearance of the essential signals which were methyl groups (-CH_3, blue lines) which are similar to compounds **7a**-**7b** and **8a**. Structure of compounds **7a**, **7b** and **8a** have been proposed following demethylated flavone on previous work[5],[31] which compounds **7a** and **7b** was demethylated on C5 position and compound **8a** was demethylated on C5 and C6 positions.

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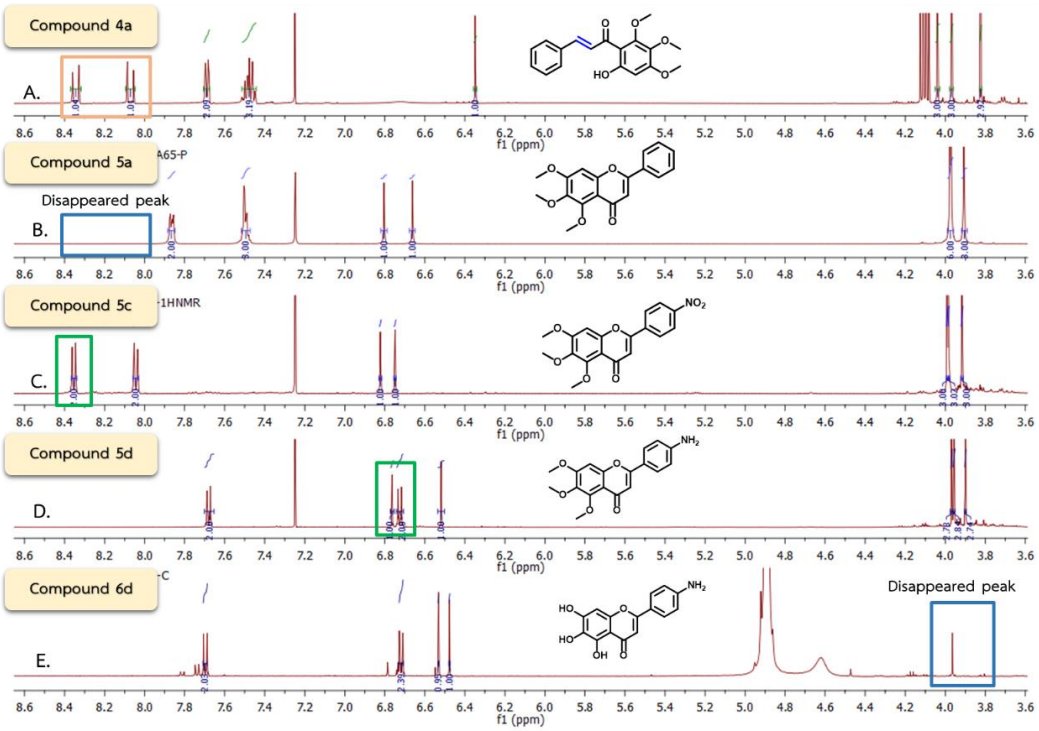


Figure 15 ^{1}H NMR examples of compound **4a**, **5a**, **5c**, **5d** and **6d**

3. Anti-dengue activity and cell toxicity

The synthetic baicalein derivatives were evaluated for their anti-dengue activity against DENV2 in LLC/MK2 cells at the concentration of 10 μM as shown in Table 2. The results demonstrate that DENV2 inhibition was increased by modification of ring A via functionalization of hydroxy group at the 5, 6 and 7 position such as compounds **1b**-**1e** except for the trimethyl baicalein analog **5a**. Substitution of bromide at the 8 position analogues such as compounds **1f** and **1g** also led to the improve of the anti-dengue activity, except for tri propionyl baicalein analog **1h**. Di and tri substitution of propionyl ester analogues (**1d**, EC_{50} = 0.070 ± 0.015 μM and **1e**, EC_{50} = 0.068 ± 0.040 μM) showed 10-folds stronger inhibitory than tri substitution of acetyl ester analog (**1c**, EC_{50} = 0.41 ± 0.56 μM) and over 350 times more active than the original baicalein (**1a**, EC_{50} = 23.9 μM[33]). However, tri acetyl ester group of brominated baicalein analog (**1g**, EC_{50} = 0.26 ± 0.14 μM) exhibited almost 4-folds of originally brominated baicalein analog (**1f**, EC_{50} = 0.88 ± 0.14 μM[29]). On the contrary, tri propionyl ester group of brominated baicalein analog was inactive.

Additionally, modification of ring B *via* substitution at the 4' position such as most of the trimethoxy analogues (**5b**-**5d**) exhibited slightly lower inhibitory effect compared to the trihydroxy analogues (**6b** and **6c**), except for the 4'-amino analog (**6d**),

in which the trihydroxy analog showed no inhibition against DENV2. The electron-withdrawing group of 4'-nitrobaicalein (**6c**, EC_{50} = 4.30 ± 0.56 μM) and 6,7-dimethyl-4'-nitrobaicalein (**7b**, EC_{50} = 4.52 ± 0.76 μM) analogues led to significant increase in the inhibitory effect compared with the original baicalein (**1a**, EC_{50} = 23.9 μM[12]). 4'-Bromobaicalein analog (**6b**, EC_{50} = 0.52 ± 0.12 μM) showed stronger inhibition of DENV2 activity than the other modified compounds on ring B. On the other hand, 7-methyl-4'-nitrobaicalein analog (**8a**) exhibited the lowest inhibition of DENV2. For cell cytotoxicity, all compounds were also nontoxic towards LLC/MK2 cell at the concentration of 10 μM with the %cell viability above 70% for all analogs excepting 8-bromo-5,6,7-triacetylbaicalein analog (**1g**) which showed cell viability about 50% to LLC/MK2 cell at the concentration of 10 μM.

Table 2 Anti-dengue activity and cytotoxicity of baicalein derivatives.

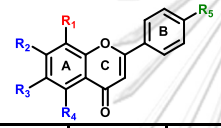
Comp.						%Inhibition[a]	EC_{50}[b] [μM]	%Viability[c]
	R^1	R^2	R^3	R^4	R^5			
1a	H	OH	OH	OH	H	75	23.9[d]	92.89±1.44
1b	H	OMe	OMe	OH	H	80.0	-[e]	97.47±2.47
1c	H	OAc	OAc	OAc	H	100	0.41±0.56	107±7.34
1d	H	OPp	OPp	OPp	H	90.0	0.070±0.015	83.79±2.61
1e	H	OPp	OPp	OH	H	91.67	0.068±0.040	89.62±5.95
1f	Br	OH	OH	OH	H	100	0.88±0.14[d]	73.36±14.80
1g	Br	OAc	OAc	OAc	H	100	0.26±0.14	50.89±3.22
1h	Br	OPp	OPp	OPp	H	NA[f]	-[e]	84.52±6.56
5a	H	OMe	OMe	OMe	H	44.44	-[e]	96.84±11.95
5b	H	OMe	OMe	OMe	Br	70.4	-[e]	101.13±11.06
5c	H	OMe	OMe	OMe	NO_2	70.0	-[e]	100.95±1.97
5d	H	OMe	OMe	OMe	NH_2	62.0	-[e]	93.00±2.36
6b	H	OH	OH	OH	Br	100	0.52±0.12	89.99±6.61
6c	H	OH	OH	OH	NO_2	100	4.30±0.56	78.55±9.87
6d	H	OH	OH	OH	NH_2	NA[f]	-[e]	108.69±2.53
7a	H	OMe	OMe	OH	Br	64.8	-[e]	96.78±5.28

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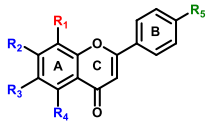
Table 3 Anti-dengue activity and cytotoxicity of baicalein derivatives. (Continued)

Comp.						%Inhibition[a]	EC_{50} [b] [μM]	%Viability[c]
	R^1	R^2	R^3	R^4	R^5			
7b	H	OMe	OMe	OH	NO_2	98.2	4.52±0.76	84.73±5.07
8a	H	OMe	OH	OH	NO_2	22.2	-[e]	91.60±0.89

[a] %viral inhibition against DENV2 at 10 μM; [b] EC_{50}: 50% effective concentration against DENV2; [c] %viability in LLC/MK2 cell at 10 μM; [d] data according to literature; [e] - = not determined; [f] NA = not active.

4. Structural-Activity Relationship (SAR) study

These compounds were established the relationship between structural modification of baicalein derivatives and anti-dengue activity, called the structure–activity relationship (SAR) as shown in Figure 16. The results showed that the 6, 7, 8 position on ring A and para position on ring B were substituted with the electron-withdrawing group giving excellent DENV2 inhibition. Modification on ring A should be substituted at C5, C6 and C7 positions with acyl group (acetyl and propionyl group) and C8 position bromo group whereas substituted propionyl group on C5, C6 and C7 positions. Modification on ring B should be substituted at C4' position with electron-withdrawing group whereas unsubstituted on C5, C6 and C7 positions. Therefore, the researcher expects that this SAR could improve anti-DENV2 activity on LLC/MK2 cells in the future.

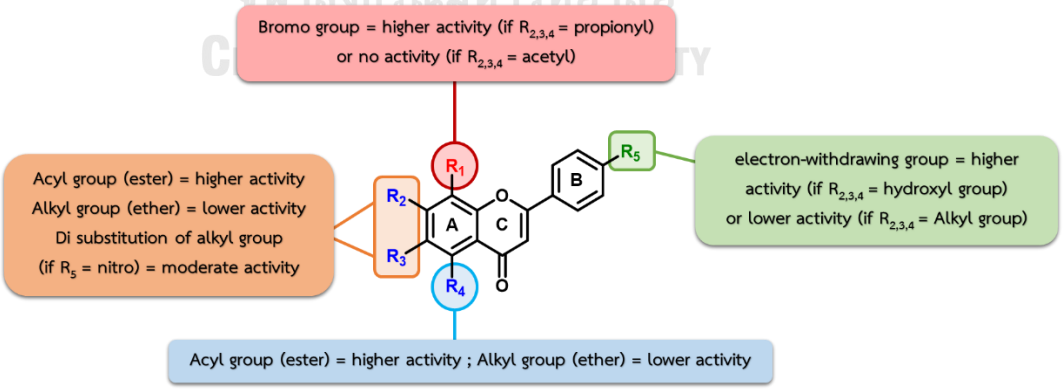


Figure 16 SAR study of modified baicalein derivatives for anti-DENV2 activity

5. Physicochemical properties prediction

The physicochemical properties and drug-likeness of the baicalein derivatives, as predicted by SwissADME, are showed in Table 3. Most of these molecules were acceptable following by Lipinski's rule of five except for compound **1h** due to its slightly higher molecular weight (more than 500) and Lipinski Violations (should be the value = 0). The lipophilicity prediction (log P) demonstrated that the acceptable values of log P is in a range of 2.34 to 4.54 which compound **1h** showed higher lipophilicity than other compounds due to non-polar propionyl group. On the other hand, the solubility prediction (log S) exhibited that the values of log S are in a range of -5.61 to -3.62 which compound **6d** showed highest hydrophilicity due to polar NH_2 group of trihydroxy derivative. The total polar surface area (TPSA) of all compounds showed acceptable values, corresponding to the values of HBD and HAD, suggesting that these compounds might have are good cell permeability. Therefore, the synthetic baicalein compounds are predicted to have good physicochemical properties and drug-likeness following by Lipinski's rule of five and Verber parameters, except for compound **1h**. Moreover, high molecular refractivity or MR demonstrated strong attractions of London dispersion forces of the drug-receptor interaction such as compound **1d**.

Table 4 Predicted physicochemical properties and drug-likeness of baicalein derivatives

Comp.	Drug-likeness parameters					Verber parameters			Others	
	MW[a]	HBD[b]	HBA[c]	Log P[d]	Lip. Vio[e]	TPSA[f]	No. of RB[g]	Ver. Vio[h]	Log S[i]	MR[j]
1a	270.24	3	5	3.16	0	90.90	1	0	-4.03	73.99
1b	298.29	1	5	3.82	0	68.90	3	0	-4.44	82.93
1c	396.35	0	8	2.44	0	109.11	7	0	-3.78	102.42
1d	438.43	0	8	3.85	0	109.11	10	0	-4.69	116.84
1e	382.36	1	7	3.82	0	103.04	7	0	-4.58	102.56
1f	349.13	3	5	3.36	0	90.90	1	0	-4.62	81.69
1g	475.24	0	8	3.13	0	109.11	7	0	-4.69	110.12
1h	517.32	0	8	4.54	1	109.11	10	0	-5.61	124.54
5a	312.32	0	5	3.09	0	57.90	4	0	-3.97	87.40
5b	391.21	0	5	3.79	0	57.90	4	0	-4.88	95.10
5c	357.31	0	7	2.92	0	103.72	5	0	-4.02	96.22

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Table 5 Predicted physicochemical properties and drug-likeness of baicalein derivatives (Continued)

Comp.	Drug-likeness parameters					Verber parameters			Others	
	MW[a]	HBD[b]	HBA[c]	Log P[d]	Lip. Vio[e]	TPSA[f]	No. of RB[g]	Ver. Vio[h]	Log S[i]	MR[j]
5d	327.33	1	5	2.41	0	83.92	4	0	-3.62	91.80
6b	349.13	3	5	3.71	0	90.90	1	0	-4.84	81.69
6c	315.23	3	7	2.85	0	136.72	2	0	-3.97	82.81
6d	285.25	4	5	2.34	0	116.92	1	0	-3.58	78.39
7a	377.19	1	5	4.36	0	68.90	3	0	-5.24	90.63
7b	343.29	1	7	3.50	0	114.72	4	0	-4.38	91.75
8a	329.26	2	7	3.18	0	125.72	3	0	-4.18	87.28

[a]MW = Molecular weight: ≤500; [b] HBD = Hydrogen bond donors: ≤5; [c] HBA = Hydrogen bond acceptors: ≤10; [d] Log P = log of octanol to water partition coefficient: ≤5; [e] Lip. Vio. = Lipinski Violations; [f] TPSA = Total polar surface area [A°]^2: ≤140; [g] No. of RB = Number of rotatable bonds: ≤10; [h] Ver. Vio = Verber Violations; [i] Log S = log of aqueous solubility (mol/L): −6 to 0; [j] MR = Molecular refractivity [cm^3/mol]: 40 to 130.

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Chapter IV

Conclusion

In conclusion, five novel and thirteen known baicalein derivatives were successfully synthesized *via* semi synthesis from baicalein with modifications at C5, C6, C7, and C8 on ring A, and a four-step total synthesis with modifications at the para position on the ring B with a range of electron-withdrawing or electron-donating groups, with the overall yield ranging from 14% to 40%. Most compounds exhibited activity against DENV2 with good %inhibition and %viability. The anti–dengue activity of these baicalein analogues against DENV2 in LLC/MK2 cells revealed that the compounds with electron-withdrawing substituents in both ring A and B such as **1b**-**1g**, **6b**-**6c** and **7b** were the most effective with the range of EC_{50} between 0.068±0.040 and 4.52±0.76 μM at concentration 10 μM. Moreover, these compounds were found to be relatively non-toxic to normal cell. Especially, the tri (**1d**, EC_{50} = 0.070 ± 0.015 μM) and di (**1e**, EC_{50} = 0.068 ± 0.040 μM) propionyl derivatives showed efficiently inhibited DENV2 activity with great viability (**1d**, viability = 83.79±2.61 and **1e**, viability = 89.62±5.95). On the other hand, 8-bromo-5,6,7-tripropionoyl (**1h**) and 4’ amino (**6d**) derivatives were not active. Moreover, the synthetic baicalein compounds have good physicochemical properties and drug-likeness according to the Lipinski's rule of five and Verber parameters except for compound **1h**. Therefore, baicalein analogs with the presence of the electron-withdrawing groups at 6, 7, and 8 position in on ring A and *para* position on ring B can become a promising candidate for further development into novel anti-dengue agents.

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จุฬาลงกรณ์มหาวิทยาลัย
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APPENDIX

จุฬาลงกรณ์มหาวิทยาลัย
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5-hydroxy-6,7-dimethoxy-2-phenyl-4H-chromen-4-one (1b)

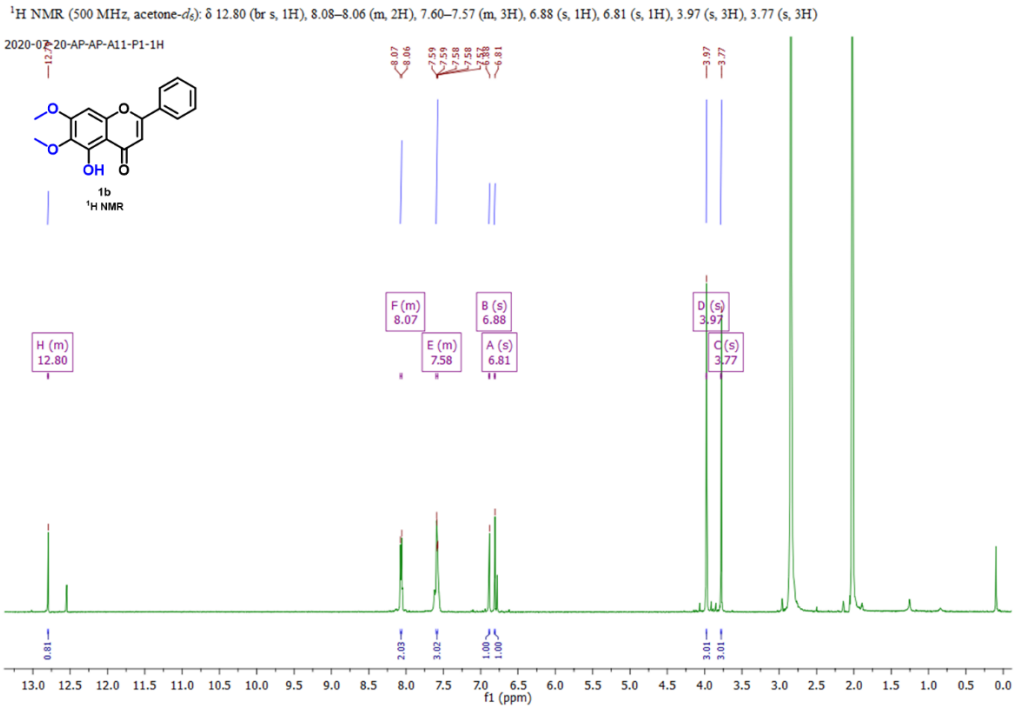

Figure 17 ^{1}H NMR spectrum of **1b**

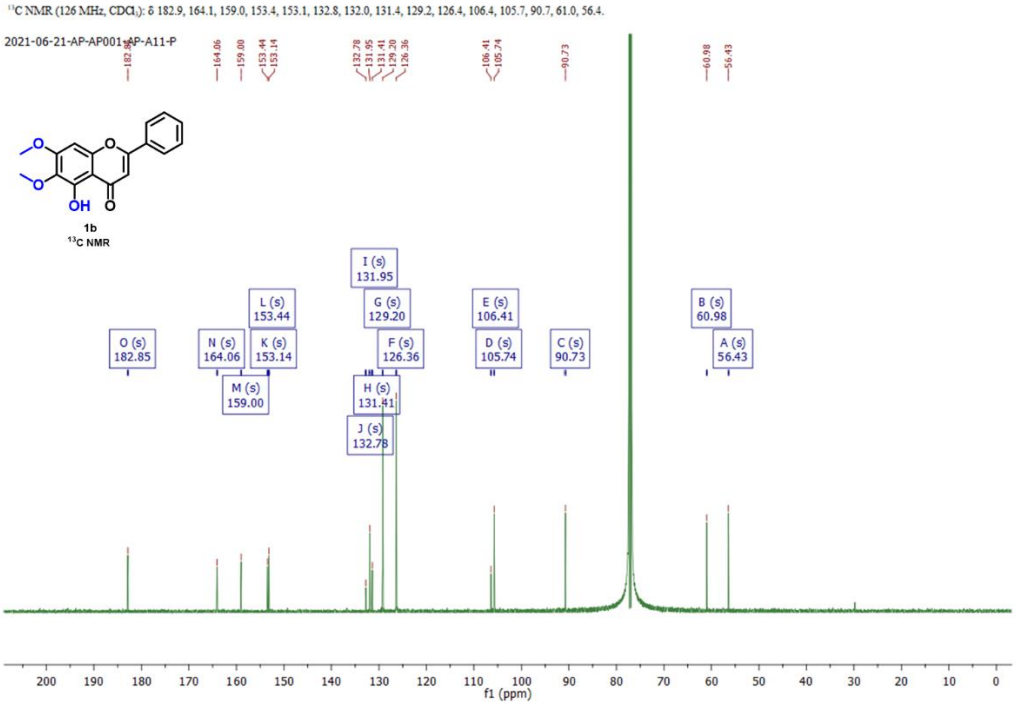

Figure 18 ^{13}C NMR spectrum of **1b**

4-oxo-2-phenyl-4H-chromene-5,6,7-triyl triacetate (1c)

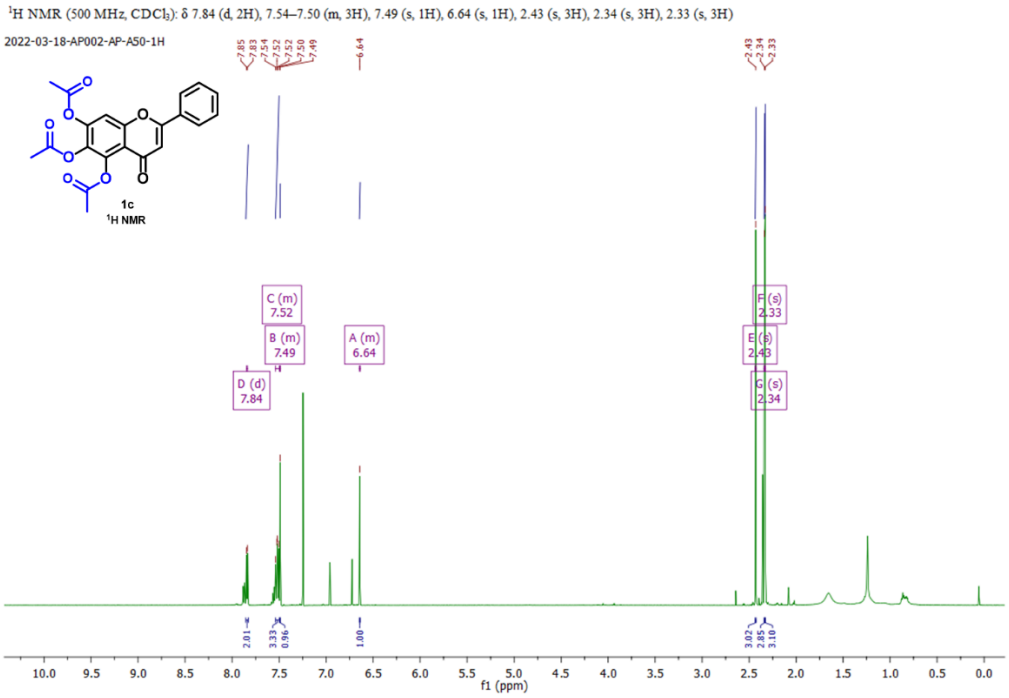

Figure 19 ^{1}H NMR spectrum of **1c**

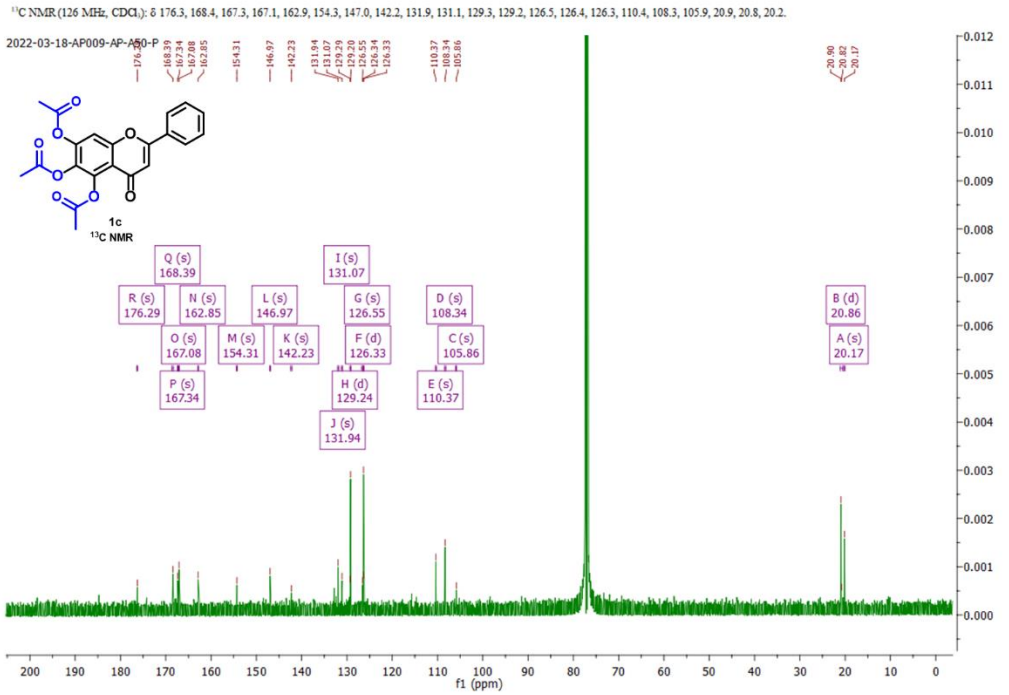

Figure 20 ^{13}C NMR spectrum of **1c**

4-oxo-2-phenyl-4H-chromene-5,6,7-triyl tripropionate (1d)

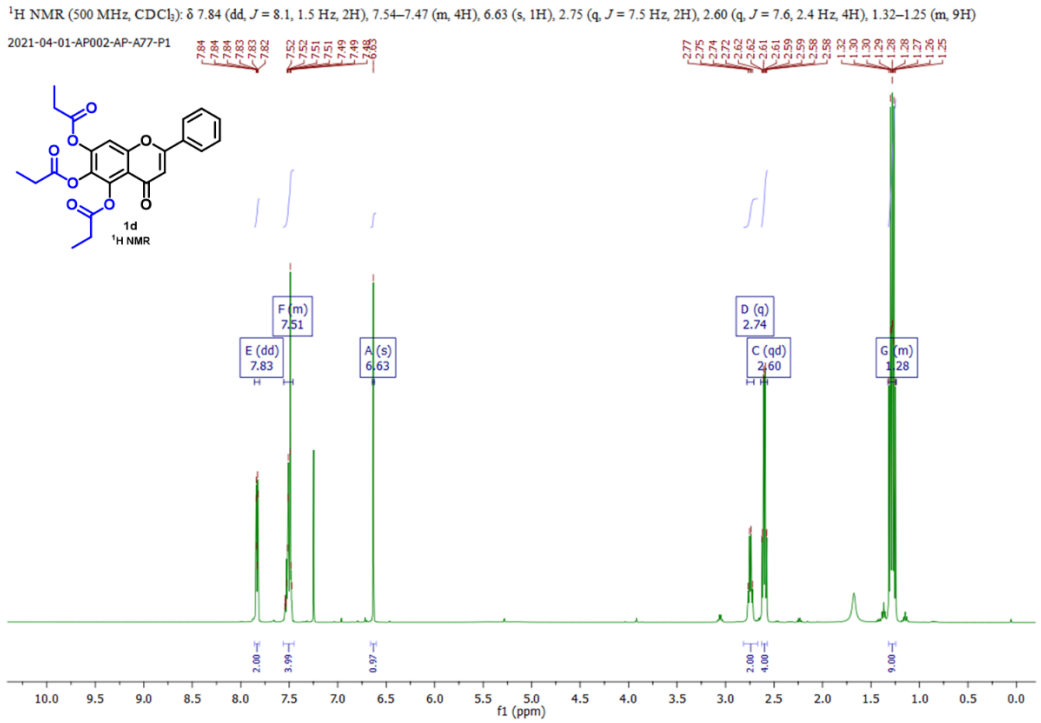

Figure 21 ^{1}H NMR spectrum of **1d**

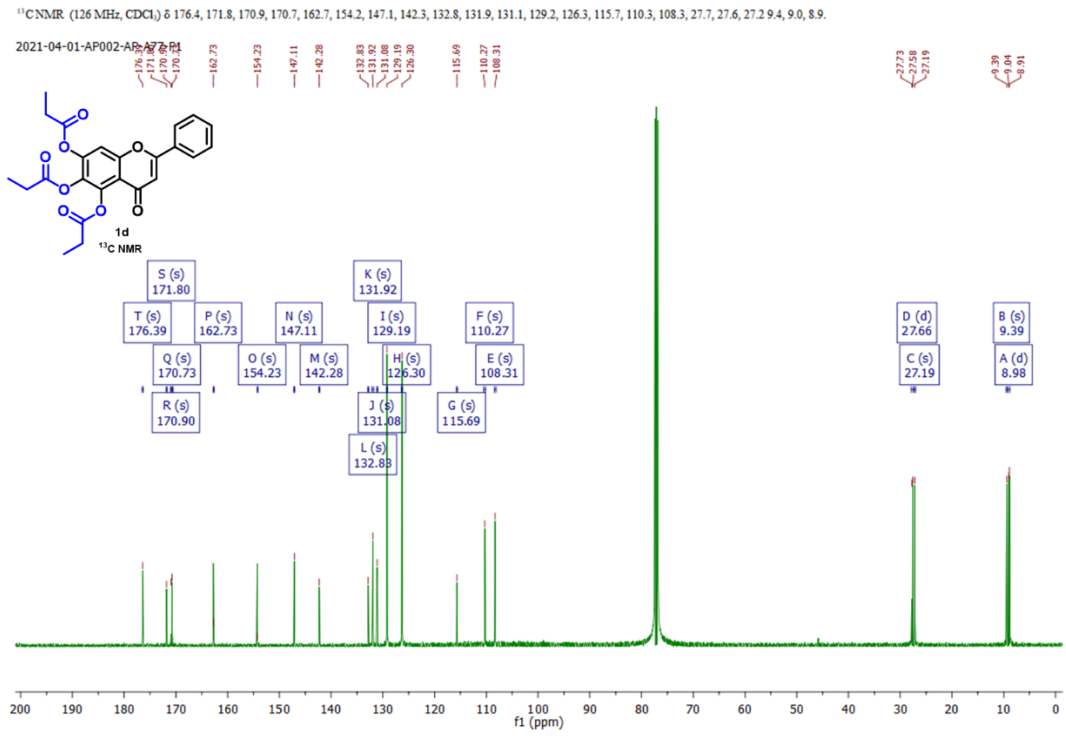

Figure 22 ^{13}C NMR spectrum of **1d**

5-hydroxy-4-oxo-2-phenyl-4H-chromene-6,7-diyl dipropionate (1e)

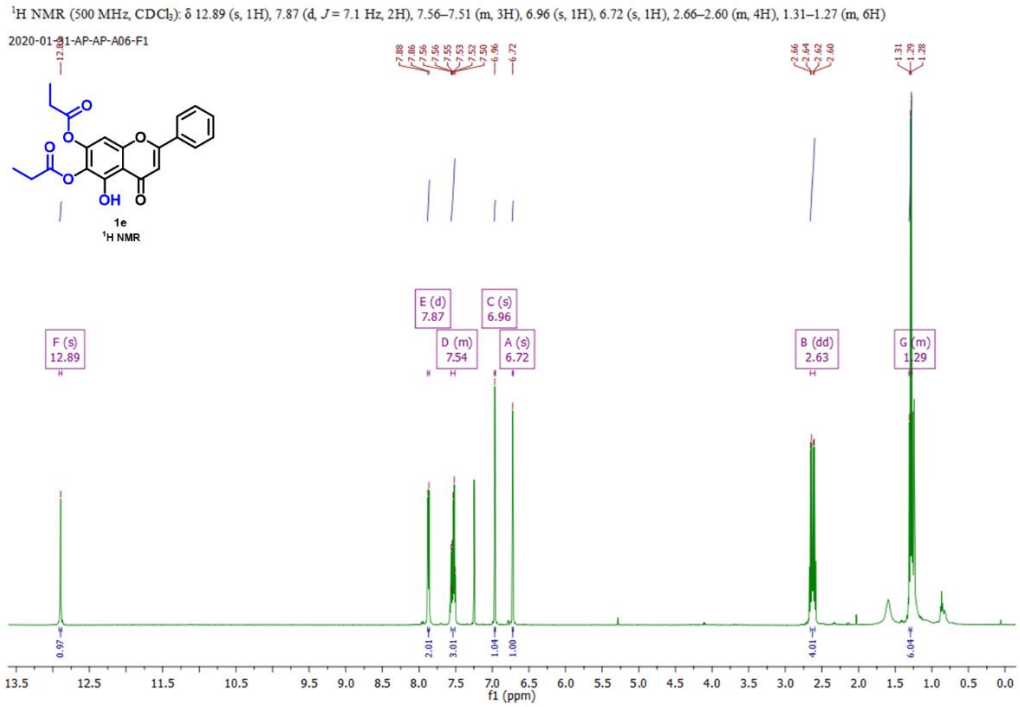

Figure 23 ^{1}H NMR spectrum of **1e**

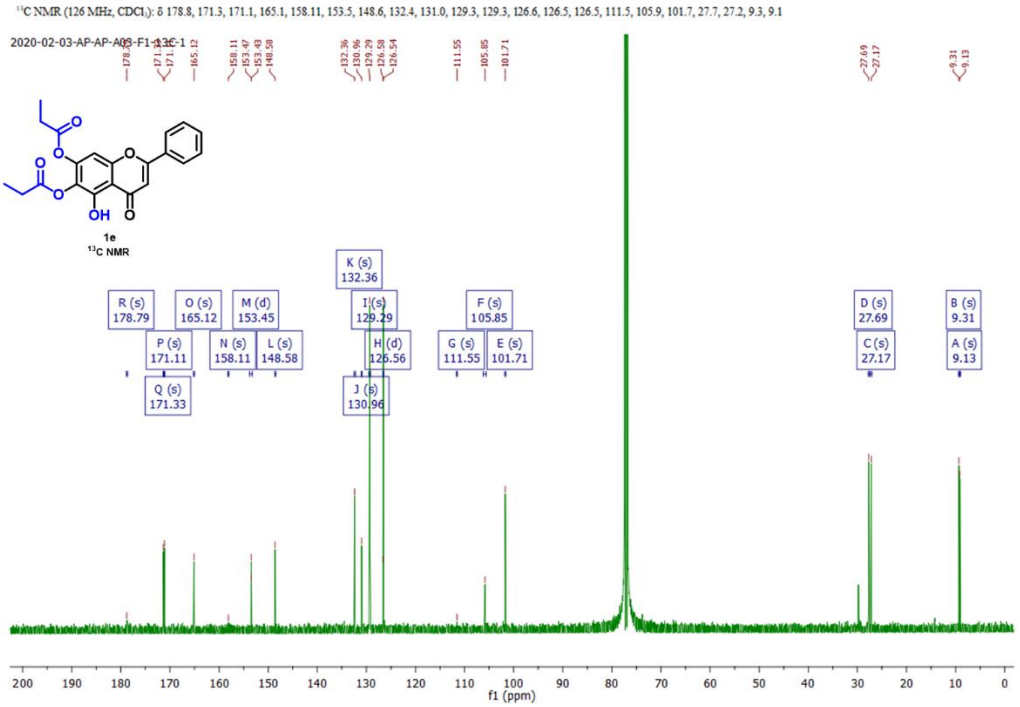

Figure 24 ^{13}C NMR spectrum of **1e**

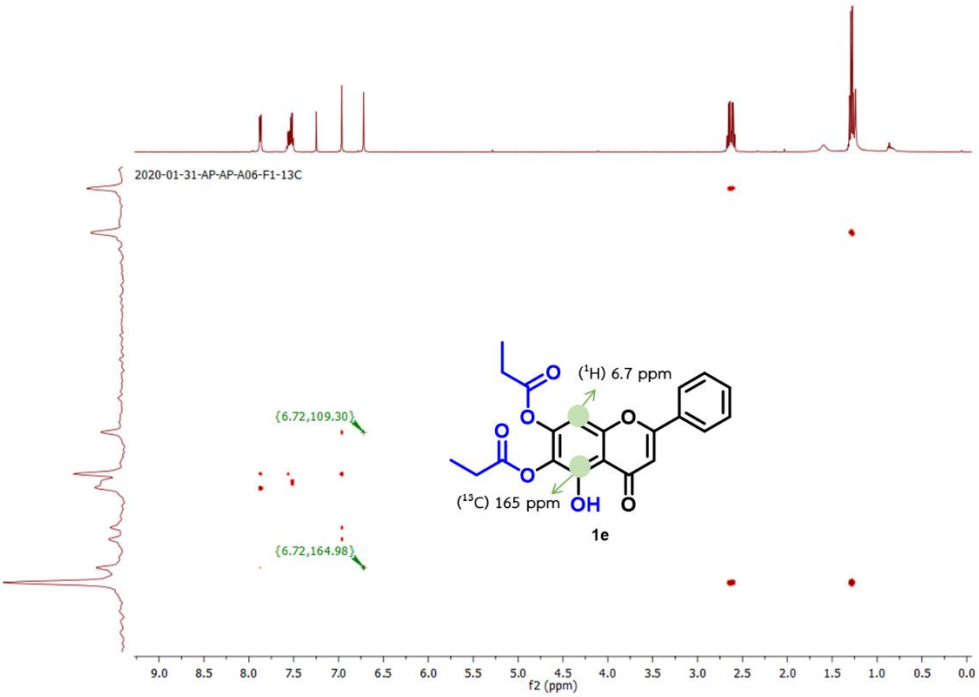

Figure 25 2D NMR (HMBC) of spectrum of **1e**

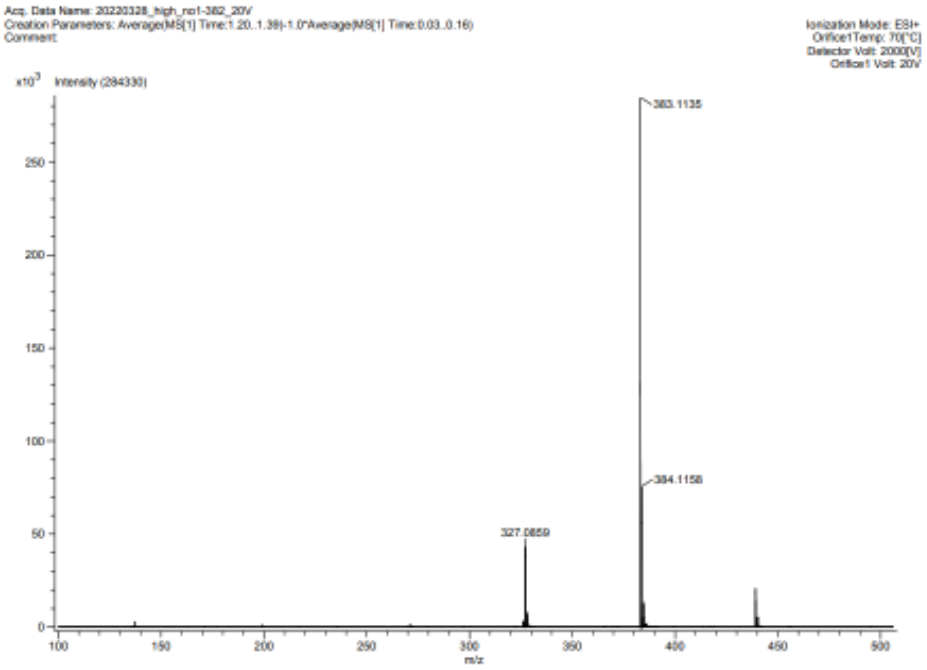

Figure 26 HSMS of **1e**

8-bromo-5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one (1f)

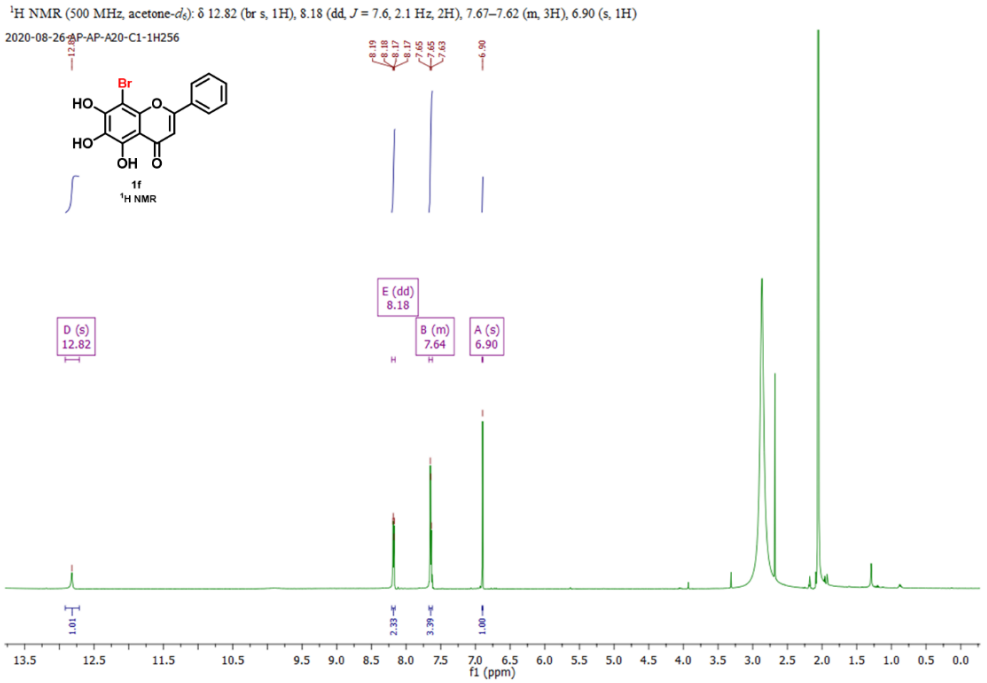

Figure 27 ^{1}H NMR spectrum of **1f**

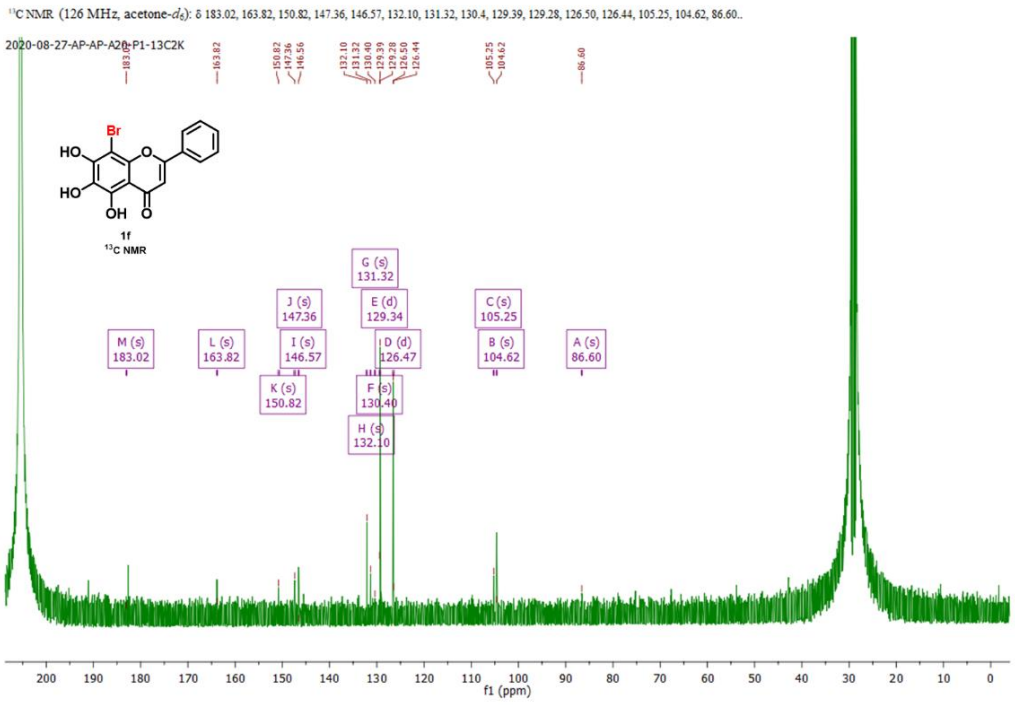

Figure 28 ^{13}C NMR spectrum of **1f**

8-bromo-4-oxo-2-phenyl-4H-chromene-5,6,7-triyl triacetate (1g)

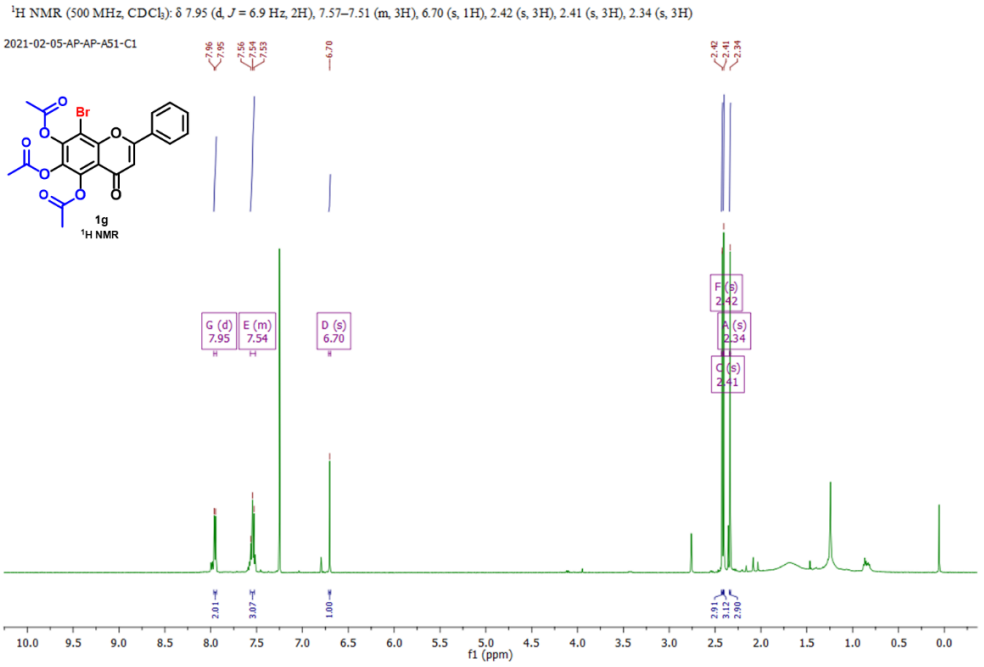

Figure 29 ^{1}H NMR spectrum of **1g**

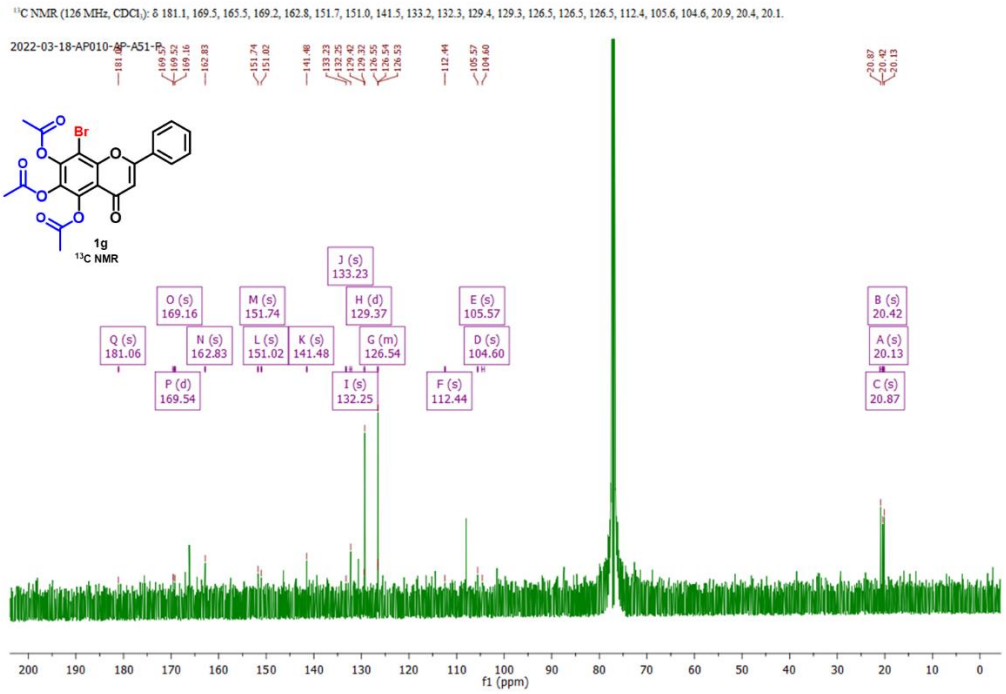

Figure 30 ^{13}C NMR spectrum of **1g**

8-bromo-4-oxo-2-phenyl-4H-chromene-5,6,7-triyl tripropionate (1h)

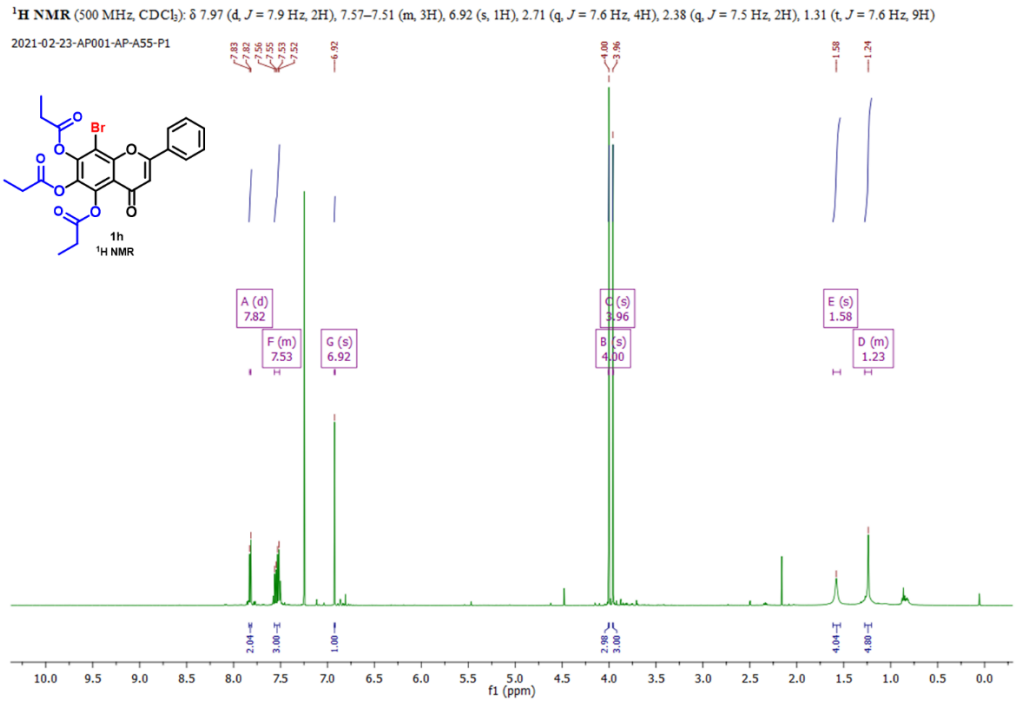

Figure 31 ^{1}H NMR spectrum of **1h**

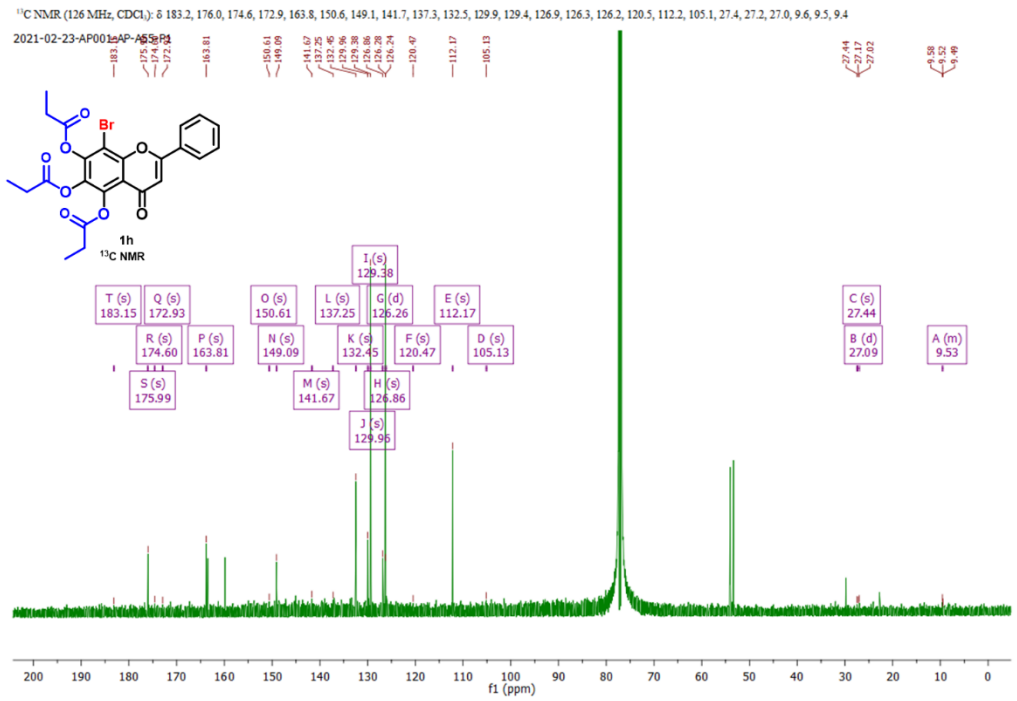

Figure 32 ^{13}C NMR spectrum of **1h**

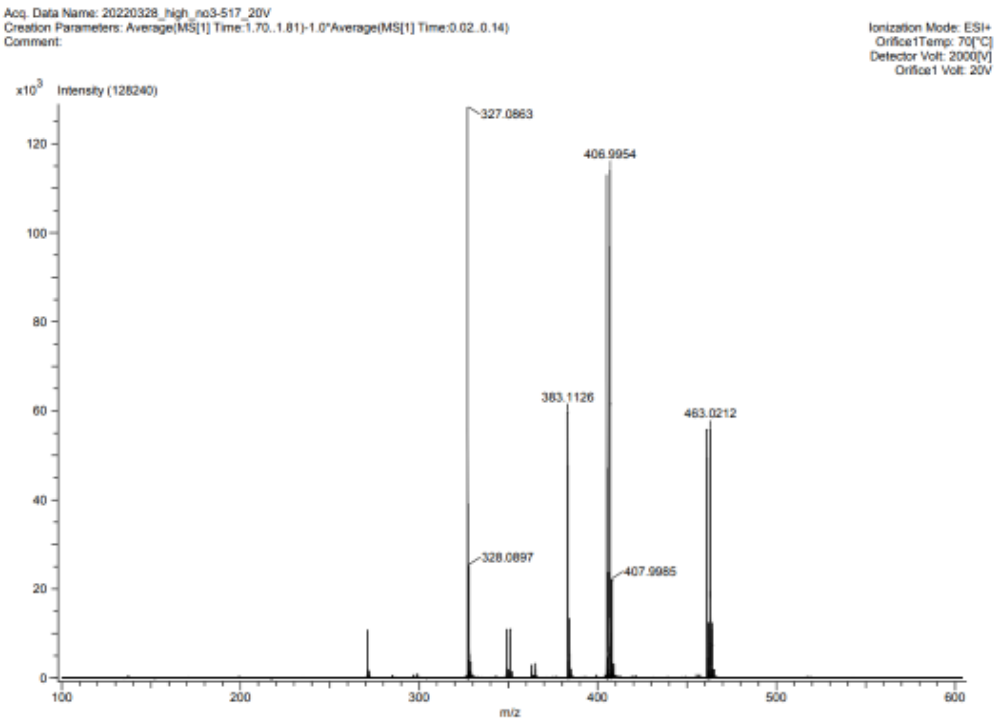

Figure 33 HSMS of **1h**

5,6,7-trimethoxy-2-phenyl-4H-chromen-4-one (5a)

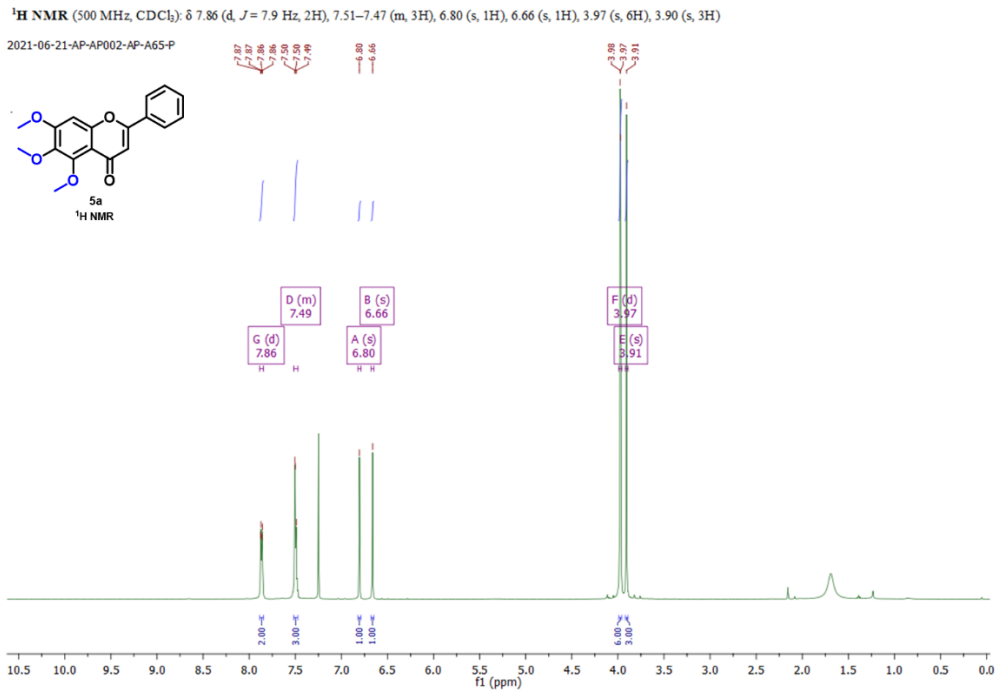

Figure 34 ^{1}H NMR spectrum of **5a**

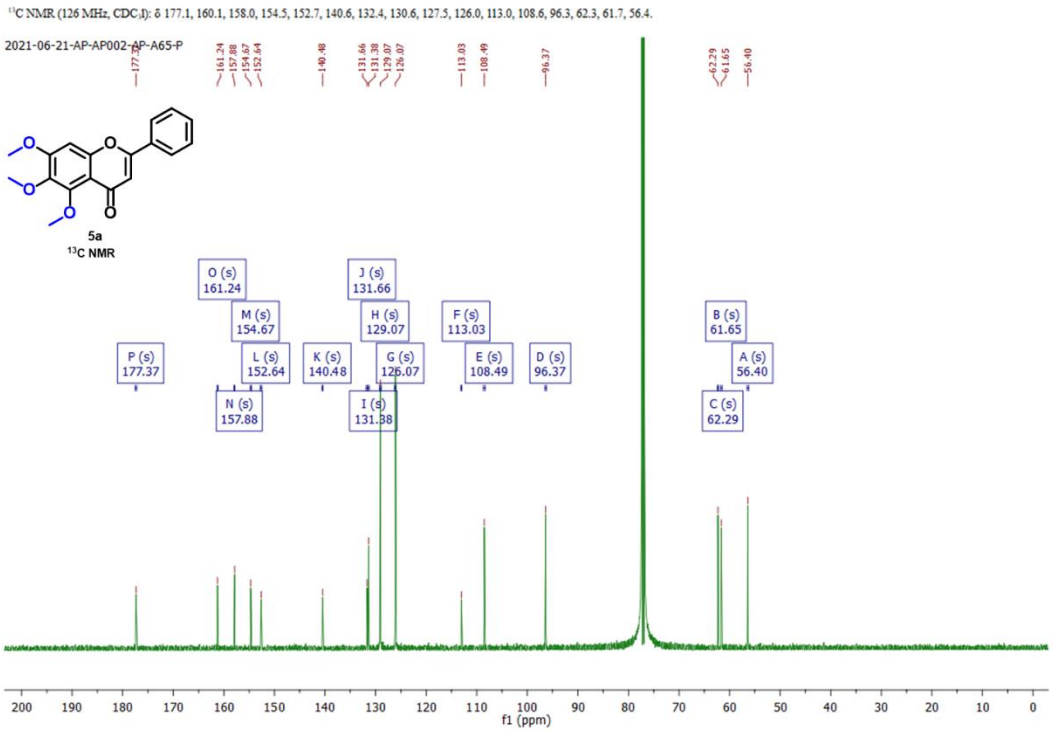

Figure 35 ^{13}C NMR spectrum of **5a**

2-(4-bromophenyl)-5,6,7-trimethoxy-4H-chromen-4-one (5b)

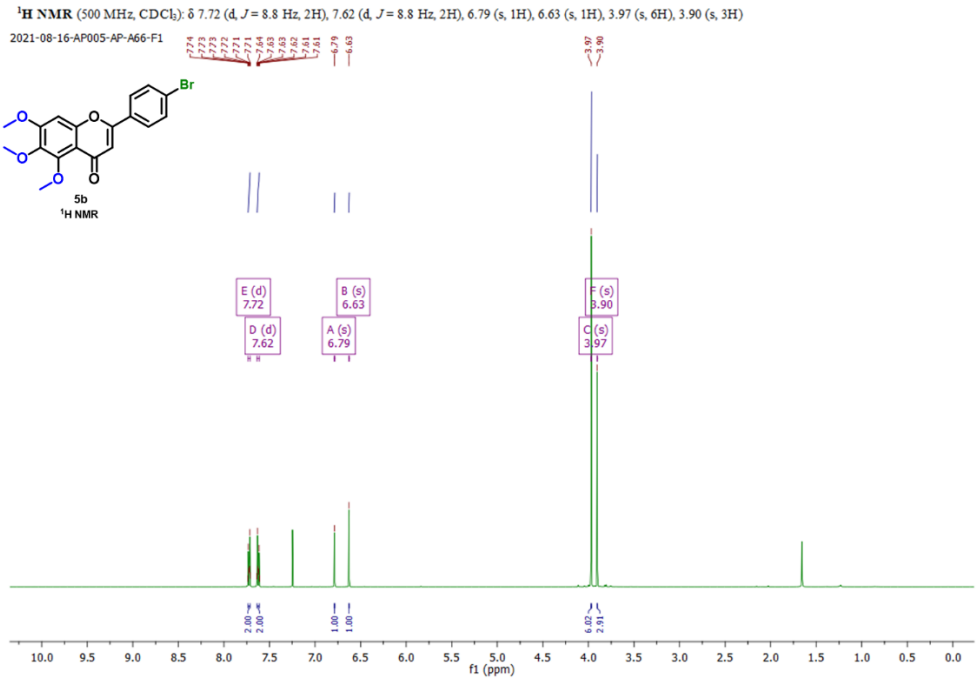

Figure 36 ^{1}H NMR spectrum of **5b**

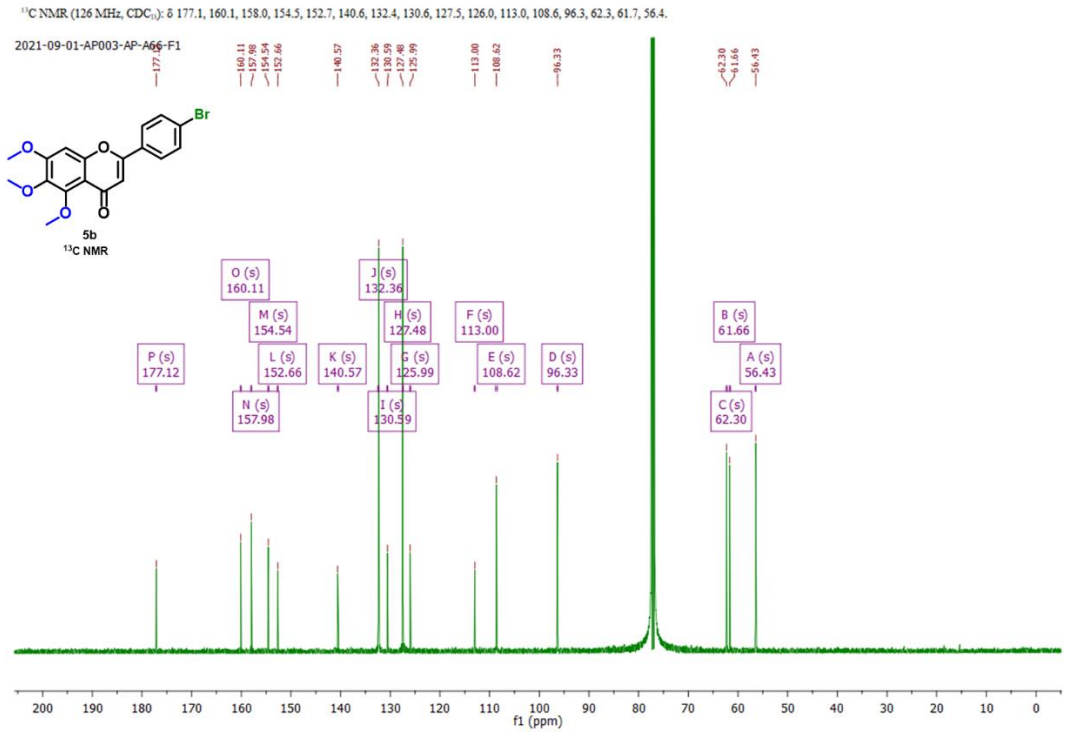

Figure 37 ^{1}H NMR spectrum of **5b**

5,6,7-trimethoxy-2-(4-nitrophenyl)-4H-chromen-4-one (5c)

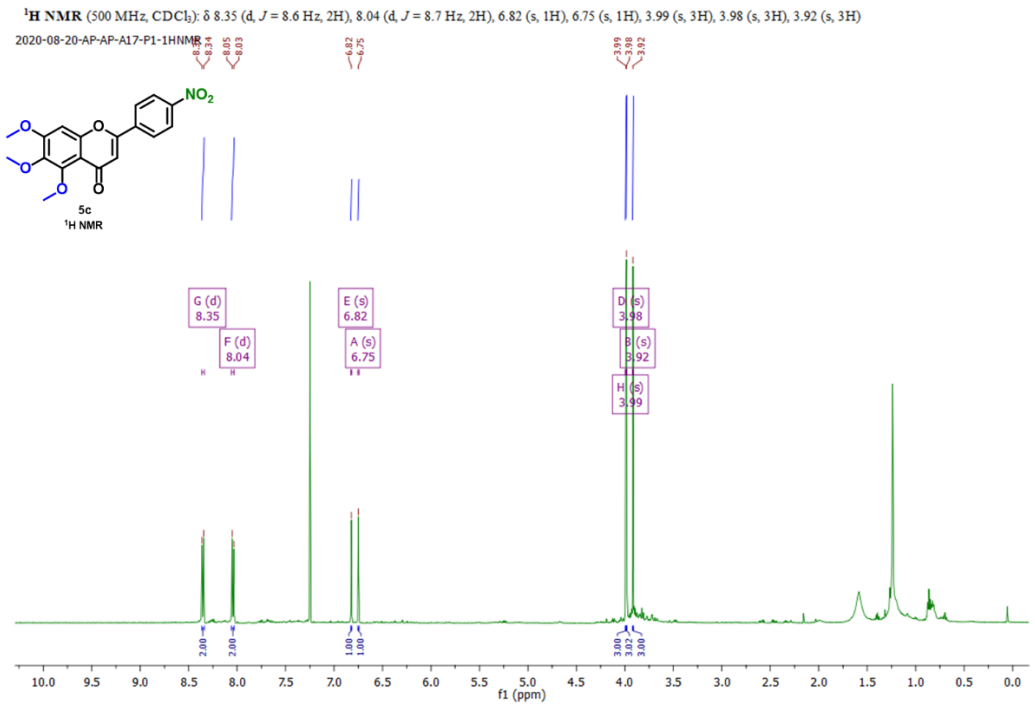

Figure 38 ^{1}H NMR spectrum of **5c**

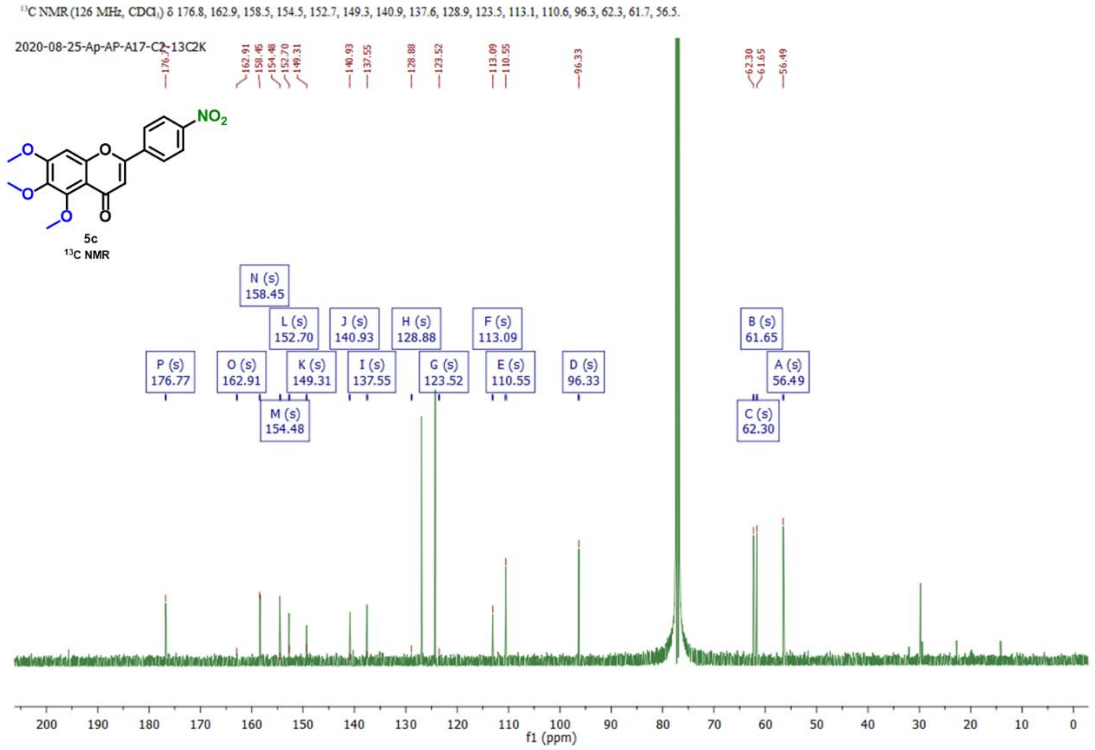

Figure 39 ^{13}C NMR spectrum of **5c**

2-(4-aminophenyl)-5,6,7-trimethoxy-4H-chromen-4-one (5d)

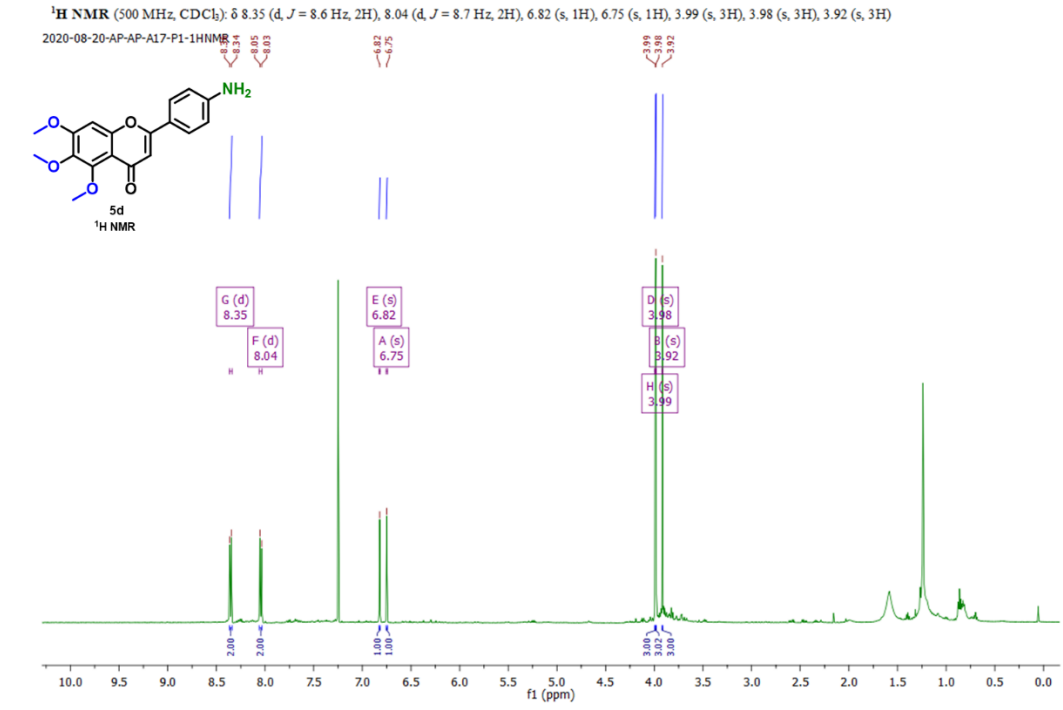

Figure 40 ^{1}H NMR spectrum of **5d**

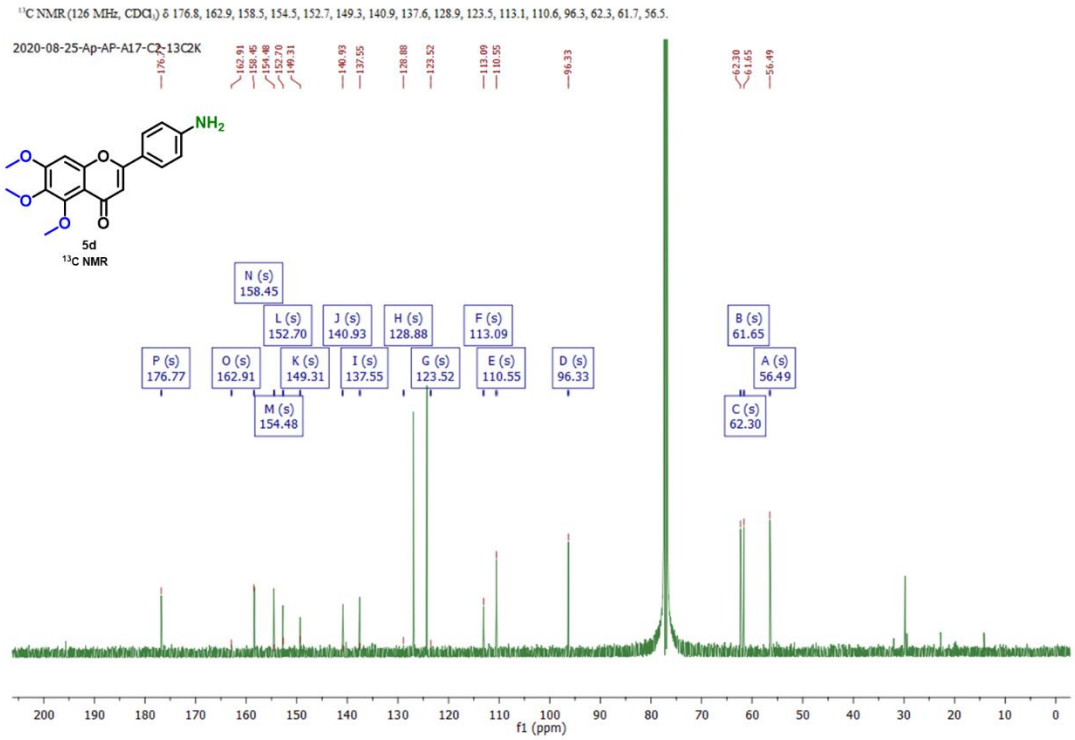

Figure 41 ^{13}C NMR spectrum of **5d**

2-(4-bromophenyl)-5,6,7-trihydroxy-4H-chromen-4-one (6b)

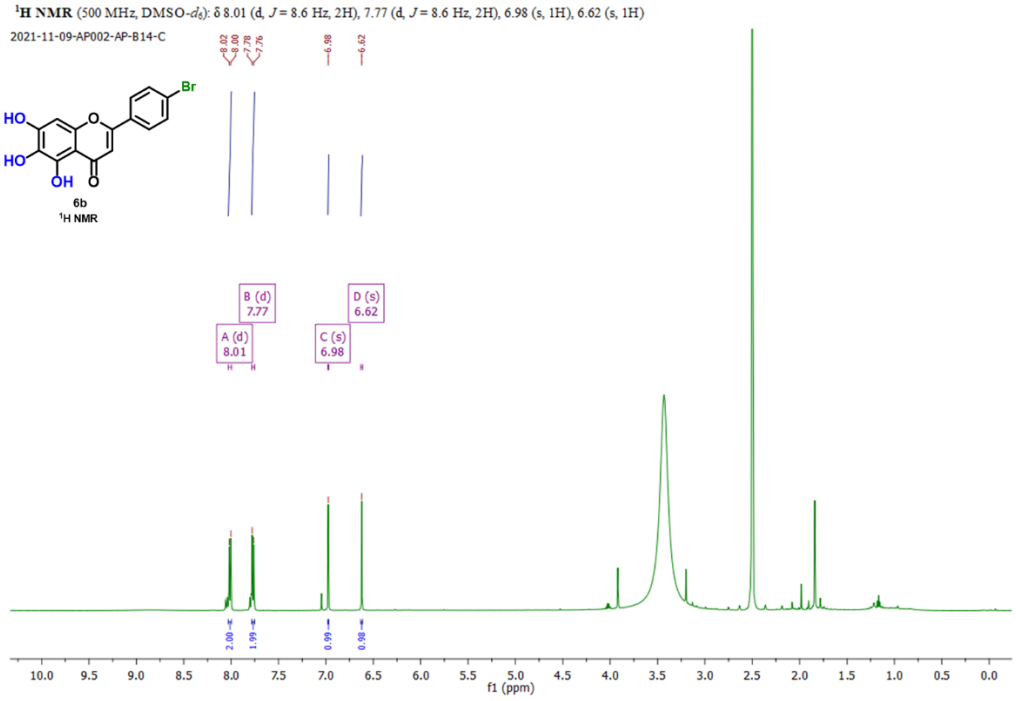

Figure 42 ^{1}H NMR spectrum of **6b**

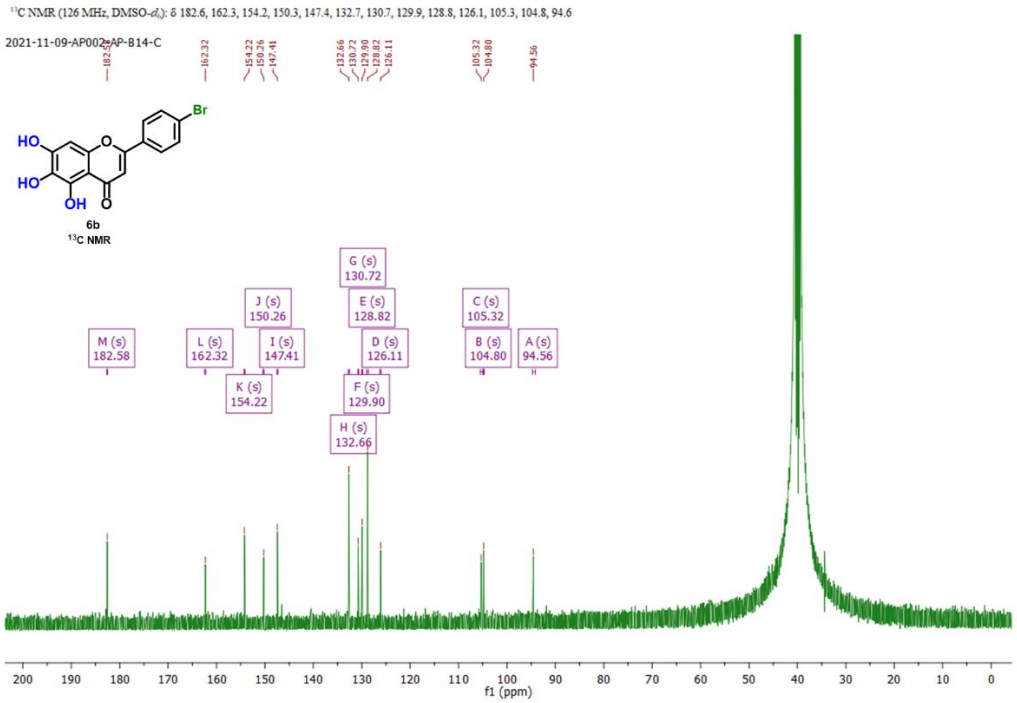

Figure 43 ^{13}C NMR spectrum of **6b**

5,6,7-trihydroxy-2-(4-nitrophenyl)-4H-chromen-4-one (6c)

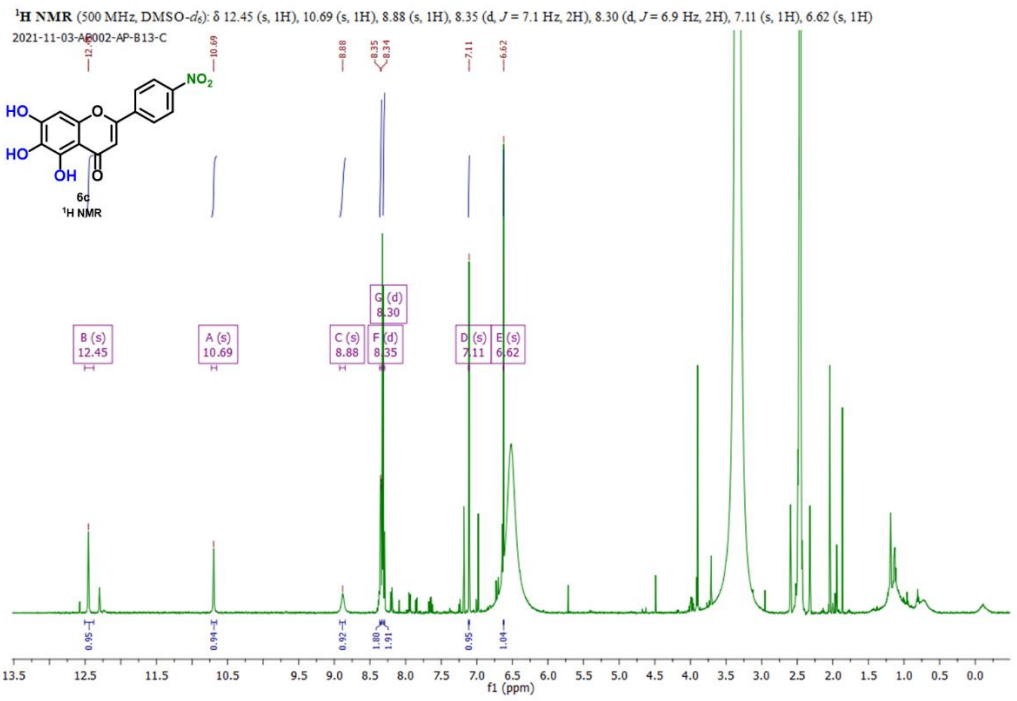

Figure 44 ^{1}H NMR spectrum of **6c**

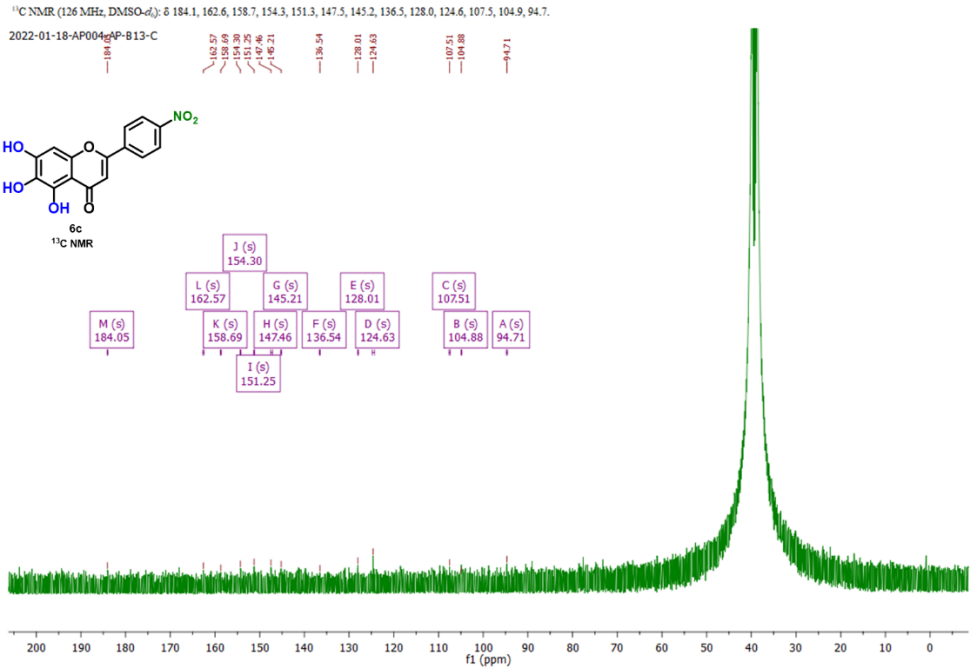

Figure 45 ^{13}C NMR spectrum of **6c**

2-(4-aminophenyl)-5,6,7-trihydroxy-4H-chromen-4-one (6d)

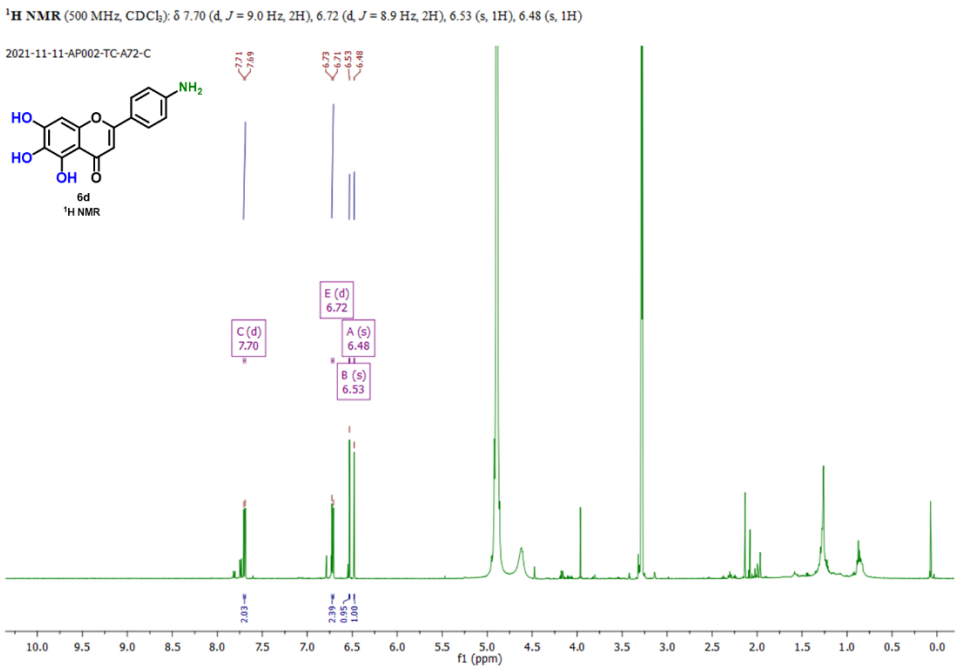

Figure 46 ^{1}H NMR spectrum of **6d**

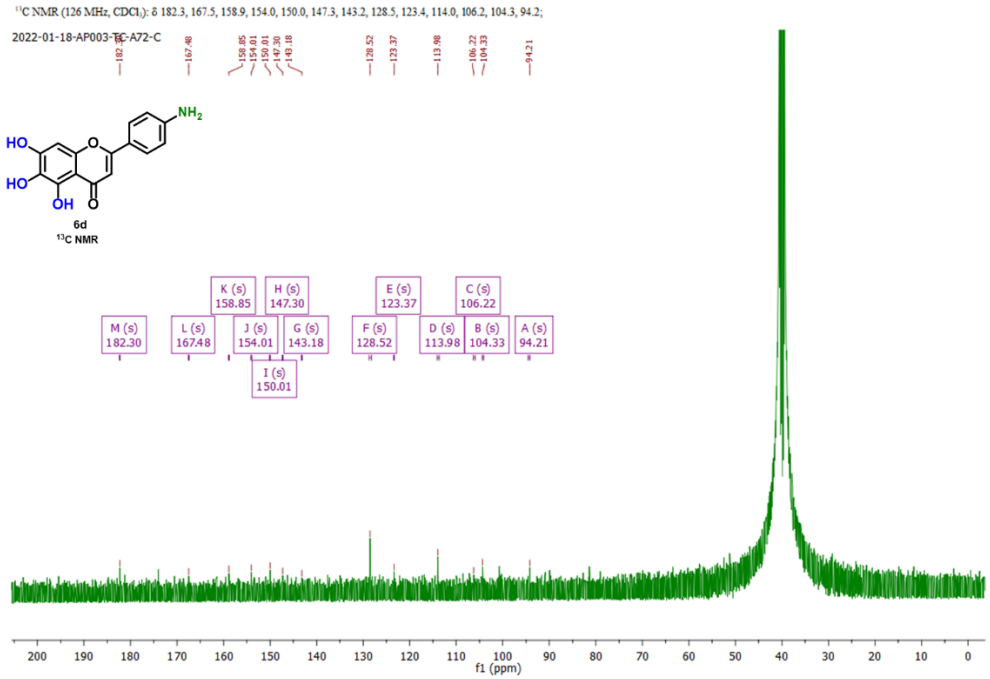

Figure 47 13C NMR spectrum of **6d**

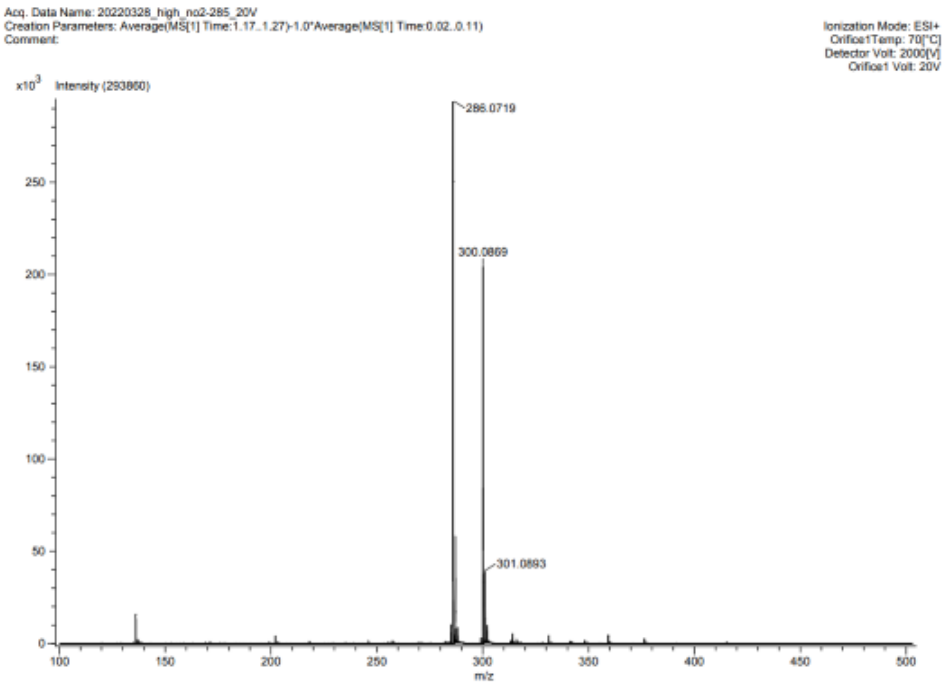

Figure 48 HSMS of **6d**

2-(4-bromophenyl)-5-hydroxy-6,7-dimethoxy-4H-chromen-4-one (7a)

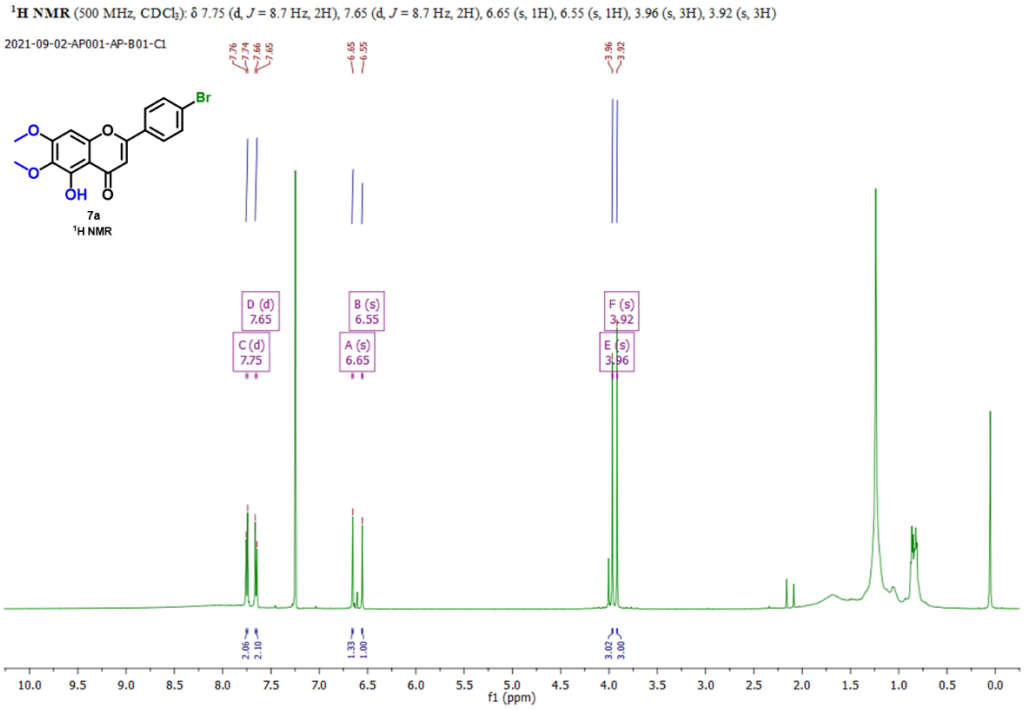

Figure 49 ^{1}H NMR spectrum of **7a**

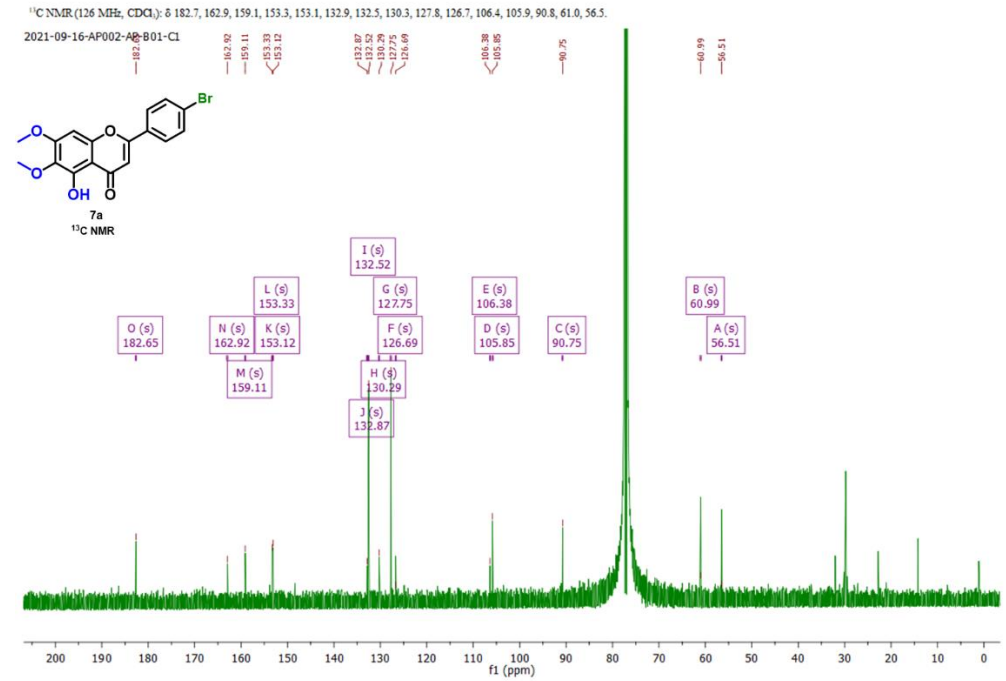

Figure 50 ^{13}C NMR spectrum of **7a**

5-hydroxy-6,7-dimethoxy-2-(4-nitrophenyl)-4H-chromen-4-one (7b)

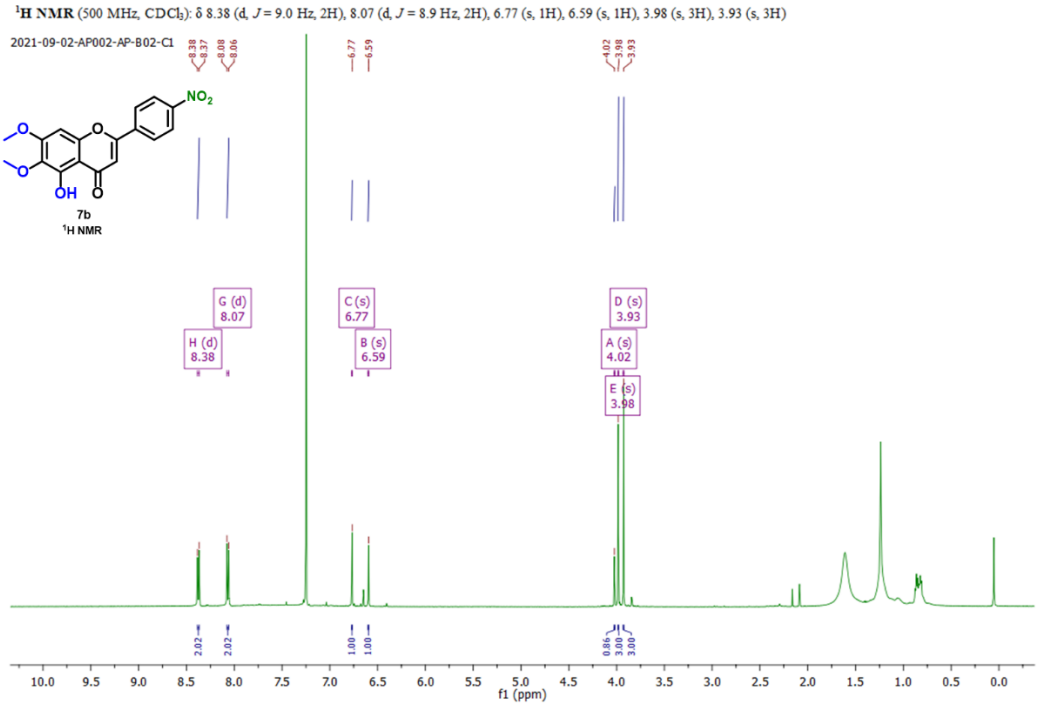

Figure 51 ^{1}H NMR spectrum of **7b**

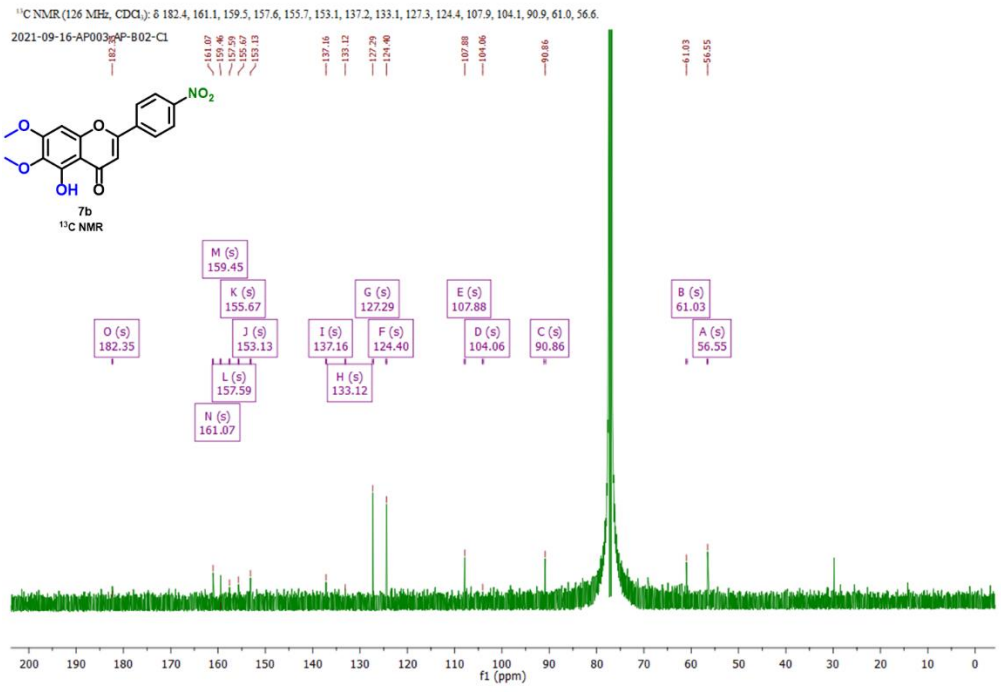

Figure 52 ^{13}CNMR spectrum of **7b**

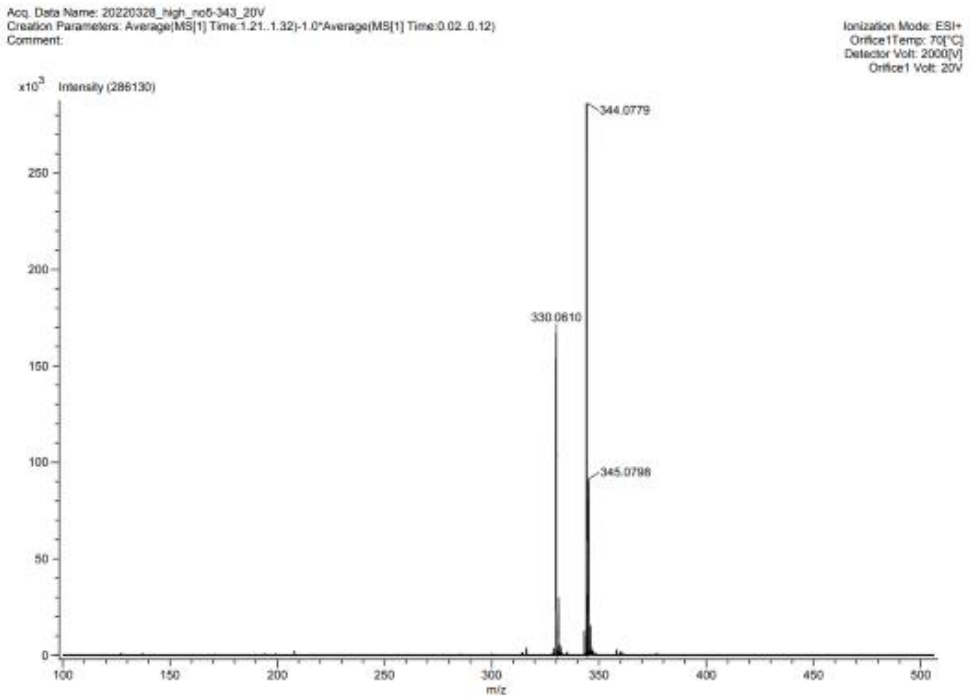

Figure 53 HSMS of **7b**

5,6-dihydroxy-7-methoxy-2-(4-nitrophenyl)-4H-chromen-4-one (8a)

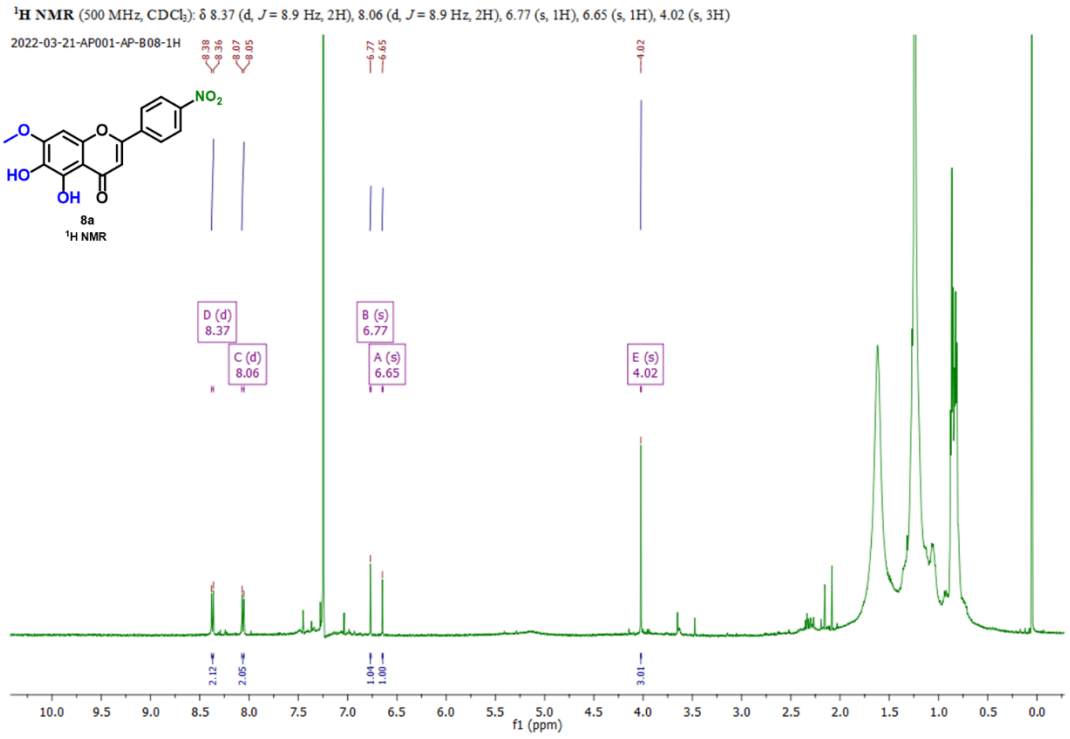

Figure 54 ^{1}H NMR spectrum of **8a**

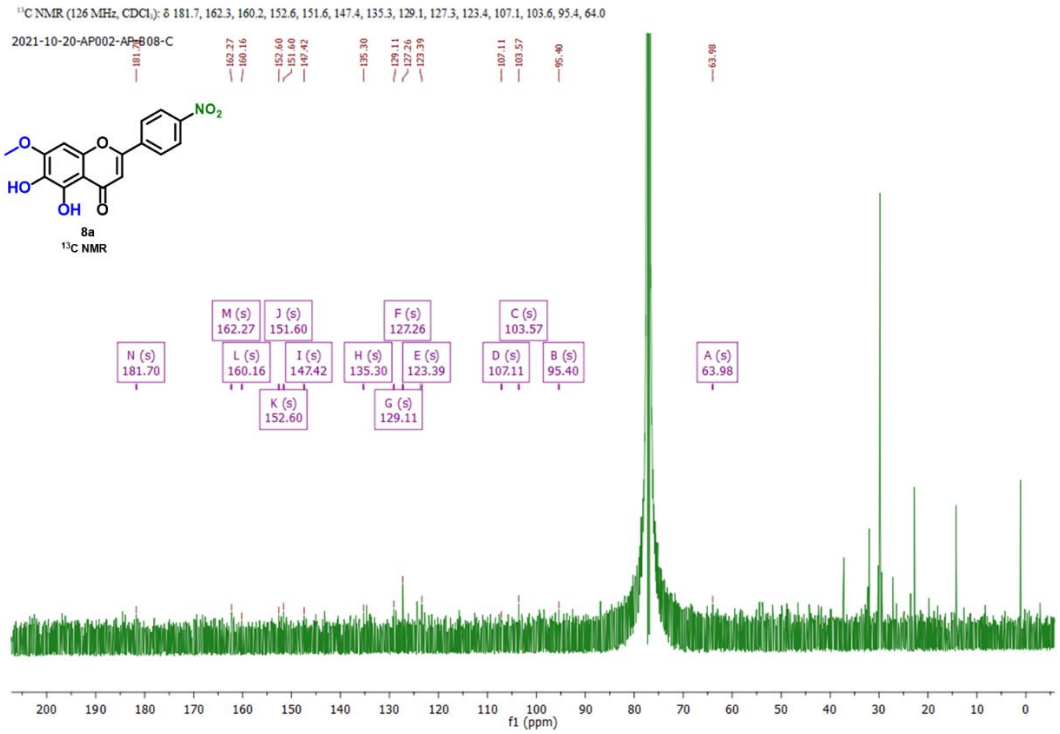

Figure 55 ^{13}C NMR spectrum of **8a**

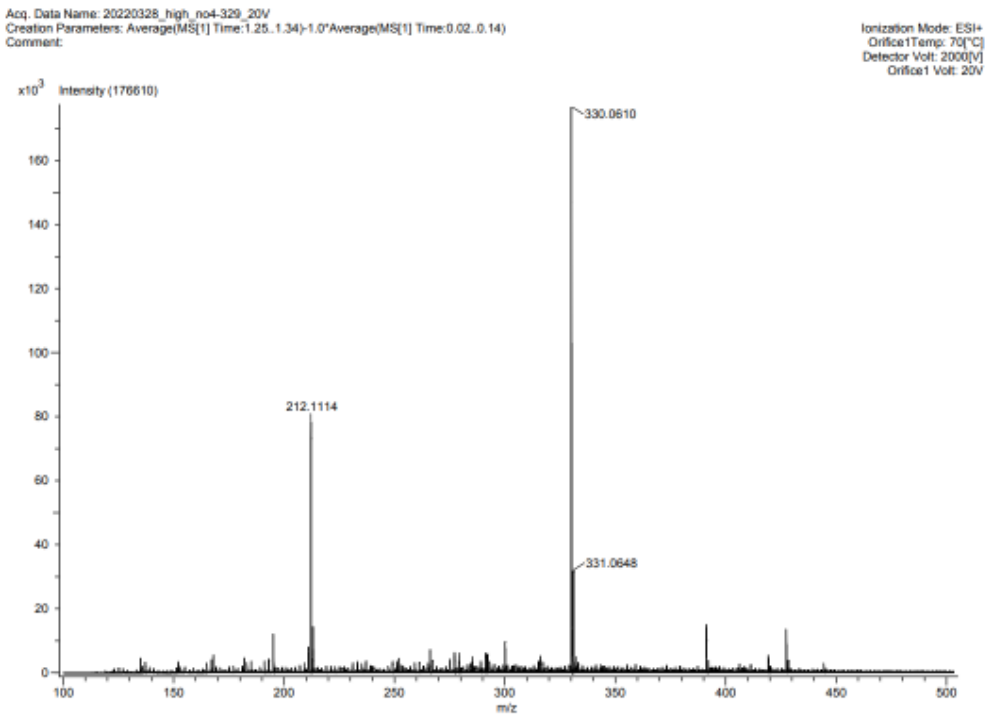

Figure 56 HSMS of **8a**

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PUBLICATION	-
AWARD RECEIVED	-

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY