SYNTHESIS OF 1,2-NAPHTHOQUINONE DERIVATIVES AS α-GLUCOSIDASE INHIBITORS

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Chemistry

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การสังเคราะห์อนุพันธ์ 1,2-แนพโทควิโนนเป็นตัวยับยั้งแอลฟากลูโคซิเดส

น.ส.ตรัง ฟาม ธิ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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| By | Miss Trang Pham Thi |
| Field of Study | Chemistry |
| Thesis Advisor | WARINTHORN CHAVASIRI |

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ตรัง ฟาม ธิ : การสังเคราะห์อนุพันธ์ 1,2-แนพโทควิโนนเป็นตัวยับยั้งแอลฟากลูโคซิเดส. ( SYNTHESIS OF 1,2-NAPHTHOQUINONE DERIVATIVES AS α-GLUCOSIDASE INHIBITORS ) อ.ที่ปรึกษาหลัก : วรินทร ชวศิริ

ในช่วงสองทศวรรษได้มีการพิจารณาฤทธิ์ทางชีวภาพผ่านการออกแบบยาที่มีพื้นฐานจากโครงสร้างควิโนน งานวิจัยนี้ ได้สังเคราะห์ 4-phenylamino-, 4-alkylamino-, 4-alkylphenylamino-, 4-phenoxy- และอนุพันธ์ของ 1,2-naphthoquinone ทดสอบฤทธิ์ต้าน α-glucosidase และศึกษาความสัมพันธ์ของโครงสร้างและฤทธิ์ทางชีวภาพ ผลการทดลองชี้ให้เห็นว่า มีหลายปัจจัยที่ส่งผลต่อการยับยั้ง α-glucosidase รวมถึงอันตรกิริยาชนิด π-π ความไม่ชอบน้ำและพันธะไฮโดรเจนกับเอนไซม์ โดยปัจจัยหลักที่มีผลต่อฤทธิ์ทางชีวภาพคือ การเกิด 1,2-dione หมู่แทนที่ที่ดึงอิเลคตรอนบนวงเฟนิลและตัวเชื่อมเฮเทอโรอะตอมที่ต่อกับโครงสร้างควิโนนทำให้ฤทธิ์ทางชีวภาพเพิ่มขึ้น สารที่แสดงฤทธิ์ดีที่สุด ได้แก่ 4-(4-nitrophenylamino)-1,2-naphthoquinone (29) และ 4-methoxy-1,2-naphthoquinone (64) มีค่า $IC_{50}$ 14.59 และ 11.39 μM ตามลำดับ ดีกว่า acarbose ซึ่งเป็นยาที่ใช้ต้านเบาหวานในปัจจุบัน ($IC_{50}$ 93.63 μM)

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| สาขาวิชา    เคมี | ลายมือชื่อนิสิต .............................................. |
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For a couple of decades the bioactivity of quinone has been considered as privileged structure-based drug design. In this research, 4-phenylamino, 4-alkylamino, 4-alkylphenylamino, 4-phenoxy and other derivatives of 1,2-naphthoquinone were synthesized, evaluated for their anti **α**-glucosidase activity and elucidated for their structure-activity relationship. The results indicated that multiple factors affecting to the **α**-glucosidase inhibition included **π-π** interaction, hydrophobic interaction and hydrogen bonding with the enzyme. In general, the main factor affecting the activity was the more favorable 1,2-dione production. The presence of electron withdrawing substituents on the phenyl ring and heteroatom linker bound to the quinone skeleton increased the activity. The most active compounds were 4-(4-nitrophenylamino)-1,2-naphthoquinone (29) and 4-methoxy-1,2-naphthoquinone (64) with $IC_{50}$ value of 14.59 μM and 11.39 μM, respectively, which were better than current anti-diabetes drug, acarbose ($IC_{50}$ = 93.63 μM).

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# ACKNOWLEDGEMENTS

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| [M+Na]+ | pseudomolecular ion |
| AGI(s) | $\alpha$-Glucosidase inhibitor(s) |
| brs | broad signal |
| d | doublet (NMR) |
| dd | doublet of doublets (NMR) |
| DMF | N,N-dimethylformamide |
| DMSO | Dimethyl sulfoxide |
| equiv | equivalent |
| ESI | Electron spray ionization |
| EtOAc | Ethyl acetate |
| EtOH | Ethanol |
| g | gram(s) |
| h | hour(s) |
| HRMS | High resolution mass spectra |
| IC | Inhibition concentration |
| $K_2CO_3$ | Potassium carbonate |
| LAPA | $\beta$-lapachone |
| m | multiplet (NMR) |
| MeOH | Methanol |

| | |
|---|---|
| MHz | Mega Hertz |
| mL | milliliter(s) |
| mmol | millimole(s) |
| NCDs | Non-communicable diseases |
| NMR | Nuclear Magnetic Resonance |
| NQs | Naphthoquinones |
| $p$-NPG | $p$-nitrophenyl - $\alpha$ - D -glucosidase |
| q | quartet (NMR) |
| quint | quintet (NMR) |
| ROS | Reactive Oxygen Species |
| s | singlet (NMR) |
| SSNQ | Sodium 3,4-dioxo-3,4-dihydronaphthalene-1-sulfonate |
| t | triplet (NMR) |
| TLC | Thin layer chromatography |
| WHO | World Health Organization |
| $\alpha$ | Alpha |
| $\delta$ | Chemical shift |
| $\delta_C$ | Chemical shift of carbon |
| $\delta_H$ | Chemical shift of proton |
| $\mu M$ | Micromolar |

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จุฬาลงกรณ์มหาวิทยาลัย

CHULALONGKORN UNIVERSITY

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CHAPTER 1

INTRODUCTION

Non-communicable diseases (NCDs), including cardiovascular disease, cancer, diabetes and chronic respiratory disease, account for 63% of all deaths globally.

Diabetes is a continuing disease in which the degree of blood glucose, or blood sugar, is extremely high. There are two chronic diabetes types: type 1 and type 2 diabetes. Type 1 diabetes (5-10%), also known as insulin-dependent diabetes, results from the destruction of insulin secreting beta cells.[1] Type 2 diabetes (90-95%), also known as non-insulin-dependent diabetes, involves the host organisms which poorly or cannot respond to insulin.[2] Besides that, there are some less common forms of diabetes based on its pathogenesis. In 2016, WHO evaluated that diabetes mellitus was the seventh most leading cause of mortality for human beings.

Nearly three quarters of all diabetes cases occur in developing countries where diagnostics and therapeutic services are deficient.[3] With rapid enhancing in diabetes, there is an urgent need to develop effective and accessible drugs with minimal side effects.

1.1 Introduction of naphthoquinones

Naphthoquinones are one of the most active natural products found in plants and microorganisms. There are two structural distinct naphthoquinones: 1,2- (1,2-NQ) and 1,4-naphthoquinones (1,4-NQ) due to the position of carbonyl groups.[3] (**Figure 1.1**). They have been reported as effective antitumor agents through a variety of

mechanisms including: DNA damage *via* the excess production of reactive oxygen species (ROS), inhibition of DNA topoisomerase II and activation of tumor suppressor factor gene p53 but the mechanism of action is still not completely elucidated.[3-5] In contrast, there are few researches of naphthoquinones with anti-diabetic activity. Those quinones utilize their anti-diabetic activity *via* wide mechanisms including the inhibition of $\alpha$-glucosidase and protein tyrosine phosphatase 1B, an increase in glucose uptake through the translocation of glucose transporter protein GLUT4 and GLUT2, the activation of peroxisome proliferator-activated receptor $\gamma$, and the normalization of liver carbohydrate metabolic enzymes. Furthermore, naphthoquinone control obesity by impeding adipogenesis, which is an important risk factor for diabetes.[1]



1,2-naphthoquinone        1,4-naphthoquinone
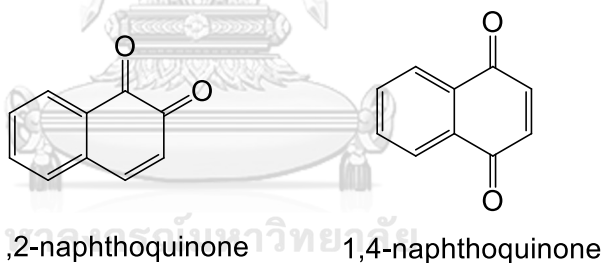
**Figure 1.1** Chemical structures of 1,2- and 1,4-naphthoquinones

Natural and synthesized naphthoquinones also represented a variety of pharmacological effects including anticonvulsant[6], anti-inflammatory[7] and MALT1 inhibition[8], as well as exhibited significant activity against *Trypanosoma cruzi*[9] and Hepatitis C virus[10].

In recent years, researches on structural modifications and potential therapeutic effects of naphthoquinones for diabetes treatment have received a great

deal of attention.[11] There are two common ways to modify quinones structure to enhance their activity including modifying the redox center and adding substitution on quinone ring.

## 1.2 Naphthoquinones and their anti-diabetic activities

For a couple of decades, the bioactivity of quinone has been considered as privileged structure-based drug design.[12] The biological activity of naphthoquinones is mainly associated with their ability to receive one or two electrons to generate the corresponding radical anion or radical dianion.[13] First, naphthoquinones are reduced to corresponding radical semiquinone by enzymes such as NADPH-cytochrome P-450 reductase or NADH:ubiquinone oxidoreductase. Under aerobic conditions, the semiquinone radical is oxidized in redox cycling to produce superoxide radical anion, all of which are believed to be responsible for most of the drug activity.[14] The ability to accept electrons of naphthoquinones can be improved by appending a substituent to the quinone ring.[15]

## 1.2.1 Natural naphthoquinones and their anti-diabetic activities

A variety of natural products and synthetic naphthoquinone chromophores have been used as medicinal drugs. Ubiquitous naturally occurring naphthoquinones such as β-lapachone **(1)**, biflorin **(2)**, plumbagin **(3) (Figure 1.2)**, all of which have several applications in human life.
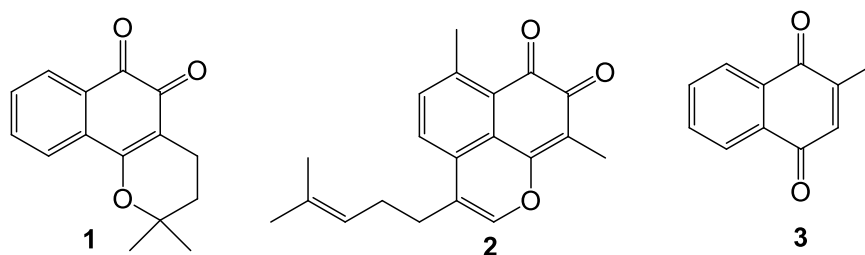
**Figure 1.2** Chemical structures of β-lapachone **(1),** biflorin **(2)** and plumbagin **(3)**

β-lapachone (2,2-dimethyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione, LAPA, **1**), a natural 1,2-naphthoquinone-based compound, possess an advanced antitumor effect and is currently intensively investigation.[16] LAPA was first obtained from the bark of Lapacho tree (*Tabebuia avellanedae*), which grows in South Africa.[17] LAPA is a promising chemotherapeutic agent that present a widely biological activities such as antivirus, antibacterial, anticancer, anti-inflammatory and also against HIV-1 replication.[7, 18, 19] However, the vital application of LAPA is associated to its cytotoxic activity against numerous tumor cells. Recently, new discovery reported that LAPA increase the phosphorylation of AMP-activated protein kinase (AMPK) and inhibit acetyl-CoA carboxylase activity in mice.[20] This result suggested that LAPA could be a new, potent agent for treatment of diabetes in the future.

Biflorin (6,9-dimethyl-3-(4-methylpent-3-en-1-yl)benzo[de]chromene-7,8-dione, **2**) is a 1,2-naphthoquinone-based compound isolated from the roots of *Capraria biflora L.* (Schrophulariaceae), which widespread grows in Mexico, Central and South America and West Indies.[21, 22] Biflorin has shown various biological activities such as antifungal, antibacterial.[23] Previous studies demonstrated that biflorin presented strong

antioxidant activity and cytotoxic, genotoxic, and antimutagenic potential against various cancer cells lines *in vitro* and *in vivo* such as Sarcoma 180, Ehrlich ascites carcinoma and melanoma tumor (B16) cells.[24-28]

The yellow pigment plumbagin (5-hydroxy-2-methyl-1,4-napthoquinone, **3**) is a pharmacologically active 1,4-napthoquinone isolated from the roots of *Plumbago zeylanica L.* plant.[29] Several researches have showed the potential anticancer activity of plumbagin against various cancer cell lines such as lung, breast, ovarian, gastric, and colon cancer.[30-33] In recent years, some publications reported that plumbagin possessed potent antidiabetic and anti-inflammatory activity.[34] Plumbagin exerted their anti-diabetic effect by enhancing insulin secretion from remaining or reproducing β-cells. Plumbagin also increased the GLUT4 translocation and reduced blood glucose level at the plasma membrane in rats.[35] Additionally, plumbagin was found to be advantage in healing diabetic wounds. Indeed, plumbagin diminished the oxidative stress and improved growth factors expressions in diabetic rats.[34]

## 1.2.2 Synthetic 1,2-naphthoquinones and their cytotoxic and anti-diabetic activities

There is a great number of synthetic anticancer agents containing quinones such as LAPA **(1),** salvicine **(4)**, napabucasin **(5)** (**Figure 1.3**).
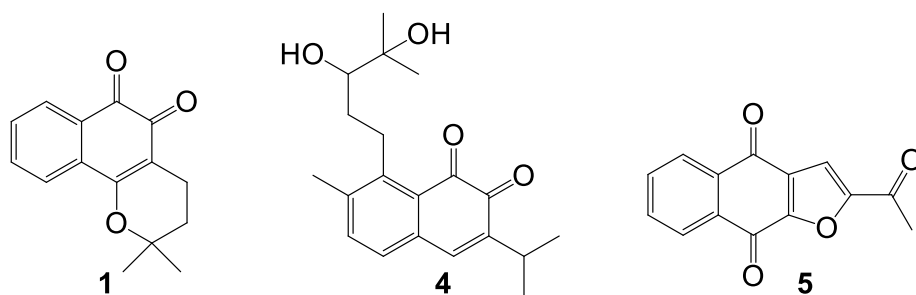
**Figure 1.3** Quinone-based drugs used in chemotherapy

Many naphthoquinones have been synthesized to improve their biological activities as well as the stability. In 2002, Ahn *et al.*[36] synthesized a series of 4-aryl-1,2-naphthoquinones with substituents at $R^4$ position as a protein tyrosine phosphatase 1B inhibitor. 1,2-Naphthoquinone was detected as a good PTP1B inhibitor after screening with $IC_{50}$ of 1.64 µM. Therefore, a wide range of substituents were introduced to the naphthoquinone ring at C-4 position to reduce the instability owing to Michael nucleophilic addition. The results suggested that the introduction of alkyl or aryl groups at $R^4$ position increased inhibitory activity **(Figure 1.4)**.
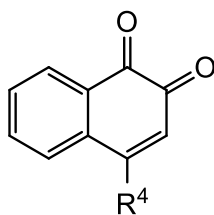
**6**: $R^4 = C_6H_5$; $IC_{50} = 0.86\ \mu M$

**7**: $R^4 = C_6H_4\text{-}4\text{-}OH$; $IC_{50} = 0.44\ \mu M$

**8**: $R^4 = C_6H_4\text{-}2\text{-}OH$; $IC_{50} = 1.60\ \mu M$

**9**: $R^4 = Cyclohexyl$; $IC_{50} = 0.32\ \mu M$

**10**: $R^4 = C_6H_3\text{-}2,5\text{-}F_2$; $IC_{50} = 0.50\ \mu M$

**11**: $R^4 = C_6H_4COOCH_3$; $IC_{50} = 1.54\ \mu M$

**12**: $R^4 = C_6H_4\text{-}2\text{-}OCH_2COOEt$; $IC_{50} = 2.15\ \mu M$

**13**: $R^4 = C_6H_4\text{-}4\text{-}OCH_2COOEt$; $IC_{50} = 1.07\ \mu M$

**14**: $R^4 = C_6H_4\text{-}2\text{-}NO_2$; $IC_{50} = 1.17\ \mu M$

**Figure 1.4** Reported 1,2-NQ-based compounds **(6-14)** with anti-diabetic

activities

Since 1,2-naphthoquinone derivatives with substituents at C-4 position exhibited higher activity, more substituents were adding to modify the 1,2-naphthoquinone structure at C-7 and C-8. The results showed that amino-linked derivatives increased the inhibitory activity and alkoxy-linked derivatives presented potent activity **(Figure 1.5)**.
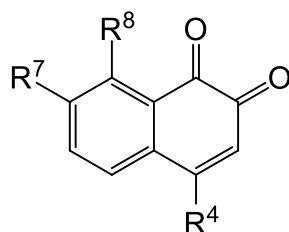
**15**: $R^4 = C_6H_5$, $R^7 = NHCOOBn$, $R^8 = H$; $IC_{50} = 2.25\ \mu M$
**16**: $R^4 = C_6H_5$, $R^7 = H$, $R^8 = NHCOOBn$; $IC_{50} = 1.34\ \mu M$
**17**: $R^4 = C_6H_5$, $R^7 = H$, $R^8 = NHCOOEt$; $IC_{50} = 0.65\ \mu M$
**18**: $R^4 = C_6H_5$, $R^7 = O(CH_2)_4CH_3$, $R^8 = H$; $IC_{50} = 0.92\ \mu M$
**19**: $R^4 = C_6H_5$, $R^7 = OMe$, $R^8 = H$; $IC_{50} = 2.04\ \mu M$
**20**: $R^4 = C_6H_5$, $R^7 = OCH(Bn)COOH$, $R^8 = H$; $IC_{50} = 2.54\ \mu M$

**Figure 1.5** Reported 1,2-NQ-based compounds **(15-20)** with anti-diabetic

activities

## 2. Diabetics disease

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia from total or relative insulin deficiency. Hyperglycemia is a state of high blood glucose levels and this condition promotes excessive insulin secretion in pancreatic β-cells. Diabetes patients who are consistently provoked by increased insulin become less responsive to insulin and eventually resulting in insulin resistance. Prolonged hyperglycemia in diabetes patients can lead to damaging eyes, blindness, kidneys, nerves, causing of cancer, severe cardiovascular disease, and nephropathy.[37] WHO predicts that the number of diabetes patients would rise to 422 million in 2030. Approximately 90-95% of all diabetes cases are diagnosed with type 2 diabetes and 5-10% are diagnosed with type 1 diabetes. The main treatment for type 1 diabetes is insulin injection or insulin infusion. Insulin is a peptide hormone produced by the β-

cells in the pancreatic islets which regulates the transportation of blood glucose to liver. However, there are several medications available for treating type 2 diabetes including glucagon like peptide-1 (GLP-1) analogues, dipeptidyl peptidase 4 (DPP-4) inhibitor, sulfonylurea, biguanides, $\alpha$-glucosidase inhibitors, thiazolidinediones and meglitinides.[38] Summarize of these chemotherapeutic are given in **Table 1**. Classically, obesity treatments are given in combination with diabetes medicines.

**Table 1.** Chemotherapeutic treatments for type 2 diabetes

| Chemotherapeutic | Efficacy | Side effects |
|---|---|---|
| Insulin | Reduce blood glucose level | Weight gain<br>Short effective time |
| GLP-1 analogues | Promote insulin secretion<br>Repress glucagon release | Ineffective to patients with insulin resistance |
| DPP4 inhibitor | Escalate GLP-1 levels | Hypoglycemia<br>Headache |
| Sulfonylurea | Provokes insulin secretion | Weight gain<br>Hypoglycemia |
| Meglitinides | Promote insulin secretion | Short effective time |
| Thiazolidinediones | Increase the sensibility of insulin receptor | Anaemia<br>Dizziness |
| Biguanides | Diminish glucose release from the liver<br>Increase the responsiveness of insulin receptor | Vomit<br>Diarrhea |
| $\alpha$-glucosidase inhibitors | Defer digestion and degrade glucose production | Diarrhea<br>Flatulence |

## 3. α-glucosidase

The principle treatment strategies for type 2 diabetes is to control blood glucose levels. The approachable tactics to maintain the near-normal blood glucose levels are delaying, controlling and preventing carbohydrate hydrolytic enzymes.[39] α-Glucosidase is a key hydrolytic enzyme which plays a critical role in carbohydrates digestion and cellular glycoproteins biosynthesis.[40] It selectively catalyzes the hydrolysis of polysaccharides and disaccharides, reducing them into absorbable monosaccharides and consequently causes hyperglycemia **(Figure 1.6)**.[39] In this respect, the inhibition of α-glucosidase may be an effective therapeutic approach for treating diabetes by decreasing the digestion and absorption of carbohydrates. Moreover, previous reports have shown that the inhibition of α-glucosidase can be linked to anticancer and antiviral treatment. In consequence, α-glucosidase is a potential target for further pharmaceutical research.[41]
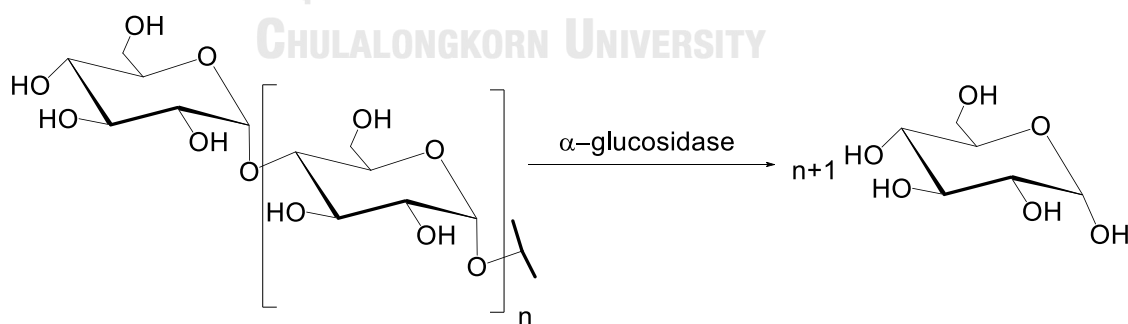


**Figure 1.6** Hydrolysis of polysaccharides and disaccharides catalyzed by α-glucosidase

Two categories of **α**-glucosidase inhibitors (AGIs) have been studied including sugar structure mimicked compounds or sugar derivatives and others compounds. On the other hand, there are few AGIs without glycosyl in their structures have been reported including alkaloids, phenolics, curcuminoids, terpenoids and anthocyanins obtained from natural plants.[42] The current and common used **α**-glucosidase inhibitors for instance acarbose **(21)**, voglibose **(22)** and miglitol **(23)** are sugar structure mimicked AGIs **(Figure 1.7)**. Though these drugs appeared on the market a couple of decades ago, no new AGIs have been approved since 1990s.
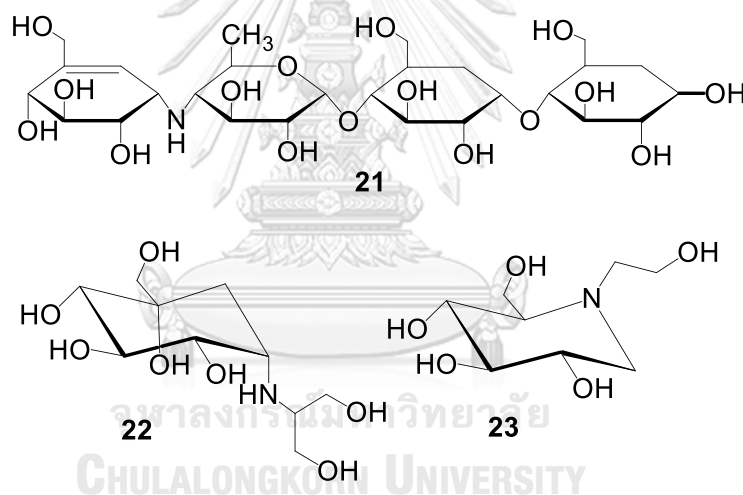


**Figure 1.7** Chemical structures of some common used **α**-glucosidase inhibitors

Most sugar structure mimicked AGIs are structurally similar to monosaccharides or polysaccharides therefore they can bind to carbohydrate active site of **α**-glucosidase and inhibit the enzyme. Acarbose was the first oral AGIs approved for the clinical treatment of diabetes. Acarbose is $10^4$ to $10^5$ times higher affinity to **α**-glucosidase than oligosaccharides therefore acarbose binding with **α**-glucosidase more

effectively **(Figure 1.8)**.[43] Acarbose is a useful and favorable medication for treating

diabetes due to its safety maintainable long-term effect. However, all AGIs causes

various side effects such as abdominal pain, liver problems, flatulence, diarrhea, and

increased lactate dehydrogenase.[44] Moreover, they are high cost and have limited

usage. Hence, there is an urgent require to explore new, effective and accessible $\alpha$-

glucosidase inhibitors.
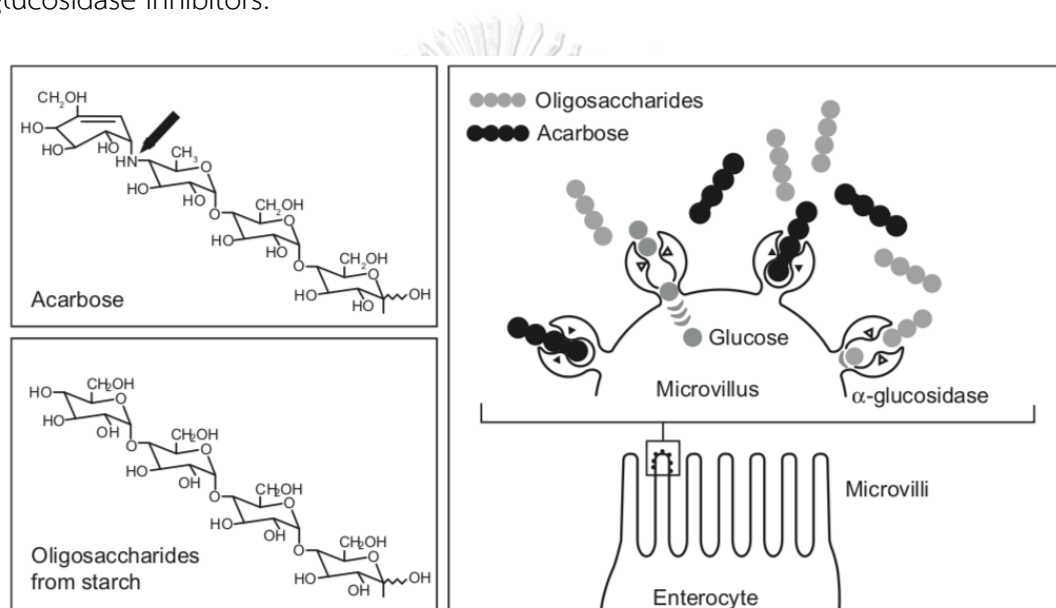


**Figure 1.8** Acarbose mechanism of $\alpha$-glucosidase inhibition

## 4. The aim of this research

Naphthoquinone is a privileged structure that exhibits a broad spectrum of

pharmacological effects such as antidiabetic, anticancer, anti-inflammatory. Hence, this

core structure was selected to develop five series of 1,2-naphthoquinone derivatives

including 4-phenylamino 1,2-NQ, 4-alkylphenylamino 1,2-NQ, 4-alkylamino 1,2-NQ, 4-

phenoxy 1,2-NQ and other 1,2-NQs to evaluate their $\alpha$-glucosidase inhibition activity.

CHAPTER 2

EXPERIMENTAL

## 2.1 Instruments

$^1$H and $^{13}$C NMR spectra were performed in CDCl$_3$, DMSO-$d_6$ or otherwise stated

and were recorded using a Bruker Ultrashield 400 Plus NMR spectrometer with an

Oxford YH400 magnet operating at 400 MHz for $^1$H and 100 MHz for $^{13}$C or a Jeol 500

MHz for $^1$H and 125 MHz for $^{13}$C NMR spectrometer. High-resolution mass spectra

(HRMS) were recorded on a Bruker Daltonics microTOF using electron spray ionization

(ESI).

## 2.2 General

All solvents used in this research were distilled prior to use except those which

were reagent grades. Thin-layer chromatography (TLC) was performed on aluminum

sheets precoated with silica gel (Merk Kieselgel 60 PF254). Silica gel (No. 7734 and

9385, Merck) was used as stationary phase for open column chromatography.

## 2.3 Preparation of 1,2-naphthoquinones with phenylamino substituents

1,2-Naphthoquinone derivatives were prepared as previously reported with

some modification.[45] Diverse anilines were reacted with sodium 1,2-naphthoquinone

4-sulfonate **(SSNQ)** in water at room temperature for 4-24 hours (TLC monitoring). In

the situation that 1,2-naphthoquinones precipitated, they were collected by filtration

and crystallization from EtOH. In other situations, the mixture was partitioned with

EtOAc. The organic layer was combined, washed with brine, and dried over anhydrous Na$_2$SO$_4$ and purified by silica gel chromatography. Eleven 1,2-naphthoquinones with phenylamino substituents are described in **Figure 2.1**.



24. R$^1$ = R$^2$ = R$^3$ = R$^4$ = H = R$^5$ = H     30. R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = OPh

25. R$^2$ = R$^3$ = R$^4$ = R$^5$ = H, R$^1$ = COOH    31. R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$=CH$_2$CH$_2$OH

26. R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = OMe     32. R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = COOH

27. R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = Me      33. R$^2$ = R$^3$ = R$^4$ = H, R$^1$ = R$^3$ = Me

28. R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = F       34. R$^2$ = R$^3$ = R$^4$ = H, R$^1$ = R$^5$ = Me

29. R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = NO$_2$

**Figure 2.1** The structures of 1,2-naphthoquinones with phenylamino substituents

**SSNQ.** (Sodium 3,4-dioxo-3,4-dihydronaphthalene-1-sulfonate). $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ (ppm) 8.41 (d, $J$ = 8.0 Hz, 1H), 7.97 (d, $J$ = 7.6 Hz, 1H), 7.74 (t, $J$ = 8.0 Hz, 1H), 7.57 (t, $J$ = 7.6 Hz, 1H), 6.74 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ (ppm) 181.8, 178.5, 156.5, 134.6, 131.9, 131.8, 130.5, 130.2, 129.0, 124.2.

**24.**[46] (4-(phenylamino)naphthalene-1,2-dione). ^{1}H NMR (DMSO-$d_6$, 400 MHz): $\delta$ (ppm) 8.32 (d, $J$ = 8.0 Hz, 1H), 8.04 (d, $J$ = 7.6 Hz, 1H), 7.86 (t, $J$ = 7.6 Hz, 1H), 7.74 (t, $J$ = 8.0 Hz, 1H), 7.49 (t, $J$ = 7.6 Hz, 2H), 7.31 (t, $J$ = 7.6 Hz, 1H), 7.24 (brs, 2H), 5.85 (s, 1H). ^{13}C NMR (DMSO-$d_6$, 100 MHz): $\delta$ (ppm) 181.3, 175.3, 155.0, 134.2, 131.6 (3C), 131.2, 129.4 (3C), 127.5, 126.1, 124.3, 124.2, 101.4.

**25.** (2-((3,4-dioxo-3,4-dihydronaphthalen-1-yl)amino)benzoic acid). ^{1}H NMR (DMSO-$d_6$, 500 MHz): $\delta$ (ppm) 11.12 (s, 1H), 8.08 (d, $J$ = 7.5 Hz, 1H), 8.04 (d, $J$ = 7.5 Hz, 1H), 7.98 (dd, $J$ = 7.5, 1.0 Hz, 1H), 7.88 (t, $J$ = 7.5 Hz, 1H), 7.81 (t, $J$ = 7.5 Hz, 1H), 7.66 (td, $J$ = 7.5, 1.5 Hz, 2H), 7.21 (dd, $J$ = 7.5, 2.0 Hz 1H), 6.56 (s, 1H). ^{13}C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 183.4, 181.6, 169.0, 155.2, 143.9, 140.1, 135.1, 133.7, 133.1, 132.4, 132.1, 130.4, 126.5, 125.5, 123.3, 120.5, 104.8. HRMS (ESI): calcd for $C_{17}H_{11}NO_4$ [M+Na]$^+$: 316.0586, found 316.0578.

**26.**[46] (4-((4-methoxyphenyl)amino)naphthalene-1,2-dione). ^{1}H NMR (DMSO-$d_6$, 400 MHz): $\delta$ (ppm) 9.82 (s, 1H), 8.29 (d, $J$ = 6.8 Hz, 1H), 8.03 (d, $J$ = 8.0 Hz, 1H), 7.86 (t, $J$ = 7.8 Hz, 1H), 7.73 (t, $J$ = 7.6 Hz, 1H), 7.31 (d, $J$ = 7.6 Hz, 1H), 7.07 (d, $J$ = 8.0 Hz, 2H), 5.56 (s, 1H), 3.80 (s, 3H). ^{13}C NMR (DMSO-$d_6$, 100 MHz): $\delta$ (ppm) 181.4, 175.7, 158.1, 155.1, 134.4, 131.6, 131.4, 130.9, 130.1, 128.2, 127.6 (2C), 123.8, 114.7 (2C), 100.1, 55.4.

**27.**[46] (4-(p-tolylamino)naphthalene-1,2-dione). ^{1}H NMR (pyridine-$d_5$, 400 MHz): $\delta$ (ppm) 8.63 (d, $J$ = 8.0 Hz, 1H), 8.34 (d, $J$ = 7.6 Hz, 1H), 7.69 (t, $J$ = 7.6 Hz, 1H), 7.64 (brs, 1H), 7.22 (brs, 2H), 7.16 (brs, 2H), 6.66 (s, 1H), 2.30 (s, 3H), ^{13}C NMR (pyridine-$d_5$, 100 MHz): $\delta$

(ppm) 182.9 (2C), 156.7, 135.6, 134.1, 132.4, 131.7 (2C), 130.7 (3C), 127.4, 125.7, 123.6, 104.6, 21.4.

**28.**[46] (4-((4-fluorophenyl)amino)naphthalene-1,2-dione). ^{1}H NMR (pyridine-$d_5$, 400 MHz): $\delta$ (ppm) 8.61 (d, $J$ = 8.0 Hz, 1H), 8.34 (d, $J$ = 7.6 Hz, 1H), 7.71 (t, $J$ = 7.2 Hz, 1H), 7.65 (t, $J$ = 8.8 Hz, 1H), 7.21 (brs, 2H), 7.19 (brs, 2H), 6.61 (s, 1H). ^{13}C NMR (pyridine-$d_5$, 100 MHz): $\delta$ (ppm) 182.8 (2C), 162.1, 160.2, 156.5, 135.7, 134.1, 132.3, 131.8, 131.7, 127.4, 125.8 (2C), 125.7, 116.9 (2C), 116.7 (2C), 104.5.

**29.**[47] (4-((4-nitrophenyl)amino)naphthalene-1,2-dione). ^{1}H NMR (pyridine-$d_5$, 400 MHz): $\delta$ (ppm) 8.56 (d, $J$ = 7.6 Hz, 1H), 8.34 (m, 2H), 8.31 (m, 1H), 7.77 (t, $J$ = 7.6 Hz, 1H), 7.70 (d, $J$ = 7.6 Hz, 1H), 7.19 (d, $J$ = 8.4 Hz, 2H), 6.48 (s, 1H). ^{13}C NMR (pyridine-$d_5$, 100 MHz): $\delta$ (ppm) 182.8 (2C), 157.9, 145.0, 135.4, 134.3, 132.3, 132.2, 127.5, 127.2, 126.5, 125.9 (2C), 121.8 (2C), 105.0. HRMS (ESI): calcd for $C_{16}H_{10}N_2O_4$ [M+Na]$^+$: 317.0538, found 317.0555.

**30.** (4-((4-phenoxyphenyl)amino)naphthalene-1,2-dione). ^{1}H NMR (DMSO-$d_6$, 500 MHz): $\delta$ (ppm) 8.32 (d, $J$ = 8.0 Hz, 1H), 8.04 (d, $J$ = 7.5 Hz, 1H), 7.86 (t, $J$ = 7.5 Hz, 1H), 7.74 (t, $J$ = 8.0 Hz, 1H), 7.43 (t, $J$ = 8.0 Hz, 2H), 7.28 (brs, 2H), 7.17 (t, $J$ = 7.5 Hz, 1H), 7.12 (d, $J$ = 8.5 Hz, 2H), 7.08 (d, $J$ = 8.0 Hz, 2H), 5.84 (s, 1H). ^{13}C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 184.7, 175.3, 156.8, 155.6, 140.1, 134.7, 133.4, 132.0, 131.4, 130.6 (2C), 130.4, 127.8, 124.4, 124.2, 119.7 (2C), 119.1 (2C), 119.0 (2C), 101.4. HRMS (ESI): calcd for $C_{22}H_{15}NO_3$ [M+Na]$^+$: 364.0950, found 364.0958.

**31.** (4-((4-(2-hydroxyethyl)phenyl)amino)naphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 500 MHz): $\delta$ (ppm) 8.30 (d, $J$ = 8.0 Hz, 1H), 8.02 (d, $J$ = 7.5 Hz, 1H), 7.84 (t, $J$ = 8.0 Hz, 1H), 7.72 (t, $J$ = 7.5 Hz, 1H), 7.33 (d, $J$ = 7.5 Hz, 2H), 7.18 (brs, 2H), 5.80 (s, 1H), 4.75 (s, 1H), 3.65 (t, $J$ = 7.0 Hz, 2H), 2.77 (t, $J$ = 7.0 Hz, 2H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 181.3 (2C), 155.0, 134.3, 131.8, 131.6 (2C), 131.2, 130.1, 129.9 (3C), 127.9, 127.7, 124.2, 101.0, 62.2, 38.6. HRMS (ESI): calcd for $C_{18}H_{15}NO_3$ [M+Na]$^+$: 316.0950, found 316.0966.

**32.** (4-((3,4-dioxo-3,4-dihydronaphthalen-1-yl)amino)benzoic acid). $^1$H NMR (DMSO-$d_6$, 500 MHz): $\delta$ (ppm) 8.34 (d, $J$ = 7.5 Hz, 1H), 8.01 (d, $J$ = 7.5 Hz, 1H), 7.91 (d, $J$ = 8.5 Hz, 2H), 7.78 (t, $J$ = 7.5 Hz, 1H), 7.69 (t, $J$ = 7.5 Hz, 1H), 7.05 (d, $J$ = 8.0 Hz, 2H), 5.95 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 182.9 (2C), 167.5, 155.7, 134.6, 134.1, 133.2, 131.5, 130.8, 130.3 (2C), 126.3, 124.7, 121.6 (2C), 101.9.

**33.** (4-((2,4-dimethylphenyl)amino)naphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 500 MHz): $\delta$ (ppm) 9.79 (s, 1H), 8.35 (brs, 1H), 8.05 (d, $J$ = 7.5 Hz, 1H), 7.87 (t, $J$ = 7.5 Hz, 1H), 7.74 (t, $J$ = 7.5 Hz, 1H), 7.21 (brs, 1H), 7.15 (brs, 2H), 5.09 (s, 1H), 2.33 (s, 3H), 2.14 (s, 3H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 182.0, 181.9, 155.8, 135.0, 134.8, 132.2, 132.0, 131.9, 130.4, 128.2, 128.1, 128.0, 127.9, 124.4, 124.3, 100.3, 21.2, 17.8.

**34.** (4-((2,6-dimethylphenyl)amino)naphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 500 MHz): $\delta$ (ppm) 8.37 (d, $J$ = 8.0 Hz, 1H), 8.05 (d, $J$ = 7.5 Hz, 1H), 7.89 (t, $J$ = 8.0 Hz, 1H), 7.75 (t, $J$ = 7.5 Hz, 1H), 7.24 (brs, 3H), 5.05 (s, 1H), 2.14 (s, 6H). $^{13}$C NMR (DMSO-$d_6$, 125

MHz): $\delta$ (ppm) 181.5 (2C), 155.0, 138.6, 134.5, 132.7, 131.7, 131.5 (2C), 130.6, 128.7, 128.5 (2C), 124.0, 123.8, 104.0, 17.6 (2C). HRMS (ESI): calcd for $C_{18}H_{15}NO_2$ [M+Na]$^+$: 300.1000, found 300.1007.

## 2.4 Preparation of 1,2-naphthoquinones with alkylamino substituents

**35** was prepared as previously reported.[48] To a solution of 1,2-naphthoquinone (1 equiv) in acetic acid (15 mL) at 40$^o$C, a solution of NaN$_3$ (1.7 equiv) in water (5 mL) was added. The mixture was stirred at 40$^o$C in 2 hours (TLC monitoring). The precipitate was collected by filtration, washed with brine, and dried over anhydrous Na$_2$SO$_4$ and purified by silica gel chromatography to give the target product.

**42** was prepared as previously reported with some modification.[49] The corresponding alkylamino (2 equiv), CeCl$_3$.7H$_2$O (5% mmol in respect to **SSNQ**), and **SSNQ** (1 equiv) in water (20 mL for 1 mmol of **SSNQ**) were stirred at room temperature for 24 hours (TLC monitoring). The mixture was then partitioned with EtOAc. The organic layer was combined, washed with brine, and dried over anhydrous Na$_2$SO$_4$ and purified by silica gel chromatography.

The naphthoquinone derivatives were prepared as previously reported with some modification.[50] The corresponding aliphatic amine (1.11 equiv), K$_2$CO$_3$ (2.02 equiv) and **SSNQ** (1 equiv) in water (10 mL for 1 mmol of **SSNQ**) were stirred at room temperature for 24-48 hours (TLC monitoring). The mixture was then partitioned with EtOAc. The organic layer was combined, washed with brine, and dried over anhydrous

Na$_2$SO$_4$ and purified by utilizing silica gel chromatography. Nine naphthoquinones with alkylamino substituents are described in **Figure 2.2.**
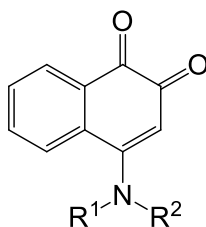


**35.** R$^1$ = R$^2$ = H                  **40.** R$^1$ = H, R$^2$ = Cyclohexyl

**36.** R$^1$ = H, R$^2$ = Et          **41.** R$^1$ = H, R$^2$ = Benzyl

**37.** R$^1$ = H, R$^2$ = Propyl      **42.** R$^1$ = Et, R$^2$ = Et

**38.** R$^1$ = H, R$^2$ = Butyl       **43.** R$^1$ = H, R$^2$ = 3-bromopropyl

**39.** R$^1$ = H, R$^2$ = Isobutyl

**Figure 2.2** The structures of 1,2-naphthoquinones with alkylamino substituents

**35.**[48] (4-aminonaphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 400 MHz): δ (ppm) 8.32 (s, 1H), 8.16 (s, 1H), 8.04 (d, *J* = 7.6 Hz, 1H), 7.96 (d, *J* = 6.8 Hz, 1H), 7.80 (t, *J* = 7.6 Hz, 1H), 7.68 (t, *J* = 7.6 Hz, 1H), 5.74 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): δ (ppm) 182.2, 174.7, 158.1, 134.2, 131.7, 131.6, 130.5, 127.8, 124.0, 101.0.

**36.**[51] (4-ethylamino)naphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 500 MHz): δ (ppm) 7.97 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.94 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.82 (td, *J* = 7.0, 1 Hz, 1H), 7.72 (td, *J* = 7.5, 1.5 Hz, 1H), 7.54 (t, *J* = 6.0 Hz, 1H), 5.66 (s, 1H), 3.21 (q, *J* = 7.0 Hz, 2H), 1.16 (t, *J* = 7.5 Hz, 3H).

**37.** (4-propylamino)naphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ (ppm) 8.45 (s, 1H), 8.13 (d, $J$ = 8.0 Hz, 1H), 7.98 (d, $J$ = 7.6 Hz, 1H), 7.80 (t, $J$ = 8.0 Hz, 1H), 7.67 (t, $J$ = 7.6 Hz, 1H), 5.69 (s, 1H), 3.34 (d, $J$ = 6.8 Hz, 2H), 1.68 (m, 2H), 0.95 (t, $J$ = 7.6 Hz, 3H), $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ (ppm) 182.1, 174.8, 155.1, 134.2, 131.4, 131.3, 130.9, 127.9, 123.4, 98.1, 44.9, 21.1, 11.5. HRMS (ESI): calcd for $C_{13}H_{13}NO_2$ [M+Na]$^+$: 238.0844, found 238.0858.

**38.** (4-butylamino)naphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ (ppm) 8.42 (s, 1H), 8.11 (d, $J$ = 8.0 Hz, 1H), 7.98 (d, $J$ = 7.6 Hz, 1H), 7.81 (t, $J$ = 7.6 Hz, 1H), 7.68 (t, $J$ = 7.6 Hz, 1H), 5.69 (s, 1H), 1.65 (quint, $J$ = 7.2 Hz, 2H), 1.38 (m, 2H), 0.92 (t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ (ppm) 175.5, 174.8, 155.0, 134.3, 131.4, 131.3, 130.9, 127.9, 123.4, 98.1, 43.0, 29.8, 19.8, 13.7.

**39.** (4-isobutylamino)naphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ (ppm) 8.47 (s, 1H), 8.15 (d, $J$ = 8.0 Hz, 1H), 7.98 (d, $J$ = 7.6 Hz, 1H), 7.81 (t, $J$ = 7.6 Hz, 1H), 7.68 (t, $J$ = 7.6 Hz, 1H), 5.70 (s, 1H), 3.20 (t, $J$ = 7.2 Hz, 2H), 2.05 (m, 1H), 0.95 (d, $J$ = 6.4 Hz, 6H), $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ (ppm) 182.1, 174.8, 155.3, 134.3, 131.4, 131.3, 130.9, 127.9, 123.4, 98.3, 50.6, 27.2, 20.3. HRMS (ESI): calcd for $C_{14}H_{15}NO_2$ [M+Na]$^+$: 252.1000, found 252.0995.

**40.** (4-cyclohexylamino)naphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ (ppm) 8.22 (d, $J$ = 8.0 Hz, 1H), 7.97 (d, $J$ = 6.8 Hz, 1H), 7.91 (d, $J$ = 7.6 Hz, 1H), 7.80 (t, $J$ = 7.6 Hz, 1H), 7.67 (t, $J$ = 7.6 Hz, 1H), 5.76 (s, 1H), 3.61 (m, 1H), 1.95 (d, $J$ = 11.6 Hz, 2H), 1.76

(d, $J$ = 12.4 Hz, 2H), 1.64 (d, $J$ = 13.2 Hz, 2H), 1.43 (m, 4H). ^{13}C NMR (DMSO-$d_6$, 100 MHz):

$\delta$ (ppm) 182.1, 174.9, 153.8, 134.0, 131.3, 131.2, 130.9, 127.8, 123.6, 98.2, 52.4, 31.5

(2C), 25.1, 24.5 (2C). HRMS (ESI): calcd for $C_{16}H_{17}NO_2$ [M+Na]$^+$: 278.1157, found 278.1161.

**41.** (4-benzylamino)naphthalene-1,2-dione). ^{1}H NMR (DMSO-$d_6$, 400 MHz): $\delta$ (ppm) 9.06

(t, $J$ = 6.0 Hz, 1H), ) 8.18 (d, $J$ = 7.6 Hz, 1H), ) 7.99 (d, $J$ = 7.6 Hz, 1H), 7.85 (t, $J$ = 7.6 Hz,

1H), 7.70 (t, $J$ = 7.6 Hz, 1H), 7.37 (m, 4H), 7.29 (m, 1H), 5.60 (s, 1H), 4.64 (d, $J$ = 6.0 Hz,

2H). ^{13}C NMR (DMSO-$d_6$, 100 MHz): $\delta$ (ppm) 181.8, 175.0, 155.1, 137.5, 134.4, 131.4,

131.3, 130.9, 128.7 (2C), 128.0, 127.4, 127.1 (2C), 123.4, 99.1, 46.3.

**42.**[52] (4-diethylamino)naphthalene-1,2-dione). ^{1}H NMR (DMSO-$d_6$, 400 MHz): $\delta$ (ppm)

8.09 (d, $J$ = 7.6 Hz, 1H), 7.61 (d, $J$ = 7.6 Hz, 1H), 7.57 (brs, 1H), 7.55 (brs, 1H), 6.00 (s, 1H),

3.51 (q, $J$ = 7.2 Hz, 4H), 1.31 (t, $J$ = 6.8, 6H). ^{13}C NMR (DMSO-$d_6$, 100 MHz): $\delta$ (ppm)

133.8, 130.8 (2C), 129.6, 126.9, 124.8, 124.2, 108.4, 46.3 (2C), 12.7 (2C).

**43.** (4-((3-bromopropyl)amino)naphthalene-1,2-dione). ^{1}H NMR (DMSO-$d_6$, 500 MHz): $\delta$

(ppm) 8.44 (t, $J$ = 5.5 Hz, 1H), 8.10 (d, $J$ = 8 Hz, 1H), 7.98 (d, $J$ = 7.5, 1.5 Hz, 1H), 7.81 (t,

$J$ = 7.5, 1.5 Hz, 1H), 7.70 (t, $J$ = 7.5 Hz, 1H), 5.74 (s, 1H), 3.64 (t, $J$ = 6.5 Hz, 2H), 3.59 (brs,

2H), 2.20 (q, $J$ = 7.0 Hz, 2H). ^{13}C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 182.0 (2C), 155.2,

134.4, 131.5, 131.3, 130.9, 128.0, 123.6, 98.3, 41.8, 32.4, 31.0. HRMS (ESI): calcd for

$C_{13}H_{12}BrNO_2$ [M+Na]$^+$: 315.9949, found 315.9942.

**2.5 Preparation of naphthoquinones with alkylphenylamino substituents**

1,2-Naphthoquinone derivatives **44** and **46** were prepared as previously reported with some modification.[49] The corresponding alkyl aniline (2 equiv), $CeCl_3.7H_2O$ (5% mmol in respect to **SSNQ**), and **SSNQ** (1 equiv) in water (20 mL for 1 mmol of **SSNQ**) were stirred at room temperature for 24-48 hours (TLC monitoring). The mixture was then partitioned with EtOAc. The organic layer was combined, washed with brine, and dried over anhydrous $Na_2SO_4$ and purified by utilizing silica gel chromatography.

Compound **45** was prepared as previously reported.[53] The corresponding 4-phenylamino 1,2-NQ (1 equiv), sodium hydride NaH (0.5g for 1.0 mmol of 4-phenylamino 1,2-NQ) and methyl iodide $CH_3I$ (3 equiv) in dry DMF (20 mL for 1 mmol of 4-phenylamino 1,2-NQ) were stirred at room temperature for 40 mins (TLC monitoring). The mixture was then partitioned with EtOAc. The organic layer was combined, washed with brine, and dried over anhydrous $Na_2SO_4$ and purified by utilizing silica gel chromatography. Three 1,2-naphthoquinones with alkylphenylamino are described in **Figure 2.3.**
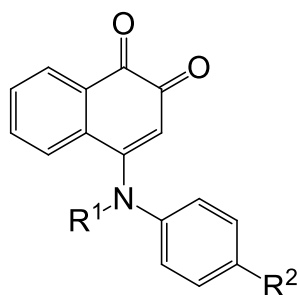
**44.** R$^1$ = Me, R$^2$ = H          **46.** R$^1$ = Et, R$^2$ = H

**45.** R$^1$ = Me, R$^2$ = OMe

**Figure 2.3** The structures of 1,2-naphthoquinones with alkylphenylamino substituents

**44.**[46] (4-(methyl(phenyl)amino)naphthalene-1,2-dione). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 8.05 (d, $J$ = 7.6 Hz, 1H), 7.33 (t, $J$ = 7.2 Hz, 3H), 7.21 (t, $J$ = 7.2 Hz, 2H), 7.11 (d, $J$ = 7.6 Hz, 2H), 7.02 (d, $J$ = 8.0 Hz, 1H), 6.27 (s, 1H), 3.49 (s, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 180.9, 178.7, 159.7, 148.0, 133.4, 132.8, 132.3, 130.1, 130.0 (2C), 129.2, 129.0, 126.5, 125.5 (2C), 111.1, 44.3.

**45.**[46] (4-((4-methoxyphenyl)(methyl)amino)naphthalene-1,2-dione). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 8.54 (d, $J$ = 8.0 Hz, 1H), 8.22 (dd, $J$ = 7.6, 1.2 Hz, 1H), 7.72 (td, $J$ = 7.6, 1.6 Hz, 1H), 7.64 (t, $J$ = 7.6 Hz, 1H), 6.98 (d, $J$ = 8.4 Hz, 4H), 6.51 (s, 1H), 3.88 (s, 3H), 3.75 (s, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 180.4 (2C), 157.5, 156.6, 155.0, 134.9, 133.4, 131.1, 131.0, 126.6, 125.4, 122.4 (2C), 114.7 (2C), 102.1, 55.9, 55.7.

**46.** (4-(ethyl(phenyl)amino)naphthalene-1,2-dione). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 8.08 (d, $J$ = 7.6 Hz, 1H), 7.37 (t, $J$ = 7.6 Hz, 3H), 7.28 (brs, 1H), 7.22 (t, $J$ = 8.0 Hz, 1H), 7.13 (d, $J$ = 8.0 Hz, 2H), 7.02 (d, $J$ = 8.0 Hz, 1H), 6.47 (s, 1H), 4.05 (q, $J$ = 7.2 Hz, 2H), 1.34

(t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 181.0 (2C), 159.2, 146.2, 133.3, 132.9, 132.6, 130.3, 130.1 (2C), 129.2, 129.1, 126.7, 126.0 (2C), 110.6, 51.0, 12.3.

## 2.6 Preparation of 1,2-naphthoquinones with phenoxy, alkoxy, phenyl substituents

1,2-Naphthoquinone with phenoxy substituents **47-63** were prepared as previously reported with some modification.[45] The corresponding phenol (1 equiv), KOH (1 equiv) and **SSNQ** (1.1 equiv) in water (27 mL for 1 mmol of **SSNQ**) were stirred at room temperature for 48 hours (TLC monitoring). The mixture was then partitioned with EtOAc. The organic layer was combined, washed with brine, and dried over anhydrous Na$_2$SO$_4$ and purified by silica gel chromatography. Fourteen naphthoquinones with phenoxy substituents and two naphthoquinones with phenyl substituents are described in **Figure 2.4**.

**64**, **65** were synthesized as previously studied with some modification.[54] To a solution of **SSNQ** (1 equiv) in MeOH or EtOH (5 mL MeOH or EtOH for 1.26 mmol of **SSNQ**), KIO$_3$ (1 equiv) and CeCl$_3$.7H$_2$O (1 equiv) were added. The reaction was stirred vigorously in 2 hours at room temperature. The mixture was evaporated under reduced pressure and partitioned with water and EtOAc. The organic layer was combined, washed with brine, and dried over anhydrous Na$_2$SO$_4$ and purified by silica gel chromatography.

**47.** $R^1 = R^2 = R^3 = R^4 = R^5 = H$

**48.** $R^2 = R^3 = R^4 = R^5 = H$, $R^1 = $ Benzyl

**49.** $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = $ OMe

**50.** $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = $ Cl

**51.** $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = $ Br

**52.** $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = $ Cumyl

**53.** $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = $ NHCOCH$_3$

**54.** $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = $ Et

**55.** $R^3 = R^4 = R^5 = H$, $R^1 = R^2 = $ Me

**56.** $R^2 = R^4 = R^5 = H$, $R^1 = R^3 = $ Me

**57.** $R^2 = R^3 = R^5 = H$, $R^1 = R^4 = $ Me

**58.** $R^2 = R^3 = R^4 = H$, $R^1 = R^5 = $ Me

**59.** $R^1 = R^4 = R^5 = H$, $R^2 = R^3 = $ Me

**60.** $R^1 = R^3 = R^5 = H$, $R^2 = R^4 = $ Me

**Figure 2.4** The structures of 1,2-naphthoquinones with phenoxy

substituents

**47.**[46] (4-phenoxynaphthalene-1,2-dione). $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm) 8.14 (dd,

$J$ = 7.5, 1.0 Hz, 1H), 8.08 (dd, $J$ = 8.0, 1.0 Hz, 1H), 7.76 (td, $J$ = 7.5, 1.5 Hz, 1H), 7.64 (td,

$J$ = 7.5, 1.0 Hz, 1H), 7.47 (t, $J$ = 8.0 Hz, 2H), 7.34 (t, $J$ = 7.5 Hz, 1H), 7.15 (d, $J$ = 7.5 Hz,

2H), 5.64 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 179.6, 179.4, 168.9, 152.4, 135.3, 132.0, 131.7, 130.6, 130.5 (2C), 129.5, 127.0, 125.0, 121.4 (2C), 106.4.

**48.** (4-(2-benzylphenoxy)naphthalene-1,2-dione). $^{1}$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm) 8.14 (dd, $J$ = 7.5, 1.5 Hz, 1H), 7.92 (dd, $J$ = 8.0, 0.5 Hz, 1H), 7.73 (td, $J$ = 8.0, 1.5 Hz, 1H), 7.64 (td, $J$ = 7.5, 1.0 Hz, 1H), 7.35 (td, $J$ = 7.0, 1.5 Hz, 2H), 7.30 (td, $J$ = 7.5, 1.5 Hz, 1H), 7.15 (m, 1H), 7.12 (m, 2H), 7.08 (m, 1H), 7.04 (dd, $J$ = 7.5, 1.5 Hz, 2H), 5.46 (s, 1H), 3.92 (s, 2H). $^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ (ppm) 179.4, 179.3, 168.1, 150.6, 139.2, 135.2, 133.4, 132.1, 132.0, 131.5, 130.6, 129.5, 129.0 (2C), 128.7, 128.6 (2C), 127.4, 126.6, 125.0, 122.1, 106.2, 36.7. HRMS (ESI): calcd for C$_{23}$H$_{16}$O$_3$ [M+Na]$^+$: 363.0997, found 363.1016.

**49.**[46] (4-(4-methoxyphenoxy)naphthalene-1,2-dione). $^{1}$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm) 8.15 (d, $J$ = 8.0 Hz, 1H), 8.08 (dd, $J$ = 8.0, 1.5 Hz, 1H), 7.76 (td, $J$ = 7.5, 1.0 Hz, 1H), 7.64 (td, $J$ = 7.5, 1.0 Hz, 1H), 7.07 (dd, $J$ = 7.0, 2.5 Hz, 2H), 6.97 (dd, $J$ = 7.0, 2.5 Hz, 2H), 5.67 (s, 1H), 3.84 (s, 3H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 179.7, 179.6, 169.5, 158.2, 145.8, 135.2, 132.0, 131.8, 130.6, 129.5, 125.0, 122.2 (2C), 115.4 (2C), 106.4, 55.9.

**50.**[46] (4-(4-chlorophenoxy)naphthalene-1,2-dione). $^{1}$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm) 8.16 (dd, $J$ = 7.5, 1.0 Hz, 1H), 8.06 (dd, $J$ = 8.0, 1.0 Hz, 1H), 7.77 (td, $J$ = 7.5, 1.5 Hz, 1H), 7.66 (td, $J$ = 7.5, 1.0 Hz, 1H), 7.45 (dd, $J$ = 9.0, 3.0 Hz, 2H), 7.12 (dd, $J$ = 8.5, 3.5 Hz, 2H), 5.63 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 179.5, 179.2, 168.6, 150.9, 135.3, 132.7, 132.2, 131.5, 130.6 (2C), 130.5, 129.6, 125.0, 122.9 (2C), 106.5.

**51.**[46] (4-(4-bromophenoxy)naphthalene-1,2-dione). $^{1}$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm)

8.18 (dd, $J$ = 8.0, 2.0 Hz, 1H), 8.06 (dd, $J$ = 7.5, 1.0 Hz, 1H), 7.78 (td, $J$ = 8.0, 1.5 Hz, 1H), 7.67 (td, $J$ = 7.5, 1.0 Hz, 1H), 7.62 (dd, $J$ = 9.0, 3.0 Hz, 2H), 7.07 (dd, $J$ = 8.5, 3.5 Hz, 2H), 5.65 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 179.5, 179.2, 168.5, 151.5, 135.3, 133.7 (2C), 132.2, 131.5, 130.6, 129.7, 125.0, 123.3 (2C), 120.3, 106.6.

**52.** (4-(4-(2-phenylpropan-2-yl)phenoxy)naphthalene-1,2-dione). $^{1}$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm) 8.04 (t, $J$ = 8.0 Hz, 2H), 7.88 (td, $J$ = 7.5, 1.0 Hz, 1H), 7.75 (td, $J$ = 8.0, 1.0 Hz, 1H), 7.38 (m, $J$ = 8.5, 3.0 Hz, 2H), 7.31 (m, 2H), 7.26 (m, 2H), 7.22 (m, $J$ = 8.5, 3.0 Hz, 2H), 7.18 (m, $J$ = 7.5 Hz, 1H), 5.35 (s, 1H), 1.68 (s, 6H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 178.8, 178.6, 167.8, 150.1, 150.0, 149.0, 135.2, 132.1, 131.1, 130.7, 128.6 (2C), 128.5, 128.2 (2C), 126.5 (2C), 125.8, 124.6, 120.8 (2C), 105.9, 42.5, 30.5 (2C). HRMS (ESI): calcd for C$_{25}$H$_{20}$O$_3$ [M+Na]$^+$: 391.1310, found 391.1316.

**53.** (N-(4-((3,4-dioxo-3,4-dihydronaphthalen-1-yl)oxy)phenyl)acetamide). $^{1}$H NMR (DMSO-$d_6$, 500 MHz): $\delta$ (ppm) 10.16 (s, 1H), 8.06 (dd, $J$ = 7.5, 0.5 Hz, 1H), 8.03 (dd, $J$ = 7.5, 1.0 Hz, 1H), 7.88 (td, $J$ = 7.5, 1.5 Hz, 1H), 7.77 (td, $J$ = 7.5, 1.5 Hz, 1H), 7.73 (m, 2H), 7.25 (d, $J$ = 9.0 Hz, 2H), 5.37 (s, 1H), 2.07 (s, 3H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 178.8, 178.6, 169.6, 168.0, 147.2, 137.9, 135.2, 132.1, 131.1, 130.7, 128.6, 125.5, 122.2 (2C), 120.6 (2C), 105.8, 24.0. HRMS (ESI): calcd for C$_{18}$H$_{13}$NO$_4$ [M+Na]$^+$: 330.0742, found 330.0745.

**54.** (4-(4-ethylphenoxy)naphthalene-1,2-dione). $^{1}$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm) 8.13 (d, $J$ = 6.5 Hz, 1H), 8.07 (d, $J$ = 8.0 Hz, 1H), 7.75 (t, $J$ = 7.5 Hz, 1H), 7.63 (t, $J$ = 7.5 Hz,

1H), 7.27 (d, $J$ = 9.0 Hz, 2H), 7.05 (d, $J$ = 8.5 Hz, 2H), 5.66 (s, 1H), 2.68 (q, $J$ = 7.5 Hz, 2H), 1.25 (t, $J$ = 7.5 Hz, 2H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 179.7, 179.5, 169.2, 150.3, 143.2, 135.2, 132.0, 131.8, 130.5, 129.7 (2C), 129.4, 125.0, 121.1 (2C), 106.4, 28.4, 15.8. HRMS (ESI): calcd for $C_{18}H_{14}O_3$ [M+Na]$^+$: 301.0841, found 301.0835.

**55.** (4-(2,3-dimethylphenoxy)naphthalene-1,2-dione). $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm) 8.17 (d, $J$ = 7.5 Hz, 1H), 8.12 (d, $J$ = 8.0 Hz, 1H), 7.78 (t, $J$ = 8.0 Hz, 1H), 7.66 (t, $J$ = 8.0 Hz, 1H), 7.18 (t, $J$ = 7.5 Hz, 1H), 7.13 (d, $J$ = 7.5 Hz, 1H), 6.92 (d, $J$ = 8.0 Hz, 1H), 5.54 (s, 1H), 2.33 (s, 3H), 2.09 (s, 3H). $^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ (ppm) 179.7, 179.5, 168.4, 150.7, 139.8, 135.3, 132.0, 131.6, 130.7, 129.5, 128.7, 128.5, 127.1, 125.0, 118.9, 106.1, 29.8, 12.3. HRMS (ESI): calcd for $C_{18}H_{14}O_3$ [M+Na]$^+$: 301.0841, found 301.0849.

**56.** (4-(2,4-dimethylphenoxy)naphthalene-1,2-dione). $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm) 8.15 (dd, $J$ = 8.0, 1.5 Hz, 1H), 8.10 (dd, $J$ = 8.0, 1.0 Hz, 1H), 7.77 (td, $J$ = 7.5, 1.5 Hz, 1H), 7.64 (td, $J$ = 8.0, 1.5 Hz, 1H), 7.10 (brs, 1H), 7.06 (dd, $J$ = 8.5, 2.5 Hz, 1H), 6.94 (d, $J$ = 8.0 Hz, 1H), 5.56 (s, 1H), 2.34 (s, 3H), 2.15 (s, 3H). $^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ (ppm) 179.7, 179.5, 168.4, 148.5, 136.9, 135.3, 132.6, 132.0, 131.6, 130.6, 129.6, 129.5, 128.4, 125.0, 121.0, 105.9, 20.9, 15.7. HRMS (ESI): calcd for $C_{18}H_{14}O_3$ [M+Na]$^+$: 301.0841, found 301.0862.

**57.** (4-(2,5-dimethylphenoxy)naphthalene-1,2-dione). $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm) 8.16 (d, $J$ = 7.5 Hz, 1H), 8.10 (d, $J$ = 8.0 Hz, 1H), 7.77 (t, $J$ = 7.5 Hz, 1H), 7.65 (t, $J$ = 7.5 Hz, 1H), 7.18 (d, $J$ = 7.5 Hz, 1H), 7.04 (d, $J$ = 8.0 Hz, 1H), 6.88 (s, 1H), 5.57 (s, 1H),

2.34 (s, 3H), 2.15 (s, 3H). $^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ (ppm) 179.7, 179.5, 168.3, 150.5, 138.1, 135.3, 132.0, 131.8, 131.6, 130.7, 129.5, 127.9, 126.7, 125.0, 121.8, 106.0, 21.0, 15.4.

**58.** (4-(2,6-dimethylphenoxy)naphthalene-1,2-dione). $^{1}$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm) 8.19 (dd, $J$ = 8.0, 3.0 Hz, 1H), 8.14 (dd, $J$ = 7.5, 3.0 Hz, 1H), 7.80 (td, $J$ = 7.5, 2.0 Hz, 1H), 7.67 (td, $J$ = 8.0, 3.0 Hz, 1H), 7.13 (brs, 3H), 5.52 (d, $J$ = 3.0 Hz, 1H), 2.18 (d, $J$ = 3.0 Hz, 6H). $^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ (ppm) 179.8, 179.5, 167.1, 149.2, 135.4, 132.1, 131.4, 130.9, 130.0 (2C), 129.7, 129.6 (2C), 127.0, 125.0, 105.2, 16.0 (2C). HRMS (ESI): calcd for C$_{18}$H$_{14}$O$_3$ [M+Na]$^{+}$: 301.0841, found 301.0864.

**59.** (4-(3,4-dimethylphenoxy)naphthalene-1,2-dione). $^{1}$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm) 8.14 (dd, $J$ = 7.5, 1.0 Hz, 1H), 8.07 (dd, $J$ = 7.5, 1.0 Hz, 1H), 7.75 (td, $J$ = 7.5, 1.5 Hz, 1H), 7.63 (td, $J$ = 7.5, 1.0 Hz, 1H), 6.95 (d, $J$ = 8.5 Hz, 1H), 6.92 (d, $J$ = 3.0 Hz, 1H), 6.87 (dd, $J$ = 8.5, 2.5 Hz, 1H), 5.67 (s, 1H), 2.28 (s, 6H). $^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ (ppm) 179.7, 179.6, 169.2, 150.4, 139.2, 135.5, 135.2, 131.9, 131.8, 131.2, 130.6, 129.4, 125.0, 122.1, 118.3, 106.4, 20.0, 19.3.

**60.** (4-(3,5-dimethylphenoxy)naphthalene-1,2-dione). $^{1}$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm) 8.15 (d, $J$ = 7.5 Hz, 1H), 8.06 (dd, $J$ = 8.0, 1.0 Hz, 1H), 7.76 (td, $J$ = 7.5, 1.0 Hz, 1H), 7.64 (td, $J$ = 7.5, 1.0 Hz, 1H), 6.95 (s, 1H), 6.76 (s, 2H), 5.69 (s, 1H), 2.34 (s, 6H). $^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ (ppm) 179.8, 179.6, 169.1, 152.4, 140.5 (2C), 135.2, 132.0, 131.9,

130.6, 129.5, 128.6, 125.0, 118.8 (2C), 106.5, 21.4 (2C). HRMS (ESI): calcd for $C_{18}H_{14}O_3$ [M+Na]$^+$: 301.0841, found 301.0862.

**61.** (4-(4-hydroxy-3-methoxyphenyl)naphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 500 MHz): $\delta$ (ppm) 8.03 (d, $J$ = 7.5 Hz, 1H), 7.71 (t, $J$ = 8.0 Hz, 1H), 7.61 (t, $J$ = 7.5 Hz, 1H), 7.44 (d, $J$ = 8.0 Hz, 1H), 7.05 (brs, 1H), 6.94 (brs, 2H), 6.35 (brs, 1H), 3.82 (s, 3H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 179.8, 179.2, 155.6, 148.4, 147.8, 135.1, 134.8, 131.9, 130.7, 129.5, 129.4, 127.3, 126.8, 121.6, 115.6, 112.5, 55.8. HRMS (ESI): calcd for $C_{17}H_{12}NO_4$ [M+Na]$^+$: 303.0633, found 303.0664.

**62.** (4-(2-hydroxy-4-methoxyphenyl)naphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 500 MHz): $\delta$ (ppm) 9.97 (s, 1H), 7.99 (dd, $J$ = 8.0, 2.0 Hz, 1H), 7.64 (td, $J$ = 7.5, 1.5 Hz, 1H), 7.55 (td, $J$ = 7.5, 1.5 Hz, 1H), 7.09 (d, $J$ = 8.0 Hz, 1H), 7.04 (d, $J$ = 7.5 Hz, 1H), 6.58 (d, $J$ = 2.5 Hz, 1H), 6.51 (dd, $J$ = 8.0, 2.0 Hz, 1H), 6.25 (s, 1H), 3.66 (s, 3H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 180.0, 179.1, 160.4, 157.6, 154.4, 135.1, 135.0, 131.3, 130.5, 130.4, 129.3, 128.9, 128.0, 116.1, 107.7, 99.6, 55.4. HRMS (ESI): calcd for $C_{17}H_{12}NO_4$ [M+Na]$^+$: 303.0633, found 303.0661.

**63.** (4-(4-hydroxy-2,6-dimethoxyphenyl)naphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 500 MHz): $\delta$ (ppm) 8.02 (d, $J$ = 7.5 Hz, 1H), 7.69 (t, $J$ = 7.5, 2.0 Hz, 1H), 7.59 (t, $J$ = 7.5 Hz, 1H), 7.44 (d, $J$ = 7.5 Hz, 1H), 6.74 (s, 2H), 6.34 (s, 1H), 3.95 (s, 6H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 180.4, 179.8, 156.6, 148.4 (2C), 137.5, 135.8, 135.2, 132.2, 131.3,

130.1, 130.0, 127.2, 126.9, 106.4 (2C), 56.6 (2C). HRMS (ESI): calcd for $C_{18}H_{14}O_5$ [M+Na]$^+$: 333.0739, found 333.0744.

**64.**[54] (4-methoxynaphthalene-1,2-dione). $^1$H NMR (DMSO-$d_6$, 500 MHz): $\delta$ (ppm) 8.11 (dd, $J$ = 8.0, 1.5 Hz, 1H), 7.86 (dd, $J$ = 7.5, 1.0 Hz, 1H), 7.69 (td, $J$ = 8.0, 1.5 Hz, 1H), 7.58 (td, $J$ = 7.5, 1.5 Hz, 1H), 5.98 (s, 1H), 4.02 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, 125 MHz): $\delta$ (ppm) 179.7. 179.6, 168.9, 135.2, 132.1, 131.7, 130.5, 129.3, 124.9, 103.2, 57.0.

**65.**[55] (4-ethoxynaphthalene-1,2-dione). $^1$H NMR (Acetone-$d_6$, 500 MHz): $\delta$ (ppm) 8.03 (d, $J$ = 7.5 Hz, 1H), 7.96 (d, $J$ = 7.5 Hz, 1H), 7.82 (t, $J$ = 7.5 Hz, 1H), 7.69 (t, $J$ = 7.5 Hz, 1H), 5.98 (s, 1H), 4.34 (q, $J$ = 7 Hz, 2H), 1.55 (t, $J$ = 7 Hz, 3H).

## 2.7 Anti α-glucosidase activity assessment

These experiments were performed by Mr. Ade Danova in Assist. Prof. Dr. Warinthorn Chavasiri's laboratory. To a solution of 0.1 M phosphate buffer (pH 6.9), α-glucosidase enzyme (0.1 unit/mL) and substrate $p$-nitrophenyl-α-D-glucopyranoside ($p$-NPG) (1 mM) were added. The test sample (10 μL) was pre-incubated with α-glucosidase (40 μL) at 37 $^0$C for 10 min. Then, substrate solution (50 μL) was added and incubated at 37 $^0$C. After 20 min of incubation, 1 M $Na_2CO_3$ solution (100 μL) was added to stop the enzymatic hydrolysis of $p$-NPG reaction. The amount of $p$-nitrophenol released **(Figure 2.5)** was quantified using ALLSHENG AMR-100 microplate reader. The absorbance was measured at 405 nm. The IC$_{50}$ value was deduced from the plot of % inhibition $vs$ concentration of test sample. Acarbose was used as standard

condition and the experiment was repeated three times. The $IC_{50}$ is the concentration

of an inhibitor that gives 50% inhibition.
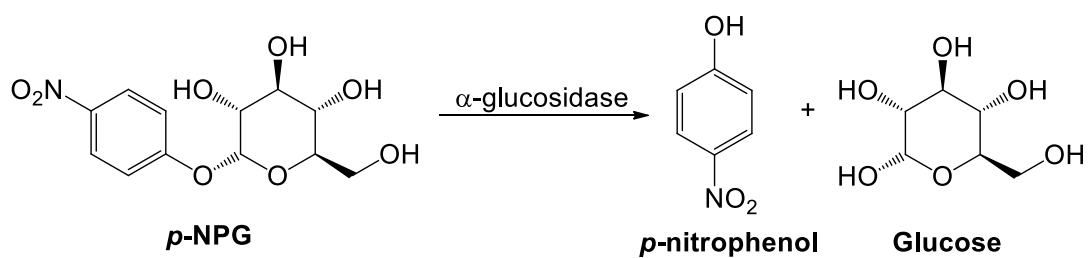


**Figure 2.5** Enzymatic hydrolysis reaction of *p*-NPG by **α**-glucosidase

CHAPTER 3

RESULTS AND DISCUSSION

In 2002, Ahn *et al.*[36] reported a series of 4-phenyl 1,2-NQs, 4-alkyl 1,2-NQs, 7-alkoxy 1,2-NQs, 8-amino-linked 1,2-NQs derivatives incorporating different substituents on 1,2-naphthoquinone ring. However, 1,2-naphthoquinone derivatives containing different alkyl and aryl groups on heteroatom linker at C-4 position were not investigated and the role of electron donating and electron withdrawing substituents on the biological activities of these derivatives were not evaluated. The effect of heteroatom linker was also not addressed. Therefore, 1,2-naphthoquinones **52-91** were synthesized and examined for their **α**-glucosidase inhibitory activity. Furthermore, the structure-activity relationship was investigated according to the $IC_{50}$ values of naphthoquinones to find some potential candidates for further modifications.

The synthesis of target naphthoquinones could be achieved in moderate to high yields. Structural elucidation of all compounds was carried out by $^1$H, $^{13}$C and 2D NMR. For known compounds, their structural interpretation was clarified by comparing with reported NMR database. In addition, HR-MS was collected for new compounds to confirm their structures.

## 3.1 Synthesis and structural elucidation of 4-phenylamino 1,2-naphthoquinones

Following the procedure of Tseng *et al.*[45] with some modification, ten 4-phenylamino 1,2-naphthoquinones were prepared and four synthesized compounds are new **(Firgure 3.1)**. Those derivatives were prepared for investigating the role of

substitution, position of substitutions on the phenyl ring to the activity, increasing the solubility and stability of the products. Phenyl ring possessed only one substituent mainly at *para* or *ortho*-position. Two naphthoquinone derivatives possessed two substituents at *ortho* and *para*-position. All products were obtained as red crystal or orange solid with moderate to high yields (42-92%) as shown in **Table 3.1**. All of them were attained by column chromatography or crystalized from EtOH. 4-Phenylamino-1,2-naphthoquinones with donating substituent on phenyl ring were achieved in higher yield because of the increment of the nucleophilicity of reactive center (nitrogen atom). On the other hand, those with withdrawing substituent on phenyl ring were achieved in moderate yield.
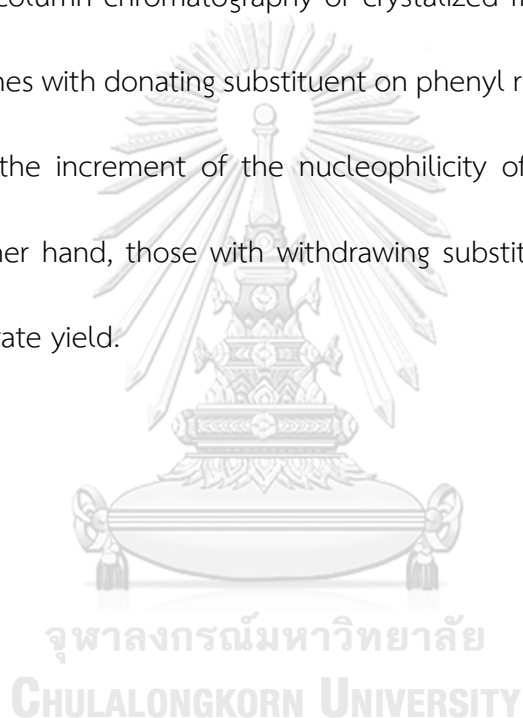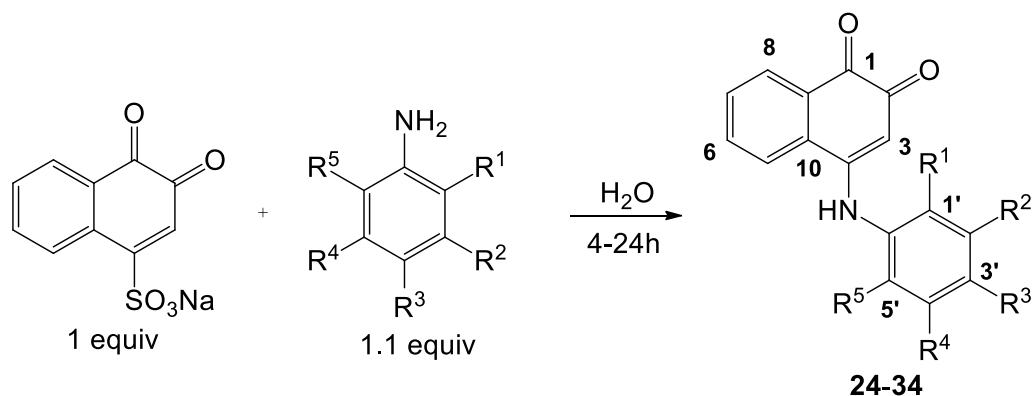
**24.** R$^1$ = R$^2$ = R$^3$ = R$^4$ = H = R$^5$ = H  **30.** R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = OPh

**25.** R$^2$ = R$^3$ = R$^4$ = R$^5$ = H, R$^1$ = COOH  **31.** R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = CH$_2$CH$_2$OH

**26.** R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = OMe  **32.** R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = COOH

**27.** R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = Me  **33.** R$^2$ = R$^3$ = R$^4$ = H, R$^1$ = R$^3$ = Me

**28.** R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = F  **34.** R$^2$ = R$^3$ = R$^4$ = H, R$^1$ = R$^5$ = Me

**29.** R$^1$ = R$^2$ = R$^4$ = R$^5$ = H, R$^3$ = NO$_2$

**Figure 3.1** The synthesis of naphthoquinones with phenylamino

substituents

The structural identification of these compounds was conducted by $^1$H and $^{13}$C-NMR analysis. The $^1$H and $^{13}$C-NMR spectra of **24-34** showed all signals of 1,2-naphthoquinone skeleton. Besides, all the proton signals of additional phenyl ring appeared in the range of at 7.07-7.69 ppm. The important signals of 1,2-naphthoquinones were downfield doublet peak of the **α**-proton of C-N group at 8.32-8.63 and the carbon peak around 155-158 ppm of vinyl carbon in quinone ring connected with nitrogen. The proton of the double bond in quinone ring appeared in a wide range of 5.05-6.66 ppm as a sharp or broad peak.

The $^{1}$H and $^{13}$C-NMR spectral assignments of sodium 1,2-naphthoquinone 4-sulfonate **(SSNQ)** and **24**-**34** are gathered in **Tables 3.2**-**3.9**. The signals in the $^{1}$H and $^{13}$C-NMR spectra of **24** and **31** were similar to each other and those of **33**-**34** were similar to each other. The signals of proton and carbon on phenyl ring would be varied due to the difference of substituents. The proton and carbon signals of the double bond in quinone ring and the proton belonging to nitrogen sometime appeared as low intensity. The $^{1}$H and $^{13}$C-NMR spectra of **27**-**29** were different due to the change of solvent used for NMR analysis.

**Table 3.1** Yield and characteristics of 1,2-naphthoquinones **24-34**

| 1,2-NQs | Appearance | Yield (%) | Remarks |
|---|---|---|---|
| **24** | Red crystal | 90 | Known |
| **25** | Red crystal | 45 | New |
| **26** | Red crystal | 92 | Known |
| **27** | Red crystal | 85 | Known |
| **28** | Red crystal | 67 | Known |
| **29** | Red crystal | 55 | Known |
| **30** | Red crystal | 42 | New |
| **31** | Red crystal | 58 | New |
| **32** | Red crystal | 57 | Known |
| **33** | Red crystal | 56 | New |
| **34** | Orange solid | 44 | Known |

Table 3.2 $^1$H & $^{13}$C NMR chemical shift assignment of **SSNQ**

| Position | $\delta$H (ppm) | $\delta$C (ppm) |
|:---:|:---:|:---:|
| 1 | - | 178.5 |
| 2 | - | 181.8 |
| 3 | 6.74, s | 124.2 |
| 4 | - | 156.5 |
| 5 | 8.41, d, $J$ = 8.0 Hz | 129.0 |
| 6 | 7.74, t, $J$ = 8.0 Hz | 130.5 |
| 7 | 7.57, t, $J$ = 7.6 Hz | 130.2 |
| 8 | 7.97, d, $J$ = 7.6 Hz | 134.6 |
| 9 | - | 131.8 |
| 10 | - | 131.9 |

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**Table 3.3** ^{1}H NMR chemical shift assignment of 1,2-naphthoquinones **24, 26, 31**

| Position | $\delta_H$ (ppm) | | |
|---|---|---|---|
| | 24 (DMSO-$d_6$) | 26 (DMSO-$d_6$) | 31 (DMSO-$d_6$) |
| 3 | 5.85, s | 5.56, s | 5.80, s |
| 5 | 8.32, d, $J$ = 8.0 Hz | 8.29, d, $J$ = 7.8 Hz | 8.30, d, $J$ = 8.0 Hz |
| 6 | 7.74, t, $J$ = 8.0 Hz | 7.73, t, $J$ = 7.6 Hz | 7.72, t, $J$ = 7.5 Hz |
| 7 | 7.86, t, $J$ = 7.6 Hz | 7.86, t, J = 7.8 Hz | 7.84, t, $J$ = 8.0 Hz |
| 8 | 8.04, d, $J$ = 7.6 Hz | 8.03, d, $J$ = 8.0 Hz | 8.02, d, $J$ = 7.5 Hz |
| 2′ | 7.24, brs | 7.07, d, $J$ = 8.0 Hz | 7.18, brs |
| 3′ | 7.49, t, $J$ = 7.6 Hz | 7.31, d, $J$ = 7.6 Hz | 7.33, d, $J$ = 7.5 Hz |
| 4′ | 7.31, t, $J$ = 7.6 Hz | - | - |
| 5′ | 7.49, t, $J$ = 7.6 Hz | 7.31, d, $J$ = 7.6 Hz | 7.33, d, $J$ = 7.5 Hz |
| 6′ | 7.24, brs | 7.07, d, $J$ = 8.0 Hz | 7.18, brs |
| NH | - | 9.82, s | - |
| $R^3$ | - | OMe 3.80, s | $CH_2CH_2OH$ $CH_2$: 2.77, t, $J$ = 7.0 Hz $CH_2$: 3.65, t, $J$ = 7.0 Hz OH: 4.75, s |

**Table 3.4** ^{13}C NMR chemical shift assignment of 1,2-naphthoquinones **24, 26, 31**

| Position | δC (ppm) | | |
|---|---|---|---|
| | 24 (DMSO-$d_6$) | 26 (DMSO-$d_6$) | 31 (DMSO-$d_6$) |
| 1 | 175.3 | 175.7 | 181.3 |
| 2 | 181.3 | 181.4 | 181.3 |
| 3 | 101.4 | 100.1 | 101.0 |
| 4 | 155.0 | 155.1 | 155.0 |
| 5 | 124.2 | 123.8 | 124.2 |
| 6 | 131.6 | 130.9 | 131.8 |
| 7 | 134.2 | 134.4 | 134.3 |
| 8 | 129.4 | 128.2 | 129.9 |
| 9 | 127.5 | 131.4 | 127.9 |
| 10 | 126.1 | 130.1 | 127.7 |
| 1′ | 131.2 | 131.6 | 131.2 |
| 2′ | 129.4 | 127.6 | 129.9 |
| 3′ | 131.6 | 114.7 | 131.6 |
| 4′ | 124.3 | 158.1 | 130.1 |
| 5′ | 131.6 | 114.7 | 131.6 |
| 6′ | 129.4 | 127.6 | 129.9 |
| R[3] | - | OMe 55.4 | CH$_2$CH$_2$OH CH$_2$: 38.6 CH$_2$: 62.2 |

**Table 3.5** ^{1}H NMR chemical shift assignment of 1,2-naphthoquinones **25, 30, 33-34**

| Position | $\delta$H (ppm) | | | |
|---|---|---|---|---|
| | 25 (DMSO-$d_6$) | 30 (DMSO-$d_6$) | 33 (DMSO-$d_6$) | 34 (DMSO-$d_6$) |
| 3 | 6.56, s | 5.84, s | 5.09, s | 5.05, s |
| 5 | 8.08, d, $J$ = 7.5 Hz | 8.32, d, $J$ = 8.0 Hz | 8.35, brs | 8.37, d, $J$ = 8.0 Hz |
| 6 | 7.81, t, $J$ = 7.5 Hz | 7.74, t, $J$ = 8.0 Hz | 7.74, t, $J$ = 7.5 Hz | 7.75, t, $J$ = 7.5 Hz, 1H |
| 7 | 7.88, t, $J$ = 7.5 Hz | 7.86, t, $J$ = 7.5 Hz | 7.87, t, $J$ = 7.5 Hz | 7.89, t, $J$ = 8.0 Hz |
| 8 | 8.04, d, $J$ = 7.5 Hz | 8.04, d, $J$ = 7.5 Hz | 8.04, d, $J$ = 7.5 Hz | 8.05, d, $J$ = 7.5 Hz |
| 2′ | - | 7.08, d, $J$ = 8.0 Hz | - | - |
| 3′ | 7.98, dd, $J$ = 7.5, 1.0 Hz | 7.28, brs | 7.15, brs | 7.24, brs |
| 4′ | 7.66, td, $J$ = 7.5, 2.0 Hz | - | - | 7.24, brs |
| 5′ | 7.66, td, $J$ = 7.5, 1.0 Hz | 7.28, brs | 7.15, brs | 7.24, brs |
| 6′ | 7.21, dd, $J$ = 7.5, 2.0 Hz | 7.08, d, $J$ = 8.0 Hz | 7.21, brs | - |
| NH | - | - | 9.79, s | - |
| R[1] | COOH 11.12, s | - | Me 2.14, s | Me 2.14, s |
| R[3] | - | O-Phenyl: 7.12, d, $J$ = 8.5 Hz, H-2″, 6″; 7.43, t, $J$ = 8.0 Hz, H-3″, 5″; 7.17, t, $J$ = 7.5 Hz, H-4″ | Me 2.33, s | - |
| R[5] | - | - | - | Me: 2.14, s |

**Table 3.6** ^{13}C NMR chemical shift assignment of 1,2-naphthoquinones **25, 30, 33-34**

| Position | $\delta$C (ppm) (DMSO-$d_6$) | | | |
|---|---|---|---|---|
| | 25 (DMSO-$d_6$) | 30 (DMSO-$d_6$) | 33 (DMSO-$d_6$) | 34 (DMSO-$d_6$) |
| 1 | 181.6 | 175.3 | 181.9 | 181.5 |
| 2 | 183.4 | 184.7 | 182.0 | 181.5 |
| 3 | 104.8 | 101.4 | 100.3 | 104.0 |
| 4 | 155.2 | 156.8 | 155.8 | 155.0 |
| 5 | 125.5 | 124.4 | 124.4 | 124.0 |
| 6 | 132.1 | 132.0 | 132.2 | 132.7 |
| 7 | 135.1 | 134.7 | 134.8 | 134.5 |
| 8 | 132.4 | 131.4 | 131.9 | 131.7 |
| 9 | 130.4 | 130.4 | 130.4 | 130.6 |
| 10 | 126.5 | 127.8 | 127.9 | 128.7 |
| 1′ | 143.9 | 133.7 | 135.0 | 138.6 |
| 2′ | 133.1 | 119.0 | 128.0 | 131.5 |
| 3′ | 140.1 | 119.1 | 132.0 | 128.5 |
| 4′ | 123.3 | 140.1 | 128.2 | 123.8 |
| 5′ | 133.7 | 119.1 | 128.1 | 128.5 |
| 6′ | 120.5 | 119.0 | 124.3 | 131.5 |
| R$^1$ | COOH<br>169.0 | - | Me<br>17.8 | Me<br>17.6 |
| R$^3$ | - | O-Phenyl<br>C1″: 155.6,<br>C2″, C6″:<br>119.7, C3″,<br>C5″: 130.6<br>C4″: 124.2 | Me<br>21.2 | - |
| R$^5$ | - | | - | Me<br>17.6 |

**Table 3.7** ^{1}H NMR chemical shift assignment of 1,2-naphthoquinones **27-29**

| Position | $\delta_H$ (ppm) | | |
|---|---|---|---|
| | 27 (Pyridine-$d_5$) | 28 (Pyridine-$d_5$) | 29 (Pyridine-$d_5$) |
| 3 | 6.66, s | 6.61, s | 6.48, s |
| 5 | 8.63, d, *J* = 8.0 Hz | 8.61, d, *J* = 8.0 Hz | 8.56, d, *J* = 7.6 Hz |
| 6 | 7.64, brs | 7.65, t, *J* = 8.8 Hz | 7.70, t, *J* = 7.6 Hz |
| 7 | 7.69, t, *J* = 7.6 Hz | 7.71, t, *J* = 7.2 Hz | 7.77, t, *J* = 7.6 Hz |
| 8 | 8.34, d, *J* = 7.6 Hz | 8.34, d, *J* = 7.6 Hz | 8.31, brs |
| 2′ | 7.16, brs | 7.19, brs | 7.19, d, *J* = 8.4 Hz |
| 3′ | 7.22, brs | 7.21, brs | 8.34, m |
| 5′ | 7.22, brs | 7.21, brs | 8.34, m |
| 6′ | 7.16, brs | 7.19, brs | 7.19, d, *J* = 8.4 Hz |
| NH | - | - | - |
| R$^3$ | Me  2.30, s | - | - |

**Table 3.8** ^{13}C NMR chemical shift assignment of 1,2-naphthoquinones **27-29**

| Position | $\delta_C$ (ppm) (Pyridine-$d_5$) | | |
|---|---|---|---|
| | 27 | 28 | 29 |
| 1 | 182.9 | 182.8 | 182.8 |
| 2 | 182.9 | 182.8 | 182.8 |
| 3 | 104.6 | 104.5 | 105.0 |
| 4 | 156.7 | 156.5 | 157.9 |
| 5 | 125.7 | 125.7 | 126.5 |
| 6 | 132.4 | 132.3 | 132.3 |
| 7 | 134.1 | 134.1 | 134.3 |
| 8 | 130.7 | 131.7 | 127.2 |
| 9 | 131.7 | 131.8 | 132.2 |
| 10 | 127.4 | 127.4 | 127.5 |
| 1' | 135.6 | 135.7 | 145.0 |
| 2' | 123.6 | 116.9 / 116.7 | 121.8 |
| 3' | 130.7 | 125.8 | 125.9 |
| 4' | 131.7 | 162.1 / 160.2 | 135.4 |
| 5' | 130.7 | 125.8 | 125.9 |
| 6' | 123.6 | 116.9 / 116.7 | 121.8 |
| R[3] | Me 21.4 | - | - |

**Table 3.9** ^{1}H & ^{13}C NMR chemical shift assignment of 1,2-naphthoquinones **32**

| Position | $\delta$H (ppm) (DMSO-$d_6$) | $\delta$C (ppm) (DMSO-$d_6$) |
|----------|------------------------------|------------------------------|
| 1 | - | 182.9 |
| 2 | - | 182.9 |
| 3 | 5.95, s | 101.9 |
| 4 | - | 155.7 |
| 5 | 8.34, d, $J$ = 7.5 Hz | 124.7 |
| 6 | 7.69, t, $J$ = 7.5 Hz | 131.5 |
| 7 | 7.78, t, $J$ = 7.5 Hz | 134.1 |
| 8 | 8.01, d, $J$ = 7.5 Hz | 130.8 |
| 9 | - | 133.2 |
| 10 | - | 126.3 |
| 1′ | - | 134.6 |
| 2′ | 7.05, d, $J$ = 8.0 Hz | 121.6 |
| 3′ | 7.91, d, $J$ = 8.5 Hz | 130.3 |
| 4′ | - | 126.2 |
| 5′ | 7.91, d, $J$ = 8.5 Hz | 130.3 |
| 6′ | 7.05, d, $J$ = 8.0 Hz | 121.6 |
| $R^3$ | - | COOH 167.5 |

## 3.2 Synthesis and structural elucidation of 4-alkylamino 1,2-naphthoquinones

The introduction of alkyl chain increases the van deer Waal interaction between tested inhibitors and cells or enzymes.[2, 56] Therefore, 4-alkylamino-1,2-naphthoquinone derivatives were synthesized. The effect of phenyl ring on the inhibitory activity was elucidated by comparing the activities of 4-phenylamino-1,2-naphthoquinone and 4-alkylamino-1,2-naphthoquinone analogues. Following the

procedure of Lim *et al.*[50] with some modification, seven 4-alkylamino-1,2-naphthoquinones were synthesized, **35** was prepared following Shukla *et al.*'s[48] procedure, **42** was prepared following Benites *et al.*'s[49] procedure **(Figure 3.2)**. The mechanism of reaction created **35** is given in **Figure 3.3**. Total nine derivatives were prepared including four new compounds. These derivatives were investigated the effect of the alkyl chain length and the effect of sugar similar structural substituent to the activity. The chain length was varied from two to four carbons. 4-Amino, 4-cyclohexylamino and 4-benzylamino 1,2-NQ derivatives were also prepared. There is only one dialkylamino derivative. **43** containing bromine on the alkyl chain was prepared but biological activities were not tested. All products were purified by column chromatography as red crystal with moderate to high yields (45-78%) as shown in **Table 3.10**.
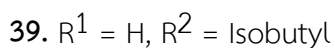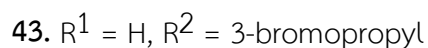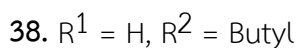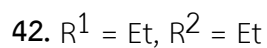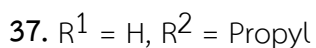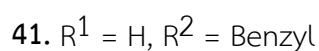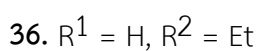
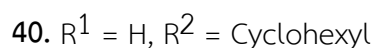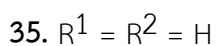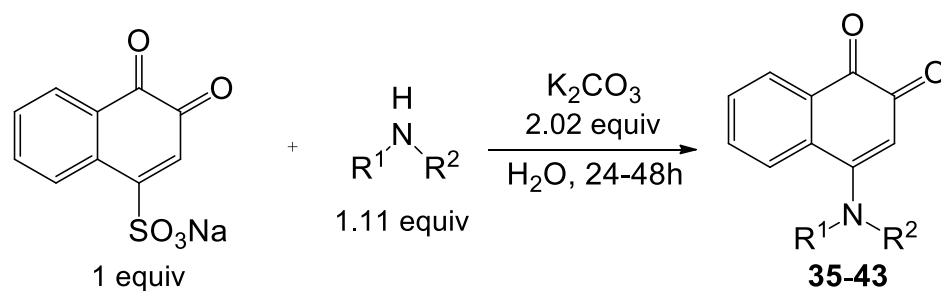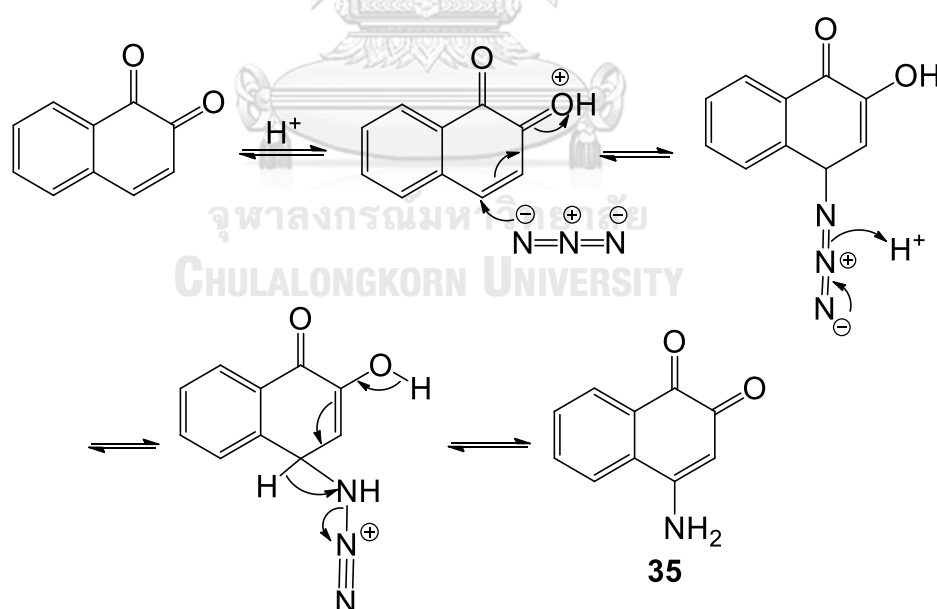**Figure 3.2** The structures of 1,2-naphthoquinones with alkylamino

substituents



**Figure 3.3** The mechanism of reaction created **35**

The reaction mechanism between aniline derivatives or aliphatic amines with

SSNQ are shown in **Figure 3.4**.[57] The first step is the Michael-like additional step. In

this step, aniline derivatives or aliphatic amines will attack to the C-4 in quinone ring. Due to the steric effect of sodium sulfonate group, steric aniline derivatives or bulky aliphatic amines would not attack to the reactive center. Therefore, bulky 4-amino 1,2-naphthoquinone scaffold was not possible. The second step is the elimination step of sodium sulfonate. The last step is the deprotonation step of the proton on the nitrogen atom. 4-Alkylamino-1,2-naphthoquinones were achieved in lower yield compared with 4-phenylamino-1,2-naphthoquinones. Although alkyl group increased the nucleophilicity of nitrogen, it decreased the acidity making hydrogen atom harder to leave. Therefore, $K_2CO_3$ was used as a base to facilitate the deprotonation step. However, **SSNQ** has three reactive centers therefore strong nucleophile reagent such as diethylamine **(42)** created several byproducts. In this respect, Lewis catalyst was used to increase the selectivity.
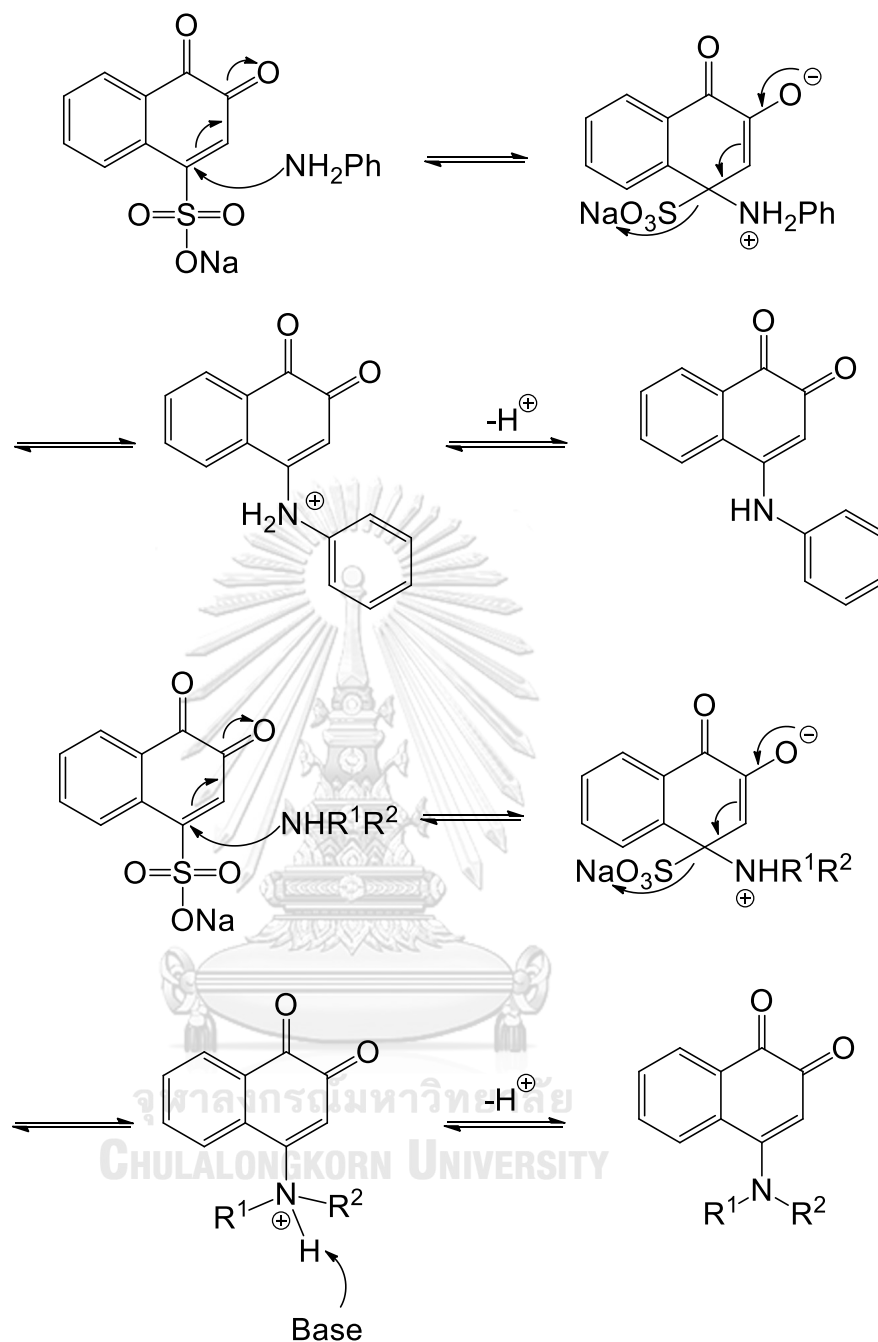
**Figure 3.4** The reaction mechanism between substituted aniline, aliphatic

amine and **SSNQ**

The structural identification of these compounds was conducted by $^1$H and $^{13}$C-

NMR analysis. The $^1$H and $^{13}$C-NMR spectra of **35-43** excepting **42** showed all signals of

1,2-naphthoquinone skeleton. Two signals of carbonyl groups **(42)** were not shown in

the ^{13}C-NMR spectra. The intensity of quaternary carbon was weak therefore the signals of carbonyl groups were hard to be seen. The important signals of 1,2-naphthoquinones were downfield doublet peak of the $\alpha$-proton with C-N group at around 8.00 ppm and the carbon peak around 150-160 ppm of vinyl carbon in quinone ring connected with nitrogen.

The ^{1}H and ^{13}C-NMR spectral assignments of **35**-**43** are shown in **Tables 3.11-3.17**. The signals in the ^{1}H and ^{13}C-NMR spectra of **35**-**41** and **43** were similar to each other. The proton signal belonging to the double bond in quinone ring appeared around 5.60 ppm as a sharp peak. The proton signal on nitrogen sometimes disappeared due to the exchange with DMSO-$d_6$.

**Table 3.10** Yield and characteristics of 1,2-naphthoquinones **35-43**

| 1,2-NQs | Appearance | Yield (%) | Remarks |
|---------|------------|-----------|---------|
| 35 | Red crystal | 59 | Known |
| 36 | Red crystal | 63 | Known |
| 37 | Red crystal | 60 | New |
| 38 | Red crystal | 54 | Known |
| 39 | Red crystal | 74 | New |
| 40 | Red crystal | 78 | New |
| 41 | Red crystal | 59 | Known |
| 42 | Red oil | 45 | Known |
| 43 | Red crystal | 61 | New |

**Table 3.11** ^{1}H & ^{13}C NMR chemical shift assignment of naphthoquinones **35, 36**

| Position | 35 (DMSO-$d_6$) | | 36 (DMSO-$d_6$) |
| --- | --- | --- | --- |
| | $\delta$H (ppm) | $\delta$C (ppm) | $\delta$H (ppm) |
| 1 | - | 174.7 | - |
| 2 | - | 182.2 | - |
| 3 | 5.74, s | 101.0 | 5.66, s |
| 4 | - | 158.1 | - |
| 5 | 8.04, d, $J$ = 7.6 Hz | 124.0 | 7.97, dd, $J$ = 8.0, 1.5 Hz |
| 6 | 7.68, d, $J$ = 7.6 Hz | 131.7 | 7.72, td, $J$ = 7.5, 1.5 Hz |
| 7 | 7.80, t, $J$ = 7.6 Hz | 134.2 | 7.82, td, $J$ = 7.0, 1.0 Hz |
| 8 | 7.96, d, $J$ = 6.8 Hz | 127.8 | 7.94, dd, $J$ = 7.5, 1.5 Hz |
| 9 | - | 131.6 | - |
| 10 | - | 130.5 | - |
| NH | 8.32, s<br>8.16, s | - | 7.54, t, $J$ = 6.0 Hz |
| $R^2$ | - | - | Et<br>CH$_2$: 3.21, q, $J$ = 7.0 Hz<br>CH$_3$: 1.16, t, $J$ = 7.5 Hz |

**Table 3.12** ^{1}H NMR chemical shift assignment of naphthoquinones **37-39**

| Position | 37 (DMSO-$d_6$) | 38 (DMSO-$d_6$) | 39 (DMSO-$d_6$) |
|---|---|---|---|
| 3 | 5.69, s | 5.69, s | 5.70, s |
| 5 | 8.13, d, $J$ = 8.0 Hz | 8.11, d, $J$ = 8.0 Hz | 8.15, d, $J$ = 8.0 Hz |
| 6 | 7.67, t, $J$ = 7.6 Hz | 7.68, t, $J$ = 7.6 Hz | 7.68, d, $J$ = 7.6 Hz |
| 7 | 7.80, td, $J$ = 8.0, 1.6 Hz | 7.81, t, $J$ = 7.6 Hz | 7.81, d, $J$ = 7.6 Hz |
| 8 | 7.98, dd, $J$ = 7.6, 1.6 Hz | 7.98, d, $J$ = 7.6 Hz | 7.98, d, $J$ = 7.6 Hz |
| NH | 8.45, s | 8.42, s | 8.47, s |
| $R^2$ | Propyl<br>$CH_2$: 3.34, d, $J$ = 6.8 Hz<br>$CH_2$: 1.68, m<br>$CH_3$: 0.95, t, $J$ = 7.6 Hz | Butyl<br>$CH_2$: 1.65, quint, $J$ = 7.2 Hz<br>$CH_2$: 1.38, m<br>$CH_3$: 0.92, t, $J$ = 7.2 Hz | Isobutyl<br>$CH_2$: 3.20, t, $J$ = 7.2 Hz<br>$CH_2$: 2.05, m<br>$CH_3$: 0.95, d, $J$ = 6.4 Hz |

**Table 3.13** ^{13}C NMR chemical shift assignment of 1,2-naphthoquinones **37-39**

| Position | 37 (DMSO-$d_6$) | 38 (DMSO-$d_6$) | 39 (DMSO-$d_6$) |
|----------|-----------------|-----------------|-----------------|
| 1 | 174.8 | 174.8 | 174.8 |
| 2 | 182.1 | 174.8 | 182.1 |
| 3 | 98.1 | 98.1 | 98.3 |
| 4 | 155.1 | 155.0 | 155.3 |
| 5 | 123.4 | 123.4 | 123.4 |
| 6 | 131.3 | 131.4 | 131.4 |
| 7 | 134.2 | 134.3 | 134.3 |
| 8 | 127.9 | 127.9 | 127.9 |
| 9 | 130.9 | 131.4 | 131.3 |
| 10 | 130.9 | 130.9 | 130.9 |
| $R^2$ | Propyl<br>$CH_2$: 44.9<br>$CH_2$: 21.1<br>$CH_3$: 11.5 | Butyl<br>$CH_2$: 43.0<br>$CH_2$: 29.8<br>$CH_2$: 19.8<br>$CH_3$: 13.7 | Isobutyl<br>$CH_2$: 50.6<br>$CH_2$: 27.2<br>$CH_3$: 20.3 |

**Table 3.14** ^{1}H NMR chemical shift assignment of 1,2-naphthoquinones **40-41**

| Position | 40 (DMSO-$d_6$) | 41 (DMSO-$d_6$) |
|---|---|---|
| 3 | 5.76, s | 5.60, s |
| 5 | 7.97, d, $J$ = 6.8 Hz | 8.18, d, $J$ = 7.6 Hz |
| 6 | 7.67, t, $J$ = 7.6 Hz | 7.70, t, $J$ = 7.6 Hz |
| 7 | 7.80, t, $J$ = 7.6 Hz | 7.85, td, $J$ = 7.6, 1.6 Hz |
| 8 | 7.91, d, $J$ = 7.6 Hz | 7.99, dd, $J$ = 7.6, 1.2 Hz |
| NH | 8.22, d, $J$ = 8.0 Hz | 9.06, t, $J$ = 6.0 Hz |
| $R^2$ | Cyclohexyl<br>CH: 3.61, m<br>$CH_2$: 1.95, d, $J$ = 11.6 Hz<br>$CH_2$: 1.76, d, $J$ = 12.4 Hz<br>$CH_2$: 1.64, d, $J$ = 13.2 Hz<br>$CH_2$: 1.43, m (4H) | Benzyl<br>$CH_2$: 4.64, d, $J$ = 6.0 Hz<br>$o$-CH: 7.37, m (2H)<br>$m$-CH: 7.37, m (2H)<br>$p$-CH: 7.29, m |

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**Table 3.15** $^{13}$C NMR chemical shift assignment of 1,2-naphthoquinones **40-41**

| Position | 40 (DMSO-$d_6$) | 41 (DMSO-$d_6$) |
|---|---|---|
| 1 | 174.9 | 175.0 |
| 2 | 182.1 | 181.8 |
| 3 | 98.2 | 99.1 |
| 4 | 153.8 | 155.1 |
| 5 | 123.6 | 123.4 |
| 6 | 131.2 | 131.4 |
| 7 | 134.0 | 134.4 |
| 8 | 127.8 | 128.0 |
| 9 | 131.3 | 131.3 |
| 10 | 130.9 | 130.9 |
| R$^2$ | Cyclohexyl<br>CH: 52.4<br>CH$_2$: 31.5 (2C)<br>CH$_2$: 24.5 (2C)<br>CH$_2$: 25.1 | Benzyl<br>CH$_2$: 46.3<br>Tertiary C: 137.5<br>$o$-CH: 128.7 (2C)<br>$m$-CH: 127.1 (2C)<br>$p$-CH: 127.4 |

For **42**, the free rotation changing into restricted rotation of carbon-nitrogen bond resulted in non-equivalence of the two ethyl groups. Signals of quaternary carbon did not show due to the small amount of sample.[52]
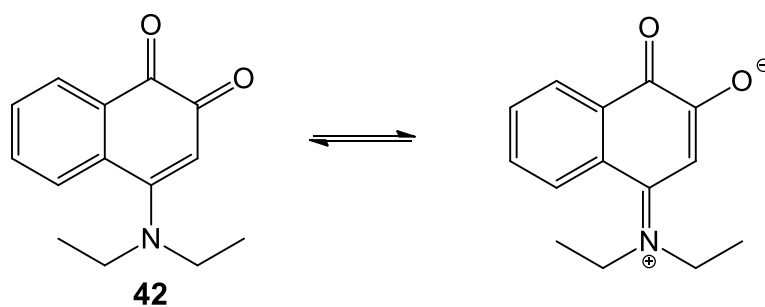
**Table 3.16** ^{1}H NMR chemical shift assignment of 1,2-naphthoquinones **42-43**

| Position | 42 (CDCl$_3$) | 43 (DMSO-$d_6$) |
| --- | --- | --- |
| 3 | 6.00, s | 5.74, s |
| 5 | 8.09, d, $J$ = 7.6 Hz | 8.10, d, $J$ = 8.0 Hz |
| 6 | 7.55, brs | 7.70, t, $J$ = 7.5 Hz |
| 7 | 7.57, brs | 7.81, td, $J$ = 7.5, 1.5 Hz |
| 8 | 7.61, d, $J$ = 7.6 Hz | 7.98, dd, $J$ = 7.5, 1.5 Hz |
| R$^1$ | Et<br>CH$_2$: 3.51, q, $J$ = 7.2 Hz<br>CH$_3$: 1.31, t, $J$ = 6.8 Hz | NH<br>8.44, t, $J$ = 5.5 Hz |
| R$^2$ | Et<br>CH$_2$: 3.51, q, $J$ = 7.2 Hz<br>CH$_3$: 1.31, t, $J$ = 6.8 Hz | CH$_2$CH$_2$CH$_2$Br<br>CH$_2$: 3.64, t, $J$ = 6.5 Hz<br>CH$_2$: 2.20, q, $J$ = 7.0 Hz<br>CH$_2$: 3.59, brs |

Table 3.17 ^{13}C NMR chemical shift assignment of 1,2-naphthoquinones **42-43**

| Position | 42 (CDCl$_3$) | 43 (DMSO-$d_6$) |
|---|---|---|
| 1 | - | 182.0 |
| 2 | - | 175.0 |
| 3 | 108.4 | 98.3 |
| 4 | - | 155.2 |
| 5 | 124.8 | 123.6 |
| 6 | 129.6 | 131.5 |
| 7 | 133.8 | 134.4 |
| 8 | 126.9 | 128.0 |
| 9 | 130.8 | 131.3 |
| 10 | 130.8 | 130.9 |
| R$^1$ | Et<br>CH$_2$: 42.4<br>CH$_3$: 11.4 | - |
| R$^2$ | Et<br>CH$_2$: 46.3<br>CH$_3$: 12.7 | CH$_2$CH$_2$CH$_2$Br<br>CH$_2$: 41.8<br>CH$_2$: 32.4<br>CH$_2$: 31.0 |

## 3.3 Synthesis and structural elucidation of 4-alkylphenylamino-1,2-naphthoquinones

Previous studies by Hatfield *et al.*[46] suggested that the methyl group on nitrogen linker played an important role on the enzyme inhibitory activity. Therefore, 4-alkyl phenylamino 1,2-naphthoquinone derivatives were prepared to investigate the role of the alkyl chain on the nitrogen linker on the inhibitory activity. The chain length was varied from one to two carbons. **44** was synthesized for comparison. Another 4-

alkylphenylamino-1,2-naphthoquinone **(45)** was prepared. **46** bearing methoxy

substituent on phenyl ring was synthesized to explore the effect of substituent on the

biological activity for this analogue. Following the procedure of Benites *et al.*[49] and

Tseng *et al.*[45] with some modification, three 4-alkylphenylamino-1,2-naphthoquinones

were prepared using secondary anilines or 4-phenylamino-1,2-NQs as starting material

**(Figure 3.5)**. All products were purified by column chromatography as red crystal or

red solid in moderate yields (12-45%) as shown in **Table 3.18**. Though alkyl group on

nitrogen increased the nucleophilicity of nitrogen, the yields were low. Alkyl groups

acted as a hindrance reducing the capacity to access to reactive carbon center on

quinone ring of aniline molecules. Therefore, Lewis acid catalyst was used to increase

the electrophilicity and selectivity of reactive carbon center. All of synthesized

compounds are known compounds. **45** was prepared using different procedure to

create 4-methylphenylamino-1,2-NQ derivatives from 4-phenylamino-1,2-NQs.

However, the deprotonation of secondary amines by NaH was not effective lead to

low yield.
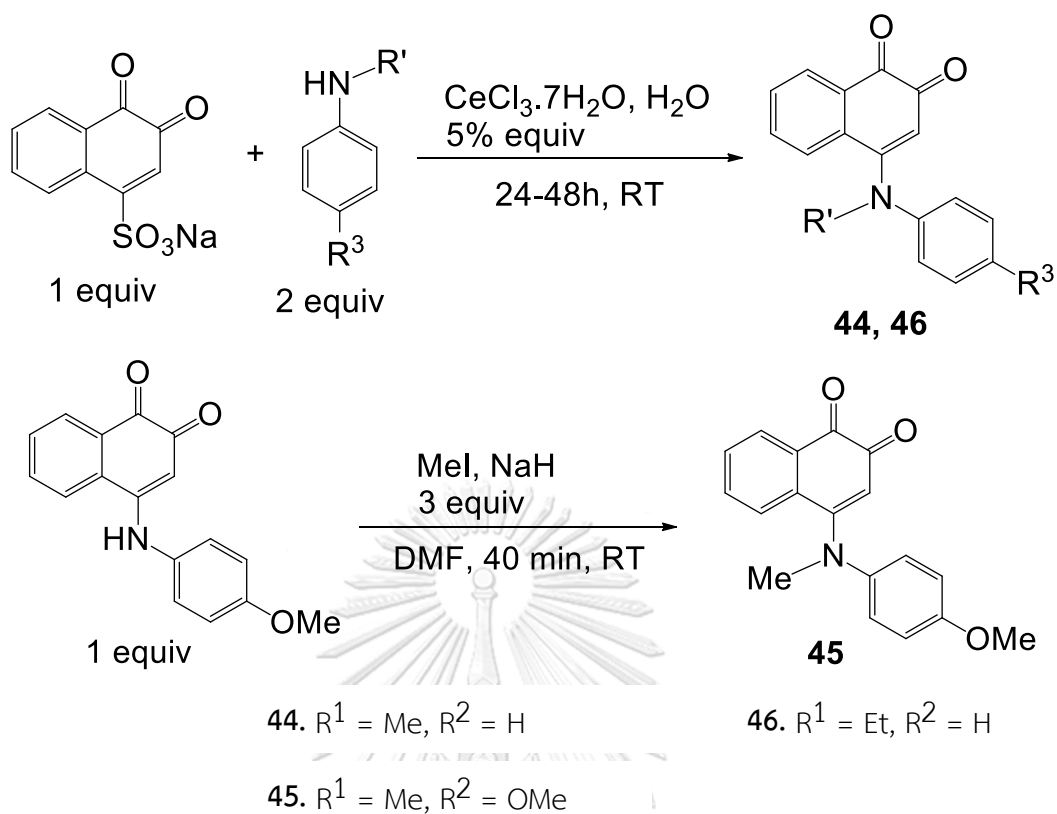
**Figure 3.5** The structures of 1,2-naphthoquinones with phenylalkylamino

substituents

The reaction mechanism between secondary aniline derivatives with **SSNQ** are

shown in **Figure 3.6**. The first step is the activation 1,2-naphthoquinone by Lewis acid

generating a positive charge on carbon at 4-position of quinone ring. The second step

is the Michael-like additional step in which secondary anilines will attack to the very

electrophilic carbon at 4-position of quinone ring. However, sodium sulfonate is a bulky

group making secondary anilines harder to attack to the reactive carbon center. The

third step is the deprotonation step. The last step is the elimination step of sodium

sulfonate group and $CeCl_3$.
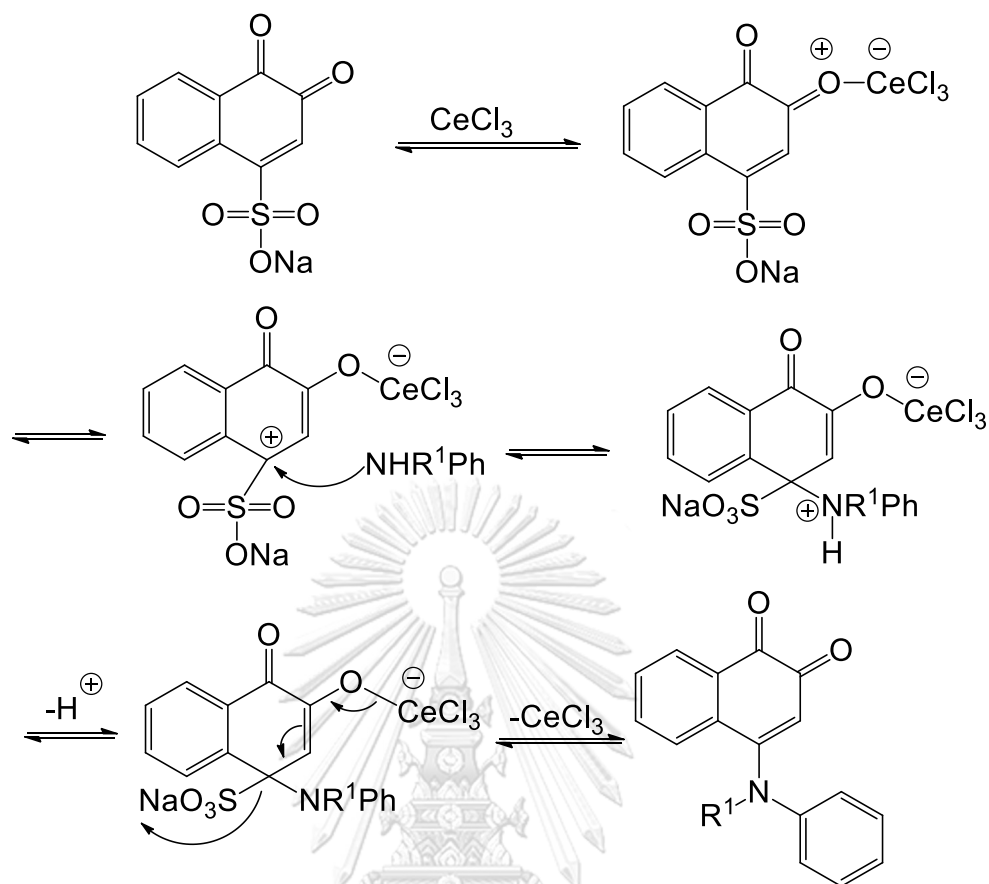
**Figure 3.6** The reaction mechanism between secondary aniline and **SSNQ**

The structural identification of these compounds was conducted by $^{1}$H and $^{13}$C-NMR analysis. The $^{1}$H and $^{13}$C-NMR spectra of **44-46** showed all signals of 1,2-naphthoquinone skeleton. Besides, all the proton signals of additional phenyl ring appeared at 6.98-7.37 ppm. The important signals of 1,2-naphthoquinones were downfield doublet peak of the **α**-proton with C-N group at around 8.05 ppm and the carbon peak around 157-159 ppm of vinyl carbon in quinone ring connected with tertiary nitrogen.

The $^{1}$H and $^{13}$C-NMR spectral assignments of **44-46** are shown in **Tables 3.19-3.20**. The signals in $^{1}$H and $^{13}$C-NMR spectra of those derivatives were similar to each

other. The peak of double bond proton in quinone ring appeared around 6.20-6.60

ppm as a sharp peak.

Table 3.18 Yield and characteristics of 1,2-naphthoquinones **44-46**

| 1,2-NQs | Appearance | Yield (%) | Remarks |
|---------|------------|-----------|---------|
| 44 | Red crystal | 45 | Known |
| 45 | Red crystal | 31 | Known |
| 46 | Red solid | 12 | Known |

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**Table 3.19** ^{1}H NMR chemical shift assignment of 1,2-naphthoquinones **44-46**

| Position | 44 (CDCl$_3$) | 45 (CDCl$_3$) | 46 (CDCl$_3$) |
|---|---|---|---|
| 3 | 6.27, s | 6.51, s | 6.47, s |
| 5 | 8.05, d, $J$ = 7.6 Hz | 8.54, d, $J$ = 8.0 Hz | 8.08, d, $J$ = 7.6 Hz |
| 6 | 7.21, t, $J$ = 7.2 Hz | 7.67, t, $J$ = 7.6 Hz | 7.22, d, $J$ = 8.0 Hz |
| 7 | 7.33, t, $J$ = 7.2 Hz | 7.75, td, $J$ = 7.6, 1.6 Hz | 7.37, t, $J$ = 7.6 Hz |
| 8 | 7.02, d, $J$ = 8.0 Hz | 8.22, dd, $J$ = 7.6, 1.2 Hz | 7.02, d, $J$ = 8.0 Hz |
| 2′ | 7.11, d, $J$ = 7.6 Hz | 6.98, d, $J$ = 8.4 Hz | 7.13, d, $J$ = 8.0 Hz |
| 3′ | 7.33, t, $J$ = 7.2 Hz | 6.98, d, $J$ = 8.4 Hz | 7.37, t, $J$ = 7.6 Hz |
| 4′ | 7.21, t, $J$ = 7.2 Hz | - | 7.28, brs |
| 5′ | 7.33, t, $J$ = 7.2 Hz | 6.98, d, $J$ = 8.4 Hz | 7.37, t, $J$ = 7.6 Hz |
| 6′ | 7.11, d, $J$ = 7.6 Hz | 6.98, d, $J$ = 8.4 Hz | 7.13, d, $J$ = 8.0 Hz |
| R$^1$ | Methyl CH$_3$: 3.49, s | Methyl 3.75, s | Ethyl CH$_2$: 4.05, q, $J$ = 7.2 Hz CH$_3$: 1.34, t, $J$ = 7.2 Hz |
| R$^2$ | - | OMe 3.88, s | - |

**Table 3.20** ^{13}C NMR chemical shift assignment of 1,2-naphthoquinones **44-46**

| Position | 44 (CDCl$_3$) | 45 (CDCl$_3$) | 46 (CDCl$_3$) |
|----------|---------------|---------------|---------------|
| 1 | 178.7 | 180.4 | 181.0 |
| 2 | 180.9 | 180.4 | 181.0 |
| 3 | 111.1 | 102.1 | 110.6 |
| 4 | 159.7 | 157.5 | 159.2 |
| 5 | 133.4 | 134.9 | 133.3 |
| 6 | 129.2 | 126.6 | 129.1 |
| 7 | 130.1 | 131.0 | 130.3 |
| 8 | 129.0 | 125.4 | 129.2 |
| 9 | 132.3 | 133.4 | 132.6 |
| 10 | 132.8 | 131.1 | 132.9 |
| 1′ | 148.0 | 155.0 | 146.2 |
| 2′ | 125.5 | 114.7 | 126.0 |
| 3′ | 130.0 | 122.4 | 130.1 |
| 4′ | 126.5 | 156.6 | 126.7 |
| 5′ | 130.0 | 122.4 | 130.1 |
| 6′ | 125.5 | 114.7 | 126.0 |
| R$^1$ | Me 44.3 | Me 55.7 | Et 51.0 12.3 |
| R$^2$ | - | OMe 55.9 | - |

## 3.4 Synthesis and structural elucidation of 4-phenoxy-1,2-naphthoquinones

From the NMR results, 1,2-naphthoquinone derivatives containing nitrogen atom as a linker with phenyl ring or alkyl chain having a strong conjugation and as a consequence, creating imine structure. To limit the formation of conjugation between

heteroatom linker with quinone moiety, oxygen atom was chosen as a linker and 4-phenoxy-1,2-naphthoquinone derivatives were synthesized. Following the procedure of Tseng *et al.*[45] with some modification, fourteen 4-phenoxyamino-1,2-naphthoquinones were prepared. However, **61**-**63** comprising phenyl substituent was created instead of phenoxy substituent. Intramolecular hydrogen bond between hydroxy group and methoxy group making it hard to created phenoxy anion. Moreover, two strong donating group created a strong electron negative charge at carbon center. As a consequence, C-alkylation was occurred instead of O-alkylation. To solve this problem, phenols were reacted with potassium hydroxide created potassium phenoxide first. Potassium phenoxide reacted with **SSNQ** generating desire O-alkylation products. **64**-**65** was synthesized using a different procedure **(Figure 3.7)**. Total nineteen derivatives were synthesized, ten synthesized compounds are new. Those derivatives were prepared to explore the role of oxygen atom on the activity and also the role of different substituent with different position on the phenyl ring to the activity. There is seven mono-substituent on the phenyl ring compounds and seven di-substituent on the phenyl ring. All products were obtained as yellow crystal except **61**-**63** with moderate yields (20-68%) as shown in **Table 3.21**. All of them were attained by using column chromatography.
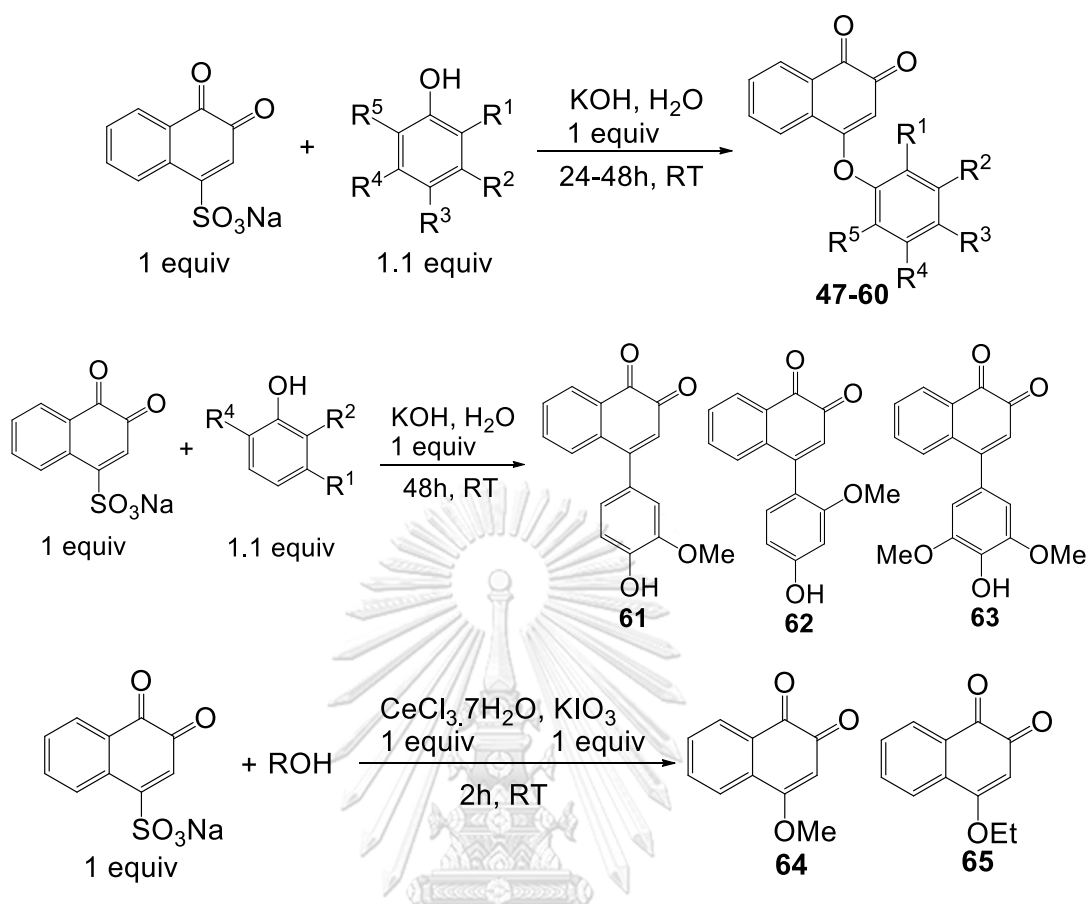
47. $R^1 = R^2 = R^3 = R^4 = R^5 = H$        54. $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Et$

48. $R^2 = R^3 = R^4 = R^5 = H$, $R^1 = Benzyl$        55. $R^3 = R^4 = R^5 = H$, $R^1 = R^2 = Me$

49. $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = OMe$        56. $R^2 = R^4 = R^5 = H$, $R^1 = R^3 = Me$

50. $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Cl$        57. $R^2 = R^3 = R^5 = H$, $R^1 = R^4 = Me$

51. $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Br$        58. $R^2 = R^3 = R^4 = H$, $R^1 = R^5 = Me$

52. $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Cumyl$        59. $R^1 = R^4 = R^5 = H$, $R^2 = R^3 = Me$

53. $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = NHCOCH_3$        60. $R^1 = R^3 = R^5 = H$, $R^2 = R^4 = Me$

**Figure 3.7** The structures of 1,2-naphthoquinones with alkylamino

substituents

**Table 3.21** Yield and characteristics of 1,2-naphthoquinones **47-65**

| 1,2-NQs | Appearance | Yield (%) | Remarks |
|---|---|---|---|
| 47 | Yellow crystal | 62 | Known |
| 48 | Yellow crystal | 68 | New |
| 49 | Yellow crystal | 42 | Known |
| 50 | Yellow crystal | 60 | Known |
| 51 | Yellow crystal | 55 | Known |
| 52 | Yellow crystal | 57 | New |
| 53 | Yellow crystal | 54 | New |
| 54 | Yellow crystal | 65 | New |
| 55 | Yellow crystal | 59 | New |
| 56 | Yellow crystal | 60 | Known |
| 57 | Yellow crystal | 61 | New |
| 58 | Yellow crystal | 45 | Known |
| 59 | Red crystal | 51 | Known |
| 60 | Yellow crystal | 55 | New |
| 61 | Red crystal | 30 | New |
| 62 | Red crystal | 23 | New |
| 63 | Yellow crystal | 20 | New |
| 64 | Yellow crystal | 67 | Known |
| 65 | Yellow crystal | 59 | Known |

The structural identification of these compounds was performed by $^{1}$H and $^{13}$C-NMR analysis. The $^{1}$H and $^{13}$C-NMR spectra of **47-65** showed all signals of 1,2-naphthoquinone skeleton. Besides, all the proton signals of additional phenyl ring appeared in a wide range of 6.35-7.45 ppm. The important signals of 1,2-naphthoquinones are downfield doublet peak of **α**- proton with C-O group at around

8.07 ppm and carbon peak around 167-169 ppm of vinyl carbon in quinone ring connected with oxygen.

The $^1$H and $^{13}$C-NMR spectral assignments of **47**-**65** are shown in **Tables 3.22-3.34**. The signals in $^1$H and $^{13}$C-NMR spectra of those derivatives were similar to each other excepting **61**-**63** where the peak of proton belonging to double bond in quinone ring was shifted downfield due to the lack of oxygen linker. The peak of double bond proton in quinone ring appeared around 6.20-6.60 ppm as a sharp peak.

The reaction mechanism is shown in **Figure 3.8**. Under basic condition, phenolic compounds were activated becoming phenoxides which have higher nucleophilicity than phenols. The first step is the Michael-like additional step. In this step, phenoxides will attack to the C-4 in quinone ring. The second step is the elimination step of sodium sulfonate group.
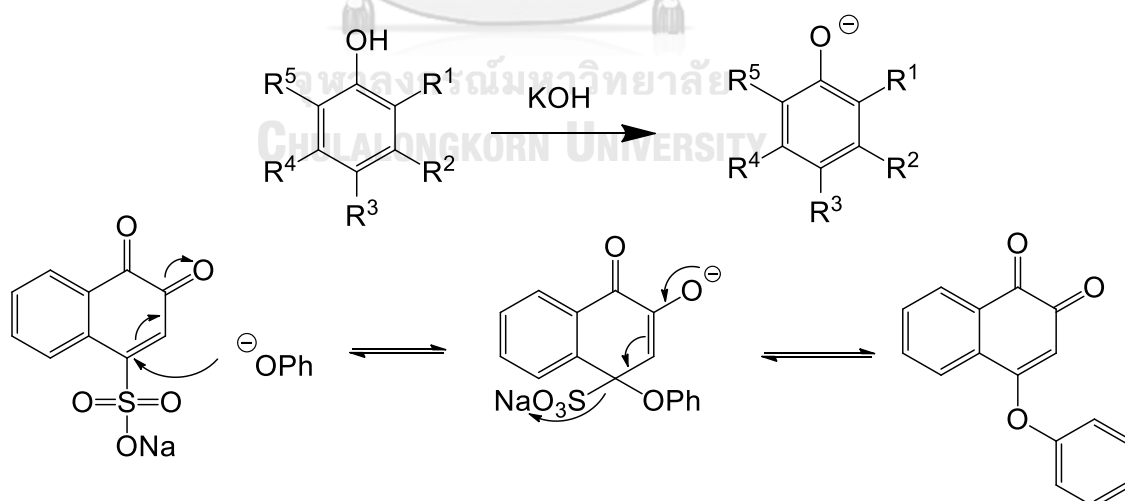
**Figure 3.8** The reaction mechanism between substituted phenol and SSNQ

Table 3.22 ^{1}H NMR chemical shift assignment of 1,2-naphthoquinones **47, 49-50**

| Position | 47 (CDCl$_3$) | 49 (CDCl$_3$) | 50 (CDCl$_3$) |
|---|---|---|---|
| 3 | 5.64, s | 5.67, s | 5.63, s |
| 5 | 8.14, dd, $J$ = 7.5, 1.0 Hz | 8.15, d, $J$ = 8.0 Hz | 8.16, dd, $J$ = 7.5, 1.0 Hz |
| 6 | 7.64, td, $J$ = 7.5, 1.0 Hz | 7.64, td, $J$ = 7.5, 1.0 Hz | 7.66, td, $J$ = 7.5, 1.0 Hz |
| 7 | 7.76, td, $J$ = 7.5, 1.5 Hz | 7.76, td, $J$ = 7.5, 1.0 Hz | 7.77, td, $J$ = 7.5, 1.5 Hz |
| 8 | 8.08, dd, $J$ = 8.0, 1.0 Hz | 8.08, dd, $J$ = 8.0, 1.5 Hz | 8.06, dd, $J$ = 8.0, 1.0 Hz |
| 2′ | 7.15, d, $J$ = 7.5 Hz | 6.97, dd, $J$ = 7.0, 2.5 Hz | 7.12, dd, $J$ = 8.5, 3.5 Hz |
| 3′ | 7.47, t, $J$ = 8.0 Hz | 7.07, dd, $J$ = 7.0, 2.5 Hz | 7.45, dd, $J$ = 9.0, 3.0 Hz |
| 4′ | 7.34, t, $J$ = 7.5 Hz | - | - |
| 5′ | 7.47, t, $J$ = 8.0 Hz | 7.07, dd, $J$ = 7.0, 2.5 Hz | 7.45, dd, $J$ = 9.0, 3.0 Hz |
| 6′ | 7.15, d, $J$ = 7.5 Hz | 6.97, dd, $J$ = 7.0, 2.5 Hz | 7.12, dd, $J$ = 8.5, 3.5 Hz |
| R$^3$ | - | OMe 3.84, s | - |

**Table 3.23** ^{13}C NMR chemical shift assignment of 1,2-naphthoquinones **47, 49-50**

| Position | 47 (CDCl$_3$) | 49 (CDCl$_3$) | 50 (CDCl$_3$) |
|---|---|---|---|
| 1 | 179.4 | 179.6 | 179.2 |
| 2 | 179.6 | 179.7 | 179.5 |
| 3 | 106.4 | 106.4 | 106.5 |
| 4 | 168.9 | 169.5 | 168.6 |
| 5 | 125.0 | 125.0 | 125.0 |
| 6 | 132.0 | 132.0 | 132.2 |
| 7 | 135.3 | 135.2 | 135.3 |
| 8 | 129.5 | 129.5 | 129.6 |
| 9 | 131.7 | 131.8 | 131.5 |
| 10 | 130.6 | 130.6 | 130.5 |
| 1′ | 152.4 | 145.8 | 150.9 |
| 2′ | 121.4 | 115.4 | 122.9 |
| 3′ | 130.5 | 122.2 | 130.6 |
| 4′ | 127.0 | 158.2 | 132.7 |
| 5′ | 130.5 | 122.2 | 130.6 |
| 6′ | 121.4 | 115.4 | 122.9 |
| R$^3$ | - | OMe 55.9 | - |

**Table 3.24** ^{1}H NMR chemical shift assignment of 1,2-naphthoquinones **51, 54**

| Position | 51 (CDCl$_3$) | 54 (CDCl$_3$) |
|---|---|---|
| 3 | 5.65, s | 5.66, s |
| 5 | 8.18, dd, $J$ = 8.0, 2.0 Hz | 8.13, d, $J$ = 6.5 Hz |
| 6 | 7.67, td, $J$ = 7.5, 1.0 Hz | 7.63, t, $J$ = 7.5 Hz |
| 7 | 7.78, td, $J$ = 8.0, 1.5 Hz | 7.75, t, $J$ = 7.5 Hz |
| 8 | 8.06, dd, $J$ = 7.5, 1.0 Hz | 8.07, d, $J$ = 8.0 Hz |
| 2' | 7.07, dt, $J$ = 8.5, 3.5 Hz | 7.05, d, $J$ = 8.5 Hz |
| 3' | 7.62, dt, $J$ = 9.0, 3.0 Hz | 7.27, d, $J$ = 9.0 Hz |
| 5' | 7.62, dt, $J$ = 9.0, 3.0 Hz | 7.27, d, $J$ = 9.0 Hz |
| 6' | 7.07, dt, $J$ = 8.5, 3.5 Hz | 7.05, d, $J$ = 8.5 Hz |
| R$^3$ | - | Ethyl<br>CH$_2$: 2.68, quint, $J$ = 7.5 Hz<br>CH$_3$: 1.25, t, $J$ = 7.5 Hz |

**Table 3.25** ^{13}C NMR chemical shift assignment of 1,2-naphthoquinones **52, 53**

| Position | 52 (CDCl$_3$) | 53 (DMSO-$d_6$) |
|---|---|---|
| 3 | 5.35, s | 5.37, s |
| 5 | 8.04, t, $J$ = 8.0 Hz | 8.06, dd, $J$ = 7.5, 0.5 Hz |
| 6 | 7.75, td, $J$ = 8.0, 1.0 Hz | 7.77, dd, $J$ = 7.5, 1.5 Hz |
| 7 | 7.88, td, $J$ = 7.5, 1.0 Hz | 7.88, td, $J$ = 7.5, 1.5 Hz |
| 8 | 8.04, t, $J$ = 8.0 Hz | 8.03, dd, $J$ = 7.5, 1.0 Hz |
| 2′ | 7.26, m | 7.25, dt, $J$ = 9.0, 3.5 Hz |
| 3′ | 7.31, m | 7.73, m |
| 5′ | 7.31, m | 7.73, m |
| 6′ | 7.26, m | 7.25, dt, $J$ = 9.0, 3.5 Hz |
| | Cumyl | |
| | 7.22, dt, $J$ = 8.5, 3.0 Hz, 2H | NHCOCH$_3$ |
| R$^3$ | 7.38, dt, $J$ = 8.5, 3.0 Hz, 2H | NH: 10.16, s |
| | 7.18, t, $J$ = 7.5 Hz, 1H | CH$_3$: 2.07, s |
| | 1.68, s, 6H | |

**Table 3.26** ^{13}C NMR chemical shift assignment of 1,2-naphthoquinones **51-54**

| Position | 51 (CDCl$_3$) | 52 (CDCl$_3$) | 53 (DMSO-$d_6$) | 54 (CDCl$_3$) |
|---|---|---|---|---|
| 1 | 179.2 | 178.6 | 178.6 | 179.5 |
| 2 | 179.5 | 178.8 | 178.8 | 179.7 |
| 3 | 106.6 | 105.9 | 105.8 | 106.4 |
| 4 | 168.5 | 167.8 | 169.6 | 169.2 |
| 5 | 125.0 | 125.8 | 125.5 | 125.0 |
| 6 | 132.2 | 132.1 | 132.1 | 132.0 |
| 7 | 135.3 | 135.2 | 135.2 | 135.2 |
| 8 | 129.7 | 128.5 | 128.6 | 129.4 |
| 9 | 131.5 | 131.1 | 131.1 | 131.8 |
| 10 | 130.6 | 130.7 | 130.7 | 130.5 |
| 1′ | 151.5 | 150.1 | 147.2 | 150.3 |
| 2′ | 123.3 | 126.5 | 120.6 | 121.1 |
| 3′ | 133.7 | 128.2 | 122.2 | 129.7 |
| 4′ | 120.3 | 149.0 | 137.9 | 143.2 |
| 5′ | 133.7 | 128.2 | 122.2 | 129.7 |
| 6′ | 123.3 | 126.5 | 120.6 | 121.1 |
| R$^3$ | - | Cumyl<br>42.5<br>30.5<br>120.8 (2C)<br>128.6 (2C)<br>124.6 | NHCOCH$_3$<br>168.0<br>24.0 | Ethyl<br>28.4<br>15.8 |

**Table 3.27** ^{1}H NMR chemical shift assignment of 1,2-naphthoquinones **55-57**

| Position | 55 (CDCl$_3$) | 56 (CDCl$_3$) | 57 (CDCl$_3$) |
|---|---|---|---|
| 3 | 5.54, s | 5.56, s | 5.57, s |
| 5 | 8.17, d, $J$ = 7.5 Hz | 8.15, dd, $J$ = 8.0, 1.5 Hz | 8.16, d, $J$ = 7.5 Hz |
| 6 | 7.66, t, $J$ = 8.0 Hz | 7.64, td, $J$ = 8.0, 1.5 Hz | 7.65, t, $J$ = 7.5 Hz |
| 7 | 7.78, t, $J$ = 8.0 Hz | 7.77, td, $J$ = 7.5, 1.5 Hz | 7.77, t, $J$ = 7.5 Hz |
| 8 | 8.12, d, $J$ = 8.0 Hz | 8.10, dd, $J$ = 8.0, 1.0 Hz | 8.10, d, $J$ = 8.0 Hz |
| 3′ | - | 7.10, brs | 7.18, d, $J$ = 7.5 Hz |
| 4′ | 6.92, d, $J$ = 8.0 Hz | - | 7.04, d, $J$ = 8.0 Hz |
| 5′ | 7.18, t, $J$ = 7.5 Hz | 7.06, dd, $J$ = 8.5, 2.5 Hz | - |
| 6′ | 7.13, d, $J$ = 7.5 Hz | 6.94, d, $J$ = 8.0 Hz | 6.88, s |
| R$^1$ | Methyl 2.33, s | Methyl 2.34, s | Methyl 2.34, s |
| R$^2$ | Methyl 2.09, s | - | - |
| R$^3$ | - | Methyl 2.15, s | - |
| R$^4$ | - | - | Methyl 2.15, s |

**Table 3.28** $^{13}$C NMR chemical shift assignment of 1,2-naphthoquinones **55-57**

| Position | 55 (CDCl$_3$) | 56 (CDCl$_3$) | 57 (CDCl$_3$) |
|---|---|---|---|
| 1 | 179.5 | 179.5 | 179.5 |
| 2 | 179.7 | 179.7 | 179.7 |
| 3 | 106.1 | 105.9 | 106.0 |
| 4 | 168.4 | 168.4 | 168.3 |
| 5 | 125.0 | 125.0 | 125.0 |
| 6 | 132.0 | 132.6 | 132.0 |
| 7 | 135.3 | 135.3 | 135.3 |
| 8 | 129.5 | 129.5 | 129.5 |
| 9 | 131.6 | 131.6 | 131.6 |
| 10 | 130.7 | 130.6 | 130.7 |
| 1′ | 150.7 | 148.5 | 150.5 |
| 2′ | 128.7 | 129.6 | 126.7 |
| 3′ | 139.8 | 128.4 | 131.8 |
| 4′ | 118.9 | 136.9 | 126.7 |
| 5′ | 127.1 | 132.0 | 138.1 |
| 6′ | 128.5 | 121.0 | 127.9 |
| R$^1$ | Methyl 29.8 | Methyl 20.9 | Methyl 21.0 |
| R$^2$ | Methyl 12.3 | - | - |
| R$^3$ | - | Methyl 15.7 | - |
| R$^4$ | - | - | Methyl 15.4 |

**Table 3.29** ^{1}H NMR chemical shift assignment of 1,2-naphthoquinones **58, 59**

| Position | 58 (CDCl$_3$) | 59 (CDCl$_3$) |
| --- | --- | --- |
| 3 | 5.52, d, $J$ = 3.0 Hz | 5.67, s |
| 5 | 8.19, dd, $J$ = 8.0, 3.0 Hz | 8.14, dd, $J$ = 7.5, 1.0 Hz |
| 6 | 7.67, td, $J$ = 8.0, 3.0 Hz | 7.63, td, $J$ = 7.5, 1.0 Hz |
| 7 | 7.80, td, $J$ = 7.5, 2.0 Hz | 7.75, td, $J$ = 7.5, 1.5 Hz |
| 8 | 8.14, dd, $J$ = 7.5, 3.0 Hz | 8.07, dd, $J$ = 7.5, 1.0 Hz |
| 2′ | - | 6.92, d, $J$ = 3.0 Hz |
| 3′ | 7.13, brs | - |
| 4′ | 7.13, brs | - |
| 5′ | 7.13, brs | 6.95, d, $J$ = 8.5 Hz |
| 6′ | - | 6.87, dd, $J$ = 8.5, 2.5 Hz |
| R$^1$ | Methyl 2.18, d, $J$ = 3.0 Hz | - |
| R$^2$ | - | Methyl 2.28, s |
| R$^3$ | - | Methyl 2.28, s |
| R$^5$ | Methyl 2.18, d, $J$ = 3.0 Hz | - |

**Table 3.30** ^{1}H NMR chemical shift assignment of 1,2-naphthoquinones **48, 60**

| Position | 48 (CDCl$_3$) | 60 (CDCl$_3$) |
|---|---|---|
| 3 | 5.46, s | 5.69, s |
| 5 | 8.14, dd, $J$ = 7.5, 1.5 Hz | 8.06, dd, $J$ = 8.0, 1.0 Hz |
| 6 | 7.64, td, $J$ = 7.5, 1.0 Hz | 7.64, td, $J$ = 7.5, 1.0 Hz |
| 7 | 7.73, td, $J$ = 8.0, 1.5 Hz | 7.76, td, $J$ = 7.5, 1.0 Hz |
| 8 | 7.92, dd, $J$ = 8.0, 0.5 Hz | 8.15, d, $J$ = 7.5 Hz |
| 2′ | - | 6.76, s |
| 3′ | 7.15, m | - |
| 4′ | 7.08, m | 6.95, s |
| 5′ | 7.30, td, $J$ = 7.5, 1.5 Hz | - |
| 6′ | 7.12, m | 6.76, s |
| R$^2$ | Benzyl 3.92, s, 2H 7.04, dd, $J$ = 7.5, 1.5 Hz 7.35, td, $J$ = 7.0, 1.5 Hz, 2H 7.12, m | Methyl 2.34, s |
| R$^4$ | - | Methyl 2.34, s |

**Table 3.31** ^{13}C NMR chemical shift assignment of 1,2-naphthoquinones **48, 58, 59-60**

| Position | 48 (CDCl$_3$) | 58 (CDCl$_3$) | 59 (CDCl$_3$) | 60 (CDCl$_3$) |
|---|---|---|---|---|
| 1 | 179.3 | 179.5 | 179.6 | 179.6 |
| 2 | 179.4 | 179.8 | 179.7 | 179.8 |
| 3 | 106.2 | 105.2 | 106.4 | 106.5 |
| 4 | 168.1 | 167.1 | 169.2 | 169.1 |
| 5 | 125.0 | 125.0 | 125.0 | 125.0 |
| 6 | 132.1 | 132.1 | 131.9 | 132.0 |
| 7 | 135.2 | 135.4 | 135.2 | 135.2 |
| 8 | 129.5 | 129.7 | 129.4 | 129.5 |
| 9 | 131.5 | 131.4 | 131.8 | 131.9 |
| 10 | 130.6 | 130.9 | 130.6 | 130.6 |
| 1′ | 150.6 | 149.2 | 150.4 | 152.4 |
| 2′ | 133.4 | 129.6 | 118.3 | 118.8 |
| 3′ | 127.4 | 130.0 | 139.2 | 140.5 |
| 4′ | 126.6 | 127.0 | 135.5 | 128.6 |
| 5′ | 132.0 | 130.0 | 131.2 | 140.5 |
| 6′ | 122.1 | 129.6 | 122.1 | 118.8 |
| R$^1$ | Benzyl<br>36.7<br>139.2<br>129.0 (2C)<br>128.6 (2C)<br>125.0 | 16.0 | - | - |
| R$^2$ | - | - | 20.0 | 21.4 |
| R$^3$ | - | - | 19.3 | - |
| R$^4$ | | - | - | 21.4 |
| R$^5$ | | 16.0 | - | - |

Table 3.32 ^{1}H NMR chemical shift assignment of 1,2-naphthoquinones **61-63**

| Position | 61 (DMSO-$d_6$) | 62 (DMSO-$d_6$) | 63 (DMSO-$d_6$) |
|---|---|---|---|
| 3 | 6.35, s | 6.25, s | 6.34, s |
| 5 | 8.03, d, $J$ = 7.5 Hz | 7.99, dd, $J$ = 8.0, 2.0 Hz | 8.02, d, $J$ = 7.5 Hz |
| 6 | 7.61, t, $J$ = 7.5 Hz | 7.55, td, $J$ = 7.5, 1.5 Hz | 7.69, td, $J$ = 7.5, 2.0 Hz |
| 7 | 7.71, t, $J$ = 8.0 Hz | 7.64, td, $J$ = 7.5, 1.5 Hz | 7.59, t, $J$ = 7.5 Hz |
| 8 | 7.44, d, $J$ = 8.0 Hz | 7.04, d, $J$ = 7.5 Hz | 7.44, d, $J$ = 7.5 Hz |
| 2′ | 6.94, brs | - | - |
| 3′ | - | 6.58, d, $J$ = 2.5 Hz | 6.74, s |
| 4′ | - | - | 6.74, s |
| 5′ | 6.94, brs | 7.09, d, $J$ = 8.0 Hz | 6.74, s |
| 6′ | 7.05, brs | 6.51, dd, $J$ = 8.0, 2.0 Hz | - |
| R[1] | - | OH 9.97, s | OMe 3.95, s |
| R[2] | OMe 3.82, s | - | - |
| R[3] | OH - | OMe 3.66, s | OMe 3.95, s |

**Table 3.33** $^{13}$C NMR chemical shift assignment of 1,2-naphthoquinones **61-63**

| Position | 61 (DMSO-$d_6$) | 62 (DMSO-$d_6$) | 63 (DMSO-$d_6$) |
|---|---|---|---|
| 1 | 179.2 | 179.1 | 179.8 |
| 2 | 179.8 | 180.0 | 180.4 |
| 3 | 126.8 | 128.9 | 126.9 |
| 4 | 155.6 | 160.4 | 156.6 |
| 5 | 129.4 | 129.3 | 130.0 |
| 6 | 130.7 | 130.4 | 131.3 |
| 7 | 135.1 | 135.0 | 135.2 |
| 8 | 129.5 | 130.5 | 130.1 |
| 9 | 134.8 | 135.1 | 135.8 |
| 10 | 131.9 | 131.3 | 132.2 |
| 1′ | 127.3 | 128.0 | 127.2 |
| 2′ | 112.5 | 154.4 | 106.4 |
| 3′ | 147.8 | 99.6 | 148.4 |
| 4′ | 148.4 | 157.6 | 137.5 |
| 5′ | 121.6 | 107.7 | 148.4 |
| 6′ | 115.6 | 116.1 | 106.4 |
| R$^1$ | - | OMe 55.4 | 56.6 |
| R$^2$ | OMe 55.8 | - | - |
| R$^3$ | - | - | - |
| R$^5$ | - | - | 56.6 |

**Table 3.34** ^{1}H & ^{13}C NMR chemical shift assignment of 1,2-naphthoquinones **64-65**

| Position | 64 (DMSO-$d_6$) | | 65 (Acetone-$d_6$) |
| --- | --- | --- | --- |
| | $\delta$H (ppm) | $\delta$C (ppm) | $\delta$H (ppm) |
| 1 | - | 179.6 | - |
| 2 | - | 179.7 | - |
| 3 | 5.98, s | 103.2 | 5.98, s |
| 4 | - | 168.9 | - |
| 5 | 8.11, dd, $J$ = 8.0, 1.5 Hz | 124.9 | 8.03, d, $J$ = 7.5 Hz |
| 6 | 7.58, td, $J$ = 7.5, 1.5 Hz | 131.7 | 7.69, t, $J$ = 7.5 Hz |
| 7 | 7.69, td, $J$ = 8.0, 1.5 Hz | 135.2 | 7.82, t, $J$ = 7.5 Hz |
| 8 | 7.86, dd, $J$ = 7.5, 1.0 Hz | 129.3 | 7.96, d, $J$ = 7.5 Hz |
| 9 | - | 132.1 | - |
| 10 | - | 130.5 | - |
| OR | OMe 4.02, s | OMe 57.0 | OEt CH$_2$: 4.34, quint, $J$ = 7 Hz<br>CH$_3$: 1.55, t, $J$ = 7 Hz |

## 3.5 Anti α-glucosidase activity evaluation of synthesized compounds

In 2002, Ahn *et al.*[36] reported a series of C4-substituted 1,2-naphthoquinone including 4-amino-1,2-naphthoquinone, 4-methoxy-1,2-naphthoquinone and 4-methlphenylamino-1,2-naphthoquinone. Those compounds showed detriment to the PTP1B inhibitory activity. In contrast, 4-substituted phenyl-1,2-naphthoquinone and 4-alkyl-1,2-naphthoquinone demonstrated good potency. However, 4-phenylamino-1,2-

naphthoquinone, 4-alkylamino-1,2-naphthoquinone and 4-phenoxy-1,2-naphthoquinone derivatives have not been evaluated. Importantly, there is no previous reports investigated the function of phenylamino, alkylphenylamino, alkylamino and phenoxy on $\alpha$-glucosidase inhibitory activity. Therefore, 1,2-naphthoquinones **24-65** were synthesized and elucidated. Moreover, the 4-alkoxy-1,2-naphthoquinone derivatives were also synthesized for comparison. All of the compounds, except **43**, were evaluated for their ability to inhibit $\alpha$-glucosidase enzyme and the results are exhibited in **Tables 3.35-3.39**. The structure activity correlation was also first discovered.

**Table 3.35 α**-Glucosidase inhibitory activity data of 4-phenylamino-1,2-naphthoquinone

derivatives **24-34**
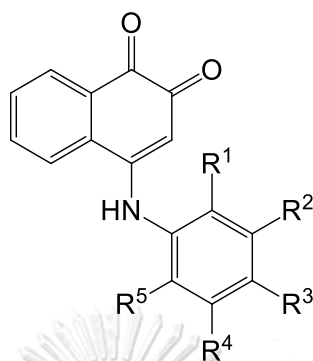


| Compounds | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $IC_{50}$ (μM) |
|-----------|-------|-------|-------|-------|-------|----------------|
| 24[a] | H | H | H | H | H | 105.63 ± 2.14 |
| 25 | COOH | H | H | H | H | 35.95 ± 2.99 |
| 26[a] | H | H | OMe | H | H | Not active |
| 28[a] | H | H | F | H | H | 86.06 ± 8.96 |
| 29 | H | H | $NO_2$ | H | H | 14.59 ± 2.27 |
| 30[a] | H | H | OPh | H | H | 34.62 ± 3.22 |
| 31 | H | H | $CH_2CH_2OH$ | H | H | 56.04 ± 3.39 |
| 32 | H | H | COOH | H | H | 58.54 ± 4.83 |
| 33 | Me | H | Me | H | H | >200 (64.67%)[b] |
| 34 | Me | H | H | H | Me | Not active |
| Acarbose | | | | | | 93.63 ± 0.49 |

a: not completely soluble in the activity test

b: % inhibition at 200μM

4-Phenylamino-1,2-naphthoquinone **(24)** and 4-substituted phenylamino-1,2-naphthoquinone derivatives **25-34** were tested for **α**-glucosidase inhibitory activity. The inhibitory activity data is exhibited in **Table 3.35**. Compounds **25, 28-32** showed better inhibition activity compared with **24** and better than acarbose. Meanwhile, **26,**

**33** and **34** displayed not good ($IC_{50} > 200$ μM) and no activity (not active). These results suggested that the presence of substituent have some effect on the anti-**α**-glucosidase activity. The electron donating group, including methoxy and methyl groups, decreased the activity. Whereas, the electron withdrawing group, including carboxylic acid, fluoro and nitro groups improved the activity. From the results, **29** containing strong withdrawing substituent showed an improving inhibitory activity compared to **26** which comprising strong donating group. The introduction of the nitrogen linker influenced the substituent effect on the phenyl ring to the quinone ring.[49] **29** possessing stronger withdrawing substituent illustrated significantly improving the potency compared to **28** which possessing weaker withdrawing substituent. Adding one more phenyl ring (**30**) resulted in enhancing the activity due to the more **π**-**π** interaction between test sample and many aromatic amino acid residues in the enzyme.[58, 59] **31** presented good activity suggested that hydroxy group might improve the activity. The formation of hydrogen bond between test sample and **α**-glucosidase might be a reason responsible for the high activity of **31**.[58-60] There are some previous studies reported that carboxylic acid establish two hydrogen bonds with amino acid residues of **α**-glucosidase.[61-63] Therefore **25**, **32** exerted potential inhibitory activity besides the withdrawing electron effect of carboxylic acid group.

To further discover the role of electron density of the nitrogen on the inhibitory activity, 4-alkylphenylamino-1,2-naphthoquinones were synthesized and tested against α-glucosidase enzyme. The results are presented in **Table 3.36**.

**Table 3.36 α**-Glucosidase inhibitory activity data of 4-alkylphenylamino-1,2-naphthoquinone derivatives **44**-**46**
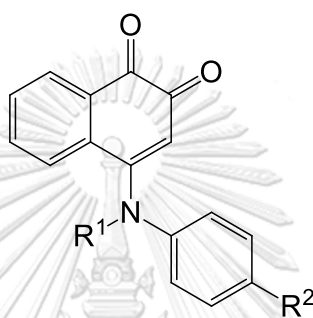


| Compounds | $R^1$ | $R^2$ | $IC_{50}$ (µM) |
|-----------|-------|-------|----------------|
| 44 | Me | H | 32.30 ± 3.30 |
| 45[a] | Me | OMe | 81.60 ± 2.76 |
| 46[a] | Et | H | 109.33 ± 1.40 |
| Acarbose | | | 93.63 ± 0.49 |

a: not completely soluble in the activity test.

The introduction of alkyl group increased the electron density on the nitrogen linker. However, the inhibitory activity of **44** ($IC_{50}$: 32.30 ± 3.30 µM) is significantly enhancing compared to **24** ($IC_{50}$: 105.63 ± 2.14 µM) which did not contain alkyl group. According Hatfield et al.[46] studies, the lack of labile hydrogen atom on nitrogen linker led to less activity. In addition, methoxy substituted phenyl-1,2-naphthoquinone (compound **45**) showed limited activity as predicted.

Several previous investigations showed that increasing the hydrophobicity led to the improving inhibitory activity by enhancing the van der Waals interaction or hydrophobic effect between tested inhibitors and many non-polar residues in the enzyme.[58, 59, 64] It seemed like hydrophobic effect might have critical influence on inhibitory activity. On the other hand, Hari Moorthy and coworkers[65] indicated that the optimal molecular hydrophilic surface was important for $\alpha$-glucosidase inhibitors and prerequisite. Indeed, the biological activities of long chain molecules amplified gradually with enlarging chain length up to a crucial peak, beyond which the activity moderated. This phenomenon was called "a cut-off effect"[66]. Exceedingly hydrophobic substances would advance self-interaction in aqueous solution rather than interacts with the enzyme.

Conversely, the trend was observed such that the longer alkyl chain compound exhibited less potent enzyme inhibition. **46** containing longer alkyl chain demonstrated less activity than **44**. This result suggested that the less hydrophilicity than the optimum conditions of **45** might be responsible for the reduction of the activity.

In order to gain further insight into how hydrophobic-hydrophilic balance affected the inhibition of substances, 4-alkylphenylamino-1,2-naphthoquinone derivatives containing two to six carbon atoms were prepared. $\alpha$-Glucosidase inhibitory activity data of those derivatives and acarbose are illustrated in **Table 3.37**.

**Table 3.37 α**-Glucosidase inhibitory activity of 4-alkylamino-1,2-naphthoquinone derivatives

**35-42**
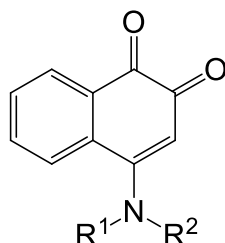


| Compounds | $R^1$ | $R^2$ | $IC_{50}$ (µM) |
|---|---|---|---|
| 35 | H | H | 39.91 ± 2.72 |
| 36[a] | H | Et | 61.08 ± 1.71 |
| 37[a] | H | Propyl | 156.93 ± 3.50 |
| 38[a] | H | Isobutyl | 187.62 ± 14.20 |
| 39[a] | H | Butyl | >200 (47.61%)[b] |
| 40[a] | H | Cyclohexyl | 122.15 ± 6.36 |
| 41[a] | H | Benzyl | >200 (40.56%)[b] |
| 42 | Et | Et | 62.68 ± 2.62 |
| Acarbose | | | 93.63 ± 0.49 |

a: not completely soluble in the activity test.

b: % inhibition at 200µM

The activity decreased progressively when escalating the alkyl chain length. Longer alkyl chain might increased the size of the substances and prevented tested inhibitors binding with **α**-glucosidase.

Comparison of the activities of 4-ethylamino-1,2-naphthoquinone **(36)** and 4-diethylamino-1,2-naphthoquinone **(42)** might elucidate the role of hydrophobic-hydrophilic balance effect on the inhibitory activity. **42** containing two positive

inductive electron group causing a strong imine production which can be seen in the NMR spectrum. **37** only encompassed one positive inductive electron group. However, the inhibition of these two inhibitors were comparable. The hydrophobic effect might account for this result. Furthermore, **41** possessed one phenyl ring which provided more π-π interactions showed not good activity indicated that "cut off effect" might be an important aspect that is responsible for the loss of inhibition. Nevertheless, the lack of 4-methylamino-1,2-naphthoquinone making it hard to completely interpret the hydrophobic-hydrophilic effect on to the inhibitory activity. Since there were other factors influencing the inhibition of tested compounds, the hydrophobic effect and "cut off effect" were not further investigated.

Generally, heteroatom like nitrogen atom bound to the quinone skeleton played an important role in α-glucosidase inhibitory activity. However, the electron lone pair on nitrogen was labile and could easily conjugate with quinone ring which made the inhibition decreasing. For this reason, 4-phenoxy 1,2-naphthoquinone derivatives were prepared and tested against α-glucosidase. Oxygen atom having higher electronegativity than nitrogen therefore the electron lone pair on oxygen was less labile. 4-phenoxy-1,2-naphthoquinone analogues were predicted to display radically increasing α-glucosidase inhibition. The inhibition data of 4-(monosubstituted)phenoxy and 4-(disubstituted)phenoxy-1,2-naphthoquinone are presented in **Tables 3.38** and **3.39**.

**Table 3.38 α**-Glucosidase inhibitory activity data of 4-(monosubstituted)phenoxy-1,2-

naphthoquinone derivatives **47-54**
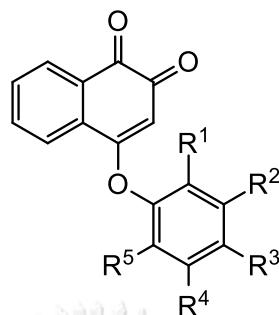


| Compounds | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $IC_{50}$ (μM) |
|-----------|-------|-------|-------|-------|-------|----------------|
| 47[a] | H | H | H | H | H | 53.20 ± 7.98 |
| 48[a] | Benzyl | H | H | H | H | 20.64 ± 1.45 |
| 49[a] | H | H | OMe | H | H | 66.59 ± 1.46 |
| 50 | H | H | Cl | H | H | 42.76 ± 2.39 |
| 51[a] | H | H | Br | H | H | 137.23 ± 12.9 |
| 52[a] | H | H | Cumyl | H | H | 39.38 ± 3.61 |
| 53 | H | H | $NHCOCH_3$ | H | H | 47.38 ± 1.74 |
| 54[a] | H | H | Et | H | H | 129.22 ± 6.05 |
| Acarbose | | | | | | 93.63 ± 0.49 |

a: not completely soluble in the activity test
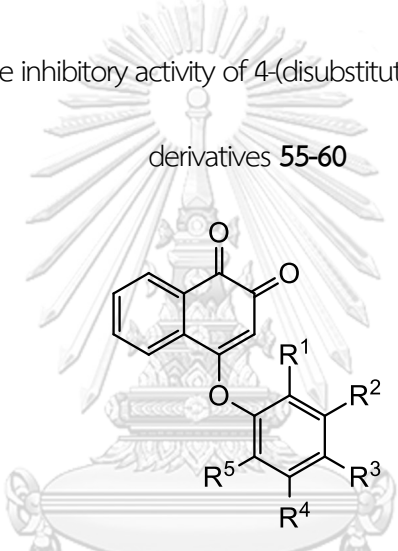
4-Phenoxy-1,2-naphthoquinone bearing one substituent on the phenyl ring
exhibited better **α**-glucosidase inhibitory activity compared to 4-phenylamino-1,2-
naphthoquinone scaffolds as hypothesized **(Figure 3.12)**. **47** presented twice stronger
inhibition than **24**. **49** showed drastic improvement in the activity whereas **26** did not
represent any inhibition at 200μM.

**24**
$IC_{50} = 105.63 \mu M$

**47**
$IC_{50} = 53.20 \mu M$

**26**
Not active

**49**
$IC_{50} = 66.59 \mu M$

**33**
$IC_{50} > 200 \mu M$ (65%)

**56**
$IC_{50} > 200 \mu M$ (88%)

**34**
Not active

**58**
$IC_{50} > 200 \mu M$ (36%)

**Figure 3.9** α-Glucosidase inhibitory activity of **24**, **26**, **33**, **34**, **47**, **49**, **56**, **58**
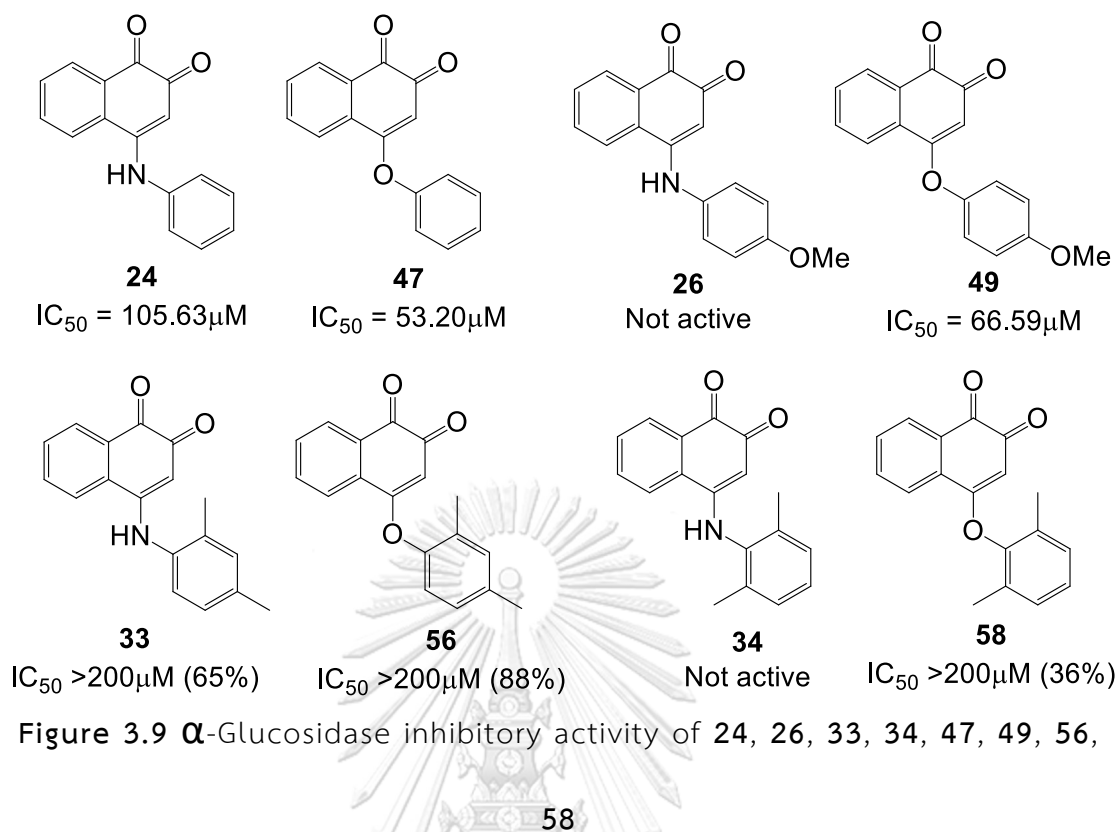
The same trend was observed in which compounds containing electron donating substituents demonstrated poor activity and in contrast, compounds bearing electron withdrawing substituents showed increasing the potency compared to **47** which did not contain any substituent. Compounds **49** and **54**, which bear electron donating substituents including methoxy and ethyl groups, showed diminishing activity compared to **47**. Compound **50** with chlorine atom exhibited higher activity compared to compound **47**. However, in 4-(disubstituted) phenoxy-1,2-naphthoquinone scaffolds, all of substances presented substantially decreasing inhibitory activity **(Table 3.39)**. Nonetheless, the effect of donating substituents on the phenyl ring to the activity was proved.

In 4-(monosubstituted)phenoxy-1,2-naphthoquinone series, **52** and **48** possessed more $\pi$-$\pi$ interaction with $\alpha$-glucosidase due to the introduction of another phenyl ring have more effective activity than **47**.[67] In addition, the presence of acetylamino group (**53**) advanced the activity by adding more hydrogen bond with the enzyme. On the contrary, bromine substituent on the phenyl ring significantly impaired the inhibition. However, the reason account for this was not clear.

**Table 3.39** $\alpha$-Glucosidase inhibitory activity of 4-(disubstituted)phenoxy-1,2-naphthoquinone derivatives **55-60**



| Compounds | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $IC_{50}$ ($\mu$M) |
|---|---|---|---|---|---|---|
| 55[a] | Me | Me | H | H | H | 125.86 ± 10.44 |
| 56 | Me | H | Me | H | H | 119.11 ± 8.04 |
| 57[a] | Me | H | H | Me | H | 103.38 ± 4.34 |
| 58[a] | Me | H | H | H | Me | >200 (36.15%)[d] |
| 59[a] | H | Me | Me | H | H | 43.67 ± 2.11 |
| 60[a] | H | Me | H | Me | H | 137.75 ± 6.36 |
| Acarbose | | | | | | 93.63 ± 0.49 |

a: not completely soluble in the activity test

d: % inhibition at 200μM

In 4-(disubstituted)-phenoxy-1,2-naphthoquinone series, **60** bearing two methyl groups at meta position of phenyl ring showed weaker activity than **59** with two methyl groups at *meta* and *para* position of phenyl ring suggested that substituent at *para* position was more favorable than *meta* position. The activity of 2,3-substituted phenoxy derivative **(55)** and 2,5-substituted phenoxy derivative **(57)** were similar. These two compounds contained two methyl group at *meta* position of phenyl ring. The substituents at *meta* position was the main factor affected to the activity rather than the different position in 3D structure. Additionally, two methyl groups at *ortho* position caused a steric effect with the quinone ring leading to the changes in 3D structure of **34** and **58**. As a consequence, the activity decreased significantly.

However, 4-phenyl-1,2-naphthoquinone derivatives **61-63** exhibited not good activity **(Table 3.40)**. It seemed like phenoxy derivatives of 1,2-naphthoquinone showed better activities than phenyl derivatives.

Table 3.40 $\alpha$-Glucosidase inhibitory activity of 4-phenyl-1,2-naphthoquinone derivatives **61-63**

| Compounds | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $IC_{50}$ ($\mu$M) |
|-----------|-------|-------|-------|-------|-------|-------|
| 61 | H | OMe | OH | H | H | 156.55 ± 2.12 |
| 62[a] | OMe | H | OH | H | H | 147.74 ± 2.24 |
| 63 | H | OMe | OH | OMe | H | 97.35 ± 4.42 |
| Acarbose | | | | | | 93.63 ± 0.49 |

4-Amino-1,2-naphthoquinone **(35)** exhibited three times better activity than acarbose encouraged to investigate the activity of small substance. 4-methoxy-1,2-

naphthoquinone **(64)** was prepared and tested against **α**-glucosidase. **SSNQ** was also

tested for comparison. The results are exhibited in **Figure 3.12**.



**SSNQ**
$IC_{50} = 75.97 \mu M$

**35**
$IC_{50} = 39.91 \mu M$

**64**
$IC_{50} = 11.39 \mu M$
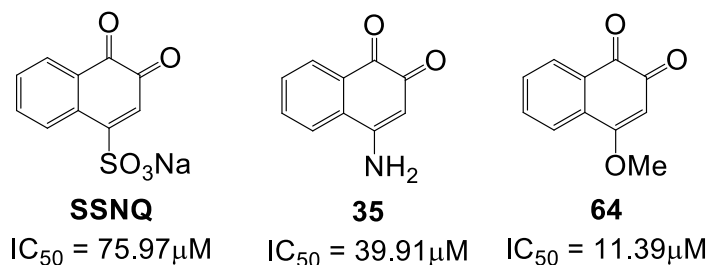
**Figure 3.10 α**-Glucosidase inhibitory activity of **SSNQ, 35, 64**

The trend was observed that higher electronegativity of heteroatom (O > N >

S) displayed better activity (**64 > 35 > SSNQ**), respectively. In previous study by Hati *et*

*al.*[68], the molecular docking and modelling revealed that small compounds bind

deeply and strongly to the binding site of **α**-glucosidase. This may responsible for

abnormally high activity of those compounds.

# CHAPTER 4

# CONCLUSION

In summary, five series of 1,2-naphthoquinone derivatives including 4-phenylamino, 4-alkylamino, 4-alkylphenylamino, 4-phenoxy and others 1,2-NQs were synthesized as enzyme $\alpha$-glucosidase inhibitors. Nevertheless, certain derivatives were proven to be potential $\alpha$-glucosidase inhibitors and better than acarbose. Forty-one derivatives of 1,2-naphthoquinone were prepared, evaluated for anti-$\alpha$-glucosidase activity and elucidated their structure activity relationship. Summary of all synthesized compounds are organized below.

24

25: R$^1$=COOH
26: R$^3$=OMe
27: R$^3$=Me
28: R$^3$=F
29: R$^3$=NO$_2$
30: R$^3$=OPh
31: R$^3$=CH$_2$CH$_2$OH
32: R$^3$=COOH
33: R$^1$=R$^3$=Me
34: R$^1$=R$^5$=Me

44: R$^1$=Me
45: R$^1$=Et
46: R$^1$=Me; R$^2$=OMe

64: R = Me
65: R = Et

35

36: R$^2$=Et
37: R$^2$=Propyl
38: R$^2$=Butyl
39: R$^2$=Isobutyl
40: R$^2$=Cyclohexyl
41: R$^2$=Benzyl
42: R$^1$=R$^2$=Et
43: R$^2$=CH$_2$CH$_2$CH$_2$Br

47

48: R$^1$=Benzyl
49: R$^3$=OMe
50: R$^3$=Cl
51: R$^3$=Br
52: R$^3$=Cumyl
53: R$^3$=NHCOCH$_3$
54: R$^3$=Et

55: R$^1$=R$^2$=Me
56: R$^1$=R$^3$=Me
57: R$^1$=R$^4$=Me
58: R$^1$=R$^5$=Me
59: R$^2$=R$^3$=Me
60: R$^2$=R$^4$=Me

61

62

63
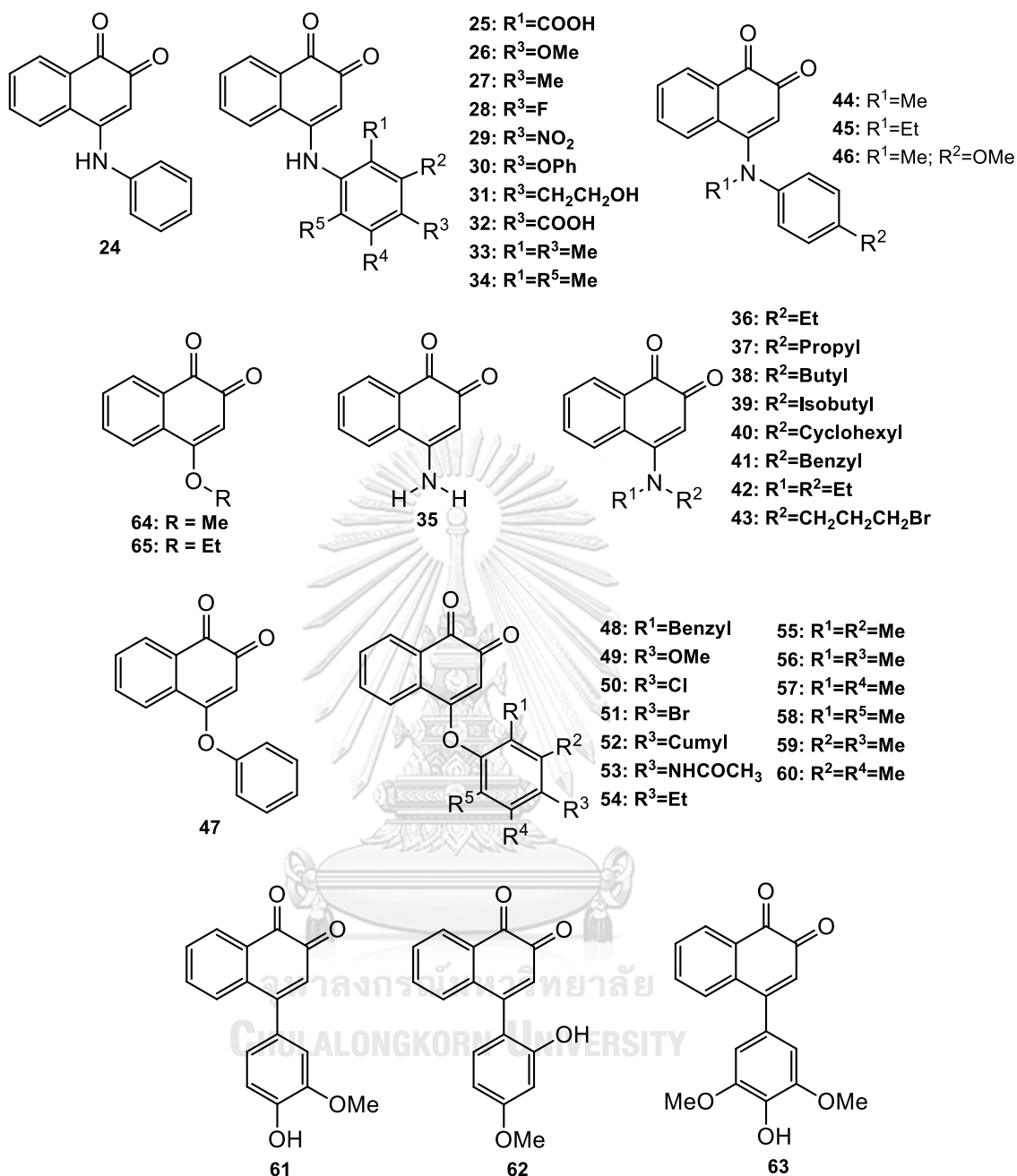
The structure activity relationships indicated that multiple factors affected to the α-glucosidase inhibition including π-π interaction, hydrophobic interaction and hydrogen bonding with the enzyme. The presence of electron withdrawing substituents on the phenyl ring and heteroatom linker bound to the quinone skeleton was important. However, depending on the structures of tested inhibitors, any of these

effects might be dominant and influence the inhibitory activity and further investigate was necessary. Those compounds showed the best activity among each group are presented below.
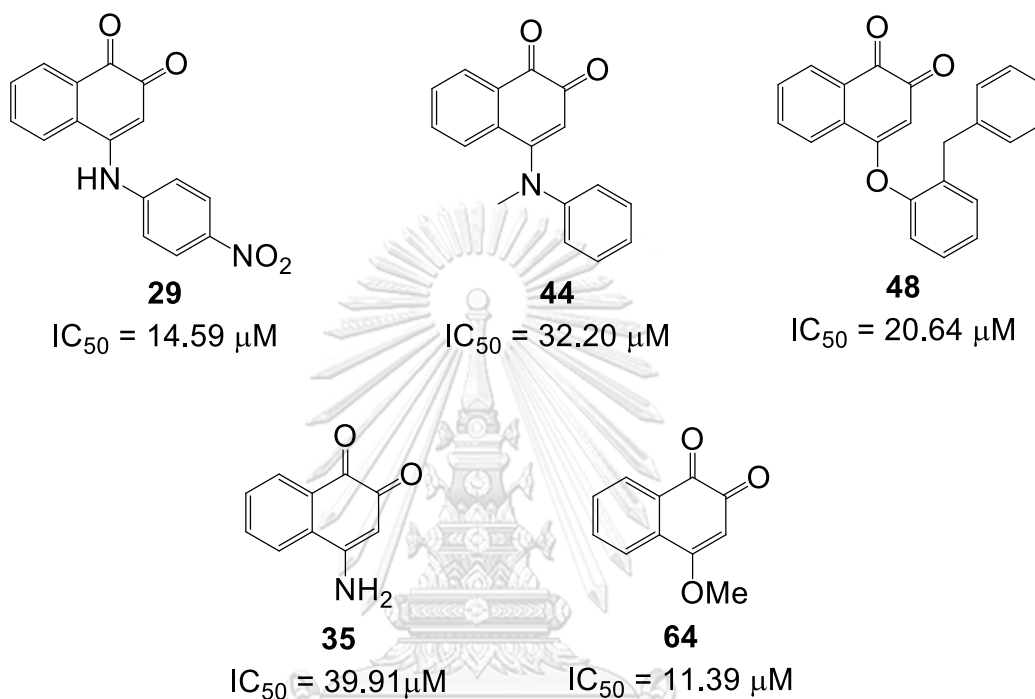


**29**
$IC_{50}$ = 14.59 $\mu$M

**44**
$IC_{50}$ = 32.20 $\mu$M

**48**
$IC_{50}$ = 20.64 $\mu$M

**35**
$IC_{50}$ = 39.91$\mu$M

**64**
$IC_{50}$ = 11.39 $\mu$M

4-(Electron withdrawing substituted)phenylamino and 4-(electron withdrawing substituted)phenoxy-1,2-naphthoquinone scaffolds displayed increasing the activity compared to 4-phenylamino and 4-phenoxy 1,2-naphthoquinone. 4-(electron donating substituted) phenylamino, 4-alkyl (electron donating substituted) phenylamino and 4-(electron donating substituted) phenoxy 1,2-naphthoquinone scaffolds showed significantly decreasing the activity compared to 4-phenylamino and 4-phenoxy-1,2-naphthoquinone. Different position of substituents on the phenyl ring played a crucial role on the activity. Adding methyl group on the nitrogen linker increased activity. Longer alkyl chain 4-alkylphenylamino and 4-alkylamino-1,2-naphthoquinone

displayed poor activity. Small molecule compounds showed excellent activity and the reason need more research to interpret.

There are eighteen new compounds, among them, four new compounds **25**, **29**, **48** and **52** exhibited superior $\alpha$-glucosidase inhibitory activities with $IC_{50}$ of 35 µM, 14 µM, 21 µM and 39 µM. All of those four compounds showed two to three-fold better activity than current anti-diabetes drug, acarbose. All of the synthesized compounds were first investigated their $\alpha$-glucosidase inhibition. Moreover, the effect of heteroatom linker bound to the quinone skeleton, the effect of electron donating substituents and electron withdrawing substituents on the $\alpha$-glucosidase were first discovered.

**Suggestion for future work**

Further investigation such as molecular docking to support structure-activity correlation analysis in this study is worth noted. Reaction kinetic study on the mechanism of $\alpha$-glucosidase inhibition of potent compounds should be discovered. Importantly, 4-(electron withdrawing substituted)phenylamino and 4-(electron withdrawing substituted)phenoxy drastically enhanced the inhibitory activity as well as solubility and stability. Hence, other type of 4-phenylamino and 4-phenoxy-1,2-naphthoquinones containing electron withdrawing substituents on the phenyl ring should be unremitting research. Furthermore, hydrogen bond interaction with the enzyme proved to improve the $\alpha$-glucosidase inhibition of 1,2-naphthoquinone

derivatives. This result suggested extensive exploration on H-bonding donor interaction of functional groups. Finally, small molecule compounds displayed favorable $\alpha$-glucosidase inhibitory activity, opening a new route of development this kind of compound as potential $\alpha$-glucosidase inhibitors.

APPENDIX
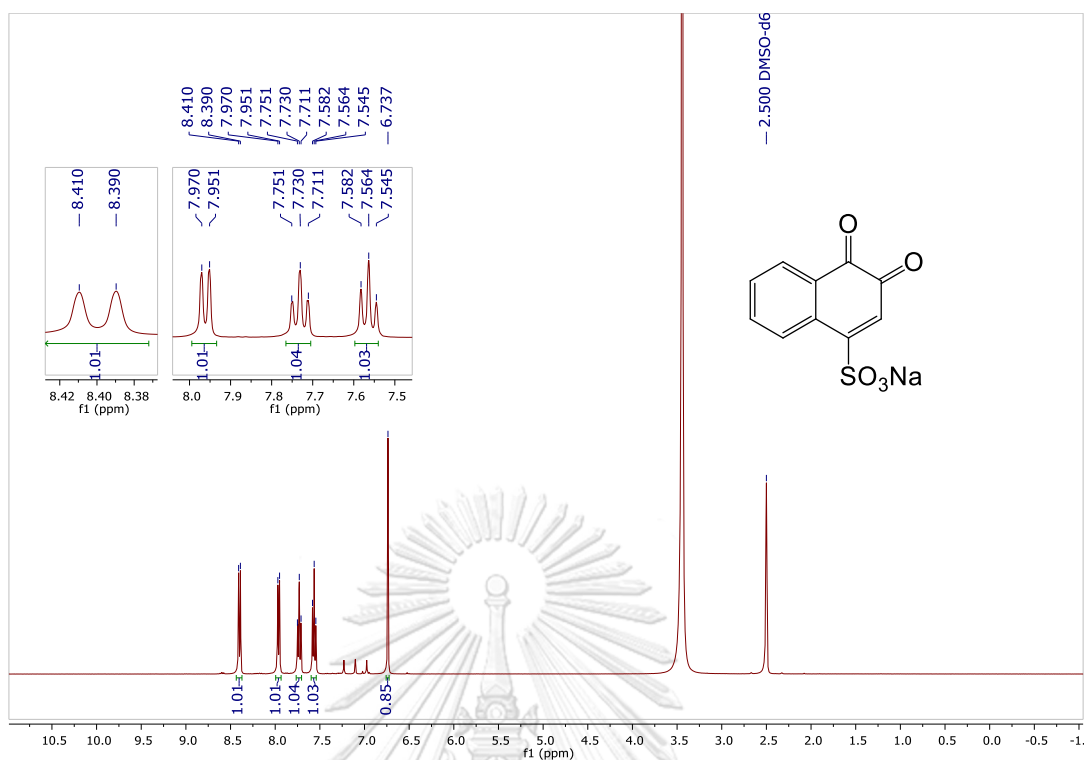
**Figure A.1** $^{1}$H-NMR (DMSO-$d_6$, 400 MHz) of **SSNQ.**
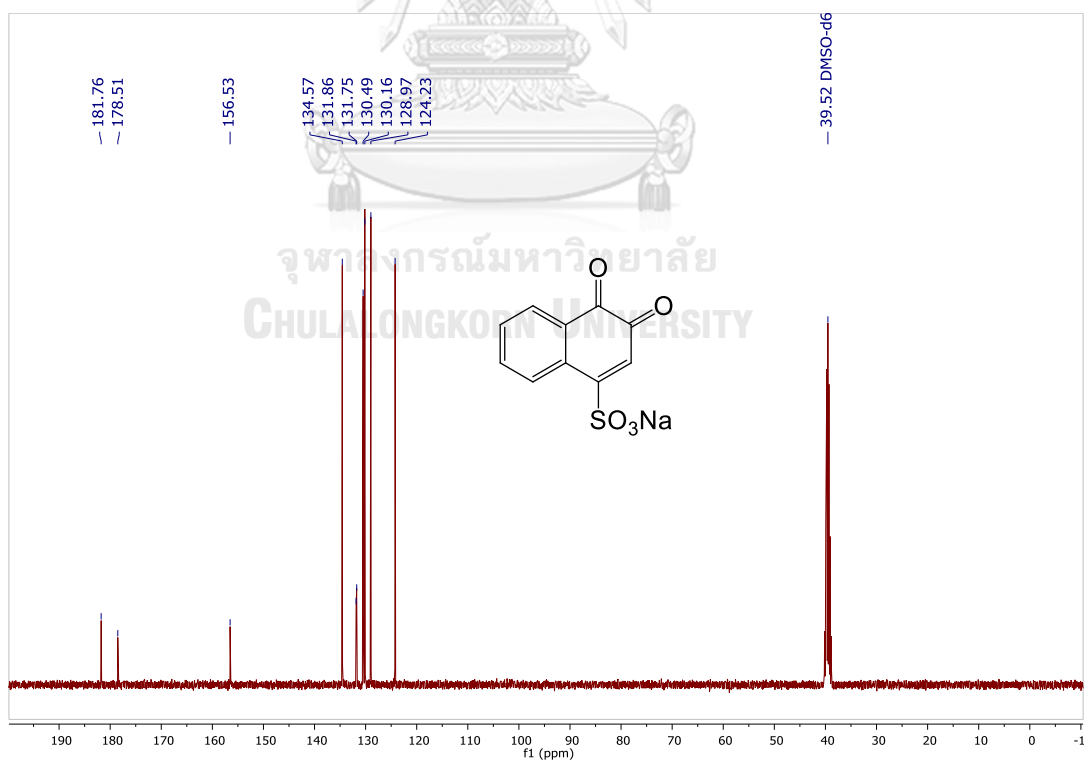


**Figure A.2** $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) of **SSNQ.**
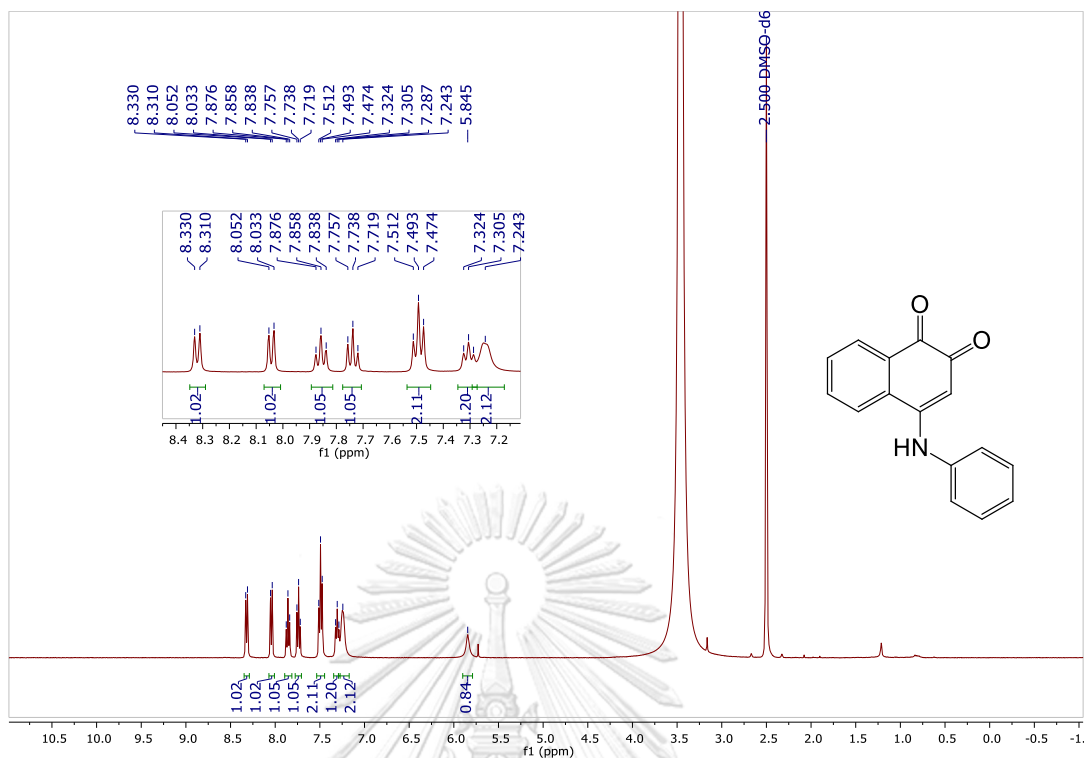
**Figure A.3** $^1$H-NMR (DMSO-$d_6$, 400 MHz) of **24.**
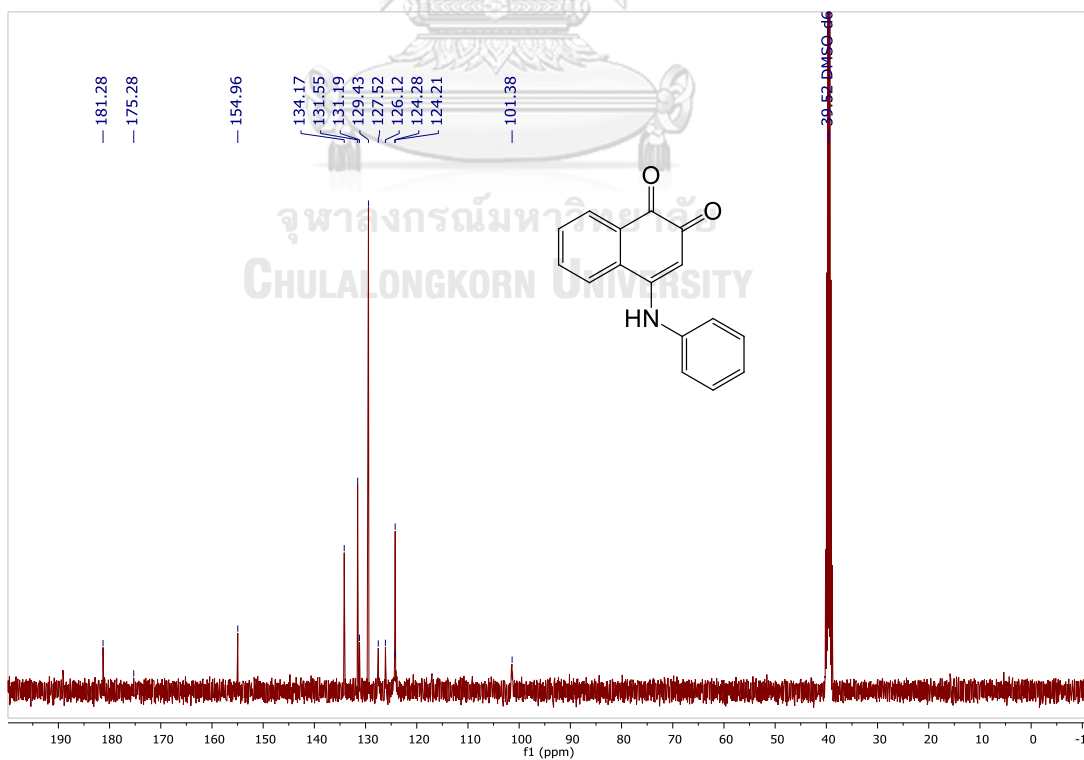


**Figure A.4** $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) of **24.**
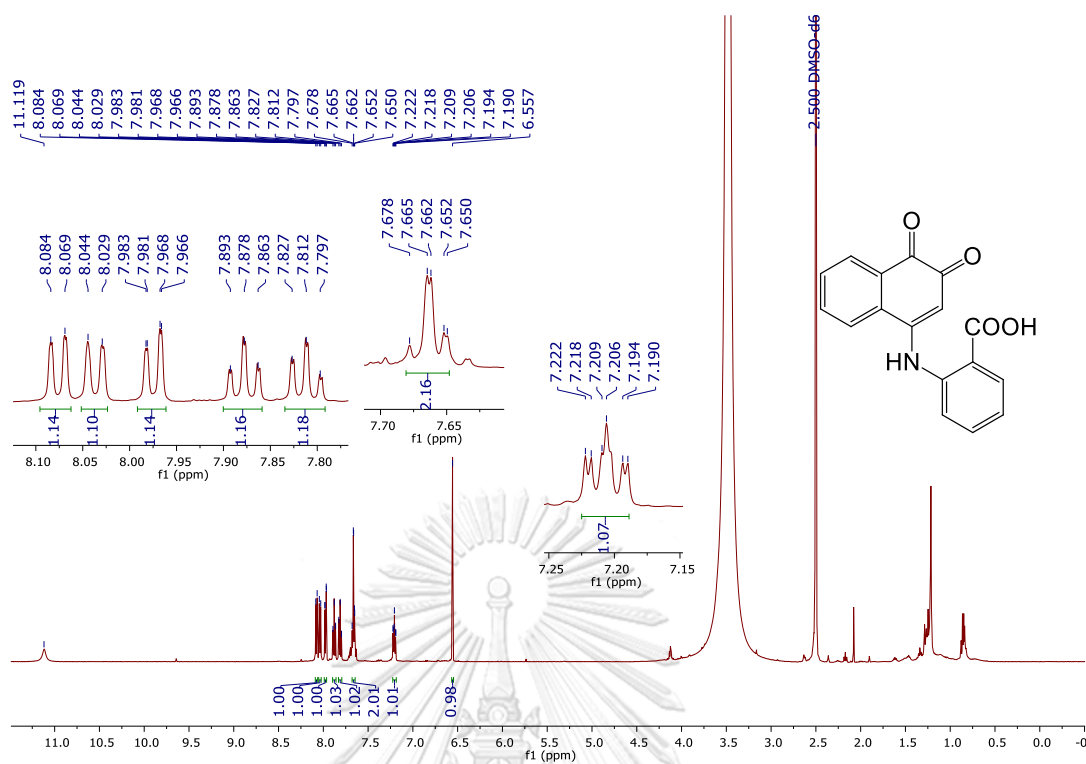
**Figure A.5** $^{1}$H-NMR (DMSO-$d_6$, 500 MHz) of **25.**
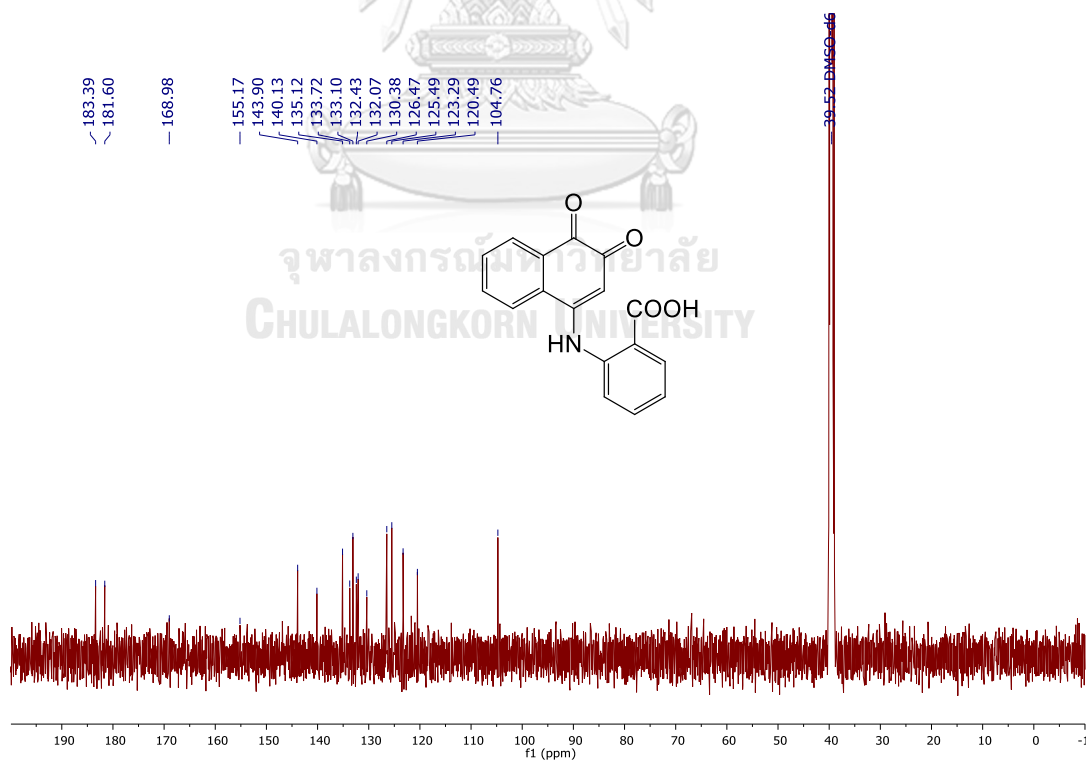


**Figure A.6** $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) of **25.**

## Generic Display Report
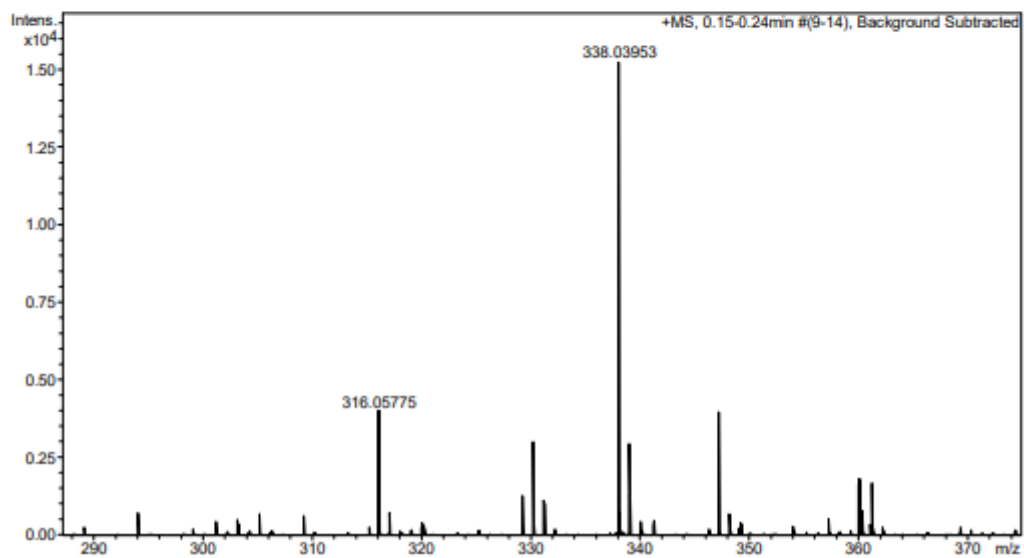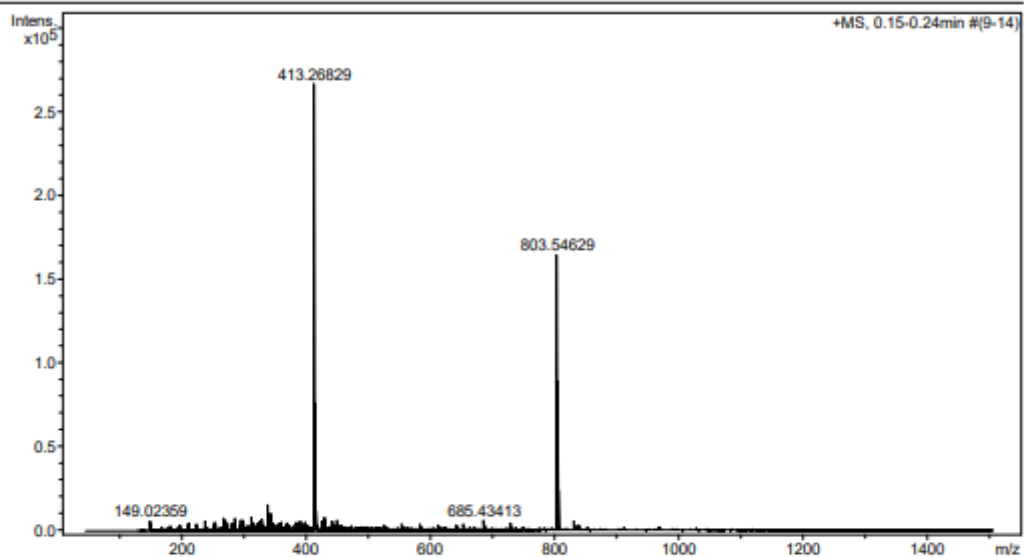
**Figure A.7** HRMS of **25.**
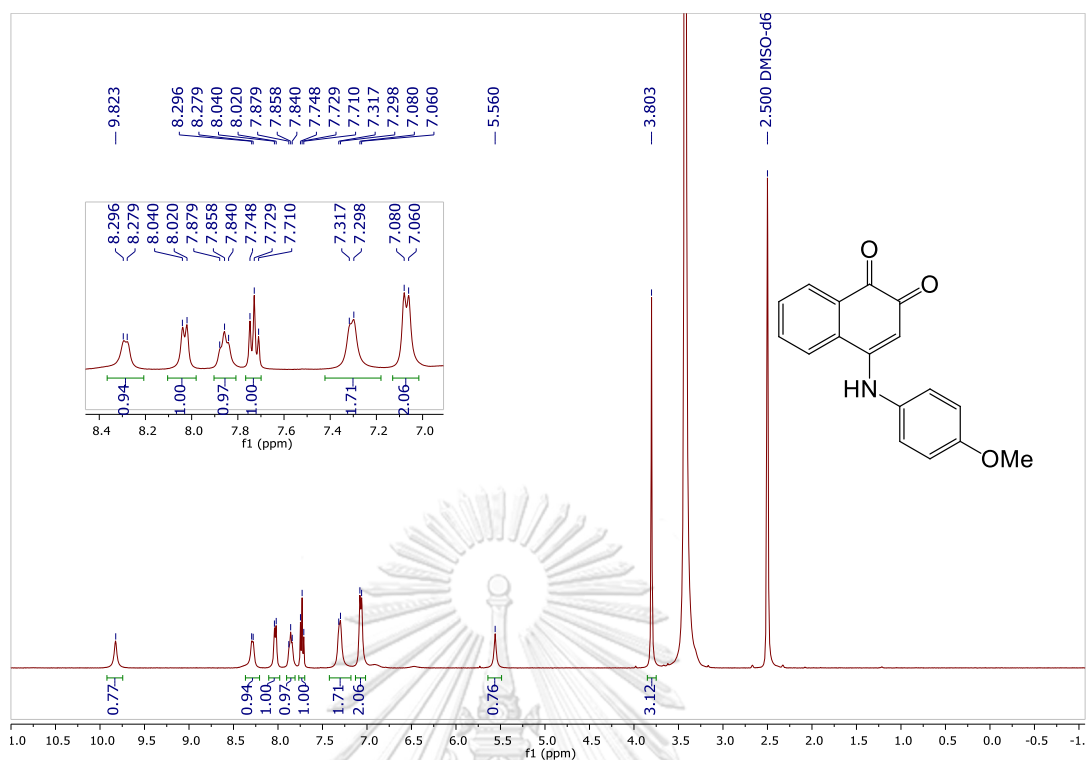
**Figure A.8** $^1$H-NMR (DMSO-$d_6$, 400 MHz) of **26.**
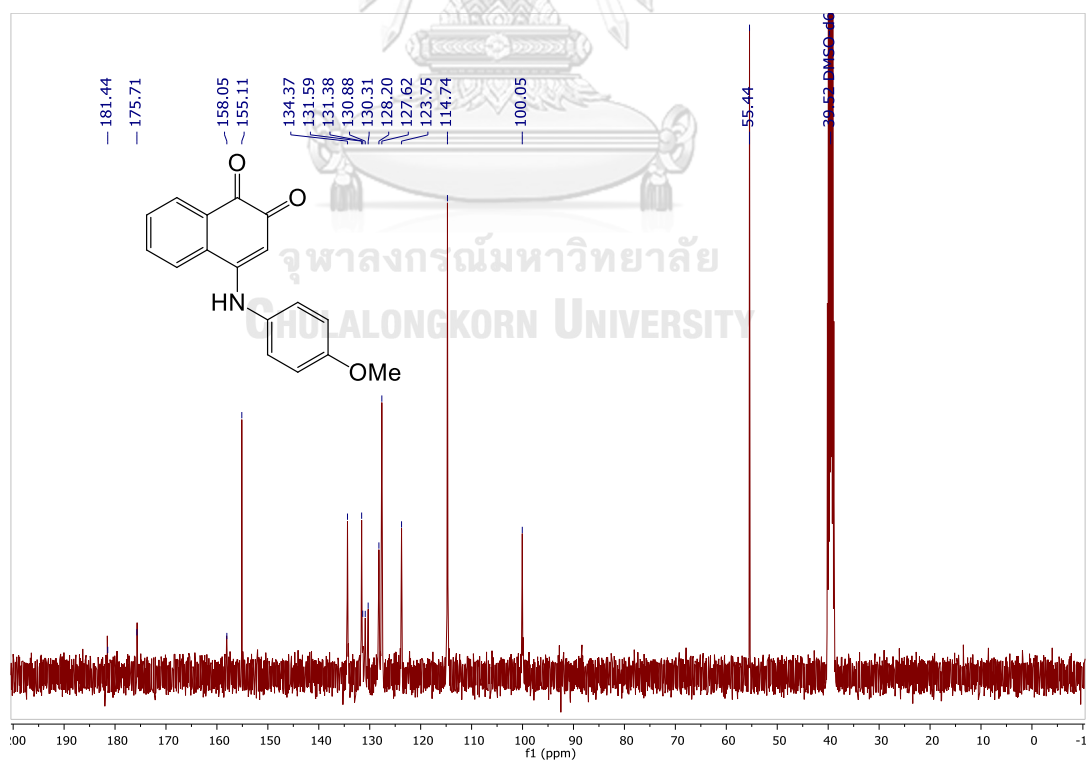


**Figure A.9** $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) of **26.**
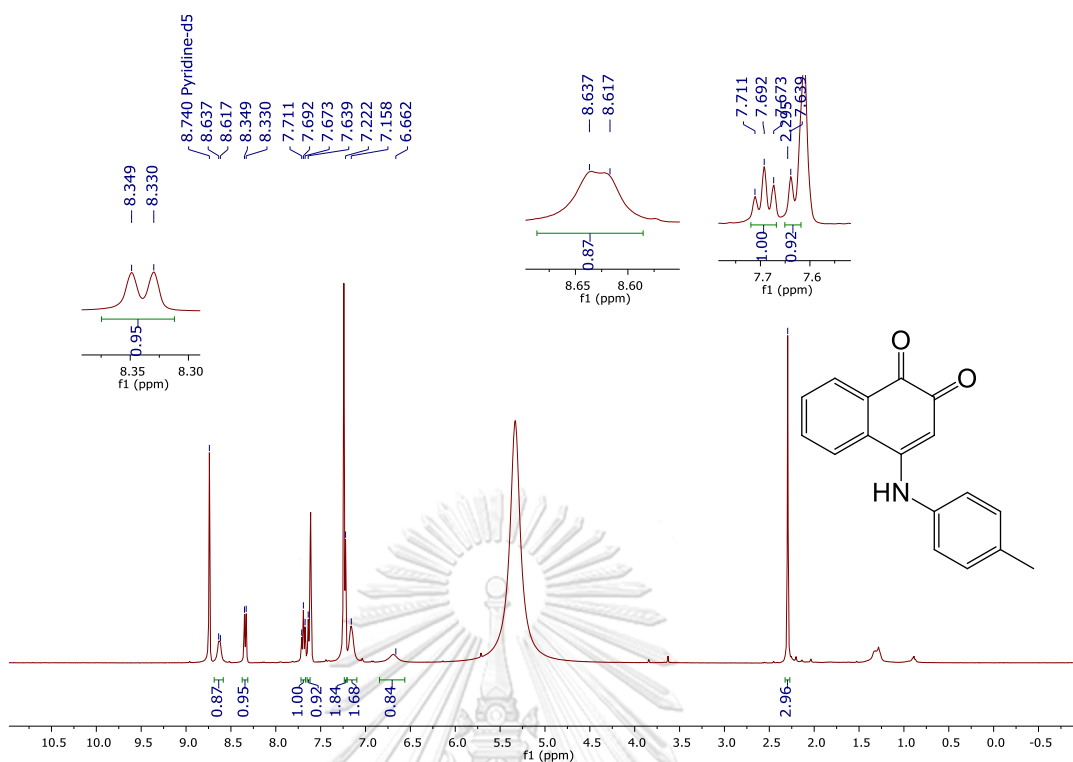
**Figure A.10** $^1$H-NMR (DMSO-$d_6$, 400 MHz) of **27.**
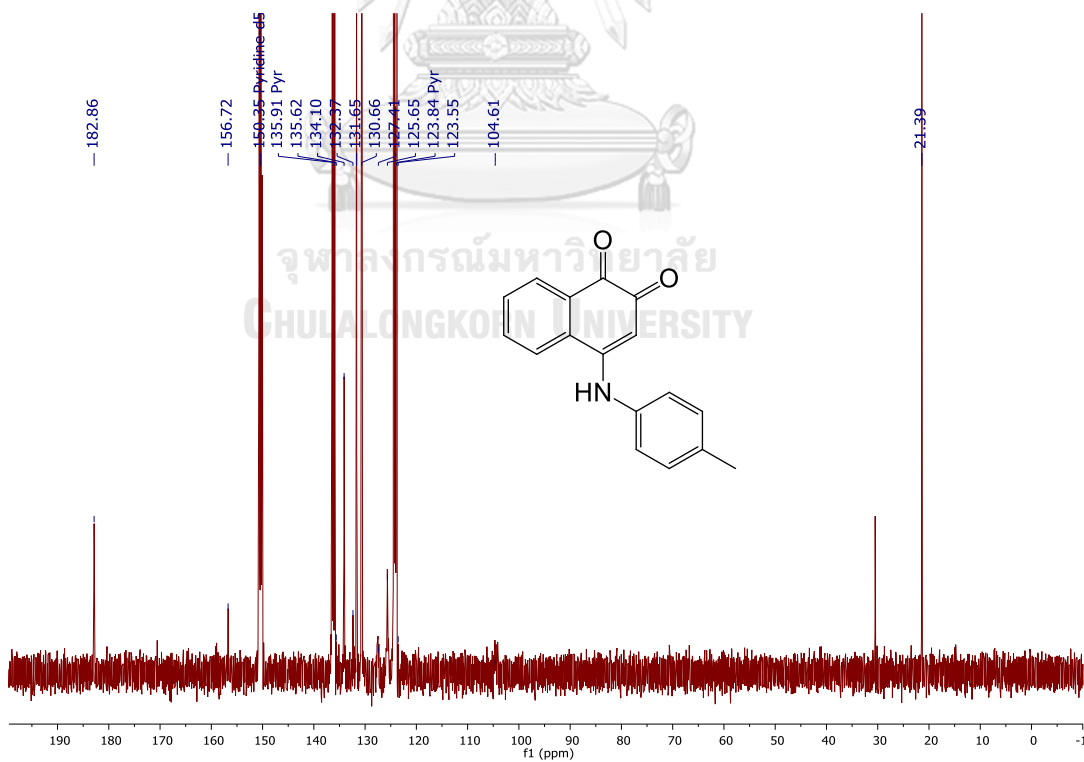


**Figure A.11** $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) of **27.**
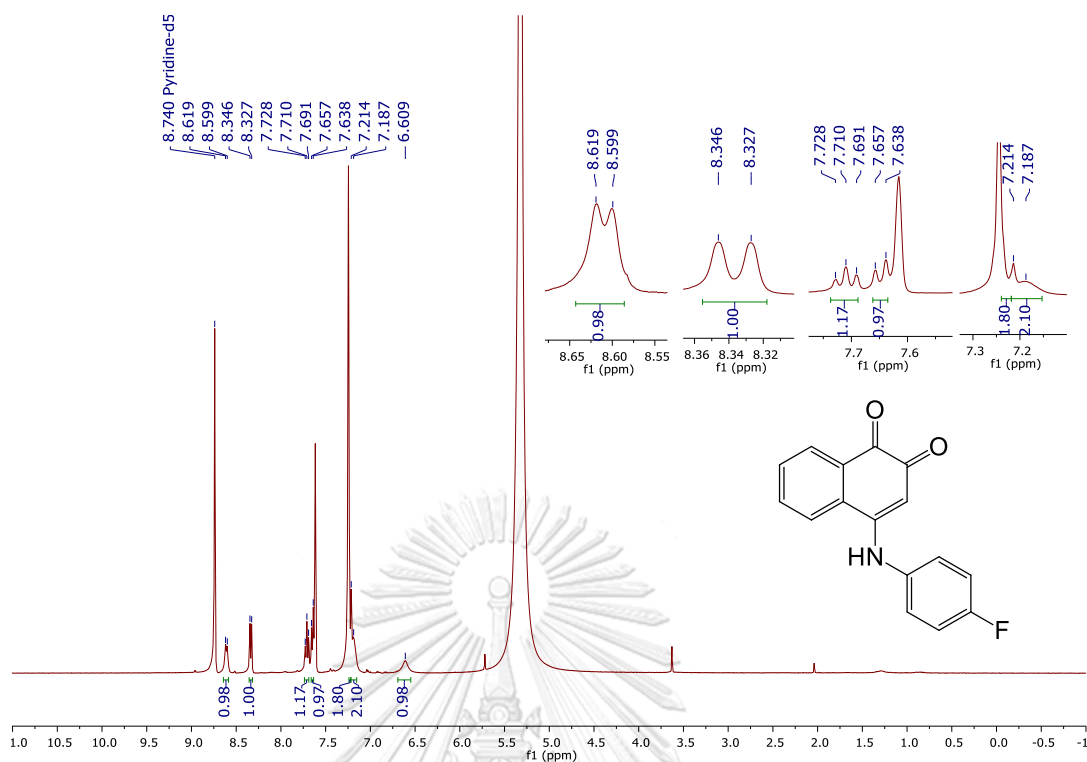
**Figure A.12** ^{1}H-NMR (DMSO-$d_6$, 400 MHz) of **28.**
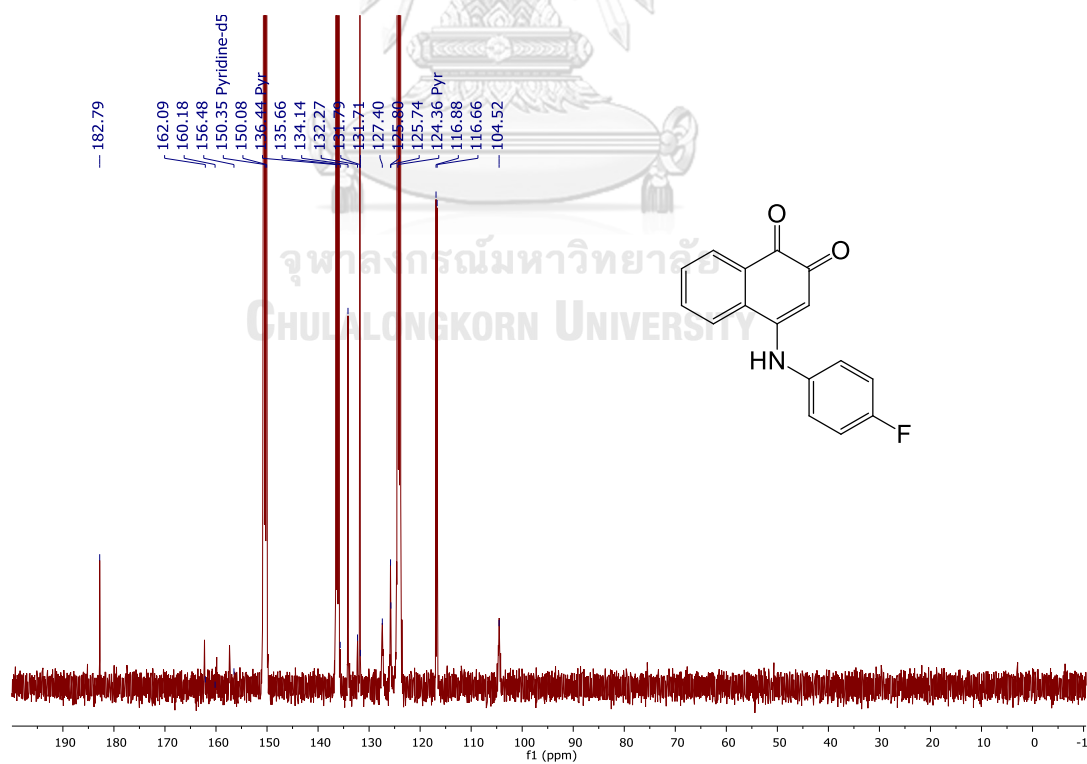


**Figure A.13** ^{13}C-NMR (DMSO-$d_6$, 100 MHz) of **28.**
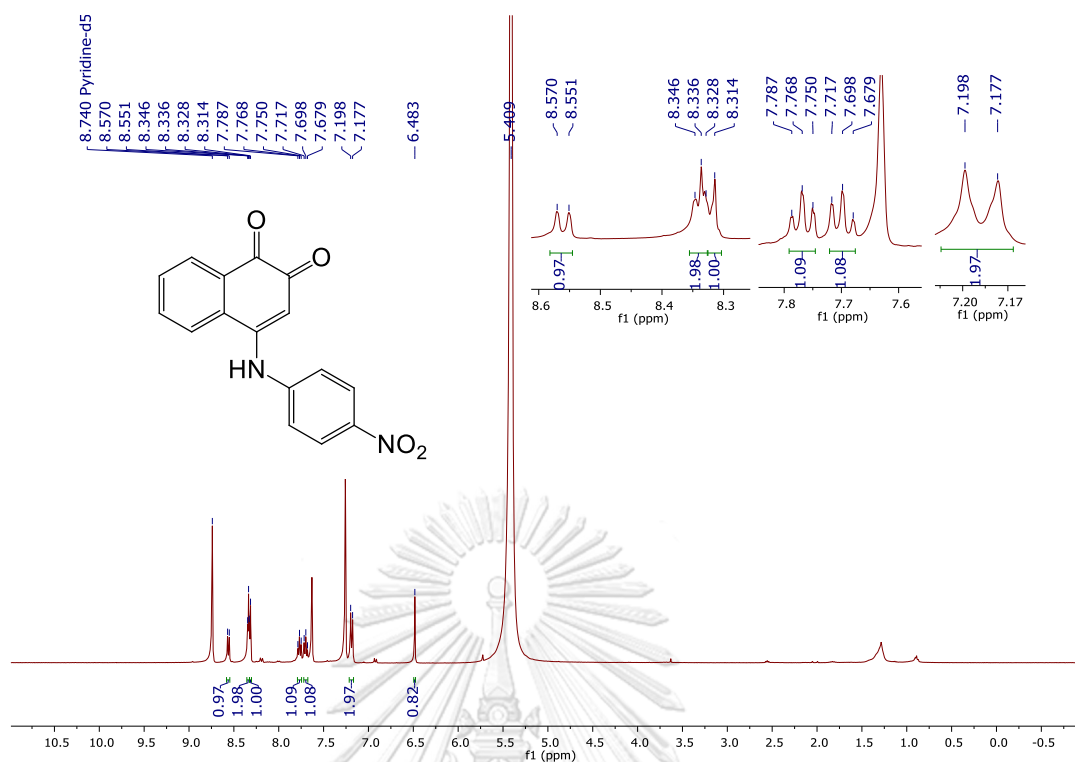
Figure A.14 $^1$H-NMR (DMSO-$d_6$, 400 MHz) of **29.**
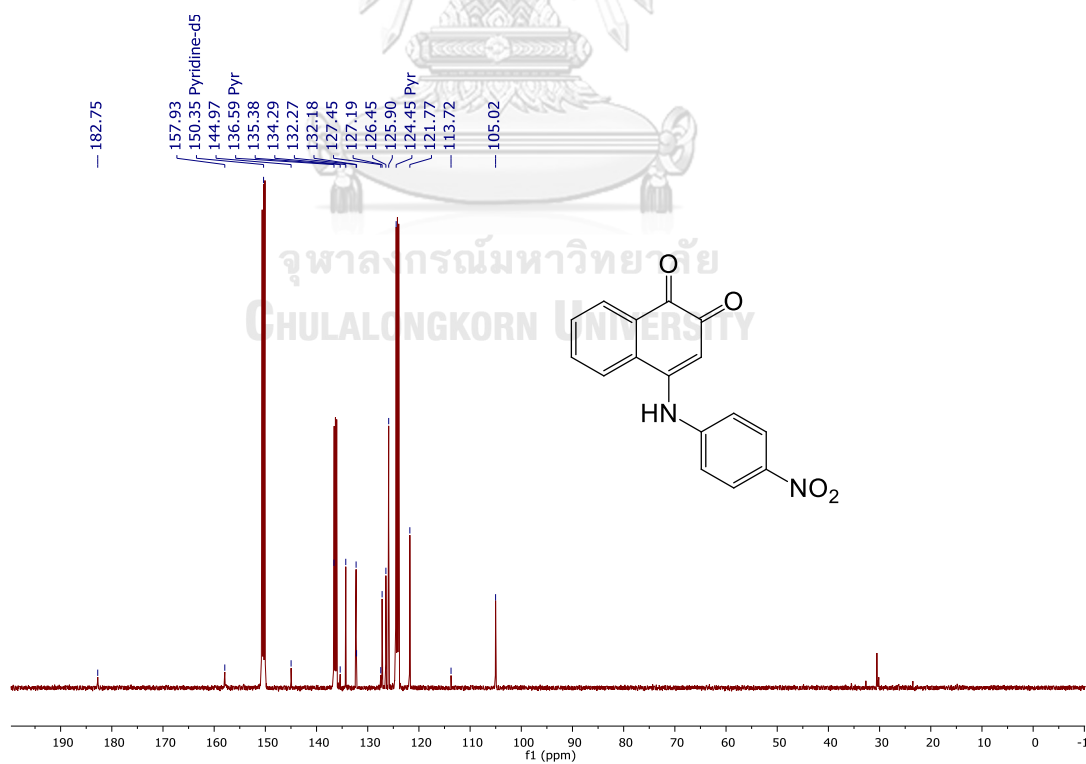


Figure A.15 $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) of **29.**

## Generic Display Report
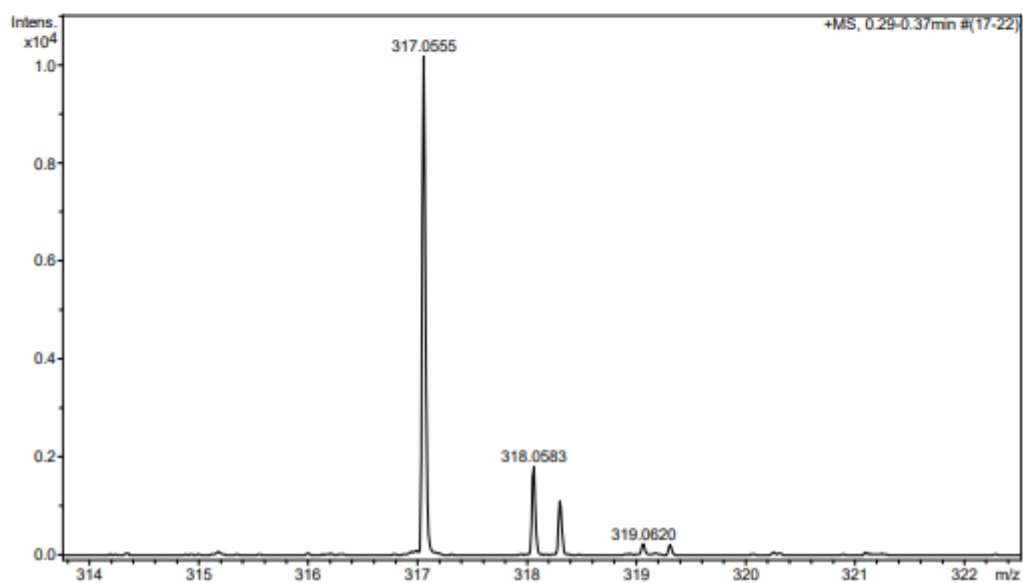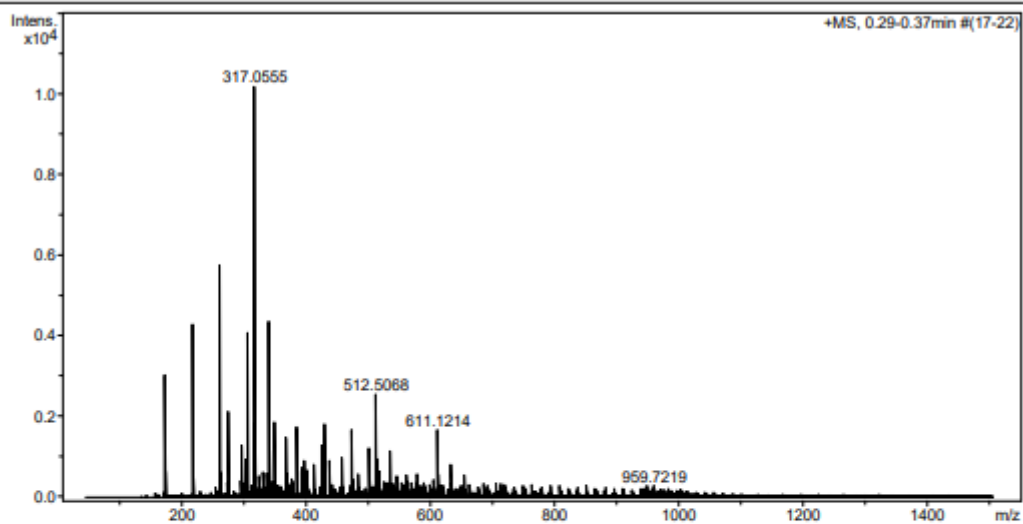
Figure A.16 HRMS of 29.
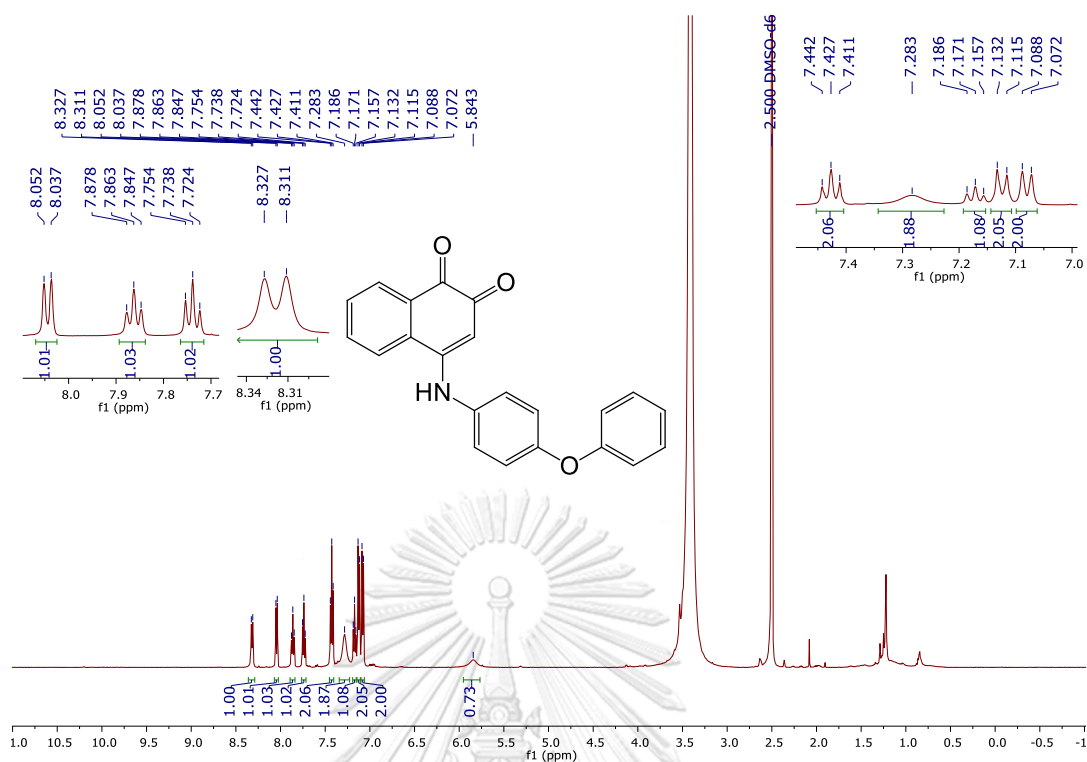
**Figure A.17** $^1$H-NMR (DMSO-$d_6$, 500 MHz) of **30.**
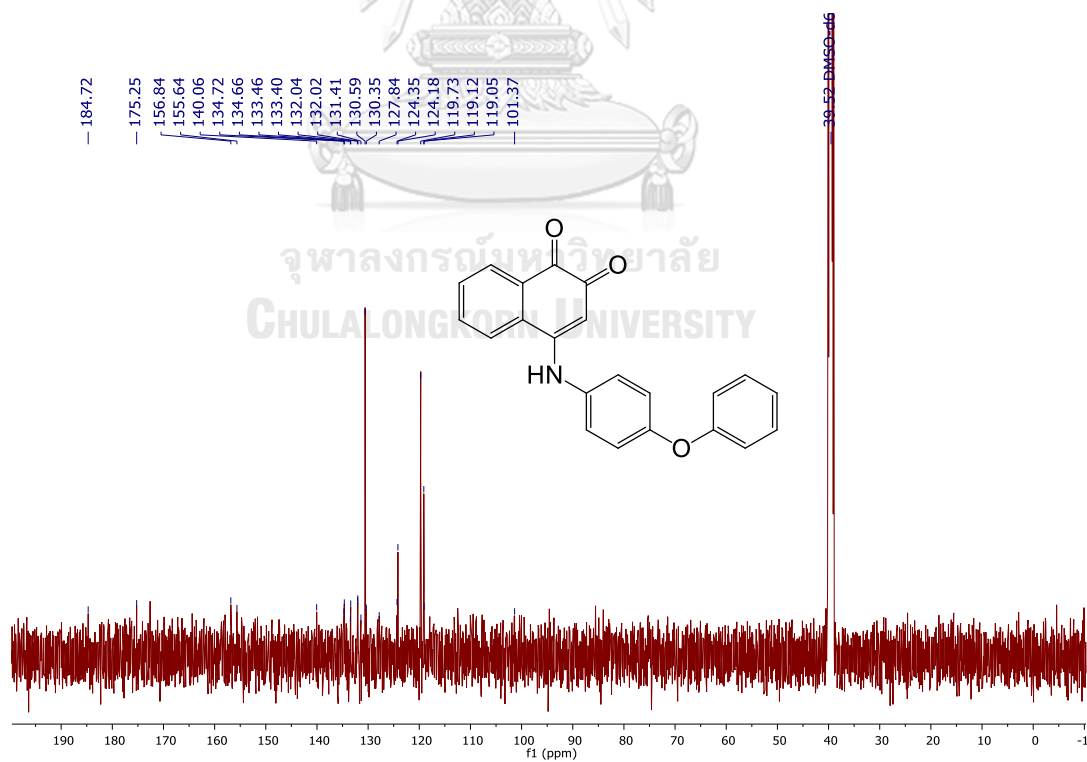


**Figure A.18** $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) of **30.**

## Generic Display Report

Figure A.19 HRMS of 30.

**Figure A.20** $^1$H-NMR (DMSO-$d_6$, 500 MHz) of **31.**
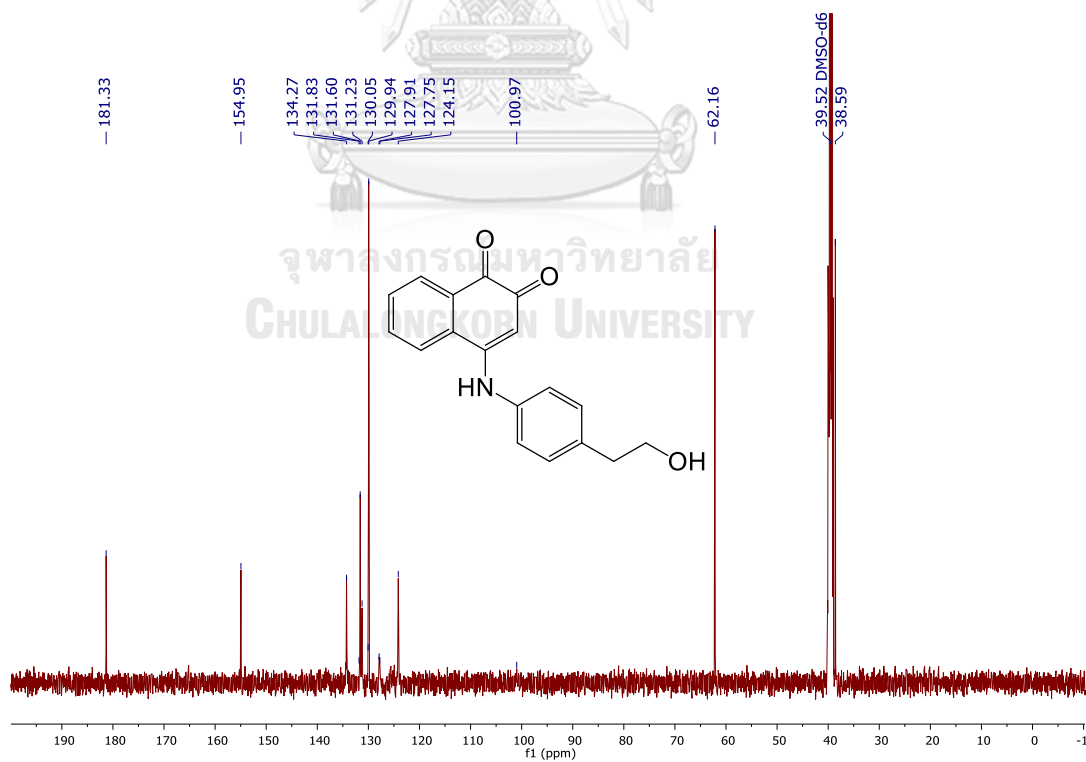


**Figure A.21** $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) of **31.**

## Generic Display Report
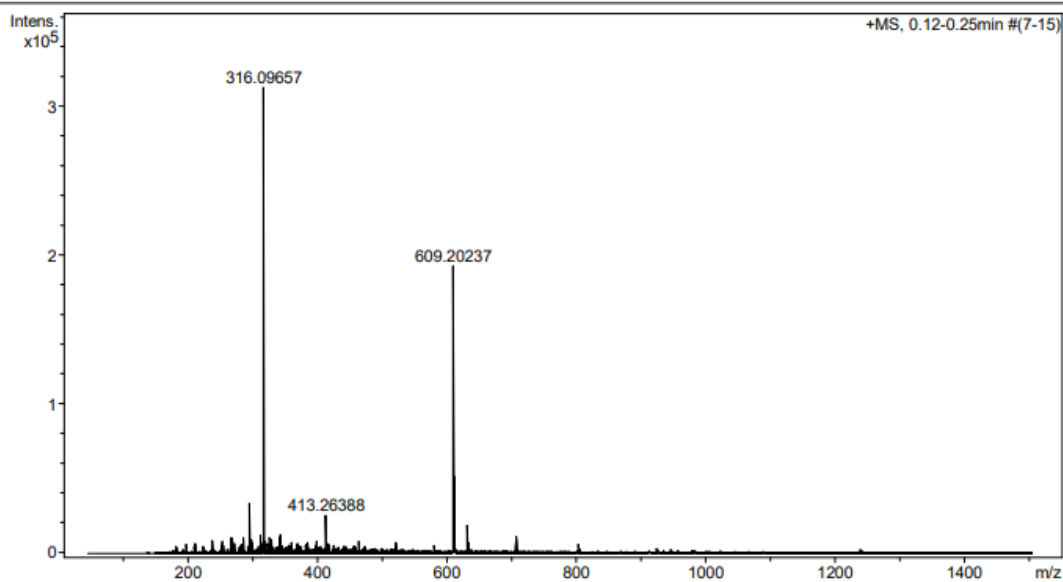
**Figure A.22** HRMS of **31.**
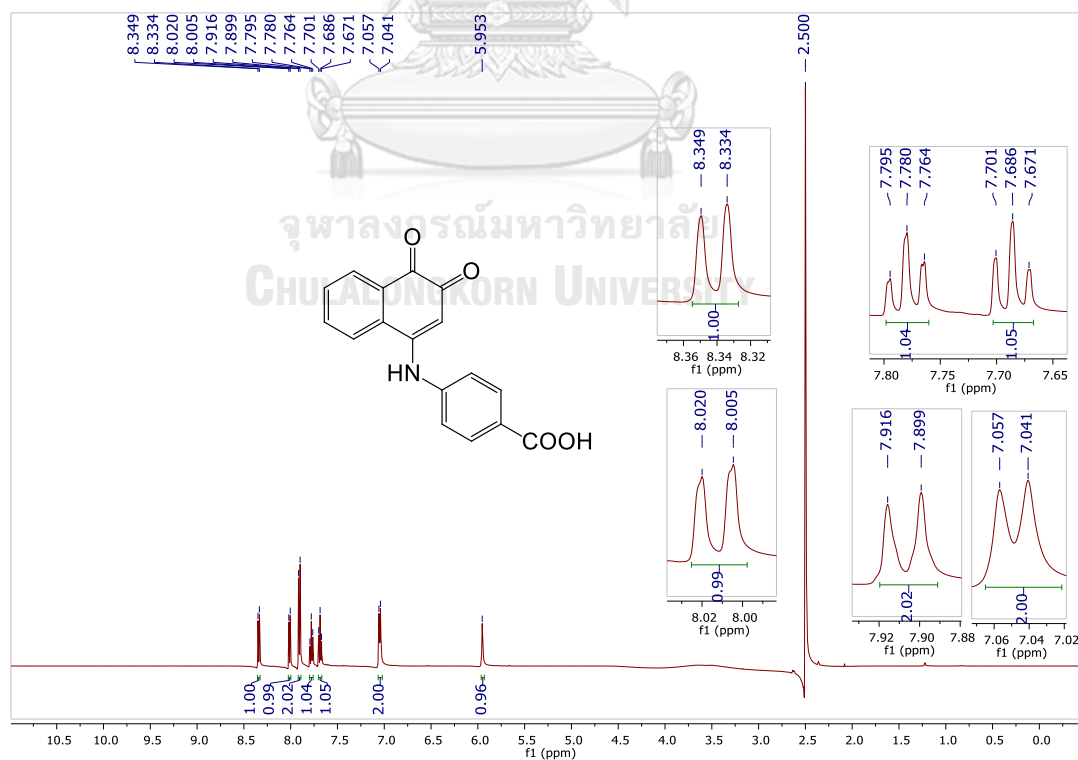


**Figure A.23** $^1$H-NMR (DMSO-$d_6$, 500 MHz) of **32.**
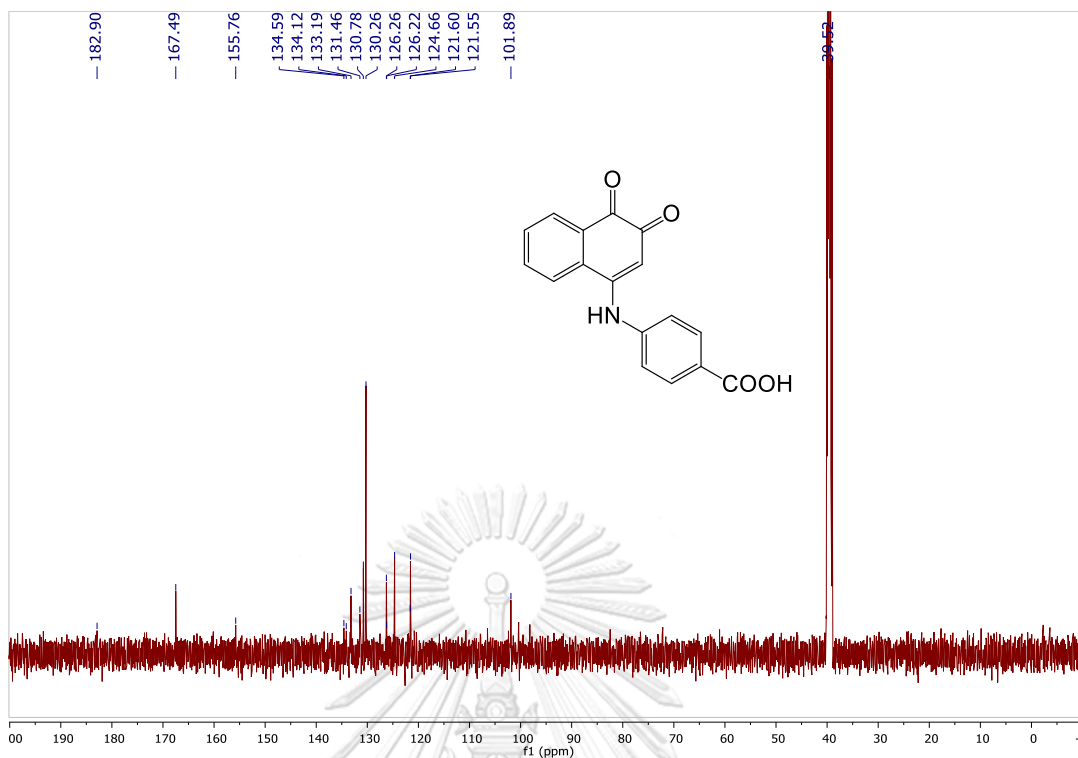
Figure A.24 $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) of **32**.
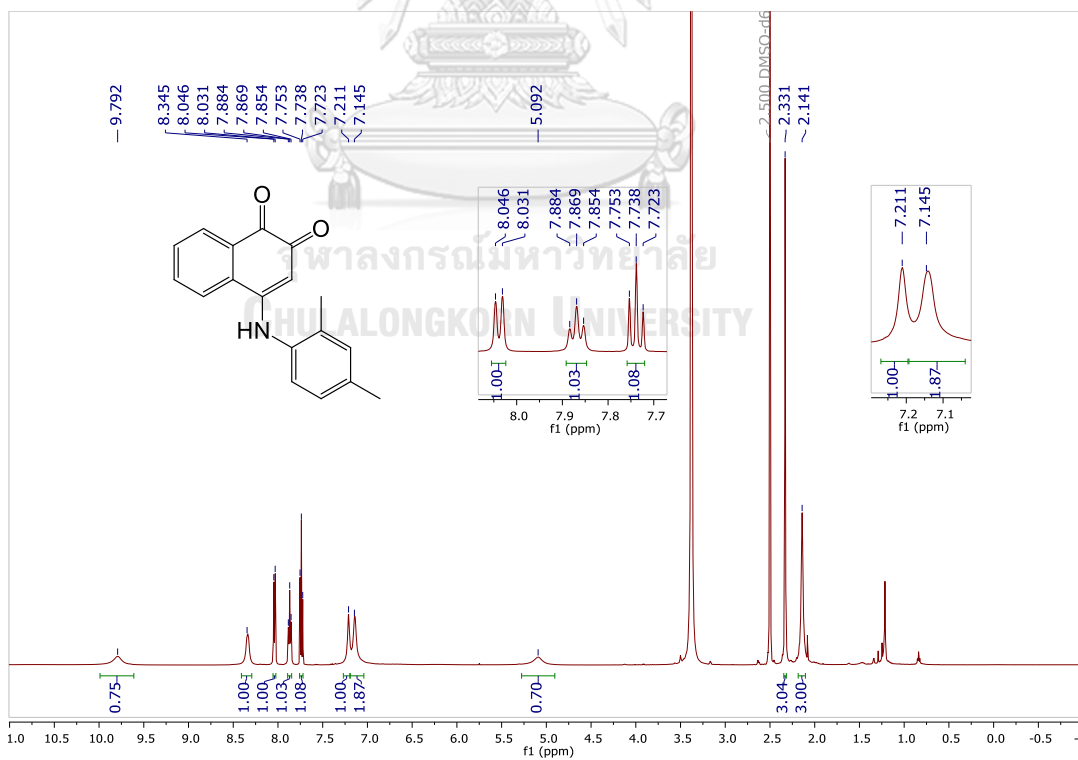


Figure A.25 $^{1}$H-NMR (DMSO-$d_6$, 500 MHz) of **33**.
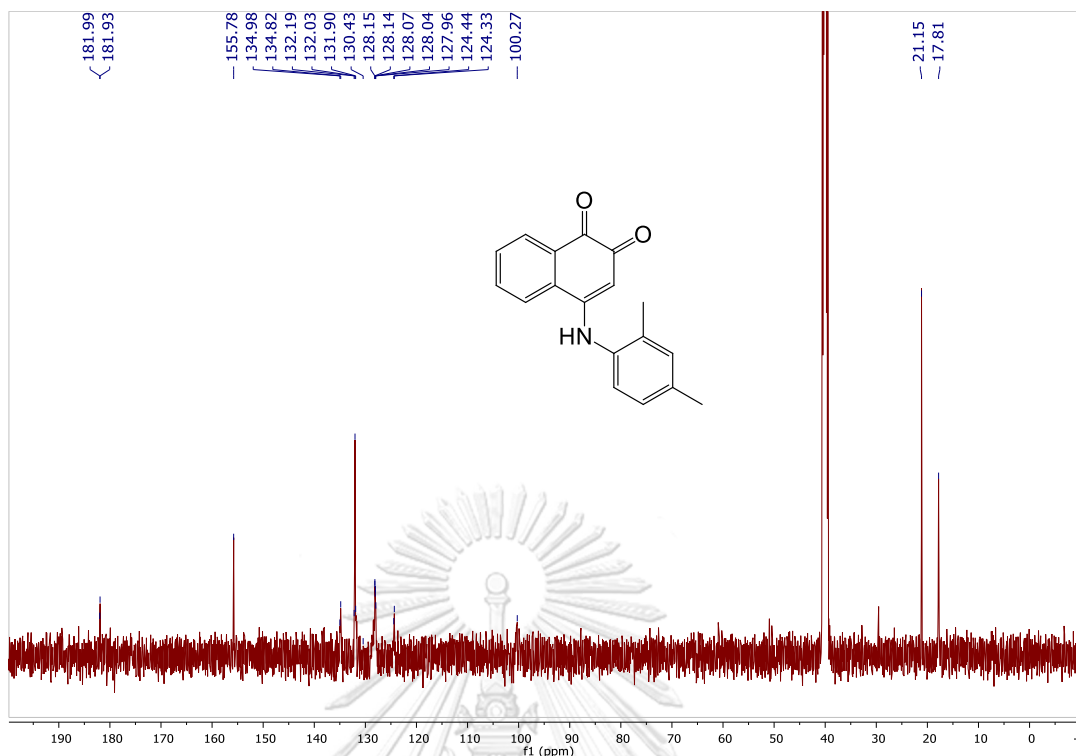
Figure A.26 $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) of **33.**
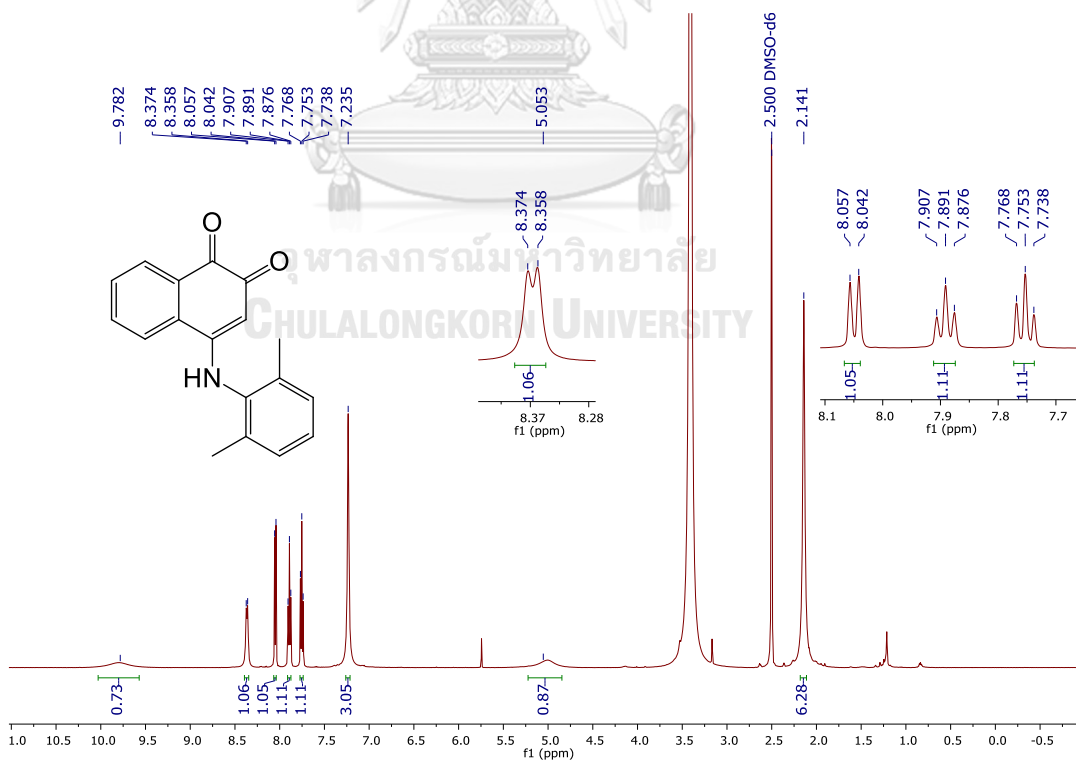


Figure A.27 $^1$H-NMR (DMSO-$d_6$, 500 MHz) of **34.**
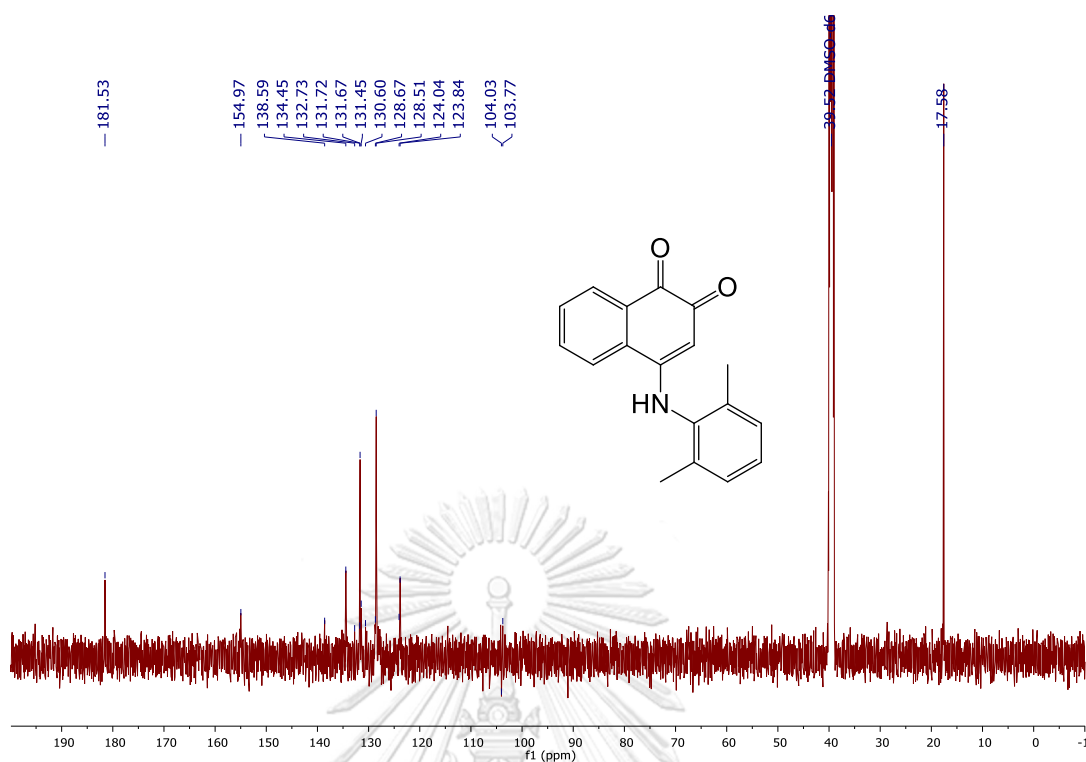
Figure A.28 $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) of **34.**

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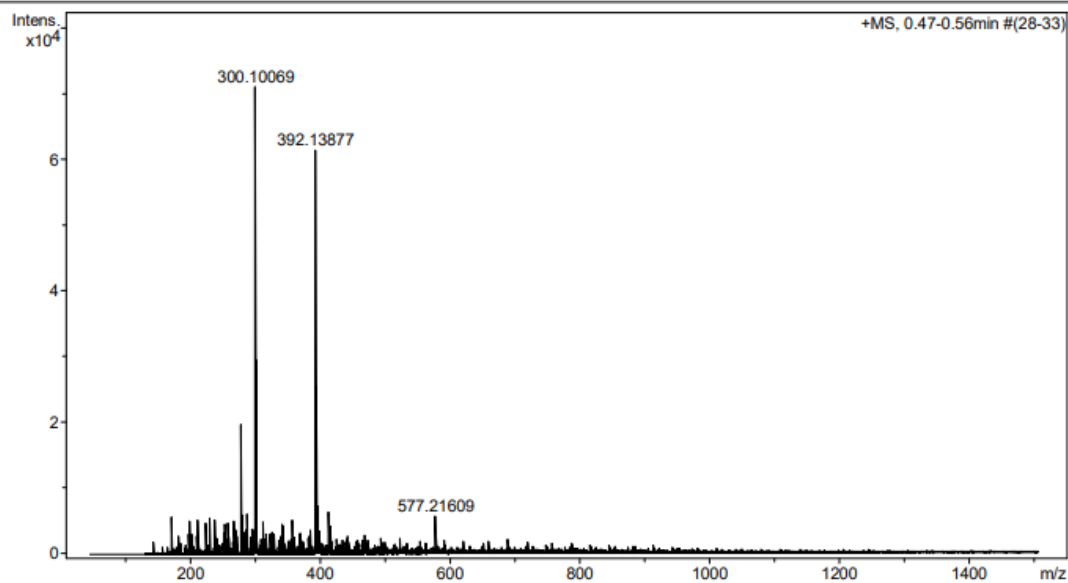


Figure A.29 HRMS of **34.**
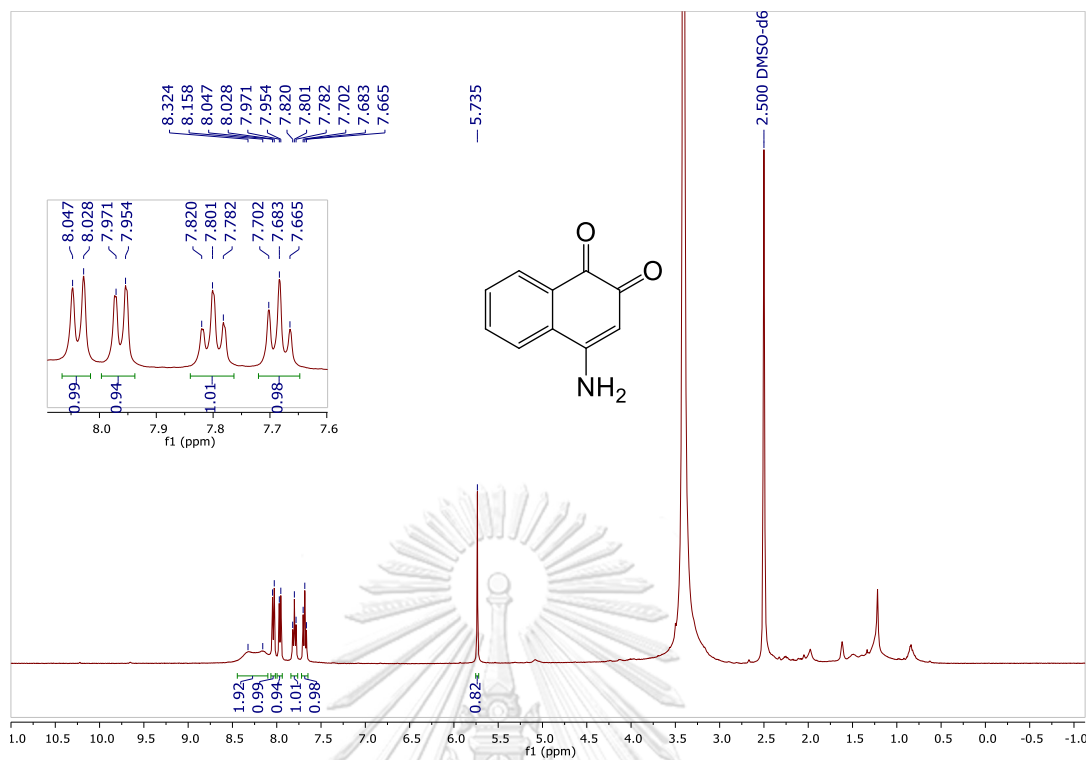
**Figure A.30** $^1$H-NMR (DMSO-$d_6$, 400 MHz) of **35.**
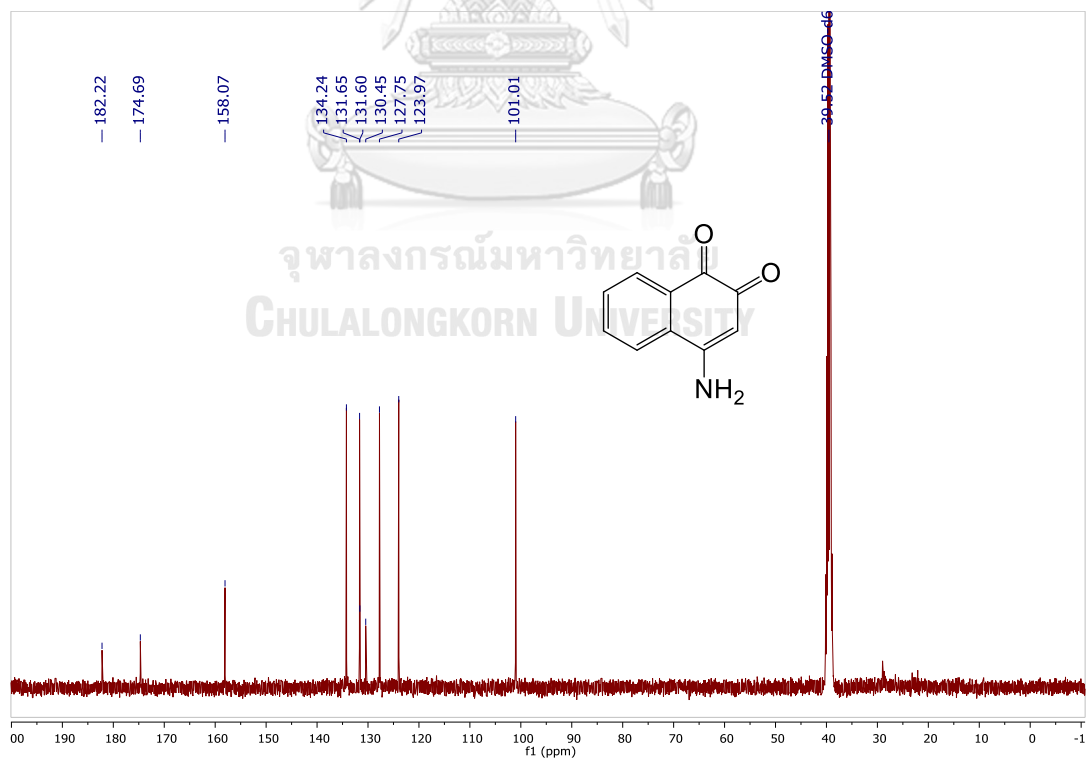


**Figure A.31** $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) of **35.**

**Figure A.32** $^{1}$H-NMR (DMSO-$d_6$, 500 MHz) of **36.**



**Figure A.33** $^{1}$H-NMR (DMSO-$d_6$, 400 MHz) of **37.**
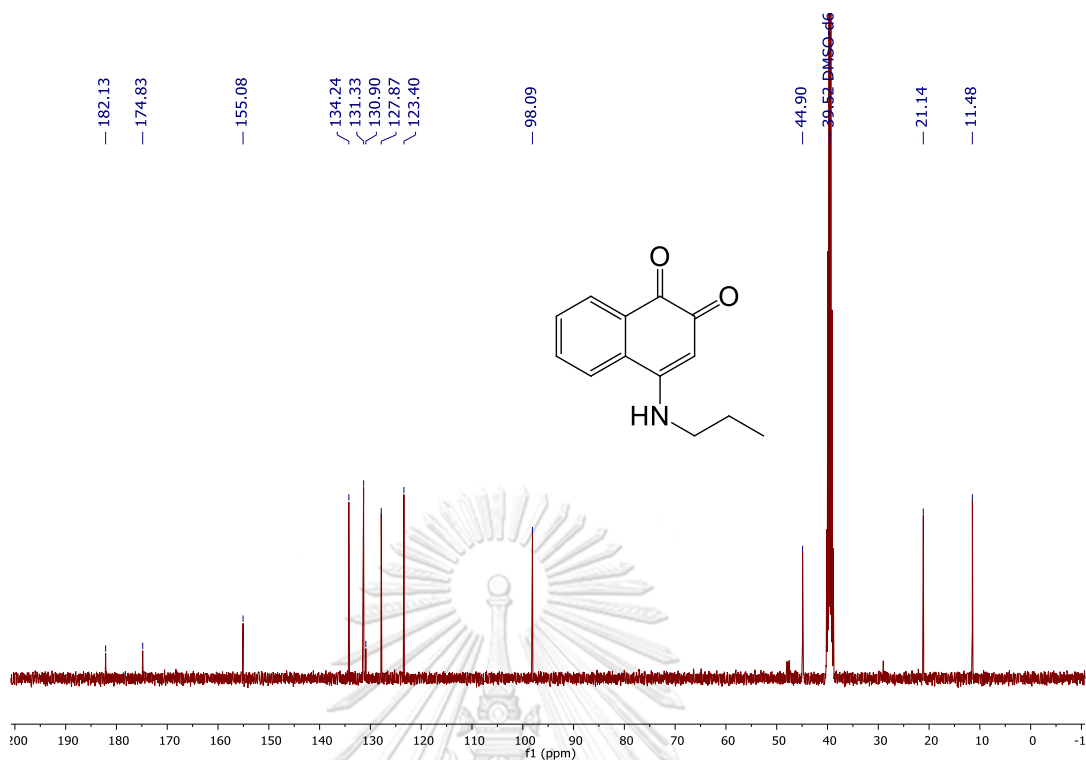
Figure A.34 $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) of **37**.

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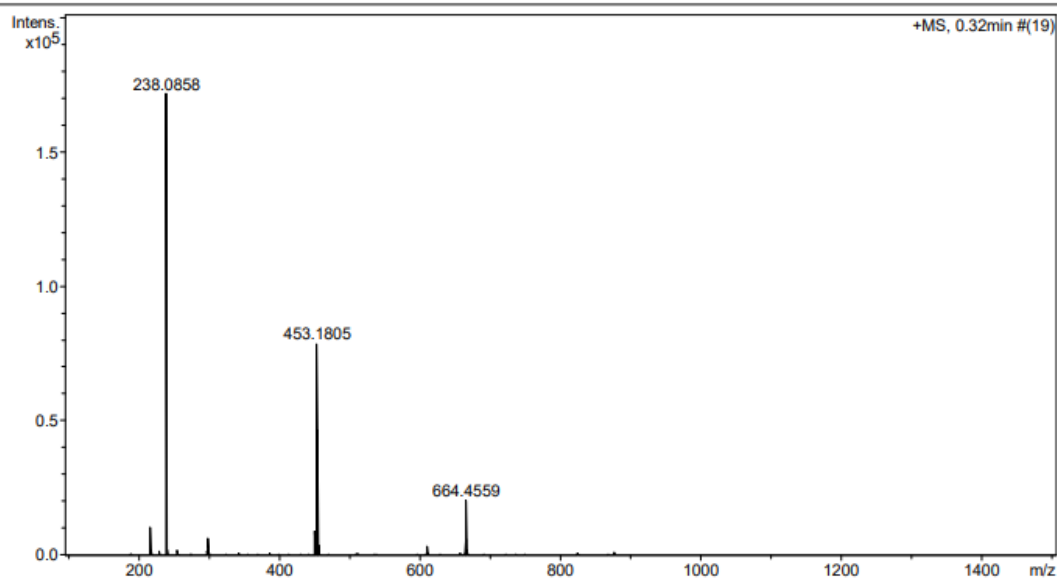


Figure A.35 HRMS of **37**.
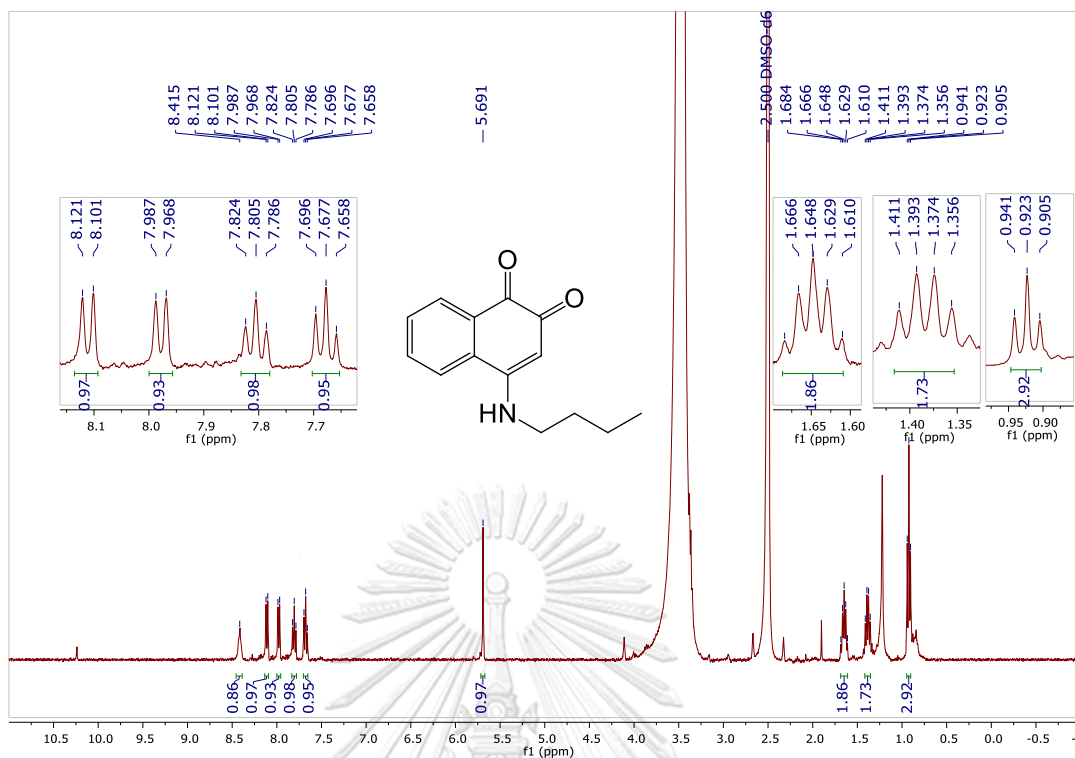
**Figure A.36** $^1$H-NMR (DMSO-$d_6$, 400 MHz) of **38.**
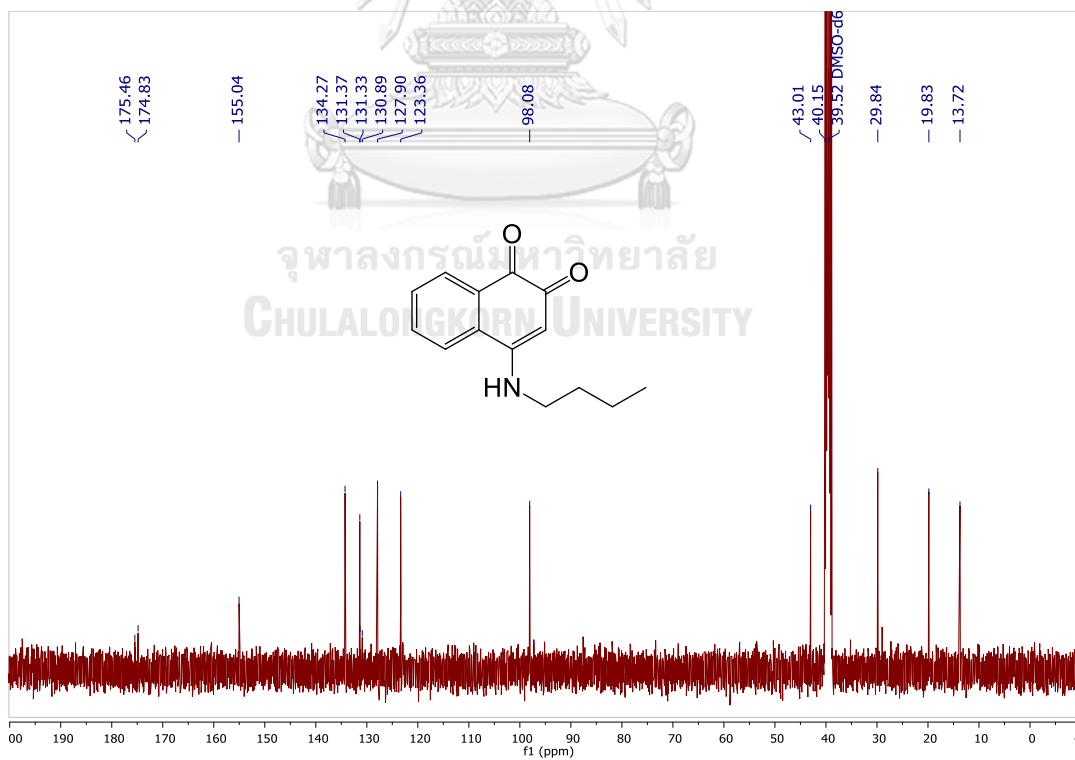


**Figure A.37** $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) of **38.**
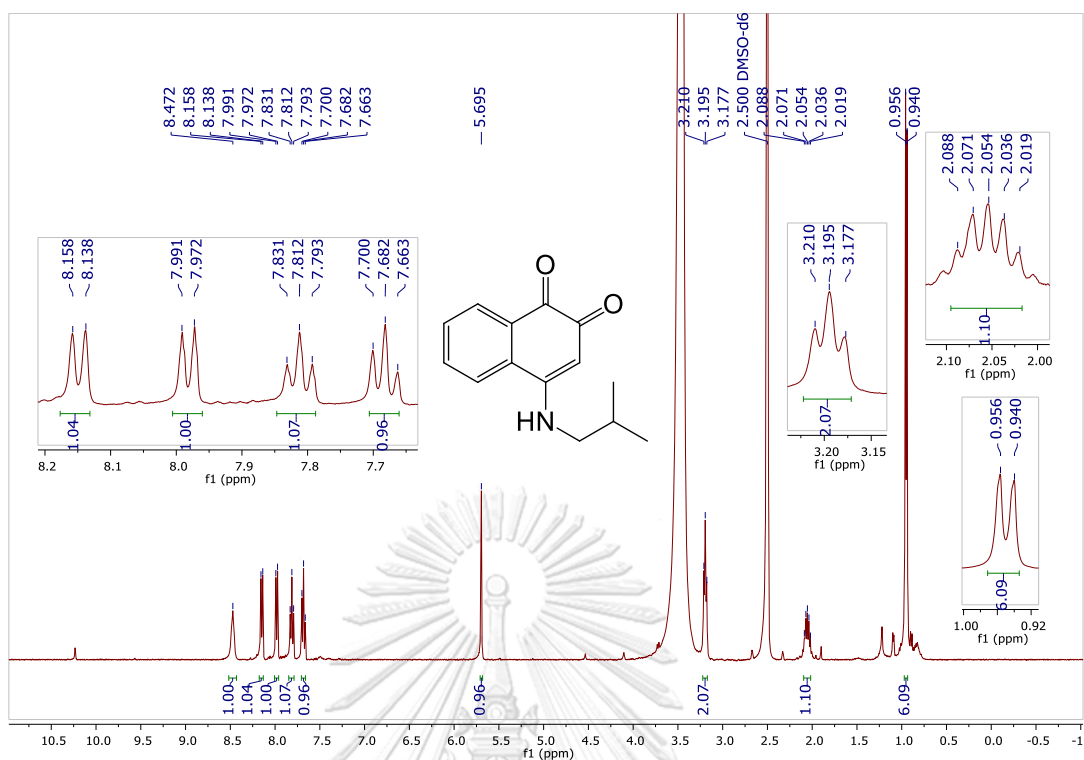
**Figure A.38** ^{1}H-NMR (DMSO-$d_6$, 400 MHz) of **39.**
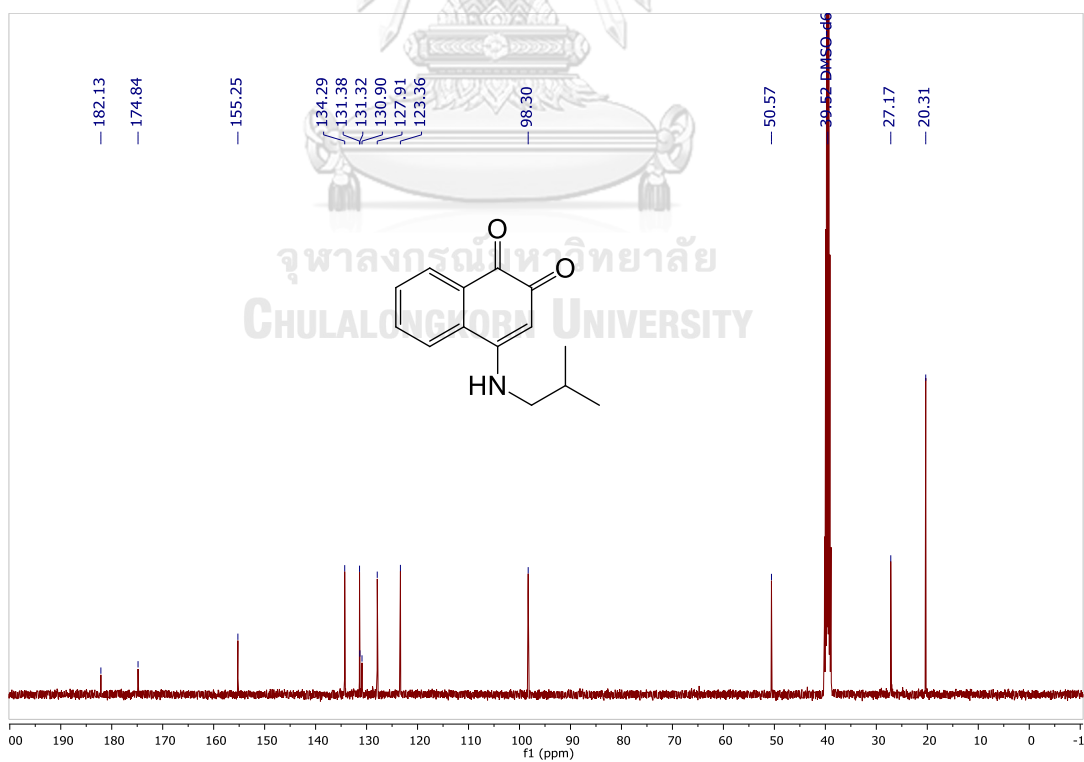


**Figure A.39** ^{13}C-NMR (DMSO-$d_6$, 100 MHz) of **39.**

## Generic Display Report
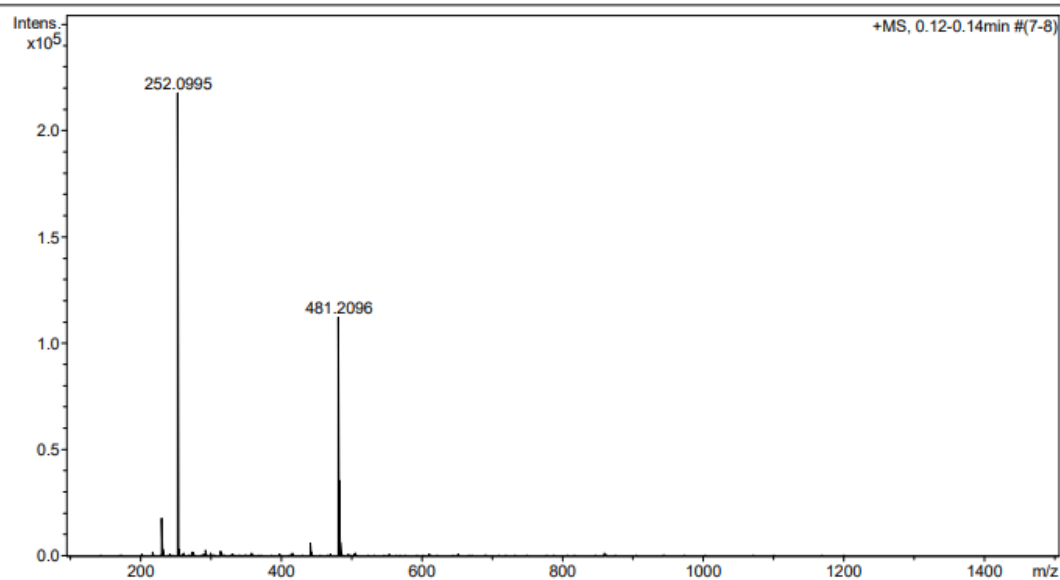
**Figure A.40** HRMS of **39.**
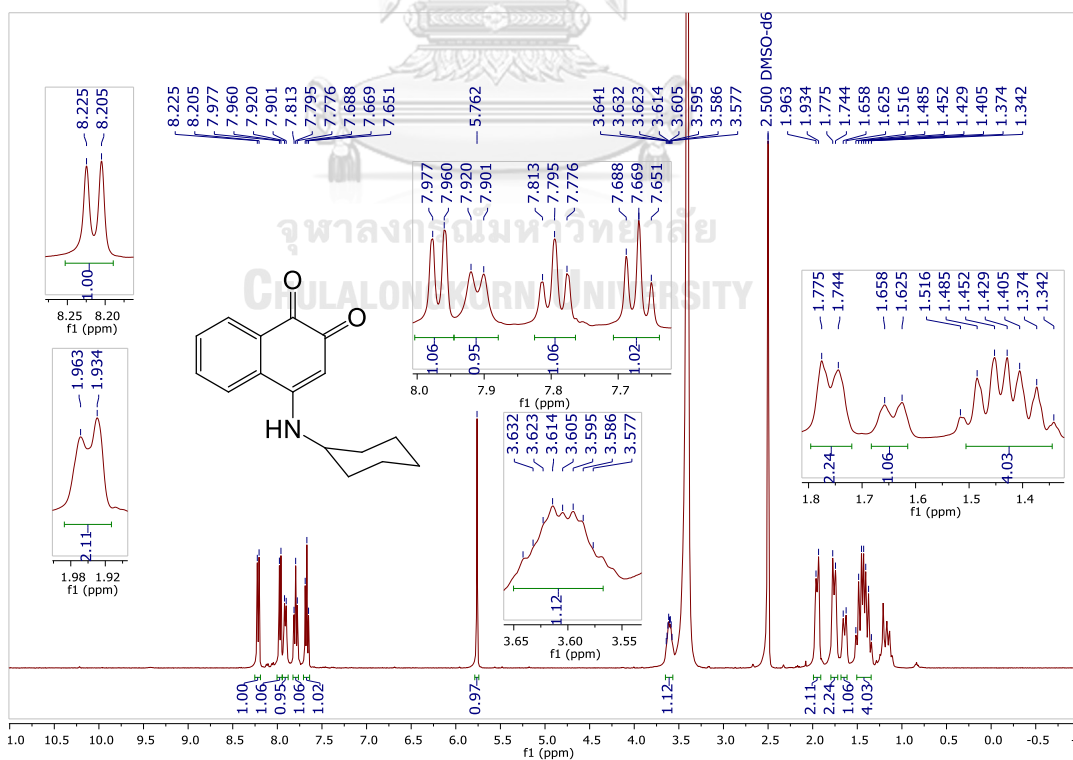


**Figure A.41** $^{1}$H-NMR (DMSO-$d_6$, 400 MHz) of **40.**
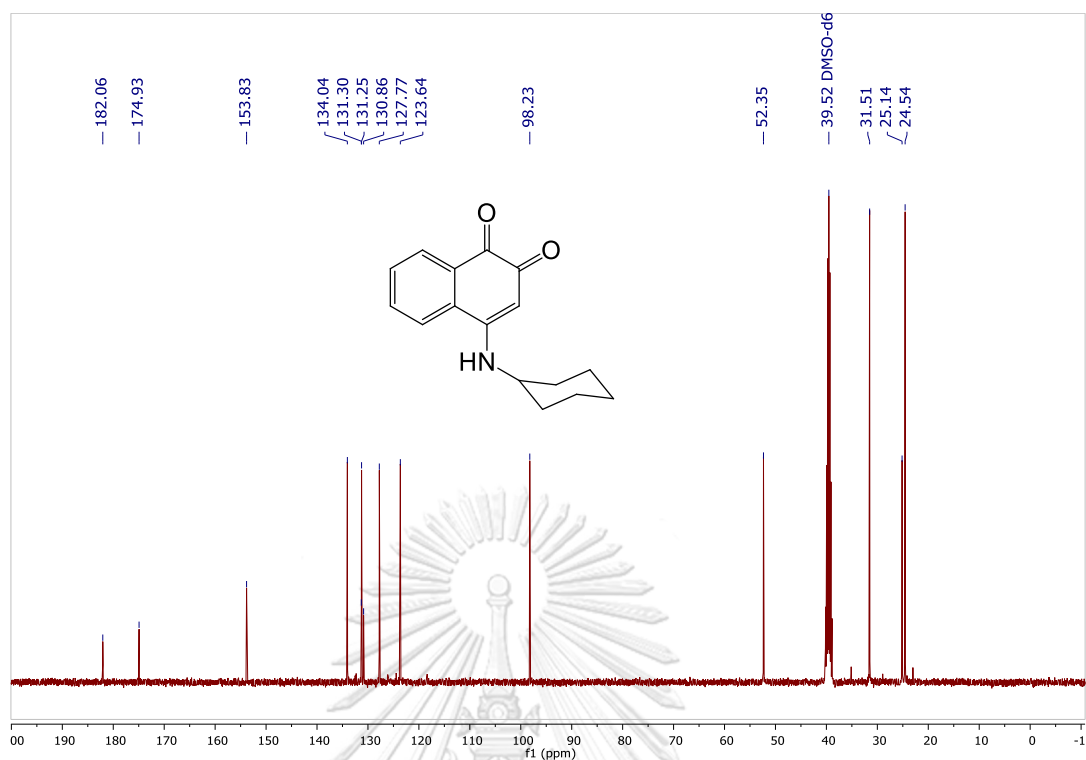
Figure A.42 $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) of **40.**
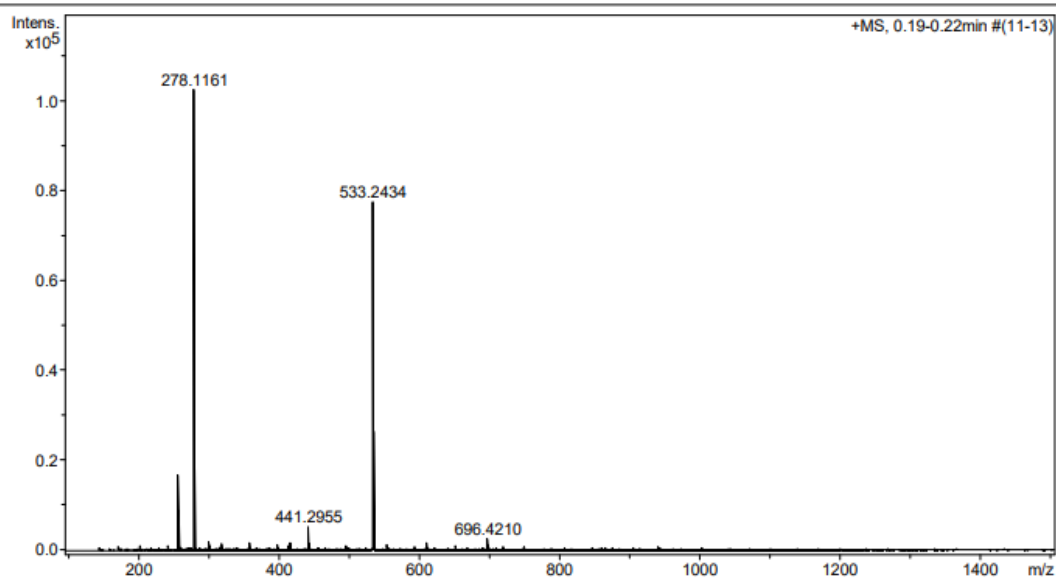


Figure A.43 HRMS of **40.**
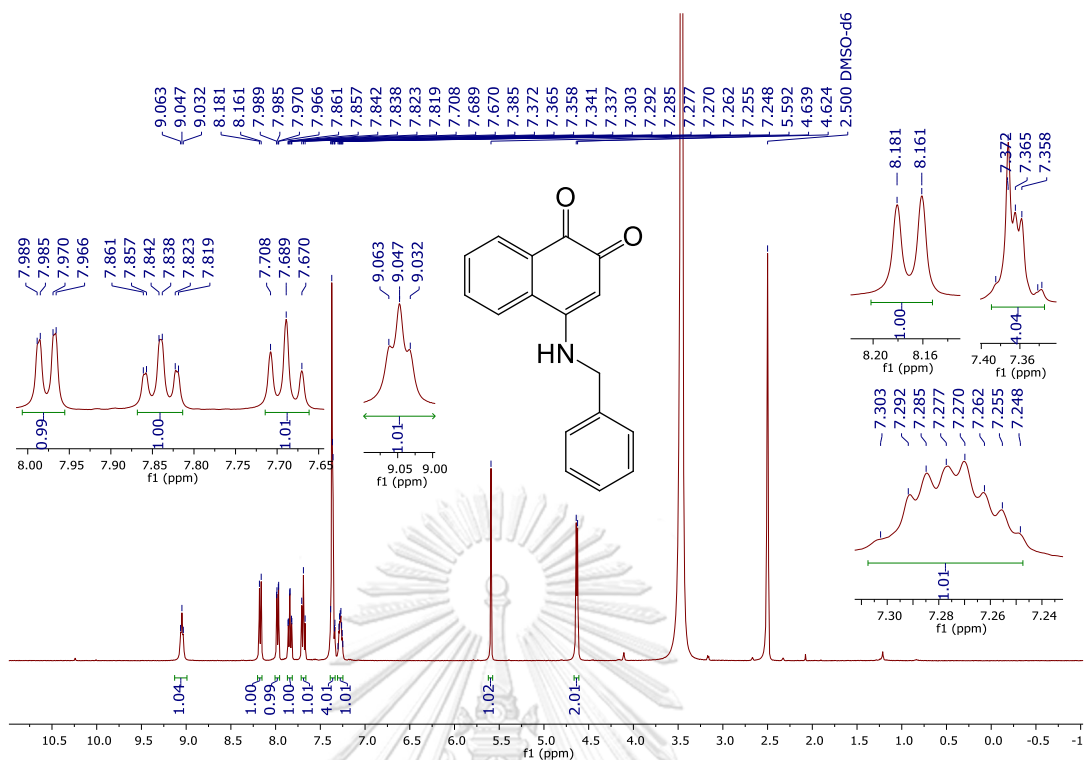
**Figure A.44** ^{1}H-NMR (DMSO-$d_6$, 400 MHz) of **41**.
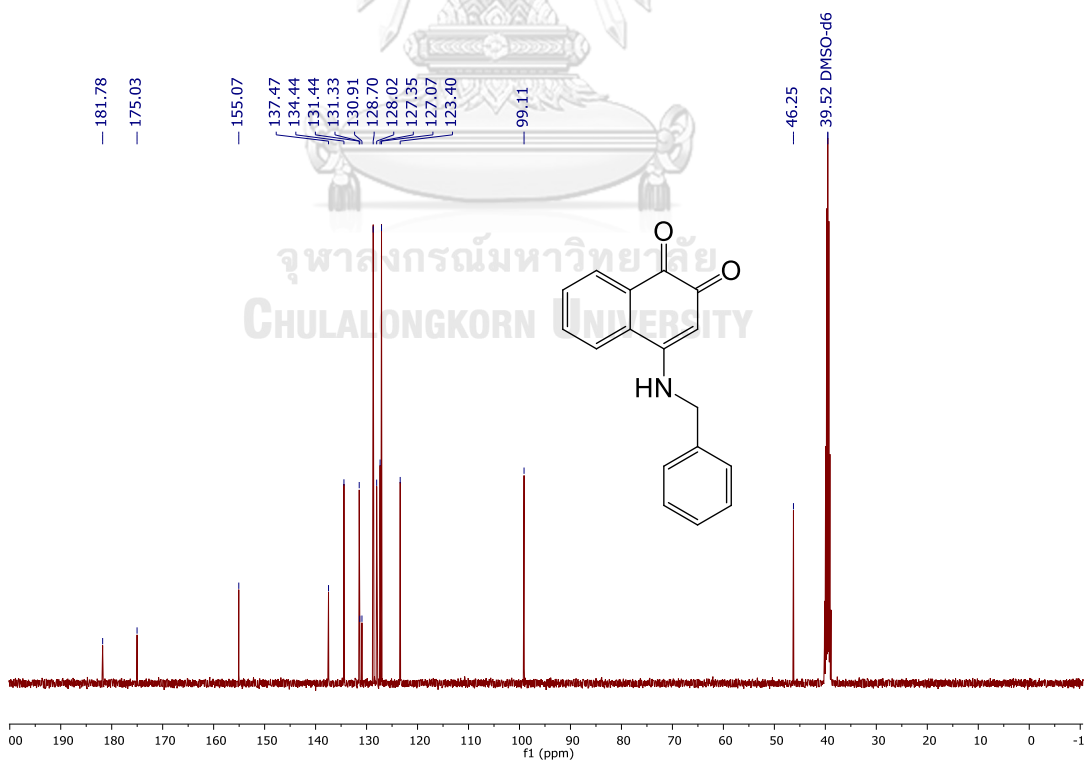


**Figure A.45** ^{13}C-NMR (DMSO-$d_6$, 100 MHz) of **41**.
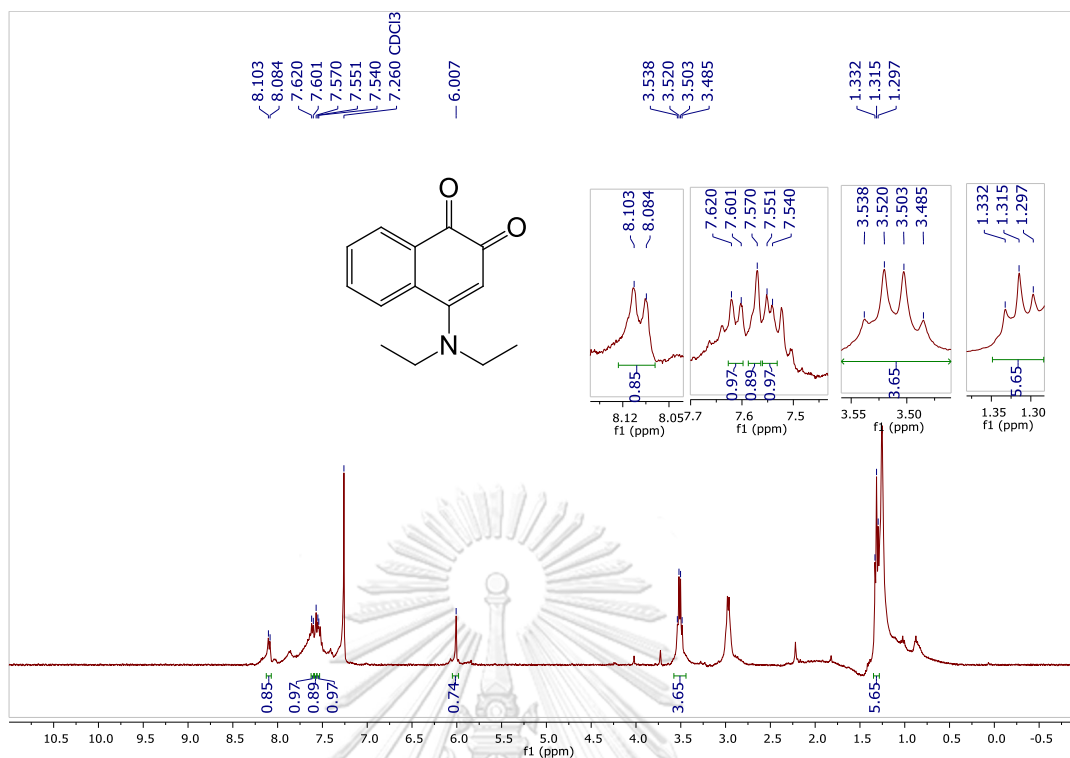
**Figure A.46** $^1$H-NMR (DMSO-$d_6$, 400 MHz) of **42.**
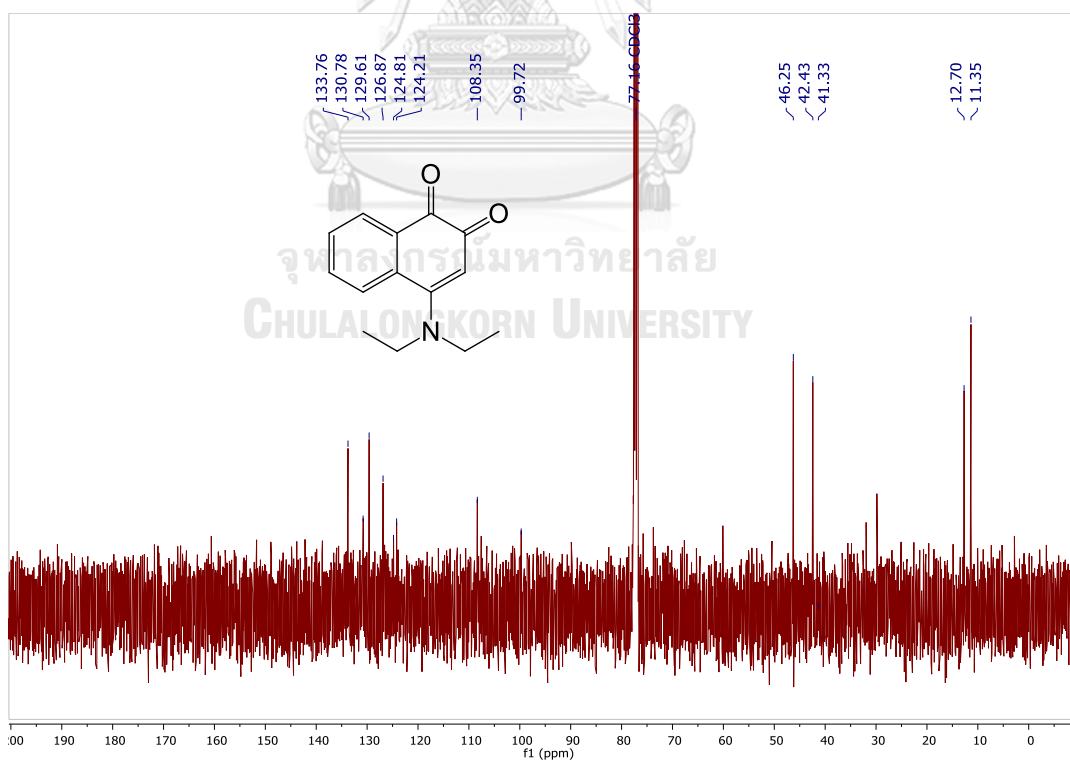


**Figure A.47** $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) of **42.**
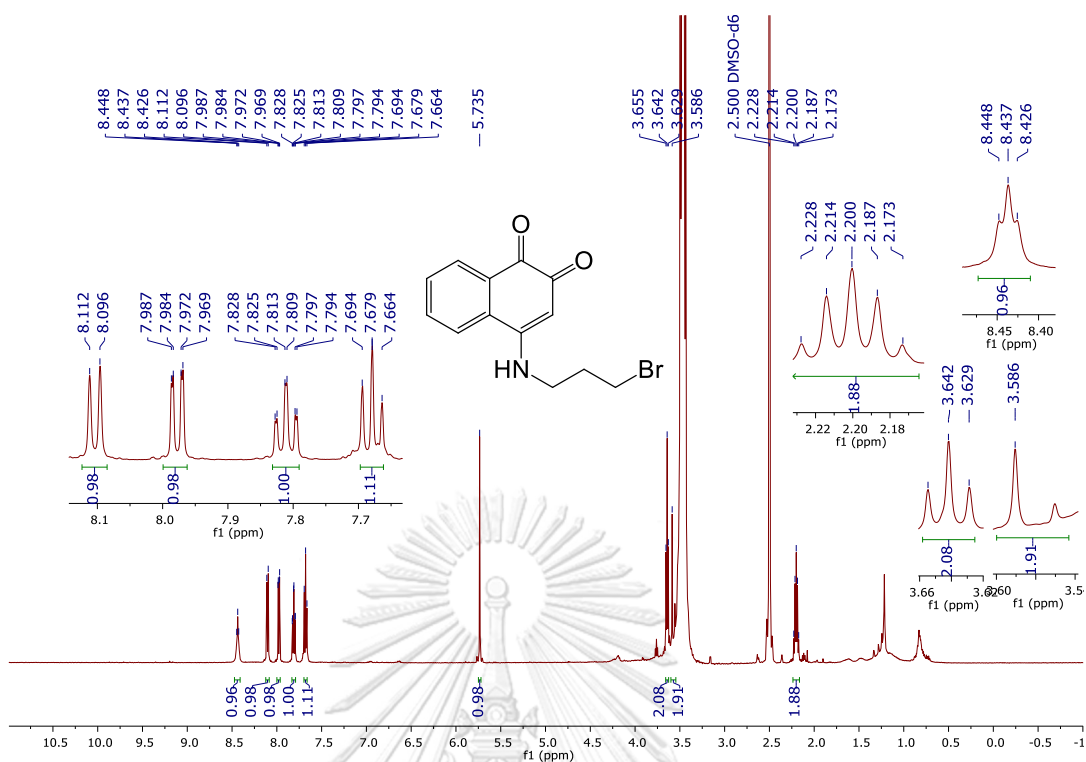
**Figure A.48** $^{1}$H-NMR (DMSO-$d_6$, 500 MHz) of **43.**
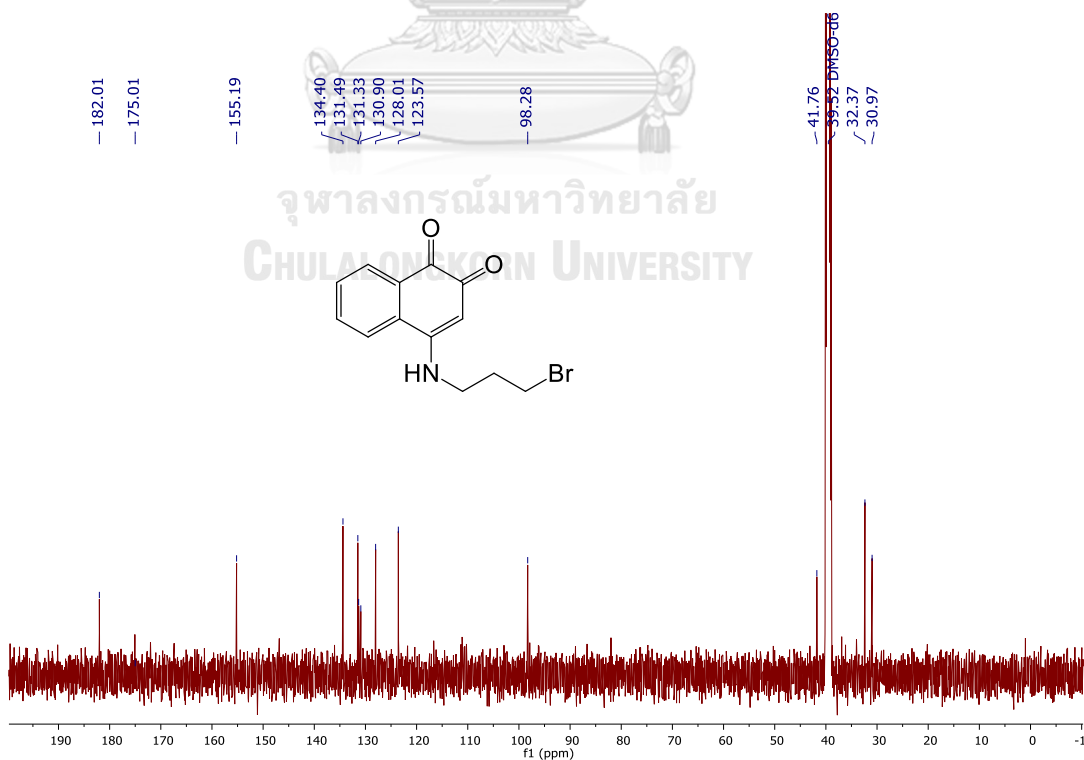


**Figure A.49** $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) of **43.**

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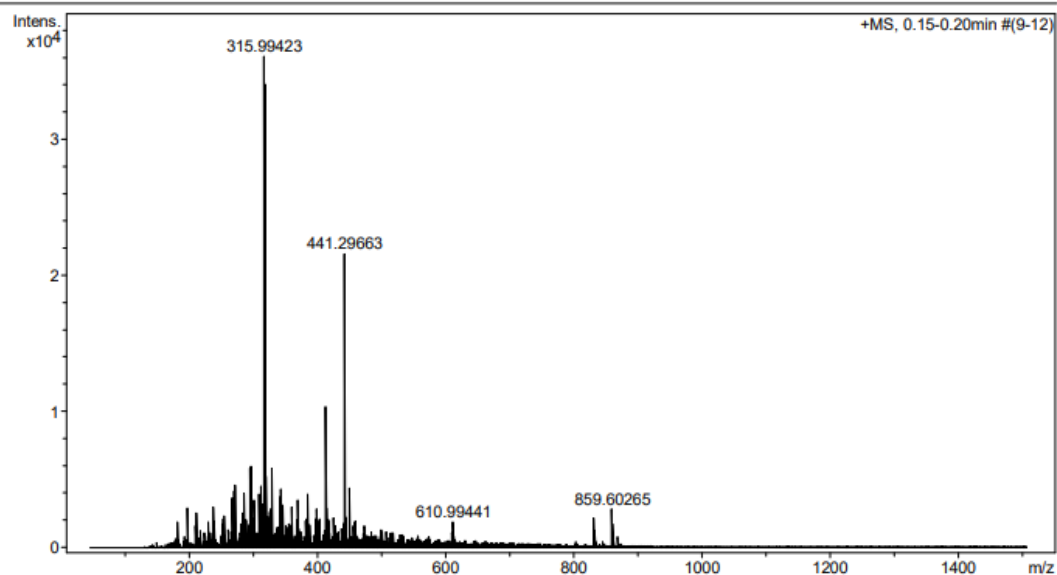
**Figure A.50** HRMS of **43.**
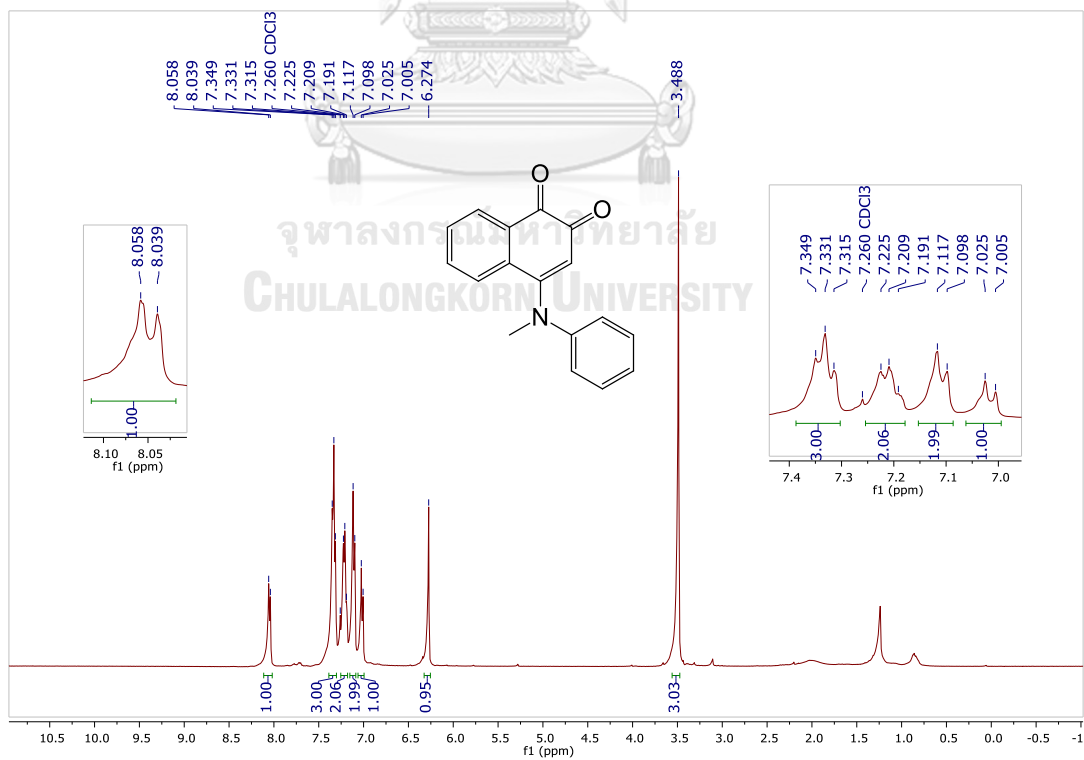


**Figure A.51** $^1$H-NMR (CDCl$_3$, 400 MHz) of **44.**
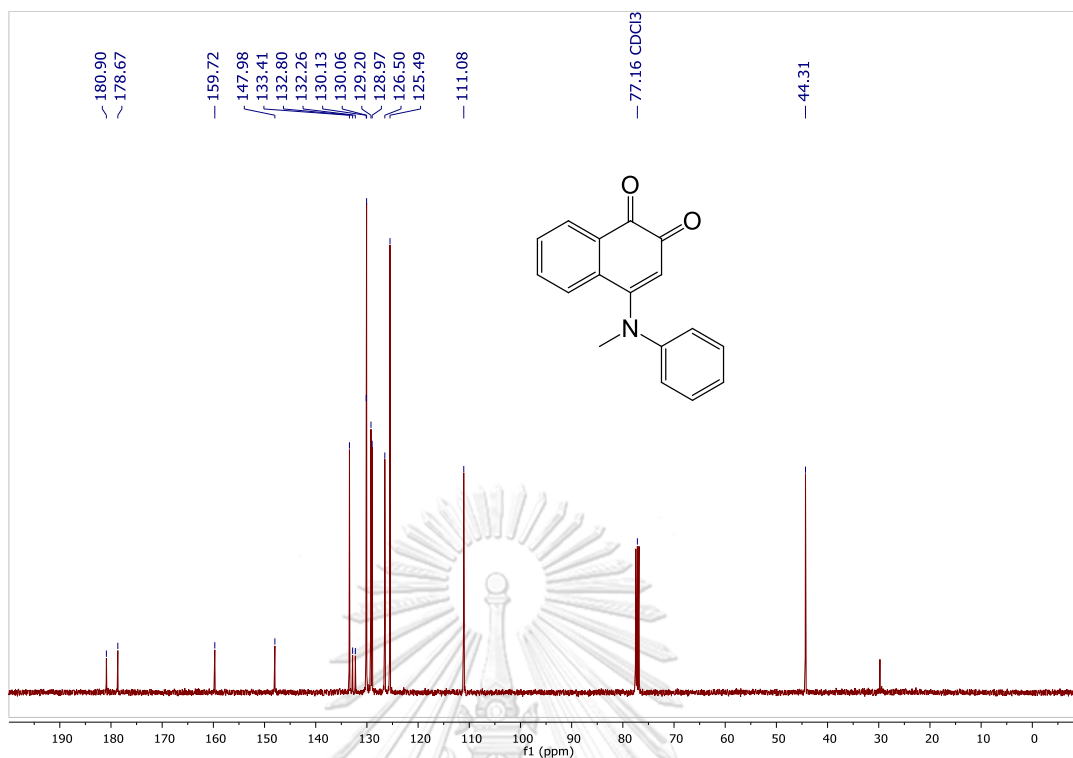
**Figure A.52** $^{13}$C-NMR (CDCl$_3$, 100 MHz) of **44.**
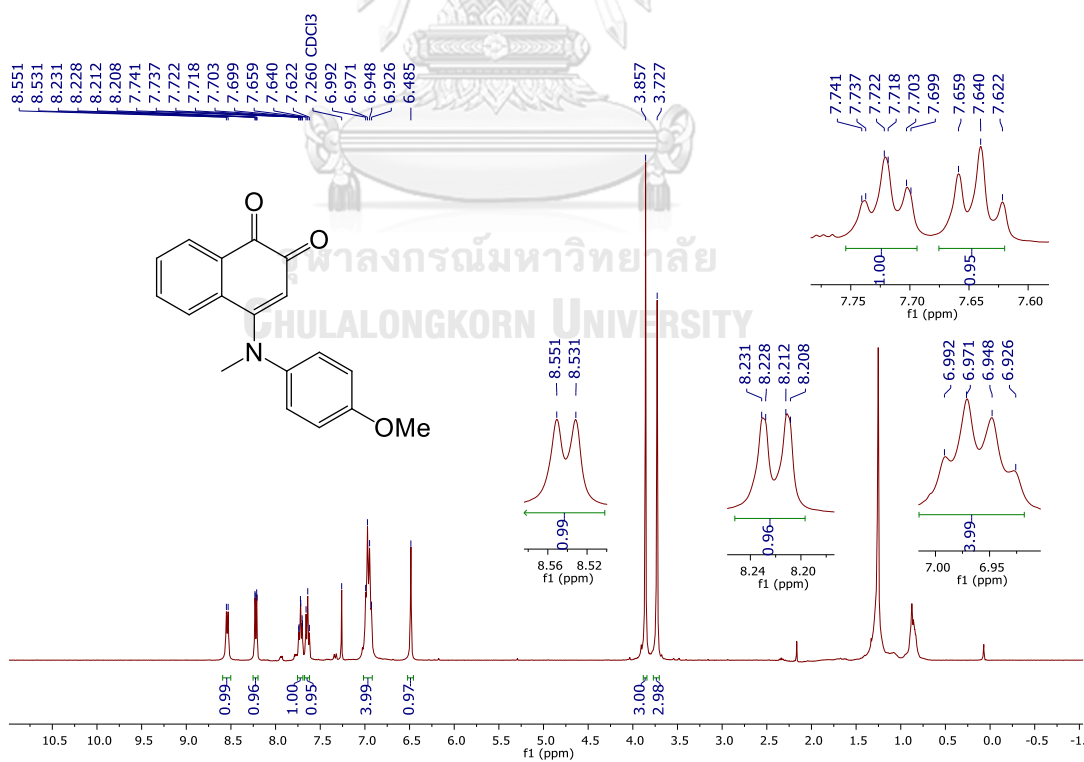


**Figure A.53** $^{1}$H-NMR (CDCl$_3$, 400 MHz) of **45.**
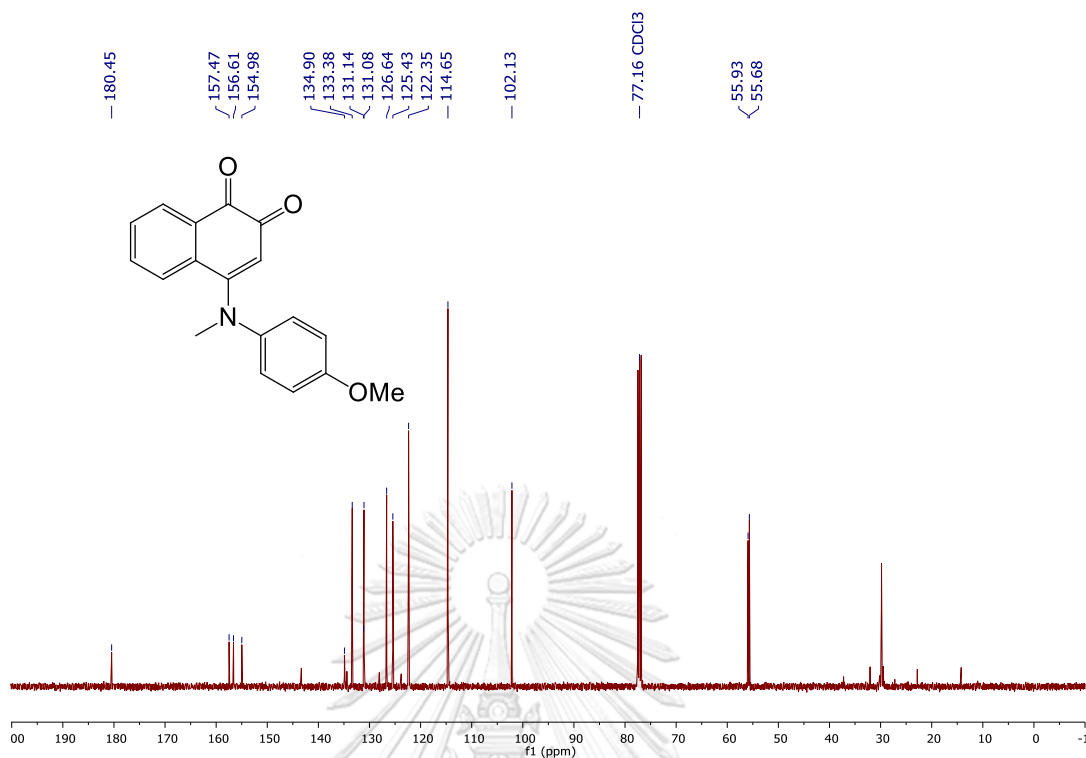
Figure A.54 $^{13}$C-NMR (CDCl$_3$, 100 MHz) of **45.**
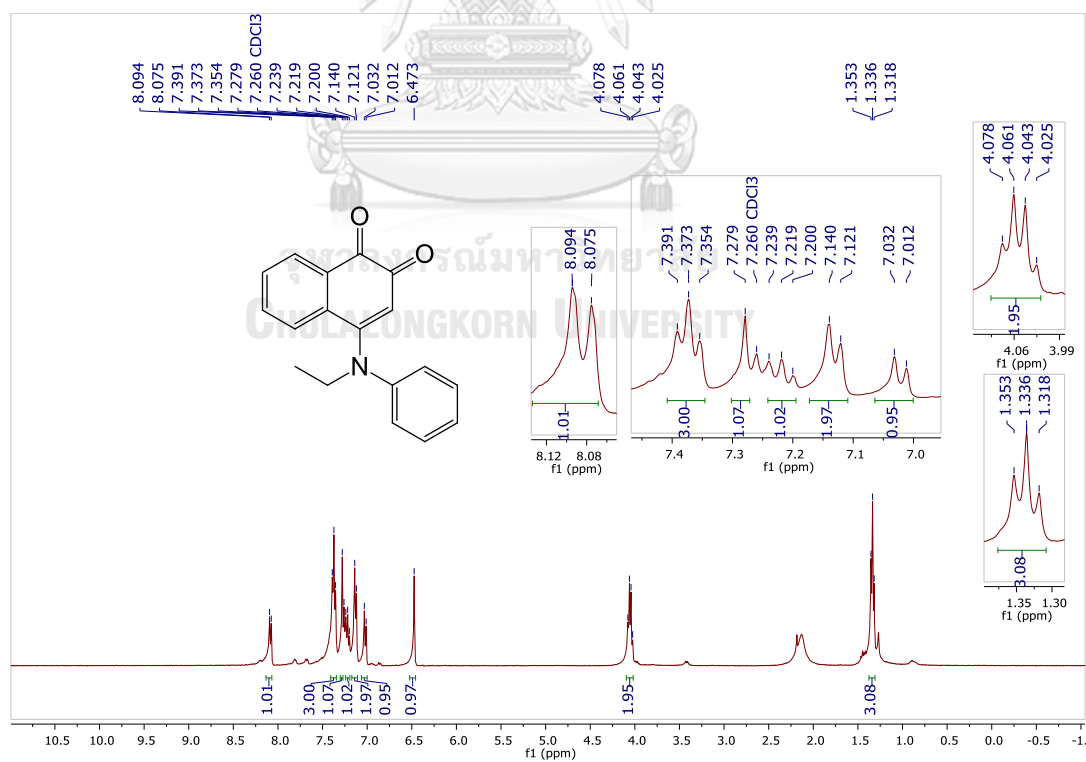


Figure A.55 $^1$H-NMR (CDCl$_3$, 400 MHz) of **46.**
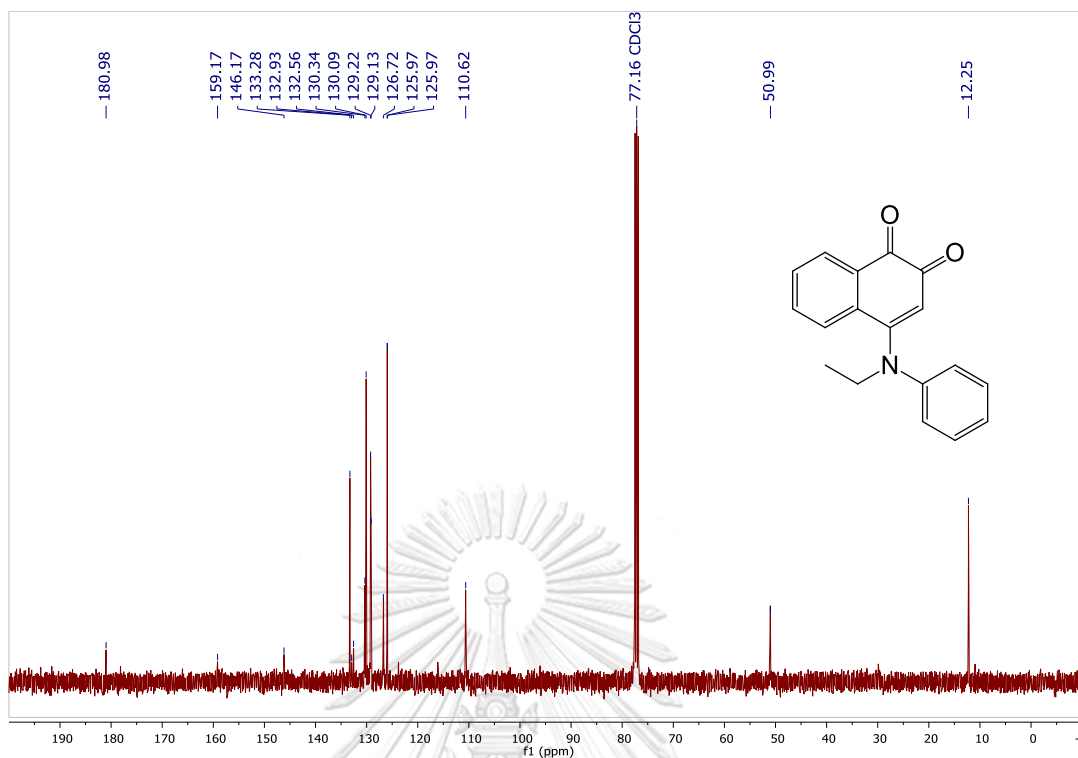
Figure A.56 $^{13}$C-NMR (CDCl$_3$, 100 MHz) of **46.**
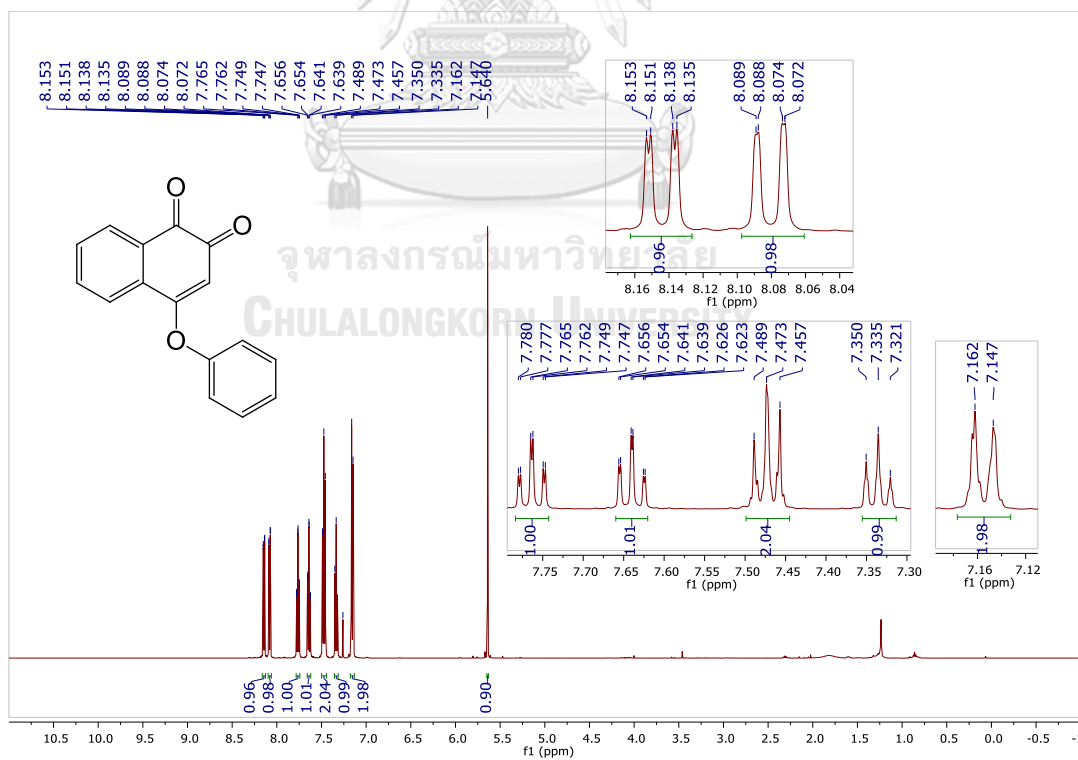


Figure A.57 $^{1}$H-NMR (CDCl$_3$, 500 MHz) of **47.**
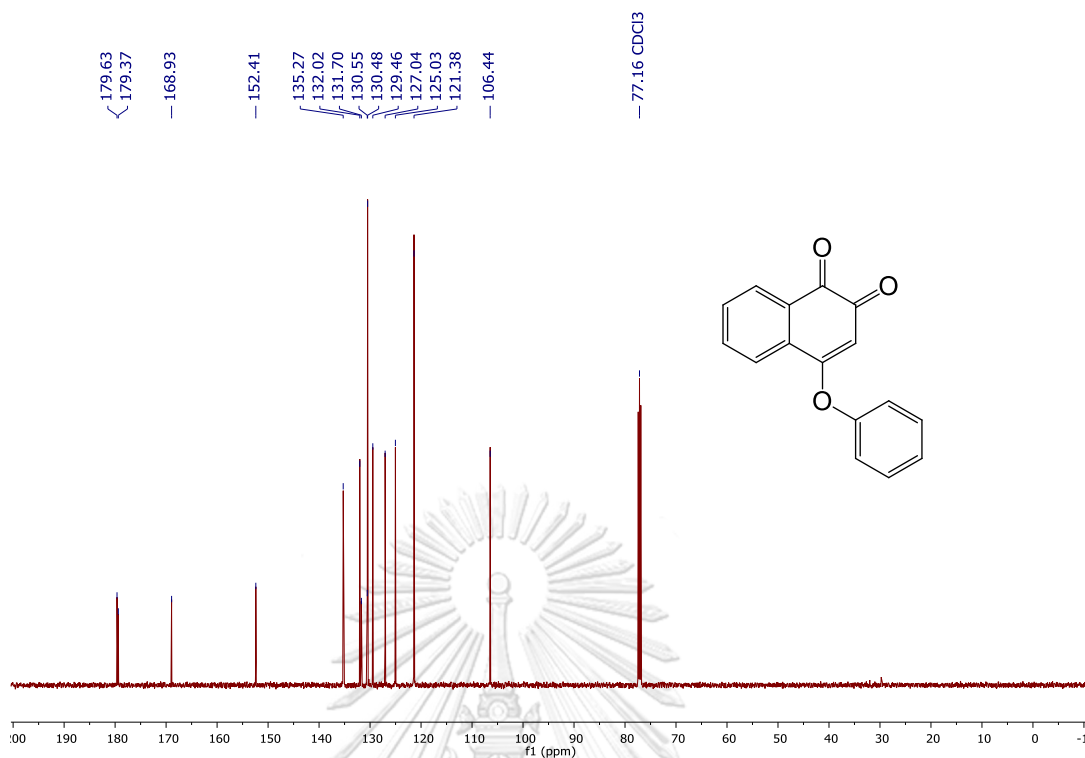
Figure A.58 $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **47**.
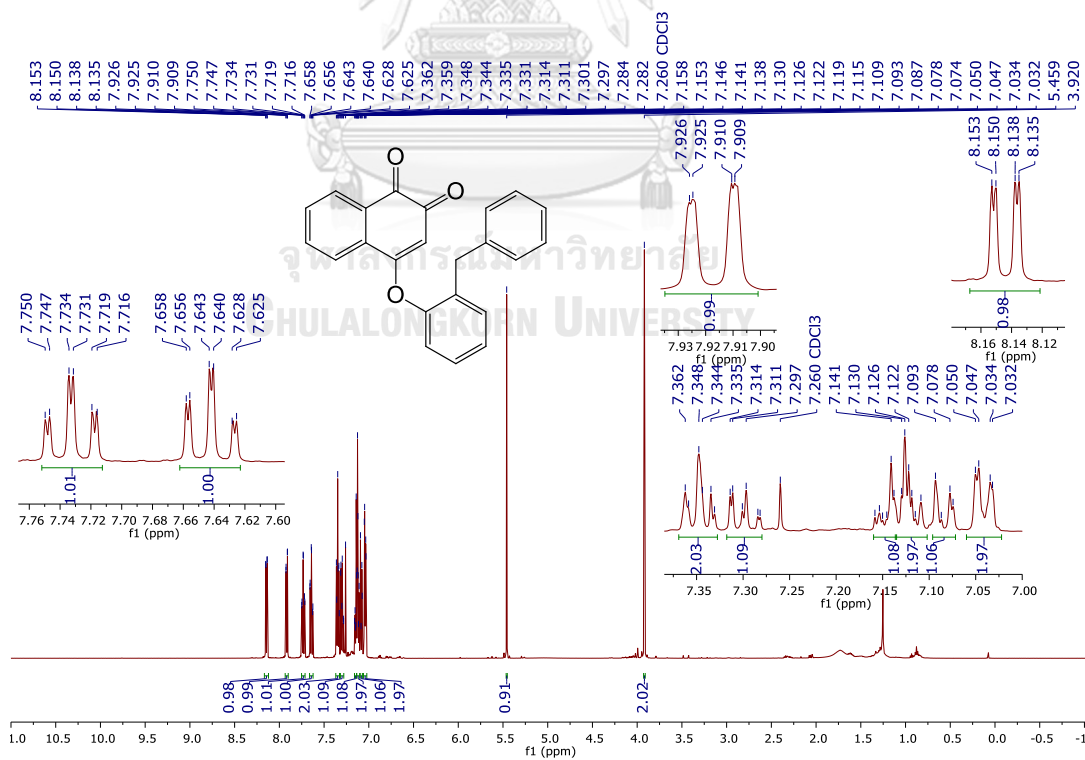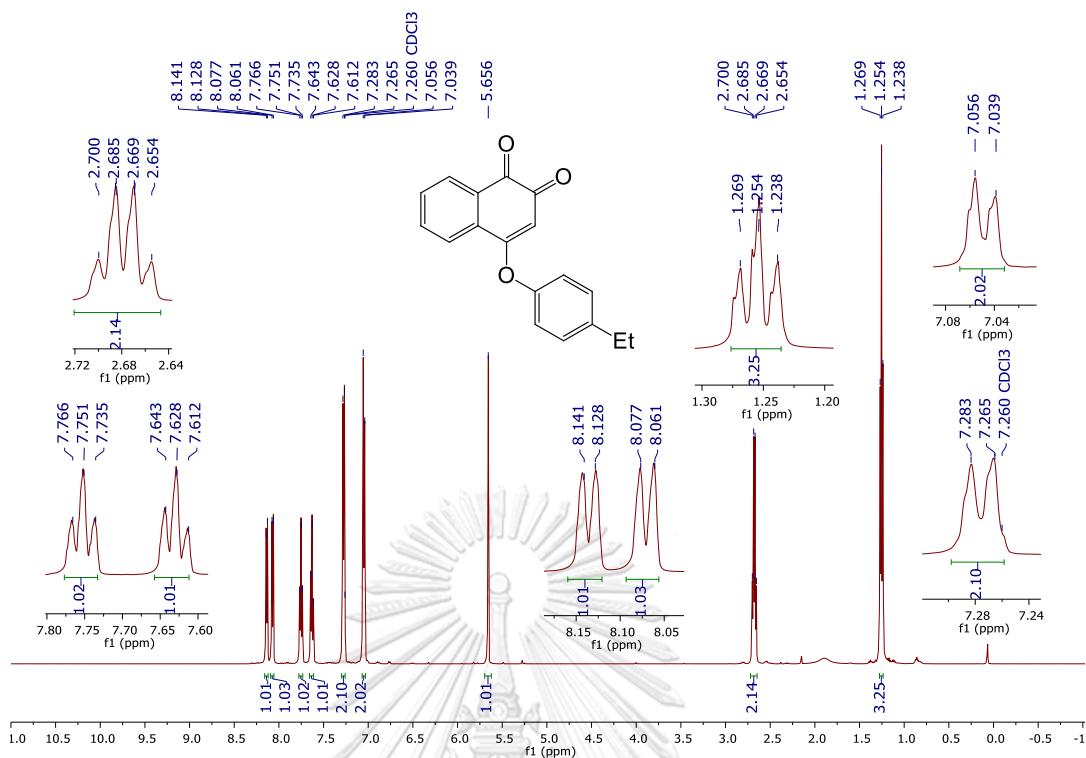


Figure A.59 $^1$H-NMR (CDCl$_3$, 500 MHz) of **48**.
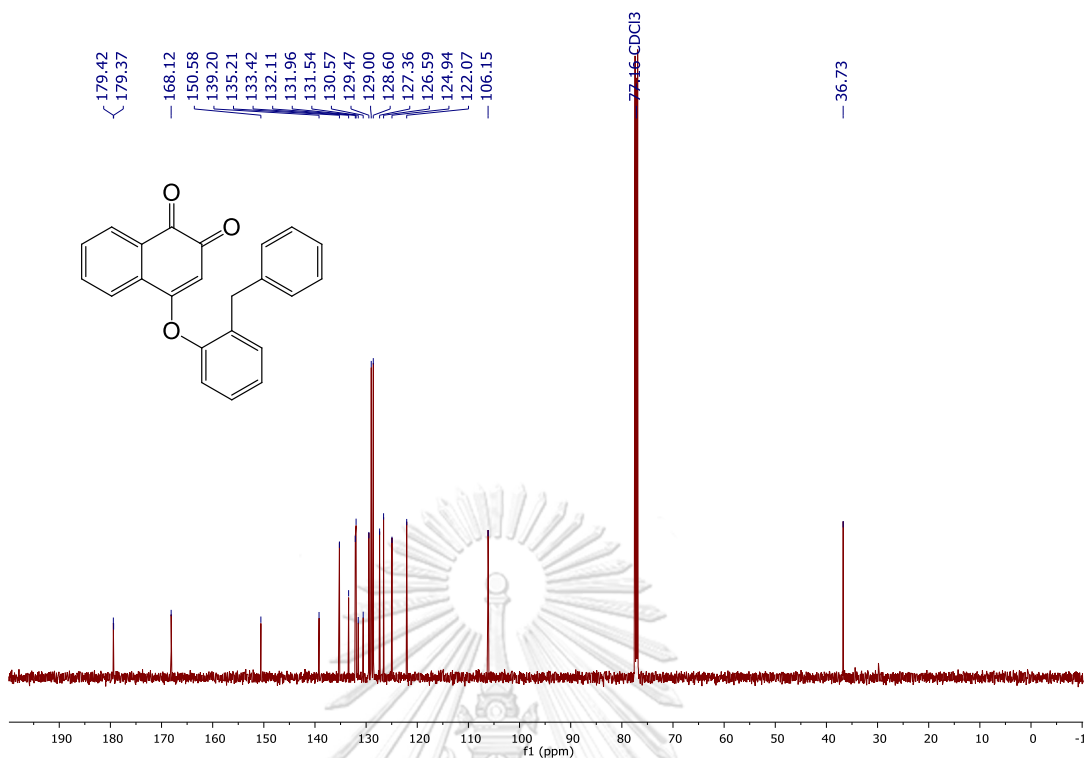
Figure A.60 $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **48**.

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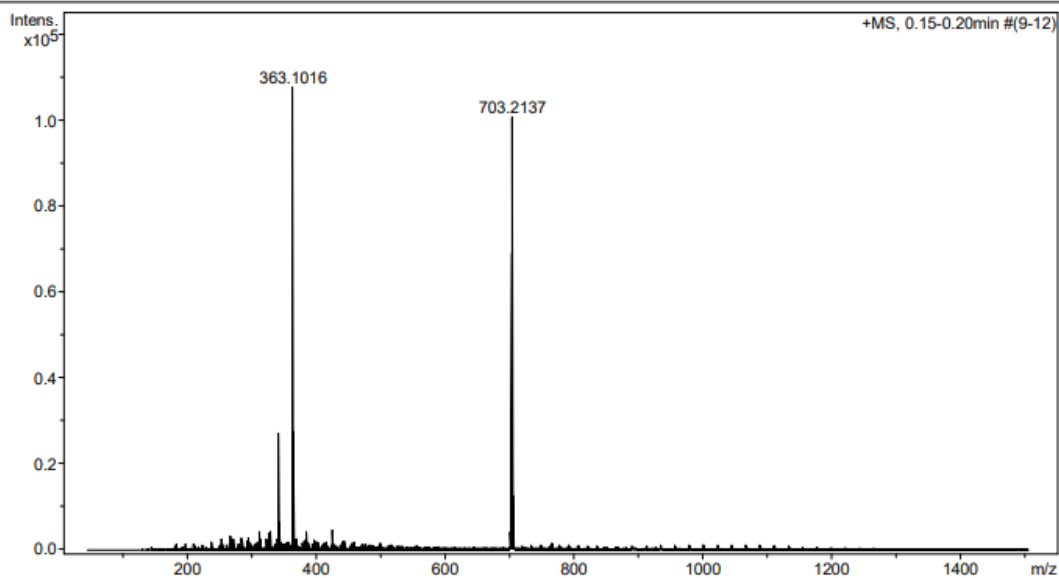


Figure A.61 HRMS of **48**.
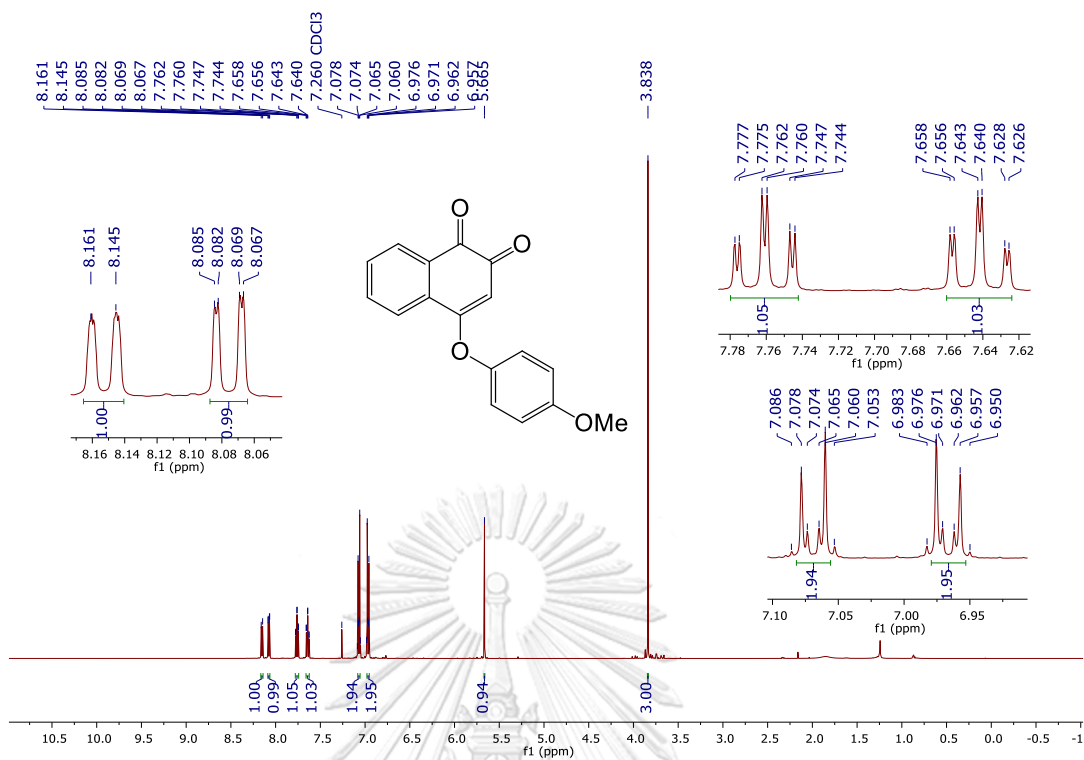
**Figure A.62** $^1$H-NMR (DMSO-$d_6$, 500 MHz) of **49.**
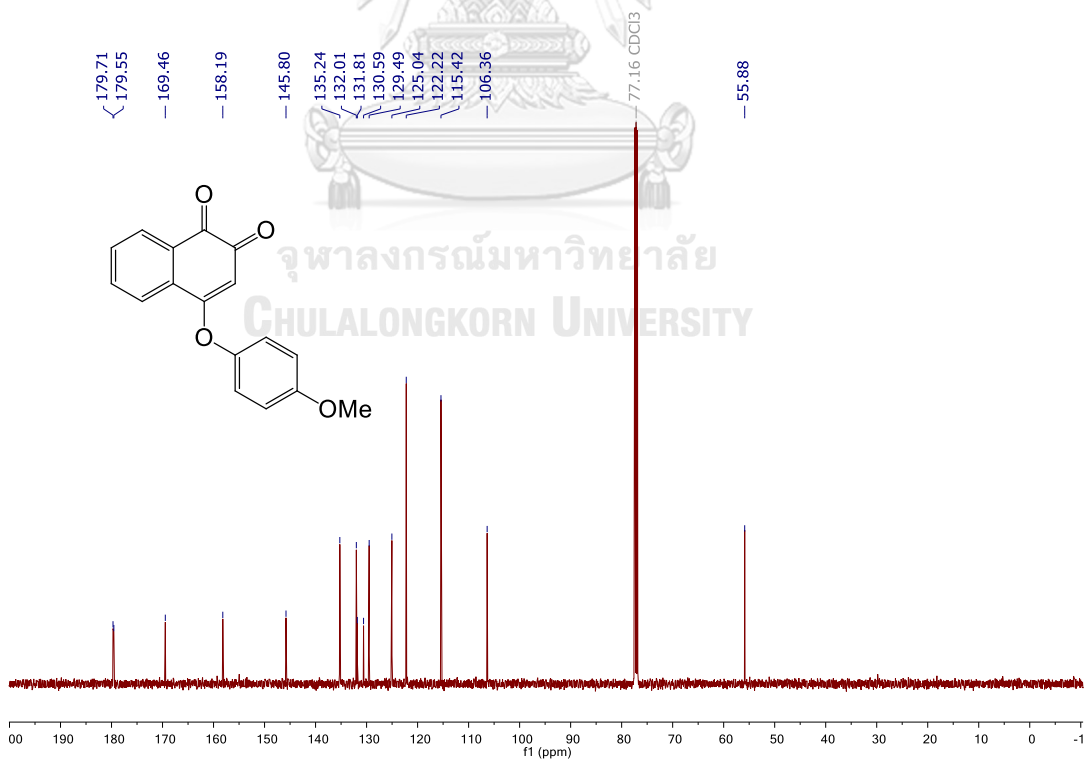


**Figure A.63** $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) of **49.**
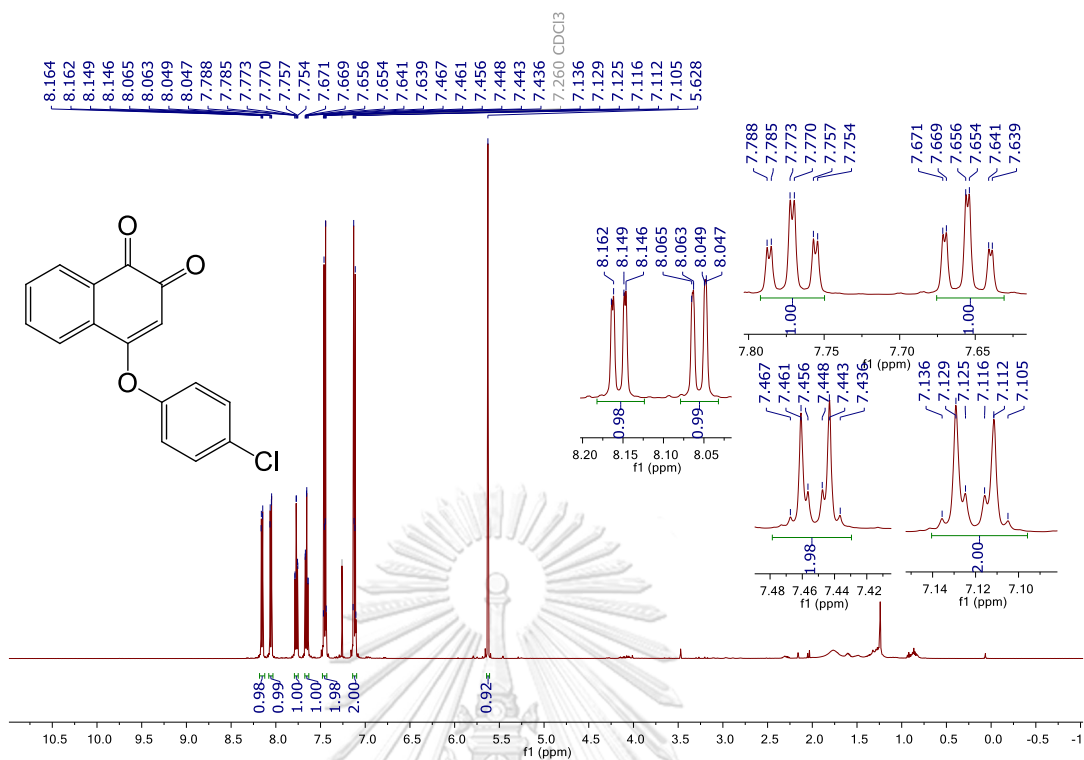
Figure A.64 $^1$H-NMR (CDCl$_3$, 500 MHz) of **50.**
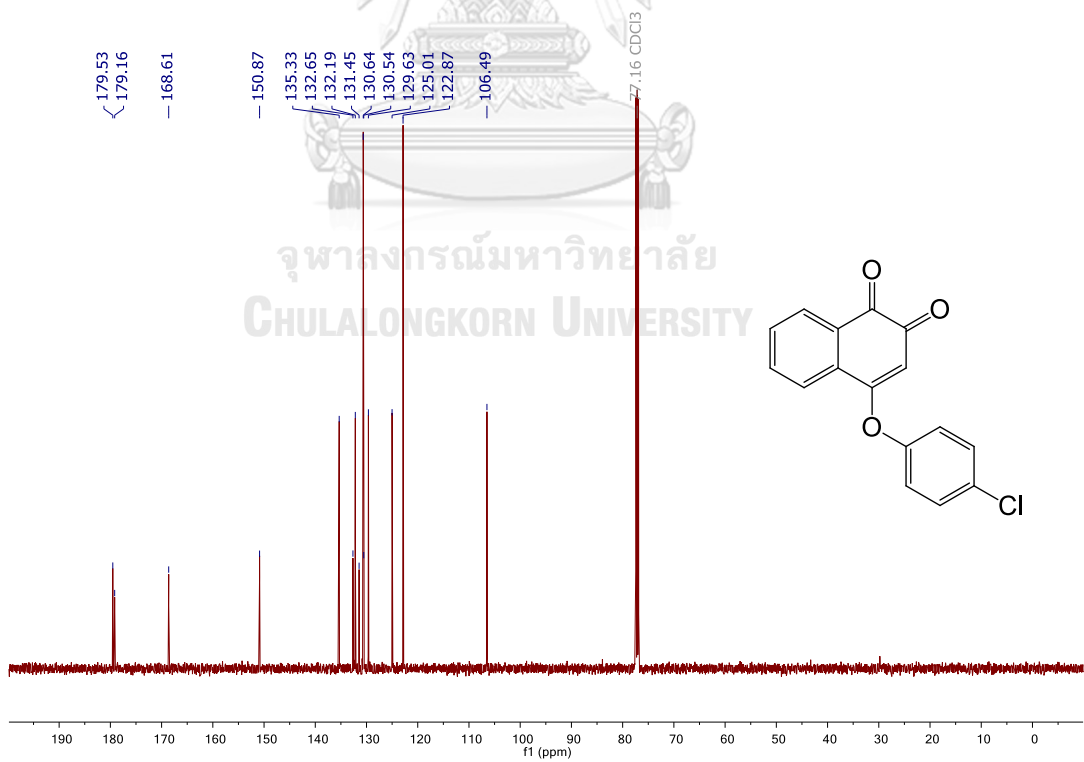


Figure A.65 $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **50.**
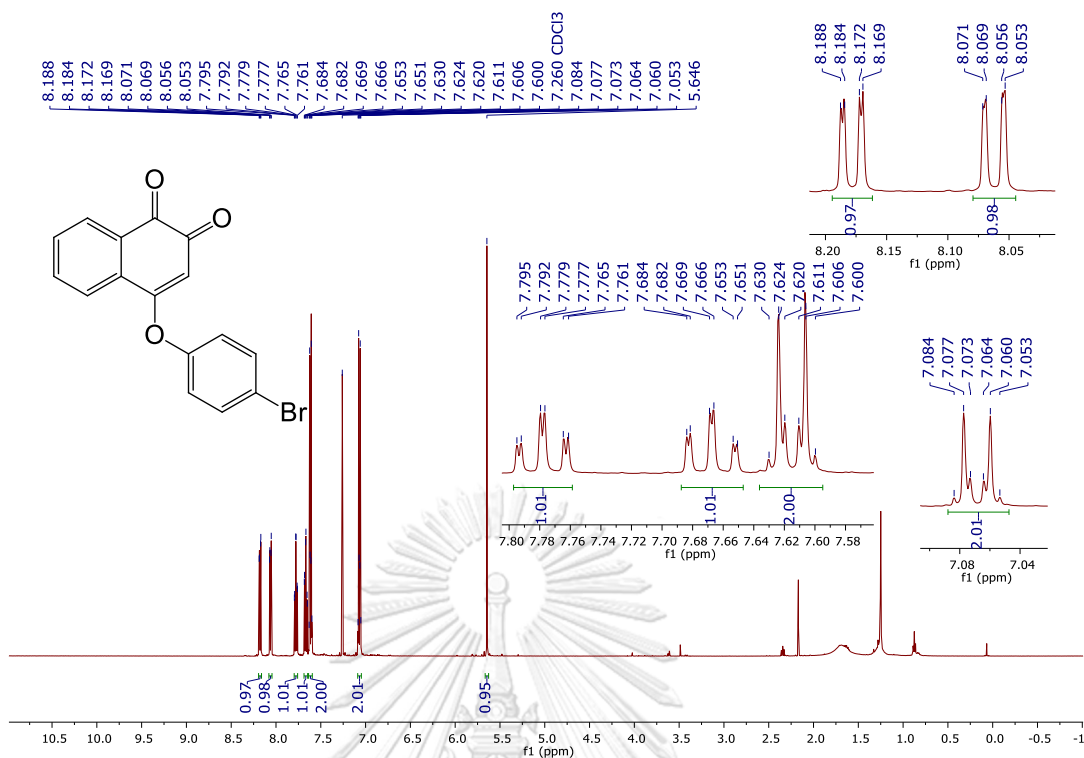
Figure A.66 $^1$H-NMR (CDCl$_3$, 500 MHz) of **51.**
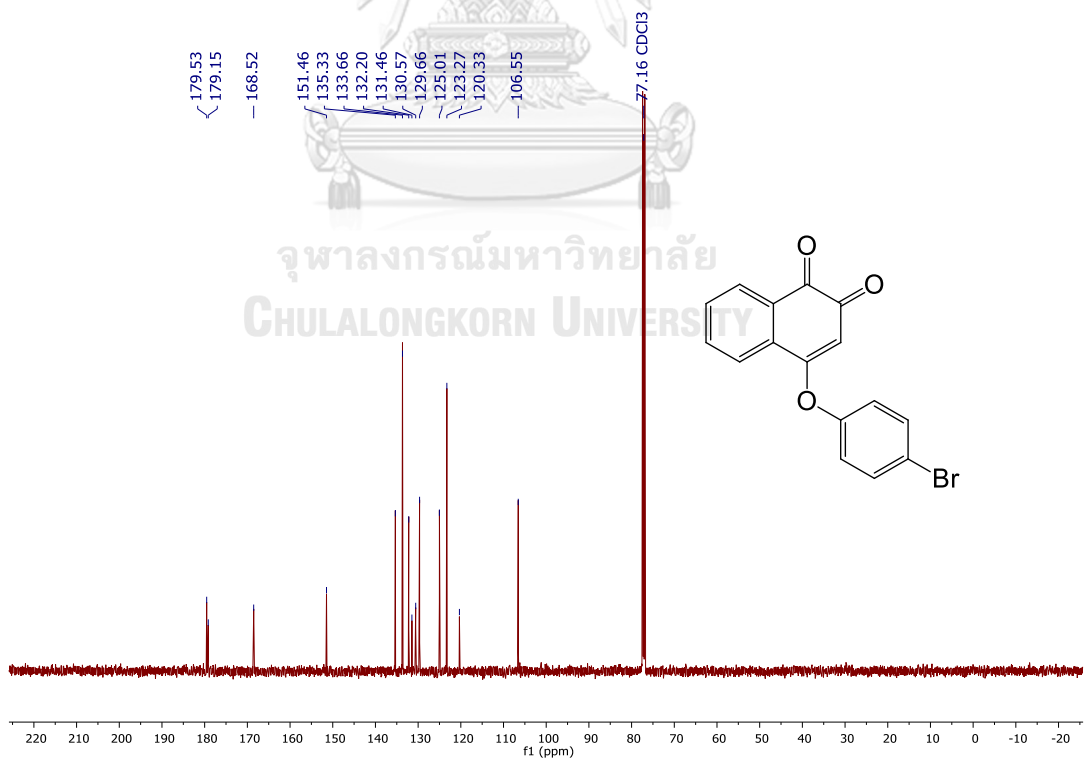


Figure A.67 $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **51.**

**Figure A.68** $^1$H-NMR (CDCl$_3$, 500 MHz) of **52.**



**Figure A.69** $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **52.**

## Generic Display Report
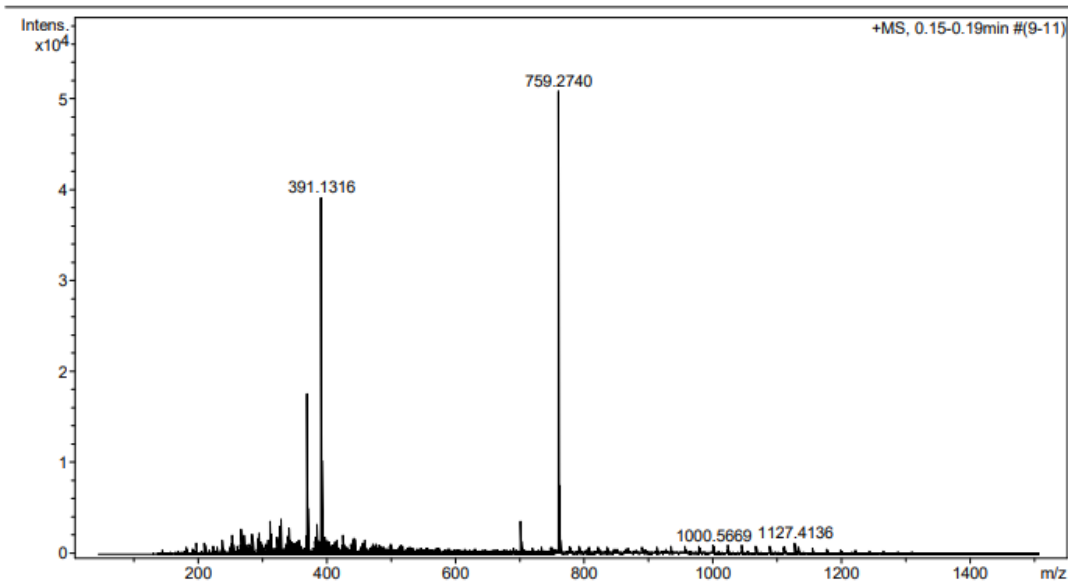
Figure A.70 HRMS of 52.
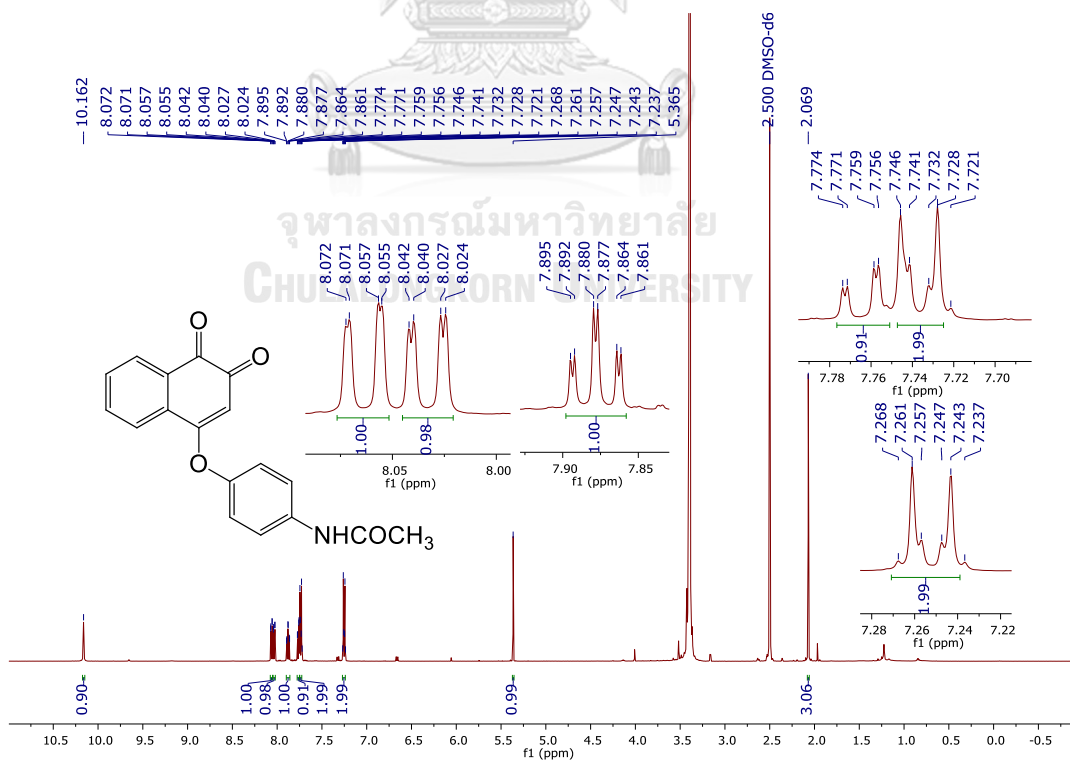


Figure A.71 $^1$H-NMR (DMSO-$d_6$, 500 MHz) of 53.
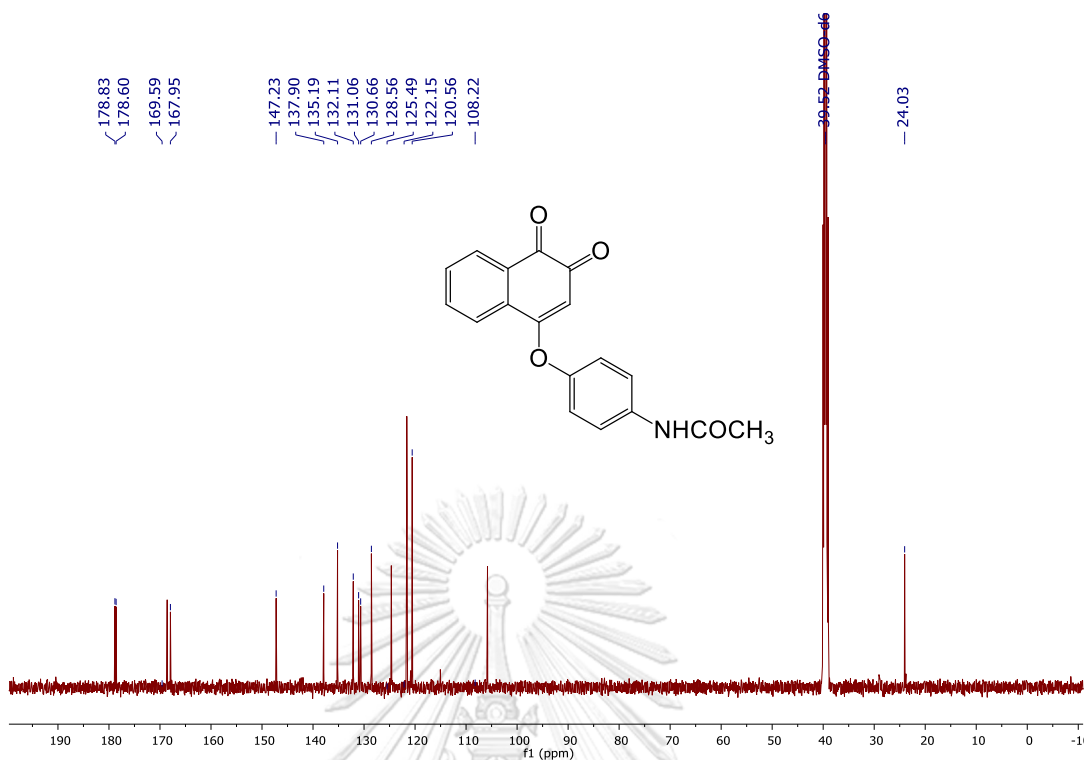
Figure A.72 $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) of **53**.
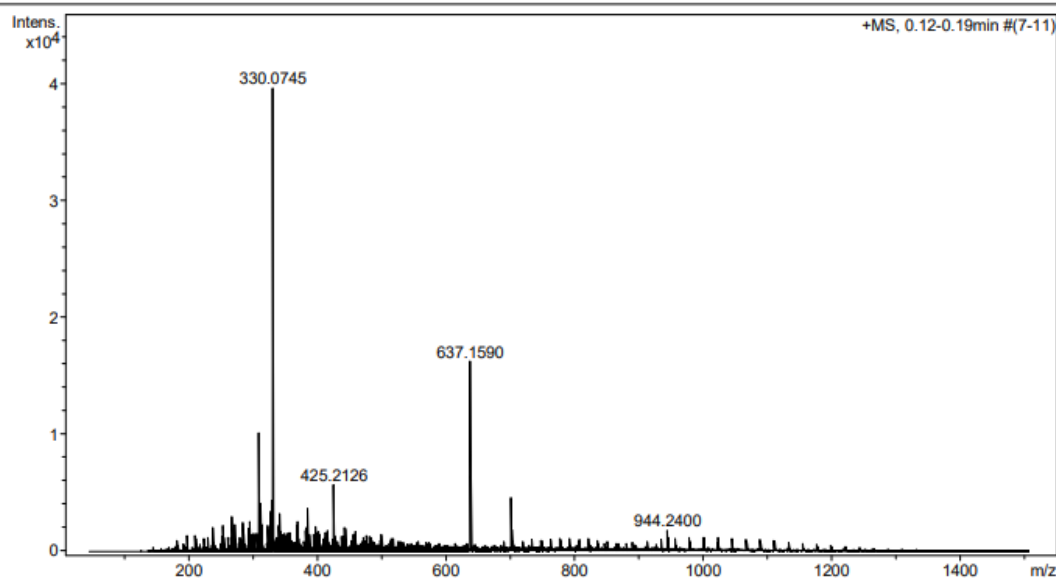


Figure A.73 HRMS of **53**.

Figure A.74 $^1$H-NMR (CDCl$_3$, 500 MHz) of **54.**
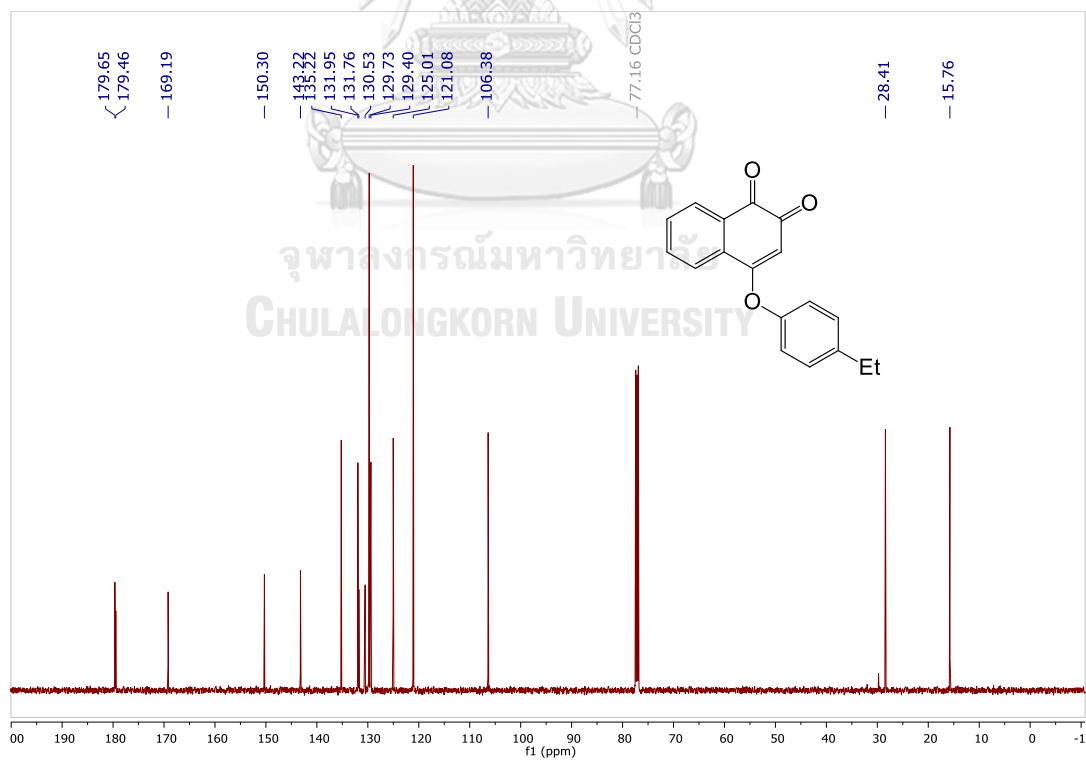


Figure A.75 $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **54.**
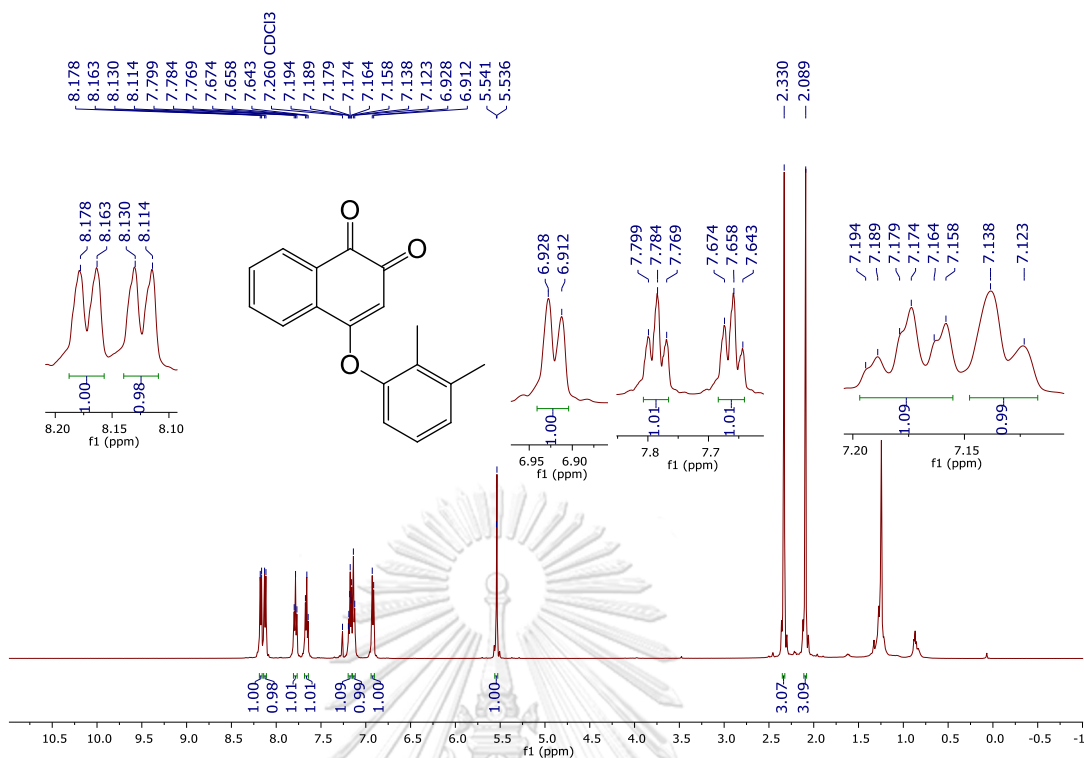
## Generic Display Report
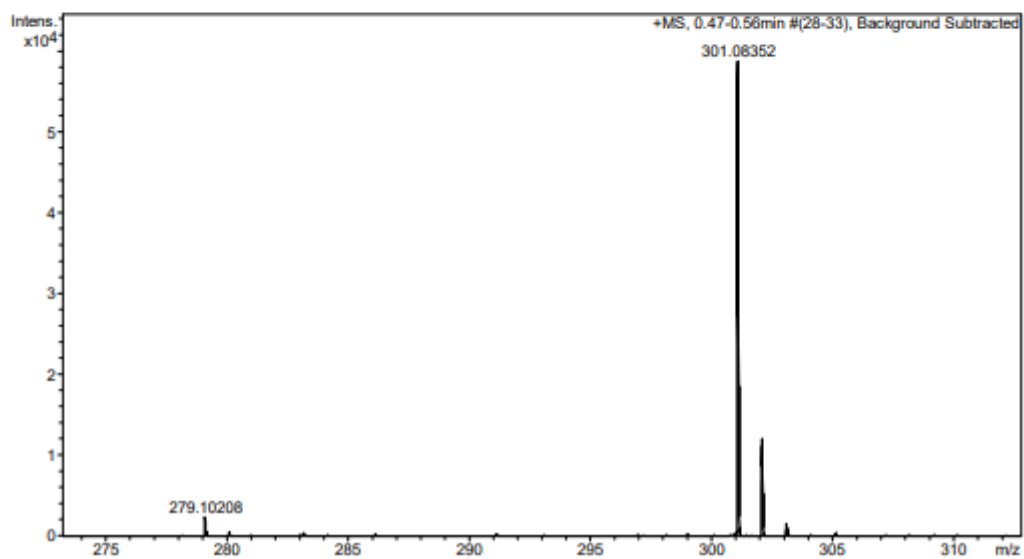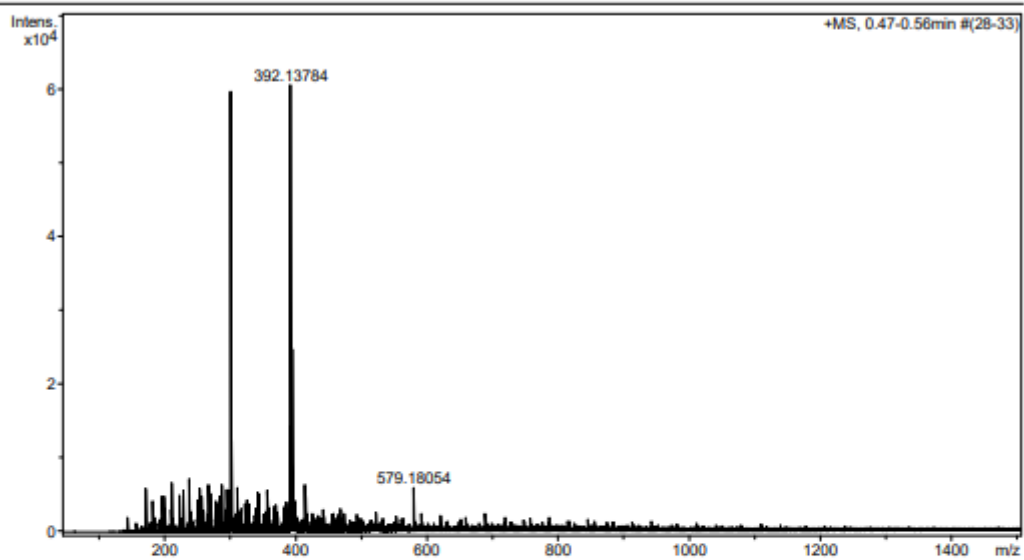
Figure A.76 HRMS of 54.

Figure A.77 $^1$H-NMR (CDCl$_3$, 500 MHz) of **55.**
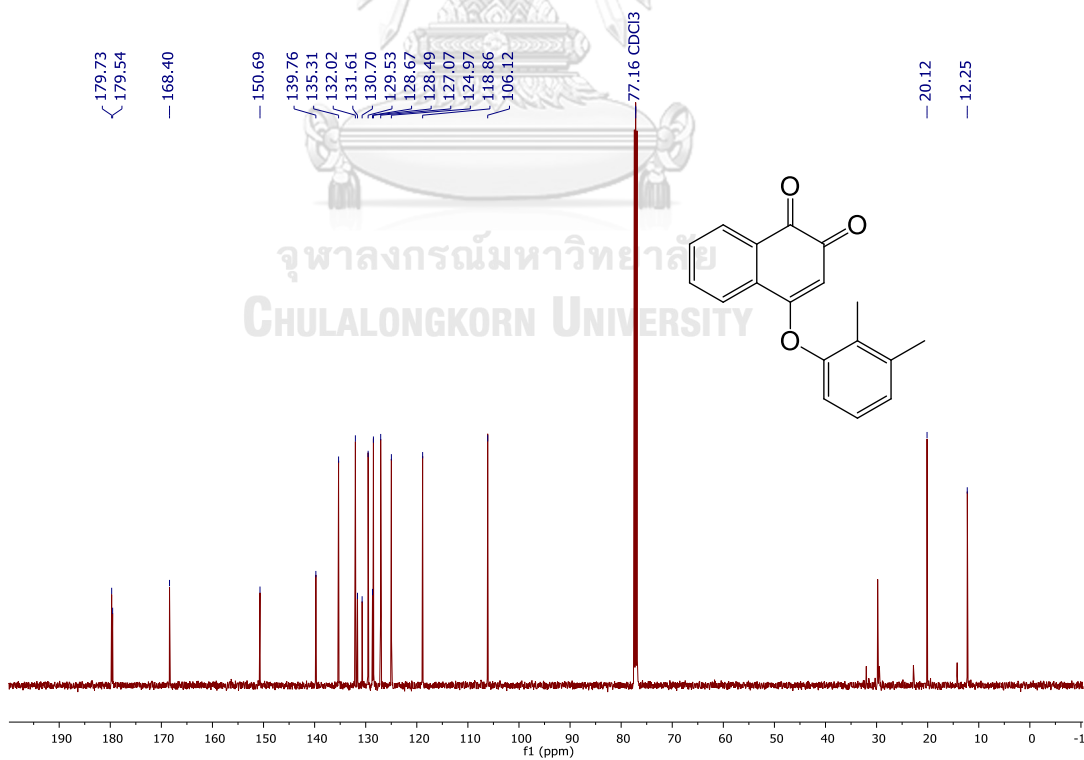


Figure A.78 $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **55.**

## Generic Display Report

**Figure A.79** HRMS of **55.**



**Figure A.80** $^1$H-NMR (CDCl$_3$, 500 MHz) of **56.**
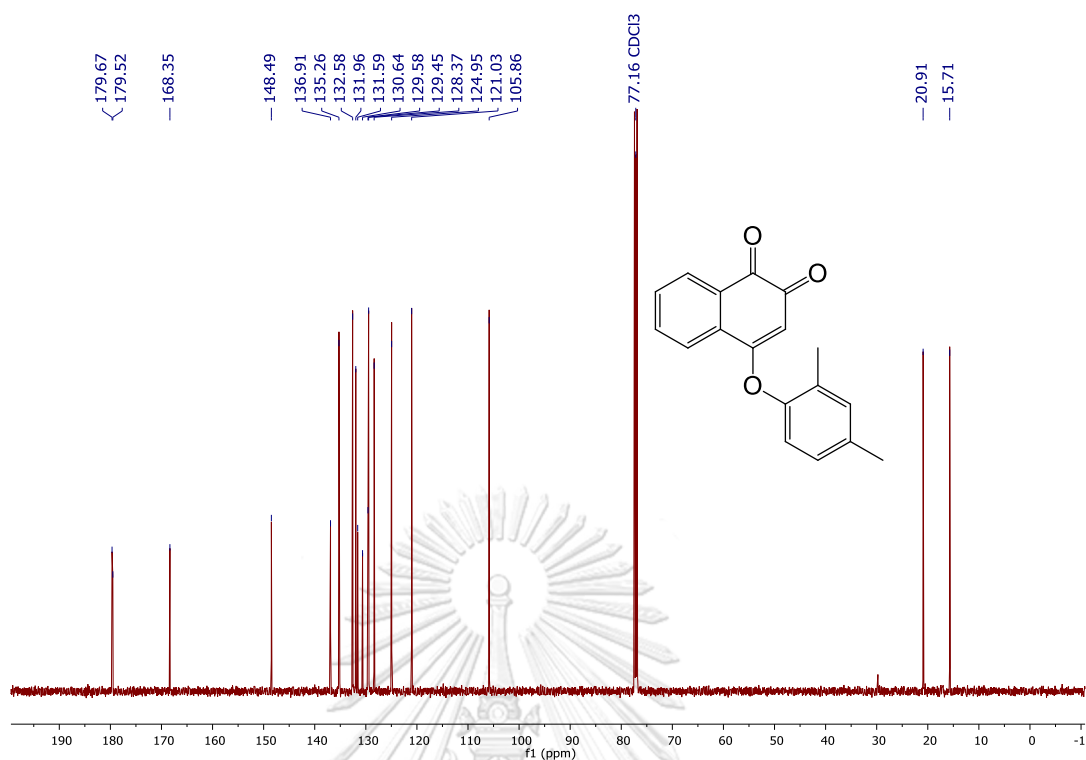
Figure A.81 $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **56**.

## Generic Display Report

**Analysis Info**

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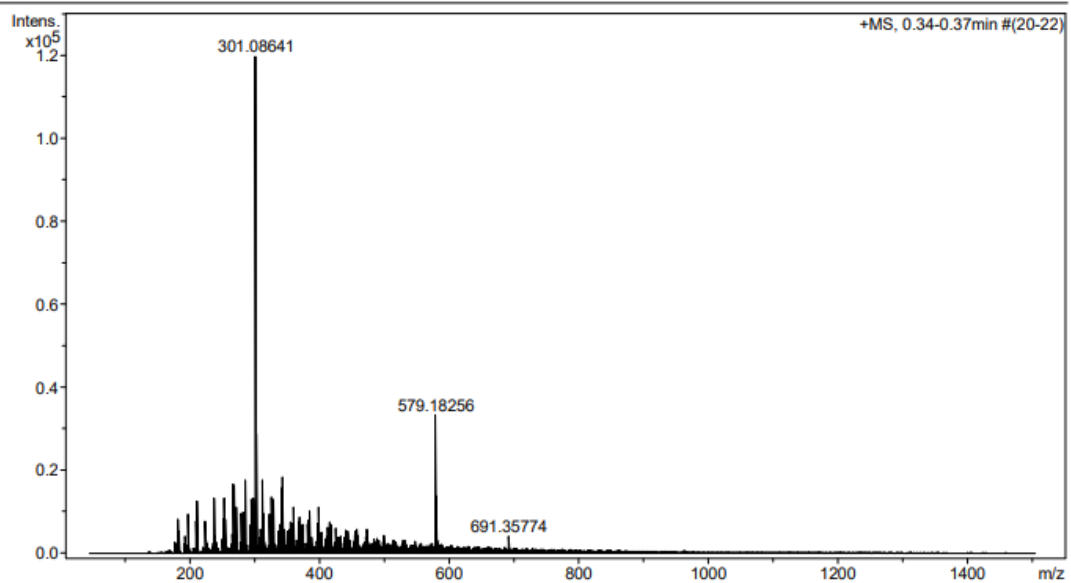


Figure A.82 HRMS of **56**.
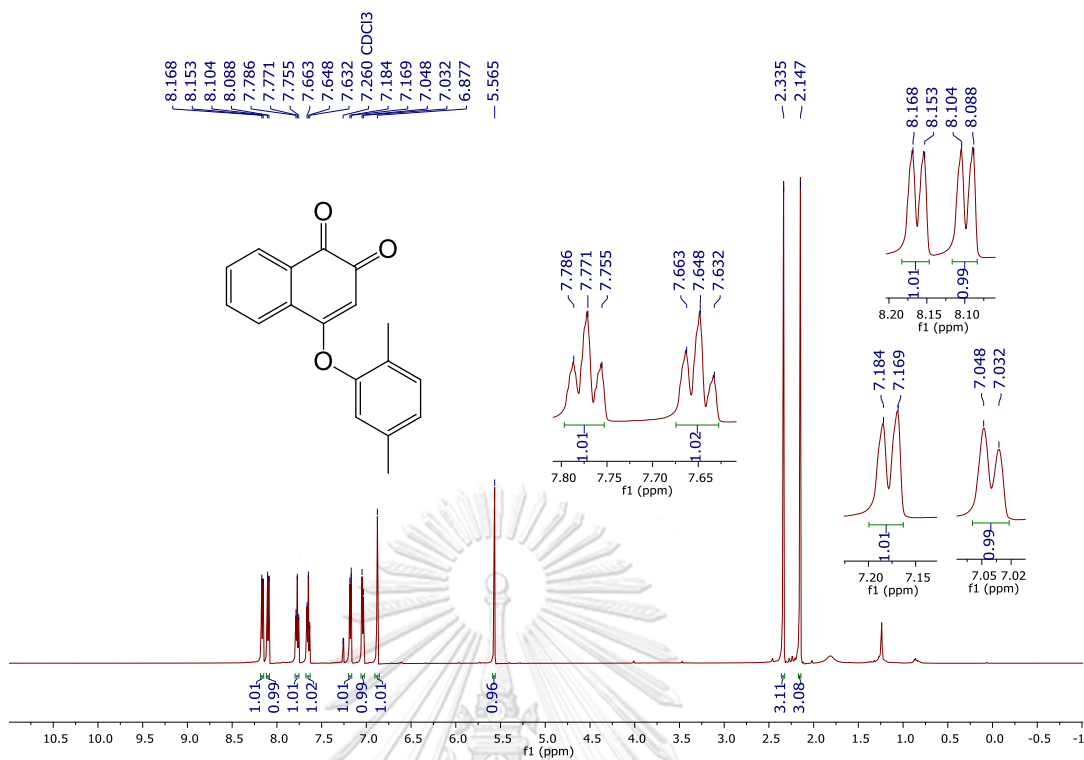
Figure A.83 $^1$H-NMR (CDCl$_3$, 500 MHz) of **57.**
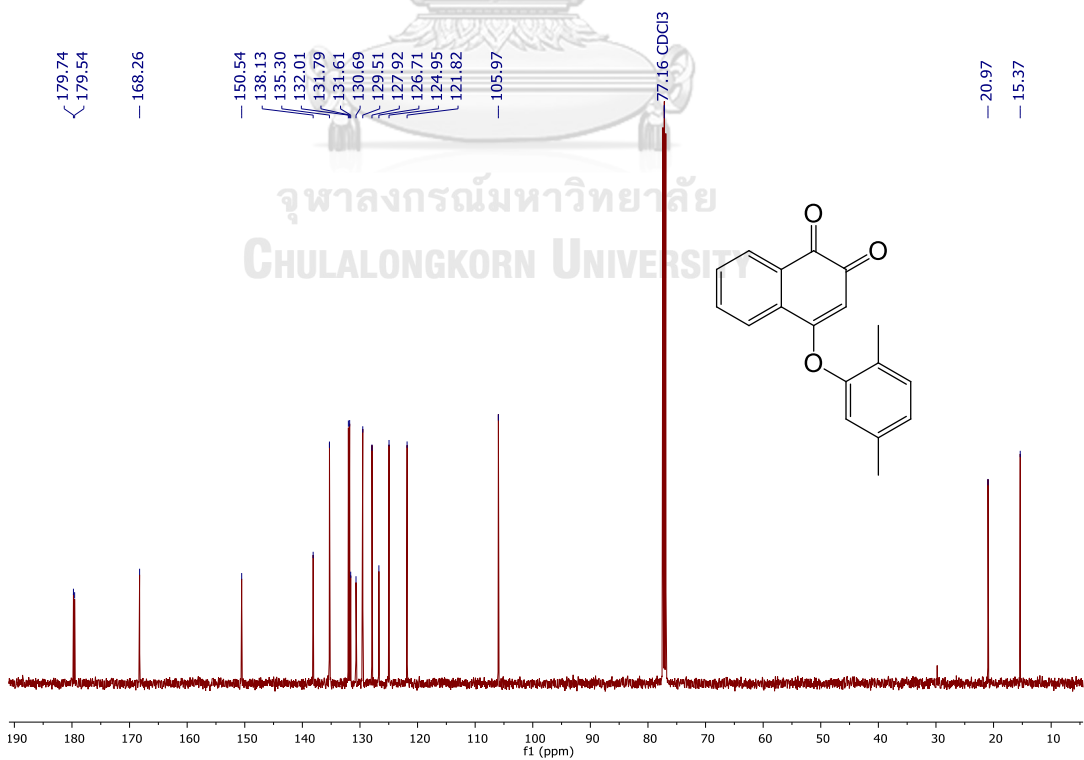


Figure A.84 $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **57.**
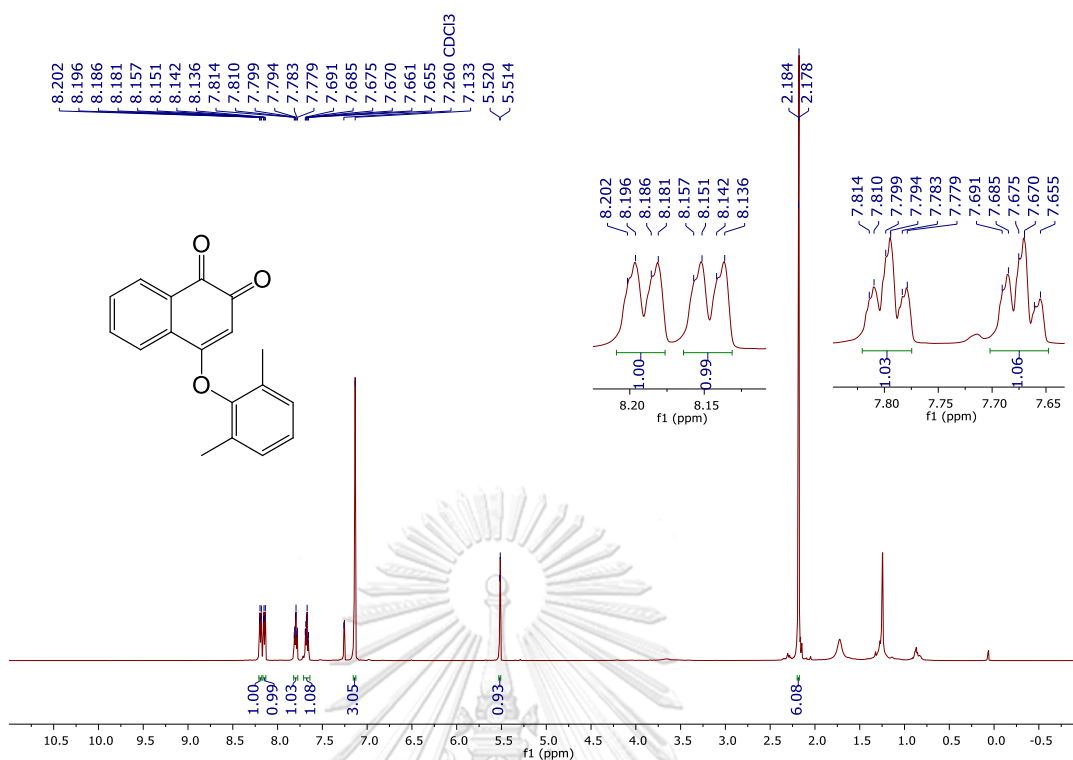
Figure A.85 $^1$H-NMR (CDCl$_3$, 500 MHz) of **58**.
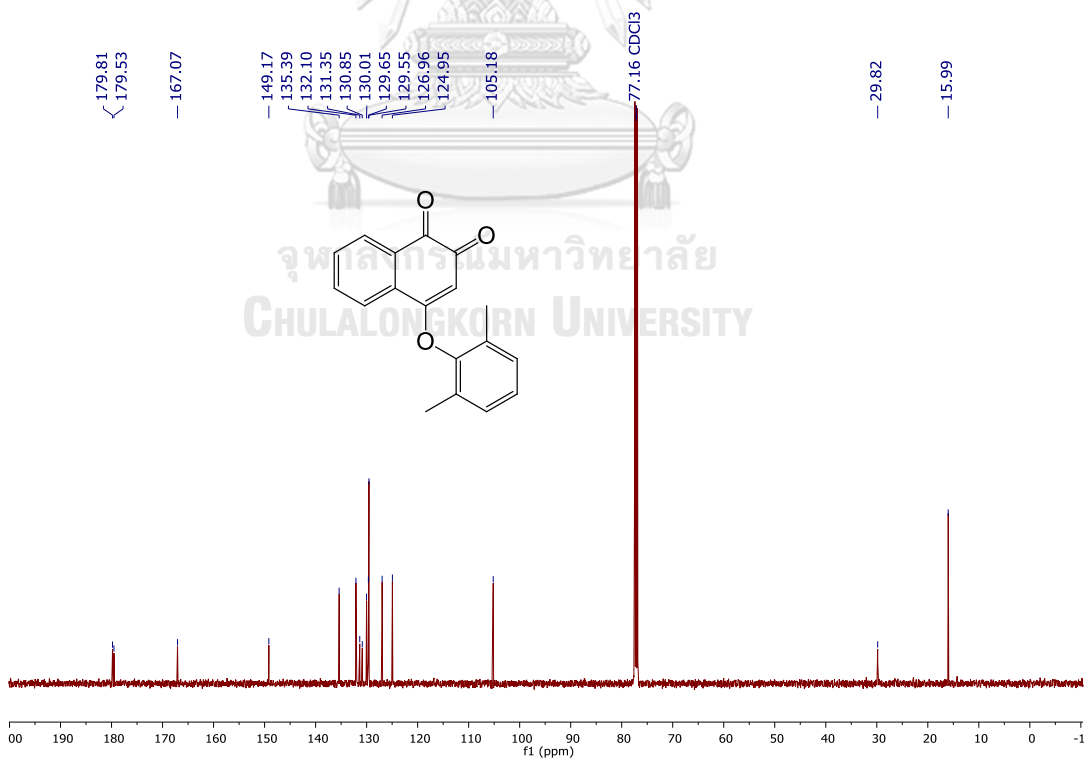


Figure A.86 $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **58**.

## Generic Display Report
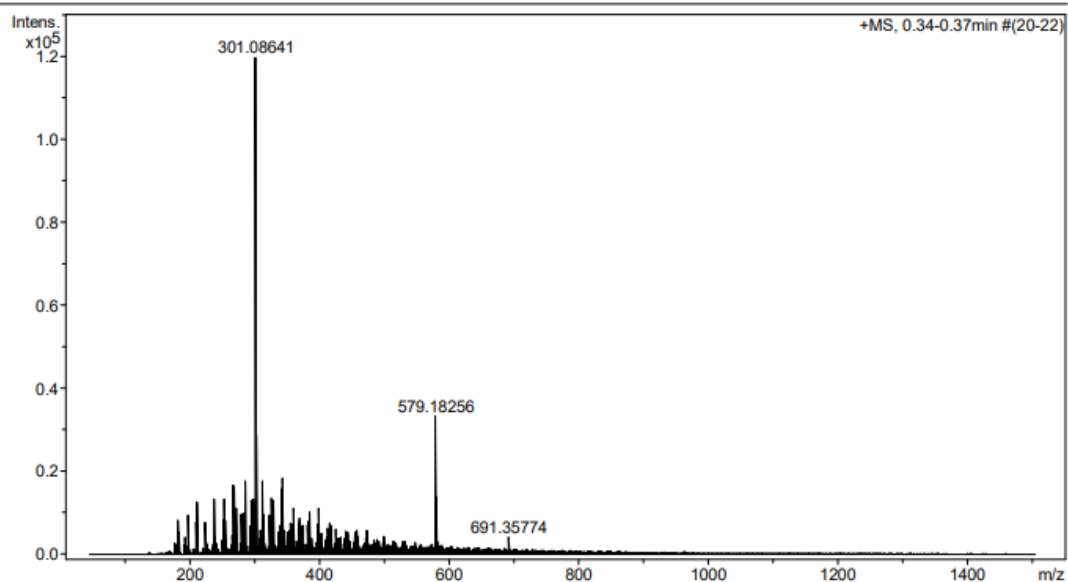
**Figure A.87** HRMS of **58.**
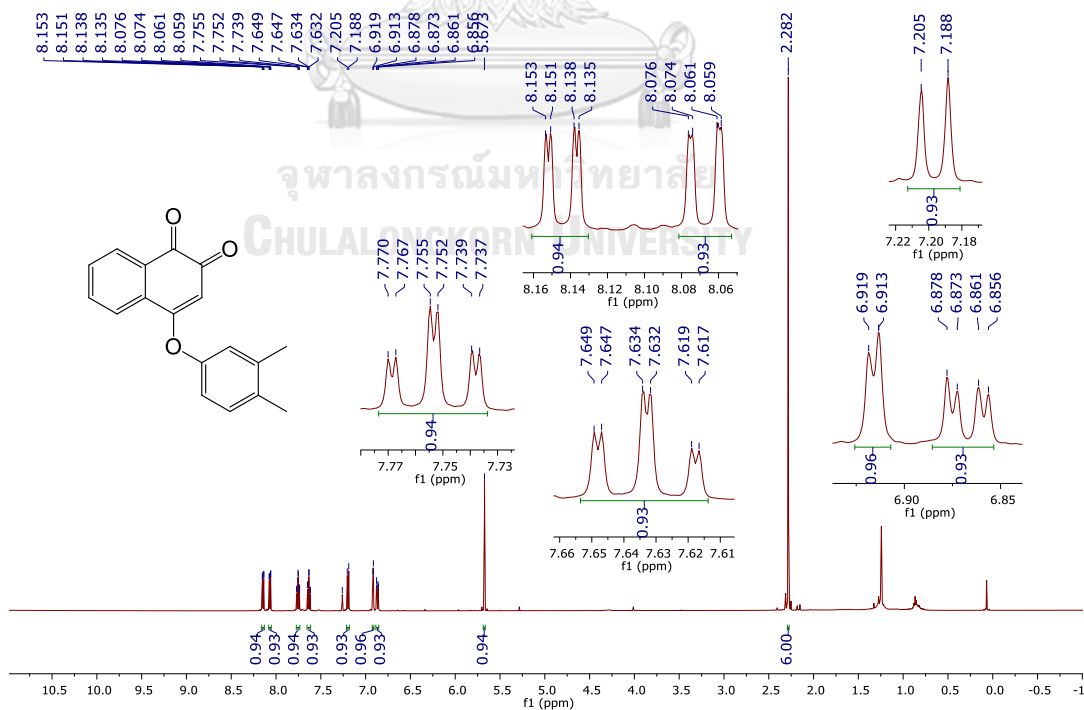


**Figure A.88** $^1$H-NMR (CDCl$_3$, 500 MHz) of **59.**
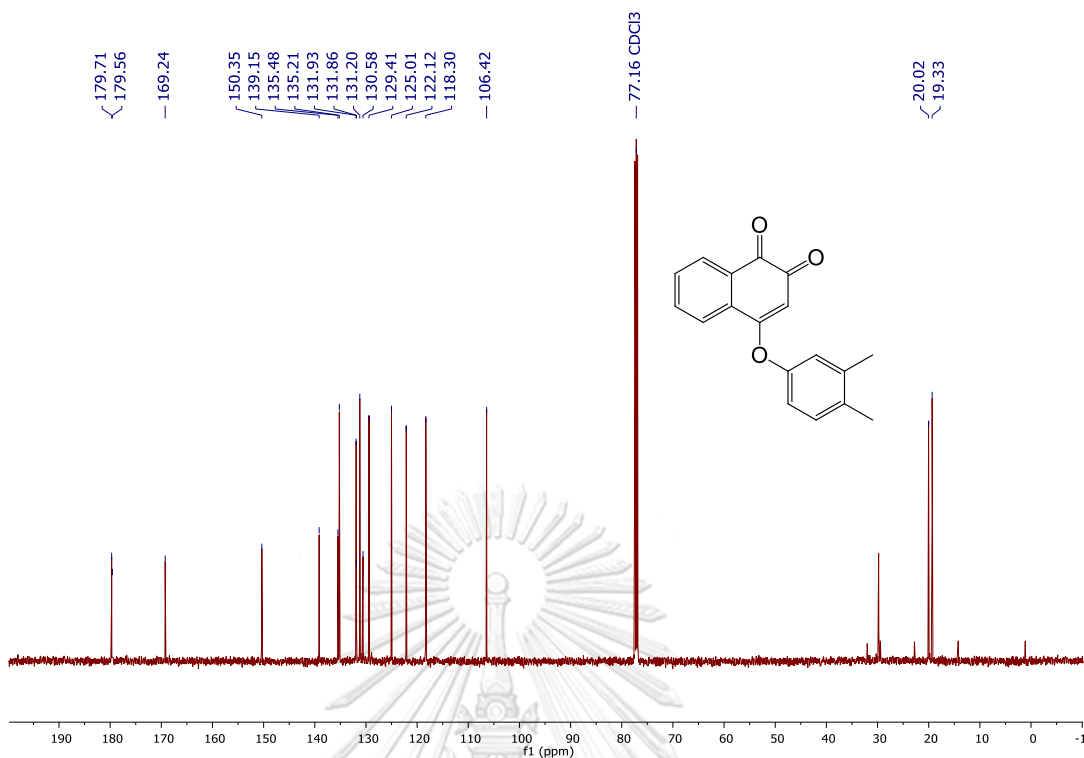
Figure A.89 $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **59.**
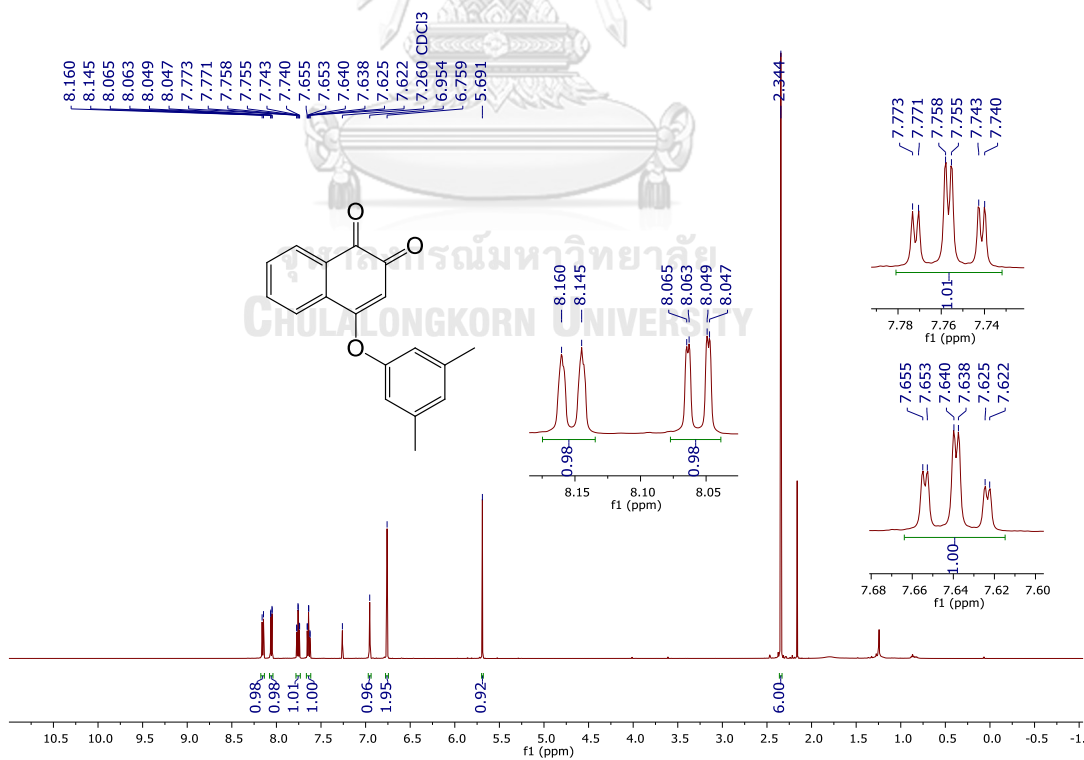


Figure A.90 $^1$H-NMR (CDCl$_3$, 500 MHz) of **60.**
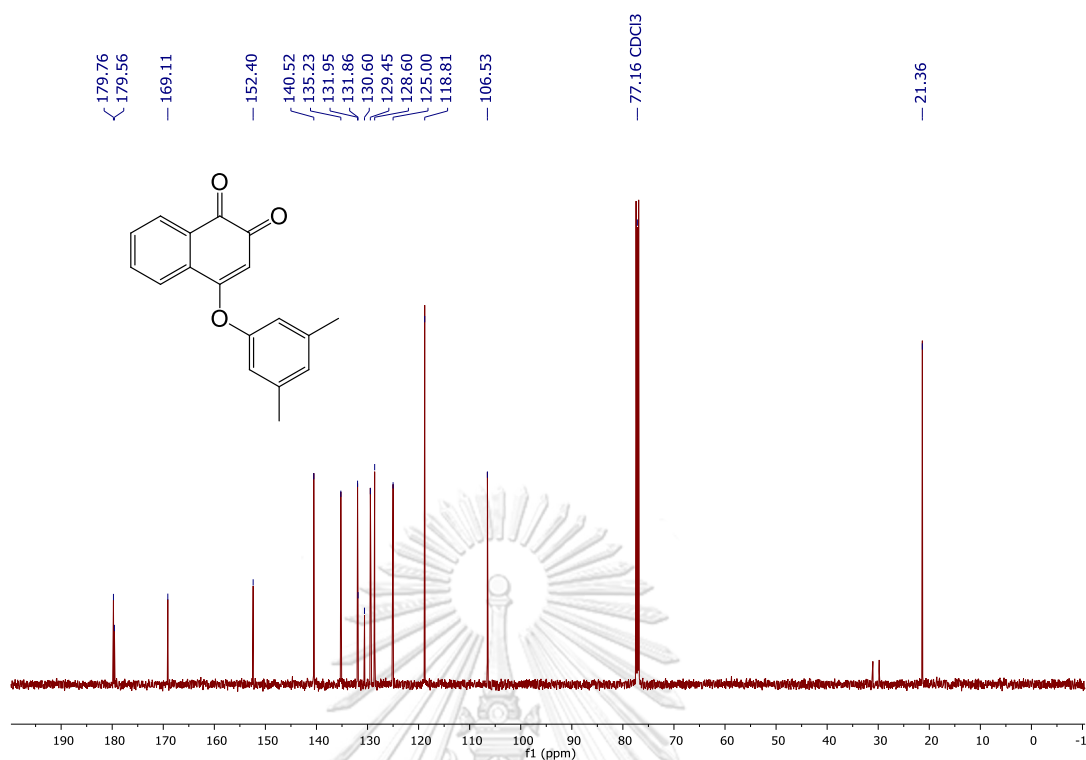
Figure A.91 $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **60**.

## Generic Display Report

**Analysis Info**

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| Comment | | | |
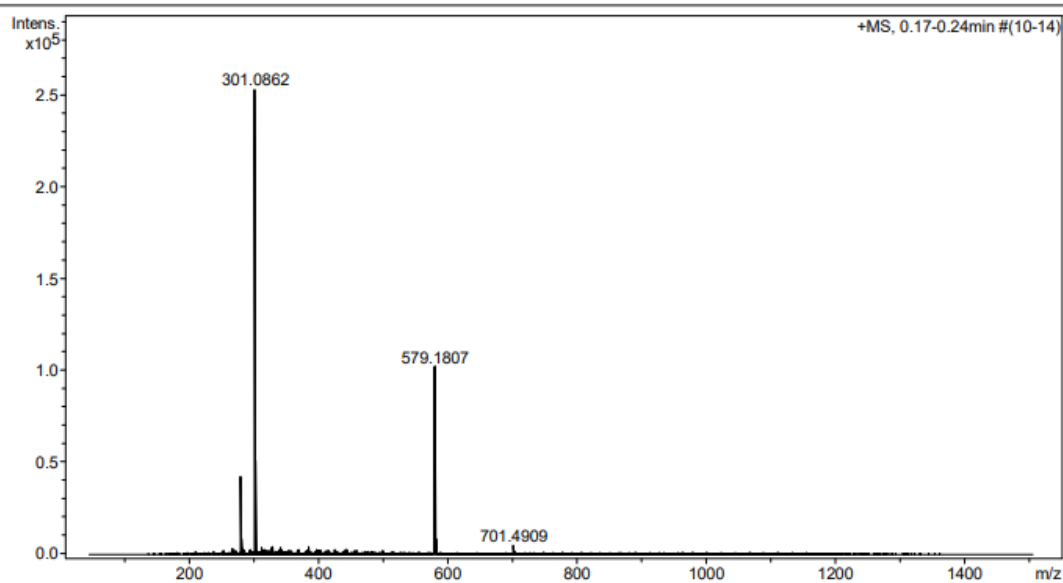


Figure A.92 HRMS of **60**.
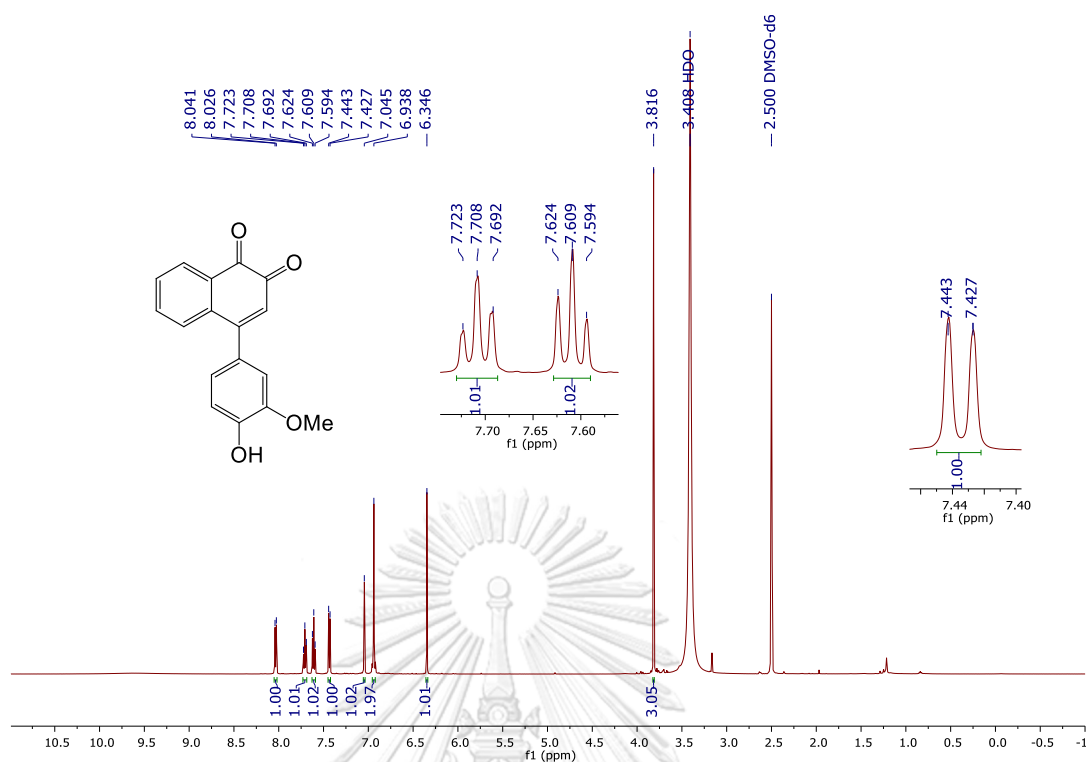
**Figure A.93** ^{1}H-NMR (DMSO-$d_6$, 500 MHz) of **61.**
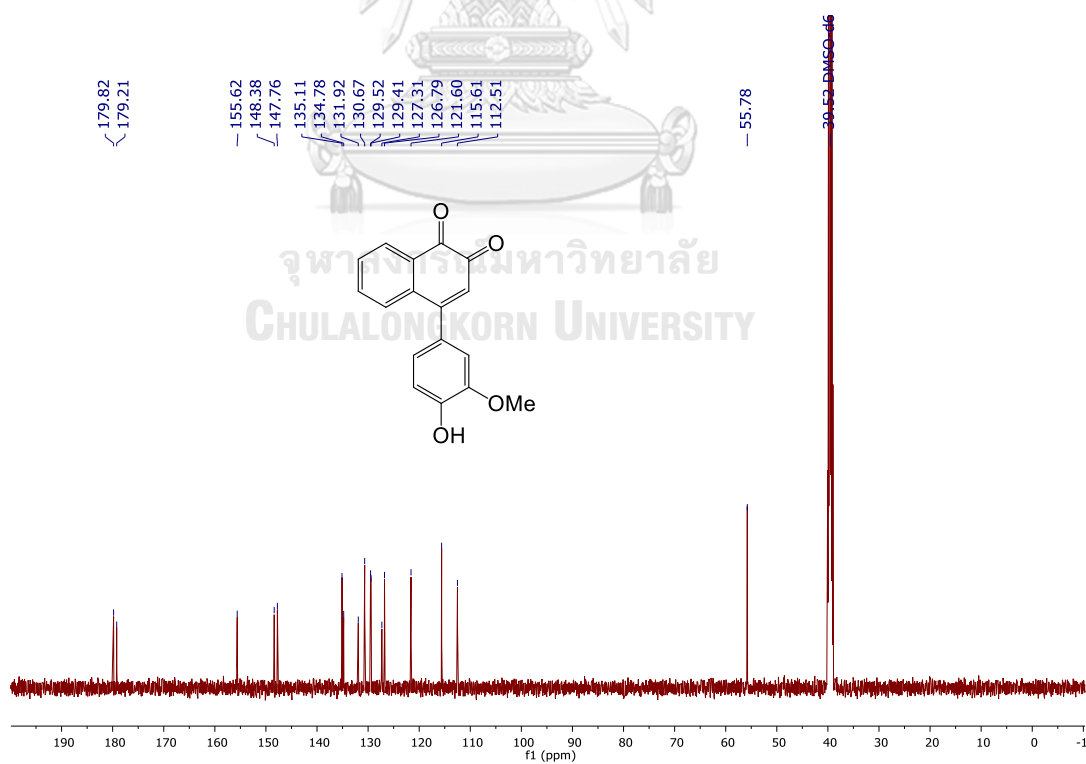


**Figure A.94** ^{13}C-NMR (DMSO-$d_6$, 125 MHz) of **61.**
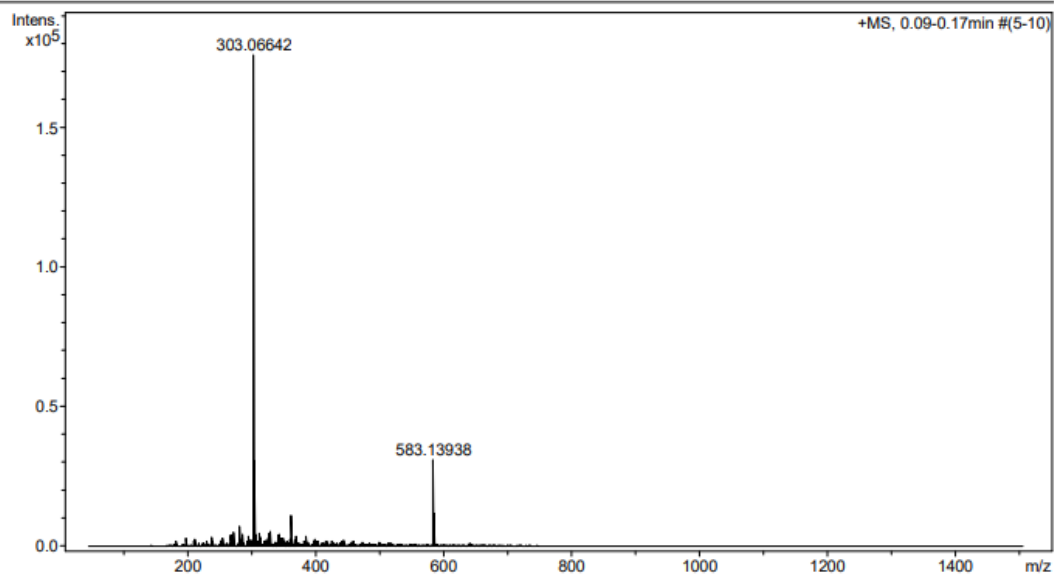
Figure A.95 HRMS of 61.
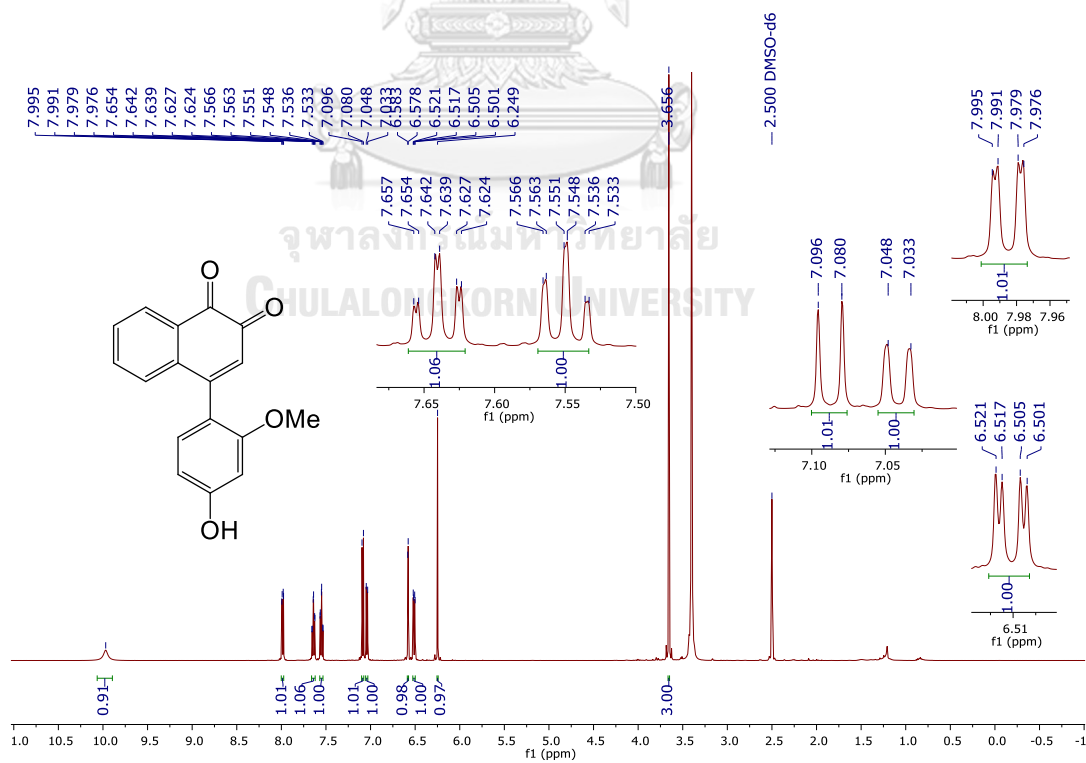


Figure A.96 $^{1}$H-NMR (DMSO-$d_6$, 500 MHz) of 62.
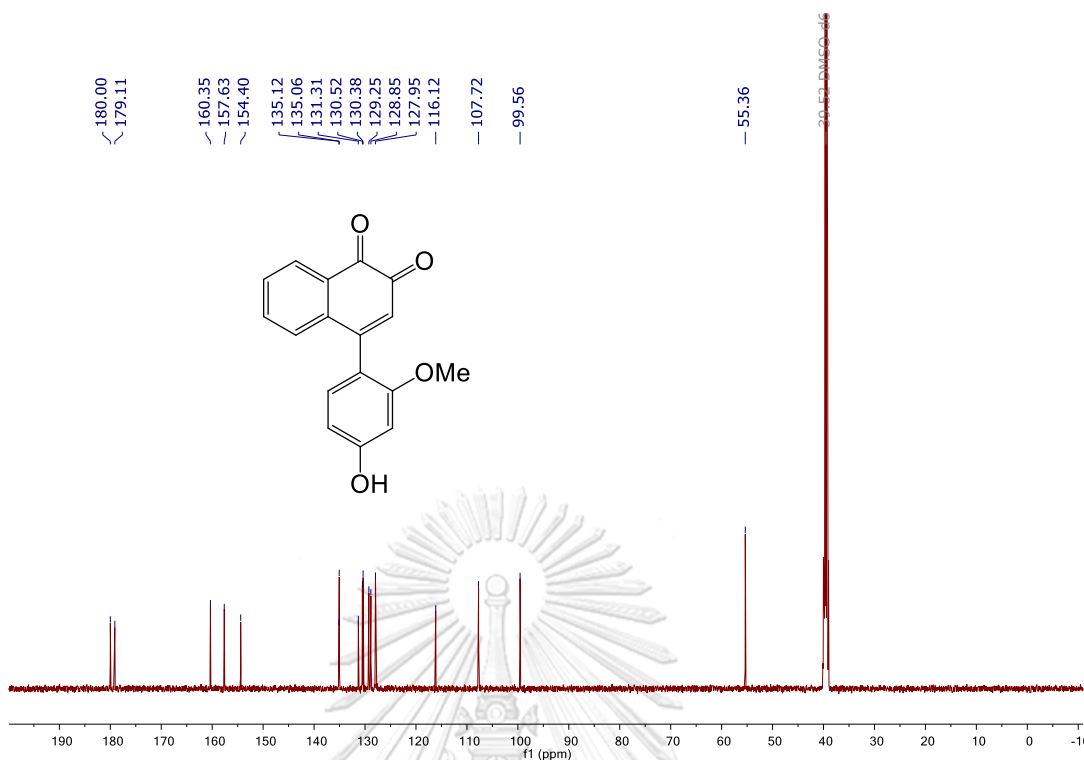
Figure A.97 $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) of **62.**
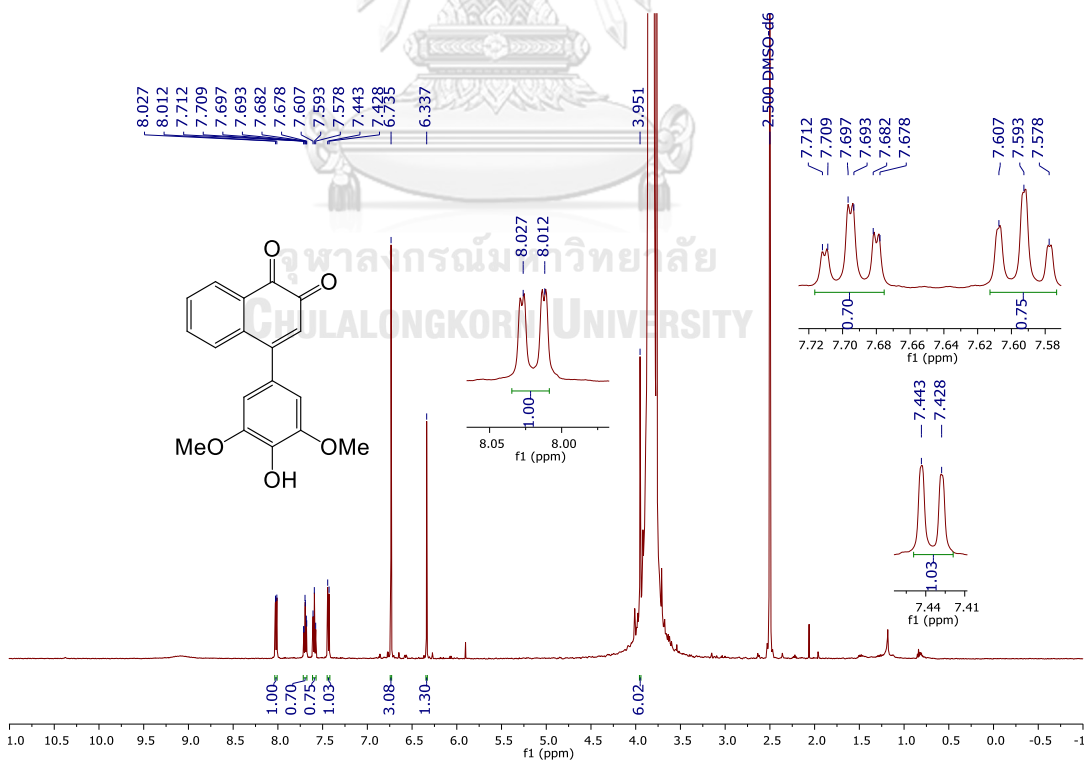


Figure A.98 $^1$H-NMR (DMSO-$d_6$, 500 MHz) of **63.**
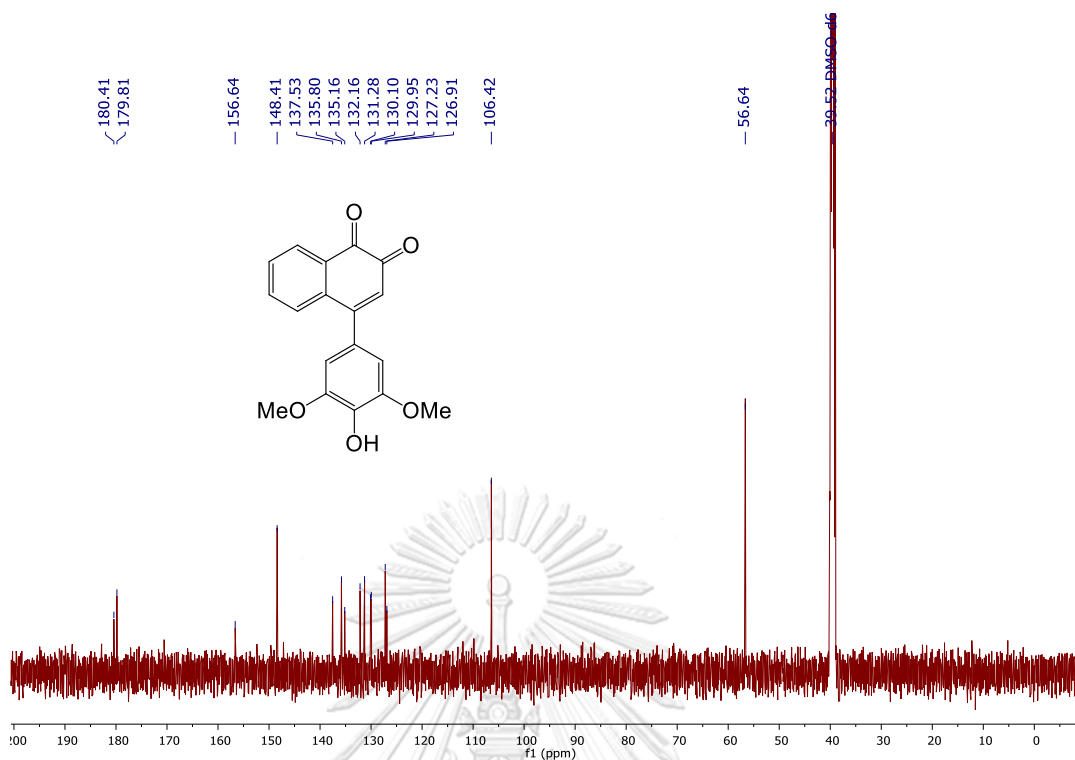
Figure A.99 $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) of **63**.

## Generic Display Report

**Analysis Info**

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| Comment | | | |
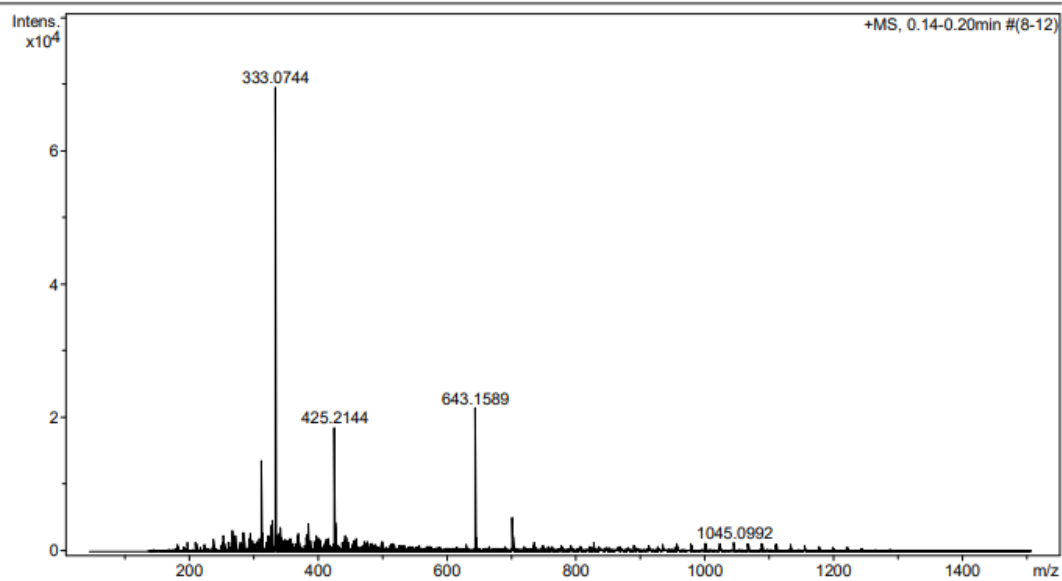


Figure A.100 HRMS of **63**.

**Figure A.101** $^{1}$H-NMR (CDCl$_3$, 500 MHz) of **64.**
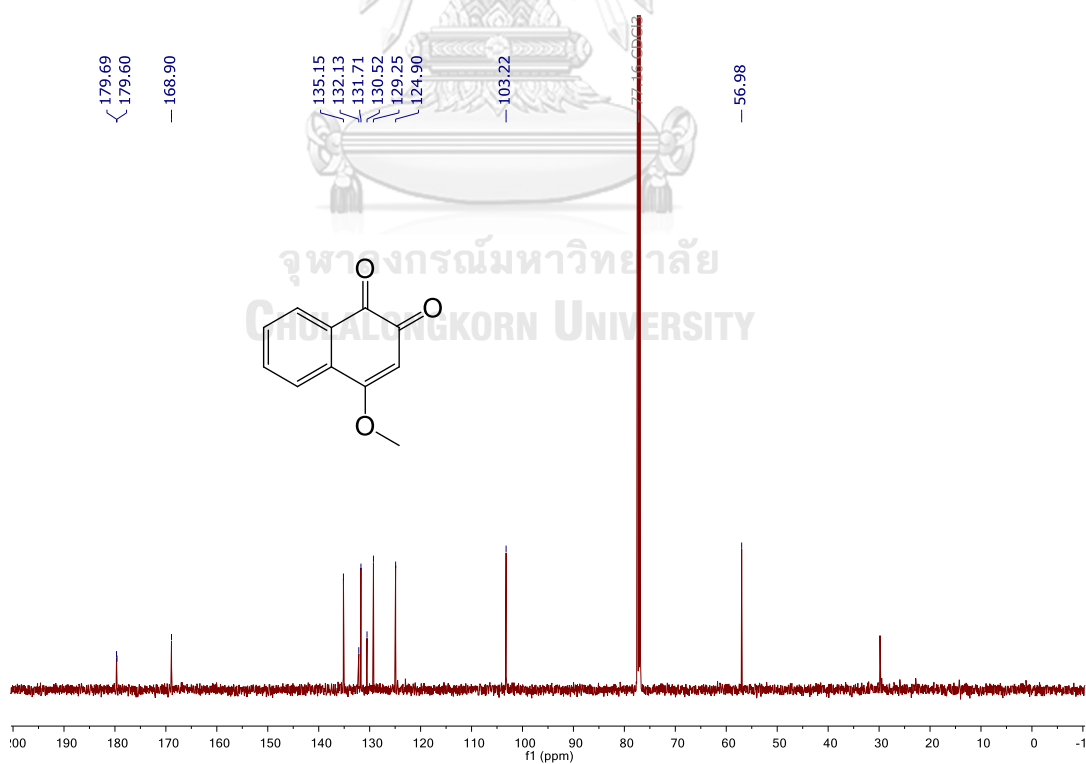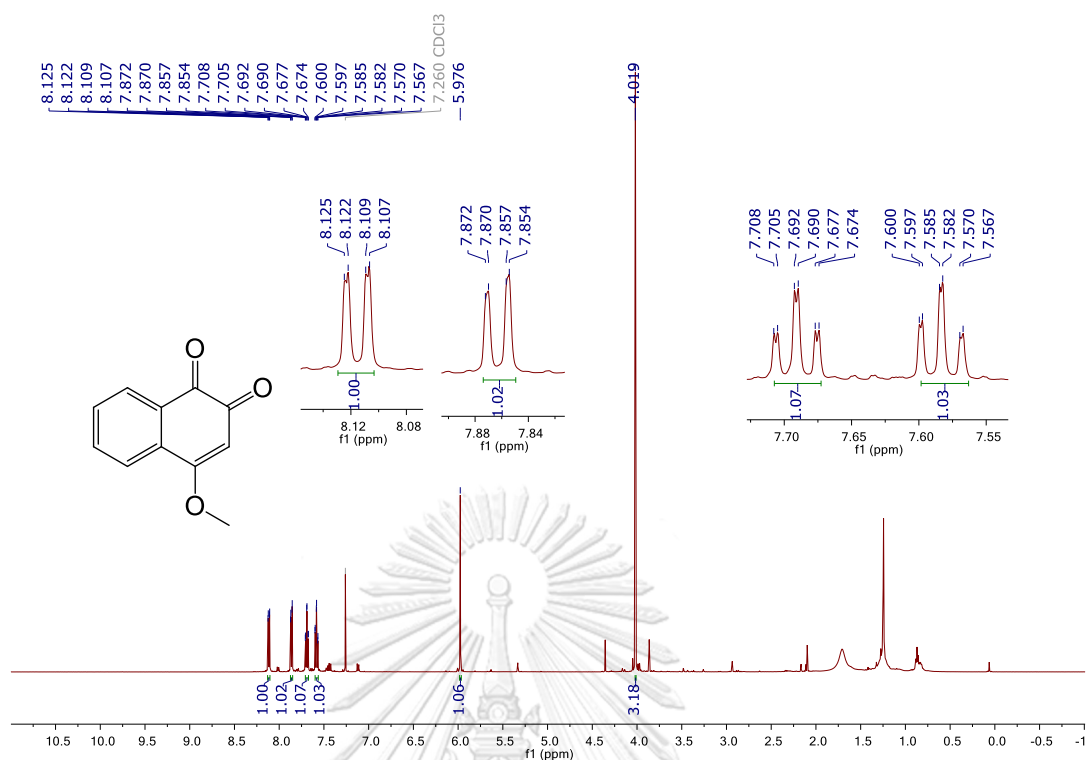


**Figure A.102** $^{13}$C-NMR (CDCl$_3$, 125 MHz) of **64.**
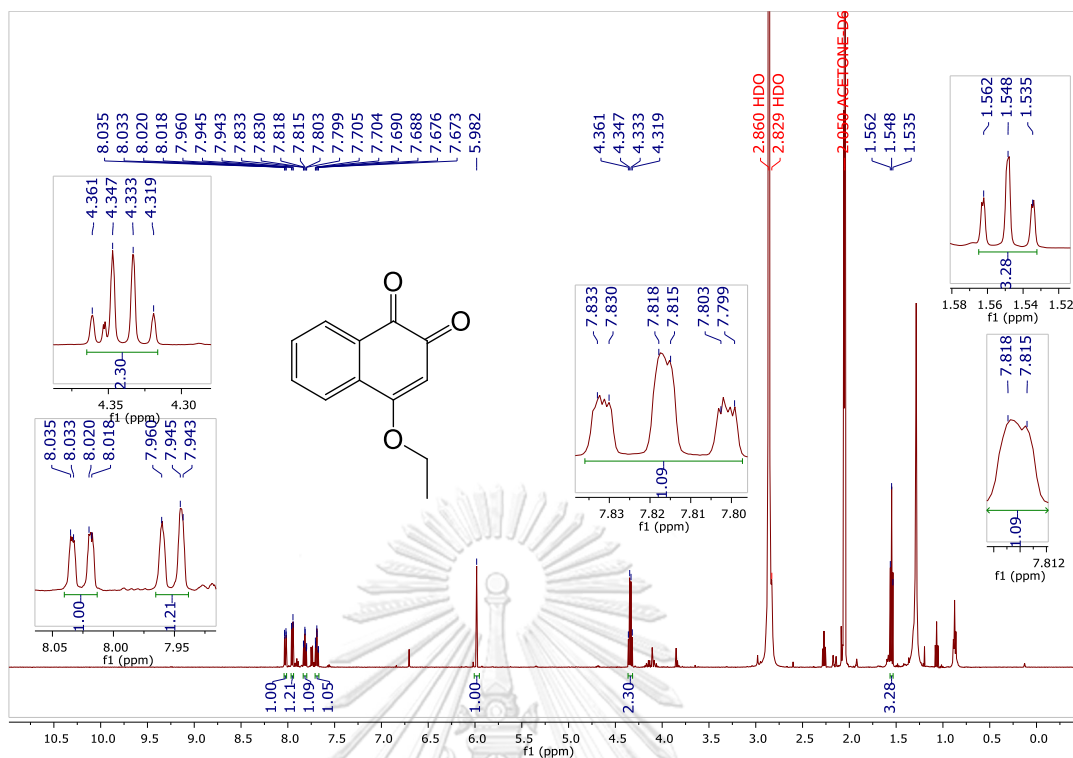
**Figure A.103** $^1$H-NMR (Acetone-$d_6$, 500 MHz) of **65.**

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