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SYNTHESIS OF QUINAZOLINE DERIVATIVES WITH ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITIES

Miss Yuparaid Uetrongchit

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Thesis Title	SYNTHESIS OF QUINAZOLINE DERIVATIVES WITH ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITIES
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ยุพเรศ เอื้อตรงจิตต์ : การสังเคราะห์อนุพันธ์ควินาโซลีนที่มีฤทธิ์ยับยั้งแอซีทิลโคลีนเอสเทอเรสและบิวทิริลโคลีนเอสเทอเรส. (SYNTHESIS OF QUINAZOLINE DERIVATIVES WITH ACETYLCHOLINESTERASEAND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITIES) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ดร. พัฒทรา สวัสดี, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม อ. ดร. สัมฤทธิ์ วัชรสินธุ์, 90 หน้า.

ควินาโซลีนเป็นสารที่น่าสนใจและมีประโยชน์ในทางเคมียาเนื่องจากมีฤทธิ์ทางยาได้แก่ ต้านแบคทีเรีย ต้านมะเร็ง ต้านเนื้องอก ต้านเบาหวาน ต้านการอักเสบ บรรเทาอาการปวด ต้านไวรัส เป็นต้น ในการศึกษานี้ได้ทำการประเมินฤทธิ์ยับยั้งเอนไซม์แอซีทิลโคลีนเอสเทอเรส (AChE) และบิวทิริลโคลีนเอสเทอเรส(BChE)ของควินาโซลีน อันดับแรกได้สังเคราะห์สารควินาโซลีนที่มีหมู่แทนที่ในตำแหน่งที่4โดยปฏิกิริยาอะมิเนชันและทดสอบฤทธิ์พบว่า 4-piperidinoquinazoline (**12e**)มีฤทธิ์ยับยั้งเอนไซม์ AChE และ BChE ได้ดีที่สุดในชุดนี้ มีค่า IC_{50} เท่ากับ 1.3 และ 7.5 μM ตามลำดับ ต่อไปนำ4-butylaminoquinazoline (**12a**), 4-piperidinoquinazoline (**12e**)และ 4-morpholinoquinazoline (**12f**) ไปปรับเปลี่ยนโครงสร้างโดยใช้หมู่ดึงและหมู่ให้อิเล็กตรอน จากผลการทดสอบฤทธิ์ของสารที่สังเคราะห์ได้ไม่พบแนวโน้มจากการแทนที่ด้วยหมู่แทนที่เหล่านี้ในวงเบนซีนของโครงสร้างหลักของ **12a**, **12e** และ **12f** สารเหล่านี้อาจมีโหมดการออกฤทธิ์ที่แตกต่างกัน อย่างไรก็ตามในการศึกษาครั้งนี้ 4-piperidinoquinazoline(**12e**) ถือได้ว่าเป็นตัวยับยั้งเอนไซม์ AChE และ BChE 6-Nitro-4-butylaminoquinazoline(**16a**)เป็นตัวยับยั้งชนิดเลือกยับยั้ง AChE ได้ดีกว่า BChE ขณะที่ 6,7-dimethoxy-4-butylaminoquinazoline(**16d**) มีฤทธิ์เลือกยับยั้ง BChE ได้ดีกว่า AChE

สาขาวิชา... เทคโนโลยีชีวภาพ... ลายมือชื่อนิสิต.....

ปีการศึกษา.....2553..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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YUPARAID UETRONGCHIT : SYNTHESIS OF QUINAZOLINE DERIVATIVES WITH ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITIES. ADVISOR : PATTARA SAWASDEE, Ph.D., CO-ADVISOR : SUMRIT WACHARASINDHU, Ph.D., 90 pp.

Quinazolines were the attractive and useful compounds in medicinal chemistry due to their pharmacology activities such as antibacterial, anticancer, antitumor, antidiabetic, anti-inflammatory, analgesic and antiviral activities. In this study, the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of quinazolines derivatives were evaluated. The 4-substitued aminoquinazolines *via* amination reaction were first synthesized and found that 4-piperidinoquinazolines (**12e**) possess the highest activity with the IC_{50} values toward AChE and BChE of 1.3 μM and 7.5 μM, respectively. Then 4-butylaminoquinazolin (**12a**), 4-piperidinoquinazolines (**12e**) and 4-morpholinoquinazoline were chosen for further modified their structures by the electron-withdrawing and electron-releasing groups. From the results, there is no activity trend from these groups as substituents on a benzene ring of the core structures of **12a**, **12e** and **12f**. These might be the cause of the different mode of action in each compound. However, this study 4-piperidinoquinazolines (**12e**) was a candidate for both AChE and BChE inhibitors. 6-Nitro-4-butylaminoquinazoline (**16a**) was a potent and selective inhibitor toward AChE over BChE while 6,7-dimethoxy-4-butylaminoquinazoline (**16d**) was a potent and selective inhibitor toward BChE over AChE.

Field of Study : ….Biotechnology…. Student's Signature .....

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Asp (D)	aspartic acid (amino acid)
Glu (E)	glutamic acid (amino acid)
His (H)	histidine (amino acid)
Ser (S)	serine (amino acid)
Trp (W)	tryptophan (amino acid)
3D	three dimension
δ	chemical shift
d	doublet (NMR)
dd	doublet of doublet (NMR)
t	triplet (NMR)
m	multiplet (NMR)
br s	broad singlet (NMR)
J	coupling constant (NMR)
$CDCl_3$	chloroform deuterium
DMSO-d_6	deuterated dimethyl sulfoxide
mmol	millimole
h	hour
IC_{50}	the half maximal inhibitory concentration
µM	micromolar (concentration)
mM	millimolar (concentration)
µL	microliter (volume)
U/mL	unit per milliliter (concentration)
A.R. grade	analytical reagent grade

CHAPTER I
INTRODUCTION

Elder people have risked to be aging diseases, for examples, Alzheimer's disease (AD), cerebrovascular disease (vascular dementia), and dementia with Lewy bodies. AD is a common dementia that causes of a gradual declining in memory and learning ability in the elder people. AD progression can be divided approximately into three stages. In the first stage, AD patient's abilities or behavior are found to be changed a little, e.g. short-term memory loss. In the middle stage, the ability and behavior loss have increased such as increasing forgetfulness, and AD patients require someone to take care of them for their daily activities, such as eating, washing, dressing or using the toilet. In the last stage, AD patients may become increasingly frail, have difficulty eating, lose memory and speech abilities, and so gradually become completely dependent on caring [1- 3].

AD is a major public health problem from the economic perspective. The World Alzheimer Report 2010 estimated that the total worldwide costs for the informal care, direct medical and social care of dementia's patient are US$ 604 billion in 2010. About 70% of AD patients were found in West Europe and North America. The highest expense per a AD patient is in North America (US$ 48,605), and the lowest is in the South Asia region (US$ 903). It expected that around 2,482,076 people in Southeast Asia region got dementia and loss 3.97 total costs in treatment this disease [1]. In the United States, the estimate cost of caring for AD patients was 172 billion dollars per year and 10.9 million unpaid caregivers [2]. In the United Kingdom (UK), a previous study estimated that 683,597 people will suffered from dementia in 2005 and the dementia cost is 23 billion pounds per year. This cost is more than the cost in treatment cancer (12 billion pounds per year) combined with that of the heart disease (8 billion pounds per year) as shown in Figure1.1 [3]. In 2010 Alzheimer's Association and Dementia 2010 of the Alzheimer's research Trust showed that approximately 5.3 million people in the United States and 821,884 people in the UK were distrurbed by AD.

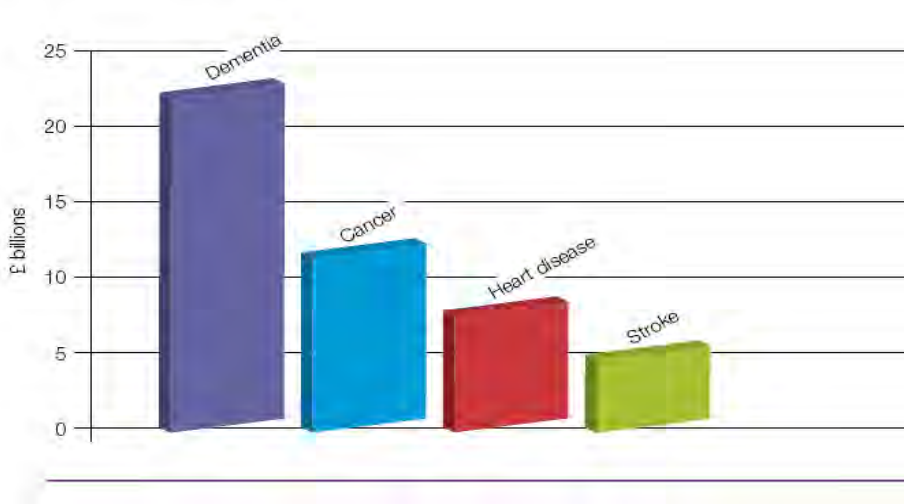


Figure 1.1 The comparison of dementia, cancer, heart disease and stroke cost in UK by the Alzheimer's Research Trust [3].

AD is a dementia that causes the loss of memory and learning ability. There are three main hypotheses of this dementia; cholinergic, amyloid, and tau hypotheses. The main problem of AD patients is the reduction of neurotransmitter acetylcholine (ACh) levels by cholinesterase (ChE). So increasing AChE is needed for the treatment. Tacrine was a non-selective acetylcholinesterase inhibitor (AChEI) but it had toxicity to the liver [4]. Quinazolinones are the fused heterocyclic compounds that their structures are quite similar to that of tacrine. Quinazolinone derivatives are considerable interestingly because of their broad spectrum of bioactivities [5]. Thus, this work aims to study the anti-cholinesterase of the 4(*3H*)-quinazolinone derivatives.

1.1 Cholinesterase

AD was first reported by Dr. Alois Alzheimer, German physician, in 1906. The neuropathology of AD is characterized by several features including extracellular aggregation of amyloid β (Aβ) peptide-occur senile plaques in the cerebral cortical regions, accompanied by the presence of intracellular neurofibrillary tangles and progressive loss of basal forebrain cholinergic neurons leading to reduction in cholinergic markers, such as, ACh levels, choline acetyltrasferase (ChAT), muscarinic and nicotinic acetylcholine receptor binding [6]. ACh is a neurotransmitter that can be the fastest

hydrolysed by two cholinesterases; acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), to obtain choline and acetate [7] as shown in Figure 1.2.

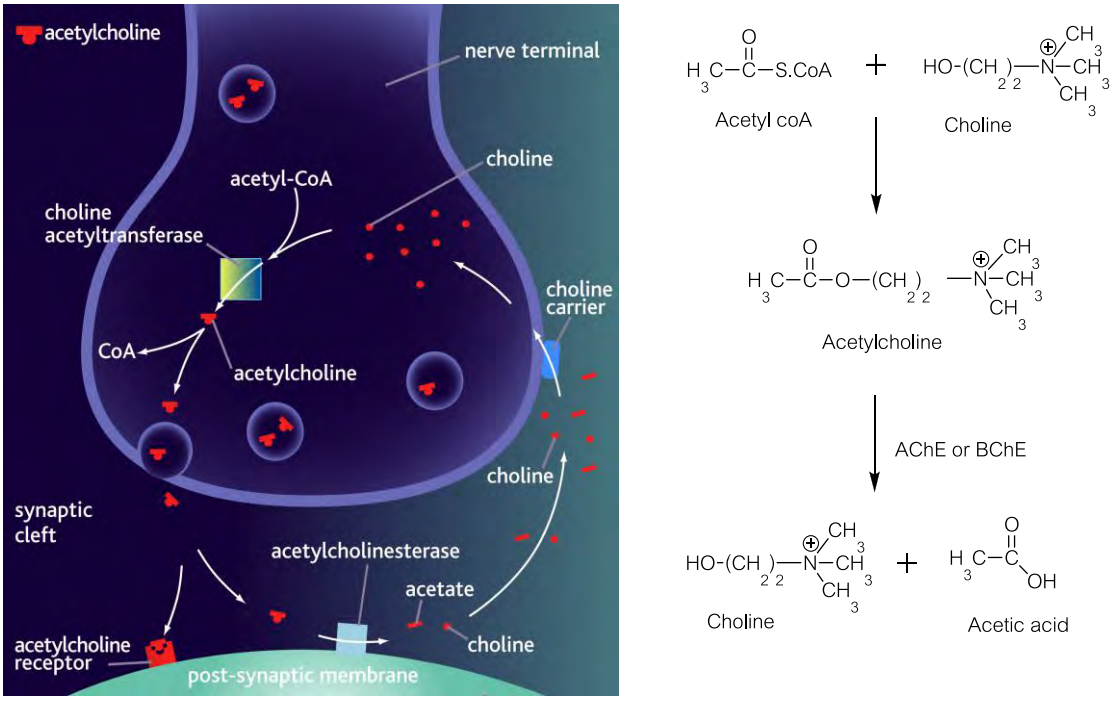


Figure 1.2 The mechanism of action of choline acetyl transferase in nervous cell [8].

AChE, EC 3.1.1.7, is a crucial enzyme mostly in nervous tissue. The major function of this enzyme is cleavage of ACh in the synaptic cleft. It terminates the impulse transmission at cholinergic synapses. The monomer of AChE with a molecular weight around 60,000 Da is an ellipsoidal molecule. The size of its molecule is approximately 45 x 60 x 65 angstroms, consisting of a 12 stranded central mixed beta sheet surrounded by 14 alpha helices. Each monomer contains one catalytic center which is composed of two compartments: the esteratic subsite containing the catalytic triad and the anionic subsite that accommodates the positive quaternary compartment of ACh. The esteratic subsite contains the catalytic machinery of the enzyme: a catalytic triad of Ser 200, His 440 and Glu 327. The anionic subsite is defined by Trp 84, Phe 330, and Phe 331. Its role is to orient the charged part of the substrate that enters the active center. This role is the main function of the Trp residual. The recent rendition of the x-ray structure for AChE places the active catalytic anionic subsite (CAS) deep within a gorge-like fold of the protein. The aromatic gorge in the protein is approximately 20 angstroms deep and penetrates halfway into the

enzyme. The active site lies at the base of this gorge only 4 angstroms above the base, leading some to label this the 'active gorge'. The 'aromatic' gorge is a more appropriate term, though, because 40% of its lining is composed of 14 aromatic residue located in the gorge which is highly conserved from different species of AChE. The high aromatic cont of the walls and floor may explain why studies have proposed hydrophobic and anionic binding sites independent of the active site. Only a few acidic residues are present within the gorge. The aromatic residues clearly play an important role in the stabilization of the complex. Electrostatic as well as hydrophobic effects are of important here. The electrostatic potential map of AChE suggests that this enzyme, possibly like other enzymes with charged substrates, steers its substrate toward its gorge and into the active site. The second anionic site of AChE, the so called 'peripheral' anionic site (PAS) is located at the active center gorge entry, encompasses overlapping binding sites for different activators and inhibitors as Figure 1.3. The peripheral anionic site consists of the residues Asp 74 and Trp 286 as a common core. Binding of ligands to these residues may be the key to the allosteric modulation of AChE catalytic activity [9]. AChE has numerous inhibitors: some, such as the organophosphates, bind exclusively to the esteratic site; others, having a quaternary ammonium group similar to ACh, bind to either of the anionic site. Bis- and tris-quaternary compounds with more than one ammonium moiety may span both sites. Yet, there are others, such as, propidium and the mamba snake venom peptide, fasciculin, bind exclusively to the peripheral site [10].

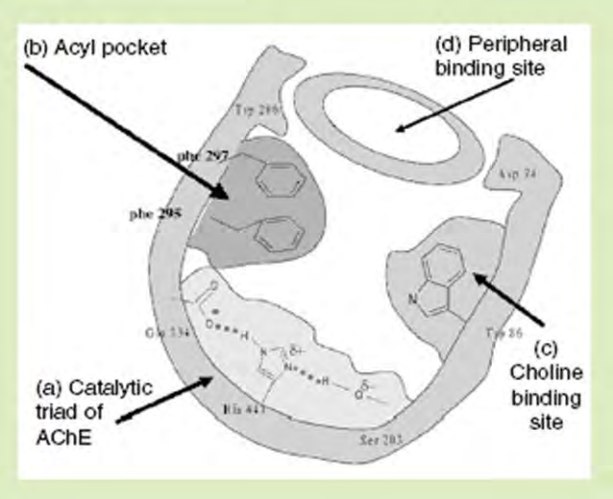


Figure 1.3 The active site of mammalian cholinesterase [11]

BChE, EC 3.1.1.8, is a serine esterase and principal plasma enzyme that catalyzes hydrolysis acetylcholine (ACh) and butyrylcholine (BCh). It was found in serum, liver, heart, and the central nervous system. Native human BChE has a half-life of about 46 h in mice or about 12 days in human plasma. BChE consists more than 95 % of tetramers that lead to the longer half-life of native BChE in plasma [12]. Human BChE's primary amino-acid sequence is 54% identical with that of *Torpedo californica* AChE. A 3D model for human BChE has been built from the known co-ordinates for the 3D structure of *T. californica* AChE. This model agrees with the general features of the recently determined X-ray structure of human BChE. In particular, most of the essential features of the catalytic site (i.e. a catalytic triad of Ser-His-Glu, an oxyanion hole, a π-cation-binding site, and an acyl-binding pocket) are the same in AChE and BChE. The acyl-binding pocket which is responsible for the difference in substrate specificity between the two enzymes is larger in BChE. The active site for both enzymes is located at the same distance. An aspartate residue (D70) of human BChE is located at the mouth of the gorge. But in *T. californica* AChE is D72 that is a part of the PAS contributes to the affinity of positively charged substrates for active site, and is a major factor in the binding of excess substrate to these enzymes [13].

1.2 Drugs for treatment Alzheimer's disease

There is a numerous research taking place into new drug treatments for Alzheimer's disease and the other dementias. Five anti-dementive drugs are approved by the United States Food and Drug Administration (FDA) for AD such as Donepezil (Aricept® or E2020), Galantamine (Reminyl® or Razadyne®), Rivastigmine, (Exelon®), Tacrine (Cognex®) and Memantine (Ebixa®, Axura®, or Namenda®) (Figure 1.4). Four drugs are AD drugs in reducing the breakdown of acetylcholine in the brain. Donepezil is an AChE inhibitor approved for treatment mild to severe AD and others are AChE inhibitors for treatment mild to moderate AD. Memantine is a drug for treatment of moderate to severe AD which modifies the function of the NMDA (*N* – methyl – D – aspartate) receptor [14-16].

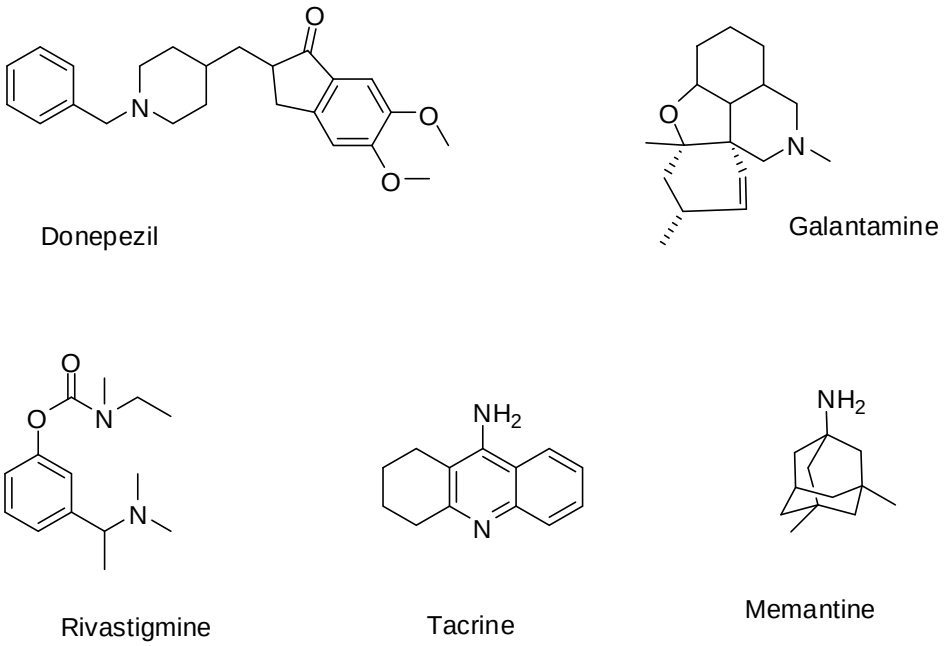


Figure 1.4 The structures of AD drugs

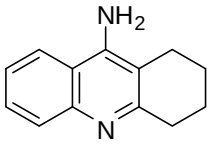
The AChE inhibitor not only increases the level of ACh in the brain, but they also can increase ACh level in the periphery causing side effect. These included the increasing of secretion of gastric acid, bronchial secretions, vagotonic effects on the heart that can exacerbate bradyarrhythmias, and the effect of succinylcholine in anesthesia. The most common gastrointestinal side effect related to cholinergic mechanisms was nausea, vomiting, anorexia and diarrhea. Anorexia and weight loss may be clinically significant problems over the long term which should be monitored and reduced medication or discontinued to assess if appetite returns. The drug types, treatment, side effect and the dose recommend of producer were summarized in Table 1.1 [14].

Table 1.1 Approved drugs by USFDA for AD treatment.

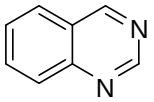
Drug type and Treatment	Brand	Approved For	Producer's recommended Dosage	Side Effects
Donepezil Cholinesterase inhibitor	Aricept	All stages	5 mg, once a day, available in tablet form. Increase after 4-6 weeks to 10 mg, once a day if well tolerated.	Nausea, vomiting, loss of appetite and Increased frequency of bowel movements.
galantamine Cholinesterase inhibitor	Razadyne Reminyl	Mild to moderate	4 mg, twice a day (8 mg/ day, available in tablet or capsule form. Increase by 8 mg/day after 4 weeks to 8 mg, twice a day (16 mg/ day) if well tolerated. After another 4 weeks, increase to 12 mg twice a day (24 mg/day) If well tolerated.	Nausea, vomiting, loss of appetite and Increased frequency of bowel movements.
Rivastigmine Cholinesterase inhibitor	Exelom	Mild to moderate	1.5 mg, twice a day (3 mg/day, available in capsule and liquid form Increase by 3 mg/day every 2 weeks to 6 mg, twice a day (12 mg/day) If well tolerated.	Nausea, vomiting, loss of appetite and Increased frequency of bowel movements.
Memantine N-methyl D-aspartate (NMDA) antagonist Blocks the toxic effects associated with excess glutamate and regulates glutamate activation.	Namenda	Moderate to severe	5 mg, once a day, available in tablet form Increase to 10 mg/day (5 mg twice a day), 15mg/day (5 mg and 10 mg as separate doses), and 20 mg/day (10 mg twice a day) at minimum of one week intervals if well tolerated.	Headache, constipation, confusion and dizziness.

1.3 Quinazolines

Quinazolines are pharmacological compounds which are used as antibacterial, anticancer, antimalarial, anticonvulsant and anti- inflammatory agents [15]. Quinazolines have the similarity of structure to tacrine which was a potent AChE and BChE inhibitors [16]. Tacrine was first described pharmacologically by Shaw and Bently (1949) in Australia as an analeptic capable of causing rapid arousal of morphinized dogs and cats. Moreover Shaw and Bently (1953) had shown that it is almost as powerful a ChE inhibitor as eserine or neostigmine, with IC_{50} of ChE at concentration of 10^{-7} M [17]. Tacrine, as Cognex, has, however, been replaced by other ChE inhibiors because of its hepatoxicity. Computational studies suggested that tacrine interacts with AChE, not only at the CAS, principally through a stacking interaction with Trp84, but also, with lower affinity, by interaction with the indole ring of Trp279 at the PAS of *T. californica* AChE [18]. Thus various quinazoline derivatives were synthesized and determined theirs ChE inhibitory activities against AChE and BChE.



tacrine



quinazoline

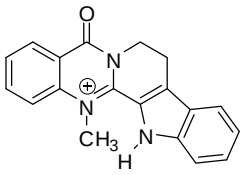
Figure 1.5 Chemical structures of tacrine and quinazoline.

In 2005-2006, Decker *et.al*. synthesized novel anticholinesterase quinazolinimines based on the structures of the alkaloids dehydroevodiamine (DHED) and rutaecarpine [19]. Alkaloid DHED was first isolated from the Chinese herb *Evodia rutaecarpa* bentham and reported to possess strong an antiamnesic activity *in vivo* and a moderate AChE inhibition *in vitro*. The search for new compounds was directed by several approaches: on the one hand, imines of the quinazolinones should be synthesized (both N-unsubstituted and N-substitued). An unsubstituted imine replaces the H-bond acceptor oxygen-atom by an H-bond donor. Furthermore this modification gives rise to strong bases

which can easily be dissolved in water in the form of their salts. Synthesis of substituted imines opened the way to introduction of different functionalities into the lead molecules, in an initial step the researcher wanted to introduce a 2-phenylethyl group giving rise to a further hydrophobic moiety within the molecule. On the other hands, in order to investigate, whether the indole moiety is essential for DHED pharmacology, compounds were synthesized, in which the indole is replaced by a benzene moiety. Since a benzene moiety is less sensitive to more drastically chemical conditions, retaining activity with the respective dibenzo-compounds might give rise to facilitate modification within the molecular structure compared to benzindolo compounds. Further modification should be achieved by reduction of the carbonyl group (in position 5) of DHED (or its dibenzo-analogue) for combinining the ChE inhibitory activities of DHED and a quinazoline as **1**. Among seven synthetic compounds, compound **4** showed the highest activity with the IC_{50} values of 3.4 and 0.5 μM towards AChE and BChE, respectively. Following by compound **3** which has a moderate AChE and BChE inhibitory effects with the IC_{50} values of 7.7 and 4.4 μM, respectively. Moreover, compounds **2**, **4** and **7** showed a 10-fold higher affinity to BChE. Compounds **2** and **7** were moderatre ChE inhibitors with a 10-fold higher affinity at BChE. Compounds **5** and **6** were dibenzo-analogues of DHED. The comparison of compounds **3**, **4**, **6** and DHED performed indole group could increase ChE inhibitory activities. The products of carbonyl reduction (**5** vs. **6**) could increase BChE inhibitory activity but reduce AChE inhibitory activity (Figure 1.6).

In 2006, Decker *et al* interested in compound **7** which had moderate to good affinities at the ChEs and its 10-fold selectivity towards BChE [20]. Since BChE possesses a larger void at the active site gorge, either changing the size of the alicyclic ring or changing the distance between the quinazolinimine moiety and the phenyl grouph might result in an improved selectivity towards BChE. So compound **7** was further modified its structure by varying the size of alicyclic ring, and the chain length. Two general trends were found from this study. Firstly, an increasing ring size lowers the affinity towards AChE, whereas the BChE affinity stays the same or-especially in the case of aniline-derivatives is even increased. These properties could be expected, because BChE has a larger void at the active site gorge. An increasing distance of the phenyl ring from the quinazolinimine

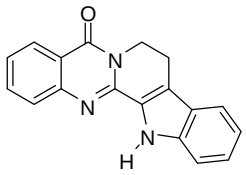
heterocycle generally increased affinity towards AChE. In a less pronounced manner, this also applies for BChE: the seven-membered ring quinazolinimine phenylethyl compound **8b** has a more than 2-fold higher affinity as that of the respective benzyl-compound **8a**. The most active compound is the compound that **n** and **m** has 2 and 1 bonds, respectively with an IC_{50} (BChE) at 0.14 μM and a selectivity of 1/100 towards BChE, Figure 1.7.



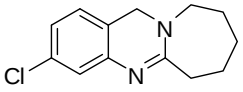
DHED

IC_{50} (AChE) = 6.3 μM

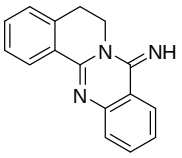
IC_{50} (BChE) = 8.4 μM



rutaecarpin



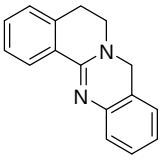
1 3-chloro-6, 7, 8, 9, 10, 12-Hexahydroazepino [2,1-b] quinazoline



2

IC_{50} (AchE) = 22.1 μM

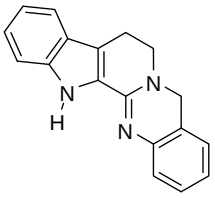
IC_{50} (BChE) = 2.0 μM



3

IC_{50} (AChE) = 7.7 μM

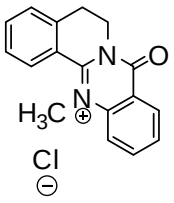
IC_{50} (BChE) = 4.4 μM



4

IC_{50} (AChE) = 3.4 μM

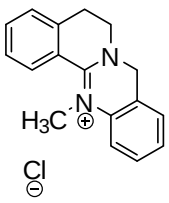
IC_{50} (BChE) = 0.5 μM



5

IC_{50} (AChE) = 12.0 μM

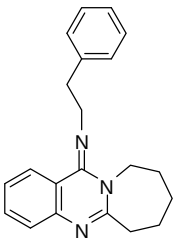
IC_{50} (BChE) = >500 μM



6

IC_{50} (AChE) = >500 μM

IC_{50} (BChE) = 2.2 μM

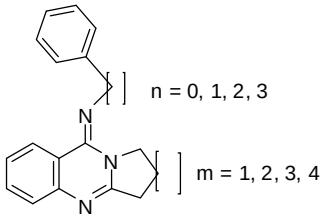


7

IC_{50} (AChE) = 27.7 μM

IC_{50} (BChE) = 2.2 μM

Figure 1.6 The chemical structure and IC_{50} values towards AChE and BChE of synthesized compounds derived from DHED and rutaecarpine.



8a n = 1, m = 3

IC_{50} (AChE) = >500 μM

IC_{50} (BChE) = 1.1 μM

8b n = 2, m = 3

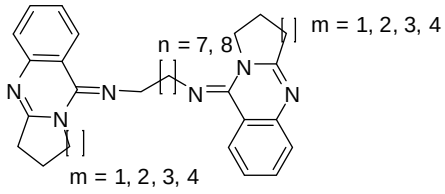
IC_{50} (AChE) = 11.5 μM

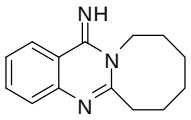
IC_{50} (BChE) = 0.62 μM

Figure 1.7 The chemical structure analogues of quinazolinimine **7** and IC_{50} values toward AChE and BChE of synthesized compounds

In the search for novel compounds with ChE inhibitory activity, Decker interested compound **2** and **7** that were identified as a micromolar ChE inhibitors with >10-fold selectivity toward BChE [21]. By variation of the distance of the phenyl ring to the heterocycle and especially by the change of size of the alicycle, it was possible to obtain either activities at both enzymes or highly selective BChE inhibitors; BChE selectivity increased with increasing size of the alicycle, through increasing activity toward BChE and/or decreasing activity at AChE. To further improve activities of quinazolinimines toward ChE, the bivalent approach was applied to this novel class of ChE inhibitors. The object of this research was to obtain more potent inhibitors with additional strong activity at BChE or even BChE selective compound. Since the fact that the quinazolinimines bind at the active site like tacrine, similar space lengths of the most potent tacrine dimmers (a heptamethylene spacer exhibits the highest activities) were applied. The imine-N-unsubstituted compound **10** is micromolar ChE inhibitors; the eight-member-ring shows a very moderate BChE selectivity (of about a factor of 5). This is in strong contrast to the compounds carry a phenyl ring at the imine-N similar to compound **7**, which are highly BChE selective. Therefore, N-substitution with phenyl, benzyl, and phenylethyl groups, respectively, and a concomitant larger alicycle size lead to BChE selectivity. The bivalent inhibitors connected by a heptamethylene spacer **9a** show a powerful increase, by a factor of 100, in inhibitory activity at both enzymes compared with the monomeric compound **10**. The comparison

heptamethylene-bridged **9a** with octamethylene-bridged **9b** were found **9a** good inhibition to both enzymes **9b** good selectivity toward BChE that is 190-fold as Figure 1.8.





9a n = 7, m = 4	**9b** n = 8, m = 4	**10**
IC_{50} (AChE) = 65 nM	IC_{50} (AChE) = 14400 nM	IC_{50} (AChE) = 14600 nM
IC_{50} (BChE) = 27 nM	IC_{50} (BChE) = 76 nM	IC_{50} (BChE) = 3000 nM

Figure 1.8 Chemical structures of the modified structure of rutaecarpine to homobivalent inhibitors with their anti-cholinesterase activities.

In 2008, V. Alagarsamy *et al* [22] reported that quinazolines and condensed quinazolines exhibit potent central nervous system (CNS) activities e.g. analgesic and anti-inflammatory activities. They developed new molecules that exhibited high analgesic and anti-inflammatory activities with minimal gastrointestinal ulceration side effects from 2-phenyl-3-substituted quinazoline **I**, 2-methyl-3- substituted quinazoline **II** and 2-methylthio-3-substitued quinazoline **III** (Figure1.9). They synthesized 15 derivatives. They evaluated the percentage of analgesic, the percentage of anti-inflammatory activities at dose of 10 and 20 mg/kg and at the reaction time of 30 min, 1 h, 2 h, and 3 h by using the reference standard diclofenac sodium. Moreover, the gastrointestinal ulceration side effects were tested ulcerogenic index by using the reference standard aspirin. This research was performed by orally testing in animal and found all of these compounds including standard gave the highest action each at 2 h. Compound **IV** exhibited 64% & 72% analgesic activityand and 46% & 60% anti-inflammatory activity at the dose of 10 mg/kg and 20 mg/kg, respectively, at the reaction time of 2 h. Compound **V** showed 55% & 69% analgesic activity and 50% & 63% at the dose of 10 mg/kg and 20 mg/kg, respectively, at the reaction time of 2 h. At the same condition diclofenac performed 45% & 62% analgesic

activity and 39% & 60% anti-inflammatory activity, respectively. Compound **IV** showed the best analgesic activity, and compound **V** showed the best anti-inflammatory. Interestingly these compounds showed one-third of ulcer index of the reference non–steroidal anti-inflammatory drugs (NSAID) aspirin and diclofenac, Figure 1.9.

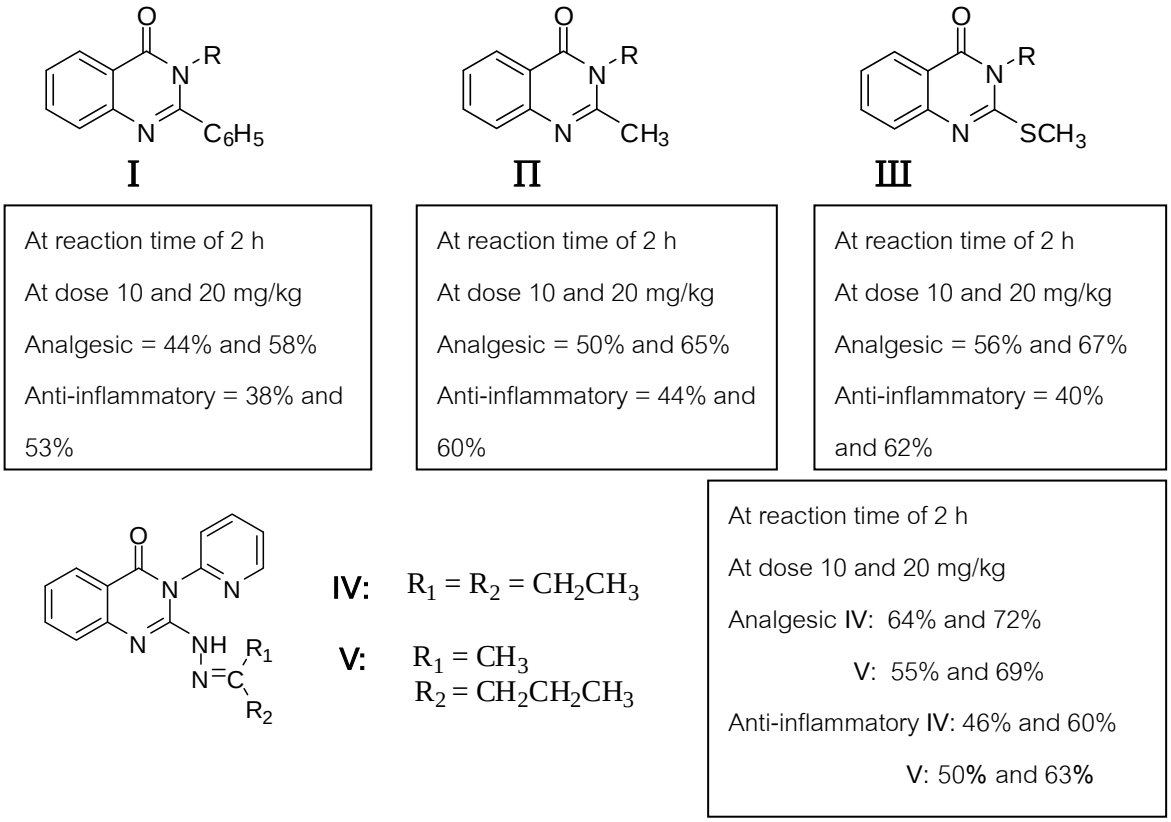


Figure 1.9 Structures of lead compounds generation quinazoline-4-ones with analgesic and inflammatory activities.

From the above–mentioned reports, the researcher is interested in the modified structure with anticholinesterase and quinazoline and condensed quinazolines with anticholinesterase. DHED and rutaecarpine that were extracted from Chinese herb have big structure quinazoline derivatives with anticholinesterase and especially BChE inhibitory activity and other quinazoline derivatives exhibit potent central nervous system (CNS) activities. Moreover their structures were like tacrine which is a powerful drug for AD treatment but it has hepatotoxic side effect. The quinazoline and its derivatives had many medicinal activities. Furthermore the quinazoline derivatives that will be synthesized in this research have not been anticholinesterase research, and some compounds are new

ynthesized compounds. Therefore the researcher will develop new class of quinazoline derivatives from 4(3H)-hydroxyquinazoline and study its anticholinesterase activities.

1.4 Scope of research

The aims of this work are to synthesize 4(*3H*)-hydroxyquinazoline derivatives to increase their AChE and BChE inhibitory activities. Firstly, the derivatives of 4–substituted aminoquinazolines were synthesized using a one pot method [23]. Then, synthesizing of 3-substitued quinazolines is treated with mukaiyama's reagent as activator for C-N promotion and used *N*,*N*-diisopropylethylamine (DIPEA) as a base [24]. All synthesized derivatives are further evaluated their anti-cholinesterase to afford lead structures. Then the substituents (electron-withdrawing and electron-donating groups) of a benzene ring of quinazolines are studied their effect related to anti-cholinesterase activity. Quinazoline derivatives with lead structures combined with the good substituents in a benzene ring are then synthesized and determined their activities. Finally, all data are analyzed to obtain the lead candidate of AD drug.

CHAPTER II

EXPERIMENTAL

2.1 Instruments and equipments for synthesis experiment

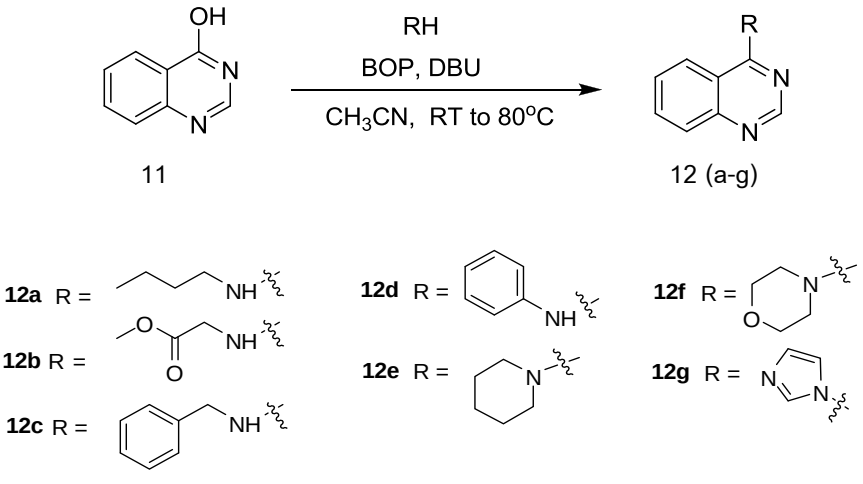
Synthesis of 4-aminoquinazoline, 3-alkylquinazolin-4-ones and substituted 4-aminoquinazoline derivatives were reported with their cholinesterase inhibitory activities. All of these were characterized by Varian Mercury^{+} 400 NMR spectrometer which operated at 399.87 MHz for ^{1}H and 100.55 MHz for ^{13}C nuclei together with ESI-Mass Spectrometer (ESIMS) model VG TRIO 2000.

2.2 Chemicals for synthesis experiment

Silica gel 60 G Art 7734 (70-230 mesh) was used for column chromatography, and thin layer chromatography (TLC) performed on aluminum back sheets pre-coated with silica gel (Merck's, Kieselgel 60 F_{254}, 0.25 mm thick layer).The solvent for column chromatography was distilled before used. The other reagents (AR grade) were purchased from Fluka, Sigma-Aldrich and Merck Companies. Deuterated solvents, $CDCl_3$-d_1 and DMSO-d_6 were used for determined the chemical structures.

2.3 Synthesis of 4(*3H*)-hydroxyquinazoline derivatives

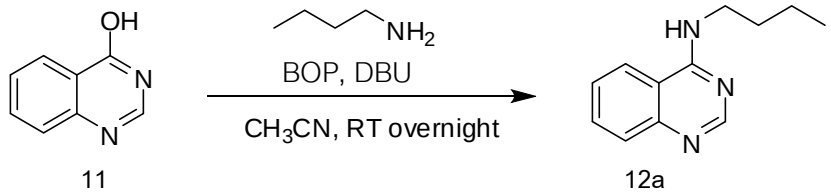
2.3.1 General procedure for synthesizing of 4-substituted aminoquinazolines (12a-g)



Scheme 2.1 General procedure for synthesizing 4-substitued aminoquinazolines.

The synthesis of 4-substitued aminoquinazolines (**12a-g**) were followed the previous procedure by Wan, Z.-K. *et al*. (2006) [23]. A mixture of the commercially available 4(*3H*)-hydroxyquinazoline (**11**), 1.1 equivalent of benzotriazolyloxytris (dimethylamino) phosphonium hexafluorophosphate (BOP), 1.5 – 4.0 equivalent of diazabicycloundec-7-ene (DBU) and 1.0 – 3.0 equivalent of amine in acetronitrile (CH_3CN) was stirred at room temperature for overnight. Then the solution was further heat up to 60°C to 80°C for 2 – 4 h if the starting chemical, 4(*3H*)-hydroxyquinazoline, remained. The product 4-aminoquinazolines (**12a-g**) were afforded after purify by a column chromatography eluted by a mixture of ethyl acetate and hexane.

2.3.1.1 4-Butylaminoquinazoline (12a):

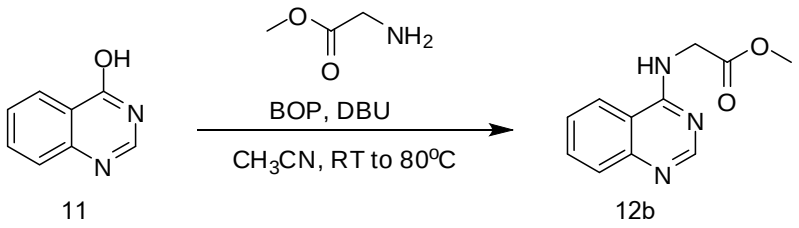


11 12a

Scheme 2.2 The procedure for synthesizing 4-butylaminoquinazoline (**12a**).

Compound **12a** was synthesized from 4-hydroxyquinazoline (54.9 mg, 0.38 mmol), BOP (165.3 mg, 0.37 mmol), DBU (150 µL, 1.0 mmol) and *n*-$BuNH_2$ (50 µL, 0.5 mmol) in CH_3CN 2 mL followed the general procedure in 2.3.1 and Scheme 2.2. The product (**12a**) is gray solid: 45 mg, 66 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.58 (s, 1H), 7.76 (m, 2H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.40 (m,1H), 6.18 (br s,1H), 3.61 (m, 2H), 1.66 (m, 2H), 1.40 (m, 2H), 0.92 (t, *J* = 8.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ (ppm) 159.6, 154.8, 148.0, 132.9, 127.6, 126.2, 120.8, 114.7, 41.3, 31.4, 20.2 and 13.9. HRMS [$(M+H)^+$]: calcd for $C_{12}H_{16}N_3$ 202.1266, found 202.1344. The NMR data of (**12a**) was in agreement with those of previous work [23].

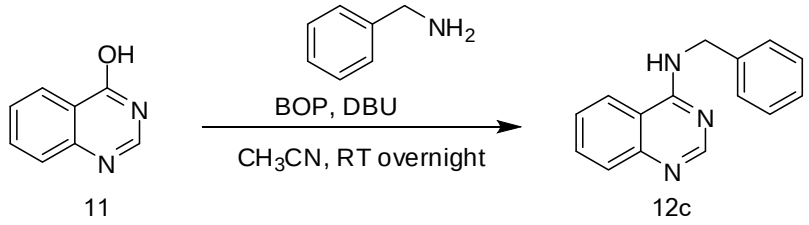
2.3.1.2 4-Glycinemethylaminoquinazoline (12b):



Scheme 2.3 The procedure for synthesizing 4-glycinemethylaminoquinazoline (**12b**).

Compound **12b** was synthesized from 4-hydroxyquinazoline (51.0 mg, 0.35 mmol), BOP (166.4 mg, 0.38 mmol), DBU (209.3 µL,1.40 mmol) and glycine methyl ester HCl (131.8 mg, 1.05 mmol) in CH_3CN 3 mL followed the general procedure in 2.3.1 and Scheme 2.3. The product (**12b**) is white solid: 66.8 mg, 88 %yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.63 (s, 1H), 7.79 (t, *J* = 8.0 Hz, 2H), 7.71 (t, *J* = 8.0 Hz, 1H), 7.43 (t, *J* = 8.0 Hz, 1H), 6.85 (br s, 1H), 4.42 (s, 2H), 3.83 (s, 3H). ^{13}C NMR ($CDCl_3$) δ (ppm) 171.0, 159.2, 154.5, 148.2, 133.2, 127.6, 126.5, 121.0, 114.6, 52.7 and 42.9. The NMR data of (**12b**) was in agreement with those of previous work [23].

2.3.1.3 4-Benzylaminoquinazoline (12c):

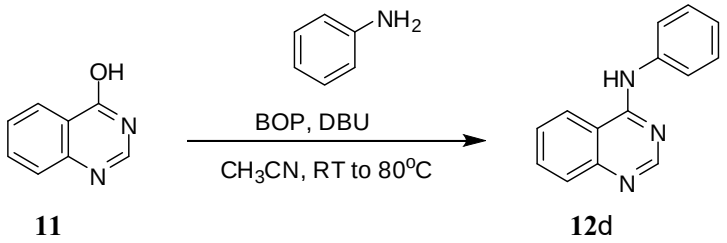


Scheme 2.4 The procedure for synthesizing 4-benzylaminoquinazoline (**12c**).

Compound **12c** was synthesized from 4-hydroxyquinazoline (50 mg, 0.34 mmol), BOP (166.1 mg, 0.38 mmol), DBU (80 µL, 0.52 mmol) and benzylamine, (45 µL, 0.41 mmol) in CH_3CN 5 mL follow general procedure in 2.3.1 and Scheme 2.4. The product (**12c**) is white powder: 50.0 mg, 62 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.58 (s, 1H), 7.82 (m, 2H), 7.65 (t, *J* = 8.0 Hz 1H), 7.38 (t, *J* = 8.0 Hz 1H), 7.28 (m, 2H), 7.25 (m, 3H), 6.80 (br s,

1H), 4.82 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ (ppm) 159.2, 155.4, 149.4, 138.0, 132.7, 128.9, 128.6, 128.0, 127.8, 126.1, 120.4, 114.8, 45.4. The NMR data of (**12c**) was in agreement with those of previous work [23].

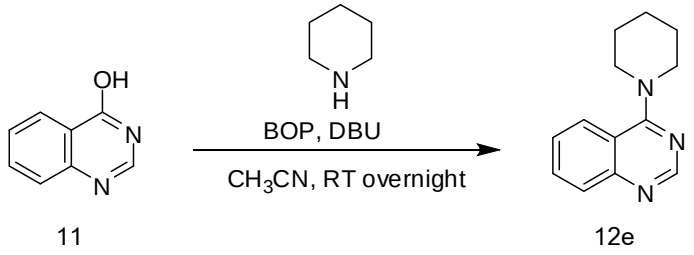
2.3.1.4 4-Anilinoquinazoline (12d):



Scheme 2.5 The procedure for synthesizing 4-anilinoquinazoline (**12d**).

Compound **12d** was synthesized from 4-hydroxyquinazoline (53 mg, 0.36 mmol), BOP (167.4 mg, 0.38 mmol), DBU (76 µL, 0.51 mmol) and aniline (48 µL, 0.51 mmol) in CH_3CN 2 mL followed the general procedure in 2.3.1 and Scheme 2.5. The product (**12d**) is brown amorphous: 54.7 mg, 65 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.72 (s, 1H), 7.86 (m, 2H), 7.75 (t, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.52 (t, *J* = 8.0 Hz, 1H), 7.37 (m, 2H), 7.14 (t, *J* = 8.0 Hz 1H). ^{13}C NMR ($CDCl_3$) δ (ppm) 157.6, 155.0, 150.0, 138.1, 133.0, 129.2 (2C), 129.0, 126.7, 124.8, 122.0 (2C), 120.3, 115.1. MS (ESI, EI^+) m/z 222.1(MH^+). The NMR data of **12d** was in agreement with those of previous work [23].

2.3.1.5 4-Piperidinoquinazoline (12e):

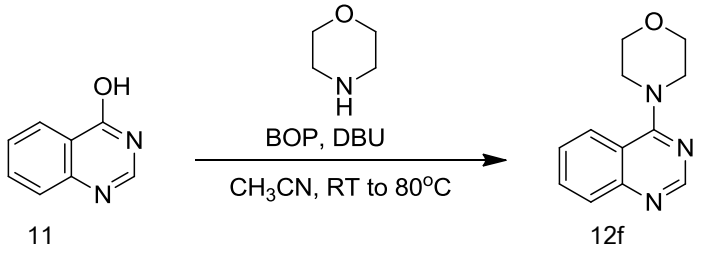


Scheme 2.6 The procedure for synthesizing 4-piperidinoquinazoline (**12e**).

Compound **12e** was synthesized from 4-hydroxyquinazoline (52.8 mg, 0.36 mmol), BOP (164.9 mg, 0.37 mmol), DBU (80.5 µL, 0.54 mmol) and piperidine (54 µL, 0.54

mmol) in 2 mL followed the general procedure in 2.3.1 and Scheme 2.6. The product (**12e**) is brown yellow oil: 64.7 mg, 84 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.66 (s, 1H), 7.83 (t, *J* = 8 Hz, 2H), 7.67 (m, 1H), 7.39 (t, *J* = 8 Hz, 1H), 3.68 (s, 4H), 1.74 (s, 6H). ^{13}C NMR ($CDCl_3$) δ (ppm) 163.8, 153.6, 151.1, 132.6, 127.7, 126.8, 125.4, 115.8, 50.9, 24.1, 22.1. MS (ESI, EI^+) m/z 214.2 (MH^+). The NMR data of (**12e)** was in agreement with those of previous work [23].

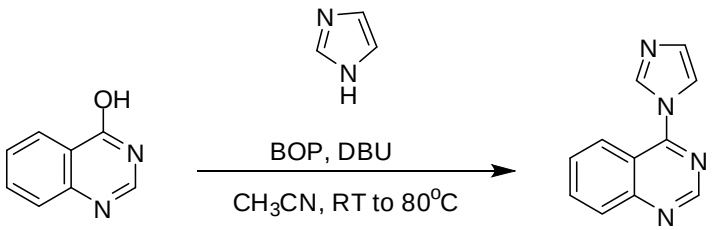
2.3.1.6 4-Morpholinoquinazoline (12f):



Scheme 2.7 The procedure for synthesizing 4-morpholinoquinazoline (**12f**).

Compound **12f** was synthesized from 4-hydroxyquinazoline (56.6 mg, 0.39 mmol), BOP (164.2mg, 0.37 mmol), DBU (203 µL, 1.36 mmol), and morpholine (45 µL, 0.35 mmol) in CH_3CN 4 mL followed the general procedure in 2.3.1 and Scheme 2.7. The product (**12f)** is pale yellow solid: 33.3 mg, 39% yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.68 (s, 1H), 7.86 (d, *J* = 8 Hz, 1H), 7.81 (m, 1H), 7.68 (m, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 3.83 (m, 4H), 3.72 (m, 4H). ^{13}C NMR ($CDCl_3$) δ(ppm) 64.6, 153.8, 151.3, 132.7, 128.5, 125.7, 124.7, 116.3, 66.7, 50.2. MS (ESI, EI^+) m/z 216.2 (MH^+). The NMR data of (**12f)** was in agreement with those of previous work [23].

2.3.1.7 4-Imidazolaminoquinazoline (12g):



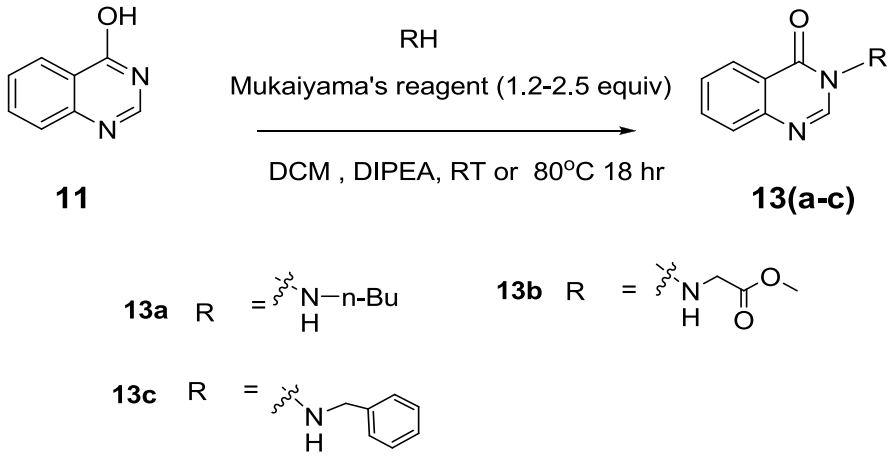
11 12g

Scheme 2.8 The procedure for synthesizing 4-imidazolaminoquinazoline (**12g**).

Compound **12g** was synthesized from 4-hydroxyquinazoline (**11**) (51.8 mg 0.35mmol), BOP (181.56 mg, 0.41 mmol), DBU (77 µL, 0.51 mmol) imidazol and (140.2 mg, 2.06 mmol) in CH_3CN 2 mL followed the general procedure in 2.3.1 and Scheme 2.8. The product (**12g**) is white powder: 6.5 mg, 10 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 9.13 (s,1H), 8.25 (s, 1H), 8.11 (t, *J* = 8.0 Hz, 3H), 7.90 (m, 1H), 7.69 (m ,2H). ^{13}C NMR ($CDCl_3$) δ (ppm) 155.0, 154.2 152.9, 137.6, 134.8, 130.7, 129.2, 129.1, 126.3, 124.0, 119.6. The NMR data of (**12f**) was in agreement with those of previous work [23].

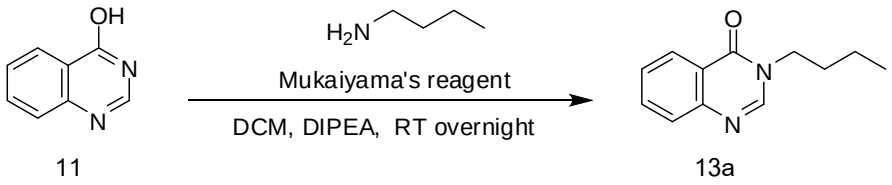
2.3.2 General procedure for synthesizing of 3-substituted quinazolinones (13a-c)

The synthesis of 3-substitued aminoquinazolinones (**13a-c**) were presented in Scheme 2.9 followed the previous procedure reported by Punthasee, P. *et al*. (2010) [24]. Mukaiyama's reagent (2-Chloro-1-methylpyridinium iodide) was added in a solution of 4-hydroxyquinazoline (**11**) in dichloromethane (DCM) 5 mL followed by the addition of *N*,*N*-Diisopropylethylamine (DIPEA) The solution was stirred at room temperature for 1 hour before adding amine nucleophiles. Then, the solution was keeping stir at room temperature for overnight, except for synthesizing compound (**13c**) that the solution was stirred at 80°C for 18 hours. After removal of solvent, the extract was purified by a column chromatography using a gradient mixture of ethyl acetate and hexane to obtain the desired product. The procedure for synthesizing (**13a-c**) is shown in Scheme 2.9.



Scheme 2.9 General procedure for synthesizing 3-substitued quinazolinones

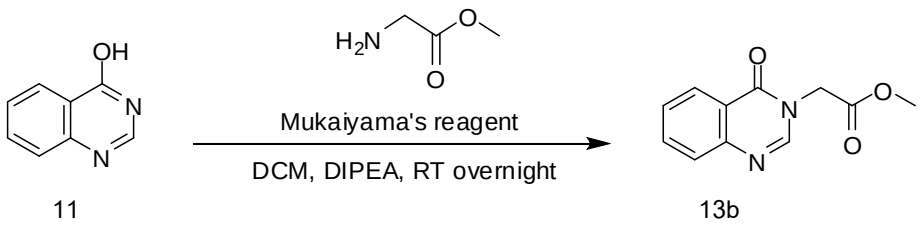
2.3.2.1 3-Butylquinazolin-4(3*H*)-one (13a):



Scheme 2.10 The procedure for synthesizing 3-butylquinazoline-4(*3H*)-one (**13a**).

Compound **13a** was synthesized from 4-hydroxyquinazoline (**11**) (71.3 mg, 0.49 mmol), 2-chloro-1-methylpyridinium iodide (249.0 mg, 0.98 mmol), DIPEA (424.0 µL, 2.44 mmol) and butan-1-amine (242.0 µL, 2.44 mmol) followed the general procedure in 2.3.2 and Scheme 2.10. The product (**13a**) is pale brown solid: 77.1 mg, 78.0 **%** yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.32 (d, *J* = 8.0 Hz, 1H), 8.03 (s, 1H), 7.81-7.65 (m, 2H), 7.51 (t, *J* = 7.4 Hz, 1H), 4.01 (t, *J* = 7.3 Hz, 2H), 1.87-1.70 (m, 2H), 1.51-1.32 (m, 3H), 0.97 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ (ppm) 160.4, 147.6, 146.2, 133.5, 126.8, 126.6, 126.1, 121.6, 46.2, 30.9, 19.4, 13.2. HRMS [$(M+H)^+$]: calcd for $C_{12}H_{15}N_2O$ 203.1106, found 203.1007. The NMR data of (**13a**) was in agreement with those of previous work [24].

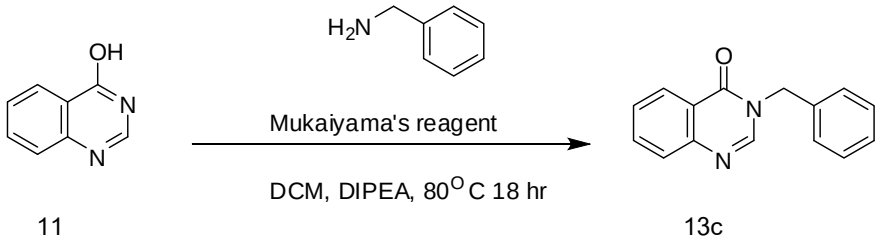
2.3.2.2 Methyl 2-(4-oxoquinazolin-3(4*H*)-yl) acetate (13b):



Scheme 2.11 The procedure for synthesizing methyl 2-(4-oxoquinazolin-3(*4H*)-yl) acetate (**13b**).

Compound **13b** was synthesized from 4-hydroxyquinazoline (**11**) (70.3 mg, 0.48 mmol), 2-chloro-1-methylpyridinium iodide (245.8 mg, 0.98 mmol), DIPEA (837.9 µL, 4.81 mmol) and 2-methoxy-2-oxoethanaminium chloride (302.0 mg, 2.41 mmol) followed the general procedure in 2.3.2 and Scheme 2.11. The product is yellow solid: 51.0 mg, 49 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.31 (d, *J* = 8.0 Hz, 1H) 7.99 (s, 1H), 7.85-7.70 (m, 2H), 7.53 (t, *J* = 7.4 Hz, 1H), 4.73 (s, 2H), 3.81 (s, 3H). ^{13}C NMR ($CDCl_3$) δ (ppm) 167.6, 160.8, 147.9, 146, 134.5, 127.5, 126.7, 121.7, 52.8, 47.2. HRMS [$(M+H)^+$]: caled for $C_{11}H_{11}N_2O_3$ 219.0619 found 219.0625. The NMR data of (**13b**) was in agreement with those of previous work [24].

2.3.2.3 3-Benzylquinazolin-4(3*H*)-one (13c):

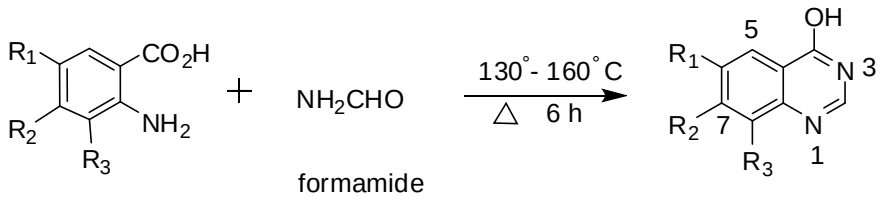


Scheme 2.12 The procedure for synthesizing 3-benzylquinazolin-4(*3H*)-one (**13c**).

Compound **13c** was synthesized from 4–hydroxyquinazoline (**11**) (69.6 mg, 0.48 mmol), 2-chloro-1-methylpyridinium iodide (183.95 mg, 0.72 mmol), DIPEA (418.0 µL,

2.40 mmol) and phenylmethanamine (262.0 mg, 2.40 mmol): followed the general procedure in 2.3.2 and Scheme 2.12. The product is pale yellow solid: 78.6 mg, 69 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.33 (dd, *J* = 8.0, 1.1 Hz,1H), 8.12 (s, 1H), 7.82-7.66 (m, 2H), 7.57-7.46 (m, 1H), 7.40-7.27 (m, 5H), 5.21 (s, 2H). ^{13}C NMR ($CDCl_3$) δ (ppm) 161.0, 147.9 135.6, 134.3, 129.0, 128.2, 127.9, 127.4, 127.3, 126.8, 122.1, 49.6. HRMS [$(M+H)^+$]: caled for $C_{15}H_{13}N_2O$ 237.0950, found 237.0892. The NMR data of (**13c**) was in agreement with those of previous work [24].

2.3.3 General procedure for synthesis of 4-hydroxquinazolines with various substituent on a benzene ring



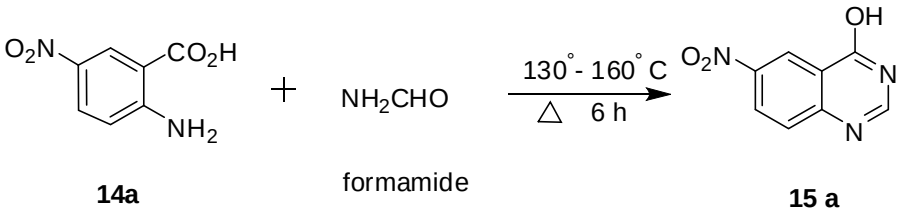
14 (a-e) **15 (a-e)**

	R_1	R_2	R_3
a	NO_2	H	H
b	Cl	H	Cl
c	F	H	H
d	OMe	OMe	H

Scheme 2.13 Synthesis procedure of 4-hydroxyquinazoline with various substituent on a benzene ring *via* Niementowski's reaction

A mixture of various substituted anthranilic acid (**14a-e**) (1 equiv) and formamide (5-13 equiv) was stirred in a seal tube at 130°C to 160°C for 6 h and then cooled to room temperature. Ethyl acetate (15 mL) was added to precipitate **15a-c** and **15e** while deionized water (15 mL) was added to precipitate **15d**, respectively. The desired compounds were filtered and washed with cold methanol.

2.3.3.1 6–Nitroquinazolin-4-ol (15a):

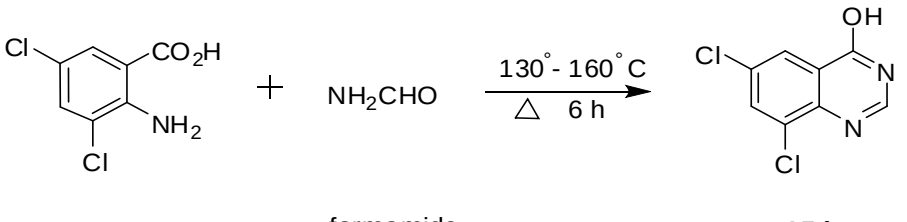


14a **15 a**

Scheme 2.14 The procedure for synthesizing 6-nitroquinazolin-4-ol (**15a**).

Compound **15a** was synthesized from 2-amino-5-nitrobenzoic acid (**14a**) (317.7 mg, 1.74 mmol) and formamide (370 µL, 9.28 mmol) followed the general procedure in 2.3.3 and Scheme 2.14. The product is pale brown powder: 271.6 mg, 82 % yield. ^{1}H NMR (DMSO-d_6) δ (ppm) 12.76 (br s, 1H), 8.75 (d, J = 4.0 Hz, 1H), 8.50 (dd, J = 2.6, 8.0 Hz, 1H), 8.26 (s, 1H), 7.82 (d, J = 8.0 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm) 160.4, 153.2, 149.2, 145.3, 129.4, 128.6, 123.0, 122.2. The NMR data of **13b** was in agreement with those of previous work [25].

2.3.3.2 6,8 – Dichloroquinazolin-4-ol (15b):



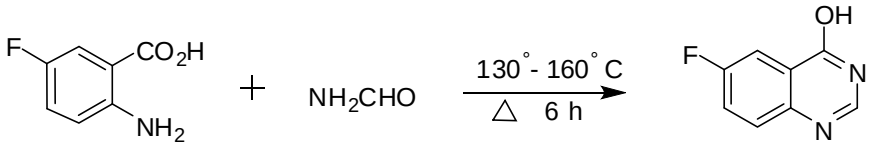
14b **15 b**

Scheme 2.15 The procedure for synthesizing 6, 8-dichloroquinazolin-4-ol (**15b**).

Compound **15b** was synthesized from 2-amino-5,7-dichlorobenzoic acid (**14b**) (200.3 mg, 0.97 mmol) and formamide (216 µL, 5.42 mmol) followed the general procedure in 2.3.3 and Scheme 2.15. The product is brown powder: 169.9 mg, 79 % yield. ^{1}H NMR (DMSO-d_6) δ (ppm) 12.69 (br s, 1H), 8.22 (s, 1H), 8.12 (d, J = 2.4 Hz, 1H), 8.00

(d, *J* = 2.4 Hz,1H). ^{13}C NMR (DMSO-d_6) δ (ppm) 159.8, 147.2, 144.7, 134.5, 132.8, 131.17, 125.46, 124.61.

2.3.3.3 6-Fluoroquinazolin-4-ol (15c)

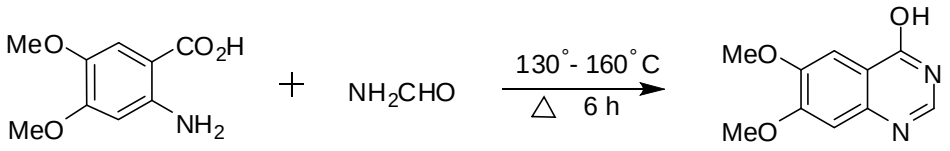


14c formamide **15 c**

Scheme 2.16 The procedure for synthesizing 6-fluoroquinazolin-4-ol (**15c**).

Compound **15c** was synthesized from 2-amino-5-fluorobenzoic acid (**14c**) (293.8 mg, 1.89 mmol), and formamide (400 µL, 10.04 mmol) followed the general procedure in 2.3.3 and Scheme 2.16. The product is brown powder: 170.1 mg, 53 % yield. ^{1}H NMR (DMSO-d_6) δ (ppm) 12.37 (br s, 1H), 8.05 (s, 1H), 7.71 (m, 3H). ^{13}C NMR (DMSO-d_6) δ (ppm) 146.0, 145.2, 130.5, 130.5, 123.4, 123.2, 111.0, 110.8.

2.3.3.4 6,7-Dimethoxyquinazolin-4-ol (15d):



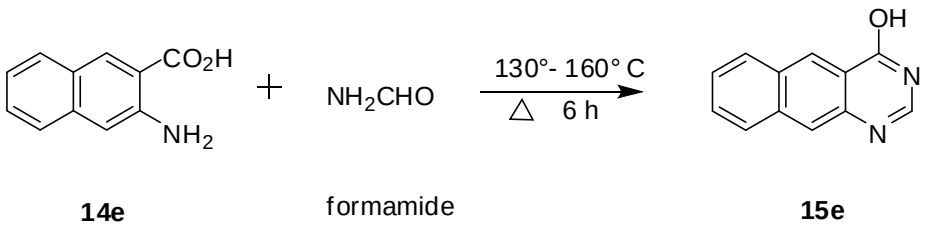
14d formamide **15d**

Scheme 2.17 The procedure for synthesizing 6,7-dimethoxyquinazolin-4-ol (**15d**)

Compound **15d** was synthesized from 2-amino-4,5- dimethoxybenzoic acid (**14d**) (502.05 g, 2.55 mmol) and formamide (1.3 mL, 32.62 mmol) followed the general procedure 2.3.3 and Scheme 2.17. The product is brown powder: 160 mg, 31 % yield. ^{1}H NMR (DMSO-d_6) δ (ppm) 12.07 (br s, 1H), 7.97 (s, 1H), 7.42 (s, 1H), 7.11 (s, 1H), 3.88 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (DMSO-d_6) δ (ppm) 160, 154.5,148.6, 144.8, 143.8, 115.5,

107.9, 104.9, 55.9, 55.7. The NMR data of **13b** was in agreement with those of previous work [25].

2.3.3.5 Benzo[g]quinazolin-4-ol (15e):

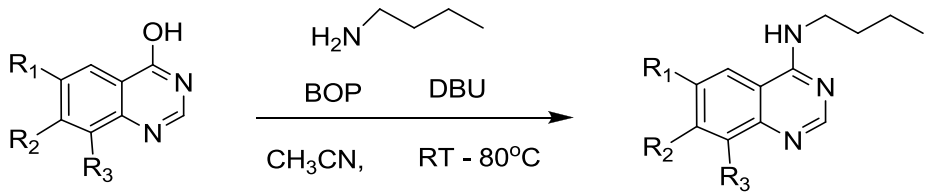


14e formamide **15e**

Scheme 2.18 The procedure for synthesizing benzo[g]quinazolin-4-ol (**15e**).

Compound **15e** was synthesized from 3-amino-2-napthoic acid (**14e**) (241.36 mg, 1.29 mmol), and formamide (500 µL, 9.73 mmol) followed the general procedure in 2.3.3 and Scheme 2.18. The product is brown powder: 205.7 mg, 81% yield. ^{1}H NMR (DMSO-d_6) δ (ppm) 12.06 (br s, 1H), 8.80 (s, 1H), 8.21 (s, 1H), 8.17 (d, J = 8.4 Hz,1H), 8.07 (d, J = 8.0 Hz 1H), 8.04 (s, 1H), 7.64 (t, J = 8.0 Hz, 1H), 7.58 (t, J = 8.0 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm) 161.7, 145.0, 144.5, 136.5, 131.3, 129.8, 129.0, 128.0, 127.7, 126.9, 125.1, 121.9.

2.3.4 General procedure for synthesizing of 4–butylaminoquinazolines with various substituents on a benzene ring.



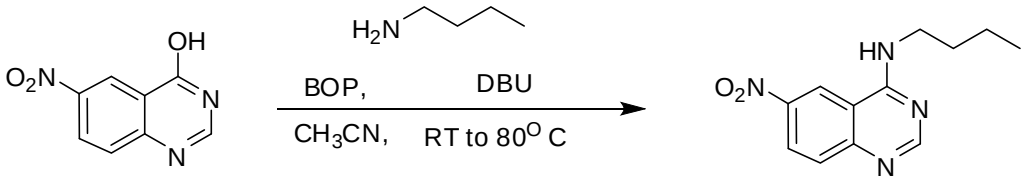
15 (a-d) 16 (a-d)

	R_1	R_2	R_3
a	NO_2	H	H
b	Cl	H	Cl
c	F	H	H
d	OMe	OMe	H

Scheme 2.19 Synthesis of 4–butylaminoquinazolines with various substituent on a benzene ring .

The synthesized compounds (**15a-d**) were used as starting chemicals and were further modified their structures to obtain (**16a–d**) followed the procedure as described in 2.3.1.

2.3.4.1 6-Nitro-4-butylaminoquinazoline (16a):

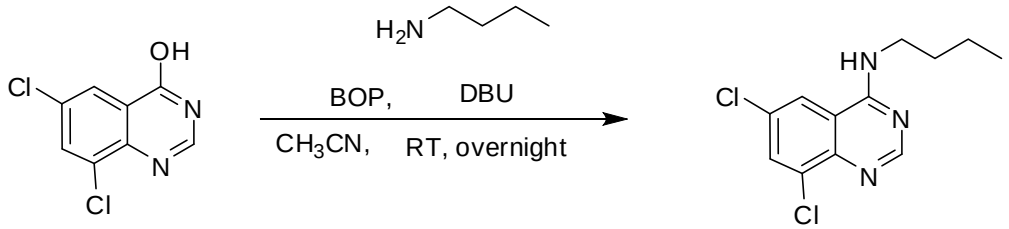


15a **16a**

Scheme 2.20 The procedure for synthesizing 6 – nitro-4-butylaminoquinazoline (**16a**)

Compound **16a** was synthesized base on general procedure 2.3.1 from **15a** (34.6 mg, 0.18 mmol), BOP (162.5 mg,0.37 mmol), DBU (87.6 µL, 0.58 mmol) and *n*-butylamine (54 µL, 0.54 mmol) in CH_3CN 2 mL at room temperature for overnight and heat 70°- 100° C for 2 h. The product is yellow solid: 15.0 mg, 40 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.76 (s, 1H), 8.68 (s, 1H), 8.42 (m, 1H), 7.88 (m, 1H), 6.42 (br s, 1H), 3.68 (m, 2H), 1.70 (m, 2H), 1.43 (m, 2H), 0.94 (t, J = 8.0 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ (ppm) 160.3, 158.3, 152.6, 144.4, 129.8, 126.3, 118.32, 113.9, 41.6, 31.1, 20.2, 13.8. MS (ESI, EI$^+$) m/z 247.2 (MH^+).

2.3.4.2 6,8-Dichloro-4-butylaminoquinazoline (16b):

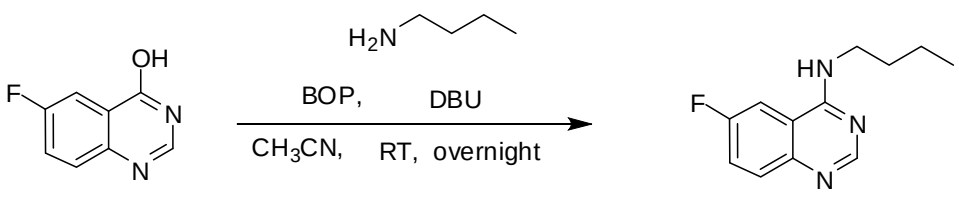


15b **16b**

Scheme 2.21 The procedure for synthesizing 6,8–dichloro-4-butylaminoquinazoline (**16b**).

Compound **16b** was synthesized base on general procedure 2.3.1 from **15b** (42.1 mg, 0.2 mmol), BOP (180.7 mg, 0.41 mmol), DBU (134 µL, 0.90 mmol) and *n*-butylamine (30 µL, 0.30 mmol) in CH_3CN 3 mL. The product is pale yellow needle: 12.4 mg, 23 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.13 (s, 1H), 8.05 (s, 1H), 7.75 (s, 1H), 5.7 (br s, 1 H), 3.94 (m, 2H), 1.70 (m, 2H), 1.35 (m, 2H), 0.90 (m, Hz, 3H), ^{13}C NMR ($CDCl_3$) δ (ppm) 159.5, 156.2, 147.3, 134.5, 132.9, 132.8, 125.0, 118.8, 47.2, 31.2, 19.8, 13.6. MS (ESI, EI$^+$) m/z 270.1 (MH^+).

2.3.4.3 6-Fluoro-4-butylaminoquinazoline (16c):

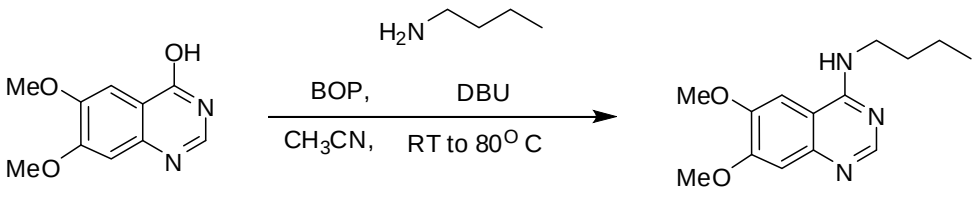


15c **16c**

Scheme 2.22 The procedure for synthesizing 6–fluoro-4-butylaminoquinazoline (**16c**).

Compound **16c** was synthesized base on general procedure 2.3.1 from **15c** (35.5 mg, 0.2 mmol), BOP (219.9 mg, 0.5 mmol), DBU (68 µL, 0.46 mmol), and n-butylamine (63 µL, 0.6 mmol) in CH_3CN 2 mL. The product is white solid: 47.0 mg 98 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.57 (s, 1H), 7.77 (m, 1H), 7.41 (m, 1H), 7.31 (d, J = 8.8 Hz, 1H), 5.70 (s, 1H), 3.59 (m, 2H), 1.64 (t, J = 8.0 Hz, 1H), 1.40 (m, 2H), 0.91 (t, J = 8.0 Hz, 3H), ^{13}C NMR ($CDCl_3$) δ (ppm) 154.9, 154.8, 131.3, 131.2, 122.1, 121.9, 105.0, 104.7. 41.3, 31.4, 20.2, 13.8. MS (ESI, EI$^-$) m/z 218.2 (MH^-).

2.3.4.4 6,7-Dimethoxy-4-butylaminoquinazoline (16d):



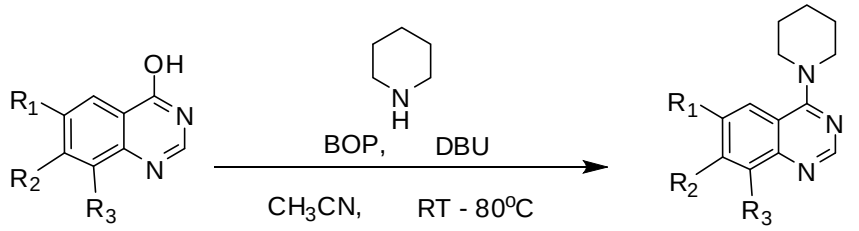
15d **16d**

Scheme 2.23 The procedure for synthesizing 6, 7–dimethoxy-4-butylaminoquinazoline(**16d**).

Compound **16d** was synthesized base on general procedure 2.3.1 from **15d** (34.9 mg, 0.17 mmol), BOP (169.8 mg, 0.38 mmol), DBU (52 µL, 0.35 mmol) and butylamine (81.5 µL, 0.82 mmol) in CH_3CN 2 mL. The product is a dark brown liquid 44.4 mg 97 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.44 (s, 1H), 7.07 (s, 1H), 6.92 (s, 1H), 5.98 (br s, 1H), 3.90 (s, 6H), 3.59 (m, 2H), 1.65 (m, 2H), 1.38 (m,3H), 0.90 (t, J = 8.0 Hz, 3H), ^{13}C

NMR ($CDCl_3$) δ (ppm) 158.6, 154.8, 152.6, 149.4, 143.5, 108.1, 105.7, 100.0, 56.3, 41.5 , 31.4, 20.2, 13.8. MS (ESI, EI^+) m/z 262.3 (MH^+).

2.3.5 General procedure for synthesizing of 4-piperidinoquinazoline with various substituent on a benzene ring.



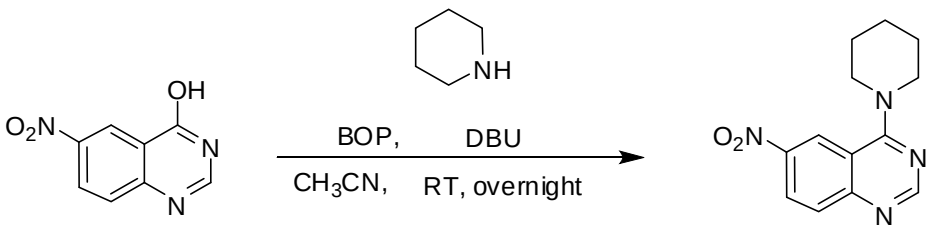
15(a-d) 17(a-d)

	R_1	R_2	R_3
a	NO_2	H	H
b	Cl	H	Cl
c	F	H	H
d	OMe	OMe	H

Scheme 2.24 Synthesis of 4 – piperidinoquinazoline derivatives with various substituent on a benzene ring .

The synthesized compounds **15a-d** were used as starting chemicals and were further modified their structure to obtain **17a–d** followed the procedure as described in 2.3.1.

2.3.5.1 6-Nitro-4-piperidinoquinazoline (17a):

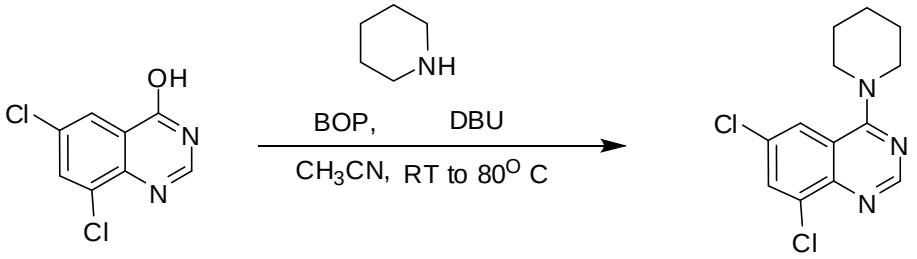


15a **17a**

Scheme 2.25 The procedure for synthesizing 6-nitro-4-piperidinoquinazoline (**17a**).

Compound **17a** was synthesized base on general procedure 2.3.1 from **15a** (50.9 mg, 0.27 mmol), BOP (270.1 mg, 0.61 mmol), DBU (56 µL, 0.56 mmol) and piperidine (81 µL, 0.82 mmol) in CH_3CN 2 mL. The product is a yellow solid: 29.6 mg ,43 % yield. ^{1}H NMR($CDCl_3$) δ (ppm) 8.77 (s,1H), 8.68 (s,1H,), 8.42 (m, 1H), 7.95 (d, *J* =9.2, 1H), 3.89 (s, 4H), 1.78 (s, 6H). ^{13}C NMR ($CDCl_3$) δ (ppm) 163.4, 155.8, 154.3, 142.3, 124.9, 121.6, 113.7, 49.8, 28.8, 25.0, 23.4. MS (ESI, EI^+) m/z 259.2 (MH^+).

2.3.5.2 6,8-Dichloro-4-piperidinoquinazoline (17b):

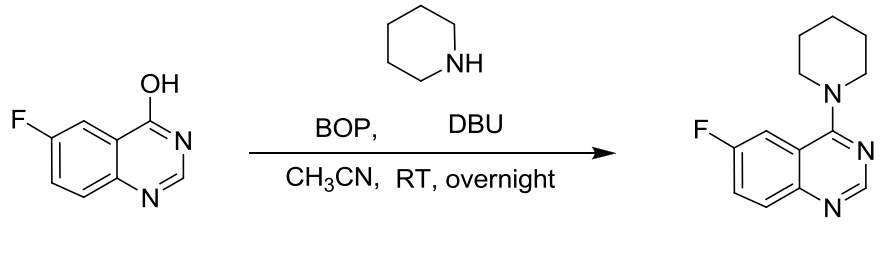


15b **17b**

Scheme 2.26 The procedure for synthesizing 6,8-dichloro-4-piperidinoquinazoline (**17b**).

Compound **17b** was synthesized base on general procedure 2.3.1 from **15b** (30.1 mg, 0.14 mmol), BOP (141.2 mg, 0.32 mmol), DBU (84 µL, 0.56 mmol) and piperidine (44 µL, 0.45 mmol) in CH_3CN 2 mL that obtain a white solid: 8.1 mg 21 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.72 (s, 1H), 7.74 (s, 1H), 7.67(s, 1H), 3.66 (m, 4H), 1.72 (m, 6H,). ^{13}C NMR ($CDCl_3$) δ (ppm) 164.0, 154.8 , 147.4, 133.8, 132.7, 129.5, 123.2, 117.9, 51.0, 25.9 , 24.5. MS (ESI, EI^+) m/z 282.2 (MH^+).

2.3.5.3 6-Fluoro-4-piperidinoquinazoline (17c):

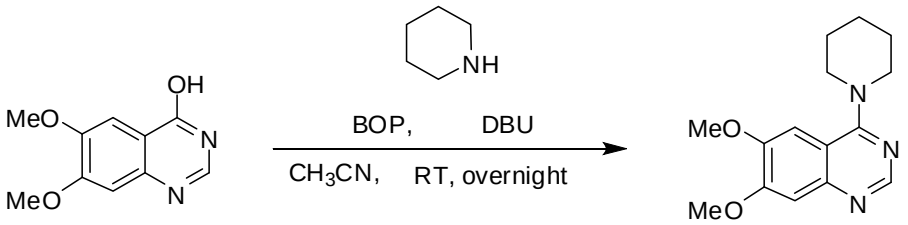


15c **17c**

Scheme 2.27 The procedure for synthesizing 6-fluoro-4-piperidinoquinazoline (**17c**).

Compound **17c** was synthesized base on general procedure 2.3.1 from **15c** (43.7 mg, 0.27 mmol), BOP (243.6 mg, 0.55 mmol), DBU (84 µL, 0.56 mmol) and piperidine (81 µL, 0.82 mmol) in CH_3CN 2 mL. The product is a red brown oil: 25.6 mg 41 % yield. ^1H NMR ($CDCl_3$) δ (ppm) 8.62 (s, 1H), 7.81 (m, 1H), 7.42 (m, 2H), 3.60 (m, 4H), 1.71 (m, 6H), ^{13}C NMR ($CDCl_3$) δ (ppm) 164.7, 160.2, 153.6, 148.6, 130.9, 122.0, 109.0, 50.9, 25.9, 24.6. MS (ESI, EI^+) m/z 232.2 (MH^+).

2.3.5.4 6,7-Dimethoxy-4-piperidinoquinazoline (17d):

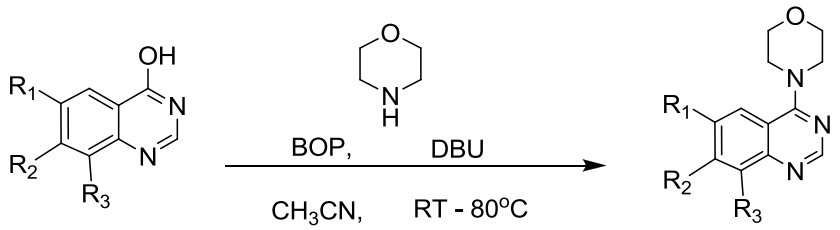


15d **17d**

Scheme 2.28 The procedure for synthesizing 6,7-dimethoxy-4-piperidinoquinazoline (**17d**).

Compound **17d** was synthesized base on general procedure 2.3.1 from **15d** (46.3mg, 0.22 mmol), BOP (198.2 mg, 0.45 mmol), DBU (70 µL, 0.47 mmol) and piperidine (67 µL, 0.68 mmol) in CH_3CN 2 mL. The product is a pale yellow solid: 47.9 mg 80 % yield. ^1H NMR ($CDCl_3$) δ (ppm) 8.62 (s, 1H), 7.20 (s, 1H), 7.08 (s, 1H), 3.98 (m, 6H), 3.57 (m, 4H), 1.77 (m, 6H). ^{13}C NMR ($CDCl_3$) δ (ppm) 164.4, 154.4, 153.2, 149.0, 148.2, 111.6, 107.4, 103.4, 56.2, 56.0, 51.0, 26.0, 24.8. MS (ESI, EI^+) m/z 274.1 (MH^+).

2.3.6 General procedure for synthesizing of 4-morpholinoquinazoline with various substituent on a benzene ring.



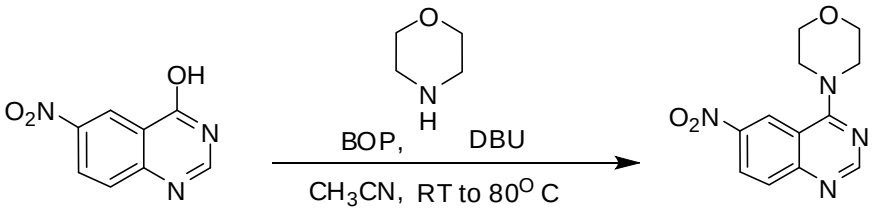
15 (a-d) 18 (a-d)

	R_1	R_2	R_3
a	NO_2	H	H
b	Cl	H	Cl
c	F	H	H
d	OMe	OMe	H

Scheme 2.29 Synthesis of 4–morpholinoquinazoline derivatives with various substituents on a benzene ring.

The synthesized compounds **15a-d** were used as starting chemicals and were further modified their structure to obtain **17a–d** followed the procedure as describe in 2.3.1.

2.3.6.1 6-Nitro-4-morpholinoquinazoline (18a):

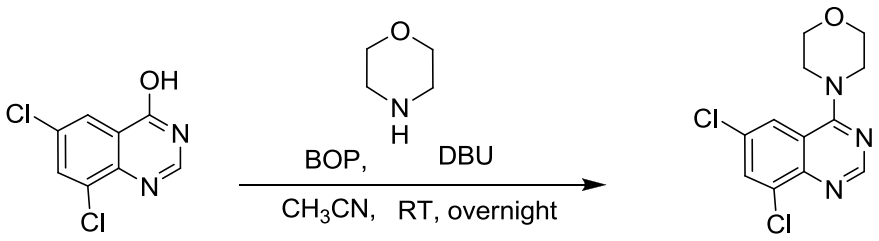


15a **18a**

Scheme 2.30 The procedure for synthesizing 6-nitro-4-morpholinoquinazoline (**18a**)

Compound **18a** was synthesized base on general procedure 2.3.1 from **15a** (30.6 mg, 0.16 mmol), BOP (136.3 mg, 0.308 mmol), DBU (50 µL, 0.335 mmol) and morpholine (50 µL, 0.57 mmol) in CH_3CN 2 mL. The product is a yellow solid: 5.5 mg, 13 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.77 (s, 1H), 8.72 (s, 1H), 8.44 (dd, *J* = 4.0, 8.0 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 3.92 (t, *J* = 4.0 Hz, 4H), 3.84 (t, *J* = 4.0 Hz, 4H), ^{13}C NMR ($CDCl_3$) δ (ppm) 164.6, 156.8, 155.3, 143.8, 130.4, 126.2, 121.9, 114.8, 66.7, 50.0. HRMS [$(M+H)^+$]: caled for $C_{15}H_{13}N_2O$ 261.0909, found 261.1011.

2.3.6.2 6, 8-Dichloro-4-morpholinoquinazoline (18b):

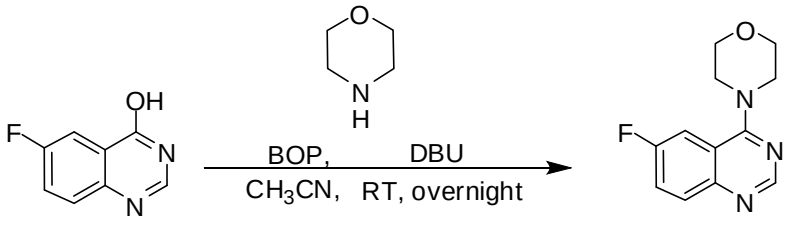


15b **18b**

Scheme 2.31 The procedure for synthesizing 6,8-dichloro-4-morpholinoquinazoline (**18b**)

Compound **18b** was synthesized base on general procedure 2.3.1 from **15b** (35.6 mg, 0.17 mmol), BOP (171.6 mg, 0.39 mmol), DBU (53 µL, 0.35 mmol) and morphorine (46 µL, 0.53 mmol) in CH_3CN 2 mL. The product is a pale yellow needle 8.0 mg 17 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.84 (s, 1H), 7.84 (d, *J* = 2.4 Hz, 1H), 7.75 (d, *J* = 2.4 Hz, 1H), 3.89 (m, 4H), 3.80 (t, *J* = 4.0 Hz, 4H). ^{13}C NMR ($CDCl_3$) δ (ppm) 163.9, 154.6, 147.4, 134.3, 133.0, 130.2, 122.7, 117.8 , 66.6, 50.4.

2.3.6.3 6-Fluoro-4-morpholinoquinazoline (18c):

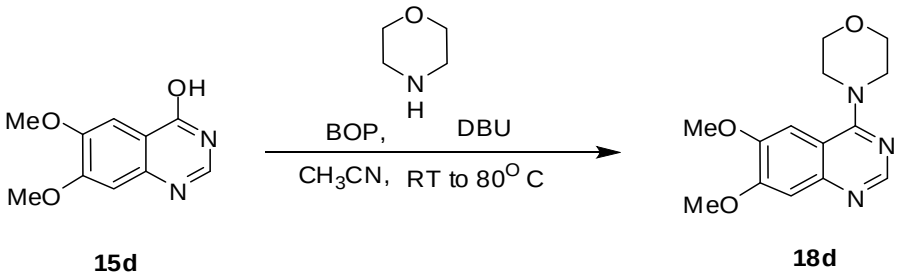


15c **18c**

Scheme 2.32 The procedure for synthesizing 6-fluoro-4-morpholinoquinazoline (**18c**)

Compound **18c** was synthesized base on general procedure 2.3.1 from **15c** (42.9 mg, 0.26 mmol), BOP (257.5 mg, 0.58 mmol), DBU (82 µL, 0.55 mmol) and morphorine (70 µL, 0.80 mmol) in CH_3CN 2 mL. The product is a white solid: 47 mg, 78% yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.69 (s, 1H), 7.87 (m, 1H), 7.46 (m, 2H), 3.84 (t, *J* = 4.0 Hz, 4H), 3.68 (t, *J* = 4.0 Hz, 4H). ^{13}C NMR ($CDCl_3$) δ (ppm) 160.6, 158.1, 153.5, 148.6, 131.4, 128.8, 122.6, 108.8, 66.7, 50.2.

2.3.6.4 6,7-Dimethoxy-4-morpholinoquinazoline (18d):



15d **18d**

Scheme 2.33 The procedure for synthesizing 6,7-dimethoxy-4-morpholinoquinazoline (**18d**).

Compound **18d** was synthesized base on general procedure 2.3.1 from **15d** (31.4 mg, 0.15 mmol). BOP (150.7 mg, 0.34 mmol), DBU (48 µL, 0.32 mmol) and morphorine (40.13 µL, 0.46 mmol) in CH_3CN 2 mL. The product is light brown solid: 43.1 mg, 103 % yield. ^{1}H NMR ($CDCl_3$) δ (ppm) 8.61 (s, 1H), 7.24 (s, 1H), 7.02 (s, 1H), 3.97 (s, 3H), 3.92 (s, 3H), 3.84 (s, 4H), 3.63 (d, *J* = 4.0 Hz, 4H). ^{13}C NMR ($CDCl_3$) δ (ppm) 163.7, 154.9, 152.6, 148.8, 148.4, 111.1, 107.1, 102.9, 66.7, 56.4, 56.1, 50.3.

2.4 Anticholinesterase evaluation

The determination of cholinesterase inhibitory activities of synthetic compounds was performed according to the modified Ellman's method (1961) and Sawasdee, P. et al. (2009) [26]. The studied compounds were tested in a 96 - well plate. This evaluation is based on the cleavage by cholinesterase of acetylthiocoline to produce thiocholine and acetate (Scheme 2.34). The thiocholine was then reacts with dithiobisnitrobenzoate ion to give 2-nitrobenzoate-5-mercaptothiocholine which is yellow

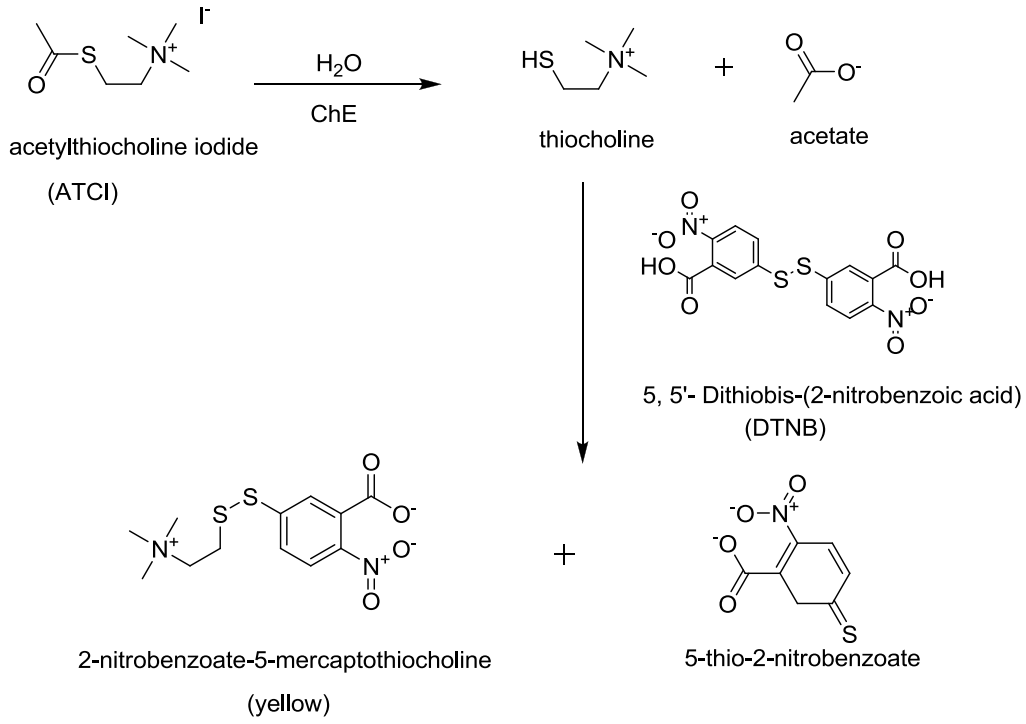
color. The yellow color was measured at 415 nm by Sunrise microplate reader (Tecan SIN 0393000 3190, P-Intertrade Equipmenta, Australia).

2.4.1 Enzyme and reagents

Acetylcholinesterase (AChE) from electric eel (Type–VI–S, EC 3.1.1.7), butyrylcholinesterase (BChE) from horse serum (EC 3.1.1.8), acetylthiocholine iodide (ATCI), 5,5´ -dithiobis-bis(2-nitrobenzoic) acid (DTNB) were purchased from Sigma (St Louis, MO, USA).

Eserine and bovine serum albumin (BSA) were purchased from Fluka BioChemika.

Tris-(hydroxymethyl)-aminomethane (*Tris*-HCl) was purchased from Merck (Darmstadt, Germany).



Scheme 2.34 Reaction of anticholinesterase assay

2.4.2 Reagent preparation

2.4.2.1 Buffers:

Buffer A (50 mM *Tris*-HCl, pH 8) was prepared by dissolving *Tris*-HCl 7.88 g in 980 mL of distilled water. The solution was adjusted to pH8 with 25% of HCl (A.R. grade) and make the total volume to be 1000 mL.

Buffer B (50 mM *Tris*-HCl, pH 8 containing 0.1% bovine serumn albumin (BSA)) was prepared by dissolving *Tris*-HCl 7.88 g and BSA 1 g in 980 mL of distilled water. The solution was adjusted to pH8 with 25% of HCl (A.R.grade) and make the total volume to be 1000 mL.

2.4.2.2 Enzymes:

Cholinesterases (AChE and BChE) were dissolved in buffer A to make 1130 U/mL stock solution in each, and further diluted to 0.25 U/mL with buffer B.

2.4.2.3 Substrate:

15 mM ATCI in Milli Q water was used for microplate assay.

2.4.2.4 Ellman's reagent:

3 mM DTNB in buffer A was used for the microplate assay.

2.4.2.5 Anticholinesterase activity assay

The 5 mM of tested compounds were prepared by 10% ethanol in buffer A. In a 96-well plate, 50 µL of buffer A, 25 µL of sample solution, 25 µL of 15 mM ATCI in Milli Q water, 125 µL of 3 mM DTNB in buffer A were added. And 25 µL of 0.25 U/mL of AChE or 25 µL of 0.25 U/mL of BChE was added and mixed well. Then the absorbance was measured at 415 nm over 2 min with a 5 s interval by kinetic mode of a Sunrise microplate reader. The evaluate AChE and BChE inhibitory activities of these compounds were calculated by comparing the mean slope for the samples relative to that for the blank (25 µL of solvent that was used to dissolve sample). The percentage of inhibitory activity was calculated follow as Equation 2.1. Eserine was used as standard compound. A determination was determined in triplicate. Finally the good inhibitors (more than 60 % inhibition) were

evaluated their IC_{50} in the interval concentration which had % inhibition between 20 to 80 by using software package Prism program (Graph Pad Inc., San Diago, CA). The example of a curve was shown in Figure 2.1. The IC_{50} values are the mean of two or three independent measurements, each performed in triplicate.

Equation 2.1: $$\text{Inhibition (\%)} = \left(\frac{MC-MS}{MC} \right) \times 100$$

MC is a mean slope of control

MS is a mean slope of sample

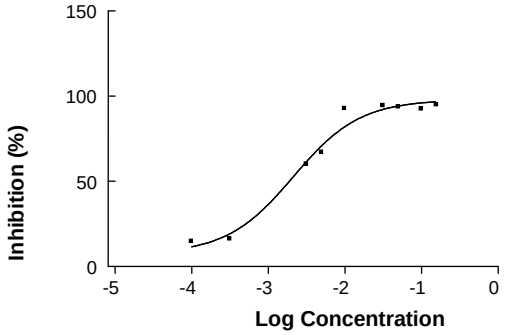


Figure 2.1 A graph for determining an IC_{50} value by using software package Prism program.

CHAPTER III
RESULTS AND DISCUSSION

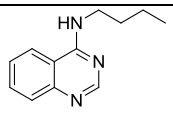
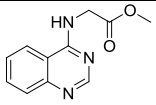
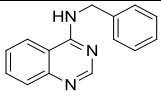
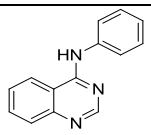
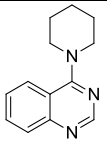
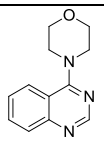
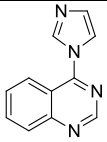
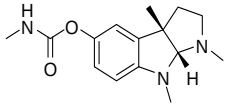
3.1 The anti-cholinesterase activities of 4-substitued aminoquinazolines (12a-g) and 3-substitued quinazolines (13a-c)

To begin with 4-substitued aminoquinazolines (**12a-g**) and 3-substitued quinazolines-4-ones (**13a-c**) were synthesized from 4-hydroxyquinazoline as described in chapter II. First series of 4-substitued aminoquinazolines (**12a-g**) were prepared by reaction between the 4-hydroxyquinazoline with various amines in the presence of BOP and DBU under the condition reported by Wan, Z.-K. *et al*. [23]. The desired compounds (**12a-g**) were isolated in the fair yields (10-88%) after column chromatography. On the other hands, 3-substitued quinazolines-4-ones derivatives (**13a-c**) were prepared from the coupling reaction between the 4-hydroxyquinazoline and various primary amines in the present of Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide). The target compounds (**13a-c**) were obtained in good yields (49-78%). Moreover, all prepared compounds were characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometry techniques which were corresponded to the previous reports (see more detail in Chapter II).

With the 10 compounds in hands, the inhibition activities of these synthesized quinazolines were evaluated for their AChE and BChE inhibitory activities at the final concentration of 0.5 mM and using eserine as standard. The results were shown in Table 3.1 and 3.2, respectively.

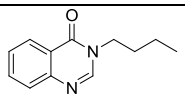
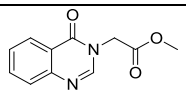
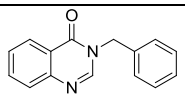
The results in Table 3.1 showed that the 4-piperidinoquinazoline (**12e**) exhibited the highest AChE and BChE inhibitory activities. The highest selectivity on BChE over AChE was observed in the 4-morpholinoquinazoline (**12f**). In addition, 4-butylaminoquinazoline (**12a**) has a moderate inhibition towards both enzymes and the rest compounds showed low activities. Moreover, the 3-substitued quinazolines (Table 3.2) seem to possess lower inhibition effects comparing with the 4-substitued quinazolines (Table 3.1), except for (**13c**) which the inhibition toward BChE increased comparing with that of (**12c**).

Table 3.1 Anti-cholinesterase activities of quinazoline derivatives (**12a-g**).

Compound	Structure (% yield)	Inhibition percentage[a] (IC_{50} , μM)	
		AChE	BChE
12a	 (59)	44.0 ± 1.8 (> 500)	66.0 ± 0.2 (38.0 ± 0.37)
12b	 (88)	27.0 ± 2.8 (>500)	24.0 ± 2.8 (>500)
12c	 (62)	43.0 ± 2.8 (>500)	24.0 ± 0.6 (>500)
12d	 (65)	4.0± 0.7 (>500)	11.0 ± 2.4 (>500)
12e	 (84)	84.6 ± 0.7 (1.3 ± 0.1)	86.0 ± 0.5 (7.5 ± 0.4)
12f	 (39)	29.2 ± 2.7 (>500)	72.8 ± 1.8 (>500)
12g	 (10)	22.0 2.2 (>500)	27.6 ± 3.4 (>500)
Eserine		93.2 ± 1.4 (0.2 ± 0.04)	94.8 ± 1.5 (0.7 ± 0.04)

[a] *final concentration at 0.5 mM.*

Table 3.2 Anti-cholinesterase activities of quinazoline derivatives (**13a-c**).

Compound	Structure (% yield)	Inhibition percentage[a] (IC_{50} , μM)	
		AChE	BChE
13a	 (78)	38.5 ± 3.7 (> 500)	33.5 ± 3.3 (> 500)
13b	 (49)	28.0 ± 5.3 (>500)	7.4 ± 3.1 (> 500)
13c	 (69)	27.0± 0.7 (> 500)	83.1± 0.8 (> 500)

[a] *final concentration at 0.5 mM.*

3.2 The anti-cholinesterase activities of quinazoline derivatives 15a-e with various substituents on a benzene ring

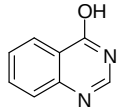
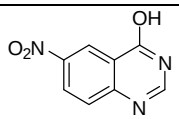
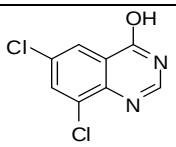
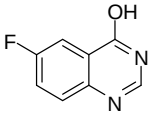
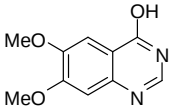
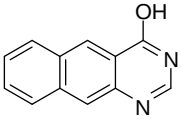
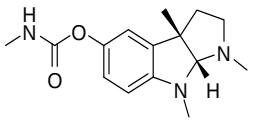
Five quinazoline derivatives with various substituents on a benzene ring (**15a-e**) were synthesized using a formamide and available anthranilic acid *via* Niementowski condensation (see Scheme 2.13). The desired compounds (**15a-e**) were isolated in the fair yields (31-82%) after precipitated and characterized their structures by spectroscopic techniques (see more detail in Chapter II).

Compounds (**15a-c**) were designed to contain an electron-withdrawing group; the nitro, chloro and fluoro groups in the benzene ring, while compounds (**15d-e**) had an electron-donating group including methoxyl and napthyl groups. All compounds were evaluated their AChE and BChE inhibitory activities at the final concentration of 0.5 mM. The results were shown in Table 3.3.

From the results in Table 3.3, 6-nitro-4(*3H*)-hydroxyquinazoline (**15a**) had the highest inhibition toward AChE followed by benzo[g]quinazoline-4-ol (**15e**) and 6,8-dichloro-4(*3H*)-hydroxyquinazoline (**15b**). These three compounds have activities higher than starting compound 4-hydroxyquinazoline (**11**), while the rest showed the

comparable activities. However, the nitro group was considered as a potential substituent candidate for further study due to the best AChE inhibitory activity.

Table 3.3 Anti-cholinesterase activities of quinazoline derivatives (**15a-e**) with various substituents on a benzene ring.

Compound	Structure (% yield)	Inhibition percentage[a]	
		AChE	BChE
11		16.6 ± 5.7	15.0 ± 2.3
15a	 (82)	50.1 ± 1.6	20.7 ± 6.0
15b	 (82)	25.0 ± 8.8	20.2 ± 6.0
15c	 (53)	19.5 ± 3.5	9.9 ± 0.4
15d	 (31)	13.0 ± 2.0	NI
15e	 (81)	33.1 ± 0.45	19.0 ± 3.8
Eserine		95.5 ± 0.9 (0.2 ± 0.04)	96 ± 0.18 (0.7 ± 0.04)

[a] *final concentration at 0.5 mM*.

NI means “no inhibition”.

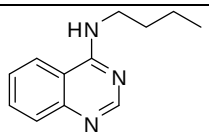
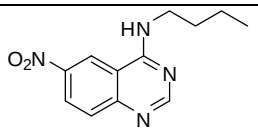
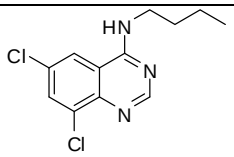
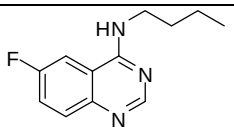
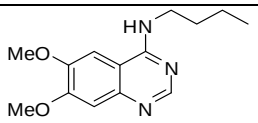
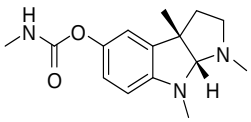
3.3 The anti-cholinesterase activities of 4-butylaminoquinazoline derivatives (16a-d) with various substituents on a benzene ring

The various substituents in a benzene ring of 4-butylaminoquinazoline (**16a-d**) were synthesized based on Niementowski reaction, which involves the fusion of anthranilic acid with formamide (Scheme 2.13). And the process was extended to 4-substitued aminoquinazolines with *n*-butylamine (Scheme 2.19). The desired compounds (**16a-d**) were isolated in the fair yields (23-98%) after column chromatography and characterized their structures by spectroscopic techniques (see more detail in Chapter II).

Compound (**12a**) was substitued with the electron withdrawing (nitro or halogen groups) and electron donating (methoxyl group) in a benzene ring and compared their AChE and BChE inhibitory activities. The results in Table 3.4, the AChE inhibitory activities of compounds (**16a-b**) which contained the electron withdrawing groups showed IC_{50} value at 1.4 and 2.9 μM, respectively but were reduced BChE inhibitory activity. In contrast fluoro group was none affected to both enzymes. Compound (**16a**), which was substituted with nitro group, exhibited higher AChE inhibitory activities than (**12a**). Especially the substituent a nitro group, its AChE inhibitory activity of (**16a**) was the highest. Compound (**16d**) which was substituted with an electron donating group (2-methoxyl) was increased BChE Inhibitory activity, IC_{50} value at 16.0 μM.

The activity results could be implied that the electron withdrawing group seem to increase AChE inhibitory activity of the substituted-4- butylaminoquinazoline by the order of NO_2 > Cl > F, but these groups reduced the BChE inhibitory activity. Moreover, the methoxyl group (the electron donating group) increased the BChE inhibitory activity.

Table 3.4 Anti-cholinesterase activities of 4-butylaminoquinazoline derivatives (**16a-d**) with various substituents on a benzene ring.

Compound	Structure (% yield)	Inhibition percentage[a] (IC_{50} , μM)	
		AChE	BChE
12a	 (68)	44.0 ± 1.8 (>500)	66.0 ± 0.2 (38.0 ± 0.37)
16a	 (34)	91.0 ± 0.8 (1.4 ± 0.05)	37.0 ± 2.1 (>500)
16b	 (23)	73.0 ± 3.5 (2.9 ± 3.3)	43.0 ± 3.5 (>500)
16c	 (98)	59.2 ± 1.1 (>500)	36.1 ± 5.8 (>500)
16d	 (97)	40.0± 5.0 (>500)	86.5 ± 1.4 (16.0 ± 2.6)
Eserine		95.5 ± 0.9 (0.2 ± 0.04)	96 ± 0.18 (0.7 ± 0.04)

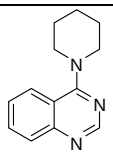
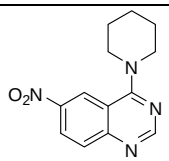
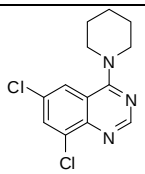
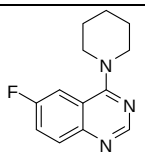
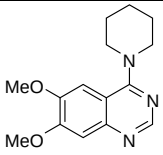
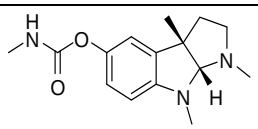
[a] *final concentration at 0.5 mM*.

3.4 The anti-cholinesterase activities of 4-piperidinoquinazoline derivatives (17a-d) with various substituents on a benzene ring

The various substituents in a benzene ring of 4-piperidino aminoquinazoline (**17a-d**) were synthesized based on Niementowski reaction, which involves the fusion of anthranilic acid with formamide (Scheme 2.13). And the process was extended to 4-substitue aminoquinazolines with piperidine (Scheme 2.24). The desired compounds (**17a-d**) were isolated in the fair yields (42-80%) after column chromatography and characterized their structures by spectroscopic techniques (see more detail in Chapter II).

The results of AChE and BChE inhibitory activities in Table 3.1, compound (**12e**) performed the highest inhibition to both enzymes with the IC_{50} values of 1.3 and 7.5 μM, respectively. In this study, four derivatives of **12e** were synthesized and evaluated their activities. The results were shown in Table 3.5, compound **17a-c** with an electron withdrawing group (nitro, dichloro and fluoro group) substituents on a benzene ring showed the reducing of both enzymes inhibitory activities. Compound **17d** substituted with electron donating group showed the decreasing both AChE and BChE inhibitory activities.

Table 3.5 Anti-cholinesterase activities of 4-piperidinoquinazoline derivatives (**17a-d**) with various substituents on a benzene ring.

Compound	Structure (% yield)	Inhibition percentage[a] (IC_{50} , μM)	
		AChE	BChE
12e	 (84)	84.6 ± 0.7 (1.3 ± 0.07)	86.0 ± 0.5 (7.5 ± 0.4)
17a	 (43)	71.5 ± 2.0 (14.0 ± 0.4)	47.4 ± 5.3 (36.0 ± 2.6)
17b	 (21)	83.3 ± 2.0 (7.3 ± 1.3)	76.9 ± 1.1 (8.5 ± 0.4)
17c	 (42)	91.7 ± 0.7 (5.2 ± 0.4)	74.6 ± 0.9 (16.5 ± 2.6)
17d	 (80)	82.1 ± 0.8 (8.6 ± 0.6)	59.6 ± 1.8 (25.0 ± 1.4)
Eserine		95.5 ± 0.9 (0.2 ± 0.04)	96 ± 0.18 (0.7 ± 0.04)

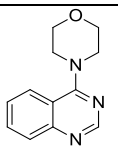
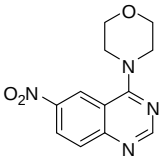
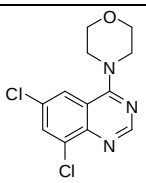
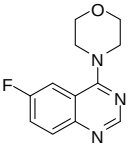
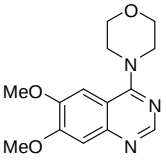
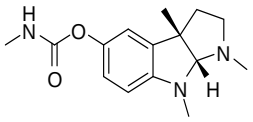
[a] *final concentration at 0.5 mM.*

3.5 The anti-cholinesterase activities of 4-morpholinoquinazoline derivatives (18a-d) with various substituents on a benzene ring

The various substituents in a benzene ring of 4-morpholinoquinazoline (**18a-d**) were synthesized based on Niementowski reaction, which involves the fusion of anthranilic acid with formamide (Scheme 2.13). And the process was extended to 4-substitue aminoquinazolines with morpholine (Scheme 2.29). The desired compounds (**18a-d**) were isolated in the fair yields (13-103%) after column chromatography and characterized their structures by spectroscopic techniques (see more detail in Chapter II).

According to the result in Table 3.1, compound **12f** showed the selectivity on BChE, thus this compound was chosen to modified structures and obtained four derivatives, **18a-d**. The anti-cholinestrerase activities of **18a-d** were shown in Table 3.6. The AChE inhibitory activity of compound **18a-c** modified with electron withdrawing groups increased by comparing with that of **12f**. While compound **18d** substituted with a donating group increase selectivity of AChE inhibition over BChE.

Table 3.6 Anti-cholinesterase activities of 4-morpholinoquinazoline derivatives (**18a-d**) with various substituents on a benzene ring.

Compound	Structure (% yield)	Inhibition (%)[a]	
		AChE	BChE
12f	 (39)	29.2 ± 2.7	72.8 ± 1.8
18a	 (13.2)	73.0 ± 1.4	40.0 ± 0.8
18b	 (17)	65.6 ± 0.9	47.1 ± 1.8
18c	 (78)	32.6 ± 3.9	18.3 ± 4.0
18d	 (103)	67.8 ± 2.5	8.7 ± 7.9
Eserine		95.5 ± 0.9 (0.2 ± 0.04)	96 ± 0.18 (0.7 ± 0.04)

[a] *final concentration at 0.5 mM.*

CHAPTER IV
CONCLUSION

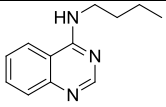
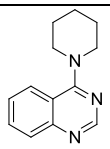
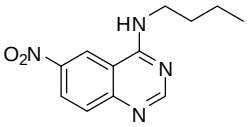
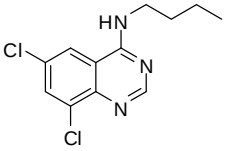
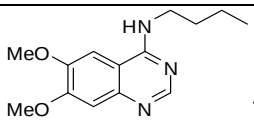
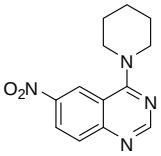
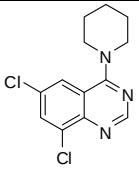
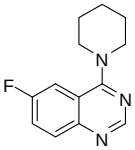
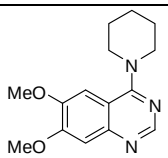
From the World Alzheimer Report of 2010 and the research of Alzheimer's association and Dementia 2010, the problem of aging disease is continually increase and becomes a major public health problem around the world. It leads to a log of procurement research to find the suitable chemical to inhibit the cause of the disease. This document focuses to verify how well the focused compounds can inhibit the AChE and BChE which are the cause of the Alzheimer's disease.

In this research the 27 synthetic compounds were determined their anticholinesterase activities by using the modification Ellman's method. Compounds which exhibited the enzyme inhibitory percentage more than 60% were further evaluated their IC_{50} values as summarized in Table 4.1. The most effective inhibition compound is 4-piperidinoquinazoline (**12e**) which can inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) with the IC_{50} values of 1.3 and 7.5 μM, respectively. However, compounds **12a**, **12e** and **12f** were chosen to further modify their structures. Various substituents were applied to a benzene ring of the core structures of **12a**, **12e** and **12f**.

When a benzene ring of **12a** had a nitro group (**16a**), the AChE inhibitiory activity increased. While it had dimethoxy group (**16d**), the BChE inhibitory activity increase. In contrast with the derivatives of **12e**, when a benzene ring contained either an electron withdrawing group (**17a-c**) or electron releasing group (**17d**), both enzyme activities slightly decreased. If the substituent on a benzene ring of **12f** was a nitro (**18a**), dichloro (**18b**) or dimethoxy (**18d**) groups, the AChE inhibitory activity increased.

In conclusion of this study, there is no trend for applying an electron withdrawing or electron releasing groups in quinazoline derivatives. That might be the different mode of reaction in any compounds. Thus we suggest further study for evaluating the mode of reaction of every potent quinazoline derivatives. However, this study 4-piperidinoquinazolines (**12e**) was a candidates for both AChE and BChE inhibitor. 6-Nitro-4-butylaminoquinazoline (**16a**) was a potent and selective inhibitor toward AChE over BChE while 6,7-dimethoxy-4-butylaminoquinazoline (16d) was a potent and selective inhibitor toward BChE over AChE.

Table 4.1 Summary of the IC_{50} values towards AChE and BChE inhibitory activities of potent quinazoline derivatives.

Compound	IC_{50} (μM)	
	AChE	BChE
 **12a**	>500	38.0
 **12e**	1.3	7.5
 **16a**	1.4	>500
 **16b**	2.9	>500
 **16d**	>500	16.0
 **17a**	14.0	36.0
 **17b**	7.3	8.5
 **17c**	5.2	17.0
 **17d**	8.6	25.0
Eserine	0.2	0.7

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Appendix

A

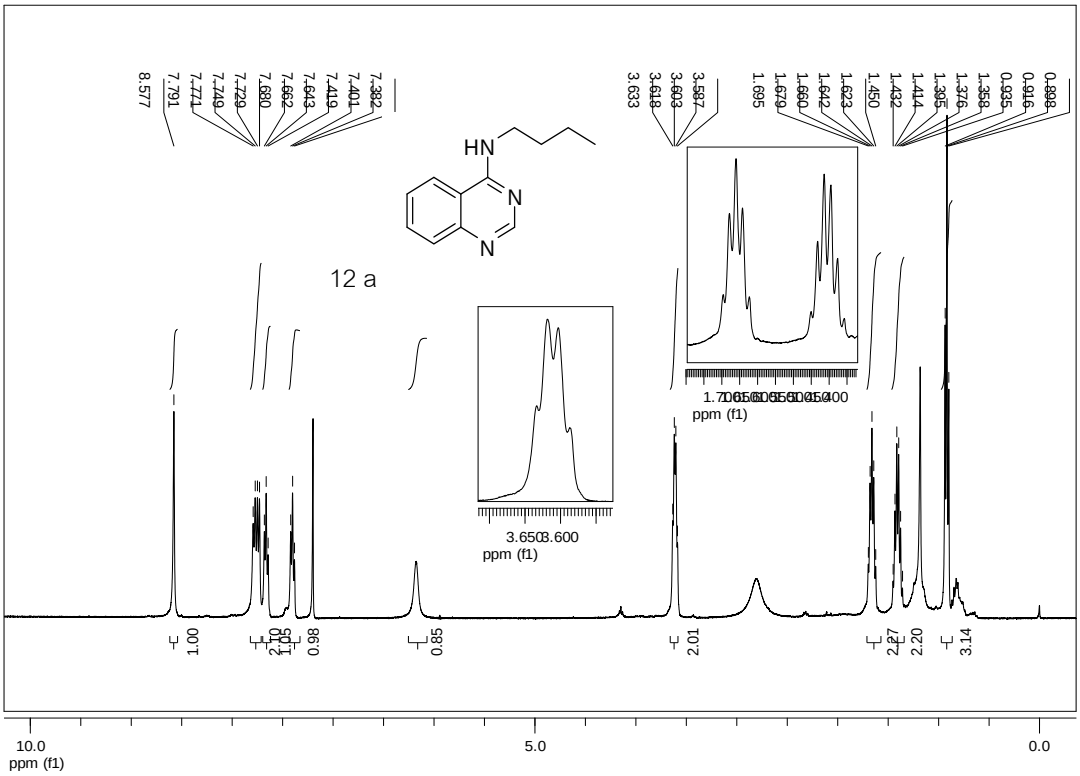

Figure 1 ^{1}H-NMR spectrum of 4-butylaminoquinazoline (**12a**).

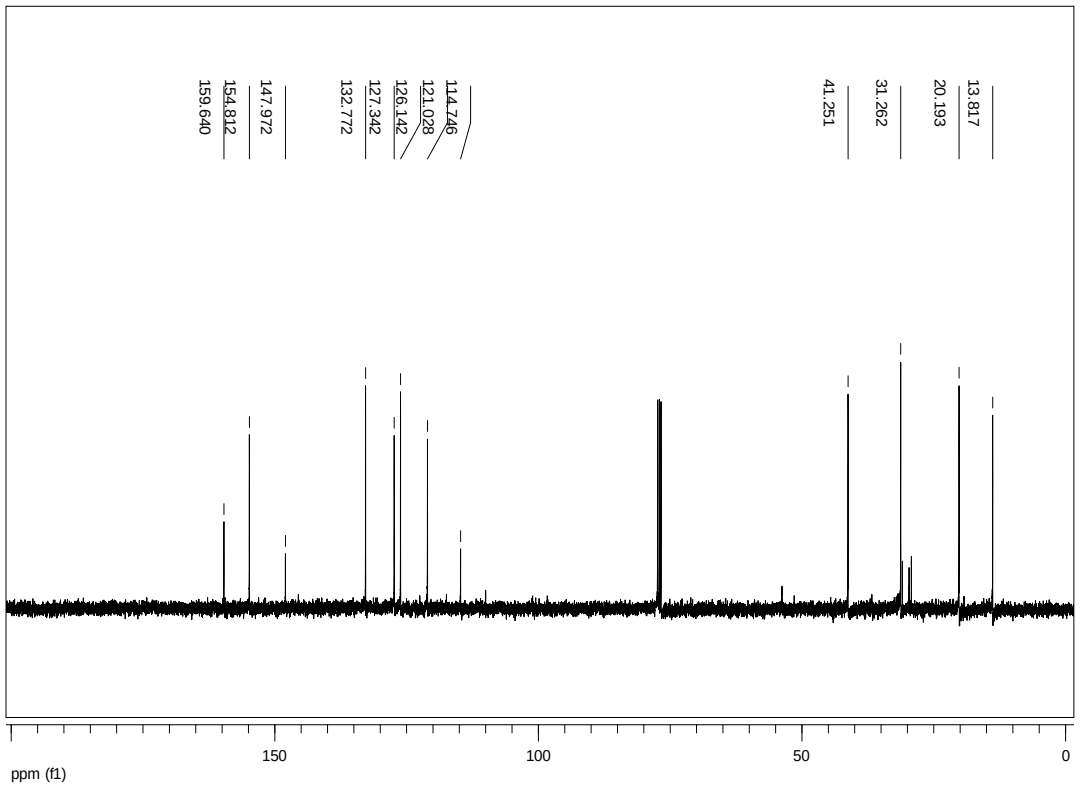

Figure 2 ^{13}C-NMR spectrum of 4-butylaminoquinazoline (**12a**).

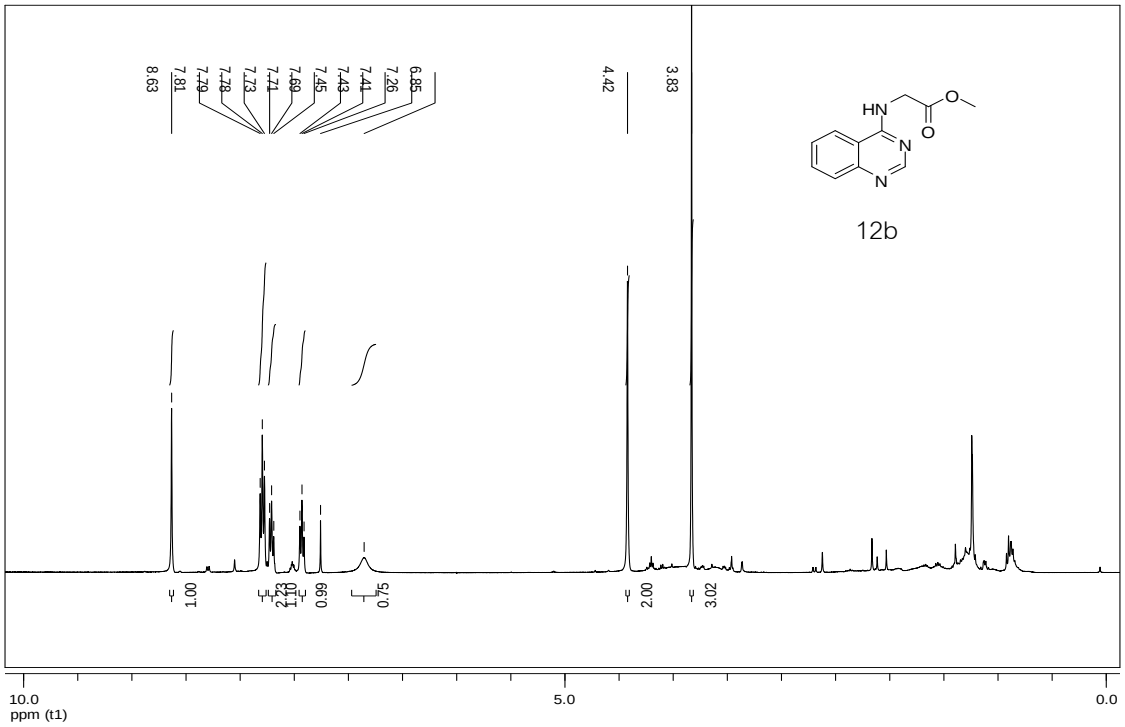

Figure 3 ^{1}H-NMR spectrum of 4-glycinmethylaminoquinazoline (**12b**).

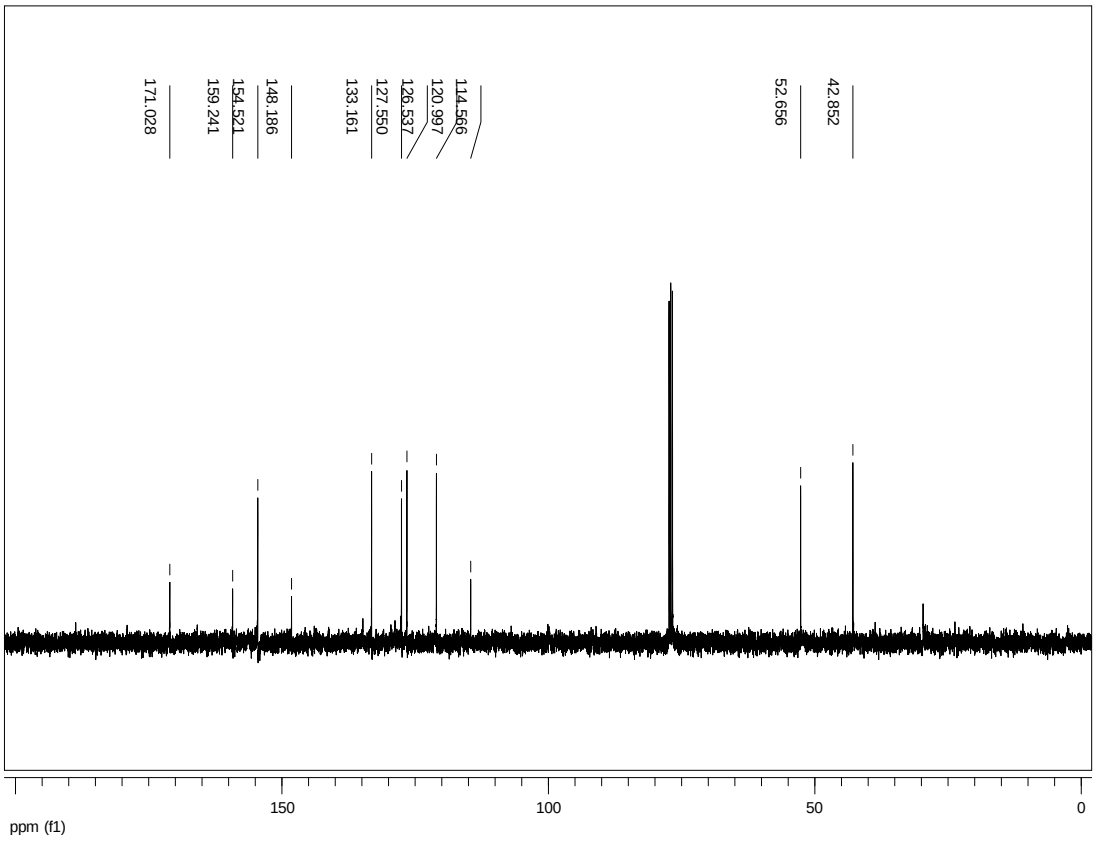

Figure 4 ^{13}C-NMR spectrum of 4-glycinmethylaminoquinazoline (**12b**).

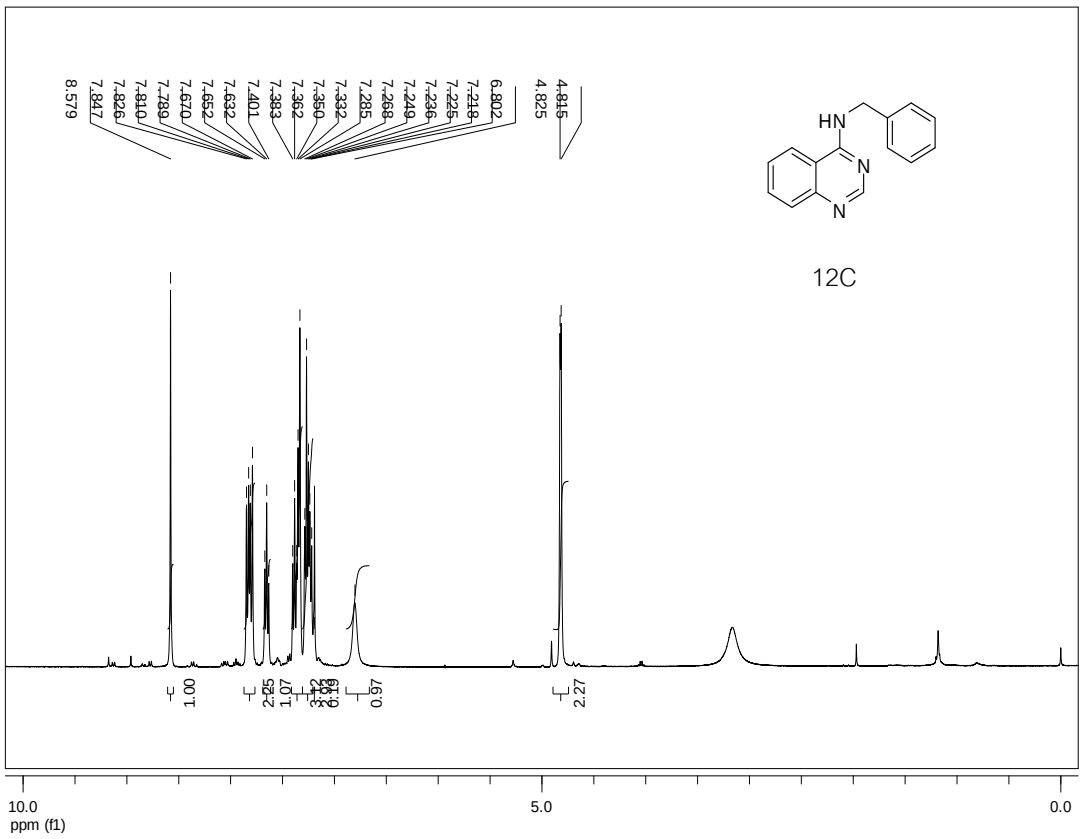

Figure 5 ^{1}H-NMR spectrum of 4-benzylaminoquinazoline(**12c**).

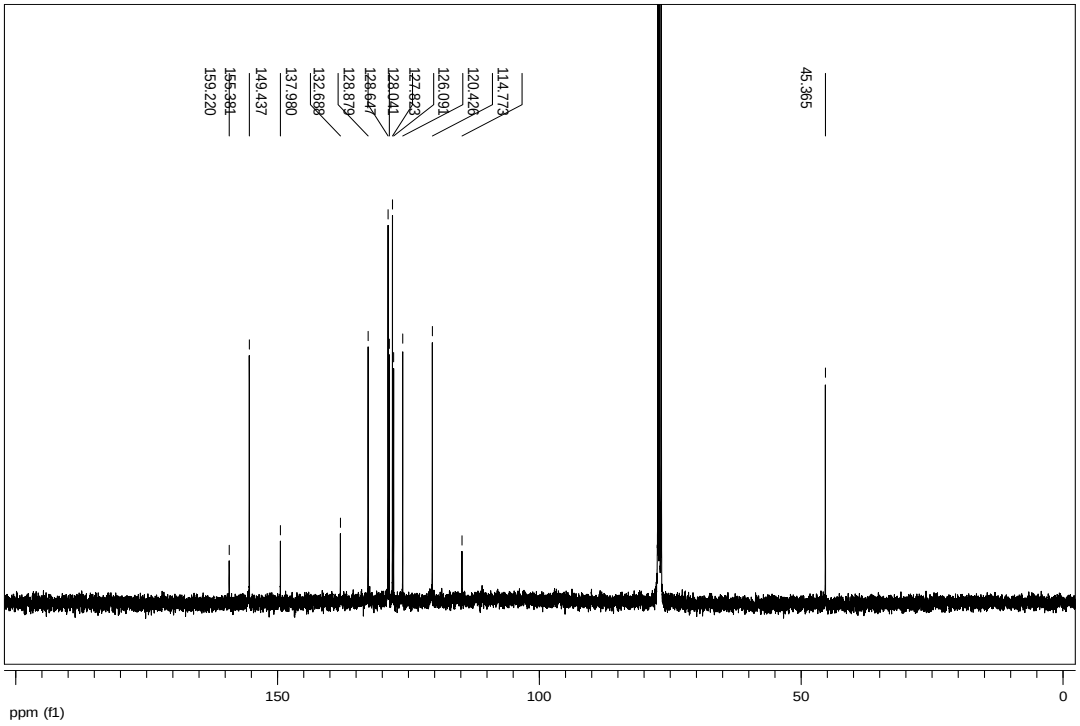

Figure 6 ^{13}C-NMR spectrum of 4-benzylaminoquinazoline(**12c**).

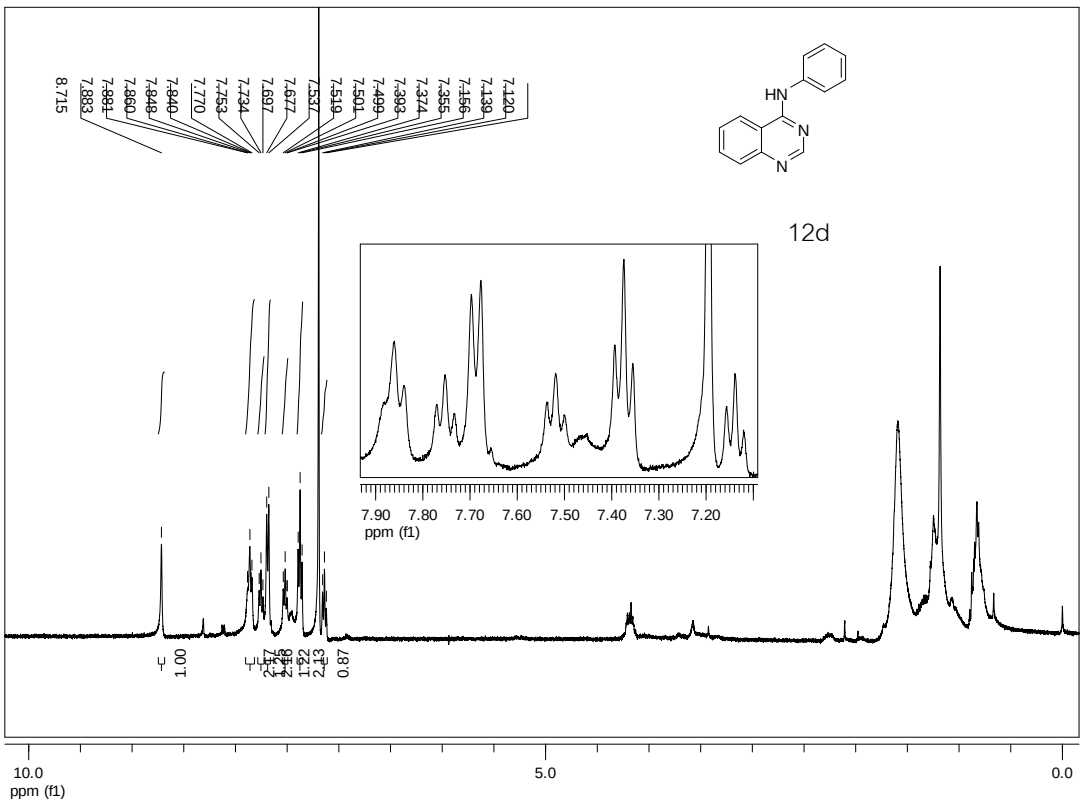

Figure 7 ^{1}H-NMR spectrum of 4-anilinoquinazoline (**12d**).

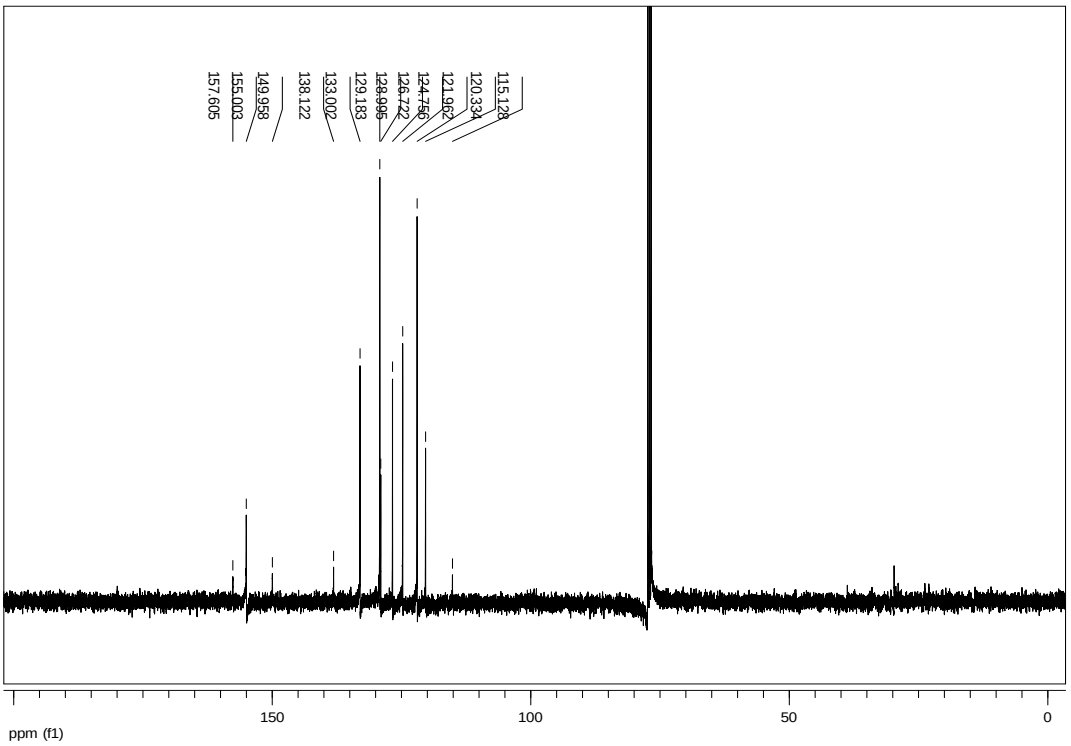

Figure 8 ^{13}C-NMR spectrum of 4-anilinoquinazoline (**12d**).

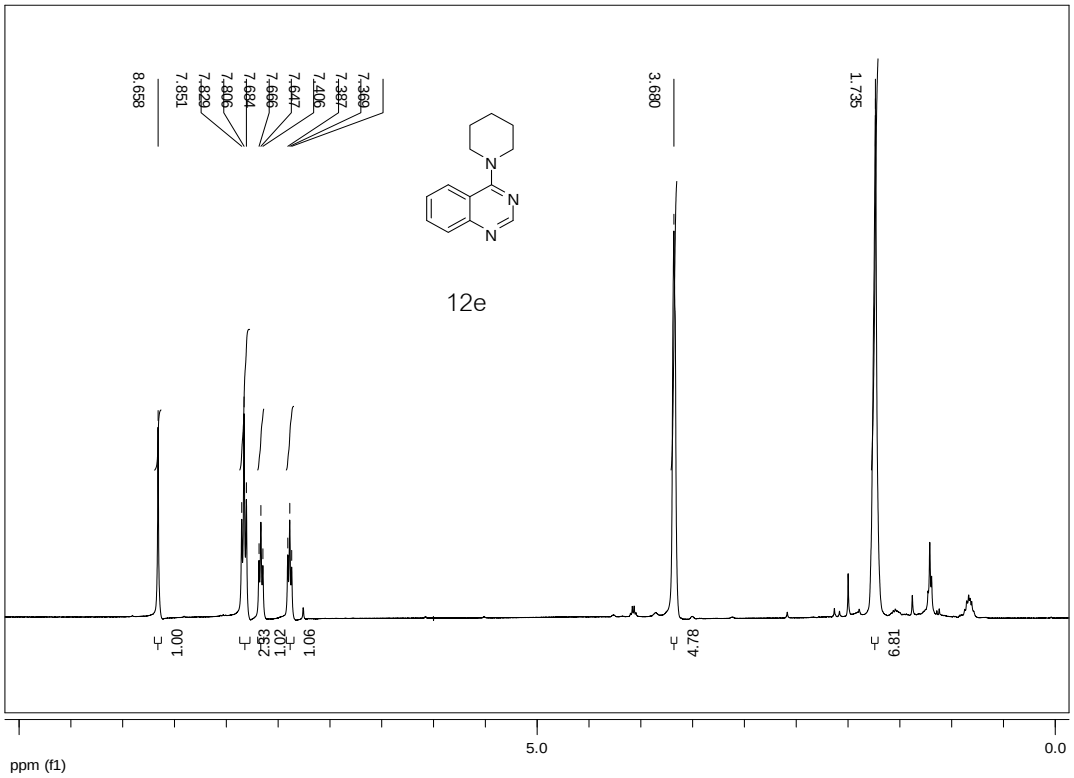

Figure 9 ^{1}H-NMR spectrum of 4-piperidinoquinazoline (**12e**).

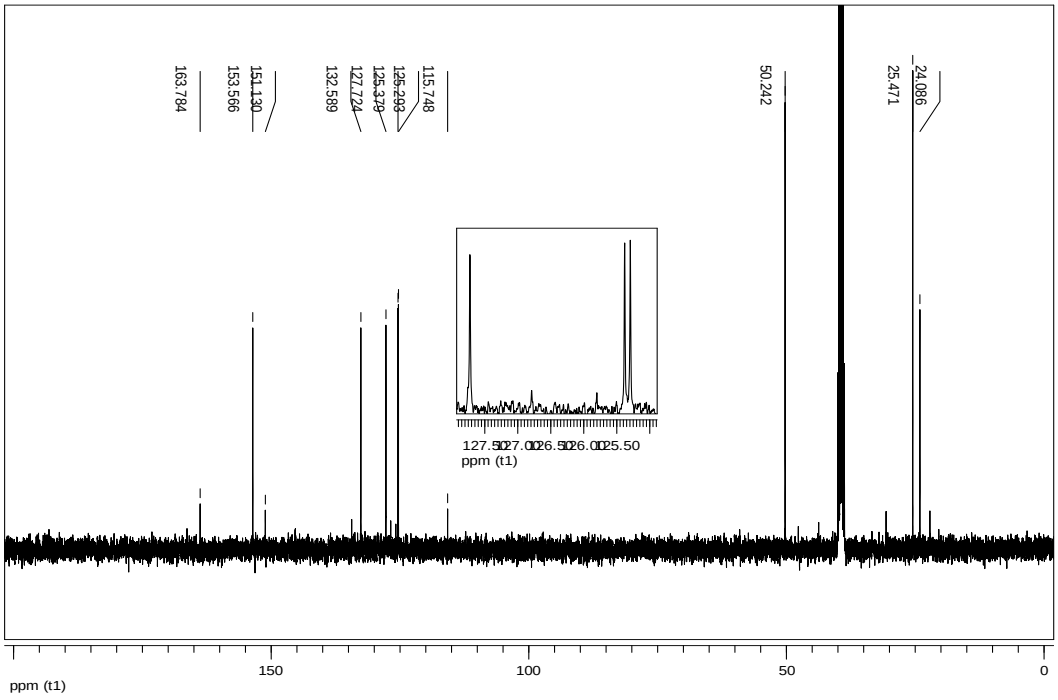

Figure 10 ^{13}C-NMR spectrum of 4-piperidinoquinazoline (**12e**).

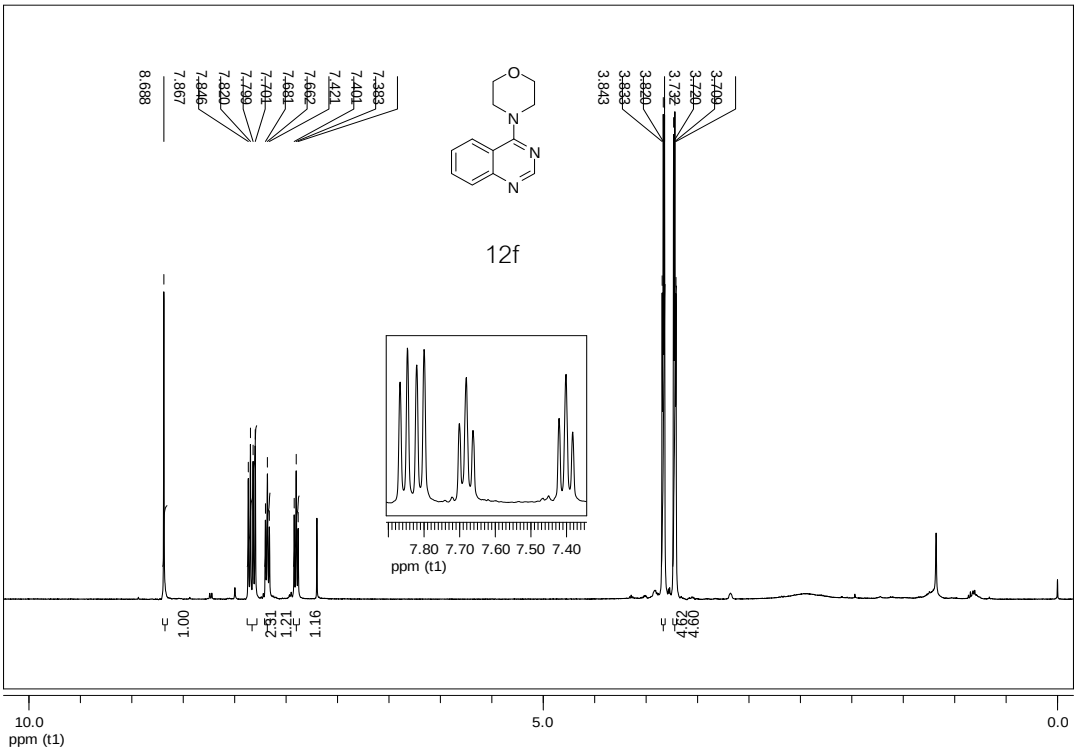

Figure 11 ^{1}H-NMR spectrum of 4-morpholinoquinazoline (**12f**).

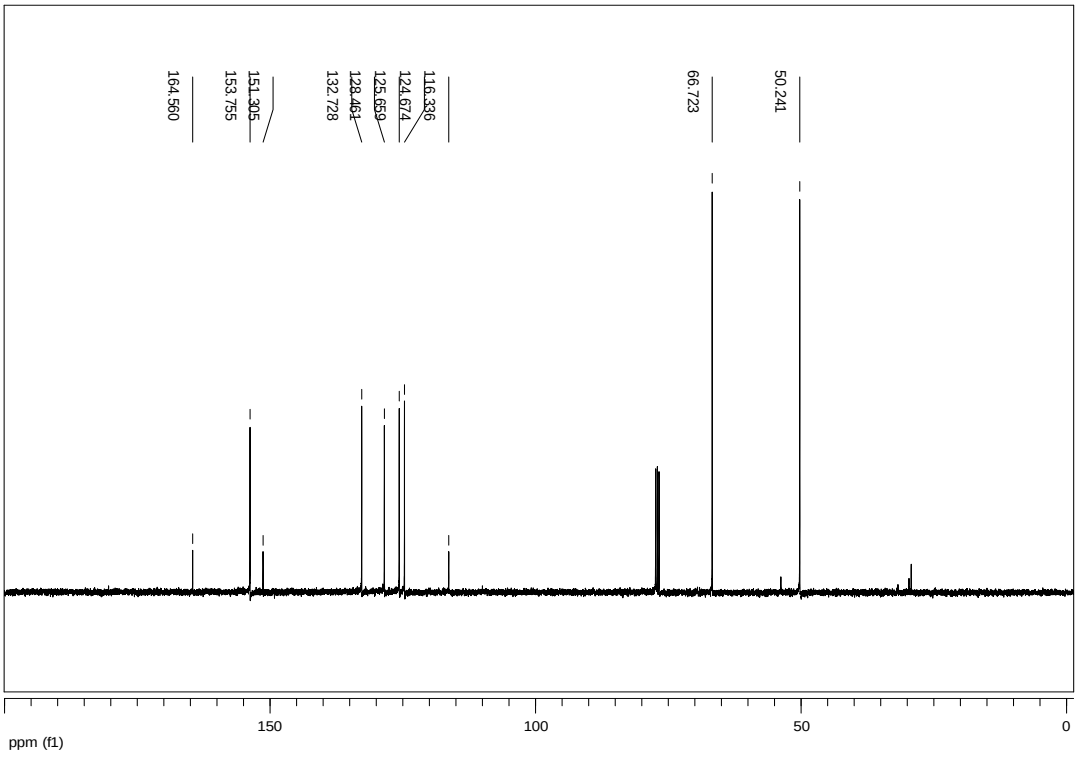

Figure 12 ^{13}C-NMR spectrum of 4-morpholinoquinazoline (**12f**).

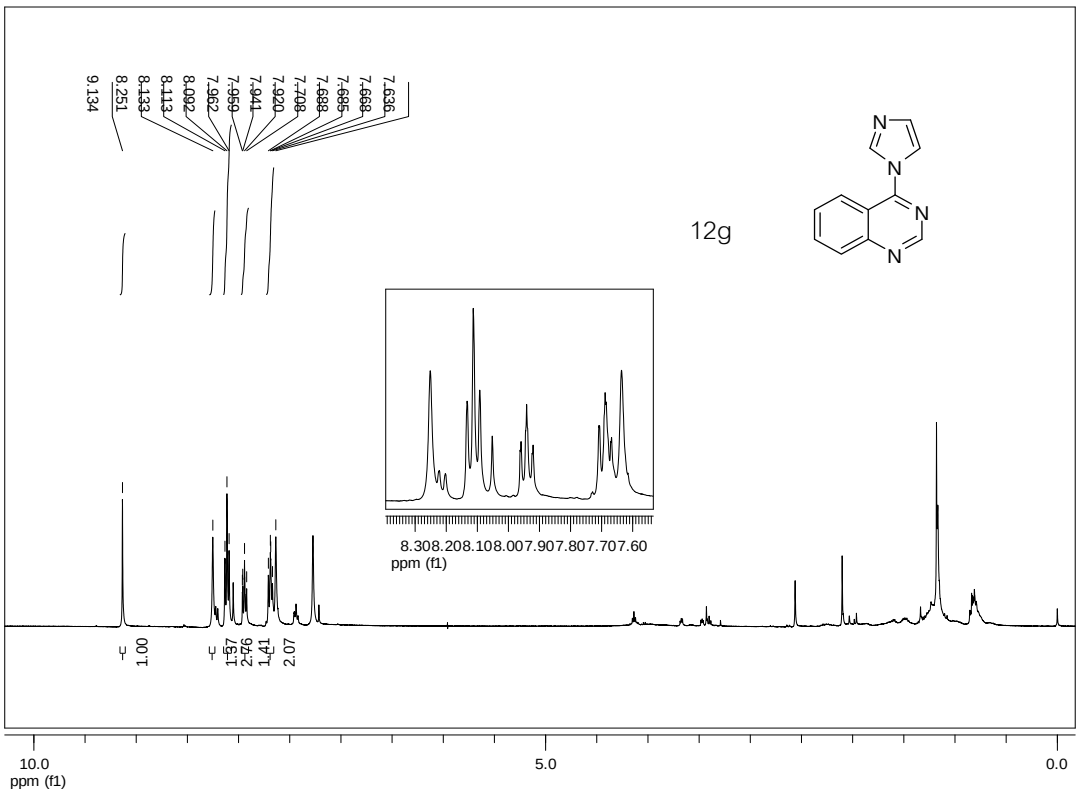

Figure 13 ^{1}H-NMR spectrum of 4-imidazolaminoquinazoline (12g).

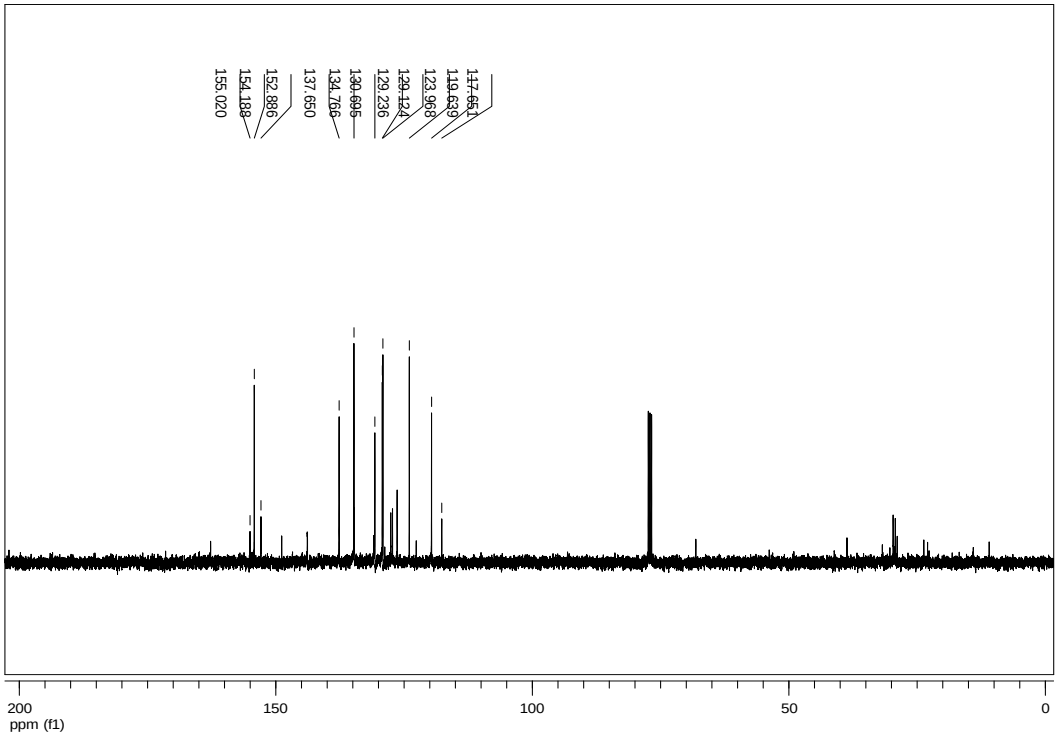

Figure 14 ^{13}C-NMR spectrum of 4-imidazolaminoquinazoline (12g).

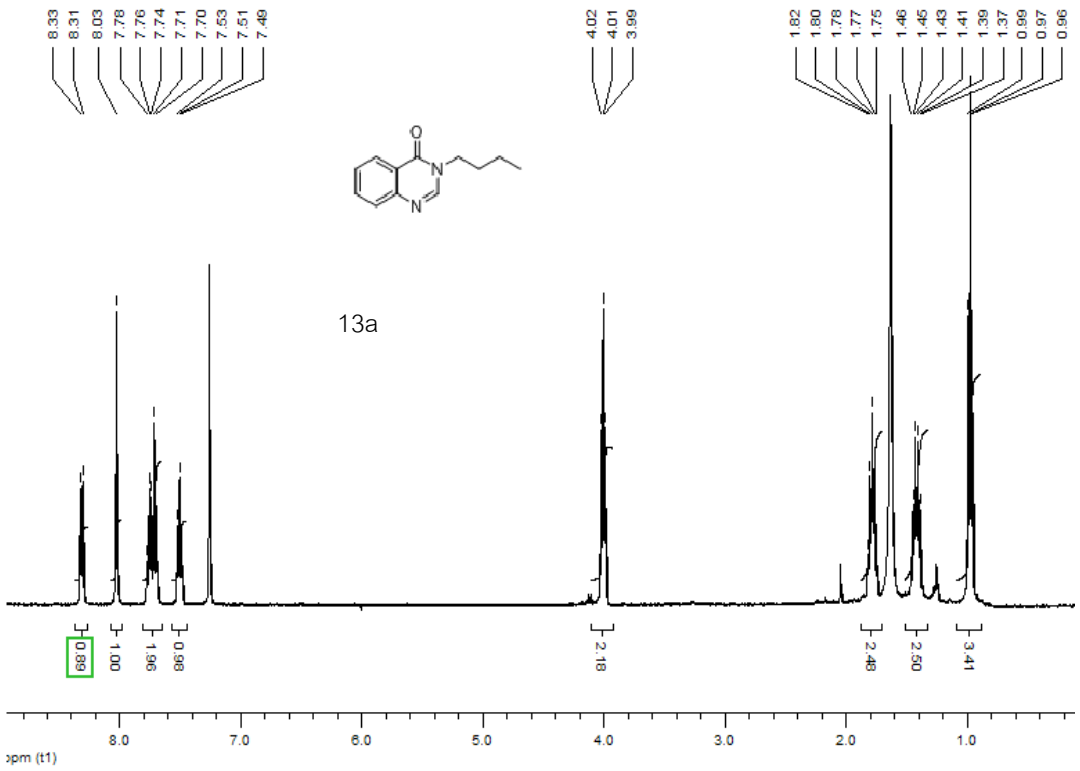

Figure 15 ^{1}H-NMR spectrum of 3-butylquinazoline-4(*3H*)–one (**13a**).

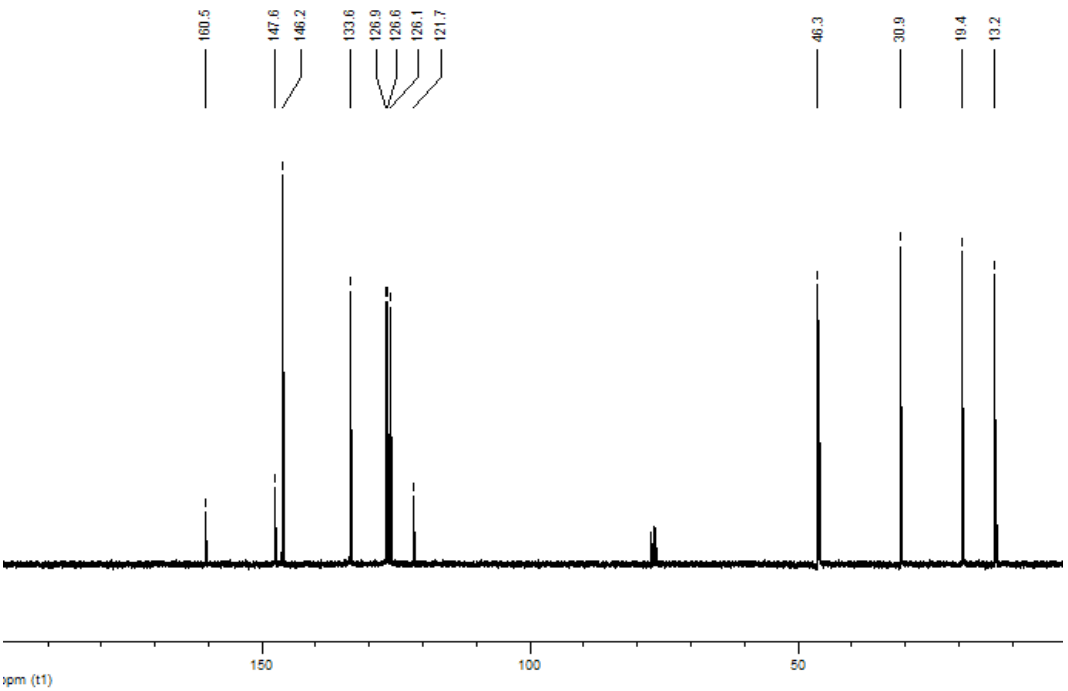

Figure 16 ^{13}C-NMR spectrum of 3- butylquinazoline-4(*3H*) –one (**13a**).

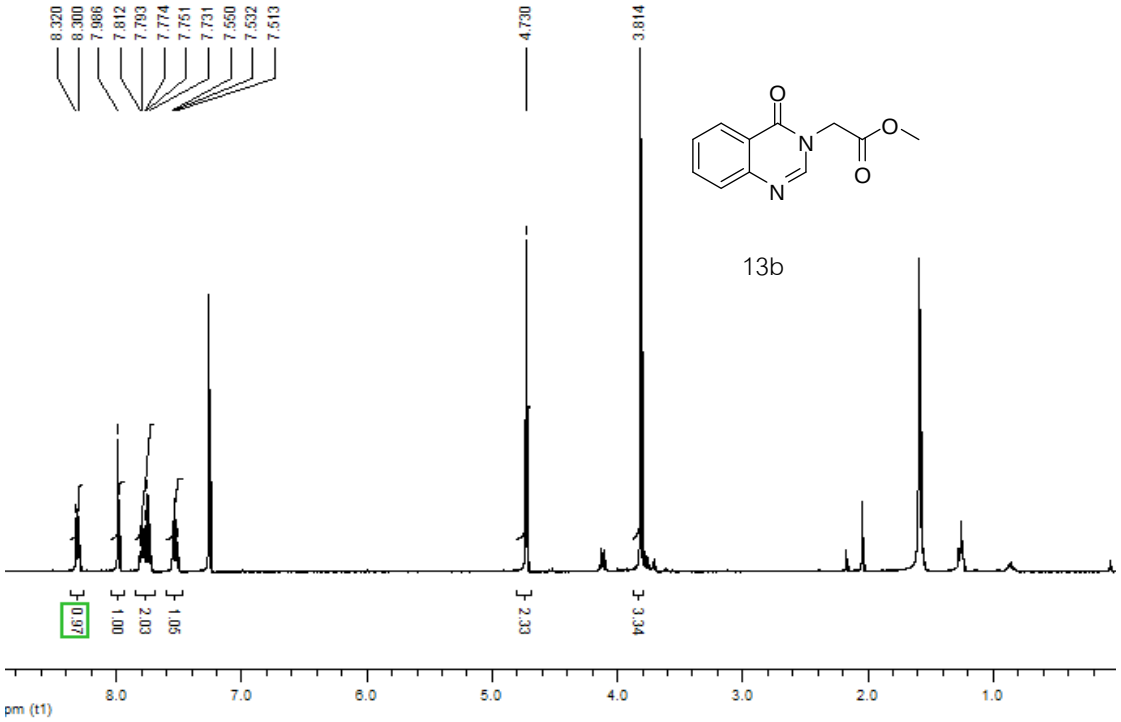

Figure 17 ^{1}H-NMR spectrum of methyl 2-(4-oxoquinazoline-3(*4H*)-yl) acetate (**13b**).

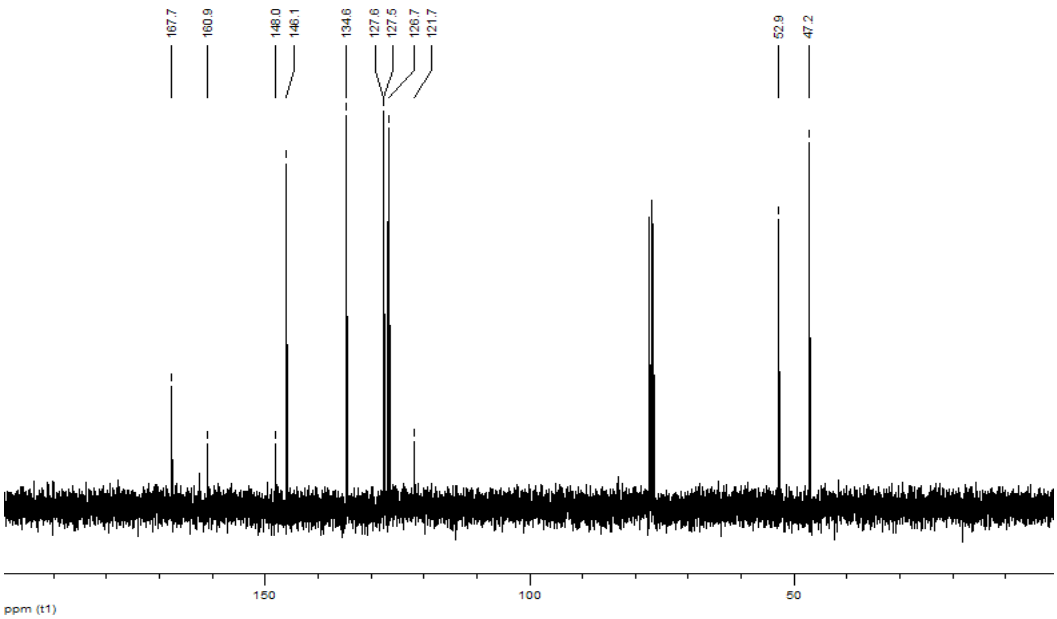

Figure 18 ^{13}C-NMR spectrum of 3-glycinemethylquinazoline-4(*3H*)–one (**13b**).

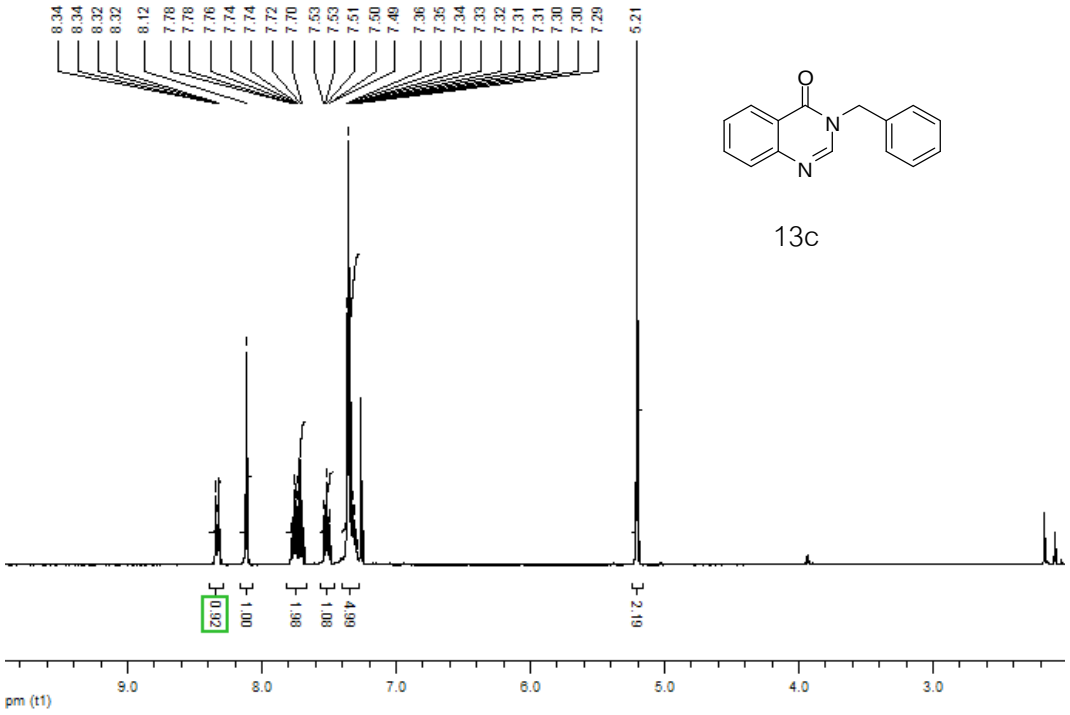

Figure 19 ^{1}H-NMR spectrum of 3-benzylquinazoline-4(*3H*)–one (**13c**).

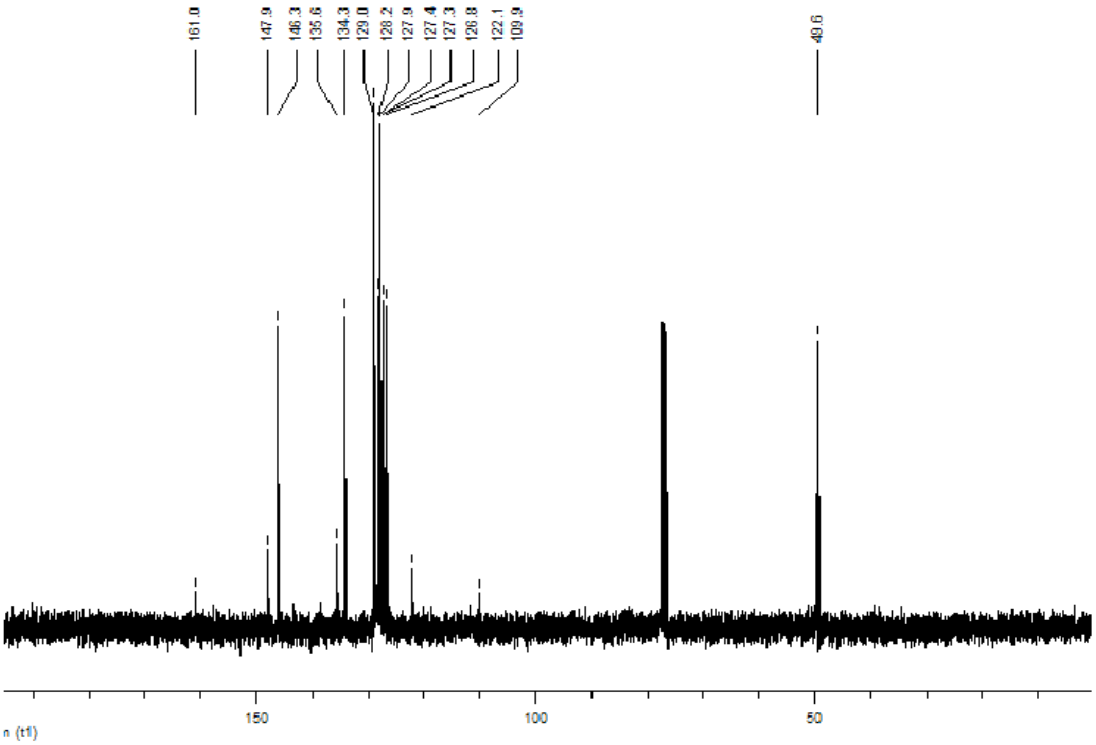

Figure 20 ^{13}C -NMR spectrum of 3- benzylquinazoline-4(*3H*) –one (**13c**).

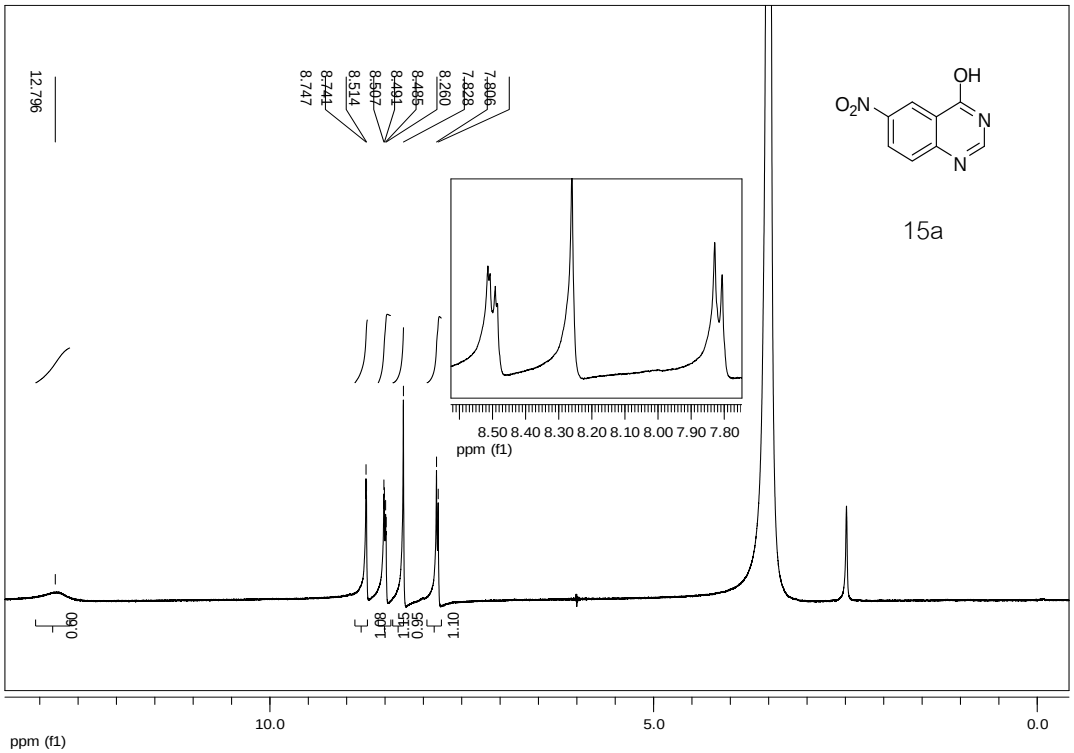

Figure 21 ^{1}H-NMR spectrum of 6- nitroquinazolin-4-ol (**15a**).

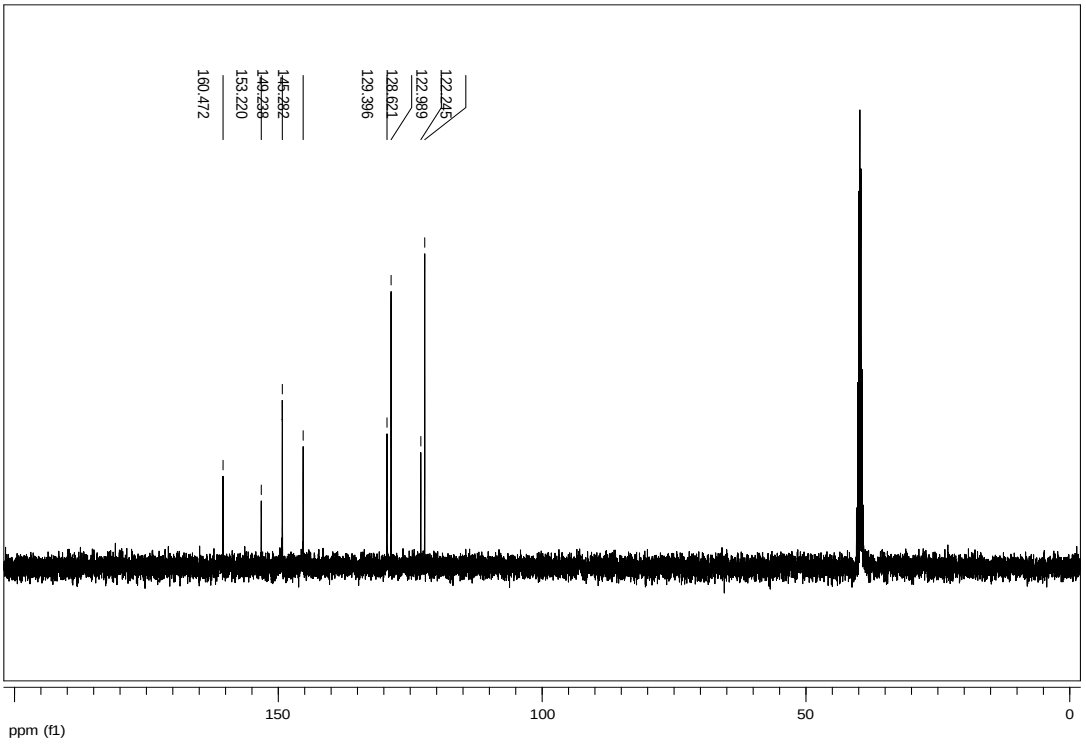

Figure 22 ^{13}C-NMR spectrum of 6- nitroquinazolin-4-ol (**15a**).

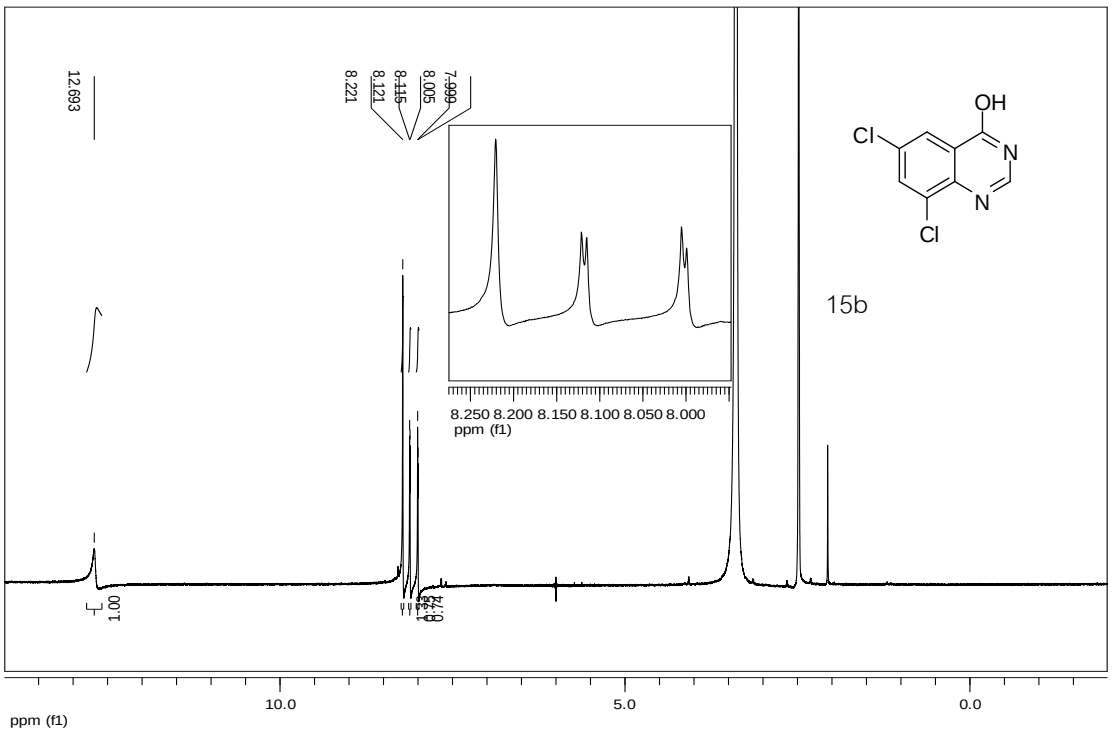

Figure 23 ^{1}H-NMR spectrum of 6, 8-dichloroquinazolin-4-ol (**15b**).

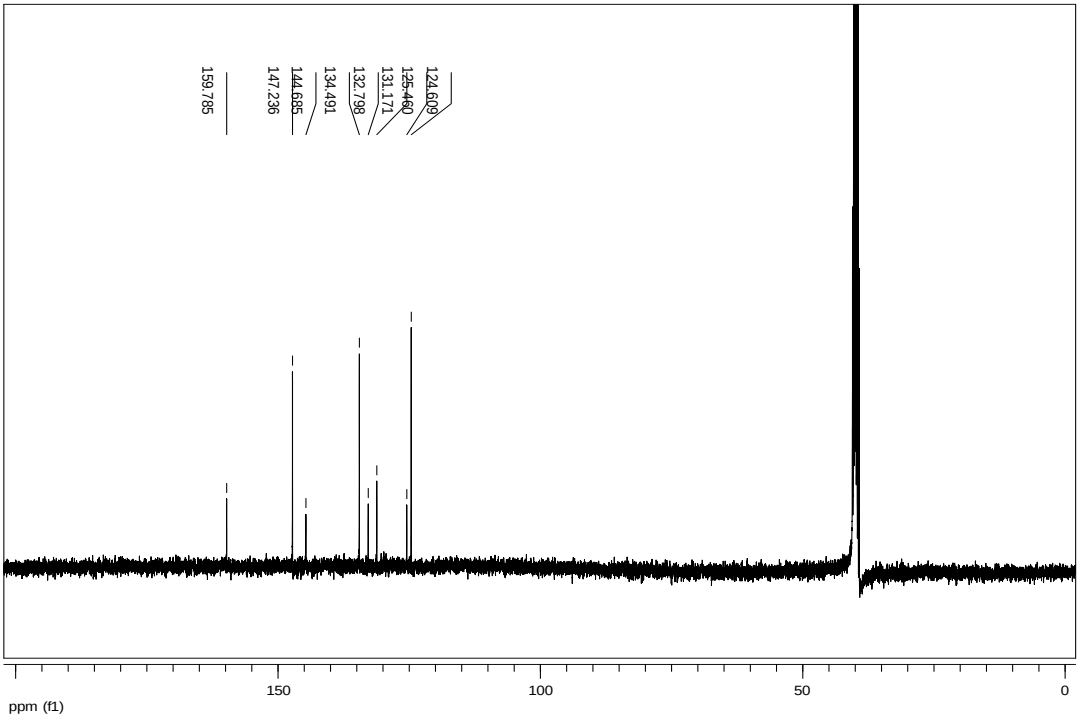

Figure 24 ^{13}C -NMR spectrum of 6, 8- dichloroquinazolin-4-ol (**15b**).

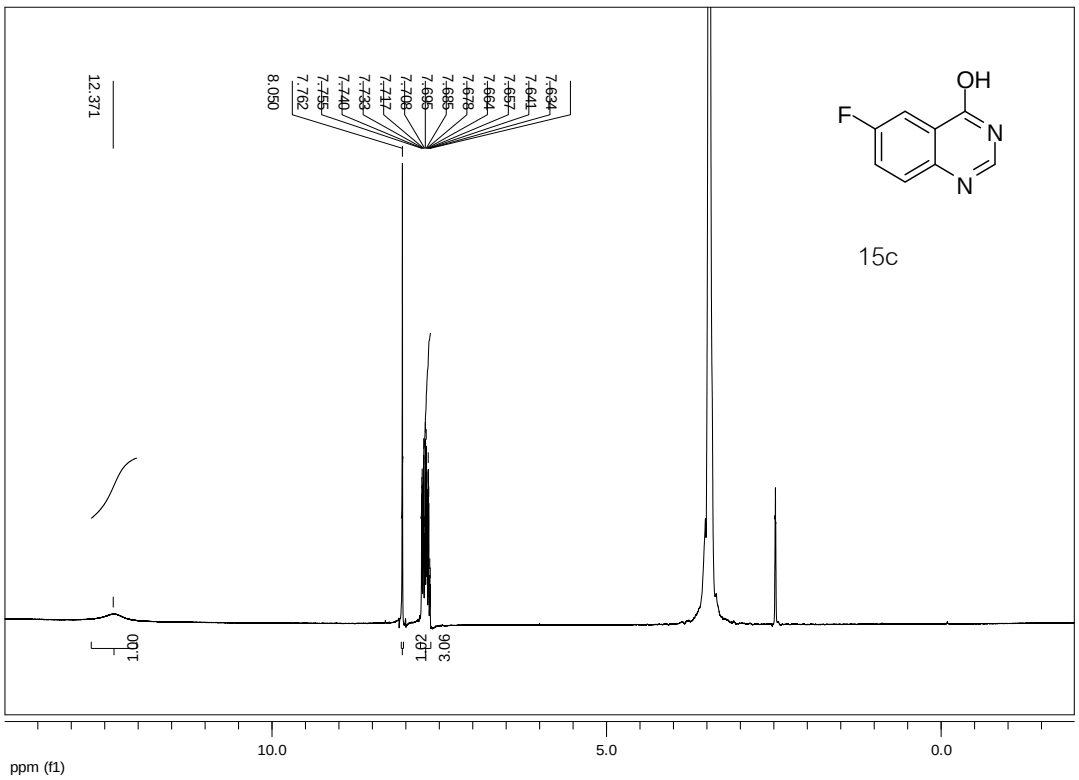

Figure 25 ^{1}H -NMR spectrum of 6- fluoroquinazolin-4-ol (**15c**).

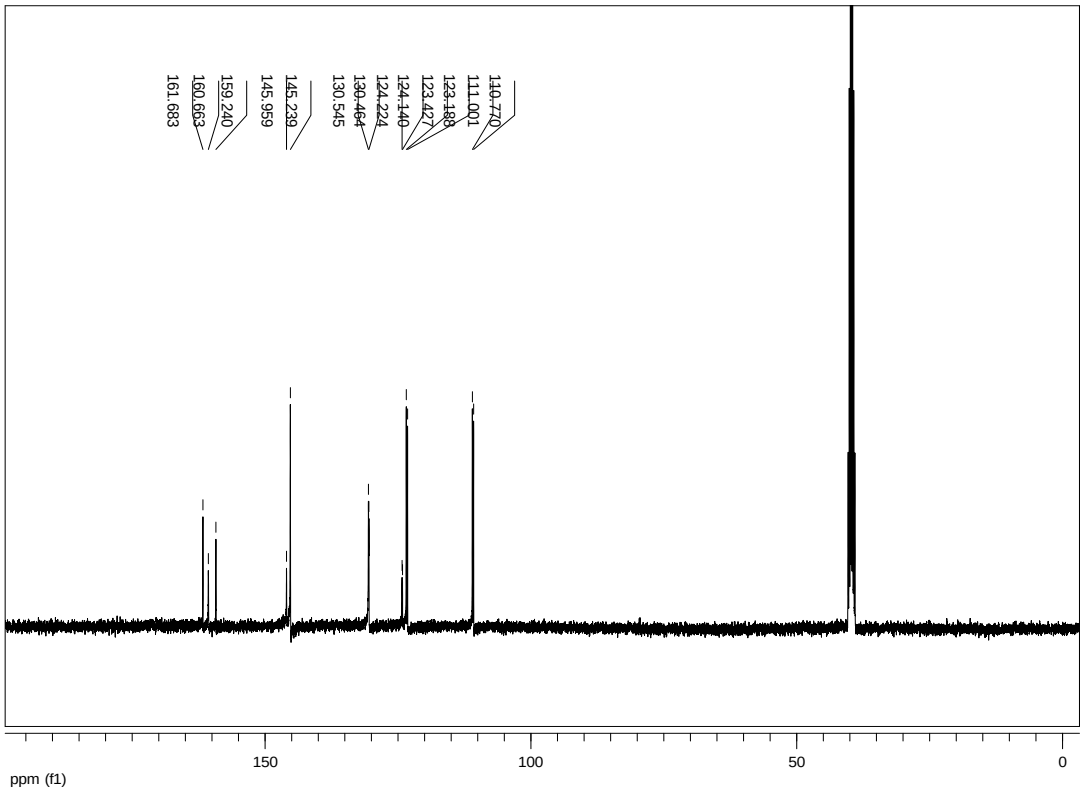

Figure 26 ^{13}C-NMR spectrum of 6- fluoroquinazolin-4-ol (**15c**).

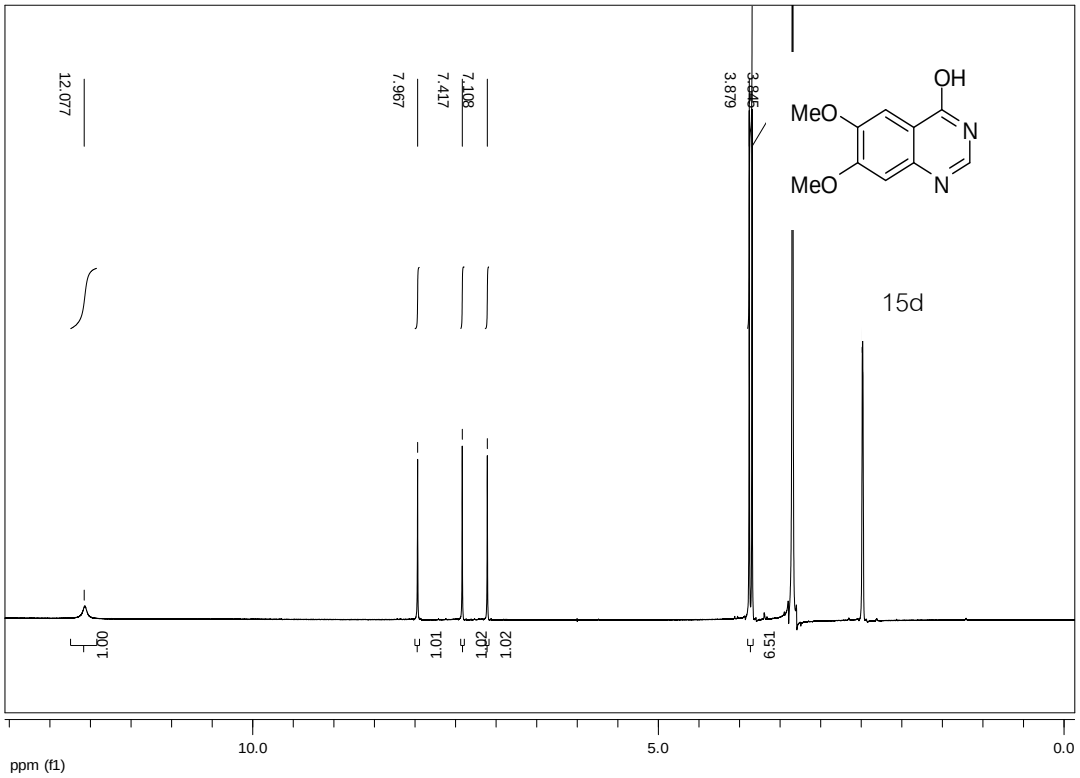

Figure 27 ^{1}H -NMR spectrum of 6, 7- dimethoxyquinazolin-4-ol (**15d**).

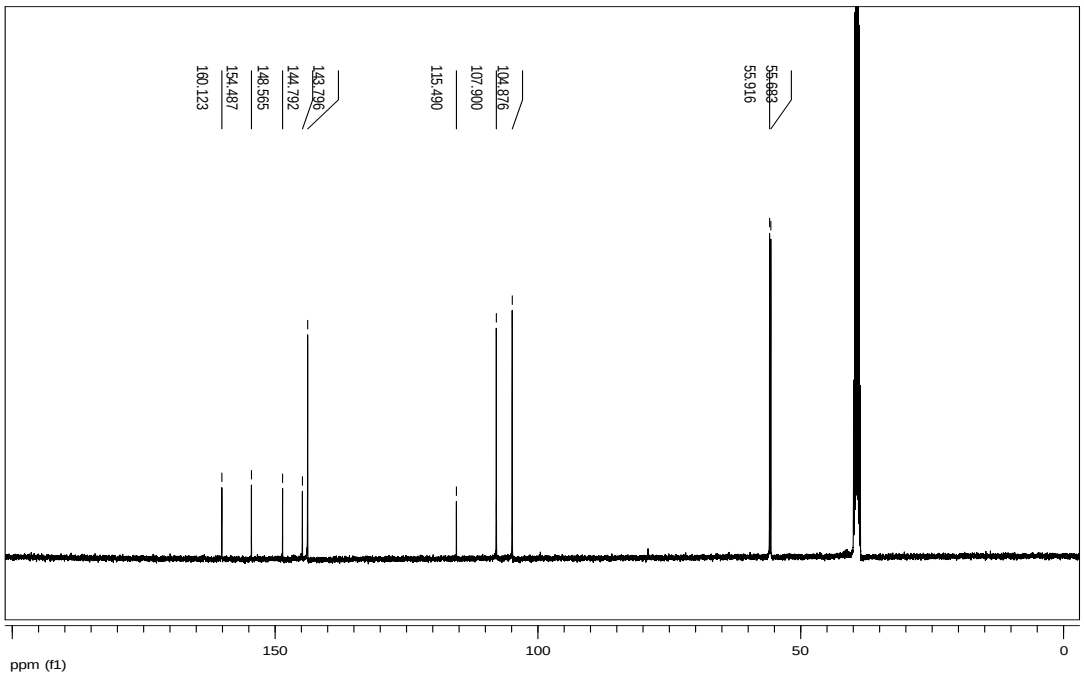

Figure 28 ^{13}C -NMR spectrum of 6, 7- dimethoxyquinazolin-4-ol (**15d**).

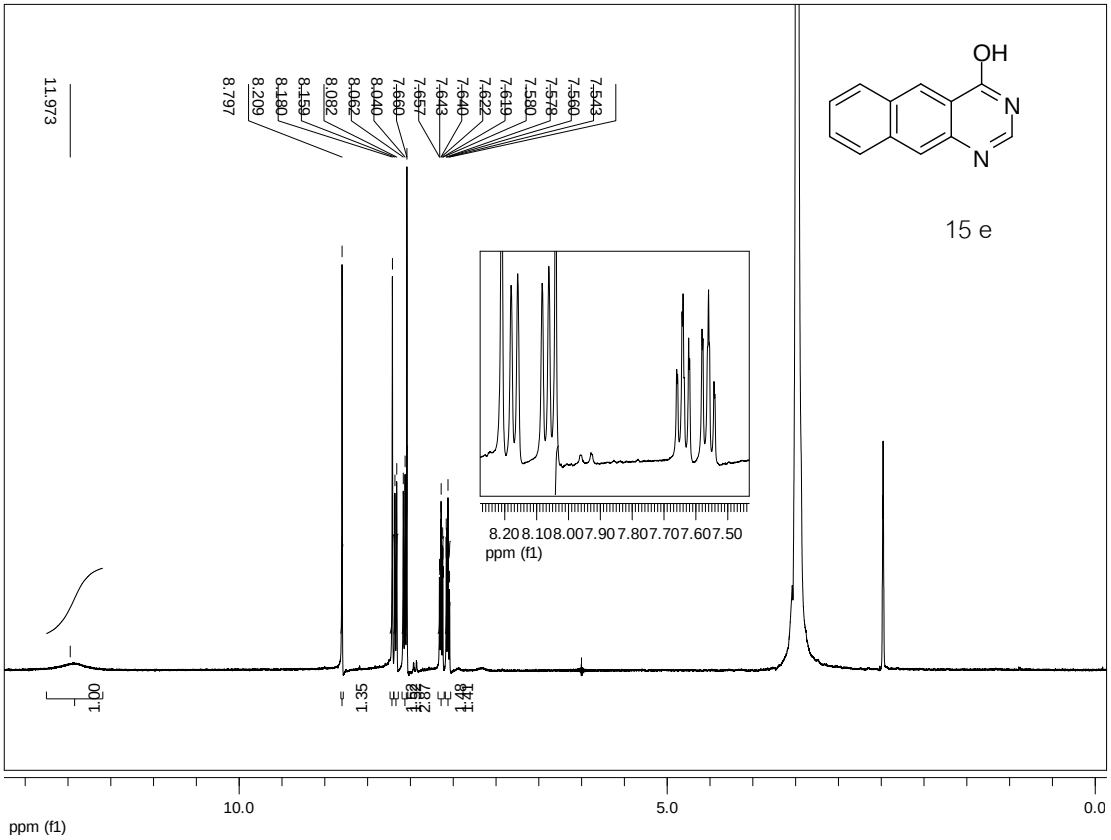

Figure 29 ^{1}H -NMR spectrum of benzo[g]quinazolin-4-ol (**15e**).

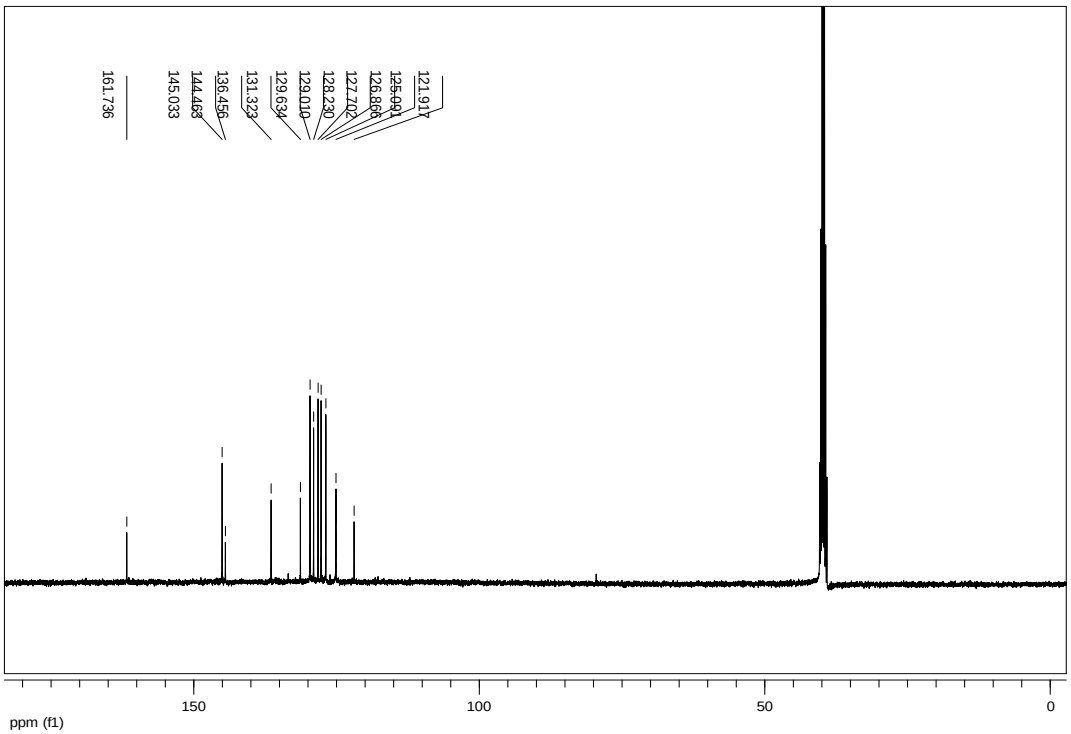

Figure 30 ^{13}C -NMR spectrum of benzo[g]quinazolin-4-ol (**15e**).

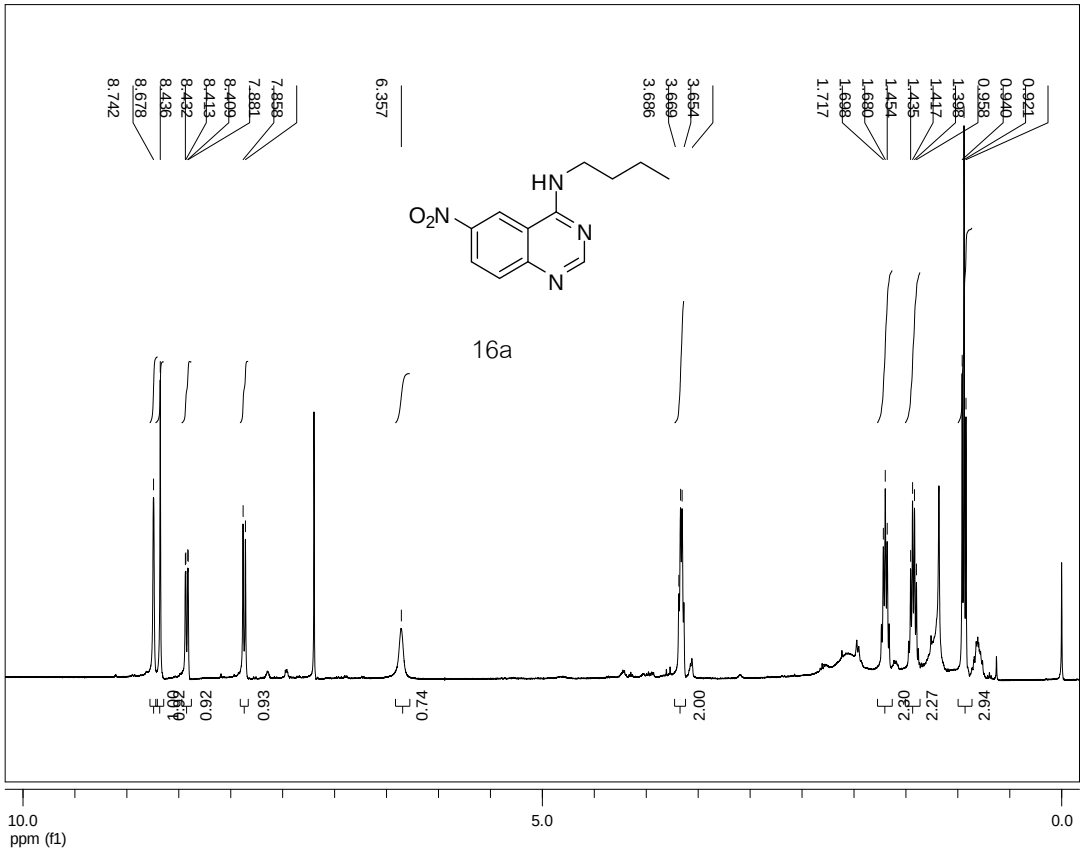

Figure 31 ^{1}H -NMR spectrum of 6 - nitro-4-butylaminoquinazoline (**16a**).

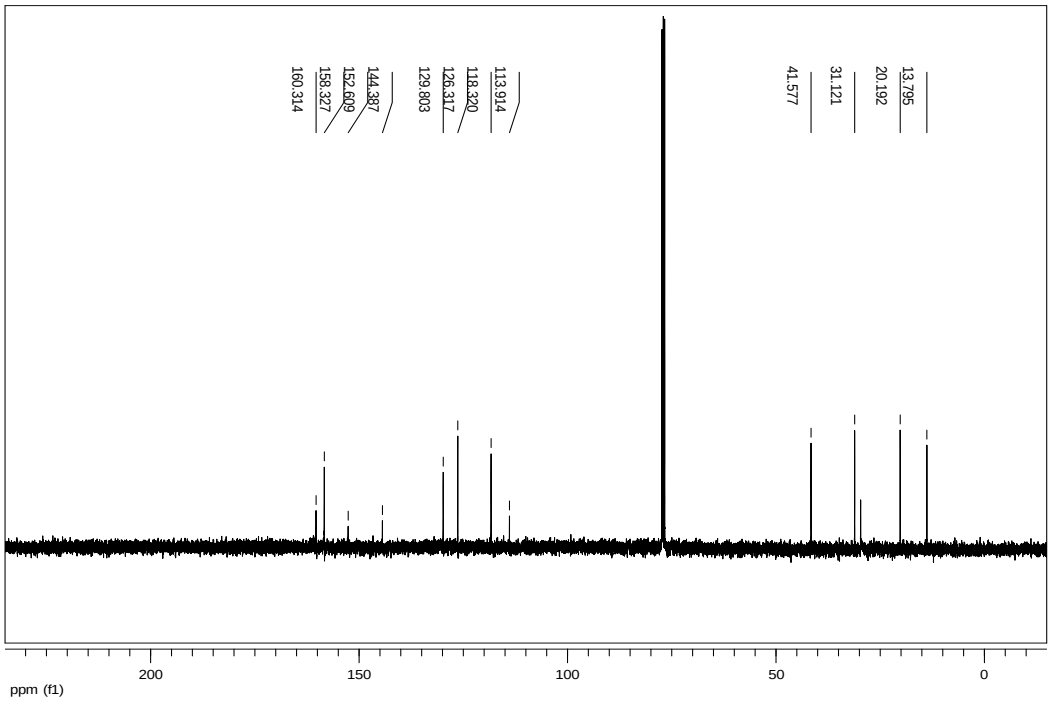

Figure 32 ^{13}C -NMR spectrum of 6 – nitro -4- butylaminoquinazoline (**16a**).

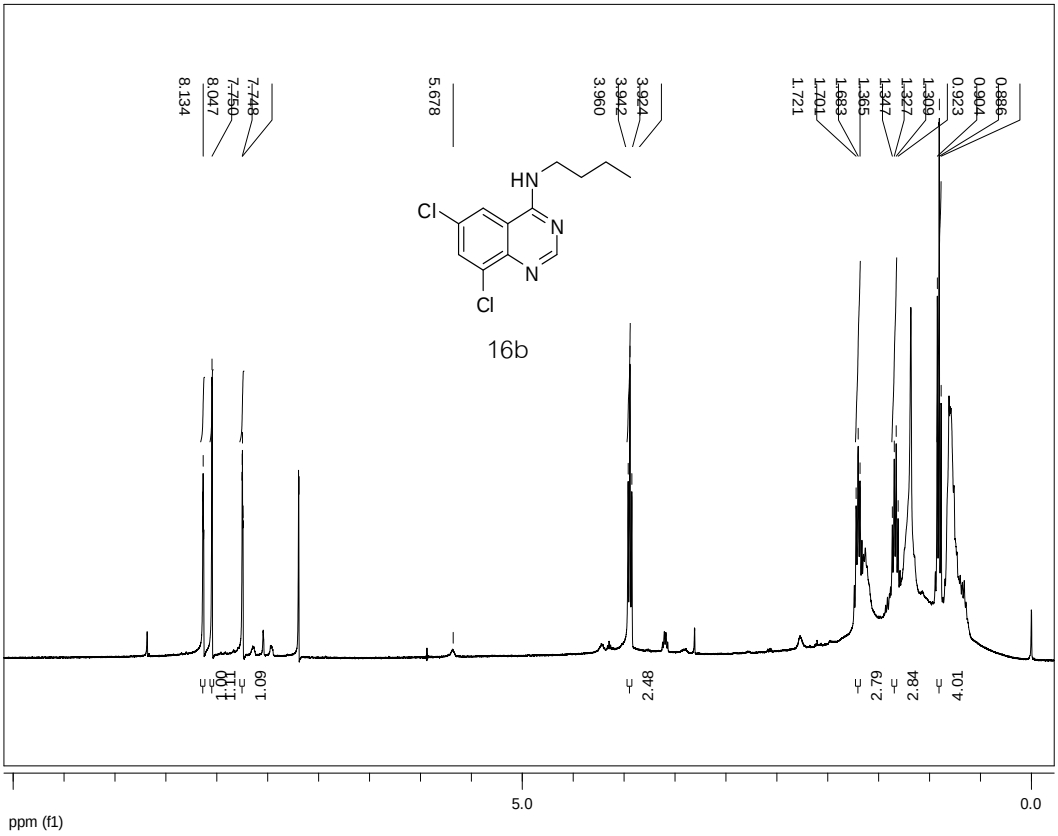

Figure 33 ^{1}H -NMR spectrum of 6, 8 – dichloro -4 –butylaminoquinazoline (**16b**).

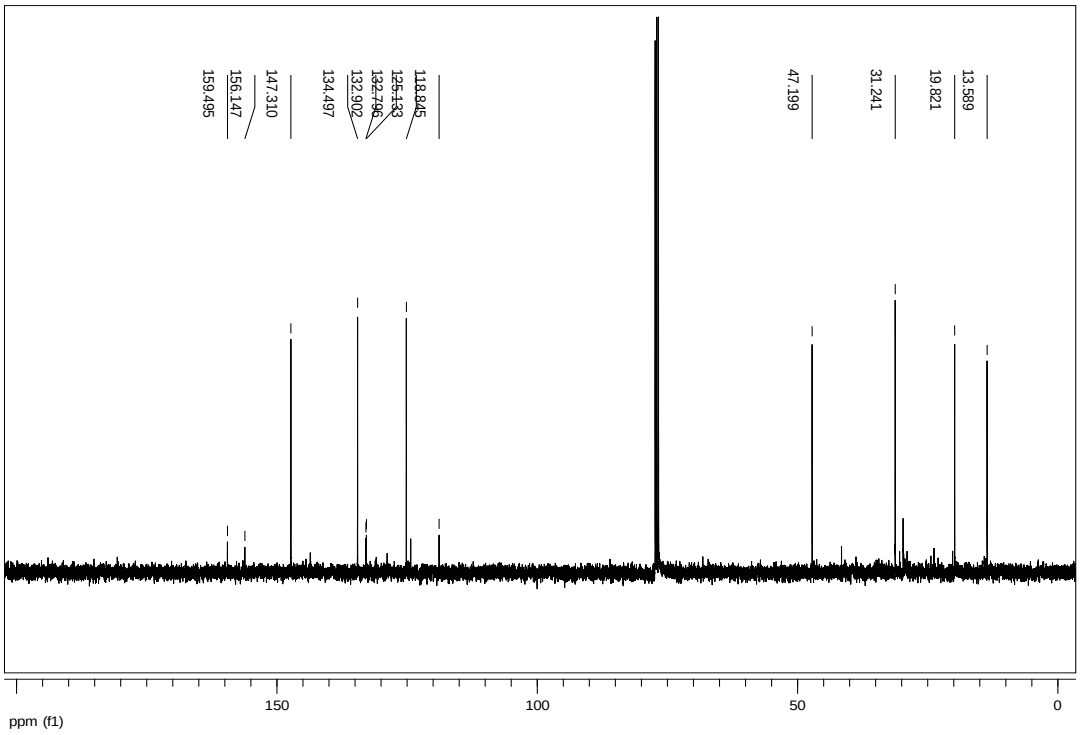

Figure 34 ^{13}C -NMR spectrum of 6, 8 – dichloro -4 –butylaminoquinazoline (**16b**).

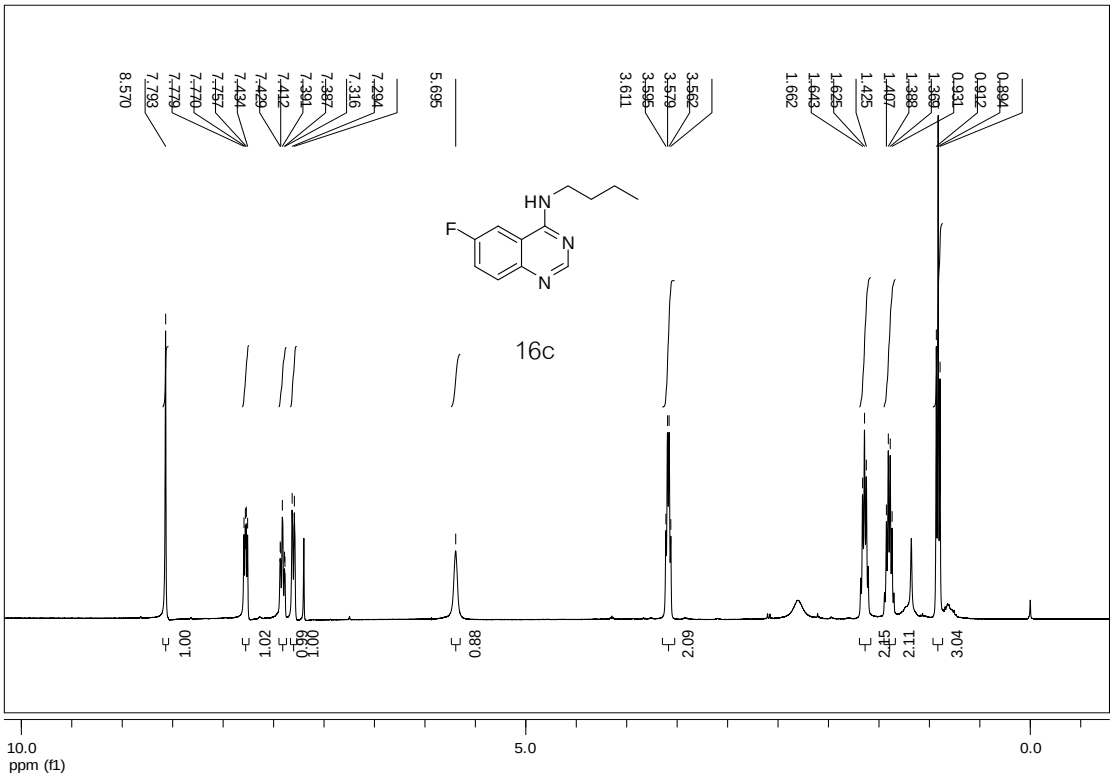

Figure 35 ^{1}H -NMR spectrum of 6 – fluoro -4 – butylaminoquinazoline (**16c**).

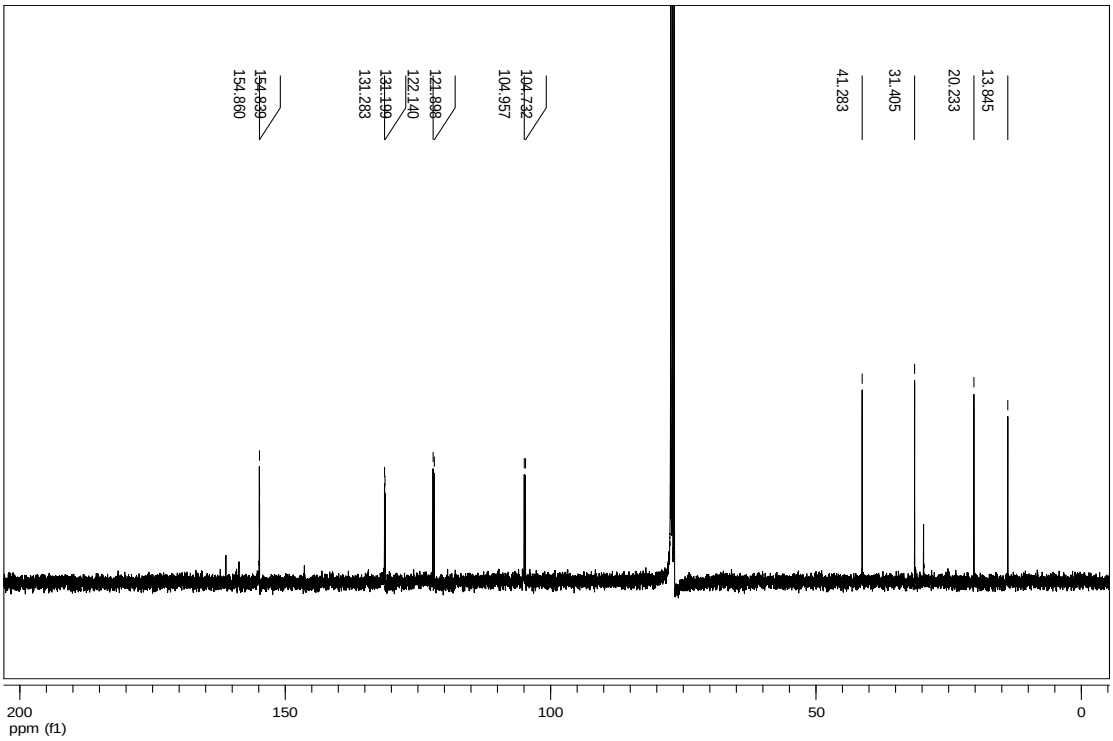

Figure 36 ^{13}C -NMR spectrum of 6 – fluoro -4- n-butylaminoquinazoline (**16c**).

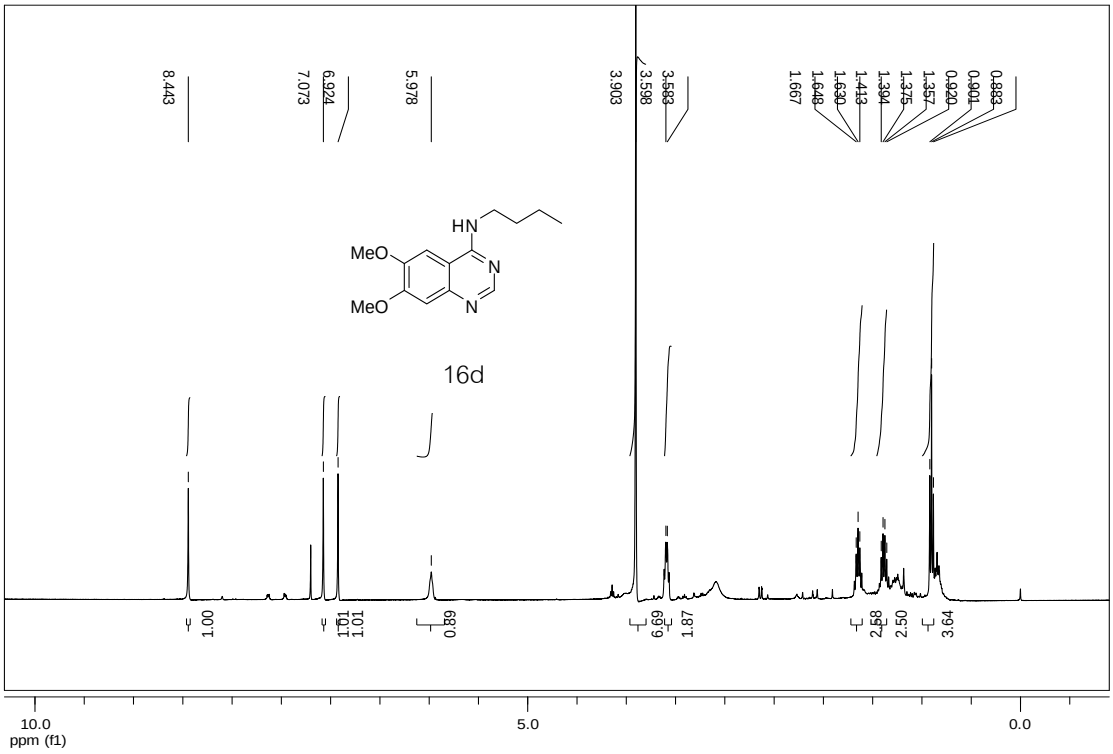

Figure 37 ^{1}H -NMR spectrum of 6,7– dimethoxy -4- butylaminoquinazoline (**16d**).

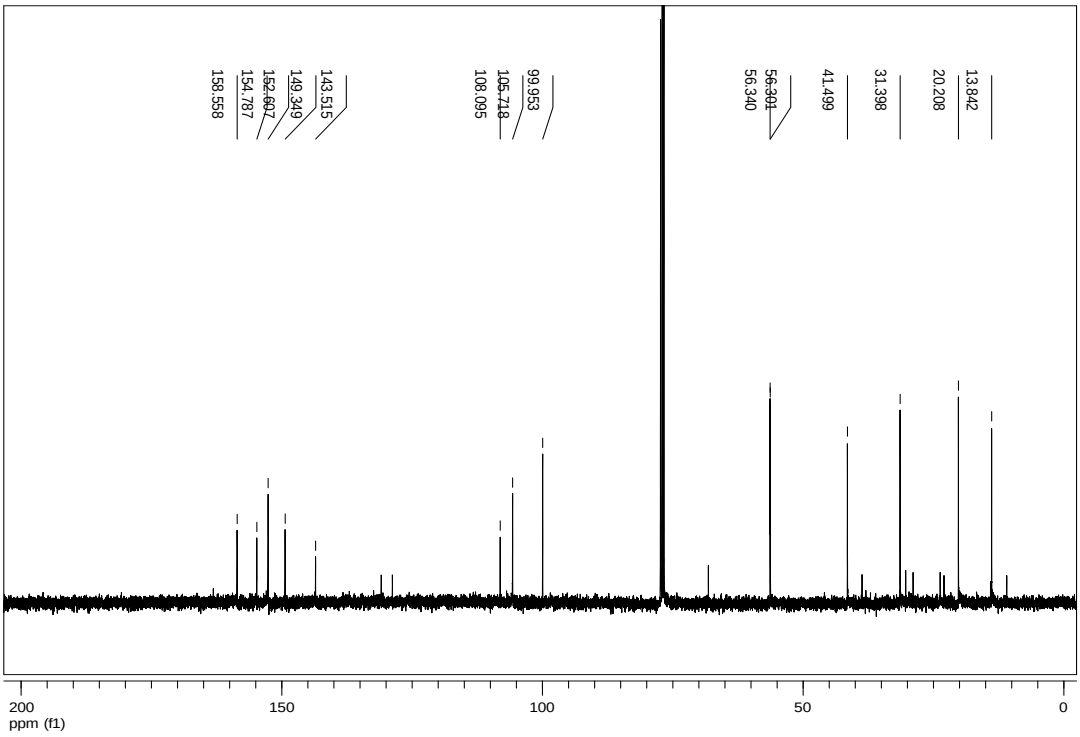

Figure 38 ^{13}C -NMR spectrum of 6,7–dimethoxy -4-butylaminoquinazoline (**16d**).

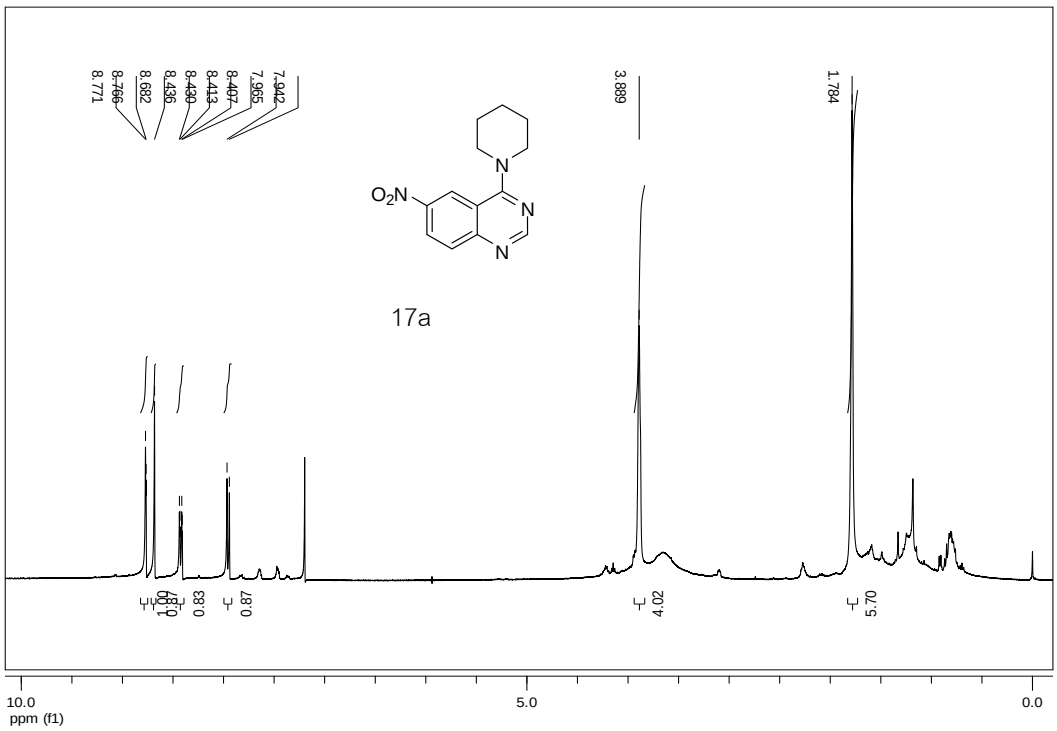

Figure 39 ^{1}H -NMR spectrum of 6 – nitro -4- piperidinoquinazoline (**17a**).

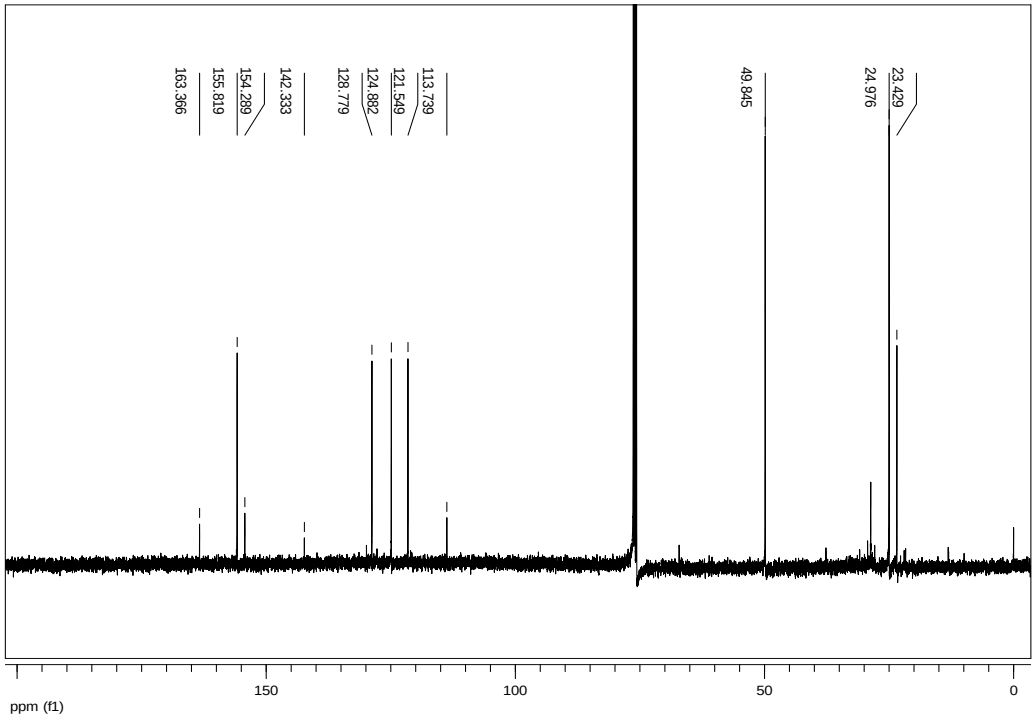

Figure 40 ^{13}C -NMR spectrum of 6 – nitro -4- piperidinoquinazoline (**17a**).

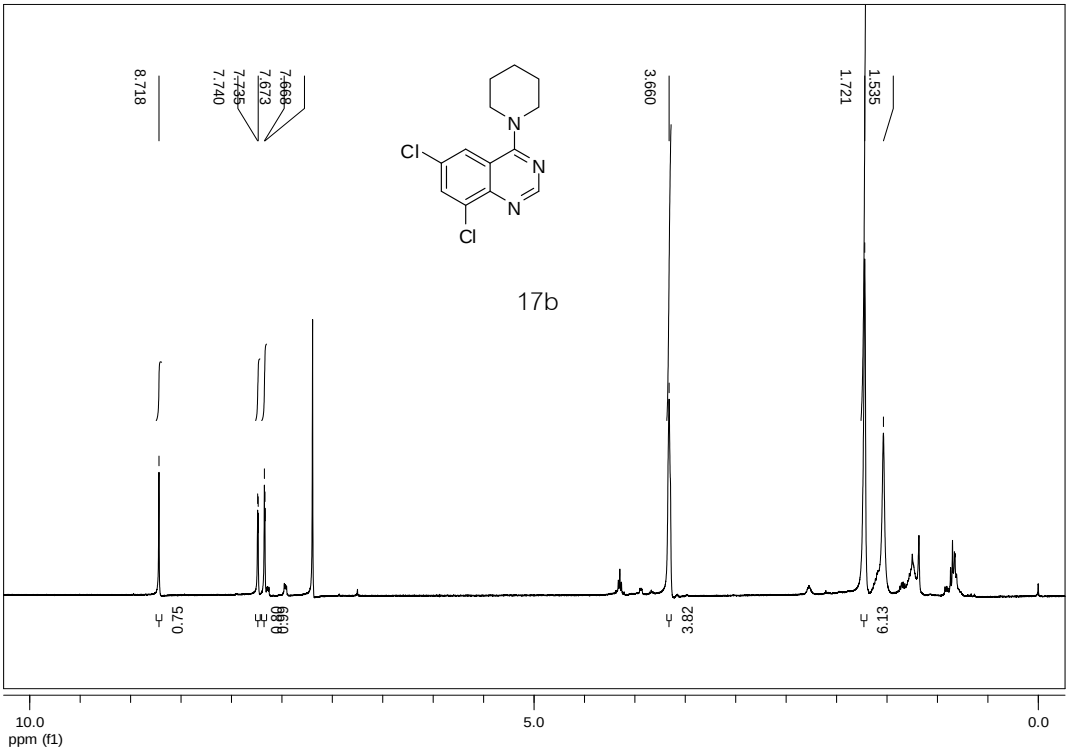

Figure 41 ^{1}H -NMR spectrum of 6, 8 – dichloro -4- piperidinoquinazoline (**17b**).

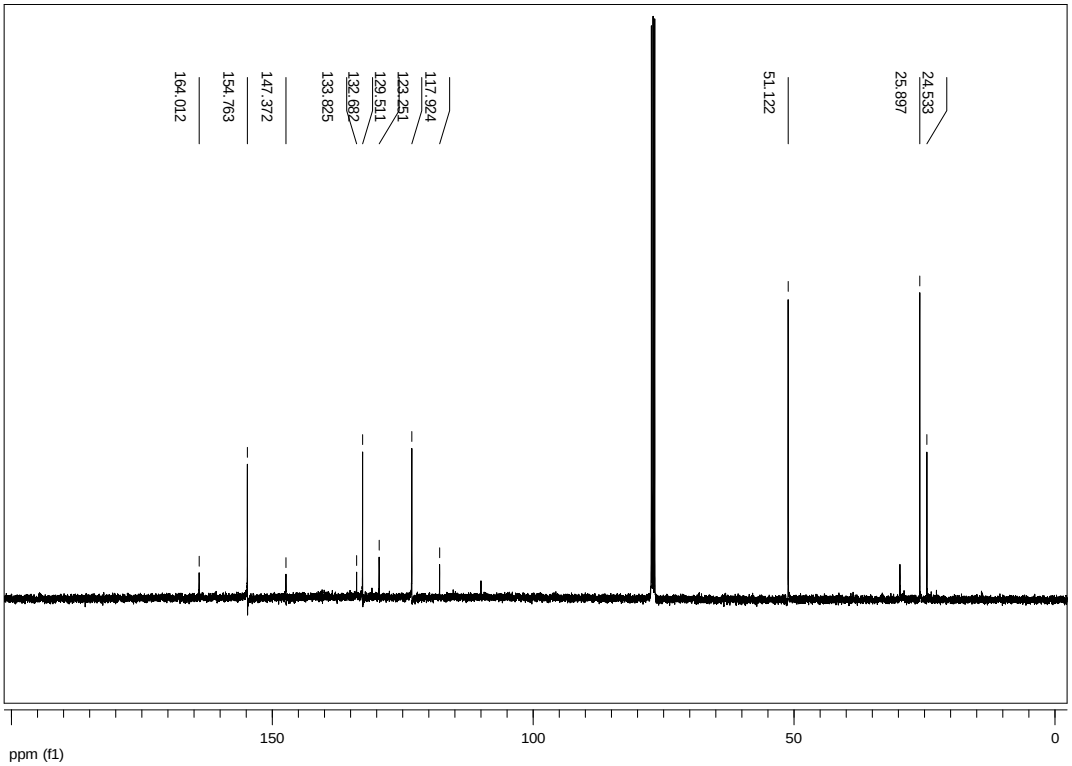

Figure 42 ^{13}C -NMR spectrum of 6, 8 – dichloro -4- piperidinoquinazoline (**17b**).

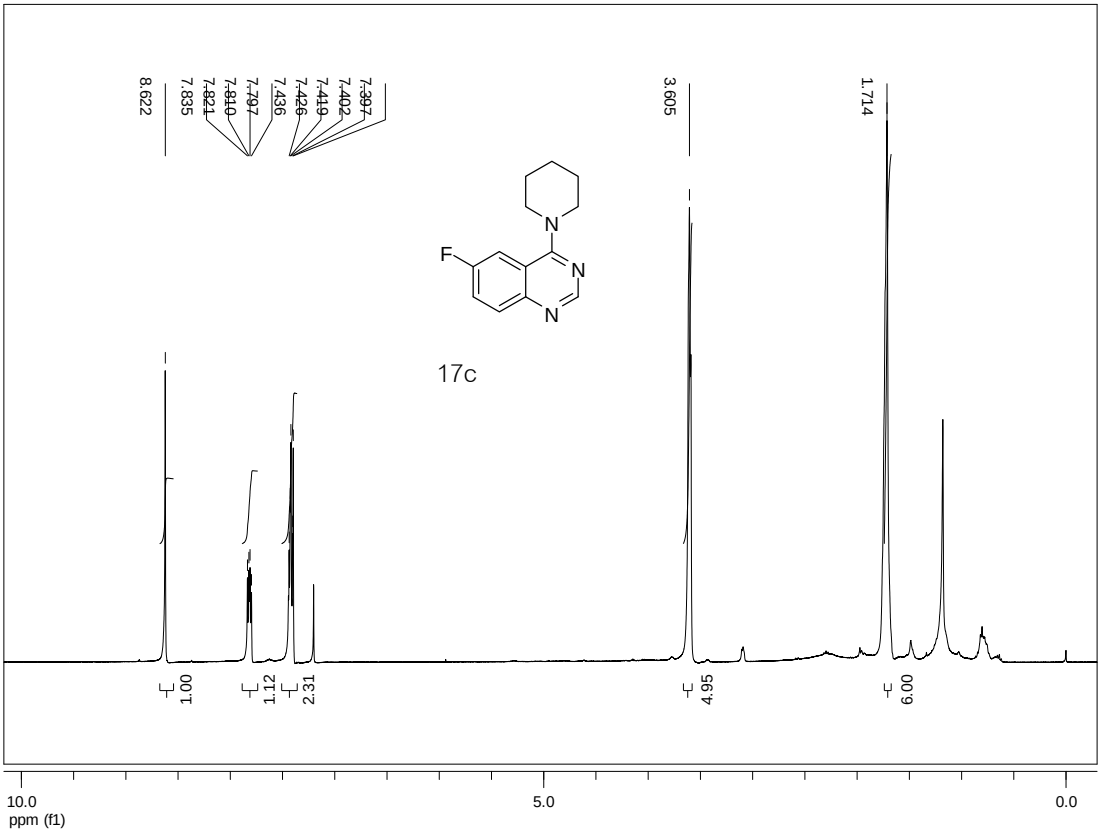

Figure 43 ^{1}H -NMR spectrum of 6- fluoro -4- piperidinoquinazoline (**17c**).

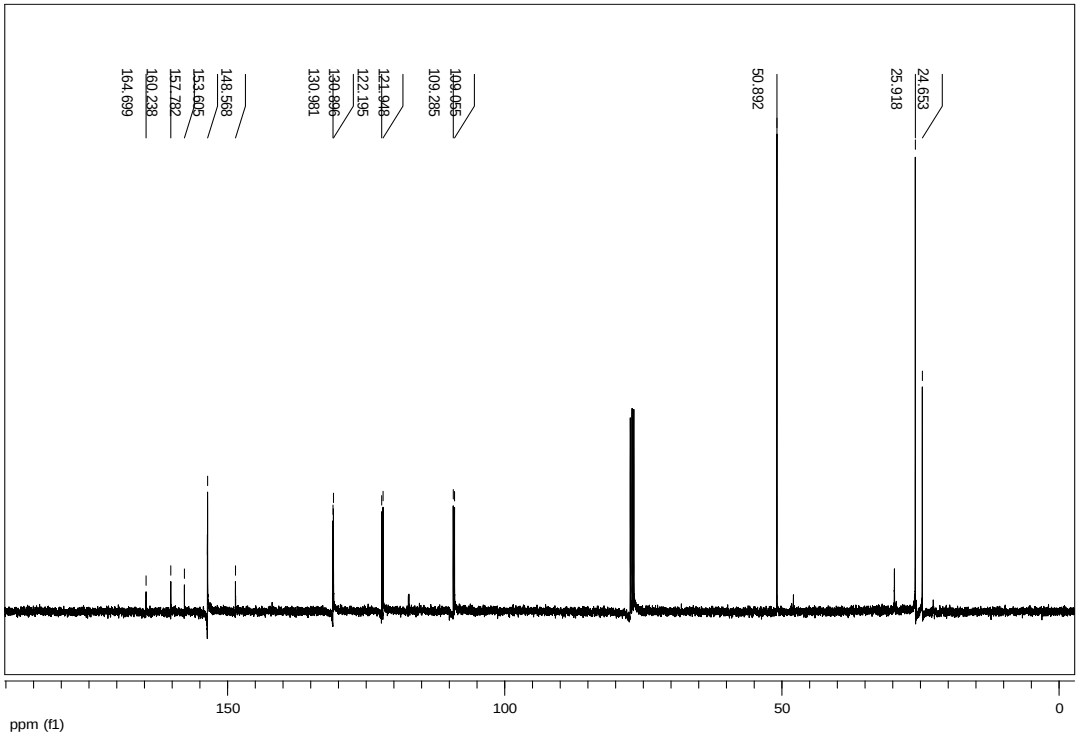

Figure 44 ^{13}C -NMR spectrum of 6- fluoro -4- piperidinoquinazoline (**17c**).

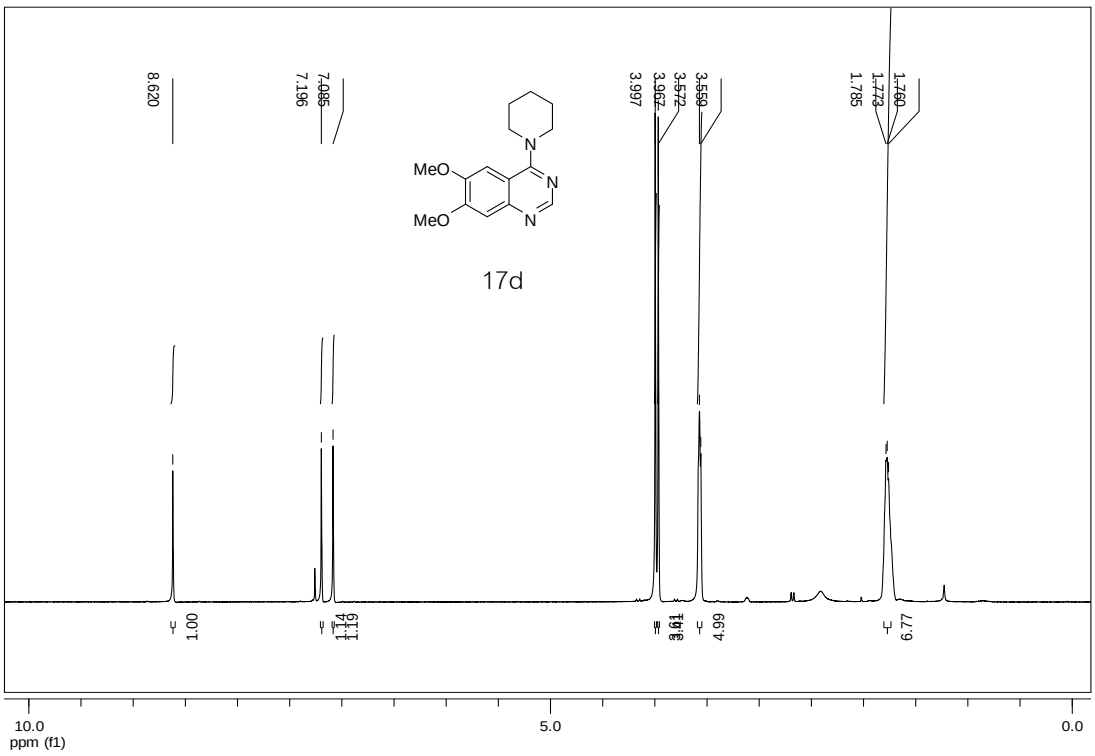

Figure 45 ^{1}H -NMR spectrum of 6, 7- dimethoxy -4- piperidinoquinazoline (**17d**).

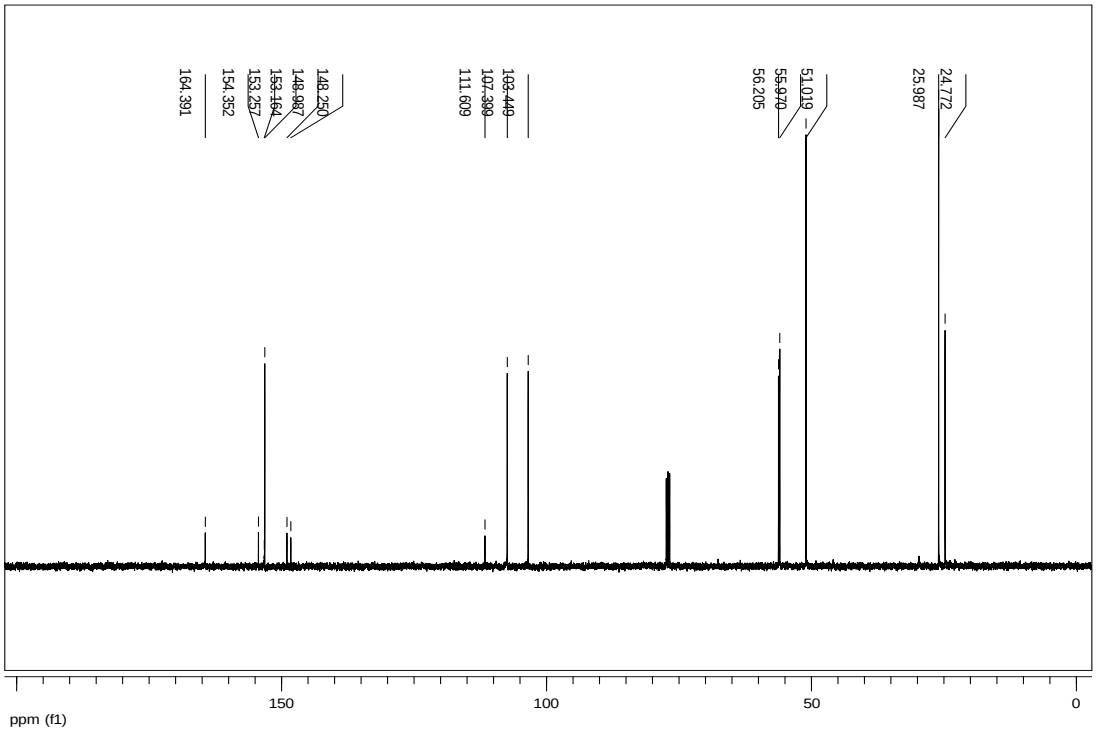

Figure 46 ^{13}C -NMR spectrum of 6, 7- dimethoxy -4- piperidinoquinazoline (**17d**).

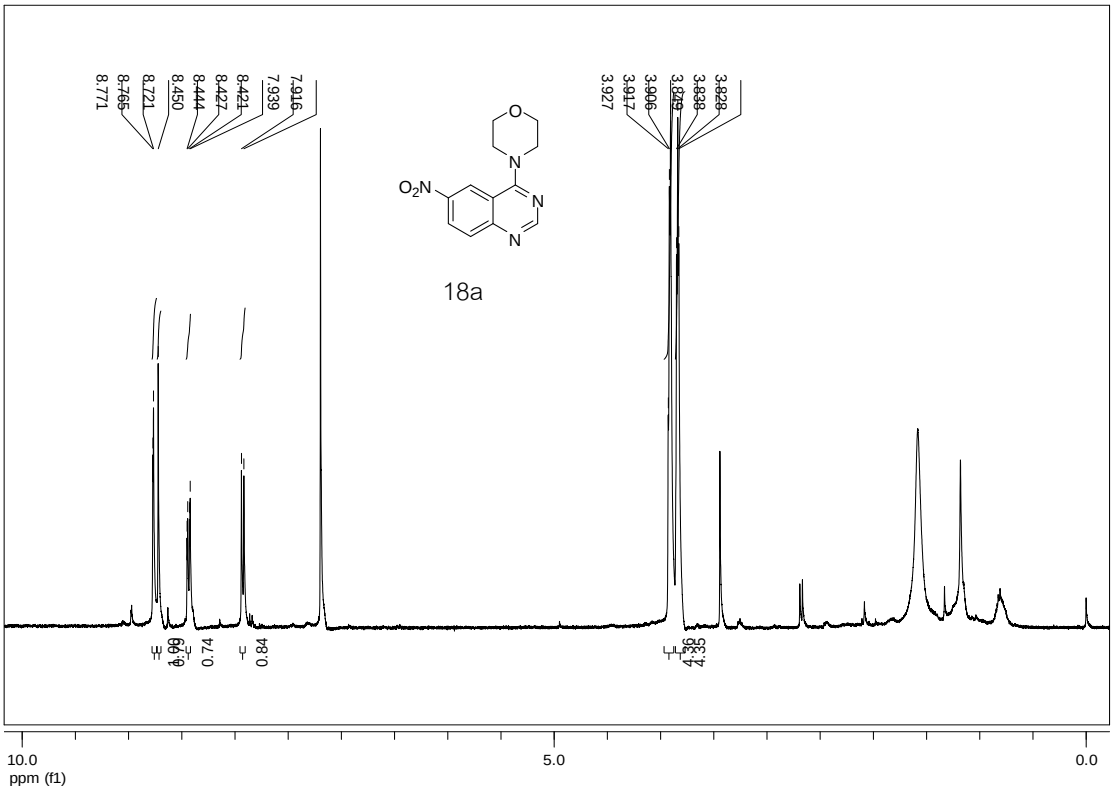

Figure 47 ^{1}H-NMR spectrum of 6 - nitro – 4 – morpholinoquinazoline (**18a**).

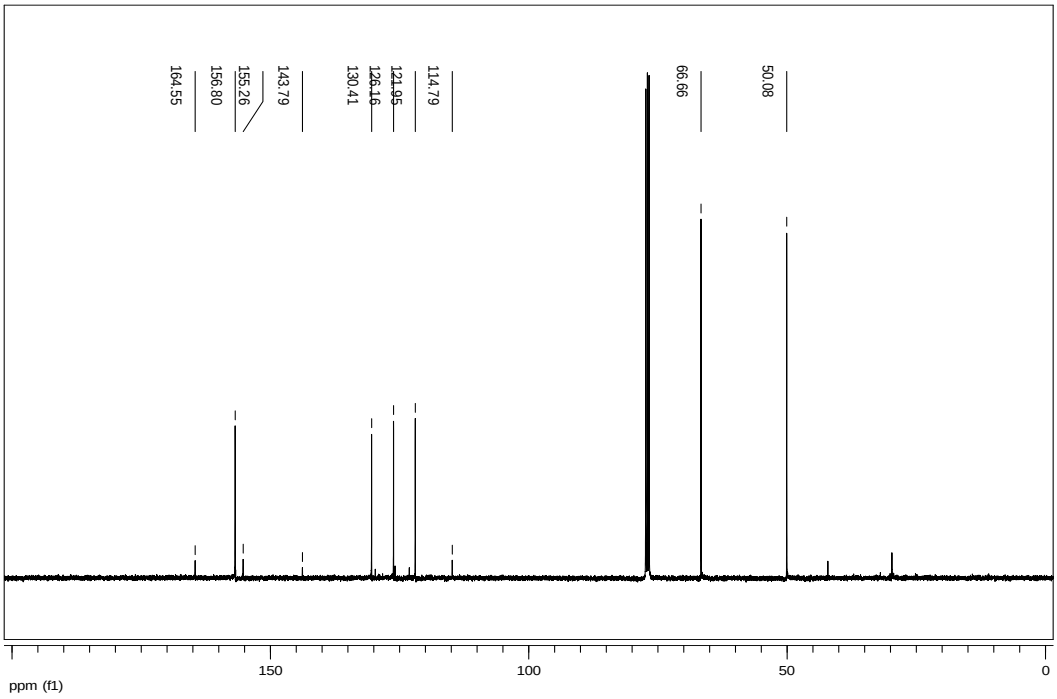

Figure 48 ^{13}C-NMR spectrum of 6 - nitro - 4 – morpholinoquinazoline (**18a**).

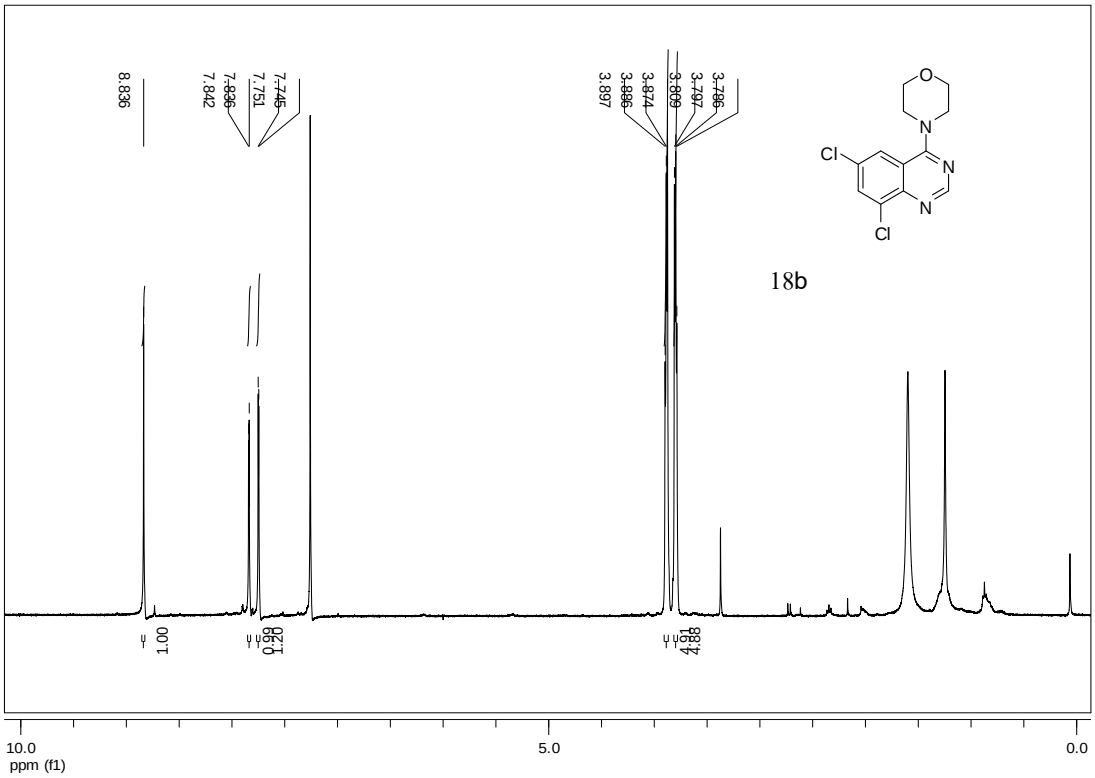

Figure 49 ^{1}H-NMR spectrum of 6, 8 - dichloro – 4 – morpholinoquinazoline (**18b**).

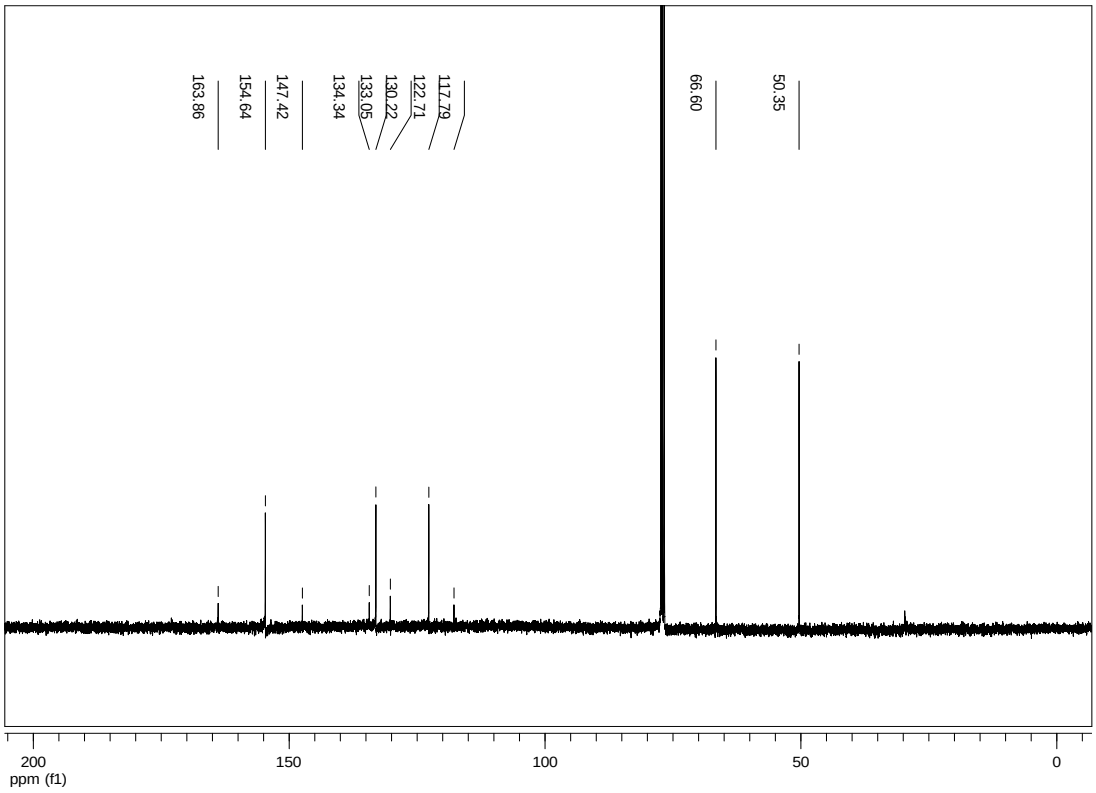

Figure 50 ^{13}C-NMR spectrum of 6, 8 - dichloro - 4 – morpholinoquinazoline (**18b**).

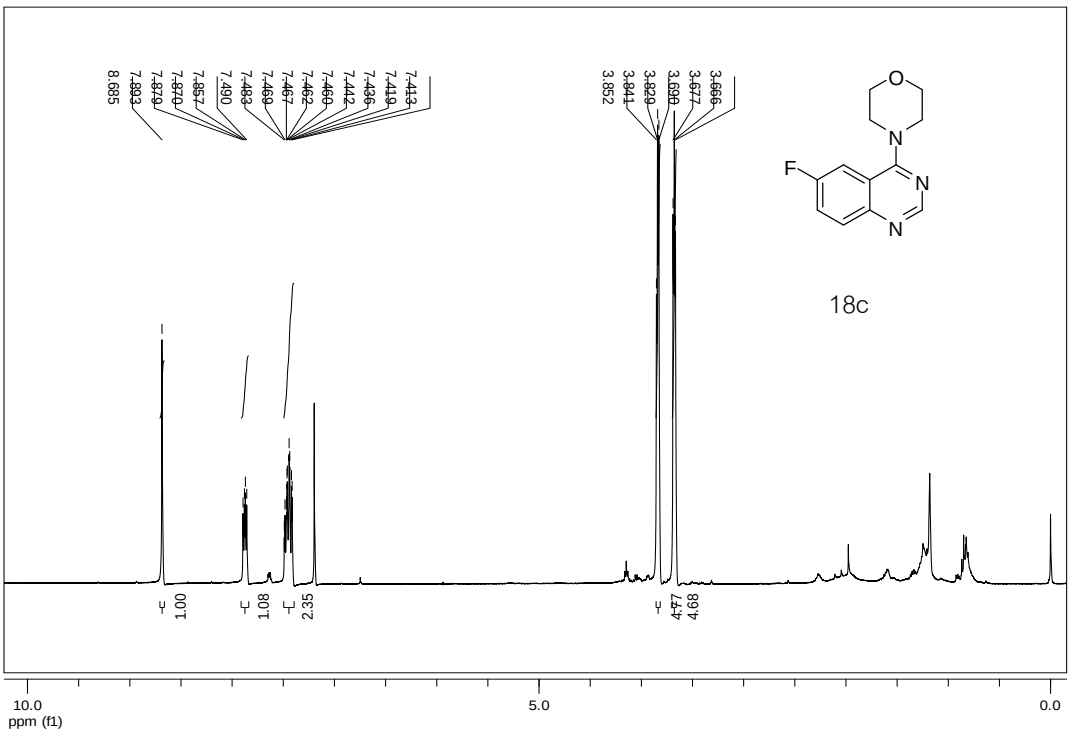

Figure 51 ^{1}H -NMR spectrum of 6 - fluoro – 4 – morpholinoquinazoline (**18c**).

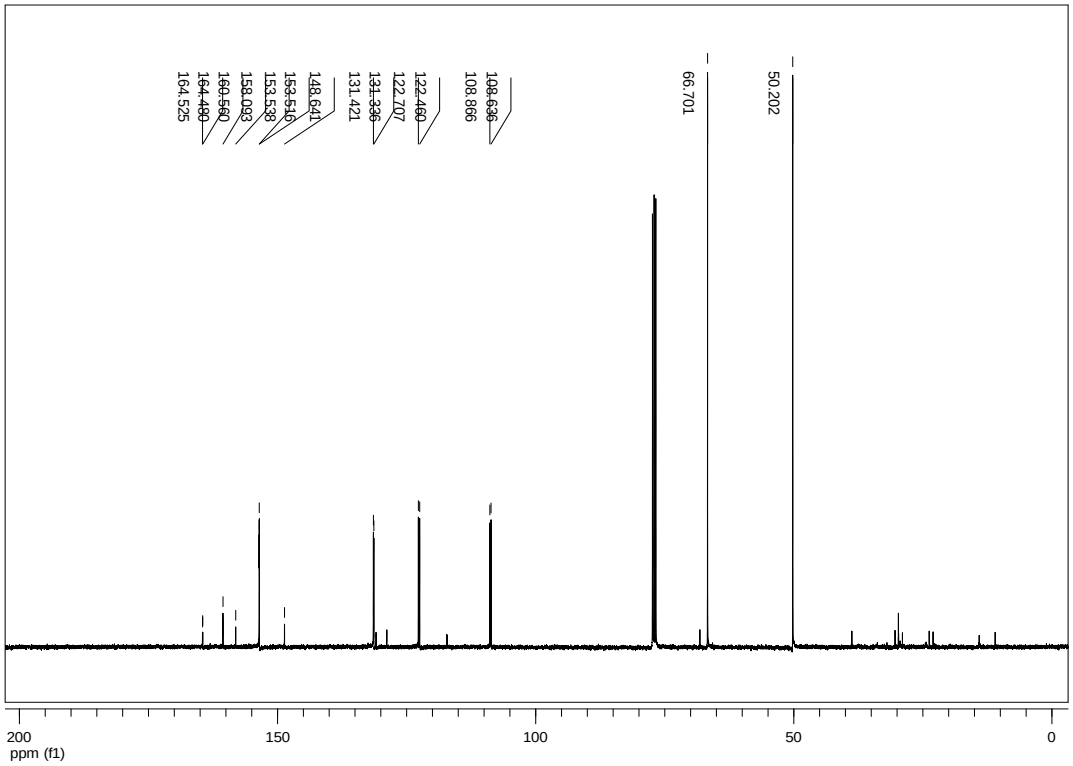

Figure 52 ^{13}C -NMR spectrum of 6 - fluoro - 4 – morpholinylquinazoline (**18c**).

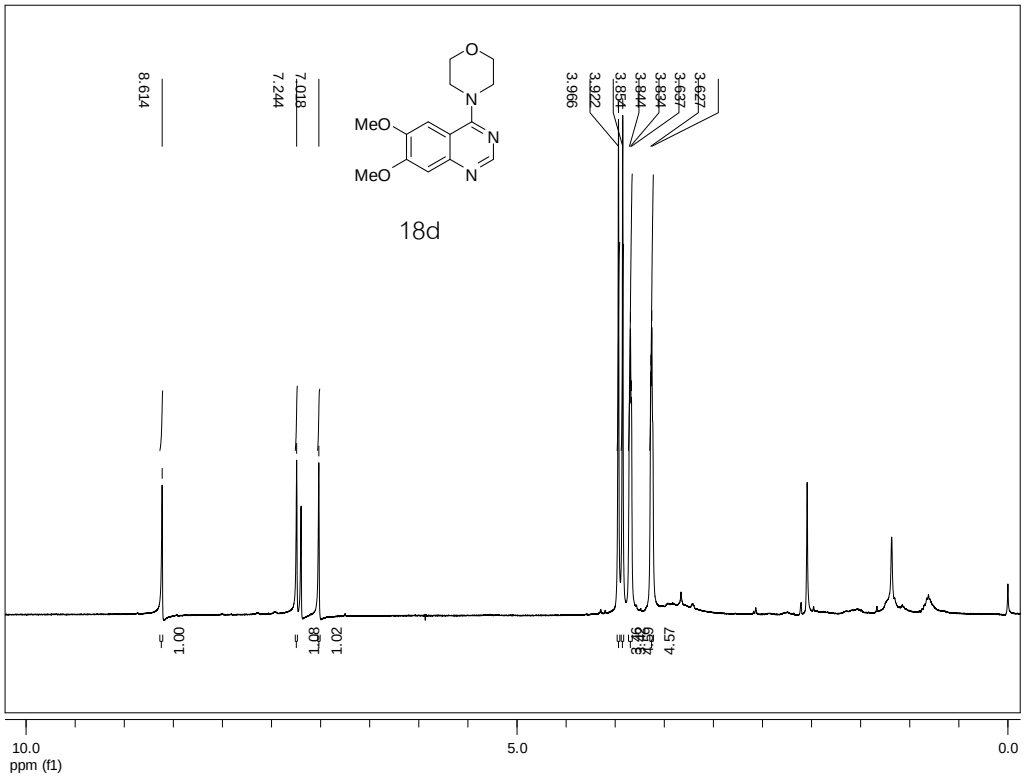

Figure 53 ^{1}H-NMR spectrum of 6, 7 - dimethoxy – 4 – morpholinoquinazoline (**18d**).

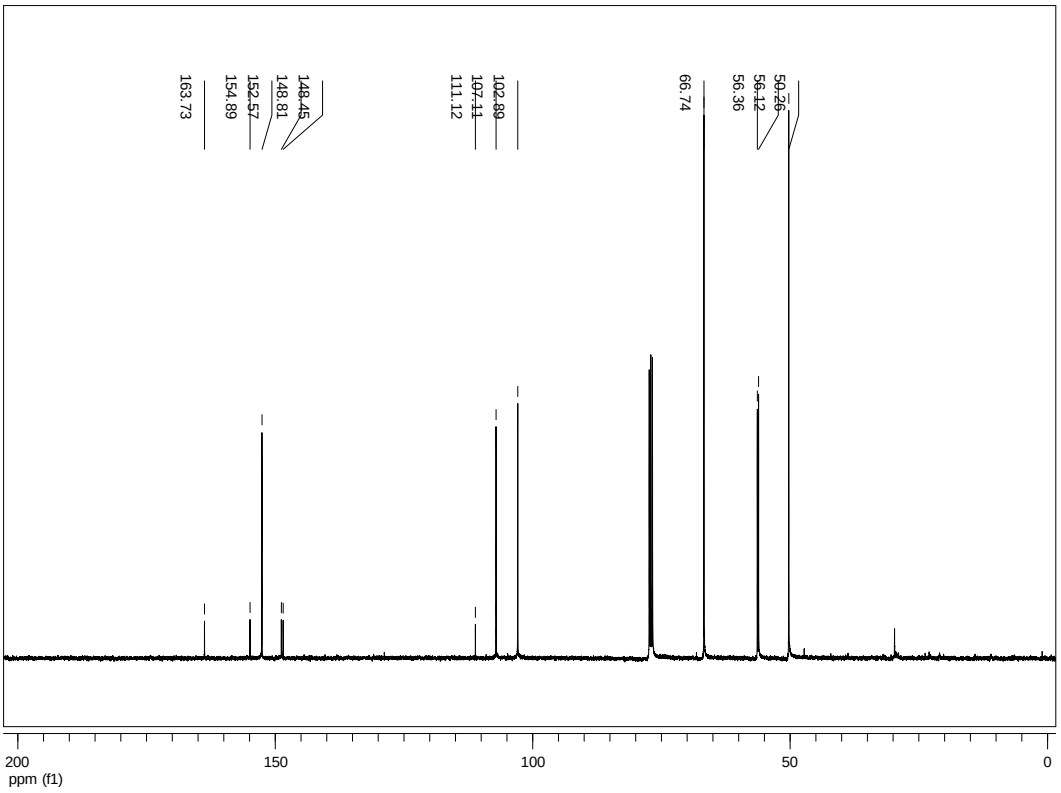

Figure 54 ^{13}C-NMR spectrum of 6, 7 - dimethoxy - 4 – morpholinoquinazoline (**18d**).

Appendix

B

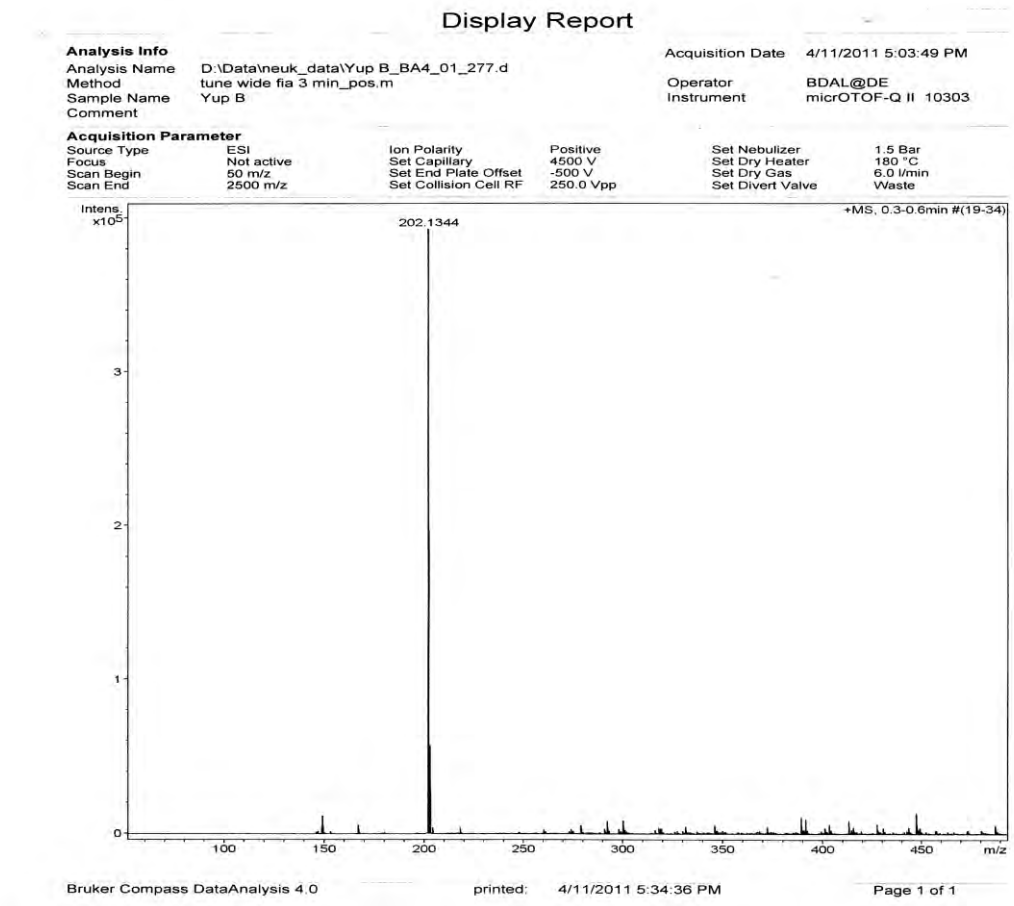

Figure 55 The high-resolution mass spectra of 4-butylaminoquinazoline (12a).

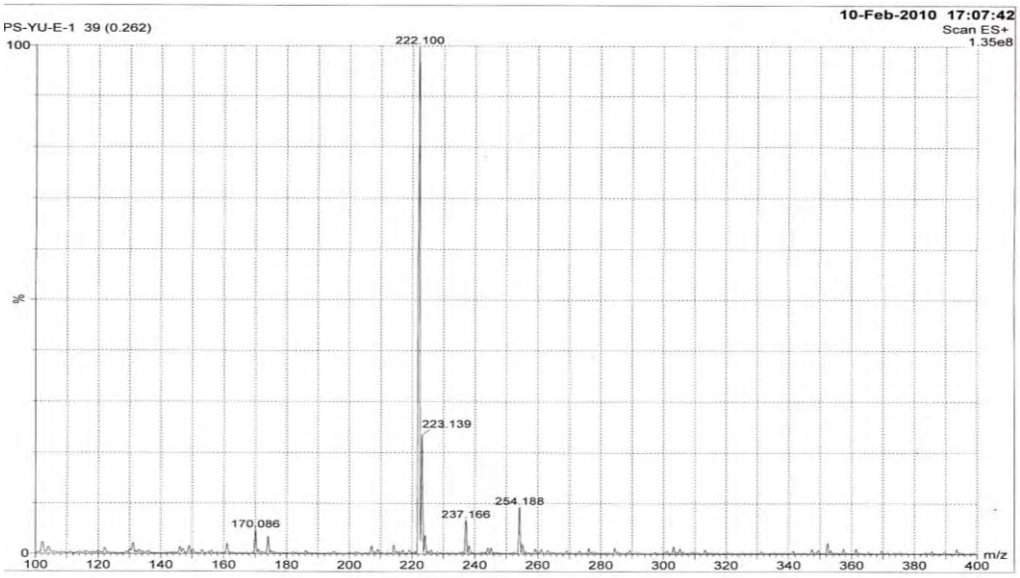

Figure 56 The low-resolution mass spectra of 4-anilinoquinazoline (12d).

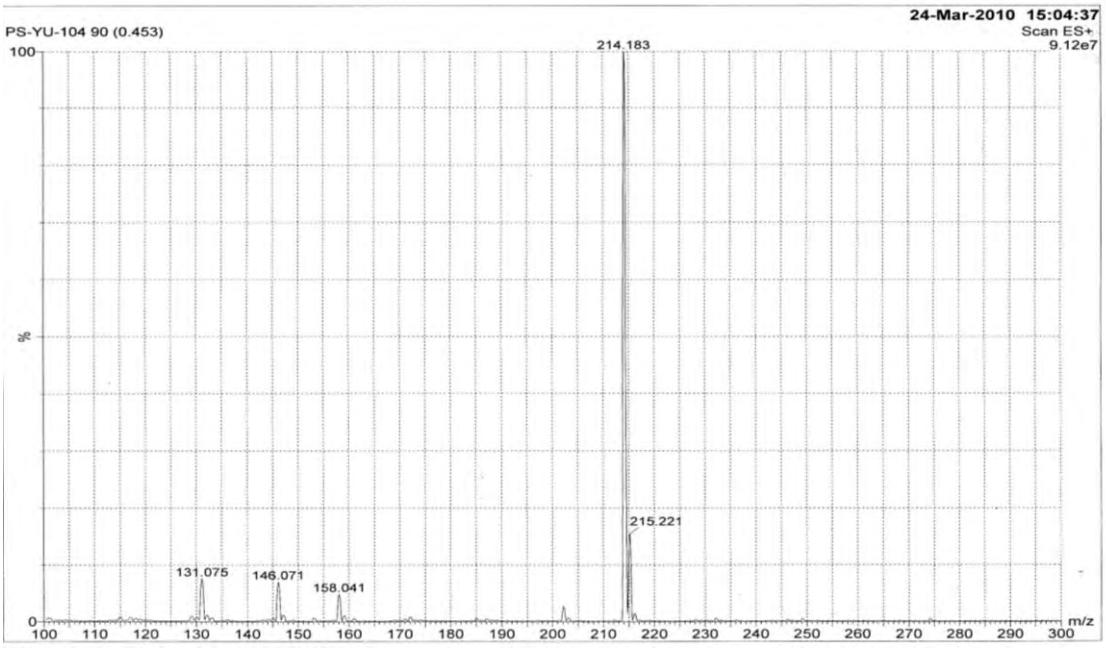

Figure 57 The low-resolution mass spectra of **4-piperidinoquinazoline** (**12e**).

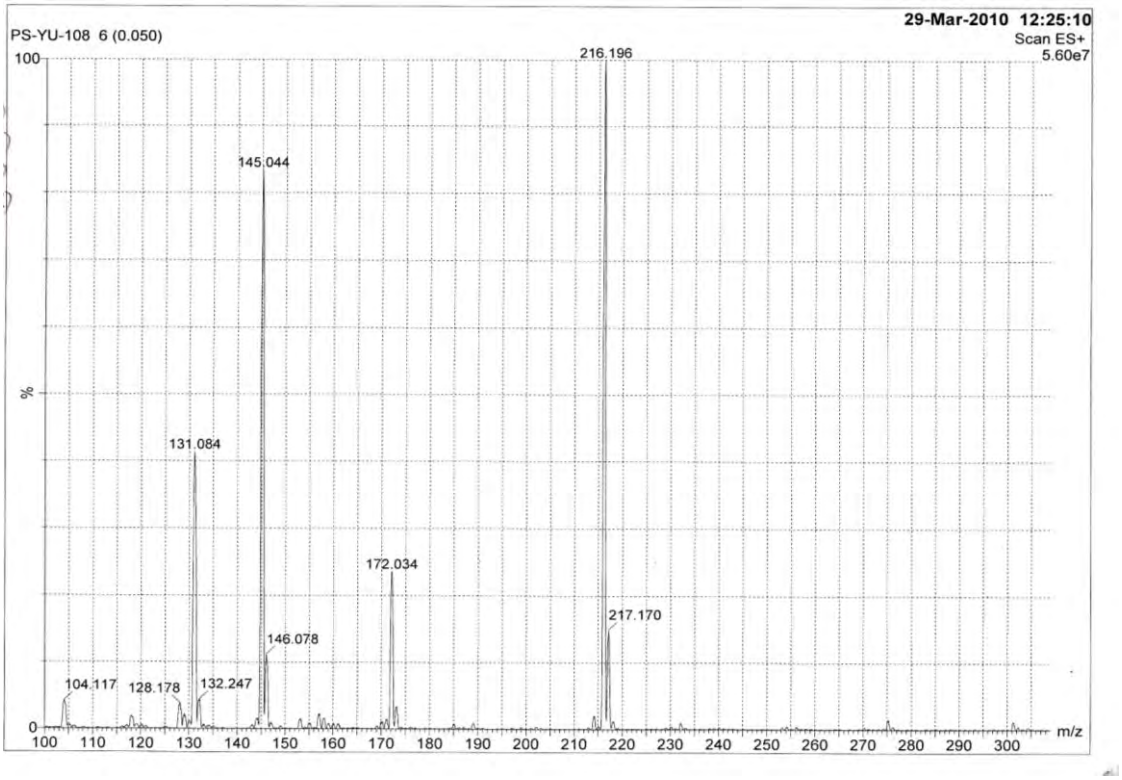

Figure 58 The low-resolution mass spectra of **4-morpholinoquinazoline** (**12f**).

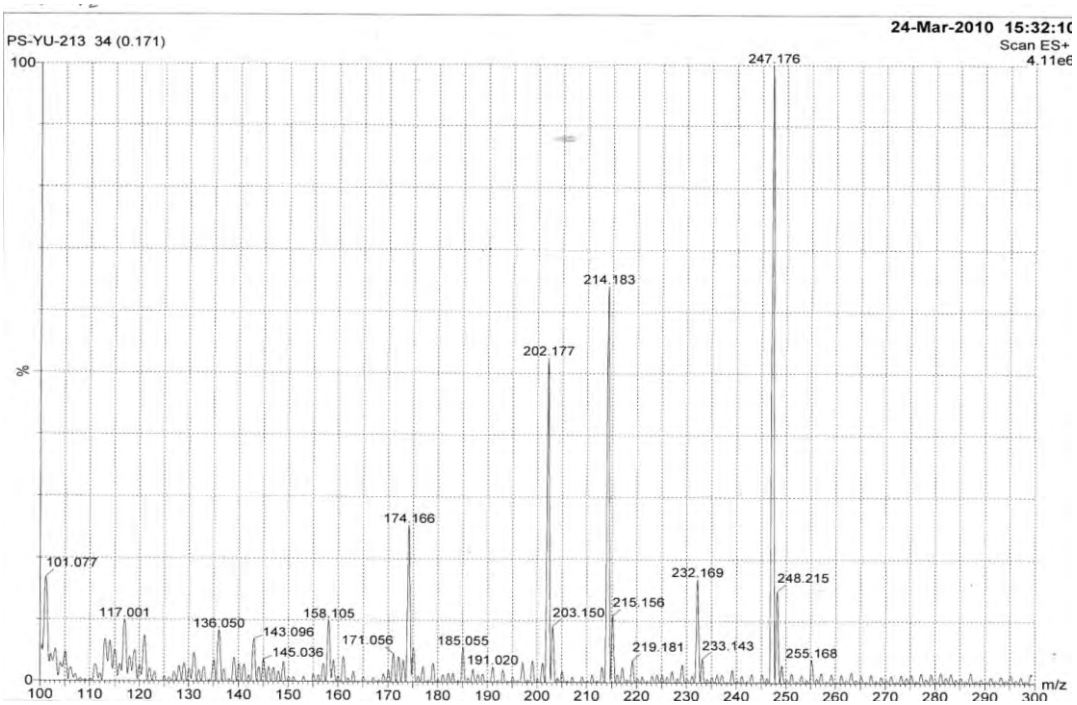

Figure 59 The low-resolution mass spectra of 6 - nitro-4-butylaminoquinazoline (16a).

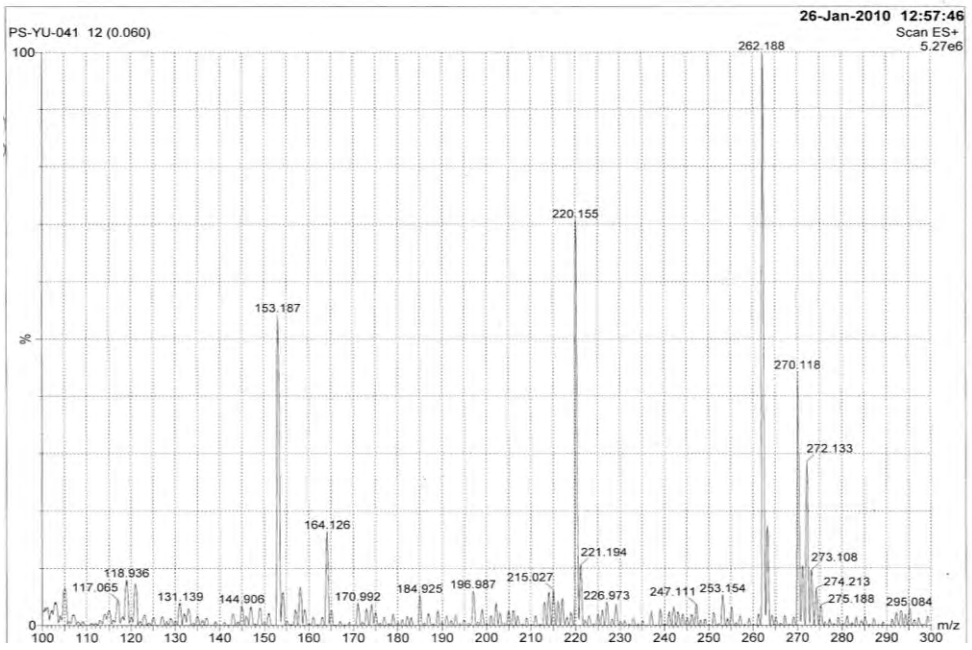

Figure 60 The low-resolution mass spectra of 6, 8-dichloro -4-butylaminoquinazoline (16b).

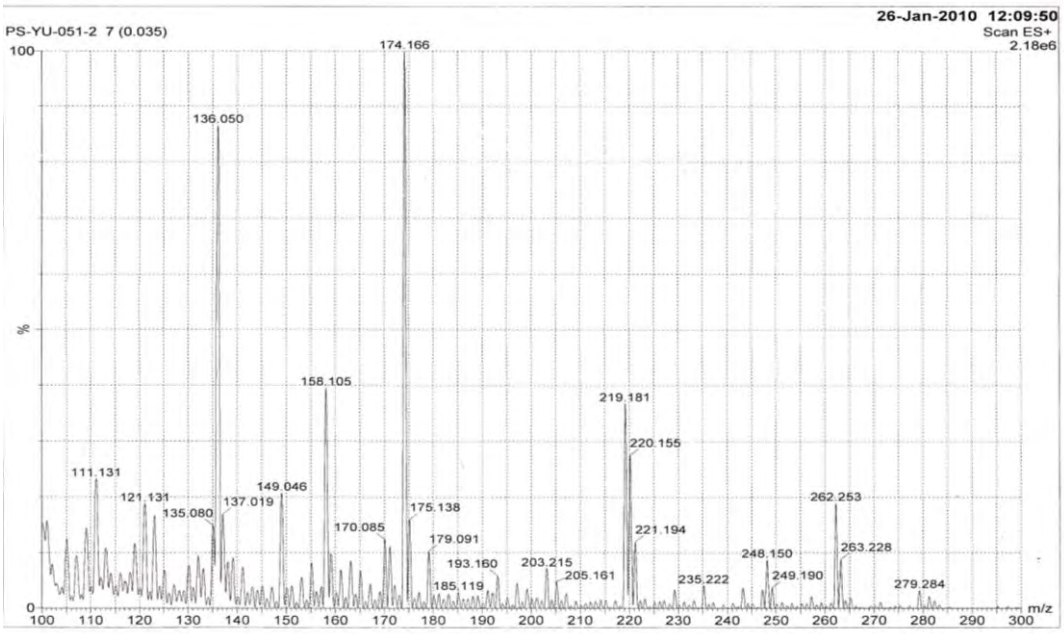

Figure 61 The low-resolution mass spectra of 6-fluoro-4-butylaminoquinazoline (**16c**).

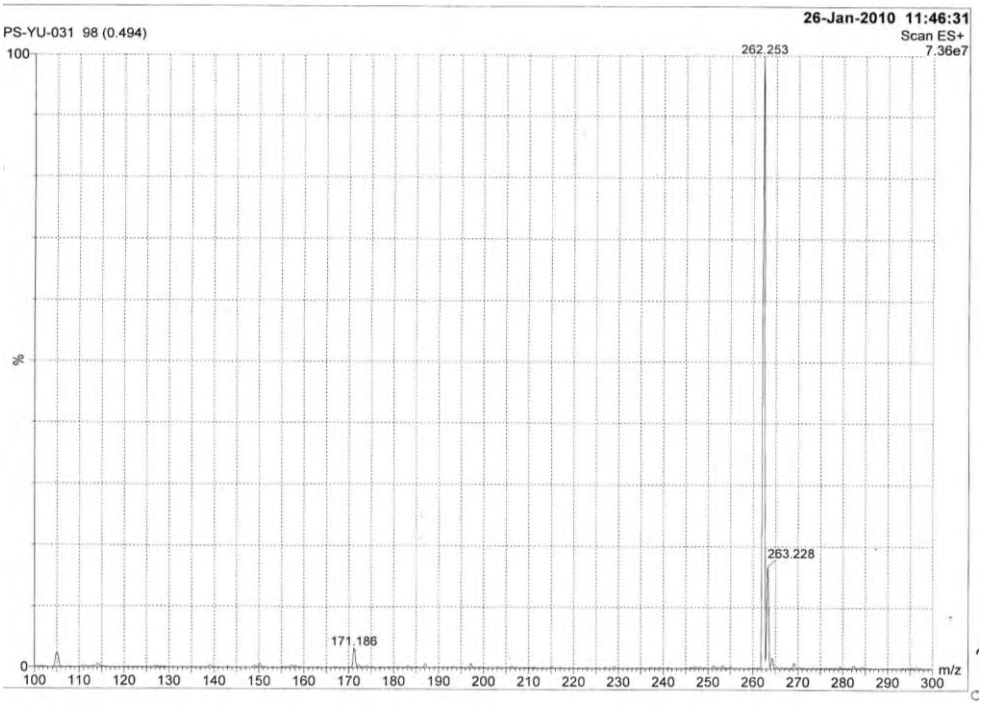

Figure 62 The low-resolution mass spectra of 6,7–dimethoxy-4- butylaminoquinazoline (**16d**).

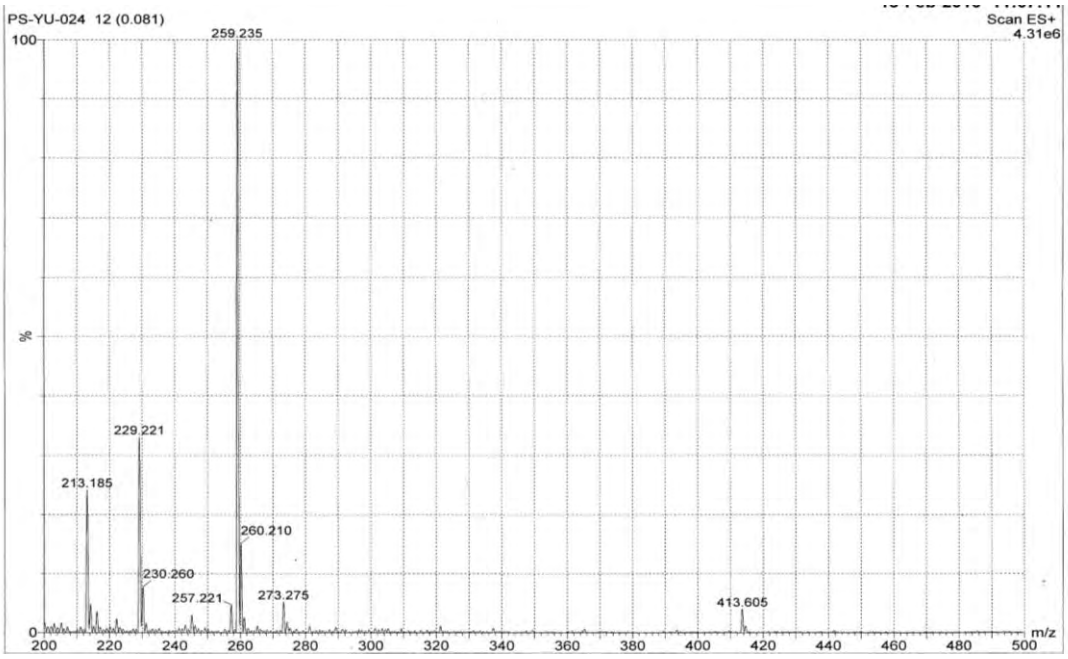

Figure 63 The low-resolution mass spectra of 6-nitro-4-piperidinoquinazoline (**17a**).

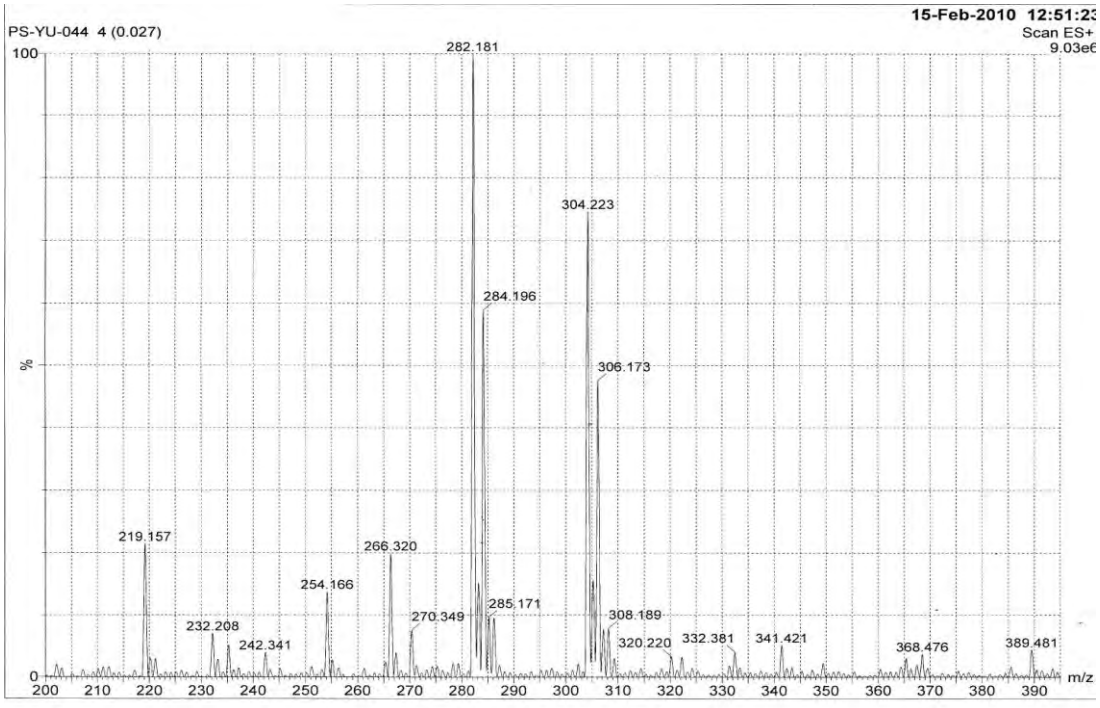

Figure 64 The low-resolution mass spectra of 6,8-dichloro-4-piperidinoquinazoline (**17b**).

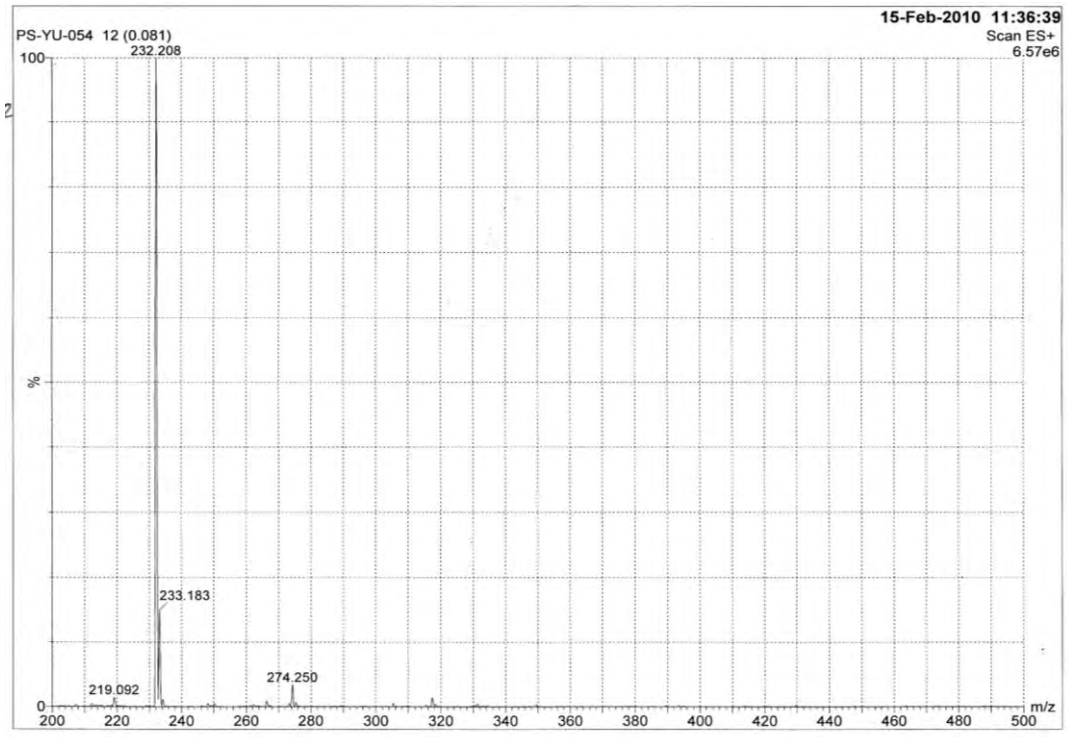

Figure 65 The low-resolution mass spectra of 6-fluoro-4-piperidinoquinazoline (**17c**).

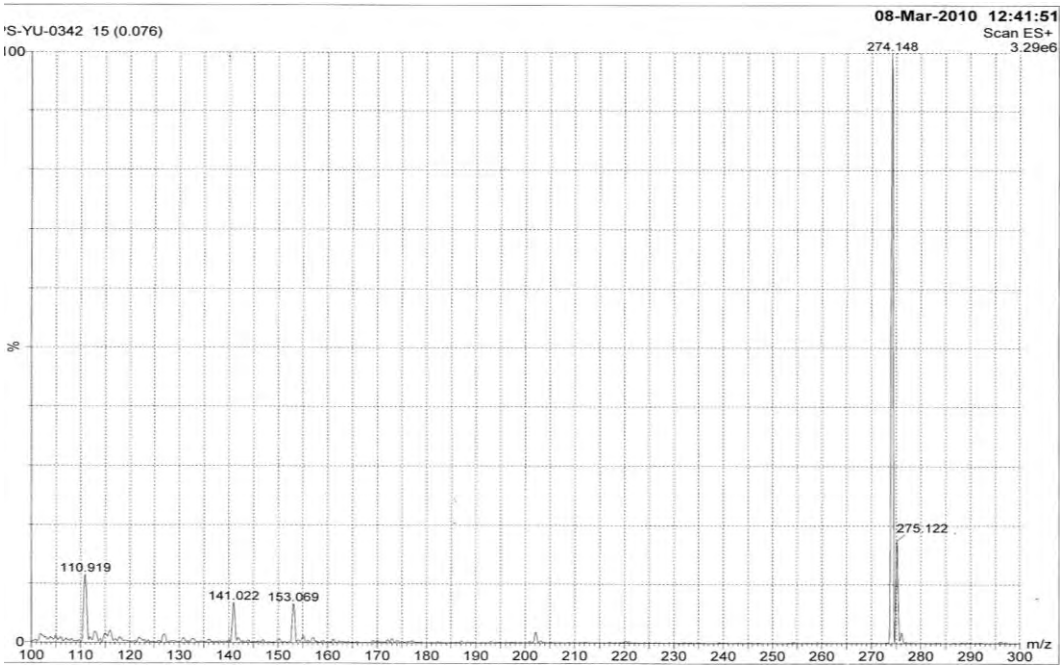

Figure 66 The low-resolution mass spectra of 6,7-dimethoxy -4-piperidinoquinazoline (**17d**).

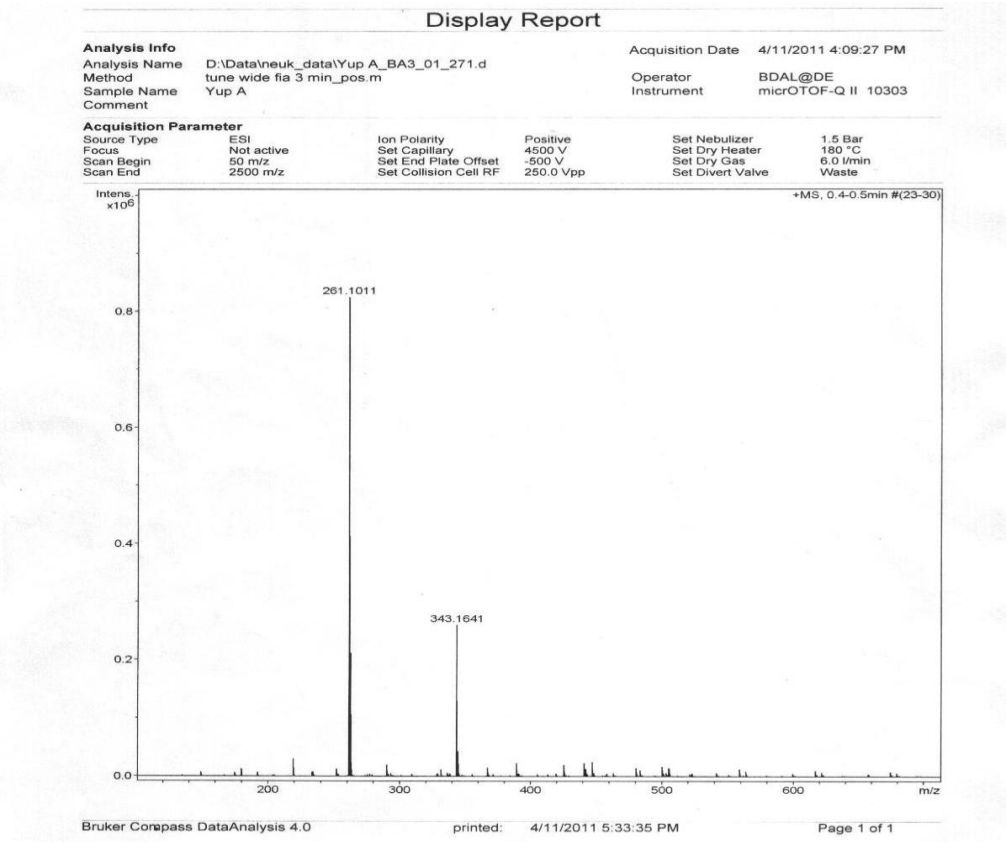

Figure 67 The high-resolution mass spectra of 6-nitro-4-morpholinoquinazoline (**18a**).

VITA

Ms. Yuparaid Uetrongchit was born on June 15, 1968 in Bangkok, Thailand. She graduated with Bachelor Degree of Science, majoring in Chemistry from Srinakharinwirot University, Prasanmitr in 1992. She works as Medical Scientist 6 or Medical Scientist, Professional Level for the Department of Medical Sciences, Ministry of Public Health. Her works involve food chemical analysis. In 2007, she began studying the Master's Degree in Biotechnology program of science faculty in Chulalongkorn University. During the course, she was awarded the outstanding poster from the Pure and Applied Chemistry International Conference 2010 (PACCON 2010) on January 21-23, 2010 at Ubon Ratchathani.

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