Synthesis and evaluation of 3,4-Dihydro-3-methyl-2(1H)-quinazolinone derivatives as monoamine oxidase inhibitors

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Dissertation submitted in partial fulfilment of the requirements for the degree *Master of Science in Pharmaceutical Chemistry* at the North-West University

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PREFACE

This dissertation is submitted in article format consisting of one article. The research article presented in this dissertation was compiled for submission to *Bioorganic & Medicinal Chemistry*. The author guidelines are included (see Appendix B, p.173). The research described in this dissertation was conducted by Ms. L Marais at the North-West University, Potchefstroom campus.

Letters of agreement from the co-authors of the research article is included.

DECLARATION

This dissertation is submitted in fulfilment of the requirements for the degree *Magister Scientiae* in *Pharmaceutical Chemistry*, at the School of Pharmacy, North-West University.

I, Leréze Marais, hereby declare that the dissertation with the title: **Synthesis and evaluation of 3,4-Dihydro-3-methyl-2(1H)-quinazolinone derivatives as monoamine oxidase inhibitors** is my own work and has not been submitted at any other university either whole or in part.

L Marais

October 2017

LETTER OF AGREEMENT

11 October 2017

To whom it may concern,

Dear Sir/Madam

CO-AUTHORSHIP ON RESEARCH PAPER

The undersigned as co-authors of the research article listed below, hereby give permission to Miss L. Marais to submit this article as part of the degree MSc in Pharmaceutical Chemistry at the North-West University.

- **The monoamine oxidase inhibition properties of C6- and N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives**
  Awaiting submission

Yours sincerely,

Prof. L.J. Legoabe

Prof. J.P. Petzer

Prof. A. Petzer

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ABSTRACT

Parkinson's disease (PD) is an age related neurodegenerative disorder. Loss of dopamine from the striatum is responsible for the motor symptoms of PD. This loss of dopamine is due to degeneration of the neurons of the substantia nigra in the brain. Mononamine oxidase B (MAO-B) is an enzyme in the brain that plays a key role in the catabolic pathway of dopamine. Inhibitors of MAO-B protect the striatum from the depletion of dopamine and the MAO-B enzyme is therefore an important target for the treatment of PD. Levodopa (L-dopa), the metabolic precursor of dopamine, is currently the treatment of choice in PD, and MAO-B are particularly useful as adjuvants to L-dopa since they may enhance the level of dopamine after administration of L-dopa.

In the present study, a series of C6-substituted and N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives were synthesised and evaluated as inhibitors of recombinant human MAO-A and MAO-B. These quinazolinones are structurally related to a series of 3,4-dihydro-2(1*H*)-quinolinone derivatives, which has previously been reported to act as MAO-B inhibitors. The 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives substituted on C6 with benzoyl, or cinnamoyl moieties were successfully synthesised by reacting 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one with acyl chloride/bromide in carbon disulphide or with an appropriate benzaldehyde in a mixture of hydrochloric acid and methanol. The N1-substituted derivatives were successfully synthesised by reacting 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one with an appropriate alkyl bromide/chloride with dimethylformamide serving as a solvent. Twenty-six C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one and eleven N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives were synthesised. The structures of these compounds were verified with NMR and MS, and the purities were estimated by HPLC.

The MAO inhibitory properties of the synthesised compounds were determined by using the recombinant human MAO-A and MAO-B enzymes. The inhibition potencies were expressed as the corresponding IC_{50} values. Lineweaver-Burk plots were constructed to determine the mode of MAO inhibition, while the reversibility of inhibition was examined by measuring the recovery of enzyme activity after dialysis of the enzyme-inhibitor mixtures. The results showed that the C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives are potent and selective MAO-B inhibitors and to a lesser extent inhibitors of MAO-A, while the N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives proved to be MAO-A inhibitors. The most potent MAO-B inhibitor, 3-methyl-6-[(2*E*,4*Z*)-5-phenylpenta-2,4-dienoyl]-3,4-dihydroquinazolin-2(1*H*)-one, displayed an IC_{50} of 0.269 μM. The results thus show that 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives are potent MAO inhibitors, with the C6-substituted derivatives being the most potent. With representative inhibitors, it was shown that these compounds are reversible inhibitors of MAO-A and MAO-B since dialysis of enzyme-inhibitor mixtures restores enzyme activity.

It may thus be concluded that the C6-substituted and N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives are promising MAO inhibitors, and thus leads for the future therapy of PD.

Keywords: Parkinson's disease; Monoamine oxidase; 3-Methyl-3,4-dihydroquinazolin-2(1*H*)-one; Selectivity; Reversible inhibition

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LIST OF ABBREVIATIONS

5-HT	5-Hydroxytryptamine/serotonin
6-OHDA	6-Hydroxydopamine
A	
AD	Alzheimer's disease
APCI	Atmospheric-pressure chemical ionization
B	
BDNF	Brain-derived neurotropic factor
C	
$CDCl_3$	Deuterochloroform
COMT	Catechol-O-methyltransferase
CSF	Cerebrospinal fluid
D	
D1	Dopamine 1
D2	Dopamine 2
D3	Dopamine 3
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DOPAC	3,4-Hydroxyphenylacetic acid
HRMS	High resolution mass spectra
F	
FAD	Flavin adenine dinucleotide
G	
GDNF	Glial-derived neurotrophic factor
H	
H_2O_2	Hydrogen peroxide
HPLC	High pressure liquid chromatography
I	

IL	Interleukins
K	
KCl	Potassium chloride
L	
LBs	Lewy bodies
L-dopa	Levodopa
LN	Lewy neurites
LRRK2	Leucine-rich repeat kinase 2
M	
MAO	Monoamine oxidase
$MgSO_4$	Magnesium sulphate
Mp	Melting point
MPP^+	1-Methyl-4-phenylpyridine
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MS	Mass spectrometry
N	
NaOH	Sodium hydorixide
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NMR	Nuclear magnetic resonance
NSAID	Non-steroidal anti-inflammatory agents
NT-3	Neurotrophin-3
P	
PD	Parkinson’s disease
PEA	Phenylethylamine
Ppm	Parts per million
R	
ROS	Reactive oxygen species
S	

SAR	Structure-activity relationships
SD	Standard deviation
SET	Single electron transfer
SI	Selectivity index
SN	Substantia nigra
SNpc	Substantia nigra pars compacta
T	
TLC	Thin layer chromatography
TNF-α	Tumor necrosis factor-alpha
U	
UCHL-1	Ubiquitin carboxyl-terminal hydrolase L1
V	
VMAT2	Vesicular monoamine transporter

CHAPTER 1

INTRODUCTION

1.1 General background

James Parkinson wrote a monograph, “An Essay on the Shaking Palsy”, in which he reported an apparent disorder present in six patients. The patients experienced involuntary tremulous motion and weakened muscular power. These patients tended to bend their trunk forward and go from a walking speed to a running speed. The father of neurology, Jean Martin Charcot, suggested that this unrecognised disorder should be called maladie de Parkinson’s, known today as Parkinson’s disease (Lees *et al.*, 2009).

Parkinson’s disease (PD) is an age-related neurodegenerative disease, which is characterised by numerous motor and non-motor features (Jankovic, 2008). The clinical symptoms of PD include, tremor while resting, bradykinesia and rigidity. These symptoms are not completely developed in early PD. Loss of postural reflex may also occur (Brooks, 2012).

The incidence of PD increases with age (Aarsland *et al.*, 2004). The predominance of PD is estimated at 1% in humans over the age of 65, and increasing to 4.3% in humans over the age of 85. The risk of PD in men is higher than in woman (Huang *et al.*, 2003).

1.2 Monoamine oxidase and monoamine oxidase inhibitors

The deamination of tyramine, dopamine, adrenaline, noradrenaline and 5-hydroxytryptamine are catalysed by monoamine oxidase (MAO). MAO is found in cells throughout the body and is present in the kidney, liver, brain and in all structures stimulated by the sympathetic nerve system (Perks, 1964).

The MAO’s are flavoproteins and exist as two isoforms, MAO-A and MAO-B. These isoforms have different functions. MAO-A deaminates serotonin (5-HT), noradrenaline and adrenaline. MAO-B deaminates 2-phenylethylamine and benzylamine. Substrates for both MAO-A and MAO-B include tyramine and dopamine (Volz & Gleiter, 1998). The activity of these iso-enzymes determines the monoaminergic tone of the brain (Ramsay, 2016).

MAO inhibitors are predominantly used for the treatment of PD and depressive disorder. MAO-B inhibitors have an important therapeutic role in the treatment of PD while MAO-A inhibitors are used in patients suffering from depression (Youdim & Bakhle, 2006). Despite the potential of these drugs, care should be taken as non-selective and irreversible MAO inhibitors can cause a hypertensive crisis, the “cheese reaction” (Yamada & Yasuhara, 2004). MAO metabolises

tyramine and other sympathomimetic amines present in food, such as cheese, beer and wine. These amines obtain access to the circulatory system when peripheral MAO is inhibited. This causes the sympathetic neurons to release noradrenaline, which can cause a hypertensive reaction (Youdim & Weinstock, 2004). The cheese reaction occurs mostly with irreversible MAO-A inhibitors and not MAO-B inhibitors since tyramine is metabolised by MAO-A in the gastrointestinal tract. Selegiline, a selective MAO-B inhibitor and moclobemide, a reversible MAO-A inhibitor, are not associated with this side effect (Yamada & Yasuhara, 2004).

Oxidative stress in the brain may initiate or enhance neurodegeneration in PD, which is a critical event, however, selective inhibition of MAO in the brain may contribute positively to lowering such stress. MAO inhibitors may lower oxidative stress in the brain by reducing the formation of hydrogen peroxide (H_2O_2), a normal by-product of MAO catalysis. This underscores the importance of developing new MAO inhibitors as such compounds may be neuroprotective in PD by lowering oxidative stress (Youdim & Bakhle, 2006).

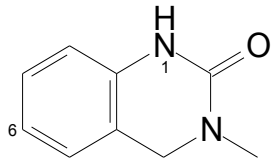
1.3 Rationale

Quinazolinones and quinazolines are nitrogen-containing heterocycles, with a wide spectrum of biological properties (Asif, 2014), including, antimicrobial, anticonvulsant, anticancer (Chandrika *et al.*, 2008), antimalarial, antihypertensive, anti-inflammatory (Alagarsamy *et al.*, 2009), anti-diabetic, antitumor, anti-cholinesterase, inhibition of dihydrofolate reductase, inhibition of cellular phosphorylation and inhibition of kinase (Khan *et al.*, 2016).

Because of the wide variety of biological activities of quinazolinones, this class will, in the present study, be explored as potential MAO inhibitors. As mentioned MAO inhibitors, particularly MAO-B inhibitors, are useful agents for the management of PD (Fernandez & Chen, 2007). It is important to note at this point that for the design of MAO inhibitors, the reversibility of inhibition is an important consideration.

Reversible inhibitors are much less likely to cause the cheese reaction than irreversible MAO inhibitors, and this study will therefore focus on the discovery of quinazolinone inhibitors that inhibit MAO reversibly.

The possibility that the quinazolinone class of compounds may act as MAO inhibitors is supported by literature which reports that 3,4-dihydro-2(1*H*)-quinolinone derivatives substituted on the C6 and C7 positions, are potent MAO inhibitors (Meiring *et al.*, 2013). Quinazolinone and 3,4-dihydro-2(1*H*)-quinolinone bear much structural resemblance.



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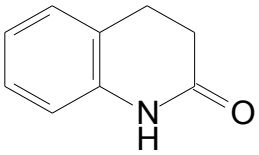
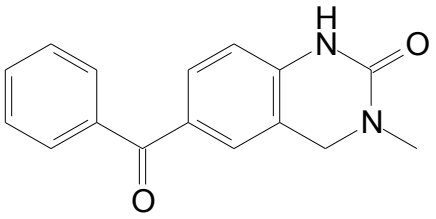
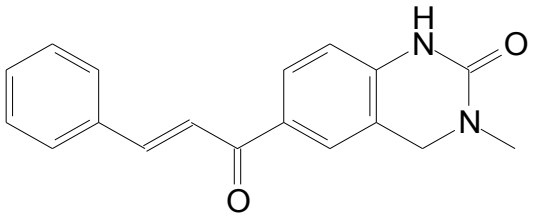
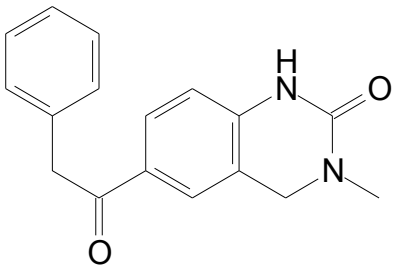
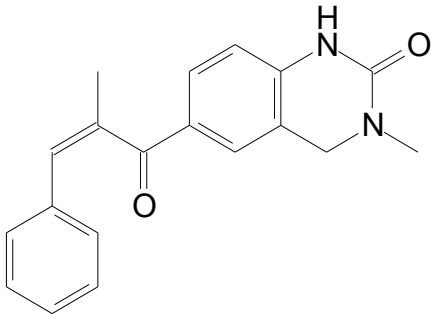


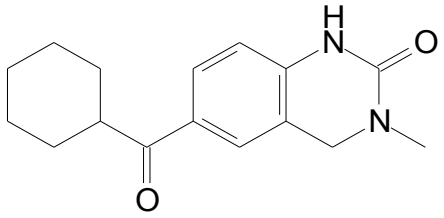
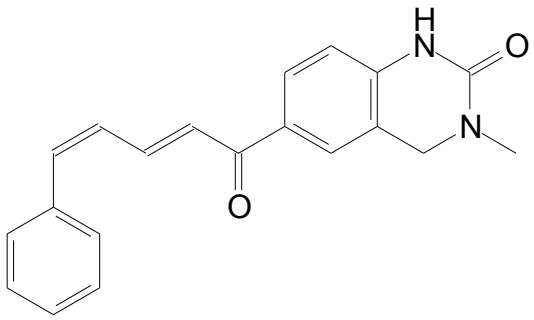
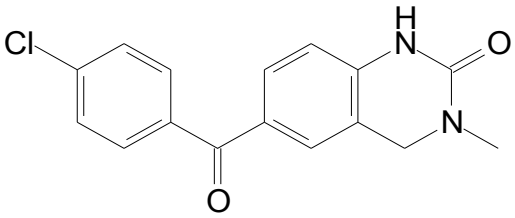
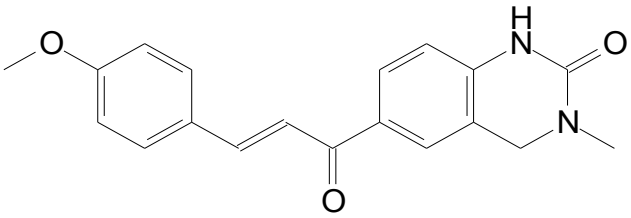
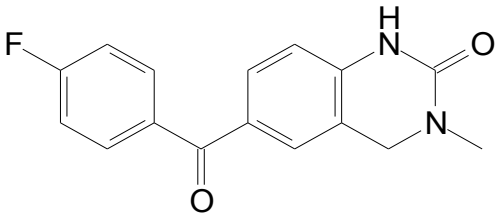
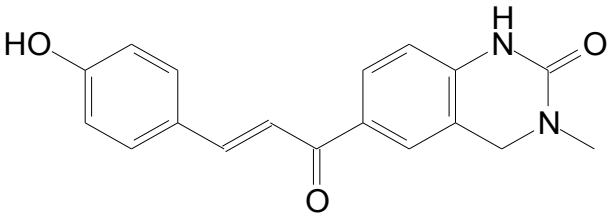
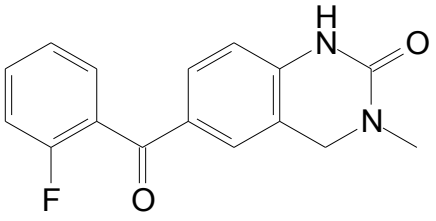
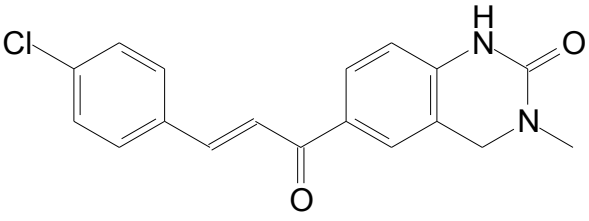
(2)

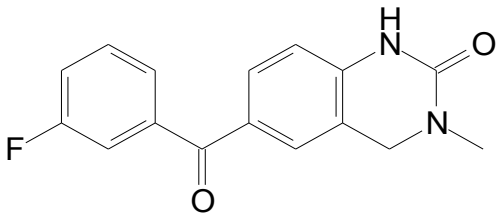
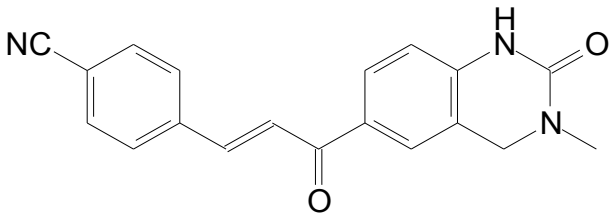
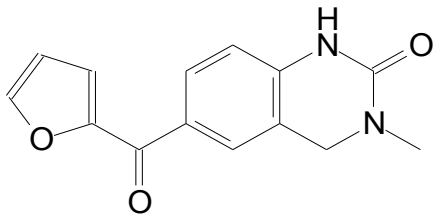
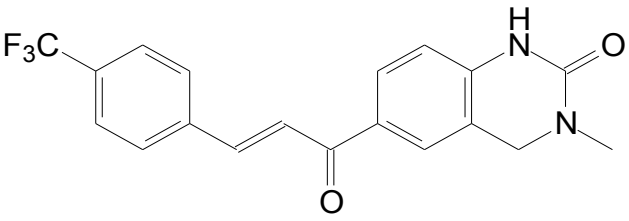
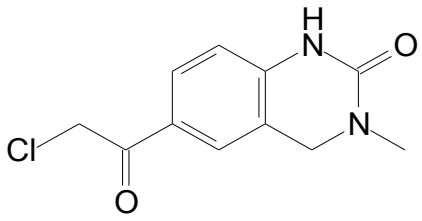
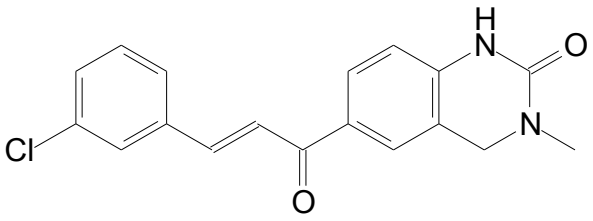
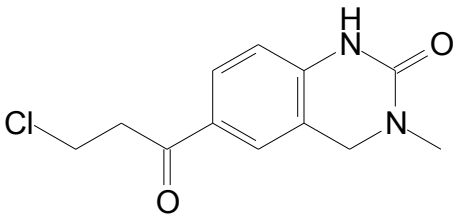
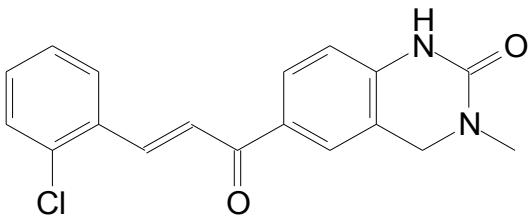
Figure 1.1: The basic structure of 2(1H)quinazolinone (1) and 3,4-dihydro-2(1H)-quinolinone (2).

The general structure of quinazolinone is shown in **figure 1.1**. In this study, quinazolinone derivatives will be synthesised with substitution at two positions. As shown in **table 1.1** and **table 1.2**, quinazolinones will be substituted at the C6 position to yield compounds **3a-m** as well as compounds **4a-m**, and at the N1 position to yield compounds **5a-e** and **6a-f**.

Table 1.1: The structures of the C6-substituted quinazolinones that will be synthesised in this study.

 **(3a)**	 **(4a)**
 **(3b)**	 **(4b)**

 **(3c)**	 **(4c)**
 **(3d)**	 **(4d)**
 **(3e)**	 **(4e)**
 **(3f)**	 **(4f)**

 **(3g)**	 **(4g)**
 **(3h)**	 **(4h)**
 **(3i)**	 **(4i)**
 **(3j)**	 **(4j)**

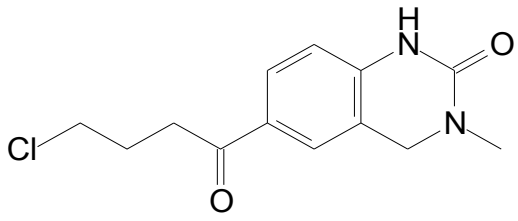
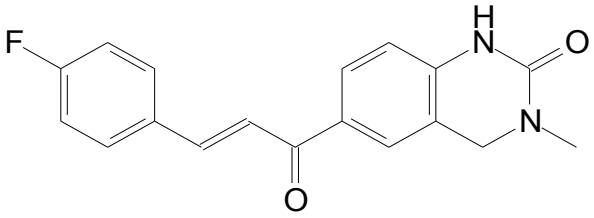
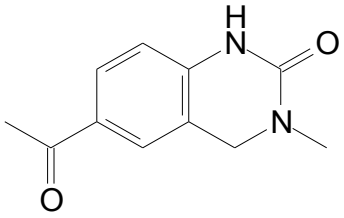
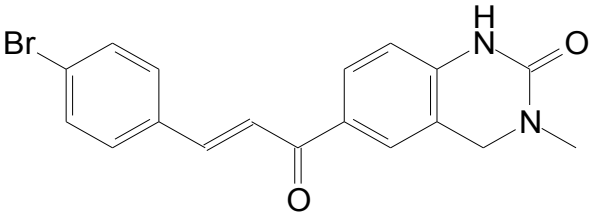
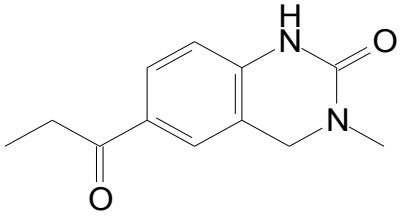
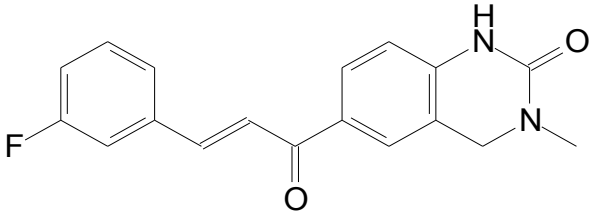
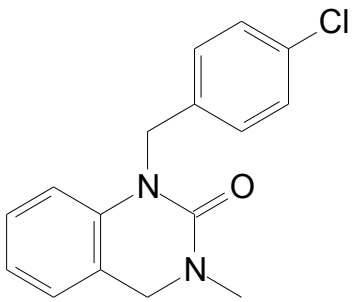
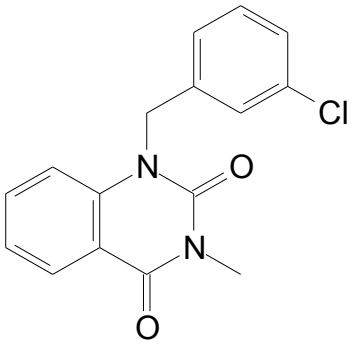
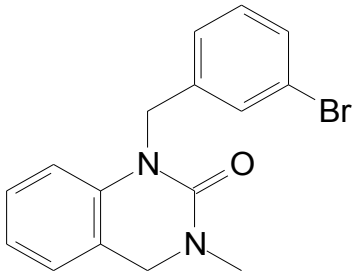
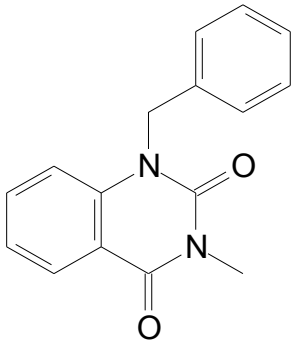
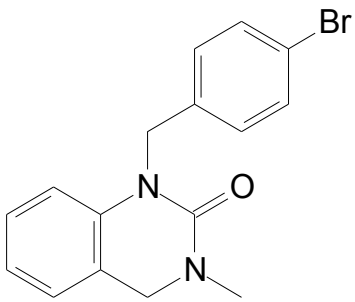
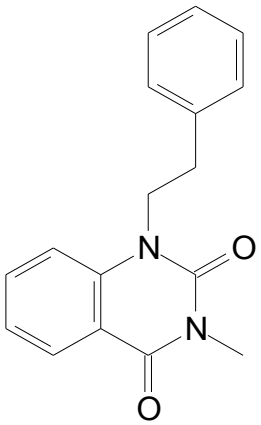
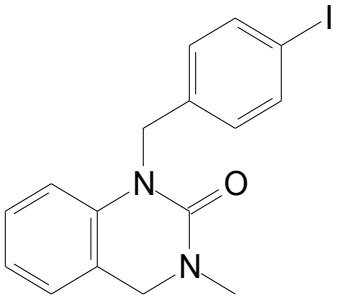
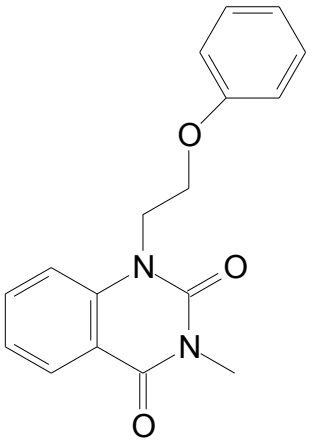
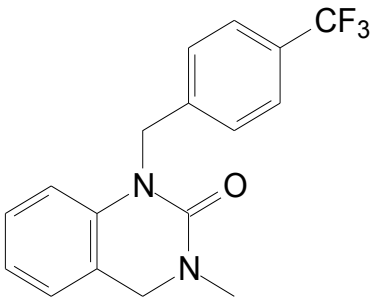
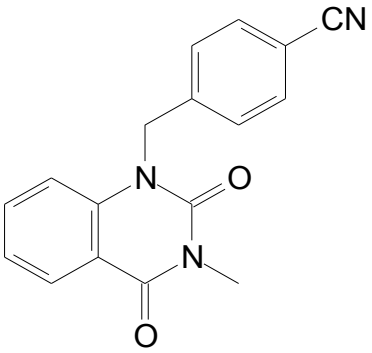
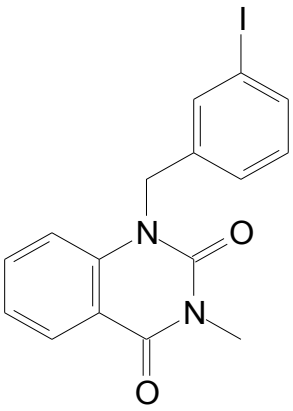
 **(3k)**	 **(4k)**
 **(3l)**	 **(4l)**
 **(3m)**	 **(4m)**

Table 1.2: The structures of N-substituted quinazolinones that will be synthesised in this study.

 **(5a)**	 **(6a)**
 **(5b)**	 **(6b)**
 **(5c)**	 **(6c)**

 **(5d)**	 **(6d)**
 **(5e)**	 **(6e)**
	 **(6f)**

1.4 Hypothesis of this study

Considering the broad biological activities of the quinazolinone class of compounds, it is hypothesised that quinazolinones may represent a promising scaffold for the design of MAO inhibitors. It is further postulated that appropriate substitution on the C6 and N1 position of quinazolinones may yield potent and possibly selective MAO inhibition.

The current study will determine which structural features are essential for MAO inhibition, while allowing for reversibility and isoform selective inhibition.

This study will thus attempt to answer the following questions:

- Are 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives inhibitors of MAO?
- Which structural features are essential for good MAO inhibition activity?

1.5 Objectives of the study

The objectives of this study are:

- Two series of novel 3,4-dihydro-3-methyl-2(1*H*)-quinazolinone derivatives will be synthesised.
- The synthesised compounds will be characterised by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), and the melting points will be measured. Purity determination will be done by high performance liquid chromatography (HPLC).
- The synthesised compounds will be investigated as potential inhibitors of MAO-A and MAO-B by using the recombinant human MAO enzymes. The concentration of an inhibitor that produces 50% inhibition (IC_{50} values) will be used to define the inhibition potency.
- To determine if the compounds are reversible MAO inhibitors, the recovery of the enzymatic activity after dialysis of the enzyme-inhibitor mixtures will be evaluated.
- A set of Lineweaver-Burk plots will be constructed to determine the mode of inhibition (e.g. competitive) of representative inhibitors.

1.6 References

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CHAPTER 2

LITERATURE REVIEW

2.1 Parkinson's disease

2.1.1 Clinical characteristics of Parkinson's disease

Parkinson disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease (AD). These neurodegenerative diseases significantly decrease the quality of life of PD patients. PD is a progressive disorder, which primarily results from the death of dopaminergic neurons in the brain (Dauer & Przedborski, 2003). Occurrence of severe nigral-cell loss and accumulation of aggregated α-synuclein in specific brain stem, spinal cord and cortical regions are characteristics of PD (Lees *et al.*, 2009). The major risk factors of PD is age and symptoms will worsen over time (Fahn, 2003).

Additional clinical characteristics of PD are tremor during rest, bradykinesia, rigidity and postural reflex deterioration. There will also be changes in mental status, such as anxiety, depression, dementia, hallucinations, psychosis and sleep disorders (Wirdefeldt *et al.*, 2011). In the late stages of PD, the face of the patient is masked and impassive, slurring of the speech may occur as well as the speech becoming monotonous, the posture is flexed, and pill rolling motion of the hands will be present. Freezing and the inability to begin a voluntary movement may occur (Lees *et al.*, 2009).

2.1.2 Neurochemical and neuropathological features

The loss of nigrostriatal dopaminergic neurons and the presence of Lewy Bodies are the main neuropathological features of PD (Dauer & Przedborski, 2003). PD presenting with Lewy Bodies (LBs) is the most common neurodegenerative movement disorder (Lee & Trojanowski, 2006). The normal nigrostriatal pathway comprises of dopaminergic neurons, with their cell bodies located in the substantia nigra pars compacta (SNpc). These dopaminergic neurons project their synapses to the striatum as well as other structures of the basal ganglia. The nigrostriatal pathway degenerates during PD, thus leading to a loss of dopaminergic innervation in the striatum (Dauer & Przedborski, 2003).

Apart from dopaminergic neuron loss, there is also neurodegeneration and LB formation in the locus coeruleus, raphe, dorsal nucleus of the vagus, the cerebral cortex, olfactory bulb and autonomic nervous system (Dauer & Przedborski, 2003). According to Dauer & Przedborski

(2003) the dementia that accompanies PD especially in the elderly is exacerbated by the degeneration of hippocampal structures and cholinergic cortical inputs.

2.1.3 Etiology of PD

The etiology of PD is still unknown, but includes both genetic and environmental factors (Wirdefeldt *et al.*, 2011). The most important risk factor for the development and progression of PD, is ageing (Reeve *et al.*, 2014). With PD there is a distinct clinical picture, whereas very mild parkinsonian signs can be associated with normal aging. Many cellular processes are affected by ageing, which leads to neurodegeneration and age-related changes in cellular functions, contributing to the pathogenesis of PD (Hindle, 2010). The substantia nigra (SN) is an important brain region that is affected by severe cell loss in PD. Dopaminergic neurons of the pars compacta are lost within the SN, which shows more pathological changes with normal ageing than any other brain region. The effect of ageing in PD is the reduction of the strength of neurons and their ability to respond to further insults (Reeve *et al.*, 2014).

Genetic factors also play a role in the development of PD. Results from genetic studies have shown that there are mutations in seven genes which could be linked to levodopa-responsive parkinsonism (Lees *et al.*, 2009). Parkinsonism that resembles idiopathic PD can be caused by mutations in five genes namely SNCA (α-synuclein), PARK2 (parkin), PARK7 (DJ-1), PINK1 and leucine rich repeat kinase 2 (LRRK2). After mutations in LRRK2, the second most common genetic cause of parkinsonism is triggered by mutations of PARK2 (Healy *et al.*, 2008). Six pathogenic mutations in LRRK2 have been described. Amongst these mutations, the Gly2019Ser mutation is the most common. A frequency of 1% in sporadic cases worldwide and 4% in patients with hereditary parkinsonism are due to Gly2019Ser mutations (Lees *et al.*, 2009).

The LRRK2 Gly2019Ser mutation can be inherited resulting in a 28% risk of developing PD at the age of 59 years, 51% risk at the age of 69 years and at the age of 79 years the risk will be 74% (Healy *et al.*, 2008). Point mutations and gene triplications of α-synuclein also can cause a parkinsonian syndrome which is difficult to distinguish from PD. Early parkinsonism (age of <40 years) can be caused by loss of function mutations in genes such as parkin, DJ-1, PINK1 and ATP13A2 (Lees *et al.*, 2009).

Factors such as caffeine consumption and cigarette smoking also have an effect on the development of PD. According to Hernán *et al.* (2002), the risk of developing PD is 60% lower in cigarette smokers compared to non-smokers. Cigarette smoke upregulate nicotinic receptors and inhibit free radical damage to nigral cells and also stimulate the release of dopamine. By inhibition of MAO-B or competitive inhibition of the activation of neurotoxins by MAO-B, cigarette smoke may protect against neuronal damage (Hernán *et al.*, 2002).

According to Hernán *et al.* (2002), the risk of PD is 30% less among coffee drinkers than non-coffee drinkers. Caffeine may protect against neurodegeneration by antagonism of adenosine A_{2A} receptors. Furthermore, caffeine acts as a reversible inhibitor of both MAO-A and MAO-B, with K_i values of 0.70 mM and 3.83 mM, respectively (Petzer *et al.*, 2013). By inhibition of MAO, caffeine also may provide neuroprotection in PD (Chen *et al.*, 2001).

Environmental toxins such as pesticides, rural living, drinking well water, heavy metal and solvent exposure, welding and mining can also increase the risk of developing PD (Hindle, 2010). PD related neurodegeneration results from the exposure to dopaminergic neurotoxins. A syndrome, nearly identical to PD can be created in animals and humans by the dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydeopyridine (MPTP) (Dauer & Przedborski, 2003).

2.1.4 Pathogenesis of PD

The pathogenesis of PD describes the events leading to neuronal cell death. Oxidative stress, impaired mitochondrial function, excitotoxicity, protein misfolding and aggregation, impaired lysosome and chaperone-mediated autophagy and the development of LBs can all cause cell death in PD. Cell loss appears in the noradrenergic locus coeruleus, the cholinergic nucleus basalis of Meynert, the serotonergic raphe nucleus and the autonomic nervous system, as well as in dopaminergic cells in the SN (Hindle, 2010).

2.1.4.1 Oxidative stress and mitochondrial dysfunction

Mechanisms that can cause oxidative stress include depletion of antioxidants, impeded mitochondrial electron transport and the presence of neurotoxins (Alam *et al.*, 1997). The metabolism of dopamine may also lead to oxidative stress by increasing the levels of dopamine-quinone, superoxide radicals and hydrogen peroxide (H_2O_2). Due to the presence of dopamine, nigral dopaminergic neurons are exposed to higher levels of oxidative stress. According to the oxidative stress hypothesis, oxidative damage to proteins, lipids and DNA are increased in PD because of the uncontrolled formation of reactive oxygen and nitrogen species (Fernandez-Espejo, 2004). In support of thus, damage of protein, lipid and nucleic acid has been found in the SN of PD patients (Yacoubian & Standaert, 2009). Oxidative stress is also caused by the neurotoxin, MPTP, which blocks the mitochondrial electron transport chain by inhibiting complex I. The inhibition of complex I leads to the production of superoxide, and superoxide, in turn, may be converted to toxic hydroxyl radicals or peroxynitrite (Dauer & Przedborski, 2003).

As mentioned, PD is associated with abnormal protein deposition. The accumulation and aggregation of proteins such as α-synuclein may promote cell death (Hindle, 2010). Accumulation of protein aggregates can cause damage by deforming the cell or interfering with intracellular trafficking in neurons. Vesicular storage of dopamine may also be affected because of

mitochondria-related energy failure. This leads to an increase in cytosolic dopamine concentration and harmful dopamine reactions and metabolism that cause cellular macromolecule damage (Dauer & Przedborski, 2003). Mutations in mitochondrial DNA, inherited or acquired, may contribute to mitochondrial dysfunction in PD (Cantuti-Castelvetri *et al.*, 2005). Free radical damage, especially in the presence of neuromelanin, is enhanced by increased iron levels found in the SN of PD patients (Yacoubian & Standaert, 2009).

2.1.4.2 Excitotoxicity

The main excitatory transmitter in the mammalian central nervous system is glutamate. Glutamate is also a primary driver in the excitotoxicity process. Glutamate causes activation of N-methyl-D-aspartate (NMDA) receptors, which causes an increase in intracellular calcium levels. Increased calcium activates cell death pathways and also stimulates peroxynitrite production through activation of nitric oxide synthase (Yacoubian & Standaert, 2009).

2.1.4.3 Protein misfolding and aggregation

Aggregation or protein misfolding is toxic to neurons. As mentioned, protein aggregates causes damage which includes deforming of cells and interfering with intracellular trafficking in neurons (Dauer & Przedborski, 2003).

The primary aggregating protein found in PD is α-synuclein (Yacoubian & Standaert, 2009) and α-synuclein is the major component of LBs and Lewy neurites (LNs). α-Synuclein also plays a role in LB-like inclusions, neuraxonal spheroids and LNs (Giasson *et al.*, 2000). Furthermore, PD is caused by gene duplications of the α-synuclein locus (Yacoubian & Standaert, 2009), while point mutations in α-synuclein have also been associated with familial PD (Masliah *et al.*, 2000).

It is thus hypothesised that overproduction or defective clearance of α-synuclein may cause PD, and that future inhibitors of α-synuclein aggregation may prevent protein aggregation and halt neurodegeneration in PD. Another strategy to reduce the harmful effects of α-synuclein aggregation is to promote the functions of ubiquitin C-terminal hydrolase L1 (UCHL-1) function or parkin, which are involved in the proteasomal or lysosomal degradation of α-synuclein (Yacoubian & Standaert, 2009).

2.1.4.4 Neuroinflammation

Neuroinflammation is a prominent neuropathological feature of PD (Hunot & Hirsch, 2003). In PD, the concentration of pro-inflammatory cytokines such as interleukins (IL-1β and IL-6) and tumour necrosis factor-alpha (TNF-α) are increased in the cerebrospinal fluid (CSF) and basal ganglia

(Yacoubian & Standaert, 2009). A neuroinflammatory reaction can be initiated by injured dopaminergic neurons (Hunot & Hirsch, 2003).

Furthermore, synergism between gene products linked to PD (α-synuclein, parkin) and neuroinflammation may occur to advance neurodegeneration of the nigrostriatal pathway (Lee *et al.*, 2009). Hallmarks of neuroinflammation includes activated microglia and reactive astrocytes in the parenchyma of the central nervous system, heightened production of cytokines, chemokines, prostaglandins, compliment cascade proteins and reactive oxygen species (ROS). This may lead to disruption of the blood brain barrier (Lee *et al.*, 2009).

Anti-inflammatory agents have been considered for their neuroprotective potential in PD. Agents that reduces dopaminergic cell death in animal and culture PD models include non-steroidal anti-inflammatory agents (NSAIDs) and minocycline, the latter also an anti-inflammatory drug that has been investigated for its potential neuroprotective effects (Yacoubian & Standaert, 2009).

2.1.4.5 Apoptosis

Apoptosis, also known as programmed cell death, has a role in neural development and also in some forms of neural injury. Evidence of both apoptotic and autophagic cell death has been discovered in the SN of PD brains (Yacoubian & Standaert, 2009). Programmed cell death activates intracellular signalling pathways which causes cell demise. Physiological programmed cell death plays an important part in normal development and acts as a homeostatic mechanism in some systems. Neurodegeneration may occur as a result of dysregulation of this pathway (Dauer & Przedborski, 2003).

In PD, both apoptotic and autophagic cell deaths are triggered by oxidative stress, protein aggregation, excitotoxicity and inflammatory processes. The activation of cell death pathways probably symbolises the end-stage processes in PD neurodegeneration (Yacoubian & Standaert, 2009).

2.1.4.6 Loss of trophic factors

Cell death recognised in PD can also occur following the loss of neurotrophic factors (Yacoubian & Standaert, 2009). Neurotrophic factors are important for neuron survival (especially during development), neuron sprouting (which is essential during regeneration) and neuron maintenance (which is vital throughout the life cycle) (Appel, 1981).

In animals and *in-vitro* models, brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) are all factors that promote dopaminergic cell growth and health (Chauhan *et al.*,

2001). In PD, the levels of BDNF, GDNF and NGF are reduced in the nigra (Yacoubian & Standaert, 2009). GDNF is a member of the transforming growth factor-β family (Lang *et al.*, 2006). GDNF possibly improves the function and delays the degree of degeneration of dopaminergic neurons in PD (Gasmi *et al.*, 2007). GDNF may thus lead to neuroprotective effects in PD (Lang *et al.*, 2006).

BDNF has more extensive neurotrophic effects, and also advances the sprouting and neurite outgrowth of human fetal dopaminergic neurons (Howells *et al.*, 2000). Because of their effect on growth stimulation and arborisation of dopaminergic neurons, tropic factors may thus be useful as neuroprotective agents in PD (Yacoubian & Standaert, 2009).

2.1.5 Animal models of PD

Animal models are used to study pathogenic mechanisms and therapeutic strategies in human diseases.

2.1.5.1 The reserpine model

The reserpine model played an important role in our understanding of the link between monoamine depletion and parkinsonian symptoms. The vesicular monoamine transporter (VMAT2) is inhibited by reserpine, which causes a loss of storage capacity and depletion of catecholamine levels in the brain (Duty & Jenner, 2011). This inhibition causes an akinetic state in rabbits. According to Betarbet *et al*. (2002), the administration of levodopa (L-dopa) will alleviate the reserpine-induced akinetic state, which proves that behavioural recovery is dopamine-dependent. The development of the reserpine model was driven by the discovery that striatal dopamine deficiency leads to PD-like symptoms. A disadvantage of reserpine is that it does not deliver morphological changes in the dopaminergic neurons, thus only displaying temporary changes. The therapeutic effects of striatal dopamine replacement agents, including L-dopa and dopamine receptor agonists, have been successfully investigated by using the reserpine model (Betarbet *et al.*, 2002).

2.1.5.2 Methamphetamine model

Methamphetamine has neurotoxic effects on the nervous system and causes functional deficits and structural alterations (Jackson-Lewis *et al.*, 2012). Although it displays minimal effects in the nigral cell bodies, the administration of methamphetamine results in dopamine depletion. Evidence suggests that methamphetamine works through the dopamine receptor and transporter, but the mechanism is still unclear (Betarbet *et al.*, 2002). According to Jackson-Lewis *et al.* (2012), the methamphetamine model is not very reliable as a PD model.

2.1.5.3 The 6-hydroxydopamine model

6-Hydroxydopamine (6-OHDA) is an agent with specific neurotoxic effects on catecholaminergic pathways. Degeneration of catecholaminergic pathways occurs with 6-OHDA, which uses the same transport system as dopamine and norepinephrine (Betarbet *et al.*, 2002). 6-OHDA is injected into the SN, nigrostriatal tract or striatum to precisely target the nigrostriatal dopaminergic pathway. After 6-OHDA is injected a slow declining degeneration of the nigrostriatal system is obtained over a period of weeks (Betarbet *et al.*, 2002). Injection of 6-OHDA directly into the brain is required because 6-OHDA does not cross the blood-brain barrier (Duty & Jenner, 2011). Side effects of the bilateral injection of this compound includes severe adipsia, aphagia, seizures and death, therefore 6-OHDA is used as a unilateral model (Jackson-Lewis *et al.*, 2012). 6-OHDA undergoes auto-oxidation once it reaches the neuron, which leads to the formation of H_2O_2 (Blandini & Armentero, 2012). Oxidative stress is thus central to the neurotoxic effects of 6-OHDA. In the presence of iron, H_2O_2 is converted to hydroxyl radicals, which damages virtually all biomolecules in the neuron. Not all the clinical and pathological features of PD, such as the formation of LBs, are however represented by the 6-OHDA model.

Neurotrophic factors and compounds that promote survival of the degenerating dopaminergic nigral neurons in PD have been evaluated with this model (Betarbet *et al.*, 2002).

2.1.5.4 The MPTP model

Clinical symptoms similar to PD in humans can be seen after injection with MPTP. MPTP is a lipophilic protoxin that crosses the blood-brain barrier (Duty & Jenner, 2011). MAO-B transforms MPTP to its active metabolite, 1-methyl-4-phenylpyridinium ion (MPP^+), after crossing the blood-brain barrier. MPP^+ blocks mitochondrial complex I activity after being transported into dopaminergic neurons of the SNpc (Blandini & Armentero, 2012). MPTP exposure leads selectively to nigrostriatal dopaminergic degeneration with more than 99% loss of dopamine in the striatum (Betarbet *et al.*, 2002).

Oxidative stress, ROS, energy failure and inflammation are key indicators of PD and the MPTP model has the ability to replicate all of these in monkeys and other mammals, but not in rats (they are resistant to this toxin). A limitation of this model is the absence of LBs (Jackson-Lewis *et al.*, 2012). This model has, however, proved the hypothesis that complex I dysfunction is lethal to dopaminergic neurons (Betarbet *et al.*, 2002).

2.1.5.5 Paraquat and Maneb model

The active metabolite of MPTP, MPP^+, has structural similarity to the herbicide 1,1'-dimethyl-4, 4'-bipyridinium (paraquat). Paraquat can cross the blood-brain barrier and causes a decrease in

dopaminergic nigral neurons and striatal dopaminergic innervation, followed by reduced ambulatory movement (Betarbet *et al.*, 2002). The mechanism of action involves the generation of oxidative stress since paraquat may act as a redox cycler. In individual dopaminergic neurons in the SNpc, paraquat increases α-synuclein content and stimulates the formation of LBs (Jackson-Lewis *et al.*, 2012).

Another compound, maneb inhibits complex III of the mitochondrial respiratory chain and thus leads to decreased locomotor activity in animals. Maneb also potentiates the toxic effects of MPTP and paraquat on the dopaminergic system of experimental animals (Betarbet *et al.*, 2002).

2.1.5.6 Rotenone model

Rotenone is a flavonoid used as a broad-spectrum pesticide. Exposure to rotenone can lead to selective degeneration of nigrostriatal dopaminergic neurons (Blandini & Armentero, 2012). Rotenone is lipophilic and crosses the blood-brain barrier where it inhibits complex I of the electron transport chain. Previous studies found that exposure of rats to rotenone lead to the inhibition of complex I throughout the rat brain (Betarbet *et al.*, 2002). Indicators of PD that are replicated by rotenone are complex I blockade, behavioural alterations, inflammation, α-synuclein aggregation, LB formation, oxidative stress and gastrointestinal problems (Jackson-Lewis *et al.*, 2012).

This model is, however, labour-intensive and has significant variability. Rotenone also shows a high degree of systemic toxicity, which produces death (Duty & Jenner, 2011).

2.1.5.7 Genetic models

α-Synuclein mutations causes a rare form of autosomal dominant PD. Pro-30, Thr-53 and Lys-46 are the three mutations that have been identified thus far. The PD syndrome caused by mutations in α-synuclein exhibits motor dysfunctions which respond to treatment with dopamine replacement therapy, and it has thus been hypothesised that PD animal models may be generated by mutations of the α-synuclein gene.

Autosomal dominant PD is also caused by mutations in the gene LRRK2 (leucine-rich repeat kinase 2). Gly-1441, Cys-1441, His-1437, Cys-4699, Ser-2019 and Thr-2020 are the six disease causing mutations in LRRK2. The overexpression of the Ser-2019 mutation causes an age-dependant reduction of the striatal content, while overexpression of the Gly-1441 mutant causes age-dependant and progressive motor-activity deficits. These deficits can be reversed by dopaminergic agents (Blandini & Armentero, 2012). Only minimal levels of neurodegeneration are observed by creating these mutations in animal models, thus making it not particularly useful (Jackson-Lewis *et al.*, 2012).

The second most common autosomal-recessive mutation in PD is mutations in PINK1 (PTEN-induced putative kinase 1) (Dawson *et al.*, 2010). Mild mitochondrial and nigrostriatal neurotransmission deficits as well as increased susceptibility to oxidative stress and the production of ROS can be present in PINK2 knockout mice (Blandini & Armentero, 2012).

The most common autosomal recessive form of PD is caused by parkin mutations. Parkin contributes to the ubiquitin proteasome system where it functions as an E3 ubiquitin ligase. Parkin loses its E3 ubiquitin ligase activity because of disease-causing mutations. Furthermore, dopaminergic oxidative and nitrosative stress in PD may also inactivate parkin. Parkin is useful to understand early abnormalities in the nigrostriatal dopamine system, which occurs due to these mutations (Dawson *et al.*, 2010).

DJ-1 is a redox-sensitive molecular chaperone protein found in the cytoplasm. Mutations causes instability of the dimeric, functional form of the protein, which leads to a decrease in the function of DJ-1. The serine protease activity of DJ-1 is also affected by mutations. Oxidative stress is higher in DJ-1 knockout mice which provides an opportunity to study the contribution of oxidative stress to the development of PD (Blandini & Armentero, 2012).

2.2 Treatment of PD

As mentioned previously, in PD the major abnormality is the loss of striatal dopamine as a result of degeneration of nigrostriatal neurons. Treatment options that are currently available primarily aim to improve the motor impairment of the patient for as long as possible. The progression of the neurodegenerative process is not halted or slowed with the use of current treatments (Singh *et al.*, 2007). Approaches providing symptomatic relief, while postponing the development of motor fluctuations and dyskinesias are the focus in patients with early PD. Treatment that minimise motor or behavioural complications are the focus in patients with more advanced PD (Martin & Wieler, 2003).

Treatment currently available for PD either elevates the levels of dopamine in the brain, or mimics the function of dopamine in the brain. Within a few years of only using a dopamine receptor agonist, levodopa will have to be added to the patient's prescription. Initially low doses are prescribed and over time the doses are titrated up, which is an approach followed to minimise side effects (Singh *et al.*, 2007).

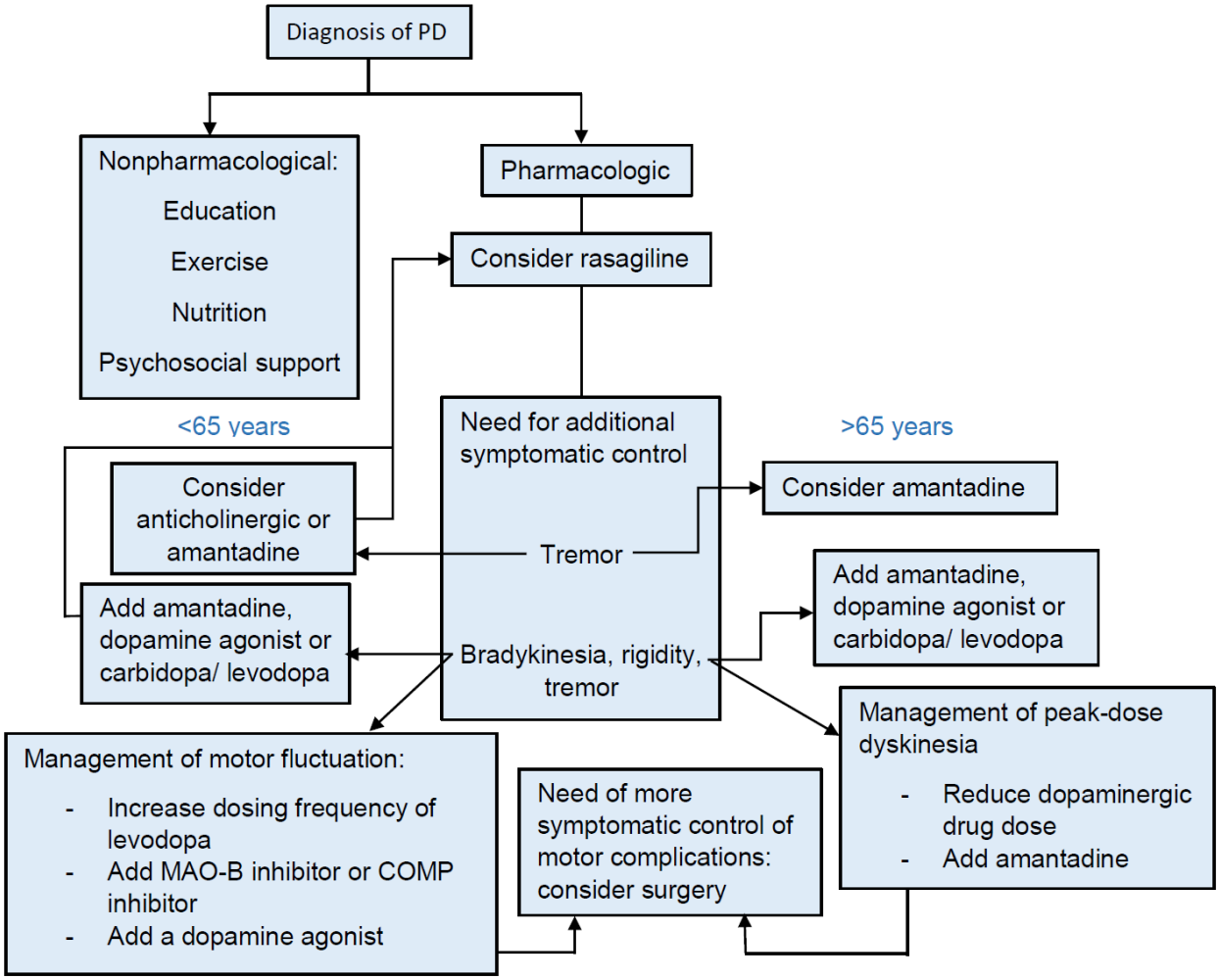


Figure 2.1: **General approach to the management of early to advanced PD (Katzung et al., 2016).**

2.2.1 Levodopa

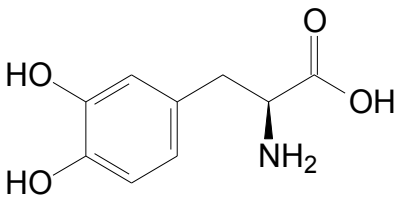
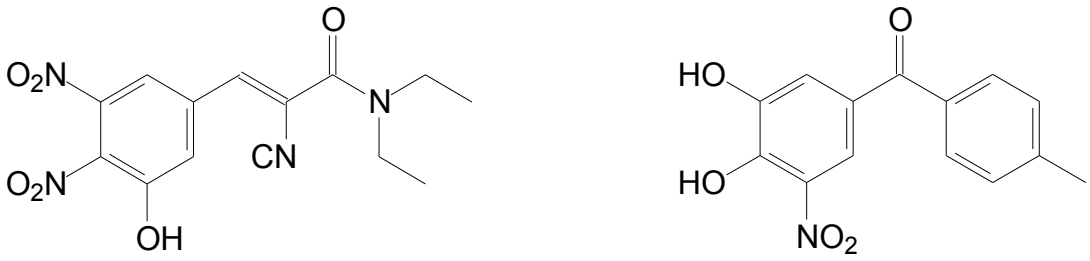


Figure 2.2: **The structure of levodopa.**

Levodopa is the metabolic precursor of dopamine. Levodopa is an amino acid that is transported across the blood-brain barrier into the brain, where it is decarboxylated to form dopamine. Nausea and vomiting may occur from activation of dopamine receptors by the excessive formation of

dopamine (Martin & Wieler, 2003). To increase the amount of levodopa reaching the brain and to lower the possibility of side effects, levodopa is administered in combination with a peripheral dopa-decarboxylase inhibitor, carbidopa or benserazide (Aminoff, 1994). In patients with a history of heart disease, levodopa may cause cardiac arrhythmias. Other side effects of levodopa are depression, insomnia, agitation and anxiety. Levodopa does not affect non-motor symptoms or halt the degeneration of dopaminergic nigral neurons, but it does reduce many of the motor symptoms of PD. The 'on-off' phenomena, wearing off, dose failure, akinesia and dyskinesias are long-term side effects of treatment with levodopa. Dyskinesias can affect speech, swallowing, respiration and balance (Singh *et al.*, 2007). Failure of dose can be due to poor absorption or competition of dietary amino acids with levodopa for transport across the blood-brain barrier. The 'on-off' fluctuations are unpredictable, but may be controlled by using controlled release formulations of levodopa (Hely *et al.*, 2000). According to Fahn *et al.* (2003), levodopa remains the standard drug with which other therapies are compared to, and is highly effective in ameliorating the symptoms of PD.

2.2.2 Catechol-O-methyl transferase (COMT) inhibitors



Entacapone Tolcapone

Figure 2.3: The structures of entacapone and tolcapone.

COMT inhibitors include entacapone and tolcapone. These inhibitors are used in combination with levodopa/carbidopa to prevent the peripheral metabolism of levodopa (Aminoff, 1994). Levodopa's bioavailability and plasma elimination half-life are increased by more or less 50%, when entacapone is used to inhibit its peripheral metabolism. Entacapone does not cause hepatotoxicity, but can cause diarrhoea and urine discoloration (Martin & Wieler, 2003). Other common side effects of the COMT inhibitors include sleep disturbances, orthostatic hypotension, dyskinesias, confusion and insomnia. Tolcapone is effective in controlling motor fluctuations but can potentially cause hepatotoxicity (Singh *et al.*, 2007).

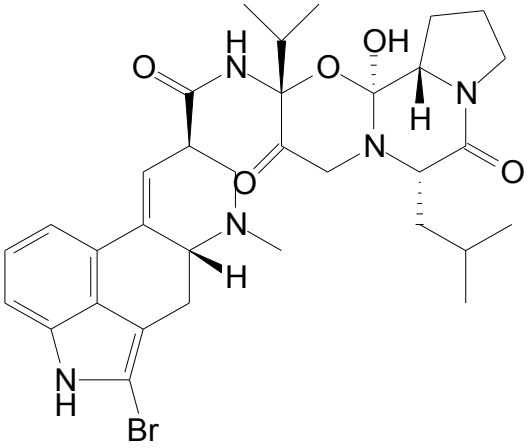
2.2.3 Dopamine agonist

Dopamine agonists stimulate dopamine receptors directly. Dopamine agonists do not have to undergo enzymatic conversions to become an active metabolite. Dopamine agonists does not compete for transport across the blood brain barrier and potentially has no toxic metabolites (Katzung *et al.*, 2016). These agonists can be divided into two groups, the ergot derivatives (bromocriptine, pergolide, lisuride and cabergoline) and the non-ergot derivatives (ropinirole, pramipexole, apomorphine and piribedil) (Singh *et al.*, 2007). Antiparkinsonian effects are experienced with drugs that activate the dopamine 2 (D2) receptors (Martin & Wieler, 2003).

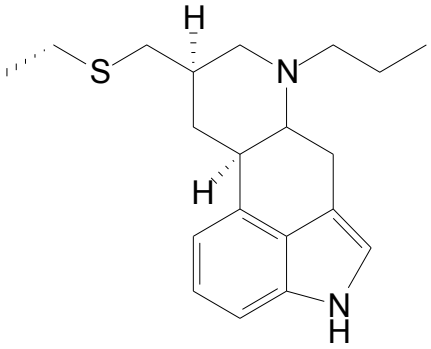
According to Katzung *et al.* (2016), dopamine agonists are associated with a lower incidence of response fluctuations and dyskinesias compared to levodopa. Patients with levodopa associated end-of-dose akinesia or on-off phenomenon may benefit from dopamine agonists (Katzung *et al.*, 2016).

Bromocriptine is a D2 agonist which decreases parkinsonian disability and allows for a reduction of the effective levodopa dosage. Unlike bromocriptine, pergolide is also a D1 agonist as well as D2 agonist. Pergolide increases motor activity and also has a levodopa sparing effect. Pergolide is beneficial in the treatment of patients who do not respond to bromocriptine, especially in patients with advanced PD (Martin & Wieler, 2003). Psychiatric disturbances and cardiovascular problems are potential adverse effects of the ergot derivatives (Singh *et al.*, 2007).

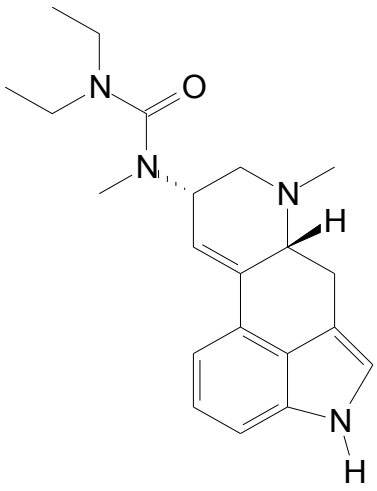
Patients with a history of psychotic illness, an active peptic ulceration or with recent myocardial infarction should avoid dopamine agonists. The ergot-derived agonists should be avoided in patients with peripheral vascular disease (Katzung *et al.*, 2016).



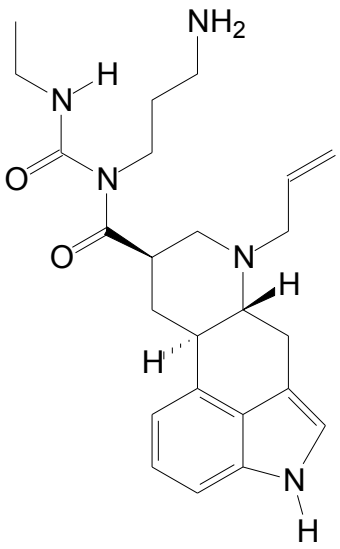
Bromocriptine



Pergolide



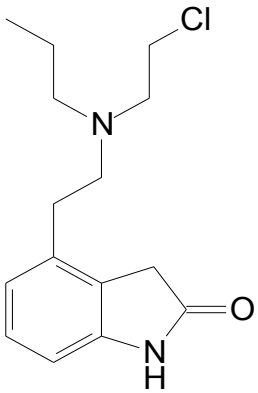
Lisuride



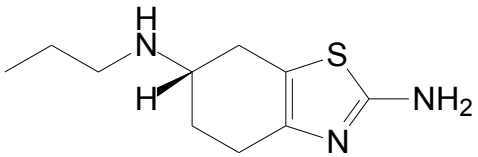
Cabergoline

Figure 2.4: The structures of bromocriptine, pergolide, lisuride and caberdoline (ergot derivatives).

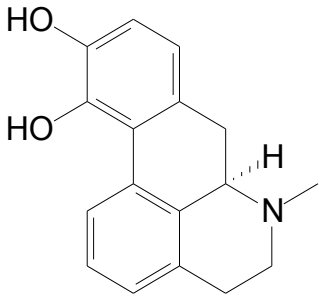
Pramipexole has affinity for the D3 family of receptors and is effective as monotherapy for mild parkinsonism as well as in patients that experience the on-off phenomenon. Pramipexole has a possible neuroprotective effect. Apomorphine, a potent dopamine agonist, is effective in the “off” periods of akinesia (Katzung *et al.*, 2016). Transdermal administration of the dopamine agonists may lead to a more constant response and less fluctuations (Aminoff, 1994).



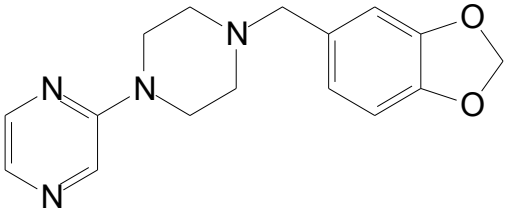
Ropinirole



Pramipexole



Apomorphine



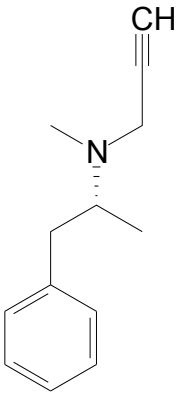
Piribedil

Figure 2.5: The structures of ropinirole, pramipexole, apomorphine and piribedil (non-ergot derivatives).

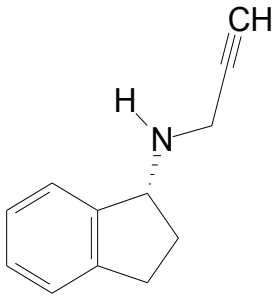
2.2.4 Monoamine oxidase inhibitors

Selegiline is a selective irreversible inhibitor of MAO-B. *In vivo,* MAO-B inhibitors block the oxidative deamination of dopamine. When selegiline is used in combination with levodopa, it enhances the antiparkinsonian effect and allows for a reduction of the dose of levodopa required for an effective therapeutic effect (Singh *et al.*, 2007). According to Katzung *et al.* (2016), selegiline only has a small therapeutic effect in PD when given as monotherapy.

Rasagiline is a more potent MAO-B inhibitor than selegiline and is used to reduce “off” time associated with motor fluctuations in patients with PD. Rasagiline and selegiline may also possess neuroprotective effects in PD (Fernandez & Chen, 2007).



Selegiline



Rasagiline

Figure 2.6: The structures of selegiline and rasagiline.

2.2.5 Amantadine

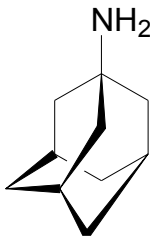


Figure 2.7: The structure of amantadine.

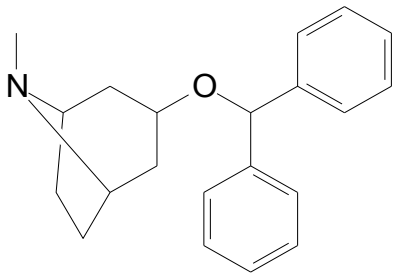
Amantadine is an antiviral agent which has an effect on the synthesis, release and reuptake of dopamine (Katzung *et al.*, 2016). Amantadine has the ability to effectively reduce dyskinesia's (Singh *et al.*, 2007). Adverse effects of amantadine include restlessness, depression, irritability, insomnia, agitation, excitement, hallucinations and confusion. Patients with a history of seizures or heart failure should use amantadine with caution. Headache, heart failure, postural hypotension, urinary retention and gastrointestinal disturbances are also side effects of amantadine (Katzung *et al.*, 2016). Amantadine possesses mild antimuscarinic activity, and enhances dopaminergic transmission (Deleu *et al.*, 2012).

2.2.6 Anticholinergic drugs

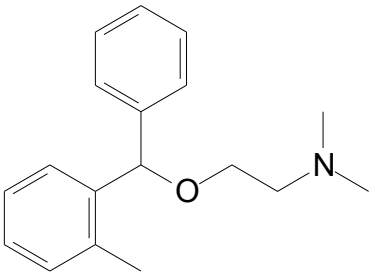
Anticholinergic drugs that are used in PD include benztropine, biperiden, orphenadrine, procyclidine and trihexphenidyl. These drugs are also known as antimuscarinic agents and may improve tremor and rigidity in PD (Katzung *et al.*, 2016).

Benztropine is a potent inhibitor of presynaptic dopamine reuptake and causes a dose-dependent inhibition of reuptake in the nerve terminal area (Deleu *et al.*, 2012). Orphenadrine has antimuscarinic, NMDA antagonistic and antihistaminic properties. Orphenadrine is used to control tremor in PD and has almost no sedative and anticholinergic effects. Procyclidine has minor adverse effects such as dizziness and sedation. According to Deleu *et al*. (2012), the parkinsonian effects of procyclidine wears off after 3 to 5 hours. Biperiden has no effect on akinesia and rigidity (Deleu *et al.*, 2012).

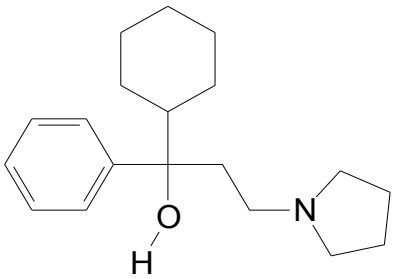
Anticholinergic drugs often display side effects such as confusion, drowsiness, agitation and hallucination. Dementia and effects on memory have also been reported with these drugs (Singh *et al.*, 2007). Anticholinergic drugs should be withdrawn gradually, since these agents may cause acute parkinsonian symptoms if therapy is abruptly terminated (Katzung *et al.*, 2016).



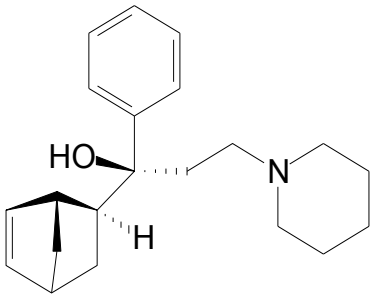
Benztropine



Orphenadrine



Procyclidine



Biperiden

Figure 2.8: Structures of benztropine, orphenadrine, procyclidine and biperiden.

2.3 Monoamine oxidase

2.3.1 Introduction

MAO is a flavin adenine dinucleotide (FAD) containing enzyme found attached to the outer mitochondrial membrane of neuronal, glial and many other cells (Patil & Bari, 2014). MAO metabolises major monoamine neurotransmitters such as serotonin (5-HT), nor-adrenaline and dopamine (Khattab *et al.*, 2015). Besides terminating the functions of these neurotransmitters, MAO also functions as a metabolic barrier in the micro-vessels of the gut wall to limit the entry of sympathomimetic amines into the systemic circulation (Legoabe *et al.*, 2012).

MAO consists of two isoenzymes, MAO-A and MAO-B. MAO-A oxidises 5-HT and norepinephrine, and MAO-B preferentially oxidises phenylethylamine (PEA), with dopamine and tyramine serving as substrates for both isoenzymes (Billett, 2004). MAO inhibitors (MAOI) are often used in the treatment of neurodegenerative and neurological disorders (Patil & Bari, 2014). MAO-A inhibitors are used as antidepressants and anxiolytics, and MAO-B inhibitors are used for the treatment of PD and AD (Khattab *et al.*, 2015). The main roles of the MAO enzymes are the metabolism of exogenous amines and the regulation of neurotransmitter levels and intracellular amine stores (Billett, 2004).

2.3.2 General background

In 1928, Mary Hare characterised the enzyme activity responsible for the oxidation of tyramine (Ramsay, 2013). Hugh Blaschko realised that tyramine oxidase, noradrenaline oxidase and aliphatic amine oxidase were all the same enzyme, which is capable of metabolising primary, secondary and tertiary amines. Zeller named this enzyme mitochondrial MAO. Diamines are not metabolised by MAO (Youdim & Bakhle, 2006).

Isoniazid, an anti-tuberculosis drug, was the first drug discovered to inhibit MAO. The first MAO inhibitor, iproniazid was used in the treatment of depressive illness. Remarkable antidepressant action was demonstrated by iproniazid in the late 1960's, but this drug exhibited side effects such as liver toxicity and the cheese reaction (Youdim & Bakhle, 2006). Tyramine, an amine found in various foods, induces the cheese reaction. Normally dietary tyramine is metabolised by MAO-A in the gut wall and liver, but when MAO-A is inhibited, this protective system is inactivated and tyramine and other monoamines present in ingested food are not normally metabolised. Tyramine enters the systemic circulation and induces the release of noradrenaline from peripheral adrenergic neurons, which may lead to a severe hypertensive response that could be, in some cases, fatal (Youdim & Bakhle, 2006). Since MAO-B is absent from the gut wall, MAO-B inhibitors do not cause a cheese reaction. This suggests that MAO-B inhibitors may be developed for the treatment of depression and other neuropsychiatric diseases, since such inhibitors would exhibit less adverse effects than MAO-A inhibitors, most notably the cheese reaction (Youdim & Bakhle, 2006).

2.3.3 Localization and tissue distribution

In the 1960's the discovery was made that MAO is not a single enzyme but exists in at least two forms. MAO-B is resistant to clorgyline inhibition and prefers benzylamine as a substrate, whereas MAO-A is inhibited by clorgyline (Youdim & Bakhle, 2006). According to Youdim & Bakhle (2006) these isoforms are differently distributed in the mammalian brain. In the brain, high concentrations of MAO-A is located in the locus coeruleus, while the highest concentrations of MAO-B is found in the raphe nuclei (Shih *et al.*, 1999).

As mentioned, MAO-A and MAO-B are associated with the mitochondrial outer membrane. MAO-A appears before MAO-B during development, with the level of the latter increasing after birth. At birth, MAO-A is almost at adult level while MAO-B activity increases with aging (Shih *et al.*, 1999).

MAO-A is present in the placenta and skin fibroblasts, and MAO-B is found in platelets and blood lymphocytes. MAO activity is absent from erythrocytes (Billett, 2004). Interestingly, MAO-A is found in adrenergic, noradrenergic and dopaminergic neurons, and MAO-B is found in serotonergic and histaminergic neurons, and also in astrocytes and ependymal cells (Nicotra *et*

al., 2004). In these regions the roles of MAO are not only to terminate the function of neurotransmitters, but also to protect neurons from stimulation by extraneous amines that can act as false neurotransmitters (Shih *et al.*, 1999). MAO-A activity is present in the following human peripheral tissues: placenta and liver, kidney and the adrenal gland, heart, lung and intestines. MAO-B activity is lower in all tissues, except skeletal muscle where MAO-B activity is the same as that of MAO-A (Billett, 2004). The main MAO isoform found in the basal ganglia is MAO-B (Youdim & Bakhle, 2006).

2.3.4 The role of MAO in neurodegeneration

In the human brain, MAO-B activity and density increase with age. The loss of dopaminergic neurons in the SN is associated with increased oxidation of dopamine by MAO. Since the catalytic activity of MAO-B generates ROS, the oxidation of dopamine by MAO-B in the basal ganglia may contribute to neurodegeneration in PD. For each mole of dopamine metabolised by MAO-B, one mole of H_2O_2 is generated. Although H_2O_2 is not considered harmful, it may react in the Fenton reaction with ferrous iron (Fe^{2+}) to yield the highly reactive hydroxyl radical, a molecule that reacts with and damages all types of biomolecules. The hydroxyl radical derived from MAO activity may thus mediate neurodegeneration in PD. In support of this, elevated MAO-B activity in the SN has been seen in patients with PD (Youdim *et al.*, 2005). Patients with PD thus may exhibit increased oxidation of dopamine by MAO-B, which may produce higher levels of oxygen radicals that trigger oxidative damage of nigrostriatal neurons (Shih *et al.*, 1999).

Also of interest is the observation that MAO-B is the catalyst responsible for the activation of MPTP to yield the neurotoxic metabolite, MPP^+ (Gerlach *et al.*, 1996). Neuronal damage in PD is similar to neurodegeneration induced by MPTP, thus suggesting that MAO-B may activate an environmental neurotoxin similar to MPTP to cause idiopathic PD. No evidence for the existence of such an environmental toxin has however been found (Shih *et al.*, 1999).

2.3.5 Known inhibitors of MAO

2.3.5.1 Irreversible inhibitors of MAO-B

An increase in striatal dopamine can be seen with the inhibition of MAO-B (Yamada & Yasuhara, 2004). Selegiline and rasagiline are both selective MAO-B inhibitors and used for the symptomatic treatment in early PD. These inhibitors enhance motor function and activities of daily living, and reduces general parkinsonian disability (Löhle & Storch, 2012).

Selegiline is a propargyl amphetamine derivative that undergoes extensive first-past hepatic metabolism. Amphetamine metabolites from selegiline are potentially neurotoxic and are associated with cardiovascular and psychiatric effects (Fernandez & Chen, 2007). Inhibition of

the oxidative deamination of dopamine, PEA and benzylamine takes place at a low dose of this compound (Youdim & Bakhle, 2006). Selegiline does not induce the cheese reaction when taken in combination with tyramine containing food (Montastruc *et al.*, 2000). As mentioned, the absence of the cheese effect is due to the absence of MAO-B in the intestine as well as the fact that tyramine is metabolised by intestinal MAO-A (Youdim *et al.*, 2006). Pathological evidence of oxidative stress in the SNpc of PD patients indicates that selegiline may be neuroprotective in PD (Mytilineou *et al.*, 1997). Since the catalytic cycle of MAO generates H_2O_2 as by-product, the inhibition of MAO-B by selegiline reduces the formation of potentially harmful oxygen radicals in the brain. Adverse effects of selegiline include anorexia and nausea, dry mouth, dyskinesia, orthostatic hypotension, musculoskeletal injuries and cardiac arrhythmias (Yamada & Yasuhara, 2004).

Rasagiline is a non-amphetamine aminoindan derivative. It has been reported that rasagiline, in animal models, is a more potent inhibitor of MAO-B in the brain compared to selegiline (Fernandez & Chen, 2007). Rasagiline enhances dopamine levels in microdialysate following L-dopa treatment (Youdim & Bakhle, 2006). Rasagiline also has neuroprotective effects against neurotoxins and serum-starved cell cultures. The apoptotic cell death initiated by the mitochondria is abolished by rasagiline (Mandel *et al.*, 2005). Amphetamine-like side effects are absent with rasagiline, because unlike selegiline, this compound is not metabolised to amphetamine derivatives (Chen & Swope, 2005).

Ladostigil, also a cholinesterase inhibitor, selectively inhibits MAO-A and MAO-B in the brain and displays an irreversible mode of inhibition (Sagi *et al.*, 2005). Since ladostigil is a cholinesterase inhibitor, memory impairment induced by scopolamine in rats can be improved with ladostigil. Central levels of dopamine, noradrenaline and 5-HT are also increased by ladostigil (Mandel *et al.*, 2005). Nigrostriatal neurodegeneration and dopamine depletion induced by the neurotoxin, MPTP, are prevented by ladostigil. By inhibiting MAO ladostigil increases brain dopamine in MPTP-treated mice. In cultures of neuronal cells, ladostigil has shown both neuroprotective and anti-apoptotic activities (Youdim & Bakhle, 2006).

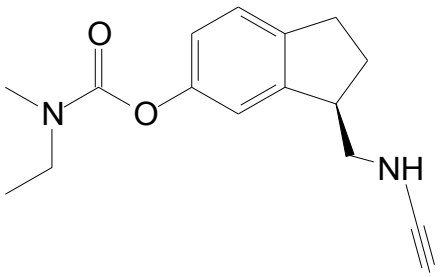


Figure 2.9: The structure of ladostigil.

2.3.5.2 Reversible inhibitors of MAO-B

Lazabemide is an example of a reversible MAO inhibitor (Kieburtz, 1993). Lazabemide protects against nigrostriatal neurodegeneration and dopamine depletion induced by the neurotoxin, MPTP, by inhibiting the MAO-B-catalysed activation of MPTP. It has been suggested that lazabemide may be used to treat therapy resistant depression, in combination with a reversible MAO-A inhibitor (Youdim & Bakhle, 2006). Unfortunately the development of lazabemide has been discontinued due to liver toxicity (Berlin *et al.*, 2002).

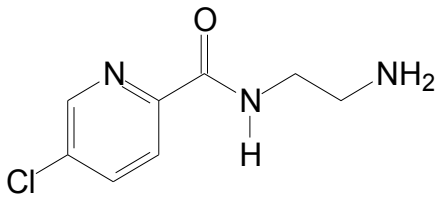


Figure 2.10: The structure of lazabemide.

Safinamide is an α-aminoamide derivative that inhibits MAO-B and reduces dopamine reuptake (Fernandez & Chen, 2007). Safinamide thus has multiple mechanisms of action that are relevant to PD and displays promising symptomatic and neuroprotective effects. Safinamide provides improvement in cognition and neuroprotection by blocking voltage-dependent sodium channels, modulating calcium channels and inhibiting glutamate release induced by abnormal neuronal activity (Onofrj *et al.*, 2008).

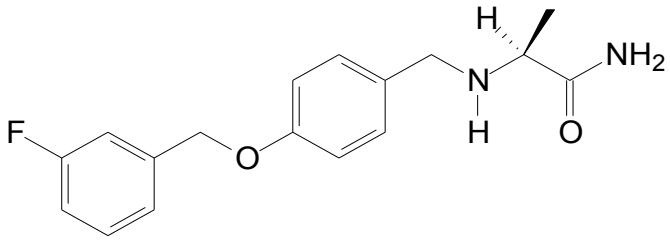


Figure 2.11: The structure of safinamide.

2.3.5.3 Irreversible inhibitors of MAO-A

Clorgyline is an irreversible MAO-A selective inhibitor that has antidepressant effects (Lotufo-Neto *et al.*, 1999). By the inhibition of MAO-A, clorgyline increases the levels of noradrenaline and 5-HT in the brain, which is responsible for the antidepressant activity of this compound. Clorgyline does not affect dopamine levels in the brain tissue of rats. Since clorgyline is an

irreversible MAO-A inhibitor, its major disadvantage is that it precipitates the cheese reaction (Youdim & Bakhle, 2006).

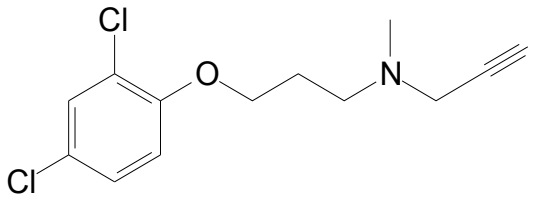
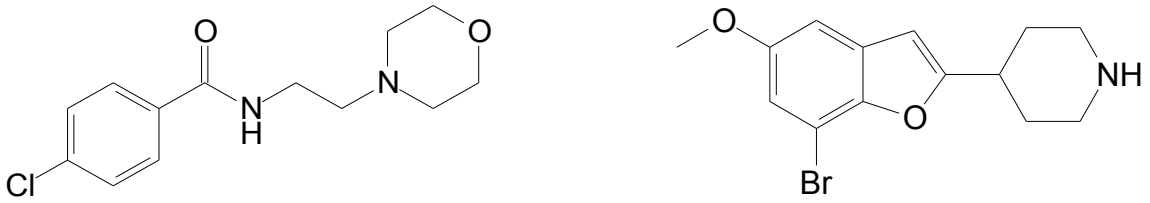


Figure 2.12: The structure of clorgyline.

2.3.5.4 Reversible inhibitors of MAO-A



Moclobemide Brofaromine

Figure 2.13: The structures of moclobemide and brofaromine.

As mentioned, MAO-A is located in the brain, small intestine, liver, portal system and peripheral adrenergic neurons (Lotufo-Neto *et al.*, 1999). MAO-A metabolises both intraneuronal and released dopamine (Youdim & Bakhle, 2006). Moclobemide and brofaromine are reversible inhibitors of MAO-A. Moclobemide is used as an antidepressant and is generally not associated with hypertensive crises (Lotufo-Neto *et al.*, 1999). Reversible inhibitors of MAO-A do not cause the cheese effect because the reversible mode of inhibition allows competition. Thus, as tyramine concentrations increases with MAO-A inhibition, the reversible inhibitor may be displaced from the enzyme by tyramine to allow for normal metabolism in the gut and liver (Youdim *et al.*, 2006). Adverse effects of moclobemide include sleep disturbances, anxiety, restlessness and headache (Yamada & Yasuhara, 2004).

Besides acting as a MAO-A inhibitor, brofaromine also inhibits 5-HT reuptake, which is the most notable difference between brofaromine and moclobemide. This pharmacodynamic effect can improve the therapeutic potency of brofaromine, but increases the risk of 5-HT toxicity (Lotufo-Neto *et al.*, 1999).

2.3.5.5 Irreversible inhibitors of both MAO-A and MAO-B

Tranylcypromine Phenelzine

Figure 2.14: The structures of tranylcypromine and phenelzine.

Tranylcypromine and phenelzine are irreversible non-selective MAO-A and MAO-B inhibitors. By inhibition of MAO-A, both compounds displayed antidepressant activity and are thus used clinically as antidepressants. Since these drugs are irreversible MAO-A inhibitors, they may cause the cheese reaction and are also associated with orthostatic hypotension. For these reasons, tranylcypromine and phenelzine are used as third and fourth-line treatments in depression (Lotufo-Neto *et al.*, 1999).

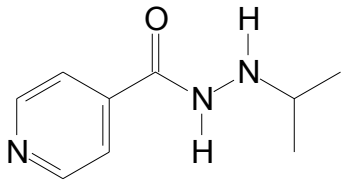


Figure 2.15: The structure of iproniazid.

Iproniazid was the first MAO inhibitor to be used as an antidepressant (Youdim *et al.*, 2006). Unfortunately, iproniazid exhibits adverse effects such as liver toxicity and also causes the cheese reaction. The observation that the hydrazine structure of iproniazid is responsible for liver toxicity led to the development of alternative MAO inhibitors such as non-hydrazine MAO inhibitors (Youdim & Bakhle, 2006).

2.3.6 The three-dimensional structures of MAO-B

The crystal structures of the MAOs are used to understand the binding of inhibitors and substrates to the active sites of the enzymes. Although MAO-A and MAO-B are similar in amino acid sequence and three-dimensional structure, their substrate and inhibitor specificities are different. The shapes of the MAO-A and MAO-B active sites are noticeably different and responsible for differing substrate and inhibitor specificities. MAO-B has a more restricted cavity with an obvious

constriction defined by Ile199 and Tyr326 (Ramsay, 2013). The crystal structure indicates that the MAO-B enzyme is dimeric [**Fig. 2.16 (B)**] (Binda *et al.*, 2002).

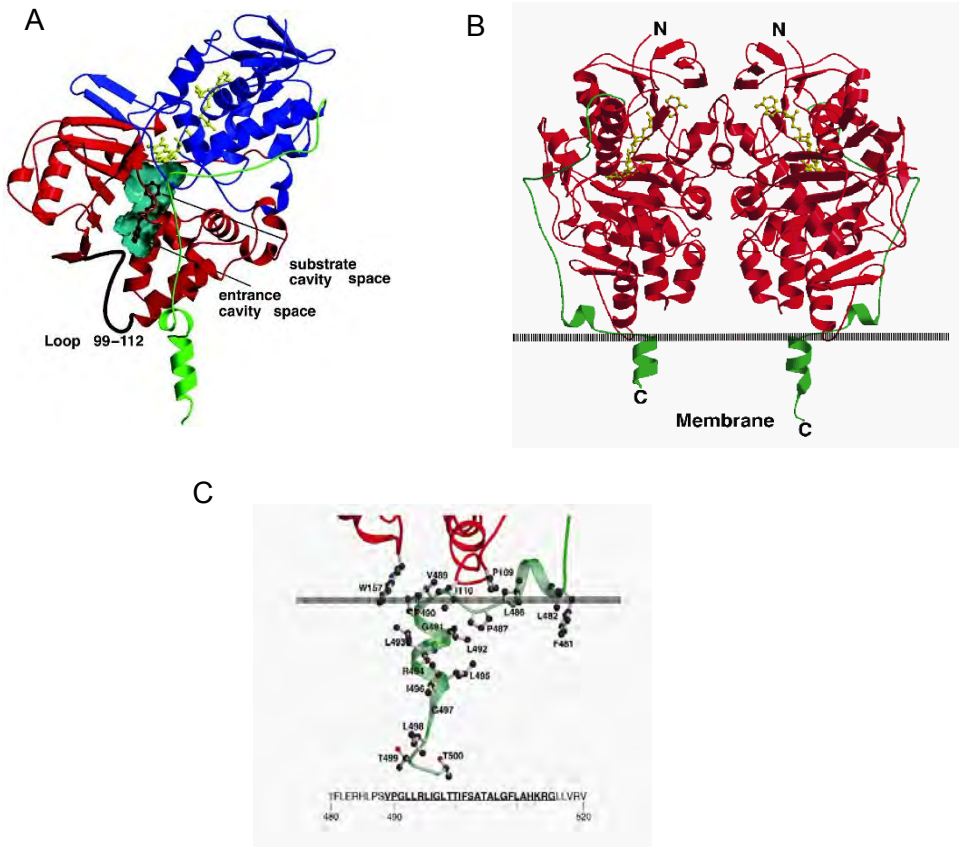


Figure 2.16: **The three-dimensional structure of human MAO-B. (A) The FAD-binding area is in blue, the substrate-binding is shown in red and the C-terminal membrane-binding region is shown in green. The inhibitor is coloured black and the FAD-cofactor is shown in yellow (Binda *et al.*, 2003). (B) The ribbon diagram of the MAO-B dimer, with monomer A on the right and monomer B on the left. (C) Close view of the binding region in monomer A. The C-terminal tail is shown in green [These structures are repeated with permission from (Binda *et al.*, 2002)].**

2.3.6.1 Structure of the membrane binding region

MAO-B is bound to the outer mitochondrial membrane. The region of MAO-B that is attached to the membrane is the C-terminal amino acids 461-520. The C-terminal residues form an extended polypeptide chain that crosses the monomer surface, terminating in an α-helix that commences at Val489. This leads to the formation of a transmembrane helical segment [**Fig. 2.16 (B&C)**]. Arg494 located in the second helix turn that undergoes electrostatic interaction with the anionic

phospholipid head groups. This is shown in **figure 2.16 (C)**. The structure in **figure 2.16 (C)** also shows that other protein regions can also be involved in membrane binding. Pro109 and Ile110 are surface-exposed and this may interact with the membrane [**Fig. 2.16 (A)**] (Binda *et al.*, 2002).

2.3.6.2 The active site

A flat cavity with a volume of 700 Å^3 forms the active site of MAO-B. This cavity is lined by numerous aromatic and aliphatic amino acids. The active site consists of two cavities, the entrance cavity and the substrate cavity. These two cavities form an access channel to the FAD, located deeper in the enzyme (De Colibus *et al.*, 2005). The substrate cavity has a volume of 420 Å^3. The entrance cavity has a volume of 290 Å^3 and is lined by residues Phe103, Pro104, Trp119, Leu164, Leu167, Phe168, Leu171, Ile199, Ile316 and Tyr326. This cavity is protected from solvent by loop 99-112. The two cavities are separated by the side chain of residues Tyr326, Ile199, Leu171 and Phe168 (Binda *et al.*, 2002). Ile199 functions as a gate to separate or fuse the two cavities (Binda *et al.*, 2004). Interestingly, movement of loop 99-112 has to occur to open access to the entrance cavity (Binda *et al.*, 2002).

2.3.6.3 The three-dimensional structure of MAO-A

Differences in substrate and inhibitor specificities can be seen between MAO-A and MAO-B. Differences in the active site cavities of MAO-A and MAO-B are responsible for these differing specificities. In the MAO-A active site, Ile180, Asn181, Phe208 and Ile335 determine substrate and inhibitor specificity while in MAO-B residues Leu171, Cys172, Ile199 and Tyr326 determine substrate and inhibitor specificity. The active site of MAO-A consists of a larger single cavity, which has the ability to accommodate larger substrates and inhibitors (Binda *et al.*, 2002).

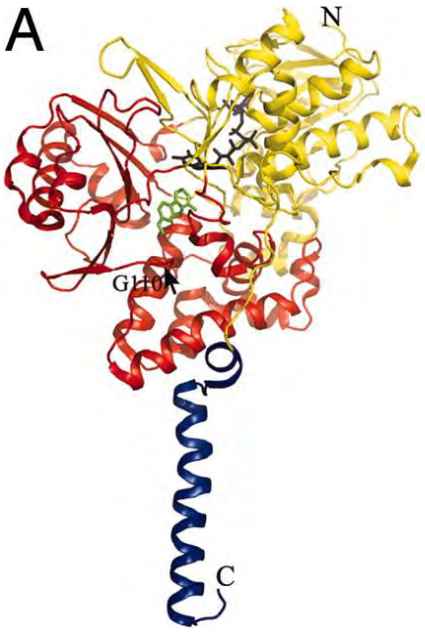


Figure 2.17: **The structure of human MAO-A. The structure is divided into domains. The yellow and red shows the extra-membrane domains, and the membrane binding domain is shown in blue. The FAD binding region (yellow) and substrate/inhibitor binding domain (red) are also shown. The stick models represent the FAD (black) and harmine (green). G110 is indicated with the black arrow [This structure is duplicated with the permission from (Son et al., 2008)].**

MAO-A crystallises as a monomer, while MAO-B crystallises as a dimer. As mentioned, in contrast to MAO-B, MAO-A has a single substrate binding cavity (Edmondson *et al.*, 2007). Human MAO-A has a cavity volume of 550 Å^3 and rat MAO-A has a cavity value of 450 Å^3 (Ramsay, 2013). In general, the substrate binding site of MAO-A is hydrophobic (Edmondson *et al.*, 2007).

When the side chain of Ileu199 is in the open configuration, the active site of MAO-B has a combined volume of 700 Å^3. MAO-B has a bipartite elongated cavity while MAO-A has a single, rounder shaped cavity, which has a larger volume for substrate binding than MAO-B. Substrates have limited ability for rotation in MAO-B compared to MAO-A. In MAO-A the cavity shaping loop is in a more extended shape, where in MAO-B it is in a more compact shape (Edmondson *et al.*, 2007). Human G110 is an important residue in MAO-A since it allows for flexibility of loop 108-118. This flexibility, in turn, allows the substrate to access the human MAO-A active site (Son et al., 2008). The active site cavities of human MAO-A and human MAO-B are compared in **figure 2.18**, and also shows the FAD at the end of a long tunnel from the surface of the protein (Ramsay, 2013).

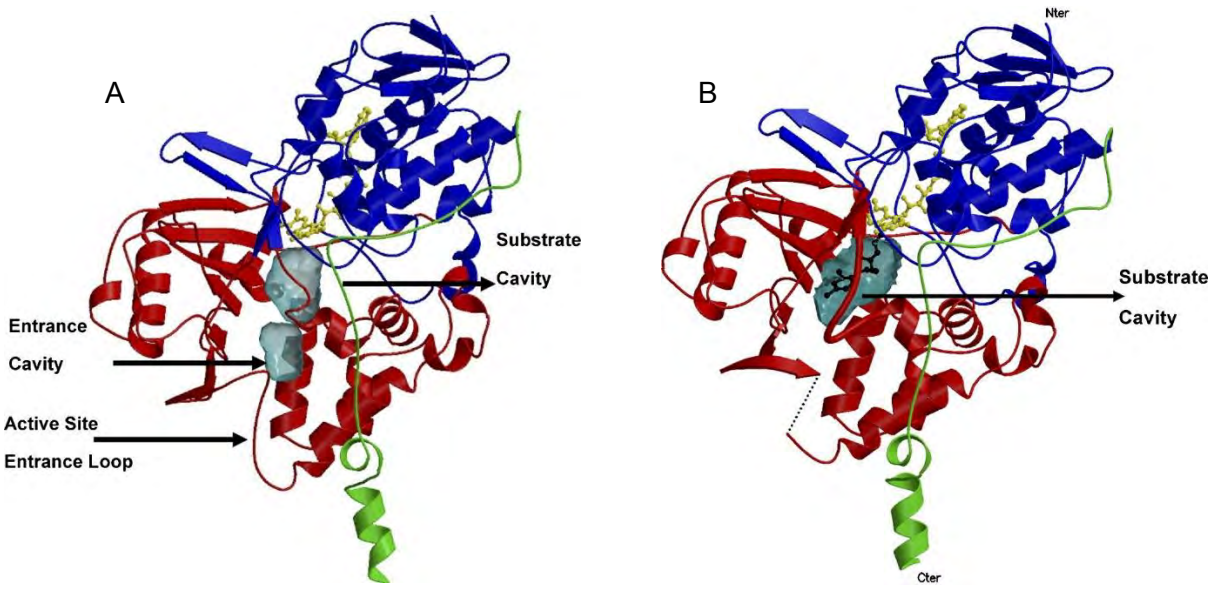


Figure 2.18: **MAO-A and MAO-B shown in ribbon form. (A) MAO-B with the covalently bound FAD shown in yellow and the flavin binding domain in blue. The red indicates the substrate domain and the green is the membrane binding domain. (B) MAO-A with the covalently bound FAD is in yellow and the covalently bound inhibitor in black. The blue indicates the flavin binding domain and the red is the substrate domain. The membrane binding domain is green. [The structures are reproduced with permission from (Edmondson *et al.*, 2007)].**

2.3.6.4 The catalytic cycle of MAO-B

MAO catalyses the oxidation of numerous primary, secondary and tertiary amines (Silverman, 1995). They also catalyse the oxidation of dietary arylalkylamines (Milczek *et al.*, 2008). In the MAO catalytic reaction, amine substrates are oxidised to yield the analogous imines. These imines are released from the enzyme and hydrolysed to the corresponding aldehydes. With the oxidation of a substrate, the FAD is reduced. To complete the cycle, the FAD is re-oxidised by molecular oxygen to yield H_2O_2 (Silverman, 1995).

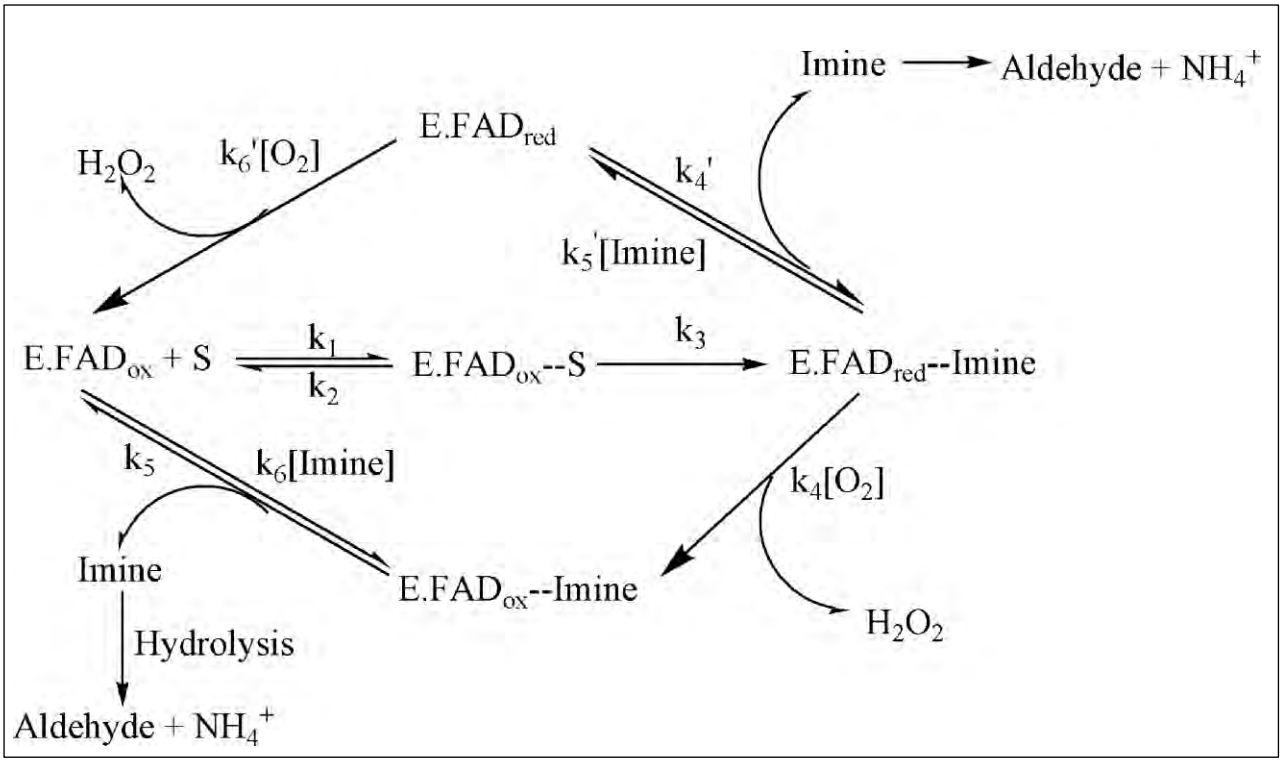
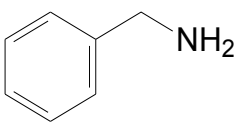
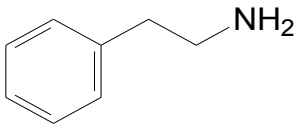


Figure 2.19: The reaction pathway for MAO catalysis [This structure is reproduced with permission from (Edmondson *et al.*, 2007)].

To form the imine and reduce FAD, respectively, the Cα-H bond of the amine substrate is cleaved with transfer of two electrons to the flavin, as seen in **figure 2.19** (Edmondson *et al.*, 2007). Benzylamine and its analogues follow the lower branch of the catalytic pathway in **figure 2.19.** PEA follows the upper branch because the imine product dissociates from the reduced enzyme (Ramsay, 1991). In MAO-A the substrate catalysis follows the lower branch of the catalytic pathway (Edmondson *et al.*, 2007).



Benzylamine



Phenethylamine

Figure 2.20: Structures of benzylamine and phenethylamine.

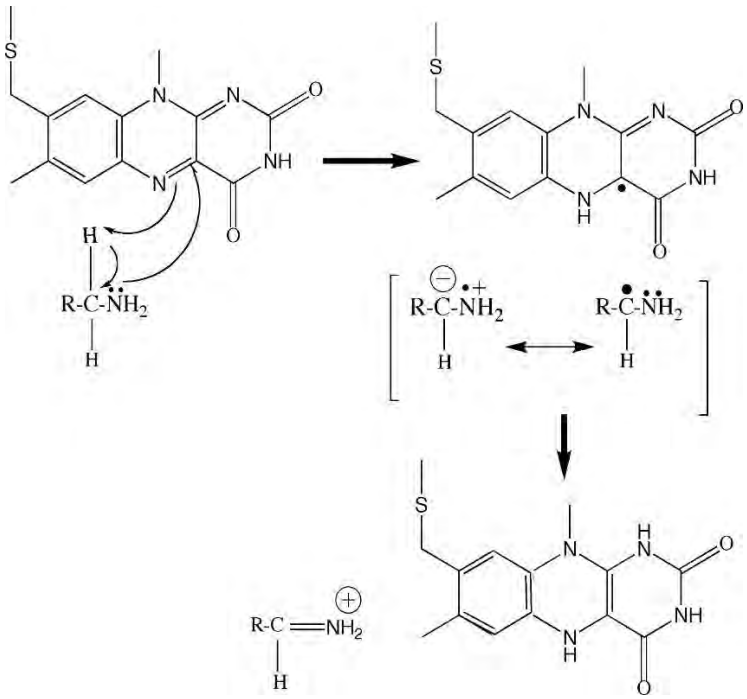


Figure 2.21: The SET mechanism of MAO catalysis (Edmondson *et al.*, 2007).

To explain substrate oxidation,two mechanisms are proposed, the single electron transfer (SET) mechanism and the polar-nucleophilic mechanism. In the SET, the first step is the one-electron oxidation to form an aminium cation radical as well as a flavin radical (Miller *et al.*, 1995). With the transfer of a second electron from the substrate to the FAD, the α-proton is abstracted by a basic residue, with the formation of the imine product and two-electron reduced FAD (**Fig. 2.21**) (Edmondson *et al.*, 2007).

During the polar-nucleophilic mechanism, the substrate amine nucleophilically attacks the FAD at the C4a position to yield a transient adduct. In the adduct, the N5 position of the FAD becomes a strong base and abstracts the α-proton from the substrate. In a concerted reaction the substrate is released to form the iminium product and reduced flavin (**Fig. 2.22**). This mechanism is more likely than the SET since an active site base is not required for proton abstraction from the α-carbon of the substrate (Edmondson *et al.*, 2007). Stuctural evidence shows that no active site base in MAO is present that could perform this function.

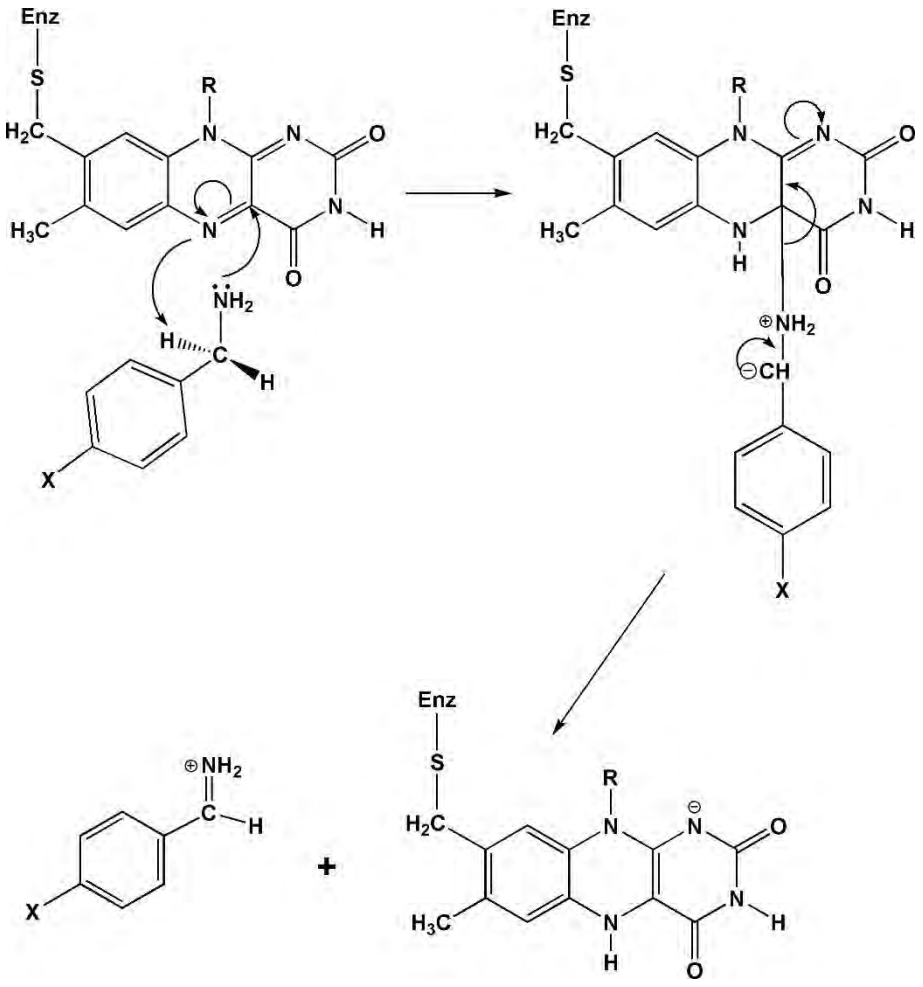


Figure 2.22: The polar nucleophilic mechanism proposed for MAO catalysis (Edmondson *et al.*, 2007).

2.4 Conclusion

This chapter provides a discussion of PD, a neurodegenerative disorder. The symptomatic treatment of PD was provided with emphasis on MAO-B inhibitors. MAO-B inhibitors are of much interest in the treatment of PD since they not only provide symptomatic relief, but also reduces the formation of potentially harmful metabolic by-products of MAO catalysis. These by-products, notably H_2O_2, may lead to oxidative damage and further neurodegeneration in PD. This suggests that the discovery of a new generation of MAO inhibitors may potentially be valuable for the future treatment of neurodegenerative disease.

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CHAPTER 3

ARTICLE

The monoamine oxidase inhibition properties of C6- and N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives

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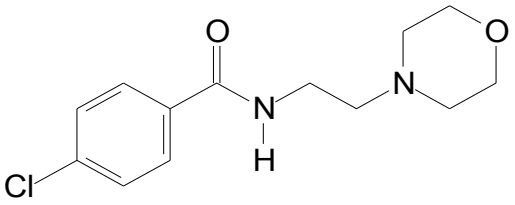
ABSTRACT

Quinazolinone compounds are of interest in medicinal chemistry since they display a wide range of biological properties. In the present study, a series of C6- and N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives were synthesised and evaluated as inhibitors of recombinant human monoamine oxidase (MAO). Some of these quinazolinones are structurally related to a series of 3,4-dihydro-2(1*H*)-quinolinone derivatives, which has previously been reported to act as specific inhibitors of MAO-B. The results document that, among 37 compounds synthesised, 7 displayed IC_{50} values < 1 μM for the inhibition of MAO-B. The most potent MAO-A inhibitor exhibit an IC_{50} value of 7.43 μM while the most potent MAO-B inhibitor possesses an IC_{50} value of 0.269 μM. Good potency MAO inhibition was only observed among C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives with N1-substitution yielding comparatively low potency inhibition. MAO-B specific inhibitors such as some of the quinazolinone compounds investigated here may act as leads for the design of therapies for neurodegenerative disorders such as Parkinson's disease (PD).

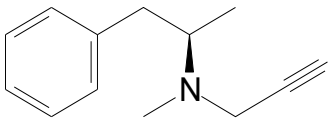
3.1 Introduction

The MAO enzymes are flavin adenine dinucleotide (FAD) containing enzymes that catalyse the oxidation of neurotransmitter and dietary amines in both the peripheral and central tissues (Shih *et al.*, 1999). Two isoforms of MAO are expressed, MAO-A and MAO-B, which are products of distinct genes. Although MAO-A and MAO-B share a high degree of sequence and structural similarity, they exhibit different substrate and inhibitor specificities. MAO-A metabolises serotonin and is specifically inhibited by the propargylamine compound clorgyline, while MAO-B metabolises the arylalkylamines, benzylamine and 2-phenethylamine, and is specifically inhibited

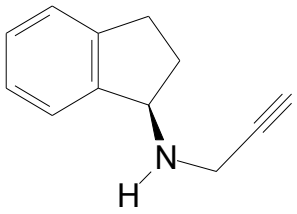
by selegiline (Shih *et al.*, 1999) (**Fig. 3.1**). Certain substrates such as dopamine, epinephrine, tyramine and norepinephrine are oxidised by both MAO isoforms, while a number of inhibitors (e.g. tranylcypromine) also do not display isoform specificity (Harfenist *et al.*, 1996). Because of their involvement in the catabolism of neurotransmitter amines, MAO-A and MAO-B are considered targets for the treatment of neurological disorders (Yamada & Yasuhara, 2004). In this respect, MAO-A inhibitors are used in the treatment of depression and anxiety, while MAO-B inhibitors are often combined with L-dopa in the treatment of PD (Yamada & Yasuhara, 2004). In PD, MAO-B inhibitors block the MAO-B-catalysed metabolism of dopamine in the brain and thus enhance dopaminergic neurotransmission. In combination with L-dopa, MAO-B inhibitors may enhance dopamine levels derived from the conversion of L-dopa to dopamine and may thus reduce the dose of L-dopa required for a therapeutic effect (Fernandez & Chen, 2007). MAO-B inhibitors may also protect against neuronal injury and neurodegeneration in PD by reducing oxidative damage mediated by hydrogen peroxide (H_2O_2), a by-product of MAO catalysis (Kaludercic *et al.*, 2014). Currently, two irreversible acting propargylamine compounds, selegiline and rasagiline, are clinically used for the management of PD, while a reversible inhibitor, safinamide, has recently been approved. MAO-A inhibitors that are used in the clinic for the treatment of depression include tranylcypromine, phenelzine, moclobemide, toloxatone and brofaromine. It should be cautioned that reversibility of MAO-A inhibition is an important consideration since irreversible inhibitors such as tranylcypromine and phenelzine can cause a severe hypertensive crisis when combined with food that contains the sympathomimetic amine, tyramine (Youdim & Weinstock, 2004). With the irreversible inhibition of MAO-A in the gut and vascular endothelium, dietary tyramine gains access to the circulatory system, which leads to the release of norepinephrine from the sympathetic neurons and subsequently a hypertensive reaction, which in some cases can be fatal (Youdim & Weinstock, 2004). This led to the development of reversible MAO-A inhibitors such as moclobemide and toloxatone, which are not associated with the hypertensive reaction (Finberg & Tenne, 1982). MAO-B specific inhibitors also do not cause tyramine-induced hypertension since MAO-B is absent from the gut tissues (Youdim & Weinstock, 2004).



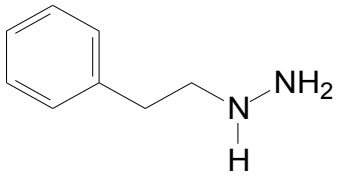
Moclobemide



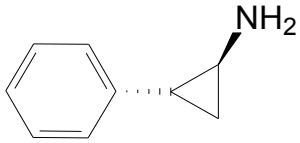
Selegiline



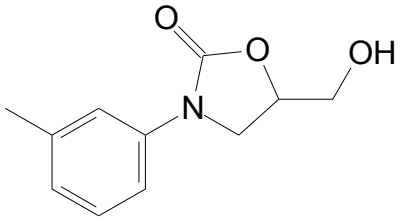
Rasagiline



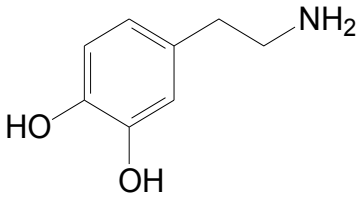
Phenelzine



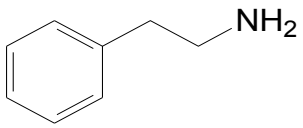
Tranylcypromine



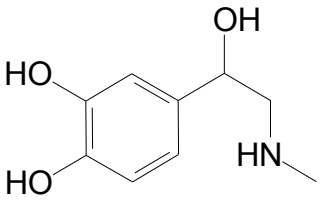
Toloxatone



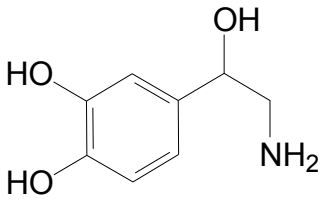
Dopamine



2-phenylethylamine



Epinephrine

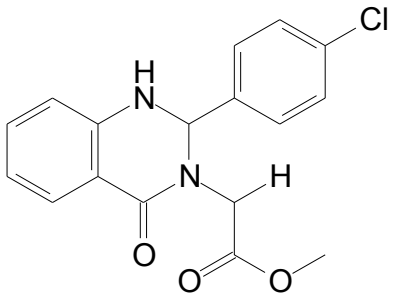


Norepinephrine

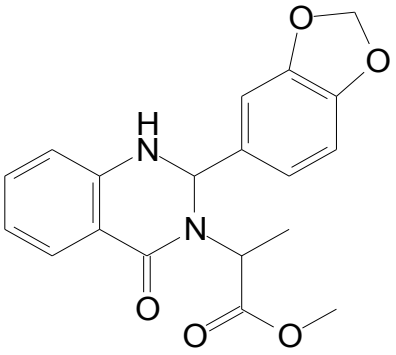
Figure 3.1: The structures of known MAO substrates and inhibitors.

Because of their biochemical and clinical significance, the discovery and development of MAO inhibitors are being actively pursued (Youdim *et al.*, 2006). Quinazolinone is a heterocyclic nitrogen containing system composed of fused phenyl and pyrimidine rings. Over the years natural and synthetic quinazolinones have attracted attention since they display a variety of biological activities (Chen *et al.*, 2006; Mhaske & Argade, 2006; Kshirsagar, 2015; Jafari *et al.*, 2016) including MAO inhibition (Gökhan-Kelekçi *et al.*, 2009). Other biological activities include antimicrobial, anticonvulsant, anticancer, antimalarial, antihypertensive, anti-inflammatory,

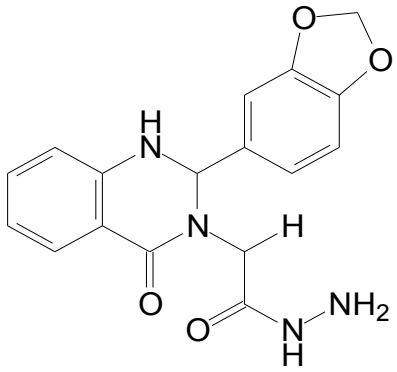
antidiabetic, antitumor, anti-cholinesterase, dihydrofolate reductase inhibition, inhibition of cellular phosphorylation and kinase inhibitory activities (Khan *et al.*, 2016). The MAO inhibitory properties of quinazolinone derivatives have also been reported (Gökhan-Kelekçi *et al.*, 2009; Khattab *et al.*, 2015). For example, Khattab and co-workers synthesised a series of 4(3*H*)-quinazolinone amino acid ester and 4(3*H*)-quinazolinone amino acid hydrazide derivatives, and evaluated them as potential inhibitors of MAO-A and MAO-B. Compounds **1** (IC_{50} = 3.6 nM), **2** (IC_{50} = 2.8 nM) and **3** (IC_{50} = 2.1 nM) were found to be most active inhibitors and displayed MAO-A inhibition that was comparable to the reference inhibitor, clorgyline (Khattab *et al.*, 2015). Quinazolinones that have been studied as MAO inhibitors are from the 4(3*H*)-quinazolinone class of compounds, while 2(1*H*)-quinazolinones have not yet been investigated. Based on promising properties of 4(3*H*)-quinazolinones we envisage that isomeric 2(1*H*)-quinazolinone derivatives may also act as MAO inhibitors. In support of this, structurally related compounds such as 3,4-dihydro-2(1*H*)-quinolinone derivatives are known to potently and specifically inhibit MAO-B. For example, among a series of 3,4-dihydro-2(1*H*)-quinolinone derivatives substituted on the C6 and C7 positions, the most active compound (**4**) exhibits an IC_{50} value of 0.086 μM (**Fig. 3.2**) (Meiring *et al.*, 2013). Based on these findings, the present study synthesises series of C6- and N1- substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives. In total, 27 derivatives with C6-substitution (**5a**-**m**; **6a**-**m**) and 11 derivatives with N1-substitution (**7a**-**e**; **8a**-**f**) were synthesised. The present study is the first investigation of MAO inhibition properties of 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives.



1



2



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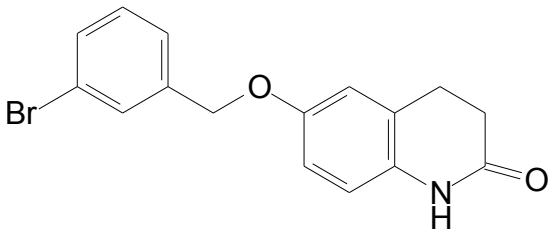

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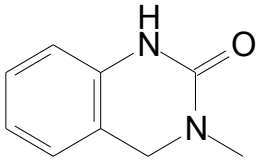


3-methyl-3,4-dihydroquinazolin-2(1*H*)-one

Figure 3.2: The structures of 4(3*H*)-quinazolinone derivatives 1-3, 3,4-dihydro-2(1*H*)-quinolinone derivative 4 and the structure of 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one.

3.2 Results and discussion

3.2.1 Chemistry

In the current study the two series of 3-methyl-3,4-dihydroquinazolin-2(1*H*)-ones were synthesised. The first series consists of C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **5a**-**m** and **6a**-**m** for which benzoyl and cinnamoyl C6-substitutions were considered, respectively. The C6-substituted benzoyl derivatives (**5**) were synthesised by Friedel-Crafts acylation as reported in literature (**Fig. 3.3**) (Dar *et al.*, 2013). To a solution of aluminium trichloride and 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one in carbon disulphide, the appropriate acyl chloride or bromide was added. The reaction mixture was heated under reflux (50 °C) for 24 h, and on completion, the reaction was terminated by adding a mixture of ice and water. The product was collected by filtration, dried in a convection oven and recrystallized from ethanol (Ogawa *et al.*, 1988). Attempts to synthesise the C6-substituded cinnamoyl derivatives by direct acylation failed, and with the exception of **6b**, the target cinnamoyl derivatives **6** were thus synthesised from 6-acetyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (**5l**). Derivative **6b** was synthesised by employing **5m** as starting material. To a solution of **5l** or **5m** in a mixture of hydrochloric acid

(32%) and methanol, an appropriate benzaldehyde was added. The reaction was heated under reflux for 24-48 h, and on completion the product was isolated and recrystallised as described above.

The target 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **5** and **6** were obtained in yields of 13-100%. The structures were determined by ^{1}H NMR, ^{13}C NMR and mass spectrometry, while purities were estimated by HPLC as cited in the experimental section. The successful synthesis of the cinnamoyl derivatives **6** were verified by the presence of two distinctive ^{1}H NMR signals of the vinylic protons of the substituent. For the *cis* configuration, the coupling constants ranged from 0-12 Hz while for the *trans* configuration the coupling constants ranged from 12-18 Hz (Xu *et al.*, 1996). Among the C6-cinnamoyl derivatives, **6a** and **6d**-**m** were found to be in *trans* configuration. Peaks present at 2,5 and 3,4 ppm in the ^{1}H NMR spectra, for derivatives **5** and **6**, are those of DMSO-d6 and water, respectively.

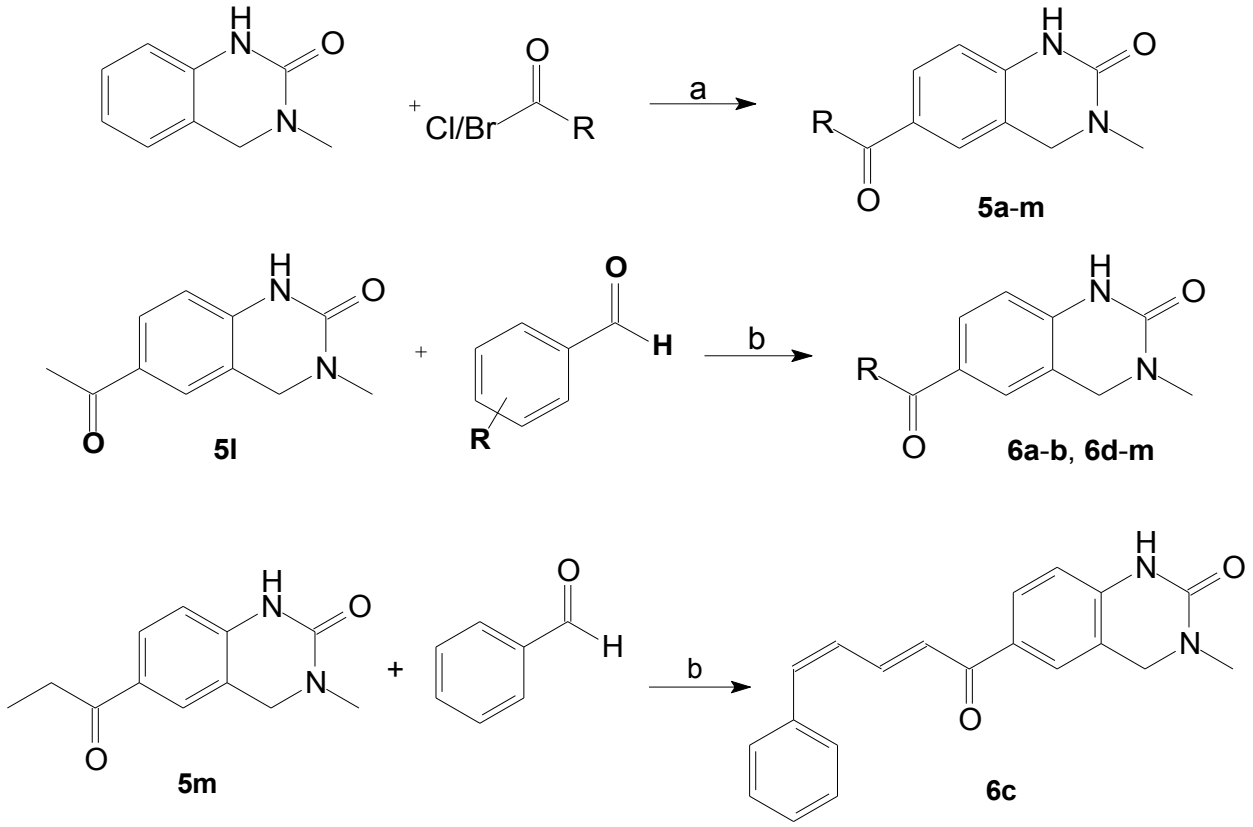


Figure 3.3: **The synthesis of C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives 5 and 6. Key (a) $AlCl_3$, CS_2, reflux, 24 h; (b) HCl/methanol, reflux, 24-48 h.**

The second series, the N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **7a**-**e** and **8a**-**f** were synthesised according to the pathway shown in **Fig. 3.4**.

3,4-Dihydro-3-methyl-2(1*H*)-quinazolinone was dissolved in dimethylformamide (DMF) and treated with sodium hydride at 0 °C. The reaction was stirred for 20 min and an appropriate alkyl bromide or alkyl chloride was subsequently added. The reaction was stirred for a further 1 h at 0 °C, and after completion was partitioned between water and ethyl acetate. The organic phase was dried over $MgSO_4$, then concentrated *in vacuo* and the resulting residue was recrystallised from ethanol, ethyl acetate or a mixture of petroleum ether and ethyl acetate. Compounds **7b**, **7c**, **7e**, **8a and 8c** were purified by column chromatography. The 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives were obtained in yields of 10-67%. The structures were verified, as cited in the experimental section, by ^{1}H NMR, ^{13}C NMR and mass spectrometry, and purity was estimated by HPLC.

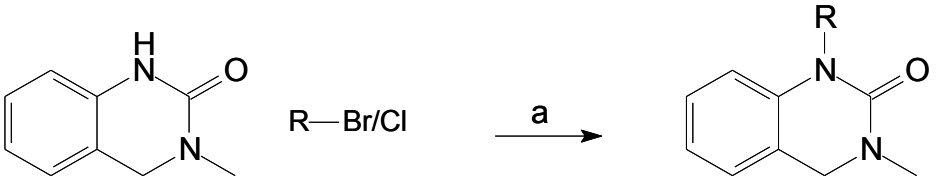


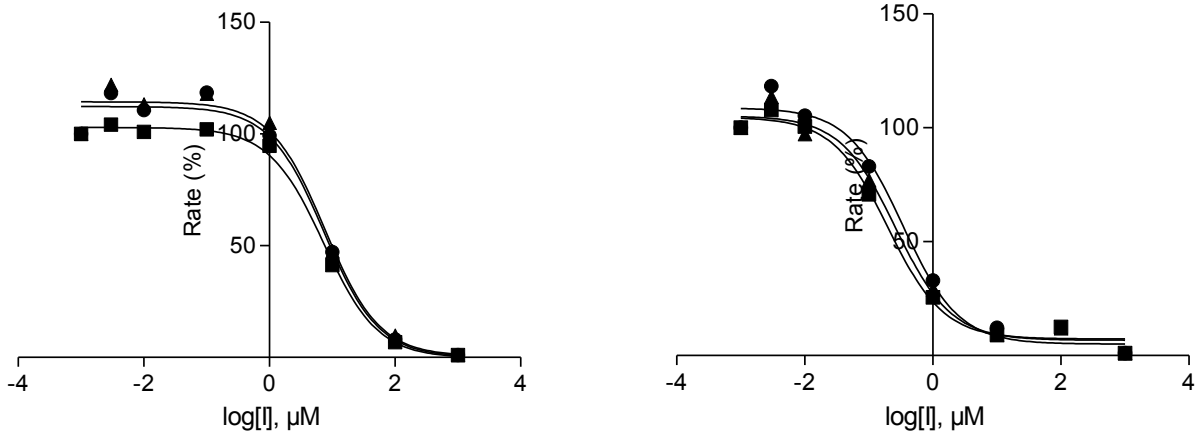
Figure 3.4: The synthesis of N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives 7 and 8. Key (a) NaH, DMF, 0 °C.

Interestingly, this procedure yielded both the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **7a**-**e** as well as the 3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-diones, compounds **8a**-**8f**. For **7a**-**e**, ^{1}H NMR spectra exhibit a singlet (2H) at approximately 4.5 ppm for the methylene protons at position 4, which was absent for compound **8a**-**f**. Furthermore, in the ^{13}C NMR spectra of **7a**-**e**, the signal of methylene C4 occurred at approximately 92 ppm, which was replaced by the signal of a carbonyl carbon at approximately 162 ppm for **8a**-**f**.

3.2.2 IC_{50} values for the inhibition of MAO

The IC_{50} values for the inhibition of MAO-A and MAO-B were determined by employing the human recombinant enzymes (Petzer *et al.*, 2013). Kynuramine, a nonspecific MAO substrate, was used to measure the catalytic rates of both enzyme isoforms. Kynuramine is non-fluorescent, and after MAO-catalysed oxidative deamination yields 4-hydroxyquinoline, a metabolite which fluoresces in alkaline media. The rate of kynuramine oxidation was thus determined by measuring the production of 4-hydroxyquinoline by fluorescence spectrophotometry (λ_{ex} = 310, λ_{em} = 400) at the end-point of the enzyme reaction. The enzyme reactions contained substrate (50 µM) and test inhibitor (0.003-100 µM) in potassium phosphate buffer (pH 7.4, 100 µM). After the reactions were initiated with the addition of the enzyme (0.0075-0.015 mg protein/ml), the reactions were incubated for 20 min at 37 °C and terminated with the addition of sodium hydroxide (2 N). Control reactions were conducted in the absence of inhibitor. IC_{50} values were estimated by constructing

sigmoidal plots of enzyme catalytic rate versus the logarithm of inhibitor concentration. An example of such plots is shown in **Fig. 3.5**



6b (MAO-A) **6c (MAO-B)**

Figure 3.5: Sigmoidal plots for the inhibition of MAO-A and MAO-B by 6b and 6c, respectively.

The IC_{50} values for the inhibition of the human MAO's by 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives are given in **table 3.1**. From the results it is evident that the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **5**-**8** are weak MAO-A inhibitors with IC_{50} >7.43 µM. Only three compounds (e.g. **6b**, **6g** and **6h**) possess IC_{50} <15 µM of the inhibition of MAO-A. Furthermore, with the exception of **5c** and **5j**, none of the compounds evaluated are specific for the MAO-A isoform. Compounds **5c** and **5j** are, however, very weak inhibitors of MAO-A. While no clear trend exists for the inhibition of MAO-A, it is noteworthy that none of the 3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-diones (**8a**-**f**) exhibited inhibition, even at a maximal tested concentration of 100 µM. Furthermore, the three most potent MAO-A inhibitors, **6b**, **6g** and **6h** are all cinnamoyl substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives.

In general, the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives are more potent MAO-B inhibitors. Among the 37 compounds synthesised, 7 displayed IC_{50} <1 µM for the inhibition of MAO-B. The most potent MAO-B inhibitor, possesses an IC_{50} value of 0.269 µM. Good potency MAO inhibition was only observed among C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives, specifically among the cinnamoyl substituted compounds **6**, which all display IC_{50} values <3.72 µM. Among the benzoyl substituted derivatives **5**, only two compounds (**5d** and **5g**) possess IC_{50} <10 µM of the inhibition of MAO-B. With N1-substitution, no MAO-B inhibition was observed, even at a maximal tested concentration of 100 µM.

The following structure-activity relationships (SARs) for MAO inhibition were observed:

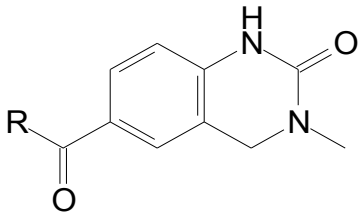
Substitution with chlorine in the *para* and fluorine in *meta* positions of the benzoyl moiety led to more potent MAO-B inhibitors (**5d** and **5g**) among the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives **5**. The position of substituent is important since *para* (**5e**) and *ortho* (**5f**) fluorine substituted derivatives are much less potent MAO-B inhibitors. Substitution of the benzoyl phenyl ring with the cyclohexyl (**5c**) led to the loss of MAO-B inhibition activity, suggesting that the aromatic ring is essential for activity. This is supported by the observation that the compounds bearing non-aromatic alkyl or alkylhalide substituents, **5i**-**m** are also devoid of MAO-B activity. Compounds substituted with heteroaromatic groups, such as furanyl, (**5h**), also do not inhibit MAO-B which indicates that heteroaromatic groups are less appropriate than the phenyl. Interestingly, phenyl and benzyl containing compounds (**5a**) (IC_{50} = 51.7 μM) and **5b** (IC_{50} = 50.9 μM) possess similar MAO-A inhibition potencies, while a loss of MAO-A inhibition was observed when the phenyl group was replaced with the cyclohexyl (**5c**) and furanyl (**5h**) groups.

In contrast to the benzoyl substituted derivative **5a** (IC_{50} = 30.8 μM), cinnamoyl substitution to yield **6a** (IC_{50} = 1.06 μM) resulted in more potent MAO-B inhibition. Compound **6c** (IC_{50} = 0.269 μM), in turn, is approximately fourfold more potent than compound **6a**, which shows that extension of conjugation (by replacing the cinnamyl group with a cinnamylidine) further enhances MAO-B inhibition activity. Recently it has been reported that compounds containing α,β,ɣ,δ-diunsaturated ketones, such as cinnamylidene acetophenones, display antioxidant and anti-inflammatory activities (Silva *et al.*, 2016). Compound **6c** may thus be a candidate for multiple biological activities relevant to PD including MAO-B inhibition, antioxidant and anti-inflammatory activities. MAO inhibitors with antioxidant and anti-inflammatory properties would be beneficial in the treatment of PD, since inflammation and oxidative stress have been linked to the pathogenesis of PD (Yacoubian & Standaert, 2009). Substitution with halogens and alkyl groups (e.g.**6f**-**j**) on the cinnamoyl phenyl ring leads to improved MAO-B inhibition activity compared to substitution with a polar hydroxyl group (e.g. **6e**). Compound **6a** is devoid MAO-A inhibition activity, however extension of conjugation (e.g. **6c**) results in a compound with MAO-A inhibition. Substitution on the cinnamoyl phenyl ring of **6a** with the *para* nitrile (**6g**) and trifluoromethyl (**6h**) groups yields the second and third most potent MAO-A inhibitors of the study. Substitution with a smaller halogen (fluorine) on phenyl moiety of cinnamoyl phenyl ring produced compounds which lacked MAO-A inhibition activity (**6k** and **6m**), although substitution with bromine and chlorine produced active MAO-A inhibitors (**6f, 6i, 6j** and **6l**). Interestingly, compound **6e**, the hydroxy substituted compound, was active as a MAO-A inhibitor (IC_{50} = 24.6 μM), although the methoxy substituted homologue (**6d**) was found to be inactive.

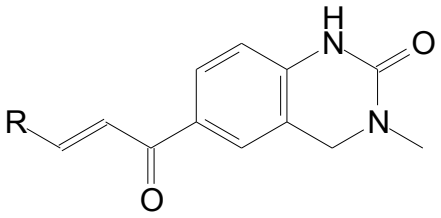
Although the N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives (**7a**-**d**) are weak MAO-A inhibitors, they exhibited specificity for the MAO-A isoform. In contrast the 3-methyl-

3,4-dihydroquinazolin-2,4(1*H*,3*H*)-diones (**8a**-**f**) where found to be inactive against both MAO isoforms. For the 3-methyl-3,4-dihydroquinazolin-2,4(1*H*,3*H*)-diones, a number of structural modifications were explored, however, no MAO inhibition was observed. It may thus be concluded that 3-methyl-3,4-dihydroquinazolin-2,4(1*H*,3*H*)-diones are not suitable for the design of MAO inhibitors. Among the N-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives, substitution with halogens at the appropriate position on the benzyl moiety produced compounds with MAO-A inhibition activity (**7a**-**d**), with compound **7c** displaying the highest inhibition potency (IC_{50} = 47.9 µM). Interestingly, the MAO-A inhibition potencies of the bromine substituted compounds (**7b** and **7c**) were within experimental error identical, which shows that the position (*para* versus *meta*) is not an important consideration.

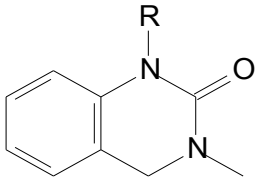
Table 3.1: The IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives 5-8.



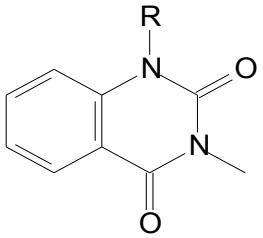
5a-**m**



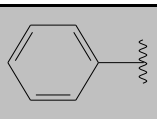
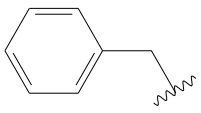
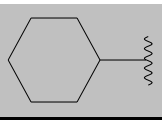
6a-**m**

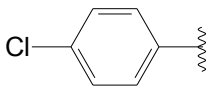
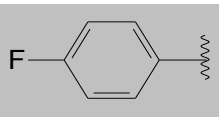
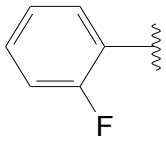
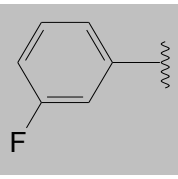
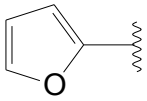
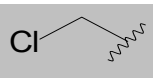
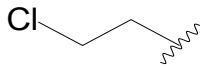
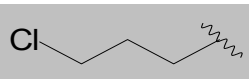
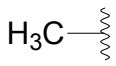
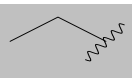
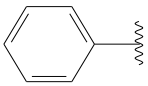
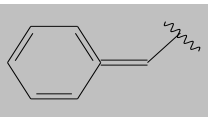
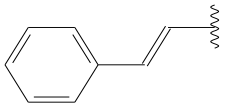
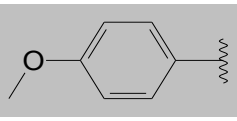
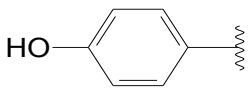
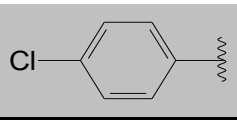


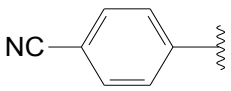
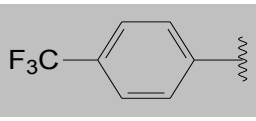
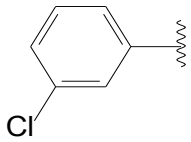
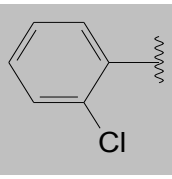
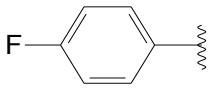
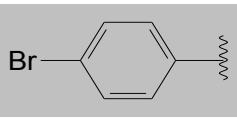
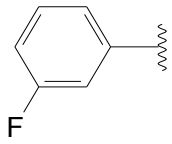
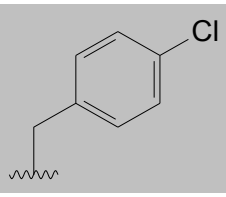
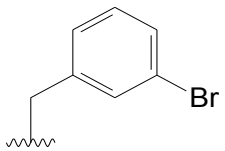
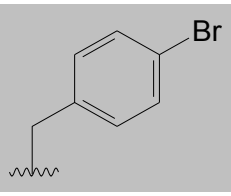
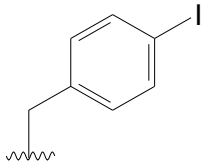
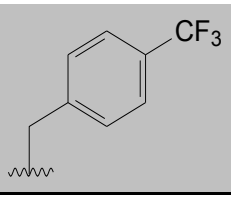
7a-**e**

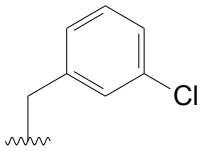
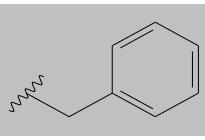
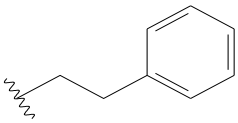
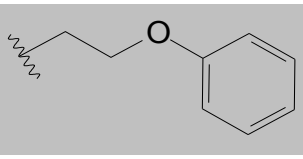
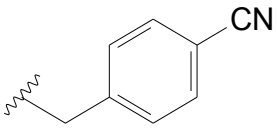
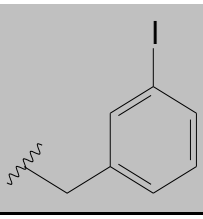


8a-**f**

	R	IC_{50} (µM)[a] MAO A	MAO B	SI[b]
5a		51.7 ± 3.70	30.8 ± 0.638	1.7
5b		50.9 ± 14.3	36.8 ± 1.334	1.4
5c		86.7 ± 4.62	No inh[c]	

5d		24.3 ± 2.84	2.22 ± 0.409	10.9
5e		54.9 ± 27.2	27.2 ± 3.104	2.0
5f		71.6 ± 12.1	20.4 ± 1.410	3.5
5g		50.0 ± 4.774	9.664 ± 0.897	5.2
5h		No inh[c]	No inh[c]	
5i		No inh[c]	No inh[c]	
5j		86.2 ± 16.2	No inh[c]	
5k		No inh[c]	No inh[c]	
5l		No inh[c]	No inh[c]	
5m		No inh[c]	No inh[c]	
6a		No inh[c]	1.06 ± 0.066	
6b		7.43 ± 0.178	2.544 ± 0.103	2.9
6c		24.7 ± 2.36	0.269 ± 0.071	91.8
6d		No inh[c]	3.72 ± 0.044	
6e		24.6 ± 3.80	3.16 ± 0.460	7.8
6f		78.1 ± 9.16	0.607 ± 0.034	128.7

6g		8.86 ± 0.532	1.23 ± 0.289	7.2
6h		11.1 ± 1.63	0.406 ± 0.024	27.3
6i		44.2 ± 7.10	0.350 ± 0.030	126.3
6j		52.6 ± 2.93	0.416 ± 0.029	126.4
6k		No inh[c]	0.446 ± 0.082	
6l		21.8 ± 0.566	1.01 ± 0.101	21.6
6m		No inh[c]	0.513 ± 0.031	
7a		86.7 ± 4.97	No inh[c]	
7b		50.2 ± 7.22	No inh[c]	
7c		47.9 ± 3.17	No inh[c]	
7d		55.8 ± 5.12	No inh[c]	
7e		No inh[c]	No inh[c]	

8a		No inh[c]	No inh[c]
8b		No inh[c]	No inh[c]
8c		No inh[c]	No inh[c]
8d		No inh[c]	No inh[c]
8e		No inh[c]	No inh[c]
8f		No inh[c]	No inh[c]

[a] All values are expressed as the mean ± standard deviation (SD) of triplicate determinations.

[b] Selectivity index (SI) = IC_{50}(MAO-A)/IC_{50}(MAO-B).

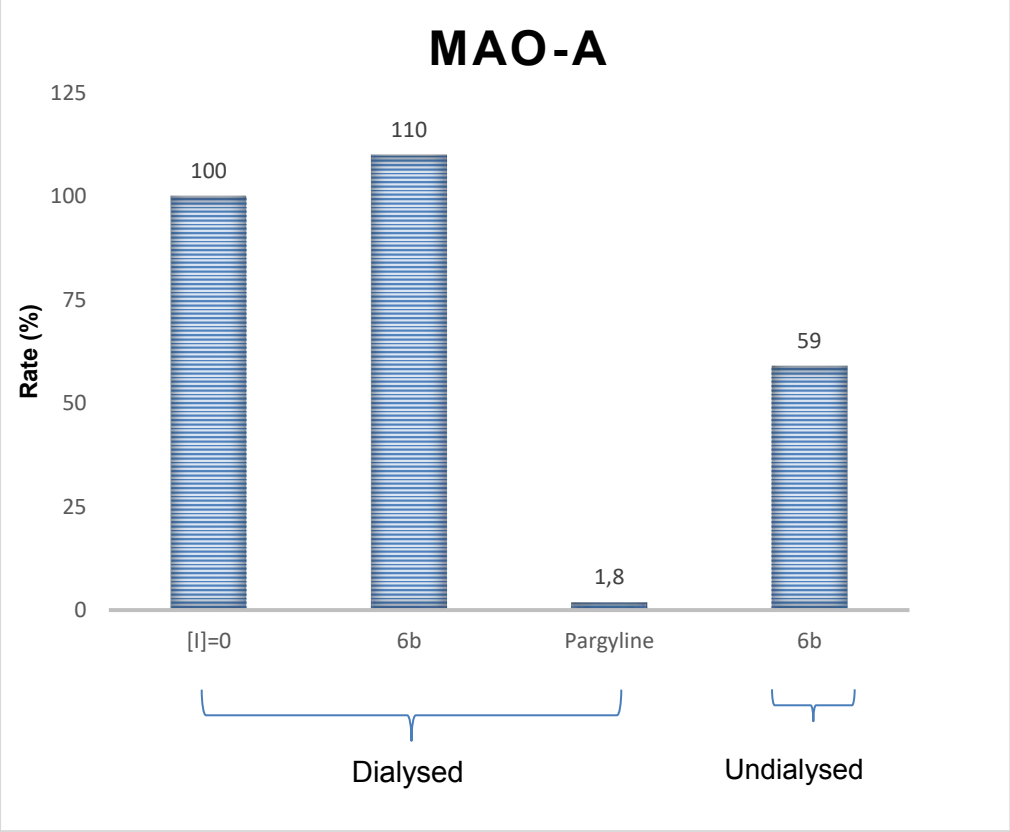
[c] No inhibition observed at a maximal tested concentration of 100 μM.

3.2.3 Reversibility of MAO inhibition

As mentioned, reversibility of MAO inhibition, especially of the MAO-A isoform, is an important consideration since irreversible MAO-A inhibitors may lead to a potentially fatal hypertensive crisis, when combined with certain food (Youdim & Weinstock, 2004). Compared to reversible inhibition, irreversible inhibitors also have the disadvantage of slow and variable rates of enzyme recovery after drug withdrawal (Legoabe *et al.*, 2012). For these reasons, we have set out to investigate the reversibility of MAO inhibition by selected 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives. Compounds **6b** and **6c** were selected to investigate the reversibility of MAO-A and MAO-B inhibition, respectively. Since these compounds do not possess functional groups (e.g. propargylamine, hydrazine, cyclopropylamine, haloallylamine) that are associated with irreversible MAO inhibition, it is expected that **6b** and **6c** would act as reversible inhibitors. Dialysis was used to investigate the reversibility of inhibition. Compounds **6b** and **6c**, at concentrations of 4 x IC_{50}, were combined with the MAO enzyme and pre-incubated for 20 min followed by dialysis for 24 h. Following dialysis, the reactions were diluted twofold with the addition of kynuramine to

yield an inhibitor concentration of 2 × IC_{50}. The residual enzyme activities were measured and plotted on histograms. Similar dialysis experiments in the presence of the irreversible inhibitors, pargyline and selegiline, served as positive controls, while dialysis of the enzymes in the absence of an inhibitor served as negative control. For comparison, the MAO activities of undialysed mixtures of the MAOs and the test inhibitors, **6b** and **6c**, were also measured.

For the negative control (absence of the inhibitor) the residual activity was set to 100% (**Fig. 3.6**). As shown by the results, **6b** and **6c** are reversible MAO inhibitors since dialysis restores MAO-A and MAO-B activity to 110% and 95%, respectively. Inhibition, however, persists in undialysed mixtures of MAO-A and MAO-B with these test inhibitors with the residual activity at 59% and 35%, respectively. Dialysis of the mixtures containing the MAOs and the irreversible inhibitors, pargyline and selegiline, does not restore activity with the residual activities at 1.8% and 3%, respectively.



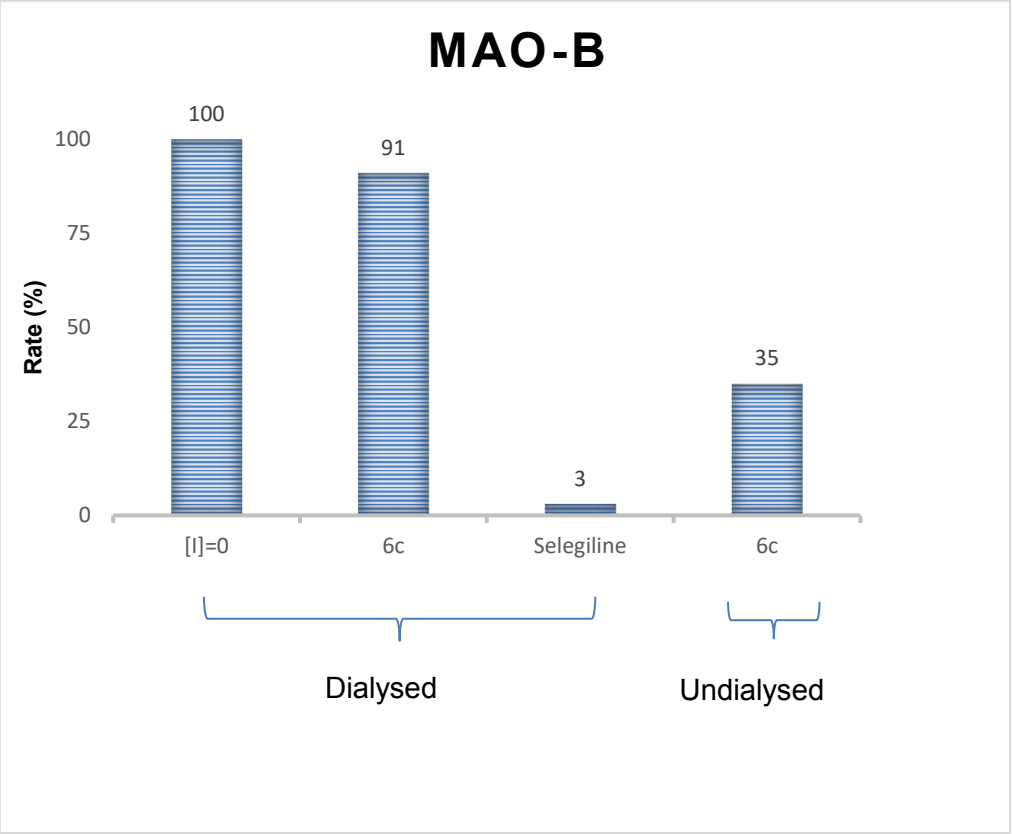
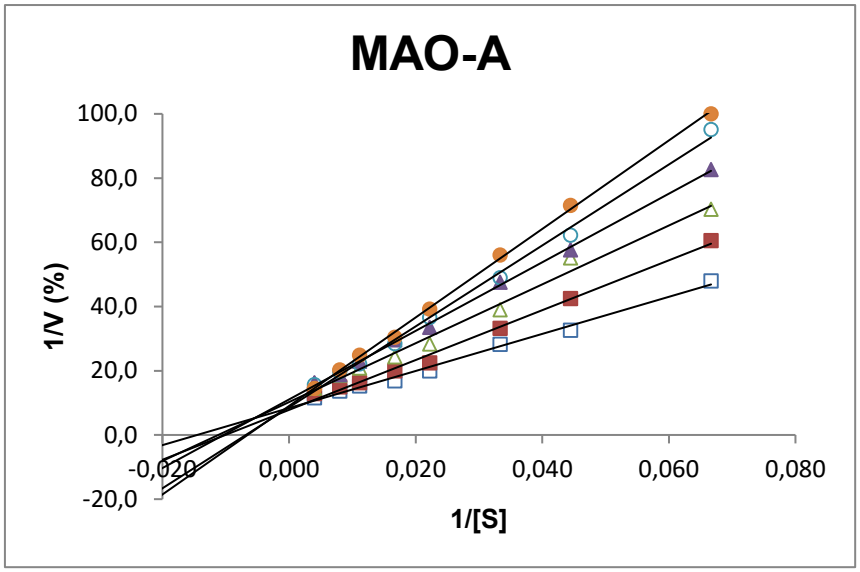


Figure 3.6: **Reversibility of inhibition of MAO-A and MAO-B by compounds 6b and 6c, respectively. MAO-A was pre-incubated in the absence of inhibitor and presence of 6b and pargyline (top), and MAO-B was pre-incubated in the absence of inhibitor and presence of 6c and selegiline (bottom). After dialysis, the residual enzyme activities were measured. For comparison, the MAO activities of undialysed mixtures of the MAOs and the test inhibitors were also measured.**

3.2.4 Mode of inhibition

Lineweaver-Burk plots were constructed to investigate the modes of MAO-A and MAO-B inhibition by **6b** and **6c**, respectively. MAO activity was measured at 8 different kynuramine concentrations (15-250 µM), in the absence and presence of 5 different concentrations of the inhibitors. As shown in **Fig. 3.7**, the Lineweaver-Burk plots for the inhibition of both MAO-A and MAO-B are linear and has a common y-intercept. From this data it may be concluded that **6b** and **6c** are competitive inhibitors of the MAO isozymes, respectively.



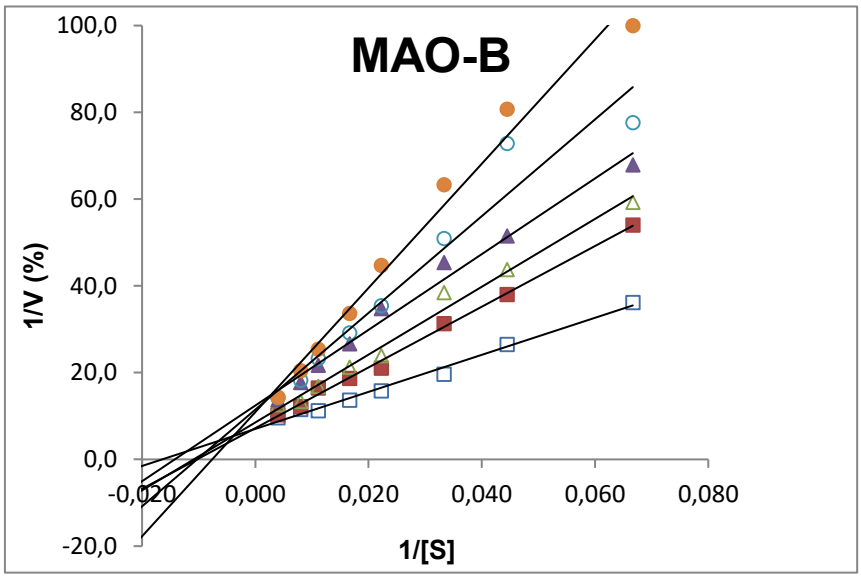


Figure 3.7: **Lineweaver-Burk plots of human MAO-A and MAO-B catalytic activities in the absence (open squares) and presence of various concentrations of 6b and 6c, respectively. For MAO-A the concentrations of 6b were: 0.185 µM (filled squares), 3.717 µM (open triangles), 5.575 µM (filled triangles), 7.433 µM (open circles) and 9.29 µM (filled circles). For the studies with MAO-B the concentrations of 6c were: 0.067 µM (filled squares), 0.134 µM (open triangles), 0.202 µM (filled triangles), 0.269 µM (open circles) and 0.336 µM (filled circles).**

3.3 Conclusion

The present study shows that a number of compounds among series of C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives act as reversible and specific MAO-B inhibitors. Such compounds are thus suitable leads for the development of selective MAO-B inhibitors that may find application in neurodegenerative disorders such as PD. The N1-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives (**7**) proved to be MAO-A specific inhibitors, although the inhibition potencies were weak. An interesting SAR for MAO-B inhibition by the C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives is the increase in potency when the conjugation is extended by replacing the cinnamyl group with a cinnamylidine group. In this respect, the cinnamylidene derivative **6c** is the most potent MAO-B inhibitor of the series. Some derivatives also exhibited MAO-A inhibition, with **6b** in particular displaying an IC_{50} value of 7.43 μM. This potency is comparable to reference inhibitors such as toloxatone, which is reported to inhibit MAO-A with an IC_{50} value of 3.92 μM (Petzer *et al.*, 2013). Since dopamine is metabolised by both MAO isoforms, nonspecific MAO inhibitors such as **6b** may be considered promising for the therapy of PD. The present study also shows that the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one class of compounds are reversible inhibitors of both MAO-A and MAO-B. This is significant since reversible MAO inhibitors are less likely to cause tyramine-induced hypertension than irreversible MAO inhibitors. Based on these findings, it may be concluded that C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives are promising MAO-B inhibitors for the treatment of PD, while nonspecific MAO inhibitors may also find application in the treatment of depression, which often is a comorbid condition of PD.

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André Joubert and Johan Jordaan of the SASOL Centre for Chemistry, North-West University, recorded the NMR and MS spectra. This work was supported by grants from the National Research Foundation and the Medical Research Council of South Africa (Grant specific unique reference numbers (UID) 85642, 96180, 916135). Opinions expressed, and conclusions arrived at, are those of the authors and therefore the NRF do not accept any liability in regard thereto.

3.5 Conflict of interest

The authors have no conflicts of interest to declare.

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3.7 Experimental section

3.7.1 Chemicals and instrumentation

Unless otherwise specified all chemicals were obtained from Sigma-Aldrich and were used without further purification. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO-*d*6) or deuterochloroform ($CDCl_3$) with a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 151 MHz, respectively. MestReNova was used to process and analyse NMR data. ^{1}H NMR data is reported by providing the chemical shift (δ), the integration and multiplicity of the signals. The chemical shifts (δ) are given in parts per million (ppm) and the coupling constants (J) in Hz. Spin multiplicities are given as singlet (s), doublet (d), doublet of doublets (dd), doublet of triplets (dt), doublet of doublet of doublets (ddd), triplet (t), triplet of doublet (td) and multiplet (m). High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-QII mass spectrometer in atmospheric-pressure chemical ionisation (APCI) mode. Melting points (mp) were determined with a Buchi B-545 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out to monitor the progress of reactions. Silica gel 60 (Merck) F_{254} sheets were used for TLC and the developed sheets were visualised under a UV254 light or by staining with iodine vapour. A Varian Cary Eclipse instrument was employed for fluorescence spectrophotometry. For the enzymology, microsomes from insect cells containing recombinant human MAO-A and MAO-B (5 mg protein/mL) and kynuramine dihydrobromide were obtained from Sigma-Aldrich.

3.7.2 General procedure for preparation of 5a-m

Friedel-Crafts acylation was employed to synthesise the C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives. Aluminium trichloride (4.62 mmol), 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (3.08 mmol) and the appropriate acyl chloride or bromide (3.08 mmol) were dissolved in carbon disulphide (6 mL). The reaction was heated under reflux (50 °C) for 24 h. The progress of the reaction was monitored by silica gel TLC with ethyl acetate as mobile phase. On completion of the reaction, 30 mL of ice water was added. The precipitate that formed was collected by filtration and dried in a convection oven overnight. The crude product was purified by recrystallisation from a suitable solvent (ethanol).

6-Benzoyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (5a)

The title compound was prepared in a yield of 39%: mp 235.6-258.4 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.69 (s, 1H), 7.66 (d, *J* = 7.6 Hz, 2H), 7.63 (t, *J* = 7.4 Hz, 1H), 7.57 (d, *J* = 8.3 Hz, 1H), 7.54 – 7.51 (m, 3H), 6.87 (d, *J* = 8.3 Hz, 1H), 4.46 (s, 2H), 2.85 (d, *J* = 11.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 194.18, 153.08, 142.20, 137.78, 132.02, 130.72, 129.47, 129.25, 128.44, 127.93, 117.72, 112.94, 49.41, 33.88; APCI-HRMS *m/z* calcd for $C_{16}H_{14}N_2O_2$ (MH^+), 267.1128, found 267.1124. Purity (HPLC): 96%.

3-Methyl-6-(2-phenylacetyl)-3,4-dihydroquinazolin-2(1*H*)-one (5b)

The title compound was prepared in a yield of 53%: mp 259.4-259.5 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.66 (s, 1H), 7.88 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.82 (s, 1H), 7.30 (t, *J* = 7.5 Hz, 2H), 7.26 (d, *J* = 7.1 Hz, 2H), 7.23 (d, *J* = 7.2 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 4.47 (s, 2H), 4.27 (s, 2H), 2.87 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 196.25, 153.49, 142.81, 135.94, 130.07, 129.83, 129.75, 128.77, 126.99, 126.87, 118.18, 113.48, 49.96, 44.71, 34.37; APCI-HRMS *m/z* calcd for $C_{17}H_{16}N_2O_2$ (MH^+), 281.1284, found 281.1284. Purity (HPLC): 92.8%.

6-(Cyclohexanecarbonyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (5c)

The title compound was prepared in a yield of 39%: mp 207.3-242.2 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.62 (s, 1H), 7.78 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.73 (s, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.47 (s, 2H), 3.29 (dd, *J* = 12.6, 9.7 Hz, 1H), 2.87 (s, 3H), 1.80 – 1.70 (m, 4H), 1.67 (d, *J* = 12.8 Hz, 1H), 1.36 (ddd, *J* = 23.4, 17.3, 10.8 Hz, 4H), 1.18 (d, *J* = 12.6 Hz, 1H). ^{13}C NMR (151 MHz, DMSO) δ 201.74, 153.56, 142.56, 129.28, 129.22, 126.69, 118.23, 113.54, 49.99, 44.41, 34.37, 29.70, 26.10, 25.69; APCI-HRMS *m/z* calcd $C_{16}H_{20}N_2O_2$ (MH^+), 273.1597, found 273.1583. Purity (HPLC): 98.8%.

6-(4-Chlorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (5d)

The title compound was prepared in a yield of 69%: mp 236.3-242.1 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.71 (s, 1H), 7.71 – 7.64 (m, 2H), 7.62 – 7.52 (m, 4H), 6.87 (d, *J* = 8.3 Hz, 1H), 4.46 (s, 2H), 2.86 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 193.04, 153.04, 142.39, 136.87, 136.47, 131.14, 130.73, 129.13, 128.56, 127.97, 117.78, 112.99, 49.39, 33.88; APCI-HRMS *m/z* calcd for $C_{16}H_{13}ClN_2O_2$ (MH^+), 301.0738, found 301.0756. Purity (HPLC): 99.4%.

6-(4-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (5e)

The title compound was prepared in a yield of 64%: mp 233.6-236.2 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.69 (s, 1H), 7.81 – 7.68 (m, 2H), 7.58 – 7.51 (m, 2H), 7.40 – 7.28 (m, 2H), 6.87 (d, *J* = 8.3 Hz, 1H), 4.46 (s, 2H), 2.85 (d, *J* = 12.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 192.85,

165.12, 163.46, 153.08, 142.24, 134.30, 132.18, 132.11, 130.68, 129.38, 127.91, 117.76, 115.57, 115.42, 112.96, 49.41, 33.89; APCI-HRMS *m/z* calcd for $C_{16}H_{13}FN_2O_2$ (MH^+), 285.1033, found 285.1059. Purity (HPLC): 98.9%.

6-(2-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (5f)

The title compound was prepared in a yield of 52%: mp 268.3-268.5 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.74 (s, 1H), 7.62 (d, *J* = 8.3 Hz, 1H), 7.58 – 7.51 (m, 2H), 7.49 (dd, *J* = 7.4, 1.7 Hz, 1H), 7.38 – 7.31 (m, 2H), 6.86 (d, *J* = 8.4 Hz, 1H), 4.45 (s, 2H), 2.84 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 190.81, 159.72, 158.08, 152.93, 143.11, 132.94, 132.89, 130.55, 130.06, 130.04, 129.50, 127.57, 127.05, 126.95, 124.74, 124.72, 117.95, 116.23, 116.09, 113.19, 49.30, 33.86; APCI-HRMS *m/z* calcd for $C_{16}H_{13}FN_2O_2$ (MH^+), 285.1033, found 285.1021. Purity (HPLC): 99.2%.

6-(3-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (5g)

The title compound was prepared in a yield of 81%: mp 243.9-354.9 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.72 (s, 1H), 7.58 (ddd, *J* = 13.7, 8.0, 4.6 Hz, 3H), 7.52 – 7.42 (m, 3H), 6.88 (d, *J* = 8.3 Hz, 1H), 4.47 (s, 2H), 2.86 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 192.78, 162.62, 160.99, 153.01, 142.52, 140.12, 140.08, 130.83, 130.67, 130.62, 128.92, 128.03, 125.41, 118.91, 118.77, 117.82, 115.74, 115.59, 113.03, 49.37, 33.87; APCI-HRMS *m/z* calcd for $C_{16}H_{13}FN_2O_2$ (MH^+), 285.1033, found 285.1017. Purity (HPLC): 99.0%.

6-(Furan-2-carbonyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (5h)

The title compound was prepared in a yield of 100%: mp 244.8-248.2 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.72 (s, 1H), 8.09 (dd, *J* = 1.7, 0.7 Hz, 1H), 7.79 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.74 (s, 1H), 7.39 (d, *J* = 3.5 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 1H), 6.78 (dd, *J* = 3.6, 1.7 Hz, 1H), 4.51 (s, 2H), 2.88 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 180.24, 153.54, 151.98, 148.40, 142.68, 130.29, 129.84, 127.68, 120.74, 118.25, 113.57, 112.99, 49.95, 34.39; APCI-HRMS *m/z* calcd for $C_{14}H_{12}N_2O_3$ (MH^+), 257.0920, found 257.0902. Purity (HPLC): 99.3%.

6-(2-Chloroacetyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (5i)

The title compound was prepared in a yield of 81.1%: mp 249.8-252.1 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.72 (s, 1H), 7.80 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.76 (s, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 5.07 (s, 2H), 4.47 (s, 2H), 2.88 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 190.11, 153.39, 143.35, 129.70, 127.66, 126.97, 118.27, 113.53, 49.87, 47.60, 34.37; APCI-HRMS *m/z* calcd for $C_{11}H_{11}ClN_2O_2$ (MH^+), 239.0581, found 239.0584. Purity (HPLC): 99.4%.

6-(3-Chloropropanoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (5j)

The title compound was prepared in a yield of 78.82%: mp 171.7-171.8 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.68 (s, 1H), 7.80 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.76 (s, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.47 (s, 2H), 3.91 (t, *J* = 6.3 Hz, 2H), 3.44 (t, *J* = 6.3 Hz, 2H), 2.88 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 195.36, 153.47, 142.99, 129.84, 129.26, 126.65, 118.15, 113.50, 49.93, 34.38; APCI-HRMS *m/z* calcd for $C_{12}H_{13}ClN_2O_2$ (MH^+), 253.0738, found 253.0736. Purity (HPLC): 96.3%.

6-(4-Chlorobutanoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (5k)

The title compound was prepared in a yield of 57%: mp 180.8-238.3 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.65 (s, 1H), 7.79 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.74 (s, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.47 (s, 2H), 3.70 (t, *J* = 6.7 Hz, 2H), 3.08 (t, *J* = 7.1 Hz, 2H), 2.87 (s, 3H), 2.08 – 2.02 (m, 2H). ^{13}C NMR (151 MHz, DMSO) δ 197.46, 153.52, 142.76, 130.03, 129.06, 126.47, 118.11, 113.47, 49.95, 45.44, 35.13, 34.37, 27.47; APCI-HRMS *m/z* calcd for $C_{13}H_{15}ClN_2O_2$ (MH^+), 267.0894, found 267.0896. Purity (HPLC): 94.5%.

6-Acetyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (5l)

The title compound was prepared in a yield of 76.1%: mp 213.3-224.8 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.65 (s, 1H), 7.77 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.72 (s, 1H), 6.83 (d, *J* = 8.3 Hz, 1H), 4.47 (s, 2H), 2.87 (s, 3H), 2.48 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 196.47, 153.53, 142.69, 130.48, 129.39, 126.74, 118.03, 113.40, 49.95, 34.37, 26.77; APCI-HRMS *m/z* calcd for $C_{11}H_{12}N_2O_2$ (MH^+), 205.0971, found 205.0973. Purity (HPLC): 98.9%.

3-Methyl-6-propionyl-3,4-dihydroquinazolin-2(1*H*)-one (5m)

The title compound was prepared in a yield of 80%: mp 301.0-312.6 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.63 (s, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.73 (s, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 4.47 (s, 2H), 2.94 (q, *J* = 7.2 Hz, 2H), 2.87 (s, 3H), 1.06 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 199.03, 153.57, 142.54, 130.16, 128.99, 126.37, 118.05, 113.42, 49.98, 34.37, 31.17, 8.83; APCI-HRMS *m/z* calcd for $C_{12}H_{14}N_2O_2$ (MH^+), 219.1128, found 219.1137. Purity (HPLC): 99.1%.

3.7.3 General procedure for preparation of compounds 6a-m

6-Acetyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (**5l**) for the synthesis of **6a** and **6c**-**m** or 3-methyl-6-propionyl-3,4-dihydroquinazolin-2(1*H*)-one (**5m**) (1.375 mmol) for the synthesis of **6b** and the appropriate benzaldehyde (1.375 mmol) were dissolved in a mixture of hydrochloric acid (5.5 mL) and methanol (3.67 mL). The reaction was heated under reflux (50 °C) for 24 to 48 h and the progress was monitored by using silica gel TLC with ethyl acetate as the mobile phase. Upon completion, 30 mL of ice water was added to the reaction. The residue was collected by

filtration and dried in a convection oven overnight. The crude product was purified by recrystallization from ethanol.

6-Cinnamoyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (6a)

The title compound was prepared in a yield of 40%: mp 249.9-250.0 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 8.04 – 7.97 (m, 2H), 7.95 – 7.85 (m, 3H), 7.71 (d, *J* = 15.6 Hz, 1H), 7.57 – 7.44 (m, 3H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.45, 153.49, 143.50, 142.85, 135.32, 131.06, 130.91, 129.80, 129.38, 129.22, 127.28, 122.42, 118.28, 113.61, 50.03, 34.42; APCI-HRMS *m/z* calcd for $C_{18}H_{16}N_2O_2$ (MH^+), 293.1284, found 293.1281. Purity (HPLC): 91.9%.

(*z*)-3-Methyl-6-(2-methyl-3-phenylacryloyl)-3,4-dihydroquinazolin-2(1*H*)-one (6b)

The title compound was prepared in a yield of 17%: mp 122.8-122.9 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.64 (s, 1H), 7.61 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.56 (s, 1H), 7.51 (d, *J* = 7.4 Hz, 2H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.38 (d, *J* = 7.3 Hz, 1H), 7.05 (s, 1H), 6.87 (d, *J* = 8.3 Hz, 1H), 4.49 (s, 2H), 2.87 (s, 3H), 2.15 (d, *J* = 1.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 197.38, 153.64, 142.16, 139.32, 136.65, 135.95, 130.74, 130.55, 130.12, 128.97, 128.92, 128.04, 118.09, 113.32, 49.96, 34.36, 15.34; APCI-HRMS *m/z* calcd for $C_{19}H_{18}N_2O_2$ (MH^+), 307.1441, found. Purity (HPLC): 91.8%.

3-Methyl-6-((2*E*,4*Z*)-5-phenylpenta-2,4-dienoyl)-3,4-dihydroquinazolin-2(1*H*)-one (6c)

The title compound was prepared in a yield of 68%: mp 243.7-247.2 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 7.87 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.83 (s, 1H), 7.60 (d, *J* = 7.3 Hz, 2H), 7.55 – 7.44 (m, 1H), 7.44 – 7.30 (m, 4H), 7.27 – 7.18 (m, 2H), 6.88 (d, *J* = 8.4 Hz, 1H), 4.51 (s, 2H), 2.89 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.48, 153.49, 143.92, 142.72, 141.64, 136.56, 131.14, 129.62, 129.43, 127.79, 127.68, 126.88, 125.86, 118.33, 113.62, 50.00, 34.40; APCI-HRMS *m/z* calcd for $C_{20}H_{18}N_2O_2$ (MH^+), 319.1441, found 319.1449. Purity (HPLC): 99.6%.

(*E*)-6-(3-(4-Methoxyphenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (6d)

The title compound was prepared in a yield of 47%: mp 220.3-331.9 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 8.03 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.98 (s, 1H), 7.91 (d, *J* = 15.6 Hz, 1H), 7.68 (d, *J* = 15.5 Hz, 1H), 7.48 – 7.40 (m, 2H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.02 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 3.83 (s, 3H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.46, 160.12, 153.48, 143.50, 142.86, 136.72, 131.05, 130.39, 129.86, 127.26, 122.67, 121.94, 118.28, 116.84, 113.93, 113.59, 55.78, 50.04, 34.41; APCI-HRMS *m/z* calcd for $C_{19}H_{18}N_2O_3$ (MH^+), 323.1390, found 323.1389. Purity (HPLC): 96.4%.

(*E*)-6-(3-(4-Hydroxyphenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (6e)

The title compound was prepared in a yield of 16%: mp 290.9-294.8 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 10.07 (s, 1H), 9.67 (s, 1H), 8.00 – 7.92 (m, 2H), 7.71 (dd, *J* = 12.0, 10.4 Hz, 3H), 7.64 (d, *J* = 15.4 Hz, 1H), 6.88 (d, *J* = 8.3 Hz, 1H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.51 (s, 2H), 2.89 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.33, 160.41, 153.53, 143.95, 142.53, 131.44, 131.28, 129.54, 127.04, 126.42, 118.83, 118.19, 116.26, 113.54, 56.51, 50.06, 34.41, 19.06; APCI-HRMS *m/z* calcd for $C_{18}H_{16}N_2O_3$ (MH^+), 309.1233, found 308.1145. Purity (HPLC): 98.9%.

(*E*)-6-(3-(4-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (6f)

The title compound was prepared in a yield of 42%: mp 244.3-256.6 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 8.02 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.98 (s, 1H), 7.92 (t, *J* = 12.1 Hz, 3H), 7.69 (d, *J* = 15.6 Hz, 1H), 7.53 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.88 (d, *J* = 13.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.32, 153.46, 142.91, 142.02, 135.35, 134.31, 130.96, 130.91, 129.86, 129.41, 127.30, 123.16, 118.29, 113.60, 50.02, 34.41; APCI-HRMS *m/z* calcd for $C_{18}H_{15}ClN_2O_2$ (MH^+), 327.0894, found 327.0864. Purity (HPLC): 75.4%.

(*E*)-4-(3-(3-Methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)-3-oxoprop-1-en-1-yl)benzonitrile (6g)

The title compound was prepared in a yield of 13%: mp 220.9-224.8 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.72 (s, 1H), 8.10 – 8.06 (m, 2H), 8.05 – 8.03 (m, 1H), 8.03 – 8.01 (m, 1H), 8.00 – 7.92 (m, 3H), 7.73 (d, *J* = 15.6 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.25, 153.42, 143.11, 141.28, 139.92, 133.19, 130.76, 130.02, 129.95, 129.78, 127.44, 125.74, 118.34, 113.64, 112.60, 50.01, 34.42; APCI-HRMS *m/z* calcd for $C_{19}H_{15}N_3O_2$ (MH^+), 318.1237, found 318.1250. Purity (HPLC): 76.5%.

(*E*)-3-Methyl-6-(3-(4-(trifluoromethyl)phenyl)acryloyl)-3,4-dihydroquinazolin-2(1*H*)-one (6h)

The title compound was prepared in a yield of 26%: mp 216.9-252.0 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.72 (s, 1H), 8.09 (d, *J* = 8.2 Hz, 2H), 8.07 – 8.02 (m, 2H), 8.00 (s, 1H), 7.81 (d, *J* = 8.3 Hz, 2H), 7.75 (d, *J* = 15.6 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.30, 153.43, 143.06, 141.49, 139.37, 130.80, 130.45, 130.24, 129.98, 129.76, 127.39, 126.16, 126.14, 125.46, 125.12, 123.66, 118.33, 113.63, 50.01, 34.41; APCI-HRMS *m/z* calcd for $C_{19}H_{15}F_3N_2O_2$ (MH^+), 361.1158, found 360.1090. Purity (HPLC): 78.7%.

(*E*)-6-(3-(3-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (6i)

The title compound was prepared in a yield of 49%: mp 257.7-257.9 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 8.08 – 8.02 (m, 2H), 7.99 (d, *J* = 15.4 Hz, 2H), 7.80 (d, *J* = 6.9 Hz, 1H), 7.68 (d, *J* = 15.6 Hz, 1H), 7.49 (t, *J* = 4.8 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.30, 153.45, 142.98, 141.78, 137.60, 134.27, 131.15, 130.90, 130.43, 129.95, 128.27, 128.24, 127.37, 123.96, 118.29, 113.61, 50.04, 34.42; APCI-HRMS *m/z* calcd for $C_{18}H_{15}ClN_2O_2$ (MH^+), 327.0894, found 327.0884. Purity (HPLC): 93.2%.

(*E*)-6-(3-(2-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (6j)

The title compound was prepared in a yield of 56%: mp 257.6-265.1 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.72 (s, 1H), 8.19 (d, *J* = 6.6 Hz, 1H), 8.01 (dd, *J* = 26.0, 10.6 Hz, 4H), 7.58 (d, *J* = 6.9 Hz, 1H), 7.47 (s, 2H), 6.90 (d, *J* = 8.2 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.20, 153.44, 143.08, 138.06, 134.72, 132.94, 132.31, 130.77, 130.52, 129.98, 128.95, 128.14, 127.41, 125.22, 118.35, 113.65, 50.01, 34.42; APCI-HRMS *m/z* calcd for $C_{18}H_{15}ClN_2O_2$ (MH^+), 327.0894, found 327.0900. Purity (HPLC): 92.3%.

(*E*)-6-(3-(4-Fluorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (6k)

The title compound was prepared in a yield of 27%: mp241.5-253.3 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.69 (s, 1H), 8.01 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.99 – 7.93 (m, 3H), 7.87 (d, *J* = 15.6 Hz, 1H), 7.71 (d, *J* = 15.6 Hz, 1H), 7.31 (t, *J* = 8.8 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.37, 164.60, 162.95, 153.47, 142.84, 142.26, 132.02, 132.00, 131.56, 131.51, 131.04, 129.81, 127.25, 122.33, 118.27, 116.46, 116.31, 113.59, 50.04, 34.41; APCI-HRMS *m/z* calcd for $C_{18}H_{15}FN_2O_2$ (MH^+), 311.1190, found 310.1112. Purity (HPLC): 95.4%.

(*E*)-6-(3-(4-Bromophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (6l)

The title compound was prepared in a yield of 15%: mp 260.3-265.0 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 8.01 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.98 (s, 1H), 7.94 (d, *J* = 15.6 Hz, 1H), 7.84 (d, *J* = 8.5 Hz, 2H), 7.66 (d, *J* = 8.3 Hz, 3H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.33, 153.46, 142.92, 142.12, 134.63, 132.33, 131.13, 130.96, 129.87, 127.31, 124.23, 123.21, 118.29, 113.61, 50.03, 34.42; APCI-HRMS *m/z* calcd for $C_{18}H_{15}BrN_2O_2$ (MH^+), 371.0389, found 372.0323. Purity (HPLC): 96.8%.

(*E*)-6-(3-(3-Fluorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (6m)

The title compound was prepared in a yield of 23%: mp 317.3-336.2 °C (ethanol). ^{1}H NMR (600 MHz, DMSO) δ 9.70 (s, 1H), 8.03 (dd, *J* = 8.4, 1.9 Hz, 1H), 8.00 – 7.95 (m, 2H), 7.83 (d, *J* = 10.1

Hz, 1H), 7.72 – 7.65 (m, 2H), 7.50 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.28 (d, *J* = 2.4 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.52 (s, 2H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 187.33, 163.79, 162.18, 153.45, 142.96, 142.02, 137.95, 137.89, 131.33, 131.28, 130.90, 129.91, 127.36, 125.93, 123.88, 118.28, 117.61, 117.47, 115.04, 114.89, 113.62, 50.03, 34.41; APCI-HRMS *m/z* calcd for $C_{18}H_{15}FN_2O_2$ (MH^+), 311.1190, found 311.1200. Purity (HPLC): 84.6%.

3.7.4 General synthesis of 7a-e and 8a-f

Commercially available 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (1.85 mmol) and sodium hydride (3.7 mmol) were dissolved in anhydrous dimethylformamide. The reaction was stirred for 20 min at 0 °C. The appropriate alkyl bromide or alkyl chloride (1.85 mmol) was added to the mixture and stirring was continued for 1 h at 0 °C. The reaction progress was monitored by TLC with petroleum ether and ethyl acetate (2:1) as the mobile phase. Upon completion, 30 mL of ice water was added and the mixture was extracted to 40 mL ethyl acetate. The organic phase was dried over $MgSO_4$, filtered and evaporated *in vacuo*. In most instances, the residue was purified by recrystallisation from ethanol or form a mixture of ethanol and petroleum ether (1:1). Compounds **7b**, **7c**, **7e**, **8a** and **8c** were purified by silica gel column chromatography with a mixture of petroleum ether and ethyl acetate (2:1) serving as mobile phase.

1-(4-Chlorobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (7a)

The title compound was prepared in a yield of 26%: mp 118.9-123.9 °C (ethanol). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.28 – 7.24 (m, 2H), 7.19 (d, *J* = 8.3 Hz, 2H), 7.13 – 7.03 (m, 2H), 6.94 (t, *J* = 7.4 Hz, 1H), 6.63 (d, *J* = 8.2 Hz, 1H), 5.08 (s, 2H), 4.46 (s, 2H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.04, 138.40, 136.38, 132.55, 128.79, 128.18, 127.79, 125.57, 121.95, 119.56, 113.69, 50.47, 46.22, 35.83; APCI-HRMS *m/z* calcd for $C_{16}H_{15}ClN_2O$ (MH^+), 287.0945, found 301.0731. Purity (HPLC): 97.4%.

1-(3-Bromobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (7b)

The title compound was prepared in a yield of 67%: mp 96.8-100.1 °C (petroleum ether: ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.3 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 1H), 7.06 (d, *J* = 7.4 Hz, 1H), 6.94 (t, *J* = 7.4 Hz, 1H), 6.62 (d, *J* = 8.2 Hz, 1H), 5.06 (s, 2H), 4.46 (s, 2H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.03, 138.39, 136.93, 131.73, 128.19, 128.16, 125.58, 121.96, 120.62, 119.56, 113.68, 50.48, 46.27, 35.83; APCI-HRMS *m/z* calcd for $C_{16}H_{15}BrN_2O$ (MH^+), 331.0440, found 331.0407. Purity (HPLC): 98.4%

1-(4-Bromobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (7c)

The title compound was prepared in a yield of 57.4%: mp 095.8-098.6 °C (petroleum ether: ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 – 7.39 (m, 2H), 7.16 – 7.03 (m, 4H), 6.94 (td, *J* = 7.4, 0.6 Hz, 1H), 6.62 (d, *J* = 8.2 Hz, 1H), 5.06 (s, 2H), 4.46 (s, 2H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.02, 138.39, 136.93, 131.73, 128.19, 128.16, 125.58, 121.96, 120.62, 119.56, 113.68, 50.47, 46.27, 35.83; APCI-HRMS *m/z* calcd for $C_{16}H_{15}BrN_2O$ (MH^+), 331.0440, found 331.0440. Purity (HPLC): 99.2%.

1-(4-Iodobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (7d)

The title compound was prepared in a yield of 11%: mp 106.8-109.1 °C (petroleum ether: ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.61 (d, *J* = 8.3 Hz, 2H), 7.12 – 7.03 (m, 2H), 7.01 (d, *J* = 8.2 Hz, 2H), 6.95 (d, *J* = 7.4 Hz, 1H), 6.62 (d, *J* = 8.2 Hz, 1H), 5.06 (s, 2H), 4.46 (s, 2H), 3.09 (s, 3H), 1.65 (s, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.02, 138.39, 137.68, 137.65, 128.44, 128.20, 125.58, 121.96, 119.56, 113.68, 92.07, 50.47, 46.35, 35.84; APCI-HRMS *m/z* calcd for $C_{16}H_{15}IN_2O$ (MH^+), 379.0301, found 379.0278. Purity (HPLC): 95.4%.

3-Methyl-1-(4-(triflouromethyl)benzyl)-3,4-dihydroquinazolin-2(1*H*)-one (7e)

The title compound was prepared in a yield of 24.8%: mp 086.4-088.9 °C (petroleum ether: ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.51 – 7.45 (m, 1H), 7.41 (d, *J* = 6.4 Hz, 2H), 7.13 – 7.05 (m, 2H), 6.95 (td, *J* = 7.4, 0.7 Hz, 1H), 6.61 (d, *J* = 8.2 Hz, 1H), 5.17 (s, 2H), 4.48 (s, 2H), 3.10 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.99, 139.01, 138.36, 129.67, 129.18, 128.27, 125.67, 123.89, 123.86, 123.21, 123.19, 123.17, 122.07, 119.58, 113.56, 50.48, 46.52, 35.86; APCI-HRMS *m/z* calcd for $C_{17}H_{15}F_3N_2O$ (MH^+), 321.1209, found 321.1240. Purity (HPLC): 99.1%.

1-(3-Chlorobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione (8a)

The title compound was prepared in a yield of 15%: mp 127.6-130.0 °C (petroleum ether: ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.57 (ddd, *J* = 8.6, 7.3, 1.6 Hz, 1H), 7.29 – 7.22 (m, 4H), 7.14 (d, *J* = 6.8 Hz, 1H), 7.06 (d, *J* = 8.4 Hz, 1H), 5.36 (s, 2H), 3.56 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.89, 151.55, 139.61, 137.85, 135.13, 134.98, 130.30, 129.13, 128.01, 126.60, 124.63, 123.30, 115.69, 114.09, 46.93, 28.73; APCI-HRMS *m/z* calcd for $C_{16}H_{13}ClN_2O_2$ (MH^+), 301.0738, found 301.0738. Purity (HPLC): 99.8%.

1-Benzyl-3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione (8b)

The title compound was prepared in a yield of 11%: mp 155.2-156.1 °C (ethanol: petroleum ether). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.25 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.54 (ddd, *J* = 8.7, 7.3, 1.6 Hz, 1H), 7.34 (t, *J* = 7.4 Hz, 2H), 7.26 (dd, *J* = 7.6, 3.3 Hz, 3H), 7.24 – 7.21 (m, 1H), 7.12 (d, *J* = 8.4 Hz,

1H), 5.39 (s, 2H), 3.56 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.01, 151.62, 139.86, 135.71, 135.00, 128.99, 127.68, 126.46, 123.08, 115.65, 114.37, 47.40, 28.70; APCI-HRMS *m/z* calcd for $C_{16}H_{14}N_2O_2$ (MH^+), 267.1128, found 267.1118. Purity (HPLC): 100%.

3-Methyl-1-phenethyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione (8c)

The title compound was prepared in a yield of 25%: mp 143.8-145.3 °C (petroleum ether: ethyl acetate). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.71 – 7.64 (m, 1H), 7.36 – 7.29 (m, 4H), 7.27 – 7.22 (m, 3H), 4.37 – 4.31 (m, 2H), 3.50 (s, 3H), 3.06 – 2.99 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.96, 150.90, 139.55, 137.74, 135.08, 129.24, 128.82, 128.81, 126.91, 122.90, 115.63, 113.32, 45.18, 33.61, 28.42; APCI-HRMS *m/z* calcd for $C_{17}H_{16}N_2O_2$ (MH^+), 281.1284, found 281.1262. Purity (HPLC): 99.6%.

3-Methyl-1-(2-phenoxyethyl)-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione (8d)

The title compound was prepared in a yield of 10%: mp 148.8-165.3 °C (ethanol). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.23 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.70 – 7.65 (m, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.30 – 7.22 (m, 3H), 6.94 (t, *J* = 7.3 Hz, 1H), 6.83 (d, *J* = 8.2 Hz, 2H), 4.55 (t, *J* = 5.8 Hz, 2H), 4.34 (t, *J* = 5.8 Hz, 2H), 3.49 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.95, 158.14, 151.25, 140.32, 134.83, 129.55, 128.87, 123.08, 121.29, 115.53, 114.42, 114.32, 65.19, 43.45, 28.43; APCI-HRMS *m/z* calcd for $C_{17}H_{16}N_2O_3$ (MH^+), 297.1233, found 297.1229. Purity (HPLC): 94.1%.

4-((3-Methyl-2,4-dioxo-3,4-dihydroquinazolin-2(1*H*)-yl)methyl)benzonitrile (8e)

The title compound was prepared in a yield of 30%: mp 194.5-281.9 °C (petroleum ether: ethanol). ^{1}H NMR (600 MHz, DMSO) δ 8.09 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.81 (d, *J* = 8.2 Hz, 2H), 7.68 – 7.62 (m, 1H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 1H), 5.47 (s, 2H), 3.37 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 161.80, 151.49, 142.78, 139.82, 135.63, 133.03, 128.49, 127.98, 123.46, 119.20, 115.72, 115.12, 110.54, 46.65, 28.79; APCI-HRMS *m/z* calcd for $C_{17}H_{13}N_3O_2$ (MH^+), 292.1080, found 292.1059. Purity (HPLC): 97.0%.

1-(3-Iodobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione (8f)

The title compound was prepared in a yield of 34%: mp 131.4-134.0 °C (ethyl acetate). ^{1}H NMR (600 MHz, DMSO) δ 8.08 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.76 (s, 1H), 7.70 – 7.61 (m, 2H), 7.33 – 7.24 (m, 3H), 7.12 (t, *J* = 7.8 Hz, 1H), 5.35 (s, 2H), 3.37 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 161.80, 151.49, 139.91, 139.55, 136.54, 135.60, 135.55, 131.25, 128.42, 126.34, 123.38, 115.68, 115.22, 95.67, 46.17, 28.80; APCI-HRMS *m/z* calcd for $C_{16}H_{13}IN_2O_2$ (MH^+), 393.0094, found 393.0084. Purity (HPLC): 96.3%.

3.7.5 The determination of IC_{50} values for MAO inhibition

The IC_{50} values were measured according to the literature procedure (Petzer *et al.*, 2013). Recombinant human MAO-A and MAO-B were used as enzyme sources and kynuramine served as enzyme substrate. The enzyme reactions were carried out to a volume of 200 µL in white polypropylene 96-well microtiter plates. The reactions consisted of substrate (50 µM) and the test inhibitors (0.003-100 µM) dissolved in potassium phosphate buffer (pH 7.4, 100 mM). The reaction was initiated with the addition of MAO-A (0.0075 mg/mL) or MAO-B (0.015 mg/mL), and were incubated for 20 min at 37 °C. At endpoint, the reactions were terminated with the addition of 80 µL NaOH (2 N) and the fluorescence was measured (λ_{ex} = 310, λ_{em} = 400). Quantitative estimations of 4-hydroxyquinoline were made by constructing a linear calibration curve (0.047-1.50 µM). From these data, the enzyme catalytic rates were calculated and the IC_{50} values were estimated by constructing sigmoidal plots of enzyme catalytic rate versus the logarithm of inhibitor concentration. For this purpose, the data were fitted to the one-site competition model incorporated into the Prism 5 software package. All IC_{50} values were measured in triplicate and are reported as the mean ± standard deviation (SD).

3.7.6 Determination of reversibility if inhibition by dialysis

Reversibility of inhibition was determined by dialysis. Dialysis was carried out with Slide-A-Lyzer dialysis cassettes with a molecular weight cut-off of 10,000 and a sample volume of 0.5-3 mL. For this purpose, the literature procedure was followed (Petzer *et al.*, 2013). MAO-A or MAO-B (0.03 mg/mL) and the test inhibitors (at concentrations of 4 × IC_{50}) were dissolved in potassium phosphate buffer (100 mM, pH 7.4) containing 5% sucrose, and pre-incubated for 20 min at 37 °C. Stock solutions of the inhibitors were prepared in DMSO and added to yield a final concentration of 4% DMSO. The samples were subsequently dialysed for 24 h at 4 °C with the dialysis buffer [potassium phosphate buffer (100 mM, pH 7.4) containing 5% sucrose] being replaced with fresh buffer at 3 h and 7 h after dialysis was started. As positive controls, MAO-A and MAO-B were pre-incubated and dialysed in the presence of the irreversible inhibitors, pargyline [IC_{50}(MAO-A) = 13 µM] and selegiline [IC_{50}(MAO-B) = 0.079 µM], respectively. The concentrations of the irreversible inhibitors were 4 × IC_{50}. Dialysis of the enzymes was also carried out in the absence of inhibitor and served as negative control (Mostert *et al.*, 2016). After 24 h, the dialysis samples were diluted twofold with the addition kynuramine to yield an inhibitor concentration of 2 × IC_{50} and a kynuramine concentration of 50 µM. These reactions were incubated for 20 min at 37 °C, terminated with the addition of NaOH (2 N) and the fluorescence of the samples was measured as described above. The residual enzyme activities were calculated and expressed as mean ± SD of triplicate determinations. For comparison, undialysed mixtures of the test inhibitors and the MAOs were maintained at 4 °C for the same time period (24 h), and the residual enzyme catalytic rates were measured.

CHAPTER 4

CONCLUSION

Parkinson's disease (PD) is characterised as a progressive neurodegenerative disease with severe motor complications. The motor symptoms includes bradykinesia, rigidity and tremor (Politis *et al.*, 2010). Non-motor symptoms of PD include anxiety, apathy, sleep disturbances, pain and depression. These symptoms may evolve with age and severity of PD (Chaudhuri & Schapira, 2009). The main treatment for PD is with the drug levodopa (L-dopa), the direct metabolic precursor of dopamine. Dopamine is not used as a drug since it does not cross the blood-brain barrier. Once in the brain, the enzyme, aromatic-L-amino-acid decarboxylase converts levodopa to dopamine, and thus increases the concentrations of dopamine in the brain (Calne, 1993). However, problems such as dyskinesia and end of dose failure arises with the use of L-dopa. Other side effects of L-dopa include postural instability, speech disturbances, dysphagia and cognitive decline (Hely *et al.*, 2000). The current treatment for PD is only symptomatic in nature (Calne, 1993).

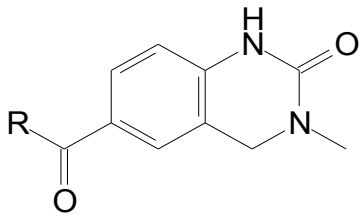
MAO is a flavoenzyme that is attached to the outer membrane of mitochondria (Billett, 2004). These enzymes are responsible for the metabolism of neurotransmitter amines in the central and peripheral tissues. As by-product of catalysis, hydrogen peroxide (H_2O_2) is generated by the MAOs (Shih *et al.*, 1999). MAO exists as two isoforms, MAO-A and MAO-B. The MAO enzymes are distinguished by their preference for different substrates. MAO-A catalyses the oxidation of 5-hydroxytryptamine (serotonin, 5-HT) and norepinephrine, whereas MAO-B oxidises 2-phenylethylamine (PEA) and benzylamine. Dopamine and the dietary amine, tyramine, are oxidised by both MAO-A and MAO-B (Volz & Gleiter, 1998). Human MAO-A has a monopartite active site cavity, whereas MAO-B has a bipartite cavity structure (Kaludercic *et al.*, 2014). MAO inhibitors have been used for the treatment of affective and neurodegenerative diseases. MAO-A inhibitors are used in the treatment of depression and anxiety, while MAO-B inhibitors are employed in the treatment of PD (Gökhan-Kelekçi *et al.*, 2009). The main MAO isoform in the human brain is MAO-B. MAO-B metabolises dopamine to yield 3,4-hydroxyphenylacetic acid (DOPAC) and homovanillic acid (Fernandez & Chen, 2007). MAO-B inhibitors inhibit the oxidative metabolism of dopamine in the brain of patients with PD, and therefore protect against further depletion of the dopamine supply. Furthermore, MAO-B inhibitors are often used in combination with L-dopa in an attempt to extend its action and to lower the dosage of L-dopa required for a therapeutic effect (Legoabe *et al.*, 2012). The predominant isoform in the intestinal tract is MAO-A. MAO-A plays an important role in the metabolism of circulating catecholamines and dietary vasopressors such as tyramine. Selective and non-selective inhibitors of MAO-A may lead to a

potentially fatal hypertensive crisis when combined with tyramine-containing food. This adverse effect occurs when tyramine concentration in the systemic circulation increases and causes the release of norepinephrine from peripheral neurons. With the arrival of reversible MAO-A inhibitors, such as moclobemide, the hypertensive crisis may be avoided. Reversibility allows competition, thus making it possible for ingested tyramine to displace the inhibitor from the enzyme and thus be metabolised normally in the gut and liver (Youdim & Bakhle, 2006). The selective MAO-B inhibitor selegiline does not have this effect with tyramine-containing food since tyramine is not metabolised by MAO-B in the gut (Rojas *et al.*, 2015). MAO-A inhibitors may also lead to a life threatening serotonin syndrome when administered with selective serotonin enhancing drugs (Fernandez & Chen, 2007).

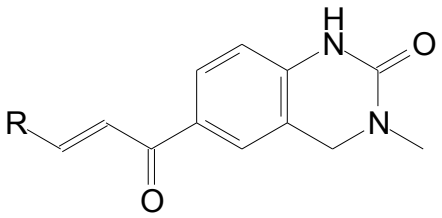
In the present study we aimed to discover MAO inhibitors with selectivity for particularly the MAO-B isoforms. C6- and N1-Substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives were synthesised and evaluated as recombinant human MAO-A and MAO-B inhibitors. Previous studies have reported that 4(3*H*)-quinazolinones act as MAO inhibitors (Gökhan-Kelekçi *et al.*, 2009). The structures of the target 2(1*H*)-quinazolinone derivatives of this study are shown in **table 4.1** and **table 4.2**.

Twenty-six C6-substituted 2(1*H*)-quinazolinone derivatives (**table 4.1**) and eleven N1-substituted 2(1*H*)-quinazolinone derivatives (**table 4.2**) were successfully synthesised. Derivatives **5a**-**m** were synthesised by employing Friedel-Crafts acylation (Dar *et al.*, 2013). Commercially available 3-methyl-3,4-dihydroquinazilon-2(1*H*)-one suspended in carbon disulphide was treated with an appropriate acyl chloride or bromide in the presence of aluminium trichloride. Compounds **6a-m** was synthesised by reacting 6-acetyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one or 3-methyl-6-propionyl-3,4-dihydroquinazolin-2(1*H*)-one with the appropriate benzaldehyde in a mixture of hydrochloric acid (32%) and methanol. The reaction mixtures were refluxed at 50 °C for 24 h. The reactions were terminated with the addition of ice water (30 mL) and the crude obtained was purified by recrystallisation.

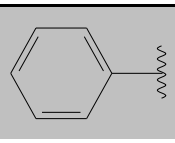
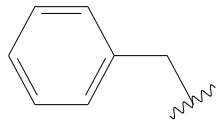
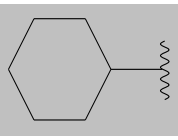
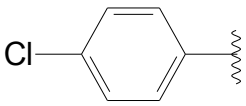
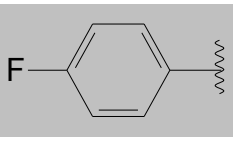
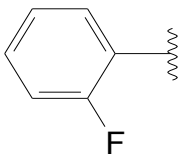
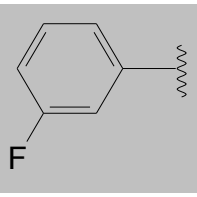
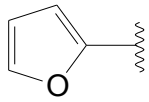
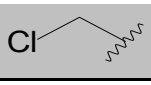
Table 4.1: **The synthesised C6-substituted 2(1*H*)-quinazolinone derivatives which were evaluated as MAO inhibitors in this study.**

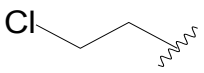
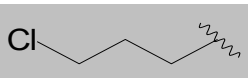
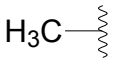
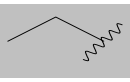
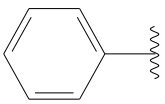
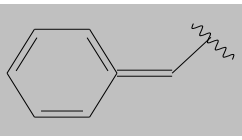
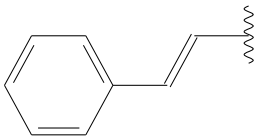
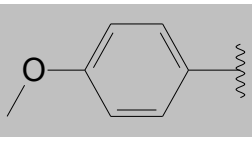
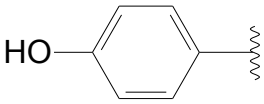
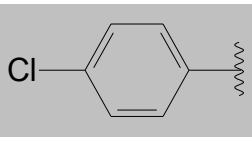
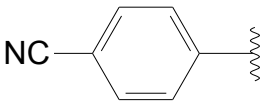
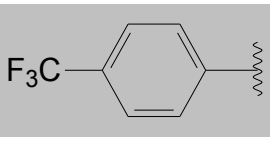
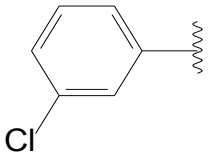
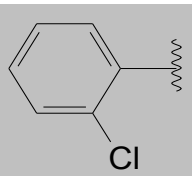


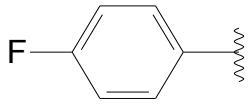
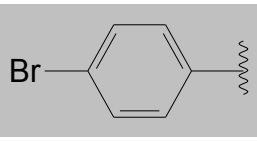
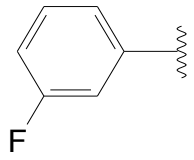
5a – m



6a – m

	R	IC_{50} (μM)[a] MAO A	MAO B	SI[b]
5a		51.7 ± 3.70	30.8 ± 0.638	1.7
5b		50.9 ± 14.3	36.8 ± 1.334	1.4
5c		86.7 ± 4.62	No inh[c]	
5d		24.3 ± 2.84	2.22 ± 0.409	10.9
5e		54.9 ± 27.2	27.2 ± 3.104	2.0
5f		71.6 ± 12.1	20.4 ± 1.410	3.5
5g		50.0 ± 4.774	9.664 ± 0.897	5.2
5h		No inh[c]	No inh[c]	
5i		No inh[c]	No inh[c]	

5j		86.2 ± 16.2	No inh[c]	
5k		No inh[c]	No inh[c]	
5l		No inh[c]	No inh[c]	
5m		No inh[c]	No inh[c]	
6a		No inh[c]	1.06 ± 0.066	
6b		7.43 ± 0.178	2.544 ± 0.103	2.9
6c		24.7 ± 2.36	0.269 ± 0.071	91.8
6d		No inh[c]	3.72 ± 0.044	
6e		24.6 ± 3.80	3.16 ± 0.460	7.8
6f		78.1 ± 9.16	0.607 ± 0.034	128.7
6g		8.86 ± 0.532	1.23 ± 0.289	7.2
6h		11.1 ± 1.63	0.406 ± 0.024	27.3
6i		44.2 ± 7.10	0.350 ± 0.030	126.3
6j		52.6 ± 2.93	0.416 ± 0.029	126.4

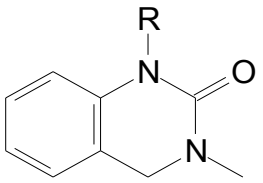
6k		No inh[c]	0.446 ± 0.082	
6l		21.8 ± 0.566	1.01 ± 0.101	21.6
6m		No inh[c]	0.513 ± 0.031	

[a] All values are expressed as the mean ± standard deviation (SD) of triplicate determinations.

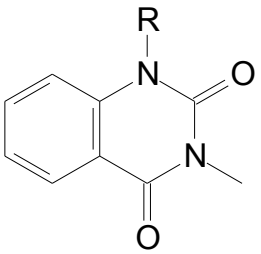
[b] Selectivity index (SI) = IC_{50}(MAO-A)/IC_{50}(MAO-B).

[c] No inhibition observed at a maximal tested concentration of 100 μM.

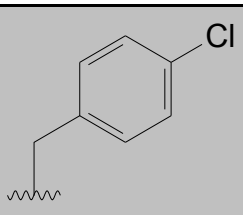
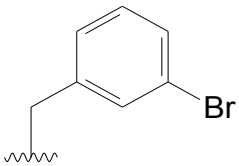
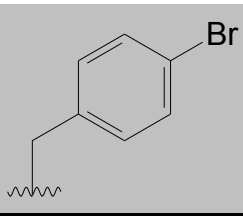
Table 4.2: The synthesised N1-substituted 2(1*H*)-quinazolinone derivatives which were evaluated as MAO inhibitors in this study.

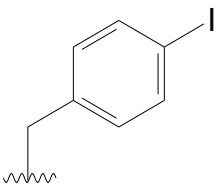
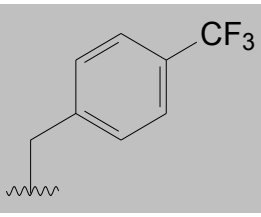
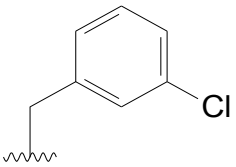
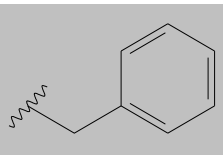
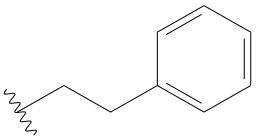
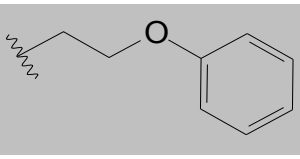
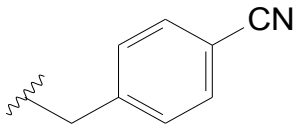
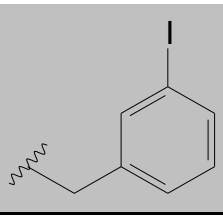


7a-e



8a-f

	R	IC_{50} (μM)[a] MAO A	MAO B
7a		86.7 ± 4.97	No inh[c]
7b		50.2 ± 7.22	No inh[c]
7c		47.9 ± 3.17	No inh[c]

7d		55.8 ± 5.12	No inh[c]
7e		No inh[c]	No inh[c]
8a		No inh[c]	No inh[c]
8b		No inh[c]	No inh[c]
8c		No inh[c]	No inh[c]
8d		No inh[c]	No inh[c]
8e		No inh[c]	No inh[c]
8f		No inh[c]	No inh[c]

[a] All values are expressed as the mean ± standard deviation (SD) of triplicate determinations.

[c] No inhibition observed at a maximal tested concentration of 100 μM.

The N1-substituted derivatives were synthesised by reacting 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one, dissolved in anhydrous dimethylformamide, with the appropriate alkyl bromide or chloride at 0 °C in the presence of sodium hydride. After completion, the reaction mixtures were extracted to ethyl acetate and the crude was purified by recrystallisation. Compounds **7b**, **7c**, **7e**, **8a** and **8c** were purified by column chromatography. The structures of the target compounds were verified by ^{1}H NMR, ^{13}C NMR and MS, and purities were estimated by HPLC analyses. Based on

the physical characterisations, it was concluded that the synthesised compounds corresponded with the proposed structures.

The 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives were evaluated as inhibitors of the recombinant human MAO-A and MAO-B enzymes. Kynuramine was employed as a MAO A/B mixed substrate to measure the MAO activities. Kynuramine is oxidised to yield 4-hydroxyquinoline, a fluorescent metabolite. Potassium phosphate buffer (100 mM, pH 7.4, made isotonic with KCl) was used as a reaction buffer. The reactions contained kynuramine, the test inhibitors at concentrations of 0.003–100 μM and the MAO enzymes. The reactions were incubated for 20 min at 37 °C and then terminated by the addition of NaOH (2 N). Concentration measurements of 4-hydroxyquinoline were carried out by fluorescence spectrophotometry (λ_{ex} = 310, λ_{em} = 400). Sigmoidal dose-response curves were constructed from the MAO activity measurements in the presence of the test inhibitors, and IC_{50} values were calculated.

The results show that the C6-substituted derivatives are specific inhibitors of the MAO-B isoform. The N1-substituted derivatives were found to be inactive against MAO-B, displaying only weak activity for MAO-A. The most potent inhibitor amongst both series was 3-methyl-6-((2*E,*4*Z*)-5-phenylpenta-2,4-dienoyl)-3,4-dihydroquinazolin-2(1*H*)-one (**6c**) with an IC_{50} value of 0.269 μM for the inhibition of MAO-B. Compound **6b** displayed the highest inhibition potency for MAO-A (7.43 μM). It was thus shown that substitution on the C6 position of the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one moiety yields compounds which are more potent MAO-B inhibitors compared to substitution on the N1 position.

Compounds **6b** and **6c**, were used to evaluate the reversibility of MAO-A and MAO-B inhibition, respectively. The results indicate that **6b** acts as a reversible inhibitor of MAO-A and compound **6c** acts as a reversible inhibitor of MAO-B. A set of Lineweaver-Burk plots were constructed, of which the lines were linear and intersected on the y-axis. This suggested that compounds **6b** and **6c** are competitive inhibitors of human MAO A and MAO-B, respectively.

Based on this study, it may be concluded that the C6-substituted 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives are reversible MAO-B specific inhibitors and suitable lead compounds for the development of drugs for the treatment of PD. Based on the discussion and results in this dissertation, it may be concluded that the objective of this study has been achieved. In addition, the hypothesis of this study has also been proven: 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one derivatives are an appropriate scaffold for the design of selective MAO inhibitors. Such compounds are promising leads for the treatment of PD and depressive illness.

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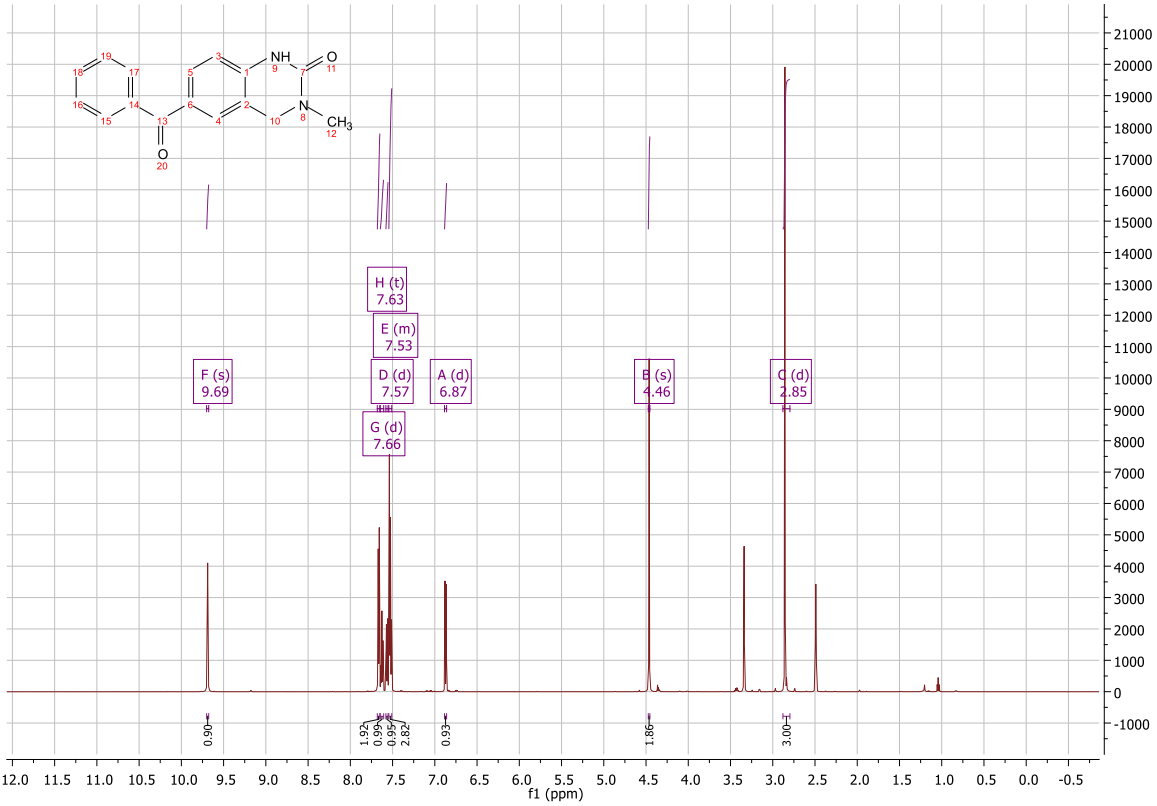
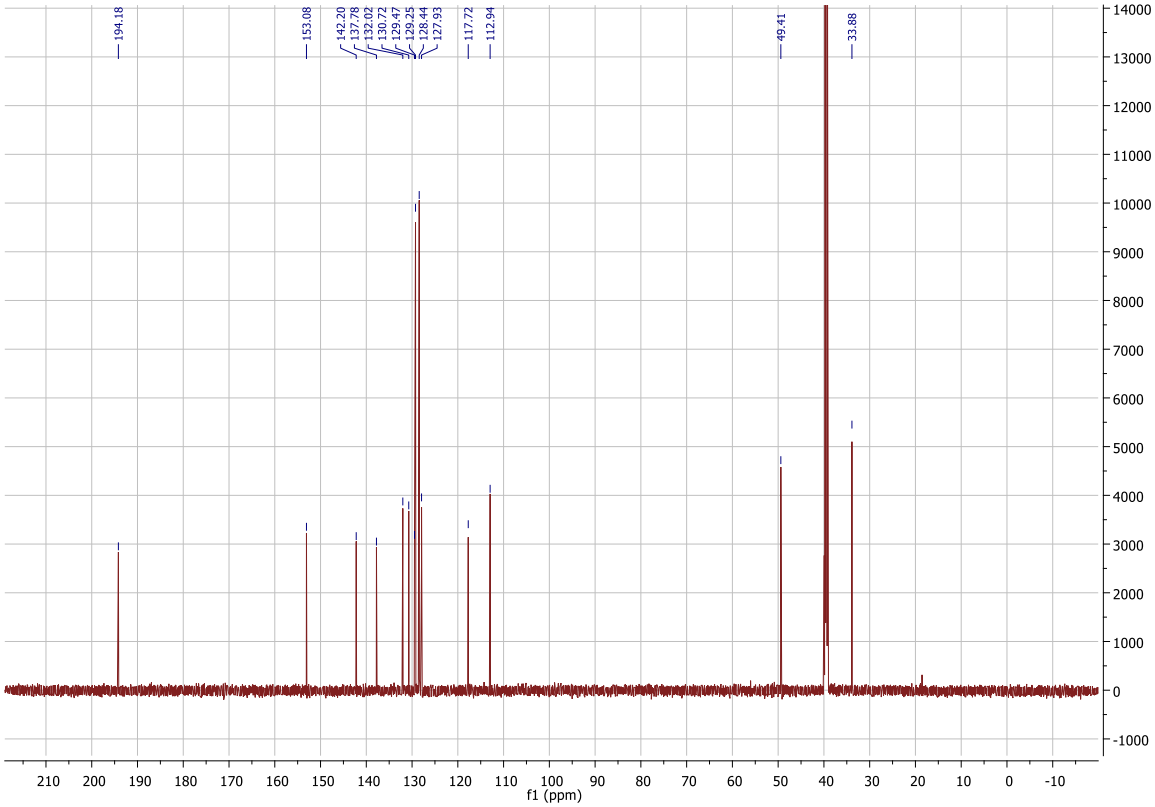
ANNEXURE A

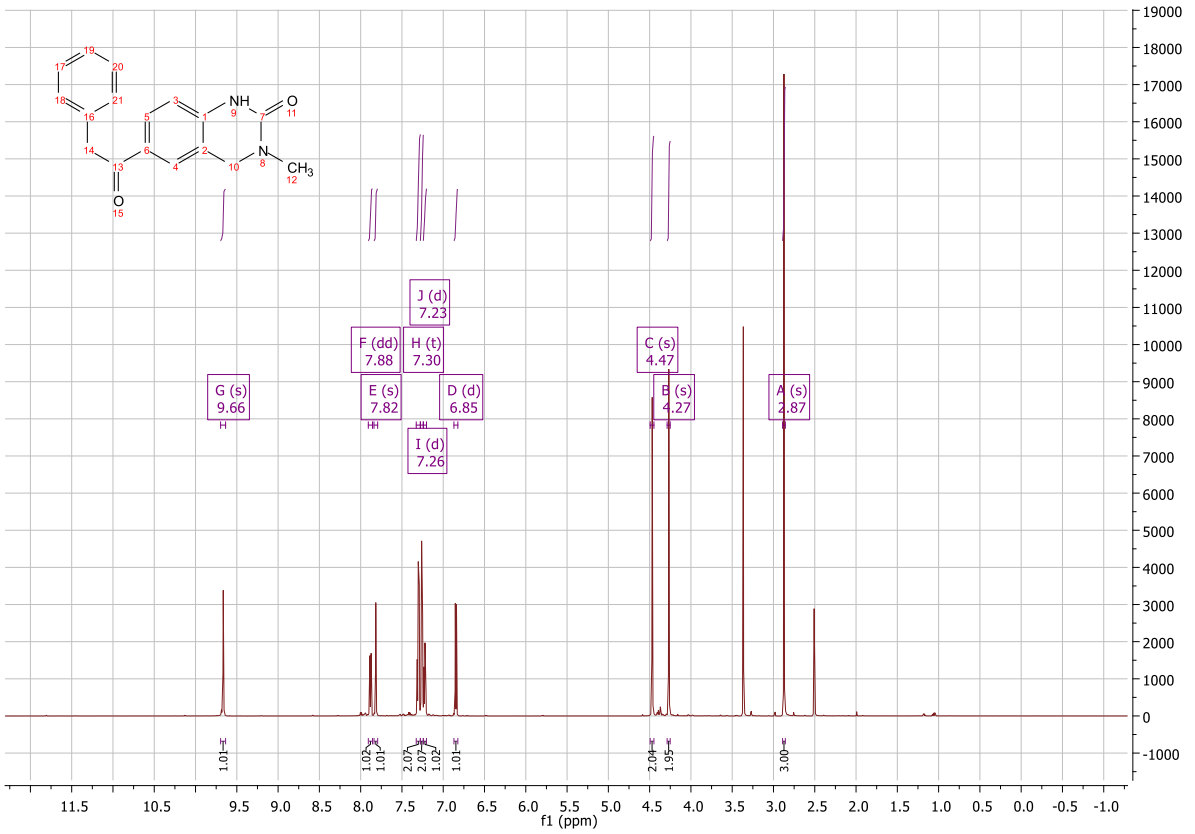
LIST OF ^{1}H-NMR AND ^{13}C-NMR SPECTRA

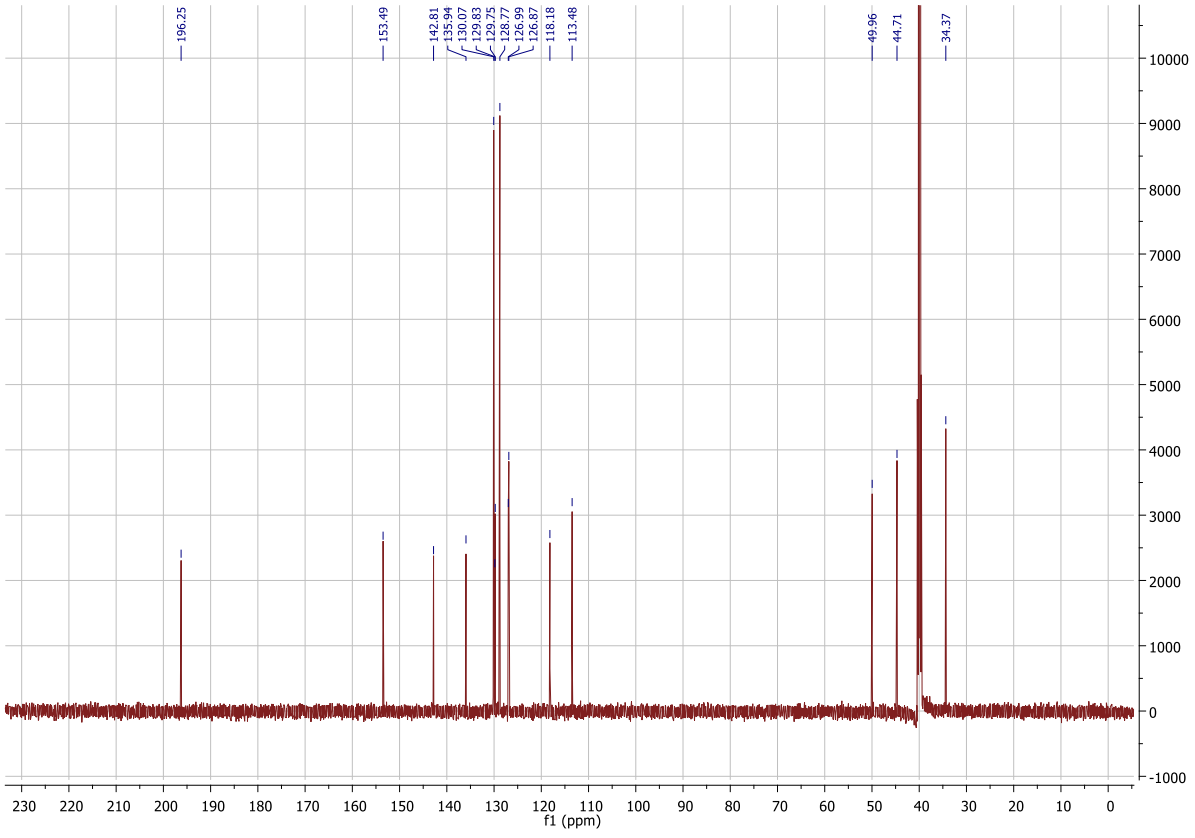
5a: 6-Benzoyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



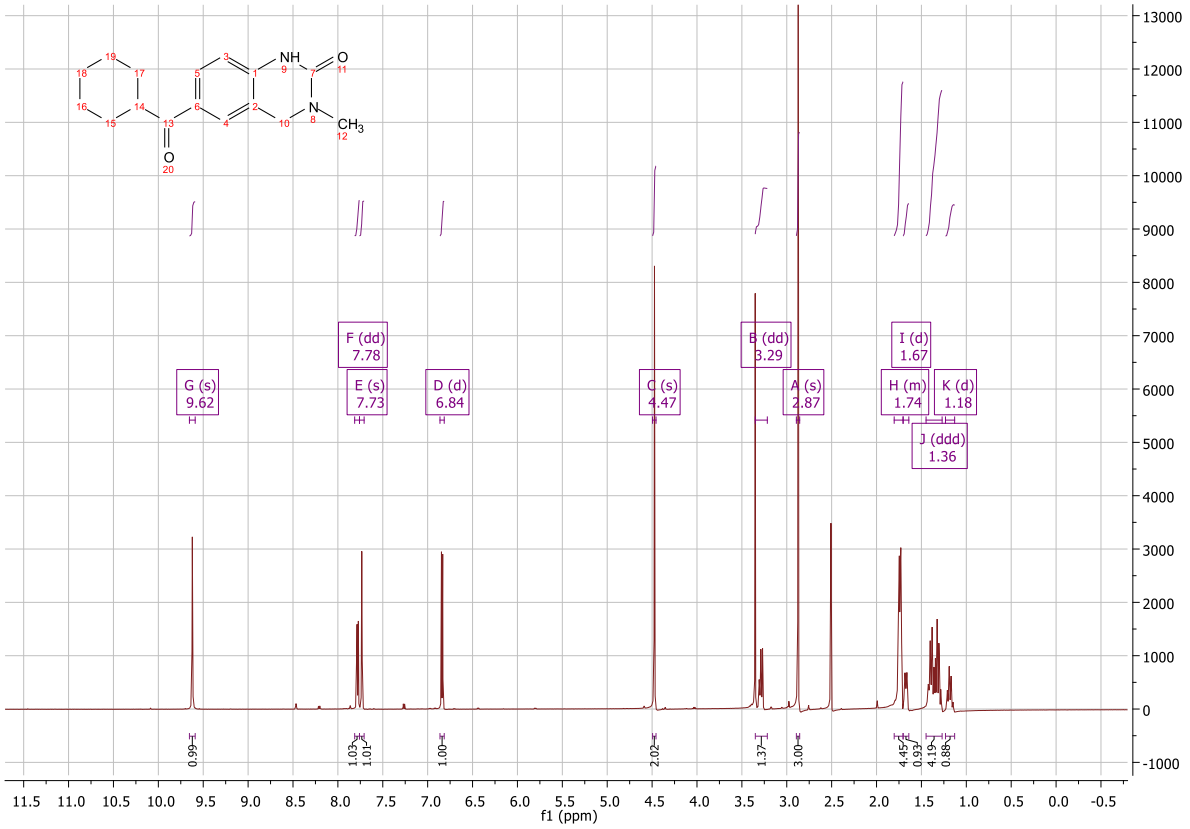


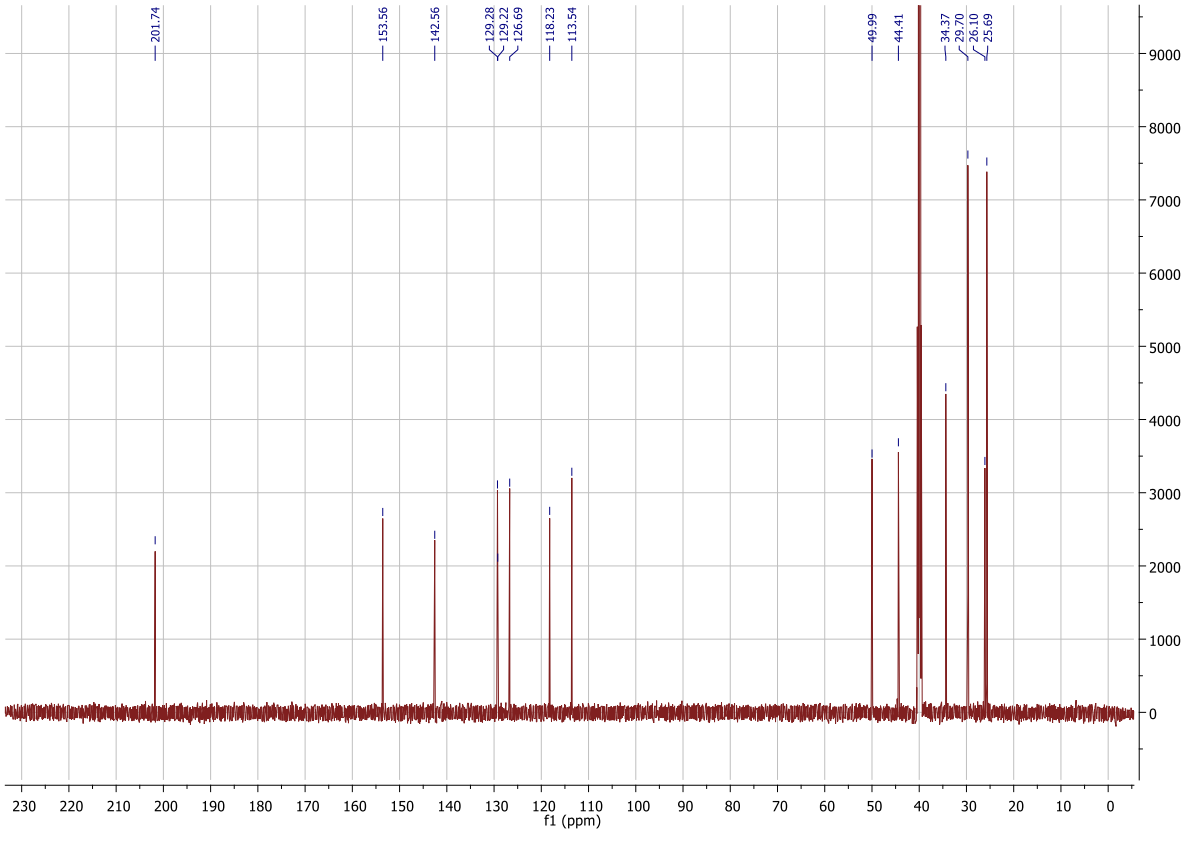
5b: 3-Methyl-6-(2-phenylacetyl)-3,4-dihydroquinazolin-2(1*H*)-one



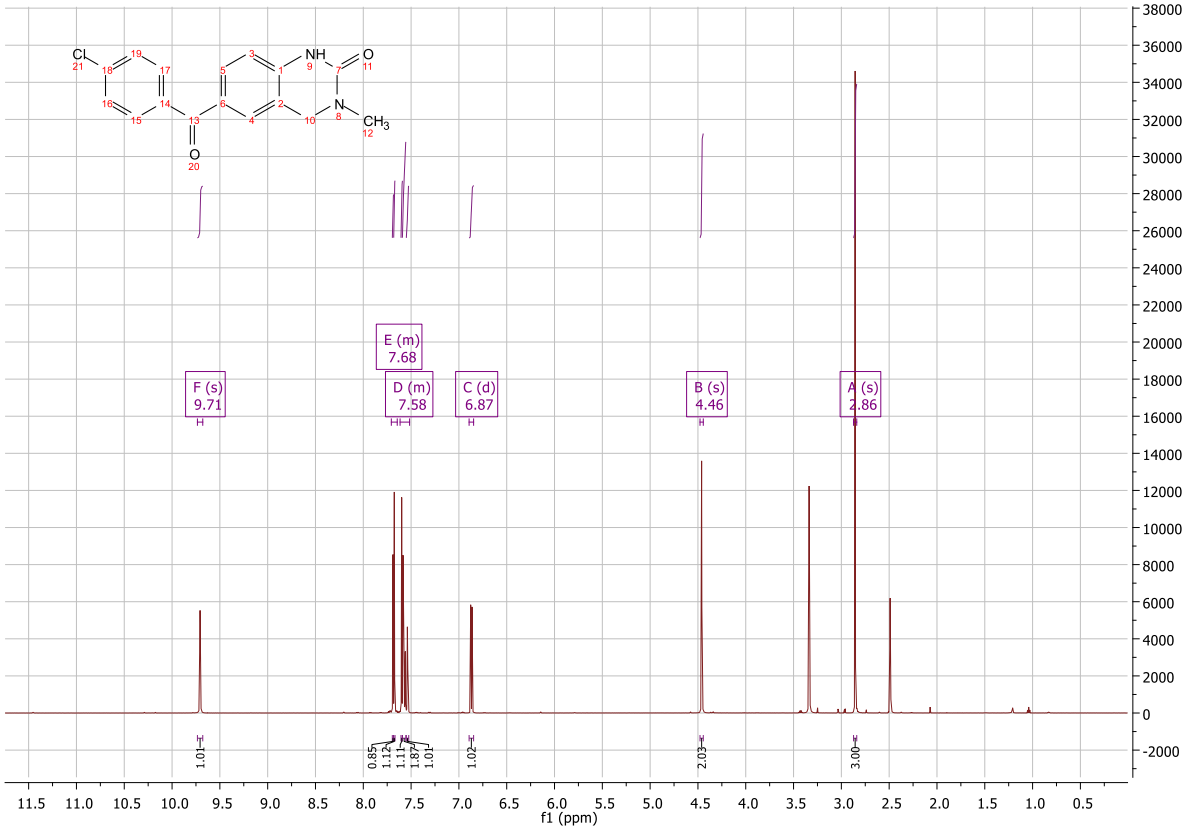


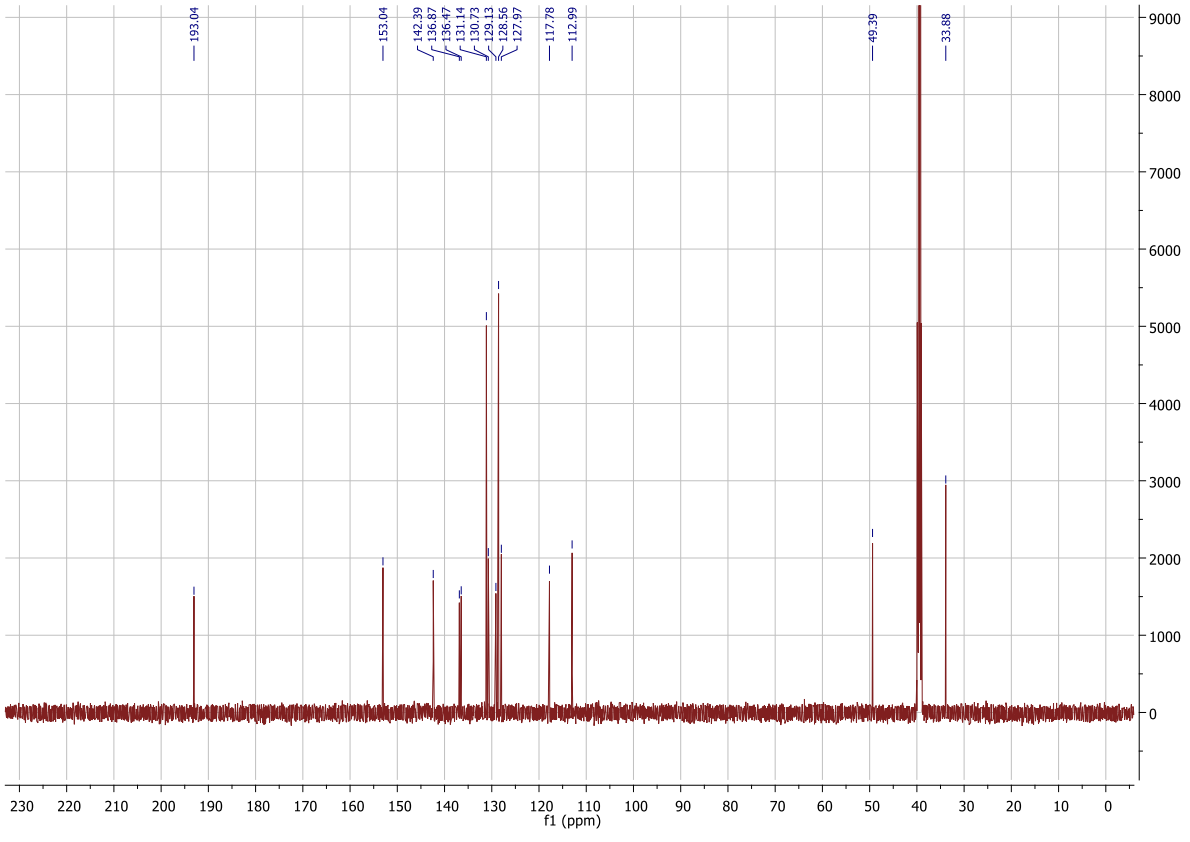
5c: 6-(Cyclohexanecarbonyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



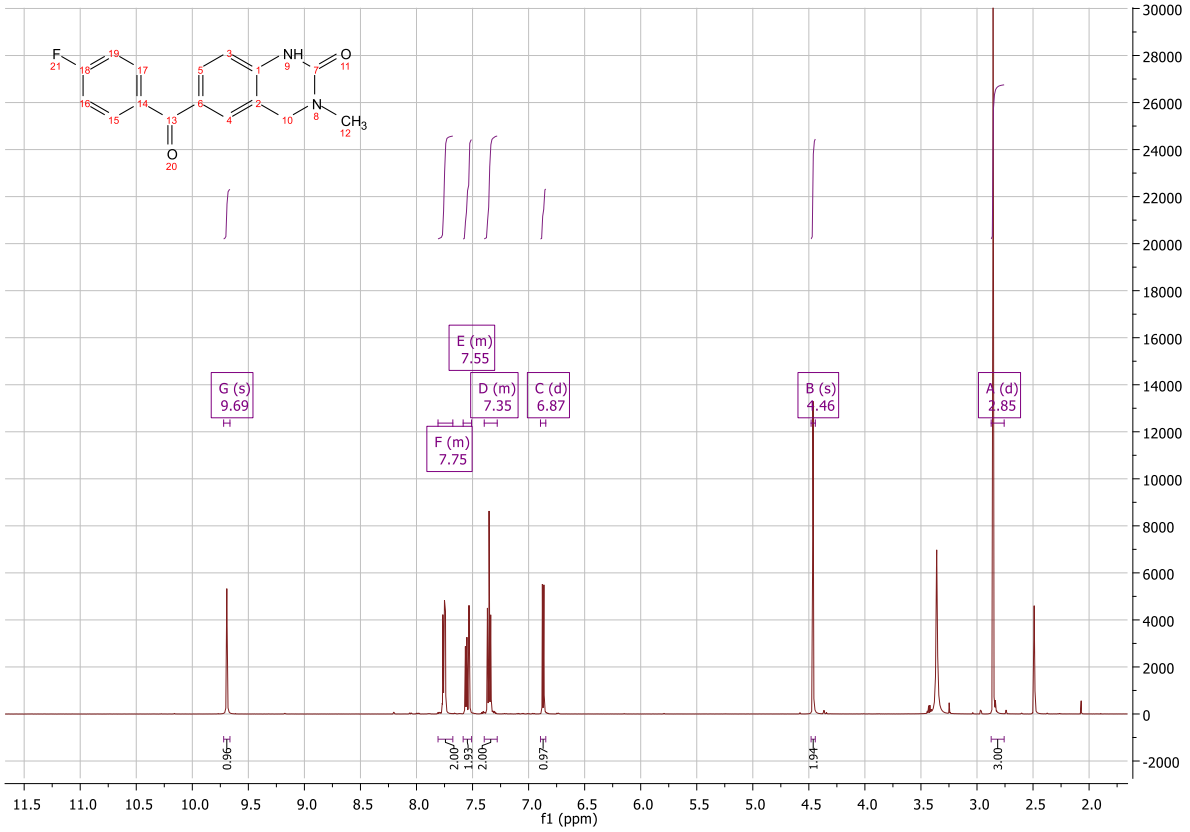


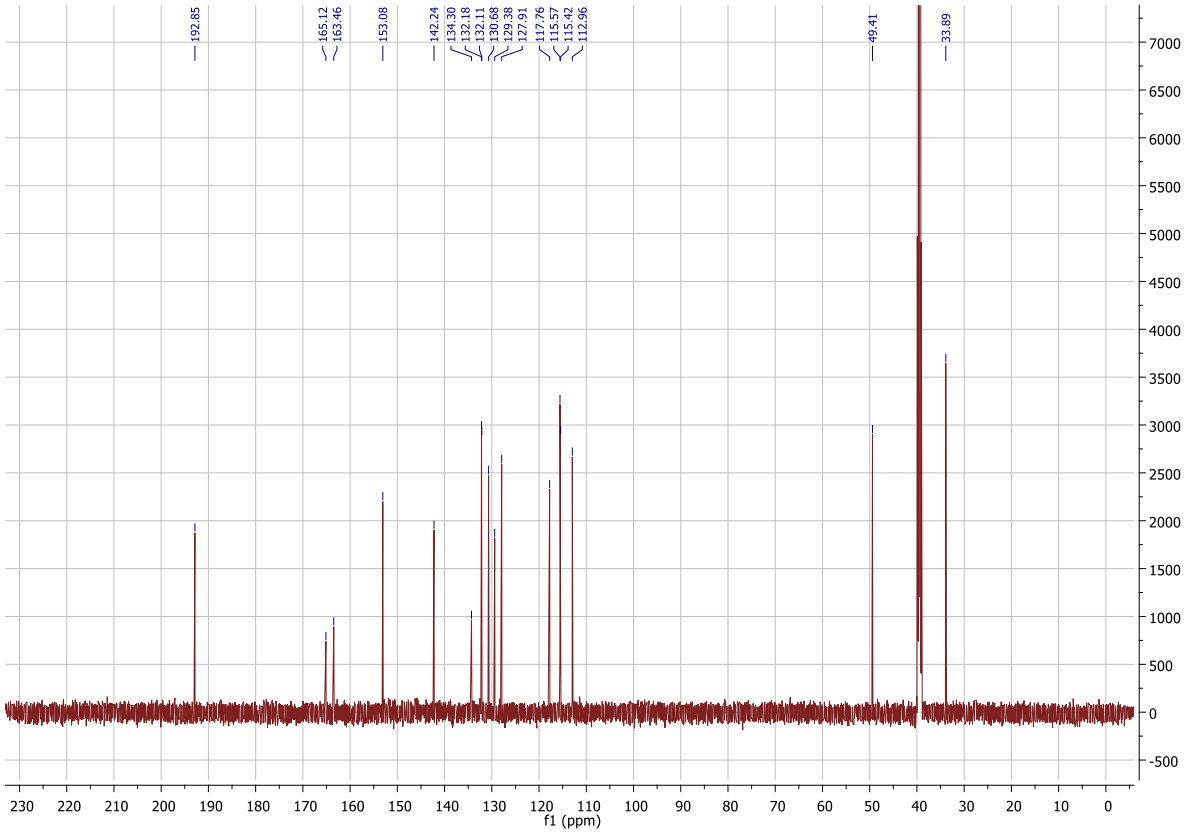
5d: 6-(4-Chlorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



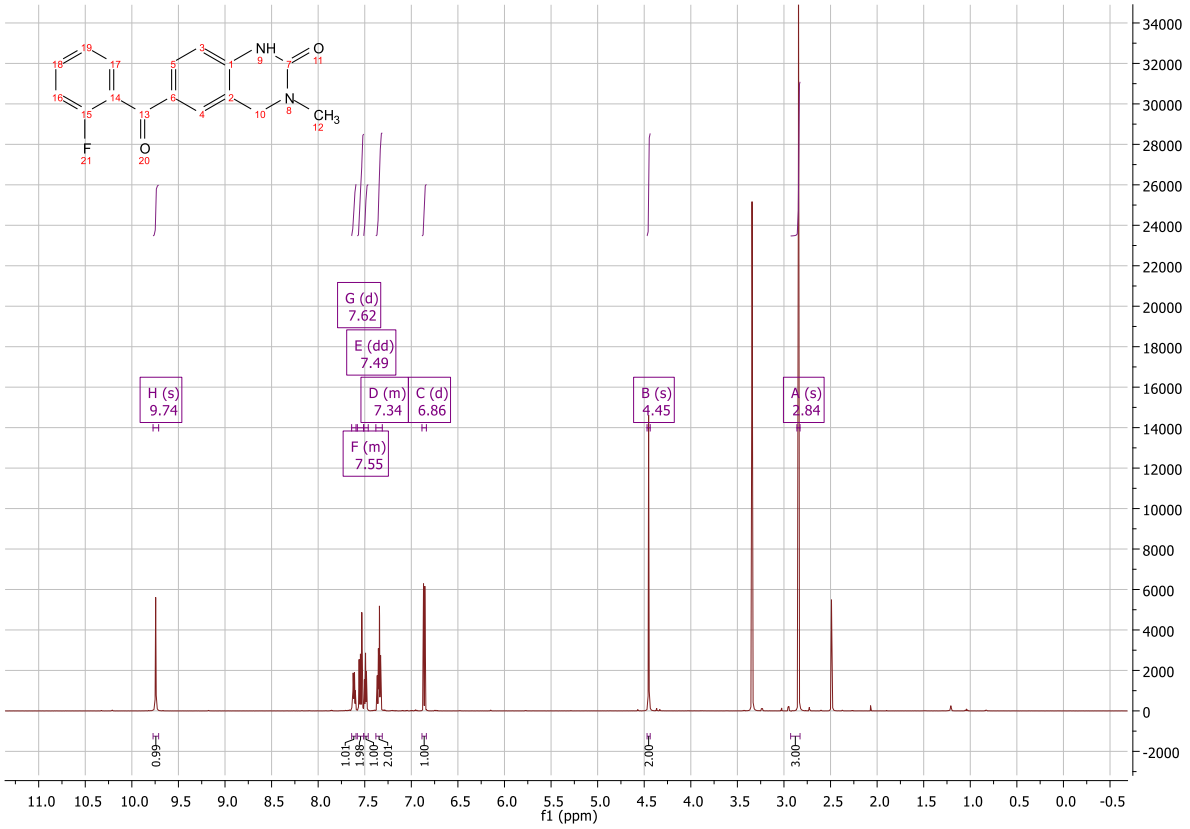


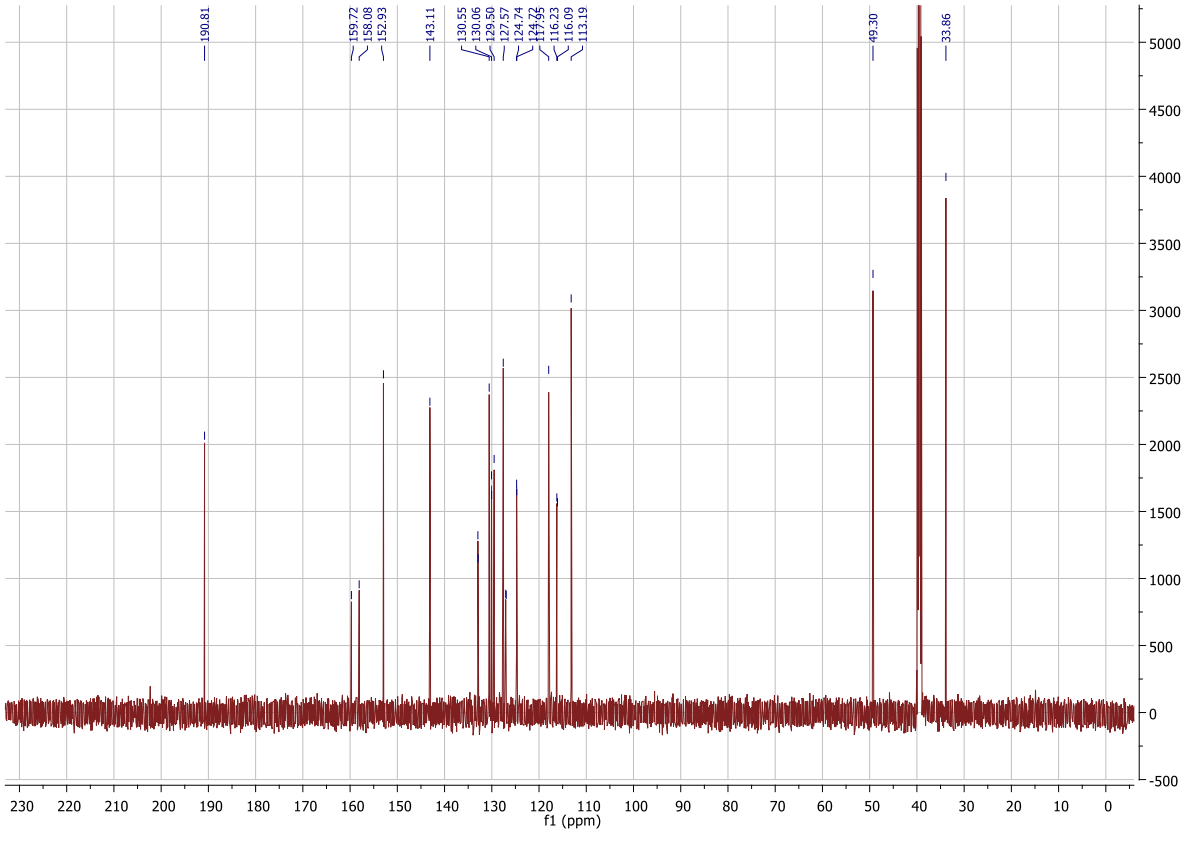
5e: 6-(4-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



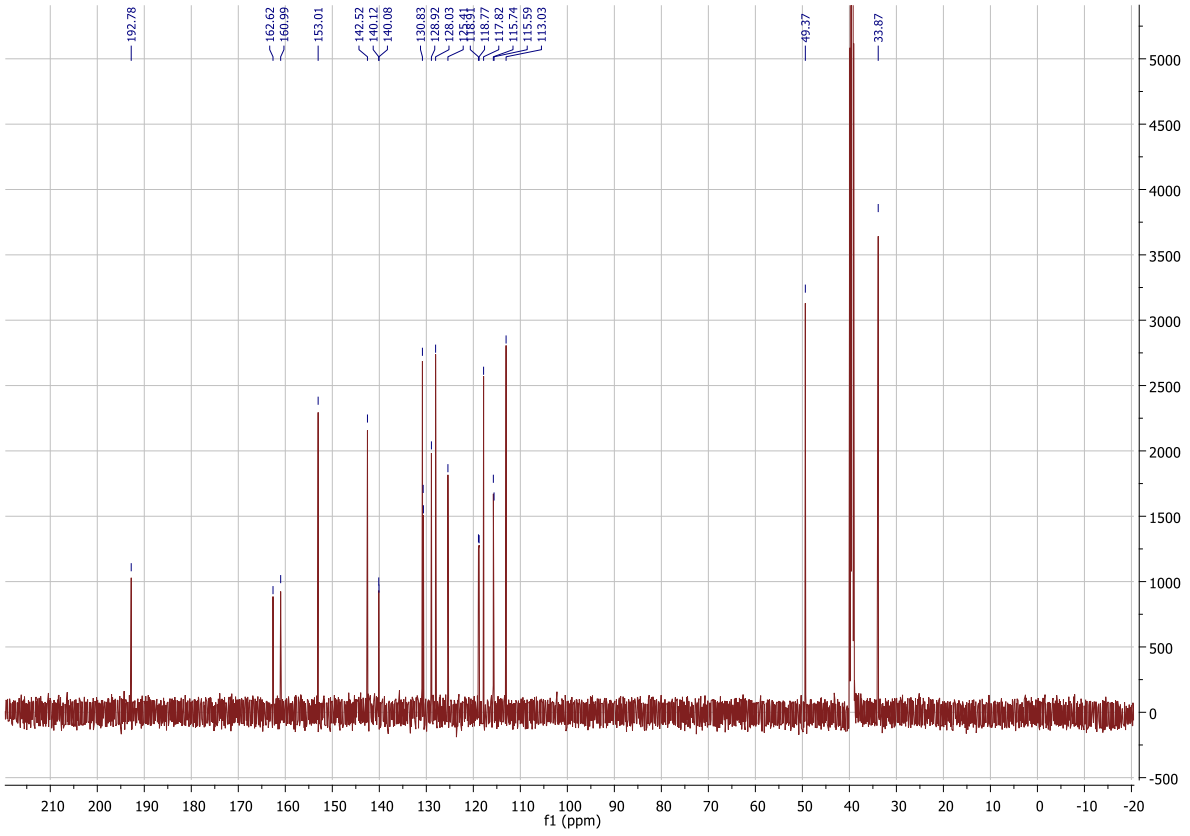


5f: 6-(2-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one

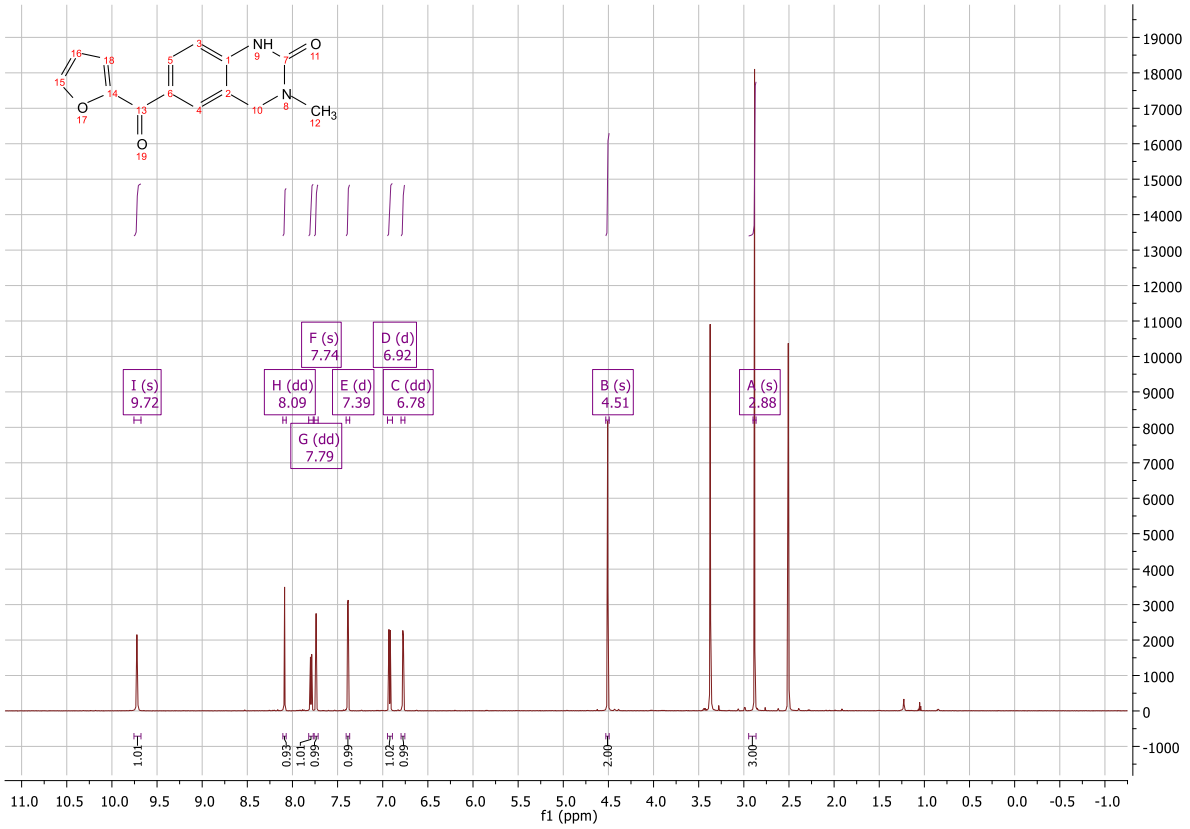


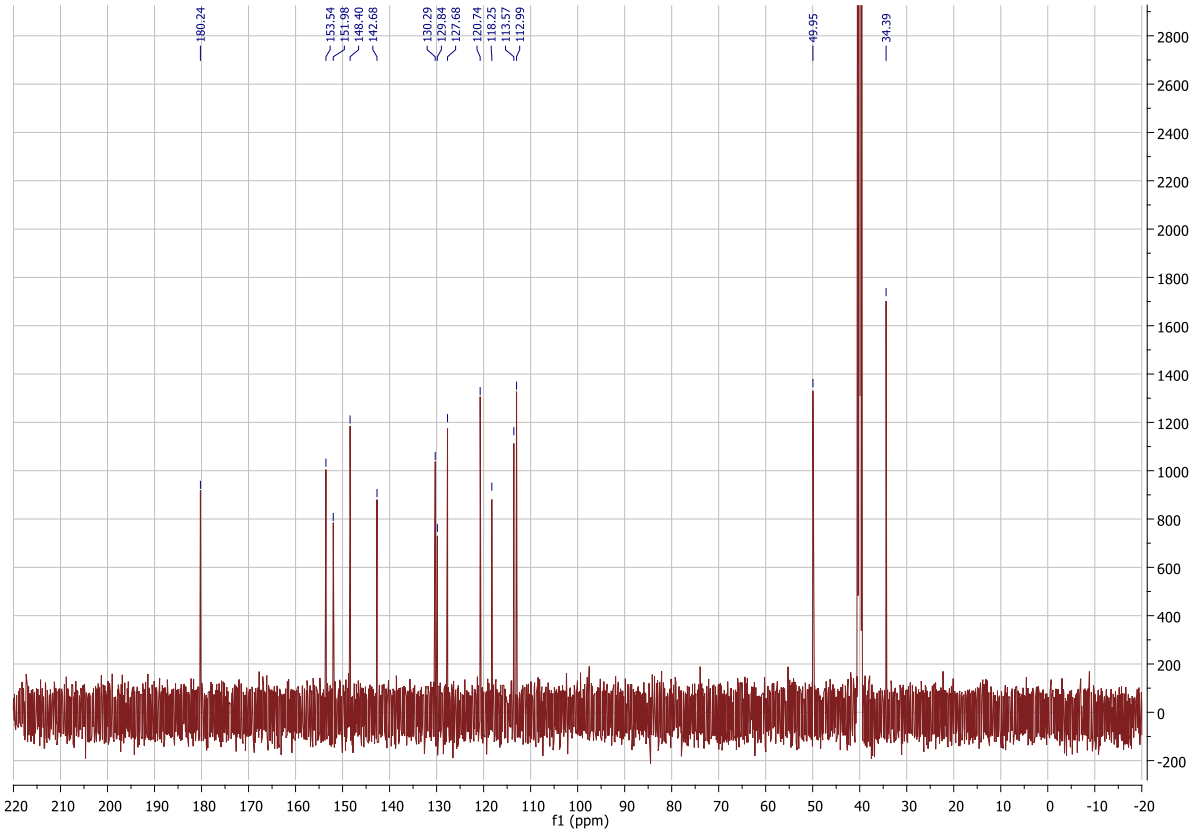


5g: 6-(3-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one

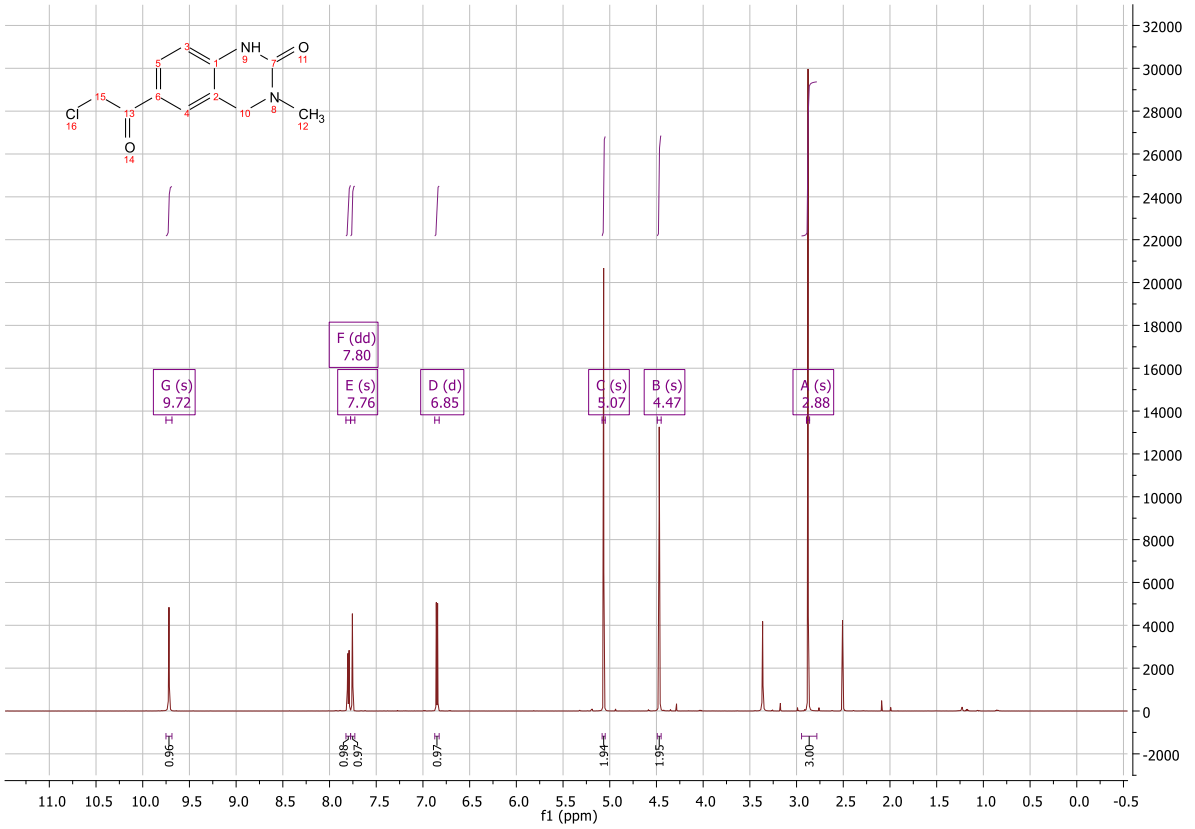


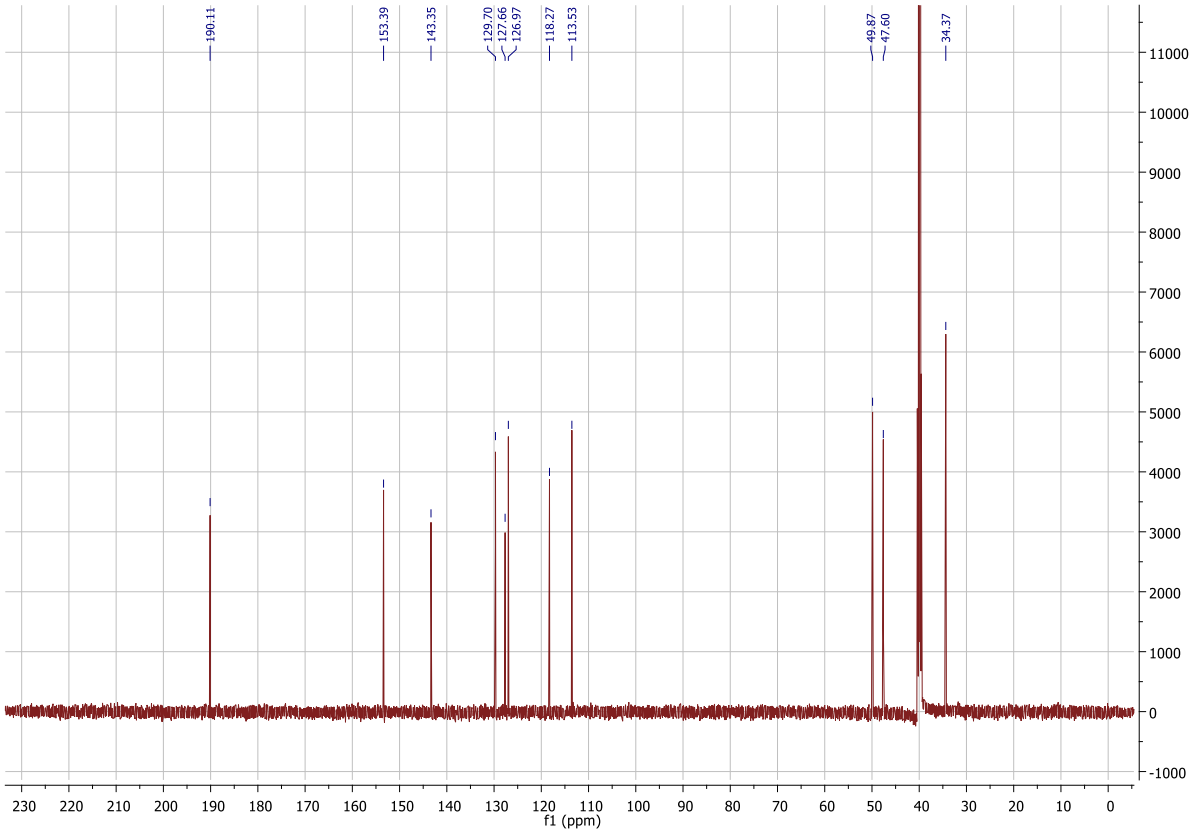
5h: 6-(Furan-2-carbonyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



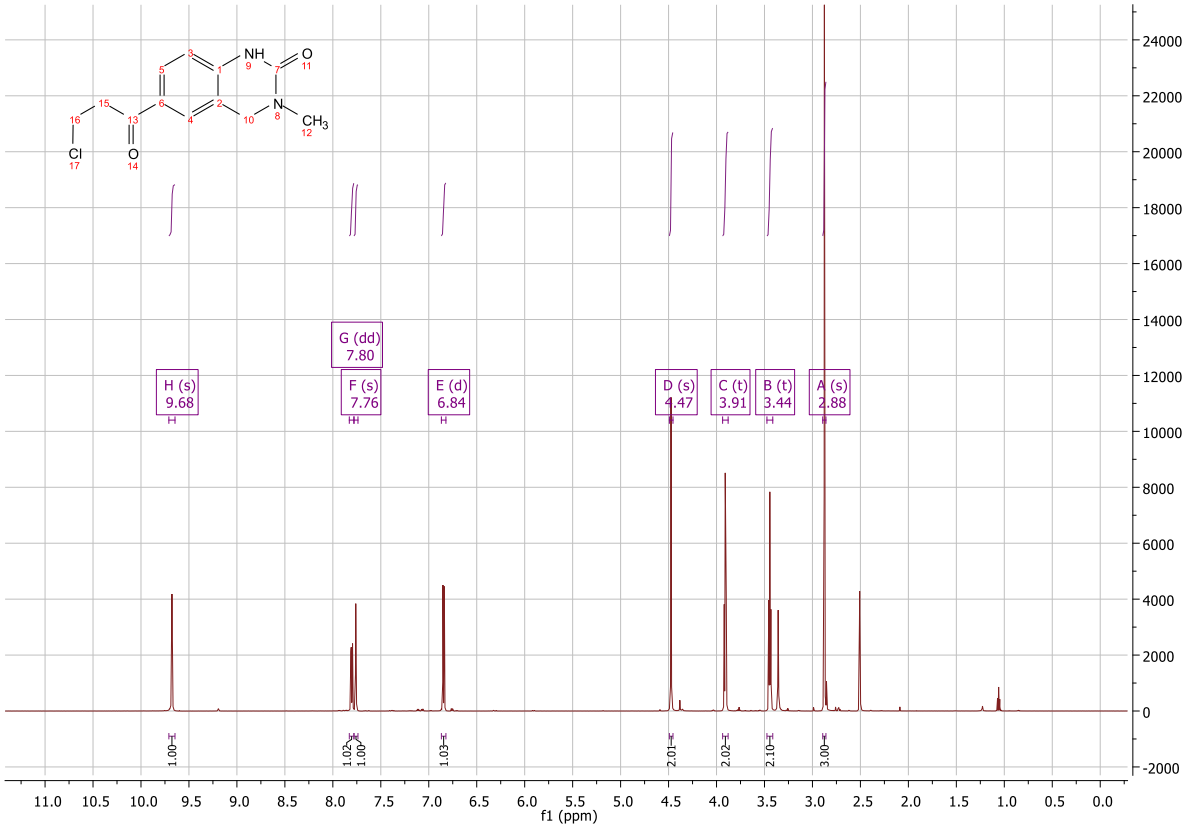


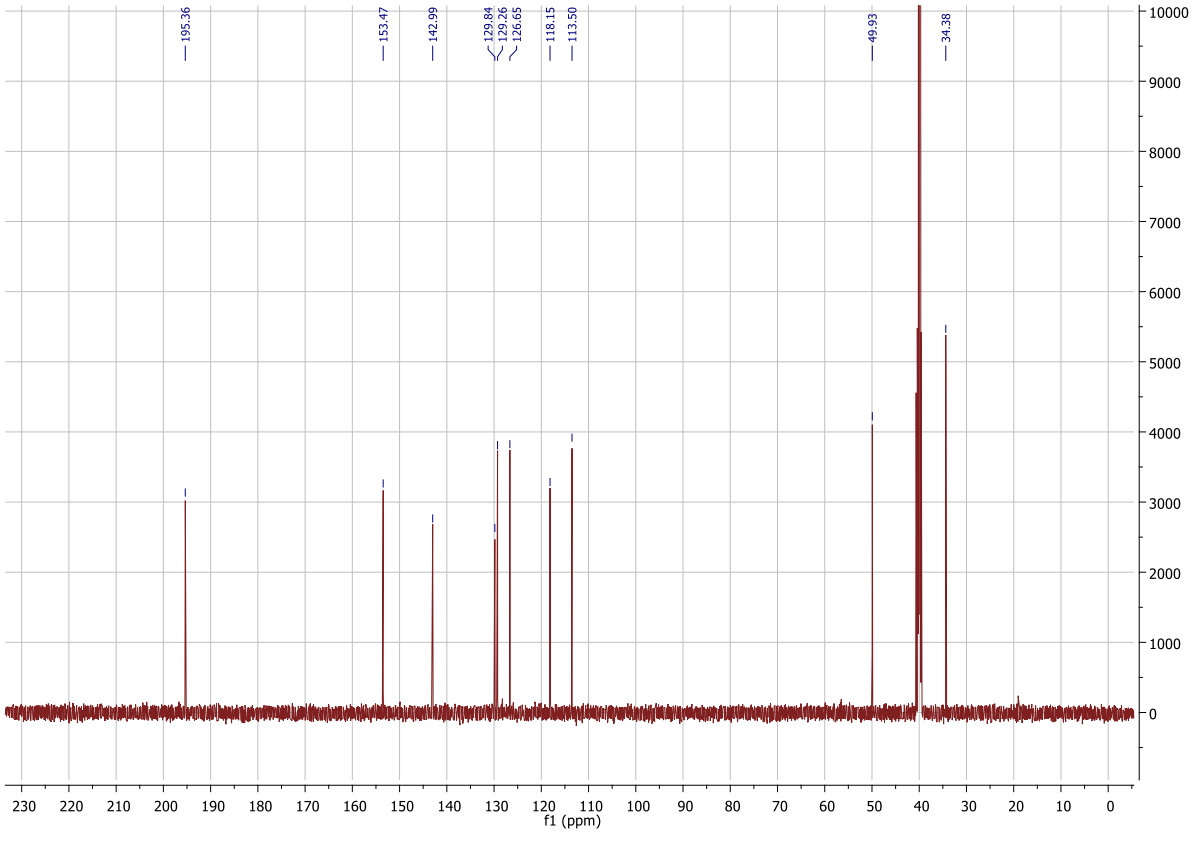
5i: 6-(2-Chloroacetyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



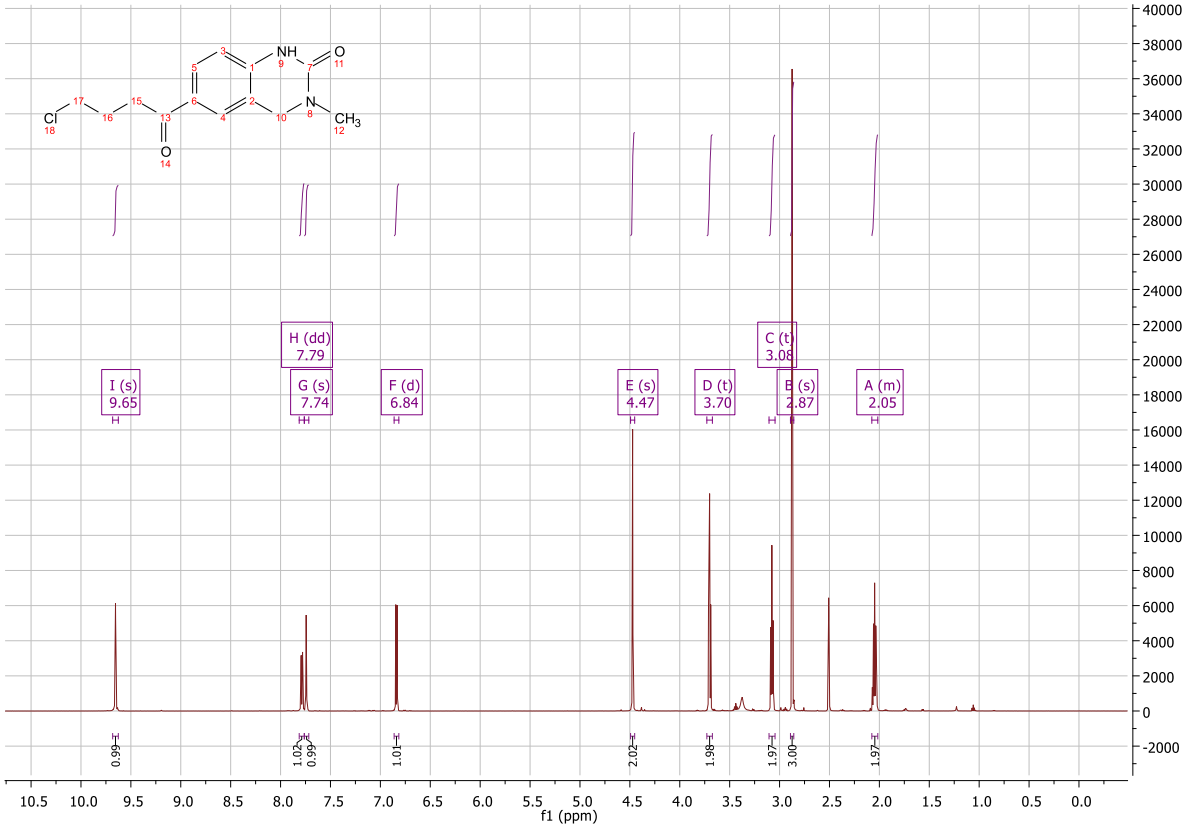


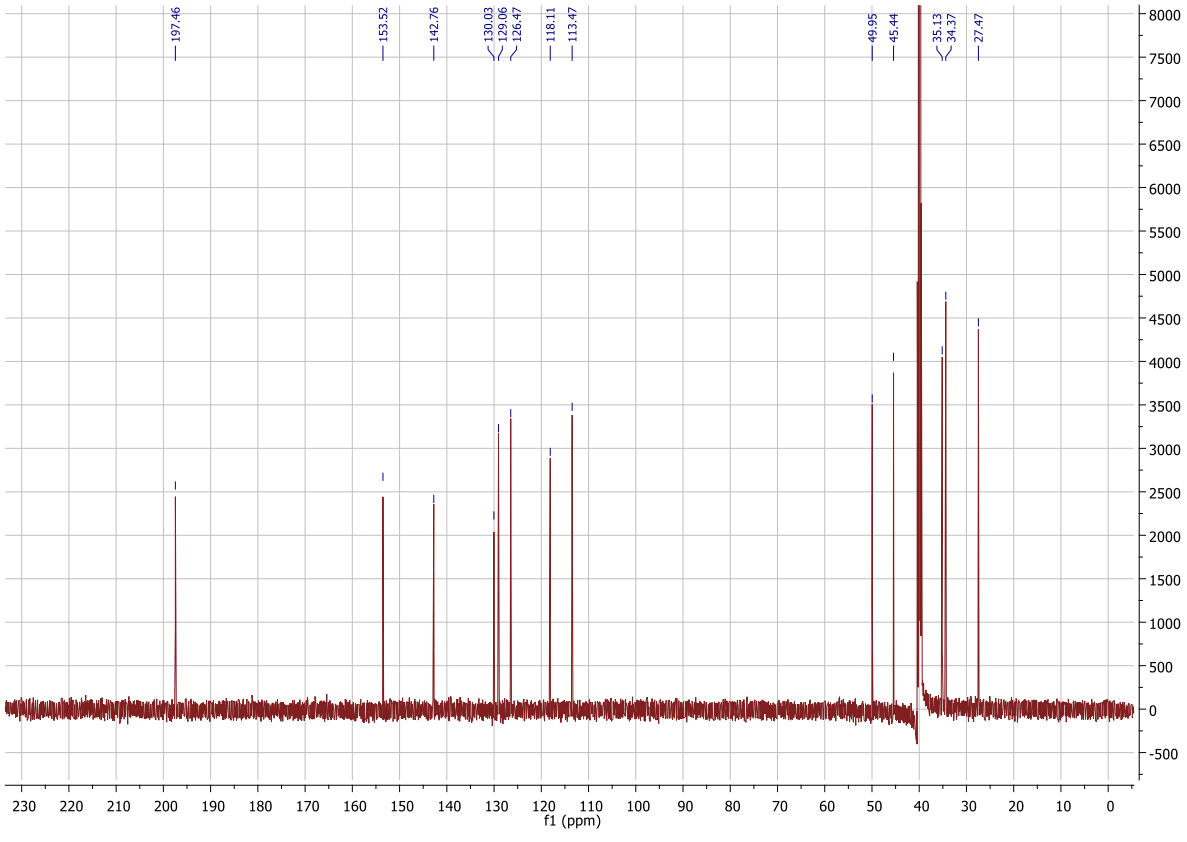
5j: 6-(3-Chloropropanoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



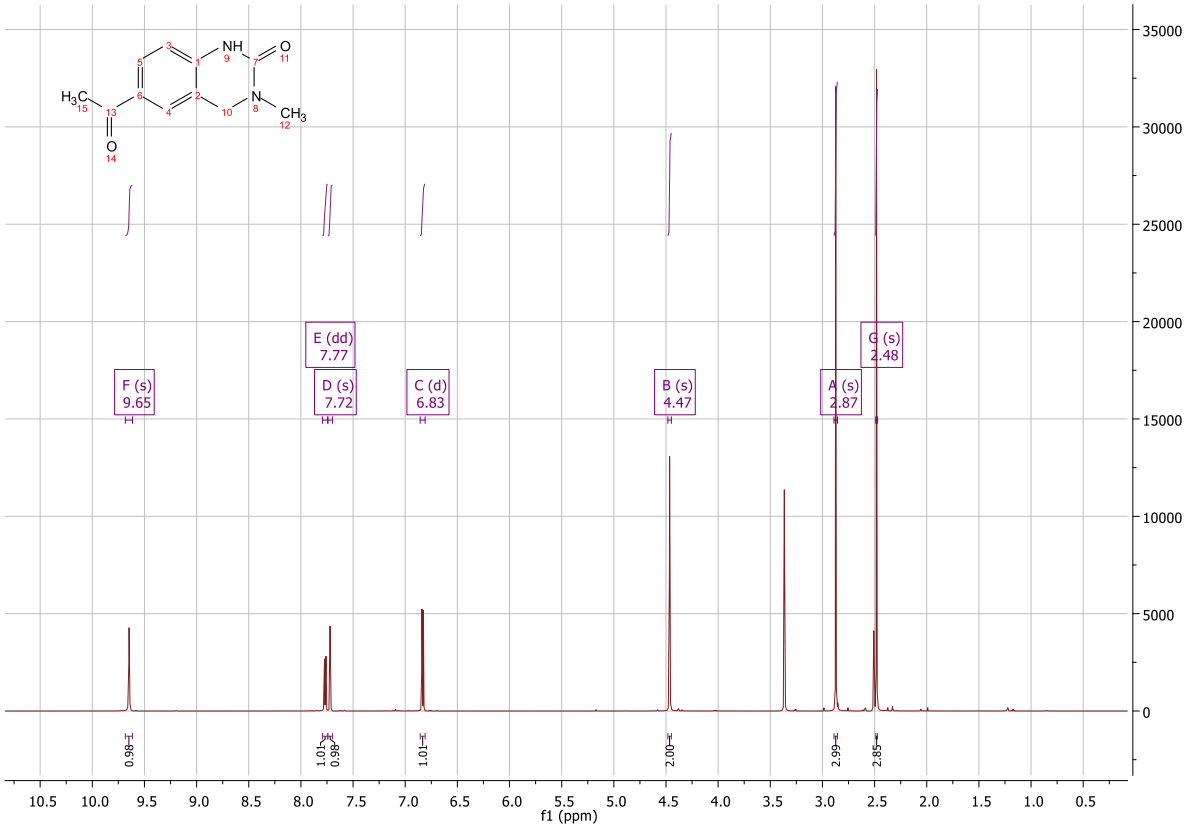


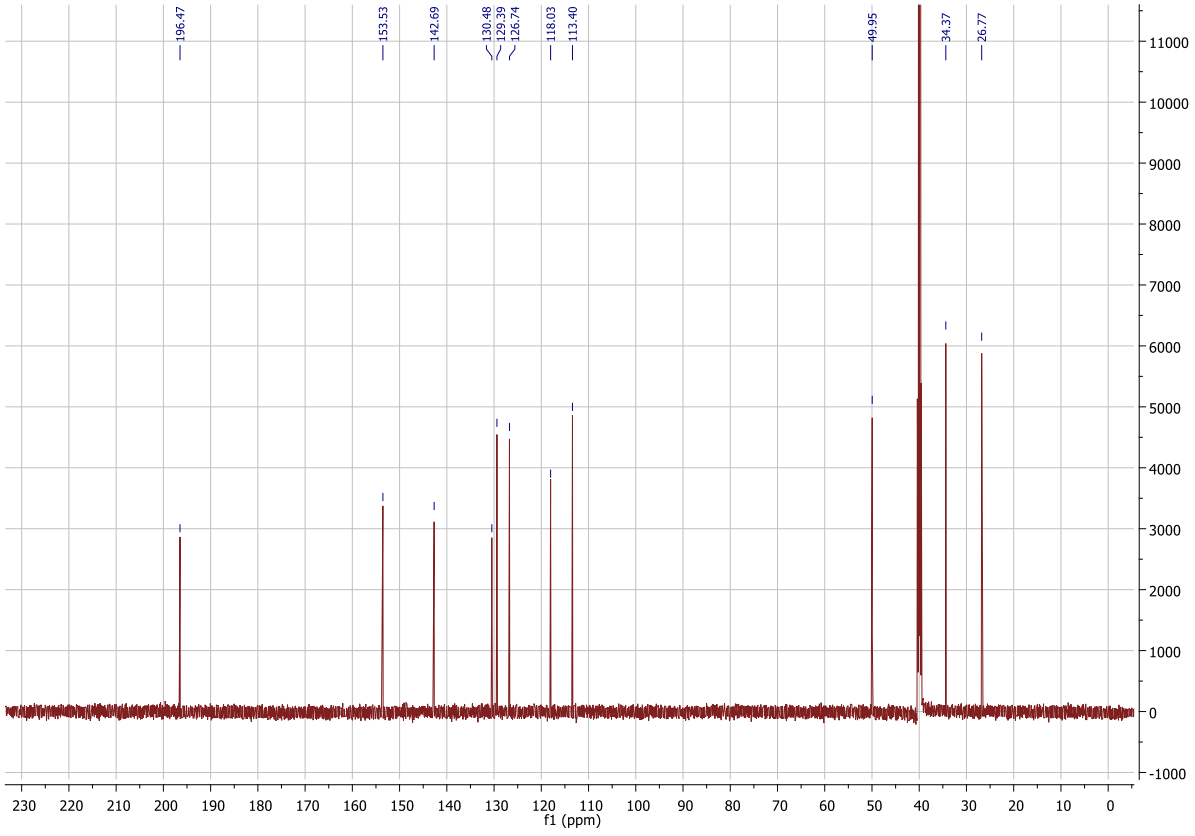
5k: 6-(4-Chlorobutanoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



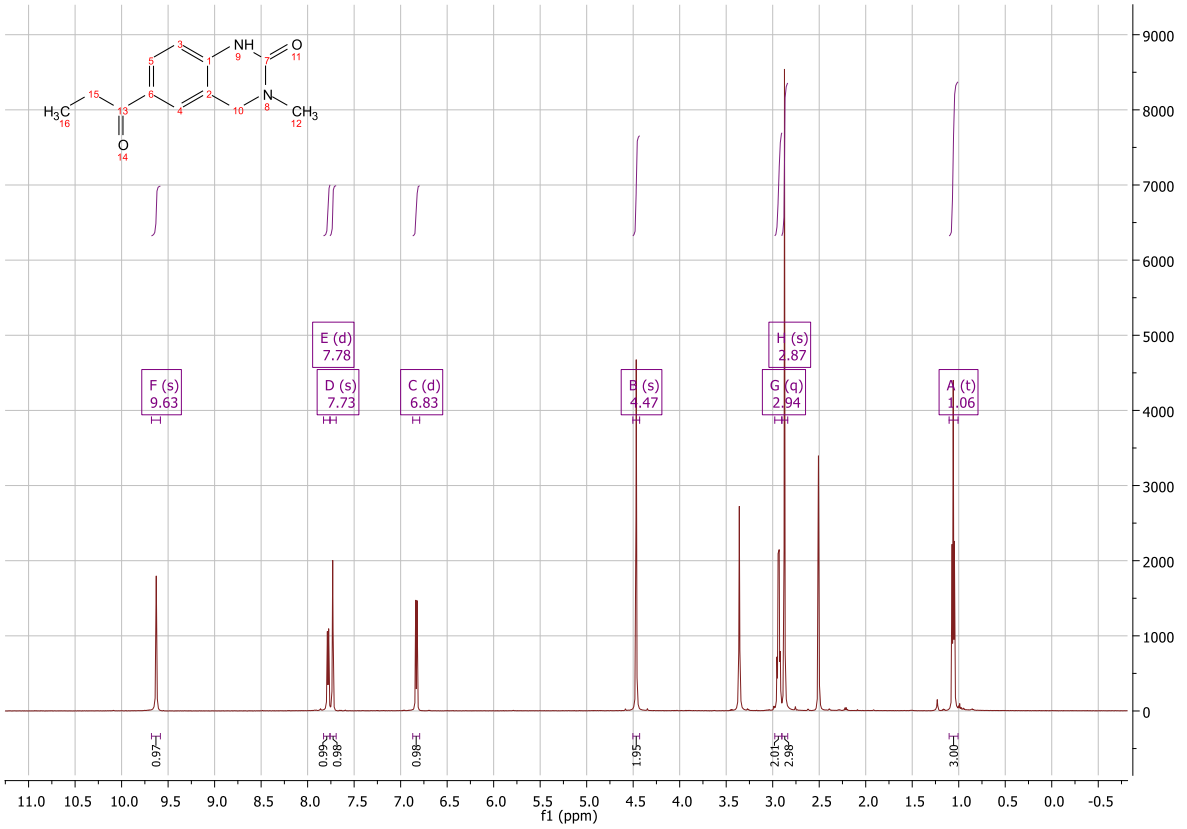


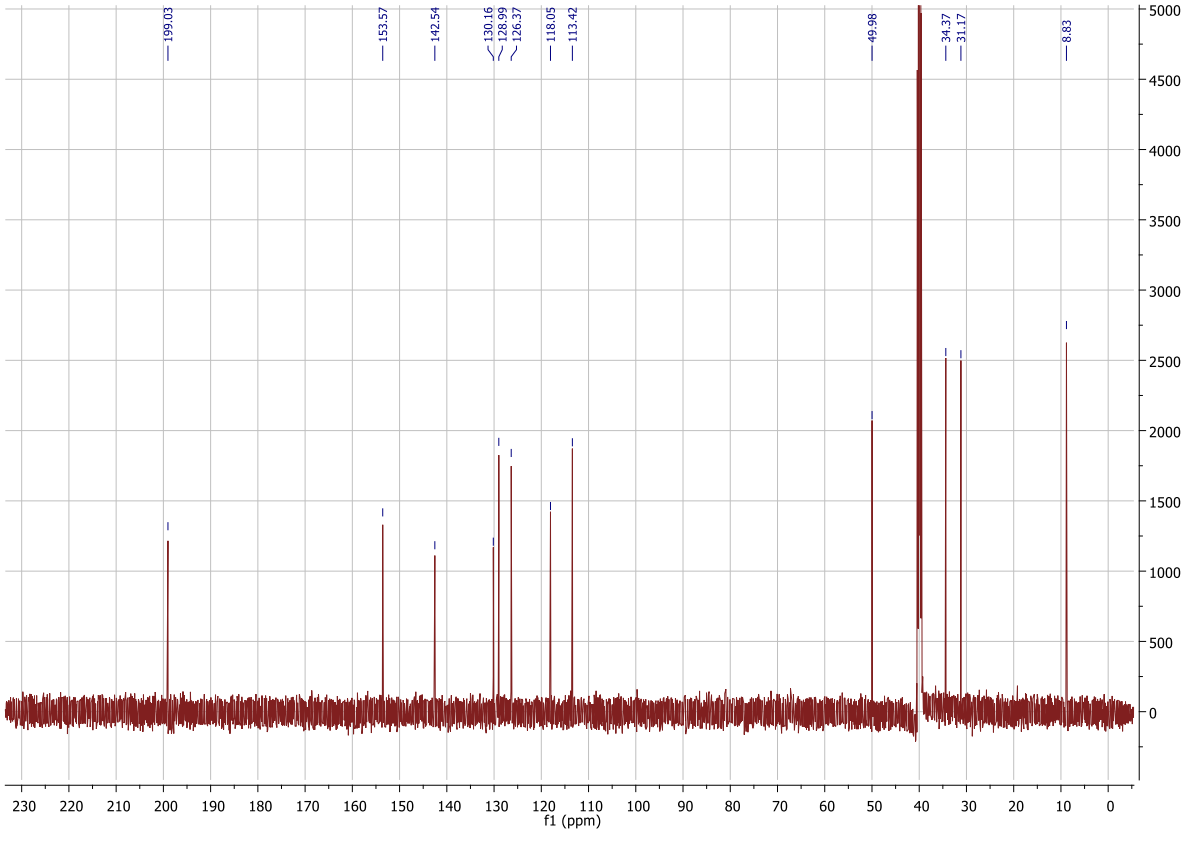
5l: 6-Acetyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



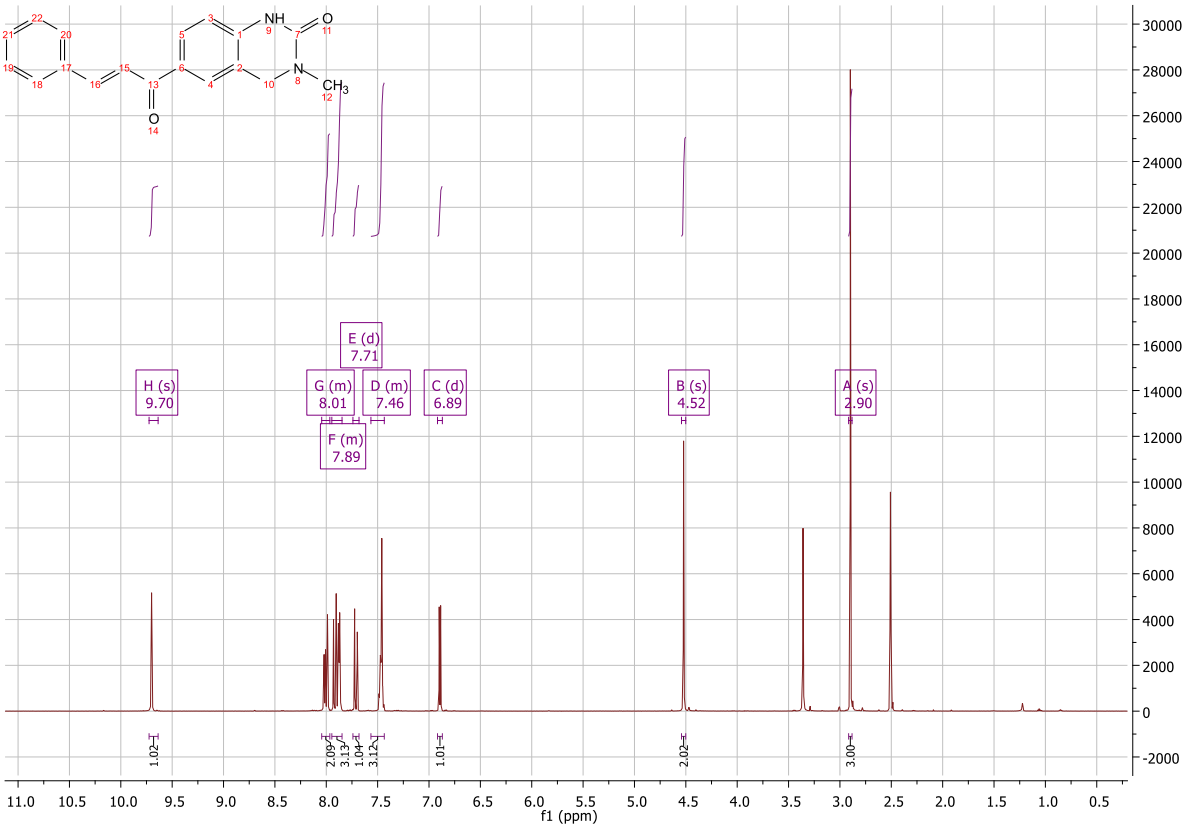


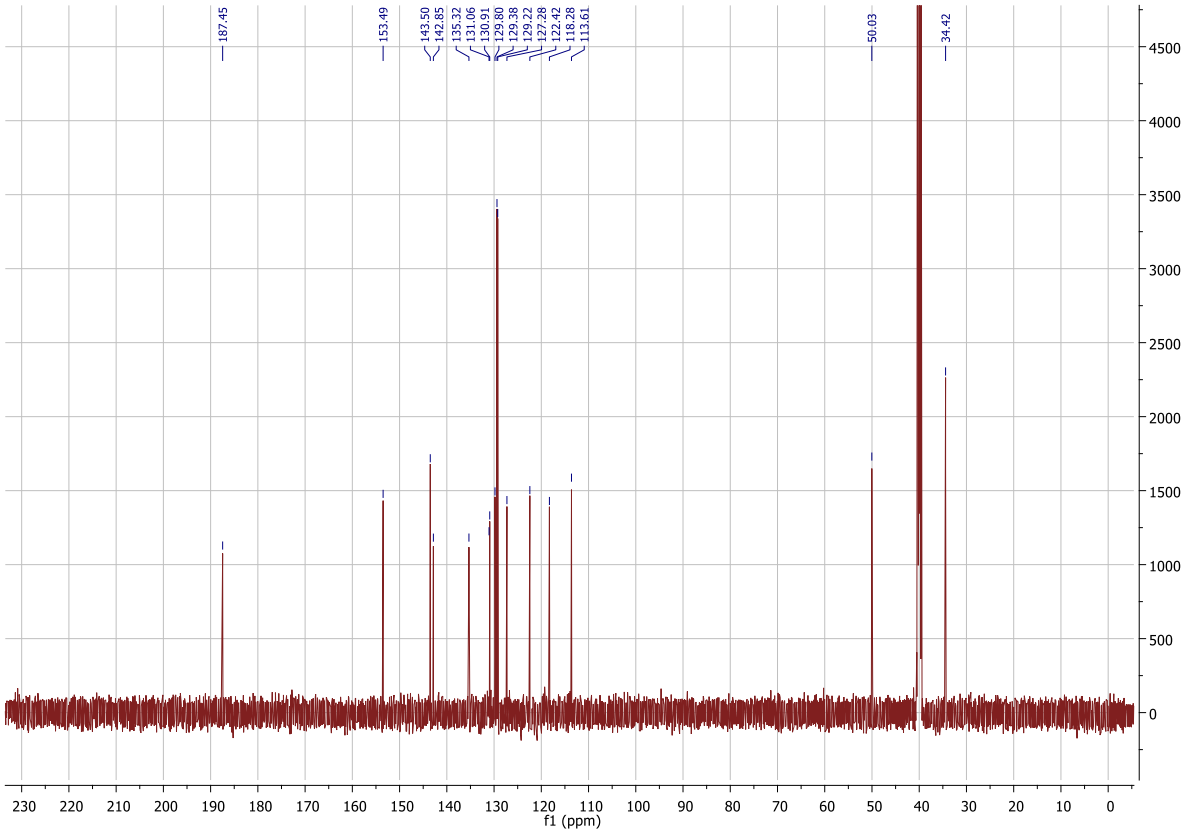
5m: 3-Methyl-6-propionyl-3,4-dihydroquinazolin-2(1*H*)-one



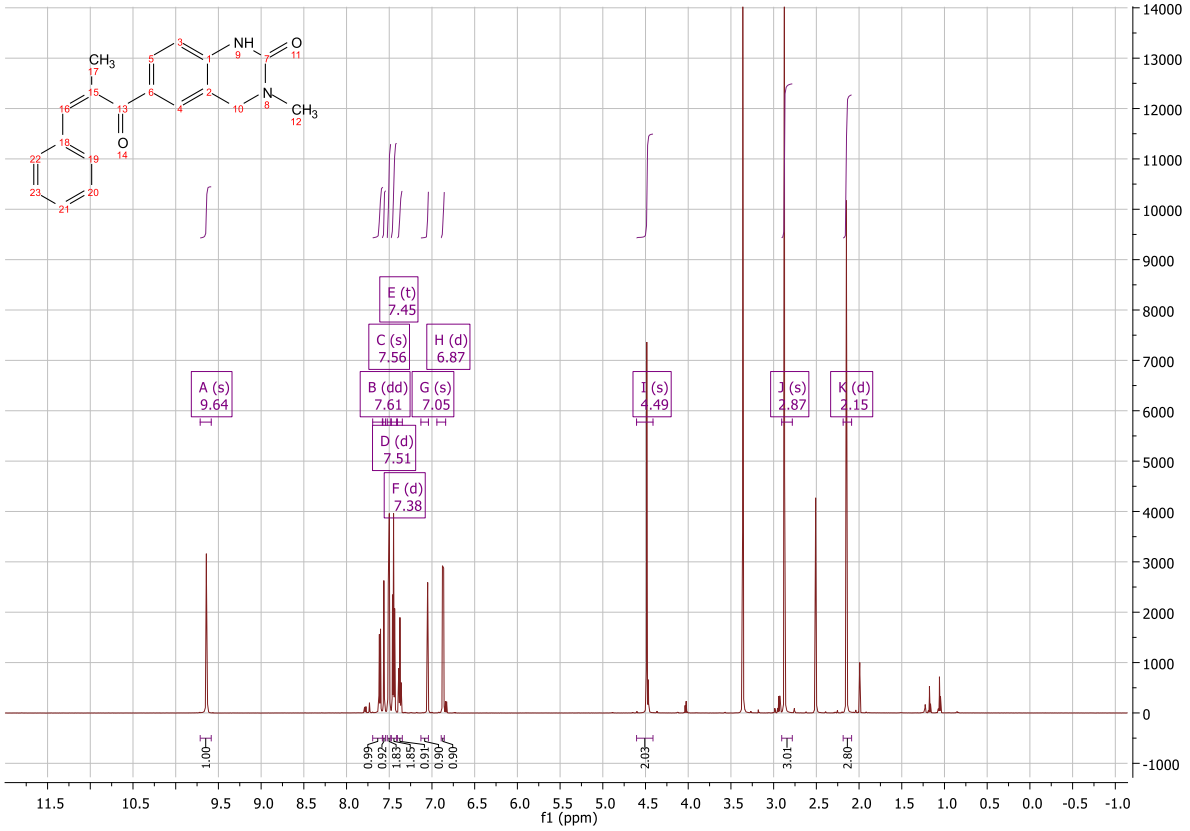


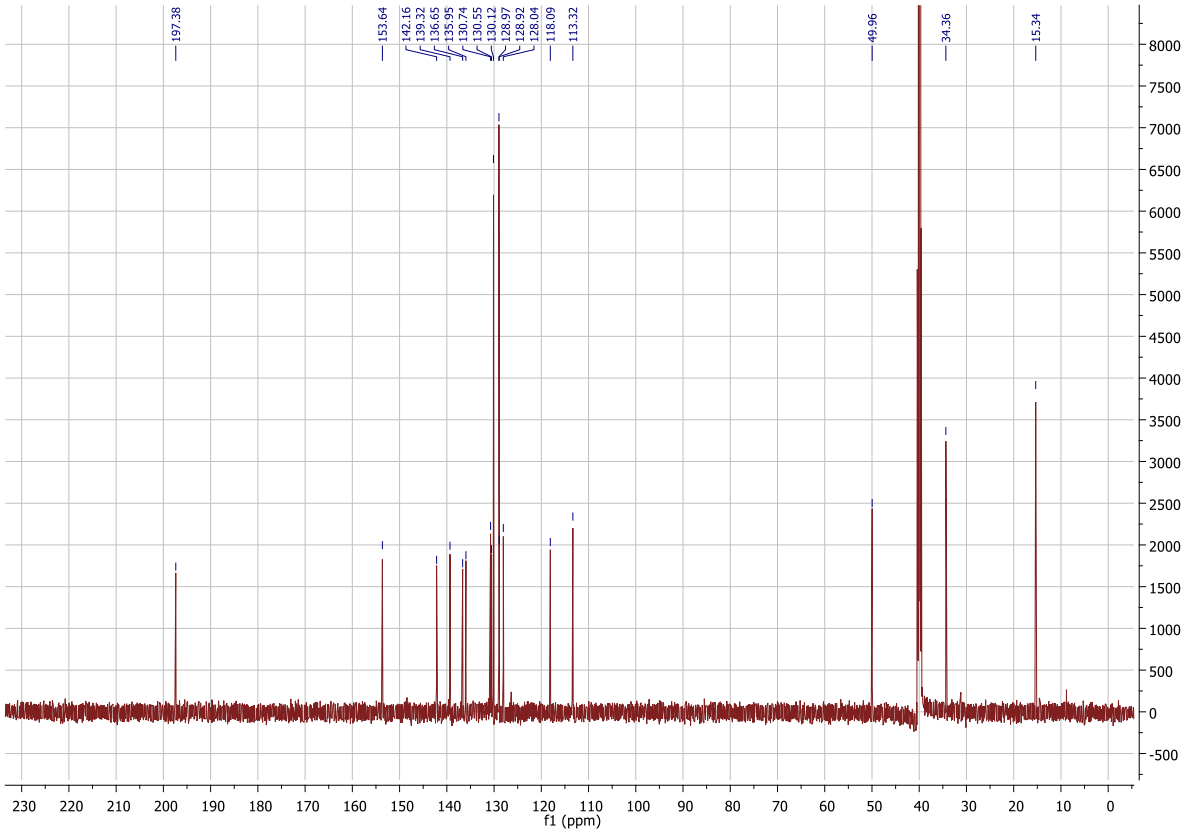
6a: 6-Cinnamoyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



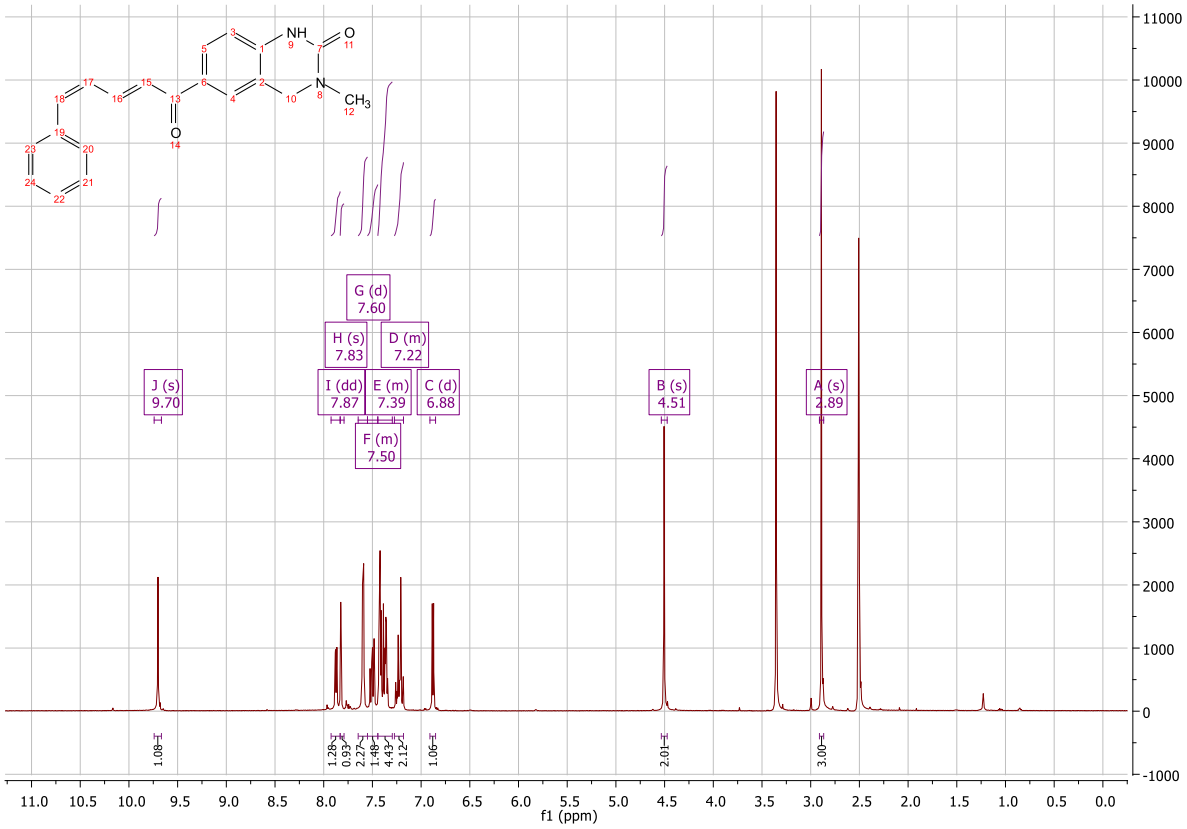


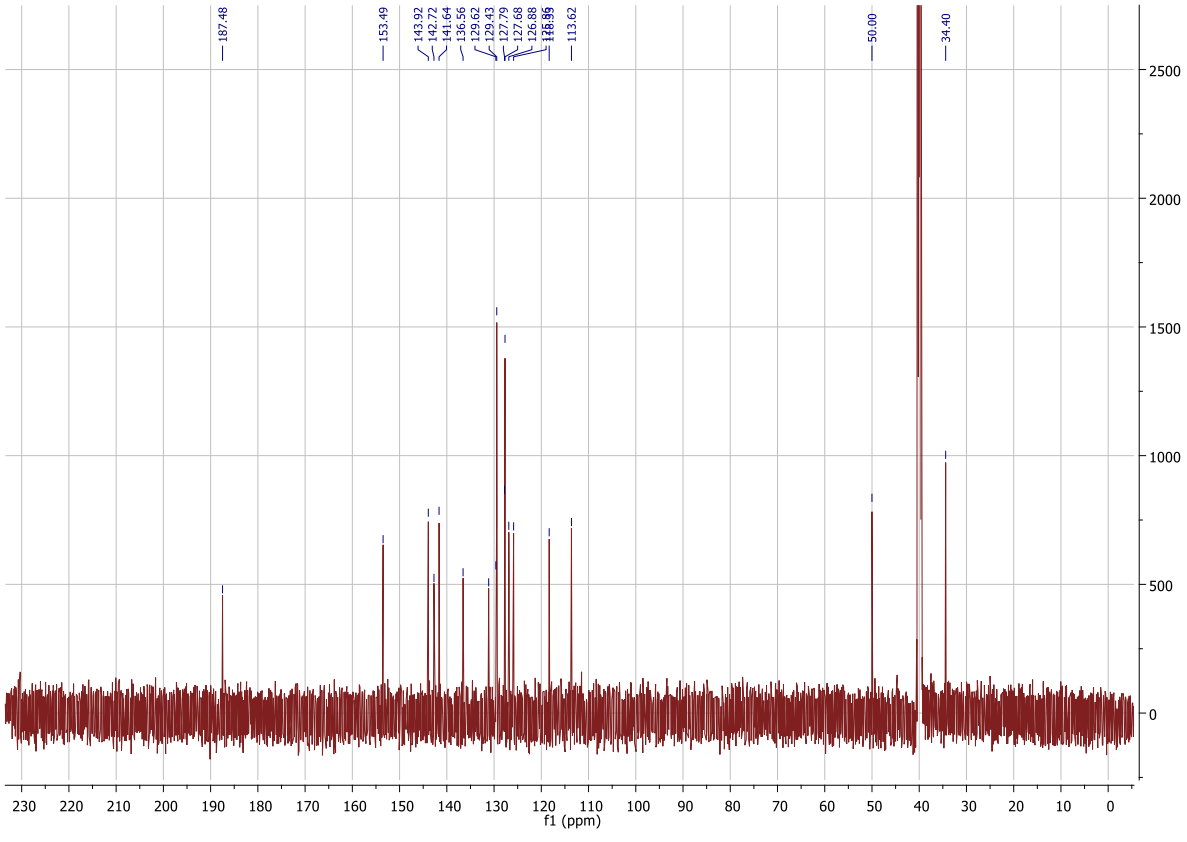
6b: (*z*)-3-Methyl-6-(2-methyl-3-phenylacryloyl)-3,4-dihydroquinazolin-2(1*H*)-one



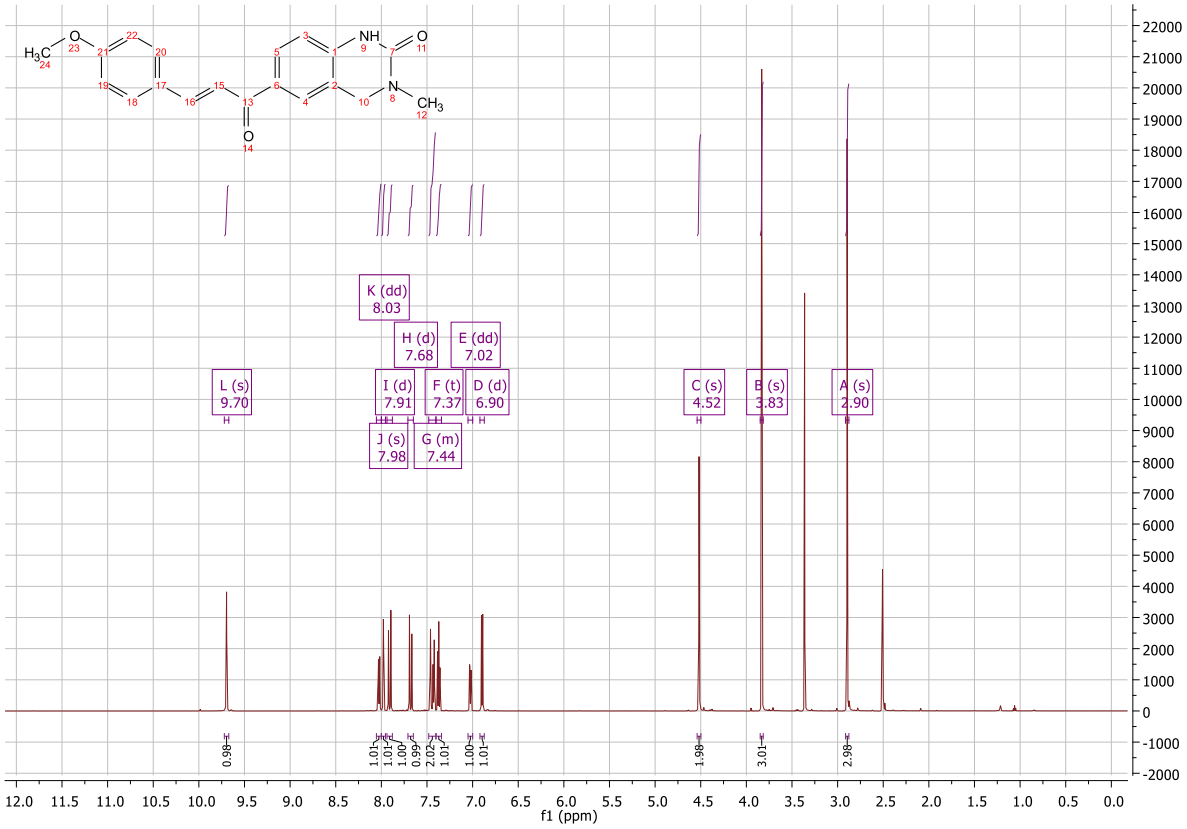


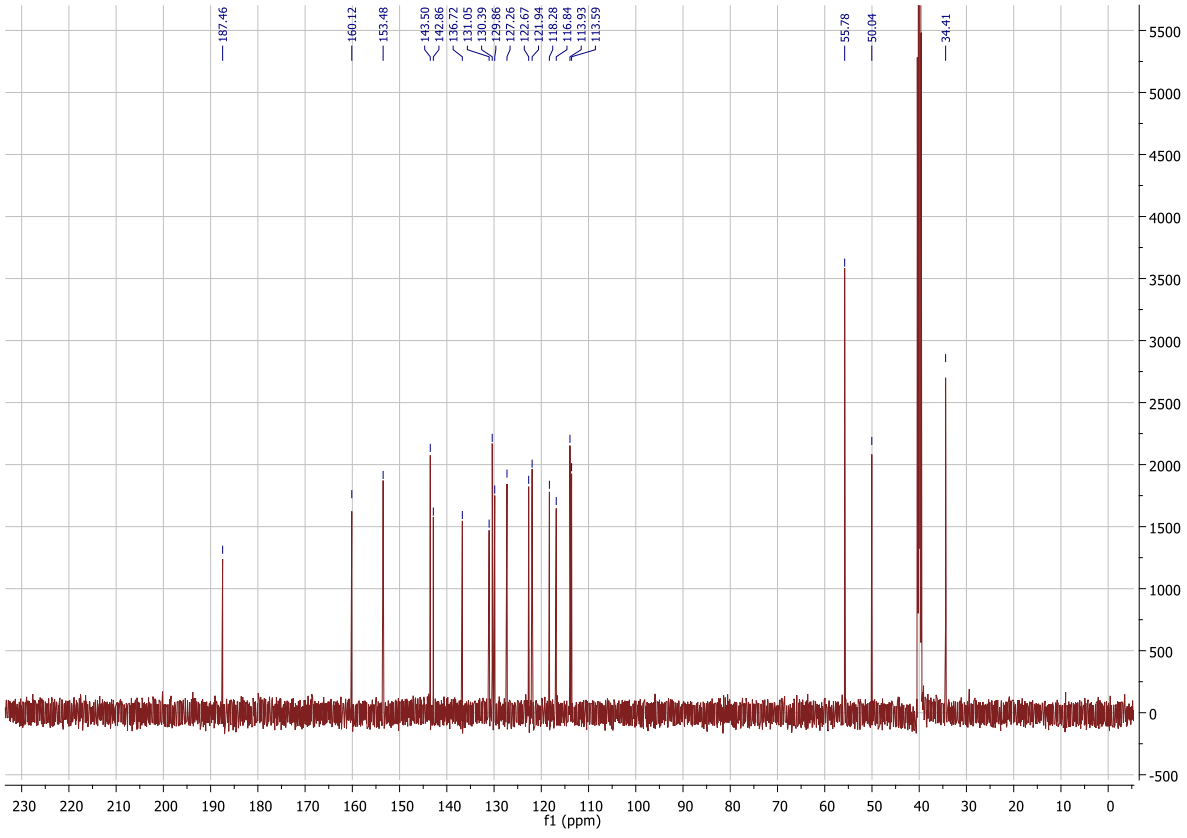
6c: 3-Methyl-6-((2*E*,4*Z*)-5-phenylpenta-2,4-dienoyl)-3,4-dihydroquinazolin-2(1*H*)-one



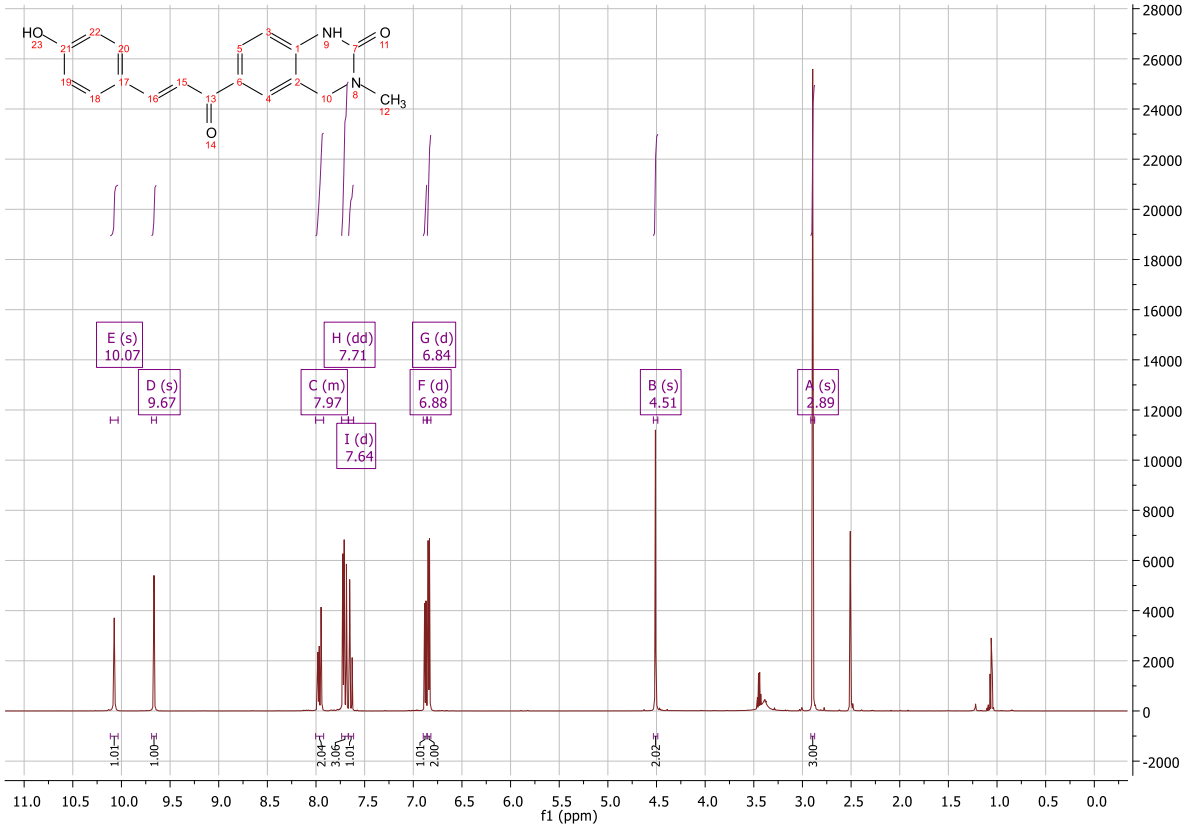


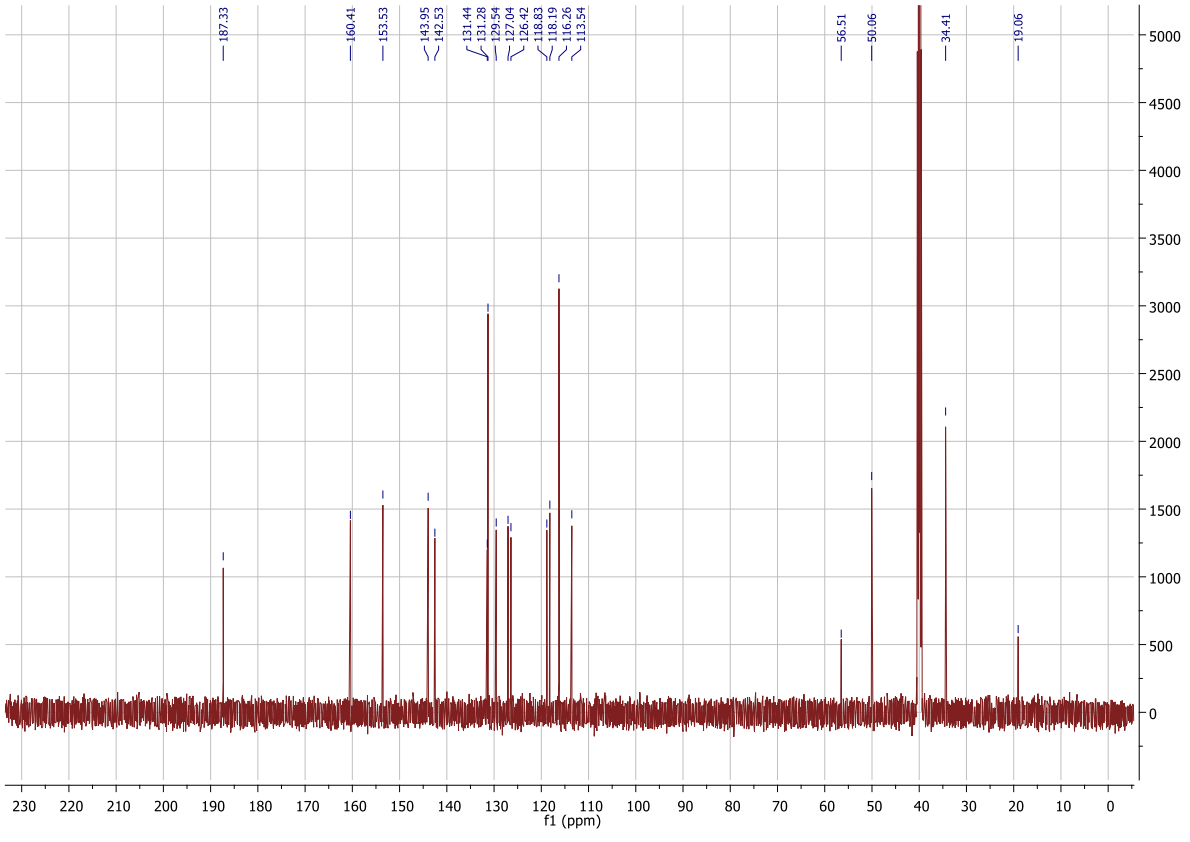
6d: (*E*)-6-(3-(4-Methoxyphenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



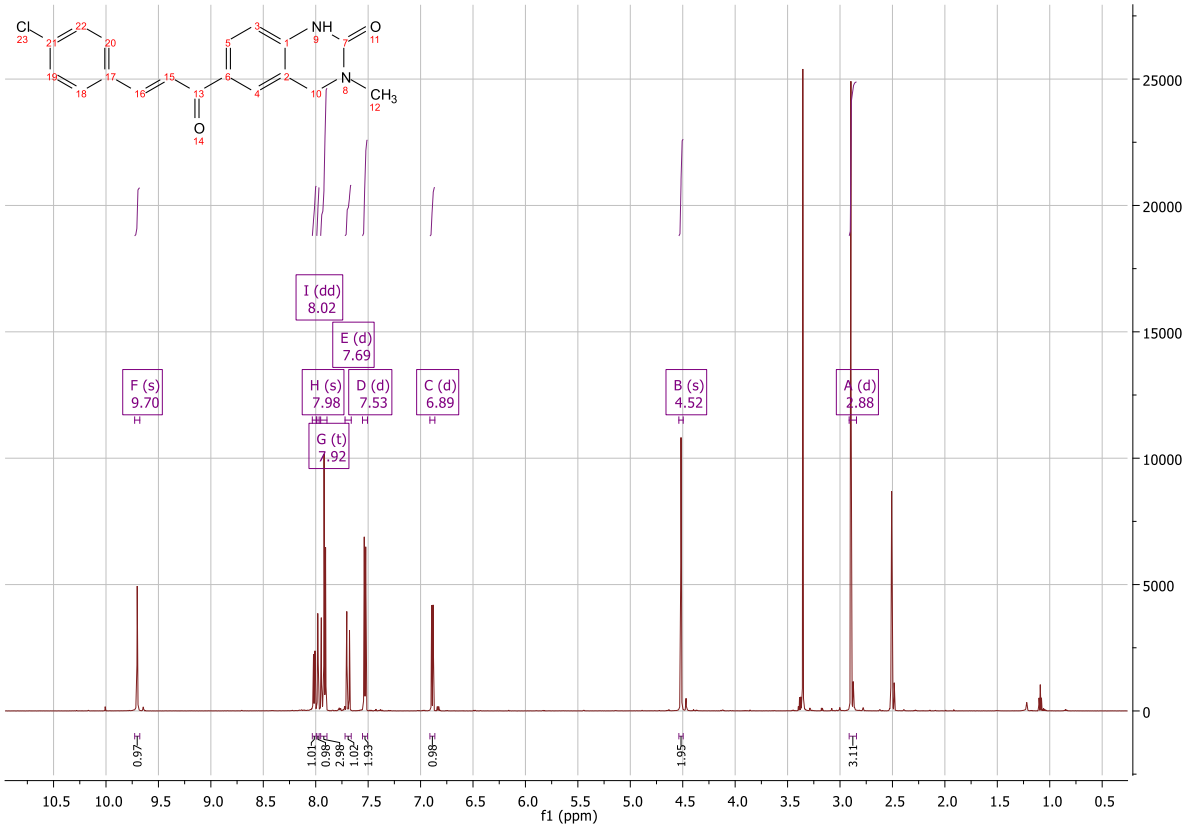


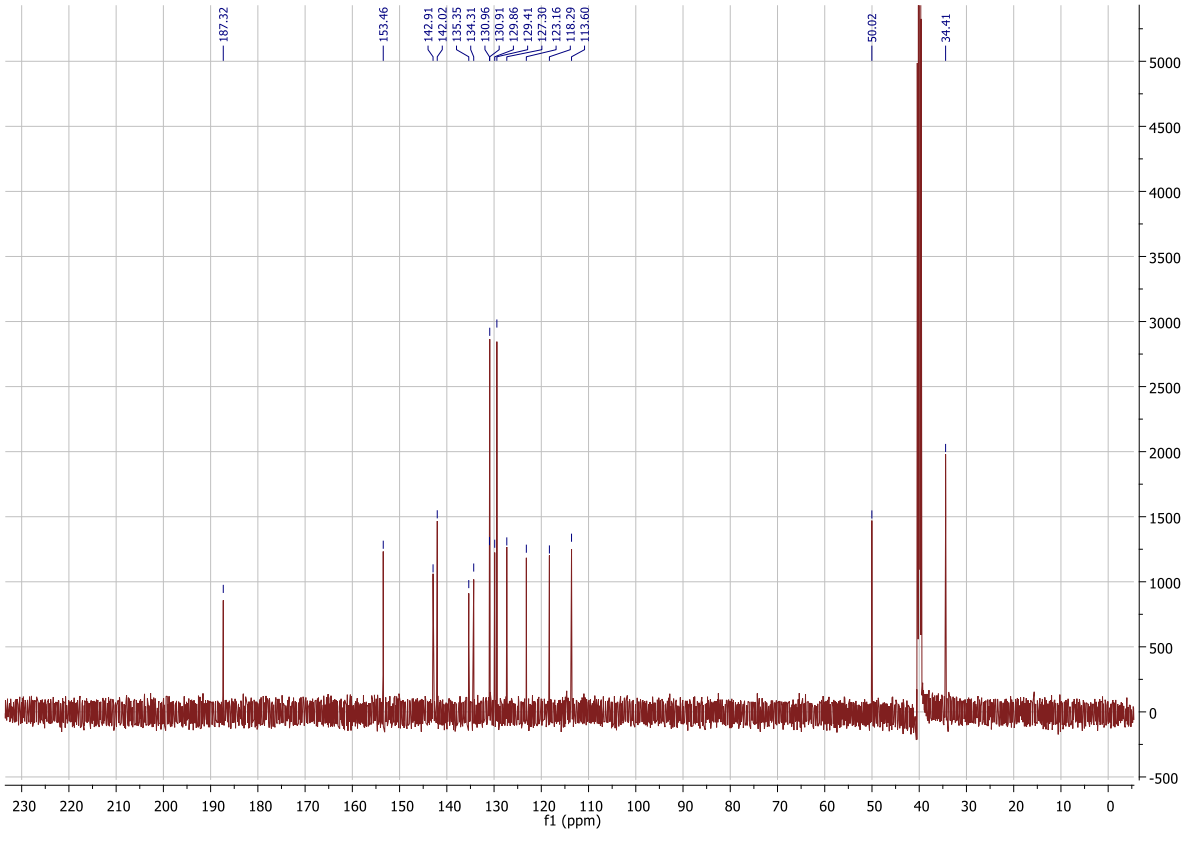
6e: (*E*)-6-(3-(4-Hydroxyphenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



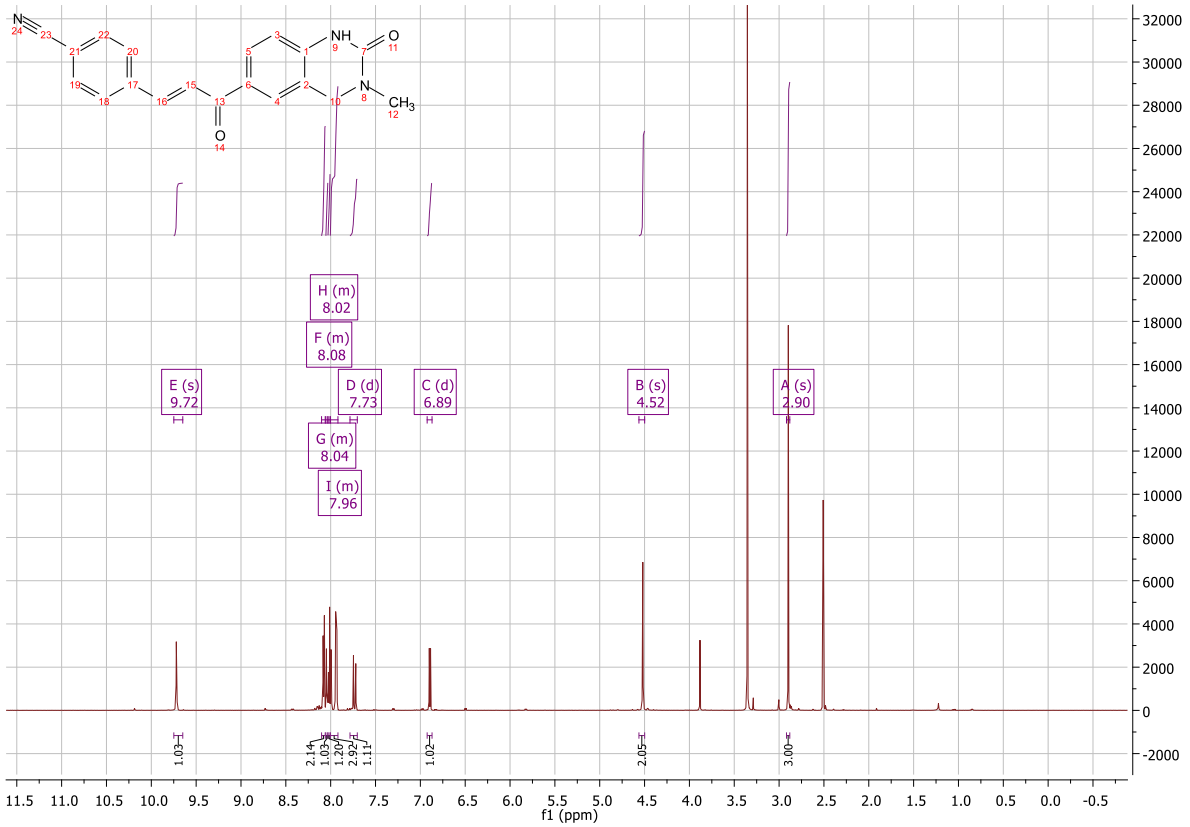


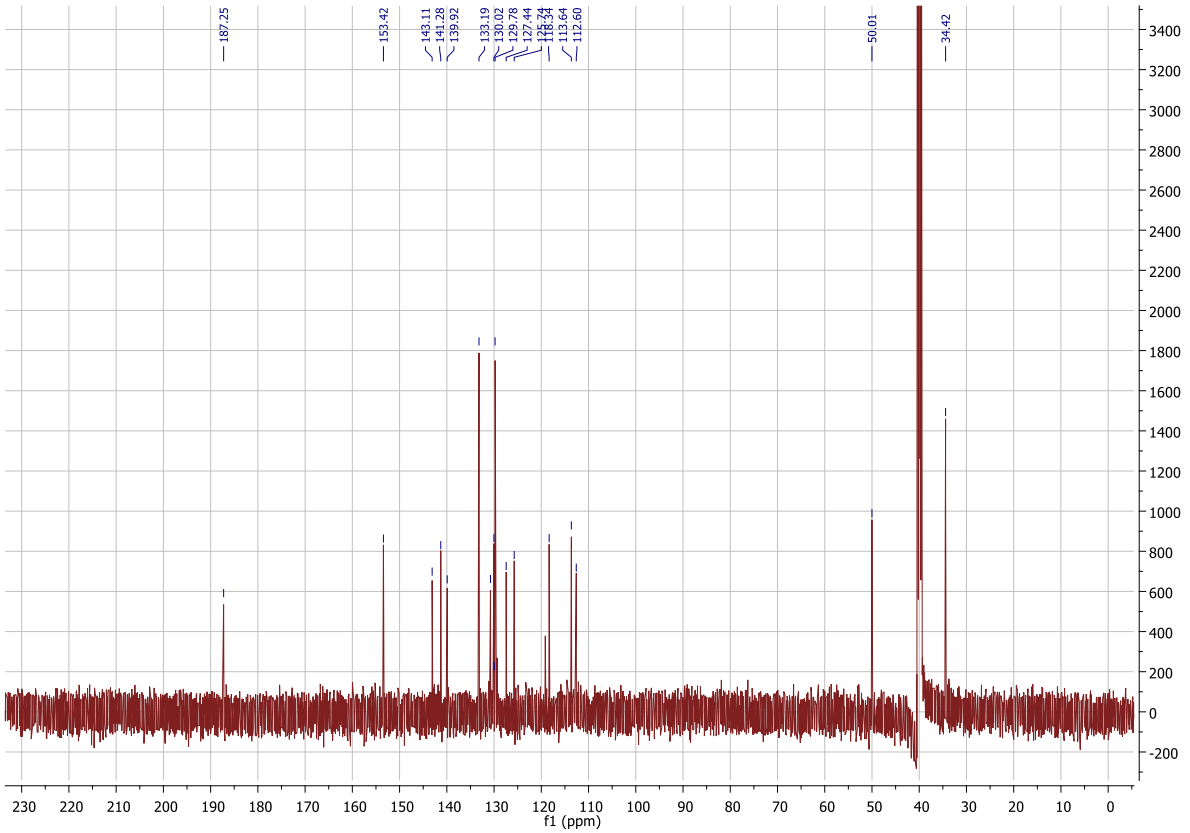
6f: (*E*)-6-(3-(4-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



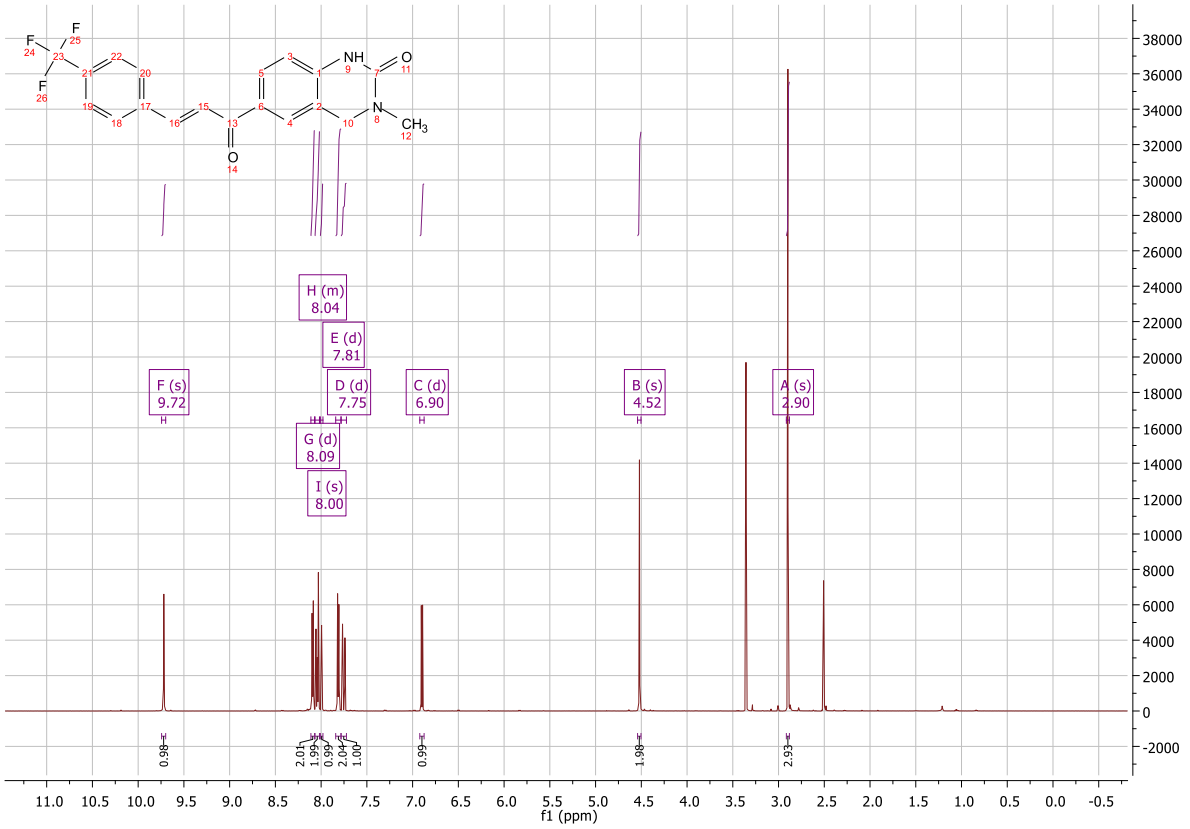


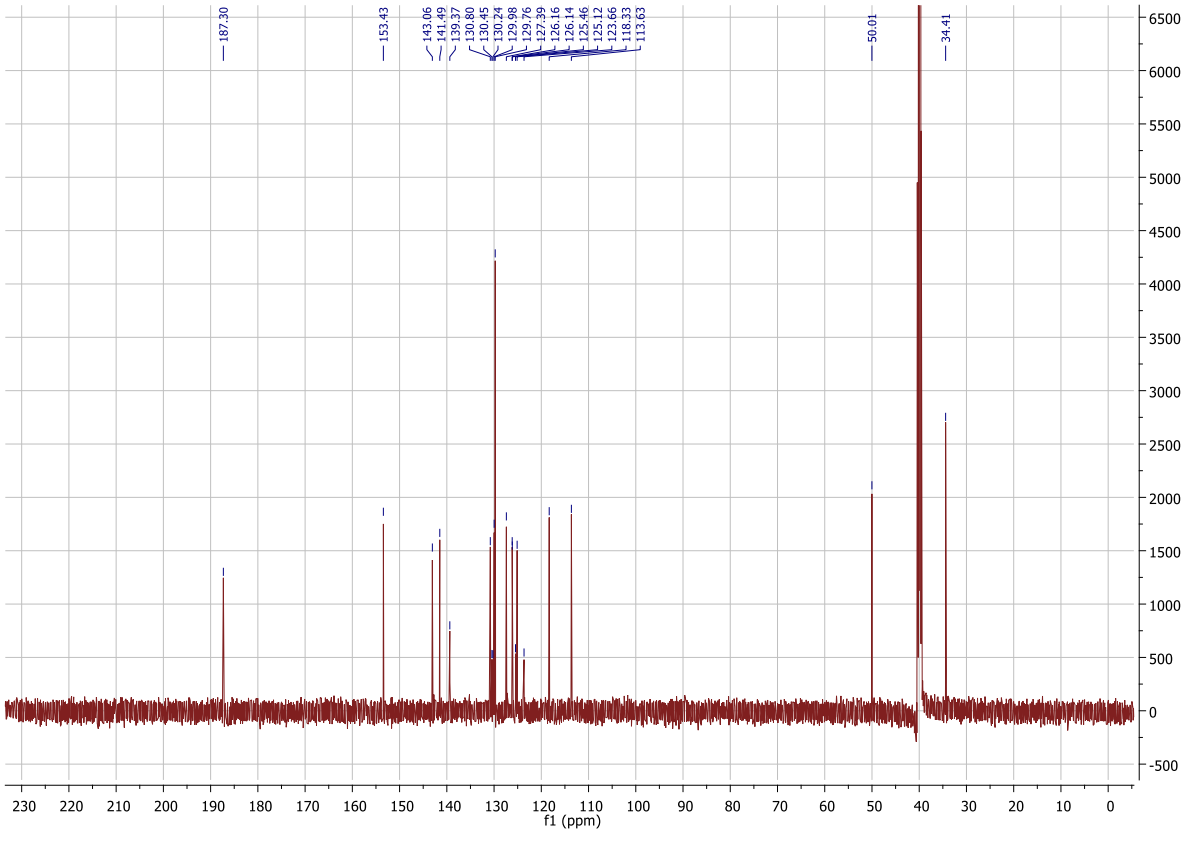
6g: (*E*)-4-(3-(3-Methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)-3-oxoprop-1-en-1-yl) benzonitrile



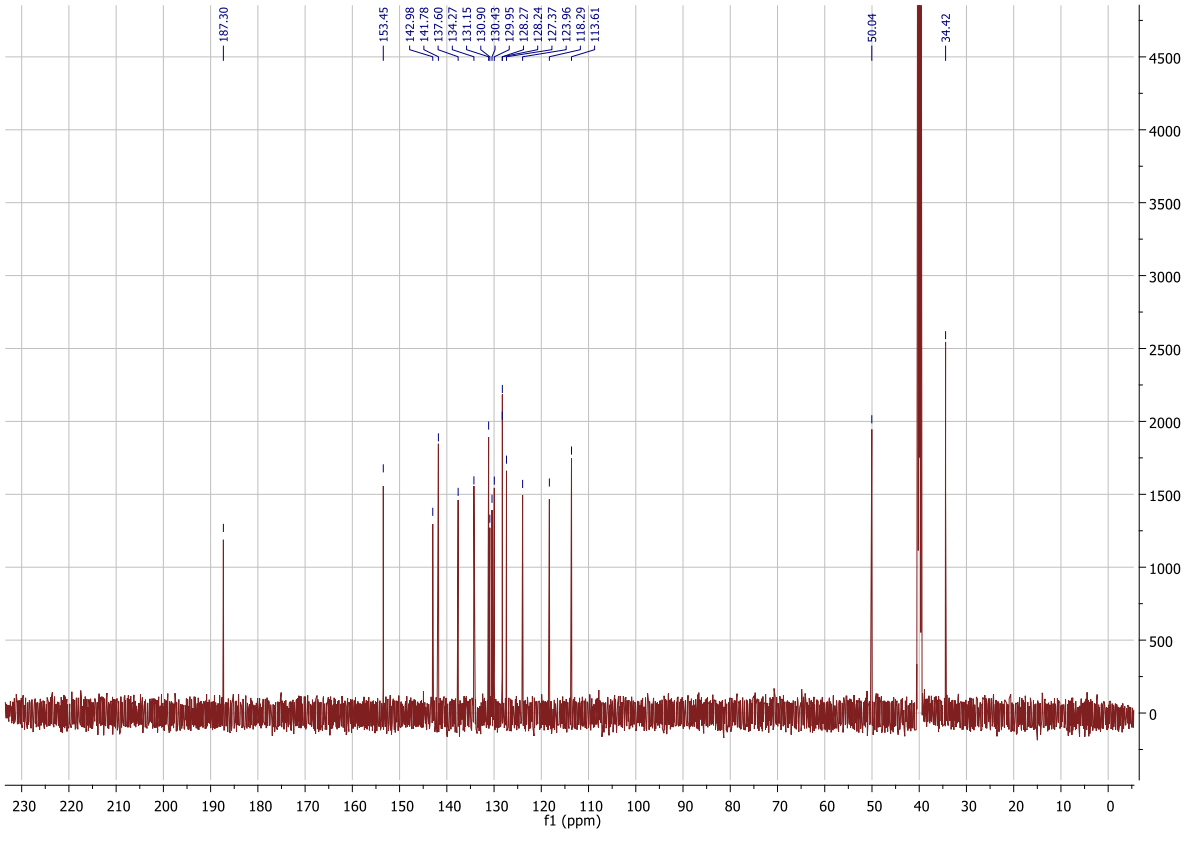


6h: (*E*)-3-Methyl-6-(3-(4-(trifluoromethyl)phenyl)acryloyl)-3,4-dihydroquinazolin-2(1*H*)-one

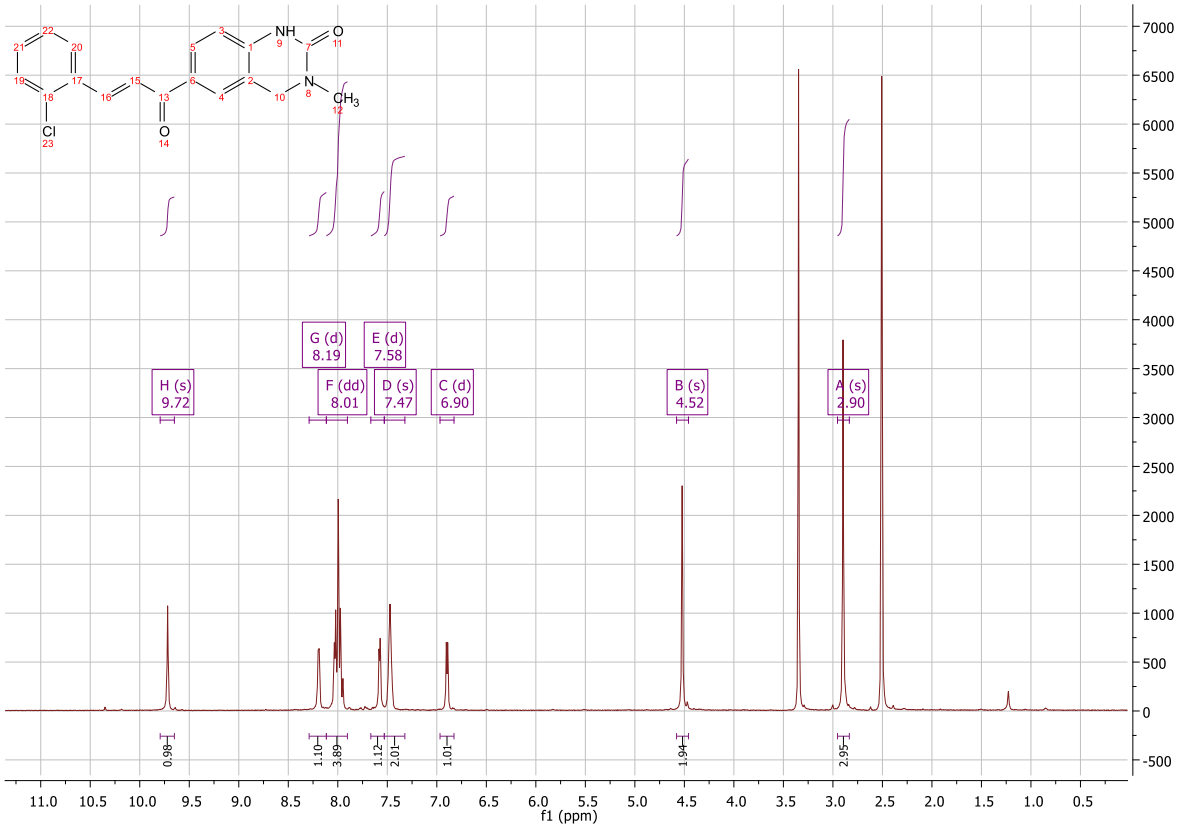


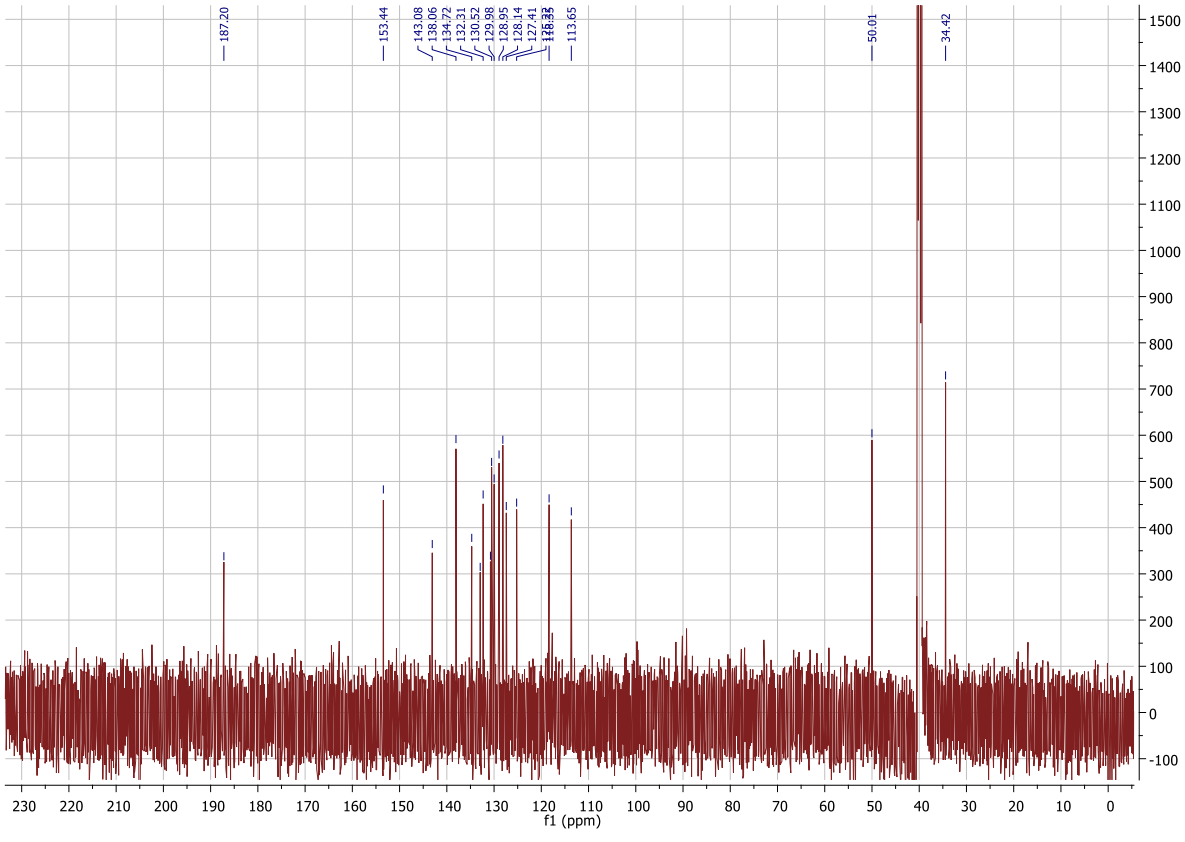


6i: (*E*)-6-(3-(3-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one

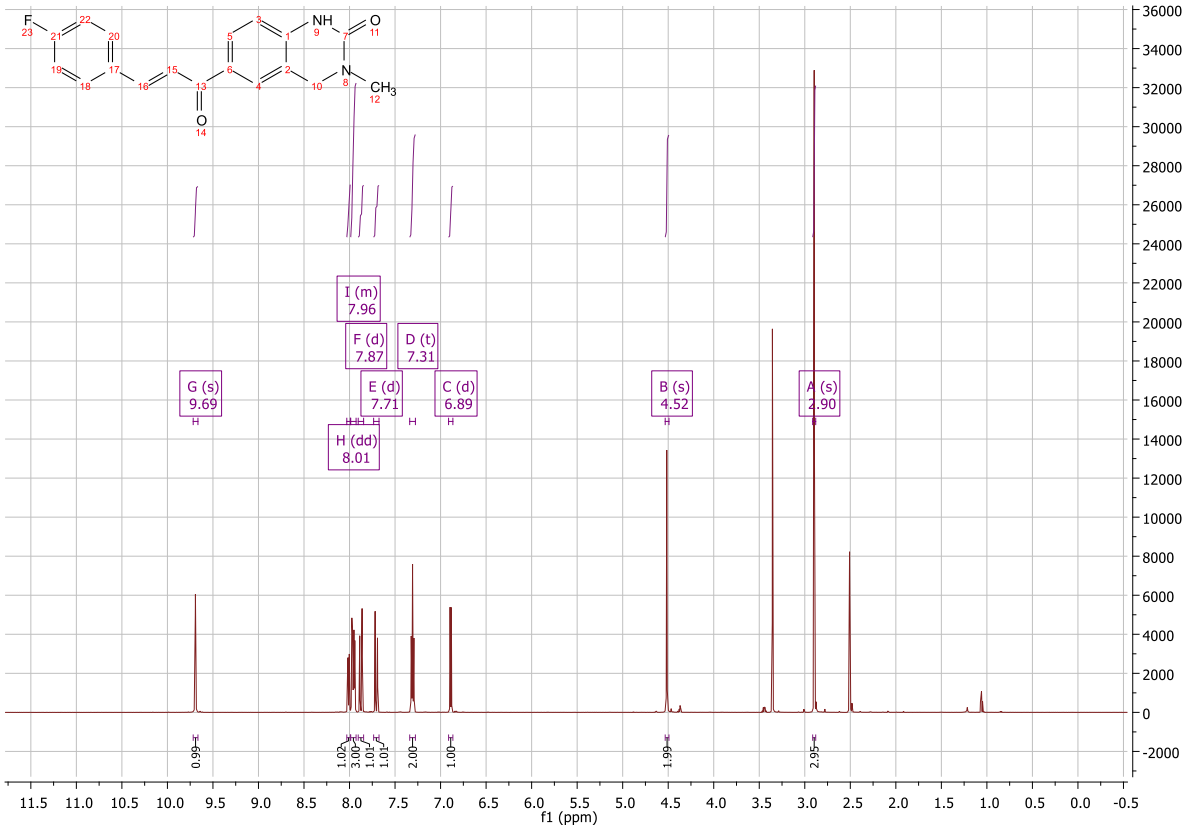


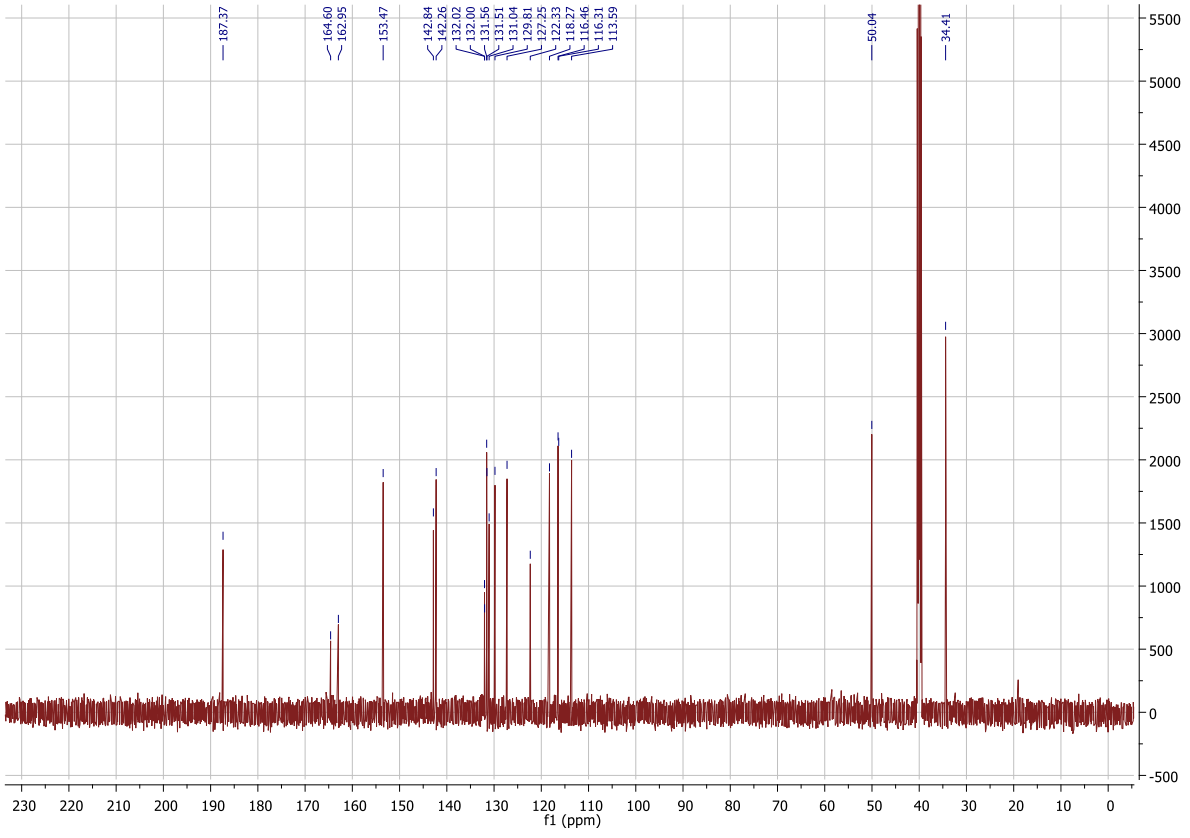
6j: (*E*)-6-(3-(2-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



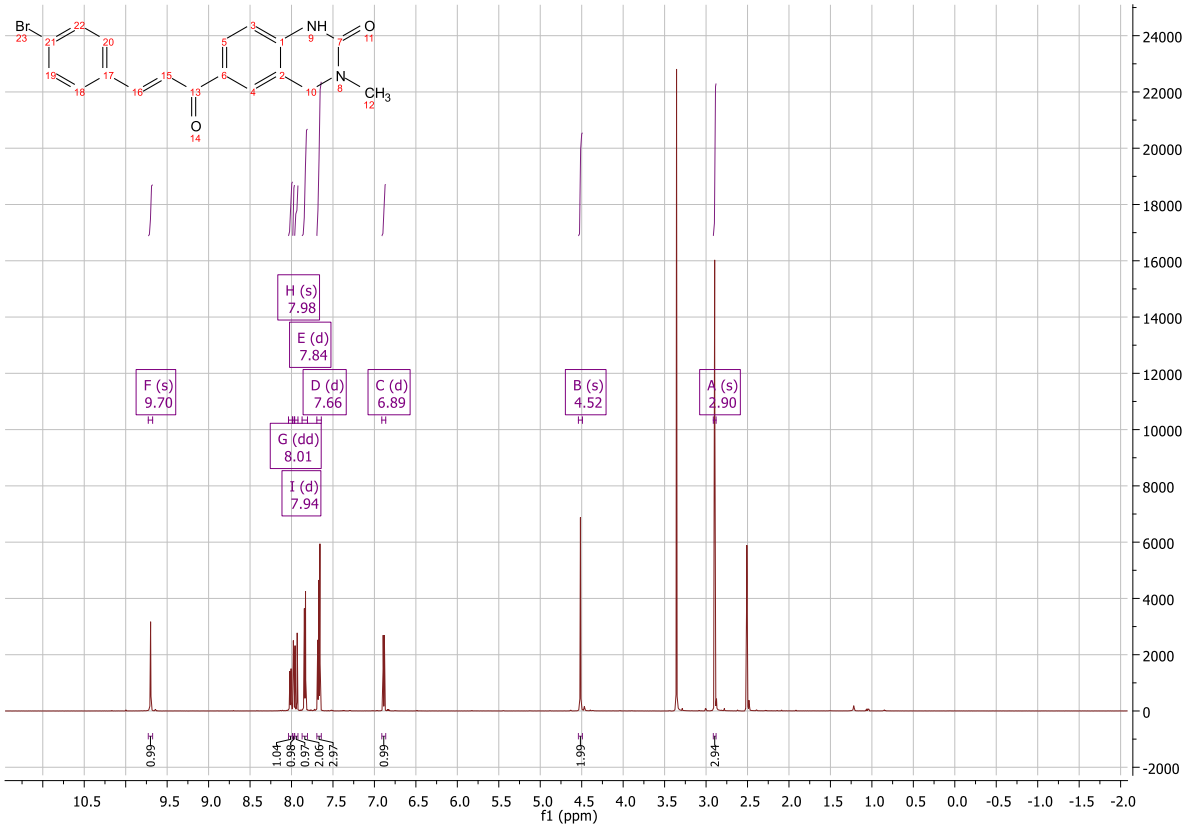


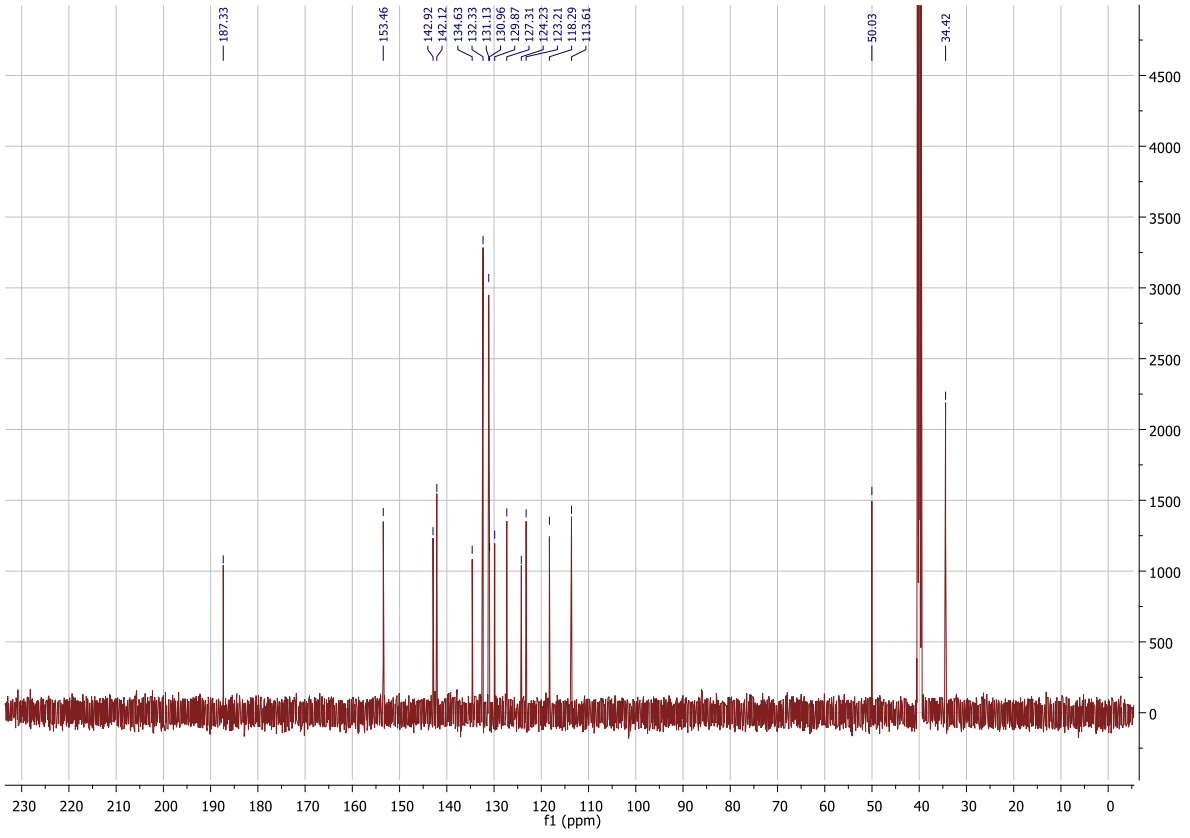
6k: (*E*)-6-(3-(4-Fluorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



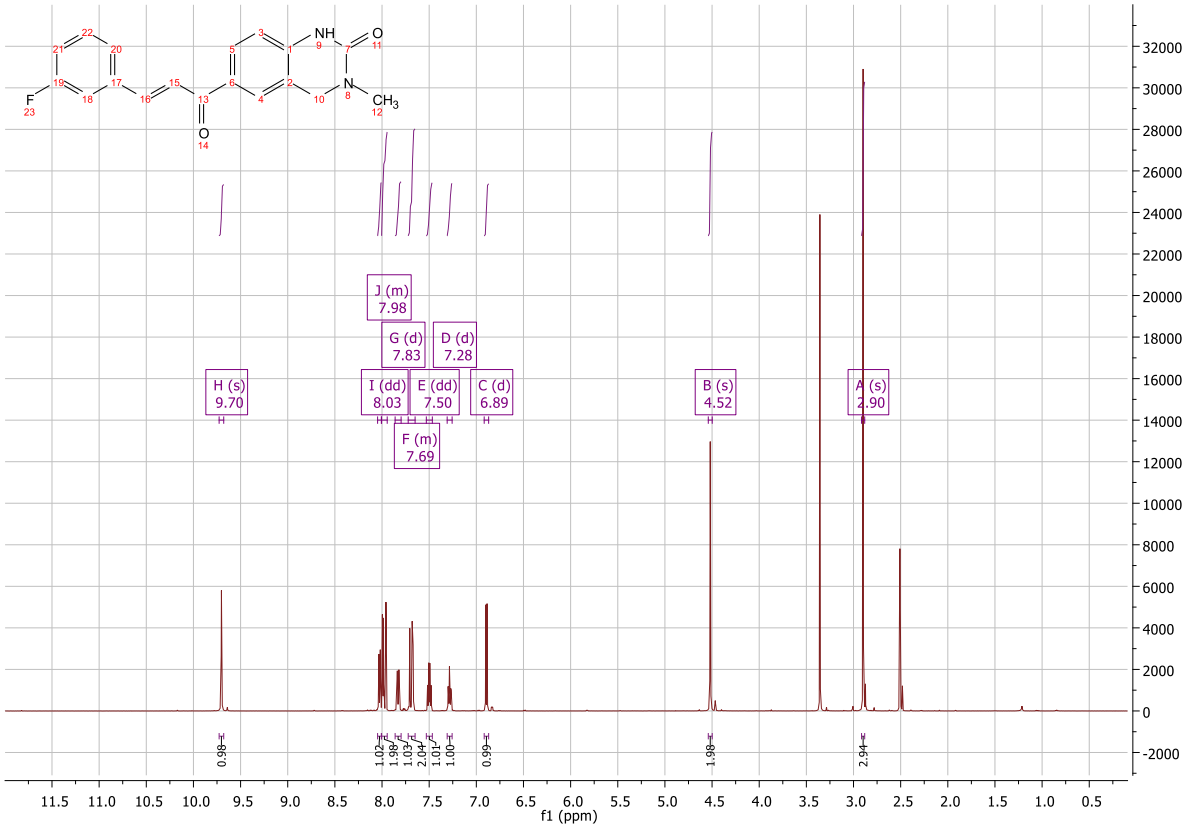


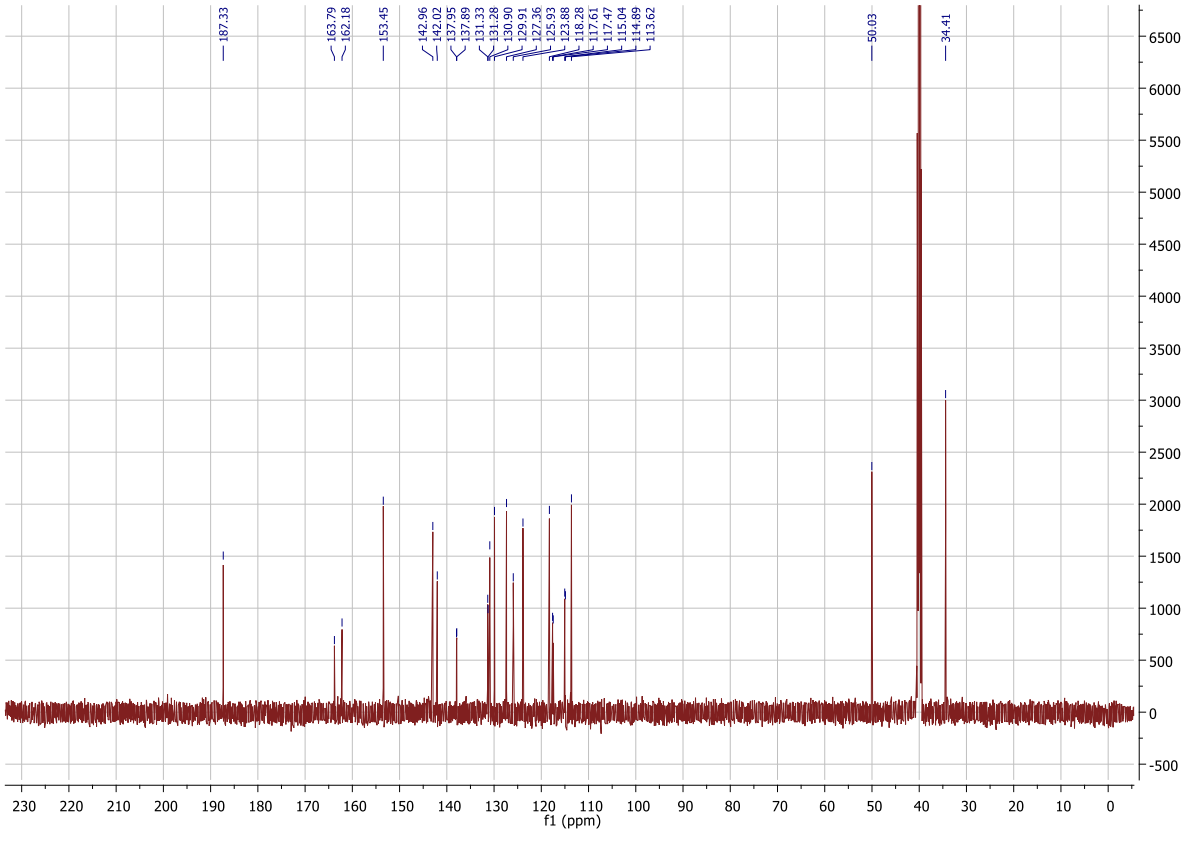
6l: (*E*)-6-(3-(4-Bromophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



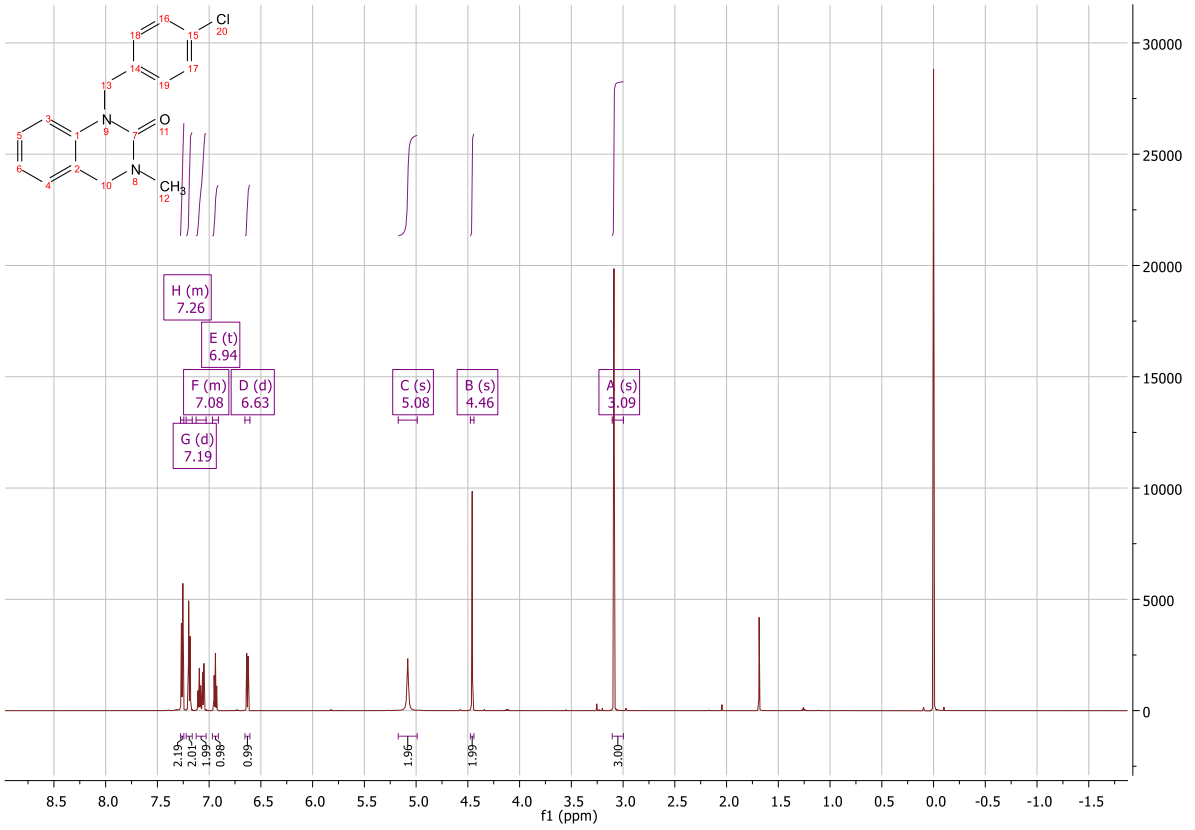


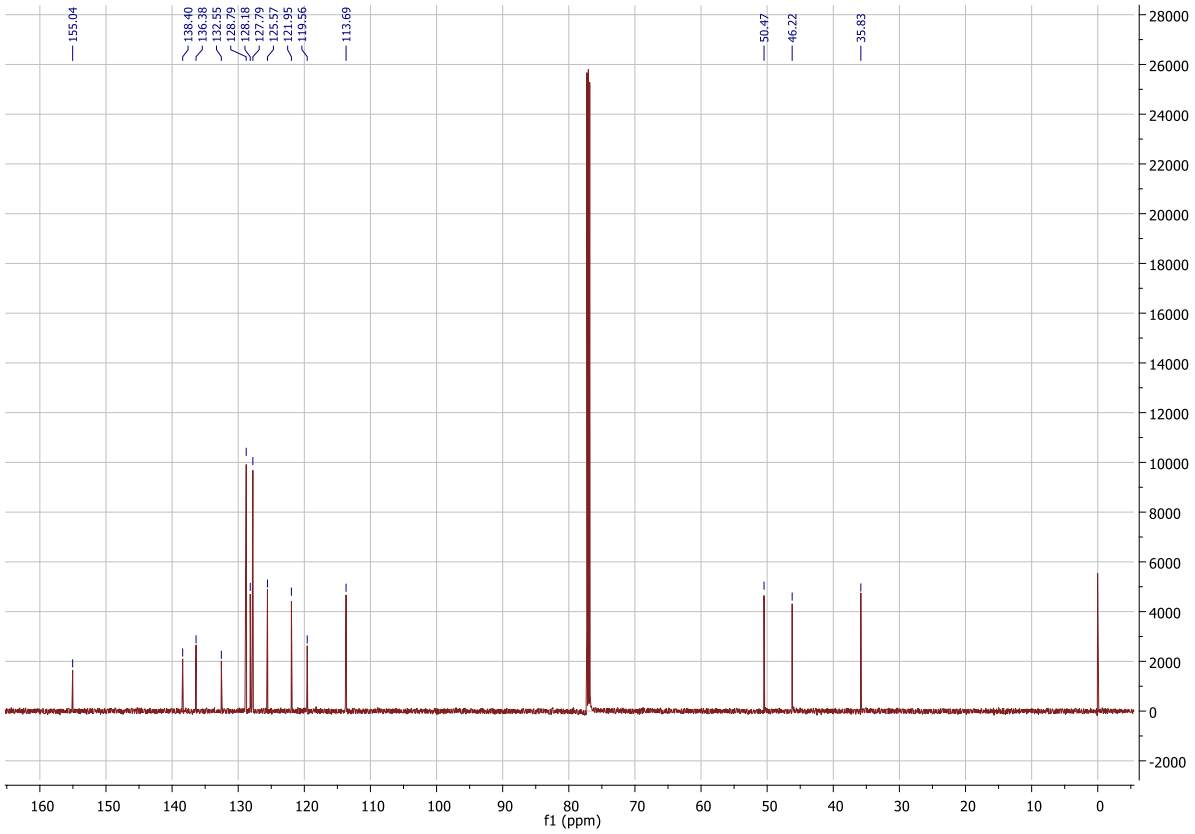
6m: (*E*)-6-(3-(3-Fluorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



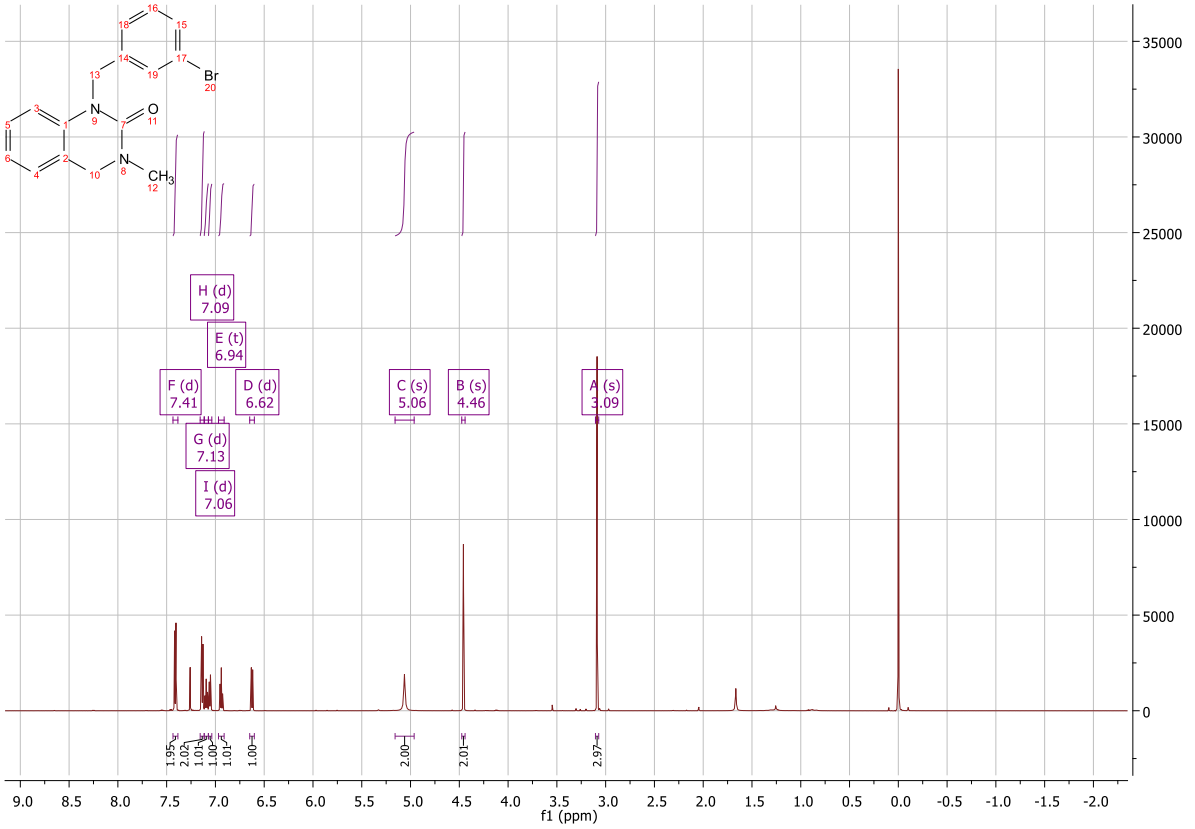


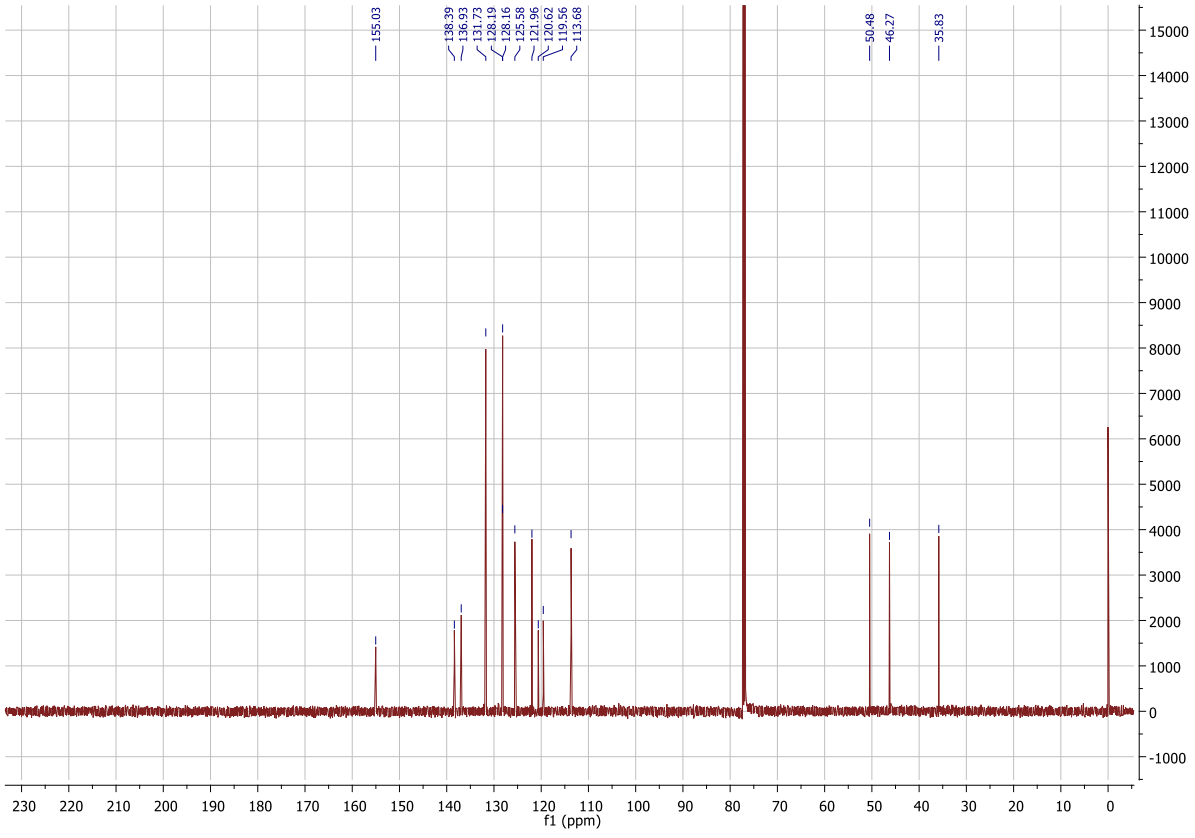
7a: 1-(4-Chlorobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



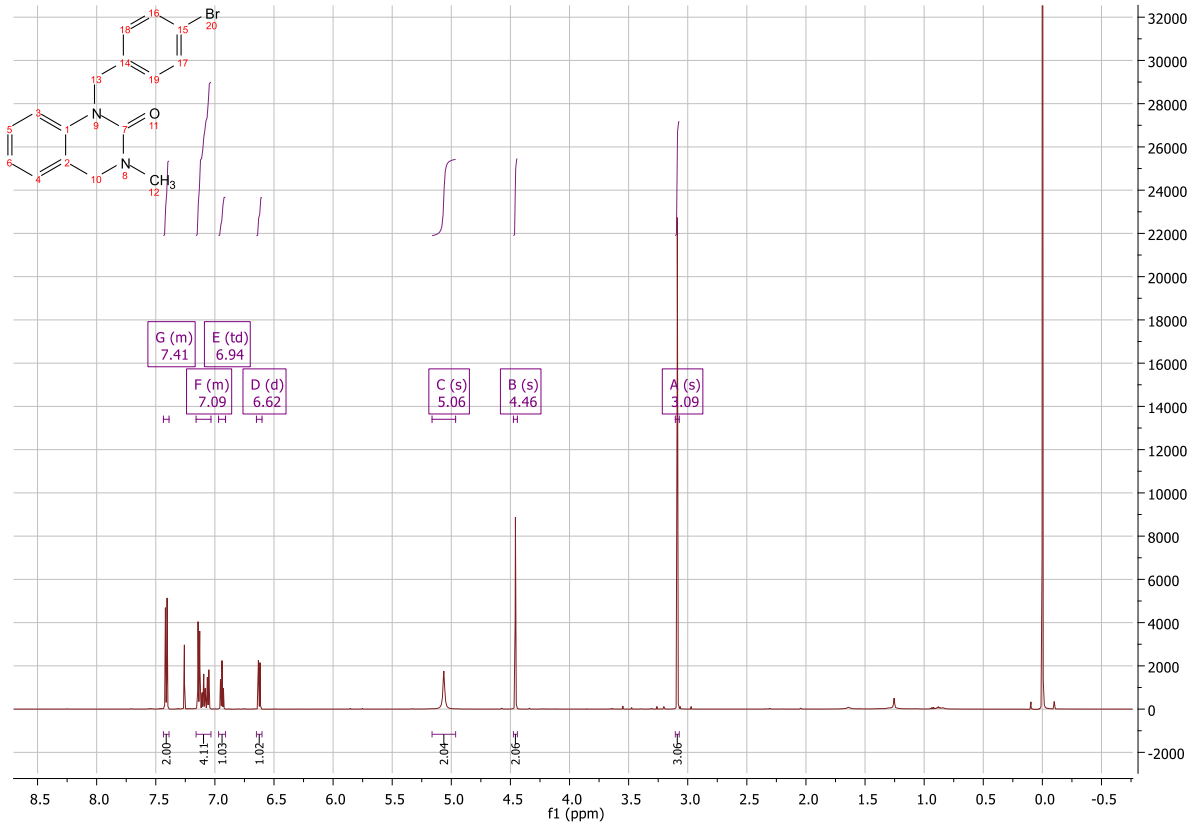


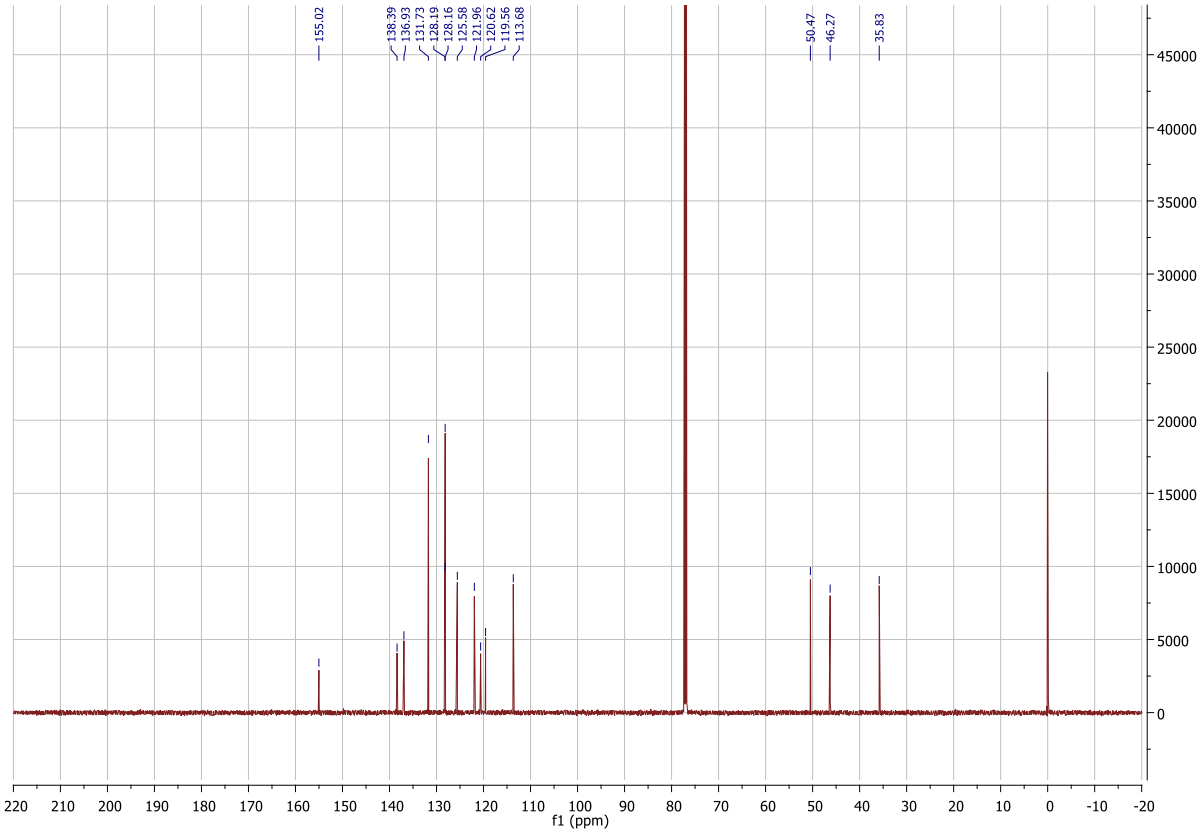
7b: 1-(3-Bromobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



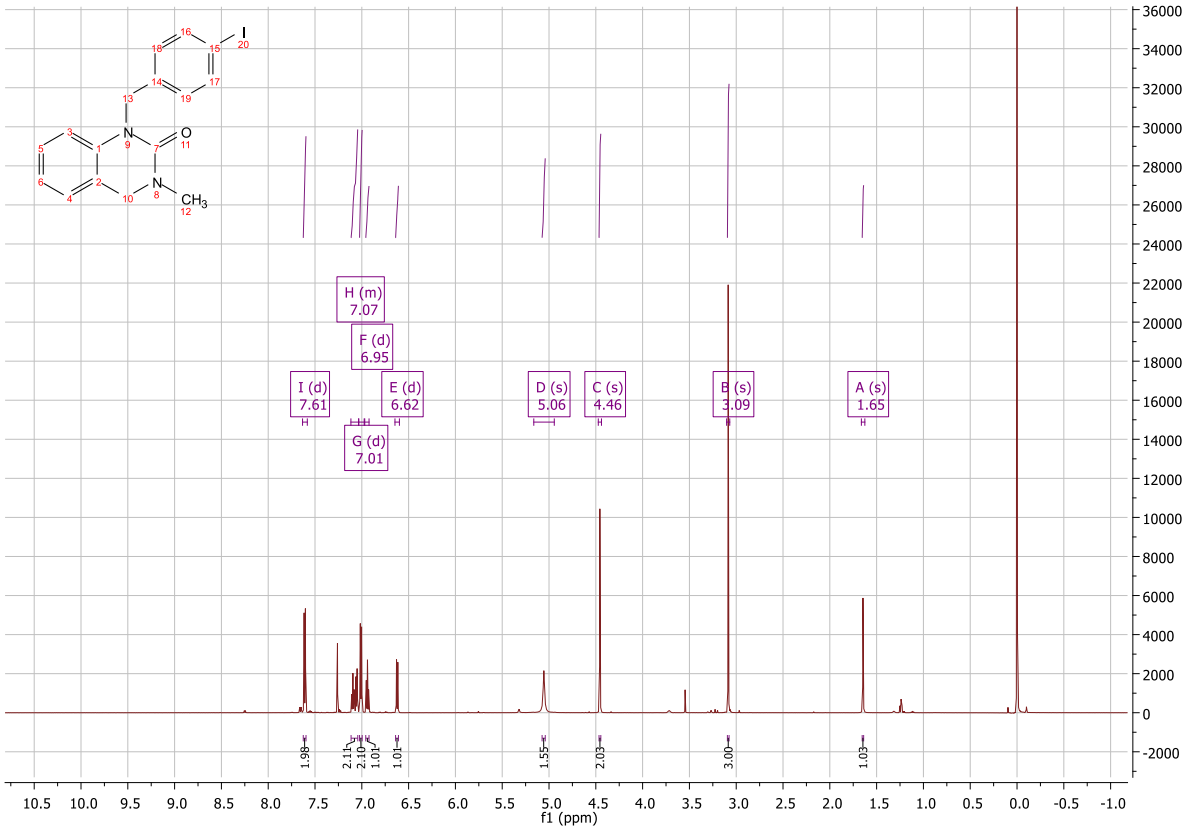


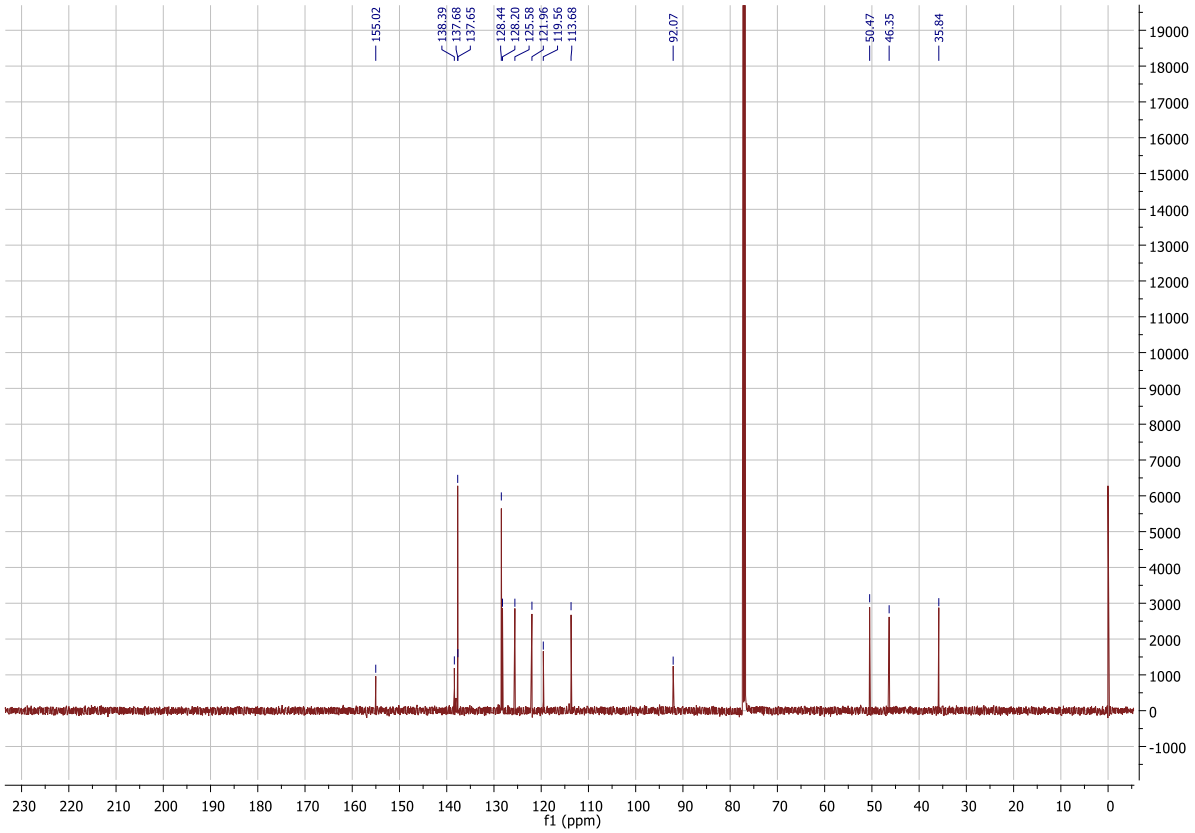
7c: 1-(4-Bromobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



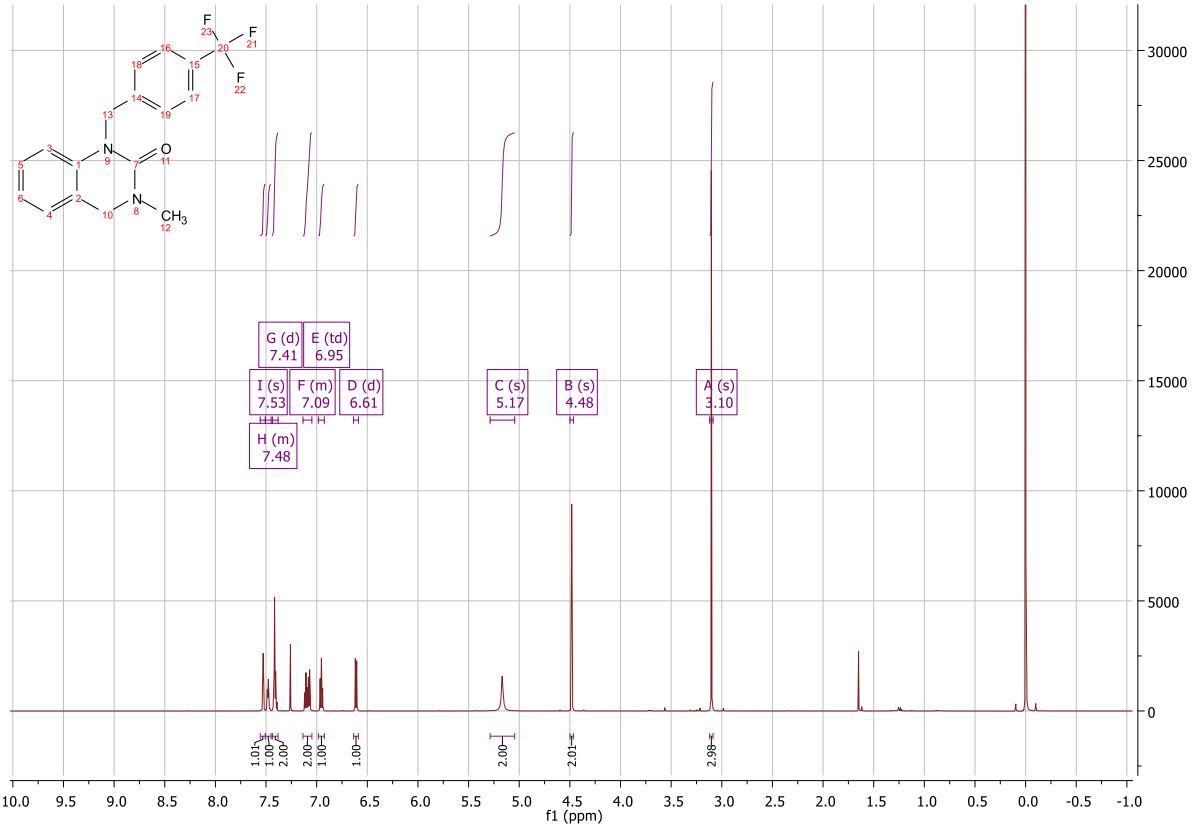


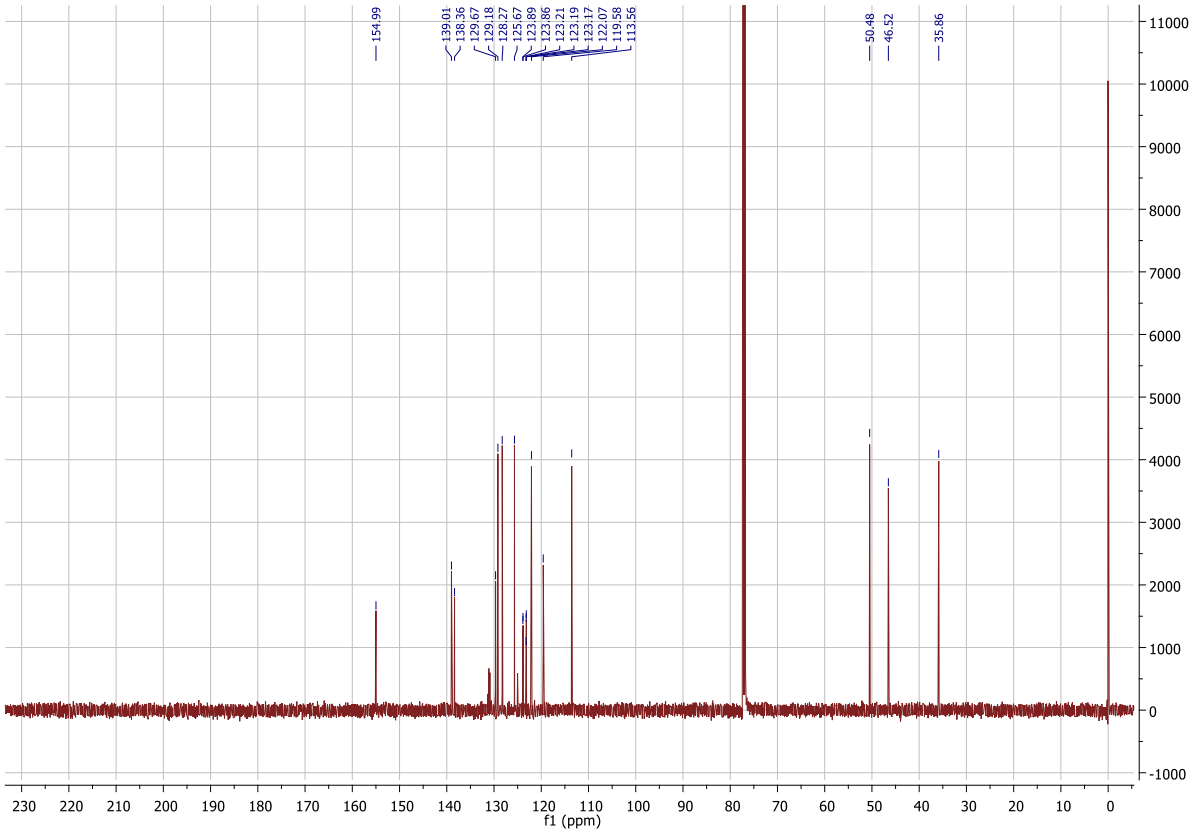
7d: 1-(4-Iodobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



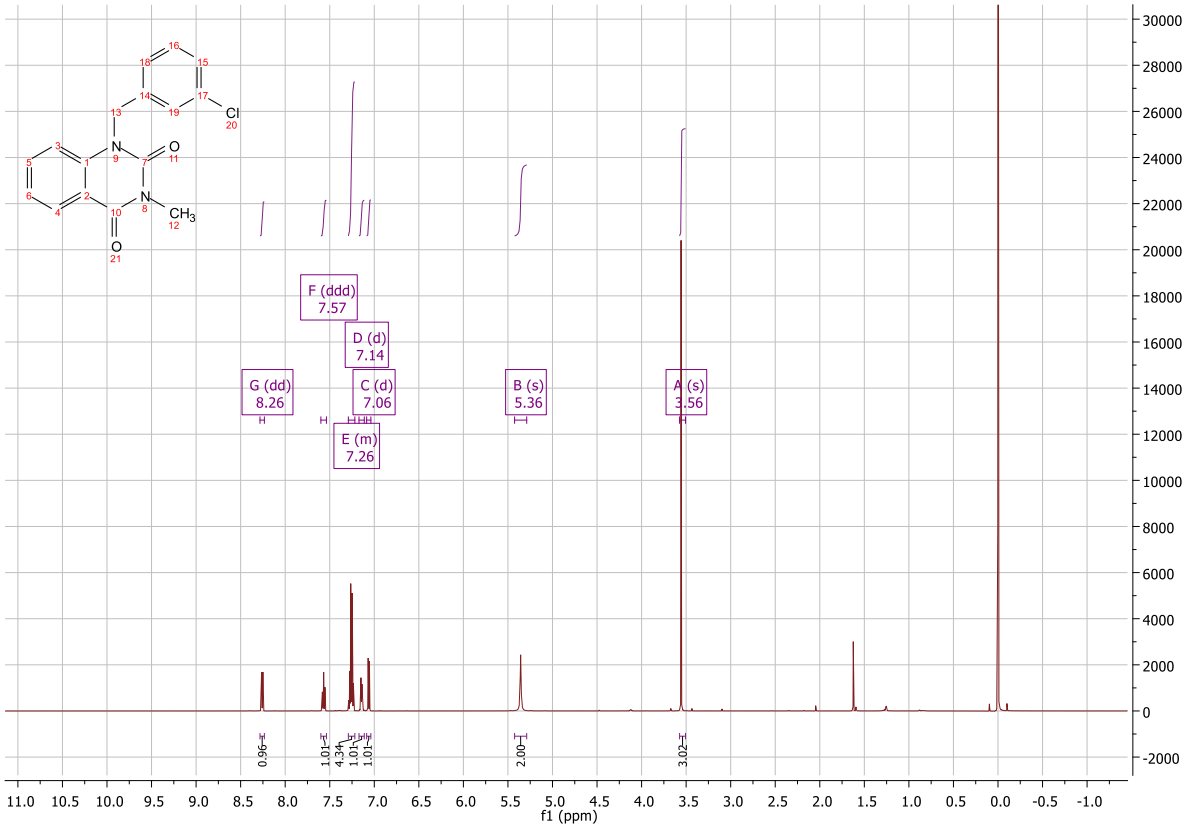


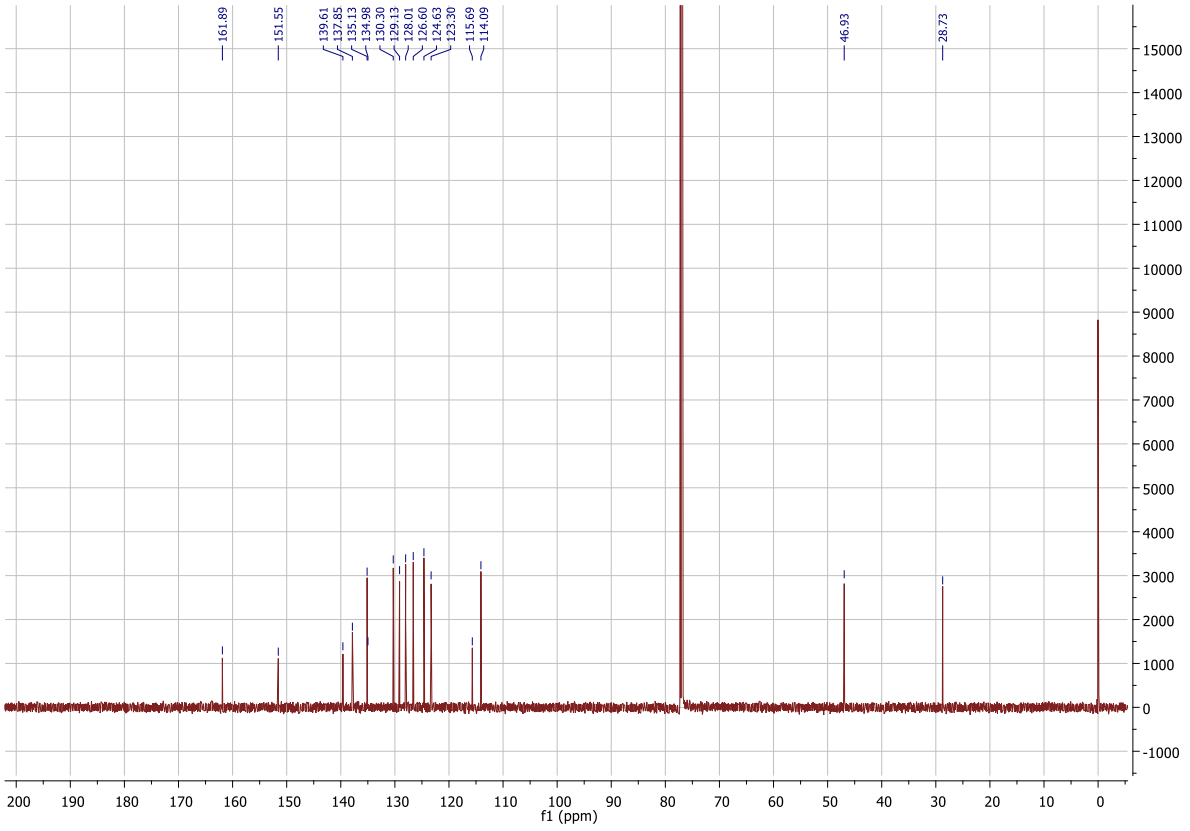
7e: 3-Methyl-1-(4-(triflouromethyl)benzyl)-3,4-dihydroquinazolin-2(1*H*)-one



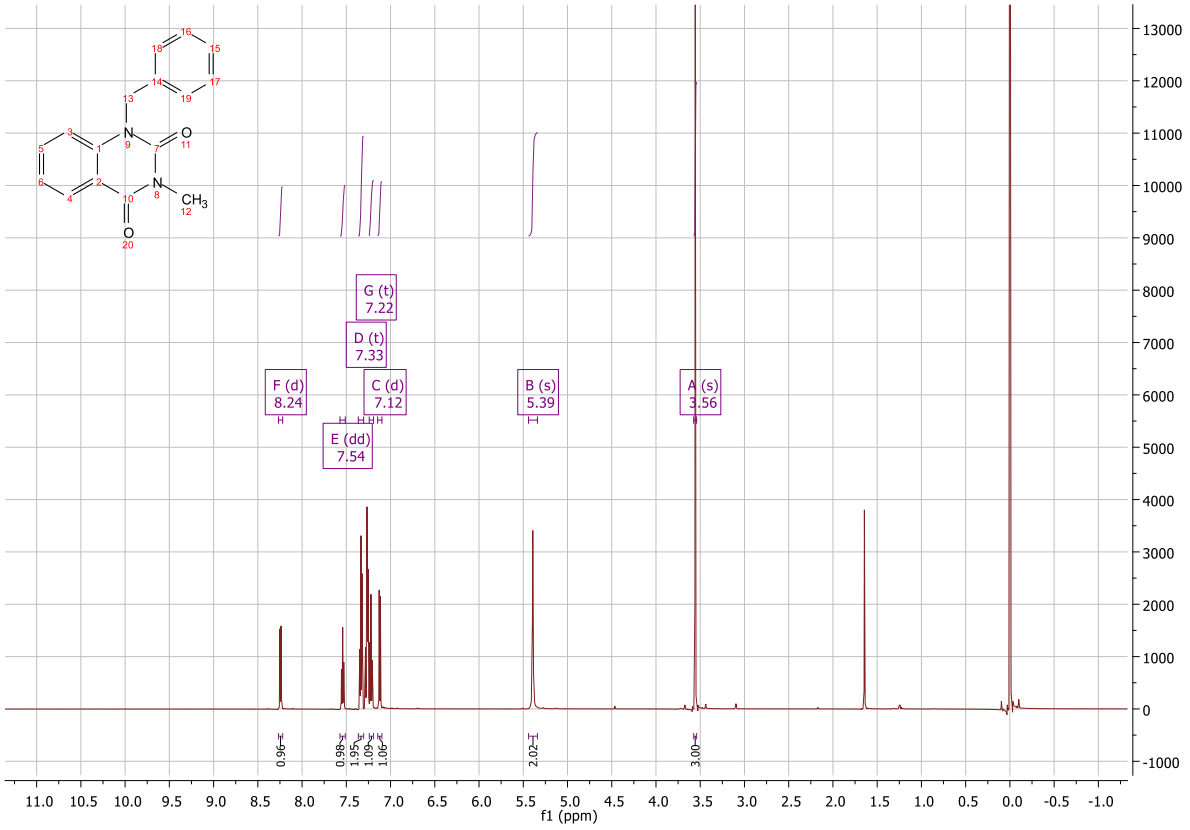


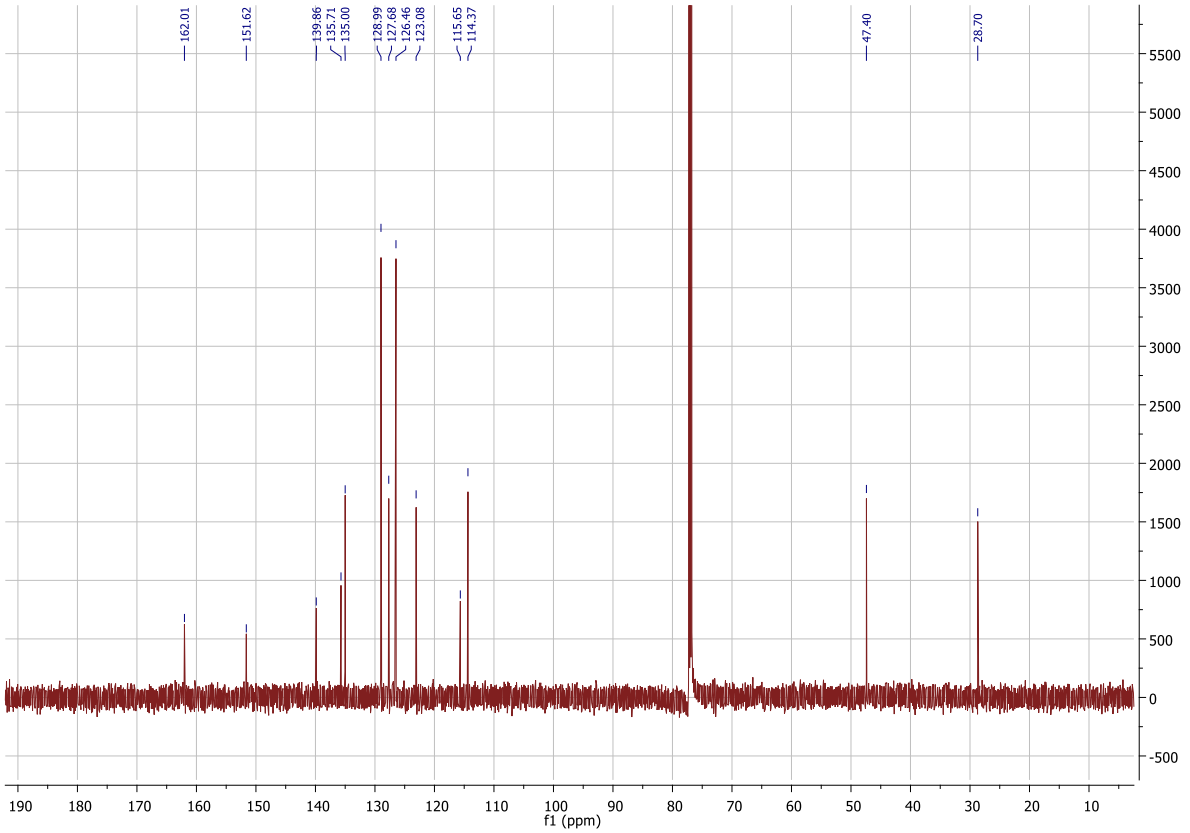
8a: 1-(3-Chlorobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



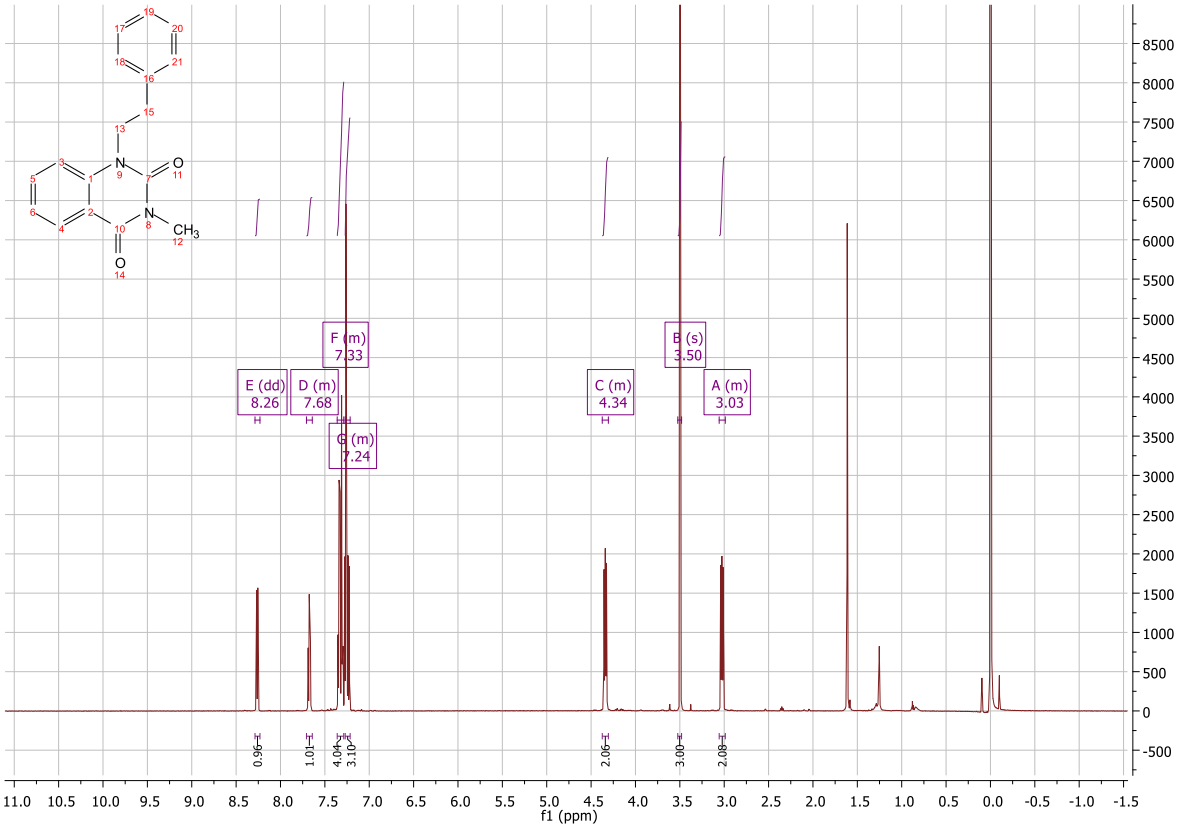


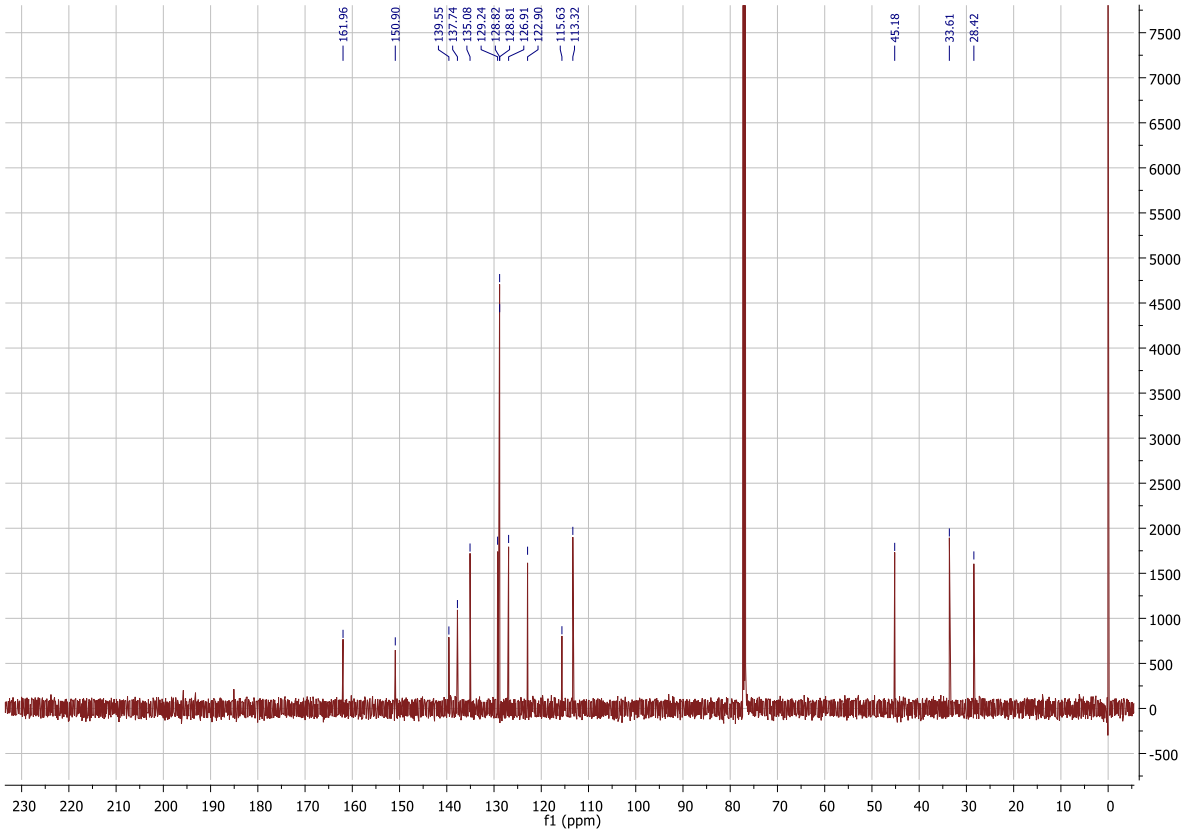
8b: 1-Benzyl-3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



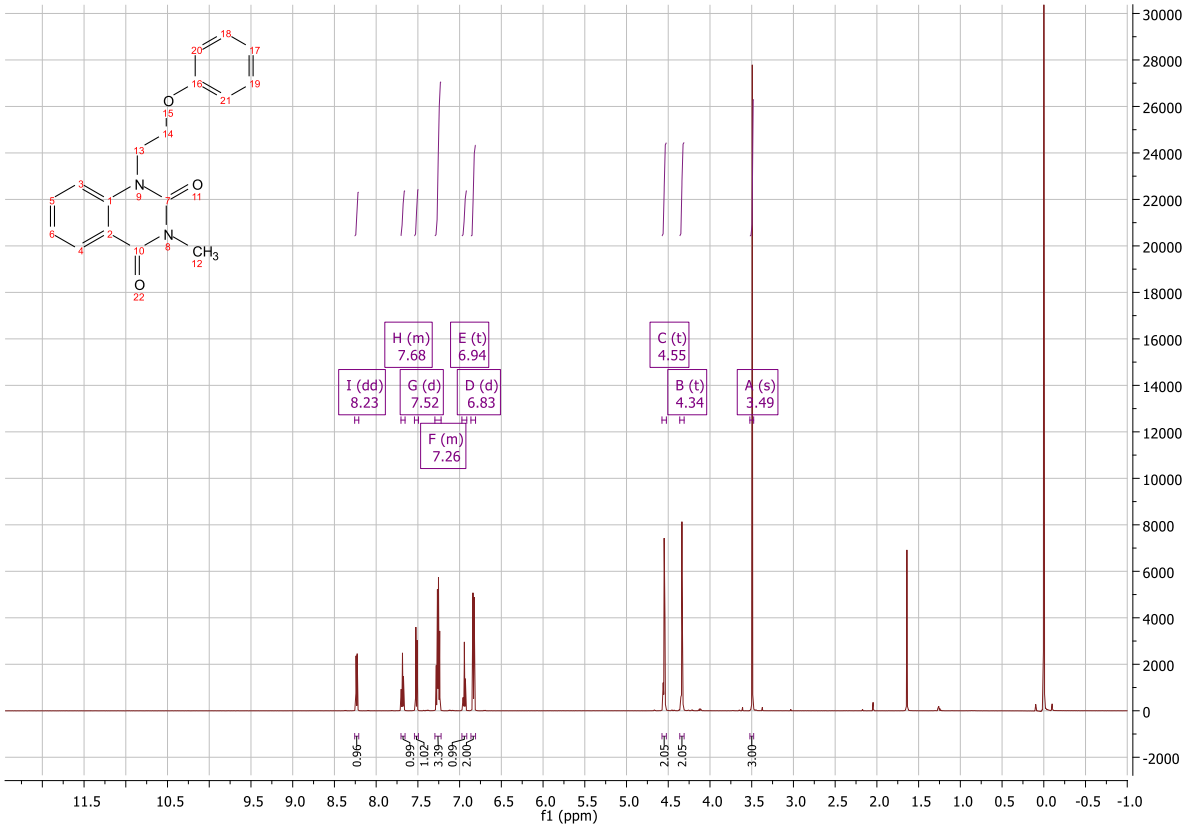


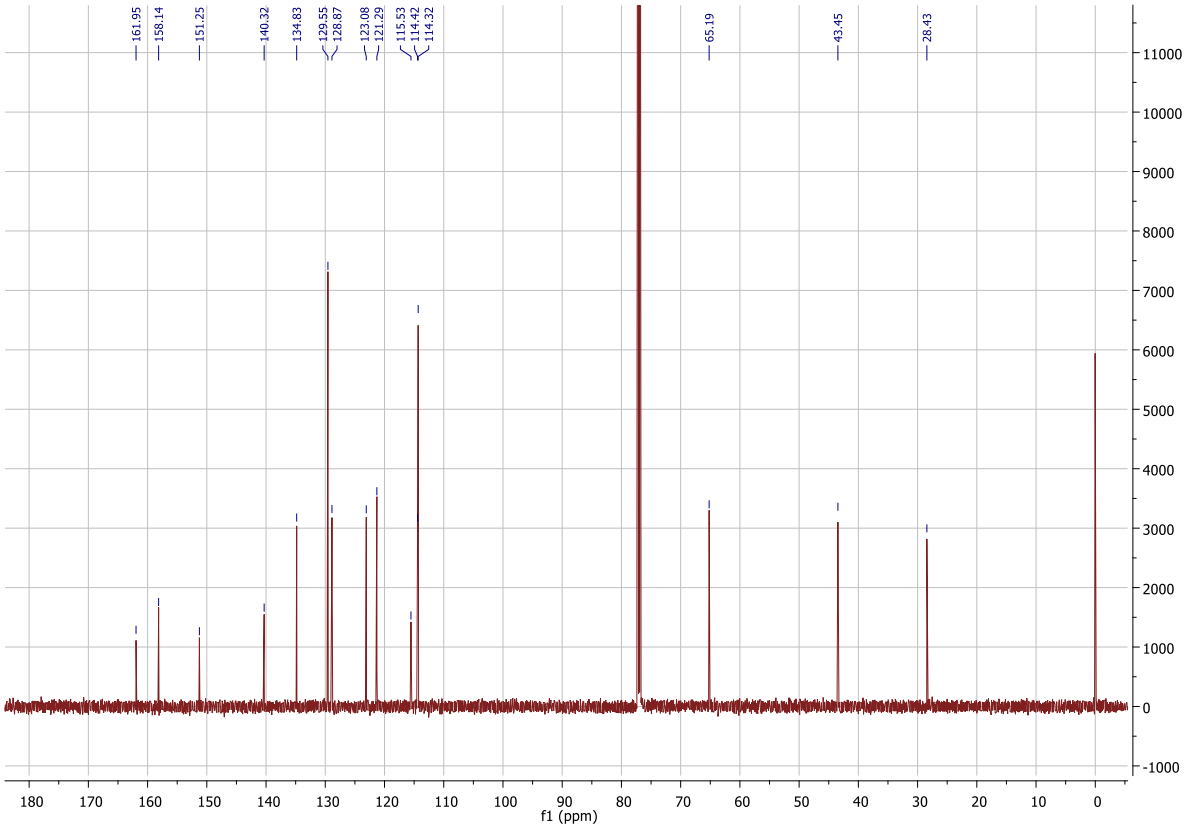
8c: 3-Methyl-1-phenethyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



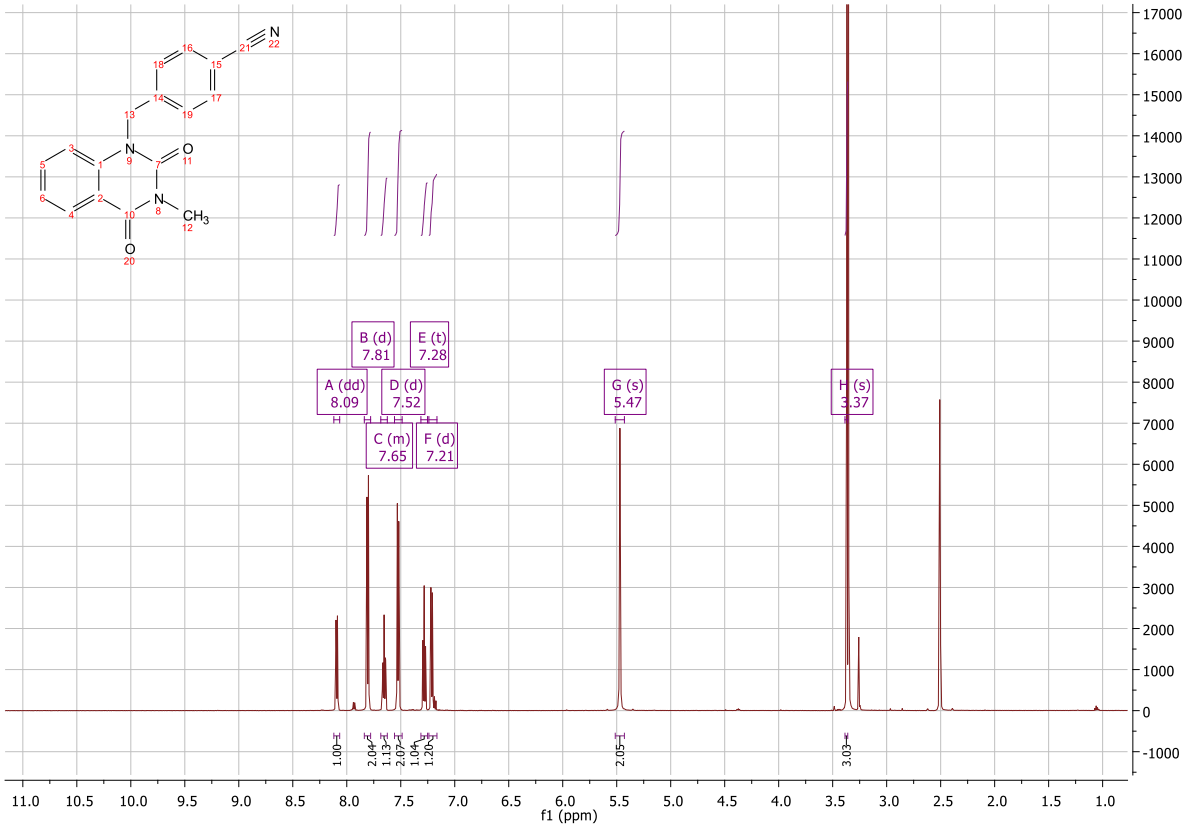


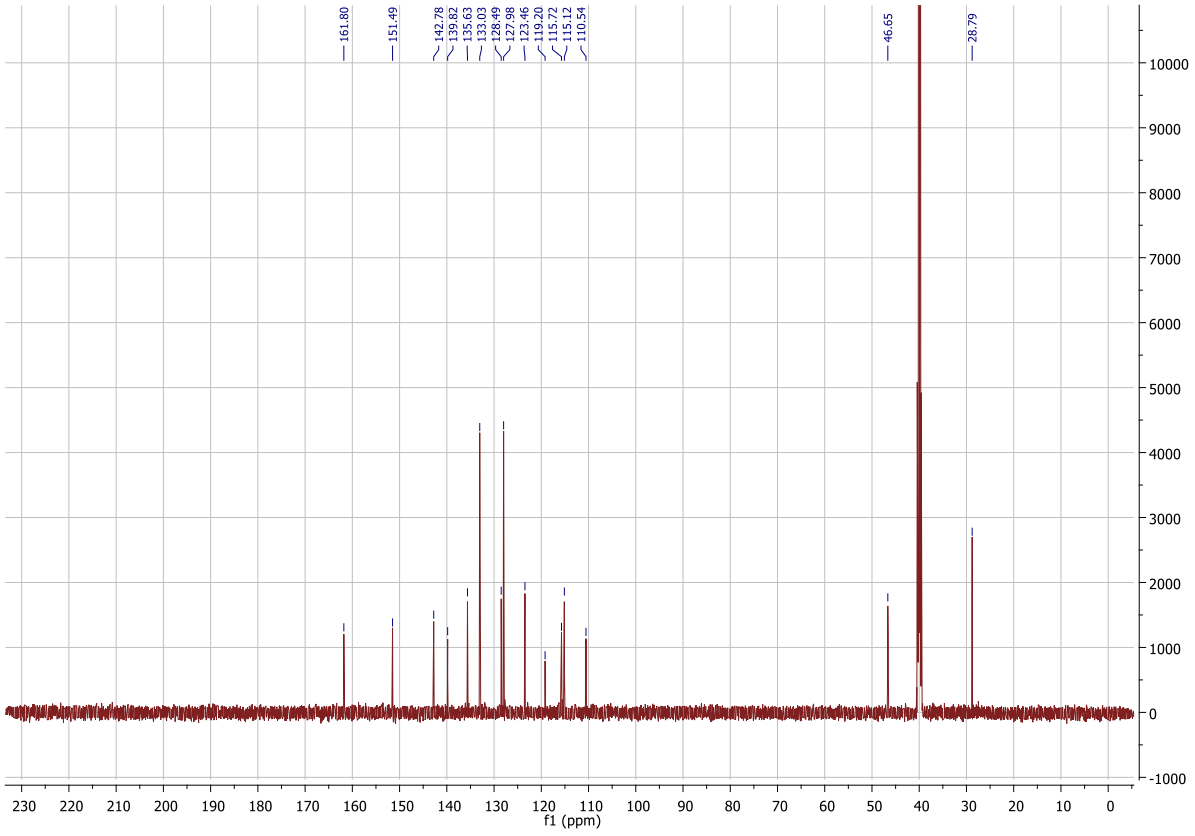
8d: 3-Methyl-1-(2-phenoxyethyl)-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



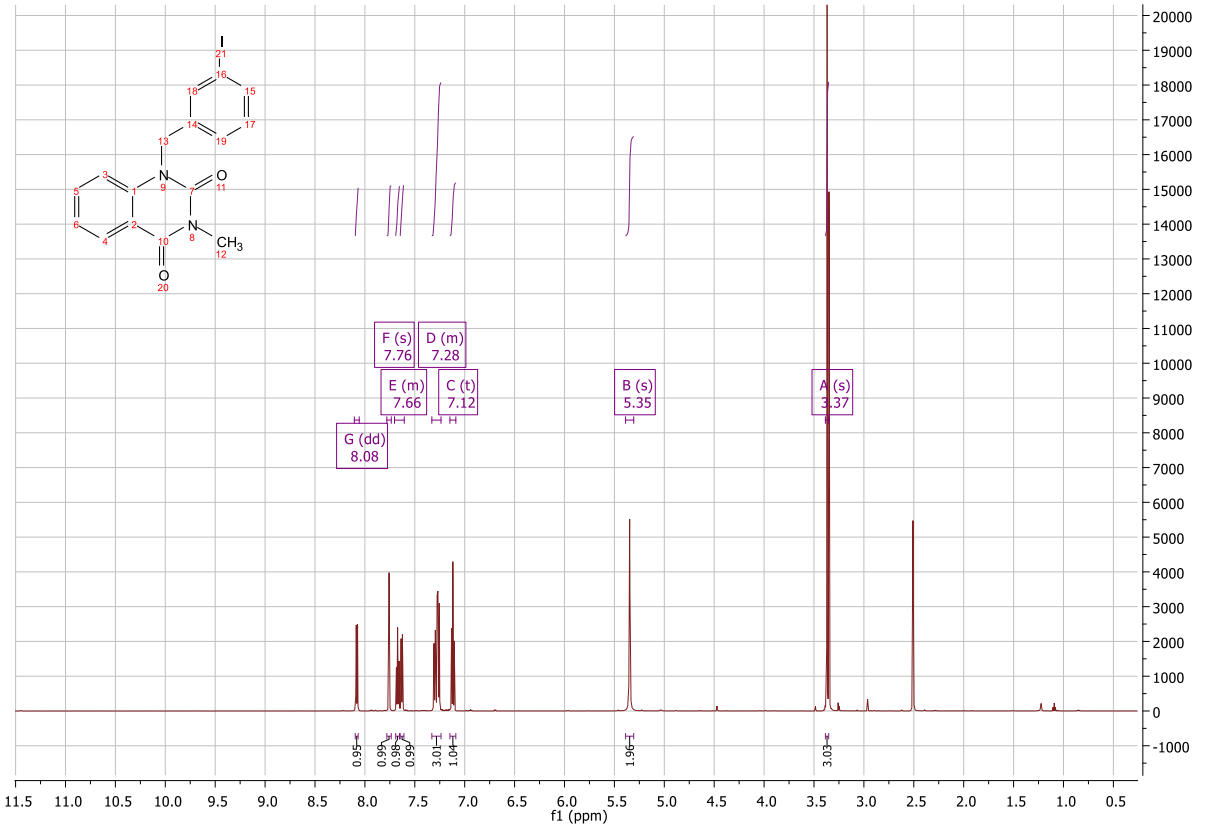


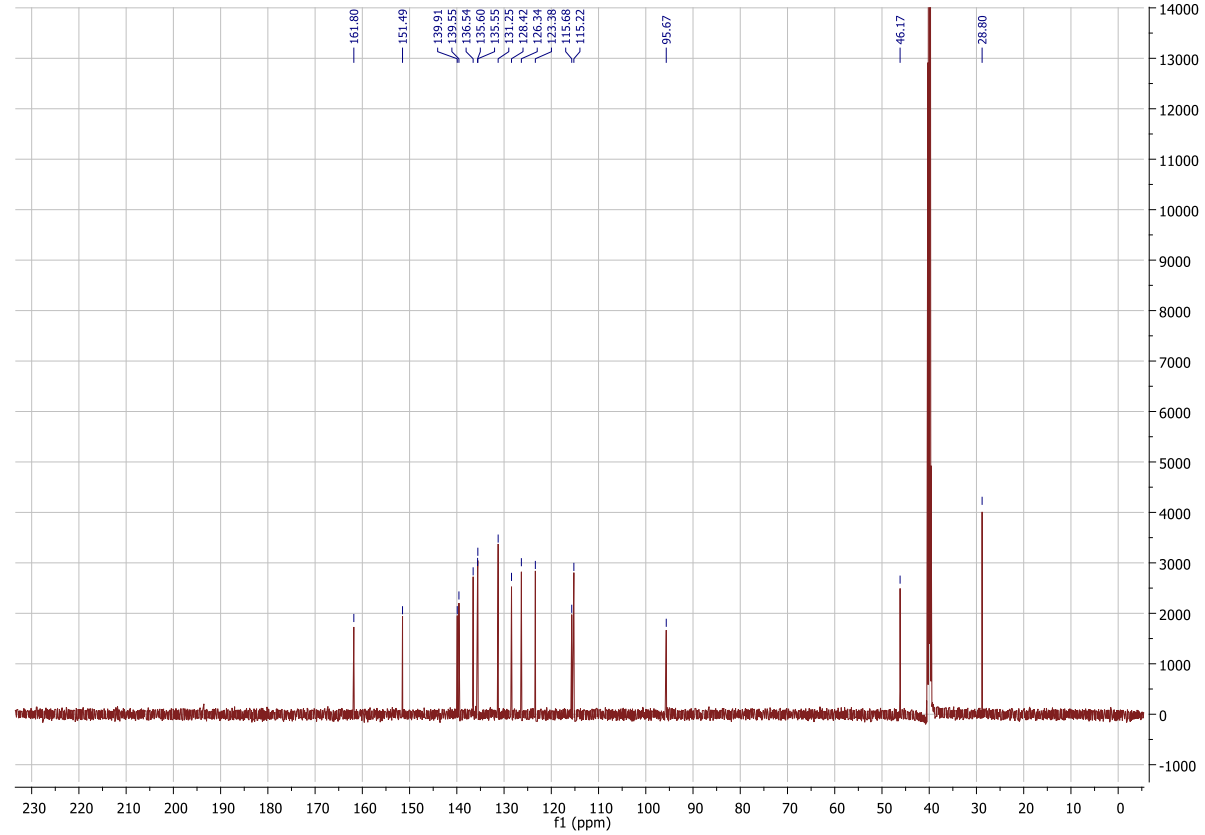
8e: 4-((3-Methyl-2,4-dioxo-3,4-dihydroquinazolin-2(1*H*)-yl)methyl)benzonitrile





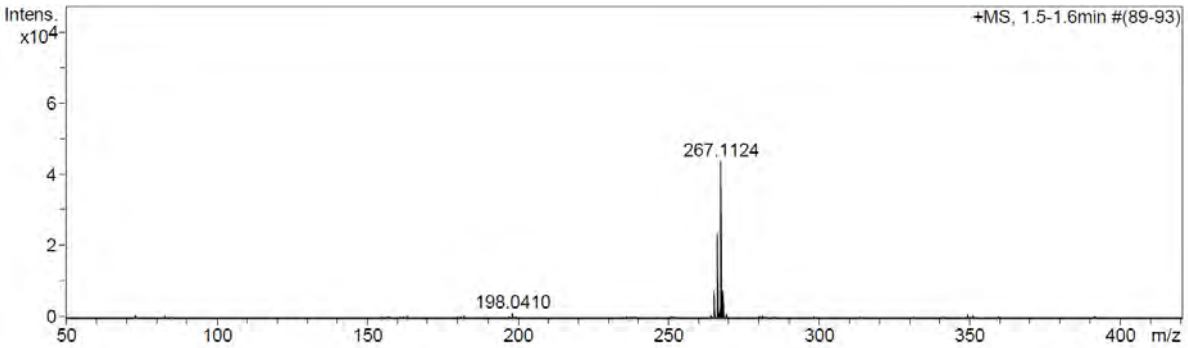
8f: 1-(3-Iodobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione





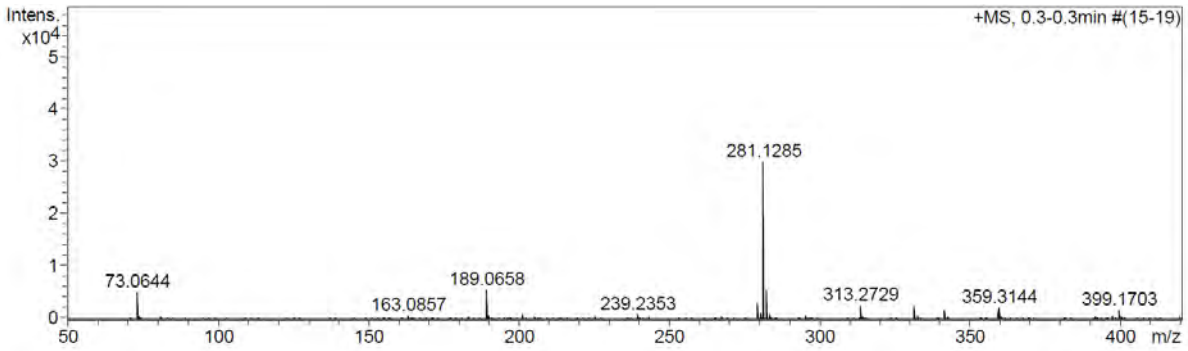
LIST OF MS SPECTROMETRY DATA

5a: 6-Benzoyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



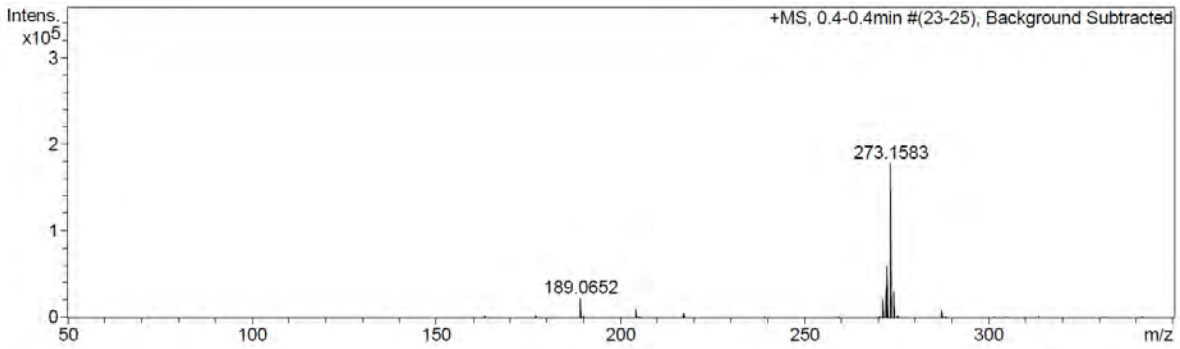
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
267.1124	1	C 16 H 15 N 2 O 2	100.00	267.1128	0.4	1.6	4.8	10.5	ok	even

5b: 3-Methyl-6-(2-phenylacetyl)-3,4-dihydroquinazolin-2(1*H*)-one



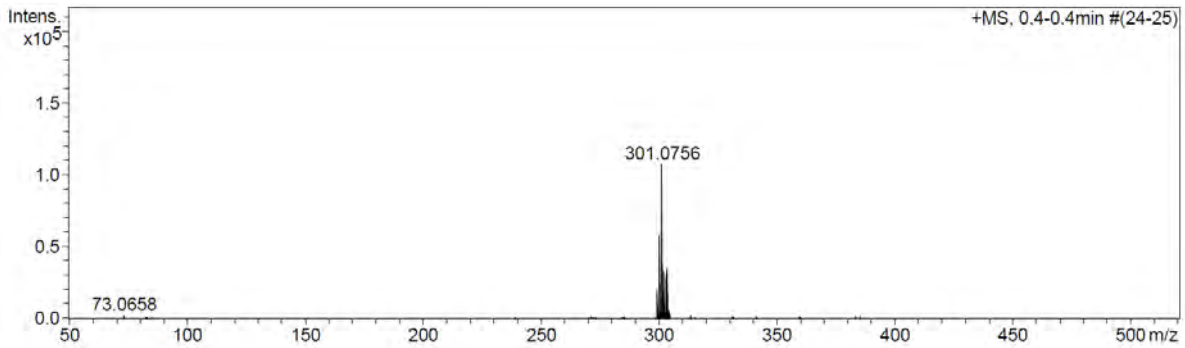
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
281.1285	1	C 17 H 17 N 2 O 2	100.00	281.1285	-0.1	-0.2	5.2	10.5	ok	even
	2	C 22 H 17	2.98	281.1325	4.0	14.1	28.7	14.5	ok	even

5c: 6-(Cyclohexanecarbonyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



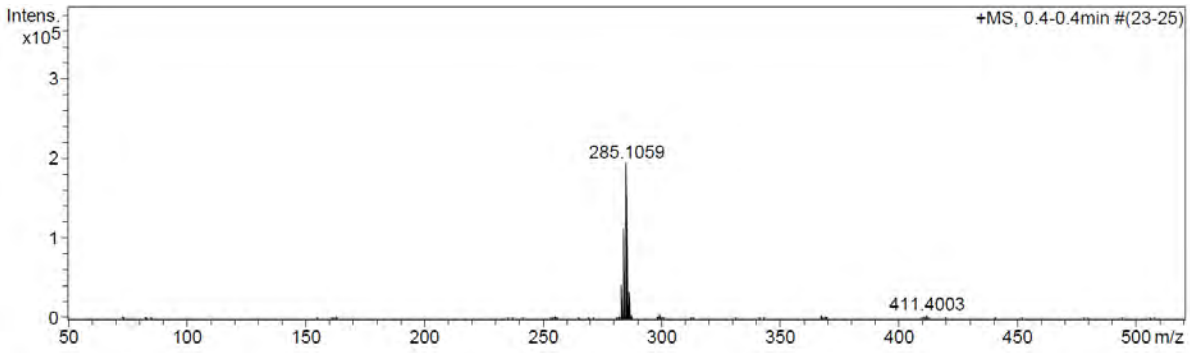
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
273.1583	1	C 16 H 21 N 2 O 2	100.00	273.1598	1.5	5.4	4.7	7.5	ok	even

5d: 6-(4-Chlorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



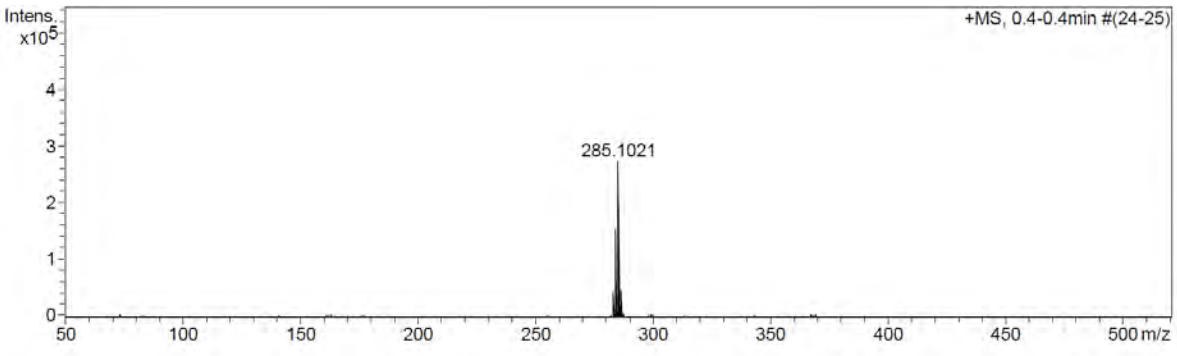
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
301.0756	1	C 16 H 14 Cl N 2 O 2	100.00	301.0738	-1.7	-5.8	58.5	10.5	ok	even

5e: 6-(4-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



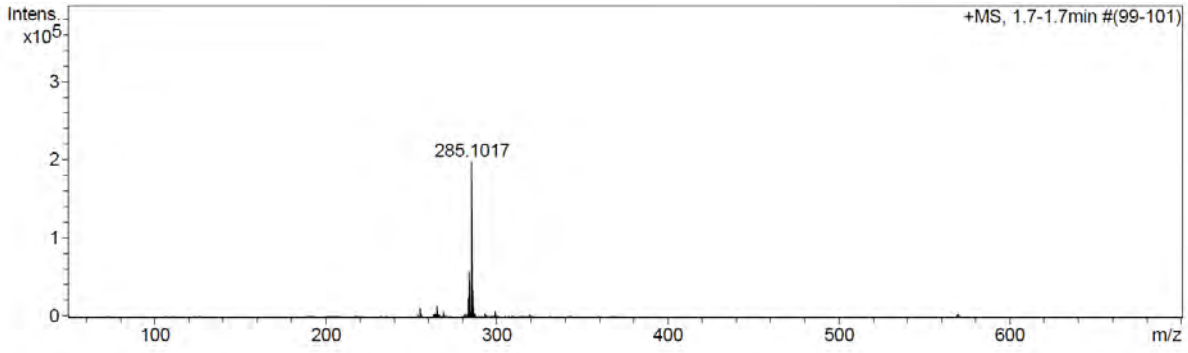
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
284.0981	1	C 15 H 18 Cl F O 2	100.00	284.0974	-0.7	-2.5	426.4	6.0	ok	odd
	2	C 11 H 21 Cl O 6	1.94	284.1021	4.0	14.2	443.5	1.0	ok	odd
	3	C 16 H 13 F N 2 O 2	0.00	284.0956	-2.5	-8.9	539.6	11.0	ok	odd
	4	C 12 H 16 N 2 O 6	0.00	284.1003	2.2	7.8	561.5	6.0	ok	odd
	5	C 9 H 17 F N 2 O 7	0.00	284.1014	3.3	11.8	578.3	2.0	ok	odd
	6	C 9 H 18 N O 9	0.00	284.0976	-0.5	-1.7	579.9	1.5	ok	even
285.1059	1	C 16 H 14 F N 2 O 2	100.00	285.1034	-2.5	-8.7	5.4	10.5	ok	even
	2	C 12 H 17 N 2 O 6	91.29	285.1081	2.3	7.9	19.6	5.5	ok	even
	3	C 15 H 19 Cl F O 2	1.54	285.1052	-0.6	-2.3	143.6	5.5	ok	even

5f: 6-(2-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



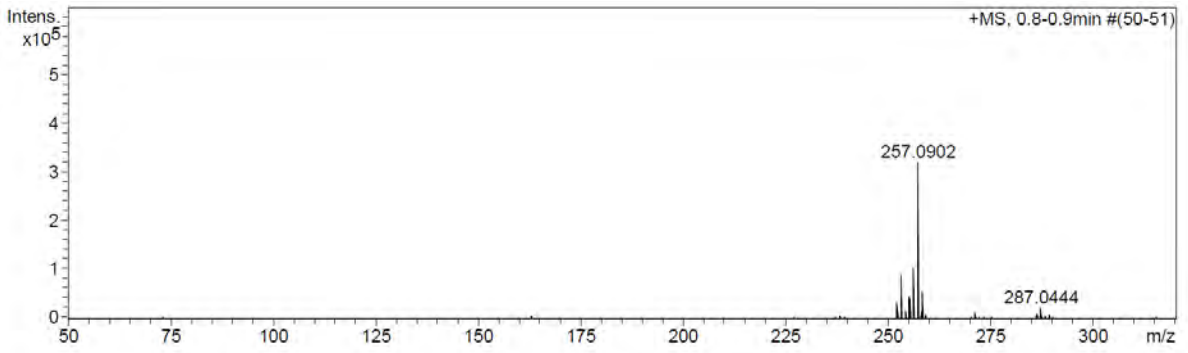
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
285.1021	1	C 16 H 14 F N 2 O 2	100.00	285.1034	1.3	4.5	4.6	10.5	ok	even

5g: 6-(3-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



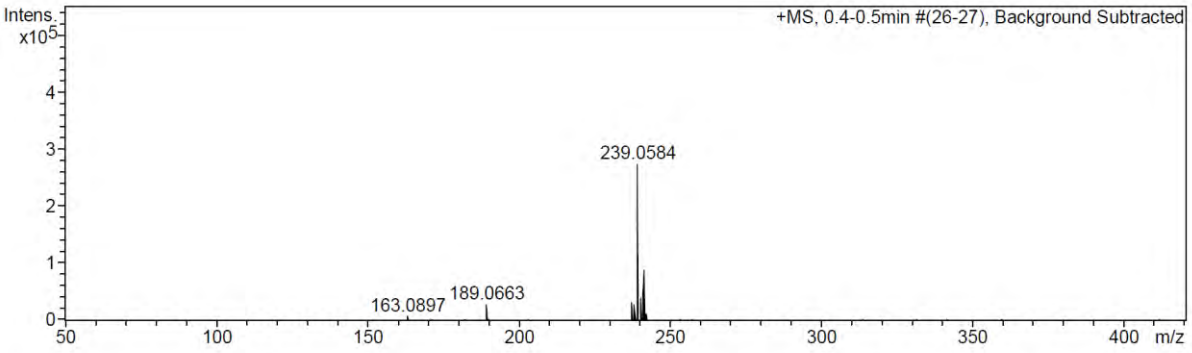
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
285.1017	1	C 16 H 14 F N 2 O 2	100.00	285.1034	1.7	5.9	3.5	10.5	ok	even
	2	C 11 H 13 F 2 N 5 O 2	80.61	285.1032	1.5	5.2	21.7	7.0	ok	odd
	3	C 9 H 13 F 4 N 4 O 2	2.05	285.0969	-4.8	-16.8	36.4	3.5	ok	even
	4	C 6 H 12 F 3 N 8 O 2	48.16	285.1030	1.3	4.5	46.8	3.5	ok	even
	5	C 8 H 15 F 6 N 2 O 2	40.72	285.1032	1.5	5.3	47.2	-0.5	ok	even
	6	C 4 H 12 F 5 N 7 O 2	0.73	285.0967	-5.0	-17.5	61.3	0.0	ok	odd

5h: 6-(Furan-2-carbonyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



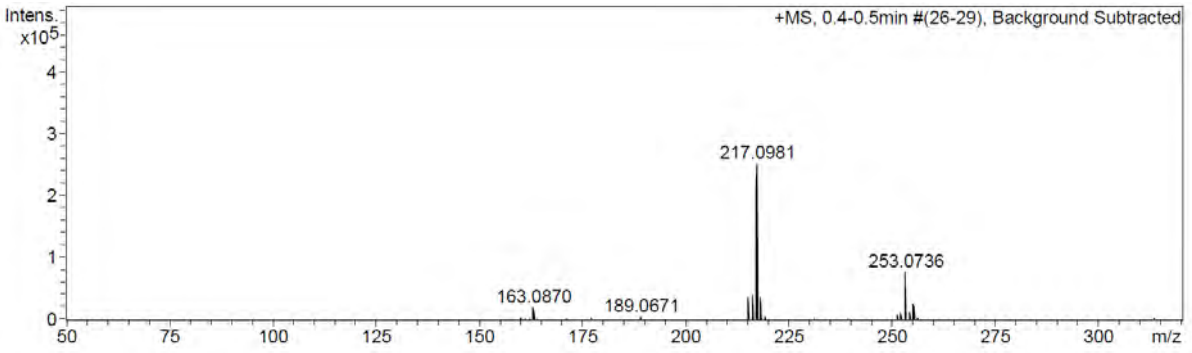
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
257.0902	1	C 14 H 13 N 2 O 3	100.00	257.0921	1.9	7.2	6.8	9.5	ok	even
	2	C 18 H 11 N O	0.19	257.0835	-6.7	-26.1	16.1	14.0	ok	odd
	3	C 19 H 13 O	0.72	257.0961	5.9	22.9	20.2	13.5	ok	even

5i: 6-(2-Chloroacetyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



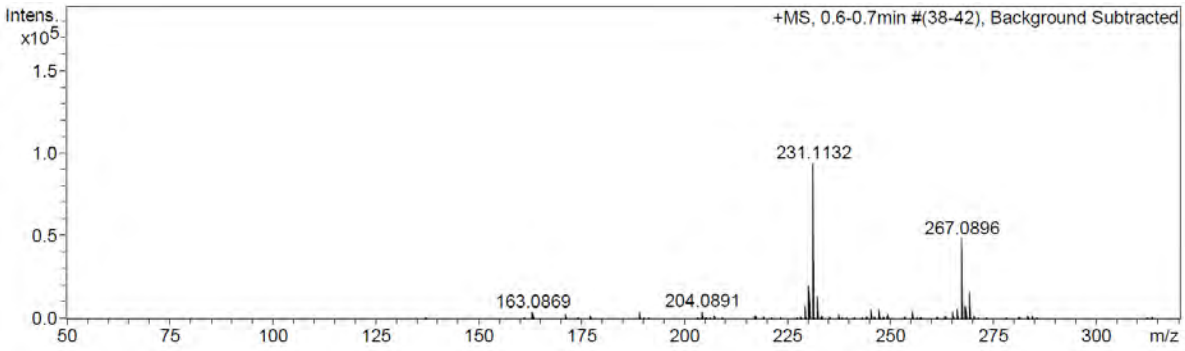
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
239.0584	1	C 11 H 12 Cl N 2 O 2	100.00	239.0582	-0.2	-0.8	9.5	6.5	ok	even
	2	C 16 H 12 Cl	5.51	239.0622	3.8	16.0	14.3	10.5	ok	even
	3	C 17 H 7 N 2	0.01	239.0604	2.0	8.4	180.8	15.5	ok	even

5j: 6-(3-Chloropropanoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



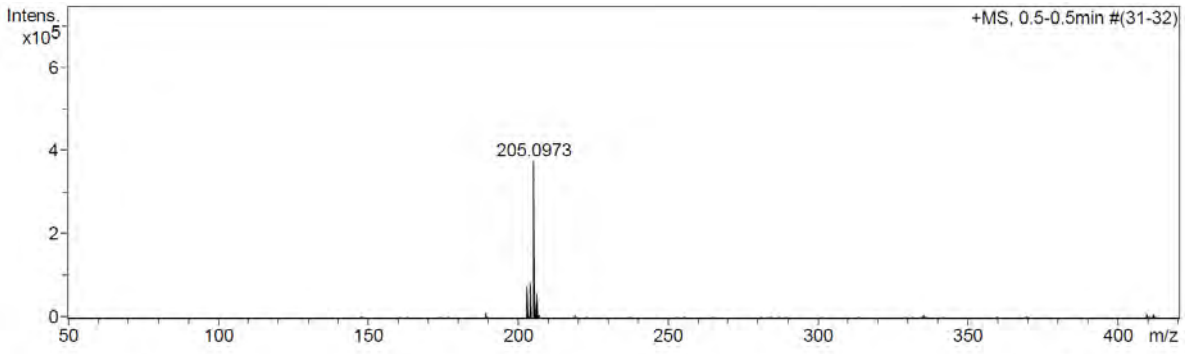
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
217.0981	1	C 12 H 13 N 2 O 2	100.00	217.0972	-1.0	-4.4	9.1	7.5	ok	even
	2	C 17 H 13	15.45	217.1012	3.1	14.1	21.1	11.5	ok	even
	3	C 9 H 16 Cl N 3 O	0.34	217.0976	-0.5	-2.1	152.6	3.0	ok	odd
	4	C 11 H 18 Cl O 2	0.24	217.0990	0.9	4.0	154.8	2.5	ok	even
253.0736	1	C 12 H 14 Cl N 2 O 2	100.00	253.0738	0.2	1.0	12.8	6.5	ok	even
	2	C 18 H 9 N 2	0.01	253.0760	2.4	9.6	182.3	15.5	ok	even

5k: 6-(4-Chlorobutanoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



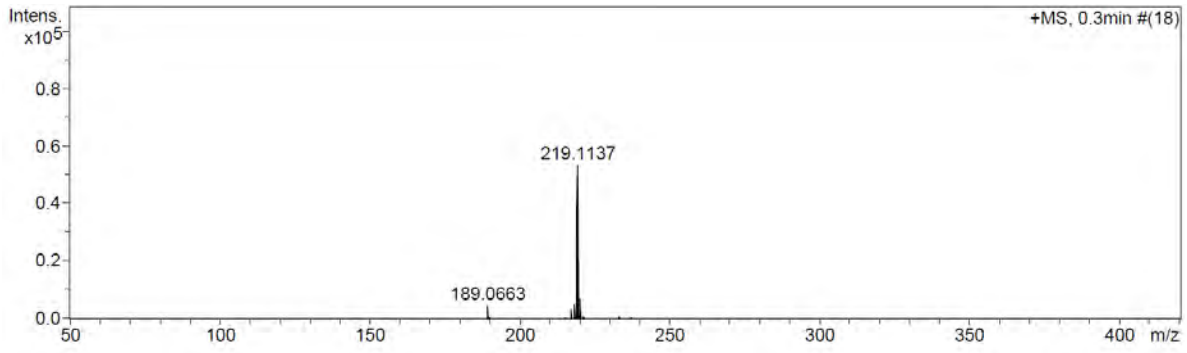
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
231.1132	1	C 13 H 15 N 2 O 2	100.00	231.1128	-0.4	-1.6	6.4	7.5	ok	even
	2	C 18 H 15	5.05	231.1168	3.7	15.8	28.2	11.5	ok	even
	3	C 10 H 18 Cl N 3 O	0.29	231.1133	0.1	0.5	152.0	3.0	ok	odd
	4	C 12 H 20 Cl O 2	0.12	231.1146	1.5	6.3	154.5	2.5	ok	even
267.0896	1	C 13 H 16 Cl N 2 O 2	100.00	267.0895	-0.1	-0.5	10.0	6.5	ok	even
	2	C 18 H 16 Cl	5.13	267.0935	3.9	14.5	13.8	10.5	ok	even
	3	C 19 H 11 N 2	0.01	267.0917	2.1	7.7	182.0	15.5	ok	even

5l: 6-Acetyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



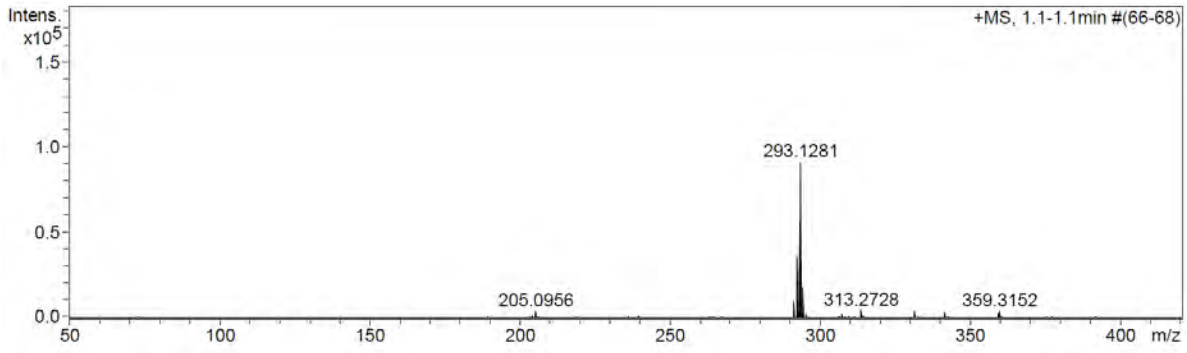
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
205.0973	1	C 16 H 13	5.44	205.1012	3.9	19.0	13.3	10.5	ok	even
	2	C 11 H 13 N 2 O 2	100.00	205.0972	-0.1	-0.6	13.7	6.5	ok	even

5m: 3-Methyl-6-propionyl-3,4-dihydroquinazolin-2(1*H*)-one



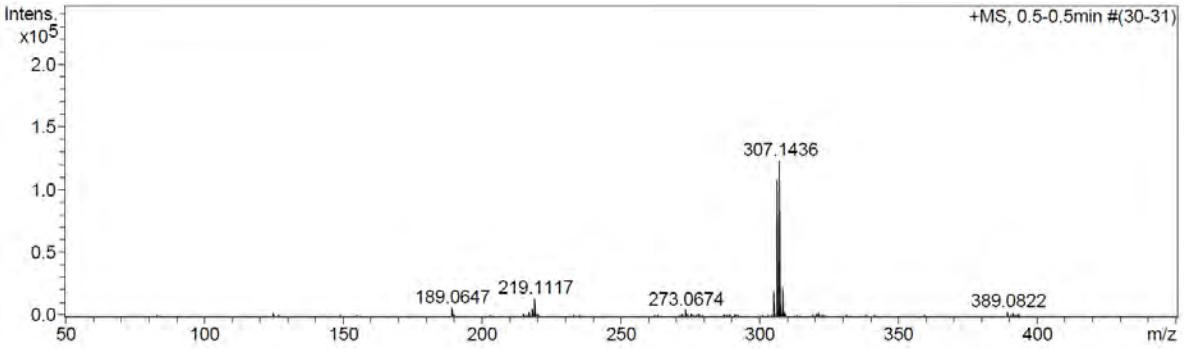
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
219.1137	1	C 12 H 15 N 2 O 2	100.00	219.1128	-0.9	-4.0	8.8	6.5	ok	even
	2	C 17 H 15	10.18	219.1168	3.2	14.4	33.0	10.5	ok	even

6a: 6-Cinnamoyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



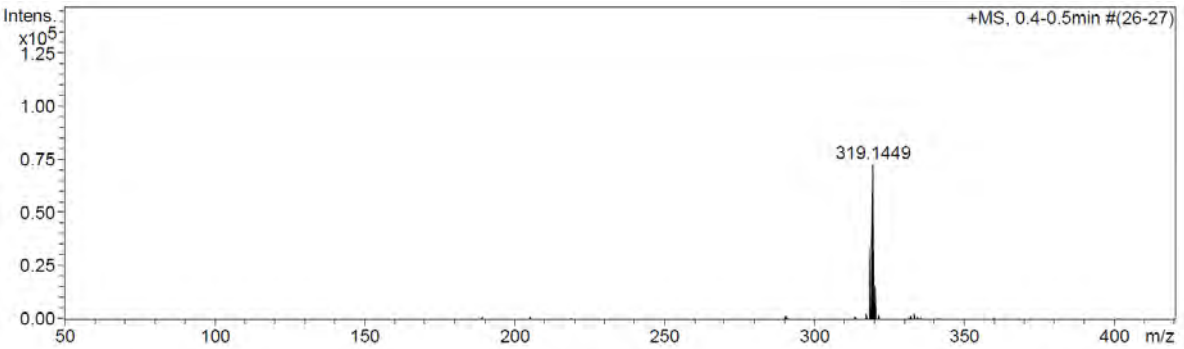
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
293.1281	1	C 18 H 17 N 2 O 2	100.00	293.1285	0.3	1.1	3.9	11.5	ok	even

6b: (*z*)-3-Methyl-6-(2-methyl-3-phenylacryloyl)-3,4-dihydroquinazolin-2(1*H*)-one



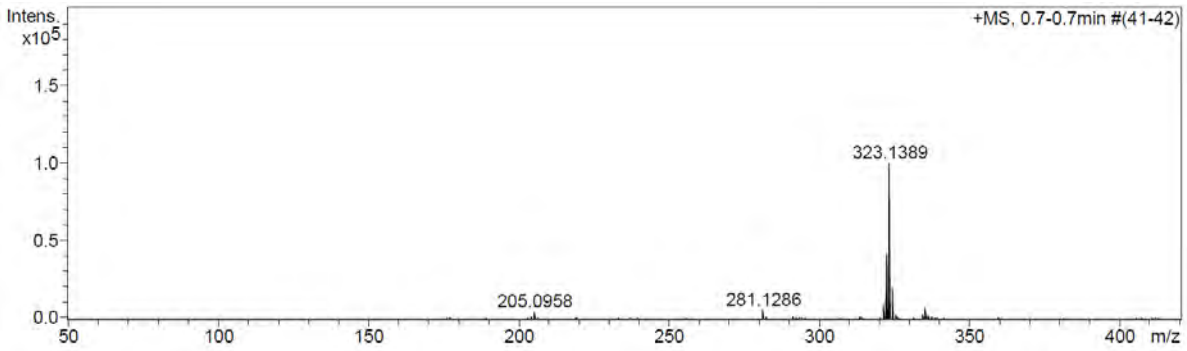
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
307.1436	1	C 19 H 19 N 2 O 2	100.00	307.1441	0.5	1.6	12.9	11.5	ok	even

6c: 3-Methyl-6-((2*E*,4*Z*)-5-phenylpenta-2,4-dienoyl)-3,4-dihydroquinazolin-2(1*H*)-one



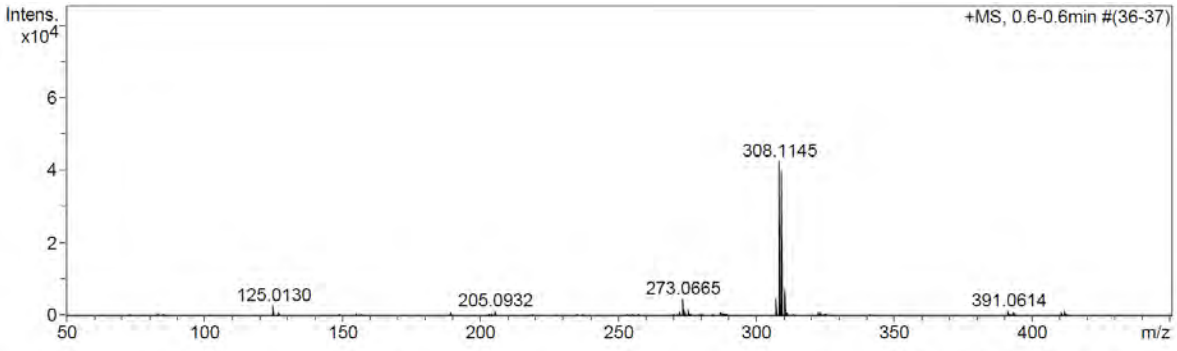
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
319.1449	1	C 20 H 19 N 2 O 2	100.00	319.1441	-0.8	-2.4	2.2	12.5	ok	even
	2	C 25 H 19	8.36	319.1481	3.3	10.2	29.4	16.5	ok	even

6d: (*E*)-6-(3-(4-Methoxyphenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



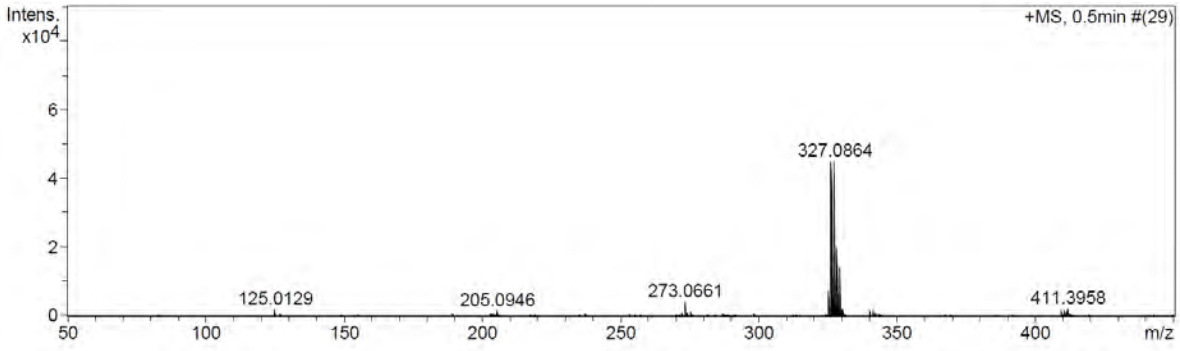
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
323.1389	1	C 19 H 19 N 2 O 3	100.00	323.1390	0.1	0.2	6.6	11.5	ok	even
	2	C 22 H 17 N 3	11.29	323.1417	2.8	8.5	27.4	16.0	ok	odd

6e: (*E*)-6-(3-(4-Hydroxyphenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



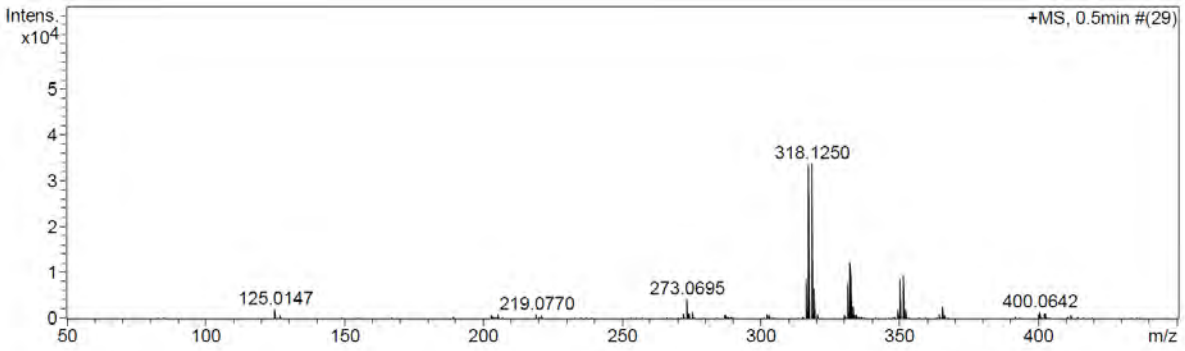
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
308.1145	1	C 18 H 16 N 2 O 3	100.00	308.1155	1.1	3.5	430.9	12.0	ok	odd
309.1216	1	C 18 H 17 N 2 O 3	100.00	309.1234	1.7	5.6	20.2	11.5	ok	even

6f: (*E*)-6-(3-(4-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



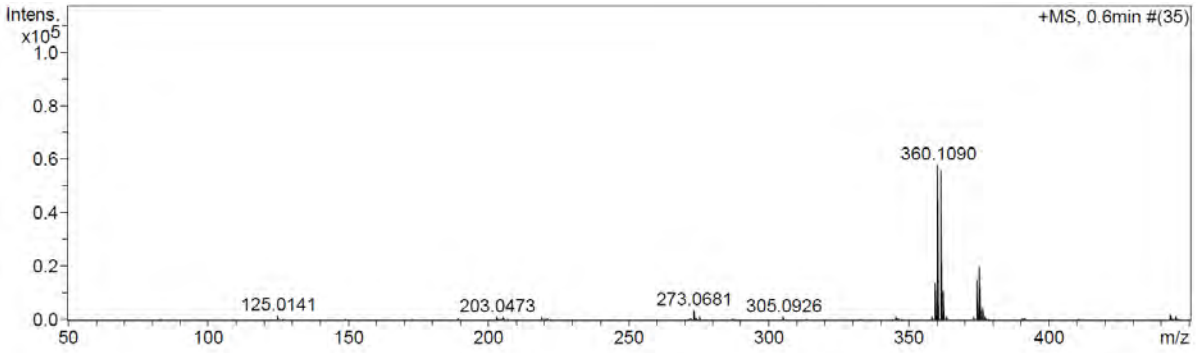
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
326.0804	1	C 18 H 15 Cl N 2 O 2	100.00	326.0817	1.2	3.8	377.2	12.0	ok	odd
327.0864	1	C 18 H 16 Cl N 2 O 2	100.00	327.0895	3.1	9.4	109.5	11.5	ok	even

6g: (*E*)-4-(3-(3-Methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)-3-oxoprop-1-en-1-yl)benzonitrile



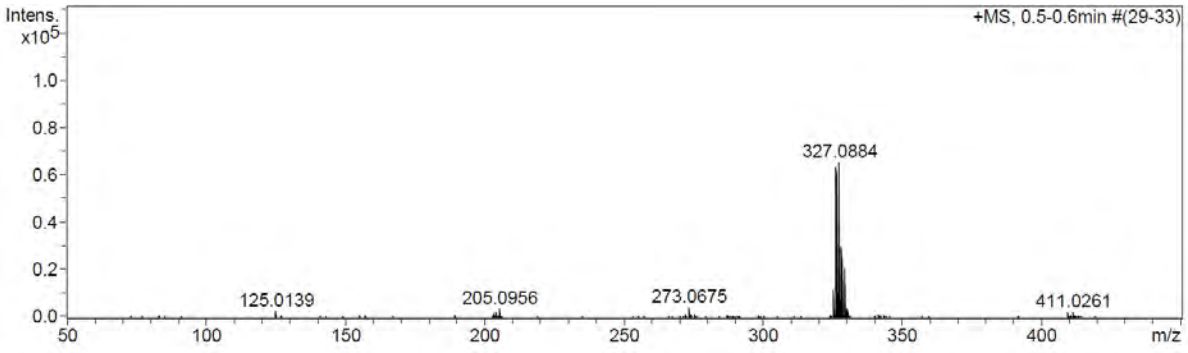
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
317.1177	1	C 24 H 15 N	100.00	317.1199	2.2	6.9	435.2	18.0	ok	odd
	2	C 21 H 17 O 3	6.94	317.1172	-0.5	-1.6	455.9	13.5	ok	even
	3	C 19 H 15 N 3 O 2	0.93	317.1159	-1.8	-5.8	462.3	14.0	ok	odd
318.1250	1	C 19 H 16 N 3 O 2	62.62	318.1237	-1.3	-4.0	20.7	13.5	ok	even
	2	C 21 H 18 O 3	100.00	318.1250	0.1	0.3	26.8	13.0	ok	odd
	3	C 24 H 16 N	10.68	318.1277	2.8	8.7	45.3	17.5	ok	even

6h: (*E*)-3-Methyl-6-(3-(4-(trifluoromethyl)phenyl)acryloyl)-3,4-dihydroquinazolin-2(1*H*)-one



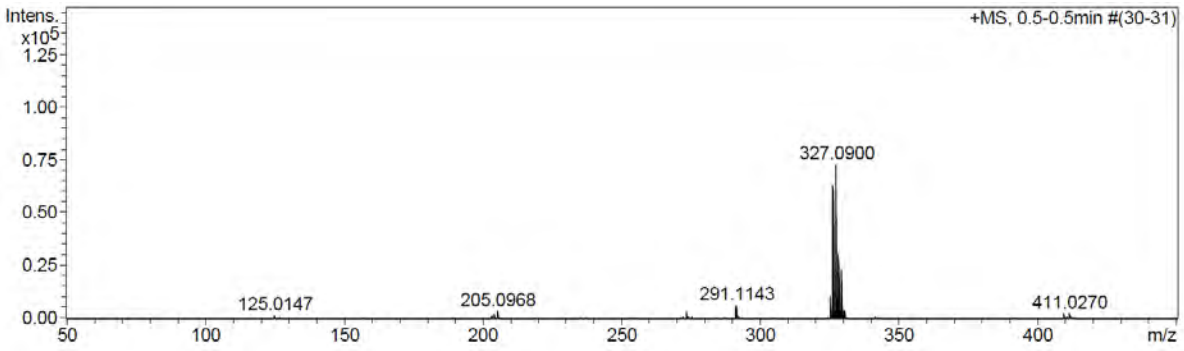
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
360.1090	1	C 19 H 15 F 3 N 2 O 2	100.00	360.1080	-0.9	-2.6	440.8	12.0	ok	odd
361.1158	1	C 19 H 16 F 3 N 2 O 2	100.00	361.1158	0.0	0.0	7.4	11.5	ok	even

6i: (*E*)-6-(3-(3-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



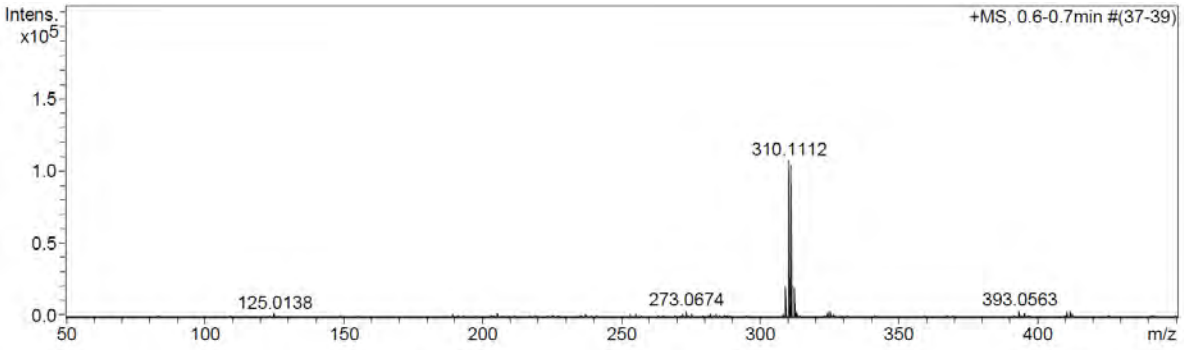
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
326.0821	1	C 23 H 15 Cl	100.00	326.0857	3.6	11.1	356.3	16.0	ok	odd
	2	C 18 H 15 Cl N 2 O 2	45.42	326.0817	-0.4	-1.2	378.2	12.0	ok	odd
	3	C 24 H 10 N 2	0.00	326.0838	1.8	5.5	486.8	21.0	ok	odd
327.0884	1	C 18 H 16 Cl N 2 O 2	100.00	327.0895	1.1	3.4	111.2	11.5	ok	even
	2	C 24 H 11 N 2	0.07	327.0917	3.3	10.1	194.5	20.5	ok	even

6j: (*E*)-6-(3-(2-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



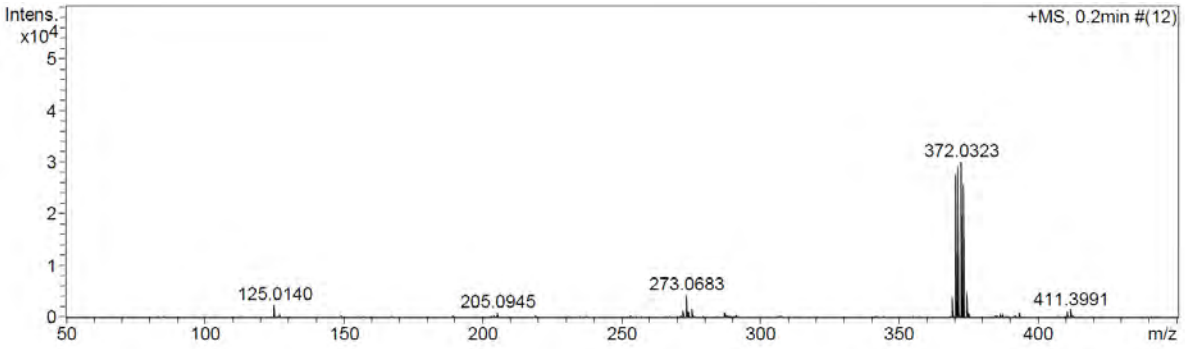
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e¯ Conf
326.0838	1	C 18 H 15 Cl N 2 O 2	100.00	326.0817	-2.1	-6.5	381.6	12.0	ok	odd
327.0900	1	C 23 H 16 Cl	24.95	327.0935	3.5	10.6	76.9	15.5	ok	even
	2	C 18 H 16 Cl N 2 O 2	100.00	327.0895	-0.6	-1.7	96.7	11.5	ok	even
	3	C 24 H 11 N 2	0.17	327.0917	1.6	5.0	187.7	20.5	ok	even
	4	C 21 H 13 N O 3	0.10	327.0890	-1.0	-3.2	199.5	16.0	ok	odd

6k: (*E*)-6-(3-(4-Fluorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



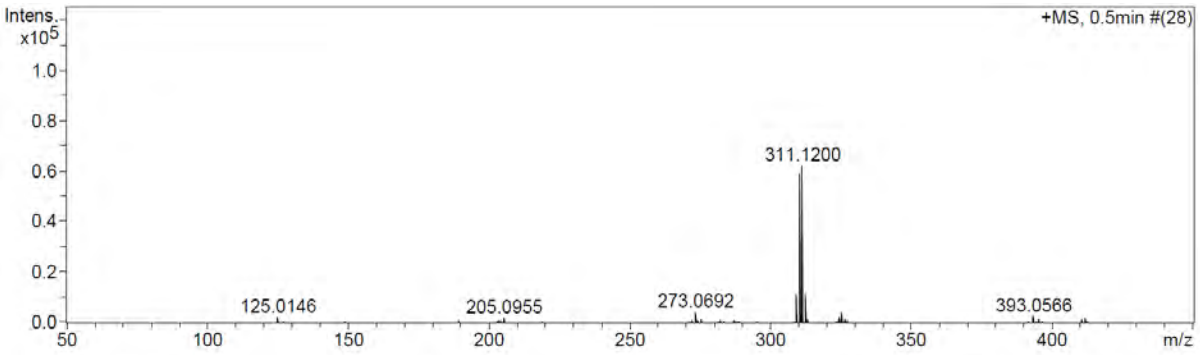
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e¯ Conf
310.1112	1	C 18 H 15 F N 2 O 2	100.00	310.1112	0.0	0.1	455.9	12.0	ok	odd
311.1179	1	C 18 H 16 F N 2 O 2	100.00	311.1190	1.1	3.7	6.8	11.5	ok	even

6l: (*E*)-6-(3-(4-Bromophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



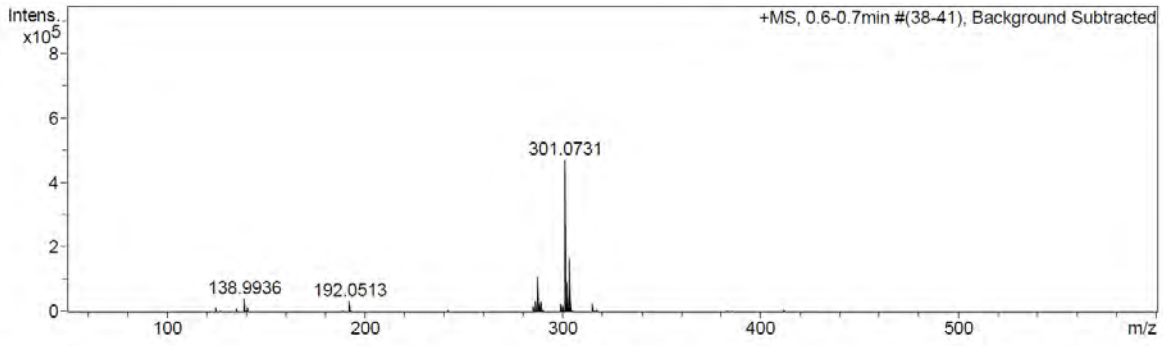
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
370.0324	1	C 23 H 15 Br	100.00	370.0352	2.7	7.4	427.7	16.0	ok	odd
	2	C 18 H 15 Br N 2 O 2	1.80	370.0311	-1.3	-3.5	456.2	12.0	ok	odd
	3	C 28 H 4 N O	0.00	370.0287	-3.7	-10.0	720.0	27.5	ok	even
371.0378	1	C 18 H 16 Br N 2 O 2	100.00	371.0390	1.1	3.1	363.3	11.5	ok	even
	2	C 28 H 5 N O	0.00	371.0366	-1.3	-3.4	538.2	27.0	ok	odd
372.0323	1	C 27 H 4 N 2 O	100.00	372.0318	-0.5	-1.4	283.0	27.0	ok	odd
	2	C 19 H 17 Br O 3	0.00	372.0356	3.2	8.7	481.2	11.0	ok	odd

6m: (*E*)-6-(3-(3-Fluorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



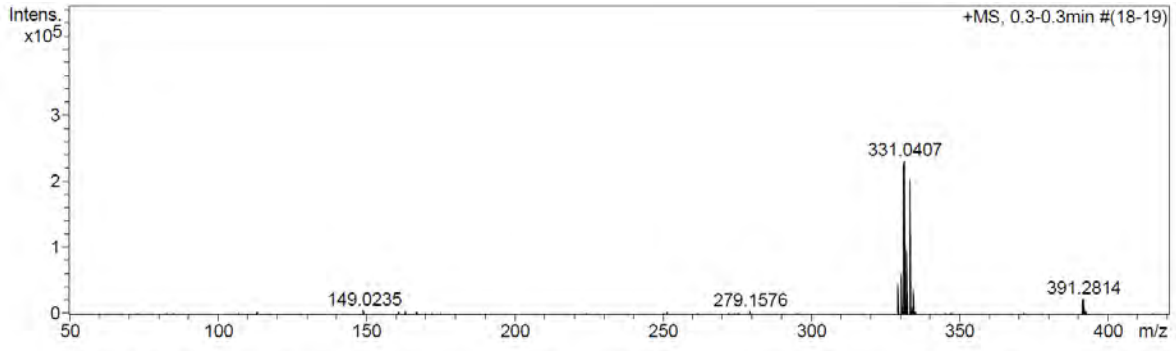
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
310.1132	1	C 23 H 15 F	100.00	310.1152	2.0	6.5	444.3	16.0	ok	odd
	2	C 21 H 14 N 2 O	7.77	310.1101	-3.2	-10.2	452.7	16.0	ok	odd
	3	C 18 H 15 F N 2 O 2	0.66	310.1112	-2.0	-6.5	471.3	12.0	ok	odd
311.1200	1	C 18 H 16 F N 2 O 2	100.00	311.1190	-1.0	-3.2	15.4	11.5	ok	even
	2	C 21 H 15 N 2 O	33.29	311.1179	-2.1	-6.8	30.1	15.5	ok	even
	3	C 23 H 16 F	12.11	311.1231	3.0	9.8	38.3	15.5	ok	even

7a: 1-(4-Chlorobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



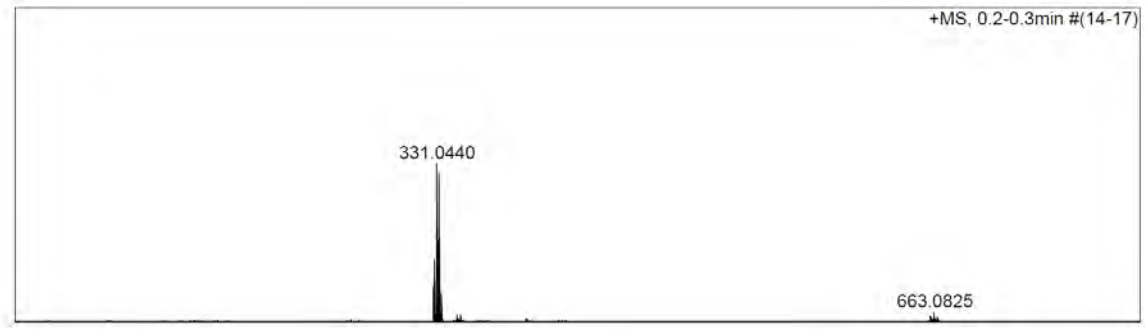
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
301.0731	1	C 16 H 14 Cl N 2 O 2	100.00	301.0738	0.7	2.4	9.2	10.5	ok	even
	2	C 13 H 16 Cl N O 5	30.67	301.0712	-2.0	-6.5	27.6	6.0	ok	odd
	3	C 16 H 13 O 6	0.01	301.0707	-2.5	-8.2	188.5	10.5	ok	even
	4	C 15 H 13 N 2 O 5	0.00	301.0819	8.8	29.2	190.1	10.5	ok	even
	5	C 12 H 15 N O 8	0.00	301.0792	6.1	20.3	192.4	6.0	ok	odd

7b: 1-(3-Bromobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



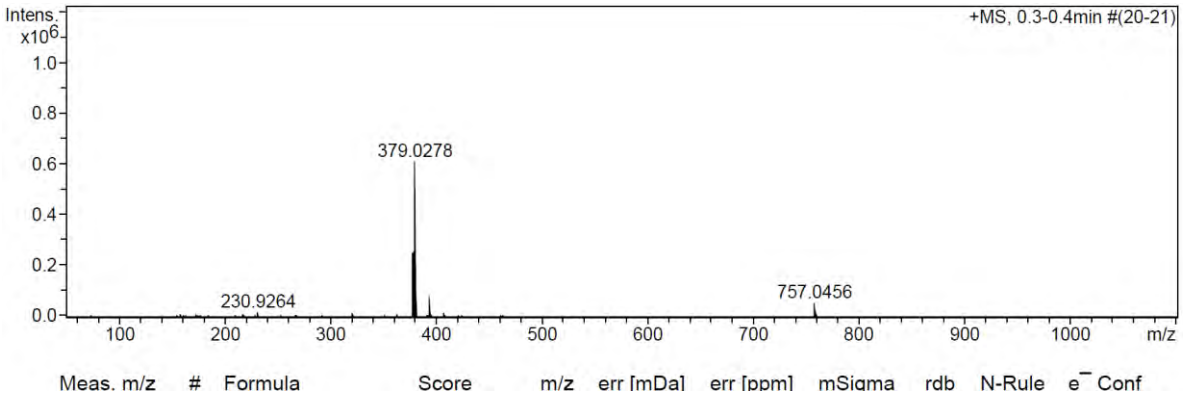
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
331.0407	1	C 16 H 16 Br N 2 O	100.00	331.0441	3.3	10.1	116.6	9.5	ok	even
	2	C 26 H 5 N	0.00	331.0417	0.9	2.8	488.6	25.0	ok	odd

7c: 1-(4-Bromobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



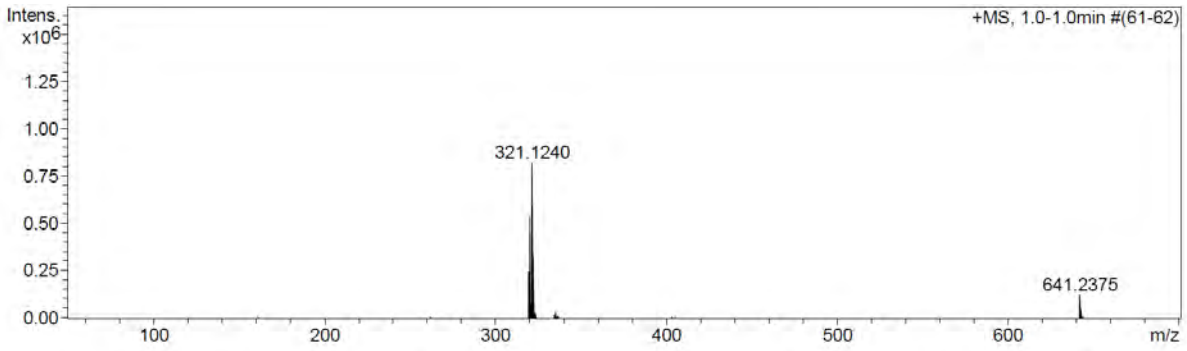
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
331.0440	1	C 16 H 16 Br N 2 O	100.00	331.0441	0.1	0.2	150.2	9.5	ok	even

7d: 1-(4-Iodobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



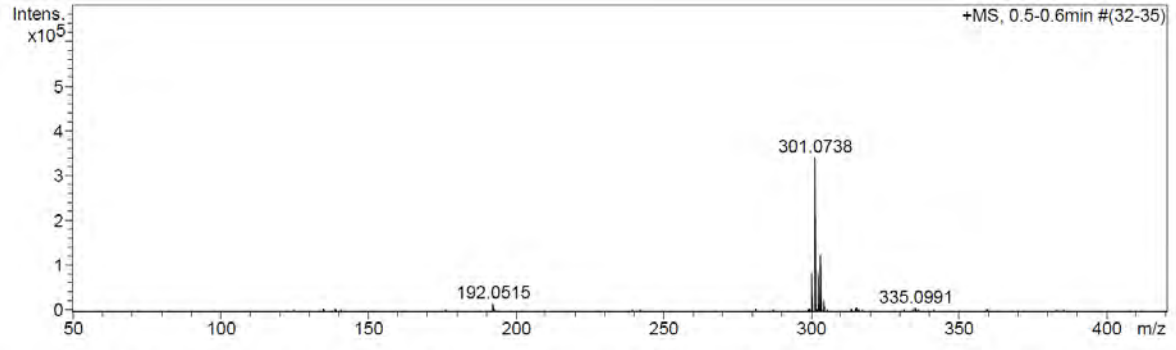
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
379.0278	1	C 17 H 18 I N O	0.00	379.0428	15.0	39.6	46.0	9.0	ok	odd
	2	C 16 H 16 I N 2 O	100.00	379.0302	2.4	6.4	50.2	9.5	ok	even

7e: 3-Methyl-1-(4-(triflouromethyl)benzyl)-3,4-dihydroquinazolin-2(1*H*)-one



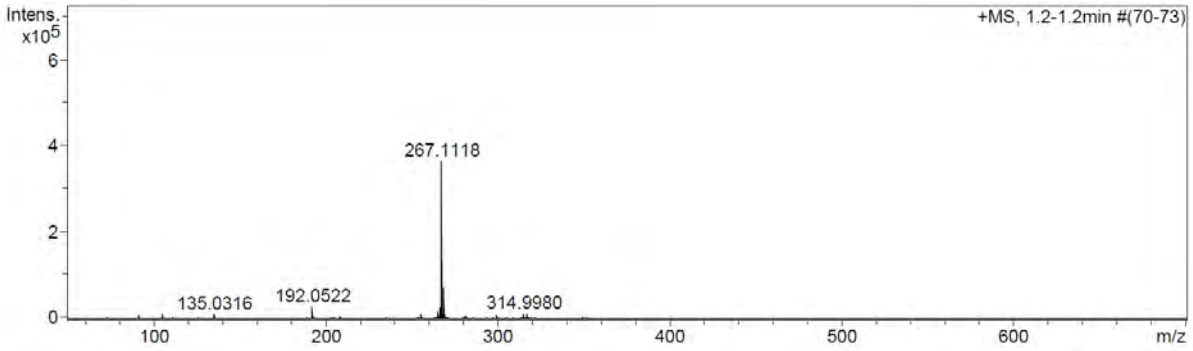
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
321.1240	1	C 24 H 17 O	100.00	321.1274	3.4	10.7	94.4	16.5	ok	even
	2	C 17 H 16 F 3 N 2 O	18.44	321.1209	-3.0	-9.4	134.4	9.5	ok	even

8a: 1-(3-Chlorobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



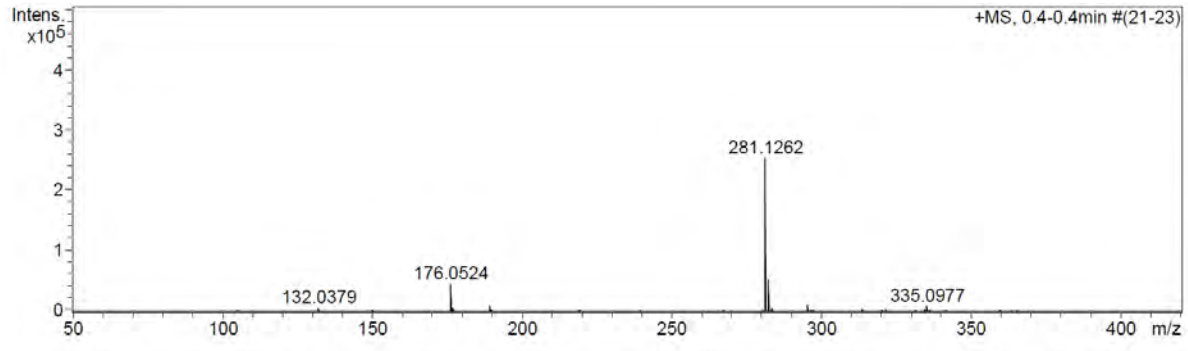
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
301.0738	1	C 16 H 14 Cl N 2 O 2	100.00	301.0738	0.1	0.2	34.2	10.5	ok	even
	2	C 22 H 9 N 2	0.01	301.0760	2.3	7.5	193.4	19.5	ok	even
	3	C 19 H 11 N O 3	0.02	301.0733	-0.4	-1.4	196.1	15.0	ok	odd

8b: 1-Benzyl-3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



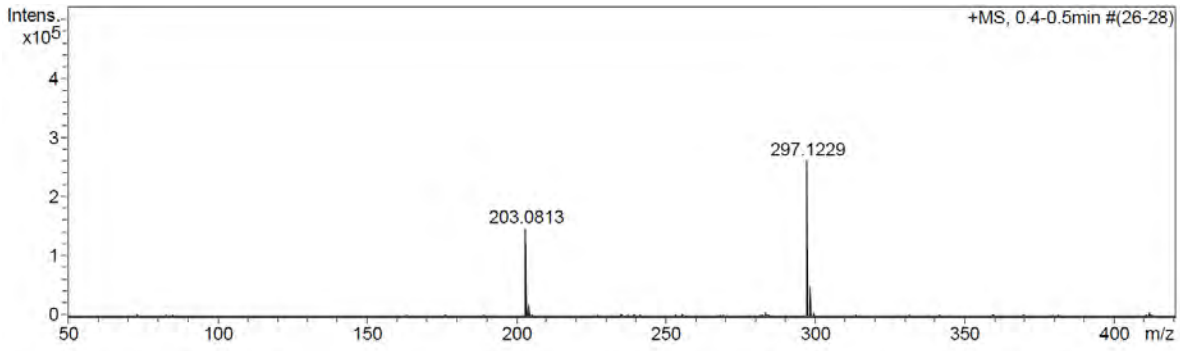
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
267.1118	1	C 17 H 17 N O 2	0.00	267.1254	13.6	51.0	3.5	10.0	ok	odd
	2	C 17 H 15 O 3	0.00	267.1016	-10.2	-38.2	4.1	10.5	ok	even
	3	C 16 H 15 N 2 O 2	100.00	267.1128	1.0	3.9	6.6	10.5	ok	even
	4	C 20 H 13 N	0.02	267.1043	-7.5	-28.1	17.8	15.0	ok	odd
	5	C 21 H 15	1.40	267.1168	5.1	18.9	21.8	14.5	ok	even

8c: 3-Methyl-1-phenethyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



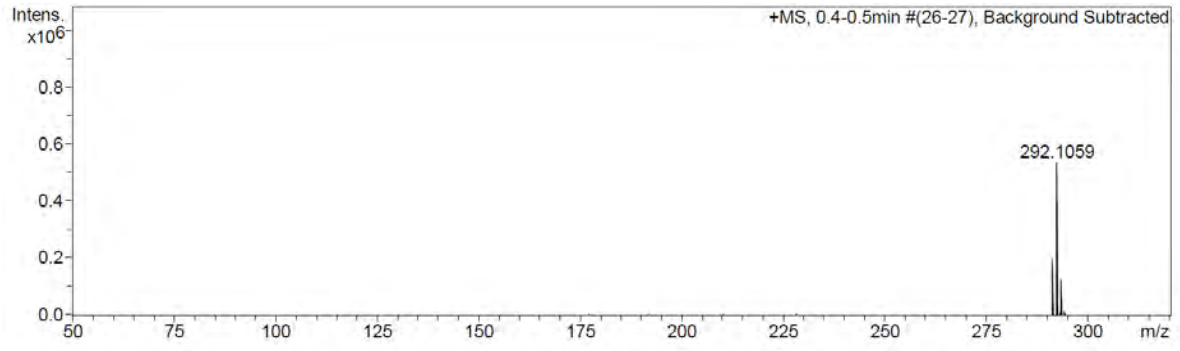
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
267.1409	1	C 16 H 17 N 3 O	31.43	267.1366	-4.3	-16.1	106.7	10.0	ok	odd
	2	C 17 H 19 N 2 O	0.03	267.1492	8.3	31.0	110.9	9.5	ok	even
	3	C 18 H 19 O 2	100.00	267.1380	-3.0	-11.1	113.1	9.5	ok	even
281.1262	1	C 17 H 17 N 2 O 2	100.00	281.1285	2.2	8.0	7.4	10.5	ok	even

8d: 3-Methyl-1-(2-phenoxyethyl)-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



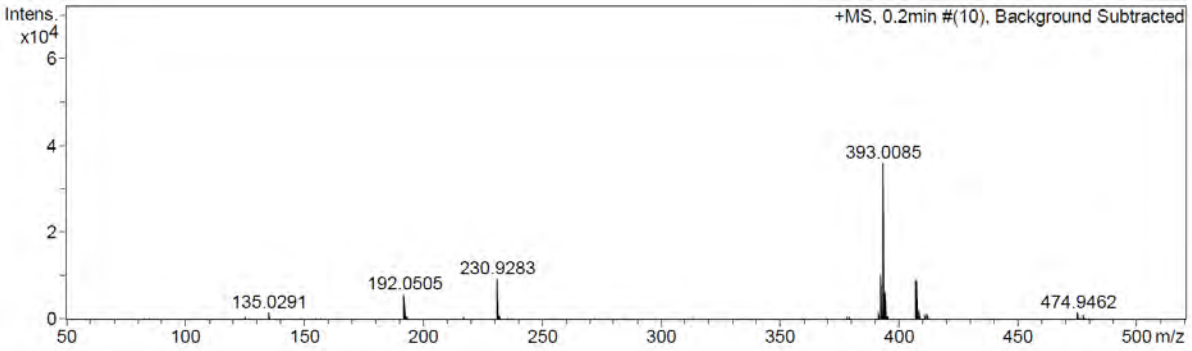
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
203.0813	1	C 11 H 11 N 2 O 2	100.00	203.0815	0.2	0.8	3.8	7.5	ok	even
	2	C 9 H 9 N 5 O	59.25	203.0802	-1.2	-5.8	4.0	8.0	ok	odd
	3	C 7 H 7 N 8	19.60	203.0788	-2.5	-12.4	10.8	8.5	ok	even
	4	C 8 H 13 N O 5	17.38	203.0788	-2.5	-12.4	17.1	3.0	ok	odd
	5	C 6 H 11 N 4 O 4	3.93	203.0775	-3.9	-19.0	23.2	3.5	ok	even
297.1229	1	C 17 H 17 N 2 O 3	100.00	297.1234	0.5	1.6	2.0	10.5	ok	even
	2	C 15 H 15 N 5 O 2	78.24	297.1220	-0.9	-2.9	4.8	11.0	ok	odd

8e: 4-((3-Methyl-2,4-dioxo-3,4-dihydroquinazolin-2(1*H*)-yl)methyl)benzonitrile



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
292.1059	1	C 19 H 16 O 3	15.40	292.1094	3.5	12.0	14.1	12.0	ok	odd
	2	C 17 H 14 N 3 O 2	47.54	292.1081	2.2	7.4	20.2	12.5	ok	even
	3	C 15 H 12 N 6 O	100.00	292.1067	0.8	2.8	26.8	13.0	ok	odd

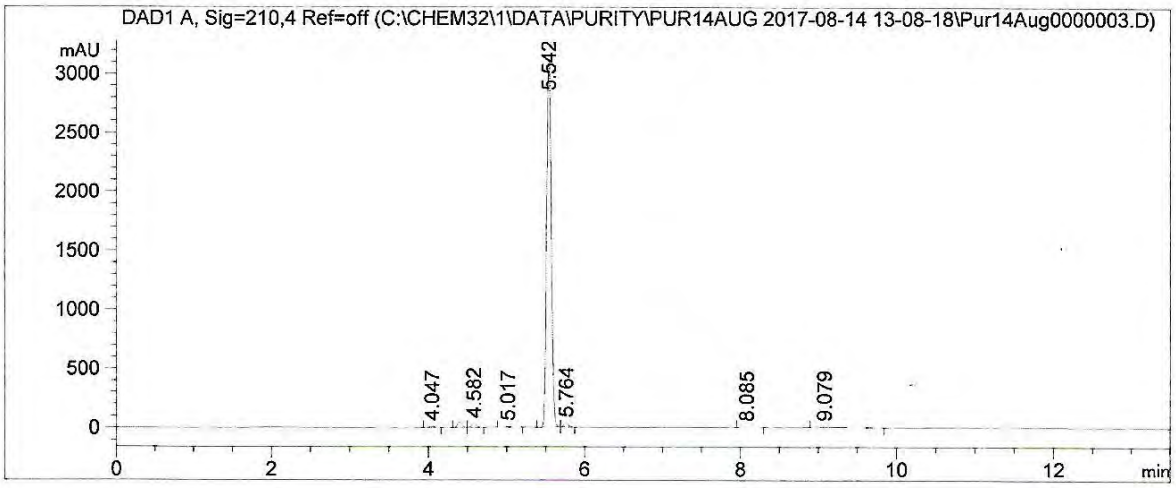
8f: 1-(3-Iodobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



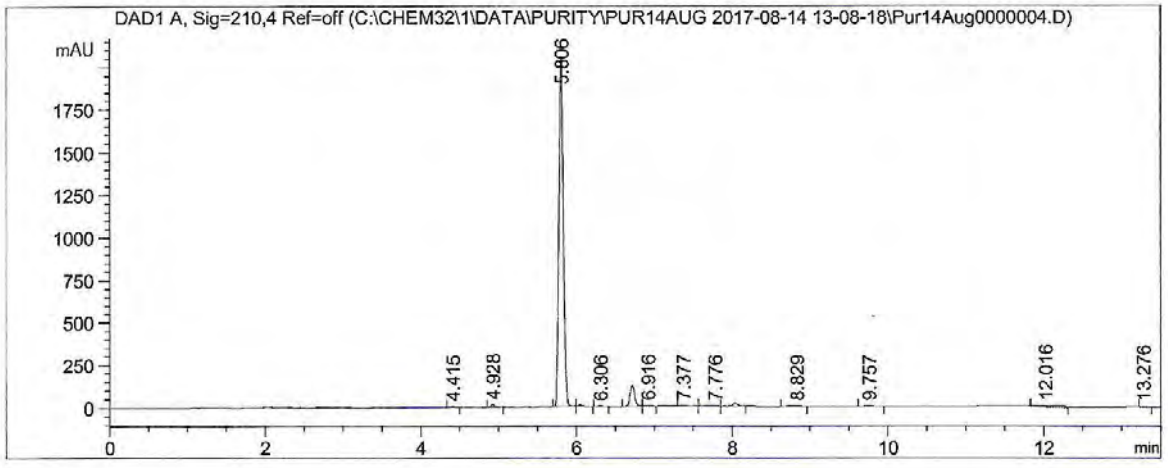
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
192.0505	1	C 9 H 8 N 2 O 3	100.00	192.0529	2.4	12.5	60.8	7.0	ok	odd
230.9283	1	C 7 H 4 I O	100.00	230.9301	1.9	8.1	3.5	5.5	ok	even
	2	C 2 H 4 I N 2 O 3	45.75	230.9261	-2.2	-9.3	30.7	1.5	ok	even
393.0085	1	C 16 H 14 I N 2 O 2	100.00	393.0094	0.9	2.3	11.7	10.5	ok	even
	2	C 29 H N 2 O	21.12	393.0083	-0.2	-0.5	77.3	30.5	ok	even
406.9878	1	C 16 H 12 I N 2 O 3	100.00	406.9887	0.9	2.3	33.0	11.5	ok	even

LIST OF HPLC DATA

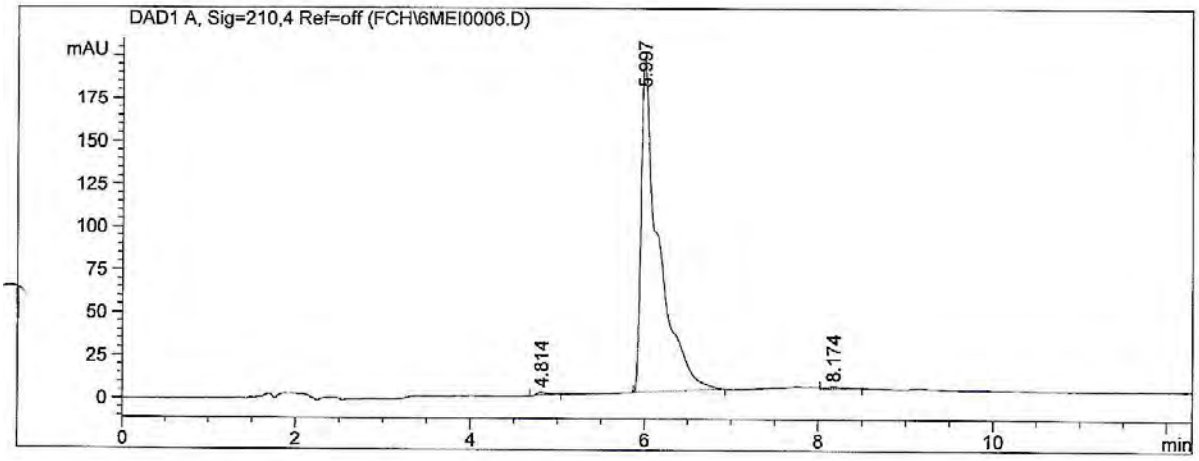
5a: 6-Benzoyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



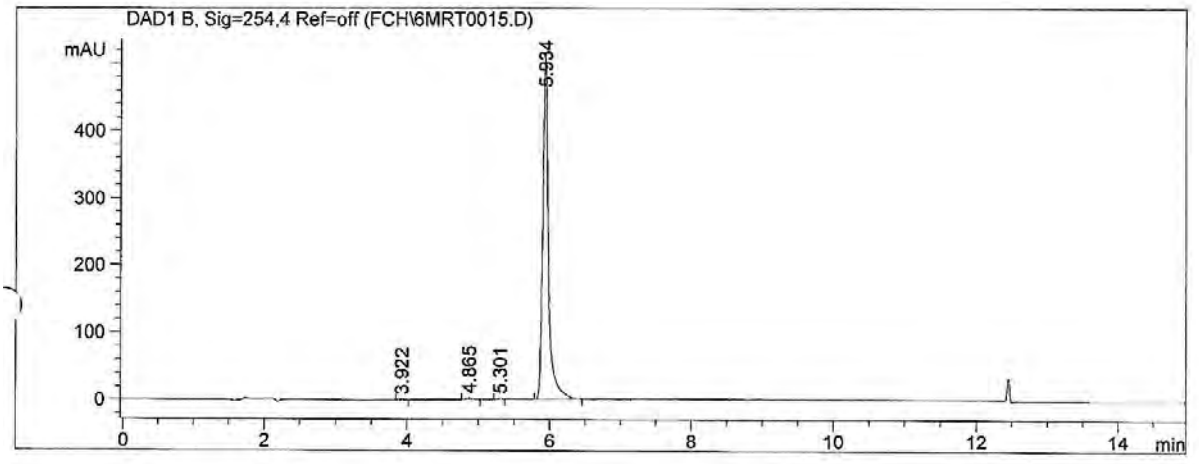
5b: 3-Methyl-6-(2-phenylacetyl)-3,4-dihydroquinazolin-2(1*H*)-one



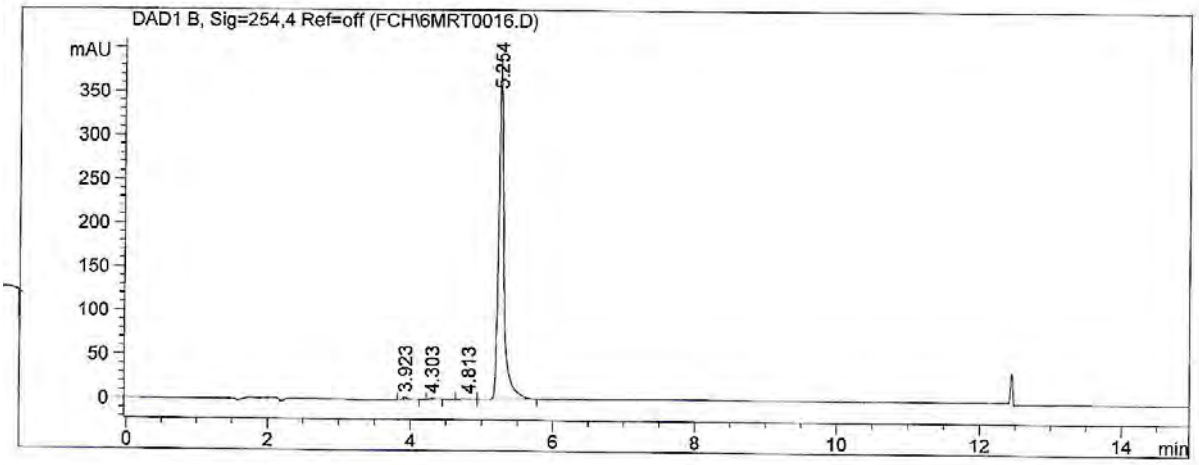
5c: 6-(Cyclohexanecarbonyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



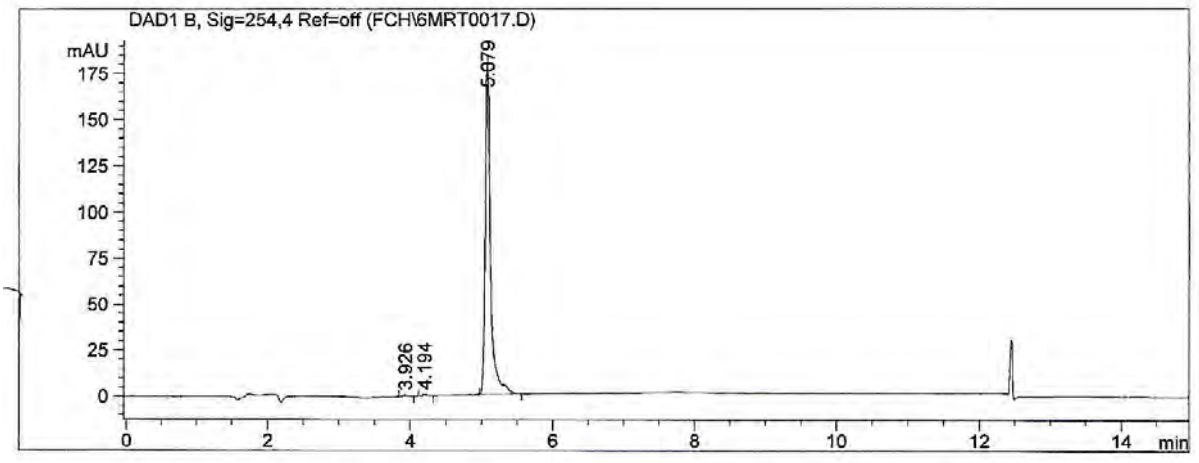
5d: 6-(4-Chlorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



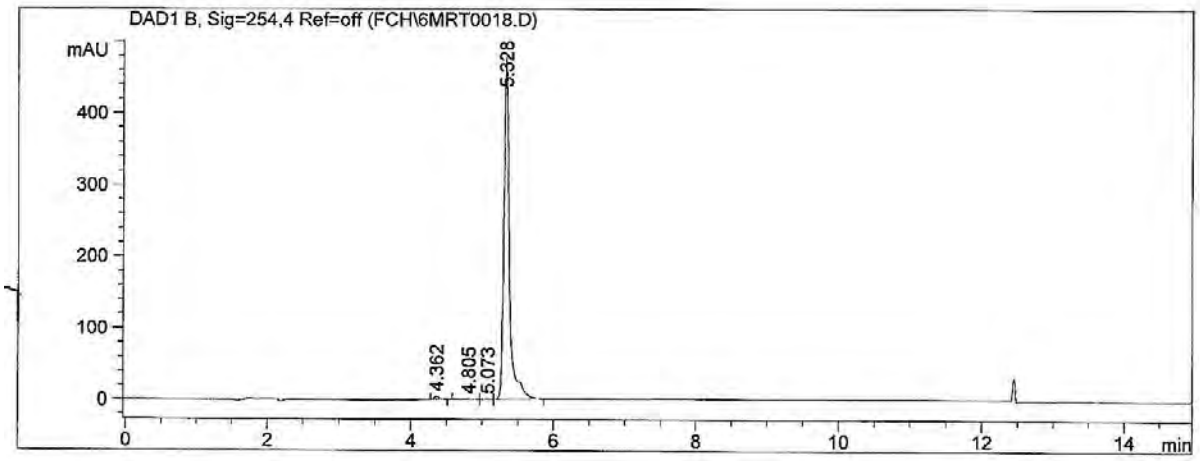
5e: 6-(4-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



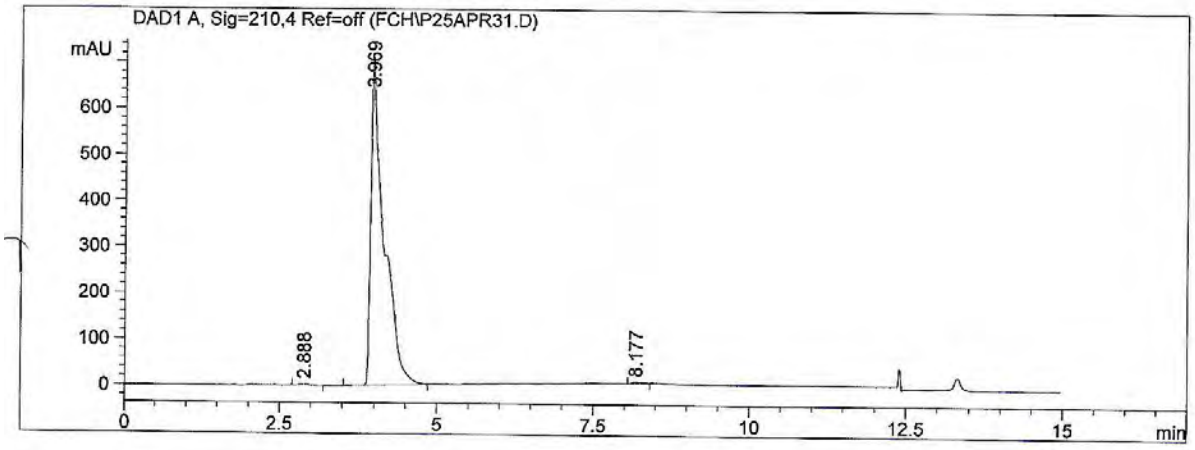
5f: 6-(2-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



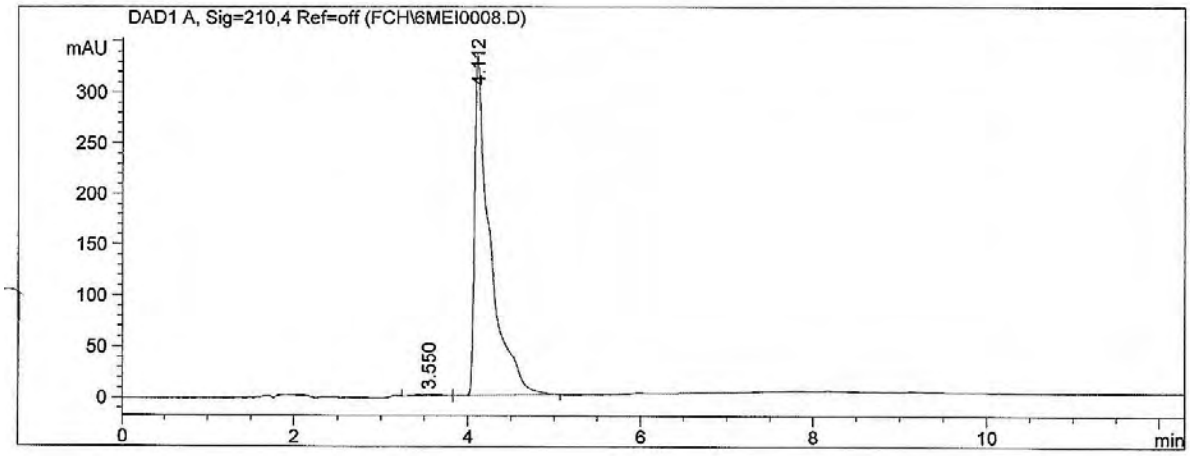
5g: 6-(3-Fluorobenzoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



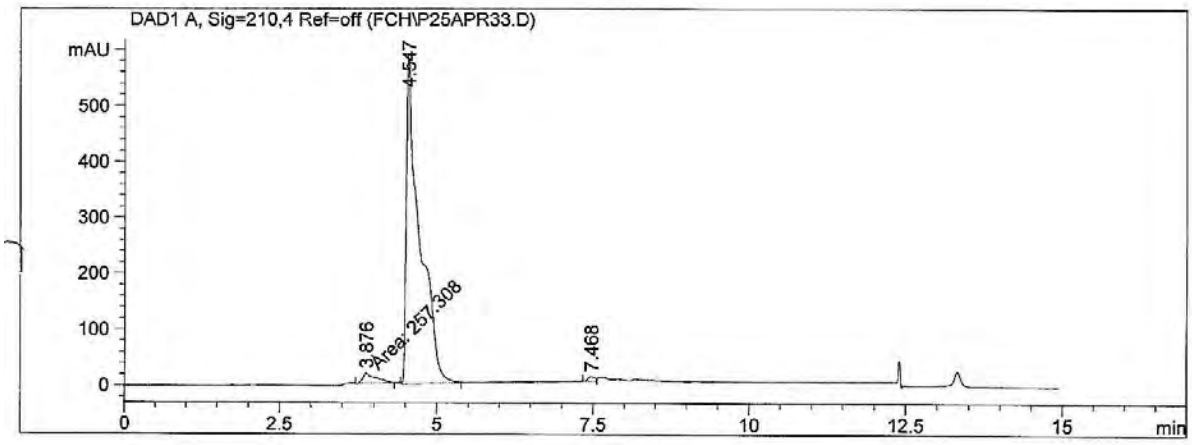
5h: 6-(Furan-2-carbonyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



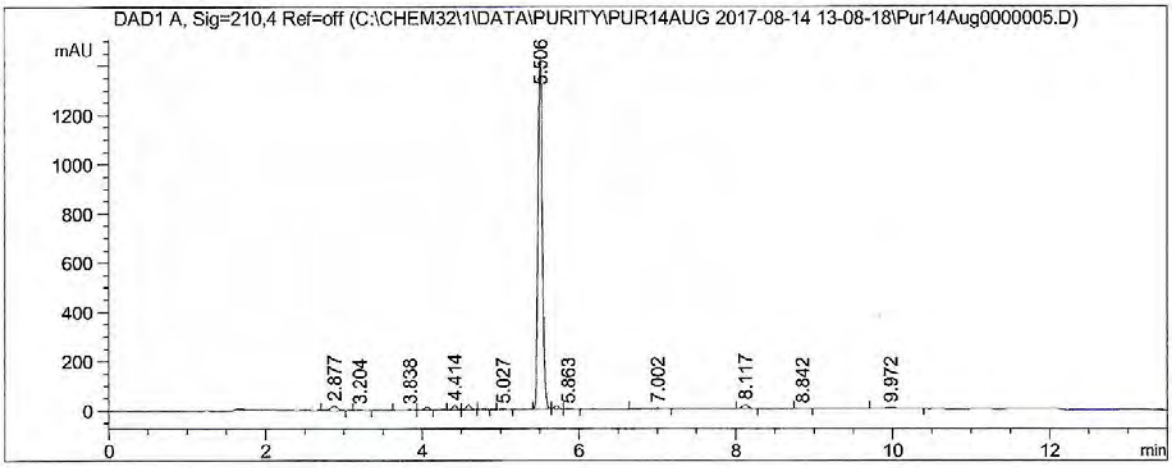
5i: 6-(2-Chloroacetyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



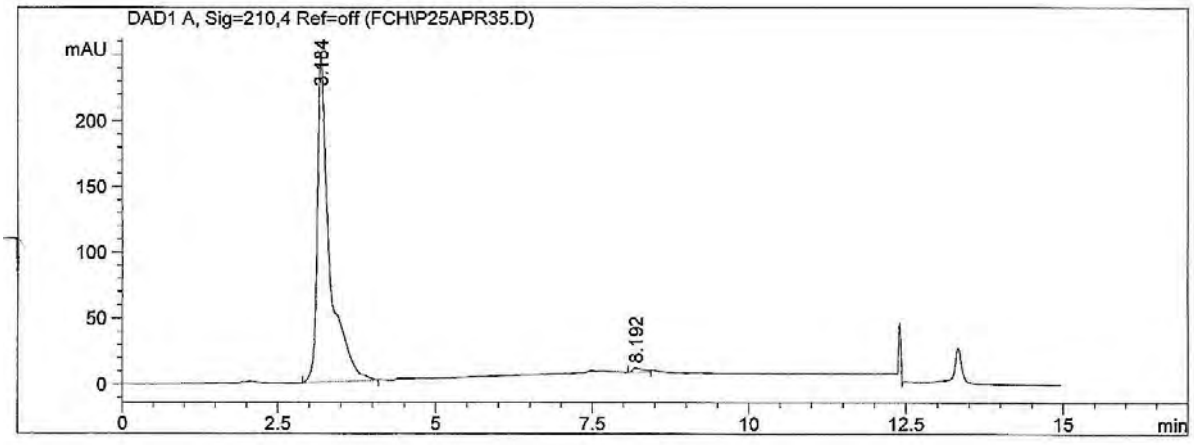
5j: 6-(3-Chloropropanoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



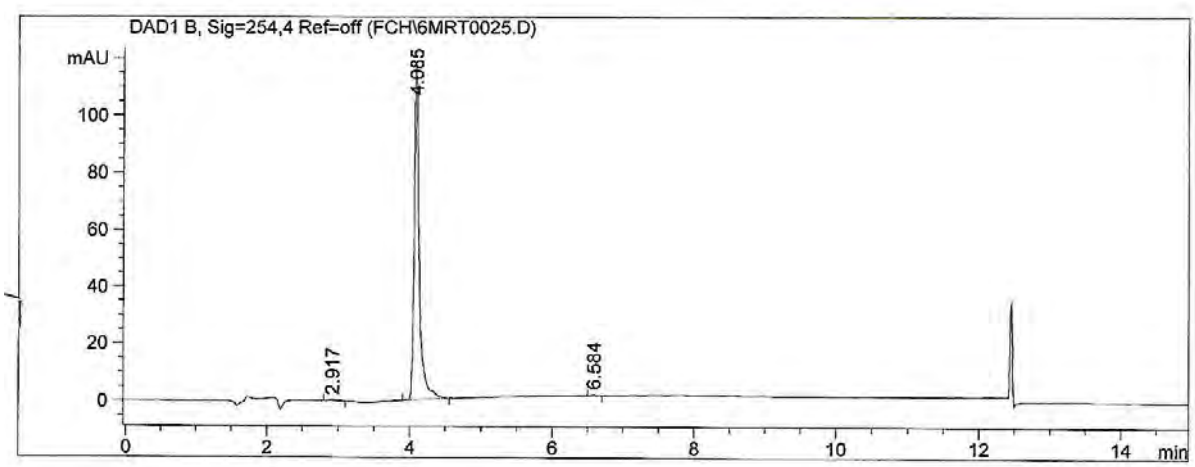
5k: 6-(4-Chlorobutanoyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



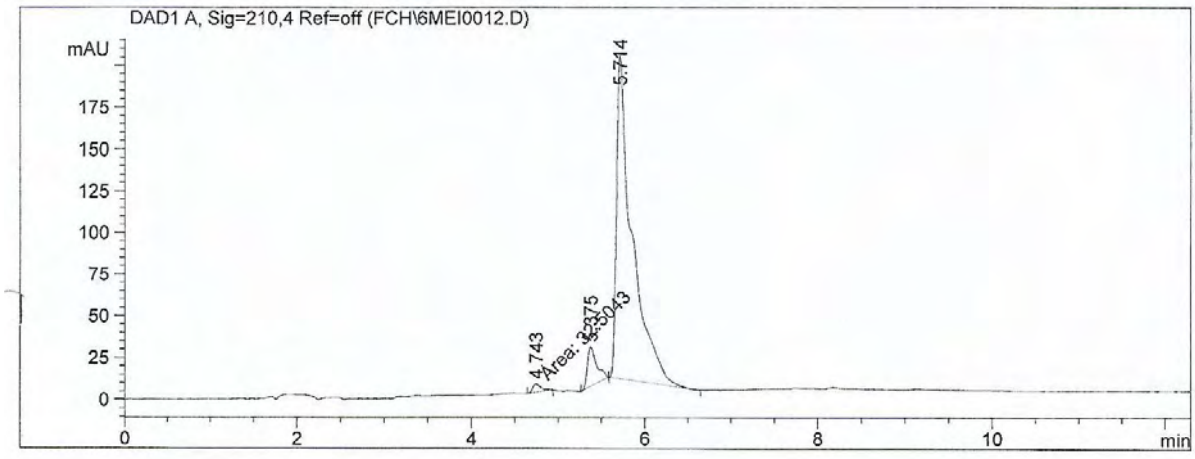
5l: 6-Acetyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



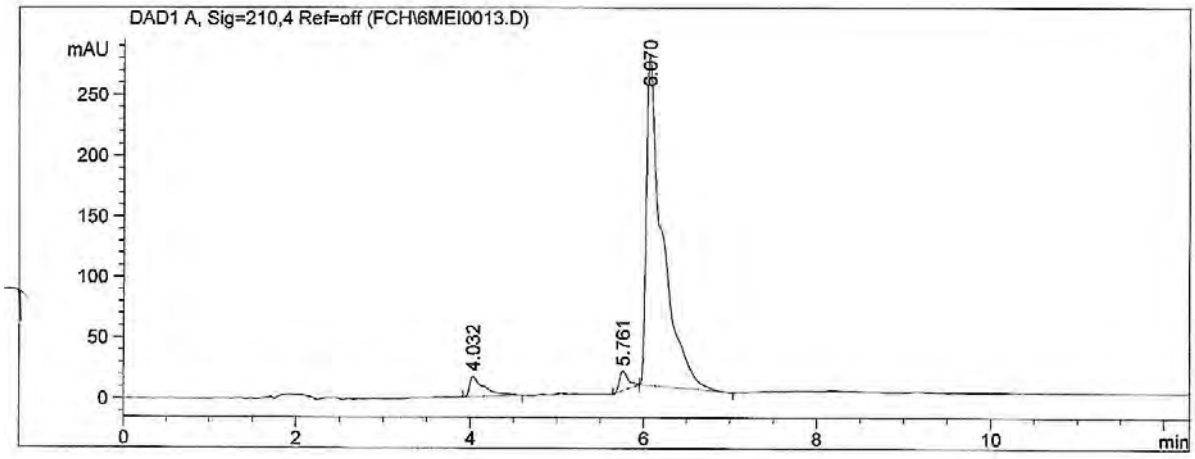
5m: 3-Methyl-6-propionyl-3,4-dihydroquinazolin-2(1*H*)-one



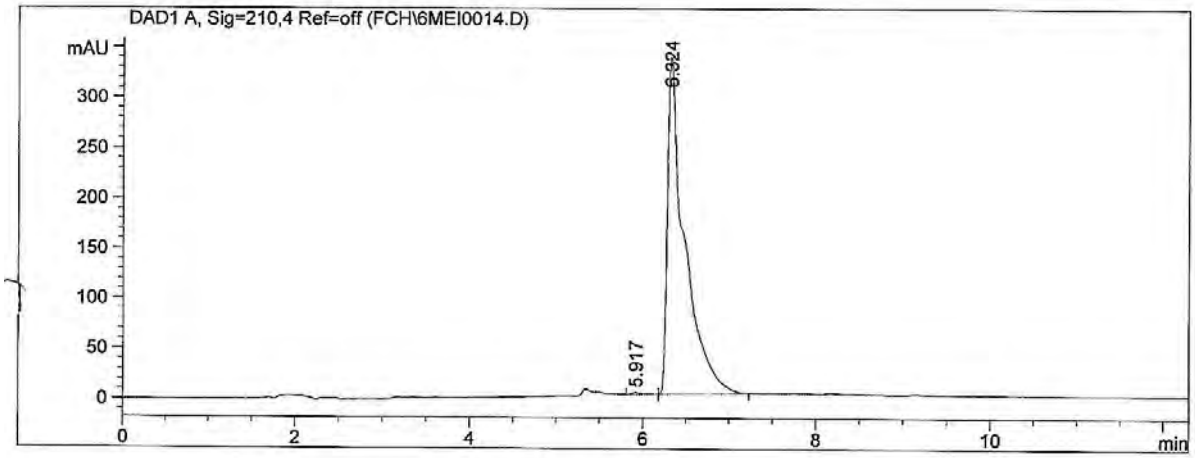
6a: 6-Cinnamoyl-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



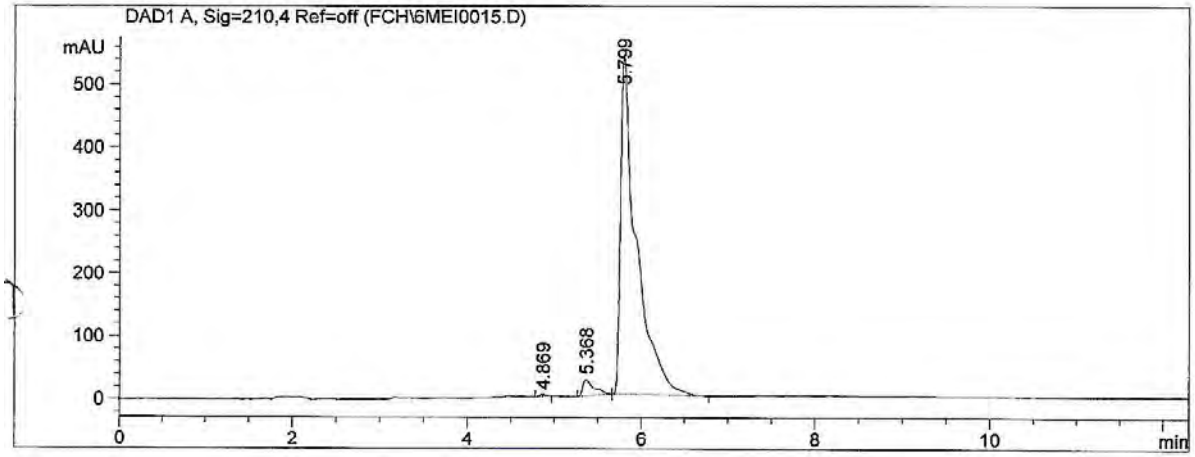
6b: (*z*)-3-Methyl-6-(2-methyl-3-phenylacryloyl)-3,4-dihydroquinazolin-2(1*H*)-one



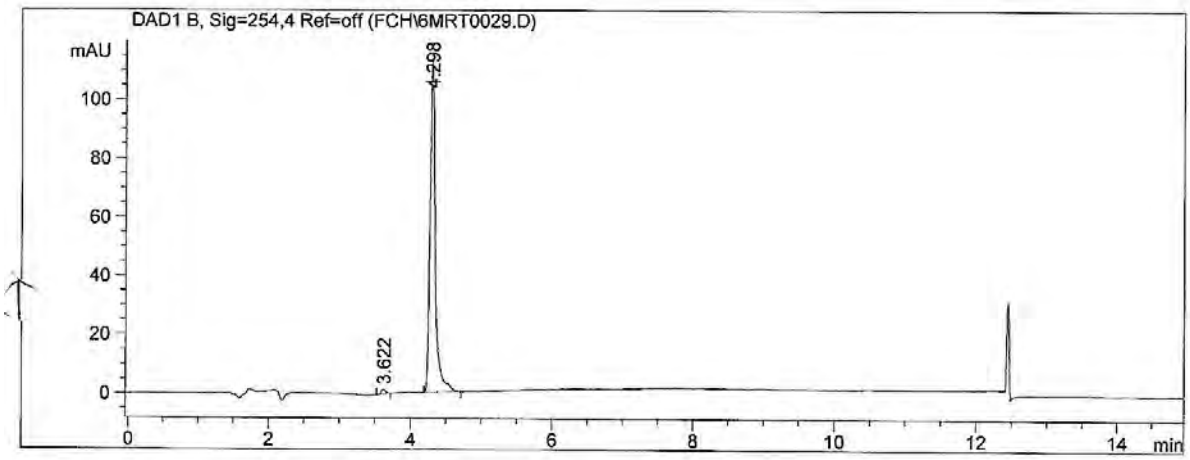
6c: 3-Methyl-6-((2*E*,4*Z*)-5-phenylpenta-2,4-dienoyl)-3,4-dihydroquinazolin-2(1*H*)-one



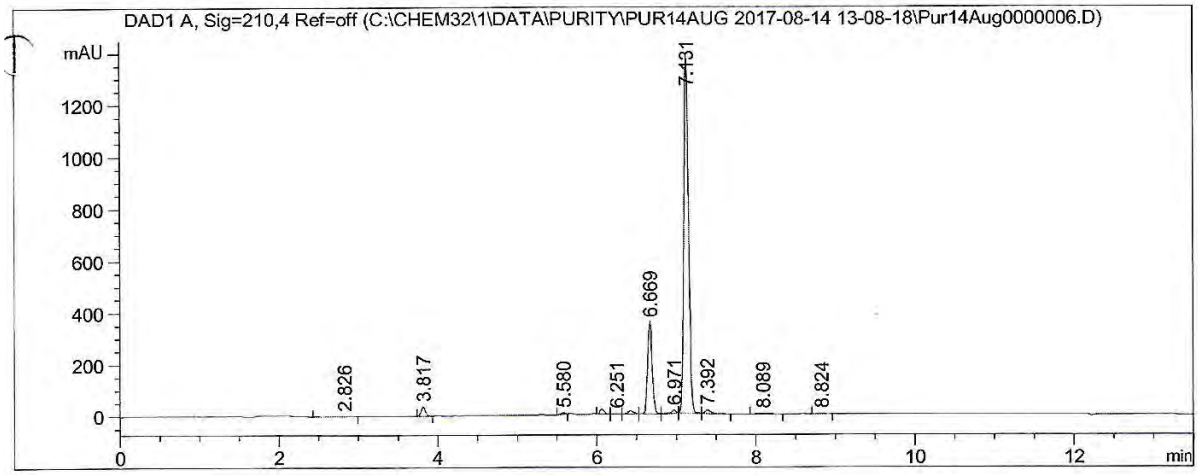
6d: (*E*)-6-(3-(4-Methoxyphenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



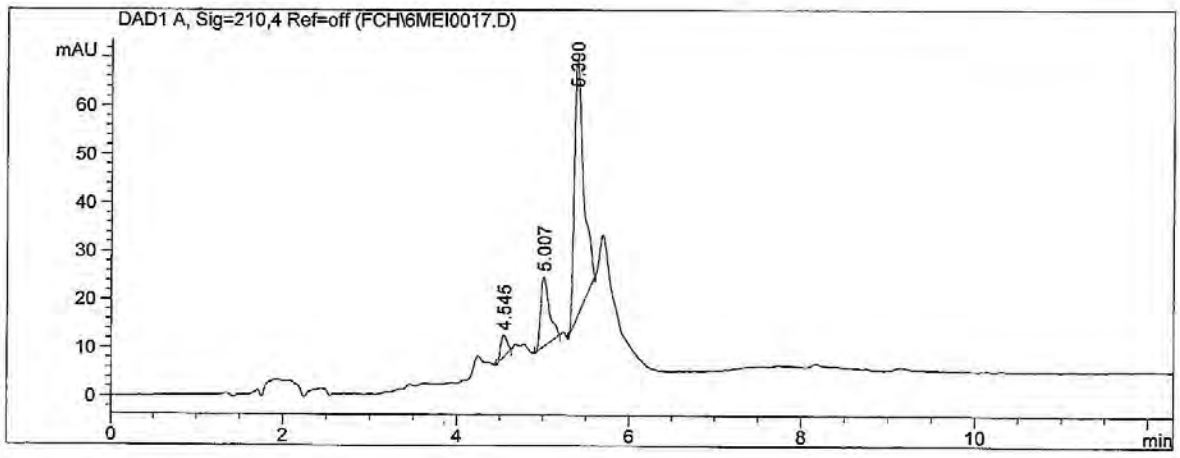
6e: (*E*)-6-(3-(4-Hydroxyphenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



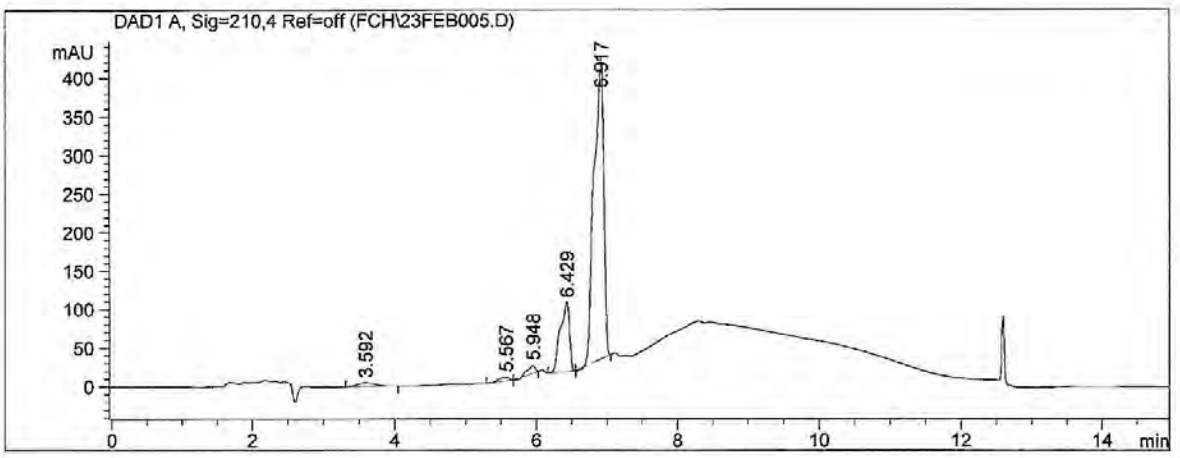
6f: (*E*)-6-(3-(4-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



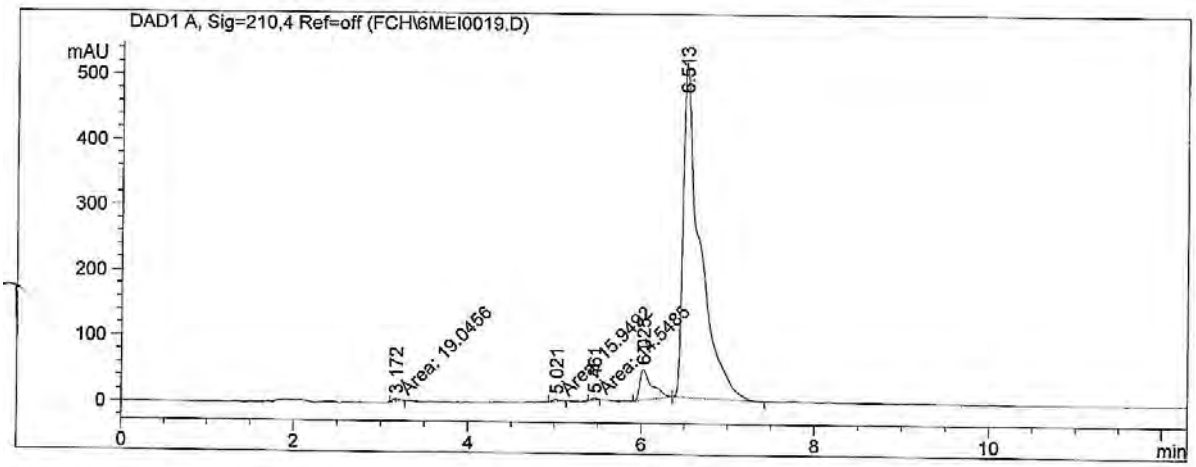
6g: (*E*)-4-(3-(3-Methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)-3-oxoprop-1-en-1-yl)benzonitrile



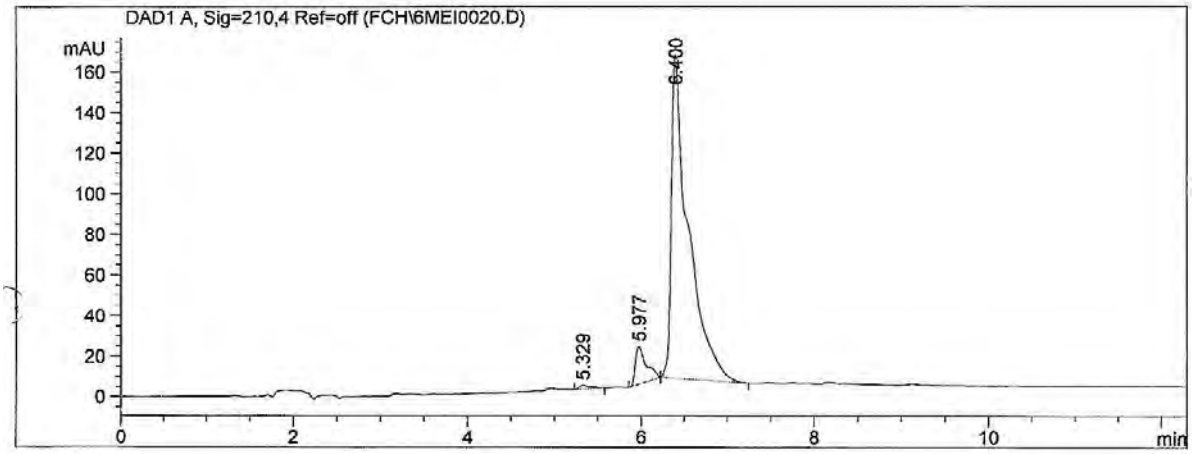
6h: (*E*)-3-Methyl-6-(3-(4-(trifluoromethyl)phenyl)acryloyl)-3,4-dihydroquinazolin-2(1*H*)-one



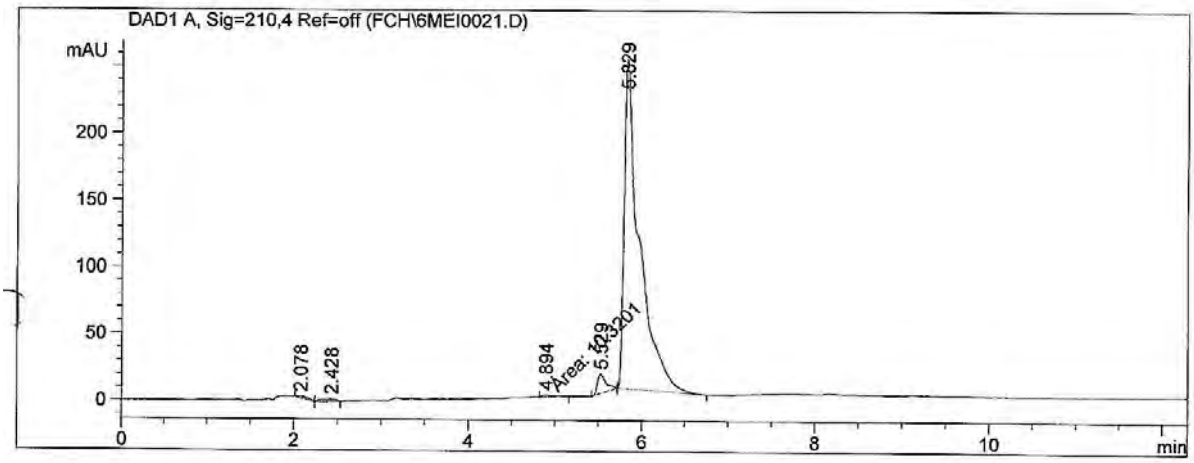
6i: (*E*)-6-(3-(3-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



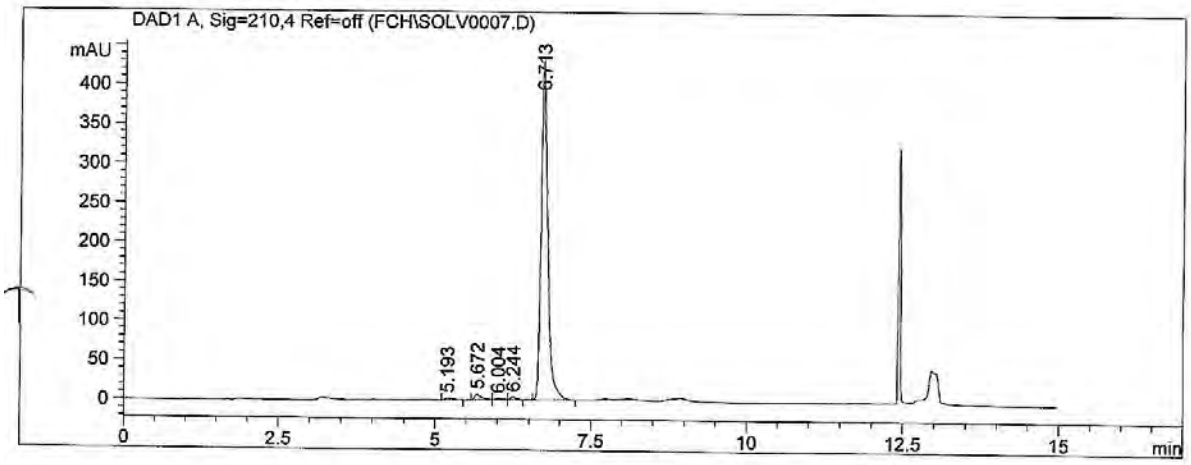
6j: (*E*)-6-(3-(2-Chlorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



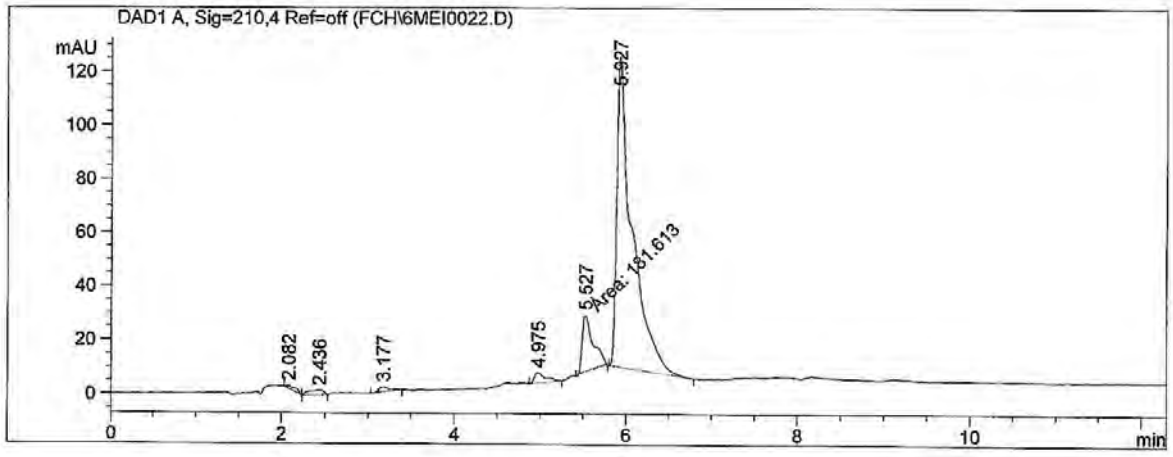
6k: (*E*)-6-(3-(4-Fluorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



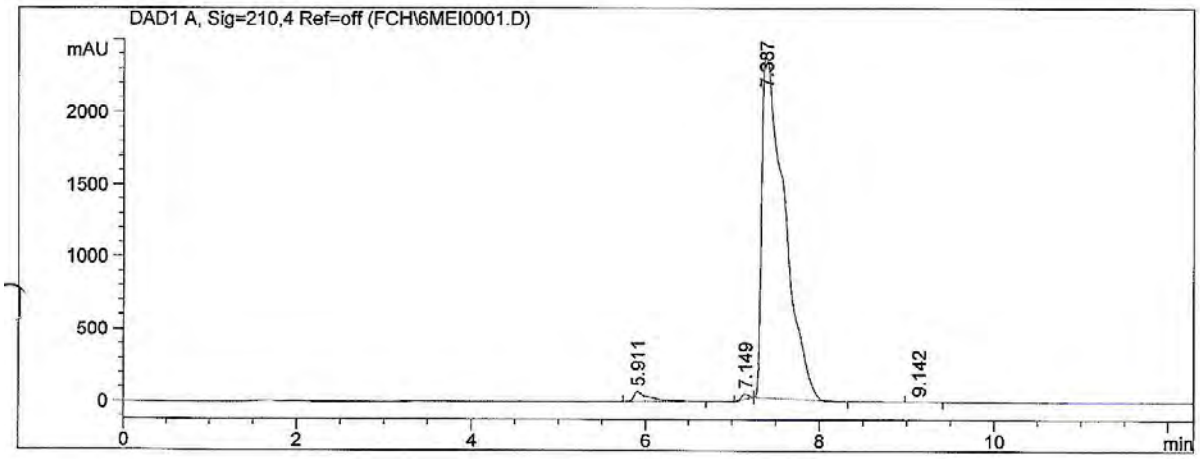
6l: (*E*)-6-(3-(4-Bromophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



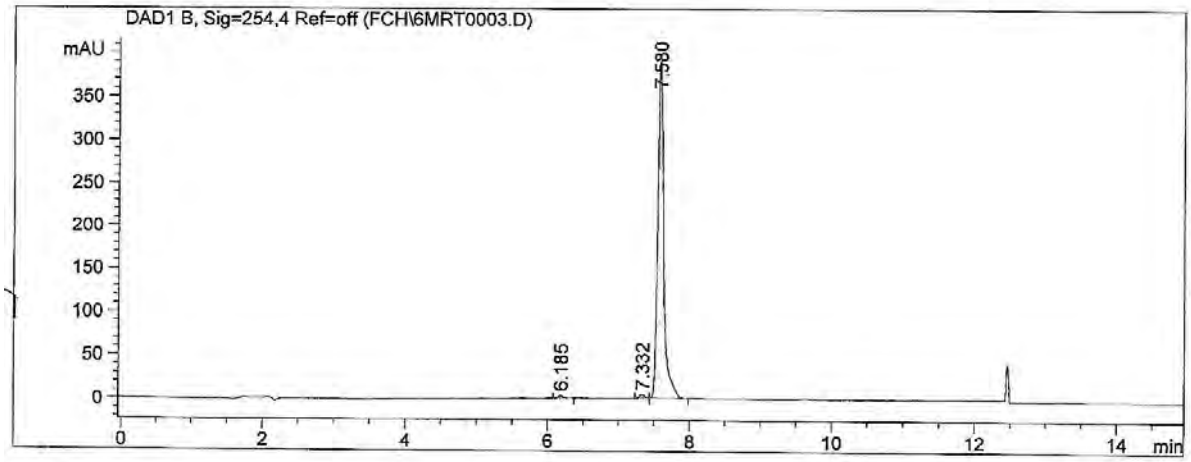
6m: (*E*)-6-(3-(3-Fluorophenyl)acryloyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



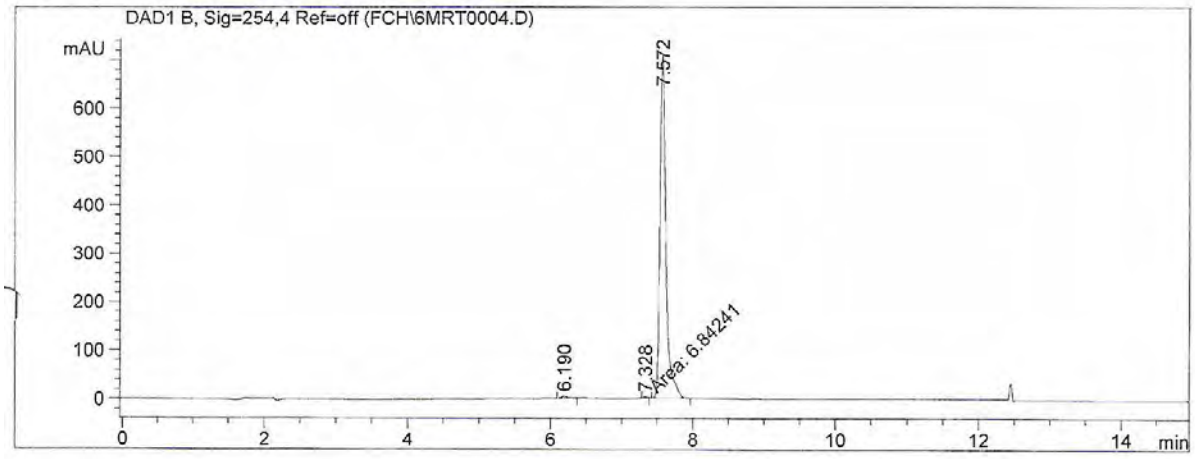
7a: 1-(4-Chlorobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



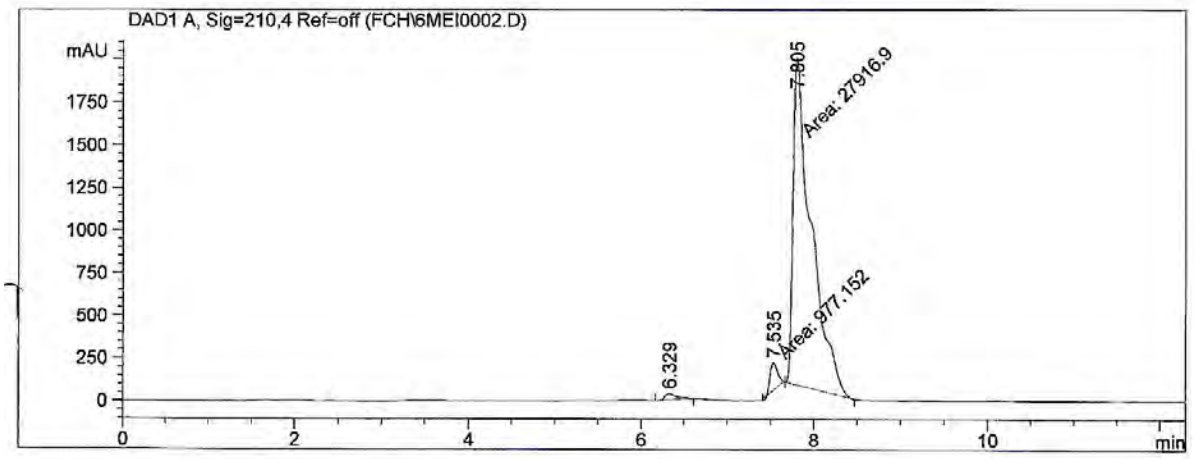
7b: 1-(3-Bromobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



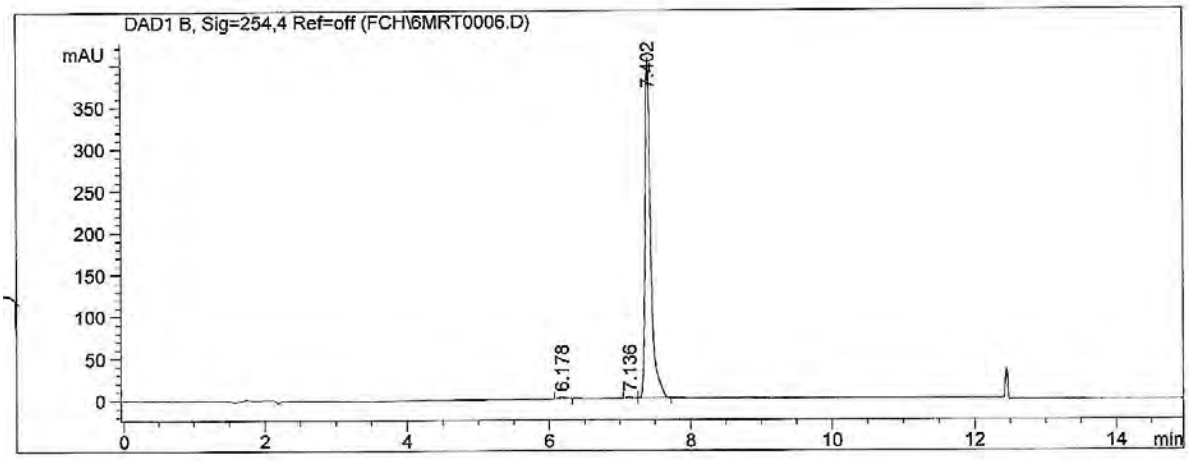
7c: 1-(4-Bromobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



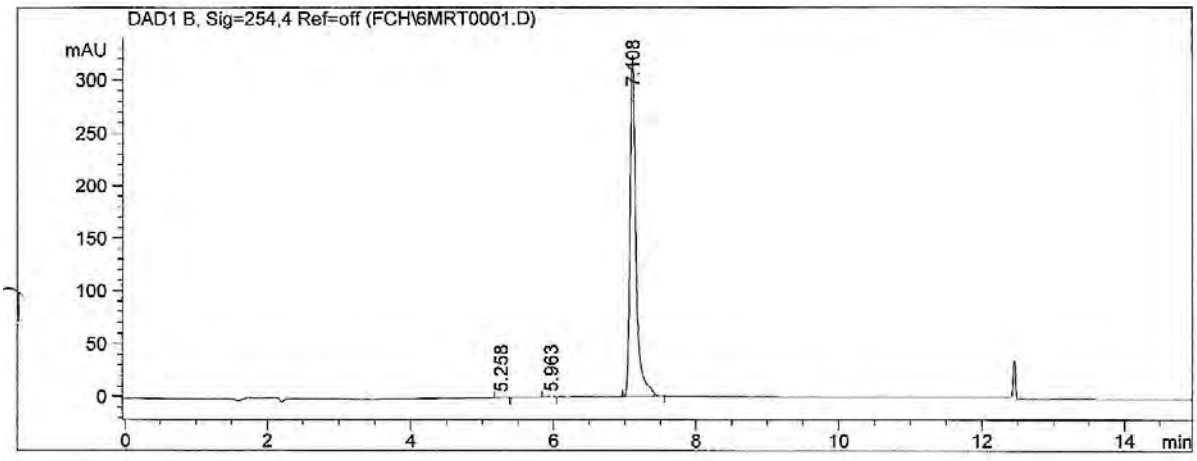
7d: 1-(4-Iodobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one



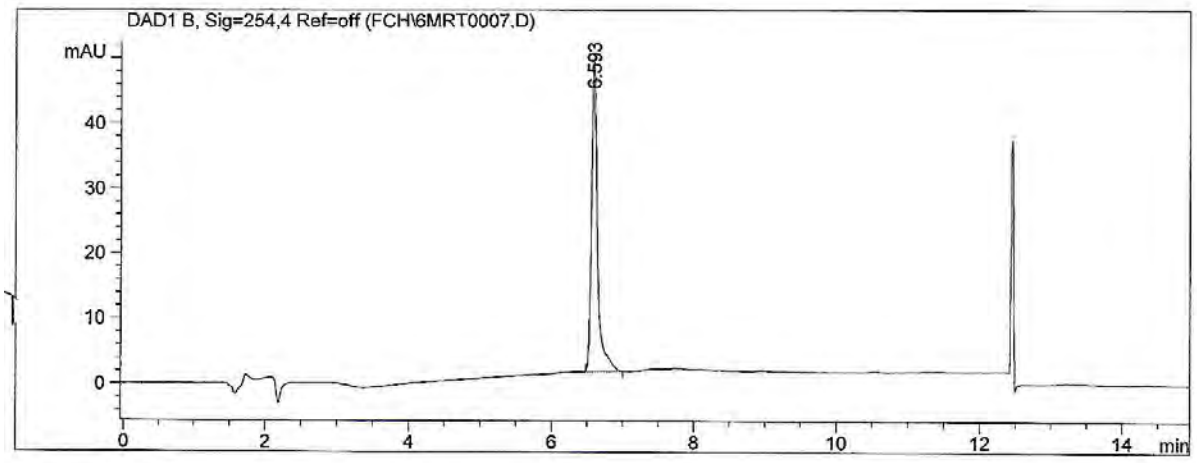
7e: 3-Methyl-1-(4-(triflouromethyl)benzyl)-3,4-dihydroquinazolin-2(1*H*)-one



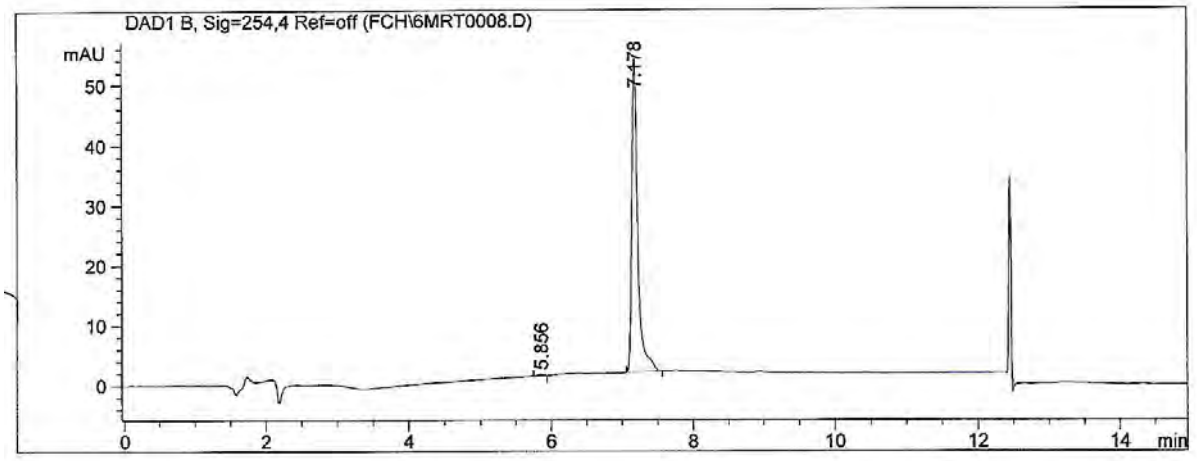
8a: 1-(3-Chlorobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



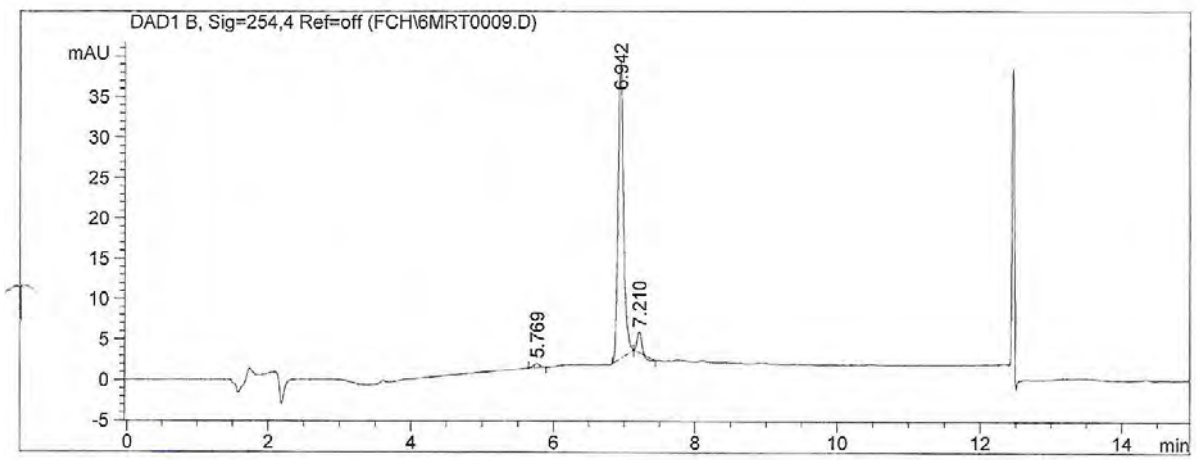
8b: 1-Benzyl-3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



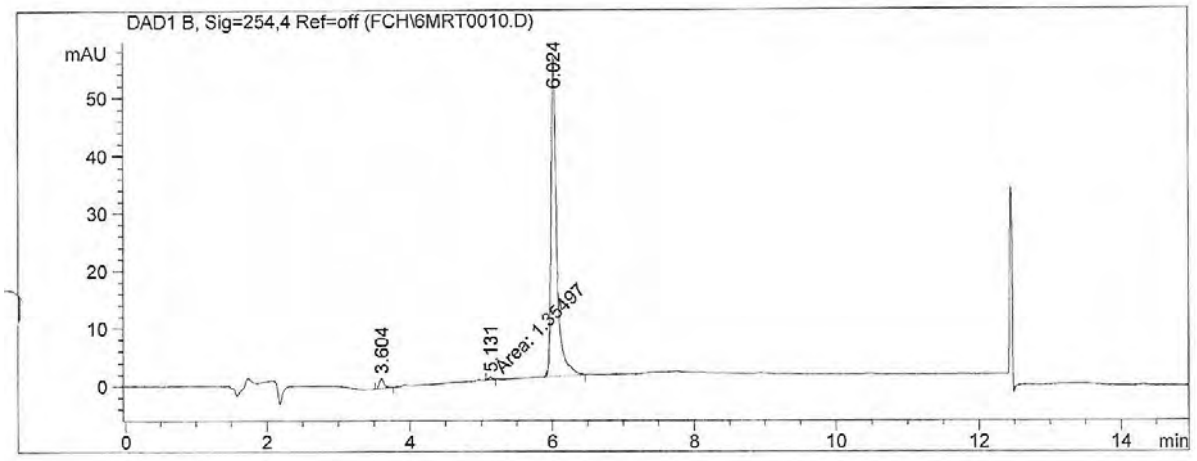
8c: 3-Methyl-1-phenethyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



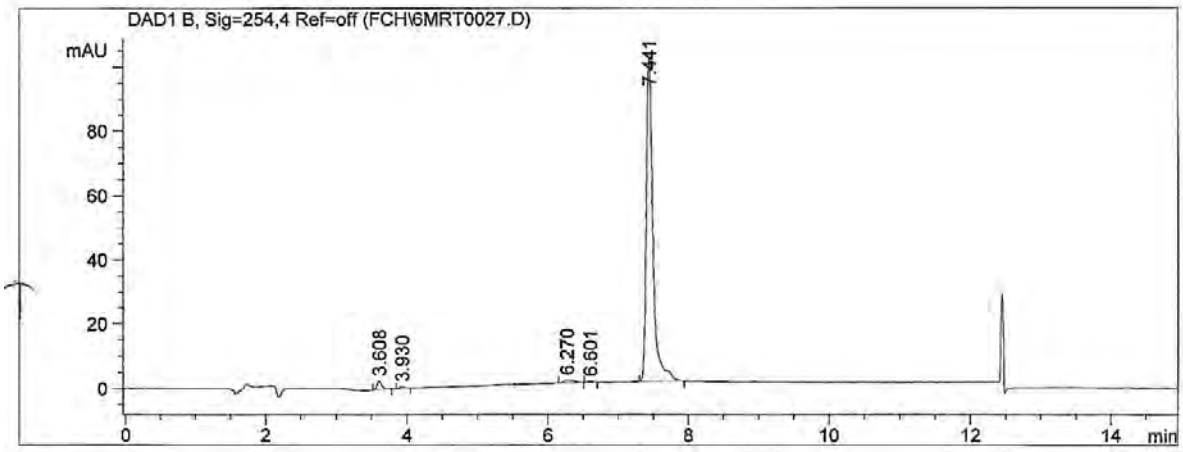
8d: 3-Methyl-1-(2-phenoxyethyl)-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



8e: 4-((3-Methyl-2,4-dioxo-3,4-dihydroquinazolin-2(1*H*)-yl)methyl)benzonitrile



8f: 1-(3-Iodobenzyl)-3-methyl-3,4-dihydroquinazolin-2(1*H*,3*H*)-dione



ANNEXURE B

AUTHOR GUIDELINES

BIOORGANIC & MEDICINAL CHEMISTRY

The Tetrahedron Journal for Research at the Interface of Chemistry and Biology

ELSEVIER

AUTHOR INFORMATION PACK

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ISSN: 0968-0896

DESCRIPTION

Bioorganic & Medicinal Chemistry publishes complete accounts of research of outstanding significance and timeliness on all aspects of molecular interactions at the interface of chemistry and biology, together with critical review articles. The journal publishes reports of experimental results in medicinal chemistry, chemical biology and drug discovery and design, emphasizing new and emerging advances and concepts in these fields. The aim of the journal is to promote a better understanding at the molecular level of life processes, and living organisms, as well as the interaction of these with chemical agents.

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Chemists, Medicinal Chemists, Pharmacologists, Biochemists, Molecular Biologists.

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GUIDE FOR AUTHORS

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