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The Synthesis and Analysis of Methaqualone and some positional and structural isomers thereof

by

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To everyone who believed in me, more than I did

To everyone who supported me, more than I deserved

To everyone who helped me, more than I can ever repay

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TABLE OF CONTENTS

SUMMARY

An extensive review of the reported synthesis of methaqualone (2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone) and some positional and structural isomers of methaqualone is given. A detailed summary of the synthetic routes reviewed is given as an appendix. Methaqualone, four previously reported, and seven until hereto unreported isomers of methaqualone were prepared using a modified, known synthetic route for quinazolinones. The modified route allowed for the rapid laboratory scale preparation of the desired quinazolinones in high yields. Melting points are given. The prepared compounds were characterized using various organic analytical techniques. NMR and IR was found to be the most specific single techniques for the effectively and unequivocally differentiation between the studied compounds. The most convenient, and equally specific, differentiation was achieved using coupled gas-liquid chromatography. The thin-layer chromatography and liquid chromatography systems evaluated proved of no value in differentiating the thirteen isomers.

OPSOMMING

'n Uitgebreide oorsig van die sintese van metakaloon (2-metiel-3-*o*-toliel-4(3*H*)-quinazolinoon), asook enkele posisionele and strukturele isomere daarvan is gedoen, en 'n gedetailleerde opsomming daarvan word gegee as 'n bylaag. Metakaloon, vier reeds gerapporteerde isomere, asook sewe ongerapporteerde isomere van metakaloon is berei volgens 'n aangepaste, bekende sintese roete vir quinazolinone. Die aangepaste roete het die vinnige laboratoriumskaal bereiding van gewenste quinazolinone in hoë opbrengs verseker. Smeltpunte word gegee. Die voorbereide verbindings is karakteriseer deur gebruik te maak van verskillende organies-analitiese tegnieke. Al dertien isomere kon geredelik met beide KMR en IR onderskei word. GC-MS het die mees gerieflike onderskeiding van die isomere bewerkstellig. Die dunlaagchromatografie en vloeistofchromatografie sisteme evalueer, kon geen sinvolle onderskeiding tussen die isomere bewerkstellig nie.

CHAPTER 1

Introduction

General Introduction

The synthesis of methaqualone (**I**, Figure 1.1, R_1 = Me, R_2 or R_6 = Me, R_3 = R_4 = R_5 = H) was first reported 1951.[1] The drug was introduced pharmaceutically as non barbiturate, nonaddictive "sleeping pills" in 1965.[2] It has been listed in the US Federal Register of March 1966 as an approved sedative-hypnotic with trade name Quaalude.[3] The abuse potential of methaqualone quickly became apparent resulting in it being listed in the 1971 United Nations (UN) Convention on Psychotropic Substances, and its subsequent banning in most member countries.[4] Methaqualone is currently listed in the UN Convention on Psychotropic Substances of 1988.

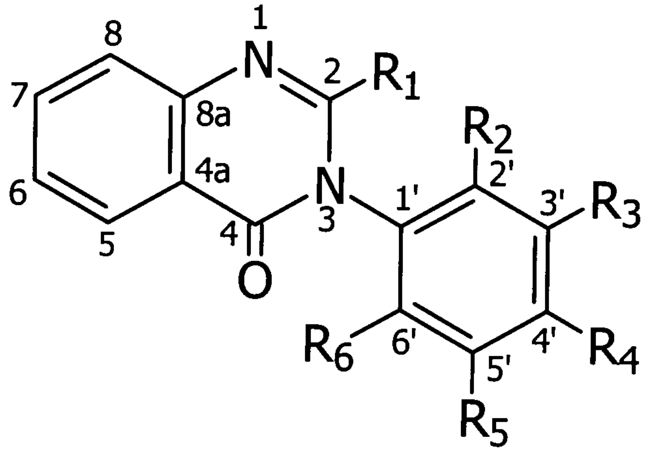


Figure 1.1 General structure of 2-alkyl-3-aryl-4(3*H*)-quinazolinones

The production, trafficking, and abuse of methaqualone are of particular forensic importance to South Africa as it remains the synthetic drug of choice amongst South African drug abusers.[5,6] This is illustrated by the fact that methaqualone-seizures amounts to more than 60% of all street-drug seizures submitted to the South African Police Service National Forensic Science Laboratories (SAPS FSL).[7] During 1999 a total of 3971 methaqualone-related cases was submitted to the SAPS FSL, with the cumulative number of dosage units exceeding three million.

Methaqualone was introduced pharmaceutically in South Africa under the trade name "Mandrax", a formulation containing methaqualone (250 mg) and diphenhydramine hydrochloride (25 mg). Following the identification of its abuse potential, methaqualone and its isomers were effectively removed from the legal market in 1971.[5]

All methaqualone seized in South Africa originates from illicit manufacturing sources in the Middle-East, South and Central Asia, as well as South and Southern Africa.[5] The product is marketed in South Africa as illicit tablet formulations usually in combination with the antihistaminic drug **diphenhydramine,** and less frequently with the benzodiazepine tranquilliser **diazepam.** The formulation of methaqualone with diphenhydramine is thought to be historic in nature with illicit producers simply mimicking the original licit "Mandrax" formulation, or by design due to the fact that diphenhydramine inhibits the metabolism of methaqualone.[8]

Methaqualone abuse gives rise to a barbiturate-type dependence.[9] The most prevalent abuse pattern observed in South Africa is in conjunction with Cannabis.[6] This involves mixing methaqualone with Cannabis and then smoking it as a so-called "witpyp", i.e. white pipe.

The synthesis of methaqualone usually involves, but is not limited to, uncomplicated one and two step reactions that are easily adapted for illicit synthesis.[10] Soliman *et al*[11] stated in 1979 that the majority of 2,3-disubstituted-4(3*H*)-quinazolinones reported in literature have been synthesized *via* the following generic routes as depicted in Figure 1.2:

i) Reacting N-acylanthranilic acid (**1**) with a primary amine in a suitable solvent in the presence of a catalyst.
ii) Reacting 3, 1, 4-benzoxazones (acylanthranils) (**2**) with amines.
iii) Thermal cyclization of o-acylamino (N-substituted) benzamides (**3**).

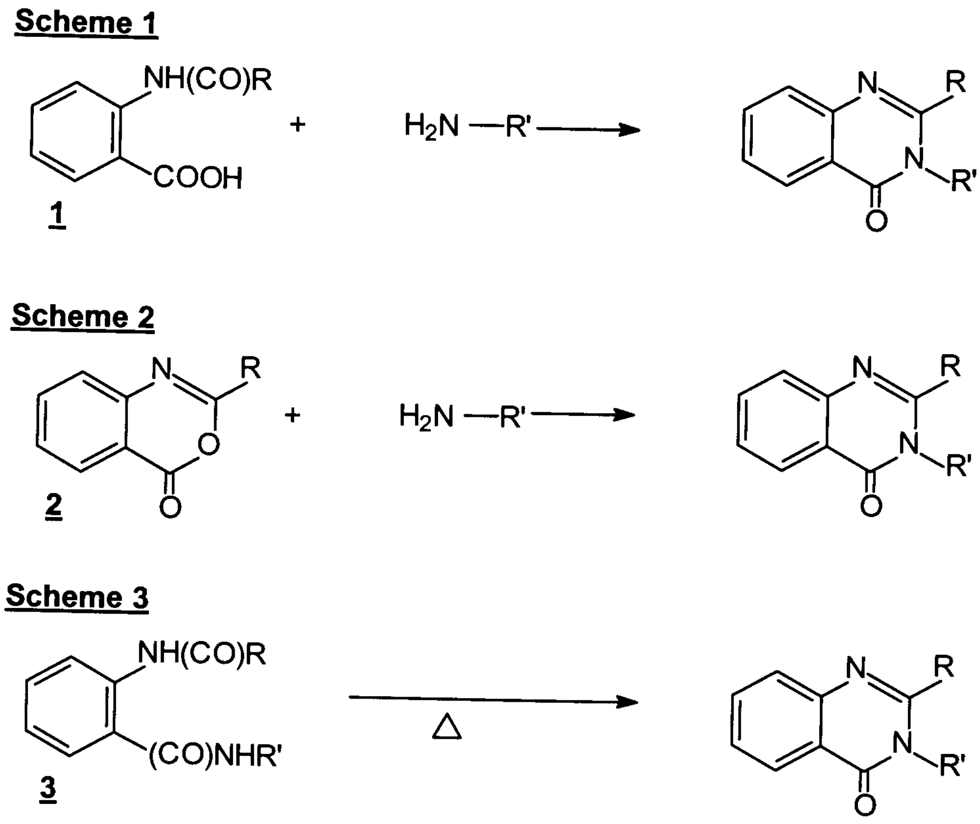


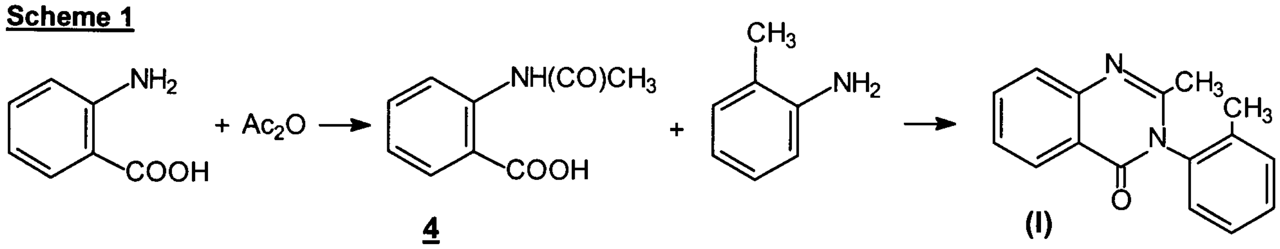
Figure 1.2 Reaction schemes for the synthesis of 2,3-disubstituted-4(3*H*)-quinazolinones[11]

In 1985 Angelos *et al*[2] reported that two basic synthetic routes for the illicit manufacture of methaqualone have been encountered - see also Figure 1.3:

(i) A two-step reaction involving the preparation of N-acetylanthranilic acid (**4**) from anthranilic acid and acetic acid anhydride, followed by condensation with *o*-toluidine in the presence of phosphorous trichloride.

(ii) A one-step reaction carried out by the reflux of anthranilic acid, acetic acid (or acetic anhydride), and *o*-toluidine. Polyphosphoric acid may be added to remove water.

Scheme 1



Scheme 2

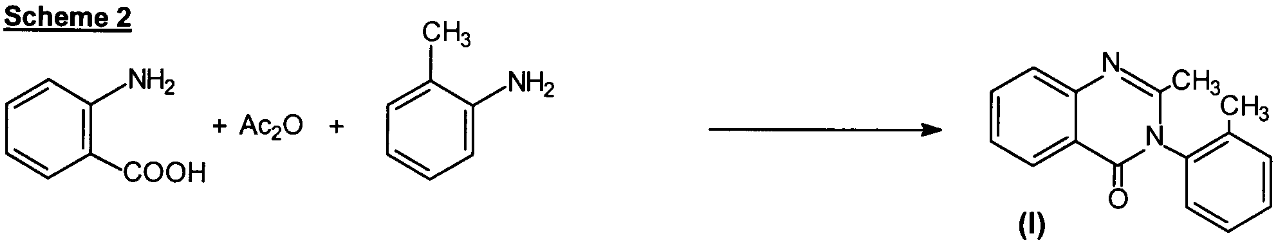


Figure 1.3 Generic reaction schemes for the synthesis of methaqualone[2]

A survey of some important synthesis is given by Ramana *et al*[12], and can also be found in limited other sources.[13,14]

Given the simplicity and ease of synthesis of methaqualone; the knowledge that it is exclusively produced in illicit laboratories; and the general lack of quality control measures in the synthesis and production thereof, it is reasonable to assume that some suspected methaqualone submitted for forensic identification may indeed be isomers of methaqualone. Such isomers may be synthesized by illicit chemists - by accident or design - through the simple substitution of precursors chemicals to create "designer" substituted 4(3*H*)-quinazolinones.

It is of cardinal importance that the forensic chemical identification of suspected drug substances be unequivocal. The importance of correctly identifying the alleged drug substance, and even more important, excluding the possibility of false-positives, takes on particular significance when considering the penalty clauses of the South African Drugs and Drug Trafficking Act, Act 140 of 1992. This Act makes provision for imprisonment of up to 25 years for offenders found guilty of trafficking in certain categories of drugs, including methaqualone.

It is thus the responsibility of the forensic chemist to anticipate possible designer isomers of drugs of abuse, and to ensure that the analytical methodology used to identify these substances, can selectively and demonstrably differentiate between such possible isomers and the actual controlled compounds.

Aim

The aim of this study was -

- to conduct a literature study on the reported synthesis and analysis of methaqualone and positional and structural isomers thereof;

- to synthesize methaqualone, some selected reported isomers of methaqualone, and some unreported anticipated possible positional and structural isomers of methaqualone;

- to collect relevant analytical data on each of the synthesized compounds;

- to evaluate the collected analytical data and to ascertain if the data are sufficiently characteristic to allow for the selective and unequivocal differentiation of methaqualone from the isomers synthesized for this study.

Scope

The target compounds considered for inclusion in this study where determined based on the following:

- All compounds must be synthesized via routes that would be suitable for methaqualone synthesis, with only systematic substitution of commercially available precursors. The rationale being that these compounds must all be of such nature that they could, intentionally or accidentally, be produced by illicit methaqualone producing laboratories.

- All compounds had to be isomers - positional and/or structural - of methaqualone. This was considered relevant as the analytical technique of choice to determine methaqualone at the SAPS FSL is coupled gas chromatography - mass spectrometry (GC-MS), with electron-impact ionization. Including such isomers would allow for the critical evaluation of the selectivity of the technique when confronted with very similar compounds of identical molecular mass.

Based on the above the specific target compounds included in this study were limited to substituted 4(3*H*)-quinazolinones as listed in Table 1.1.

Table 1.1 Target substituted 4(3*H*)-quinazolinones considered in this study (see Fig. 1.1, p. 2)

No.	Compound	MM	R₁	R₂	R₃	R₄	R₅	R₆
I	2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone	250	CH_3	CH_3	H	H	H	H
II	2-methyl-3-*m*-tolyl-4(3*H*)-quinazolinone	250	CH_3	H	CH_3	H	H	H
III	2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone	250	CH_3	H	H	CH_3	H	H
IV	3-(2,3-dimethylphenyl)-4(3*H*)-quinazolinone	250	H	CH_3	CH_3	H	H	H
V	3-(2,4-dimethylphenyl)-4(3*H*)-quinazolinone	250	H	CH_3	H	CH_3	H	H
VI	3-(2,5-dimethylphenyl)-4(3*H*)-quinazolinone	250	H	CH_3	H	H	CH_3	H
VII	3-(2,6-dimethylphenyl)-4(3*H*)-quinazolinone	250	H	CH_3	H	H	H	CH_3
VIII	3-(3,4-dimethylphenyl)-4(3*H*)-quinazolinone	250	H	H	CH_3	CH_3	H	H
IX	3-(3,5-dimethylphenyl)-4(3*H*)-quinazolinone	250	H	H	CH_3	H	CH_3	H
X	2-ethyl-3-phenyl-4(3*H*)-quinazolinone	250	CH_2CH_3	H	H	H	H	H
XI	3-*o*-ethylphenyl-4(3*H*)-quinazolinone	250	H	CH_2CH_3	H	H	H	H
XII	3-*m*-ethylphenyl-4(3*H*)-quinazolinone	250	H	H	CH_2CH_3	H	H	H
XIII	3-*p*-ethylphenyl-4(3*H*)-quinazolinone	250	H	H	H	CH_2CH_3	H	H

A survey of reported synthesis of target substituted 4(3*H*)-quinazolinones

This survey encompasses 32 published papers and registered patents, detailing 39 reported synthesis. In 1946 Grimmel *et al*[15] reported the synthesis of *inter alia* compound **III**, starting from N-acetylanthranilic acid and *p*-toluidine in the presence of PCl_3. This general procedure of condensing a N-acylanthranilic acid with a substituted or unsubstituted aromatic amine, usually in the presence of PCl_3, $POCl_3$, or polyphosphoric acid is reported a further 11 times in literature.[1,10-12,16-22]

Similar to the above route is synthesis of **I** starting with the hydrochloride salt of *o*-toluidine which was reported in Dutch Patent 295501 in 1965 [23], or alternatively by starting with the sodium salt of the N-acylanthranilic acid as reported by Rawat *et al* in 1988.[24]

Synthesis starting from anthranilic acid which is acylated and reacted with an aromatic amine was reported in1960[16], with a further four reports since.[18,25-27] These proceed via either a one-, or a two-step route, with the intermediates being either N-acylanthranilic acid or acylanthranil depending on the work-up.

Acetanthranil as a precursor for synthesis was reported on in 1963 by Boltze *et al*[17], with two more reports since.[28,29] These routes all involved condensation with a substituted primary aromatic amine to yield the target 4(3*H*)-quinazolinone.

Manhas *et al*[30] reported the synthesis of **I.HCl** starting from isatoic anhydride, *o*-toluidine, and an acetylating agent, detailing a one- and a two-step route. It was subsequently reported twice.[31,32]

The synthesis of **III** from *p*-methylacetophenone oxime and methylanthranilate was reported by Stephen *et al*[33] in 1956. This reaction proceeded via the di-*o*-tolylacetamidine intermediate and $SOCl_2$ was used as a reagent.

In 1961 Grammaticakis [34] reported on the synthesis of **I**, **II**, and **III** starting from the corresponding N-tolyl-o-nitrobenzamide and an acetylating agent. The synthesis proceeded via the N-substituted-o-aminobenzamide and the N-substituted-*o*-acylaminobenzamide. Miyata *et al* [35] in 1997 reported on the synthesis of **I** starting from N-*o*-tolyl-*o*-aminobenzamide and an acetylating agent.

In Austrian Patent 235839, 1964 Ecsery *et al* [36] reported on the preparation of **I** starting with N-acetylanthranilic acid and various N-o-tolyl compounds, including isocyanate, isothiocyanate, urea, thiourea, thiourethane, and dithiourethane.

The synthesis of **I** from methylanthranilate, $(MgBr)_2$-N-*o*-toluidine, and acetic anhydride via N-o-tolylanthranilamide was reported in 1965.[37] In 1967 Hurmer *et al* [38] reported the synthesis of **XI.HCl** from *o*-ethylphenylanthranilamide and *o*-ethylformate, as well as from anthranilic acid and N-formyl-*o*-ethylaniline. Kozhevnikov *et al* [39] subsequently reported the synthesis of **X** from N-propionyl-*o*-methylanthranilate and N,N-dimagnesiumhalidoaniline in 1969.

The preparation of **I**-d_4 from phtalimide-3,4,5,6-d_4, acetic anhydride and *o*-toluidine was described by Fentiman *et al* [40] in 1976. The synthesis proceeded via anthranilic acid and N-acetylanthranilic acid intermediates.

In 1980 Nielsen *et al* [41] reported on the synthesis of **I** from N-acetylanthranilate and *o*-toluidine hydrochloride in the presence of N,N-dimethylcyclohexylamine. Hilmy *et al* [42] reported on the synthesis of **I**, **II**, and **III** from *o*-toluidine hydrochloride and 2-acetylaminobenzonitrile in the presence of N,N-dimethylcyclohexylaminehydrochloride.

The most frequent reported synthetic route for target 4(3*H*)-quinazolinones considered in this study, involve the condensation of a primary aromatic amine, or salts thereof, with acylanthranilic acid, or acylanthranil. These compounds are either used as precursors, or prepared as intermediates, or *in situ* from anthranilic acid.

The second most reported route involves the reaction of isatoic anhydride with a primary aromatic amine and an acylating agent in either a one, or a two-step reaction. A third type of synthesis involves the cyclization of o-acylamino (N-substituted) benzamides. Some other more exotic synthetic approaches have been reported in literature, and many more should be possible. Due to the intricate and/or tedious nature of such routes, the author is of the opinion that it is unlikely that these will be encountered at illicit laboratories.

A complete summary of the synthesis of some 4(3*H*)-quinazolinones of interest to this study is given in **Appendix A**.

Five of the target compounds listed in Table 1.1 have been reported in literature surveyed, namely **I** - **III** and **X-XI** .

CHAPTER 2

Synthesis of methaqualone and target isomers

Introduction

Compounds **I** - **XIII** were synthesized through the condensation of the required primary aromatic amine with the corresponding acylanthranilic acid indicated in Table 2.1. A modified procedure based on that reported by Klosa and Stark[18] was followed. All aromatic amines used were commercial synthetic grade reagents. Commercial synthetic grade N-acetylanthranilic acid was used in the synthesis of **I** - **III**. Melting points were determined in open glass capillaries using a Gallenkamp 889339 melting point apparatus, and are uncorrected.

Table 2.1 Precursors used in the synthesis of Compounds **I** to **XIII** (see Fig. 1.1, p. 2)

No.	Compound	Aromatic amine	Acylanthranilic acid
I	2-Methyl-3-*o*-tolyl-4(3*H*)-quinazolinone	*o*-Toluidine	N-Acetylanthranilic acid
II	2-Methyl-3-*m*-tolyl-4(3*H*)-quinazolinone	*m*-Toluidine	N-Acetylanthranilic acid
III	2-Methyl-3-*p*-tolyl-4(3*H*)-quinazolinone	*p*-Toluidine	N-Acetylanthranilic acid
IV	3-(2,3-dimethylphenyl)-4(3*H*)-quinazolinone	*o*-3-Xylidine	N-Formylanthranilic acid
V	3-(2,4-dimethylphenyl)-4(3*H*)-quinazolinone	*o*-4-Xylidine	N-Formylanthranilic acid
VI	3-(2,5-dimethylphenyl)-4(3*H*)-quinazolinone	*o*-5-Xylidine	N-Formylanthranilic acid
VII	3-(2,6-dimethylphenyl)-4(3*H*)-quinazolinone	*o*-6-Xylidine	N-Formylanthranilic acid
VIII	3-(3,4-dimethylphenyl)-4(3*H*)-quinazolinone	*m*-4-Xylidine	N-Formylanthranilic acid
IX	3-(3,5-dimethylphenyl)-4(3*H*)-quinazolinone	*m*-5-Xylidine	N-Formylanthranilic acid
X	2-Ethyl-3-phenyl-4(3*H*)-quinazolinone	Aniline	N-Propanoylanthranilic acid
XI	3-*o*-Ethylphenyl-4(3*H*)-quinazolinone	*o*-Ethylbenzene	N-Formylanthranilic acid
XII	3-*m*-Ethylphenyl-4(3*H*)-quinazolinone	*m*-Ethylbenzene	N-Formylanthranilic acid
XIII	3-*p*-Ethylphenyl-4(3*H*)-quinazolinone	*p*-Ethylbenzene	N-Formylanthranilic acid

Synthesis of selected precursors[43]

N-formyl- and N-propanoylanthranilic acid were prepared from commercial synthetic grade anthranilic acid and the corresponding acid anhydride. Conversion of the mono-carbonyl containing anthranilic acid to the di-carbonyl acylanthranilic acid were verified using IR spectral data collected using general experimental procedures as outlined in **CHAPTER 3 Analytical Methodology**. The synthesis of both these anthranilic acid derivatives were based on the general procedure for the preparation of N-formyl-*o*-toluidine as describe on page 1158 of the reference.

Synthesis of N-formylanthranilic acid

Anthranilic acid (20 mmoles) was added to an excess of concentrated formic acid. The mixture was refluxed on a water bath with stirring for 30 minutes. The resulting viscous liquid was allowed to cool to room temperature, upon which rapid crystallization ensued. An excess of cold distilled water was added, and the product repeatedly washed with cold distilled water. Upon filtration, and drying at 90 °C, a pure white product with melting point 158 - 162 °C was obtained. Yield = 64%.

IR(cm^{-1}): **1705** (C = O, aryl acid); **1693** (C = O, aryl amide)

Synthesis of N-propanoylanthranilic acid

N-propanoylanthranilic acid was similarly prepared from anthranilic acid and propanoic acid anhydride to yield a yellowish product with melting point 119 -120 °C. Yield = 88%.

IR(cm^{-1}): **1706** (C = O, aryl acid); **1667** (C = O, aryl amide)

General method for the preparation of the salts of I - XIII

To 10 mmoles of the acylanthranilic were added an equimolar amount of aromatic amine and approximately 10 ml of $POCl_3$. The mixture was slowly heated resulting in a vigorous reaction and the formation of a viscous liquid that solidified on cooling. The resulting solid was dissolved in a minimum volume (50 - 200 ml) of hot methanol, boiled over activated charcoal and filtered. The filtrate was evaporated to a minimum volume. On the addition of diethyl ether a precipitate formed. The precipitate was filtered to yield the hydrochloride salt of the desired compound.

In the preparation of **VII** no precipitate formed on addition of diethyl ether. On repeating the preparation, the methanol solution was made basic (pH 9) through the addition of concentrated ammonium hydroxide solution. This was followed with the addition of 10 ml of 10% (v/v) dilution of concentrated sulphuric acid. The solution was evaporated until dryness and subsequently taken up in methanol. The addition of diethyl ether resulted in the formation of a precipitate. The precipitate was filtered to yield the sulphate salt of **VII**. The yield and physical appearance of the prepared compounds are given in Table 2.2

Table 2.2 % Yields and physical appearance of salts of **I** - **XIII** (see Fig. 1.1, p. 2)

No.	%Yield(HCl)	%Yield(HCl) - literature	Appearance
I	71.1	76[21];72[23]	Fine, white crystals
II	79.5	-	Off-white crystals
III	65.2	-	Fine, light yellow crystals
IV	61.0	-	Brilliant white, flakes
V	63.9	-	Fine, white crystals
VI	52.7	-	Fine, white crystals
VII	60.5 as sulphate	-	Yellow crystals
VIII	71.1	-	Light yellow flakes
IX	45.9	-	Fine, off-white crystals
X	51.6	-	Fine, white crystals
XI	61.3	-	Fine, white crystals
XII	27.6	-	Fine, white crystals
XIII	63.7	-	Off-white crystals

Purification and isolation of I - XIII as bases

Thin-layer chromatographic evaluation of the salts - using System TB as described in **CHAPTER 3 Analytical Methodology** - revealed the presence of precursor compounds and other residual contaminants. Each of the salts of compounds **I** - **XIII** were subsequently subjected to an individual clean-up procedure as described below and isolated as the free base. Purification was continued until no residual contaminants could be detected in the final product. The yield, melting point and physical appearance of each of the isolated compounds are given in Table 2.3 below.

*2-Methyl-3-o-tolyl-4(3H)-quinazolinone (**I**)*

A portion of **I.HCl** was dissolved in a minimum volume of a of 10%(v/v) solution of concentrated hydrochloric acid. The resulting solution was extracted thrice using diethyl ether. The aqueous phase was adjusted to pH 9 with a 10%(v/v) solution of concentrated ammonium hydroxide solution, and extracted thrice with chloroform. The chloroform extracts were combined, dried over CaO, filtered, and evaporated to dryness. The residue was recrystallized from methanol with the addition of cold distilled water. The resulting crystals were washed several times with cold distilled water, filtered, and dried at 60 °C to yield **I**.

*2-Methyl-3-m-tolyl-4(3H)-quinazolinone (**II**)*

Similar as for **I**, except a final recrystallization from isopropyl alcohol and water to yield fine yellowish crystals. A TLC-evaluation indicated that the resulting product was contaminated with unknown trace impurities. The product was subsequently dissolved in a minimum volume of pentane, boiled over activated charcoal, and filtered. The filtrate was solvent stripped under vacuum, and the resulting residue recrystallized from methanol through the addition of cold distilled water. The resulting crystals were washed several times with cold distilled water, filtered, and dried at 60 °C to yield **II**.

*2-Methyl-3-m-tolyl-4(3H)-quinazolinone (**III**)*

A portion of **III.HCl** was dissolved in a minimum volume of pentane with the addition of methanol. Activated charcoal was added and the solution boiled for several minutes, after which it was filtrated. The filtrate was evaporated to a minimum volume, and an equal quantity of cold distilled water was added. The pH was adjusted to pH 10. Stirring the solution in an ice bath resulted in crystal formation. The crystals was washed with distilled water, filtered and dried at 60 °C to yield **III**.

*3-(2,3-Dimethylphenyl)-4(3H)-quinazolinone (**IV**)*

A portion of **IV.HCl** was made into a paste with methanol. Concentrated ammonium hydroxide solution was added until a clear solution resulted. The addition of methanol resulted in crystal formation. Crystallization was taken to completion through the addition of cold distilled water. The crystals were washed with cold distilled water, filtered, and dried at 60 °C to yield **IV**.

*3-(2,4-Dimethylphenyl)-4(3H)-quinazolinone (**V**)*

Similar as for **IV**, but on addition of the ammonia the product collected as small droplets against the sides of the holding vessel. The mixture was extracted thrice with chloroform, dried, and the chloroform stripped under vacuum. The resulting residue was recrystallized from pentane on an ice bath. The crystals were washed with pentane, collected through filtration, and dried at 50 °C to yield **V**.

*3-(2,5-Dimethylphenyl)-4(3H)-quinazolinone (**VI**)*

Similar as for **V**, except that after vacuum stripping the residue was first taken up in methanol, boiled over activated charcoal, filtered, evaporated to dryness, and then recrystallized from pentane.

*3-(2,6-Dimethylphenyl)-4(3H)-quinazolinone (**VII**)*

Similar as for **IV**, except that crystal growth was only observed after leaving the methanolic solution overnight.

3-(3,5-Dimethylphenyl)-4(3H)-quinazolinone ***(VIII)***

Similar as for **IV**, except that crystal formation only started upon addition of cold distilled water to the methanolic solution. The final product was dried at at 50 °C.

3-(3,5-Dimethylphenyl)-4(3H)-quinazolinone ***(IX)***

Similar as for **VIII**.

2-Ethyl-3-phenyl-4(3H)-quinazolinone ***(X)***

Similar as for **VIII**, except that the methanolic solution was boiled over activated charcoal, filtered, and then cold distilled water was added.

3-o-Ethylphenyl-4(3H)-quinazolinone ***(XI)***

Similar as for **V**, except that upon vacuum stripping the chloroform, a clear viscous oil remained. Several attempts to crystallize the oil proved futile. The oil failed to crystallize after several months.

3-m-Ethylphenyl-4(3H)-quinazolinone ***(XII)***

Similar as for **VIII**, except that the final product was dried at 20 °C under vacuum.

3-p-Ethylphenyl-4(3H)-quinazolinone ***(XIII)***

Similar as for **X**.

All compounds were fully characterized using IR, MS, and NMR data as described in **CHAPTER 3 Analytical Methodology**.

Table 2.3 %Yield, melting points, and physical appearance of **I** - **XIII** (see Fig. 1.1, p. 2)

No.	%Yield	%Yield - Literature	M.P.(°C.)	M.P.(°C.) - Literature	Appearance
I	33.1	60[11] 75[12] 22.8[22]	113 - 115	113-114[12] 114-115[22] 113-114[32] 116.5-117.5[35]	Fine, white crystals
II	22.6	60[2] 40[28]	126 - 128	127-129[10] 126-132[34]	Fine, white crystals
III	21.2	69[4] 33[28] 80[31]	149 - 151	149-151[10] 148-150[12] 147-148[22] 151-152[29]	Fine, white crystals
IV	55.4	-	159 - 161	-	White, crystalline needles
V	32	-	96 - 98	-	Fine, white crystals
VI	11.7	-	89 - 91	-	Fine, white crystals
VII	53	-	94 - 96	-	Fine, white crystals
VIII	32.5	-	133 - 135	-	Fine, white crystals
IX	21.3	-	118 - 120	-	Fine, white crystals
X	23.5	80[2] 91[11] 85[16] 80[31]	126 - 128	126-127[1] 125-126[23] 133-134[25] 126-127[39]	Fine, white crystals
XI	27.2	-	Liquid at 0 °C	Semi-solid[38]	Clear, viscous liquid
XII	19.8	-	48 - 50	-	Fine, white crystals
XIII	45.3	-	136 - 138	-	Off-white crystals

Discussion

The modified synthetic route employed to prepare the compounds yielded products within a few minutes and in acceptable yields. The major modification to the method as described by Klosa *et al*[18], was the use of $POCl_3$ instead of polyphosphoric acid. The use of $POCl_3$ in the preparation of quinazolinones is well known and reported in **Chapter 1: Introduction**. All these reported methods however make use of fairly small quantities of $POCl_3$ in a reaction medium like toluene, requiring preparation times in the order of 2 - 3 hours. Introduction of an excess of $POCl_3$ directly to an intimate mixture of the precursors as described in this study, resulted in virtually instantaneous formation of the product, with the reaction completed within less than 10 minutes (depending on the quantities of precursors used, and the rate of heating). The exothermic nature of the reaction and the resulting foaming may however limit application to the research laboratory only.

With the exception of **XI**, all isomers could be isolated as solid substances. In general the higher melting point compounds proved easier to manipulate and recrystallize. The reported yields for the free bases of each of **I** - **XIII** is significantly lower than what may have been obtained with alternative sample purification techniques. The preparation and purification of the base compounds proved tedious and resulted in a high loss of product. Product purification using column chromatography may proved to be a more viable alternative for the rapid isolation and purification of compounds **I** - **XIII**.

CHAPTER 3: Analytical Methodology

Part 1: Chromatography

Introduction

Chromatography is the analytical technique of choice employed by the SAPS FSL to screen for the presence of illicit drugs. The value of such screening is limited by the selectivity of the chromatographic technique used. All multiple dosage units of suspected methaqualone (**I**) containing exhibit material received by the FSL are screened using two uncorrelated thin-layer chromatography (TLC) systems. Once, using both TLC systems, it has been established that all dosage units in the selected random sample contains a component with identical Rf values as **I**, the presence of methaqualone is unequivocally confirmed using an interfaced gas liquid chromatography - mass spectrometry (GC - MS) method. Acceptance criteria for a positive GC - MS determination include a retention time and mass spectrum match with a certified reference standard of **I**. Liquid chromatography (LC) is infrequently used as a quantitative technique for illicit drugs. The inherent significance and importance of chromatography in the routine determination of methaqualone is thus evident.

Various sources have reported on the use of TLC [4,44,46], LC [4,44,46], GC [4,44 - 47], and more recently micellar electrokinetic capillary chromatography (MECC)[47] for the separation and quantitation of **I** in physical and biological matrices.

Daenens *et al*[48] described the separation of **I** from mecloqualone and nitromethaqualone using TLC and GC and concluded that the two technique's usefulness are limited to determining the probable identity of the compounds.

The enrichment of the enantiomers of **I** using LC with triacetylcellulose as stationary phase was reported by Mannschreck *et al.* [49]

A GC method for the differentiation of **I** from mecloqualone and nitromethaqualone has been described.[50] In the United Nations ST/NAR/15 publication of 1988[4], *"Recommended methods for testing methaqualone/mecloqualone"*, it is suggested that a positive confirmation of methaqualone can be made using inter alia two uncorrelated TLC systems.

This is in stark contrast with Dal Cason *et al*[10] who in 1981 conclude that *"TLC, using the systems examined, has no value as an identification tools ...* (for inter alia **I**, **II**, and **III**)*... since Rf values vary so little...".*

The same authors also evaluated the GC separation of **I**, **II**, and **III**, using packed columns and concluded that GC may be used to screen for these compounds, and also to quantitate them once they have been *"conclusively identified".*

Given the analytical protocols followed to identify methaqualone at the SAPS FSL it was decided to evaluate the selectivity of TLC, LC, and GC with regard to compounds **I** - **XIII**. It is of crucial importance that chromatographic methods utilized in forensic chemistry be highly selective towards actual illicit target compounds - in this instance **I** - in the presence of "designer analogs".

Experimental

Thin-layer chromatography (TLC)

TLC was performed using Macherey-Nagel Alugram SIL G/UV$_{254}$ aluminium back 200 x 200 mm Silica Gel 60 plates with a layer thickness of 0.20 mm. All solvents used were analytical grade. Standard ascending TLC techniques were employed to analyse compounds **I** to **XIII**. Mobile phases as recommended for basic nitrogenous drugs were evaluated - see Table 3.1.1:[45]

Table 3.1.1 TLC mobile phases evaluated

Mobile Phase	Composition
TA	Methanol: Ammonium hydroxide (25%) (100:1.5)
TB	Cyclohexane: Toluene: Diethylamine (75:15:10)
TC	Ethyl acetate: Methanol: Ammonium hydroxide (strong) (85:10:5)
TD	Chloroform: Methanol (90:10)
TE	Chloroform: Acetone (4:1)

Approximately 1-2 μl of a 1 mg/ml solution of each of compounds **I** to **XIII** were applied in spots to the plates. Solvent blanks were also applied. Each plate was developed to a minimum distance of 150 mm. Upon drying the compounds were visualized under ultraviolet light at 254 nm and Rf values calculated.

Systems **TB** and **TC** as described are the mobile phases currently in use at the SAPS FSL to screen submitted exhibit material for the presence of methaqualone.

Liquid chromatography (LC)

LC analysis was performed using a model W600 liquid chromatograph fitted with a model 996 PDA photodiode array detector (Microsep/Waters) scanning from 210-400 nm. One microlitre of sample (ca. 30ng/μl per analyte) was injected using a model 717plus automatic autosampler (Waters). For separation a Symmetry C18, 5 μm, 4.6 x 250 mm LC column from Waters was installed. An initial flow rate of 1.5 ml/min. of 100% **Buffer 1** (60% Acetonitrile in H_2O + 4 ml triethylammonium phosphate solution (1 M in H_2O)) was on injection - over a ten minute period - developed over a linear gradient to a 100% flow of **Buffer 2** (10% Acetonitrile in H_2O + 4 ml triethylammonium phosphate solution (1 M in H_2O)). A flow rate of 1.5 ml/min. of **Buffer 2** was maintained for 15 minutes, upon which the gradient was reversed over a one minute period to a 100% flow of **Buffer 1**. This flow was maintained for a total run time of 30 minutes.

The method described is the method currently in use at the SAPS FSL to screen for the presence of basic nitrogenous drugs.

Gas-liquid chromatography (GC)

GC analysis were conducted using a model HP 6890 gas chromatograph interfaced with a model HP 6890 mass selective detector (Hewlett-Packard). One microlitre of sample (*ca.* 30ng/μl per analyte) was injected using a model 7673 automatic autosampler (Hewlett-Packard). The injector port temperature was set at 250 °C for all injections. Separation was achieved using a 30 m x 0.25 mm DB - 5MS column with a film thickness of 0.25 μm (J & W Scientific). Helium was used as carrier gas at a constant flow of 1.0 ml/min. The transfer line to the mass spectrometer was set at 280°C, and the source temperature kept at 156°C. Total ion chromatograms were collected in full-scan positive ion electron impact mode scanning from 40 to 550 amu at 1.5 scan/s.

The oven parameters evaluated are given in Table 3.1.2:

Table 3.1.2 GC oven parameters evaluated

	Method A	**Method B**	**Method C**
Initial temperature (°C)	120	50	100
Initial time (min.)	0	1	0
Ramp (°C/min.)	30	30	50
Final temperature (°C)	290	110	200
Hold time (min.)	2.3	2	0
Ramp (°C/min.)		17	2.5
Final temperature (°C)		280	250
Hold time (min.)		10	0
Run time (min.)	7.97	25	22

Method A described is the general routine method currently used by the SAPS FSL for the GC-MS separation and identification of methaqualone in exhibit materials suspected to contain methaqualone. **Method B** is the general routine method currently used by the SAPS FSL to screen for basic drugs in unknowns. **Method C** was specifically developed for this study.

Results and discussion

Thin-layer chromatography (TLC)

With the exception of **TB**, all solvent systems evaluated were unable to effect any differentiation between the studied compounds. The Rf values obtained with **TB** are listed in Table 3.1.3. From the Rf-values reported it is clear that, although **TB** separates the studied compounds over a Rf(x100) spread of 14, it does not allow for the differentiation of the studied compounds. Thin-layer chromatography, using the systems evaluated, has no diagnostic value in differentiating compounds **I** - **XIII**.

Table 3.1.3 Thin-layer chromatography data for **I** - **XIII**: Mobile phase TB

Compound	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**
Rf (x100)	56	55	53	64	64	66	64	63	67	60	64	67	65

Liquid chromatography (LC)

The LC method evaluated yielded sharp well defined peaks for compounds **I** - **XIII** when injected individually, thus proving its suitability for the type of compounds studied. Retention times (Rt) and relative retention times (RRt) obtained are listed in Table 3.1.4.

Table 3.1.4 HPLC data for **I** - **XIII**

	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**
Rt	14.94	15.11	15.15	15.99	16.31	16.30	15.89	16.49	16.79	16.10	16.12	16.76	16.88
RRt	1.00	1.01	1.01	1.07	1.09	1.09	1.06	1.10	1.12	1.08	1.08	1.12	1.13

The results obtained indicated that sets of *o* -, *m* -, and *p* - isomers, eluted in that sequence, with **I** (*o* - methyl substituted) having the smallest Rt of all compounds studied. **XIII** (*p* - ethyl substituted) eluted last, with the rest of the compounds showing no predictable elution order.

From the observed retention data, as well as the analysis of mixtures of **I** - **XIII**, it is clear that the evaluated LC method could not differentiate between **I** and its positional isomers **II** and **III**. The method evaluated may be of limited value to separate selected structural isomers, and possibly to quantitate compounds **I** - **XIII** respectively if it could unequivocally be proven that such a compound is individually present in a sample. In general the method is not selective enough, thus limiting its practical application.

Gas-liquid chromatography (GC)

The total ion chromatograms (TIC) obtained when a mixture of **I** - **XIII** (*ca.* 20ng/μl per analyte in $CHCl_3$) was chromatographed using each of methods **A**, **B**, and **C** are given in Figures 3.1.1 - 3.1.3. Relevant chromatographic data obtained using each of the methods are tabulated in Table 3.1.5.

From the sharp well defined peak shapes obtained with all three chromatographic methods evaluated, it is clear that all are suited for the chromatographic separation of the class of compounds studied. As expected the sequence of elution is identical for all three methods. Sets of *o* -, *m* -, and *p* - isomers, eluted in that sequence, with **I** (*o* - methyl substituted) before **XI** (*o* - ethyl substituted). Both **XII** (*m* - ethyl substituted) and **XIII** (*p* - ethyl substituted), eluted after **III** (*p* - methyl substituted). The dimethyl substituted compounds showed the following elution order: **VII** (2,6 - dimethyl); **VI** (2,5 - dimethyl); **V** (2,4 - dimethyl); **IV** (2,3 - dimethyl); **IX** (3,5 - dimethyl); **VIII** (3,4 - dimethyl).

All three methods allowed for effective separation of **I**. Only **Method C** however achieved baseline-to-baseline separation of **I** from **VII**. **Method A** proved to be particularly useful as separation is achieved in less than 7 minutes. Given that this method also utilizes a fairly high initial oven temperature, it would be ideally suited for rapid high throughput routine applications where effective separation of the rest of the isomers from each other is of no consequence.

Both **Method A** and **B** failed to separate **III** and **IV**. In the TIC obtained using **Method A** these two compounds appears as a single peak. In **Method B** compound **IV** appears as a shoulder to the left of **III**. **Method A** furthermore failed to separate **II** and **V**, as well as **IX** and **XII**. Although **Method B** showed improved separation over **Method A**, it offers no advantage over **Method C**, and is thus of limited use for the separation of the isomers studied.

Method C proved to be of superior selectivity with effective separation of all thirteen isomers studied being achieved in under 16 minutes. Utilizing **Method C** it would be possible to selectively identify compounds **I** - **XIII** based on their retention data. Such identification would be presumptive in nature, and would require unequivocal confirmation using interfaced molecular spectroscopy detection techniques such as mass spectrometry or infrared spectroscopy.

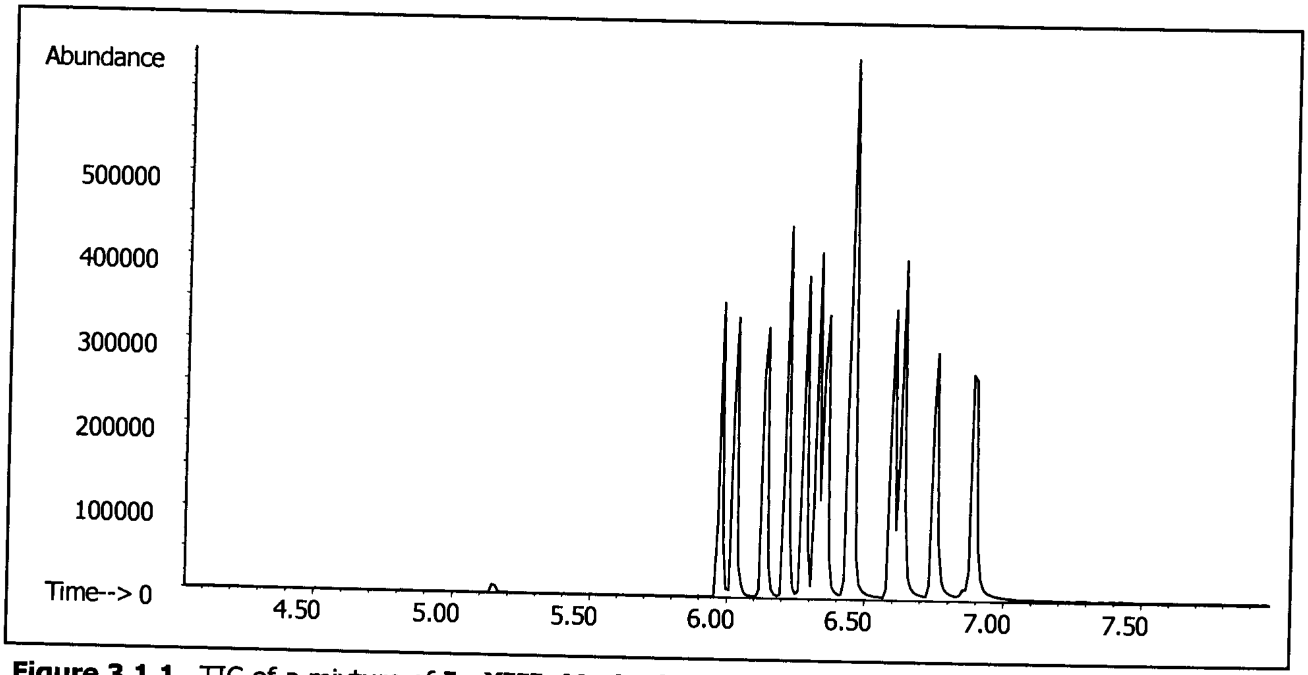


Figure 3.1.1 TIC of a mixture of **I** - **XIII**: **Method A**

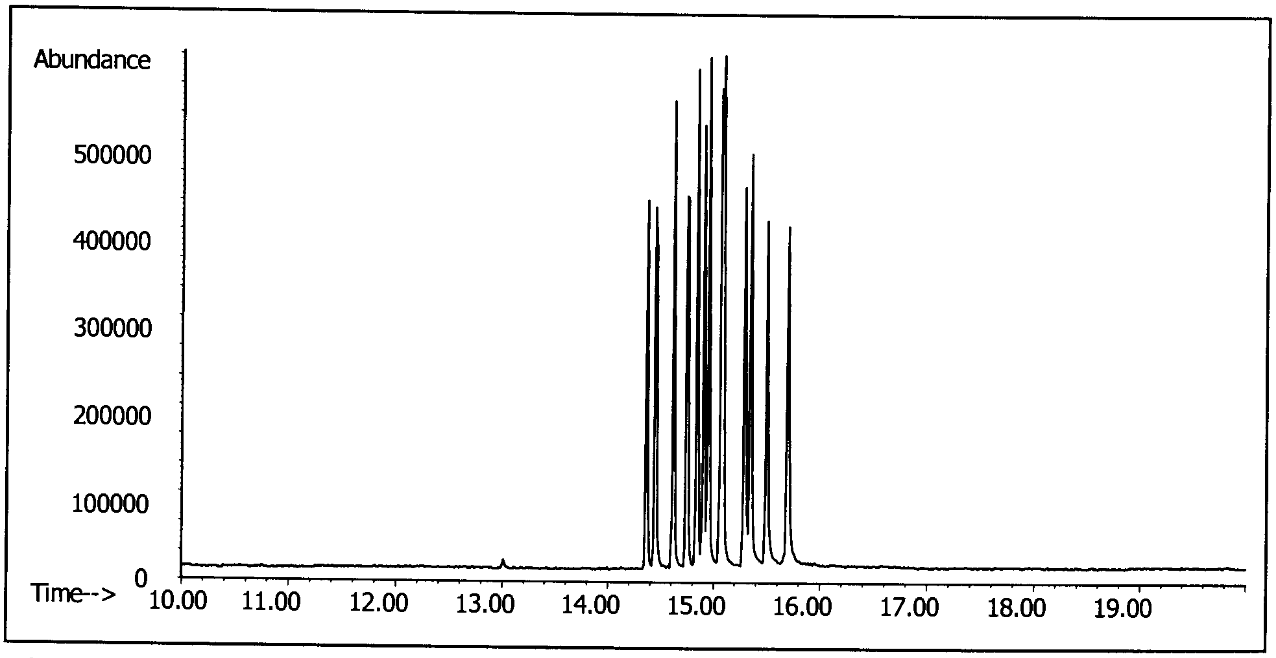


Figure 3.1.2 TIC of a mixture of **I** - **XIII**: **Method B**

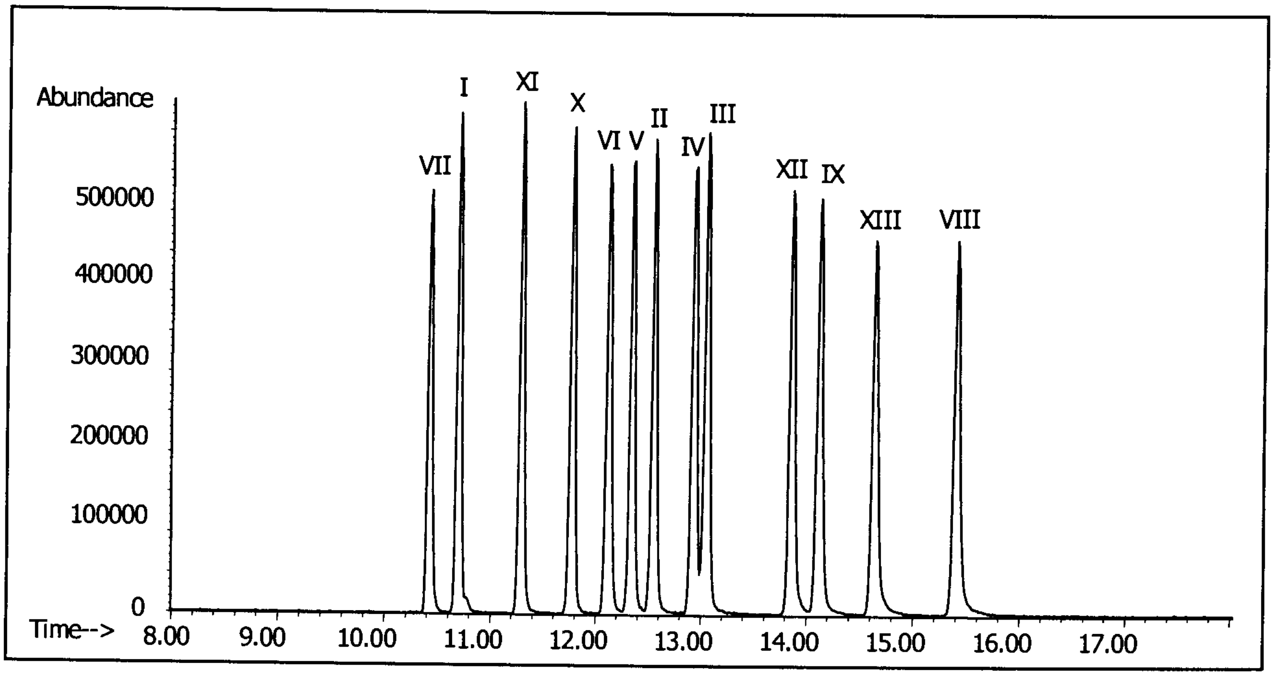


Figure 3.1.3 TIC of a mixture of **I** - **XIII**: **Method C**

Table 3.1.5 GC retention times (**Rt**) and relative retention times (**RRt**) for Compounds **I** - **XIII**

Compound	**Method A**		**Method B**		**Method C**	
	Rt	**RRt**	**Rt**	**RRt**	**Rt**	**RRt**
I	6.03	1.000	14.45	1.000	10.70	1.000
II	6.36	1.055	14.96	1.035	12.56	1.174
III	6.45	1.070	15.08	1.044	13.06	1.221
IV	6.45	1.070	15.07	1.043	12.94	1.209
V	6.33	1.050	14.91	1.032	12.35	1.154
VI	6.29	1.043	14.85	1.028	12.12	1.133
VII	5.98	0.992	14.37	0.994	10.43	0.975
VIII	6.90	1.144	15.70	1.087	15.41	1.440
IX	6.64	1.101	15.35	1.062	14.12	1.320
X	6.22	1.032	14.75	1.021	11.78	1.101
XI	6.14	1.018	14.62	1.012	11.30	1.056
XII	6.60	1.095	15.29	1.058	13.86	1.295
XIII	6.76	1.121	15.50	1.073	14.65	1.369

Conclusion

The superior selectivity of GC **Method C** evaluated allowed for the rapid differentiation of all thirteen compounds studied based on retention data. Such retention time based differentiation however is of a presumptive nature. GC was found to be a powerful separation technique that would prove extremely useful when screening for the presence of compound **I** - **XIII** in physical and biological matrices. Even more important is the ability of GC to be interfaced with highly selective detection systems such as mass selective or infrared spectral detectors.

TLC and LC, employing the experimental conditions described, have no practical value in differentiating the isomers studied.

CHAPTER 3: Analytical Methodology

Part 2: Ultraviolet Spectroscopy

Introduction

Due to its inherent lack of selectivity ultraviolet spectroscopy (UV) is neither a used nor a recognized technique for the confirmatory identification of methaqualone by the SAPS FSL. As the LC method evaluated for the differentiation of **I** - **XIII** as described in **Chapter 3: Part 1** of this study made use of a photodiode array (PDA) detector scanning from 200 to 400 nm, it was decided to consider the ability of UV-Vis spectroscopy to differentiate between the studied compounds.

Patel *et al* [44] reported that **I** exhibits characteristic ultraviolet absorption properties in different solvents. The UV spectra, as well λ_{max} and λ_{min}, for **I** in methanol and ethanol are given. A range of 2-substituted styryl-3-alkyl (aryl)-4(3*H*)-quinazolinones was prepared and the UV spectra collected in absolute ethanol.[51] All compounds studied exhibit two strong absorption in the 220 - 254 nm, and 263 - 345 nm regions respectively. UV spectroscopy was used in an attempt to differentiate between **I**, mecloqualone, and nitromethaqualone in alkaline ethanol solutions.[48] All three compounds showed strong absorption at ca. 225 nm, 265 nm, 304 nm, and 315 nm. These absorptions the authors attributed to the quinazolinone nucleus, and concluded that the data *"make identification of the individual products impossible".*

Dal Cason *et al* [10] evaluated the ability of UV spectroscopy to differentiate between inter alia **I**, **II**, and **III**. It was found that the absorption wavelength maxima of all three compounds were near identical.

UV spectral data for **I** is given in limited other sources.[46,52]

Experimental

Experimental procedures as described under *Liquid Chromatography* in **Chapter 3: Part 1** were followed. Spectra were collected using the mentioned PDA UV detector scanning from 200 - 400 nm.

Results and discussion

The spectra obtained for **I** is given in Figure 3.2.1. All isomers studied exhibited near identical spectra with strong absorption near 225 nm; 265 nm; and 305 nm. Detailed spectral data are given in Table 3.2.1. The observed λ_{max} is characteristic for the quinazolinone nucleus[48] , and is reminiscent of the UV spectral data for naphtalene.[43]

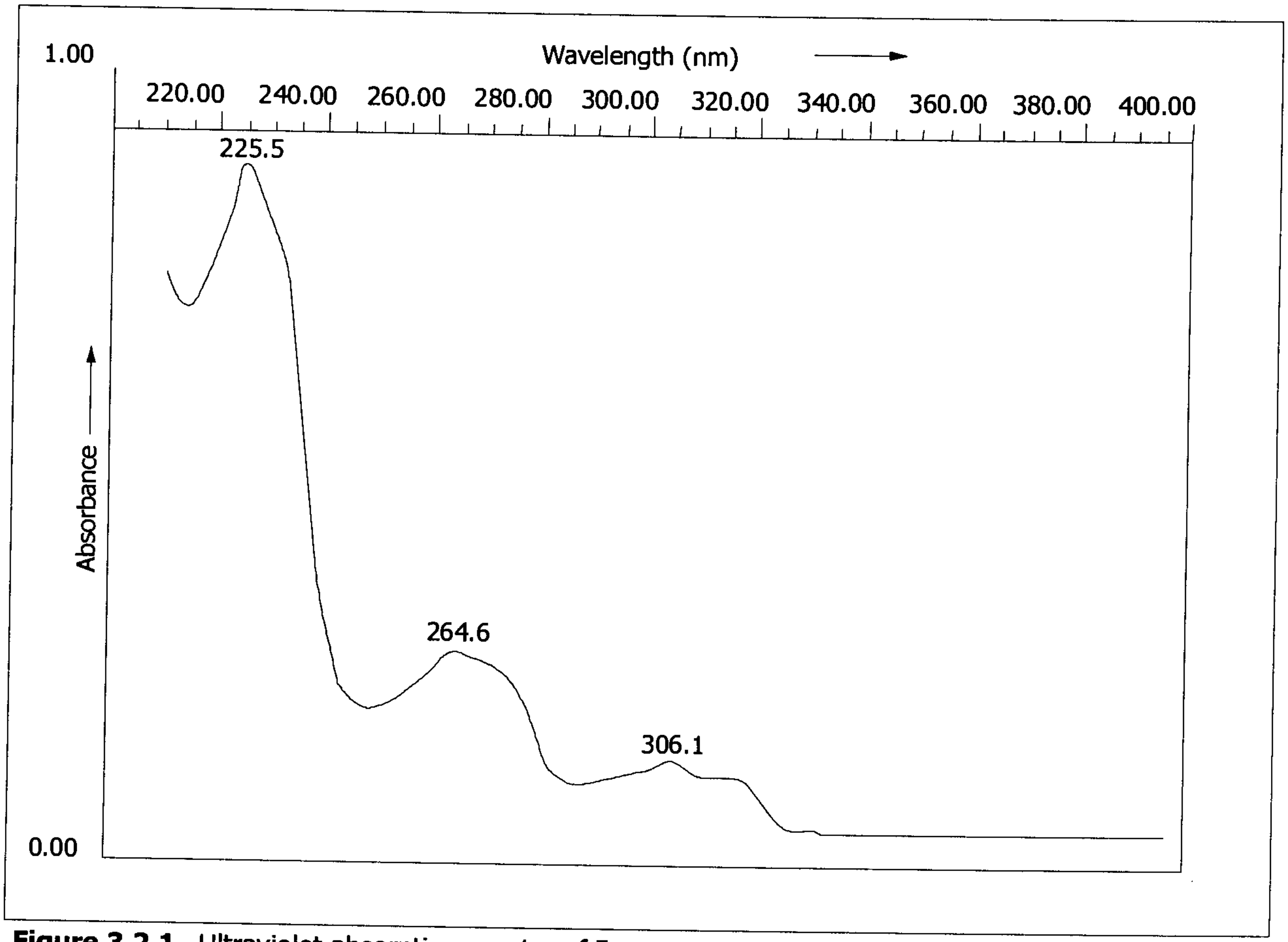


Figure 3.2.1 Ultraviolet absorption spectra of **I**

Table 3.2.1 UV Spectral data: **I** - **XIII**

	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**
$\lambda_{max\ a}$	226	226	226	226	226	226	226	226	226	226	226	226	226
$\lambda_{max\ b}$	265	265	265	266	266	266	266	267	267	266	266	267	267
$\lambda_{max\ c}$	306	305	305	301	302	302	302	303	303	302	305	303	303

Conclusion

UV spectroscopy has no diagnostic value in differentiating **I** - **XIII**. The technique should be refrained from for the determination of **I**, or any of its isomers or analogs. UV spectroscopy may be suitable to detect and quantify the studied isomers, where such isomers are individually present in samples.

CHAPTER 3: Analytical Methodology

Part 3: Infrared Spectroscopy

Introduction

Infrared spectroscopy (IR) is recognized by the SAPS FSL as an accepted confirmatory technique for the identification of **I** due to its high specificity. As it does not have the selectivity associated with chromatographic techniques, it is not the technique of choice for the routine determination of **I**. It is however from time-to-time used as a secondary and/or an alternative technique for the confirmatory analysis of **I**. It is thus of critical importance to evaluate the techniques ability to differentiate between **I** - **XIII**.

The most comprehensive assignments of the infrared absorptions of **I** is given by Patel *et al.*[44] The infrared spectrum of **I** was collected from a 0.5% dispersion in KBr. The actual spectrum, principal peaks, as well as peak assignments are given: **3049, 2994 and 2907 cm $^{-1}$** (CH stretching); **1672 cm $^{-1}$** (amido carbonyl stretching); **1603, 1595, and 1471 cm $^{-1}$** (aromatic skeletal vibrations); **1340 and 1326 cm $^{-1}$** (aromatic C - N stretching); **778, 760, 720, and 703 cm $^{-1}$** (CH deformation of aromatic ring (out-of-plane bending)).

Daenens *et al*[48] in 1975 reported on the differentiation of **I**, nitromethaqualone, and mecloqualone. Spectra of the free bases were collected using the standard potassium halide disk method and are reported. No band assignments were made, but the authors concluded that although the spectra are similar, they contain sufficient detail to allow differentiation.

A range of 2-methyl-3aryl-4(3*H*)-quinazolinones - i.a. **I** and **III** - were prepared and their structural assignments verified by their infrared spectra.[29] The following assignments are given: sharp band at ca **5.9 μ (1695 cm-1)** (C = O); sharp band at ca. 6.1 μ **(1639 cm-1)** (C = N). The infrared spectrum of N-Propanoylanthranilic acid - the precursor for **X** - were also collected: Broad absorption from **3.3** to **4.5 μ (3030 - 2222 cm-1)** (O - H acid); very sharp band at **5.9 μ (1695 cm-1)** (C = O acid); very sharp band at **6.1 μ (1639 cm-1)** (C = O amide); sharp band at **6.6 μ (1515 cm-1)** (N - H).

A brief survey of infrared studies on quinazolinones is given by Anwar *et al.*[53] The following peak assignments are quoted from literature: **1700 cm^{-1}** (C = O, stretch); **1625 cm^{-1}** (C = N, stretch); **1628-1618 cm^{-1}** Quinazolinone I; **1581-1566 cm^{-1}** Quinazolinone II; **1517-1478 cm^{-1}** Quinazolinone III. A number of 2, 3 - disubstituted-4(3*H*)-quinazolinones were prepared and their solid state spectra (KBr disk) were collected. No significant absorptions were observed in the 2200-1720 region. The following assignments and discussion are given:

C - H

All quinazolinone ring systems gave rise to C - H stretching absorptions near 3100 cm^{-1} and 3030 cm^{-1}. Aliphatic C - H asymmetric and symmetric stretches near 2960 cm^{-1} and 2860 cm^{-1} respectively.

C = O, C = N, and C = C

All compounds showed strong absorption in the vicinity of 1670 cm^{-1} assigned to the quinazolinone C = O stretch vibrations. A shoulder or strong band near 1650 cm^{-1} (dependent on the substituent at the 2 position) were assigned to the C = N stretch vibration.

Skeletal Vibrations

The so-called Quinazolinone vibrations were assigned as follows:
1610 - 1585 cm^{-1} Quinazolinone 1; **1580 -1550 cm^{-1}** Quinazolinone 2;
1520 - 1480 cm^{-1} Quinazolinone 3.

1500 - 1000 cm^{-1} Region

Assigned to C - N stretches, various C - C stretching modes, and aromatic C - H in-plane bending.

1000 - 650 cm^{-1} Region

Primarily assigned to out-of-plane aromatic C - H bending. Others peaks assigned to various C - C and C - O stretching modes.

I was synthesized and its infrared (KBr) spectrum was collected.[19] The following assignments: **3100-2700 cm^{-1}** (C - H); **1670 cm^{-1}** (C = O); band split at **1600, 1590**, and **1560 cm^{-1}** (C = N, C = C, and aromatics). The infrared (nujol) spectra of a range of 2,3-disubstituted-4(3*H*)-quinazolinones, including **I**, were collected. All collected spectra showed a C = O absorption at **1670-1680 cm $^{-1}$**.[11] Badr *et al*[54] prepared a range of 6-bromo-2,3 - disubstituted-4(3*H*)-quinazolinones. The infrared spectra collected showed the following characteristic absorptions at **1720 -1680 cm^{-1}** (C = O) and **1630 - 1615 cm^{-1}** (C = N).

An in-depth study on the identification of some chemical analogues and homologues of methaqualone were reported by Dal Cason *et al* in 1980.[10] *Inter alia* **I**, **II**, and **III** were prepared and the infrared spectra of both the free bases and hydrochlorides were collected using the standard KBr disk method. The actual spectra are given, but is of limited use due to their reduced representation and the absence of peak annotation. The authors concluded that *"although spectra are similar, and in some cases identical, careful examination will reveal a sufficient number of unique features to permit differentiation of the synthesized compounds."*

Clarke prepared a range of aryl substituted 2-methyl-(2-metoxy-aryl)-4(3*H*)-quinazolinones.[50,55] Infrared spectra of the free bases were collected and are given. Differentiation of all prepared compounds, including methaqualone were possible.

The infrared spectra of **X** , **X.HCl** and **N – propanoylanthranilic acid** are given[56,57], and the principal peaks listed:[57] **X** (cm $^{-1}$) - **1675, 1585, 770, 690**; **X.HCl** (cm $^{-1}$) - **1720, 1640, 760, 690**.

The collection of the infrared spectrum of methaqualone and mecloqualone using the standard halide disk method is described.[4] Spectra of **I**, **I.HCl** and mecloqualone and mecloqualone HCl are given with tables of significant peaks, no band assignments are given: **I** (cm $^{-1}$) - 1676, 1609, 778, 1469, 1271, 3012; **I. HCl** (cm $^{-1}$) - 1725, 762, 1649, 579, 1484, 1580.

A range of 2-alkyl-3-aryl-4(3*H*) quinazolinones were prepared:[58] **1685 cm $^{-1}$** (C = O); **1280 cm $^{-1}$** (C - N tertiary amine); **3010 cm $^{-1}$** (C - H stretch).

Ramana *et al*[12] reported on the preparation of a range of 2-alkyl-3-aryl-4(3*H*)-quinazolinones - *inter alia* **I, III** and **X**. Their infrared spectra all exhibited carbonyl absorptions at **1676 - 1692 cm $^{-1}$**. Infrared spectra were collected in chloroform: **I** (cm $^{-1}$) **1683** (C = O); **III** (cm $^{-1}$) **1676** (C = O); **X** (cm $^{-1}$) **1681** (C = O). The spectrum of 2-methyl-3-phenyl-4(3*H*)-quinazolinone exhibited the followings absorptions: **1684 cm $^{-1}$** (C= O); **1606 cm $^{-1}$** (C= N).

Infrared spectral data on **I** are given in several literature references.[50,52] Principal peaks for methaqualone are listed:[50] (cm $^{-1}$, KBr) **1682**; **1599**; **1565**; **770**; **1265**; **697**. The transmittance spectrum of **I** is given[52], with characteristic peaks in the 900 - 690 cm $^{-1}$ region annotated: (cm $^{-1}$) 780; 759; 723; 702.

A detailed survey on the theory of infrared spectral interpretation is given by Pavia *et al.*[59] Table 3.3.1 gives a summary of base values of bonds occurring in the target compounds considered for this study.

Table 3.3.1 Infrared absorption values of bonds occurring in compounds **I** - **XIII**[59]

Bond	**Vibration type**	**Wavenumber (cm^{-1})**	**Intensity**
C - H	aromatic, stretch	3150 - 3050	s
C - H	- CH_3, asymmetric stretch	2962	s
C - H	- CH_2 -, asymmetric stretch	2926	s
C - H	- CH_3, symmetric stretch	2872	s
C - H	- CH_2 -, symmetric stretch	2853	s
Aromatic	overtone and combination	2000 - 1667	w

Table 3.3.1 Infrared absorption values of bonds occurring in compounds **I** - **XIII**[59]

Bond	**Vibration type**	**Wavenumber (cm^{-1})**	**Intensity**
C = O	amide, stretch	1670 - 1640	s
C = N	imine, stretch	1690 - 1640	w - s
C = C	aromatic, stretch	1600 and 1475	m - w
C - H	- CH_2 -, in-plane bend	1450	m
C - H	-CH_3, symmetric bend	1450 and 1375	m
C - N	aromatic amine, stretch	1350 - 1250	m - s
C - H	aromatic, out-of-plane bend	900 - 720	s
C = C	aromatic, out-of-plane ring bend	720 - 667	s
C - H	aromatic, out-of-plane bend, mono-substitution	750	s, s
C = C	aromatic, out-of-plane ring bend, mono-substitution	690	s
C - H	aromatic, out-of-plane bend, *o*-disubstitution	750	s
C - H	aromatic, out-of-plane bend, *m*-disubstitution	880 and 780	m, s
C = C	aromatic, out-of-plane ring bend, *m*-disubstitution	690	s
C - H	aromatic, out-of-plane bend, *p*-disubstitution	850 - 800	s
C - H	aromatic, out-of-plane bend, 1,2,3-trisubstitution	780	s
C = C	aromatic, out-of-plane ring bend, 1,3,5-trisubstitution	700	m
C - H	aromatic, out-of-plane bend, 1,2,4-trisubstitution	880 and 810	m, s
C - H	aromatic, out-of-plane bend, 1,3,5-trisubstitution	840 - 910	s
C = C	aromatic, out-of-plane ring bend, 1,3,5-trisubstitution	650 -700	m

s = strong; m = medium; w = weak

Experimental

The infrared spectra of **I** - **XIII** were collected using a model Magna-IR 750 Series II Fourier-transform infrared spectrophotometer fitted with a KBr beamsplitter and a DTGS KBr detector (Nicolet). Samples were prepared as dispersions in KBr so as to give spectra with an absorbance between 0.2 and 1. Spectra were collected in the 4000 - 400 cm^{-1} region using 32 scans at a resolution of 4 cm^{-1}. A background spectrum, using the same collection parameters, was collected before each sample spectrum. Wavelength accuracy were verified using a commercial 0.1 mm polystyrene reference film (Nicolet).

Results

The infrared spectra collected for each of compounds **I** - **XIII**, as well as the expanded spectra (*ca.* 1800 - 400 cm^{-1}), are given in Figures 3.3.1 to 3.3.26. Detailed spectral data and band assignments for **I** - **XIII** are given in Tables 3.3.2 to 3.3.14.

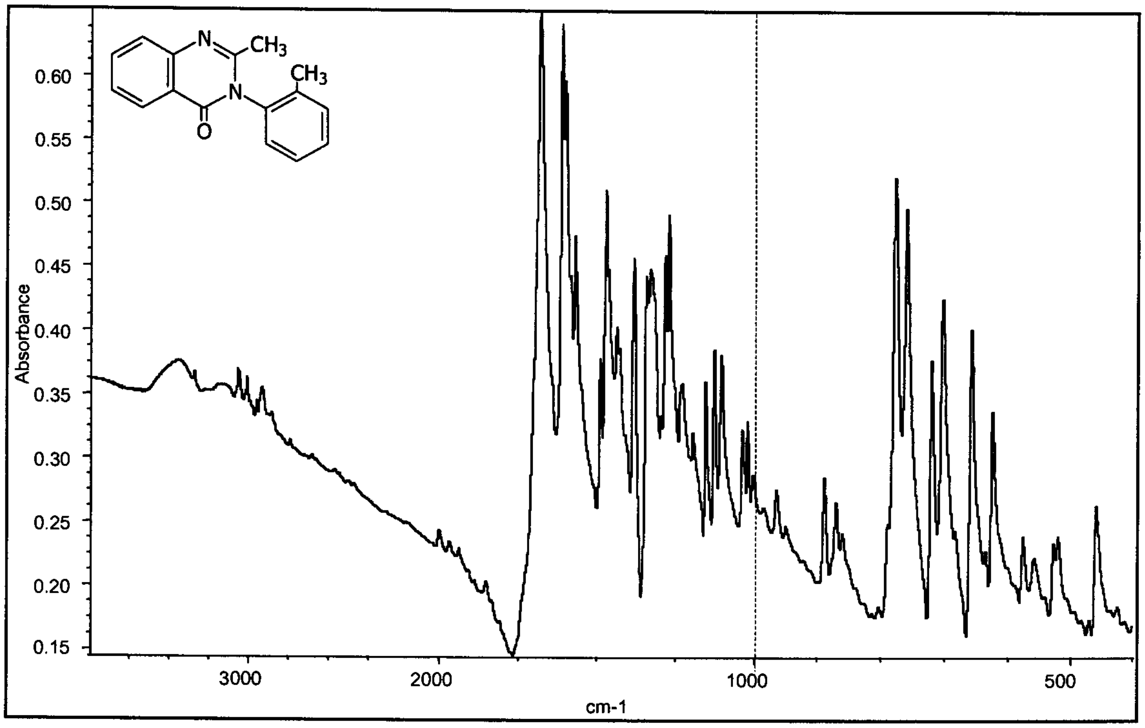


Figure 3.3.1 Condensed phase infrared spectrum of **I**

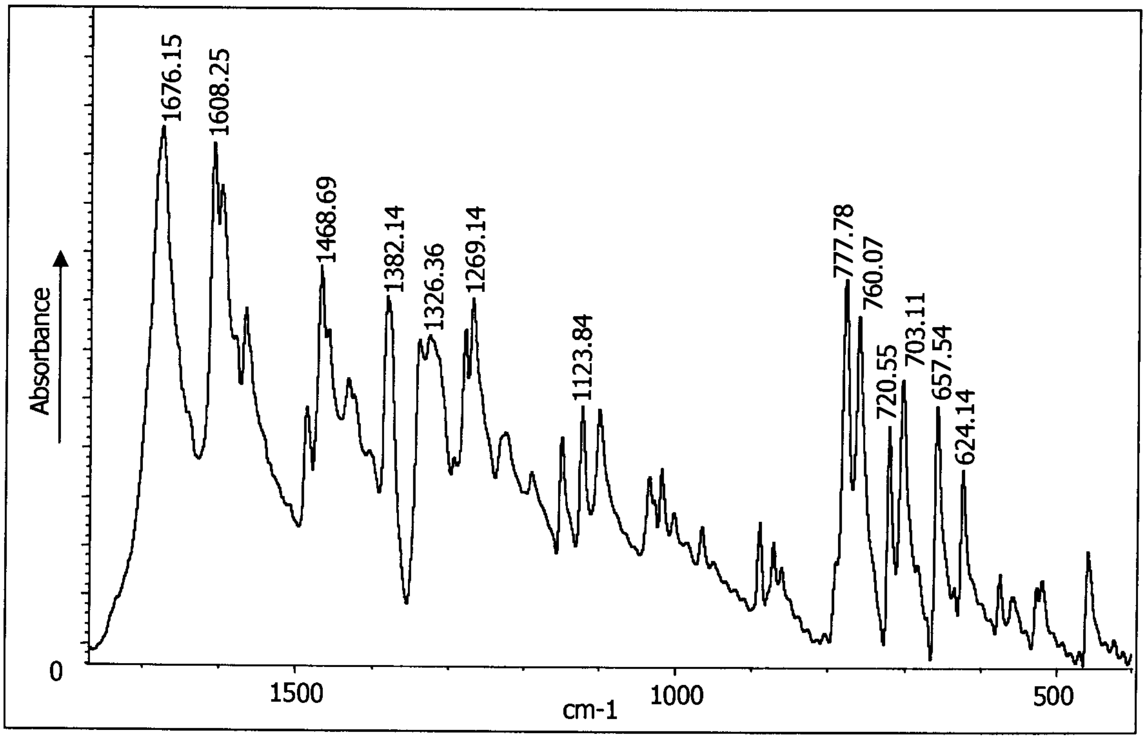


Figure 3.3.2 Expanded condensed phase infrared spectrum of **I**

Table 3.3.2 Infrared spectral data for **I**

Absorption (cm^{-1})	Bond	Band assignment
3080 - 3010	C - H	aromatic, stretch
2950 - 2850	C - H	- CH_3, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1676	C = O	amide, stretch
1608	C = N	imine group, stretch
1598 and 1468	C = C	aromatic, stretch
1383	C - H	- CH_3, symmetric bend
1300 - 1000	C - H	aromatic, in-plane bend
1340 and 1326	C - N	aromatic, stretch
778 and 760	C - H	aromatic, out-of-plane bend
721 and 703	C = C	aromatic, out-of-plane ring bend

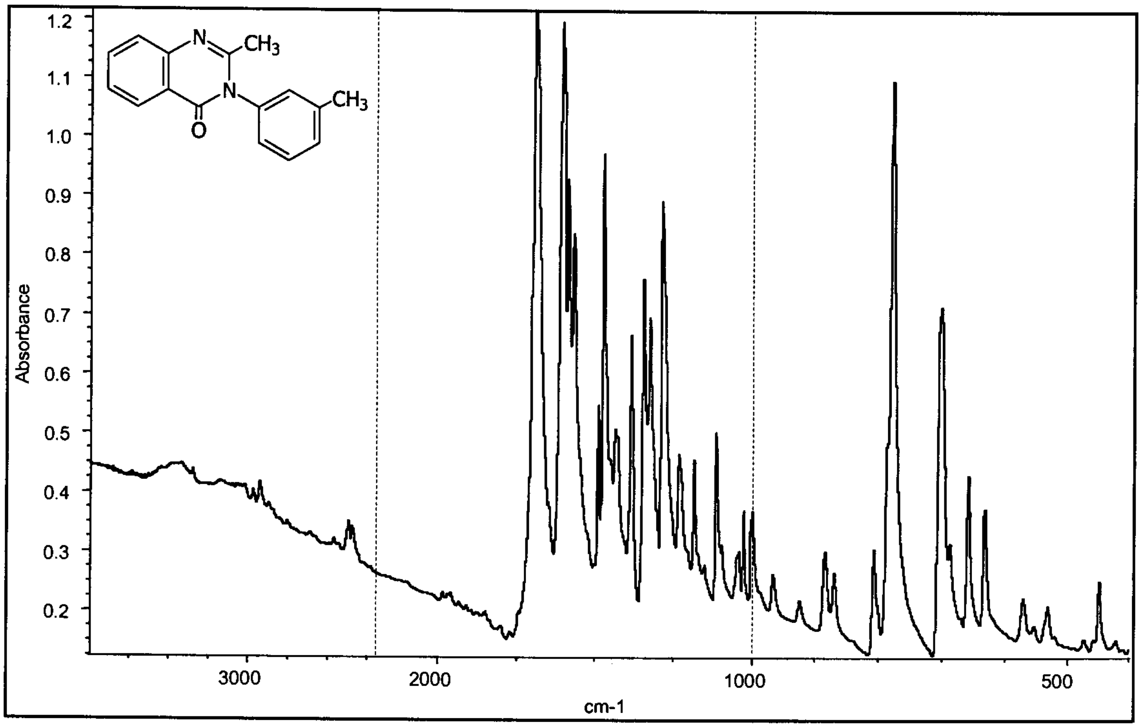


Figure 3.3.3 Condensed phase infrared spectrum of **II**

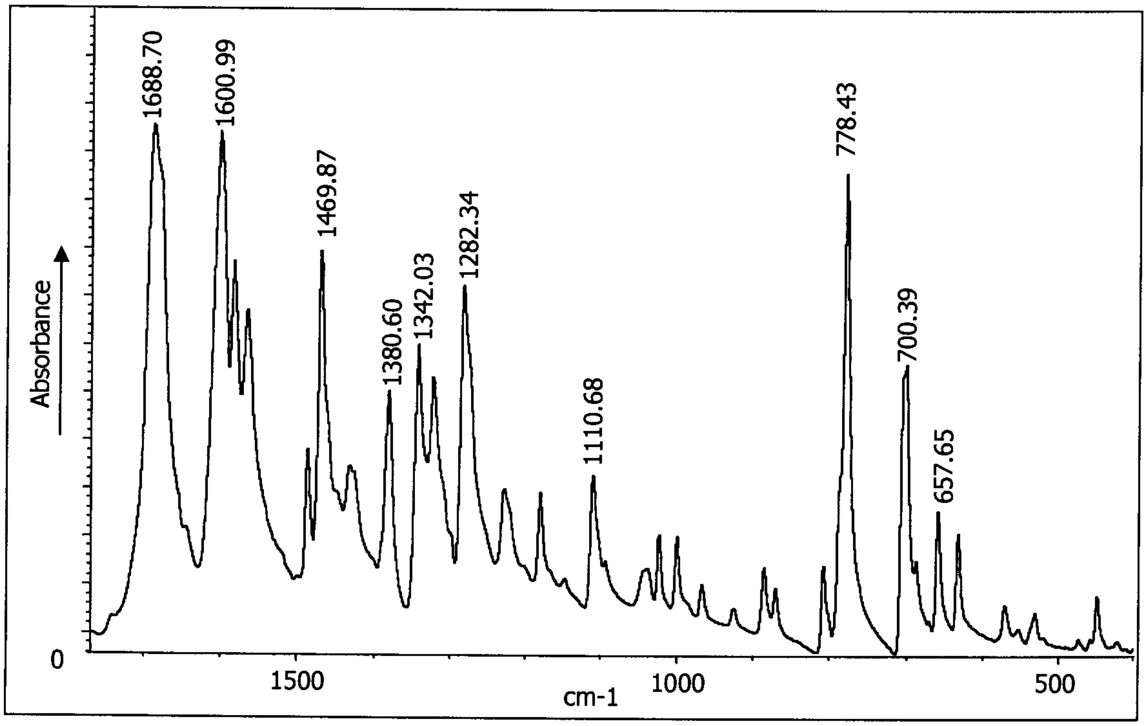


Figure 3.3.4 Expanded condensed phase infrared spectrum of **II**

Table 3.3.3 Infrared spectral data for **II**

Absorption (cm^{-1})	**Bond**	**Band assignment**
3080 - 3010	C - H	aromatic, stretch
2965 - 2800	C - H	- CH_3, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1689	C = O	amide, stretch
1601	C = N	imine group, stretch
1583 and 1470	C = C	aromatic, stretch
1381	C - H	- CH_3, symmetric bend
1342 and 1322	C - N	aromatic, stretch
1300 - 1000	C - H	aromatic, in-plane bend
786 (shoulder)	C - H	aromatic, out-of-plane bend
778	C - H	aromatic, out-of-plane bend
703 (not resolved)	C = C	aromatic, out-of-plane ring bend
700	C = C	aromatic, out-of-plane ring bend

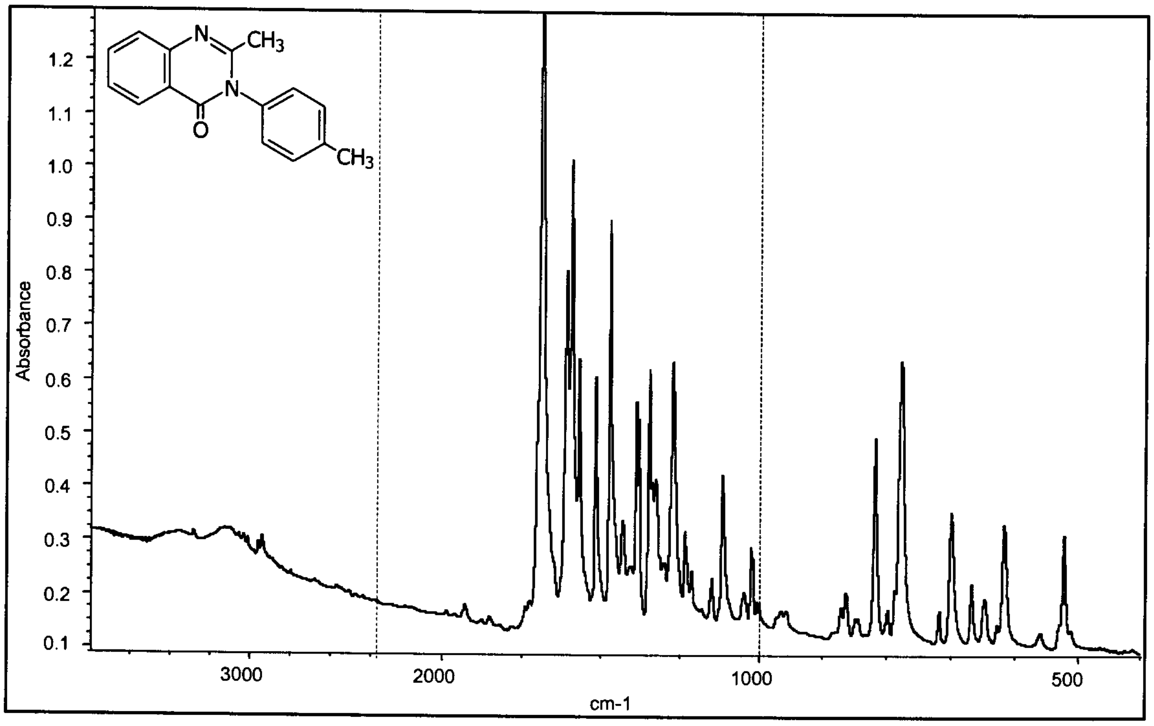


Figure 3.3.5 Condensed phase infrared spectrum of **III**

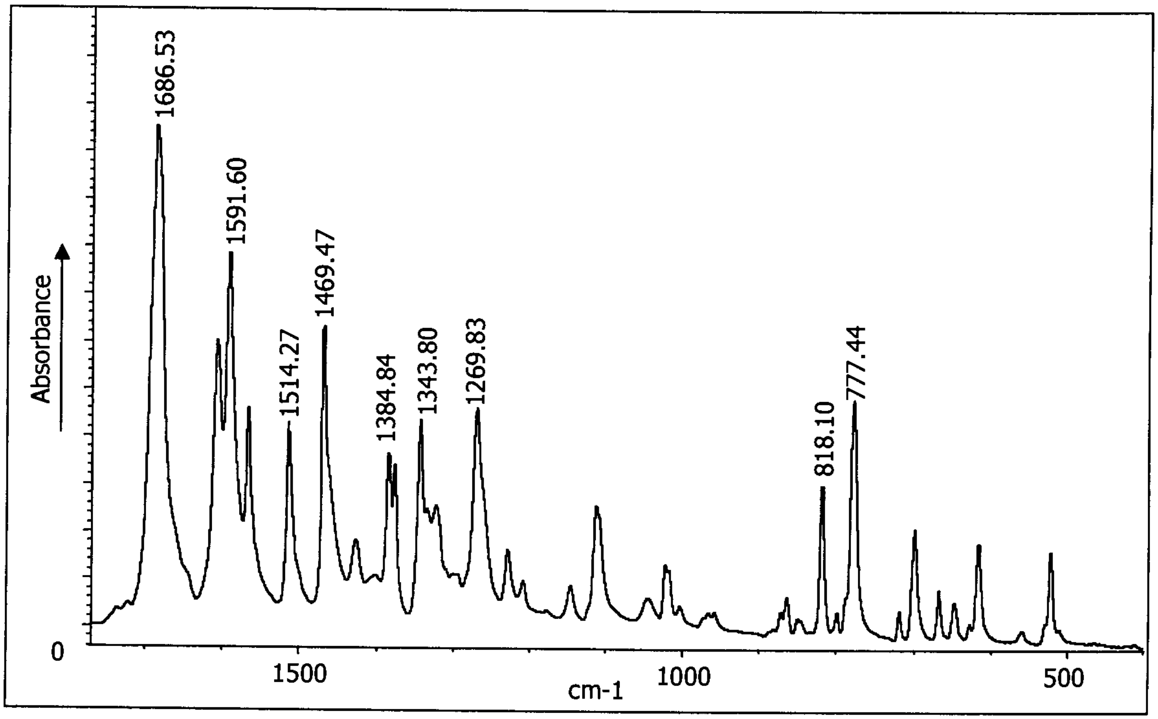


Figure 3.3.6 Expanded condensed phase infrared spectrum of **III**

Table 3.3.4 Infrared spectral data for **III**

Absorption (cm^{-1})	**Bond**	**Band assignment**
3080 - 3010	C - H	aromatic, stretch
2950 - 2800	C - H	- CH_3, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1686	C = O	amide, stretch
1608	C = N	imine group, stretch
1591 and 1469	C = C	aromatic, stretch
1381 and 1377	C - H	- CH_3, symmetric bend
1344 and 1323	C - N	aromatic, stretch
1300 - 1000	C - H	aromatic, in-plane bend
818 and 777	C - H	aromatic, out-of-plane bend
699	C = C	aromatic, out-of-plane ring bend

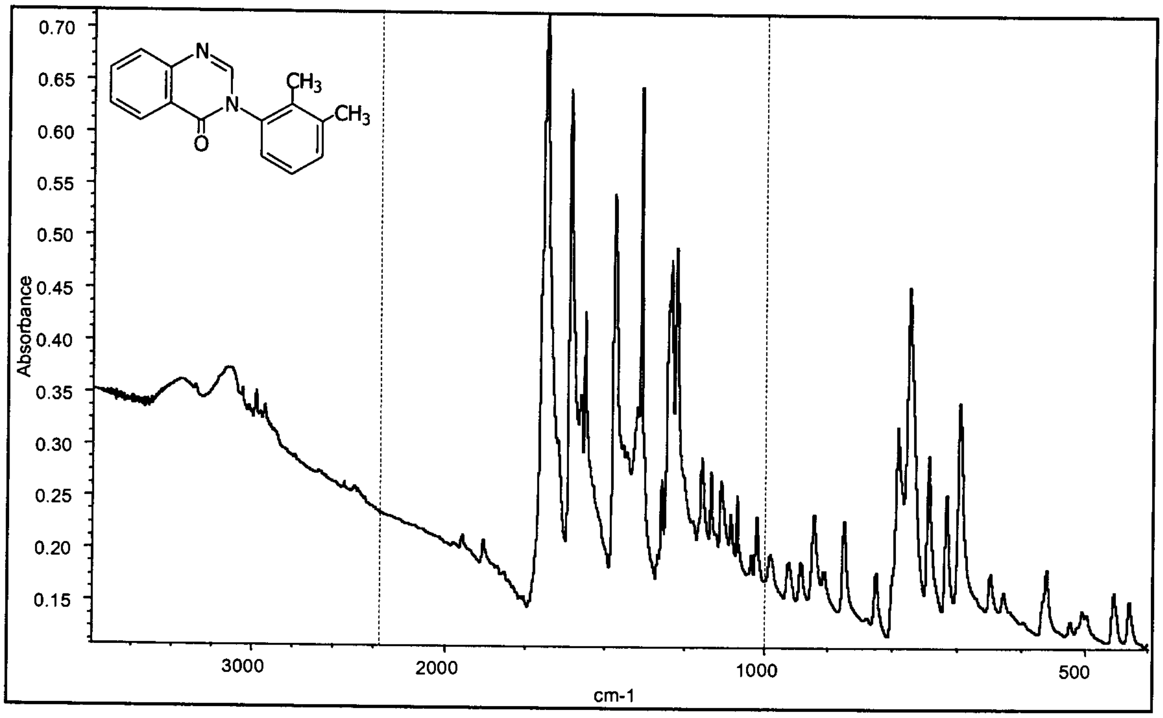


Figure 3.3.7 Condensed phase infrared spectrum of **IV**

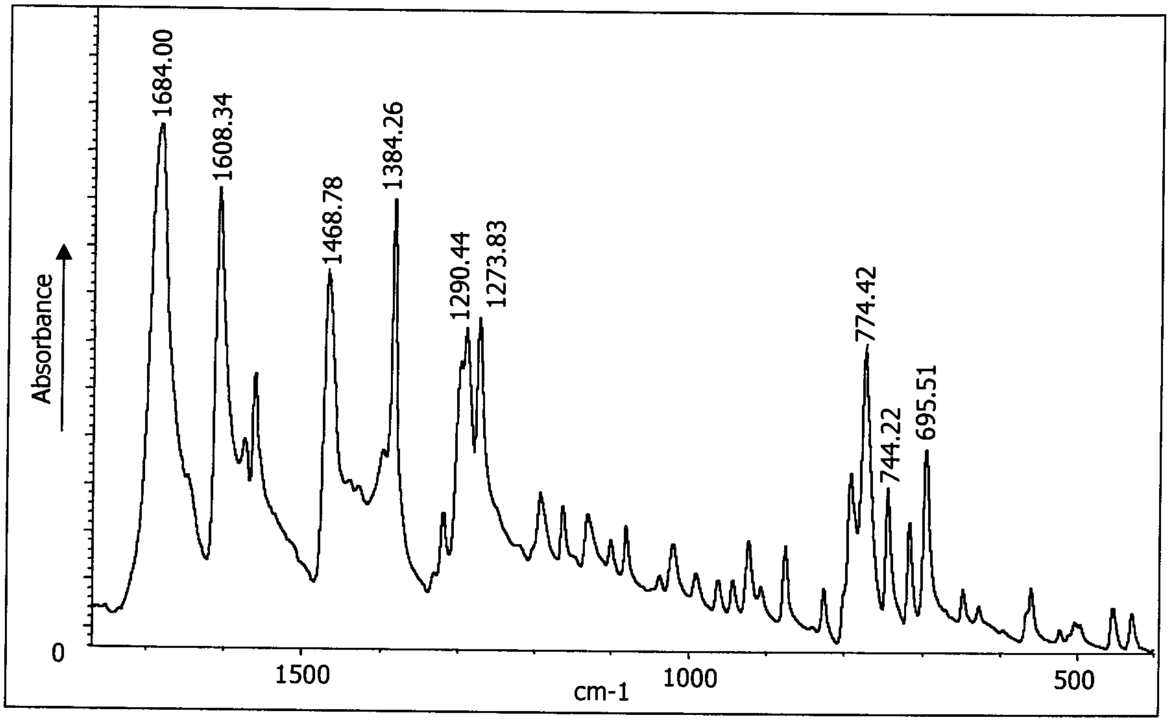


Figure 3.3.8 Expanded condensed phase infrared spectrum of **IV**

Table 3.3.5 Infrared spectral data for **IV**

Absorption (cm^{-1})	**Bond**	**Band assignment**
3080 - 3005	C - H	aromatic, stretch
2980 - 2800	C - H	- CH_3, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1684	C = O	amide, stretch
1608	C = N	imine group, stretch
1574 and 1469	C = C	aromatic, stretch
1384	C - H	- CH_3, symmetric bend
1300 - 1000	C - H	aromatic, in-plane bend
774 and 744	C - H	aromatic, out-of-plane bend
716 and 696	C = C	aromatic, out-of-plane ring bend

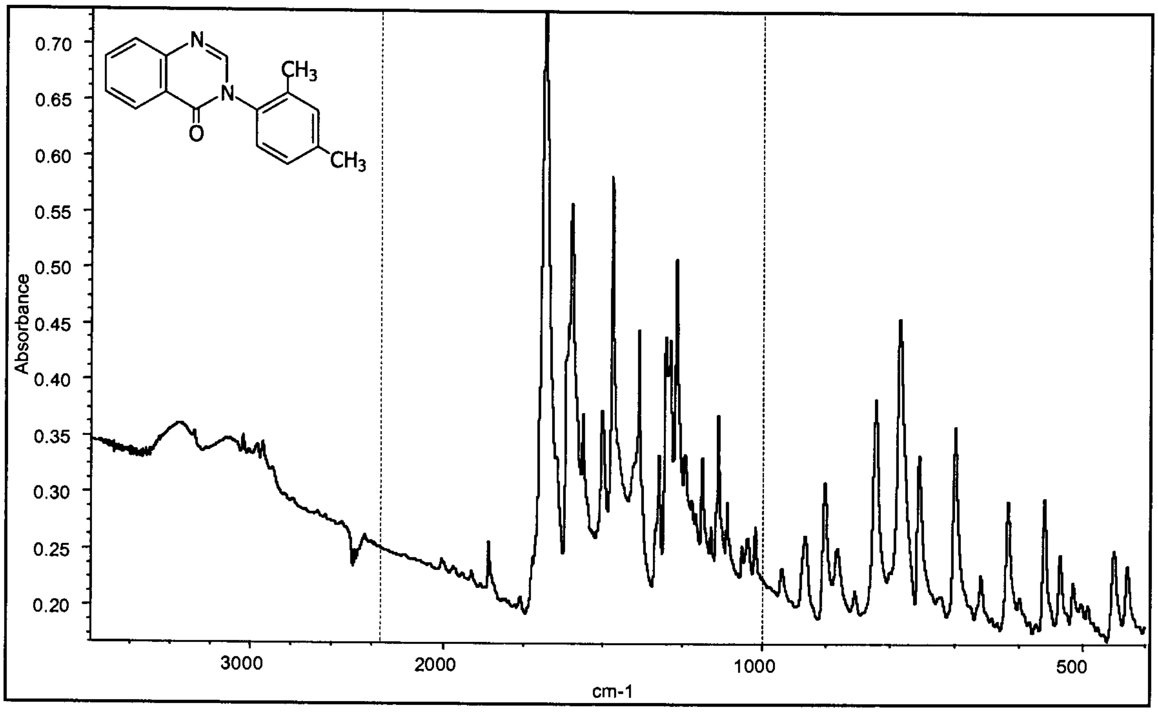


Figure 3.3.9 Condensed phase infrared spectrum of **V**

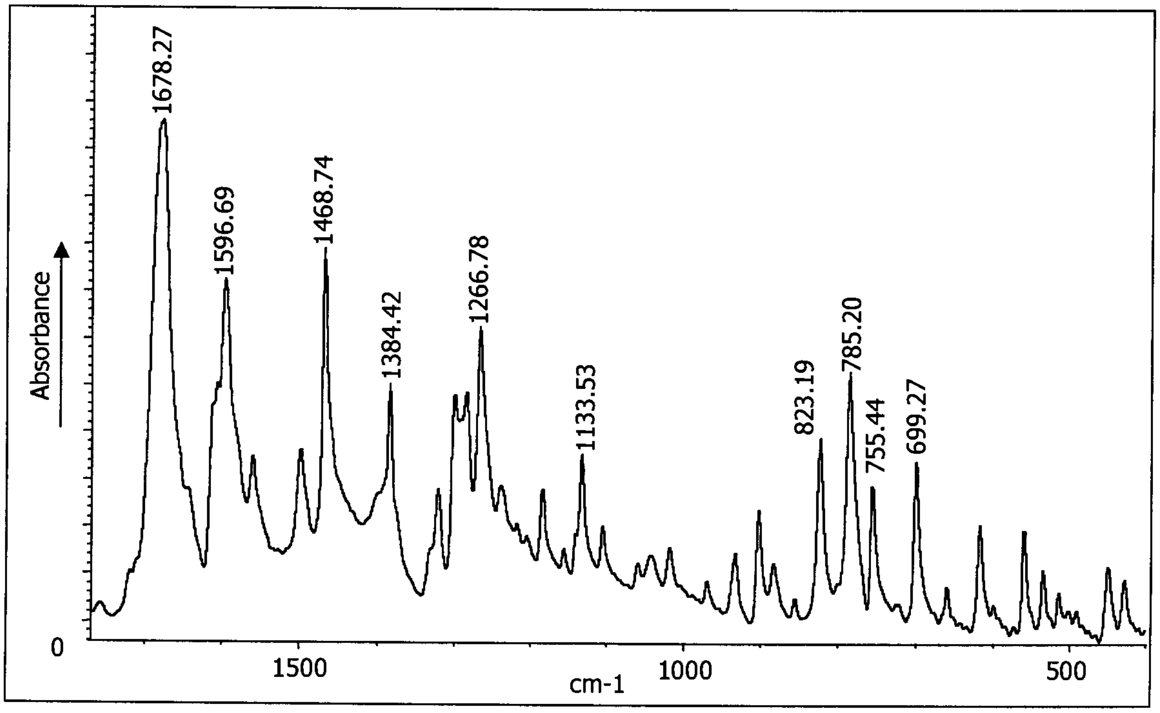


Figure 3.3.10 Expanded condensed phase infrared spectrum of **V**

Table 3.3.6 Infrared spectral data for **V**

Absorption (cm^{-1})	**Bond**	**Band assignment**
3080 - 3005	C - H	aromatic, stretch
2980 - 2800	C - H	- CH_3, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1678	C = O	amide, stretch
1607	C = N	imine group, stretch
1597 and 1469	C = C	aromatic, stretch
1384	C - H	- CH_3, symmetric bend
1350 - 1000	C - H	aromatic, in-plane bend
823 and 785	C - H	aromatic, out-of-plane bend
699	C = C	aromatic, out-of-plane ring bend

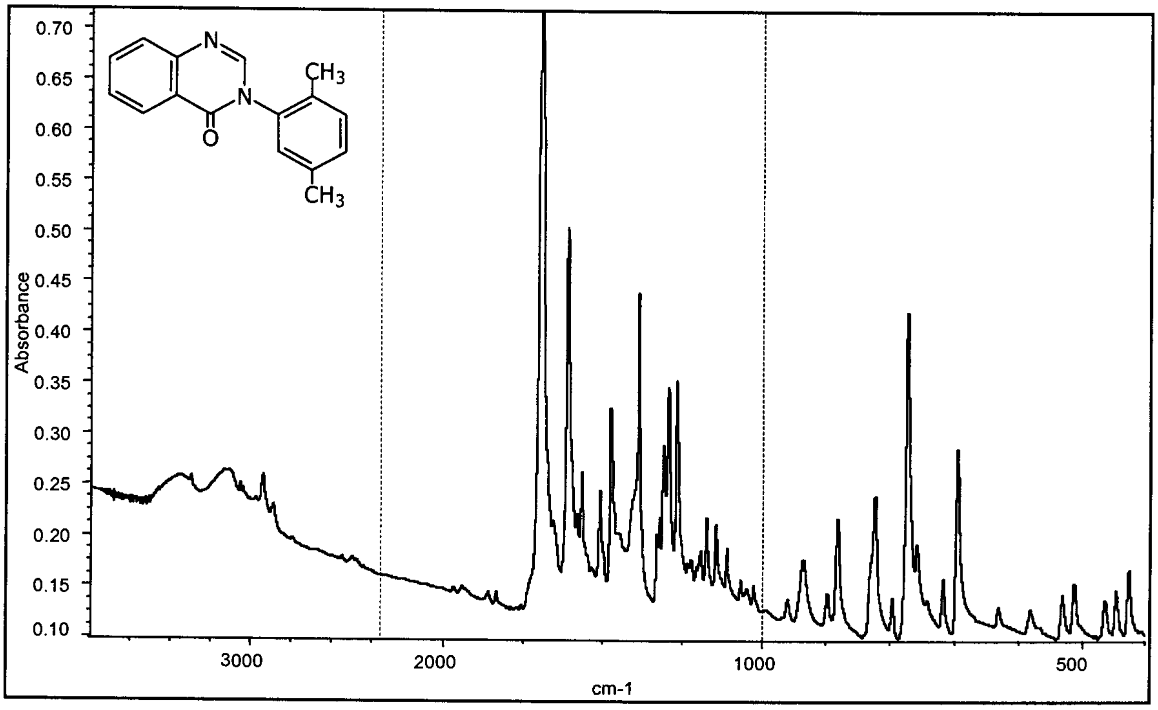


Figure 3.3.11 Condensed phase infrared spectrum of **VI**

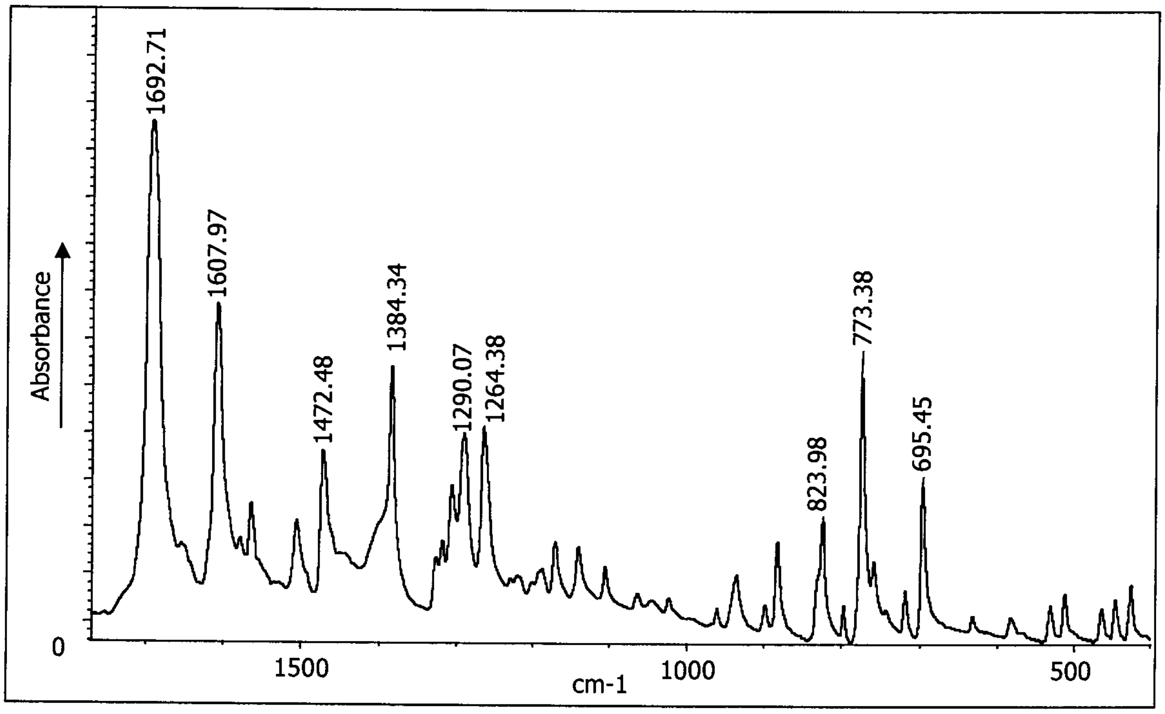


Figure 3.3.12 Expanded condensed phase infrared spectrum of **VI**

Table 3.3.7 Infrared spectral data for **VI**

Absorption (cm^{-1})	**Bond**	**Band assignment**
3080 - 3003	C - H	aromatic, stretch
2980 - 2850	C - H	- CH_3, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1693	C = O	amide, stretch
1608	C = N	imine group, stretch
1578 and 1472	C = C	aromatic, stretch
1384	C - H	- CH_3, symmetric bend
1350 - 1000	C - H	aromatic, in-plane bend
882 and 823	C - H	aromatic, out-of-plane bend
695	C = C	aromatic, out-of-plane ring bend

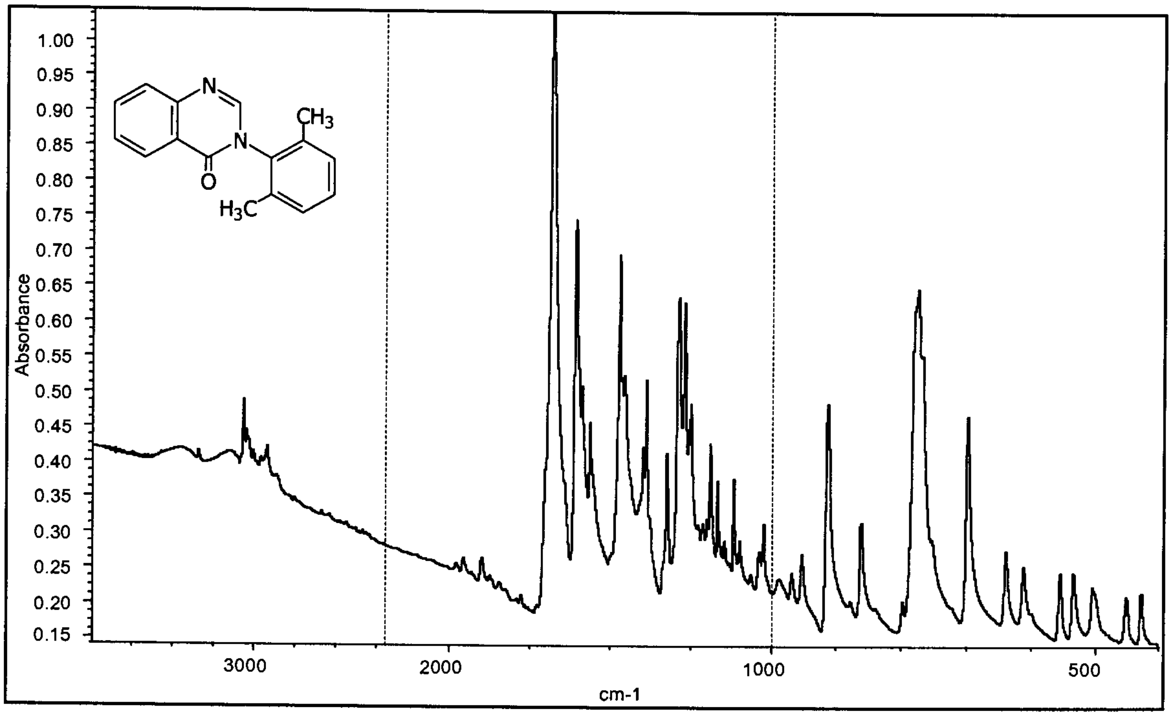


Figure 3.3.13 Condensed phase infrared spectrum of **VII**

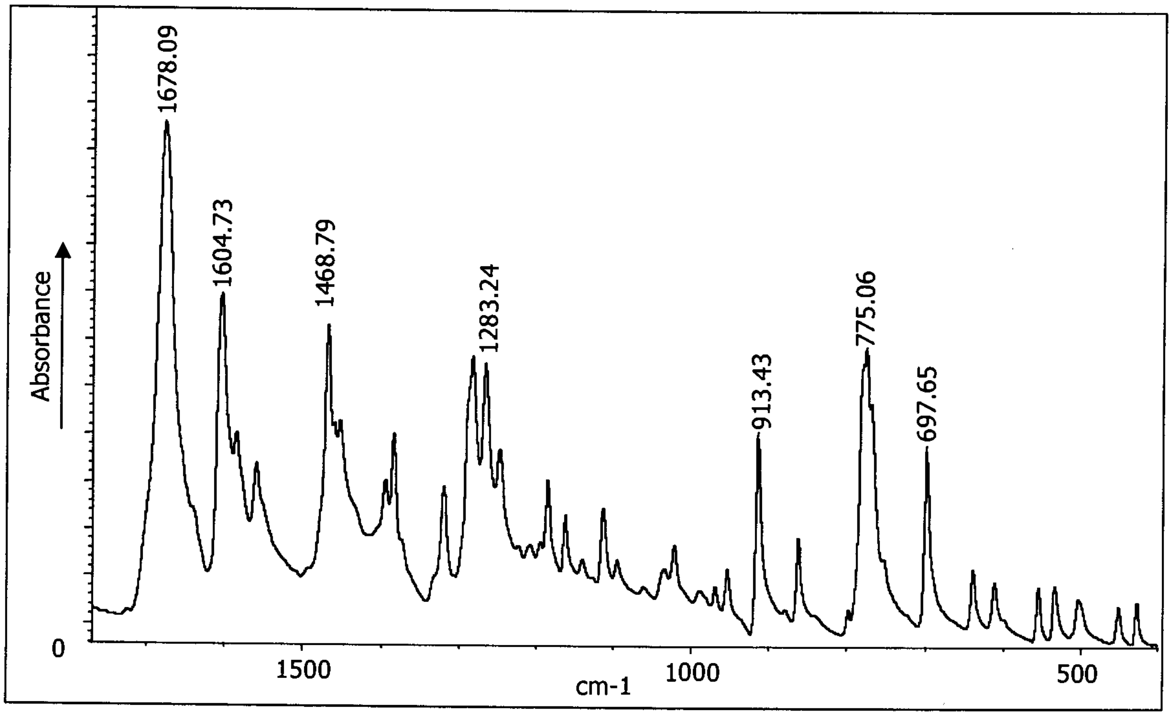


Figure 3.3.14 Expanded condensed phase infrared spectrum of **VII**

Table 4.3.8 Infrared spectral data for **VII**

Absorption (cm^{-1})	**Bond**	**Band assignment**
3080 - 3000	C - H	aromatic, stretch
2970 - 2850	C - H	- CH_3, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1678	C = O	amide, stretch
1604	C = N	imine group, stretch
1585 and 1469	C = C	aromatic, stretch
1384	C - H	- CH_3, symmetric bend
1350 - 1000	C - H	aromatic, in-plane bend
775	C - H	aromatic, out-of-plane bend
698	C = C	aromatic, out-of-plane ring bend

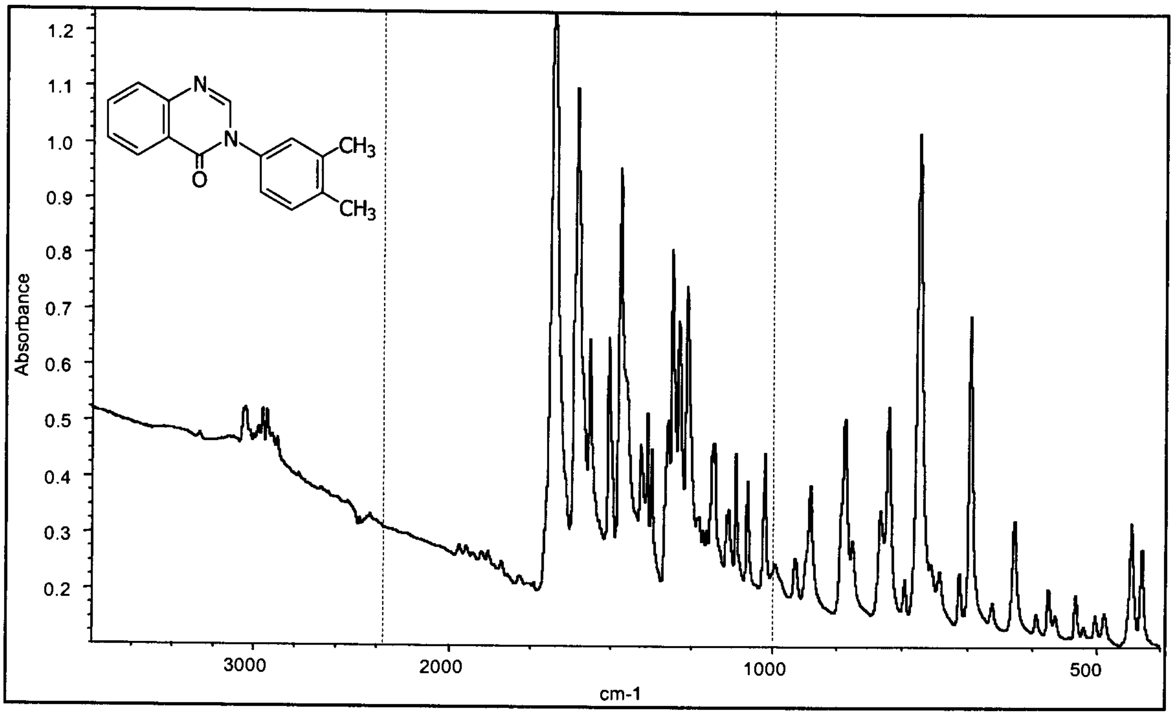


Figure 3.3.15 Condensed phase infrared spectrum of **VIII**

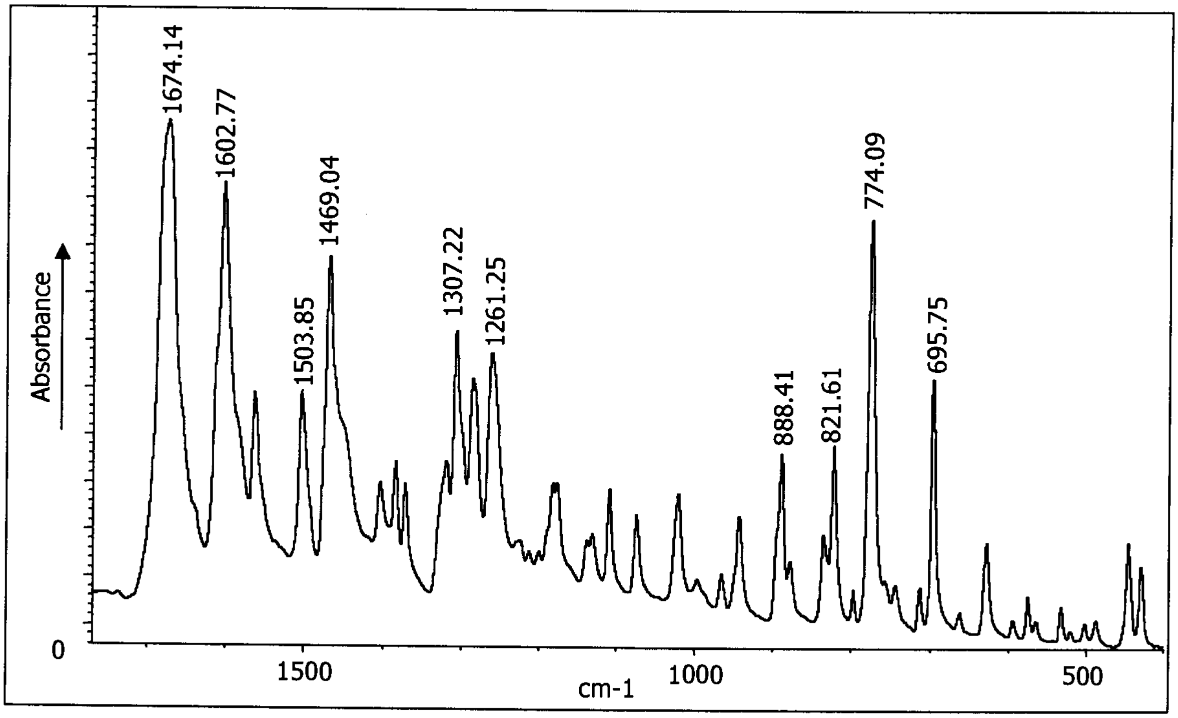


Figure 3.3.16 Expanded condensed phase infrared spectrum of **VIII**

Table 3.3.9 Infrared spectral data for **VIII**

Absorption (cm^{-1})	**Bond**	**Band assignment**
3080 - 3000	C - H	aromatic, stretch
2980 - 2850	C - H	- CH_3, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1674	C = O	amide, stretch
1603	C = N	imine group, stretch
1469	C = C	aromatic, stretch
1384	C - H	- CH_3, symmetric bend
1350 - 1000	C - H	aromatic, in-plane bend
888 and 821	C - H	aromatic, out-of-plane bend
696	C = C	aromatic, out-of-plane ring bend

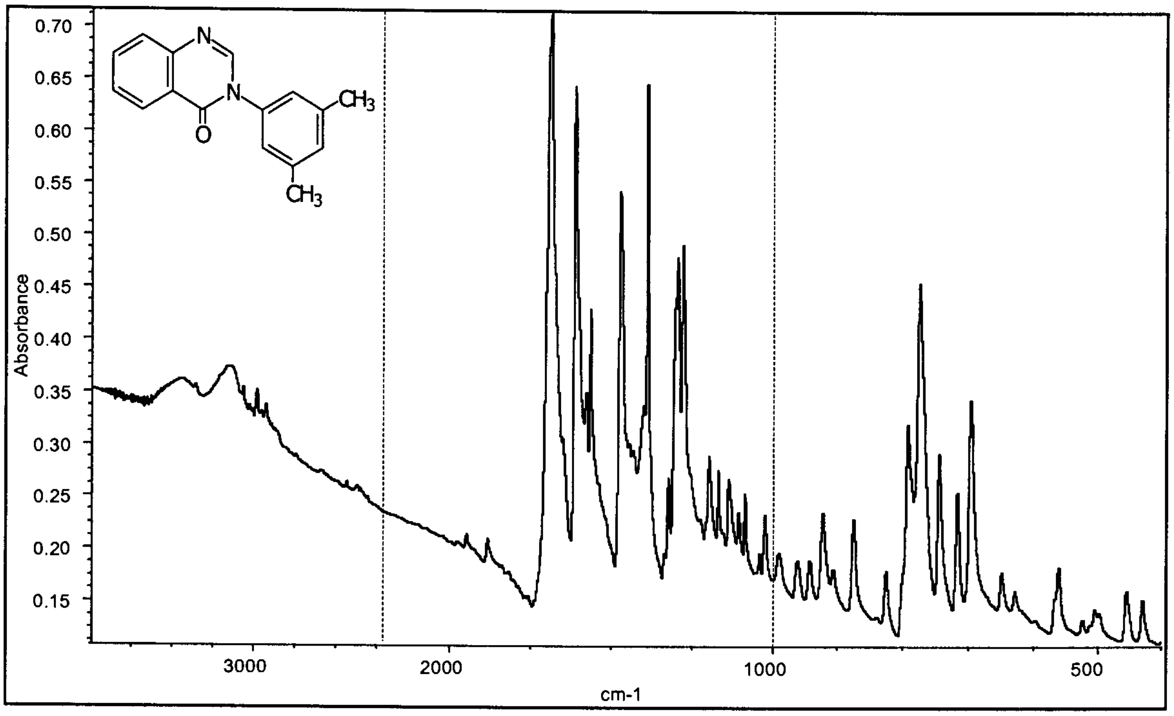


Figure 3.3.17 Condensed phase infrared spectrum of **IX**

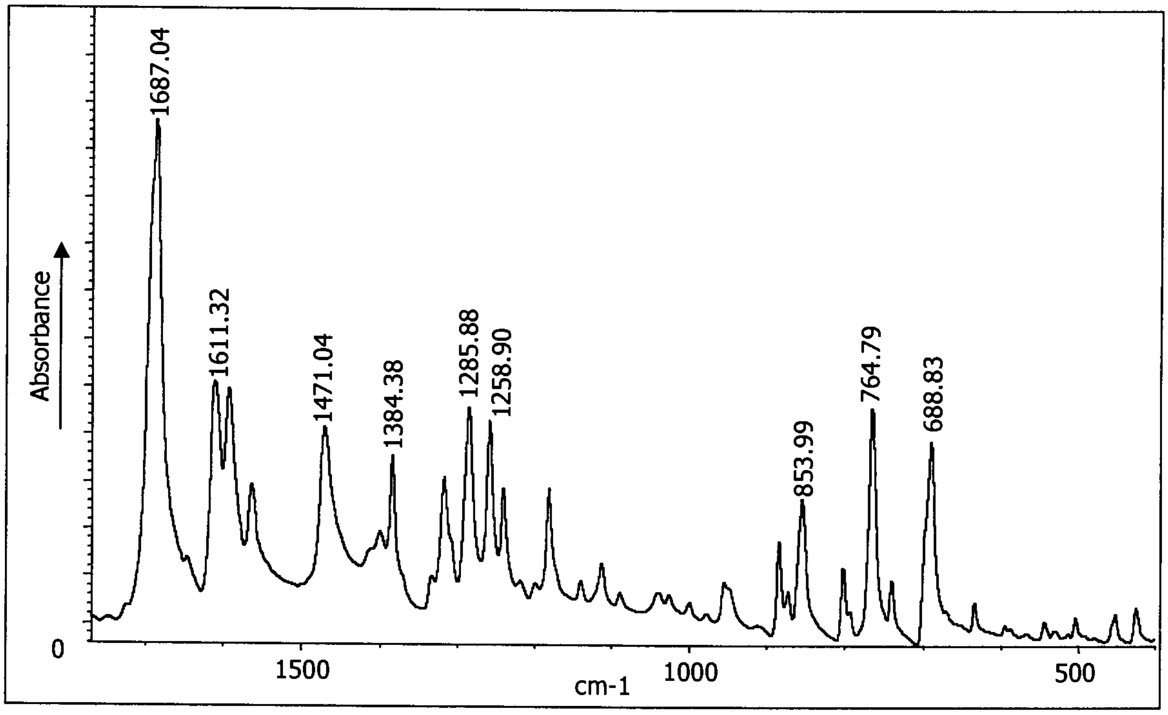


Figure 3.3.18 Expanded condensed phase infrared spectrum of **IX**

Table 4.3.10 Infrared spectral data for **IX**

Absorption (cm^{-1})	Bond	Band assignment
3080 - 3005	C - H	aromatic, stretch
2980 - 2850	C - H	- CH_3, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1687	C = O	amide, stretch
1611	C = N	imine group, stretch
1593 and 1471	C = C	aromatic, stretch
1384	C - H	- CH_3, symmetric bend
1350 - 1000	C - H	aromatic, in-plane bend
853, and 764	C - H	aromatic, out-of-plane bend
689	C = C	aromatic, out-of-plane ring bend

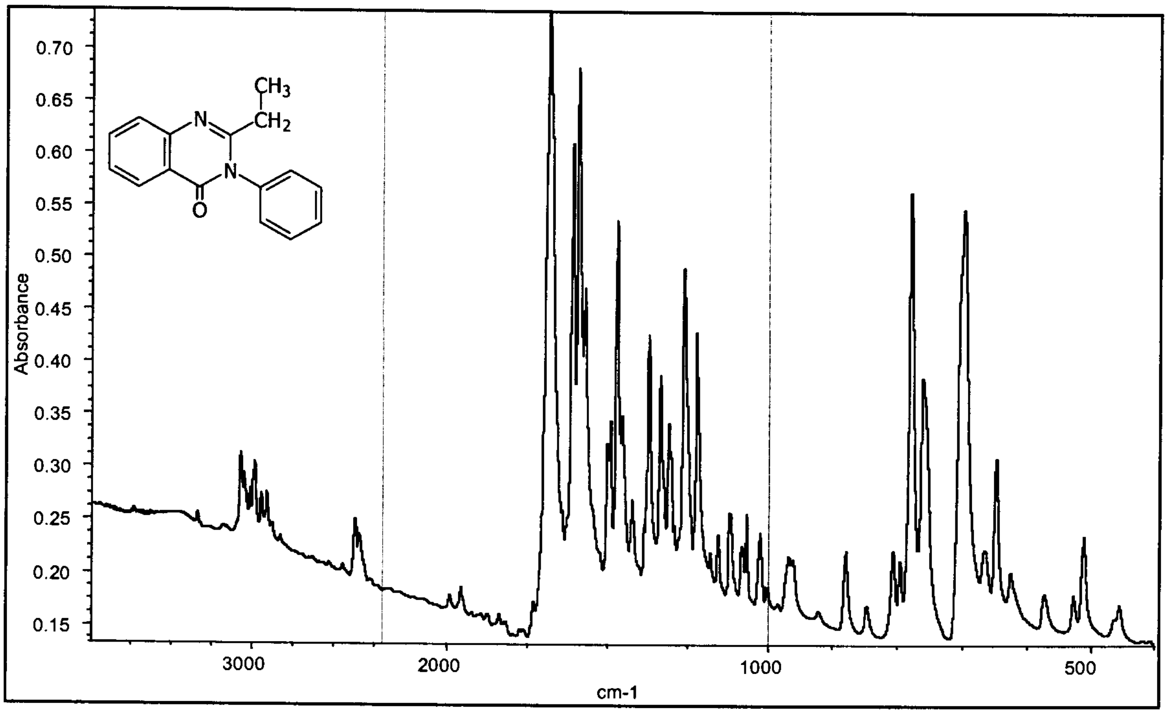


Figure 3.3.19 Condensed phase infrared spectrum of **X**

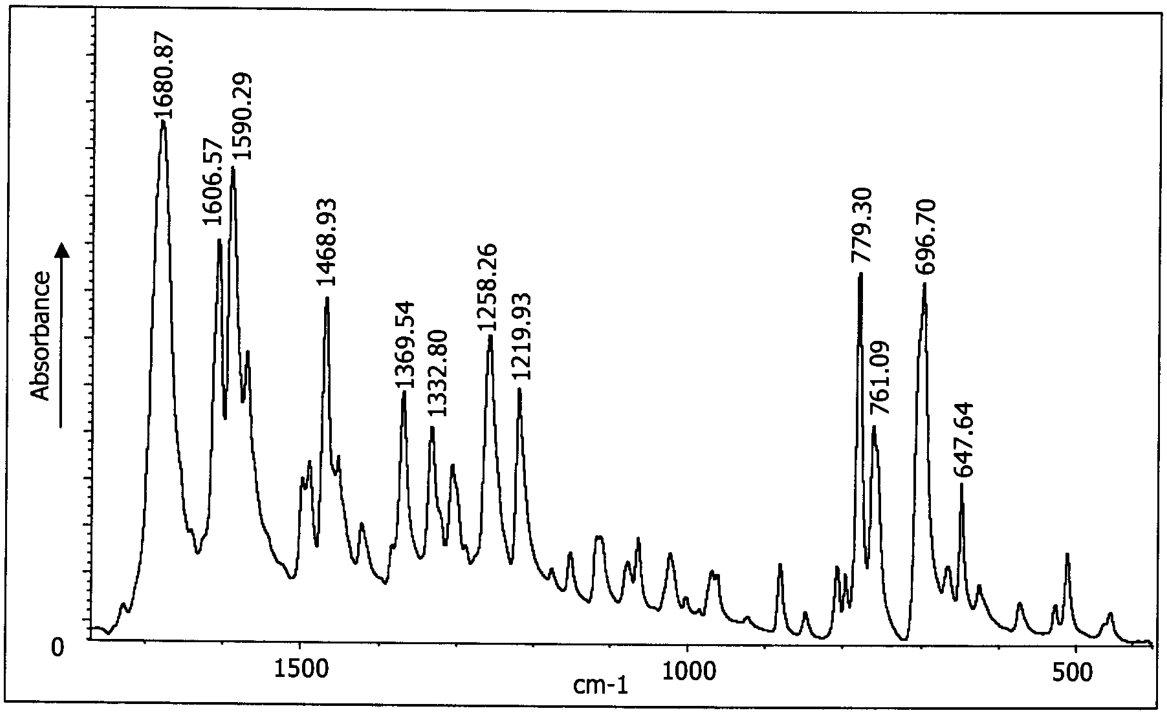


Figure 3.3.20 Expanded condensed phase infrared spectrum of **X**

Table 3.3.11 Infrared spectral data for **X**

Absorption (cm^{-1})	Bond	Band assignment
3080 - 3010	C - H	aromatic, stretch
2990 - 2820	C - H	- CH_3, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1681	C = O	amide, stretch
1607	C = N	imine group, stretch
1590 and 1468	C = C	aromatic, stretch
1453	C - H	- CH_2 -
1383 and 1370	C - H	- CH_3, symmetric bend
1350 - 1000	C - H	aromatic, in-plane bend
779 and 761	C - H	aromatic, out-of-plane bend
697	C = C	aromatic, out-of-plane ring bend

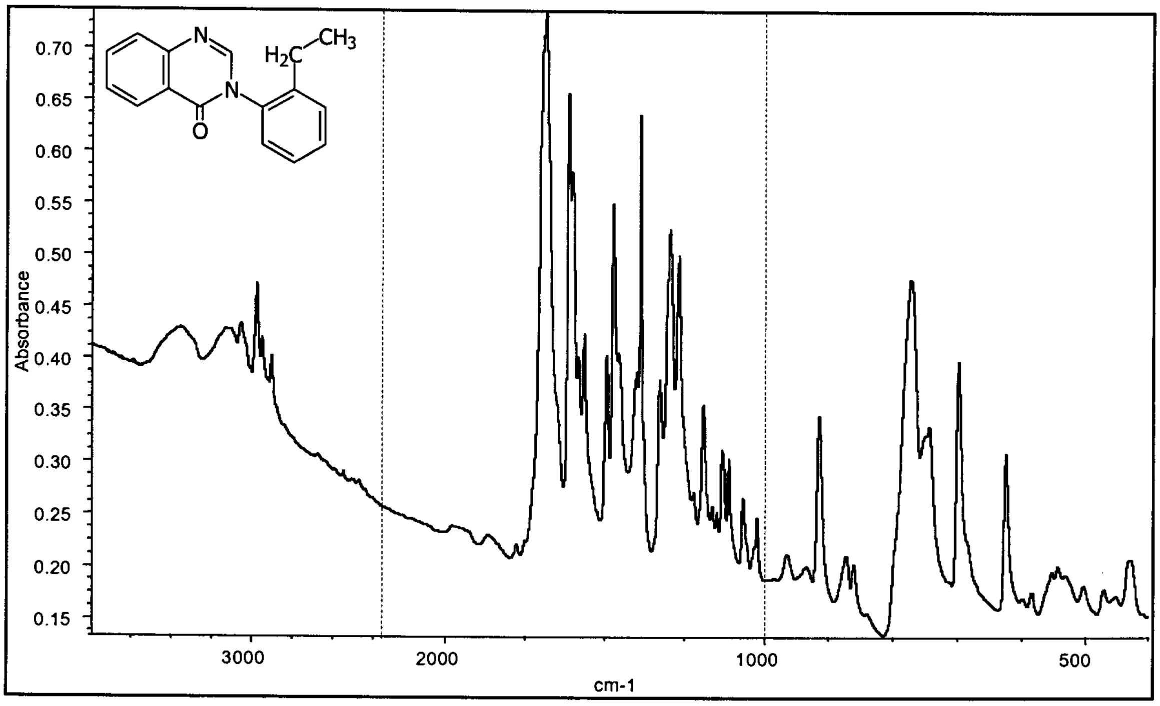


Figure 3.3.21 Condensed phase infrared spectrum of **XI**

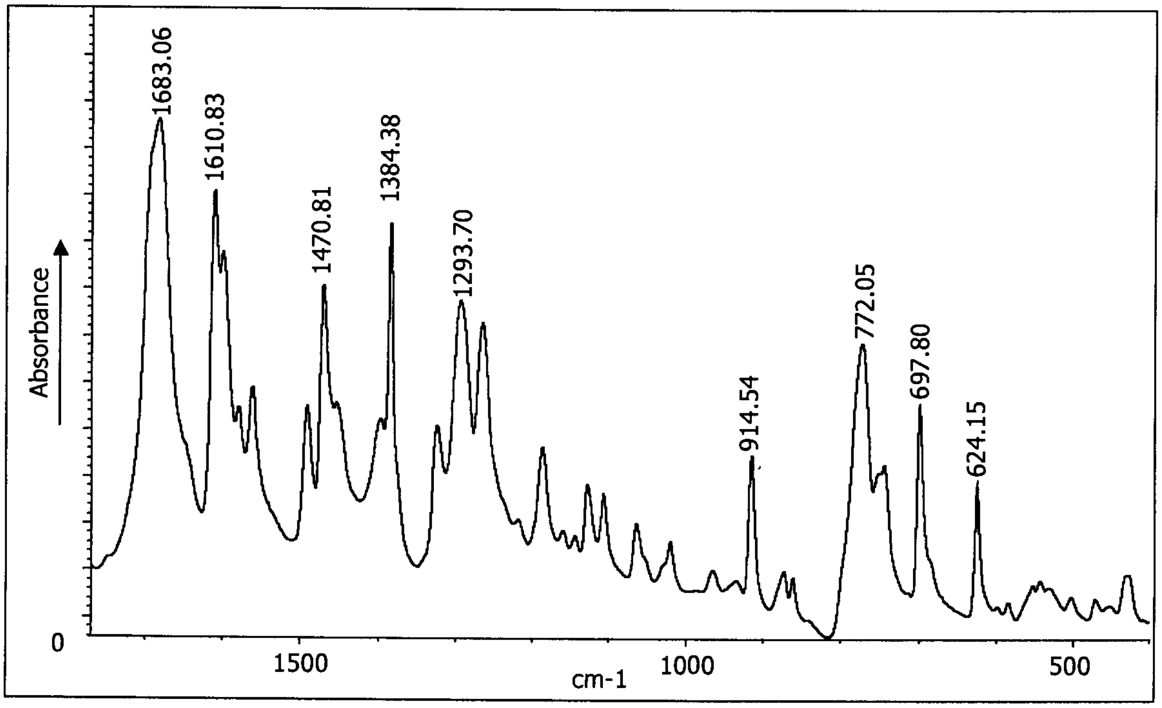


Figure 3.3.22 Expanded condensed phase infrared spectrum of **XI**

Table 3.3.12 Infrared spectral data for **XI**

Absorption (cm^{-1})	**Bond**	**Band assignment**
3080 - 3005	C - H	aromatic, stretch
2968 and 2895	C - H	- CH_3, asymmetric and symmetric stretch
2933 and 2874	C - H	- CH_2 -, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1683	C = O	amide, stretch
1610	C = N	imine group, stretch
1600 and 1471	C = C	aromatic, stretch
1454	C - H	- CH_2 -
1384	C - H	- CH_3, symmetric bend
1350 - 1000	C - H	aromatic, in-plane bend
772 and 743	C - H	aromatic, out-of-plane bend
698	C = C	aromatic, out-of-plane ring bend

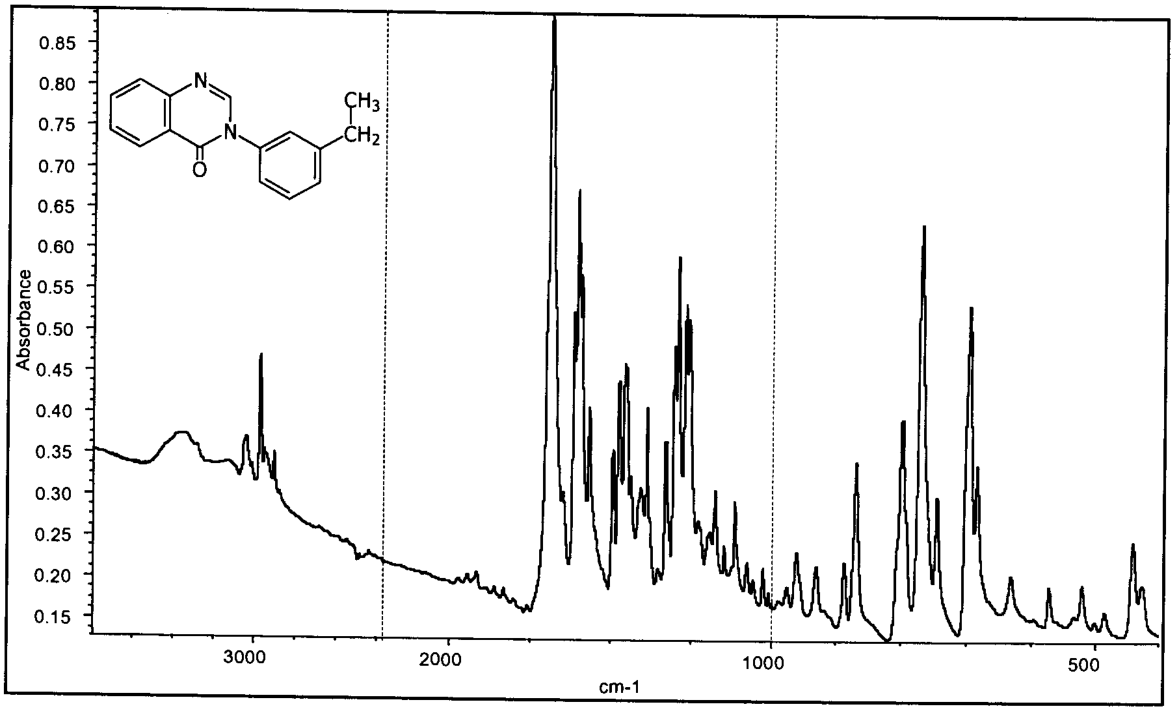


Figure 3.3.23 Condensed phase infrared spectrum of **XII**

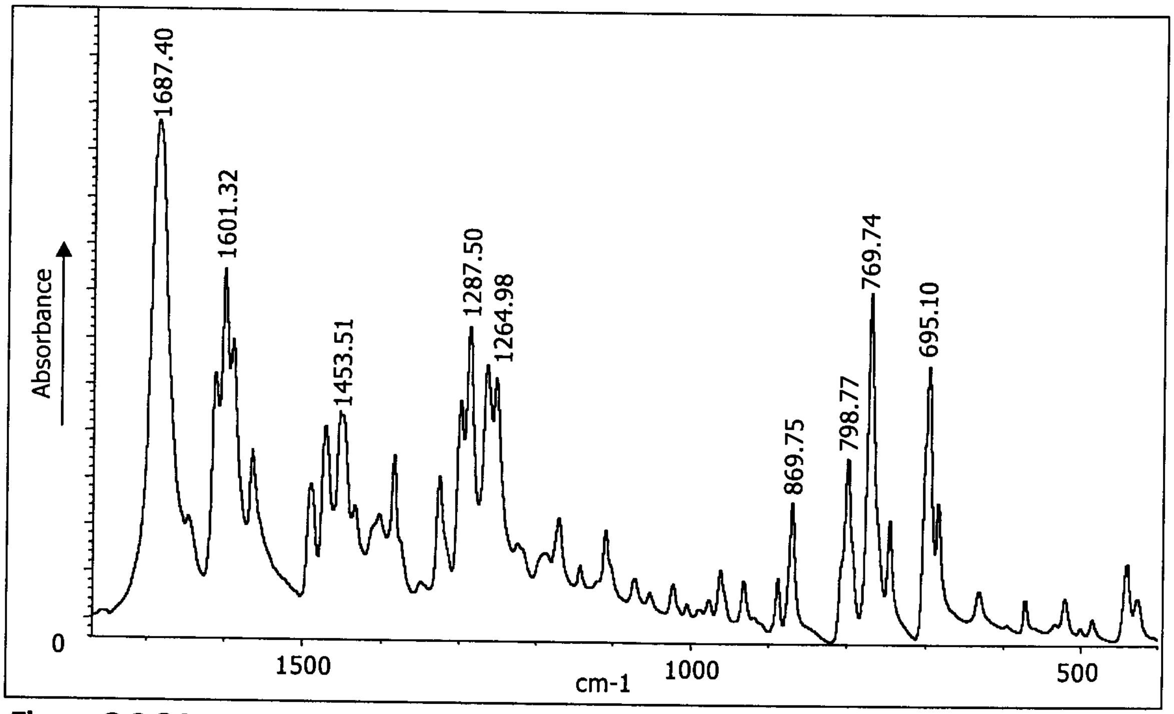


Figure 3.3.24 Expanded condensed phase infrared spectrum of **XII**

Table 3.3.13 Infrared spectral data for **XII**

Absorption (cm^{-1})	**Bond**	**Band assignment**
3080 - 3005	C - H	aromatic, stretch
2964 and 2876	C - H	- CH_3, asymmetric and symmetric stretch
2935 and 2843	C - H	- CH_2 -, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1687	C = O	amide, stretch
1614	C = N	imine group, stretch
1601 and 1472	C = C	aromatic, stretch
1454	C - H	- CH_2 -
1384	C - H	- CH_3, symmetric bend
1350 - 1000	C - H	aromatic, in-plane bend
870, 799, and 770	C - H	aromatic, out-of-plane bend
695	C = C	aromatic, out-of-plane ring bend

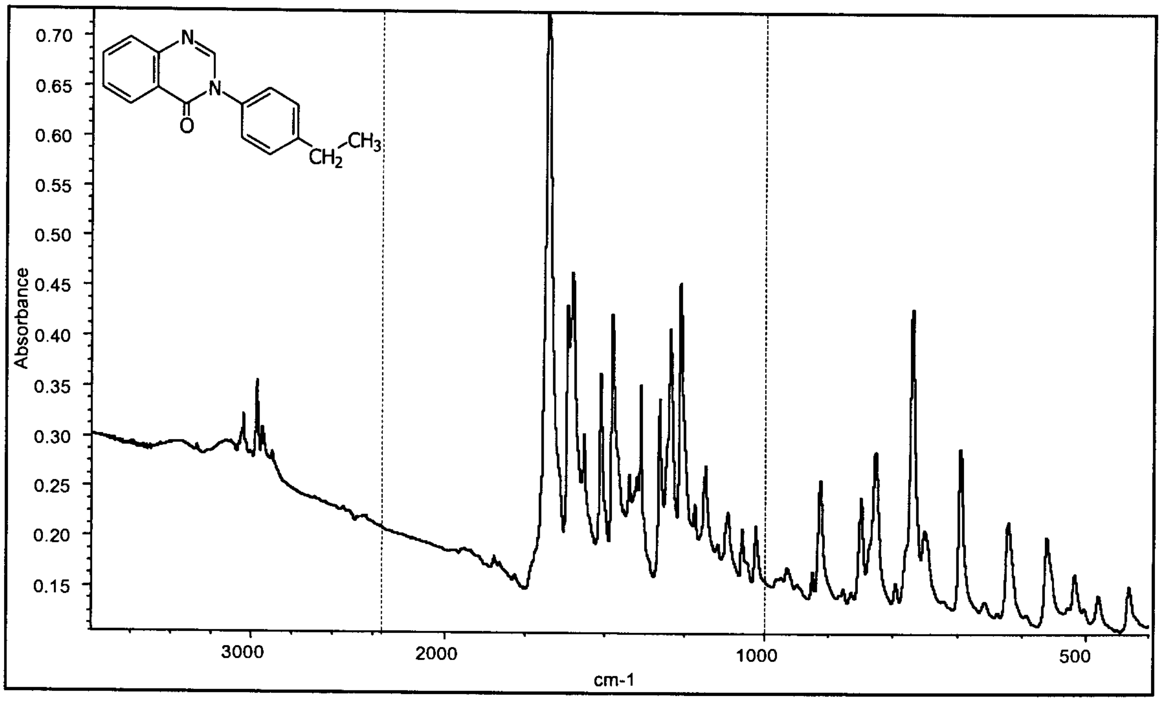


Figure 3.3.25 Condensed phase infrared spectrum of **XIII**

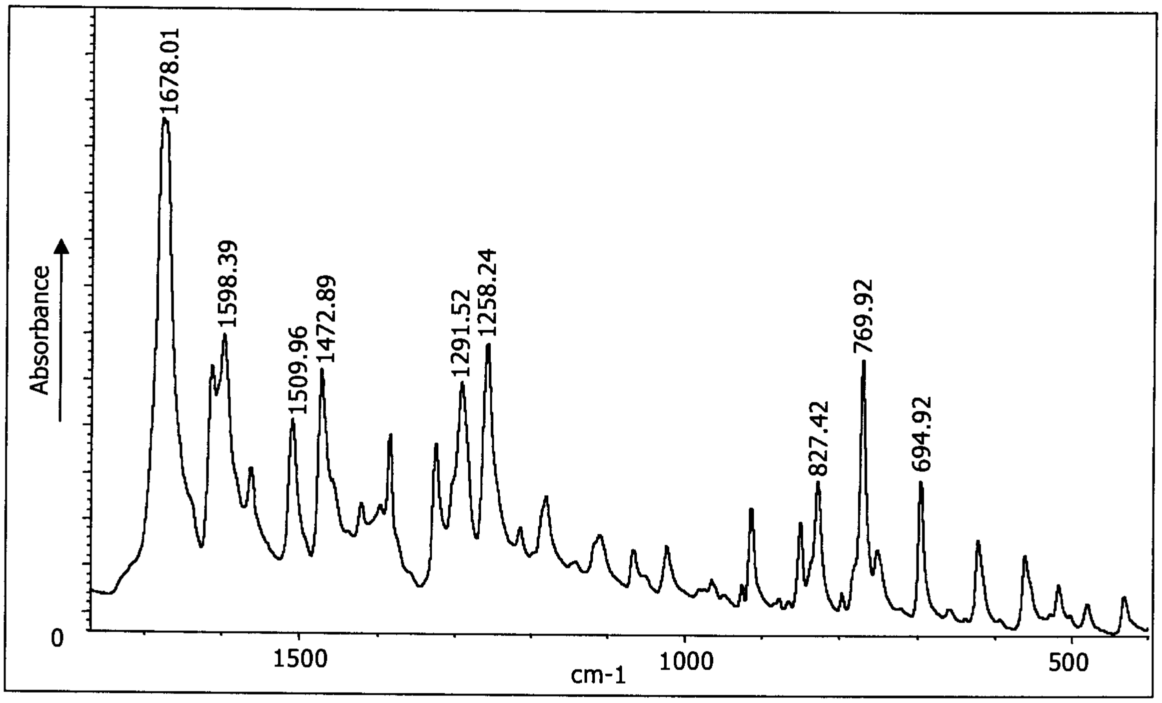


Figure 3.3.26 Expanded condensed phase infrared spectrum of **XIII**

Table 3.3.14 Infrared spectral data for **XIII**

Absorption (cm^{-1})	**Bond**	**Band assignment**
3080 - 3005	C - H	aromatic, stretch
2963 and 2888	C - H	- CH_3, asymmetric and symmetric stretch
2929 and 2869	C - H	- CH_2 -, asymmetric and symmetric stretch
2000 - 1750	Aromatic ring	overtone and combination
1687	C = O	amide, stretch
1614	C = N	imine group, stretch
1598 and 1473	C = C	aromatic, stretch
1452 shoulder	C - H	- CH_2 -
1384	C - H	- CH_3, symmetric bend
1350 - 1000	C - H	aromatic, in-plane bend
850, 827, and 770	C - H	aromatic, out-of-plane bend
695	C = C	aromatic, out-of-plane ring bend

Discussion

All target compounds absorbed strongly in the mid-infrared region (4000 - 400 cm^{-1}), displaying complex absorption patterns. With the exception of the weak absorption in the 2000 - 1750 cm^{-1} region, no significant absorption bands were observed in the 2800 - 1750 cm^{-1} region.

The spectra of all compounds studied showed overlapping C - H stretching bands in the region 3100 - 3000 cm^{-1}. These absorptions arise from the aromaticity of the fundamental quinazolinone ring system, as well as the aryl substituent in the 3 - position of each of the compounds. The aromatic C - H stretch absorption have no diagnostic value in differentiating the target compounds.

The spectra of compounds **I** - **IX** also showed overlapping bands from just below 3000 cm^{-1} to about 2850 cm^{-1}, arising from the aliphatic asymmetric and symmetric C - H stretches of the two methyl groups characteristic of these compounds. Compounds **IX** - **XIII**, having only one methyl group each, exhibited two discrete aliphatic methyl C - H stretch absorptions at approximately 2960 cm^{-1} and 2890 cm^{-1}. In addition, these compounds also had asymmetric and symmetric methylene C - H stretch absorptions at approximately 2930 cm^{-1} and 2870 -2840 cm^{-1} arising from the methylene group in either the ethyl group in the 2 - position, or the ethylaryl substituent in the 3 - position. The aliphatic C - H stretch absorptions allow for the effective differentiation between those isomers containing two methyl groups (**I** - **IX**), and those containing an ethyl group (**X** - **XIII**).

The C = O stretching vibration of the quinazolinone ring system gave rise to a strong absorption band near 1685 cm^{-1}. The C = O stretch absorptions for each of compounds **I** - **XIII** are given in Table 3.3.15. The observed absorption of the C = O bond at frequencies below 1715 cm^{-1} is characteristic of amides, and results due to conjugation of the unshared electrons of the nitrogen atom and the carbonyl group, thus increasing the single bond character of the C = O bond.[59] Anwar *et al*[53] reported on 2,3-disubstituted -4(3*H*)-quinazolinone C = O stretching absorptions ranging from 1680 - 1665 cm^{-1}. Given that the C = O bond of compounds **I**,**V**, **VII**, and **VIII** all absorbs within a range of 5 cm^{-1}, it is clear that the carbonyl stretch absorption is of limited value in differentiating **I** from these compounds. The C = O stretch absorptions for **IX**, **XII**, and **XIII** are all observed at 1687 cm^{-1}, further highlighting the limited diagnostic value thereof.

Table 3.3.15 Condensed phase infrared data for **I** - **XIII** (cm^{-1})

Compound	C = O	C = N	C = C	Aromatic out-of-plane bends
I	1676	1608	1598 and 1468	778; 760; 721; 703
II	1689	1601	1583 and 1470	786; 778; 703; 700
III	1686	1608	1591 and 1469	818; 777; 699
IV	1684	1608	1574 and 1469	792; 774; 744; 716; 696
V	1678	1607	1597 and 1469	823; 785; 755; 699
VI	1693	1608	1578 and 1472	882; 823; 773; 695
VII	1678	1604	1585 and 1469	913; 861; 775; 698
VIII	1674	1603	1469	888; 821; 774; 696
IX	1687	1611	1593 and 1471	883; 853; 764; 689
X	1681	1607	1590 and 1468	779; 761; 697
XI	1683	1610	1600 and 1471	772; 743; 698
XII	1687	1614	1601 and 1472	870; 799; 770; 695
XIII	1687	1614	1598 and 1473	850; 827; 770; 695

All compounds studied showed a strong C = N stretch absorption near 1605 cm^{-1}. Absorption frequencies are given in Table 3.3.15. It was reported by Anwar *et al*[53] that the position of the C = N stretch absorption of 2,3 - disubstituted - 4(3*H*)-quinazolinones are directly influenced by the substituent in the 2 - position. It is significant that in the current study **I**, that has a methyl substituent in the 2 - position, and e.g. **IV** or **VI**, with no substituent in the 2 - position, all show a strong C = N absorption at 1608 cm^{-1}. Also of interest is the observed shift in the C = N absorption between compound **I** and **II**. Both are 2-methyl substituted, but differ with regard to the aromatic substitution on the 3-aryl substituent.

In general, the observed C = N stretch frequencies are significantly lower than those reported in literature, with literature values ranging from 1625 to 1655 cm^{-1}. [12,29,53,54] Given the narrow range of absorption, the C = N stretch absorptions observed in this study are of limited diagnostic value, but considered characteristic of this type of compounds, i.e. 4(3*H*)-quinazolinones.

Aromatic skeletal C = C stretch absorption were observed in the spectra of all compounds at approximately 1600 - 1570 cm^{-1} and 1470 cm^{-1}. The observed similarity once again render these absorption of limited diagnostic value in differentiating between the compounds studied.

All spectra showed a strong absorption near 1380, assigned to the symmetric methyl C - H bend vibration of the methyl group(s) present in all compounds.

Absorptions in the 1450 to 1000 cm^{-1} region were assigned to various skeletal C - N stretch, and C - H bending vibrations. No rigorous assignments were made, but the absorption pattern in this region, though similar, is sufficiently unique to allow for the differentiation of **I** - **XIII**.

As expected the spectra of all compounds exhibited characteristic absorption patterns in the 900 - 690 cm^{-1} range. Significant absorptions in this area are listed in Table 3.3.15. These absorptions arise from aromatic C - H out-of-plane bending vibrations (900 - 720 cm^{-1}), and C = C aromatic skeletal deformations, and are characteristic for various aromatic substitution patterns.[59] The observed spectra all exhibited fairly characteristic absorption patterns in this region, although differentiation between some compounds of which the spectra is fairly similar, e.g. **VI**, **VIII**, and **IX** would required very close inspection. Given the complex structures of the compounds studied, the observed absorptions in this region did not follow the general rules for aromatic substitution.[59]

Conclusion

In general, the observed infrared spectral data of all compounds studied were very similar, but still sufficiently unique to allow for the specific differentiation of **I** - **XIII**. Differentiation should be based on a close and in-depth comparison of spectra over the complete absorption region. The most noticeable differences occur in the 1500 - 690 cm^{-1} range.

A prerequisite to the differentiation **I** - **XIII** based on infrared spectral data, is the availability of sufficient quantities of the pure compounds of interest. This may be a severe limitation where the forensic identification of trace quantities of **I** is required.

CHAPTER 3: Analytical Methodology

Part 4: Mass Spectrometry

Introduction

Electron impact (EI) ionization mass spectrometry is the analytical technique of choice used by the SAPS FSL for the unequivocal identification **I**. Mass spectra are collected using integrated gas chromatography - mass spectrometry (GC - MS), thus combining the high selectivity of capillary gas chromatography with the specificity of the mass spectrometer. Given the inherent value attached by the SAPS FSL to the use of mass spectrometry to identify **I**, the ability of the technique to differentiate **I** from the **II** to **XIII** thus needed to be evaluated.

The EI mass spectrum of **I** is well known and widely reported on.[44,46,52] The forensic use of GC - MS to identify **I** has been reported as early as 1974.[60] Mass spectrometric differentiation between *inter alia* **I**, **II**, and **III**, as well as a range of halogenated analogs have been reported.[10] The collected mass spectra are given. Differentiation between positional isomers were based on the relative ion abundances observed in the normalized spectra. The authors concluded that the use of GC - MS proved to be the most efficient way of differentiating the studied compounds. The relative intensity ratios utilized for differentiation are given in Table 3.4.1.

Table 3.4.1 Relative EI mass spectral ion abundances used for differentiating **I** - **III**[10]

Compound	**m/z 132**	**:143**	**m/z 142**	**:143**	**:144**	**m/z 234**	**:235**	**:236**
I	3	: 2	0	: 4	: 1	1	: 4	: 2
II	2	: 3	1	: 40	: 8	1	: 14	: 3
III	1	: 3	1	: 38	: 6	1	: 15	: 3

In response Lui *et al*[61] presented what they called a *"clear and less complicated correlation between the intensities of properly selected ions and the position of substitution"*. The data given in Table 3.4.2 were presented. The authors noted that intensity correlations of the [M - R']$^+$ ion, as defined in Table 3.4.2, cannot readily be established because of the two methyl groups in the structure of **I** both contributing to the formation of this fragment.

Table 3.4.2 Relative EI mass spectral ion abundances used for differentiating **I** - **III**[61]

Compound	**%[M - R']$^+$**	**Intensity ratios -**				
	(R' = R_2/R $_3$/R_4 = CH_3) **(R 1 = CH_3)**	**[M - R']$^+$:**	**[M - 15]$^+$:**	**M+**	**: [R'+ 76]$^+$:**	**143**
I	100	1	:1	: 0.86	: 1.0	: 0.33
II	93	1	:1	: 0.70	: 1.1	: 0.03
III	81	1	:1	: 0.96	: 1.2	: 0.97

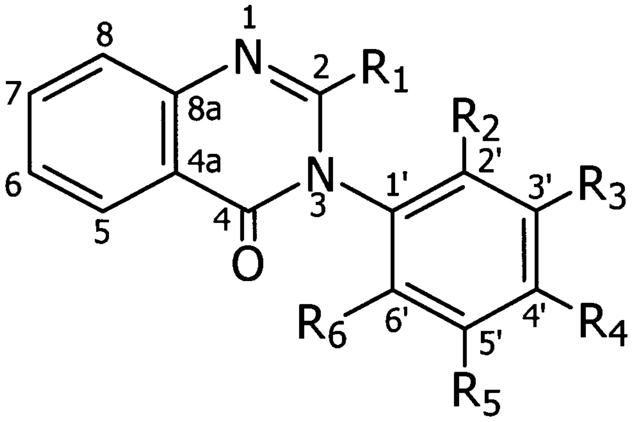


Figure 3.4.1 General structure of 2-alkyl-3-aryl-4(3*H*)-quinazolinones

The EI mass spectrometric differentiation of **I**, mecloqualone, and nitromethaqualone using sample introduction *via* direct insertion has been described.[48] Mass spectra and a fragmentation pattern for *inter alia* **I** are given - see Figure 3.4.2.

The EI mass spectrum of **X** has been reported [56, 57], and methaqualone was differentiated from nitromethaqualone and positional isomers of nitromethaqualone.[50] Difficulty was experienced to differentiate between the various positional isomers of nitromethaqualone. An analog (2-methyl-3-(2,4-dimethyl)-4(3*H*)-quinazolinone) of **I** was differentiated from **I** using *inter alia* EI mass spectrometry.[62]

The electron impact (EI) ionization mass spectra of substituted 4(3*H*)-quinazolinones have widely been reported.[63] Studies of its simplest forms were reported as early as 1967.[64] Subsequently more than twenty papers reporting on electron-impact ionization studies of substituted 4(3*H*)-quinazolinones were published, including three review papers.[63]

Daenens *et al*[48] reported simple fragmentation pathways for **I** accounting for the formation of mass fragments *m/z* = 235, *m/z* = 132, and *m/z* = 91 as outlined in Figure 3.4.2. No mechanisms were provided, but expulsion of the 2-methyl (R_1) was favoured.

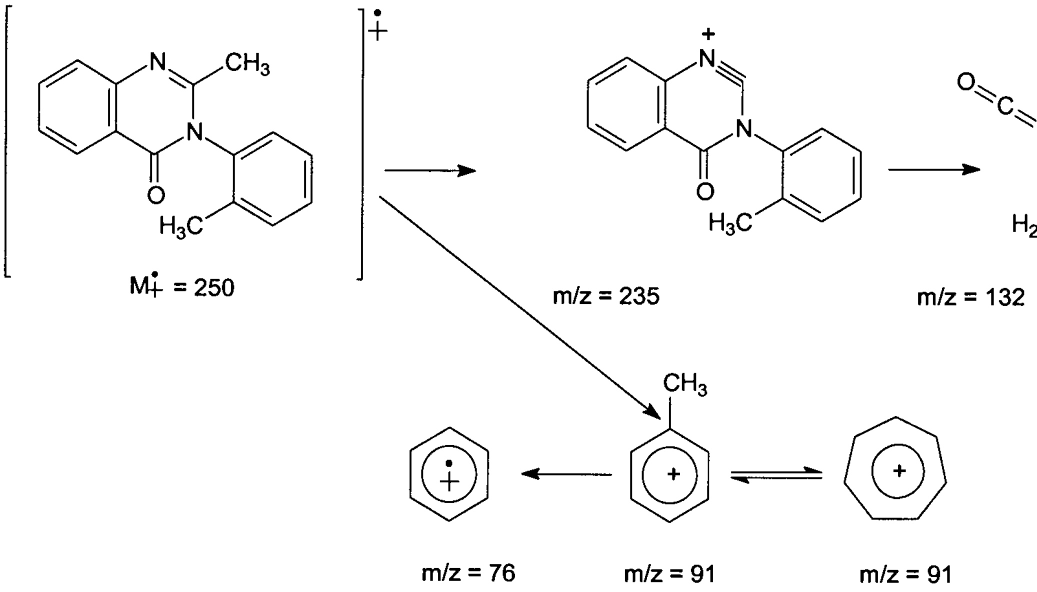


Figure 3.4.2 Fragmentation pathways for **I** reported by Daenens *et al.* [48]

The EI mass spectra of nine 2,3-disubstituted-4(3*H*)-quinazolinones, *inter alia* **I** and **III** have been discussed.[65] The introduction of an aryl substituent at the 3 - position was reported to increase the stability of the $[M\text{-}R_1]^+$ ion. In the mass spectrum of **III** the molecular ion appeared as the base peak, whereas the spectrum for **I** had a base peak of $[M\text{-}CH_3]^+$ (*m/z* = 235). Fragmentation pathways for **III** and 2-methyl-3-phenyl-4(3*H*)-quinazolinone were given as represented in Figure 3.4.3.

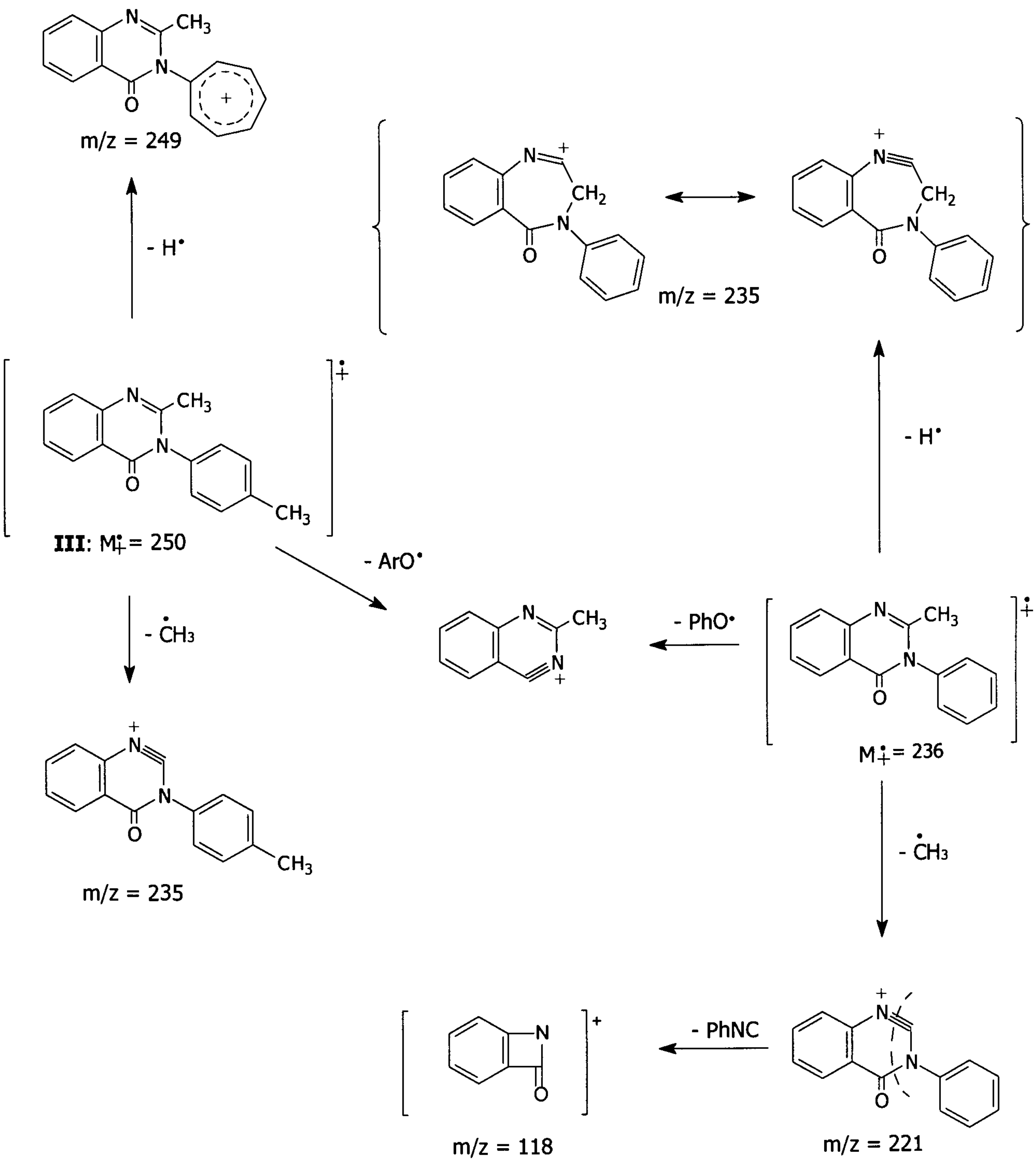


Figure 3.4.3 EI induced fragmentation pathways of 2,3 disubstituted 4(3*H*)-quinazolinones reported by Kirmani *et al*[65]

The electron impact ionization mass spectra of 2,3-disubtituted-4(3*H*)-quinazolinones are strongly characterised by rearrangement processes induced by the presence of the substituents in the 2- and 3- positions.[63]

Ramana *et al* [66] reported on a study of dual *ortho* interaction in 2-substitued-3(2-methylphenyl)-4(3*H*)-quinazolinones under EI conditions. The dual ortho interaction involves the carbonyl oxygen group and the methyl group in the *ortho* position of the 3-aryl moiety, resulting in the competitive loss of an **•OH** radical and the **2'/6'- methyl** group (R_2/R_6) from the molecular ion - see Figure 3.4.4. Ortho interaction in the absence of a **2'/6'- methyl** group (R_2/R_6) leads to the loss of a **•H** radical.

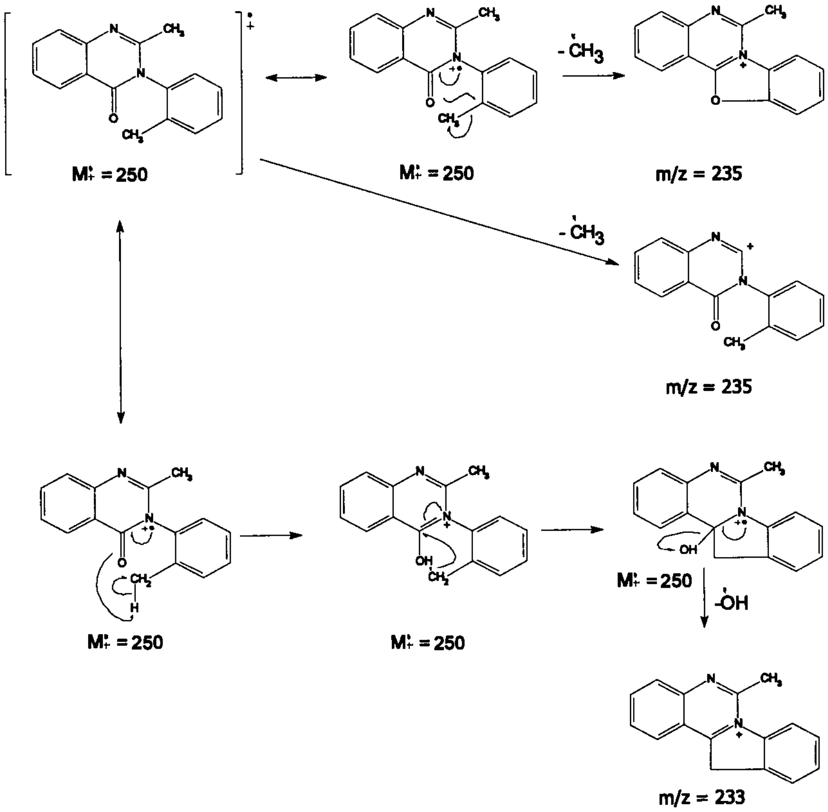


Figure 3.4.4 EI induced dual *ortho* interaction proposed for **I**[66]

The formation of the $[M - OH]^+$ ion for **I** along a similar mechanism was earlier reported by Bogentoft *et al.*[67] Analogously, the formation of the $[M - CH_3]$+ ion due to the ejection of the *ortho* substituent was first reported by Preuss *et al*[68], and subsequently by other workers.[63]

Formation of the $[M-CH_3]^+$ ion due to the expulsion of the 2-methyl group from **I** was also postulated[66], and the competitive loss of the 2- and 2'-methyl groups were quantitatively determined.[67] According to evidence presented by Ramana *et al*[66], expulsion of 2'-methyl group is the preferred fragmentation pathway for **I**.

The elimination of the ArO-radical from either 3-aryl, or 3-heteroaryl-4(3*H*)-quinazolinones have been reported in literature, and several possible mechanism have been suggested.[63] Ramana *et al*[66] suggested a mechanism as outlined in Figure 3.4.5, while Agozzino *et al*[63] argued more convincingly for a mechanism involving a retro-Chichibabin rearrangement as outlined in Figure 3.4.6. The resulting structure ***b***, or ***b'***, or an equilibrium between these two structures have been hypothesized, with unpublished work by the authors indicating that **b'** is thermodynamically more stable.

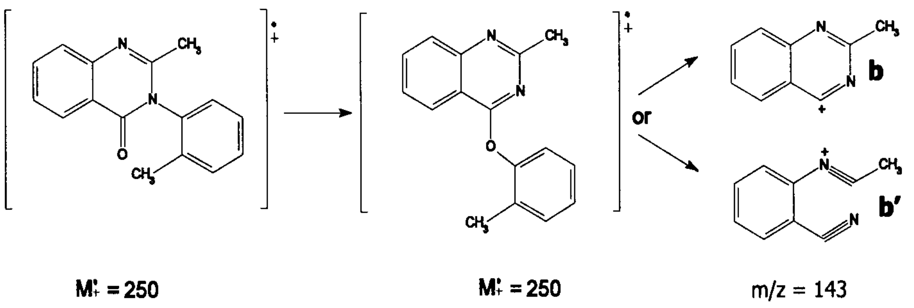


Figure 3.4.6 EI induced ArO• loss from **I** via retro - Chichibabin rearrangement[63]

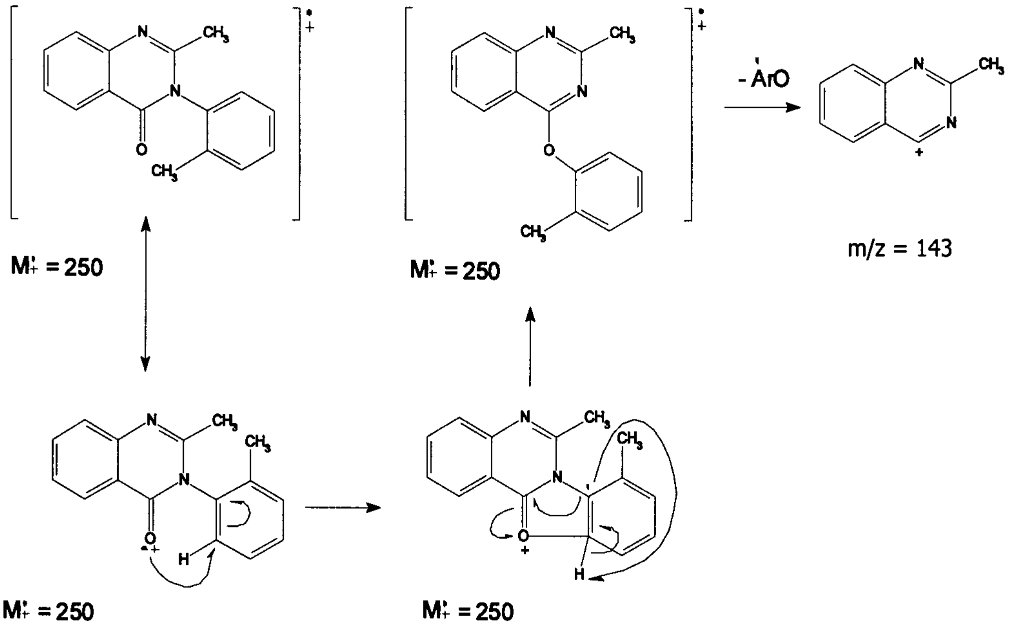


Figure 3.4.5 EI induced loss of the ArO•-radical from **I** [66]

Experimental

Experimental conditions as described in **CHAPTER 3 Part 1 Experimental Gas-liquid Chromatography** were utilized. Mass spectra were collected in positive ion electron impact mode at an ionization potential of 70 eV.

Results

The EI induced mass spectra collected for compounds **I** - **XIII** are given in Figures 3.4.7 - 3.4.19. Selected mass spectral data are given in Table 3.4.3

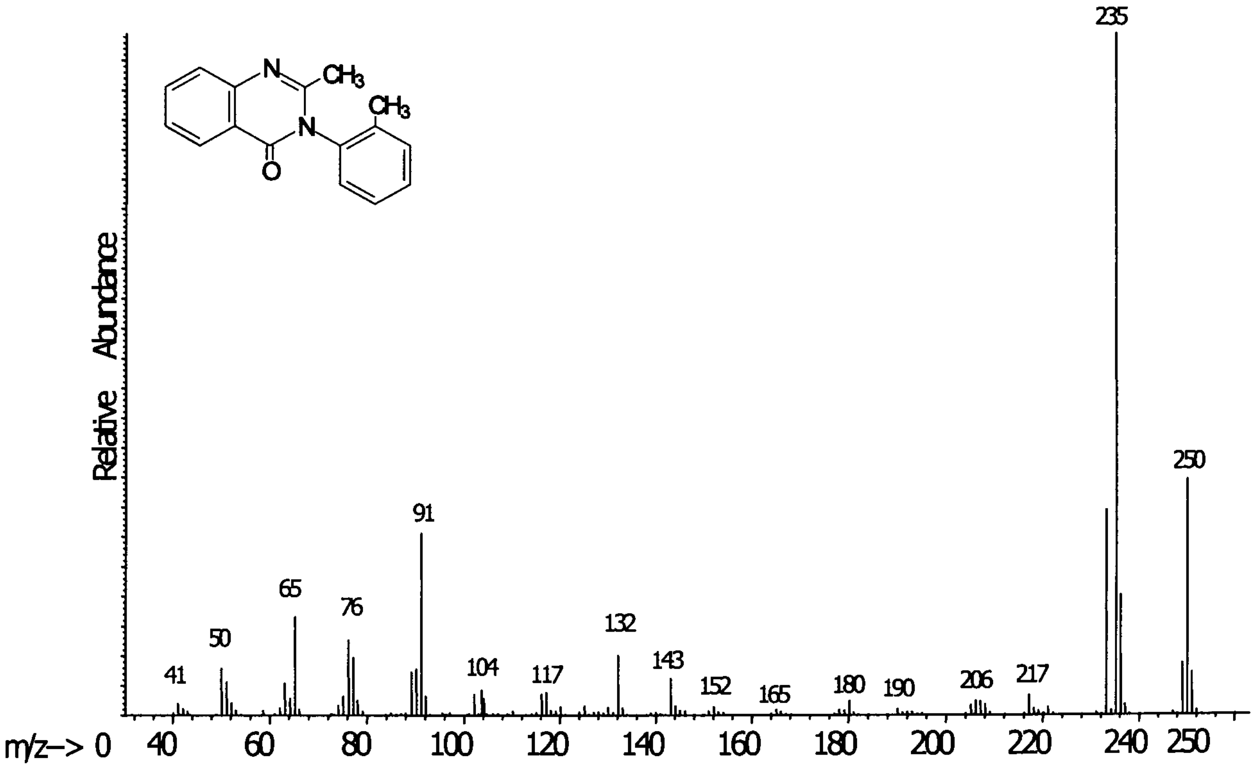


Figure 3.4.7 EI induced (70 eV) mass spectrum of **I**

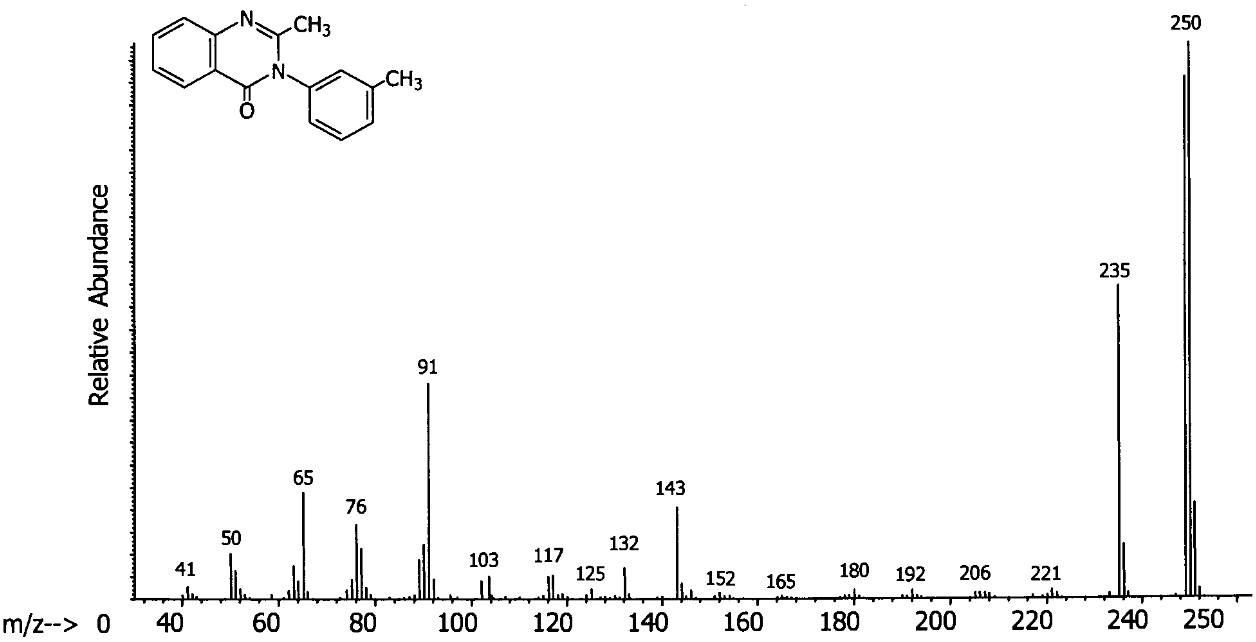


Figure 3.4.8 EI induced (70 eV) mass spectrum of **II**

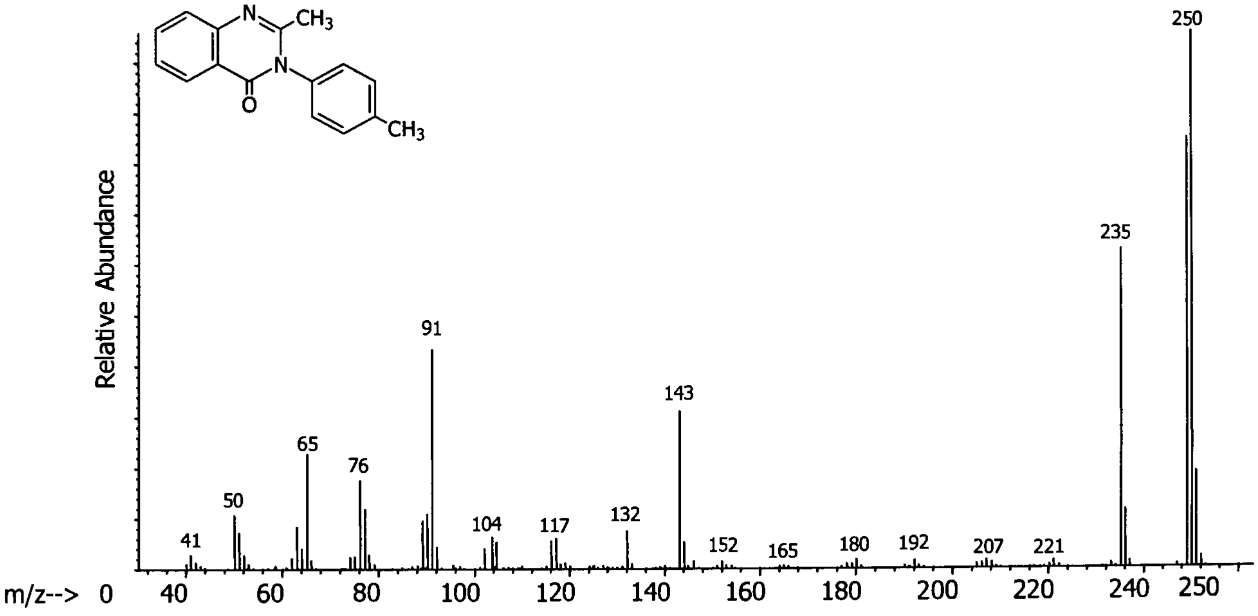


Figure 3.4.9 EI induced (70 eV) mass spectrum of **III**

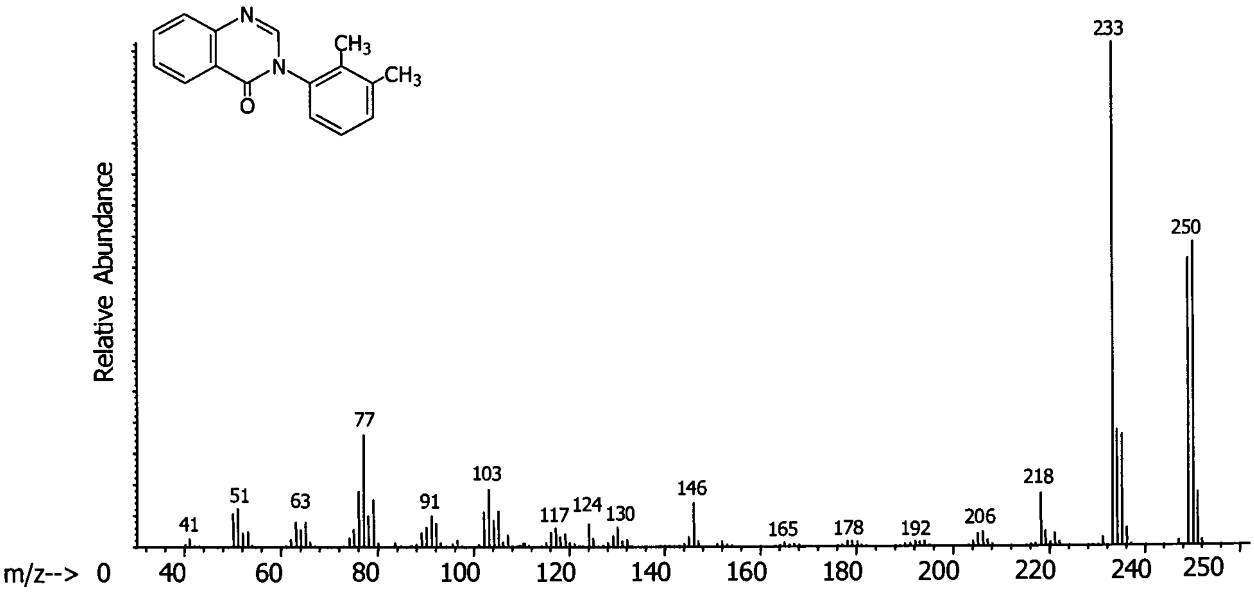


Figure 3.4.10 EI induced (70 eV) mass spectrum of **IV**

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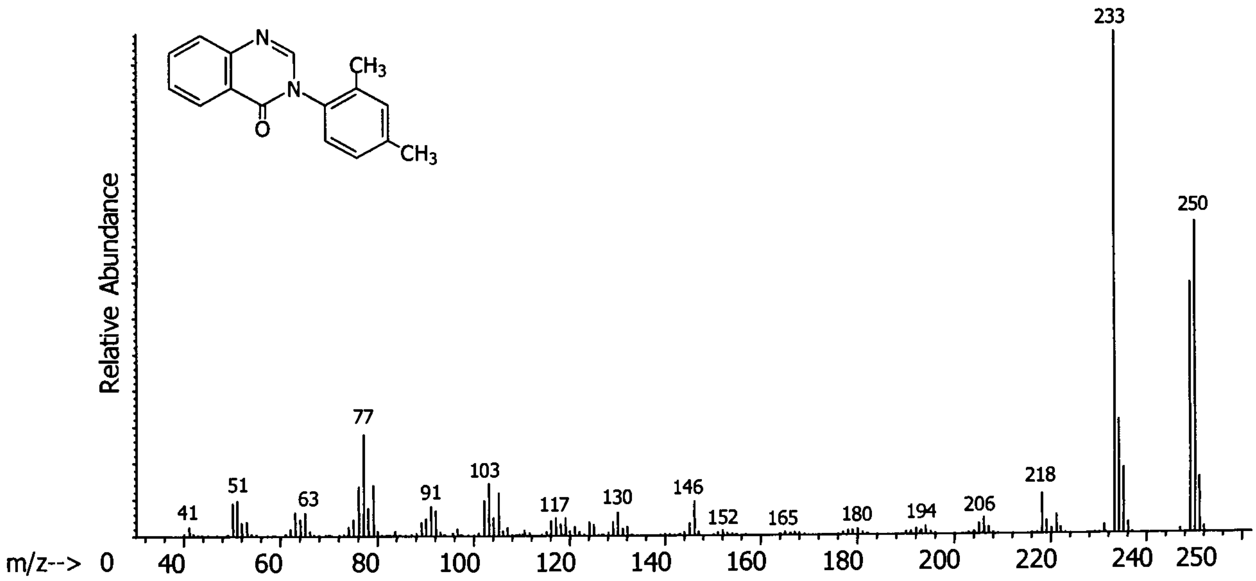


Figure 3.4.11 EI induced (70 eV) mass spectrum of **V**

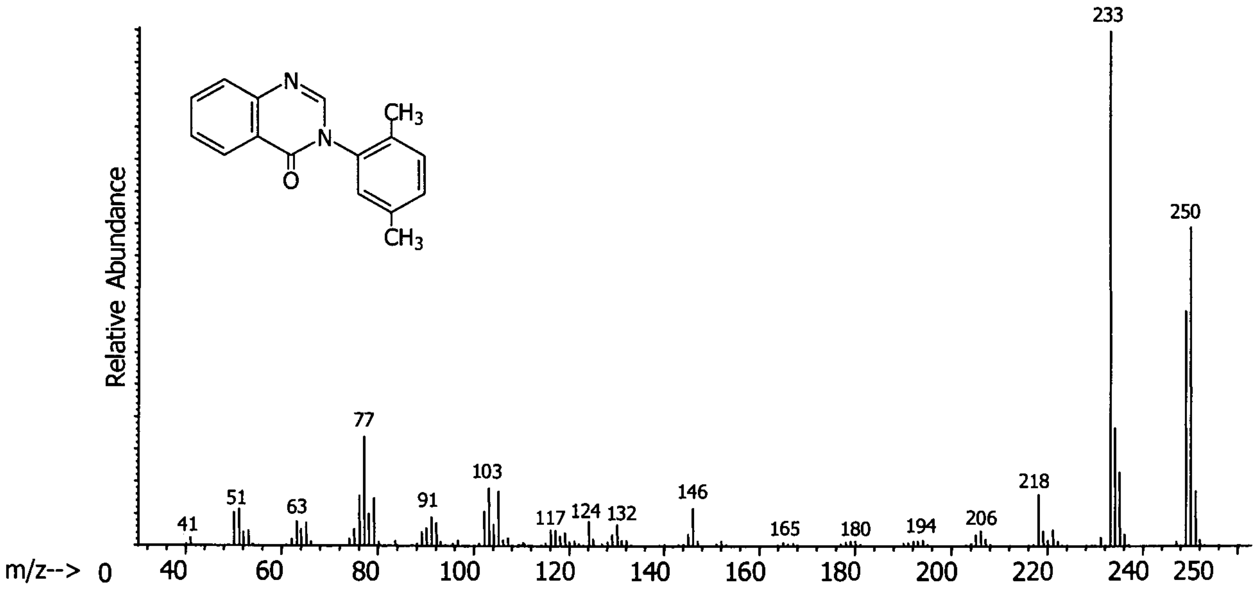


Figure 3.4.12 EI induced (70 eV) mass spectrum of **VI**

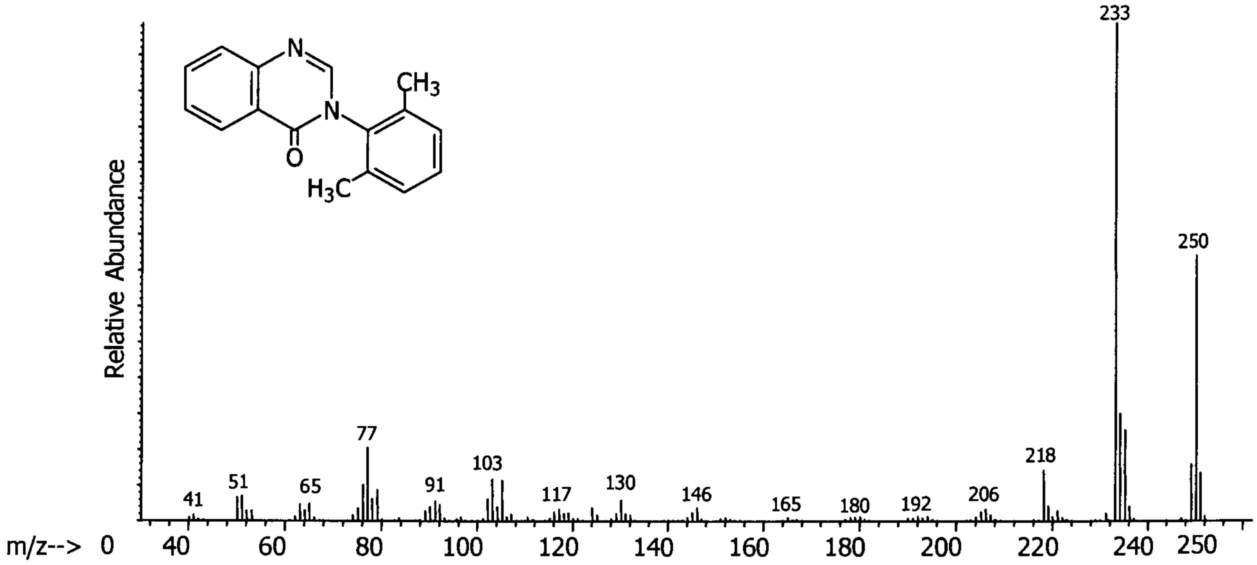


Figure 3.4.13 EI induced (70 eV) mass spectrum of **VII**

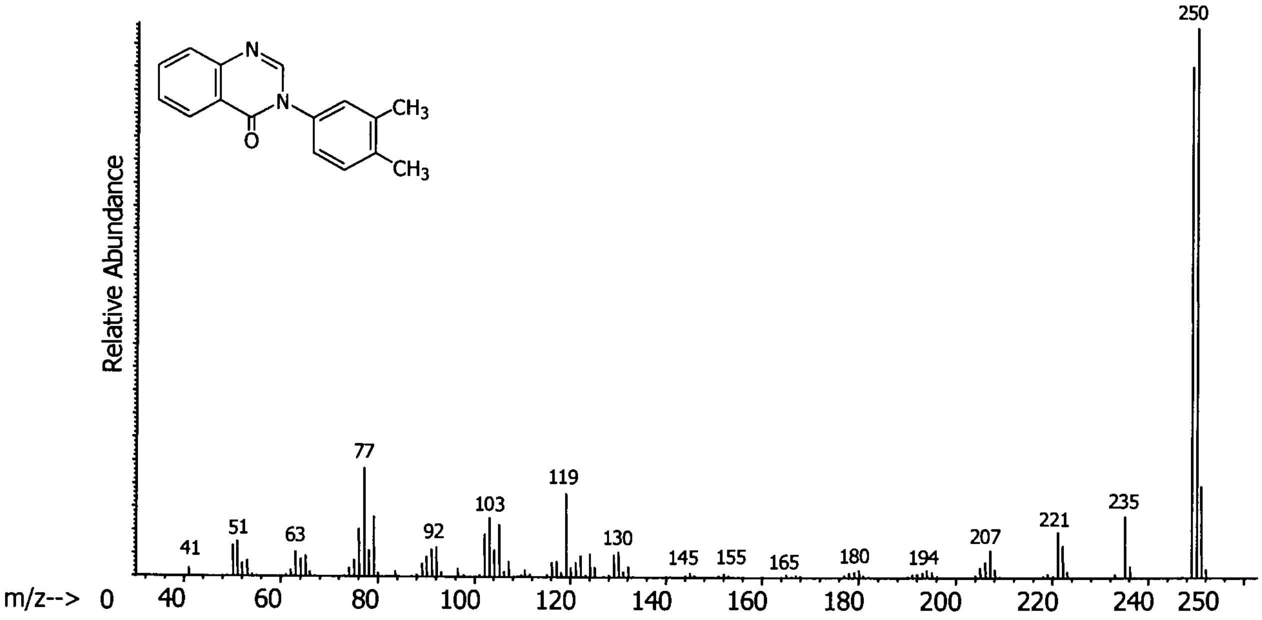


Figure 3.4.14 EI induced (70 eV) mass spectrum of **VIII**

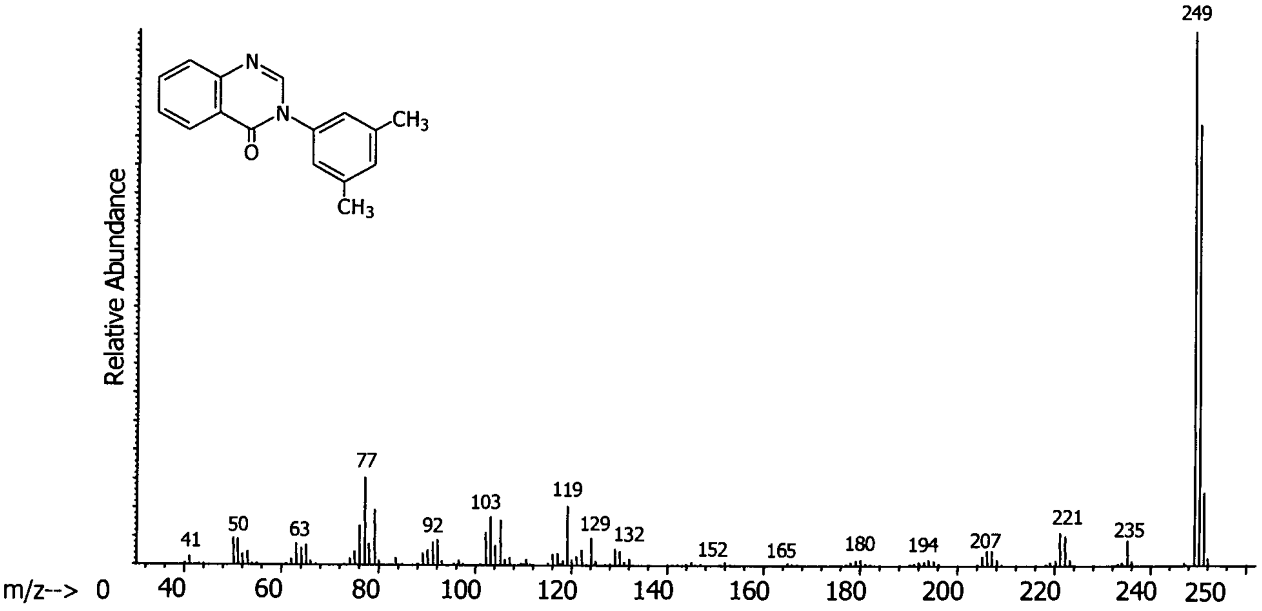


Figure 3.4.15 EI induced (70 eV) mass spectrum of **IX**

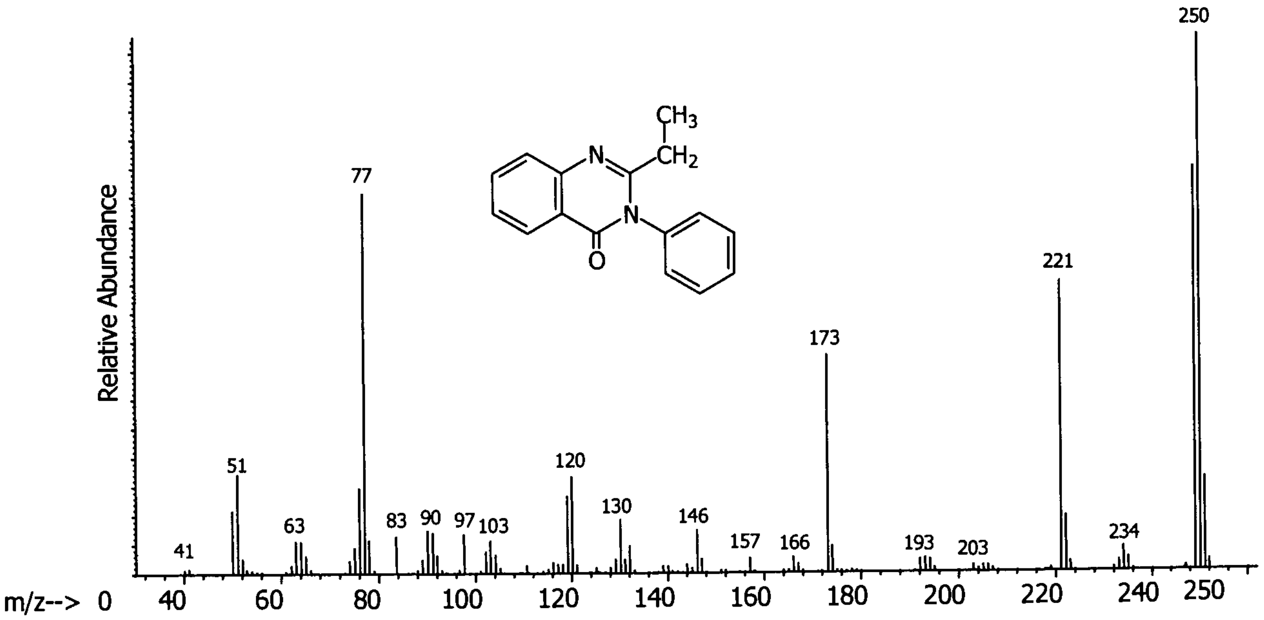


Figure 3.4.116 EI induced (70 eV) mass spectrum of **X**

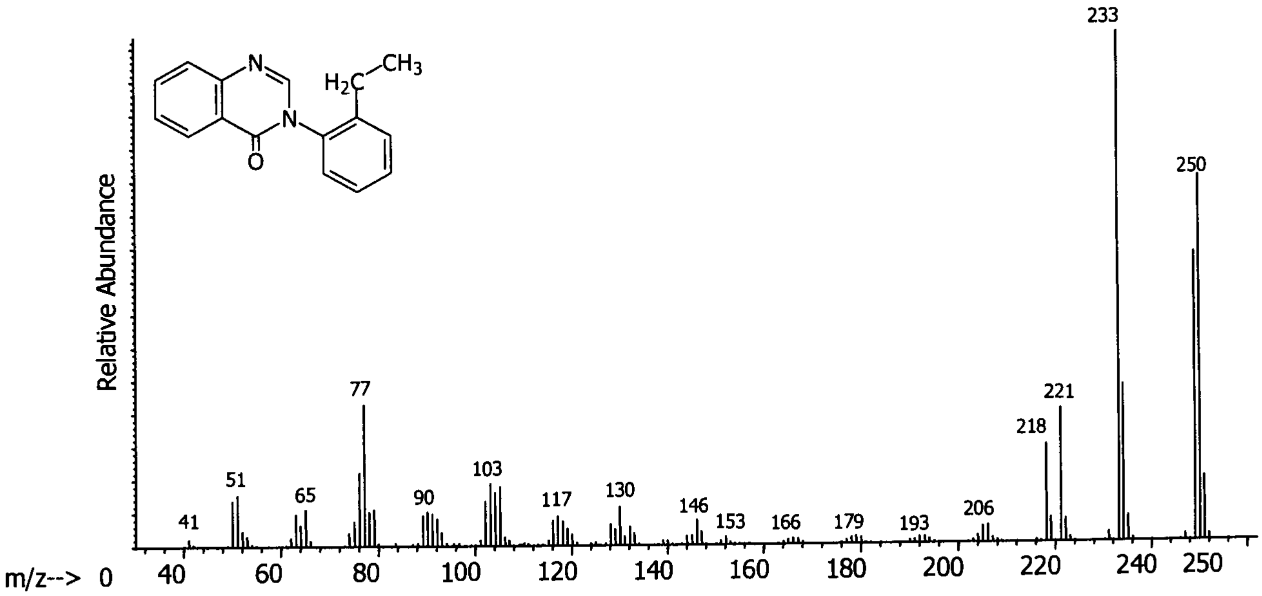


Figure 3.4.17 EI induced (70 eV) mass spectrum of **XI**

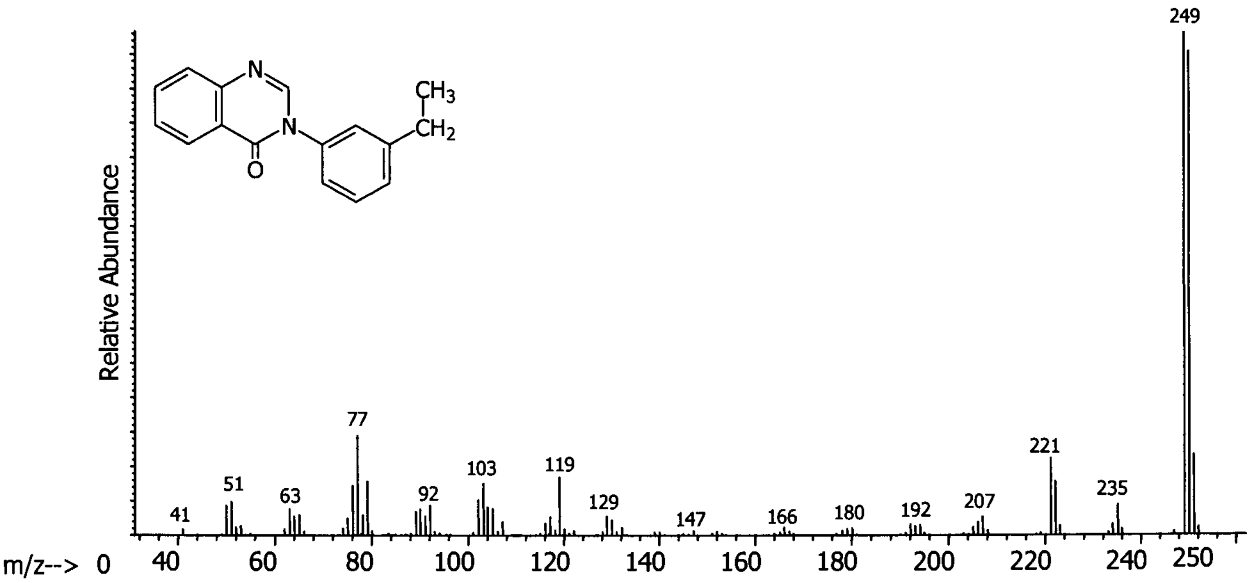


Figure 3.4.18 EI induced (70 eV) mass spectrum of **XII**

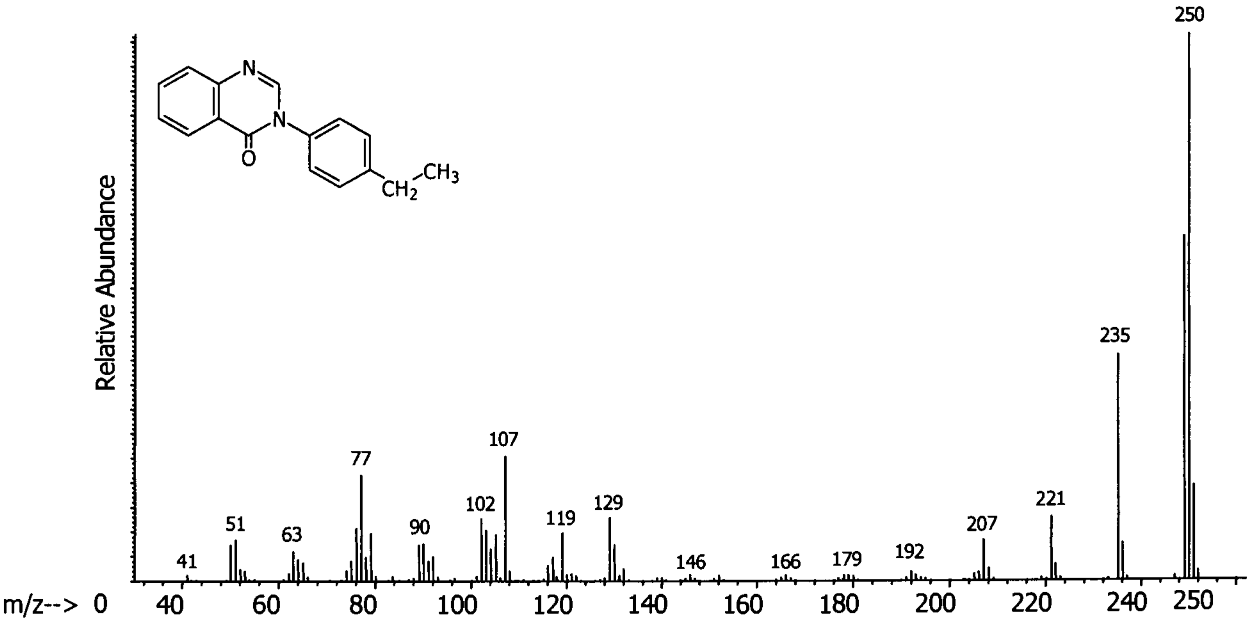


Figure 3.4.19 EI induced (70 eV) mass spectrum of **XIII**

Table 3.4.3 Selected EI induced mass spectral data (*m/z* and relative abundance) for **I** - **XIII**

No.	$M^{+\cdot}$	a	b	c	d	e	f	g	Other significant peaks						
I	250*	249	235	233	-***	217	-	143	132	-	117	-	103	91	76
	*34***	*8*	*100*	*30*	-	*3*	-	*5*	*9*	-	*3*	-	*4*	*27*	*11*
II	250	249	235	-	-	-	-	143	132	-	117	-	103	91	76
	100	*94*	*56*	-	-	-	-	*17*	*6*	-	*4*	-	*4*	*40*	*14*
III	250	249	235	-	-	-	-	143	132	-	117	-	103	91	76
	100	*80*	*59*	-	-	-	-	*29*	*7*	-	*6*		*6*	*41*	*17*
IV	250	249	234	233	-	218	146	130	-	124	117	105	103	91	77
	60	*57*	*23*	*100*	-	*10*	*9*	*4*	-	*5*	*4*	*7*	*11*	*6*	*22*
V	250	249	234	233	-	218	146	130	-	124	117	105	103	91	77
	62	*50*	*22*	*100*	-	*8*	*7*	*5*	-	*3*	*3*	*8*	*10*	*6*	*20*
VI	250	249	234	233	-	218	146	130	-	124	117	105	103	91	77
	61	*45*	*23*	*100*	-	*10*	*7*	*4*	-	*5*	*3*	*11*	*12*	*6*	*22*
VII	250	249	234	233	-	218	146	130	-	124	117	105	103	91	77
	52	*10*	*22*	*100*	-	*10*	*3*	*5*	-	*3*	*3*	*10*	*11*	*6*	*20*
VIII	250	249	235	-	221	-	-	130	-	124	119	105	103	91	77
	100	*93*	*11*	-	*8*	-	-	*4*	-	*4*	*15*	*9*	*10*	*5*	*19*
IX	250	249	235	-	221	-	-	129	-	124	119	105	103	92	77
	83	*100*	*5*	-	*6*	-	-	*3*	-	*5*	*11*	*9*	*9*	*5*	*17*
X	250	249	234	-	221	-	173	157	130	-	120	-	103	90	77
	100	*75*	*4*	-	*54*	-	*43*	*3*	*10*	-	*18*	-	*6*	*8*	*71*
XI	250	249	234	233	221	-	146	130	-	-	117	-	103	90	77
	72	*57*	*31*	*100*	*26*	-	*5*	*7*	-	-	*6*	-	*12*	*7*	*28*
XII	250	249	235	-	221	-	-	129	-	-	119	-	103	92	77
	96	*100*	*6*	-	*15*	-	-	*4*	-	-	*12*	-	*10*	*6*	*20*
XIII	250	249	235	-	221	-	-	129	-	-	119	107	102	90	77
	100	*63*	*40*	-	*12*	-	-	*12*	-	-	*9*	*23*	*11*	*7*	*20*

a = $[M^{+\cdot} - H]^+$; **b** = $[M^{+\cdot} - Me]^+$; **c** = $[M^{+\cdot} - OH]^+$; **d** = $[M^{+\cdot}$ - Et or $(Me + Me)]^+$; **e** = $[$**c** $- Me]^+$; **f** = $[M^{+\cdot} - Ar]^+$; **g** = $[M^{+\cdot} - ArO]^+$

Notes: * figures in normal font denote m/z values; ** figures in italics denote relative abundance; *** hyphens denotes peaks absent or of low intensity

Discussion

The mass spectral data collected for **I**, **III** and **X** correspond with that reported in literature - see **Chapter 3: Part 4 - Introduction**. All mass spectra are characterized by the presence of a fairly strong to strong molecular ion (> 10%) at *m/z* 250, with the molecular ion appearing as the base peak in the spectra of **II**, **III**, **VIII**, **X**, and **XIII**. Similarly all spectra exhibit a $[M^{+\bullet} - H]^+$ ion at *m/z* 249. This ion was relatively more abundant in the spectra of those isomers that were not *ortho* substituted, denoting a loss of an *ortho*-aryl proton due to *ortho* interaction with the carbonyl oxygen of the quinazolinone moiety.[66] In the spectra of both **IX** (*meta-, meta*-dimethyl) and **XII** (*m*-ethyl) *m/z* 249 appears as the base peak, allowing differentiation of these two isomers from remaining group. In general spectral data seems to suggest that, for the class of compound studied, increased *meta*-alkyl substitution seems to increase the relative abundance of $[M^{+\bullet} - H]^+$ in the absence of *ortho* substituents. This is due to increased activation of the *ortho*-aryl protons towards interaction with the carbonyl oxygen.

Only methaqualone (**I**) exhibited a base peak at *m/z* 235 allowing for its rapid and unequivocal differentiation from the other isomers studied. The peak at *m/z* 235 corresponds to $[M^{+\bullet} - CH_3]^+$ and appears in the spectra of all isomers studied, but is less intense than *m/z* 234 in the spectra of **IV** - **VII**, **X**, and **XI**.

The observed high intensity of *m/z* 235 in the mass spectrum of **I** is attributed to the respective loss of the 2-methyl, and the *ortho*-methyl substituents resulting in a high abundance of $[M^{+\bullet} - CH_3]^+$. The spectra of **II** (2-methyl; *meta*-methyl) and **III** (2-methyl; *para*-methyl), the only other two 2-methyl substituted isomers, does not show a base peak at *m/z* 235, but rather at *m/z* 250. This indicate that the high *m/z* 235 intensity in the spectrum of **I** is attributable to a significantly increased loss of the *ortho* -methyl substituent due to *ortho* interaction.[66] Further evidence of the *ortho* effect can be found in the observed relative high abundance of *m/z* 234/235 in other *ortho*-methyl substituted isomers (**IV** - **VII**) compared to isomers that are only *meta* and *para* substituted (**VIII** - **IX**).

Evidence of the dual-*ortho* effect as reported by Ramana *et al*[66] can also be found in the presence of *m/z* 233 in the spectra of all *ortho* substituted isomers studied. The ion at *m/z* 233 is attributed to the loss of an •OH radical in a competitive pathway to the loss of the •CH3 radical from the *ortho* position. The mass spectra of compounds **IV** - **VII** show *m/z* 233 as the base peak, clearly indicating that expulsion of the •OH radical is favoured. This is also observed in the spectrum **XI**, the only *ortho*-ethyl substituted isomer studied, indicating that the reported dual-*ortho* effect is not limited to *ortho*-methyl substituents only. The *m/z* 233 ion also features prominently in the mass spectrum of **I**. This, combined with the fact that *m/z* 233 is absent in all *metha-* and *para-* only substituted isomers, indicate that the presence of *m/z* 233 in this class of compound, i.e. 3-aryl-4(3*H*)-quinazolinones, is diagnostic for *ortho* substitution. All dimethylaryl substituted compounds having an *ortho*-methyl substituent , exhibited peaks at *m/z* 218. This is probably due to the expulsion of a methyl group from the $[M^{+\bullet} - OH]^+$ at *m/z* 233, and may be diagnostic for these type of 3-dimethylaryl-4(3*H*)-quinazolinones.

Compounds **X** - **XIII** showed $[M^{+\bullet} - CH_2CH_3]^+$ ions at *m/z* 221. As expected the relative abundance of *m/z* 221 is the highest for **X** supporting the preferred loss of the 2-alkyl substituent in the absence of aryl substituents. The observed relative abundance of *m/z* 221 decreased in the sequence **XI** (*ortho*-ethyl), **XII** (*meta*-ethyl), and **XIII** (*para*-ethyl). A low intensity peak at *m/z* 221 was also observed in the spectra of **XIII** (*metha-*, *para-*dimethyl) and **IX** (*meta-*, *meta*-dimethyl).

The spectra of compounds **IV** - **VII** exhibited *m/z* values corresponding to $[M^{+\bullet} - Ar]^+$ at *m/z* 146. This was not observed in the spectra of **VIII** and **IX**. Given that **VIII** and **IX** exhibit peaks at m/z 221 corresponding to $[M^{+\bullet} - (dimethyl)]^+$, and this is not observed in the spectra of **IV** - **VII**, this indicates the preferred expulsion of two methyl radicals from the *meta-*, *para*-dimethyl and *meta-*, *meta*-dimethyl substituted isomers. In contrast dimethyl substituted isomers with at least one methyl substituent in the *ortho* position tended to be more stable in this regard, with the loss of the complete aryl moiety favoured. Expulsion of the intact aryl moiety were also observed for **X** (phenyl), and **XI** (*ortho*-ethyl).

All compounds studied showed peaks at *m/z* values corresponding to the loss of •ArO. The diagnostic value of this ion is limited to differentiating groups of isomers with differences in the number and type of atoms substituted onto the aryl moiety. **I**, **II**, and **III** exhibited diagnostic and characteristic peaks at *m/z* 132, whereas all the dimethylaryl substituted isomers (**IV** - **IX**) exhibited peaks at *m/z* 124 and *m/z* 105. All spectra exhibited peaks at *m/z* 117 -120, 103, 90-92, and 76-77. The high relative abundance of *m/z* 77 (71%) in the spectrum of **X** is diagnostic in differentiating this compound.

The studied isomers can be differentiated based on their EI-MS spectra. Differentiation is based on the presence and also the relative abundance of specific ions. Initial differentiation of a specific isomer can be done using the scheme given in Figure 3.4.20. Once the initial identification is done, the identity of a specific compound can be unequivocally established by a detailed comparison to the spectral data given in Table 3.4.3.

Differentiation between compounds **IV** to **VII**, and especially **IV**, **V**, and **VI** requires very close inspection of their mass spectral data. In addition to the ratio of 1:1-1.1 for *m/z* 249:250, the spectra of **IV** is also characterized by *m/z* 235:234 ratio of 1:1-1.1. These ratios is fairly higher for both **V** and **VI**. The spectra of **V** in turn has a **m/z** 124:130 and 124:117 ratio of 1:>1, whereas **VI** is characterized by these ratios always being 1:<1. Differentiation between **II** and **III** is based on the relative intensities of *m/z* 132 and 143. The ration between these two *m/z* values is ~ 1:3 for **II** and ~ 1:4 for **III**.

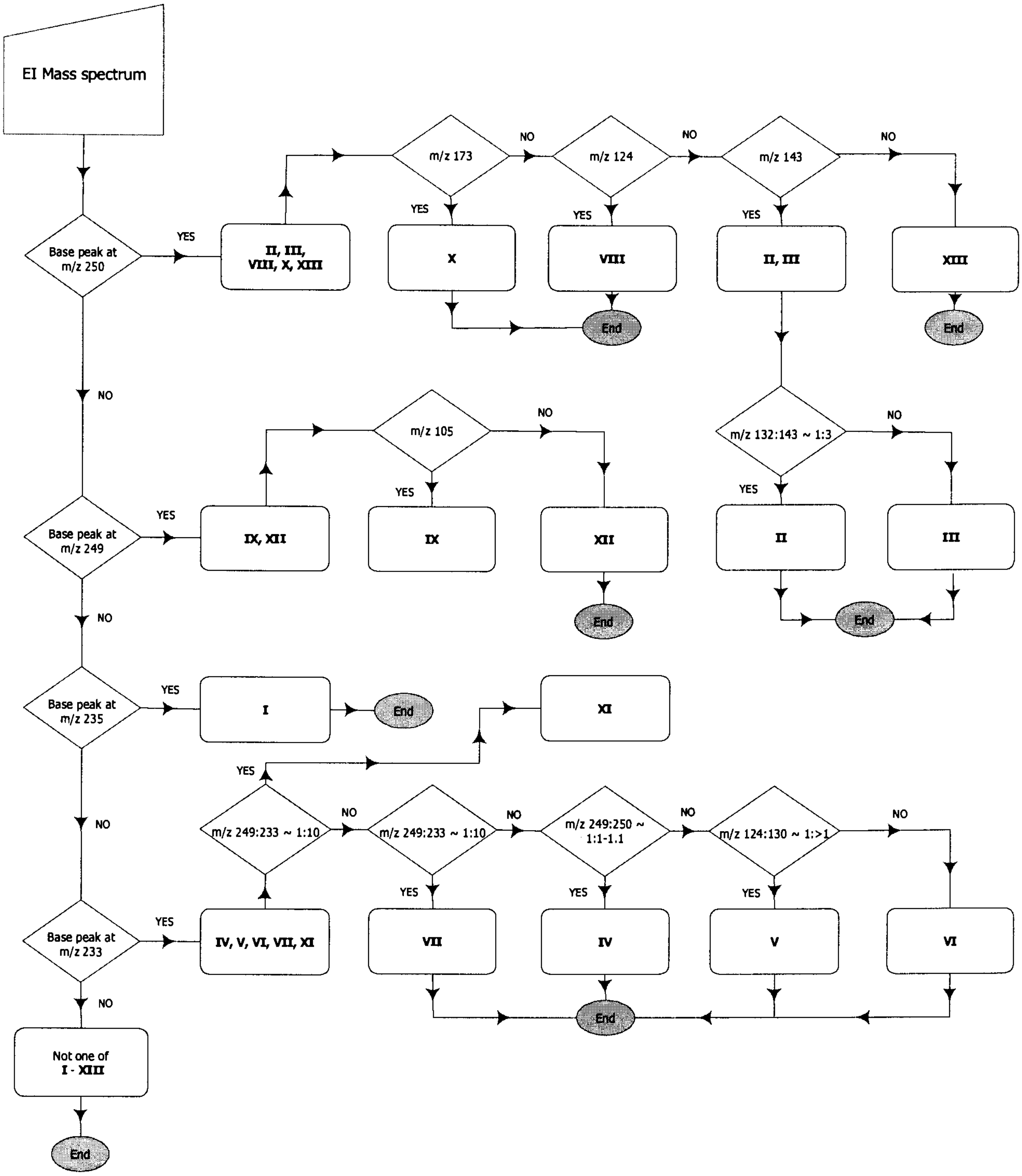


Figure 3.4.20 Differentiation scheme for **I** - **XIII** based on EI-MS spectral data

Conclusion

EI-MS, using the conditions described, allows for the unequivocal differentiation of **I** from the rest of the isomers studied. Close inspection of the spectral data over the complete mass range also allows for the differentiation of the remaining twelve target isomers. Utilizing a selective separation technique like GC, as described in **Chapter 3: Part 1**, interfaced with the specific detection capabilities of EI-MS allows for the convenient and unequivocal differentiation of the isomers studied.

The observed fragmentation patterns provide ample support for the dual *ortho* interaction reported by Ramana *et al* [66], but also that the effect is not limited to *ortho*-methyl substituents. A distinct *meta* effect was observed for the class of compound studied, with increased *meta*-alkyl substitution increasing the relative abundance of $[M^{+\bullet} - H]^+$ in the absence of *ortho* substituents. This is due to increased activation of the *ortho*-aryl protons towards interaction with the carbonyl oxygen.

CHAPTER 3

Part 5: Nuclear Magnetic Resonance Spectroscopy

Introduction

Methaqualone (**I**) has been the subject of numerous ^1H and ^{13}C nuclear magnetic resonance (NMR) studies, detailing partial and rigorous assignments of resonance signals. A survey of relevant studies prior to 1990 is given by Blumenstein *et al*, with the authors concluding that the studies surveyed reported conflicting and ambiguous assignments.[69]

Limited ^1H NMR spectral data for quinazolinones, including **I**, was reported by Daenens *et al.*[48] The ^1H NMR spectra of **I**, mecloqualone, and nitromethaqualone are given with δ values reported relative to tetramethylsilane (TMS). Experimental conditions were not reported. Singlets appearing at 2.1 ppm and 2.18 ppm in the spectrum of **I** were assigned to the C_2 - CH_3 and $C_{2'}$ - CH_3 groups respectively, but no preferred assignments for were made. The signals in the 7 - 8.4 ppm range were assigned to aromatic protons of the two aromatic ring structures. The authors concluded that should sufficient pure product be available ^1H NMR will allow for quick differentiation between the compounds studied.

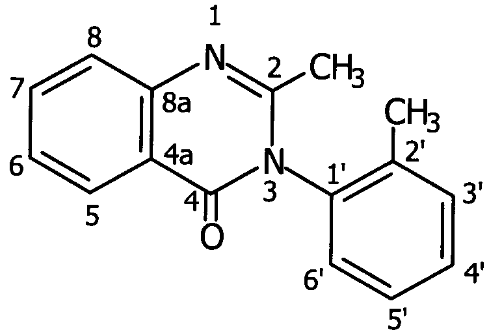


Figure 3.1 Methaqualone (**I**)

Rücker *et al*[70] studied the ^1H NMR spectra of **I** at 60 MHz and assigned the C_2 - CH_3 to the more high-field singlet, and the $C_{2'}$ - CH_3 to the low-field singlet appearing in the spectrum.

The ^1H NMR spectra of inter alia **I**, **II**, **III** were collected as part of a study to differentiate **I** from some isomers and analogs.[10] Limited experimental conditions are reported, and actual spectra are given. The resonance singlet appearing at 2.15 ppm in the spectrum of **I** was assigned to $C_{2'}$ - CH_3, and the singlet at 2.25 ppm to C_2 - CH_3. The singlet appearing at 2.45 ppm in the spectra of **II** and **III** was respectively assigned to $C_{3'}$ - CH_3 and $C_{4'}$ - CH_3. A slight downfield shift of the C_2 - CH_3 signal at 2.3 ppm was also reported. The overlapped complex absorption patterns observed in the 7.2 - 8.8 ppm range of all spectra were assigned to phenyl ring protons. ^1H NMR spectral data of all compounds studied were sufficiently unique to allow identification.

Angelos *et al*[2] collected the ^1H NMR spectra of inter alia **I**, **I.HCl**, mecloqualone, and mecloqualone hydrochloride in deuterated DMSO using a 90 MHz spectrometer. Actual spectra is given, but no assignments or other spectral data is given.

The most in-depth NMR study of **I** was reported by Blumenstein *et al.*[69] The 300MHz ^1H and 75MHz ^{13}C NMR spectra of **I** have been extensively studied using one and two-dimensional techniques, and rigorous assignments for the methyl groups and the quinazolinone nucleus were made. Achiral and chiral lanthanide shift reagent experiments were conducted providing potential for direct optical purity determinations. Selective deuteration experiments were performed. Experimental conditions and detailed assignments is given in Table 5.5.1.

Assigned ^1H NMR signals for **I** is given by Patel *et al*[44] : δ_H (ppm) 2.06 (3H, s) **C_2 - CH_3**; 2.15 (3H, s) **$C_{2'}$ - CH_3**; 7 - 8.4 (8H, m) **aromatic protons**. The ^1H NMR spectrum for **I** is given and the following relevant signals tabulated[52] : δ_H (ppm) 2.13 (s), 2.19 (s).

In a more recent ^1H NMR study of **I**, methyl assignments conflicting with that of Blumenstein *et al* were reported.[62] The upfield signal at 2.139 ppm was assigned to C_2 - CH_3, and the signal at 2.801 to $C_{2'}$ - CH_3. Experimental data and detailed assignments are given in Table 3.5.1.

Ramana *et al*[12] reported the 400MHz ^{1}H NMR data for inter alia **I**, **III**, and **X**. $CDCl_3$ was used as a solvent and δ reported relative to TMS:

I: δ_H (ppm) 2.24 (s,3H,C_2 - CH_3); 2.56 (s,3H,$C_{2'}$ - CH_3); 7.12 - 7.84 (m,7H,Ar); 8.26 (q,1H,C_5 - H)
III: δ_H (ppm) 2.24 (s,3H,C_2 - CH_3); 2.56 (s,3H,$C_{2'}$ - CH_3); 7.12 - 7.84 (m,7H,Ar); 8.26 (q,1H,C_5 - H)
X: δ_H (ppm) 1.06 (t,3H,C_2 - Et(CH_3)); 2.31 (q,2H,3H,C_2 - Et(CH_2)); 6.98 - 7.94 (m,8H,Ar); 8.26 (q,1H, C_5 - H)

The reported data indicate that identical ^{1}H NMR spectra was obtained for both **I** and **III**. Considering the structures of these two compounds this is impossible, and thus also cast doubt on the usefulness of the data reported for **X**.

The ^{1}H NMR spectra of **I** in the presence of the optically active auxiliary compound $(+)_{589}$ - tris[heptaflourobutyryl-D-camphorato]europium (III) showed two $C_{2'}$ - CH_3 signals, persisting up to at least 363K.[49] This was attributed to hindered rotation of the 3-aryl around the aryl C - N bond, and thus the existence of **I** as two distinct rotamers, **(M)-** and **(P)-I**.

The NMR study of restricted internal rotation in 2,3-disubstituted-4(1H) quinazolinones, specifically **I**, was reported.[71] Steric interaction between the 3-aryl and the heterocyclic moiety was found to produce sufficient restriction to rotation around the aryl C - N bond to allow for the existence of torsional isomers at room temperature.

Throughout this study the assignments as reported by Blumenstein *et al*[69] were favoured and used as a basis to infer expected substituent shifts when assigning the resonance signals in the spectra of those compounds for which no NMR data has been reported in literature. Selected ^{13}C and ^{1}H assignments for **I** are given in Table 3.5.1 on the following page.

Table 3.5.1 NMR chemical shift assignments for **I** reported in literature

	δ_C (ppm)				δ_H (ppm)		
Reference	[72]	[73]	[74]	[69]	[10]	[69]	[62]
Frequency (Hz)	15.00	25.00	25.00	75.00	90	300	300
Solvent	DMSO-$\underline{d}_6$	DMSO-$\underline{d}_6$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$
Temperature (K)	305.00	"ambient"	"ambient"	300.00	not given	300	300
<u>Nucleus</u>							
$C_{2'}$ - CH_3	16.70	16.89	17.30	17.33	2.15 3H, s	2.13 3H, s	2.80 3H,s
C_2 - CH_3	23.40	23.57	17.30	23.82	2.25 3H, s	2.19 3H, s	2.14 3H,s
2	154.20	154.30	154.00	154.22	-	-	
4	160.70	160.68	161.20	161.55	-	-	
4a	120.30	120.31	120.40	120.71	-	-	
5	126.80^a	126.36$_a$	126.70	127.07	-	8.29 1H, dd J = 8.01, 1.15	8.24 1H, dd J = 8.0, 1.6
6	126.40$_a$	126.50$_a$	126.50	126.50	-	7.47 1H, approx. t J = 7.48	7.42 1H, td J = 6.9, 1.1
7	134.60^b	134.65	134.20	134.49	-	7.77 1H, approx. t J = 7.96	7.73 1H, td J = 6.9, 1.6
8	126.40^a	126.75$_a$	126.30	126.74	-	7.69 1H, approx. d J = 7.72	7.65 1H, br d J = 6.9
8a	147.50	147.42	147.20	147.62	-	-	-
1'	136.80^b	136.84	136.40	136.78^a	-	-	-
2'	135.00^b	135.04	135.00	135.30^a	-	-	-
3'	131.00^c	131.04	131.20	131.46	} -	7.30 - 7.42 3H, m	7.37 - 7.29 3H, m
4'	128.30^c	128.40	127.60	129.50^b			
5'	127.30^c	127.38	127.30	127.57^b			
6'	129.30^c	129.28	129.20	127.87	-	7.16 1H, d J = 6.73	7.12 1H, br d J = 6.8

s = singlet; dd = doublet of doublets; approx. t = approximate triplet; td = triplet of doublets; approx. d = approximate doublet; br d = broad doublet; m = multiplet; d = doublet

Note: Within each column superscript alpha characters designate groups of nuclei within which the assignments may be reversed.

Experimental

NMR spectra were recorded using model AC-300 FT-NMR (Bruker). ^{1}H NMR spectra were recorded at 300.135 MHz and ^{13}C spectra at 75.477 MHz, with both experiments being performed at 300K. Samples were dissolved in $CDCl_3$, and chemical shifts reported relative to the signal of the deuterated solvent, i.e. ^{1}H $CDCl_3$ 7.24 ppm and ^{13}C $CDCl_3$ 77.0 ppm.

Results

The ^{1}H NMR spectra, as well as the expanded spectra, of compounds **I** - **XIII** are given in Figures 3.5.2 to 3.5.27. ^{1}H NMR and ^{13}C NMR spectral data as well as the preferred signal assignments based on expected chemical shift are summarized in Tables 3.5.2 to 3.5.27.

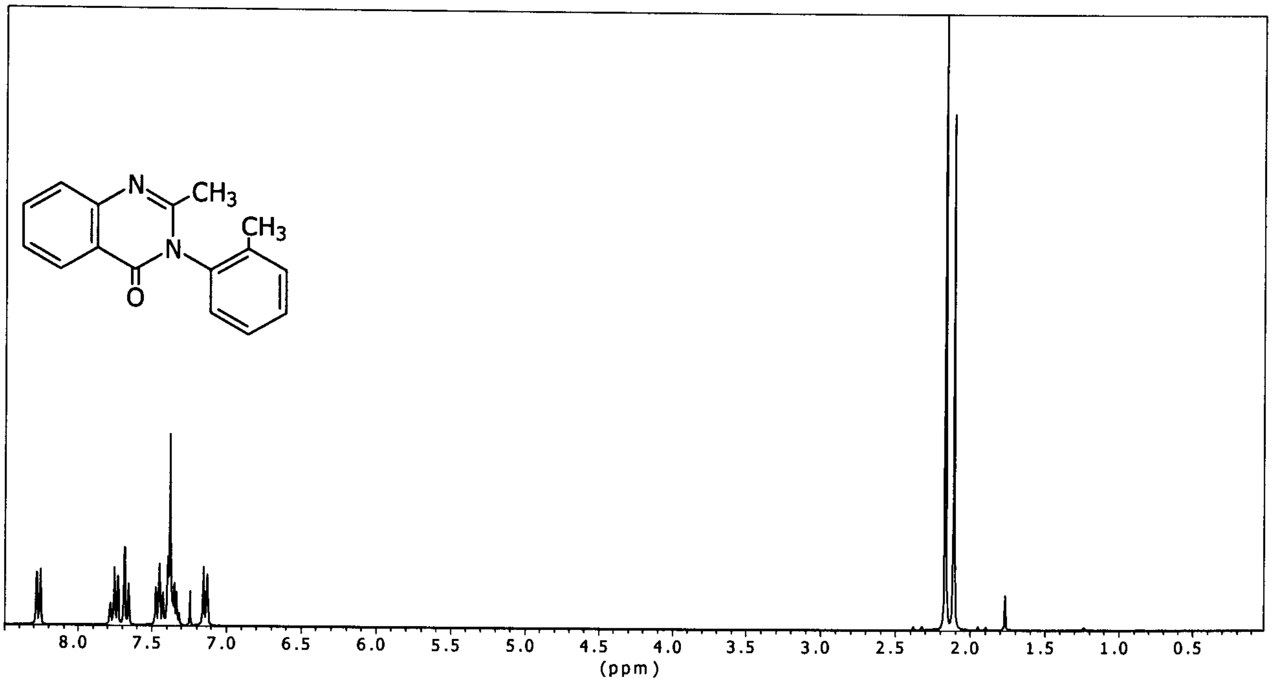


Figure 3.5.2 ^{1}H NMR spectrum of **I**

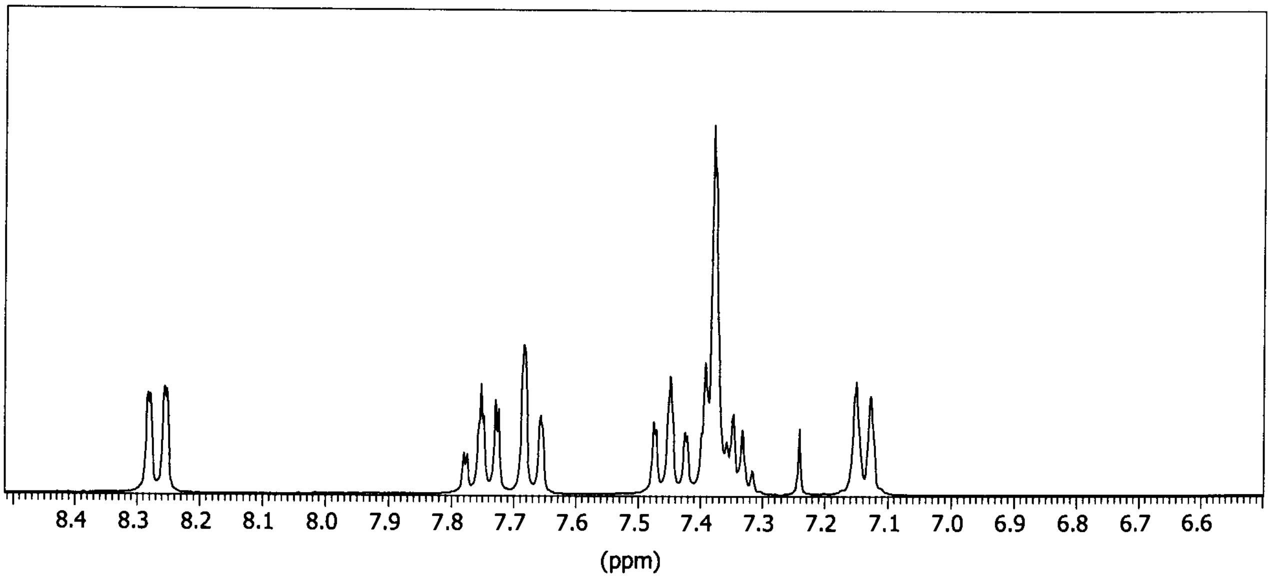


Figure 3.5.3 Expanded ^{1}H NMR spectrum of **I**

Table 3.5.2 Proton NMR chemical shift assignments for **I** (300MHz, $CDCl_3$, 300K)

Signal No.	Nuclei	δ_H (ppm)	Pattern	J (Hz)	Assignment
1	3H	2.11	s	-	$C_{2'}$ - CH_3
2	3H	2.16	s	-	C_2 - CH_3
3	1H	7.14	d	6.86	$C_{6'}$ - H
4	3H	7.32 - 7.39	m	-	$C_{3'}$ - H $C_{4'}$ - H $C_{5'}$ - H
5	1H	7.45	td	7.54, 1.15	C_6 - H
6	1H	7.67	d	8.01	C_8 - H
7	1H	7.74	td	7.63, 1.46	C_7 - H
8	1H	8.27	dd	7.92, 1.05	C_5 - H

Table 3.5.3 ^{13}C NMR chemical shift assignments for **I** (75MHz, $CDCl_3$, 300K)

Signal No.	δ_C (ppm)	Assignment
1	17.3	CH_3 ($C_{2'}$)
2	23.8	CH_3 (C_2)
3	120.8	C_{4a}
4	126.5	C_6
5	126.8	C_8
6	127.2	C_5
7	127.6	$C_{5'}$
8	127.9	$C_{6'}$
9	129.5	$C_{4'}$
10	131.5	$C_{3'}$
11	134.5	C_7
12	135.4	$C_{2'}$
13	136.9	$C_{1'}$
14	147.7	C_{8a}
15	154.3	C_2
16	161.6	C_4

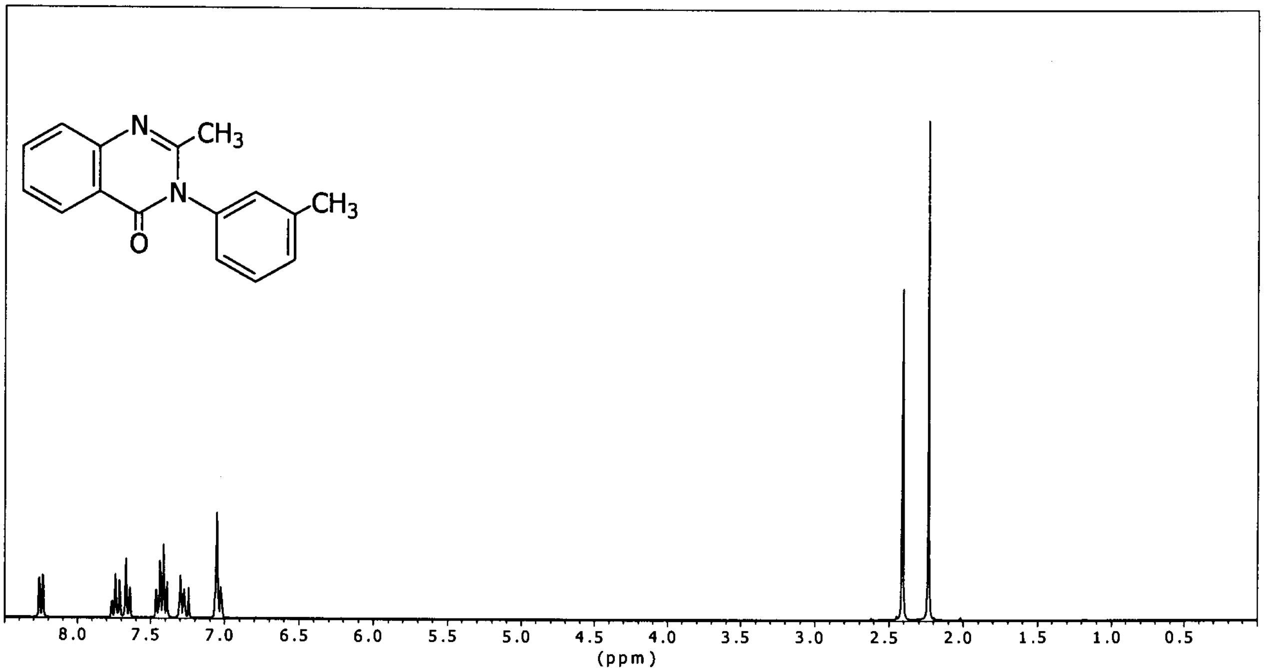


Figure 3.5.4 ^{1}H NMR spectrum of **II**

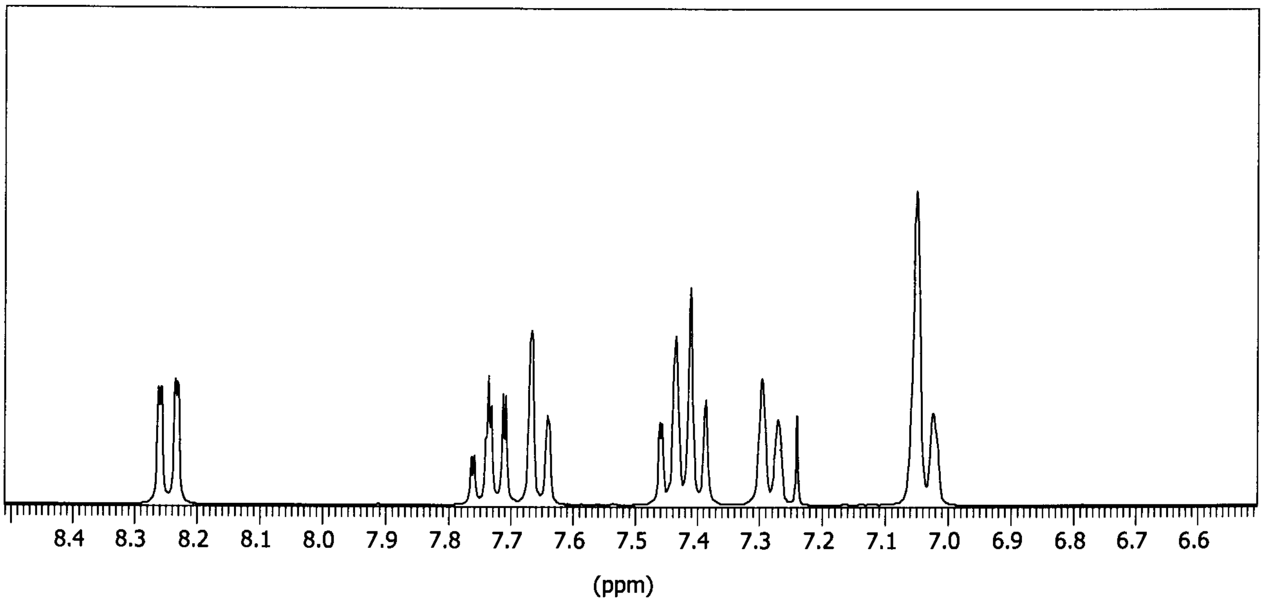


Figure 3.5.5 Expanded ^{1}H NMR spectrum of **II**

Table 3.5.4 Proton NMR chemical shift assignments for **II** (300MHz, $CDCl_3$, 300K)

Signal No.	Nuclei	δ_H (ppm)	Pattern	J (Hz)	Assignment
1	3H	2.23	s	-	C_2 - CH_3
2	3H	2.40	s	-	$C_{3'}$ - CH_3
3	2H	7.02 to 7.05	m	-	$C_{2'}$ - H $C_{6'}$ - H
4	1H	7.280	d	7.44	$C_{4'}$ - H
5	2H	7.37 to 7.46	m	-	$C_{5'}$ - H C_6 - H
6	1H	7.65	d	7.44	C_8 - H
7	1H	7.74	td	7.54, 1.52	C_7 - H
8	1H	8.25	dd	7.82, 1.15	C_5 - H

Table 3.5.5 ^{13}C NMR chemical shift assignments for **II** (75MHz, $CDCl_3$, 300K)

Signal No.	δ_C (ppm)	Assignment
1	21.3	CH_3 ($C_{3'}$)
2	24.3	CH_3 (C_2)
3	120.8	C_{4a}
4	124.9	$C_{2'}$ or $C_{6'}$
5	126.5	C_6
6	126.7	C_8
7	127.1	C_5
8	128.5	$C_{6'}$ or $C_{2'}$
9	129.7	$C_{4'}$
10	130.1	$C_{3'}$
11	134.5	C_7
12	137.7	$C_{1'}$
13	140.1	$C_{5'}$
14	147.5	C_{8a}
15	154.3	C_2
16	162.2	C_4

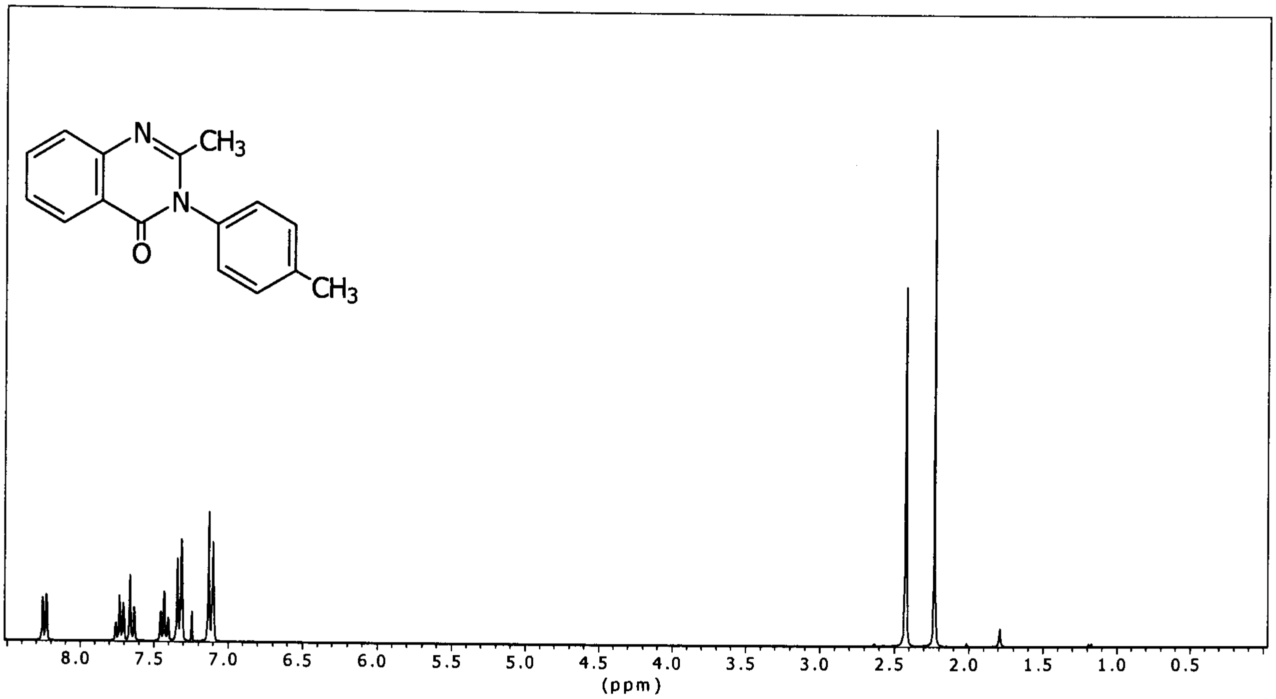


Figure 3.5.6 ^{1}H NMR spectrum of **III**

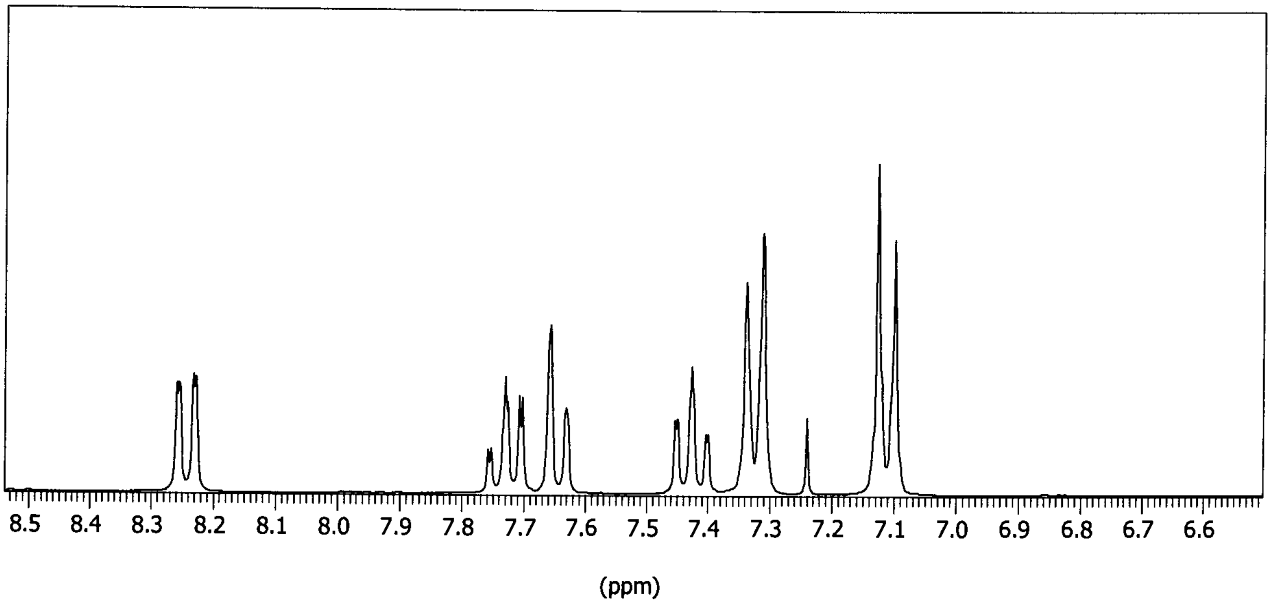


Figure 3.5.7 Expanded ^{1}H NMR spectrum of **III**

Table 3.5.6 Proton NMR chemical shift assignments for **III** (300MHz, $CDCl_3$, 300K)

Signal No.	Nuclei	δ_H (ppm)	Pattern	J (Hz)	Assignment
1	3H	2.23	s	-	$C_2 - CH_3$
2	3H	2.42	s	-	$C_{4'} - CH_3$
3	2H	7.11	d	8.20	$C_{2'} - H$ $C_{6'} - H$
4	2H	7.32	d	8.01	$C_{3'} - H$ $C_{5'} - H$
5	1H	7.43	td	7.54, 1.14	$C_6 - H$
6	1H	7.64	d	7.63	$C_8 - H$
7	1H	7.73	td	7.63, 1.46	$C_7 - H$
8	1H	8.24	dt	8.01, 0.43	$C_5 - H$

Table 3.5.7 ^{13}C NMR chemical shift assignments for **III** (75MHz, $CDCl_3$, 300K)

Signal No.	δ_C (ppm)	Assignment
1	21.2	CH_3 ($C_{4'}$)
2	24.3	CH_3 (C_2)
3	120.8	C_{4a}
4	126.5	C_6
5	126.7	C_8
6	127.1	C_5
7	127.7	$C_{2'}$; $C_{6'}$
8	130.6	$C_{3'}$; $C_{5'}$
9	134.4	C_7
10	135.1	$C_{4'}$
11	139.3	$C_{1'}$
12	147.5	C_{8a}
13	154.4	C_2
14	162.3	C_4

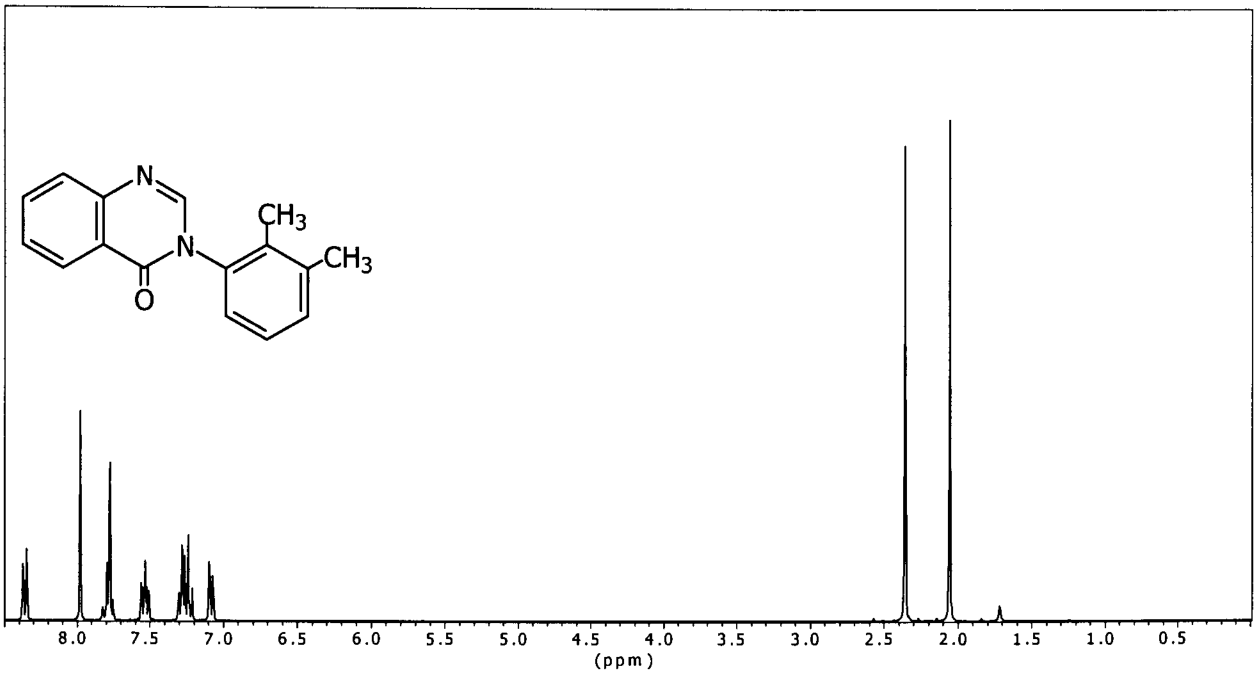


Figure 3.5.8 ^{1}H NMR spectrum of **IV**

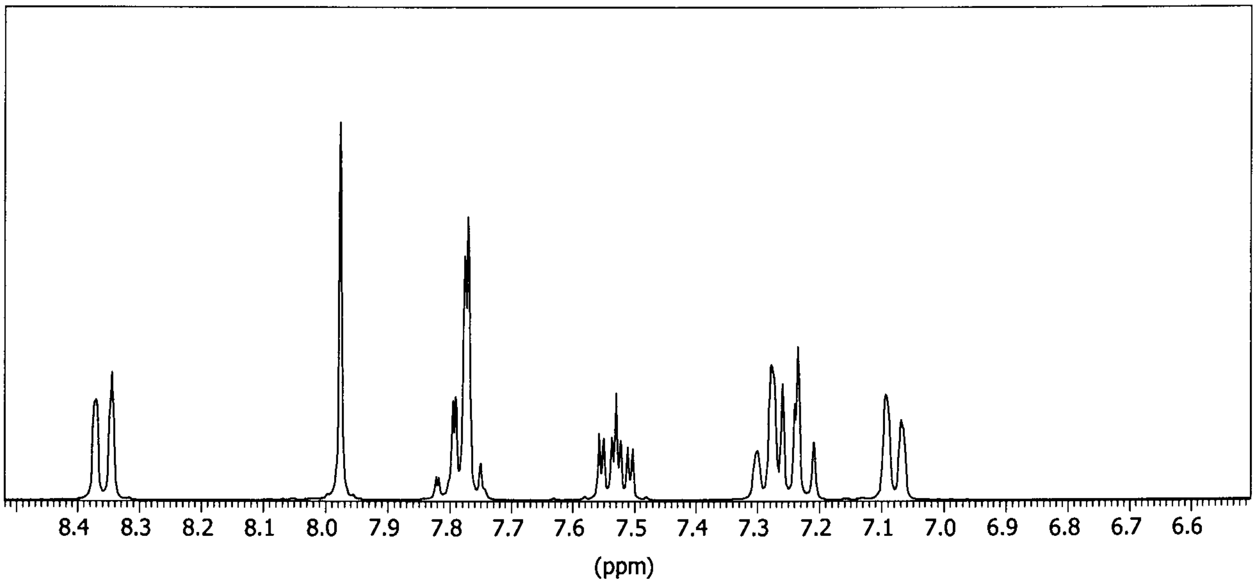


Figure 3.5.9 Expanded ^{1}H NMR spectrum of **IV**

Table 3.5.8 Proton NMR chemical shift assignments for **IV** (300MHz, $CDCl_3$, 300K)

Signal No.	Nuclei	δ_H (ppm)	Pattern	J (Hz)	Assignment
1	3H	2.05	s	-	$C_{2'}$ - CH_3
2	3H	2.36	s	-	$C_{3'}$ - CH_3
3	2H	7.08	d	7.44	$C_{6'}$ - H
4	2H	7.21 to 7.30	m	-	$C_{4'}$ - H $C_{5'}$ - H
5	1H	7.53	tm	7.06	C_6 - H
6	2H	7.74 to 7.82	m	-	C_7 - H C_8 - H
7	1H	7.98	s	-	C_2 - H
8	1H	8.39	dm	7.82	C_5 - H

Table 3.5.9 ^{13}C NMR chemical shift assignments for **IV** (75MHz, $CDCl_3$, 300K)

Signal No.	δ_C (ppm)	Assignment
1	14.3	CH_3 ($C_{2'}$)
2	20.3	CH_3 ($C_{3'}$)
3	122.5	C_{4a}
4	125.4	$C_{6'}$
5	126.6	C_6
6	127.0	C_8
7	127.3	C_5
8	127.5	$C_{2'}$
9	131.1	$C_{3'}$
10	134.4	C_7
11	134.5	$C_{4'}$ or $C_{1'}$
12	136.7	$C_{1'}$ or $C_{4'}$
13	138.7	$C_{5'}$
14	146.5	C_{8a}
15	148.2	C_2
16	160.6	C_4

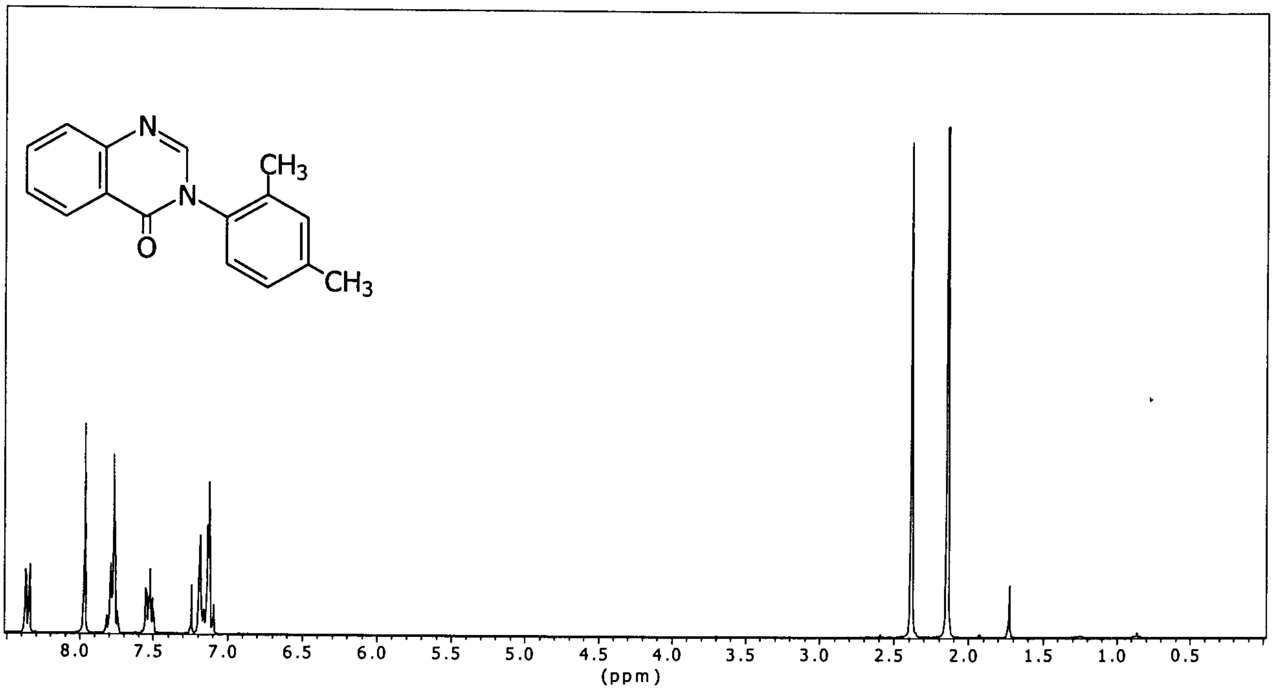


Figure 3.5.10 ^{1}H NMR spectrum of **V**

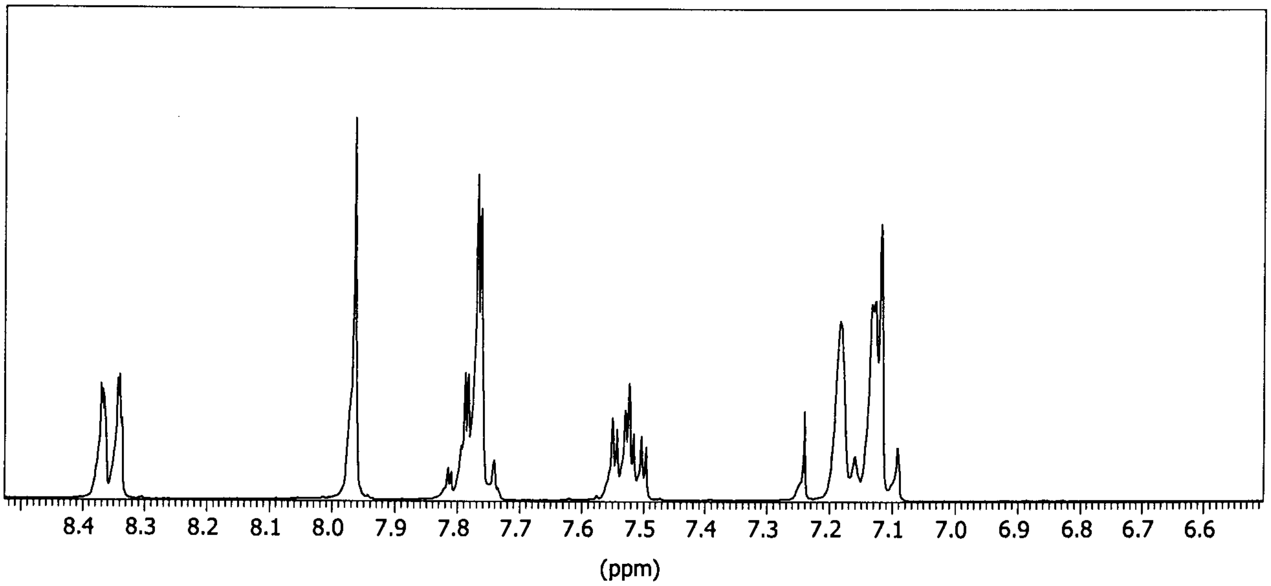


Figure 3.5.11 Expanded ^{1}H NMR spectrum of **V**

Table 3.5.10 Proton NMR chemical shift assignments for **V** (300MHz, $CDCl_3$, 300K)

Signal No.	**Nuclei**	**δ_H (ppm)**	**Pattern**	**J (Hz)**	**Assignment**
1	3H	2.14	s	-	$C_{2'}$ - CH_3
2	3H	2.38	s	-	$C_{4'}$ - CH_3
3	3H	7.09 to 7.18	m	-	$C_{3'}$ - H $C_{5'}$ - H $C_{6'}$ - H
4	1H	7.52	tm	5.92	C_6 - H
5	2H	7.74 to 7.82	m	-	C_7 - H C_8 - H
6	1H	7.96	s	-	C_2 - H
7	1H	8.35	dm	7.82	C_5 - H

Table 3.5.11 ^{13}C NMR chemical shift assignments for **V** (75MHz, $CDCl_3$, 300K)

Signal No.	**δ_C (ppm)**	**Assignment**
1	17.6	CH_3 ($C_{2'}$)
2	21.1	CH_3 ($C_{4'}$)
3	122.5	C_{4a}
4	127.0	C_8 or C_6
5	127.3	C_6 or C_8
6	127.5	C_5
7	127.6	$C_{5'}$ or $C_{6'}$
8	127.9	$C_{6'}$ or $C_{5'}$
9	132.0	$C_{3'}$ or $C_{4'}$
10	134.1	$C_{4'}$ or $C_{3'}$
11	134.4	C_7
12	135.4	$C_{1'}$ or $C_{5'}$ or $C_{4'}$
13	139.8	$C_{5'}$ or $C_{1'}$ or $C_{4'}$
14	146.6	C_{8a}
15	148.2	C_2
16	160.6	C_4

Table 3.5.12 Proton NMR chemical shift assignments for **VI** (300MHz, $CDCl_3$, 300K)

Signal No.	Nuclei	δ_H (ppm)	Pattern	J (Hz)	Assignment
1	3H	2.13	s	-	$C_{2'}$ - CH_3
2	3H	2.36	s	-	$C_{5'}$ - CH_3
3	1H	7.05	s	-	$C_{6'}$ - H
4	2H	7.18 to 7.27	m	-	$C_{3'}$ - H $C_{4'}$ - H
5	1H	7.53	tm	5.91	C_6 - H
6	2H	7.75 to 7.82	m	-	C_7 - H C_8 - H
7	1H	7.96	s	-	C_2 - H
8	1H	8.36	dm	8.01	C_5 - H

Table 3.5.13 ^{13}C NMR chemical shift assignments for **VI** (75MHz, $CDCl_3$, 300K)

Signal No.	δ_C (ppm)	Assignment
1	17.2	CH_3 ($C_{2'}$)
2	20.7	CH_3 ($C_{5'}$)
3	122.5	C_{4a}
4	127.3	C_5 or C_8 or C_6
5	127.5	C_8 or C_5 or C_6
6	127.6	C_6 or C_5 or C_8
7	128.3	} $C_{2'}$ and $C_{3'}$ and $C_{6'}$
8	130.5	
9	131.1	
10	132.5	$C_{4'}$ or $C_{1'}$
11	134.5	C_7
12	136.4	$C_{1'}$ or $C_{4'}$
13	137.3	$C_{5'}$
14	146.4	C_{8a}
15	148.1	C_2
16	160.5	C_4

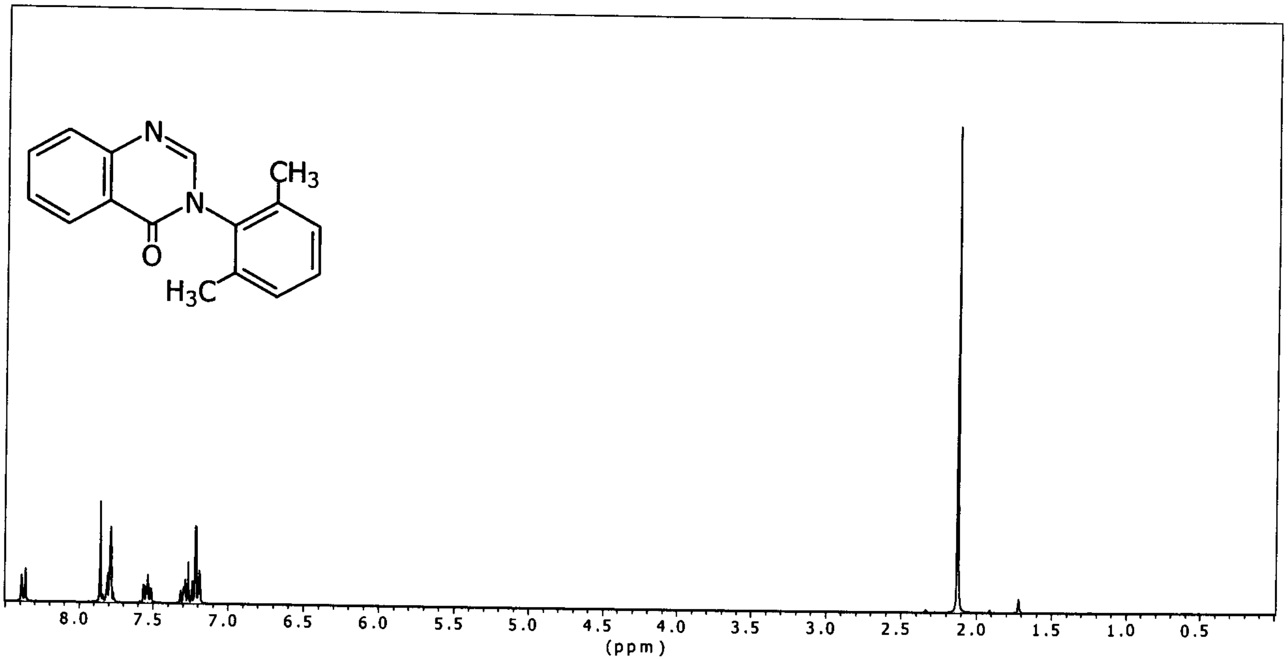


Figure 3.5.14 ^{1}H NMR spectrum of **VII**

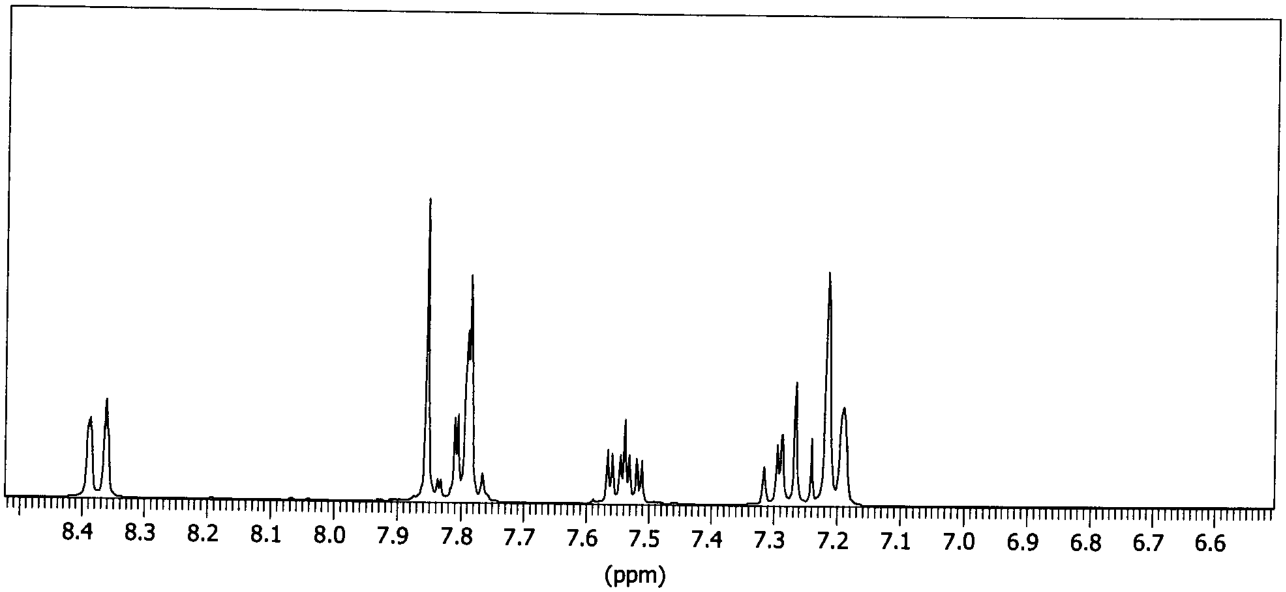


Figure 3.5.15 Expanded ^{1}H NMR spectrum of **VII**

Table 3.5.14 Proton NMR chemical shift assignments for **VII** (300MHz, $CDCl_3$, 300K)

Signal No.	**Nuclei**	**δ_H (ppm)**	**Pattern**	**J (Hz)**	**Assignment**
1	6H	2.13	s	-	$C_{2'}$ - CH_3 $C_{6'}$ - CH_3
2	2H	7.20	d	7.63	$C_{3'}$ - H $C_{5'}$ - H
3	1H	7.29	tm	7.44	$C_{4'}$ - H
4	1H	7.54	tm	6.96	C_6 - H
5	2H	7.77 to 7.84	m	-	C_7 - H C_8 - H
6	1H	7.85	s	-	C_2 - H
7	1H	8.37	dm	7.82	C_5 - H

Table 3.5.15 ^{13}C NMR chemical shift assignments for **VII** (75MHz, $CDCl_3$, 300K)

Signal No.	**δ_C (ppm)**	**Assignment**
1	17.9	2 xCH_3 ($C_{2'}$ and $C_{6'}$)
2	122.6	C_{4a}
3	127.5	C_5 ; C_6 ; C_7 ; C_8
4	127.6	
5	128.8	
6	129.5	
7	134.5	$C_{2'}$ and $C_{6'}$
8	135.7	$C_{3'}$ and $C_{5'}$
9	135.8	$C_{1'}$
10	146.5	C_{8a}
11	148.3	C_2
12	160.1	C_4

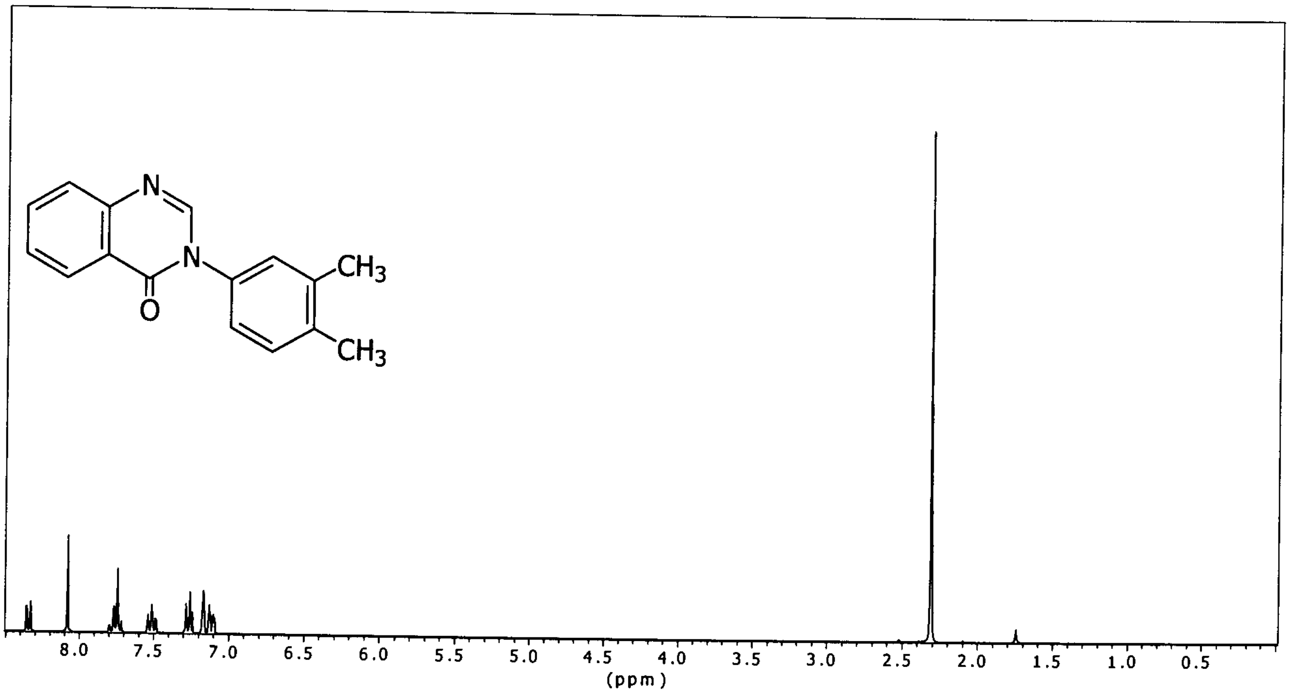


Figure 3.5.16 ^{1}H NMR Spectrum of **VIII**

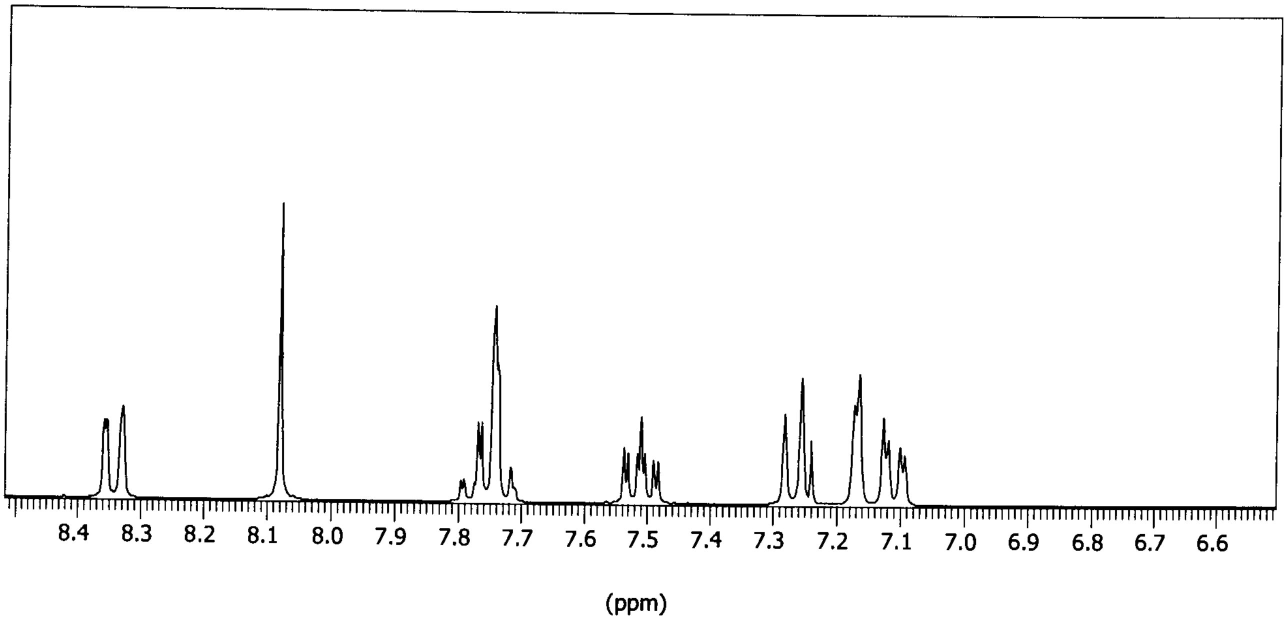


Figure 3.5.17 Expanded ^{1}H NMR Spectrum of **VIII**

Table 3.5.16 Proton NMR chemical shift assignments for **VIII** (300MHz, $CDCl_3$, 300K)

Signal No.	**Nuclei**	**δ_H (ppm)**	**Pattern**	**J (Hz)**	**Assignment**
1	6H	2.31	s	-	$C_{3'} - CH_3$ $C_{4'} - CH_3$
2	1H	7.11	dd	7.92, 2.19	$C_{6'}$ - H
3	1H	7.17	s	-	$C_{2'}$ - H
4	1H	7.27	d	7.82	$C_{5'}$ - H
5	1H	7.51	tm	7.16	C_6 - H
6	2H	7.71 to 7.79	m	-	C_7 - H C_8 - H
7	1H	8.08	s	-	C_2 - H
8	1H	8.34	dm	7.82	C_5 - H

Table 3.5.17 ^{13}C NMR chemical shift assignments for **VIII** (75MHz, $CDCl_3$, 300K)

Signal No.	**δ_C (ppm)**	**Assignment**
1	19.4	CH_3 ($C_{3'}$ or $C_{4'}$)
2	19.8	CH_3 ($C_{4'}$ or $C_{3'}$)
3	122.4	C_{4a}
4	124.1	$C_{6'}$
5	127.0	C_6 ; C_8 ; C_5
6	127.4	
7	127.5	
8	127.9	$C_{2'}$ or $C_{3'}$
9	130.6	$C_{3'}$ or $C_{2'}$
10	134.4	C_7
11	135.2	$C_{1'}$ or $C_{4'}$
12	137.9	$C_{4'}$ or $C_{1'}$
13	138.2	$C_{5'}$
14	146.3	C_{8a}
15	148	C_2
16	160.9	C_4

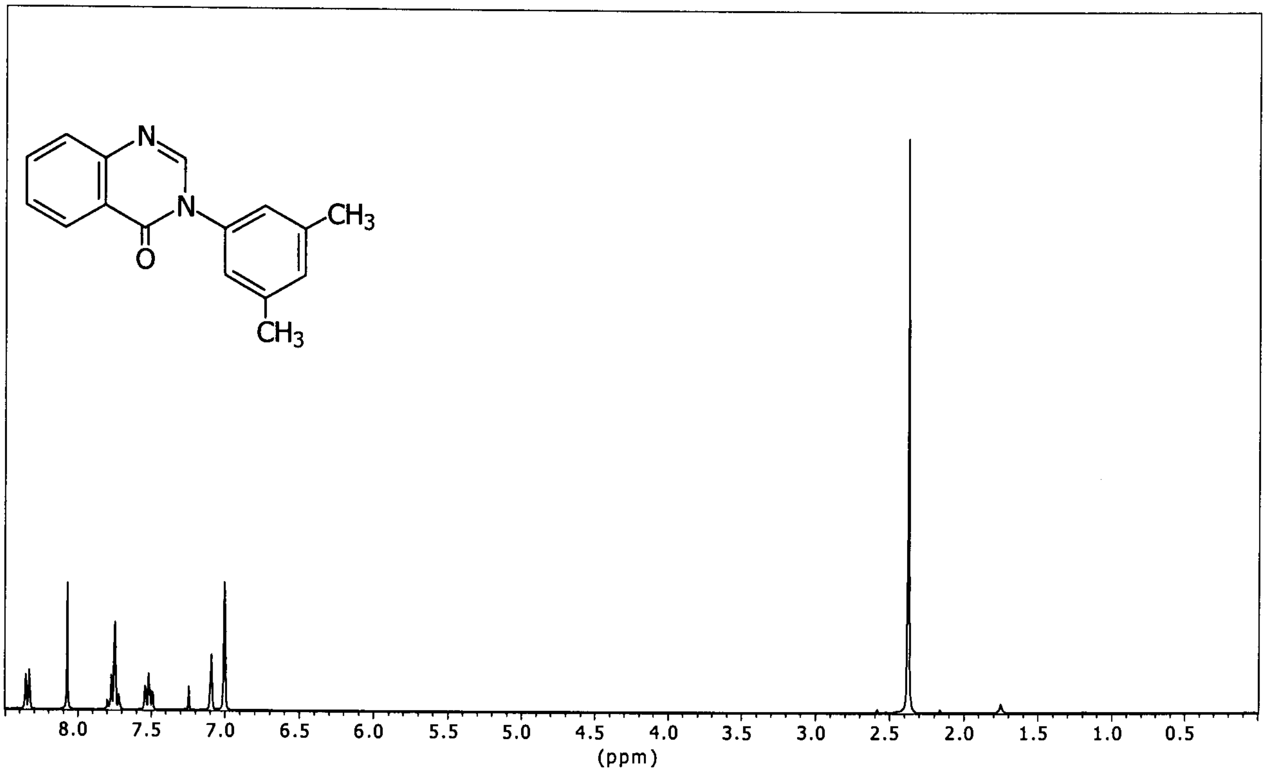


Figure 3.5.18 ^{1}H NMR Spectrum of **IX**

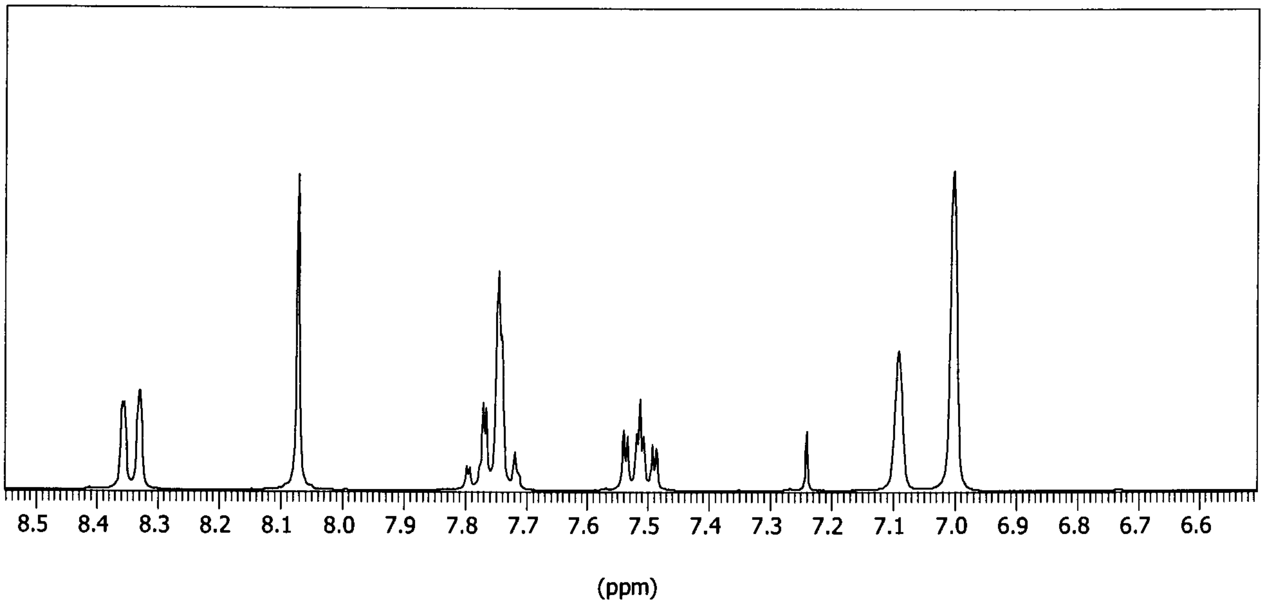


Figure 3.5.19 Expanded ^{1}H NMR Spectrum of **IX**

Table 3.5.18 Proton NMR chemical shift assignments for **IX** (300MHz, $CDCl_3$, 300K)

Signal No.	Nuclei	δ_H (ppm)	Pattern	J (Hz)	Assignment
1	6H	2.37	s	-	$C_{3'}$ - CH_3 $C_{5'}$ - CH_3
2	2H	7.00	s	-	$C_{2'}$ - H $C_{6'}$ - H
3	1H	7.09	s	-	$C_{4'}$ - H
4	1H	7.51	tm	7.15	C_6 - H
5	2H	7.71 to 7.79	m	-	C_7 - H C_8 - H
6	1H	8.07	s	-	C_2 - H
7	1H	8.34	dm	8.10	C_5 - H

Table 3.5.19 ^{13}C NMR chemical shift assignments for **IX** (75MHz, $CDCl_3$, 300K)

Signal No.	δ_C (ppm)	Assignment
1	21.6	2 x CH_3 ($C_{3'}$; $C_{5'}$)
2	122.9	C_{4a}
3	125.1	$C_{1'}$
4	127.7	
5	127.9	} C_6 ; C_8 ; C_5
6	131.2	
7	134.8	C_7
8	137.8	$C_{4'}$
9	140.0	$C_{2'}$; $C_{6'}$
10	146.7	C_{8a}
11	148.4	C_2
12	161.3	C_4

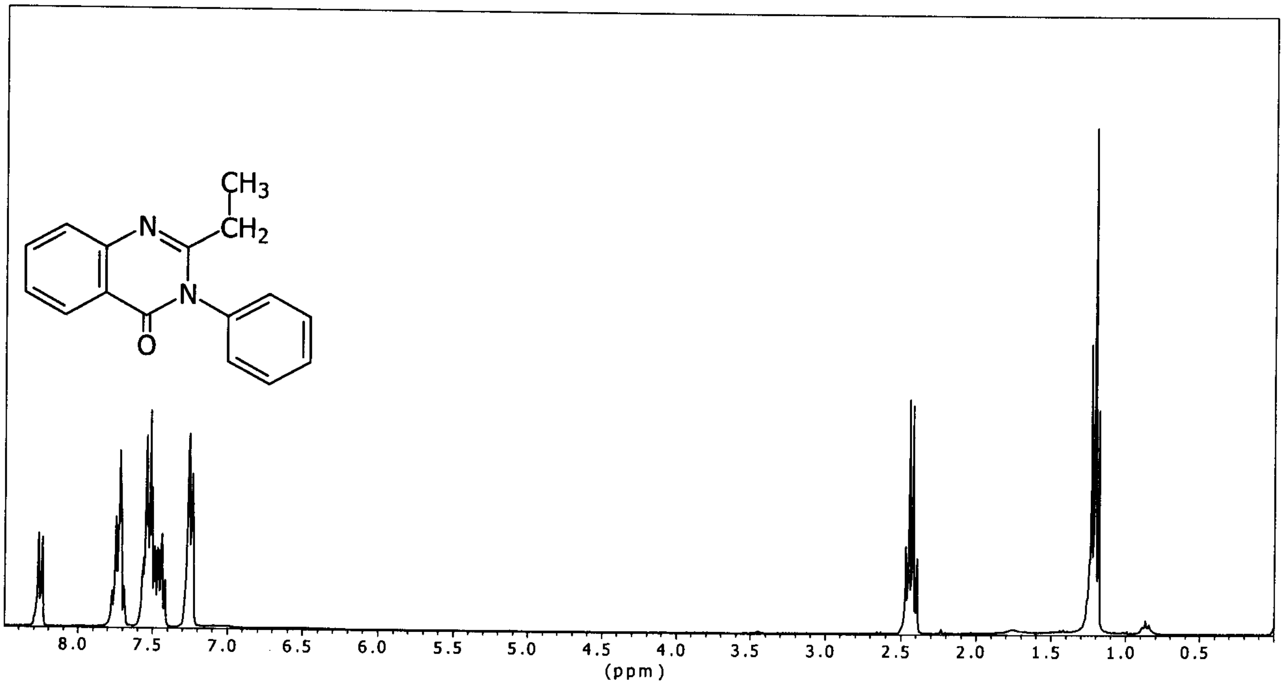


Figure 3.5.20 ^{1}H NMR Spectrum of **X**

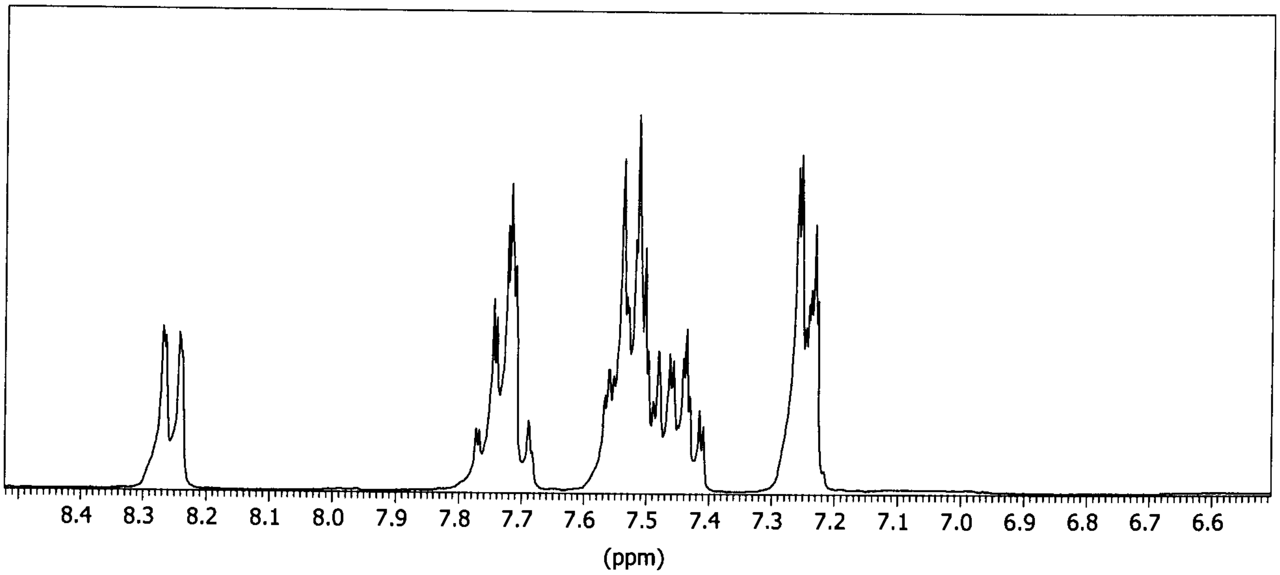


Figure 3.5.21 Expanded ^{1}H NMR Spectrum of **X**

Table 3.5.20 Proton NMR chemical shift assignments for **X** (300MHz, $CDCl_3$, 300K)

Signal No.	**Nuclei**	**δ_H (ppm)**	**Pattern**	**J (Hz)**	**Assignment**
1	3H	1.19	t	7.35	C_2 - Et(CH_3)
2	2H	2.42	q	7.38	C_2 - Et(CH_2)
3	2H	7.21 to 7.25	m	-	$C_{2'}$ - H $C_{6'}$ - H
4	4H	7.40 to 7.56	m	-	$C_{3'}$ - H $C_{4'}$ - H $C_{5'}$ - H C_6 - H
5	2H	7.67 to 7.67	m	-	C_7 - H C_8 - H
6	1H	8.25	d of t	8.01, 0.67	C_5 - H

Table 3.5.21 ^{13}C NMR chemical shift assignments for **X** (75MHz, $CDCl_3$, 300K)

Signal No.	**δ_C (ppm)**	**Assignment**
1	11.2	CH_3 (Ethyl on C_2)
2	29.3	CH_2 (C_2)
3	120.8	C_{4a}
4	126.5	C_6
5	127.1	C_5 or C_8
6	128.3	C_8 or C_5
7	129.2	$C_{2'}$; $C_{3'}$; $C_{4'}$
8	129.8	
9	134.4	C_7
10	137.4	$C_{1'}$
11	147.6	C_{8a}
12	157.7	C_2
13	162.4	C_4

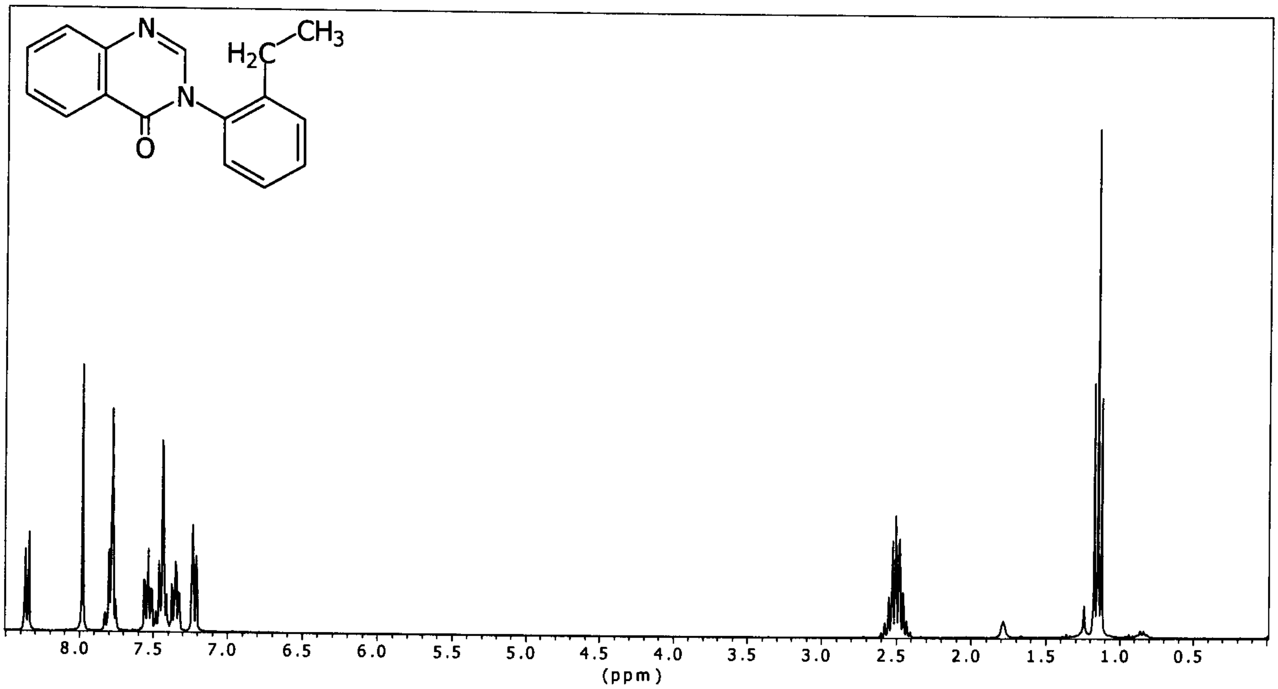


Figure 3.5.22 ^{1}H NMR Spectrum of **XI**

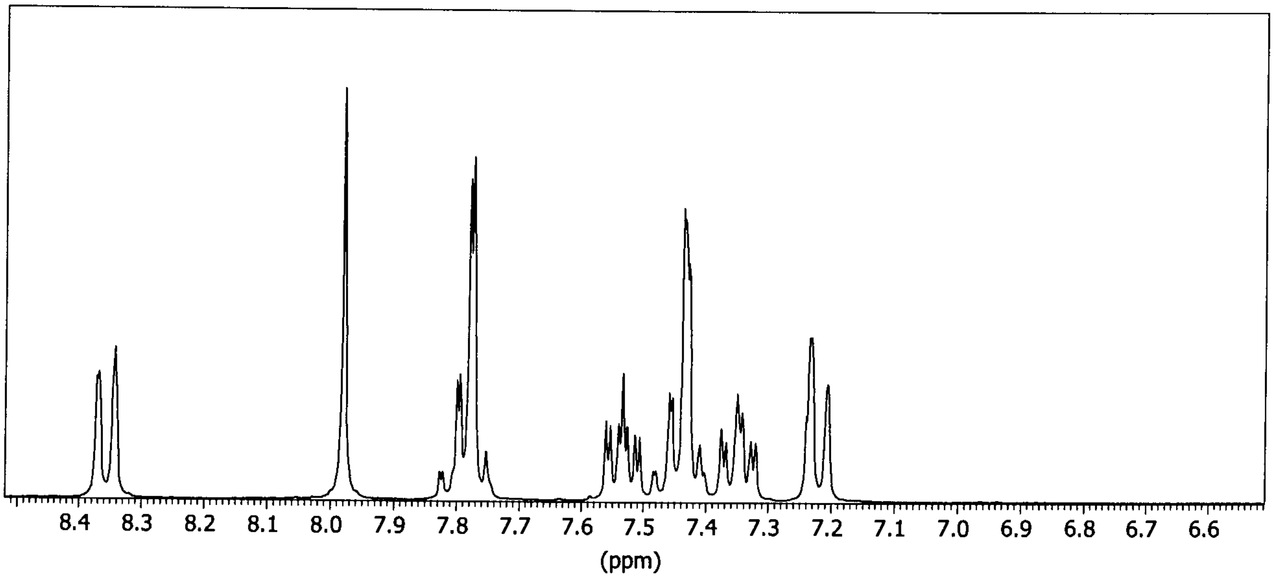


Figure 3.5.23 Expanded ^{1}H NMR Spectrum of **XI**

Table 3.5.22 Proton NMR chemical shift assignments for **XI** (300MHz, $CDCl_3$, 300K)

Signal No.	Nuclei	δ_H (ppm)	Pattern	J (Hz)	Assignment
1	3H	1.15	t	7.63	$C_{2'}$ - Et(CH_3)
2	2H	2.50	m	7.39	$C_{2'}$ - Et($CH_{2)}$
3	1H	7.22	d	7.82	$C_{6'}$ - H
4	1H	7.35	td	7.10, 2.24	$C_{4'}$ - H
5	2H	7.40 to 7.48	m	-	$C_{3'}$ - H $C_{5'}$ - H
6	1H	7.53	tm	8.11	C_6 - H
7	2H	7.56 to 7.83	m	-	C_7 - H C_8 - H
8	1H	7.98	s	-	C_2 - H
9	1H	8.36	dm	8.20	C_5 - H

Table 3.5.23 ^{13}C NMR chemical shift assignments for **XI** (75MHz, $CDCl_3$, 300K)

Signal No.	δ_C (ppm)	Assignment
1	14.2	CH_3 (Ethyl on $C_{2'}$)
2	24.0	CH_2 ($C_{2'}$)
3	122.5	C_{4a}
4	127.3	} C_6 ; C_8 ; C_5
5	127.6	
6	128.1	
7	129.5	} $C_{1'}$; $C_{3'}$; $C_{4'}$; $C_{5'}$ some overlap
8	130.0	
9	134.5	C_7
10	136.1	} $C_{2'}$; $C_{6'}$
11	141.4	
12	146.6	C_{8a}
13	148.1	C_2
14	160.7	C_4

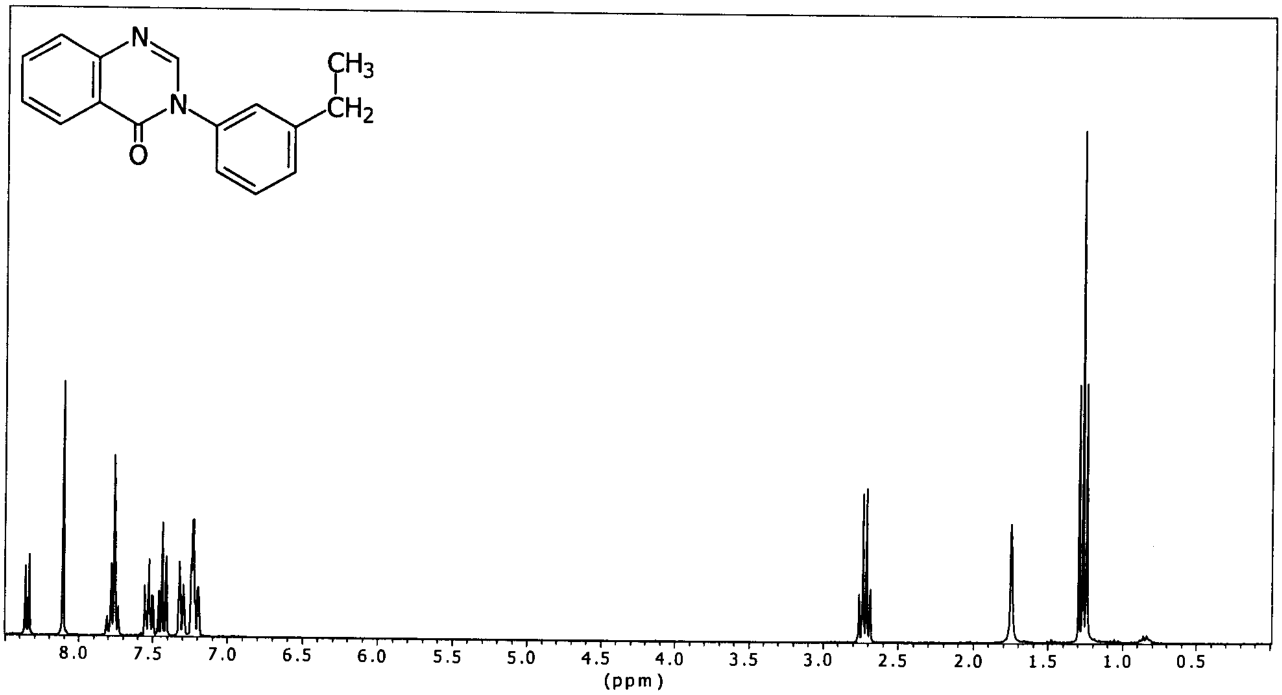


Figure 3.5.24 ^{1}H NMR Spectrum of **XII**

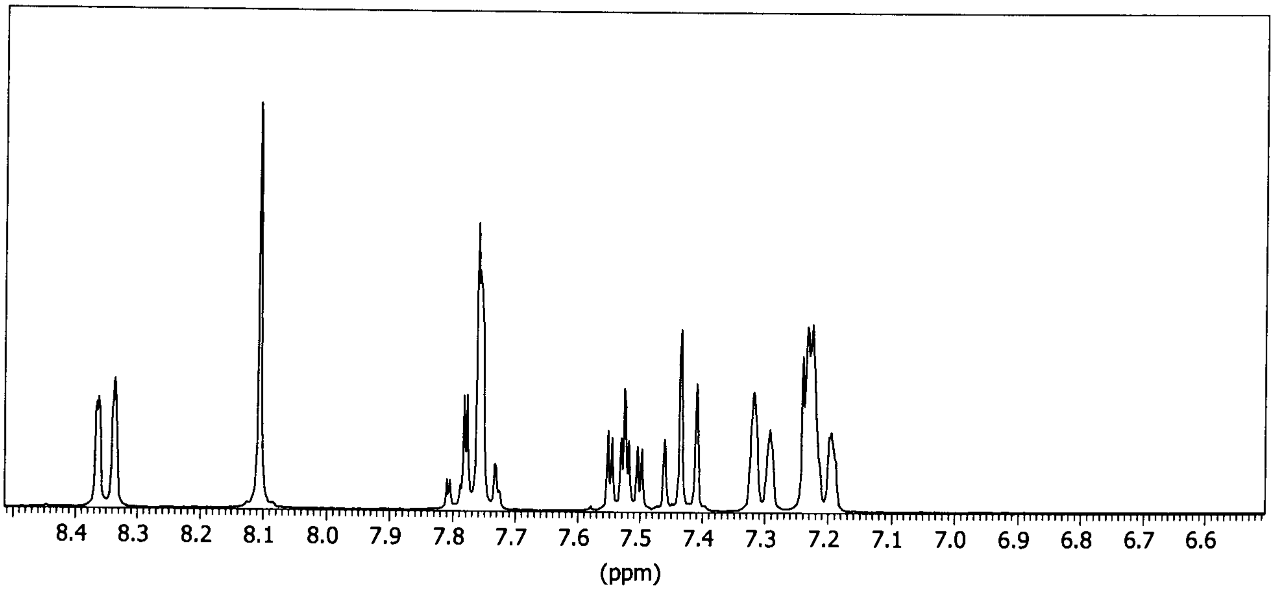


Figure 3.5.25 Expanded ^{1}H NMR Spectrum of **XII**

Table 3.5.24 Proton NMR chemical shift assignments for **XII** (300MHz, $CDCl_3$, 300K)

Signal No.	Nuclei	δ_H (ppm)	Pattern	J (Hz)	Assignment
1	3H	1.27	t	7.63	$C_{3'}$ - $Et(CH_3)$
2	2H	2.72	q	7.63	$C_{3'}$ - $Et(CH_{2)}$
3	2H	7.19 to 7.23	m	-	$C_{2'}$ - H $C_{6'}$ - H
4	1H	7.30	d	7.82	$C_{4'}$ - H
5	1H	7.43	t	7.73	$C_{5'}$ - H
6	1H	7.53	t	7.59	C_6 - H
7	2H	7.72 to 7.78	m	-	C_7 - H C_8 - H
8	1H	8.12	s	-	C_2 - H
9	1H	8.35	dm	8.11	C_5 - H

Table 3.5.25 ^{13}C NMR chemical shift assignments for **XII** (75MHz, $CDCl_3$, 300K)

Signal No.	δ_C (ppm)	Assignment
1	15.2	CH_3 (Ethyl on $C_{3'}$)
2	28.6	CH_2 ($C_{3'}$)
3	122.4	C_{4a}
4	124.2	$C_{1'}$; $C_{2'}$; $C_{3'}$; $C_{4'}$; $C_{5'}$; $C_{6'}$ *
5	126.4	C_6 ; C_8 ; C_5
6	127.5	
7	128.7	
8	129.5	$C_{1'}$; $C_{2'}$; $C_{3'}$; $C_{4'}$; $C_{5'}$; $C_{6'}$ *
9	134.5	C_7
10	137.5	$C_{5'}$ (probably)
11	146.1	C_{8a}
12	147.8	C_2
13	160.8	C_4

* = significantly overlapped

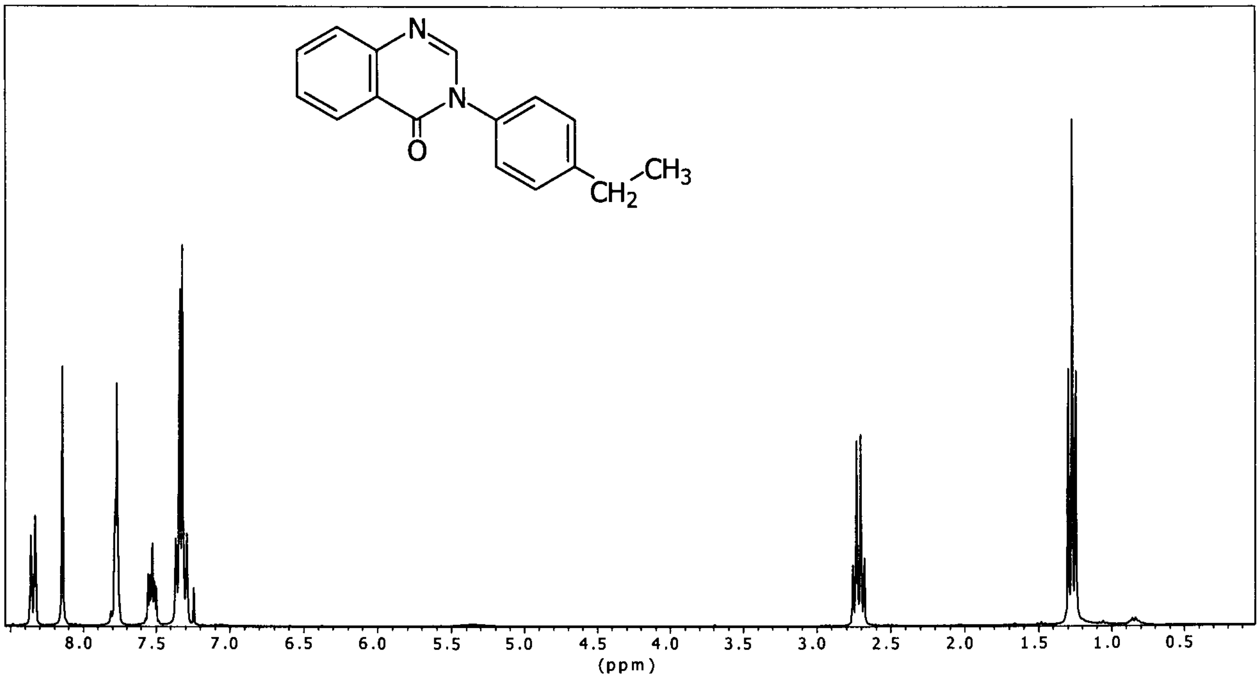


Figure 3.5.26 ^{1}H NMR Spectrum of **XIII**

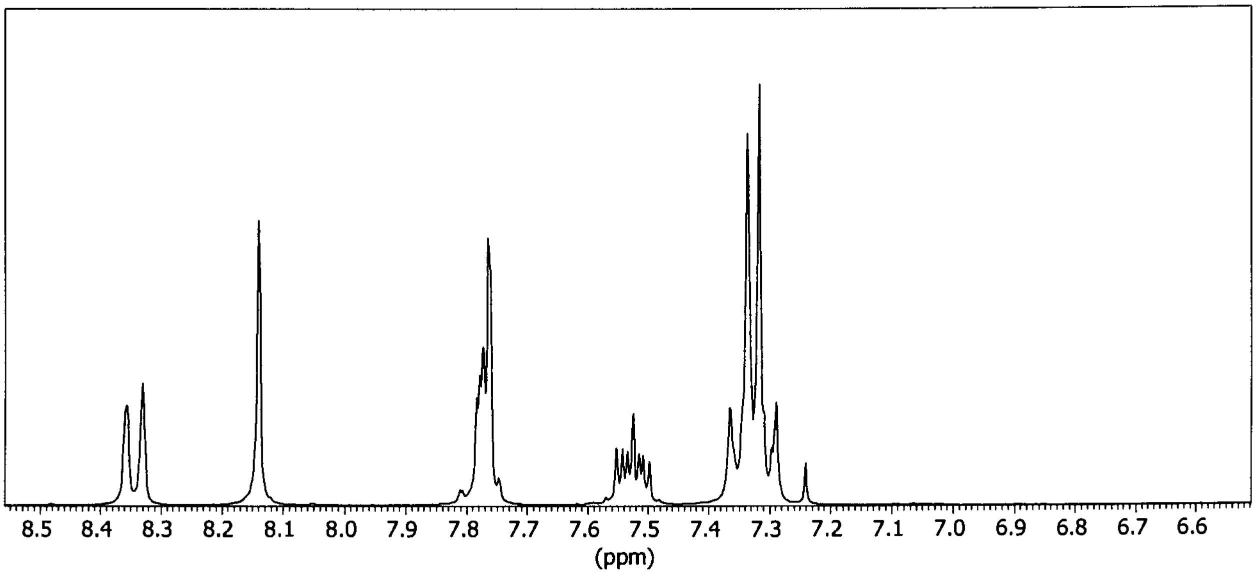


Figure 3.5.27 Expanded ^{1}H NMR Spectrum of **XIII**

Table 3.5.26 Proton NMR chemical shift assignments for **XIII** (300MHz, $CDCl_3$, 300K)

Signal No.	**Nuclei**	**δ_H (ppm)**	**Pattern**	**J (Hz)**	**Assignment**
1	3H	1.27	t	7.63	$C_{4'}$ - Et(CH_3)
2	2H	2.71	q	7.57	$C_{4'}$ - Et(CH_2)
3	4H	7.29 to 7.36	m	-	$C_{2'}$ - H $C_{3'}$ - H $C_{5'}$ - H $C_{6'}$ - H
4	1H	7.51	tm	-	C_6 - H
5	2H	7.75 to 7.81	m	-	C_7 - H C_8 - H
6	1H	8.14	s	-	C_2 - H
7	1H	8.34	d	8.01	C_5 - H

Table 3.5.27 ^{13}C NMR chemical shift assignments for **XIII** (75MHz, $CDCl_3$, 300K)

Signal No.	**δ_C (ppm)**	**Assignment**
1	15.3	CH_3 (Ethyl on $C_{4'}$)
2	28.5	CH_2 ($C_{4'}$)
3	122.3	C_{4a}
4	126.7	C_6 ; C_8 ; C_5
5	127.2	
6	127.7	
7	129.1	$C_{1'}$; $C_{2'}$; $C_{3'}$; $C_{4'}$ *
8	134.6	C_7
9	134.9	$C_{1'}$; $C_{2'}$; $C_{3'}$; $C_{4'}$ *
10	145.5	
11	146.5	C_{8a}
12	147.4	C_2
13	160.7	C_4

Discussion

The $_1$H and $_{13}$C NMR spectra of all thirteen isomeric substituted 4(3*H*)-quinazolinones were recorded and a tentative assignment of the individual signals were made. Spectral interpretation were guided by the comprehensive analysis of the NMR spectrum of **I**, reported by Blumenstein and coworkers.[69] The study by Blumenstein *et al* involved the use of highly sophisticated homonuclear and heteronuclear two-dimensional NMR techniques, so the assignments made for **I** can be taken as unambiguous, and can be used as a standard of reference. Spectra of **I** obtained in this study agree very well with those reported in reference 69. Perhaps a rather surprising result obtained in the ^{13}C NMR spectrum of **I** was that the signals for the C_5, C_6, and C_8 carbon atoms of the quinazolinone moiety appear at <u>higher</u> fields (δ_C 126.5 - 127.2) than those of the aromatic ring substituent at N(3) (δ_C 127.6 - 136.9). Simple prediction based on the electronic effects of substituents present at both rings would indicate an opposite trend in the shift values.

For identification purposes, the signals of the hydrogen atoms attached to the aromatic carbons, as well as signals of the those aromatic carbons (^{13}C NMR spectra), proved to be of little value. In the ^{1}H NMR spectra, the signals appear as multiplets (d, dt, m, etc.), and in any mixture of the isomers extensive overlap would make any identification very difficult without additional spectroscopic experiments. Similarly, in the ^{13}C NMR spectra, the carbons of each isomer give rise to up to fourteen signals of the sp^2 hybridized carbons, subjected to weak and difficult to predict substituent effect. The observed NMR signals of some of those carbons (C_4, C_{8a}, C_{4a}) stay remarkably constant through the series, with others (C_5, C_6, C_7, C_8, $C_{7'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$) varying in such a way that the unambiguous differentiation of **I** - **XIII**, based on these signals alone, would prove very difficult.

Of much higher analytical value are the signals derived from the alkyl (methyl and ethyl) substituents. As can be seen from Tables 3.5.2 - 3.5.26, the methyl groups at C_2 yield singlets at δ_H 2.16 - 2.23, the methyl groups at $C_{2'}$ yield singlets at δ_H 2.05 - 2.14, the methyl groups at $C_{3'}$ yield singlets at δ_H 2.36 - 2.40, and the methyl groups at $C_{4'}$ yield singlets at δ_H 2.31 - 2.42. These ranges of δ_H values therefore offer some means of identification and differentiation of individual isomers.

For **X** - **XIII** the proton NMR signals of the ethyl groups yield a **"t + q"** pattern, but again the triplets of the methyl groups appear at their characteristic δ_H values: 1.19; 1.15; 1.27; 1.27, for **X**, **XI**, **XII**, and **XIII** respectively.

The ^{13}C NMR signals can also be used for the (limited) identification of **I** - **XIII**. The δ_C for the methyl group at C_2 is 23.8 - 24.3; at $C_{2'}$ is 14.3 - 17.9; at $C_{3'}$ is 19.8 - 21.6; and at $C_{4'}$ is 19.4 - 21.2. For the ethyl derivatives (**X** - **XIII**), the δ_C values for the methyl group are: 11.2 (**X**); 14.2 (**XI**); 15.2 (**XII**); and 15.3 (**XIII**), thus differing sufficiently to allow differentiation between **X** and **XI**, and also to differentiate **X** and **XI** from **XII** or **XIII**.

Conclusion

In conclusion, although the high-resolution ^1H and ^{13}C spectra of compound **I** - **XIII** display many similarities, it can provide useful information in many instances. All shift differences discussed above would increase with the increase of frequency of the instrument used, and thus improve its usefulness. A detailed comparison of spectra over the full resonance range, taking into account both observed chemical shifts, as well as resonance patterns, allowed for the unequivocal differentiation of all isomers studied.

CHAPTER 4

Final Conclusion

General Review and Comments

All target compounds included in this study could be prepared using the modified synthetic route described. The modified route lends itself well to the rapid laboratory scale preparation of quinazolinones. The isolation and purification procedures described allowed for the isolation of sufficiently pure samples of all target compounds to enable effective characterization using molecular spectroscopic methods. Eight previously unreported "designer" isomers of **I** were prepared and fully characterized using IR, EI-MS, ^{1}H-NMR, and ^{13}C-NMR. Melting points are given.

IR, EI-MS, and NMR allowed for the unequivocal differentiation of all thirteen isomers studied. EI-MS was found to be the least specific of these methods, with differentiation of groups of positional isomers based on the relative abundance of selected ions. All three molecular spectroscopy techniques unambiguously differentiated methaqualone (**I**) from the other isomers studied.

The combined selectivity and specificity of automated GC-MS allowed for the rapid separation and unequivocal identification of all isomers. This, together with the inherent sensitivity of the technique, makes it the technique of choice for the routine, high volume forensic identification of methaqualone in seized materials. A prerequisite for the use of GC-MS in this manner however would be the analysis of authenticated standards of **I** - **XIII**, and identification based on both retention time and mass spectral data. It is further advised that the selectivity of any GC-MS method employed to determine **I**, be validated for **I** in the presence of **II** - **XIII**. In the absence of authenticated standards of **I** - **XIII**, the identity of an unknown may be unequivocally established through comparison of the NMR and/or IR spectral data with the data reported in this study.

The TLC , LC and UV spectroscopy proved of no value in differentiating the isomers studied. LC and UV spectroscopy may have application in the quantitation of the isomers, once their identity has been unambiguously established.

The NMR (^{1}H and ^{13}C) spectroscopy gave interesting results, indicating that in some cases the technique could prove useful in both identification of the individual isomers, as well as distinguishing between two (or more) of the isomers present in a mixture.

Future Work

Future work expanding on this study should include -

- characterizing the newly reported compounds using elemental analysis and crystallographic techniques;
- conducting more advanced NMR experiments at higher frequencies to allow for the rigorous assignment of all observed signals in the spectra of **I** - **XIII**;
- conducting low-temperature NMR studies to evaluate the hindered restriction of the rotation of the various substituted aryl moieties around the C - N bond;
- enantiomeric separation and isolation of the various possible rotational isomers;
- evaluating the biological activity of the isolated isomers of **I** - **XIII**;
- preparing analogous isomers to **I** - **XIII** of the type:

  2-aryl-4(3*H*)-quinazolinones
  2-aryl-3-alkyl-4(3*H*)-quinazolinone
  1-aryl-4(1*H*)-quinazolinones
  1-aryl-2-alkyl-4(1*H*)-quinazolinone
  2-aryl-4(1*H*)-quinazolinones
  2-aryl-1-alkyl-4(1*H*)-quinazolinone

APPENDIX A

Part 1

TABLE 1: Summary of reported synthesis of some 4(3H)-quinazolinones

Table 1: Summary of reported synthesis of some 4(3H)-quinazolinones

Nr	Ref	Year	Target	Precursors	Intermediates	Reagents & Solvents	Isolation	Recryst. Solvent	Duration (H)	Yield (%)	MP (° C)
1	15	1946	III	N-Acetylanthranilic acid *p*-Toluidine	-	MePh PCl_3 Na_2CO_3	Basified with Na_2CO_3(aq). Steam distilled. Filtered. Washed with water. Dried at 60 ° C.	-	3 - 5	68	147 - 149
2	1	1951	I II X	N-Acetylanthranilic acid *o*-Toluidine *m*-Toluidine N-Propanoylanthranilic acid Aniline	-	MePh PCl_3 Na_2CO_3 EtOH	Basified with Na_2CO_3(aq). Steam distilled. I & II were semi-solid. Isolated by triturating with EtOH, or as hydrochlorides by passing HCl gas through ethereal or ethanolic solutions. Bases regenerated with cold dilute ammonium hydroxide. Recrystallized from EtOH.	EtOH	3 - 5	I;48 II;60 X;80	I;115 - 116 II;126 X;126 - 127
3	33	1956	III	*p*-Methylacetophenone oxime Methylanthranilate	di-*o*-tolylacetamidine	$SOCl_2$ $CHCl_3$ Alkalising agent	Made alkaline and steam distilled	-	14 - 16	69	148 - 150
4	16	1960	I	N-Acetylanthranilic acid *o*-Toluidine	-	MePh $POCl_3$ NaOH HCl EtOH	Cooled, filtered, and dissolved in dilute HCl. Cooled, made alkaline with NaOH to let viscous oil separate that crystallized after few hours. Allowed to stand overnight, crystals collected and recrystallized from EtOH.	EtOH	14 - 16	80	114 - 115
5	16	1960	I	Anthranilic acid Acetic anhydride *o*-Toluidine	Acetanthranil	HCl NaOH Carbon EtOH	Cooled to 100 ° C. Dilute HCl added, mixture boiled and stirred. Neutralized with NaOH with stirring. Crystals dissolved in EtOH and decolourised over activated carbon. Recrystallized twice from EtOH.	EtOH	4 - 6	70	114 - 115
6	34	1961	I II III	N-*o*-tolyl-*o*-nitrobenzamide N-*m*-tolyl-*o*-nitrobenzamide N-*p*-tolyl-*o*-nitrobenzamide Acetylating agent	N-substituted-*o*-aminobenzamide N-substituted-N-*o*-acylaminobenzamide	$SOCl_2$, or H_2SO_4, or $(CH_3COO)_2O$	-	-	-	-	I;118 - 119 II;126 - 132 III;149 - 151
7	17	1963	I	Acetanthranil *o*-Toluidine	-	Benzene/MePh/MeCl K_2CO_3 EtOH/*i*-PropOH	Solvent distilled under vacuum, and water added to residue. Mixture steam distilled. Product isolated and recrystallized from EtOH or *i*-PropOH.	EtOH or *i*-PropOH	4 - 6	74	115
8	17	1963	I	N-Acetylanthranilic acid *o*-Toluidine	-	MePh PCl_3 EtOH/*i*-PropOH	Cooled. Product recrystallized from EtOH or *i*-PropOH.	EtOH or *i*-PropOH	3 - 5	74	115
9	36	1964	I	N-Acetylanthranilic acid N-*o*-tolylisocyanate, or N-*o*-tolylisothiocyanate, or N-*o*-tolylurea, or N-*o*-tolylthiourea, or N-*o*-tolylthiourethane, or N-*o*-tolyldithiourethane.	-	DiMePh or NitroPh	-	-	-	-	114 - 115
10	28	1965	I	Acetylanthranil *o*-Toluidine	-	None	Slowly heated to 150 ° C to remove excess *o*-toluidine	-	-	45	-

APPENDIX A: Part 1 - Table 1 Summary of reported synthesis of some 4(3H)-quinazolinones cont.

Nr	Ref	Year	Target	Precursors	Intermediates	Reagents & Solvents	Isolation	Recryst. Solvent	Duration (H)	Yield (%)	MP (°C)
11	18	1965	I	N-Acetylanthranilic acid *o*-Toluidine	-	H_3PO_4 Activated C Na_2CO_3 MeOH	Water was added and mixture stirred over activated C. Filtered. Basified with Na_2CO_3(aq) to pH 8-9. Product filtered of, washed, dried, and recrystallized from hot MeOH.	MeOH	3 - 5	-	-
12	18	1965	I	Anthranilic acid Acetic acid *o*-Toluidine	-	H_3PO_4 Activated C Na_2CO_3 MeOH	Reaction mixture poured into water and stirred over activated C. Filtered. Filtrate basified with Na_2CO_3(aq) to give oil that crystallized on standing. Filtered, washed with water, dried and recrystallized from hot MeOH	MeOH	-	62-	113 - 115
13	25	1965	I X	Anthranilic acid Acetic anhydride *o*-Toluidine Propionic anhydride Aniline	Acetanthranil Formylanthranil	PhCl $POCl_3$ NaOH Na_2CO_3 HCl Activated C	Cooled and water added. NaOH(aq) and Na_2CO_3(aq) was added to Brilliant Yellow alkalinity. Steam distilled, cooled to yield crude product. Dissolved in dilute HCl and treated with activated C for 1 hour. Filtered with filtrate allowed to run into water basified with NaOH. Filtered, washed with water, and dried.	-	8 - 10	I;90 X;91.5	I;116 - 117 X;133-134
14	23	1965	I I.HCl	N-Acetylanthranilic acid *o*-Toluidine.HCl	-	HCl NaOH EtOH Et_2O	Dissolved in concentrated HCl, filtered, and hot solution made alkaline with 50% NaOH(aq). Cooled with stirring. Precipitate collected and water washed until neutral. Dried and dissolved in EtOH. Addition of EtOH HCl caused precipitation. Completed by addition of Et_2O. Filtered and washed with 1:1 Et_2O/EtOH to yield HCl. Freebase prepared from salt.	Acidic EtOH/Et_2O	2 - 4	I.HCl;72	I.HCl; 250 -256 I;113 - 114
15	37	1965	I	Methylanthranilate N,N-dimagnesiumbromido-*o*-toluidine Acetic anhydride	N-*o*-tolylanthranilamide	Na-acetate (Calcinated) NaOH EtOH	Reaction mixture poured into water. Crystalline NaOH added to weakly alkaline, and resulting precipitate filtered off. Recrystallized from EtOH.	EtOH	3 - 4	95.3	113 - 114
16	26	1966	I	Anthranilic acid Acetic anhydride *o*-Toluidine	N-acetylanthranilic acid	H_3PO_4 P_2O_5 Na_2CO_3 HCl Activated C NH_4OH	Cooled reaction mixture poured into water and neutralized with Na_2CO_3. The precipitate washed and dissolved in 10% HCl. After clarifying over activated C, concentrated HCl was added and mixture allowed to stand overnight to yield I.HCl. Salt mixed with 10% NH_4OH to liberate bases	-	12 - 15	-	115 - 116
17	38	1967	XI.HCl	N-formylanthranilic acid *o*-Ethylaniline	-	MePh $POCl_3$ EtOH HCl	Solvent decanted off and the product taken up in EtOH. Solution acidified with concentrated HCl and cooled in fridge. Crystals collected through filtration.	EtOH	3 - 4	-	XI.HCl; 165 - 170
18	38	1967	XI.HCl	Anthranilic acid N-formyl-*o*-ethylaniline	-	Na_2CO_3 HCl	Na_2CO_3 was added, boiled for several minutes, cooled, and filtered to yield semi-solid base. Base was converted to HCl - salt with concentrated HCl.	-	3 - 4	-	XI.HCl; 165 - 170
19	38	1967	XI.HCl	*o*-Ethylphenylanthranilamide *o*-Ethylformate	-	HCl	Reaction mixture treated with concentrated HCl. Base was semi-solid.	-	3 - 4	-	XI.HCl; 165 - 170
20	39	1969	X	N-propionyl-o-methylanthranilate N,N-dimagnesiumhalidoaniline	N-propionyl-*o*-phenylanthranilamide	None	-	-	-	85	126 - 127

APPENDIX A: Part 1 - Table 1 Summary of reported synthesis of some 4(3H)-quinazolinones cont.

Nr	Ref	Year	Target	Precursors	Intermediates	Reagents & Solvents	Isolation	Recryst. Solvent	Duration (H)	Yield (%)	MP (°C)
21	27	1969	I	Anthranilic acid Acetic anhydride *o*-Toluidine	Acetanthranil	MePh PCl_3 NaOH EtOH	Mixture neutralized with NaOH. Organic layer washed with water until neutral. Steam distilled, and product recrystallized from EtOH	EtOH	-	87	114 - 116
22	29	1976	I III	Acetanthranil *o*-Toluidine *p*-Toluidine	-	Benzene, or Et_2O Basifying agent	Taken directly to dryness at elevated temperature and under vacuum. Residue leached with aqueous base, and extract allowed to stand overnight.	-	12 - 15	-	I;113 - 114 III;151-152
23	40	1976	I-d	Phtalimide - 3,4,5,6-d_4 Acetic anhydride *o*-Toluidine	Anthranilic acid - 3,4,5,6-d_4	$NaOH/Br_2/HCl$ Acetic acid $MePh/POCl_3$ $Na_2CO_3/MeOH$ Activated C Hexane	Reaction mixture poured into Na_2CO_3 solution - solid washed into solution with MeOH. Steam distilled to leave oil that crystallized on standing. Crystals washed with water, taken up in hot MeOH, treated with activated C, and filtered. To boiling filtrate was added water until turbid. Crystals washed with water and chromatographed on basic alumina with $CHCl_3$ as eluent. Recrystallized from hexane/MeOH.	Hexane/ MeOH	10 - 12	-	115 - 116
24	30	1977	I.HCl	Isatoic anhydride *o*-Toluidine Acetylacetone	N-(*o*-tolyl)-anthranilamide	Et_2O CH_2Cl_2/Hexane EtOH HCl	Reaction mixture cooled.	-	6 - 8	85	I.HCl; 235 - 237
25	30	1977	I.HCl	Isatoic anhydride *o*-Toluidine Acetylacetone	N-(*o*-tolyl)-anthranilamide	MePh HCl	Solvent evaporated.	-	4 - 6	80	-
26	19	1978	I	N-Acetylanthranilic acid *o*-Toluidine	-	BrPh Benzene HCl Et_2O NaOH Benzene/Light Petroleum Ether	Half of solvent distilled off, benzene added, and filtered. Filtrate extracted with 1:1 aqueous HCl. Acidic layer extracted with ether and made alkaline with NaOH. Product solidified on standing. Filtered, washed with water, dried, and recrystallized from benzene/light petroleum ether.	Benzene/ Light Petroleum Ether	6 - 8	48.4	112.5-113
27	20	1979	I	N-Acetylanthranilic acid *o*-Toluidine	-	$POCl_3$ MePh Basifying Agent	Product isolated as salt and dissolved in water. Repeatedly extracted with toluene, and basified.	-	-	-	-
28	11	1979	I	N-Acetylanthranilic acid *o*-Toluidine	-	-	Recrystallized after cooling.	-	2 - 3	60	112.5-113
29	31	1980	I	Isatoic anhydride Acetylating agent *o*-Toluidine	Anthranilic acid	Basifying agent Acidifying agent	-	-	-	-	-
30	31	1980	I	Isatoic anhydride *o*-Toluidine Acetic anhydride	N-(*o*-tolyl)-anthranilamide	$POCl_3$	-	-	-	-	-

APPENDIX A: Part 1 - Table 1 Summary of reported synthesis of some 4(3H)-quinazolinones cont.

Nr	Ref	Year	Target	Precursors	Intermediates	Reagents & Solvents	Isolation	Recryst. Solvent	Duration (H)	Yield (%)	MP (°C)
31	41	1980	I	N-Acetylanthranilate *o*-Toluidine.HCl	-	P_2O_5 N,N-dimethyl-cyclohexylamine NaOH CH_2Cl_2/EtOH	Cooled to 100 ° C and 2 M NaOH(aq) added until alkaline. Stirred for 1 hour and aqueous phase extracted thrice with CH_2Cl_2. Evaporated and distilled at 10mmHg. Residue recrystallized from EtOH.	EtOH	3 - 5	84	113 -114
33	21	1981	I II III	N-Acetylanthranilic acid *o*-Toluidine *m*-Toluidine *p*-Toluidine	-	MePh PCl_3 MeOH $CHCl_3$ HCl/NaOH	Cooled to room temperature. Reaction product dissolved in hot MeOH and filtered. Distilled water added until turbid, and allowed to sit to complete crystal formation. Additionally - $CHCl_3$ extracted from acidic or alkaline aqueous solution.	MeOH/H_2O	3 - 5	-	I;115 - 117 II;127 - 129 III;149 - 151
34	42	1984	I.HCl	N-Acetylanthranilic acid *o*-Toluidine	-	MePh $CHCl_3$/$POCl_3$ MeOH/Acetone	Mixture decomposed with MeOH and acetone. Allowed to sit to complete crystal formation. Filtered and washed with acetone.	-	3 - 5	I.HCl;76.7	-
35	24	1987	I II III	*o*-Toluidine.HCl 2-Acetylaminobenzonitrile	-	P_2O_5 N,N-diMeCyclHex-amine.HCl NaOH CH_2Cl_2/MeOH	Cooled to 100 ° C and 2 M NaOH(aq) added until alkaline. Stirred until reaction cake digested and aqueous phase extracted thrice with CH_2Cl_2. Evaporated and distilled at 10mmHg. Residue recrystallized from MeOH.	MeOH	3 - 5	I;53 II;40 III;33	-
36	20	1988	I	Sodium-N-Acetylanthranilate *o*-Toluidine	-	MePh PCl_3	-	-	-	-	-
37	12	1990	I II	N-Acetylanthranilic acid *o*-Toluidine *p*-Toluidine	-	MePh/PCl_3 $NaHCO_3$ $CHCl_3$/ $MgSO_4$ *i*-PropOH	Cooled to room temperature and solid collected. Suspended in water and neutralised with aqueous $NaHCO_3$. Water added and suspension extracted twice with $CHCl_3$. Organic extract dried and solvent stripped to leave viscous oil that crystallized on standing. Product recrystallized from *i*-PropOH.	*i*-PropOH	4 - 6	I;22.8	I;114 - 115 II;147 - 148
38	32	1994	I II X	N-Acetylanthranilic acid *o*-Toluidine *m*-Toluidine N-Propionylanthranilic acid Aniline	2-Acylamino-(N-substituted)-benzamide	TosCl/Pyridine $NaHCO_3$ CH_2Cl_2/Na_2SO_4 n-Heptane	Reaction mixture poured in aqueous $NaHCO_3$ with stirring. Solution extracted thrice with CH_2Cl_2 washed with water, and dried. Solvent evaporated and solid recystallized.	N-Heptane	3 - 5	I;75 III;80 X;68	I;113 - 114 III;148 -150 X;125 - 126
39	35	1997	I	Isatoic anhydride *o*-Toluidine Acetylating agent	2-Amino-(N-o-tolyl)-benzamide 2-Methylamido-(N-o-tolyl)-benzamide	AcCN/Benzene Activated C TsOH/$NaHCO_3$ Petroleum Ether	Cooled to room temperature and residue filtered off. Organic layer washed with dilute $NaHCO_3$ solution, solvent stripped and crude product recrystallized	Benzene Benzene/ Petroleum Ether	18 - 20	54	113 - 114
40	32	1997	I	2-Amino-(N-o-tolyl)-benzamide Acetylating agent	2-Methylamido-(N-o-tolyl)-benzamide	Halogenated trialkylsilane Base	-	-	-	-	116.5 - 117.5

Note: Recryst. Solvent = Recrystallization Solvent

APPENDIX A

Part 2

Reported synthesis of some 4(3*H*)-quinazolinones of interest to this study

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 1[15]

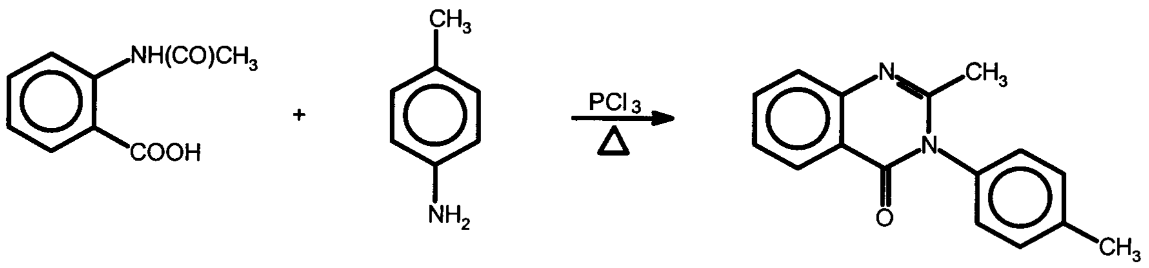
Target compounds relevant to this study

a) 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone

Method

In a 500 millilitre 3-necked flask equipped with stirrer, reflux condenser, dropping funnel and mounted in an oil-bath were placed 17.9 gram (0.1 mole) of N-acetylanthranilic acid **(I)**, an equimolar amount of *p*-toluidine **(II)**, and 175 millilitre of toluene. The mixture was stirred and 4.6 gram (0.0334 mole) PCl_3 in 25 millilitre toluene was added dropwise over a period of fifteen minutes. The resulting suspension was refluxed with stirring for 2 hours. The reaction mixture was transferred to a bigger flask and treated with 200 millilitre of a 10% (m/v) aqueous sodium carbonate solution. Toluene was removed through steam distillation and the crude product obtained through filtration. The product was washed with water and dried at 60 ° C to yield 68% 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone **(III)** , with melting point 147-149 ° C.

Reaction scheme



I II III

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 2[1]

Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone
b) 2-methyl-3-*m*-tolyl-4(3*H*)-quinazolinone
c) 2-ethyl-3-phenyl-4(3*H*)-quinazolinone

Method

In a 500 millilitre 3-necked flask equipped with mechanical stirrer, reflux condenser, dropping funnel and mounted in an oil-bath were placed 0.05 mole of N-acylanthranilic acid **(I)**, an equimolar amount of primary aromatic amine **(II)**, and 180 millilitre of dry toluene. The mixture was stirred and a mixture of 0.017 mole PCl_3 in 12 millilitre toluene was added dropwise over a period of fifteen minutes. The resulting suspension was refluxed with stirring for 2 hours in an oil bath at 130-135 ° C. The reaction mixture was transferred to a bigger flask and treated with approximately 100 millilitre of a 10% (m/v) aqueous sodium carbonate solution, and the toluene removed through steam distillation. After cooling the supernatant was removed via filtration or decantation, and the crude product collected. Products resulting from *o*- and *m*-substituted aromatic amines formed as semi-solids, and were isolated after triturating it with ethanol, or as crystalline hydrochlorides by passing dry HCl through ethanolic or ethereal solutions of the semi-solid product. The bases were regenerated by treating the hydrochlorides with cold dilute ammonium hydroxide solution, and recrystallization from dilute ethanol.

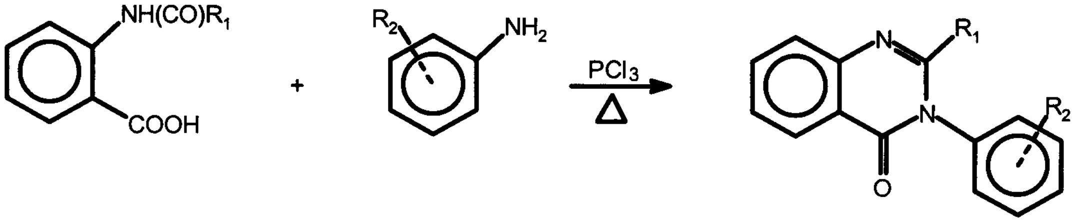
The reaction of N-acetylanthranilic acid with *o*-toluidine yielded 48% 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**, with melting point 115-116 ° C.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

The reaction of N-acetylanthranilic acid with *m*-toluidine yielded 60% 2-methyl-3-*m*-tolyl-4(3*H*)-quinazolinone **(IV)**, with melting point 126 ° C.

The reaction of N-propionylanthranilic acid with aniline yielded 80% 2-ethyl-3-phenyl-4(3*H*)-quinazolinone **(V)**, with melting point 126-127 ° C.

Reaction scheme



I

II

III $R_1 = CH_3$, $R_2 = o\text{-}CH_3$

IV $R_1 = CH_3$, $R_2 = m\text{-}CH_3$

V $R_1 = CH_2CH_3$, $R_2 = H$

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 3 [33]

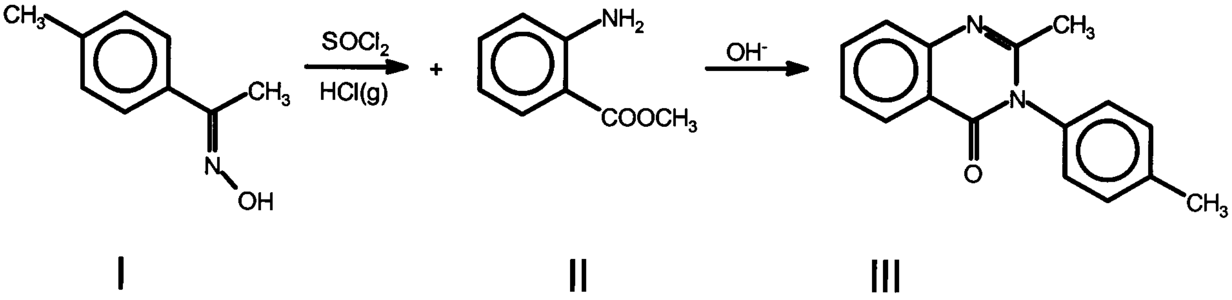
Target compounds relevant to this study

a) 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone

Method

4-Methylacetophenone oxime **(I)** in chloroform was warmed to 35 ° C with $SOCl_2$. Dry HCl was passed through the solution for 1-2 hours until it had acquired a deep yellow colour. The resulting precipitate was filtered off. To the filtrate was added methyl-anthranilate **(II)**. The mixture was kept for 12 hours at room temperature, made alkaline and steam distilled to yield 69% 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone **(III)**, with melting point 148-150 ° C.

Reaction scheme



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 4[16]

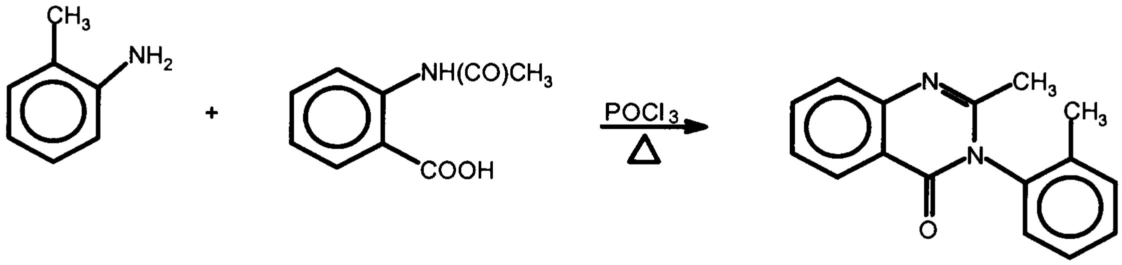
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

o-Toluidine **(I)** (1 part) was mixed with a solution of N-acetylanthranilic acid **(II)** (2 parts) in toluene (3 parts) in a vessel with a stirrer and a means of cooling. A solution of phosphorus oxychloride (1 part) in toluene (3 parts) was added dropwise with stirring and the temperature raised to boiling point for 2 hours with further stirring. After cooling the precipitate was filtered of, dried, and dissolved in boiling dilute hydrochloric acid. On cooling and making alkaline with with NaOH, a viscous oil separated that crystallized after a few hours. The crystals was allowed to collect overnight, after which it was collected and purified by recrystallization from alcohol to yield 80% 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)** with melting point 114-115 °C.

Reaction scheme



I II III

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 5[16]

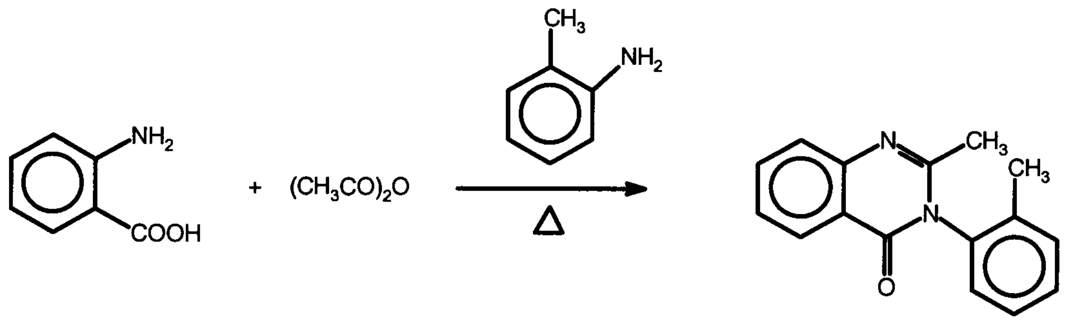
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

Anthranilic acid **(I)** (1 part) was dissolved in acetic anhydride **(II)** (2 parts) and the temperature raised progressively to 190-200 ° C while distillation took place. The last traces of acetic acid were removed under vacuum, and after the mixture was allowed to cool down to 50-60 ° C, *o*-toluidine **(III)** (1 part) was added in portions. The temperature was raised to 170-200 ° C and maintained for 2 hours. After allowing the mixture to cool down to about 100 ° C, dilute hydrochloric acid (3 parts) was added and the mixture boiled and stirred. The solution was then neutralized with with NaOH with stirring. The product that crystallized was collected and recrystallized twice from ethanol after decolourising with carbon to yield 70 % 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(IV)** with melting point 114-115 ° C.

Reaction scheme



I II III IV

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 6[34]

Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone
b) 2-methyl-3-*m*-tolyl-4(3*H*)-quinazolinone
c) 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone

Method

N-substituted-*o*-nitrobenzamide **(I)** was hydrogenated over Raney Ni to give the corresponding N-substituted-*o*-aminobenzamide **(II). II** was acelyted to give the N-substituted- N-acylaminobenzamide **(III). III** was dehydrated with $SOCl_2$, H_2SO_4, or acetic anhydride to yield the corresponding 3-substituted-2-methyl-4(3*H*)-quinazolinone.

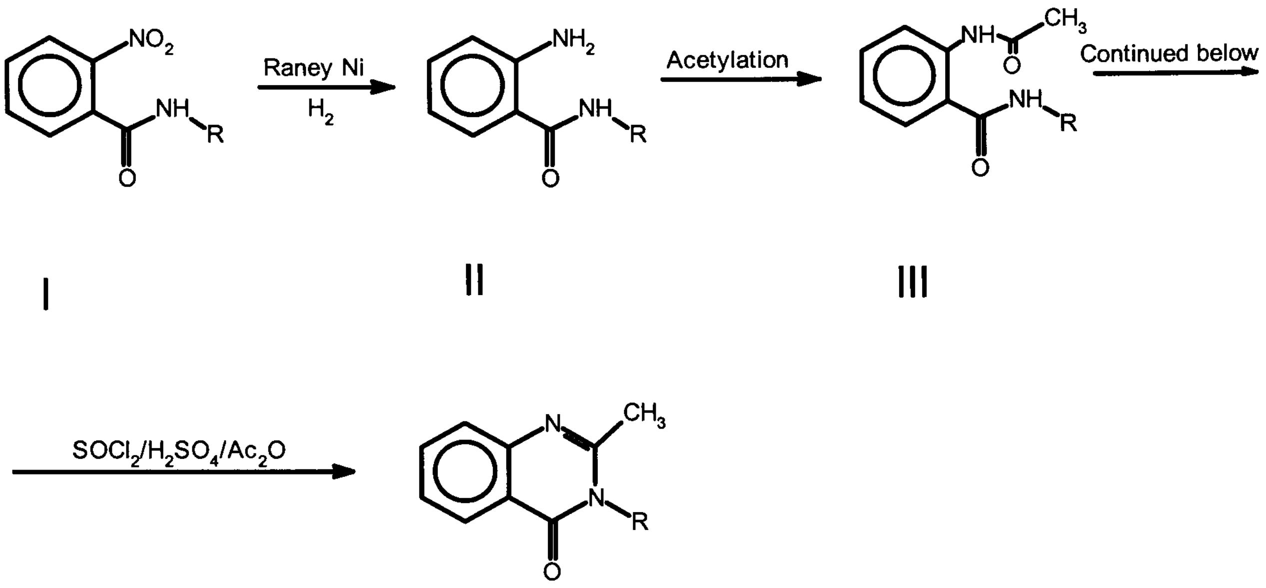
The reaction of N-*o*-tolyl-*o*-nitrobenzamide yielded 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(IV)** with melting point 118-119 ° C.

The reaction of N-*m*-tolyl-*o*-nitrobenzamide yielded 2-methyl-3-*m*-tolyl-4(3*H*)-quinazolinone **(V)** with melting point 126-132 ° C.

The reaction of N-*p*-tolyl-*o*-nitrobenzamide yielded 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone **(VI)** with melting point 149-151 ° C.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Reaction scheme: Synthesis 6



IV R = o–C_6H_4Me

V R = m–C_6H_4Me

VI R = p–C_6H_4Me

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 7[17]

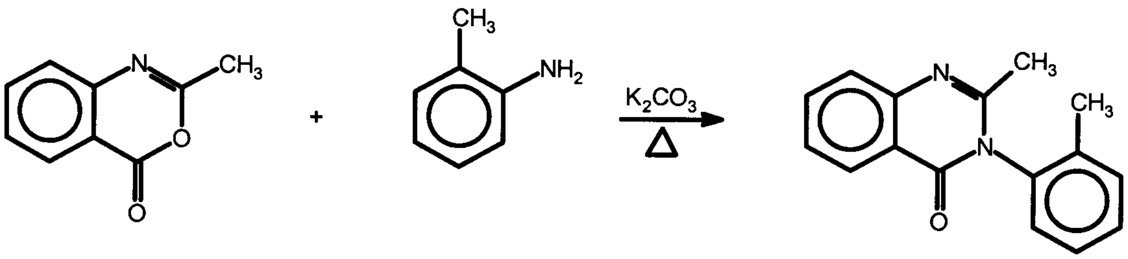
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

Acetylanthranil **(I)** (0.3 mole) was suspended in an inert solvent like benzene, toluene, or dichloromethane, with K_2CO_3 (0.08 mole). *o*-Toluidine **(II)** (0,35 mole) was added and the mixture boiled for 1-3 hours. The solvent was distilled of under vacuum, and water was added to the residue. The resulting mixture was steam distilled to remove residual solvent. The product obtained was isolated and re-crystallised from ethanol or isopropanol to yield 74% 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)** with melting point 115 ° C.

Reaction scheme



I II III

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 8[17]

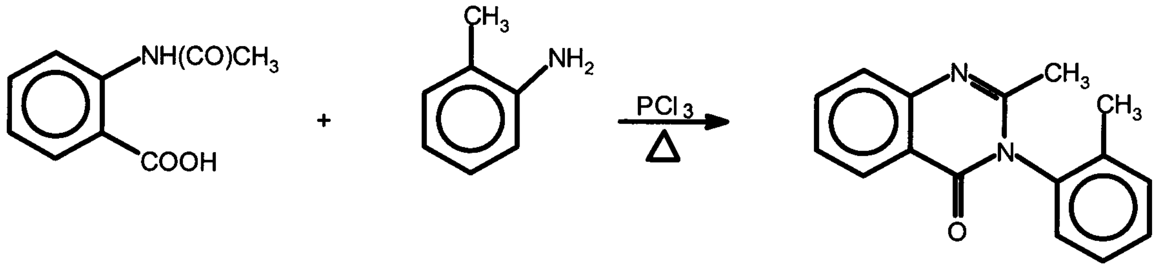
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

A suspension of N-acetylanthranilic acid **(I)** (0.25 mole) and *o*-toluidine **(II)** (0.25 mole) in absolute toluene was heated to 60 ° C with constant stirring. A solution of 0.2 mole PCl_3 in 20 millilitre of toluene was added dropwise and the mixture boiled under reflux for 2 hours. The mixture was allowed to cool and the product isolated and re-crystallised from ethanol or isopropanol to yield 74 % 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)** with melting point 115 ° C.

Reaction scheme



I II III

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 9[36]

Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

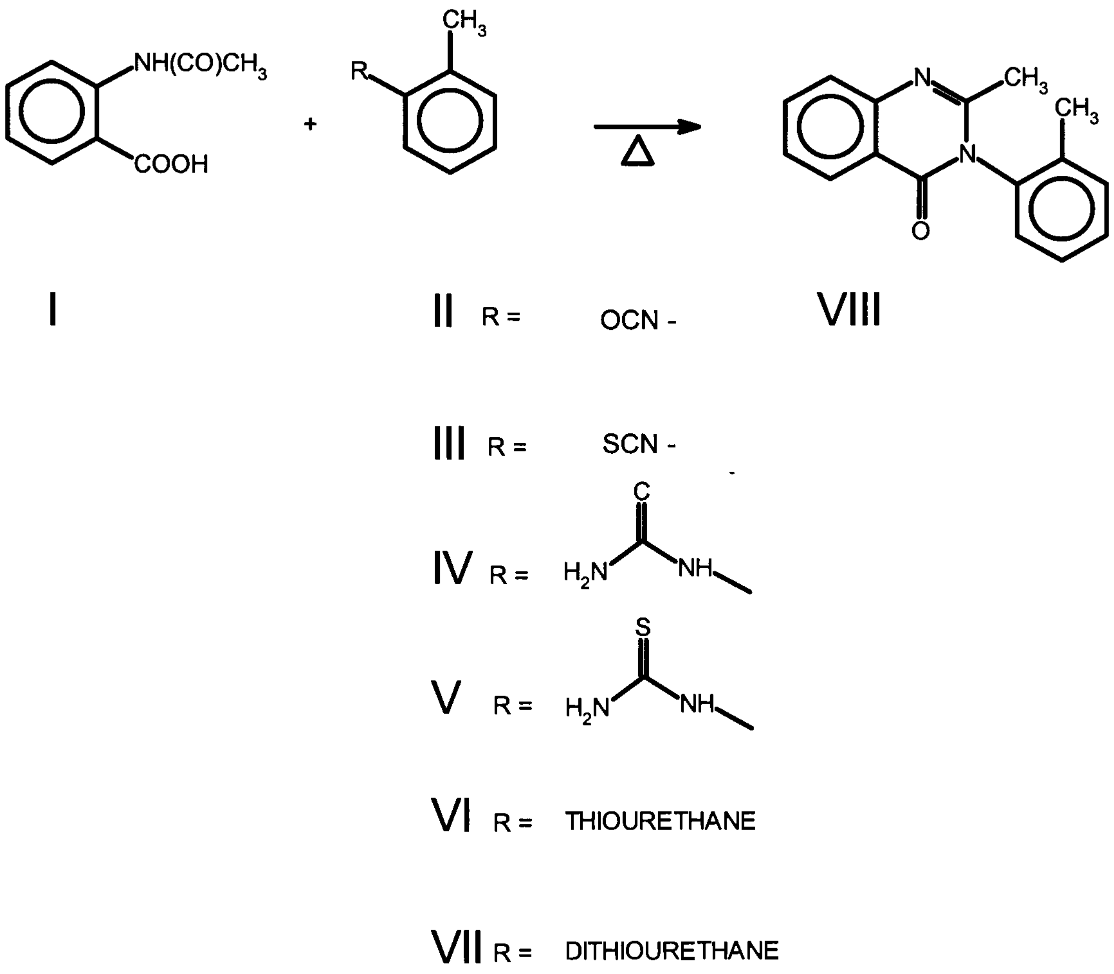
To a suspension of N-acetylanthranilic acid **(I)** in xylene or nitrobenzene, was added any of the following:

N-*o*-tolylisocyanate **(II)**
N-*o*-tolylisothiocyanate **(III)**
N-*o*-tolylurea **(IV)**
N-*o*-tolylthiourea **(V)**
N-*o*-tolylthiourethane **(VI)**
N-*o*-tolyldithiourethane **(VII)**

The reagents were allowed to react at 120-220 ° C to yield 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(VIII)** with melting point 114-115 ° C.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Reaction scheme: Synthesis 9



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 10[28]

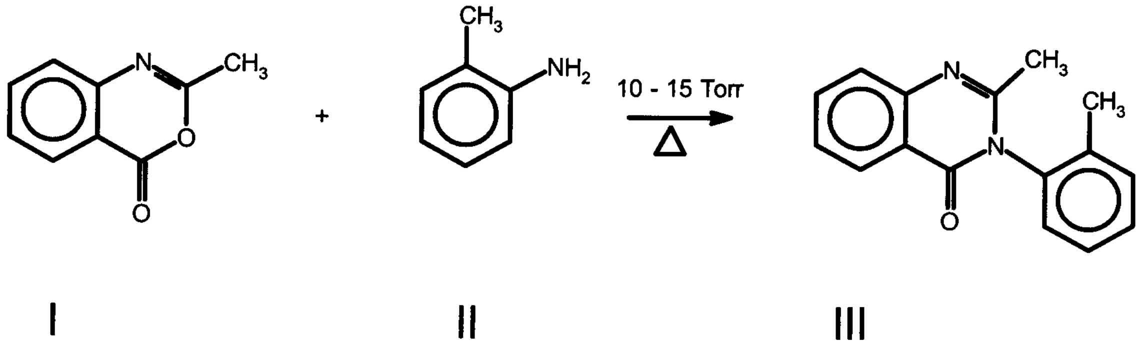
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

N-acetylanthranil **(I)** (30 kilogram) was melted, allowed to solidify, covered with *o*-toluidine **(II)** (25 kilogram), and heated to 60-70 ° C. Evolved water vapour was removed *in vacuo* at 10-15 Torr. The temperature remained below 80 ° C until all the solid reacted. The mixture was then slowly heated to 100 ° C to allow the reaction to complete. Excess *o*-toluidine was removed by slow heating to 150 ° C to yield 30 kilogram 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**.

Reaction scheme



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 11[18]

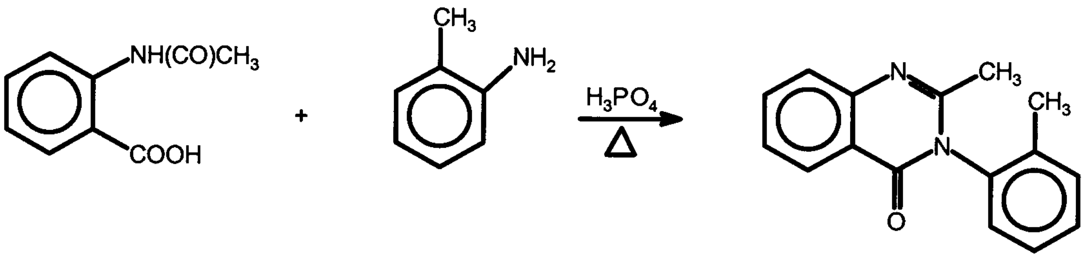
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

N-acetylanthranilic acid **(I)** (179 gram) was stirred with *o*-Toluidine **(II)** (107 gram). To the solidified mass was added 800 - 1000 gram polyphosphoric acid and the mixture heated to 100 ° C to obtain an easily stirred mass. The mixture was heated to 160-180 ° C with constant stirring, after which it was cooled to 100 ° C. Water (1.5 litres) was slowly added to avoid boiling. The solution was stirred over activated charcoal for 30 - 40 minutes, and filtrated. The filtrate was adjusted to pH 8 - 9 with sodium carbonate, and the resulting product filtered off. The product was washed with water, dried, and recrystallized from hot methanol to yield 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**. The hydrochloride salt was also prepared.

Reaction scheme



I II III

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 12[18]

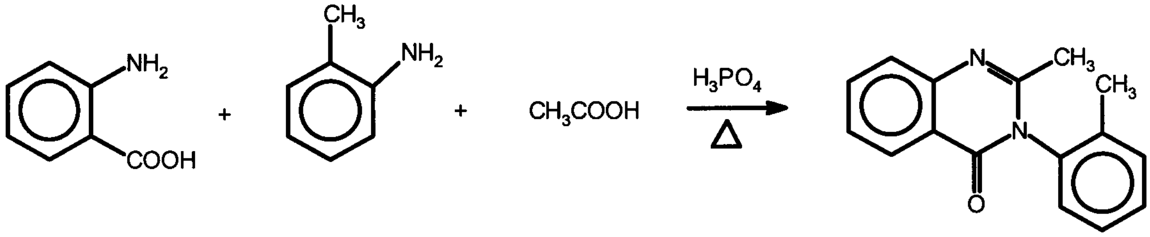
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

Anthranilic acid **(I)** (13.7 gram) was stirred with *o*-Toluidine **(II)** (11 gram) and acetic acid **(III)** (4 millilitre). To the solidified mass was added 30 - 40 gram polyphosphoric acid and the mixture heated to 100 ° C to obtain an easily stirred mass. The mixture was heated to 160-180 ° C with constant stirring, after which it was cooled. The resulting brown mass was poured into 100 -150 millilitre water. The solution was stirred over activated charcoal at room temperature, and filtrated. The filtrate was basified with sodium carbonate, to give an oil that crystallized on standing. The resulting product was filtered off, washed with water, dried, and recrystallized from hot methanol to yield 16 gram 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(IV)** with melting point 113 - 115 ° C.

Reaction scheme



I II III IV

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 13[25]

Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone
b) 2-ethyl-3-phenyl-4(3*H*)-quinazolinone

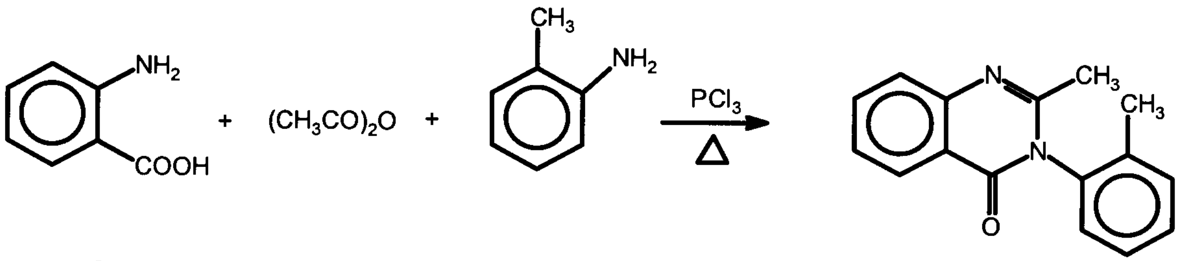
Method

Anthranilic acid **(I)** (54.8 gram; 0.4 mole) was slurried to smoothness in 100 gram (0.88 mole) of chlorobenzene. Acetic anhydride **(II)** (44.8 gram; 0.44 mole) was added over a period of 1 hour. During addition the temperature was maintained at 45 - 50 ° C. After addition the mixture was kept at the same temperature and stirred for ½ hour, followed by addition of *o*-toluidine **(III)** (100 gram; 0.94 mole). Phosphorus trichloride (46.0 grams; 0.33 mole) was slowly added over the course of 1 ½ hours. The temperature rose to about 126 ° C. After addition the mixture was refluxed for 1 hour, allowed to cool to 100 ° C, and 100 millilitre of water was added. Sodium hydroxide (68 gram as a 30% by weight solution) was carefully added, followed by the addition of sodium carbonate to Brilliant Yellow alkalinity. The chlorobenzene and excess *o*-toluidine was removed by steam distillation, the mixture stirred and cooled down to 50 ° C. The crude product crystallized as slightly pink, hard, sandy crystals. The crude product was dissolved at 30 ° C in 2000 millilitre of water acidified with hydrochloric acid (150 gram of 31.5% by weight), and treated with activated charcoal for 1 hour. After treatment it was filtered, with the filtrate being allowed to slowly, over the course of 1 hour, run into 600 millilitre of water containing 58 gram of sodium hydroxide. Brilliant Yellow alkalinity was maintained at all times. The product precipitated as soft, fine white crystals. The crystals was isolated, washed neutral with cold water, and dried at 80 ° C to yield 90% 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(IV)** with melting point 116-117 ° C.

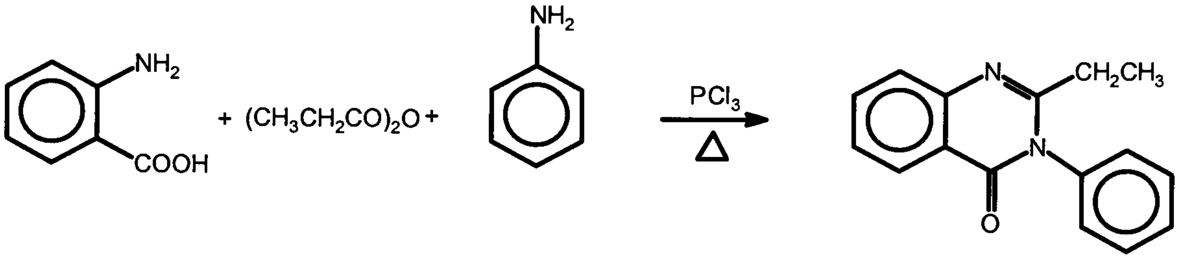
Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Similarly was prepared from aniline **(V)** (87.5 gram), propionic anhydride **(VI)** (57.2 gram), and anthranilic acid (54.8 gram) 2-ethyl-3-phenyl-4(3*H*)-quinazolinone **(VII)** in 91.5% yield with melting point 133-134 ° C.

Reaction scheme



I II III IV



I V VI VII

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 14[23]

Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-HCl

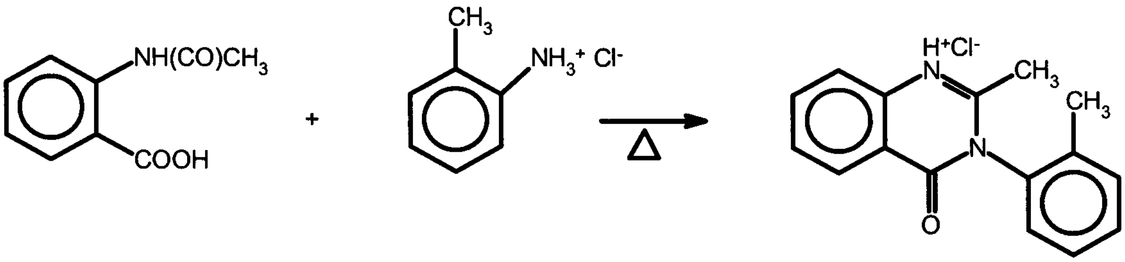
b) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

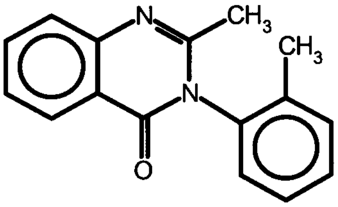
N-Acetylanthranilic acid **(I)** (18 gram) and *o*-toluidine-HCl **(II)** (14.5 gram) were mixed and heated together at 170 ° C for 5 minutes. The mixture melted at approximately 130 ° C, and started to boil vigourously. After boiling for 3 to 5 minutes the mixture instantaneously solidified. The solidified mixture was dissolved in a mixture of 150 millilitre of water and 10 millilitre of concentrated hydrochloric acid and filtered. The hot solution was made alkaline with 50% sodium hydroxide solution, and and allowed to cool whilst still being vigorously stirred. The resulting precipitate was collected and washed with water untill neutral. After drying the product was dissolved in 40 millilitre of 96% ethanol and filtered. After addition of 20 millilitre of 20 % ethanolic hydrochloric acid the a precipitate started forming. Precipitation was completed by the addition of 60 millilitre of diethyl ether to the cooled mixture. The precipitate was filtered and washed with 60 millilitre of1:1 diethyl ether and ethanol to yield 19.8 gram (71.5%) of 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-HCl **(III)** with melting point 250 - 256 ° C. The free base of **III,** 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(IV),** had a melting point of 113-114 ° C.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Reaction scheme: Synthesis 14



I II III



IV

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 15[37]

Target compounds relevant to this study

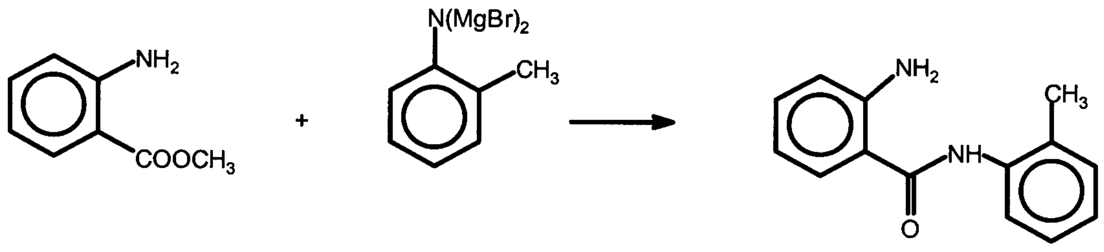
a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

Methylanthranilate (I) was reacted with N,N-dimagnesiumbromido-*o*-toluidine (II) to yield the 2-methylanalide of anthranilic acid (III). III (0.01 mole) was treated with acetic anhydride (IV) (0.03 mole), and freshly calcinated sodium acetate. The mixture was boiled for 2.5 hours, after which it was poured into 30 millilitre of water. Crystalline sodium hydroxide was added until the mixture was weakly alkaline, and the resulting precipitate filtered off. The precipitate was recrystallized from ethanol to yield 95.3% 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone (V) with melting point 113-114 °C.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

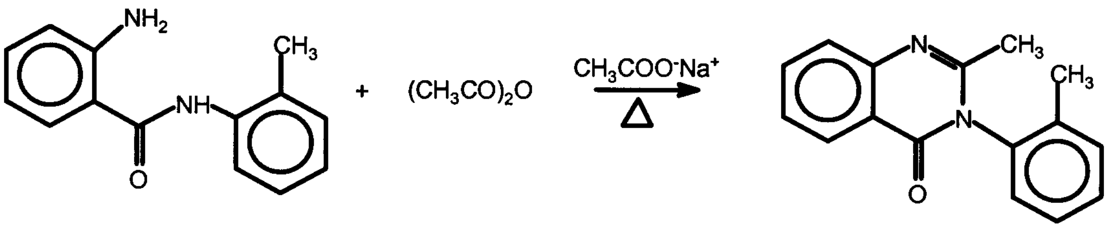
Reaction scheme: Synthesis 15



I

II

III



III

IV

V

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 16[26]

Target compounds relevant to this study

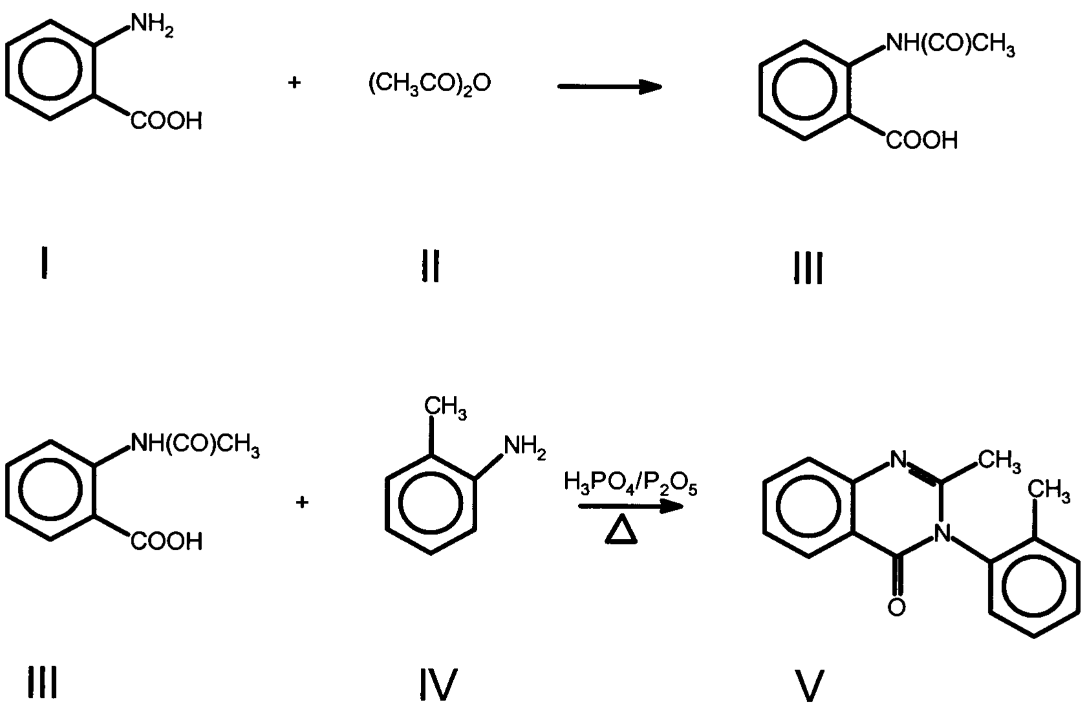
a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

Anthranilic acid **(I)** and acetic anhydride **(II)** was reacted to give N-acetyl anthranilic acid **(III)**. **III**, PFK (a blend of 85% H_3PO_4 with P_2O_5), and *o*-toluidine **(IV)** was heated at 185-195° C for 1 hour. The cooled product was poured into water and neutralized with Na_2CO_3, the precipitate washed, and dissolved in 10% hydrochloric acid. After clarifying over activated charcoal concentrated hydrochloric acid was added and the mixture allowed to stand overnight to give the hydrochloride salt of 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(V)**. The hydrochloride salt was mixed with 10% aqueous ammonium hydroxide to give **V** with melting point 115-116°C.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Reaction scheme: Synthesis 16



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 17[38]

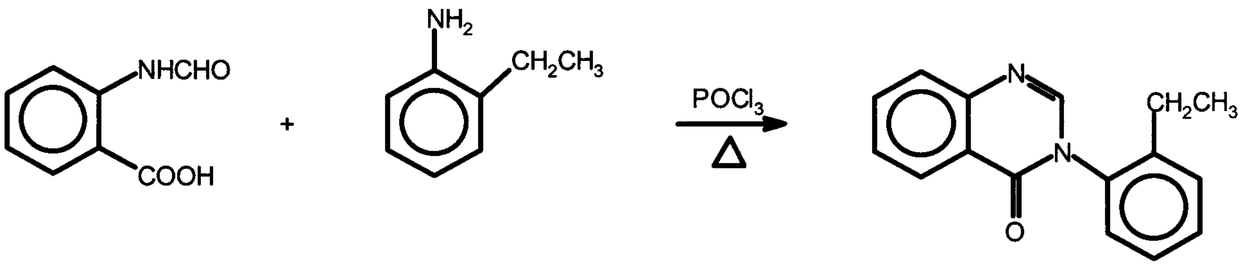
Target compounds relevant to this study

a) 3-*o*-ethylphenyl-4(3*H*)-quinazolinone-HCl

Method

o-Ethylaniline **(I)** (12.5 gram) was added to a stoichiometric quantity of N-formyl-*o*-aminobenzoic acid **(II)** suspended in 150 millilitre of toluene. The mixture was heated with stirring on a water bath at 75 ° C and 15.5 gram of phosphorous oxychloride. The reaction mixture was refluxed for 2 hours, cooled, and the toluene decanted off. The reaction product was taken up in ethanol and the solution acidified with concentrated hydrochloric acid. After cooling in a fridge the crystals that had formed were collected by filtration to yield 3-*o*-ethylphenyl-4(3*H*)-quinazolinone-HCl **(III)**, with melting point 165-170 ° C. The base was semi-solid.

Reaction scheme



I II III

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 18[38]

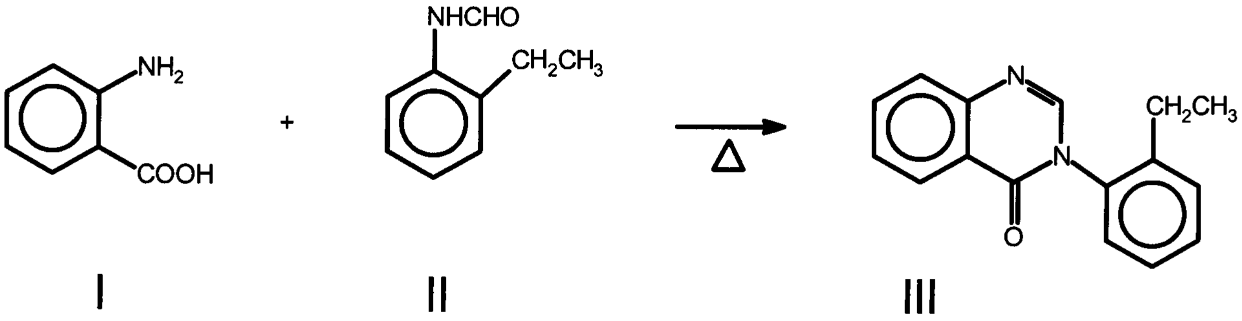
Target compounds relevant to this study

a) 3-*o*-ethylphenyl-4(3*H*)-quinazolinone-HCl

Method

Anthranilic acid **(I)** (13.5 gram) was mixed with a stoichiometric quantity of N-formyl-*o*-ethylaniline **(II)**. The mixture was heated on a oil bath at 200 ° C for 2 hours, and then at 220 ° C for 20 minutes. After cooling 100 millilitre of 15 % sodium carbonate solution was added. The mixture was boiled for several minutes and then cooled, and filtered. The product obtained was converted to the hydrochloride with concentrated hydrochloric acid to yield 3-*o*-ethylphenyl-4(3*H*)-quinazolinone-HCl **(III)**, with melting point 165-170 ° C. The base was semi-solid.

Reaction scheme



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 19[38]

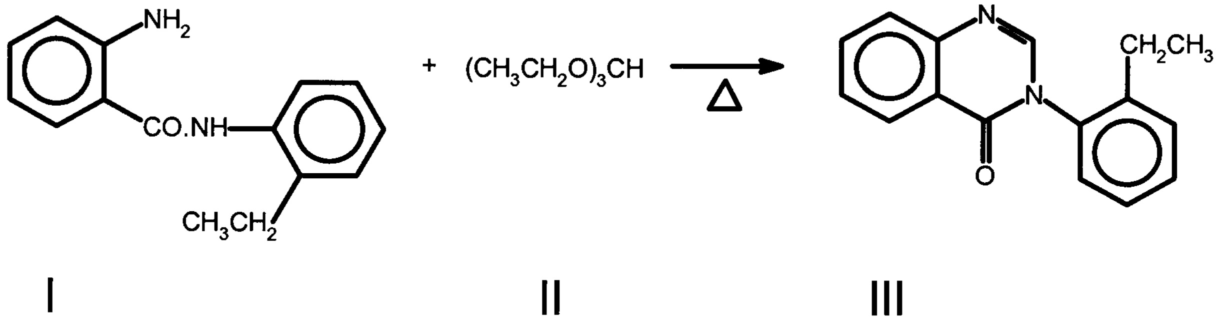
Target compounds relevant to this study

a) 3-*o*-ethylphenyl-4(3*H*)-quinazolinone-HCl

Method

o-Ethylphenylanthranilamide **(I)** was refluxed for 2 hours with an excess of ethyl orthoformate **(II)**. The excess ethyl orthoformate and ethanol formed was removed in vacuo. The reaction mixture was treated with concentrated hydrochloric acid to yield 3-*o*-ethylphenyl-4(3*H*)-quinazolinone-HCl **(III)**, with melting point 165-170 ° C. The base was semi-solid.

Reaction scheme



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 20[39]

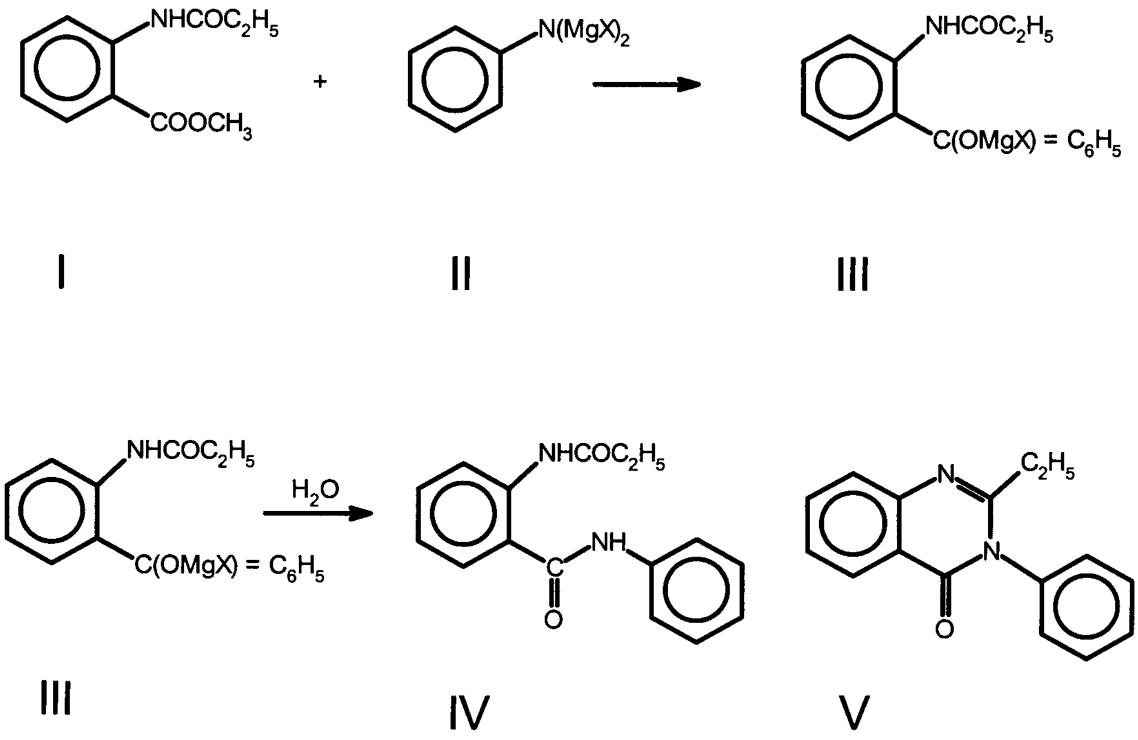
Target compounds relevant to this study

a) 2-ethyl-3-phenyl-4(3*H*)-quinazolinone

Method

N-propionyl-*o*-methylanthranilate **(I)** was reacted with N,N-dimagnesiumhalido-aniline **(II)** to yield **(III)**. **III** was hydrolysed to yield N-propionyl-*o*-phenyl-anthranilamide **(IV)**. **IV** was cyclized to yield 85% 2-ethyl-3-phenyl-4(3*H*)-quinazolinone **(V)**, with melting point 126-127 ° C.

Reaction scheme



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 21[27]

Target compounds relevant to this study

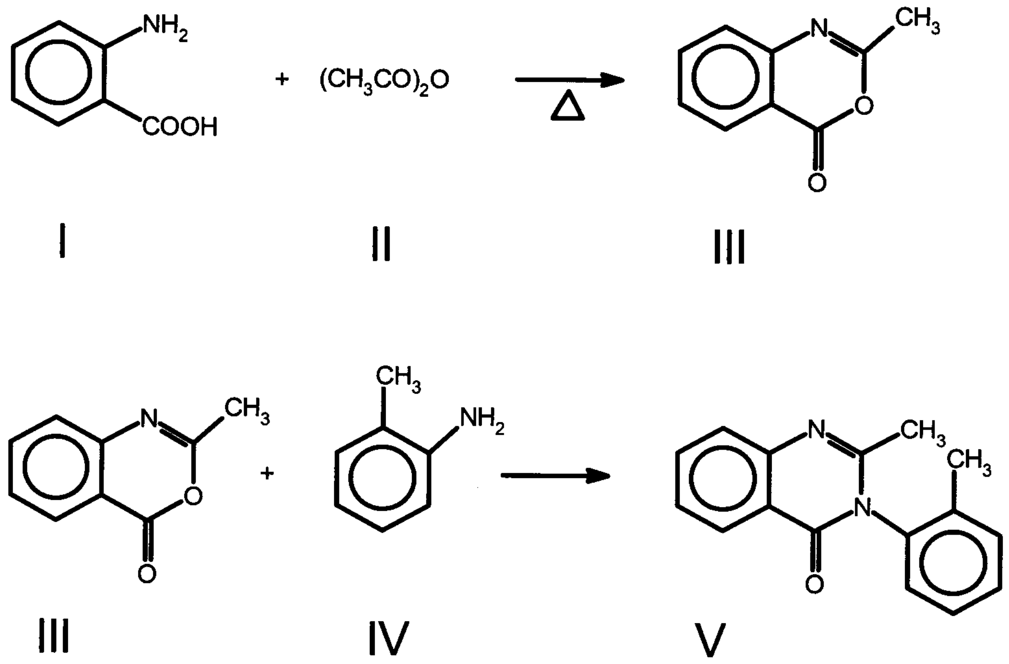
a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

Anthranilic acid **(I)** (137 gram) was heated with acetic anhydride **(II)** (204 gram) at 100 ° C. Excess acetic acid were removed under vacuum. The resulting acetanthranil **(III)** was dissolved in 1000 millilitre of toluene, and *o*-toluidine **(IV)** (107 gram) was added. PCl_3 (50 gram) or $POCl_3$ was added drop wise over a period of time. The mixture was then neutralized with with NaOH and the organic layer washed with water until neutral. Excess toluene and unreacted **IV** were removed through steam distillation. The product was recrystallized from ethanol to yield 87%
2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(V)** with melting point 114-116 ° C.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Reaction scheme: Synthesis 21



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 22[29]

Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone
b) 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone

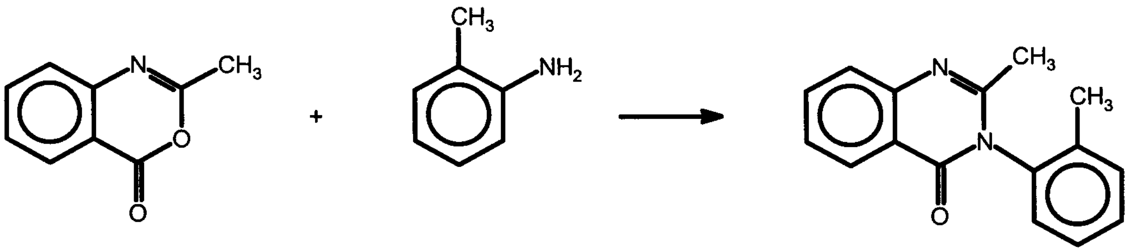
Method

Acetylanthranil **(I)** (0.03 mole) and equivalent weight of *o*-toluidine **(II)** were dissolved in 50 millilitre of an inert solvent like benzene or diethyl ether. The reaction was allowed to take place overnight at room temperature. If the acetamidine salt separated from the solution, it was removed through filtration. The solution was taken directly to dryness at approximately 60 ° C under vacuum. The residue was leached with dilute aqueous base, and the alkaline extract allowed to stand overnight at room temperature to precipitate 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)** with melting point 113 -114 ° C.

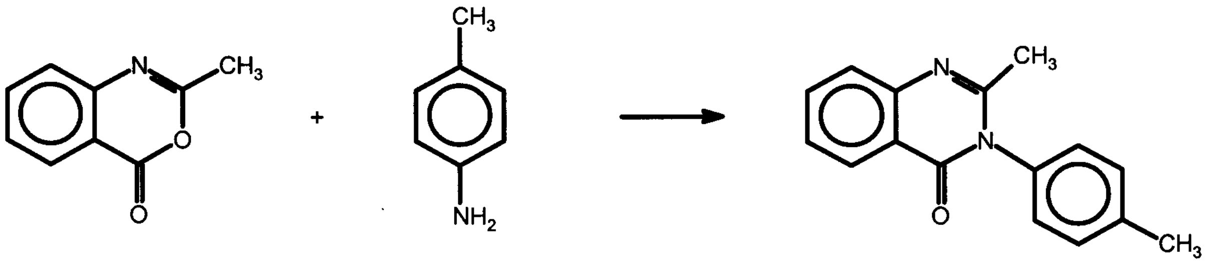
Similarly was prepared from **(I)** and *p*-toluidine **(IV)**, 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone **(V)** with melting point 151-152 ° C.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Reaction scheme: Synthesis 22



I II III



I IV V

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 23[40]

Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone - 5, 6, 7, 8 - d_4

Method

To a cooled solution of sodium hydroxide (10 gram in 50 millilitre of water) was added bromine (8.0 gram). The resulting solution was cooled -10 ° C and phthalimide - 3, 4, 5, 6 - d_4 **(I)** (7.5 gram), as a slurry in about 10 millilitre of water, was added over about 15 minutes with strong stirring. The freezing mixture was removed and the reaction mixture allowed to slowly warm up to 10 ° C (about 30 minutes), at which point all the solid had dissolved. Fine sodium hydroxide (6 gram) was added and the temperature rose to 45 ° C. The solution was heated to 80 ° C, cooled, and then neutralized with concentrated hydrochloric acid (16 millilitre). The resulting solution was acidified with acetic acid (8 millilitre) and allowed to stand at room temperature. The solid that crystallized was filtered, washed with cold water, and dried to yield 4.85 gram (69%) anthranilic acid - 3, 4, 5, 6, - d_4 **(II)**, with melting point 144-145 ° C.

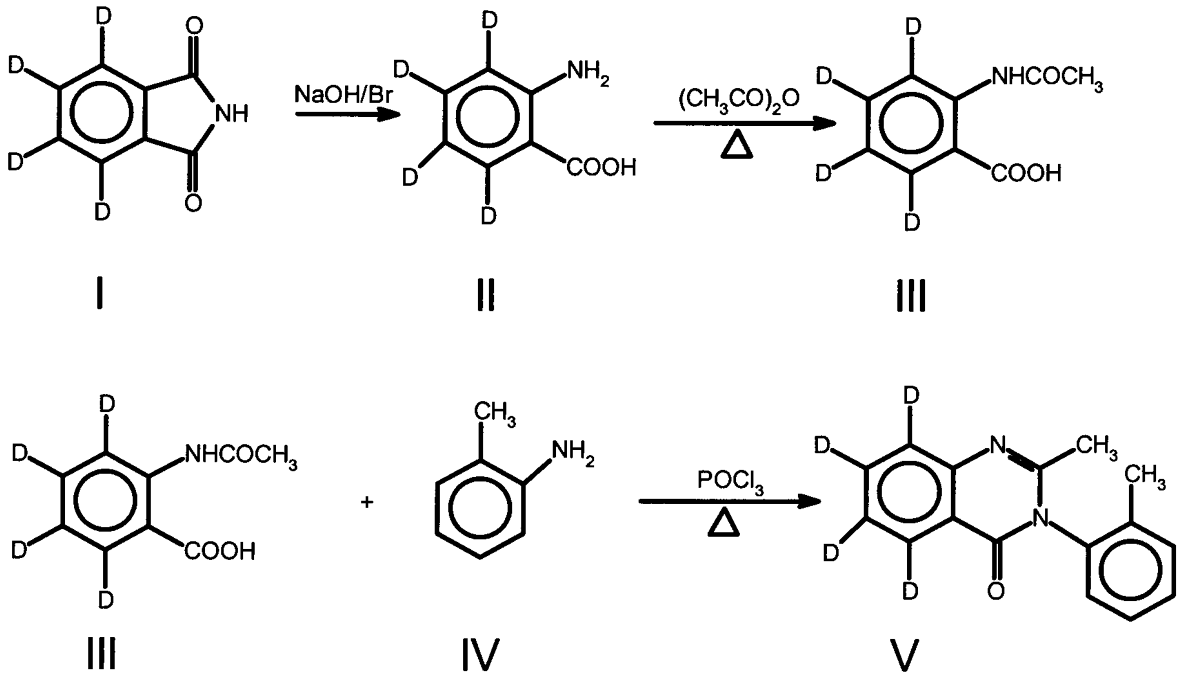
To **II** (3.3 gram) was added acetic anhydride (10 millilitre), and the mixture was refluxed for 1 hour. The solution was then cooled, and 3.5 millilitre of water added, and the solution again brought to reflux. At reflux temperature, 10 millilitres of water was added, and the solution allowed to cool. The resulting crystals were collected and washed several times with water to yield 3.9 gram (91%) N-acetyl anthranilic acid - 3, 4, 5, 6, - d_4 **(III)**, with melting point 183-186 ° C.

To **(III)** (3.0 gram) in 10 millilitre of toluene was added *o*-toluidine **(IV)** (1.8 gram). To this mixture was added $POCl_3$ (3.0 gram in 10 millilitre of toluene) drop wise over 15 minutes with stirring. The mixture was refluxed for 2 hours with mechanical stirring.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

The reaction mixture was cooled and poured into 100 millilitre of a 10% sodium bicarbonate solution. Any solid was washed into the sodium bicarbonate solution using methanol. Methanol and toluene was removed through steam distillation, and after the remaining oil had crystallized, it was filtered off, washed with water. The crystals was taken up in hot methanol, treated with activated charcoal, and the solution filtered. To the boiling filtrate was added water untill the solution became turbid, and the product was allowed to crystallize. The product was washed with water and further purified by chromatography on basic alumina with chloroform as the eluent. The chromatographed product was then crystallized from hexane/methanol to yield 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone - 5, 6, 7, 8 - d_4 **(V)**, with melting point 115-116 ° C.

Reaction scheme



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 24[30]

Target compounds relevant to this study

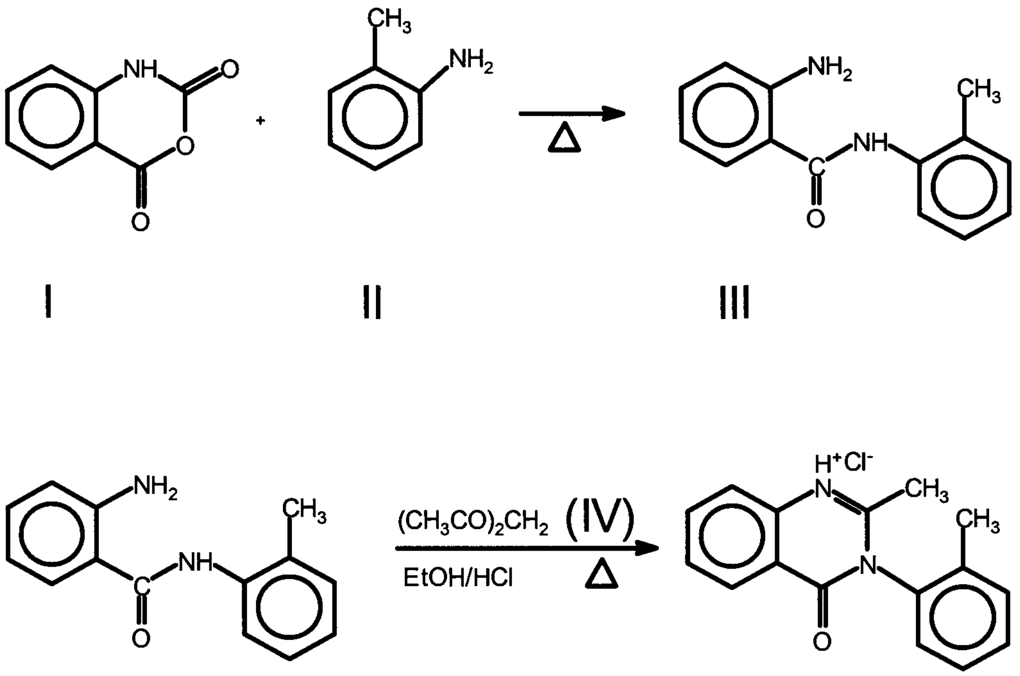
a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-HCl

Method

A mixture of isatoic anhydride **(I)** (1.6 gram, 0.01 mole) and *o*-toluidine **(II)** (1.1 gram, 0.01 mole) was heated at 120 ° C for 2 hours. After cooling, the reaction mixture was triturated with ether, and the resulting solid collected by suction and recrystallization from dichloromethane/hexane to yield 75% N-(*o*-tolyl)-anthranilamide **(III)**, with melting point 110 ° C. A mixture of **III** (0.5 gram, 0.0025 mole) and acetylacetone **(IV)** (0.39 gram, 0.0025 mole) in ethanol, acidified with a few drops of hydrochloric acid, was refluxed for 1 hour. On cooling the mixture yielded 0.59 gram (85%) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-HCl **(V)**, with melting point 235-237 ° C.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Reaction Scheme: Synthesis 24



III V

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 25[30]

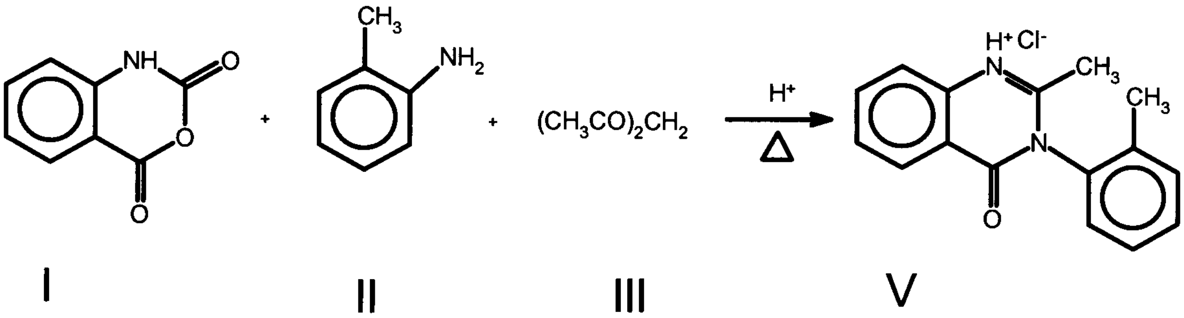
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-HCl

Method

A mixture of isatoic anhydride **(I)** (1.6 gram, 0.01 mole) and *o*-toluidine **(II)** (1.1 gram, 0.01 mole) in toluene (200 millilitre) was refluxed for 2 hours. Acetylacetone **(III)** (0.5 gram), acidified with a few drops of hydrochloric acid, was added and the mixture refluxed for 1 hour. Evaporation of the solvent yielded 80% 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-HCl **(IV)**.

Reaction scheme



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 26[19]

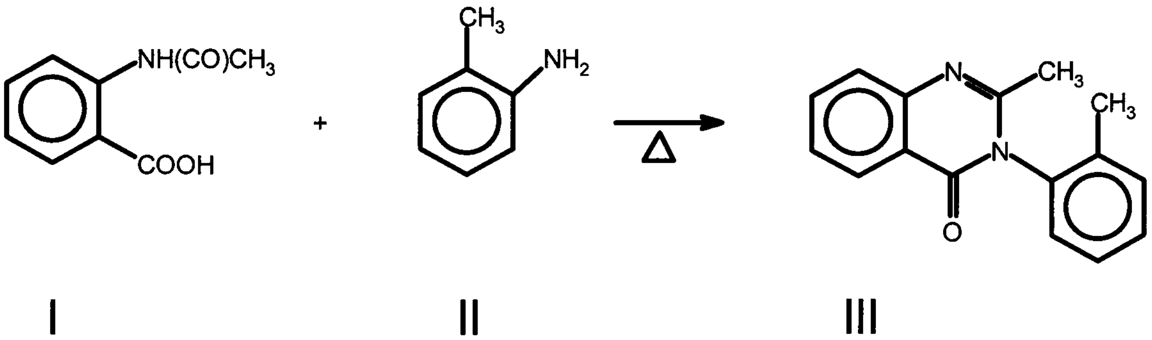
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

N-acetylanthranilic acid **(I)** (3.6 gram, 0.02 mole) and freshly distilled *o*-toluidine **(II)** (2.14 gram, 0.02 mole) in bromobenzene (10 millilitres) were refluxed for 2.5 hours. Subsequently, approximately half of the volume of solvent was distilled, and the dark reaction mixture was mixed with benzene (30 millilitre). The solution was filtered, and the filtrate was extracted with 3 x 10 millilitre of hydrochloric acid (1:1). The acidic layer was purified by extraction with ether, and then made alkaline (pH8) with sodium hydroxide (25%). The crude semisolid product obtained solidified on standing. The product was filtered, washed with water, dried, and recrystallized from benzene-light petroleum ether to yield 2.3 gram (48.4%) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**, with melting point 112.5-113 ° C.

Reaction scheme



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 27[20]

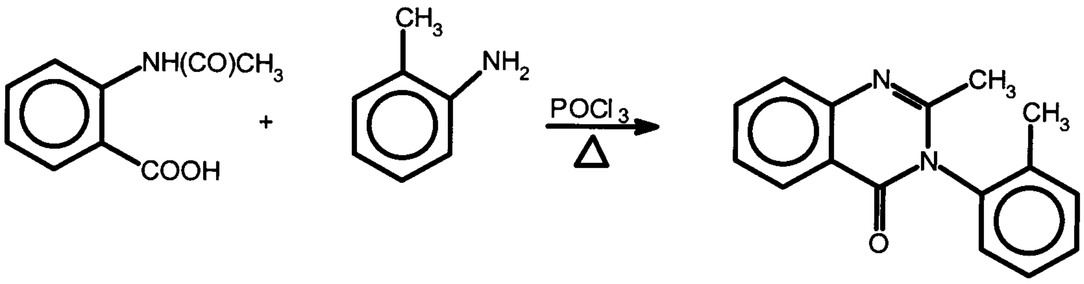
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

N-acetylanthranilic acid **(I)** was condensed with *o*-toluidine **(II)** in the presence of $POCl_3$. The resulting product was isolated as the hydrochloride salt of 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone. The salt was dissolved in water and the solution repeatedly extracted with toluene. Alkalization yielded 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**.

Reaction scheme



I II III

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 28[11]

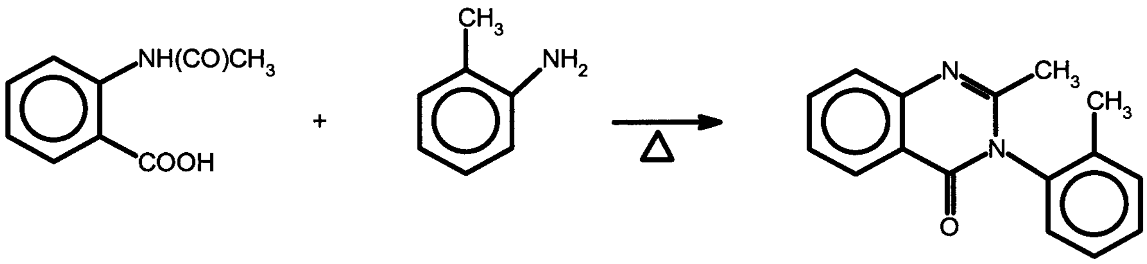
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

An intimate mixture of N-acetylanthranilic acid **(I)** (0.01 mole) and an equimolar amount of *o*-toludine **(II)** was fused at 150-190 ° C for 30-60 minutes. The crude product obtained was recrystallized after cooling to yield 60% 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**, with melting point 112.5-113 ° C.

Reaction scheme



I II III

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 29[31]

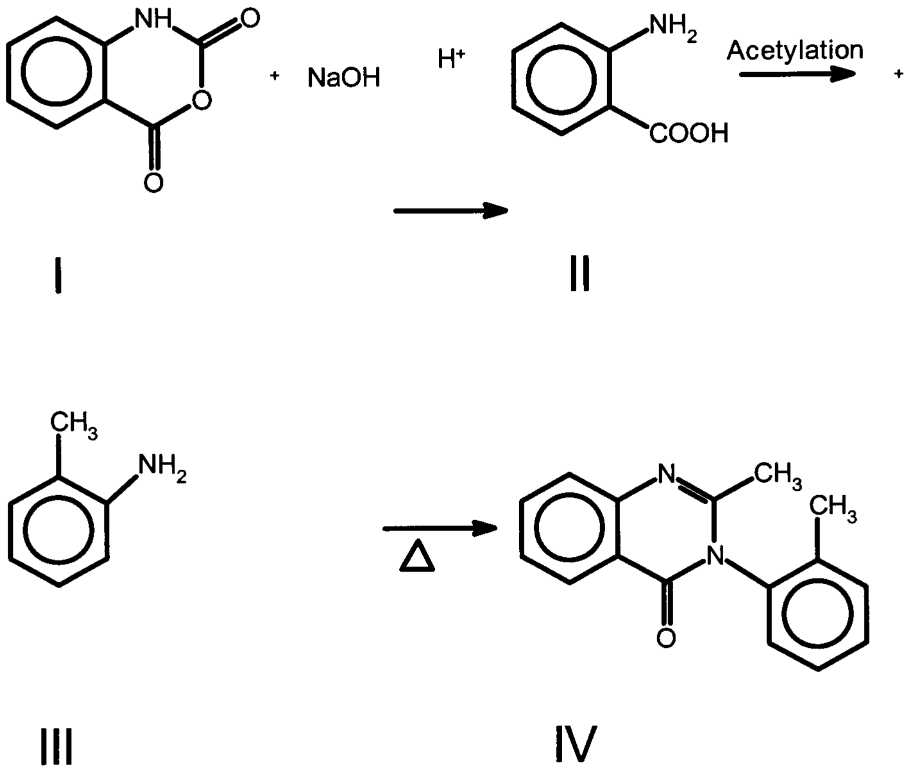
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

Isatoic anhydride **(I)** was subjected to alkaline hydrolysis, followed by acidification, to yield anthranilic acid **(II)**. Acetylation of **II** after drying, followed by reaction with *o*-toluidine **(III)** and ring closure yielded 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(IV)**.

Reaction Scheme



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 30[31]

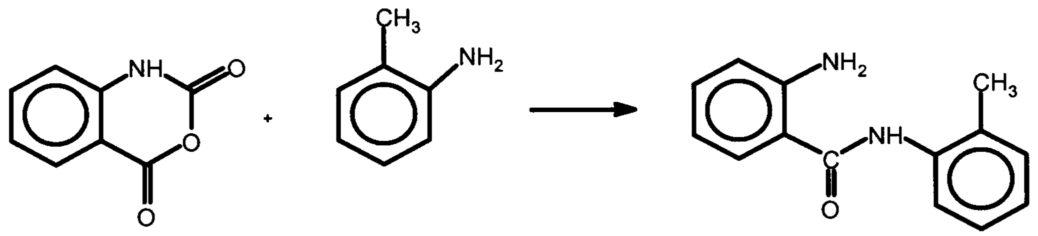
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

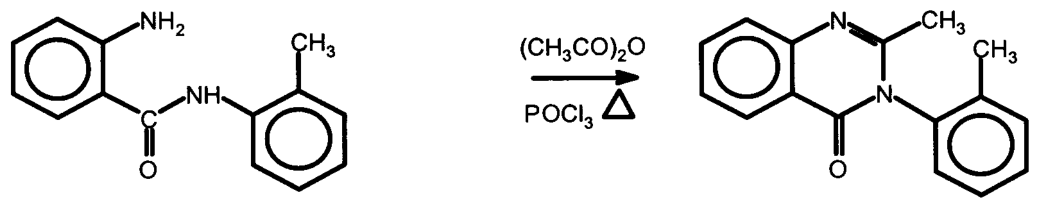
Method

Isatoic anhydride **(I)** was reacted with *o*-toluidine **(II)** resulting in the opening of the anhydride ring, and the formation of N-(*o*-tolyl)-anthranilamide **(III)**. **III** was acetylated with acetic anhydride, followed by ring closure with $POCl_3$ to yield 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(IV)**.

Reaction scheme



I II III



III IV

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 31[41]

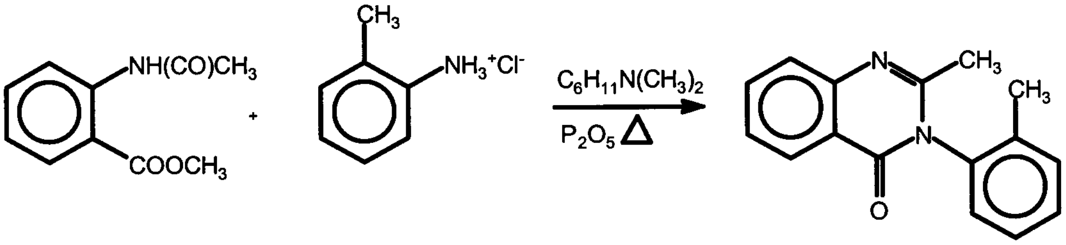
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

A mixture of methyl N-acetylanthranilate **(I)** (0.2 mole), *o*-toluidine HCl **(II)** (0.2 mole), P_2O_5, and N,N-dimethylcyclohexylamine was heated on a silicone-oil bath at 180 ° C for 45 minutes. The mixture was allowed to cool to 100 ° C and 2 molar NaOH was poured into the reaction mixture until alkaline. Stirring was continued for 1 hour. The aqueous phase was extracted with 3 x 100 millilitre dichloromethane. The dichloromethane was evaporated off and the N,N-dimethylcyclohexylamine distilled of at 10 mmHg. The residue was recrystallized from ethanol to yield 84%
2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**, with melting point 113-114 ° C.

Reaction scheme



I II III

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 32[10]

Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone
b) 2-methyl-3-*m*-tolyl-4(3*H*)-quinazolinone
c) 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone

Method

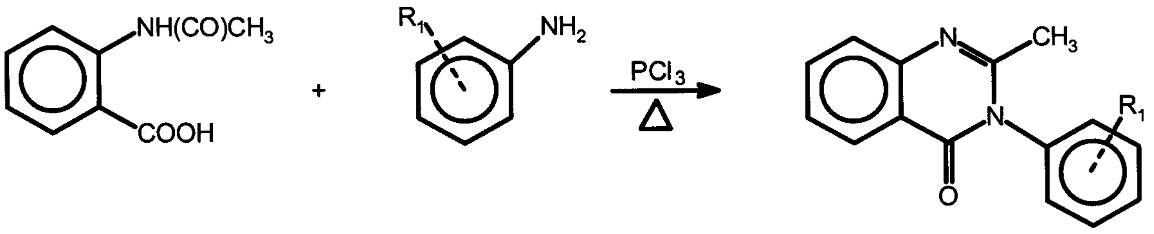
A toluene solution of *o*-toluidine **(I**, R_1 = *o*-CH_3**)** was reacted in a 1:1 molar ratio with N-acetylanthranilic acid **(II)** in the presence of PCl_3. PCl_3 (3 millilitre in 15 millilitre toluene) was added drop wise over a period of 10 minutes. The mixture was refluxed for 60 minutes and allowed to cool to room temperature. The reaction mixture was dissolved in hot methanol and filtered. Distilled water was added until a constant turbidity was obtained, and the solution allowed to sit until crystal formation was completed. This process was repeated to obtain white crystal. If impurities persisted, the product was chloroform extracted from hydrochloric or sodium hydroxide aqueous solutions to yield 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**, with melting point 115-117 ° C, and its hydrochloride with melting point 243-246 ° C.

Similarly was prepared from m-toluidine, 2-methyl-3-*m*-tolyl-4(3*H*)-quinazolinone **(IV)**, with melting point 127-129 ° C, and its hydrochloride with melting point 246-249 ° C.

Also prepared from p-toluidine, 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone **(V)**, with melting point 149-151 ° C, and its hydrochloride with melting point 250-253 ° C..

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Reaction scheme: Synthesis 32



I

II

III R_1 = o–CH_3

IV R_1 = m–CH_3

V R_1 = p–CH_3

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 33[21]

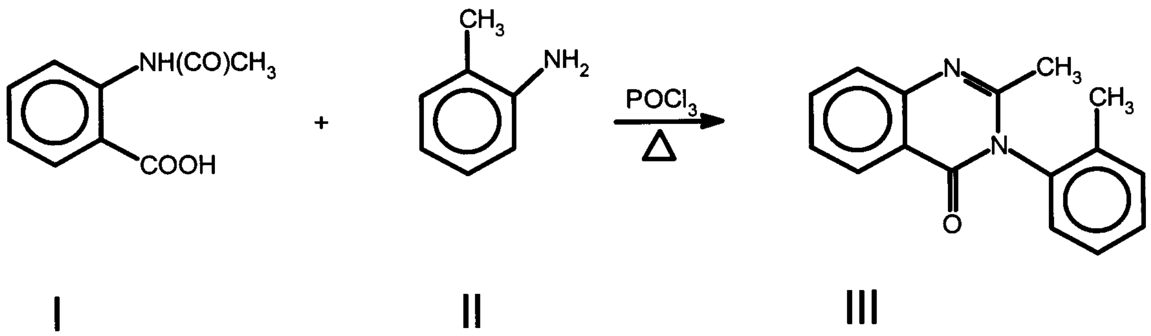
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-HCl

Method

N-acetylanthranilic acid **(I)** (179.0 gram, 1 mole) was suspended in 300 millilitre of toluene mixed with 50 millilitres of chloroform. *o*-Toluidine **(II)** (107.0 gram, 1 mole) was added at 30° C, followed by $POCl_3$ (205.0 gram). The heterogeneous mixture was then heated at 90° C for 3 hours, causing the required product to be released from the oily reaction mixture. The mixture was decomposed by methanol (200 millilitre) and acetone (300 millilitre). The mixture was allowed to stand in order for impurities to dissolve, and the crystals that formed on subsequent cooling was filtered of and washed with acetone to yield 76.7% 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-HCl **(III)**.

Reaction scheme



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 34[42]

Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone
b) 2-methyl-3-*m*-tolyl-4(3*H*)-quinazolinone
c) 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone

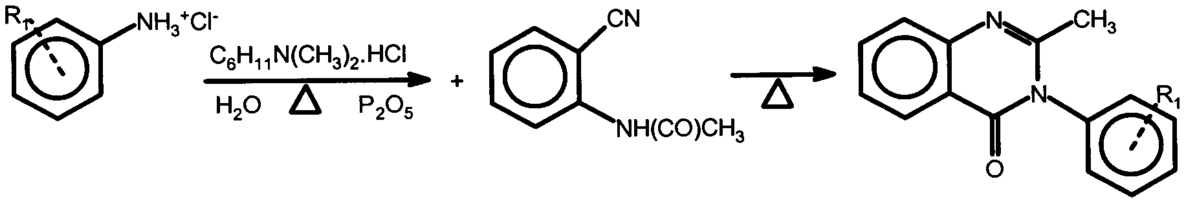
Method

o-Toluidine-HCl **(I**, R_1 = *o*-CH_3**)** (60 millimole) was mixed together with P_2O_5 (8.5 gram, 60 millimole), N,N-dimethylcyclohexylamine-HCl (9.8 gram, 60 millimole), and water (2.16 gram, 120 millimole) in a flask fitted with a mechanical stirrer and a reflux condensor fitted with a drying tube. The mixture was heated in an oil bath at 200-220 ° C until a homogeneous mixture was obtained (approximately 30 minutes). The oil bath temperature was lowered to 180 ° C, and 2 -acetylaminobenzonitrile (II) (15 millimole) was added and allowed to react for 53 minutes. The reaction mixture was allowed to cool to about 100 ° C and 2 molar aqueous NaOH (250 millilitre) was added until alkaline reaction (pH 9-11). The mixture was stirred at room temperature until the reaction cake was digested, and then extracted with dichloro-methane (3 x 100 millilitre). The dichloromethane was stripped off and the , N,N-dimethylcyclohexylamine distilled of at 10 mmHg. Excess *o*-toluidine was distilled off at 1 mmHg at 100 ° C The crude product was recrystallized from methanol to yield 53% 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**.

Similarly was prepared 2-methyl-3-*m*-tolyl-4(3*H*)-quinazolinone **(IV)**, in a 40% yield, and 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone **(V)**, in a 33% yield.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Reaction scheme: Synthesis 34



I

II

III R_1 = o–CH$_3$

IV R_1 = m–CH$_3$

V R_1 = p–CH$_3$

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 35[24]

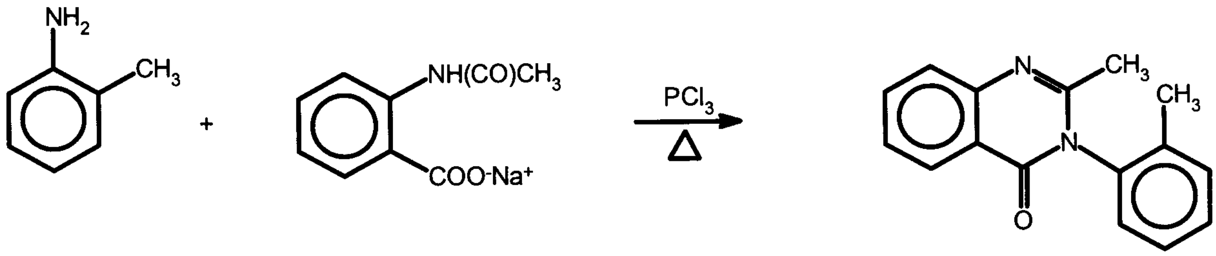
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

o-Toluidine **(I)** was condensed with the sodium salt of N-acetylanthranilic acid **(II)** in toluene with PCl_3 to yield 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**.

Reaction scheme



I II III

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 36[22]

Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone
b) 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone

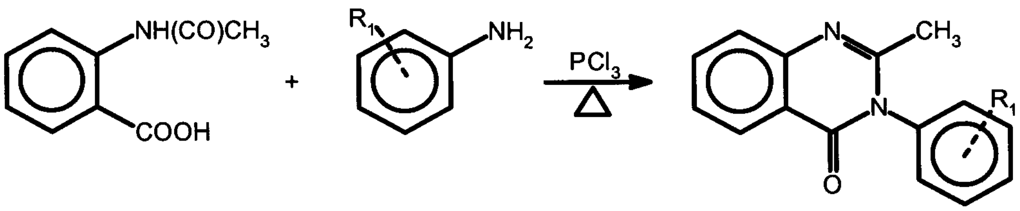
Method

To a vigorously stirred suspension of N-acetylanthranilic acid **(I)** (24.8gram, 139 millimole) and freshly distilled *o*-toluidine **(II,** R_1 = *o*-CH_3**)** (14.8 gram, 139 millimole) in 450 millilitres of toluene was added dropwise, over a period of 15 minutes, PCl_3 (9.2 gram, 69 millimole) in toluene. When addition was completed, the reaction mixture was heated at reflux for 3 hours. It was allowed to cool to room temperature and the crude solid collected by filtration. This solid was suspended in 50 millilitre of water and neutralised with saturated sodium bicarbonate. An additional 250 millilitres of water was added and the resulting suspension was extracted twice with 200 millilitre portions of chloroform. The organic extract was dried over anhydrous magnesium sulphate, and the solvent removed under reduced pressure to give a viscous oil, which crystallized on standing. The crude product was recrystallized from isopropyl alcohol to yield 22.8 gram (66%) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**, with melting point 114-115 ° C.

Similarly was prepared 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone **(IV)**, with melting point 147-148 ° C.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Reaction scheme: Synthesis 36



I

II

III R_1 = o–CH_3

IV R_1 = p–CH_3

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 37[12]

Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone
b) 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone
c) 2-ethyl-3-phenyl-4(3*H*)-quinazolinone

Method

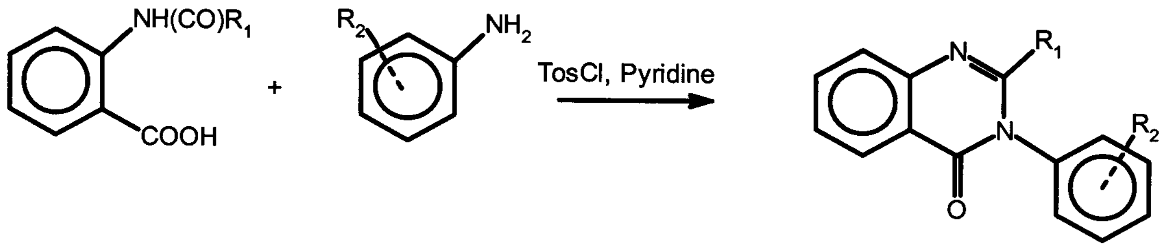
To a solution of N-acetylanthranilic acid **(I)** (10 millimole) in pyridine (10 millilitre) was added tosyl chloride (1.89 gram, 10 millimole) with stirring at room temperature. After 10 minutes *o*-toluidine **(II)** (10 millimole) was added slowly at room temperature and with vigorous stirring. Stirring was continued for 2 hours at room temperature and the reaction mixture was poured into a saturated aqueous solution of sodium bicarbonate (100 millilitre) with stirring. The resulting solution was extracted with dichloromethane (3 x 50 millilitre). The combined extracts were washed with distilled water and the organic layer dried over anhydrous sodium sulphate. The solvent was removed to yield 75% 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**, with melting point 113-114 ° C.

Similarly was prepared from p-toluidine **(IV)** 80% 2-methyl-3-*p*-tolyl-4(3*H*)-quinazolinone **(V)**, with melting point 148-150 ° C.

Also prepared from N-propionylanthranilic acid **(VI)** and aniline **(VII)** was 80% 2-ethyl-3-phenyl-4(3*H*)-quinazolinone **(VIII)**, with melting point 148-150 ° C.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Reaction scheme: Synthesis 37



I

II

III $R_1 = CH_3$, $R_2 = o\text{-}CH_3$

IV $R_1 = CH_3$, $R_2 = p\text{-}CH_3$

V $R_1 = CH_2CH_3$, $R_2 = H$

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 38[32]

Target compounds relevant to this study

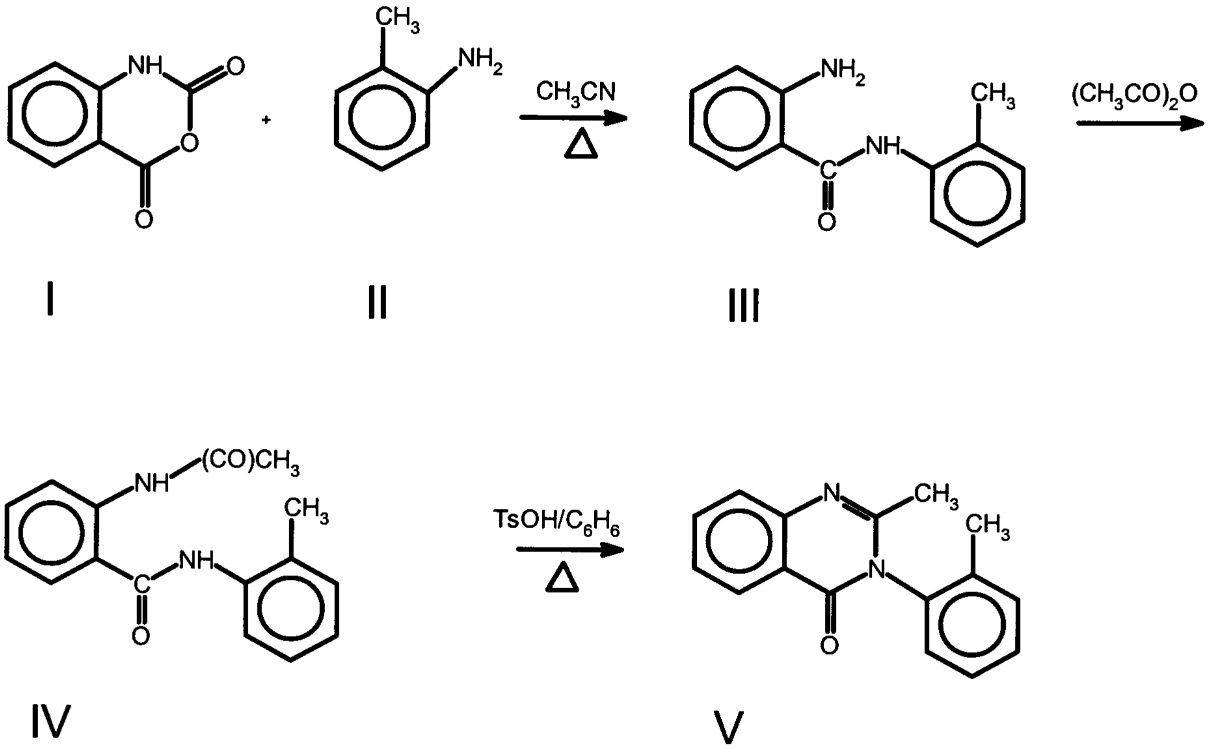
a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

Isatoic anhydride **(I)** (0.02 mole) and *o*-toluidine **(II)** (0.02 mole) were refluxed in acetonitrile (50 millilitres) for 12 hours. The reaction mixture was cooled to room temperature, and all insoluble material filtered off. Evaporation of the solvent gave a crude product which was dissolved in hot benzene, boiled with charcoal for 1-2 minutes and filtered hot. Concentration of the hot filtrate followed by cooling to room temperature yielded 61% 2-amino (N-*o*-tolyl) benzamide **(III)**, with melting point 109-110 °C. **III** was acetylated to give 2-methylamido (N-*o*-tolyl) benzamide **(IV)**. **IV** (3.2 millimole) was refluxed in benzene (25 millilitres) with p-toluenesulphonic acid (TsOH) (1 millimole) for 5 hours. The reaction mixture was allowed to cool to room temperature, any residue was filtered off, and the organic layer washed successively with dilute sodium bicarbonate solution and water until neutral. The organic layer was dried, and the solvent stripped. The crude product obtained was recrystallized from benzene or benzene-petroleum ether to yield 54% 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(V)**, with melting point 113-114 °C.

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

Reaction scheme: Synthesis 38



Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

SYNTHESIS 39[35]

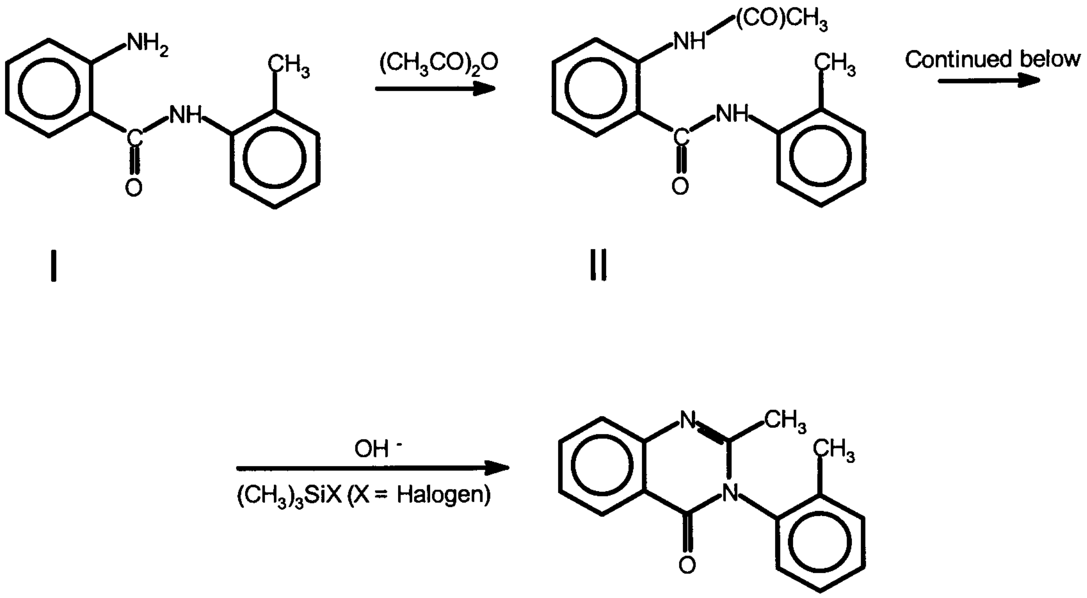
Target compounds relevant to this study

a) 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone

Method

2-methylamido (N-*o*-tolyl) benzamide **(I)** was obtained through the acetylation of 2-amino (N-*o*-tolyl) benzamide **(II)**. **II** was cyclized by dehydration in the presence of a halogenated trialkylsilane in the presence of a base to yield 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone **(III)**, with melting point 116.5-117.5 ° C.

Reaction scheme



I

II

III

Note: Roman numeral designation in **Appendix A: Part 2** has no bearing on, or correlation with the use of Roman numeral throughout the rest of this document.

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