Synthesis of Novel-1,3,5-Triazine-Based-Anti-Tuberculosis Drugs

By

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Declaration

This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

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STATEMENT 1

This dissertation is being submitted in partial fulfilment of the requirements for the degree of MSc.

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This dissertation is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by explicit references. The views expressed are my own.

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Abstract

Identification of unique leads represents a significant challenge in drug discovery. This challenge is widely visible in neglected diseases such as tuberculosis, which is an infectious disease caused by bacillus Mycobacterium tuberculosis. The urgent need in search of new biological entities to fight back TB and drug resistant TB is a drive behind this project. Several specific synthetic protocols have been developed using 1,3,5-triazines due to the important biological properties which they display. The chemistry and an extensive spectrum of biological activities of *s*-triazines have been examined since several decades and this heterocyclic core has received emerging consensus. Hence, the aim of this project was to synthesize novel anti-TB drugs total with the usage of 1,3,5-triazine as a linker between known anti-TB drugs together with different types of amines. A total of 20 compounds were synthesized, 3 compounds were mono-substituted with an average yield of 75 %, 6 compounds were di-substituted with an average yield of 63 % and 11 compounds were tri-substituted with an average yield of 93 %. Out of 10 compounds which were analysed for biological activity 8 of which showed biological activity against *M.smegmatis*. Furthermore compound **26** which was hybridized with an amine and a known anti-TB drug inhibited better biological activity. In conclusion the influence of cyanuric chloride in combination with pyrrolidine and anti-TB drugs deserves further study. The newly synthesized compounds were characterized by IR, melting point, GC-MS, biological testing, ^{1}H and ^{13}C NMR.

Acknowledgements

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Abbreviations

AIDS: Acquired Immunodeficiency Syndrome

$CDCl_3$: Chloroform

CD4: Cluster of Differentiation 4

$CD4^+$ T: Cluster of Differentiation 4 T helper cells

DCM: Dichloromethane

DHFS: Dihydrofolate Synthase

DHFR: Dihydrofolate reductase

DHPS: Dihydropteroate Synthase

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic Acid

EMA: European Medical Agency

et al: and others

Hz: Hertz

HIV: Human Immunodeficiency Virus

HMM: Hexamethylmelamine

HMPMM: Hydroxymethylpentamethylmelamine

InhA: Enoyl-acyl carrier protein reductase

IUPAC: International Union of Pure and Applied Chemistry

IR: Infrared radiation

KatG: Bacterial catalase-perioxidase enzyme

MAOS**:** Microwave Assisted Organic Synthesis

MS: Mass spectrometry

MCC: Medicines Control Council

MDR-TB: Multi-drug resistant tuberculosis

MH: Molecular Hybridization

MIC: Minimum inhibitory concentration

m.p.**:** Melting point

M.tb: Mycobacterium tuberculosis

NAD^+: Nicotinamide Adenine Dinucleotide

NADH: Nicotinamide Adenine Dinucleotide Hydrogen

NI:	Not inhibited at the highest concentration tested
PAS:	*p*-Aminosalicyclic Acid
ppm:	Parts per million
RNA:	Ribonucleic Acid
rRNA:	Ribosomal Ribonucleic Acid
SIV:	Simian immunodeficiency virus
TB:	Tubercle bacillus (Tuberculosis)
TDR-TB:	Total drug resistant-TB
tRNA:	transfer Ribonucleic Acid
THF:	Tetrahydrofuran
TOF:	Time of Flight
USFDA:	United States Food & Drug Administration
WHO:	World Health Organization
XDR-TB:	Extensively drug resistant-TB
^{13}C-NMR:	13-Carbon Nuclear Magnetic resonance
^{1}H-NMR:	Proton nuclear magnetic resonance

Contents

Chapter 1
Introduction

Tubercle bacillus, abbreviated as TB, is a highly contagious pulmonary infection caused by bacillus *Mycobacterium tuberculosis*.[1] This mycobacterium is slow-growing and usually attacks the lungs (known as pulmonary TB), though it can also affect other organs such as the kidney, spine, brain and the heart.[1] TB has stayed a serious contagious disease among developing countries with the simultaneous appearance of MDR and XDR-TB strains which have further amplified death rates worldwide and has become a pressure for frontline therapeutics.[2]

There are two types of TB that can be found in patients, namely latent and active TB. A patient is said to be diagnosed with latent TB when he or she has been infected with TB bacteria but does not show any TB symptoms. Latent TB is not known to be contagious. On the other hand, patients with active TB experience symptoms such as pain in the chest, fever, night sweats, coughing up blood and the most common symptom been a bad cough that lasts longer than three weeks. Active TB can be passed on simply when a patient with this type of TB coughs, sneezes, sings or even talks.

There is a 10% chance of latent TB becoming active TB, but this risk is much higher for people who have weaker immune systems, for example people who have human immunodeficiency virus (HIV) or people who smoke.[2] HIV suppresses the immune system, making it harder for the body to control TB bacteria. People who are infected with HIV and have latent TB are around 20-30% more likely to develop active TB than HIV negative people.[1]

Anti-TB drugs commonly used to treat TB were first developed in the early 1940s while some were discovered in the early 1950s.[2] These drugs can be categorised as first line and second line anti-TB drugs. Examples of drugs classified as first line anti-TB drugs are isoniazid **1**, rifampicin (rifadin, rimactane) **2**, ethambutol **3** and pyrazinamide **4 (figure 1)**.

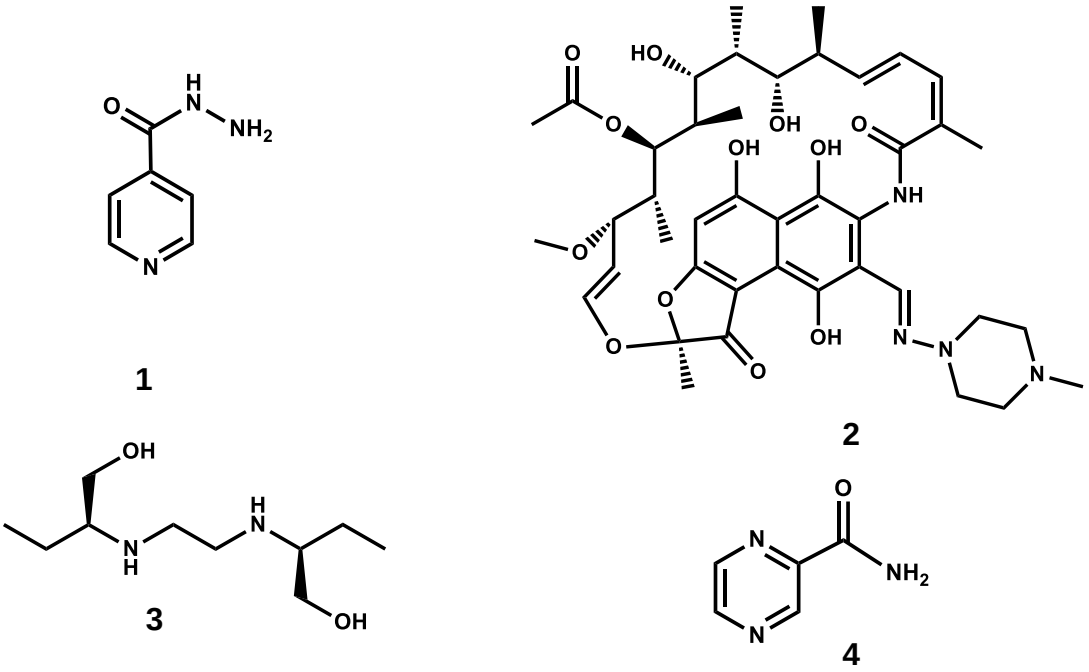


Figure 1: Structures of first line anti-TB drugs

Isoniazid **1** was first discovered in 1912 by Hans Meyer and Josef Mally, however its activity against tuberculosis was first reported in 1959.[3] This drug acts as a bactericidal (substance that kills bacteria) for active TB. This prodrug must be activated by KatG (bacterial catalase-perioxidase enzyme) which combines the isonicotinic acyl with NADH to form isonicotinic acyl-NADH complex.[4] KatG is able to bind tightly to the enoyl-acyl carrier protein reductase (InhA).[4] The main purpose of this mechanism is to prevent the synthesis of mycobacterial cell wall. Side effects associated with using isoniazid as an anti-TB drug include nausea, fever, rash, and vomiting.

Rifampicin **2** was first isolated from bacteria in 1959 by Lepetit Pharmaceuticals.[5] It acts as a bactericidal for active TB. This drug develops bacterial DNA-dependent RNA synthesis by inhibiting bacterial DNA-dependent RNA polymerase.[5] Side effects associated with using rifampicin as an anti-TB drug include hepatitis, liver failure, breathlessness, vomiting, nausea, fever and abdominal cramps.[5]

Ethambutol **3** was first synthesized in 1965.[6] This drug acts as a bacteriostatic against actively growing TB bacilli, preventing the formation of cell wall. Ethambutol is well absorbed from the gastrointestinal tract and well distributed in body tissues and fluids.[6] Ethambutol can be used in combination with other drugs such as isoniazid **1** and rifampicin **2** in the treatment of *Mycobacterium tuberculosis*. Side effects associated with using ethambutol as an anti-TB drug ethambutol include blurred vision, eye pain, joint pain, skin rash, weakness in hands or feet, burning pain and liver problems.[6]

Pyrazinamide **4** was first synthesized in the 1950s.[7] It is a prodrug that stops the growth of mycobacterium tuberculosis. Pyrazinamide diffuses in *M. tuberculosis*, where the enzyme pyrazinanidase converts pyrazinamide into the active form pyrazinoic acid.[7] Side effects associated with using this drug as an anti-TB drug includes hepatotoxicity which is dose related. Other side effects include nausea, vomiting, anorexia sideroblastic anemia, skin rash, and fever.[8]

On the other hand, drugs classified as second line anti-TB drugs are *para* aminosalicylic acid **5**, kanamycin **6**, capreomycin **7**, ciprofloxacin **8**, ethionamide **9** and streptomycin **10**.

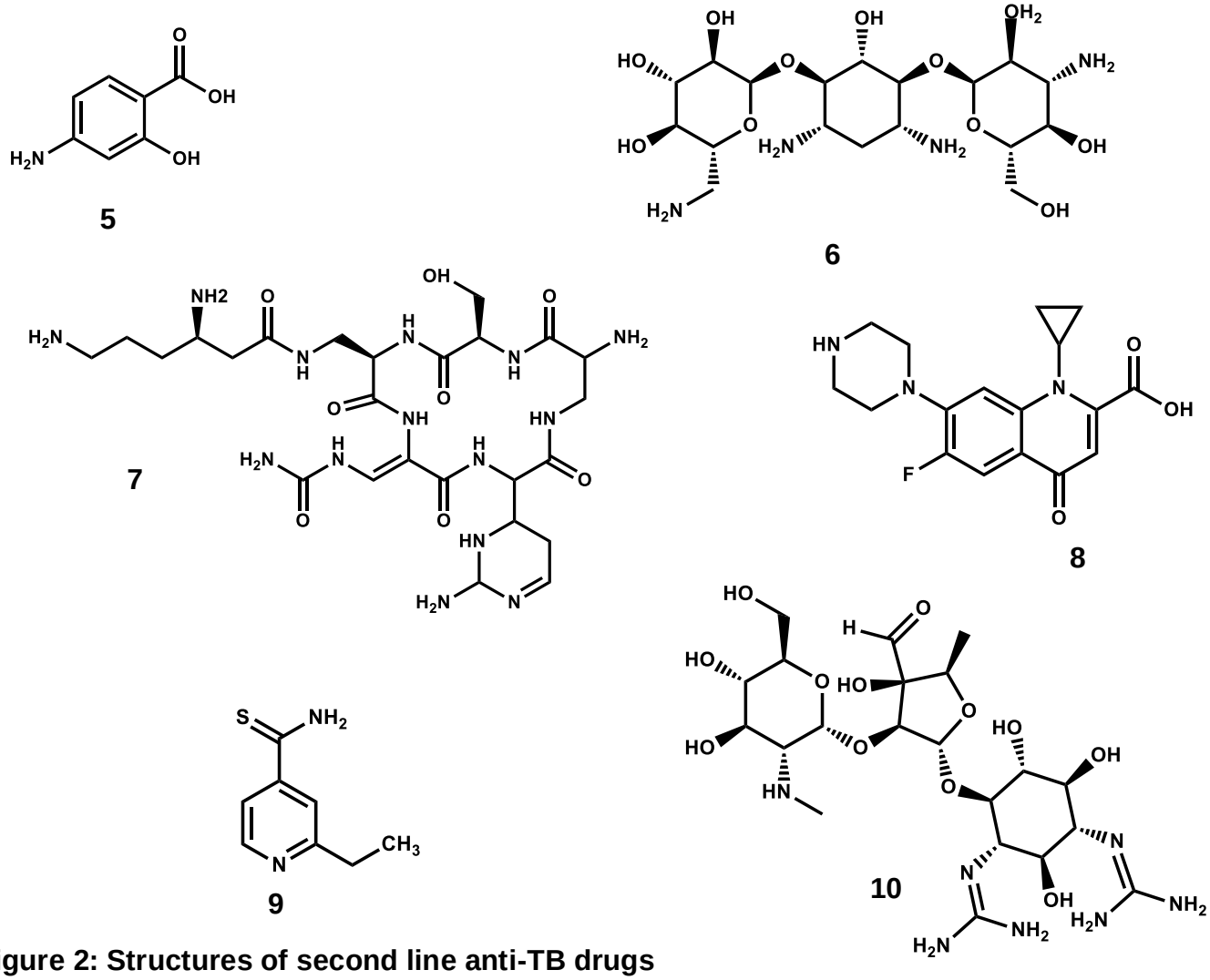


Figure 2: Structures of second line anti-TB drugs

Para aminosalicylic acid **5** was first synthesized in 1902 by Seidel and Bittner.[9] This is a pro-drug which is incorporated into the folate pathway by dihydropteroate synthase (DHPS) and dihydrofolate synthase (DHFS) to generate a hydroxyl dihydrofolate antimetabolite, which in turn inhibits dihydrofolate reductase (DHFR) enzymatic activity.[10] PAS **5** is one of the antimycobacterial drugs currently used for the treatment of multidrug-

resistant tuberculosis. Side effects associated with using PAS as an anti-TB drug include nausea, vomiting, diarrhoea and hepatitis.[11]

Kanamycin **6** was first synthesized in the 1950s by Hamao Umezawa.[12] This drug acts as an aminoglycoside bacteriocidal antibiotic. Side effects associated with using this drug as an anti-TB drug include pain or irritation where the injection was given, mild skin rash, headache, fever, nausea, and vomiting.[12]

Capreomycin **7** was discovered in 1960 by Bayers Corporation, isolated from streptomyces capreolus.[13] This drug is thought to inhibit protein synthesis by binding to the 70S ribosomal unit. It also binds to components in the bacterial cell which result in the production of abnormal proteins. These proteins are necessary for the bacteria's survival. Therefore the production of these abnormal proteins is ultimately fatal to the bacteria. Side effects associated with using this drug as an anti-TB drug include nephrotoxicity and 8th cranial auditory vestibular nerve toxicity.

Ciprofloxacin **8** was first discovered in the 1980s by Bayer Corporation.[14] it is a second-generation fluoroquinolone.[15] It is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria.[16] The bactericidal action of this drug results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercoiling repair, and recombination.[17] Patients who use this drug as an anti-TB drug may experience side effects such as headaches, dizziness, insomnia, nausea, diarrhoea, abnormal liver function tests and vomiting.[17]

Ethionamide **9** was first synthesized in 1956.[18] It belongs to the class of organic compounds known as pyridines and derivatives. It is activated by the enzyme EthA, a mono-oxygenase in *Mycobacterium tuberculosis*, and binds NAD+ to form an adduct which inhibits InhA in the same way as isoniazid **1**.[18] It is used as part of treatment regimens, generally involving medicines to treat MDR and XDR TB. Patients who use this drug as an antibiotic may experience side effects such as headaches, dizziness, insomnia, nausea, diarrhoea, abnormal liver function tests and vomiting.

Streptomycin **10** was first synthesized in 1943 by Waksman.[19] It was the first clinically useful anti-tuberculosis agent synthesized However a few years after its introduction, streptomycin-resistant strains emerged.[19] This is an antibiotic that inhibits both Gram-positive and Gram-negative bacteria.[20] As a protein synthesis inhibitor this drug binds to the small 16S rRNA of the 30S subunit of the bacterial ribosome, interfering with the binding of formyl-methionyl-tRNA to the 30S sub-unit.[21] This results in an unstable

ribosomal-mRNA complex, leading to cell death. Side effects which patients might encounter include hearing loss, kidney damage, numbness, tingling or pain of hands. The use of streptomycin as an anti-TB drug may also have a toxic effect on brain or spinal cord function and toxicity to organs of hearing.[22]

1.1 Newest known anti-TB drugs

Latest drugs to be approved by United States FDA for the treatment of tuberculosis are compound **11**, **12** and **13 (figure 3)**.

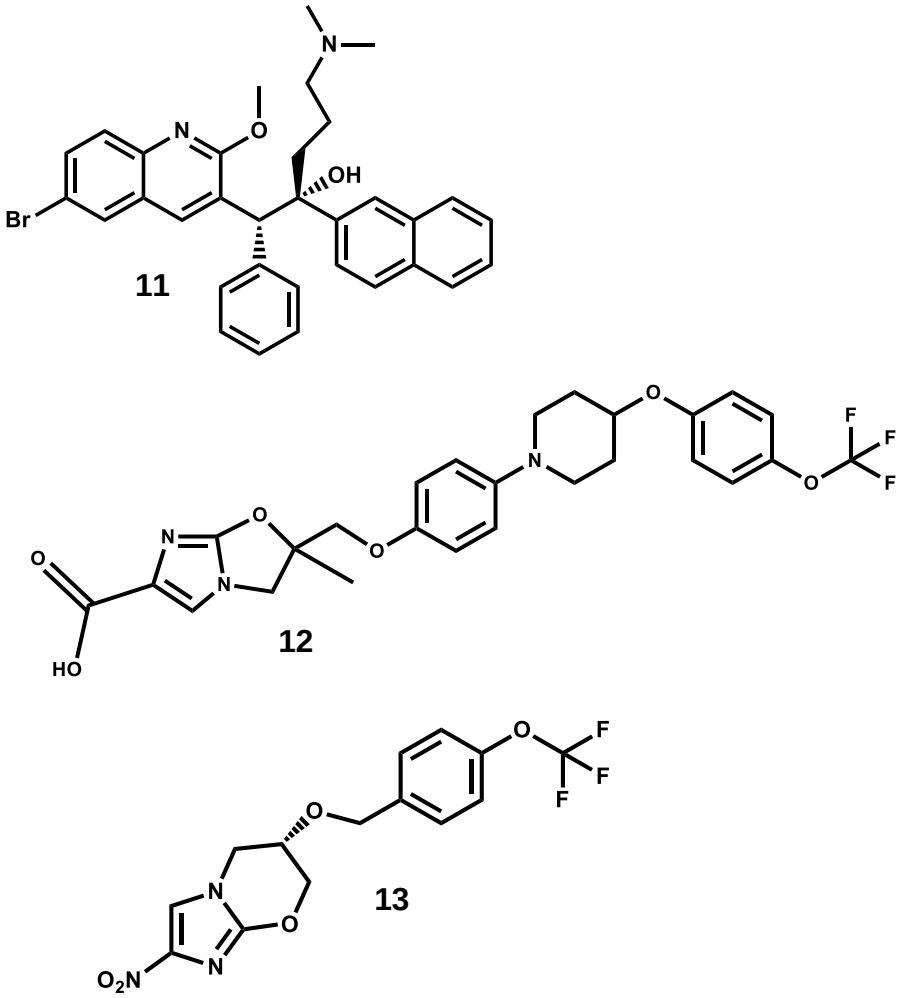


Figure 3: Structures of newest known anti-TB drugs

Bedaquiline **11** was developed by Jansen pharmaceutical and approved by FDA in 2012 is said to be active against XDR-TB and MDR-TB. Bedaquiline's most common side effects are: headache, nausea, spitting up blood, vomiting and skin rash.[23] Delamanid **12** was developed by Otsuka pharmaceutical and is given to adults with pulmonary TB that is affecting the lungs and is said to be active against MDR-TB.[23] Delamanid's most common

side effects are: nausea, vomiting and dizziness.[23] The most troubling side effect of delamanid, is also caused by other MDR-TB drugs like bedaquiline.[23]

Pretomanid **13** was developed by Pathogenesis Corporation and later transferred to the TB Alliance, where it is currently undergoing Phase III clinical trials.[23] The mode of action of pretomanid showed a puzzling mixed effect both on genes responsive to cell wall inhibition, like isoniazid (**1**). [24]

Both bedaquiline **11** and delamanid **12** can be used for the treatment of drug resistant TB when there are no other alternatives.[23] Bedaquiline and delamanid must also not be used with other drugs that the patient might already be resistant too. If this is done the patient could become resistant to bedaquiline and/or delamanid as well.[23]

1.2 Treatment of tuberculosis

Currently, the most widely used combinations of antibiotics against tuberculosis employ isoniazid **1**, rifampicin **2**, *p*ara amino salicylic acid **5** or ethambutol **3**. The current short course TB therapy used to treat drug susceptible *M.tuberculosis* consists of 2 months' treatment with four so called first line drugs including rifampin, isoniazid, pyrazinamide and ethambutol, followed by 4 months' treatment with rifampicin and isoniazid. Despite more than 50 years of use, combination therapy with isoniazid **1** is still a first-line treatment for tuberculosis.

Ethambutol **3** is highly successful when taken in combination with isoniazid **1** since the two act on different aspects of cell wall biosynthesis of the bacterium. This is made possible by disrupting the synthesis of the arabinogalactan leading to an increase in permeability of the cell wall.[10]

Pyrazinamide **4** is only used when taken with other drugs such as isoniazid **1** and rifampicin **2** in the treatment of *Mycobacterium tuberculosis*.[8]

The risk or severity of adverse effects can be increased when ethionamide **9** is taken with eycloserine.[25] The serum concentration of Isoniazid **1** can be increased when it is combined with ethionamide **9**.[25]

1.3 Drug resistance in anti-tuberculosis drugs

It came as an unpleasant surprise shortly after the introduction of each of the various classes of antibiotics new pathogenic strains emerged that were no longer susceptible. The emergence of the resistant microorganisms is now known to be predictable due to the

widespread antibiotic use and misuse. The drug regimen must be strictly followed to avoid the emergence of resistant strains.

Anti-TB drug resistance is a major public health problem that threatens progress made in the attempt to obtain faster and better drugs for TB. Different strains of resistant TB are known, namely, multi-drug resistant TB (MDR-TB), extensively drug-resistant TB (XDR-TB) and extremely drug-resistant tuberculosis abbreviated as XXDR-TB. MDR-TB is defined as a resistant to more than one of the most powerful first line anti-TB drugs for example rifampicin **2** and isoniazid **1**.[1] MDR-TB usually occurs when the anti-TB drugs which are currently in use are mismanaged and therefore becoming ineffective. XDR-TB is a TB strain that is resistant to both the first and second line anti-TB drugs. XXDR-TB strain that is resistant to all the anti-TB drugs Due to the major problem of TB drug resistance and side effects of some of the current TB drugs, new anti-TB drugs are always needed. This together with the problem of the interactions of the current TB drugs with the antiretroviral drugs taken by HIV positive people, shows that there is an urgent need for the development of existing and new anti-TB drugs.

1.4 Tuberculosis in South Africa

South Africa has the third highest burden of disease in the world after India and China with an estimated incidence of 530 000 case of active TB in 2014, an increase of 40% over the last 15 years An estimated 60-73% of the 530 000 incident cases have both HIV and TB infection, given that 30% of the global incident cases of TB-HIV co-infection occur in SA.[26] The incidence of Multidrug-resistant (MDR) and extensively drug-resistant TB are increasing and South Africa has the second highest number of reported multi-grug-resistant TB (MDR-TB) cases globally.[26] Schools and clinics have long been recognized as sites of TB transmission.[27] Recently a study from the Kwazulu Natal province in South Africa used site-based CO_2 measurement and calculated annual probabilities of acquiring infection using estimates of exposure durations.[28] Both the classroom and also the clinic were found to have the highest annual probabilities of acquiring infection for school attendees and clinic staff respectively.[28]

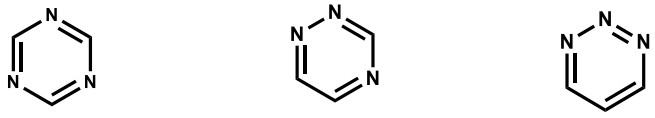
In 2017 Kigozi and co-workers reported females of advancing age (>24 years), and being on TB treatment for longer than 2 months were protective against treatment default. Furthermore, female cases were 40% less likely to default treatment compared to their male counterparts.[29]

1.5 Molecular hybridization

The rational planning of new synthetic prototypes for anti-cancer, anti-microbial, anti-HIV, cardioactive agents, anti-tumoral agents, anti-infectious agents, anti-diabetic, neuroactive and anti-inflammatory agents have been developed using a new and useful tool called molecular hybridization (MH).[30] MH was first proposed in Holland on the basis of biological evolution to explain the chemical evolution.[31] This strategy is based on the combination of different moieties of different bioactive substances to produce a new hybrid compound.[31] The combination of different moieties occurs through the recognition of pharmacophoric sub-unit in the molecular structure of the parent molecule I and parent molecule II which presents the substitutions.[20] This strategy normally results in new hybrid compounds with different modes of action and less side effects to that of the parent drugs. If the tool is carried out correctly a new drug which is more selective, effective and efficient is produced. Hybrid drugs have been proposed as new alternatives for the regulation of dependency and tolerance to opioid pharmaceuticals.[32] Opioid are medications that reduce the intensity of pain signals reaching the brain and affect those brain areas controlling emotions. The MH strategy is interesting for the development of new prototypes for diseases whose treatment is restricted to commercial drugs. The other reason is that there are drugs which are being discovered but have high toxicity or pharmacokinetic and pharmacodynamics restrictions. If the effect of the discovered drug has a tremendous negative impact on humans the drug is most likely to be rejected by the MCC, EMA and the USFDA which are regulatory bodies. As a result, this project was aimed at hybridizing different known anti-TB drugs together with amines to develop novel anti-TB drugs.

1.6 Types of triazines

A triazine is defined as a benzene ring that contains three nitrogen atoms and three carbon atoms. Three different types of triazines are known (**figure 4).**



14 **15** **16**

Figure 4: Different types of triazines

A triazine that contains nitrogen atoms at positions 1,3,5 of the benzene ring is known as 1,3,5-triazine **14** (*s*-triazine). A triazine that contains nitrogen atoms at positions 1,2,4 of

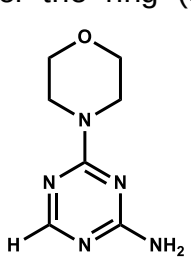
the benzene ring is known as 1,2,4-triaizine **15** (sec triazine). The last known triazine contains nitrogen atoms at positions 1,2,3 of the benzene ring and is known as 1,2,3-triazine **16**. 1,3,5-triazine is the most abundant and demonstrates ease of substitution of chlorine atoms by various nucleophiles under controlled conditions as compared to 1,2,4-triazine and 1,2,3-triazine.

1.6.1 Chemistry of 1,3,5-triazine

The compound was first synthesized by Nef in 1895 by reacting hydrogen cyanide with ethanol in an ether solution saturated with hydrogen chloride. The resultant mixture was then treated with base and distilled to produce 1,3,5-triazine in low yields of 10 %.[33] Nef incorrectly identified the product as a dimeric species. However, in 1954, Grundmann and Kreutzberger proved the compound to be a trimer of hydrogen cyanide, *s*-triazine.[34] *S*-triazine (1,3,5-triazine) is quite stable, aromatic in character and susceptible to nucleophilic attack and it rapidly decomposes in water.[35] Its reactivity and properties have been studied most extensively. It is thermally stable up to 600 °C and readily undergoes nucleophilic substitution.[36]

1.6.2 Known drugs containing 1,3,5-triazine

1,3,5-triazine derivatives constitute a group of compounds which still continue to be an object of considerable interest for medicinal chemists due to their broad biomedical value as therapeutics. The compound has provided the basis for the design of compounds with a wide variety of properties useful in medicinal and agricultural applications. Substituted *s*-triazine derivatives have attracted much synthetic interest among chemists due to the wide range of biological activities such as anti-microbial,[37] anti-malaria[37] and anti-cancer.[38] Also, it was reported that some of these compounds possess potent antibacterial and antifungal activities.[39] 1,3,5-triazine is also being used as a core in the synthesis of many compounds due to its reactivity towards nucleophilic substitution, high symmetry and the increased stability of the ring (82.5 Kcal/mol) compared to that of the benzene (39 Kcal/mol). [40]



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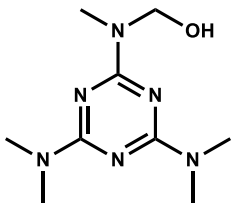

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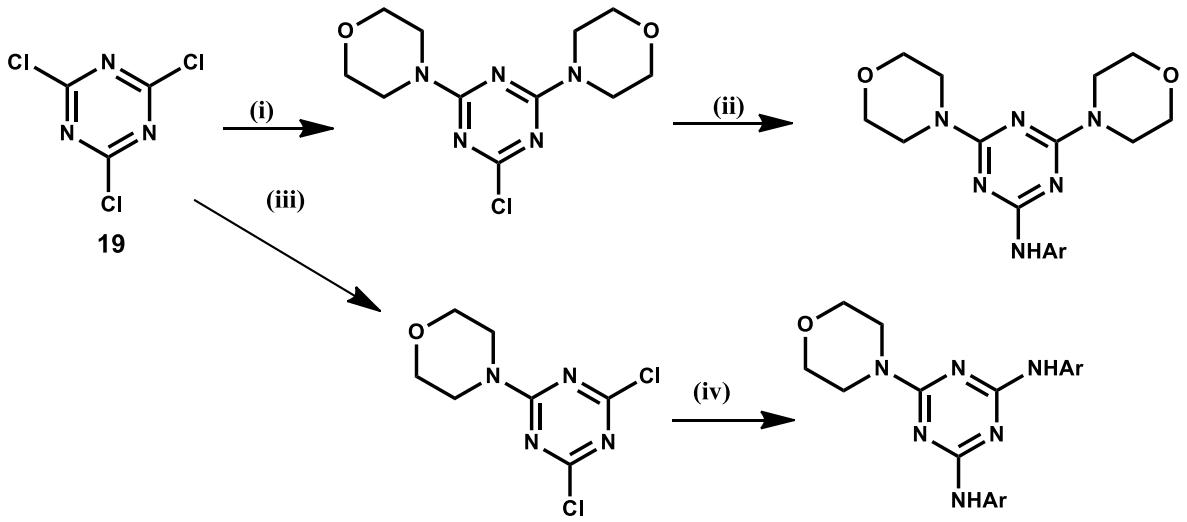
Figure 5: Structures of 1,3,5-triazines containing compounds

A study by Sunduru and co-workers has confirmed that several *s*-triazines derivatives bearing morpholine and piperidine moieties are effective against *Mycobacterium tuberculosis*.[41] *p*-Amino benzonitrile moiety has been found to possess an enhanced antimicrobial profile, improved anti-tubercular and profound anti-cancer activity.[42] Newly synthesized 1,3,5-triazine-isonicotino hybrazide based thiazole derivatives exhibit great antimicrobial potency when fluro and nitro substituents bearing derivatives are used. This is because these particular derivatives are electron withdrawing which causes enhancement in activity against most test microorganisms.[43]

Among several other substituted 1,3,5-triazine polyamines tested, some compounds show impressive *in vitro* activity against the protozoan parasite (trypanosome brucei), which has been identified to be the causative organism of human African trypanosomasis.[44] Other 1,3,5-triazines containing compounds like 2-amino-4-morpholine-1,3,5-triazine **17** are used clinically to treat lung, breast and ovarian cancer due to their antitumor property.[45] Hydroxymethylpentamethylmelamine (HMPMM) **18** is the hydroxylated metabolite which corresponds to the major form of HMM and is used clinically for antitumor activity in human cancer and muribe leukemia cell lines.

1.6.3 Known synthesis methods

There are a number of known ways in which triazine derivatives can be synthesized either through the use of conventional method or microwave. In 2007 Doktorov conducted research on microwave assisted solventless synthesis of melaminines with flexible aromatic substituents (**scheme 1)**.[46]



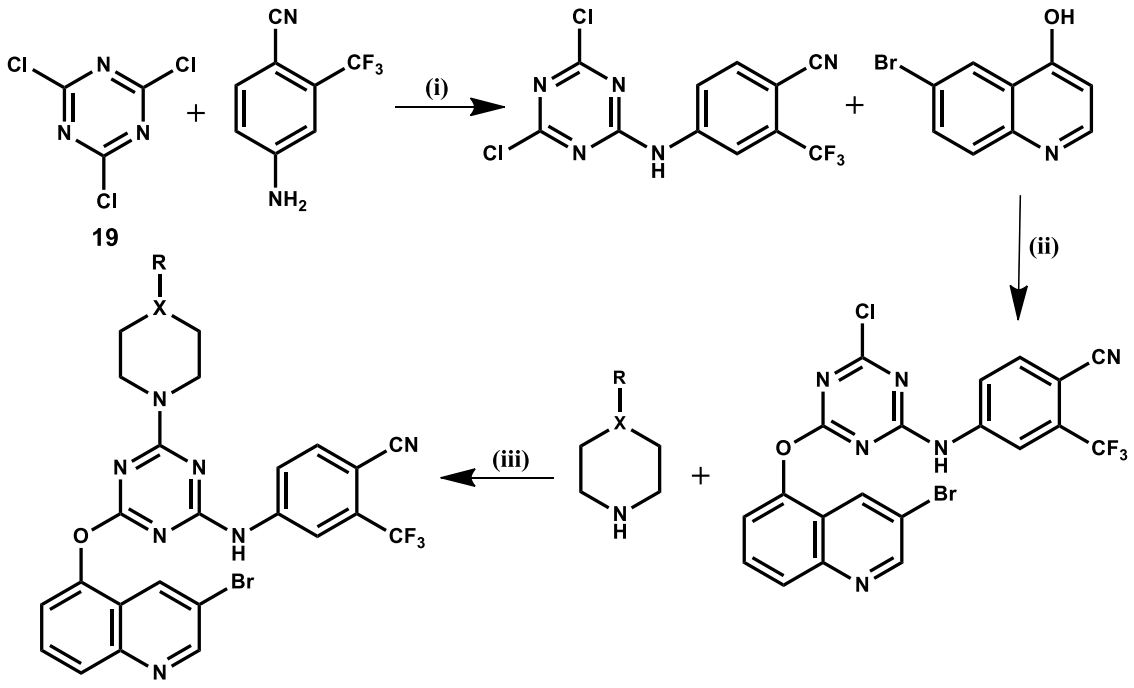
Ar = Ph, 4-MePh, 4-MeOPh, 4-BrPh, 4-CNPh, 1-naphthyl, 2-naphthyl

(i) morpholine (4 equiv), DCM, R.T; (ii) $ARNH_2$ (2 equiv), silica gel (2 g/mmol), MWI, 800W, DCM, 3 min; (iii) morpholine (1 equiv), Na_2CO_3 (1equiv), DCM, 0°C; (iv) $ArNH_2$ (4 equiv), silica gel (2 g/mmol), MWI, 800W, 3 min

Scheme 1: Synthesis of melamines with flexible aromatic substituents

The synthesized *s*-triazine derivatives with different amines were done in order to prove that both the microwave and silica gel accelerate the transformation studied. The conversion was complete within 3 min, which leads to a serious energy saving.[45] Hydrogen chloride was the only by-product, which makes the procedure an atom economy friendly.[45] Furthermore the by-product was quenched as ammonium salts during the reaction, thus preventing its release into the environment.[46]

Patel *et al* designed and synthesised novel series based upon 4-amino-2-trifluoromethyl-benzonitrile and 6-bromo-4-hydroxyquinoline-incoporated *s*-triazines **(scheme 2)**.[47] Furthermore they introduced a similar piperazine base in both systems in order to identify the difference between the biological profiles of the resistant series, in which activity was found to increase significantly against most of the studied strains of bacteria and fungi in terms of MIC.[48] It was then reported that the presence of halogens in a compound decreases the MIC levels significantly therefore increasing the biological activity, furthermore the presence of piperidine or piperazine bases in a compound also provide constant activity against *M.tuberculosis* .[48]



X= N or H or O
R= H

i) THF, Et_3N; ii) THF, NaH; iii) 1,4-dioxane, K_2CO_3

Scheme 2: Synthesis of *s*-triazine derivatives

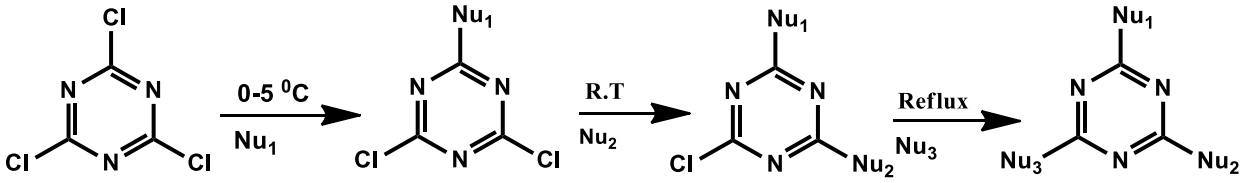
In the case of this project, both microwave and conventional method were used to synthesis *s*-triazine derivatives. However, microwave irradiation was used to synthesize mainly tri substituted compounds, while the conventional methods was used to synthesize the mono .and di substituted derivatives.[48] Furthermore instead of substituting the cyanuric chloride with only amines we also substituted known anti-TB drugs with the hope of increasing the biological activity.

1.7 Aims of the project

Identification of unique leads represents a significant challenge in drug discovery. This challenge is widely visible in neglected diseases such as tuberculosis. The urgent need in search of new biological entities to fight back TB and drug resistant TB is a drive behind this project.

Sunduru and co-workers recently reported that 2,4,6-trisubstituted triazine derivatives have shown promising biological activities, such as enhanced antimicrobial profile, improved anticancer and anti-tuberculosis activity.[41] With this in mind this project attempted to synthesize novel anti-TB drugs using molecular hybridization as a tool to achieve our aim. This tool was visible by using cyanuric chloride which is a derivative of

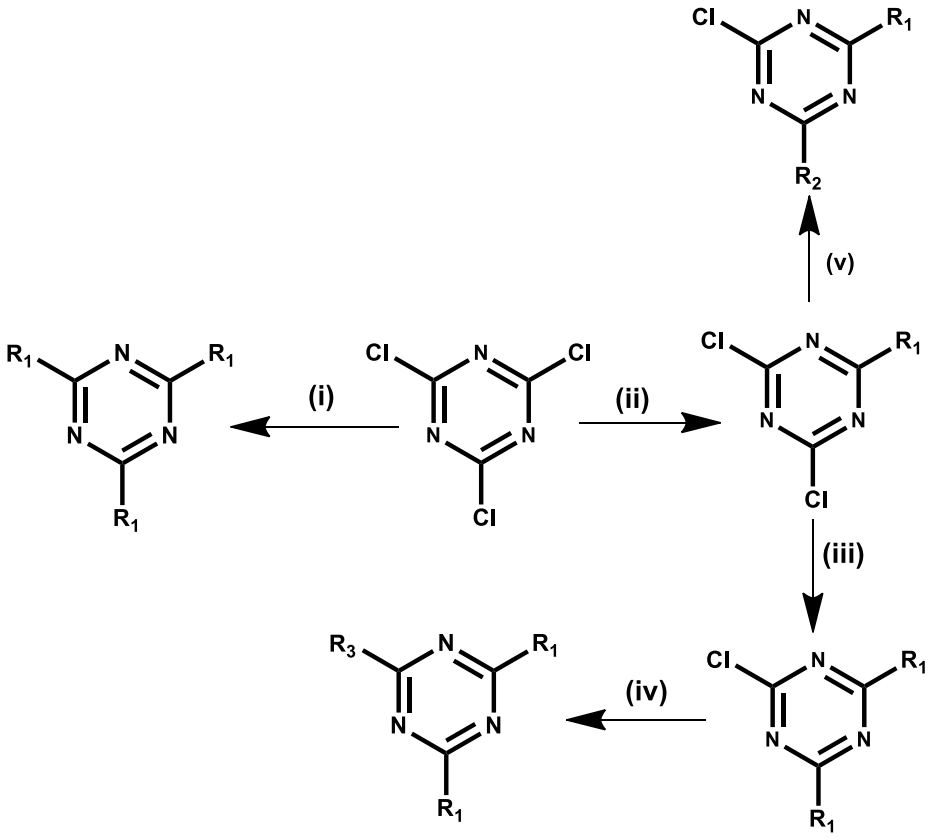
1,3,5-triazine as the starting material, substituted by known anti-TB and different heterocyclic amines.[49] 1,3,5-triazine is an inexpensive, commercially available reagent used for the preparation of variety of *s*-triazine derivatives. The ease of substitution of chlorine atoms in cyanuric chloride by various nucleophiles enhances the utility of this reagent for the preparation of mono, di and tri substituted 1,3,5-triazine derivatives under controlled temperature conditions **(scheme 3)**.[50] Mono aminosubstituted 1,3,5-triazine derivatives can be achieved by reacting cyanuric chloride an one molar equivalent of an amine at 0 °C. Di-substituted derivatives by reacting cyanuric chloride with two molar equivalent at room temperature and tri-substituted derivatives by reacting three molar equivalent of an amine under reflux.



Scheme 3: Replacement of chlorines

Chapter 2
Results and discussion

This project was aimed at synthesizing tri-amino-substituted-1,3,5-triazine as depicted in the reaction **scheme 4** below. These compounds were synthesized from cyanuric chloride **19** or from diamino-substituted-monochloro-1,3,5-triazine. Both reactions were done under microwave irradiation in a closed vessel with a power of 800 W. In turn diamino-substituted monochloro-1,3,5-triazine compounds were synthesized from monoamino-substituted dichloro-1,3,5-triazine. These reactions were performed at room temperature. Monoamino-substituted 1,3,5-triazines would be synthesized from reactions of cyanuric chloride and amines at 0°C. The structures of the products were confirmed by IR, ^{1}H NMR and ^{13}C NMR spectroscopy's together with GC/MS spectrometry.

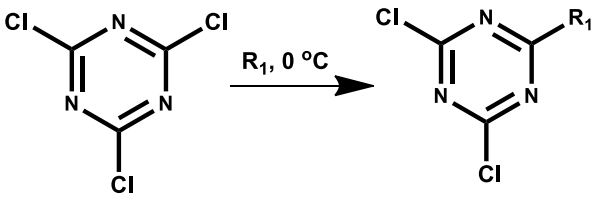


R_1 = morpholine, piperidine, pyrrolidine,ethionamide, PAS, isoniazid; R_2 = ethionamide, PAS
R_3 = morpholine, piperidine, ethionamide, PAS, isoniazid;

(i) 3 × R_1, MW ; (ii) R_1, 0°C ; (iii) R_1, R.T; (v) R_2, R.T; (iv) R_3, MW

Scheme 4: General reaction

2.1 Synthesis of mono-substituted triazines



19 **20, 21, 22**

R_1 = morpholine, piperidine, pyrrolidine

Scheme 5: Synthesis of compounds 20, 21 and 22: Conventional method

Table 1: Results of compounds 20, 21, 22: Conventional method

Compound	Yield[a] (%)	Melting point (°C)
20, R_1=morpholine	74	162 – 166 (154 – 156 °C)[53]
21, R_1=piperidine	75	180 – 182 (176 – 178)[53]
22, R_1=pyrrolidine	77	76 – 79 (80 - 85°C)[54]

[a]After recrystallization from hot ethanol

Envisaged products were obtained by reacting cyanuric chloride with one molar equivalent of a secondary amine. Secondary amines of choice were morpholine, piperidine and pyrrolidine. Products were obtained in good yields as shown in **(table 1)** above and were confirmed by NMR spectroscopy.

^{1}H NMR spectra of all three compounds (**20**, **21**, **22**) were characterized by appearance of peaks in the aliphatic to heteroatomic regions (1.70 – 3.90 ppm). Compound **20** showed two signals appearing as triplets and accounting for four protons each at 3.72 ppm and 3.90 ppm respectively. Also ^{1}H NMR spectrum of compound **22** showed two signals in the aliphatic to heteroatomic regions. These two signals were observed as multiplets accounting for four protons each. First multiplet was observed at 1.93 ppm and the second one at 3.54 ppm. Furthermore ^{1}H NMR spectrum of compound **21** showed three signals in the aliphatic to heteroatomic regions. These three signals were observed as multiplets. First multiplet accounting for two protons was observed at 1.60 ppm, the second one, accounting for four protons at 1.75 ppm and the third one, also accounting for four protons was observed at 3.78 ppm. Signals of the unreacted piperidine were reported at 1.47 ppm, 1.67 ppm and 2.82 ppm respectively. [51]

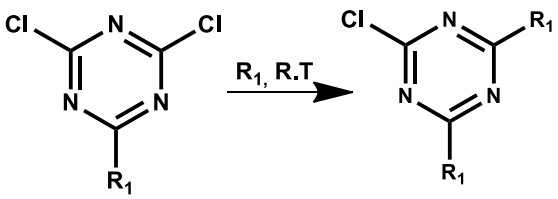
The ^{13}C NMR results were more revealing about our products. All our three products (**20**, **21**, **22**) showed two quaternary peaks in the aromatic regions (160.0 – 171.0 ppm) of their spectra. More revealing was the intensities of the two peaks. Peaks observed at ~164.0 ppm, indicating carbons bonded to amines were about half the size of the peaks observed at ~170.0 ppm, indicating carbon bonded to chlorine. These peaks were observed at 170.4

ppm (2 × C-Cl) and 164.1 ppm (C-N) for compound **20** and at 169.0 ppm (2 × C-Cl) and 163.6 ppm (C-N) for compound **22** and at 170.1 ppm (2 × C-Cl) and 163.6 ppm (C-N) for compound **21**. Also present in the ^{13}C NMR spectra of our compounds were the methylene carbon peaks in the aliphatic to heteroatomic regions (24.0 – 70.0 ppm). For compound **20**, two methylene carbon peaks were observed at 44.6 ppm and 66.3 ppm. Also compound **22**, showed two methylene carbon peaks at 19.8 ppm and 47.4 ppm. Furthermore compound **21**, showed three methylene carbon peaks at 24.6 ppm, 25.8 ppm and 45.3 ppm. Signals of the unreacted piperidine were reported at 25.5 ppm, 27.2 ppm and 47.5 ppm respectively.[51] In addition the mass spectra results showed three lines in the molecular region (M+, M+2 and M+4) with gaps of 2 m/z units between them which confirmed that compounds **20, 21 and 22** contain two chlorines atoms, indicating that these compounds are mono-substituted and the product was formed.

2.2 Synthesis of di-substituted triazines

Two different types of di-substituted triazines were synthesized in this project. The first type of di-substituted triazines were synthesized by reacting monoamino-substituted triazines **20**, **21**, **22** with secondary amines and the second type were synthesized by reacting compounds **20**, **21**, **22** with known anti-TB drugs such as PAS and ethionamide.

2.2.1 Di-substituted triazines from similar amines



20, 21, 22 **23, 24; 25**

R_1= morpholine, piperidine, pyrrolidine

Scheme 6: Synthesis of compounds 23, 24, 25: Conventional method

Table 2: Results of compounds 23, 24, 25: Conventional method

Compound	Yield[a] (%)	Melting point (°C)
23, R_1=morpholine	73	177 – 180 (173 -174)[55]
24, R_1=piperadine	62	118 – 120 (114 – 117)[55]
25, R_1=pyrrolidine	68	107 – 110 (112 – 115)[55]

[a]After recrystallization from hot ethanol

Diamino-1,3,5-triazines **23**, **24**, **25** were synthesized by reacting compounds **20**, **21** and **22** with morpholine, piperidine and pyrrolidine respectively. Products were obtained in good yields as shown above **(table 2)** and were confirmed by NMR spectroscopy.

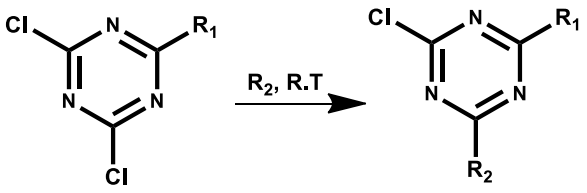
^{1}H NMR spectra of all three compounds (**23**, **24**, **25**) were characterized by appearance of peaks in the aliphatic to heteroatomic regions (1.50 – 3.70 ppm). Compound **23** showed two signals appearing as triplets and accounting for eight protons each. First triplet was observed at 3.64 ppm and second one at 3.71 ppm as compared to the mono-substituted starting material which showed these peaks at 3.72 ppm and 3.90 ppm respectively, thus the change in chemical shift serves as evidence that the desired product was obtained. Also ^{1}H NMR spectrum of compound **25** showed two signals in the aliphatic to heteroatomic regions. These two signals were observed as multiplets accounting for eight protons each. First multiplet was observed at 1.86 ppm and the second one at 3.43 ppm as compared to the mono-substituted starting material which showed these peaks at 1.93 ppm and the second one at 3.54 ppm, thus the change in chemical shift serves as evidence that the desired product was obtained. Furthermore ^{1}H NMR spectrum of compound **24** showed three signals in the aliphatic to heteroatomic regions. These three signals were observed as multiplets. First one, accounting for four protons was observed at 1.57 ppm, the second one, accounting for eight at 1.70 ppm and the third also accounting for eight protons at 3.30 ppm as compared to the mono-substituted starting material which showed these peaks 1.60 ppm, 1.75 ppm and the third one, at 3.78 ppm, thus the change in chemical shift serves as evidence that the desired product was obtained. Signals of the unreacted piperidine were reported at 1.47 ppm, 1.67 ppm and 2.82 ppm respectively.[51]

The ^{13}C NMR results were more revealing about our products. ^{13}C NMR spectrum.[51] All our three products showed two quaternary peaks in the aromatic regions (160.0 – 170.0

ppm) of their spectra. More revealing was the intensities of the two peaks. Peaks observed at ~169.0 ppm, indicating carbons bonded to chlorines were about half the size of the peaks observed at ~163.0 ppm, indicating carbon bonded to nitrogen. These peaks were observed at 169.7 ppm (C-Cl) and 164.5 ppm (2 × C-N) for compound **22** and at 168.1 ppm (C-Cl) and 162.5 ppm (2 × C-N) for compound **24** and at 169.7 ppm (C-Cl) and 163.8 ppm (2 × C-N) for compound **23**. Also present in the ^{13}C NMR spectra of our compounds were the methylene carbon peaks in the aliphatic to the heteroatomic regions (20.0 – 70.0 ppm). For compound **23**, two methylene carbon peaks were observed at 43.9 ppm and 66.7 ppm. Also two methylene carbons peaks were observed for compound **24** at 25.1 ppm and 46.4 ppm. Signals of the unreacted pyrrolidine were reported at 25.7 ppm and 47.1 ppm respectively.[50] Furthermore three methylene carbons peaks were observed for compound **25** at 24.4 ppm, 25.7 ppm and 44.9 ppm. Signals of the unreacted piperidine were reported at 25.5 ppm, 27.2 ppm and 47.5 ppm respectively.[51] In addition the mass spectra results showed two lines in the molecular region (M+ and M+2) with gaps of 2 m/z units between them which confirmed that compounds **23, 24** and **25** contain one chlorine atom indicating that these compounds are di-substituted and the product was formed.

2.2.2 Di-substituted triazines from mono-substituted derivatives

In keeping to our research topic, synthesis of 1,3,5-triazine based anti-TB drugs, we looked to incorporate known anti-TB drugs onto 1,3,5-triazine. This was done by reacting monoamino-substituted products **20**, **21** and **22** with known anti-TB drugs ethionamide and PAS as shown below **(scheme 7)**



20, 21, 22 **26, 27, 28**

R_1= morpholine, pyrrolidine, piperadine
R_2= PAS, ethionamide

Scheme 7: Synthesis of compound 26, 27, 28 using conventional method

Table 3: Results of compound 26, 27, 28 using conventional method

Compound	Yield[a] (%)	Melting point (°C)
26,R_1=pyrrolidine,R_2=ethionamide	57	211 – 215
27,R_1=piperidine,R_2=ethionamide	51	223 – 226
28, R_1=morpholine, R_2=PAS	69	232 – 236

[a]After recrystallization from hot ethanol

Products were obtained in average yields as shown above **(table 3)** and were confirmed by NMR spectroscopy

Compounds **26** and **27** were synthesized by reacting monoamino-substituted compounds **21** and **22** with ethionamide. ^{1}H NMR spectra of these compounds (**26** and **27**) were characterized by appearance of peaks in the aliphatic to aromatic regions (1.10 – 8.00 ppm), confirming the attachment of the ethionamide to compounds **21** and **22**. Quartets accounting for two protons were observed at ~2.80 ppm and triplets accounting for three protons were observed at ~1.20 ppm. Furthermore, the change in chemical shifts of the peaks observed in the aliphatic to heteroatomic regions from the starting material compared to the product serves as evidence that the desired product was obtained. In the 1H NMR of both compounds, unreacted ethionamide shows quartet at 1.01 ppm, triplet at 2.86 ppm. More assuring were the 13C spectra of compounds 26 and 27. The quaternary carbon peaks at ~170.0 ppm (C-Cl) have the same intensity as quaternary carbons at ~ 164.0 ppm (C-N). This confirms that we no longer have (2 × C-Cl) bonds in our molecules. Also, we observed the appearance of aromatic C-H peaks in the 13C NMR spectra, confirming the attachment of the ethionamide onto compounds 21 and 22.

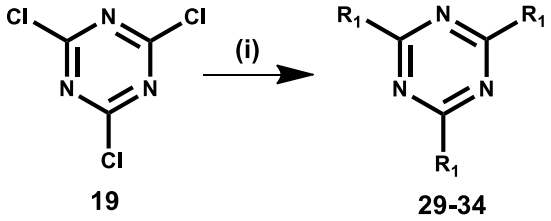
On the other hand, compound **28** was synthesized by reacting morpholino-triazine **20** with a known anti-TB drug, PAS. ^{1}H NMR spectra of compound **28** was characterized by appearance of peaks aromatic region (6.00 – 7.50 ppm), confirming the attachment of the PAS onto compounds **20**. Furthermore, the change in chemical shifts of two peaks observed in the aliphatic to heteroatomic region from the starting material at 3.72 ppm and 3.90 ppm to 1.06 ppm and 3.45 ppm for the product serves as evidence that the desired product was obtained. Certainty was obtained from the ^{13}C NMR spectrum whereby the intensity of a quaternary carbon peak at ~170.0 ppm (C-Cl) in compound **28** is no more double but equal to the intensity of a quaternary carbons at 164.2 ppm (C-N). This

confirms the replacement of one of the C-Cl bonds with C-N bond since we now observe the quaternary carbon peaks (C-N) at 163 9 ppm and 164.9 ppm.

2.3 Synthesis of tri-substituted triazines

The different types of tri-substituted triazines were synthesized. The first type were triazines that were synthesized using three similar amines, second type were triazines that were synthesized using two different amines. Both these types of tri-substituted triazines are discussed below. Tri-substituted compounds were carried out using microwave irradiation and silica gel.

2.3.1 Tri-substituted triazines from cyanuric chloride



19 29-34

R_1= morpholine, piperidine, pyrrolidine, ethionamide, PAS, Isoniazid

i) R_1, silica gel, 85 - 164 °C, 3 - 8 min

Scheme 8: Synthesis of compounds 29 - 34 using the microwave

Table 4: Results of compounds 29, 30, 31, 32, 33, 34 using the microwave

Compounds	Yield[a] (%)	Melting point (°C)
29, R_1=morpholine	98	293-296 (284 – 289)[56]
30, R_1=piperidine	95	222 – 225 (219 – 221)[56]
31, R_1 =pyrrolidine	96	179 – 184 (175 – 177)[57]
32, R_1 = PAS	92	328 – 333
33, R_1 = ethionamide	95	299 – 301
34, R_1 = Isoniazid	90	252 – 256

[a]After recrystallization from hot ethanol

Envisaged products **29**, **30**, **31** were obtained by reacting cyanuric chloride with three molar equivalent of secondary amines (morpholine, piperidine and pyrrolidine) whereas products **32**, **33**, **34** where obtained by reacting cyanuric chloride **19** with three molar equivalent known anti-TB drugs (PAS, ethionamide and isoniazid). Products were

obtained in excellent yields as shown above **(table 4)** and were confirmed by NMR spectroscopy.

^{1}H NMR spectra of compounds (**29**, **30**, **31**) were characterized by appearance of peaks in the aliphatic to heteroatomic regions (1.50 - 3.95 ppm). Compound **29** showed two signals observed as triplets accounting for twelve protons each. The first triplet was observed at 3.18 ppm and the second one at 3.93 ppm. These signals confirm attachment of morpholine onto 1,3,5-triazine. Signals of the unreacted morpholine were reported at 2.64 ppm and 3.64 ppm respectively.51 Also 1H NMR spectrum of compound 31 showed two signals in the aliphatic to heteroatomic regions. These two signals were observed as multiplets accounting for twelve protons each. First multiplet was observed at 1.84 ppm and the second one at 3.40 ppm. Signals of the unreacted pyrrolidine were reported at 1.75 ppm and 3.04 ppm respectively.51 1H NMR spectrum of compound 30 showed three signals in the aliphatic to heteroatomic regions. These three signals were observed as multiplets. First one, accounting for six protons was observed at 1.52 ppm, the second one, accounting for twelve at 1.59 ppm and the third multiplet also accounting for twelve protons was observed at 3.40 ppm. Signals of the unreacted piperidine were reported at 1.47 ppm, 1.67 ppm and 2.82 ppm respectively.[51]

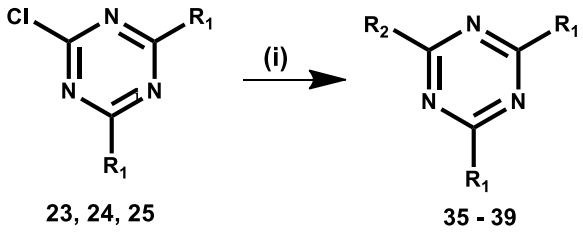
Moreover ^{1}H NMR spectrum of compound **32** showed three signals in the aromatic regions (6.10 – 7.80 ppm). These three signals were observed as doublets accounting for three protons each. First double was observed at 6.10 ppm, the second one at 7.12 ppm and the third at 7.20 ppm. Signals of the unreacted PAS were reported at 6.22 ppm, 6.61 ppm and 7.78 ppm respectively.[51] Furthermore, ^{1}H NMR spectrum of compound **33** showed five signals in the aliphatic to aromatic regions (1.30 – 8.75 ppm). One of these signals was observed as a triplet and the second one as a quartet. First triplet, accounting for nine protons was observed at 1.30 ppm and the second quartet, accounting for six protons was observed at 3.09 ppm. Furthermore, the other three signals were observed as doublets accounting for three protons each. The third was observed at 7.91 ppm the fourth one at 8.70 ppm and the fifth one at 8.75 ppm. Signals of the unreacted ethionamide were reported at 1.01 ppm, 2.86 ppm, 7.51 ppm, 7.64 ppm, and 8.62 ppm respectively.[51] In addition ^{1}H NMR spectrum of compound **34** showed two signals in the aromatic regions (7.20 – 9.00 ppm). These two signals were observed as multiplets accounting for six protons each respectively. First multiplet was observed at 8.12 ppm, the

second multiplet at 8.15 ppm and the third at 7.20 ppm. Signals of the unreacted isoniazid were reported at 8.72 ppm and 7.88 ppm respectively.[51]

The 13C NMR results were more revealing about our products. All our six products showed one quaternary peak in the aromatic regions (99.0 – 164 ppm) of their spectra. More revealing was the disappearance of the peak at ~169.0 ppm indicative of the carbon bonded to the chlorine atom. These peaks were observed at 165.3 ppm (3 × C-N) for compound 29, 162.6 ppm (3 × C-N) for compound 31, 165.4 ppm (2 × C-N) for compound 30. 163.8 ppm (3 × C-N) for compound 32, 164.8 ppm (3 × C-N) for compound 33 and 163.7 ppm (3 × C-N) for compound 34. Also present in our compounds were methylene carbons, aromatic carbons and methyl carbons observed for compound **29**, two methylene carbon peaks were observed at 43.4 ppm and 66.8 ppm. The unreacted morpholine peaks are seen at 46.7 ppm and 68.1 ppm respectively.[51] Compound **31**, showed two methylene carbon peaks at 25.0 ppm and 46.6 ppm. Signals of the unreacted pyrrolidine were reported at 47.1 ppm and 25.7 ppm respectively.[51] Furthermore compound **30**, showed three methylene carbon peaks at 24.1 ppm, 25.8 ppm and 43.9 ppm. Signals of the unreacted piperidine were reported at 25.5 ppm, 27.2 ppm and 47.5 ppm respectively.[51] Compound **32**, showed six aromatic carbon peaks at 99.9 ppm, 101.5 ppm, 107.3 ppm, 131.5 ppm, 132.0, 144.1 ppm and a C=O peak at 172.5 ppm. Signals of the unreacted PAS were reported at 100.3 ppm, 102.1 ppm, 107.9 ppm, 133.2 ppm 147.1 ppm, 165.0 ppm respectively, and a C=O peak at 172.1.[50] Compound **33**, showed one methyl carbon peak, one methylene carbon peak, and five aromatic carbon peaks at 13.7 ppm, 31.2 ppm, 122.6 ppm, 123.3 ppm, 143.8 ppm 152.5 ppm, 160.3 ppm respectively, and a C=S at 196.6 ppm. Signals of the unreacted ethionamide were reported at 13.4 ppm, 31.4 ppm, 117.8 ppm, 119.5 ppm 146.9 ppm, 149.5 ppm,164.3 ppm respectively, and a C=S peak at 200.9.[51] Compound **34**, showed three aromatic carbon peaks at 123.5 ppm, 147.9 ppm,148.8 ppm and a C=O peak at 164.0 ppm. Signals of the unreacted isoniazid were reported at 121.0 ppm, 140.3 ppm, 150.0 ppm respectively, and C=O peak at 164.0 ppm.50 Furthermore, from the IR spectra it is observed that there was no C-Cl stretch peak at ~700 cm-1 which we expected since all the chlorines have been substituted by an amine. In addition, the mass spectra results did not show any line in the chlorine molecular region which confirmed that compounds **29, 30 and 31** do not contain any chlorine atom indicating that these compounds are tri substituted and the product was formed.

2.3.2 Tri-substituted triazines from di-substituted compounds

In keeping to our research topic, synthesis of novel-1,3,5-triazine-based-anti-TB drugs, we looked to incorporate known anti-TB drugs onto 1,3,5-triazine. This was done by reacting mono-substituted products **23**, **24** and **25** with a known anti-TB drug ethionamide, PAS and isoniazid in the presence of silica gel as a catalyst as shown below **(scheme 8).**



23, 24, 25 **35 - 39**

R_1,= morpholine, piperidine,pyrrolidine;
R_2,= morpholine, piperidine, ethionamide, PAS, isoniazid
i) R_2, silica gel, 85 - 160 °C, 3 - 8 min

Scheme 9: **Synthesis of compounds 35, 36, 37, 38, 39 using the microwave**

Table 5: **Results of compounds 35 - 39 using the microwave**

Compounds	Yield[a] (%)	Melting point (°C)
35, R_1= pyrrolidine; R_2=morpholine,	91	167 – 170
36, R_1= pyrrolidine; R_2=piperidine,	89	252 – 255
37, R_1= piperidine, R_2= PAS	94	248 – 251
38, R_1= morpholine, R_2= ethionamide,	93	259 – 263
39, R_1= morpholine, R_2= isoniazid,	85	240 – 244

[a]After recrystallization from hot ethanol

Envisaged products were obtained by reacting di-substituted cyanuric chloride with two molar equivalent of primary amines or a known drug. Known anti-TB drugs of choice were ethionamide, PAS and isoniazid. Products were obtained in excellent yields as shown above **(table 5)** and were confirmed by NMR spectroscopy.

Compound **35** showed four signals appearing as multiplets and accounting for eight, four, eight and four protons each at 1.92 ppm, 3.51 ppm, 3.68 ppm and 3.72 ppm respectively. Also ^{1}H NMR spectrum of compound **36** showed five signals appearing as multiplets and accounting for two, eight, four, eight, four protons each at 1.62 ppm, 1.89 ppm, 2.18 ppm, 3.58 ppm and 3.72 ppm respectively. Furthermore, it was observed that there was a

change in chemical shifts of the two peaks from the starting material which were observed in the aliphatic to heteroatomic regions at 1.93 ppm and 3.54 ppm serving as evidence that the desired compounds were obtained. ^{13}C NMR results were more revealing about our products (**35** and **36**). All two products showed two quaternary peaks at ~164.0 ppm (C-N), this is because all the (C-Cl) bonds were substituted by two different amines. More revealing was the intensities of the quaternary peaks, (C-$N_{pyrrolidine}$) bond is double the size of the intensity of the (C-$N_{morpholine}$) bond for compound **35.** Also for compound **36** the intensities of the quaternary peaks, (C-$N_{pyrrolidine}$) bond is double the size of the intensity of the (C-$N_{piperidine}$) bond. On the other hand, compound **37** was synthesized by reacting compound **24** with PAS. ^{1}H NMR spectra of compound **37** was characterized by appearance of peaks in the aromatic region (6.90 – 7.80 ppm), confirming the attachment of the PAS onto compounds **24**. Furthermore, it was observed that there was a change in chemical shifts of the three peaks from the starting material in the aliphatic to heteroatomic region at 1.60 ppm, 1.75 ppm and 3.78 ppm which serves as evidence that the desired compounds were obtained. Certainty was obtained from the ^{13}C NMR spectrum whereby there is no longer a carbon peak at ~170.0 ppm (C-Cl), but rather two quaternary carbon peaks at ~ 164.0 ppm (C-N) which have different intensities. (C-$N_{morpholine}$) bond is double the size of the intensity of a quaternary carbons of the (C-N_{PAS}) bond. This confirms the replacement of (C-Cl) bond with (C-N) bond. Furthermore, compound **39** was synthesized by reacting compound **23** with isoniazid. ^{1}H NMR spectra of compound **39** was characterized by appearance of peaks in the aromatic region (8.10 - 8.40 ppm), confirming the attachment of the isoniazid onto compounds **23**. In addition the change in chemical shifts of the peaks in the aliphatic to heteroatomic region from the starting material at 1.60 ppm and 1.75 ppm was observed compared to 1.24 ppm and 2.50 ppm of the product which serves as evidence that the desired product was obtained. Signals of the unreacted isoniazid were reported at 8.72 ppm and 7.88 ppm respectively.[51] Certainty was obtained from the ^{13}C NMR spectrum whereby there is no longer a carbon peak at ~170.0 ppm (C-Cl), but rather two quaternary carbon peaks at ~164.0 ppm (C-N) which have different intensities. (C-$N_{morpholine}$) bond is double the size of the intensity of a quaternary carbons of the (C-$N_{isoniazid}$) bond. This confirms the replacement of (C-Cl) bond with (C-N) bond. In the ^{1}H NMR of compound **39**, signals of the unreacted isoniazid were reported at 8.72 ppm and 7.88 ppm respectively.[51] ^{1}H NMR spectra of compound **38** was

characterized by appearance of peaks in the aromatic regions (7.60 – 8.55 ppm), confirming the attachment of the ethionamide to compound **23**. Quartets accounting for two protons were observed at 2.50 ppm and triplets accounting for three protons were observed at ~1.23 ppm. In addition a change in chemical shift of the peaks in the aliphatic to heteroatomic region of the starting material at 1.60 ppm and 1.75 ppm was observed compared to 1.66 ppm and 2.19 ppm of the product serves as evidence that the desired product was obtained. In the ^{1}H NMR of compound **38**, unreacted ethionamide shows quartet at 1.01 ppm, triplet at 2.86 ppm. More assuring were the 13C spectra of compounds 38 and 39. We know see that the there are no longer quaternary carbon peaks at~ 170.0 ppm (C-Cl) but rather two quaternary carbons peaks with the same intensity at ~164.0 ppm (C-N).

2.4 Biological evaluation

Some of the compounds synthesized in this project were subjected to anti mycobacterial testing against *M.smegmatis* using a microdilution broth method with prestoblue, at various concentration range from 1000 - 7.8125 µg/ml. The lowest concentration which showed no growth after spot subculture was considered as MIC for each drug. The standard drug used in the present study was Ciprofloxacin for evaluating biological activity.

Table 7: Biological activity of known anti-TB drugs

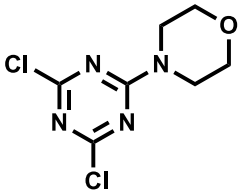
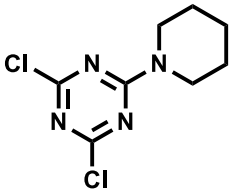
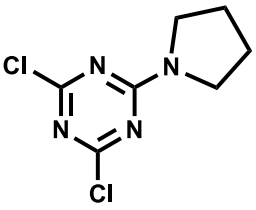
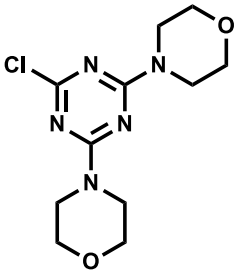
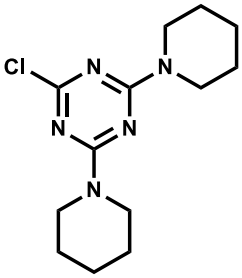
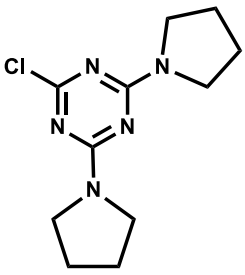
Samples	**MIC[a] (µg/ml)**
Ethionamide	500
PAS	500
Isoniazid	250
Ciprofloxacin	1.25

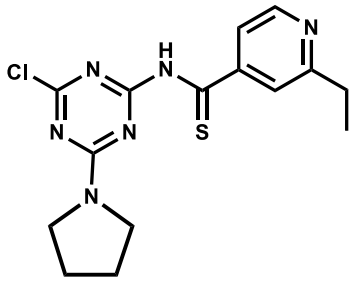
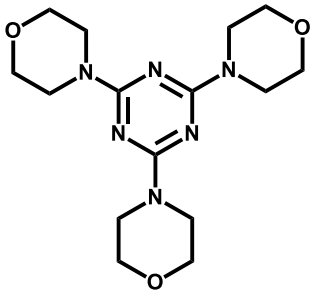
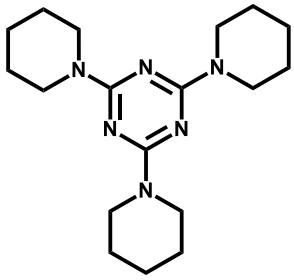
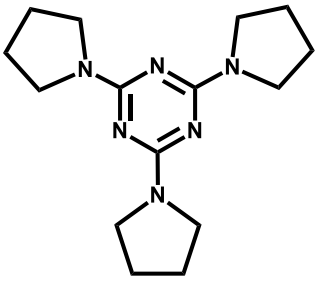
[a]MIC - Minimum inhibitory concentration

[b]NI – Not inhibited at the highest concentration tested

Concentration range was from 1000 - 7.8125 µg/ml

Table 8: Results of Biological activity

Compounds	Structure	MIC[a] (µg/ml
20		250
21		250
22		1000
23		Ni[b]
24		500
25		500

26		125
29		NI[b]
30		1000
31		250
Ciprofloxacin		1.25

[a]MIC - Minimum inhibitory concentration

[b]NI – Not inhibited at the highest concentration tested

Concentration range was from 1000 - 7.8125 µg/ml

Mono-substituted analogues **20** bearing morpholine substituents inhibit anti-TB activity at 250 µg/ml, which was expected since it is known that most potent analogues bearing chlorine atom with morpholine substituent have shown moderate toxicity values and an important lead for antituberculosis drug discovery.[52] Piperidine bearing compound **21** also inhibit activities at the 250 µg/ml, while pyrrolidine bearing compound **22** showed activity at 1000 µg/ml. Di-substituted analogues **23** bearing morpholine substituents did not inhibit anti-TB activity. In addition, di-substituted compound **24** bearing piperidine substituents

inhibited poor anti-TB activity at 500 µg/ml as compared to the mono substituted compound **21**. This is because the number of chlorines in the compound have decreased (one chlorine vacant), which in actual fact confirms Rahul and co-worker's findings that most potent analogues bearing chlorine atom with morpholine and piperidine substituent inhibit better anti-TB.[51] On the contrary di analogues bearing pyrrolidine substituents compound **25** increased in biological activity at 500 µg/ml when the chlorines are substituted. In addition, the di-substituted compound **26** bearing pyrrolidine and ethionamide substituents inhibit better anti-TB activity at 125 µg/ml as compared to the mono-substituted compound **22** bearing pyrrolidine substituent and even better anti-TB activity as compared to the known drug ethionamide.

Tri-substituted compound **29** bearing morpholine substituents did not inhibit anti-TB activity at the highest concentration tested, whilst tri-substituted compound **30** bearing piperidine inhibit poor anti-TB activity at 1000 µg/ml. These results confirm that the more chlorines substituted, the poorer the anti-TB activity when substituted by only morpholine and piperidine. On the contrary tri-substituted analogue bearing only pyrrolidine substituents compound **31** inhibit better anti-TB activities at 250 µg/ml as compared to the mono and di substituted compounds **25** and **22**. This indicates that compound with pyrrolidine as a substituent inhibit better biological activity when the chlorines are substituted.

Chapter 3
Conclusion and future work

Development of antimycobacterial agents based on identified targets and their mechanisms of action is a productive approach for the lead generation. Structural construction of 1,3,5-triazine ring in terms of replacement of three reactive chlorine atoms have been contemplated towards each biological target and specific conversation on beneficial replacement design is revealed. In this perception, we have chosen to explore 1,3,5-triazine moiety. In case of synthesizing novel anti-TB drugs, 1,3,5-triazine ring was substituted with morpholine, piperidine, pyrrolidine, PAS, ethionamide and isoniazid. With this aim, we designed and synthesized easily accessible mono, di and tri substituted 1,3,5-triazines derivatives and biological testing was carried out.

A range of anti-TB drugs incorporating a triazine core have been synthesised and characterised. Cyanuric chloride was used to prepare a number of triazine derivatives, di-chloro-1,3,5-triazine derivatives **20**, **21** and **22** were obtained by the reaction of morpholine, piperidine and pyrrolidine respectively with cyanuric chloride at 0°C. These compounds were obtained in 74 %, 75 % and 77 % yield respectively. Mono-chloro-1,3,5-triazine derivatives **23**, **24**, **25**, **26**, **27** and **28** were synthesized by either reacting the same amine as that was used to mono substitute the first chloride or by a known anti-TB drug such as ethionamide or PAS. These compounds were obtained in good to excellent yields 73 %, 62 %, 68 %, 57 %, 51 % and 69 % respectively. Tri-substituted-1,3,5-triazine compounds were prepared by reacting cyanuric chloride with three molar equivalents of an amine or known anti-TB drugs to obtain **29**, **30**, **31**, **32**, **33** and **34**. These compounds were obtained in excellent yields 98 %, 95 %, 96 %, 92 %, 95 % and 90% respectively. Furthermore tri-substituted derivatives were also prepared by reacting mono-chloro-1,3,5-triazine derivatives **23**, **24** and **25** with an amine or known anti-TB drugs to obtain **35**, **36**, **37**, **38**, **39**. These compounds were obtained in excellent yields 91 %, 89 %, 94 %, 93 % and 85 % respectively. The use of microwave irradiation component reactions for tri-substituted derivatives is highly recommended. This is because these two methods, minimize waste production, save time, and allowing an ecologically and economically favourable process, in addition these methods gave good yields (90 – 98 %).

Compounds **20**, **21** were found to be moderately active against *M.smegmatis*. This is because of the presence of piperidine and morpholine and two other chlorines. However compounds **23**, **29** showed no activity against *M.smegmatis,* compounds **24** and **30** showed moderated activity against *M.smegmatis.* This is because the presence of chlorine decreases. On the contrary compounds **22**, **25** and **31** increase activity against *M.smegmatis* as the chlorines are substituted.[52] In addition compound **26** showed better activity against *M.smegmatis* due to the presence of a known drug ethionamide.

Furthermore, the molecular systems presented in this dissertation would assist the process of further derivation which may eventually lead to the more active class of compounds to be considered as clinical trial candidates. The influence of cyanuric chloride in combination with amines, other anti-TB drugs and anti-HIV drugs deserves further study.

Moreover, we believe that findings of the present study will have a good impact on medicinal chemistry to synthesize similar compounds which will probably indicate better biological potency.

Future work includes exploring a wider range of the known anti-TB drugs. Furthermore further biological testing against *M.tuberculosis* as a strain on each compound to see which combination is more active. In addition, send all remaining samples for GC-MS.

Chapter 4
Experimental procedure

4.0 General procedure

All experiments were performed using standard glassware. All chemical reagents (cyanuric chloride, morpholine, piperidine, pyrrolidine, PAS, ethionamide and isoniazid) used were Analytical Grade Reagents from Fluka and Sigma Aldrich.

The microwave irradiated reactions (MWI) were performed in CEM Discover microwave DU8470 that has a maximum power of 800 W and maximum temperature of 300 ºC using 10 ml vials. Merck silica gel 60 (0.040 - 0.063 mm) was employed as a solid support.

Thin Layer Chromatography was carried out on Macherey-nagel UV_{254} plates. Detection was done under ultra violet light at 254 nm. The melting points of the synthesized compounds were determined by using a BÜCHI Melting point B-540 apparatus and were uncorrected. The Infrared spectra were run on the Bruker Fourier IR spectrometer using KBr pellet technique. Absorption maxima are reported in wavenumbers (cm^{-1}).

NMR spectroscopic analysis was done on Bruker Ultrashield 400 MHz. The frequency at which ^{1}H NMR spectra were reported was 400.02 MHz (rounded to 400 MHz). These spectra are reported as parts per million (ppm), with s = singlet, d = doublet, dd = doublet of doublet, t = triplet, m = multiple. The chemical shifts are given as dimensionless δ values and are frequency referenced relative to TMS. Coupling constants values are given in hertz (Hz) as positive values regardless of their real individual signs. The ^{13}C NMR spectra were reported at a frequency of 100.63 (rounded to 100 MHz) using $CDCl_3$ at 77.00 ppm or DMSO at 39.52 ppm as solvents.

The mass spectra were performed using a waters GCT with a column of Restek Rxi-5Sil MS Wintegra Guard (15 m, 0.25 mm ID, 0.25 µm film thickness; maximum temperature, 350 °C). Ultrahigh purity helium (99.99%) was used as carrier gas at a constant flow rate of 1.0 mL/min. The injection, transfer line and ion source temperatures were all at 280 °C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from auto tune. The oven temperature was programmed from 100 °C (hold for 2 min) to 300 °C at a rate of 15 °C/min. The samples were diluted with appropriate solvent (acetone or dichloromethane) (1/100, v/v) and filtered. The particle-free diluted crude extracts (1 µL) were taken in a syringe and injected into injector with a split ratio 10:1. All data was

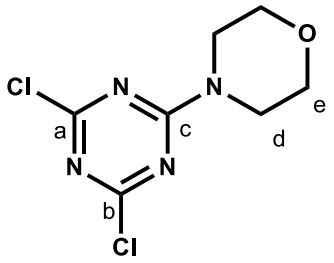
obtained by collecting the full-scan mass spectra within the scan mass range 35 - 650 m/z. Scan time 0.15 min, Inter-scan delay 0.05 min and the mass mode (TOF MS EI+). The percentage composition of the crude extract constituents was expressed as a percentage by peak area. The identification and characterization of chemical compounds in various crude extracts was based on GC retention time. The mass spectra were computer matched with those of standards available in mass spectrum libraries.

4.1 Synthesis of 1,3,5-triazine based anti-TB drugs

4.1.1 Mono-substituted 1,3,5-triazine derivatives: General procedure (1)

To a solution of cyanuric chloride **19** (1 mmol) in dichloromethane/acetone (1:1) an amine (1 mmol) and Na_2CO_3 (1 mmol) were added successively. The mixture was stirred for 8h at 0°C, over which time a suspension was formed. The solid phase was filtered off and washed with dichloromethane/acetone. The solvent was removed in *vacuo* to obtain products as solids. Recrystallization from hot ethanol gave the product as crystalline solid.

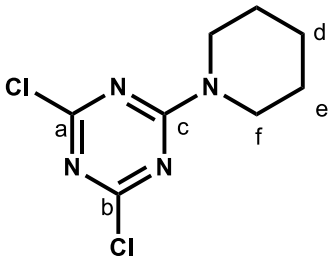
4.1.1.1. 2,4-dichloro-6-morpholino-1,3,5 triazine **(20)**



20

Using general procedure (1), cyanuric chloride (4.00 g, 21.69 mmol) was reacted morpholine (1.87 mL, 21.69 mmol) in the presence of Na_2CO_3 (2.30 g, 21.69 mmol) using DCM (40 mL) as a solvent. Recrystallized product **20** was obtained as a white crystalline solid (3.79 g, 74 %); m. p = 162 - 166 °C (lit. 154 – 156 °C);[53] ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 3.72 (4H, t, *J* = 4.8 Hz, 2 × C_dH_2); 3.90 (4H, t, *J* = 4.8 Hz, 2 × C_eH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 44.5 (2 × C_dH_2), 66.3 (2 × C_eH_2), 164.1 (C_c-N), 170.4 ($C_{a,b}$-Cl); IR (KBr) ϑ_{max} (cm^{-1}): 787.41 (C-Cl), 1068.00 (C-O), 3021.29 (C-H); TOF MS (EL+) m/z: $C_7H_8ON_4Cl_2$ calculated 235.0702 found 234.0050 (M^+ - H)

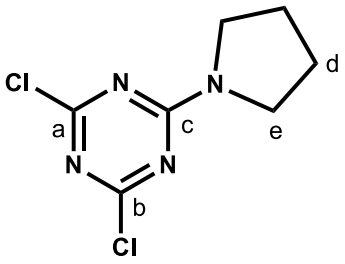
4.1.1.2. 2,4-dichloro-6-(piperidin-1-yl)-1,3,5-triazine **(21)**



21

Using general procedure (1), cyanuric chloride (2.00 g, 10.00 mmol) was reacted with piperidine (0.98 mL, 10.00 mmol) in the presence of Na_2CO_3 (1.06 g, 10.00 mmol) using acetone (40 mL) as a solvent. Recrystallized product **21** was obtained as a yellow crystalline solid (1.76 g, 75%); m. p. 180 - 182 °C (lit. 176 – 178 °C);[54] ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 1.70 (2H, m, C_dH_2); 2.19 (4H, m, 2 × C_eH_2); 3.78 (4H, m, 2 × C_fH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 24.60 (C_dH_2), 25.8 (2 × C_eH_2), 45.3 (2 × C_fH_2), 163.5 (C_c-N) 170.1 ($C_{a,b}$-Cl); IR (KBr) ϑ_{max} (cm^{-1}): 652.00 (C-Cl), 2999.10 (C-H); TOF MS (EL+) m/z: $C_8H_{10}N_4Cl_2$ calculated 233.8682 found 232.0234 (M^+ - H)

4.1.1.3. 2,4-dichloro-6-(pyrrolidin-1-yl)-1,3,5-triazine **(22)**



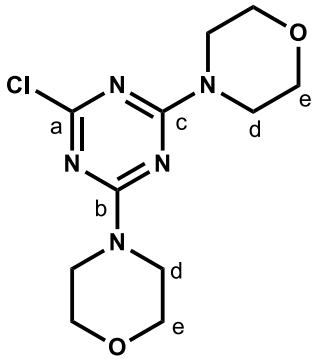
22

Using general procedure (1), cyanuric chloride (2.00 g, 10.00 mmol) was reacted with pyrrolidine (0.82 mL, 20.00 mmol) in the presence of Na_2CO_3 (1.06 g, 10.00 mmol) using acetone (40 mL) as a solvent. Recrystallized product **22** was as a yellow crystalline solid (1.69 g, 77%); m. p. 76 - 79 °C (lit. 80 - 85°C);[54] ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 1.93 (4H, m, 2 × C_dH_2); 3.54 (4H, m, 2 × C_eH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 19.8 (2 × C_dH_2), 47.4 (2 × C_eH_2), 163.6 (C_c-N), 169.0 ($C_{a,b}$-Cl); IR (KBr) ϑ_{max} (cm^{-1}): 691.66 (C-Cl), 2966.82 (C-H); TOF MS (EL+) m/z: ($C_7H_8N_4Cl_2$) calculated 225.0712 found 225.0770

4.1.2 Di-substituted 1,3,5-triazine derivatives: General procedure (2)

To a solution of mono substituted derivatives (**20**, **21**, **22**) (1 mmol) in a solvent (DCM, THF or acetone) was added an amine (2 mmol) and Na_2CO_3 (1 mmol) successively. The mixture was stirred overnight at R.T, over time a suspension was formed. The solid phase was filtered off and washed with a solvent (DCM, THF or acetone). The solvent was removed in *vacuo* to obtain products as solids. Recrystallization from hot ethanol gave the product as crystalline solid.

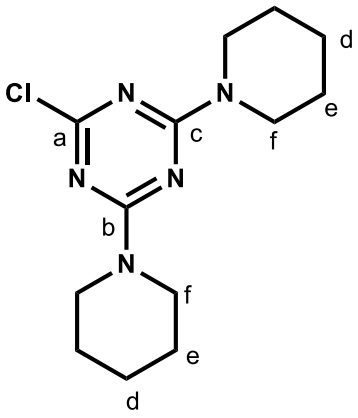
4.1.2.1. 2-chloro-4,6-dimorpholino-1,3,5-triazine **(23)**



23

Using general procedure (2), compound **20** (0.50 g, 2.11 mmol) was reacted with morpholine (0.36 g, 4.11 mmol) in the presence of Na_2CO_3 (0.22 g, 2.11 mmol) using DCM (20 mL) as a solvent. Recrystallized product **23** was obtained as a white crystalline solid (0.44 g, 73%); m. p. 177 - 180°C (lit. 173 -174°C);[55] ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 2.64 (8H, t, J = 4.8 Hz, 4 × C_dH_2), 3.71 (8H, t, J = 4.8 Hz, 4 × C_eH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 43.9 (4 × C_dH_2), 66.7 (4 × C_eH_2), 164.5 ($C_{b,c}$-N), 169.7 (C_a-Cl); IR (KBr) ϑ_{max} (cm^{-1}): 653.11 (C-Cl), 1062.24 (C-O), 2999.80 (C-H); TOF MS (EL+) m/z: $C_{11}H_{16}O_2N_5Cl$ calculated 285.7392 found 285.0986

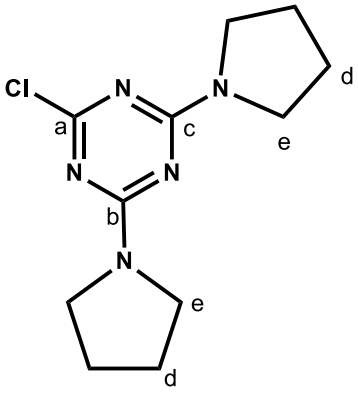
4.1.2.2. 2-chloro-4,6-di(piperidin-1-yl)-1,3,5-triazine **(24)**



24

Using general procedure (2), compound **21** (0.50 g, 2.13 mmol) was reacted with piperidine (0.41 mL, 4.16 mmol) in the presence of Na_2CO_3 (0.22 g, 2.13 mmol) using acetone (20 mL) as a solvent. Recrystallized product **24** was obtained as a yellow crystalline solid (0.37 g, 62%). m. p. 118 - 120 °C (lit. 114 - 117°C);[56] ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 1.57 (4H, m, 2 × C_dH_2); 1.66 (8H, m, 4 × C_eH_2); 3.30 (8H, m, 4 × C_fH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 24.4 (2 × C_dH_2), 25.7 (4 × C_eH_2), 44.9 (4 × C_fH_2), 163.8 ($C_{b,c}$-N) 169.7 (C_a-Cl); IR (KBr) ϑ_{max} (cm^{-1}): 664.64 (C-Cl), 3001.57 (C-H); TOF MS (EL+) m/z: $C_{13}H_{20}N_5Cl$ calculated 281.7844 found 281.1405

4.1.2.3. 2-chloro-4,6-di(pyrrolidin-1-yl)-1,3,5-triazine **(25)**

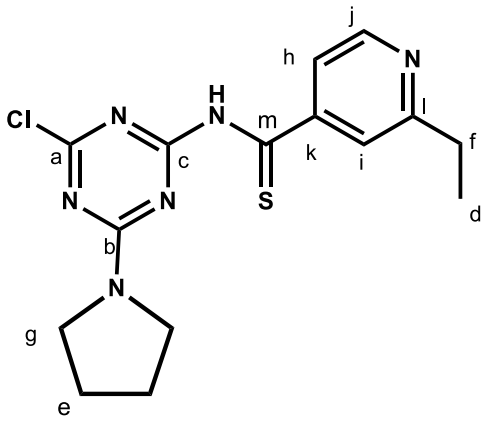


25

Using general procedure (2), compound **22** (0.50 g, 2.27 mmol) was reacted pyrrolidine (0.40 mL, 4.54 mmol) in the presence of Na_2CO_3 (0.24 g, 2.27 mmol) using acetone (20 mL) as a solvent. Recrystallized product **25** was obtained as a yellow crystalline solid (0.40 g, 68%); m. p. 107 – 110 °C (lit. 112 - 115°C);[55] ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: ^{1}H NMR (400 MHz, DMSO) δ_H ppm: 1.86 (8H, m, 4 × C_dH_2), 3.43 (8H, m, 4 × C_eH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 25.1 (4 × C_dH_2), 46.4 (4 × C_eH_2), 162.5

($C_{b,c}$-N), 168.1 (C_a-Cl); IR (KBr) ϑ_{max} (cm^{-1}): 793.06 (C-Cl), 2970.21 (C-H); TOF MS (EL+) m/z: $C_{11}H_{16}N_5Cl$ calculated 253.7312 found 253.1085

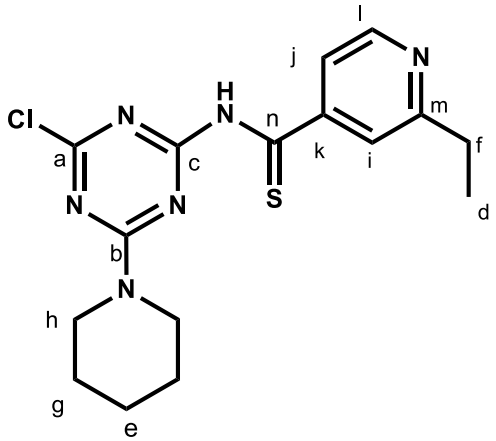
4.1.2.4. 2-chloro-4-(pyrrolidin-1-yl)-6-((2-ethyl)-thioisonicotinamide)-1,3,5-triazine **(26)**



26

Using general procedure (2), compound **22** (0.10 g, 0.45 mmol) was reacted ethionamide (0.21 g, 0.90 mmol) in the presence of Na_2CO_3 (0.05 g, 0.45 mmol) using acetone (20 mL) as a solvent. Recrystallized product **26** was obtained as yellow crystalline solids (0.08 g, 57%); m.p. 211 - 215 °C; ^{1}H NMR (400 MHz, DMSO) δ_H ppm: 1.23 (3H, t, *J* = 7.2 Hz, C_dH_3); 2.08 (4H, m, 2 × C_eH_2), 2.79 (2H, q, *J* = 7.6 Hz, C_fH_2), 3.51 (4H, m, 2 × $C_{10}H_2$), 7.52 (1H, d, *J* = 1.6 Hz, $C_{11}H$), 7.57 (1H, d, *J*= 5.2 Hz, $C_{12}H$), 8.53 (1H, d, *J* = 5.4 Hz, $C_{14}H$); ^{13}C NMR (100 MHz, DMSO) δ_C ppm: 14.1 (C_dH_3), 24.5 (2 × C_eH_2), 25.7 (2 × C_fH_2), 44.4 (C_gH_2),118.8 (C_iH), 119.4 (C_hH), 147.3 (C_{jH}), 149.5 (C_k), 163.6 (C_l), 163.6 (C_c-N), 163.9 (C_b-N), 169.2 (C_a-Cl), 199.2 (C=S); IR (KBr) ϑ_{max} (cm^{-1}): 750.00 (C-Cl), 1580.00(C=S), 3001.00 (C-H)

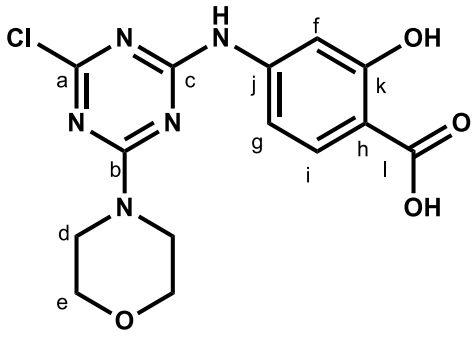
4.1.2.5. 2-chloro-4-(piperidin-1-yl)-6-((2-ethyl)-thioisonicotinamide)-1,3,5-triazine **(27)**



27

Using general procedure (2), compound **21** (0.10 g, 0.42 mmol) was reacted with ethionamide (0.14 g, 0.85 mmol) in the presence of Na_2CO_3 (0.04 g, 0.42 mmol) using acetone (20 mL) as a solvent. Recrystallized product **27** was obtained as yellow crystalline solids (0.07, 51%); m.p. 223 - 226 °C; ^{1}H NMR (400 MHz, DMSO) δH ppm: 1.25 (3H, t, *J* = 7.4 Hz C_dH_3); 1.59 (2H, m, C_eH_2), 2.50 (4H, m, 2 × C_fH_2), 2.79 (2H, q, *J* = 7.6 Hz, C_gH), 3.64 (4H, m, 2 × C_hH_2). 7.51 (1H, d, *J* = 1.6 Hz, C_iH), 7.57 (1H, d, *J* = 5.2 Hz, C_jH), 8.53 (1H, d, *J* = 5.4 Hz, C_lH); ^{13}C NMR (100 MHz, DMSO) δ_C ppm: 13.6 (C_dH_3), 24.1 (C_eH_2), 25.3 (C_fH_2), 30.6 (2 × C_gH_2), 43.9 (2 × C_hH_2), 118.4 (C_iH), 119.0 (C_jH), 146.8 (C_k), 149.1 (C_lH), 163.1 (C_m), 163.5 (C_c-N), 164.6 (C_b-N), 168.8 (C_a-Cl), 198.7 (C=S); IR (KBr) ϑ_{max} (cm^{-1}): 1584.91 (C=S), 709.00 (C-Cl), 2932.87 (C-H); 2962.11 (N-H)

4.1.2.6. 4-((2-chloro-4-morpholino-1,3,5-triazin-2-yl)amino)-6-hydroxylbenzoic acid **(28)**



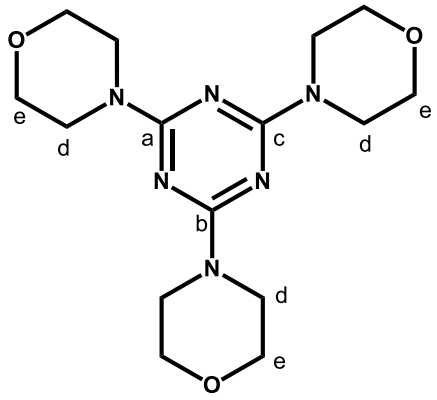
28

Using general procedure (2), compound **20** (0.50 g, 2.11 mmol) was reacted with PAS (0.65 g, 4.22 mmol) in the presence of Na_2CO_3 (0.22 g, 2.11) using THF (20 mL) as a solvent. Recrystallized product **28** was obtained as a brown crystalline solid (0.90 g, 69%), m.p 232 - 236 °C; ^{1}H NMR (400 MHz, DMSO) δ_H ppm: 1.06 (4H, t, *J* = 7.2 Hz, 2 × C_dH_2), 3.45 (4H, t, *J* = 7.0 Hz, 2 × C_eH_2), 6.09 (1H, d, *J* = 1.6 Hz, C_fH), 7.05 (1H, dd, *J* = 7.6 Hz, C_gH), 7.43 (1H, d, *J* = 8.4 Hz, C_iH); ^{13}C NMR (100 MHz, DMSO) δ_C : 44.0 (2 × C_dH_2), 66.3 (2 × C_eH_2), 99.1 (C_fH), 100.7 (C_gH), 106.8 (C_h), 131.9 (C_iH), 156.0 (C_j), 163.8 (C_k-OH), 163.9 (C_c-N), 164.9 (C_b-N), 169.23 (C_a-Cl), 172.54 (C=O); IR (KBr) ϑ_{max} (cm^{-1}): 758.26 (C-Cl), 1645.05 (C=O), 2862.43 (C-H), 3430.13 (OH)

4.1.3 Tri-substituted 1,3,5-triazine derivatives from cyanuric chloride: General procedure (3)

To a solution of cyanuric chloride **19** (1 mmol) in a solvent (DCM, THF or acetone) was added an amine (3 mmol) and silica gel (1 mmol) successively in a 10 mL vessel. 7 mL of the mixture was then transferred into a 10 mL vial. The mixture was irradiated in a microwave oven with a power of 300 W to obtain products as solids. Recrystallization from hot ethanol gave the products as crystalline solids.

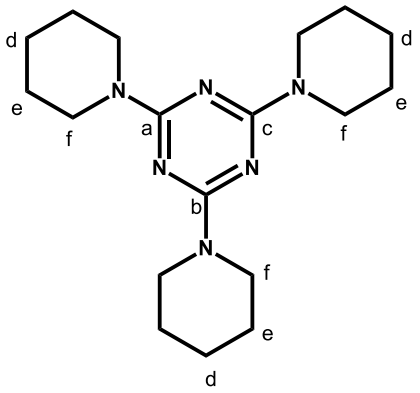
4.1.3.1. 2,4,6-tri(morpholino-1-yl)-1,3,5-triazine **(29)**



29

Using general procedure (3), cyanuric chloride **19** (1.00 g, 5.00 mmol) was reacted with morpholine (2.58 mL, 15 mmol) in the presence of silica gel (0.03 g, 5.00 mmol) using DCM (5 mL) as a solvent. The mixture was irradiated in microwave oven for 3 min at 160 ºC. Recrystallized product **29** was obtained as a white crystalline solid (1.66 g, 98%). m. p. 293 - 296 °C (lit. 284 - 289 °C);[56] ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 3.18 (12H, t, J = 4.8 Hz, 6 × C_dH_2); 3.93 (12H, t, J = 4.8 Hz, 6 × C_eH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 43.4 (6 × C_dH_2), 66.8 (6 × C_eH_2), 165.3 ($C_{a,b,c}$-N); IR (KBr) ϑ_{max} (cm^{-1}):1069.43 (C-O), 2999.45 (C-H); MS (EL+) m/z: ($C_{12}H_{24}O_3N_6$) calculated 336.3510 found 336.1772

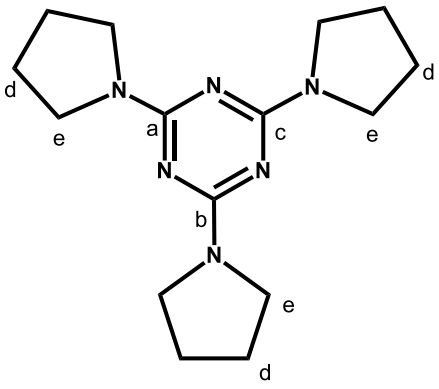
4.1.3.2. 2,4,6-tri(piperdin-1-yl)-1,3,5-triazine **(30)**



30

Using general procedure (3), cyanuric chloride **19** (1.00 g, 5.00 mmol) was reacted with piperidine (0.98 mL, 15.00 mmol) in the presence of silica gel (0.03 g, 5.00 mmol) using DCM (5 mL) as a solvent. The mixture was irradiated in microwave oven for 3 min at 160 ºC. Recrystallized product **30** was obtained as a yellow crystalline solid (1.58 g, 95 %); m. p. 221 - 225 °C (lit. 219 - 221 °C);[57] ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 1.52 (6H, m, 3 × C_dH_2); 1.59 (12H, m, 6 × C_eH_2), 3.40 (12H, m, 6 × C_fH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 24.10 (3 × C_fH_2), 25.8 (6 × C_eH_2), 43.9 (6 × C_fH_2), 165.4 ($C_{a,b,c}$-N); IR (KBr) ϑ_{max} (cm^{-1}): 2947.12 (C-H) ; TOF MS (EL+) m/z: 333.36 ($C_{18}H_{33}N_6$) calculated 333.3601 found 330.2524

4.1.3.3. 2,4,6-tri(pyrrolidin-1-yl)-1,3,5-triazine **(31)**

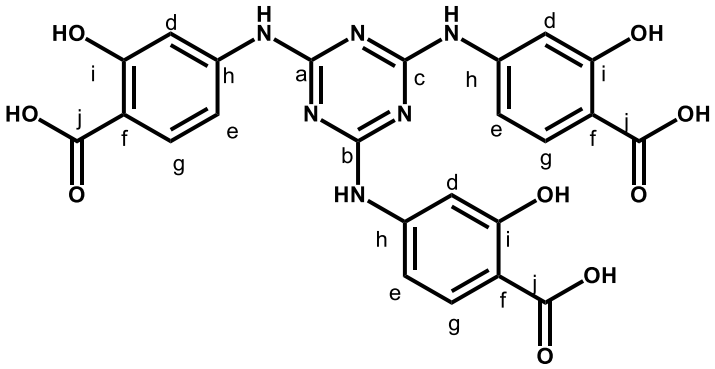


31

Using general procedure (3), cyanuric chloride **19** (2.00 g, 10.00 mmol) was reacted with pyrrolidine (2.05 mL, 30.00 mmol) in the presence of silica gel (0.06 g, 10.00 mmol) using DCM (5 mL) as a solvent. The mixture was irradiated in microwave for 3 min at 160 ºC. Recrystallized product **31** was obtained as a yellow crystalline solid (2.80 g, 96 %); m. p. 179 - 180 °C (lit. 175 - 177°C);[57] ^{1}H NMR (400 MHz, $CDCl_3$) δ_H

ppm: 1.84 (12H, m, 6 × C_dH_2); 3.40 (12H, m, 6 × C_eH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 25.0 (6 × C_dH_2), 46.59 (6 × C_eH_2), 162.6 ($C_{a,b,c}$-N); IR (KBr) ϑ_{max} (cm^{-1}): 2976.58 (C-H); TOF MS (EL+) m/z: ($C_{15}H_{24}N_6$) calculated 288.3912 found 288.2064

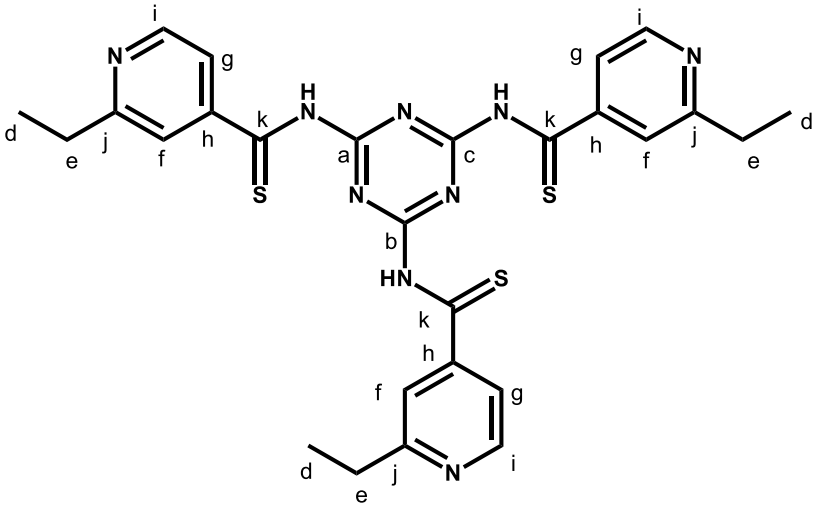
4.1.3.4. 4,4',4''-((1,3,5-triazine-2,4,6-triyl)tris(azanediyl))tris(2-hydroxybenzoic acid) **(32)**



32

Using general procedure (3), cyanuric chloride **19** (1.00 g, 5.00 mmol) was reacted with PAS (2.30 g, 15 mmol) in the presence of silica gel (0.03 g, 5.00 mmol) using THF (5 mL) as a solvent. The mixture was irradiated in microwave oven for 4 min at 162 ºC. Recrystallized product **32** was obtained as a brown crystalline solid (2.50 g, 92%), m. p. 328 - 333 °C; ^{1}H NMR (400 MHz, DMSO) δ_H ppm: 6.10 (3H, d, *J* = 1.8 Hz, 3 × C_dH) 7.12 (3H, dd, *J* = 1.6, 7.0 Hz, 3 × C_eH), 7.20 (3H, d, *J* = 7.0 Hz, 3 × C_gH); ^{13}C NMR (100 MHz, DMSO) δ_C ppm: 99.9 (3 × C_dH), 101.5 (3 × C_eH), 107.3 (C_f), 131.5 (3 × C_gH), 132.0 (3 × C_h), 144.1 (3 × C_i-OH) 163.8 ($C_{a,b,c}$-N), 172.5 (3 × C_{13}=O); IR (KBr) ϑ_{max} (cm^{-1}): (C=O) 1761.65, (C-H) 2937.60, 3836.23 (O-H)

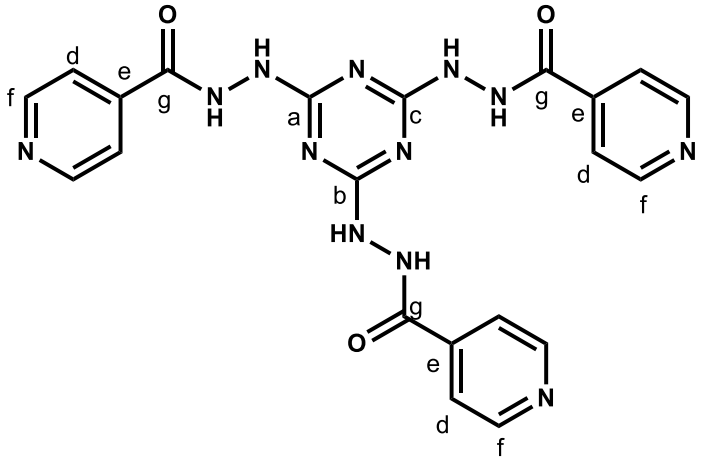
4.1.3.5. 2,4,6-tri((2-ethyl)-thioisonicotinamide)-1,3,5-triazine **(33)**



33

Using general procedure (3), cyanuric chloride **19** (1.00 g, 5.00 mmol) was reacted ethionamide (2.40 g, 15 mmol) in the presence of silica gel (0.03 g, 5.00 mmol) with acetone (5 mL) as a solvent. The mixture was irradiated in microwave oven for 8 min at 85 ºC. Recrystallized product **33** was obtained as a yellow crystalline solid (2.71 g, 95 %); m. p. 299 - 301°C; ^{1}H NMR (400 MHz, DMSO) δ_H ppm: 1.30 (9H, t, *J* = 7.6 Hz, 3 ×C_dH_3), 3.09 (6H, q, *J* = 7.6 Hz, 3 × C_eH_2), 7.91 (3H, d, *J*, 1.4 Hz, 3 × C_fH), 8.70 (3H, d, *J* = 5.4 Hz, 3 × C_gH), 8.75 (3H, d, *J* = 6.0 Hz, 3 × C_iH); ^{13}C NMR (100 MHz, DMSO) δ_C ppm: 13.7 (3 × C_dH_3), 31.2 (3 × C_eH_2), 122.6 (3 × C_fH), 123.3 (3 × C_gH), 143.8 (3 × C_h), 152.5 (3 × C_iH), 160.3 (3 × C_j), 164.8 ($C_{a,b,c}$-N), 196.6 (3 × C_{14}=S); IR (KBr) ϑ_{max} (cm^{-1}): 1851.02 (C=S), 3049.06 (C-H)

4.1.3.6. 2,4,6-tri(isonicotinohydrazide)-1,3,5-triazine **(34)**



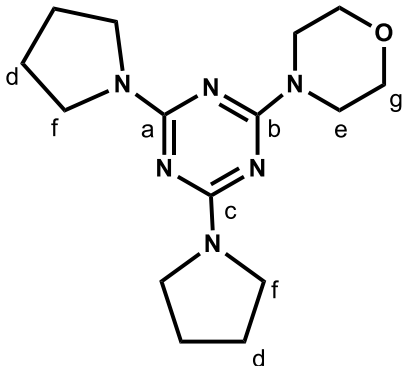
34

Using general procedure (3), cyanuric chloride **19** (1.00 g, 5.00 mmol) was reacted with isoniazid (2.05 g, 15 mmol) in the presence of silica gel (0.03 g, 5.00 mmol) using acetone (5 mL) as a solvent. The mixture was irradiated in microwave oven for 8 min at 85 ºC. Recrystallized product **34** was obtained as a orange crystalline solid (2.94 g, 90 %); m. p. 252 – 256 °C; ^{1}H NMR (400 MHz, DMSO) δ_H ppm: 8.12 (6H, m, 6 × C_dH), 8.95 (6H, m, 6 × C_fH); ^{13}C NMR (100 MHz, DMSO) δ_C ppm: 123.5 (6 × C_dH), 147.9 (3 × C_e), 148.8 (6 × C_fH), 163.7 ($C_{a,b,c}$-N), 164.0 (3 × C=O); IR (KBr) ϑ_{max} (cm^{-1}): 1692.22 (C=O), 3030.44 (C-H)

4.1.4 Tri-substituted 1,3,5-triazine derivative from di-substituted products: General procedure (4)

To a solution of 1,3,5-trazine derivatives (1 mmol) in a solvent (DCM, THF and acetone) was added an amine (2 mmol) and silica gel (1 mmol) successively in a 10 ml vessel. 7 mL of the mixture was then transferred into a 10 ml vial. The mixture was irradiated in a microwave oven with a power of 300 W to obtain products as solids. Recrystallization from hot ethanol gave the product as crystalline solid.

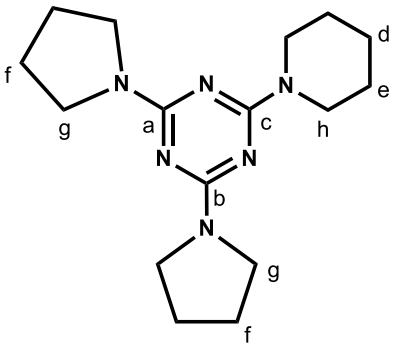
4.1.4.1. 6-(morpholino-1-yl)-2,4-di(pyrrolidin-1-yl)-1,3,5-triazine **(35)**



35

Using general procedure (4), compound **25** (0.50 g, 1.95 mmol) was reacted morpholine (0.33 mL, 3.90 mmol) in the presence of silica gel (0.12 g, 1.95 mmol) with DCM (5 mL) as a solvent. The mixture was irradiated in microwave oven for 3 min at 160 ºC. Recrystallized product **35** was obtained as a yellow crystalline solid (0.55 g, 91%); m.p.167 - 170 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 1.92 (8H, m, 4 × C_dH_2), 3.51 (4H, m, 2 × C_eH_2), 3.63 (8H, t, *J* = 5.4 Hz, 4 × C_fH_2), 3.72 (4H, t, *J* = 4.6 Hz, 2 × C_gH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 25.3 (4 × C_dH_2), 43.6 (2 × C_eH_2), 47.2 (4 × C_fH_2), 67.0 (2 × C_gH_2), 163.8 ($C_{a,b}$-N), 165.1 (C_c-N); IR (KBr) ϑ_{max} (cm^{-1}): 972.37 (C-O), 2779.59 (C-H); TOF MS (EL+) m/z: ($C_{15}H_{24}N_6O$) calculated 304.2000 found 301.9962

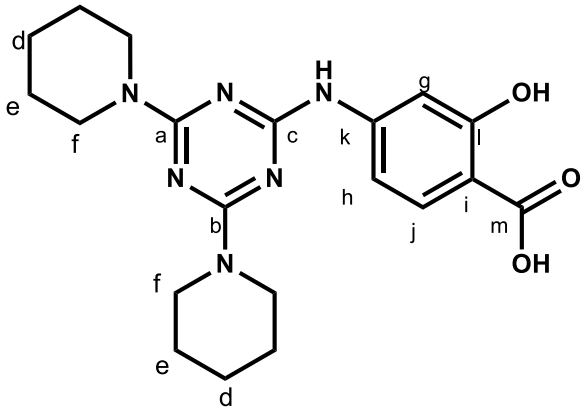
4.1.4.2. 6-(piperidin-1-yl)-2,4-di(pyrrolidin-1-yl)-1,3,5-triazine **(36)**



36

Using general procedure (4), compound **25** (0.50 g, 1.95 mmol) was reacted with piperidine (0.38 mL, 3.90 mmol) in the presence of silica gel (0.12 g, 1.95 mmol) using DCM (5 ml) as a solvent The mixture was irradiated in microwave oven for 3 min at 160 ºC. Recrystallized product **36** was obtained as a yellow crystalline solid (0.58 g, 89%). m.p 252 - 255 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 1.62 (2H, m, C_dH_2), 1.89 (4H, m, 2 × C_eH), 2.18 (8H, m, 4 × C_fH_2), 3.55 (8H, m, 4 × C_gH_2), 3.72 (4H, m, 2 × C_hH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm: 25.1 (C_dH_2), 25.8 (2 × C_eH_2), 44.1 (4 × C_fH_2), 46.2 (4 × C_gH_2), 46.2 (2 × C_hH_2), 162.7 ($C_{a,b}$-N), 165.4 (C_c-N); IR (KBr) ϑ_{max} (cm^{-1}): 2780.13 (C-H); TOF MS (EL+) m/z: ($C_{15}H_{24}N_6$) calculated 302.1220 found 302.9810

4.1.4.3. 4-((2,4-di(piperidin-1-yl)-1,3,5-triazin-2-yl)amino)-6-hydroxybenzoic acid **(37)**

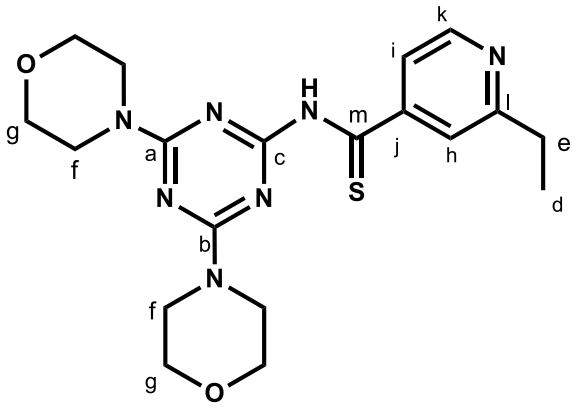


37

Using general procedure (4), compound **25** (0.50 g, 1.76 mmol) was reacted with PAS (0.53 g, 3.52 mmol) in the presence of silica gel (0.105 g, 1.76 mmol) using THF (5 mL) as a solvent. The mixture was irradiated in a microwave oven for 4 min at 162 °C. Recrystallized product **37** was obtained as a brown crystalline solid (0.66 g, 94%); m.p

248 - 251 °C; ^{1}H NMR (400 MHz, DMSO) δ_H ppm: 1.66 (4H, m, 2 × C_dH_2), 2.19 (8H, m, 4 × C_eH_2); 3.79 (8H, m, 4 × C_fH_2), 6.98 (1H, d, J = 2.0 Hz, C_gH), 7.30 (1H, d, J = 7.2 Hz ,C_hH), 7.75 (1H, d, J = 7.4 Hz, C_jH); ^{13}C NMR (100 MHz, DMSO) δ_C: 24.9 (C_dH_2), 25.8 (C_eH_2), 44.5 (C_fH_2),106.1 (C_gH), 109.4 (C_hH), 112.0 (C_i), 131.0 (C_jH), 144.6 (C_k), 162.3 (C_l-OH), 164.1 (C_c-N), 164.8 ($C_{a,b}$-N), 175.5 (C=O); IR (KBr) ϑ_{max} (cm^{-1}): 1650.08 (C=O), 2912.67 (C-H), 3010.04 (N-H), 3480.93 (OH)

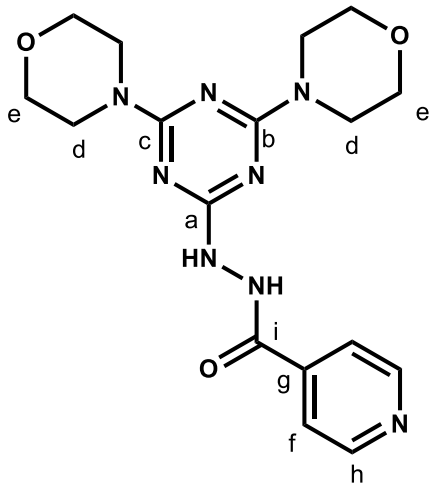
4.1.4.4. 2,4-(dimorpholino-1-yl)-6-((2-ethyl)-thioisonicotinamide)-1,3,5-triazine **(38)**



38

Using general procedure (4), compound **24** (0.50 g, 1.73 mmol) in acetone (20 mL) was added ethionamide (0.58 g, 3.48 mmol) and silica gel (0.10 g, 1.73 mmol) successively. The mixture was irradiated in a microwave for 10 min at 85 °C. Recrystallized product **38** was obtained as a brown crystalline solid (0.97 g, 93 %); m.p. 259 - 263 °C ;^{1}H NMR (400 MHz, DMSO) δ_H ppm: 1.24 (3H, t, J = 7.6 Hz, C_dH_3), 2.50 (2H, q, J = 7.8 Hz, C_eH_2), 2.98 (8H, t, J = 4.8 Hz, 4 × C_fH_2), 3.72 (8H, t, J = 4.8 Hz, 4 × C_gH_2), 7.65 (1H, d, J = 1.6 Hz, C_hH), 7.76 (2H, d, J = 5.6 Hz, C_iH), 8.52 (1H, d, J = 5.2 Hz, C_kH); ^{13}C NMR (100 MHz, DMSO) δ_C ppm: 13.5 (C_dH_3), 30.5 (C_eH_2), 43.9 (C_fH_2), 64.1 (C_gH_2), 118.4 (C_hH), 119.7 (C_iH), 146.8 (C_j) 149.1 (C_kH), 163.0 (C_l), 163.1 (C_c-N), 164.4 ($C_{a,b}$-N), 198.7 (C_{16}=S); IR (KBr) ϑ_{max} (cm^{-1}): 1524.69 (C=S), 2987.12 (C-H), 3001.21 (N-H)

4.1.4.5. 2,4-(dimorpholino-1-yl)-6-(isonicotinohydrazide)-1,3,5-triazine **(39)**



39

Using general procedure (4), compound **24** (0.50 g, 1.73 mmol) in acetone (20 mL) was added isoniazid (0.47 g, 3.48 mmol) and silica gel (0.10 g, 1.73 mmol) successively. The mixture was irradiated in a microwave for 10 min at 85 °C. Recrystallized product **39** was obtained as brown crystalline solids (0.42 g, 85%); m.p. 240 - 244 °C ;^{1}H NMR (400 MHz, DMSO) δ_H ppm: 2.14 (8H, t, *J* = 4.6 Hz, 4 ×C_dH_2), 2.57 (8H, t, *J* = 4.8 Hz, C_eH_2), 8.12 (2H, m, C_fH_2), 8.31 (2H, m, 2 × C_hH_2); ^{13}C NMR (100 MHz, DMSO) δ_C ppm: 44.3 (4 × C_dH_2), 66.2 (4 × C_eH_2), 124.7 (2 × C_fH), 145.3 (C_g), 148.8 (2 × C_hH), 160.9 (C_a-N), 163.1 ($C_{b,c}$-N), 164.0 (C=O); IR (KBr) ϑ_{max} (cm^{-1}): 1708.09 (C=O), 2859.15 (C-H)

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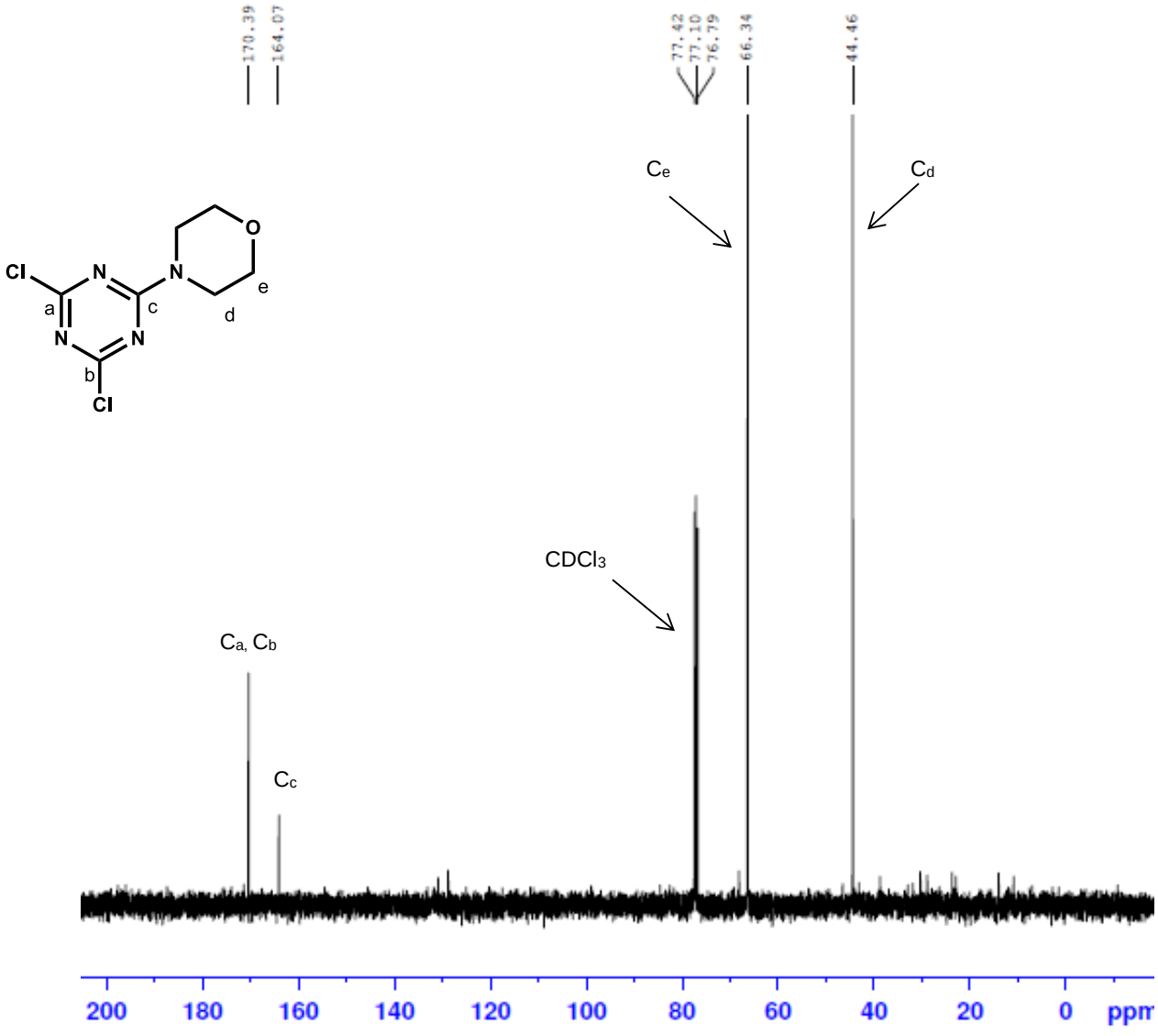
Appendix

NMR Spectra

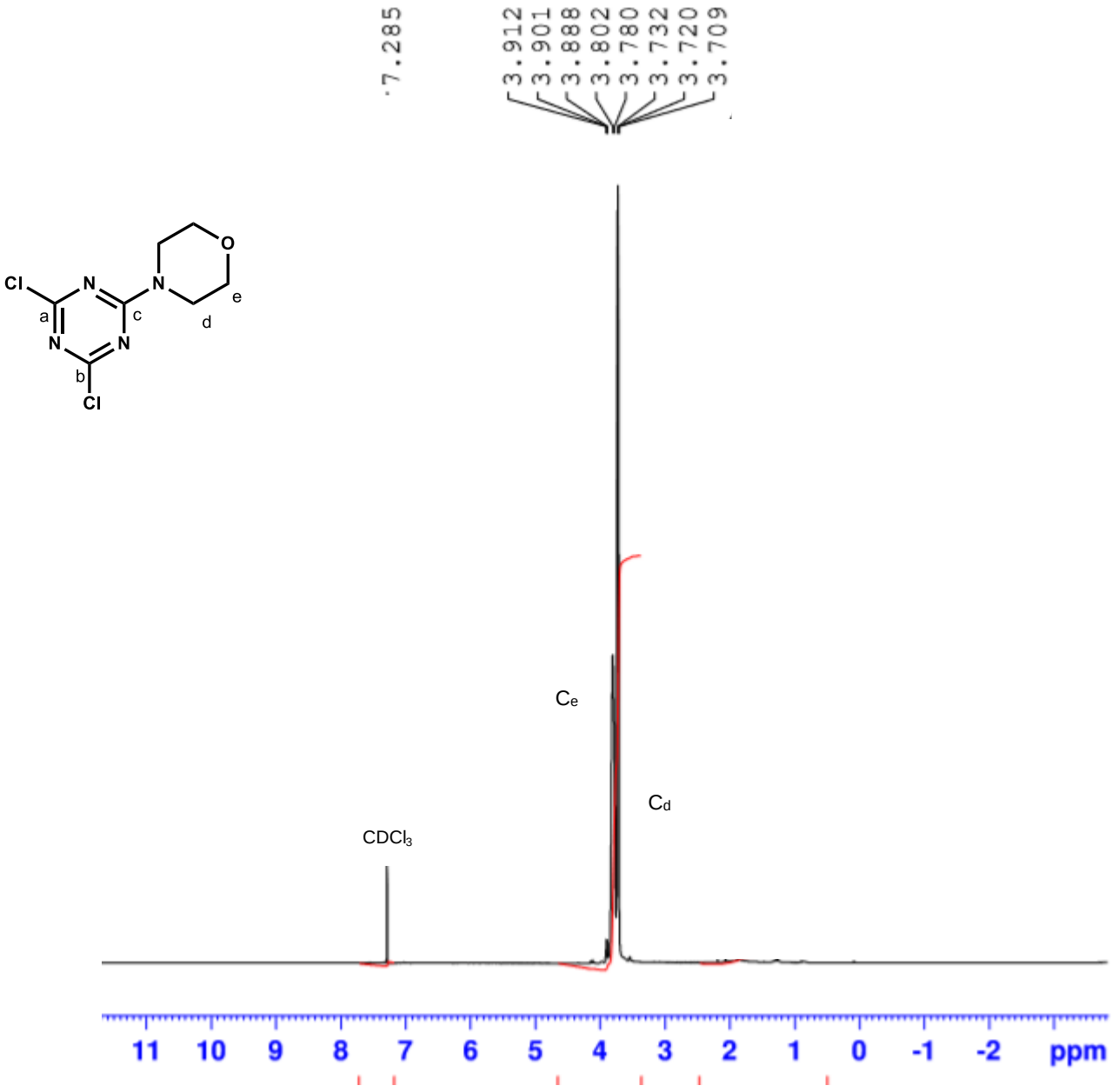
^{13}C NMR spectra

2-chloro-4,6-dimorpholino-1,3,5-triazine (**20)**



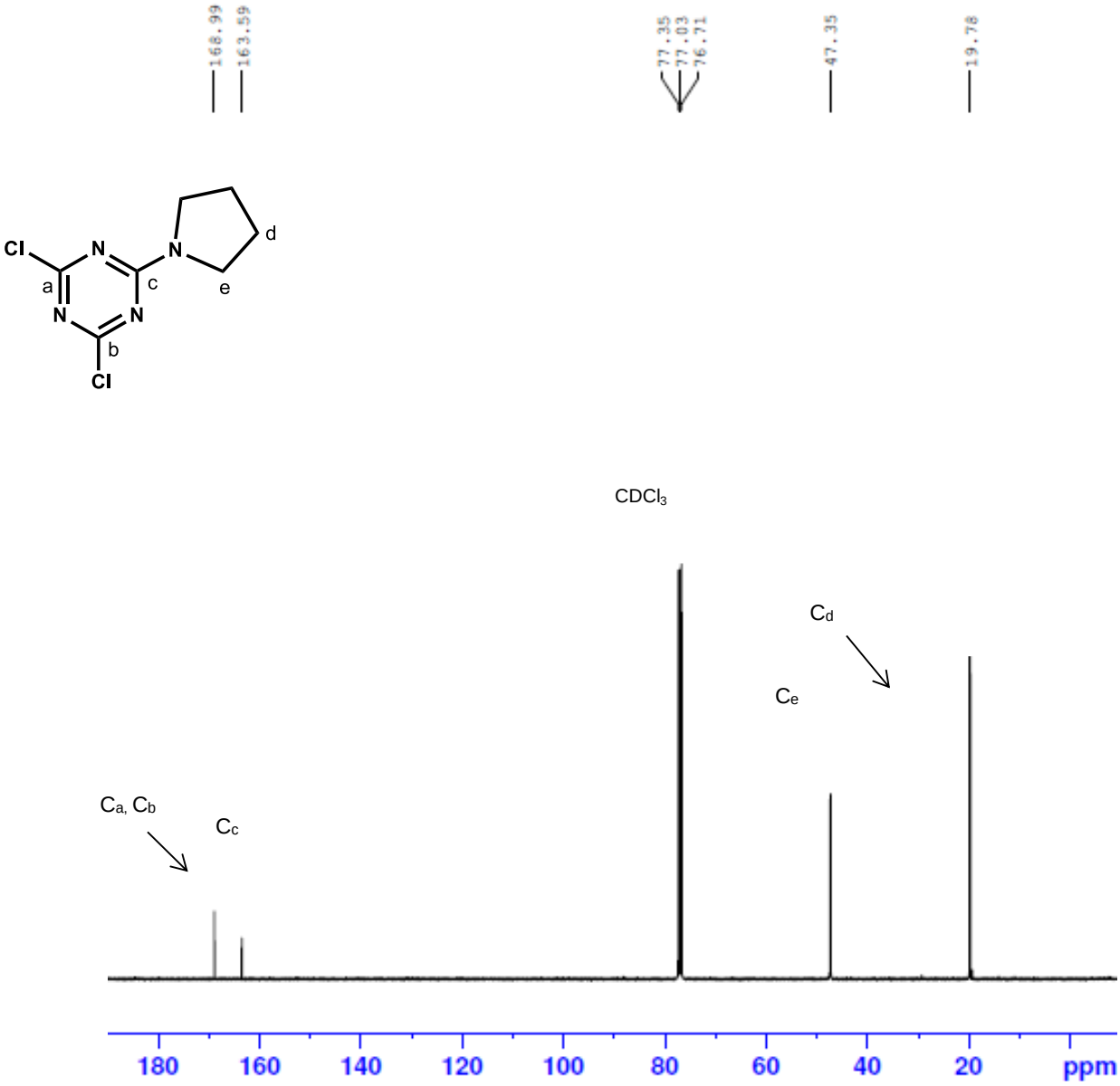
^{1}H NMR spectra

2-chloro-4,6-dimorpholino-1,3,5-triazine **(20)**



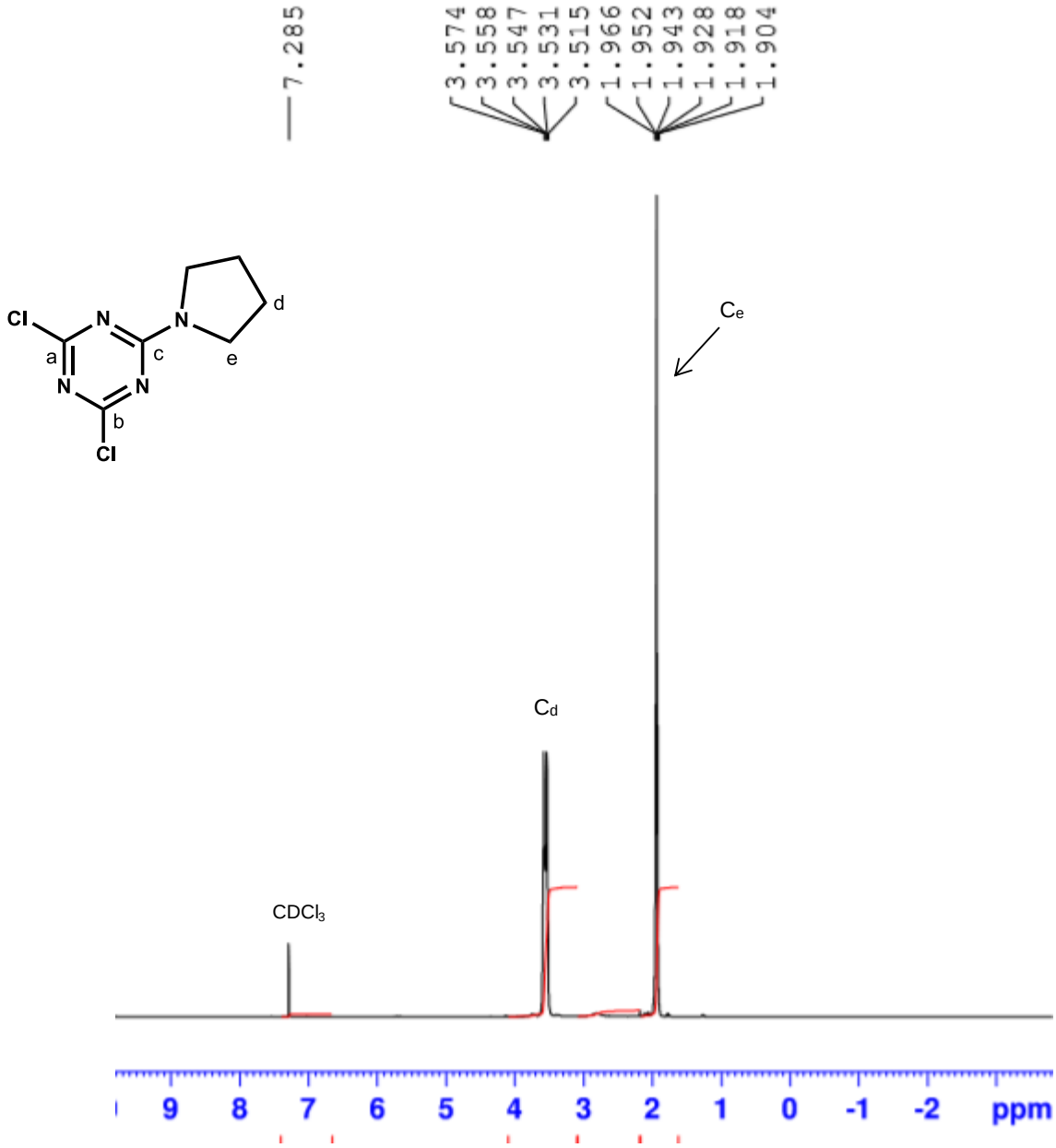
^{13}C NMR spectra

2,4-dichloro-6-(pyrrolidin-1-yl)-1,3,5-triazine **(22)**



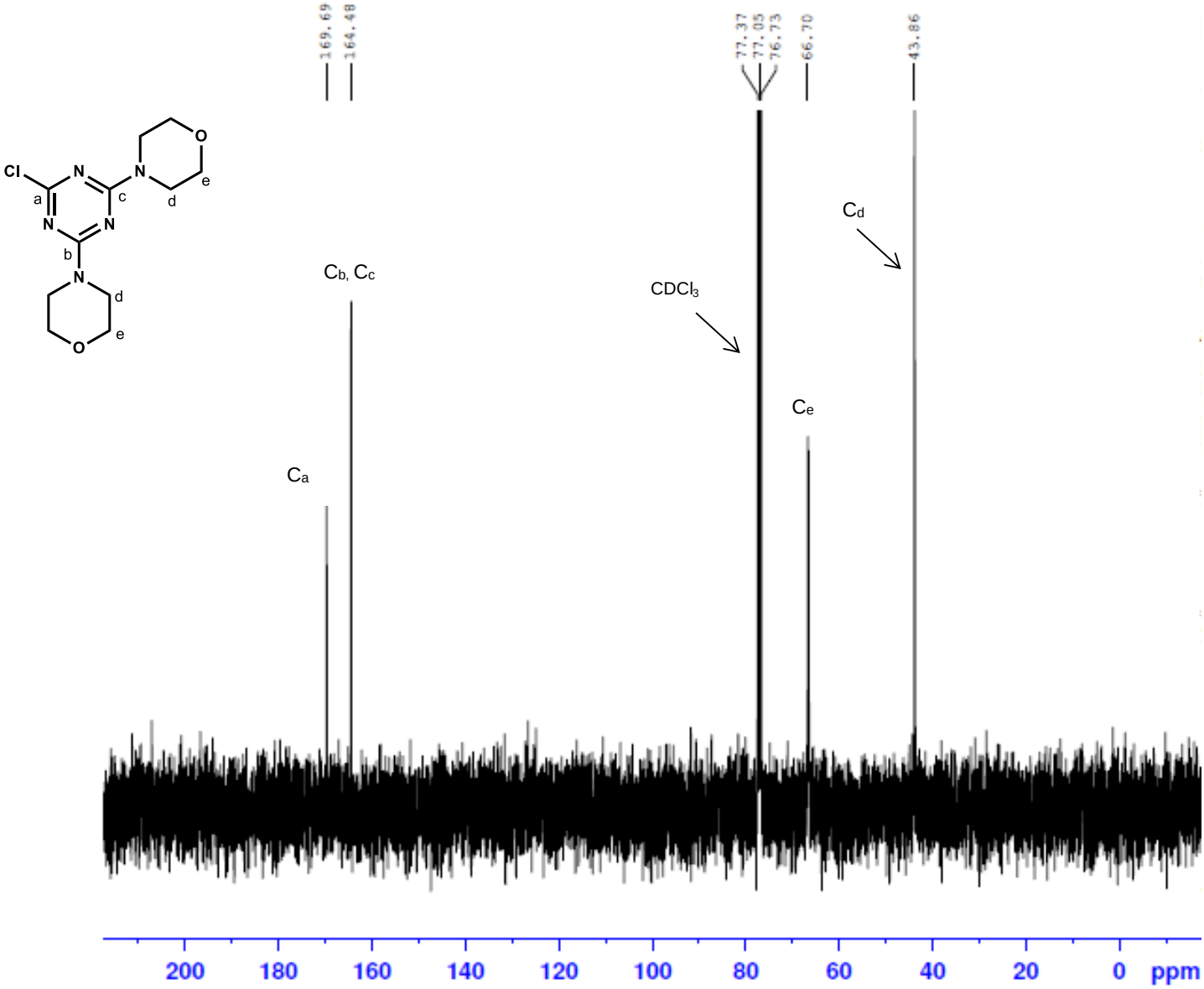
^{1}H NMR spectra

2,4-dichloro-6-(pyrrolidin-1-yl)-1,3,5-triazine **(22)**



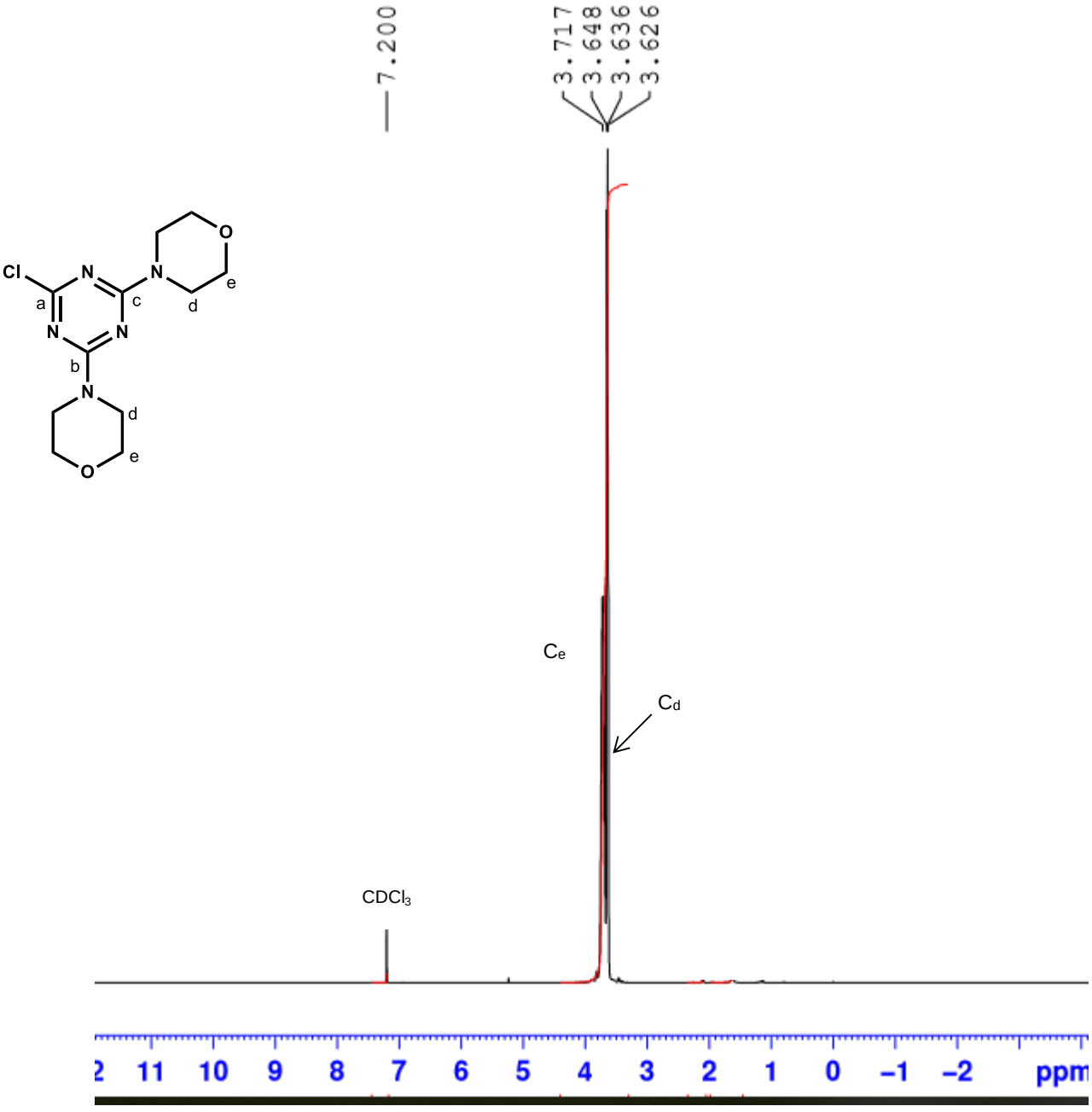
^{13}C NMR spectra

2,4-dichloro-6-morpholino-1,3,5-triazine **(23)**



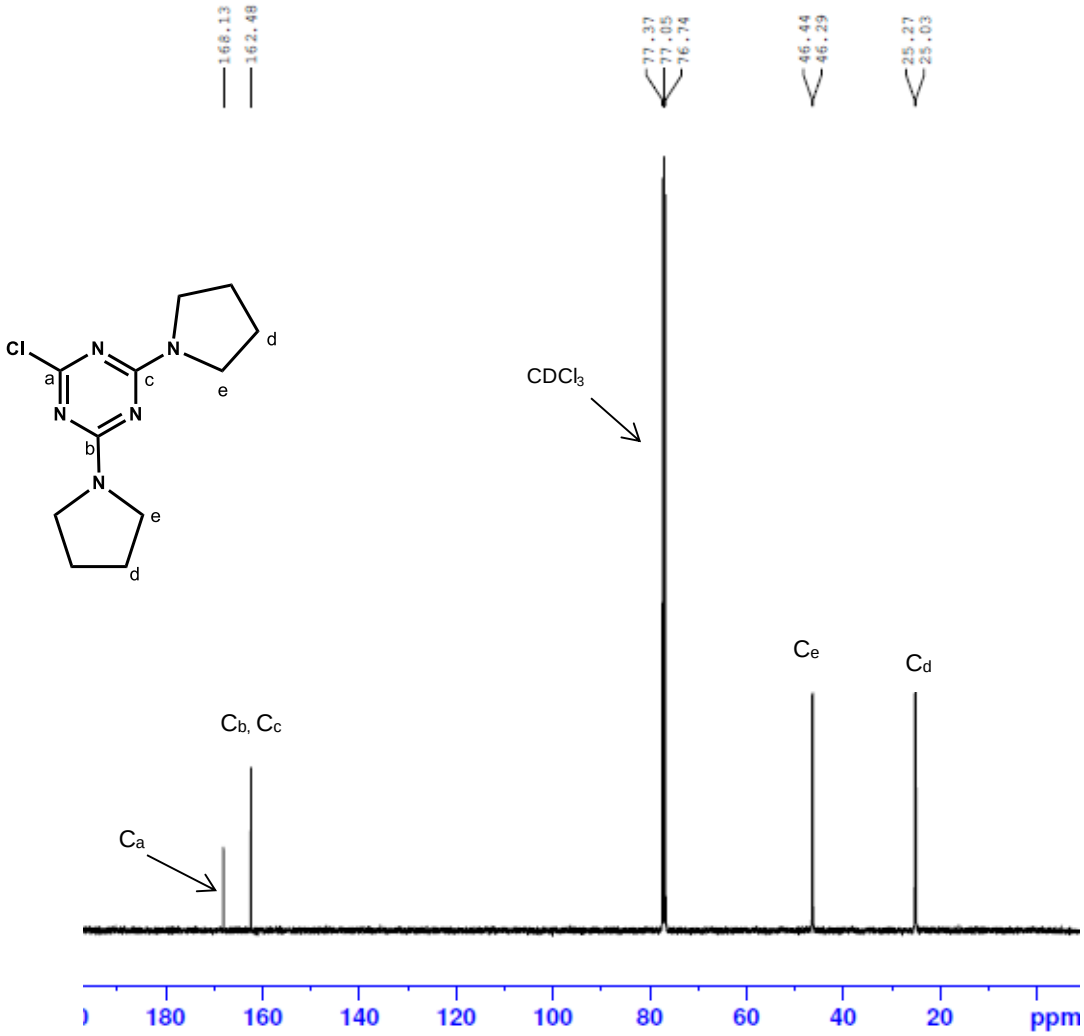
^{1}H NMR spectra

Synthesis of 2,4-dichloro-6-morpholino-1,3,5 triazine **(23)**



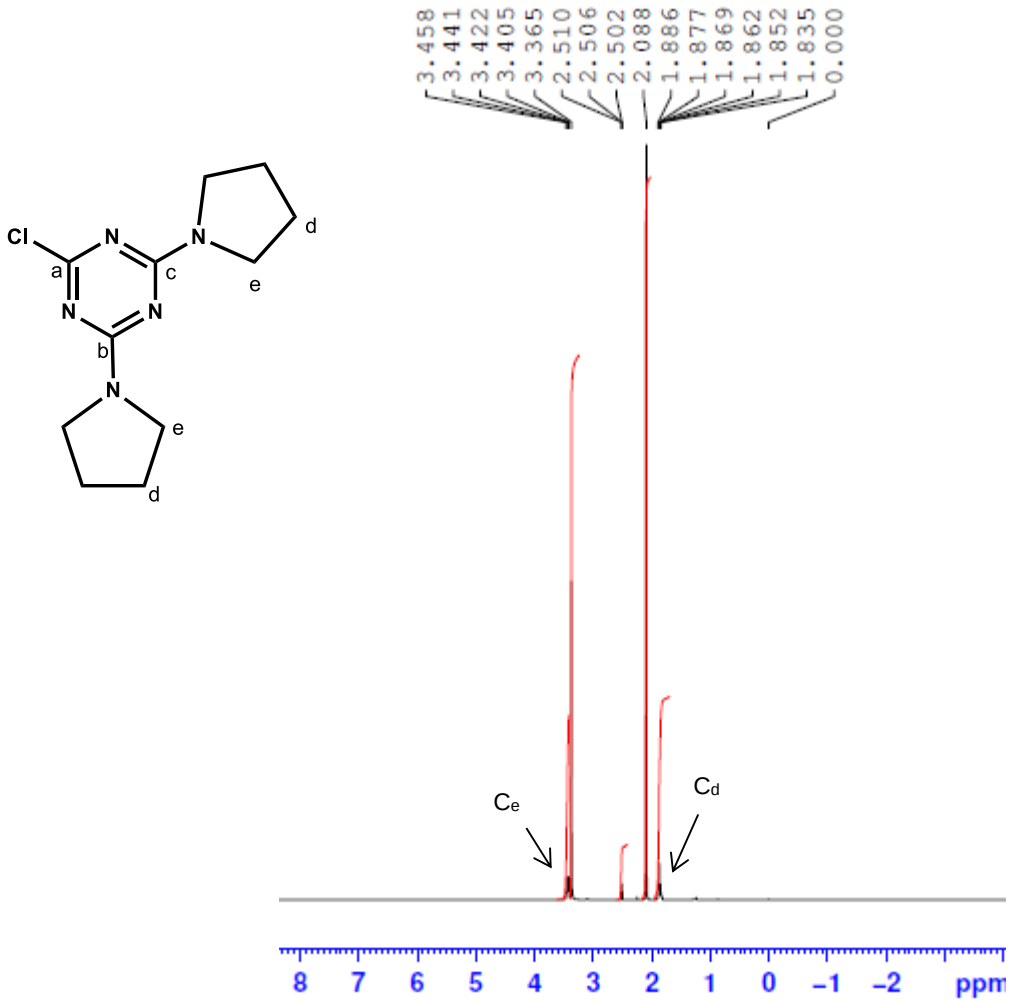
^{13}C NMR spectra

2-chloro-4,6-(dipyrrolidin-1-yl)-1,3,5-triazine **(25)**



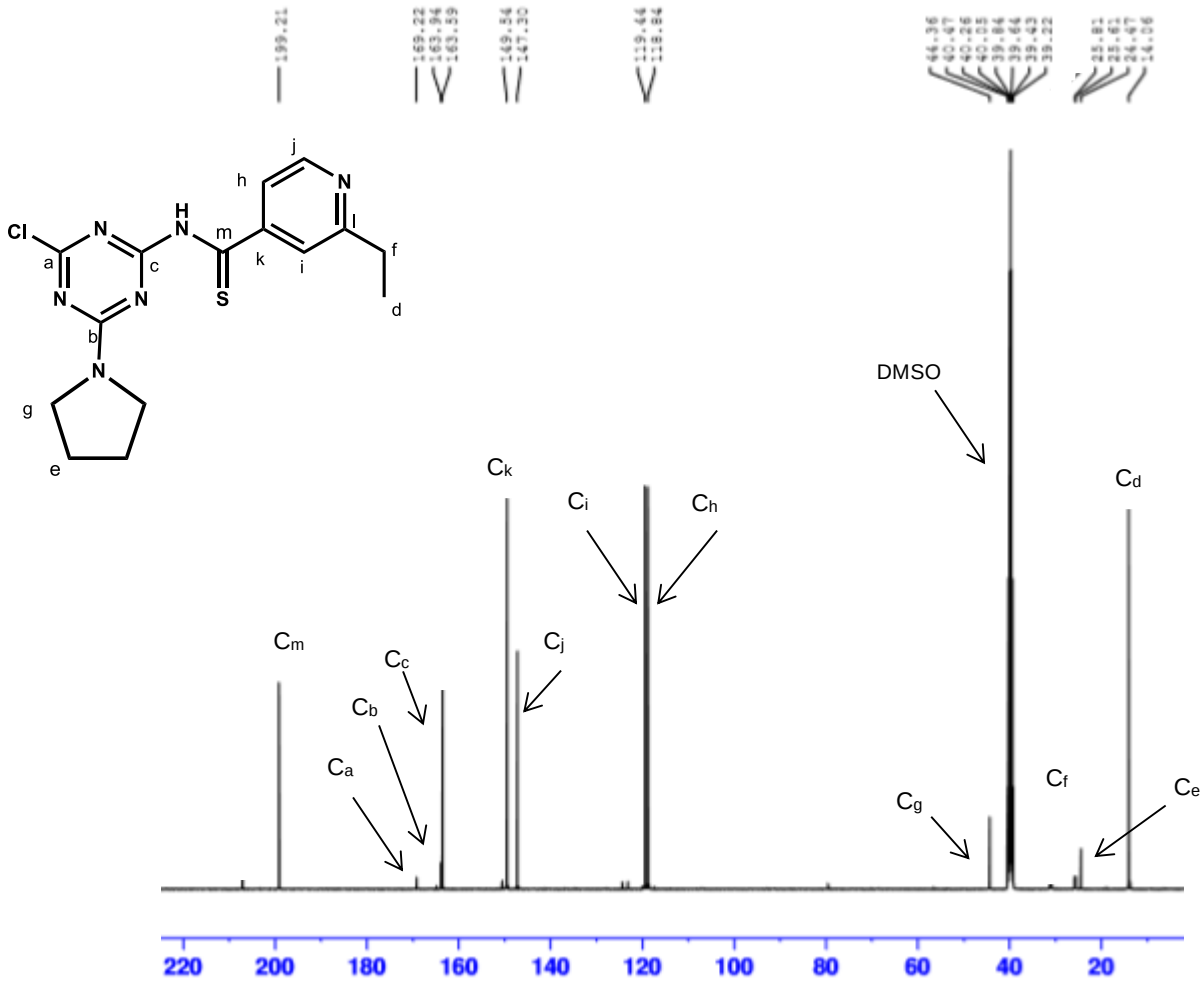
^{1}H NMR spectra

2-chloro-4,6-(dipyrrolidin-1-yl)-1,3,5-triazine **(25)**



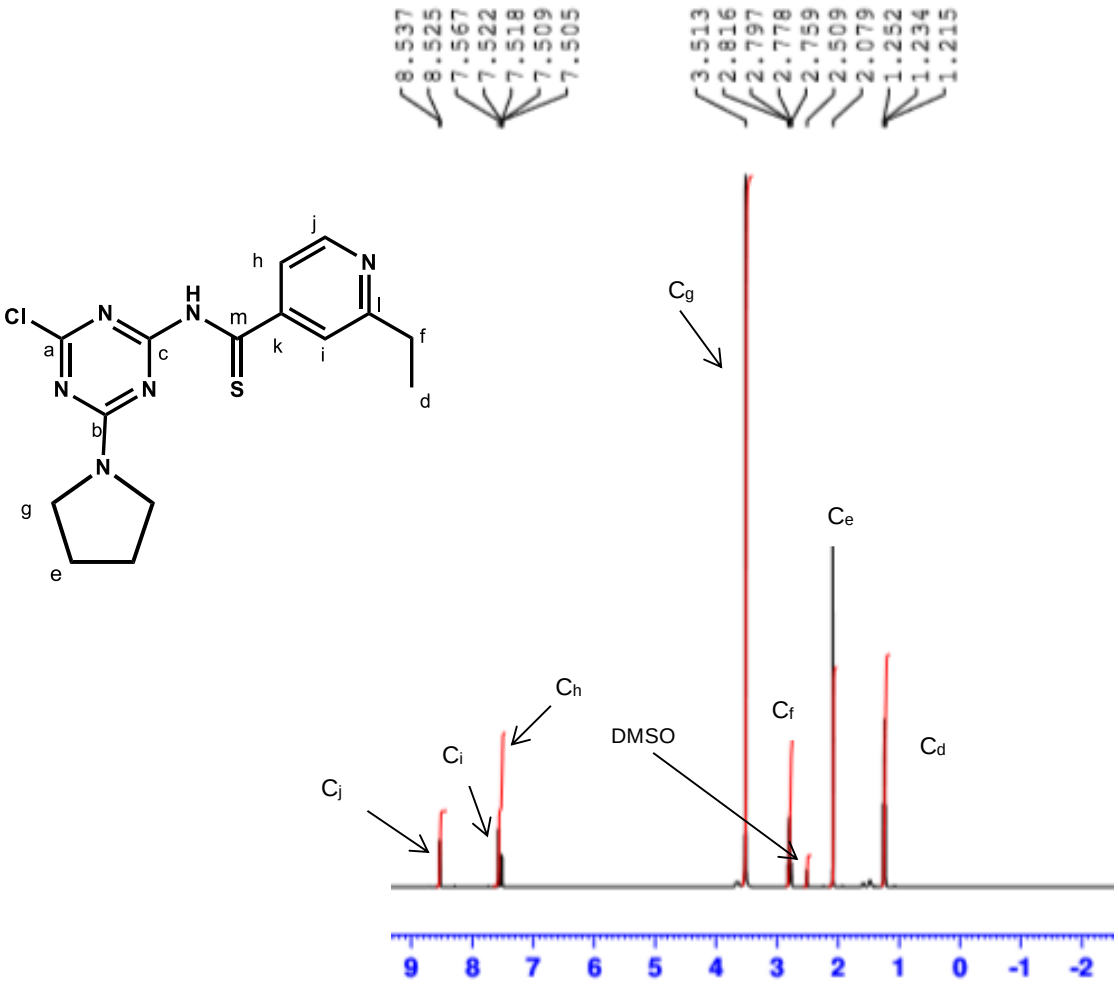
^{13}C NMR spectra

2-chloro-4-(pyrrolidin-1-yl)-6-((2-ethyl)-thioisonicotinamide)-1,3,5-triazine **(26)**



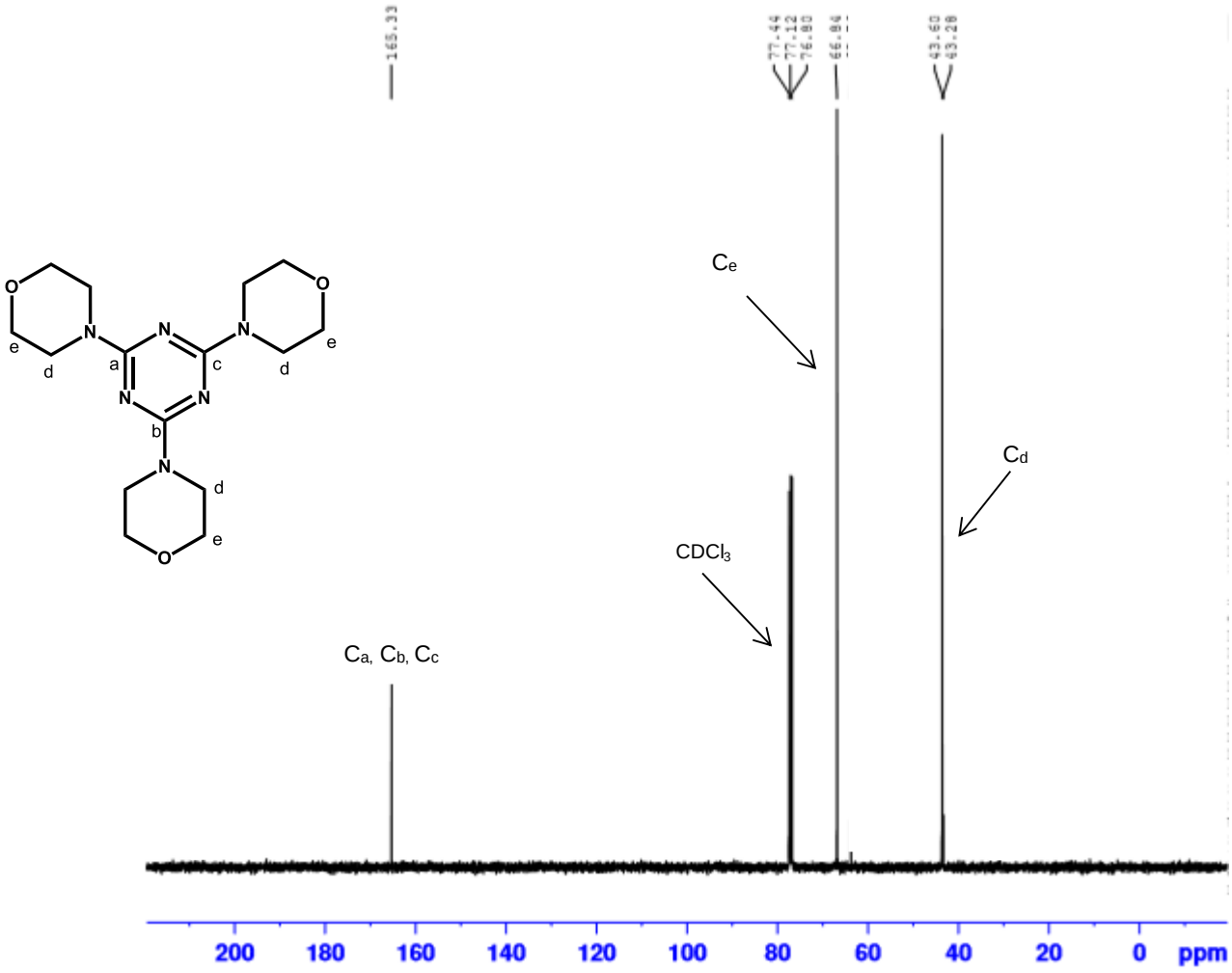
^{1}H NMR spectra

2-chloro-4-(pyrrolidin-1-yl)-6-((2-ethyl)-thioisonicotinamide)-1,3,5-triazine **(26)**



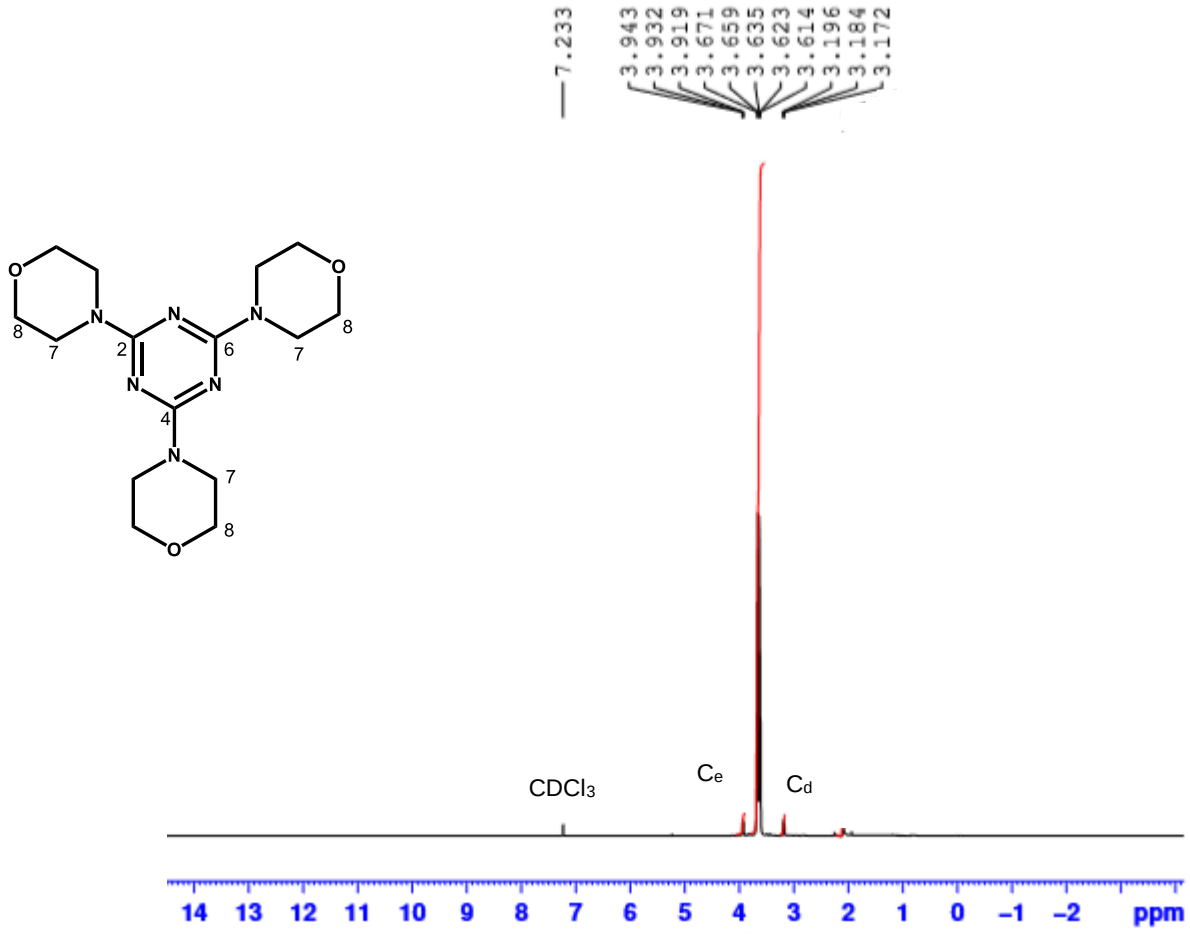
13C NMR spectra

2,4,6-tri(morpholino-1-yl)-1,3,5-triazine **(29)**



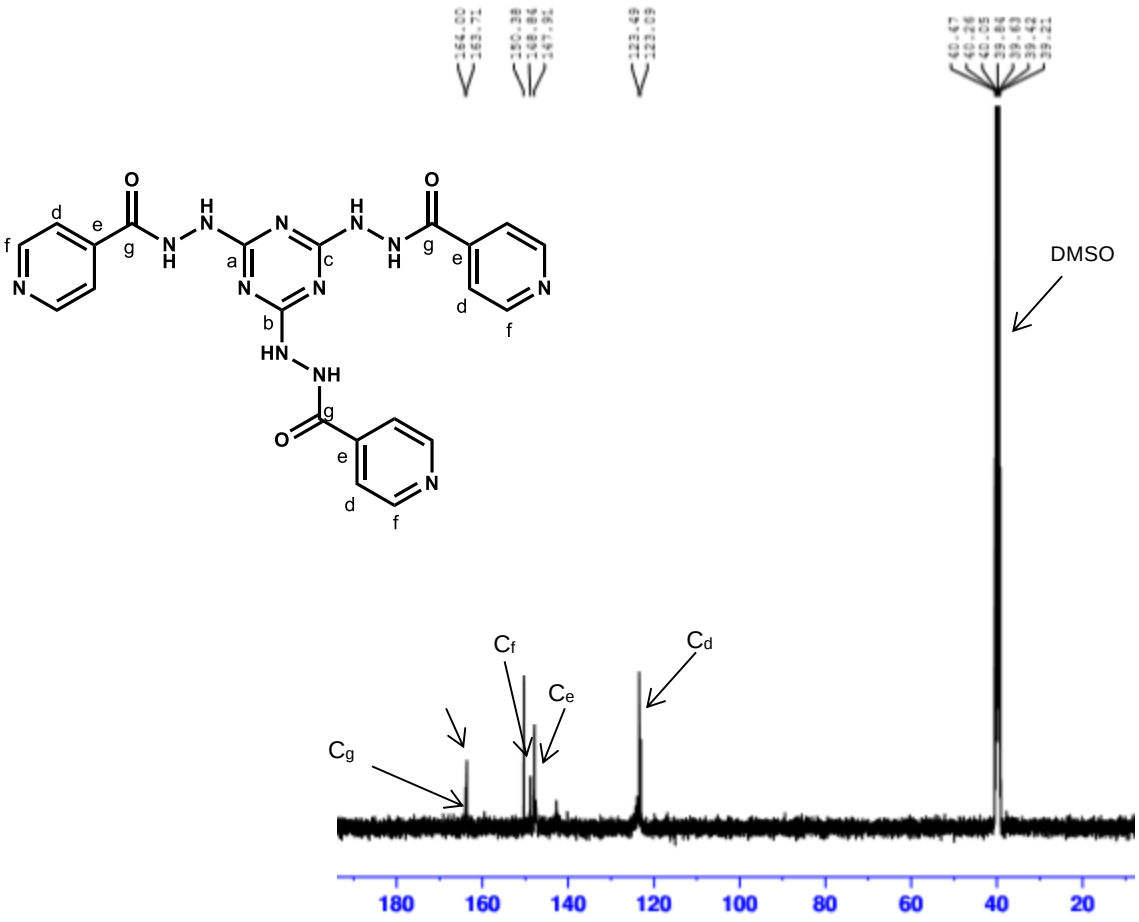
1H NMR spectra

2,4,6-tri(morpholino-1-yl)-1,3,5-triazine **(29)**



13C NMR spectra

2,4,6-tri(isonicotinohydrazide)-1,3,5-triazine **(34)**



1H NMR spectra

2,4,6-tri(isonicotinohydrazide)-1,3,5-triazine **(34)**