DERIVATIZATION OF TRIAZINES AND THEIR EVALUATION AS ANTIFUNGAL AGENTS

Thesis

Submitted to the Punjab Agricultural University
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In
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(Minor Subject: Biochemistry)

By

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CERTIFICATE I

This is to certify that the thesis entitled **“Derivatization of triazines and their evaluation as antifungal agents”** submitted for the degree of **5-year Integrated M.Sc. (Hons.) Programme**, in the subject of **Chemistry** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Kiranveer Kaur (L-2014-BS-54-IM)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled, **"Derivatization of triazines and their evaluation as antifungal agents"** submitted by **Kiranveer Kaur** (**L-2014-BS-54-IM**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **5-year Integrated M.Sc. (Hons.) Programme**, in the subject of **Chemistry** (Minor subject: **Biochemistry**) has been approved by the Student's Advisory Committee along with External Examiner after an oral examination on the same.

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ABSTRACT

Seven mono-, di- and tri-substituted derivatives of *s*-triazine were synthesized by condensation reaction of s-triazine with different aromatic amines at different temperature conditions. The characterization of synthesized derivatives was done using ^{1}H NMR, D_2O exchange, ^{13}C NMR and IR spectroscopy. Among all the synthesized triazine derivatives, 6-chloro-N^2,N^4-*bis*(4-bromophenyl)-1,3,5-triazin-2,4-diamine, N^2, N^4-*bis*(4-nitrophenyl)-N^6-(2,4,6-trichlorophenyl)-1,3,5-triazin-2,4,6-triamine and 6-chloro-N^2,N^4-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine were found to be most effective compound against *Helminthosporium oryzae, Rhizoctonia solani* and *Alternaria brassicae*, respectively with 100% mycelial growth inhibition where as compound 4,6-dichloro-*N*-(2,4-dinitrophenyl)-1,3,5-triazin-2-amine, triazine (1) was found to be least effective with ED_{50} value 188, 230 and 258 μg/ml.

Keywords: Triazine derivatives, aromatic amines, antifungal activity, *Helminthosporium oryzae, Rhizoctonia solani* and *Alternaria brassicae*

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ਟਰਾਈਜ਼ੀਨ ਅਤੇ ਐਰੋਮੈਟਿਕ ਅਮੀਨ ਦੀ ਵੱਖਰੇ-ਵੱਖਰੇ ਤਾਪਮਾਨ ਉੱਤੇ ਕੰਨਡੰਸ਼ੇਸ਼ਨ ਨਾਲ ਸੱਤ ਮੋਨੋ, ਡਾਈ ਅਤੇ ਟਰਾਈ ਡੈਰੀਵੇਟਿਵਜ਼ ਬਣਾਏ ਗਏ। ਬਣਾਏ ਗਏ ਡੈਰੀਵੇਟਿਵਜ਼ ਦੀ ਵਿਸ਼ੇਸ਼ਤਾ ਪ੍ਰੋਟੋਨ ਐੱਨ.ਐੱਮ.ਆਰ., ਡੀ$_2$ਓ, ^{13}ਸੀ ਐੱਨ.ਐੱਮ.ਆਰ. ਅਤੇ ਆਈ.ਆਰ. ਦੁਆਰਾ ਕੀਤੀ ਗਈ। ਬਣਾਏ ਗਏ ਮਿਸ਼ਰਣਾ ਦੀ *ਹੈਲਮਿੰਨਥੋਸਪੌਰਿਅਮ ਓਰਾਈਜ਼ੇ, ਰਾਈਜੋਕਟੋਨੀਆਂ ਸੋਲਾਨੀ* ਅਤੇ *ਐਲਟਰਨੇਰੀਆ ਬਰਾਸੀਕੇਏ* ਵਿਰੁੱਧ ਉੱਲੀਨਾਸ਼ਕ ਗਤੀਵਿਧੀ ਦਾ ਮੁਲਾਂਕਣ ਕੀਤਾ ਗਿਆ। ਬਣਾਏ ਗਏ ਡੈਰੀਵੇਟਿਵਜ਼ ਵਿੱਚੋਂ 6-ਕਲੋਰੋ, ਨ^2, ਨ^4-ਬਿਸ-(4-ਬਰੋਮੋਫਨਾਈਲ)-1,3,5-ਟਰਾਈਜੀਨ-2,4,6- ਟਰਾਈਅਮੀਨ, ਨ^2, ਨ^4-ਬਿਸ-(4-ਨਾਈਟਰੋਫਨਾਈਲ)-ਨ^6-(2,4,6-ਟਰਾਈਕਲੋਰੋਫਨਾਈਲ)-1,3,5-ਟਰਾਈਜੀਨ-2,4,6- ਟਰਾਈਅਮੀਨ ਅਤੇ 6-ਕਲੋਰੋ, ਨ^2, ਨ^4-ਬਿਸ-(4-ਨਾਈਟਰੋਫਨਾਈਲ) -1,3,5-ਟਰਾਈਜੀਨ-2,4,6-ਡਾਈਅਮੀਨ ਸਭ ਤੋਂ ਵੱਧ ਪ੍ਰਭਾਵਸ਼ਾਲੀ ਉੱਲੀਨਾਸ਼ਕ ਪਾਏ ਗਏ। ਮੁਲਾਂਕਣ ਦੌਰਾਨ ਇਹਨਾਂ ਦੀ ਦੀ.ਡੀ.$_{50}$ ਦੀ ਮਾਤਰਾ 4,7 ਅਤੇ 9 µg/ml ਪਾਈ ਗਈ ਅਤੇ ਇਹਨਾਂ 500 µg/ml ਦੀ ਮਾਤਰਾ ਉੱਤੇ ਸੰਪੂਰਨ ਤੌਰ ਉੱਤੇ (100%) ਉੱਲੀ ਦਾ ਖਾਤਮਾ ਕਰ ਦਿੱਤਾ। ਬਣਾਏ ਗਏ ਮਿਸ਼ਰਣਾ ਵਿੱਚੋਂ 4,6-ਡਾਈਕਲੋਰੋ-ਨ-(2,4-ਡਾਈਨਾਈਟਰੋਫਨਾਈਲ)-1,3,5-ਟਰਾਈਜ਼ੀਨ-2-ਅਮੀਨ ਅਤੇ ਟਰਾਈਜ਼ੀਨ (1) ਨੂੰ ਸਭ ਤੋਂ ਘੱਟ ਪ੍ਰਭਾਵਸ਼ਾਲੀ ਪਾਇਆ ਗਿਆ ਕਿਉਂਕਿ ਇਸ ਉੱਲੀਨਾਸ਼ਕ ਦੀ ਈ ਡੀ$_{50}$ ਦੀ ਮਾਤਰਾ 188, 230 ਅਤੇ 258 µg/ml ਪਾਈ ਗਈ। 6-ਕਲੋਰੋ ਮਿਸ਼ਰਣਾ ਦੀ । ਇਸ ਵਾਸਤੇ ਪੋਏਜ਼ਨ ਫੂਡ ਟੇਕਨੀਕ ਦਾ ਪ੍ਰਯੋਗ ਕੀਤਾ ਗਿਆ।

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ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

CONTENTS

LIST OF TABLES

LIST OF FIGURE

CHAPTER-I

INTRODUCTION

Heterocyclic compounds are cyclic compounds holding hetero atoms other than carbon atom such as oxygen, nitrogen and sulfur in the ring (Kaur *et al* 2019). Heterocycles have shown their wide range of applications in coordination compounds, photo sensitizers, dyes, polymeric materials and other fields (Kumar *et al* 2018). The chemistry of heterocyclic compounds is an interesting area of research due to their specific chemical reactivity and natural abundance (Anamika *et al* 2018, Utreja *et al* 2019). Several heterocyclic compounds such as indole, benzothiazole, triazole, pyrrole, imidazole, thiophene, pyrolidine, tetrazole, benzimidazole, triazine are known to exhibit remarkable pharmacological activities such as central nervous system depressant, neuroleptic and tuberculostatic *etc* (Sharma *et al* 2016).

Nitrogen atom bearing heterocyclic compounds are of immense importance in synthetic organic and medicinal chemistry due to their wide existence as bioactive molecules (Baldaniya *et al* 2009). Among nitrogen containing heterocycles, triazine is an important scaffold consisting of benzene ring in which three carbon atoms are substituted by three nitrogen atoms (Arsad *et al* 2014). The three isomers of triazine are distinguished by positions of their nitrogen atoms in the benzene ring: 1,2,3-triazine; 1,2,4-triazine; 1,3,5-triazine or *s*-triazine among which the last one is most common, oldest and most extensively studied isomeric form (Shah *et al* 2014). 1,3,5-Triazine is known as *s*-triazine due to the symmetry of three nitrogen atoms in the ring (Kumar *et al* 2017). Triazine, also known as cyanuric chloride, was first synthesized by Serullas in 1827, by converting cyanogen chloride to cyanuric chloride in the presence of sunlight. It is considered to be trichloride of cyanogen for long time (Hutmacher and Most 2005).

The triazine molecule can undergo a variety of reactions like Lossen rearrangement, Sulfonyl chloride preparation, Friedel craft acylation, Swern oxidation, sulfide to sulfone oxidation, promotes Beckman rearrangement and also undergo displacement reactions like carboxylic acids into amides, peptides, esters and sulfonic acids into sulfonamides (Siddalingamurthy *et al* 2013). The most important attribute of cyanuric chloride is its ability to undergo nucleophilic aromatic substitution reaction and also used as an acid chloride in various organic transformations including chlorination, dehydration and coupling reactions such as peptide coupling (Liu *et al* 2007). Embarked attention gained by triazine is due to the different reactivity of its chlorine atoms at different temperature conditions (Patel *et al* 2014).

Triazine and its derivatives play a vital role in agricultural and pharmaceutical industry (Giacomelli *et al* 2004). Most of the triazine herbicides such as Simazine, Anilazine, Cyromazine and Atrazine are formed by simple displacement of chlorine atoms with nucleophiles such as amines (Patel *et al* 2012). These herbicides inhibit the photosynthetic electron transport in some plants. Ammeline, Acetoguanide, Acetoguanamine, Cyanuric acid, Melamine, Altretamine, Tretamine, Cycloguanil and Almitrine are the 1,3,5-triazine moiety containing important drugs which are used clinically due to their antitumor action against lung, breast, ovarian and epidermoid cancers (Gorka *et al* 2005). Quinolone linked triazine derivatives are found to be effective against malarial diseases (Rana *et al* 2014).

From past few years, there is a significant increase in the use of 1,3,5-triazine derivatives and have found wider applications in the textile, rubber, plastic industries and also used as pesticides, optical bleaches, explosives, dyestuffs and surface active agents (Comins and O' Connor 1988). Triazines can also be used as chromophore group in colorants and in formulating bactericides and fungicides (Anupama *et al* 2015). Moreover in recent years, new 1,3,5-triazine derivatives are known to possess their medicinal and biological properties such as anti-AIDS and anticancer (Ali and El Sayed 2009), antitumor (Schmidt *et al* 2009), anticonvulsant (Eddington *et al* 2002), analgesic (Karabasanagouda *et al* 2009), antioxidant (Padmaja *et al* 2009), antidepressant (Andres *et al* 2003), herbicidal (Zhou *et al* 2003 and Nishimura *et al* 2001), insecticidal (Upadhyay *et al* 2010), fungicidal (Kai *et al* 2000), antibacterial (Ringott *et al* 2011), anti-HIV (Liu *et al* 2010) and antiinflammatory activities *etc* (Selvam *et al* 2005). Other properties include antitrypanosomal (Bacchi *et al* 1998), antileishmanial (Sunduru *et al* 2009), antitomato mosaic virus (TMV) (Pandey and Kumar 2006), electron transport material for OLEDs (Chen *et al* 2009) and molecular imprinting (Padalkar *et al* 2014, Karunakaram *et al* 2012).

During the past several decades, increase of invasive fungal infections have been observed in various agricultural crops (Vibha *et al* 2018). Among various plant pathogens, fungi are the major cause of crop loss worldwide whereas antifungal diseases are very common all over the world (Baldaniya and Patel 2009). Fungal infections lead to yield loss of at least 125 million tons of top five food crops *viz.* rice, wheat, soyabean, potatoes and maize. The fungus attacks aerial parts, stems and roots of plant and affects its translocation system, resulting in permanent dying of plant (Soylu *et al* 2010).

Plant fungal pathogens are major cause of outburst of diseases in various agricultural crops (Shuping and Eloff 2017). Phytopathogens have developed different mechanisms and ways to attack host plant (Knogge 1996) and take nutrients from attacked plant for their growth and development (Horbach *et al* 2009). These pathogens can reproduce asexually or sexually and can overcome plant defence system (Thomma *et al* 2011; Zvereva and Pooggin 2012). These fungi collectively are responsible for 80% of the plant diseases (El Hussein *et al* 2014). About 8000 fungal species cause nearly 100,000 diseases in plants (Agrios G N 1997). Plant fungal pathogens also pose a serious threat to human health by deteriorating plant quality and quantity. These phytopathogens cause enormous problems for farmers, policy makers, researchers and consumers due to their prevailing resistance for various agrochemicals (Liu *et al* 2010). Various synthetic antifungal agents are used in order to overcome the diseases caused by them but it leads to the development of resistance in concerned fungi and have various side effects (Daferera *et al* 2003). *Rhizoctonia solani*, *Alternaria brassicae* and *Helmithosporium oryzae* cause sheath blight, brown leaf spot and black spot. At least 20% of agricultural loses are caused by *Alternaria* species, most other losses may reach up to 80% of yield (Wan *et al* 2018). Globally, rice is the staple food of over half the world's population, especially in Asia (Fairhurst and Dobermann 2004). Currently, India is the second largest producer of rice crop in the world after China and it contributes total 90% share in global rice production. According to FAO, it is the agricultural commodity with the third-highest worldwide production *i.e.* 741.5 million tones after sugarcane 1.9 billion tones and maize 1.0 billion tones. The productivity of these cereal crops is decreasing day by day due to fungal attack (Kaur *et al* 2016). *R. Solani* causes sheath blight of rice and have wide host range. But till now no complete resistance source has been identified for this disease from phytochemicals or botanicals, so management relies on chemical control (Goyal *et al* 2018). Under favourable environmental conditions, sheath blight fungus can reduce yield up to 50 % (Chen and Zhu 2011). *A. brassicae*, the disease caused by *Alternaria* are the major factors responsible for low production of cucurbitaceous, brassicaceous and solanaceous family crops. Hence, there is a strong need to effectively control this polyphagus, destructive group of fungi (Schultz and Nicholas 2000). Brown spot of rice caused by *H. oryzae* is known to occur in all the rice growing states (Mahlein 2015). The control of these plant diseases is extremely important for achieving high crop productivity (Kaur *et al* 2017, Anamika *et al* 2018). The need to save agriculture and environment made it vital to discover, design and synthesize novel scaffolds as antifungal agents, which can help in the battle against various fungal microorganisms. Efforts are made by researchers for synthesizing new chemicals to control the fungal diseases (Singh *et al* 2013).

Due to the proficient nature of triazine as an antifungal agent (Sharma *et al* 2016), it has always been the most crucial part of research. There have been constant efforts to examine newer chemicals to get adequate control of fungi. The synthesis of more triazine derivatives as an antifungal drug will be a contributive research accomplishment. The work was undertaken with the following objectives:

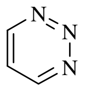
1. To synthesize and characterize derivatives of triazine
2. To evaluate antifungal activity of synthesized triazine derivatives against *Rhizoctonia solani, Helminthosporium oryzae* and *Alternaria brassicae*

This thesis comprises of chapterwise division of review of literature, materials and methods, results and discussion followed by summary. The chapter II covers review of literature of triazine derivatives and their biological activities. The various methods employed, different chemicals and reagents used in the synthesis of triazine derivatives are mentioned in chapter III. In chapter IV, results and discussion regarding our investigations have been described. Chapter V includes summary of the research work carried out. The references cited in the text are alphabetically arranged at the end of the thesis.

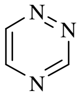
CHAPTER-II

REVIEW OF LITERATURE

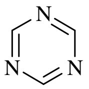
Triazine ($C_3Cl_3N_3$), also known as cyanuric chloride, belongs to the class of simple aromatic organic compound of heterocyclic series. Triazine is an organic compound composed of three carbons, three chlorine and three nitrogen atoms present in adjacent positions of a six membered ring (Besedia *et al* 2011). Isomers of triazines are distinguished from each other by different arrangement of three nitrogens in the benzene ring: 1,2,3-triazine, 1,2,4-triazine and 1,3,5-triazine (Arshad *et al* 2014).



1,2,3-Triazine

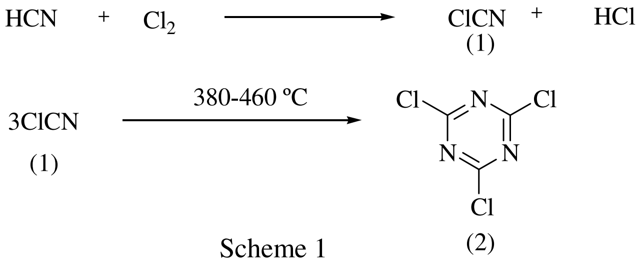


1,2,4-Triazine



1,3,5-Triazine

Among the three isomers, the derivatives of 1,3,5-triazine are most extensively used to disinfect water and as a stabilizer to minimize the consumption of chlorine in swimming pools (Chien *et al* 2016). It is also used as a precursor of atrazine which is a well-known herbicide. Cyanuric chloride also named as tricyanogen chloride, cyanuric trichloride and cyanuryl chloride, is a white powdery, easy hydrolytic degrading monocyclic crystal of pungent odour, inexpensive and non-volatile compound (Siddalingamurthy *et al* 2013) having molecular formula $C_3N_3Cl_3$, molecular weight 81.078 g/mol and melting point in the range of 81-82 ºC. It is readily soluble in acetone, acetonitrile, ether and dichloromethane (Yan *et al* 2008). Trichloro triazine has much weaker resonance energy than benzene, therefore, nucleophilic substitution is preferred than electrophilic substitution (Comins and O' Connor 1988). Among the various high yielding methods employed for the synthesis of triazine, the most widely used method is the reaction of cyanuric chloride (ClCN) (1) in the gaseous phase at 350-750 ºC in the presence of an activated charcoal as a catalyst as shown in scheme 1. Further the reaction was improved by operating with a molar excess of chlorine, preferably at a temperature of 380-460 ºC (Huthmacher and Most 2005).

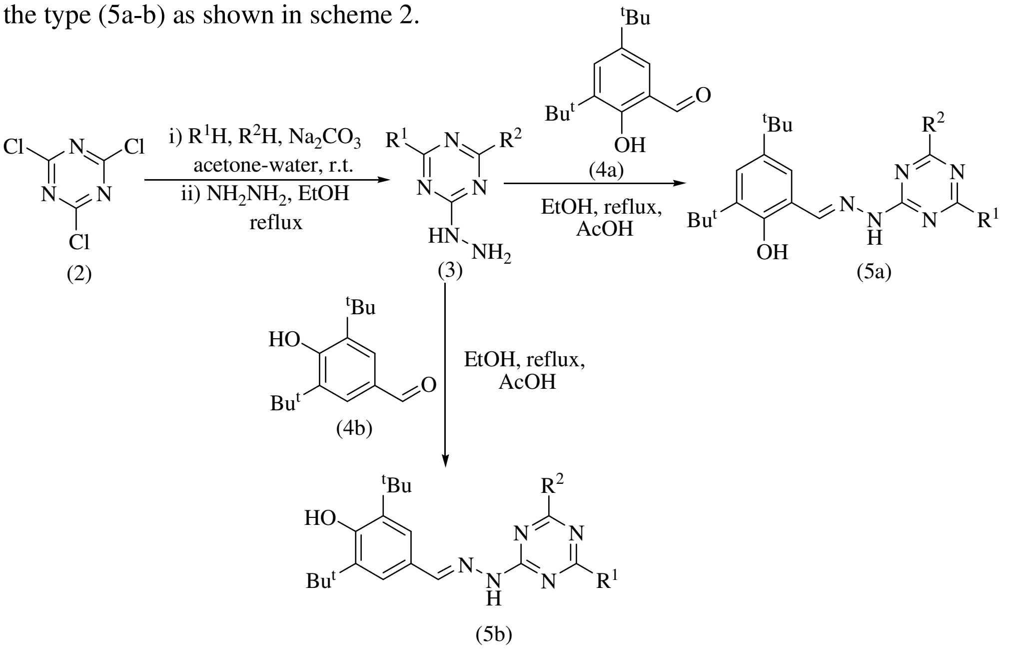


Scheme 1

2,4,6-Trichloro-1,3,5-triazine has been used for the activation of carboxyl group for the preparation of esters, amides, acyl azides as well as peptides because of its condensing nature (Nezhad *et al* 2007). Moreover, mono-, di- or tri-substituted derivatives of 1,3,5-triazine can be synthesized, due to the high reactivity of chlorine atoms present in cyanuric chloride towards nucleophilic substitution reactions (El-Samahy *et al* 2016). The sequential substitution of chlorine atoms can be done by replacing or controlling temperature to move in a stepwise process. This substitution will depend upon the structure of nucleophile, the steric factors, basic strength, the substituents already present on the benzene ring and the nature of the solvent used (Blonty 2006).

Most of triazine substituted derivatives exhibit a broad range of biological properties which includes antiprotozoal (Klenke *et al* 2001), antimicrobial (Patel *et al* 2010), antimalarial (Manohar *et al* 2010), antituberculosis (Sunduru *et al* 2009), anticancer (De Hoog *et al* 2002), antiviral (Desai *et al* 2015), antitumour (Schmidt *et al* 2009), analgesic (Yaguchi *et al* 2006), herbicidal (Zhou *et al* 2003), insecticidal (Upadhyay *et al* 2010), antifungal (Pfaller and Diekema 2004), antibacterial (Bhat and Al Daihan 2014) and cyclooxygenase inhibitor (Selvam *et al* 2005) *etc*. Many scientists have been doing extensive efforts to construct a number of triazine substituted derivatives with a wide range of biological properties through developing versatile and efficient synthetic strategies. In the present review, we shall discuss some of the reported procedures for the synthesis of triazine derivatives and the biological activities exhibited by them.

Barakat *et al* (2019) synthesized novel Schiff base derivatives of triazine hydrazides of the type (5a-b) as shown in scheme 2.

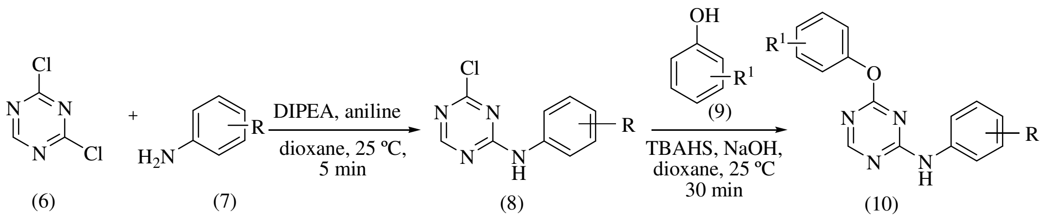


R^1, R^2 = piperidine, morpholine, diethylamine, methoxy, benzylamine, aniline

Scheme 2

The scheme was initiated by reacting triazine (2) with two different amines in the presence of sodium bicarbonate (Na_2CO_3) at room temperature for 2-3 h which on further refluxing with hydrazine in ethanol (EtOH) furnished *s*-triazine derivatives (3). Further, the refluxing of intermediate (3) with hydroxybenzaldehyde derivatives (4a-b) and acetic acid (AcOH) in EtOH yielded target compounds (5a-b). All the synthesized compounds were screened for their antiproliferative activity against two different cancer cell lines including breast cancer (MCF-7) and colon cancer (HCT-116). The two compounds (5a-b) with hydroxy group in benzylidine ring and substituents on the *s*-triazine moiety showed excellent activity against both the cell lines with IC_{50} values ranging between 17µM to 20µM as compared to Cisplatin (IC_{50} = 10µM) as standard drug.

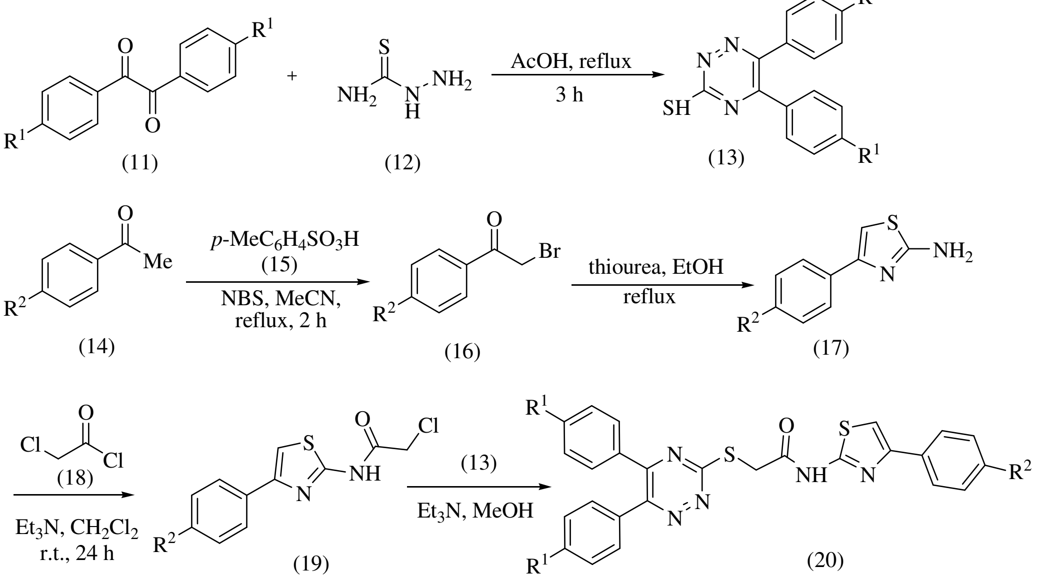
Variety of disubstituted triazine derivatives of the type (10) were synthesized by Salado *et al* (2018) as shown in scheme 3. The synthetic scheme begins with the reaction of di-chloro triazine (6) with substituted amine (7) in the presence of diisopropylethylamine (DIPEA) in dioxane to afford 4-chloro-*N*-(4-fluorophenyl)-1,3,5-triazin-2-amine (8) which on further addition of phenol derivatives (9) in the presence of phase transfer catalyst tetrabutylammoniumhydrogensulfate (TBAHS) and NaOH in dioxane at 25 ºC yielded the desired compound *N*-(4-fluorophenyl)-4-(mesityloxy)-1,3,5-triazin-2-amine (10). The synthesized compounds were found to show potent antitrypanosomal activity and microsomal activity against bacteria *Trypanosoma cruzi*, *Leishmania infantum*, *Plasmodium falciparum* and *Trypanosoma brucei*. Among the synthesized compounds, the compound (10) bearing (R = 4-CN and R^1 = 4-F) groups (IC_{50} = 39.83 µM) showed more potent activity against *T. brucei* as compared to standard drug Suramin (IC_{50} = 0.03 µM).



R = H, 4-F, 4-Cl, 4-CF_3, 3,5-di F, 2,6-$(F)_2$, 4-CN, 4-Me,
R^1 = 2,4,6-$(Me)_3$, 4-F, H, 2-Cl, 4-Me, 2,6-$(Br)_2$-4-Me, 2,6-$(Me)_2$-4-F

Scheme 3

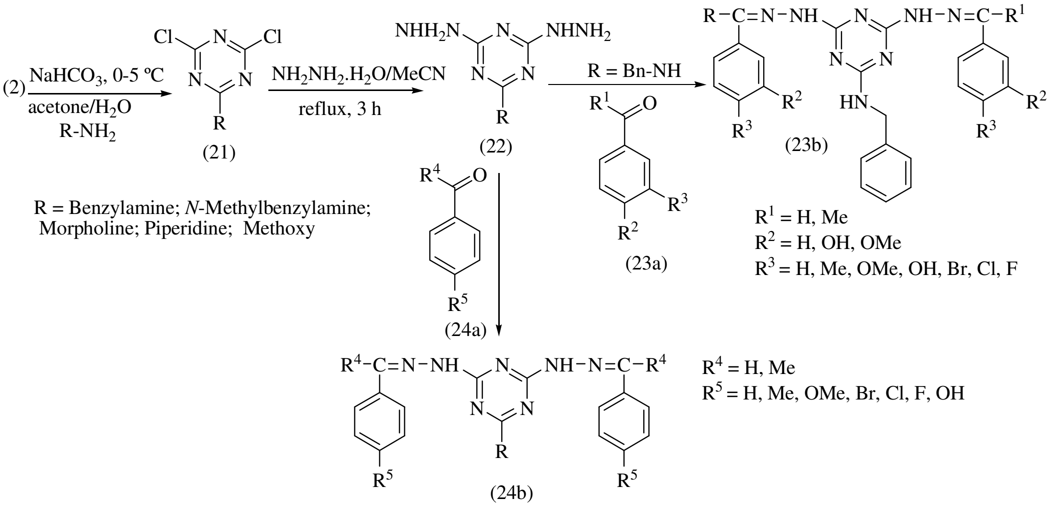
Wang *et al* (2018) synthesized novel 5,6-diaryl-1,2,4-triazine thiazole derivatives of type (20) and screened them for their *α*-glycosidase inhibitory activity as shown in scheme 4. The synthetic scheme begins with the condensation of substituted benzyl (11) with thiosemicarbazide (12) in the presence of AcOH to afford 5,6-diaryl-1,2,4-triazine-3-thiols (13). Also, the compound (14) was reacted with *N*-bromosuccinimide (NBS) in the presence of *p*-tolouenesulfonic acid (15) to yield bromoacetophenone (16) which on further refluxing with thiourea in EtOH furnished 4-arylthiazol-2-amines (17). The synthesized compound (17) was reacted with 2-chloroacetylchloride (18) in the presence of triethylamine (Et_3N) as a base to afford the intermediates (19). Finally, condensation of (19) with (13) in Et_3N and dry methanol (MeOH) furnished the target compounds 5,6-diaryl-1,2,4-triazine thiazole (20). The synthesized compound (20) showed potent α-glycosidase inhibitory activity with IC_{50} value ranging between 2.85±0.13 μM to 14.19±0.23 μM as compared to standard drug Acarbose (IC_{50} = 817.38±6.27 μM). The compound (20) with (R^1 = Br, R^2 = OMe) showed highest activity with IC_{50} values of 2.85±0.13 μM.



R^1 = H, Br, F
R^2 = H, Me, MeO, F, Cl, Br, H,

Scheme 4

A novel series of 2,4-*bis*(hydrazine)-6-substituted-1,3,5-triazine and their Schiff base derivatives were synthesized by Al Rasheed *et al* (2018) as shown in scheme 5. Triazine (2) was reacted with different amines (7) in the presence of $NaHCO_3$ at 0-5 ºC to afford dichloro derivatives (21) which on further refluxing with hydrazine in acetonitrile (MeCN) yielded 2,4-dihydrazino-6-substituted-1,3,5-triazine derivatives (22).



R = Benzylamine; *N*-Methylbenzylamine; Morpholine; Piperidine; Methoxy

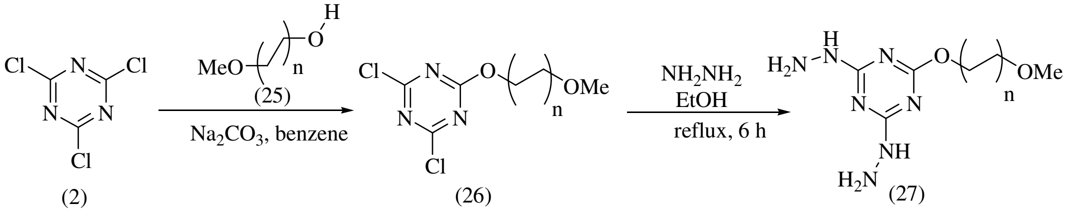
R^1 = H, Me
R^2 = H, OH, OMe
R^3 = H, Me, OMe, OH, Br, Cl, F

R^4 = H, Me
R^5 = H, Me, OMe, Br, Cl, F, OH

Scheme 5

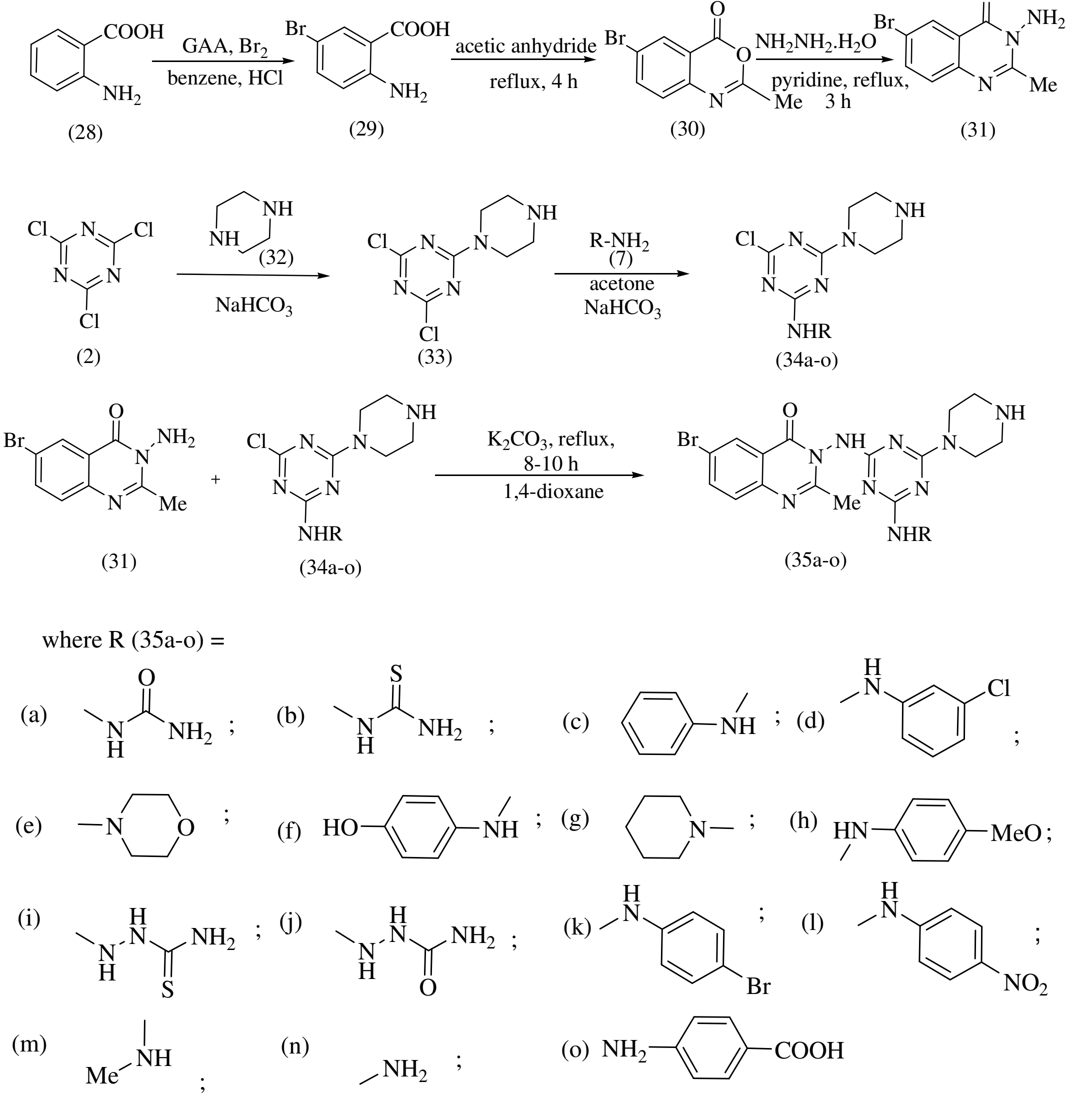
Finally, the condensation of hydrazine derivative (22) with different aldehydes and susbstituted acetophenones (23a) and (23b) in the presence of dry EtOH furnished the final products (24a) and (24b) respectively. Most of the synthesized compounds were screened for their antimicrobial activity against gram+ve and gram-ve bacteria and showed excellent results against *Staphylococcus aureus* at 62.5 µg/ml, whereas some hydroxy and methoxy substituted compounds showed good activity against *Streptococcus mutans* and *Escherichia coli* with MIC values at 31.25 µg/ml and 62.5 µg/ml respectively. It was concluded that presence of substituents in benzylidene and triazine ring increased its activity as antimicrobial agent.

El Faham *et al* (2018) synthesized a variety of triazine hydrazides of the type (27) by using solid phase technique as shown in scheme 6. Triazine (2) was reacted with methoxypolyethylene glycol (25) in benzene followed by addition of sodium carbonate (Na_2CO_3) to furnish 6-(methoxypolyethyleneglycol)-1,3,5-triazine (26). Refluxing of ethanolic solution of hydrazine with (26) in the presence of EtOH at room temperature resulted in the formation of target compound 2,4-dihydrazino-6-(methoxypolyethylene glycol)-1,3,5-triazine (27). Epoxy coatings were screened for their anticorrosion activity and showed excellent inhibition even at low concentrations.



Scheme 6

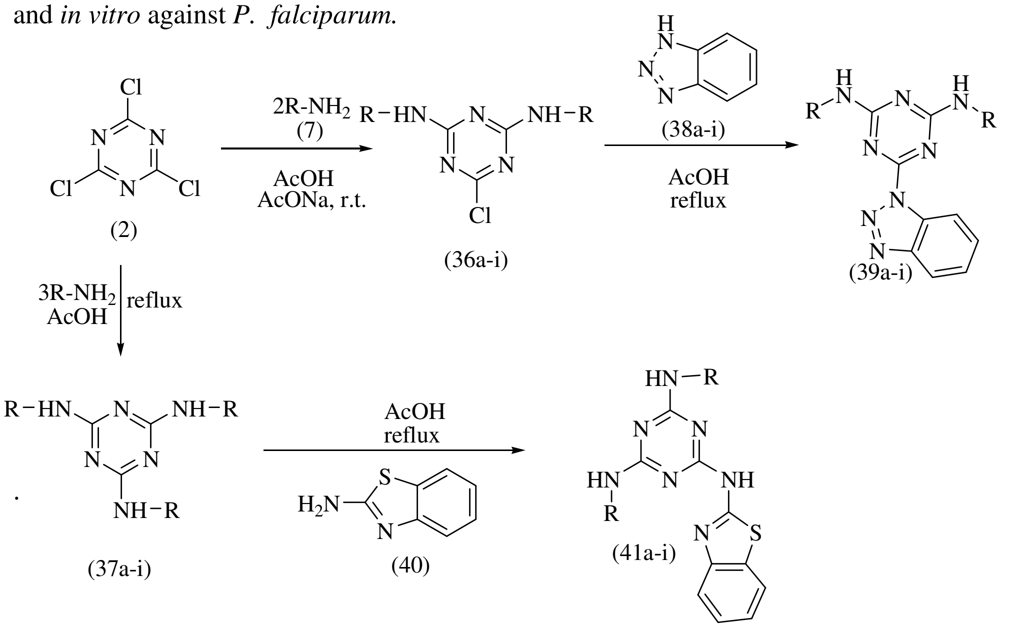
A novel series of quinazoline containing 1,3,5-triazine derivatives of the type (35a-o) were synthesized by Pathak *et al* (2018) as shown in scheme 7. In the first step, the mono-substituted anthranilic acid (29) was synthesized by bromination of (28) in glacial acetic acid (GAA) in the presence of benzene and hydrochloric acid (HCl) which on further refluxing with acetic anhydride afforded 6-bromo-2-methyl-4*H*-benzo[d][1,3]oxazine (30). Refluxing of (30) in the presence of hydrazine hydrate and pyridine yielded 6-bromo-2-methyl-4*H*-quinazolin-3-ylamine (31). In the second step, the reaction of cyanuric chloride (2) with piperazine (32) in the presence of sodium hydrogen carbonate ($NaHCO_3$) as a base yielded 4,6-dichloro-2-piperazin-1-yl-[1,3,5]triazine (33) which on stirring with various amines (7) in the presence of acetone furnished 2-chloro-4-substituted amino-6-piperazin-1-yl-[1,3,5]triazine derivatives (34a-o). Further, the reaction of (34a-o) with (31) in the presence of potassium carbonate (K_2CO_3) and 1,4-dioxane afforded the target compounds (35a-o). All the synthesized compounds were screened for their *in vitro* anticancer activity against HeLa (human cervical cancer), MCF-7 (human breast cancer cell) using Vandetinib as standard drug. Among the synthesized compounds (35a-o), the compounds (35d), (35i), (35m) showed excellent anticancer activity with minimum IC_{50} value of 07.176±1.17 μM, 09.54±0.33 μM and 09.52±0.27 μM respectively. It was concluded that compounds containing electron withdrawing group showed potent biological activity through increased plasma protein binding due to balanced SlogP value.



Scheme 7

Pore and Mahajan (2018) had synthesized a new class of symmetrical and unsymmetrical triamino derivatives of triazine (41a-i) in a sequence of reactions as shown in scheme 8. The synthetic scheme begins with the reaction of cyanuric chloride (2) with various aromatic amines (7) in AcOH to afford disubstituted triazine derivatives 6-chloro-*N*,*N'*-*bis*(2-chlorophenyl)-1,3,5-triazine-2,4-diamine (36a-i) which on further refluxing with benzotriazole (38a-i) in AcOH furnished 6-(1*H*-benzothiazol-2-yl)-N^4-N^6-diphenyl-1,3,5-triazine-2,4,-diamine (39a-i), while the same reaction under reflux condition selectively afforded symmetrical trisubstituted triazine derivatives *N*,*N'*,*N''*-triphenyl-1,3,5-triazine-2,4,6-triamine (37a-i). The final product N^2-(1,3-benzothiazol-1-yl)-N^4-N^6-diphenyl-1,3,5-

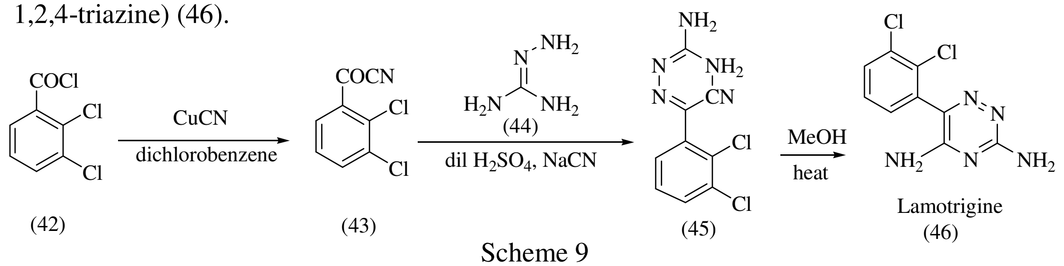
triazine-2,4,6-triamine (41a-i) was obtained on refluxing (37a-i) with 2-amino benzothiazole (40) in good synthetic yields. The synthesized compounds were evaluated for *in vivo* antimalarial activity against *Plasmodium berghei, Plasmodium ovale, Plasmodium malariae* and *in vitro* against *P. falciparum.*



R = (a) Ph; (b) F-C_6H_4; (c) 3-FC_6H_4; (d) 5-FC_6H_4; (e) 4-ClC_6H_4; (f) 5-ClC_6H_4; (g) 6-ClC_6H_4; (h) 4-MeOC_6H_4; (i) 2-MeOC_6H_4

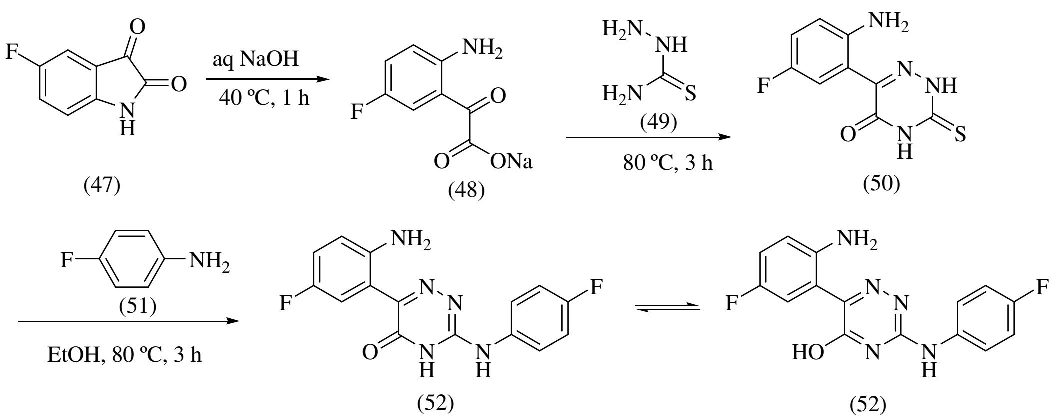
Scheme 8

Makki *et al* (2018) reported the synthesis of novel Lamotrigine antiepileptic drug (46) and their fluorine substituted lamotrigine analogues in sequential steps as shown in scheme 9. The scheme was initiated by reacting 2,3-dichloro-benzoyl chloride (42) with metal cyanide in dichlorobenzene to yield 2,3-dichloro-benzoyl cyanide (43) which on further reaction with amino guanidine salt (44) in H_2SO_4 and NaCN afforded intermediate (45). Refluxing of (45) in MeOH yielded the target compound Lamotrigine (3,5-diamino-6-(2'3'-dichloro-phenyl)-1,2,4-triazine) (46).



Scheme 9

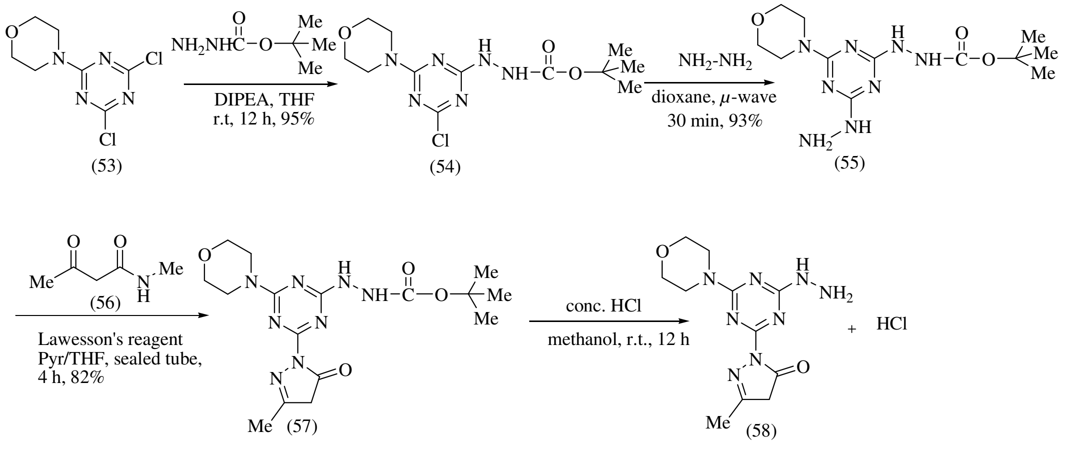
In another reaction scheme 10, the reaction involves the treatment of 5-fluoroindolin-2,3-dione (47) with NaOH at 40 ºC and resulted in the formation of sodium 2-(2-amino-5-fluorophenyl)-2-oxoacetate (48) which on reaction with thiosemicarbazide (49) furnished the fluorinated Lamotrigine analogues 6-(2'-amino-5'-fluorophenyl)-3-thioxo-1,2,4-triazin-5-(2*H*,4*H*)-one (50). The product (50) on refluxing with 4-fluoroaniline (51) in EtOH afforded the compound 3-(4'-fluorophenyl)amino-6-(2'-amino-5'-fluorophenyl)-1,2,4-triazine-5-(2*H*)-one (52) which lies in equilibrium with its enolic form.



Scheme 10

The synthesized compounds (46), (48) and (52) mentioned in schemes 9 and 10 were screened for their anti-inflammatory activity using standard Indomethacin. It was observed that the compound (52) with higher number of fluorine atoms showed excellent anti-inflammatory activity with inhibition rate of 39.39% and 45.45% while the compound (46) with no fluorine atoms showed poor anti-inflammatory activity as compared to standard Indomethacin.

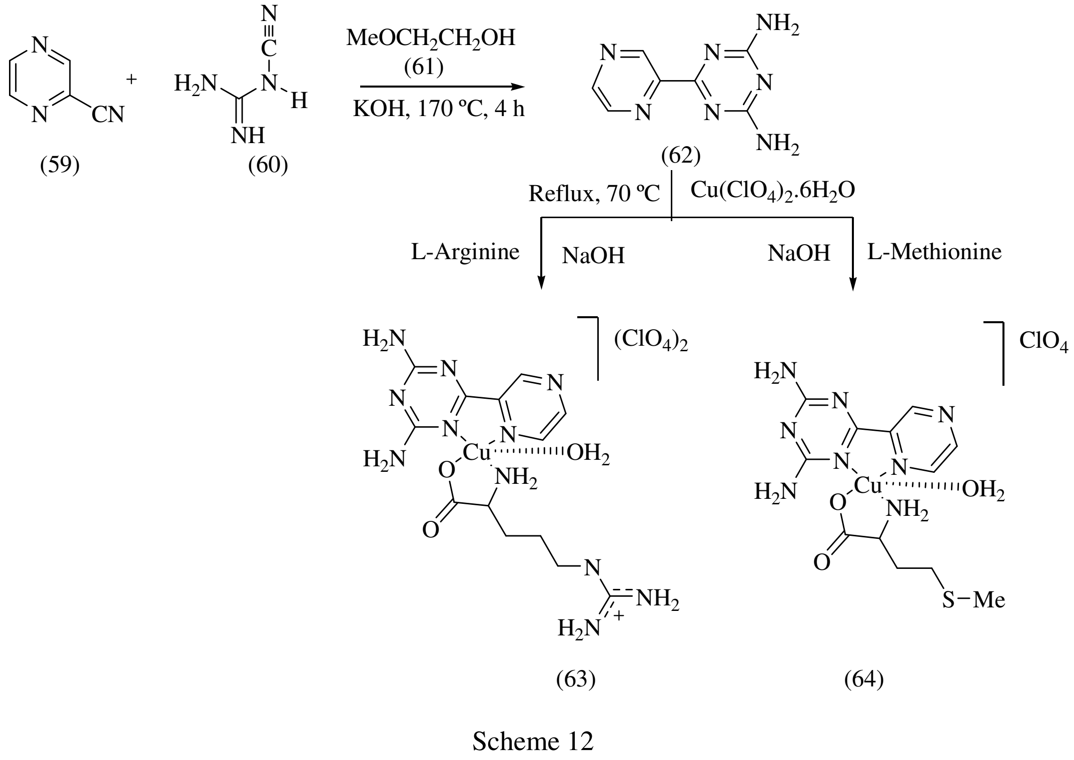
Yepremyan *et al* (2018) carried out the synthesis of novel triazine based ligands of the type (58) as shown in scheme 11. The synthetic scheme begins with the nucleophilic substitution of 4-(4,6-dichloro-1,3,5-triazin-2-yl)morpholine (53) with *tert*-butoxycarbonyl hydrazine (NH_2NHBOC) in the presence of DIPEA and tetrahydofuran (THF) to furnish 4-(4-chloro-6-BOC-hydrazine-1,3,5-triazin-2-yl)morpholine (54). Subsequently, dropwise addition of hydrazine to (54) under microwave irradiation yielded 4-(4-hydrazine-6-BOC-hydrazine-1,3,5-triazin-2-yl)morpholine (55).



Scheme 11

Further reaction of (55) with acetoacetamide (56) in the presence of Lawesson's reagent afforded the compound 4-(4-methylpyrazolone-6-BOC-hydrazine-1,3,5-triazin-2-yl)morpholine (57) which on treatment with HCl and MeOH furnished the target compound 4-(4-methylpyrazolone-6-hydrazine HCl-1,3,5-triazin-2-yl)morpholine (58). It was found that synthesized compound (58) showed potent therapeutic properties in cellular toxicity studies with EC_{50} value of 1.183 ± 0.002 mM.

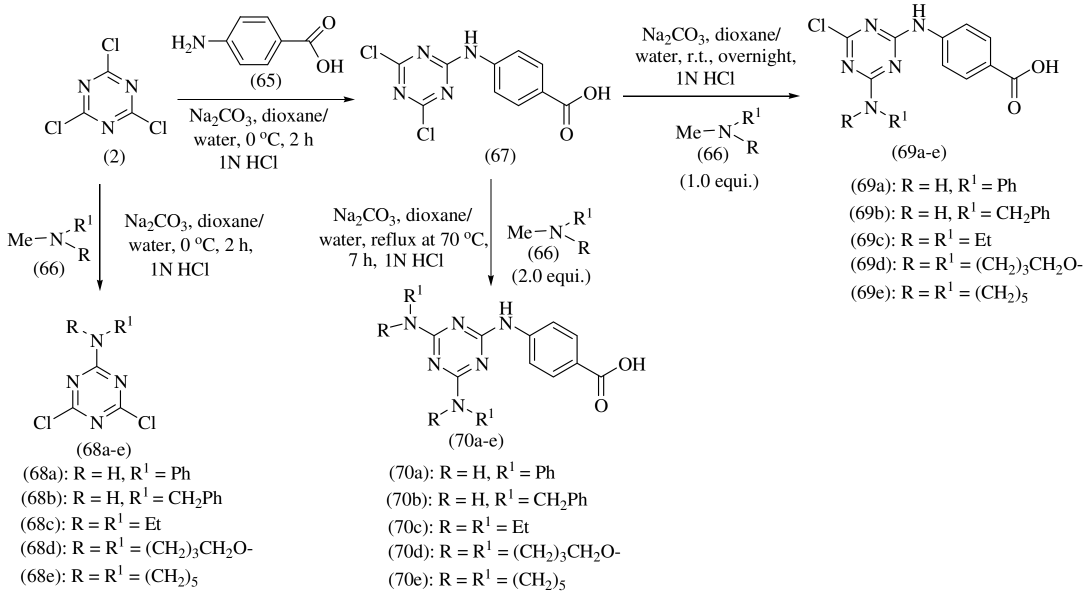
Zhang *et al* (2018) synthesized the copper complexes of 6-(pyrazinyl-2-yl)-1,3,5-triazine-2,4-diamine (pzta) and amino acids, [Cu(pzta)(*L*-ArgH)(H_2O)](ClO_4)$_2$ (63) and [Cu(pzta)(*L*-Met)(H_2O)](ClO_4).3H_2O (64) as shown in scheme 12. The reaction was initiated by condensation of pyrazine-2-carbonitrile (59) with 1-cyanoguanidine (60) in the presence of 2-methoxyethanol (61) at high temperature to furnish 6-(pyrazinyl-2-yl)-1,3,5-triazine-2,4-diamine (pzta) (62). Further, refluxing of (62) with an aqueous solution of amino acids L-Arginine and L-Methionine in the presence of copper perchlorate hexahydrate yielded the target compounds [Cu(pzta)(*L*-ArgH)(H_2O)](ClO_4)$_2$ (63) and [Cu(pzta)(*L*-Met)(H_2O)] (ClO_4.3H_2O) (64) respectively. The synthesized complexes were evaluated for their *in vitro* cytotoxic activity against A549 (pulmonary), PC-3 (prostatic) and HeLa (cervical) cell lines by using standard 5-Fluorouracil. It was found that the complex (64) showed more potent inhibitory effect against A549 cell line with IC_{50} value 51.29 µM than complex (63) (IC_{50} = 89.13 µM) while the complex (63) showed maximum cytotoxicity against HeLa and PC-3 cell lines with IC_{50} value 131.83 µM and 85.11 µM respectively.



Scheme 12

The synthesis of mono, di and tri-substituted-triazine derivatives were reported by Al-Zaydi *et al* (2017) as shown in scheme 13. The monosubstituted 1,3,5-triazine derivatives was synthesized by replacing one chlorine atom of cyanuric chloride (2). The compound (2) was reacted with 4-aminobenzoic acid (65) and (66) in the presence of sodium carbonate (Na_2CO_3) to yield 4-((4,6-dichloro-1,3,5-triazin-2yl)amino)benzoic acid derivative of the type

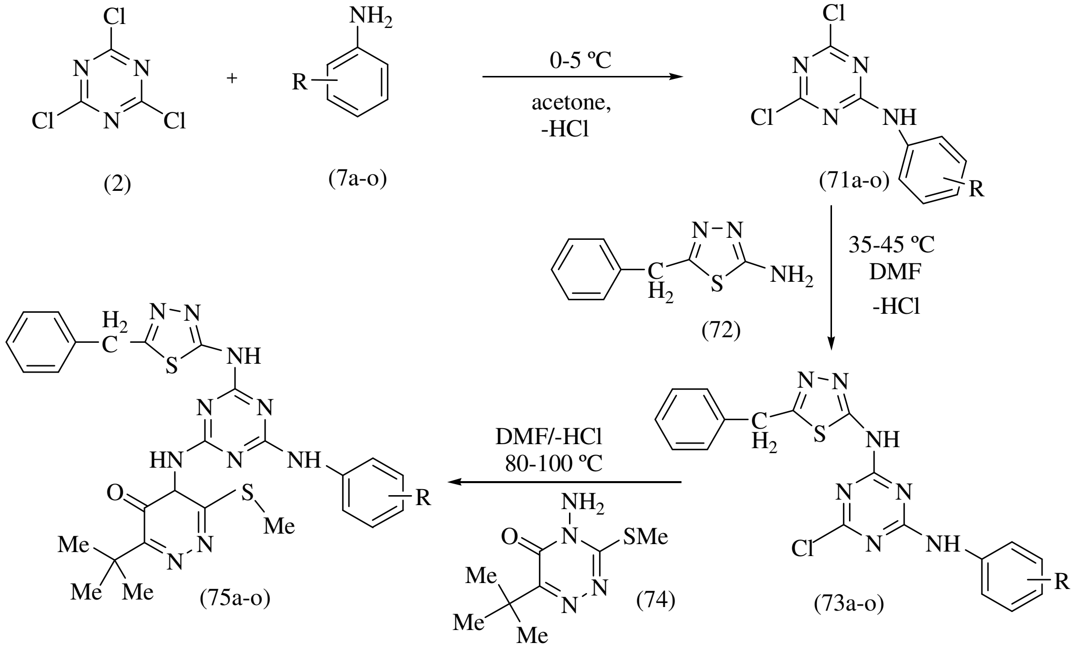
(67) and (68a-e) respectively. Further, a series of disubstituted 1,3,4-triazine derivatives (69a-e) were synthesized by replacing second chlorine atom of (67) with amines (66) in the presence of Na_2CO_3. Similarly, another trisubstituted series of 1,3,5-triazine derivatives (70a-e) were afforded by refluxing (67) with (66) in the presence of Na_2CO_3. All the synthesized compounds were screened for their antimicrobial activity against *S. aureus* by using Ampicillin as a standard drug. It was found that compounds (68d) and (69c) showed more potent activity against *E. coli* and *in vitro* cytotoxicity results showed that these compounds were nontoxic upto concentration 250 µg/ml (SI = 10) and 125 µg/ml (SI = 5) respectively.



Scheme 13

Malik and Patel (2017) reported a series of 1,2,4-triazine and thiadiazole linked triazine derivatives (75a-o) that showed better activity against gram+ve bacteria *S. aureus* and *Staphylococcus pyogenes* as compared to standard Ampicillin as shown in scheme 14. Triazine (2) was reacted with primary aromatic amine (7a-o) in acetone to afford 4,6-dichloro-*N*-phenyl-1,3,5-triazin-2-amine (71a-o) which was further reacted with 5-benzyl-1,3,4-thiadiazol-2-amine (72) in the presence of dimethylformamide (DMF) to furnish N^2-(5-benzyl-1,3,4-thiadiazol-2-yl)-6-chloro-N^4-phenyl-1,3,5-triazine-2,4-diamine (73a-o). The products so obtained (73a-o) on condensation with 4-amino-6-(*tert*-butyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one (74) in the presence of DMF at 80-100 ºC yielded the final product 4-((4-((5-benzyl-1,3,4-thiazol-2-yl)amino)-6-(phenyl amino) 1,3,5-triazin-2-yl)amino)-6-(*tert*-butyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one (75a-o). The synthesized compounds were evaluated for their *in vitro* anitbacterial activity against gram +ve (*S. aureus* and *S. pyogenes*) and gram -ve (*E. coli* and *Pseudomonas aeruginosa*) bacterial strains by using micro dilution technique. The evaluation for antifungal activity was also carried out against fungal species

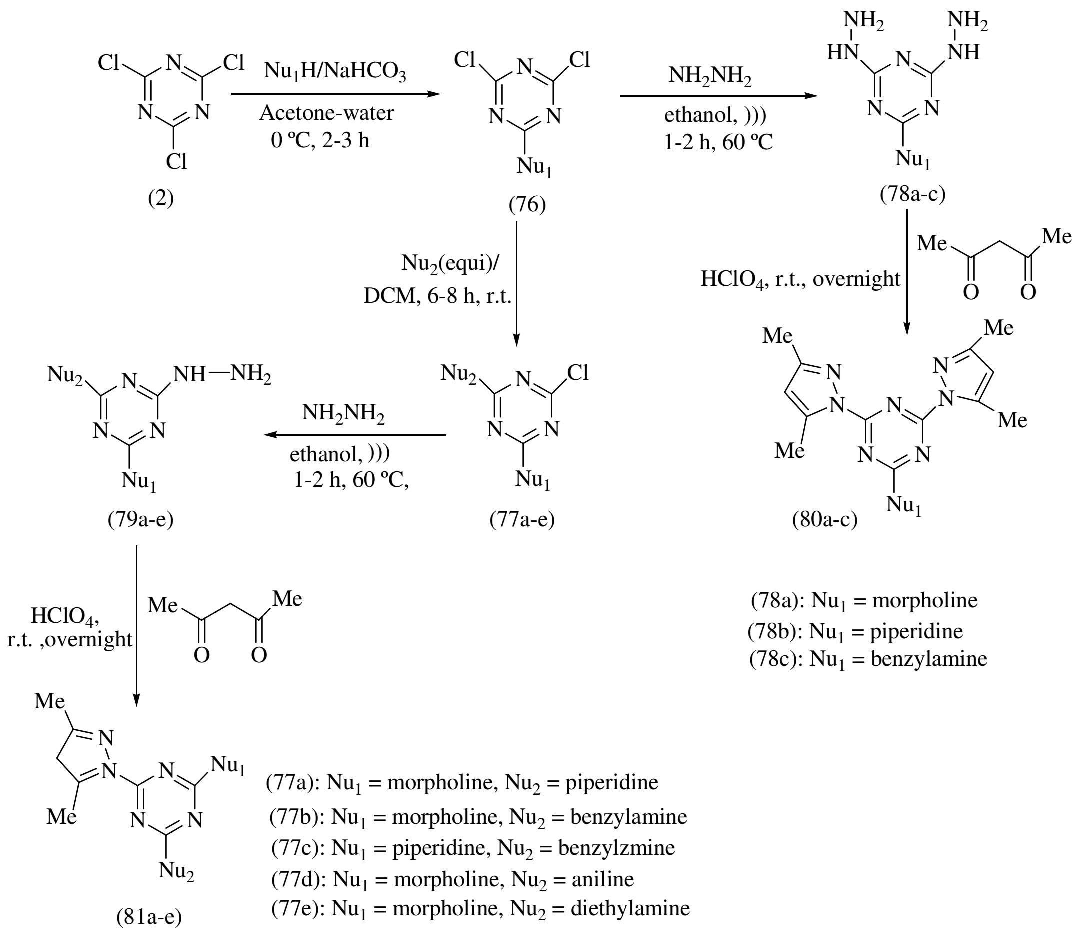
(*Candida albicans*, *Aspergilllus niger* and *Aspergillus clavatus*) by using Griseofulvin as a standard. Amongst all the synthesized compounds (75b), (75e) and (75j) showed good antibacterial activity against *E. coli* with zone of inhibition (500 µg/ml, 250 µg/ml and 125 µg/ml), *P. aeruginosa* (500 µg/ml, 500 µg/ml and 500 µg/ml), *S. aureus* (31.25 µg/ml, 62.5 µg/ml and 62.5 µg/ml) and *S. pyogenes* (125 µg/ml, 500 µg/ml and 1000 µg/ml) as compared to standard drug Ampicillin and Chloramphenicol.



where R = (a) 4-Cl; (b) 4-F; (c) 4-Br; (d) 4-Me; (e) 2-OMe; (f) 2-Me; (g) H; (h) 4-OMe; (i) 4-COMe; (j) 1-NH; (k) 4-NO_2; (l) 3-Cl; (m) -Ph; (n) 2,5-Cl; (o) 4-Cl,2-NO_2

Scheme 14

Sharma *et al* (2017) synthesized pyrazole containing triazine derivatives of type (81a-e) by using ultrasound assisted technique as shown in scheme 15. The synthetic scheme begins with the nucleophilic substitution of first chlorine atom of cyanuric chloride (2) by using different amines (morpholine, piperidine or benzylamine) at 0-5 ºC in the presence of $NaHCO_3$ as a base to afford (76) and another chlorine atom was replaced by using amine at room temperature to furnish (77a-e). Then, compounds (76) and (77a-e) were reacted with hydrazine (80%) in ethanol for 1-2 h to afford the hydrazine derivatives (78a-c) and (79a-e) respectively. The compounds (78a-c) and (79a-e) were then reacted with acetyl acetone in the presence of $HClO_4$ to furnish the target compounds (80a-c) and (81a-e) in good synthetic yield. The synthesized compounds were investigated for their antimicrobial activity against gram -ve (*E. coli*, *P. aeruginosa*) bacteria, gram +ve (*Micrococcus luteus*, *S. aureus*) bacteria and fungi *C. albicans*. Antifungal screening data revealed that compound (81a) showed modest activity against fungi *C. albicans* with inhibition at 9 mm as compared to standard used Tobramycin, Fusidic acid and Canesten.

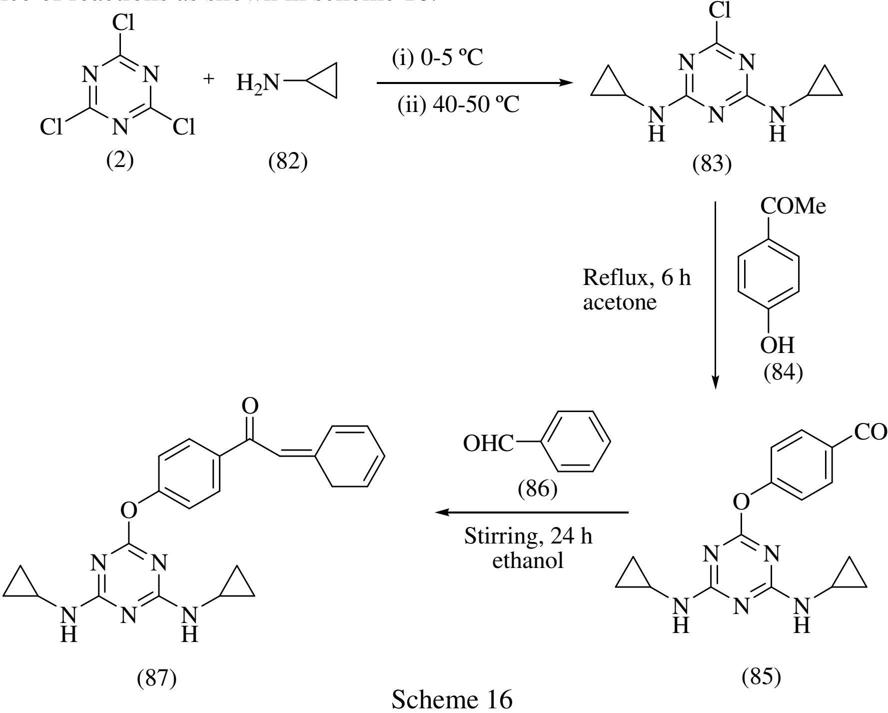


(78a): Nu_1 = morpholine
(78b): Nu_1 = piperidine
(78c): Nu_1 = benzylamine

(77a): Nu_1 = morpholine, Nu_2 = piperidine
(77b): Nu_1 = morpholine, Nu_2 = benzylamine
(77c): Nu_1 = piperidine, Nu_2 = benzylzmine
(77d): Nu_1 = morpholine, Nu_2 = aniline
(77e): Nu_1 = morpholine, Nu_2 = diethylamine

Scheme 15

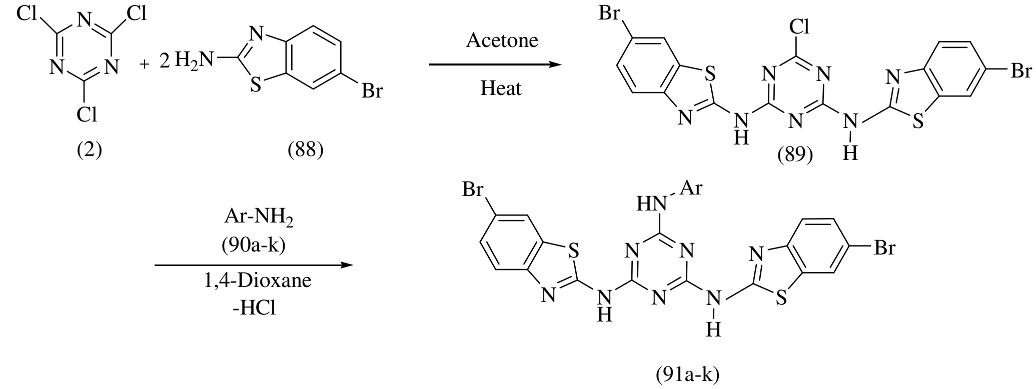
Hashmi and Kishore (2016) had synthesized a novel series of compounds in a sequence of reactions as shown in scheme 16.



Scheme 16

The synthetic scheme begins with reaction of cyanuric chloride (2) with cyclopropylamine (82) to yield 6-chloro-N^2,N^4-dicyclopropyl-1,3,5-triazine-2,4-diamine (83). Refluxing of compound (83) with *p*-hydroxyacetophenone (84) in the presence of acetone afforded 1-(4-(4,6-*bis*(cyclopropylamino)-1,3,5-triazin-2yloxy)phenyl)ethanone) (85). The product (85) formed was reacted with (86) in the presence of absolute alchohol to furnish the complex (87) with trinuclear structure in good synthetic yield.

Patel and Baldaniya (2016) carried out the synthesis of a series of N^2,N^4-*bis*(6-bromo-1,3-benzothiazol-2-yl)-N^6-aryl-1,3,5-triazine-2,4,6-triamine (91a-k) indole fused triazine derivatives as shown in scheme 17. Initially, reaction of triazine (2) with 2 moles of 6-bromo-1,3-benzothiazol-2-amine (88) in acetone resulted in formation of intermediate 6-chloro-*N,N'*-*bis*(6-bromo-1,3-benzothiazol-2-yl)-N^6-aryl-1,3,5-triazine-2,4-diamine (89). Further, the compound (89) was condensed with different aryl amines (90a-k) in the presence of 1,4-dioxane as a base to furnish the target compounds (91a-k).

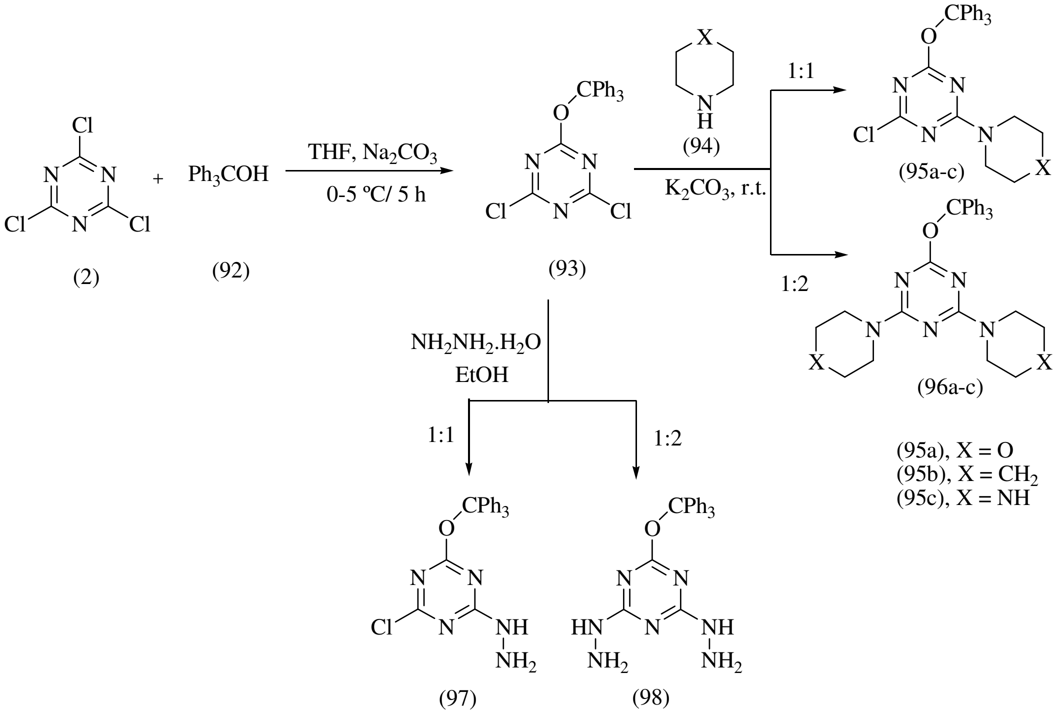


Ar (90 a-k)= (a) Ph (b) 3-ClC_6H_4 (c) 4-ClC_6H_4 (d) 3-$NO_2C_6H_4$ (e) 4-$NO_2C_6H_4$ (f) 4-BrC_6H_4 (g) 4-FC_6H_4 (h) 2-$C_5H_4N_4$ (i) 4-$C_5H_4N_2$ (j) N-Me-Ph (k) 4-Me-C_6H_4

Scheme 17

Most of the synthesized compounds showed good to average antibacterial activity when tested against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenus* at 1000, 500, 200, 100, 50, 25 and 12.5 µg/ml. The antifungal activity was also carried out against *C. albicans*, *A. clavatus* and *A. niger* at maximum fungal concentration of 1000, 500, 200 and 100 µg/ml.

El-Samahy *et al* (2016) synthesized a series of di- and tri-substituted 1,3,5-triazine derivatives of types (95a-c) and (96a-c) as shown in scheme 18. The reaction underwent by treating cyanuric chloride (2) with triphenylmethanol (92) in the presence of dry tetrahydrofuran (THF) as in anhydrous Na_2CO_3 to afford 2,4-dichloro-6-(trityloxy)-1,3,5-triazine (9). Further, the reaction of (93) with cyclic amine (94) in the presence of K_2CO_3 as a base in different ratios *i.e.* (1:1) and (1:2) at room temperature furnished the compounds (95a-c) and (96a-c) respectively. Also, the reaction of compound (93) with hydrazine hydrate in (1:1) and (1:2) ratios in absolute alcohol at room temperature yielded 2-chloro-4-hydrazinyl-6-(trityloxy)-1,3,5-triazine (97) and dihydrazinyl-6-(trityloxy)-1,3,5-triazine (98) respectively.

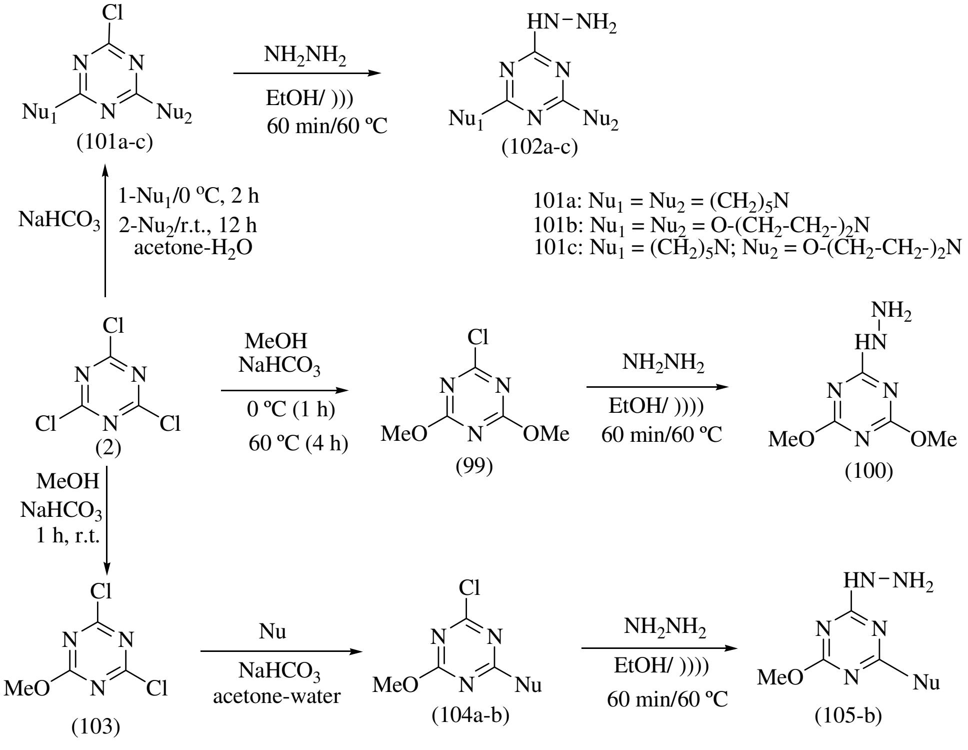


Scheme 18

The synthesized compounds (95a-c), (96a-c), (97) and (98) were evaluated for their antimicrobial activity against gram +ve bacteria *S. aureus* and *C. albicans* using Cephradine as standard. Amongst the synthesized compounds, compounds (93) and (95a-c) showed greater potency against *C. albicans* but were not effective against *S. aureus*. It was also observed that compounds (97) and (98) did not show activity against any tested bacteria.

A novel series of *s*-triazine-Schiff base derivatives were synthesized by El-Faham *et al* (2016) by employing ultrasonic irradiation method as shown in scheme 19. The synthetic scheme begins with the reaction of cyanuric chloride (2) with MeOH in the presence of $NaHCO_3$ to afford the triazine derivative (99) which on further condensation with hydrazine (80%) in ethanol for 1 h at 60º C by employing ultrasonic irradiation furnished hydrazine derivative (100). The compounds (101a-c) were obtained with the reaction of cyanuric chloride (2) with piperidine and morpholine in the presence of $NaHCO_3$ which on further refluxing with hydrazine in the presence of ethanol afforded hydrazine derivatives (102a-c). Finally, the reaction of cyanuric chloride (2) with MeOH furnished dichloro-methoxy derivative (103) which on further condensation with morpholine and piperidine yielded (104a-b) derivatives. The ethanolic solution of compounds (104a-b) was refluxed with excess of hydrazine to afford (105a-b) by employing ultrasonic irradiation. The synthesized compounds were evaluated for their anti-proliferative effect against different cancer cell line including the lung carcinoma cell line A549, the hepatocellular carcinoma cell line HepG2 and the Adenocarcinoma cell line MCF7. It was found that compound (105a) was able to inhibit the growth of two cancer cell lines including A549 and HepG2 with IC_{50} value (5.6

μg/ml and 6.5 μg/ml) respectively.

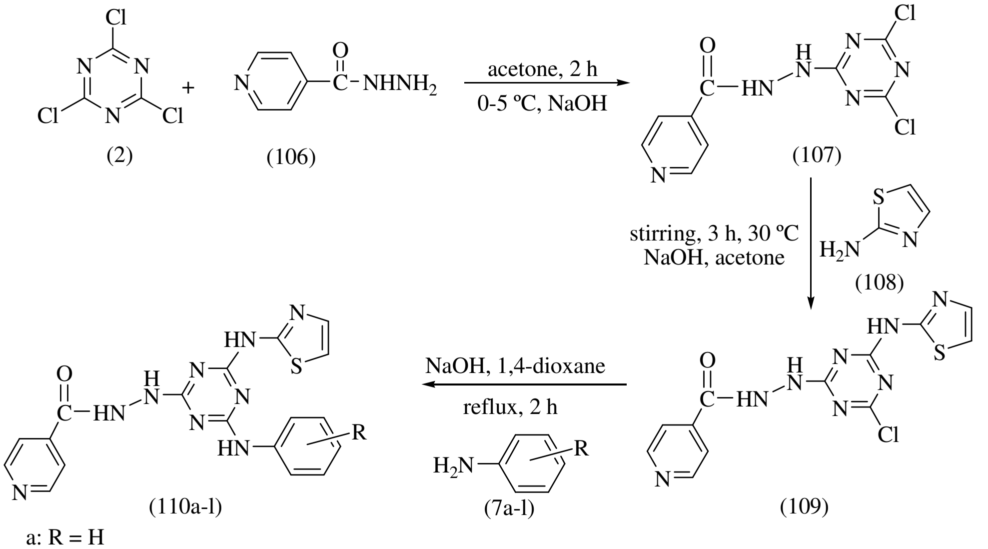


101a: $Nu_1 = Nu_2 = (CH_2)_5N$
101b: $Nu_1 = Nu_2 = O\text{-}(CH_2\text{-}CH_2\text{-})_2N$
101c: $Nu_1 = (CH_2)_5N$; $Nu_2 = O\text{-}(CH_2\text{-}CH_2\text{-})_2N$

104a: $Nu = (CH_2)_5N$
104b: $Nu = O\text{-}(CH_2\text{-}CH_2\text{-})_2N$

Scheme 19

Desai *et al* (2015) synthesized sulfonamide derivatives of triazine (110a-l) as shown in scheme 20.

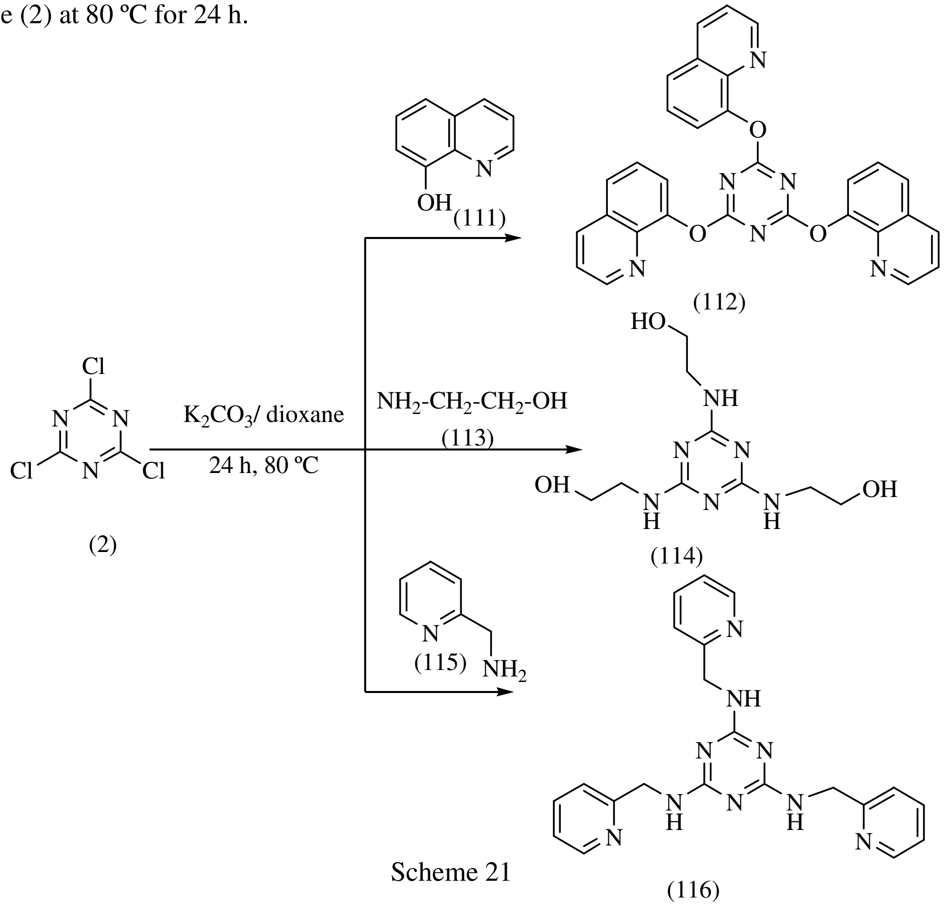


a: R = H
b: R = 4-Br
c: R = 2-Cl
d: R = 3-Cl
e: R = 4-Cl
f: R = 2,5-$(Cl)_2$
g: R = 2,6-$(Cl)_2$
h: R = 4-F
i : R = 2-Me
j: R = 4-Me
k: R = 2-NO_2
l: R = 4-NO_2

Scheme 20

The synthetic scheme began with the addition of isoniazid (106) to triazine (2) in acetone in the presence of NaOH as base and resulted in formation of *N'*-(4,6-dichloro-1,3,5-triazin-2-yl)isonicotinohydrazide (107) which on further reaction with thiazol-2-amine (108) at room temperature with constant stirring for 3 h furnished intermediate *N'*-(4-chloro-6-(thiazol-2-ylamino)-1,3,5-triazin-2-yl)isonicotinohydrazide (109). Further, this compound (109) was reacted with substituted aromatic amine (7a-l) to yield the final products *N'*-(4-(arylamino)-6-(thiazol-2-ylamino)-1,3,5-triazin-2-yl)isonicotinohydrazides (110a-l). The synthesized compounds were tested for their antibacterial activity against *S. aureus*, *S. pyogenes*, *E. coli* and *P. aeruginosa* using Ampicillin as standard drug. It was found that compounds having 2,6-dichloro, 4-fluoro, 4-nitro and 2-nitro substituents were more active against bacterial strains.

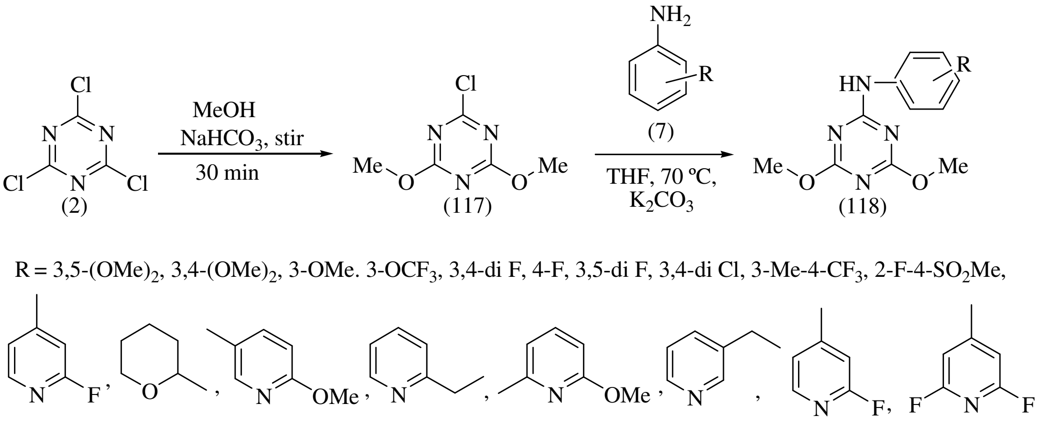
El-Faham *et al* (2016) in another report, mentioned the synthesis of triazine derivatives such as 2,4 ,6-*tris*(quinolin-8-yloxy)-1,3,5-triazine (112), $2,2^{'},2^{''}$ -[(1,3,5-triazine -2,4,6-triyl)*tris*(azanediyl)]*tris*(ethan-1-ol) (114) and N^2,N^4,N^6 -*tris*(pyridine-2-ylmethyl)-1,3,5-triazine-2,4,6-triamine (116) and also showed their inhibition of steel corrosion in HCl solution by electrochemical techniques as shown in this scheme 21. The three inhibitors (112), (114) and (116) were synthesized by reacting nucleophiles (111), (113) and (115) with triazine (2) at 80 ℃ for 24 h.



Scheme 21

It was observed that the corrosion protection by synthesized compounds was increased at higher concentration up to 98% at 250 ppm. The efficiency of tested intermediates was affected by the nature of side chain present in the triazine ring and it was concluded that the compound (114) exhibited least inhibition while (112) and (116) exhibited higher and almost the same inhibition.

Shinde and Salunke (2015) synthesized amine derivatives of triazine (118) as shown in scheme 22. Reaction began with addition of methanol to triazine (2) in the presence of $NaHCO_3$ to yield 2-chloro-4,6-dimethoxy-1,3,5-triazine (117) which on further reaction with substituted aniline (7) in the presence of tetrahydrofuran (THF) and K_2CO_3 afforded the final product 4,6-dimethoxy-*N*-phenyl-1,3,5-triazin-2-amine (118). The synthesized compounds were screened for their antibacterial activity by using agar cup plate method using Chloramphenicol and Griseofulvin as standards against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa*. Majority of the compounds were found to be effective antibacterial agents with zone of inhibition = 11 mm to 25 mm.

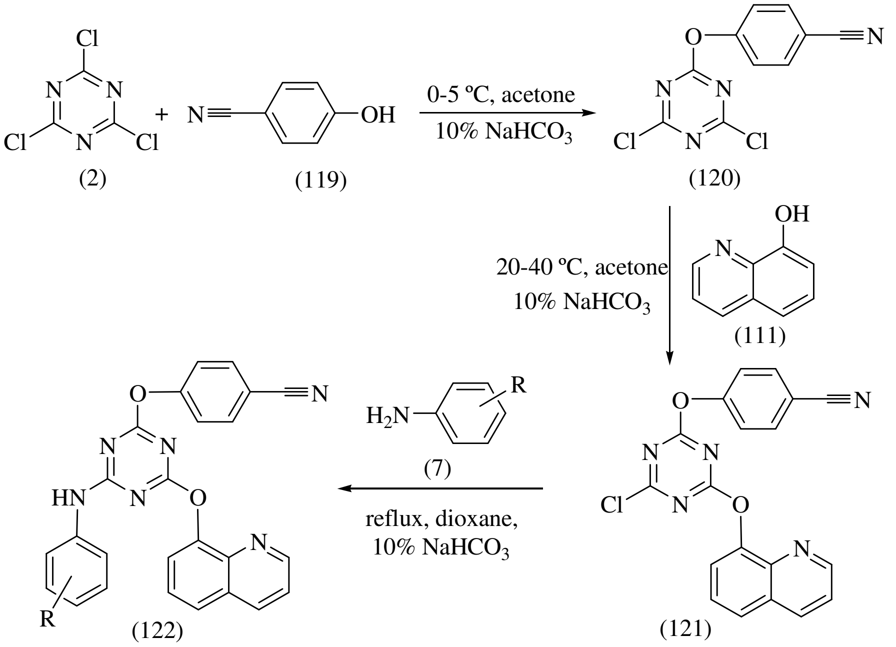


(2) (117) (7) (118)

R = 3,5-$(OMe)_2$, 3,4-$(OMe)_2$, 3-OMe. 3-OCF_3, 3,4-di F, 4-F, 3,5-di F, 3,4-di Cl, 3-Me-4-CF_3, 2-F-4-SO_2Me,

Scheme 22

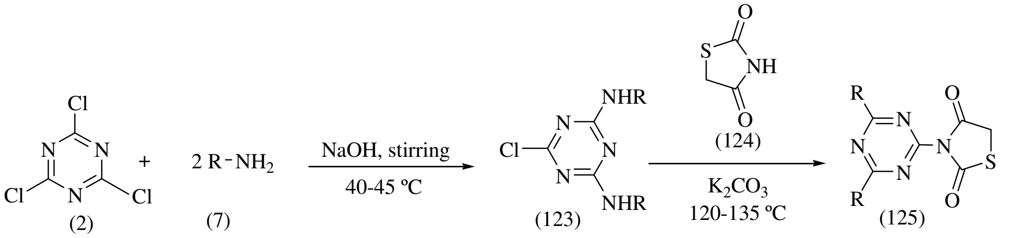
Mahyavanshi *et al* (2015) synthesized aromatic amine substituted derivatives of triazine (122) as shown in scheme 23. The scheme was initiated by reacting triazine (2) with 4-hydroxy benzonitrile (119) in the presence of $NaHCO_3$ base to afford 4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]benzonitrile (120) which on further condensation with 8-hydroxy quinolone (111) in acetone in the presence of $NaHCO_3$ selectively yielded 4-{[4-chloro-6-(quonoline-4-yl)-1,3,5-triazin-2-yl)oxy]benzonitrile (121). Further, refluxing of (121) with various substituted aromatic amines (7) in dioxane in the presence of $NaHCO_3$ furnished the target product 4-{[4-(arylamino)-6-(quinolin-8-yloxy)-1,3,5-triazin-2-yl]oxy}benzonitrile (122). The synthesized compounds were tested for their antibacterial activity against *Bacillus pumilus, B. cereus* and *E. coli* using Ciprofloxacin as reference drug. It was found that the resulted compounds showed average antibacterial activity.



R = 3-Cl, 4-Me, 3-Me, F, 4-Cl, 3-Br, 4-NO_2, 4-N$(Me)_2$, 4-OMe, 3-Cl-4-F, 4-NH(Me)

Scheme 23

Shrivastava *et al* (2015) reported the synthesis of thiazolidine derivatives of triazine (125) as shown in scheme 24. Triazine (2) was stirred with different substituted amines (7) in the presence of sodium hydroxide (NaOH) at 40-45 ºC to afford mono chloro di-substituted 1,3,5-triazine (123) which was further condensed with thiazolidin-2,4,5-dione (124) to furnish the final product 3-(4,6-dihydrazinyl-1,3,5-triazin-2-yl)thiazolidine-2,5-dione (125).



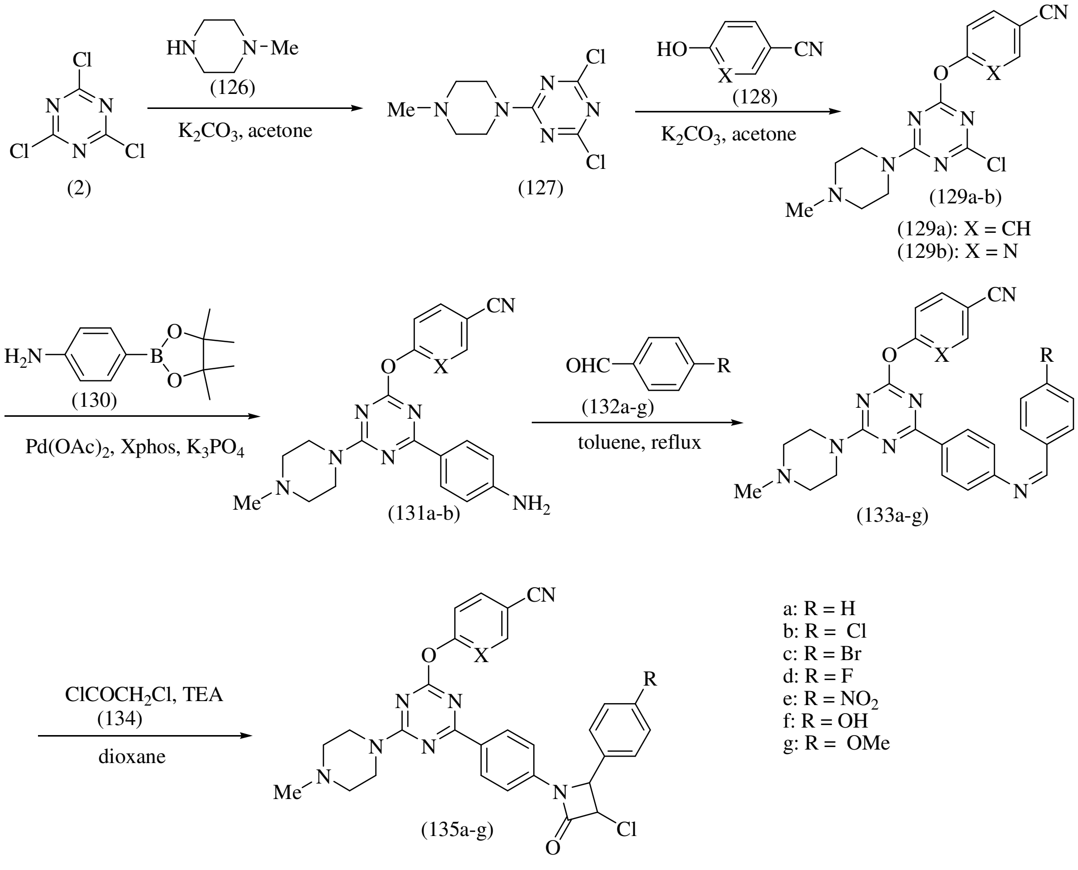
R = -$NHNH_2$, PhNH-, 2-$NH_2C_6H_4$NH-, 2-$NO_2C_6H_4$NH-, 3-$NO_2C_6H_4$NH-, 4-$NO_2C_6H_4$NH-, 2-ClC_6H_4NH-, 3-ClC_6H_4NH-, 4-ClC_6H_4NH-, 4-FC_6H_4NH-,4-BrC_6H_4NH-

Scheme 24

The synthesized compounds (125) were evaluated for their antibacterial efficiency against *B. subtilis, B. cereus*, *S. aureus, E. coli*, *P. vulgaris* and *P. aeruginosa* using Cefixime as reference drug. It was found that the compound with nitro group showed significant antibacterial activity against all tested organisms at 32.25 µg/ml except *P. vulgaris* and *P. aeruginosa.*

Patel *et al* (2015) synthesized triazine derivatives (135a-g) by sequence of reactions as shown in scheme 25. The synthetic scheme began with condensation of triazine (2) with *N*-methylpiperazine (126) in acetone in the presence of a K_2CO_3 to yield 2,4-dichloro-6-(4-

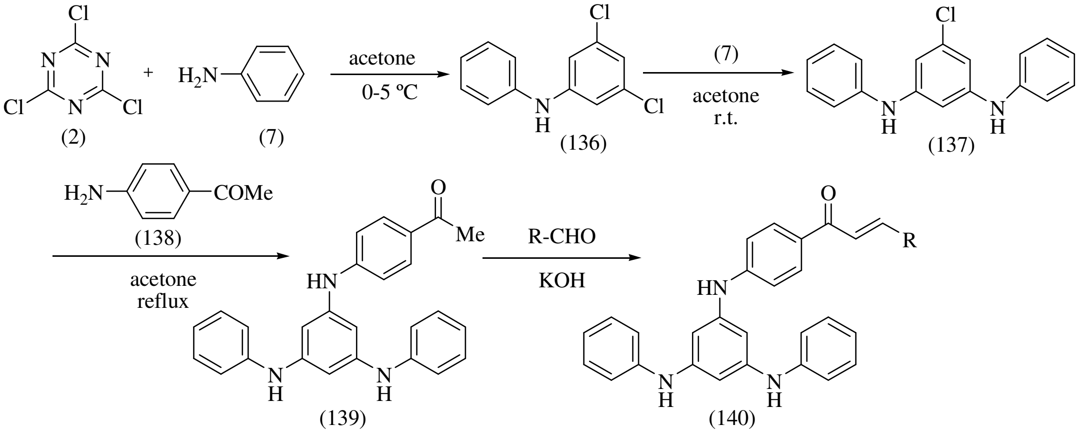
methylpiperazin-1-yl)-1,3,5-triazine (127) which on further reaction with 4-hydroxybenzonitrile (128) afforded 4-(4-chloro-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yloxy)benzonitrile (129a-b). The reaction of (129a-b) with 4-aminophenylboronic acid pinacol ester (130) in the presence of Pd(OAC)$_2$, ligand Xanthophonas (Xphos) and K_3PO_4 afforded (131a-b). The products 4-{[4-[4-aminophenyl]-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yloxy)benzonitrile (131a-b) so obtained were condensed with various aldehydes (132a-g) to yield Schiff bases (133a-g) which on further cyclization with chloroacetylcholine (134) in the presence of triethylamine (TEA) and dioxane afforded the target azetidin-2-one derivatives (135a-g). The synthesized compounds were screened for their antibacterial (*B. cereus* and *S. aureus*), antifungal and antimycobacterial activity against gram +ve bacteria. Amongst the synthesized compounds, (135c) and (135d) showed best activity against *B. cereus* (MIC = 6.25 µg/ml). On the other hand, analog (135b) showed good antifungal activity against *A. niger* (MIC = 6.25 µg/ml) using Ciprofloxacin as a standard.



Scheme 25

Shah *et al* (2014) synthesized a series of new chalcones containing core *s*-triazine derivatives of type (140) as shown in scheme 26. Triazine (2) was condensed with aniline (7) in acetone with continuous stirring at 0-5 ºC resulting in the formation of *N*-phenyl-6-dichloro 1,3,5-triazine-2-amine (136) which on further reaction with another aniline (7) yielded 2,6-

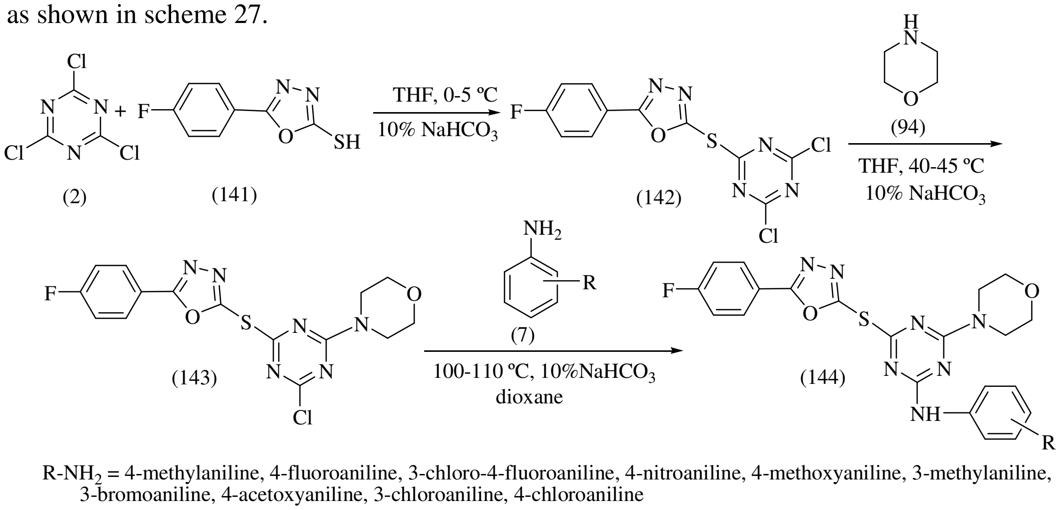
chloro-*N*,*N'*diphenyl-1,3,5-triazine-2,4-diamine (137). The compound (137) on further refluxing with 4-aminoacetophenone (138) in acetone furnished 1-{4-[(4,6-dianilino-1,3,5-triazin-2-yl)amino]phenyl}ethanone (139) which finally afforded 1-{4-[(4,6-dianilino-1,3,5-triazin-2-yl)amino]phenyl}-3-(5-methylfuran-2-yl)prop-2-en-1-one (140) on reaction with different substituted aldehydes in the presence of KOH and ethanol by employing ultrasonicator. Most of the synthesized compounds showed good antibacterial activity when tested against gram +ve (*B. subtilis, S. aureus*) and gram -ve (*E. coli, P. aeruginosa* and *Klebsiella aerogenes*) bacteria at 200 µg/ml. It was found that compound (140) showed excellent activity against all gram+ve and gram-ve bacteria in the range of inhibition at 5-18 mm except against *E. coli* as compared to Amoxycillin and Streptomycin as reference drugs.



R-CHO= 5-methylfurfural, 3-nitrobenzaldehyde, 2-chlorobenzaldehyde, 2-bromobenzaldehyde, 3-bromobenzaldehyde, 4-bromobenzaldehyde

Scheme 26

Rathavi *et al* (2014) reported the synthesis of oxadiazole derivatives of triazine (144) as shown in scheme 27.

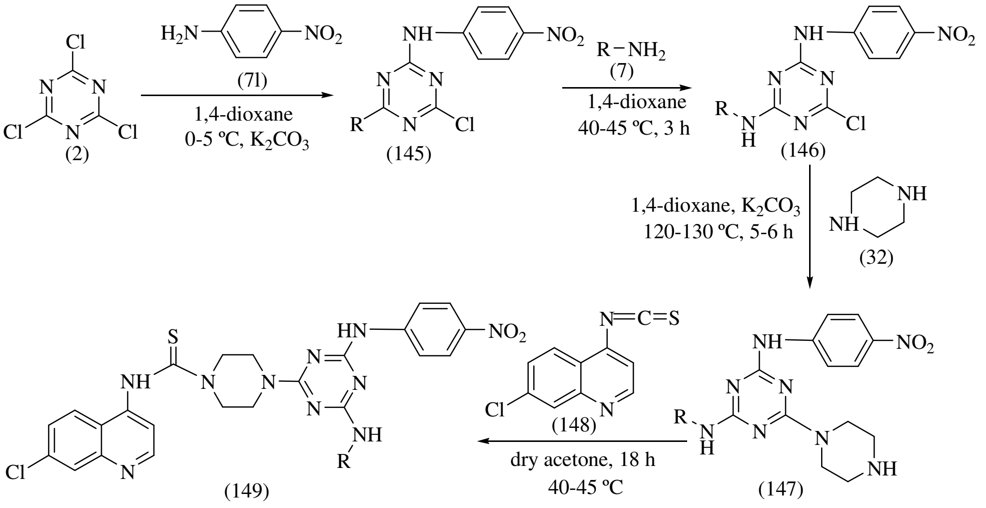


R-NH_2 = 4-methylaniline, 4-fluoroaniline, 3-chloro-4-fluoroaniline, 4-nitroaniline, 4-methoxyaniline, 3-methylaniline, 3-bromoaniline, 4-acetoxyaniline, 3-chloroaniline, 4-chloroaniline

Scheme 27

This multistep synthetic scheme was initiated by reacting triazine (2) with 5-(4-fluorophenyl)-1,3,4-oxadiazole-2-thiol (141) in the presence of THF and $NaHCO_3$ to yield 2-(4-flourophenyl-1,3,4-oxadiazolyl)-5-thio-4,6-dichloro-*s*-triazine (142) which on further treatment with morpholine (94) furnished 2-(4-flourophenyl-1,3,4-oxadiazolyl)-5-thio-4-(morpholino)-6-chloro-*s*-triazine (143). Finally, the resulted compound (143) was refluxed with aryl amine (7) in dioxane in the presence of $NaHCO_3$ to yield the target compound 2-(4-flourophenyl-1,3,4-oxadiazolyl)-5-thio-4-(morpholino)-6-(arylamino)-s-triazine (144). The evaluation of the synthesized compounds was carried out against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* using Ciprofloxacin, Ampicillin and Chloramphenicol as reference drugs. It was found that majority of the compounds showed good inhibition effect against all bacterial strains as compared to standard drugs.

Bhat *et al* (2012) synthesized quinoline derivatives of triazine (149) as shown in scheme 28.

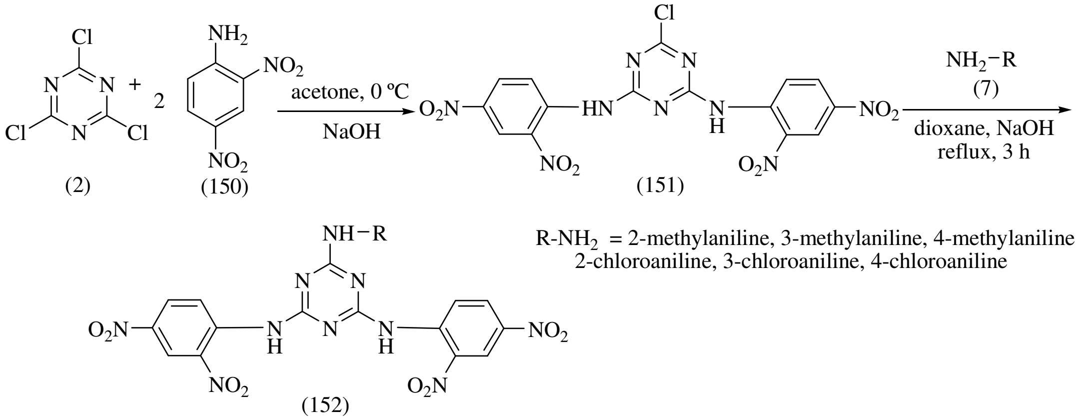


R = morpholine, 2-methylaniline, 1,3-diaminepropane, hydrazine hydrate, aniline, *p*-hydroxyaniline, thio-semicarbazide, semicarbazide, toulene

Scheme 28

The synthetic scheme begins with the condensation of triazine (2) with *p*-nitro aniline (71) resulted in formation of 4,6-dichloro-*N*-(4-nitrophenyl)-1,3,5-triazin-2-amine (145) which on reaction with substituted amines (71) yielded 4-chloro-6-morpholino-*N*-(4-nitrophenyl)-1,3,5-triazin-2-amine (146) followed by its reaction with piperazine (32), which furnished trisubstituted triazine derivative (147). The product (147) so obtained further reacted with 7-chloro-4-isothiocyanatoquinoline (148) and afforded final compound (149). The synthesized compounds were evaluated for their antibacterial activity against *B. subtilis, B. cereus, S. aureus, P. aeruginosa, E. coli, P. mirabilis* and *P. vulgaris* using Ofloxacin as reference compound. It was found that the synthesized compounds showed average to good activity against tested bacteria with MIC = 3.125 µg/ml to 25.0 µg/ml.

Indorkar *et al* (2013) carried out the synthesis of new amine derivatives of triazine as shown in scheme 29. Triazine (2) on reaction with 2 moles of 4-dinitroaniline (150) furnished 2-chloro 4',6-*bis*-(2,4 dinitro-aniline)-*s*-triazine (151) which on further reaction with different amines (7) afforded the target compounds 4',6-*bis*-(2,4-dinitro-aniline)-(2'-aryl-amine)-*s*-triazine (152). The resulted compounds (152) were found to be moderately effective when evaluated for their antibacterial activity against *E. coli, B. subtilis, S. aureus* and *Shigella.*



R-NH_2 = 2-methylaniline, 3-methylaniline, 4-methylaniline
2-chloroaniline, 3-chloroaniline, 4-chloroaniline

Scheme 29

Conclusion

From the above literature, it was concluded that triazine and its derivatives possess a broad range of biological properties but little information is available on their antifungal activity against *R. solani*, *H. oryzae*, and *A. brassicae,* therefore, it was required to be explored further. In the following chapters, procedure for the derivatization of triazine and their antifungal activity studies have been discussed.

CHAPTER-III

MATERIALS AND METHODS

3.1 Chemicals used

Chemicals used during experimental procedure, were obtained from following sources. All reagents used were of analytical grade and the solvents utilized were either 99% pure or dried/purified by established procedures.

Chemical	**Source**
Acetone	: S. D. Fine Chemicals Ltd., Mumbai
Acetic acid	: S. D. Fine Chemicals Ltd., Mumbai
4-Bromoaniline	: S. D. Fine Chemicals Ltd., Mumbai
2-Chloroaniline	: HiMedia Laboratories Pvt. Ltd., Mumbai
4-Chloroaniline	: HiMedia Laboratories Pvt. Ltd., Mumbai
Calcium carbonate	: S. D. Fine Chemicals Ltd., Mumbai
Dichloromethane	: S. D. Fine Chemicals Ltd., Mumbai
Diethyl ether	: Thermo Fischer Scientific Pvt. Ltd., Mumbai
2,4-Dinitroaniline	: S. D. Fine Chemicals Ltd., Mumbai
Dioxane	: S. D. Fine Chemicals Ltd., Mumbai
Ethanol	: S. D. Fine Chemicals Ltd., Mumbai
Ethyl acetate	: S. D. Fine Chemicals Ltd., Mumbai
n-Hexane	: S. D. Fine Chemicals Ltd., Mumbai
Methanol	: S. D. Fine Chemicals Ltd., Mumbai
2-Nitroaniline	: S. D. Fine Chemicals Ltd., Mumbai
4-Nitroaniline	: S. D. Fine Chemicals Ltd., Mumbai
Potassium carbonate	: SAM TECH Chem Industry, Vadodara
Potassium hydroxide	: SAM TECH Chem Industry, Vadodara
Sodium hydroxide	: SAM TECH Chem Industry, Vadodara
Silica Gel G	: Molychem, Mumbai
s-Triazine	: TCI Pvt. Ltd., Japan
1,3,5-Trichloroaniline	: S. D. Fine Chemicals Ltd., Mumbai

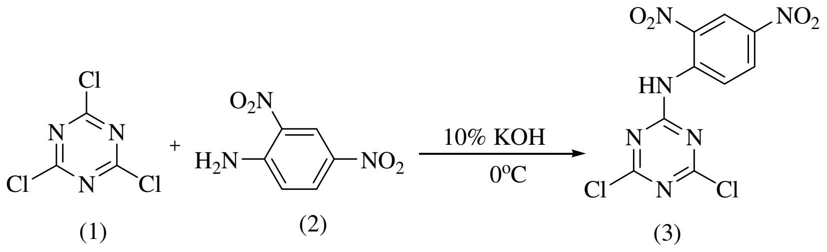
3.2 Instruments used

This chapter provides information regarding the various chemicals used and experimental procedures for synthesis of triazine derivatives and their characterization. Meltingspoints of various synthesized triazine derivatives were recorded by using digital melting point apparatus in an open capillary tube. A very small amount of samples were taken in capillary tubes with one closed end to obtain the melting points. Then, capillary tubes were placed in digital melting point apparatus to determine their melting points. The spectral studies *i.e.* ^{1}HNMR, D_2O exchange and ^{13}CNMR of synthesized triazine derivatives were recorded by using solvents DMSO-d_6 and internal standard trimethylsilane (TMS) on a BRUKER ADVANCE II 500 MHz NMR spectrometers at room temperature. The chemical shifts were denoted by δ and expressed in terms of ppm. IR spectral studies of the triazine derivatives were carried out on a Perkin Elmer Spectrum Two-IR Fourier-Transform spectrophotometer using KBr pellets ranging from 400-4000 cm^{-1}. The ^{1}HNMR, ^{13}CNMR and FT-IR were analyzed from Sophisticated Analytical Instrumentation Facility (SAIF), Panjab University, Chandigarh.

3.3 General procedure for the synthesis of triazine derivatives (3), (5a-c), (7a-b) and (8)

3.3.1 Synthesis of mono substituted triazine derivative (3)

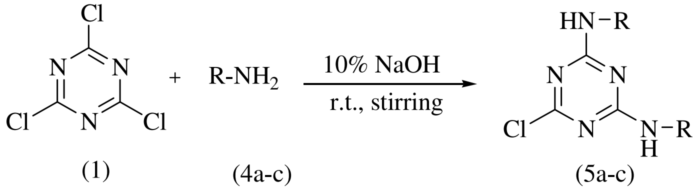
To the stirring solution of *s*-triazine (1) (0.1 mol) in dry acetone (6.0 ml), an aqueous solution of 10% KOH was added at 0-5 °C. Further, 2,4-dinitroaniline (2) (0.1 mol) dissolved in acetone (10.0 ml) was added dropwise to the above stirring solution and the mixture was continued to stir till the completion of the reaction (Scheme 1) (TLC). After completion of reaction, the reaction mixture was poured onto crushed ice and precipitates so formed were filtered, washed with water and diethyl ether to remove the unreacted amine left in the reaction. The recrystallization from acetone offered the pure product (3).



Scheme 1

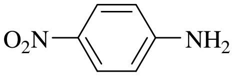
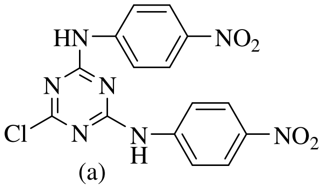
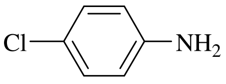
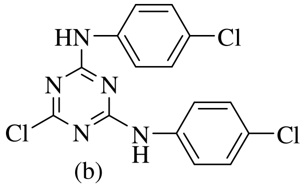
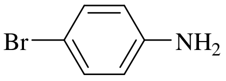
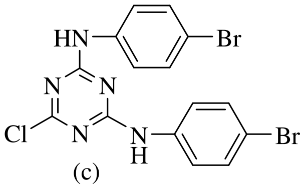
3.3.2 Synthesis of disubstituted triazine derivatives (5a-c)

A solution of *s*-triazine (1) (0.1 mol) dissolved in acetone (6.0 ml) was mixed with solution of aromatic amines (4a-c) (0.2 mol) in acetone (6.0 ml) with continuous stirring at room temperature. Further, a slow addition of 10% NaOH (4 drops) was made and reaction was continued to stir till the completion of reaction (Scheme 2) (TLC). After completion of reaction, the reaction mixture was poured onto crushed ice and solid so formed was filtered, dried and recrystallization from acetone furnished the pure products (5a-c). Table 3.1 depicts the amines and disubstituted products formed using this procedure.



Scheme 2

Table 3.1 Synthesized disubstituted amine derivatives of triazine (5a-c)

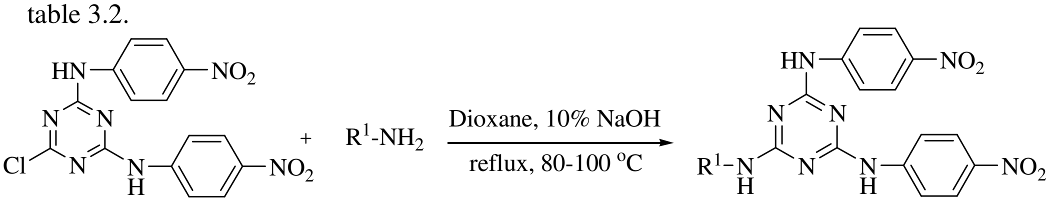
S. No	$R\text{-}NH_2$ (4a-c)	Product (5a-c)
1	 (a)	 (a)
2	 (b)	 (b)
3	 (c)	 (c)

3.3.3 Synthesis of trisubstituted triazine derivatives (7a-b) and (8)

Trisubstituted triazine derivatives were synthesized by two different ways (i) by reacting disubstituted triazine derivative with aromatic amines (ii) by direct reaction of triazine with aromatic amine under reflux conditions.

3.3.3.1 Synthesis of trisubstituted triazine derivatives (7a-b) from disubstituted triaizne derivatives (5a)

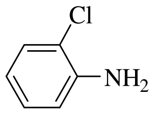
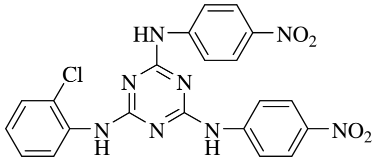
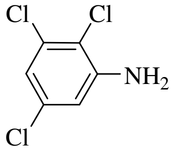
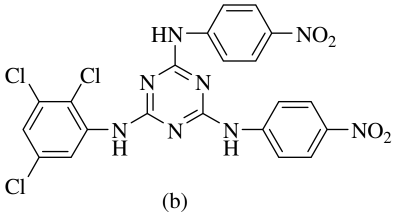
To a solution of disubstituted triazine derivative (5a) (0.1 mol) dissolved in dioxane (6.0 ml), solution of aromatic amines (6a-b) (0.1 mol) dissolved in dioxane (3.0 ml) was added. Dropwise addition of an aqueous solution of 10% NaOH (6.0 ml) was made within 2 h and refluxed at 80-100 °C (Scheme 3). On completion of reaction (TLC), mixture so obtained was poured onto crushed ice followed by neutralization with 10% NaOH (pH = 7). Precipitates so formed were filtered, dried and recrystallization from methanol offered the pure products (7a-b). Trisubstituted compounds synthesized using this procedure are listed in table 3.2.



(5a) (6a-b) (7a-b)

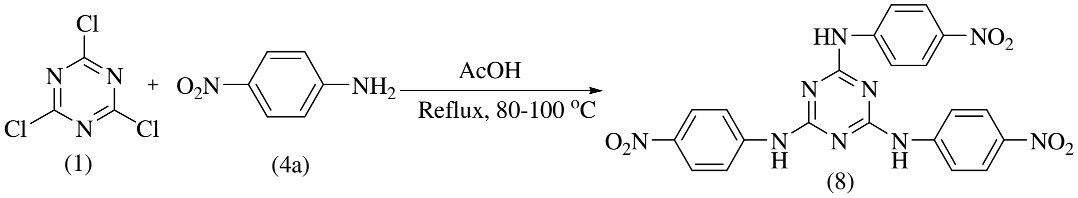
Scheme 3

Table 3.2 Synthesized trisubstituted amine derivatives of triazine (7a-b)

S. No.	R^1–NH_2 (6a-b)	Product (7a-b)
1	 (a)	 (a)
2	 (b)	 (b)

3.3.3.2 Synthesis of trisubstituted triazine derivatives (8) from triazine (1)

A solution of *s*-triazine (1) (0.1 mol) and 4-nitroaniline (4a) (0.3 mol) in acetic acid (20.0 ml) was refluxed at 80-100 °C. After completion of reaction (TLC), the reaction mixture was poured onto crushed ice followed by neutralization with 10% NaOH (pH = 7) (Scheme 4). The precipitates so formed were filtered and washed with water to remove the unreacted reactant from the crude mixture obtained. Recrystallization from acetone and methanol offered the pure product (8).



Scheme 4

3.4 Antifungal activity of triazine and its derivatives against *Rhizoctonia solani, Helminthosporium oryzae* and *Alternaria brassicae*

All the synthesized compounds were evaluated *in vitro* against different fungal pathogens *viz. Helminthosporium oryzae, Rhizoctonia solani* and *Alternaria brassicae* employing poison food technique (Grover and Moore, 1962). Stock solutions of the synthesized compounds (a.i. basis) were prepared in absolute alcohol in 250 ml flask containing potato dextrose agar medium. Final concentrations 5, 10, 50, 100, 250 and 500 µg/ml were prepared from the stock solution. The compounds amended medium was poured in sterilized Petri plates of 90 mm size. After solidification of medium, 5 mm mycelial disc were cut with the help of sterilized cork borer from 7 days old culture of test pathogens inoculated and placed in the centre of each Petri plate in the inverted position. The Petri plates containing unamended medium, with test pathogens served as control. The fungicides, Propiconazole (Tilt 25 EC) was taken as standard check against *R.* s*olani and H. oryzae*, whereas, Mencozeb 75 WP was used as standard against *A. brassicae*. Each treatment was replicated thrice, the inoculated Petri plate were sealed with paraffin' film to minimize the chances of contamination and incubated at 25±2 °C. The colony diameter was measured from two perpendicular sides and the average values were calculated after 5-7 days of incubation. The efficacy of synthesized compounds was expressed as percent inhibition in mycelial growth over control, calculated by using the following formula (Singh and Tripathi 1999):

$$\mathrm{Pi} = \frac{\mathrm{C-T}}{\mathrm{C}} \times 100$$

Whereas Pi = Percent inhibition in colony growth

C = Colony growth in control (mm)

T = Treatment growth in test concentration (mm)

3.5 Statistical Analysis

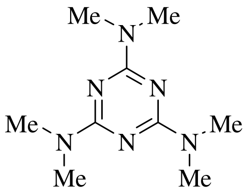
The effect of factors, synthesized 'compounds' and 'concentration' and their interaction on Percent disease inhibition were analysed by using CPCS1 software. The ED_{50} value of each compound was also calculated by plotting percent inhibition in colony growth against each concentration of the synthesized compound.

CHAPTER-IV

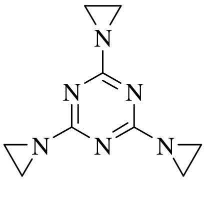
RESULTS AND DISCUSSION

Owing to wide range of biological properties, nitrogen containing heterocyclic compounds have gained immense importance among chemists for designing new pharmaceutical drugs with improved drug resistance profile (Mewada *et al* 2014, Jain *et al* 2019a, Jain *et al* 2019b). 1,3,5-Triazine, a six membered, heterocyclic ring is one of the oldest organic compound available with vast biological activities (Patel *et al* 2007, Kaur *et al* 2018, Kaur *et al* 2019). *s*-Triazine derivatives represent an important class of compounds due to their broad spectrum of activities not only in life sciences but also in analytical chemistry, medicinal chemistry and as a herbicide or pesticide components in agricultural field (Zeng *et al* 2016). Triazine, a privileged scaffold forms a basis for the discovery of large number of biologically active drugs due to their symmetrical shape, high therapeutic value and unique binding affinity to enzymes; it serves as potential nucleus in many bioactive compounds (Agarwal *et al* 2005, Srinivas *et al* 2009).

Altretamine, a 1,3,5-triazine containing drug also known as hexalen, act as a antineoplastic agent. Hexalen was first approved by United State of Food and Drug Administration (US-FDA) in 1990 (Moreno *et al* 2018). Altrertamine, a less toxic drug is used in treatment of refractory ovarian cancer against various cancer cell lines (Shah *et al* 2014). Another *s*-triazine based drug, triethylamine deals with retinoblastoma treatment and generally used in chemotherapy as anticancer agent. But due to its instability it is no longer in use (Rakshit 1913, Malik 2001).

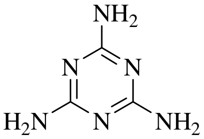


Altretamine

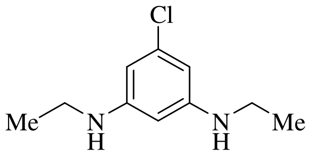


Triethylenemelamine (TEM)

Melamine is an organic base and a trimer of cyanamide, with a 1,3,5-triazine skeleton (Stewart *et al* 2004). Melamine reacts with formaldehyde to form melamine formaldehyde resins which have wide range of applications such as glues, adhesives, molding compounds, decorative purposes, leather tanning agents, concrete additives, wood preservatives, ion exchange resins and strengtheners for building material and so forth (Baliani *et al* 2009). Besides from this, melamine is one of the major component of pigment yellow 150 which is used as a colorant in inks, paper and plastics (Klenke *et al* 2001, Guan *et al* 2007). Simazine is a selective triazine herbicide available in wettable powder, water dispersible granule, liquid and granular formations and is used to control broad-leaves weeds and annual grasses in fields, fruits, vegetables, orchids, vineyards and ornamental crops (Desai *et al* 2013).

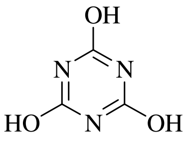


Melamine

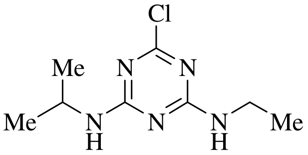


Simazine

The most commonly used triazine based drug, cyanuric acid contains three hydroxyl groups on the place of three chlorine atoms present in cyanuric chloride. It occurs naturally in soil humus. It acts as disinfectant agent in water and as stabilizer to minimize the consumption of chlorine in swimming pool (Chien *et al* 2016). In additions, cyanuric acid is commercially used in household bleech, dishwasher detergents, industrial cleaners and sanitizers (Bernhard *et al* 2012). Atrazine, a disubstituted triazine derivative is widely used herbicide to control soil erosion (Waghmare and Tayade 2016). It is used to control pre- and post-emergence broad leaf and grassy weeds in many crops. Although it has been banned in the European countries but still it is one of the most widely used herbicide in the world (Menicagli *et al* 2004).

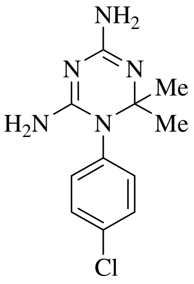


Cynauric acid

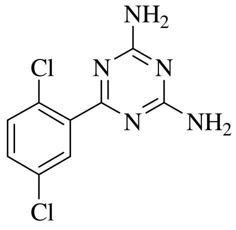


Atrazine

Dihydrofolate reductase inhibitor, known as cycloguanil act as antimalarial drug (Shrivastava *et al* 2015, Solankee and Tailor 2016). Cycloguanil is a commercially available drug, extensively used drug alone or in combination with other antimalarial agents for the treatment of malaria in human beings (Xiong *et al* 2008).



Cycloguanil

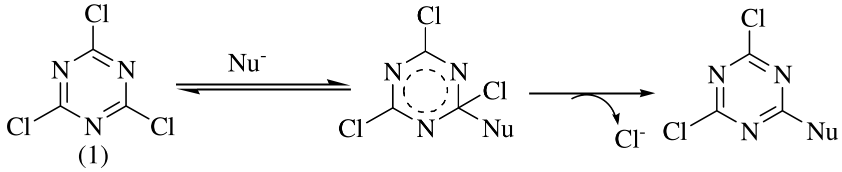


Irsogladine

Irsogladine is a mucosal protective drug which is used in the treatment of acute gastritis and peptic ulcer disease (Zhang *et al* 2018). It also acts as active antibacterial and anticancer therapeutic agent. Beside from this, it is also found to suppress tumor genesis in mice (Junaid *et al* 2019). Triazine serves as an ubiquitous constituent in many natural products, peptides, herbicides, pesticides, fungicides and pharmacologically active drugs (Kumar *et al* 2018, Steffensen *et al* 2006).

The most important attribute of cyanuric chloride is its ability to undergo nucleophilic substitution reactions (Schelz *et al* 2006). It acts as an acid chloride in many organic transformations including halogenation, dehydration and peptide coupling reactions (Lowe *et al* 2001). A large number of synthetic routes have been employed to carry out the mono-, di- and tri-substituted 1,3,5-triazine including conventional and modern methods like ultrasonication (Mewada *et al* 2016), microwave irradiation (Patel *et al* 2010), solid phase technique (Rajanarendar *et al* 2011) and solvent free synthesis using phase transfer catalyst (Shah and Deliwala 1968). Likewise, various types of alkaline medium used for substitution involves sodium hydroxide (NaOH) (Maarouf *et al* 2012), potassium hydroxide (KOH) (Sareeen *et al* 2006), potassium carbonate (K_2CO_3) (Gavade *et al* 2012), potassium hydride (KH) (Solankee *et al* 2010), sodium hydride (NaH) (Ashour *et al* 2013), sodium carbonate (Na_2CO_3) (Koc *et al* 2013) and sodium bicarbonate ($NaHCO_3$) (Al Rasheed *et al* 2018). The reactivity of cyanuric chloride for replacement of chlorine atoms with various nucleophiles varies with suitable temperature conditions (Saleh *et al* 2010). Generally, reactivity depends upon the type of nucleophile used in reaction under temperature controlled conditions. The formation of mono-substituted derivatives takes place at extremely low temperature *i.e.* 0 ^{o}C, di-substitution reaction takes place at room temperature *i.e.* 25-28 ^{o}C, and tri-substituted product at high temperature *i.e.* 80-90 ^{o}C conditions (Solankee and Tailor 2016, Patel and Chikhalia 2003). This attributes to substitution of three different types of nucleophiles onto the same molecule which leads to a series of triazine derivatives (Singh *et al* 2012). In the view of active participation of *s*- triazine and its derivatives in various biological activities, we have planned to synthesize mono-, di- and tri-substituted triazine derivatives and evaluated their antifungal activity against *Rhizoctonia solani, Helminthosporium oryzae* and *Alternaria brassicae*.

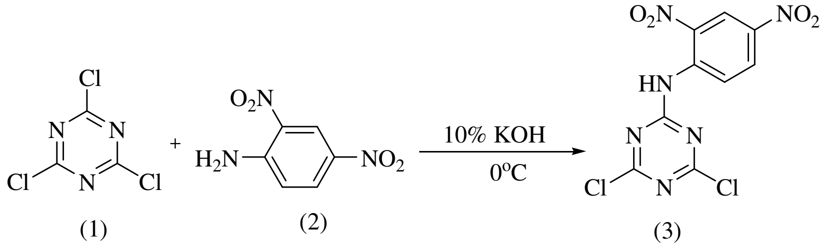
Novel series of compounds have been synthesized by following nucleophilic aromatic substitution mechanism (S_NAr) in which 2,4,6-trisubstituted-1,3,5-triazine was reacted with various nucleophilic reagents like primary amines (Scheme 1). The mechanism involves addition of nucleophile followed by elimination of leaving group under suitable conditions to afford mono, di and tri substituted triazine derivatives.



Scheme 1: Nucleophilic aromatic substitution mechanism (S_NAR) for the synthesis of triazine derivatives

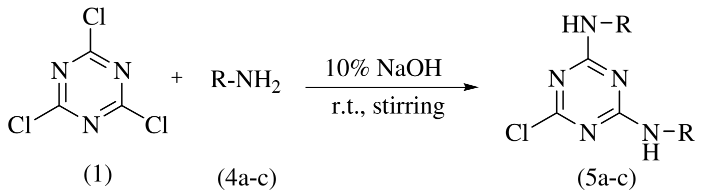
4.1 Synthesis of mono-, di- and tri-substituted triazine derivatives (3), (5a-c), (7a-b) and (8)

The mono-substituted triazine derivatives were synthesized by reaction of *s*-triazine (1) with aromatic amine (2) in the presence of base KOH at 0 °C to afford the corresponding mono-substituted derivative of triazine (3) as shown in scheme 2. All the synthesized derivatives were obtained in good to average yield and their identity was confirmed by various spectral techniques.



Scheme 2: Synthesis of mono-substituted triazine derivative (3)

The di-substituted triazine derivatives (5a-c) were obtained by stirring solution of triazine (1) in acetone with different aromatic amines (4a-c) in the presence of base NaOH at room temperature as shown in scheme 3. Considerable variations in nature of substituent (R) was introduced so as to understand the scope and limitation of reaction.

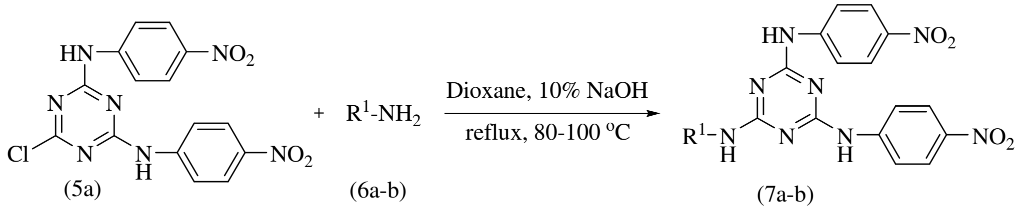


(4a): $R\text{-}NH_2$ = 4-nitroaniline
(4b): $R\text{-}NH_2$ = 4-chloroaniline
(4c): $R\text{-}NH_2$ = 4-bromoaniline

Scheme 3: Synthesis of di-substituted triazine derivative (5a-c)

Further, trisubstituted derivatives of triazine were obtained by reacting disubstituted derivative with amine and direct reaction of triazine with amines under reflux conditions.

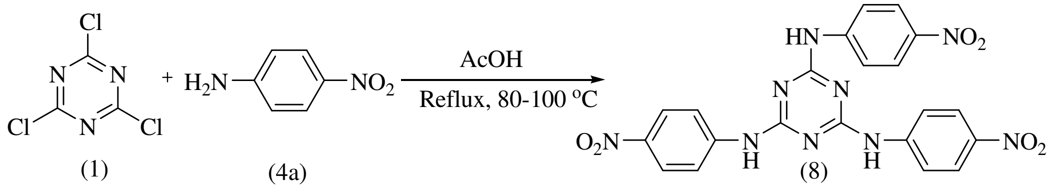
In the first approach, di-substituted triazine derivative (5a) was refluxed with different aromatic amines (6a-b) by using base NaOH and solvent dioxane at 80-100 °C to furnish the target tri-substituted triazine derivatives (7a-b) as outlined in scheme 4. To understand the scope and limitation of reaction considerable variation in electron withdrawing groups (mono and trisubstituted) were introduced and synthesized compounds were isolated in good synthetic yield.



(6a): R^1-NH_2 = 2-chloroaniline
(6b): R^1-NH_2 = 2,3,5-trichloroaniline

Scheme 4: Synthesis of tri-substituted triazine derivatives (7a-b)

In second approach for the synthesis of trisubstituted triazine derivatives, acetic acid was added to a solution of *s*-triazine (1) and 4-nitroaniline (4a) and refluxed at 80-100 °C for 4 h (Scheme 5). After completion of reaction (TLC), reaction mixture was poured onto crushed ice followed by neutralization with 10% NaOH (pH = 7). Precipitates so formed were filtered and washed with water to remove excess of unreacted amine left in the reaction. Recrystallization of crude solid from acetone offered the pure product (8).



Scheme 5: Synthesis of tri-substituted triazine derivative (8)

4.2 General discussion

All the synthesized triazine derivatives were found to be stable in air and highly soluble in acetone, ethanol, methanol and dimethylsulfoxide.

4.2.1 Physical characteristics

Compounds were observed visually for their physical properties. Table 4.1 depicts color, yield and melting point of the synthesized compounds (3, 5a-5c, 7a-7b and 8)

Table 4.1 Physical parameters of synthesized triazine derivatives (3, 5a-c, 7a-b and 8)

Compound No.	Molecular Formula	Color	Yield (%)	M.pt. (°C)
3	$C_9H_4Cl_2N_6O_4$	White	80	280-282
5a	$C_{15}H_{10}ClN_7O_4$	Grey	90	297-300
5b	$C_{15}H_{10}Cl_3N_5$	Off-white	85	185-188
5c	$C_{15}H_{10}Br_2ClN_5$	White	85	276-278
7a	$C_{21}H_{15}ClN_8O_4$	White	83	280-284
7b	$C_{21}H_{13}Cl_3N_8O_4$	White	80	288-290
8	$C_{21}H_{15}N_9O_6$	Yellow	88	270-271

Triazine (1) (0.01 mol) dissolved in acetone was taken in 250 ml round bottom flask, followed by addition of an aqueous solution of KOH (10%) at 0 °C. Further, aromatic amine *i.e.* 2,4-dinitroaniline (0.01 mol) dissolved in acetone (10.0 ml) was added dropwise to the above stirring solution. After completion of reaction, the reaction mixture was poured on to crushed ice and the precipitates so formed were filtered. The crude solid was washed with diethyl ether to remove the unreacted amine present in reaction and recrystallization from acetone offered the pure product. ^{1}HNMR (DMSO-d_6, 500 MHz) spectrum of this product showed multiplet signal at δ 8.61-8.64 ppm corresponding to one aromatic proton. Two doublets were observed at δ 8.05 and 8.82 ppm due to two aromatic protons with coupling constants J = 9.16 Hz and J = 8.0 Hz respectively. The unique feature of this spectrum include deuterium exchangeable singlet at δ 11.40 ppm due to –NH proton. The peaks in its ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum appeared at δ 119.74, 123.38, 128.63, 129.30, 135.07, 149.79 and 149.89 ppm which corroborated well with its ^{1}HNMR spectral assignment. IR spectrum of this compound exhibited bands at υ_{max}: 800 (C-Cl str), 1259 (C-N str), 1331 (N-O str), 1405 (C=C str), 1585 (N=O str), 1699 (C=N str) and 3076 (=C-H str) cm^{-1}. The spectral analysis confirmed the structure, 4,6-dichloro-*N*-(2,4-dinitrophenyl)-1,3,5-triazin-2-amine (3), assigned to this product.

For the synthesis of disubstituted triazine derivatives, slow addition of 4-nitroaniline (0.2 mol) was made to the stirring solution of triazine (1) (0.1 mol) in acetone. Further, 10% NaOH was added to the above solution and after completion of reaction, reaction mixture was poured onto crushed ice. The solid so obtained was immediately filtered, washed with water and recrystallization from acetone offered the pure product. In its ^{1}HNMR (DMSO-d_6, 500 MHz) spectrum, multiplet signals appeared at δ 7.89-7.91 and 8.30-8.32 ppm corresponding to eight aromatic protons. The main feature of this spectrum is deuterium exchangeable singlet at δ 11.68 ppm due to two –NH protons. In its ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum peaks appeared at δ 119.63, 122.74, 124.30, 124.30, 131.46, 137.46, 141.09 and 163.63 ppm. IR spectrum of this compound exhibited band at υ_{max}: 794 (C-Cl str), 1228 (C-N str), 1381 (N-O str), 1425 (C=C str), 1574 (N=O str), 1616 (C=N str) and 3348 (N-H str) cm^{-1}. The spectral analysis confirmed the structure 6-chloro-N^2,N^4-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a), assigned to this product.

Likewise, reaction of 4-chloroaniline (0.2 mol) with solution of triazine (1) (0.1 mol) in acetone afforded off-white solid. The ^{1}HNMR (DMSO-d_6, 500 MHz) spectrum of this product exhibited multiplet signals at δ 7.46-7.48 and 7.62-7.65 ppm corresponding to eight aromatic protons. The characteristic feature of this spectrum included deuterium exchangeable singlet at δ 11.26 ppm due to two –NH protons. In its ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum peaks appeared at δ 122.65, 122.74, 122.84, 127.98, 128.66, 128.73, 129.78, 130.04, 135.75, 135.86, 136.09, 149.81, 153.94, 163.55 and 168.71 ppm. IR spectrum of this off-white solid exhibited bands at υ_{max}: 790 (C-Cl str), 1220 (C-N str), 1375 (N-O str), 1417 (C=C str), 1536 (N=O str) and 3394 (N-H str) cm^{-1}. The spectral analysis confirmed the structure 6-chloro-N^2,N^4-*bis*(4-chlorophenyl)-1,3,5-triazin-2,4-diamine (5b), assigned to this product.

Also, 4-bromoaniline (0.2 mol) was reacted with triazine (1) (0.1 mol) to carry out variation in the R-group. Recrystallization from acetone furnished the pure product. The ^{1}HNMR (DMSO-d_6, 500 MHz) spectrum of this prodcut exhibited multiplet signal at δ 7.49-7.63 ppm corresponding to four aromatic protons. A singlet was observed at δ 7.74 ppm due to four aromatic protons. The main feature of this spectrum is deuterium exchangeable singlet at δ 9.88 ppm due to two –NH protons. In its ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum peaks appeared at 114.42, 115.91, 122.55, 122.78, 131.11, 131.53, 131.73, 136.61, 138.41, 153.98 and 162.14 ppm. IR specral bands for this compound appeared at υ_{max}: 501 (C-Br str), 789 (C-Cl str), 1228 (C-N str), 1373 (N-O str), 1441 (C=C str), 1587 (N=O str), 1689 (C=N str), 3039 (=C-H str) and 3248 (N-H str) cm^{-1}. The spectral analysis confirmed the structure 6-chloro-N^2,N^4-*bis*(4-bromophenyl)-1,3,5-triazin-2,4-diamine (5c), assigned to this product.

For the synthesis of tri-substituted triazine derivative, di-substituted triazine derivative *i.e.* 6-chloro-N^2,N^4-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a) (0.1 mol) was dissolved in dioxane in round bottom flask. Further aromatic amine *i.e.* 2-chloroaniline (0.1 mol) dissolved in dioxane was added to the above refluxing solution at 80-100 °C followed by dropwise addition of base 10% NaOH within 2 h. After completion of reaction (TLC), neutralization followed by extractive workup and recrystallization from methanol offered the pure product. The ^{1}HNMR (DMSO-d_6, 500 MHz) spectrum of the compound showed multiplet signal at δ 7.39-7.85 ppm corresponding to four aromatic protons. A doublet with coupling constant J = 8.5 Hz was observed at δ 7.97 ppm due to four aromatic protons. Antother doublet signal appeared at δ 8.28 ppm (J = 9.0 Hz) corresponding to four aromatic protons. The characteristic feature of the spectrum consisted D_2O exchangeable broad signal at δ 10.35 and 10.80 due to three –NH protons. In its ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum, the signals appeared at δ 119.77, 122.96, 123.14, 124.25, 124.93, 125.02, 128.05, 133.75, 135.04, 139.10, 142.44, 144.04, 145.07, 149.70, 154.04, 161.15, 163.96, 165.04, 169.05, 180.17 and 182.79 ppm. IR spectrum of this compound exhibited bands at υ_{max}: 749 (C-Cl str), 1255 (C-N str), 1314 (N-O str), 1417 (C=C str), 1556 (N=O str), 1605 (C=N str), 3125 (=C-H str) and 3383 (N-H str) cm^{-1}. The spectral analysis confirmed the structure N^2-(2-chlorophenyl)-N^4,N^6-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4,6-triamine (7a), assigned to this product.

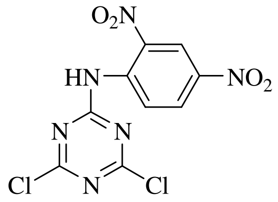
Further, 2,3,5-trichloroaniline (0.1 mol) was reacted with 6-chloro-N^2,N^4-*bis*(4-

nitrophenyl)-1,3,5-triazin-2,4-diamine (5a) (0.1 mol) to afford tri-substituted triazine derivatives. Recrystallization from methanol offered the pure product. In ^{1}HNMR (DMSO-d_6, 500 MHz) spectrum of this product showed singlet at δ 10.49 due to two aromatic protons. Two doublets were observed at δ 7.97 ppm and 8.20 ppm with coupling constant J = 9.0 Hz and J = 9.0 Hz respectively, corresponding to eight aromatic protons. The characteristic feature of this spectrum included deuterium exchangeable singlet at δ 13.02 ppm due to three –NH protons. The peaks in its ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum appeared at δ: 115.24, 116.19, 116.44, 119.75, 123.37, 128.64, 129.27, 129.76, 129.90, 135.07, 145.07 and 149.80 ppm. The correct spectral analysis confirmed the structure N^2, N^4-*bis*(4-nitrophenyl)-N^6-(2,4,6-trichlorophenyl)-1,3,5-triazin-2,4,6-triamine (7b), assigned to this product.

Also, refluxing of triazine (1) (0.1 mol) with 4-nitroaniline (0.3 mol) in the presence of acetic acid at 80-100 ^{o}C afforded the tri-substituted triazine derivative. After completion, neutralization followed by extractive work up and recrystallization afforded yellow solid. The ^{1}HNMR (DMSO-d_6, 500 MHz) spectrum of this yellow solid exhibited a singlet signal at δ 7.96 ppm due to six aromatic protons. A doublet signal was observed at δ 8.26 ppm with coupling constant J = 9.5 Hz due to six aromatic protons. A broad signal appeared at δ 10.97 ppm corresponding to three –NH protons. The peaks in its ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum appeared at δ 119, 120.05, 124.69, 142.24, 144.63, 163.76 and 168.60 ppm which corroborated well with its ^{1}HNMR spectral assignment. IR spectral bands for this compound appeared at υ_{max}: 794 (C-Cl str), 1239 (C-N str), 1317 (N-O str), 1416 (C=C str), 1551 (N=O str), 1617 (C=N str), 3076 (=C-H str) and 3346 (N-H str) cm^{-1}. The spectral analysis confirmed the structure, N^2, N^4, N^6-*tris*(4-nitrophenyl)-1,3,5-triazin-2,4,6-triamine (8), assigned to this product, obtained in 88 % yield.

4.3 Analytical data of substituted triazine derivatives

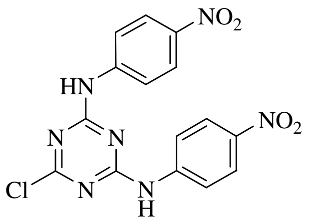
4,6-Dichloro-*N*-(2,4-dinitrophenyl)-1,3,5-triazin-2-amine (3)



White solid, R$_f$: 0.65 (hexane:ethylacetate/70:30), Yield: 80%, acetone, m.p. 280-282 ^{o}C. IR (KBr) υ_{max}: 800 (C-Cl str), 1259 (C-N str), 1331 (N-O str), 1405 (C=C str), 1585 (N=O str), 1699 (C=N str) cm^{-1} and 3076 (=C-H str) cm^{-1}. ^{1}HNMR (DMSO-d_6, 500 MHz) δ: 8.05 (d, J = 9.16 Hz, 1H, Ar-H), 8.64 (m, 1H, Ar-H), 8.82 (d, J = 8.0 Hz, 1H, Ar-H) and 11.40 (s, 1H, D$_2$O exchangeable –NH) ppm. ^{13}CNMR (DMSO-d_6, 125 MHz) δ: 119.74, 123.38, 128.63, 129.30, 135.07, 149.79 and 149.89 ppm (Refer annexure I).

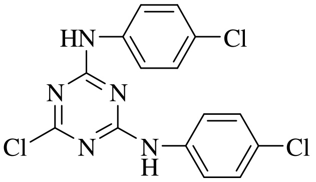
6-Chloro-N^2,N^4-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a)

Grey solid, R$_f$: 0.7 (hexane:ethylacetate/80:20), Yield: 90%, acetone, m.p. 297-300 ^{o}C. IR (KBr) υ_{max}: 794 (C-Cl str), 1228 (C-N str), 1381 (N-O str), 1425 (C=C str), 1574 (N=O str), 1616 (C=N str) and 3348 (N-H str) cm^{-1}. ^{1}HNMR (DMSO-d_6, 500 MHz) δ: 7.89-7.91 (m, 4H, Ar-H), 8.30-8.32 (m, 4H, Ar-H), and 11.68 (s, 2H, D_2O exchangeable -NH) ppm. ^{13}CNMR (DMSO-d_6, 125 MHz) δ: 119.63, 122.74, 124.30, 131.10, 137.46, 141.09 and 163.63 ppm (Refer annexure I).



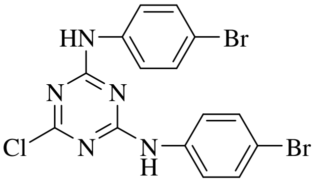
6-Chloro-*N*^{2},*N*^{4}-bis(4-chlorophenyl)-1,3,5-triazin-2,4-diamine (5b)

Off-white crystalline solid, R_f: 0.65 (hexane:ethylacetate/80:20), Yield: 85%, acetone, m.p. 185-188 °C. IR (KBr) υ_{max}: 790 (C-Cl str), 1220 (C-N str), 1375 (N-O str), 1417 (C=C str), 1536 (N=O str) and 3394 (N-H str) cm^{-1}. ^{1}HNMR (DMSO-d_6, 500 MHz) δ: 7.46-7.48 (m, 4H, Ar-H), 7.62-7.65 (m, 4H, Ar-H) and 11.26 (s, 2H, D_2O exchangeable –NH) ppm. ^{13}CNMR (DMSO-d_6, 125 MHz) δ: 122.65, 122.74, 122.84, 127.98, 128.66, 128.73, 129.78, 130.04, 135.75, 135.86, 136.09, 149.81, 153.94, 163.55 and 168.71 ppm (Refer annexure I).



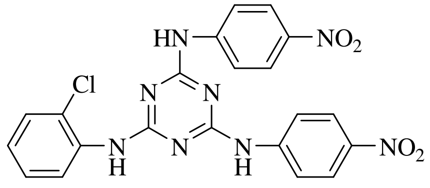
6-Chloro-*N*^{2},*N*^{4}-*bis*(4-bromophenyl)-1,3,5-triazin-2,4-diamine (5c)

White solid, R_f: 0.65 (hexane:ethylacetate/80:20), Yield: 85%, acetone, m.p. 276-278 °C. IR (KBr) υ_{max}: 501 (C-Br str), 789 (C-Cl str), 1228 (C-N str), 1373 (N-O str), 1441 (C=C str), 1587 (N=O str), 1689 (C=N str), 3039 (=C-H str) and 3248 (N-H str) cm^{-1}. ^{1}HNMR (DMSO-d_6, 500 MHz) δ: 7.49-7.63 (m, 4H, Ar-H), 7.74 (s, 4H, Ar-H) and 9.88 (s, 2H, D_2O exchangeable –NH) ppm. ^{13}CNMR (DMSO-d_6, 125 MHz) δ: 114.42, 115.91, 122.55, 122.78, 131.11, 131.53, 131.73, 136.61, 138.41, 153.98 and 162.14 ppm (Refer annexure I).



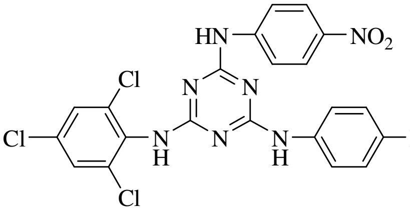
***N*^{2}-(2-Chlorophenyl)-*N*^{4},*N*^{6}-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4,6-triamine (7a)**

White solid, R_f: 0.72 (hexane:ethylacetate/80:20), Yield: 83%, methanol, m.p. 280-284 °C. IR (KBr) υ_{max}: 749 (C-Cl str), 1255 (C-N str), 1314 (N-O str), 1417 (C=C str), 1556 (N=O str), 1605 (C=N str), 3125 (=C-H str) and 3383 (N-H str) cm^{-1}. ^{1}HNMR (DMSO-d_6, 500 MHz) δ: 7.39-7.85 (m, 4H, Ar-H), 7.97 (d, J = 8.5 Hz, 4H, Ar-H), 8.28 (d, J = 9.0 Hz, 4H, Ar-H), 10.35 (bs, 2H, D_2O exchangeable –NH) and 10.80 (bs, 1H, D_2O exchangeable –NH) ppm. ^{13}CNMR (DMSO-d_6, 125 MHz) δ: 119.77, 122.96, 123.14, 124.25, 124.93, 125.02, 128.05, 133.75, 135.04, 139.10, 142.44, 144.04, 145.07, 149.70, 154.04, 161.15, 163.96, 165.04, 169.05, 180.17 and 182.79 ppm (Refer annexure I).



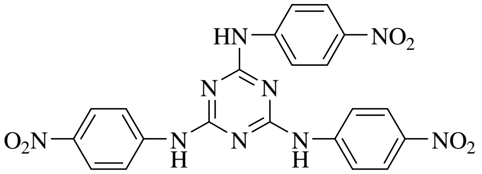
***N*^{2}, *N*^{4}-*bis*(4-Nitrophenyl)-*N*^{6}-(2,4,6-trichlorophenyl)-1,3,5-triazin-2,4,6-triamine (7b)**

White solid, R_f: 0.68 (hexane:ethylacetate/80:20), Yield: 80%, methanol, m.p. 288-290 °C. IR (KBr) υ_{max}: 761 (C-Cl str), 1262 (C-N str), 1332 (N-O str), 1462 (C=C str), 1589 (N=O str), 1697 (C=N str) and 3065 (=C-H str) cm^{-1}. ^{1}HNMR (DMSO-d_6, 500 MHz) δ: 7.97 (d, J = 9.0 Hz, 4H, Ar-H), 8.20 (d, J = 9.0 Hz, 4H, Ar-H), 10.49 (s, 2H, Ar-H) and 13.02 (s, 3H, D_2O exchangeable –NH) ppm. ^{13}CNMR (DMSO-d_6, 125 MHz) δ: 115.24, 116.19, 116.44, 119.75, 123.37, 128.64, 129.27, 129.76, 129.90, 135.07, 145.07 and 149.80 ppm (Refer annexure I).



N^2, N^4, N^6-*tris*(4-Nitrophenyl)-1,3,5-triazin-2,4,6-triamine (8)

Yellow solid, R_f: 0.6 (hexane:ethylacetate/70:30), Yield: 88%, acetone, m.p. 270-271 °C. IR (KBr) υ_{max}: 794 (C-Cl str), 1239 (C-N str), 1317 (N-O str), 1416 (C=C str), 1551 (N=O str), 1617 (C=N str), 3076 (=C-H str) and 3346 (N-H str) cm^{-1}. ^{1}HNMR (DMSO-d_6, 500 MHz) δ: 7.96 (s, 6H, Ar-H), 8.26 (d, J = 9.5 Hz, 6H, Ar-H) and 10.97 (s, 3H, D_2O exchangeable –NH) ppm. ^{13}CNMR (DMSO-d_6, 125 MHz) δ: 120.05, 124.69, 142.24, 144.63, 163.76 and 168.60 ppm (Refer annexure I).



4.4 Antifungal activity of triazine and its derivatives

Triazine (1) was selected for its antifungal potential because it is a well known compound with wide range of pharmacological properties. Triazine (1) and its derivatives *viz.* (3), (5a), (5b), (5c), (7a), (7b) and (8) were evaluated for their *in vitro* antifungal potential against different pathogens *viz. Helminthosporium orzyae*, *Rhizoctonia solani* and *Alternaria brassicae* by employing posion food technique at different concentration levels (5, 10, 50, 100, 250 and 500 µg/ml). The results have also been expressed in terms of ED$_{50}$ values *i.e.* effective dose where 50 percent of inhibition of mycelial growth of pathogens occurs.

4.4.1 *In vitro* efficacy of triazine and its derivatives against *Helminthosporium orzyae*

The per cent mycelial inhibition of colony growth in case of *H. orzyae* was calculated at different concentrations of tested compounds and is represented in Table 4.2 and Fig. 4.2. Among all the tested compounds, 6-chloro-N^2,N^4-*bis*(4-bromophenyl)-1,3,5-triazin-2,4-diamine (5c) was found most effective resulting in 56.28 %, 62.05 %, 67.46 %, 73.03 %, 80.82 % and 100.00 % inhibition of mycelial growth at 5, 10, 50, 100, 250, and 500 µg/ml concentration, respectively. While, triazine (1) was found least effective with 52.3 %, 32.45 %, 36.36 %, 38.97 %, 51.66 % and 76.94 % mycelial growth inhibition, respectively. At concentration level 500 µg/ml, the maximum per cent mycelial growth inhibition (100 %) was shown by five compounds *viz.* (3), (5b), (5c), (7b) and (8) whereas, minimum per cent mycelial growth inhibition was shown by compound 7a (85.16 %) followed by 5a (82.28 %)

and triazine (76.94 %). At concentration level 250 µg/ml, compounds (7b) and (5c) showed maximum activity with per cent mycelial growth inhibition 83.88 % and 80.82%, respectively. Compound 5a (78.31 %), 5b (77.30 %), 8 (72.69 %), 3 (72.69 %) and 7a (67.86 %) had shown decrease in per cent inhibition at same concentration.

At concentration level 100 µg/ml, compound (7b) exhibited maximum per cent mycelial growth inhibition (80.37 %) followed by 5c (73.03 %) after control (100 %) where as minimum was shown by compound 3 (62.62 %) followed by 8 (68.42 %), 7a (66.47 %), 5a (65.68 %) and 5b (63.06 %), respectively.

Compound 6-chloro-N^2,N^4-*bis*(4-bromophenyl)-1,3,5-triazin-2,4-diamine (5c), *i.e.* disubstituted triazine derivative had exhibited lowest ED_{50} value (4 µg/ml) and was found to be most effective compound among all the synthesized triazine derivatives against *H. oryzae* (Fig. 4.1). This may be attributed to the electron withdrawing effect of the bromo group attached to the moiety (Gopalakrishnan *et al* 2008). Compounds (8), (3), (5a), (7a), (7b) and (5b) had ED_{50} value of 5, 13, 13, 14, 20 and 83 µg/ml, respectively. All the synthesized compounds were less effective as compared to standard check (Propiconzole) at all the concentrations.

Table 4.2 *In vitro* evaluation of triazine and its derivatives against *H. oryzae*

Compound No.	**Per cent mycelial growth inhibition**						
	Concentrations (µg/ml)						
	5	**10**	**50**	**100**	**250**	**500**	**ED_{50}**
3	46.29	49.24	59.04	62.62	72.69	100.00	13
5a	35.01	47.29	58.57	65.68	78.31	82.28	13
5b	30.26	36.58	47.97	63.06	77.30	100.00	83
5c	56.28	62.05	67.46	73.03	80.82	100.00	4
7a	44.71	48.31	64.19	66.47	67.86	85.16	14
7b	46.85	49.09	64.63	80.37	83.88	100.00	20
8	50.22	52.46	66.96	68.42	72.69	100.00	5
Triazine 1	5.23	32.45	36.36	38.97	51.66	76.94	230
Propiconazole	-	-	-	100.00	-	-	2
CD (5 %)	1.71	2.22	3.95	2.14	1.29	0.91	--

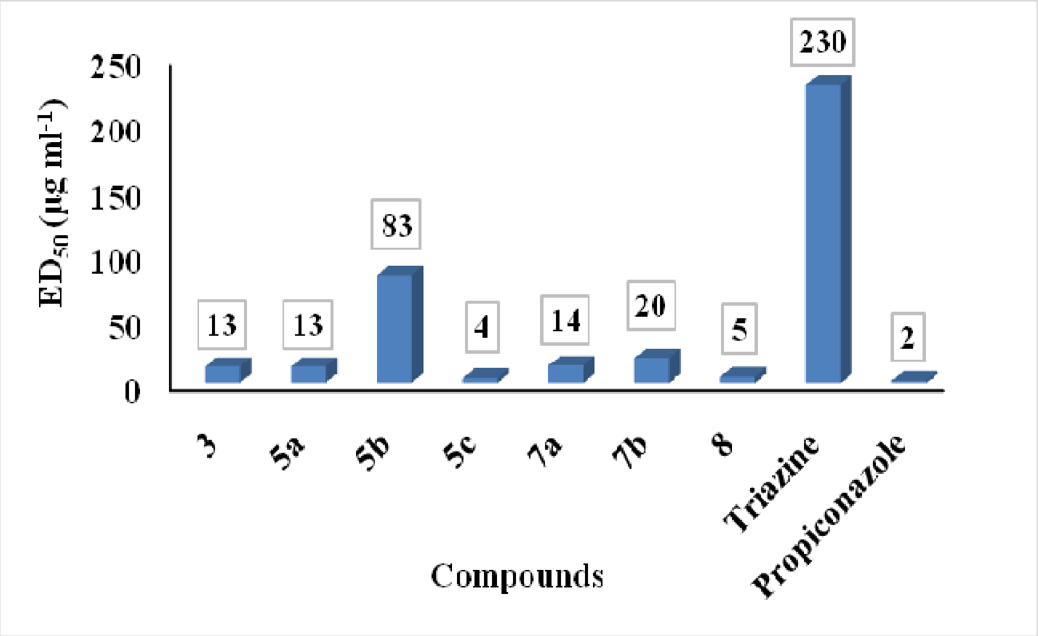


Figure 4.1 ED$_{50}$ values of triazine and its derivatives against *H. orzyae*

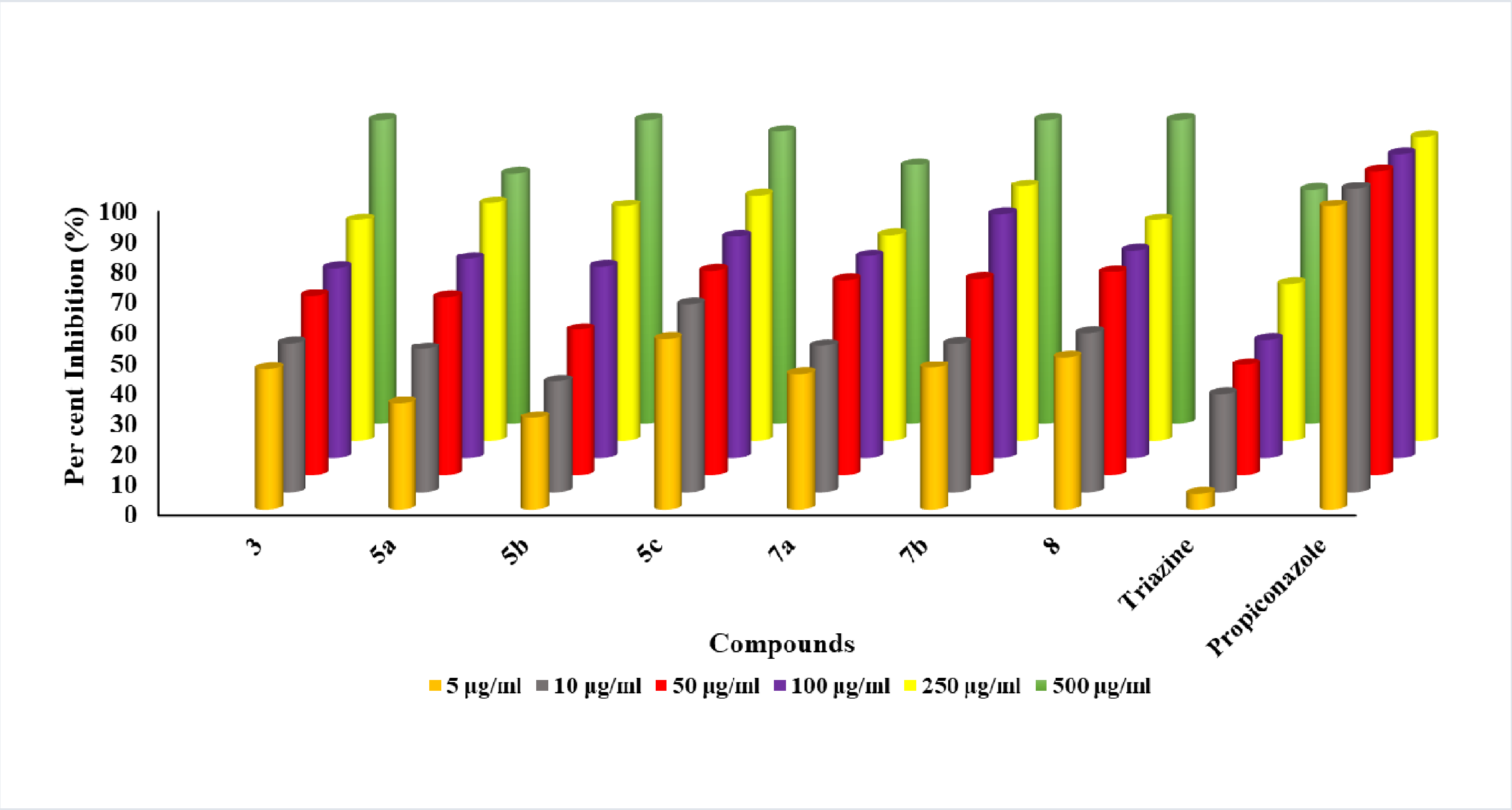


Figure 4.2 Per cent mycelial growth inhibition of triazine and its derivatives at different concentrations against *H. oryzae*

4.4.2 Antifungal activity of triazine and its derivatives against *Rhizoctonia solani*

The per cent mycelial inhibition of colony growth in case of *R. solani* was calculated at different concentrations of tested compounds and is represented in Table 4.3 and Fig. 4.4. Among all the tested compounds, compound (8) bearing three nitro groups at all para positions and compound (7b) bearing three chlorine atoms in one ring and two nitro groups at para positions was found to be most effective resulting in 100 %, 88.76 %, 85.23 %, 71.79 %, 69.66 %, 47.07 % inhibition of mycelial growth at 500, 250, 100, 50, 10 and 5 µg/ml concentration, respectively. While, (7a) was found to be least effective with 98.68 %, 73.81 %, 16.40 %, 12.54 %, 7.15 % and 5.27 % mycelial growth inhibition. At concentration level 500 µg/ml, the maximum per cent mycelial growth inhibition (100 %) was shown by five compounds *viz.* (7b), (8), (5a) ,(5c) and triazine whereas, minimum per cent mycelial growth inhibition was shown by compound 3 (98.68 %) followed by 7a (86.92 %) and 5b (34.59 %). It was also observed that with variation in concentrations the activity of compounds (7b) and (8) varies but activity was found to be at par (ED_{50} value of 8 and 7µg/ml respectively). All the other compounds were found to be less effective or insignificant at lower concentrations.

Compound, *N*^{2}, *N*^{4}-*bis*(4-nitrophenyl)-*N*^{6}-(2,4,6-trichlorophenyl)-1,3,5-triazin-2,4,6-triamine (7b) had exhibited ED_{50} value of 7 µg/ml and was found to be most effective compound among all the synthesized triazine derivatives against *R. solani*. *N*^{2}, *N*^{4}, *N*^{6}-*tris*(4-nitrophenyl)-1,3,5-triazin-2,4,6-triamine (8) showed effective antifungal potential with 100 per cent mycelial growth inhibition at 500 µg/ml with ED_{50} values of 8 µg/ml. Compounds (5a) and (5b) also showed significant antifungal potential with ED_{50} values, 21 µg/ml and 23 µg/ml, respectively (Fig. 4.3). Compounds (7a) and (3) had shown moderate antifungal potential against the tested fungus with ED_{50} values at 184 µg/ml and 188 µg/ml, respectively. Propiconazole (Tilt 25 EC) was taken as standard against *R. solani* and showed 100 per cent mycelial inhibition at concentration 100 µg/ml. All the synthesized compounds were less effective as compared to standard check (Propiconzole) at all the concentrations.

Table 4.3 ***In vitro*** **evaluation of triazine and its derivatives against** ***R. solani***

Compound No.	**Per cent mycelial growth inhibition**						
	Concentrations (µg/ml)						
	5	**10**	**50**	**100**	**250**	**500**	**ED$_{50}$**
3	5.27	7.15	12.54	16.40	73.81	98.68	188
5a	40.10	45.95	61.19	70.44	79.66	100.00	21
5b	1.24	1.86	4.61	12.73	23.81	34.59	--
5c	13.53	45.72	58.45	70.06	81.20	100.00	23
7a	12.80	16.13	20.10	27.40	67.71	86.92	184
7b	36.69	69.66	71.45	85.23	86.54	100.00	7
8	47.07	52.31	71.79	74.79	88.76	100.00	8
Triazine 1	29.23	52.73	49.37	55.28	100.00	100.00	55
Propiconazole	-	-	-	100.00	-	-	5
CD (5 %)	1.48	1.27	2.01	2.93	3.57	1.69	--

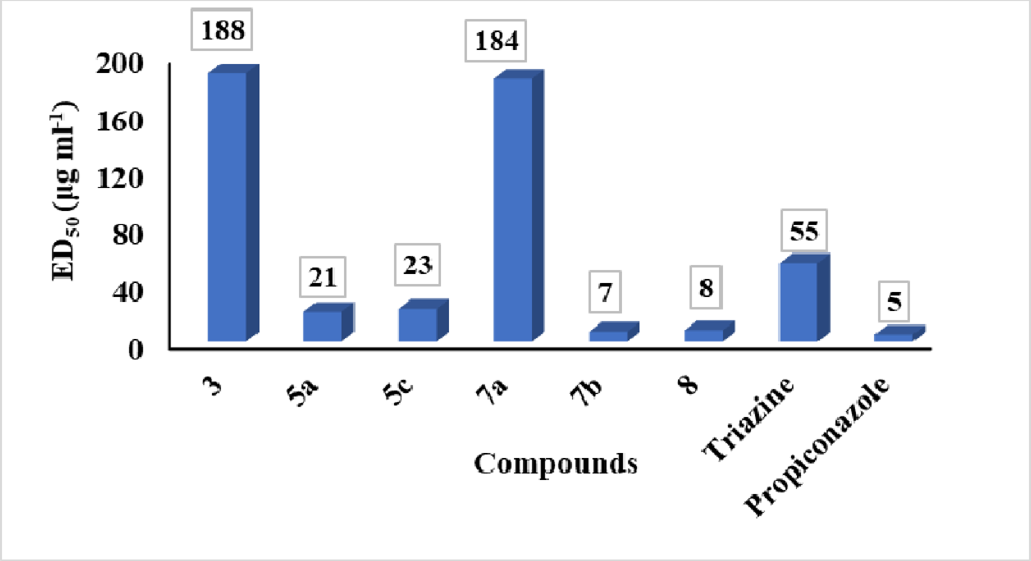


Figure 4.3 ED$_{50}$ values of triazine and its derivatives against ***R. solani***

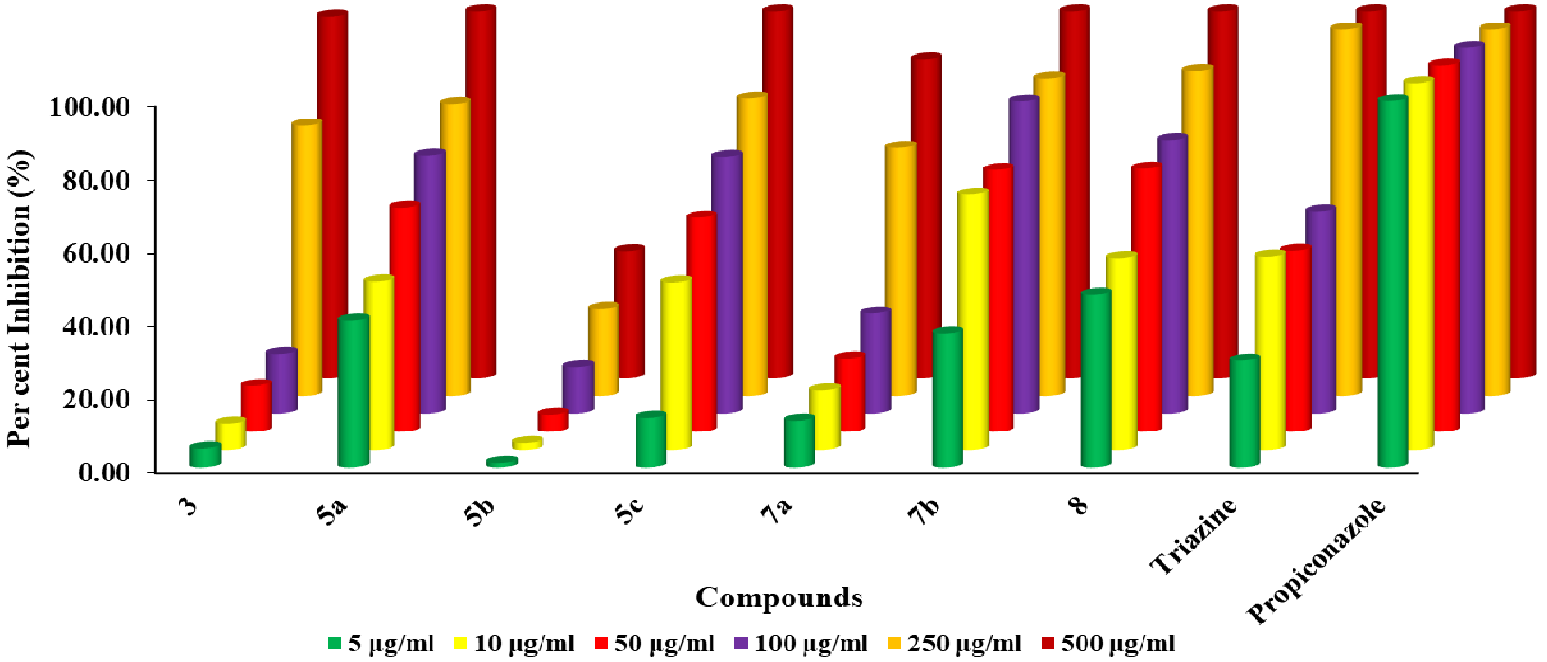


Figure 4.4 Per cent mycelial growth inhibition of triazine and its derivatives at different concentrations against *R. solani*

4.4.3 Antifungal activity of triazine and its derivatives against *Alternaria brassicae*

The per cent mycelial inhibition of colony growth in case of *A. brassicae* was calculated at different concentrations of tested compounds and is represented in Table 4.4 and Fig. 4.6. Among all the tested compounds, compound 6-chloro-*N*^{2},*N*^{4}-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a) was found most effective resulting in 38.08 %, 52.31 %, 63.70 %, 69.43 %, 72.95 % and 100.00 % inhibition of mycelial growth at 5, 10, 50, 100, 250, and 500 µg/ml concentration, respectively. While, triazine (1) was found least effective with 16.62 %, 24.64 %, 31.90 %, 37.95 %, 49.06 % and 79.79 % mycelial growth inhibition, respectively. At concentration level 500 µg/ml, the maximum per cent mycelial growth inhibition (100%) was shown by four compounds *viz.* (5a), (5b), (5c) and (7b) whereas, minimum per cent mycelial growth inhibition 8 (89.03 %), 7a (88.06 %), 3 (80.25 %) and 1 (79.79 %) respectively. Also, compound 6-chloro-*N*^{2},*N*^{4}-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a) and 6-chloro-*N*^{2},*N*^{4}-*bis*(4-chlorophenyl)-1,3,5-triazin-2,4-diamine (5b) showed 72.95 and 90.18 per cent mycelial growth inhibition potential at concentration 250 µg/ml. All the compounds were found to be less effective or insignificant at lower concentrations**.** 6-Chloro-*N*^{2},*N*^{4}-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a) showed better antifungal potential with ED$_{50}$ value of 9 µg/ml. 6-Chloro-*N*^{2},*N*^{4}-*bis*(4-chlorophenyl)-1,3,5-triazin-2,4-diamine (5b) showed effective antifungal potential with 100 per cent mycelial growth inhibition at 500 µg/ml with ED$_{50}$ values of 10 µg/ml. Compounds (5c) and (7a) also showed significant antifungal potential with ED$_{50}$ values of 40 µg/ml and 73 µg/ml, respectively. Compounds (8) and (3) had shown moderate antifungal potential against the tested fungus with ED$_{50}$ values at 93 µg/ml and 217 µg/ml, respectively. None of the treatments showed antifungal potential greater than standard *i.e.* Mancozeb (75WG) which completely inhibited mycelial growth at 250 µg/ml with ED$_{50}$ value 2 µg/ml (Figure 4.5).

Decreasing order of ED$_{50}$ values of all synthesized triazine derivatives against *A. brassicae* was as follows:

Mancozeb (75WG)> 5a> 5b> 5c> 7b> 7a> 8> 3> Triazine

From the results, it was found that 6-chloro-*N*^{2},*N*^{4}-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a) was most effective against *A. brassicae* followed by the 6-chloro-*N*^{2},*N*^{4}-*bis*(4-chlorophenyl)-1,3,5-triazin-2,4-diamine (5b) and 6-chloro-*N*^{2},*N*^{4}-*bis*(4-bromophenyl)-1,3,5-triazin-2,4-diamine (5c). It was therefore concluded that replacement of two Cl groups of triazine with para substituted bromo and chloro anilines *i.e.* di-substituted derivatives of triazine were more effective than mono- and trisubstituted triazine derivatives.

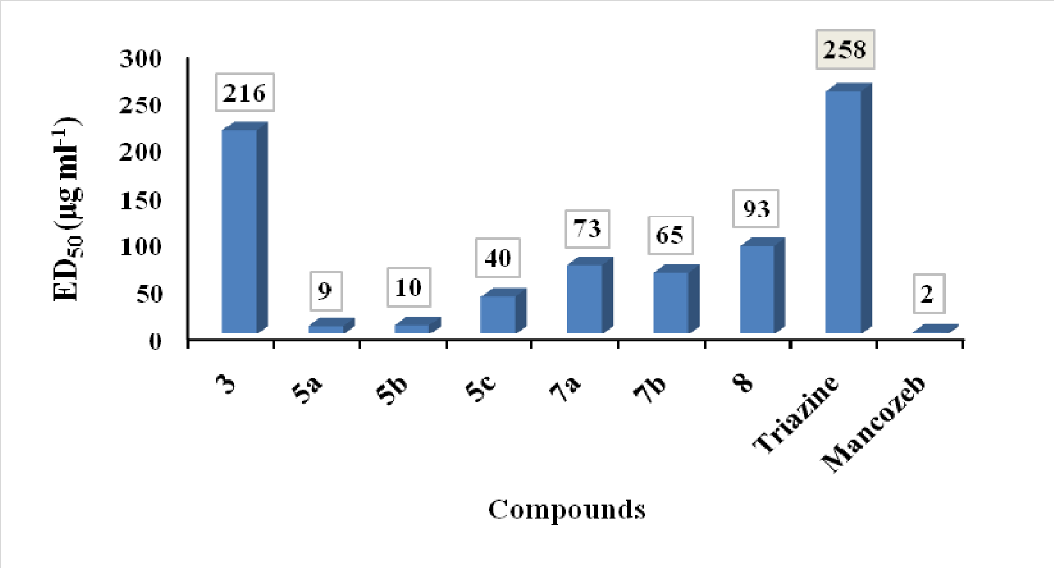


Figure 4.5 ED_{50} **values of triazine and its derivatives against** ***A. brassicae***

Table 4.4 ***In vitro*** **evaluation of triazine and its derivatives against** ***A. brassicae***

Compound No.	**Per cent mycelial growth inhibition**						
	Concentrations (□g/ml)						
	5	**10**	**50**	**100**	**250**	**500**	ED_{50}
3	11.15	20.14	29.39	37.97	53.47	80.25	217
5a	38.08	52.31	63.70	69.43	72.95	100.00	9
5b	40.59	50.18	68.94	81.49	90.18	100.00	10
5c	32.50	40.59	52.85	68.46	81.76	100.00	40
7a	12.83	30.56	37.26	64.79	72.17	88.06	73
7b	17.22	28.51	35.73	81.68	85.45	100.00	65
8	11.15	20.14	36.48	52.05	69.91	89.03	93
Triazine 1	16.62	24.64	31.90	37.95	49.06	79.79	258
Mancozeb	76.50	80.76	82.43	96.90	100.00	100.00	2
CD (5%)	1.85	2.01	2.32	1.81	2.35	2.22	--

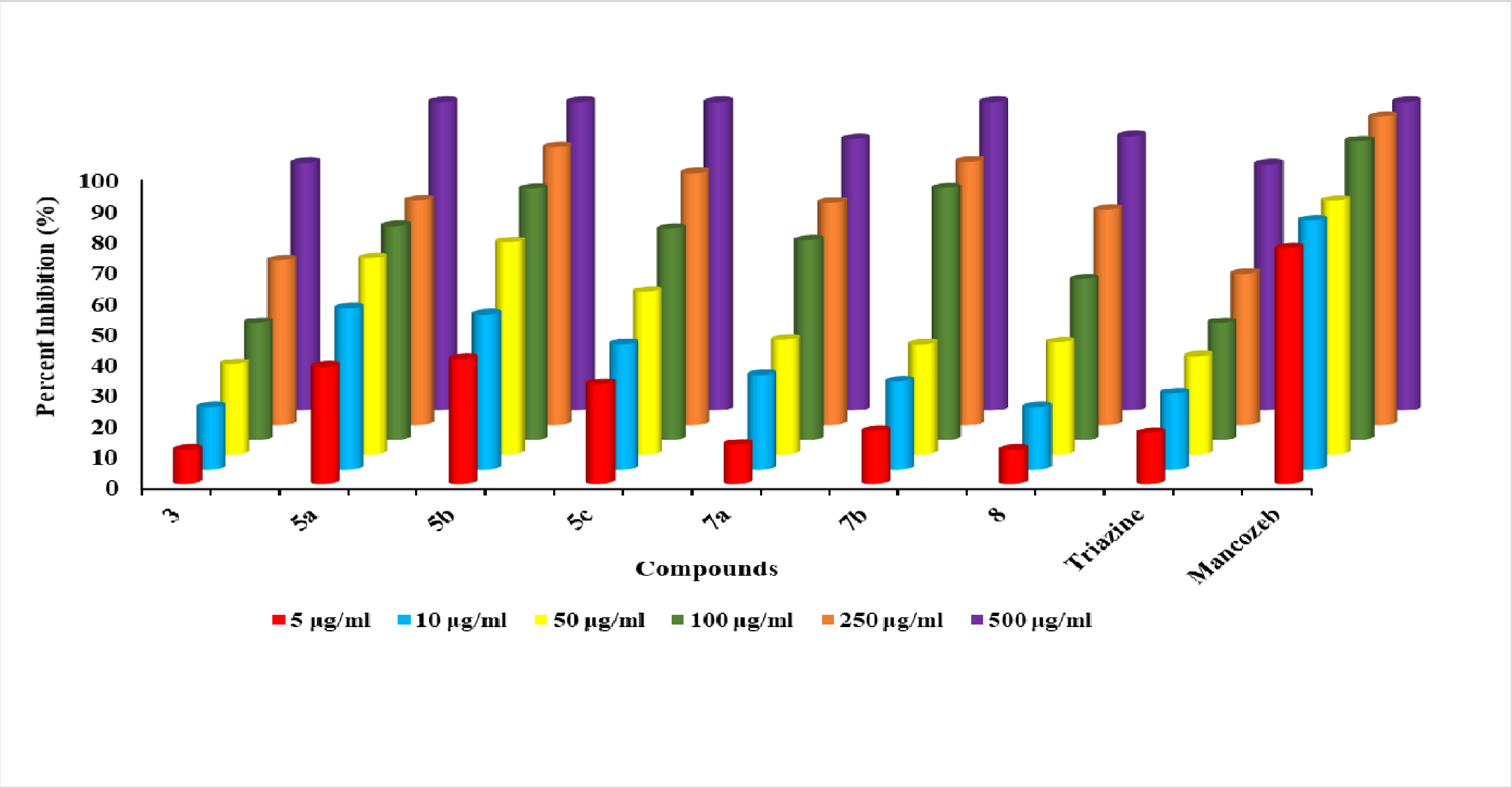


Figure 4.6 Per cent mycelial growth inhibition of triazine and its derivatives at different concentrations against *A. brassicae*

Table 4.5 depicts the ED_{50} values of all triazine synthesized derivatives against different fungal pathogens *viz. H. oryzae, R. solani* and *A. brassicae*. It was found that compound 5c, 7b and 5a was the most effective compound with lowest ED_{50} value at 4, 7 and 9 µg/ml. Order of ranking of ED_{50} values of all synthesized compounds against *H. oryzae* as follows: Propiconazole> 5c> 8> 3> 5a> 7a> 7b> 5b> Triazine which revealed that compound (5c) was found to be the most effective and compound (5b) was the least effective. However, in case of *R. solani* order of ranking of all synthesized compounds was Propiconazole> 7b> 8> 5a> 5c> Triazine> 7a> 3 which showed that compound (7b) was the most effective and compound (5b) was least effective. Decreasing order of ED_{50} values of all synthesized triazine derivatives against *A. brassicae* was as follows: Mancozeb(75WG)> 5a> 5b> 5c> 7b> 7a> 8> 3> Triazine

Table 4.5 Comparison of ED_{50} values of different treatment against different fungi

Treatments	**ED_{50} Values (µg/ml)**		
	Rhizoctonia solani	***Helminthosporium oryzae***	***Alternaria brassicae***
3	13	188	217
5a	13	21	**9**
5b	83	-	10
5c	**4**	23	40
7a	14	184	73
7b	20	**7**	65
8	5	8	93
Triazine 1	230	55	258
Standard	2	5	2

4.5 Conclusions

- A convenient thermally controlled approach was used for the synthesis of mono-, di- and tri substituted triazine derivatives by using base sodium hydroxide and solvent acetone with easy analysis of reaction progress and purification of products.
- Apart from the simplicity of the reaction procedure, ability to tolerate functional groups variations is an important feature of the reaction.
- Reaction procedure allows the synthesis of products out from laboratory scale to large scale.

- Among all the synthesized triazine derivatives, (5c), (7b) and (5a) were found to be most effective compound against *H. oryzae, R, solani* and *A. brasicae*, respectively with 100% mycelial growth inhibition at concentration level 500 µg/ml with ED_{50} value at 4, 7 and 9 µg/ml, where as compound (3), triazine (1) was found to be least effective with ED_{50} value 188, 230 and 258 µg/ml.

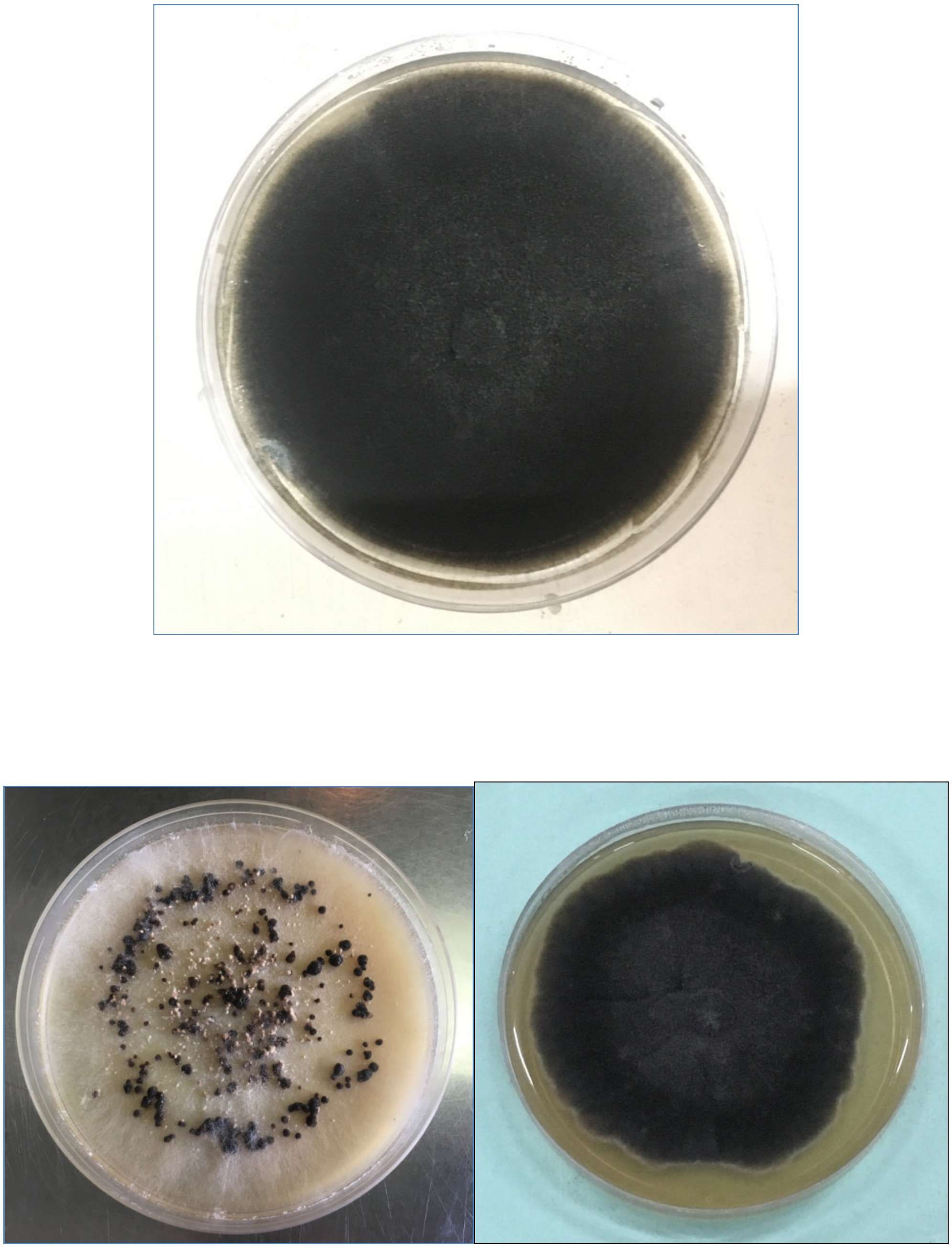
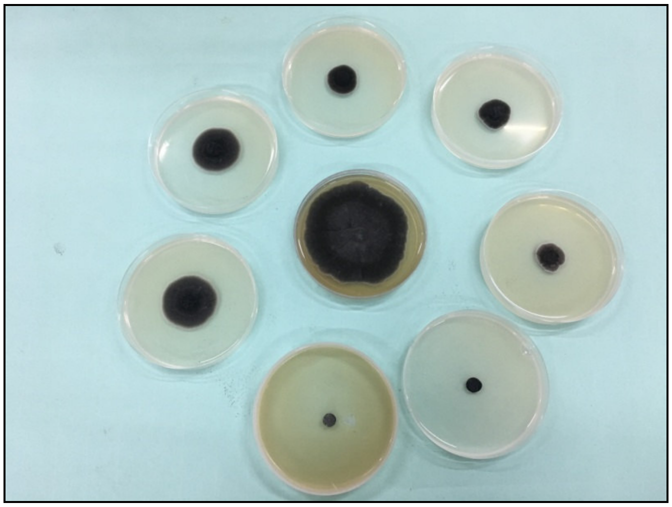
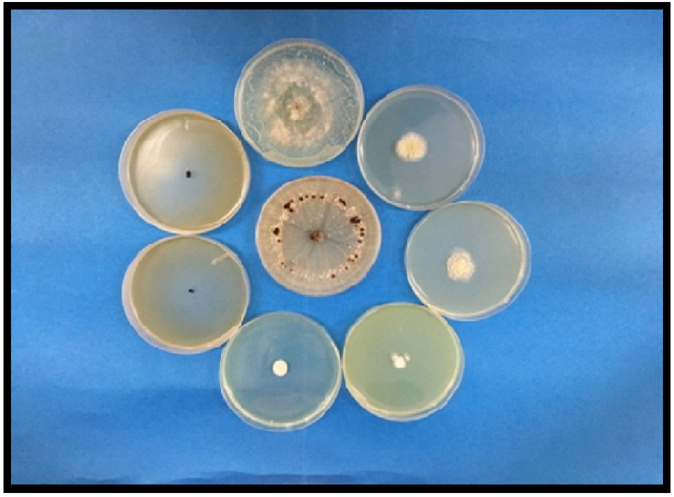


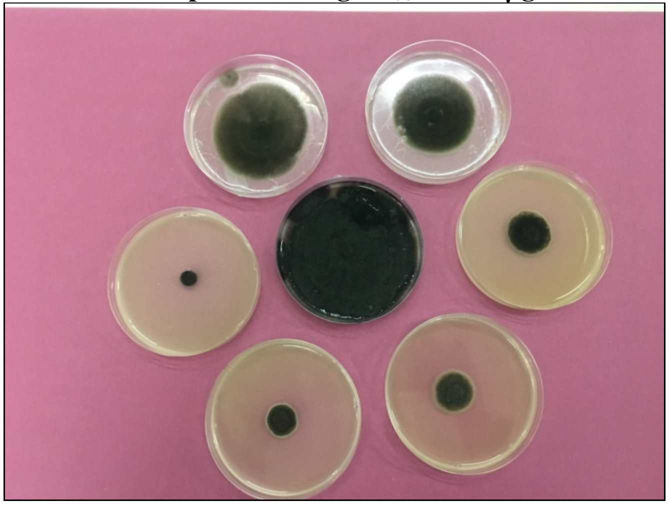
Plate I : Isolation and maintenance of pure cultures of *Helminthosporium oryzae, Rhizoctonia solani* and *Alternaria brassicae*



5c compound having ED_{50} value 4 μg/ml



7b compound having ED_{50} value 7 μg/ml



5a compound having ED_{50} value 9μg/ml

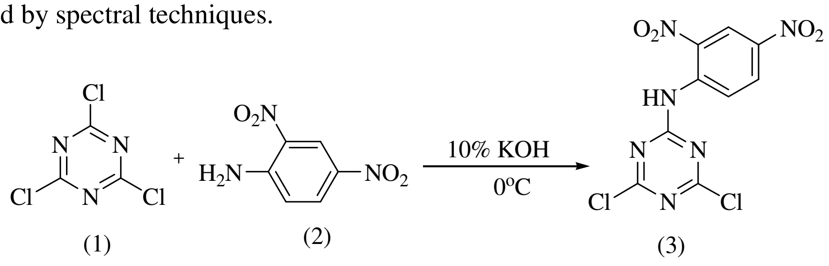
Plate II : Compound 5c, 7b and 5a showed maximum activity against *Helminthosporium oryzae, Rhizoctonia solani* and *Alternaria brassicae*

CHAPTER-V

SUMMARY

The chemistry of heterocyclic compounds has become an interesting field for long time because of their specific chemical reactivity and natural abundance. Various nitrogen containing heterocyclic compounds had gained immense importance in organic and medicinal chemistry due to their wide existence in natural bioactive molecules and proved as useful therapeutic agents (Baldaniya and Patel 2009). Triazine being one of the most important nitrogen containing heterocyclic compound attracted the considerable interest of many researchers due to its variable biological and physiochemical properties. Recently, it has been reported that 70% of triazine is used for the preparation of triazine a class pesticides. Most of the triazine herbicides such as simazine, anilazine, cyromazine and atrazine are synthesized from triazine by simple displacement of chlorine atoms with nucleophiles such as amines. Moreover, triazine and its derivatives play a vital role in agrochemicals, bioactive drugs and medicinal chemistry. Triazines are selective herbicides useful for the control of wide spectrum of grass and broad leaf weeds in cereal, oilseed and horticultural crops. Embarked attention gained by triazine is due to the different reactivity of its chlorine atoms at different temperature conditions (Patel *et al* 2012). Generally, reactivity depends upon the type of nucleophile used in reaction under temperature controlled conditions. The formation of mono-substituted derivatives takes place at extremely low temperature *i.e.* 0 °C, di-substitution reaction takes place at room temperature *i.e.* 25-28 °C, and tri-substituted product at high temperature *i.e.* 80-90 °C conditions (Solankee and Tailor 2016, Patel and Chikhalia 2003). Novel series of compounds have been synthesized by following nucleophilic aromatic substitution mechanism (S_NAr) in which 1,3,5-triazine was reacted with various nucleophilic reagents like primary amines to synthesize mono-, di- and trisubstituted triazine derivatives respectively.

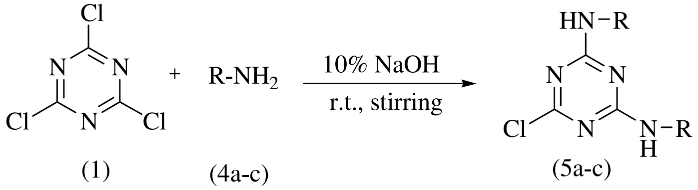
The mono-substituted triazine derivatives was synthesized by reaction of equimolar concentration of triazine (1) with 2,4-dinitroaniline (2) in the presence of base 10% KOH at 0 °C in an ice bath for 5 h to afford the corresponding mono-substituted derivative of triazine (3) (Scheme 1). The synthesized derivative was obtained in 80% yield and its identity was confirmed by spectral techniques.



Scheme 1: Synthesis of mono-substituted triazine derivative (3)

^{1}HNMR (DMSO-d_6, 500 MHz) spectrum of the compound showed multiplet signal at δ 8.61-8.64 ppm corresponding to one aromatic proton. Two doublets were observed at δ 8.05 and δ 8.82-8.86 ppm due to two aromatic protons with coupling constants J = 9.16 Hz and J = 8.0 Hz respectively. The unique feature of this spectrum include deuterium exchangeable singlet at δ 11.40 ppm due to –NH proton. The peaks in its ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum appeared at δ 119.74, 123.38, 128.63, 129.30, 135.07, 149.79 and 149.89 ppm which corroborated well with its ^{1}HNMR spectral assignment. Thus, the spectral analysis confirmed the structure, 4,6-dichloro-*N*-(2,4-dinitrophenyl)-1,3,5-triazin-2-amine (3), assigned to this product.

The di-substituted triazine derivatives (5a-c) were obtained by stirring solution of triazine (1) (0.1 mol) in acetone with different aromatic amines (4a-c) (0.2 mol) in the presence of base NaOH at room temperature as shown in scheme 2. Considerable variation in the nature of substituent (R) was introduced so as to understand the scope and limitation of reaction.



(4a): R-NH$_2$ = 4-nitroaniline
(4b): R-NH$_2$ = 4-chloroaniline
(4c): R-NH$_2$ = 4-bromoaniline

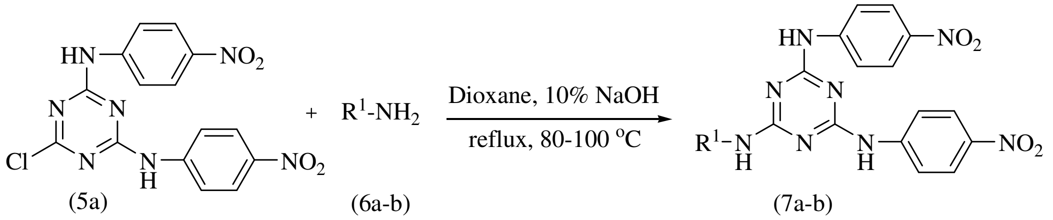
Scheme 2: Synthesis of di-substituted triazine derivative (5a-c)

The confirmation of di-substituted triazine derivatives (5a-c) was made from spectral data. In ^{1}HNMR (DMSO-d_6, 500 MHz) spectrum of the compound obtained by reacting triazine (1) (0.1 mol) and 4-nitroaniline (4a) (0.2 mol), multiplet signals appeared at δ 7.89-7.91 and 8.30-8.32 ppm corresponding to eight aromatic protons. The main feature of this spectrum is deuterium exchangeable signal appeared at δ 11.68 ppm due to two –NH protons. In its ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum, peaks appeared at δ 119.63, 122.74, 124.30, 124.30, 131.46, 137.46, 141.09 and 163.63 ppm. The spectral analysis confirmed the structure 6-chloro-N^2,N^4-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a), assigned to this product.

Likewise in ^{1}HNMR (DMSO-d_6, 500MHz) spectrum of the compound obtained by reacting triaizne (1) (0.1 mol) and 4-chloroaniline (4b) (0.2 mol), exhibited multiplet signals at δ 7.46-7.48 and 7.62-7.65 ppm corresponding to eight aromatic protons. The characteristic feature of this spectrum included deuterium exchangeable singlet at δ 11.26 ppm due to two –NH protons. In its ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum peaks appeared at δ 122.65, 122.74, 122.84, 127.98, 128.66, 128.73, 129.78, 130.04, 135.75, 135.86, 136.09, 149.81, 153.94, 163.55 and 168.71 ppm. The spectral analysis confirmed the structure 6-chloro-N^2,N^4-bis(4-chlorophenyl)-1,3,5-triazin-2,4-diamine (5b), assigned to this product.

Similarly, the ^{1}HNMR, ^{13}CNMR and IR confirmed the structure 6-chloro-*N*^{2},*N*^{4}-bis(4-bromophenyl)-1,3,5-triazin-2,4-diamine (5c) obtained by reacting triazine (1) (0.1 mol) with 4-bromoaniline (4c) (0.2 mol) at room temperature reaction conditions.

Trisubstituted triazine derivatives were synthesized by using two different approaches. In the first approach, disubstituted triazine derivative (5a) was reacted with different amines (6a-b) in the presence of 10% NaOH base under reflux conditions as shown in scheme 3.



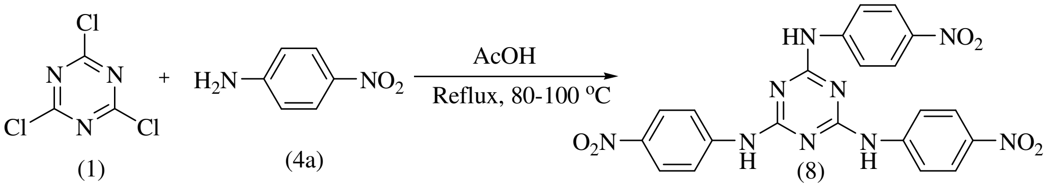
(6a): R^1-NH$_2$ = 2-chloroaniline
(6b): R^1-NH$_2$ = 2,3,5-trichloroaniline

Scheme 3: Synthesis of tri-substituted triazine derivatives (7a-b)

Reaction of 6-chloro-*N*^{2},*N*^{4}-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a) with 2-chloro aniline (6a) in equimolar concentration furnished white solid compound. The ^{1}HNMR (DMSO-d_6, 500 MHz) spectrum of this compound showed multiplet signal at δ 7.39-7.85 ppm corresponding to four aromatic protons. A doublet with coupling constant *J* = 8.5 Hz was observed at δ 7.97 ppm due to four aromatic protons. Another doublet signal appeared at δ 8.28 ppm (*J* = 9.0 Hz) corresponding to four aromatic protons. The characteristic feature of the spectrum consisted D_2O exchangeable broad signal at δ 10.35 and 10.80 ppm due to three –NH protons. In its ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum, the signals appeared at δ 109.55, 119.09, 124.30, 124.63, 127.58, 128.12, 129.06, 129.65, 134.33 and 141.19 ppm. In its IR (KBr) bands appeared at υ_{max}: 749 (C-Cl str), 1255 (C-N str), 1314 (N-O str), 1417 (C=C str), 1556 (N=O str), 1605 (C=N str), 3125 (=C-H str) and 3383 (N-H str) cm^{-1}. The correct NMR and IR spectral analysis confirmed the structure *N*^{2}-(2-chlorophenyl)-*N*^{4},*N*^{6}-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4,6-triamine (7a), obtained in 83% yield.

In a similar way, characterization of compound obtained by reacting equivalent concentration of (5a) with (6b) at 80-100 °C temperature, was made with the help of spectral analysis and *N*^{2}, *N*^{4}-*bis*(4-nitrophenyl)-*N*^{6}-(2,4,6-trichlorophenyl)-1,3,5-triazin-2,4,6-triamine (7b), was assigned to this compound, obtained in 80% yield.

In the second approach, synthesis of trisubstituted triazine derivative (8) was carried out by reacting triazine (1) (0.1 mol) with 4-nitroaniline (4a) (0.3 mol) in the presence of acetic acid under reflux conditions as shown in scheme 4.



Scheme 4: Synthesis of tri-substituted triazine derivative (8)

The ^{1}HNMR (DMSO-d_6, 500 MHz) spectrum of the product exhibited a singlet signal at δ 7.96 ppm due to six aromatic protons. A doublet signal was observed at δ 8.26 ppm with coupling constant J = 9.5 Hz due to six aromatic protons. A broad signal appeared at δ 10.97 ppm corresponding to three –NH protons. The peaks in its ^{13}CNMR (DMSO-d_6, 125 MHz) spectrum appeared at δ 119, 120.05, 124.69, 142.24, 144.63, 163.76 and 168.60 ppm which corroborated well with its ^{1}HNMR spectral assignment. The spectral analysis confirmed the structure, N^2, N^4, N^6-*tris*(4-nitrophenyl)-1,3,5-triazin-2,4,6-triamine (8), assigned to this product.

In vitro, antifungal evaluation of triazine (1) and its derivatives (3), (5a-c), (7a-b) and (8) were carried out using poison food technique against the fungal pathogens *Helminthosporium oryzae*, *Rhizoctonia solani and Alternaria brassicae.* The percent mycelial growth inhibition of triazine and its derivatives were observed at different concentrations *i.e* 5, 10, 50, 100, 250 and 500 µg/ml. The antifungal activity results are expressed in terms of ED_{50} values. Each test was replicated thrice. Among all the synthesized triazine derivatives, 5c, 7b and 5a were found to be most effective compound against *H. oryzae, R, solani* and *A. brasicae*, respectively with 100% mycelial growth inhibition where as compound (3), triazine (1) was found to be least effective with ED_{50} value 188, 230 and 258 µg/ml. Order of ranking of ED_{50} values of all synthesized compounds against *H. oryzae* as follows: Propiconazole> 5c> 8> 3> 5a> 7a> 7b> 5b which revealed that compound (5c) was found to be the most effective and compound (5b) was the least effective. However, in case of *R. solani* order of ranking of all synthesized compounds was Propiconazole> 7b> 8> 5a> 5c> 7a> 7b> 5b which showed that compound (7b) was the most effective and compound (5b) was least effective. Compound (5a) was found to be most active synthesized triazine derivative against *A. brassiace*

It may be concluded that compounds bearing electron withdrawing groups such as halogens and nitro showed better antifungal potential (Gopalkrishnan *et al* 2008). Also, it was observed that presence of halogens at the para position increases the activity of synthesized compounds.

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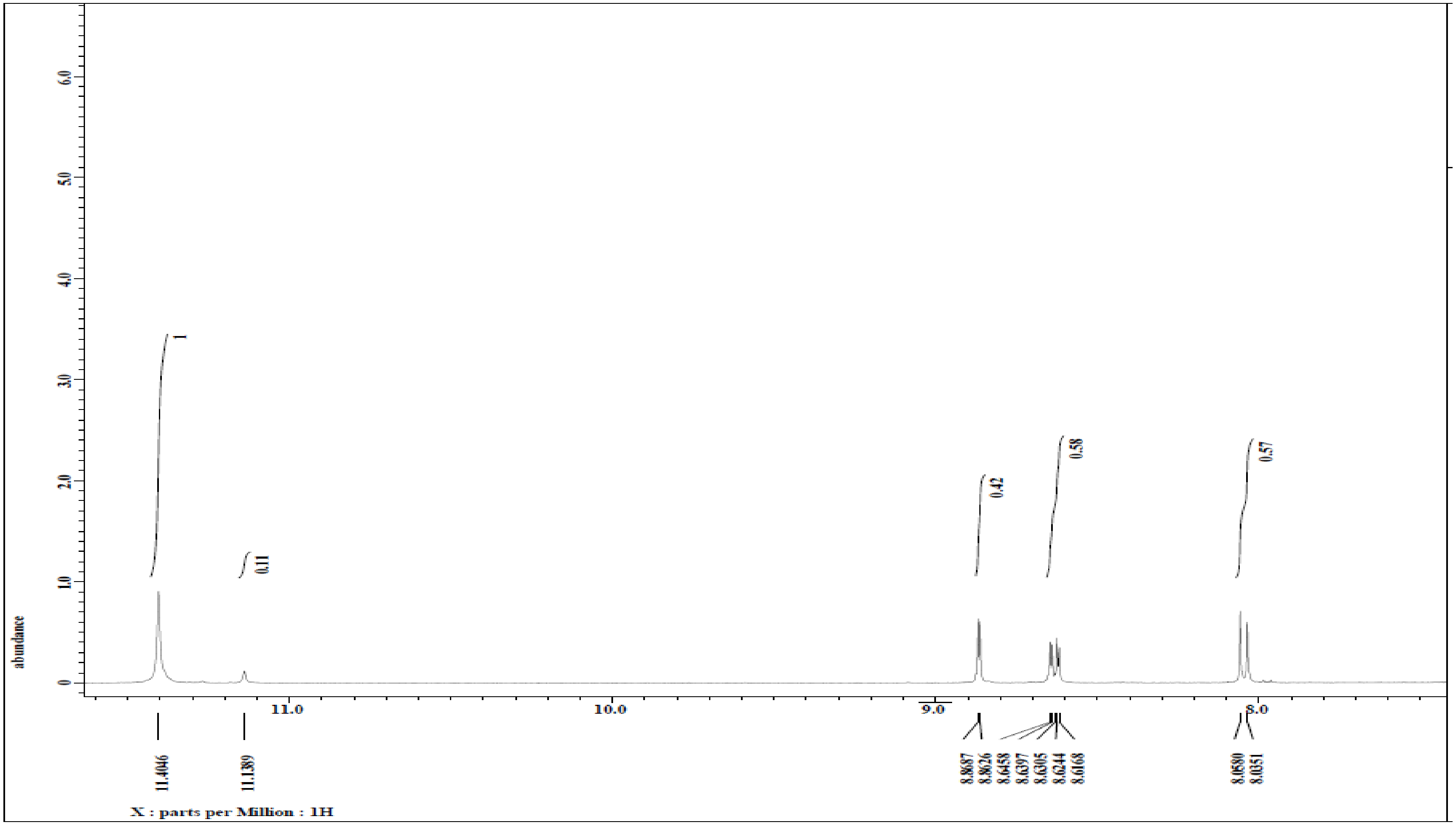
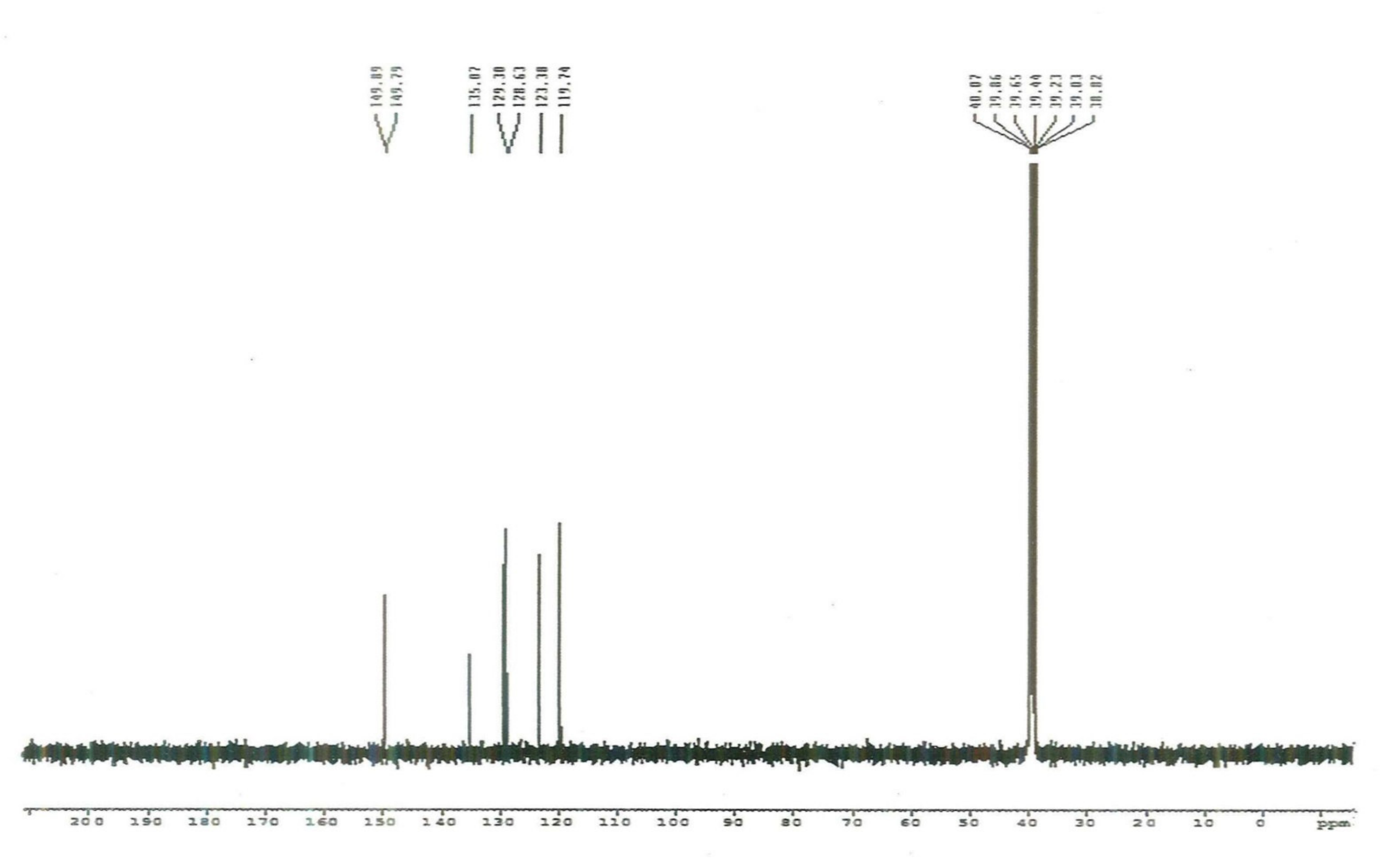
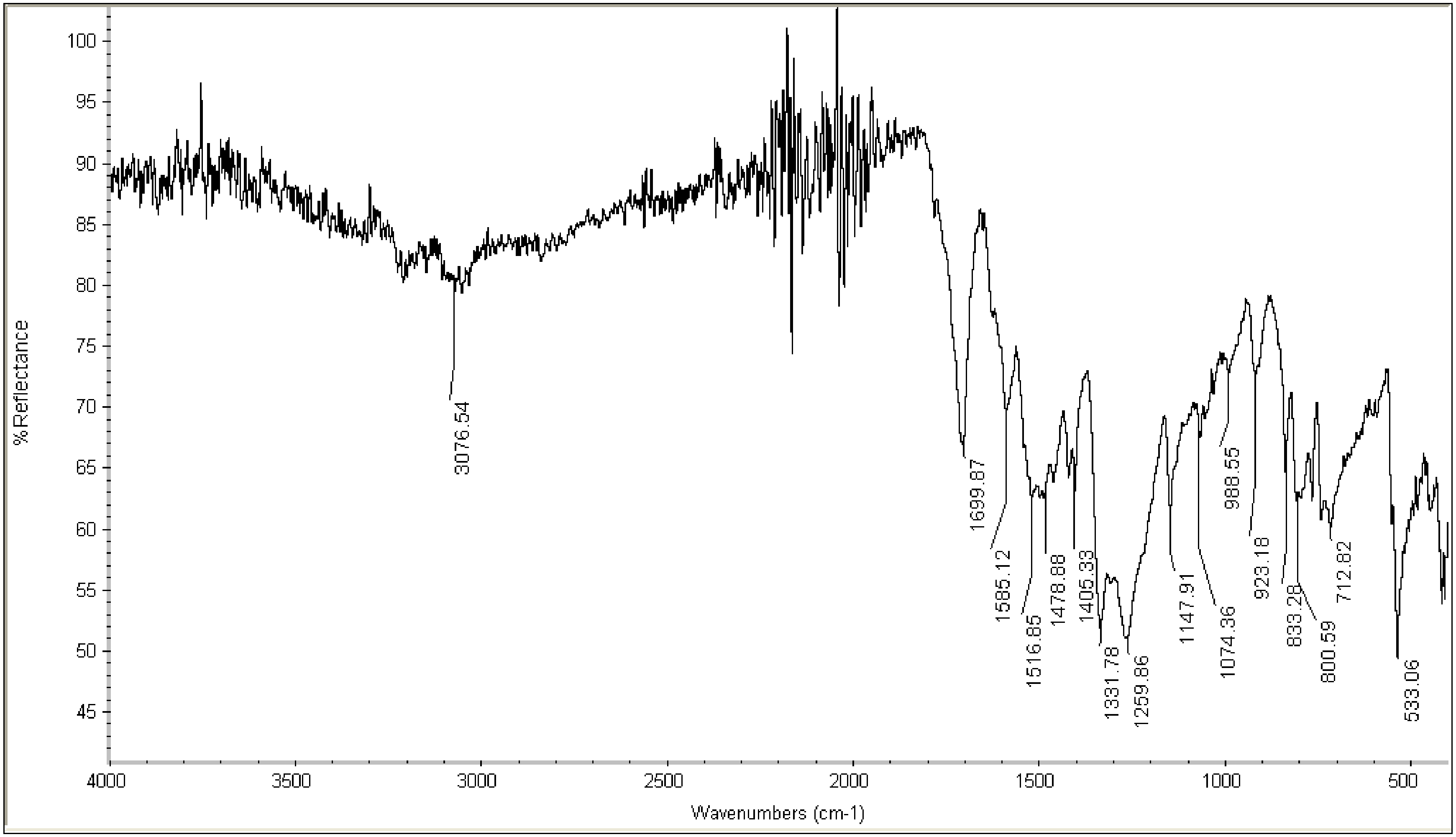
ANNEXURE - I



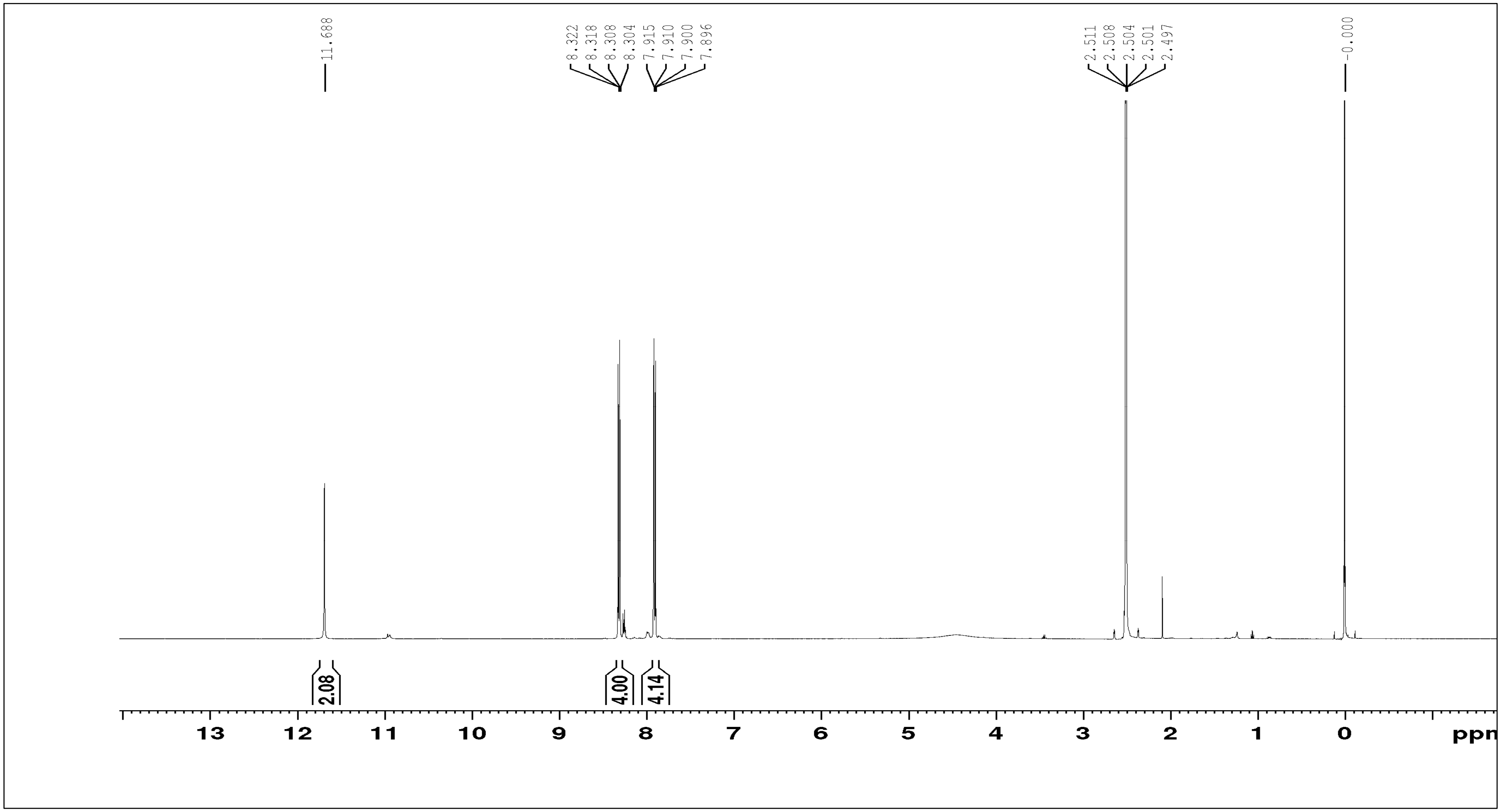
^{1}H NMR spectrum of 4,6-Dichloro-*N*-(2,4-dinitrophenyl)-1,3,5-triazin-2-amine (3)



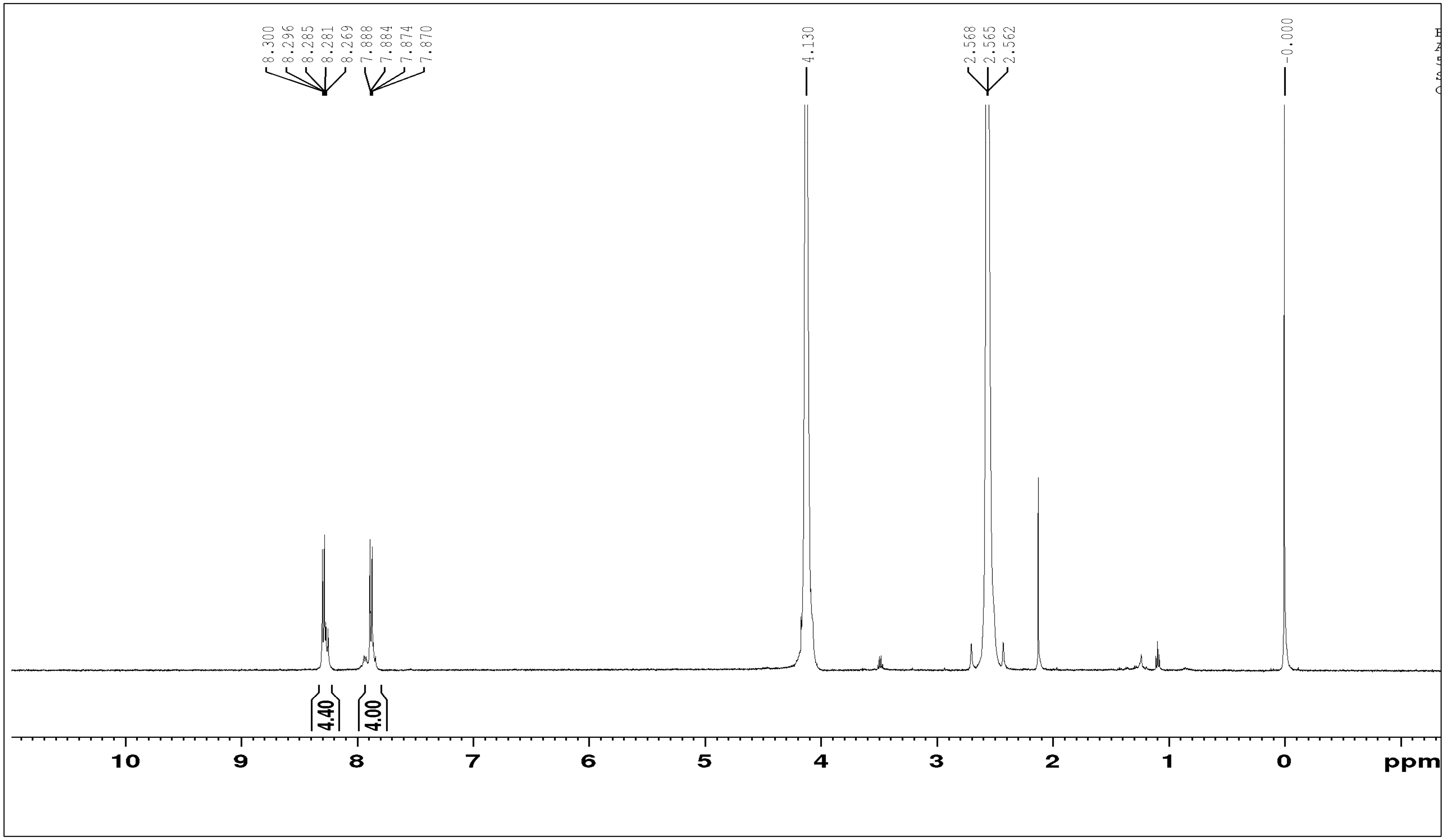
^{13}**C NMR Spectrum of 4,6-Dichloro-*N*-(2,4-dinitrophenyl)-1,3,5-triazin-2-amine (3)**



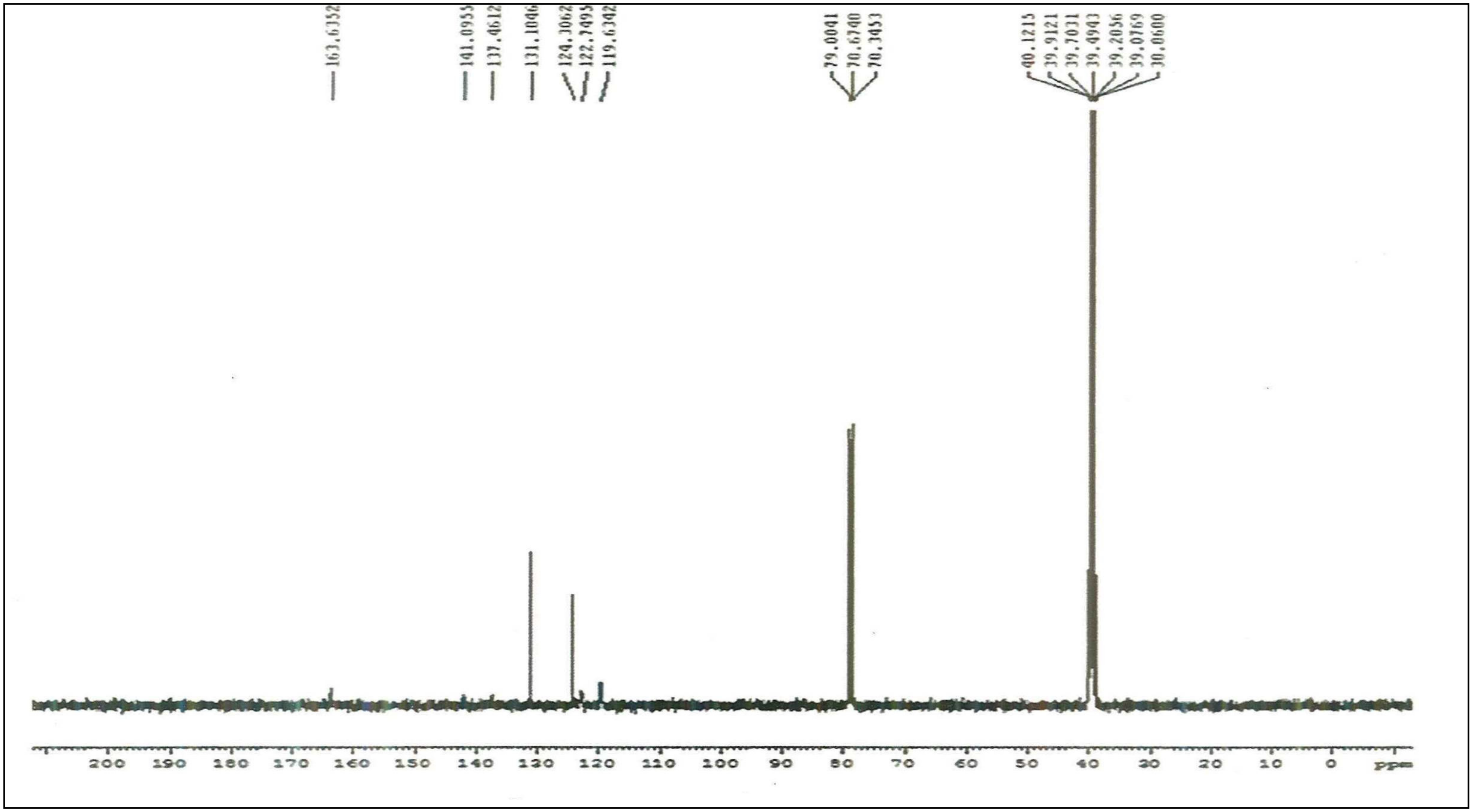
IR spectrum of 4,6-Dichloro-*N*-(2,4-dinitrophenyl)-1,3,5-triazin-2-amine (3)



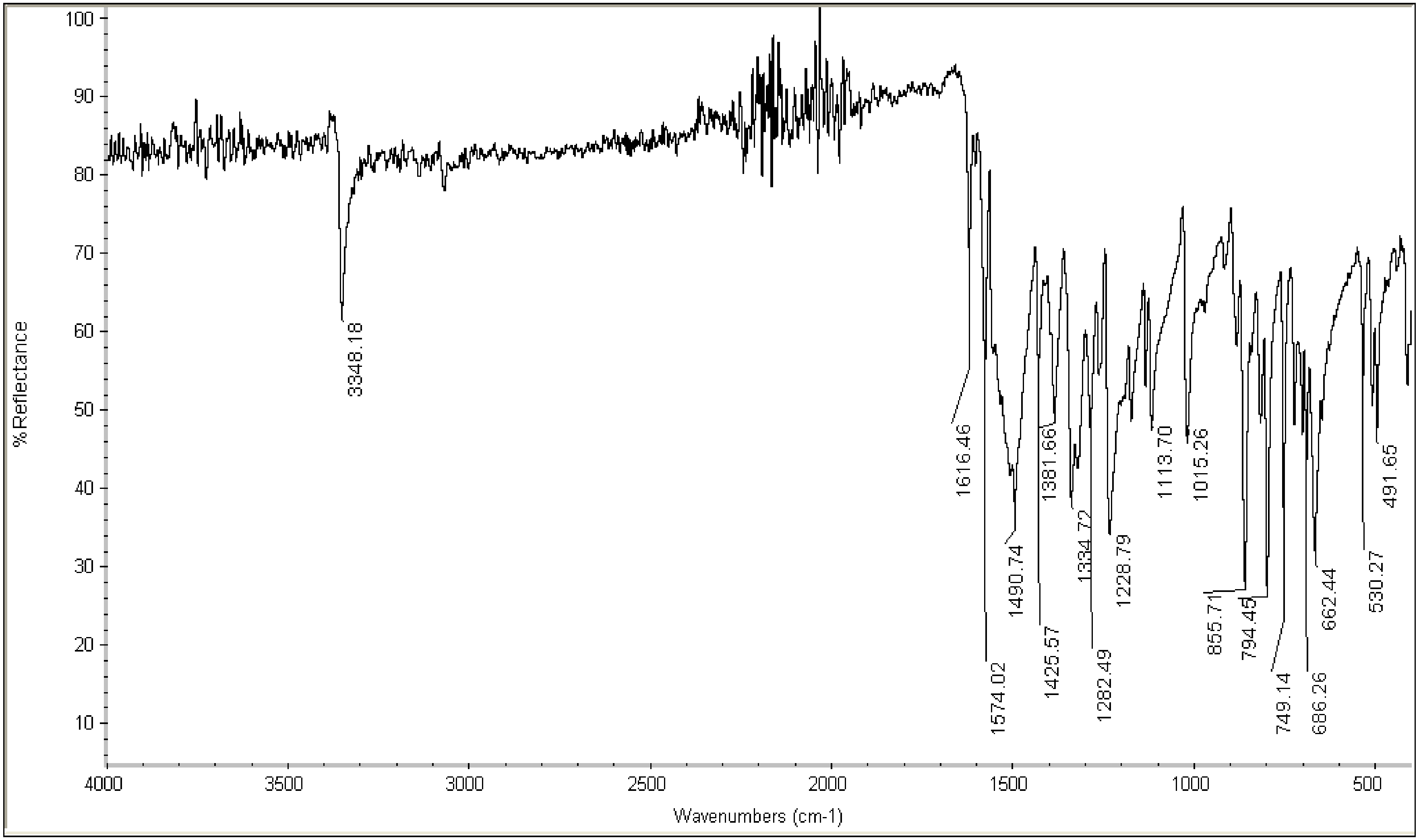
^{1}H NMR spectrum of 6-Chloro-*N*^{2},*N*^{4}-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a)



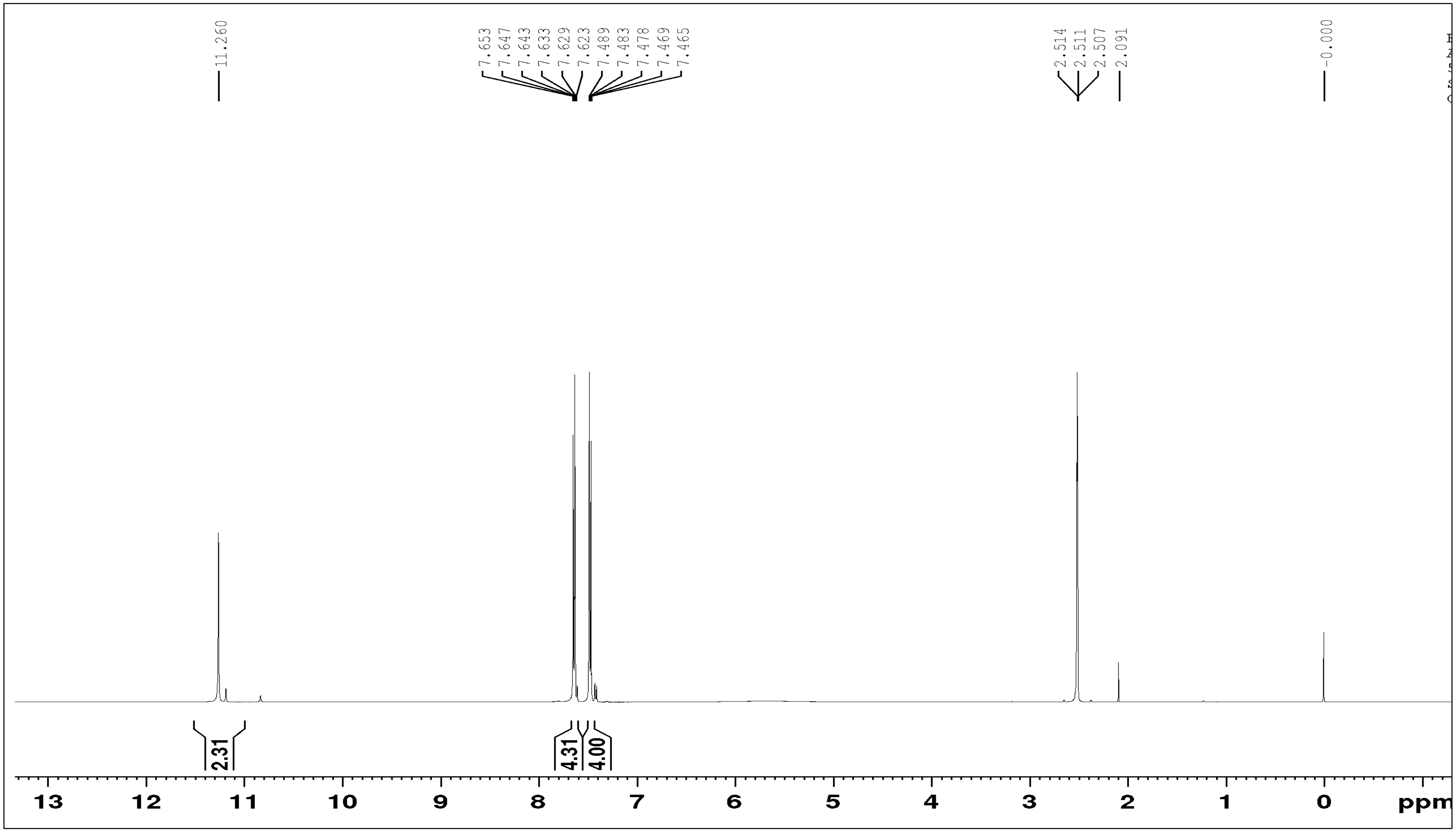
^{1}H NMR - D$_2$Oexchange spectrum of 6-Chloro-N^2,N^4-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a)



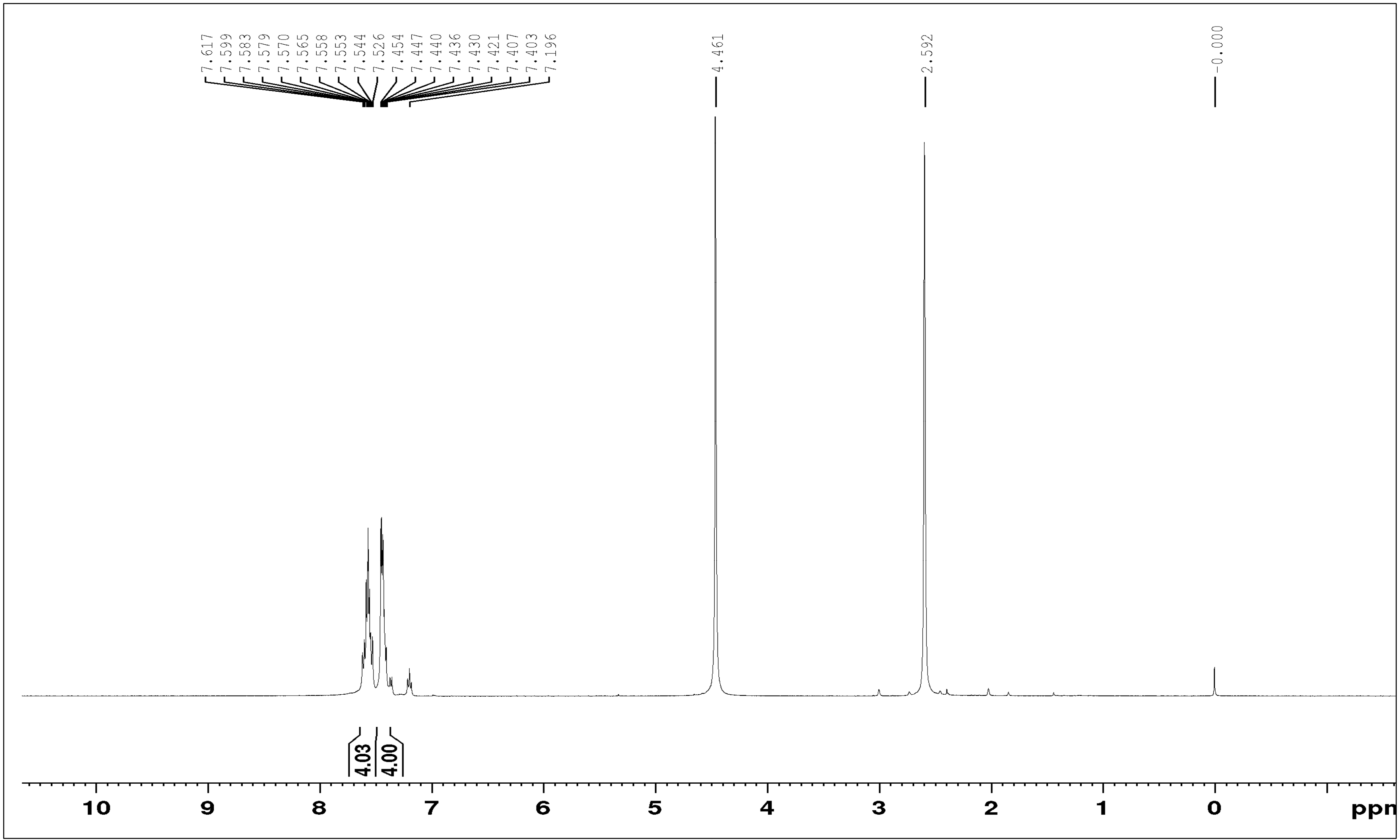
^{13}C NMR Spectrum of 6-Chloro-N^2,N^4-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a)



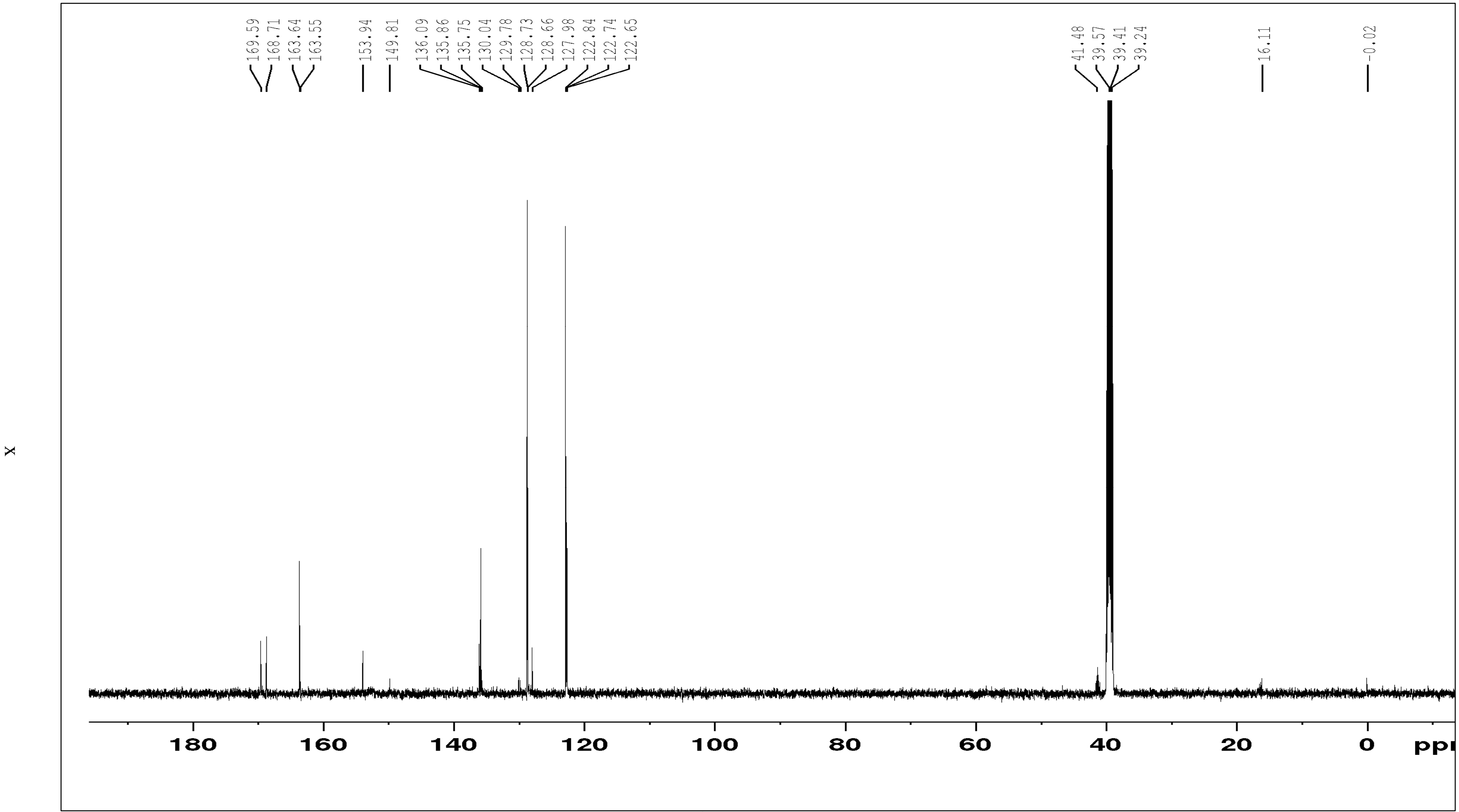
IR spectrum of 6-Chloro-N^2,N^4-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4-diamine (5a)



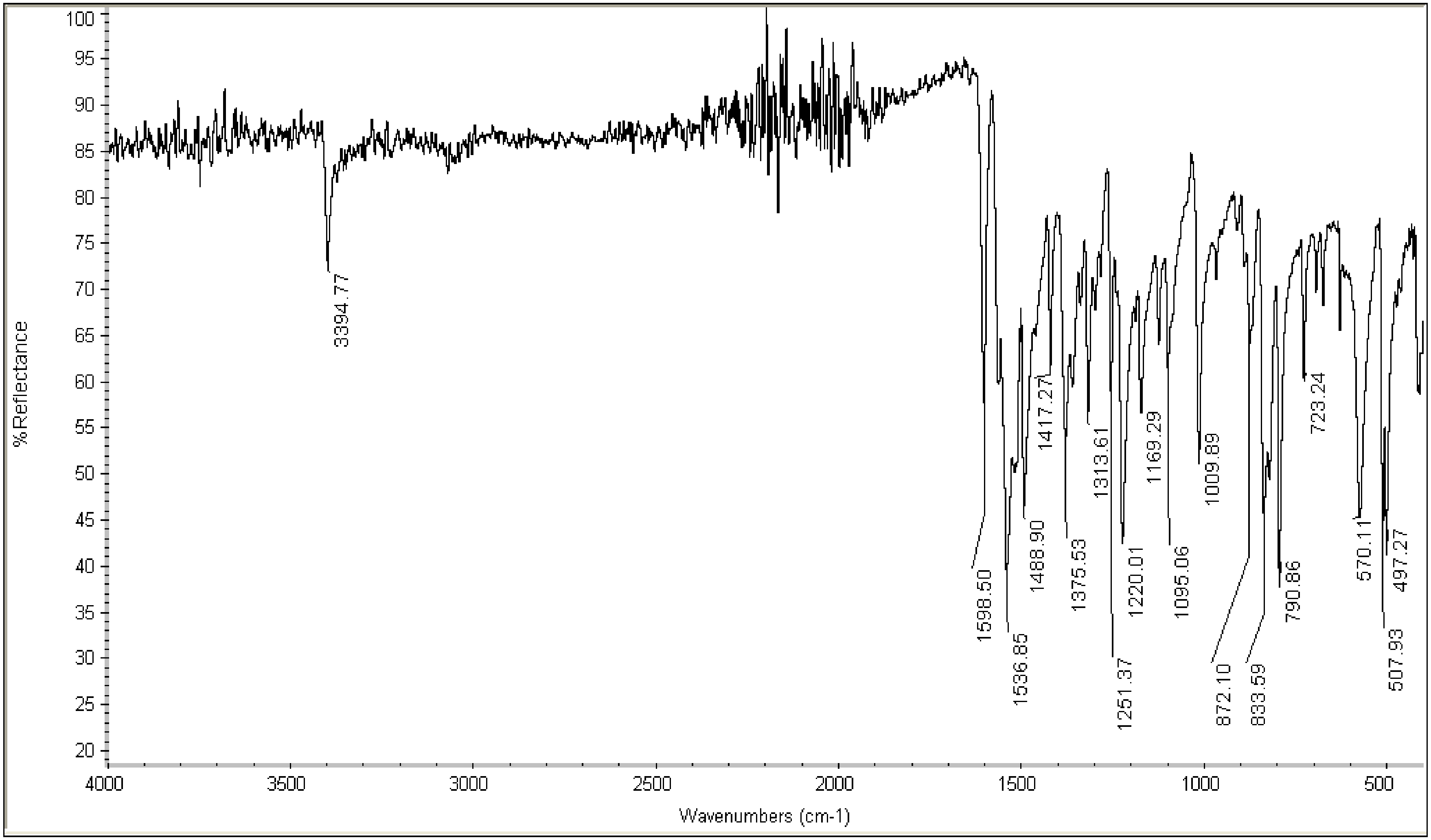
^{1}H NMR spectrum of 6-Chloro-*N*^{2},*N*^{4}-bis(4-chlorophenyl)-1,3,5-triazin-2,4-diamine (5b))



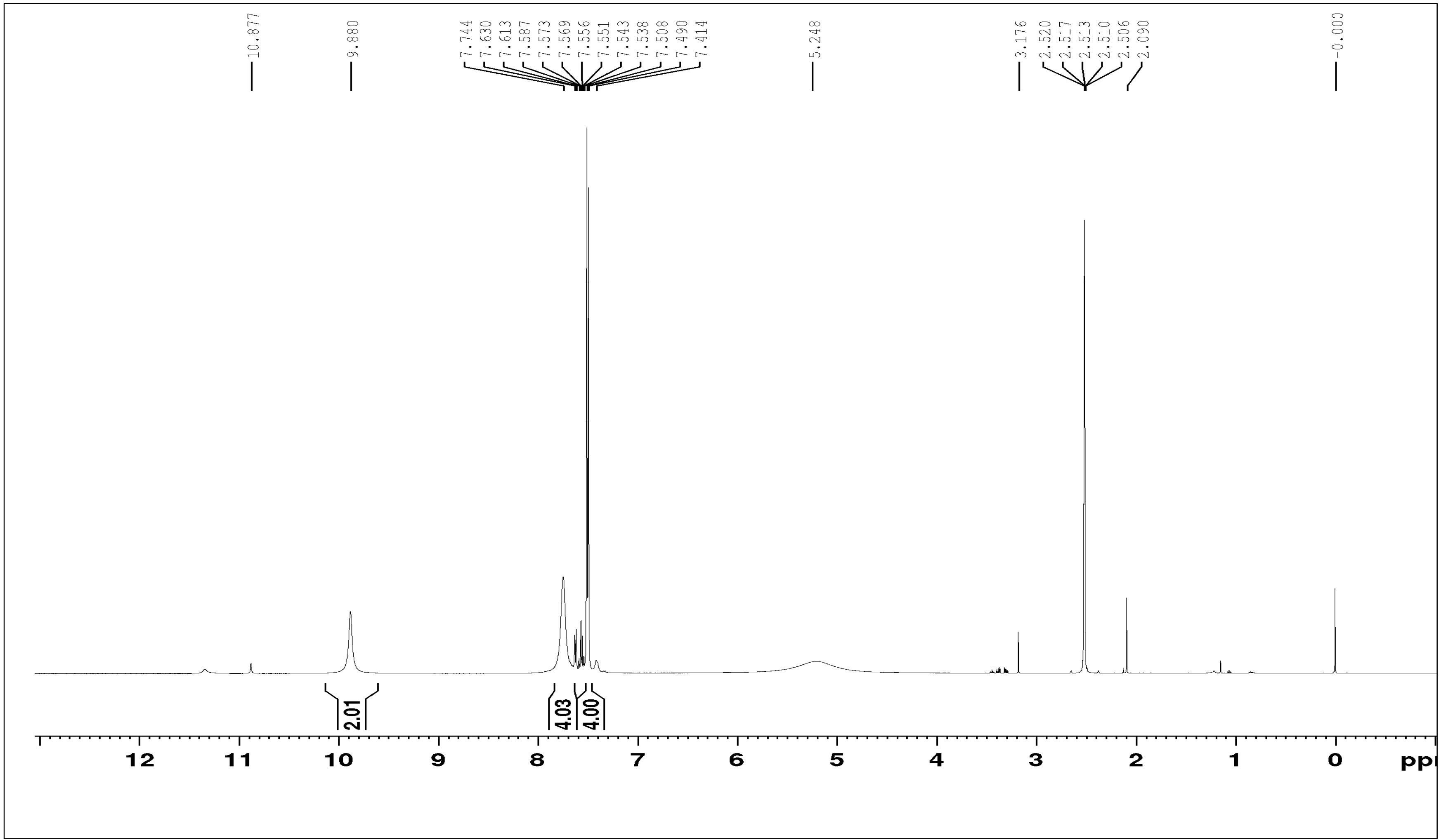
^{1}H NMR - D_2O exchange spectrum of 6-Chloro-*N*^{2},*N*^{4}-bis(4-chlorophenyl)-1,3,5-triazin-2,4-diamine (5b)



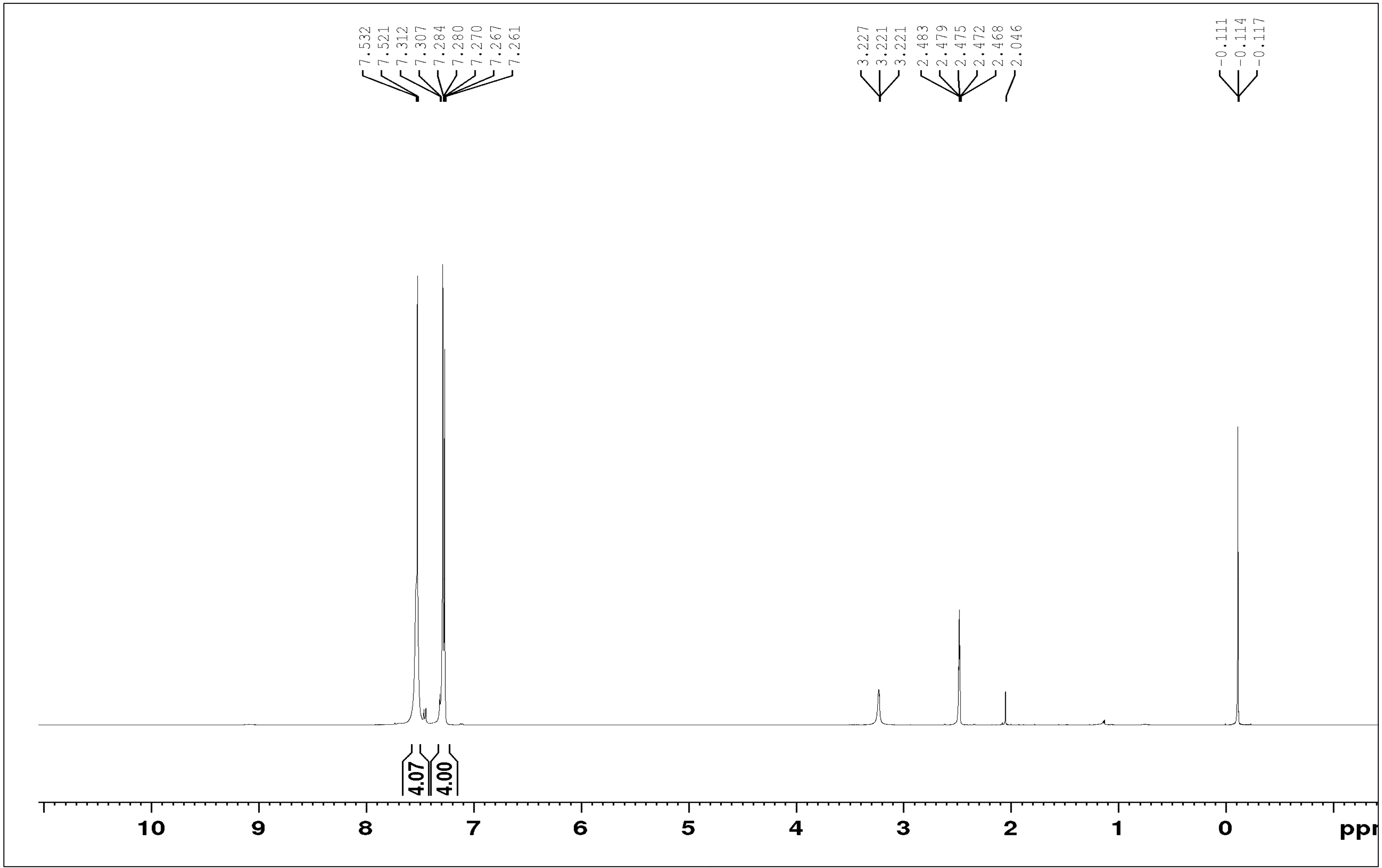
^{13}C NMR Spectrum of 6-Chloro-*N^2*,*N^4*-bis(4-chlorophenyl)-1,3,5-triazin-2,4-diamine (5b)



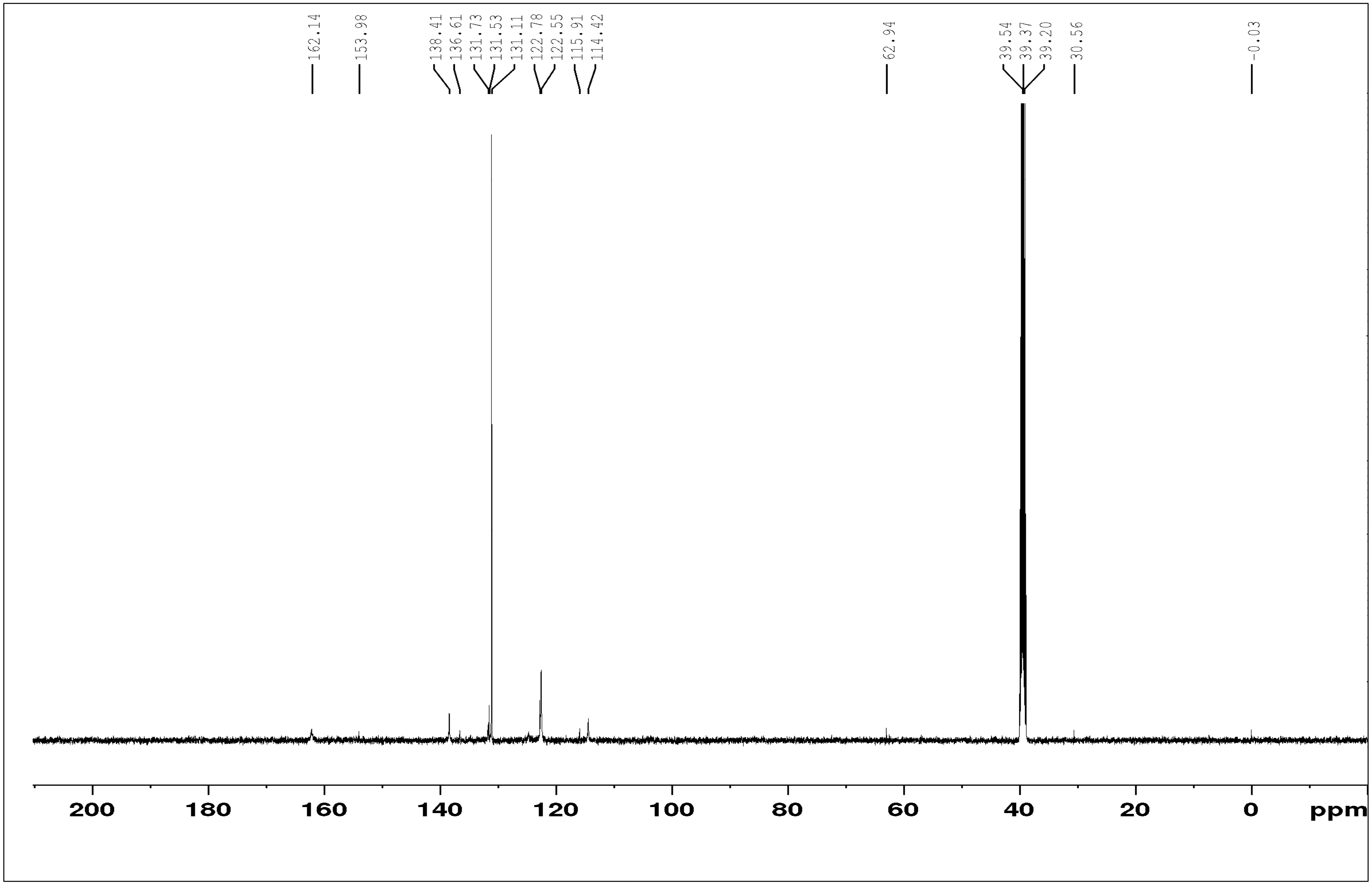
IR spectrum of 6-Chloro-*N*^{2},*N*^{4}-bis(4-chlorophenyl)-1,3,5-triazin-2,4-diamine (5b)



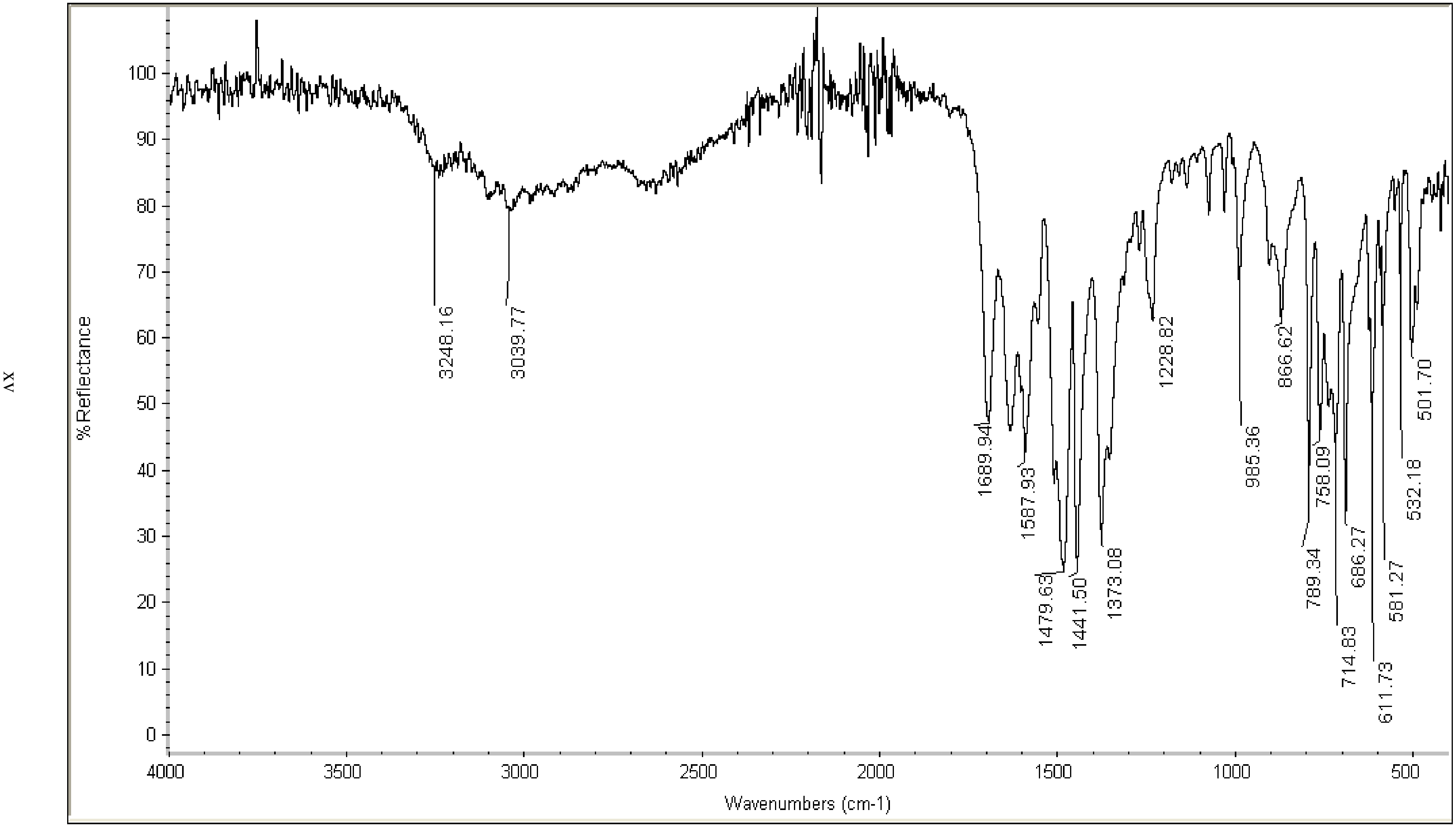
^{1}H NMR spectrum of 6-Chloro-*N*^{2},*N*^{4}-*bis*(4-bromophenyl)-1,3,5-triazin-2,4-diamine (5c)



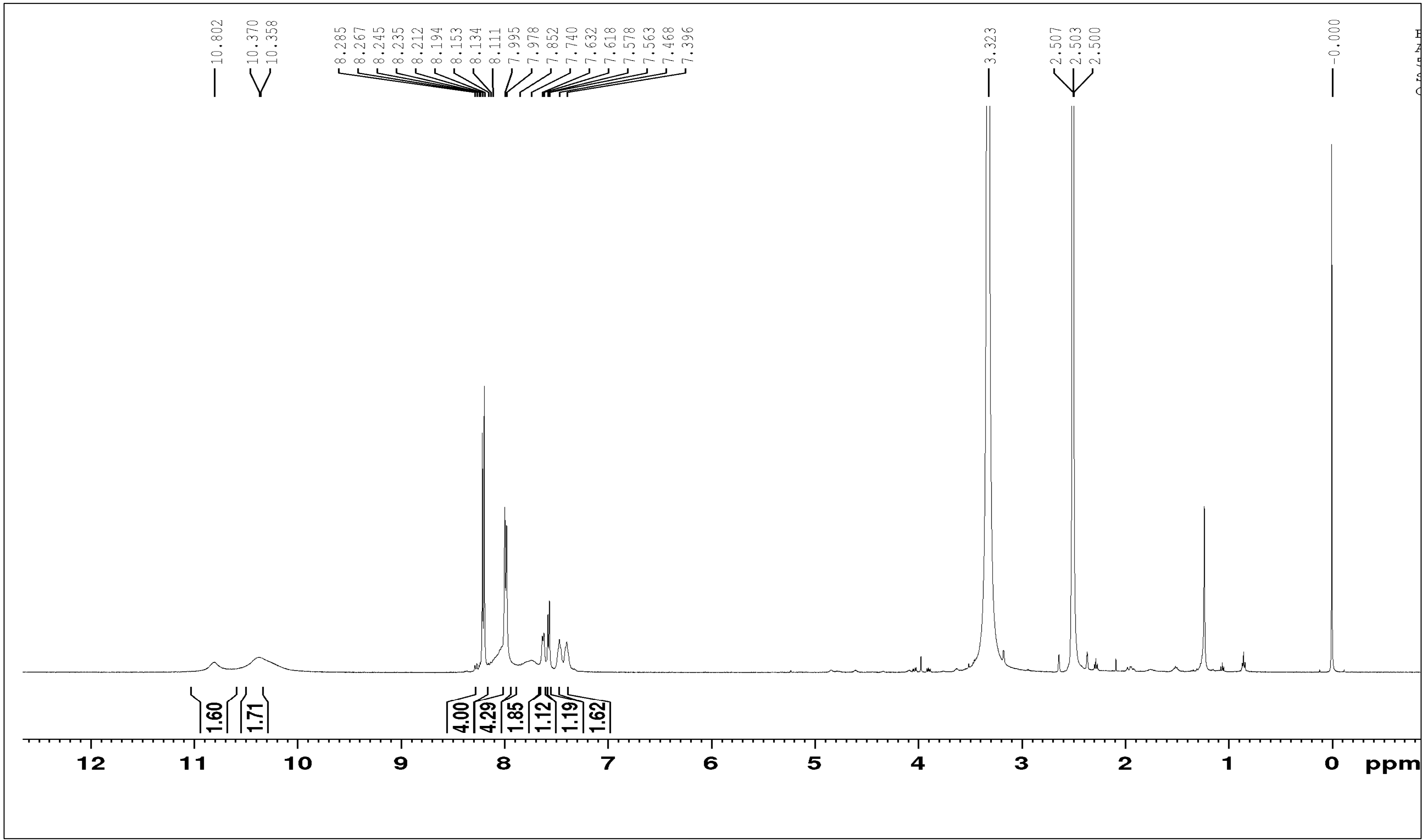
^{1}H NMR - D$_2$O exchange spectrum of 6-Chloro-*N*^{2},*N*^{4}-*bis*(4-bromophenyl)-1,3,5-triazin-2,4-diamine (5c)



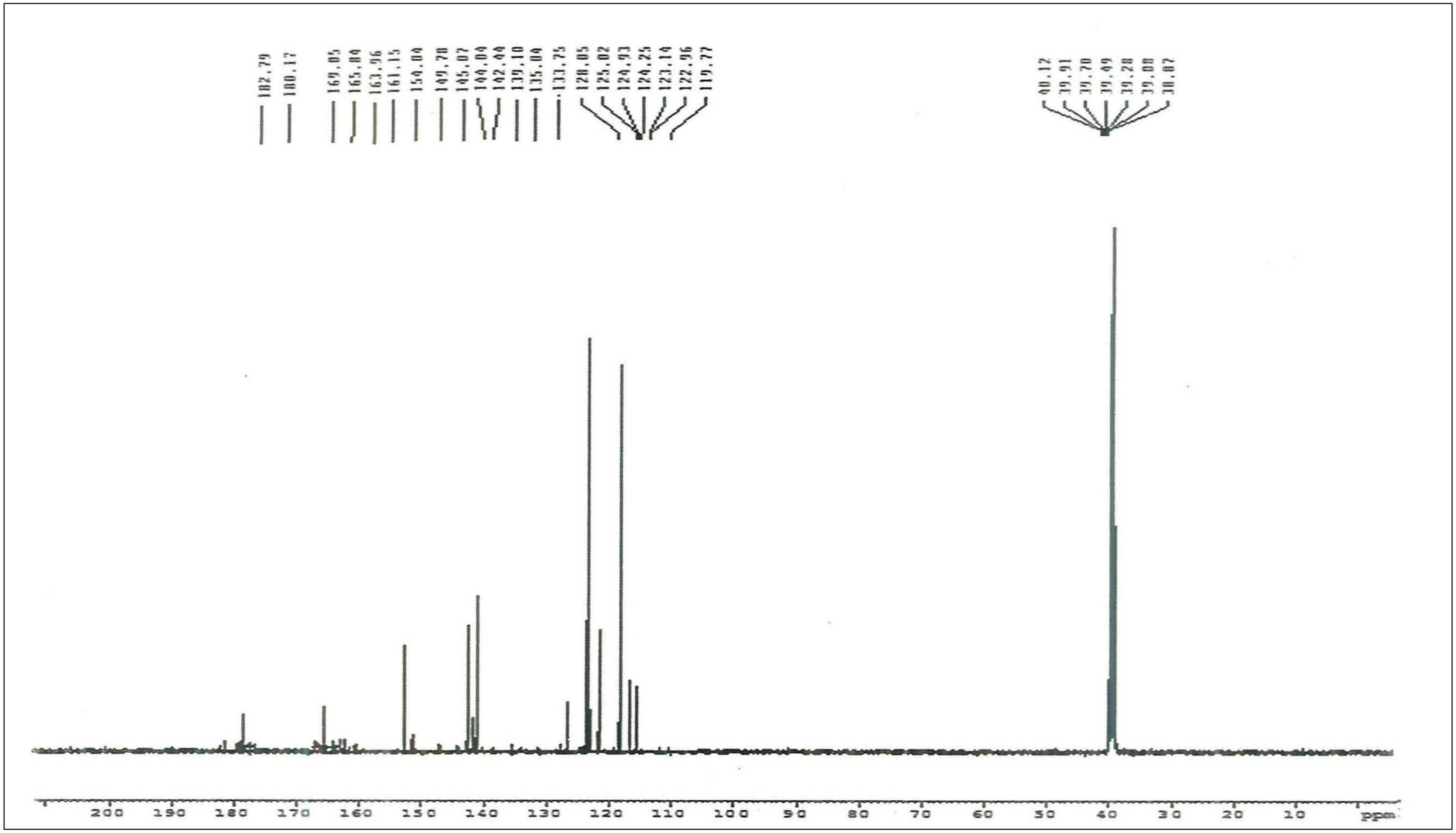
^{13}C NMR Spectrum of 6-Chloro-*N*^{2},*N*^{4}-*bis*(4-bromophenyl)-1,3,5-triazin-2,4-diamine (5c)



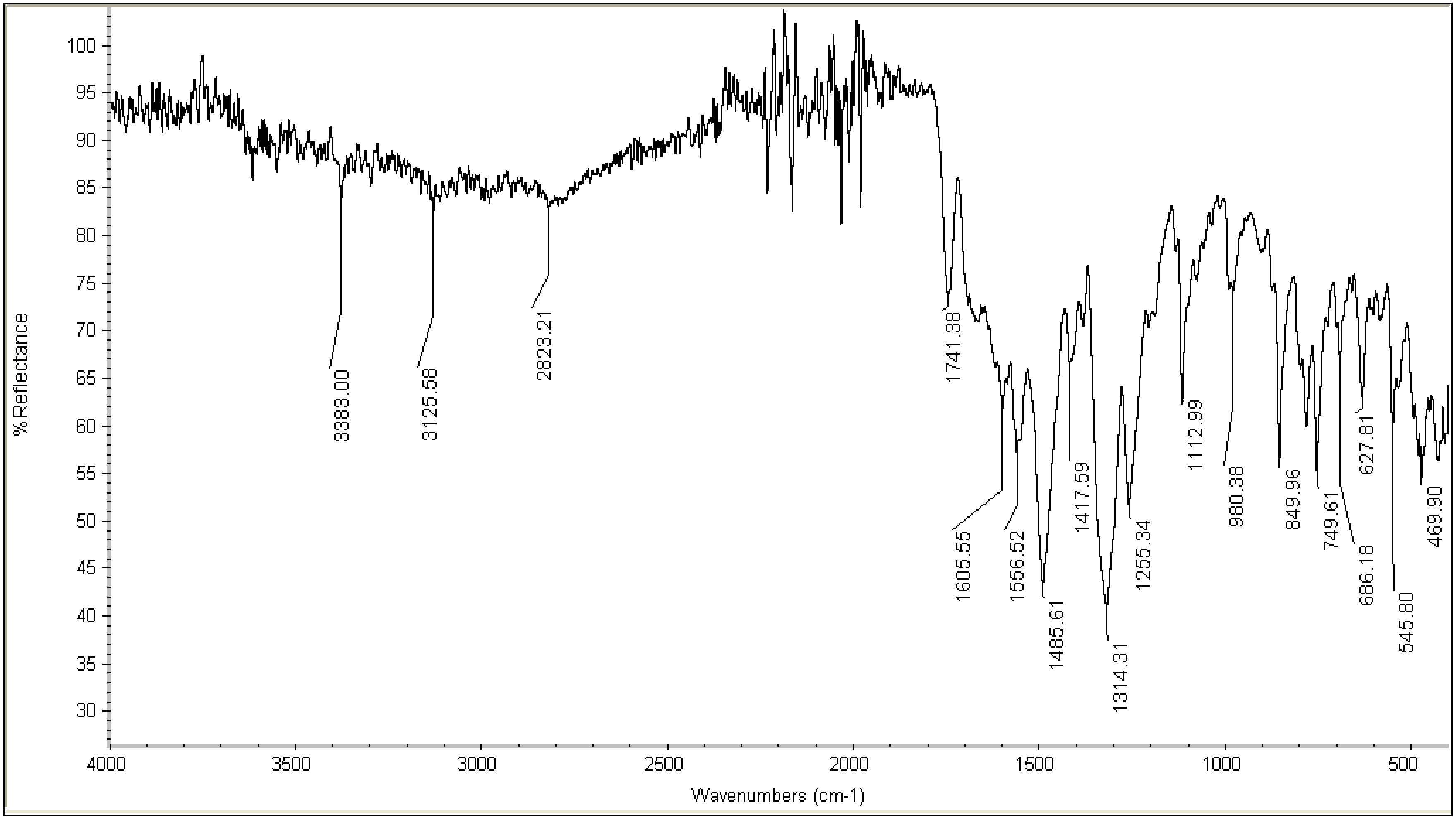
IR spectrum of 6-Chloro-N^2,N^4-*bis*(4-bromophenyl)-1,3,5-triazin-2,4-diamine (5c)



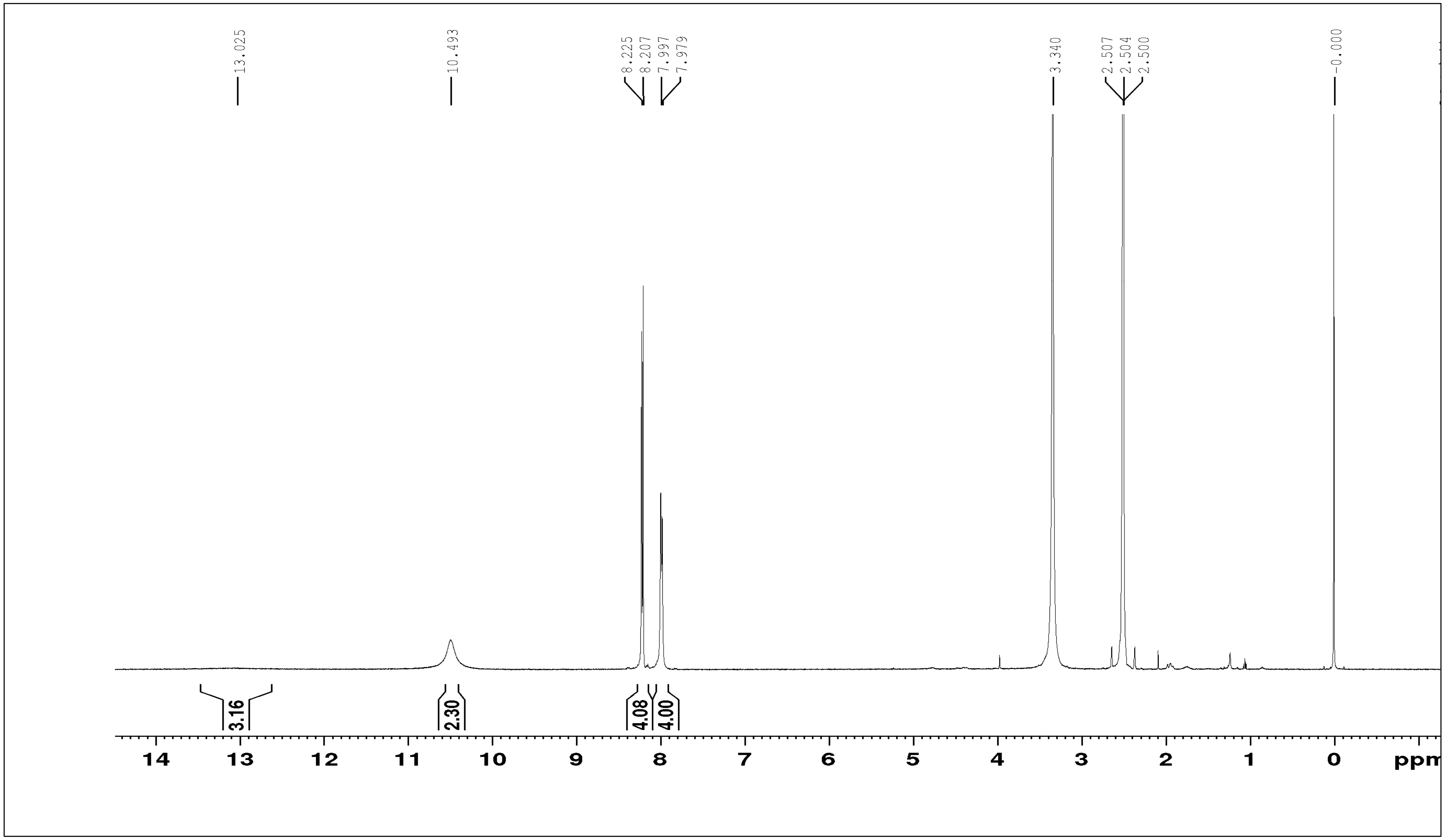
^{1}H NMR spectrum of *N*^{2}-(2-Chlorophenyl)-*N*^{4},*N*^{6}-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4,6-triamine (7a)



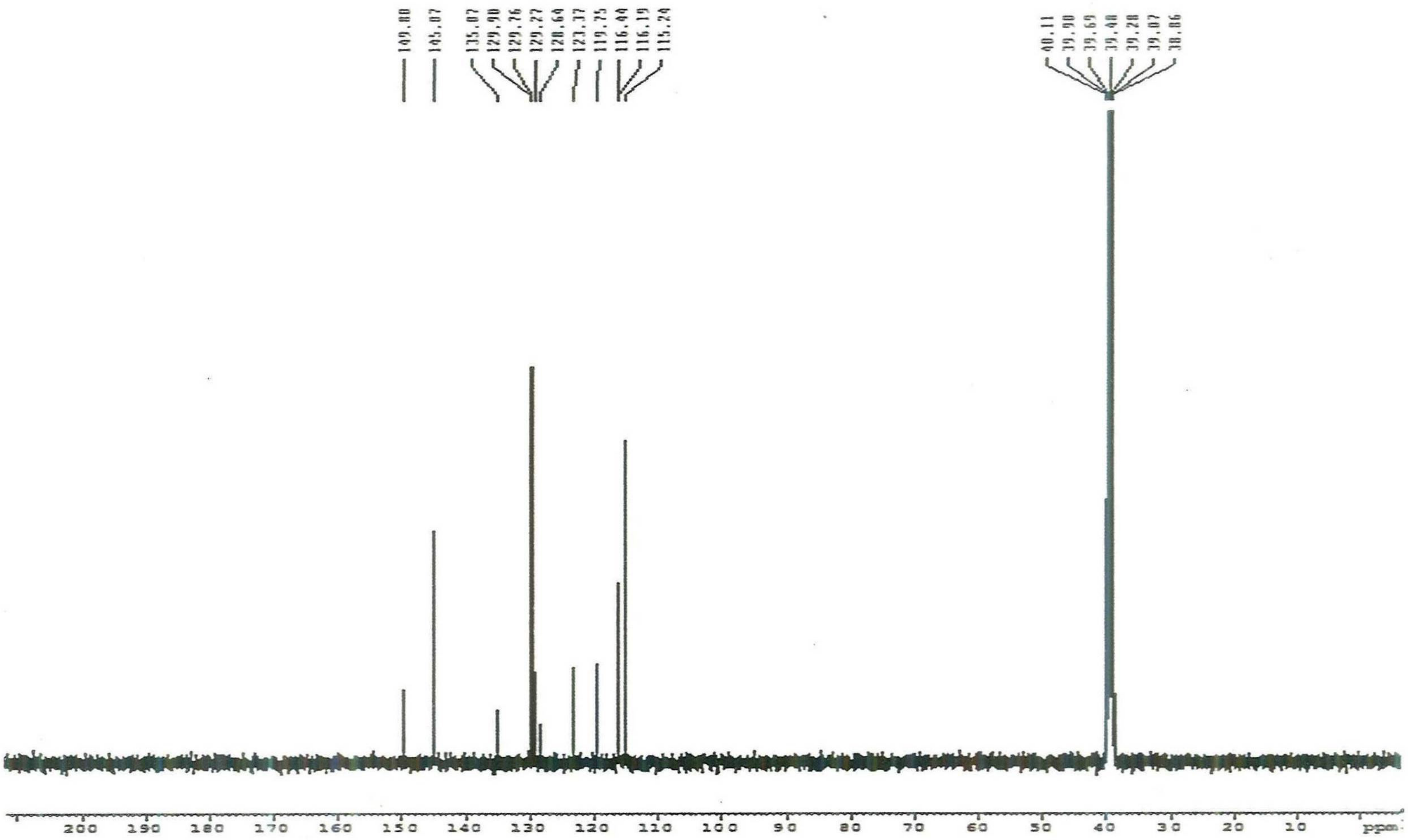
^{13}C NMR Spectrum of *N*^{2}-(2-Chlorophenyl)-*N*^{4},*N*^{6}-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4,6-triamine (7a)



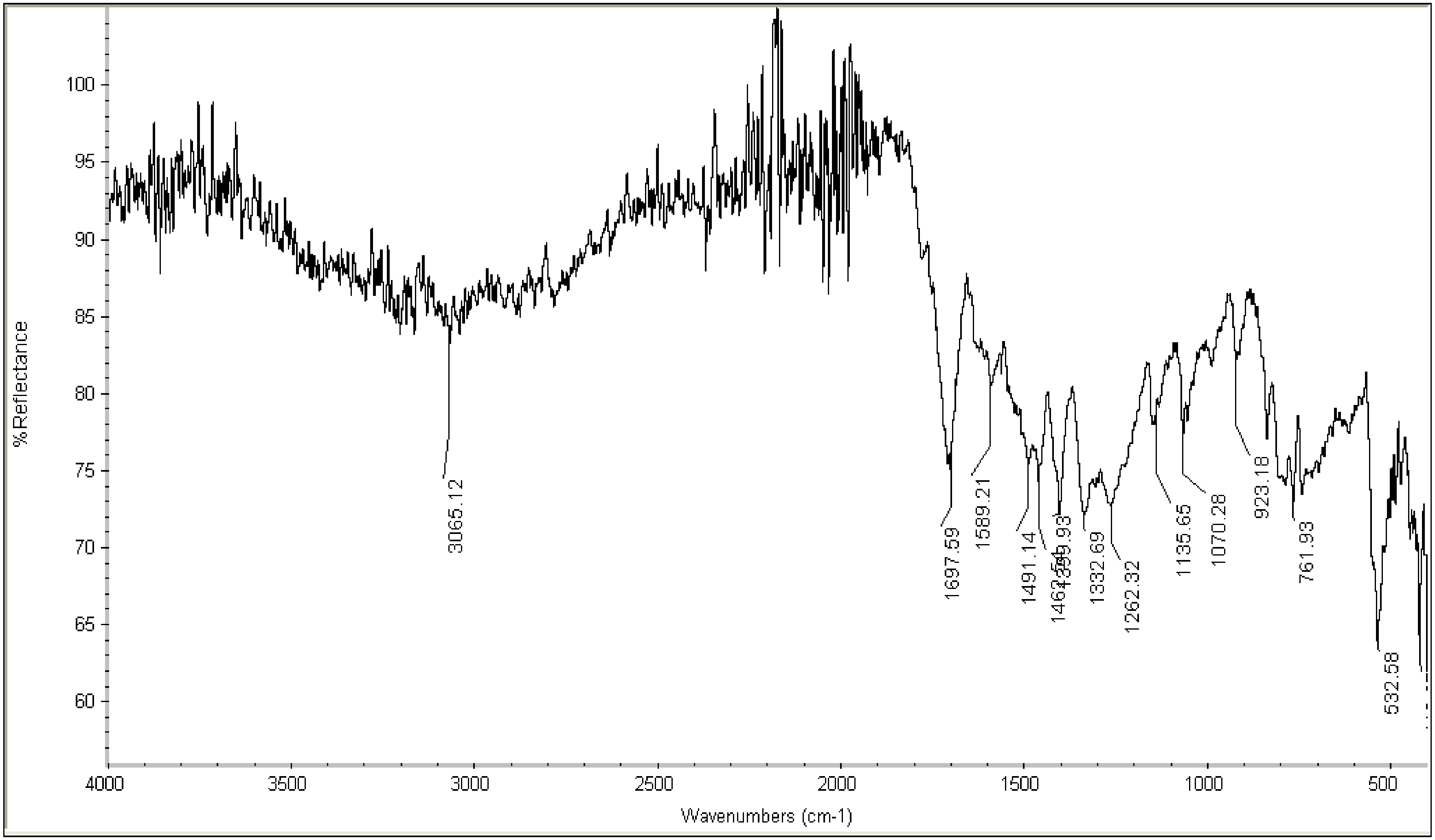
IR spectrum of N^2-(2-Chlorophenyl)-N^4,N^6-*bis*(4-nitrophenyl)-1,3,5-triazin-2,4,6-triamine (7a)



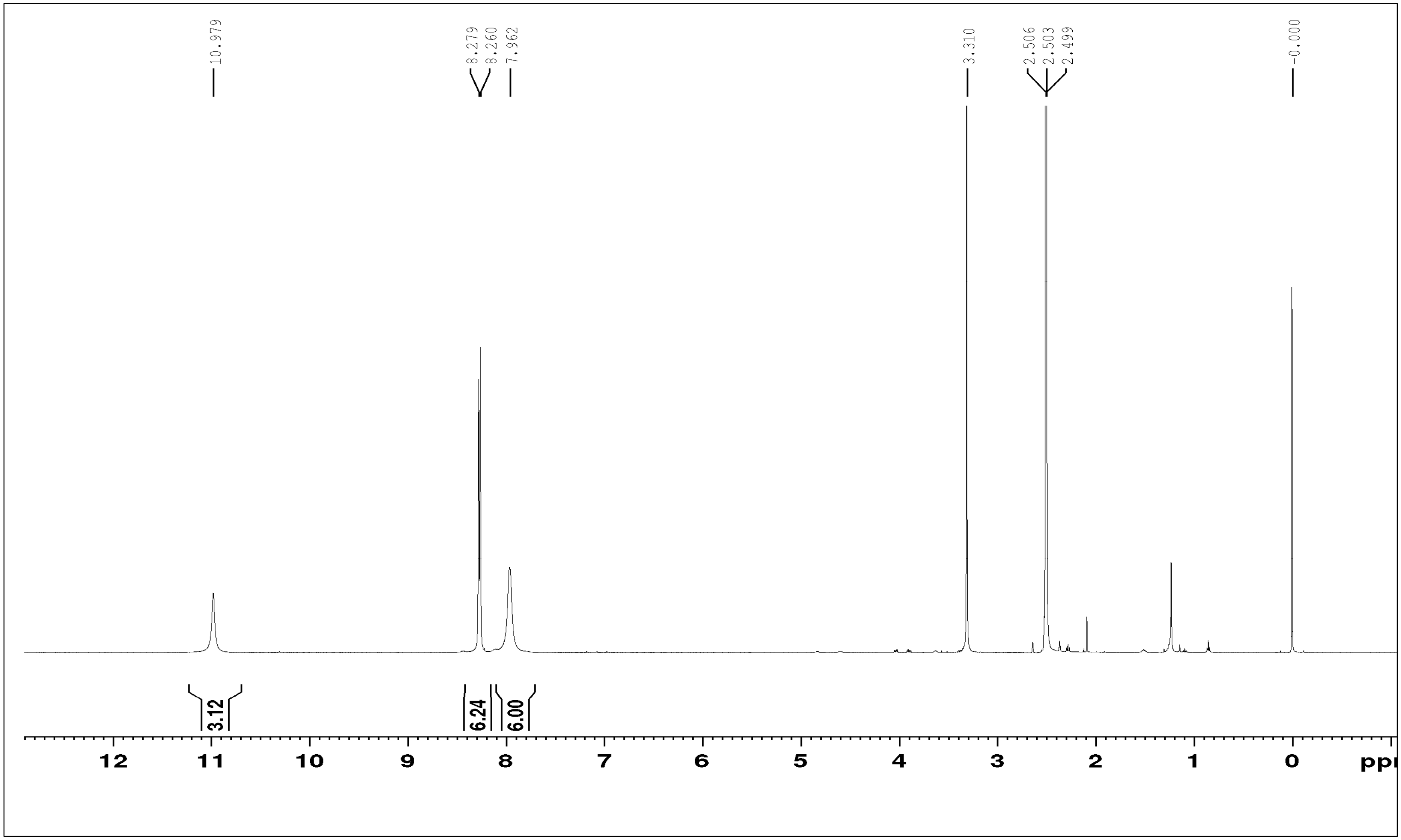
^{1}H NMR spectrum of N^2, N^4-*bis*(4-Nitrophenyl)-N^6-(2,4,6-trichlorophenyl)-1,3,5-triazin-2,4,6 triamine (7b)



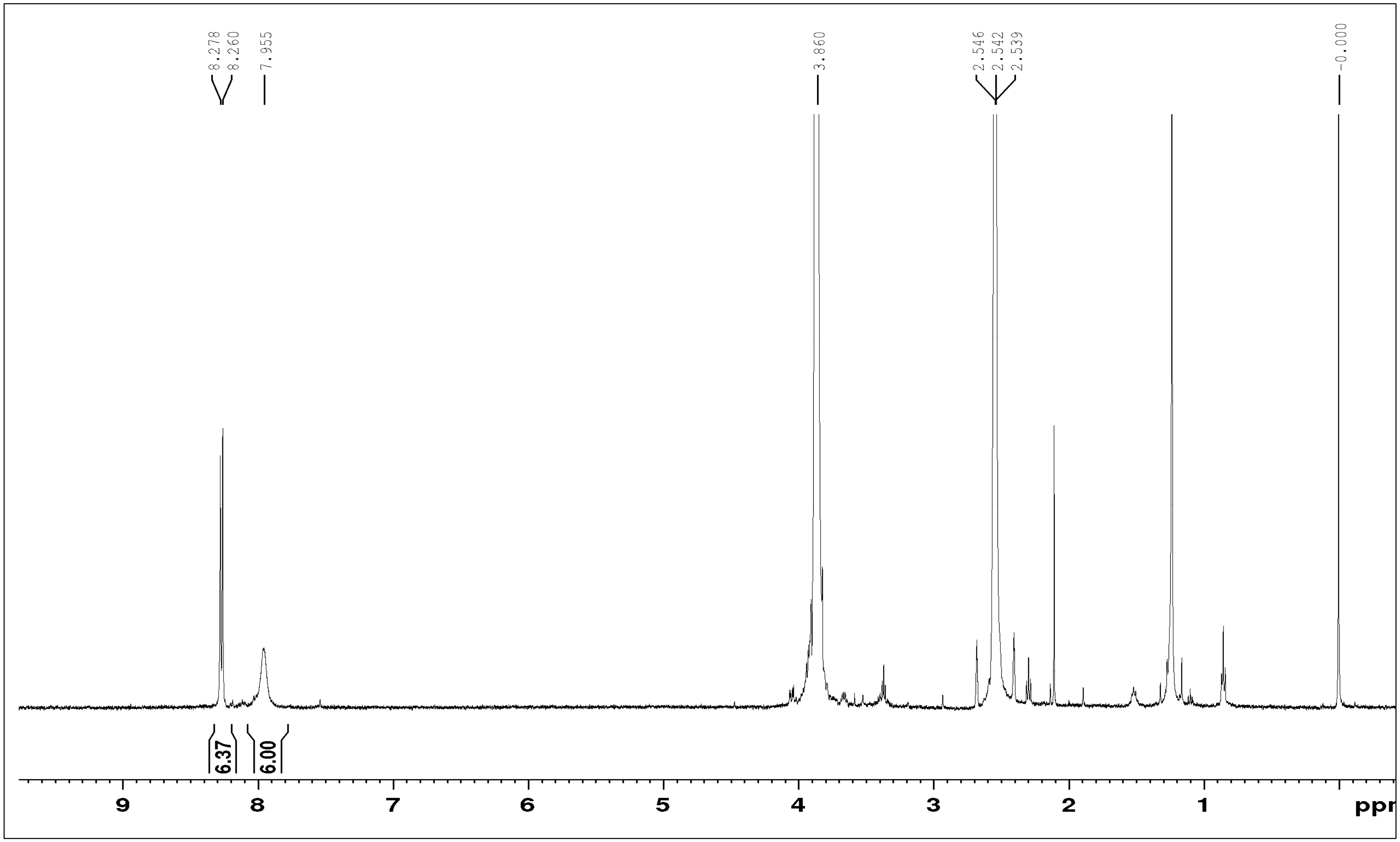
^{13}C NMR Spectrum of *N*^{2}, *N*^{4}-*bis*(4-Nitrophenyl)-*N*^{6}-(2,4,6-trichlorophenyl)-1,3,5-triazin-2,4,6 triamine (7b)



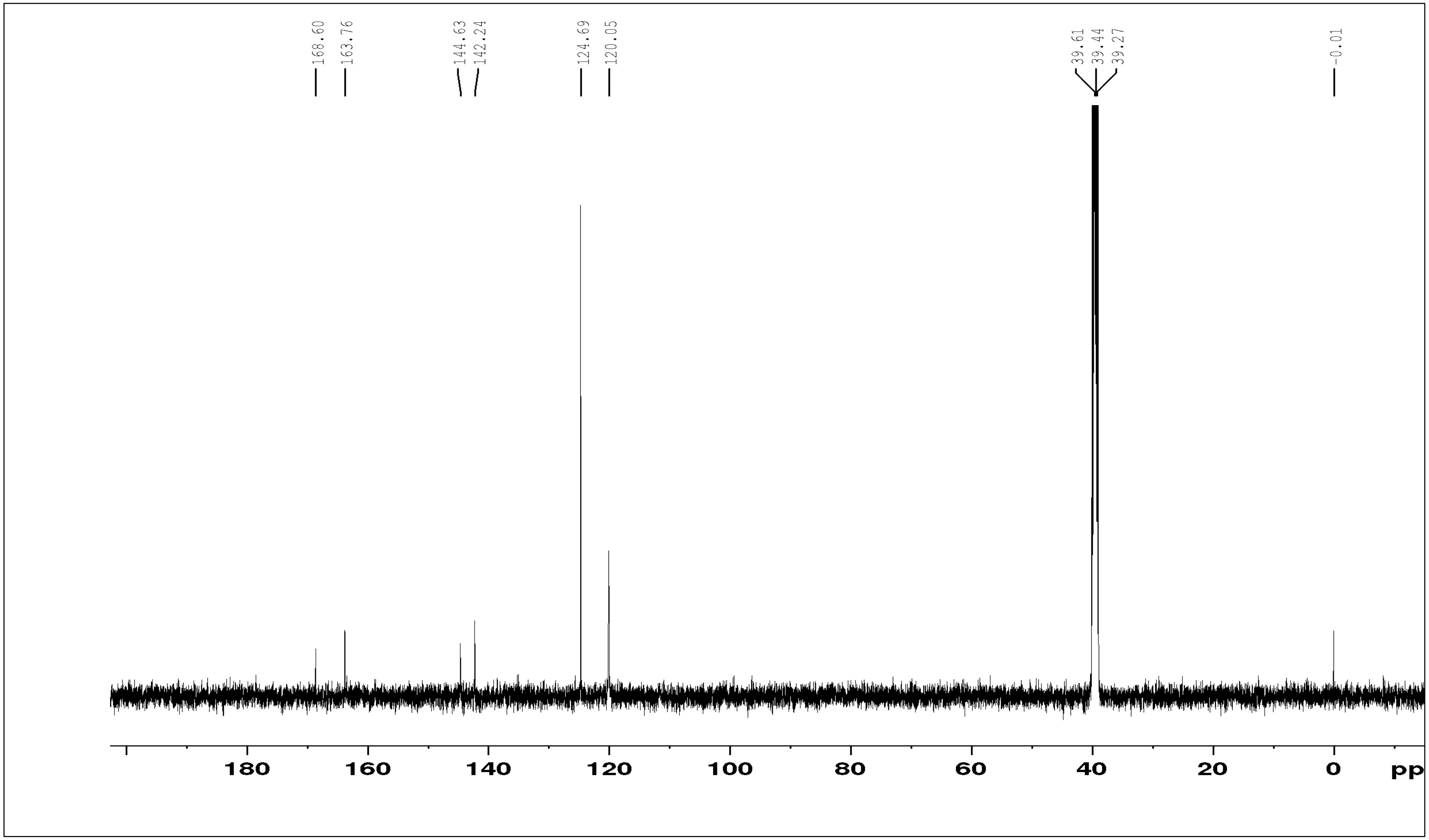
IR spectrum of N^2, N^4-*bis*(4-Nitrophenyl)-N^6-(2,4,6-trichlorophenyl)-1,3,5-triazin-2,4,6-triamine (7b)



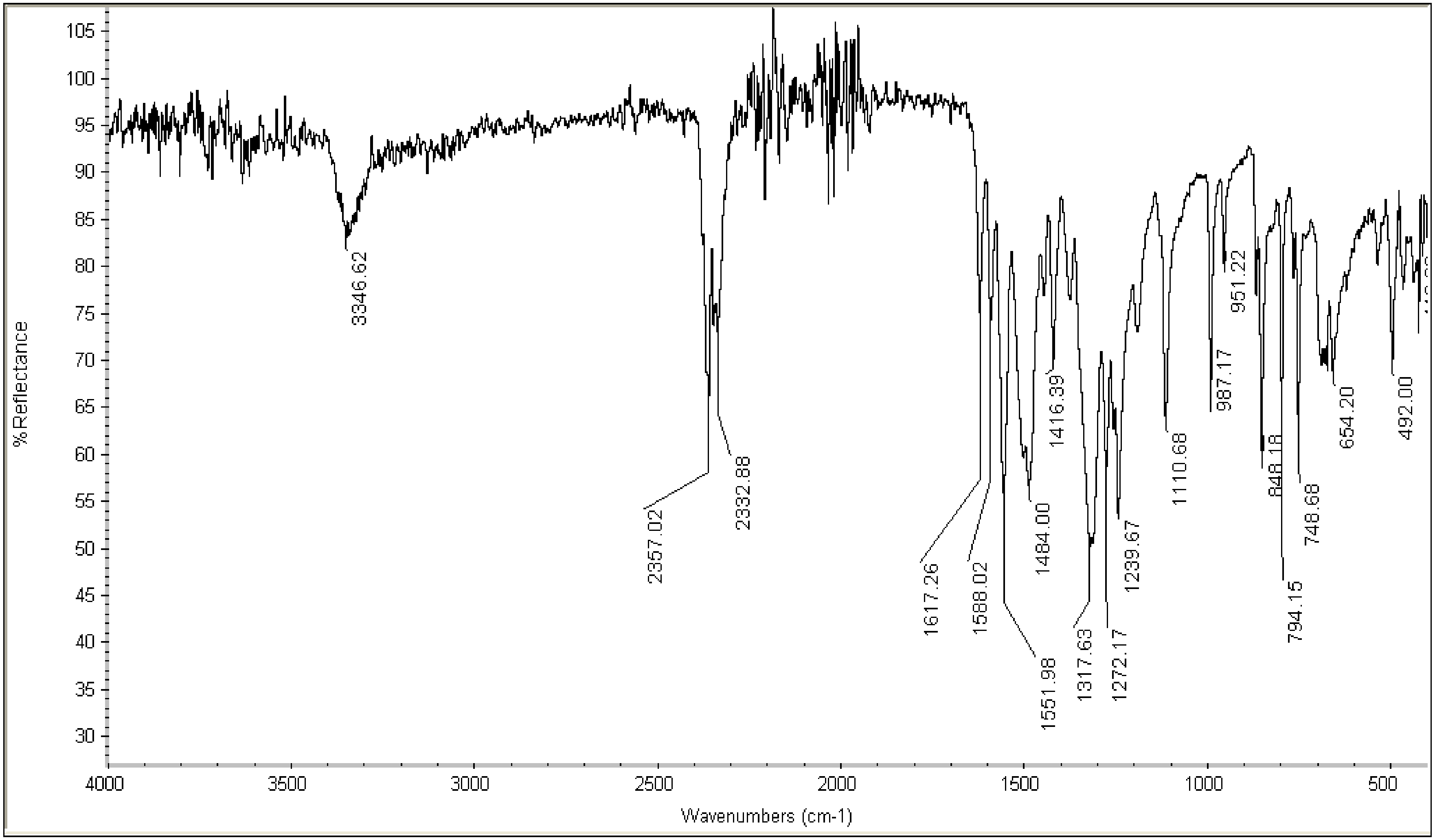
^{1}H NMR spectrum of N^2, N^4, N^6-*tris*(4-Nitrophenyl)-1,3,5-triazin-2,4,6-triamine (8)



^{1}H NMR - D_2O exchange spectrum of *N*^{2}, *N*^{4}, *N*^{6}-*tris*(4-Nitrophenyl)-1,3,5-triazin-2,4,6-triamine (8)



^{13}C NMR Spectrum of *N*^{2}, *N*^{4}, *N*^{6}-*tris*(4-Nitrophenyl)-1,3,5-triazin-2,4,6-triamine (8)



IR spectrum of N^2, N^4, N^6-*tris*(4-Nitrophenyl)-1,3,5-triazin-2,4,6-triamine (8)

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