Synthesis of Novel 6-Amino-Substituted-1,2,4-Triazines scaffolds as Potential Antibacterial Agents

By

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ABSTRACT

1,2,4-Triazine and its derivatives have proven their importance in the biological world since they are used in the treatment of diseases like cancer and HIV/AIDS. Thus this project was aimed at synthesizing novel 1,2,4-triazine-containing compounds, 6-aryl-1,2,4-triazine-3-amines and 6-amino-1,2,4-triazine-3,5-(2H,4H)-diones compounds in particular. Synthesis of 6-amino-1,2,4-triazine-3,5-(2H,4H)-diones started by the bromination of 6-azauracil which was subsequently reacted with different amines like acyclic primary amines, aryl primary amines and secondary amines. Products were obtained in good to excellent yields. Two different classes of 6-aryl-1,2,4-triazine-containing compounds were synthesized. The first class contained the 6-aryl-1,2,4-triazine-3-amine moiety whereas the second class contained the 6-aryl-1,2,4-triazine-3,5-diamine moiety. Synthesis of the first class of compounds commenced by the bromination of 1,2,4-triazine-3-amine which was subsequently reacted with different aryl boronic acids under Suzuki coupling reaction conditions using tetrakis(triphenylphosphine)palladium(0) as a catalyst. Products were obtained in poor to excellent yields. The second class of these novel compounds were obtained by firstly aminating 1,2,4-triazine-3-amine using the Chichibabin reaction followed by the bromination step. The resultant 6-bromo-1,2,4-triazine-3,5-diamine was Suzuki coupled with different aryl boronic acids to obtain desired products in moderate to excellent yields.

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My dear Parents Mr Muvhango Baldwin Mutshaeni and My Late Mother Mrs Khathutshelo Diana Mutshaeni, I know it has always been your wish for me to excel in life and this is token of appreciation to you, “For everything that am starts with you”.

This would not all be possible without God and I know he will continuously provide for me. Amen

DEDICATION

This research is dedicated to the memory of my Mother Mrs Khathutshelo Diana Mutshaeni, She would so proud of my achievements, My late Aunt Professor Humbulani Nancy Mutshaeni, who always encouraged to continuously further my studies, My Dad Mr Muvhango Baldwin Mutshaeni and My Wife Mrs Ndivho Mutshaeni.

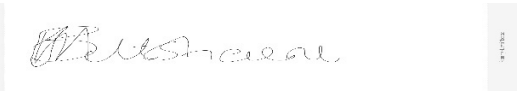
ACRONYMS AND ABBREVIATIONS

BNF	British National Formulary
COX-2	Cyclooxygenase
CYP3A4 DNA	Cytochrome P450 3A4
DNA	Deoxyribonucleic acid
DLD1	Human colon cancer
GABA	γ-Amino butyric acid
FDA	Food and Drug Administration
HA22T	Human liver cancer
HEPG2	Human liver cancer
HONE1	Nasopharyngeal carcinoma
MCF7	Human breast cancer
HIV	Human immunodeficiency Virus
HSF	Human skin fibroblast cells
IFN-γ	Interferon
IL-4 and IL-5,	Interleukin
MAOS	Microwave assisted organic synthesis
MWI	Microwave Irradiation
MES	Maximum Electric Shock
MIC	Minimum Inhibitory Concentration
NSAID	Non-steroidal anti-inflammatory drugs

NCE	Novel Chemical Entities
NUGC	Human gastric cancer
PTC	Phase transfer catalyst
RT	Reverse transcriptase inhibitors
ScPTZ	Subcutaneous pentylenetrazole
SiHa	Uterus cancer cells
TNF-α	Tumor necrosis factor
TH1 and TH2	T-lymphocytes
URAT1	Urate anion exchange transporter 1
WHO	World Health Organization

DECLARATION

I, Fhumulani Baldwin Mutshaeni, declare that this research project is my original work and has not been submitted for any degree at any other university or institution. The project does not contain other persons' writing unless specifically acknowledged and referenced accordingly.

Signed:  Date: 11 March 2022

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1. INTRODUCTION

1.1 HETEROCYCLES

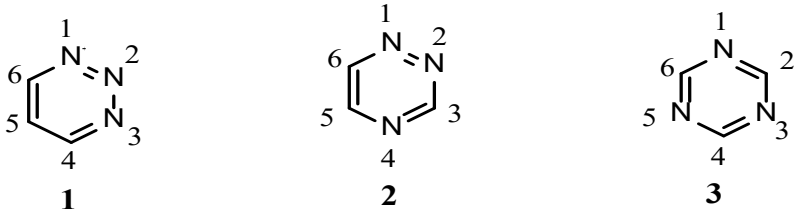
One of the most versatile disciplines of organic chemistry is heterocyclic chemistry. Heterocyclic compounds, both naturally occurring and synthesized, frequently exhibit significant biological activity. (*Bur & Padwa,* 2004). Heterocycles are one of the most important classes of compounds for which experimental data suitable for a basic framework is required in the majority of currently marketed pharmaceuticals, and their inherent complexity, versatility, and unique physicochemical properties have positioned them as true cornerstones of medicinal chemistry. (Martins *et al.,* 2015) (Verevkin *et al.,* 2011). Heterocycles occupy a prominent place in chemistry due to their wide range of applications in the fields of drug design, photochemistry, agrochemicals, dyes and etc, (Sravanthi & Manju., 2016). The presence of heterocycles in all kinds of organic compounds of relevance in electronics, biology, optics, pharmacology, material sciences, and other fields is widely known. (Gupta & Kant., 2013). These are main class compounds which include more than 50% of all well-known organic compounds and can be drugs, vitamins and natural products (Saini *et al*., 2013), Furthermore, heterocycles are by far the most important of the traditional divisions of organic chemistry, and they have a wide range of biological and industrial applications. (Patel, *et al*., 2007), and are found in a wide variety of natural and manufactured medications, as well as being employed as building blocks in organic synthesis. (Marin-Ocampo *et al.,*2019). Over 90% of new drugs contain at least one heterocyclic fragment in their structures (Zhang *et al.,* 2018).

Compounds that contain one or more diverse ring atoms (other than carbon like N, O, S, P, B, Si, As and Se), are known as heterocycles. Amongst them the five and six-membered rings are the most pivotal heterocyclic systems (Kumar *et al.,* 2017). The most frequent heteroatoms are nitrogen, oxygen, and sulfur, but heterocyclic rings containing additional heteroatoms are also well-known. (Al-Mulla *et al.,* 2017).

According to Salih *et al.,* 2008 & Ashardi *et al.,* 2017, nitrogen-containing heterocycles are of higher interest since they are a diverse group of natural and synthetic compounds with a wide range of physicochemical and pharmacological properties. Researchers have paid close attention to nitrogen-containing heterocycles in recent decades due to their promising medicinal potential (Doan et al., 2016). Elsewhere, the synthesis of heterocyclic compounds containing nitrogen has been attracting growing interest due to their use to identify biological receptors with a high degree of binding (Banerjee *et al.,* 2016). Heterocycles containing nitrogen or sulphur have shown to be particularly fascinating due to their physio-chemical properties that are relevant in the development of new medications and materials (Garcia-Valverde *et al.,* 2005). Triazine derivatives are a form of nitrogen-containing heterocycle with a wide range of biological applications (including analgesic, anti-tuberculosis, anti-fungal, anti-cancer, anti-protozoal, antimalarial, antiviral, anti-microbial, and anti-inflammatory activity). As a result, they are regarded as an essential heterocycle scaffold in medicinal chemistry (Marin-Ocampo *et al.,*2019).

1.1.1 What are Triazines?

Triazine is a heterocyclic six membered ring with three carbon atoms substituted by nitrogen atoms, similar to six-membered benzene ring. Theoretically, three different triazines are possible and are represented in **Figure 1**. A triazine with nitrogen atoms at positions 1,2,3 of the benzene ring is known as 1,2,3-triazine. The parent compound of the 1,2,3-triazine series has structure **(1)** and is numbered as indicated. In chemical abstracts it is called 1,2,3-triazine or v-triazine. Triazine with nitrogen atoms at positions 1,2,4 is known as 1,2,4 - or as-triazine **(2)** and the one with nitrogen atoms at positions 1,3,5 is known as 1,3,5 - or s-triazine **(3)**.



1 **2** **3**

Figure 1: Structures of three different triazines.

Although 1,2,3-triazine has had the least research done on it, its derivatives are the most clinically acceptable due to its high efficacy and low adverse effects (Marin-Ocampo *et al.,* 2019).

Among stable azines, 1,2,4,5-tetrazines are the only heterocyclic compounds with the most nitrogen atoms in the ring (Tolshchina *et al.,* 2013). Due to the presence of four electron-withdrawing heteroatoms in the ring, these compounds have unique properties, such as increased electrophilicity, decreased aromaticity, significant azadiene characteristics, and a proclivity for ring opening (Hurst *et al.,* 1995).

1.1.2 1,3,5-Triazine or s-triazine

1,3,5-Triazine, also known as s-triazine, is a versatile, oldest known organic compound, and highly stable, six-membered heterocyclic compound, broadly used as a lead structure. It finds widespread application in the field of construction, transportation, electronics, packaging, industrial machinery, aerospace and consumer products (Mishra & Vasava *et al.,* 2020). Recent developments in synthetic approaches have been explored to produce different substituted s-triazine derivatives. The symmetrical shape of the *s*-triazine derivatives is the major reasons of compounds showing many pharmacological activities and is responsible for its excellent biological profile, also providing exceptional framework for the design of biologically potent molecules (Kumar *et al.,* 2017). Meanwhile, the 1,3,5-triazine moiety allows for the sequential introduction of various substituents in the preparation process of mono-, di-, and tri-substituted 1,3,5-triazines due to its specific structure and electronic properties (Marin-Ocampo *et al.,* 2019). The most frequent reaction of 1,3,5-triazine is the selective nucleophilic displacement reactions of Cl atoms by nitrogen (N), oxygen (O) or sulphur (S) under temperature control (Pal *et al.,* 2005 & Majeed Ganai *et al.,* 2021). 1,3,5-Triazine is the basic structure of some commercial herbicides like atrazine **(4)**, cyanazine **(6)**, and resin modifiers like melamine **(5).**

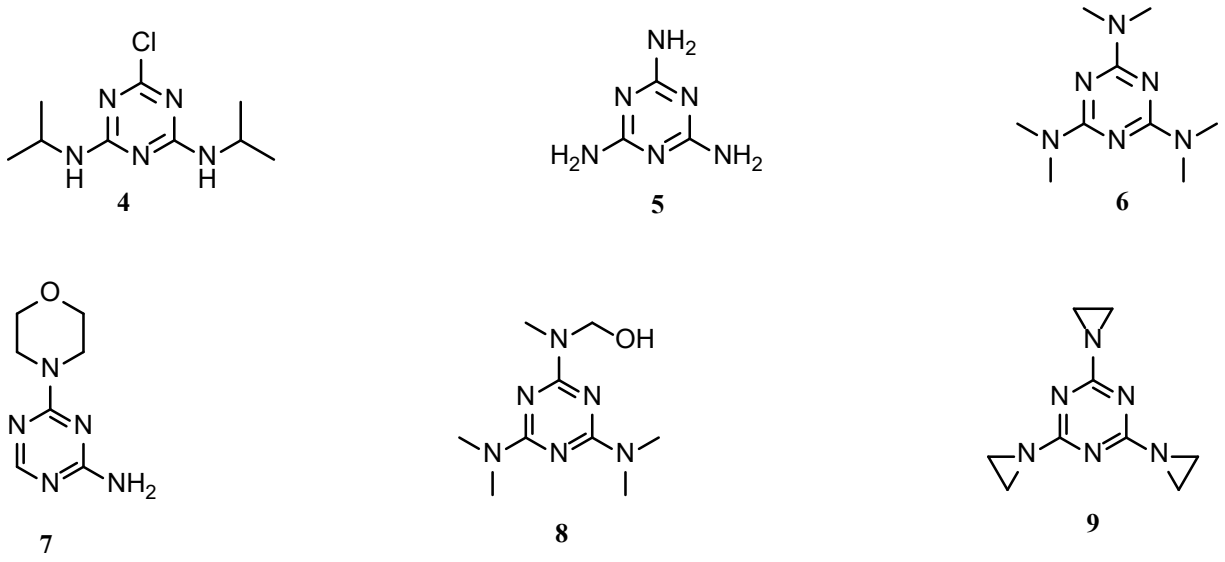


Figure 2: Drugs with anticancer activity that contain 1,3,5-triazine**.**

S-triazine is a well-known family of chemicals that can work as a building block or linker for biologically active materials, dyeing, carbohydrate, protein modifiers, dendrimers, gene therapy and polymer synthesis as a result of their various applicability in various domains (Pal et *al.,* 2005). For breast, lung, and ovarian malignancies, hexamethyl melamine (Altretamine, HMM), **(6)** is a potent anticancer drug. Clinically, the 2-amino-4-morpholino-s-triazine **(7)** is used to treat lung, breast, and ovarian malignancies because of its anticancer characteristics. The hydroxylated metabolite hydroxy methyl pentamethyl melamine **(8)** corresponds to the primary active form of HMM. Triethylenemelamine (Tretamine), **(9)** is injected into the carotid artery as an adjuvant to retinoblastoma radiation therapy. It's also used to treat malignant neoplasms as a palliative treatment (Kumar *et al.,* 2014).

1.1.3 1,2,3-Triazine

There is a small body of literature dealing with uncondensed, aromatic, or hydrogenated 1,2,3-triazines, and most of it focuses on physical factors. Although no attempts at synthesis of unsubstituted 1,2,3-triazine have been recorded, theoretical calculations indicate that some degree of electron delocalization is present, and it may be assumed that it will be a stable product based on the stability of the 4,5,6-trimethyl-1,2,3-triazine

(Neunhoeffer & Wiley.,1978). V-triazine or beta triazine can also be found in ancient literature with the name 1,2,3-triazine. The 1,2,3-triazine nucleus is becoming the preferred choice of researchers for further research due to its clinical output and safety margin (Kumar *et al.,* 2014). Currently, 1,2,3-triazines are a class of biologically active compounds that represent a widely used lead structure with a variety of interesting applications in a variety of pharmacological fields, including antibacterial, antifungal, antiviral, antiproliferative, analgesic, and anti-inflammatory properties (Cascioferro *et al.,* 2017).

To date a diverse number of pharmacological actions that have been documented and investigated include and are not limited to, v-triazine containing compounds like tubercidin **(10)**, toyocamycin **(11)**, sangivamycin **(12)** and 2-azaadenosine **(13)** are the important active moieties in pharmaceutical field (Kumar *et al.,* 2017). Elsewhere the systematic approach towards 1,2,3-triazine derivatives possessing antitumor activity combined with an ordinary synthesis confers to these molecules an immense potential as building blocks for the development of antitumor compounds with potent efficacy (Cascioferro *et al.,* 2017).

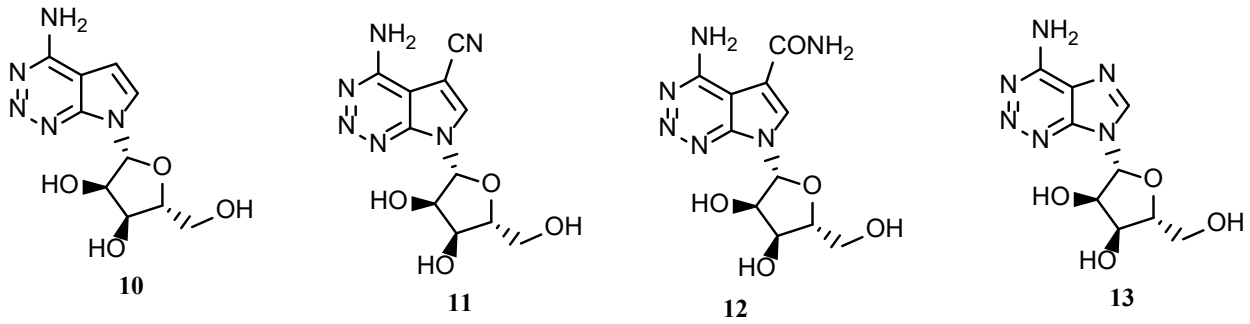


Figure 3: Drugs containing 1,2,3-triazine moiety.

1,2,3-Triazines, also known as vic-triazines or v-triazines, are the least studied of the three isomers. This is likely due to the ring system being the least stable of the three. Instead, they have piqued the interest of researchers due to their potent biological features. Structural investigations have been carried out for 1,2,3-triazine, and respective modified triazines, including pyridazine and triazine which have not been reported (Yamaguchi *et al.,* 1983). Beside the antitumor activity, as previously stated, monocyclic

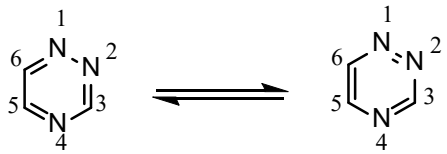
1,2,3-triazines have no additional biological data besides antibacterial, antiviral, analgesic, antiinflammatory, antihistaminic, antiangiogenic, and antifungal properties (Cascioferro *et al.,* 2017).

Tubercidin **(10)**, Toyocamycin **(11)**, and Sangivamycin **(12)**, have substantial pharmacological actions, are examples of drugs with a 1,2,3-triazine ring that come from synthetic source. Tubercidin inhibits the growth of a variety of bacteria. Toyocamycin is a well-known anticancer antibiotic that has a unique antitumor effect. Sangivamycin has been shown to be effective against leukemia and Lewis lung carcinoma in humans, as well as colon cancer, gall bladder cancer, and acute myelogenous leukemia (Kumar *et al.,* 2014).

Research around this non-symmetrical 1,2,3-triazine ring has gained momentum due to the ring's ability to easily give up the N2 molecule in a reaction, has led to tremendous interest in its synthetic reactions. Although there are no natural occurring compounds containing this ring have been discovered to date (Zvarych et al., 2019). The 1,2,3-isomer has been assigned due to similarities in band patterns in the microwave spectra of the isomeric 1,2,3- and 1,2,4-triazines (Morina *et al.,* 2019).

1.1.4 1,2,4-Triazine

According to, Neunhoeffer & Wiley., 1978 "the 1,2,4-triazine or as-triazine, and iso-triazine as it is called In the Ring *Index*, are names also found in older literature. For the purposes of this study we will refer to it as 1,2,4-triazine, and the two Kekulê structures, **(14a)** and **(14b)** shows the ring occurs in nature. Of the two structures it has been proven through theoretical studies that structure **(14a)** is more stable than **(14b)** and as such **(14a)** is preferred for synthetic reaction. For that particular reason, structure **(14a)** will be used throughout this document to express the aromatic 1,2,4-triazines.



14a **14b**

Figure 4: Kekulê structures of 1,2,4-triazine.

The most widely studied compounds for therapeutic potential and pharmacological action are those having a 1,2,4-triazine nucleus (Marin-Ocampo *et al.,* 2019). 1,2,4-Triazine ring has been extensively explored for its biological and pharmacological applications and according to Kidwai *et al.,* 2001 it has diverse pharmacological activities such as antihypertension, antileukemic and anti-inflammatory. The 1,2,4-triazine nucleus is a significant chemical synthon with a wide range of medicinal properties, including cyclooxygenase (COX-2) inhibition and anticancer activity. The synthesis of novel chemical entities (NCEs) containing this essential ring system remains a topic of ongoing interest due to a variety of proven biological functions linked with it (Banerjee *et al.,* 2016

1.2 Known Triazine-containing Drugs

1.2.1 Drugs containing 1,2,4–triazine ring

There have been twenty-two investigations on the anti-inflammatory activity of 1,2,4-triazines to date, with a total of 197 derivatives published. 67 of these were thought to be promising anti-inflammatory drugs (Marin-Ocampo *et al.,* 2019). Because of their considerable potential for pharmacological actions, 1,2,4-triazine and its analogs play an important role in modern medicinal chemistry (Kumar *et al.,* 2014). Since some of these compounds have been discovered to exhibit interesting biological properties and have been employed in medicinal chemistry as medicines (Nyffenegger *et al.*, 2007), Azapropazone **(15)** is an anti-inflammatory, analgesic, antipyretic, and uricoustic drug used to treat rheumatoid arthritis, gout, osteoarthritis, and ankylosing spondylitis are all conditions that affect the joints. Gastrointestinal (GI) adverse effects such as nausea, epigastric discomfort, and dyspepsia make it unsuitable for use. However, azapropazone **(15)** should only be used in circumstances where other NSAIDs have failed (Banerjee *et al.,* 2016).

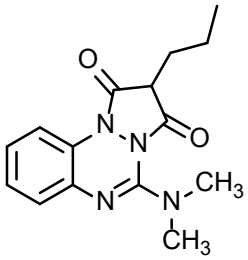


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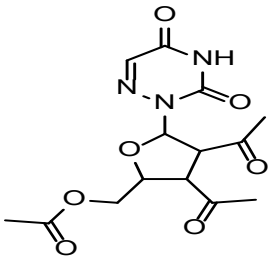
Figure 5: Structure of azapropazone.

Morina *et al.,* 2019 states that a plethora of 1,2,4-triazine derivatives has garnered attention in the pharmaceutical and other industries, rekindling the researchers previous spectroscopic interest in the compound. 1,2,4-Triazine derivatives used in clinical trials are 6-azacytosine, 6-azauracil, ceftriaxone, pymetrozine, fervenulin (planomycin), reumycin and toxoflavin (panthothricin (Kumar *et al.,* 2017).

Azaribine **(16)** and Lamotrigine **(17) (Figure 6)** are examples of medicines with a 1,2,4-triazine ring that come from both natural and synthetic sources and have major biological activity. Azaribine **(16)** is a well-known antiviral whereas, Lamotrigine **(17),** a 6-phenyl-1,2,4-triazine derivative, is used as an antiepileptic medication with a broad spectrum of action (Sztanke *et al.,* 2006).



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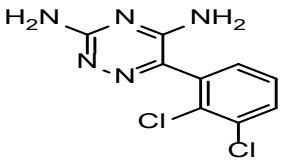

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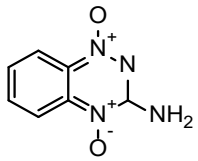
Figure 6: Structures of Azaribine and Lamotrigine.

Following one-electron bioreductive activation, Tirapazamine **(18)** is a promising anticancer drug that preferentially causes DNA damage in hypoxic tumor cells (Fuchs *et al.,* 2001). The free radical mechanism by which the anticancer medication tirapazamine (3-amino-1,2,4-benzotriazine 1,4-dioxide) generates hypoxia-selective cytotoxicity is seen as a promising avenue for developing clinically relevant bioreductive medicines

against chemo- and radiation-resistant hypoxic tumor cells (Anderson *et al.,* 2005 & Costa *et al.,* 1989). Dihydromethyl furalazine **(19)** exhibited antibacterial efficacy against a wide range of Gram-positive and Gram-negative pathogenic pathogens. Gram-negative rods like Shigella and Salmonella have been discovered to be very sensitive to the antibiotic (Kobari *et al.,* 1970). One of the compounds of nitrofuran, dihydroxymethyl furalazine, has been investigated as one of the medications that could answer the purpose and investigation of furalazine in terms of mode of action and efficacy carried out in order to clarify the mechanism of cholera therapy (Nakatomi ., 1971).



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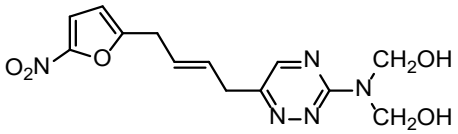

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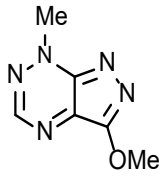
Figure 7: Structures of Tirapazamine (**18**) and Dihydromethyl furalazine (**19**)

Pseudomonas fluorescens var.pseudoidinum and Nostoc *spongiaeforme* create a family of naturally purine analogues that have attractive spectroscopic and biochemical properties for application in cancer and viral treatment. The pyrazolo [4,3-e][1,2,4]triazine ring system has a relatively unusual bicyclic nitrogen-rich skeleton, and the compounds have a variety of brilliant colors. Pseudoiodinine **(20),** fluviol A **(21),** and nostocine A **(22),** which correspond to structures in **Figure 8**, are the most important members of this family (Kumar *et al.,* 2014).



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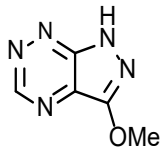

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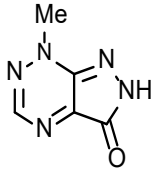

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Figure 8: Structures of Pseudoiodinine**,** fluviol A**,** and nostocine A .

1.2.2 Anticancer, Antimicrobial, Anti-inflamatory, Anti-inhibitory and Antiviral Activity of Drugs containing 1,2,4-triazine ring.

Numerous small molecules possessing a 1,2,4-triazine scaffold have been shown to exhibit a great variety of pharmacological effects (Krauth *et al.,* 2010). Compound **(23)** displayed strong inhibitory effects against SiHa and LS180 tumor cells while also being non-toxic to HSF cells, a human normal cell line. It is also effective at breaking the Deoxyribonucleic acid (DNA) strands of the cancer cells studied, has a strong affinity for SiHa cancer cells, and has statistically significant apoptotic activity in T47D carcinoma cells (Sztanke *et al.,* 2008). In vitro tests were performed on all of the substances to see if they have antitumor and antimetastatic properties. Furthermore, their cytotoxicities against HSF cells, a human normal cell line, were determined in order to highlight some structure–activity connections (Sztanke *et al.,* 2009).

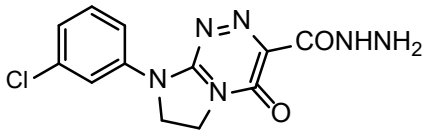

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Figure 9: Structure of 8-(3-chlorophenyl)-4-oxo-7,8-dihydro-6H-4l5,5l4-imidazo[2,1-c][1,2,4]triazine-3-carbohydrazide

The analgesic effectiveness of some new 1,2,4-triazine analogs was tested utilizing an acetic acid-induced writhing experiment. The activity of all synthesized compounds was comparable to that of indomethacin (Amin *et al.,* 2009). Some produced compounds were tested in vivo for anti-inflammatory action using a conventional acute carrageenan-induced paw edema technique. In rats, the most potent anti-inflammatory drugs were tested for ulcerogenic potential against indomethacin and celecoxib as reference standards (El-Moghazy *et al.,* 2012). Triazino analog **(24)** It had the most activity when compared to its 5-membered imidazo and triazolo analogs (Amin *et al.,* 2009).

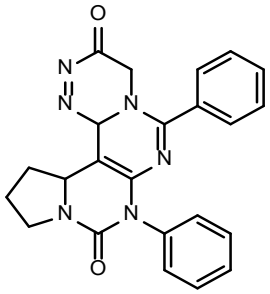

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Figure 10: Structure of triazino analog.

Novel compounds were synthesized containing the disc agar diffusion method, used to test the antibacterial activity of 1,2,4-triazine analogs. In comparison to the conventional medication, compound **(25)** showed moderate effectiveness against B. subtilis trimetroprim (Kardry *et al.,* 2008*)*.

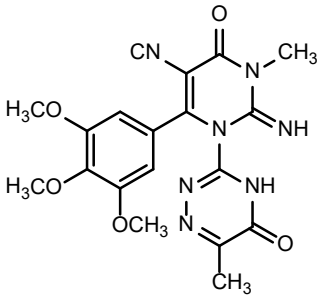

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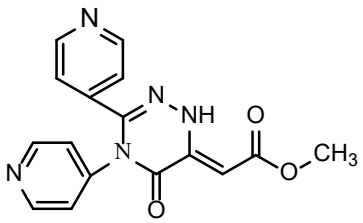
Figure 11: Structure of 3-(2-imino-5-isocyano-3-methyl-4-oxo-6-(3,4,5-trimethoxyphenyl)-3,4-dihydropyrimidin-1(2H)-yl)-6-methylw-1,2,4-triazin-5(4H)-one

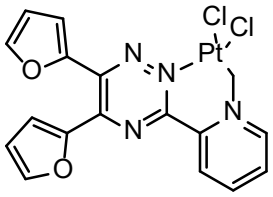
Ucherek *et al.,* 2008*,* New methyl 2-[5-oxo-3,4-di-(2-pirydyl)-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene] acetate analogs were synthesized and tested for antibacterial activity in vivo. Compound **(26)** had equal antibacterial activity against all investigated bacterial strains, 20 Candida albicans strains, and 7 Candida non-albicans strains, with a MIC of 512 g/mL. Compound **(26)** showed considerable antifungal activity against three Candida strains (Jirků *et al.,* 1981).



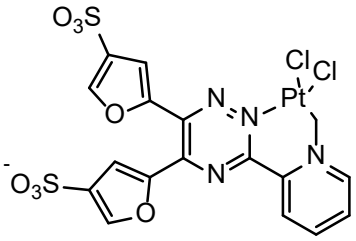
26

Figure 12: Antimicrobial Activity of Methyl (Z)-2-(5-oxo-3,4-di(pyridin-4-yl)-4,5-dihydro-1,2,4-triazin-6(1H)-ylidene)acetate.

Compounds **(27 and 28)** were synthesized and investigated for HIV-1 resistance (Human Immune Virus Type-1). At 100 M conc, none of them showed any action against HIV-1 (El-barby *et al.,* 2005). Metal-containing medications are referred to as "inorganic medicinal agents" (Orvig & Abrams., 1999). Metal chelates comprising Cu(I) and Cu(II), which undergo redox reactions and fast ligand exchange, are commonly used to assess antiviral activity of such drugs. These characteristics may play a role in the antiviral process, however determining the mechanism of action is difficult due to redox and fast exchange reactions. Inorganic pharmaceutical compounds with aromatic sulfonic acid groups have received very limited antiviral testing (Lebon et al., 2002; Clercq., 1997). Previously, a pair of comparable d10 Cu(I) cationic and anionic compounds were shown to be toxic/inactive and non-toxic/active, respectively (Davis *et al.,* 1995). Others considered drugs with the 3-(2-pyridyl)-1,2,4-triazine group **(Figure 13)**, often with aromatic sulfonic acid groups at the 5 and/or 6 positions, and the exchange-inert, redox-stable Pt(II) metal core **(27–29)** as microbicides for HIV prevention. This group of chemicals, many of which are new, will be referred to as ptt. Evaluation of the viricudial and antiviral activity of these ptt compounds against HIV-1, as well as their impact on viral Env protein fusion activity and RT activity. Infection was shown to be inhibited in 96-99 percent of cases using the most active ptt drugs. The findings suggest that some ptts have antiviral activity against R5 viruses. The findings highlight the significance of the ligand, particularly its pheripheral charge (Vzorov *et al*., 2005).



27



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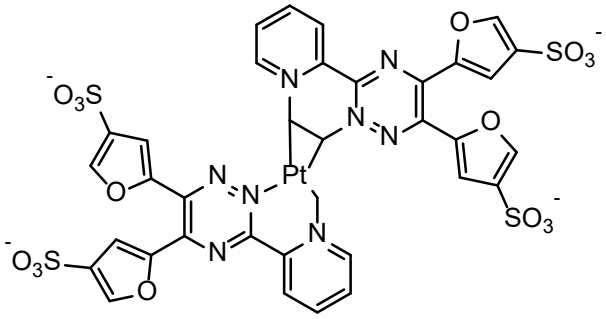
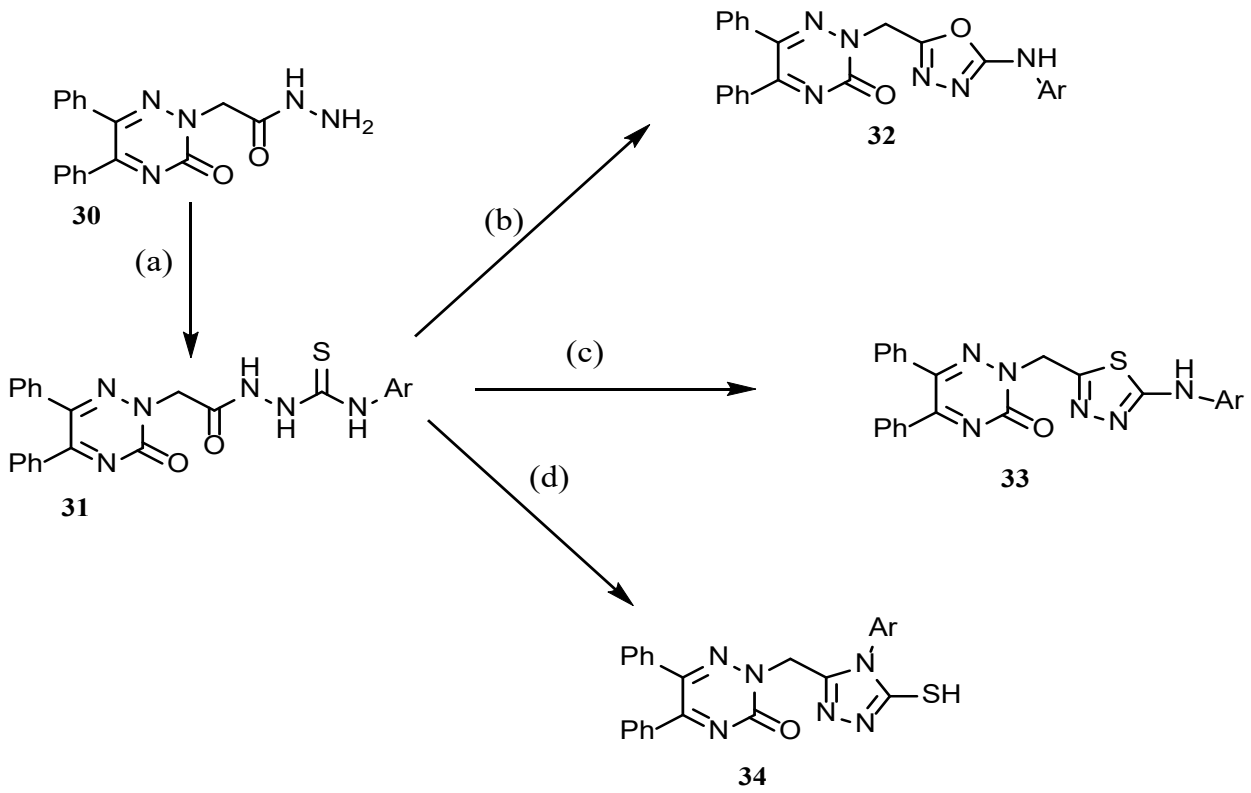


29

Figure 13: "Proposed structures of three related and representative (platinum) compounds tested against HIV-1."

Synthesis of novel 5,6-diphenyl-1,2,4-triazin-3(2H)-ones (30) and structural hybridization with 5-substituted 1,3,4-oxadiazole/thiadiazole **(32 and 33)** or 1,2,4-triazole nucleus **(34).** The hybrids were created with the structural requirements for selective COX-2 inhibitory action in mind. It had the diaryl composition, which is reported to serve a vital role in anchoring the molecule within the COX-2 active site via hydrophobic contact. Because the diaryl structural moiety was attached to a core 1,2,4-triazine ring, resulting in a diaryl heterocyclic skeleton, it performs a role as a selective COX-2 inhibitor (e.g. celecoxib, rofecoxib, valdecoxib, and etoricoxib). This basic skeleton was subsequently hybridized with substituted five membered heterocycles using a methylene linker, with the goal of giving the overall hybridized structure more flexibility (Banerjee *et al.,* 2016). When the computed energies and structures for the alternate azaphilic and nucleophilic addition pathways were compared, it was discovered that the azaphilic addition is favored kinetically over the nucleophilic addition, although the nucleophilic addition is frequently preferred thermodynamically (Lorincz *et al.,* 2010).

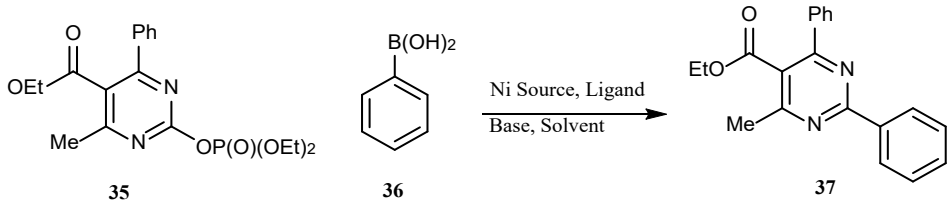


Scheme 1: "Synthesis of target compounds: (a) Phenyl/substituted phenyl isothiocynate, Ethanol (75% v/v), reflux 6h (b) KI/I_2, aq.NaOH (5N), Reflux 1h (c) Cold H_2SO_4, Stirring 4-6 h (d) aq.NaOH (4N), Reflux 2h."

1.2.3 What is a Suzuki Coupling reaction

Palladium-catalysed coupling reactions constitute one group of robust reactions with high generality, suitable for combinatorial chemistry (Larhead *et al.,* 1996). The Suzuki coupling of an aryl halide with aryl boronic acid (palladium catalyzed coupling) is often regarded as the most effective approach for creating a C(sp2)–C(sp2) bond. In the synthesis of natural products, physiologically active compounds, and materials research, the reaction has been widely used (Xing *et al.,* 2015). For the synthesis of unsymmetrical biaryl compounds, By far the most versatile synthetic approach available is the Suzuki reaction. Unlike most other palladium-catalyzed coupling reactions, this one is water and functional group tolerant, uses easily available starting materials, and produces harmless by-products (Baxendale *et al.,* 2006). The fact that the Suzuki-Miyaura coupling reaction may be carried out utilizing phase transfer catalyst (PTC) instead of hazardous ligands

and organic solvents is a significant step forward in synthetic organic chemistry, especially given the growing interest in green chemical processes. The phase transfer catalyst allows substances to be transferred from one system to another in a chemical reaction between two non-miscible, heterogeneous systems, each of which has both functional sites that may be dissolved in both systems (Jose *et al.,* 2020).



35 36 37

Scheme 2: "Optimization of the Suzuki reaction of pyrimidin-2-yl phosphate and arylboronic acid."

They found the Suzuki–Miyaura cross-coupling reaction of pyrimidin-2-yl phosphate (35) with arylboronic acid (36) to be an optimal reaction after benchmarking numerous catalysts to find the most suited catalyst with the highest yield. Ni was obviously critical to the entire process. The yield was low when Ni(acac)2 and DPE-Phos were introduced to the system. However, using Ni(PPh3)2Cl2, Ni(PPh3)2Cl2/PCy3, or Ni(PPh3)2Cl2/DPE-Phos as a catalyst did not significantly improve the yield of (37) Inevitably, more than 76 percent of the C–C cross-coupling product was generated by using Ni(dppb)Cl2 (Xing *et al.,* 2015).

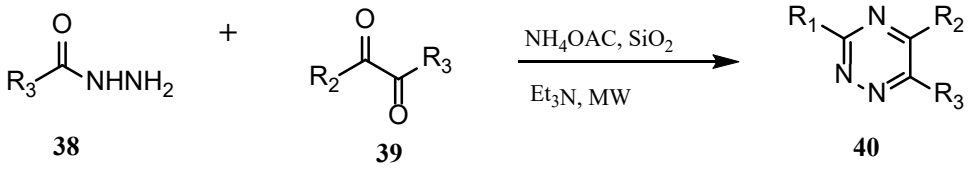
1.2.4 Microwave assisted Suzuki-coupling of 1,2,4–Triazine ring

Although fire is now rarely used in synthetic chemistry, until Robert Bunsen invented the burner in 1855, the energy from this heat source could not be delivered to a reaction vessel in a focused manner. The Bunsen burner was eventually supplanted as a source of heat for chemical reactions by the isomantle, oil bath, or hot plate. In recent years, microwave radiation has been a hot topic in the scientific community for heating and speeding up chemical reactions. This unusual heating method is gradually transforming from a laboratory curiosity to a widely used technique in academia and industry. One of the numerous advantages of "microwave flash heating" is that it cuts reaction times significantly (from days and hours to minutes and seconds). Microwave irradiation is

electromagnetic irradiation in the frequency range of 0.3 to 300 GHz (Stuerga & Delmotte *et al.,* 2002). All household "kitchen" microwave ovens and all dedicated microwave reactors for chemical synthesis operate at a frequency of 2.45 GHz (equivalent to a wavelength of 12.24 cm) to avoid interference with telecommunication and cellular phone frequencies (Gabriel et al., 1998). The energy of the microwave photon in this frequency region (0.0016 eV) is too low to break chemical bonds and is also lower than the energy of Brownian motion (Baghurst *et al.,* 1991). It is therefore clear that microwaves cannot induce chemical reactions (Mingos *et al.,* 2004). Since the first reports on the use of microwave heating to accelerate organic chemical transformations by the groups of Gedye and Giguere/Majetich in 1986, and initial slow uptake of the technology in the late 1980s and early 1990s has been attributed to its lack of controllability and reproducibility, combined with a general lack of understanding of microwave dielectric heating fundamentals (Kappe *et al.,* 2004). Microwave assisted organic synthesis (MAOS) has had a substantial impact on synthetic chemistry, and it continues to have an impact on chemistry progress (Rauf *et al.,* 2007). Elsewhere microwave irradiation (MWI) has sparked a lot of interest in the fast synthesis of a range of heterocyclic molecules (Kidwai *et al.,*1998). Microwave irradiation is a well-established method for improving selectivity, increasing rate, and reducing thermal breakdown byproducts in organic synthesis (Rauf *et al.,* 2007). MWI has become a highly valuable instrument in organic synthesis, according to Rostamizadeh & Sadeghi., 2002, and a large number of classical chemical reactions have been successfully carried out using microwave heating technology. Others have devised techniques to quickly access a variety of heterocylic frameworks with significantly reduced reaction times and yields compared to traditional thermal approaches (Zhao *et al.,* 2003). Researchers (Larhed & Hallbeg., 2001) have proved that speed is crucial in the realms of combinatorial and automated medicinal chemistry to meet the growing demand for novel compounds for drug development. As a result, the usefulness of microwave flash-heating chemistry in dramatically reducing reaction times (from days and hours to minutes and seconds) has recently been proved in a variety of organic chemical fields. Microwave-assisted organic synthesis, unlike conventional organic synthesis, is characterized by substantial increases in chemical transformation yields. Furthermore, working with open vessels and scaling up reactions is easier with

microwave-assisted reactions in solvent-free conditions (Abdel-Jalil et al., 2005). Others evaluated the catalytic activity of benzothiazole-based Pd(II)-complexes in Suzuki–Miyaura and Heck–Mizoroki C–C cross-coupling reactions of aryl chlorides and bromides with olefins and arylboronic acids in microwave irradiation conditions in water (Dawood *et al.,* 2007). Microwave-assisted flow chemistry is one of the highly crucial contributors to bulking up efforts in microwave synthesis, Although developing "clean" solutions for the chemical industry has been one of the major concerns in chemical research in recent years (Beregszászi, T. and Molnár *et al.,* 1997). Organic synthesis Microwave-assisted flow chemistry encompasses more selective transformations, the use of more active catalysts with the possibility of recycling, mild reaction conditions with an easy and waste-free work-up procedure, reduced energy consumption, the use of bio-based feedstock, and environmental applications, and the use of bio-based feedstock, and environmental applications fall into the realm of Microwave-assisted flow chemistry (Mooney & Torok *et al.,* 2021).

Microwave irradiation has proven to be a highly valuable tool in organic synthesis, with a large variety of traditional organic processes being successfully carried out using the microwave heating technique. (Rostamizadeh, & Sadeghi., 2002).



Scheme 3: Microwave irradiation to prepare 1.2.4-triazines.

The following procedures were used to create these products: (a) Condensation of acid hydrazides with benzil in acetic acid containing ammonium acetate gives 5,6-diphenyl-1,2,4-triazine with various aromatic and heterocyclic groups attached at the 3 position. In alcoholic ammonia, aliphatic, aromatic, and heterocyclic acid hydrazides are cyclized under pressure with 1,2-diketone monoacylhydrazones. (c) Condensation of monohydrazones of aromatic (but not aliphatic) 1,2-diketones yields 5,6-disubstituted 1,2,4-triazines. (d) Condensation of acid hydrazide and -halo acetophenone in the presence of NaOAc or AgOAc. (e) -diketone reactions involving amidrazones and S-

methylthiosemicarbazide are also mentioned in the literature. The 1,2,4-triazines **(40)** were synthesized in one pot. Acid hydrazides **(38),** ammonium acetate, and dicarbonyl compounds **(39)** condense on the surface of a silica gel in the presence of microwave irradiation. (Rostamizadeh, & Sadeghi., 2002).

1.3 Origins and aims of this project

This study aims to explore further applications of 1,2,4-triazine as a linker by selectively synthesizing a range of intermediates for both screening and also further synthesis to obtain target compounds. The synthetic intermediates are primary, secondary and tertiary amines, as well as aryl substituents respectively.

The study is an Attempt to aggregate research studies focusing on synthetic analogues of 1,2,4-triazine, SAR investigations, and pharmacological actions related with them. The major goals are to find a variety of medicinal combinations.

Our interest in the 1,2,4-triazine nucleus originates from the fact that there is currently no other NSAID on the market that contains the core 1,2,4-triazine heterocycle, with the exception of azapropazone **(15)**, which is only available in select parts of Europe)..

The research attempts to accomplish the following goals and objectives:

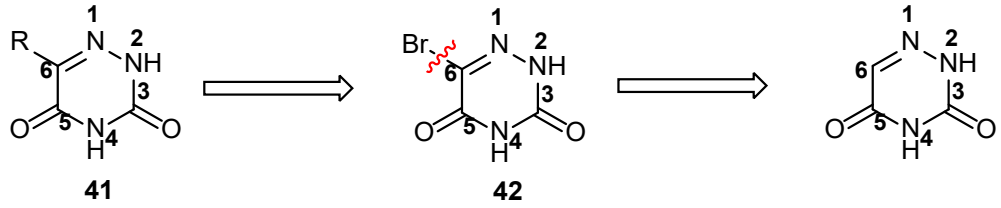
- Synthesis of 6-substituted 1,2,4-triazine-3,5(4H,6H)-dione.
- Synthesis of 6-subtituted-3-amino-1,2,4-triazine via Suzuki coupling reaction.
- Synthesis of 6-subtituted-3,5-diamino-1,2,4-triazine via Suzuki coupling reaction.
- Analysis and characterization of synthetic triazine derivatives by spectroscopic methods.
- Biological analysis of synthetic triazine derivatives.

CHAPTER 2

RESULTS AND DISCUSSION

2.1 Synthesis of 6-amino-1,2,4-triazine-3,5-diones

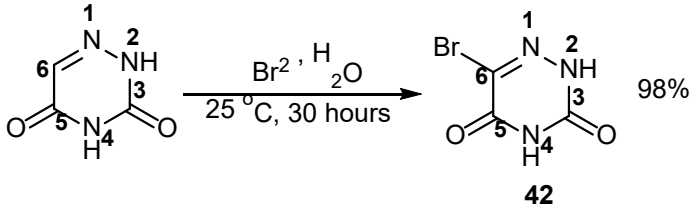
2.1.1. Retrosynthesis of 6-amino-1,2,4-triazine-3,5-(2H,4H)-diones



Scheme 4: Retrosynthesis of 6-amino-1,2,4-triazine-3,5-(2H,4H)-diones

6-amino-1,2,4-triazine-3,5-(2H,4H)-diones (**41**) could be retrosynthesized to 6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (**42**) by the replacement of amino groups with the bromine atom. Compound **42** could in turn be retrosynthesized to the commercially available 6-azauracil by the cleavage of C6-Br bond as indicated in Scheme 4 above.

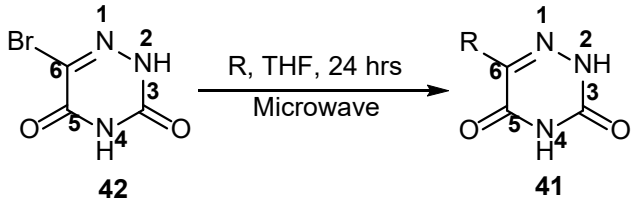
2.1.2. Synthesis of 6 bromo-1,2,4-triazine-3,5-(2H,4H)-dione (42)



Scheme 5: Synthesis of 6 bromo-1,2,4-triazine-3,5-(2H,4H)-dione (**42**)

6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (**42**) was synthesized by treating a commercially available 6-azauracil with bromine and water combination at room temperature for 30 hours as indicated in Scheme 5 above. The resultant yellow product was confirmed by NMR spectroscopy and had similar results as reported in literature (Mitran *et al.,* 2010).

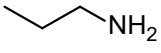
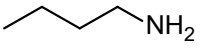
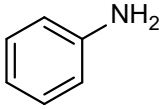
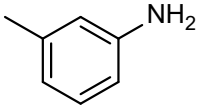
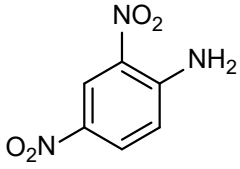
2.1.3. Amination of 6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (42)

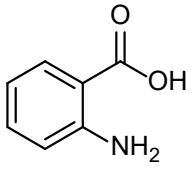
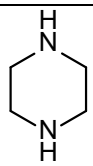
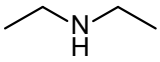
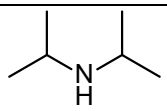
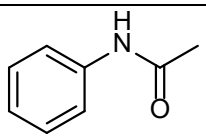
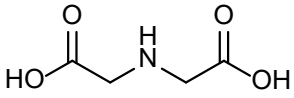


Scheme 6: Synthesis of 6-amino-1,2,4-triazine-3,5-(2H,4H)-diones (**41**)

Synthesis of 6-amino-1,2,4-triazine-3,5-(2H,4H)-diones (**41**) was achieved by reacting compound **42** with different amines in refluxing THF overnight as indicated in scheme 6 above. Initially, only moderate yields were obtained. However, when reflux was done in open vessel microwave reaction, products were obtained as evidenced by the good to outstanding yields in Table 1 below.

Table 1: Results obtained from reacting 6 bromo-1,2,4-triazine-3,5-(2H,4H)-dione with different amines.

Amine used	Compound #	% Yield
Propylamine, 	**41a**	83.4
Butylamine, 	**41b**	73.5
Isopropylamine, 	**41c**	69.5
Aniline, 	**41d**	52.3
3-Methylaniline, 	**41e**	89.6
2,4-Dinitroaniline, 	**41f**	92.7

Anthranilic acid, 	**41g**	80.6
Piperazine, 	**41h**	77.6
Diethylamine, 	**41i**	63.4
Diisopropyamine, 	**41j**	75.6
N-phenylacetamide, 	**41k**	72.4
Iminodiacetic acid, 	**41l**	98.2

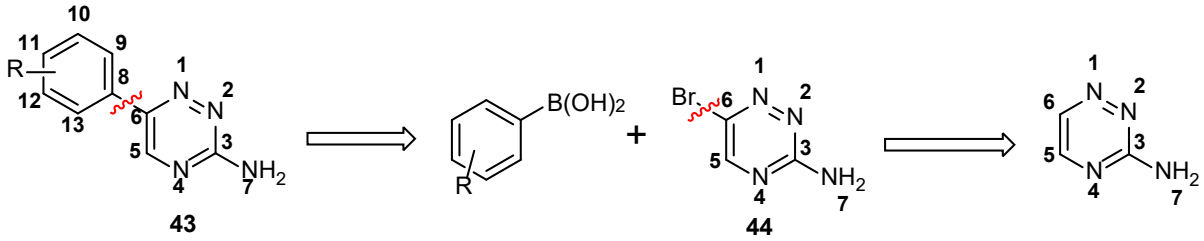
Products obtained were characterized with ^{1}H NMR, ^{13}C NMR and IR spectroscopies. ^{1}H NMR spectra of compounds (**41a-b**) which were obtained from a reaction of 6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (**42**) with linear primary amines (propylamine and butylamine) were characterized by the presence of a broad triplet peak accounting for one proton ~6 ppm confirming the presence of N-H. The same peak was observed at 3.26 ppm when isopropyl amine (a branched primary amine) was used. Two broad singlet peaks accounting for one proton each were observed at 12.6 ppm and 12.3 ppm, ascribed to H-2 and H-4 respectively in scheme 6, confirmed the other two N-H bonds. ^{13}C NMR spectra were distinguished by the presence of a new quaternary carbon peak ~131 ppm confirming that C-6 is now bonded to a nitrogen and no longer bromide atom where C-6 was observed at 129.6 ppm. Also present in these spectra were carbonyl carbons peaks, C-3 and C-5 observed ~161 ppm and ~157 ppm respectively.

^{1}H NMR spectra of compounds (**41d-g**) which were obtained from a reaction of 6 bromo-1,2,4-triazine-3,5-(2H,4H)-dione (**42**) with aromatic primary amines (anilines) were characterized by the presence of a broad singlet peak accounting for one proton ~5.8 ppm confirming the presence of N-H bond. These spectra were further characterized by two more broad singlet peaks accounting for one proton each observed at 12.6 ppm and 12.3 ppm, confirming H-2 and H-4 Scheme 6 respectively. ^{13}C NMR spectra were distinguished by the presence of a new quaternary carbon peak ~138 ppm confirming that C-6 is now bonded to a nitrogen atom and no longer bromide atom where C-6 was observed at 129.6 ppm. Also present in these spectra were carbonyl carbons peaks, C-3 and C-5 observed ~154 ppm and ~149 ppm respectively.

Infrared spectra of all obtained products were characterized with the presence of the N-H stretch ~3400 cm^{-1}, C=O stretch ~1700 cm^{-1} and C=C stretch ~1600 cm^{-1}.

2.2 Synthesis is of 6-aryl-1,2,4-triazine-3-amines.

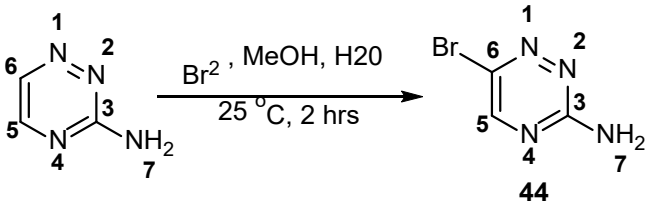
2.2.1 Retrosynthesis of 6-(substituted-phenyl)-1,2,4-triazine-3-amines (43)



Scheme 7: Retrosynthesis of 6-(substituted-phenyl)-1,2,4-triazine-3-amines.

6-(substituted-phenyl)-1,2,4-triazine-3-amines (**43**) could be retrosynthesized to different boronic acids and 6-bromo-1,2,4-triazine-3-amine (**44**) by the cleavage of the C-6 and C-8 bond of compounds **43**. Compound **44** could in turn be retrosynthesized to the commercially available 1,2,4-triazine-3-amine by the cleavage of C6-Br bond as indicated in scheme 7 above.

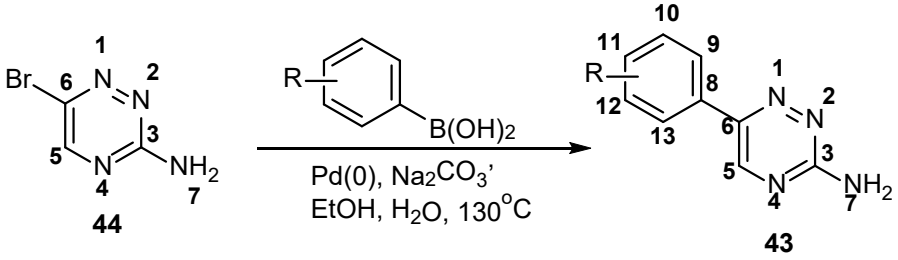
2.2.2. Synthesis of 6-bromo-1,2,4-triazine-3-amine (44).



Scheme 8: Synthesis of 6-bromo-1,2,4-triazine-3-amine

6-bromo-1,2,4-triazine-3-amine (**44**) was synthesized by treating a commercially available 1,2,4-triazine-3-amine with bromine in a room-temperature combination of methanol and water for 2 hours as indicated in scheme 8 above. The resultant yellow solid was confirmed by NMR spectroscopy and had similar results as reported in literature (Kamber *et al.,* 2015).

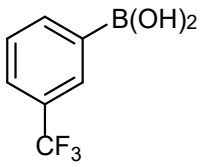
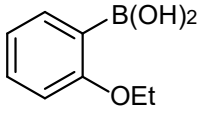
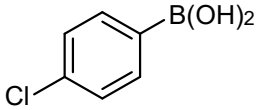
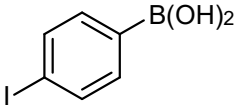
2.2.3. Suzuki coupling reactions of compound 44 with different phenylboronic acids



Scheme 9: Synthesis of compounds 3 via Suzuki coupling reactions with compound **44**

Suzuki coupling reactions of compound **44** with different phenylboronic acids In the presence of a Pd(0) catalyst, was accomplished under aqueous conditions as indicated in scheme 9 above. Products were initially obtained in very low yields. However, when reflux was done in open vessel microwave reaction, products were obtained as evidenced by the poor to exceptional yields in Table 2 below.

Table 2: Results obtained from Suzuki coupling of compound 44 with different boronic acids.

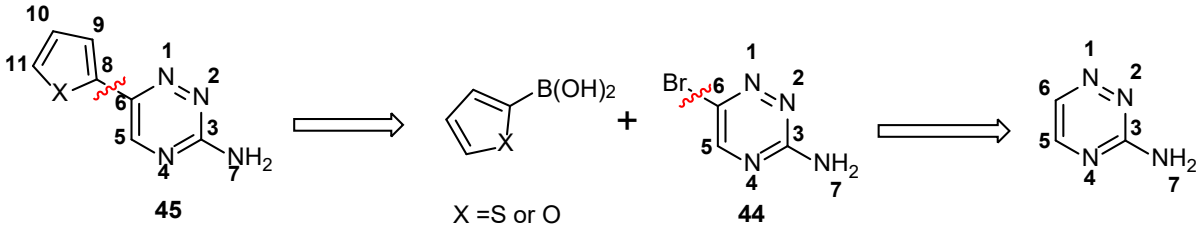
Boronic acid used	Compound #	% Yield
3-(trifluoromethyl)phenylboronic acid, 	**43a**	71.8
2-ethoxyphenylboronic acid, 	**43b**	94.3
4-chlorophenylboronic acid, 	**43c**	52.2
4-iodophenylboronic acid, 	**43d**	9.9

Products obtained were characterized with ^{1}H NMR, ^{13}C NMR and IR spectroscopies. ^{1}H NMR spectra of compounds (**43a-b**) which were obtained from a reaction of 6-bromo-1,2,4-triazine-3-amine (**44**) with 3-(trifluoromethyl) phenylboronic acid and 2-ethoxyphenylboronic acid respectively were characterized with five peaks in their aromatic region, with four different peaks accounting for one proton each coming from the phenylboronic acid moiety and the singlet accounting for one proton ~8.4 ppm coming from compound **44** moiety. ^{1}H NMR spectra of compounds (**43c-d**) which were obtained from a reaction of 6-bromo-1,2,4-triazine-3-amine (**44**) with para substituted phenylboronic acids (4-chloro or 4-iodo phenylboronic acid) were characterized by three peaks in the aromatic region, i.e. two doublets accounting for two protons each (confirming the phenyl protons from the boronic acid moiety) and a singlet accounting for one proton ~8.8 ppm confirming H-5. Conspicuous in the ^{13}C NMR all of these chemicals' spectra were a quaternary carbon peak observed ~148 ppm, ascribed to C-6 and

confirming the new C-C bond. This peak was observed at 135.9 ppm (C6-Br) in compound **44**.

Infrared spectra of all obtained products were characterized with the presence of the N-H stretch ~3300 cm^{-1} and C=C stretch ~1630 cm^{-1}.

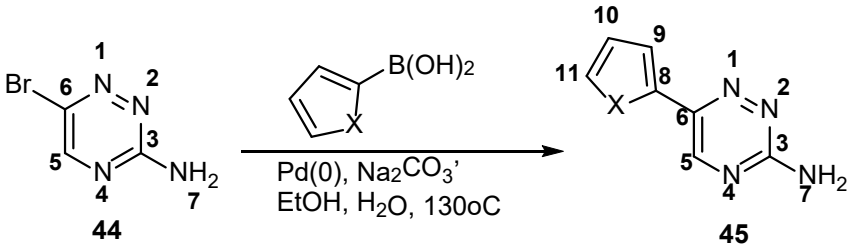
2.3 Retrosynthesis of 6-thiophenyl or furanyl-1,2,4-triazine-3-amines (45)



Scheme 10: Retrosynthesis of 6-thiophenyl or furanyl-1,2,4-triazine-3-amine

6-thiophenyl or furanyl-1,2,4-triazine-3-amine (**45**) could be retrosynthesized to 2-thiophenyl or 2-furanyl boronic acids and 6-bromo-1,2,4-triazine-3-amine (**44**) by the breaking of the C-6 and C-8 bond of compounds **45**. Compound **44** could in turn be retrosynthesized to the commercially available 1,2,4-triazine-3-amine by the breaking of the C6-Br bond, as shown in Scheme 10 above.

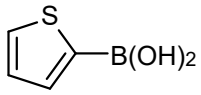
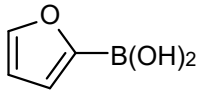
2.3.1. Suzuki coupling reactions of compound 4 with 2-thiophenyl or 2-furanylboronic acids



Scheme 11: Synthesis of compounds 5 via Suzuki coupling reactions with compound **44**

Suzuki coupling reactions of compound **44** with 2-thiophenyl or 2-furanyl boronic acids In the presence of a Pd(0) catalyst, was accomplished under aqueous conditions as indicated in scheme 11 above. Products were obtained in average yields. However, when reflux was done in open vessel microwave reaction, products were obtained as evidenced by the poor to exceptional yields in Table 3 below.

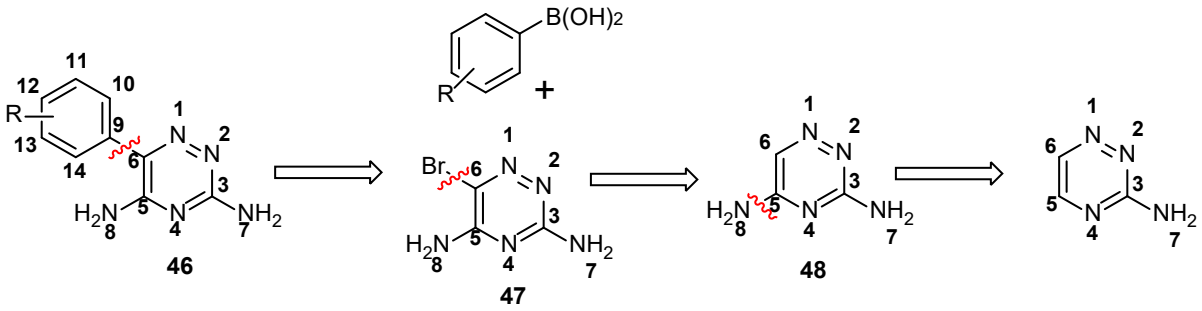
Table 3: Results obtained from Suzuki coupling of compound 4 with 2-thiophenyl and 2-furanylboronic acids.

Boronic acid used	Compound #	% Yield
2-thiophenylboronic acid, 	**45a**	94.2
2-furanylboronic acid, 	**45b**	66.7

Products obtained were characterized with ^{1}H NMR, ^{13}C NMR and IR spectroscopies. Five peaks in the aromatic area were seen in the **^{1}H** NMR spectra of these compounds **(45a-b)**. A singlet accounting for one proton (H-5) was observed ~8.4 ppm, another singlet accounting for two protons (H-7) was observed ~6.6 ppm, two doublets accounting for one proton each (H-11 and H-9) were observed ~7.7 ppm and 7.6 ppm respectively. The last peak was a doublet of doublets accounting for one proton (H-10) was observed ~7.1 ppm.

^{13}C NMR spectra of these compounds showed seven peaks, three quaternary carbon (C-3, C-6 and C-8) peaks and four methine carbon (C-5, C-9, C-10 and C-11) peaks.

2.4 Retrosynthesis of 6-(substituted-phenyl)-1,2,4-triazine-3,5-diamines (46)

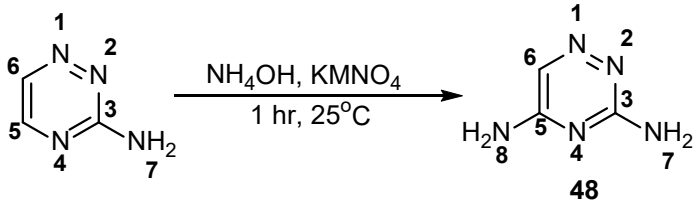


Scheme 12: Retrosynthesis of 6-(substituted-phenyl)-1,2,4-triazine-3,5-diamines.

6-(substituted-phenyl)-1,2,4-triazine-3,5-diamines (**46**) could be retrosynthesized to different boronic acids and 6-bromo-1,2,4-triazine-3,5-diamine (**47**) by the breaking of the

C-6 and C-9 bond of compounds **46**. Compound **47** could in turn be retrosynthesized to 1,2,4-triazine-3,5-diamine **48** by the cleavage of C6-Br bond as indicated in scheme 12 above. Compound **48** could in turn be retrosynthesized to the commercially available 1,2,4-triazine-3-amine by the cleavage of C5-NH_2.

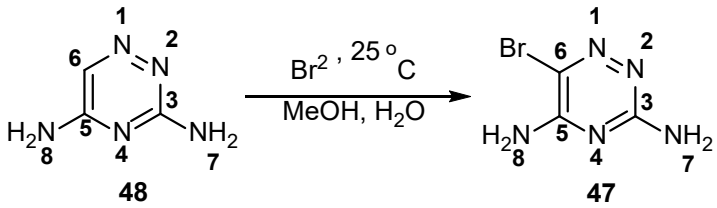
2.4.1. Synthesis of 1,2,4-triazine-3,5-diamine (48)



Scheme 13: Synthesis of 1,2,4-triazine-3,5-diamine

1,2,4-triazine-3,5-diamine (**48**) was synthesized by treating a commercially available 1,2,4-triazine-3-amine with a mixture of liquid ammonia and potassium permanganate at room temperature for 1 hour as indicated in Scheme 13 above. The resultant yellow solid was confirmed by NMR spectroscopy and had similar results as reported in literature (Rykowski & Van der Plas *et al.,* 1980).

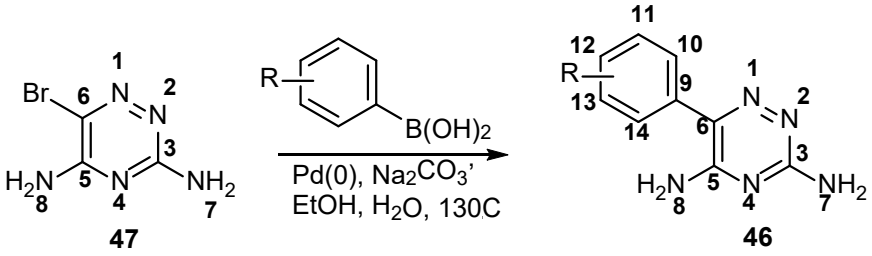
2.4.2. Synthesis of 6-bromo-1,2,4-triazine-3,5-diamine (47)



Scheme 14: Synthesis of 6-bromo-1,2,4-triazine-3,5-diamine

6-Bromo-1,2,4-triazine-3,5-diamine (**47**) was synthesized by treating compound **48** with bromine in a mixture of methanol and water at room temperature for 2 hours as indicated in Scheme 14 above. The resultant yellow solid was confirmed by NMR spectroscopy and had similar results as reported in literature ((Rykowski & Van der Plas *et al.,* 1980).

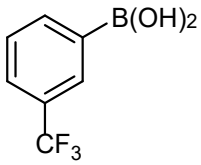
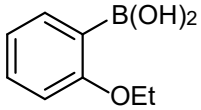
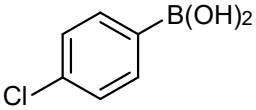
2.4.3. Suzuki coupling reactions of compound 47 with different phenylboronic acids

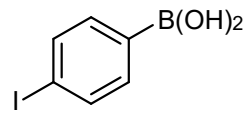
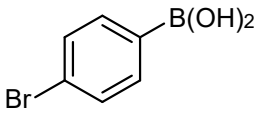


Scheme 15: Synthesis of compounds **46** via Suzuki coupling reactions with compound **47**

Suzuki coupling reactions of compound **47** with different phenylboronic acids In the presence of a Pd(0) catalyst, was accomplished under conditions as indicated in scheme 15 above. Products were obtained in low yields. However, when reflux was done in open vessel microwave reaction, products were obtained as evidenced by the poor to exceptional yields in Table 4 below.

Table 4: Results obtained from Suzuki coupling of compound 7 with different boronic acids.

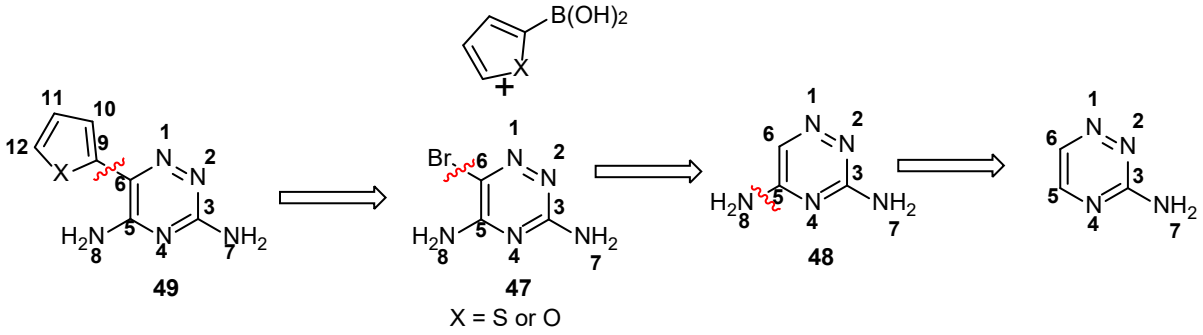
Boronic acid used	**Compound #**	**% Yield**
3-(trifluoromethyl)phenylboronic acid, 	**46a**	46.3
2-ethoxyphenylboronic acid, 	**46b**	15.4
4-chlorophenylboronic acid, 	**46c**	83.0

4-iodophenylboronic acid, 	**46d**	34.0
4-bromophenylboronic acid, 	**46e**	38.5

Products obtained were characterized with ^{1}H NMR, ^{13}C NMR and IR spectroscopies. ^{1}H NMR spectra of compounds (**46a-b**) which were obtained from a reaction of 6-bromo-1,2,4-triazine-3,5-diamine (**47**) with 3-(trifluoromethyl) phenylboronic acid and 2-ethoxyphenylboronic acid respectively were characterized with four peaks accounting for one proton each in their aromatic regions. Also present in these spectra were two broad singlets accounting for two protons each observed ~6.2 ppm (H-8) and ~5.2 ppm (H-7) ^{1}H NMR spectra of compounds (**46c-e**) which were obtained from a reaction of 6-bromo-1,2,4-triazine-3,5-diamine (**47**) with para substituted phenylboronic acids (4-chloro, 4-iodo or 4-bromophenylboronic acid) were characterized by two doublet peaks accounting for two protons each in the aromatic region. Conspicuous in the ^{13}C NMR spectra of all these compounds was a quaternary carbon peak observed ~154 ppm, ascribed to C-6 and confirming the new C6-C9 bond. This peak was observed at 125.5 ppm in compound 7 (C6-Br) bond.

Infrared spectra of all obtained products were characterized with the presence of the N-H stretch ~3300 cm^{-1} and C=C stretch ~1630 cm^{-1}.

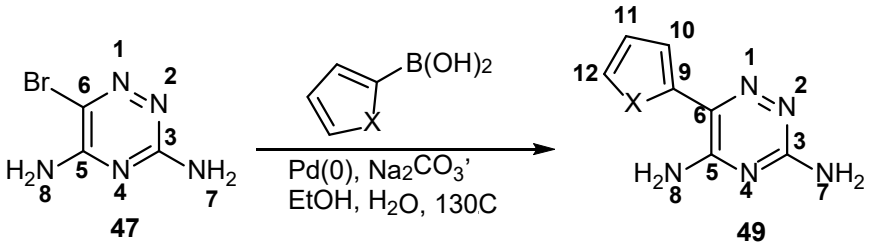
2.5 Retrosynthesis of 6-thiophenyl or furanyl-1,2,4-triazine-3,5-diamines (49



Scheme 16: Retrosynthesis of 6-thiophenyl or furanyl-1,2,4-triazine-3,5-diamine

6-thiophenyl or furanyl-1,2,4-triazine-3,5-diamines (**49**) could be retrosynthesized to 2-thiophenyl or 2-furanyl boronic acids and 6-bromo-1,2,4-triazine-3,5-diamine (**47**) by the breaking of the C-6 and C-9 bond of compounds **49**. Compound **47** could in turn be retrosynthesized to the 1,2,4-triazine-3,5-diamine **48** by the cleavage of C6-Br bond as indicated in Scheme 16 above. Compound **48** could in turn be retrosynthesized to the commercially available 1,2,4-triazine-3-amine by the cleavage of C5-NH_2.

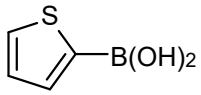
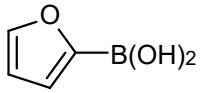
2.5.1. Suzuki coupling reactions of compound 47 with 2-thiophenyl or 2-furanylboronic acids



Scheme 17: Synthesis of compounds 9 via Suzuki coupling reactions with compound 7

Suzuki coupling reactions of compound **47** with 2-thiophenyl or 2-furanyl boronic acids in the presence of a Pd(0) catalyst, was accomplished under aqueous conditions as indicated in Scheme 17 above. Products were obtained in low yields. However, when reflux was done in open vessel microwave reaction, products were obtained as evidenced by the poor to exceptional yields in Table 5 below.

Table 5: Results obtained from Suzuki coupling of compound 7 with 2-thiophenyl and 2-furanylboronic acids.

Boronic acid used	Compound #	% Yield
2-thiophenylboronic acid, 	**49a**	32.3
2-furanylboronic acid, 	**49b**	49.5

Products obtained were characterized with ^{1}H NMR, ^{13}C NMR and IR spectroscopies. ^{1}H NMR spectra of these compounds (**49a-b**) were characterized by three peaks in the aromatic region, two doublets accounting for one proton each (H-12 and H-10) were observed ~7.6 ppm and 7.5 ppm respectively and a doublet of doublets accounting for one proton (H-11) was observed ~7.4 ppm. Two singlets accounting for two proton each were observed ~6.4 ppm and ~6.9 ppm and were ascribed to H-7 and H-8 respectively. ^{13}C NMR spectra of these compounds showed seven peaks, four quaternary carbon (C-3, C-5, C-6 and C-9) peaks and three methine carbon (C-10, C-11 and C-12) peaks.

CHAPTER 3

BIOLOGICAL EVALUATIONS

ANTIBACTERIAL ACTIVITIES

3.1 Introduction

In keeping with one of the project's goals, the novel 1,2,4-triazine derivatives synthesized in the study were submitted for antibacterial biological testing against two microorganisms, a pathogenic bacteria *Escherichia coli* (*E. coli*) and a multidrug-resistant bacteria *Pseudomonas aeruginosa (P. aeruginosa)*. MIC (minimum inhibitory concentration) (which indicate a compound's toxicity) and antimycobacterial tests using *Escherichia coli* (0157:H7) ATCC (43888) and *Pseudomonas aeruginosa* ATCC (35554) were the methods employed in the experiment.

3.1.1 Escherichia coli

Escherichia coli continues to be one among the most popular common factors of bacterial illnesses in both people and animals (Allocati *et al.,* 2013). E. coli causes enteritis, urinary tract infection, septicaemia, and other clinical illnesses such newborn meningitis. In pets and farm animals, *E. coli* is frequently linked to diarrhoea (kaper *et al.,* 2004 ; Yooh *et al.,* 2009). Antimicrobial resistance has put the pharmacotherapy treatment of Infections with *E. coli* in jeopardy (Bilinski *et al.,* 2012). Multidrug-resistant *E. coli* strains are becoming more common all over the world, owing to the expansion of mobile genetic components like plasmids. In Europe, multidrug-resistant *E. coli* bacteria are also on the rise (Qadri *et al.,*2005). As a result, the spread of *E. coli* resistance has become a growing public health concern in European countries (Abri *et al.,* 2005; Weintraub *et al.*, 2007).

3.1.2 Pseudomonas aeruginosa

Pseudomonas aeruginosa is a bacterium found in the environment that induces opportunistic human infections. It is the most prevalent Gram-negative bacterium linked with nosocomial infections and is a human opportunistic pathogen. Sixteen percent of nosocomial pneumonia cases, twelve percent of hospital-acquired urinary tract infections, eight percent of surgical wound infections, and ten percent of bloodstream infections are caused by *P. aeruginosa* (Van Delden and Iglewski 1998; Barbieri and Sun et al., 2004)

This pathogen can infect immunocompromised people, such as those with cystic fibrosis, neutropenic cancer, burn wounds, and bone marrow transplants, and the incidence of multi-drug resistance strains limits antibiotic therapy (Blocker et al. 2001). Thanks to a wide range of metabolic pathways and regulatory genes, this bacteria can grow in a wide range of conditions. This bacteria is extremely difficult to eradicate from ill people, particularly cystic fibrosis patients' lung infections, due to its nutritional diversity, large number of virulence factors, and significant antibiotic resistance (Wu et al., 2015; Thii et al., 2020).

3.1.3 Biological Results and discussion

The 1,2,4-triazine derivatives compounds, prepared in this study, were evaluated per standard protocols for their MIC antibacterial activity against E. *coli* and P. *aeruginosa*. For control purposes Gentamycin Solution, was used as a standard antibacterial drug due to its high sensitivity to E. *coli* and P. *aeruginosa* (Mashilo., 2020). **(Figure 14).** All the MIC activity of the synthesized compounds are listed in the (**Table 6)** with their results. Of all the compounds synthesized only nine of the tested compounds namely**:41c, 41d, 41g, 41h, 41f, 43d, 46c, 47 and 49b** showed significant anti-microbial activity effects with values of high activity in the region MIC of ≤ 0.1 mg/mL, *P. aeruginosa* were affected to a much lesser extent with regards to susceptibility compared to *E. coli.* Compounds **41c, 41d, 41f 41g, 41h 43d, 46c, 47 and 49b** showed better anti-microbial activity against *E. coli even at lower concentrations*, whilst only compounds **41f, 41g and 43d** show moderate activity with lower concentrations against *P. aeruginosa*. Among these derivatives, compound **41d, 41g, 43d, 46c and 49b** exhibited equivalent anti-microbial effect to the standard Gentamycin against E. *coli* strain Per literature, kamel et al., 2014, while some of the compounds were not the most potent, their activity against *E. coli* and *P. aeruginosa* makes them promising candidates for antibacterial medication development.

From literature it is apparent that drugs containing 1,2,4-triazine moiety have antimicrobial activity eg; ceftriaxone which has broad spectrum antimicrobial activity (Lemke et al., 2010; Ucherek et al., 2008) and Dihydromethyl furalazine with widespread antibacterial activity. Most compounds reported in this study have shown potent

antibacterial/antimicrobial activity against at lower concentration. While previous literature has focus on activity of the drugs this study proved the activity of straight chain amines, aryl amines substituted 1,2,4-triazine-3,5-(2H,4H)-diones **(41)** and Suzuki coupled 6-aryl-1,2,4-triazine-3-amines (**43**) and 6-aryl-1,2,4-triazine-3,5-diamines **(47).**

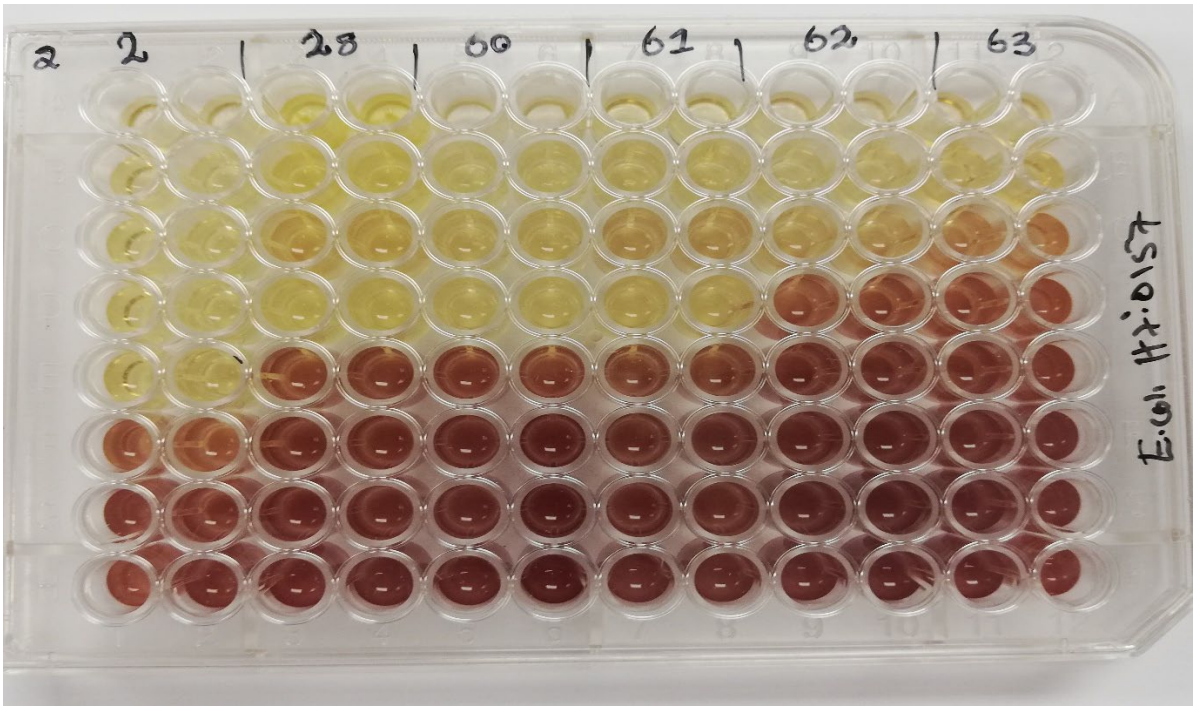


Plate A:

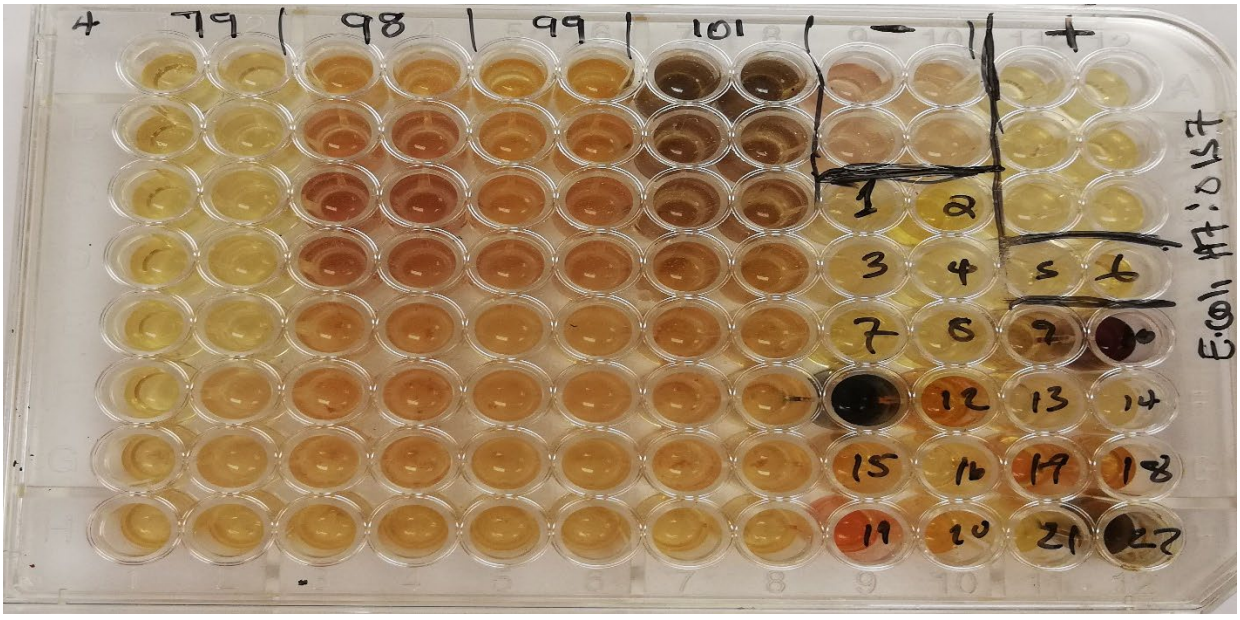
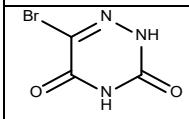
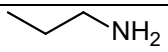
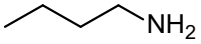
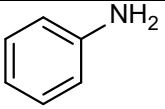
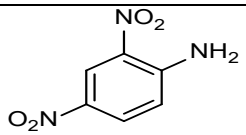
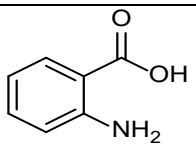
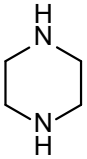


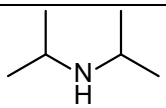
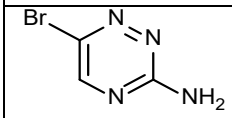
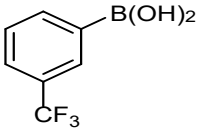
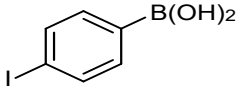
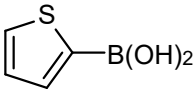
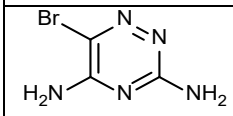
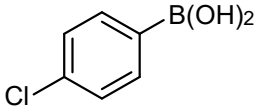
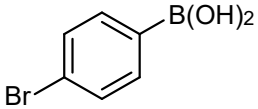
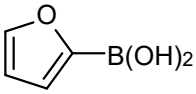
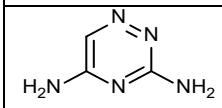
Plate B:

Figure 14: "A & B; (A) 96 microwell plate depicting results of microdilution of E. *coli* test. (B) consist of (A) with a control test; (10 mg/mL Gentamicin solution (Sigma-Aldrich, St, MO, USA) were used as a positive control, whereas distilled water was used as a negative

control). Working concentration varied as per the individual's compound *against Escherichia coli* (0157:H7) ATCC (43888). A two-fold serial dilution which resulted in 8 varied (A-H) concentration (1/2) was conducted e.g for 100mg/mL A-100/2 = 50mg/mL same applies to the rest".

Table 6: Antibacterial activity of 1,2,4-triazine derivatives

Compound	**R**	**E.coli (mg/mL)**	***P. aeruginosa* (mg/mL)**
			
41a		25.00	25.00
41b		14.00	14.00
41c		6.50	26.00
41d		0.16	n/a
41f		3.34	6.69
41g		0.63	10.00
41h		6.75	13.50

41j		15.63	31.25
			
43a		6.25	25.00
43d		0.25	3.94
45a		6.25	12.50
			
46c		0.32	41.5
46e		34.50	n/a
49b		0.43	n/a
			
47	Br	4.25	17.00
Gentamycin (10 mg/mL)		Susceptible	Susceptible

CHAPTER 4

CONCLUSIONS

This study was focused on the synthesis of novel 6-amino-1,2,4-triazine-3,5-(2H,4H)-diones (**41**) and 6-aryl-1,2,4-triazine-3-amines (**43**) and 6-aryl-1,2,4-triazine-3,5-diamines (**46**). Twelve 6-amino-1,2,4-triazine-3,5-(2H,4H)-diones (**41**) were synthesized from a reaction of 6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (**42**) with different amines in yields ranging from 52.3% to 98.2%. Different primary amines used ranged from linear ones like propyl-and butylamines to branched one like isopropylamine and different anilines. Secondary amines used ranged from cyclic one like piperazine to acyclic ones like diethylamine and diisopropylamine.

Two classes of 6-aryl-1,2,4-triazine containing compounds were synthesized, namely 6-aryl-1,2,4-triazine-3-amines (**43**) and 6-aryl-1,2,4-triazine-3,5-diamines (**46**). These compounds were synthesized via Suzuki coupling reaction between different aryl boronic acids and 6-bromo-1,2,4-triazine-3-amine (**44**) and 6-bromo-1,2,4-triazine-3,5-diamine (**47**) respectively. In total six compounds of the 6-aryl-1,2,4-triazine-3-amine (**43**) class were synthesized in yields ranging from 9.9% to 94.3%. On the other hand seven compounds of the class of 6-aryl-1,2,4-triazine-3,5-diamine (**47**) were synthesized in yields ranging from 32.2% to 83.0%. in total seven boronic acids, namely 2-ethoxyphenylboronic acid, 3-(trifluoromethyl)phenylboronic acid, 4-chlorophenylboronic acid, 4-iodophenylboronic acid, 4-bromophenylboronic acid, 2-thiophenylboronic acid and 2-furanylboronic acid, were used during the Suzuki coupling reactions.

Nine of the tested compounds namely: the 6-amino-1,2,4-triazine-3,5-(2H,4H)-diones (**41**), 6-aryl-1,2,4-triazine-3-amines (**43**) and 6-aryl-1,2,4-triazine-3,5-diamines (**46**), 6-bromo-1,2,4-triazine-3,5-diamine (**47**) and 6-thiophenyl or furanyl-1,2,4-triazine-3,5-diamines (**49**) showed significant anti-microbial activity with MIC values …. mg/mL.

Among these derivatives compound **41d, 41g, 43d, 46c and 49b** exhibited equivalent anti-microbial activity like the standard Gentamycin against *E. coli* (MIC ≥0.16 mg/mL), *P. aeruginosa* were affected to a much lesser extent (MIC ≥ 3.94 mg/mL). The obtained results suggest that these compounds may serve as lead chemical entities. Their efficacy

may be improved by adding more complex amino acids or boronic acids. Other options could be to explore the possibility of hybridizing the compounds with known drugs to improve their efficacy. The benefit of employing molecular hybridization is that it allows a single molecule to activate several sites, increasing therapeutic efficacy while also improving bioavailability (Viegas-Jr *et al.,* 2007).

CHAPTER 5

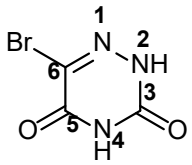
EXPERIMENTAL PROCEDURES

5.1. General procedures

"All reagents and solvents used were Analytical Grade Reagents commercially sourced from Merck, Sigma-Aldrich, and BDH. Thin Layer Chromatography was carried out on Merck TLC Silica Gel 60 F_{254} Aluminium Sheets 20 x 20 cm. Detection was done under ultra violet light at 254 nm with Spectroline UV lamp model ENF-240C/FE. For column chromatography, Machery-Nagel Silica Gel (0.063 – 0.200 mm) with (70 – 230 mesh) was used, with gel mass 30 times that of sample, eluting with stated solvent mixtures. Melting points were determined on Bruker melting point apparatus M-560. Infrared spectra were run on the Bruker Platinum ATR spectrometer. Absorption maxima are reported in wavenumbers (cm^{-1}), with s = strong, m = medium and w = weak. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker 400 MHz spectrometer using DMSO-d_6 as solvents and TMS at 0.00 ppm as an internal standard. Values for the chemical shifts are expressed in parts per million (ppm). LC-MS analysis was conducted on an LC–quadrupole time of flight (QTOF)–MS, model LC-MS 9030 instrument with a Shim Pack Velox C18 column (100 mm × 2.1 mm with particle size of 2.7 mm) (Shimadzu, Kyoto, Japan), placed in a column oven set at 40 °C. A binary solvent mixture, consisting of 0.1% formic acid in water (Eluent A) and 0.1% formic acid in acetonitrile (Eluent B) was used at a constant flow rate of 0.4 mL/min. A mass spectrometer detector was used for monitoring analyte elations, under the following conditions: ESI (electrospray ionization) negative modes; interface voltage of 3.5 kV; nitrogen gas was used as nebulizer at flow rate 3 L/min, heating gas flow at 10 L/min;

heat block temperature at 400 °C, CDL temperature at 250 °C; detector voltage of 1.70 kV and the TOF temperature at 42 °C. Tandem MS experiments. For tandem MS (MSMS) experiments, a mass calibration solution of sodium iodide (NaI) was used to obtain typical mass accuracies with a mass error below 1 ppm, and a range of m/z 100 to 1000 was used for high resolution. Argon gas was used as a collision gas for MSMS experiments along with MSE mode using collision energy ramp of 12 eV to 25 eV for generation of fragments."

5.2. Synthesis of 6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (42)

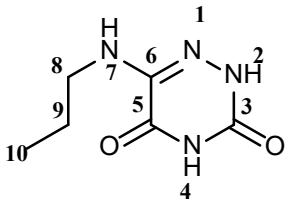


A mixture of 6-azauracil (2.00 g, 18 mmol), bromine (2 mL, 39 mmol) and water (20 mL) was stirred at room temperature for 30 hours. The mixture turned yellow over this time and a precipitate was formed, which was collected by filtration. The product was obtained as an off-white solid (2.14 g, 62.0 %), mp = 233.4 °C (lit mp = 232 - 234 °C) (Jirků et al., 1981), **^{1}H NMR (400 MHz, (DMSO-d$_6$)**: **δ_H** (ppm) 12.55 (1H, s, H-4), 12.28 (1H, s, H-2); **^{13}C NMR (100 MHz, (DMSO-d$_6$)**: **δ_C** (ppm) 154.1 (C-5), 149.5 (C-3), 129.5 (C-6); **IR ν_{max} (cm^{-1}):** 3254 (N-H stretch), 1728 (C=O stretch), 1689 (C=C stretch), 633 (C-Br stretch); **HR-ESI-MS:** Calculated m/z of $C_3H_2BrN_3O_2$ = 190.9330 [M^++H], Found m/z = 191.0910 [M^++H].

5.3. General method for the amination of 6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (41)

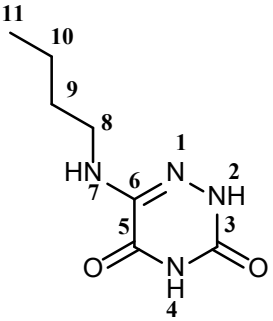
To a solution of 6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (1 mmol) in THF was added a solution of an amine (2 mmol) in THF dropwise. The resultant mixture was refluxed overnight over which time a precipitate was obtained. The products were obtained by filtration as yellow to white solids.

5.3.1 6-(Propylamino)-1,2,4-triazine-3,5(4H,6H)-dione (41a)



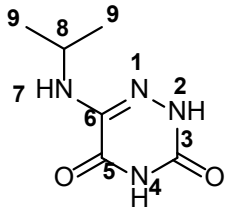
6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (1.00 g, 6 mmol) was reacted with propan-1-amine (0.52 mL, 6 mmol) in THF (5 mL) to obtain 6-(propylamino)-1,2,4-triazine-3,5(4H,6H)-dione (**41a**) as a yellow solid (0.95 g, 83.6 %), mp = 187.0 °C, **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ_H (ppm) 12.55 (1H, s, H-4), 12.28 (1H, s, H-2), 5.90 (1H, t , *J* = 7.6 Hz, H-7), 2.50 - 2.72 (2H, m, H-8), 1.30 - 1.59 (2H, m, H-9), 0.83 (3H, t, *J* = 7.4 Hz, H-10); **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 161.8 (C-3), 157.8 (C-5), 131.3 (C-6), 40.8 (C-8), 20.8 (C-9), 11.3 (C-10); **IR ν_{max} (cm^{-1}):** 3397 (N-H stretch), 2960 (C-H stretch), 1690 (C=O stretch), 1545 (C=C stretch); **HR-ESI-MS:** Calculated m/z of $C_6H_{10}N_4O_2$ = 170.0804 [M$^+$+H], Found m/z = 170.1488 [M$^+$+H].

5.3.2 6-(Butylamino)-1,2,4-triazine-3,5(4H,6H)-dione (41b)



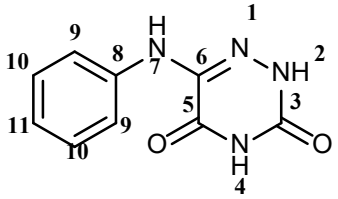
6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (1.00 g, 6 mmol) was reacted with butan-1-amine (0.52 mL, 6 mmol) in THF (5 mL) to obtain 6-(butylamino)-1,2,4-triazine-3,5(4H,6H)-dione (**41b**) as a yellow solid (1.37 g, 73.5 %), mp = 188.4 °C, **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ_H (ppm) 12.55 (1H, s, H-4), 12.28 (1H, s, H-2), 5.56 (1H, t, *J* = 7.6 Hz, H-7), 2.62 - 3.22 (2H, m, H-8), 1.09 - 1.70 (4H, m, H-9 & H-10), 0.84 (3H, t, *J* = 7.3 Hz, H-11); **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 161.1 (C-3), 157.3 (C-5), 131.3 (C-6), 39.8 (C-8), 29.5 (C-9), 19.5 (C-10), 13.9 (C-11); **IR ν_{max} (cm^{-1}):** 3314 (N-H stretch), 2959 (C-H stretch), 1650 (C=O stretch), 1574 (C=C stretch); **HR-ESI-MS:** Calculated m/z of $C_7H_{12}N_4O_2$ = 184.0960 [M$^+$+H], Found m/z = 184.9863 [M$^+$+H].

5.3.3. 6-(Isopropylamino)-1,2,4-triazine-3,5-(2H,4H)-dione (41c)



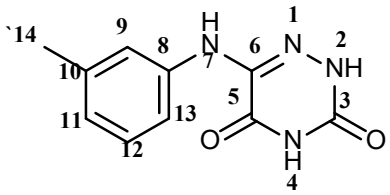
6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (1.00 g, 6 mmol) was reacted with isopropyl amine (0.52 mL, 12 mmol) in THF (5 mL) to obtain 6-(isopropylamino)-1,2,4-triazine-3,5-(2H,4H)-dione (**41c**) as yellow solid (0.91 g, 69.5 %), mp = 167.5 °C. **^{1}H NMR (400 MHz, (DMSO-d$_6$)**: **δ**$_H$ (ppm) δ$_H$ 12.55 (1H, s, H-4), 12.28 (1H, s, H-2), 3.30 - 3.31 (1H, m, H-8), 3.26 (1H, s, H-7), 1.18 (6H, d, *J* = 6.5 Hz , H-9), **^{13}C NMR (100 MHz, (DMSO-d$_6$)**: **δ**$_C$ (ppm) 165.7 (C-3), 159.7 (C-5), 130.9 (C-6), 43.5 (C-8), 20.8 (C-9); **IR ν$_{max}$ (cm^{-1}):** 3437 (N-H stretch), 3047 (C-H stretch), 1702 (C=O stretch), 1566 (C=C stretch); **HR-ESI-MS:** Calculated m/z of $C_6H_{10}N_4O_2$ = 170.0804 [M$^+$+H], Found m/z = 170.1481 [M$^+$+H].

5.3.4 6-(Phenylamino)-1,2,4-triazine-3,5(4H,6H)-dione (41d)



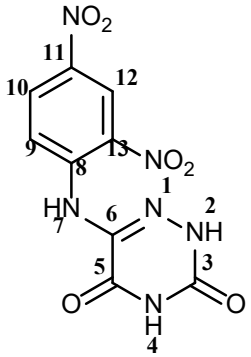
6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (1.00 g, 6 mmol) was reacted with aniline (0.52 mL, 6 mmol) in THF (5 mL) to obtain 6-(phenylamino)-1,2,4-triazine-3,5(4H,6H)-dione (**41d**) as an orange solid (0.89 g, 52.3 %), mp = 182.8 °C, **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ$_H$ (ppm) 12.60 (1H, s, H-4), 12.33 (1H, s, H-2), 7.03 (2H, dd, *J* = 7.7 Hz, *J* = 7.9 Hz, H-9), 6.56 - 6.58 (2H, m, H-10), 6.48 (1H, dd, *J* = 7.5 Hz, *J* = 7.3 Hz , H-11), 5.73 (1H, s, H-7); **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ$_C$ (ppm) 154.2 (C-3), 149.6 (C-5), 148.9 (C-8), 139.6 (C-6), 129.2 (C-9), 116.2 (C-11), 114.4 (C-10.); **IR ν$_{max}$ (cm^{-1}):** 3366 (N-H stretch), 3032 (C-H stretch), 1677 (C=O stretch) , 1600 (C=C stretch); **HR-ESI-MS:** Calculated m/z of $C_9H_8N_4O_2$ = 204.0647 [M$^+$+H], Found m/z = 205.0738 [M$^+$+H].

5.3.5 6-(m-tolylamino)-1,2,4-triazine-3,5(2H,4H)-dione (41e)



6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (1.00 g, 6 mmol) was reacted with m-toluidine (0.52 mL, 6 mmol) in THF (5 mL) to obtain 1,2,4-triazine-3,5(4H,6H)-dione (**41e**) as a purple solid (1.44 g, 89.6 %), mp = 181 °C, **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ_H (ppm) 12.60 (1H, s, H-4), 12.33 (1H, s, H-2), 7.00 (1H, dd, *J* = 7.6 Hz, *J* = 7.7 Hz, H-12), 6.54 (1H, d, *J* = 2,2 Hz, H-13), 6.49 (1H, s, H-9), 6.48 (1H, d, *J* = 7.4 Hz, H-11), 5.71 (1H, s, H-7), 2.18 (3H, s, H-13); **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 154.1 (C-3), 149.8 (C-5), 145.5 (C-8), 138.5 (C-6), 129.6 (C-12), 119.3 (C-10), 116.6 (C-11), 113.3 (C-9), 112.8 (C-13), 21.5 (C-14); **IR ν_{max} (cm^{-1}):** 3345 (N-H stretch), 3051 (C-H stretch), 1674 (C=O stretch), 1574 (C=C stretch); **HR-ESI-MS:** Calculated m/z of $C_{10}H_{10}N_4O_2$ = 218.0804 [M^++H], Found m/z = 219.1745 [M^++ H].

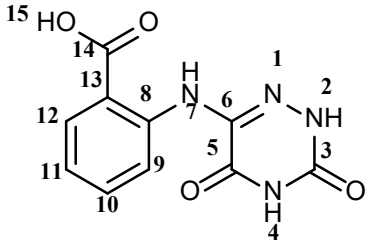
5.3.6 6-(2-(2,4-dinitrophenyl)hydrazinyl)-1,2,4-triazine-3,5(4H,6H)-dione (41f)



6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (1.00 g, 6 mmol) was reacted with 2,4-dinitroaniline (0.52 mL, 6 mmol) in THF (5 mL) to obtain 6-(2-(2,4-dinitrophenyl)hydrazinyl)-1,2,4-triazine-3,5(4H,6H)-dione (**41f**) as a yellow solid (2.17 g, 92.7 %), mp = 199.8 °C, **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ_H (ppm) 12.60 (1H, s, H-4), 12.33 (1H, s, H-2), 7.12 (1H, s, H-12), 6.96 (1H, d, *J* = 7.8 Hz, H-10), 6.57 (1H, d, *J* = 7.6 Hz, H-9), 5.88 (1H, s, H-7); **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 154.2 (C-3),

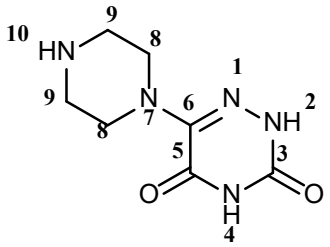
149.6 (C-5), 146.0 (C-8), 137.1 (C-6), 129.0 (C-11), 118.3 (C-13), 114.6 (C-12), 113.1 (C-10), 112.7 (C-9); **IR ν_{max} (cm^{-1}):** 3310 (N-H stretch), 3082 (C-H stretch), 1729 (C=O stretch), 1637 (C=C stretch); **HR-ESI-MS:** Calculated m/z of $C_9H_6N_6O_6$ = 294.0349 [M^++H], Found m/z = 294.2871 [M^++H].

5.3.7 2-((3,5-dioxo-3,4,5,6-tetrahydro-1,2,4-triazin-6-yl)amino)benzoic acid (41g)



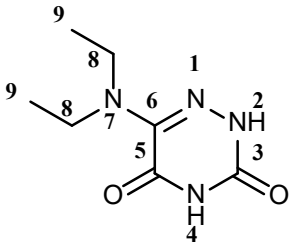
6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (1.00 g, 6 mmol) was reacted with anthranilic acid (0.52 mL, 6 mmol) in THF (5 mL) to obtain 2-((3,5-dioxo-3,4,5,6-tetrahydro-1,2,4-triazin-6-yl) amino) benzoic acid (**41g**) as a yellow solid (1.59 g, 80.7 %), mp = 216.0 °C,lit mp = (215 - 218) °C, (Mitran *et al.,* 2010)**; ^{1}H-NMR (400 MHz, DMSO-d_6):** δ_H (ppm) 13.56 (1H, s, H-15)**,** 12.44 (1H, s, H-4), 12.03 (1H, s, H-2), 7.70 (1H, d, *J* = 7.9 Hz , H-12), 7.00 (1H, dd, *J* = 7.7 Hz, *J* = 7.4 Hz , H-10), 6.57 (1H, dd, *J* = 7.5 Hz, *J* = 7.3 Hz, H-9), 6.49 (1H, d, *J* = 7.4 Hz, H-11), 5.94 (1H, s, H-7); **^{13}C-NMR (100 MHz, DMSO-d_6):** δ_C (ppm) 170.1 (C-14), 154.1 (C-3), 149.5 (C-5), 146.0 (C-8), 138.4 (C-6), 129.7 (C-10), 119.1 (C-12), 116.7 (C-9), 113.1(C-11) & (13); **IR ν_{max} (cm^{-1}):** 3690 (O-H stretch), 3462 (N-H stretch), 2958 (C-H stretch), 1686 (C=O stretch), 1636 (C=C stretch); **HR-ESI-MS:** Calculated m/z of $C_{10}H_8N_4O_4$ = 248.0546 [M^++H], Found m/z = 249.9713 [M^++H].

5.3.8 6-(piperazin-1-yl)-1,2,4-triazine-3,5(2H,4H)-dione (41h)



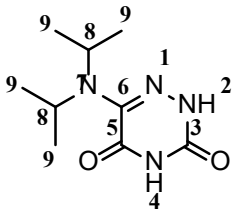
6-bromo-1,2,4-triazine (1.00 g, 6 mmol) was reacted with piperazine (0.50 g, 6 mmol) in 10 mL THF to obtain 6-(piperazin-1-yl)-1,2,4-triazine-3,5(2H,4H)-dione (**41h**) as a yellow solid (1.07 g, 77.6 %), mp = 262.6 °C, **^{1}H-NMR (400 MHz, (DMSO-d$_6$):** δ_H (ppm) 12.55 (1H, s, H-4), 12.28 (1H, s, H-2), 3.65 (4H, t, *J* = 7 Hz, H-8), 2.91 (4H, t, *J* = 7 Hz, H-9) 1,07 (1H, s, H-10); **^{13}C-NMR (100 MHz, (DMSO-d$_6$):** δ_C (ppm) 160.5 (C-3), 156.6 (C-5), 131.1 (C-6), 63.2 (C-9), 43.4 (C-8); **IR ν_{max} (cm^{-1}):** 3385 (N-H stretch), 3150 (C-H stretch), 1765 (C=O stretch), 1556 (C=C stretch); **HR-ESI-MS:** Calculated m/z Of $C_7H_{11}N_5O_5$ =197.0913 [M$^+$+H], Found m/z = 197.9932 [M$^+$+H].

5.3.9 6-(diethylamino)-1,2,4-triazine-3,5(4H,6H)-dione (41i)



6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (1.00 g, 6 mmol) was reacted with diethyl amine (0.52 mL, 6 mmol) in THF (5 mL) to obtain 6-(diethylamino)-1,2,4-triazine-3,5(4H,6H)-dione (**41i**) as an orange solid (2.16 g, 63.5 %), mp = 133.2 °C, **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ_H (ppm) 12.55 (1H, s, H-4), 12.28 (1H, s, H-2); 2.88 (4H, q, H-8), 1.12 (6H, t, *J* = 7.5 Hz, H-9); **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 159.9 (C-3), 155.9 (C-5), 130.8 (C-6), 41.8 (C-8), 11.3 (C-9); **IR ν_{max} (cm^{-1}):** 3415 (N-H stretch), 2981 (C-H stretch), 1625 (C=O stretch), 1580 (C=C stretch); **HR-ESI-MS:** Calculated m/z $C_7H_{12}N_4O_2$ = 184.0960 [M$^+$+H], Found m/z = 184.9849 [M$^+$+H].

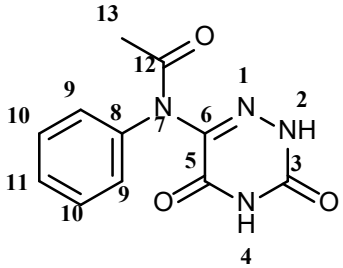
5.3.10 6-(diisopropylamino)-1,2,4-triazine-3,5(4H,6H)-dione (41j)



6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (1.00 g, 6 mmol) was reacted with diisopropyl amine (0.52 mL, 12 mmol) in THF (5 mL) to obtain 6-(diisopropylamino)-1,2,4-triazine-

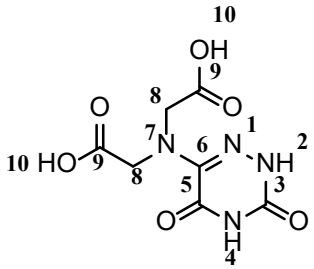
3,5(4H,6H)-dione (**41j**) as an off-white solid (1.16 g, 75.8 %), mp = 183.0 °C , **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ_H (ppm) 12.55 (1H, s, H-4), 12.28 (1H, s, H-2), 3.31 - 3.35 (2H, m, H-8), 1.16 (12H, d, *J* = 6.5 Hz, H-9), **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 160.4 (C-3), 156.6 (C-5), 131.0 (C-6), 46.5 (C-8),19.4 (C-9); **IR ν_{max} (cm^{-1}):** 3480 (N-H stretch), 3047 (C-H stretch), 1652 (C=O stretch), 1595 (C=C stretch); **HR-ESI-MS:** Calculated m/z of $C_9H_{16}N_4O_2$ = 212.1273 [M$^+$+H], Found m/z = 212.9430 [M$^+$+H].

5.3.11 N-(3,5-dioxo-3,4,5,6-tetrahydro-1,2,4-triazin-6-yl)-N-phenylacetamide (41k)



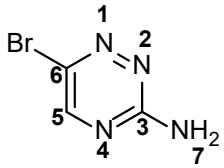
6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (1.00 g, 6 mmol) was reacted with acetanilide (0.52 mL, 6 mmol) in THF (5 mL) to obtain N-(3,5-dioxo-3,4,5,6-tetrahydro-1,2,4-triazin-6-yl)-N-phenylacetamide (**41k**) as a brown solid (2.71 g, 72.4 %), mp = 216.6 °C, **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ_H (ppm) 12.59 (1H, s, H-4), 12.33 (1H, s, H-2), 7.55 (2H, dd, *J* = 8.0 Hz, *J* = 7.7 Hz, H-9), 7.23 – 7.27 (2H, m, H-10), 7.01 (1H, dd, *J* = 7.5 Hz, *J* = 7.4 Hz, H-11), 2.02 (3H, s, H-13); **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 168.8 (C-12), 154.1 (C-3), 149.5 (C-5), 139.7 (C-8), 133.1 (C-6), 129.0 (C-9), 123.4 (C-11), 119.4 (C-10), 24.4 (C-13); **IR ν_{max} (cm^{-1}):** 3381 (N-H stretch), 2982 (C-H stretch), 1701 (C=O stretch), 1597 (C=C stretch); **HR-ESI-MS:** Calculated m/z of $C_{11}H_{10}N_4O_3$ = 246.0753 [M$^+$+H], Found m/z = 246.9834 [M$^+$+H]$^+$.

5.3.12 2,2'-((3,5-dioxo-3,4,5,6-tetrahydro-1,2,4-triazin-6-yl)azanediyl)diacetic acid (41l)



6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione (1.00 g, 6 mmol) was reacted with imminodiacetic acid (0.52 mL, 6 mmol) in THF (5 mL) to obtain 2,2'-((3,5-dioxo-3,4,5,6-tetrahydro-1,2,4-triazin-6-yl)azanediyl)diacetic acid (**41l**) as an off-white solid (1.92 g, 98.2 %), mp = 228.4 °C, **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ_H (ppm) 13.56 (2H, s, H-10)**,** 12.58 (1H, s, H-4), 12.10 (1H, s, H-2), 3.57 (4H, s, H-8), **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 178.8 (C-9), 155.5 (C-3), 146.8 (C-5), 144.9 (C-6), 56.1 (C-8); **IR ν_{max} (cm^{-1}):** 3461 (O-H stretch), 3353 (N-H stretch), 1689 (C=O stretch), 1636 (C=C stretch); **HR-ESI-MS:** Calculated m/z of $C_7H_8N_4O_6$ = 244.0444 [M$^+$+H], Found m/z = 245.0195 [M$^+$+ H].

5.5. Synthesis of 6-bromo-1,2,4-triazin-3-amine (44)



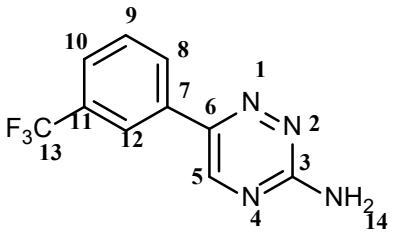
To a solution of 1,2,4-triazin-3-amine (10.00 g, 104.1 mmol) in MeOH:H_2O (1:1) (100 mL) was added bromine (5.32 mL, 104.1 mmol) drop-wise. The reaction mixture was stirred at room temperature for 2 hours. Excess solvent was removed on a rotary evaporator before the mixture was treated with 10% solution of $NaHCO_3$ (20 mL) and extracted with ethyl acetate (3 × 50 mL). Organic extracts were combined and dried ($MgSO_4$), filtered and excess solvent removed on a rotary evaporator to obtain a brown oil that was purified by column chromatography using 10% ethyl acetate/dichloromethane) as an eluent. Product was obtained as a yellow solid (5.17 g, 28.0 %), mp = 233.5 °C, **^{1}H NMR (400 MHz, DMSO-d$_6$)**: **δ_H** (ppm) 8.39 (1H, s, H-5), 7.48 (2H, s, H-7); **^{13}C NMR (100 MHz, DMSO-d$_6$)**: **δ_C** (ppm) 162.7 (C-3), 153.0 (C-5), 135.9 (C-6); **IR ν_{max} (cm^{-1}):** 3302 (N-H stretch), 3163 (C-H stretch), 1636 (C=C stretch), 525 (C-Br stretch); **HR-ESI-MS:** Calculated m/z of $C_3H_3BrN_4$ = 173.9541 [M$^+$+H], Found m/z = 174.850 [M$^+$+H].

5.6. General method for the Suzuki coupling of 6-bromo-1,2,4-triazin-3-amine

To a solution of 6-bromo-1,2,4-triazin-3-amine (1 mmol) in 1,2-dioxane and palladium catalyst (0.3 mmol) was added a deoxygenated solution of boronic acid (2 mmol) in

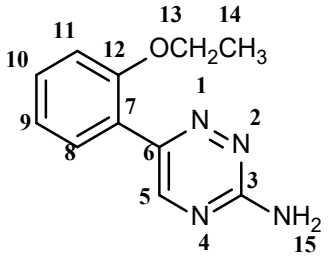
ethanol. Finally a deoxygenated solution of aqueous sodium carbonate was added to the reaction mixture. The resultant mixture refluxed for 60 minutes in a microwave. The products obtained after extraction as yellow to orange solids.

5.6.1 6-(3-(trifluoromethyl)phenyl)-1,2,4-triazin-3-amine (43a)



6-bromo-1,2,4-triazin-3-amine (0.50 g, 3 mmol) in dioxane (20 mL) and $[Pd(PPh_3)_4]$ (0.3040 g, 0.3 mmol) was reacted with 3-(trifluoromethyl) phenyl boronic acid (0.81 g, 3 mmol) in EtOH (10 mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(3-(trifluoromethyl) phenyl)-1,2,4-triazin-3-amine (**43a**) as a yellow solid (0.79 g, 71.8 %), mp = 249.0 °C, **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ_H (ppm) 8.39 (1H, s, H-5), 8.05 (1H, s, H-12), 7.63 (1H, d, *J* = 7.5 Hz , H-10), 7.56 (1H, dd, *J* = 7.6, 1,6 Hz , H-9), 7.48 (1H, d, *J* = 7.3 Hz , H-8), 6.50 (2H, s, H-14); **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 162.9 (C-3), 158.2 (C-6), 148.9 (C-5), 146.9 (C-13), 135.9 (C-7), 135.1 (C-11), 132.3 (C-10), 131.9 (C-12), 129.5 (C-9), 125.6 (C-8), **IR ν_{max} (cm^{-1}):** 3391 (N-H stretch), 3146 (C-H stretch), 1657 (C=C stretch), 1116 (C-F stretch); **HR-ESI-MS:** Calculated m/z of $C_{10}H_7F_3N_4$ = 240.0623 $[M^+ + H]$, Found m/z = 241.0699 $[M^+ + H]$.

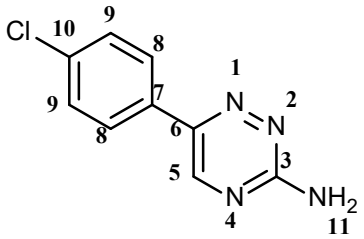
5.6.2 6-(2-ethoxyphenyl)-1,2,4-triazin-3-amine (43b)



6-bromo-1,2,4-triazin-3-amine (0.50 g, 3 mmol) in dioxane (20 mL) and $[Pd(PPh_3)_4]$ (0.3040 g, 0.3 mmol) was reacted 2-ethoxy-phenyl boronic acid (0.65 g, 3 mmol) in EtOH (10 mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(thiophen-2-yl)-1,2,4-triazin-3-amine (**43b**) as a yellow solid (076 g, 94,3 %), mp = 184.8 °C, **^{1}H-NMR (400 MHz, DMSO-d$_6$):**

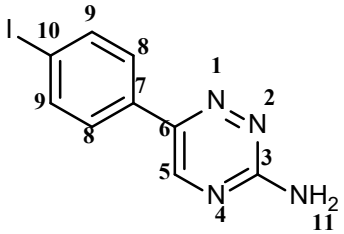
δ_H (ppm) 8.83 (H, s, H-5), 7,72 (1H, d, *J* = 7.7 Hz, H-8), 7.69 (1H, dd, *J* = 8.9 Hz, *J* = 1.8 Hz, H-9), 7.37 (1H, dd, *J* = 7.5, 1.8 Hz, H-10), 7.26 (1H, d, *J* = 7.4 Hz, H-11), 6.68 (2H, s, H-15), 3.94 (2H, q, *J* = 6.9 Hz, H-13), 1.09 (3H, t, *J* = 6.9 Hz, H-14); **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 162.0 (C-3), 156.0 (C-12), 151,4 (C-5), 148.0 (C-6), 129.73 (C-11), 124.6 (C-7), 123.96 (C-8), 120.99 (C-10), 112.90 (C-9), 63.89 (C-13), 14.95 (C-14); **IR ν_{max} (cm^{-1}):** 3347 (N-H stretch), 3026 (C-H stretch), 1574 (C=C stretch), 1232 (C-O stretch); **HR-ESI-MS:** Calculated m/z of $C_{11}H_{12}N_4O$ = 216.1011 [M$^+$+H], Found m/z = 217.1072 [M$^+$+H].

5.6.3 6-(4-chlorophenyl)-1,2,4-triazin-3-amine (43c)



6-bromo-1,2,4-triazin-3-amine (0.50 g, 3 mmol) in dioxane (20 mL) and [Pd(PPh$_3$)$_4$] (0.3040 g, 0.3 mmol) was reacted 4-chloro-phenyl boronic acid (0.23 g, 3 mmol) in EtOH (10 mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(4-chlorophenyl)-1,2,4-triazin-3-amine (**43c**) as a yellow solid mass = (0.49 g, 52.2 %), mp = 185.1 °C, **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ_H (ppm) 8.80 (1H, s, H-5), 8.01 (2H, d, *J* = 7.6 Hz, H-8), 7.54 (2H, d, *J* = 7.8 Hz, H-9), 6.77 (2H, s, H-11); **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 162.7 (C-3), 156.7 (C-6), 148.5 (C-5), 147.2 (C-7), 134.0 (C-10), 129.5 (C-9), 127.1 (C-8); **IR ν_{max} (cm^{-1}):** 3304 (N-H stretch), 3184 (C-H stretch), 1638 (C=C stretch), 801 (C-Cl stretch); **HR-ESI-MS:** Calculated m/z of $C_9H_{17}ClN_4$ = 206.0359 [M$^+$+ 2H], Found m/z = 208.9440 [M$^+$+ 2H].

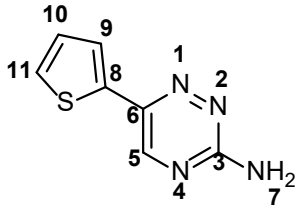
5.6.4 6-(4-iodophenyl)-1,2,4-triazin-3-amine (43d)



6-bromo-1,2,4-triazin-3-amine (0.50 g, 3 mmol) in dioxane (20 mL) and [$Pd(PPh_3)_4$] (0.3040 g, 0.3 mmol) was reacted 4-iodo-phenyl boronic acid (0.97 g, 3 mmol) in EtOH (10 mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(4-iodophenyl)-1,2,4-triazin-3-amine (**43d**) as a yellow solid (0.07 g, 9.9 %), mp 132.5 °C, **^{1}H-NMR (400 MHz, DMSO-d_6):** δ_H (ppm) 8.37 (1H, s, H-5), 8.32 (2H, s, H-11), 7.62 (2H, d, *J* = 8.0 Hz , H-9), 7.37 (2H, d, *J* = 7.9 Hz, H-8);**^{13}C-NMR (100 MHz, DMSO-d_6):** δ_C (ppm) 162.7 (C-3) ,153.0 (C-5), 138.3 (C-9), 135.9 (C-7) & C-10), 133.3 (C-6), 127.4 (C-8); **IR ν_{max} (cm^{-1}):** 3386 (N-H stretch), 3176 (C-H stretch), 1633 (C=C stretch), 687 (C-I stretch); **HR-ESI-MS:** Calculated m/z of $C_9H_7IN_4$ = 297.9715 [M^++H], Found m/z = 298.1101 [M^++H].

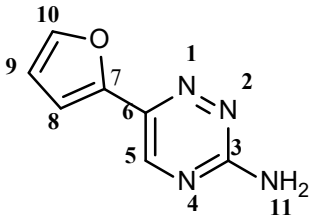
5.7. Suzuki coupling reactions of 6-bromo-1,2,4-triazin-3-amine with 2-thiophenyl or 2-furanylboronic acids

5.7.1 6-(thiophen-2-yl)-1,2,4-triazin-3-amine (45a)



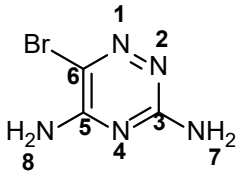
6-bromo-1,2,4-triazin-3-amine (0.50 g, 3 mmol) in dioxane (20 mL) and [$Pd(PPh_3)_4$] (0.3040 g, 0.3 mmol) was reacted with 2-thionyl boronic acid (0.55 g, 3 mmol) in EtOH (10 mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(thiophen-2-yl)-1,2,4-triazin-3-amine (**45a**) as a yellow solid (0.79 g, 94,2 %), mp = 153.4 °C, **^{1}H NMR (400 MHz, DMSO-d_6)** **δ_H** (ppm) 8.83 (1H, s, H-5), 7.68 (1H, d, *J* = 4 Hz, H-11), 7.58 (1H, d, *J* = 5.3 Hz, H-9), 7.15 (1H, dd, *J* = 5.1, 3.6 Hz, H-10), 6.63 (2H, s, H-7); **^{13}C NMR (100 MHz, DMSO-d_6)** **δ_C** (ppm) 162.5 (C-3), 147.6 (C-5), 145.4 (C-6), 139.1 (C-8), 128.7 (C-9), 127.5 (C- 11), 124.6 (C-10); **IR ν_{max} (cm^{-1}):** 3315 (N-H stretch), 3036 (C-H stretch), 1620 (C=C stretch), 996 (C-S stretch); **HR-ESI-MS:** Calculated m/z of $C_7H_6N_4S$ = 178.0313 [M^++H], Found m/z = 179.0372 [M^++H].

5.7.2 6-(furan-2-yl)-1,2,4-triazin-3-amine (45b)



6-bromo-1,2,4-triazin-3-amine (0.50 g, 3 mmol) in dioxane (20 mL) and [$Pd(PPh_3)_4$] (0.3040 g, 0.3 mmol) was reacted 2-furanyl-boronic acid (0.44 g, 3 mmol) in EtOH (10 mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(furan-2-yl)-1,2,4-triazin-3-amine (**45b**) as a yellow solid (0.46 g, 66.7 %), mp = 124.5 °C, **^{1}H-NMR (400 MHz, DMSO-d$_6$):** δ_H (ppm) 8.54 (1H, s, H-5), 8,20 (1H, d, *J* = 7.9 Hz , H-10), 7.61 (1H, dd, *J* = 11.6, 7.6 Hz, H-9), 7.54 (1H, d, *J* = 8.2 Hz , H-8), 7.02 (2H, s, H-11); **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 163.7 (C-3), 150.4 (C-5), 141.1 (C-10), 133.4 (C-6), 132.4 (C-7), 131.9 (C-9), 129.2 (C-8); **IR ν_{max} (cm^{-1}):** 3303 (N-H stretch), 3058 (C-H stretch), 1532 (C=C stretch), 860 (C-O stretch); **HR-ESI-MS:** Calculated m/z of $C_7H_6N_4O$ = 162.0542 [M^++H], Found m/z = 163.1330 [M^++H].

5.8. Synthesis of 6-bromo-1,2,4-triazin-3,5-diamine (47)



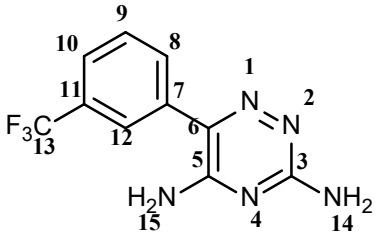
To a yellow mixture of 6-bromo-1,2,4-triazin-3-amine (1.00 g, 6 mmol) in dry liquid ammonia (250 mL) was added potassium permanganate (1.64 g, 12 mmol) in one portion. The resultant dark purple mixture was stirred for 30 minutes at room temperature before being extracted with warm isopropanol (3 × 20 mL) to give a yellow crude product that was purified by column chromatography using 10% MeOH/EtOAc as an eluent. The product was obtained as a yellow solid (0.91 g, 92.9 %), mp = 220.3 °C, **^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H** (ppm) 7.44 (2H, s, H-7), 6.45 (2H, s, H-8); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C** (ppm) 162.4 (C-3), 154.4 (C-5), 125.5 (C-6); **IR ν_{max} (cm^{-1}):** 3324 (N-H

stretch), 1638 (C=C stretch), 559 (C-Br stretch); **HR-ESI-MS:** Calculated m/z $C_3H_4BrN_5$ = 188.9650 [M^++H], Found m/z = 188.9875 & 190.9656 [M^++H].

5.9. General method for the Suzuki coupling of 3.5-diamino-6-bromo-1.2.4-triazine

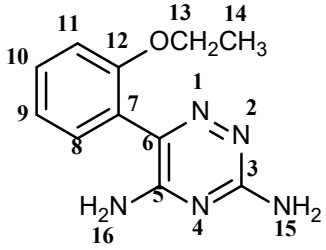
To a solution of 3.5-diamino-6-bromo-1.2.4-triazine (1 mmol) in 1,2-dioxane and [$Pd(PPh_3)_4$] (0.3 mmol) was added a deoxygenated solution of boronic acid (2 mmol) in ethanol. Finally, a deoxygenated solution of aqueous sodium carbonate was added. The resultant mixture was refluxed for 60 minutes in a microwave. The products obtained after extraction as yellow to orange solids.

5.9.1 6-(3-(trifluoromethyl)phenyl)-1,2,4-triazine-3,5-diamine (46a)



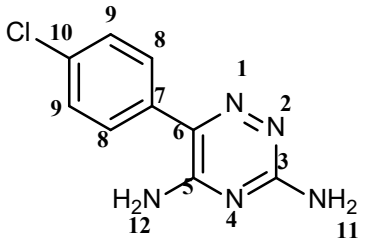
3.5-diamino-6-bromo-1.2.4-triazine (0.50 g, 3 mmol) in dioxane (20 mL) and [$Pd(PPh_3)_4$] (0.3008 g, 0.3 mmol) was reacted 3-(trifluoromethyl) phenyl boronic acid (0.81 g, 3 mmol) in EtOH (10 mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(3-(trifluoromethyl)phenyl)-1,2,4-triazine-3,5-diamine (**46a**) as a yellow solid (0.41 g, 46.3 %), mp = 243.6 °C, **^{1}H-NMR 400 MHz, (DMSO-d$_6$):** δ_H (ppm) 7.83 (1H, s, H-12), 7.75 (1H, dd, *J* = 7.7, 1,5 Hz , H-9), 7.64 (1H, d, *J* = 7.6 Hz, H-10), 7.57 (1H, d, *J* = 7,5 Hz , H-8), 6.83 (2H, s, H-15), 6.44 (2H, s, H-14); **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ_C (ppm) 162.0 (C-3), 154.8 (C-5) & (C-6), 136.9 (C-7), 132.5 (C-10), 131.9 (C-12), 130.1 (C-11), 129.2 (C-8), 125.1 (C-9), 124.9 (C-13); **IR ν_{max} (cm^{-1}):** 3327 (N-H stretch), 3035 (C-H stretch), 1660 (C=C stretch), 698 (C-F stretch); **HR-ESI-MS:** Calculated m/z of $C_{10}H_8F_3N_5$ = 255.0732 [M^++H], Found m/z = 256.0800 [M^++H].

5.9.2 6-(2-ethoxyphenyl)-1,2,4-triazine-3,5-diamine (46b)



3.5-diamino-6-bromo-1.2.4-triazine (0.50 g, 3 mmol) in dioxane (20 mL) and [$Pd(PPh_3)_4$] (0.3008 g, 0.3 mmol) was reacted 2-ethoxy-phenyl boronic acid (0.65 g, 3 mmol) in EtOH (10 mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(2-ethoxyphenyl)-1,2,4-triazine-3,5-diamine (**46b**) as a yellow solid (0.12 g, 15.4 %), mp = °C, **^{1}H-NMR (400 MHz, DMSO-d_6):** δ_H (ppm) 7.41 (1H, dd, *J* = 7.9, 1.8 Hz, H-10), 7.26 (1H, d, *J* = 7.7 Hz, H-11), 7.03 (1H, dd, *J* = 7.5, 1.8 Hz, H-9), 6.22 (2H, s, H-16), 5,52 (2H, s, H-15), 4.09 (2H, q, *J* = 6,9 Hz, H-13), 1.33 (3H, t, *J* = 6.9 Hz , H-14); **^{13}C-NMR (100 MHz, DMSO-d_6):** δ_C (ppm) 162.0 (C-3), 156.7 (C-5), 155.8 (C-12), 147.2 (C-6), 135.9 (C-7), 121.2 (C-11), 131.6 (C-8), 120.8 (C-10), 130.4 (C-9), 113.0 (C-12), 66.8 (C-13),15.1 (C-14); **IR ν_{max} (cm^{-1}):** 3464 (N-H stretch), 3057 (C-H stretch), 1660 (C=C stretch), 1009 (C-O stretch); **HR-ESI-MS:** Calculated m/z of $C_{11}H_{13}N_5O$ = 231.1120 [M^++H], Found m/z = 232.1196 [M^++H].

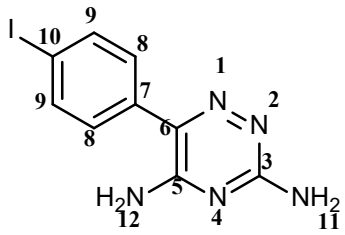
5.9.3 6-(4-chlorophenyl)-1,2,4-triazine-3,5-diamine (46c)



3.5-diamino-6-bromo-1.2.4-triazine (0.28 g, 2 mmol) in dioxane (20 mL) and [$Pd(PPh_3)_4$] (0.3008 g, 0.3 mmol) was reacted 4-chloro-phenyl boronic acid (0.23 g, 2 mmol) in EtOH (10 mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(4-chlorophenyl)-1,2,4-triazine-3,5-diamine (**46c**) as a yellow solid mass = (0.66 g, 83.0 %), mp = 220.0 °C (lit mp = 219 - 222 °C) (Hitchings *et al,.* 1952), **^{1}H-NMR (400 MHz, DMSO-d_6):** δ_H (ppm) 7.67 (2H, d, *J* = 7.8 Hz, H-8), 7.55 (2H, d, *J* = 7.6 Hz , H-9), 7.19 (2H, s, H-12), 6.78 (2H, s, H-11); **^{13}C-NMR (100 MHz, DMSO-d_6):** δ_C (ppm) 161.9 (C-3), 154.7 (C-5), 138.78 (C-6), 134.70 (C-

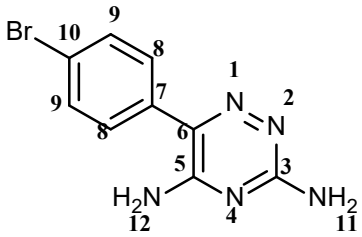
7), 133.43 (C-10), 131.98 (C-8), 129.31 (C-9); **IR ν_{max} (cm^{-1}):** 3232 (N-H stretch), 3184 (C-H stretch), 1644 (C=C stretch), 605 (C-Cl stretch); **HR-ESI-MS:** Calculated m/z of $C_9H_8ClN_5$ = 221.0468 [M^++H], Found m/z = 221.1532 [M^++H].

5.9.4 6-(4-iodophenyl)-1,2,4-triazine-3,5-diamine (46d)



3.5-diamino-6-bromo-1.2.4-triazine (0.50 g, 3 mmol) in dioxane (20 mL) and [$Pd(PPh_3)_4$] (0.3008 g, 0.3 mmol) was reacted 4-iodo-phenyl boronic acid (0.96 g, 3 mmol) in EtOH (10 mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(4-iodophenyl)-1,2,4-triazine-3,5-diamine (**46d**) as a yellow solid (0.35 g, 34.0 %), mp = 214.6 °C, **^{1}H-NMR (400 MHz, DMSO-d_6):** δ_H (ppm) 8.37 (2H, d, *J* = 7.7 Hz , H-8), 7.94 (2H, d, *J* = 7.5 Hz, H-9), 6.92 (2H, s, H-12), 6.66 (2H, s, H-11); **^{13}C-NMR (100 MHz, DMSO-d_6):** δ_C (ppm) 162.7 (C-3), 156.0 (C-5), 138.3 (C-8), 135.9 (C-7) & (C-10), 133.3 (C-6), 132.5 (C-9); **IR ν_{max} (cm^{-1}):** 3301 (N-H stretch), 3125 (C-H stretch), 1651 (C=C stretch), 583 (C-I stretch); **HR-ESI-MS:** Calculated m/z of $C_9H_8IN_5$ = 312.9824 [M^++H], Found m/z = 313.1547 [M^++H].

5.9.5 6-(4-bromophenyl)-1,2,4-triazine-3,5-diamine (46e)

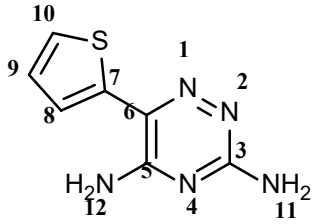


3.5-diamino-6-bromo-1.2.4-triazine (0.50 g, 3 mmol) in dioxane (20 mL) and [Pd(PPh3)4] (0.3008 g, 0.3 mmol) was reacted 4-bromo-phenyl boronic acid (0.79 g, 3 mmol) in EtOH (10 mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(4-bromophenyl)-1,2,4-triazine-3,5-diamine (**46e**) as a yellow solid mass = (0.35 g, 38.5 %), mp = 268.8 °C, **^{1}H-NMR (400 MHz, DMSO-d_6):** δ_H (ppm) 7.63 (2H, d, *J* = 7.9 Hz, H-8), 7.52 (2H, d, *J* = 7.7 Hz , H-9), 7.22 (2H, s, H-12) 6.38 (2H, s, H-11); **^{13}C-NMR (100 MHz, DMSO-d_6):** δ_C (ppm) 162.4

(C-3), 154.4 (C-5), 138.6 (C-6), 135.1 (C-7), 131.9 (C-8), 129.2 (C-9), 125.10 (C-10); **IR ν_{max} (cm^{-1}):** 3484 (N-H stretch), 3046 (C-H stretch), 1644 (C=C stretch), 692 (C-Br stretch); **HR-ESI-MS:** Calculated m/z Of $C_9H_8BrN_5$ = 264.9963 [M^++H], Found m/z = 265.1786 & 267.1935 [M^++ H]$^+$.

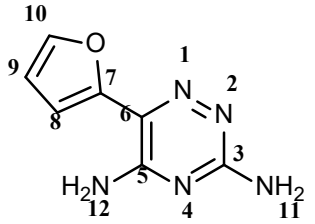
5.10. Suzuki coupling reactions of 3.5-diamino-6-bromo-1.2.4-triazine with 2-thiophenyl or 2-furanylboronic acids

5.10.1 6-(thiophen-2-yl)-1,2,4-triazine-3,5-diamine (49a)



3.5-diamino-6-bromo-1.2.4-triazine (0.50 g, 3 mmol) in dioxane (20 mL) and [$Pd(PPh_3)_4$] (0.3008 g, 0.3 mmol) was reacted 2-thionyl boronic acid (0.50 g, 3 mmol) in EtOH (10 mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(thiophen-2-yl)-1,2,4-triazine-3,5-diamine (**49a**) as an orange solid mass = (0.23 g, 32.3 %), mp = 257.7 °C, **^{1}H-NMR (400 MHz, DMSO-d_6):** δ_H (ppm) 7.64 (1H, d, *J* = 4.9 Hz, H-8), 7.56 (1H, d, *J* = 4.4 Hz, H-10), 7.43 (1H, dd, *J* = 4.7, 2.9 Hz, H-9), 6.92 (2H, s, H-12), 6,48 (2H, s, H-11); **^{13}C-NMR (100 MHz, DMSO-d_6):** δ_C (ppm) 162.4 (C-3), 147.5 (C-5), 145.0 (C-6), 138.9 (C-7), 128.3 (C-10), 127.5 (C-8), 124.4 (C-9); **IR ν_{max} (cm^{-1}):** 3474 (N-H stretch), 3049 (C=H stretch), 1664 (C=C stretch), 947 (C-S stretch); **HR-ESI-MS:** calculated m/z of $C_7H_7N_5S$ = 193.0422 [M^++H], Found m/z = 194.9692 [M^++H].

5.10.2 6-(furan-2-yl)-1,2,4-triazine-3,5-diamine (49b)



3.5-diamino-6-bromo-1.2.4-triazine (0.50 g, 3 mmol) in dioxane (20 mL) and [$Pd(PPh_3)_4$] (0.3008 g, 0.3 mmol) was reacted 2-furanyl-boronic acid (0.44 g, 3 mmol) in EtOH (10

mL) and Na_2CO_3 in H_2O (10 mL) to obtain 6-(furan-2-yl)-1,2,4-triazine-3,5-diamine (**49b**) as a yellow solid mass = (0.34 g, 49.5 %), mp = 140.5 °C, **^{1}H-NMR (400 MHz, DMSO-d_6):** δ_H (ppm) 7.64 (1H, d, *J* = 11.3 Hz, H-10), 7.56 (1H, d, *J* = 7.2 Hz, H-8), 7.53 (1H, dd, *J* = 8.0, 5.7 Hz, H-9), 6.98 (2H, s, H-12), 6.47 (2H, s, H-11); **^{13}C-NMR (100 MHz, DMSO-d_6):** δ_C (ppm) 162.3 (C-3), 154.4 (C-5) & (C-6), 132.6 (C-7), 131.9 (C-10), 129.3 (C-9), 125.1 (C-8); **IR ν_{max} (cm^{-1}):** 3299 (N-H stretch), 3056 (C-H stretch), 1589 (C=C stretch), 1118 (C-O stretch); **HR-ESI-MS:** Calculated m/z = 177.0651 [M^++H], Found m/z = 177.0358 [M^++H].

5.11 BIOLOGICAL EVALUATION

"The minimum inhibitory concentration (MIC) for the investigation of synthesized compounds were determined by the use of broth microdilution susceptibility assay adopted from (Eloff *et al.,* 1999) with slight modifications. The test was performed in the Mueller Hinton broth (MH) broth medium (Sigma-Aldrich, St, MO, USA). A volume of 100 µL of the MHI medium broth was added into each well of 96 microwell plate (NUNC, Rochester, USA) and 100 µL of the different fractions as well as synthetic derivatives with a starting concentration of 50 mg/mL was added in all the wells of the first row; followed by two-fold serial dilution and resulted into various concentrations.

Then 100 µL of 0.5 McFarland inoculum was added in the plates. The plates were incubated overnight 1t 37 °C. After incubation 40 µL of 0.4 mg/mL of Iodo-nitro-tetrazolium (INT) (Sigma-Aldrich, St, MO, USA) was added into each well and incubated for approximately 2-3 hours at 37 °C.

Following incubation, the colour change in the plates was observed and MIC was recorded as the lowest concentration of the synthetic derivatives that inhibited the visible bacterial growth. The 10 mg/mL Gentamicin solution (Sigma-Aldrich, St, MO, USA) was used as a positive control, whereas distilled water was used as a negative control."

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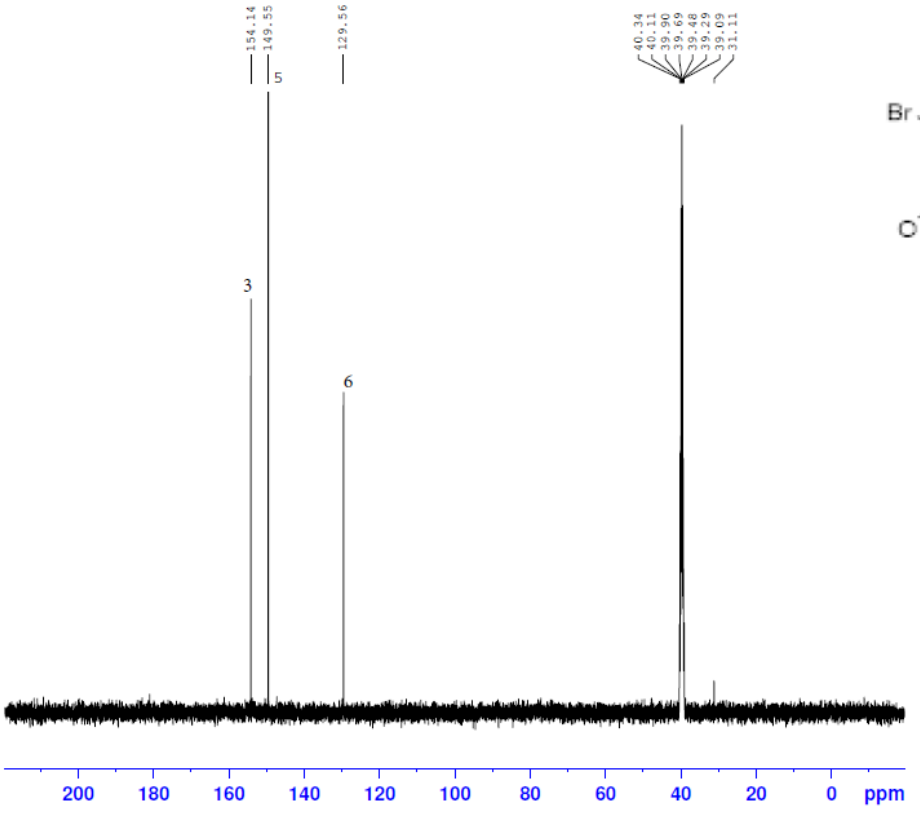
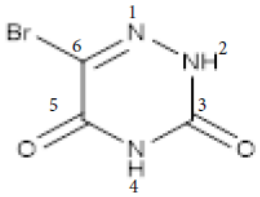
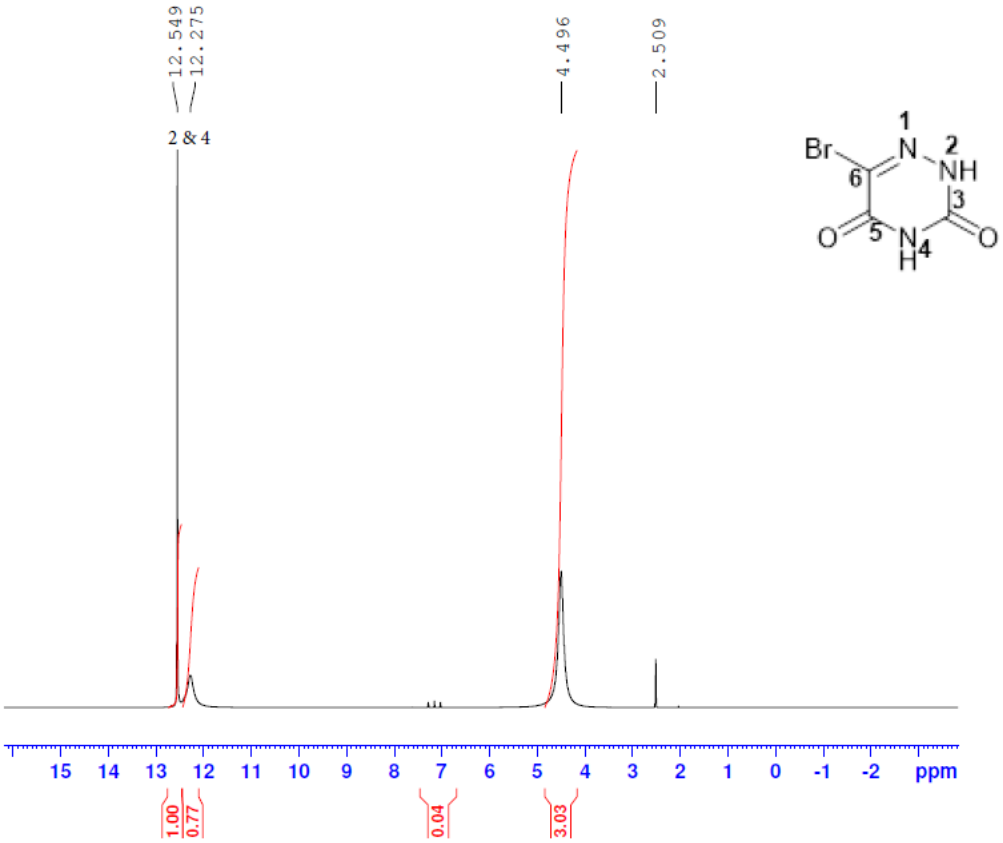
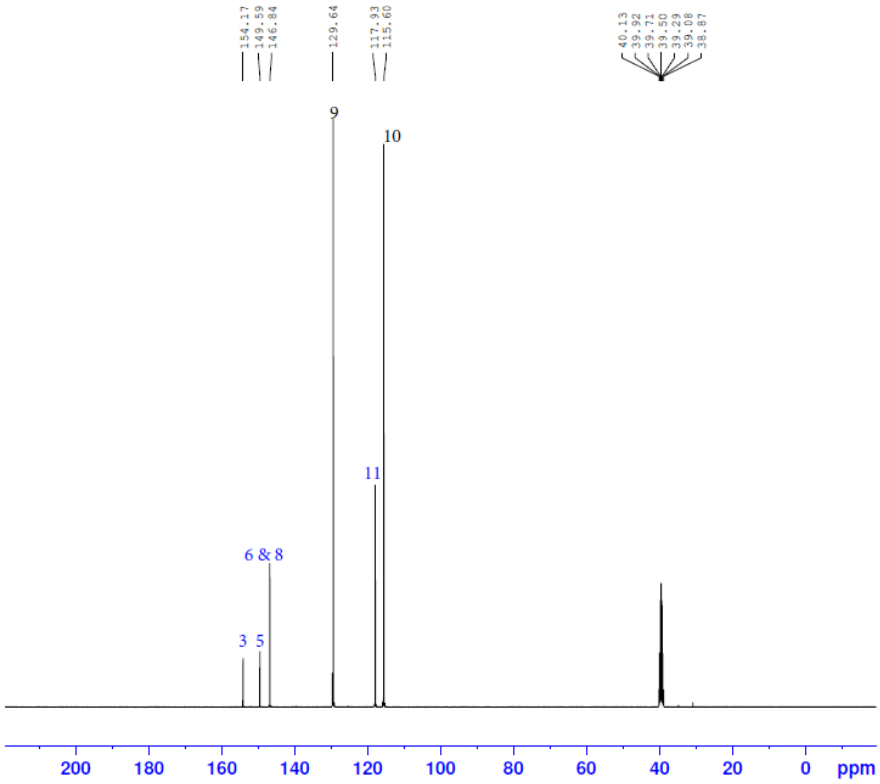
APPENDIX A

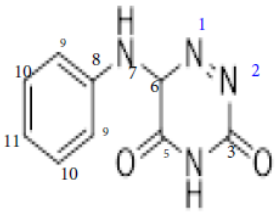
NMR SPECTRA

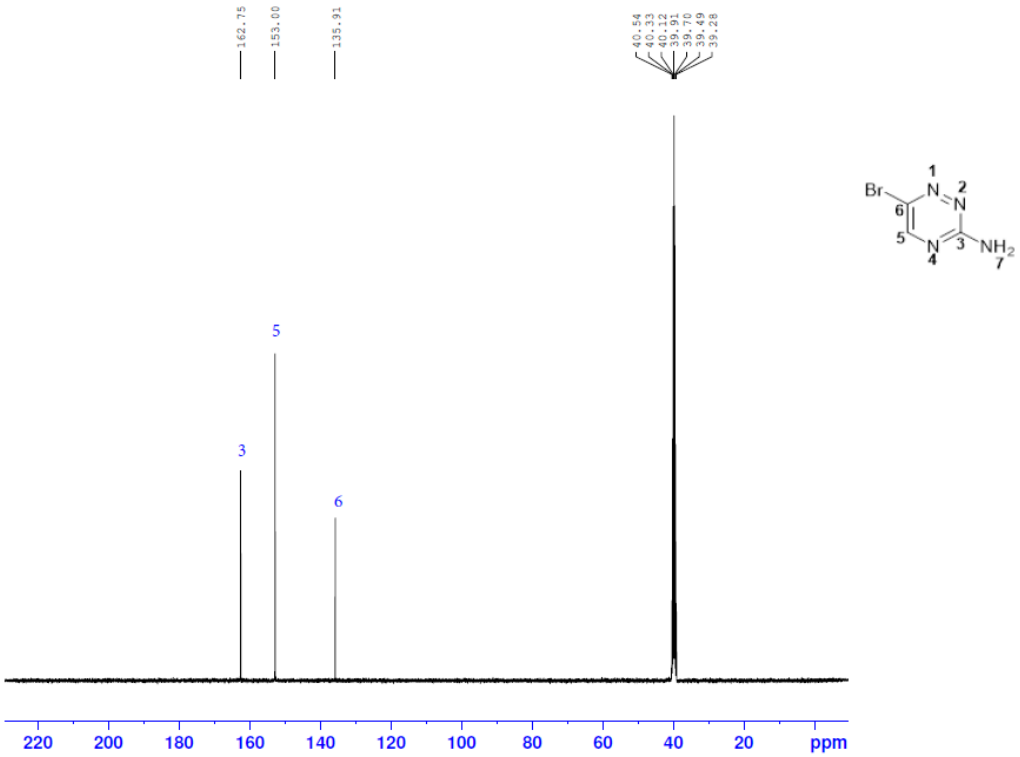


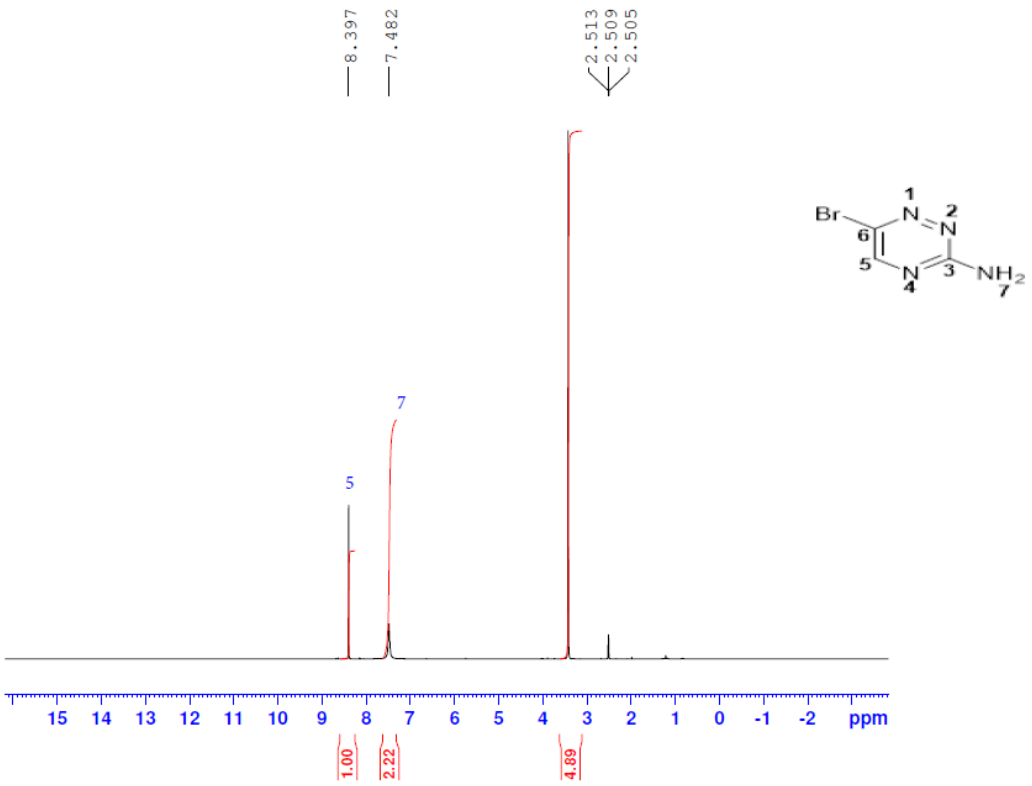


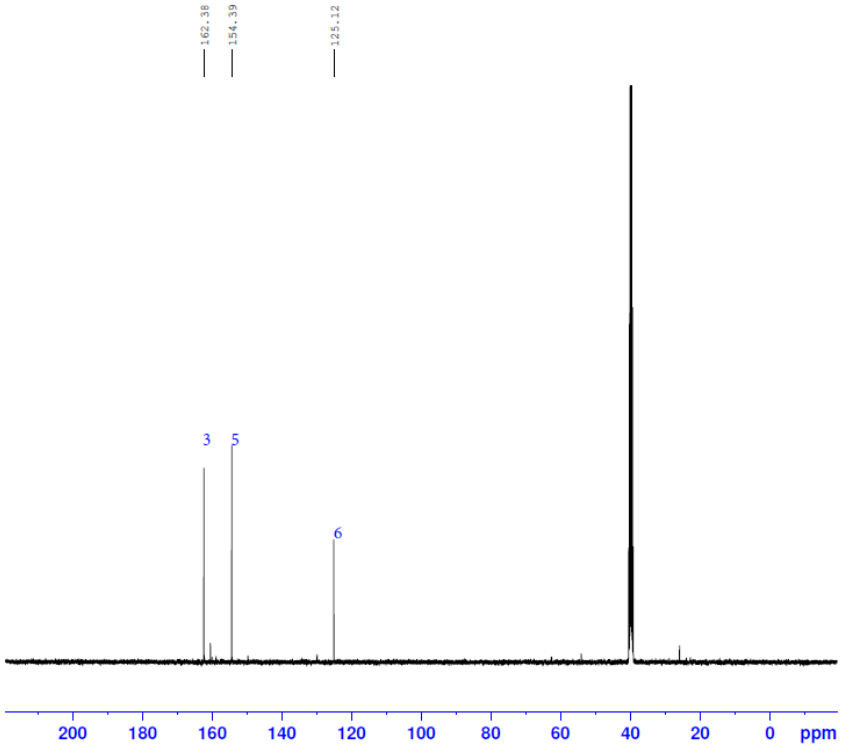


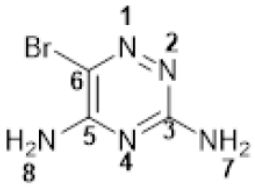


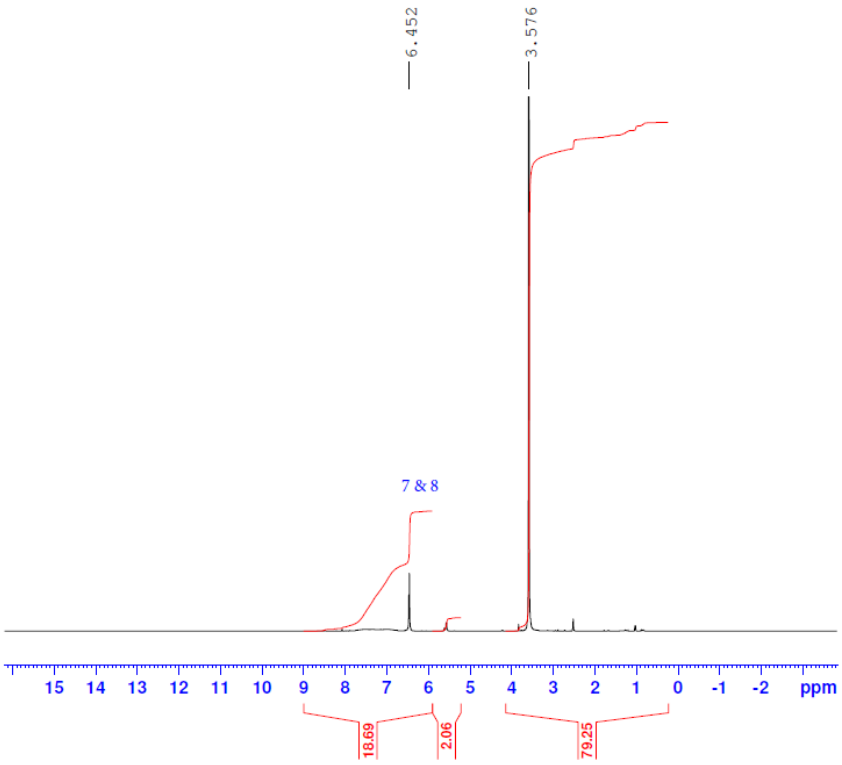


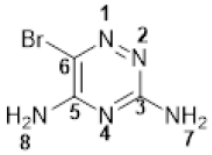


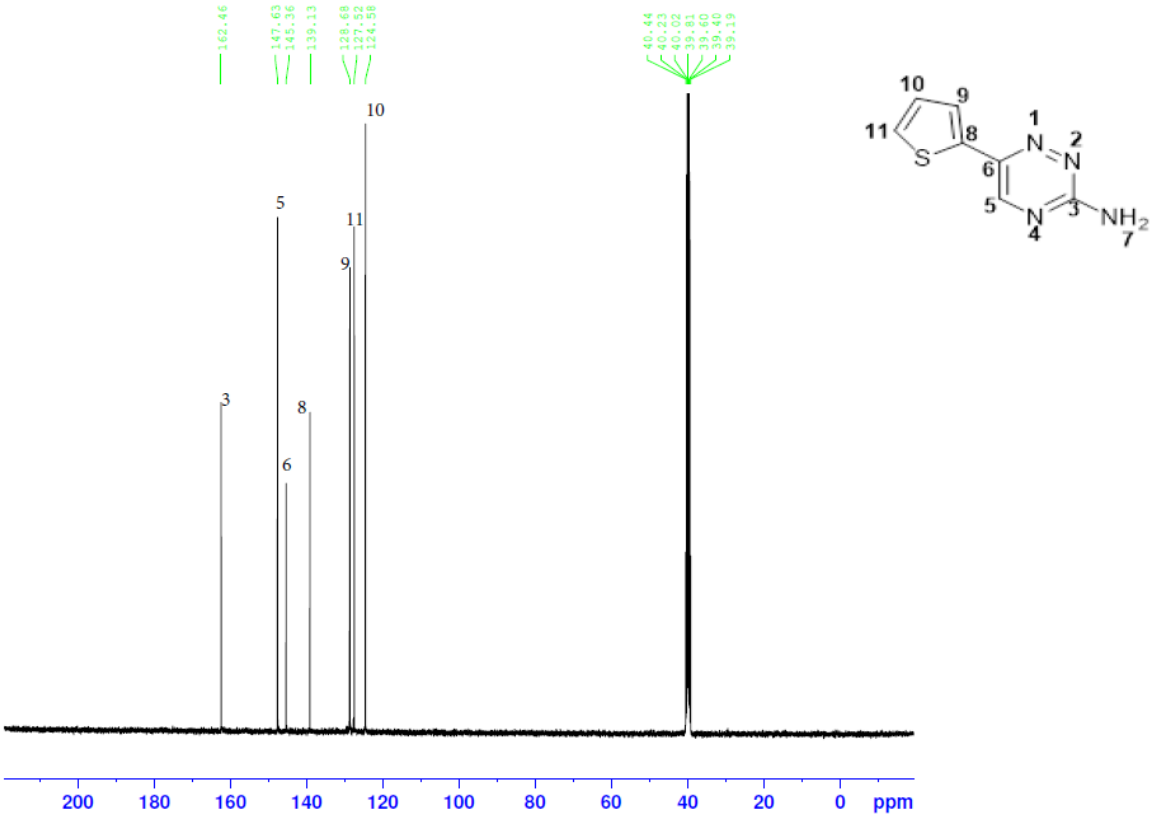


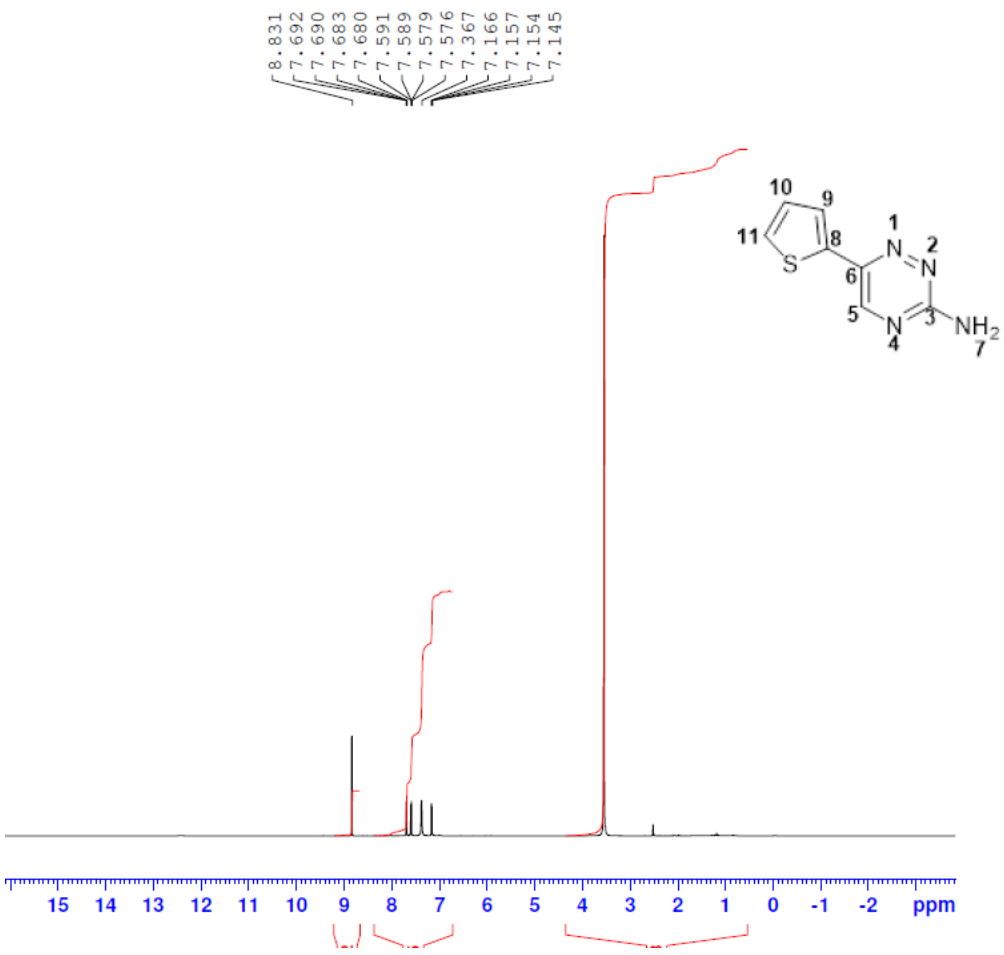


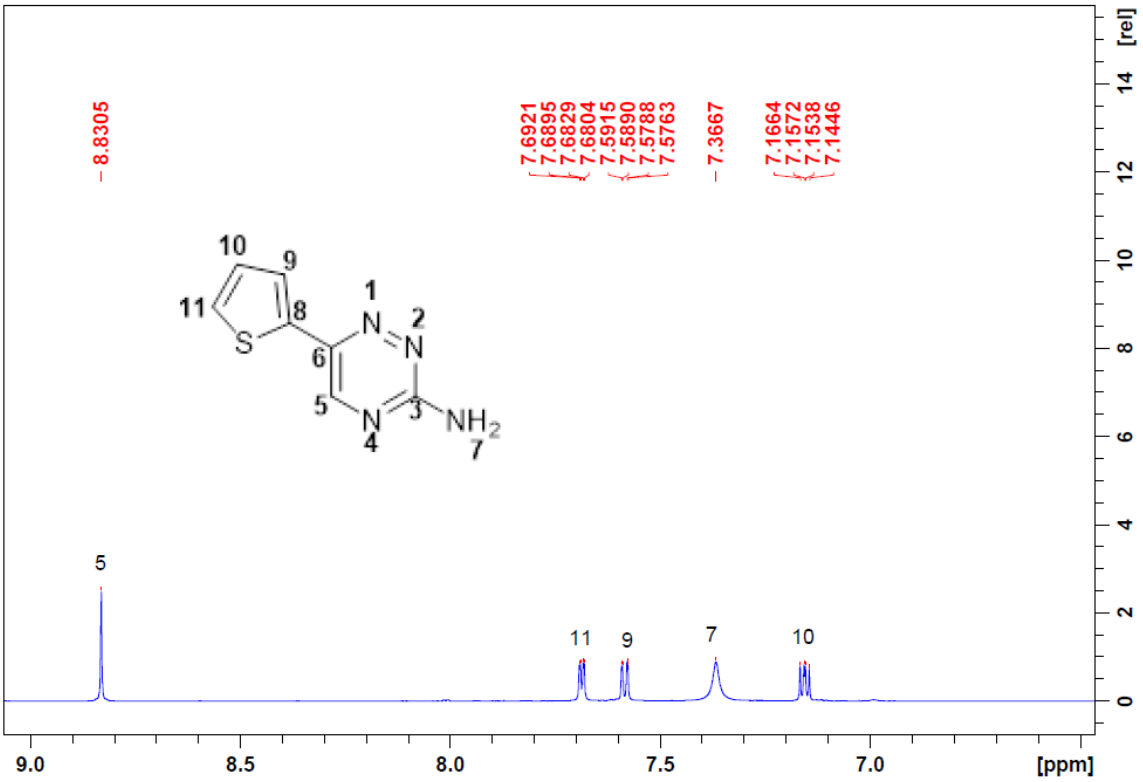


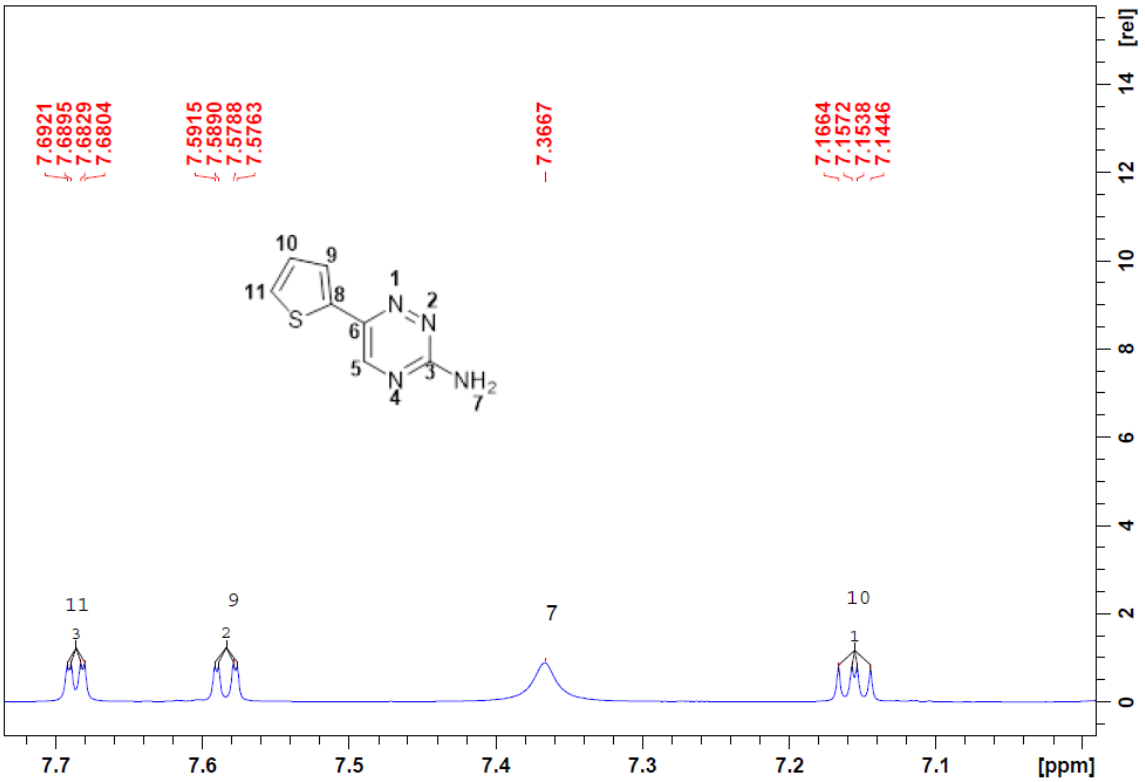






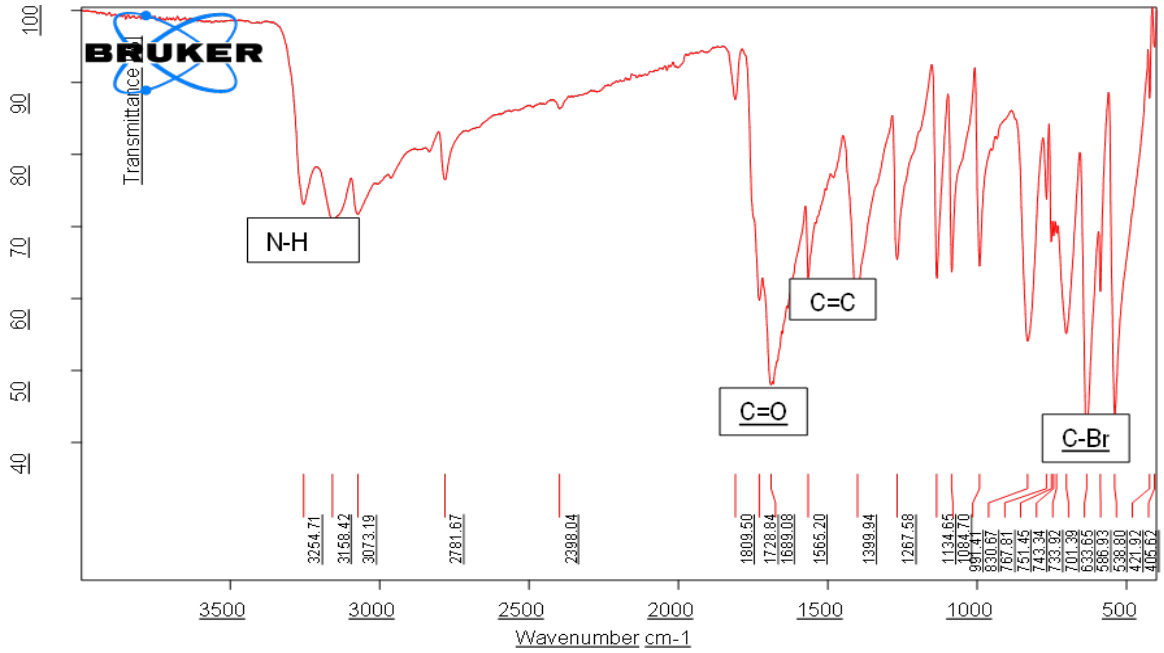






APPENDIX B

FTIR



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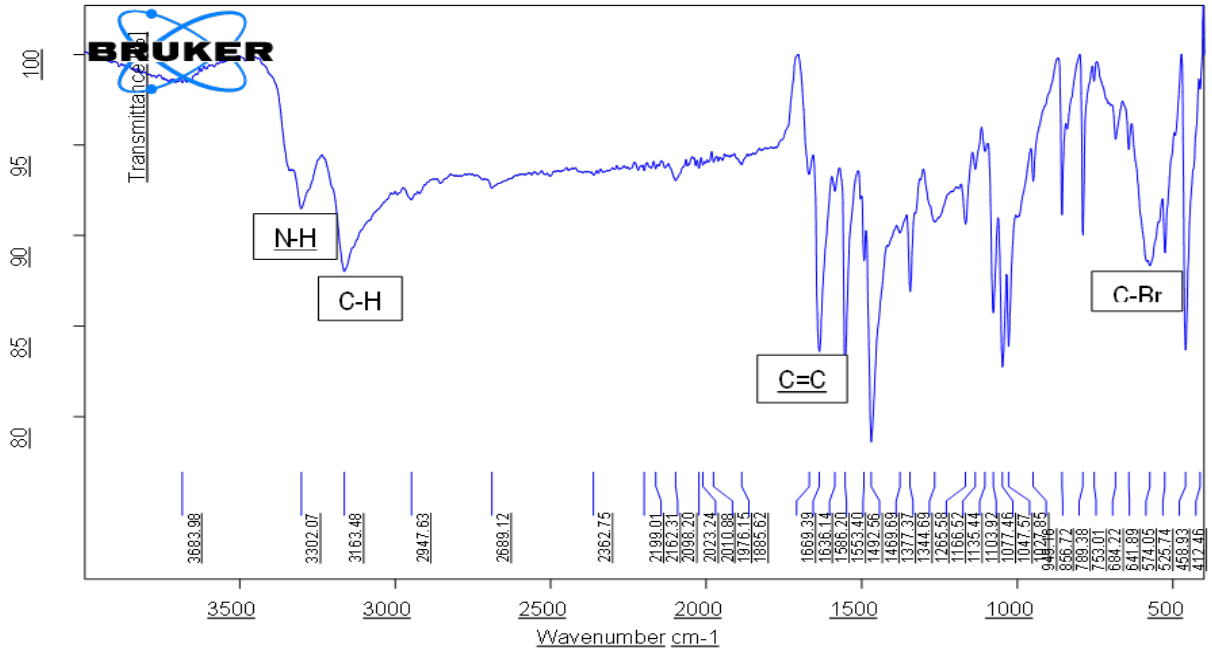

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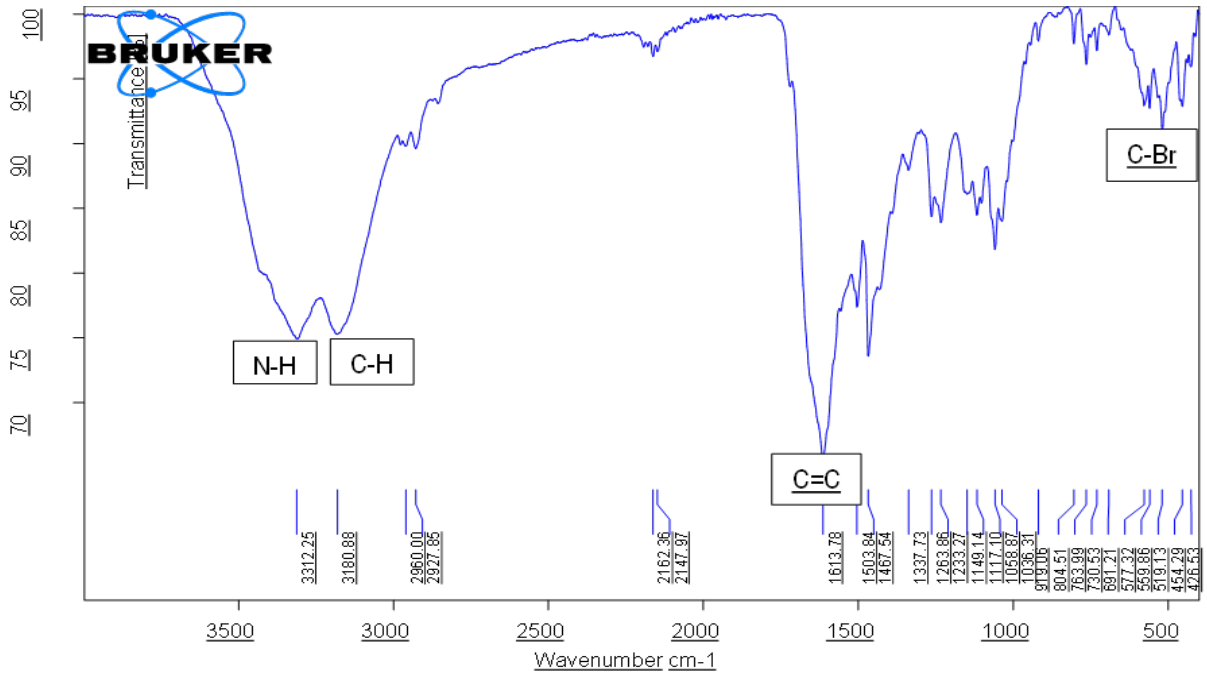
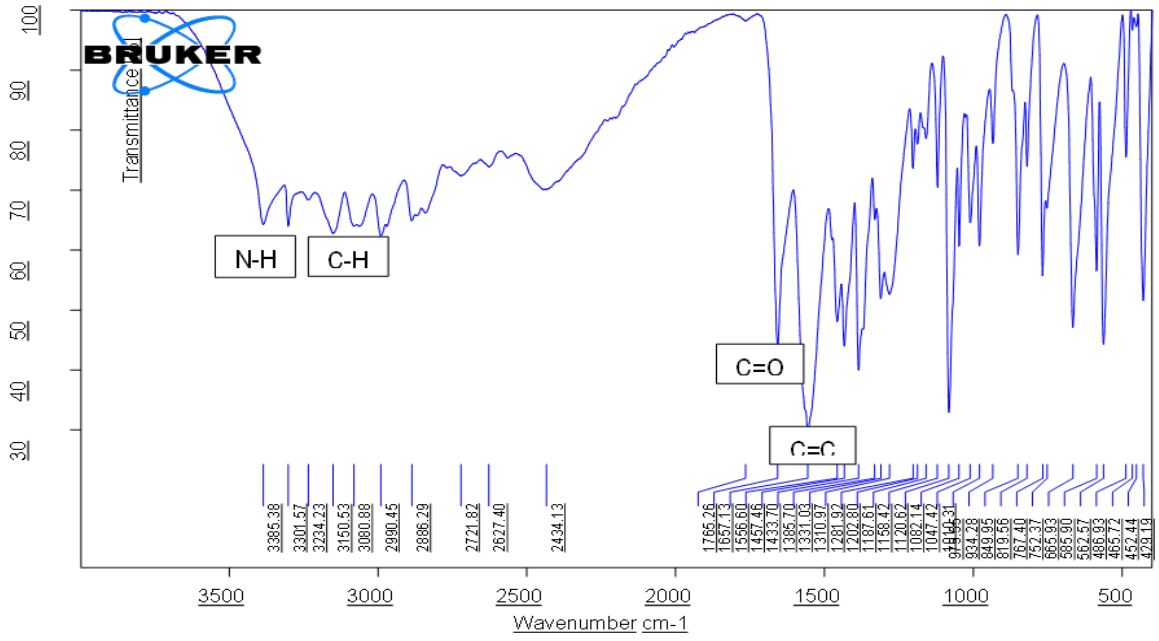


6-bromo-1,2,4-triazin-3,5-diamine



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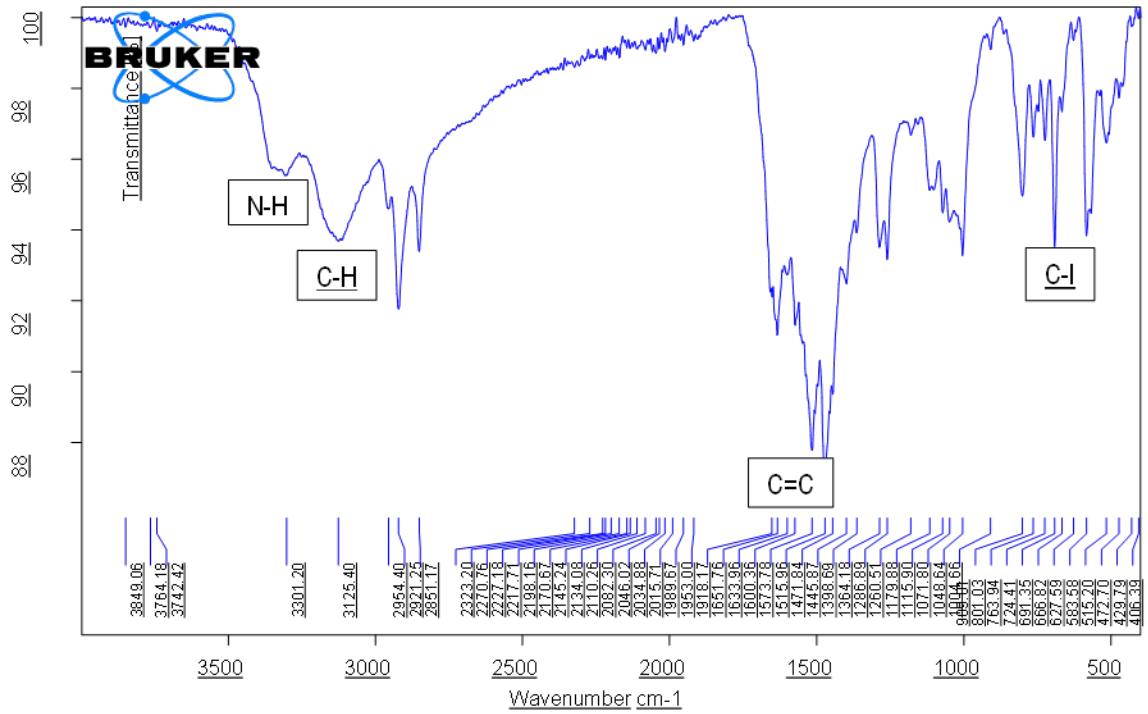
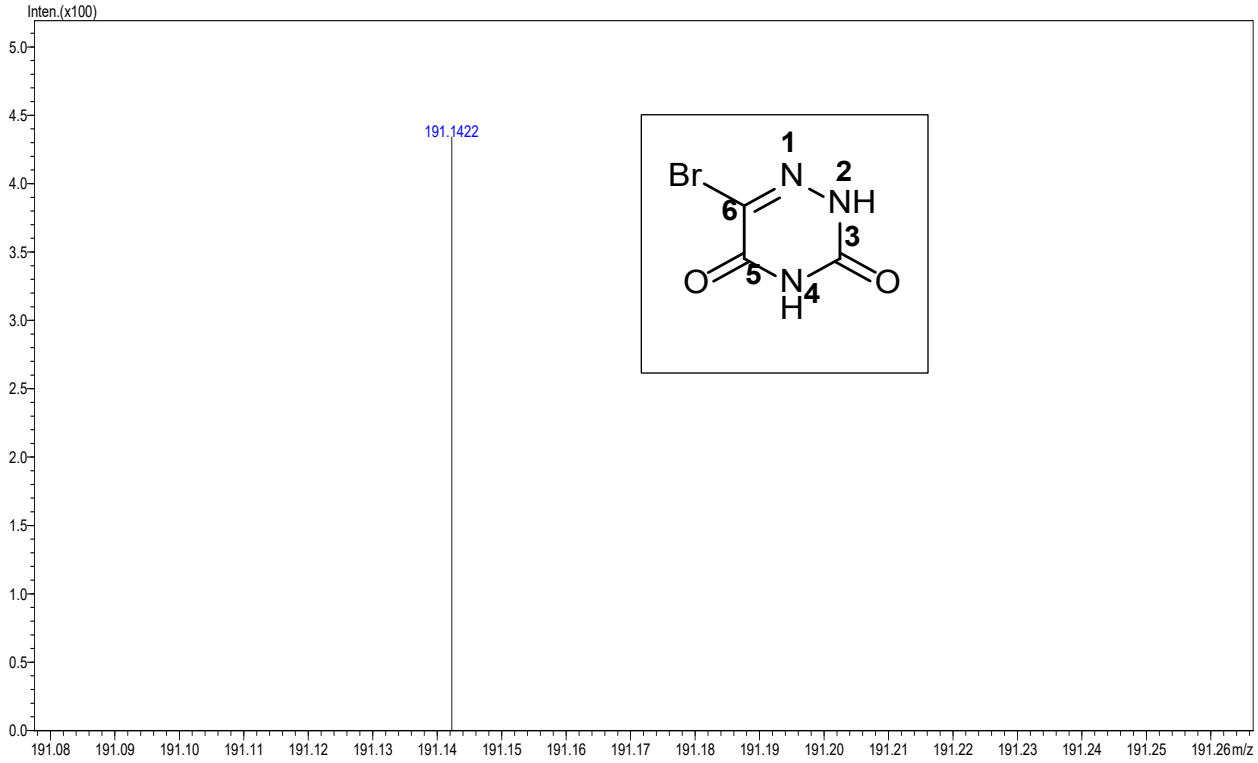


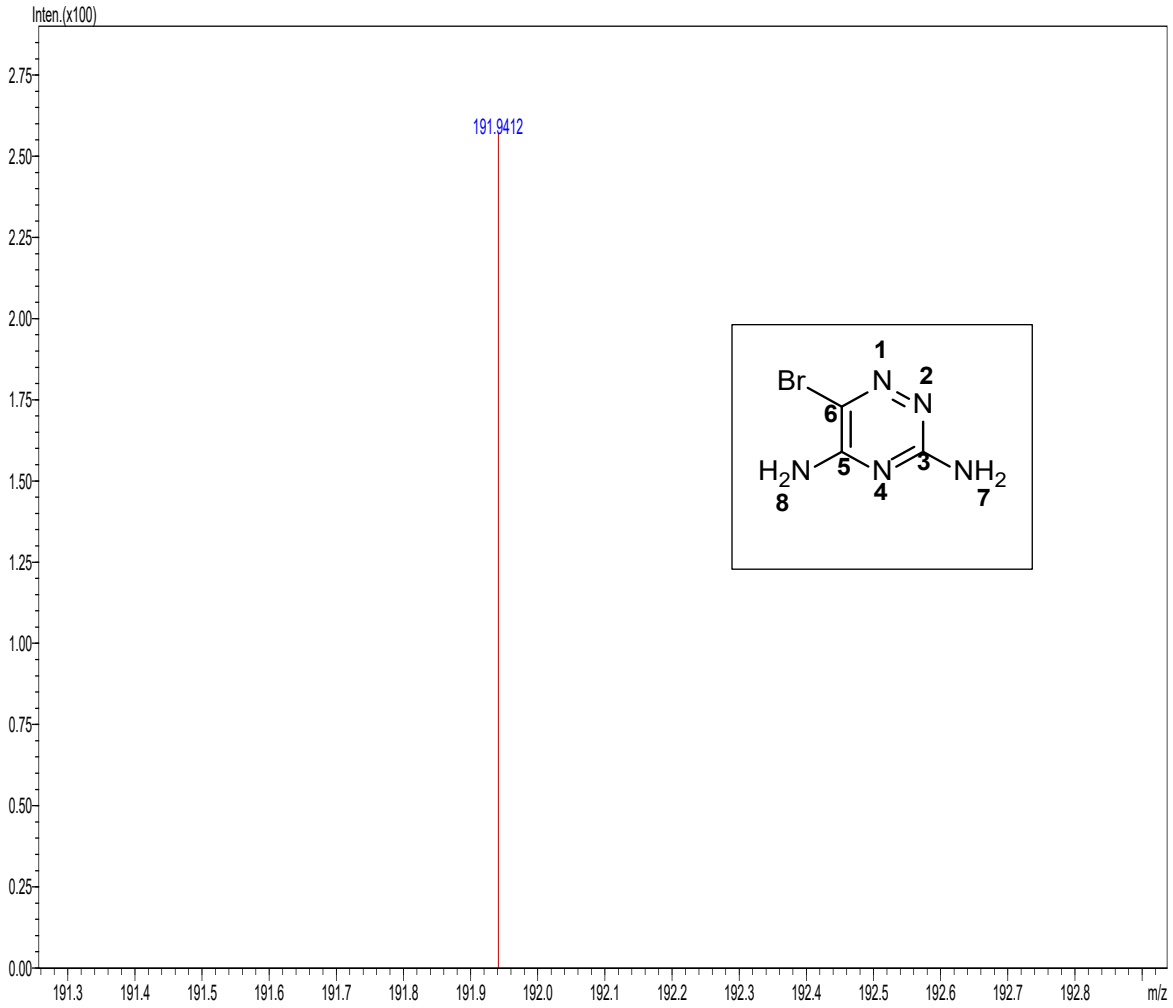
6-(4-iodophenyl)-1,2,4-triazine-3,5,diamine

APPENDIX C

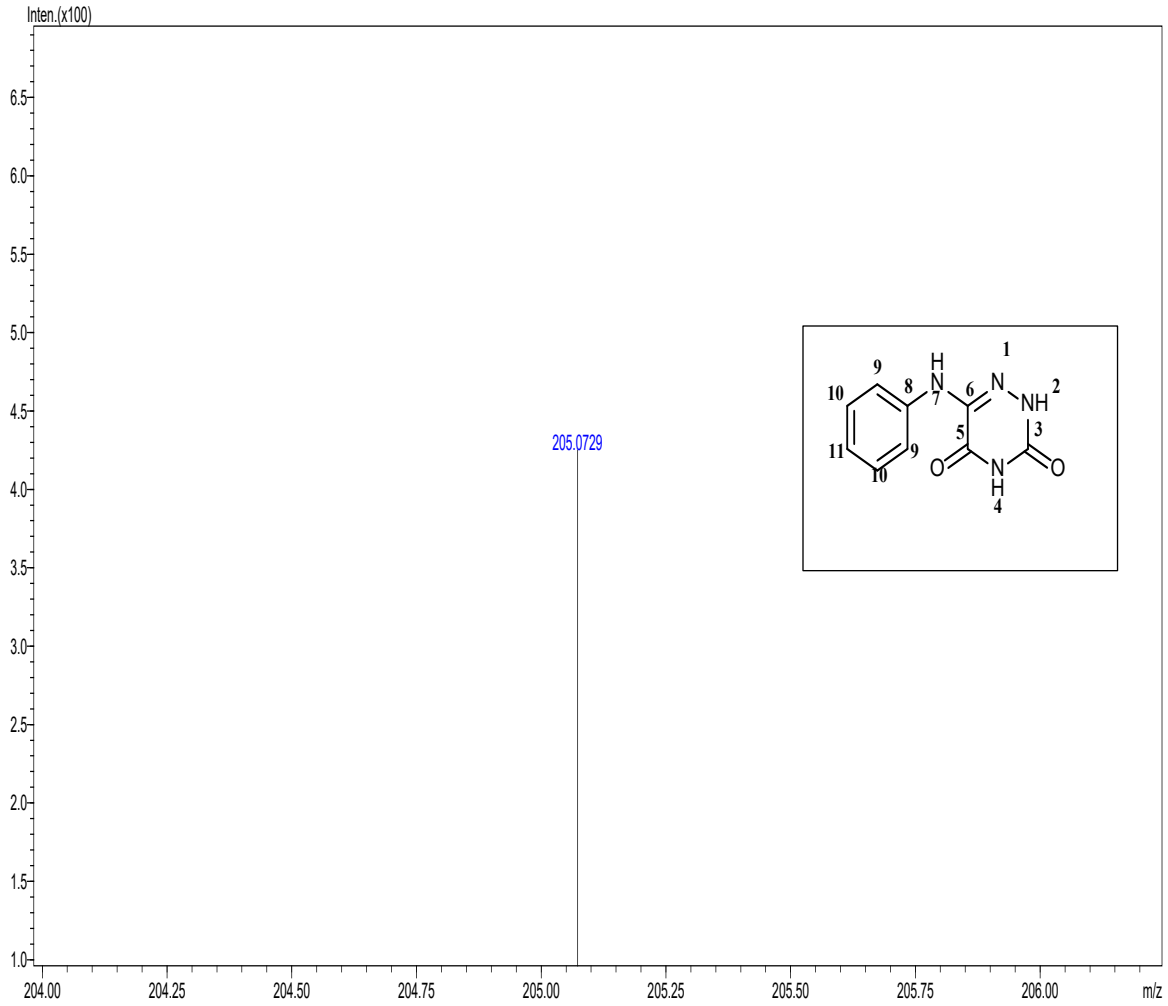
MASS SPECTRA



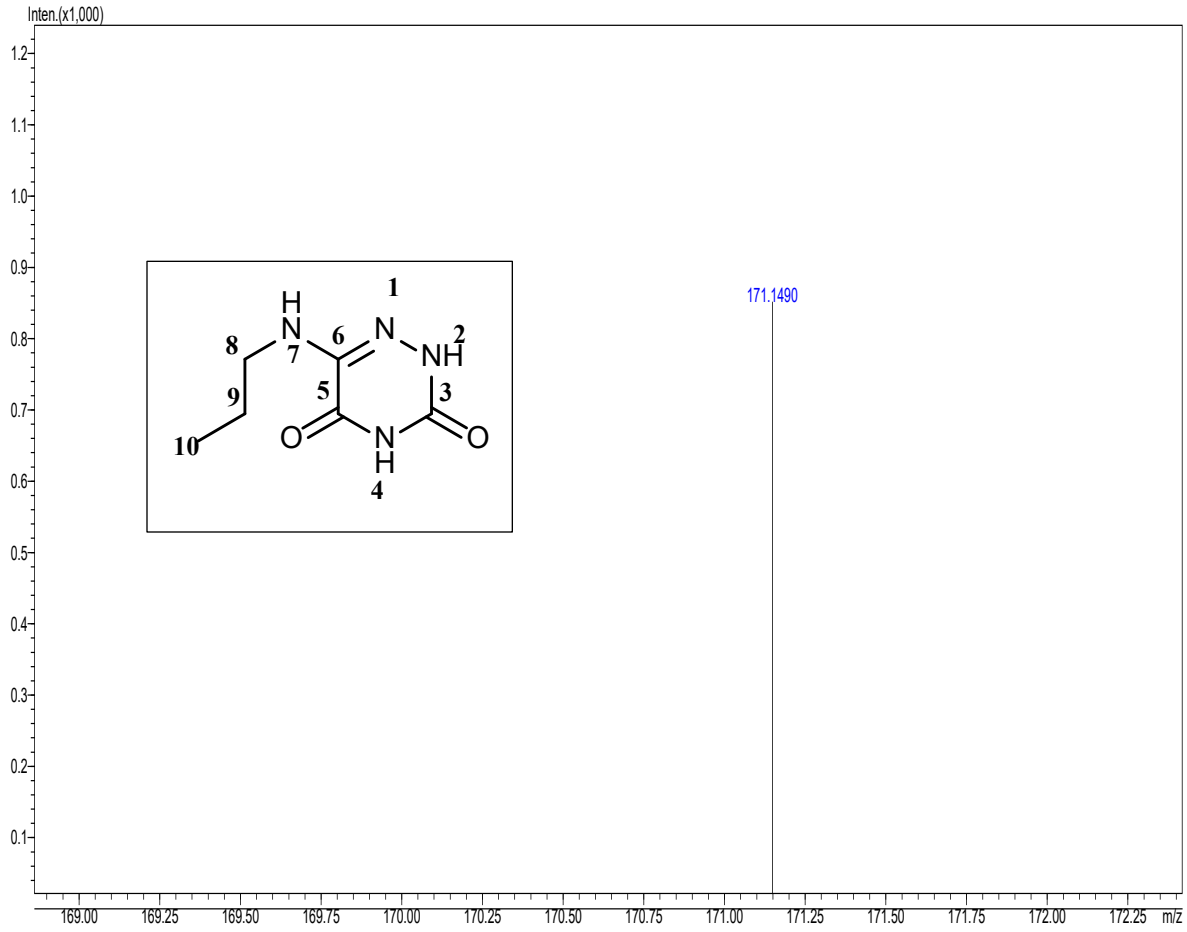
$C_3H_2BrN_3O_2$ Mass Spectra with molecular ion at m/z = 191.1422



$C_3H_4BrN_5$ Mass Spectra with molecular ion at m/z = 191.1912



$C_9H_8N_4O_2$ Mass Spectra with molecular ion at m/z = 205.0729



$C_6H_{10}N_4O_2$ Mass Spectra with molecular ion at m/z = 171.1490.