UNIVERSITY OF KWAZULU-NATAL

SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF THIADIAZOLOPYRIMIDINONES

2018

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2018

A thesis submitted to the School of Chemistry and Physics in the College of Agriculture, Engineering and Science for the fulfilment of the degree of Master of Science.

Dedication

I would like to dedicate this body of work to my daughter Oluhle Alwande Ntshele, you have been my greatest blessing and because of you I am a better version of myself. May this inspire you to always work towards fulfilling your dreams and goals. I Love You.

Preface

I hereby declare that the thesis entitled "**Synthesis, characterisation and antibacterial activity of thiadiazolopyrimidinone hecterocyclic hybrids**" submitted to the University of KwaZulu-Natal for the award of degree of Master of Science in Chemistry under the supervision of Dr Parvesh Singh and Professor Neil A. Koorbanally represents original work by the author and has not been submitted in full or part for any degree or diploma at this or any other University. Where use was made of the work of others it has been duly acknowledged in the text. This work was carried out at the School of Chemistry and Physics, University of KwaZulu-Natal, Westville campus, Durban, South Africa.

Signed: _____

Thandokazi Ntshele, BSc (HONS) (UKZN)

As the candidate's supervisors, we have approved this dissertation for submission

Signed:_____

Dr Parvesh Singh, PhD

Signed: _____

Prof. Neil A. Koorbanally, PhD

Declaration - Plagiarism

I, **Thandokazi Ntshele**, declare that:

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2. This thesis has not been submitted for any degree or examination at any other university.

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Acknowledgements

- Firstly I would like to thank the Lord Almighty for everything, for his blessings and mercy, for being with me and guiding me throughout this process of obtaining my MSc degree.
- Secondly I would like to thank my parents Mr S. Ntshele and Mrs T. P. Ntshele for being the parents that they are, for being my pillars of strength, for working so hard to give us a better tomorrow with better opportunities and for letting me continue pursuing my studies supporting me always.
- I would like to thank my supervisors Dr P. Singh and Prof. N. A. Koorbanally for giving me this chance to work with you, to learn from you, gaining knowledge and wisdom from you and for your guidance throughout my degree while making NPRG feel like a family.
- To thank my whole family especially my sisters Nolizwi Ntshele and Yonela Ntshele for their support and faith in me all the time, and a special thanks to my baby girl Oluhle Ntshele for being the best thing that ever happened to me, for being God's blessing to me and giving me a purpose in life.
- To thank Mr S. L. Makhaye for being one of my pillars and a shoulder to cry on during tough times, for supporting me and for being there for me always through my studies.
- My colleagues at NPRG who helped me with my work and the friends I made throughout my research: Silungile Mhlongo, Bongisiwe Shelembe, Dr Nomfundo Mahlangeni, Joel Mavuso, Kimona Kisten, Mxolisi Sokhela, Lamla Thungatha, Nobuhle Hadebe, Nonhlanhla Joyisa, Nyameka Diko, Noxolo Mqadi, Awolade Paul and Zamokuhle Buthelezi.
- Lastly I would like to thank the technical staff of the Chemistry department; Dillip, Vuyo, Miler, Gregory, Unathi. I would also like to thank Dr Chunderika Mocktar from the College of Health Sciences for assisting me with antibacterial testing.

List of Abbreviations

°C	degrees Celsius
^{1}H NMR	proton nuclear magnetic resonance spectroscopy
^{13}C NMR	carbon-13 nuclear magnetic resonance spectroscopy
bd	broad doublet
bs	broad singlet
$CDCl_3$	deuterated chloroform
COSY	correlation spectroscopy
d	doublet
dd	doublet of doublets
ddd	doublet of doublet of doublets
DMF	dimethylformamide
DMF-DMA	dimethylformamide-dimethylacetal
DMS	Dimethylsulfate
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
FTIR	Fourier transform infrared
HMBC	heteronuclear multiple bond correlation
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
Hz	hertz
IR	infrared
HR-MS	high resolution mass spectrometry
MBC	minimum bactericidal concentration
mp	melting point
MHA	Mueller-Hinton agar
MHz	megahertz
NOESY	nuclear overhauser effect spectroscopy
$POCl_3$	Phosphorylchloride
rt	room temperature
s	singlet
t	triplet
TEA	Triethylamine
TLC	thin layer chromatography
TMS	tetramethylsilane

Abstract

Seven thiadiazolopyrimidinone hybrid derivatives were successfully synthesized in a three step reaction that involved synthesis of 5-substituted phenyl-1,3,4-thiadiazole-2-amines, condensation of the amines with DMF-DMA (dimethylformamide-dimethylacetal), and the [4+2] cycloaddition reaction of the imidoformamides and phenoxyketene. The DMF-DMA used in the second step was synthesized using sodium methoxide, DMF and dimethylsulfate (DMS). The synthesized compounds were all novel derivatives. Structural elucidation of all synthesized compounds was carried out using both 1D and 2D NMR spectroscopy together with mass spectrometry. All synthesized compounds were subjected to antibacterial testing against both Gram positive and Gram negative strains and showed excellent activity against Gram negative strains but no activity against Gram positive bacteria. In comparison to the two standards used, Levoflaxocin and Ciproflaxocin, compounds **3a** (MBC = 0.19 μM) and **3e** (MBC = 0.15-3.92 μM) showed better activity against *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Salmonella typhimurium* while compounds **3b**, **3d**, **3g** and **3h** showed better activity than these standards for three of the four strains.

Keywords: Thiadiazolopyrimidinone, antibacterial assay, [4+2] Cycloaddition reaction, pyrimidinone, thiadiazole

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Chapter 1 Introduction

There has been a recent trend of growing antibacterial resistance due to overuse of antibiotics. Although there a number of antibiotics on the market, antibiotic resistance may render these drugs useless, which could result in a global health crisis.

1.1 Antibiotics and Antibacterial Resistance

Bacteria are single-celled microorganisms found almost everywhere and are part of the unicellular life-form known as prokaryotes. These microorganisms are identified by their morphological characteristics such as shapes and sizes and are categorised into two groups, Gram negative and Gram positive bacteria, according to their cell wall composition. Gram positive bacteria have cell walls consisting of a thick peptidoglycan layer with teichoic acid (made from polysaccharide chains cross-linked by D-amino acids) while Gram negative bacteria have thin peptidoglycan cell walls surrounded by a lipopolysaccharide and lipoprotein containing lipid membrane (van Heijenoort, 2001).

Bacteria such as *Lactobacillus acidophilus* (also known as gut flora) is found in the intestine of humans preventing growth of harmful microbes while others such as *Clostridium*, *Escherichia coli* and *Staphylococcus aureus* can cause life threatening infections and diseases (Guarner and Malagelada, 2003). Antibacterial agents are drugs that prevent and treat bacterial infections. These antibacterial agents either interfere with bacterial growth, interfere with their ability to reproduce or simply kill the bacteria. The first antibiotic was discovered in 1928 by Alexander Fleming and introduced in the late 1940s. This was Penicillin from a fungal species identified as *Penicillum notatum*.

Antibiotics are further classed into different categories according to their mode of action, chemical structure and spectrum of activity. The mode of action classifies them into bactericidal or bacteriostatic. Bactericidal antibiotics target and interfere with cell wall synthesis (e.g. penicillin), cell membrane synthesis (e.g. polymyxins) and essential bacterial enzymes (e.g. rifamycins) while bacteriostatic antibiotics are drugs that inhibit protein synthesis and metabolic pathways (e.g. tetracyclines) (Koch, 2003; Finberg et al., 2014). Antibiotics are classified by their chemical structure according to their core structural framework. For example, the penicillins all have a chemical backbone related to penicillin, while the fluoroquinolones all have a fluoroquinolone core structural framework. The drugs may have different modifications within these frameworks. Spectrum of activity refers to the number of bacterial types the drug is able to inhibit or kill. Broad spectrum antibiotics are able to kill or inhibit a wide range of Gram negative and Gram positive bacteria, while narrow spectrum antibiotics are limited to only a few strains of bacteria and generally either target Gram positive or Gram negative bacteria but not both.

Over time, overuse and misuse of antibiotics led to development of drug resistance which is seen as a serious worldwide threat by the World Health Organization (WHO, 2014). Through deletions and mutations, the bacterial genome has evolved, creating "superbugs" resistant to commonly used first and second line antibiotics (Levy and Marshal, 2004). Thus, antibiotics are becoming less effective and the rate of resistance is gradually increasing to hazardous levels (WHO, 2014).

Another mechanism by which bacteria acquire resistance is genomic duplication (for example in mammalian cancers and parasites) whereby certain genes are amplified leading to over expression of multidrug targets and transporters. The bacteria eventually build up a defence

system and mechanism against the antibiotics acting against these targets (Albertson, 2006). In 1998, Mathews and Steward showed that genomic amplification affected the susceptibility of *S. aureus* to methicillin causing resistance, resulting in methicillin resistant *Staphylococcus aureus* (MRSA) (Mathews and Steward, 1998). Antibiotic resistance can also occur as a result of modification of the structure of the antibiotics by enzymes such as *β*-lactamase (enzymes that degrade antibiotics) and macrolides & aminoglycosides, modifying proteins that chemically transform antibiotics in non-active forms (Alekshun and Levy, 2007).

It is quite certain that a wide repertoire of antibiotics is needed in order to combat the problem of drug resistance and prevent a global crisis, which if left unchecked could threaten human survival.

1.2 Introduction to Thiadiazoles

Thiadiazoles are 5-membered ring compounds that contain two nitrogen and one sulfur atom and has four isomers (**Figure 1.1**). It is a very useful moiety that displays a wide spectrum of biological activities such as antimicrobial (antibacterial, antifungal, antimycobacterial) (Foroumadi et al., 2003a; Foroumadi et al., 2003b; Thomasco et al., 2003), antileishmanial, analgesic and anti-inflammatory (Amir and Shika, 2004), antidepressant and antipsychotic (Sharma et al., 2011) and anticonvulsant (Kumar et al., 2012).

Past and present research has shown that the 1,3,4-thiadiazole isomer is the one that has been greatly investigated in depth over other isomers, which is mainly due to this isomer being a mesoionic system with properties and characteristics that allow it to cross over cellular membranes, hence achieving good interactions with biological targets. The aromaticity of this ring system which leads to it having good *in vivo* stability and minimal toxicity results in the broad spectrum of biological activities of this moiety (Hu et al., 2014; Kushwaha et al., 2012).

Many drugs containing the thiadiazole nucleus are available on the market (**Figure 1.2**) such as acetazolamide, methazolamide and sulfamethazole as shown below. The 1,3,4-thiadiazole was been chosen as the research focus for this project due to its attributes, properties and broad spectrum activity.

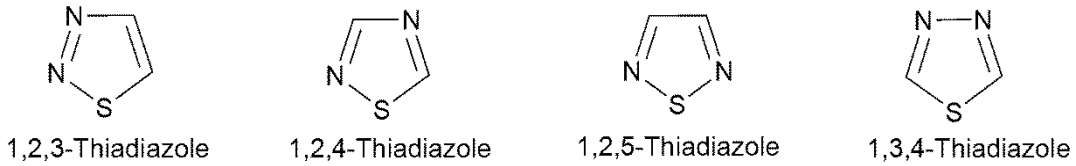


Figure 1.1 Isomers of the thiadiazole moiety

The bioactivity of the thiadiazole moiety has led to the discovery of many current commercialized thiadiazole-containing drugs. Examples are acetazolamide and methazolamide (carbonic anhydrase inhibitors), cefazolin (CFZL) and cefazedon (CFZD) (first-generation cephalosporins), timolol (antiglaucoma and antihypertensive drugs) and megazol (an antiparasitic drug) (**Figure 1.2**) (Li et al., 2013b).

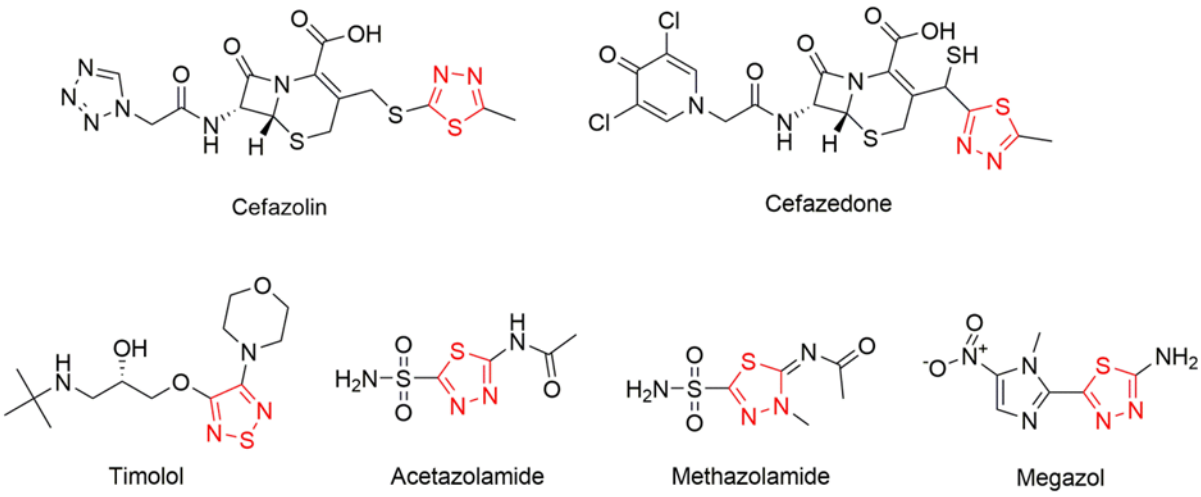


Figure 1.2 Structures of some commercialized 1,3,4-thiadiazole containing drugs

1.2.1 1,3,4-Thiadiazoles

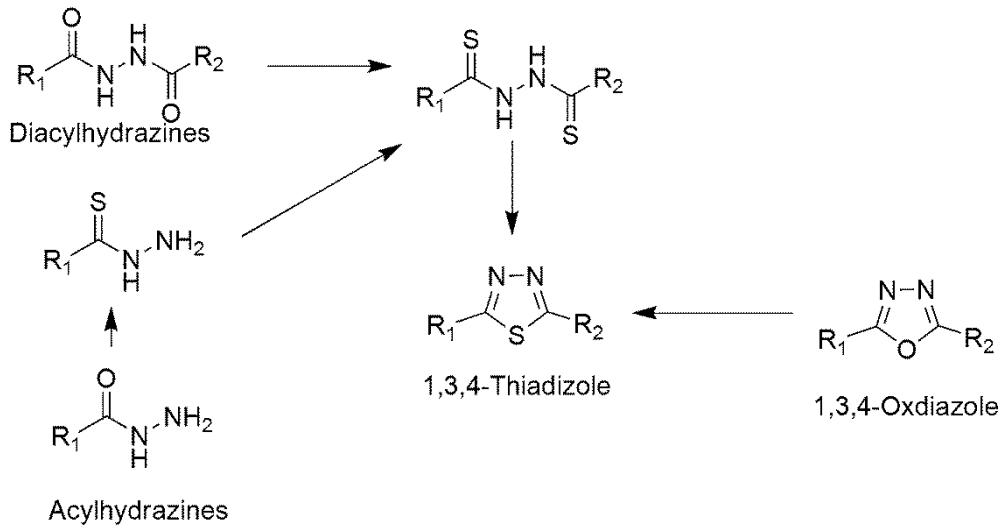
The 1,3,4-thiadiazole isomer has the sulphur atom at position 1 and two nitrogen atoms at positions 3 and 4. This is the most popular isomer with the broadest spectrum of bioactivity. The ring of this isomer is a weak base due to the inductive effect of the sulfur atom and compared to other similar compounds such as oxadiazoles, oxazole and benzene (whereby substituting any of the mentioned cores with the 1,3,4-thiadiazole cores has resulted in improved activity) (Li et al, 2013b). The lower toxicity and *in vivo* stability of the 1,3,4-thiadiazole nucleus are attributed to its aromaticity. It has been reported to have anti-inflammatory (Kadi et al., 2010), anticancer (Rzeski et al., 2007), antitubercular (Foroumadi et al., 2003a) antimicrobial (Kadi et al*.,* 2010), analgesic (Hafez et al., 2008), antiepileptic (Kumar et al, 2012), antidepressant (Clerici and Pocar, 2001), antioxidant (Sunil et al., 2010) and radioprotective activities and several pharmacophores with 1,3,4-thiadiazole rings have been reported with potential anticancer activity (Hu et al., 2014; Kushwaha et al., 2012).

This moiety is less aromatic than benzene, thiophene, and pyridine. The aromatic character is measured by π electron delocalization which decreases in the following order 1,2,5-thiadiazole > thiophene > thiazole > 1,3,4-thiadiazole. Being an azole, 1,3,4-thiadiazole is a weak base due to the inductive effect of the heteroatoms in the ring. It is relatively stable in aqueous acidic solution but undergoes cleavage in basic solution. Due to the electronegative nitrogen atoms in the ring, the carbon atoms in the moiety rarely undergo electrophilic attack but are often subject to nucleophilic substitution. On the other hand, the nitrogen atoms in the ring experience electrophilic attack due to tautomerization. Unlike the nitrogen atoms, the sulfur atom rarely experiences electrophilic attack (Hu et al., 2014).

The 1,3,4-thiadiazole core skeleton is subject to various substitution reactions with alkyl halides, acid chlorides, and sulfonyl chlorides to afford various drug like 2-amino-substituted 1,3,4-thiadiazole derivatives. This 1,3,4-thiadiazole system is also known to be a mesoionic system, which is a polyatomic system made up of a 5-membered heterocyclic ring and having conjugated pi electrons with a combination of positively and negatively charged regions. The electron dense and highly polarisable nature of this ring makes interaction of this moiety with cellular membranes and other biological entities possible as the sulphur atom improves the liposolubility of this moiety enhancing interaction with the phospholipids of the cell membrane allowing compounds to cross over (Hu et al., 2013).

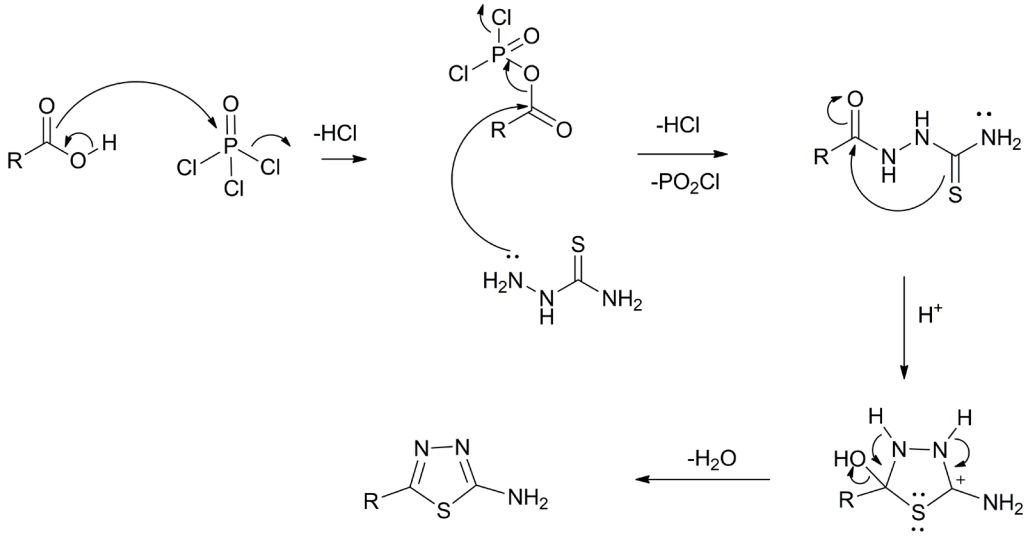
1.2.2 Synthetic routes to the 1,3,4-thiadiazole scaffold

Many different routes have been employed to synthesize the 1,3,4-thiadiazole scaffold. This includes cyclization of acylhydrazines or transformation of 1,3,4-oxodiazoles (Hu et al., 2014; Kushwaha et al., 2012). The most common method is based on the cyclization of thiohydrazines or its equivalents (thiosemicarbazides, thiocarbazides, dithiocarbazates, thioacylhydrazines and bithioureas) (**Scheme 1.1**). Each of these derivatives of thiohydrazines introduce different groups to the thiadiazole moiety allowing a variety of 1,3,4-thiadiazoles to be synthesised (Hu et al., 2014).



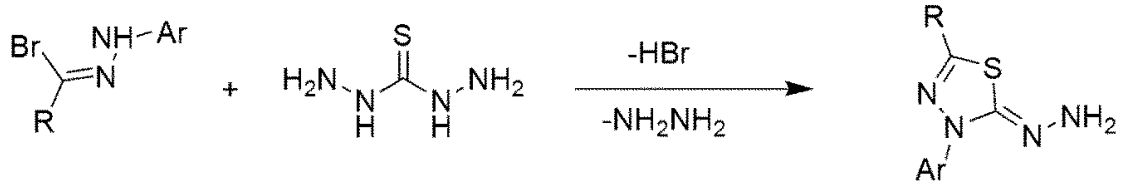
Scheme 1.1 General preparation of 1,3,4-thiadiazoles from thiohydrazines (Hu et al., 2014)

The condensation of thiosemicarbazides with carboxylic acids, acyl chlorides or esters using cyclising agents such as phosphorus chloride and sulphuric acid yields 2-amino-1,3,4-thiadiazoles. This occurs by a condensation reaction after the acid is activated by phosphoryl chloride. The primary amino group then initiates cyclisation and once cyclised, the thiadiazole ring is formed by dehydration and dehydrogenation steps (**Scheme 1.2**) (Gazieva and Kravchenko, 2012; Hu et al., 2014).



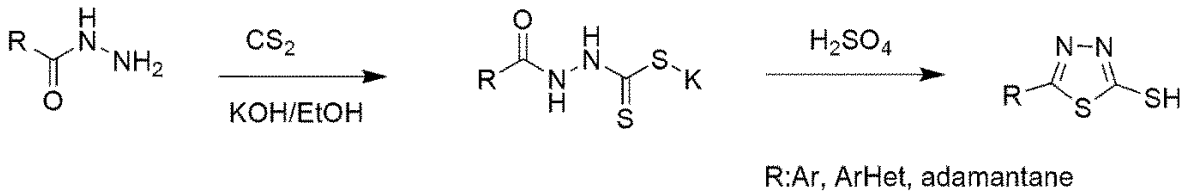
Scheme 1.2 Mechanism for the formation of 1,3,4-thiadiazole from thiosemicarbazides and benzoic acids with $POCl_3$ as cyclising agent

With thiocarbazides, also known as carbonothioic dihydrazides, a similar mechanism to that of the thiosemicarbazides is followed. In this method, the thiocarbazide is heated with the appropriate hydrazonoyl halide to afford 1,3,4-thiadiazoles with a hydrazine moiety at position 2 rather than an amino group (**Scheme 1.3**) (Hu et al., 2013).



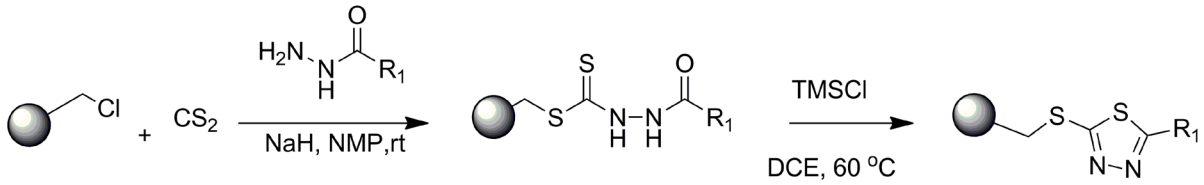
Scheme 1.3 Reaction for synthesis of 1,3,4-thiadiazoles from thiocarbazides

Kadi et al (2010) reported the synthesis of 1,3,4-thiadiazoles using acyl hydrazides. This consisted of the synthesis of the dithiocarbazate using hydrazine, hydrazide or hydrazone and CS_2 (used as sulphur source) and the dicarbazate undergoing cyclisation using concentrated sulphuric acid to catalyse dehydration and forming a 2-thiol-1,3,4-thiadiazole (**Scheme 1.4**). Aryanasab et al. (2010) and Polshettiwar and Varma (2008) reported greener methods for the synthesis of 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives, reporting solvent free one-pot condensations of acid hydrazides with triethylorthoalkanoates using microwave radiation, and using aqueous conditions for the condensation of acid hydrazides and dithiocarbamates.



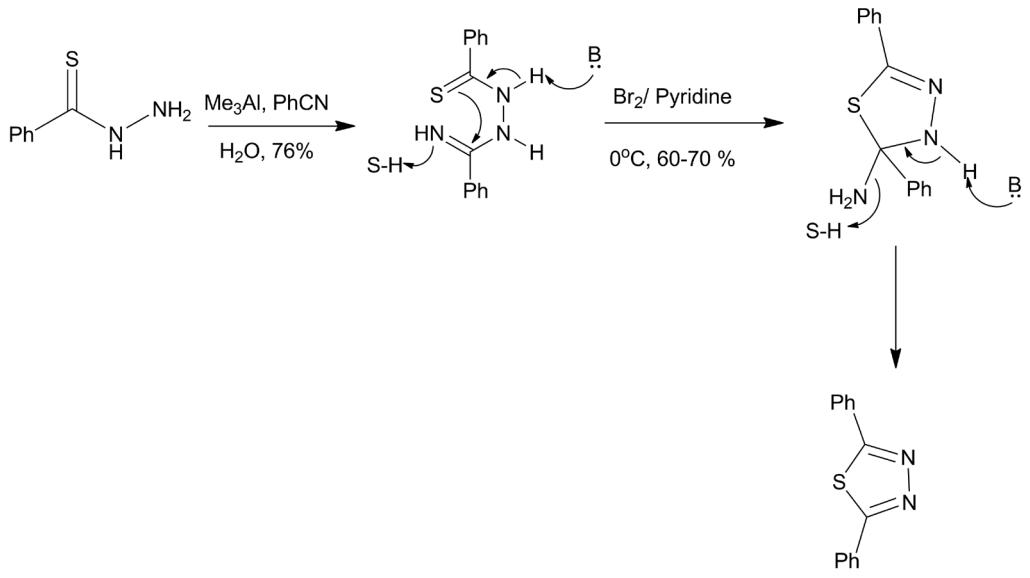
Scheme 1.4 Synthesis of 1,3,4-thiadiazoles from acyl hydrazides

The solid-phase synthesis of 1,3,4-thiadiazoles using selective reagent based cyclisation of acyldithiocarbazates were also reported. In this method CS_2 is used in the presence of sodium hydride at room temperature affording various acyldithiocarbazate resins, which are subsequently cyclised, dehydrated and dehydrogenated (**Scheme 1.5**) (Gong and Lee, 2010).



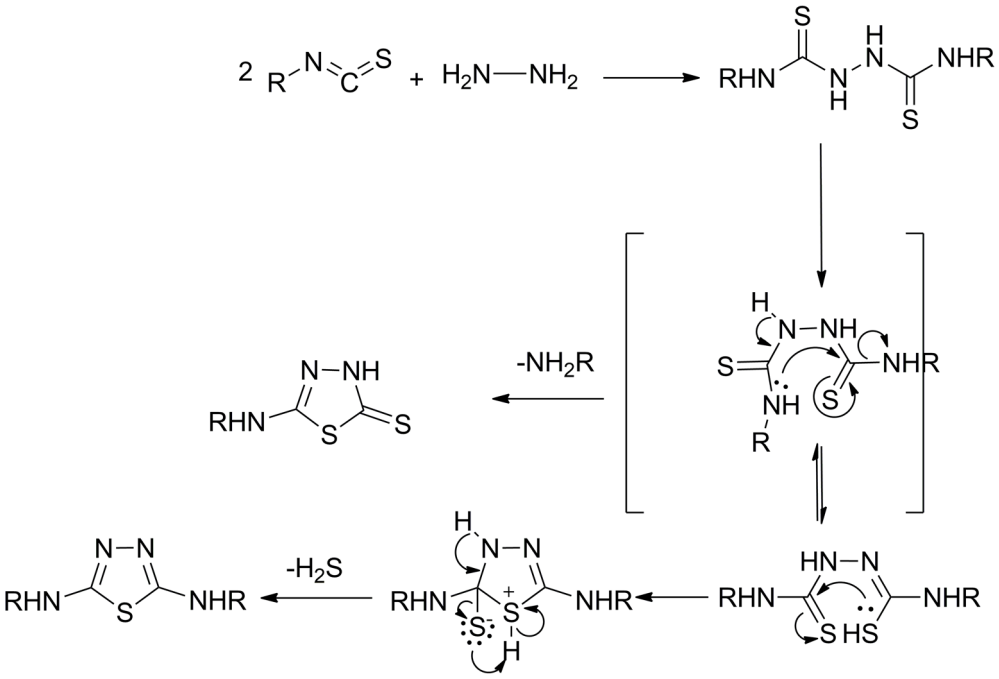
Scheme 1.5 Solid phase synthesis of 1,3,4-thiadiazoles

The condensation of aromatic thiohydrazides with benzaldehydes forming Schiff base intermediates, which are then cyclised by treating the intermediates with pyridine under acidic conditions to afford 2,5-diphenyl-1,3,4-thiadiazole compounds (Farrar et al., 2000). In this mechanism, only a dehydrogenation step is necessary after the cyclised intermediate forms (**Scheme 1.6**).



Scheme 1.6 Reaction scheme for synthesis of 1,3,4-thiadiazoles using thiohydrazides

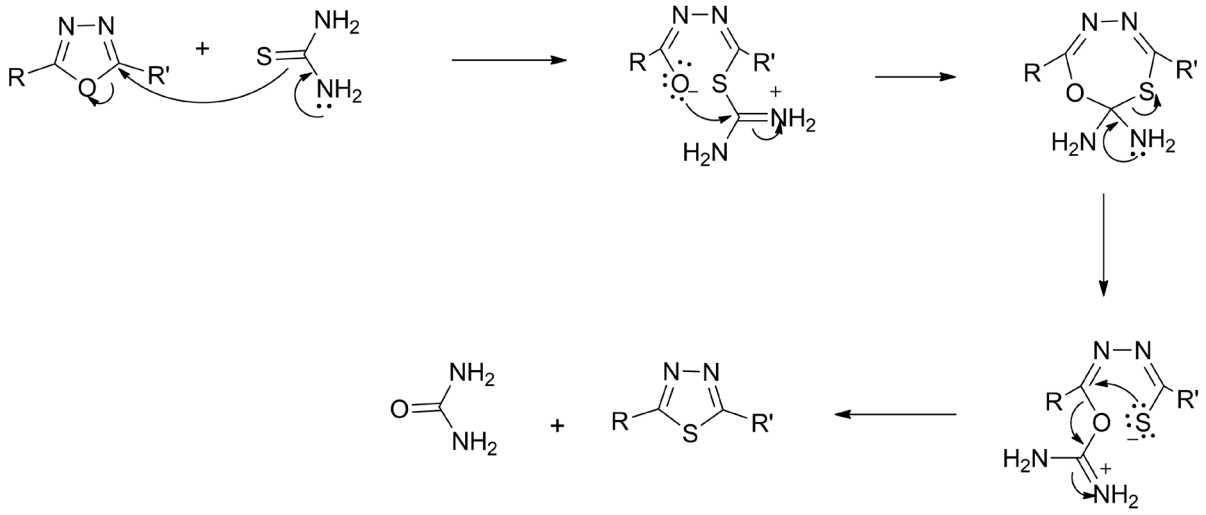
The bithiourea method is also a two-step reaction. The first step is the synthesis of bithiourea (having 2 α-amino groups on each end) from hydrazines or thiosemicarbazides and isothiocyanates. The second step is the cyclisation step forming 2,5-bis-substituted-1,3,4-thiadiazoles. After the ring is initially formed, the leaving group could either be the RNH or HS group affording either 5-substituted amino-3*H*-[1,3,4]thiadiazole-2-thiones (removal of RNH) or an *N*,*N*-disubstituted-[1,3,4]thiadiazole-2,5-diamine (**Scheme 1.7**) (Hu et al., 2014).



Scheme 1.7 Cyclization mechanism for the synthesis of 1,3,4-thiadiazole via the bithiourea method

The 1,3,4-oxadiazole ring can also be transformed to 1,3,4-thiadiazole rings. These two moieties are structurally similar with the only difference being the sulfur atom in the thiadiazole as opposed to the oxygen in the oxadiazole. This reaction was carried out by the addition of 1,3,4-oxadiazoles to thiourea, forming the 1,3,4-thiadiazoles in THF in very high yields (Linganna and Lokanatha Rai, 1998; Padmavathi, 2008; Padmavathi, 2010; Padmavathi, 2011). The mechanism occurs by the addition of thiourea to the oxadiazole forming a

thiouronium salt, which cyclises again to a seven membered intermediate, which opens again to a mesomeric oxouronium salt that ultimately forms the thiadiazole and expels urea (**Scheme 1.8**).

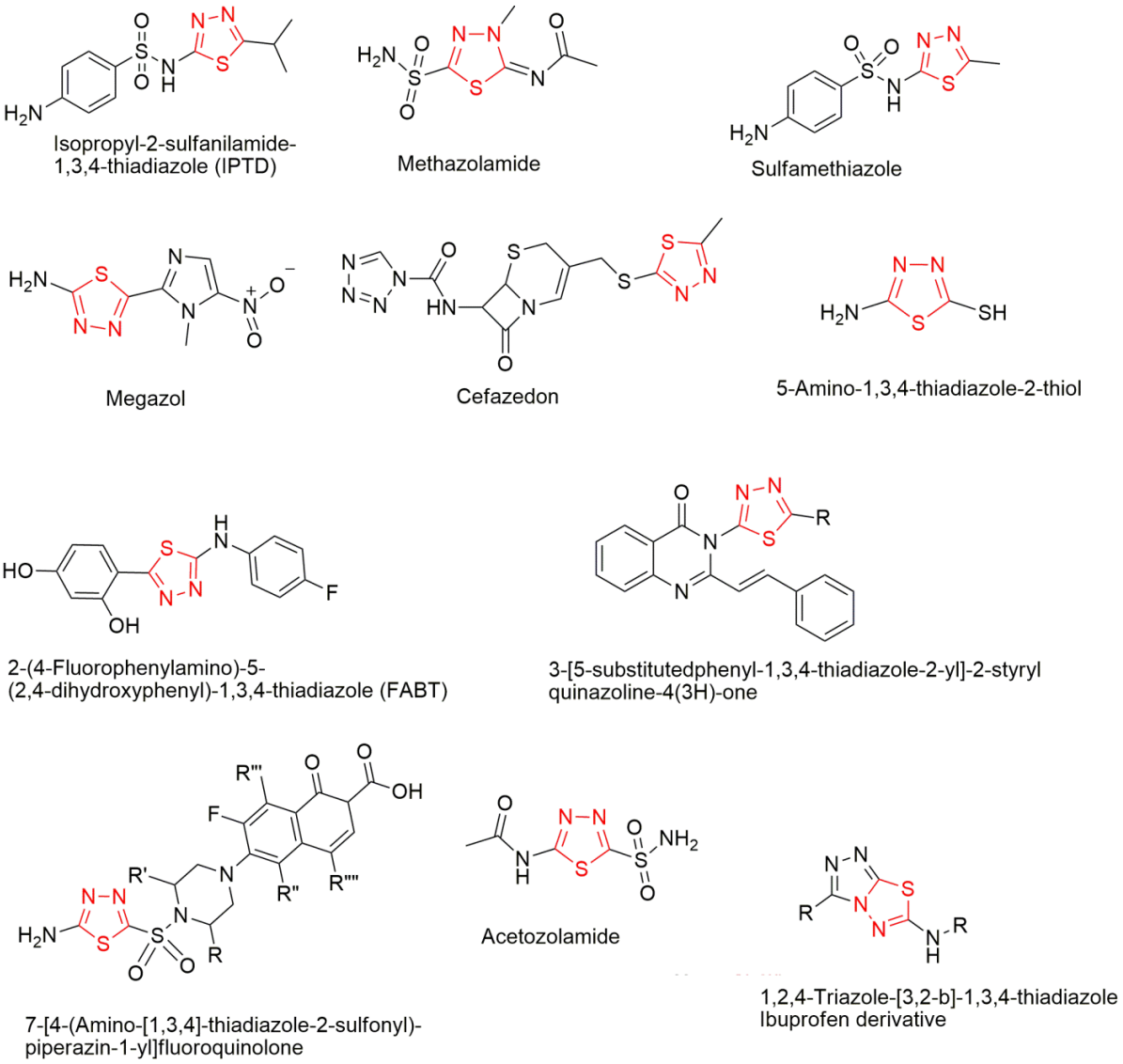


Scheme 1.8 Mechanism for conversion of 1,3,4-oxadiazole to 1,3,4-thiadiazole using thiourea

1.2.3 Bioactivities of 1,3,4-thiadiazoles

Like most heterocycles occurring in nature, the 1,3,4-thiadiazole moiety has shown a wide range of biological activities such as antimicrobial (Bhat et al., 2011), anti-inflammatory (Amir et al., 2007), anticancer (Rzeski et al., 2007) and anticonvulsant activities (Kaur et al., 2010). Compounds with the 1,3,4-thiadiazole moiety has found applications in pharmaceuticals, agro medicine medicine (the application of medicinal and agricultural science to promote the health and safety of humans related to/ or involved in agriculture) and material chemistry (Hu et al., 2013). This moiety also forms part of many commercialized drugs (**Figure 1.3**).

Figure 1.3 **Examples of 1,3,4-**

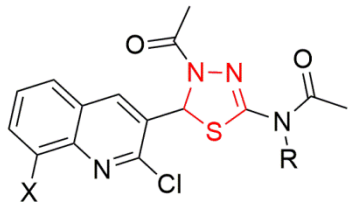


thiadiazole containing bioactive compounds currently commercialized as therapeutic drugs for different conditions

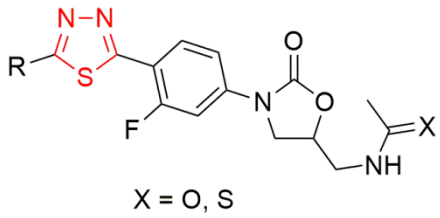
Antimicrobial activity

Quinolines coupled with 1,3,4-thiadiazoles (1,3,4-thiadiazolequinolone derivatives) (**Figure 1.4**) was found to exhibit better antimicrobial activity than amoxicillin (Bhat et al., 2011). The activity of ciprofloxacin and norfloxacin were also enhanced against Gram +ve bacteria by hybridizing these pharmacophores with 1,3,4-thiadiazoles (Foroumadi et al., 2003b).

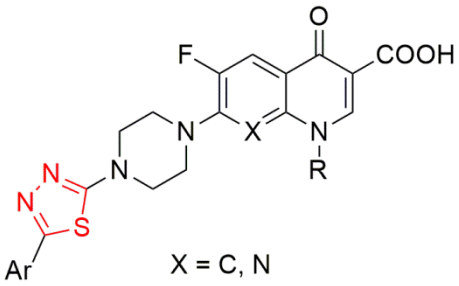
Hybridization of the 1,3,4-thiadiazole moiety with 1,2,4-triazoles (Swamy et al., 2006) and 1,3,4-oxazolodinone (Thomasco et al., 2003) scaffolds (**Figure 1.4**) have resulted in compounds with good bioactivity, even better than some commercialised drugs. For example replacing the morpholine moiety with a 1,3,4-thiadiazolyl ring in the antibiotic Linezolid resulted in increased antimicrobial potency.



1,3,4-thiadiazolequinolone derivatives

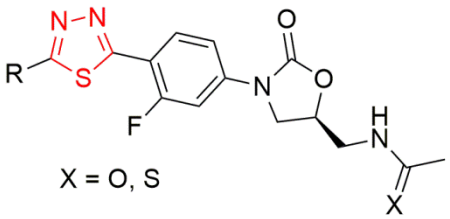


1,3,4-thiadiazoleoxazolidinone derivatives



N-(1,3,4-thiadiazole)-substituted piperazinylquinoline derivatives

1,2,4-triazolo-1,3,4-thiadiazole derivatives



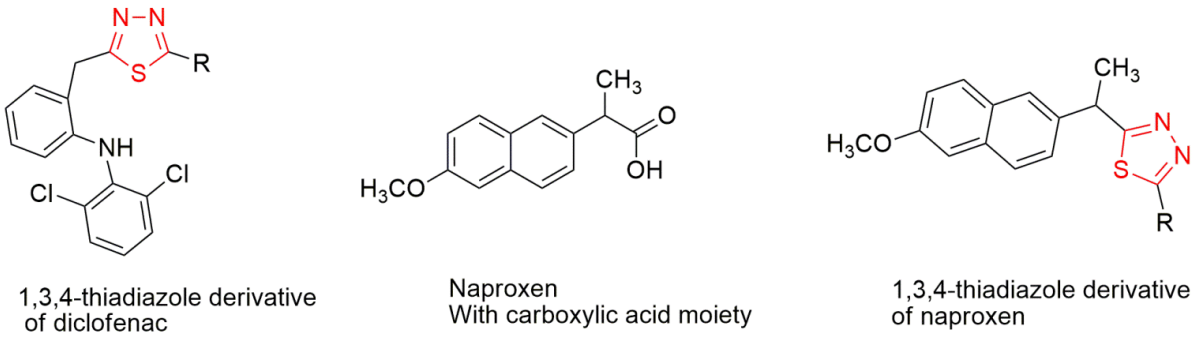
1,3,4-Thiadiazole Linezolid derivative

Figure 1.4 Antimicrobial 1,3,4-thiadiazoles

Anti-Inflammatory and Analgesic activity

The anti-inflammatory drug naproxen was derivatised by replacing the carboxyl group with a *N*-(4-bromophenyl)-1,3,4-thiadiazol-2-amine and comparing its activity with that of the parent drug. Results indicated that the 1,3,4-thiadiazole group had a slight improvement in activity than the carboxyl derivative (**Figure 1.5**) (Amir et al., 2007b).

Replacing the carboxyl group of naproxen with with 1,3,4-thiadiazole, 1,2,4-triazole and/or 1,3,4-oxadiazole resulted in increased anti-inflammatory and analgesic activity and reduced ulcerogenecity and lipid peroxidation (reduced side effects) compared to the parent compound, naproxen (Amir et al., 2007a, 2007b). Similar results were published by Amir and Shika (2004) when they replaced the carboxyl group of diclofenac with azole moieties. The azole derivatised diclofenac demonstrated increased anti-inflammatory and analgesic activity with reduced toxicity (ulcerogenic and lipid peroxidation).



1,3,4-thiadiazole derivative of diclofenac

Naproxen
With carboxylic acid moiety

1,3,4-thiadiazole derivative of naproxen

Figure 1.5 Anti-inflammatory and Analgesic 1,3,4-thiadiazoles

Anticancer activity

The thiadiazole moiety has been shown to target proliferation and metastasis of cancer cells (Hu et al., 2014), as well as attacking proteins and molecules in cancer cells essential for proliferation, such as the tyrosine kinases, carbonic anhydrases (CA) and histone deacetylases (HDALs) to name a few. Some known CA inhibitors include acetazolamide, benzolamide and

methazolamide, all of which are sulfonamide 1,3,4-thiadiazole derivatives (**Figure 1.6**) (Hu et al., 2014). Another example of an anticancer thiadiazole is 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT), which has shown antiproliferative activity against human tumour cell lines both *in vitro* and *in vivo*. These drugs were also found to be non-toxic to normal cell lines (Rzeski et al., 2007).

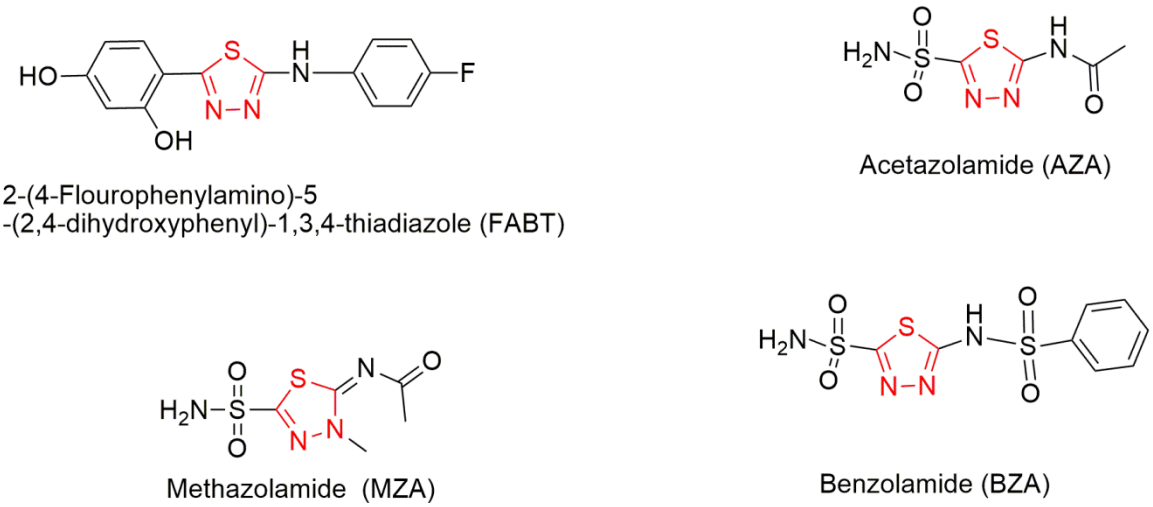


Figure 1.6 Anticancer 1,3,4-thiadiazoles

Antiparasitic activity

Diseases such as malaria, trypanosamiasis, chagas and leishmaniasis are caused by parasites and generally have a huge impact in developing and underdeveloped countries. Compounds containing the 1,3,4-thiadiazole moiety have been used as potent antiparasitic agents (Coura and Castro, 2002). Derivatization and optimization of the drug Magazol (1-methyl-2-(5-amino-1,3,4-thiadiazole)-5-nitroimidiazole) was carried out to decrease its toxicity and mutagenicity (Coura and Castro, 2002; Poli et al., 2002; Carvalho et al., 2004).

The 2-amino-1,3,4-thiadiazole containing a 5-methyl-imidiazoyl group, megazol (**Figure 1.7**) showed significant activity against *T. gondii*, a parasite that causes taxoplasmosis, a common infection that affects patients with compromised immune systems. *T. gondii* also affects foetuses through mother to foetus infection during pregnancy and has become resistant to current antitoxoplasmosis medication, especially in pregnant woman (Blader et al., 2009). The 1-methyl-2-(5-amino-1,3,4-thiadiazole)-5-nitroimidiazole derivatives demonstrated a decrease in infected cells compared to sulfodiazine and hydrourea, standard antiparasitic drugs (Carvalho et al., 2004).

Derivatives of megazol has also shown good antiparasitic activity. For example, a heterocyclic ring attached to the amino group of megazol (**Figure 1.7**) showed good activity against leishmaniasis (Foroumadi et al., 2005; Poorrajab et al., 2009) and an arylhydrazone of megazol, formed by the reaction of megazol and a guanylhydrazone derivative showed good anti-trypanocidal activity (Carvalho et al., 2004; Foroumadi et al., 2005; Poorrajab et al., 2009).

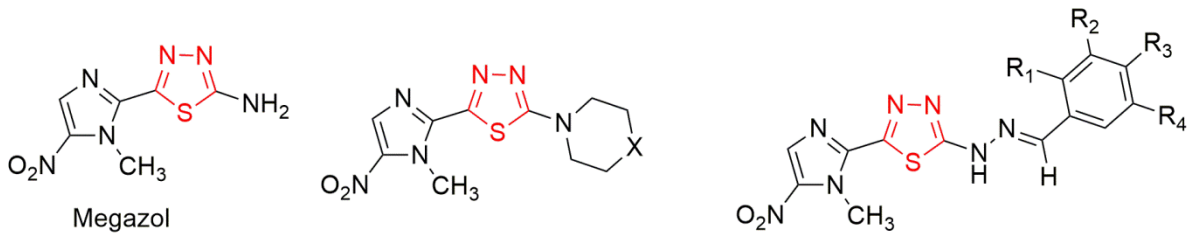


Figure 1.7 Antiparasitic 1,3,4-thiadiazoles

Anticovulsants/anti-epileptic activity

Recent research has focused on the modification of a 1,3,4-thiadiazole derivative acetazolamide (AZA), a 2-acetylamido-1,3,4-thiadiazole-5-sulfonamide discovered in 1949 and introduced in 1952 as an anti-epileptic drug (Barbara and Edwin, 1956). Since current anticonvulsant and anti-epileptic drugs have severe negative side-effects and high toxicity (Lopes, 2000), there has been a continued search for less toxic drugs with minimised side-effects. For example, the acyl group at the amino moiety of AZA was replaced with a cyclohexyl group and the sulfonamide group with a thiourea group (**Figure 1.8**) (Karakus et al., 2009). These compounds showed promising activity as anti-epileptic alternatives. Kumar et al. (2012) successfully synthesized thiadiazoloquinolines by replacing the sulfonamide moiety in AZA with a quinoline methylthio group (**Figure 1.8**) resulting in compounds with less neurotoxicity than phenytoin, a drug used to prevent seizures in epilepsy. Hybrid molecules of carbazole and thiadiazole imines have also shown promising anticonvulsant and antipsychotic activity (Kaur et al., 2010).

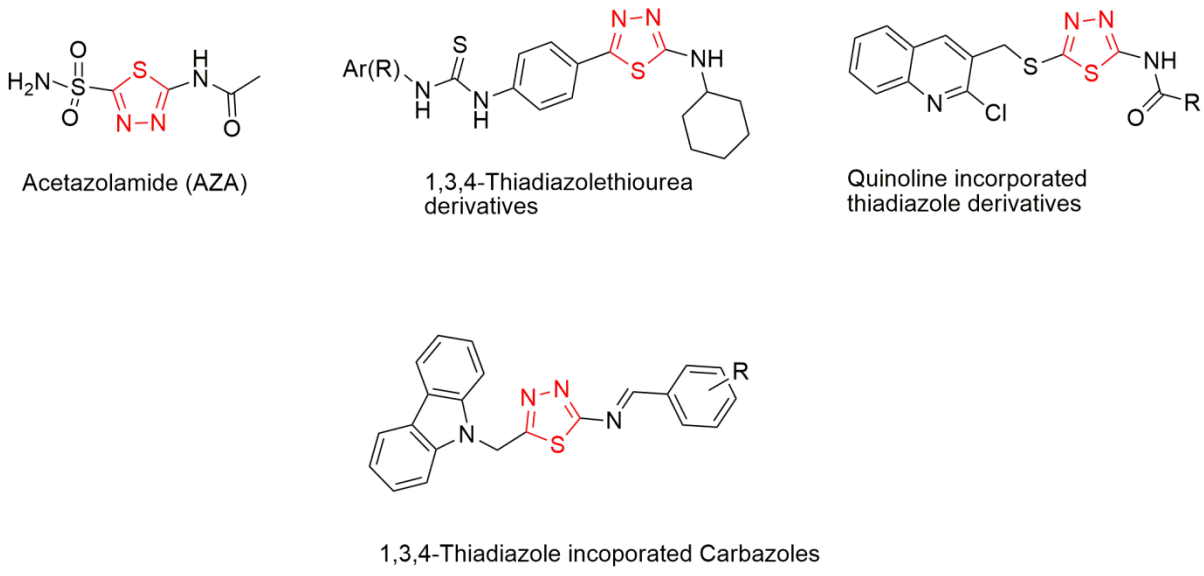
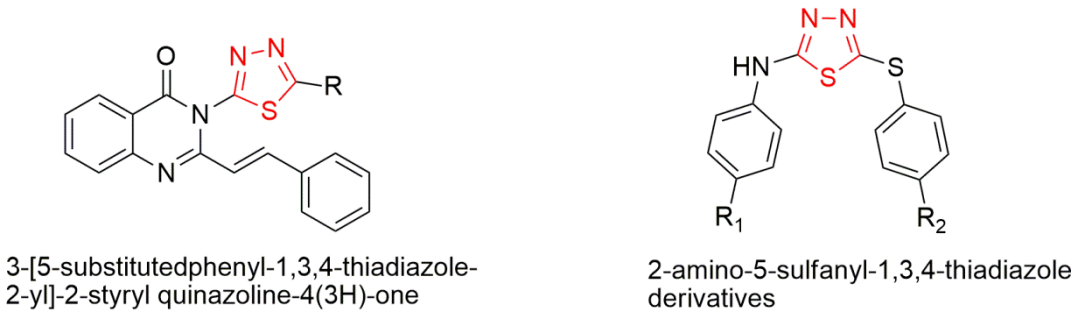


Figure 1.8 Anticonvulsant and anti-epileptic thiadiazoles

Antidepressant activity

Substituted thiadiazoles such as 2-amino-5-sulfanyl-1,3,4-thiadiazoles have exhibited good antidepressant activity (**Figure 1.9**) (Sharma et al., 2011). Combining this moiety with other pharmacophores such as quinazolines (**Figure 1.9**) has enhanced antidepressant activity synthesizing 3-[5-substituted 1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-ones (Jatav et al., 2008).



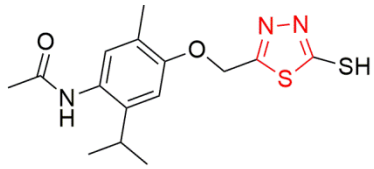
3-[5-substitutedphenyl-1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-one

2-amino-5-sulfanyl-1,3,4-thiadiazole derivatives

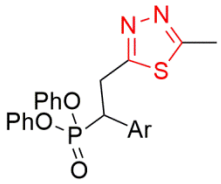
Figure 1.9 Antidepressant 1,3,4-thiadiazole derivatives

Antioxidant activity

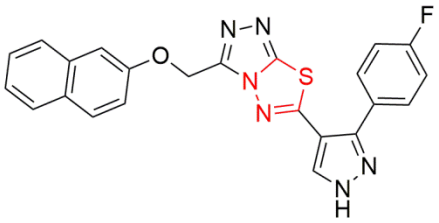
The 1,3,4-thiadiazole moiety has been shown to have antioxidant activity, not only preventing damage to DNA and cells but also preventing carcinogenesis (Conklin, 2000). The incorporation of this moiety with other pharmacophores has led to the improvement of commercial drugs such as Carvacol by hybridizing this moiety with 1,3,4-thiadiazole and oxadiazole (**Figure 1.10**) (Suresh et al., 2016). 1,3,4-Thiadiazoles containing α-amino phosphonates also showed good antioxidant activity (Azaam et al., 2017). Hybrizidization of thiadiazole with triazole and pyrazole moieties resulted in two triazolo-thiadiazoles, 6-[3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-3-[(2-naphthyloxy)methyl][1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (FPNT) and 6-[3-(4-chlororophenyl)-1*H*-pyrazol-4-yl]-3-[(phenyloxy)methyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (CPPT) which demonstrated significant antioxidant activity (**Figure 1.10**) (Sunil et al., 2010).



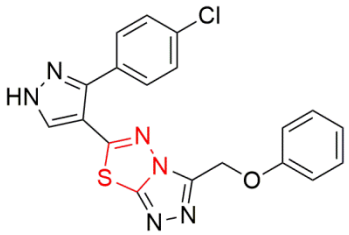
Cavarcol containing thiadiazole derivatives



Thiadiazole containing amino phosphonate derivatives



6-[3-(4-Fluorophenyl)-1H-pyrazol-4-yl]-3-[(2-naphthyloxy)methyl][1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (FPNT).



6-[3-(4-Chlorophenyl)-1H-pyrazol-4-yl]-3-[(phenyloxy)methyl]-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole (CPPT)

Figure 1.10 Antioxidant 1,3,4-thiadiazoles

1.3 Introduction to Pyrimidinones

Pyrimidinones, also known as pyrimidones are a group of 6-membered heterocyclic ketones containing a pyrimidine core that consist of 2 nitrogen and 4 carbon atoms (**Figure 1.11**). This moiety exists in many bioactive compounds and has attracted a great deal of interest due to its biological activities, which include antitumor (Rashad et al, 2012; Li et al., 2013a), antiviral (Botta et al., 1999), antibacterial (Edrees et al., 2010), antifungal (Banothu and Bavanthule, 2012) and anti-inflammatory activities (Dinakaran et al., 2012).

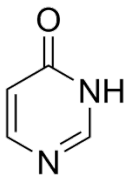


Figure 1.11 Structure of pyrimidin-4-one

Pyrimidinones form part of many natural products such as thiamine (vitamin B1) and 4-thiouridine, isolated from *E. coli* (**Figure 1.12**). They are also found in nucleic acids (DNA and RNA) and hormones. The pyrimidinone moiety is found in a number of synthetic drugs such as barbiturates, raltegravir and lamvudine (anti-retrovirals), thiadiazolopyrimidinone (anti-oesteoporosis) and allopurinol (antimetabolite enzyme inhibitor and free radical scavenger) (**Figure 1.13**) (Maurya and Gupta, 2014).

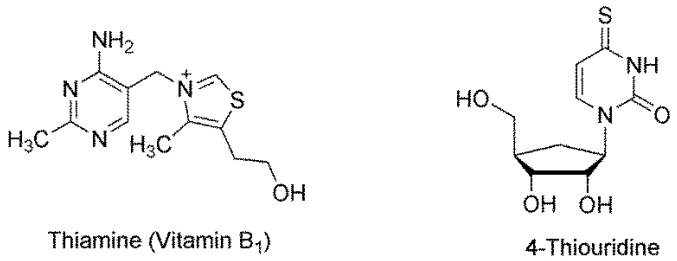


Figure 1.12 Structures of thiamine and 4-thiouridine

Since these molecules have a pyrimidine backbone, the building blocks of DNA and RNA, they have also been tried as substitutes for pyrimidine to mimic it as a base (Subach et al., 2004; Micheel et al., 2015). Subach et al. (2004) incorporated 2-pyrimidinones into the DNA sequence replacing cytosine to study the mechanism of EcoRII DNA methyltransferase – substrate interaction and Micheel et al. (2015) used the 2-pyrimidinone moiety to study DNA photodamage.

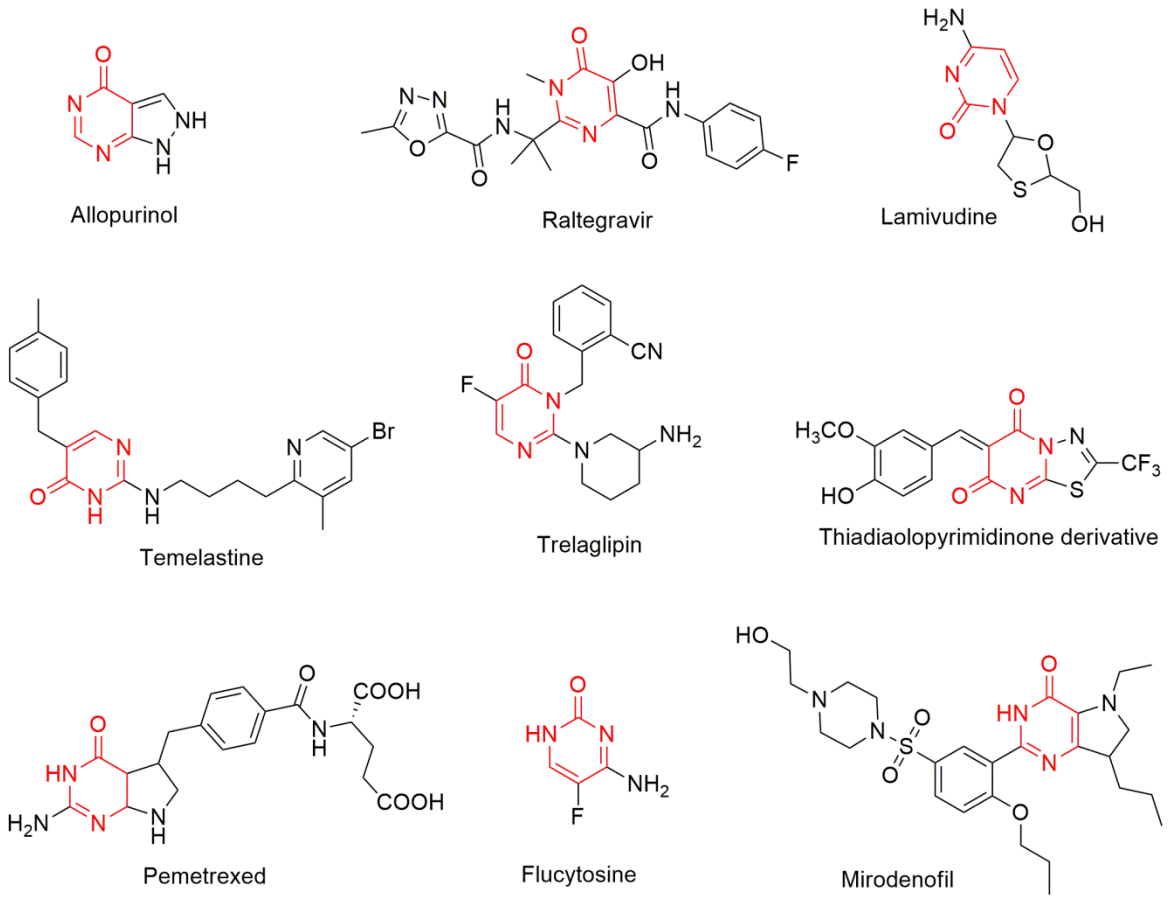
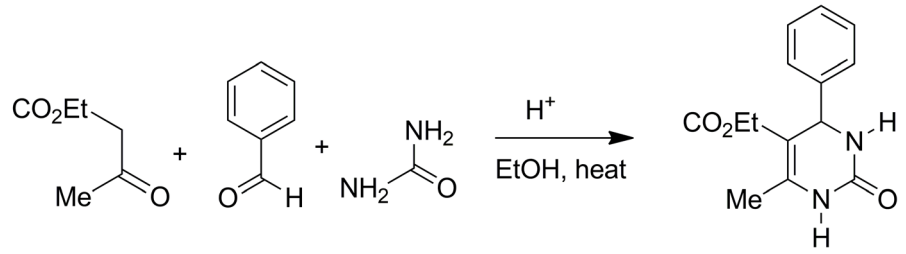


Figure 1.13 Examples of drugs containing the pyrimidinone moiety

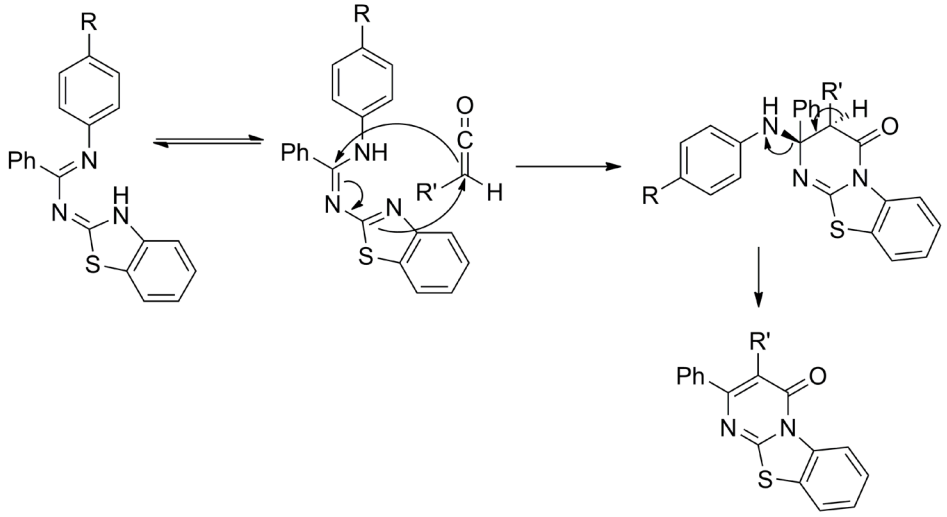
1.3.1 Synthetic routes to pyrimidinones

Biginelli reported the synthesis of dihydropyrimidinone in 1893 in a one-pot three component reaction between aldehydes, *β*-ketoesters and urea catalyzed by strong acid (**Scheme 1.9**). The reaction had some disadvantages such as very low yields, harsh conditions and long reaction times. Since then, organic chemists have been trying to improve this reaction by using different catalysts (acids, bases, metal oxides and nanoparticles), different solvents, and by using microwave and ultrasound irradiation and substituting solvents with ionic liquids.



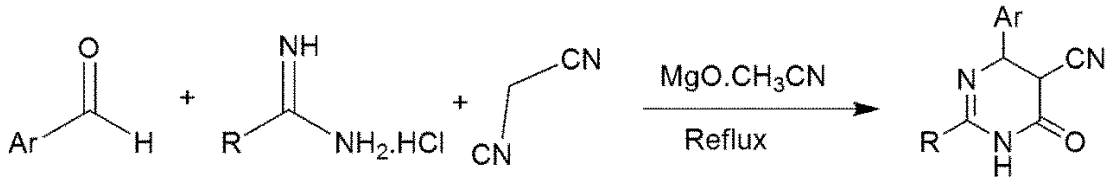
Scheme 1.9 The Biginelli reaction for the synthesis of pyrimidin-2-ones

Another route for the synthesis of pyrimidinones is the [4+2] cycloaddition reaction of 1,3-butadienes with ketenes or acid chlorides (which can also be generated *in situ*) to afford fused pyrimidin-4-one compounds (**Scheme 1.10**) (Sharma and Mahajan, 1997; Jayakumar et al., 2004).



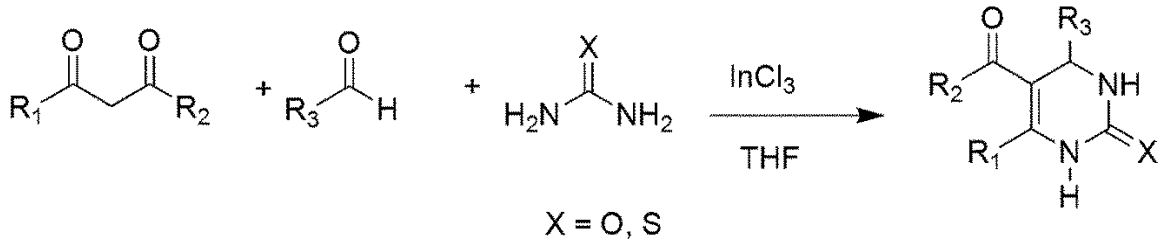
Scheme 1.10 The [4+2] cycloaddition mechanism

Sheibani et al. (2009) investigated using a heterogeneous basic MgO catalyst in a 3-component reaction with aldehydes, amidines, and malonitrile to synthesize pyrimidinone derivatives in short reaction times and high conversions (**Scheme 1.11**).



Scheme 1.11 The synthesis of pyrimidin-4-one derivatives from aldehydes, amidines and malonitrile using a MgO base

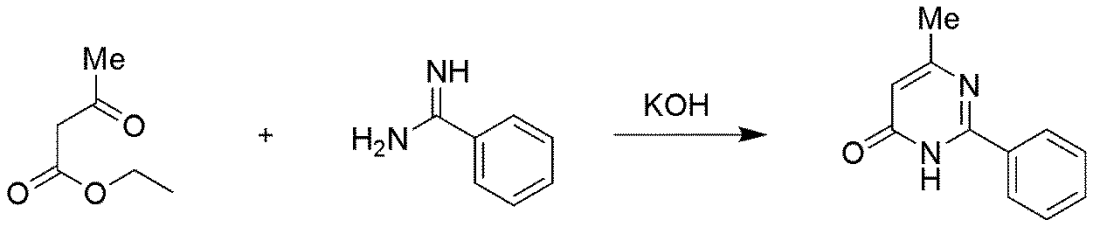
An improvement of the Bignelli reaction was reported using indium (III) chloride as a catalyst in the condensation of aldehydes, urea and 1,3-dicarbonyl compounds. The reaction conditions were milder and this method also enabled the synthesis of a variety of substituted molecules (**Scheme 1.12**) (Ranu et al., 2000).



Scheme 1.12 Synthetic scheme to pyrimidin-2-ones using aldehydes, urea and 1,3-dicarbonyl compounds with a $InCl_3$ catalyst

The same reaction was carried out by Zhang et al. (2015), but using a 5 mol% Bronsted acidic ionic liquid [Btto][*p*-TSA] as a catalyst instead of $InCl_3$, and by Patil et al (2011) using lemon juice (acidic, pH-2) as a catalyst and a solvent in one, providing a milder and greener alternative.

Pyrimidin-4-ones were also synthesised from benzamidamides and ethyl 3-oxobutanoate in aqueous medium with a strong base (KOH) (**Scheme 1.13**) (Maurya and Gupta, 2014).



Scheme 1.13 Synthetic scheme to pyrimidinones using a 1,3-dicarbonyl ester, benzamides and a KOH base in aqueous medium

1.3.2 Bioactivities of Pyrimidinones

Pyrimidinones have a broad spectrum of bioactivity including antimicrobial, antidiabetic, anticancer, antitubercular and anti-epileptic activity. These moieties were also incorporated into currently used drugs against a number of diseases and medical conditions (**Figure 1.13**). Some examples are allopurinol for the treatment of epilepsy, inflammatory bowel disease, tumor tysis syndrome and gout; raltegravir, an antiretroviral drug; lamivudine, an antiretroviral and Hepatic B drug; the anticancer drug, pemetrexed; mirodenofil for the treatment of erectile dysfunction; temelastine, a H-1 receptor antagonist; thiadiazolopyrimidinone, an anti-angiogenic drug; Trelaglipin, an antidiabetic drug and flucytosine, an antifungal (**Figure 1.13**).

Antimicrobial activity

A small library of ethanehydrazonoyl pyrimidinone derivatives (**Figure 1.14**) showed good activity against both Gram +ve and –ve strains of bacteria, with ED_{50} values between 0.173 – 0.698 µg kg^{-1}, comparable to the standard Anastrozole, with an ED_{50} of 1.09 µg kg^{-1} (Shawali and Farghaly, 2004; Edrees et al., 2010). Pyrimidinone-coumarin derivatives synthesised from ionic liquids were also shown to have good antibacterial and antifungal activity with MIC values between 12.5 - 25 µg mL^{-1} (**Figure 1.14**) (Banothu and Bavanthule, 2012). Good antiviral activity was also shown by 2-methoxy and 2-methylthio-6-alkylaminopyrimidinones

(**Figure 1.14**) against two positive strains (Rubella and Sindbis virus) and one negative strain (Vesicular stomatisis virus) with the highest activity being demonstrated against Rubella virus having an IC_{50} value of 3.9 μg mL^{-1} (Botta et al., 1999).

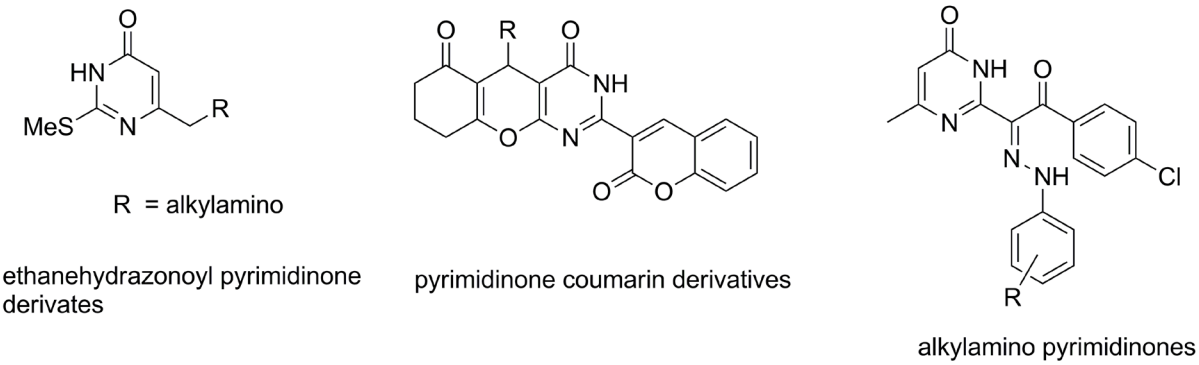


Figure 1.14 Antimicrobial pyrimidinones

Anticancer activity

Due to the structural similarity of the pyrimidinones to the pyrimidines, bases in DNA and RNA, these compounds can interact with DNA and RNA transcriptase interfering with cancer gene coding and having the potential to inhibit cancer cell replication (Subach et al., 2004). Thieno[2,3-d]pyrimidinone derivatives showed good anticancer activity, with the lead compound, a *para* bromophenyl derivative (**Figure 1.15**) having IC_{50} values of 14.96, 78.88 and 45.01 μM against HepG2 human hepatocellular liver carcinoma, MCF-7 human breast adenocarcinoma and BCG-823 human gastric cancer cell lines respectively (Li et al., 2013a). Benzofuropyrimidinones with a *para* fluorophenyl group and ethyl thioacetate group (**Figure 1.15**) were also seen to possess similar anticancer activity having IC_{50} values of 14.8, 78.8 and 53.5 μM against the Human A549 lung carcinoma, HepG2 hepatocellular carcinoma and HeLa cervical adenocarcinoma cell lines respectively (Li et al., 2015). Fused triazolo- and thienopyrimidinones (**Figure 1.15**) also exhibited good anticancer activity against human

breast adenocarcinoma (MCF7) cell lines with the most active compounds having IC_{50} values between 8.00-18.00 µg mL^{-1} (Rashad et al., 2012).

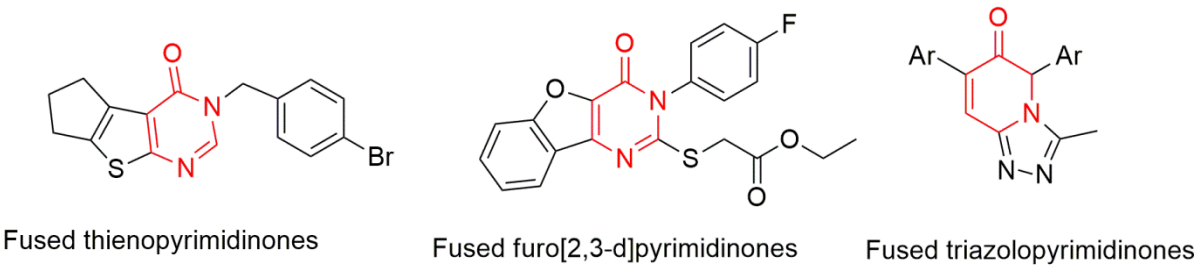


Figure 1.15 Anticancer pyrimidinones

Anti-inflammatory and Analgesic activity

The 5-acyl- and 5-aminoalkyl-3-phenyl-6-methyl-4(3*H*)-pyrimidinones (**Figure 1.16**) showed improved anti-inflammatory and analgesic activity compared to the standard drugs Tiaramide and Phenylbutazone (Ueda et al., 1983). 4-Aryl-6-alkylpyrimidin-2(1*H*)-ones (**Figure 1.16**) also showed better anti-inflammatory activity compared to the commercialized Ibuprofen (Dinakaran et al., 2012).

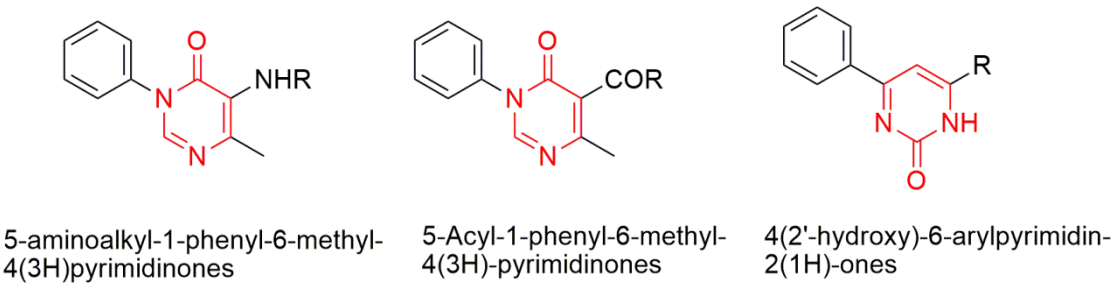
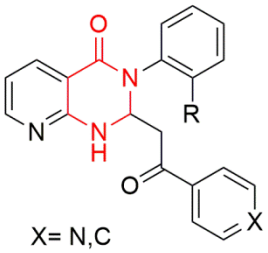


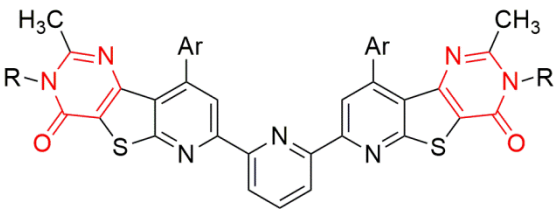
Figure 1.16 Anti-inflammatory and analgesic pyrimidinones

Anti-epileptic and anticonvulsant activity

Pyrimidinones are known to interact well with the central nervous system (McCoull et al., 2013), and research has been carried out on their anti-epileptic and anticonvulsant activity (Amr et al., 2003; White et al., 2004). Thioethyl and thioglycolic acid pyrimidinone derivatives (**Figure 1.17**) showed good activity against Yohinobine induced seizures, comparable to the anti-epileptic drug carbamazebene (Amr et al., 2003). A series of 2-substituted-3-arylpyrido[2,3-d]pyrimidinones (**Figure 1.17**) also showed potent anti-epileptic and anti-convulsant activity comparable to phenobarbital a commercialized anti-epileptic drug (White et al., 2004).



2-substituted-3-arylpyrido[2,3-d]pyrimidinones



Thioethyl/thioglycolic acid pyrimidinone derivatives

Figure 1.17 Anti-epileptic pyrimidinones

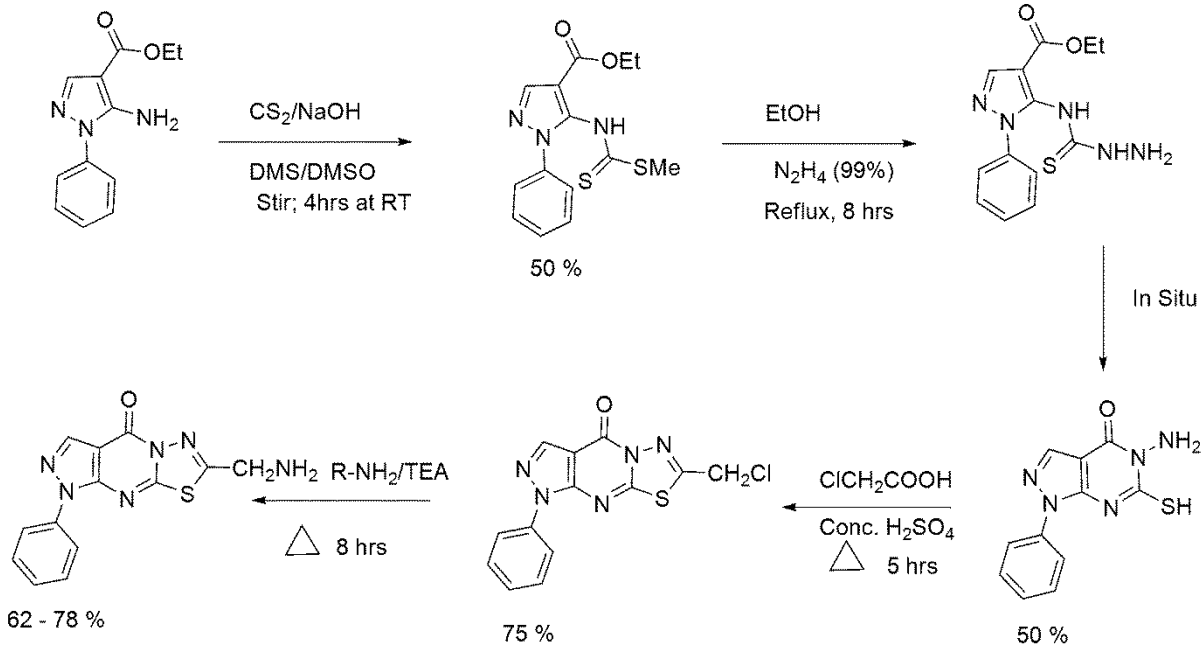
1.4 Thiadiazolopyrimidinone hybrid molecules

Molecular hybridization where two or more pharmacophores are chemically combined into a single molecule has proved useful in improving the activity of each of the individual pharmacophores on its own. This has made a significant impact in the pharmaceutical indsustry (Viegas et al., 2017). Through moleculear hybridisation, new series of potential bioactive molecules with enhanced activity and reduced side effects were synthesized (Hu et al., 2014; Viegas et al., 2007).

For example, Carvalho et al. (2004) hybridised Megazol (a 5-nitroimidiazole antimicrobial and trypanocidal agent with toxic and mutagenic effect on animals) with guanylhydrazone derivatives resulting in a new series of 1,3,4-thiadiazole-2-arylhydrazone derivatives. These hybrids showed good activity against *Trypanosma cruzi* that causes Chagas disease. The most active derivative exhibited a IC_{50} value of 3.5 μM, which was twice as active as the parent megazol (IC_{50}=9.9 μM). The hybrid molecule also demonstrated reduced toxicity (Calvalho et al., 2004).

Examples of molecular hybrids in the literature include pyrazole, thiadiazole, quinoline, oxadiazole, triazole and pyrimidinone moieties (Amir et al., 2003; Li et al., 2015).The hybridization of new thieno[2,3-d]pyrimidin-4(3*H*)-ones with 1,2,4-triazoles and 1,3,4-thiadiazole demonstrated cytotoxicity against four cancer lines (human colorectal cancer cell line HT-29, breast cancer cell line MDA-MB-231, cervical cancer cell line HeLa, human liver carcinoma HepG2 and human normal diploid cell line Lep3). The results showed that the hybridized compounds had improved activity to that of the parent thienopyrimidinone compounds with the triazole derivatives having IC_{50} values of 0.46-1.8 μM while the thiadiazole containing compounds had IC_{50} values of 0.037-3.8 μM (Mavrova et al, 2014).

Pyrazole, thiadiazole and pyrimidinone moieties have been hybridised into a single molecules starting from amino pyrazole ester precursors, converting them to thiazole intermediates and then thioureas before forming the pyrimidinones. The thiadiazoles were then formed by cyclising the thiol functionality onto the pyrimidinone core structure (Ranganath et al, 2012) (**Scheme 1.14**). These (2-aminomethyl-8-phenyl-pyrazolo[3,4-d][1,3,4]thiadiazolo[3,2-a]pyrimidin-4(1*H*)-one) showed broad spectrum antibacterial activity (Ranganath, 2012).



Scheme 1.14 Synthetic scheme for pyrazole thiadiazole pyrimidinone hybrid molecules (Ranganath et al., 2012)

1.5 Hypothesis and Aims

Hypothesis

Since both 1,3,4-thiadiazole and pyrimidinone moieties have demonstrated good bioactivity in a wide range of infections and medical conditions and have led to a number of commercialised drugs, combining the two entities and synthesising novel molecules may lead to the discovery of new antibiotics. These compounds may also find application in other diseases such as cancer, HIV and tuberculosis.

Aim

To identify compounds that could be developed into antibacterial agents.

Objectives

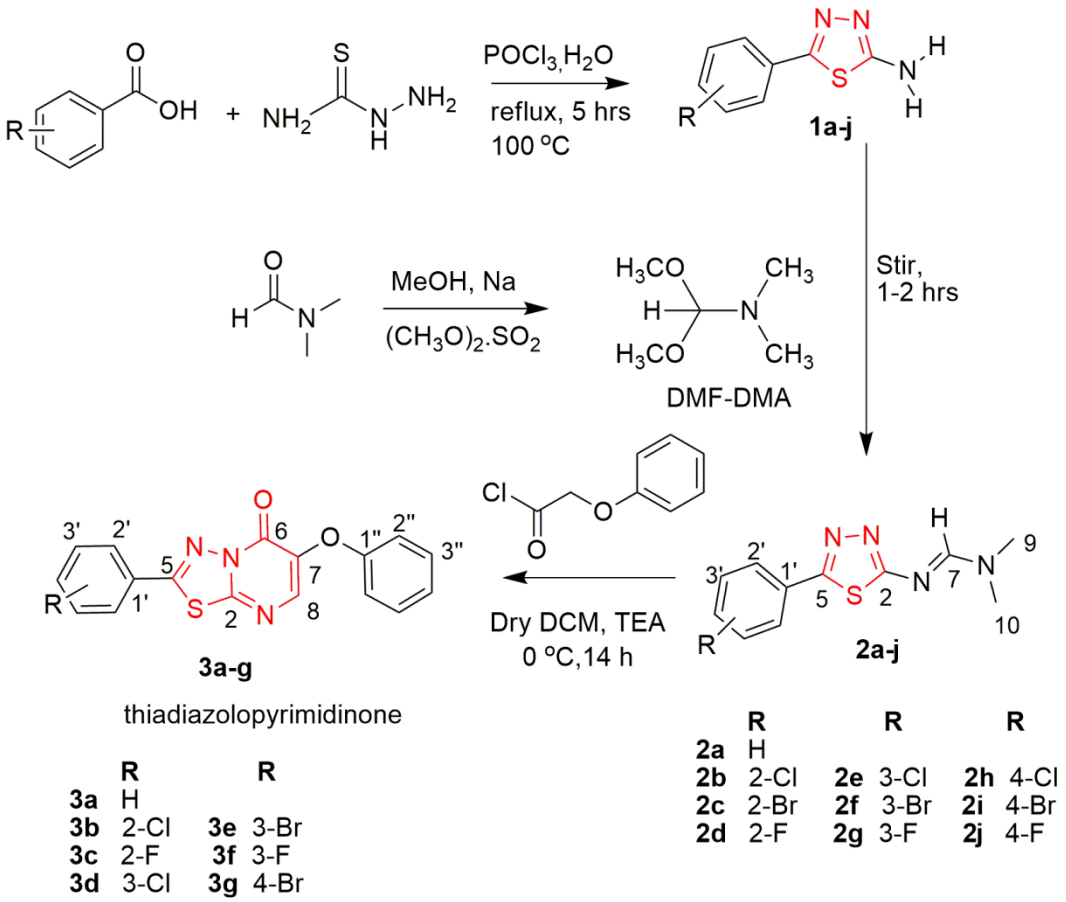
1. To synthesis a small series of thiadiazole-pyrimidinone based hybrid molecules and characterise them using NMR spectroscopy and mass spectrometry.
2. To determine the antibacterial activity of the synthesized compounds.
3. To study the effect of different substituents on the hybrid scaffold.

Chapter 2 Results and Discussion

2.1 Chemistry

A small library of seven novel pyrimidinone fused thiadiazole compounds was synthesized in a three step reaction starting by the formation of substituted 5-phenyl-1,3,4-thiadiazole-2-amines (**1a-j**) by the condensation of thiosemicarbazides and substituted carboxylic acids in the presence of phosphoryl chloride (**Scheme 2.1**). Dimethylformamide-dimethylacetal (DMF-DMA), was synthesized in parallel by the reaction of sodium methoxide (generated in situ), dimethyl sulfate and dimethyl formamide and was then reacted with thiadiazole amines to form dimethylaminoimino intermediates (**2a-j**). In the final step of the reaction, the dimethylaminoimino intermediates (**2a-j**) were cyclised to thiadiazolopyrimidinones (**3a-e**, **3g** and **3i**) via a 4+2 cycloaddition reaction in dry conditions with phenoxyacetyl chloride in the presence of triethylamine with yields of 40-80 %.

Bromo, chloro and fluoro substituents in the *ortho*, *meta* and *para* positions of the phenyl substituent provided variation in these molecules molecules (1,3,4-thiadiazole formation with other substituted benzoic acids such as nitro, methoxy and methyl substituted was unsuccessful). Only seven of the ten target molecules formed (**3a-e**, **3g** and **3i**). Formation of the 3-Br (**3f**), 4-Cl (**3h**) and 4-F (**3j**) pyrimidin-6-one derivatives was not successful on reaction with phenoxyacetyl chloride, despite several attempts. This was an extremely difficult reaction, which required dry conditions for the cyclisation. It is postulated that the reaction conditions were not ideal for these starting materials and hence only side products were observed. Structural elucidation of the synthesised molecules was carried out by 1D and 2D NMR spectroscopy and mass spectrometry.



Scheme 2.1 Reaction scheme for the synthesis of thiadiazolopyrimidinones **3a-g**

Empirical evidence supports that Diels-Alder cycloaddition reaction progresses with good yields in the presence of electron-rich dienes and electron-deficient dienophiles **(**Carruthers, 2013). Results from the present reaction revealed that a high yield of the corresponding pyrimidinone was obtained with the unsubstittuted thiadiazole based diene while inferior yields were obtained when electron withdrawing halogens were installed on the phenyl ring in the diene substrate. Furthermore, the electronic effect of the halogens on the diene also resulted in the low yields observed in these substrates; the 4-Br and 3-F derivative having the lowest yields in the series (**Table 2.1**).

Table 2.1 Yields of the synthesized compounds 3a-g

Compounds	% Yield
3a (R = H)	75
3b (R = 2Cl)	63
3c (R = 2F)	49
3d (R = 3Cl)	68
3e (R = 3Br)	58
3f (R = 3F)	42
3g (R = 4Br)	42

The [4+2] cycloaddition was carried out under cold conditions to prevent polymerisation of the ketene. The ketene is formed by triethylamine (TEA) abstracting a proton from the α-carbon of phenoxyacetyl chloride, resulting in the elimination of Cl^- (**Figure 2.1**). This ketene then acts as the dienophile with the thiadiazole aminoimino intermediate as the diene. A 4+2 cycloaddition reaction between these two molecules result in the thiadiazolopyrimidinone after an internal abstraction of a proton and elimination of dimethylamine. This occurs either via a concerted [4+2] (Path I) or non-concerted [4+2] (Path II) mechanism (Jayakumar et al, 2004).

The intermediates **1a-j** were confirmed by the aromatic protons between δ 7.40 to 7.80 and the two amino protons at δ 5.11 having the correct integral ratios. An example of this can be seen in the intermediate **1a**, where a broad singlet at δ 5.11 and the three proton and two proton multiplets at δ 7.40 (H-3'-5') and δ 7.78 (H-2'/6') are present (**Figure 2.2**). This was quite a challenging reaction at first as it involves $POCl_3$, a corrosive and hazardous reagent that vigorously reacts with water. Hence, precautions had to be taken during addition of H_2O after refluxing for the first hour. It was also important that the mixture was cool enough to prevent bubbling, spillage & loss of reactants. The cold temperature had to be maintained during the basification step as well, since this was an exothermic reaction. Nevertheless, these reactions produced good yields of between 88-97%.

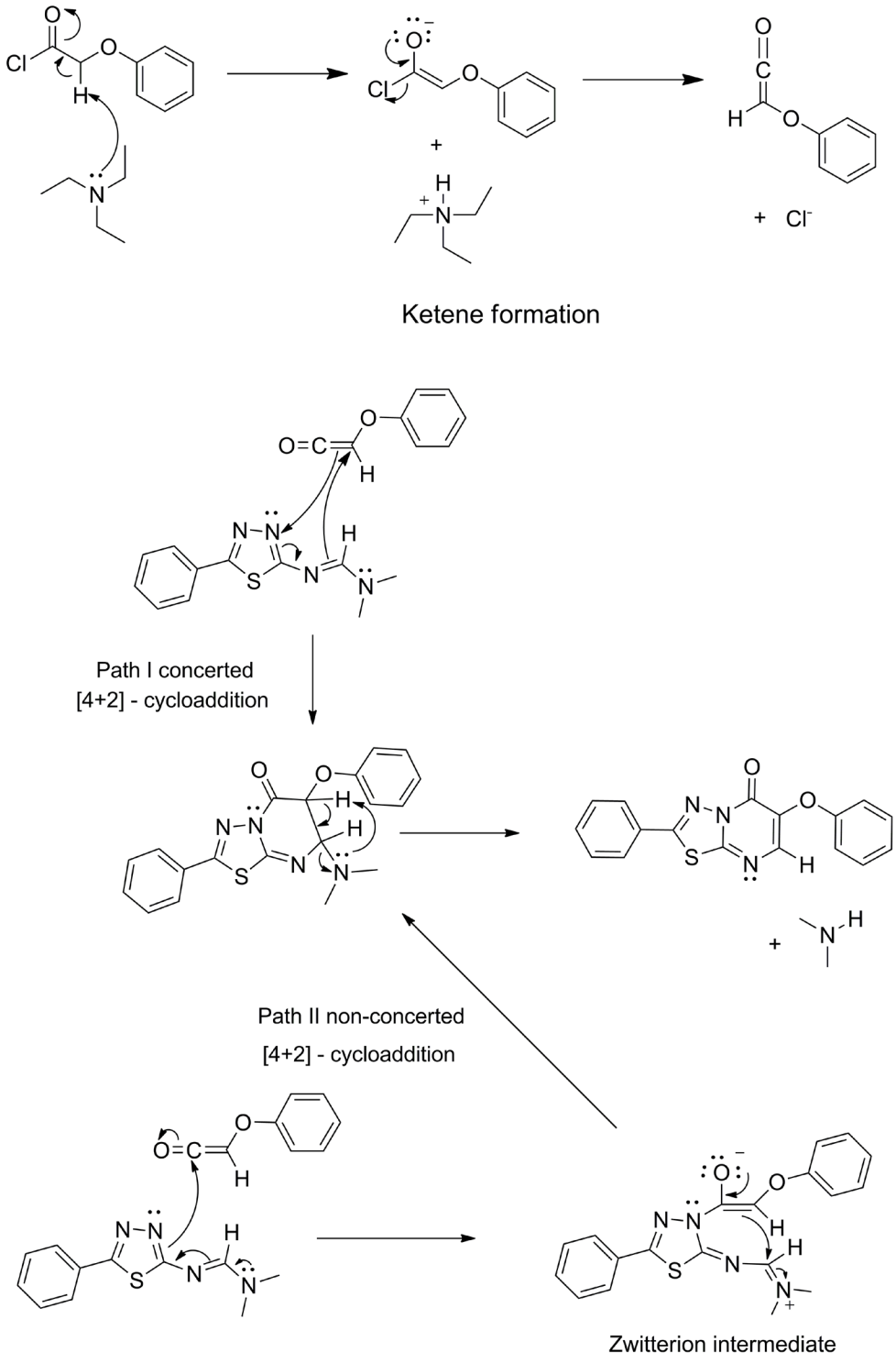


Figure 2.1 Postulated mechanism for the formation of thiadiazolopyrimidinone from phenoxyacetyl chloride and thiadiazole dimethylaminoimino intermediates

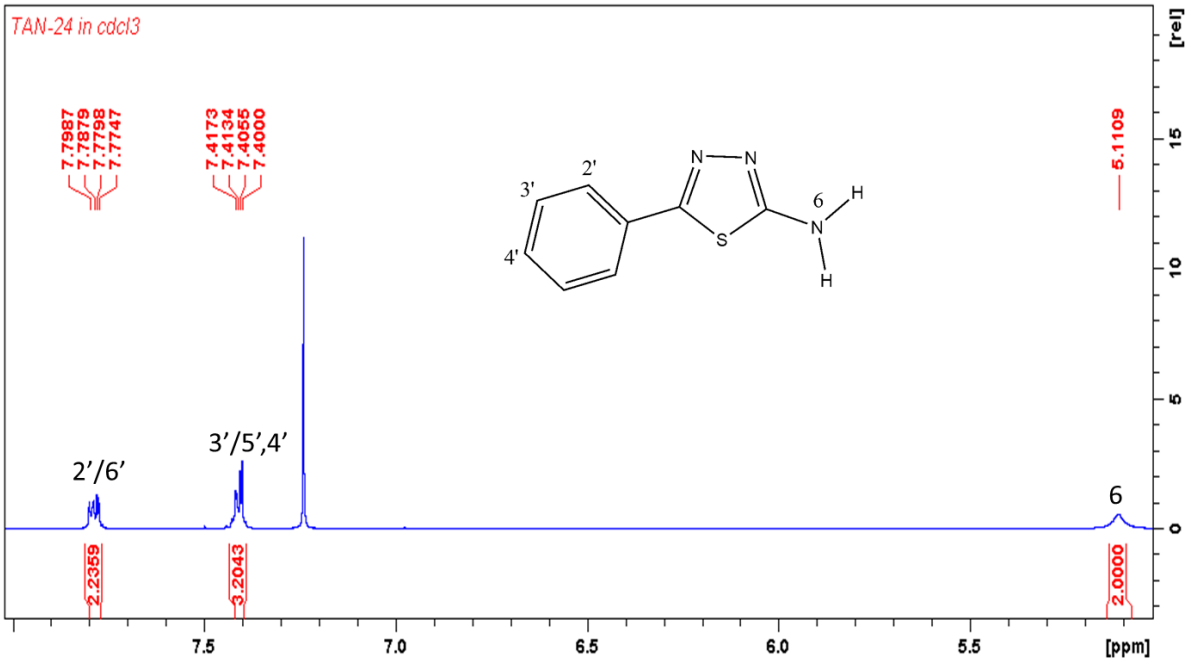


Figure 2.2 ^{1}H NMR spectrum of *5-phenyl-1,3,4-thiadiazole-2-amine* (**1a**)

Conversion to the *N*,*N*-dimethyl-*N*'-(5-phenyl-1,3,4-thiadiazol-2-yl)imidoformamides **2a-j** was then indicated by the disappearance of this amino resonance and the appearance of the imino proton H-7 at δ 8.32 as a sharp singlet and two *N*-methyl proton resonances, CH_3-9 and CH_3-10 at δ 3.16 and 3.13 respectively for **2a** (**Figure 2.3**). These were in perfect integral ratios with the aromatic protons of the phenyl moiety at δ 7.43 (3H, m, H-3'/5', 4') and δ 7.88 (2H, d, J = 7.9 Hz, H-2'/6'). The ^{13}C NMR spectrum (**Figure 2.4**) showed the imine carbon resonance C-7 at δ 156.4, four aromatic carbons resonances for the phenyl moiety at δ 127.1 (C-2'/6'), 128.9 (C-3'/5'), 130.0 (C-4') and δ 131.4 (C-1'), and the two methyl carbon resonances, CH_3-9 and CH_3-10 at δ 40.9 and 34.9 respectively. This reaction was relatively easy and was a matter of adding the thiadiazole amine (**1a-j**) to the synthesized DMF-DMA, with stirring at room temperature for between 1-2 hours. The products formed in good yields between 80-96%.

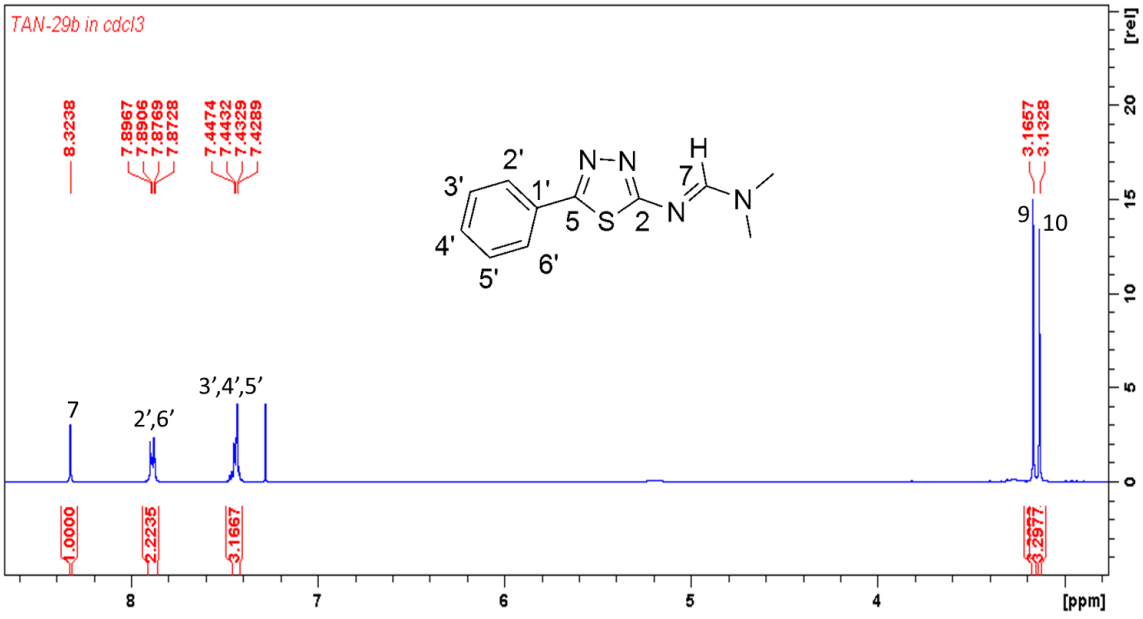


Figure 2.3 ^{1}H NMR spectrum of *N,N-dimethyl-N'-(5-phenyl-1,3,4-thiadiazol-2-yl)imidoformamide* (**2a**)

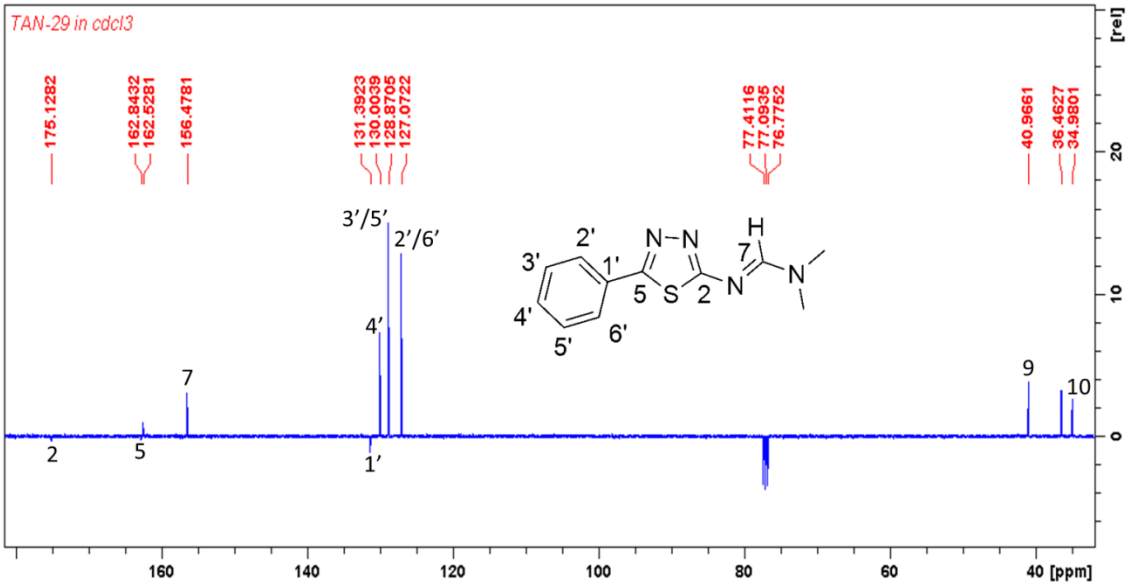


Figure 2.4 ^{13}C NMR spectrum of *N,N-dimethyl-N'-(5-phenyl-1,3,4-thiadiazol-2-yl)imidoformamide* (**2a**)

Formation of the pyrimidin-6-one ring with phenoxyacetyl chloride is a moisture and temperature sensitive reaction and quite a difficult reaction to carry out in the laboratory. It was therefore important that all solvents were properly dried and that the reaction was carried out

in an evacuated flask under nitrogen. It was also important to maintain the temperature at 0 °C to prevent polymerization of the ketene. Side reactions reduced the yields of the products and in some cases yields below 50% were recorded for **3c** (2-F), **3f** (3-F) and **3g** (4-Br). The formation of pyrimidinones through the cycloaddition reaction between 1,3-diazabutadiene and ketenes has been already been documented in literature whereby a similar reaction was reported by Jayakumar et al. (2004) where various 1,3-diazabuta-1,3-dienes were reacted with a variety of ketenes to produce pyrimidinones in yields of 42 - 75%.

Using **3a** as an example, the thiadiazole pyrimidin-6-ones were confirmed by a characteristic sharp singlet at δ 7.84 assigned to the pyrimidinone proton H-8 (**Figure 2.5**). This was the only proton on the thiadiazolepyrimidinone ring. The other proton resonances were that of the two aromatic rings, which clearly separated from each other, allowing them to be assigned using 2D NMR techniques.

The proton resonances of aromatic ring attached to the thiadiazole ring was more deshielded than that of the phenoxy moiety attached to the pyrimidinone scaffold. These resonances occurred as a doublet at δ 7.95 (2H, J = 7.6 Hz, H-2'/6') and two triplets at δ 7.60 (1H, J = 7.6 Hz, H-4') and δ 7.52 (2H, J = 7.6 Hz, H-3'/5'). They were attributed to the phenyl ring attached to the thiadiazole moiety as the H-2'/6' resonance showed HMBC correlations to the C-5 resonance at δ 160.7, the most deshielded resonance in the ^{13}C NMR spectrum (**Figure 2.6**). Incidently, this was the only carbon resonance of the thiadiazolepyrimidine scaffold that did not show a correlation to H-8.

Three deshielded carbon resonances, C-2 (δ 140.4), C-6 (δ 153.6) and C-7 (δ 156.7) all showed HMBC correlations to H-8. The C-7 resonance in turn was further differentiated by HMBC

correlations to H-2" and H-6", which occurred as a doublet at δ 7.05. The other aromatic resonances, H-3"/5" and H-4" were assigned based on COSY correlations to H-2"/6". The C-2 and C-6 resonances were differentiated based on chemicals shifts as C-6, the carbonyl resonance of pyrimidine occurred typically at δ 153.6. The two carbon resonances that remained at δ128.3 and δ156.6 were assigned to C-1' and C-1" respectively and confirmed by HMBC correlations to H-3'/5' and C-3"/5" respectively (**Figure 2.7**).

The other compounds of the series were elucidated in the same manner and only differed in the proton and carbon resonances of the aromatic ring attached to the thiadiazole moiety, as this is where the variation occurred. ^{1}H and ^{13}C overlaid spectra of the various compounds in the series are shown in **Figure 2.8** to **Figure 2.11**.

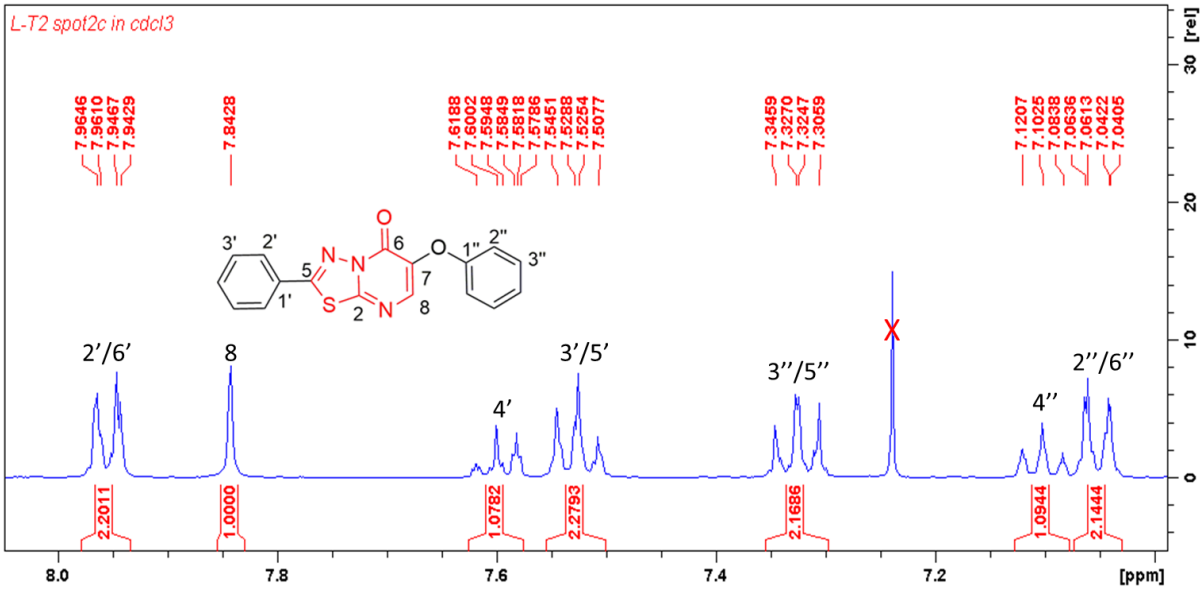


Figure 2.5 ^{1}H NMR spectrum for 7-phenoxy-5-phenyl-8*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-6-one (**3a**), 400 MHz

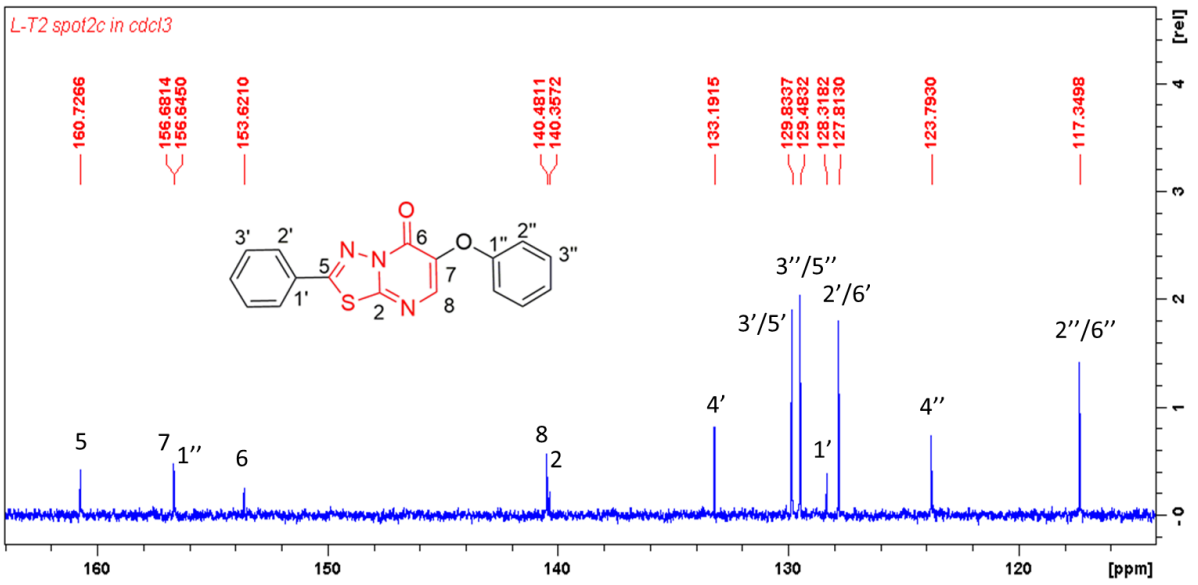


Figure 2.6 ^{13}C NMR of 7-phenoxy-5-phenyl-8*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-6-one compounds (**3a**), 100 MHz

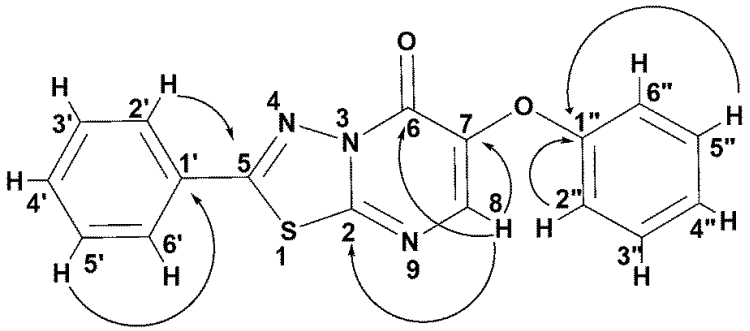


Figure 2.7 Key HMBC correlations used in the structural elucidation of **3a**

Resonance effects result in the 3' and 5' positions having greater electron density. This is evident in the overlaid spectra of the 2-Cl and 2-F derivatives together with the unsubstituted derivative, where both the H-3' and H-5' can be seen more shielded in the order of the following substituents H < Cl < F (fluoro derivative had the most shielded resonance). The H-6' resonance is the other resonance worth noting. This resonance becomes more deshielded in moving from H to Cl to F. One would expect the same to occur with H-4', however, this is not

the case. The thiadiazolopyrimidine ring removes electron density from H-6' and this is increased when the electronegative Cl or F is introduced at C-2'. In the ^{13}C NMR spectra, the resonances of the 2-Cl derivative did not change much, however for the 2-F derivative, the C-3' and C-5' resonances are more shielded as expected duet to resonance effects. Surprisingly and unlike the H-6' resonance, C-6' is more shielded, probably due to electron donation by the thiadiazolopyrimidinone ring.

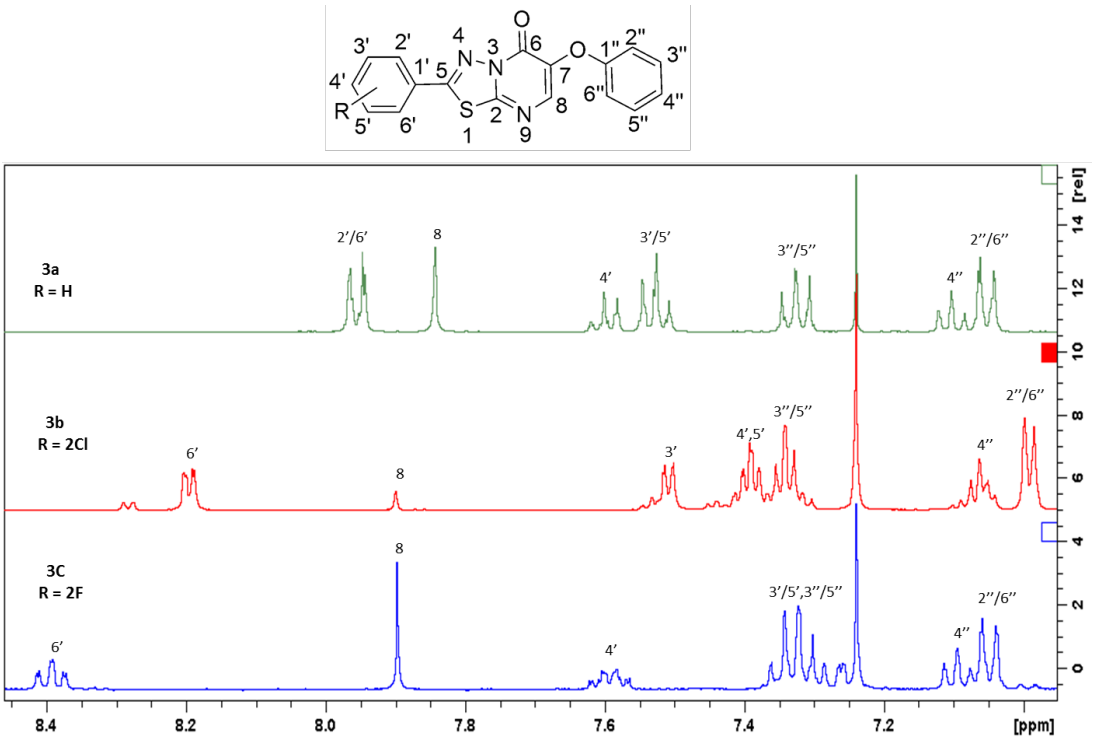


Figure 2.8 Overlay of ^{1}H NMR spectra of compounds **3a-3c**, 400 MHz

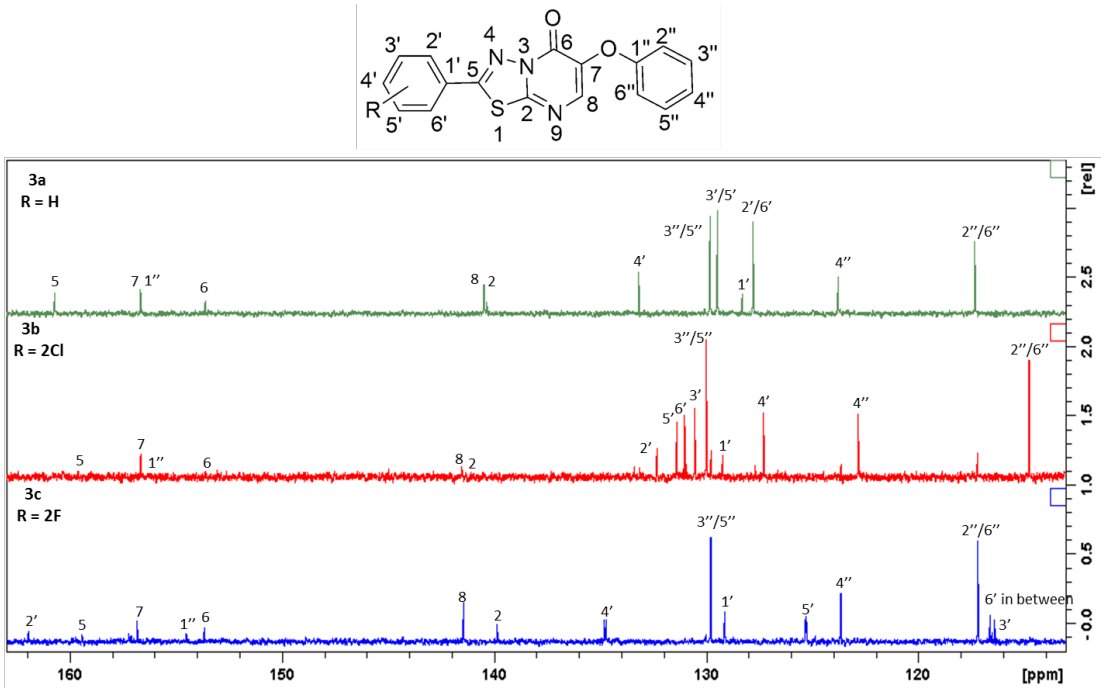


Figure 2.9 Overlay of ^{13}C NMR spectra of compounds **3a-3c**, 100 MHz

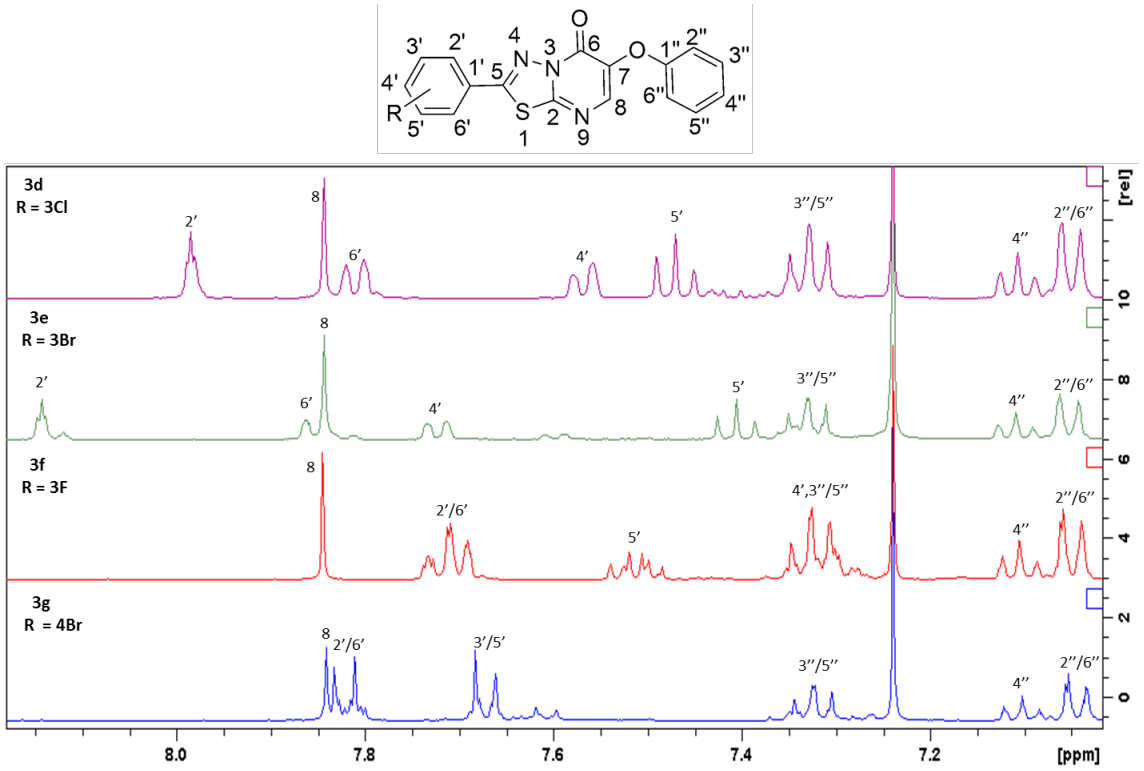


Figure 2.10 Overlay of ^{1}H NMR spectra of compounds **3d-3g**, 400 MHz

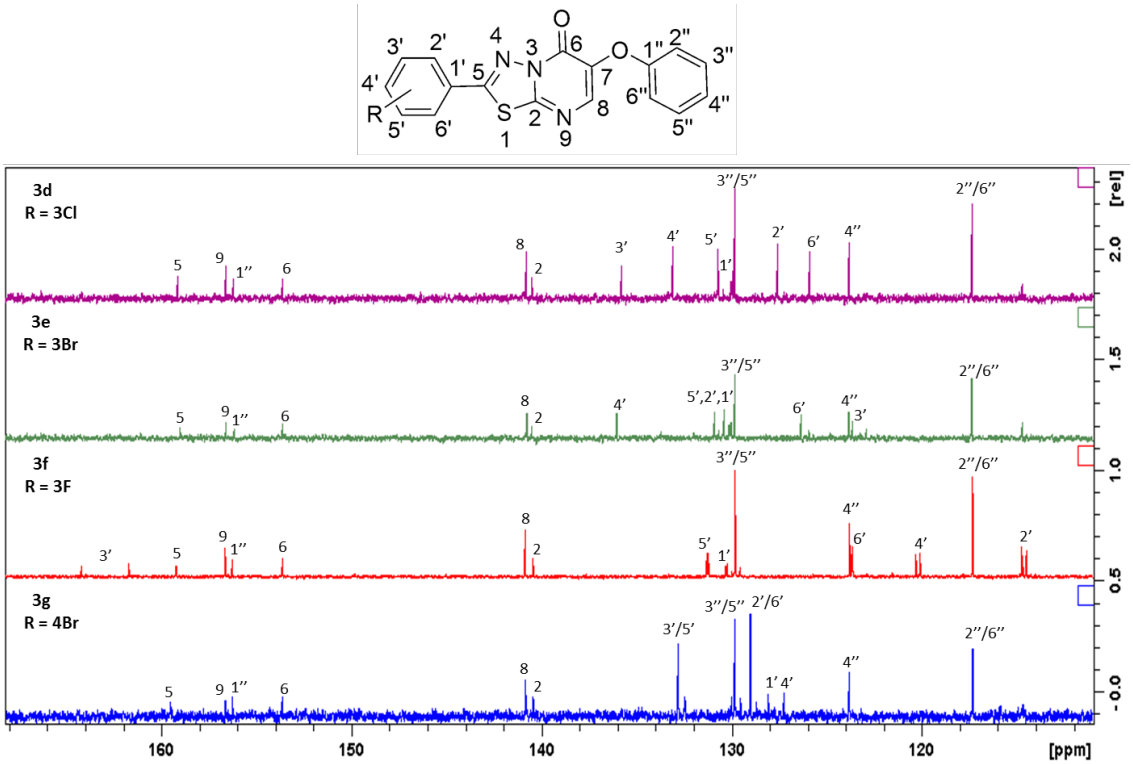


Figure 2.11 Overlay of ^{13}C NMR spectra of compounds **3d-3g**, 100 MHz

High Resolution Mass spectrometry confirmed that the structures of the molecules were correct. In addition, the IR spectrum indicated that the pyrimidinone carbonyl C=O band was present at approximately 1697 cm^{-1}.

2.2 Antibacterial activity of compounds 3a-g

The thiadiazolopyrimidinones **3a-g** were tested for their antibacterial activity against two Gram +ve and four Gram –ve bacterial strains, being initially screened by the disk diffusion method and then by the broth microdilution method to determine the MBC values of the active compounds. The commercial drugs, ciprofloxacin and levofloxacin were used in the same assays for comparison. While the seven compounds tested were inactive against the Gram +ve *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus* (MRSA), all compounds were very active against the four Gram –ve strains.

The fact that the compounds are active against the Gram –ve bacteria and not the Gram +ve requires further studies. The mechanism of action may well be enzymatic, meaning that the Gram –ve bacteria may have certain enzymes that the Gram +ve do not, which may interact with the compounds.

All the compounds were mostly active against *E. coli* except for the 2-chloro derivative (**3b**). These compounds exhibited activity between 0.15 to 0.19 μM, which was better than both the standards ciprofloxacin and levofloxacin (**Table 2.2**). Compounds (**3a**), (**3b**) and (**3e**), the unsubstituted, the 2-chloro and 3-bromo derivatives were also very active against *Klebsiella pneumonia* with activities of 0.15 to 0.19 μM. The most active compound with the broadest spectrum of activity against the Gram –ve strains was the unsubstituted compound **3a** having an MBC of 0.19 μM against all four Gram –ve strains. This was followed by the 3-bromo derivative (**3e**) having an inhibitory concentration of 0.15 μM against *E. coli* and *K. pneumonia* and under 4.0 μM against *P. aeruginosa* and *S. typhimurium*, while **3c** (2-fluoro derivative) was active at 0.18 μM against *E. coli* and *P. aeruginosa*. In addition, the 2-chloro derivative **3b** had an MBC of 0.34 μM against *Salmonella typhimurium*.

The results indicated that placing halogenated substituents on the aromatic ring attached to the thiadiazole moiety did not enhance activity. In fact, in many cases, this reduced activity of the parent compound **3a**. However, better activity was seen by derivatives where the halogen was placed at C-2' in comparison to when it was substituted at C-3' or C-4'. Since the compounds have better activity than ciprofloxacin and levofloxacin, these are good scaffolds for further studies into potential antibiotics against Gram –ve strains of bacteria.

Over the years, the disc diffusion assay has been the method of choice for antimicrobial evaluation mainly due its low cost compared to the MIC assay. However, the method is not without its intrinsic limitations such as the long period of incubation time (~18 hours), its inefficiency in evaluating some microorganism, reported inconsistencies in the results and 70-95 % accuracy (Davis and Stout, 1971; Lehtopolku et al., 2012). Albeit, this work adopted the disc diffusion assay, hence, the limitations are inherited. Also, the type and number of bacteria strains tested, two Gram +ve and four Gram –ve did not allow a broader evaluation of the antimicrobial spectrum of the synthesized molecules. This is revealed in the inactivity against the only Gram +ve strain –*Staphylococcus aureus* and its resistant strain.

A series of 8-phenyl-2-substituted aminomethylpyrazolo[3,4-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4(1*H*)-ones (**Figure 2.12**) were also shown to have antibacterial activity against *Klebsiella*, *E. coli*, *S. aureus* and *B. subtilis* using the Kirby Bauer method, however the zones of inhibition were approximately half that of the positive control, ampicillin (Raganath et al., 2012).

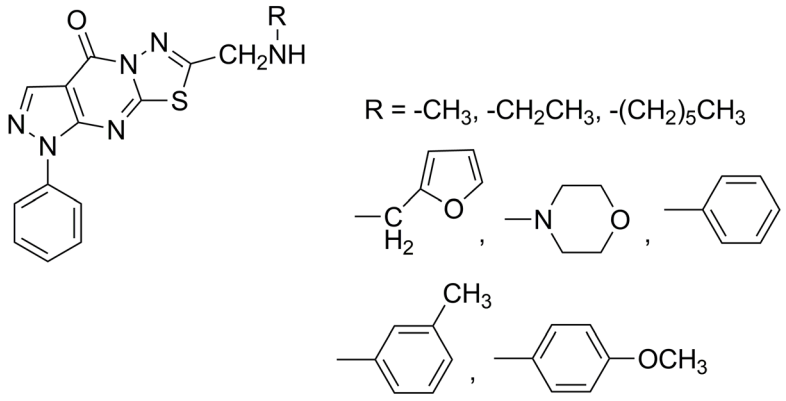
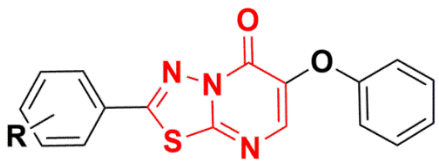


Figure 2.12 Aminomethylpyrazolo[3,4-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4(1*H*)-ones with antibacterial activity

Table 2.2 Antibacterial activity of thiadiazolopyrimidinones **3a-g** (MBCs in µM)



No.	R	Gram +ve		Gram -ve			
		Sa	***MRSA***	***Ec***	***Pa***	***Kp***	***St***
3a	H	-	-	0.19	0.19	0.19	0.19
3b	2-Cl	-	-	5.5	11	0.17	0.34
3c	2-F	-	-	0.18	0.18	58	92
3d	3-Cl	-	-	0.17	4.4	22	5.5
3e	3-Br	-	-	0.15	3.9	0.15	2.5
3f	3-F	-	-	0.18	11	12	4.6
3g	4-Br	-	-	0.15	4.9	4.9	281
Cip		94	189	2.9	187	12	2.9
Lev		22	87	0.34	345	22	21

Sa = *Staphyllococcus aureus*; MRSA = Methicillin resistant *Staphyloccocus aureus*; Ec = *Escherichia coli*; Ps = *Pseudomonas aeruginosa*; St = *Salmonella typhimurium*; Kp = *Klebsiella pneumonia*; Cip = ciprofloxacin; Lev = levofloxacin. "-" denotes MBC greater than 500 µM.

Chapter 3 Experimental

3.1 Chemicals and Reagents

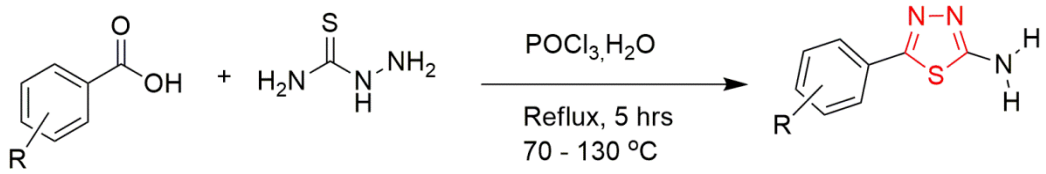
All chemicals and reagents used were purchased from Sigma Aldrich and Merck and used without further purification. Organic solvents were distilled according to standard procedures. TLC analysis was carried out with silica gel backed on aluminium 60 F_{254} plates purchased from Merck, South Africa. The organic compounds were purified by column chromatography using a silica gel (60- 120 mesh) stationary phase and a mobile phase of varying ratios of ethyl acetate and hexane. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) analysis was carried out on a BRUKER AVANCE 400 MHz spectrometer. Chemical shifts (δ) were reported in parts per million (ppm) and coupling constants (J) in hertz. Deuterated chloroform ($CDCl_3$) and dimethyl sulfoxide-d_6 (DMSO-d_6) were used as NMR solvents and referenced at δ 7.24 for ^{1}H and δ 77.23 for ^{13}C NMR in $CDCl_3$, and δ 2.50 for ^{1}H and δ 39.51 for ^{13}C NMR in DMSO-d_6. NMR data was analyzed using TopSpin 3.1 software (Bruker). Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer with universal ATR sampling accessory. High resolution mass spectra of the novel thiadiazolopyrimidinones was performed on a Bruker microTOF-Q II ESI instrument operated at ambient temperatures. Melting points were determined on an electrothermal melting point apparatus (Electrothermal IA9100).

3.2 Synthesis

3.2.1 Synthesis of substituted 5-phenyl-1,3,4-thiadiazol-2-amines (1a-j)

A volume of 5 mL phosphoryl chloride ($POCl_3$) was added to a mixture of substituted benzoic acids (0.01 mol) and thiosemicarbazide (0.75 g; 0.01 mol) and stirred under reflux at 70-130 °C (depending on the benzoic acids used) for 1 hr. The higher temperatures were for the

brominated acids, since they took longer to dissolve. The mixture was then cooled to room temperature and 10 mL water added slowly to the mixture and the contents refluxed for a further 4 hrs. The mixture was cooled again and basified to pH 8 with aqueous KOH. The precipitate formed was filtered, washed with H_2O and vacuum dried to yield the 5-phenyl-1,3,4-thiadiazole-2-amines (**1a-j**) in yields of between 88-97% (**Scheme 3.1**).

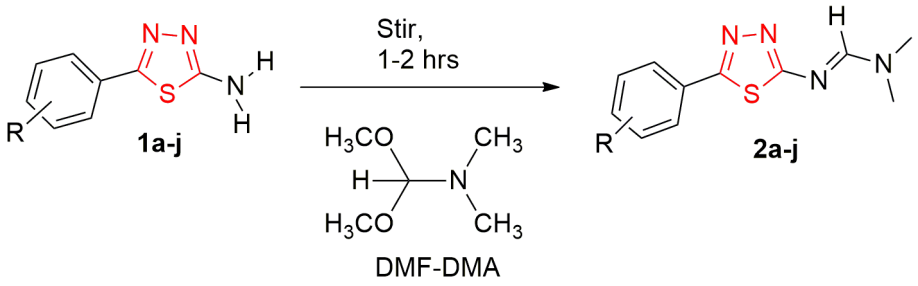


Scheme 3.1 Synthesis of substituted 5-phenyl-1,3,4-thiadiazole-2-amines (**1a-j**)

3.2.2 Synthesis of substituted *E*-*N*,*N*-dimethyl-(5-phenyl-1,3,4-thiadiazol-2-yl)imidoformamides (2a-j)

Sodium metal (18 g) was slowly dissolved in analytical grade CH_3OH (250 mL) while stirring in an ice-bath. A mixture of dimethylformamide (DMF) (60 mL) and dimethyl sulfate (DMS) (74 mL) was preheated at 60 ºC for 10 minutes and slowly added to the sodium methoxide solution, which was then stirred for 2 hr forming dimethylformamide-dimethylacetal (DMF-DMA), which was distilled at 100-105 ºC and 200 mL collected as a light and air sensitive colourless liquid with an unpleasant smell.

The respective substituted 5-phenyl-1,3,4-thiadiazol-2-amines (**1a-j**) (0.43 mmol) were dissolved in DMF-DMA (5 mL) and stirred at room temperature for 1-2 hrs. The reaction was monitored by TLC and upon completion, was concentrated *in vacuo*, removing any unreacted DMF-DMA in the process. The product was precipitated by the addition of hexane and filtered and dried to yield **2a-j** in the range of 80-96% (**Scheme 3.2**).



Scheme 3.2 Stepwise reaction for the synthesis of substituted 5-phenyl-1,3,4-thiadiazol-2-yl)imidoformamides (**2a-j**)

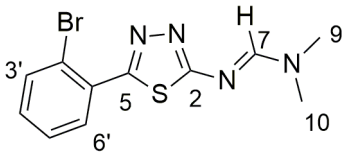
N,N-dimethyl-N'-(5-phenyl-1,3,4-thiadiazol-2-yl)imidoformamide (2a)

off-white crystalline solid; 90% yield; mp 130-132 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.28 (1H, s, H-7), 7.85 (2H, dd, J = 8.0, 2.5 Hz, H-2'/6'), 7.40 (3H, m, H-3'/4'/5'), 3.16 (3H, s, CH_3-9), 3.12 (3H, s, CH_3-10); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.1 (C-2), 162.9 (C-5), 156.4 (C-7), 131.3 (C-1'), 130.0 (C-4'), 128.8 (C-3'/5'), 127.0 (C-2'/6'), 41.0 (C-9), 35.0 (C-10).

N,N-dimethyl-N'-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide (2b)

cream white solid; 88% yield; mp 106-107 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.30 (1H, s, H-7), 8.15 (1H, dd, J = 7.6, 2.8 Hz, H-6'), 7.44 (1H, dd, J = 7.8, 2.6 Hz, H-3'), 7.31 (2H, m, H-4',5'), 3.12 (3H, s, CH_3-9), 3.09 (3H, s, CH_3-10); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 176.7 (C-2), 158.3 (C-5), 156.4 (C-7), 132.0 (C-1'), 130.7 (C-2'), 130.6 (C-4'), 130.4 (C-3'), 130.2 (C-6'), 127.1 (C-5'), 42.0 (C-9), 35.0 (C-10).

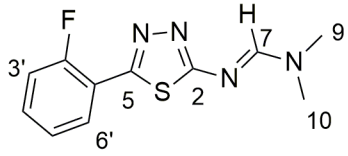
N,N-dimethyl-N'-(5-(2-bromophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide (2c)



Brown crystalline solid; 89% yield; mp 109-110 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.34 (1H, s, H-7), 8.03 (1H, dd, J = 8.0, 1.6 Hz, H-6'), 7.67 (1H, dd, J = 8.1, 1.3 Hz, H-3'), 7.40 (1H, td, J = 7.7, 1.2 Hz, H-5'), 7.27 (1H, td, J = 8.0, 1.8 Hz, H-4'), 3.16 (3H, s, CH_3-9), 3.12 (3H, s, CH_3-10), ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.6 (C-2), 161.2 (C-5), 156.5 (C-7), 133.3 (C-1'), 132.8 (C-3'), 130.4 (C-4'), 129.8 (C-6'), 125.7 (C-5'), 123.0 (C-2'), 41.0 (C-9), 35.0 (C-10).

N,N-dimethyl-N'-(5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide (2d)

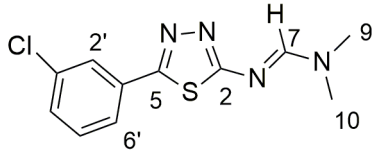
Light brown solid residue; 93% yield; mp 100-110 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.27



(1H, s, H-7), 8.25 (1H, td, J = 9.5, 2.0 Hz, H-5'), 7.36 (1H, m, H-4'), 7.22 (1H, td, J = 8.1, 1.3 Hz, H-6'), 7.13 (1H, dd, J = 10.9, 8.5 Hz, H-3'), 3.11 (3H, s, CH_3-9), 3.08 (3H,s , CH_3-10); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 176.6 (C-2), 158.6 (d, J = 253.7 Hz, C-2'), 156.4 (C-7), 155.0 (d, J = 8.2 Hz, C-5), 131.3 (d, J = 8.9 Hz, C-4'), 128.4 (d, J = 3.0 Hz, C-5'), 124.6 (d, J = 3.3 Hz, C-6'), 119.4 (d, J = 12.7 Hz, C-1'), 116.1 (d, J = 21.8 Hz, C-3'), 41.0 (C-9), 35.0 (C-10).

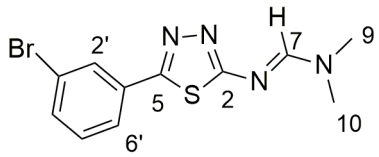
N,N-dimethyl-N'-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide (2e)

Pale yellow solid; 89% yield; mp 112-113 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.26 (1H, s, H-



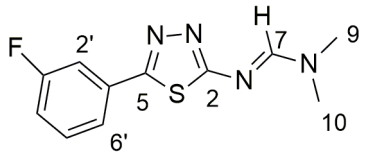
7), 7.85 (1H, d, J = 2.0 Hz, H-2'), 7.71 (1H, dt, J = 8.3, 1.9 Hz, H-4'), 7.33 (2H, m, H-5',6'), 3.12 (3H, s, CH_3-9), 3.08 (3H, s, CH_3-10); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.6 (C-2), 161.4 (C-5), 156.4 (C-7), 134.9 (C-1'), 133.1 (C-3'), 130.1 (C-5'), 129.9 (C-4'), 126.9 (C-6'), 125.2 (C-2'), 41.0 (C-9), 35.0 (C-10).

N,N-dimethyl-N'-(5-(3-bromophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide (2f)



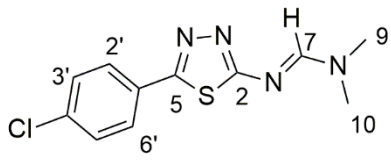
red solid; 93% yield; mp 102-104 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.28 (1H, s, H-7), 8.01 (1H, t, J = 2.7 Hz, H-2'), 7.74 (1H, d, J = 7.9 Hz, H-4'), 7.49 (1H, dd, J = 7.6, 1.2 Hz, H-6'), 7.27 (1H, t, J = 8.0 Hz, H-5'), 3.13 (3H, s, CH_3-9), 3.09 (3H, s, CH_3-10); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.7 (C-2), 159.8 (C-5), 156.4 (C-7), 133.8 (C-1'), 132.2 (C-2'), 131.5 (C-5'), 130.9 (C-6'), 127.6 (C-4'), 122.0 (C-3'), 41.0 (C-9), 35.0 (C-10).

N,N-dimethyl-N'-(5-(3-fluorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide (2g)



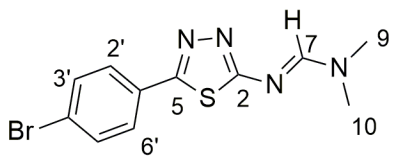
Brown solid residue; 96% yield; mp 105-106 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.27 (1H, s, H-7), 7.58 (2H, m, H-2', 6'), 7.35 (1H, td, J = 8.1, 5.2 Hz, H-5'), 7.06 (1H, td, J = 8.4, 2.3 Hz, H-4'), 3.12 (3H, s, CH_3-9), 3.08 (3H, s, CH_3-10);^{13}C-NMR ($CDCl_3$, 100 MHz) δ 175.6 (C-2), 162.9 (d, J = 248.9 Hz, C-3'), 161.5 (d, J = 3.6 Hz, C-5), 156.6 (C-7), 133.5 (d, J = 8.2 Hz, C-1'), 130.5 (d, J = 8.6 Hz, C-5'), 122.9 (d, J = 3.2 Hz, C-6'), 116.8 (d, J = 21.7 Hz, C-2'), 113.8 (d, J = 23.7 Hz, C-4'), 41.0 (C-9), 35.0 (C-10).

N,N-dimethyl-N'-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide (2h)



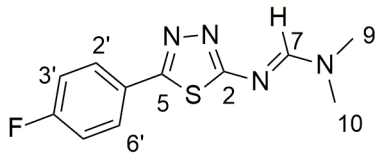
Pale yellow solid; 93% yield; mp 142-144 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.31 (1H, s, H-7), 7.80 (2H, d, J = 8.6 Hz, H-2'/6'), 7.41 (2H, d, J = 8.6 Hz, H-3'/5'), 3.12 (3H, s, CH_3-9), 3.08 (3-H, s, CH_3-10); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.4 (C-2), 161.7 (C-5), 156.4 (C-7), 135.9 (C-4'), 129.9 (C-1'), 129.1 (C-3'/5'), 128.3 (C-2'/6'), 41.0 (C-9), 35.0 (C-10).

N,N-Dimethyl-N'-(5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide (2i)



Brown solid residue; 80% yield; mp 142-143 °C ; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.28 (1H, s, H-7), 7.71 (2H, d, J = 8.6 Hz, H-2'/6'), 7.53 (2H, d, J = 8.6 Hz, H-3'/5'), 3.13 (3H, s, CH_3-9), 3.09 (3H, s, CH_3-10); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 175.5 (C-2), 161.8 (C-5), 156.4 (C-7), 132.1 (C-3'/5'), 130.4 (C-1'), 128.5 (C-2'/6'), 124.2 (C-4'), 41.0 (C-9), 35.0 (C-10).

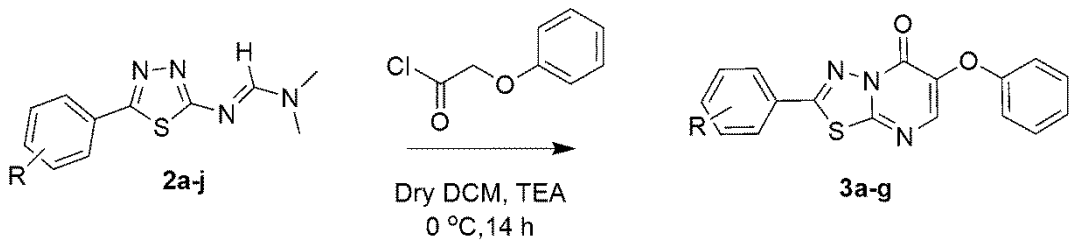
N,N-dimethyl-N'-(5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide (2j)



Brown solid residue; 92% yield: mp 145-146 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.26 (1H, s, H-7), 7.82 (2H, dd,J = 8.8, 5.3 Hz, H-2'/6'), 7.09 (2H, t, J = 8.8 Hz, H-3'/5'), 3.12 (3H, s, CH_3-9), 3.09 (3H, s, CH_3-10); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 173.3 (C-2), 163.8 (d, J = 251.8 Hz, C-4'), 162.5 (C-5), 156.4 (C-7), 129.0 (d, J = 9.2 Hz, C-2'/6'), 127.8 (C-1'), 116.0 (d, J = 22.2 Hz, C-3'/5'), 41.0 (C-9), 35.0 (C-10).

3.2.3 Synthesis of substituted 7-phenoxy-5-phenyl-8*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-6-ones (3a-g)

The thiadiazole imidoformamides (**2a-j**) were dissolved in dry dichloromethane (DCM) and triethylamine (TEA) (0.363 g) added. Phenoxyacetyl chloride was dissolved in analytical grade DCM and added dropwise to the reaction mixture maintaining the temperature at 0 °C in an ice-water slurry (**Scheme 3.3**). The reaction was monitored to completion using TLC. The duration of the reactions was typically between 12-14 hrs. Upon completion, the reaction was basified with calcium hydrogen carbonate to neutralise any acid present and the product extracted with DCM and washed with water. The DCM was then removed *in vacuo* and the product purified by column chromatography (hexane:ethyl acetate, 70:30).

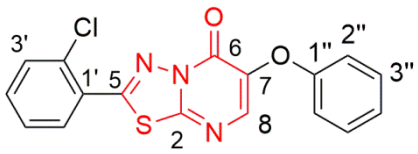


Scheme 3.3 Synthetic route to the 6-phenoxy-2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-ones (**3a-g**)

6-Phenoxy-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (3a)

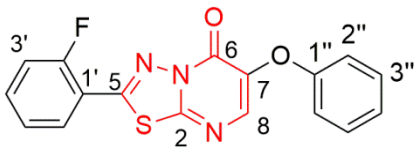
White powder; 75% yield; mp 189-190 °C; IR υ_{max} 1697 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (2H, dd, J = 7.5 Hz, H-2'/6'), 7.84 (1H, s, H-8), 7.60 (1H, t, J = 7.5 Hz, H-4'), 7.52 (2H, t, J = 7.5 Hz, H-3'/5'), 7.32 (2H, dd, J = 8.5, 7.6 Hz, H-3"/5"), 7.09 (1H, t, J = 7.9 Hz, H-4"), 7.05 (2H, d, J = 7.9 Hz, H-2"/6"); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 160.7 (C-5), 156.7 (C-7), 156.6 (C-1"), 153.3 (C-6), 140.5 (C-8), 140.4 (C-2), 133.2 (C-4'), 129.8 (C-3'/5'), 129.5 (C-3"/5"), 128.3 (C-1'), 127.8 (C-2'/6'), 123.8 (C-4"), 117.3 (C-2"/6"); HRMS (pos) (*m/z*): 344.0480 [M+Na], calculated for $C_{17}H_{11}N_3O_2SNa$: 344.0470.

6-Phenoxy-2-(2-chlorophenyl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (3b)



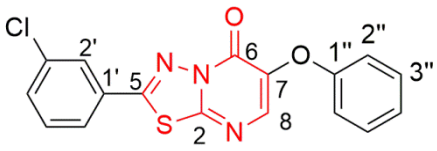
Off-white powder; 63% yield; mp 157-159 °C; IR υ_{max}: 1697 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.19 (1H, d, J = 7.6 Hz, H-6'), 7.89 (s, H-8), 7.51 (1H, dd, J = 8.6, 1.3 Hz, H-3'), 7.39 (2H, m, H-4', 5'), 7.34 (2H, t, J = 7.5 Hz, H-3"/5"), 7.06 (1H, t, J = 14.7, 7.5 Hz, H-4"), 6.99 (2H, d, J = 8.1 Hz, H-2"/6"); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 166.2 (C-5), 156.6 (C-7), 156.2 (C-1"), 153.6 (C-6), 140.8 (C-8), 140.5 (C-2), 132.3 (C-2'),131.4 (C-5'), 131.0 (C-6'), 130.5 (C-3'), 130.0 (C-3"/5"), 129.7 (C-1'), 127.3 (C-4'), 122.8 (C-4"), 114.7 (C-2"/6"); HRMS (pos) (*m/z*): 378.0092 [M+Na], calculated for $C_{17}H_{10}N_3O_2SClNa$: 378.0080.

6-Phenoxy-2-(2-fluorophenyl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (3c)



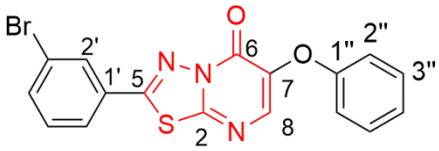
Off-white solid residue; 49% yield; mp 168-170 °C; IR υ_{max}: 1695 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.39 (1H, td, J = 8.1, 1.9 Hz, H-6'), 7.89 (1H, s, H-8), 7.60 (1H, m, H-4'), 7.32 (4H, m, H-3', H-5', H-3"/5"), 7.09 (1H, t, J = 7.5 Hz, H-4"), 7.05 (2H, d, J = 8.1 Hz, H-2"/6"); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 158.2 (d, J = 253.4 Hz, C-2'), 157.2 (d, J = 11.9 Hz, C-5), 156.8 (C-7), 154.5 (C-1"), 153.6 (C-6), 141.4 (C-8), 139.8 (C-2), 134.7 (d, J = 10.3 Hz, C-4'), 129.7 (C-3"/5"), 129.1 (d, J = 22.8 Hz, C-1'), 125.3 (d, J = 3.6 Hz, C-5'), 123.6 (C-4"), 117.2 (C-2"/6"), 116.6 (d, J = 9.2 Hz, C-6'), 116.5 (d, J = 22.7 Hz, C-3') ; HRMS (pos) (*m/z*): 362.0374, calculated for $C_{17}H_{10}N_3O_2SF$: 362.0375.

6-Phenoxy-2-(3-chlorophenyl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (3d)



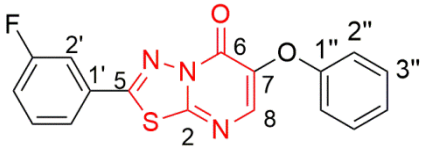
Yellow powder; 68% yield; mp 158-169 °C; IR υ_{max}: 1697 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (1H, t, J = 1.9 Hz, H-2'), 7.84 (1H, s, H-8), 7.81 (1H, d, J = 8.0 Hz, H-6'), 7.56 (1H, d, J = 8.0 Hz, H-4'), 7.47 (1H, t, J = 7.8 Hz, H-5'), 7.32 (2H, t, J = 8.0 Hz, H-3"/5"), 7.10 (1H, t, J = 7.5 Hz, H-4"), 7.05 (2H, d, J = 8.0 Hz, H-2"/6"); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.5 (C-5), 156.6 (C-7), 156.2 (C-1"), 153.6 (C-6), 140.8 (C-8), 140.5 (C-2), 135.8 (C-3'), 133.2 (C-4'), 130.7 (C-5'), 130.0 (C-1'), 129.8 (C-3"/5"), 127.5 (C-2'), 125.9 (C-6'), 123.8 (C-4''), 117.3 (C-2"/6"); HRMS (pos) (*m/z*): found: 378.0080, calculated for $C_{17}H_{11}N_3O_2SCl$ $(M + Na)^+$: 378 .0081.

6-Phenoxy-2-(3-bromophenyl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (3e)



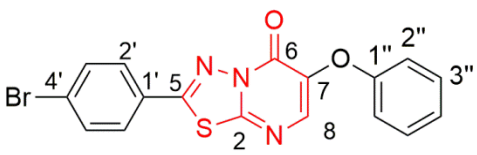
White solid residue; 58% yield; mp 170-172 °C; IR υ_{max}: 1698 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.14 (1H, t, J = 1.5 Hz, H-2'), 7.86 (1H, d, J = 8.5 Hz, H-6'), 7.86 (1H, s, H-8), 7.72 (1H, d, J = 8.1 Hz, H-4'), 7.40 (1H, t, J = 7.8 Hz, H-5'), 7.33 (2H, dd, J = 7.9, 7.5 Hz, H-3"/5"), 7.10 (1H, t, J = 7.5 Hz, H-4"), 7.05 (2H, d, J = 7.9 Hz, H-2"/6"); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.1 (C-5), 156.6 (C-7), 156.2 (C-1"), 153.6 (C-6), 140.8 (C-8), 140.5 C-2), 136.0 (C-4'), 130.9 (C-5'), 130.4 (C-2'), 130.0 (C-1'), 129.8 (C-3"/5"), 126.3 (C-6'), 123.8 (C-4"), 123.6 (3'), 117.2 (C-2"/6"); HRMS (pos) (*m/z*): 421.95 85, calculated for $C_{17}H_{10}N_3O_2SBrNa$: 421.9575

6-Phenoxy-2-(3-fluorophenyl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (3f)



Cream-white solid residue; 42% yield; mp 142-143 °C; IR υ_{max}: 1697 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84 (1H, s, H-8), 7.71 (2H, dd, J = 8.5, 2.6 Hz, H-2'/6'), 7.50 (1H, ddd, J = 8.1, 8.1, 5.7 Hz, H-5'), 7.32 (3H, m, H-4',3"/5"), 7.10 (1H, t, J = 7.7 Hz, H-4"), 7.05 (2H, d, J = 8.0 Hz, H-2"/6"); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.7 (d, J = 251.2 Hz, C-3'), 159.2 (d, J = 3.6 Hz, C-5), 156.6 (C-7), 156.2 (C-1"), 153.6 (C-6), 140.9 (C-8), 140.4 (C-2), 131.2 (d, J = 8.5 Hz, C-5'), 129.5 (d, J = 9.8 Hz, C-1'), 129.8 (C-3"/5"), 123.8 (C-4"), 123.6 (d, J = 3.2 Hz, C-6'), 120.2 (d, J = 21.2 Hz, C-4'), 117.2 (C-2"/6"), 114.6 (d, J = 24.2 Hz, C-2'); HRMS (pos) (*m/z*): 362.0377, calculated for $C_{17}H_{10}N_3O_2SFNa$: 362.0375.

6-Phenoxy-2-(4-bromophenyl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (3g)



Yellow powder; 42% yield; mp 167-169 °C; IR υ_{max}: 1697 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84 (1H, s, H-8), 7.81 (2H, d, J = 8.6 Hz, H-2'/6'), 7.67 (2H,

d, *J* = 8.6 Hz, H-3'/5'), 7.32 (2H, dd, *J* = 9.1, 7.6 Hz, H-3"/5"), 7.10 (1H, t, *J* = 7.5 Hz, H-4"), 7.04 (2H, dd, *J* = 8.6, 1.3 Hz, H-2"/6"); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.3 (C-5), 156.6 (C-7), 156.2 (C-1"), 153.6 (C-6), 140.8 (C-8), 140.4 (C-2), 132.8 (C-3'/5'), 129.8 (C-3''/5''), 129.0 (C-2'/6'), 128.1 (C-1'), 127.3 (C-4'), 123.8 (C-4"), 117.2 (C-2"/6"); HRMS (pos) (*m/z*): 421.9583, calculated for $C_{17}H_{10}N_3O_2SBr$: 421.9575.

3.3 *In-vitro* antibacterial assays

Two Gram +ve strains (*Staphylococcus aureus* ATCC 25923 and Methicillin resistant *S. aureus* ATCC BAA-1683) and four Gram –ve strains (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14026, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumonia* ATCC 314588) were used for antibacterial studies. Levofloxacin and Ciprofloxacin were used as positive controls for comparison.

3.3.1 Disc diffusion assay

The synthesised compounds were initially screened using the disc diffusion assay to determine whether or not they had any activity against the bacterial strains. The bacterial strains were grown overnight in nutrient broth (Biolab, South Africa) at 37 °C and adjusted to a 0.5 McFarland standard. Muller-Hinton Agar (MHA) plates were prepared by dissolving agar (38 g) in water (1 L), and pouring it into sterile petri dishes, allowing them to cool and set at room temperature. The different bacterial strains were inoculated onto the agar plates by streaking a swab dipped into the bacterial broth over the surface of the agar. A 5 µL aliquot of the synthesised compounds (1 mg in 1 mL DMSO) was spotted onto 12 mm antibiotic discs and placed in the inoculated MHA plates, which were then incubated for 24 h at 37 °C. All

compounds exhibiting a zone of inhibition in at least three of the six strains were tested in the minimum bactericidal concentration (MBC) assay.

3.3.2 Minimum bactericidal concentration assay

The synthesised compounds were dissolved in DMSO at a concentration of 1 mg mL^{-1} and serially diluted in 1 mL Eppendorf tubes using DMSO, down to a concentration of 0.12 μg mL^{-1}. A 5 μL aliquot of the different concentrations of the test compounds were pipetted directly on the MHA plate (prepared as above with the different bacterial strains) and incubated for 24 h at 37 °C. The MBC was taken as the lowest concentration showing inhibition of bacterial growth around the compound. DMSO was used as a negative control and showed no zone of inhibition in the MHA plates. All experiments were carried out in triplicate and an average of the readings recorded.

Chapter 4 Conclusion

A series of novel thiadiazolopyrimidinone compounds (**3a-g**) was successfully synthesized in average yields in a four step reaction. The most difficult steps were the first and last steps, formation of the aminothiadiazole and the [4+2] cycloaddition reaction involving phenoxyacetyl chloride and the thiadiazole imidoformamides. The difficulty in the first step was handling the corrosive phosphoryl chloride, a reagent which reacts vigorously with water. Since this reaction was exothermic, it was also essential for a cool temperature to be maintained for the duration of the reaction. However, the yields produced here were quite high (88-97%). The [4+2] cycloaddition reaction involving phenoxyacetyl chloride and thiadiazole imidoformamide was the most difficult and troublesome reaction that resulted in both low (42-58%) and high yields (63-75%). Some of the reactions also did not work. This reaction was moisture and temperature sensitive and produced many side products in the reaction mixture. Thus, it was important to use very dry solvents and maintain the temperature at 0 °C in order to minimise side reactions.

Formation of dimethylformamide-dimethylacetal by reaction of dimethylformamide, dimethyl sulfate and sodium methoxide and collecting the product by distillation was an easy step to perform, as was the addition of this reagent to the aminothiadiazole forming the thiadiazole imidoformamide. The yields for this step were also quite high at 80-96%.

The structures of the seven compounds synthesized were elucidated by NMR spectroscopy and confirmed by mass spectroscopy. Using 2D NMR techniques, the ^{1}H and ^{13}C NMR resonances were unequivocally assigned. This NMR data will serve as a basis for the synthesis and identification of similar compounds with the same scaffold.

All the synthesised thiadiazolopyrimidinones showed excellent antibacterial activity against all Gram negative bacterial strains but were not active against Gram positive strains. Compound **3a** (with an unsubstituted phenyl ring) showed the best activity across all four Gram negative strains with MBC values of 0.19 μM better than standard drugs Ciprofloxacin and Levofloxacin. All other compounds showed good activity with MBC values better than the two standards for two or three of the test strains. These compounds can therefore be considered as hit compounds, which can be developed further into antibiotics.

Future work will involve carrying out cytotoxicity assays on these compounds to see whether or not they are cytotoxic to normal mammalian cells. Molecular docking studies will also be carried out on the most active compounds to determine a possible mechanism of action for their antibacterial activity against Gram negative compounds.

Chapter 5 References

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UNIVERSITY OF KWAZULU-NATAL

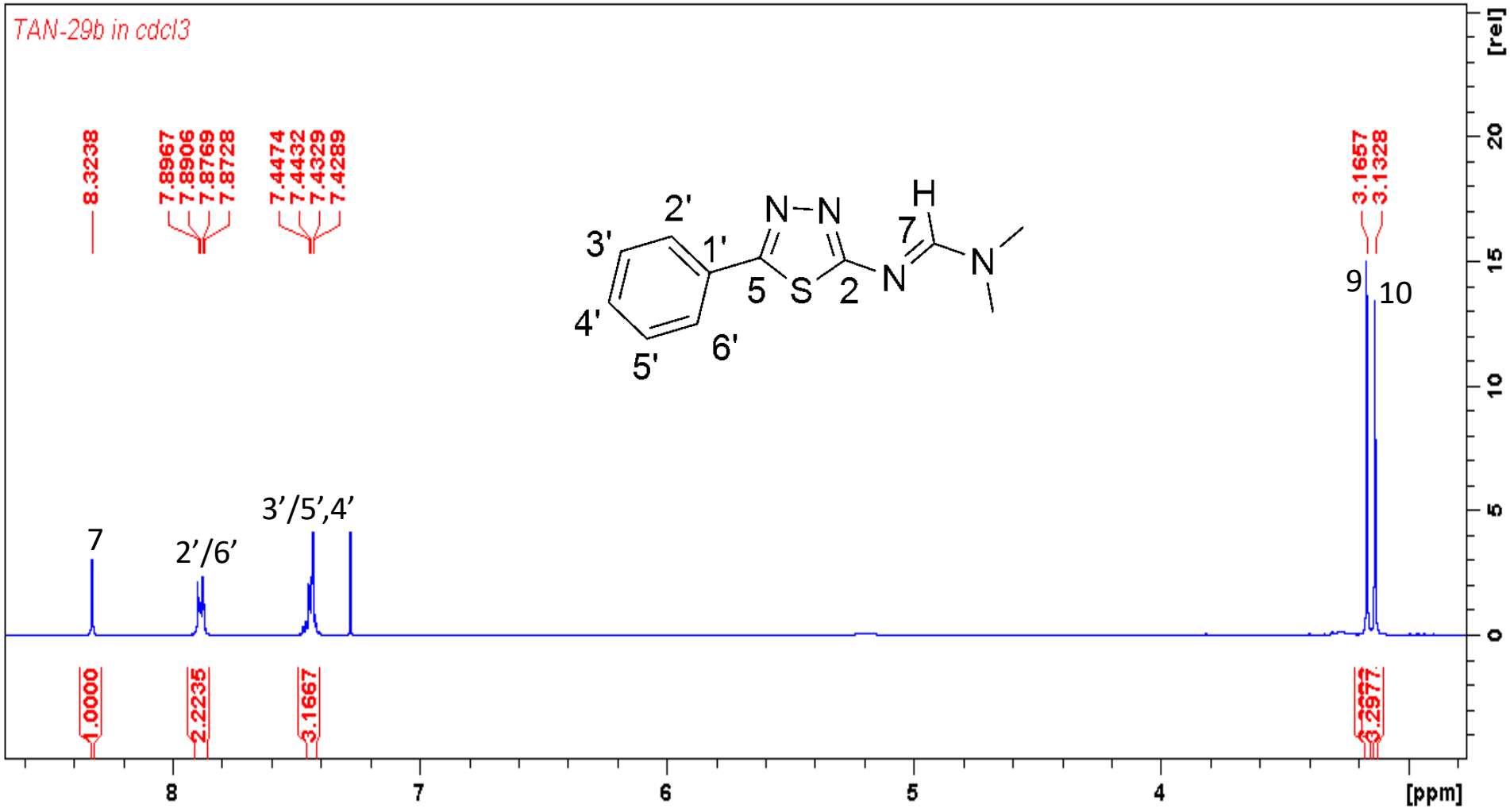
SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF THIADIAZOLOPYRIMIDINONE HETEROCYCLIC HYBRIDS

APPENDIX

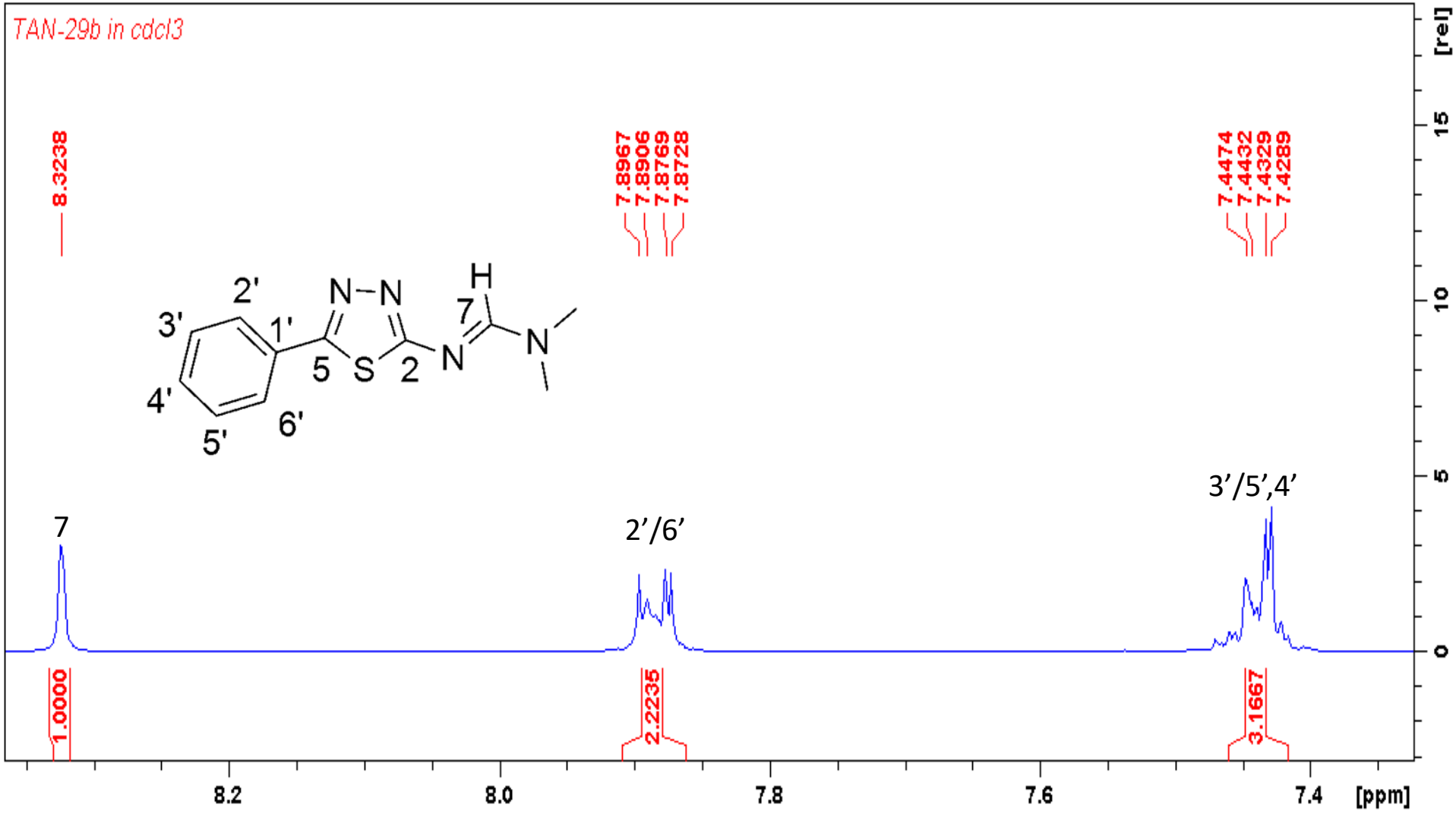
2018

THANDOKAZI ANDISWA NTSHELE

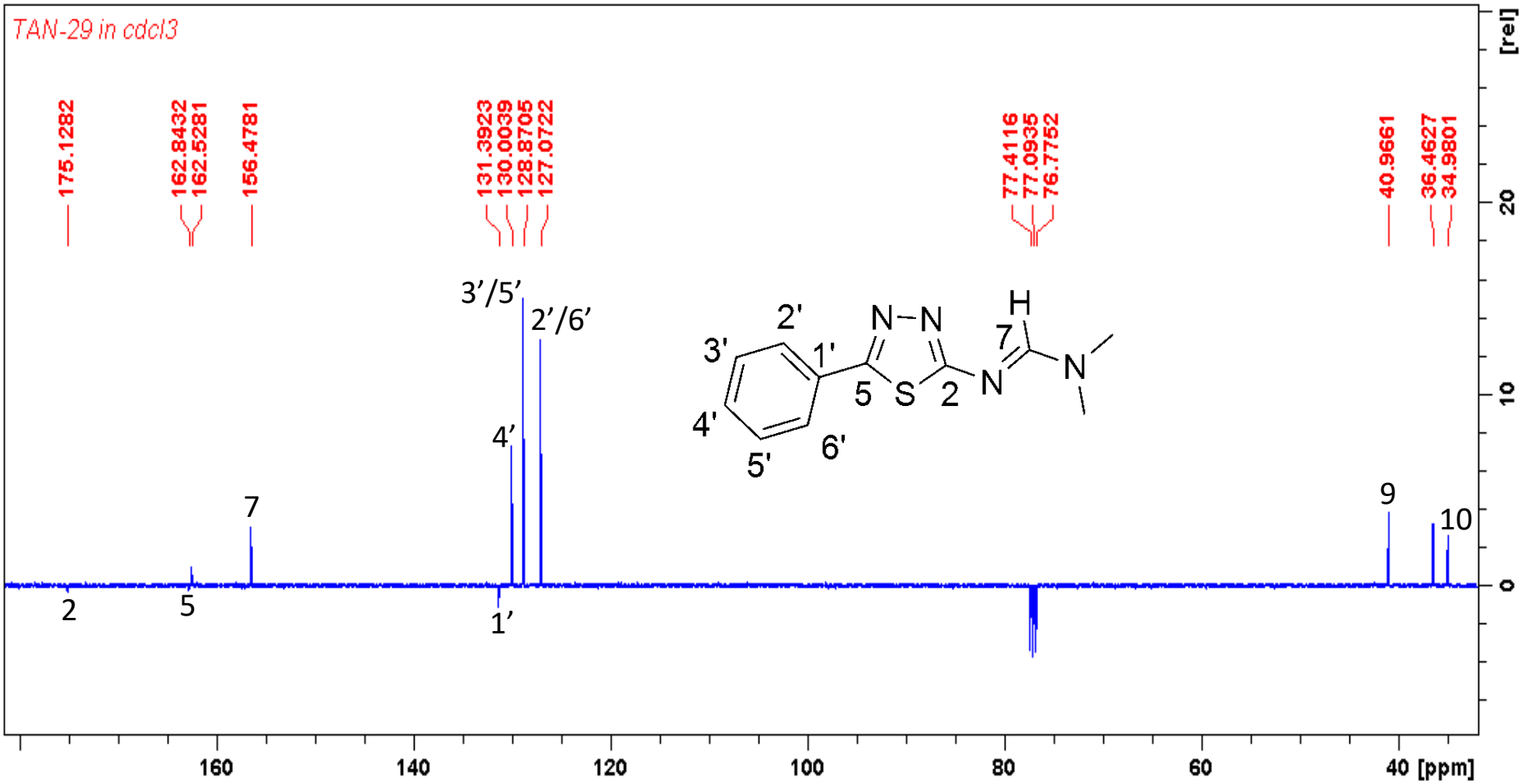
Compounds 2a-j NMR Spectra



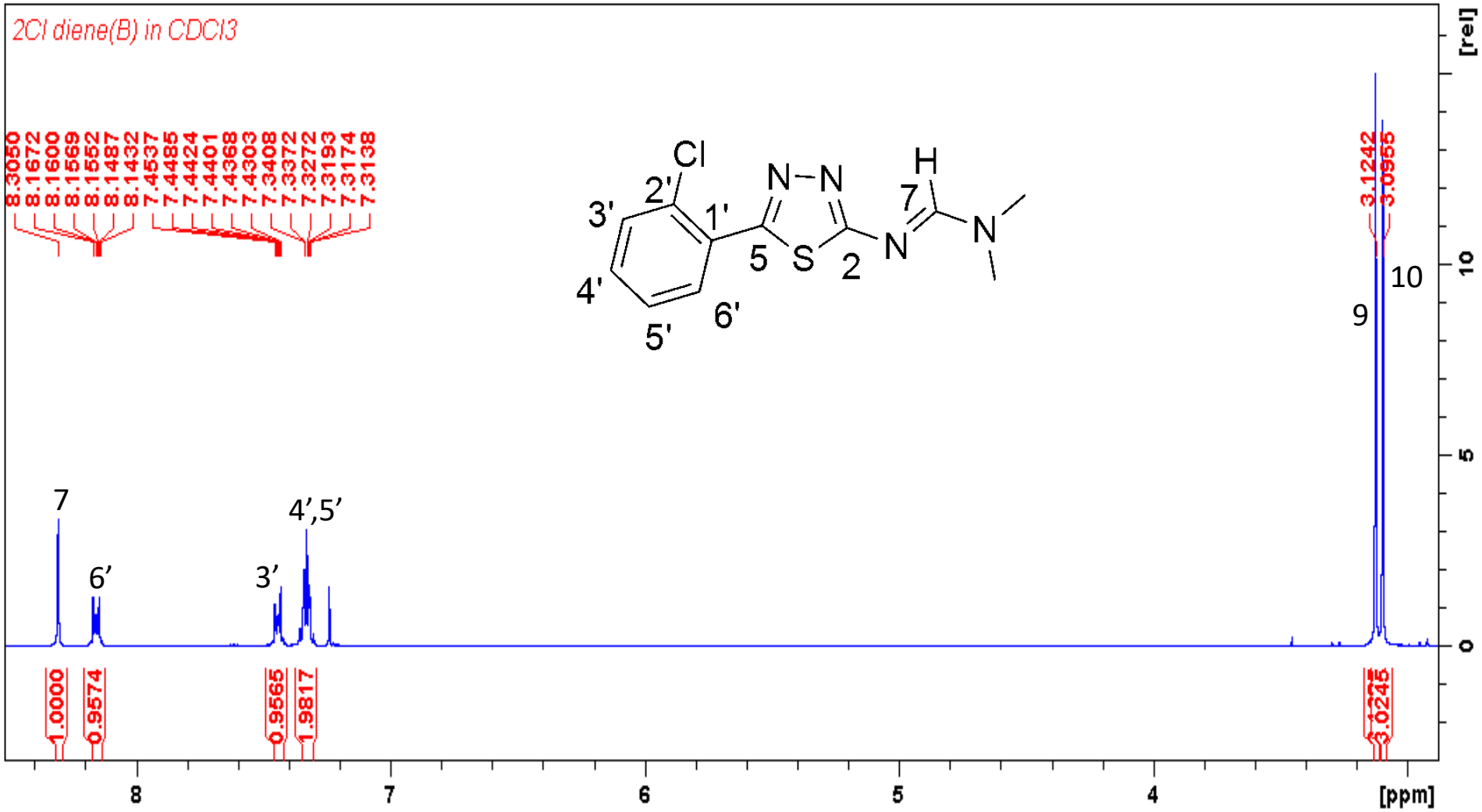
The ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-phenyl-1,3,4-thiadiazol-2-yl)imidoformamide **(2a)**



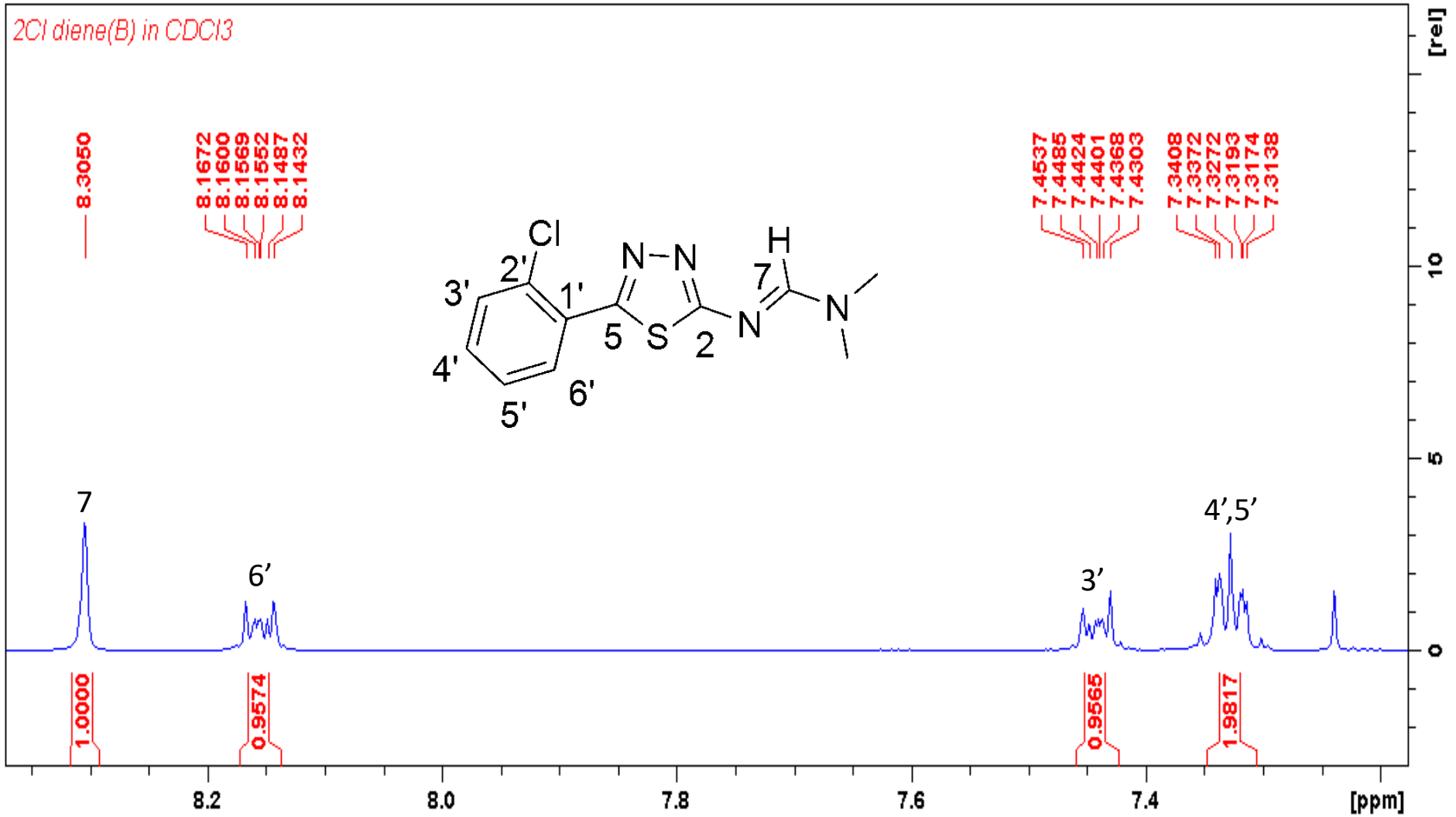
The expanded ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-phenyl-1,3,4-thiadiazol-2-yl)imidoformamide **(2a)**



The ^{13}C NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-phenyl-1,3,4-thiadiazol-2-yl)imidoformamide **(2a)**



The ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2b)**



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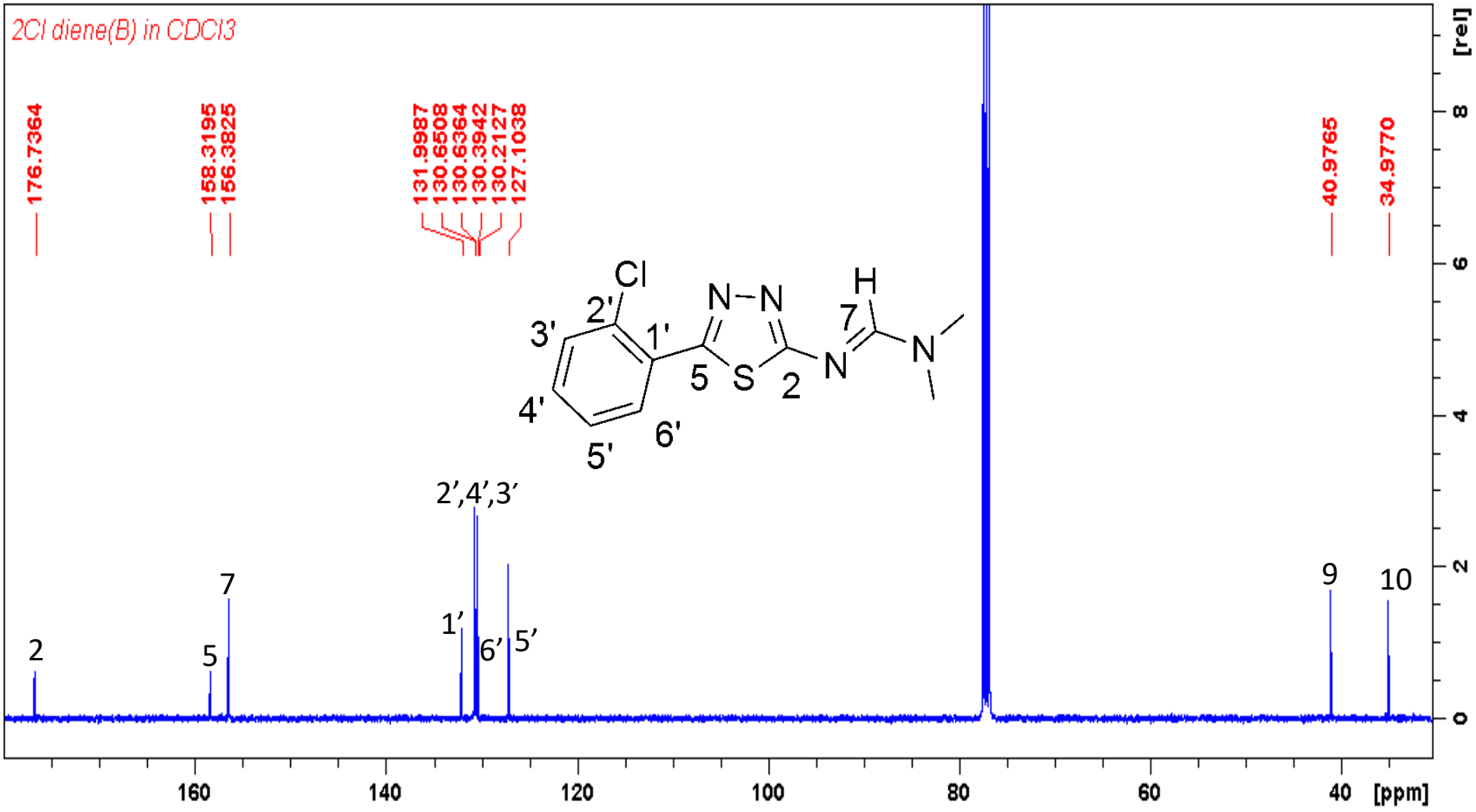
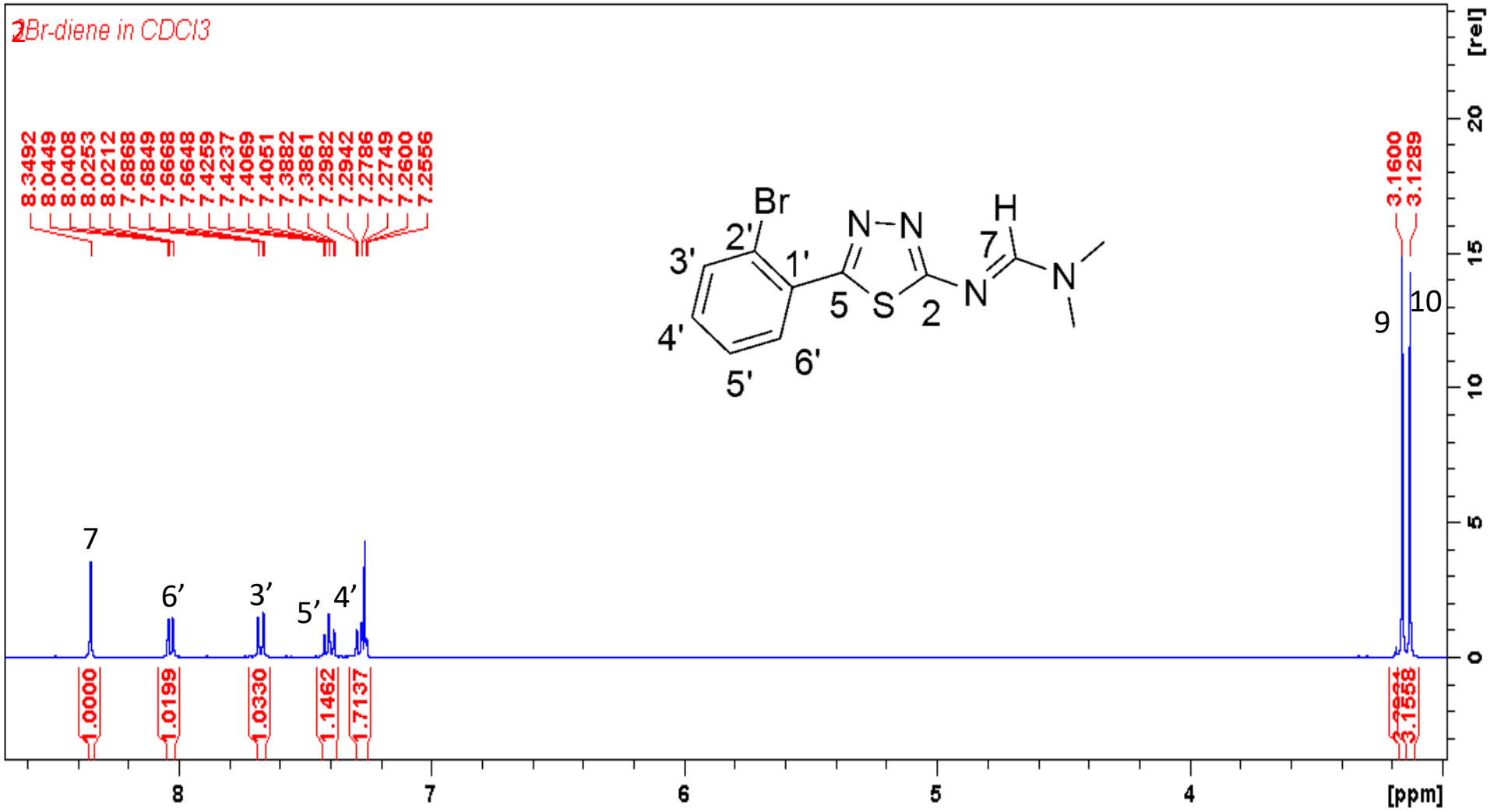


The ^{13}C NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2b)**



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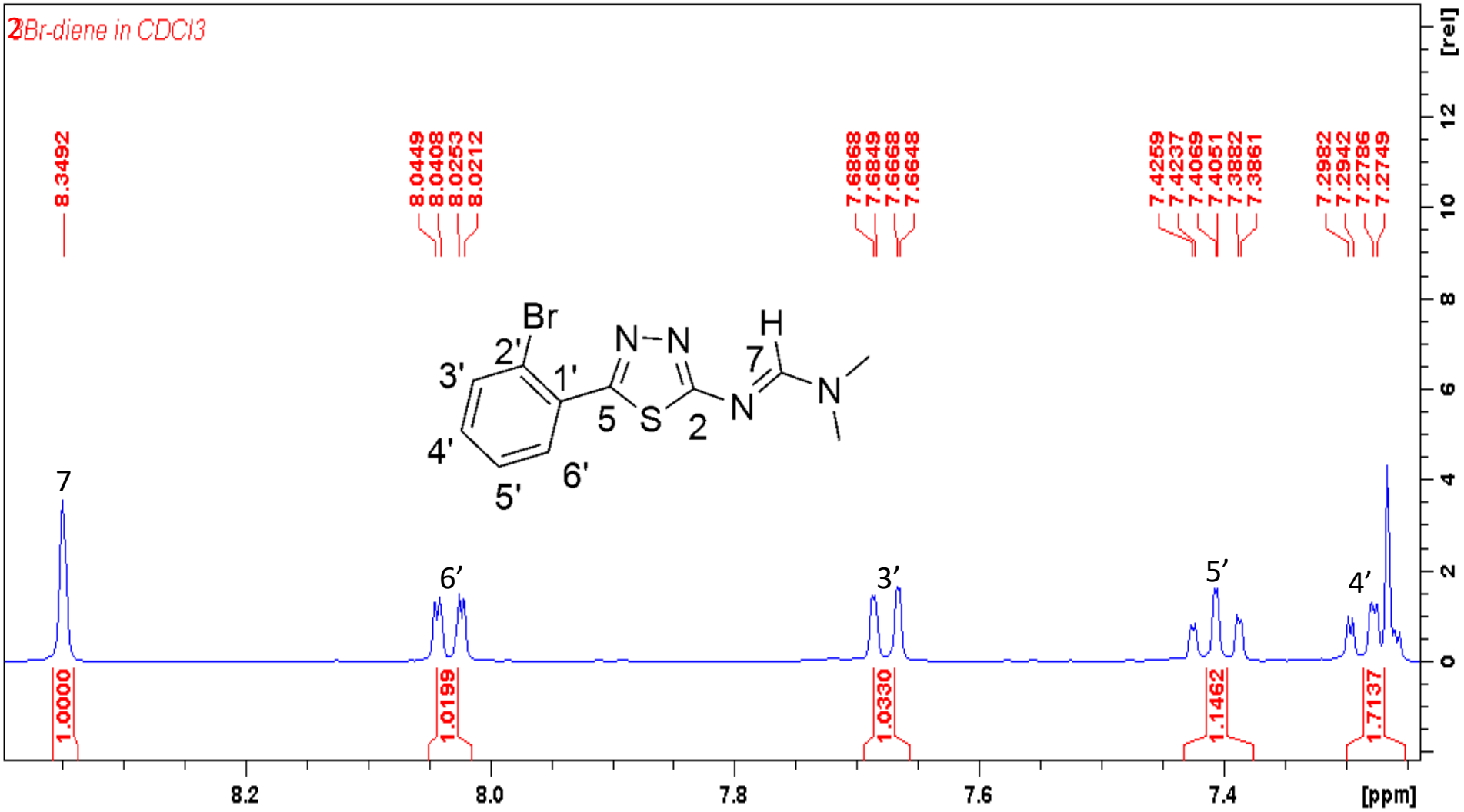
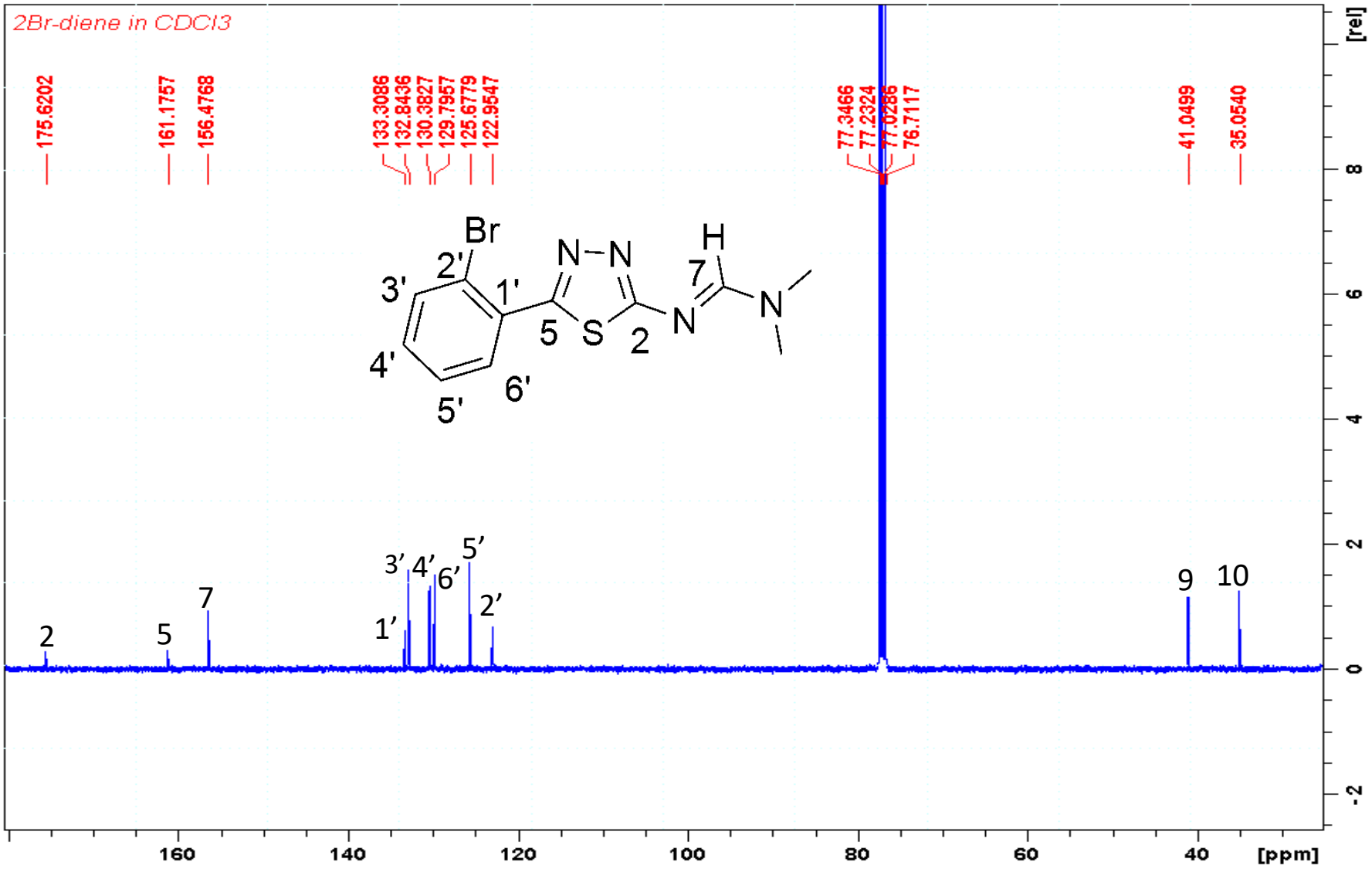
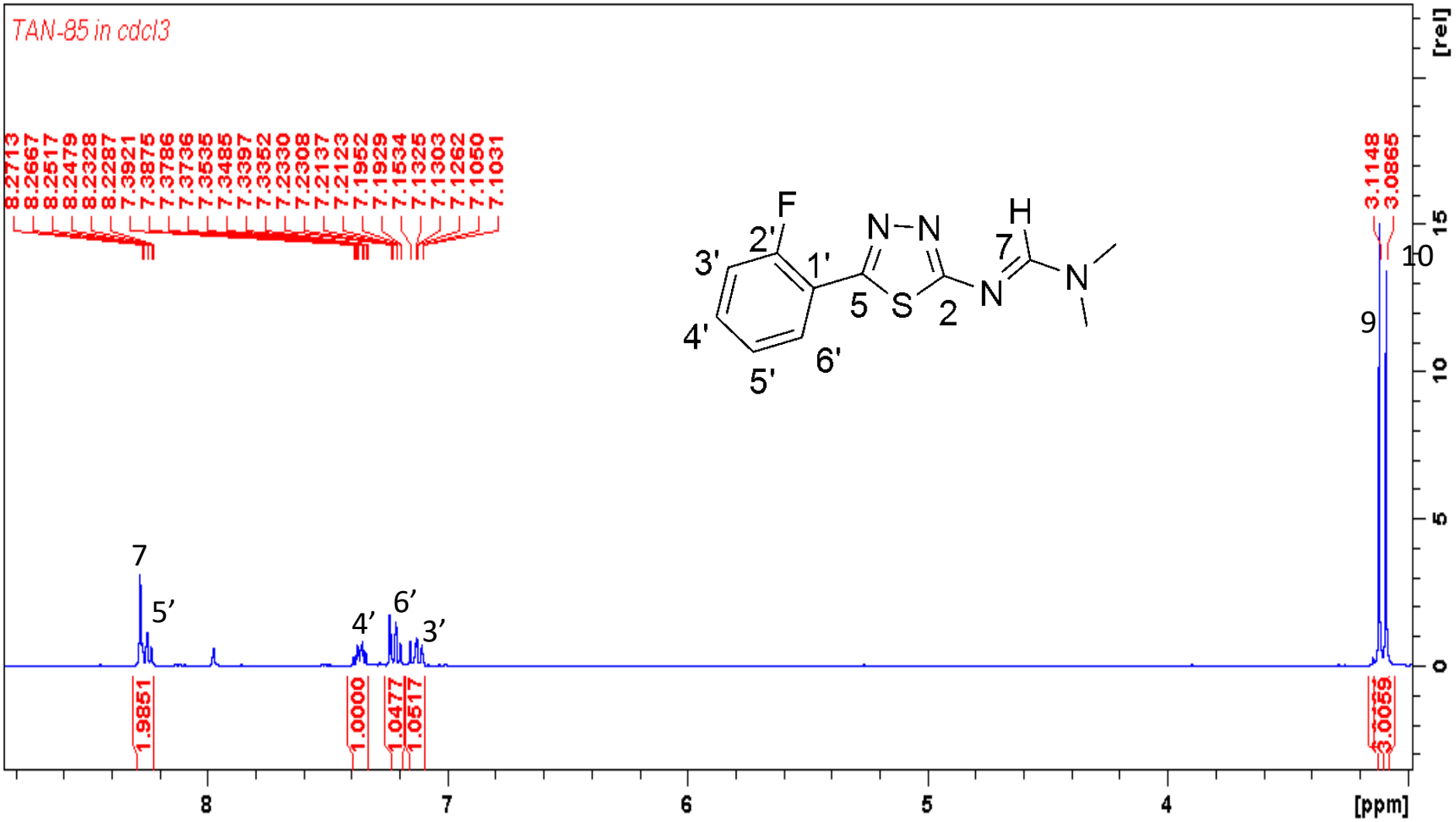


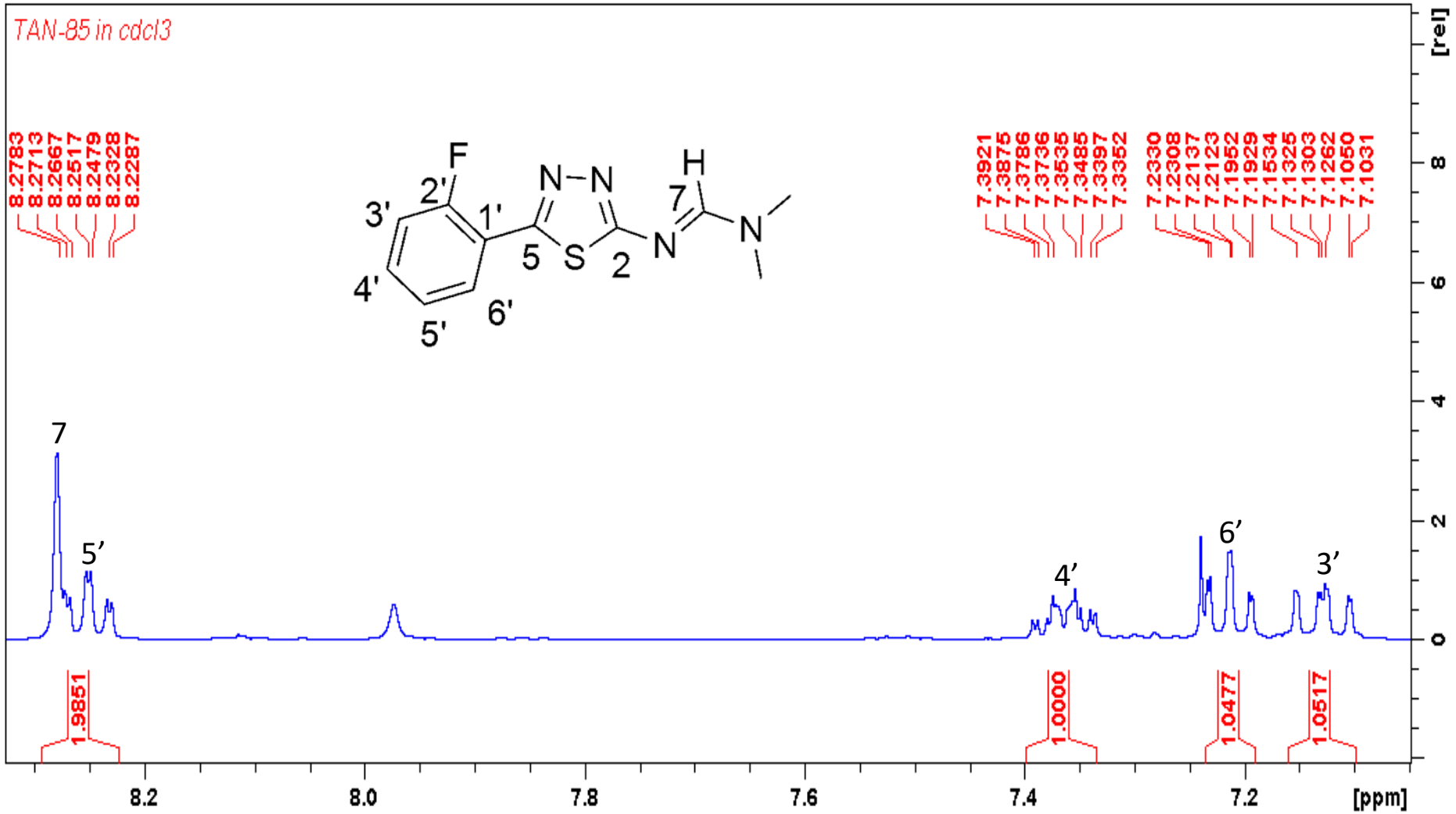
The expanded ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(2-bromophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2c)**



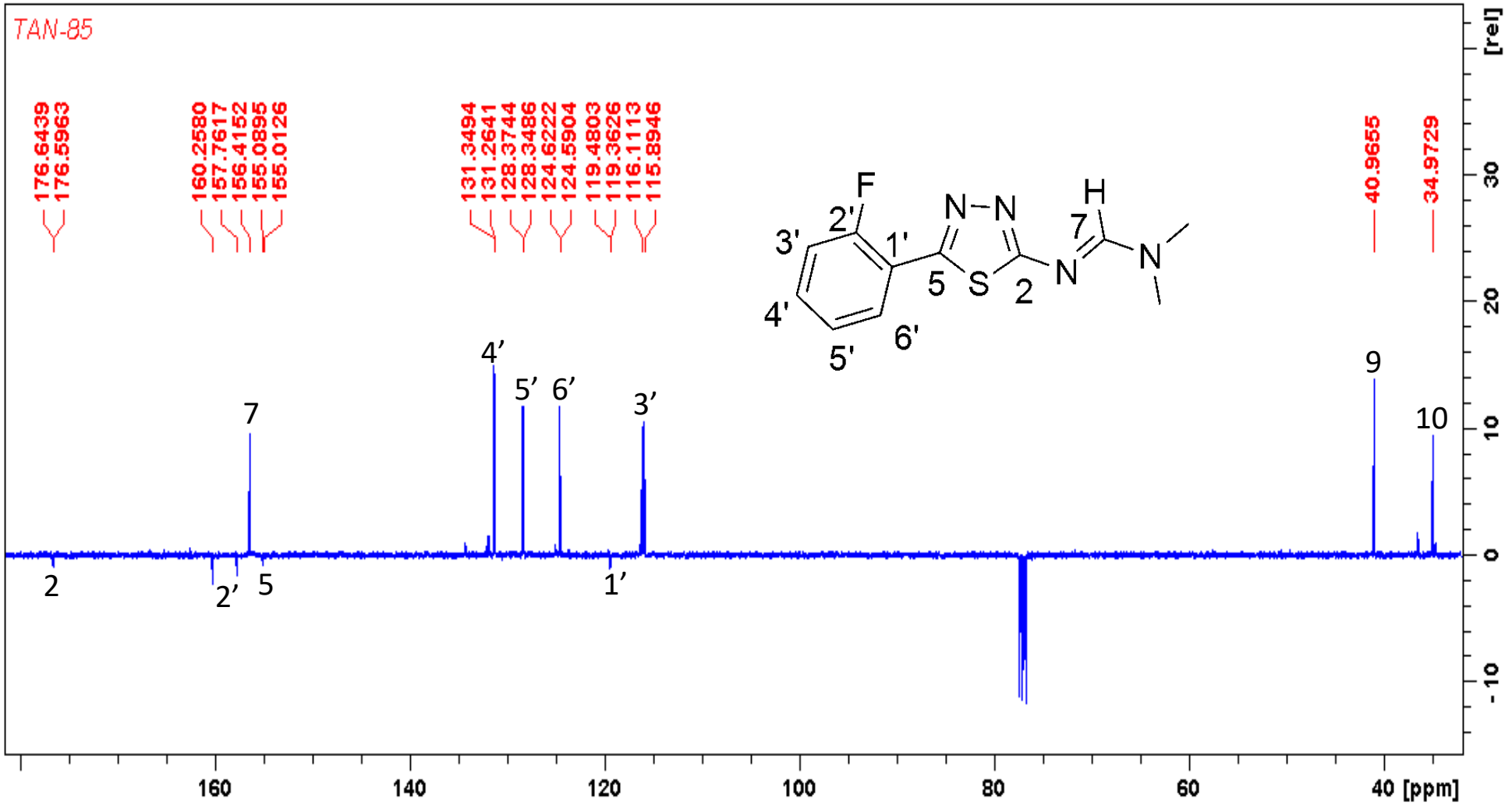
The ^{13}C NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(2-bromophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2c)**



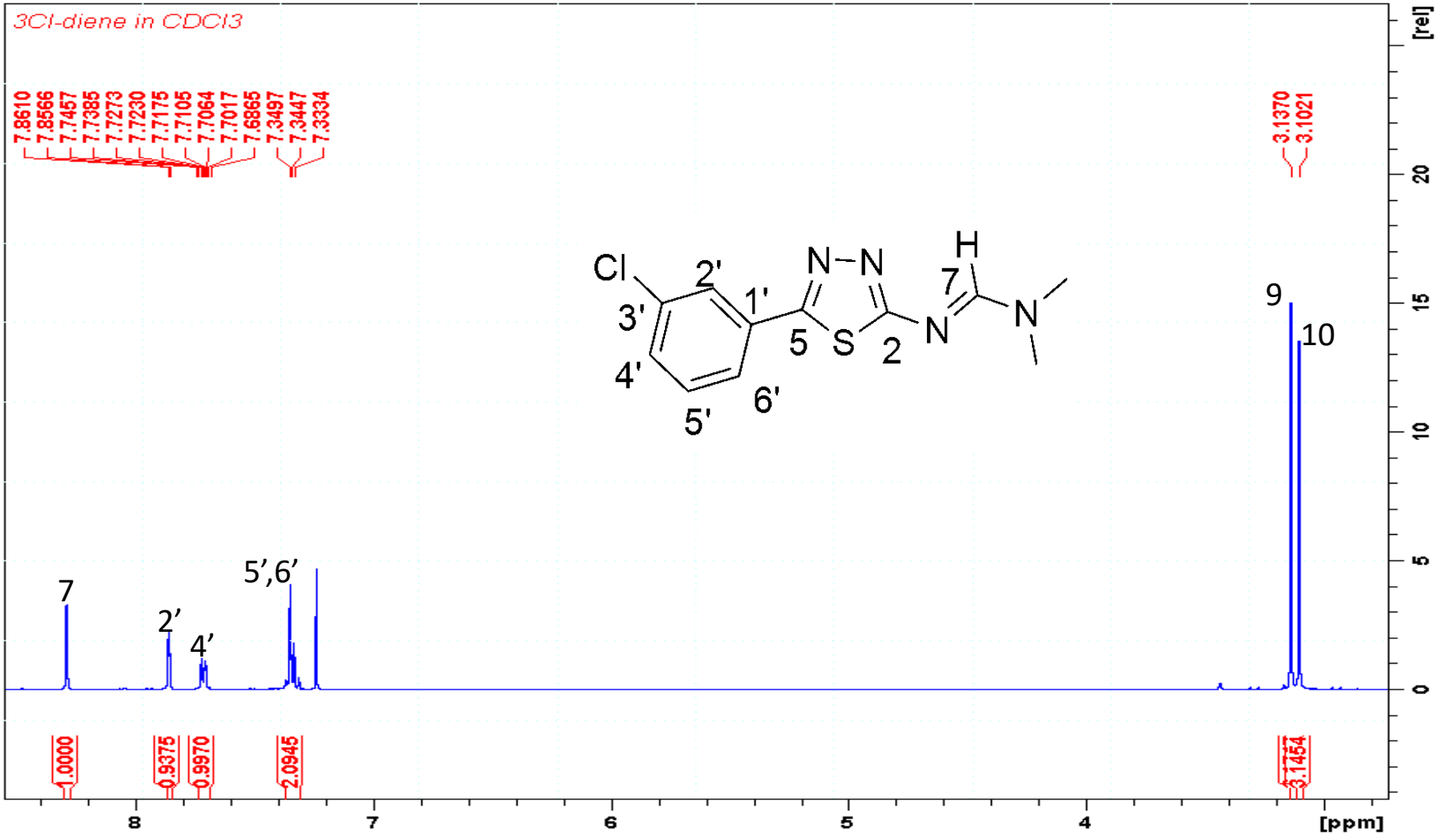
The ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2d)**



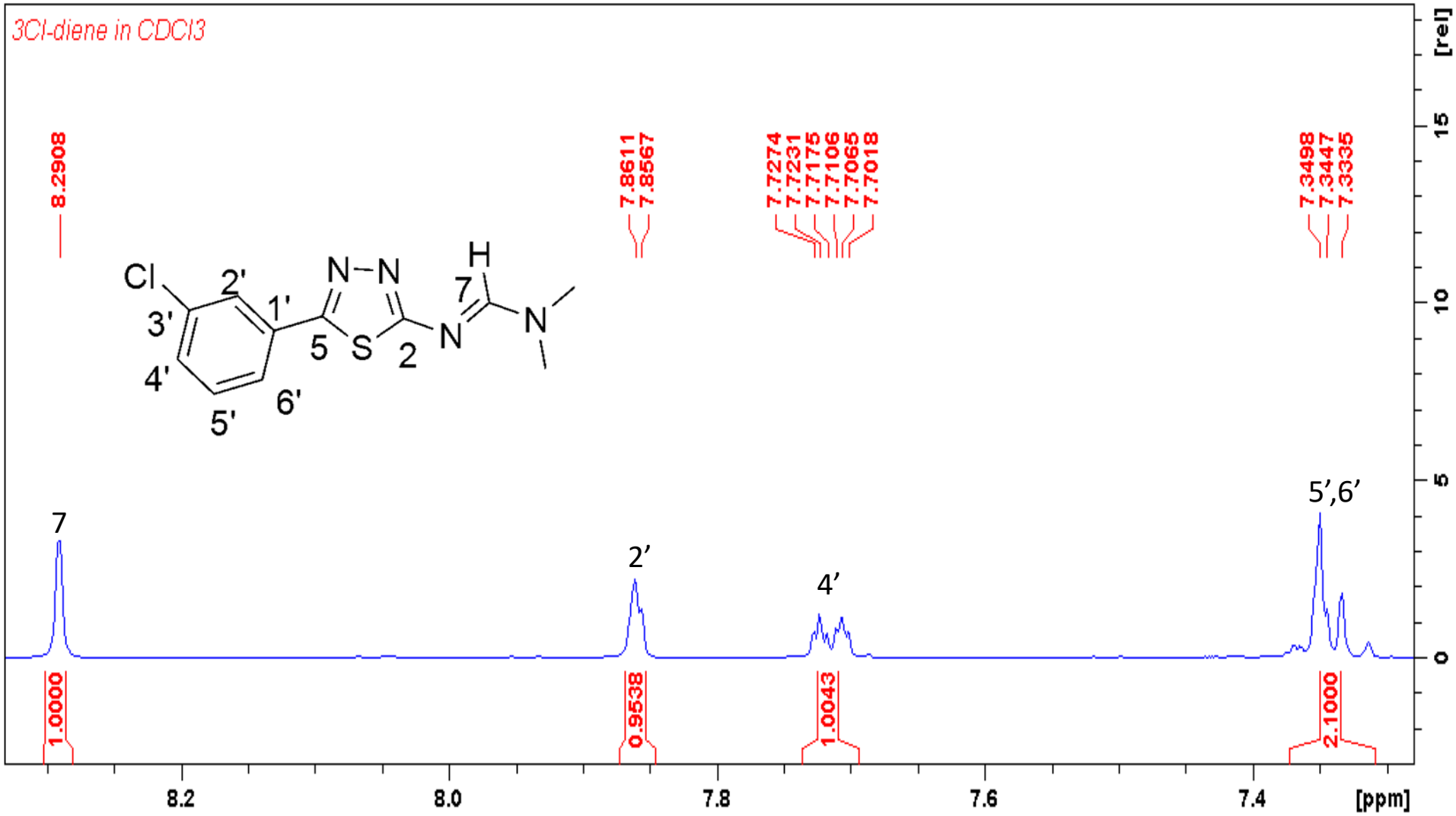
The expanded ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2d)**



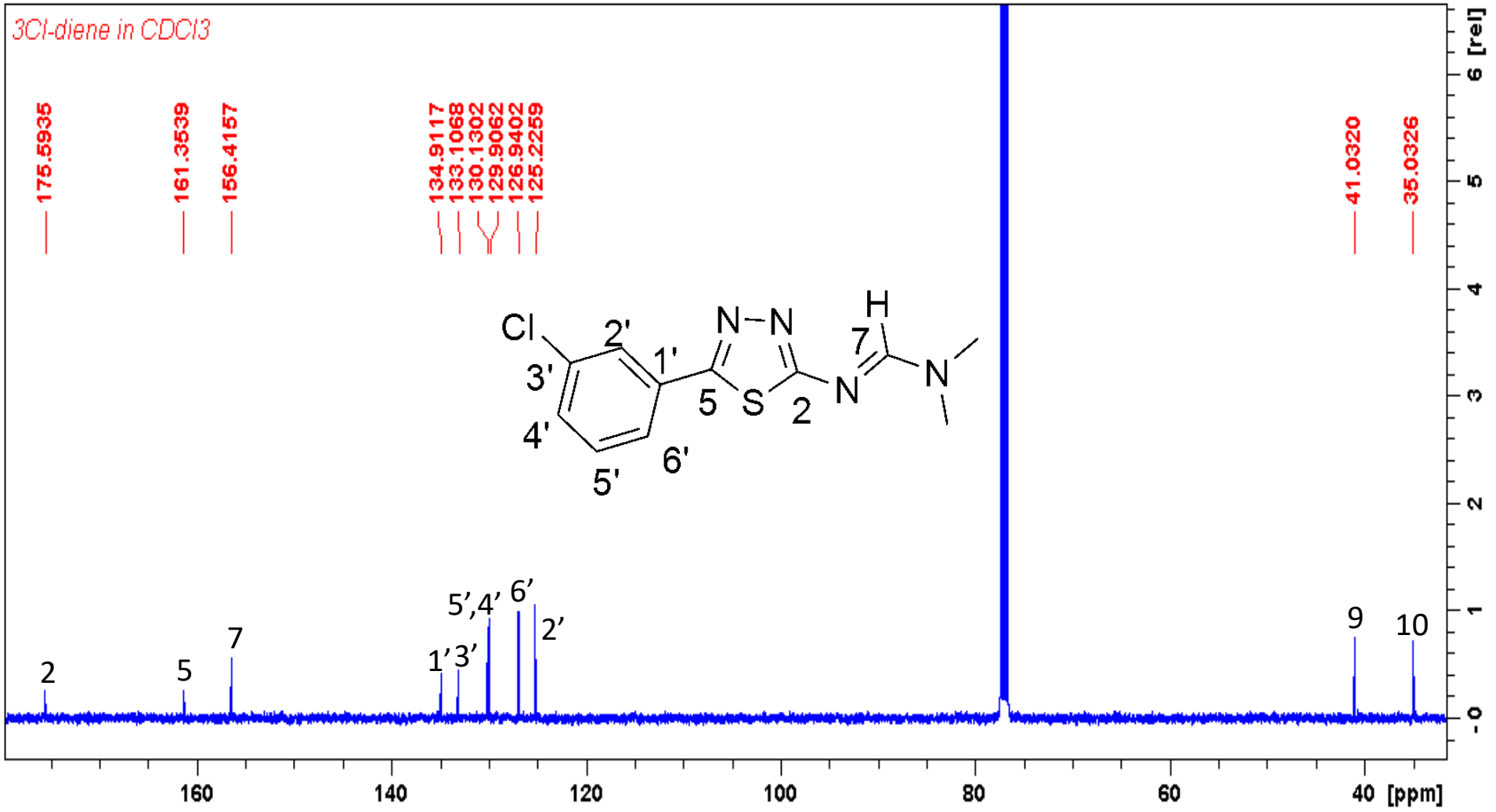
The ^{13}C NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2d)**



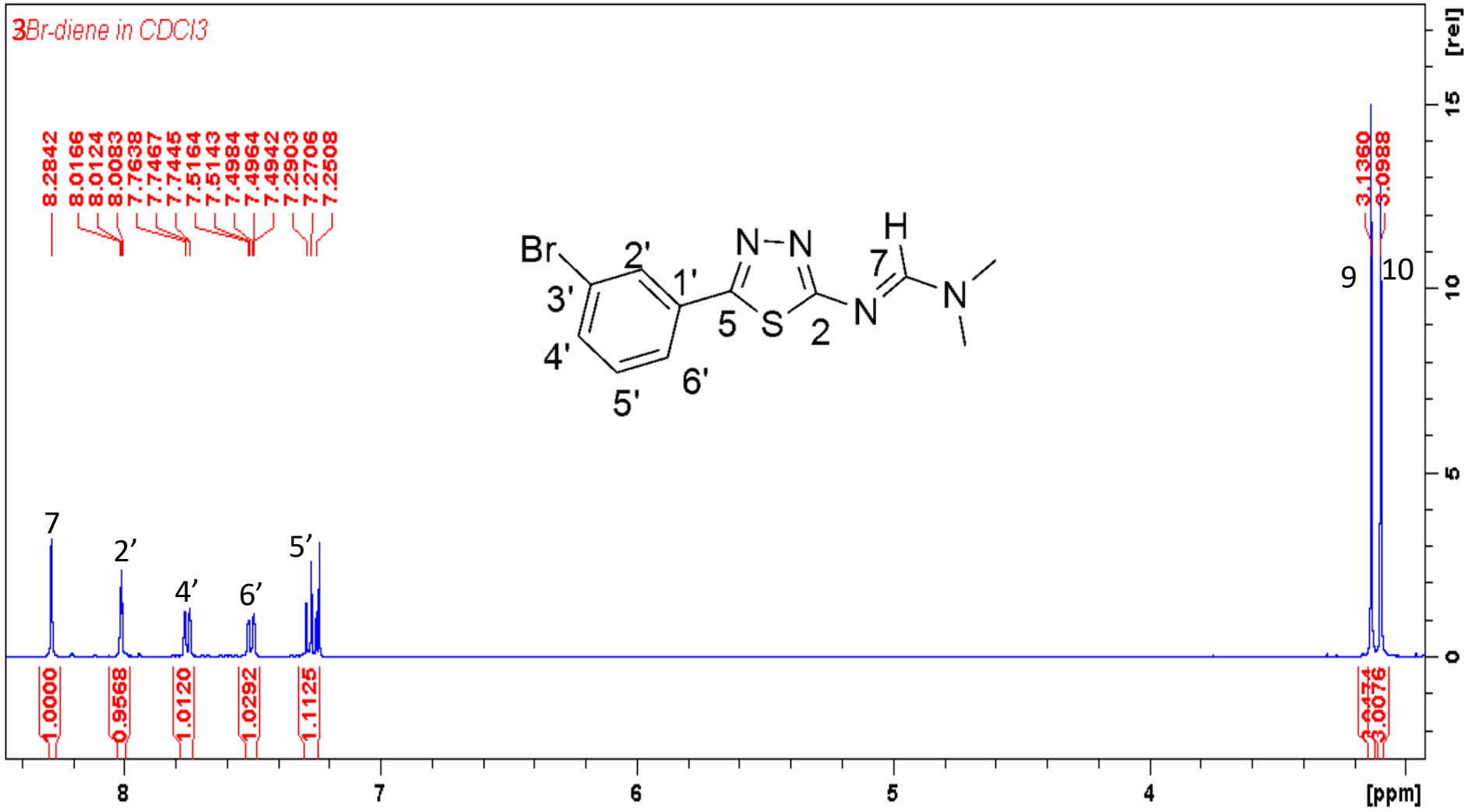
The ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2e)**



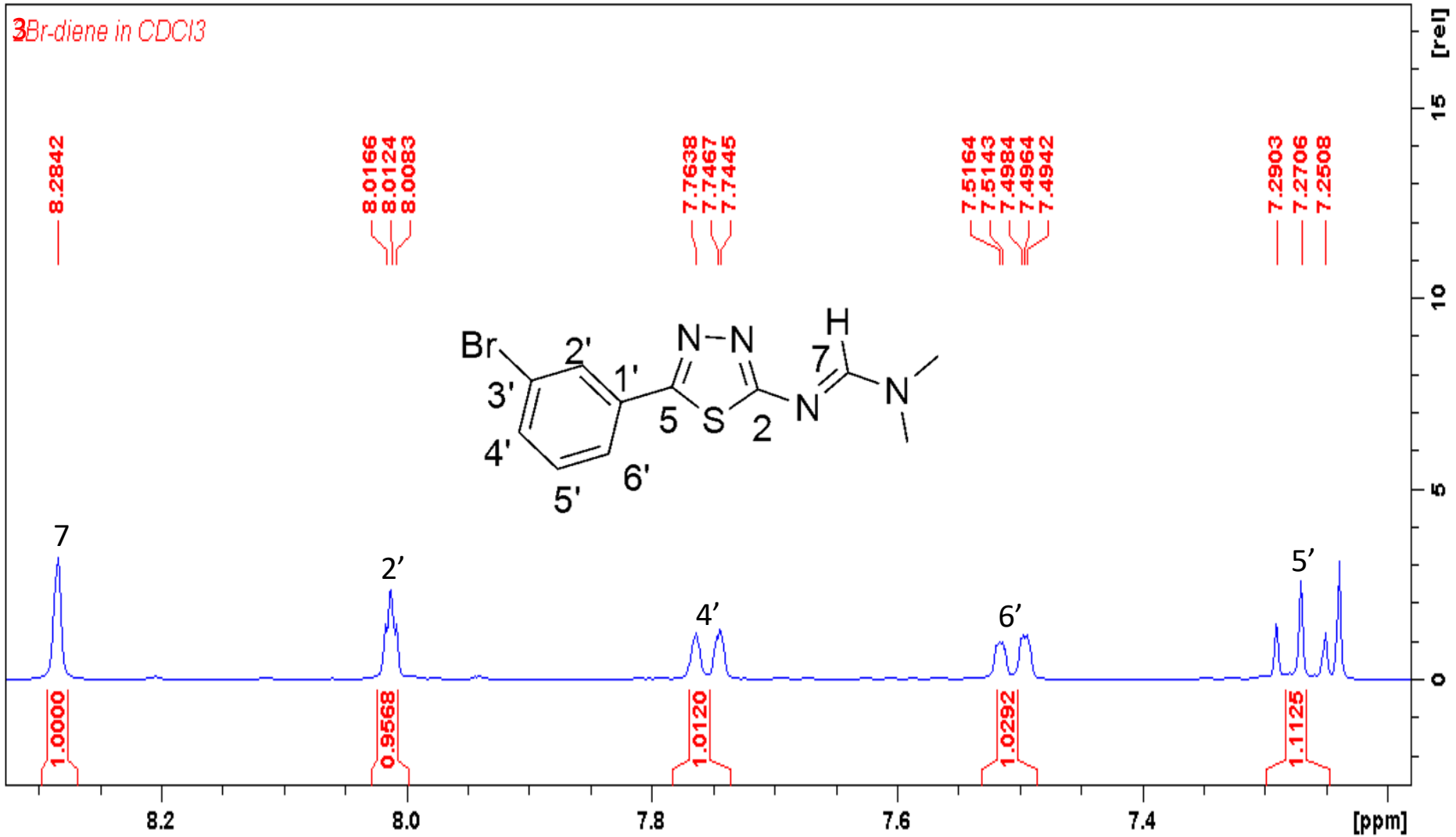
The expanded ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2e)**



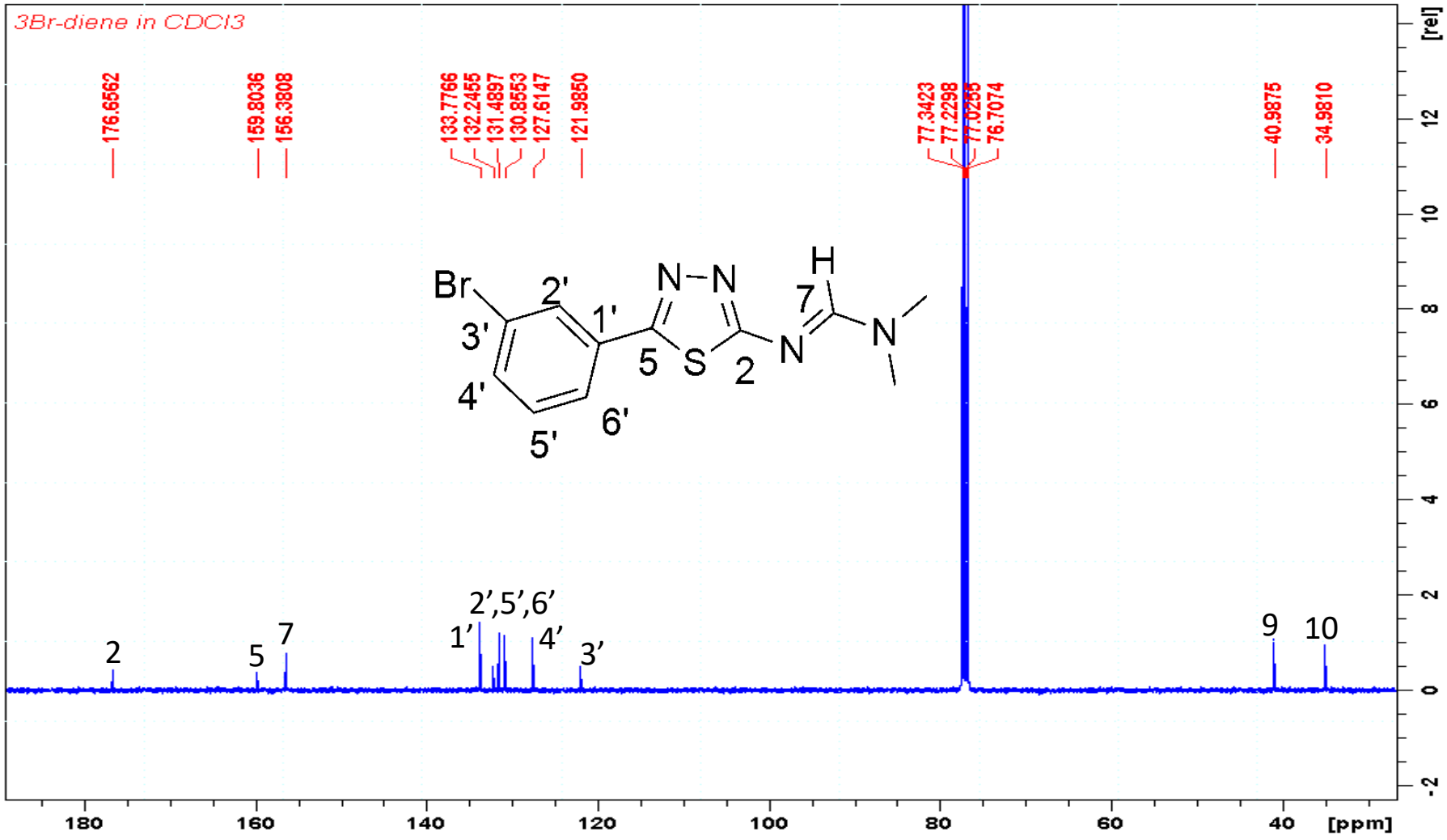
The ^{13}C NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2e)**



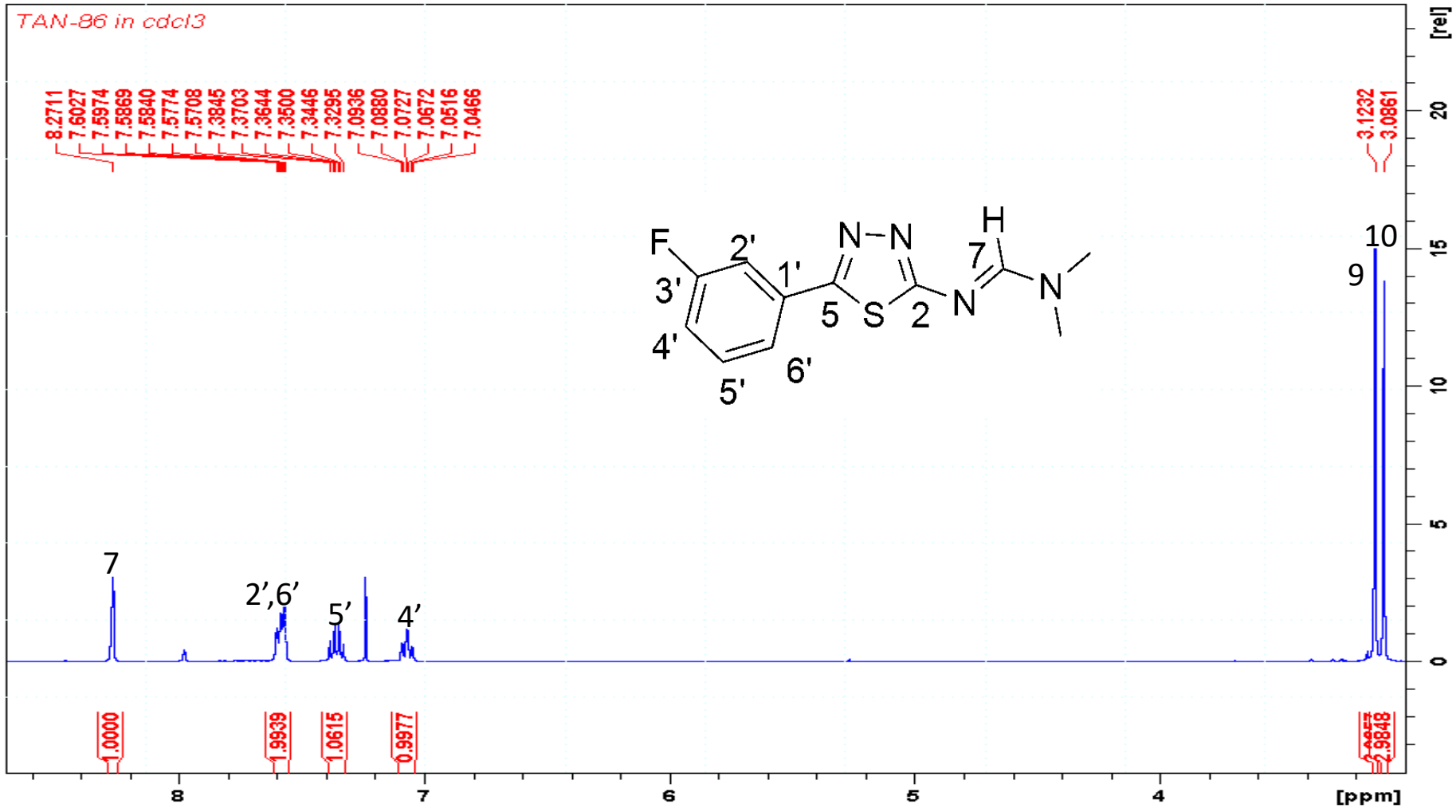
The ^{1}H NMR spectrum of N,N-dimethyl-N'-(5-(3-bromophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2f)**



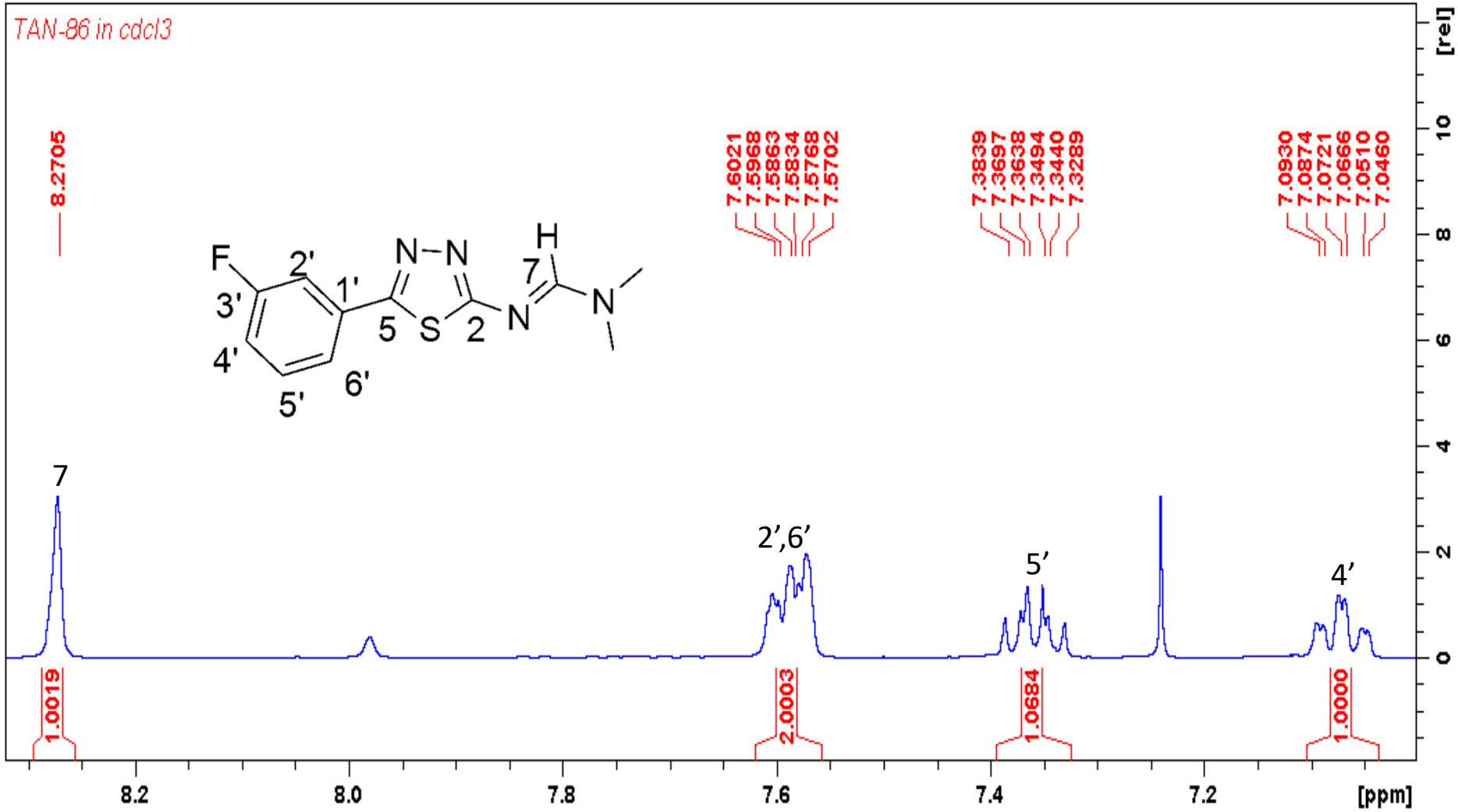
The expanded ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(3-bromophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2f)**



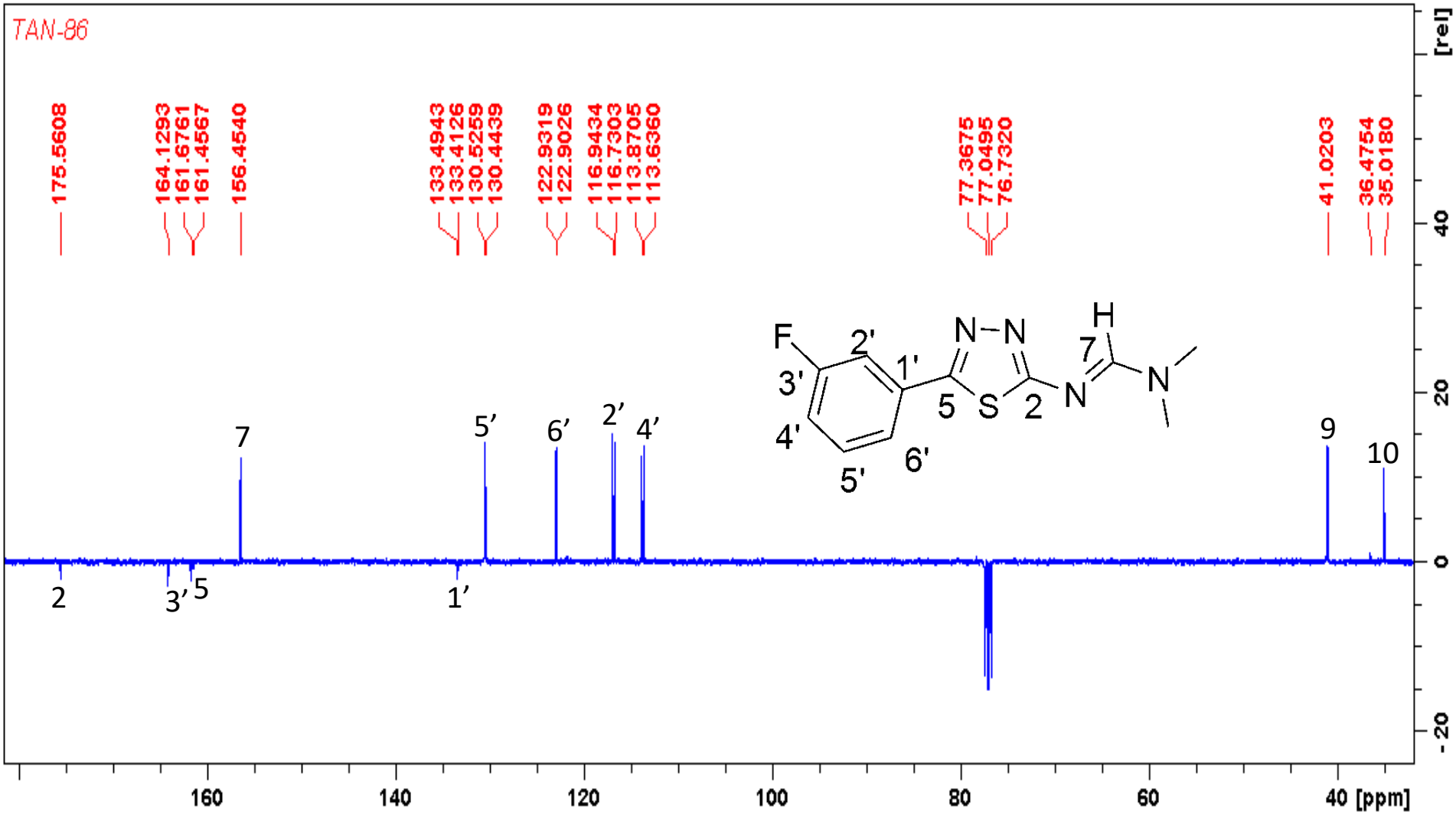
The ^{13}C NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2f)**



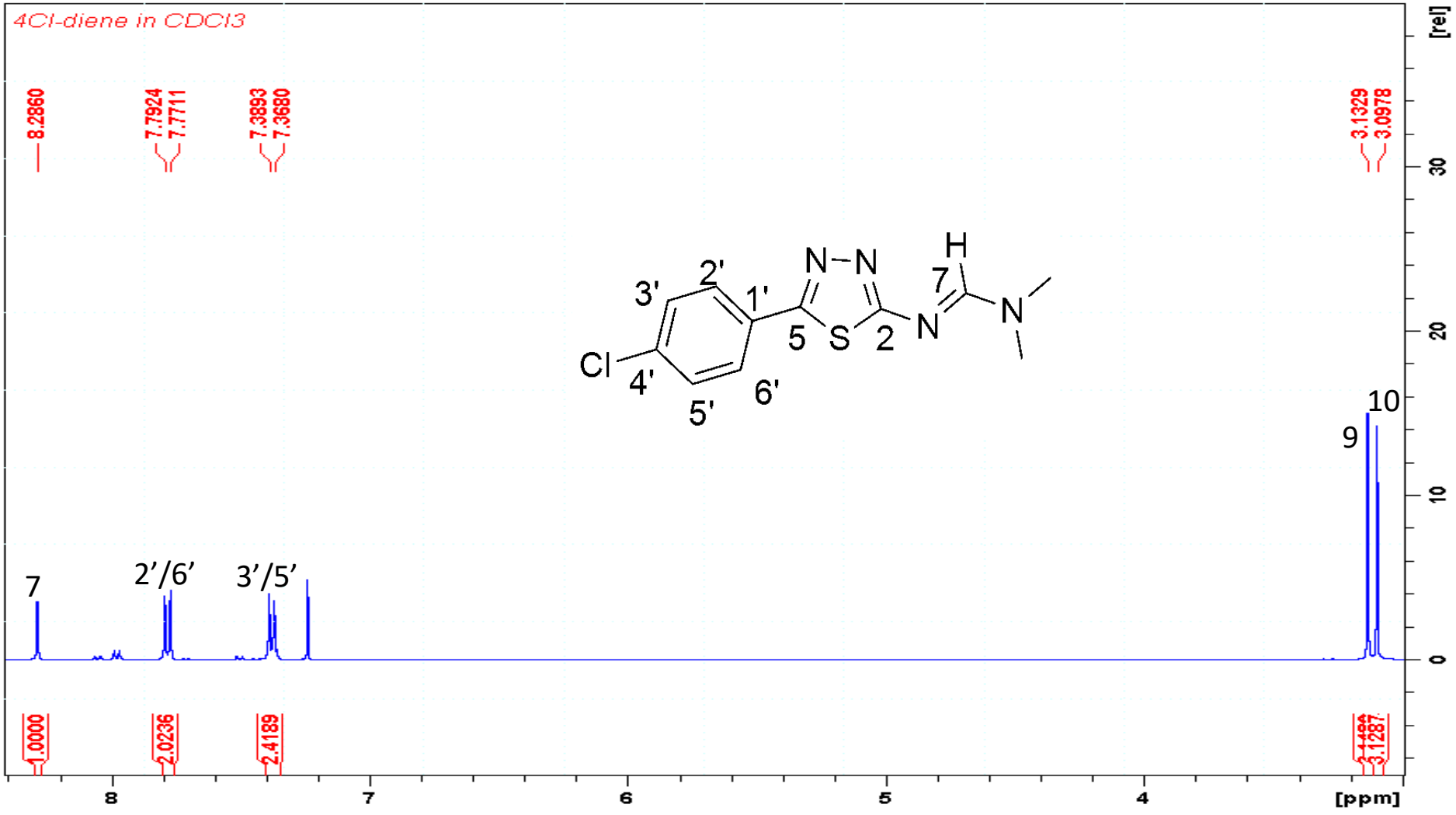
The ^{1}H NMR spectrum of N,N-dimethyl-N'-(5-(3-fluorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2g)**



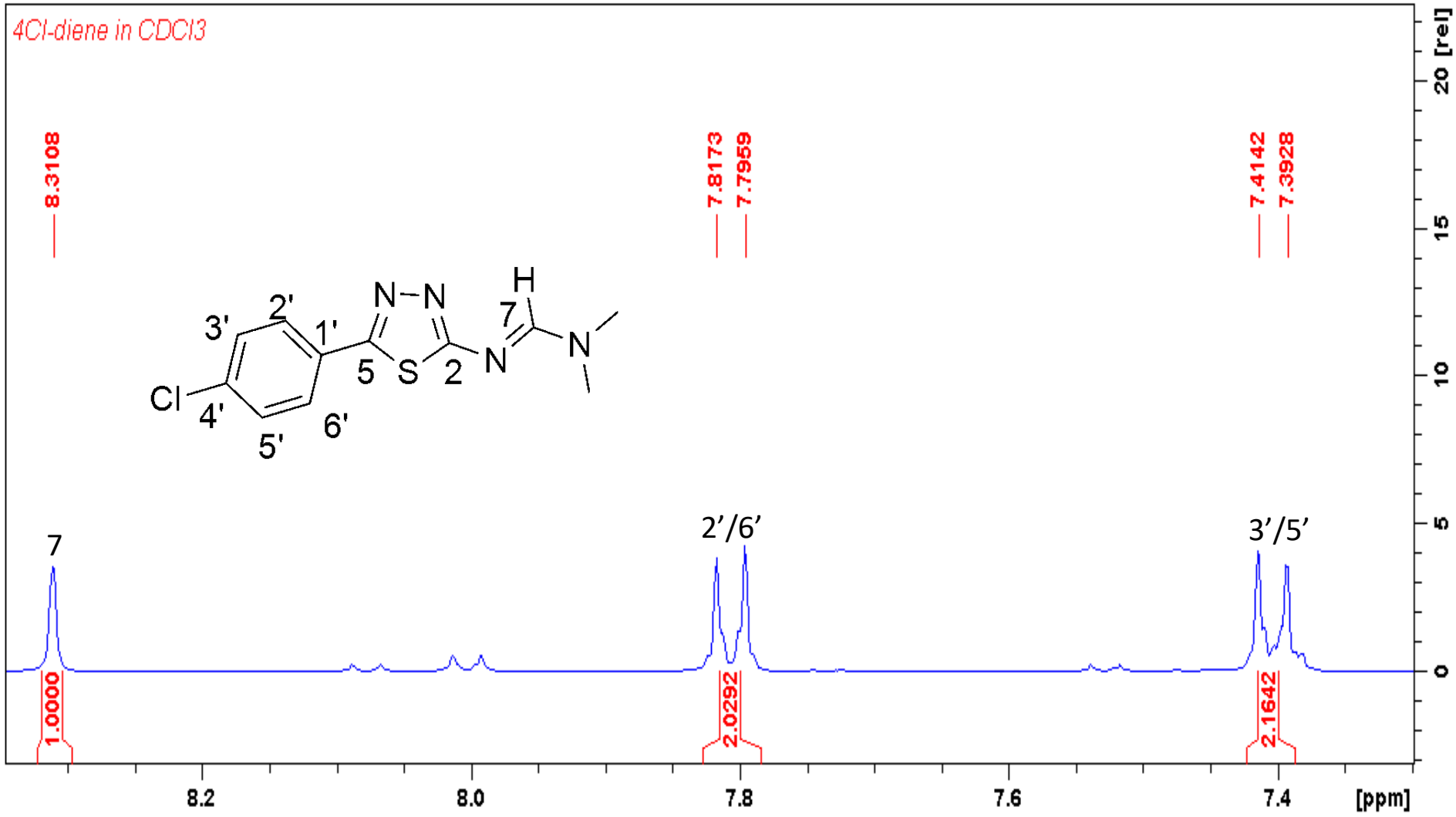
The expanded ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(3-fluorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2g)**



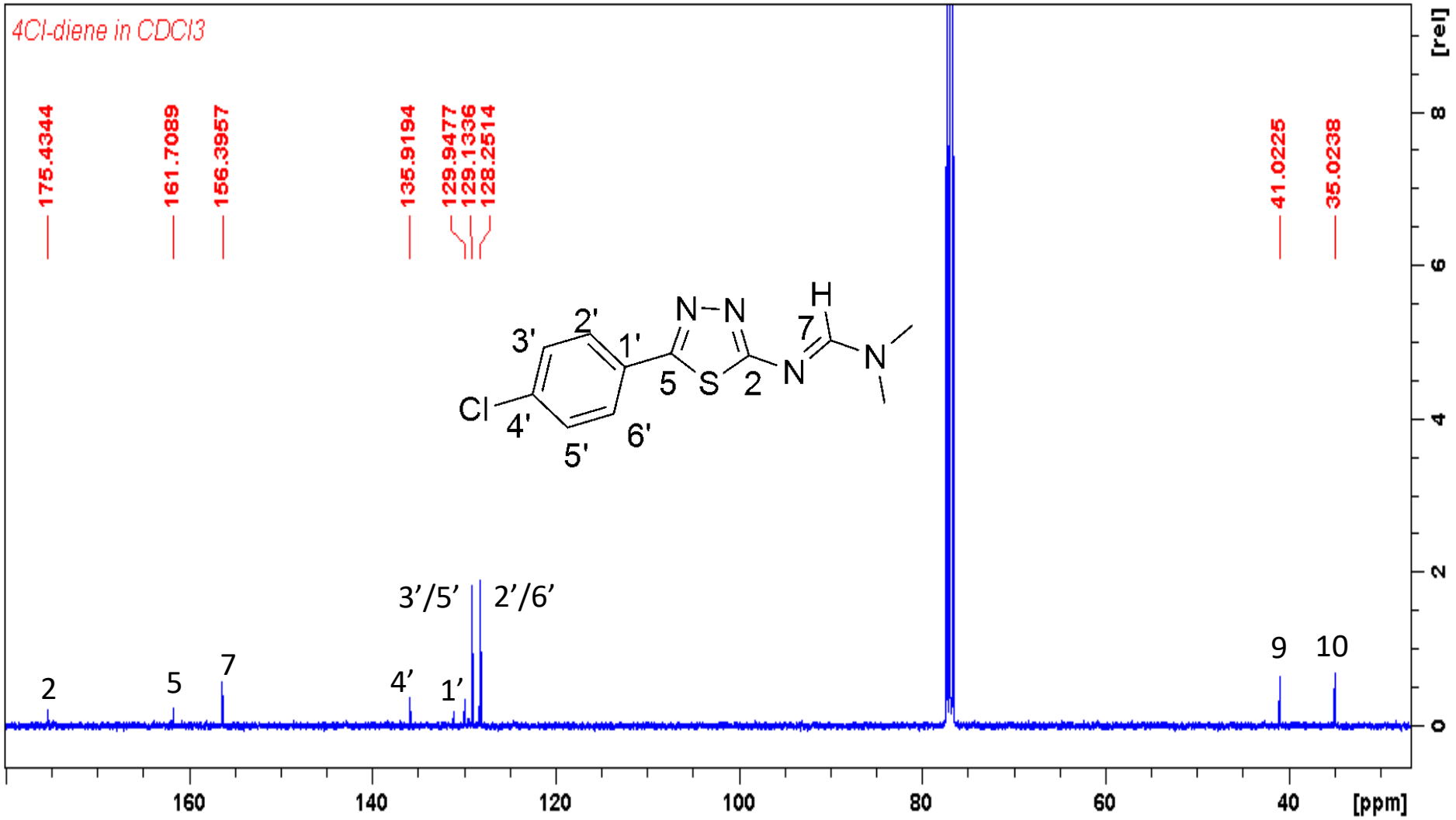
The ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(3-fluorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2g)**



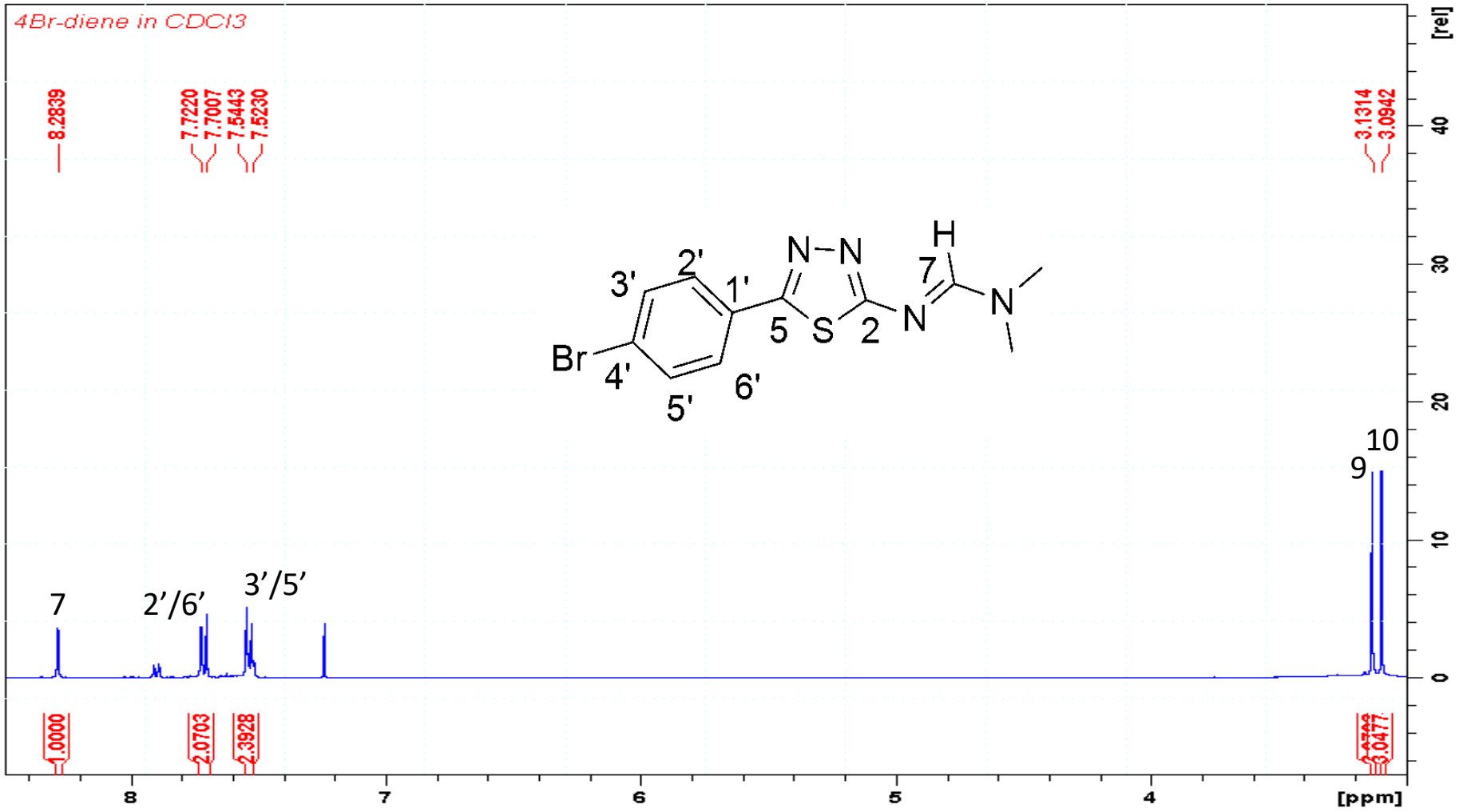
The ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2h)**



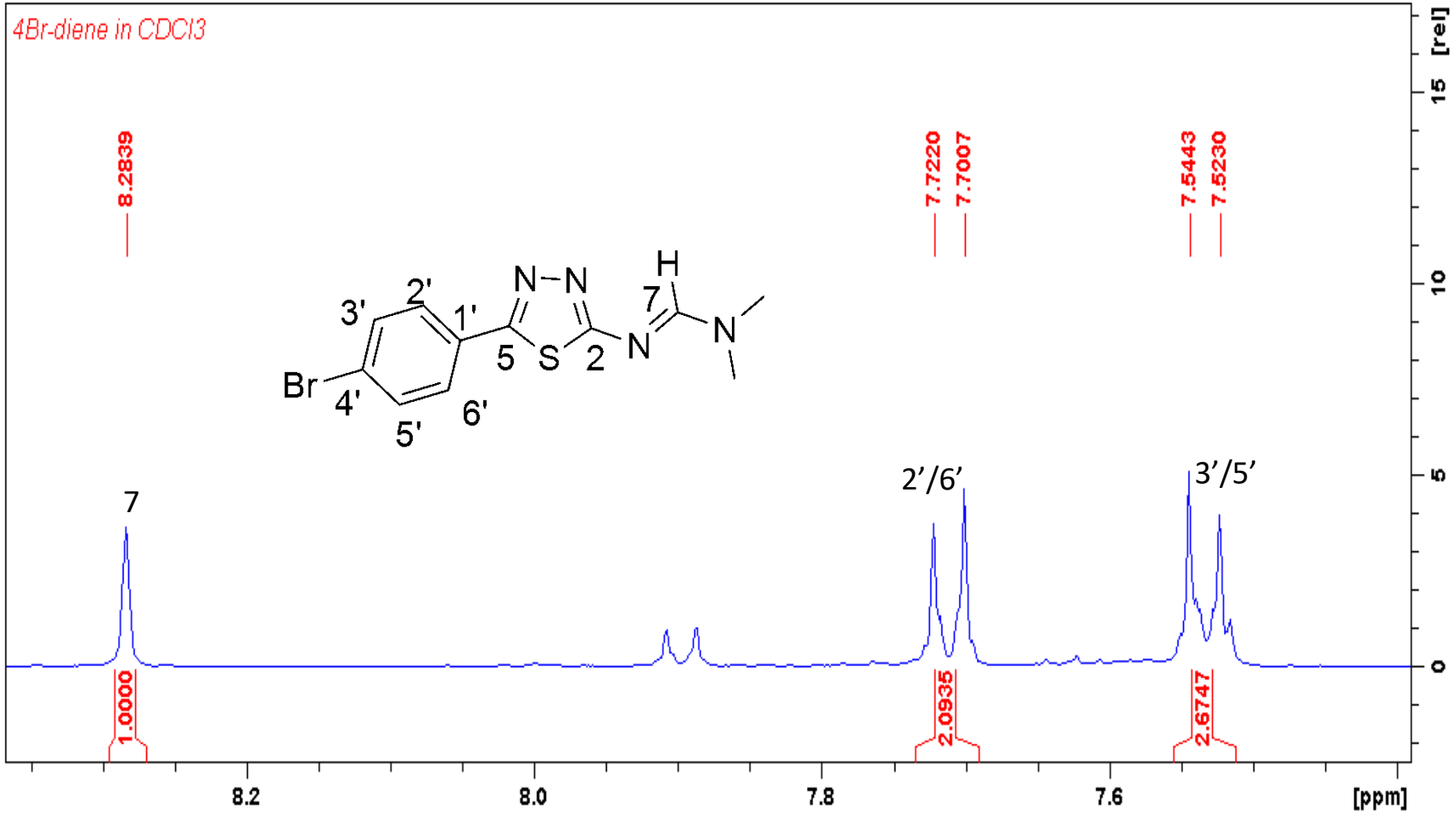
The expanded ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2h)**



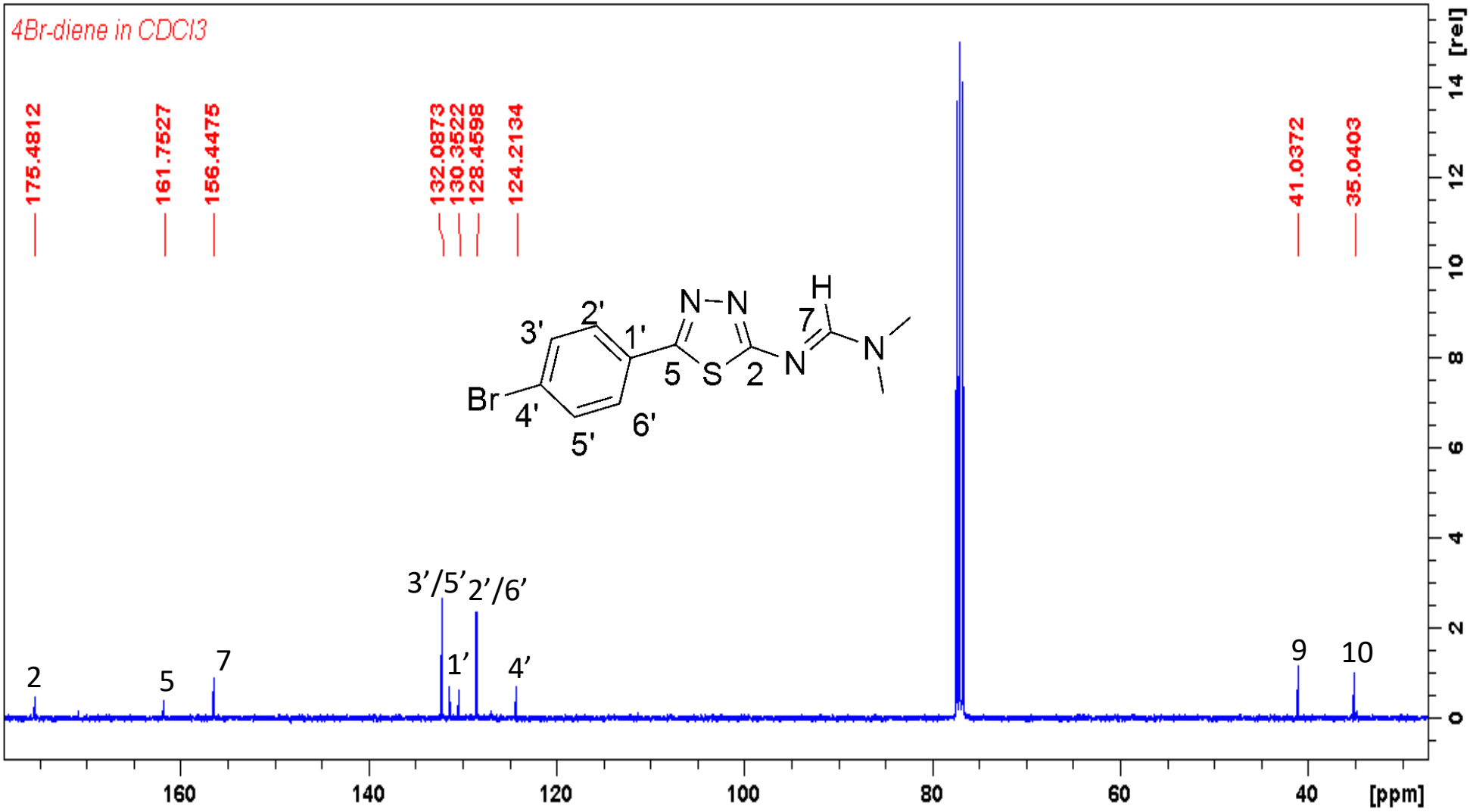
The ^{13}C NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2h)**



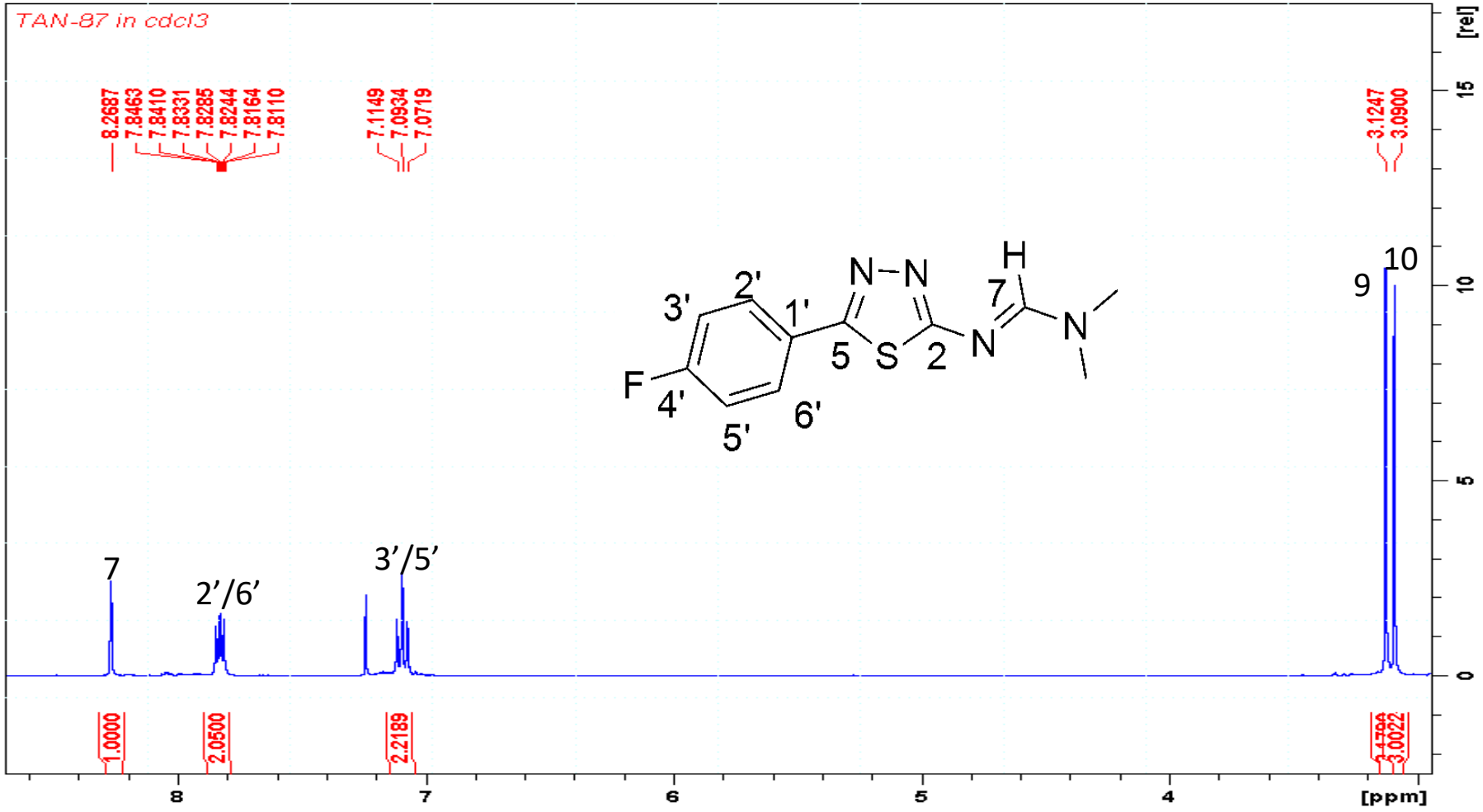
The ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(4-brorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2i)**



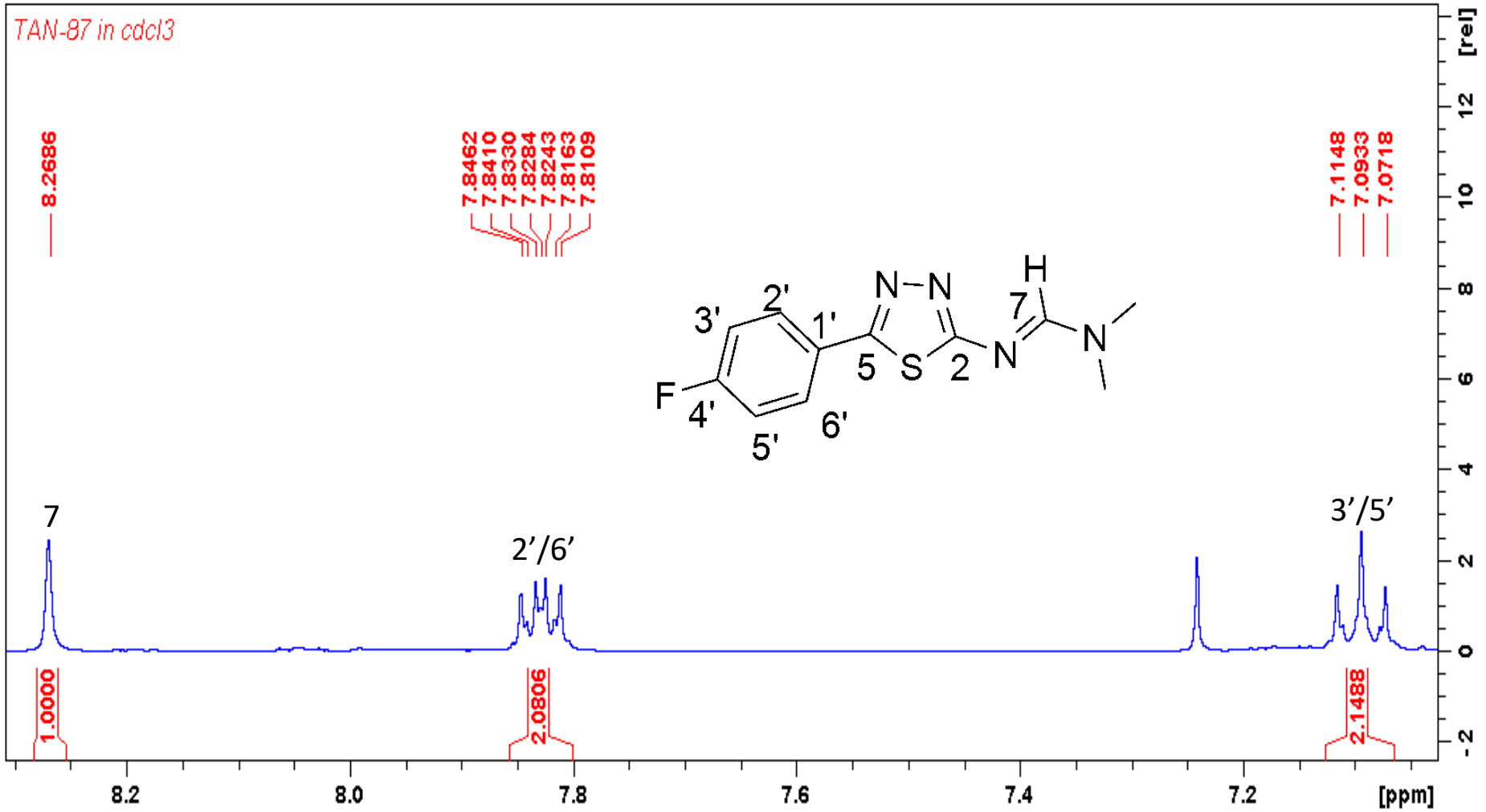
The expanded ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2i)**



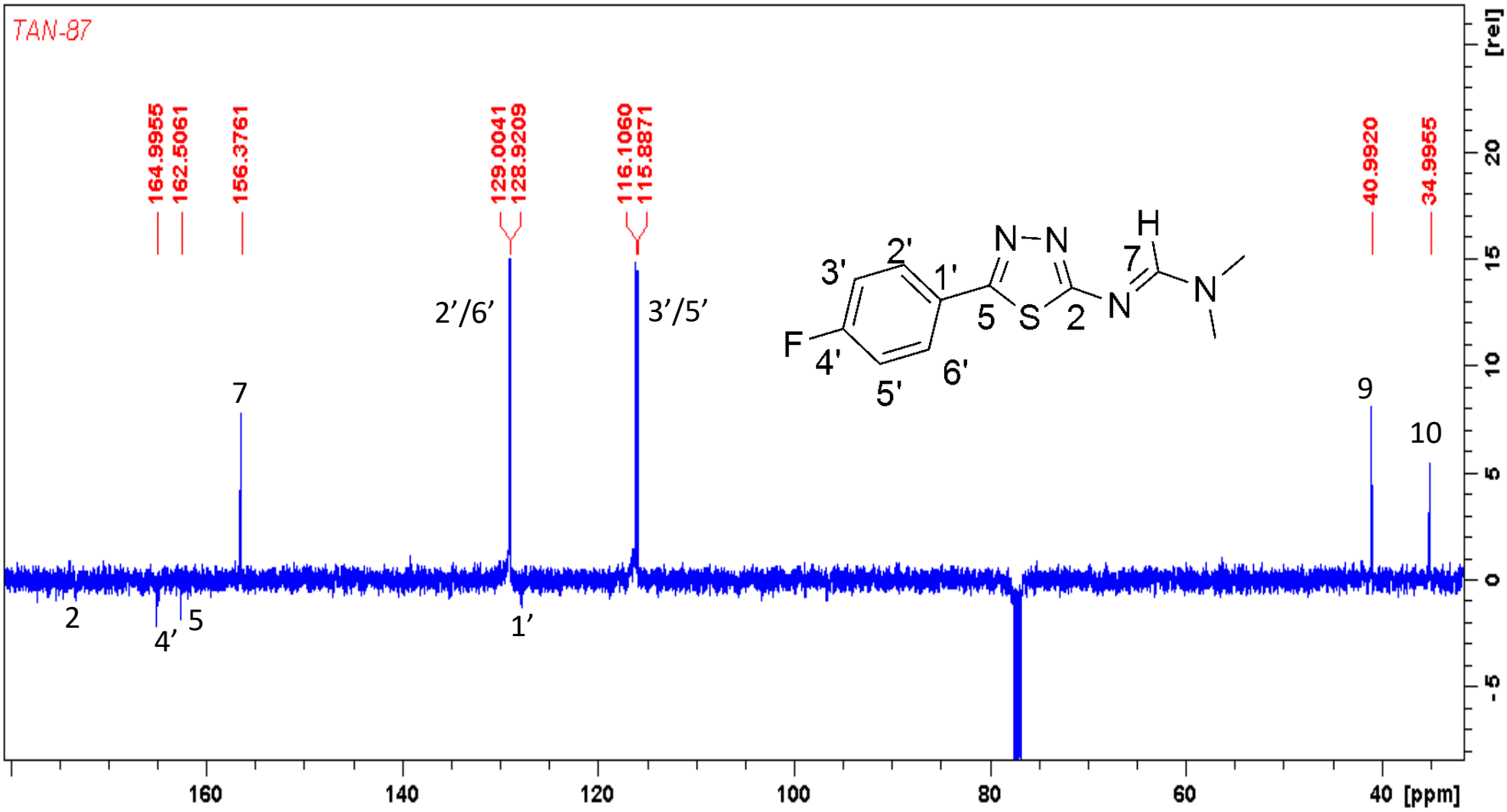
The ^{13}C NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2i)**



The ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2j)**

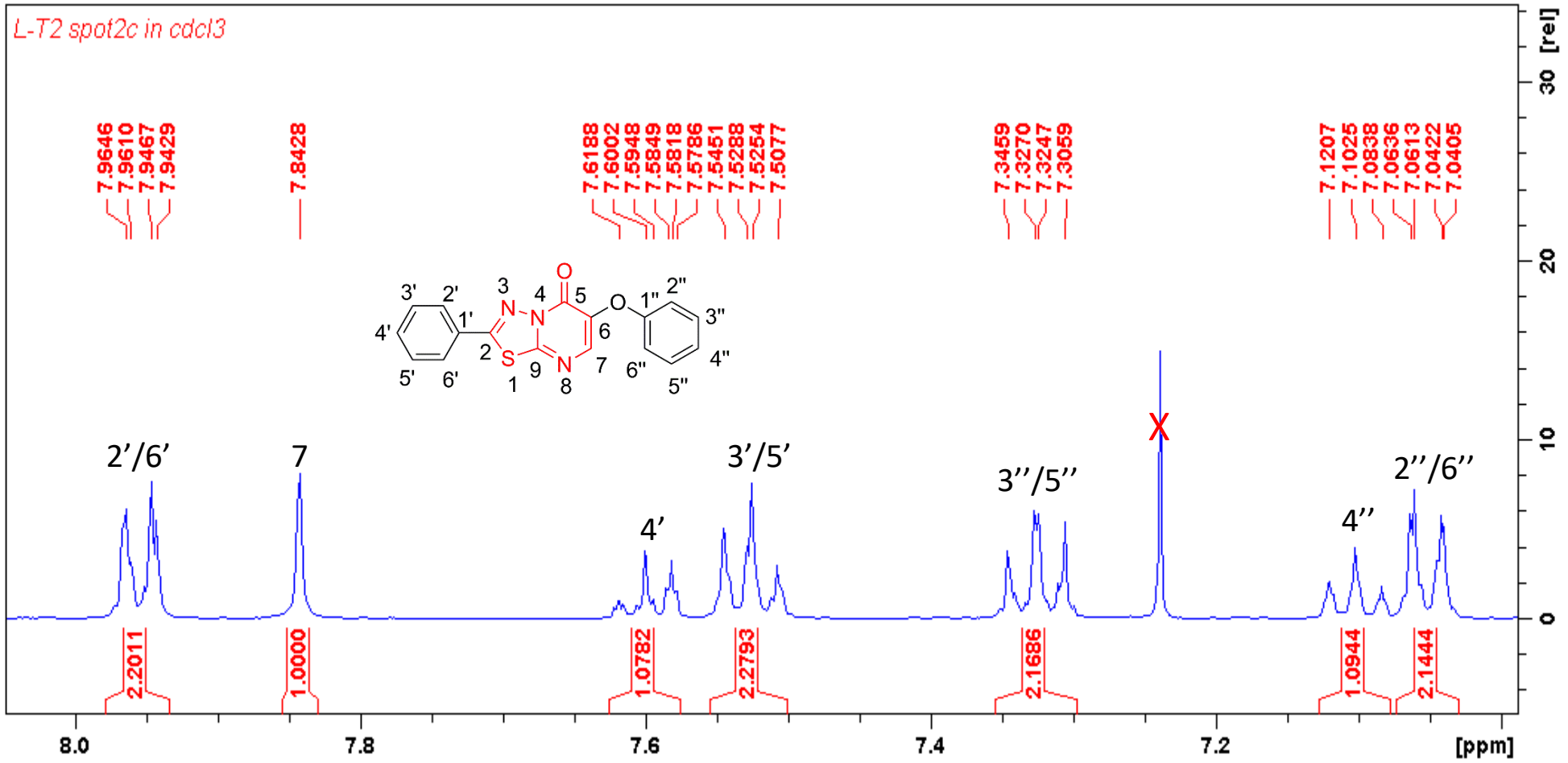


The expanded ^{1}H NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2j)**

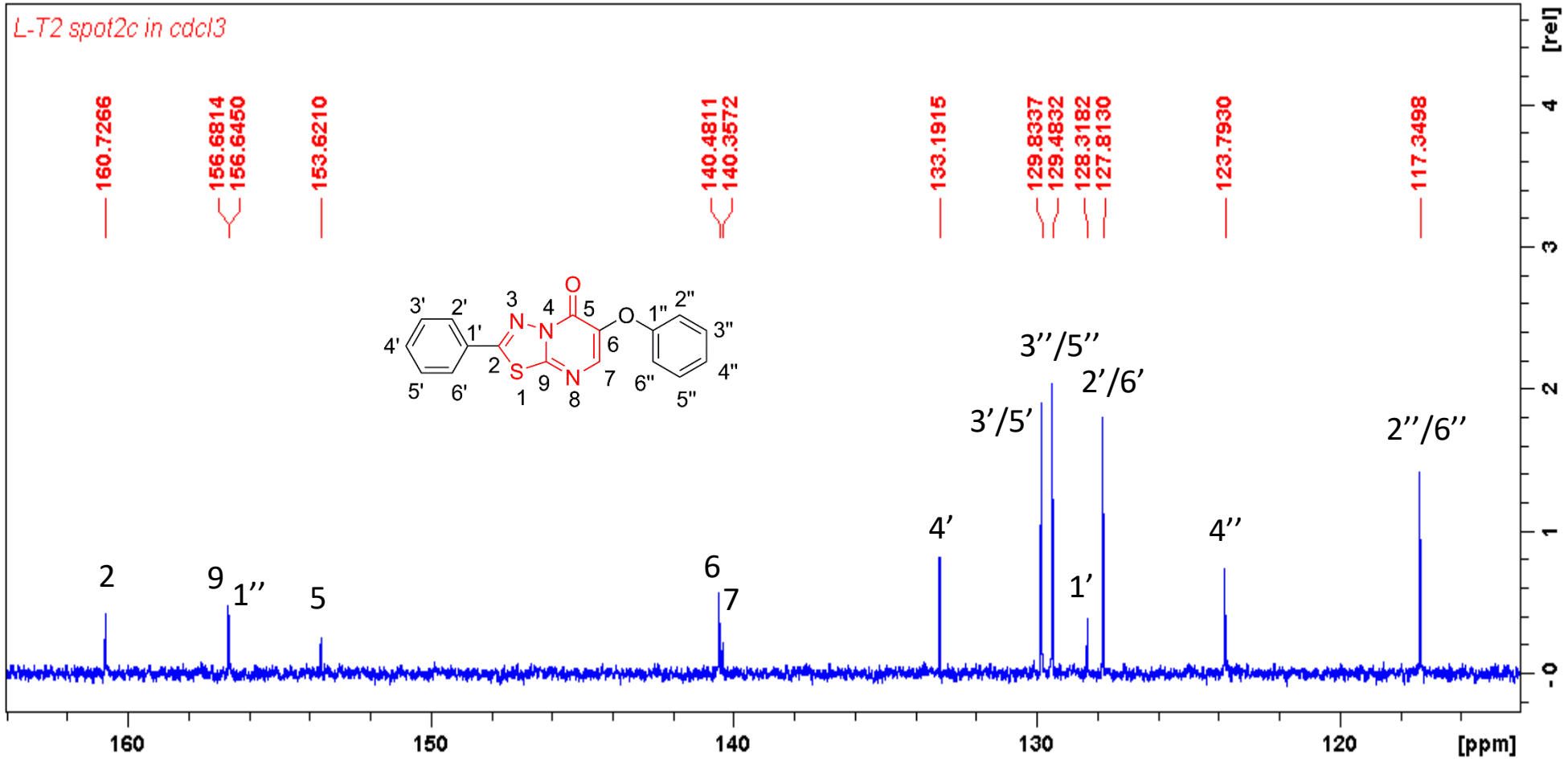


The ^{13}C NMR spectrum of *N*,*N*-dimethyl-*N*'-(5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)imidoformamide **(2j)**

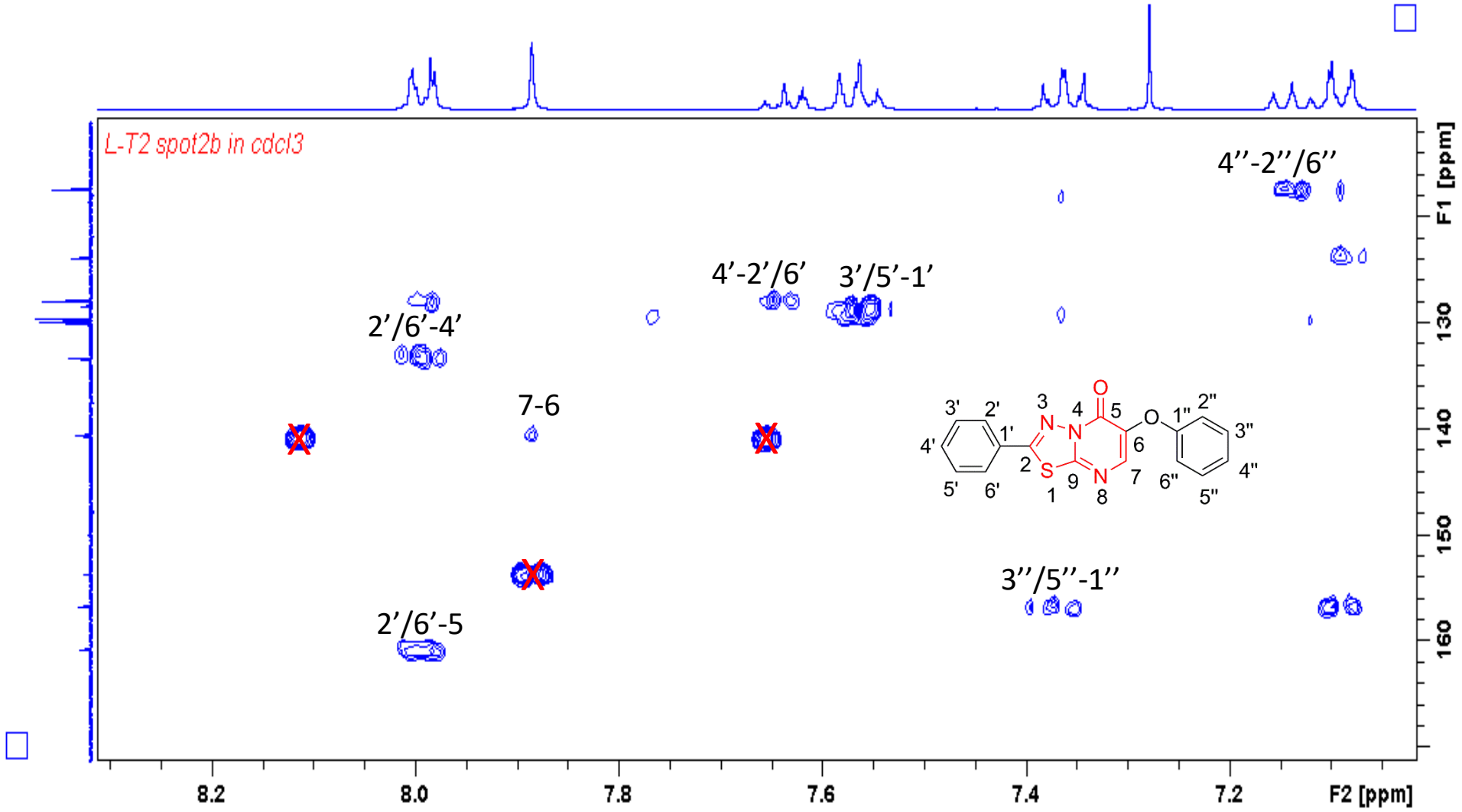
Compounds 3a-g NMR Spectra



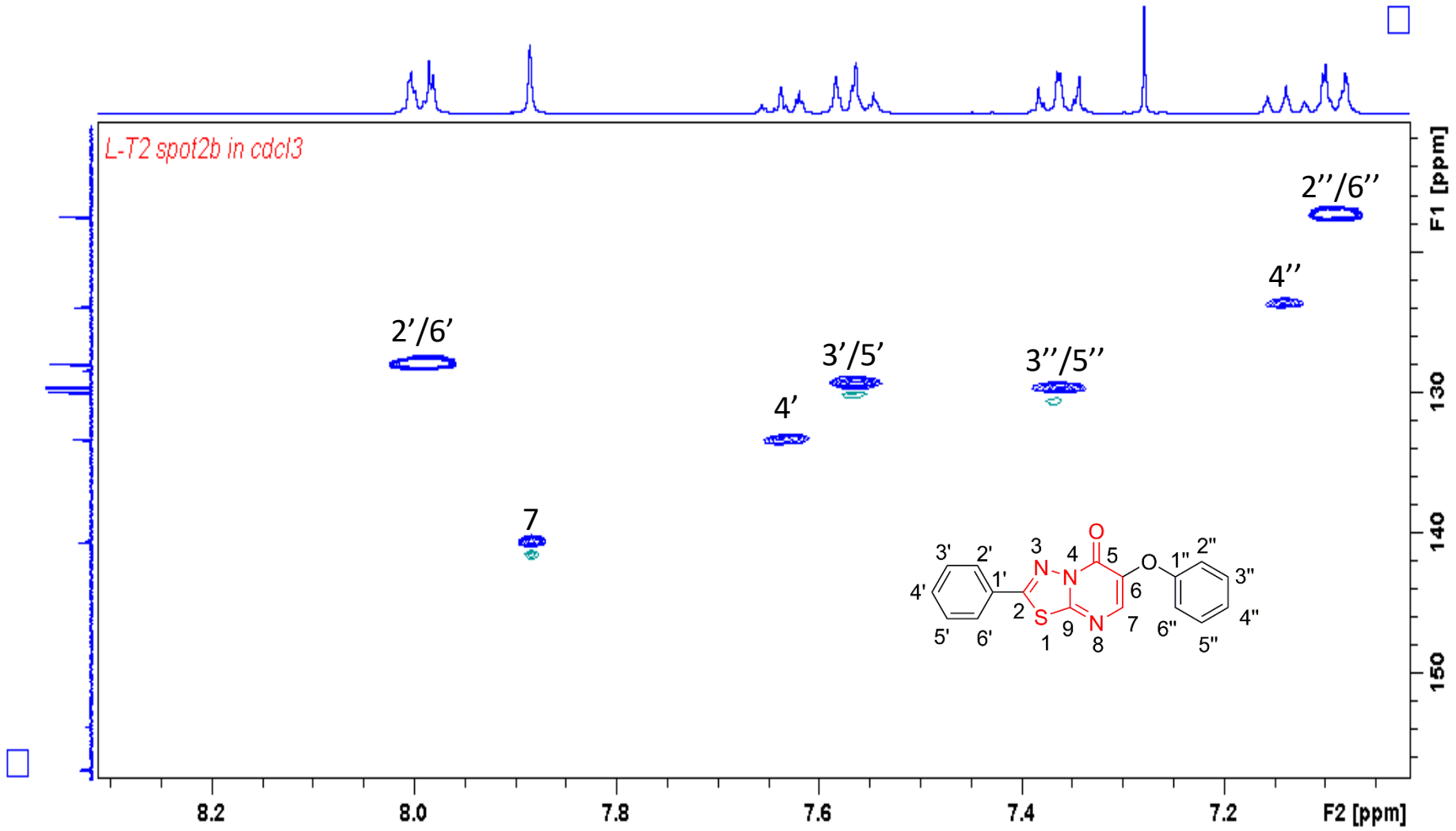
The ^{1}H NMR spectrum of 6-phenoxy-2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3a**



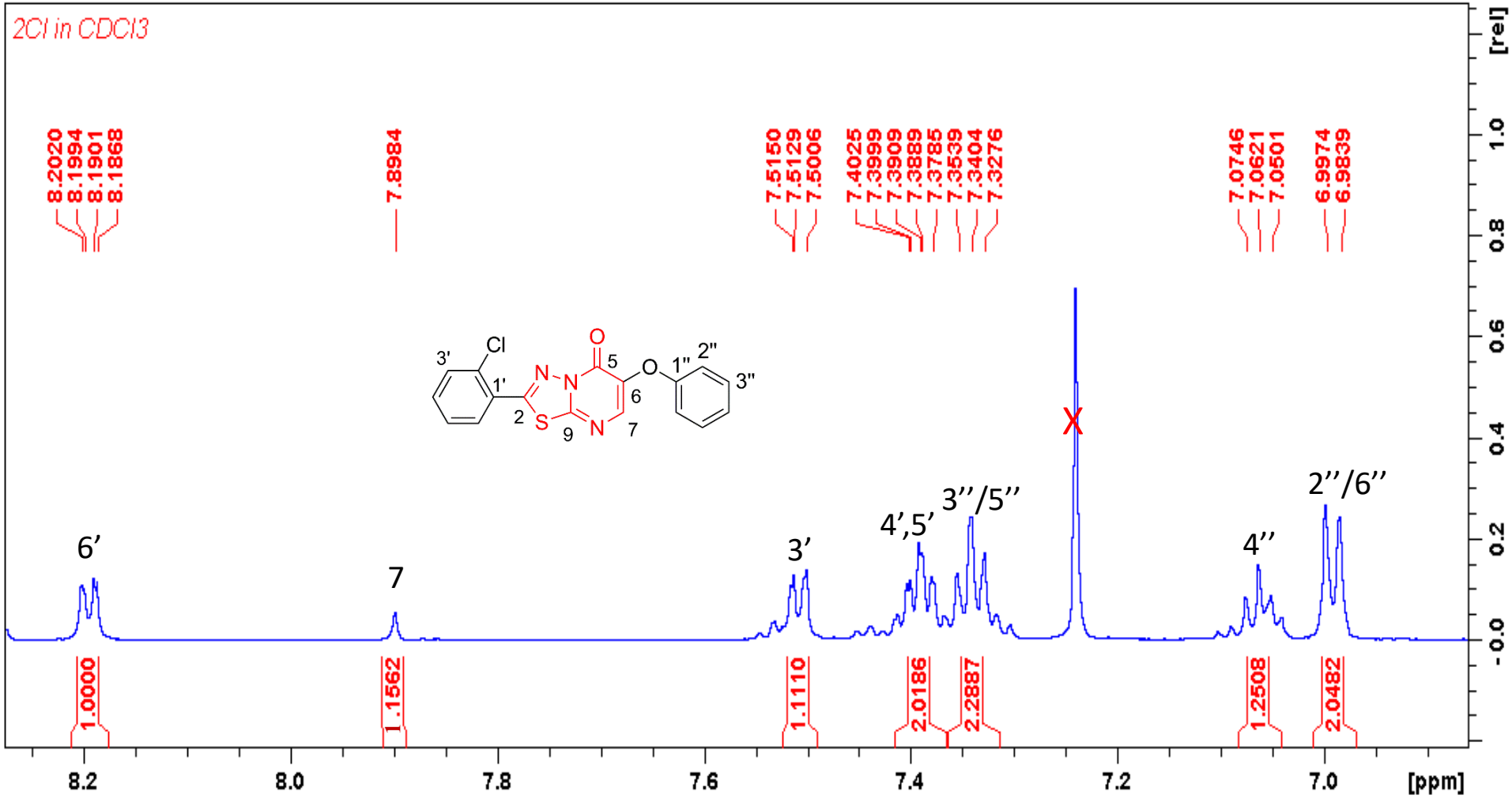
The ^{13}C NMR spectrum of 6-phenoxy-2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3a**



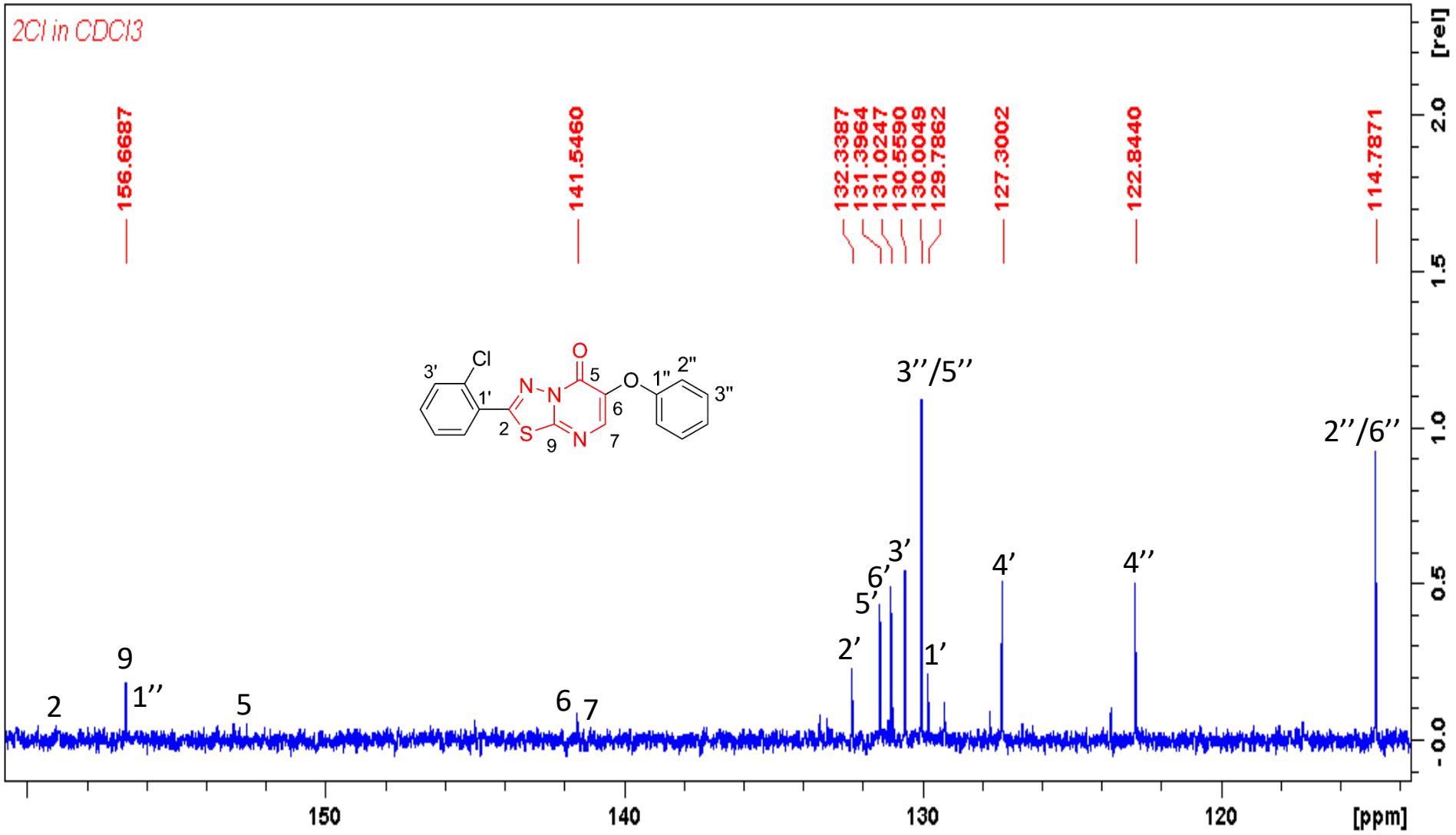
The HMBC (**C→H correlation**) NMR spectrum of 6-phenoxy-2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3a**



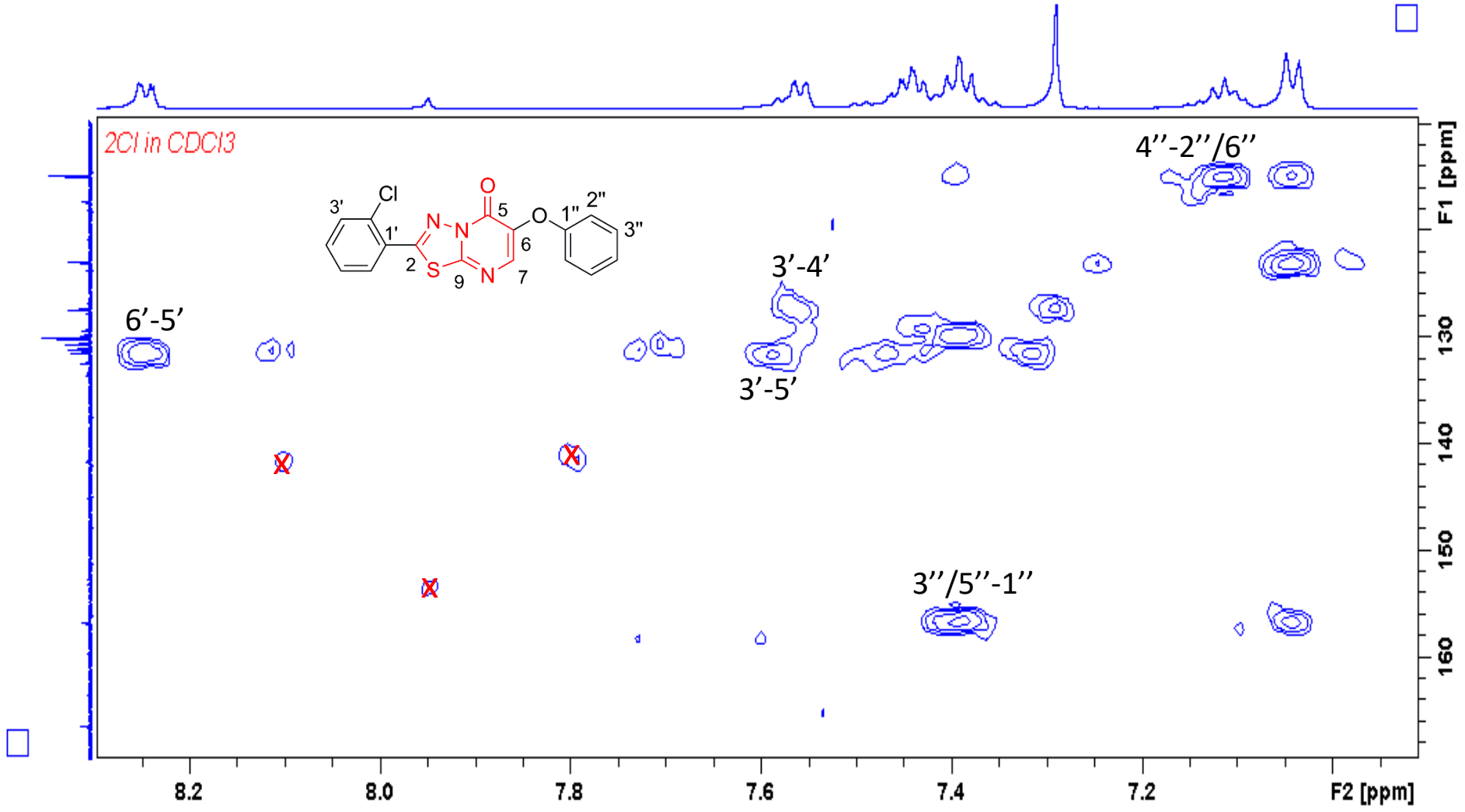
The HSQC (**C-H bond**) NMR spectrum of 6-phenoxy-2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3a**



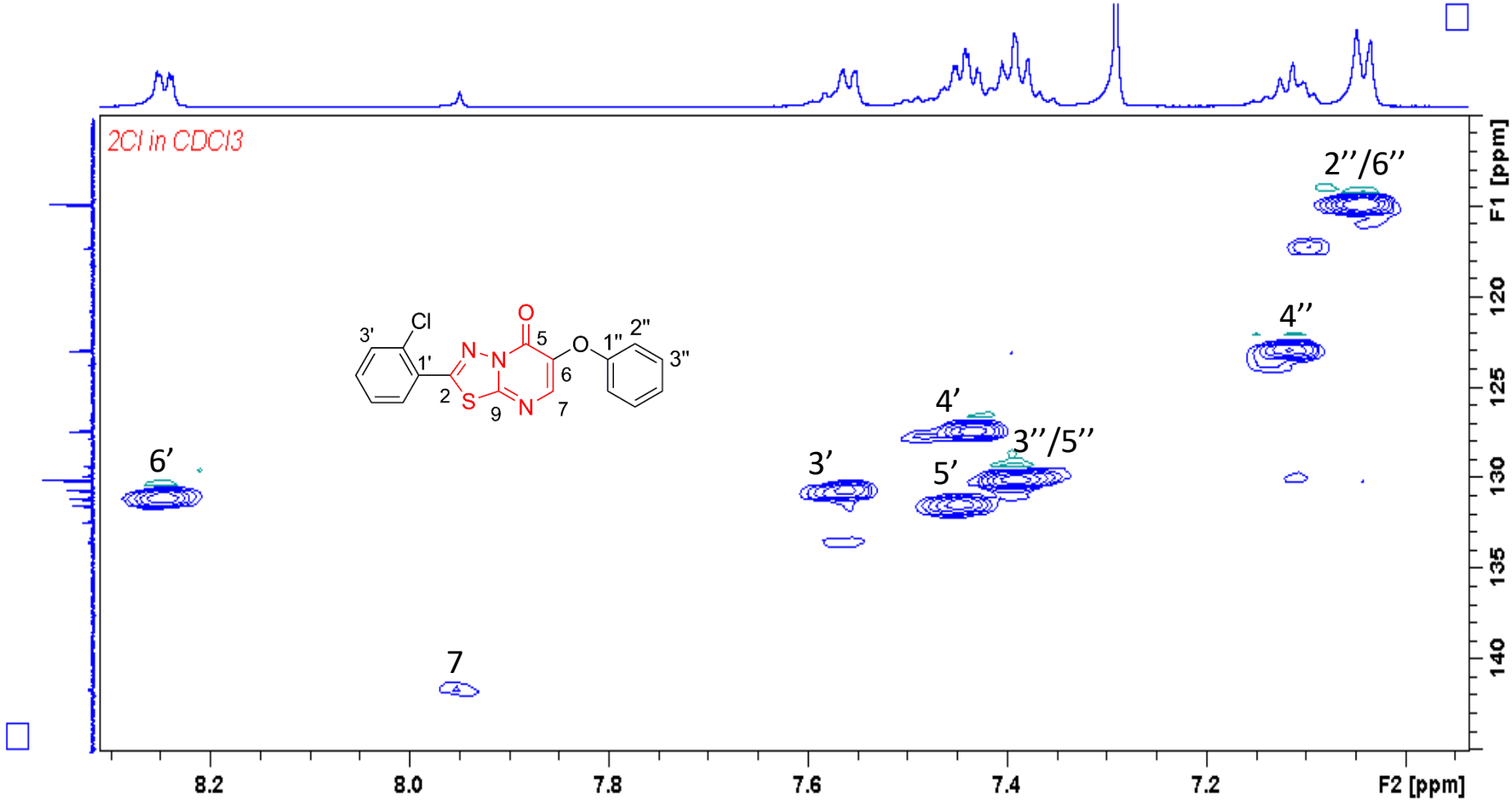
The ^{1}H NMR spectrum of 6-phenoxy-2-(2-chlorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3b**



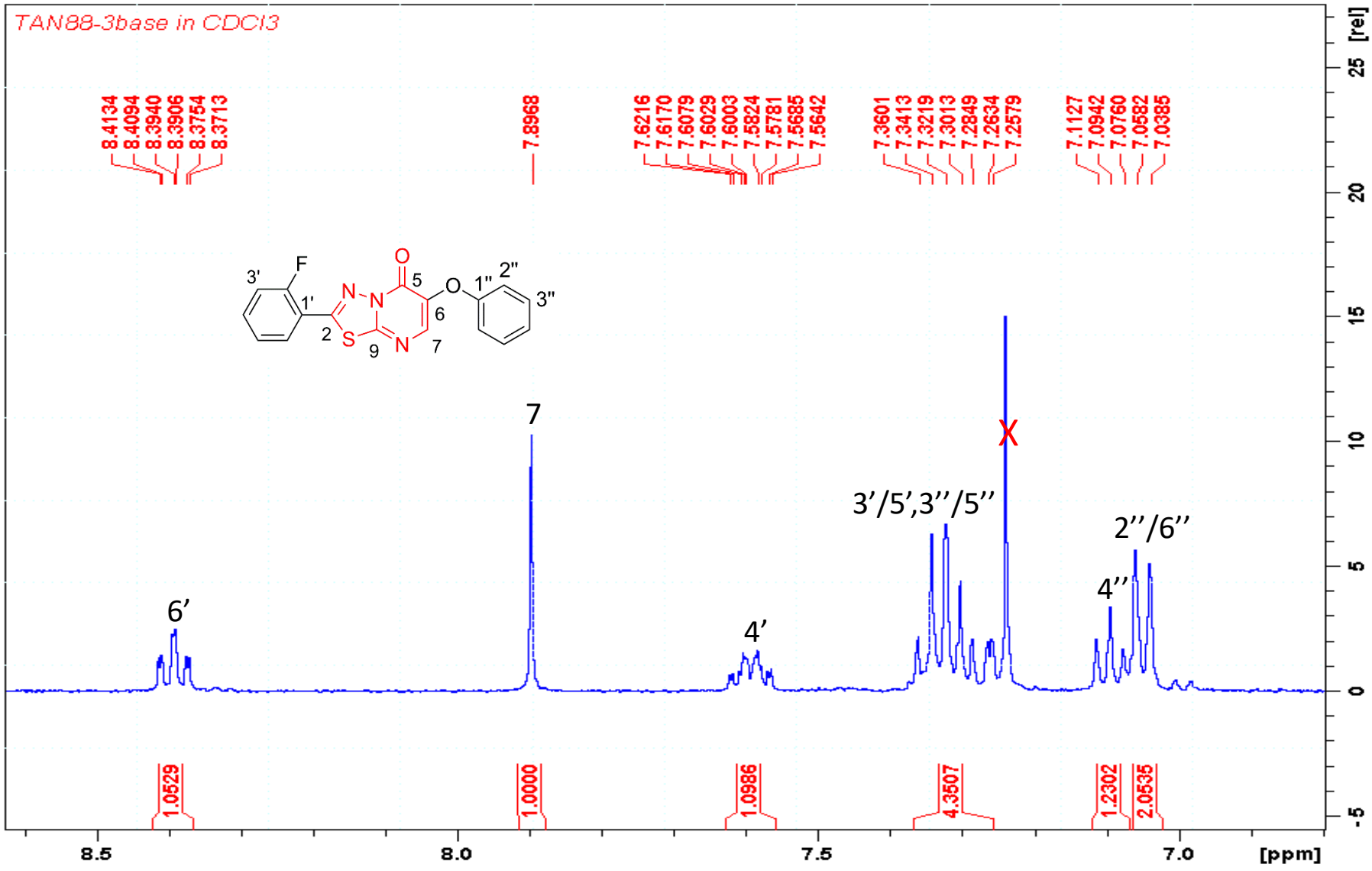
The ^{13}C NMR spectrum of 6-phenoxy-2-(2-chlorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3b**



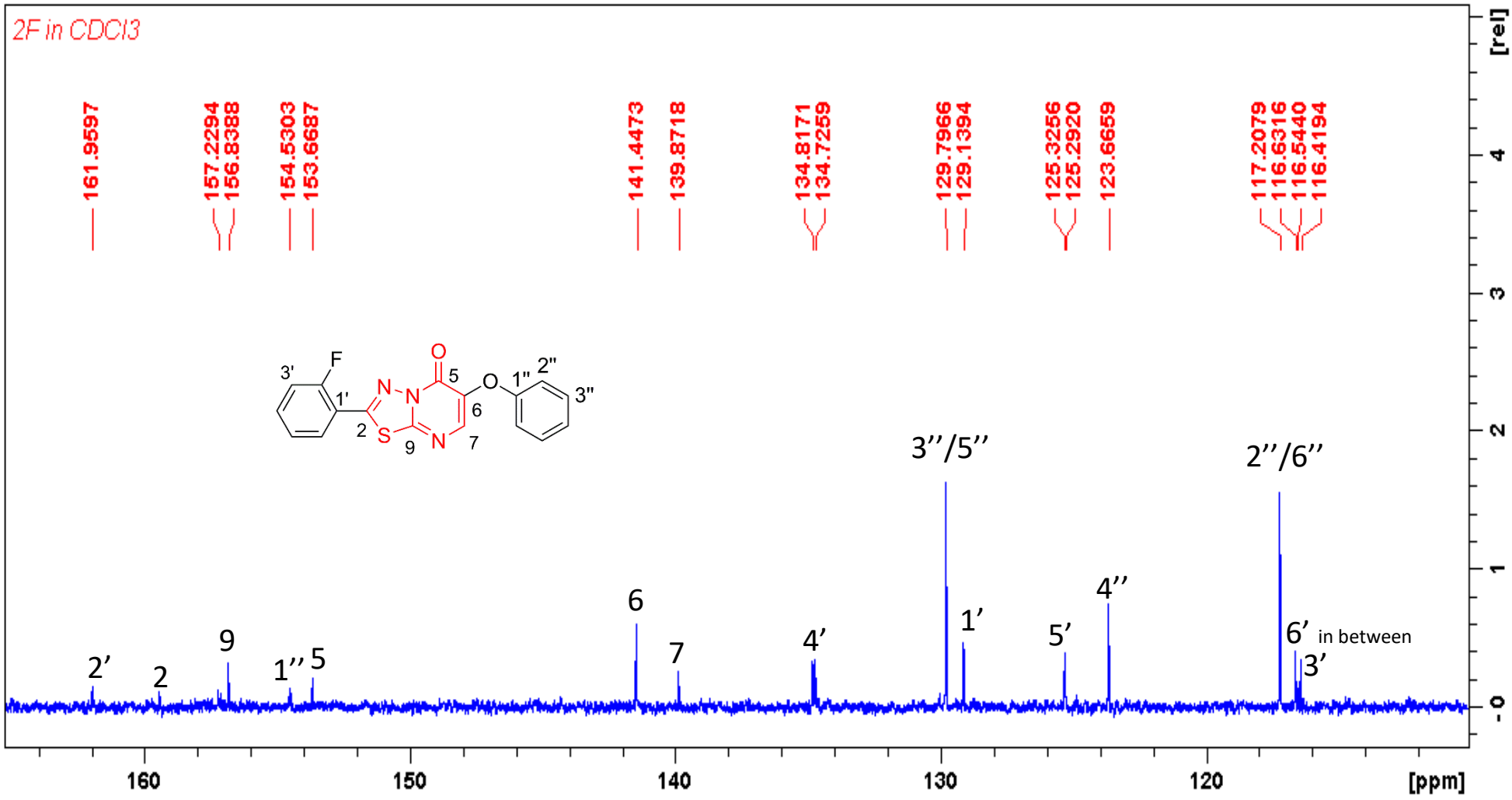
The HMBC (**C→H correlation**) NMR spectrum of 6-phenoxy-2-(2-chlorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3b**



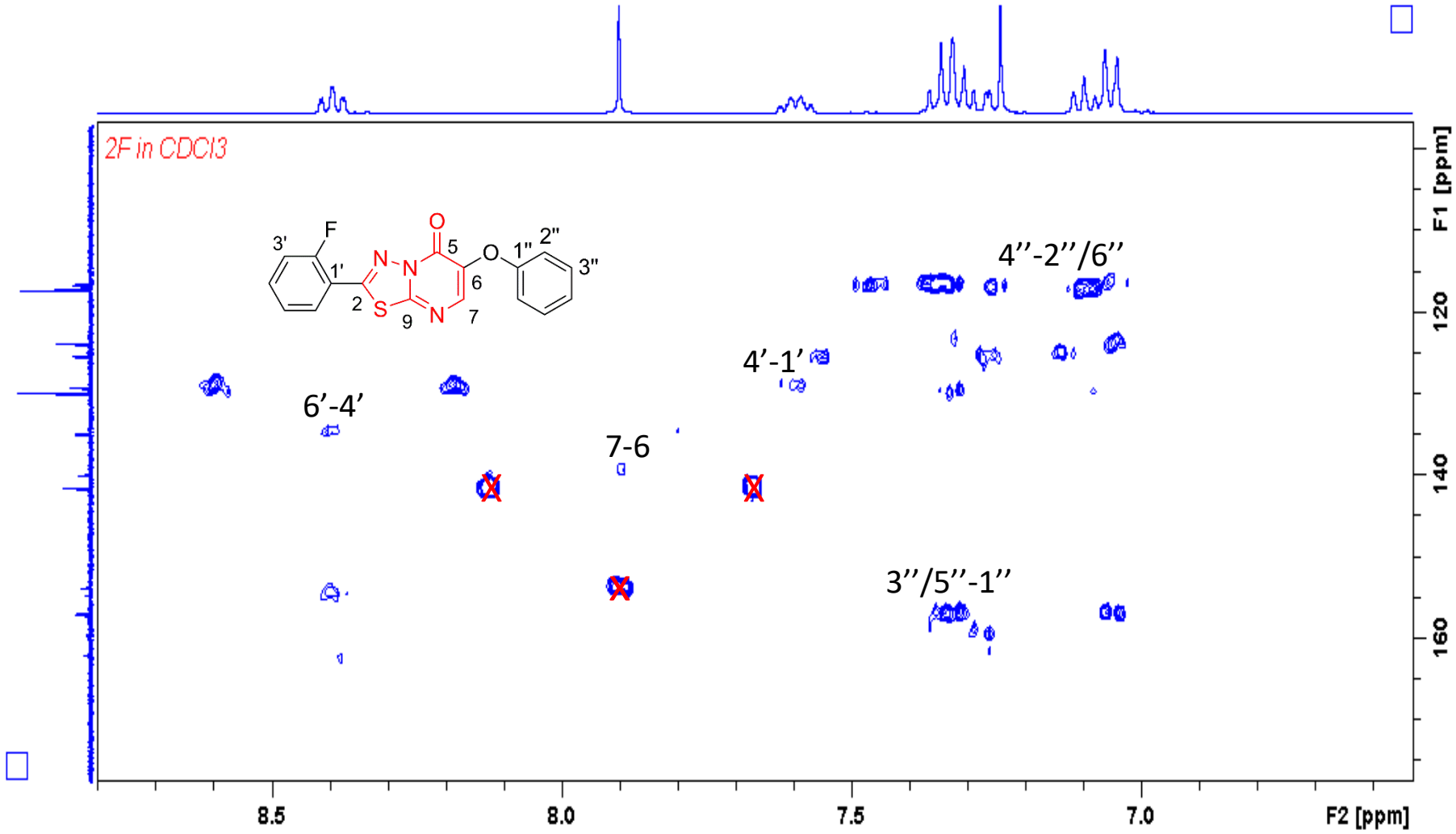
The HSQC (**C-H bond**) NMR spectrum of 6-phenoxy-2-(2-chloroophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3b**



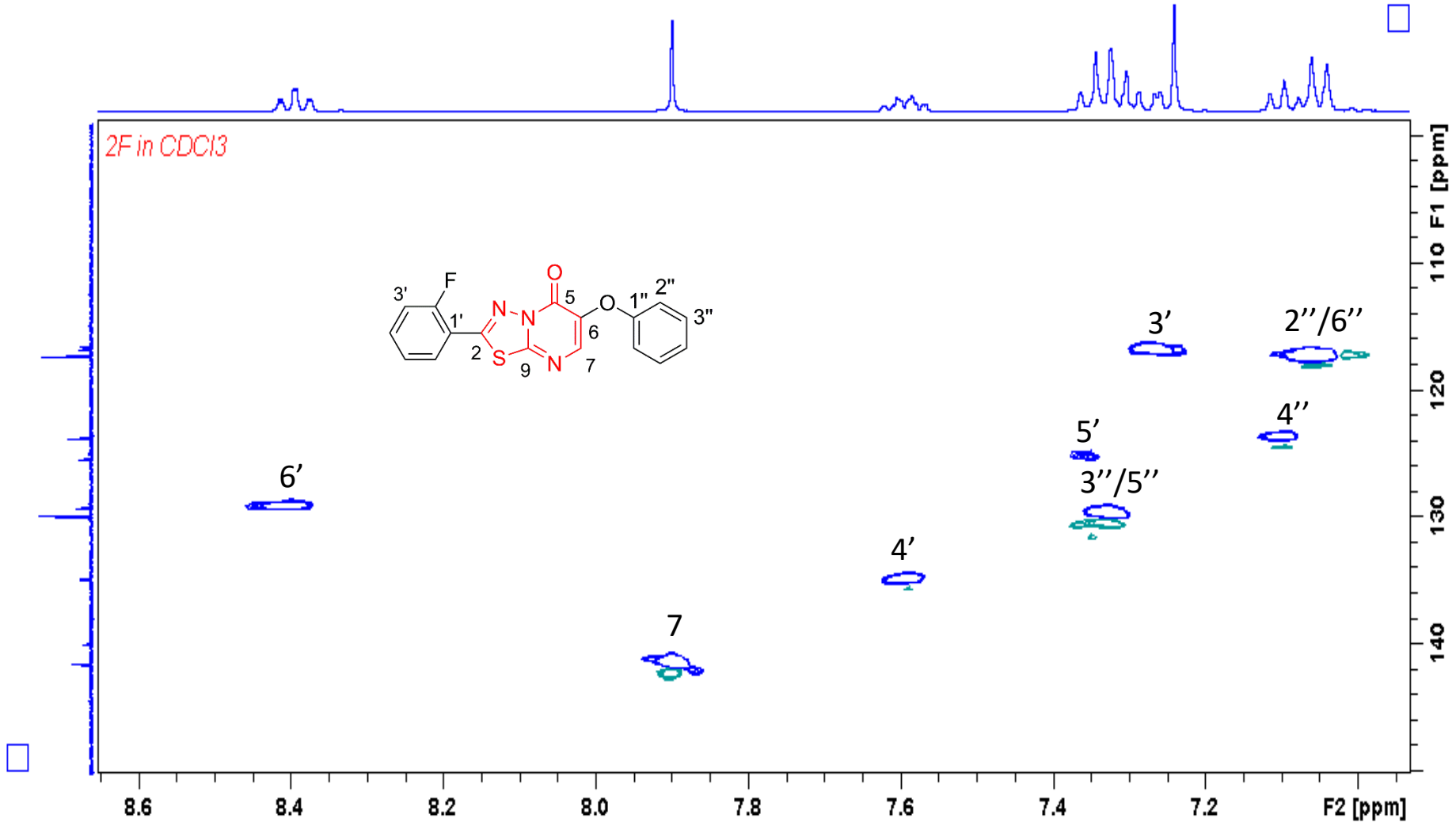
The ^{1}H NMR spectrum of 6-phenoxy-2-(2-fluorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3c**



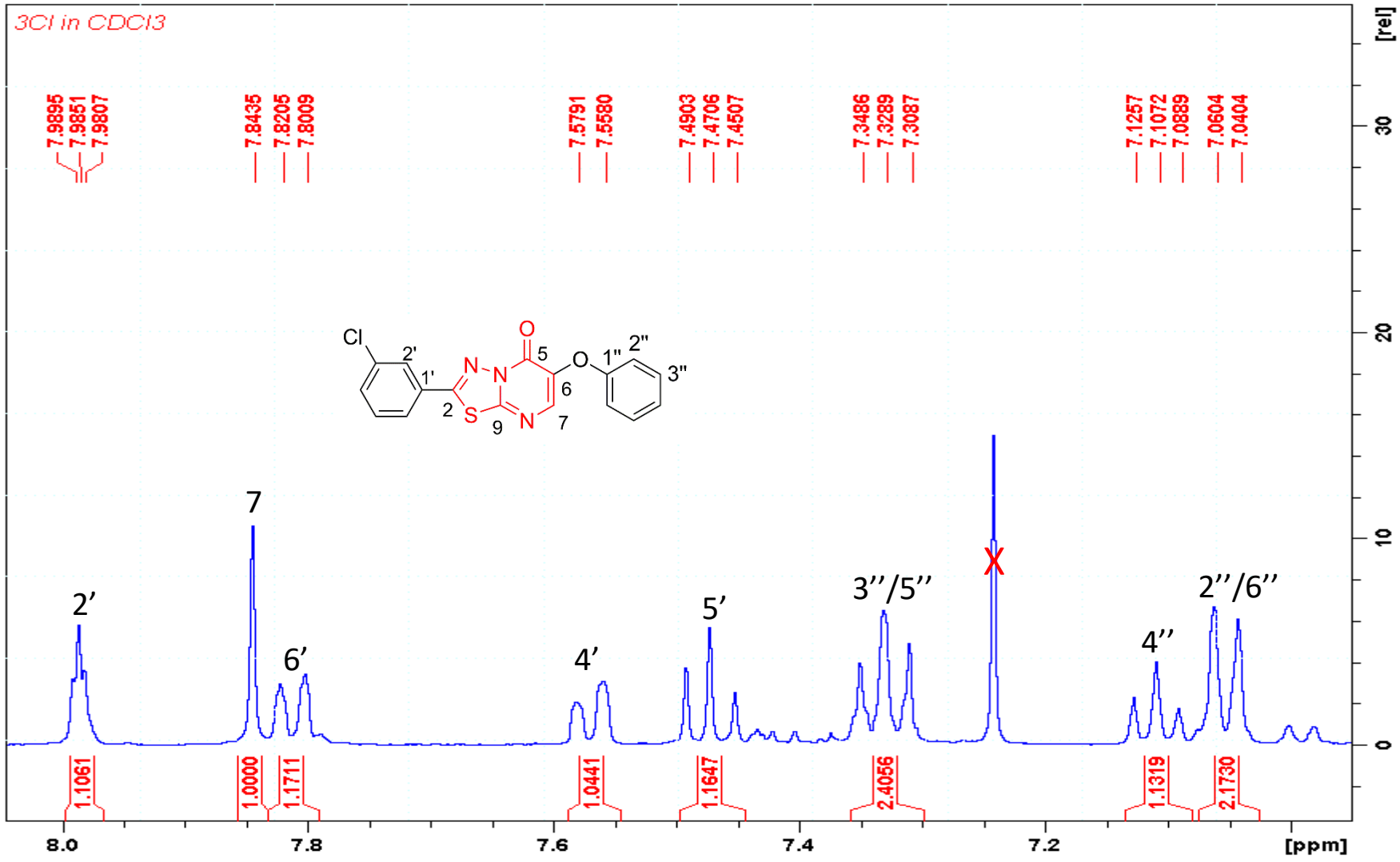
The ^{13}C NMR spectrum of 6-phenoxy-2-(2-fluorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3c**



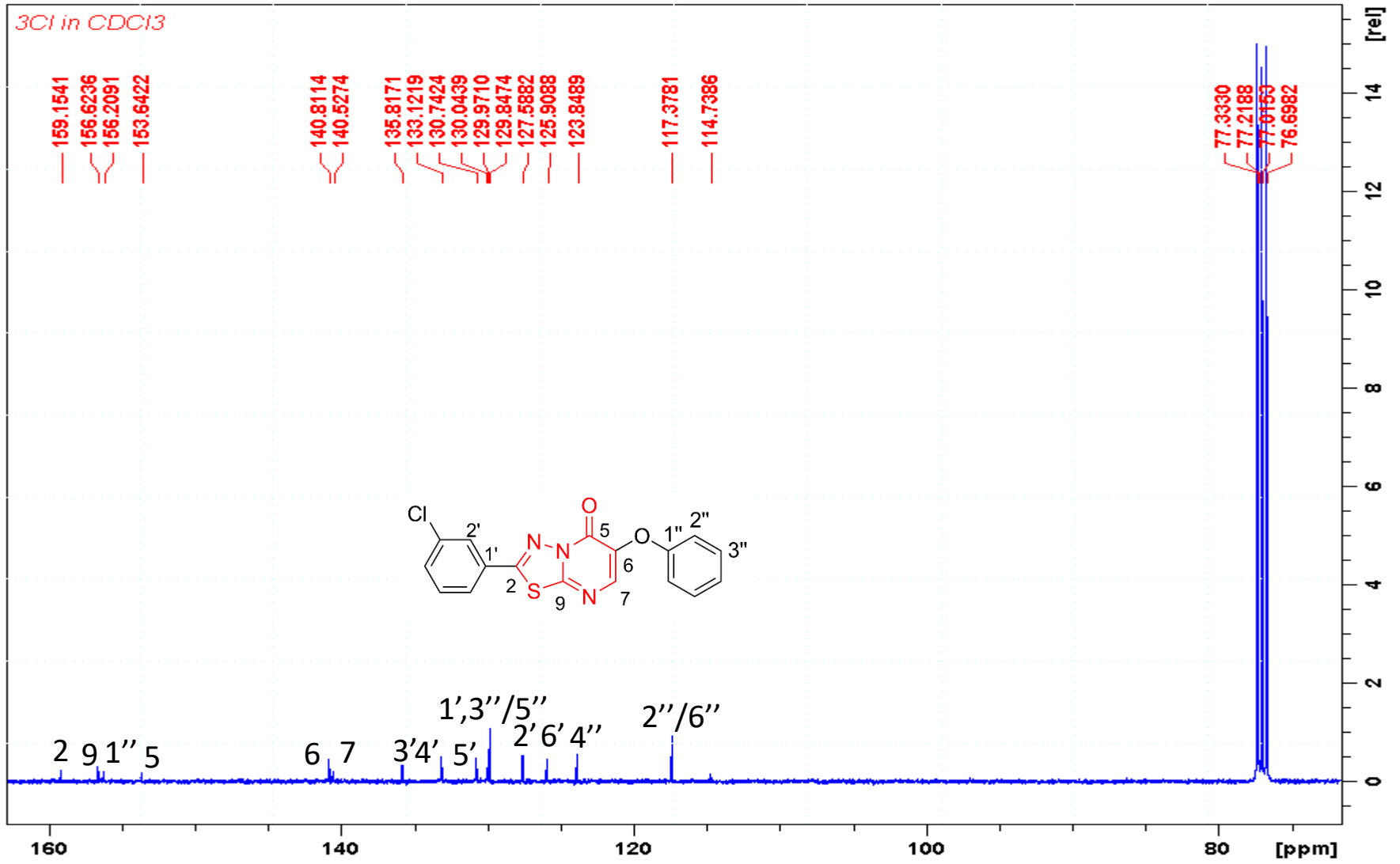
The HMBC (**C→H correlation)** NMR spectrum of 6-phenoxy-2-(2-fluorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3c**



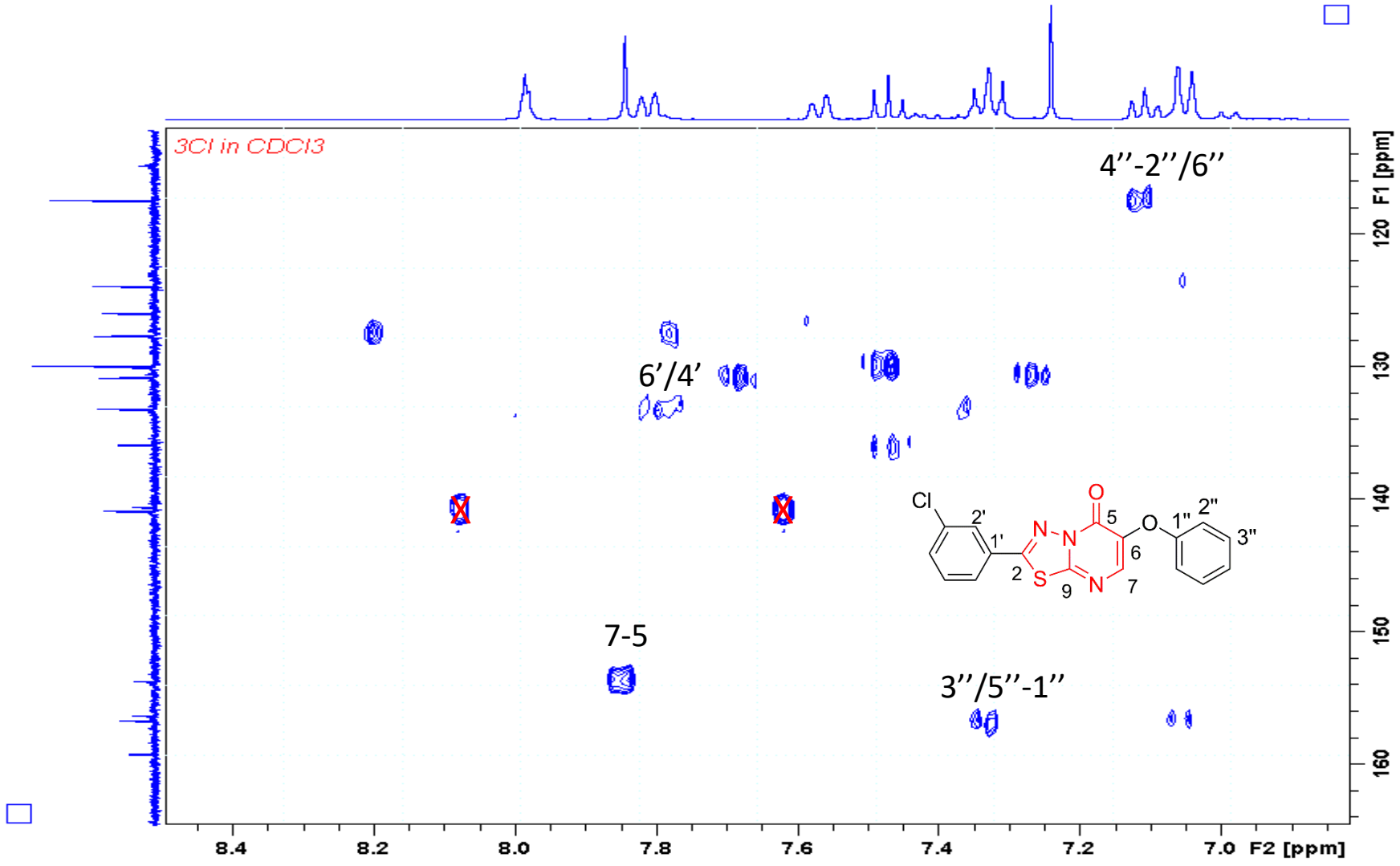
The HSQC (**C-H bond)** NMR spectrum of 6-phenoxy-2-(2-fluorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3c**



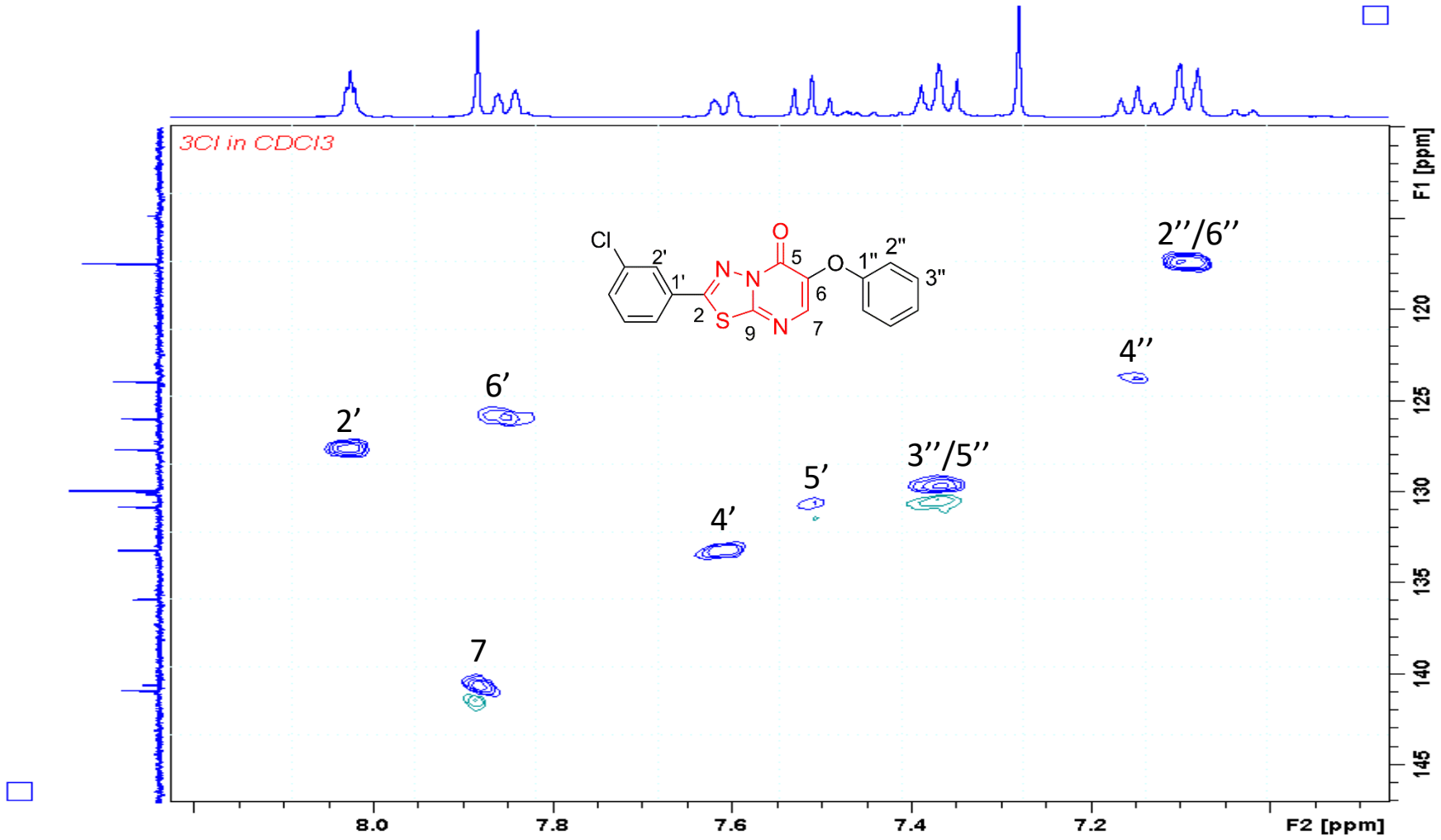
The ^{1}H NMR spectrum of 6-phenoxy-2-(3-chlorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3d**



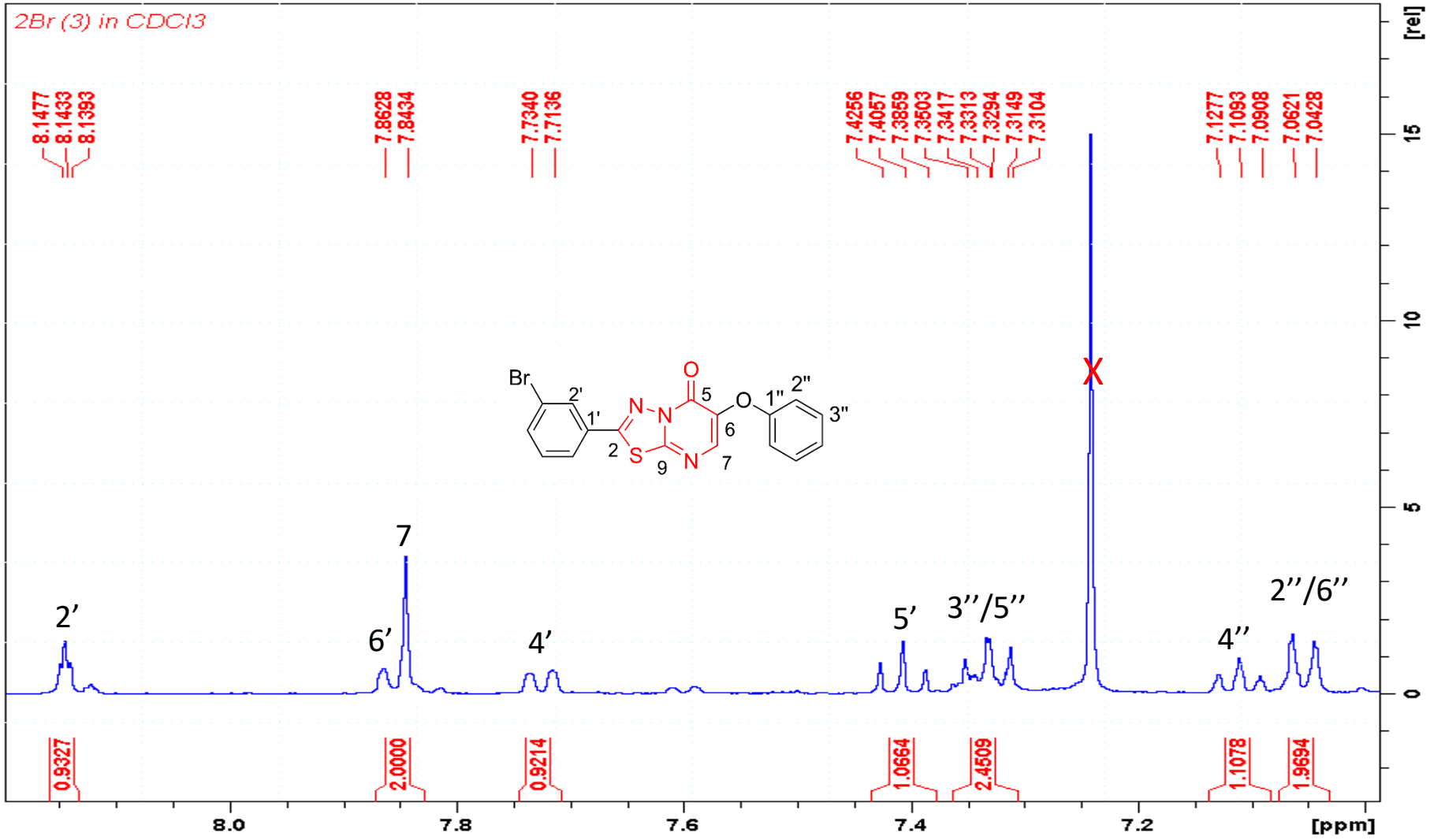
The ^{13}C NMR spectrum of 6-phenoxy-2-(3-chlorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3d**



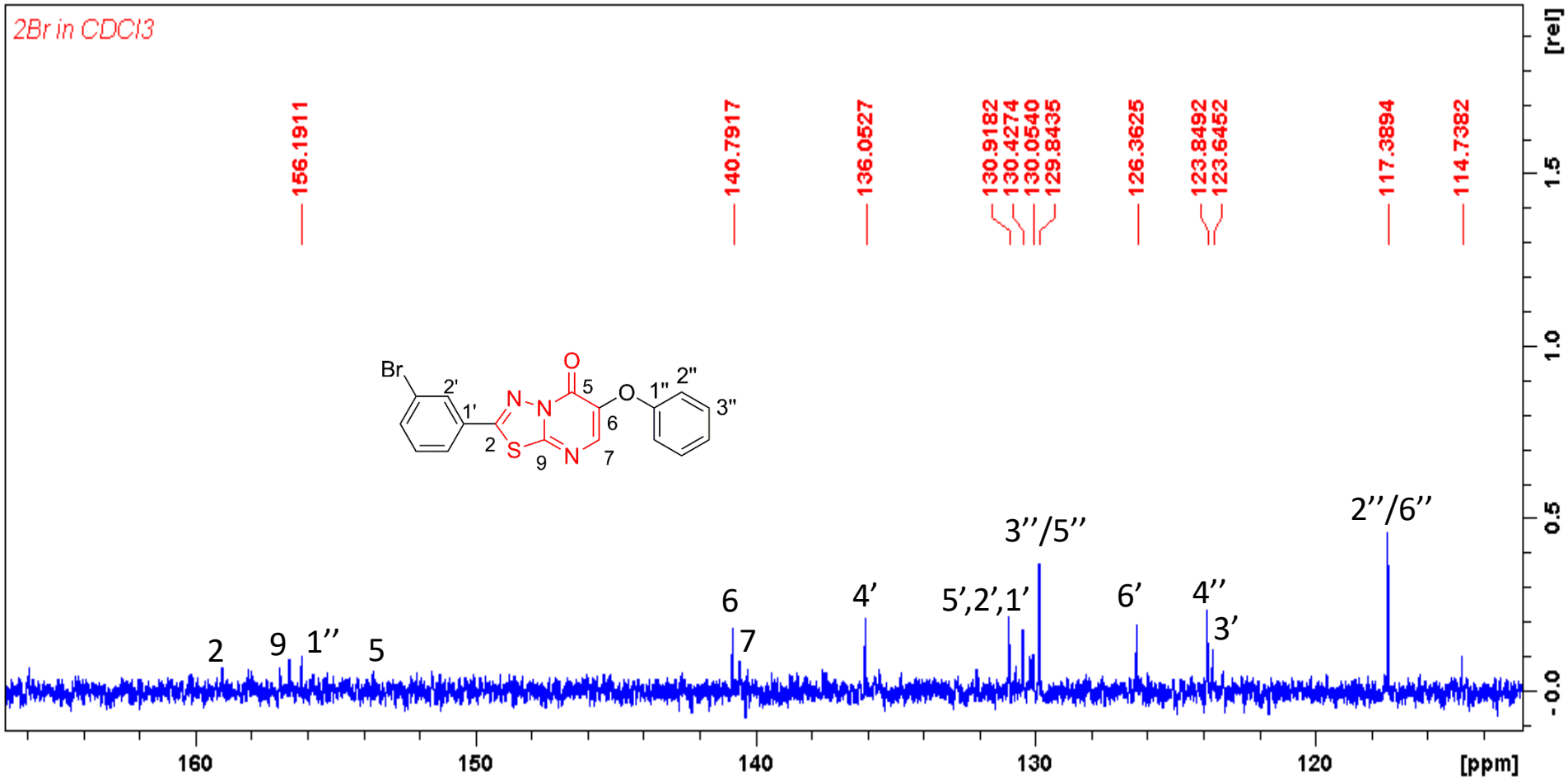
The HMBC (**C→H correlation)** NMR spectrum of 6-phenoxy-2-(3-chlorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3d**



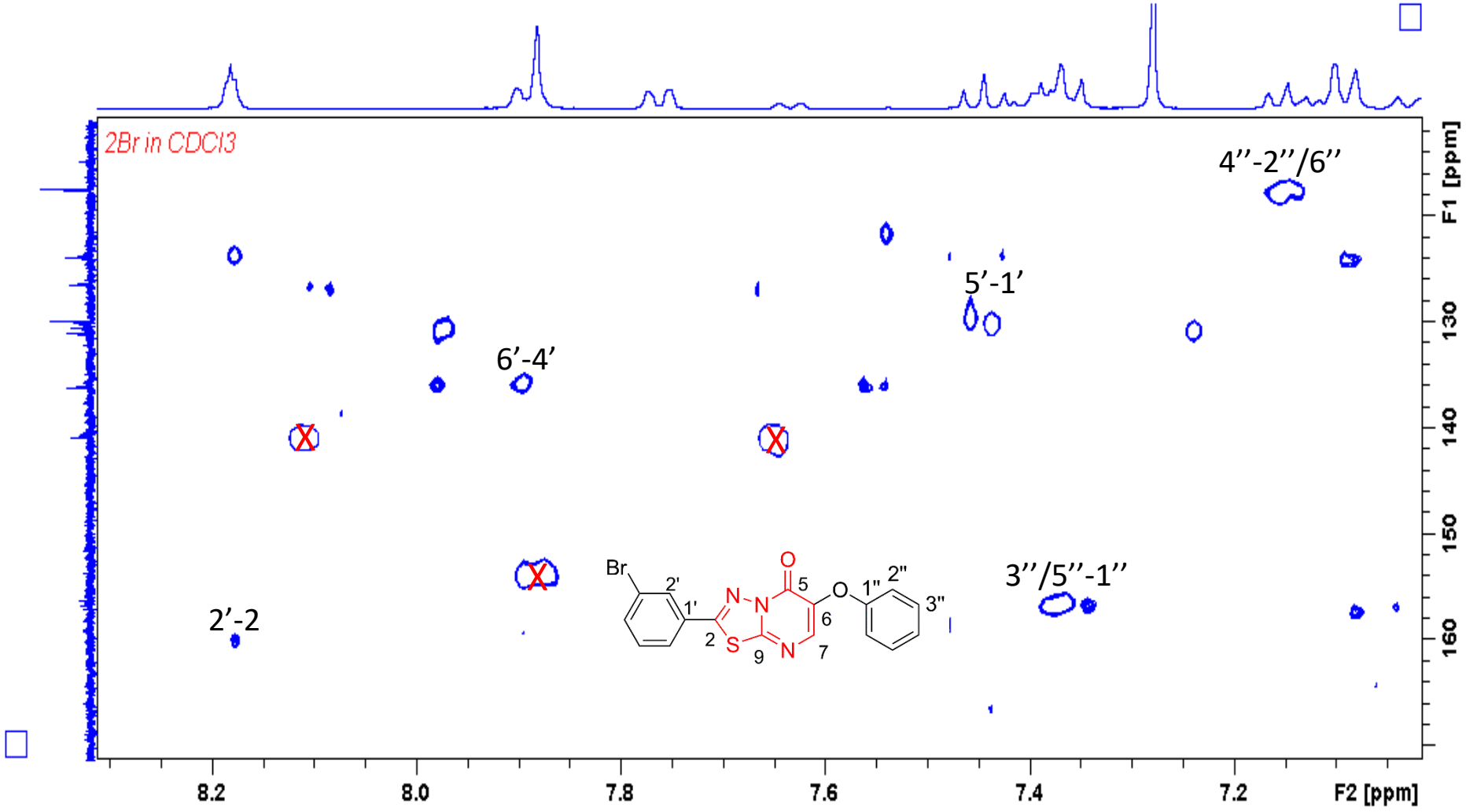
The HSQC (**C-H bond**) NMR spectrum of 6-phenoxy-2-(3-chlorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3d**



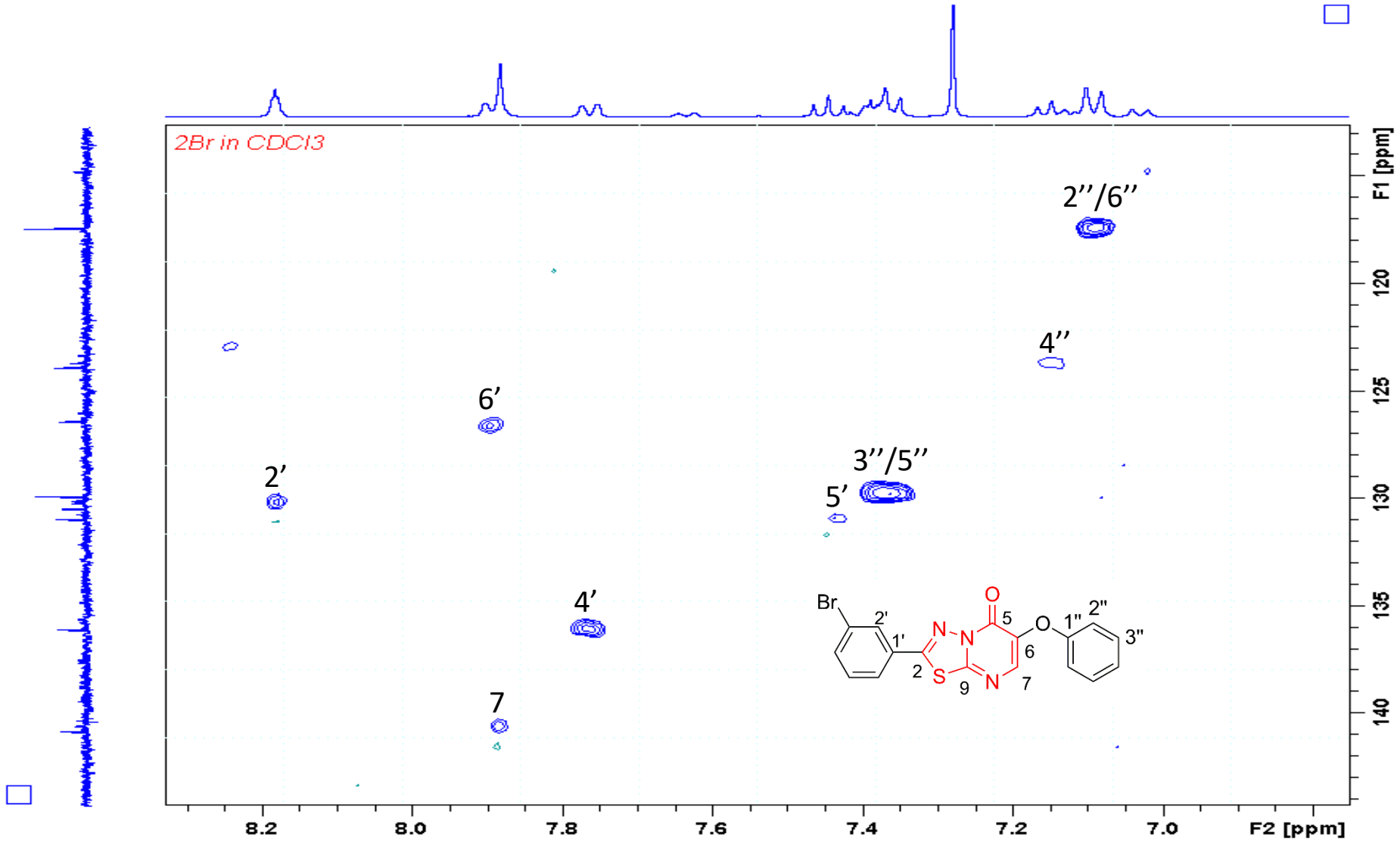
The ^{1}H NMR spectrum of 6-phenoxy-2-(2-bromophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3e**



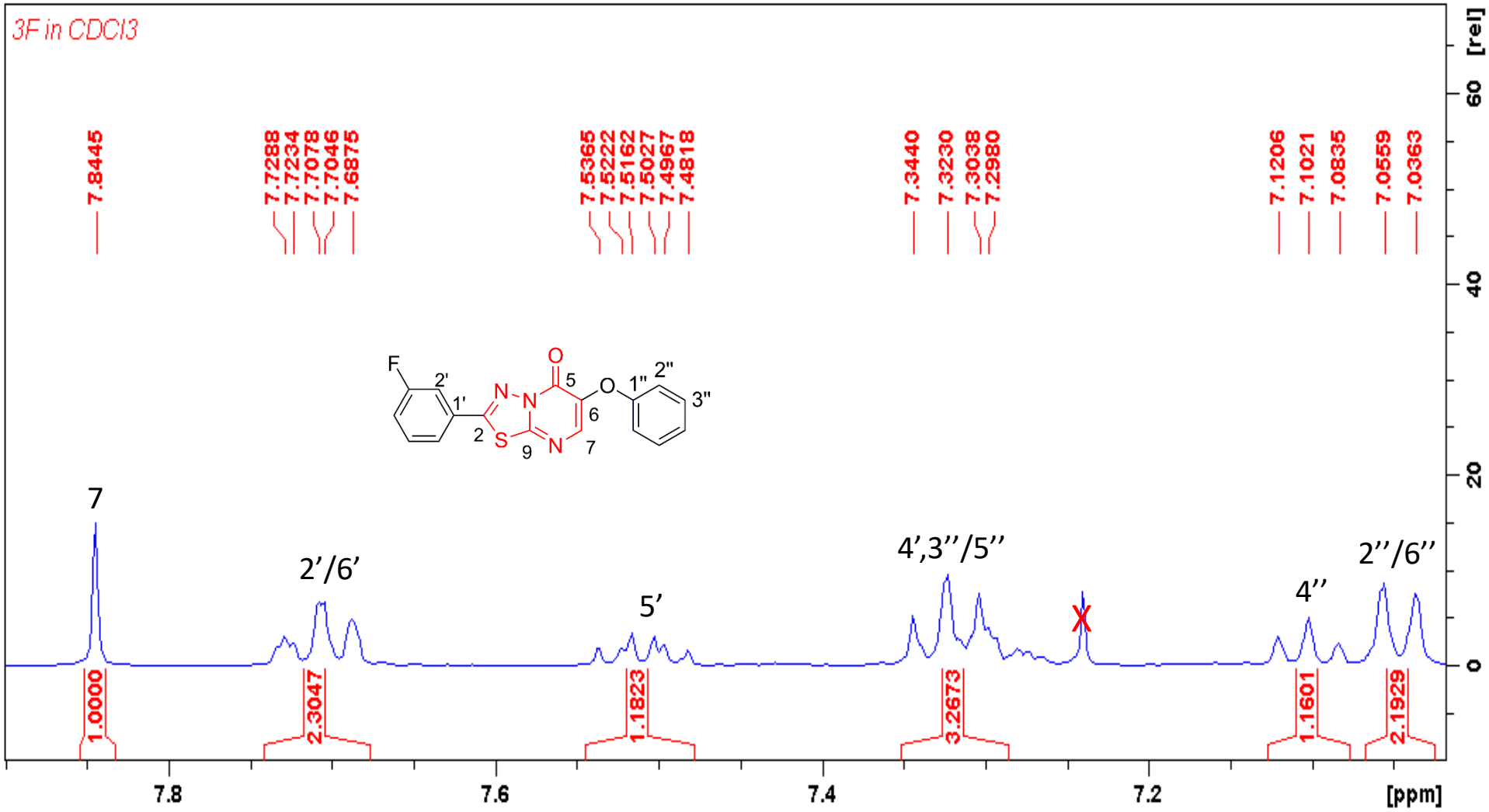
The ^{13}C NMR spectrum of 6-phenoxy-2-(2-bromophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3e**



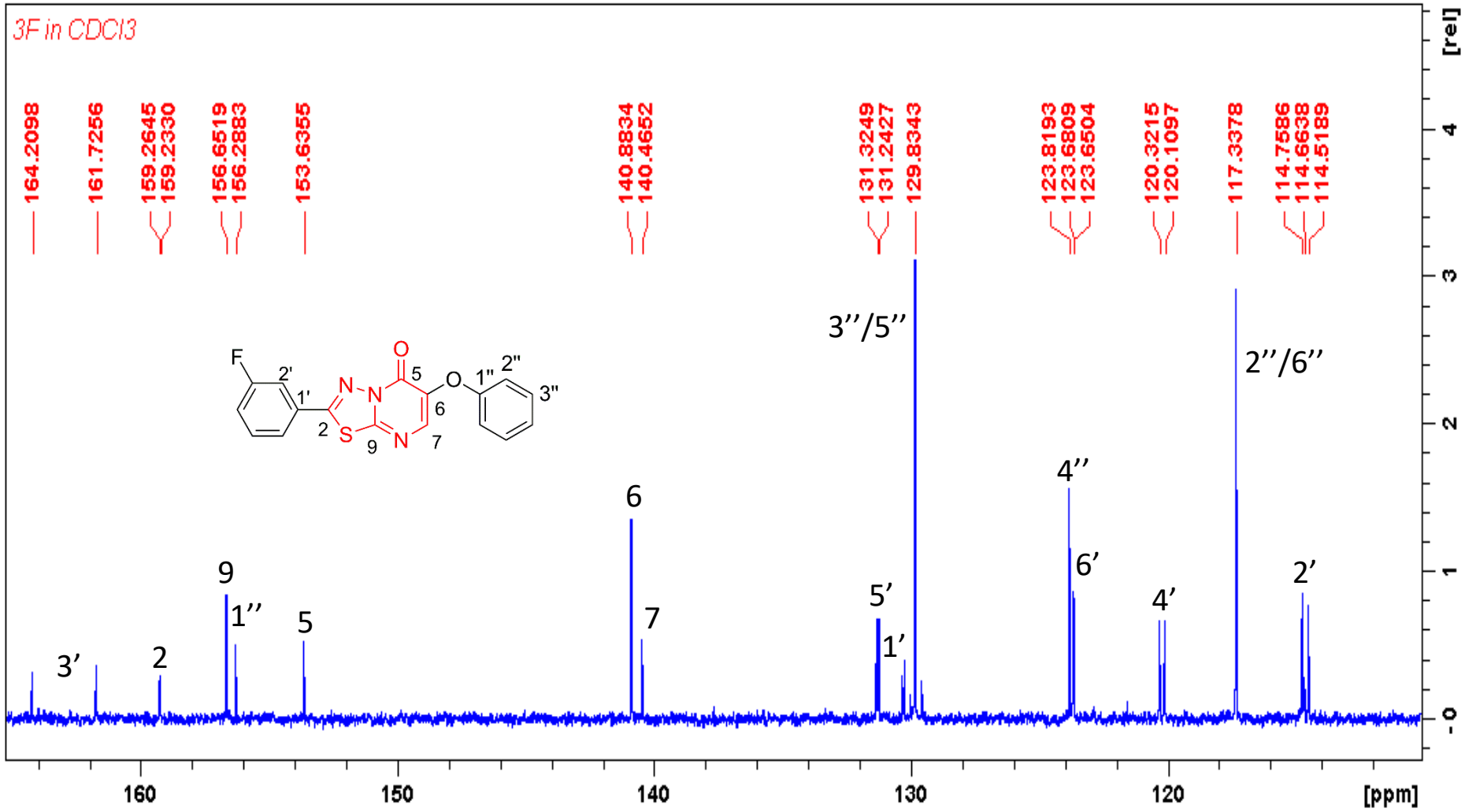
The HMBC (**C→H correlation**) NMR spectrum of 6-phenoxy-2-(2-bromophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3e**



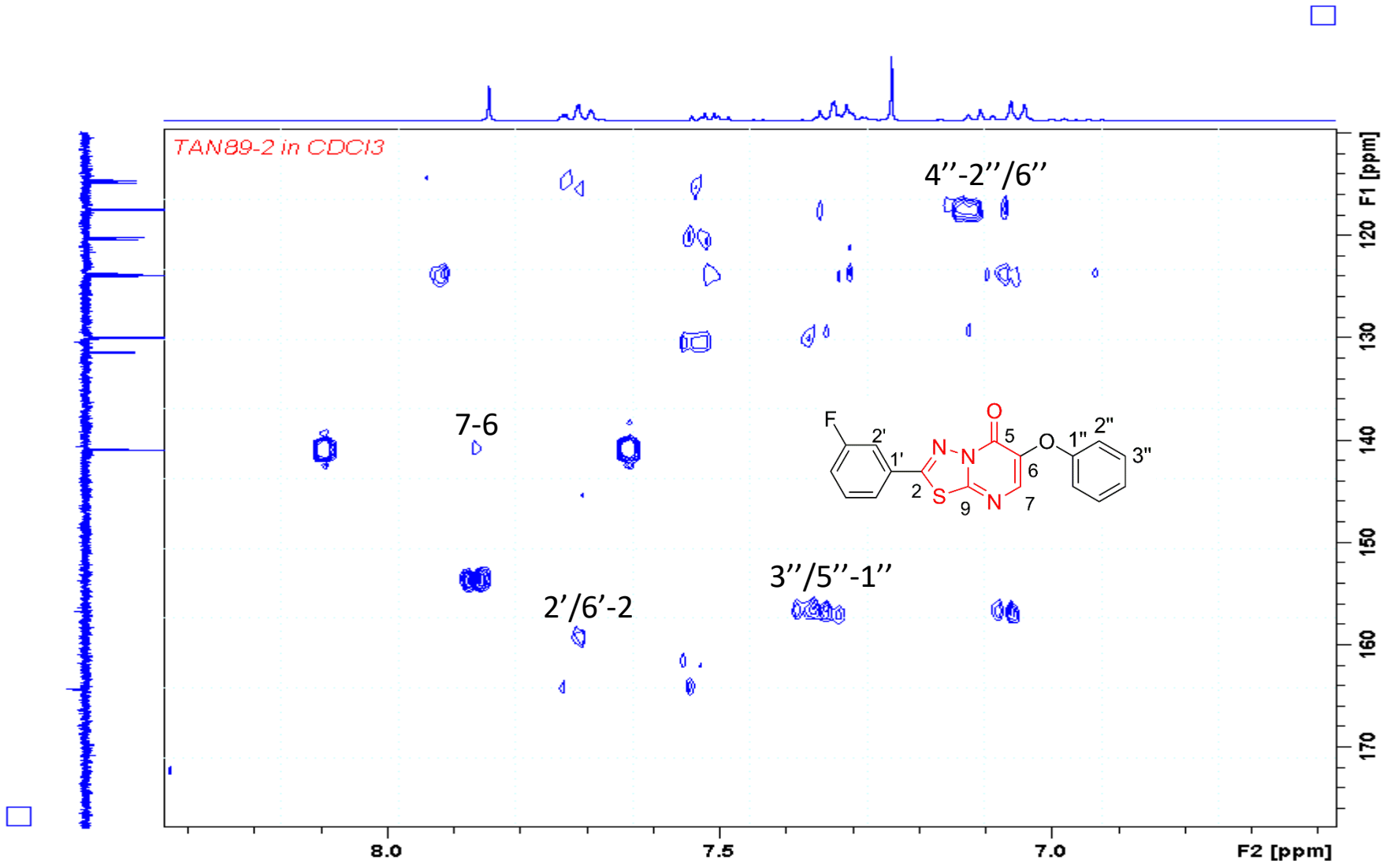
The HSQC (**C-H bond**) NMR spectrum of 6-phenoxy-2-(2-bromophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3e**



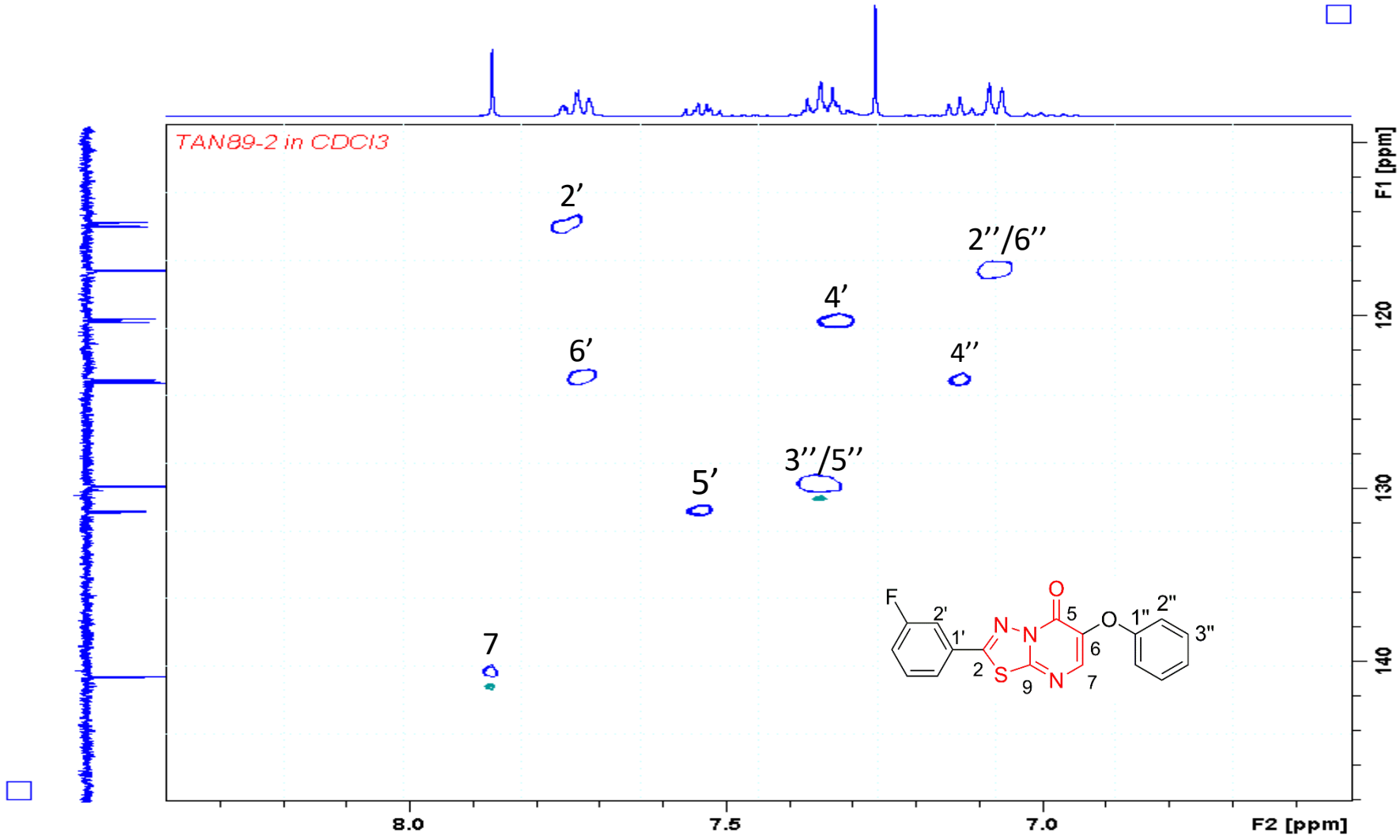
The ^{1}H NMR spectrum of 6-phenoxy-2-(3-fluorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3f**



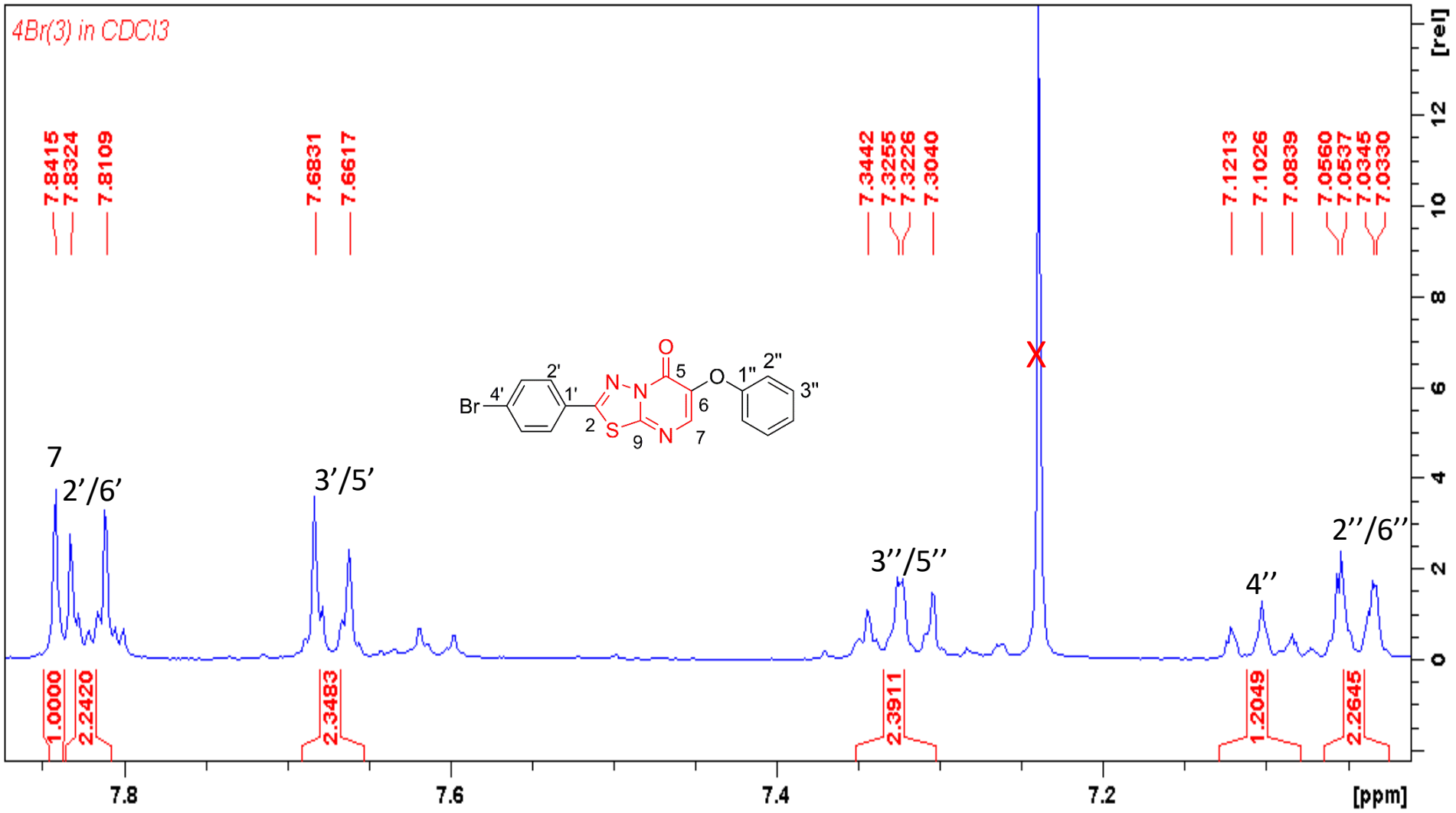
The ^{13}C NMR spectrum of 6-phenoxy-2-(3-fluorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3f**



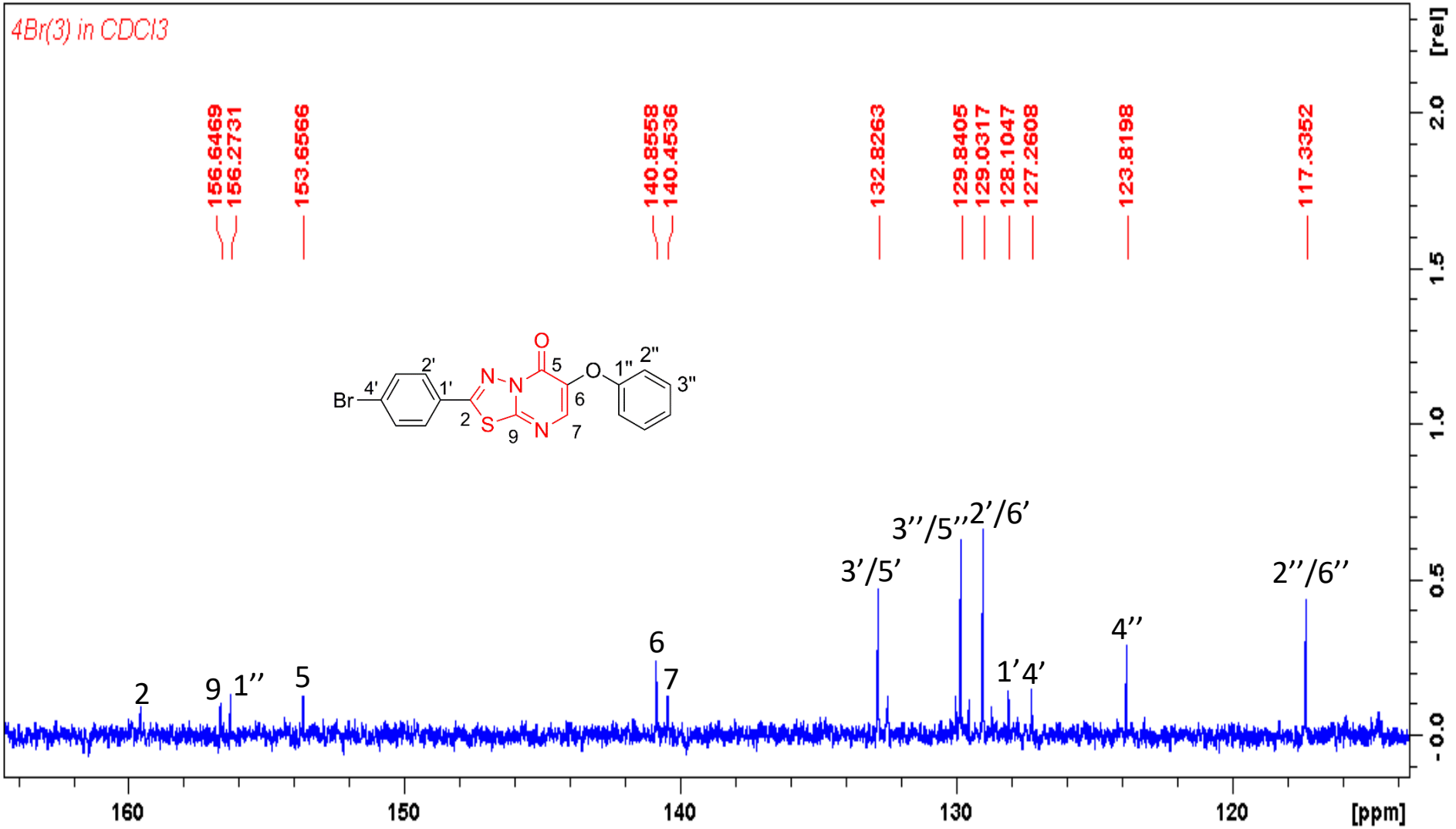
The HMBC (**C→H correlation**) NMR spectrum of 6-phenoxy-2-(3-fluorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3f**



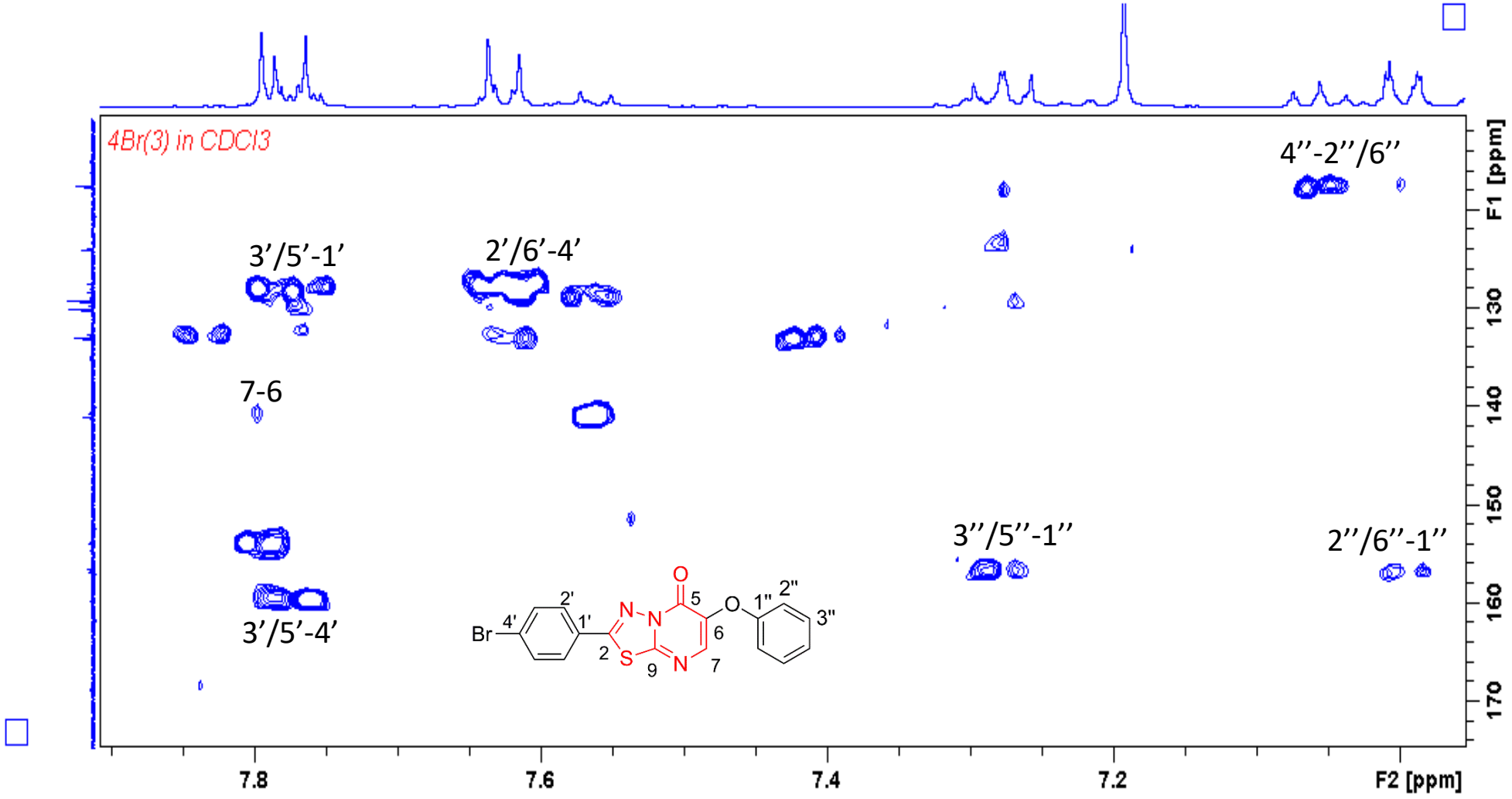
The HSQC (**C-H bonds**) NMR spectrum of 6-phenoxy-2-(3-fluorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3f**



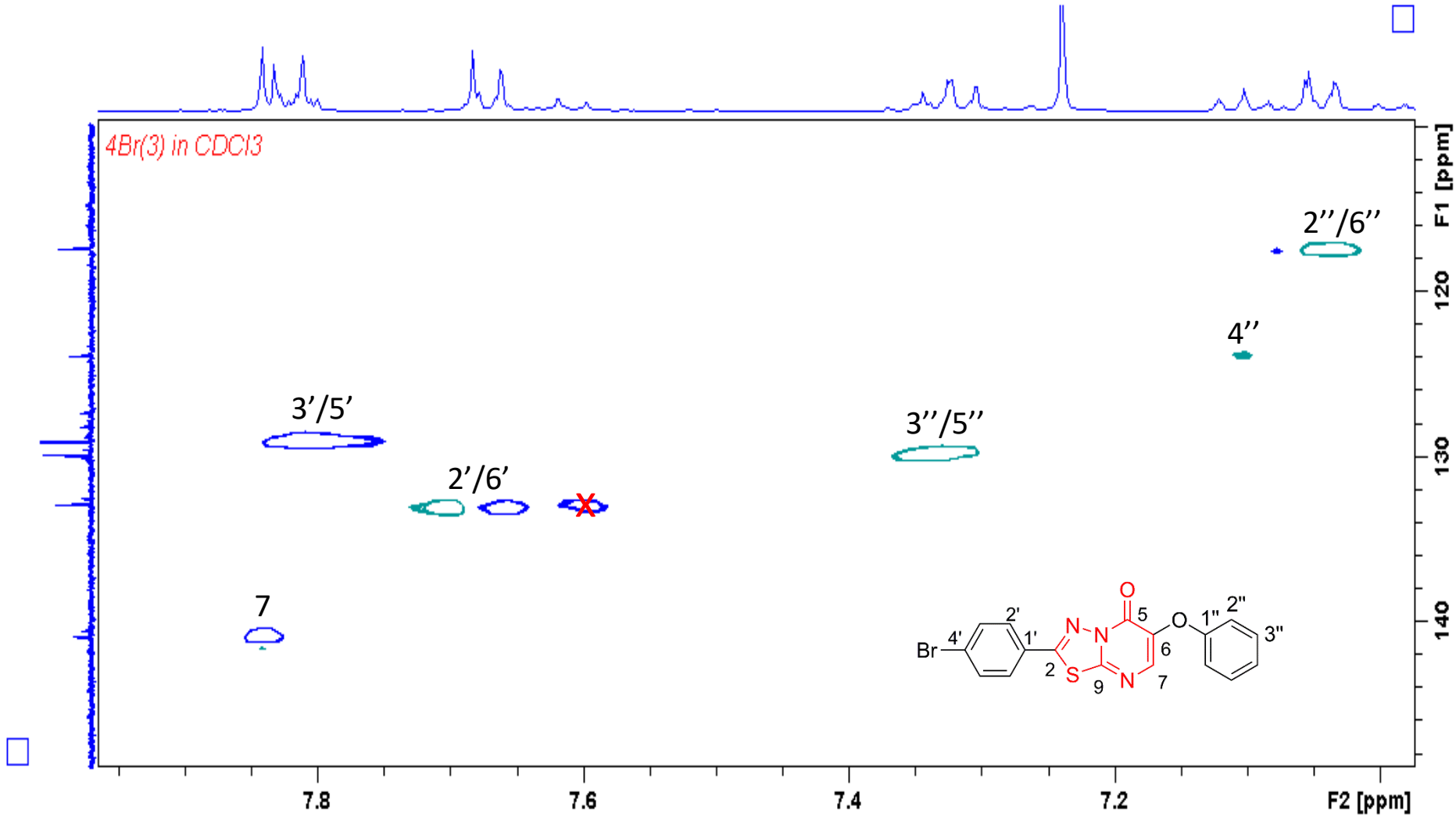
The ^{1}H NMR spectrum of 6-phenoxy-2-(4-bromophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3g**



The ^{13}C NMR spectrum of 6-phenoxy-2-(4-bromophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3g**



The HMBC (**C→H correlation**) NMR spectrum of 6-phenoxy-2-(4-bromophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3g**



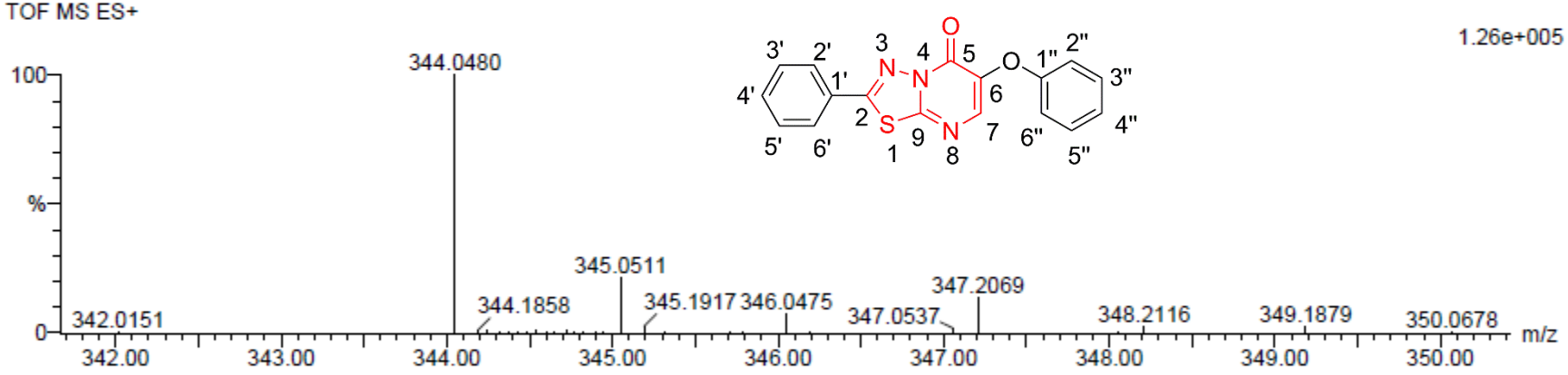
The HSQC (**C-H bond**) NMR spectrum of 6-phenoxy-2-(4-bromophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3g**

Compound 3a-g HRMS Spectra

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
30 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 S: 0-1 Na: 1-1

3A 34 (1.114) Cm (1:61)
TOF MS ES+

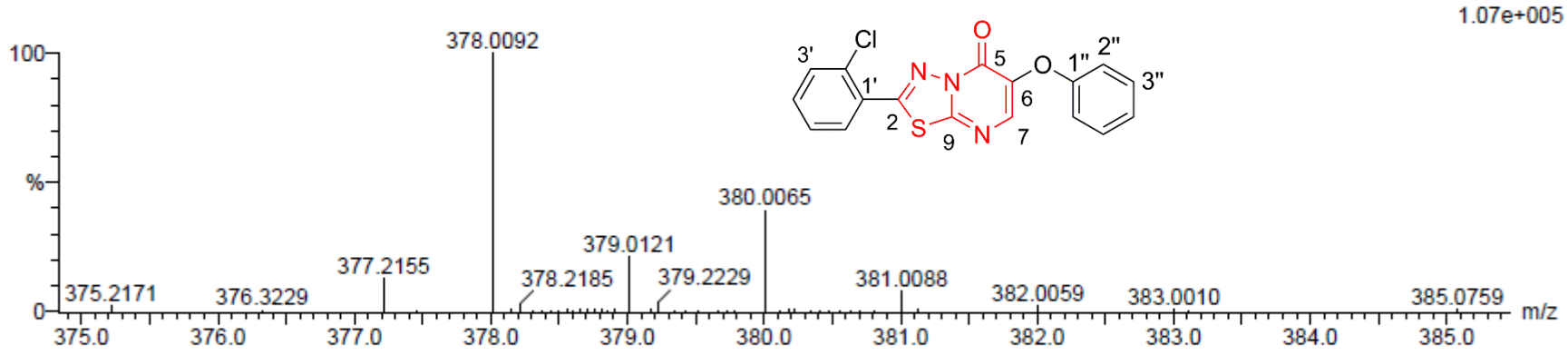


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
344.0480	344.0470	1.0	2.9	13.5	216.1	0.0	C17 H11 N3 O2 S Na

The HRMS spectrum of 6-phenoxy-2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3a**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
67 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 5-10 N: 0-5 O: 0-5 Na: 1-1 S: 0-1 Cl: 0-1
3B 33 (1.079) Cm (1:61)
TOF MS ES+



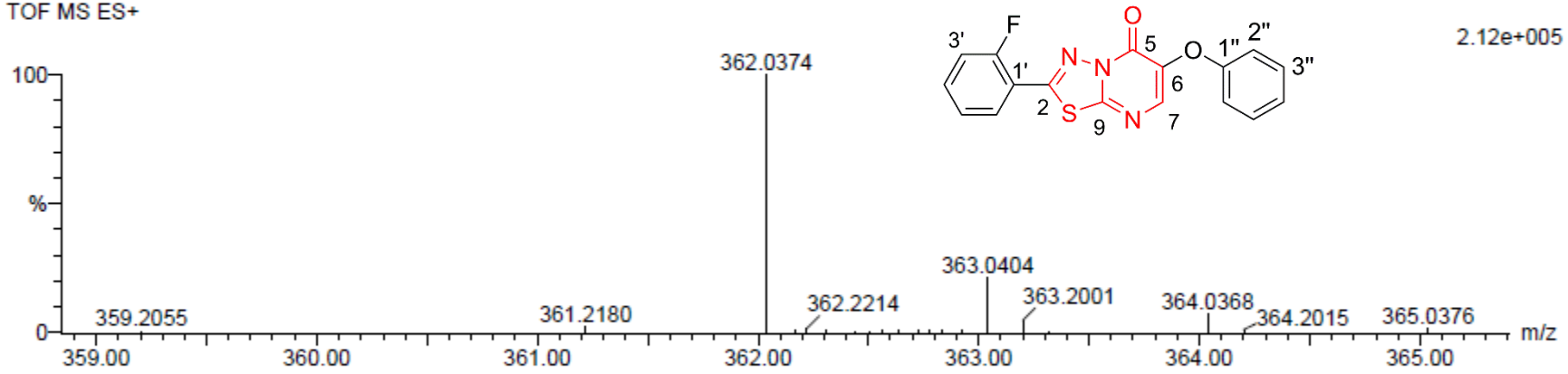
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
378.0092	378.0080	1.2	3.2	13.5	240.9	0.0	C17 H10 N3 O2 Na S Cl

The HRMS spectrum of 6-phenoxy-2-(2-chlorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3b**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
31 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 5-10 N: 0-5 O: 0-5 F: 1-1 Na: 1-1 S: 0-1

3D 2 (0.017) Cm (1:60)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
362.0374	362.0375	-0.1	-0.3	13.5	195.1	0.0	C17 H10 N3 O2 F Na S

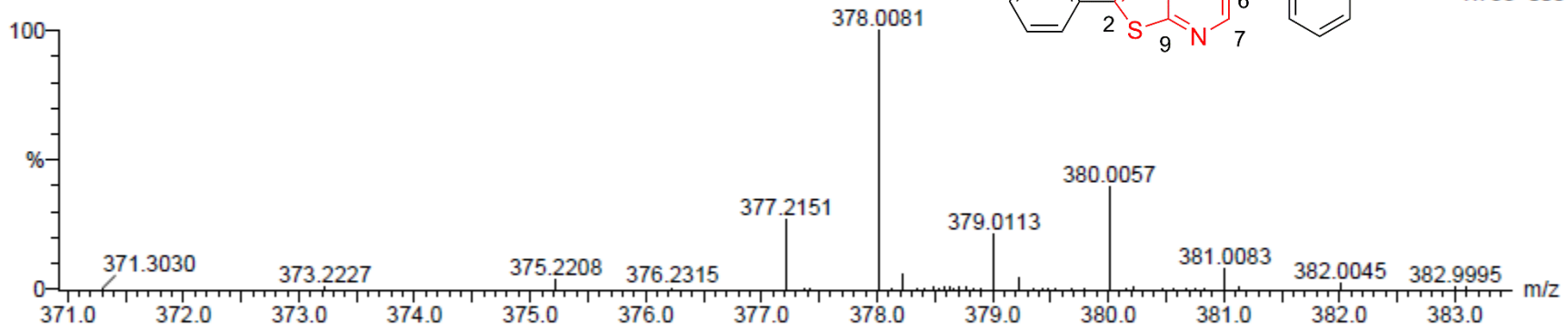
The HRMS spectrum of 6-phenoxy-2-(2-fluorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3c**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
67 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 5-10 N: 0-5 O: 0-5 Na: 1-1 S: 0-1 Cl: 0-1

3E 7 (0.203) Cm (1:61)
TOF MS ES+

1.78e+005



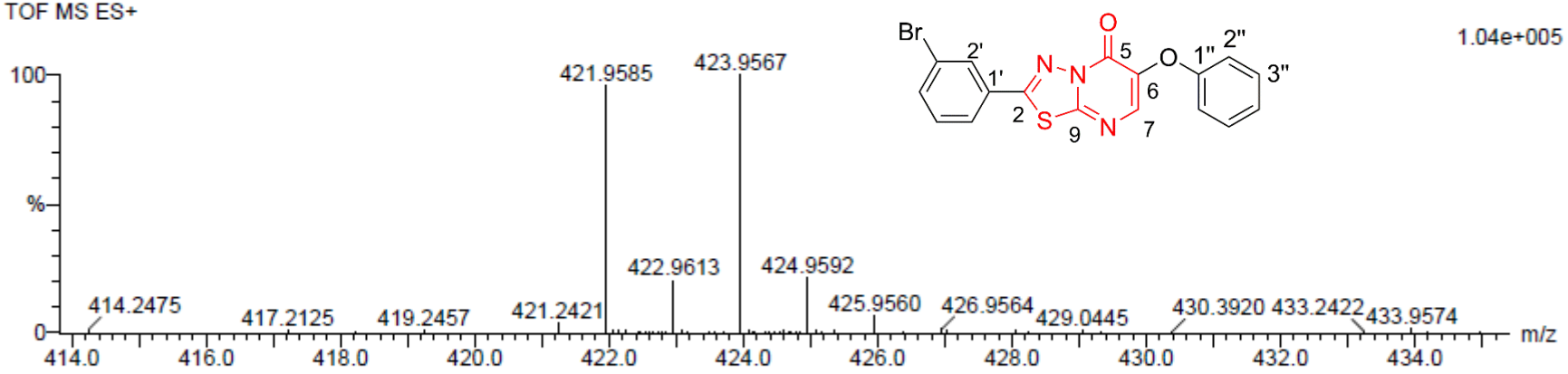
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
378.0081	378.0080	0.1	0.3	13.5	243.6	0.0	C17 H10 N3 O2 Na S Cl

The HRMS spectrum of 6-phenoxy-2-(3-chlorophenyl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3d**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
79 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 5-10 N: 0-5 O: 0-5 Na: 1-1 S: 0-1 Br: 0-1

3C 58 (1.923) Cm (1:61)
TOF MS ES+



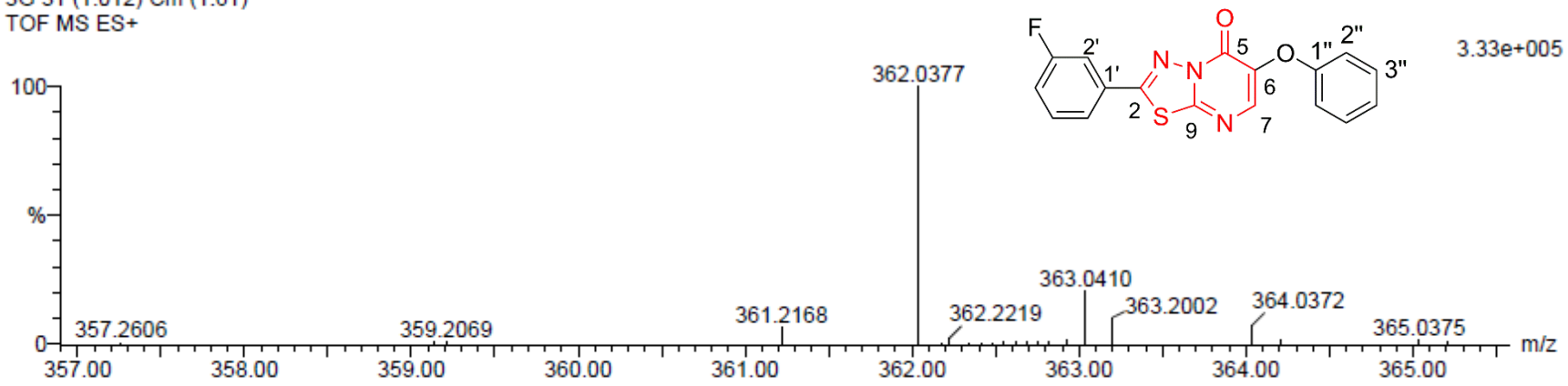
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
421.9585	421.9575	1.0	2.4	13.5	170.2	0.0	C17 H10 N3 O2 Na S Br

The HRMS spectrum of 6-phenoxy-2-(3-bromophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3e**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
31 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 5-10 N: 0-5 O: 0-5 F: 1-1 Na: 1-1 S: 0-1

3G 31 (1.012) Cm (1:61)
TOF MS ES+



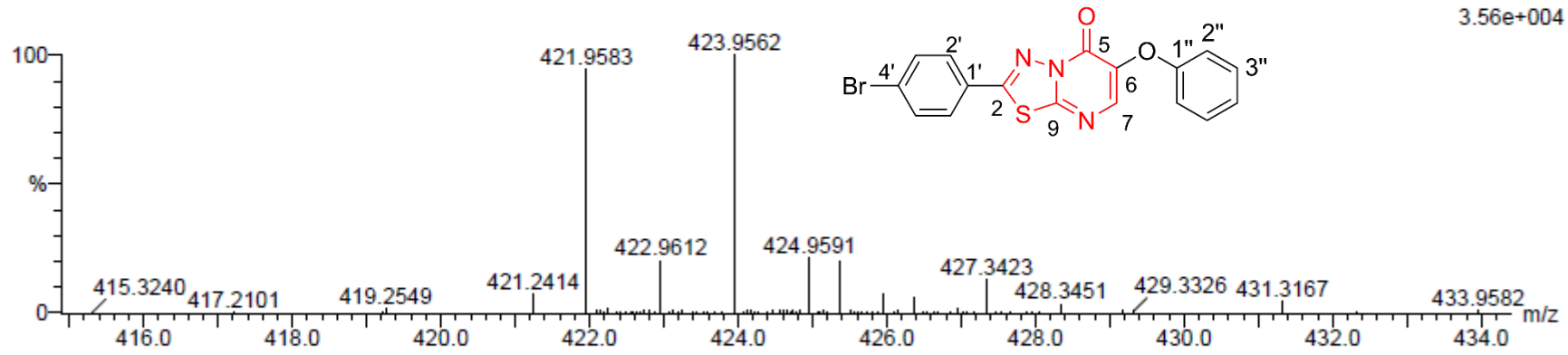
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
362.0377	362.0375	0.2	0.6	13.5	197.2	0.0	C17 H10 N3 O2 F Na S

The HRMS spectrum of 6-phenoxy-2-(3-fluorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3f**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
79 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 5-10 N: 0-5 O: 0-5 Na: 1-1 S: 0-1 Br: 0-1

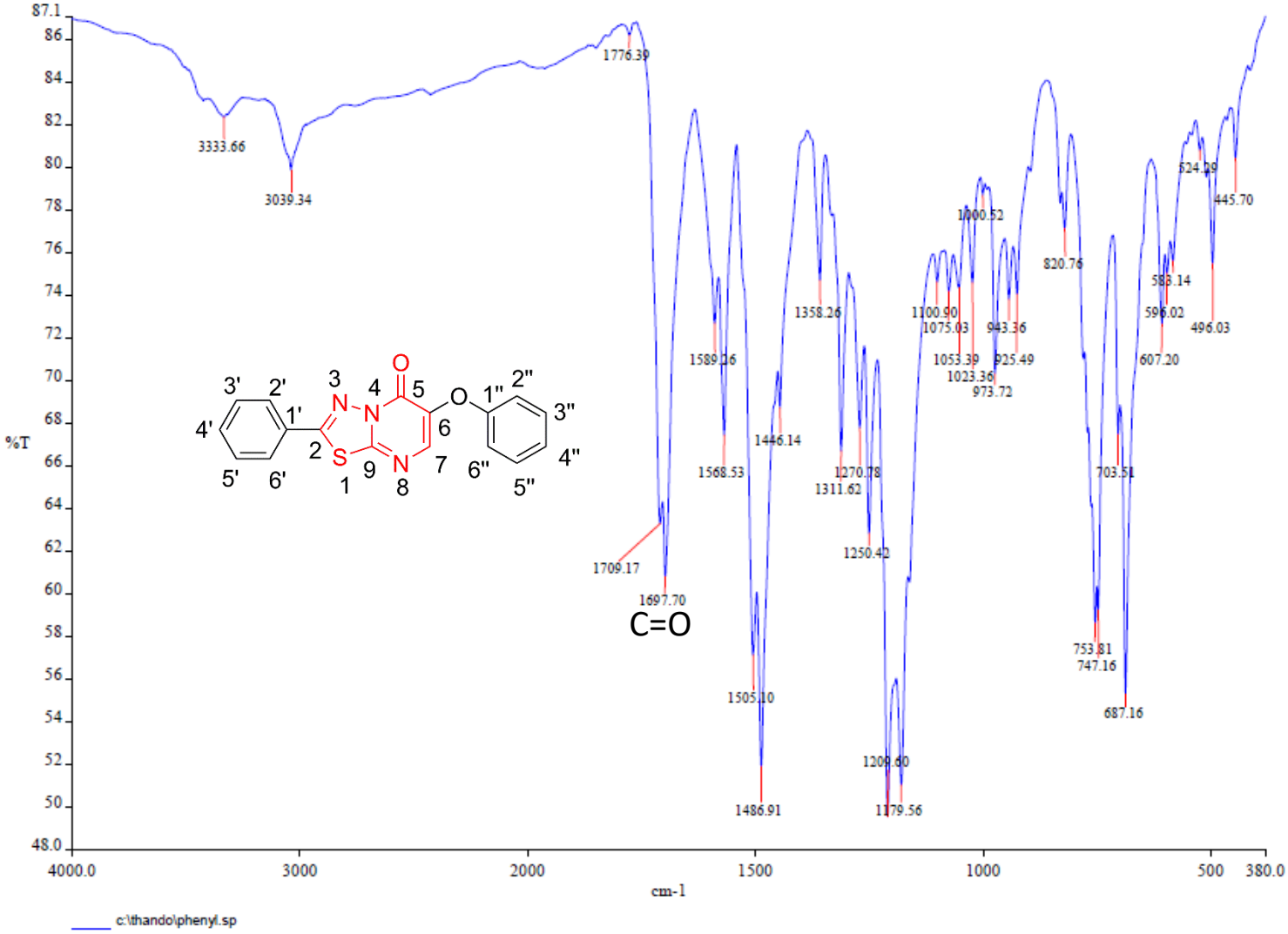
3l 2 (0.034) Cm (1:61)
TOF MS ES+



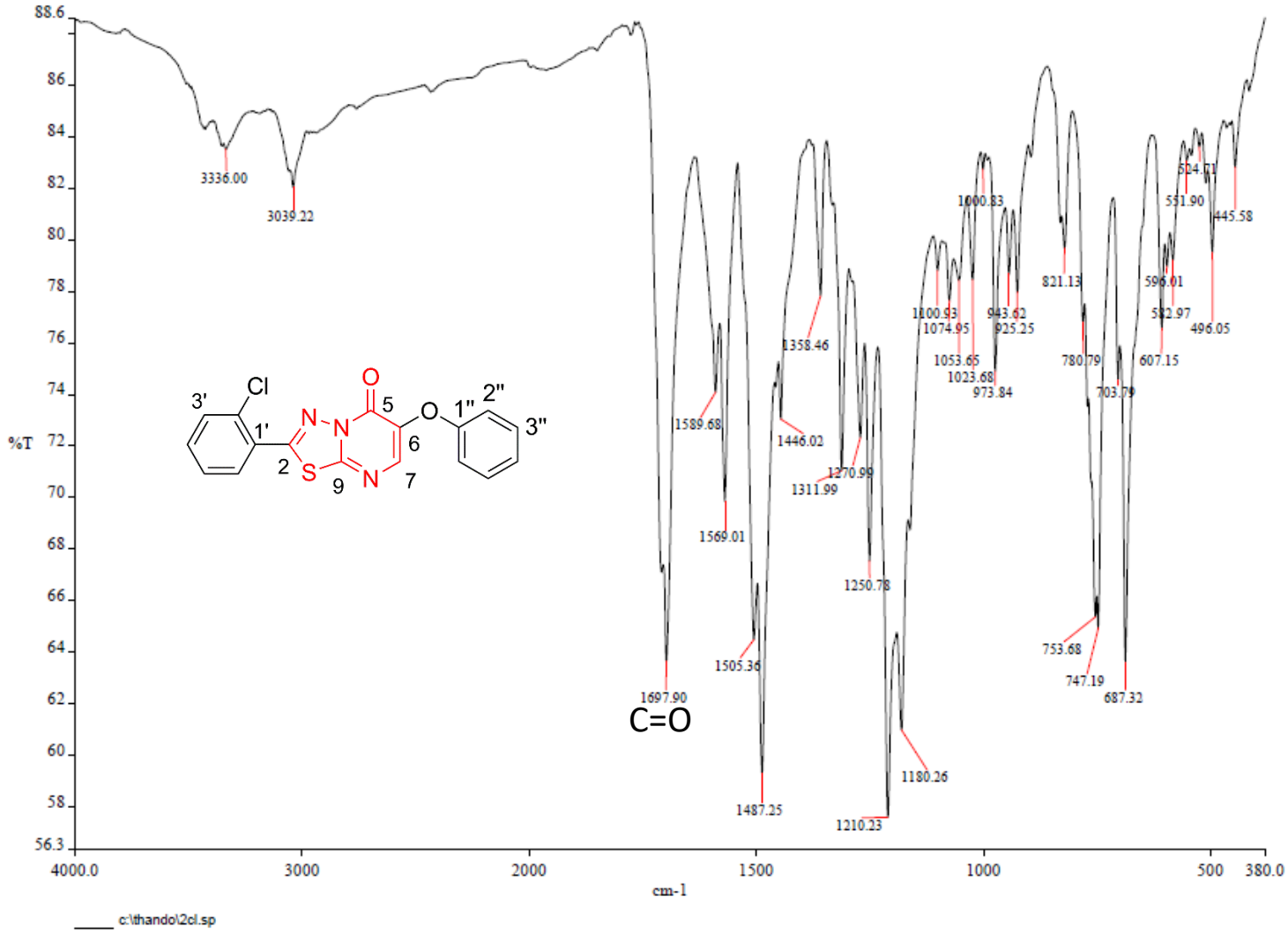
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
421.9583	421.9575	0.8	1.9	13.5	219.0	0.0	C17 H10 N3 O2 Na S Br

The HRMS spectrum of 6-phenoxy-2-(4-bromophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3g**

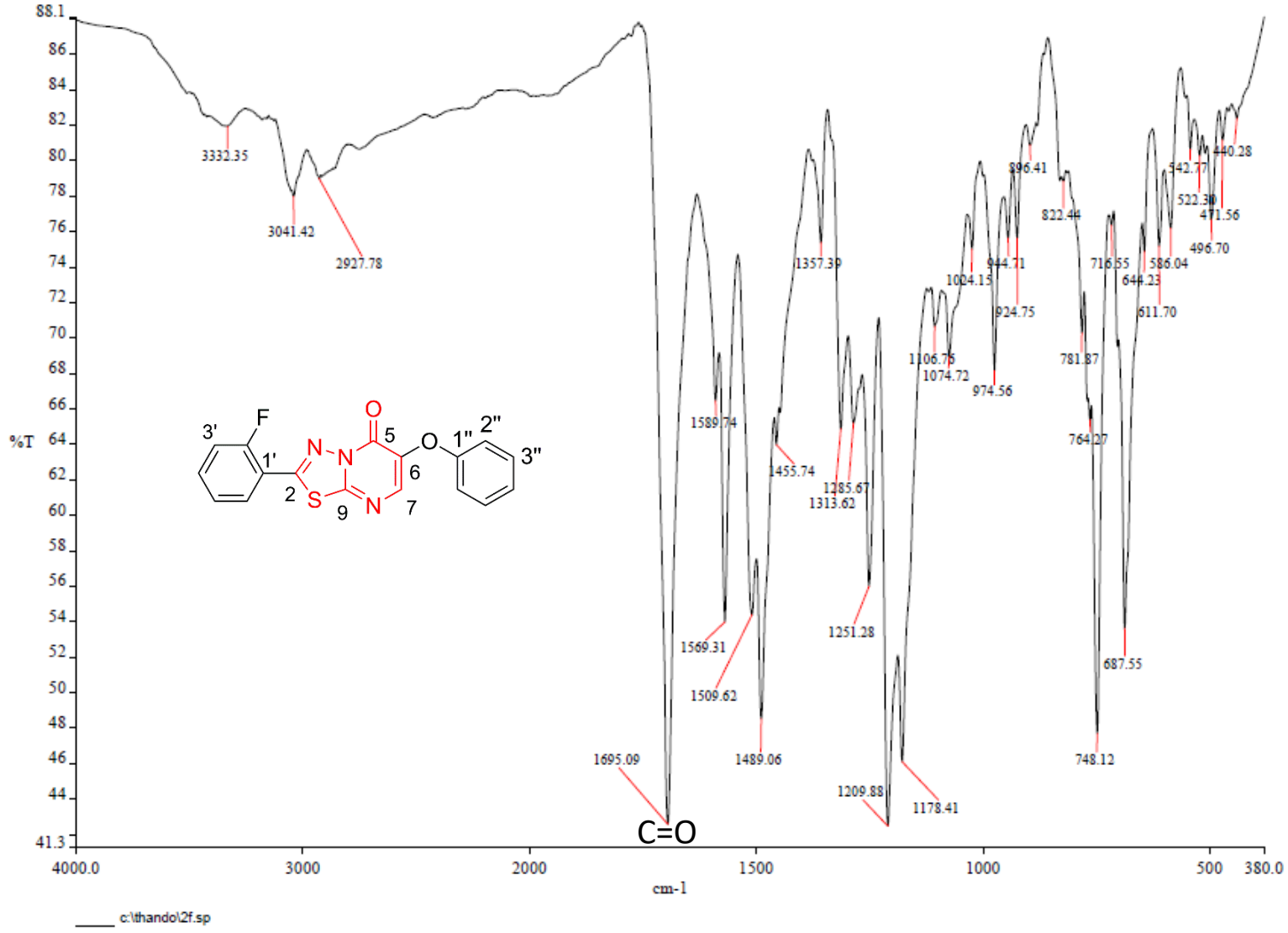
Compound 3a-g IR spectra



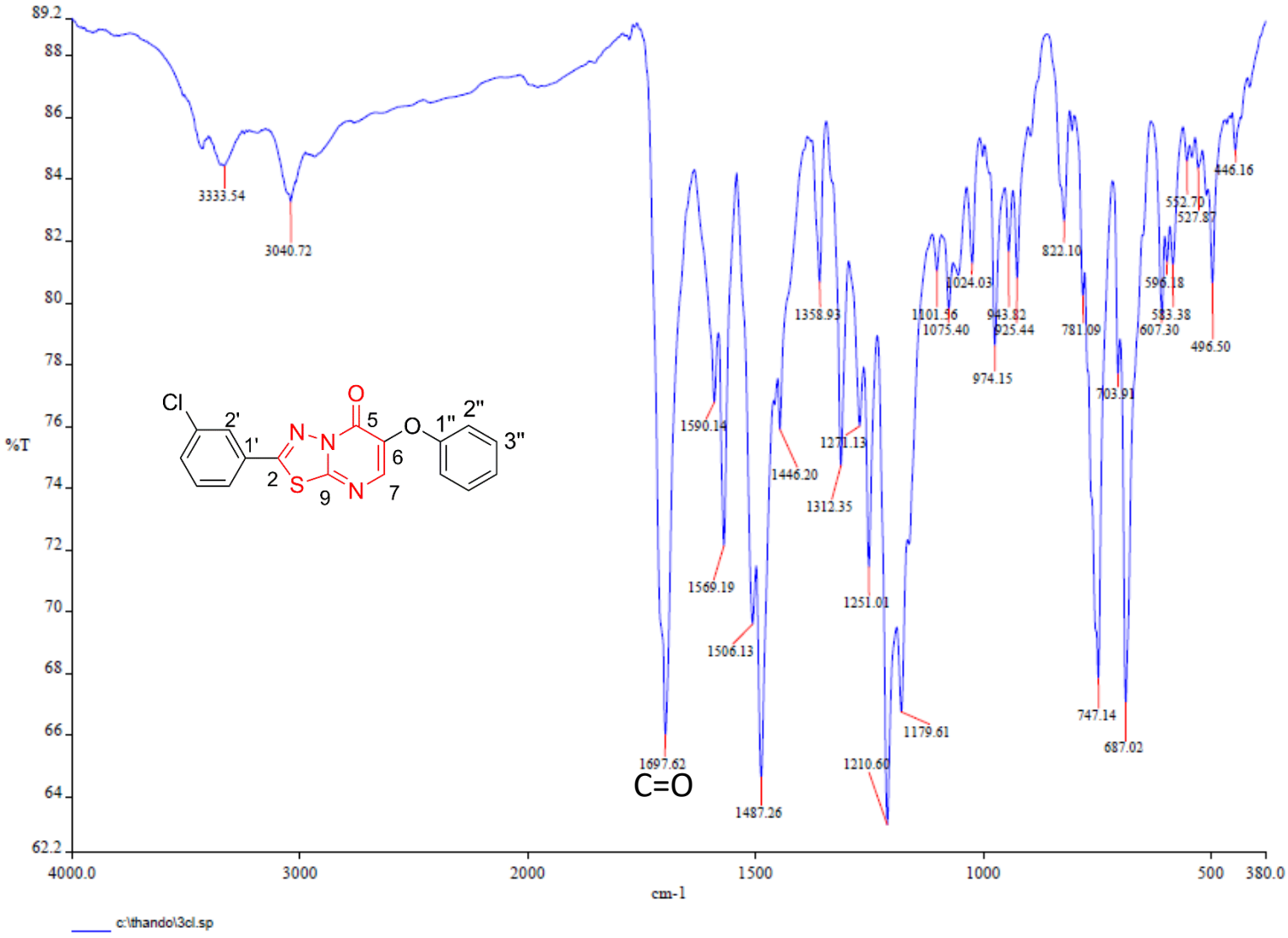
The IR spectrum of 6-phenoxy-2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3a**



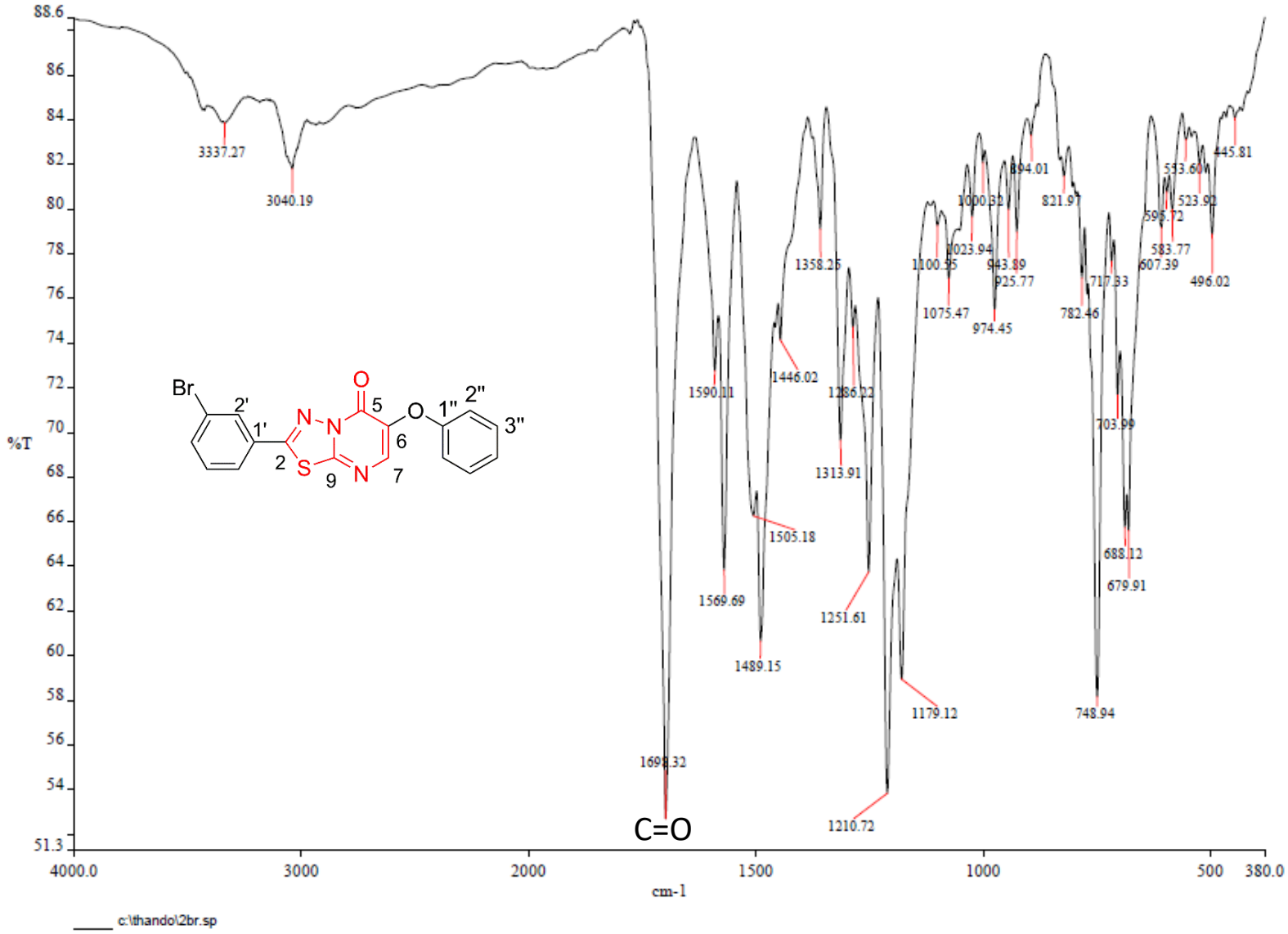
The IR spectrum of 6-phenoxy-2-(2-chlorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3b**



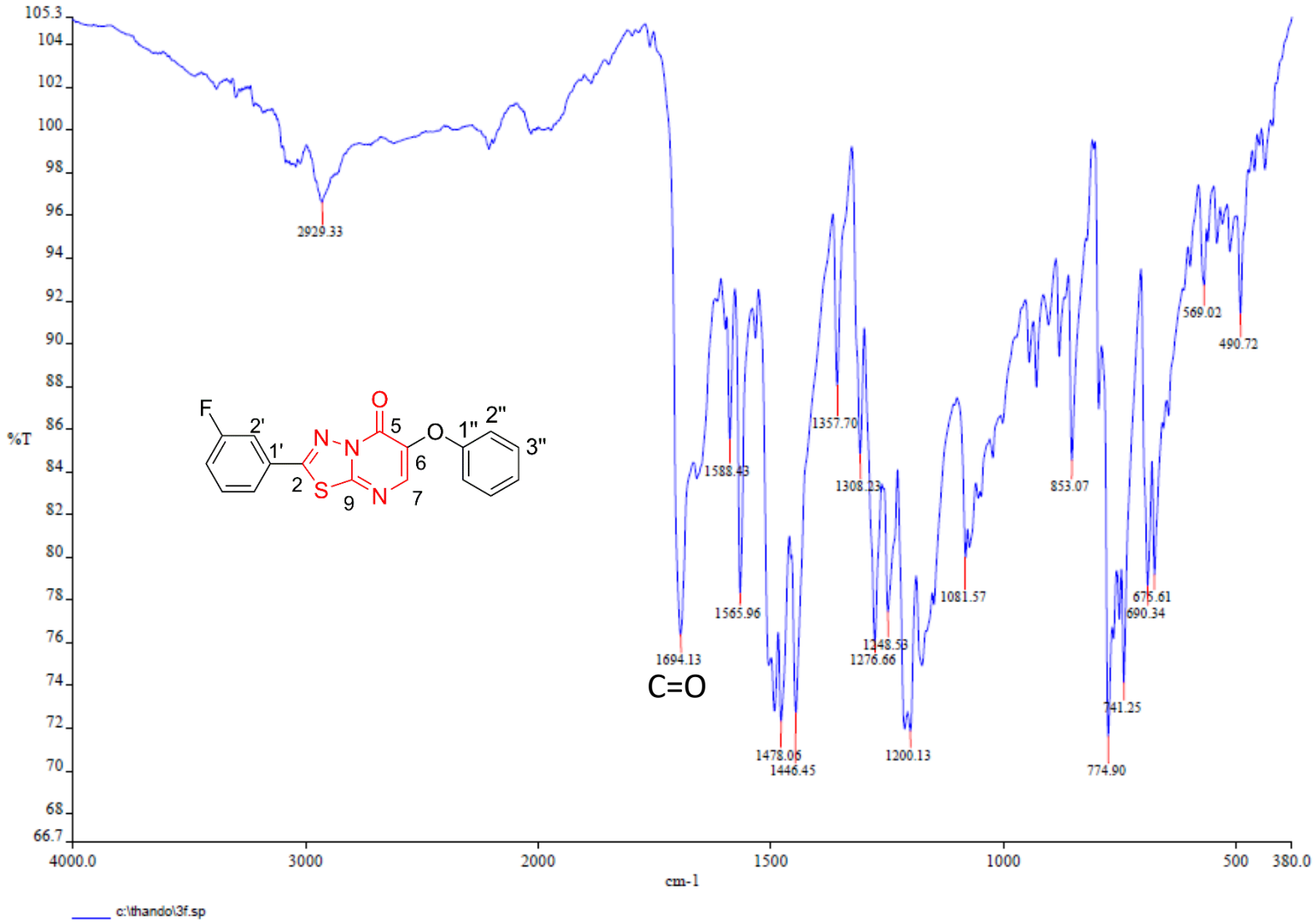
The IR spectrum of 6-phenoxy-2-(2-fluorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3c**



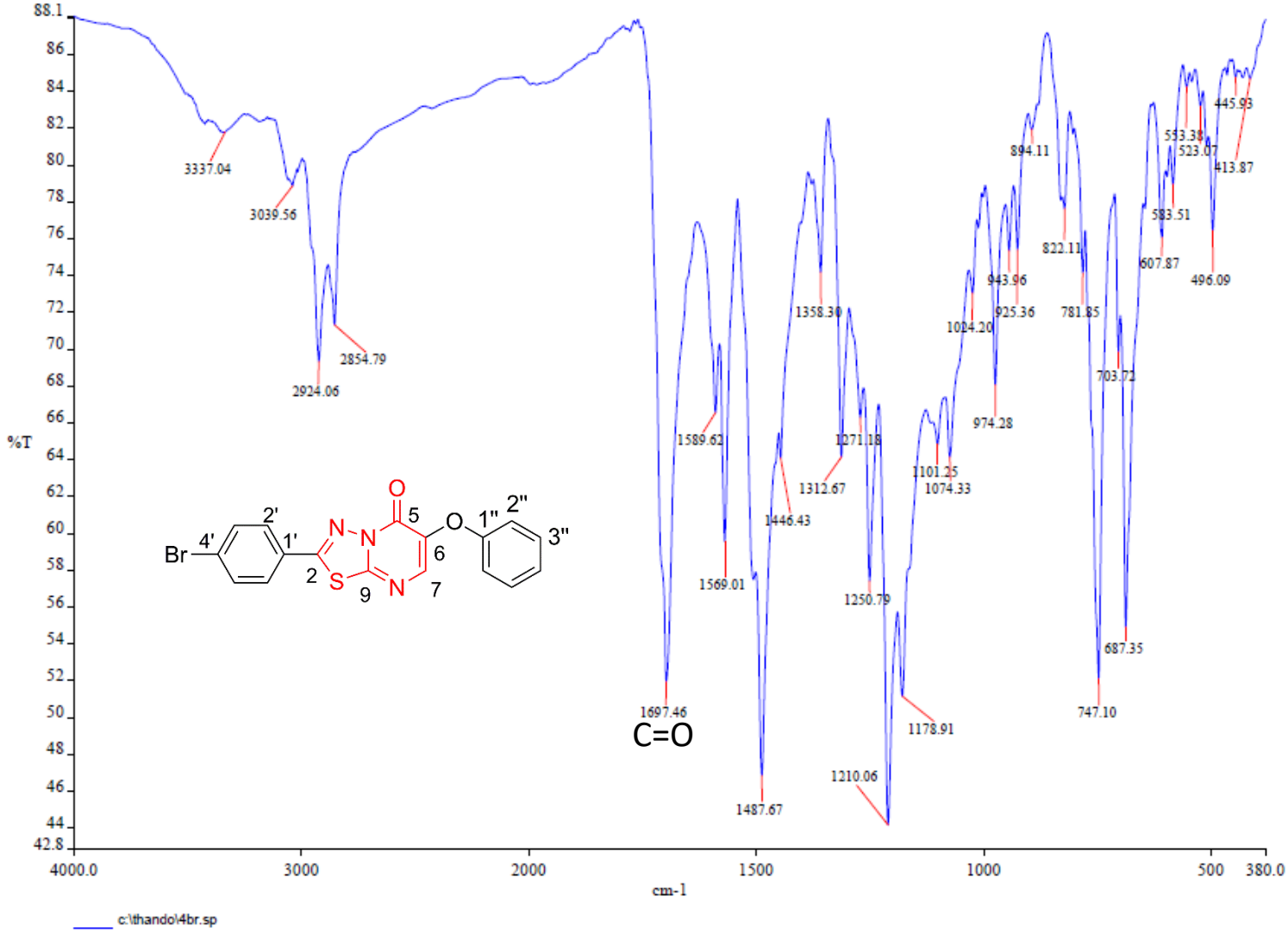
The IR spectrum of 6-phenoxy-2-(3-chlorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3d**



The IR spectrum of 6-phenoxy-2-(3-bromophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3e**



The IR spectrum of 6-phenoxy-2-(3-fluorophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3f**



The IR spectrum of 6-phenoxy-2-(4-bromophenyl)-5*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one **3g**