Amino acids based Sulphonamides and their hybridization with Substituted Piperazine and Metronidazole drug; Synthesis, Characterization and their Antibacterial potential

A Dissertation Submitted to the Department of Chemistry,

Quaid-i-Azam University Islamabad, Pakistan, in the fulfillment of the requirements for the Degree of

Master of Philosophy

in

Organic Chemistry

By

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Quaid-i-Azam University Islamabad, Pakistan

2022

DECLARATION

This is to certify that this dissertation entitled **"Amino acid-based Sulfonamide and their Hybridization with Substituted Piperazine and Metronidazole Drug; Synthesis, Characterization and their Antibacterial Potential"** submitted by **Ms. Sana Khadim,** is accepted in its present form by the Department of Chemistry, Quaid-i-Azam University, Islamabad, as satisfying the dissertation requirements for the degree of ***Master of Philosophy in Organic Chemistry.***

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"O My Lord! Expand for me my chest and make my work easy for me loosen the knot from my tongue so they may understand my speech"

(Ta'Ha, 25-28)

The Prophet Muhammad (P.B.U.H) said "ALLAH, His angles and all those in heavens and on the earth, even ants in their hills and fish in the water, call down blessings on those who instruct others in beneficial knowledge"
(Al-Tirmidhi, Hadith 422)

DEDICATED TO MY LOVING

GRANDMOTHER

Who had a passionate desire to see me entering professional life yet unfortunately passed away during the currency of my MPhil programme. Already miss her immensely.

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*By the grace of **Almighty Allah**, the Most Gracious, the most beneficent and source of ultimate knowledge, who enabled me to complete this contribution with the due time. All the respect for the **Holy Prophet (PBUH)**, the soul and the cause of the creation of the universe, the greatest Educator and Teacher of humanity who brought the educational revolution in this world.*

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Thank you very much.

Sana Khadim

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List of Abbreviation

^{13}C	Carbon-13
COVID 19	Corona virus disease 2019
CDI	Carbonyldiimidazole
DCC	*N, N'*-Dicyclohexyl carbodiimide
DCE	1,2-Dichloroethane
DCM	Dichloromethane
DIC	*N, N'*-Diisopropyl carbodiimide
DMAP	*N, N*-Dimethylaminopyridine
DMF	*N, N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
EC50	Half maximal effective concentration
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EGFR	Epidermal growth factor receptor
et al.	et alii (and others)
FDA	Food and Drug administration
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus
^{1}H	Proton
IC_{50}	Half maximal inhibitory concentration
J	Coupling constant
LC_{50}	Lethal concentration 50%
m.p.	Melting point
MIC	Minimum inhibitory concentration

MTZ	Metronidazole
NMR	Nuclear Magnetic resonance
PABA	*p*-Aminobenzoic acid
ppm	Parts per millions
SAR	Structure activity relationship
$TMSN_3$	Trimethyl silyl azide
TLC	Thin layer chromatography
TMV	Tobacco mosaic Virus
TEA	Triethylamine
U.V	Ultraviolet

Abstract

Sulfonamide and carboxamide functionalities play a vital role in pharmaceutical industry, polymer chemistry and agrochemicals. Keeping in view the importance of these functionalities, several modern and efficient methodologies have been developed for their synthesis. Moreover nitroimidazole-based compounds exhibit plethora of biological importance. The aim of this research work is the synthesis of a number of amino acids-based sulfonamides and their hybridization with metronidazole and substituted piperazine through ester and amide linkages, in view of finding more potent compounds.

Sulfonamides were synthesized in the aqueous medium from sulphonyl chlorides and amino acids, while esters and carboxamides were synthesized using acyl transfer reagents EDC and DMAP. These compounds were obtained in moderate yield and characterized by ^{1}H NMR, ^{13}C NMR and other physical parameters. The synthesized compounds containing different R-groups are the result of tweaking chemical structures of existing and reported potential drug through hybridization, which may seem to have antibacterial properties.

CHAPTER 1

INTRODUCTION

In pharmacology, a drug is a chemical material used in the treatment, cure, prevention, or diagnosis of a disease to improve physical or mental well-being. Molecular hybridization is a new concept of rational approach in drug design and development and is based on a combination of different pharmacophoric species of different bioactive substances to produce a new hybrid compound with improved affinity, biological activity and usefulness, when compared to the parent drug. Additionally, this type of approach can result in compound presenting modified selectivity profile, different or dual modes of action and reduced side effects.[1]

The pace of research and the development of "new" pharmaceutical substances has slowed down over the last few decades. First, a stringent empirical and rational approach to medication design; second, high requirements of safety and therapeutic value; third, clinical trials; and finally, enormously increased expenditures of research and development may all be contributing to this particular trend in drug development. Drug development seeks to create a substance with a strong chemotherapeutic repertoire and targeted activity. It is a continuous endeavor to create a medicine as rationally as possible, minimizing the need of the trial-and-error method. Essentially, it entails the investigation of a drug's biodynamics in addition to the interactions of drug molecules with molecules similar to other drugs that make up biological objects.[2]

In essence, drug design can be thought of as an integrated process that entails a number of steps, including chemical synthesis, activity-spectrum assessment, toxicological studies, drug metabolism, or biotransformation and the examination of several metabolites produced, evaluation procedures, and formulation and biopharmaceutics. The term "drug design" in a broader sense refers to the random evaluation of synthetic and natural products in bioassay systems, the development of newer drug molecules based on biologically-active-prototypes from either the plant or animal kingdom, the fundamental ideas of isosterism and bioisosterism, and finally the precise design of a drug to allow it to interact with a compatible receptor site effectively.[3]

1.1 Prodrug approach

In recent years, researchers have come on a common platform in order to design a new approach, 'Prodrug approach', for drug modification. Albert is credited for creating the term prodrug.[4] He defined it as a pharmacologically inactive compound that the mammalian system transforms into an active substance through either chemical or

metabolic processes. The process of chemically moderating a biologically active substance to create a parent compound is known as drug latentiation.[5] The idea behind prodrugs is that they are bio-reversible compounds that are to be transformed through biotransformation into an active pharmacophore. The two distinct pharmacophores work together to produce symbiotic activity or may aid in directing the active medication toward its intended target. Prodrug approach supersedes the issues associated with prodrug development, such as boosting solubility, bioavailability, chemical stability, pre-systemic metabolism, site-specific delivery, masked toxicity, increasing patient acceptance, or eliminating unfavorable side effects. There are several uses for the prodrug strategy. It plays a big part in drug development. Numerous scientists created diverse prodrugs with a variety of objectives in mind, such as the improvement of organoleptic or chemical qualities, pharmacokinetics parameter correction, or all three. Here are a few illustrations taken from Notari.[6]

Table 1.1: Examples of Parent drug with prodrug and their purpose of modification

Parent drug	**Prodrug**	**Reason of modification**
Amoxicillin	Sarmoxicillin	Increase distribution
Cycloserine	Pentizidone	Stability
Chloramphenicol	Chloramphenicol succinate ester	Water solubility
Carbencillin	Carfecillin, Carindacillin	Enhance Bioavailability
Estradiol	Estradiol cypionate	Extend duration
Metronidazole	Amino acid esters, Benzoyl derivatives	Water Solubility Mask taste

1.1.1 Classification of Prodrug

Prodrugs can be broadly classified into following two major categories:

1. Carrier linked Prodrugs

2. Bio-precursors

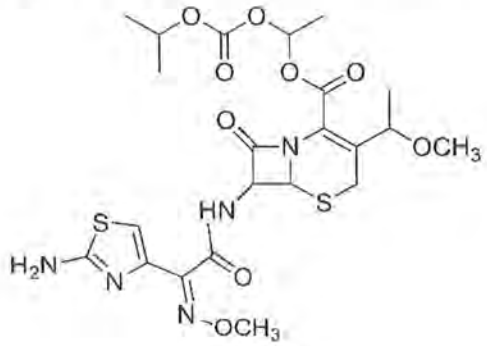
1.1.1.1 Carrier linked prodrugs or Simple drugs

An inert transporter or carrier that is covalently connected with the active medication makes up a carrier-linked prodrug. They are linked by ester or amide. They underwent

chemical or enzymatic biotransformation, releasing the active medication.[7,8] Prodrugs that are related to carriers should not be harmful. The undesirable side effects must to be concealed. Furthermore, it can be divided into the following categories based on the carrier used:

a) Pro-Prodrug / Double Prodrug

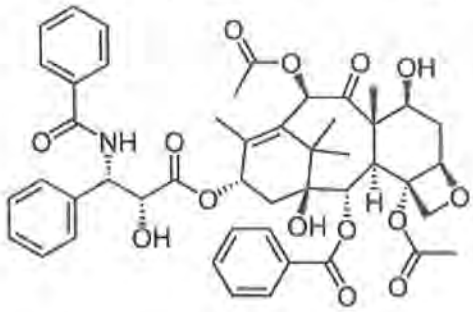
In this type, the prodrug is derivatized for a site specific enzymatic hydrolysis, which would then result in the spontaneous release of parent drug at the target site.[9] For example Cefpodoxime proxetil **(1)**.



(1)

b) Macromolecular prodrug

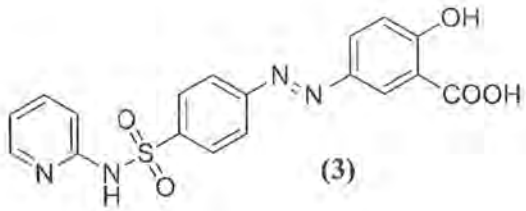
Large-molecular-weight substances, such as polysaccharides, polymers, and proteins, are used as the carriers in this case. e.g., anticancer drug such as Paclitaxel **(2)**.



(2)

c) Site specific prodrug

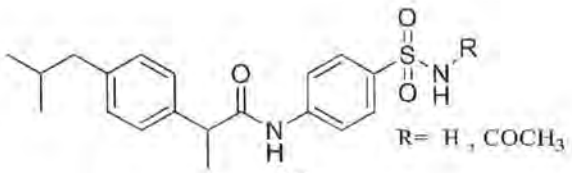
A prodrug like this one is basically employed to target the active medication at a particular place, such as sulfasalazine **(3)**, which is made up of two active components, one is 5-aminosalicylic acid and other is sulfapyridine.[10] Both substances are pharmaceutically effective drugs joined by an azo linkage. The colon releases the 5-aminosalicylic acid. The benefit of this strategy is that it releases the medication when it is needed.



(3)

d) Mutual prodrug

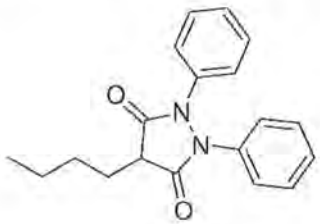
This type consists of two pharmaceutically effective drugs attached to one another. In order to achieve the same pharmacological efficacy with less time spent and fewer side effects, this strategy avoids co-administration of two drugs. For example, mutual prodrugs of ibuprofen and various sulfa drugs **(4)** are used as anti-inflammatory agents.[11]



(4)

1.1.1.2 Bio-precursors

By redox transformation with the help of enzymes, the parent drug is obtained here. Bio-precursors are devoid of any carrier or promoiety. Prodrug is the outcome of chemical alteration to the parent drug moiety. Chemical processes that are involved in the chemical transformation of bio-precursor prodrug includes hydration (e.g., lactones such as some statins), reduction or oxidation or metabolically to the active agent.[12-14] There is no general change in the lipophilicity. As an illustration, phenylbutazone **(5)**, a Oxyphenbutazone's metabolically active prodrug.

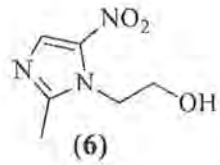


(5)

1.2 Metronidazole

Metronidazole [2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethan-1-ol, Flagyl, Pfizer] **(6)** is the first synthetic derivative of 5-nitroimidazole. In 1960, it was developed particularly to treat trichomoniasis, a genital tract infection which is caused by *Trichomonas*

vaginalis.[15] Metronidazole (MTZ) has a bioavailability of more than 90% when taken orally.[16] Metronidazole derivatives have broad spectrum of biological activities. The MTZ has remarkable value in the field of medicine for the development of novel therapeutic compounds. It is very effective to treat protozoal infections like trichomoniasis (*Trichomonas vaginalis)*, amoebiasis (*Entamoeba histolytica*), Crohn's disease, and bacterial infections (*Bacteroides fragilis, H. pylori, Fusobacterium,* and *Clostridium* sp.).[17]



(6)

1.2.1 History

After the isolation of azomycin (2-nitroimidazole) from a *Streptomyces* bacteria, researchers at Rh0ne-Poulenc tried to synthesize 2-nitroimidazole but unfortunately, all attempts were unsuccessful. After that, they focused on the synthesis of its regioisomer (5-nitroimidazole), which coincidentally led to the discovery of metronidazole in 1960, with even higher activity than azomycin.[18] In 1962, the anti-bacterial potential of metronidazole was found serendipitously, when a patient with ulcerative gingivitis and T. vaginalis was effectively treated for both illnesses.[19]

1.2.2 Mode of Action of Metronidazole

Metronidazole **(6)** is a prodrug that requires the nitro group to be bioactivated in order to have a cytotoxic action. It enters the cell through passive diffusion. MTZ captures an electron from ferredoxin and reduces the nitro group which is attached to imidazole ring, into a nitro radical anion species **(7)**. These short-lived radical species interact with cellular components, resulting in breakage of DNA strands and cell death. On the other hand, in the presence of oxygen, the toxic nitro radical anion is rapidly re-oxidized to its parent drug and the bactericidal effects are reduced.[20]

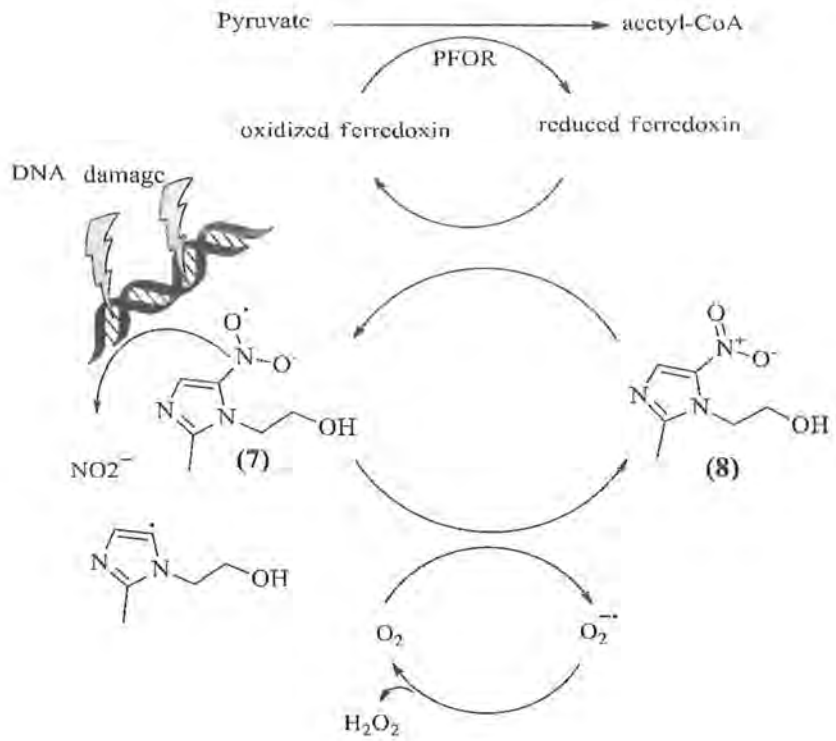
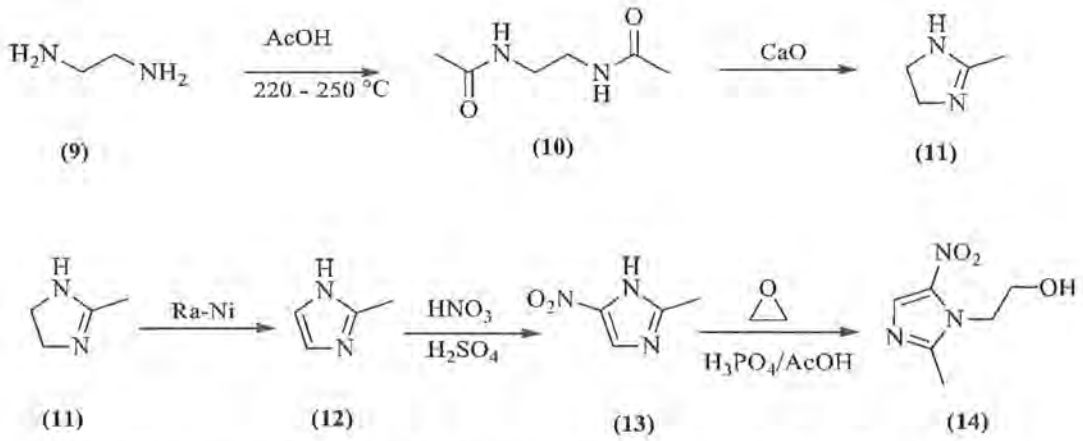


Figure 1.1: Mechanism of drug action

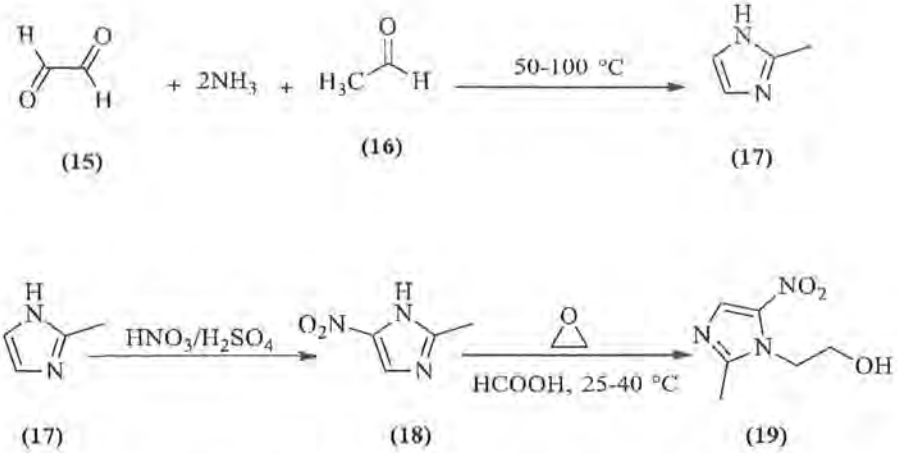
1.2.3 Synthesis of Metronidazole

Kraft *et al.* developed a procedure to synthesize metronidazole from the simplest and easily available starting material, ethylenediamine **(9)**, which was treated with acetic acid to yield *N*, *N'*-diacetylethylenediamine **(10)**, followed by the reaction of **(10)** and **(11)** with quick lime (CaO) and Raney nickel, respectively which delivered 2-methylimidazole **(12).** The nitration of 2-methylimidazole **(12)** was carried out with HNO_3 and H_2SO_4. Finally, hydroxyethylation of 2-methyl-5-nitroimidazole **(13)** with ethylene oxide provided MTZ **(14)**.[21] The schematic representation is depicted in **Scheme 1.1**.



Scheme 1.1: Synthesis of metronidazole from ethylenediamine

Ebel *et al.* reported the synthesis of 2-methylimidazole **(17)** via Debus-Radziszewski imidazole synthesis, followed by nitration to yield a 2-methyl-5-nitroimidazole intermediate **(18)**. Then the addition of ethylene oxide afforded the synthesis of MTZ **(19)**.[22]



(15) (16) (17)

(17) (18) (19)

Scheme 1.2: Synthesis of metronidazole from glyoxal

1.2.4. Side effects and Drug Resistance

Despite the significant potency of MTZ, considerable toxicity (neurotoxicity and genotoxicity) and side effects have been reported.[23] Gastrointestinal discomfort is the most frequent adverse effect, which includes anorexia, diarrhea, vomiting, abdominal pain, nausea, headaches, swollen tongue, and peripheral neuropathy.[24] MTZ clinical resistance has also been reported in anaerobic bacteria, most likely as a result of reduced drug absorption and down regulation of reductive enzymes in the specific receptor site.[25]

1.2.5 Molecular Hybridization of Metronidazole

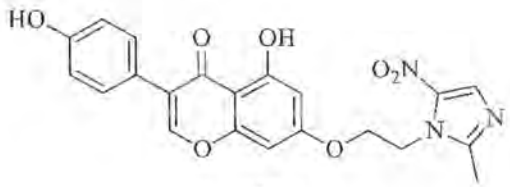
Metronidazole is hybridized with other active scaffolds to overcome adverse effects, clinical resistance, and to increase its effectiveness. Due to the crucial role of the nitro group, various structural changes have been made on MTZ without altering the nitro group. Different novel derivatives of MTZ have demonstrated a wide spectrum of biological activities, including anti-microbial,[26] anti-cancer,[27] anti-diabetic,[28] anti-HIV,[29] anti-leishmanial,[30] anti-parasitic,[31] and anti-inflammatory.[32]

1.2.6 Biological Activities of Metronidazole Hybrids

1.2.6.1 Anti-bacterial Activity

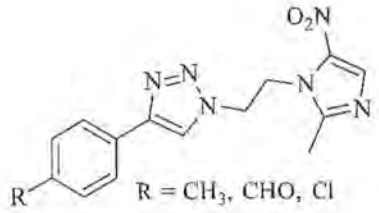
Li *et al.* synthesized a library of novel metronidazole-flavonoid conjugates. These conjugates were screened for anti-microbial potential against *H. pylori*. Among all

compounds, the genistein-metronidazole derivative **(20)** showed enhanced activity against *H. pylori* (MIC = 0.39 μg/mL).[33]



(20)

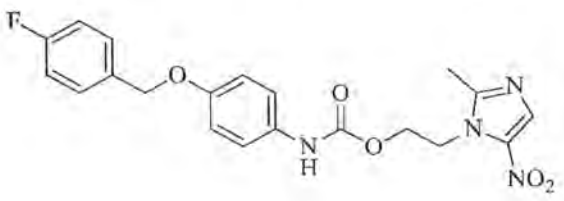
Rawat *et al.* synthesized novel metronidazole-triazole hybrids. These hybrids were assessed for anti-bacterial potential against *S. aeureus*, *P. aerogenosa*, and *E. coli*. The chloro substituted compound **(21)** was the most active again *E. coli* (IC_{50} = 0.003 μg/mL) and *P. aerogenosa* (IC_{50} = 0.020 μg/mL) than the reference compound (tetracycline). This **(21)** was the only compound that showed activity against *S. aeureus* (IC_{50} = 0.130 μg/mL). The SAR studies exhibited that the substitution at the fourth position of the benzene ring of **(21)** by a group like -CH_3, -CHO, and -Cl is pivotal for anti-bacterial activity.[34]



(21)

1.2.6.2 Anti-amoebic Activity

A library of different aryl carbamate-metronidazole hybrids has been synthesized and assessed for their anti-amoebic potential against *Entamoeba histolytica*. Among all the compounds, the fluoro substituted derivative **(22)** showed significant anti-amoebic potential with an IC_{50} value equal to 0.08 μM as compared to the commercial drug metronidazole with an IC_{50} = 1.78 μM.[35]



(22)

Duan *et al.* developed a series of isoquinoline and styryl tethered nitroimidazole derivatives. All these compounds were evaluated for telomerase inhibitory potential as well as anti-proliferative activities on various cancer cell lines (HepG-2, A-549, U-251, and Hela). In comparison to staurosporine, compounds **(26)** and **(27)** were shown to be the most effective telomerase inhibitors. The observed IC_{50} values were 0.98 and 1.92 μM, respectively.[39]

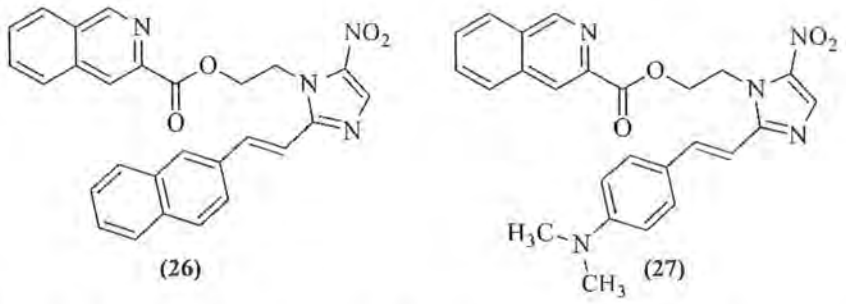


Figure 1.2: Isoquinoline and styryl tethered nitroimidazole derivatives as anti-cancer agent

1.2.6.4 Anti-diabetic Activity

Diabetics are more likely to have macro-vascular problems like unfavorable cerebrovascular events, stroke, hypertension, and myocardial ischemia.[40] Taha *et al.* synthesized metronidazole-aryl ether derivatives and evaluated them for *α*-amylase inhibitory potential. Two compounds **(28)** and **(29)** exhibited remarkable activity (IC_{50} = 0.38 and 1.6 μM, respectively), in contrast to positive control acarbose (IC_{50} =1.66 ± 0.1 μM).[41]

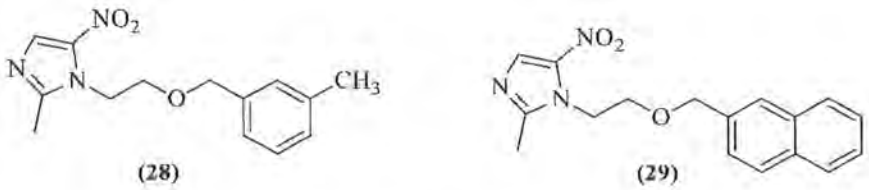
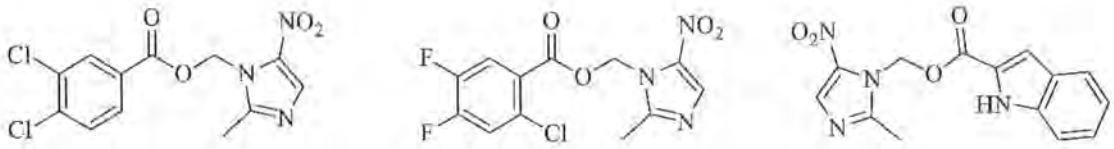


Figure 1.3: Anti-diabetic ether derivatives of metronidazole

Several derivatives of metronidazole and aryl carboxylate have been developed and tested for *β*-glucuronidase inhibitory efficacy. Many compounds have shown promising inhibitory activity in contrast to reference D-saccharic acid lactone (DSL). Compounds **(30, 31, 32)** demonstrated superior inhibitory activity (IC_{50} = 3.60, 1.20, 2.10 μM, respectively) than DSL. So compounds with -F, -Cl, -Br, and -NO_2 groups on the phenyl ring have higher inhibition efficacy against the *β*-glucuronidase enzyme.[28]

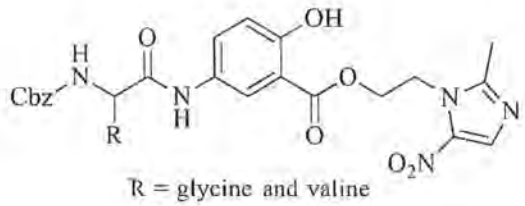


(30) (31) (32)

Figure 1.4: Some metronidazole based anti-diabetic compounds

1.2.6.5 Anti-inflammatory Activity

Naumov *et al.* synthesized novel mesalazine-metronidazole hybrids **(33)** and screened them for anti-inflammatory potential at 10 mg/kg dose in rats. These conjugates showed comparable anti-inflammatory activity with parent drugs *viz.* metronidazole, mesalazine, and indomethacin. The potent compounds possessing valine and glycine amino acids showed enhanced efficacy due to the amino acid group.[42]

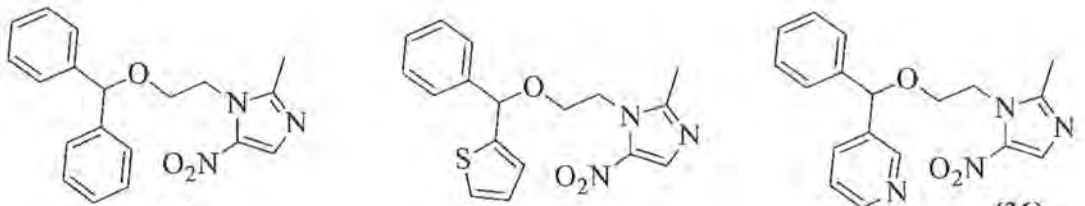


R = glycine and valine

(33)

1.2.6.6 Anti-HIV Activity

Silvestri *et al.* reported ether conjugates of MTZ **(34-36)** as anti-HIV which are effective at sub-micromolar quantities.[43] Substitution of one phenyl group of compound **(34)** with heterocyclic moiety, like pyridinyl and thienyl, led to the development of DAMNIs with enhanced potency.[44] Compounds **(35)** and **(36)** with EC_{50} values of 0.03 and 0.08 μM, respectively, were found more active than compound **(34)** against the HIV-1 strain.

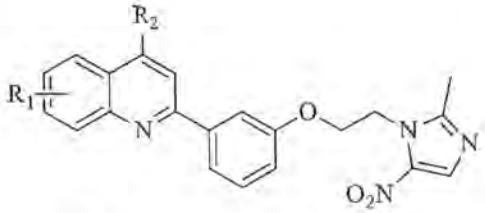


(34) (35) (36)

Figure 1.5: Ether derivatives of metronidazole with anti-HIV activity

1.2.6.7 Anti-leishmanial Activity

Visceral leishmaniasis is a life-threatening and potentially fatal chronic illness induced by *Leishmania donovani* parasites. Each year, around 400,000 people worldwide contract visceral leishmaniasis. *N*-heterocycles have been discovered to be a promising class in the development of anti-leishmanial drugs.[45] Upadhyay *et al.* synthesized derivatives of quinoline and metronidazole and screened them against *L. donovani* parasites.[46] An *in vitro* assay showed that compound **(37)** exhibits significant antileishmanial activity.



R^1 = H, 6-Cl, 6-Br, 6-NO_2, 7,8-diCl

R^2 = H, -C_6H_5

(37)

1.2.7 Metronidazole and COVID 19

Several studies show that the cytokine storm is one of the most common causes of mortality in COVID-19 infection. Cytokines are small immunoglobular proteins released by immune cells that regulate immunity and inflammation. Cytokine storm is the excessive and uncontrolled release of cytokines, which causes multisystem organ failure and death. MTZ decreases the levels of cytokines. In this way, MTZ due to its immunopharmacological behavior might be a potential candidate to combat most of the immunopathological characteristics of the COVID-19.[47]

1.3 Sulfonamides

The amides of sulfonic acids are commonly known as sulfonamides. This class of compounds comprises -SO_2NH_2 functional group. The general formula of sulfonamides is RSO_2NH_2 where R may be alkyl or aryl.[48] Sulfonamides are amide derivatives of sulfonic acids, which are one of the classes of organosulfur compounds. They make a class of synthetic, broad-spectrum bacteriostatic antibiotics *i.e.*, they inhibit bacterial multiplication but do not actively kill bacteria. Sulfonamides have been used as drugs of choice for uncomplicated urinary tract infections, especially for the patients unable to tolerate penicillin or are allergic to it.[49] Sulfonamides are also used as topical

medicines for treatment of eye infections. Sulfonamides are used synergistically with other drugs to treat various infections. For example, mixture of sulfamethaxazole - trimethoprim (septran)[50] is preferably used in recurrent urinary infections and especially opportunistic infections in patients suffering from AIDS. The sulfonamide functional group has a great contribution in organic chemistry and drug discovery.

1.3.1 History of sulfonamides

The foundation of chemotherapeutic drug design and medicinal chemistry began in early twenty century. Modern chemotherapy started with the work of Paul Ehrlich[51] when he discovered the curative properties of a dye trypan red in 1907. In between 1909-1935, Ehrlich and others tested tens of thousands of chemicals including many dyes in search of magic bullets, but few compounds produced desired effects. In 1935. Gerhard Domagk gave his daughter, who was suffering from streptococcal infection and neared death, an oral dose of dye named Prontosil. The girl recovered in a short time span. This was the beginning of the spectacularly productive phase in modem chemotherapy. Domagk was awarded with Nobel Prize for medicine in 1939).[52]

Thus, credit of discovery of sulfanilamide as a first chemotherapeutic agent must be given to Gerhard Domagk, who proposed the testing of prodrug Prontosil in 1935. Later on Jacques and Therese Trefouel along with Federico Nitti and Daniel Bovet at Pasteur Institute discovered that the active component is 4-aminobenzenesulfonamide.[52] They proclaimed that the azo linkage of prontosil is reduced by azo reductase producing sulfanilamide which is active against streptococci. Thus, the first synthesized sulfonamide was sulfanilamide.

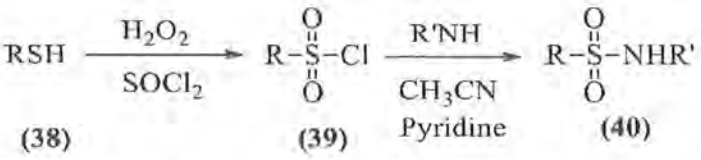
Sulfonamides unleashed a revolution in medicine to rationally design new therapeutic drugs. Thousands of variations were carried out on this moiety which resulted in the discovery of new compounds with varying pharmacological properties. Sulfonamides constitute an important class of compounds for the treatment of infectious diseases.

1.3.2 Synthesis of Sulfonamides

Usually, Sulfonamides can be synthesized from sulphonyl chlorides and primary or secondary amines. However, many other synthetic routes are also available for sulfonamide synthesis.

1.3.2.1 From Thiols

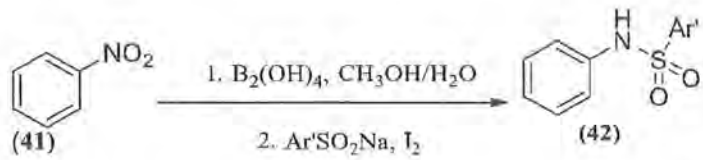
Bahrami *et al.*, (2009) reported a method in which H_2O_2 reacts with thiols **(38)** in the presence of thionyl chloride to give highly reactive sulphonyl chloride **(39)** with excellent yield. Further treatment of sulphonyl chloride with amines gave sulfonamides **(40)** with excellent yield in very short reaction time. The appropriate solvents used in this method are acetonitrile and pyridine while pyridine acts as a base.[53]



Scheme 1.3: Conversion of RSH to thionyl chloride and sulfonamide

1.3.2.2 From Sodium Sulfinates and Nitroarenes

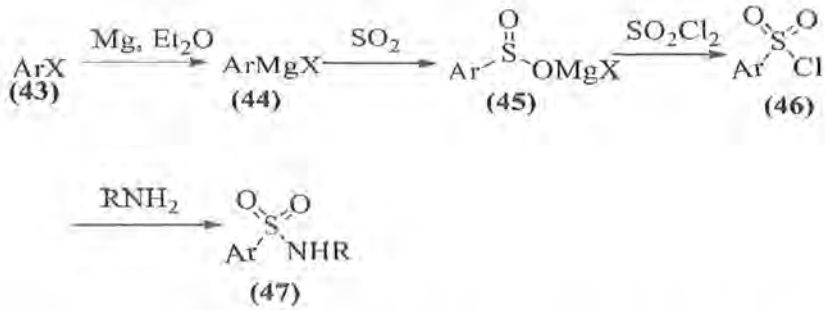
Zhou *et al.*, reported a method in which sulfonamides **(42)** can be prepared metal free one pot synthesis by the reaction of sulphonyl chlorides and nitroarenes **(41)** in the presence of diboronic acid as a reductant and water as a solvent.[54]



Scheme 1.4: N-Arylsulfonamides synthesis via sodium sulfinates and nitroarenes

1.3.2.3 One-pot Synthesis of Sulfonamides via Grignard Reagent

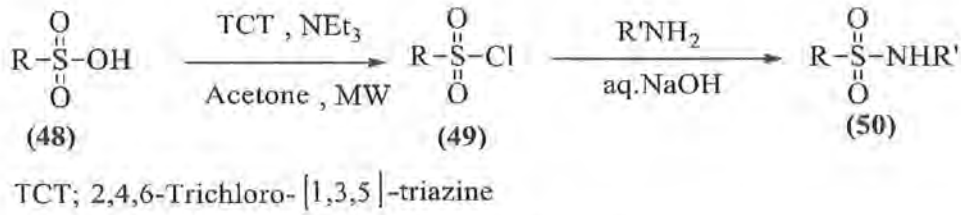
Barret *et al.* (2003) prepared a series heteroarene sulfonamides **(47)** in one pot from aryl and heteroaryl bromides *via* reaction with magnesium in the presence of diethyl ether as a solvent followed by the reaction with sulfur dioxide, sulfuryl chloride and an amine.[55]



Scheme 1.5: One-pot synthesis of sulfonamides *via* Grignard reagent

1.3.2.4 From Sulphonic Acid *via* Microwave-Assisted Synthesis

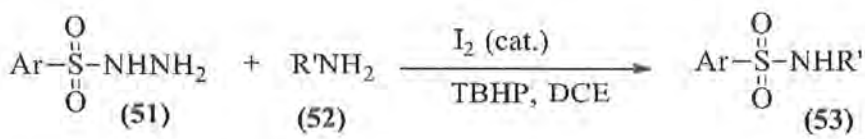
Porcheddu *et al.*, (2014) synthesized sulfonamides **(50)** by reacting the amines with substituted sulphonyl chloride **(49)** with a buffering base in the presence of aprotic solvent.[56]



Scheme 1.6: Sulfonamides synthesis from sulphonic acid via microwave-assisted synthesis

1.3.2.5 Iodine-Catalyzed Sulfonamides Synthesis from Sulfonyl Hydrazides

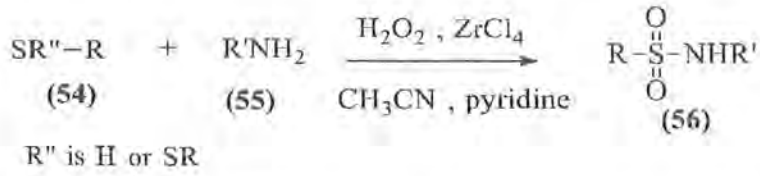
Yotphan *et al.*, (2016) prepared a series of sulphonamides **(53)** by iodine catalyzed sulphonylation of amines **(52)** with aryl sulphonyl hydrazides **(51)**. They used mild reaction conditions and brief reaction time to obtain moderate to excellent yield.[57]



Scheme 1.7: Iodine-catalyzed sulfonamides synthesis form sulfonyl hydrazides

1.3.2.6 Sulfonamides Synthesis from Disulfides and Thiols

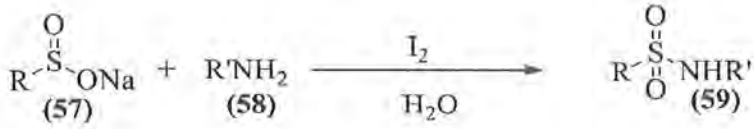
Bahrami *et al.*, (2010) utilized an efficient, economic and environmentally safe reagent $H_2O_2ZrCl_4$ for rapid sulphonamide synthesis from thiols and disulphides with excellent yield at room temperature.[58]



Scheme 1.8: Sulfonamides synthesis from disulfides and thiols

1.3.2.7 Iodine-Catalyzed Synthesis of Sulfonamides from Sodium Sulfinate

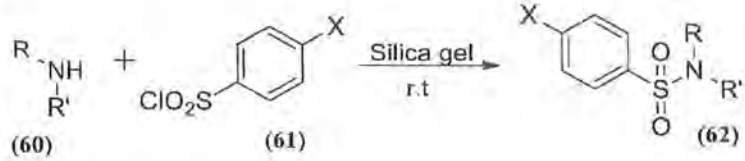
Pan *et al.*, (2015) prepared sulphonamides in a very convenient and environment friendly method by using water as a solvent in the presence of iodine as catalyst with the advantage of facile purification of product.[59]



Scheme 1.9: Iodine-catalyzed synthesis of sulfonamides from sodium sulfinate

1.3.2.8 From Sulphonyl Chlorides

An economical, safe and green method was reported by M. Jafarpour *et. al.*[60] for the catalytic synthesis of sulfonamides **(62)** through the condensation of amines **(60)** with sulfonyl chlorides **(61)** in the presence of silica gel as a heterogeneous catalyst under solvent-free conditions at room temperature. In this procedure inactive aromatic amines were also used successfully.



R, R'= H, alkyl, aryl; X= H, Me, NO_2, Br

Scheme1.10: Synthesis of Sulfonamides via condensation with amine and sulfonyl chlorides

1.3.3 Pharmacological Activities of Sulphonamides

Sulphonamides exhibit a wide range of pharmacological activities and are the most effective chemotherapeutic agents. They can be used as anti-hypertensive, anti-bacterial, antiprotozoal, anti-cancer, anti-inflammatory and anti-diuretic agent.[61]

1.3.3.1 Anti-Bacterial Activity

Sulfonamides are bacteriostatic in nature that is why they exhibit good anti-bacterial activity. Some microbes need *p*-amino benzoic acid (PABA) for the synthesis of folic acid which is considered as an essential ingredient for DNA and RNA synthesis. Sulfonamides (such as sulphanilamide) competitively inhibit the microbial growth due to its resemblance with PABA, resulting in folic acid deficiency and inhibition of bacterial growth and cell division.[62] Sulfonamides exhibited excellent anti-bacterial activity[63] against both Gram-negative and Gram-positive bacteria. Hence, sulfonamide moieties are used as drug to treat septicemia, bacillary dysentery, tonsillitis and urinary tract infections.[64]

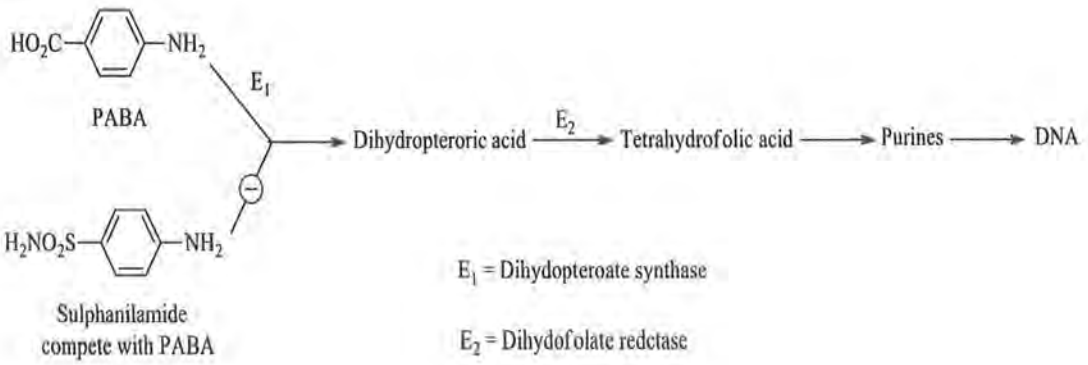


Figure 1.6: Sulfonamide action in bacteriostatic activity

1.3.3.2 Hypoglycemic Activity

Sulphonyl urea drugs having sulfonamide functional group are used as hypoglycemic agent and as diabetes mellitus type-2 treating agents by activating beta cells of pancreas to release insulin.[65]

1.3.3.3 Diuretic Activity

Sulfonamides can be used as diuretics as well as for the treatment of hypertension and edema. Acetazolamide **(64)** is used to inhibit carbonic anhydrase enzyme that is responsible for ions and water excretion, for lowering of blood pressure.[61]

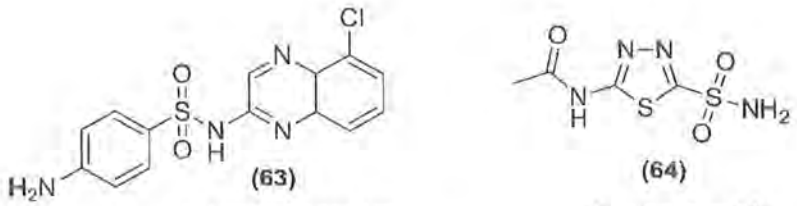


Figure 1.7: Diuretic activity of sulfonamides

1.3.3.4 Anti-Cancer Activity

Rani *et al.*, (2014) studied sulphonamide activities and promoted the designing of sulphonamides derivatives **(65-a & 65-b)** as anticancer drugs. In-silico studies confirmed the anticancer activity by exhibiting the presence of protein-ligand interactions, that have affinity ranges from -6.8 to -8.6 Kcal/mol, that are quite similar to human topoisomerase II inhibitor.[66]

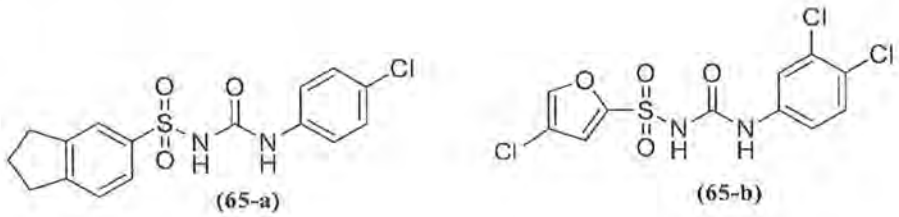


Figure 1.8: Antitumor diarylsulphonylurea

1.3.3.5 Anti-Protozoal Activity

Zaidi *et al.*, (2016) synthesized sulphonamide piperazine fused with thienopyridine scaffolds as dihydrofolate reductase (DHFR) inhibitor and evaluated their activity against *Plasmodium falciparum* and *Entamoeba histolytica.*[67]

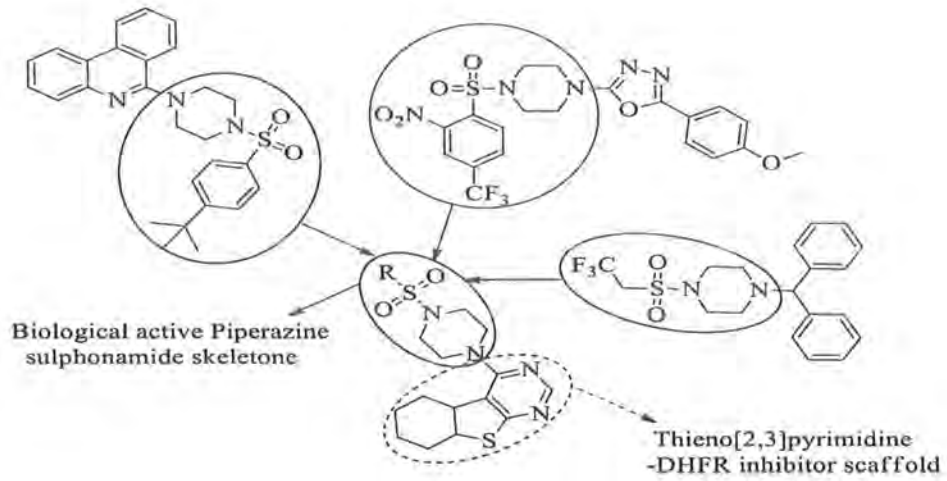
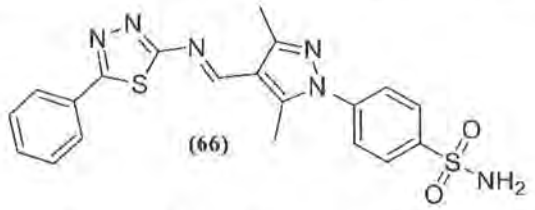


Figure 1.9: Sulfonamides containing piperazine nucleus act as anti-protozoal agent

1.3.3.6 Anti-Inflammatory Activity

Alam *et al.* (2015) reported the synthesis of a series of thiadiazole linked pyrazole benzene sulphonamides by the condensation of aldehydic pyrazole with aryl substituted thiadiazole amine followed by Schiff base reaction. The resulted sulphonamides **(66)** showed the most significant *in vivo* anti-inflammatory activity of about 72.33 %.[68]



Designed molecule having simlilarity with Celecoxib

Figure 1.10: Sulfonamides containing pyrazole nucleus act as anti-inflammatory agent

1.3.3.7 Anti-Retroviral Activity

Stranix *et al.*, (2006) prepared a series of sulphonamide **(67)** derivatives that inhibit the HIV protease and viral replication. A series of *N*-isobutyl-*N*-arylsulphonamido (*N*-acyl aromatic amino acid) lysinol derivatives was also prepared.[69]

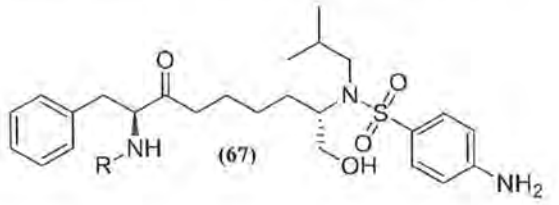


Figure 1.11: Sulfonamide active against HIV

1.3.4 Structure activity relationship of Sulfonamide

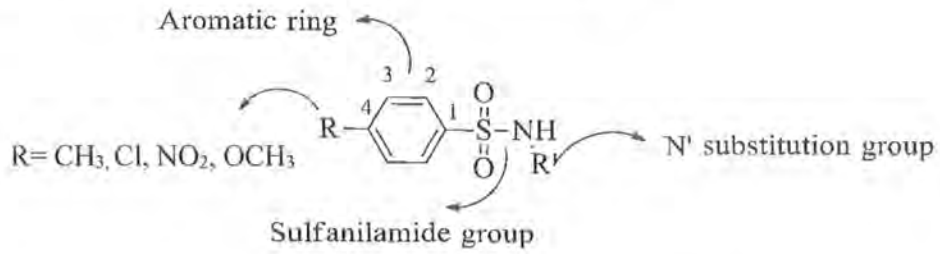


Figure 1.12: General Structure of Sulfonamide

1. The **R** and **sulphonyl groups** on the phenyl ring are necessary & should be on position 1 & 4.

2. Replacing the aromatic ring by other ring systems or the introducing additional substituents on it decrease the activity.

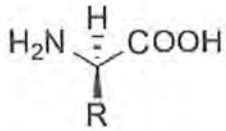
3. Substitution of aromatic heterocyclic nuclei at *N'*- yields highly potent and effective compounds.

4. Exchange of the **-SO_2NH** group by **–CO-NH** reduce the activity.

5. N'–Disubstitution in general leads to inactivity.[70]

1.4 Amino acids

The term amino acid is usually used for alpha-amino carboxylic acids **(68)**. These are the building blocks of proteins which play important role in cell structure and function.

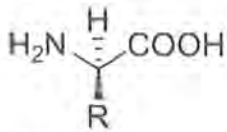


(68)

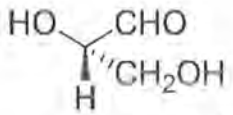
Figure 1.13: General Structure of Amino Acids

Amino acids differ by the side chain present on the alpha carbon atom. The simplest amino acid is glycine in which R=H. Except glycine, all other amino acids are chiral. All naturally occurring amino acids have S configuration except cysteine. Their

configuration is similar to that of L-glyceraldehyde's that is why S-amino acids are referred to as L-amino acids[71] **(Figure 1.14)**



S-amino acid **(69)**



(70) L-Glyceraldehyde

Figure 1.14: Configuration comparison of S-Amino Acid and L-Glyceraldehyde

1.4.1 Classification of Amino Acids

There are twenty amino acids, all having L-configuration. Their classification is done on different characteristics.

On the basis of their need in the body, amino acids are divided into two groups.

- Essential
- Non-essential

Based on the physical characteristics amino acids are divided into two groups;

- Hydrophobic
- Hydrophilic

Alanine, methionine, isoleucine, proline, phenylalanine, valine and tyrosine are hydrophobic amino acids while arginine, aspartic acid, cysteine, glutamic acid, asparagine, glycine, histidine, lysine, serine and threonine are the hydrophilic amino acids.

1.5 Esterification

Esterification is the process of conversion of carboxylic acids into esters. Esters have their utility in laboratory as well as in industry. These are the major backbone of a variety of natural products and organic compounds. These are broadly used in industry as well in organic synthesis for the protection of carboxylic acids.[72]

The major problem encountered in esterification is the equilibrium. For the reaction to go to product side one must either add one reactant in excess or remove products from

the reaction mixture. This problem can be solved by using activated reactants such as acid anhydride, halides, etc. but still these are not general methods.[73]

Esterification can be carried out without using activators. For example, esterification without catalyst and solvent is reported using high temperature or pressure. Although these methods are 'Green' but they are difficult to use.[73]Esterification is generally carried out using acid or base as a catalyst.

1.5.1 Acid Catalyst:

The acids used for esterification can be classified into two;

1. Bronsted acids
2. Lewis acids

1.5.1.1 Bronsted acids:

The Bronsted acids which are used for esterification are Hydrochloric acid, Phosphoric acid, Sulphuric acid, HBF_4 etc. **(Figure 1.15).**

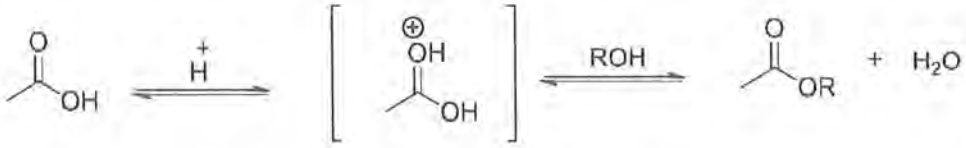


Figure 1.15: Acid Catalyzed Esterification of Carboxylic acid

These acids are good esterification catalyst for acid resistant products. Sometime reaction is very slow with these acids. In that case we have to add some activators such as molecular sieves, H_3BO_4 etc. These can also be activated using microwave or ultrasonic irradiation as activator.[73]

1.5.1.2 Lewis acids

Lewis acids are also used as catalyst for esterification. These acids are bulkier than proton so they provide a template effect. Lewis acids of Boron, Aluminium, Copper, Nickle, and Zinc are commonly used for this purpose.[73]

1.5.2 Base Catalyst

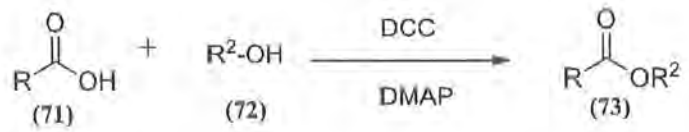
Base catalysis for the production of ester is not very popular because the esters are hydrolyzed when aqueous work-up is done. So another method is provided by the use of DMAP and Et_3N.[73]

1.5.3 Carbodiimide Activator

The use of DCC for esterification is a renowned method of esterification. It has advantage over other esterification methods in that it is a mild reaction and occurs at room temperature. But the disadvantages of this method are that DCC is skin irritant, gives poor yield and unwanted side products.[73]

1.5.4 The Steglich Esterification

This reaction was first reported by Steglich in 1978.[74] It is a modification of already reported reaction of esterification with DCC. In this reaction along with DCC, *N, N*-dimethylaminopyridine (DMAP) is used. DMAP is an acyl transfer catalyst.[75]



Scheme 1.11: Esterification of Carboxylic Acid with DCC and DMAP

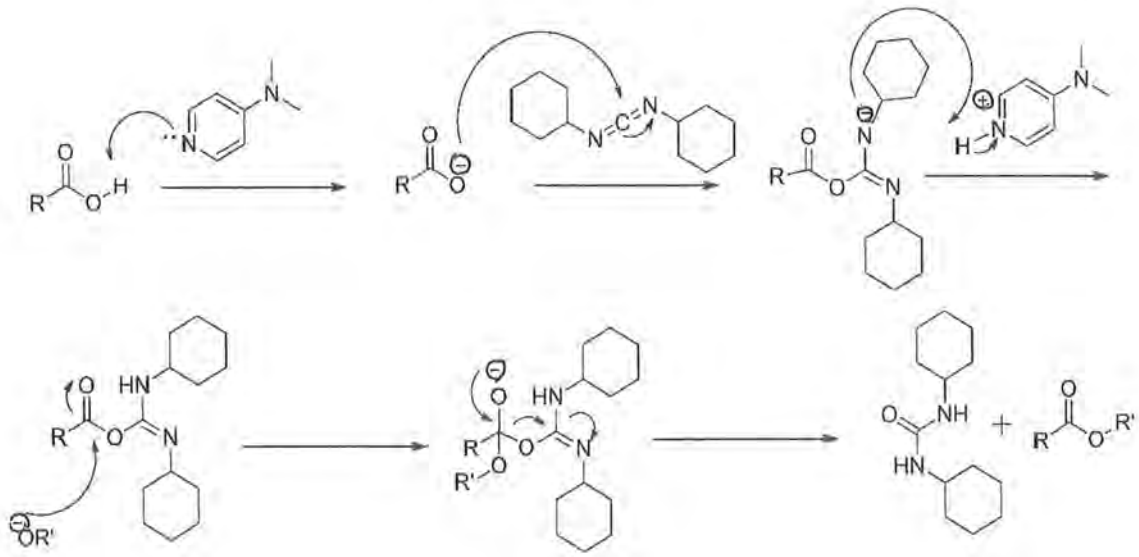


Figure 1.16: Mechanism of Steglich Esterification

1.5.5 Mitsunobu Reaction:

This is a method of esterification of Carboxylic acid in a presence of DEAD (diethylazidocarboxylate) and triphenylphosphine (PPh_3).[76]

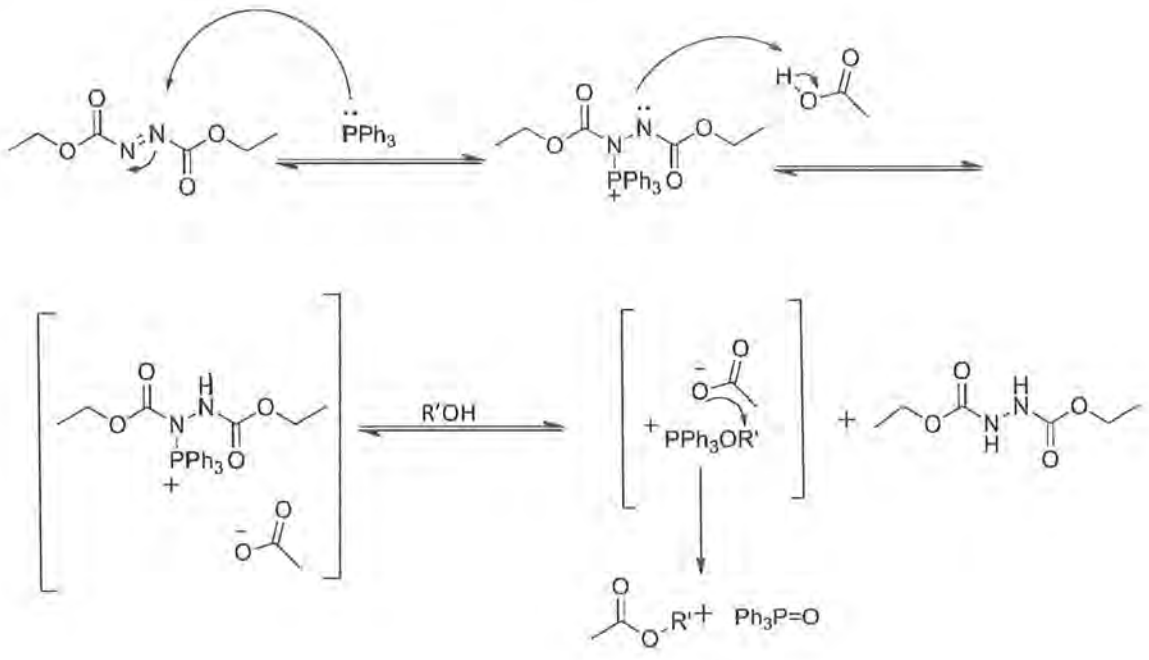
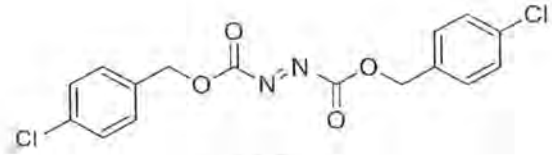


Figure 1.17: Mechanism of Mitsonobu Reaction

This reaction is highly regioselective and chemoselective. But the disadvantage of this method is that the large amount of reagent is necessary. And the production of triphenylphosphine oxide and diethylhydrazinedicarboxylate makes the isolation of product tedious.

Recently DCAD **(74)** (di-p-chlorobenzyl azodicarboxylate) has been introduced as a substitute of DEAD. With this reagent the product produced is hydrazine which is readily separable.[77]



(74)

1.6 Esterification of Amino Acids

Esterification of amino acids is an important step in the synthesis of peptides and many other organic reactions. It can be carried out with thionyl chloride,[78] DMAP in combination with DCC or EDC,[79] Trimethyl chlorosilane,[80] Protic acids,[81] Methane sulphonic acid in absence of solvents,[82] Ion exchange resins (Amberlyst^{TM}-15)[83] etc.

1.7 Biological Application of Amino Acid Esters:

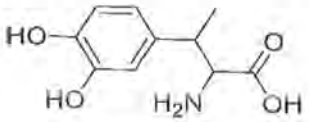
Amino acid esters are biologically active compounds and in some cases it has been observed that the esters have higher or good biological activity than the parent amino acids. Some of the examples of biological applications of esters are as follows;

Amino acid esters inhibit the concentration of unicellular protists such as astasia and euglena at a particular concentration. Experiments have proved that amino acid esters stop the growth of these species by interfering the corresponding aminoacyl tRNA synthetase. This enzyme is used to condense the adenosine monophosphate with the carboxylic group of amino acid in the first step of protein synthesis. Amino acid esters are analogues of amino acids so instead of amino acid they can be attached with activating enzymes. So they block the activating site of enzyme thus inhibiting protein formation in astasia and euglena.[84]

Amino acid esters are also used for pest control. It has been experimentally proved that they are very effective in controlling the aphids, leafhopper, flies, mosquitoes, fleas etc. and other insects including ticks and mites of different families. These esters are particularly effective against the soil inhabiting pests. These active ingredients i.e. amino acid esters are employed with diluents. Diluents are any material, liquid or solid, in which the active ingredient is present and which can be easily applied e.g. kaolin, talc, water etc.[85]

L-alpha Methyl-3,4-dihydroxy phenylalanine which is also known as dopa **(75)** or methyldopa **(Figure 1.18)** is a well-known compound used for the treatment of hypertension. Now chemists have made their amino acid ester derivatives which are

useful in emergency treatment of hypertension. Some ester derivatives of dopa have not only higher activity but also require less dosage than the known compounds.[86]



(75)

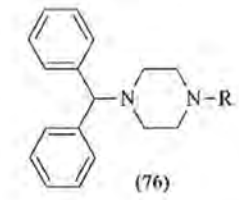
Figure 1.18: L-alpha Methyl-3,4-dihydroxy phenylalanine (dopa)

1.8 Piperazine and its biological importance

The largest traditional subfield of organic chemistry, heterocyclic chemistry is extremely significant from both a biological and industrial standpoint. Among heterocycles, aza-heterocyclic chemicals have made major contributions to society's advancement, comprehension of biological processes, and in the actions taken to raise quality of life. The majority of biologically active drugs and agricultural chemicals have aza-heterocyclic core. Considering the chemistry of Aza-heterocycles, a unique class of heterocyclic compounds of significant biological and pharmacological significance is the pyrimidine family, which also includes pyrazines and piperazines.

Piperazine is an azocycloalkane containing two nitrogen atom in its six membered skeleton. Substituted piperazine like Monoaryl- and diarylpiperazines are important class of heterocyclic compounds for clinical chemistry.[87] Piperazine's capacity to cause the flaccid paralysis of the parasite's muscles accounts for its anthelmintic activity.[88] Gene transfer reactions involving piperazines have been documented,[89] and quaternary piperazinium salts have demonstrated spasmolytic, anthelmintic, and germicidal activity. Some piperazine derivatives have high biological activity to combat cancer's multidrug resistance.[90] Piperazines and their substitutes are significant pharmacophores that are present in a variety of commercially available medications, such as the Merck HIV protease inhibitor Crixivan,[91] and drugs under development.[92] Ciprofloxacin dimers coupled to piperazine have been found to be effective antibacterial against resistant strains,[93] a novel class of mixed D2/D4 receptor antagonists,[94] dual calcium antagonist[95], and potential antipsychotic agents.[96] As an antifungal agent, piperazine derivatives with tetrazole nucleus have recently been identified.[97] Figure 1 depicts the chemical structures of biologically significant compounds that contain 1,4-piperazines with different substituents.[98-101] Additionally, as shown in figure 1, bis(fluorophenyl)-

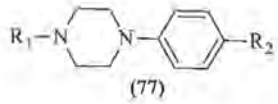
piperazine compounds were noted to have biological activity, such as anti-inflammatory and anti-eczematic action, in the US-patent.



(76)

R= alkyl, aryl

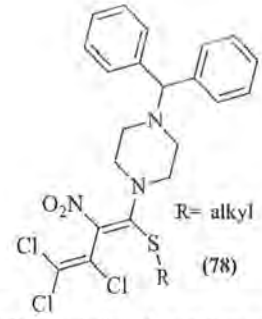
Antihistaminic and Antihelmintic



(77)

R1= alkyl, R2=aryl

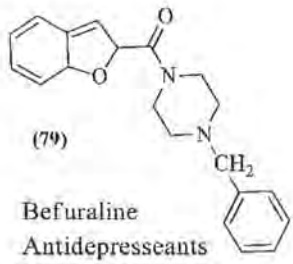
Antimicrobial



R= alkyl

(78)

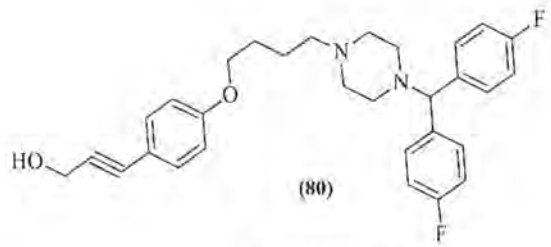
antibacterial and antifungal



(79)

Befuraline

Antidepresseants



(80)

Antieczematic

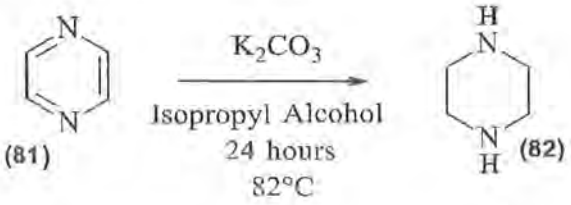
Figure 1.19: Chemical structures and biological activities of some 1,4-substituted piperazines

1.8.1 Piperazine synthesis

Despite the fact that a variety of piperazine derivatives are produced naturally, piperazine must be produced artificially. This can be done by reducing pyrazine, mixing sodium and ethylene glycol with ethylene diamine hydrochloride,[102] or combining alcoholic ammonia with 1,2-dichloroethane.[103]

1.8.1.1 Synthesis by pyrazine reduction

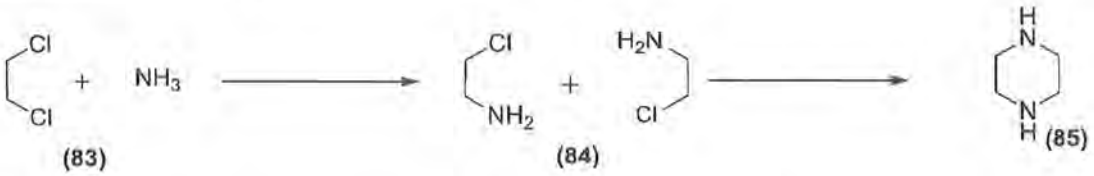
Piperazine **(82)** can be synthesized by pyrazine **(81)** reduction using potassium carbonate and Isopropyl alcohol.



Scheme 1.12: Synthesis of piperazine by pyrazine reduction

1.8.1.2 Synthesis by alcoholic ammonia and 1, 2-dichloroethane

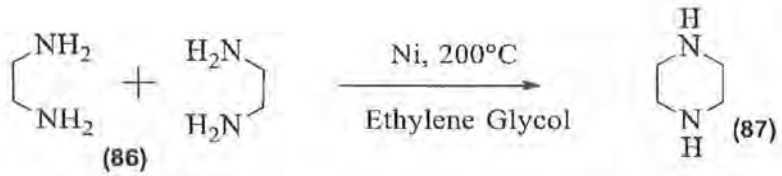
Another method to synthesize piperazine **(85)** involves the use of 1,2-dichloroethane **(83)** and ammonia as starting materials.



Scheme 1.13: Synthesis of piperazine from alcoholic ammonia and 1, 2-dichloroethane

1.8.1.3 Synthesis by ethylene diamine hydrochloride

Ethylene diamine **(86)** can be converted to piperazine **(87)** at an elevated temperature in the presence of nickel.

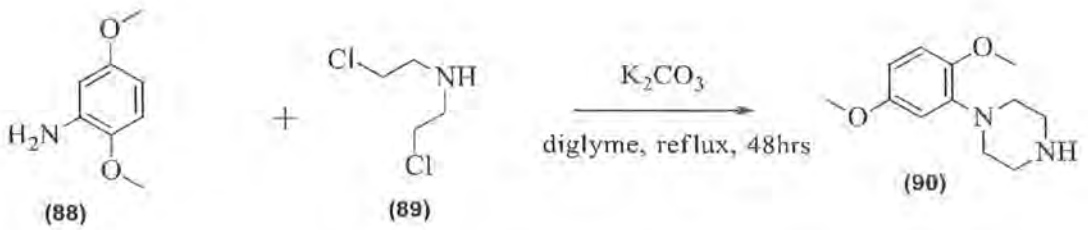


Scheme 1.14: Synthesis of piperazine from ethylene diamine

1.8.2 Synthetic pathways for phenyl piperazine

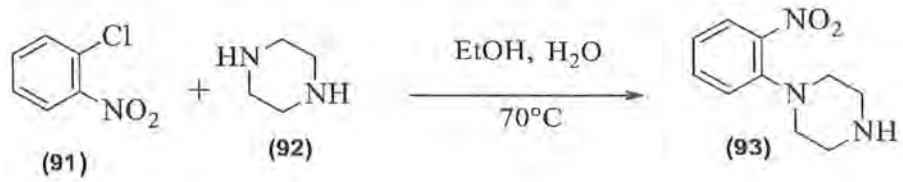
N-phenylpiperazine is a type of significant compound in pharmaceutical chemistry. Its industrial production process is divided into two types:

The first type involves cyclization in various solvents and the use of aniline **(88)** and 2-(2-halogenated ethyl) amine hydrochlorate **(89)**.[104]



Scheme 1.15: Synthesis of phenyl piperazine from aniline and 2-(2-halogenated ethyl) amine hydrochlorate.

The second type involves the use of various halogeno-benzenes and a condensation reaction to produce piperazine **(93)**.[105]



Scheme 1.16: Synthesis of phenyl piperazine from halogeno-benzene

1.9 Plan of Work

Modification of pendant hydroxyl group of metronidazole is a common strategy to develop novel antibacterial agents.[106] Since Sulfonamides and its derivatives are important pharmacophores that were reported as potent antibacterial agents against resistant and non-resistant strains.

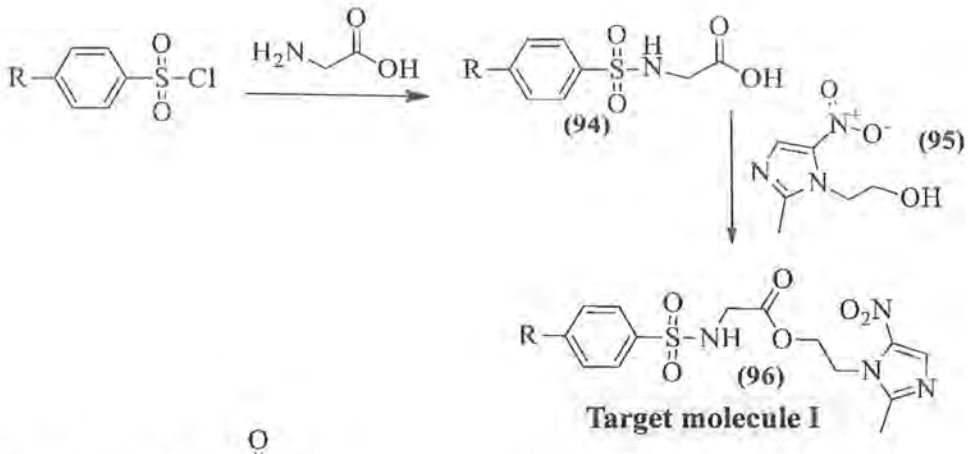
We planned the synthesis of two different Target Molecules.

Synthesis of **Target Molecule I** (part1, scheme1.17) will start with the commercially available different sulphonyl chlorides reacted with Glycine to afford different sulfonamides. These sulfonamides will then be reacted with Metronidazole to yield the desired hybrid molecule.

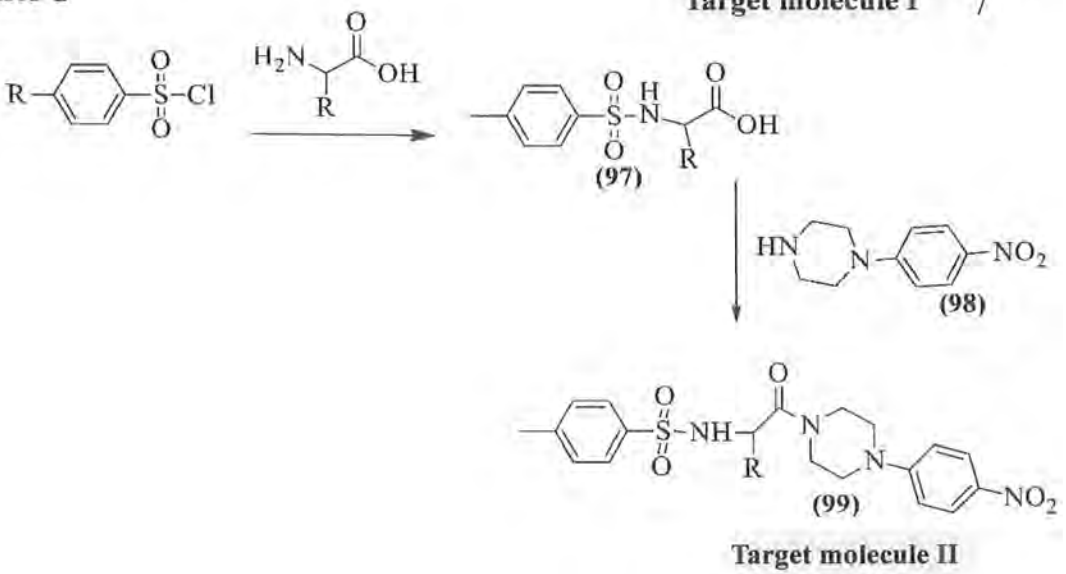
Similarly, synthesis of **Target Molecule II** (part2, scheme1.17) will start with the commercially available *p*-toluene sulfonyl chloride reacted with different amino acids to afford sulfonamides which will then be reacted with substituted piperazine to yield amide linked target compound.

The synthetic strategy is shown in **Scheme: 1.17**

PART 1



PART 2



Scheme 1.17: Synthesis of Amino acids-based sulfonamides and their hybridization with metronidazole and substituted piperazine

1.9.1 Characterization of the Synthesized Compounds

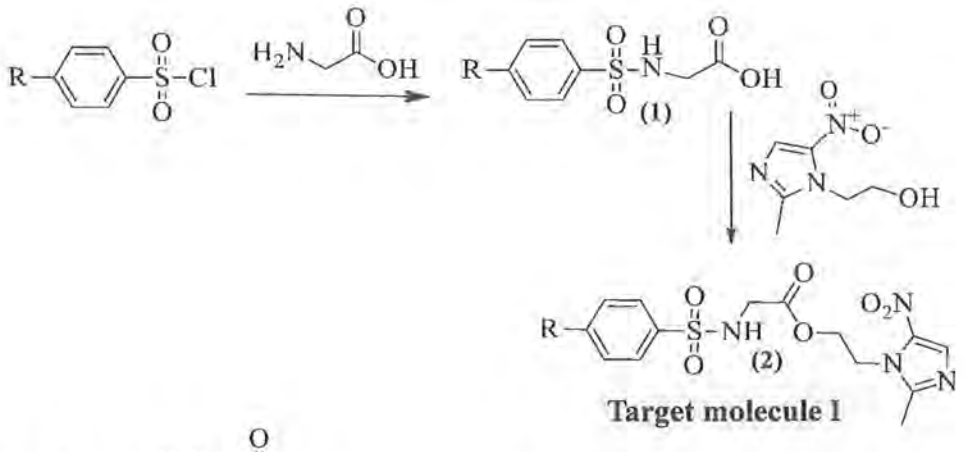
All the synthesized compounds will be characterized by using IR, ^{1}H, and ^{13}C NMR spectroscopy and mass spectrometry.

CHAPTER 2
RESULTS AND DISCUSSION

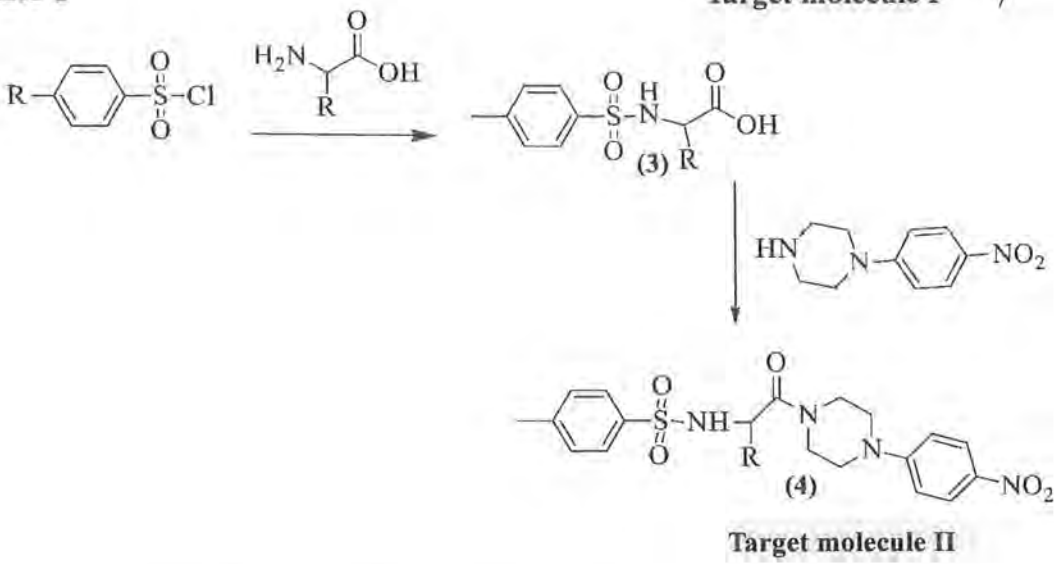
2.0 Sequential Synthesis:

Emergence of multiple drug resistance bacterial strains which represents a serious threat to public health, we planned to synthesize the Ester linked Conjugates of Sulphonamides with Metronidazole and Amide linked hybrids of sulfonamides using substituted piperazine through linear approach. We started with sulphonamide synthesis followed by esterification in **Part 1** and carboxamides synthesis in **Part 2.**

PART 1



PART 2



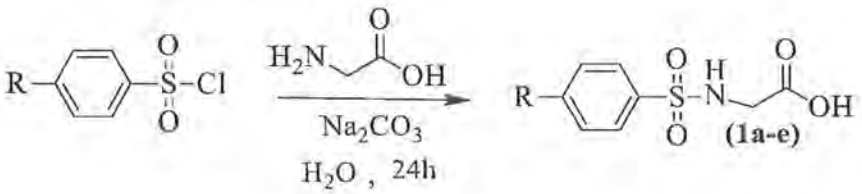
Scheme 2.1: Sequential Synthesis to design Target molecule (I) and (II)

2.1 Sulphonamide:

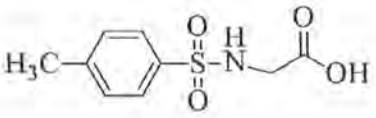
The amides of sulphonic acids are commonly known as sulfonamides. This class of compounds carries **-SO_2NH_2** functional group. Usually sulphonamides can be synthesized from sulphonyl chlorides and primary or secondary amines.[48]

2.1.1 Synthesis of 2-(4-substituted phenylsulfonamido) acetic acid (1a-1e)

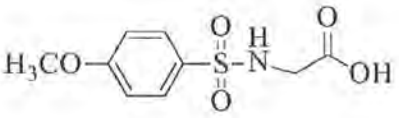
Sulphonamide synthesis is carried out by following green chemistry protocol. Different derivatives of 2-(4-substituted phenylsulfonamido) acetic acid were synthesized by reaction of different substituted sulphonyl chlorides with the amino acid i.e., Glycine in the presence of water as a solvent. The base Na_2CO_3 was also added as HCl scavenger. After completion of the reaction, mixture was then acidified using dilute HCl at pH 3 to obtain the desired product. Sulphonamides were further purified through recrystallization.[107]



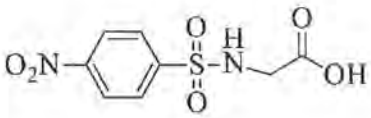
Where R= CH_3, OCH_3, NO_2, Cl, Naphthalene



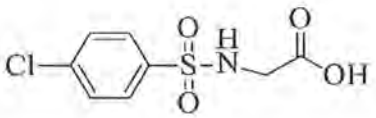
1a-92%



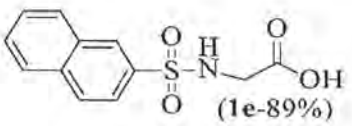
1b-90%



1c- 87%



1d- 88%



Scheme 2.2: Synthesis of 2-(4-substituted phenylsulfonamido) acetic acid:

2.1.2 Characterization by physical parameters

Physical data of synthesized derivatives of 2-(4-substituted phenylsulfonamido) acetic acid is shown in **Table 2.1**

Table 2.1: Physical data of derivatives of 2-(4-substituted phenylsulfonamido)acetic acid.

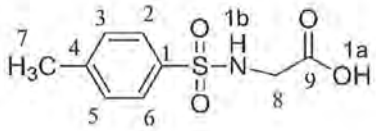
Compd.	Colours	R*f**	Melting points (°C)	Yield (%)
1a	White	0.43	146-147 (Lit. 147)	92
1b	White	0.41	149-150 (Lit. 149)	90
1c	Off White	0.38	156-159 (Lit. 157)	87
1d	White	0.40	127-129 (Lit. 128)	88
1e	Off white	0.45	161-163(Lit. 164)	89

*($CHCl_3$:MeOH::9:1) on pre coated silica gel plates $60F_{254}$ visualized under UV light at 254nm

2.1.3 Characterization of compound (1a) as representative example

2.1.3.1 Characterization by ^{1}H NMR spectroscopy

^{1}H NMR data of the compound (1a) is depicted in the table (2.2) which represents that a broad singlet appeared at 12.5 ppm corresponding to the strongly deshielded acidic proton (H-1a) while a singlet appeared at 7.95 ppm corresponding to sulfamoyl proton (H-1b), which is a characteristic signal. A doublet appeared at 7.66 ppm corresponding to aromatic protons (H-2 and H-6) while another doublet resonated at the chemical shift value of 7.36 ppm corresponding to the two protons (H-3 and H-5) of the aromatic ring. The two aliphatic protons (H-8) present next to electron withdrawing group appeared as a doublet at 3.51 ppm. A singlet for three protons (H-7) resonating at 2.37 ppm refers to the methyl group attached with benzene ring.



(1a)

Table 2.2: ^{1}H NMR analysis of the synthesized sulphonamide **(1a)**

Protons	δ (ppm)	Multiplicity	Integration	*J* (Hz)
1a	12.5	*s*	1H	---
1b	7.95	*s*	1H	---
2 and 6	7.65-7.68	*d*	2H	$^3J = 8.1$
3 and 5	7.35-7.38	*d*	2H	$^3J = 8.1$
7	2.37	*s*	3H	---
8	3.51	*d*	2H	$^3J = 10.8$

2.1.3.2 Characterization by ^{13}C NMR spectroscopy

The structure of the compound (1a) is further confirmed by ^{13}C NMR spectroscopy. ^{13}C NMR data of the compound (1a) is given in the table (2.3). The most deshielded signal appeared at 170.7ppm corresponding to carboxylic carbon (C-9). The signal at 143 ppm referred to ipso carbon (C-4), it is more deshielded than ipso carbon (C-1), which gave a signal at 138.2 ppm. Aliphatic carbon (C-8) resonated at 44.2 ppm. Signal for methyl carbon (C-7) resonated at 21.4 ppm. Other aromatic carbons appeared in their characteristic aromatic regions.

Table 2.3: ^{13}C NMR analysis of the synthesized compound (1a)

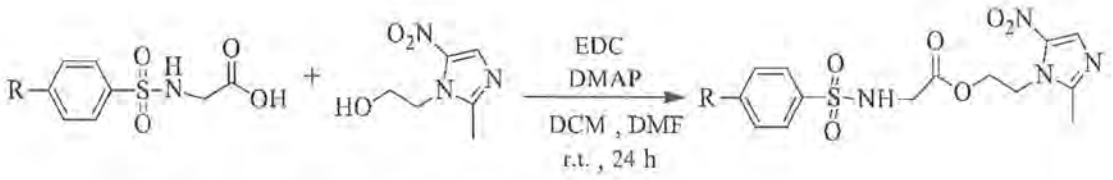
Carbons	C-9	C-4	C-1	C-3 and C-5	C-2 and C-6	C-8	C-7
δ (ppm)	170.7	143	138.2	129.9	127.0	44.2	21.4

2.2 Esterification:

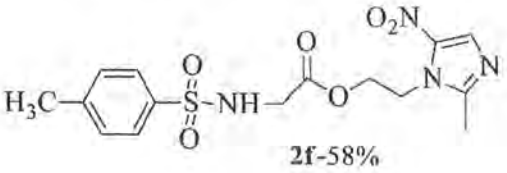
As Esters can be synthesized through numerous methods but we used acyl transfer reagents EDC and DMAP. These played a significant role of activation of carboxylic acid. EDC consumed in the reaction while DMAP behaved as a catalyst.[75] These acyl transfer reagents efficiently increase the rate of reaction and crucial for developing ester linkage.

2.2.1 Synthesis of Ester linked Conjugates of Metronidazole and Sulphonamides (2f-j)

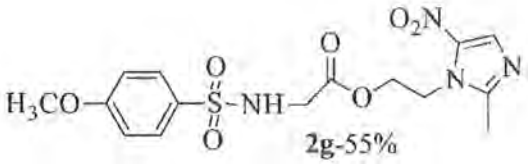
A variety of ester linked conjugates of Metronidazole with sulphonamides is synthesized by using EDC and DMAP. 2-(4-substituted phenylsulfonamido) acetic acid was dissolved in the dichloromethane (DCM) and *N,N*-Dimethylformamide (DMF), then DMAP, EDC and Metronidazole were added in the reaction flask and allowed to stir in the inert atmosphere for 24 hours. After the completion of reaction, the urea (by product) and DMAP were removed by extraction with aqueous-ethyl acetate under acidic conditions. The pale yellow precipitates obtained were recrystallized using chloroform to obtain a pure product as off-white crystals.



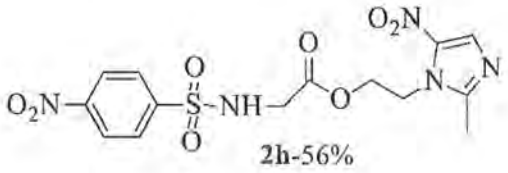
Where R= CH_3, OCH_3, NO_2, Cl, Naphthalene



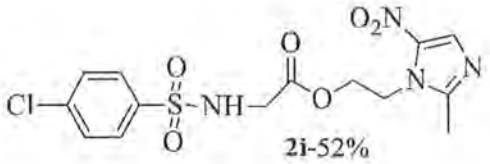
2f-58%



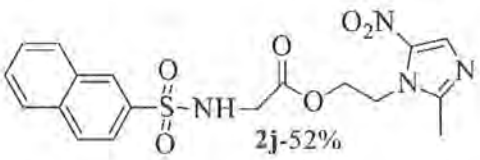
2g-55%



2h-56%



2i-52%



2j-52%

Scheme 2.3: Synthesis of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl-2-(4-substitutedphenylsulfonamido)acetate

2.2.1.1 Mechanism for the synthesis of Ester linked Conjugates of Metronidazole and Glycine based Sulfonamides

In the first step, DMAP acts as a base and abstracts the acidic proton from carboxylic acid **(a)** to form carboxylate ion **(b)**. This carboxylate ion acts as a nucleophile and attacks on carbodiimide **(c)** to give intermediate **(d)** which takes the proton from cation of DMAP **(e)** to form *O*-acylisourea **(f)**. Now DMAP acts as a nucleophile and attacks on *O*-acylisourea and as a result urea **(g)** is formed as by-product and the desired intermediate **(h)** is also formed which is in resonance with other intermediate **(i)**. The OH-bearing moiety attacks on intermediate **(i)** to give desired ester linked product **(j)** and DMAP is regenerated. The fate of coupling reagents is that EDC is consumed in the reaction while DMAP is regenerated.[108]

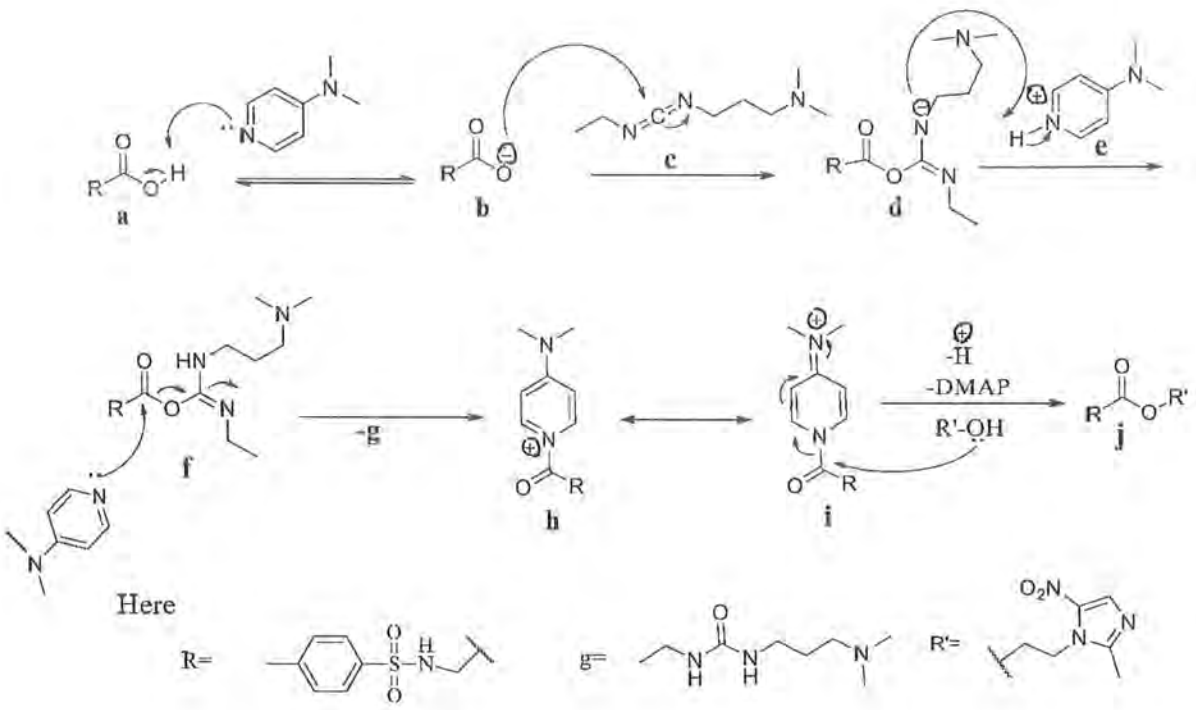


Figure 2.1: Mechanism for developing ester linkage via O-acylisourea

2.2.2 Characterization by physical parameters

Physical data of synthesized Ester linked conjugates of metronidazole and glycine based sulfonamides is given in Table (2.4).

Table 2.4: Ester linked conjugates of metronidazole and glycine based sulfonamides

Compd.	R	R_f*	Colors	Melting Points (°C)	Yield (%)
2f	CH_3	0.40	Off white	184-186	58
2g	OCH_3	0.38	White	192-195	55
2h	NO_2	0.33	White	180-182	56
2i	Cl	0.35	White	207-210	52
2j	Naphthyl	0.39	Off white	219-221	52

*($CHCl_3$:MeOH::9:1) on pre coated silica plates $60F_{254}$ visualized under UV light at 254 nm

2.2.3 Characterization of compound (2f) as representative example

2.2.3.1 Characterization by ^{1}H NMR spectroscopy

^{1}H NMR data of the compound **(2f)** is depicted in the table (2.5) which represented the characteristic signal of the proton (H-4) of the imidazole ring in the deshielded region at 8.04 ppm. This signal appeared deshielded as compared to other aromatic protons due to inductive effect of nitrogen atom and mesomeric effect of the nitro group. Similarly, disappearance of the signal of carboxylic acid's proton also confirmed the product formation via ester linkage. A triplet for one proton resonating at 8.13 ppm refers to proton of sulfonamide (H-1a). Two aliphatic protons (H-7) next to oxygen atom give triplet at 4.51 ppm. Next two aliphatic protons (H-6) resonated at 4.30 ppm to give a triplet. A doublet of two protons at 3.60 ppm corresponds to protons of CH_2 group (H-9). A singlet for three protons resonating at 2.50 ppm refers to the methyl group (H-14) attached to the imidazole ring. Another singlet for three protons at 2.36 ppm corresponds to methyl group (H-15) attached with benzene ring. All other aromatic protons appeared in their respective regions.

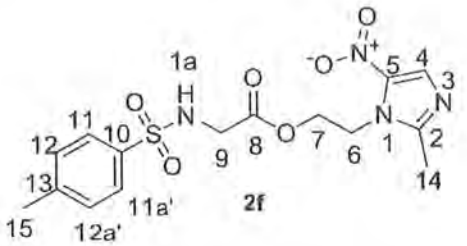


Table 2.5: ^{1}H NMR analysis of the synthesized compound

Protons	δ (ppm)	Multiplicity	Integration	*J* (Hz)
1a	8.13	*t*	1H	$^3J = 6.0$
4	8.04	*s*	1H	-----
7	4.51	*t*	2H	$^3J = 4.8$
6	4.30	*t*	2H	$^3J = 4.8$
9	3.60	*d*	2H	$^3J = 6.0$
14	2.50	*s*	3H	------
15	2.36	*s*	3H	------

2.2.3.2 Characterization by ^{13}C NMR spectroscopy

Spectral data of the synthesized compound **(2f)** is given in table. The most deshielded carbon resonated at 169.1 ppm corresponds to Carbonyl carbon (C-8). The characteristics signals for methyl carbons (C-15 and C-14) appeared at 21.3 ppm and 14.4 ppm, respectively. Aliphatic carbons (C-7, C-9 and C-6) resonated at 63.5 ppm, 45.0 ppm and 44.0 ppm, respectively. All other aromatic carbons appeared in characteristic aromatic regions.

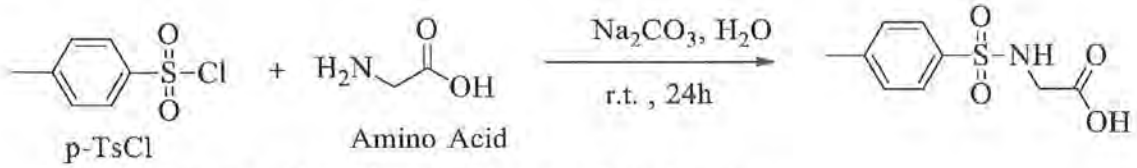
Table 2.6: ^{13}C NMR analysis of the synthesized compound **(2f)**

Carbons	C-8	C-2	C-5	C-4	C-7	C-9	C-6	C-15	C-14
δ (ppm)	169.1	152.1	143.2	133.5	63.5	45.0	44.0	21.3	14.4

2.3 Carboxamide:

Carboxamides can be synthesized through numerous methods but use of coupling reagents is considered as more efficient way to develop an amide linkage. Here we used acyl transfer reagents EDC and DMAP. These played a significant role of activation of carboxylic acid. EDC consumed in the reaction while DMAP behaved as a catalyst. These acyl transfer reagents efficiently increase the rate of reaction and crucial for synthesis of primary, secondary, and tertiary carboxamides.

2.3.1 Synthesis of 2-(4-methylphenlysulfonamido)acetic acid (3k-m)



Where Amino acids= Glycine, Alanine, Phenylalanine

Scheme 2.4: Synthesis of 2-(4-methylphenylsulfonamido)acetic acid

Synthetic protocol was same as described in the previous scheme of sulphonamide[48] but the only difference here is that we used different amino acids which are shown in following figure.

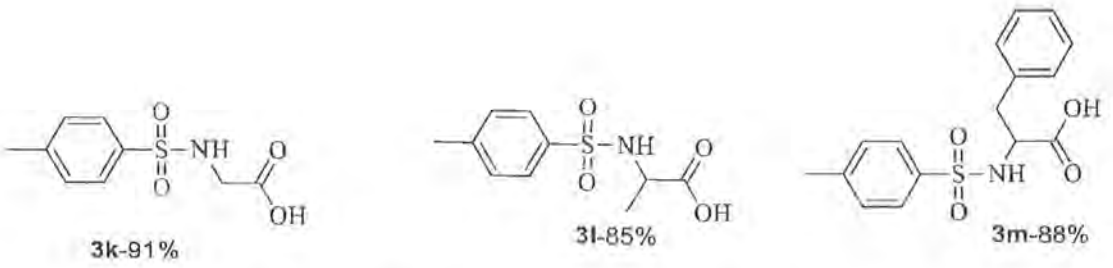


Figure 2.2: Amino acids based sulfonamides

2.3.2 Characterization of synthesized compounds by Physical parameters:

Physical data of the synthesized derivatives is shown in **table 2.7**

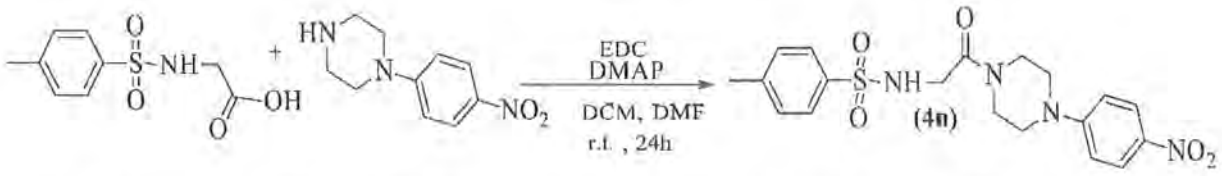
Table 2.7: Physical data of amino acids based sulfonamides

Compd.	R_f*	Colors	Melting Points (°C)	Yield (%)
3k	0.43	White	146-148 (Lit. 146-147)	91
3l	0.40	White	135-137 (Lit.138-139)	85
3m	0.59	White	164-165 (Lit.164-166)	88

*($CHCl_3$: MeOH::9:1) on pre coated silica gel plates $60F_{254}$ visualized under UV light at 254 nm

2.3.3 Synthesis of Carboxamides using amino acids based sulphonamides and substituted piperazine (4n-p)

A variety of carboxamides is synthesized by using EDC and DMAP.[109] Reaction was carried out under room temperature. 2-(4-Methylphenlysulfonamido)acetic acid dissolved in the dichloromethane (DCM) and *N,N*-Dimethylformamide (DMF), then DMAP, amine and EDC were added in the reaction flask and allowed to stir in the inert atmosphere for 24 hours. An undesired product i.e., urea formed from EDC during the reaction. After the completion of reaction, the urea and DMAP were removed by solvent extraction with aqueous-ethyl acetate under acidic conditions. Carboxamides further purified by column chromatography.



Scheme 2.5: Synthesis of Carboxamides using Amino acids base sulfonamides and substituted piperazine

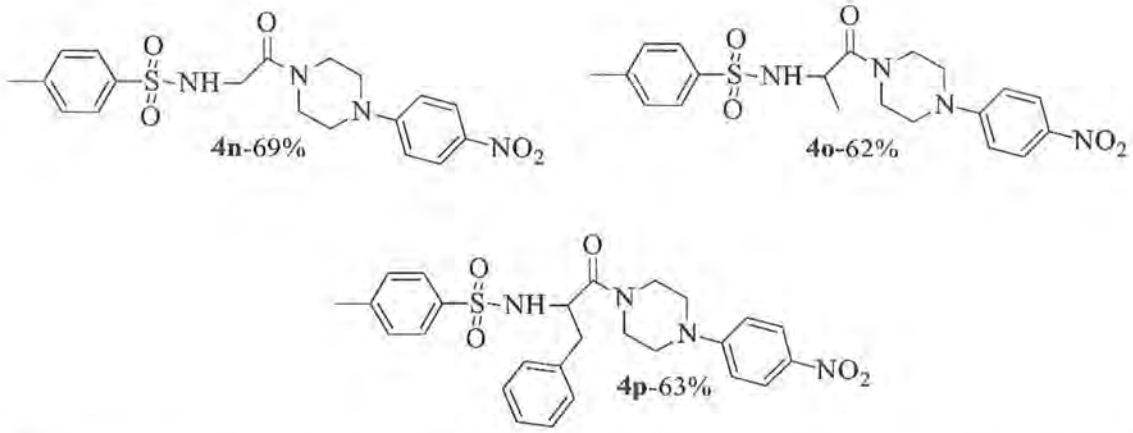


Figure 2.3: Various synthesized sulfonamides-carboxamides via sequential synthesis

2.3.4 Characterization by physical parameters

Physical data of amide linked derivatives of amino acids based sulfonamides and substituted piperazine is given in table (2.8)

Table 2.8: Physical data of synthesized carboxamides

Compd.	R_f*	Colors	Melting Points (°C)	Yield (%)
4n	0.33	Yellow	216-218	69
4o	0.39	Yellow	254-256	62
4p	0.41	Yellow	271-273	63

*(n-hex:EtOAc::1:1) on pre coated silica gel plates $60F_{254}$ visualized under UV light at 254nm

2.3.5 Characterization of compound (4n) as representative example

2.3.5.1 Characterization by ^1H NMR Spectroscopy

The spectral data of compound (4n) given in table indicates a doublet for two protons in the most deshielded region at 8.06 ppm which corresponds to the two aromatic protons (H-13 and H-13a') of phenyl ring attached to piperazine ring. A multiplet of two protons at 7.68 ppm refers to the aromatic protons (H-2 and H-2a') of benzene ring

next to sulfonamide group. Presence of these aromatic protons of two different substituted phenyl rings confirms the formation of product. A singlet of one proton resonated at 7.72 ppm corresponds to proton (H-1a). A multiplet of 8 protons at 3.51 ppm corresponds to protons (H-8, H-8a', H-9, H-9a') of piperazine ring. Remaining aromatic protons lie in their respective region.

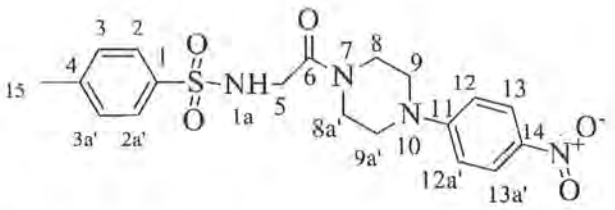


Table 2.9: ^{1}H NMR analysis of the synthesized carboxamide **(4n)**

Protons	δ (ppm)	Multiplicity	Integration	*J* (Hz)
H-13 and H-13a'	8.06	*d*	2H	-------
H-3 and H-3a'	7.36	*d*	2H	-------
H-1a	7.72	*s*	1H	-------
H-12 and H-12a'	6.99	*d*	2H	-------
H-5	3.75	*d*	2H	$^{3}J = 6.0$
H-8, 8a' and H-9, 9a'	3.51	*m*	8H	-------
H-15	2.36	*s*	3H	-------

2.3.5.2 Characterization by ^{13}C NMR spectroscopy

The structure of the compound (4n) is further confirmed by ^{13}C NMR spectroscopy. ^{13}C NMR data of the compound (4n) is depicted in the table (2.10), which represented the characteristic signals of carboxamide. The more deshielded signal appeared at 166.4 ppm corresponding to amide carbon (C-6). Signal at 154.7 ppm corresponds to the ipso carbon (C-11) of phenyl ring attached with piperazine ring. Four carbons (C-8, C-8a', C-9, C-9a') of piperazine ring resonated at the chemical shift value of 43.7 ppm, 44.4

ppm, 46.0 ppm, 46.2 ppm respectively. A signal at 21.4 ppm corresponds to methyl carbon (C-15) attached with phenyl ring. Remaining signals for aromatic carbons appear in their characteristic regions

Table 2.10: ^{13}C NMR analysis of the synthesized carboxamide **(4n)**

Carbons	C-6	C-11	C-4	C-12, 12a'	C-9a'	C-8	C-5	C-15
δ (ppm)	166.4	154.7	143.1	112.9	46.2	43.7	41.2	21.4

CHAPTER 3

EXPERIMENTAL

3.1 General Considerations

All the experiments were performed in washed, rinsed, and dried apparatus. Before configuring the reactions, the solvents were dried and distilled. Nitrogen inert atmosphere was maintained for the reactions occurring in non-aqueous solvents. Anhydrous sodium sulphate was used for moisture removal. Reaction progress was monitored by thin layer chromatography (TLC). Pre-coated silica gel-60 F_{254} plates having 0.2 nm thickness were used for chromatographic analysis. UV-active compounds were visualized under UV lamp at 254 nm wavelength while UV-inactive compounds were spotted by using different spraying reagents such as anisaldehyde, ninhydrin and potassium permanganate (KMnO4). The purification of carboxamides was accomplished by flash column chromatography using 200-300 mesh sized silica gel as stationary phase.

3.2 Instrumentation

Melting point of the synthesized compounds was ascertained by Gallenkamp melting point apparatus MPD350.BM3.5 (UK). The synthesized compounds were characterized by ^{1}H NMR and ^{13}C NMR spectroscopy using Bruker Avance 300 MHz and 75MHz NMR spectrophotometer (Switzerland). The chemical shift (δ) values were mentioned in ppm while coupling constant *(J)* was calculated in Hertz (Hz).

3.3 Drying and distillation of solvents

Moisture free organic solvents were employed to ensure the completion of reactions. So, desiccants were used to dry and distill them. A brief description for drying and distillation of organic solvents is given below which is widely employed in the organic synthesis.

3.3.1 *N*, *N*-Dimethylformamide (DMF)

The drying of DMF is difficult since it has a boiling point of 153 °C and high-water miscibility, so vacuum distillation was employed. The solvent was stirred overnight with anhydrous calcium hydride, filtered, and finally distilled under reduced pressure. The solvent was then stored over activated 4 Å molecular sieves.

3.3.2 Dichloromethane (DCM)

The boiling point of dichloromethane is 39.6 °C and it contains low water content, so its drying is not difficult. To accomplish the drying of this solvent, analytical grade DCM was refluxed over anhydrous CaH_2, filtered, then distilled and the fraction having

boiling point 40 – 41 °C was collected. Freshly dried DCM was stored over activated 4 Å molecular sieves in a brown bottle.

3.3.3 Tetrahydrofuran (THF)

THF has boiling point of 66 °C and it contains water content as it is water soluble organic solvent. It was refluxed with sodium metal followed by the addition of benzophenone as an indicator. The drying was progressed in an inert atmosphere. Dark blue color appeared due to disodium benzophenone complex which indicated the completion of drying of tetrahydrofuran. Dried THF was then stored over pre-activated 4 Å molecular sieves.

3.4 Staining Agents

3.4.1 Ninhydrin Stain

The ninhydrin stain was used to detect the NH and NH_2 group in the synthesized compounds. The ninhydrin was prepared by the following protocol;

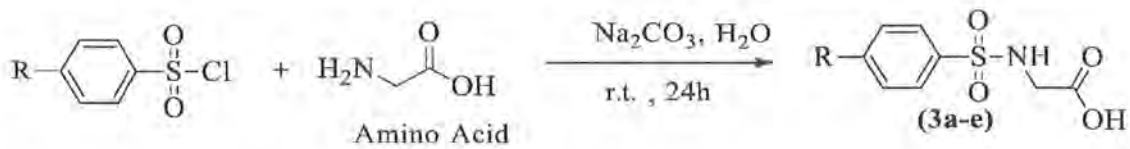
1.5g ninhydrin reagent and 3 cm^3 glacial acetic acid were dissolved in o.1L n-butanol. The resultant staining solution was kept in a glass bottle covered with a lid and wrapped in aluminium foil.

3.4.2 *p*-Anisaldehyde Stain

This stain is widely used to differentiate between various functional groups, e.g., carbonyl and ester-containing compounds. Nucleophilic functional groups are typically susceptible to it. The stain was prepared by the following protocol;

p-anisaldehyde (3.6 ml), glacial acetic acid (1.4 ml), and conc. sulfuric acid (4.5 ml) was added to 130 ml of ethanol. The solution was subjected to stirring for a few minutes at room temperature. The resulting staining was kept in a glass bottle covered with a lid and wrapped in aluminium foil.

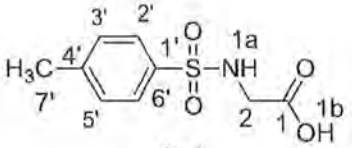
3.5 General procedure for the synthesis of Sulfonamide Carboxylic acid (3a-e)



To an aqueous combination of an amino acid (10 mmol) and Na_2CO_3 (12 mmol) in water (50 mL), *p*-substituted benzenesulfonyl chloride (12 mmol) was added over the

course of 15 minutes. Following complete addition of reagents, reaction mixture in the flask was allowed to stir for the time period of 4-6 hours at ambient temperature before being acidified with dilute HCl. The precipitates obtained were filtered, purified, dried properly and then recrystallized using ethylacetate-n-hexane to get the desired product.[107]

3.5.1 2-(4-Methylphenylsulfonamido)acetic acid (3a)

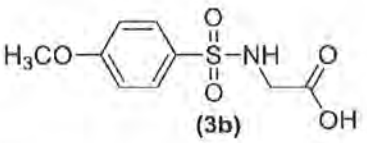


(3a)

White shiny solid; **Yield:** 91 %; **m.p. :** 146-148 °C (Lit. 147-149 °C)

R_f : 0.43 (Chloroform: Methanol :: 9 : 1); **^{1}H NMR:** (300 MHz, DMSO-d_6): δ (ppm) 12.5 (1H, s, H-1b), 7.95 (1H, s, H-1a), 7.65 - 7.66 (2H, d, ^3J=8.1 Hz, H-2' & 6'), 7.35-7.38 (2H, d, ^3J=8.1 Hz, H-3' & 5'), 3.50 -3.53 (2H, d, ^3J=10.8 Hz, H-2), 2.37 (3H, s, H-7'); **^{13}C NMR:** (75 MHz, DMSO-d_6): δ (ppm) 170.7 (C-1), 143.0 (C-4'), 138.2 (C-1'), 129.9 (C-3'& 5'), 127.0 (C-2'& 6'), 44.2 (C-2), 21.4 (C-7').

3.5.2 2-(4-Methoxyphenylsulfonamido)acetic acid (3b)

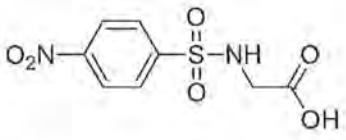


(3b)

White solid **Yield:** 89% **m.p.** : 143-149 °C (Lit. 149)

R_f: 0.41 (Chloroform: Methanol :: 9:1)

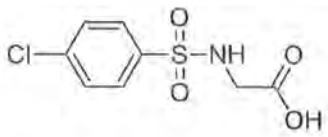
3.5.3 2-(4-Nitrophenylsulfonamido)acetic acid (3c)



White solid **Yield:** 87% **m.p.** : 156-159 °C (Lit. 157)

R_f: 0.38 (Chloroform: Methanol :: 9:1)

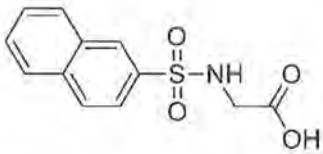
3.5.4 2-(4-Chlorophenylsulfonamido)acetic acid (3d)



White solid **Yield:**92% **m.p.** 124-131 °C (Lit. 128)

R_f: 0.40 (Chloroform: Methanol :: 9:1)

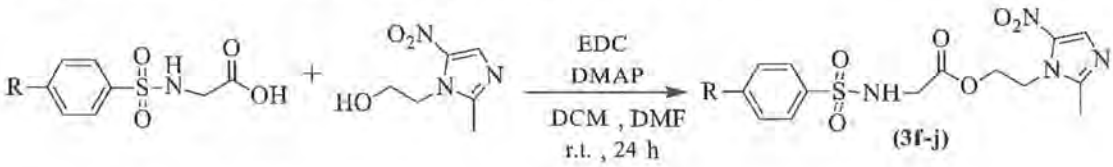
3.5.5 2-(Naphthalene-2-sulfonamido)acetic acid (3e)



White solid **Yield:** 85% **m.p.** : 161-163 °C (Lit. 164)

R_f: 0.45 (Chloroform: Methanol :: 9:1)

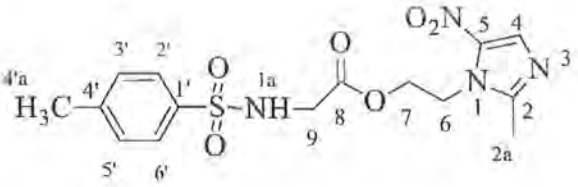
3.6 General procedure for the synthesis of Ester linked conjugates of metronidazole and sulfonamide carboxylic acid (3f-j)



Where R= CH_3, OCH_3, NO_2, Cl, Naphthalene

1 equivalent of 2-(4-substitutedphenylsulfonamido)acetic acid was dissolved in DCM and DMF (10:1), then 0.2 equivalent of DMAP, 1 equivalent of metronidazole bearing OH moiety and 1 equivalent of EDC were added in the reaction flask and allowed to stir in an inert atmosphere for 24 hours at the room temperature. After the completion of reaction, the urea and DMAP were removed by solvent extraction with ethyl acetate under acidic conditions. The pale yellow precipitates obtained were recrystallized using chloroform to afford the pure product as off white crystals.

3.6.1 2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2-(4-methylphenylsulfonamido) acetate



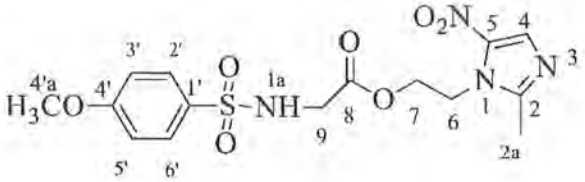
(3f)

Off white crystals; **Yield:**58% ; **m.p.** : 184-186 °C ;

R*f* : 0.40 (Chloroform: Methanol :: 9:1)

^{1}H NMR: (300 MHz, DMSO-d$_6$): δ (ppm) 8.13 (1H, t, ^3J =6 Hz, H-1a), 8.04 (1H, s, H-4), 7.61 (2H, d, H-2'& 6'), 7.34 (2H, d, H-3'& 5'), 4.51 (2H, t, ^3J =4.8 Hz, H-7), 4.30 (2H, *t*, ^3J =4.8 Hz, H-6), 3.60 (2H, d, ^3J =6 Hz, H-9), 2.43 (3H, s, H-2a), 2.36 (3H, s, H-4'a) ; **^{13}C NMR:** (75 MHz, DMSO-*d*$_6$)**:** δ (ppm) 169.1 (C-8), 152.1 (C-2), 143.2 (C-5), 138.8 (C-4'), 138.0 (C-1'), 133.5 (C-4), 129.9 (C-3'& 5'), 126.9 (C-2'& 6'), 63.5 (C-7), 45.0 (C-9), 44.0 (C-6), 21.3 (C-4'a), 14.4 (C-2a)

3.6.2 2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl2-(4-methoxyphenyl sulfonamido)acetate

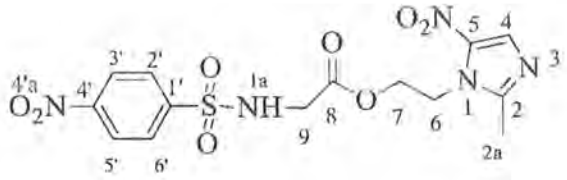


(3g)

White solid; **Yield:** 55%; **m.p.** : 192-195 °C; **R*f*** : 0.38 (Chloroform: Methanol :: 9:1)

^{1}H NMR: (300 MHz, DMSO-d$_6$): δ (ppm) 7.95 (1H, s, H-4), 7.75 (2H, d, H-2'& 6'), 6.96 (2H, d, H-3'& 5'), 5.56 (1H, t, H-1a), 4.57 (2H, t, ^3J=4.8 Hz, H-7), 4.41 (2H, t, ^3J=4.8 Hz, H-6), 3.87 (3H, s, H-4'a), 3.72 (2H, d, ^3J=6 Hz, H-9), 2.51 (3H, s, H-2a); **^{13}C NMR:** (75 MHz, DMSO-*d*$_6$)**:** δ (ppm) 168.7 (C-8), 163.1 (C-4'), 150.9 (C-2), 138.3 (C-5), 132.6 (C-1'), 130.6 (C-4), 129.3 (C-2'& 6'), 114.3 (C-3'& 5'), 63.6 (C-7), 55.6 (C-4'a), 44.8 (C-9), 43.9 (C-6), 14.2 (C-2a).

3.6.3 2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2-(4-nitrophenylsulfonamido) acetate

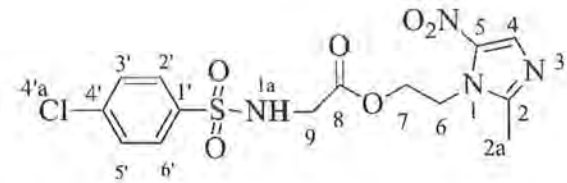


(3h)

White solid; **Yield:** 56%; **m.p.** : 180-182 °C ; **R*f***: 0.33 (Chloroform: Methanol :: 9:1)

^{1}H NMR: (300 MHz, DMSO-d$_6$): δ (ppm) 8.65 (1H, s, H-4), 8.77 (2H, m, H-3'& 5'), 8.01 (3H, m, H-2',6'& 1a), 4.51 (2H, *t*, ^3J=4.8 Hz, H-7), 4.33 (2H, t, ^3J=4.8 Hz, H-6), 3.77 (2H, s, H-9), 2.42 (3H, s, H-2a) ; **^{13}C NMR:** (75 MHz, DMSO-*d*$_6$)**:** δ (ppm) 169.1 (C-8), 152.1 (C-2), 150.0 (C-4'), 146.7 (C-1'), 138.8 (C-5), 133.5 (C-4), 128.4 (C-2'& 6'), 124.89 (C-3'& 5')), 63.6 (C-7), 45.0 (C-9), 44.0 (C-6), 14.4 (C-2a)

3.6.4 2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2-(4-chlorophenylsulfonamido) acetate

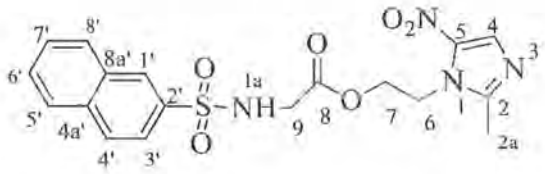


(3i)

White solid; **Yield:** 52%; **m.p.** : 207-210 °C ; **R*f***: 0.35 (Chloroform: Methanol :: 9:1)

^{1}H NMR: (300 MHz, DMSO-d$_6$): δ (ppm) 8.36 (1H, t, ^3J=6 Hz, H-1a), 8.05 (1H, s, H-4), 7.80 (2H, m, H-2' & 6'), 7.62 (2H, m, H-3' & 5'), 4.52 (2H, t, ^3J=4.9 Hz, H-7), 4.33 (2H, t, ^3J=4.9 Hz, H-6), 3.68 (2H, d, ^3J=6 Hz, H-9), 2.44 (3H, s, H-2a) ; **^{13}C NMR:** (75 MHz, DMSO-*d*$_6$)**:** δ (ppm) 169.1 (C-8), 152.1 (C-2), 140.4 (C-1'), 138.8 (C-5), 137.8 (C-4'), 133.4 (C-4), 129.6 (C-3'& 5'), 128.9 (C-2'& 6'), 63.5 (C-7), 45.07 (C-9), 44.0 (C-6), 14.4 (C-2a)

3.6.5 2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2-(naphthalene-2-sulfonamido)acetate



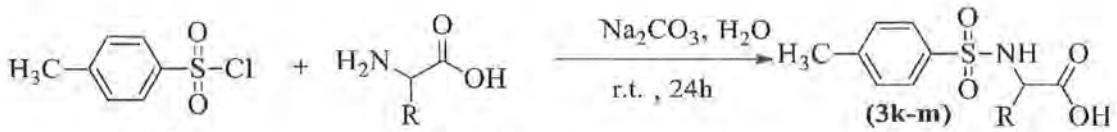
(3j)

Off White solid **Yield:** 52% **m.p.** : 217-221 °C ;

R_f : 0.39 (Chloroform: Methanol :: 9:1)

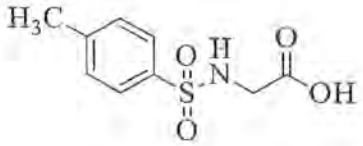
^{1}H NMR: (300 MHz, DMSO-d_6): δ (ppm) 8.80 (1H, m, H-1'), 8.40 (1H, m, H-4'), 8.13 (1H, m, H-3'), 8.00 (2H, m, H-5', 8'), 7.81 (1H, s, H-4), 7.74 (1H, s, H-1a), 7.59 (2H, m, H-6',7'), 4.56 (2H, t, ^3J =4.9 Hz, H-7), 4.44 (2H, t, ^3J=4.9 Hz, H-6), 3.81 (2H, s, H-9), 2.48 (3H, s, H-2a) ; **^{13}C NMR:** (75 MHz, DMSO-d_6): δ (ppm) 169.0 (C-8), 152.1 (C-2), 138.8 (C-5), 137.9 (C-4a'), 134.6 (C-2'), 133.5 (C-8a'), 132.0 (C-4), 129.7 (C-4'), 129.5 (C-5'), 129.2 (C-8'), 128.2 (C-6'), 128.0 (C-7'), 127.6 (C-1'), 122.6 (C-3'), 63.5 (C-7), 44.9 (C-9), 44.1 (C-6), 14.3 (C-2a).

3.7 General procedure for the synthesis of *N*-(*p*-toluene sulfonyl)amino acids (3k-m)



To an aqueous combination of an amino acid (10 mmol) and Na_2CO_3 (12 mmol) in water (50 mL), *p*-toluenesulfonyl chloride (12 mmol) was added slowly over the course of 15 minutes. Following complete addition of reagents, the reaction mixture in the flask was allowed to stir for the time period of 4-6 hours at ambient temperature before being acidified with dilute HCl. The precipitates obtained were filtered, purified, dried, and then recrystallized from ethyl acetate-*n*-hexane to get the desired product.[107]

3.7.1 *N*-(*p*-Toluenesulfonyl)glycine (3k)

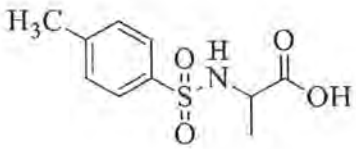


(3k)

White solid **Yield:**91% **m.p.** : 146-148 °C (Lit. 146-147 °C)

R_f: 0.43($CHCl_3$: CH_3OH:: 9:1)

3.7.2 *N*-(*p*-Toluenesulfonyl)alanine (3l)

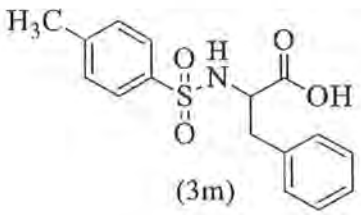


(3l)

White solid **Yield:** 85% **m.p.** : 135-137 °C (Lit. 138-139 °C)

R_f: 0.40($CHCl_3$: CH_3OH:: 9:1)

3.7.3 *N*-(*p*-Toluenesulfonyl)phenylalanine (3m)



(3m)

White solid **Yield:**88% **m.p.** : 164-169 °C (Lit. 164-165 °C)

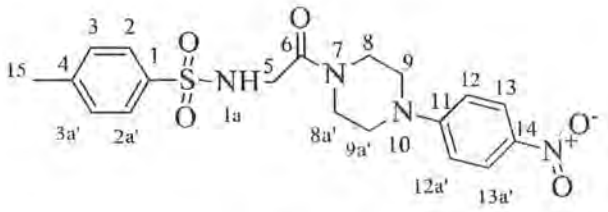
R_f: 0.59($CHCl_3$: CH_3OH:: 9:1)

3.8 General procedure for the synthesis of Carboxamides (3n-3p)

1 equivalent of *N*-(*p*-Toluenesulfonyl)-amino acid was dissolved in DCM and DMF (10:1), then 0.2 equivalent of DMAP, 1 equivalent of an amine and 1 equivalent of EDC were added in the flask containing reaction mixture and allowed to stir in an inert atmosphere for 24 hours at the room temperature. After the completion of reaction, the

urea and DMAP were removed by solvent extraction with ethyl acetate under acidic conditions. Carboxamides were further purified by flash column chromatography employing silica gel as stationary phase and n-hexane:ethyl acetate as mobile phase.

3.8.1 4-Methyl-*N*-(2-(4-(4-nitrophenyl)piperazin-1-yl)-2-oxoethyl)benzene sulfonamide



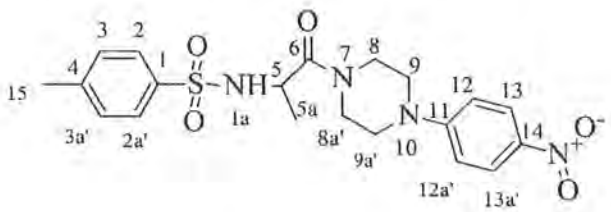
(3n)

Yellow solid **Yield:** 69% **m.p.** : 216-218°C

R_f : 0.33 (*n*-hexane: Ethyl acetate :: 1:1)

^{1}H NMR: (300 MHz, DMSO-d_6): δ (ppm) 8.06 (2H, d, H-13 & 13a'), 7.68 (3H, m, H-2,2a' & 1a), 7.36 (2H, d, H-3 & 3a'), 6.99 (2H, d, H-12 & 12a'), 3.75 (2H, d, ^3J=6 Hz, H-5), 3.51 (8H, m, H-8,8a'& 9,9a'), 2.36 (3H, s, H-15); **^{13}C NMR:** (75 MHz, DMSO-d_6)**:** δ (ppm) 166.4 (C-6), 154.7 (C-11), 143.1 (C-4), 137.8 (C-14), 137.4 (C-1), 129.9 (C-3 & 3a'), 127.1 (C-2 & 2a'), 126.2 (C-13 & 13a'), 112.9 (C-12& 12a'), 46.2 & 46.0 (C-9 & 9a'), 44.4 & 43.7 (C-8 & 8a'), 41.4 (C-5), 21.4 (C-15).

3.8.2 4-Methyl-*N*-(1-(4-(4-nitrophenyl)piperazin-yl)-1-oxo-3-phenylpropan-2-yl)benzenesulfonamide



(3o)

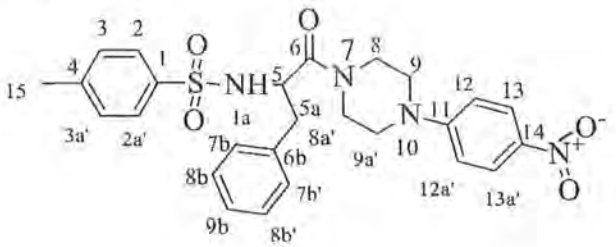
Yellow solid **Yield:** 62 % **m.p.** : 254-257 °C

R_f : 0.39 (*n*-hexane: Ethyl acetate :: 1:1);

^{1}H NMR: (300 MHz, DMSO-d_6): δ (ppm) 8.06 (2H, m, H-13 & 13a'), 7.66 (2H, m, H-2 & 2a'), 7.40 (2H, m, H-3 & 3a'), 7.01 (2H, m, H-12 & 12a'), 4.11 (1H, q, ^3J=6 Hz,

H-5), 4.00 (1H, s, H-1a), 3.88 (2H, t, ^{3}J=3 Hz, H-8 & 8a'), 3.65 (2H, t, H-8 & 8a'), 3.21 (4H, t, ^{3}J=3 Hz, H-9 & 9a'), 2.43 (3H, s, H-15), 1.29 (3H, d, ^{3}J=3 Hz, H-5a); **^{13}C NMR:** (75 MHz, DMSO-d_6): δ (ppm) 169.8 (C-6), 155.3 (C-11), 140.5 (C-4), 137.1 (C-1), 136.9 (C-14), 130.1 (C-2 & 2a'), 128.5 (C-3 & 3a'), 125.8 (C-13 & 13a'), 112.9 (C-12& 12a'), 53.3 (C-5), 47.9 (C-9 & 9a'), 46.8 (C-8 & 8a'), 21.5 (C-15), 18.7 (C-5a).

3.8.3 4-Methyl-*N*-(1-(4-(4-nitrophenyl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)benzenesulfonamido



(3p)

Yellow solid **Yield:** 63% **m.p.** : 268-273 °C

R$_f$: 0.41 (*n*-hexane: Ethyl acetate :: 1:1)

^{1}H NMR: (300 MHz, DMSO-d$_6$): δ (ppm) 8.04 (2H, m, H-13 & 13a'), 7.66 (2H, m, H-2 & 2a'), 7.39 (4H, m, H-3 & 3a'), 7.18 (5H, m, H-7b, 7b', 8b, 8b' & 9b), 7.01 (2H, m, H-12 & 12a'), 6.46 (1H, s, H-1a), 4.44 (1H, t, ^{3}J=6 Hz, H-5), 3.69 (2H, t, H-8, 8a'), 3.39 (2H, t, H-8, 8a'), 3.21 (4H, m, H-9, 9a'), 2.91 (2H, dd, ^{3}J=4.5 Hz, H-5a), 2.13 (3H, s, H-15); **^{13}C NMR:** (75 MHz, DMSO-d_6): δ (ppm) 171.0 (C-6), 155.1 (C-11), 141.6 (C-4), 139.1 (C-1), 137.3 (C-14), 137.3 (C-6b), 129.1 (C-7b & 7b'), 128.9 (C-8b & 8b'), 128.5 (C-3 & 3a'), 127.0 (C-9b), 126.6 (C-2 & 2a'), 125.9 (C-13 & 13a'), 112.5 (C-12 & 12a'), 61.3 (C-5), 47.9 (C-9 & 9a'), 46.3 (C-8 & 8a'), 38.3 (C-5a), 21.1 (C-15).

Conclusions

Sulfonamides and metronidazole scaffold has been investigated for many important biological properties. Utility of these important cores instigated the present study. Amino acids-based sulfonamides were used as core substrates. Different substituted sulfonamides were synthesized in aqueous medium and purified through recrystallization and obtained in good yields. Synthesized sulfonamides were functionalized in different ways by developing ester linkage with pendent hydroxyl group of metronidazole and amide linkage using substituted piperazine. Esters and carboxamides were synthesized using acyl transfer reagents EDC and DMAP. These acyl transfer reagents play a significant role in the activation of carboxylic acids. Carboxamides were purified through column chromatography. The synthesized compounds with different R-groups were obtained in moderate yield and then characterized by ^{1}H NMR, ^{13}C NMR spectroscopy and their physical parameters were also determined. All the synthesized compounds are in the process of screening for their potential as antimicrobial agents.

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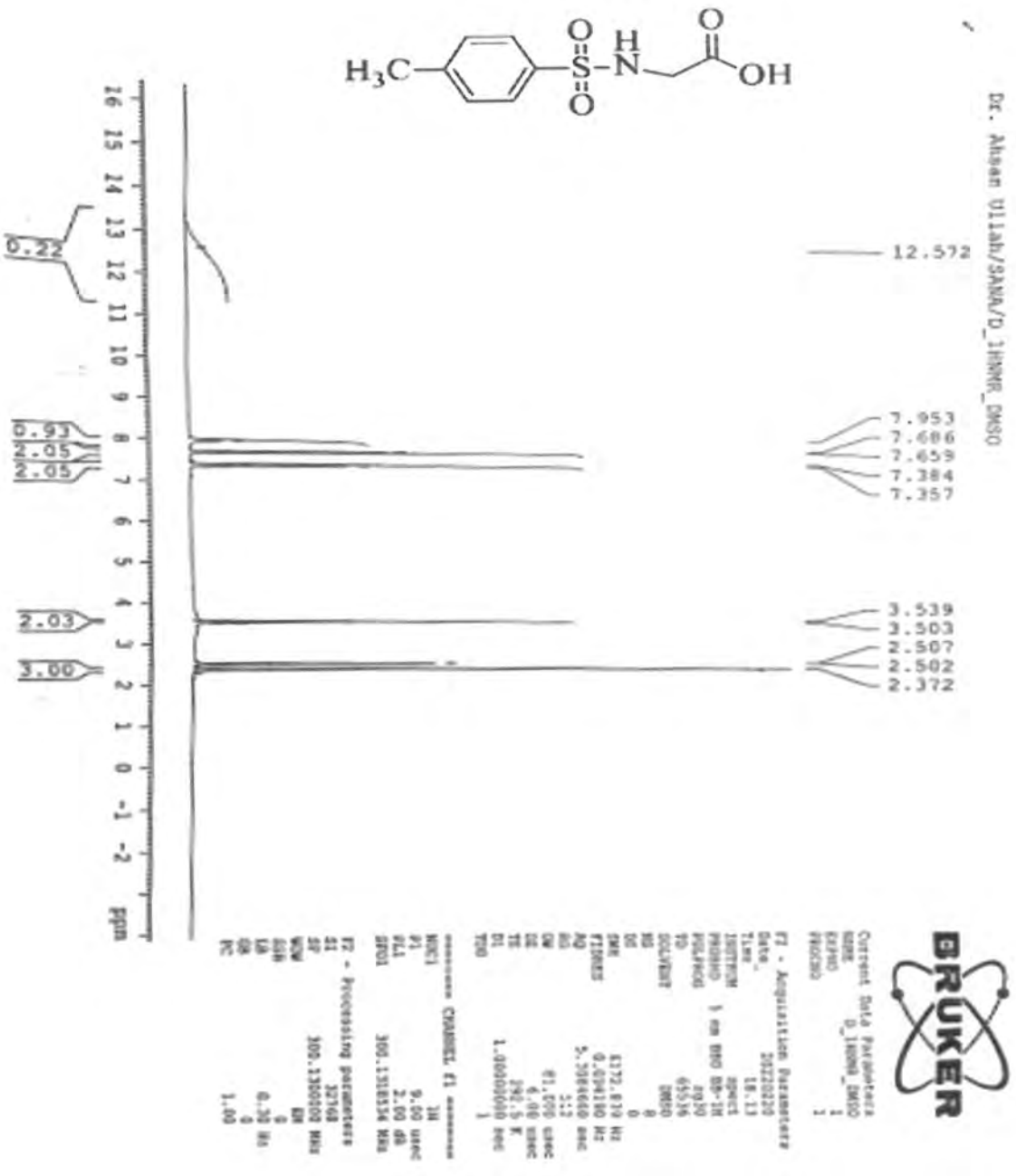
Appendix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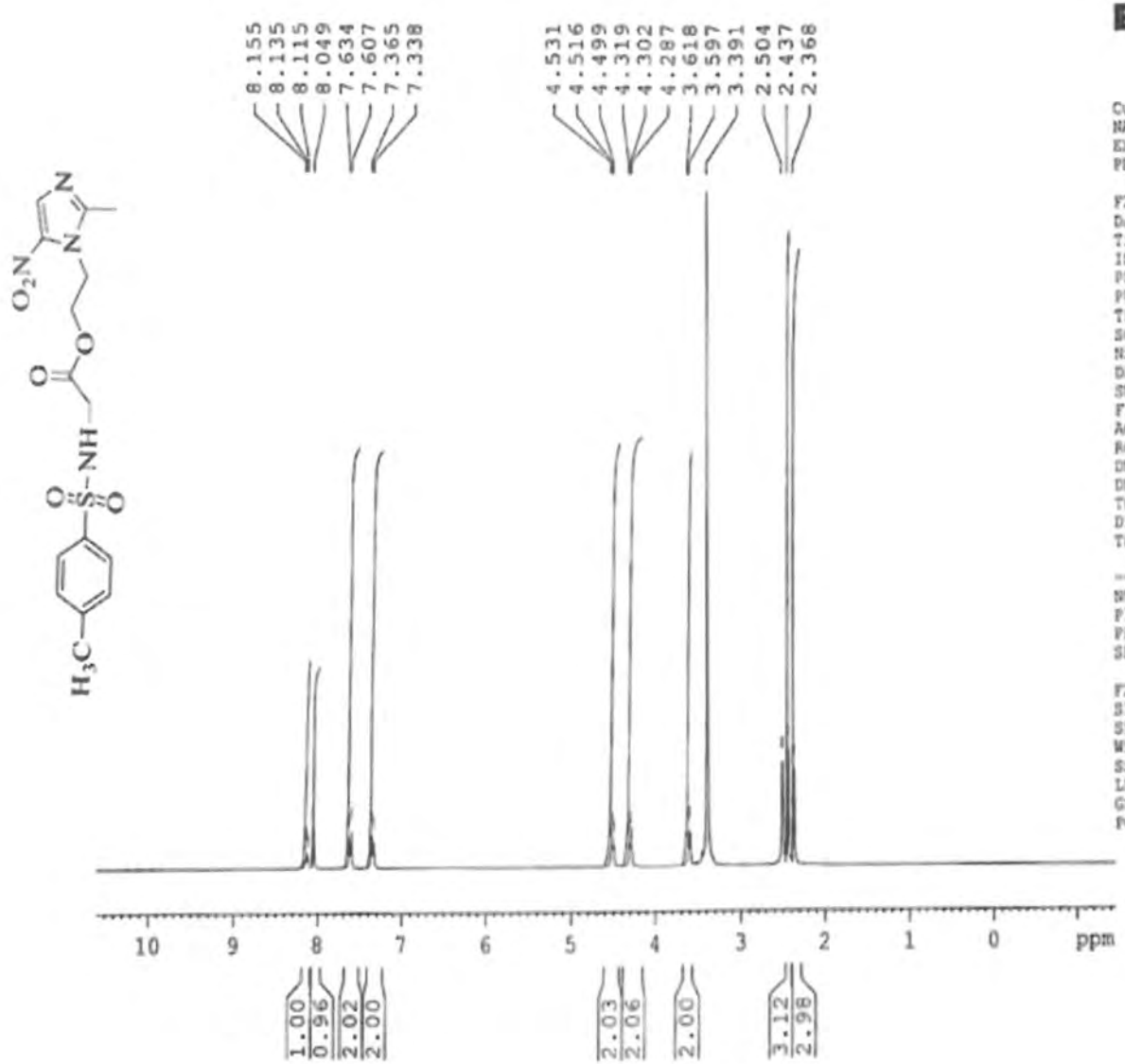
BRUKER

Dr. Ahsan Ullah/SANA/SB-044K_1HNMR_DMSO



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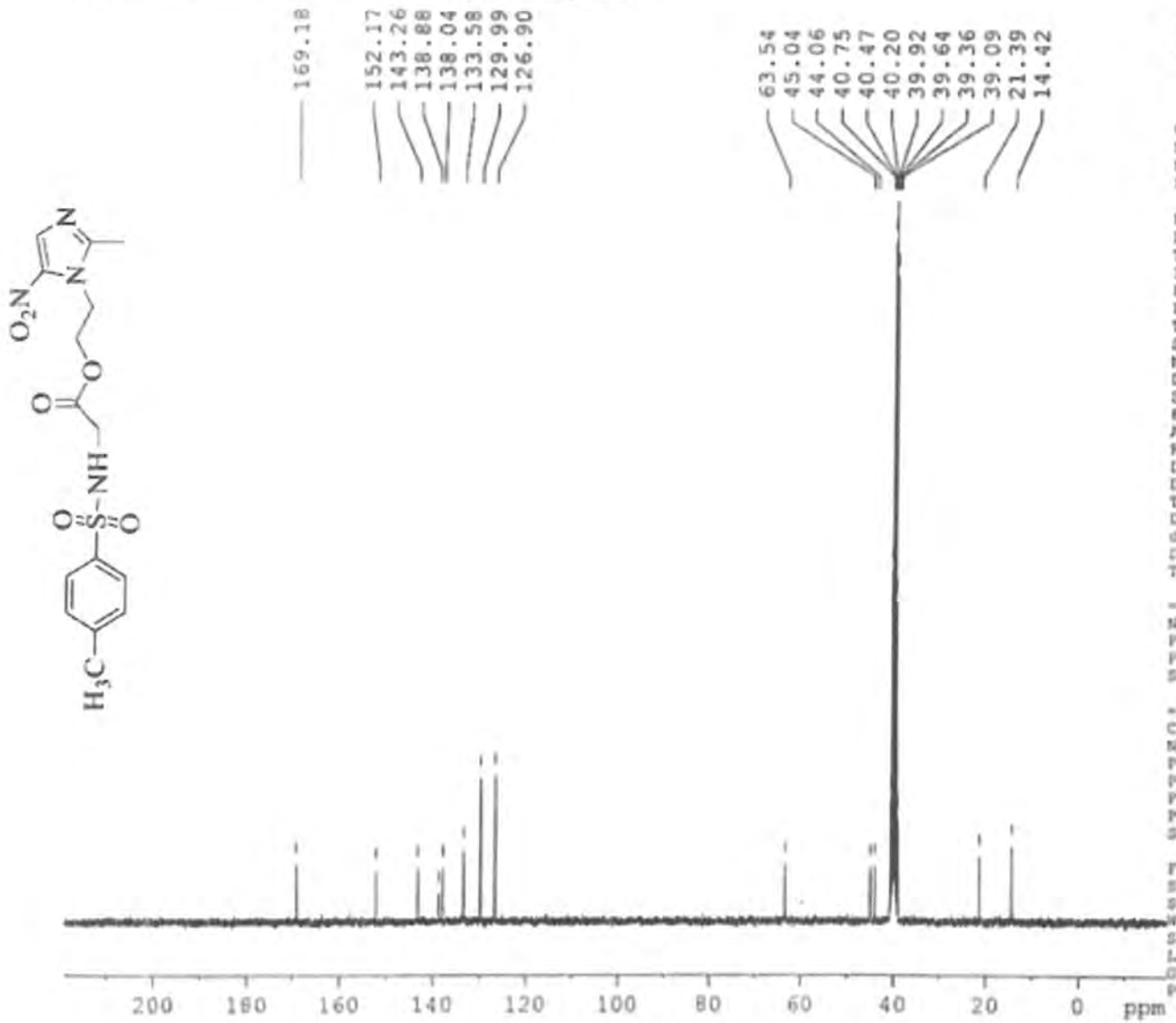
Dr. Ahsan Ullah/SANA/SB-044K_13CNMR_DMSO



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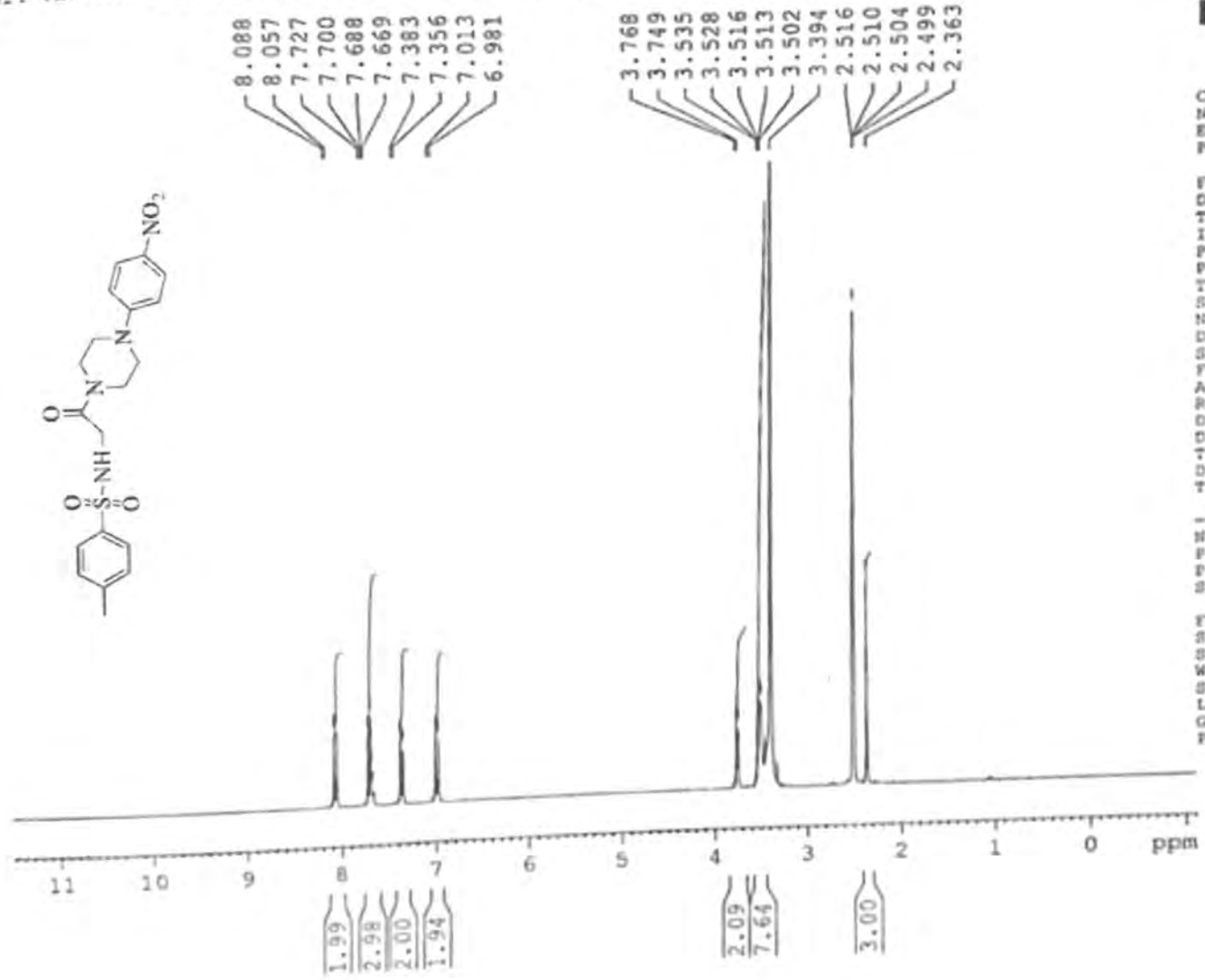

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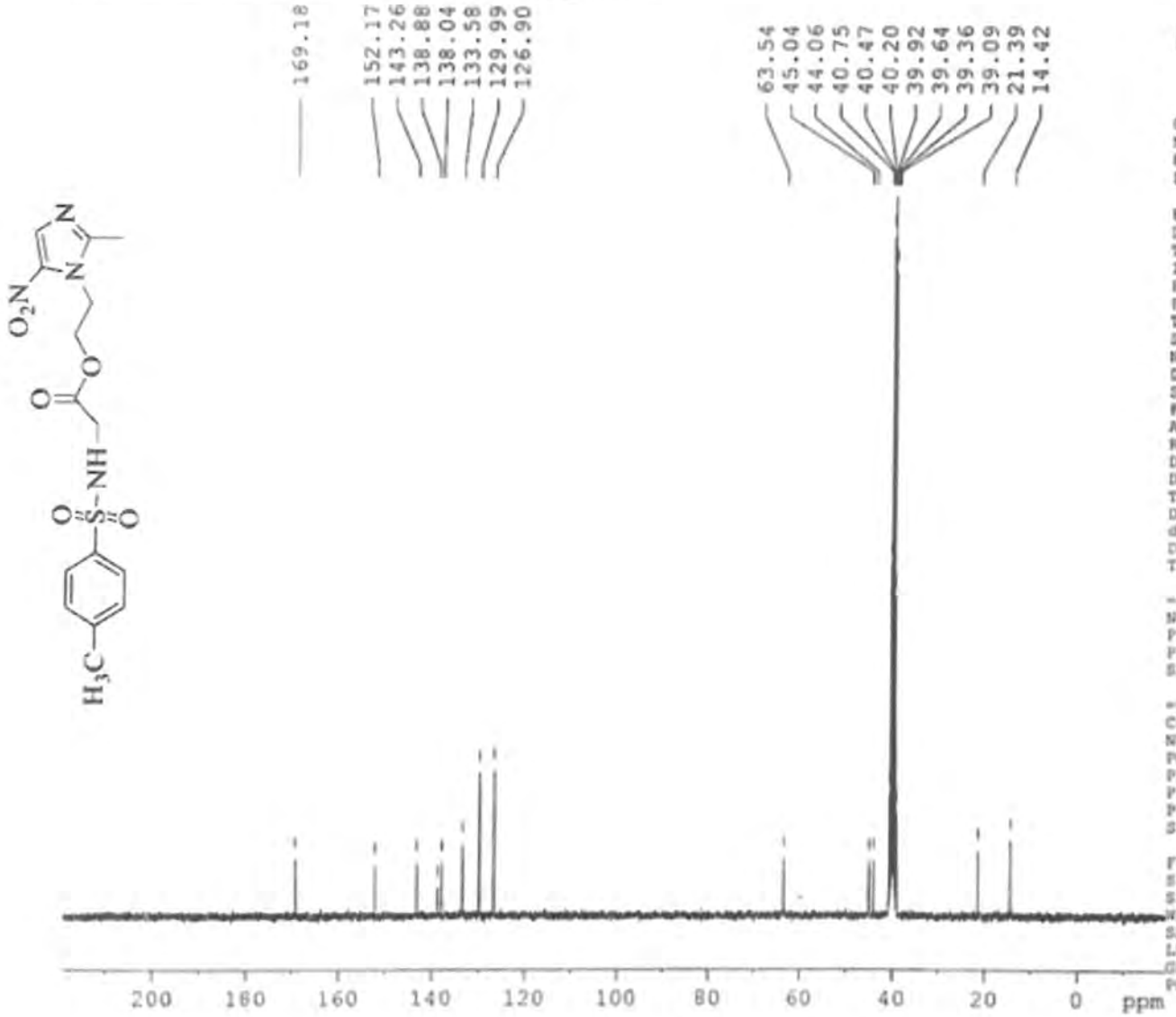
Dr. Ahsan Ullah/SANA/SB-044K_13CNMR_DMSO



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TD                  35968
SOLVENT              DMSO
NS                    688
DS                      0
SWH           17985.611 Hz
FIDRES         0.500045 Hz
AQ             0.9999604 sec
RG                 2298.8
DW                27.800 usec
DE                 6.00 usec
TE                296.6 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
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======== CHANNEL f1 ========
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PL1               -5.00 dB
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======== CHANNEL f2 ========
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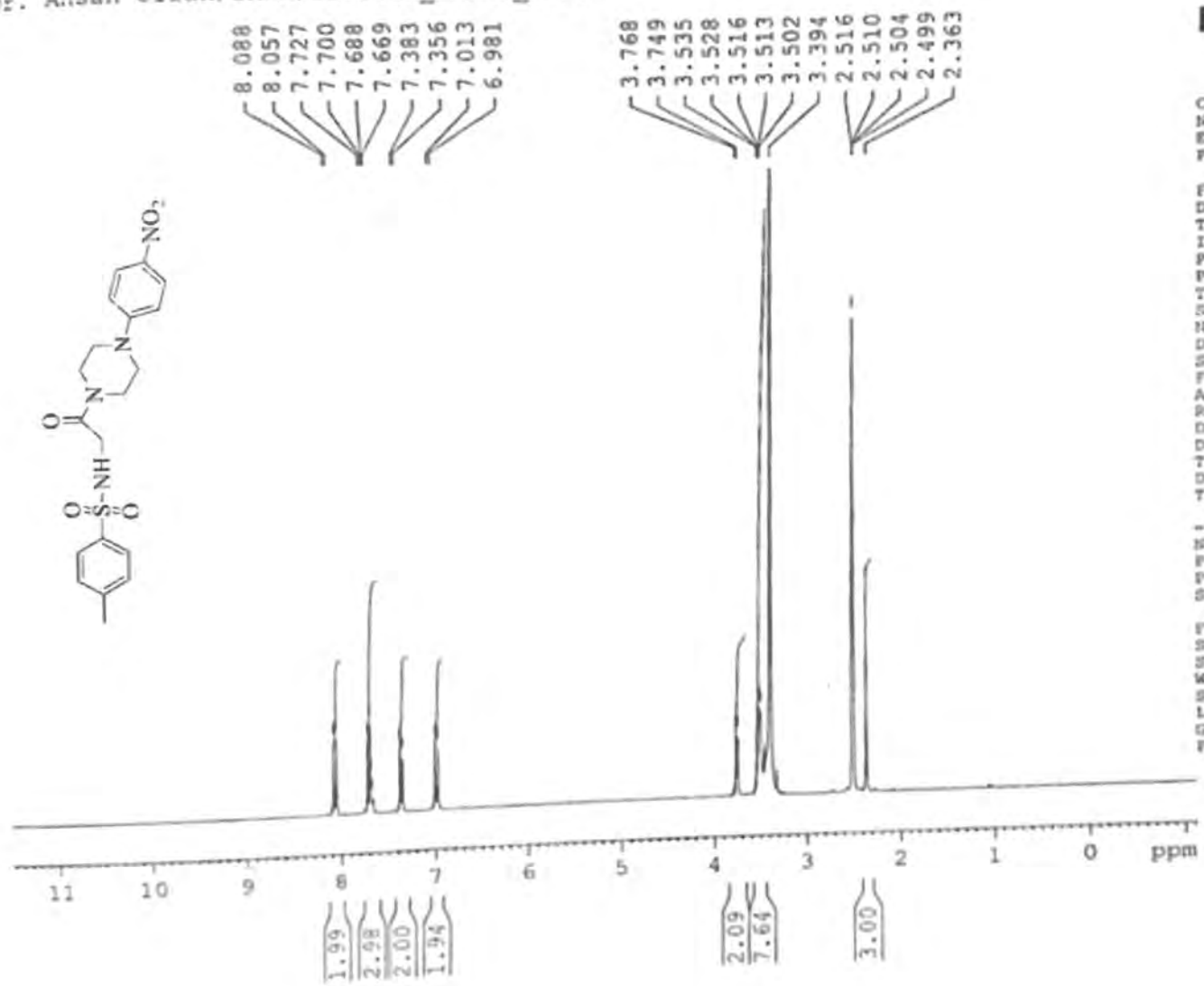
Dr. Ahsan Ullah/SANA/SB-042K_1HNMR_DMSO



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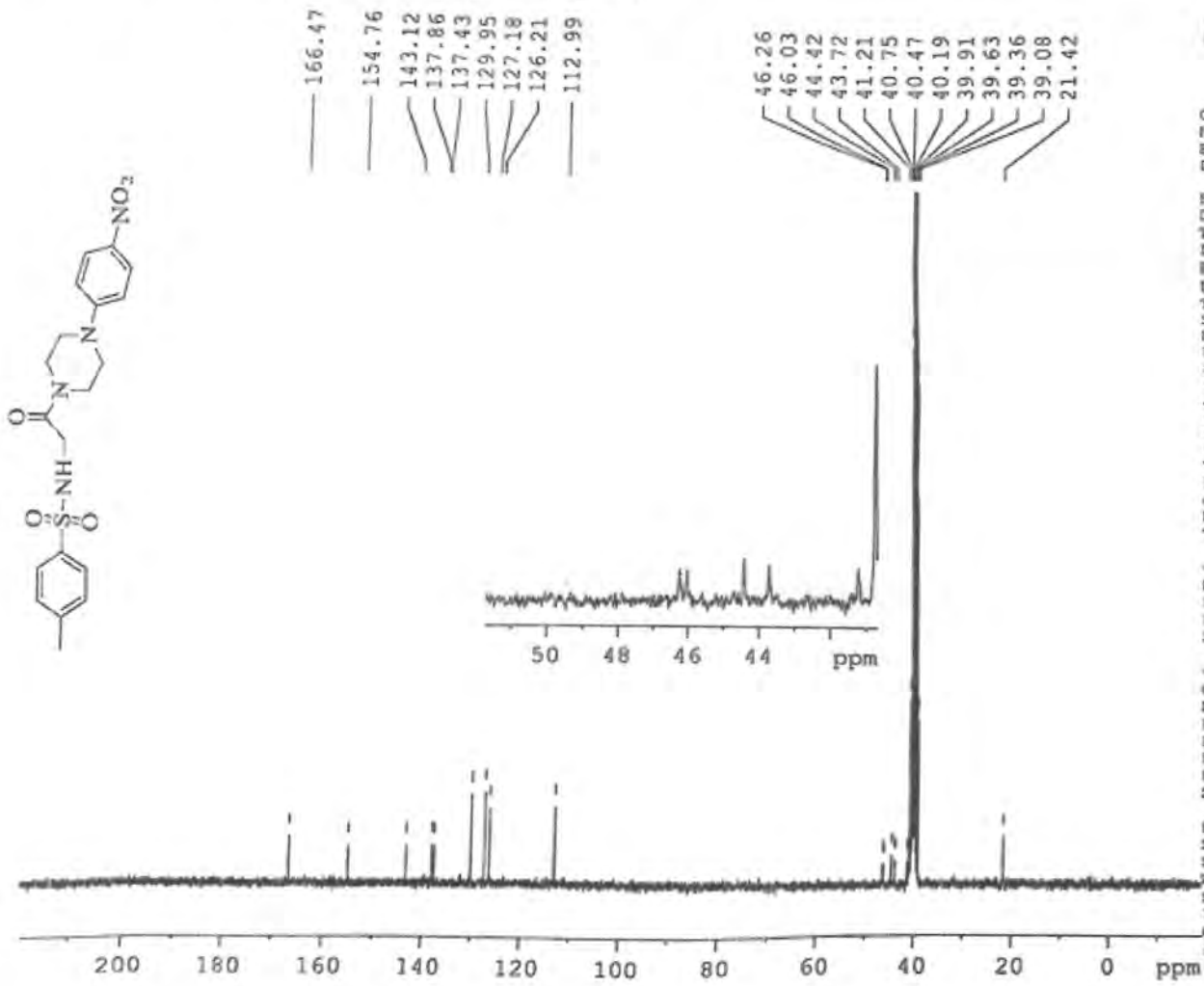

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d11 0.03000000 sec
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