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SYNTHESIS CHARACTERIZATIONS & EVALUATIONS OF NEW ANTIMICROBIALS

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ABSTRACT

Antibiotics are agents produced by microorganisms, which suppress the growth of or kill other microorganisms at very low concentrations. The term antimicrobial agent is used to designate synthetic as well as naturally obtained drugs that inhibit the growth of microorganisms. The use of antimicrobial drugs in human medicine has resulted in spectacular gains in human health and life expectancy. The main objective of the present study is to develop new chemical entities with potential antibacterial, which are superior to the currently available drugs and also which has an improved spectrum of activity even towards the most resistant organisms. So, in the introduction currently available anti-tubercular agents, antibacterial agents, antifungal agent's problems associated with the current therapy and current trends in the field of anti-microbial research On the biases of target based approach for the synthesis of new drugs by which a sufficient grade of selectivity can be achieved for anti-microbial activity. Various new molecules were synthesized by using bezothiazoles nucleus. After biological evolutions it was found that **(8a-i) (Benz)** exhibited antibacterial activity at tested concentration less 1000µg/ml, After characterization & biological evaluation it was found that the some compound of benzothiazole **8d(Benz)** exhibited activity at lower concentration <20 µg/ml. & the Compounds like **8h(Benz)**, was not exhibited activity higher concentration up to 1000 µg/ml against both gram positive and gram negative organisms.

KEYWORDS: Improved spectrum of activity Microorganisms are becoming resistant Need for the development Excessive metabolism of the drug Increased efflux of drug.

1. INTRODUCTION

Anti-microbial agents are those which indulgence contagion by repress or devastate the contributory germs such as bacteria, mycobacterium, fungi, protozoa or viruses without significant effect on host tissues.[74]

The first truly effective antimicrobial agent had a date back of the sulfonamides in the mid of 1930. Then the commercial are use of penicillin in 1940. afterward the discovery and development of streptomycin, chloramphenicol in 1944 &1947, chlortetracycline in 1948 Macrolides in 1952 engineered penicillin's, cephalosporins and glycopeptides 1958 onwards, streptogramins and quinolones fluoroquinolones, at last oxazolidinones and cationic peptides in the 1990.

No new class of antibacterial comes into existent, though many analogues of existing classes with improved profiles have been improvised. However, this bright picture of antimicrobial therapy has darker side because of the increasing impact of microbial resistance. Microorganisms are becoming resistant more quickly than new drugs are being discovered. This can be combated by the discovery of new drugs acting by novel mechanisms of action.

Incidence of microbial infections is increasing worldwide. Even after all the efforts aimed to treat infections, effective treatment still remains a major challenge. The main reason for the failure of treatment remains to be resistance. Antimicrobial resistance has long been recognized and is considered as a serious health problem.

Resistance could occur by either of the following mechanisms:

1) Decreased uptake of drug
2) Increased efflux of drug
3) Genetic modifications (such as mutation) in the target site preventing binding of the drug
4) Excessive metabolism of the drug

Due to resistance, some diseases are either difficult to treat or even rendered untreatable. Resistant strains of pathogens create havoc especially in hospitals where patients are already immuno-compromised. So Increasing in resistance as of multi-drug microbial infections in the last ten year, create great problem in health. e.g. evolving of the stains having multi-medicate safe in Gram-positive bacterial pathogens and Gram-ve pathogens Like MRSA *(Methicillin resistant Staphylococcus aureus).*

Occurrence of Such group of acquired methicillin resistant stain *S. aureus* was explained in year of 1980's. Because, the epidemic of methicillin- resistant *S. aureus* has run out rife through many hospitals in various countries and was responsible for morbidity and mortality.[5]

As a result of the need to combat drug resistant pathogens and the increasing failure of available drugs, there has been a resurgent interest in discovering newer anti-infective agents.

Traditionally most of the drugs were discovered serendipitously. There has been a great improvement in the treatment of infectious diseases due to the advances in molecular biology and medicinal chemistry. This provides a better thoughtful of the molecular origin of infection & rational growth of the agents to treat the causative pathogens. This in turn, requires identification of a target within the pathogen.

1.1 Classification of Anti-microbial Agents

Anti-microbial agents can be broadly classified as follows[3,4] (**Figure 1.1**).

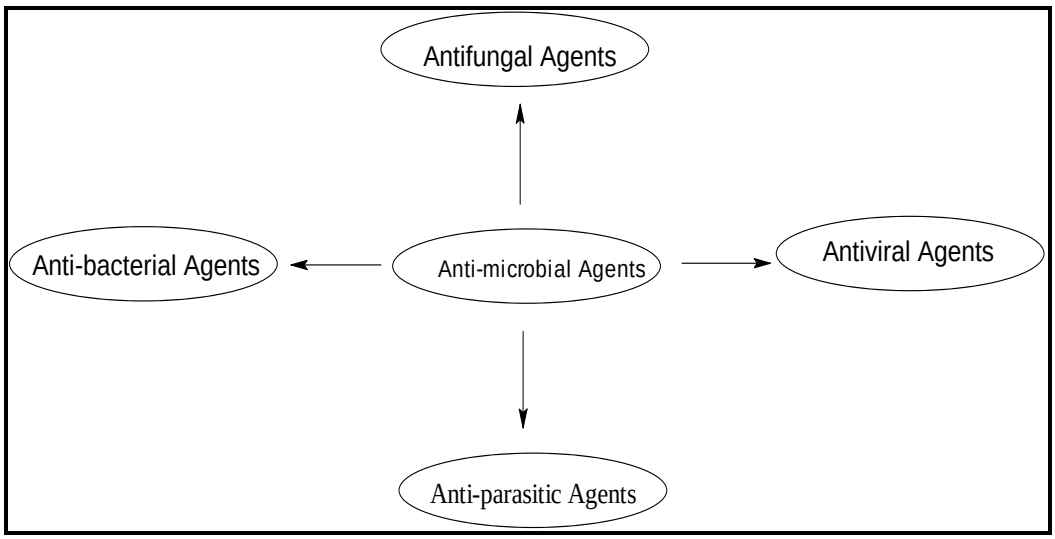


Figure 1.1: Classification of anti-microbial agents

The main objective of the present study is to develop new chemical entities with potential antibacterial, which are superior to the currently available drugs and also which has an improved spectrum of activity even towards the most resistant organisms.

So, in the introduction currently available anti-tubercular agents, antibacterial agents, antifungal agents problems associated with the current therapy and current trends in the field of anti-microbial research are described briefly.

Antifungal Agents[3,4]

Antifungal chemotherapy depends on biochemical differences between fungi and mammals. Unlike bacteria, which are prokaryotes, both fungi and mammals are eukaryotes and the biochemical differences between them are not as significant. However, there are some differences (in structure & metabolism) and the focus is on these differences which act as targets for development of new antifungal agents. Classification of antifungal agents on based mechanism of action is given in **Table 1.3**.

Table 1.1: Classification of antifungal agents

Antifungal Agents			
Drugs	**Mechanism of Action**	**Spectrum of Activity**	**Adverse Effects**
Nonsynthetic Antifungal Agents			
Amphotericin B (Polyene antibiotic)	Binds to sterols in fungal cell membrane and interferes with permeability and with transport functions	It is fungicidal at high and static at low conc. It has broad spectrum of antifungal activity	Renal toxicity, Anemia, CNS toxicity, Acute reactions
Griseofulvin	Acts by interacting with microtubules and interfering with mitosis	Active against most dermatophytes	Gastrointestinal disturbances, Photosensitivity
Nystatin (Polyene antibiotic)	Same as Amphotericin B	It is active primarily against the *Candida* sp	Nausea and bad taste in mouth
Synthetic Antifungal Agents			
Flucytosine	It is converted to antimetabolite 5-fluorouracil in fungal cells which inhibits thymidylate synthetase and thus fungal DNA synthesis	It has narrow spectrum fungi static activity	Leucopenia, Thrombocytopenia, mild anemia, Gastrointestinal disturbances
Imidazoles & Triazoles- Clotrimazole, Miconazole Ketoconazole, Fluconazole, Itraconazole	They inhibit fungal cytochrome P450 enzyme lanosterol 14-demethylase and thus impairs ergo sterol synthesis in fungal cell membrane	They have broad spectrum of antifungal activity	Local irritation, Gastrointestinal disturbances
Terbinafine	Selectively inhibits squalene epoxidase, which is involved in the synthesis of ergosterol from squalene in fungal cell wall.	It has fungicidal action; active against wide range of dermatophytes and *Candida* sp	Gastric upset, Rashes, Erythema, Urticaria, Taste disturbances
Butenafine	Same as terbinafine	It has fungicidal action	Rashes,

			Urticaria,
Naftifine	Same as terbinafine	It has fungistatic action	Gastric upset, Rashes
Tonaftate	Used as topical antifungal	Active against dermatophytes	Little irritation
Cyclopirox olamine	It causes intracellular depletion of amino acids and ions necessary for normal cellular function.	Active against dermatophytes	Sensitization occurs occasionally
Caspofungin	Cell wall lysis, by being a competitive inhibitor of 1,3-ß-D-glucan synthase	Fungicidal	-
Haloprogin (iodinated acetylene)	Appears to lead to non-specific metabolic disruption. It interferes with DNA biosynthesis and cell respiration.	Active against dermatophytes. Useful for topical application	-

1.2 Need for the development of Novel Anti-microbial Agents

The problem of antibiotic resistance is becoming increasingly apparent as more and more strains of pathogenic microorganisms are untreatable with commonly used antimicrobials.[8] The problem has increased dramatically in recent years with the emergence of multi-resistant strains of bacteria. This problem can be attributed to a variety of factors including overuse of antibiotics in agriculture and medicine and misuse of antibiotics by consumers. In addition, antibiotic resistance is often plasmid-borne. Infections remain one of the leading causes of death worldwide including India. Due to dramatic changes in society, technology and the environment, in addition to the reduced effectiveness of previous approaches to disease control, the spectrum of infectious diseases is expanding today. Diseases once believed to be conquered are increasing, as pathogens evolve and spread.

Surprisingly, some diseases which are not previously recognized as being infectious have been found to have a microbial etiology. The term "emerging infectious diseases" refers to these phenomena.[9,10] There are no treatments available for infections caused by many of the antibiotic-resistant bacteria and resistance to commonly used antibiotics is steadily increasing.[11] There are numerous new challenges confronting microbiologists, virologists, and infectious disease experts. The widespread use and misuse of antimicrobial drugs have produced drug-resistant organisms. Public water supplies and food products have been contaminated by infectious agents (e.g., *Cryptosporidium, E. coli, hepatitis A virus*), putting

entire communities at risk. Travel and commerce have fostered the worldwide spread of pathogens, such as HIV, cholera, influenza, and West Nile virus. We now recognize that emerging infections can affect persons in geographically dispersed areas, regardless of cultural or ethnic background or socioeconomic status. Concern over the possible use of infectious agents by bioterrorists also lurks in the background.[11]

Limitations of existing classes of anti-microbial agents include:

1) Narrow spectrum of activity of some agents
2) Low potency of some agents
3) Emergence of resistance
4) Adverse effects

On the positive side, drug discovery research has undergone a revolution. Proteomics, Genomics and Genetics have allowed the rapid identification of new targets and molecular biology has provided tools to clone and characterize the targets; Robotics have allowed high throughput analysis of large chemical compound files; and combinatorial chemistry has permitted the rapid synthesis of large novel compound files for the identification of lead compounds and the rapid analoging of lead structures. Integration of these advances into the search for new antimicrobial agents can be used for developing the next generation of antibacterials.[12]

1.3 Current Status of Antimicrobial Drug development

One of the current research trends is to develop new synthetic chemical classes of agents in order to overcome limitations of the existing classes and also to obtain potent drugs for clinical use. Many promising novel anti-microbial agents are in different stages of drug development and some have been introduced into the market. They include drugs such as:

Altabax

The U.S. Food and Drug Administration (FDA) on February 16, 2006 approved Altabax (retapamulin)(Glaxo) a bacterial protein synthesis inhibitor belonging to a class of compounds called pleuromutilins. These compounds act by inhibiting the initiation of protein synthesis at the level of bacterial 50S ribosome. This binding site involves ribosomal protein L3 and is in the region of the ribosomal P site and peptidyl transferase center. By virtue of binding to this site, pleuromutilins inhibit peptidyl transfer, block P-site interactions, and prevent the normal formation of active 50S ribosomal subunits.[13]

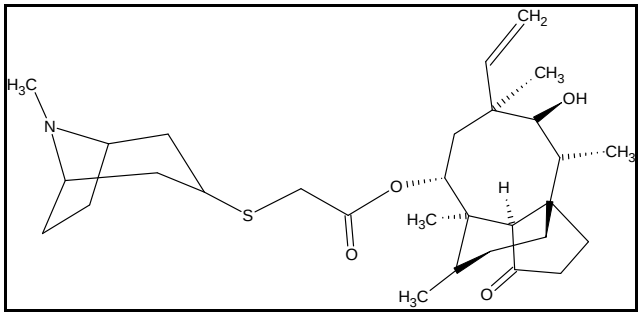


Figure 1.3: Structure of Altabax

Tigecycline

The U.S. Food and Drug Administration (FDA) on June 30, 2005 approved Tigecycline (Tygacil), a novel intravenous antibiotic with a broad spectrum of antimicrobial activity, including activity against the drug-resistant bacteria methicillin-resistant *Staphylococcus aureus* (MRSA).

Tigecyclin is the first antibiotic approved in a new class called glycylcyclines and is indicated for the treatment of complicated intra-abdominal infections and complicated skin and skin structure infections in adults. Approval of this first-in-class product comes at a time when the need for new antibiotic options to combat serious, resistant infections is increasing.[14]

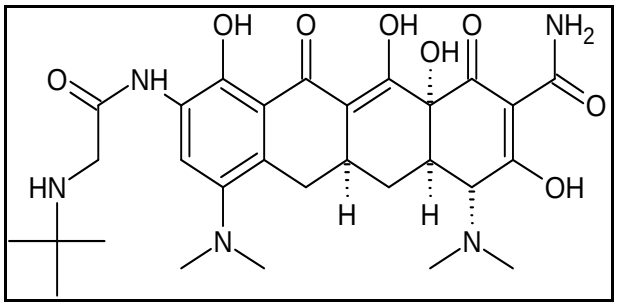


Figure 1.4: Structure of Tigecycline

Gardasil

The U.S. Food and Drug Administration (FDA) on February 16, 2006 approved, is a non-infectious quadrivalent recombinant vaccine, which delivers the major capsid (L1) protein of human papillomavirus (HPV) types 6, 11, 16 and 18 in highly purified virus-like particles, in combination with an aluminum-containing vaccine adjuvant. Gardasil delivers HPV-6, -11, -16 and -18 L1 protein, and conferring protection against these HPV strains, presumably through induction of humoral immune response. These strains are responsible for the majority of cases of cervical cancer, cervical adenocarcinoma in situ (AIS), cervical

intraepithelial neoplasia (CIN) and vulvar intraepithelial neoplasia (VIN) and for a number of cases of vaginal intraepithelial neoplasia (VaIN) and genital warts.[15]

Eraxis

The U.S. Food and Drug Administration (FDA) on February 16, 2006 approved Eraxis **(Anidulafungin)**, is an echinocandin semi-synthetic lipopetide, designed to eradicate fungal infections through disruption of enzyme synthesis pathways in these cells without affecting human tissues. Eraxis is specifically indicated for the treatment of several types of fungal infections: Candidemia; intra-abdominal abscess and peritonitis caused by the *Candida* species; and esophageal candidiasis. Anidulafungin is a semi-synthetic echinocandin designed to inhibit glucan synthase, an enzyme present in fungal (but not mammalian) cells. Inhibition of glucan synthase disrupts formation of 1,3-ß-D-glucan, an essential component of the fungal cell wall.[16]

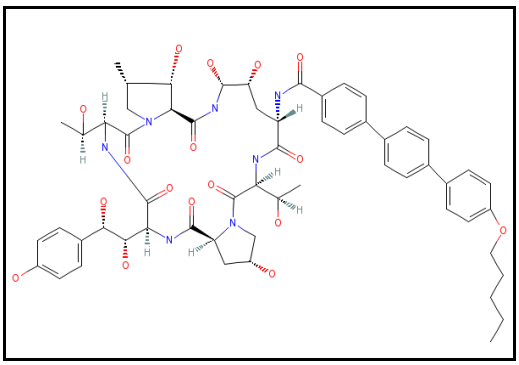


Figure 1.5: Structure of Eraxis

Dalbavancin

It is a new lipoglycopeptide antibiotic approved in March 2007 for the treatment of resistant gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Dalbavancin has a long fatty acyl moiety in an amide linkage to a glucosamine component. This fatty acyl chain is purported to improve activity by anchoring the molecule to the bacterial cell membrane, which prolongs the interaction of the agent with the bacteria.[17]

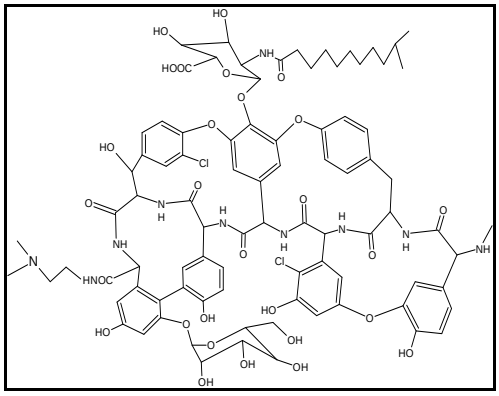


Figure 1.6: Structure of Dalbavancin

This agent exerts its bactericidal activity by binding to the terminal D-alanyl-D-alanine moiety of peptidoglycan precursors, thus blocking enzymes involved in the final stages of peptidoglycan synthesis and cell wall formation**.** It has been postulated that dalbavancin exerts its bactericidal effect on bacteria through more than one mechanism.

In addition to blocking enzymes involved in the final stages of peptidoglycan synthesis, dalbavancin may have a secondary mechanism of action independent of peptide binding. Researchers suggest that dalbavancin may inhibit transglycosylases, such as *S aureus* penicillin-binding protein-2 (PBP-2), by direct interaction with enzymes involved in the final stages of peptidoglycan synthesis.[18]

Doripenem

The U.S. Food and Drug Administration (FDA) on june, 6, 2006 approved is a novel, broad-spectrum parenteral carbapenem antimicrobial. The structure confers β-lactamase stability and resistance to inactivation by renal dehydropeptidases. Doripenem has a spectrum of activity and potency against Gram-positive cocci most similar to classical carbapenems such as imipenem and ertapenem and a Gram-negative activity most like meropenem (two- or four-fold superior to imipenem).[19]

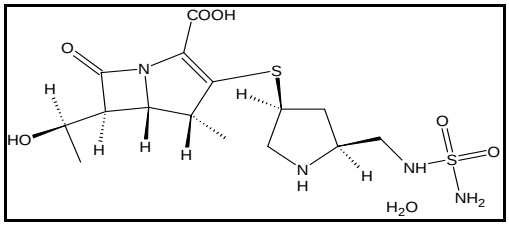


Figure 1.7: Structure of Doripenem

Ceftobiprole

It is a 5th generation cephalosporin antibiotic with activity against methicillin-resistant *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumonia*, *Pseudomonas aeruginosa* and Enterococci.[20] It was discovered by basilica pharmaceutica and was developed by Johnson and Johnson pharmaceutical research and development. It was approved by the U.S. Food and Drug Administration (FDA) on June, 2009. It is marketed under the trade name Zeftera, Ceftobiprole inhibits the 2a penicillin-binding protein (pbp) of *Methicillin-resistant Staphylococcus aureus* and the 2x pbp of *Streptococcus pneumonia* as well as the classic PBP-2 of MSSA. Ceftobiprole is resistant to staphylococcal β-lactamase. It is used in adults for the treatment of complicated skin and skin structure infection.[21]

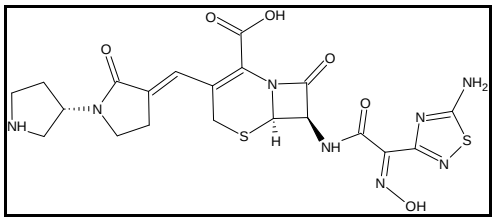


Figure 1.8: Structure of Ceftobiprole

Telavancin

It was approved by U.S. Food and Drug Administration (FDA) on sept.11, 2009. Telavancin (trade name Vibativ) is a bactericidal lipoglycopeptide for use in MRSA and treatment of complicated skin and skin structure infection (cSSSI) caused by gram-positive bacteria. It is a synthetic derivative of vancomycin. The drug was invented by Theravance Pharmaceuticals targeting super bug, like MRSA, VISA etc and co-developed with Astellas Pharmaceuticals. Vibativ inhibits bacterial cell wall synthesis by interfering with the polymerization and cross-linking of peptidoglycan. It also binds to the bacterial membrane and disrupts membrane barrier function by depolarization.[22]

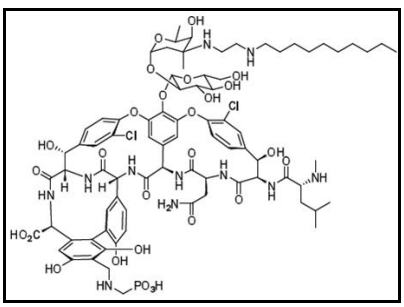


Figure 1.9: Structure of Telavancin

Besifloxacin

It was approved by U.S. Food and Drug Administration (FDA) on may 29, 2009. Besifloxacin is a fourth-generation fluoroquinolone antibiotic. It was developed by SSP, Co. Ltd., Japan and marketed by Bausch & Lomb under the trade name Besivance (0.6% Besifloxacin ophthalmic suspension) to treat bacterial conjunctivitis, also known as pink eye. It is the first fluoroquinolone specifically developed for ophthalmic use and is the first and only ophthalmic fluoroquinolone with no previous systemic use. The mode of action of Besifloxacin is to inhibit the production of pro-inflammatory cytokines *in vitro*.

Other representative fluoroquinolone class of antibiotics developed for systemic uses is ciprofloxacin, levafloxacin and moxifloxacin. Besifloxacin's chemical name is (+)-7-[(3R)-3-aminohexahydro1H-azepin-1-yl]-8-chloro-1-cyclopropyl-6-fluoro-4oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride.[23]

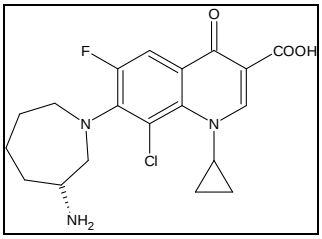


Figure 1.10: Structure of Besifloxacin

Ceftaroline

It was approved by U.S. Food and Drug Administration (FDA) on Oct 29, 2010. Ceftaroline is a fifth-generation cephalosporin antibiotic. It is marketed under the trade name Teflaro by Forest Laboratories. Ceftaroline having a broad spectrum activity against methicillin-resistant *Staphylococcus aureus* (MRSA), community-acquired bacterial pneumonia and complicated skin and skin structure infections caused by various gram positive and gram negative bacteria. Ceftaroline has a bicyclic ring with four member β-lactam ring fused to a six member cephem ring. Ceftaroline and Ceftobiprole inhibit bacterial peptidoglycan synthesis by binding the penicillin binding proteins (PBPs) in the bacterial cell wall. Inhibition of PBPs leads to irregularities in cell wall structures, such as elongation, lesions, loss of selective permeability, and eventual cell death and lysis.[24]

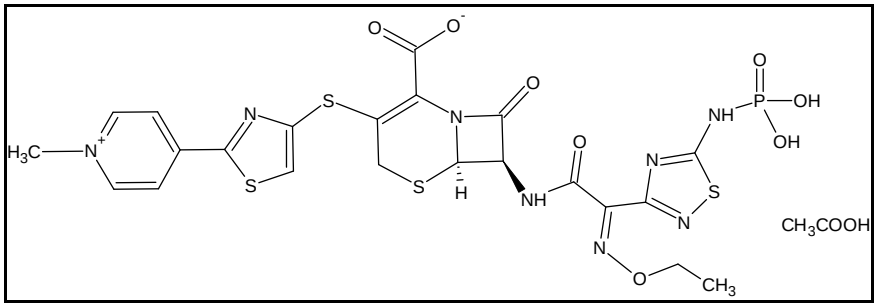


Figure 1.11: Structure of Ceftaroline

1.4 CONCLUSION

This overview of new anti-microbials in the process of drug development reflects the increased interest in the field of infectious diseases and demonstrates that, although some progress has been made, further efforts are necessary to develop more promising agents. Hopefully, these agents will overcome limitations of existing classes and will achieve the delicate balance between broad spectrum of activity and target selectivity.

Also, as mentioned above, current trend in drug discovery and development can be

1) Towards identification of novel molecules of new chemical classes

2) Synthesis of analogs of known chemical classes, to improve their biological properties.

2. MATERIALS

Table 2.1: Materials

S.No.	Name of Item	Specification (grade, pack size)	Quantity required
1	Para-toluene sulfonic acid	500gm	100gm
2	Sodium sulphite	500gm	500gm
3	Benzaldehyde	500gm	100gm
4	Nicotinic acid	100gm	100gm
5	Silica gel	1.0kg	1.0kg
6	Acetic acid	500ml	500ml
7	Sulphuric acid	500ml	250ml
8	Hydrochloric acid	500ml	500ml
9	Pyridine	500gm	100ml
10.	TLC plate	25M No.	4-5 plates
11.	Melting point capillary	100 No.	100 No.
12.	Acetophenone	500ml	100ml

3. METHODS

The purity of the starting materials used in the reaction was confirmed by melting point/boiling point and thin layer chromatography. The purity and structures of the

synthesized compounds were confirmed by melting point/boiling point, thin layer chromatography, infrared spectroscopy and nuclear magnetic resonance spectroscopy.

The melting points of the compounds synthesized were uncorrected and recorded by open glass capillary method on "Janki Melting Point Apparatus" and compared with the reported melting points wherever applicable. ^{1}H-NMR, ^{13}C-NMR spectra of reference molecule was recorded on "Bruker Avance II 400 NMR" spectrometer in DMSO-D_6 using as a solvent. Chemical shifts were expressed in parts per million (δ, ppm). IR spectra were recorded using "Brucker Alpha Infrared Spectrometer". Analytical thin layer chromatography (TLC) was carried out on precoated plates (silica gel G 254).

All solvents were distilled before use. All the starting materials were obtained from S. D. Fine Chemicals Ltd/ (CDH) and (SRL). The following are the chemicals used for biological testing:

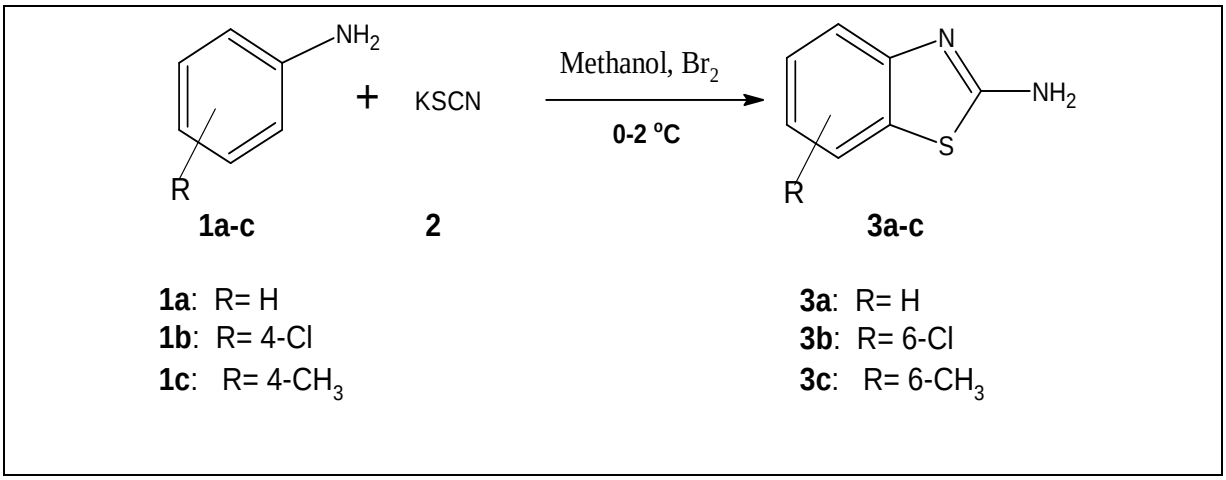
1) Dimethylsulphoxide (AR Grade, CDH, Central Drug House Pvt. Ltd., New Delhi).
2) Streptomycin, standard drugs for antibacterial testing were obtained from CDH.
3) Nutrient broth and Nutrient agar for antibacterial testing were of microbiology grade, and obtained from CDH, Central Drug House Pvt. Ltd., New Delhi.

The strains used for testing and their sources are as:

1) Gram positive: *Staphylococcus aureus*
2) Gram negative: *Escheria coli* for antibacterial testing.

i) Synthesis of intermediate (3a-c)

General scheme



Scheme 4.1: General synthetic scheme for intermediate

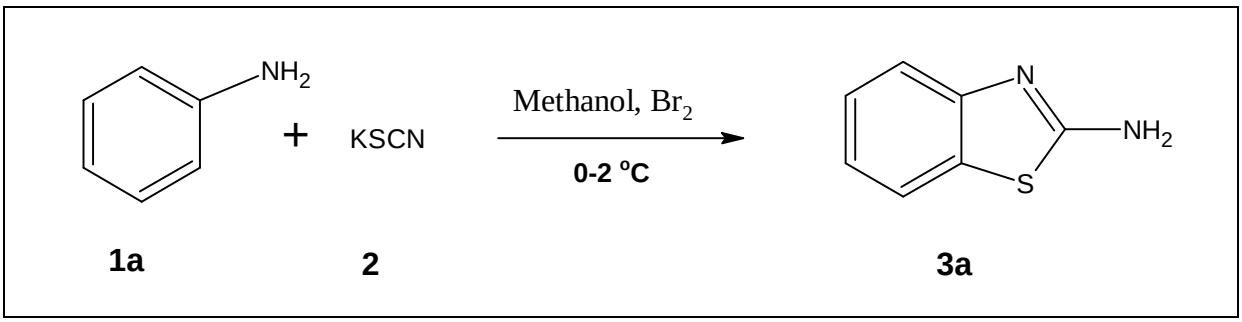
General procedure[49]

An equimolar concentration of potassium thiocyanate and substituted aniline was dissolved into in methanol (50-60 ml) and stirred the mixture for 30 min to bring reaction mixture temperature 0-2°C in a ice bath. After achieving the desired temperature, equal molar concentration of bromine was slowly added in to the stirred mixture. The temperature of the mixture was maintained between 0-2°C while addition of bromine. After completion of bromine addition, the mixture was stirred for 2-4 hr at the cold temperature.

Work up

The precipitated solid was collected by filtration and washed with cold methanol. The white solid was collected and dried in an oven; the crude product was used without further purification for next step.

ii) Synthesis of 1, 3-benzothiazol-2-amine (3a)



Scheme 4.2: Synthesis of 1, 3-benzothiazol-2-amine

Procedure

To a stirred suspension of potassium thiocyanate (25.16 g, 0.257 moles) and aniline (19.6 ml, 0.214 moles) in methanol (75 ml) was slowly added bromine (11.41 ml, 0.214 moles) in a round bottom flask while the temperature was maintained below 0°C. After addition, the mixture was stirred for 3 hrs at the same temperature. The reaction was monitored by TLC. The remainder of the workup is similar to that explained in the general procedure.

Yield : 81.12%

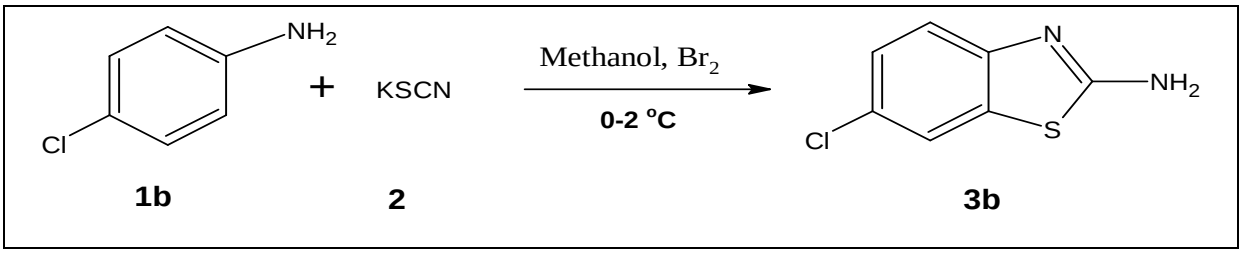
Melting point : >250°C

TLC : Silica gel G; Hexane: Ethyl acetate (1:1)

$R_f = 0.714$

IR **(Spectrum 1)**: 3368, 3319, 3033, 2996, 1558, 1521, 1497, 1457, 1386, 1187, 1078, 855 Cm^{-1}

iii) Synthesis of 6-chloro-1, 3-benzothiazol-2-amine (3b)



Scheme 4.3: Synthesis of 6-chloro-1, 3-benzothiazol-2-amine

Procedure

To a stirred suspension of potassium thiocyanate (18.38g, 0.187 moles) and p-chloro aniline (20 gm, 0.156 moles) in methanol (75ml) was slowly added bromine (8.3ml, 0.156 moles) in a round bottom flask while the temperature was maintained below 0°C. After addition, the mixture was stirred for 3 hrs at the same temperature. The reaction was monitored by TLC. The remainder of the workup is similar to that explained in the general procedure.

Yield : 79.50%

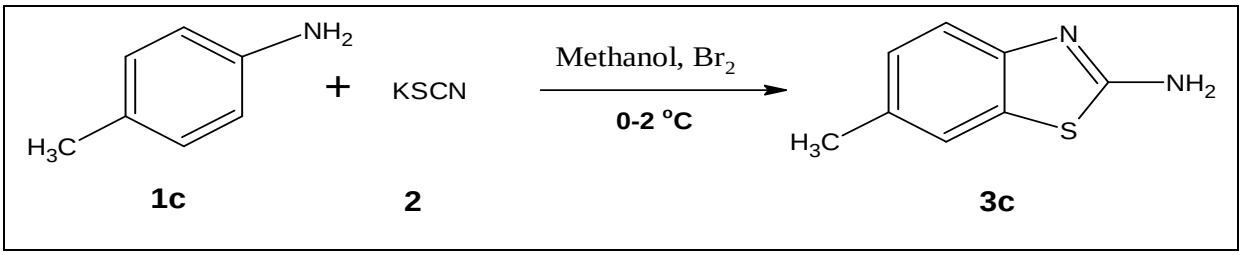
Melting point : >250°C

TLC : Silica gel G; Hexane: Ethyl acetate (1:1)

$R_f = 0.695$

IR**(Spectrum 2)**: 3420, 3291, 3259, 3020, 2990, 1683, 1558, 1404, 1207, 1015, 756 Cm^{-1}

iv) Synthesis of 6-methyl-1, 3-benzothiazol-2-amine (3c)



Scheme 4.4: Synthesis of 6-methyl-1, 3-benzothiazol-2-amine

Procedure

To a stirred suspension of potassium thiocyanate (21.94g, 0.224 moles) and p-Toludine (20gm, 0.186 moles) in methanol (75ml) was slowly added bromine (9.63ml, 0.186 moles) in a round bottom flask while the temperature was maintained below 0°C. After addition, the mixture was stirred for 3 hrs at the same temperature. The reaction was monitored by TLC. The remainder of the workup is similar to that explained in the general procedure.

Yield : 88.54%

Melting point : >250°C

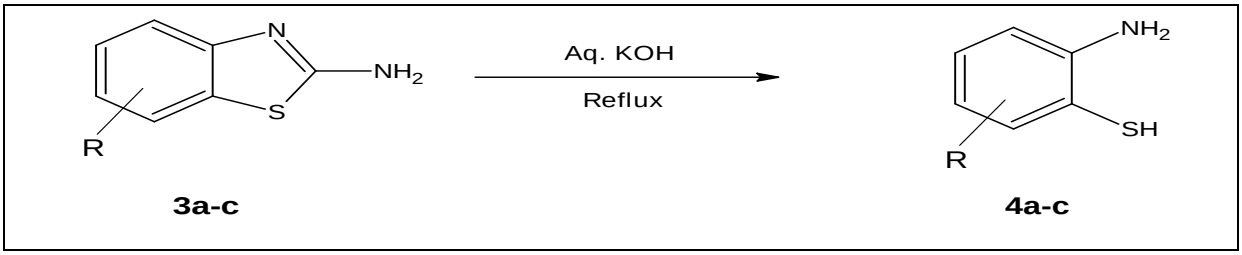
TLC : Silica gel G; Hexane: Ethyl acetate (1:1)

$R_f = 0.629$

IR**(Spectrum 3)** : 3445, 3368, 3337, 3033, 2981, 2894, 1652, 1575, 1464, 1396, 1318 Cm^{-1}

SYNTHESIS OF INTERMEDIATE (4a-c)

General scheme



Scheme 4.5: General synthetic scheme for intermediate (4a-c)

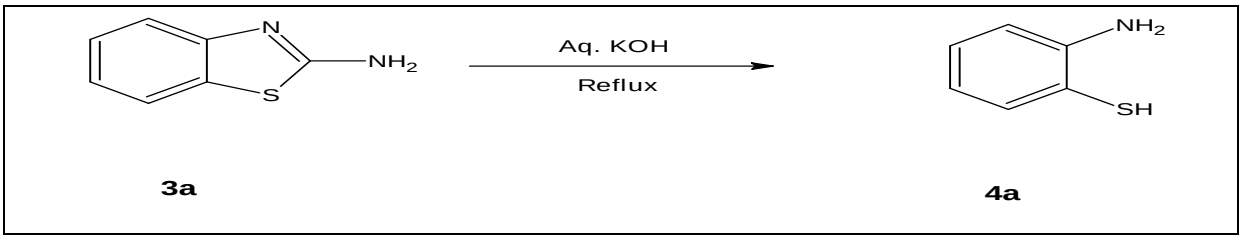
General procedure[49]

A suspension of intermediate (**3a-c**) and potassium hydroxide in water (50-70ml) was shielded from light (aluminum foil) and then refluxed for 4-5 hrs. After the mixture was cooled to room temperature, concentrated hydrochloric acid (30-45ml) was added drop wise.

Work up

The precipitated compound was filtered under vacuum and dried in oven at 50-60°C. The product was stored under light and oxygen-free conditions.

1) Synthesis of 2-aminobenzenethiol (4a)



Scheme 4.6: Synthesis of 2-aminobenzenethiol

Procedure

A suspension of 3a (3.00 g, 0.020 moles) and potassium hydroxide (15.00 g, 0.267 moles) in water (25 ml) was shielded from light (aluminum foil) and then refluxed for 5 h. After the mixture was cooled to room temperature, concentrated hydrochloric acid (35 ml) was added drop wise. The resulting mixture was cooled up to 5°C and stirred for 30 min. reaction was monitored by TLC. The remainder of the workup is similar to that explained in the general procedure.

Yield : 82.24%

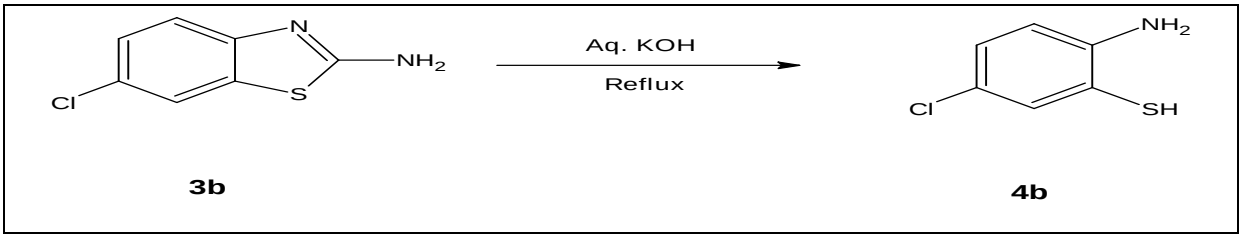
Melting point : >250°C

TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

R_f = 0.738

IR**(Spectrum 4)** : 3227, 3184, 3055, 2493, 1558, 1507, 1418, 1374, 1097 Cm^{-1}

2) Synthesis of 2-amino-5-chlorobenzenethiol (4b)



Scheme 4.7: Synthesis of 2-amino-5-chlorobenzenethiol

Procedure

A suspension of 3b (3.00 g, 0.0162 moles) and potassium hydroxide (15.00 g, 0.267 moles) in water (25 ml) was shielded from light (aluminum foil) and then refluxed for 5 h. After the mixture was cooled to room temperature, concentrated hydrochloric acid (35 ml) was added drop wise. The resulting mixture was cooled up to 5°C and stirred for 30 min. reaction was monitored by TLC. The remainder of the workup is similar to that explained in the general procedure.

Yield : 84.72%

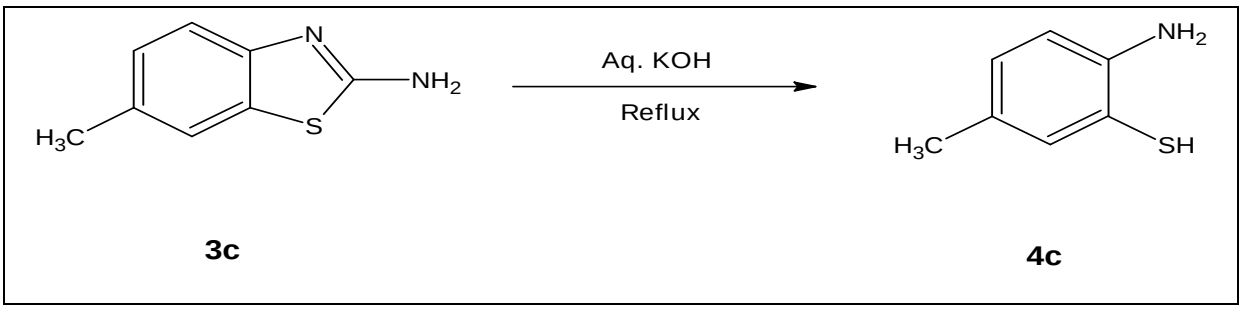
Melting point : >250°C

TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

R_f = 0.715

IR**(Spectrum 5)** : 3272, 3251, 3006, 2950, 2520, 1558, 1533, 1464, 1318, 1243, 1174, 879 Cm^{-1}

3) Synthesis of 2-amino-5-methylbenzenethiol (4c)



Scheme 4.8: Synthesis of 2-amino-5-methylbenzenethiol

Procedure

A suspension of 3c (3.00 g, 0.0182 moles) and potassium hydroxide (15.00 g, 0.267 moles) in water (25 ml) was shielded from light (aluminum foil) and then refluxed for 5 h. After the mixture was cooled to room temperature, concentrated hydrochloric acid (35 ml) was added drop wise. The resulting mixture was cooled to 5°C and stirred for 30 min. reaction was monitored by TLC. The remainder of the workup is similar to that explained in the general procedure.

Yield : 91.24%

Melting point : >250°C

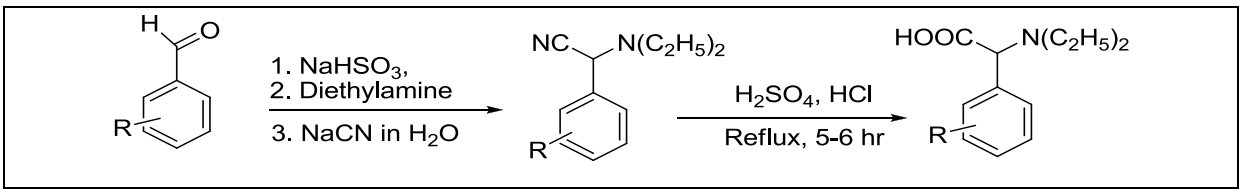
TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

$R_f = 0.794$

IR**(Spectrum 6)** : 3348, 3242, 3020, 2990, 2521, 1652, 1568, 1540, 1507, 1418 Cm^{-1}

Synthesis of intermediate (7a-b)

General scheme



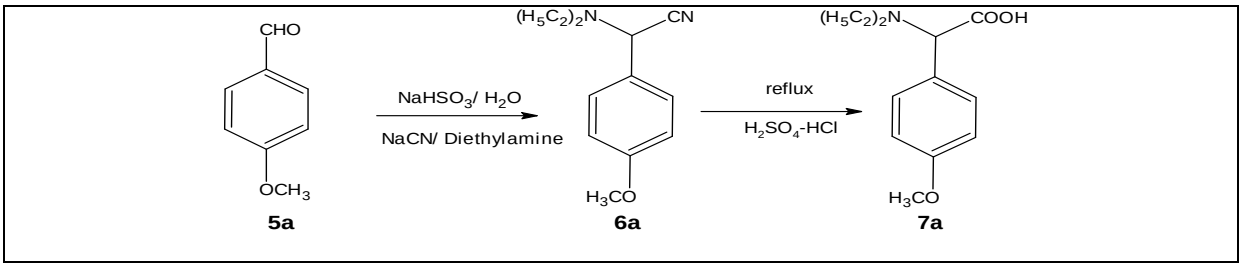
Scheme 4.9: General synthetic scheme for 2-(diethylamino)-2-(phenyl) acetic acid

General procedure[59]

Step I: Aldehyde (**1**) was added to a solution of sodium bisulphite in 25-30mL water drop wise over a period of 30 min (1:1 equivalent). During addition of aldehyde, temperature of reaction mixture was increased (40-50^{o}C) to dissolve the precipitated solid. After completion of addition, Diethylamine (1.5 equivalent) was added to reaction mixture and heated at 50-60^{o}C for about 1hr. After 1 hr, reaction mixture was cooled to 5-10^{o}C and a solution of sodium cyanide in 15 ml water (1.25 equivalent) was added drop wise over 30 min. The reaction mixture was then warmed to 30^{o}C for 3 hr. After 3 hr, an organic layer separated out. The reaction mixture was diluted with 100mL water and upper organic layer separated. Aqueous layer was extracted with EtOAc (2 ×25mL). The combined organic layers were washed with brine (50mL), dried over sodium sulphate (Na_2SO_4) and concentrated in vacuum. Liquid obtained as 2-(diethylamino)-2-phenylacetonitrile was obtained in yield ranging from 40.0-90.5%.

Step II: The intermediate obtained in step I was dissolved in equal quantities of water (10mL) H_2SO_4 and conc. HCl (10mL) and refluxed for 4-5 hrs. After this, the reaction mixture was cooled to room temperature and concentrated in vacuum. A solid residue was obtained. This was dissolved in ethanol and warmed on a water bath. It was then chilled on an ice bath to remove the salts. The mixture was filtered and the salts were removed. The solid precipitated was filtered and recrystallised from methanol.

1.) Synthesis of (diethyl amino)(phenyl)acetic acid (7a)



Scheme 4.10: Synthesis of (diethylamino)(4-methoxyphenyl)acetic acid

Procedure

Anisaldehyde (11.0ml, 0.0887 moles), sodium cyanide (4.9g, 0.1 moles in 20mL of H_2O) and diethylamine (10.39ml) were added into a solution of sodium bisulphite (10.4g, 0.1 moles in 50mL of H_2O) and reacted according to step I general procedure, to yield (diethylamino)(4-methoxyphenyl)acetic acid.

Yield : 83.78%

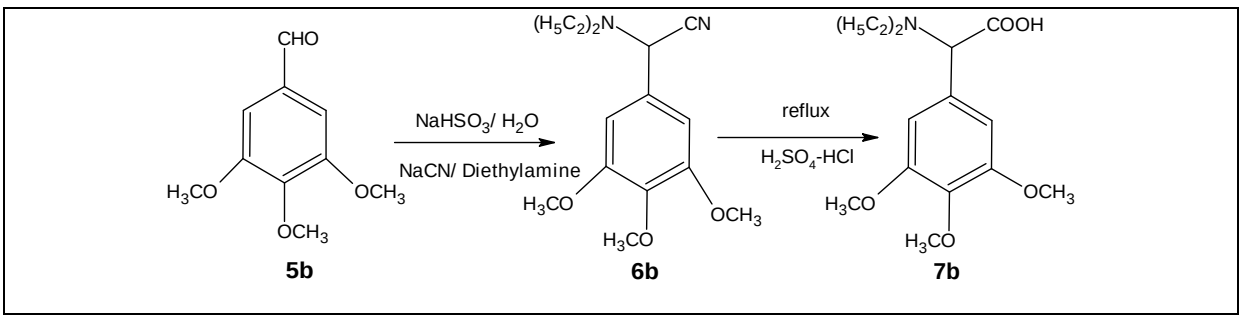
Melting point : >250°C

TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

$R_f = 0.727$

IR**(Spectrum 7)** : 3369, 3012, 1662, 1623, 1558, 1533, 1497, 1374, 1246, 1105, 1025 Cm^{-1}

2.) Synthesis of (diethyl amino)(3,4,5-trimethoxyphenyl)acetic acid (7b)



Scheme 4.11: Synthesis of (diethyl amino)(3,4,5-trimethoxyphenyl)acetic acid

Procedure

Trimethoxy benzaldehyde (5 gm, 0.0255 moles), sodium cyanide (1.5g, 0.0306 moles in 20mL of H_2O) and diethylamine (3.30ml) were added into a solution of sodium bisulphite (3.5g, 0.0336M in 25mL of H_2O) and reacted according to step I general procedure, to yield (diethylamino)(3,4,5-trimethoxyphenyl)acetic acid.

Yield : 77.06%

Melting point : 105-108°C

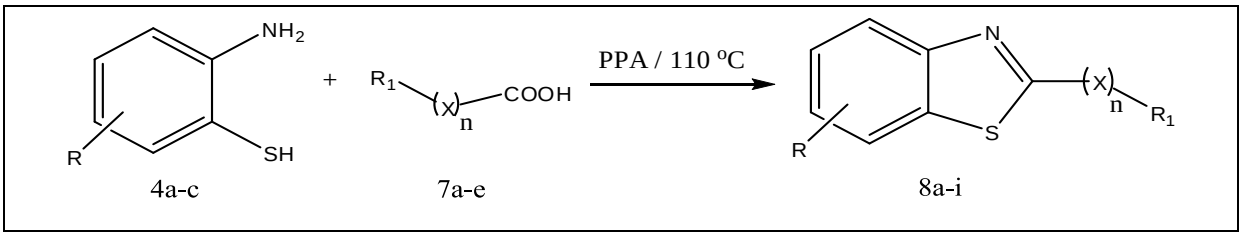
TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

$R_f = 0.618$

IR**(Spectrum 8)** : 3411, 2951, 2924, 2854, 1716, 1698, 1647, 1540, 1216, 1119 Cm^{-1}

SYNTHESIS OF FINAL PRODUCT (8a-i)

General scheme



Scheme 4.12: General synthetic scheme for final product

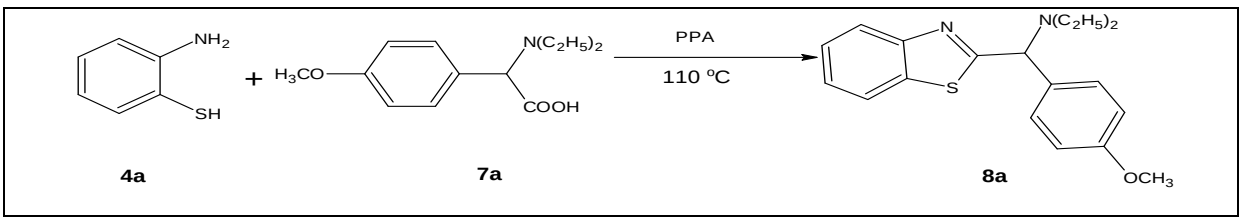
General procedure: [56]

Carboxylic acid derivative (7a-e) was dissolved in polyphosphoric acid (10 g) at 110°C. Substituted 2-aminothiophenol (4a-c) was added and the resulting solution stirred at 110°C for 1-2 hrs. After cooling, the reaction mixture was poured into aqueous ammonia (30-50 ml). A white precipitate was formed.

Workup

The precipitate was collected and washed with water (50 ml). The product was purified by column chromatography (Ethylacetate: hexane) to give the final product.

I) Synthesis of [1,3-benzothiazol-2-yl(4-methoxyphenyl)methyl]diethylamine (8a)



Scheme 4.13: Synthesis of [1,3-benzothiazol-2-yl(4-methoxyphenyl)methyl]diethylamine

Procedure

(diethylamino)(4-methoxyphenyl)acetic acid (0.908 gm, 0.004 moles) was dissolved into polyphosphoric acid (9.52 ml) at 110°C. 2-Aminothiophenol (0.5gm, 0.004 moles) was added and the resulting solution stirred at 110°C for 2 hrs. After cooling, the reaction mixture was poured into aqueous ammonia (35 ml). A white precipitate was formed. The precipitate was collected and washed with water (50 ml). Then dried in the oven. The product was purified by column chromatography.

Yield : 86.76%

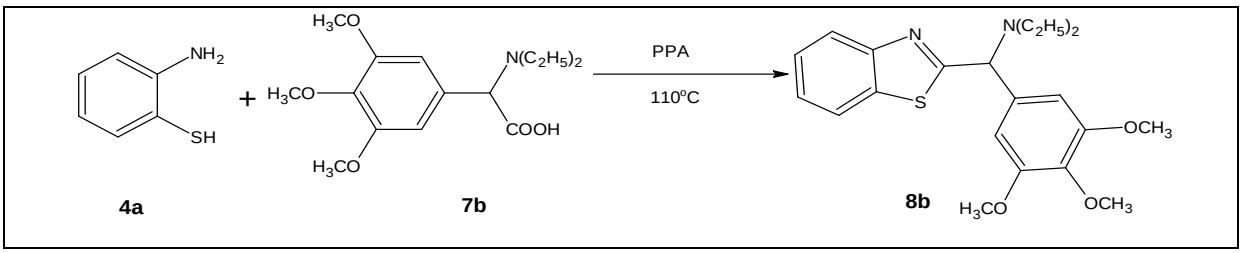
Melting point : 200-204°C

TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

$R_f = 0.727$

IR**(Spectrum 9)** : 2992, 2884, 2803, 1682, 1661, 1506, 1435, 1206, 1161, 1049, 948, 891 Cm^{-1}

II) Synthesis of [1,3-benzothiazol-2-yl(3,4,5-trimethoxyphenyl)methyl]diethylamine (8b)



4a **7b** **8b**

Scheme 4.14: Synthesis of [1,3-benzothiazol-2-yl(3,4,5-trimethoxyphenyl)methyl]diethylamine

Procedure

(Diethyl amino)(3, 4, 5-trimethoxyphenyl) acetic acid (1.18 gm, 0.004 moles) was dissolved into polyphosphoric acid (9.52 ml) at 110°C. 2-Aminothiophenol (0.50 gm, 0.004 moles) was added and the resulting solution stirred at 110°C for 90 min. After cooling, the reaction mixture was poured into aqueous ammonia (45 ml). A white precipitate was formed. The precipitate was collected and washed with water (50 ml). Then dried in the oven. The product was purified by column chromatography.

Yield : 82.20%

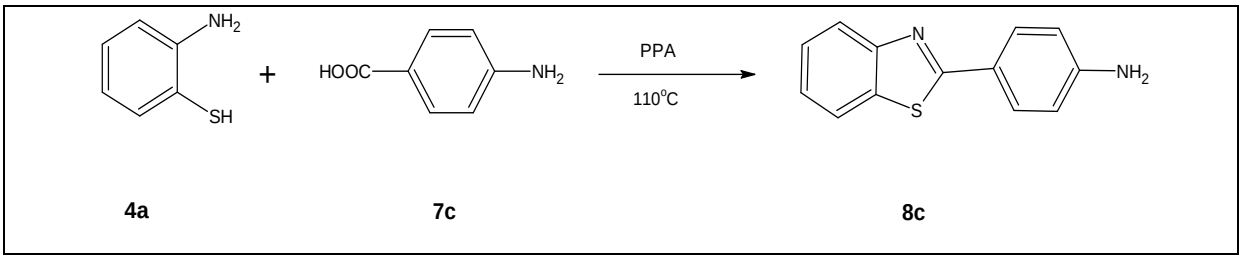
Melting point : 190-192°C

TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

$R_f = 0.618$

IR**(Spectrum 10)** : 3013, 2868, 2790, 1507, 1455, 1397, 1202, 1052, 949, 892 Cm^{-1}

III) Synthesis of [4-(1,3-benzothiazol-2-yl)phenyl]amine (8c)



Scheme 4.15: Synthesis of [4-(1,3-benzothiazol-2-yl)phenyl]amine

Procedure

Para-amino benzoic acid (PABA) (1.1 gm, 0.008 moles) was dissolved in polyphosphoric acid (19.04 ml) at 110°C. 2-Aminothiophenol (1 gm, 0.008 moles) was added and the resulting solution stirred at 110°C for 90 min. After cooling, the reaction mixture was poured into aqueous ammonia (40 ml). A white precipitate was formed. The precipitate was collected and washed with water (50 ml). Then dried in the oven. The product was purified by column chromatography.

Yield : 64.44%

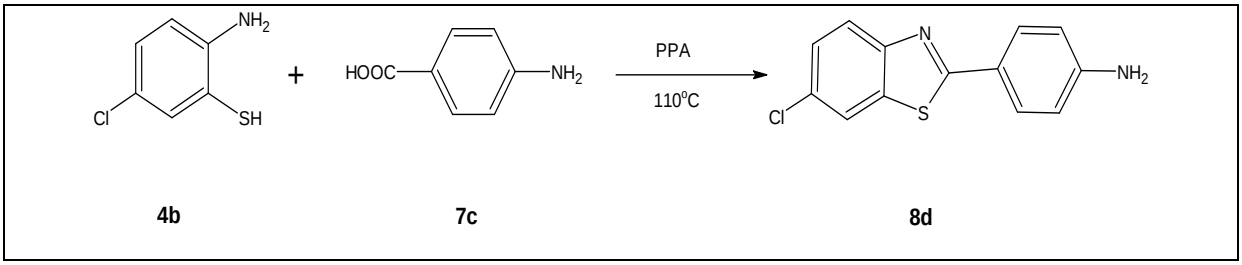
Melting point : 175-180°C

TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

$R_f = 0.615$

IR**(Spectrum 11)** : 3361, 3294, 2924, 2852, 1647, 1558, 1507, 1436, 1076 Cm^{-1}

IV) Synthesis of [4-(6-chloro-1,3-benzothiazol-2-yl)phenyl]amine (8d)



Scheme 4.16: Synthesis of [4-(6-chloro-1,3-benzothiazol-2-yl)phenyl]amine

Procedure

Para-amino benzoic acid (PABA) (0.85 gm, 0.00625 moles) was dissolved in polyphosphoric acid (14.88 ml) at 110°C. 2-Amin-5-chloroothiophenol (1 gm, 0.00625 moles) was added and the resulting solution stirred at 110°C for 90 min. After cooling, the reaction mixture was

poured into aqueous ammonia (35 ml). A precipitate was formed. The precipitate was collected and washed with water (50 ml). Then dried in the oven. The product was purified by column chromatography.

Yield : 62.56%

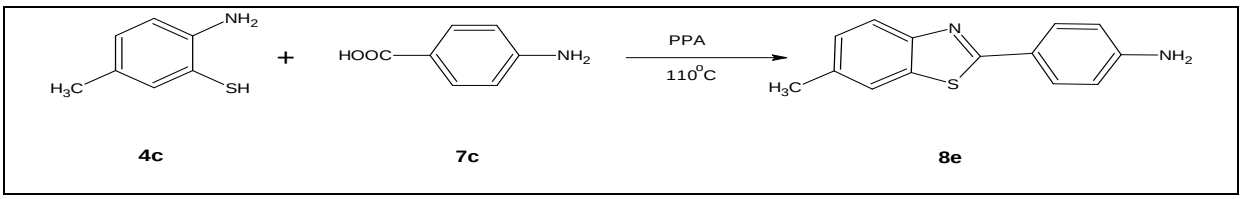
Melting point : 195-197°C

TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

$R_f = 0.698$

IR**(Spectrum 12)** : 3324, 3202, 2924, 2829, 1652, 1558, 1457, 1396, 1120, 737 Cm^{-1}

V) Synthesis of [4-(6-methyl-1,3-benzothiazol-2-yl)phenyl]amine (8e)



Scheme 4.17: Synthesis of [4-(6-methyl-1,3-benzothiazol-2-yl)phenyl]amine

Procedure

Para-amino benzoic acid (PABA) (0.98 gm, 0.00719 moles) was dissolved in polyphosphoric acid (17.11 ml) at 110°C. 2-Amino-5-methylthiophenol (1 gm, 0.00719 moles) was added and the resulting solution stirred at 110°C for 90 min. After cooling, the reaction mixture was poured into aqueous ammonia (40 ml). A white precipitate was formed. The precipitate was collected and washed with water (50 ml). Then dried in the oven. The product was purified by column chromatography.

Yield : 76.16%

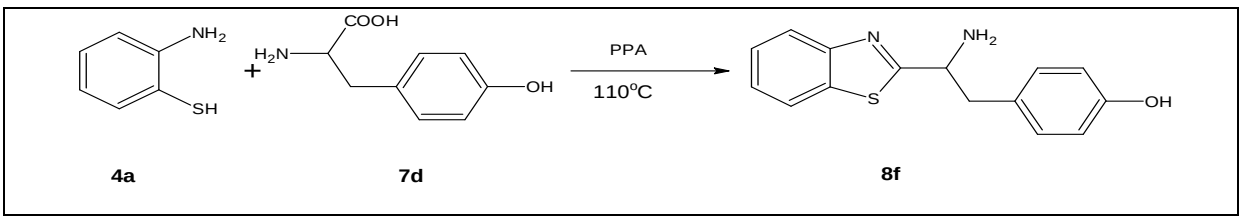
Melting point : 195-197°C

TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

$R_f = 0.535$

IR**(Spectrum 13)** : 3279, 3192, 3029, 2898 1647, 1558, 1540, 1418, 1150 Cm^{-1}

VI) Synthesis of 4-[2-amino-2-(1,3-benzothiazol-2-yl)ethyl]phenol (8f)



Scheme 4.18: Synthesis of 4-[2-amino-2-(1,3-benzothiazol-2-yl)ethyl]phenol

Procedure

L-Tyrosine (1.45 gm, 0.008 moles) was dissolved in polyphosphoric acid (19.04 ml) at 110°C. 2-Aminothiophenol (0.008 moles) was added and the resulting solution stirred at 110°C for 90 min. After cooling, the reaction mixture was poured into aqueous ammonia (35 ml). A white precipitate was formed. The precipitate was collected and washed with water (50 ml).Then dried in the oven. The product was purified by column chromatography.

Yield : 82.34%

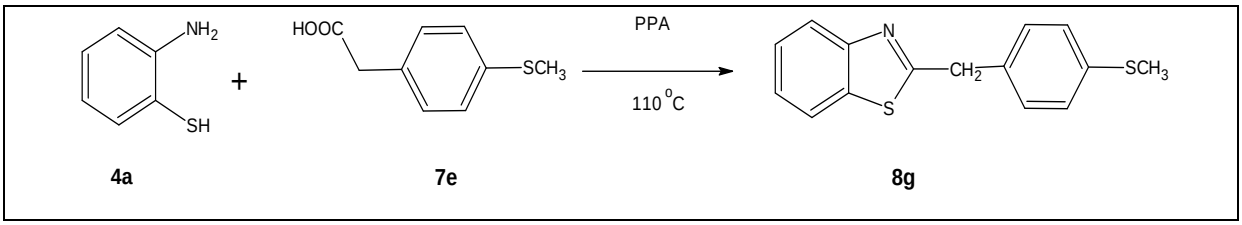
Melting point : 190-192°C

TLC : Silica gel G; Hexane: Ethyl acetate (1:1)

R_f = 0.740

IR**(Spectrum 14)** : 3196, 3178, 2850, 2688, 1520, 1448, 1396, 1044, 891 Cm^{-1}

VII) Synthesis of 2-[4-(methylthio) benzyl]-1,3-benzothiazole (8g)



Scheme 4.19: Synthesis of 2-[4-(methylthio) benzyl]-1,3-benzothiazole

Procedure

4-thiomethyl phenyl acetic acid (1.456 gm, 0.008 moles) was dissolved into polyphosphoric acid (19.04 ml) at 110°C. 2-Aminothiophenol (1 gm, 0.008 moles) was added and the resulting solution stirred at 110°C for 90 min. After cooling, the reaction mixture was poured into aqueous ammonia (35 ml). A precipitate was formed. The precipitate was collected and washed with water (50 ml). Then dried in the oven. The product was purified by column chromatography.

Yield : 84.94%

Melting point : 135-138°C

TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

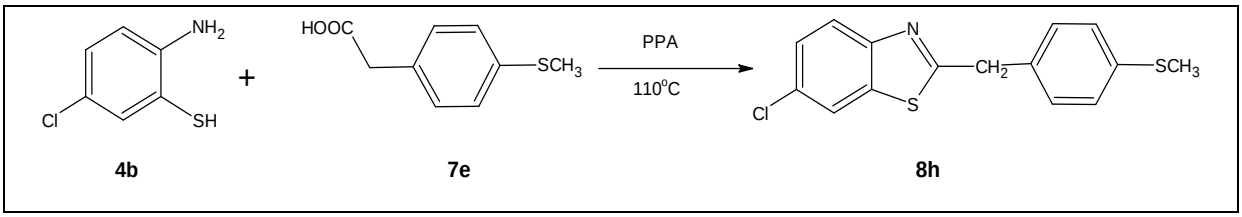
R_f = 0.642

IR(Spectrum 15) : 3012, 2920, 1699, 1652, 1576, 1489, 1418, 1218, 1093, 1014 Cm^{-1}

^{1}H NMR (Spectrum 16): δ 6.75-8.06 (m, 8H, Ar-H), 2-3.7(s, 3H, -CH_2-), 2.44(s, 3H, -SCH3)

^{13}C NMR (Spectrum 17): δ ppm. 172.8, 161.96, 130.05-126, 14.72, 40.20

VIII) Synthesis of 6-chloro-2-[4-(methylthio) benzyl]-1,3-benzothiazole (8h)



Scheme 4.20: Synthesis of 6-chloro-2-[4-(methylthio) benzyl]-1,3-benzothiazole

Procedure

4-thiomethyl phenyl acetic acid (1.137 gm, 0.00625 moles) was dissolved into polyphosphoric acid (19.04 ml) at 110°C. 2-Amino-5-chlorothiophenol (1 gm, 0.00625 moles) was added and the resulting solution stirred at 110°C for 90 min. After cooling, the reaction mixture was poured into aqueous ammonia (40 ml). A precipitate was formed. The precipitate was collected and washed with water (50 ml). Then dried in the oven. The product was purified by column chromatography.

Yield : 85.86%

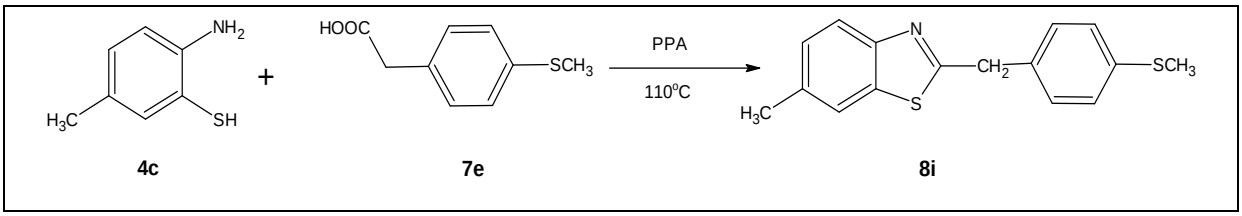
Melting point : 200-205°C

TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

$R_f = 0.705$

IR**(Spectrum 18)** : 3014, 2933, 1651, 1595, 1431, 1231, 1158, 1067, 1054, 801 Cm^{-1}

IX) Synthesis of 6-methyl-2-[4-(methylthio)benzyl]-1,3-benzothiazole (8i)



Scheme 4.21: Synthesis of 6-methyl-2-[4-(methylthio)benzyl]-1,3-benzothiazole

Procedure

4-thiomethyl phenyl acetic acid (1.31 gm, 0.00719 moles) was dissolved into polyphosphoric acid (19.04 ml) at 110°C. 2-Amino-5-methylthiophenol (1 gm, 0.00719 moles) was added and the resulting solution stirred at 110°C for 2 hrs. After cooling, the reaction mixture was poured into aqueous ammonia (40 ml). A precipitate was formed. The precipitate was collected and washed with water (50 ml). Then dried in the oven. The product was purified by column chromatography.

Yield : 84.87%

Melting point : 150-152°C

TLC : Silica gel G; Ethyl acetate: Methanol (50:50)

$R_f = 0.750$

IR**(Spectrum 19)** : 2993, 2874, 2814, 1667, 1434, 1272, 1078, 1010, 914 Cm^{-1}

4. BIOLOGICAL SCREENING

Anti-microbial testing

Activity of anti-infective agents may be demonstrated under suitable conditions by their inhibitory effect on microorganisms. The anti-microbial activity of the synthesized compounds was carried out by standard procedure using broth dilution method and minimum inhibitory concentration was determined by visual comparison with the negative control tubes.

Detailed test procedure

1. Stock Solutions of test compounds and standard drug

Compounds were taken as test samples along with a standard Streptomycin sample. Weight taken in the range of 8-20 mg of each test compound and was dissolved in 1 ml of DMSO. For preparing stock solution of Streptomycin, 10 mg of Streptomycin was dissolved in 1 ml of water.

2. Test organism

The organisms employed in the *in vitro* testing of the compounds were *Escherichia coli* and *S. aureus*. All the cultures were maintained on nutrient broth agar (Microbiology grade, CDH Pvt. Ltd. New Delhi.) medium by periodic sub culturing.

3. Preparation of Innoculum

Procedure for the preparation of innoculum for both the strains was same. The innoculum was prepared from a 24-hours old growth of organism on nutrient broth agar slant. To the agar slant, saline solution was added to obtain O.D value of 0.1 on photoelectric optical colorimeter. 0.5ml of this solution was further diluted to 20ml with use of saline.

4. Preparation of Medium

1.3 gms of nutrient broth (Microbiology grade, CDH Pvt. Ltd. New Delhi.) was dissolved in 100 ml of sterile distilled water.

5. Addition of drug, innoculum solution to medium

From diluted innoculum solution prepared, 100µl was added to separate test tube each containing 0.9ml of medium. 25 µl solution of test stock solution was added in four separate test tube containing 0.9ml of medium with 100 µl innoculum. The tests were carried out in duplicate. Apart from putting the controls of standard drug (Streptomycin), controls with dimethylsulphoxide (DMSO) were used. DMSO (positive control) is DMSO inoculated with organisms and dimethylsulphoxide (negative control) is plain DMSO. For incubation, test tubes were kept in incubator at 35°C for 24 hours.

6. Observations

At the end of incubation period, the results were interpreted by comparison with negative control. The lowest concentration of test compound which showed inhibitory effect on growth on visual distinction was taken as minimum inhibitory concentration (MIC) and visual turbidity was consider for MIC of the test molecules; standard drug and DMSO positive and negative control visual turbidity were recorded.

5. RESULTS AND DISCUSSIONS

In this project, the synthesis of benzothiazole derivative was carried out. 2-Aminobenzothiazole derivative were synthesized in very first step and then they were refluxed in presence of aqueous potassium hydroxide to form 2-Amino thiol derivative. Reaction of these thiol derivatives with various aromatic acids in the presence of polyphosphoric acid results in the formation of various benzothiazole derivatives.

1. Synthesis of 2-substituted benzothiazole derivatives

All molecules were synthesized using the common starting material-aniline. In all compounds, an intermediate was first formed by 2-aminobenzothiazole (**3**) using substituted aniline (**1**) and potassium thiocyanate and then it was hydrolyzed to get 2-amino thiol (**4**), which was directly coupled with substituted aromatic acid (**7**). The general scheme utilized for the synthesis of 2-substituted benzothiazole derivatives (**8**) are outlined below in **Figure** 5.1.

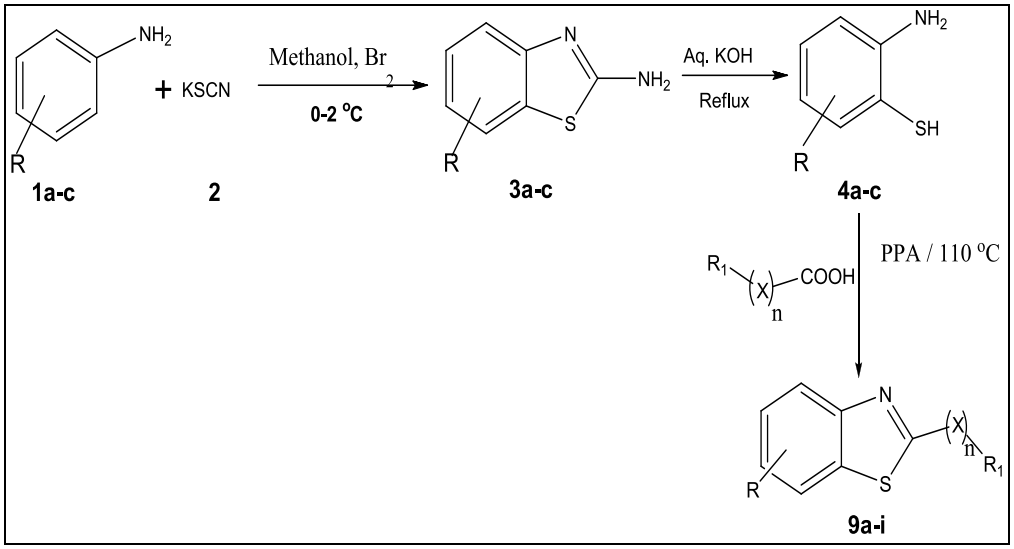


Figure 5.1: General synthetic scheme for 2-substituted benzothiazole derivatives

One of the representative molecules of the synthesized compounds is characterized by spectroscopically (IR, ^{1}H NMR, ^{13}C NMR spectroscopy) and remaining molecules are characterized by IR spectroscopically and evaluated for their biological activity using standard testing procedures.

1. Synthesis of 2-Aminobenzothiazole Derivative

2-Aminobenzothiazoles were synthesized using substituted aniline and potassium thiocyanate as starting material and bromine was used as cyclizing agent at chilled condition (**Figure 5.2).** The solvent used for this reaction was the methanol.

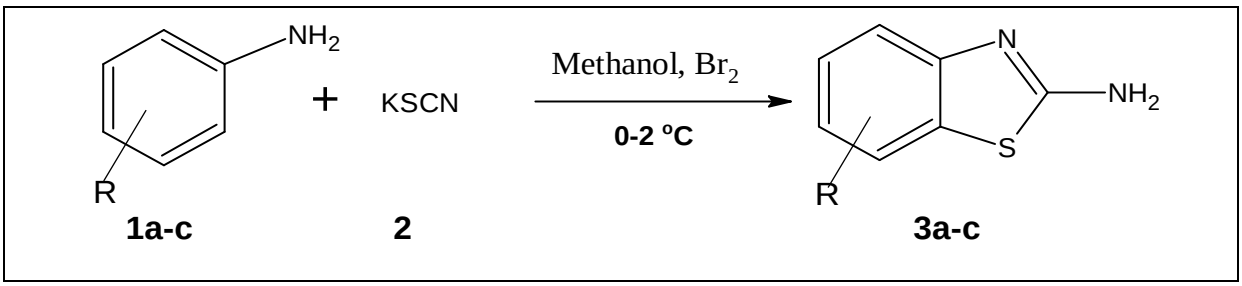


Figure 5.2: General synthetic scheme for 2-amino benzothiazole derivatives

DISCUSSION

Initially 2-aminobenzothiazole, was tried to synthesized using two step procedure, in which first thiourea have to prepare and then cyclisation to get 2-aminobenzothiazole using Br_2 and chloroform as solvent. But it was not worked properly. So that a one step procedure was used to synthesize the intermediate. In which, equimolar concentration of potassium thiocyanate and substituted aniline was dissolved into in methanol and cooled the mixture. Equal molar

concentration of bromine was slowly added in to the stirred mixture, while addition of bromine reaction mixture temperature was maintained between 0-2°C. The solid was precipitated after 4 hr stirring and precipitated solid was collected by filtration and purified by washing with cold methanol.

We noticed that in case of reaction, aniline and 4-Cl aniline when used as starting material, the yield of the 2-aminobenzothiazole was less in comparison of 4-methylaniline that could be possible due to electron releasing substitution enhance the rate of reaction for formation of the 2-aminobenzothiazole.

Possible mechanism of the reaction

The possible reaction mechanism is as follows: -

Step 1: Nucleophilic attack on nitrile carbon

Nucleophilic attack by aniline nitrogen to the carbon of nitrile followed by a proton transfer from nitrogen to oxygen leads to compound (**I**) which protonated and convert to compound (**II**).

Step 2: Bromination at ortho position of substituted aniline

In second step, bromination will take place at o-position of the benzene and form compound (**III**) and it will get cyclized and yield the substituted 2-aminobenzothiazole.

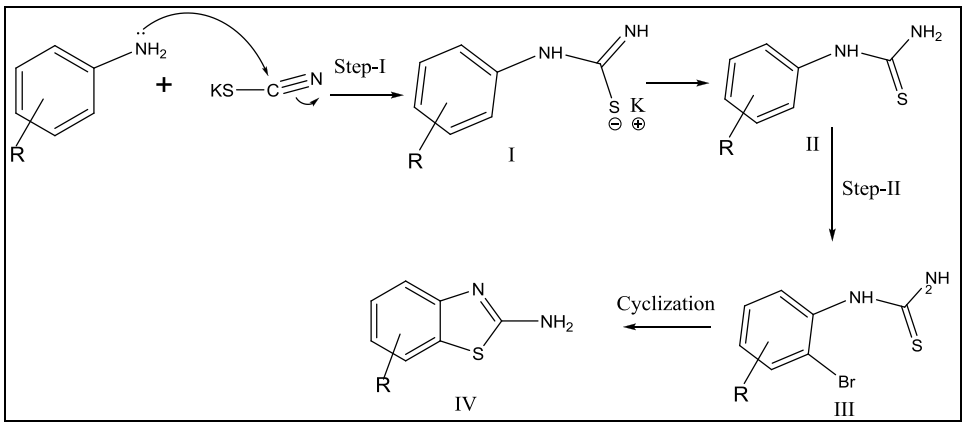


Figure 5.3: Mechanism of the reaction for synthesis of 2-aminobenzothiazole

Possible side- products for this reaction

The side-products that could from in course of the reaction are **A** and **B** (**Figure5.4**).

The compound (**II**) could be side-product which is formed during first step of aminobenzothiazoles synthesis if it is not convert in to compound (**III**). Another could be compound (**III**) if it is not cyclized into desired compound.

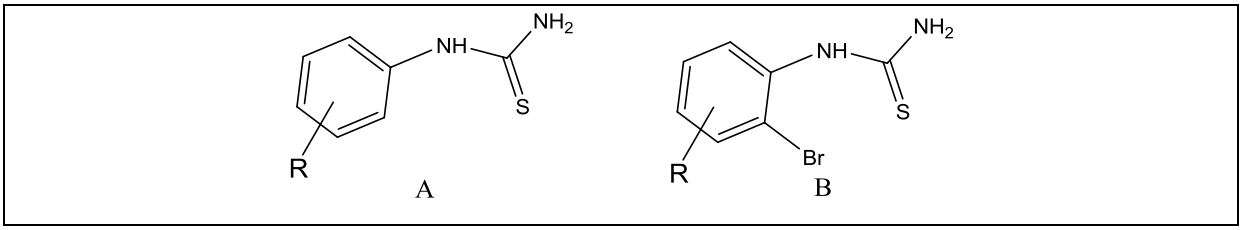


Figure 5.4: Possible side product for synthesis of 2-aminobenzothiazole

Characterization of the 2-amino benzothiazole intermediate

IR: The spectrum showed characteristic NH_2 stretching peak corresponding to the primary amine between in the range of 3300-3150cm^{-1}. The Melting point of the isolated molecules, matched with reported 2-aminobenzothiazole.[49]

2. Synthesis of 2-amino thiol derivatives

2-Aminothiol derivatives were synthesized by using a suspension of intermediate (**3a-c**) and potassium hydroxide in water and refluxed it for 4-5 hrs (**Figure 5.5**). After cooling, con. Hydrochloric acid was added. The reaction was shielded from light by aluminum foil.

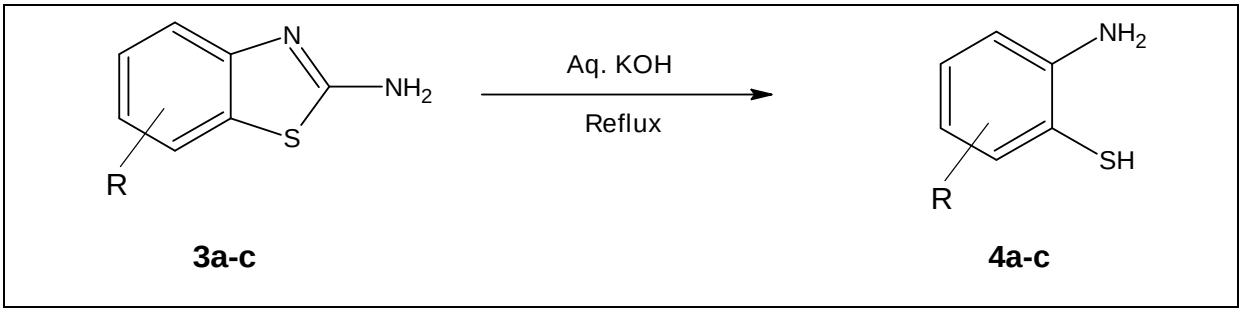


Figure 5.5: General synthetic scheme for 2-amino thiol derivatives

DISCUSSION

The reported protocol was used for the synthesis of 2-aminothiol derivatives. There was not any change in the procedure during the synthesis of 2-aminothiol derivatives. There was no possibility for side product, 2-aminobenzothiazole derivatives were converted into 2-aminothiol derivatives by hydrolysis in the presence of aqueous potassium hydroxide.

Characterization of the 2-amino benzenethiol intermediate

IR: The spectrum showed characteristic NH_2 stretching peak corresponding to the primary amine between in the range of 3300-3150cm^{-1} and the S-H stretching peak in the range of 2500-2600cm^{-1} The Melting point of the isolated molecules, matched with reported 2-aminothiol.[49]

3. Synthesis of substituted (diethylamino)(phenyl)acetic acid

For the synthesis of substituted (diethylamino)(phenyl)acetic acid, first cyanoamination reaction was carried out using substituted aldehyde and diethylamine as starting material to get the intermediate. Further, the intermediate was subjected for nitrile hydrolysis using HCl and H_2SO_4 at reflux reaction condition to yield the (diethylamino)(phenyl)acetic acid. The general scheme utilized for the synthesis of substituted (diethylamino)(phenyl)acetic acid) is given in **Figure 5.6.**

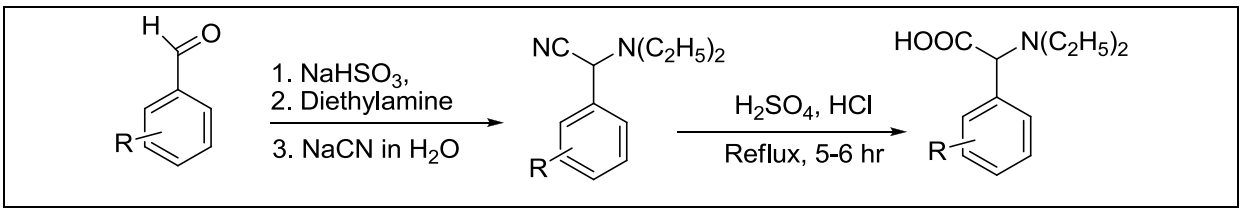


Figure 5.6: General synthetic scheme for (diethylamino)(phenyl)acetic acid

DISCUSSION

The reported protocol was used for the synthesis of (diethylamino)(phenyl)acetic acid. There was not any change in the procedure for the synthesis of (diethylamino)(phenyl)acetic acid derivative.

Characterization of the diethylaminophenyl acetic acid

IR: The spectrum showed characteristic C=O stretching peak of –COOH in the range of 1660-1700cm^{-1} and the -CHO absorption peak of the starting material which appeared in the range of 2700-2750cm^{-1} is disappeared.[59]

4. Synthesis of benzothiazole derivatives

Benzothiazole derivatives (8a-i) were synthesized by dissolving Carboxylic acid derivative (7a-e) in polyphosphoric acid at 110°C and added substituted 2-aminothiophenol (4a-c) and the resulting solution stirred at 110°C for 1-2 hrs. The synthetic scheme which was used for the synthesis of benzothiazole derivatives is given in **Figure 5.7.**

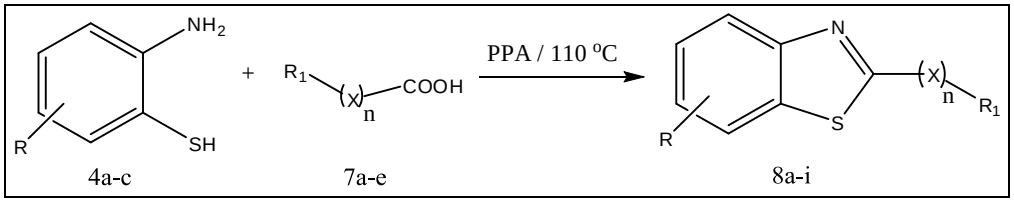


Figure 5.7: General synthetic scheme for final product

DISCUSSION

For the synthesis of 2-aryl benzothiazole initially reaction was carried out using PPA at **220°C** as per Kim Serdons et al. procedure. In the reaction conditions which are reported by Kim Serdons et al. the product as well as starting material was became dark-black brown colour. Letter than **Hutchinson et al.** protocol used for the synthesis of final molecules and it work very well. Obtained crude product was purified using column chromatography.[56]

Mechanism of the reaction

Mechanism of reaction for the synthesis of 2-arylbenzthiazol from 2-aminothiol and substituted aryl acid using polyphosphoric acid is given in **figure 5.8.**

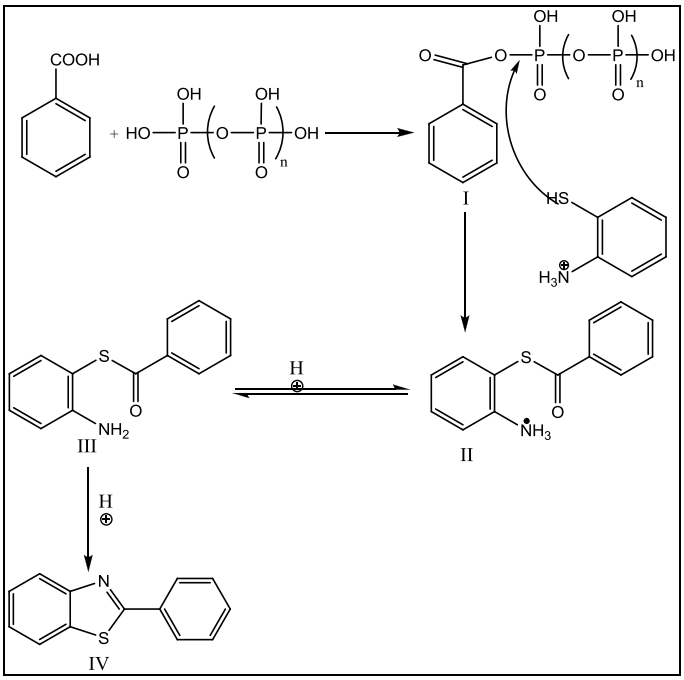
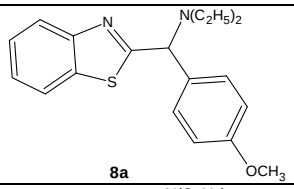
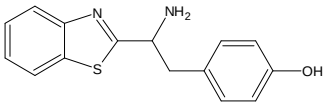
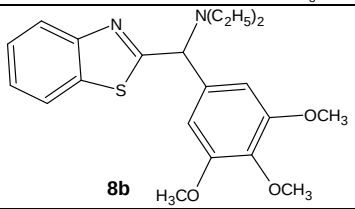
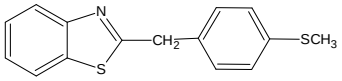
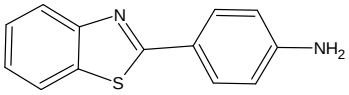
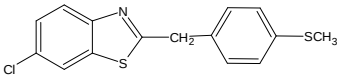
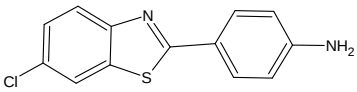
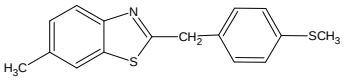
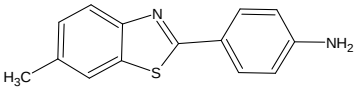


Figure 5.8: General synthetic scheme for final product List of synthesized molecules is given in Table 5.1:

Table 5.1: Synthesized final molecules

Sr. No.	Mol. ID.	Structure	Sr. No.	Mol. ID.	Structure
1	8a	 8a	6	8f	 8f
2	8b	 8b	7	8g	 8g
3	8c	 8c	8	8h	 8h
4	8d	 8d	9	8i	 8i
5	8e	 8e	-	-	-

Characterization of the benzothiazole derivatives

IR: The spectrum showed characteristic absorption band accordingly of the presence of functional group. The Ar-C-H stretching showed the absorption band in the range of 3010-3150 cm^{-1}. The –C=N stretching band appeared in the range of 1630-1660 cm^{-1}, -C=C- stretching peak comes in the range of 1400-1500 cm^{-1}, alkyl C-H stretching peak comes in the range of 2820-2860 cm^{-1}. Alkoxy group containing compounds **8a** and **8b**, showed –OCH_3 stretching in the range of 1100-1200 cm^{-1}. Compound **8c,** 8d **and 8e** contain aromatic -NH_2, which showed N-H stretching band in the range of 3150-3300 cm^{-1}

One representative molecule Compound **8g** was characterized ^{1}H NMR and ^{13}C NMR spectroscopy**.**

^{1}H NMR: The methylene protons appeared as a singlet at around δ 3.7. The SCH_3 protons appeared at around δ 2.44. The aromatic protons appeared as multiple singlets between δ 6.75-8.06. Representative example is **8g.**

Table 5.2: Comparison of ^{1}H NMR values

Sr. No.	No of proton	Predicted (δ in ppm)	Experimental (Spectrum 16)
1	-CH2-	3.8	3.7
2	-SCH3	2.5	2.44
3	Ar-H	7.13-8.18	6.75-8.06

^{13}C NMR

The ^{13}C NMR values for compound **8g** is listed and compared with the predicted values in the table below (Chembio office Chemdraw Ultra 11.0) (**Table 5.3**). Representative example is **8g** (**Spectrum 17).**

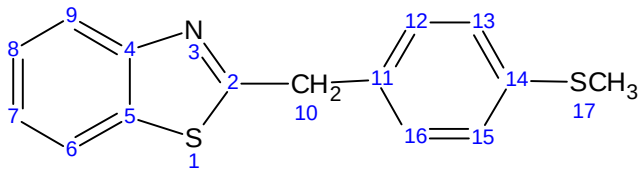


Figure 5.9: Structure of compound 8g

Table 5.3: Comparison of ^{13}C NMR values

Sr. No.	No of carbon	Predicted	Experimental
1	C-2	171	172.8
2	C-4	152	161.96
3	C-11	132	130.05
4	C-17	14.8	14.72
5	C-10	39.5	40.20

6. BIOLOGICAL EVALUATION

The synthesized NCEs were subjected to antimicrobial evaluation against *Escherichia coli* and *S. aureus* microorganism using broth dilution method keeping appropriate positive and negative controls simultaneously.

Visual turbidity of evaluated compounds is given in **Table 6.1**

Table 6.1: Visual turbidity of evaluated compounds

Sr. No.	Compounds	Visual Turbidity (*E. Coli*)	Visual Turbidity (*S. Aureus*)
1.	**8a**	-	-

2.	**8b**	-	-
3.	**8c**	-	-
4.	**8d**	+	-
5.	**8e**	-	-
6.	**8f**	-	-
7.	**8g**	+	-
8.	**8h**	+	+
9.	**8i**	-	-
10	Streptomycin	-	-

- = No Turbidity (No bacterial growth), + = Turbidity (Bacterial growth)

Based on the visual turbidity, the MIC of the evaluated molecules is given in **Table 6.1** the evaluation concentration was used single therefore, the exact MIC could not determined and results are represented in less than and more than format. To get more exact MIC of the tested molecules need to be evaluated at low concentration. The evaluation results of the single concentration is tabulated in **Table6.2.**

Table 6.2: MIC of evaluated compounds

Sr. No.	Compounds	*E. Coli* (MIC in µg/ml)	*S. Aureus* (MIC in µg/ml)
1.	**8a**	<475	<475
2.	**8b**	<450	<450
3.	**8c**	<950	<900
4.	**8d**	>400	<200
5.	**8e**	<900	<450
6.	**8f**	<950	<900
7.	**8g**	>600	<450
8.	**8h**	>900	>1000
9.	**8i**	<475	<475
10.	Streptomycin	<450	<450

Results of antibacterial testing

The compounds (8a, 8b and 8c) exhibited bacterial growth inhibition when tested at concentration of less than 500µg/ml against both microorganisms. Also, compounds (8d and 8g) exhibited growth inhibition of *S. aureus* organism less than 200 µg/ml and <475 µg/ml respectively. Other compounds exhibited activity at higher concentrations. Further testing for compounds (8a, 8b and 8c) at lower concentrations is required to compare their activity with standard Streptomycin at its MIC to get exact MIC the synthesized compounds.

7. CONCLUSION

Thus it can be concluded that designed benzothiazole derivatives were synthesized successfully and (**8a-i**) exhibited antibacterial activity at tested concentration less 1000µg/ml and some of compound **8d** exhibited activity at lower concentration <200 µg/ml. Compounds **8h,** was not exhibited activity higher concentration up to 1000 µg/ml against both gram positive and gram negative organisms. Thus, synthesized benzothiazole derivatives can therefore act as a lead for development of broad spectrum anti-infective agents.

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