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SYNTHESIS CHARACTERIZATIONS & EVALUATIONS OF NEW ACRIDINES AS ANTIMICROBIALS

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ABSTRACT

Resistance to anti-infective agents is a serious problem. Bacterial resistance continues to develop and poses a significant threat, both in hospitals and in the community.[8] Owing to the extended use and misuse of antibiotics, the number of bacteria that are resistant to antimicrobial agents is rapidly increasing. The rising prevalence of multi-drug resistance has become a serious health care problem and one of the organisms rapidly developing resistance is *S. aureus*. Infections caused by *S aureus* are as follows: septicemia, endocarditis, urinary tract infections, impetigo, abscesses, boils, carbuncles, meningitis, osteomyelitis, pneumonia, empyema, arthritis, enteritis, endophthalmitis. *S. aureus* accounts for more than 10% of nosocomial infections and about 20% of food-borne disease outbreaks. In hospitals, *S. aureus* causes 10% of infections on surgical services, 20% on pediatric services, and 35% in nurseries. The most important community-acquired staphylococcal infection is food poisoning. It is evident from the statistics that *S aureus* has significant pathogenic activity. it was observed that 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.

KEYWORDS: Broad spectrum of activity, Target selectivity, Sufficient grade of selectivity, Need of new agents, Drug resistance, Overuse of antibiotics.

1. INTRODUCTION

Anti-microbial agents are those which treat infection by suppressing or destroying the causative microorganisms such as bacteria, mycobacteria, fungi, protozoa or viruses without significant effect on host tissues.[1]

The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. Microorganisms have become opportunistic pathogens responsible for severe and often fatal infections in humans. Bacteria, fungi, and viruses are responsible for almost all of the common infectious diseases from athlete's foot, to AIDS, to ulcers. The emergence of antimicrobial resistance pathogens is rapidly becoming a major concern in human medicine. Emergence of multi-drug resistant strains of pathogenic organisms and difficulty in treating immuno-compromised patients has caused rapid increase in the incidence of microbial infections. This emergence situation calls for the need to develop selective target oriented drugs with higher potency, expanded spectrum of activity, improved safety profile and activity against the multi-drug resistant strains.

There has been a substantial growth in all the areas of antimicrobial in the last decade. While new chemical entities have steadily been described in the literature against molecular targets, many challenges remain in the future. Invasive infections, in patients with compromised immune systems brought on by cancer chemotherapy, organ transplants, surgery and inflictions with HIV/AIDS etc are limiting the arsenal of the existing anti-infective agents.[2] The increasing resistance of pathogens to current classes of anti-microbial drugs is an urgent public health concern. Thus newer agents are required to compliment current strategies and advances in this area. The latest strategy used is a target-based approach of anti-microbial action. 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.[3,4]

1.1. Based on Mechanism of action[6]

Antibiotics are very commonly used substances to eradicate bacterial infections by bacteriostatic or even bactericidal effect. They act at a very specific stage (target), although other less important or secondary interactions can occur. Penicillin disturbs the cell wall synthesis and more accurately the glycopeptide (or murein) formation, a substance giving

rigidity or shape to bacteria. Amino sides, particularly Streptomycin, link themselves to 30 S subunit of bacterial ribosome. In this case, it seems that it is a 3"OH function which reacts with lysine (from S 12 protein part of 30S subunit). The consequence is an alteration in the RNA messenger lecture, and a false transduction and consequently protein biosynthesis stops with a decrease of polyribosomes and of the formation of inert 70S ribosome. Rifampcin and particularly Rifampicin act by inhibition of RNA messenger synthesis.

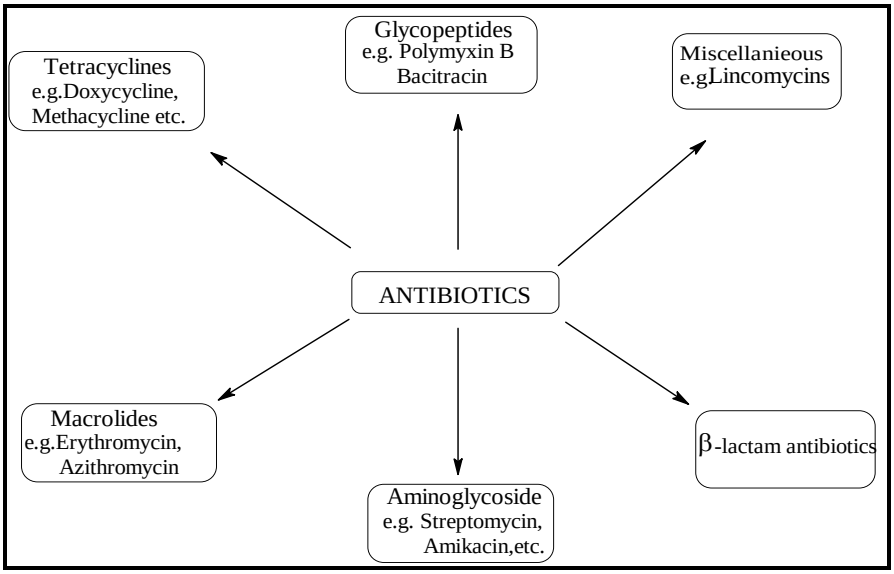


Figure 1.1: Classification of antibiotics

Major classes of synthetic antibacterials [7] is given in Table 1.1

Table 1.1 List of synthetic antibacterial

Sr. No	Class	Mechanism of Action	Spectrum of Activity	Examples of Drugs	Adverse Effects
1	**Sulfon-amides**	These are structural analogs and competitive antagonists of PABA and thus prevent normal bacterial utilization of PABA for the synthesis of folic acid.	Sulfonamides are primarily bacteriostatic; active against many Gram-positive and Gramnegative bacteria	Sulfadiazine, Sulfisoxazole, Sulfametho-xazole Sulfamoxole, Sulfadoxine, Sulfasalazine etc	Crystalluria, Acute haemolytic anaemia, Agranulocytosis Aplastic anaemia, Hypersensitivity reactions. Kernicterus
2	**Sulfones**	Same as sulphonamides	Leprostatic at low conc.	Dapsone	Mild haemolytic anaemia, Hyper-sensitivity reactions
3	**Quinol-ones**	Inhibits the bacterial enzyme DNA gyrase (topoisomerase II)	Highly active against enteric Gramnegative bacilli	Ciprofloxacin, Ofloxacin, Lomefloxacin, Sparfloxacin etc	Hypersensitivity reactions, Photo-toxicity
4	**Oxazoli-dinones**	Inhibition of early ribos-	Active against	Linezolid	Nausea, Vomiting,

		omal protein synthesis, without directly inhibiting DNA or RNA synthesis.	*S.pneumoniae* *S. aureus* *E. faecium*	(**ZYVOX**)	Thrombocytopenia (2.4 percent), Myelosuppression
5	**DHFR-Inhibi-tors**	Inhibition of Bacterial enzyme of dihydrofolate reductase and Causes sequential block of bact-erial folate metabolism	It has broad spectrum of antibacterial activity	Trimethoprim, Pyrimethamine	Megaloblastosis, Leucopenia, throm-bocytopenia, Bone marrow toxicity in elderly

1.2 Antitubercular Agents[1-7]

Tuberculosis is a chronic granulomatous disease and a major health problem in developing countries. *Mycobacterium tuberculosis* is responsible for at least 2 million deaths per year worldwide, and 30 million people are at a risk of dying from tuberculosis (TB) in the next 10 years. Drugs used to treat tuberculosis suppress or kill the slow-growing mycobacteria that cause this disease. Antitubercular agents though belonging to the category of antibacterials, but due to the increased prevalence and importance of the disease and since the organism is not affected by general antibacterials, need to be mentioned separately. Because the causative organisms tend to develop resistance to any single drug, combination drug therapy has become standard in the treatment of tuberculosis.

1.3 Need of new agents[9]

Despite the tremendous progress in human medicine, infectious diseases represent one of the greatest challenges to mankind in the 21st century. According to WHO, infectious diseases account for nearly a third of global deaths. AIDS, malaria, tuberculosis and respiratory infections were among the top eight leading causes of death in 2004. The burden of infectious diseases falls particularly on the less developed countries due to the relative unavailability of medicines and the emergence of widespread drug resistance. In developing countries, a high infectious disease burden commonly co-exists with rapid emergence and spread of microbial resistance. The growing threat of emerging diseases such as SARS and influenza A (H^1N^1) has served as a wake-up call to public health services, pharmaceutical industry and academia. Because the evolution of drug resistance is likely to compromise every drug in time, research on new anti-infective agents must be continued and all possible strategies should be explored. Therefore, there is urgent need of development of novel potential anti-infective agents.

There are several mechanisms for antibiotic resistance and these relate to the sites of antimicrobial activity.[10]

These mechanisms includes[6]

1. Altered receptors for the drug.
2. Decreased entry into the cell.
3. Destruction or inactivation of the drug.
4. Development of alternate metabolic pathway.
5. Failure to metabolize a prodrug.

Limitations of existing classes of anti-infective agents include

1) Narrow spectrum of activity.
2) Reduced potency.
3) Emergence of resistance.
4) Adverse effects.

There is therefore a pressing need to develop new antibiotics and novel antimicrobial agents. The need for research directed toward development of new antibiotics has never been greater. Development of new antimicrobial agents has been focused on modifying agents within the known classes or to block resistance mechanism to provide improvements in activity against resistant organisms.

2. MATERIALS

Table 2.1: Materials

S.No.	Name of Item	Specification (grade, pack size)	Quantity required
1	Aniline	100gm	50gm
2	Potassium thiocyanate	500gm	500gm
3	Potassium hydroxide	500gm	500gm
4	Para-toluene sulfonic acid	500gm	100gm
5	Sodium sulphite	500gm	500gm
6	Benzaldehyde	500gm	100gm
7	Nicotinic acid	100gm	100gm
8	Silica gel	1.0kg	1.0kg
9	Acetic acid	500ml	500ml
10.	Sulphuric acid	500ml	250ml
11.	Hydrochloric acid	500ml	500ml
12.	Pyridine	500gm	100ml
13.	TLC plate	25M No.	4-5 plates
14.	Melting point capillary	100 No.	100 No.
15.	Acetophenone	500ml	100ml
16	Sodium isatinate	50 gm	10
17	Potassium 2-iodobenzoate	100gm	50gm

18	anthranilic acid	100gm	50gm
19	diglyme	250 mL	250mL

3. METHODS

SYNTHESIS OF INTERMEDIATES

A. Preparation of Acridine-4-carboxylic Acid.

i) Synthesis of 2-(3-(carboxylatocarbonyl)phenylamino)benzoate. General reaction.

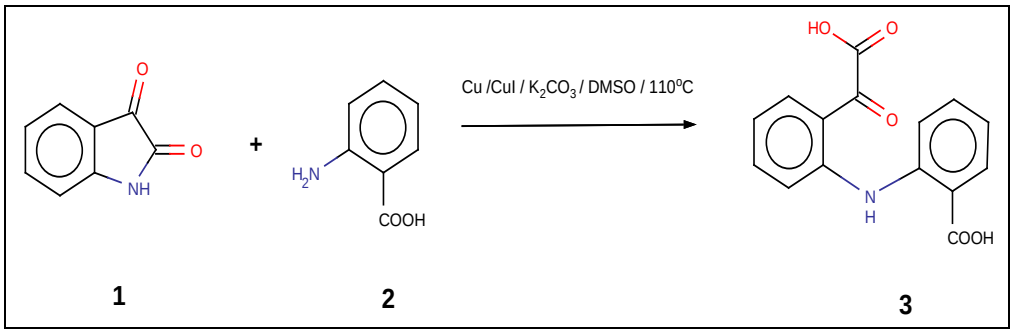


Figure 3.1: General reaction for synthesis of 2-(3-(carboxylatocarbonyl) phenylamino) benzoic acid

General Method[61]

Sodium isatinate (1.69 g, 10mmol), Potassium 2-iodobenzoate (3.74 g, 13 mmol), CuI (0.2g), Cu powder (0.2 g), and K_2CO_3 (1.38 g, 10 mmol) in DMSO(15 mL) was heated at 110 °C for 1 h under N_2. The cooled (solidified) reaction mixture was diluted with water (150 mL), and the mixture was acidified to pH 1 with concentrated HCl.

Work up

The mixture was extracted with EtOAc, and the two-phase mixture was filtered to remove copper salts. The organic layer was separated, dried (Na_2SO_4), and evaporated to give crude2-[*N*-(2-carboxyphenyl) amino]phenyl pyruvic acid.

(ii). Synthesis of 2-(3-(carboxylatocarbonyl) phenylamino) benzoate.

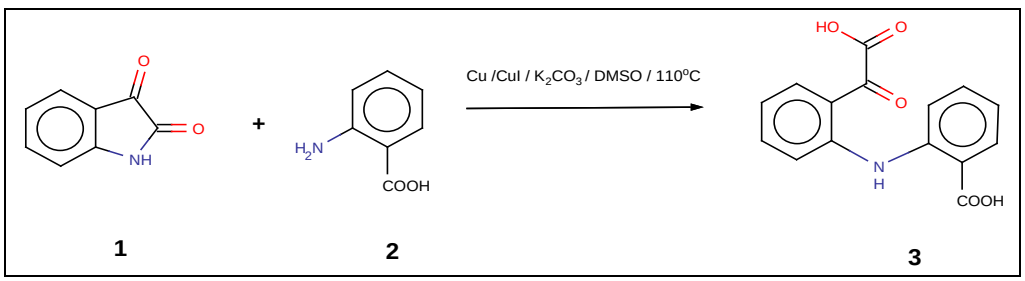


Figure 3.2: Synthetic scheme for Synthesis of 2-(3-(carboxylatocarbonyl) phenylamino) benzoate

Procedure

A mixture of isatin (**1**) (1.69 g, 10mmol), antharanilic acid(**2**) (3.74 g, 13mmol), CuI (0.2g), Cu powder (0.2 g), and K_2CO_3 (1.38 g, 10mmol) in DMSO(15mL) was mixture refluxed at 110 °C for 1 h on a sand bath with vigorous stirring. The reaction was monitored by TLC. Initially, the color of reaction mixture was yellow while reaction proceeded till reaction mixture became dark. Reaction mixture was cooled and diluted with water (150mL), and acidified to pH 1 using concentrated HCl.

Work up

The precipitated compound was filtered under vacuum and dried in oven at 50-60°C. The product(**3**) was stored under light free conditions.

TLC : Silica gel G; 100% Ethyl acetate

Yield:- : 58%, 1.68 g

Melting point : 207 ^{o}C Reported melting point: [95] 204-206 ^{o}C

(iii). Synthesis of acridine-4,9-dicarboxylic acid.

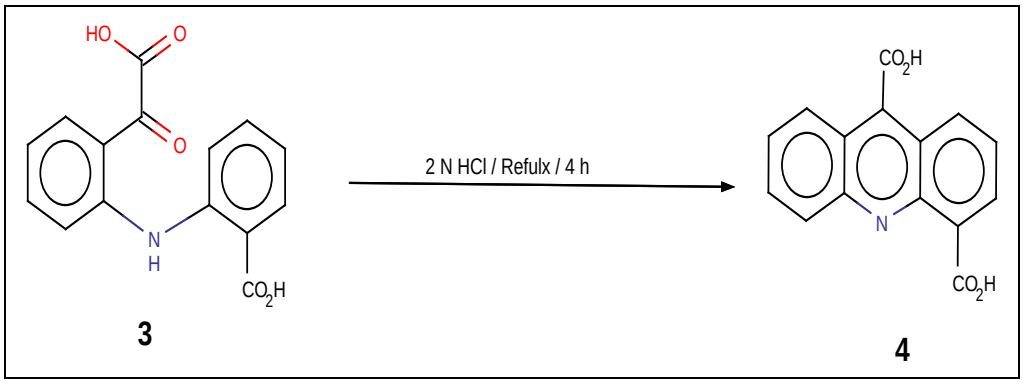


Figure 3.3: General reaction for Synthesis of acridine-4,9-dicarboxylic acid

Procedure

A solution of crude compound (**3**) (2.0 g, 6.8 mmol) was heated under reflux in 2 N HCl for 4 h and reaction was monitored by TLC.

Workup

After completion of the reaction, the mixture was cooled, neutralized with aqueous ammonia and filtered the precipitated product and dried in oven. The residue thus obtained was dissolved into ethyl acetate and washed with water twice. Ethyl acetate layer was separated and dried by anhydrous sodium sulphate. The solvent was evaporated under reduced pressure and the residue (acridine-4, 9-dicarboxylic acid) (4) thus obtained was used for next step.

TLC : Silica gel G; 100% Ethyl acetate

Yield : 1.26 g (73%)

Melting point : > 250 ^{o}C, Reported melting point: [95] 258 ^{o}C

IR **(Spectrum 1) :** 3086, 3035, 1633, 1534, 1473, 1306, 1276, 1099, 1049, 860, 811, 763, 681, 624, 565cm^{-1}

Synthesis of acridine 4-carboxylic acid.

General reaction.

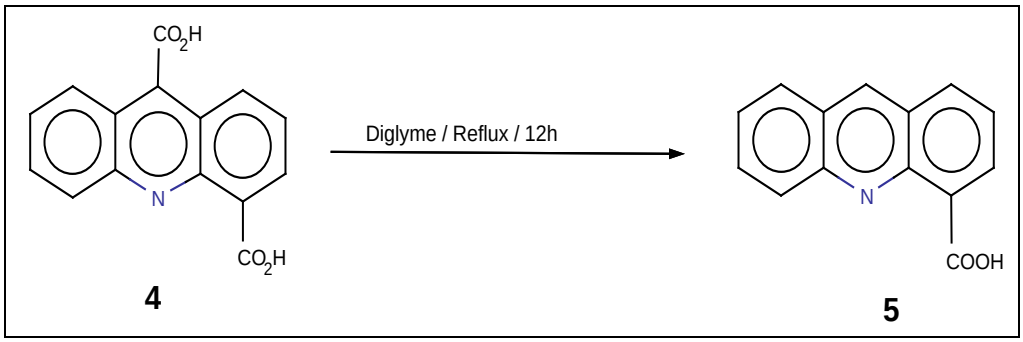


Figure 3.4: General reaction for Synthesis of acridine 4-carboxylic acid

Procedure

A suspension of acridine-4,9-dicarboxylic acid(**4**)(1.0 g, 3.75 m mol) and in dry diglyme (20 mL) was refluxed at 110 °C for 12 h on a sand bath with vigorous stirring. The reaction was monitored by TLC. Reaction proceeded till reaction mixture became dark brown in color.

Workup

After completion of the reaction mixture, solvent was removed under reduced pressure, and the residue was purified by Colum chromatography on silica gel. Elution with EtOAc containing a trace of AcOH gave acridine- 4-carboxylic acid (**5**).

TLC : Silica gel G; 100% Ethyl acetate

Yield : 0.57 g (68 %)

Melting point : 203 ^{0}C Reported melting point: [95] 202-204 ^{0}C

IR **(Spectrum 2)** : 2955, 2919, 2872, 1681, 1592, 1564, 1508, 1454, 1395, 1315, 750, 668, 649 cm^{-1}

(iv). Synthesis of methyl acridine-4-carboxylate.

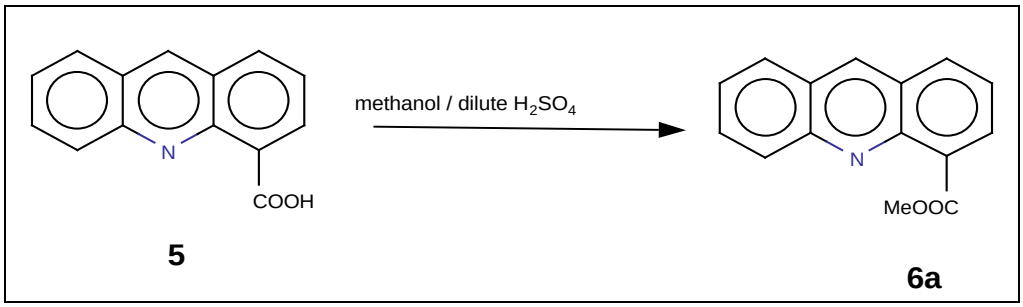


Figure 3.5: Synthetic scheme for Synthesis of methyl acridine-4-carboxylate

Procedure

Acridine 4-carboxylic acid (**5**) (1.5 g, 6.72 mmol) in) was dissolved into 30 ml methanol. 4-5 drop of conc. H_2SO_4 was added. The mixture was refluxed for 4 hr on a sand bath with vigorous stirring. The reaction was monitored by TLC. Residue (methyl acridine-4-carboxylate) (**6a**) thus obtained was used for next step.

Work up

After completion of the reaction mixture, solvent was removed under reduced pressure, and the residue was purified by Colum chromatography using ethyl acetate and hexane as solvent system.

TLC : Silica gel G; 100% Ethyl acetate

Yield : 1.5 g (90%)

Melting point : 205 – 207 ^{o}C

(v). Synthesis of acridine-4-carbohydrazide.

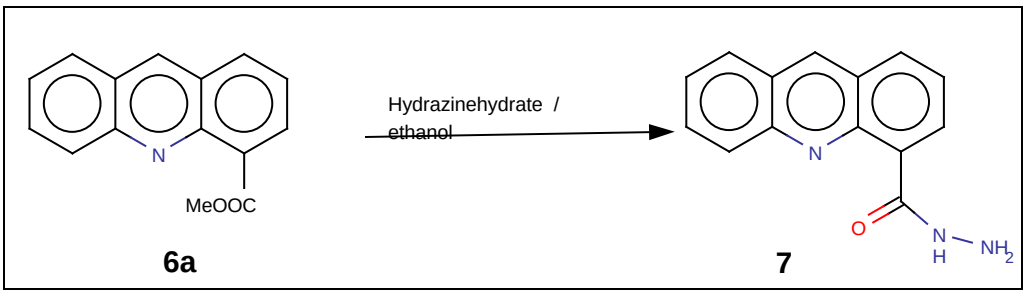


Figure 3.6: **Synthetic scheme for synthesis of acridine-4-carbohydrazide**

Procedure

Acridine methyl 4-carboxylate (**6a**) (1.5 g, 6.72 mmol) and 1ml of hydrazine hydrate were added into 30 ml methanol contained in a round bottom flask. The mixture was refluxed for 8 h on a sand bath with vigorous stirring. The reaction was monitored by TLC.

Work up

After completion of the reaction mixture, solvent was removed under reduced pressure, and the residue acridine-4-carbohydrazide (**7**) was purified by Colum chromatography using ethylacetate and hexane as solvent system.

TLC : Silica gel G; 100% Ethyl acetate

Yield : 0.96 g (90%)

Melting point **:** 273 ^{o}C

B. Synthesis of substituting side ring.

General reaction

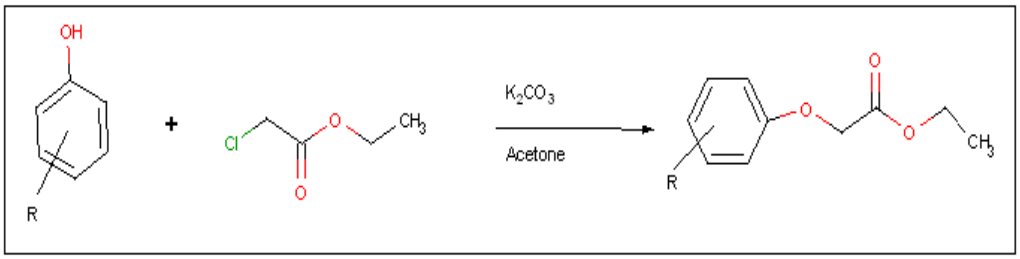


Figure 3.7: General synthetic scheme for substituted ethyl 2-phenoxyacetate

General Method[87]

A mixture of equimolar amounts of the substituted phenol and ethylchloroacetate were, contained in a round bottom flask and suspended in 50-60 ml acetone and anhydrous potassium carbonate (1-2gm) was added in the mixture. The mixture was refluxed on a sand bath with vigorous stirring. The reaction was monitored by TLC. The reaction was continued till the substituted phenol was consumed completely. Initially, the color of reaction mixture was color less in case of phenol while in other phenols light yellow and reaction proceeded till reaction mixture became dark in color.

Workup

The reaction mixture, when cooled, was filtered under vacuum to remove solid potassium carbonate and the filtrate thus obtained was evaporated under vaccum. The residue thus obtained was dissolved into ethyl acetate and washed with water twice. Ethyl acetate layer was separated and dried over anhydrous sodium sulphate. The solvent was evaporated under vacuum and the residue (liquid product) thus obtained was used for next step.

(i). Synthesis of ethyl 2-(2,4-dichlorophenoxy)acetate. (3'b)

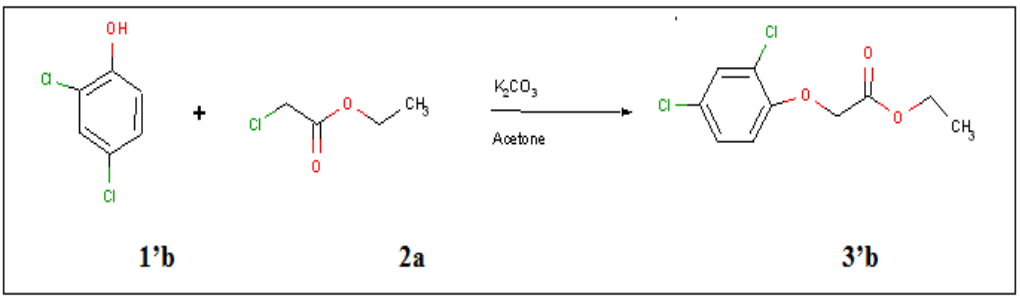


Figure 3.8: Synthetic scheme for Synthesis of ethyl 2-(2,4-dichlorophenoxy)acetate

Procedure

The solution of 2,4-dichlorophenol **(1'b)** (2.4 g, 2.3 ml, 0.02 moles), ethylchloroacetate **(2a)** (3.32 g, 0.02 moles), in acetone (20ml) and anhydrous potassium carbonate (2g) was added. The mixture was refluxed on a sand bath with vigorous stirring for 20hour. The reaction was monitored by TLC.

Workup

The reaction mixture, when cooled, was filtered under vacuum to remove solid potassium carbonate and the filtrate thus obtained was evaporated under vacuum. The residue thus obtained was dissolved into ethyl acetate and washed with water twice. Ethyl acetate layer was separated and dried over anhydrous sodium sulphate. The solvent was evaporated under vacuum and the residue (liquid product) thus obtained was used for next step.

Yield : 92.53%

TLC : Silica gel G; Hexane: Ethyl acetate (8:2)

$R_f = 0.78$

IR **(Spectrum 3) :** 3094.58, 3020.30, 2985.44, 1732.39, 1478.56, 1375.14, 1279.05, 1249.56, 1214.26, 1105.25, 1046.92, 929.20, 854.11, 744.60 cm^{-1}

(ii). Synthesis of ethyl 2-(4-nitrorophenoxy)acetate. (3'c)

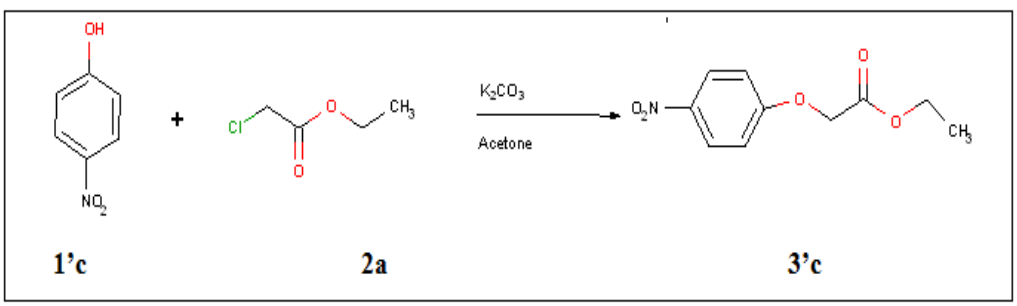


Figure 3.9: **Synthetic scheme for Synthesis of ethyl 2-(4-nitrorophenoxy)acetate (3c)**

Procedure

The solution of 4-nitrophenol (**1'c)** (2.4 g, 2.3 ml, 0.02 moles), ethylchloroacetate (**2a)** (3.32 g, 0.02 moles), in acetone (20ml) and anhydrous potassium carbonate (2g) was added. The mixture was refluxed on a sand bath with vigorous stirring for 20hour. The reaction was monitored by TLC.

Workup

The reaction mixture, when cooled, was filtered under vacuum to remove solid potassium carbonate and the filtrate thus obtained was evaporated under vaccum. The residue thus obtained was dissolved into ethyl acetate and washed with water twice. Ethyl acetate layer was separated and dried over anhydrous sodium sulphate. The solvent was evaporated under vacuum and the residue (liquid product) (**3c**) thus obtained was used for next reaction.

Yield in % : 90.97%

TLC : Silica gel G; Hexane: Ethyl acetate (8:2)

$R_f = 0.56$

IR **(Spectrum 4)** : 3062.74, 3019.59, 1749.28, 1716.97, 1592.05, 1338.83, 1213.97, 951.53, 848.51 cm^{-1}

Synthesis of substituted ethyl 2-phenoxyacetate.

General reaction

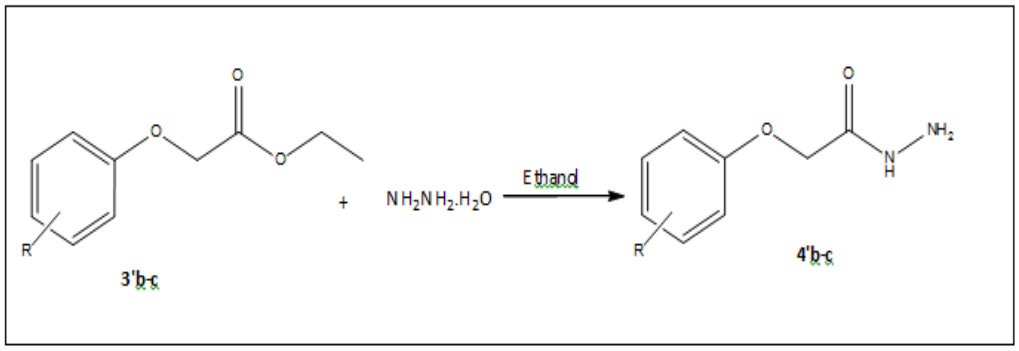


Figure 3.10: General synthetic scheme for substituted ethyl 2-phenoxyacetate

General method[87]

A solution of substituted ethyl 2-phenoxyacetate (1 mol) and hydrazine hydrate (1.5 mol) were contained in a round bottom flask and suspended in 50-60 ml ethanol. The mixture was refluxed on a sand bath with vigorous stirring. The reaction was monitored by TLC. The reaction was continued till the substituted ethyl 2-phenoxyacetate was consumed completely.

Initially, the color of reaction mixture was color less in case of phenol while in other phenols light yellow and reaction proceeded till reaction mixture became dark in color.

Workup

The reaction mixture, when cooled, was filtered under vaccum to remove solid substituted 2-phenoxyacetohydrazide. The solid thus obtained was washed with ethanol, dried and used for next step.

(i). Synthesis of phenoxyformohydrazide. (4'a)

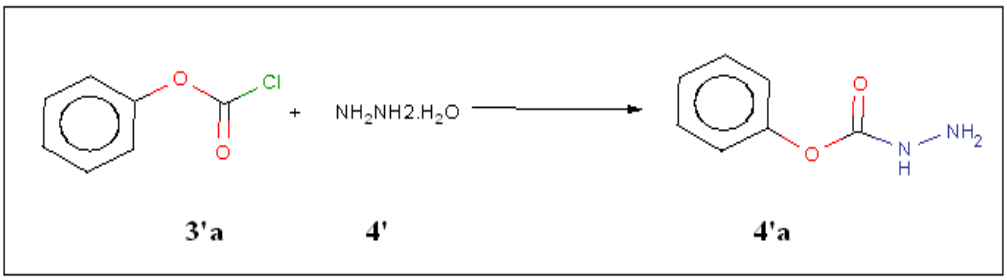


Figure 3.11: Synthetic scheme for substituted phenoxyformohydrazide.

Procedure

The solution of phenyl chloroformate (2.4 g, 2.3 ml, 0.02 moles), hydrazine hydrate (3.32 g, 0.02 moles), in ethanol (20ml) was added. The mixture was refluxed on a sand bath with vigorous stirring for 5 hour. The reaction was monitored by TLC**.** The remainder of the workup is similar to that explained in the general procedure.

Yield : 96.87%

Melting point : 100-105^{o}C

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

R_f = 0.34

(ii). Synthesis of 2-(2,4-dichlorophenoxy)acetohydrazide.

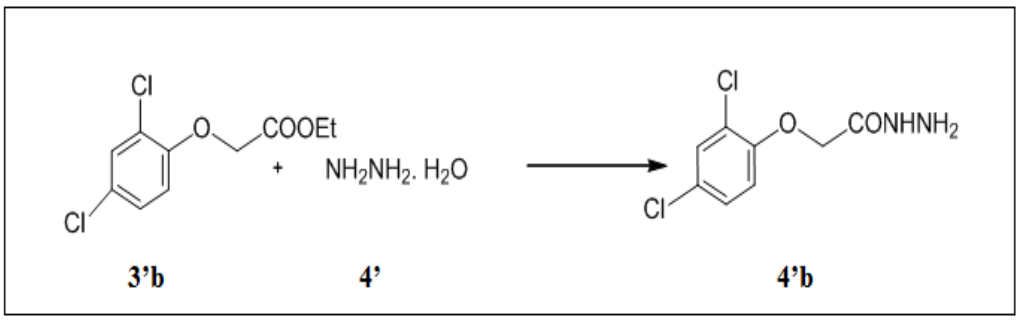


Figure 3.12: Synthetic scheme 2-(2,4-dichlorophenoxy)acetohydrazide

Procedure

The solution of 2-(2,4-dichlorophenoxy)acetate(**3'b)** (2.4 gms, 2.3 ml, 0.02 moles), hydrazine hydrate (**4')** (3.32 gms, 0.02 moles), in ethanol (20ml) was added. The mixture was refluxed on a sand bath with vigorous stirring for 5 hour. The reaction was monitored by TLC. The remainder of the workup is similar to that explained in the general procedure.

Yield : 77.75%

Melting point : 150-155^{o}C

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

R_f = 0.59

IR **(Spectrum 5):** 3315.48, 3259.87, 3226.55, 3012.11, 1683.60, 1647.12, 1558.37, 1520.75, 1497.24, 1456.97, 1387.27, 1245.18, 1138.64, 1046.29, 791.81 cm^{-1}

(iii). Synthesis of 2-(4-nitrophenoxy)acetohydrazide.

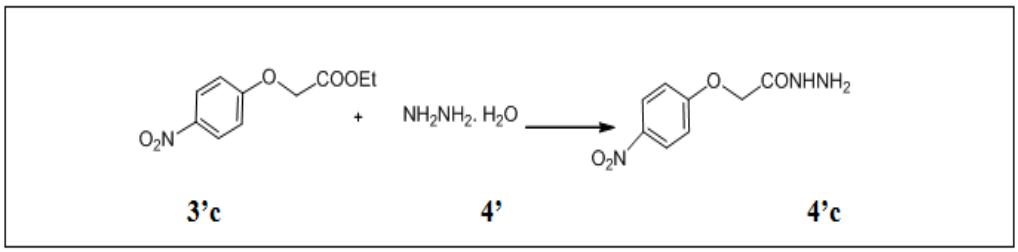


Figure 3.13: Synthetic scheme of 2-(4-nitrophenoxy)acetohydrazide

Procedure

The solution of ethyl 2-(4-nitrophenoxy)acetate (**3′c)** (2.4 gms, 2.3 ml, 0.02 moles), hydrazine hydrate (**4')** (3.32 gms, 0.02 moles), in ethanol (20ml) was added. The mixture was refluxed on a sand bath with vigorous stirring for 5 hour. The reaction was monitored by TLC. The remainder of the workup is similar to that explained in the general procedure.

Yield in % : 95.58%

Melting point : 180-185^{o}C

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

R_f = 0.47

IR **(Spectrum 6):** 3290.42, 3117.25, 3011.92, 2946.03, 2868.98, 1653.19, 1540.35, 1490.22, 1418.99, 1334.62, 1267.14, 1112.39, 1001.04, 825.07, 749.67, 699.30 cm^{-1}**.**

Synthesis of 1, 3-benzothiazol-2-amine.

General Reaction

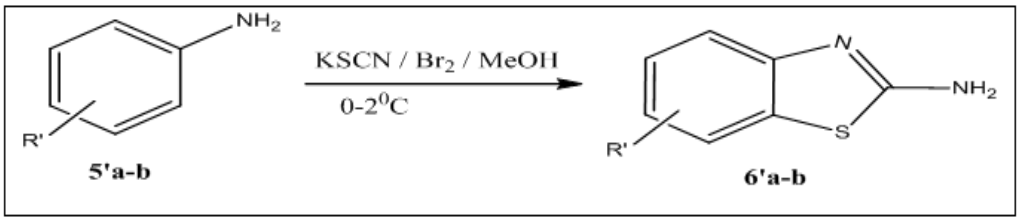


Figure 3.14: Synthetic scheme for Synthesis of 1, 3-benzothiazol-2-amine

General Method[78]

An equimolar concentration of potassium thiocyanate and substituted aniline was dissolved into in methanol (50-75 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.

(iv). Synthesis of 1, 3-benzothiazol-2-amine.(6'a)

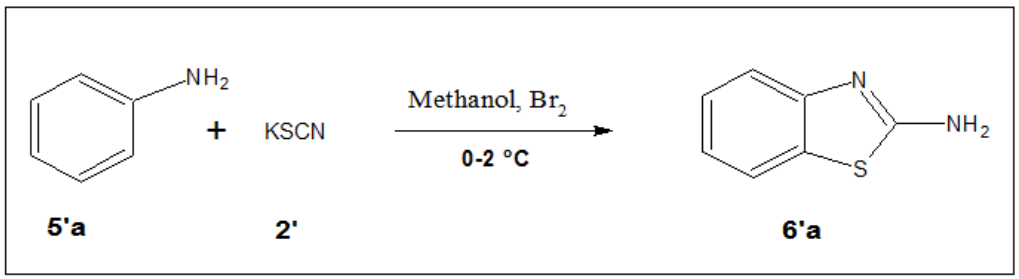


Figure 3.15: Synthetic scheme for Synthesis of 1, 3-benzothiazol-2-amine

Procedure

To a stirred suspension of potassium thiocyanate (25.16 g, 0.257mol) and aniline (19.6 ml, 0.214 mol) in methanol (75 ml) was slowly added bromine (11.41 ml, 0.214 mol) 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 : 85.12%

Melting point : >250°C

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

R_f = 0.714

IR **(Spectrum 7)** : 3368, 3319, 3033, 2996, 1558, 1521, 1497, 1457, 1386, 1187, 1078, 855 Cm^{-1}

(v). Synthesis of 6-chloro-1, 3-benzothiazol-2-amine. (6'b)

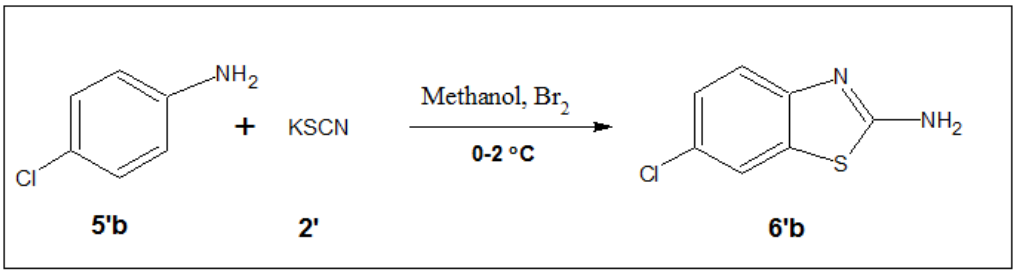


Figure 3.16: Synthetic scheme for Synthesis of 6-chloro-1, 3-benzothiazol-2-amine

Procedure

To a stirred suspension of potassium thiocyanate (18.38g, 0.187 mol) and p-chloro aniline (20 gm, 0.156 mol) in methanol (75 ml) was slowly added bromine (8.3ml, 0.156 mol) 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 : 66.50%

Melting point : >250°C

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

R_f = 0.695

IR **(Spectrum 8)** : 3420, 3291, 3259, 3020, 2990, 1683, 1558, 1404, 1207, 1015, 756 Cm^{-1}

General synthetic scheme for aminobenzenethiols. (7'a-c)

General reaction

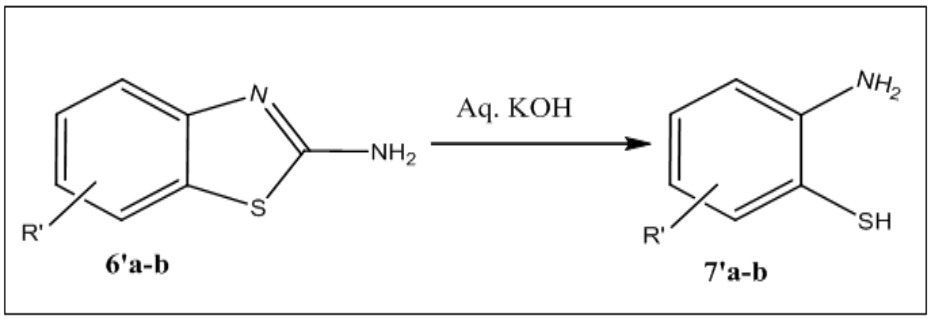


Figure 3.17: General synthetic scheme for intermediate (7'a-b)

General method[78]

A suspension of substituted 2-amino benzentiol 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.

(vi). Synthesis of 2-aminobenzenethiol. (7'a)

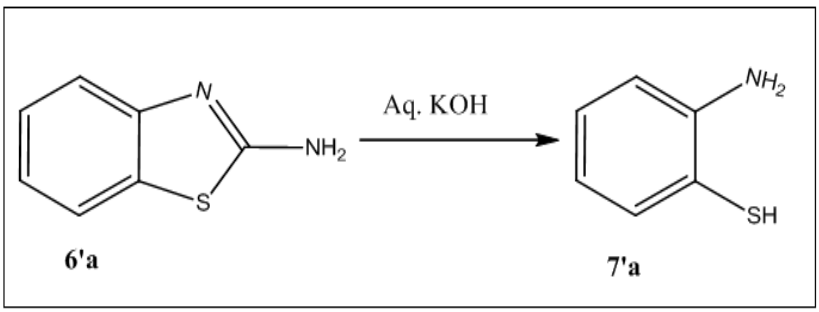


Figure 3.18: Synthetic scheme for Synthesis of 2-aminobenzenethiol

Procedure

A suspension of 6"a (3.00 g, 0.020 mol) and potassium hydroxide (15.00 g, 0.267 mol) 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 : 86.24%

Melting point : >250°C

TLC : Silica gel G; Methanol (100%)

R_f = 0.738

IR **(Spectrum 9)** : 3227, 3184, 3055, 2493, 1558, 1507, 1418, 1374, 1097 Cm^{-1}

(vii). Synthesis of 2-amino-5-chlorobenzenethiol. (7'b)

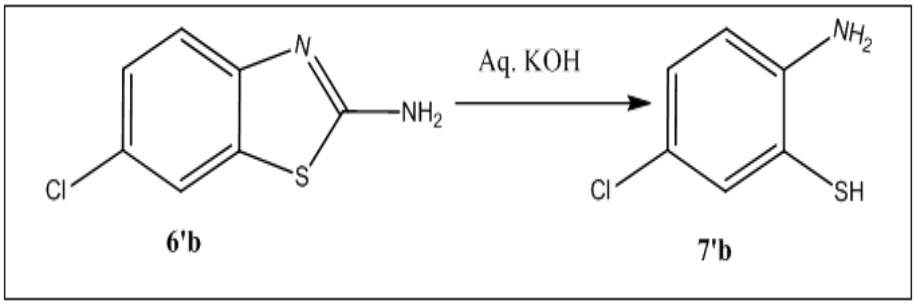


Figure 3.19: Synthesis of 2-amino-5-chlorobenzenethiol

Procedure

A suspension of 6'b (3.00 g, 0.0162mol) and potassium hydroxide (15.00 g, 0.267 mol) 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 : 89.72%

Melting point : >250°C

TLC : Silica gel G; Methanol (100%)

R_f = 0.715

IR **(Spectrum 10)** : 3272, 3251, 3006, 2950, 2520, 1558, 1533, 1464, 1318, 1243, 1174, 879 Cm^{-1}

SYNTHESIS OF ACRIDINE DERIVATIVES

I). Synthesis 0f substituted -1,3,4-oxadiazole acridine derivatives.(8b-c)

General reaction.[87]

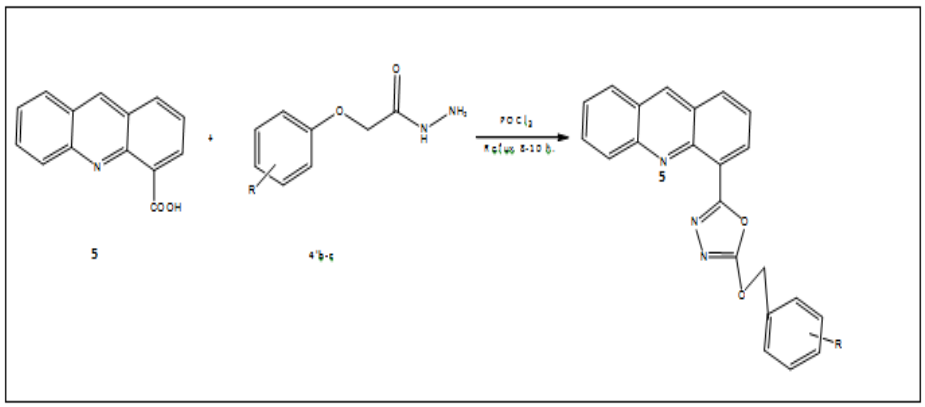


Figure 3.20: General synthetic scheme for 2,5-disubstituted -1,3,4-oxadiazole

General method[87]

A mixture of equimolar amounts of the substituted 2-phenoxyacetohydrazide and substituted acridine 4-carboxylic acid were, contained in a round bottom flask and suspended in 5 ml phosphoryl trichloride. The mixture was refluxed on a sand bath with vigorous stirring. The reaction was monitored by TLC. The reaction was continued till the substituted 2-phenoxyacetohydrazide was consumed completely.

Workup

The reaction mixture, when cooled, was slowly quenched into crushed ice and neutralizes it with solid sodium bicarbonate. The solid was filtered under vacuum and washed with cold water to remove solid sodium bicarbonate. The filtered thus obtained was dried.

1. Synthesis of 4-(5-phenoxy-1,3,4-oxadiazol-2-yl) acridine (8a).

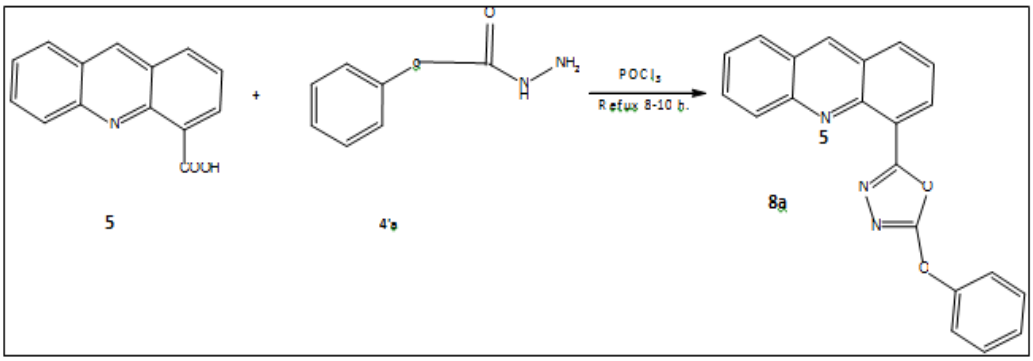


Figure 3.21: Synthetic scheme for Synthesis of 4-(5-phenoxy-1,3,4-oxadiazol-2-yl) acridine

Procedure

0.5 g of (2.29 mmol) phenoxy formohydrazide (**4′a**) and acidine 4- carboxylic acid (0.4717g, 2.11mmol) was dissolved into 2 ml $POCl_3$. Reaction mixture was refluxed for 12 hr and monitored by TLC. The remainder of the workup is similar to that explained in the general procedure.

TLC : Silica gel G; Ethyl acetate: Hexane (1:0) or 100% Ethyl acetate

Yield : 1 g (98%)

Melting point **:** > 250 ^{o}C

IR **(Spectrum 11)** : 3109, 3069, 3032, 1651, 1600, 1538, 1488, 1270, 1219, 1140, 886, 846, 772 cm^{-1}

^{1}H NMR **(Spectrum 12): δ** 6.95-7.99 (m, 13H, Ar-H)

Mass **(Spectrum 13) :** 240 (M+1)

2. Synthesis of 4-[5-(2,4-dichlorophenoxy)-1,3,4-oxadiazol-2-yl]acridine. (8b).

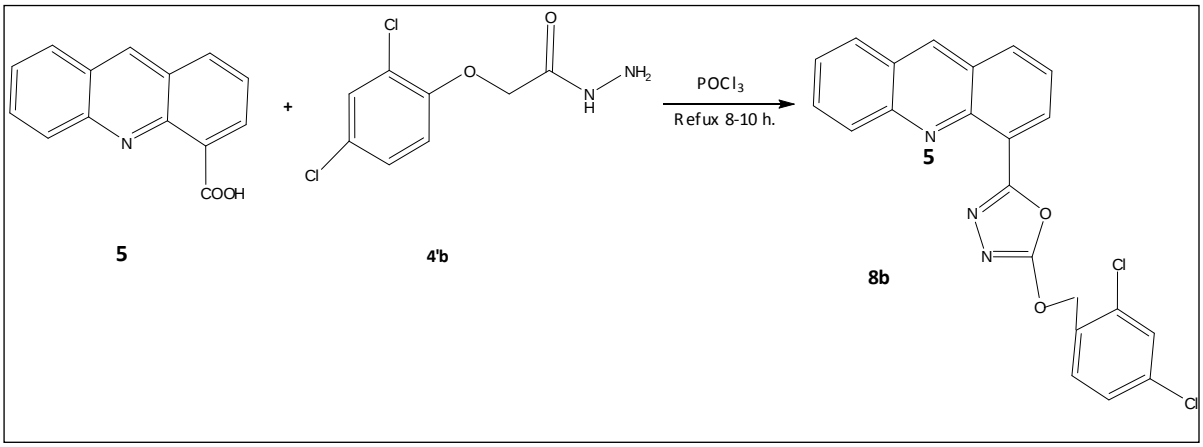


Figure 3.22: Synthetic scheme for Synthesis of 4-[5-(2,4-dichlorophenoxy)-1,3,4-oxadiazol-2-yl]acridine

Procedure

0.5 g of (2.21mmol) 2-(2,4-dichlorophenoxy)ethoxy]hydrazine (**4′b**) and acidine 4-carboxylic acid (0.4717g, 2.11 mmol) was dissolved into 2 ml $POCl_3$. Reaction mixture was refluxed for 8 hr and monitored by TLC. The remainder of the workup is similar to that explained in the general procedure.

TLC : Silica gel G; 100% Ethyl acetate

Yield : 0.78 g (91%)

Melting point **:** > 250 ^{o}C

IR **(Spectrum 14)** **:** 3054, 2996, 2926, 1575, 1532, 1476, 1392, 1231, 1092, 997, 773cm^{-1}

3. Synthesis of 4-[5-(4-nitrophenoxy)-1,3,4-oxadiazol-2-yl]acridine (8c).

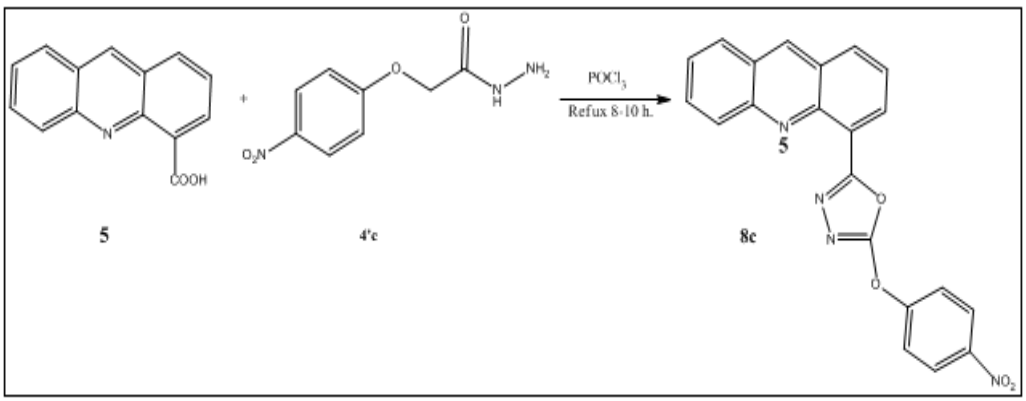


Figure 3.23: Synthetic scheme for Synthesis of 2-(4-nitrophenoxy)ethoxy]hydrazines

Procedure

1 g (3.69mmol) of 2-(4-nitrophenoxy)ethoxy]hydrazine (**4′c**) and acidine-4-carboxylic acid (0.823g, 3.69mmol) was dissolved into 2 ml $POCl_3$. Reaction mixture was refluxed for 10 hr and monitored by TLC. The remainder of the workup is similar to that explained in the general procedure.

TLC : Silica gel G; 100% Ethyl acetate

Yield : 1.5 g 93%

Melting point **:** > 250 ^{o}C

IR **(Spectrum 15)** : 3102, 3055, 1699, 1684, 1558, 1533, 1497, 1339, 1225, 1097, 983, 912, 751, 565 cm^{-1}

II. Synthesis of 5-(acridin-4-yl)-2,5-dihydro-1,3,4-oxadiazole-2-thiol.

General reaction.

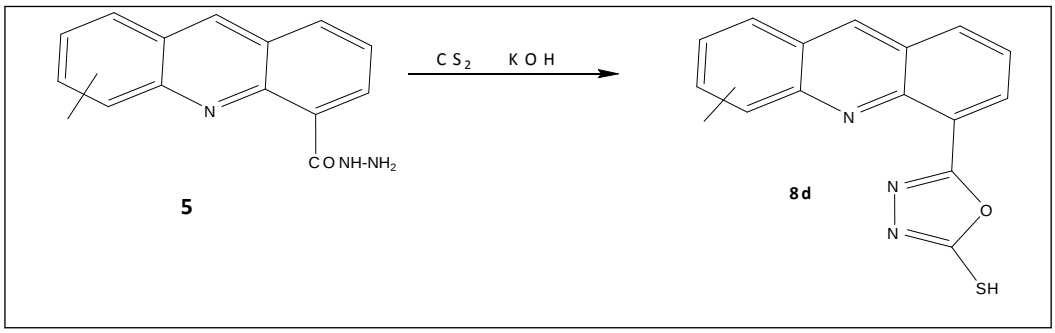


Figure 3.24: Synthetic scheme for Synthesis of 5-aryl-1,3,4-oxadiazole-2-thiol.

General method[88]

The hydrazide (**5**) obtained in the previous step was suspended in 30 ml ethanol. KOH pellets and CS_2 (Molar ratio of hydrazide: KOH: CS_2 – 1:1:2) were added. The mixture was refluxed for 8-10 hrs till the evolution of hydrogen sulfide gas ceases.

Workup

After the completion of reaction**,** the reaction mixture was cooled at room temperature and diluted with water (30ml). On acidification with dilute hydrochloric acid (10%) (15-18ml), the required oxadiazole (**8d**) was precipitated. It was filtered, thoroughly washed with cold water and recrystallised by using absolute ethanol.

4. Synthesis of 5-(acridin-4-yl)-2,5-dihydro-1,3,4-oxadiazole-2-thiol. (8d)

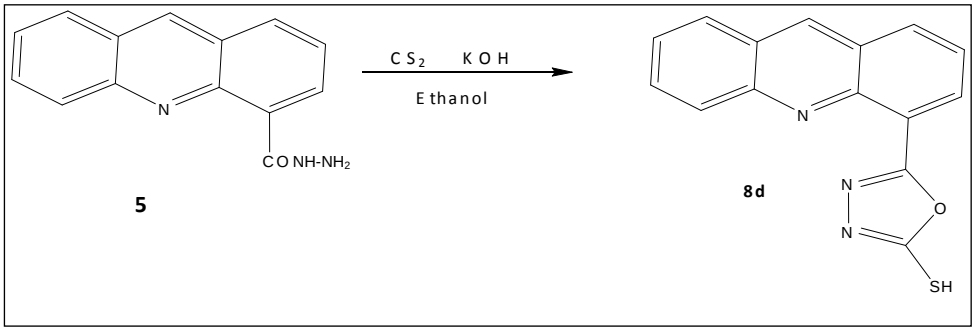


Figure 3.25: Synthetic scheme for Synthesis of 5-(acridin-4-yl)-2,5-dihydro-1,3,4-oxadiazole-2-thiol.

Procedure

acridine-4-carbohydrazide 0.5 g (2.24mmol) (**5**) obtained in the previous step was suspended in 30 ml ethanol. after mixining reactin mixture KOH pellets and 3ml CS_2 (Molar ratio of hydrazide: KOH: CS_2 – 1:1:2) were added. The mixture was refluxed for 10 hrs till the evolution of hydrogen sulfide gas ceases. The remainder of the workup is similar to that explained in the general procedure.

TLC : Silica gel G; Ethyl acetate : Hexane(1:0) or 100% Ethyl acetate

Yield : 0 .68 g (90%)

Melting point : > 250 ^{o}C

IR **(Spectrum 16)** : 3221.57, 2995.74, 2552.43, 1690.54, 1644.47, 1510.48, 1429, 1352, 1183,1156, 1018.35, 1002.39, 775.46 cm^{-1}

III. Synthesis of substituted 1,3-benzothiazol-2-yl acridine derivatives. (8e-f)

General reaction

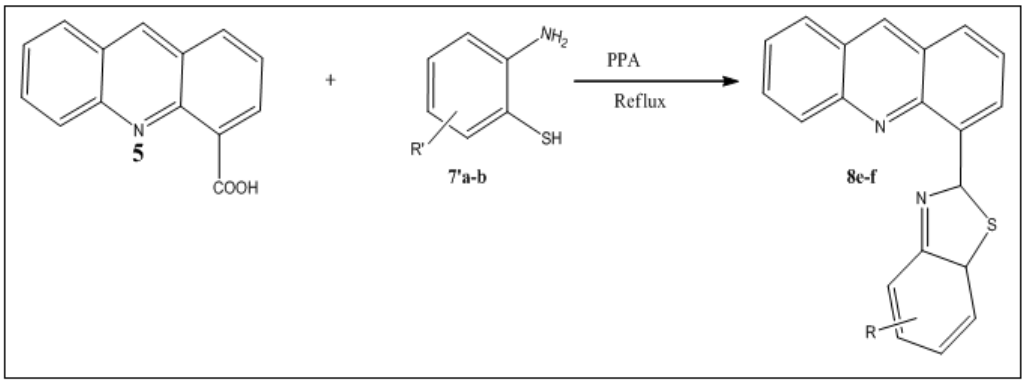


Figure 3.26: General synthetic scheme for 1,3-benzothiazol-2-yl acridine derivatives.

General method.[85]

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

Workup

The precipitate was collected and washed with water (50 ml). Then dried in the oven. The product was purified by column chromatography.

5. Synthesis of 4-(2,3-dihydro-1,3-benzothiazol-2-yl)acridine. (8e)

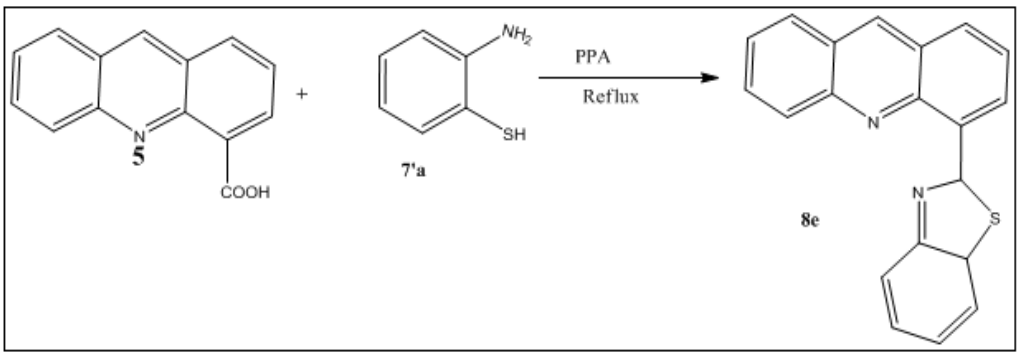


Figure 3.27: Synthetic scheme for Synthesis of 4-(2,3-dihydro-1,3-benzothiazol-2-yl)acridine

Procedure

2-aminobenzene-1-thiol (0.5 gm, 0.008 mole) was dissolved in polyphosphoric acid (20 ml) at 110 °C. acidine-4-carboxylic acid (1 gm,.008 mol) 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.

TLC : Silica gel G; Ethyl acetate: Hexane (9:1)

$R_f = 0.69$

Yield : 1.2 g (92 %)

Melting point : > 250 °C

IR **(Spectrum 17)** : 3077, 3032, 2943, 1682, 1622, 1557, 1494, 1362, 1270, 1197, 975, 913, 847, 772 Cm^{-1}

6. Synthesis of 4- (6-chloro-2,3-dihydro-1,3-benzothiazol-2-yl)acridine. (8f)

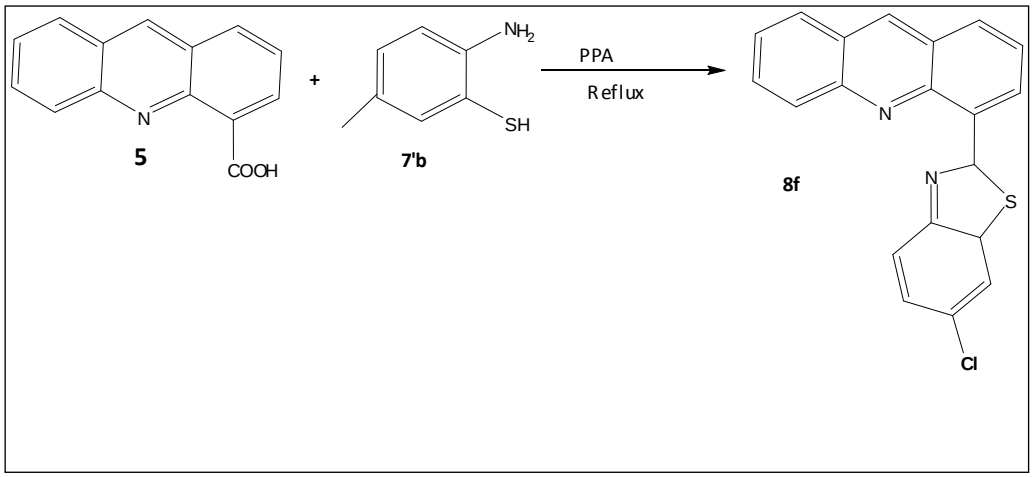


Figure 3.28: Synthetic scheme for Synthesis of 4- (6-chloro-2,3-dihydro-1,3-benzothiazol-2-yl)acridine

Procedure

0.5 g (3.144 mmol) of 2-amino-5-chlorobenzenethiol was taken & add in a hundred ml round bottom flask containing 16 ml PPA & 0.697 g or .004 mol of acidine 4- carboxylic acid dissolved previously. Reflux for 30-60 minute. Monitored the TLC in whole process. After cooling the reaction mixture, neutralized the reaction mixture using aques ammonia solution (35 ml). At the end of neutralization precipitate was formed. Filtered the precipitate , dry and weighed the product.

TLC : Silica gel G; Ethyl acetate: Hexane (9:1) or

90% Ethyl acetate 10 % hexane

Yield : 0.856 g (96 %)

Melting point : > 250 ^{o} C

IR **(Spectrum 18)**: 3416**,** 1683, 1635, 1598, 1520, 1488, 1361, 1201, 1073, 814, 771, 720 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 Inoculums

Procedure for the preparation of inoculums for both the strains was same. The inoculums 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, inoculums solution to medium**

From diluted inoculums 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 inoculums. The tests were carried out in duplicate. Apart from putting the controls of standard drug (Streptomycin), controls with dimethyl sulphoxide (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 DISCUSSION

Design of acridine 4-heteroaryl derivatives

A set of acridine 4-heteroaryl derivatives was designed on the basis of detailed studies on the biological activities of acridine and other various heterocyclic ring systems**.** For the synthesis of the designed molecules, a synthetic scheme for synthesis of various intermediates and final molecules was design.

1. Synthesis of acridine 4-heteroaryl derivatives

All molecules of this of acridine 4-heteroaryl derivatives were synthesized using the common starting acridine 4-carboxylic acid. In all compounds, intermediates were first formed by ring-opening of isatin using anthranilic acid to get intermediate compound (**3**). It was subjected for cyclization and followed by selective decarboxylation to get acridine 4-carboxylic acid (5). For the synthesis of acridine-4-carboxylic acid, this was directly coupled with acidhydrazide (3'a-c) or 2-aminothiol (7'a-b) to get compound (8a-f). The general scheme utilized for the synthesis of acridine 4-heteroaryl derivatives is outlined below in **Figure 5.1.**

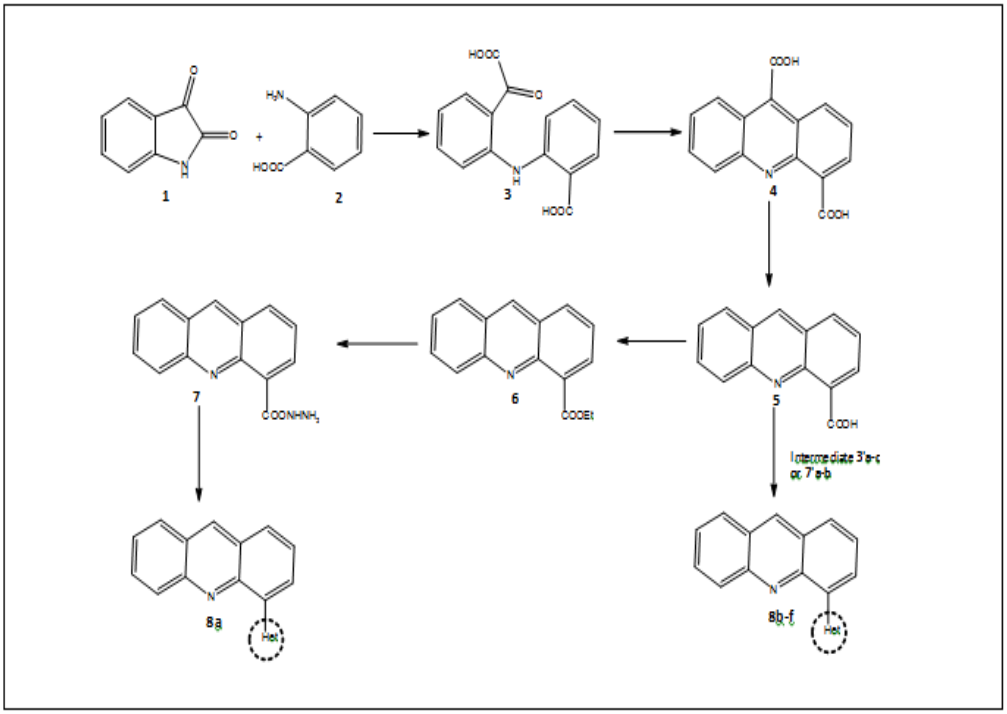


Figure 5.1: General synthetic scheme for synthesis of acridine 4-heteroaryl derivatives

All the compounds synthesized were characterized by IR spectroscopy and evaluated for their biological activity using standard testing procedures.

I. a) Synthesis of substituted ethyl 2-phenoxyacetate (3′a-c)

Ethyl 2-phenoxyacetate was synthesized using substituted phenol and ethyl chloroacetate as starting material in the presence of K_2CO_3 and acetone under reflux condition. The synthetic scheme is given in **Figure 5.2**.

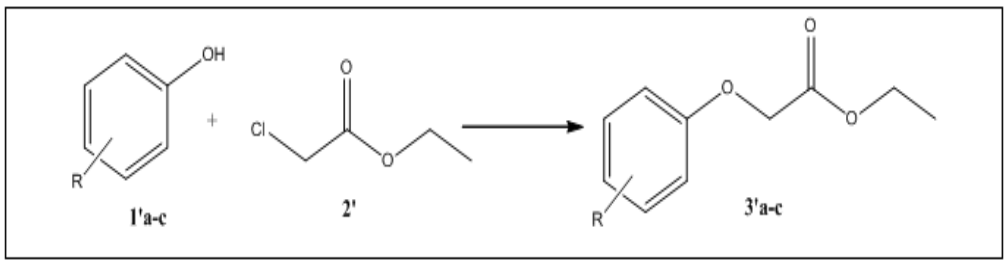


Figure 5.2: General synthetic scheme for synthesis of substituted ethyl 2-phenoxyacetate

DISCUSSION

For the synthesis of the substituted ethyl 2-phenoxyacetate has, reported procedure was used which worked properly and yield the compound. Therefore, modification of the process was not required and there was no possibility for side product.

Mechanism of reaction substituted ethyl-2-phenoxyacetate synthesis:

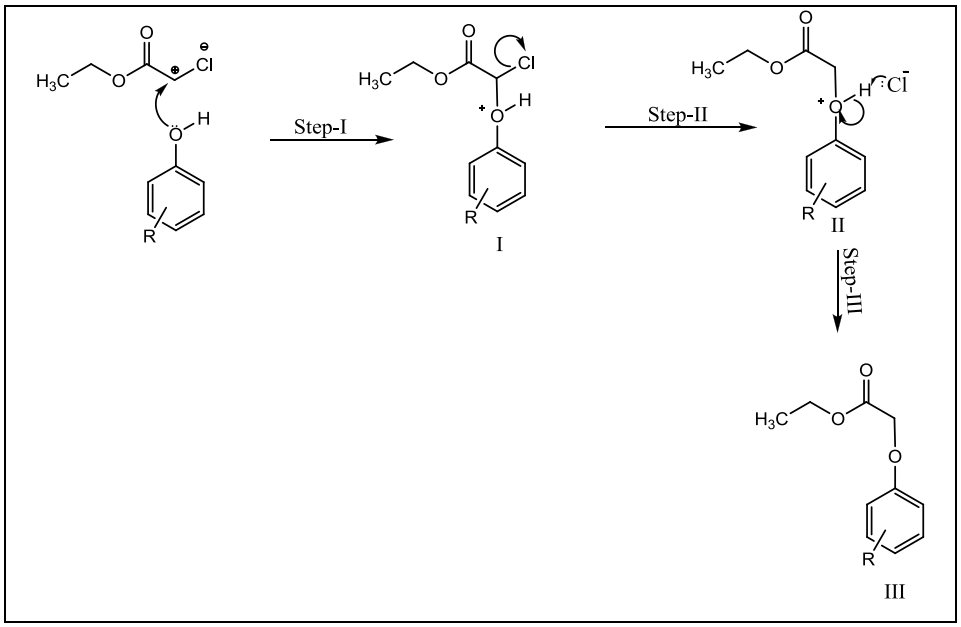


Figure 5.3: Mechanism of substituted ethyl 2-phenoxyacetate synthesis

Step-I: In this stage, involves the nucleophilic attack on the positive carbon atom of ethylchloroacetate by lone pair of electrons on the oxygen of substituted phenol molecule and form the compound-I.

Step-II: In this stage, chloride ion is pushed off with pair of electrons and the carbon-oxygen bond forms.

Step-III: In the third stage, removal of a hydrogen ion by the chloride ion occurs and yields the ester and hydrogen chloride.

Characterization of Intermediate

IR: The spectrum showed characteristic ester band in the range 1749-1720cm^{-1} and C-Cl stretching peak at in the range of 750-780cm^{-1}. The –OH stretching peak corresponding to the phenol in the range of 3450-3400cm^{-1} was disappeared in the spectrum thus it confirming the formation of the desired intermediate. Also, the values are in agreement with those reported ethyl 2-phenoxyacetate[86]

b). Synthesis of substituted 2-phenoxy acetohydrazide (4′a-c)

The intermediate of substituted 2-phenoxy acetohydrazide (**4′a-c**) was synthesized by using hydrazine hydrate (4) and substituted ethyl 2-phenoxyacetate in the presence of ethanol under reflux condition (**Figure 5.4**). The monosubstituted intermediates were used for further reaction to synthesized 1,3,4-oxadiazoles.

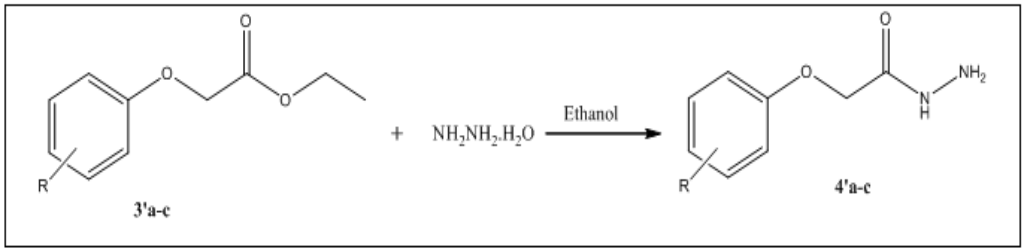


Figure 5.4: General synthetic scheme for substituted 2-phenoxy acetohydrazide

DISCUSSION

The reported procedure was used for the synthesis of substituted 2-phenoxy acetohydrazide. There was not any change in the procedure for the synthesis of substituted 2-phenoxy acetohydrazide derivative.

Mechanism of reaction

Mechanism of reaction for the acid hydrazide synthesis, first nucleophile will attack on carbonyl carbon followed by proton transfer and further it will loss one molecule of ethanol and convert into acid hydrazide. The mechanism of acid hydrazide synthesis reaction is given in **Figure 5.5.**

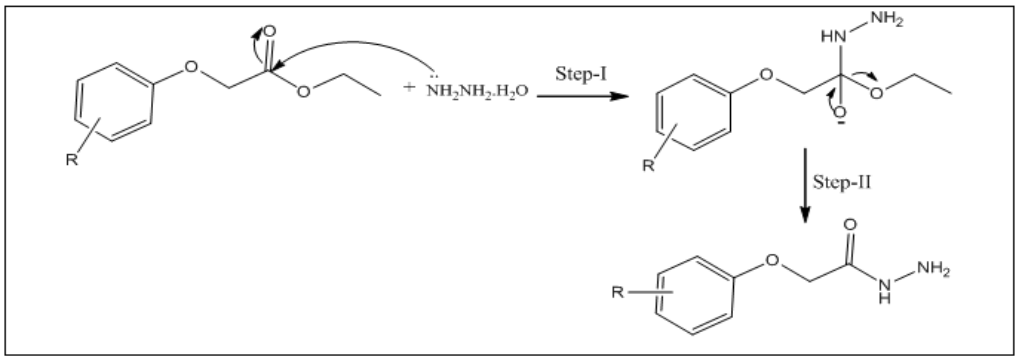


Figure 5.5: Mechanism of substituted ethyl 2-phenoxyacetate synthesis

Possible side- products for this reaction

The side-products that could be from in course of the reaction is A, which is given in Figure 5.6

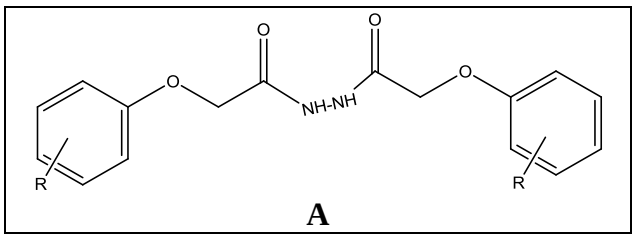


Figure 5.6: Possible side product for synthesis of substituted 2-phenoxy acetohydrazide

Characterization of Intermediate

The spectrum showed characteristic amide band in the range of 1640-1680cm^{-1} and the –ester (COOR) stretching peak in the range of 1749-1720cm^{-1} was disappeared thus confirming formation of the desired intermediate. Also, the values are in agreement with those reported 2-phenoxyacetohydrazide[76]

c). Synthesis of 2-aminobenzothiazole Derivative (6′a-b)

2-Aminobenzothiazoles were synthesized using substituted aniline and potassium thiocyanate as starting material and bromine was used as cyclizing agent at chilled condition (**Figure 5.7**). The solvent used for this reaction was the methanol.

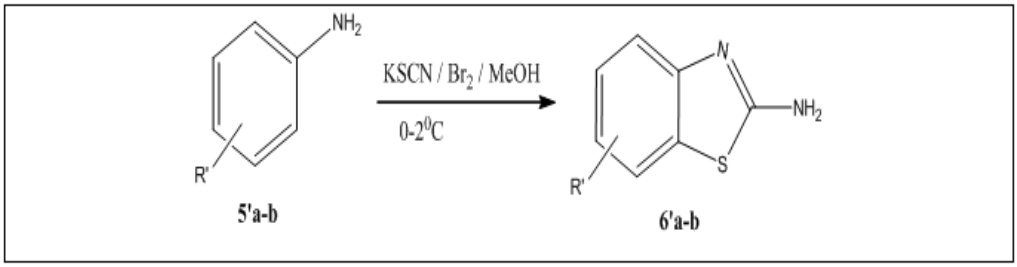


Figure 5.7: General synthetic scheme for 2-amino benzothiazole derivatives

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. Therefore, 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 4-6hr stirring and precipitated solid was collected by filtration and purified by washing with cold methanol.

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.

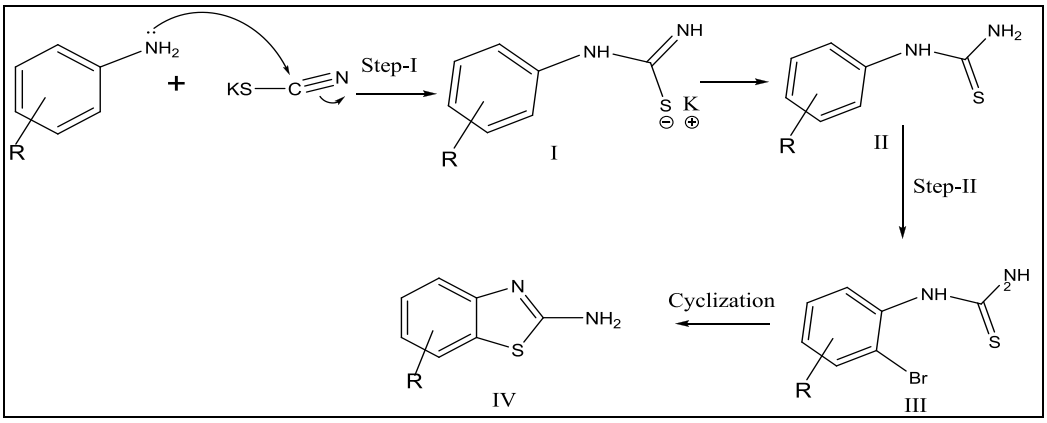


Figure 5.8: Mechanism of the reaction for synthesis of 2-aminobenzothiazole

***Possible side- products for this reaction*:**

The side-products that could from in course of the reaction are **A** and **B** (**Figure5.9**). The compound (II) could be side-product which is formed during first step of aminobenzthiazole synthesis if it is not convert in to compound (III). Another could be compound (III) if it is not cyclized into desired compound.

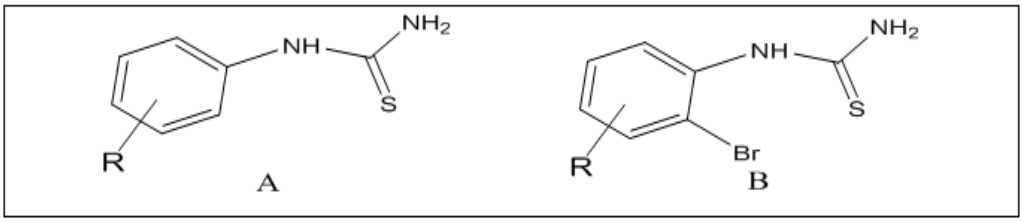


Figure 5.9: Possible side product for synthesis of 2-aminobenzothiazole

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.[86]

d). Synthesis of 2-aminothiol derivatives (7′a-b)

2-Aminothiol derivatives were synthesized by using a suspension of intermediate (**6′a-b**) and potassium hydroxide in water and refluxed it for 4-5 hrs (**Figure 5.10**). After cooling, con. hydrochloric acid was added. The reaction was shielded from light by aluminum foil.

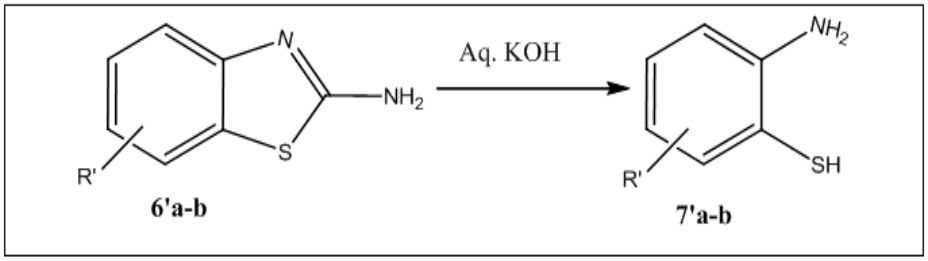


Figure 5.10: General synthetic scheme for 2-aminothiol derivatives

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 SH peak in the range of 2500-2600cm^{-1} The Melting point of the isolated molecules, matched with reported 2-aminothiol.[102]

II. a). Synthesis of 2,5-disubstituted -1,3,4-oxadiazole (8a-c)

2,5-disubstituted -1,3,4-oxadiazole (**8a-c**) were synthesized by using substituted acetohydrazide (5a-c) and acridine carboxylic acid in the presence of phosphoryl trichloride under reflux conditions.

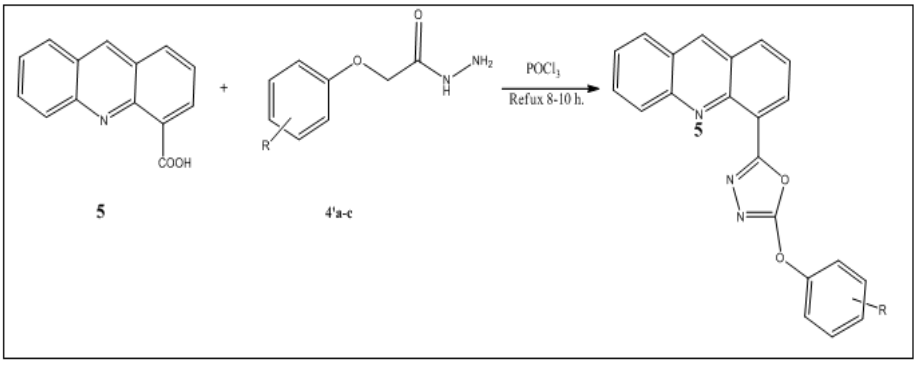


Figure 5.11: General synthetic scheme for 2,5-disubstituted -1,3,4-oxadiazole

DISCUSSION

For the synthesis of Synthesis of 2,5-disubstituted -1,3,4-oxadiazole as a final molecule, reported procedure was used and it work very well. Obtained crude product was purified using column chromatography.

Mechanism of 2,5-disubstituted -1,3,4-oxadiazole synthesis:

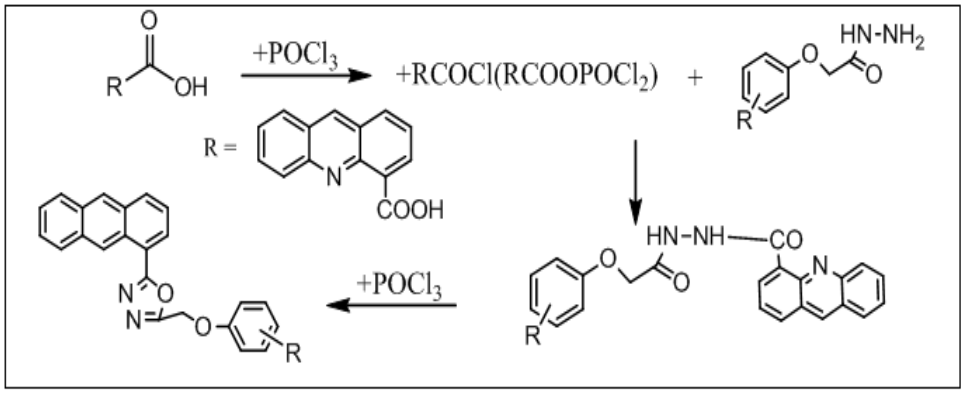
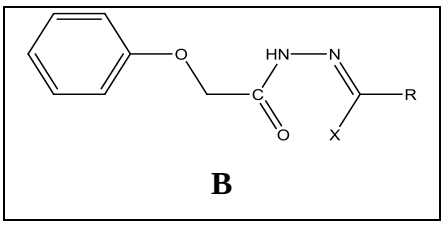


Figure 5.12: ***Mechanism of 2,5-disubstituted -1,3,4-oxadiazole synthesis.***

Possible side- products for this reaction

The side-products that could from in course of the reaction is **B** (**Figure 5.13**).



B

Figure 5.13: Possible side product for synthesis of substituted 2,5-disubstituted -1,3,4-oxadiazole

Characterization of 2,5-disubstituted -1,3,4-oxadiazole

IR: In the solid state, oxadiazole predominated the tautomeric thione (C=S). The characteristic SH peak was seen 2550-2590 cm^{-1}. The melting points of all the synthesized 2-mercapto- 1,3,4-oxadiazoles were found to be in agreement with reported values.

III). Synthesis of 1,3-benzothiazol-2-yl acridine derivatives

Acridine 4-Carboxylic acid derivatives (8e-f) were synthesized by dissolving Benzothiazole in polyphosphoric acid at 110 °C and added substituted 2-aminothiophenol (7a-c) and the resulting solution stirred at 110 °C for 1-2 hrs **Figure 5.14**

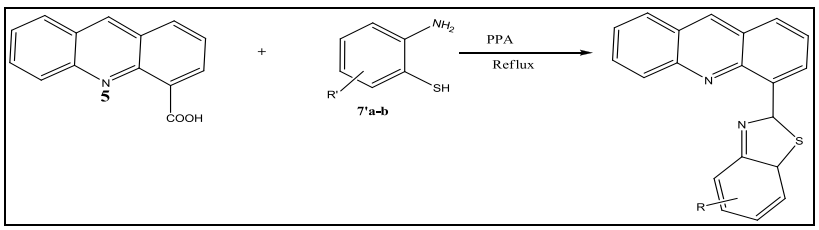


Figure 5.14: General synthetic scheme for final product

DISCUSSION

For the synthesis of 1,3-benzothiazol-2-yl acridine derivatives initially reaction was carried out using PPA at **220°C** as per Kim Serdons et al. procedure. The product as well as starting material was became dark-black brown color. 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.

Mechanism of the reaction

Mechanism of reaction for the synthesis of 1,3-benzothiazol-2-yl acridine derivatives from 2-aminothiol and substituted aryl acid using polyphosphoric acid is given in **figure 5.15**

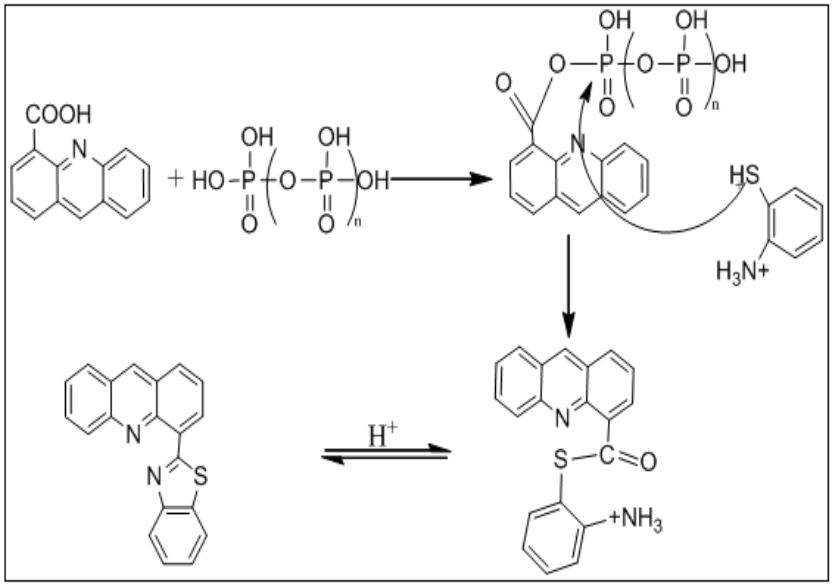


Figure 5.15: General synthetic scheme for final product

Synthesis of the 5-(acridin-4-yl)-2, 5-dihydro-1,3,4-oxadiazole-2-thiol

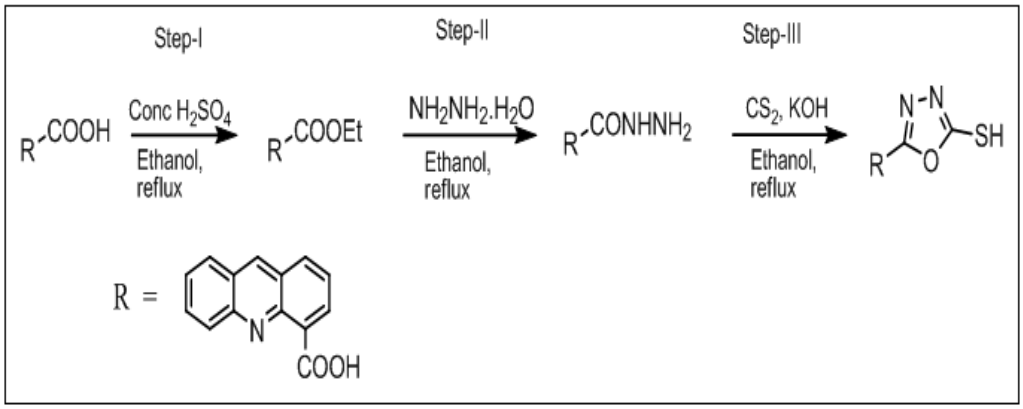


Figure 5.16: General synthetic scheme for 5-(acridin-4-yl)-2, 5-dihydro-1,3,4-oxadiazole-2-thiol.

Discussion

The esterification and the hydrazide formation reactions were clean and posed no problems with the substrates. In case of the final cyclization reaction, a trend was noted. Aromatic acids having electron withdrawing substituent's (-NO_2, Cl, Br) formed the oxadiazoles faster and with complete consumption of starting material. In case of para hydroxyl and ortho hydroxyl substitution, there was little or no formation of the oxadiazole.

Mechanism of 5-(acridin-4-yl)-2, 5-dihydro-1,3,4-oxadiazole-2-thiol synthesis

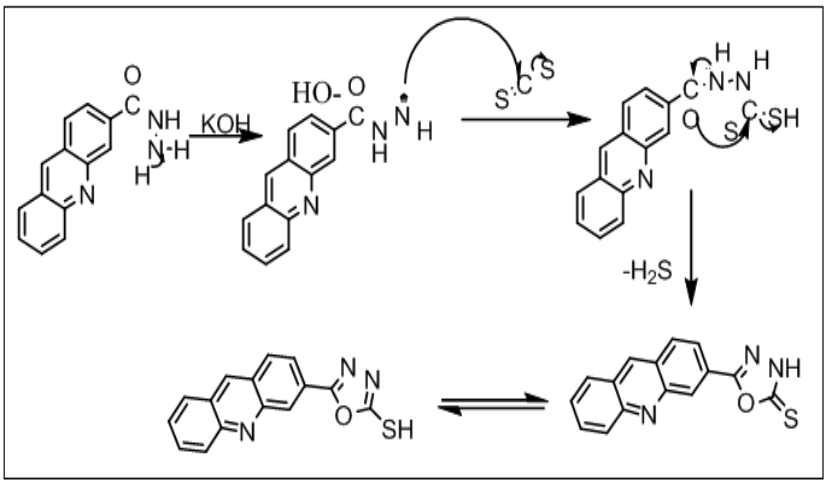


Figure 5.17: Mechanism of 5-(acridin-4-yl)-2, 5-dihydro-1,3,4-oxadiazole-2-thiol synthesis

Characterization of 5-(acridin-4-yl)-2, 5-dihydro-1,3,4-oxadiazole-2-thiol

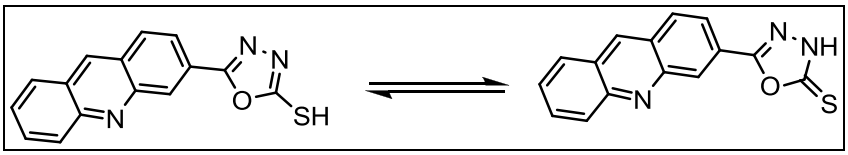


Figure 5.18: Tautomeric form of 5-(acridin-4-yl)-2, 5-dihydro-1,3,4-oxadiazole-2-thiol.

IR: In the solid state, the thiol (SH) form of the oxadiazole predominated tautomeric thione (C=S). The characteristic SH peak was seen 2550-2590 cm^{-1}. The melting points of all the synthesized 5-(acridin-4-yl)-2, 5-dihydro-1,3,4-oxadiazole-2-thiol were found to be in agreement with reported values.

Characterization of 1, 3-benzothiazol-2-yl acridine derivatives

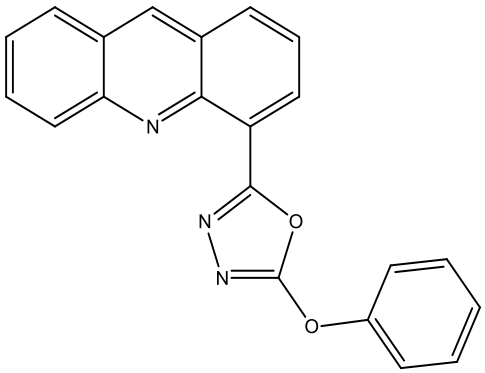
IR: The spectrum showed characteristic Peak as per presence of functional group. The Melting point of the isolated molecules, matched with reported acridine derivatives. [Ref]

One of the two molecules synthesized, was completely characterized using IR, ^{1}H NMR (300MHz), and Mass spectrometry.

IR: Aromatic CH stretch was seen at around 3009 cm^{-1} and alkyl -CH stretch was observed at 2895 cm^{-1}.

^{1}H NMR: The aromatic protons appeared as multiple singlets between δ 7.995-6.996. Representative example is **8a.**

The ^{1}H NMR values for compound **8a** is listed and compared with the predicted values in the table below (Chembio office Chemdraw Ultra 11.0) (**Table 5.1**). Representative example is



8a

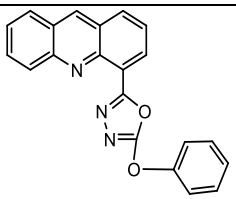
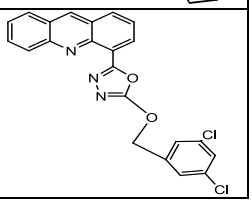
Figure 5.12: Structure of compound 2-(acridin-4-yl)-5-phenoxy-1,3,4-oxadiazole (8a).

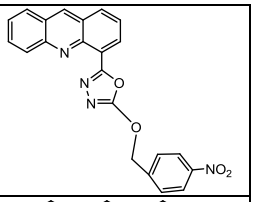
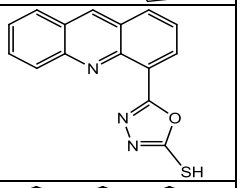
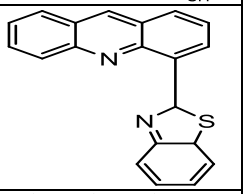
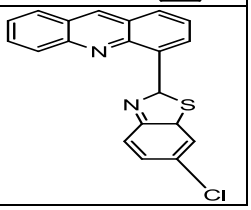
Table 5.1: Comparison of ^{1}H NMR values

Sr. No.	No of carbon	Predicted	Experimental (8a)
1	Ar-H	6.95-8.20	6.996-7.95

MS: **M+1** peak was seen at 340 for the compound **8a.**

List of synthesized molecules is given in **Table 5.2**

Sr. No.	Mol. ID.	Structure
1	**8a**	
2	**8b**	

3	**8c**	
4	**8d**	
5	**8e**	
6	**8f**	

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.	Compound	Visual Turbidity (E. Coli)	Visual Turbidity (S. Aureus)
1.	8a	+ ve	- ve
2.	8b	- ve	- ve
3.	8c	- ve	- ve
4.	8d	- ve	- ve
5.	8e	+ ve	- ve
6.	8f	+ ve	- ve
7.	Streptomycin	- ve	- ve

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 are tabulated in **Table 6.2.**

Table 6.2: Evaluation results of the single concentration.

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

Results of antibacterial testing

The compounds (8a, 8b, 8c, 8d, 8e) exhibited bacterial growth inhibition when tested at concentration. Other compounds exhibited activity at higher concentrations. Further testing for compounds (8a, 8b, 8e) at lower concentrations is required to compare their activity with standard Streptomycin at its MIC.

7. CONCLUSION

Thus it can be concluded that designed acridine derivatives were synthesized successfully and (**8a-f**) exhibited antibacterial activity at tested concentration less 1000µg/ml and some of compound **8a** exhibited activity at lower concentration <200 µg/ml. Thus, these acridine derivatives can therefore act as a lead for development of broad spectrum anti-infective agents.

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