SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF BRANCHED BENZOXAZINOPHENOTHIAZINES AND THEIR DERIVATIVES

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UNIVERSITY OF NIGERIA, NSUKKA

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TITLE PAGE

UNIVERSITY OF NIGERIA, NSUKKA
DEPARTMENT OF PURE AND INDUSTRIAL CHEMISTRY

RESEARCH PROJECT
SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF BRANCHED BENZOXAZINOPHENOTHIAZINES AND THEIR DERIVATIVES

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BY

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PROJECT SUPERVISOR: DR. B.E. EZEMA

AUGUST, 2014

APPROVAL PAGE

This work has been approved by the Department of Pure and Industrial Chemistry, University of Nigeria Nsukka.

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Project Supervisor	Head of Department
Date: _____	Date _____

_____

External Examiner

CERTIFICATION

This is to certify that the research work titled ñSynthesis and Antimicrobial Activity of Branched Benzoxazinophenothiazines and their Derivativesò was carried out by **IKE, OZOEMENA CHRISTIAN** (PG/M.Sc/12/62074) and has been approved by the undersigned of the Department of Pure and Industrial Chemistry University of Nigeria Nsukka, submitted in partial fulfillment for the requirement of the award of M.Sc in Organic Chemistry.

_____	_____
Dr. B.E. EZEMA	Dr. A.E. OCHONOGOR
Project Supervisor	Head of Department
Date: _____	Date _____

DEDICATION

This work is dedicated to my loving parents Mr. Sunday Ike and Mrs. Chibuzo Ike for their encouragement, support both morally and financially towards my educational achievement.

ACKNOWLEDGMENT

I am exceeding grateful to God Almighty for making it possible for me to achieve this educational height in academics. I acknowledge his loving grace that enabled me to pass through this programme successfully for his divine enablement is marvelous in my sight.

I am highly indebted to my supervisor Dr. B.E. Ezema for his immeasurable support, encouragement, patience and fatherly advice throughout the period of the research work.

I appreciate in a very special way these colleagues of mine: Adekola Ojo Emma, Bright, Nneka, Oluchi, Peter Oz, Priscilla . Thanks for the cooperation and time we spent together.

I remain ever thankful to my sisters and brothers, Ebere, Nkem, Ekwutosi, Emmanuel for their non-stop encouragement, unflinching support both morally, materially and being there for me.

Ike, Ozoemena Christian

ABSTRACT

The syntheses of benzoxazinophenothiazine was thoroughly explored. Condensation of 2,3-dichloro-1,4-naphthoquinone with 2-amino-4-nitrophenol, 4,5-diamino-6-hydroxypyrimidin-2-thiol and 2-amino-6-chlorophenol, respectively in alkaline medium yielded the intermediates: 6-chloro-10-nitrobenzo[a]phenoxazin-5-one(136), 11-amino-6-chloro-9-thiol-8,9-diazabenzo[a]phenoxazin-5-one (148) and 6,8-dichlorobenzo[a]phenoxazin-5-one (160). Further treatment of the intermediates with 2-aminothiophenol and 3-amino-6-methoxypyridin-2-thiol gave the complex derivatives: 14-nitrobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (137), 15-amino-13-thiol-12,14-diazabenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (149) and 12-chloro-9-methoxy-8-azabenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (161). The compounds were characterized by UV/Visible, FT-IR, (^{1}H and ^{13}C-NMR) spectroscopy and elemental analysis. The synthesized compounds were screened against eight (8) micro-organisms viz: *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus,Pseudomonas aeruginosa, Escherichia coli, Kebsiella pneumonia, Asperigellus niger and Candida albicans* using agar-cup diffusion method. The results showed that the complex derivatives were significantly active against the micro-organisms when compared to anti-bacterial and anti-fungal drugs.

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Asp.niger : Asperigellus niger

B.Cereus : Bacillus cereus

B.subtilis : Bacillus subtilis

C.albicans : Candida albians

CNS : Central nervous system

DMF : N,N dimethyl formamide

DMSO : Dimethyl sulfoxide

E.coli : Echerichia coli

KBR : Pottassium bromide

K.pneumonia : Klebsiella pneumonia

MIC : Minimum inhibitory concentration

NMR : Nuclear magnetic Resonance

Nph : Phenylimine

P.areuginosa : Pseudomonia aeruginosa

S.aureus : Staphylococcus aureus

UV : Ultraviolet

Chemical Symbols

^{o}C : degree Celsius

g : gramme

mg/mL : Milligramme per milliliter

mL : Milliliter

mmole : millimole

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CHAPTER ONE

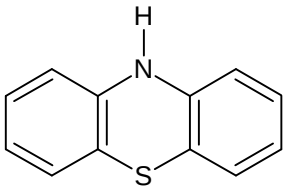
INTRODUCTION

1.1 BACKGROUND OF STUDY

The application of phenothiazine and phenoxazine compounds and their derivatives in medicine, textiles, agriculture and other related industries has been of interest to chemists for well over a century.

The chemistry of nitrogen-sulphur heteroatom-containing aromatic compounds is becoming more popular as an area of research[1]. Phenothiazine derivatives have been shown to possess a broad spectrum of pharmacological activity depending like antiparkinsonian[2-3], tranquilizer[4], anti-inflamatory[5-7], antimalarial[8-9], antipsychoti[10-12], antimicrobial [13-15], anti-tubercular [16-18], antitumor[19-20], antihistaminic[21-22], analgesic [23], prion disease drug[24] . In textile, paint and plastic industries, they are used as dyes and pigments[25-26] and in agricultural industry as insecticides[27]. In the petroleum industry, they are found useful as antioxidants in lubricants and fuels[28]. It has been observed that some phenothiazines inhibit intracellular replication of viruses including human immunodeficiency viruses (HIV) [29]. On the other hand, some have been reported to exhibit significant anticancer activity[30-31]

Similarly, phenoxazine derivatives are compounds of great interest because of their several successful applications as dyes and drugs. They exhibit strong biological activities ranging from antidepressant[32], antitutour[33], anticancer[34], antibacterial[35], antituberculosis[36] and schizophrenia agents[37]. Among other several industrial applications of phenoxazine derivatives is their use as acid-base indicator[38], biological stains[39], laser dyes[40] and chromophonic compounds[41].



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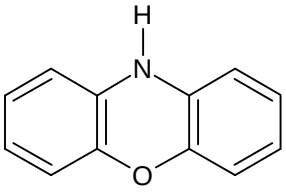

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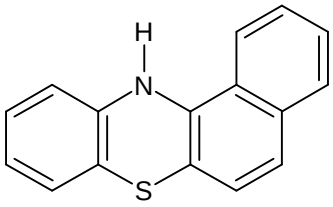
Owing to the wide range of applications of phenothiazines, intensive research has been in progress in the search for more derivatives with highly improved pharmacological and biological activities. Hence, several papers describing the synthesis of these derivatives had been reported especially on the angular derivatives including the non-aza and the congeneric aza-analogues. However, there exists limited literature on the complex derivatives of this phenothiazine ring system. Among the non-aza angular compounds that have been reported were benzo[a]phenothiazines of the type (3)[42] and benzo[c]phenoxazine of the type (4)[43] and benzo[a]phenothiazine and benzo[c]phenoxazine of types (5)[44] and (6),[45] respectively.



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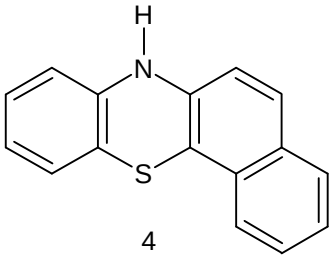

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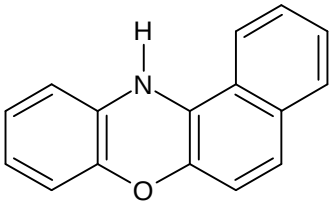

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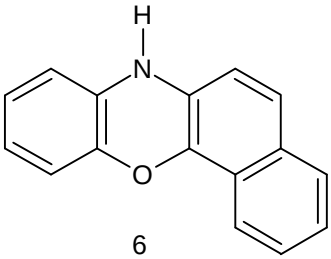

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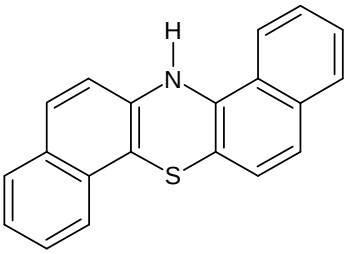
Such molecular modification had yielded other derivatives such as (7)[46], (8)[47]and (9)[48].



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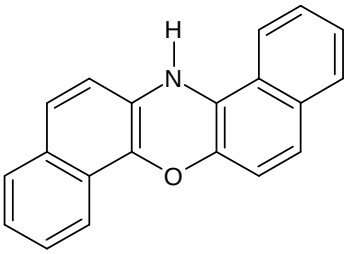

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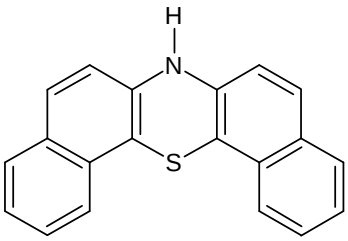

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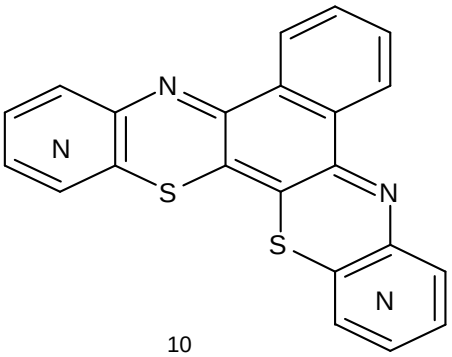
Okafor[49] also reported the synthesis of the first three branched diazaphenothiazine and diazaphenoxazine dyes of the type (10) and (11).



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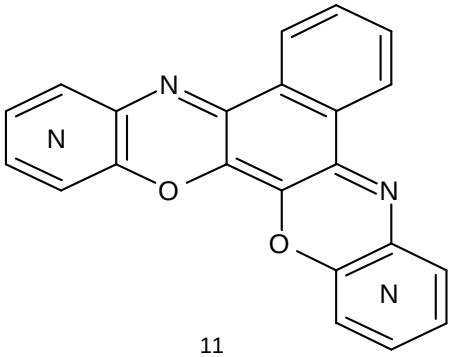

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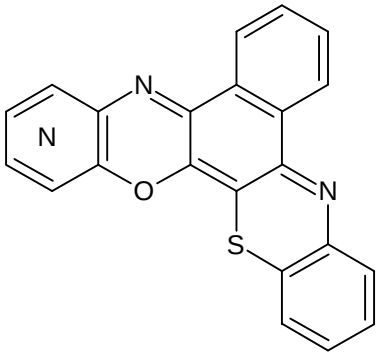
The first three-branched benzoxazinophenothiazine ring systems of monoaza, diaza and triaza analogues of the types (12), (13), and (14) were also reported by the same author[49].



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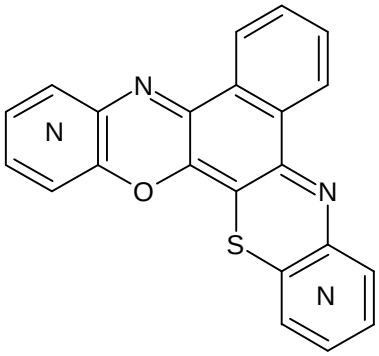

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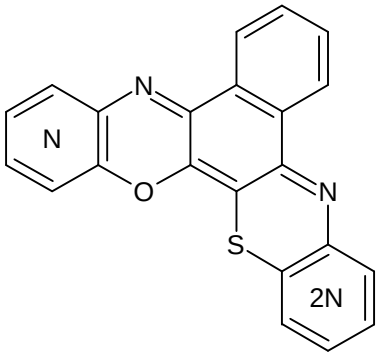

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1.2 STATEMENT OF THE PROBLEM

Owing to the wide range of applications of phenothiazine derivatives with highly improved pharmacological and biological activities, several papers describing the successful synthesis of these derivatives had been reported especially on the angular derivatives, including the non-aza and congeneric aza-analogues. However, there is still limited literature on the complex derivatives of this phenothiazine ring system and hence, modification of the existing ones is necessary. The past research done was based on their dye and pigment properties. Not much is known of anti-microbial properties of these complex phenothiazine derivatives.

1.3 OBJECTIVES OF THE STUDY

The specific objectives of the study are to:

i. Synthesize derivatives of complex benzoxazinophenothiazine.

ii. Characterize the compounds synthesized using UV-Visible, FTIR, ^{1}H-NMR and ^{13}C-NMR spectroscopy and elemental analysis.

iii. Carry out antimicrobial screening on phenothiazine and phenoxazine compounds and their derivatives.

1.4 JUSTIFICATION OF THE STUDY

Due to wide pharmaceutical activities of phenothiazine and phenoxazine compounds, it became necessary to evaluate the antimicrobial activity of the synthesized derivatives which may have better and more desirable pharmacological properties.

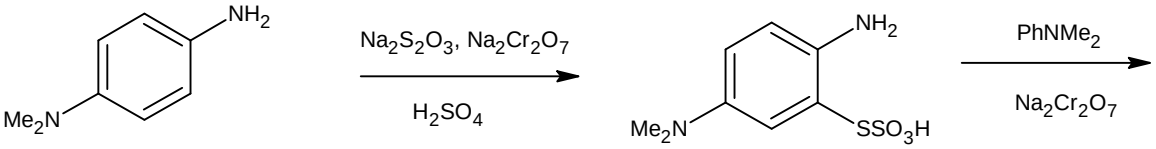
CHAPTER TWO

LITERATURE REVIEW

2.0 LINEAR PHENOTHIAZINES

Linear phenothiazines are defined as phenothiazine derivatives in which variation in the parent phenothiazine ring system (1) does not alter the linear structure of the ring system. The earliest derivatives of phenothiazine are used mainly in the dye industry where they constitute an important class of sulphur dyes[50].

In 1883, methylene blue were obtained using Bernthsenôs thiosulphate method[51] in which 4-aminoï *N,N*-dimethylaniline (15) was oxidized with sodium dichromate in the presence of sodium thiosulphate in sulphuric acid to give the arenethio-sulphuric acid derivative (16). Oxidation of intermediate (16) with sodium dichromate in the presence of *N,N*-dimethylaniline gave the indaminethiosulphuric acid derivative (17) which was subsequently oxidized with air in hot water to give methylene blue (18).



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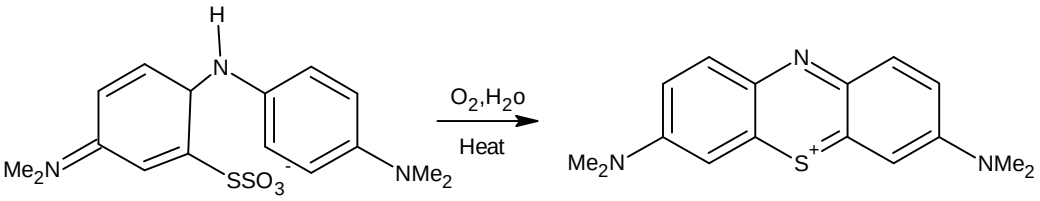

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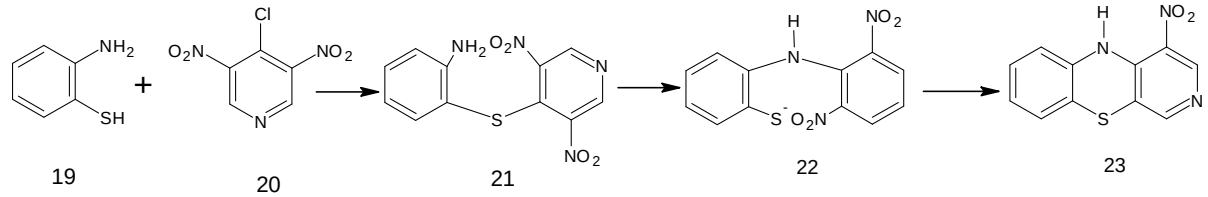
Petrow and Rewalds[52] reported the synthesis of 1-nitro-3-azaphenothiazine (23) by refluxing a mixture of 3,5-dinitro-4-chloropyridine (20) with *o*-aminothiophenol (19) in excess alcoholic sodium ethoxide in the presence of sodium acetate. The sulfide (21) formed quickly rearranged and cyclized without the isolation of the intermediate (22) to give compound (23).



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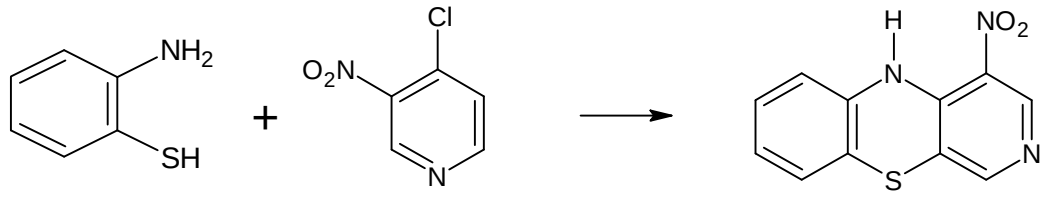
Using 3-nitro-4-chloropyridine (24) and *o*-aminothiophenol (19) as reactants, the unsubstituted 3-azaphenothiazine (25) was obtained in 62% yield.



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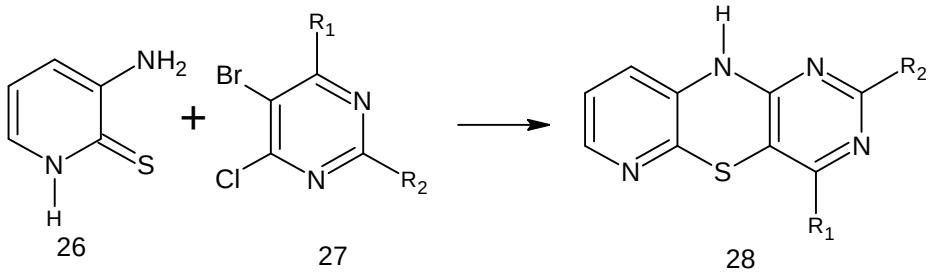
2.1 SYNTHESIS OF TRIAZAPHENOTHIAZINES

However, there was no report on any of the possible twenty isomeric triazaphenothiazines system until 1973 when Okafor[53] reported the synthesis of the first compound in this series. The compound 1,3,6-triazaphenothiazine (28) was obtained in yields from 11% to 95% in the acid condensation of 3-amino-[1*H*]-pyridine-2-thione (26) with 4,5-dihalogenopyrimidines (27). Compound (28) showed appreciable CNS-depressant activities when tested.



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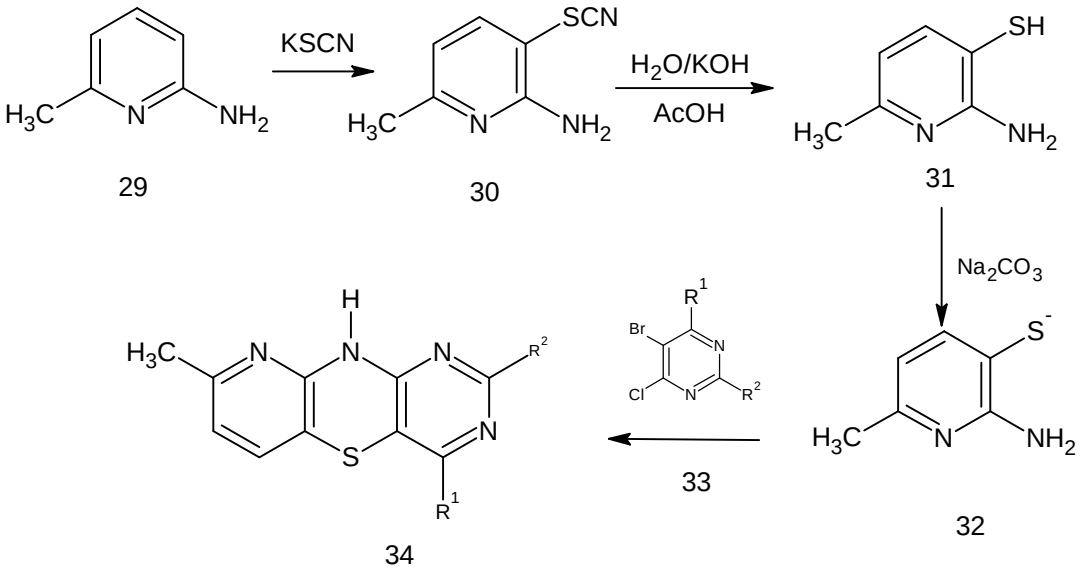
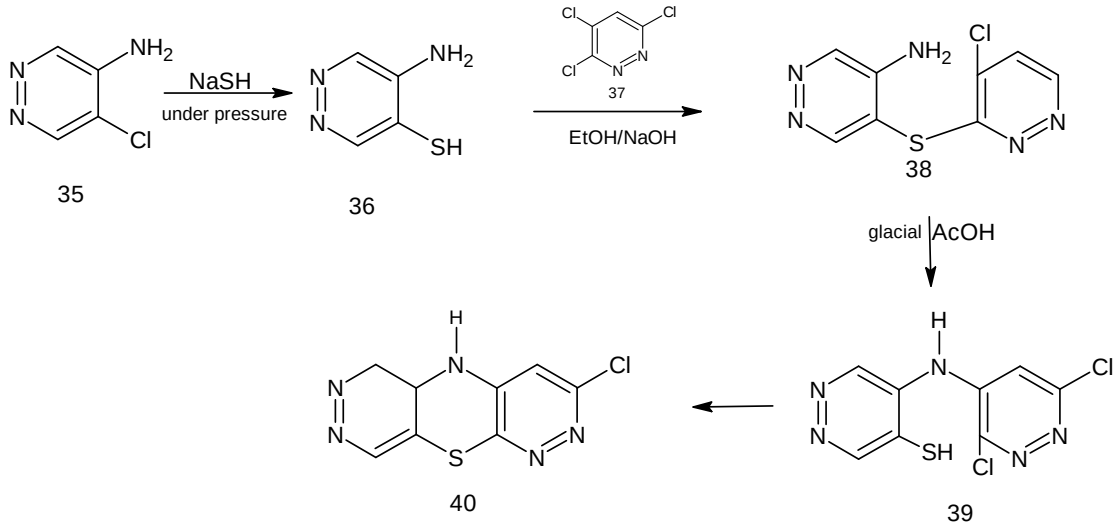
The synthesis of 1,3,9-triazaphenothiazine (34) was described. This was achieved by the acid condensation of 2-amino-6-methyl-3-pyridinethiol (32) with 4,5-dihalogenopyrimidine (33). The precursor to (32), 2-amino-6-methyl-3-thiolpyridine (31) was obtained by Haufmanôs thiocyanation of 2-amino-6-methylpyridine (29) followed by based catalyzed hydrolysis of the resulting 3-thiocyanato derivative (30) and subsequent acidification.



When compound (32) was treated with 5-bromo-4-chloro-2,6-dimethyoxyrmidine (33) (where R^2 and R^3 are ï OCH_3) in diluted sulphuric acid, 68% yield of 2,4-dimethoxyl-8-methyl-1,3,9-triazaphenothiazine (34) was obtained.

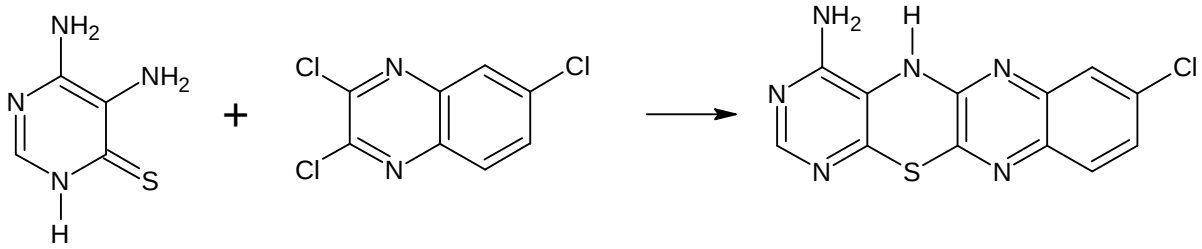
2.2 SYNTHESIS OF TETRAPHENOTHIAZINES

Wise and Castle[54] have reported the synthesis of five out of the thirty five possible isomeric tetra-azaphenothiazine rings. In these reactions, 4-amino-5-chloropyridazine (35) was converted to 4-aminopyridazine-5-thiol (36) in 58% yields by heating sodium hydrosulfide under pressure. The resulting product (36) was treated with 3,4,6-trichloropyridazine (37) in ethanolic sodium hydroxide to obtain dihydrazinyl sulfide (38), which was refluxed in the presence of glacial acetic acid to give 3-chloro-1,2,7,8-tetra-azaphenothiazine (40) melting at 234-235 ^oC.



The conversion of 4-(5-aminopyridazenyl-4-thio)-3,6-dichloropyridazine (39) to tetra-azaphenothiazine (40) was rationalized as proceeding via Smileôs rearrangement followed by cyclization. The same product (40) was obtained by heating the starting material (35) and (36) at 50 ^{o}C for 3 h in ethanolic KOH.

In their search for new pharmacoactive phenothiazine compounds, the synthesis of 1,4,6,8-tetra-azabenzo[b]phenothiazine ring (41) was reported by Wise and Castle [54]. This was achieved by the reaction of 4,5-diaminopyrimidine-6[1*H*]-thione (41) with 2,3,6-trichloroquinoxaline (42) in *N,N*-dimethylformamide, in the presence of a near stoichiometric amount of sodium hydroxide to produce a yellow crystalline solid melting above 300 ^{o}C, identified as 9-amino-11-chloro-1,4,6,8-tetraazabenzo[b]phenothiazine (43).



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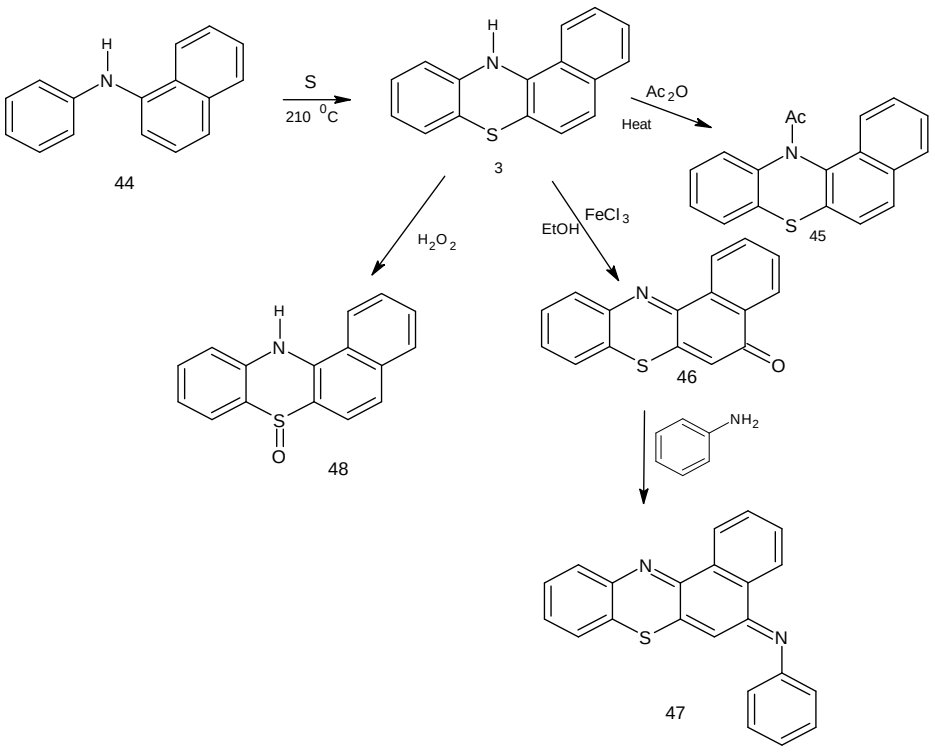
2.3 ANGULAR PHENOTHIAZINES

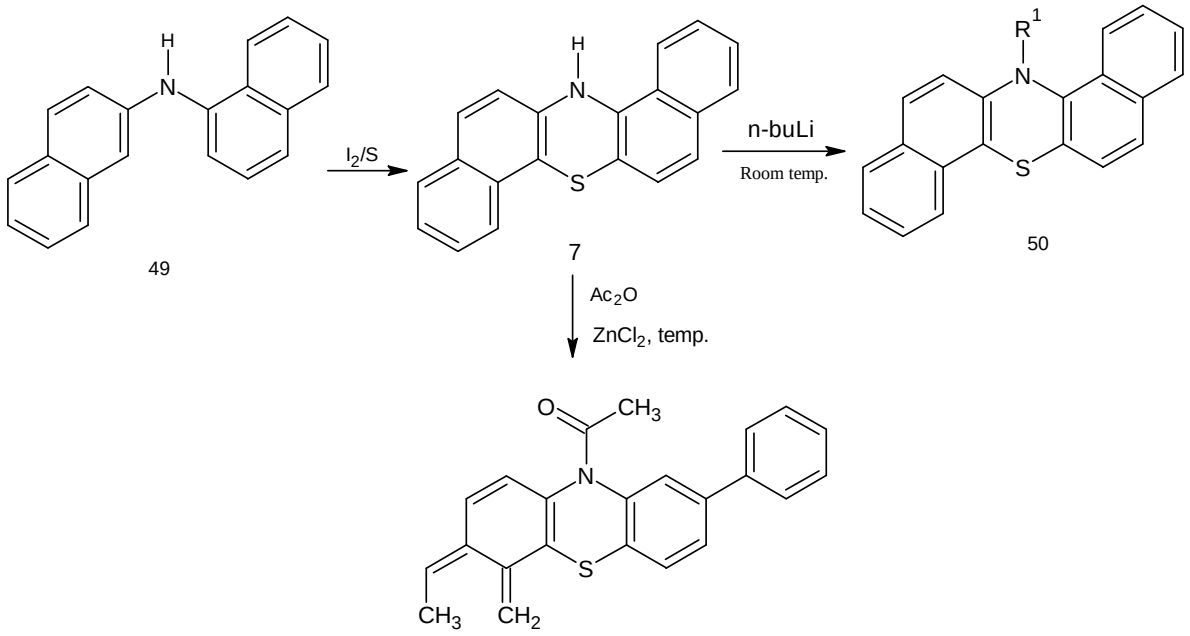
The application of angular phenothiazines as dyes and pigments for the paint and textile industries was recognized from the beginning of the 20th century. Angular phenothiazines are polynuclear phenothiazines which have a non-linear arrangement of the ring system.

In 1890, Kym[55] reported the synthesis of the first angular phenothiazine ring system, benzo[a]phenothiazine (3). This was achieved in 40% yield by heating 1-anilinonapthalene (44) with sulphur at a temperature of 210 ^{o}C for 8 h. In 1958, improved yields and possibly pure products were reported by Talukdar and Shirley[56], who prepared the same compound (3) in 80% yield by adding catalytic amount of iodine at a lesser temperature in much lesser time.

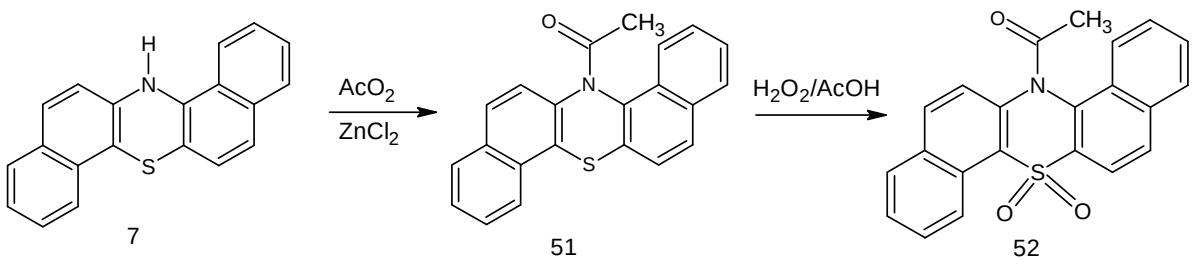
The acetyl derivative (45) was prepared by refluxing (3) in acetic anhydride in the presence of a few drops of pyridine. Ferric chloride or hydrogen perioxide oxidation of (3) gave benzo[a] phenothiazine-5-one (46) while in the presence of aniline, the product was anil (47)[55].



Dibenzo[a,h]phenothiazine (7) was obtained in 55% yield by the pyrolysis of 2-naphthyl-1-naphthylamine (49) in sulfur by Kehrmann and co-workers[46] in the presence of a small catalytic quantity of iodine. Alkylation of (7) was achieved by treatment with n-butyl lithium followed by the action of alkyltosylates. Treatment of compound (7) with acetic anhydride and anhydrous zinc chloride at room temperature for over 24 h gave 14-acetyl-[14*H*] ï dibenzol[a,h]phenothiazine (50)

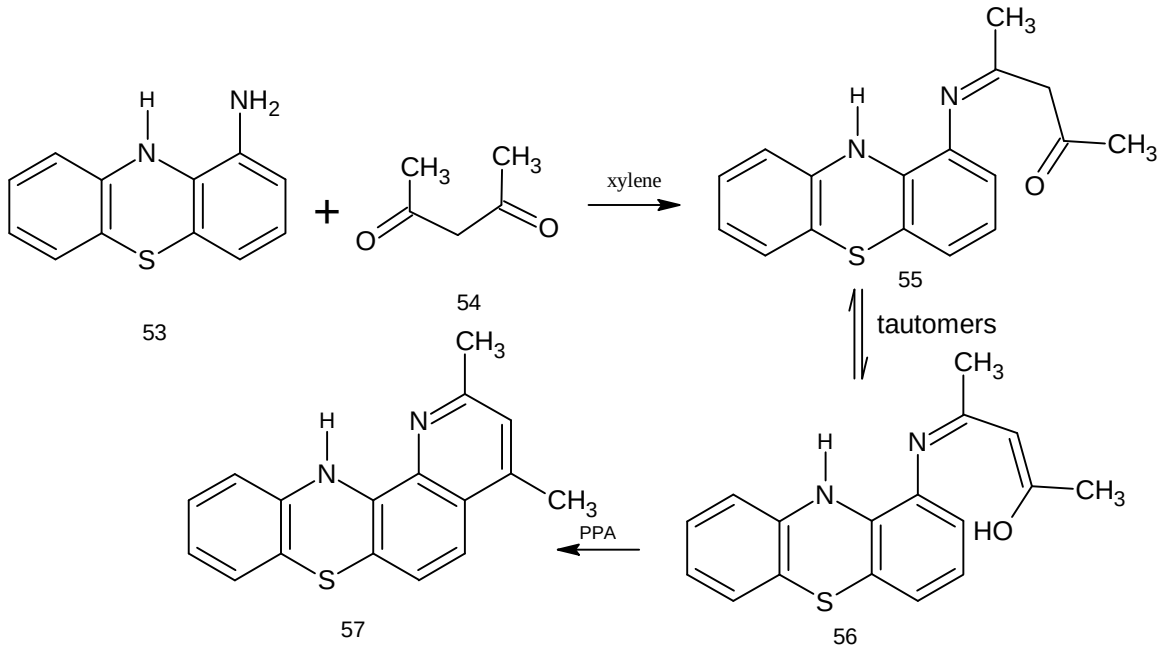


In a further search for more derivatives of angular phenothiazine compounds, Shirley and co-workers[56] reacted compound (7) with acetic anhydride and anhydrous zinc chloride at room temperature for 24 h to give 14-acetyl-[14*H*]-dibenzo[a,h]phenothiazine (51) which on oxidation with hydrogen peroxide in acetic acid gave the sulfone (52)

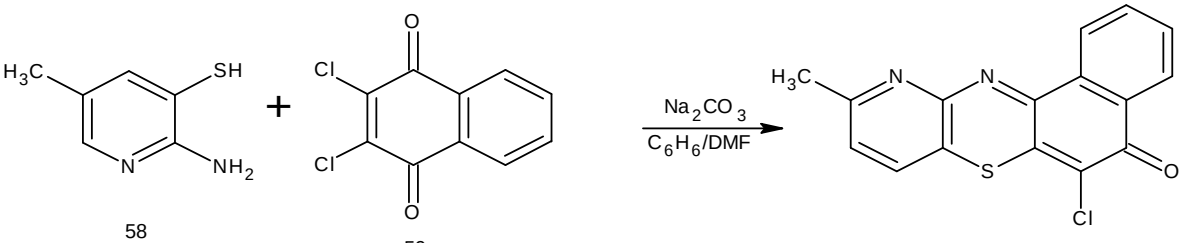


2.4 AZA ANALOGUES OF ANGULAR PHENOTHIAZINES

Interest in the synthesis of novel and more useful aza analogues of non-linear phenothiazine led to the investigation of these heterocyclic compounds. The first reported work on the synthesis of aza angular phenothiazine was by Gritsenk *et al.*[57] in which 1-aminophenothiazine (53) was reacted with pentan-2,4-dione (54) in xylene to give the tautomeric mixture of compound (55) and (56) which on cyclisation with polyphosphoric acid (PPA) at 40-60 ^{o}C gave 2,4-dimethylpyrido[2,3-a]phenothiazine (57) in 64% yield.

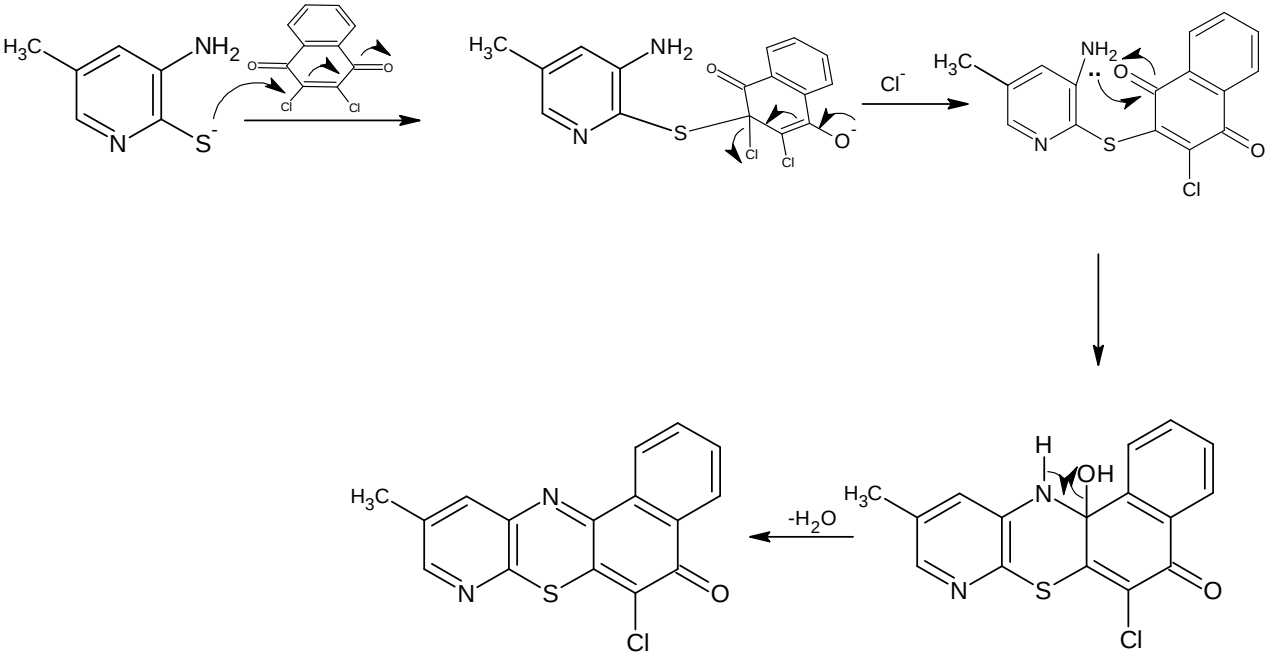


Okafor and co-workers[58] described the synthesis of 6-chloro-10-methylbenzo[a]-11-azaphenothiazine-5-one (60), obtained by refluxing 2,3-dichloro-1,4-naphthoquinone (59) with 2-amino-5-methyl-3-pyridinethiol (58) in benzene/DMF and in the presence of anhydrous sodium carbonate**.**

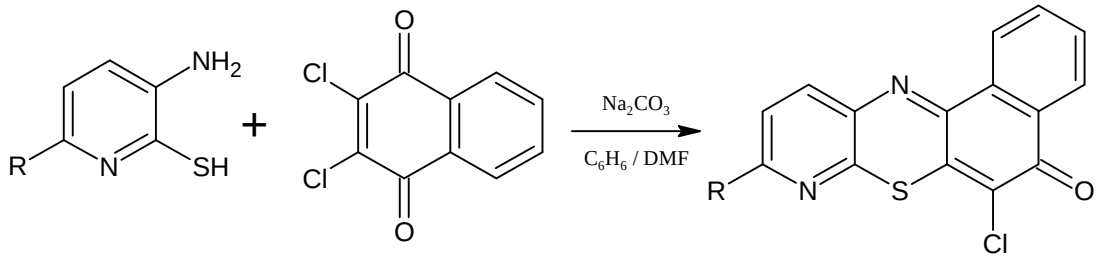


58 59 60

These compound (60) are probably formed through the mechanistic pathway shown below:

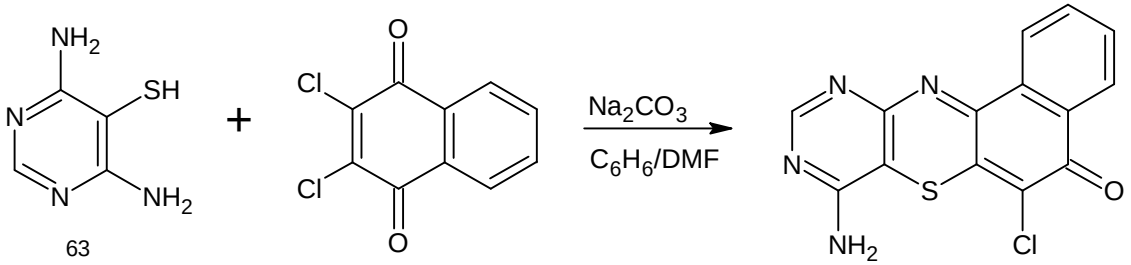


In a related development, Okafor[58] reported the synthesis of the aza analogue of 5*H*-benzo[a]phenothiazin-5-one (62), by heating 3-aminopyridine-2[1*H*]-thione (61) with 2,3-dichloro-1,4-naphthoquinone (59) in a basic medium containing anhydrous sodium carbonate.



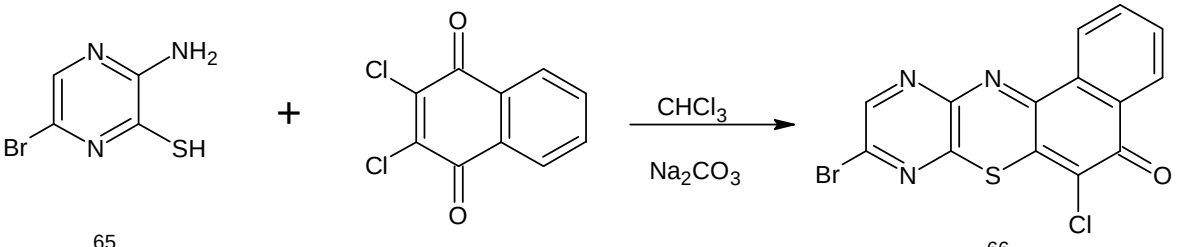
61 59 62

The synthesis of 8-amino-6-chloro-9,11-diaza-5*H*-benzo[a]phenothiazine-5-one (64) was reported by Umio and Kishimeto[59]. Compound (64) was obtained by condensing 2,3-dichloro-1,4-naphthoquinone (59) with 4,6-diaminopyrimidine-5-thiol (63) in benzene/DMF in the presence of anhydrous sodium carbonate.



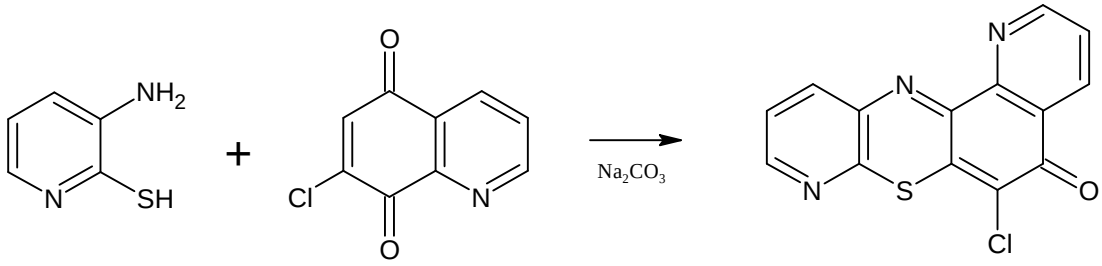
63 59 64

In another development, 9-Bromo-6-chloro-8,11,12-triazabenzo[a]anthracen-5-one (66)[60] was obtained by reacting 2-amino-5-bromopyrazine-3[4*H*]-thione (65) with 2,3-dichloro-1,4-naphthoquinone (59) in chloroform in the presence of anhydrous sodium carbonate.



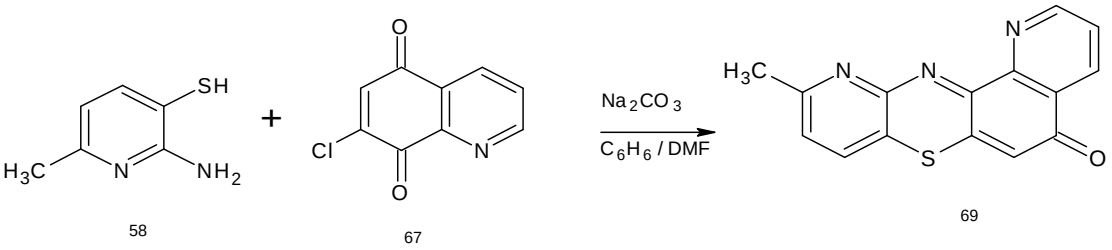
65 59 66

Okoro and Ezema[61] reported the synthesis of 1,8-diazabenzo[a]phenothiazin-5-one (68) by the base catalyzed reaction of 3-aminopyridine-2-[1*H*]-thione (26) with 7-chloro-5,8-dioxoquninoline (67).



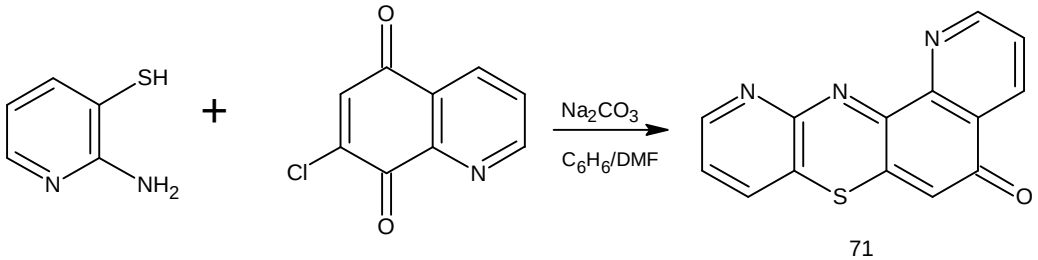
26 67 68

The new aza non-linear polycyclic phenothiazine; 10-methyl-1,11-diazabenzo[a]phenothiazin-5-one (69) was obtained by condensing 2-amino-6-methylpyridine-3-thiol (58) with 7-chloro-5,8-dioxoquinoline (67) in benzene/DMF and in the presence of anhydrous sodium carbonate[62].



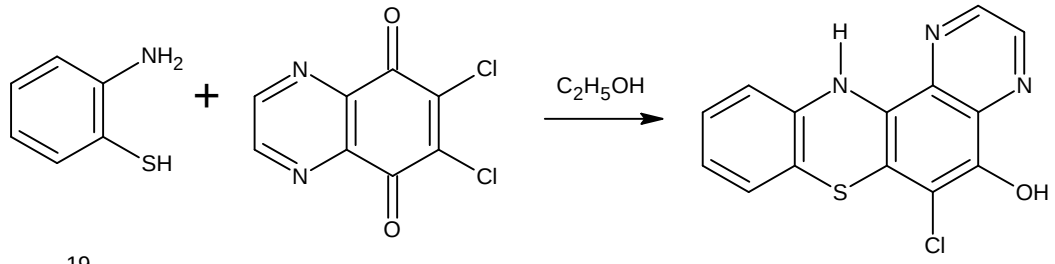
58 67 69

Ofoefule[63] synthesized another aza angular phenothiazine compound, 1,11-diaza-5*H*-benzo[a]phenothiazin-5-one (71) by refluxing a mixture of 2-aminopyridin-3-thiol (70) with 7-chloro-5,8-dioxoquinoline (67) in benzene/DMF and in a basic medium of anhydrous sodium carbonate.



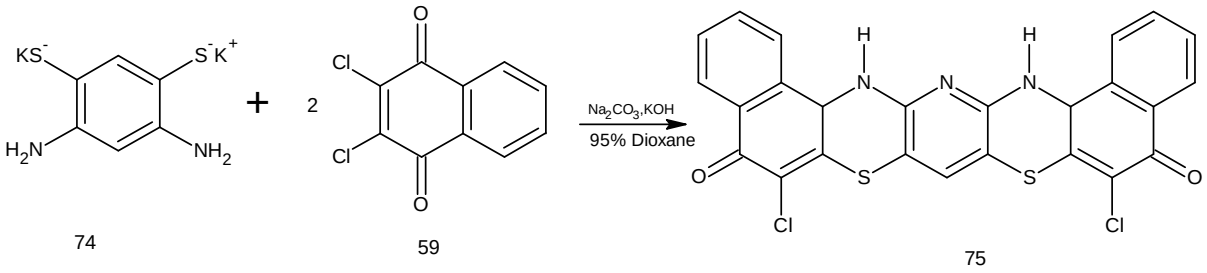
70 67 71

Won Yoo and co-workers[64] described the synthesis of another new angular phenothiazine, 6-chloro-5-hydroxy-12*H*-pyrazino[2,3-a]phenothiazine (73), obtained by refluxing 2-aminothiophenol (19) with 6,7-dichloroquinoxaline-1,4-dione (72) in ethanol.



19 72 73

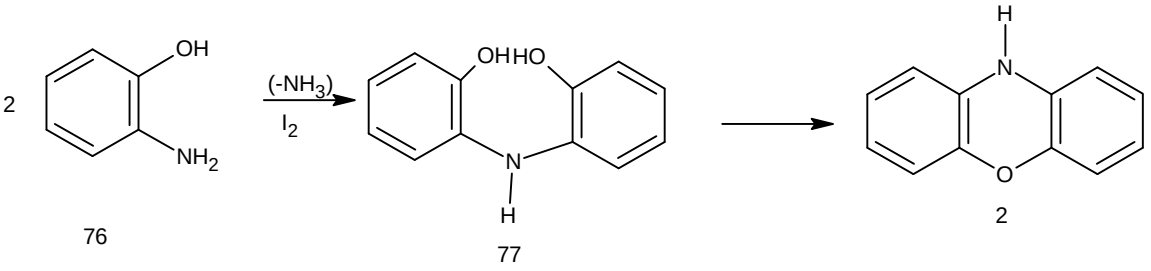
Okoro[65] reported the synthesis of 6,10-dichloro-17-azadibenzo[a,n]triphenodiathiazine-5*H*-dione (75). The compound was obtained by treating potassium thiolate (74) with 2 moles of 2,3-dichloro-1,4-naphthoquinone (59) in 95% dioxane, potassium hydroxide and sodium sulfite.



74 59 75

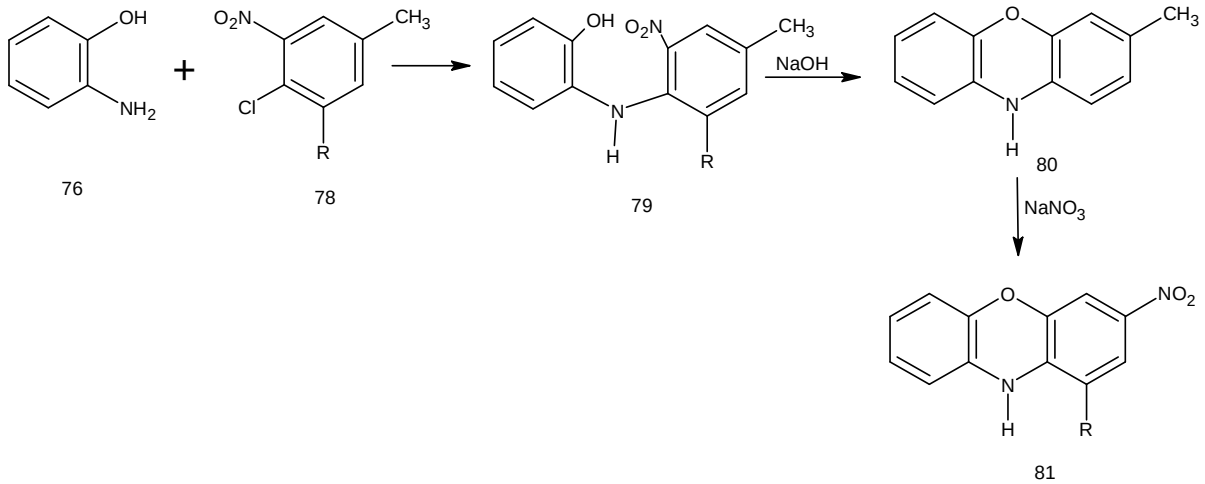
2.5 LINEAR PHENOXAZINES

Kehrmann and Neil[66] pointed out that phenoxazines may be obtained in good yield by heating an equimolar mixture of *o*-aminophenol and *o*-aminophenohydrochloride without cathechol. Also the autocondensation of *o*-aminophenol (76) in the presence of iodine with elimination of ammonia and water gave unsubstituted phenoxazine (2).



76 77 2

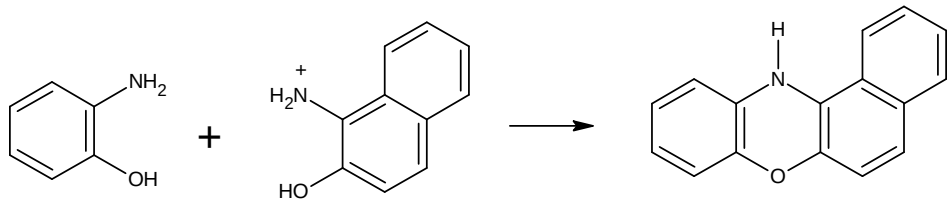
According to Turpin[67], substituted phenoxazine was obtained from the autocondensation of o-aminophenol (76) with picryl chloride (78).



76 78 79 80 81

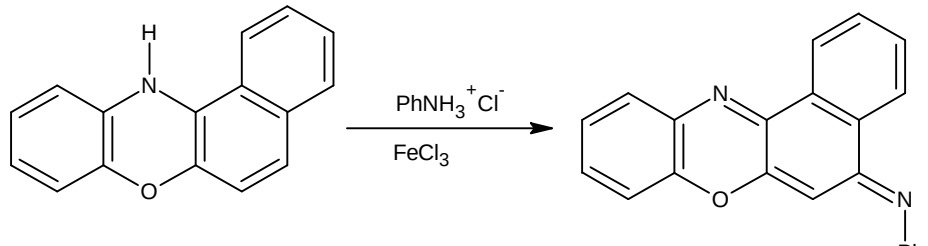
2.6 ANGULAR PHENOXAZINES

Goldstein and Semelitech[43] reported the first synthetic angular phenoxazine, benzo[a]phenoxazine in 1919. Compound (4) was obtained by the pyrolysing *o*-aminophenol (76) with 1-amino-2-naphtholhydrochloride (82) at 260 ^{o}C



76 82 4

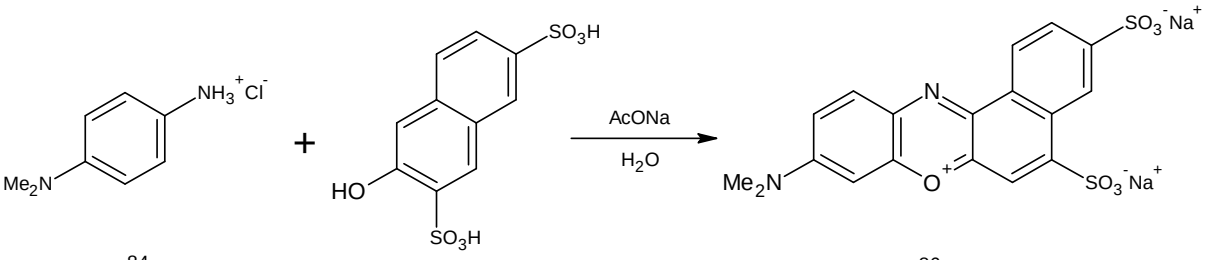
When benzo[a]phenoxazine was treated with aniline hydrochloride followed by the action of excess ferric chloride as the oxidizing agent, a more intensely coloured benzo[a]phenoxazine-5-phenylimine (83) was formed[43].



4 83

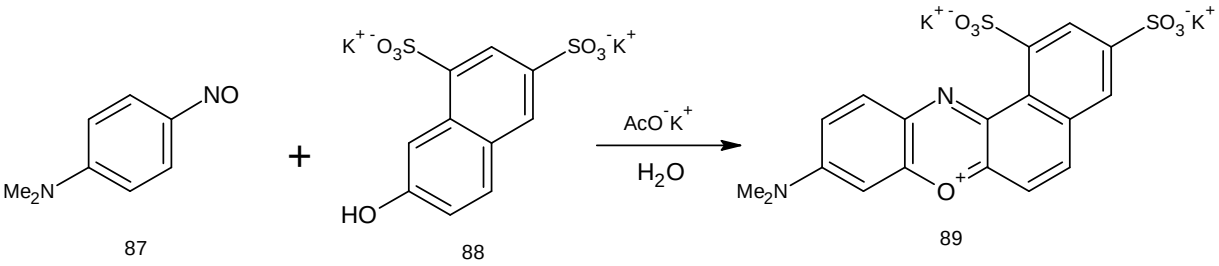
Eggers and Dickmann[68] prepared a derivative of phenoxazine (86) which is used as an indicator for redox titration. The compound was synthesized by cyclisation condensation of P-

N,N-dimethylaniline hydrochloride (84) with 2-hydroxynapthalene-3,6-disulphuric acid salt (85) in the presence of aqueous sodium ethanoate



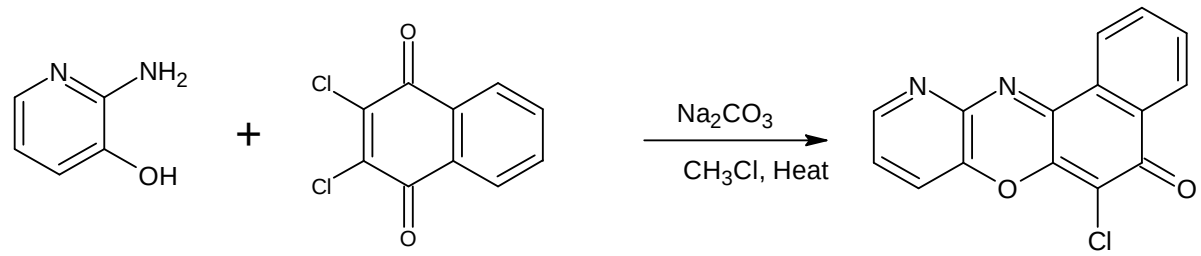
84 85 86

Similarly, compound (89) was synthesized by the condensation of 4-nitroso-1-dimethylaniline (87) with 2-hydroxynaphthalene dipotassium-6,8-sulphonate (88) in the presence of aqueous potassium ethanoate.



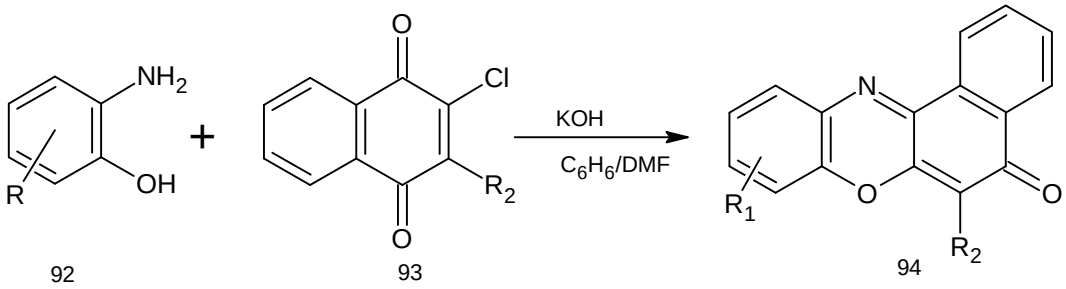
87 88 89

Agarwal and Schafar[54] reported the synthesis of 6-chlorobenzo[a]-11-azaphenoxazin-5-one (91) by heating a mixture of 2-amino-3-hydroxypyridine (90) and 2,3-dichloro-1,4-naphthoquinone (59) in the presence of anhydrous sodium carbonate.



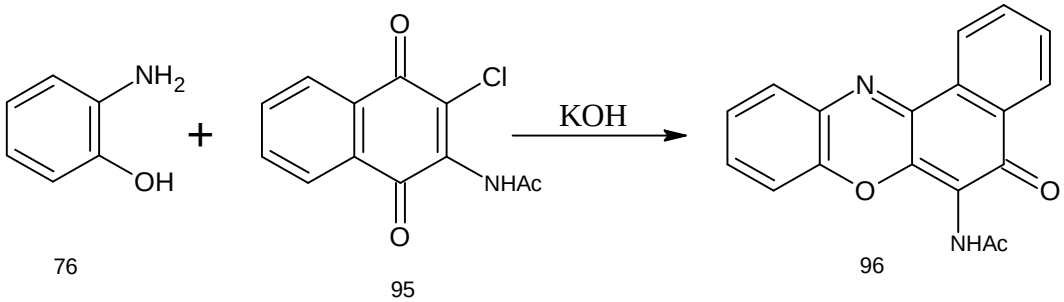
90 59 91

The synthesis of substituted benzo[a]phenoxazine-5-one (94) was achieved by refluxing substituted 2-aminophenol (92) with substituted 2-chloro-1,4-naphthoquinone (93) in anhydrous potassium hydroxide and benzene/DMF by Agarwal and Schafar[54].



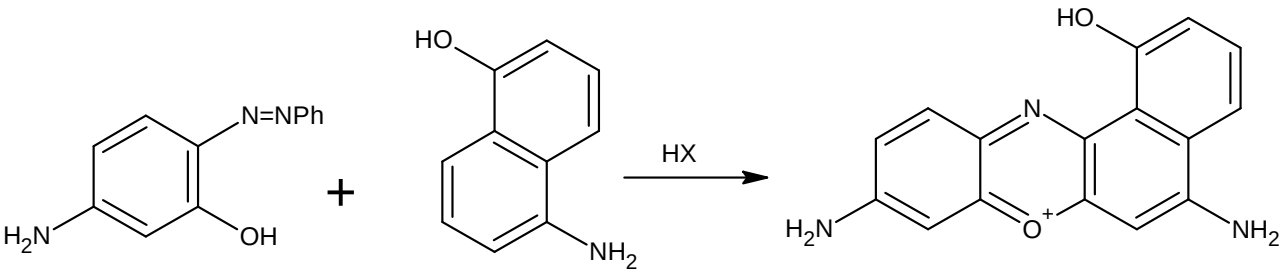
92 93 94

In a further search for new angular phenoxazines, the synthesis of 6-acetamidobenzo[a]phenoxazine (96) was achieved by the condensation of 2-acetamido-3-chloro-1,4-naphthoquinone (95) with 2-aminophenol (76) in the presence of anhydrous potassium hydroxide to produce 6-acetamidobenzo[a]phenoxazin-5-one[69].



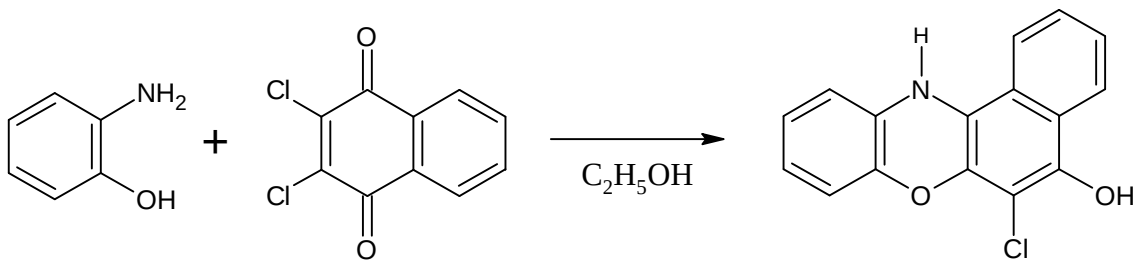
76 95 96

Muller and Psaar[70] reported the synthesis of 5,9-diamino-1-hydroxybenzo[a]phenoxazine (99) by refluxing 4-amino-2-hydroxyazobenzene (97) with amino-5-naphthol (98).



97 98 99

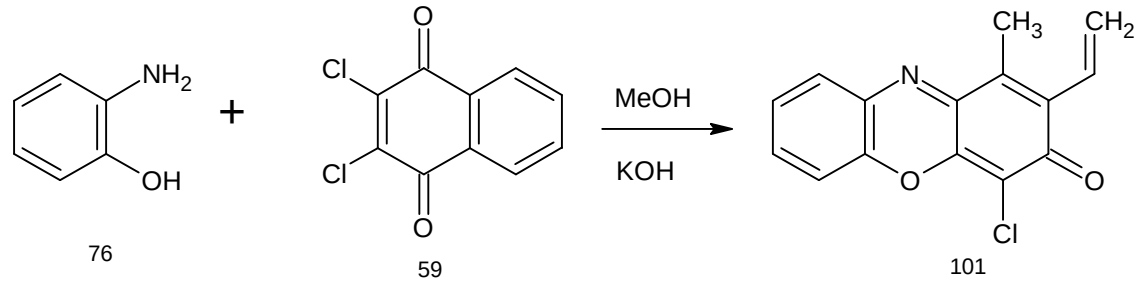
Van Allan and co-workers[71] reported the synthesis of 6-chloro-5-hydroxybenzo[a]phenoxazine (100) by reacting 2,3-dichloro-1,4-naphthoquinone (59) with 2-aminophenol (76).



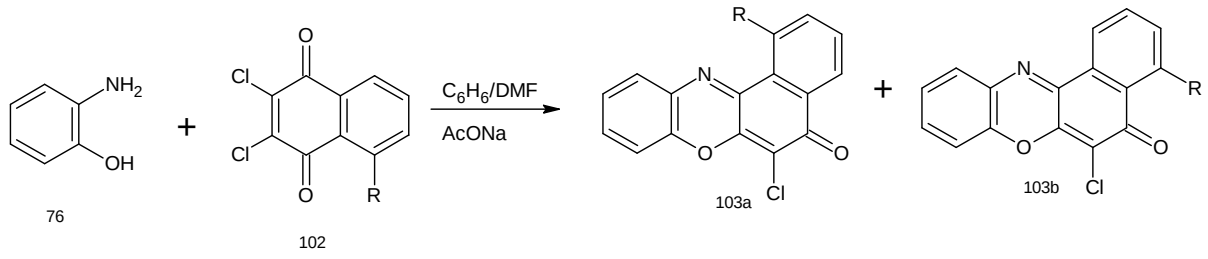
76 59 100

Also reported by the same author[71] was the reaction of 2-aminophenol (76) and 2,3-dichloro-1,4-naphthoquinone (59) in methanol and anhydrous potassium hydroxide to afford

6-chloro-5[1*H*]-benzo[a]phenoxazin-5-one (101)



In a similar development, 1- and 4-substituted 5*H*-benzo[a]phenoxazin-5-one (103a) and (103b)[72], respectively, were prepared by the condensation of 2-aminophenol (76) with 5-substituted 2,3-dichloro-1,4-napthoquinone (102) in benzene/DMF in the presence of sodium acetate.

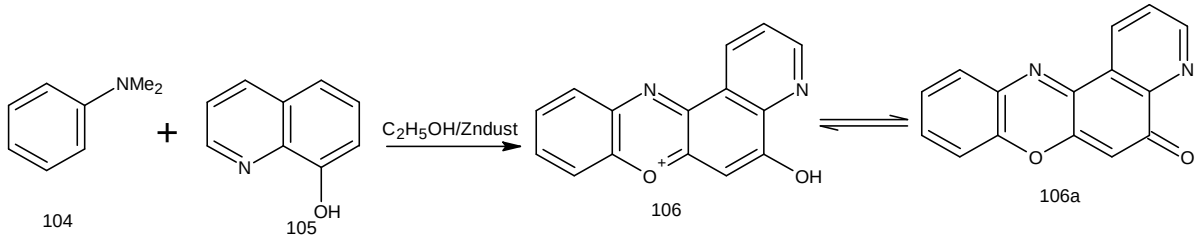


2.7 Aza-Analogue of Angular Phenoxazine Ring Systems

Research into aza analogues of phenoxazines has grown over years. Some angular aza-phenoxazines such as pyrido[3,2-a]phenoxazine occur in nature. Naturally occurring angular

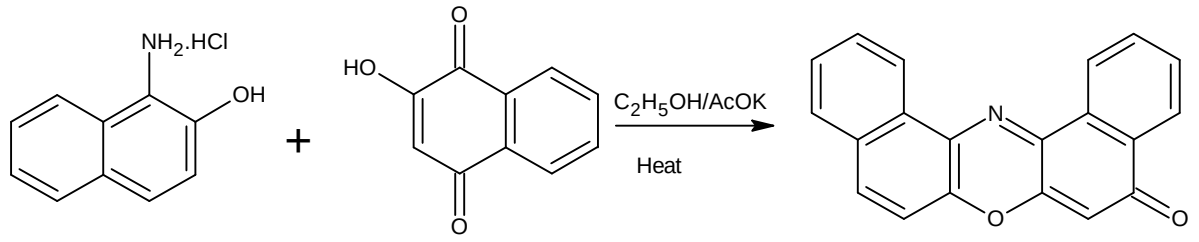
phenoxazine pigments known as ommochromes such as rhodomatin and ommatin, have been isolated from the wings of different arthropods.

Noelting[73] in 1922 reported the synthesis of the angular aza-phenoxazine, 5-hydroxypyrido[3,2-a]phenoxazine (106), obtained by heating a mixture of 8-hydroquinoline (104) and *o*-hydroxy-*N,N*-dimethylaniline (105) in ethanol in the presence of zinc dust



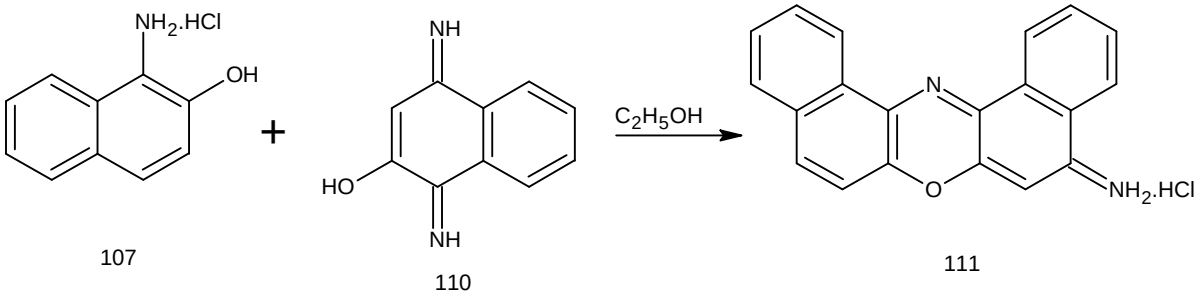
104 105 106 106a

Goldstein and co-workers[74] reported the synthesis of dibenzo[a,j]phenoxazin-5-one (109) by refluxing 1-amino-2-naptholhydrochloride (107) with 2-hydroxy-1,4-napthoquione (108) in ethanol and potassium acetate.



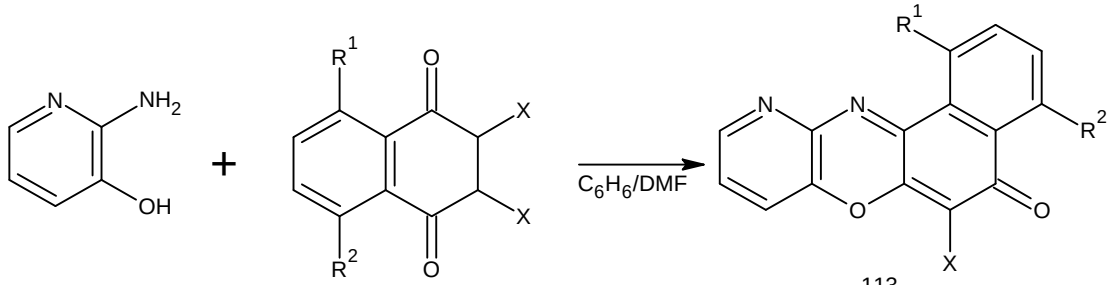
107 108 109

In a similar development, dibenzo[a,j]phenoxazin-5-imine hydrochloride (111) was prepared[74] by refluxing 1-amino-2-naphthol (107) with 2-hydroxy-1,4-naphthoquinoneimine (110) in ethanol.



107 110 111

Nanya and co-workers[75] reported the synthesis of 1,6- and 4,6-disubstituted-11-aza-5*H*-benzo[a]phenoxazine-5-one (113) by condensing 2-amino-3-hydroxypyridine (90) with 5-substituted 2,3-dihalogeno-1,4-naphthoquinone (112) in benzene/ethanol as the solvent in the presence of potassium acetate.

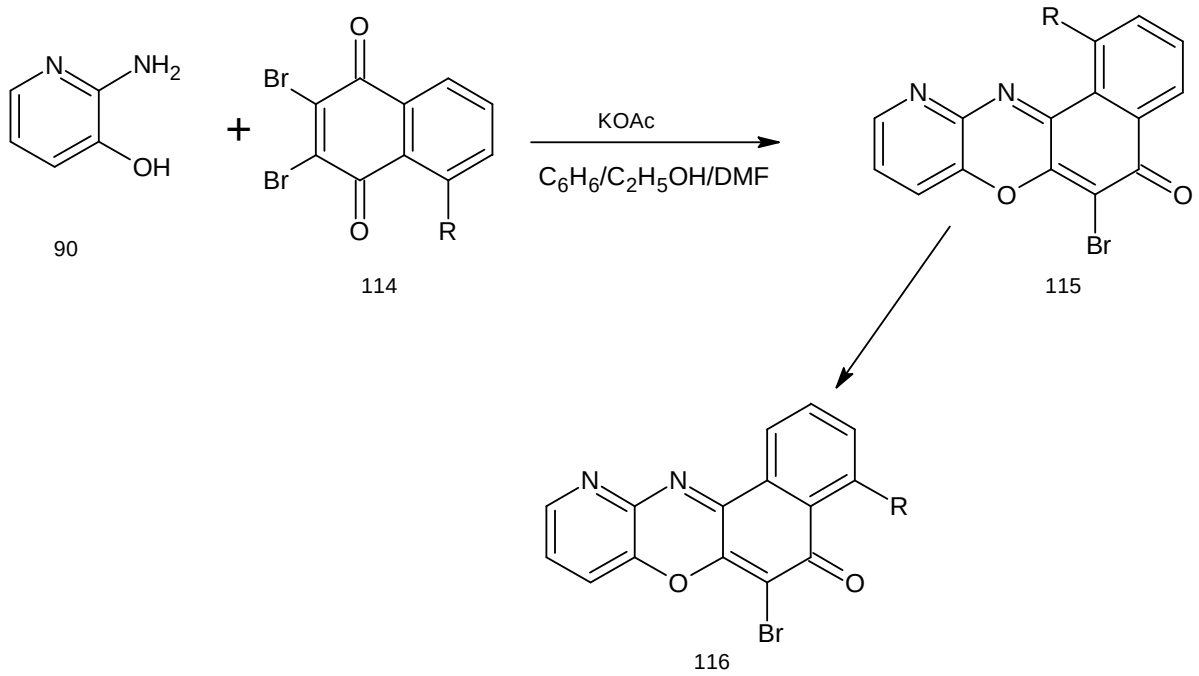


90 112 113

X=Hal, R^2=H

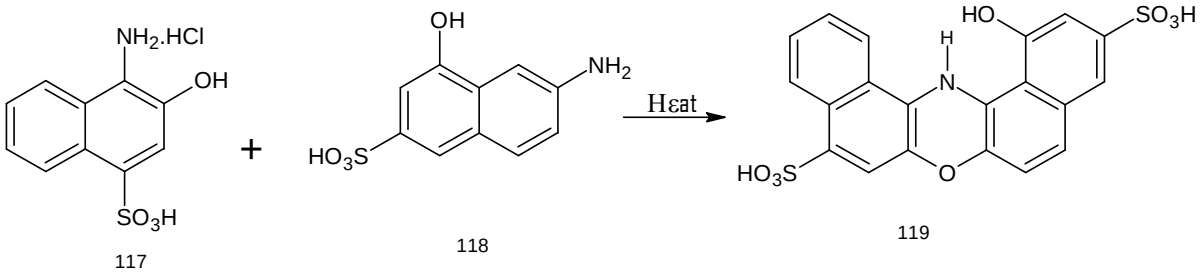
R^1 = H

The condensation of 2-amino-3-hydroxypyridine (90) with 6,7-dibromo-5,8-dioxoquinoline (114) in benzene/ethanol in the presence of potassium acetate at room temperature gave 6-substituted-11-aza-5*H*-pyrido[2,3-a]phenoxazin-5-one (115) and 6-substituted-11-aza-5*H*-pyrido[3,2-a]phenoxazin-5-one (116)[75] .



90 114 115 116

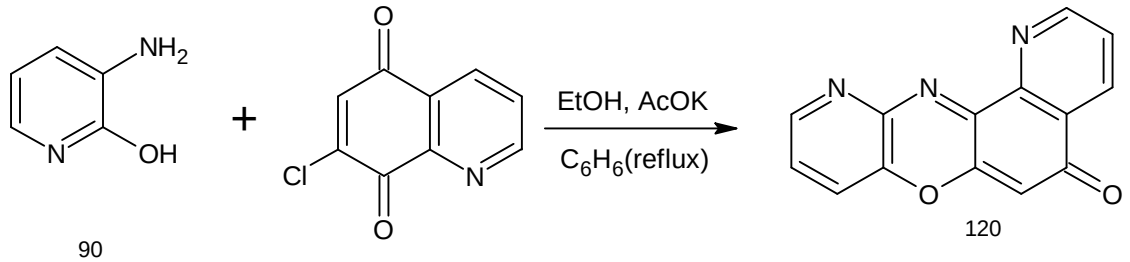
Levchenko[76], in 1960, prepared a disulphonic acid derivative of dibenzo[a,j]phenoxazine, 1-hydroxydbenzo[a,j]phenoxazin-3,9-disulphonic acid (119), by condensing 1-amino-2-naphthol-4-sulphonic acid (117) with 2-amino-8-naphthol-6-sulphonic acid (118).



117 118 119

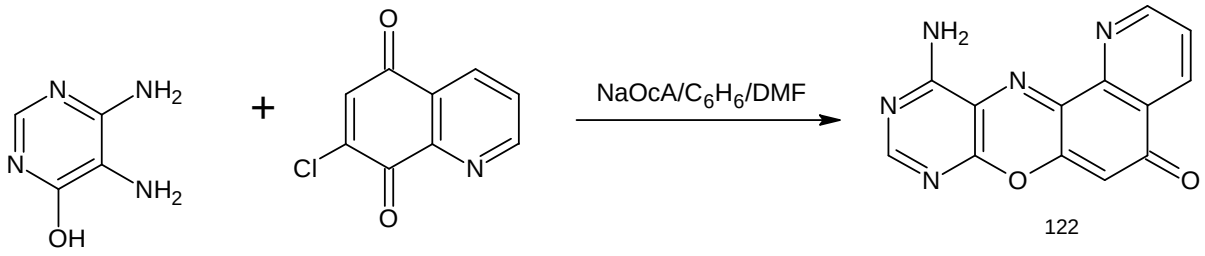
In a further attempt to synthesize diaza-analogues of non-linear phenoxazine compounds, Ugwuoke[77] reported the synthesis of 1,11-diaza-5H-benzoxazin-5-one (120) by reacting 2-

amino-3-hydroxypyridine (90) with 7-chloro-5,8-quinolinequinone (67) in anhydrous potassium acetate in benzene.



90 67 120

In a similar development, the synthesis of another new angular azaphenoxazine, 1,8,10-triazabenzo[a]phoxazine-5-one (122)[77], was achieved by condensing 4,5-diamino-6-hydroxpyrimidine (121) with 7-chloro-5,8-dioxoquinoline (67) in a mixture benzene/DMF as the solvent and in the presence of anhydrous sodium acetate on refluxing for 7 h

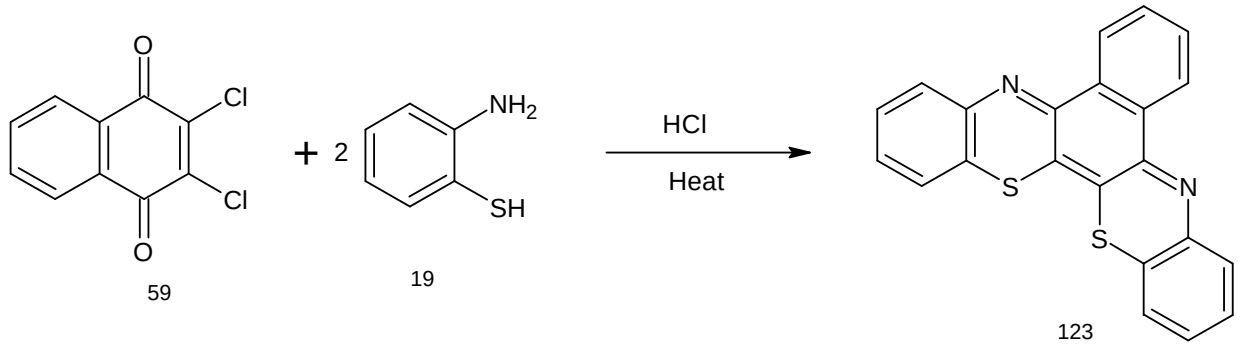


121 67 122

2.8 Branched Phenothiazine and Phenoxazine Compounds

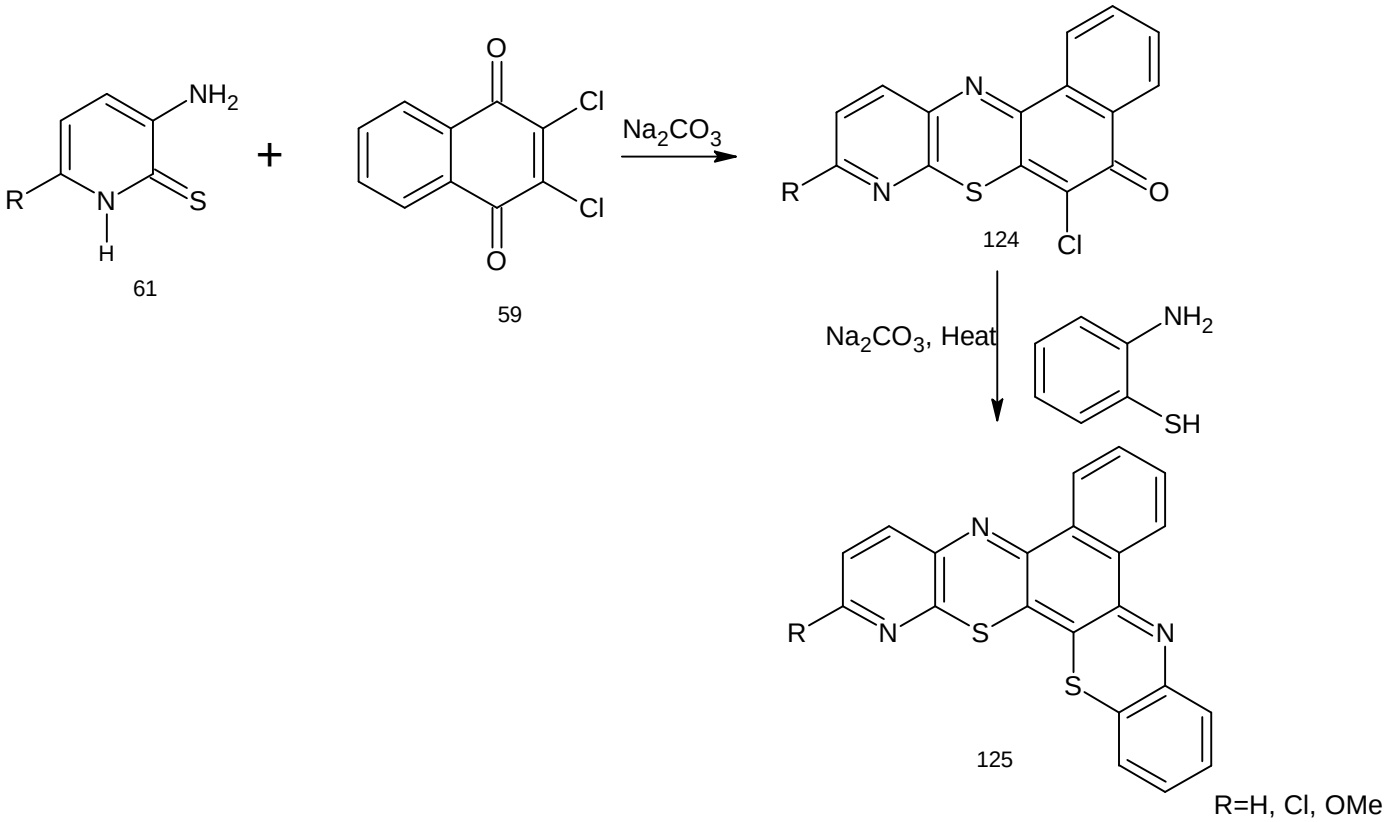
There have been reports on the synthesis of three-branched phenothiazine and phenoxazine derivatives as a result of further structural modification of the parent phenothiazine and phenoxazine compounds. For example, Akatuska and Yoshinaga[78] reported the synthesis of

a three branched phenothiazine by heating 2 moles of 2-aminothiophenol (19) with 2,3-dichloro-1,4-naphthoquinone (59) in hydrochloric acid to obtain benzo[a][1,4]benzothiazino[3,2-c]phenothiazine (123).

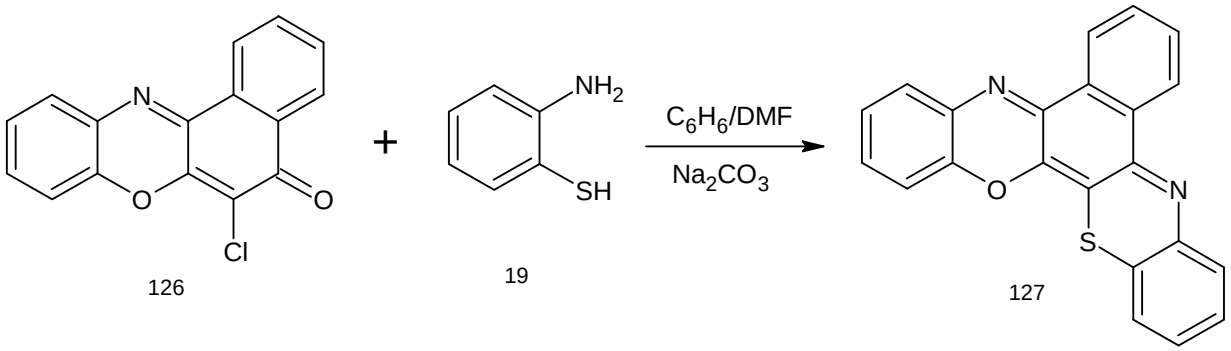


59 19 123

Okafor and Okoro[60] reported the synthesis of substituted three branched phenothiazine. The compound, 15,16-diathia-1,5,10-triazabenzo[h]pentaphene (125) was prepared by condensing 2,3-dichloro-1,4-naphthoquinone (59) with substituted 3-aminopyridine-2[1*H*]-thione (61) in the presence of anhydrous sodium carbonate to produce substituted 6-chlorobenzo[a]phenothiazin-5-one (124). Further reaction of (124) with an alkaline solution of an equimolar amount of 2-aminothiophenol under strong heating gave compound (125)

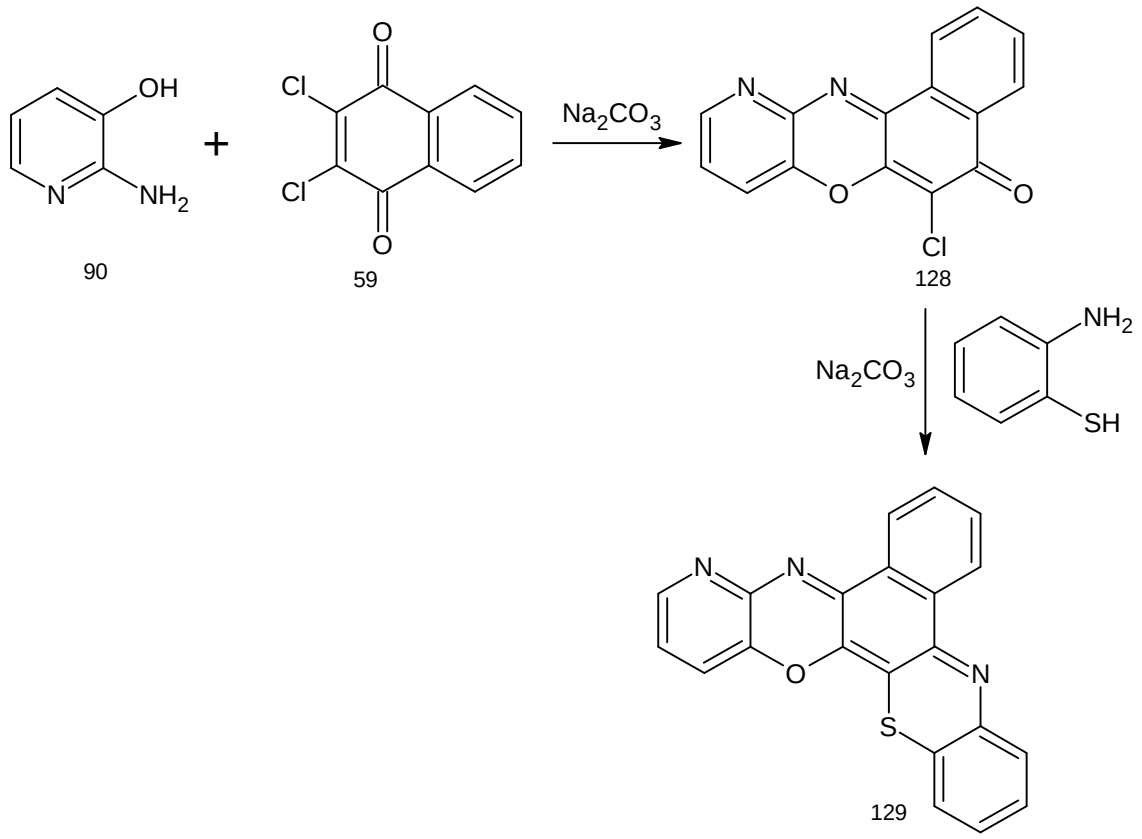


Okafor[79] also reported the first three-branched benzoxazinophenothiazine and its aza analogues. He obtained the first compound, benzo[a][1,4]benzoxazino[3,2-c]phenothiazine (127), by refluxing a mixture of 2-aminothiophenol **(19)** and 6-chlorobenzo[a]phenoxazin-5-one (126) in benzene/DMF in the presence of anhydrous sodium carbonate.



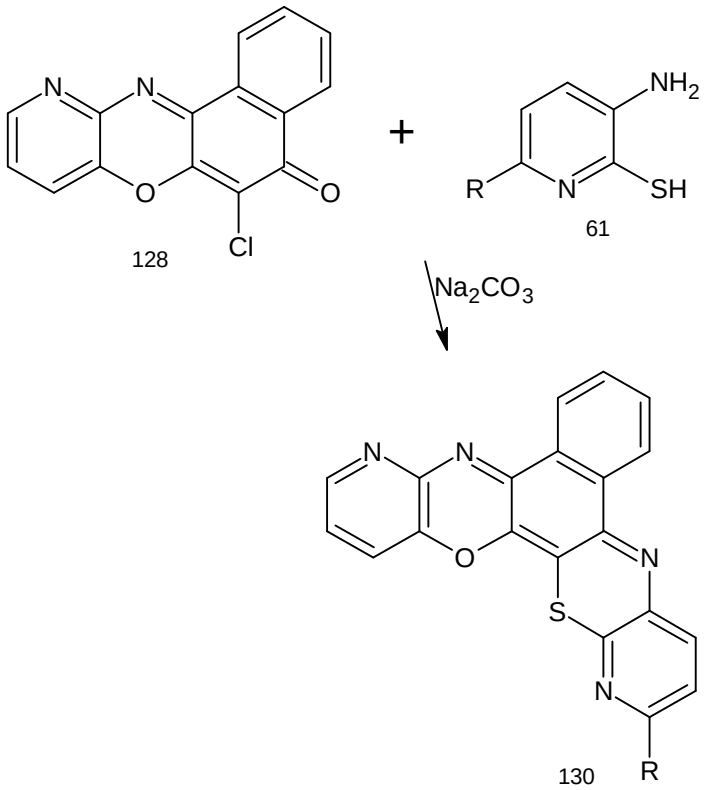
126 19 127

Okafor[79] subsequently prepared the aza analogue of (127), 16-oxa-15-thia-4,5,10-triazabenzo[h]pentaphene (129) by first refluxing a mixture of 2-amino-3-hydroxypyridine (90) and 2,3-dichloro-1-4-naphthoquinone (59) in alkaline medium to give 6-chloro-7-oxa-11,12-diazabenzo[a]anthracen-5-one (128). Further treatment of (128) with a second molecule of 2-aminothiophenol furnished compound (129).

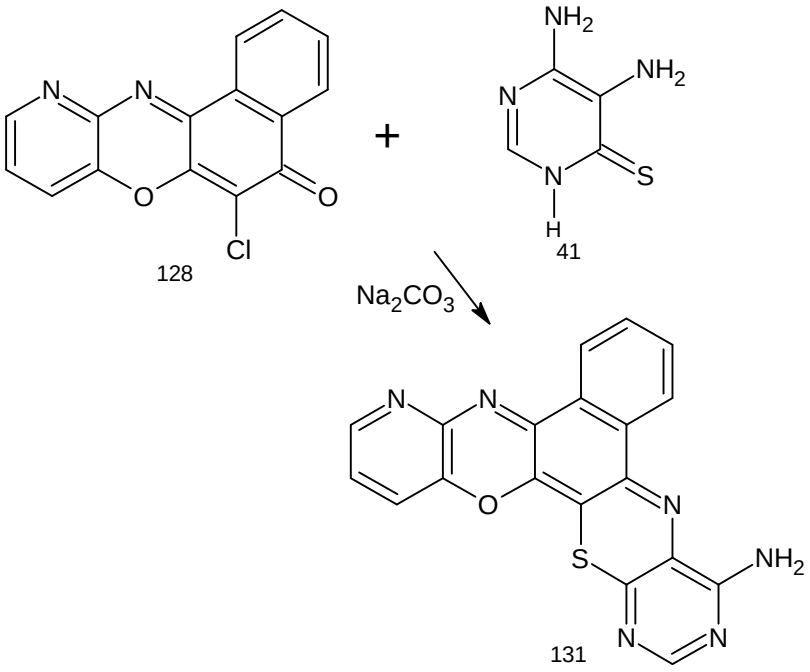


90 59 128 129

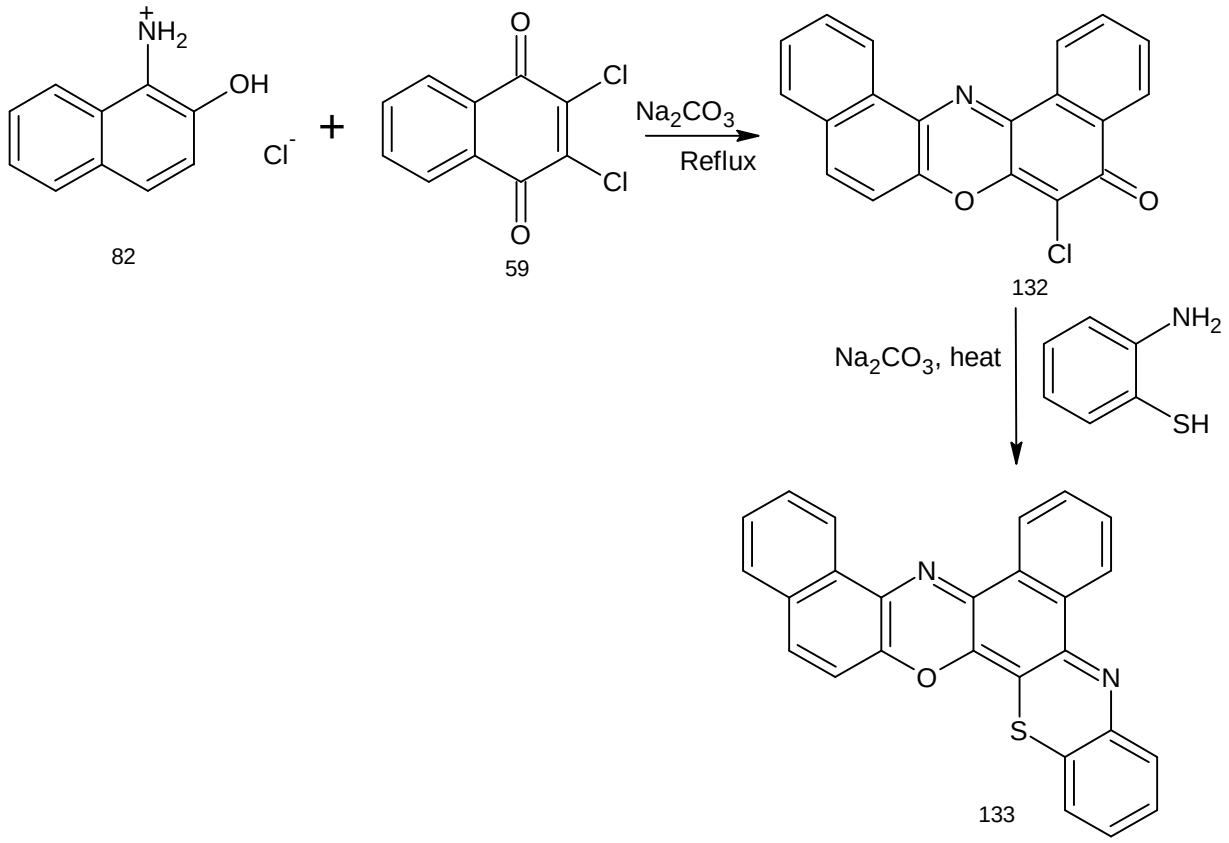
The same author[79] reported the synthesis of another derivative, 16-oxa-15-thia-4,5,10,14-tetraazabenzo[h]pentaphene (130). Compound (130) was obtained by treating 6-chloro-7oxa-11,12-diazabenzo[a]anthracen-5-one (128) with 3-aminopyridine-2[1*H*]-thione (61) in a basic medium of anhydrous sodium carbonate for 10 h.



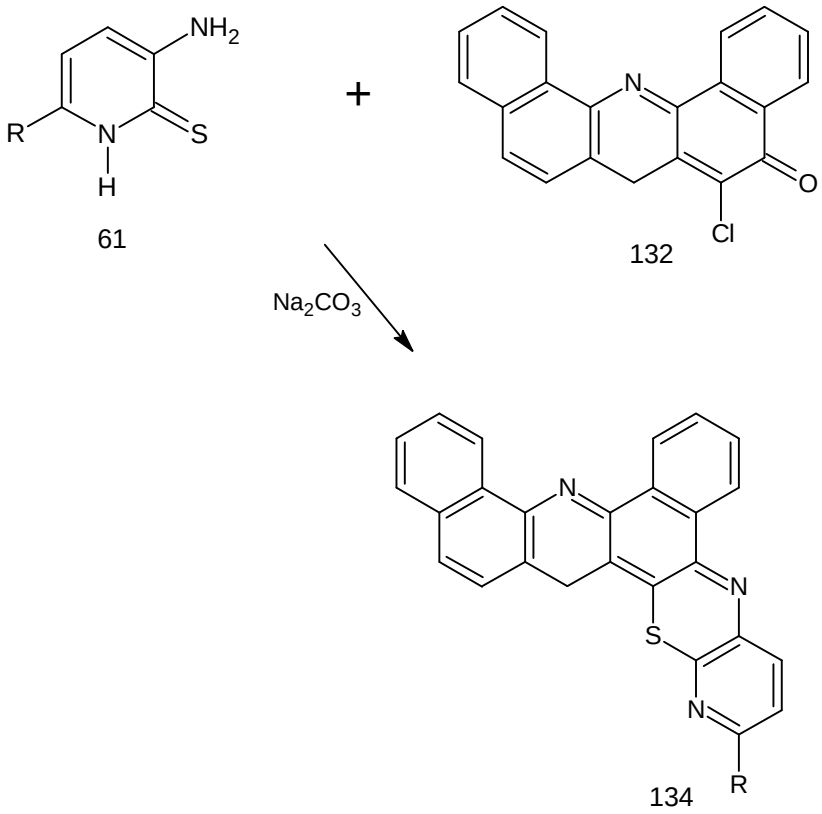
Finally, Okafor[79] reported the synthesis of 11-amino-16-oxa-15-thia-4,5,10,12,14-pentaazabenzo[h]pentaphene (131) by refluxing 6-chloro-7oxa-11,12-diazabenzo[a]anthracen-5-one (128) with 4,5-diaminopyrimidine-6[1*H*]-thione (41) in benzene/DMF in the presence of anhydrous sodium carbonate for 9 h.



Further search for more derivatives of branched phenothiazine compounds resulted in the reports of the synthesis of some new Y-shaped benzothiazinophenoxazine ring systems by Okafor *et al*[7]. The derivative, dibenzo[a, j][1,4]benzothiazino[3,2-c]phenoxazine (133), was obtained by the condensation of 2,3-dichloro-1,4-naphthoquinone (59) with 1-amino-2-napthol hydrochloride (82) in the presence of excess anhydrous sodium carbonate to give 6-chlorodibenzo[a, j][1,4]phenoxazin-5-one (132). Further treatment of (132) with 2-aminothiophenol in nitrobenzene for 11 h yielded compound (133).



Also reported by the same authors[79] was the aza derivative, 11-oxa-10-thia-5,9,18-triazadibenzo[a,r]pentaphene (134) which was obtained by refluxing a mixture of compound (132) and 3-aminopyridine-2[1*H*]-thione (61) in nitrobenzene for 21 h in the presence of anhydrous sodium carbonate.


NH2
R
N
H
S
61
+
N
O
Cl
132
Na2CO3
N
N
S
N
R
134

CHAPTER THREE

EXPERIMENTAL

3.0 GENERAL INFORMATION

All chemicals used were of laboratory grade and were purchased from Sigma Aldrich. Melting points were determined with a Fischer Johnôs apparatus and were uncorrected. UV/Visible spectra were recorded on UV-25500PC series spectrophotometer using matched 1cm quartz cells. The IR spectra were recorded on 8400S FT-IR spectrometer using KBr disc. ^{1}H-NMR and ^{13}C-NMR spectra were scanned at the University of Stratchclyde, Scottland on Joel FX 90Q spectrometer using TMS as internal standard (chemical shift in ŭ). Microanalysis was carried out on a CHN rapid analyzer. The antimicrobial screening was done at the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka .

3.1 6-Chloro-l0-nitro-5*H*-benzo[a]phenoxazin-5-one (136)

A mixture of 2-amino-4-nitrophenol (2.0 g, 13 mmol) and anhydrous sodium acetate (2.0 g, 24 mmol) was suspended in a solution of benzene (100 mL) and DMF (10 mL) in a 250 mL two-necked reaction flask fitted with a magnetic stirrer, a thermometer and a reflux condenser. The suspension was warmed in a water bath for 1 h with stirring for complete dissolution. 2,3-Dichloro-I,4-naphthoquinone (3.0 g, 13 mmol) was added and the stirring continued under reflux for 6 h at 70-78 ^{o}C. At the end of reaction, benzene was distilled off in vacuum while the slurry was poured into water and stirred to dissolve the inorganic salt. The mixture was left overnight and then filtered and recrystallized from methanol-acetone to give 6-chloro-10-nitro-5*H*-benzo[a]phenoxazin-5-one as a brown powder; m.p > 260 ^{o}C (6.3 g, 80%). UV-visible (MeOH) λ_{max} (nm) log(Ů: 304 (2.569), 344 (2.593), 711 (1.736), 759 (1.750); IR

(KBr) V_{max}: 3097 (aromatic C-H str.), 1653 (C=O str.), 1581 (-NO_2 str.), 1533, 1458 (Ar C=C str.), 1329, 1250 (C-N str.); ^{1}H-NMR (DMSO) ŭ: 8.10 -7.92 (4H, m); ^{13}C-NMR (DMSO) ŭ: 117.4, 119.1, 121.2, 122.0, 124.7, 126.4, 130.8, 131.1, 131.5, 134.5, 137.3, 140.5, 143.4, 175.3. Analysis: Calculated for $C_{16}H_7N_2O_4Cl$: C, 58.82; H, 2.16; Cl, 10.35; N, 8.57; Found: C, 58.95; H, 2.21; Cl, 10.98; N, 8.70.

3.2 14-Nitrobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (137)

2-Aminothiophenol (1.0 g, 8 mmol) and anhydrous sodium carbonate (1.0 g, 9 mmol) were weighed into a 250 mL reaction flask attached with a reflux condenser and thermometer. A solution of benzene (100 mL) and DMF (10 mL) was added and the mixture warmed in a water bath with stirring for 1 h for complete dissolution. 6-Chloro-10-nitrobenzo[a]phenoxazin-5-one (2.42 g, 8 mmol) was later added and the mixture was refluxed with constant stirring for 9 h. Benzene was distilled off and the slurry was poured into water, heated to near boiling with stirring to dissolve the inorganic salt. The mixture was chilled and filtered to obtain yellow residue which was recrystallized from methanol-acetone to give 14-nitrobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine as dark brown powder; mp > 300 ^{o}C (2.4 g, 90%). The UV-visible (MeOH) λ_{max} (nm) log (Ů: 343 (1.887), 670 (1.371), 758 (1.329); IR (KBr) V_{max}: 3381, 3300 (-NH str.), 3091(aromatic C-H str.), 1640 (C=N str.), 1587 (-NO_2) 1519, 1465 (ArC=C str.), 1321, 1244, 1140, 1076, 1076, 685 cm^{-1}; ^{1}H-NMR (DMSO) ŭ: 7.87 (2H, d), 7.08 (3H, m); ^{13}C-NMR (DMSO) ŭ: Few peaks, due to insolubility of the compound. Analysis: Calculated for $C_{22}H_{11}N_3O_3S$: C, 66.49; H, 2.79; N, 10.57; S, 8.07; Found: C, 66.70; H, 2.82; N, 10.63; S, 8.13.

3.3 11-Amino-6-chloro-9-thiol-8,10-diazabenzo[a]phenoxazin-5-one (148)

4,5-Diamino-6-hydroxypyrimidin-2-thiol (3 g, 18 mmol) and anhydrous sodium acetate (1.7g, 20 mmol) were suspended in a solution of benzene (80 mL) and DMF (10 mL) in a 250 mL two-necked reaction flask equipped with a magnetic stirrer and a reflux condenser. The mixture was warmed in a water bath for 1 h. 2,3-Dichloro-1,4-naphthoquinone (4.3 g, 18 mmol) was added and the stirring continued with refluxing for 6 h at 70-78 ^{o}C. The colour of the reaction mixture changed from brown to intense red as the reaction progressed. The solvent was distilled off and the slurry was poured into water and stirred for 20 min to dissolve the inorganic salt. The mixture was chilled overnight, filtered and recrystallized from methanol-acetone to afford 11-amino-6-chloro-9-thiol-8,10-diazabenzo[a]phenoxazin-5-one as reddish crystals; m.p > 290 ^{o}C (1.5 g, 70%). UV- visible (MeOH) ə-max (nm) log (Ů): 327 (1.08), 418 (1.36), 507 (1.00); IR (KB_r) V_{max}: 3404, 3308 (-NH_2), 3075 (aromatic C-H str), 1645 (C=O), 1573 (C=N str), 1450 (ArC=C str), 1037, 899, 748 cm^{-1}. Analysis: Calculated for $C_{14}H_7N_4O_2SCl$: C, 50.79; H, 2.12; Cl, 10.73; N, 16.93; S, 9.68; Found: C, 51.02; H, 2.01; Cl, 10.79; N, 16.70; S, 9.68.

3.4 15-Amino-13-thiol-12,14-diazabenzo[a][1,4]benzoxazino[3,2-c]phenothazine (149)

2-Aminothiophenol (1.0 g, 8 mmol) and anhydrous sodium carbonate (1.0 g, 9 mmol) were suspended in a solution of benzene (80 mL) and DMF (10 mL) in a 250 mL reaction flask fitted with a magnetic stirrer and a reflux condenser. The mixture was boiled for 1 h with stirring in a water bath for dissolution. 11-Amino-6-chloro-9-thiol-8,10-diazabenzo[a]phenoxazin-5-one (2.2 g, 8 mmol) was then added and the entire mixture refluxed for 9 h with continuous stirring. At the end of the reflux period, benzene was distilled off and the slurry was poured into water, heated to near boiling, filtered and the residue recrystallized

from methanol-acetone to give 15-amino-13-thiol-12,14-diazabenzo[a][1,4]benoxazino[3,2-c]phenothiazine as purple red crystals; m.p > 320 ^{o}C (2.0 g, 80%). UV-visible (MeoH) λ_{max} (nm) log (Ů): 314 (2.668), 373 (2.472), 379 (2.472), 482 (2.452); IR (KBr) V_{max}: 3398, 3320 (-NH_2), 3061 (aromatic C-H str.),1635 (C=N str.), 1506, 1443 (ArC=C str.), 1304, 1250, 759, 681, 546 cm^{-1} ; ^{1}H-NMR (DMSO) ŭ: 7.08-7.00 (4H, m), 4 .45 (SH, 1H, s); ^{13}C-NMR (DMSO) ŭ: There were few peaks due to difficulty in the solubility of the compound. Analysis: Calculated for $C_{20}H_{11}N_5OS_2$: C, 59.83; H, 2.76; N, 17.44; S, 15.97; Found: C, 59.96; H, 2.98; N, 17.56; S, 15.60.

3.5 6,8-Dichlolorobenzo[a]phenoxazin-5-one (160)

2-Amino-6-chlorophenol (3.0 g, 21 mmol) and anhydrous sodium acetate (1.7 g, 20 mmol) were suspended in a solution of benzene (100 mL) and DMF (10 mL) in a 250mL reaction flask equipped with a magnetic stirrer and a reflux condenser. The mixture was boiled for 1 h at 75-80 ^{o}C. 2,3-Dichloro-1,4-naphthoquinone (4.8 g, 21 mmol) was added and the entire solution was refluxed with continuous stirring for 6 h at 75- 80 ^{o}C . The solvent was removed by distillation, the slurry was poured into water and stirred to dissolve the inorganic salt. The mixture was chilled, filtered and recrystallized from methanol-acetone to give 6,8-chlorobenzo[a]phenoxazin-5-one (2.83 g, 80%) as orange powder with melting point of 265 ^{o}C. Uv-visible λ_{max} (MeOH) (nm) log(Ů): 283 (2.00), 352 (2.57); IR (KBr) V_{max}: 3320 (N-H str.), 3090 (C-H str.), 1663 (C=O str.), 1572 (C=N str.), 1477 (ArC=C str.), 1269, 1130, 824 cm^{-1}. Analysis: Calculated for $C_{16}H_7NO_2Cl$: C, 60.76; H, 2.22; Cl, 22.47; N, 4.40; Found: C, 59.92; H, 2.31; Cl, 22.51; N, 4.40.

3.6 12-Chloro-9-methoxy-8-azabenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (161)

A mixture of 3-amino-6-methoxypyridin-2-thiol (1.5 g, 10 mmol) and sodium carbonate (1.0 g, 9 mmol) were poured into a 250 mL reaction flask equipped with a magnetic stirrer, thermometer and a reflux condenser. A solution of benzene (80 mL) and DMF (10 mL) was added. The mixture was boiled in a water bath for 1 h. 6,8-Dichlorobenzo[a]phenoxazin-5-one (2.8 g, 10 mmol) was added and the entire mixture was refluxed with constant stirring for 9 h at 75-80 ^{o}C. Benzene was distilled off while the slurry was poured into water, boiled and stirred to dissolve inorganic materials. The mixture was left overnight, filtered and the orange residue recrystallized from methanol-acetone to give 12-chloro-9-methoxy-8-azabenzo[a][1,4]benzoxazino[3,2-c]pheonthiazine as orange yellow powder; m.p > 350 ^{o}C. UV-visible (MeOH) λ_{max} (nm) log(ε) : 284 (1.46), 336 (2.15), 467 (2.27); IR (KBr) V_{max}: 3080 (aromatic C-H str), 1641, 1586 (C=N str), 1279, 1080, 1017, 469 cm^{-1}; ^{1}H-NMR (DMSO) δ: 8.66- 8.10 (4H, m), 7.92 (4H, s); ^{13}C-NMR (DMSO) δ: 150.31-115.36 for aromatic carbons. Analysis: Calculated for $C_{22}H_{12}N_3O_2ClS$: C, 63.23; H, 2.89, Cl, 8.48, N, 10.06, S, 7.6; Found: C, 63.46; H, 2.93; Cl, 8.64; N, 10.12; S, 7.60.

3.7 Antimicrobial Activity

The antimicrobial screening of the synthesized compounds was carried out in form of the general sensitivity test and minimum inhibitory concentration (MIC) with respect to freshly cultured targeted micro-organisms. The micro-organisms used in this study were three gram positive bacteria *(Bacillus cereus, staphylococcus aureus, Bacillus subtilis*), three gram negative bacteria *(Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa) and* two fungi *(Asperigellus niger and Candida albicans)*.

3.8 Sensitivity testing of compounds

The agar cup diffusion method as described by Vincent (2005)[80] was used to determine the antimicrobial activity of the synthesized compounds. The sensitivity test agar plates were seeded with 0.15 mL of overnight culture of the relevant micro-organism. The seeded plates were allowed to set after which cups were made in each sector previously drawn on the backside of the plate using a marker. Using a sterile pipette, each cup was filled with six drops of their corresponding synthesized compounds (2mg/mL). The solubilizing solvent was DMF and all the plates were incubated at 40 ^{o}C for 24 h for bacteria and 48 h for fungi. Zones of clearance around each showed inhibition and the diameter of such zones was measured. The procedure was repeated for ciprofloxacin, ketoconazole and solvent (DMF). Muller hinton agar was used for the fungi in place of nutrient agar for bacteria.

3.9 Minimum inhibitory concentration of compounds (mg/mL)

Agar well diffusion method as described by Vincent (2005)[80] was used to determine the minimum inhibitory concentration (MIC) of the ciprofloxacin and ketoconazole. Serial dilutions of the synthesized compounds were prepared from 2mg/mL solution of the derivatives to give 2.0-0.125mg/ml. Six drops of each dilution were added to the corresponding cup of seeded microorganism and the agar marked previously. The plates were incubated at appropriate incubation temperature and time .The diameter of zone of inhibition was recorded and the value subtracted from the diameter of the borer (8 mm) to give the inhibition zone diameter (IZD). The graph of IZD^2 against the logarithm of concentration was plotted for each plate containing a specific compound and a micro-organism. The anti-logarithm of the intercept on X-axis gave the MIC.

CHAPTER FOUR

RESULTS AND DISCUSSION

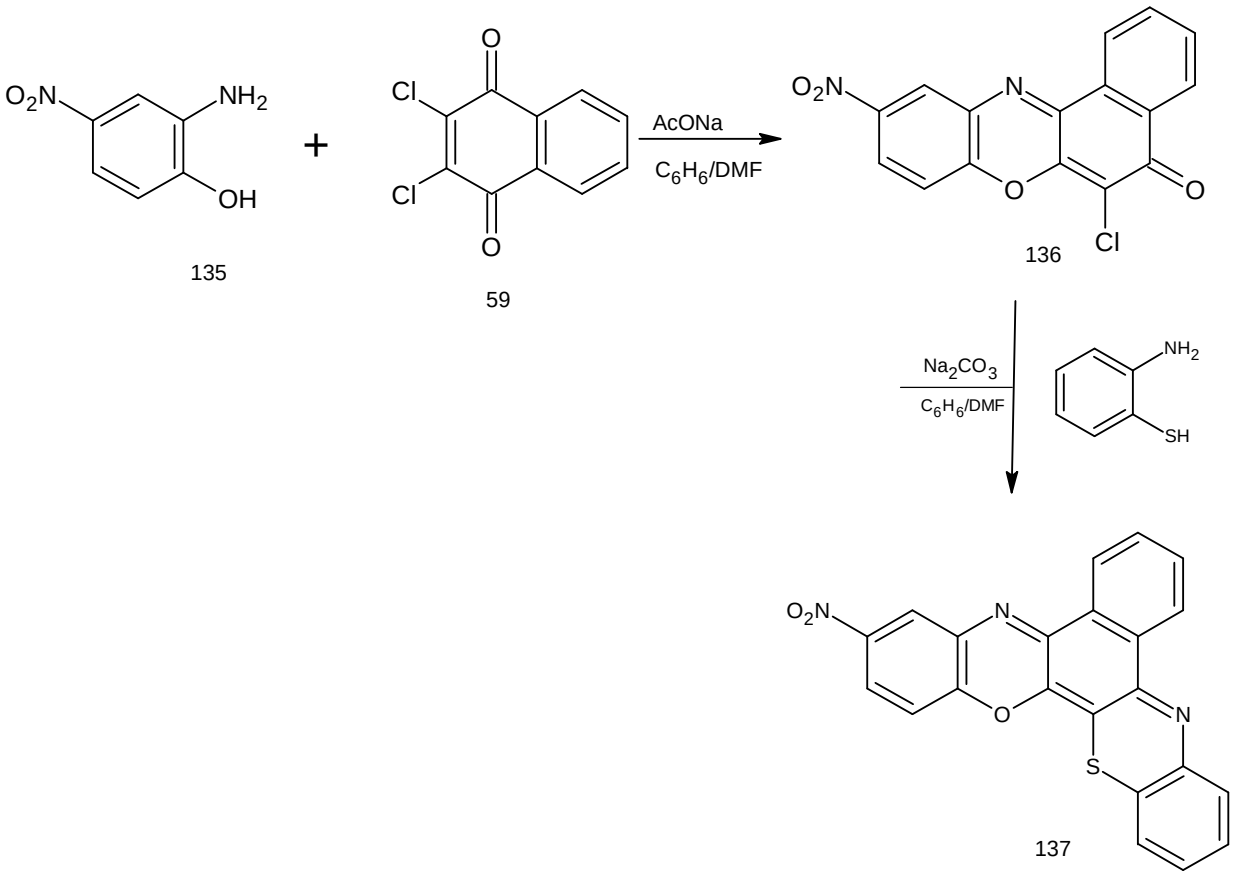
All the reactions that resulted in the formation of the intermediates and the desired complex derivatives were carried out in alkaline medium.

4.1 6-Chloro-10-nitro-5*H*-benzo[a]phenoxazin-5-one (136)

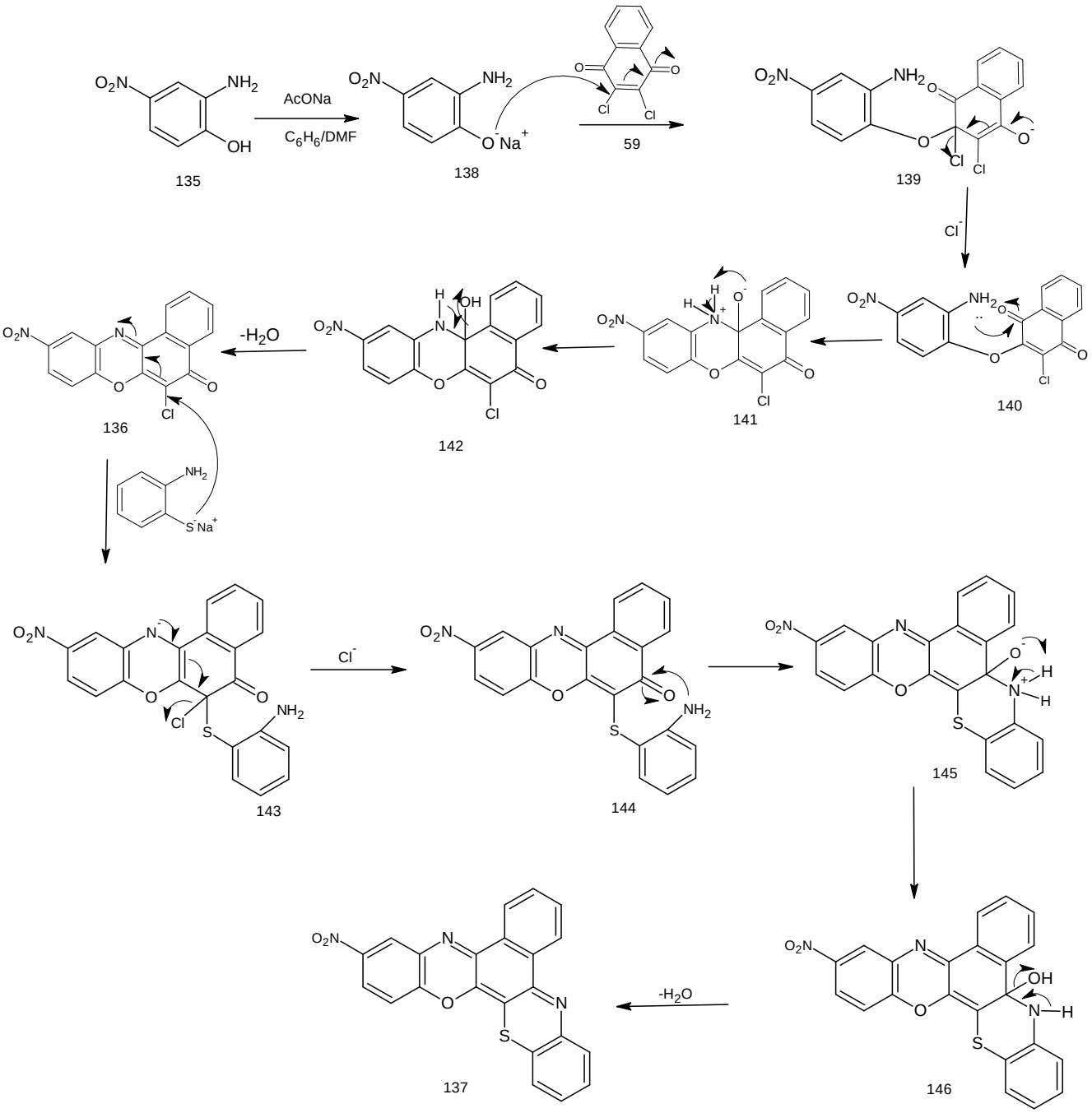
When 2-amino-4-nitro-thiophenol (135) was treated with 2,3-dichloro-1,4-naphthoquinone (59) in basic medium, 6-chloro-10-nitro-5*H*-benzo[a]phenoxazin-5-one (136) was obtained.

The assigned structure agreed with the spectral data. UV-visible maximum absorption band at 759 nm (logŮ1.750) was in agreement with its colouration due to extensiveconjugation. The infrared spectrum gave signals at 3097 cm^{-1} (aromatic C-H str.), 1653 cm^{-1} (C=O str.), 1581 cm^{-1} (-NO_2 str.), 1533, 1458 cm^{-1} (Ar C=C str.). In ^{1}H-NMR, the absorption bands at 8.10 -7.92 (4H, m) were assigned to aromatic protons. In the ^{13}C-NMR, a visible peak at 176 showed the presence of a carbonyl group C=O; the peaks at 142-127 were assigned to aromatic carbons. The elemental analysis was consistent with the molecular formula, $C_{16}H_7N_2O_4Cl$.

Further treatment of (136) with 2-aminothiophenol gave the complex derivative, 14-nitrobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (137). The UV-visible absorption band at 758 nm (logŮ1.329) was as result of its extensive conjugation. In the IR spectra, the band at 3381 cm^{-1} (-NH), 3092 cm^{-1} (aromatic C-H), 1640 cm^{-1} (C=N), 1587 cm^{-1} (-NO_2) were in agreement with the assigned structure. The disappearance of band due to C=O was an evidence for a second condensation and cyclization leading to the formation of complex derivatives. In ^{1}H-NMR, the peaks at 7.87 (2H, d) were due to aromatic proton. The elemental analysis was in good agreement with the molecular formula, $C_{22}H_{11}N_3O_3S$.


O2N
NH2
OH
135
Cl
Cl
O
O
59
AcONa
C6H6/DMF
O2N
N
O
O
Cl
136
Na2CO3
C6H6/DMF
NH2
SH
O2N
N
O
N
S
137

Scheme 1



Scheme 2

The mechanism of reactions involves proton abstraction from 2-amino-4-nitrophenol by base to generate the phenoxide (138), which then initiated a nucleophilic attack on 2,3-dichloro-

1,4-naphthoquinone to form charged intermediate (139), leading to loss of chloride ion to form the diaryl intermediate (140). A second nucleophilic attack from the amino group on the carbonyl group resulted in cyclisation to generate structure (142), followed by elimination of a molecule of water to give 6-chloro-10-nitro-5*H*-benzo[a]phenoxazin-5-one (136). Again, (136) was attacked by 2-aminothiophenol to generate a resonance stabilized (143) as an intermediate followed by elimination of a chloride ion to form (144). The intramolecular nucleophilic attack from the amino group resulted in cyclisation to generate structure (146), which on elimination of water molecule yielded the derivative (137). (**Scheme 2).**

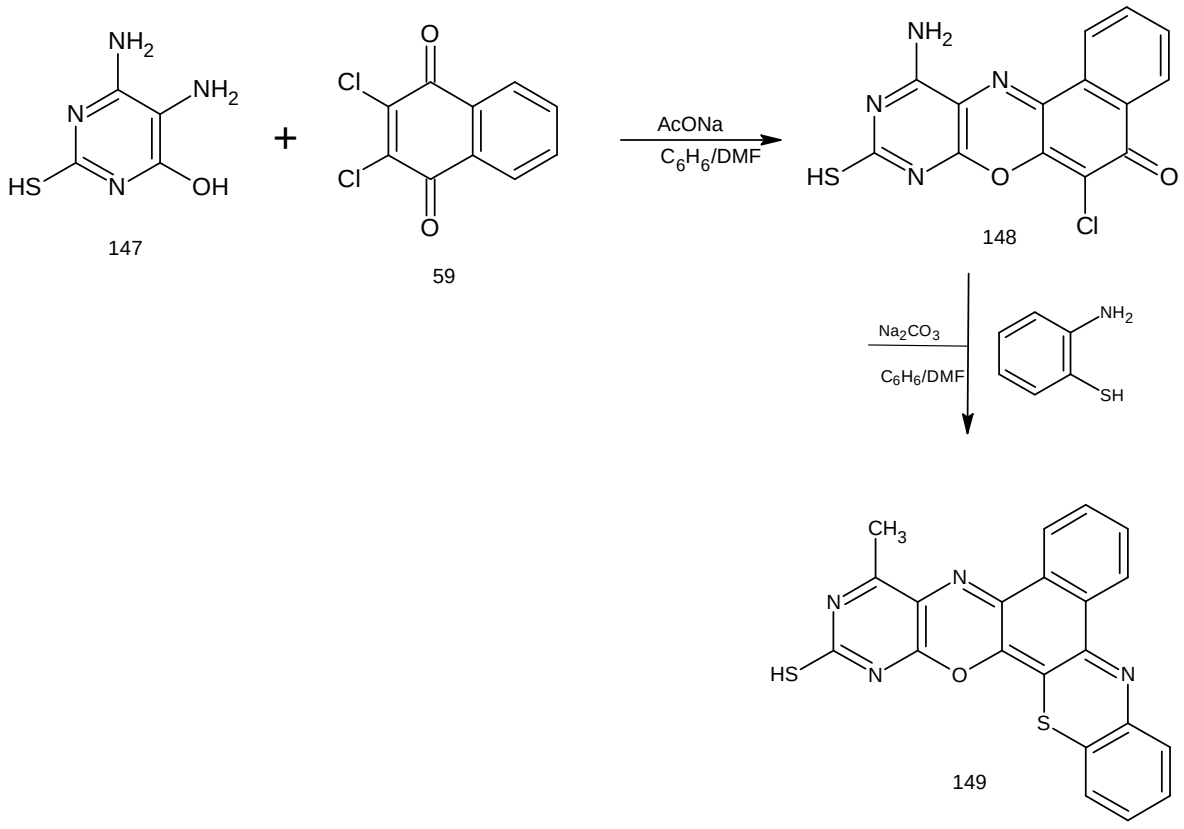
4.2 11-Amino-6-chloro-9-thio-8,10-diabenzo[a]phenoxazin-5-one (148)

When 4,5-diamino-6-hydroxypyrimidin-2-thiol (147) was refluxed with 2,3-dichloro-1,4-naphthoquinone (59) in basic medium, 11-amino-6-chloro-9-thiol-8,10-diazabenzo[a]phenothiazine (148) was furnished.

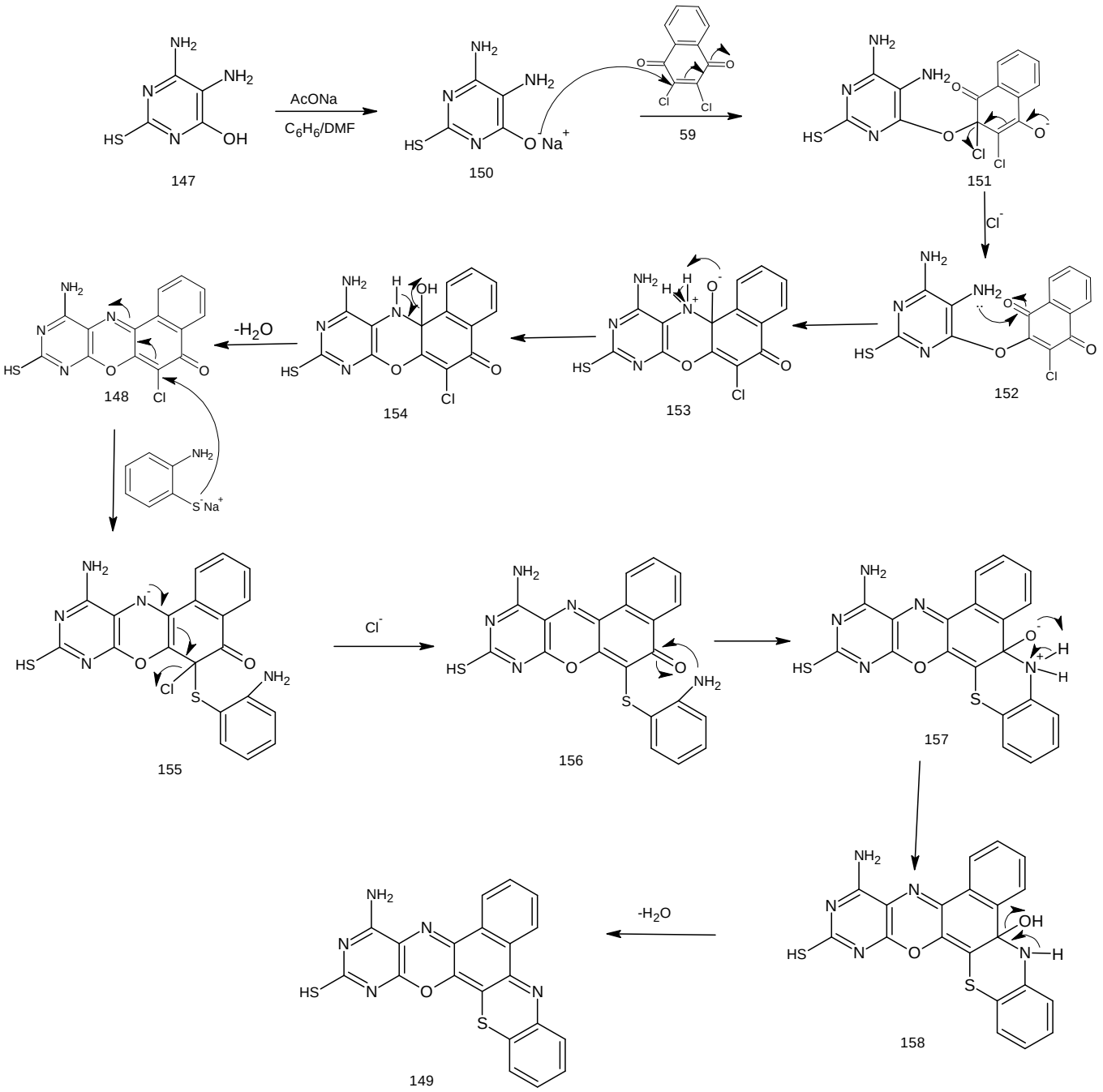
The structure was assigned based on the following spectral information and elemental analysis. UV maximum absorption band at 507nm (logŮ1.00) agreed with the observed colour. In the IR spectrum, the bands at 3404, 3308 (-NH_2), 3075 (aromatic C-H str), 1645 (C=O), 1573 (C=N str), 1450 (ArC=C str) were in agreement with the assigned structure. The result obtained from the elemental analysis of the compound is in agreement with the molecular formula, $C_{14}H_7N_4O_2SCl$.

Further treatment of (148) with 2-aminothiolphenol gave the complex derivative, 15-amino-13-thiol-12,14-diazabenzo[a][1,4]benzoxazino[3,2-c]phenothiazine (149). UV-visible absorption band at 482 nm (logŮ2.452) agreed with the colour. The IR spectrum showed peaks at 3398 cm^{-1} (NH_2), 3061 cm^{-1} (aromatic C-Hstr.), 1636 cm^{-1} (C=N str.), 1506 cm^{-1} (ArC=C str.) that were

consistent with the structure of the compound. In the ^{1}H-NMR, the peak at ŭ7.08 (4H, m) were due to aromatic protons; ŭ12.15 and ŭ8.53 were assigned to the proton of ï SH and ï NH_2 groups respectively. The elemental analysis of the compound was in agreement with the molecular formula, $C_{20}H_{11}N_5OS_2$.



Scheme 3


AcONa
C6H6/DMF
59
147
150
151
152
153
154
148
155
156
157
158
149
-H2O
-H2O
Cl-

Scheme 4

The mechanism of the reaction followed the same pattern of proton abstraction from 4,5-diamino-6-hydroxypyrimidine-2-thiol (147) by the base leading to the formation of aryloxide (150) which mounted a nucleophilic attack on the 2,3-dichloro-1,4-naphthoquinone (59) by displacing the reactive halogen group to form the diaryl intermediate (152). This is followed by another nucleophilic attack of the carbonyl carbon centre by the amino group resulted in cyclisation and formation of another intermediate (154), which on elimination of water molecule gave 11-amino-6-chloro-9-thiol-8,10-diabenzo[a]phenoxazin-5-one (148). Structure (148) was attacked by 2-amiothiophenol ion to form a resonance stabilized (155), followed by elimination of chloride ion to generate (156). The intramolecular attack from the amino group on the carbonyl group led to cyclisation and formation of structure (158). Final elimination of water molecule gave the desired compound (149). (**Scheme 4).**

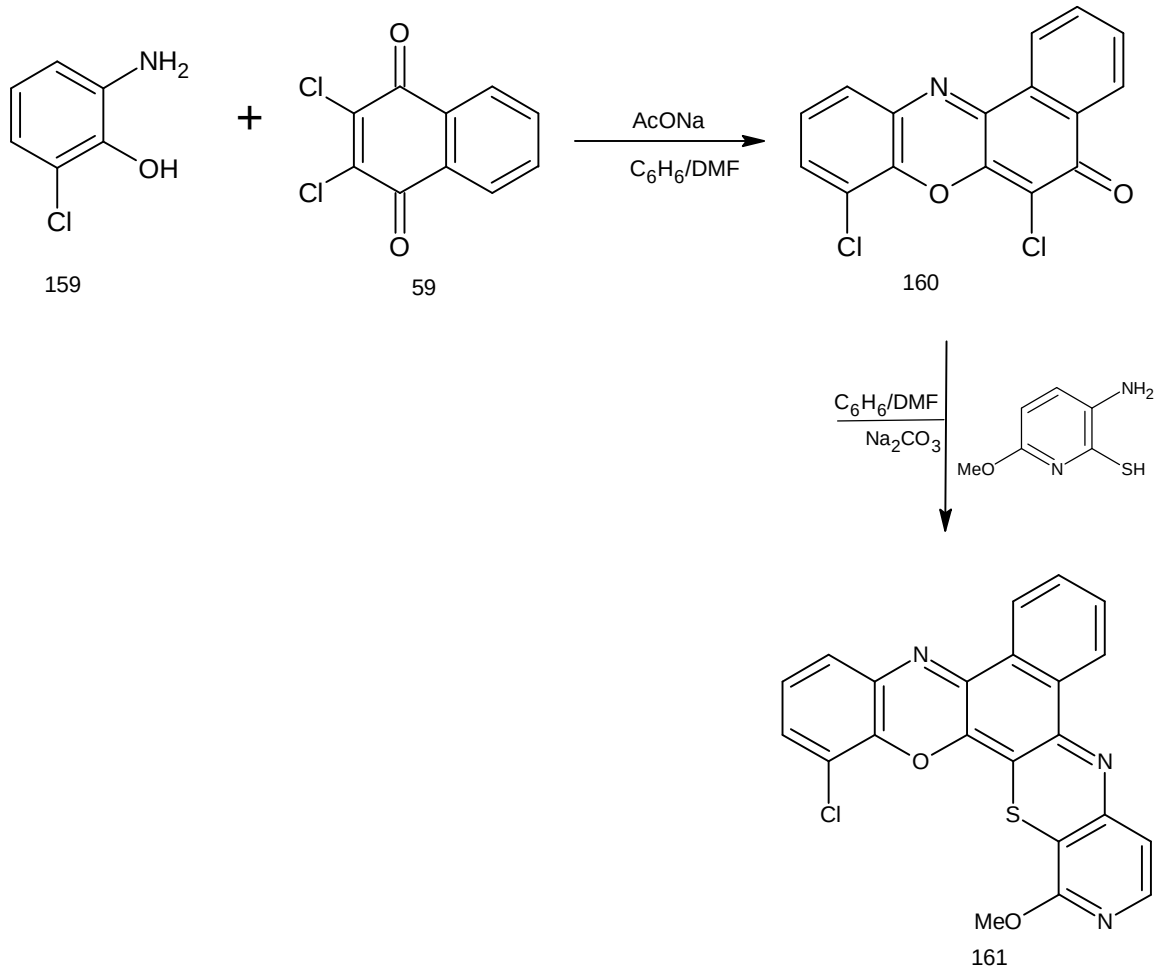
4.3 6,8-Dichlorobenzo[a]phenoxazin-5-one (160)

6,8-Dicholorobenzo[a]phenoxazin-5-one (160) was obtained when 2-amino-6-chlorophenol (159) was treated with 2,3-dichloro-1,4-naphthoquinone (59) in a basic medium.

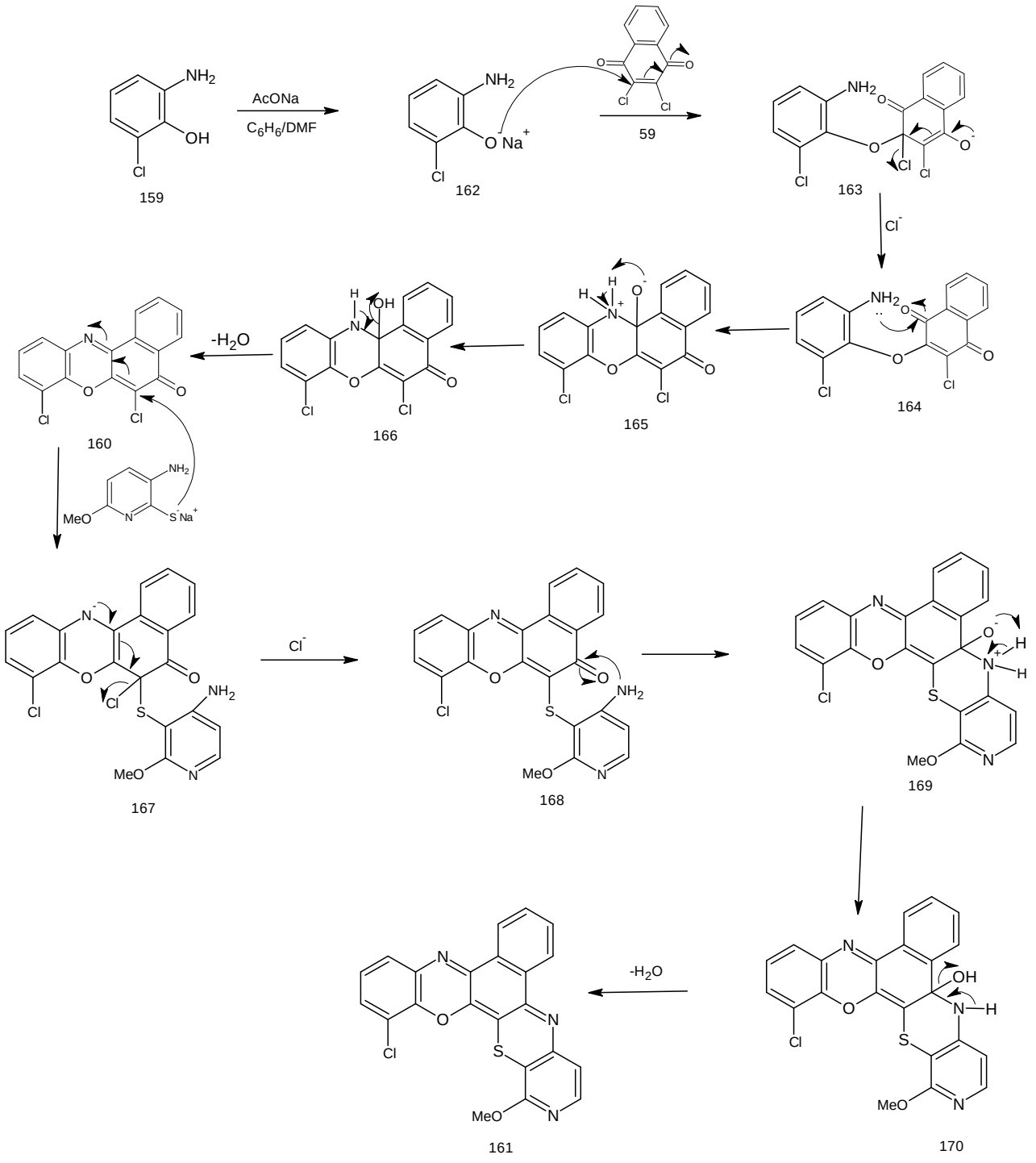
The UV-visible absorption band at 352 nm (logŮ2.57) agreed with the colour. The IR spectrum showed peaks at 3320 cm^{-1} (NH_2), 3090 cm^{-1} (aromatic C-H str.), 1636 cm^{-1} (C=O str.), 1572 cm^{-1} (C=N str) that were consistent with the structure. The analysis of the compound also agreed with the molecular formula, $C_{16}H_7NO_2Cl$.

Further treatment of (160) with 3-amino-6-methoxypyridine-2-thiol gave the complex compound, 12-chloro-9-methoxy-8-azabenzo[a][1,4]benzoxazino[3,2-c] (161). The visible maximum absorption band at 467 nm (logŮ2.27) was as a result of high conjugation. In the infrared spectrum, the bands at 3080 cm^{-1} (aromatic C-H str.) were in agreement with the structure. In ^{1}H-NMR, the peaks at 8.66 (4H, m) were for aromatic protons. The peaks due to the two protons in pyridine ring of the compound (161) occurred between ŭ7.66-7.01 and the -

OCH_3 protons in the compound showed a singlet in the region of ŭ2.40-4.28 (3d, s). In ^{13}C-NMR, the absorption band at ?135.22-127.64 due to aromatic carbons. The elemental analysis of the complex derivative is in agreement with the molecular formula, $C_{22}H_{12}N_3O_2ClS$.



Scheme 5


AcONa
C6H6/DMF
159
162
59
163
164
165
166
160
-H2O
167
168
169
170
161
-H2O

Scheme 6

The reaction mechanism followed the usual pattern of proton abstraction from 2-amino-6-chlorophenol by the base and formation of aryloxide (162), which initiated a nucleophilic attack on the C-Cl center of 2,3-dichloro-1,4-naphthtoquinone by displacing the chloride ion to form the diaryl intermediate (164). Cyclisation took place by a second nucleophilic attack from the amino group on the carbon atom of the carbonyl group to generate the second intermediate (166), followed by elimination of water molecule to furnish 6,8-dichlorobenzo[a]phenoxazin-5one (160). Structure (160) was attacked by the mercaptide ion to generate a resonance stabilized (167), which on elimination of chloride ion formed (168). The intramolecular nucleophilic attack from the amino group on the carbonyl group resulted in cyclisation of structure (170) with the elimination of water molecule to furnish the expected derivative (161). (**Scheme 6).**

4.4 Results of Antimicrobial sensitivity test of synthesized compounds (Table 1)

Compounds	Gram-negative Bacteria			Gram-positive Bacteria			Fungi Organism	
	B.cereus	*B. subtilis*	*S. aureaus*	*E. coli*	*P. aeruginosa*	*K pneumonia*	*C. albicans*	*A. niger*
16	++	+	+	++	+	-	++	-
17	+++	+	+	+	+	-	+++	+
19	+	++	-	-	++	-	+	+
24	++	+	-	-	+	-	-	++
27	-	+	++	+++	++	++	+	+
44	+	++	+	+	+	+	+	+++
Rf 1	+	++	+	+++	++	+++	-	-
Rf 2	-	-	-	-	-	-	+++	++

+ = Sensitive	Rf I, Rf 2 (reference drugs)
++ = Moderately Sensitive	Rf 1 (ciprofloxacin, antibacterial)
- = Resistance	Rf 2 (ketoconazole, antifugal)

The assay was conducted by applying agar-well diffusion method[80] using a concentration of 20 mg/mL of each compound. From the result (Table 1**)**, most of the compounds showed significant activity against the test organisms except *K.pneumonia* which was only sensitive to compound **27** and **44.** *B.subtili*s was sensitive to all the compounds. *S.aureaus* and *E.coli* were resistant to compound **19** and **24** but were sensitive to other compounds.

4.5 Minimum Inhibitory Concentration (MIC) Determination

This was carried out using agar dilution following the procedure outlined by Chemical Laboratory Standards Institute (CLSI) [33] using the following serial dilutions: 1.0, 0.5, 0.25, 0.125 and 0.0625 mg/mL. Almost all the synthesized derivatives were active against the micro-organisms even at very low concentrations, consistent with the dictum that the lower the MIC values, the higher the activity. Compound **17** has the lowest MIC values against bacteria (*B cereus, B. subtitis, S. aureus and E. coli*) which ranged from 0.0457- 0.1040 mg/mL (Table 2**)**. There was no MIC for compound **19** and **24** against *S.aureaus* and *E.coli.* In fungi organisms, *C.albican* was only resistant to compound **24** but others were active against the organism and all the compounds were active against *A.niger* except compound **16**. All the MIC values of the synthesized compounds were well above the reference drugs except for compound **27** where its value was below against *P. aeruginosa.* (see table 2).

4.6 Results of minimum inhibitory concentration of compounds (mg/ml)

Compound	Gram-negative Bacteria			Gram-positive Bacteria			Fungi Organism	
	B.cereus	*B. subtilis*	*S. aureaus*	*E. coli*	*P. aeruginosa*	*K.. pneumonia*	*C. albicans*	*A. niger*
16	0.1660	0.1380	0.1318	0.144	0.1738	-	0.151	-
17	0.0457	0.0758	0.0794	0.104	0.1258	-	0.085	0.1320
19	0.1254	0.1324	-	-	0.6996	-	0.085	0.1311
24	0.1445	0.1850	-	-	0.0920	-	-	0.069
27	-	0.1905	0.0501	0.1250	0.0661	0.1580	0.158	0.082
44	0.1585	0.1445	0.1820	0.0865	0.1819	0.1240	0.120	0.1546
Rf 1	0.0335	0.0567	0.0212	0.021	0.1677	0.0389	-	-
Rf 2	-	-	-	-	-	-	0.073	0.2220

CHAPTER FIVE

CONCLUSION

The complex benzoxazinophenothiazine derivatives were synthesized. The compounds were characterized by UV-visible, FT-IR, (^{1}H and ^{13}C-NMR) spectroscopy and elemental analysis. The complex derivatives prepared in this work could served as dyes due to its conjugation and are intensely coloured. The antimicrobial screening of the compounds were carried out on freshly cultured targeted micro-organisms using agar cup diffusion method and the results showed that the complex derivatives were active against the micro-organisms when compared with ciprofloxacin as anti-bacterial agent and ketoconazole as an anti-fungal agent. Therefore we can conclude that the compounds which showed high activity can be recommended for preclinical screening.

REFERENCES

1. Sharma, R., Samadhhiya, P. and Srivastava, S.D., (**2011**); Synthesis and biological activity of 2-oxo-azetidine derivatives of phenothiazin, *Org Commun*, 4(2):42-51.

2. Khanna, R., Palit, G., Srivastava, V.K. and Shanker, K., (**1990**); Newer Heterocycles of phenothiazine and their Antiparkinsonian Activity, *India Chem*, 29B:556-560.

3. Meghasham, N.N., Mahesh, K.G. and Pravin, K.G., (**2014**); Microwave mediated Synthesis of Pharmacological Active Substituted Derivatives of 2-94-Phenothiazinyl Pyrazoyl) Pyrrols. *World Journ of Pharm Res*, 3(4):1064-1073.

4. El-Said, M.K., (**1981**); Synthesis of New Phenothiazine Derivatives as potential tranquilizers and sedatives, Pharmazie, 36: 678.

5. Tilak, S.R., Tyagi, R., Goel, B. and Saxena, K.K., (**1988**); Synthesis and Anti-inflammatory activity of some potential cyclic phenothiazines, *India Drugs* 35: 221.

6. Sadanandam, Y.S., Shetty, M.M. and Rao, A.B., (**2009**); Rambabau Y, 10H-Phenothiazines: A new class of enzyme inhibitors for inflammatory diseases, *Eur J Med Chem*, 44:197-202.

7. Rajasekaran, A. and Thampi, P.P., (**2003**); Synthesis and anti-inflammatory activity of some 10 [(1-acyl-1htetrazol-5yl)]ethyl-10-phenothiazine, *Acta Pharm*, Turcica, 4:227-232.

8. Dominguez, J.N., Lopez, S., Iarruso, L., Lobo, G., Semenow, A., Olson, J.E., and Rosenthal, P.J., (**1997**); Synthesis and Antimalarial effects of phenothiazine inhibitors of Plasmodium Falciparium Cystein Protease, *J Med Chem*. 40:2726-2732.

9. Kalkanidis, M., Klonis, N., Tiley, L. and Deady, L.W., (**2002**); Novel phenothiazine Antimalarial: Synthesis, antimalarial activities and inhibition of th formation of beta-haematin. *Biochem Pharmcol***,** 63(5):833.

10. Lin, G. and Medha, K.K., (**1991**); Synthesis of the pipperidinone metabolites of Piperidine-type phnothiazine Antipsychotic Drugs via Ruthenium tetraoxide oxidation, *J Heterocycl Chem*, 28:215.

11. Mohamed, F.A., Mohamed, H.A., Hussein, S.A. and Ahmed. S.A., (**2005**); A Validated spectrofluorimetric method for determination of some psychoactive drugs, *J Pharm Biomed Analysis*, 39:139-146.

12. Wen, B. and Zhou, M., (**2009**); Metabolic activation of the phenothiazine antipsychotics chlorpromazine and thioridazine to electrophilic iminoquinone species

in human liver microsomes and recombinant P450s, *Chemico-Biol Interac*, 181:220-226.

13. Raval, J. and Desai. K.K., (**2005**); Synthesis and Antimicrobial activity of new Triazolopyrindnyl phenothiazines ARKIVOC, 8:21.

14. Srivastava, S.K., Dua, R. and Srivastava, S.D., (**2010**); Synthesis and antimicrobial activity of [*N*1-*N*-substituteddiarylides-hydrazin0)-acetyl]-2-methyl-imidazoles and *[N1*-(4-substitutedaryl-3-chloro-2-oxo-1-azetidinyl-amino)-acetyl]-2-methyl-imidazoles, *Proc. Nat, Acad Sci India, Sec A, Phys Sci,* 80:117-121.

15. Trivedi, P.B., Undavia, N.K., Dave, A.M., Bhatt, K.N. and Desai, N.C., (**1993**); Synthesis and antimicrobial activity of 4-oxothiazolidines, 4-oxoazetidines, malonailic acid hydrazine and pyrazoline derivatives of phenothiazine, *India J Chem*, 32B **(7)**:760-765.

16. Viveos, M. and Amaral, L., (**2001**); Design, Synthesis, characterization and Antitubarcular activity of some 2-Heterocycle-substituted phenothiazine, *Int J Antimicrob Ag,* 17:225.

17. Amaral, L. and Kristiansen., (**2001**); Phenothiazines: An Alternative to conventional therapy for the intial management of suspected multidrug resistant tubercular activity of some 2-heterocycle-substituted phenothiazines, *Int J Antimicrob Ag***,** 17:225.

18. Madrid, P.B., Polgar, W.E., Toll, L. and Tanga, M.J., (**2007**); Synthesis and antitubercular activity of phenothiazines with reduced binding to dopamine and serotonin receptors, *Bioorg Med Chem Let***,** 17:3014-3017.

19. Motohasho, N., Kawase, M., Saito, S. and Sakagami, H., (**2000**); Antitumor potential and possible targets of phenothiazine-related compounds, *Curr Drug Targets*, 1:237-245.

20. Motohasho, N., Kawase, M., Saito, S., Kurihara, T., Satoh, K., Nakashima, H. and Molmar, J., (**2000**); Synthesis and biological activity of N-acylphenothiazine, *Int J Antimicrob Ag*, 14(3):203-207.

21. Leancer, D. and Mitscher, L.A., (**1977**); Organic Chemistry of Drug Synthesis, Vol. I, John Wiley & sons, New York, pp 372-392

22. Kubota, K., Kurebayashi, H., Miyachi, H., Tobe, M., Onishi, M. and Isobe, Y., (**2009**); Synthesis and structure-activity, relationships of phenothiazine carboxylic acids having pyrimidine-dione as novel histamine H(1) antagonists*, Bioorg Med Chem Lett*, 19 (10):2766-2771.

23. Borbely, A.A. and Hinkkanen, M.L., (**1979**); Synthesis and antifugal activity of some substituted phenothiazine and related compounds *Mod Pharmacol Toxicol*, 16:403-426.

24. Korth, L.H., (**2001**); Acridine and phenothiazine derivatives as Pharmacotherapeutics for Prion disease, *National Academy of Science USA*, 98(17):9836-9841.

25. Okafor, C.O., Okerelu, I.O. and Okeke, S.I., (**1986**); Vat Dyes from Three New Heterocyclic Ring, *Dyes and Pigments*, 8:11-24.

26. Porcal, G.V., Previtali, C.M. and Bertolohi, S.G., (**2009**); Industrial Dyes: Chemistry, properties and application, 80:206.

27. Mitchell, S.C., (**1982**); Mammallian metabolism of administered phenothiazine: Drug metabolism Rev, 13:319-343.

28. Murphy, C.M., Rarner, H. and Smith, N.L., (**1950**); Action of phenothiazine-type antioxidant, *Ind Eng Chem*., 42:4279.

29. Floyd, R.A., Scheider, J.E., Zhu, Y.Q., North, T.W. and Schinazi, F., (**1993**); Synthesis of 1,3-Dipole Cycloaddition reactions of *N*-Acetylazido-2-chlorophenothiazine, *Proc Am Assoc Cancer Res,* 34:359.

30. Kurihara, T., Motohashi, N., Sakagami, H. and Molnar, H., **(1999**); Relation between cytotoxin activity and dipole moment for phthalimido and chloroethyl-phenothiazines*, J Anticancer Res*, 19:4081.

31. Kurihara, T., Nojima, K., Sakagami, H., Molnar, H. and Motohashi, H., (**1999**); Electronic structure and cytotoxin activity of half-mustard type Phenothiazine by MM3 and PM3 Methods, *J Anticancer Res***,** 19:3895.

32. Ribbentrop, A. and Schaumann, W., (**1964**); Central Nervous Action of 2-Acetylated phenothiazine and phenoxazines, Chem Abstract, *Arch. Intern. Phermalodyn* 61:9924.

33. Perez, C., Pauli, M. and Bazerque, P., **(1990);** Antibiotic Assay by the Agar-well Diffusion Method- *Acta Biologiae et Medicine Experimentalis*, 15:113-155.

34. Motohashi, N., Sakagami, H. and Molnar, H. and Kurihara, T., (**1999**); Relationship between cytotoxin activity and dipole moment for phthalimido and chloroethyl-phenothiazines, *J. Anticancer Res,* 19:4081.

35. Shimoizu, S., Tomoda, A., Arai, S., Taugachi, H., Hanawa, T. and Kamiya, S., (**2004**); Phenoxazine compounds produced by the reactions with bovine haemoglobin

show antimicrobial activity against non-tuberculosis mycobacteria, Tohoku J. Exp Med 203:47-52.

36. Shimamoto, T., Tomoda, Ishida, R. and Ohyashi, K., (**2001**); Antitumor effects of phenoxazine derivatives on Human Leukemia Cells (AACR), *Amer. Assoc. for Cancer Research, 7:704-708.*

37. Horton, K., Thimmaiah, K.N., Harwood, F.C., Kuttesch, J.F. and Houghton, P. J., (**1993**); Pharmacological characterization of *N*-substituted phenoxazines directed towards reversing Vinca Alkaloid resistance in multidrug resistance cells, 44:552-559.

38. Chu-Daniel, T.U.S., (**1986**); Pyrido[3,2,1-k]phenoxazine and antibacterial use, *Chem. Abstr*., 104,109663K.

39. Holmes, W.C., (**1928**); Certified Biological stains. *Amer. Dyestuff Rept*., 17:626.

40. Martina, H., Jan, S., Anthony, J., Kenneth, I., Thomas, J .and Uwe, H., **(2007**); Phenothiazine synthesis and metallo-chromic properties, *J. of Org Chem.,* 72(18):6714-6725.

41. Mass, H., Khatyr, A. and Calzaferrl, G., (**2003**); Phenoxazine dyes in Zeolite L, synthesis and properties, *Microporous and Mesoporous Material,* 65:233-242.

42. Okafor, C.O., (**1986**); The Chemistry and Applications Of Angular Phenothiazine. *Dyes and Pigments*, **7**(4):249-287.

43. Kehrmann, F. and Christopoulus. T.C., (**1921**); Uber Azithioniumsaize der Naphthaline, *Ber*. Deut.Chem, 15:2879.

44. Goldstein, H. and Indwig-Semelitch, Z., (**1919**); Science of synthesis: Houben-weyl method of molecular transformations, *Chem Abstract*, 2:655.

45. Kehrmann, F. and Goldstein, H., (**1920**); Chemistry of Heterocyclic Compounds, *Arch. Sg* .phys.Nat**.** *Chem. Abstract*,14: 178.

46. Kehrmann, F., Gressly, A. and Chiffere, W., Ram, M., (**1923**); Synthesis of Dibenzo[a,h]phenothiazine, *Beru, Dent, Chem*, 56:469.

47. Lantz, R., (**1934**); Synthesis of Dibenzo[a,h]phenoxazine*, Ann, Chem Abstract*, 29:2745.

48. Shirley, D., Gilmer, J.C. and Water, W.D., **(1964**); Synthesis of Dibenzo[c,h]phenoxazine*, J. Chem. Soc*., 52:60.

49. Okafor, C.O., Akpuaka, M.U. and Eluwa, I.A., **(1992**); Synthesis of Benzothiazinophenoxazine Ring System, *Dyes and Pigment***,** 81-87.

50. Rys, P. and Zollinger, H., (*1972*); Fundamentals of Chemistry and applications of Dye, *Wiley Int. Sci. London*, 17:110-113.

51. Berthsen, A., (**1883**); Zur kenntniss des Methylene blau and Verwandter farbstoffe. *Beru*. *Chem. Ges*, 16:2896-2904.

52. Petrow, V.A. and Rewald, E.I., (**1945**); Synthesis of Heterocyclic compounds (IV): 3-azaphenothiazine*, J. Chem*. *Soc*., 40:591.

53. Okafor, C.O., Uche, I.O. and Akpanisi, L.E.S., (**1981)**; Heterocyclic Chemistry, *J. Chem.,* 18:1589.

54. Wise, D.S. and Castle, R.N., **(1983**); Uniequivocal Synthesis of triaza, tetraaza and pentaazaphenothiazine Heterocyclic*, J. Chem***,** 20:1047.

55. Kym, O. (**1890);** Synthesis of benzo[a]phenothiazine, *Beru. Chem. Ges*., 23:2458.

56. Shirley, D.A. and Talukda, P.B., **(1958**); Synthesis of 12-(dialkylaminoalkyl)benzo[a]phenothiazine*, J. Chem. Soc*., 80:3462-3460.

57. Gristsenko, A.N., Ermakoa, Z.T., Mozhaeva, T.Y., Troitskaya, V.S. and Zhniravley, S.V., (**1973**); Synthesis of Phenoxazine XXXIX, DimethylPyridophenothiazine, *Khrm*. *Ges. Chem. Abstract*, 83:9942.

58. Okafor, C.O. and Okoro, U.C., (**1990**); Uniequivocal Synthesis of benzo[a,][1,4]benzothiazino[3,2-c], *J. National academy of science*, 2:77-83.

59. Umio, S. and Kisimoto, J., (**1968**); 10-substituted-2-azaphenothiazine-2-oxide, *Japanese Patents, Chem. Abstr*., 6809228.

60. Okoro, U.C. and Okafor, C.O., (**1988**); A New Synthesis of three branched diaazaphenothiazinedye, Dyes and Pigment, 9:427-442.

61. Okoro, U.C. and Ezema, B.E., (**2006**); Synthesis of a non-linear Diazaphenothiazine ring system. *Int. J. Chem***.,** 16(2):115-120.

62. Okoro, U,C. and Ijeoma, A.O., (**2005);** Synthesis of non-linear polycyclic Diazaphenothiazine ring system. *International Journal of Chem*., 16(4):245-250.

63. Ofoefula, A.U., (**2005**); Synthesis of a new non-linear diazaphenothiazine ring system, M.Sc Research Project, UNN, 40-41.

64. Hee-won, Y., Myung, E.S., Kye, J.S. and Sng-won, P., (**1997**); The Reaction of 6,7-dichloro-5,8-quinoxaline with aromatic and aliphatic dinucleophiles *bull koream Chem. Soc*., 18:241-243.

65. Okoro, U.C., (**1991**); The synthesis of first aza-analogue of dibenzotriphenothiazine ring system, *India J. Chem.*, 30B:22-24.

66. Kehrmann, F. and Neil, A.A., (**1994**); Synthese in der Gruppe der Azoxine, *Beru. Chem*.**,** 47:3102-3109.

67. Turpin, G.S. and Brathroyal, B., (**1914**); The Action of Picric chloride on Amines in the presence of Alkali*, J. Chem. Soc*., 59:714.

68. Eggers, H. and Deckmann, H., (**1943**); Synthesis of angular phenoxazine. *Biochem.Z.Chem Abstr.,* 37:3436.

69. Agarwal, N.L. and Mital, R.L., (**1976**); Angular Heterocycles: The reaction of acetamide with aromatic amine*, Ann, Org. Chem. Abstr*., 31B, 84:121.

70. Muller, W. and Pssaar, H., (**1959**); Synthesis of new angular phenoxazine, Farbenfabrunken Bayer A.G, *U.S Patent. Chem Abstr*., 54,2906589.

71. Van Allan, J.A. and Reyolds, G.A., (**1963**); Polynuclear Heterocyclics VII: Reactions of 2,3-dichloro-1,4-naphthoquinone with aromatic amines, *J.Org.Chem*., 28:1019.

72. Ishii, S., (**1961**); Reactions of substituted 2,3-dichloro-1,4-naphthoquinone with 2-aminophenol, Chiza Drug manufacturing co, *Japanese Patent***.**

73. Noelting. E., (**1922**); Synthesis of new angular aza-phenoxazine ring system, *Chimic et industries, Chem. Abstr*., 17:473.

74. Goldstein, H. and Warnery, A., (**1928**); Studies on Quinones: Reactions of naphthoquinone with 2-amino-3-naphthol, *Helv.Chem.Abstra*., 22:1777.

75. Nanya, S., Kang, W.B., Maekawa, E. and Ueno, Y., (**1968**); Synthesis of 2,3,5,8-tetrasubstituted-1,4-naphthoquinone with 2-amino-3hydroxypyridine, *Chem. Abstr*., 23:1697.

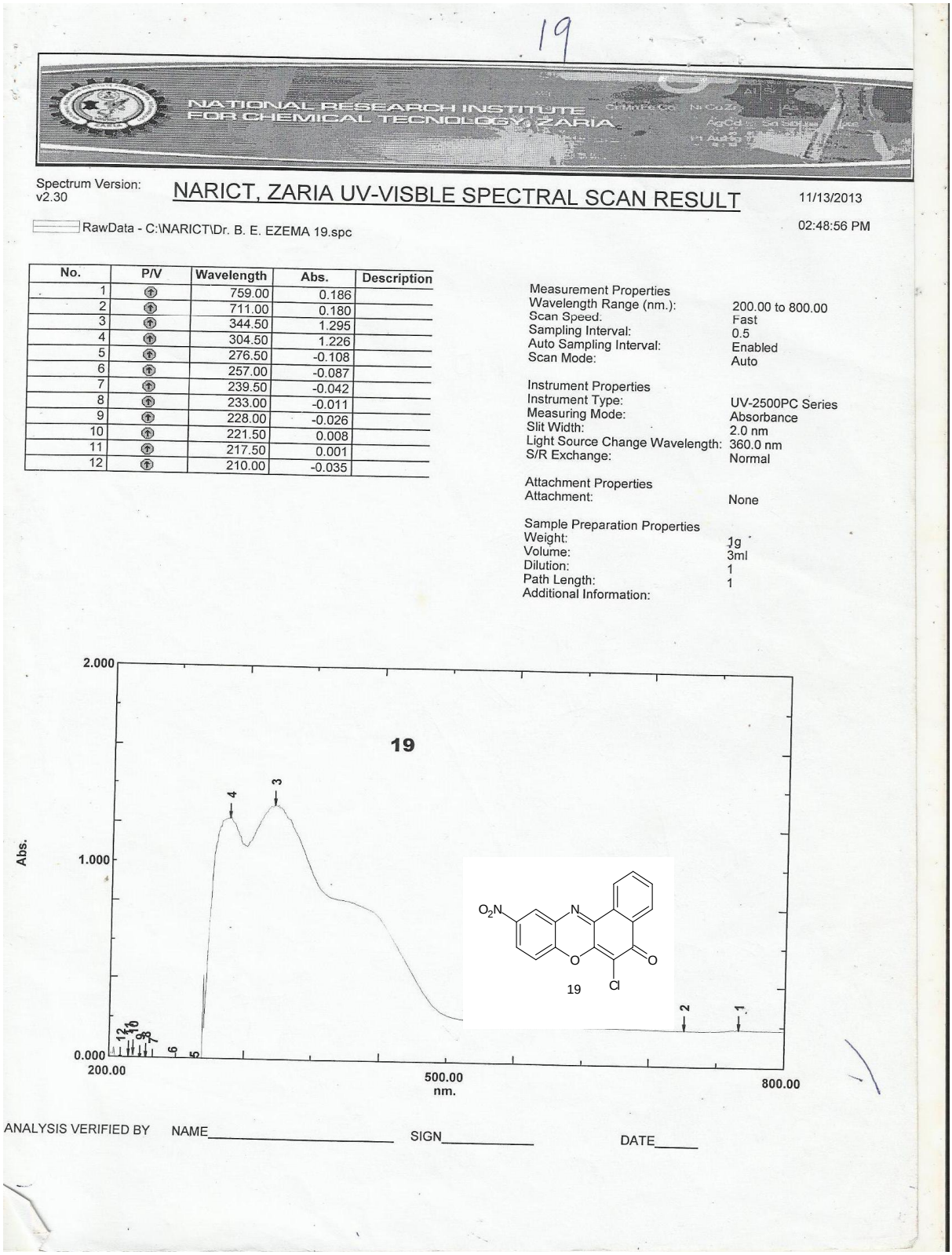
76. Levchenko, A., (**1960**); Synthesis of Dibenzo[a,j]phenoxazine. Izvvest Vyschikh Uchb Zavedeni, *Khrm Technol .Chem*. Abstract, 55:8865C.

77. Ugwuoke, R.O., (**2007**); Synthesis of Angular Triazabenzophenothiazine Ring System, M.Sc Project Report, UNN, 55.

78. Akatauka, M. and Yoshinaga, S., (**1970**); Reaction of naphthoquinone derivatives with 2-aminothiophenol.*Chem Abstract*, 72, 100625n.

79. Okafor, C.O., (**1988**); The first Branched Benzoxazinophenothiazine ring system and its Analogues tetrahedron, 44:1187-1194.

80. Vincent, C.O., (**2005**); Principles of Pharmaceutical application of antimicrobial agent, pharmaceutical microbiology, [EL DEMART] (publishers) Enugu State Nigeria, 171-177.



19

NATIONAL RESEARCH INSTITUTE FOR CHEMICAL TECNOLOGY, ZARIA

Spectrum Version: v2.30

NARICT, ZARIA UV-VISBLE SPECTRAL SCAN RESULT

11/13/2013

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4	↑	304.50	1.226	
5	↑	276.50	-0.108	
6	↑	257.00	-0.087	
7	↑	239.50	-0.042	
8	↑	233.00	-0.011	
9	↑	228.00	-0.026	
10	↑	221.50	0.008	
11	↑	217.50	0.001	
12	↑	210.00	-0.035	

Measurement Properties
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Scan Speed: Fast
Sampling Interval: 0.5
Auto Sampling Interval: Enabled
Scan Mode: Auto

Instrument Properties
Instrument Type: UV-2500PC Series
Measuring Mode: Absorbance
Slit Width: 2.0 nm
Light Source Change Wavelength: 360.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight: 1g
Volume: 3ml
Dilution: 1
Path Length: 1
Additional Information:

ANALYSIS VERIFIED BY NAME_____ SIGN_____ DATE_____

FIG 1 : Uv-spectral chart of 6 chloro-10-nitro-5H-benzo[a]phenoxazin-5-one

16

NATIONAL RESEARCH INSTITUTE FOR CHEMICAL TECNOLOGY, ZARIA

Spectrum Version: v2.30

NARICT, ZARIA UV-VISBLE SPECTRAL SCAN RESULT

11/12/2013
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4	↑	243.50	-2.391	

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Scan Mode: Auto

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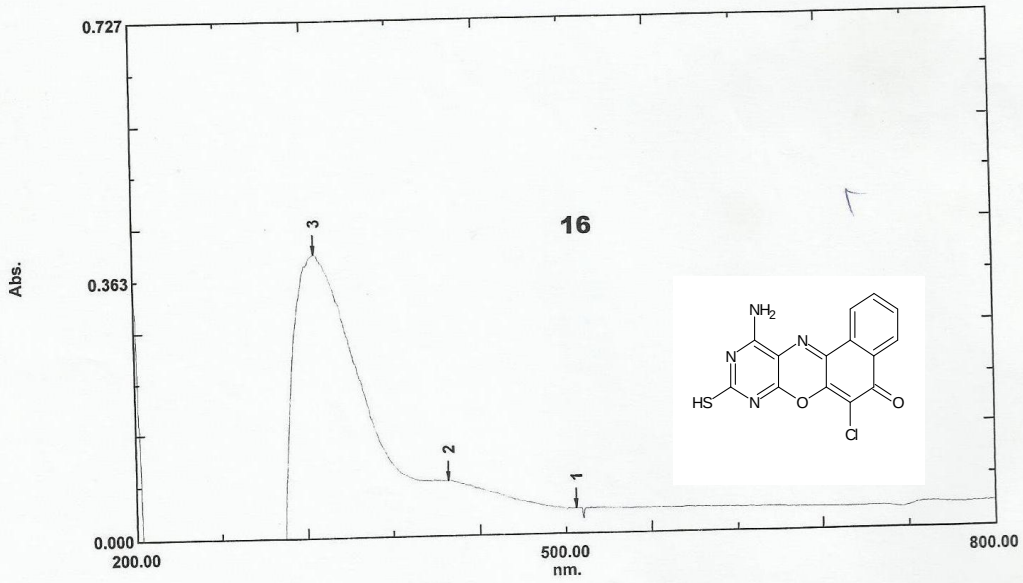


ANALYSIS VERIFIED BY NAME_____ SIGN_____ DATE_____

FIG 2: UV-spectral chart of 11-amino-6-chloro-9-thiol-8,10-diazabenzo[a]phenoxazin-5one

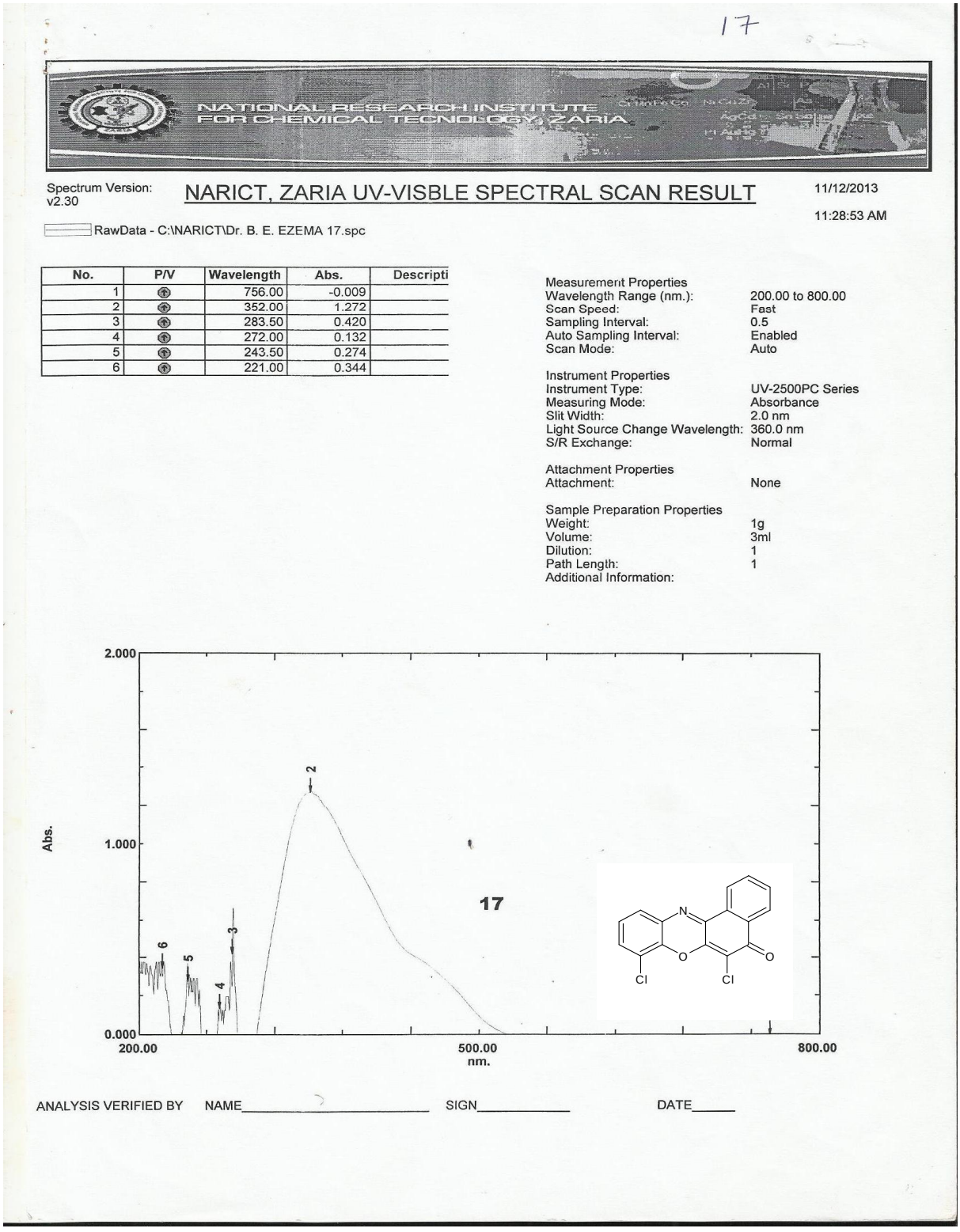


FIG 3: Uv-spectral chart of 6,8-dichlorobenzo[a]phenoxazine-5-one

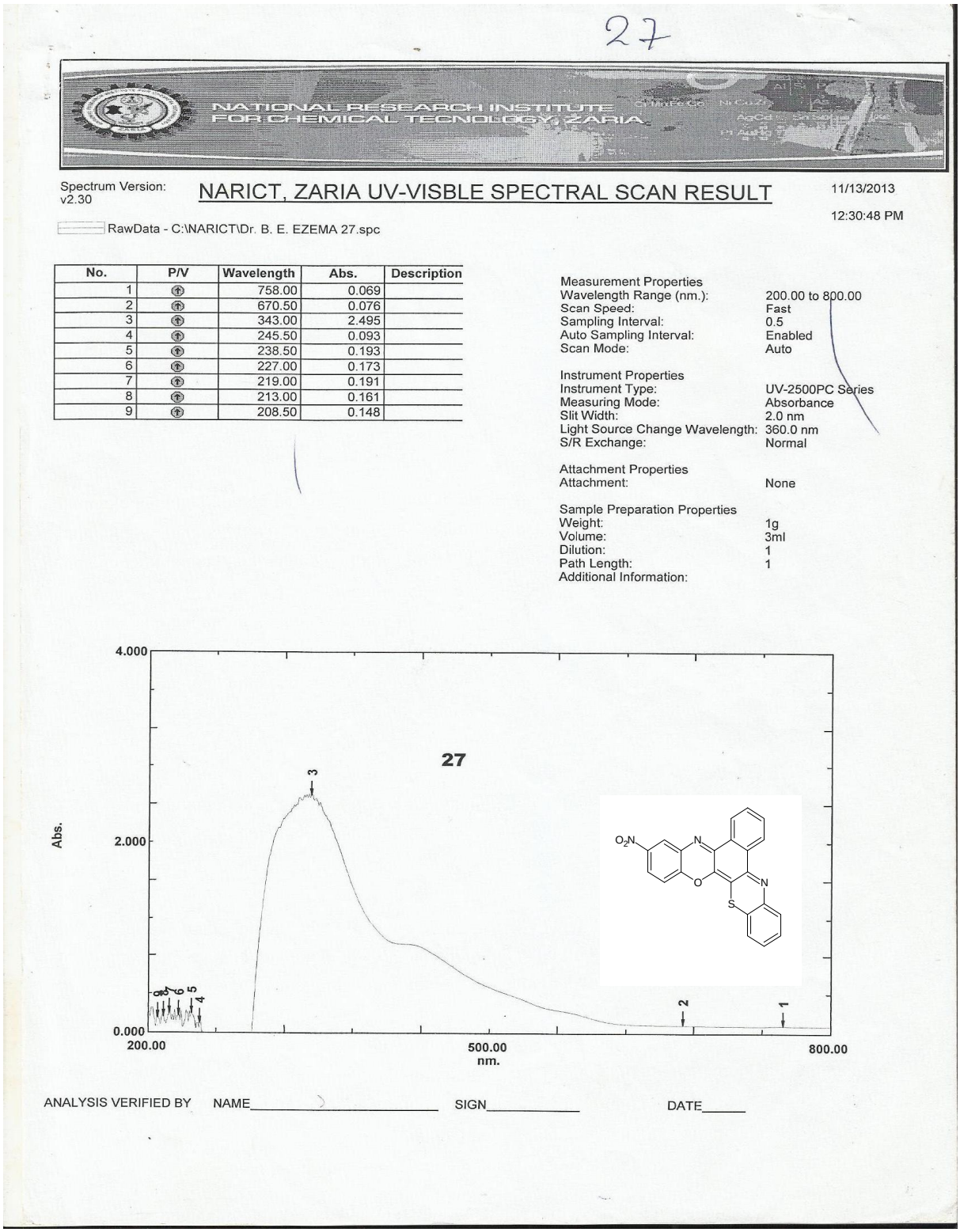


FIG 4 : Uv-spectral chart of 14-nitrobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine

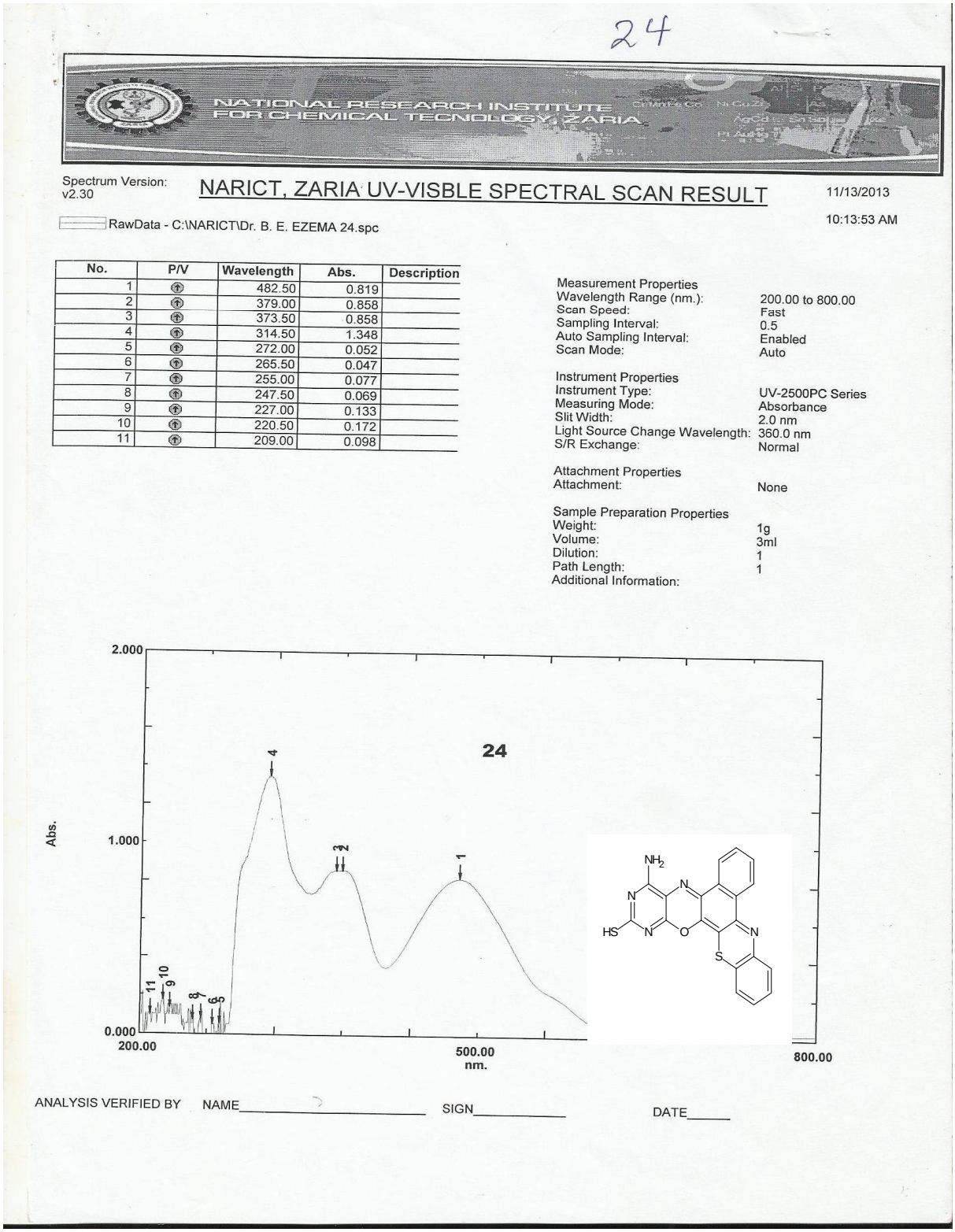


FIG 5: Uv-spectral chart of 15-amino-13-thiol-12,14-diazabenzo[a]benzo[a][1,4]benzoxazino [3,2-c]phenothiazine

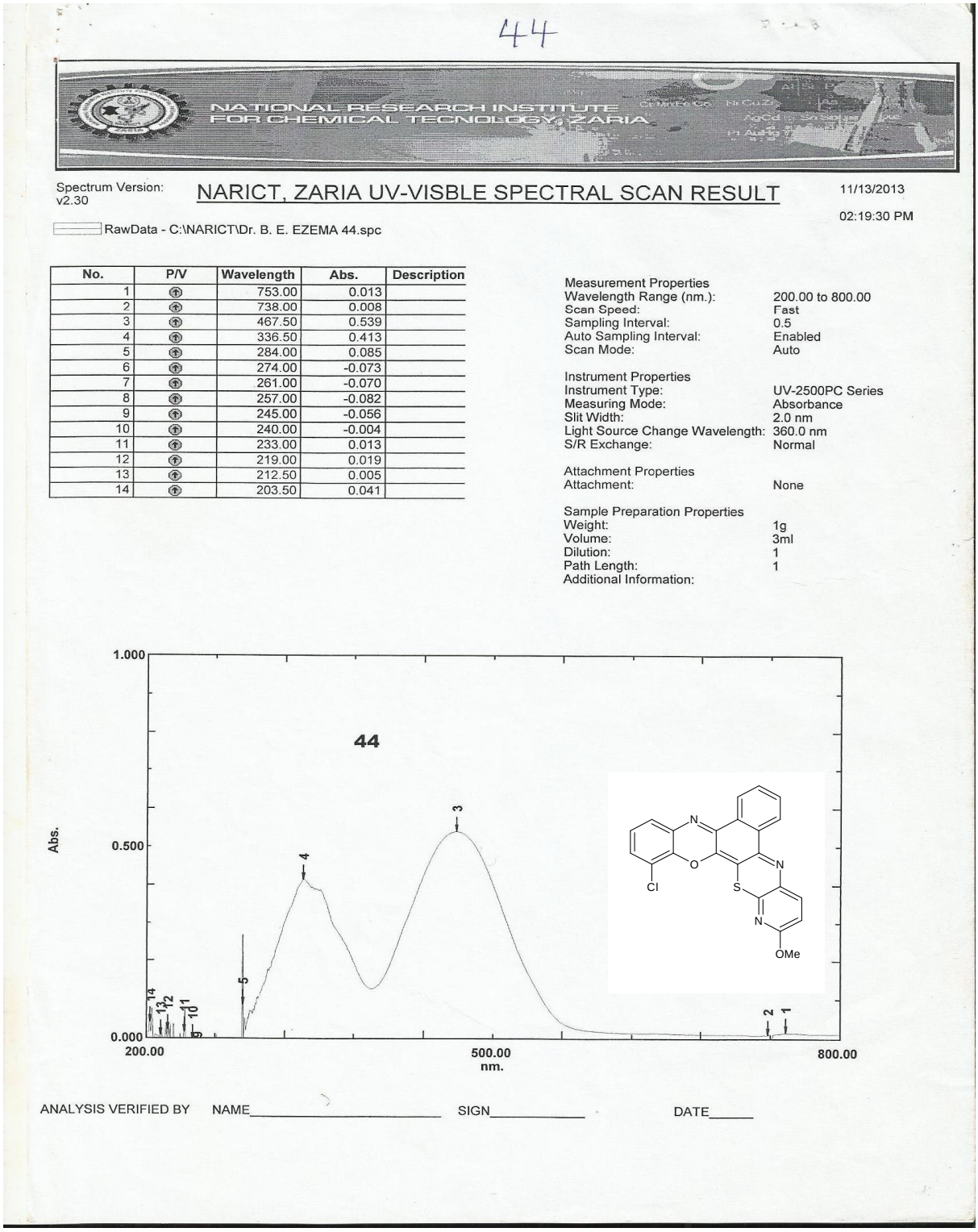
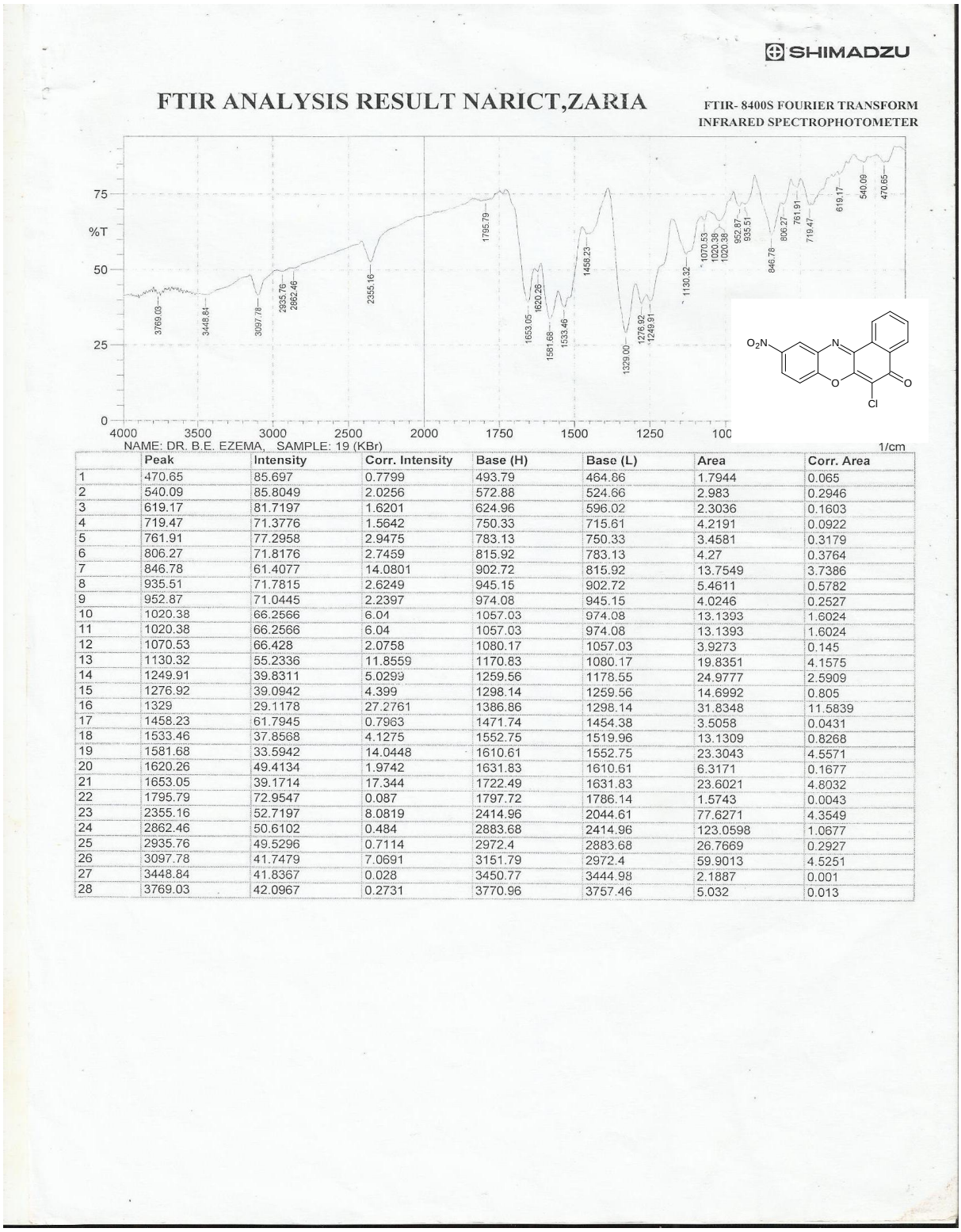


FIG 6: Uv-spectral chart of 12-chloro-9-methoxy-8-azabenzo[a][1,4]benzoxazino [3,2-c]phenothiazine



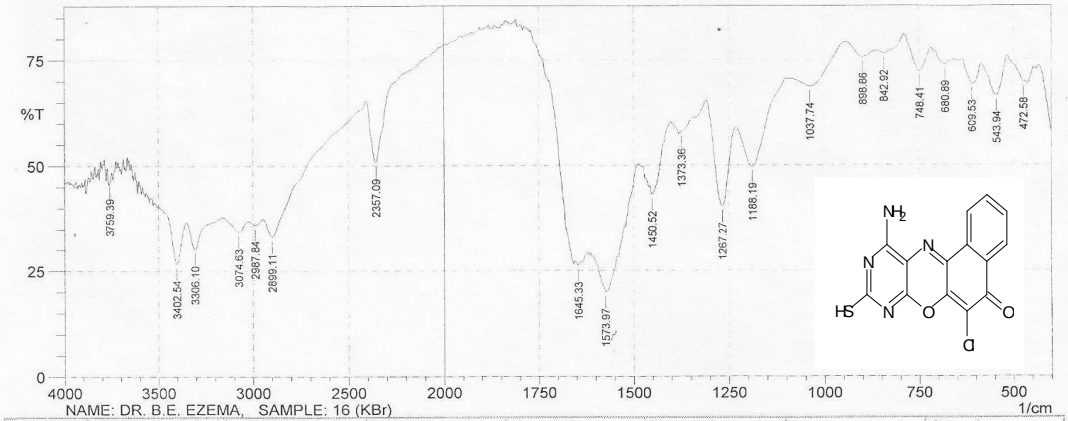
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	470.65	85.697	0.7799	493.79	464.86	1.7944	0.065
2	540.09	85.8049	2.0256	572.88	524.66	2.983	0.2946
3	619.17	81.7197	1.6201	624.96	596.02	2.3036	0.1603
4	719.47	71.3776	1.5642	750.33	715.61	4.2191	0.0922
5	761.91	77.2958	2.9475	783.13	750.33	3.4581	0.3179
6	806.27	71.8176	2.7459	815.92	783.13	4.27	0.3764
7	846.78	61.4077	14.0801	902.72	815.92	13.7549	3.7386
8	935.51	71.7815	2.6249	945.15	902.72	5.4611	0.5782
9	952.87	71.0445	2.2397	974.08	945.15	4.0246	0.2527
10	1020.38	66.2566	6.04	1057.03	974.08	13.1393	1.6024
11	1020.38	66.2566	6.04	1057.03	974.08	13.1393	1.6024
12	1070.53	66.428	2.0758	1080.17	1057.03	3.9273	0.145
13	1130.32	55.2336	11.8559	1170.83	1080.17	19.8351	4.1575
14	1249.91	39.8311	5.0299	1259.56	1178.55	24.9777	2.5909
15	1276.92	39.0942	4.399	1298.14	1259.56	14.6992	0.805
16	1329	29.1178	27.2761	1386.86	1298.14	31.8348	11.5839
17	1458.23	61.7945	0.7963	1471.74	1454.38	3.5058	0.0431
18	1533.46	37.8568	4.1275	1552.75	1519.96	13.1309	0.8268
19	1581.68	33.5942	14.0448	1610.61	1552.75	23.3043	4.5571
20	1620.26	49.4134	1.9742	1631.83	1610.61	6.3171	0.1677
21	1653.05	39.1714	17.344	1722.49	1631.83	23.6021	4.8032
22	1795.79	72.9547	0.087	1797.72	1786.14	1.5743	0.0043
23	2355.16	52.7197	8.0819	2414.96	2044.61	77.6271	4.3549
24	2862.46	50.6102	0.484	2883.68	2414.96	123.0598	1.0677
25	2935.76	49.5296	0.7114	2972.4	2883.68	26.7669	0.2927
26	3097.78	41.7479	7.0691	3151.79	2972.4	59.9013	4.5251
27	3448.84	41.8367	0.028	3450.77	3444.98	2.1887	0.001
28	3769.03	42.0967	0.2731	3770.96	3757.46	5.032	0.013

FIG 7 : IR-spectral chart of 6-chloro-10-nitro-5H-benzo[a]phenoxazin-5-one

SHIMADZU

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM
INFRARED SPECTROPHOTOMETER



NAME: DR. B.E. EZEMA, SAMPLE: 16 (KBr)

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	472.58	69.6888	0.8593	515.01	466.79	6.7258	0.069
2	543.94	66.5351	8.5396	584.45	515.01	10.6066	1.8972
3	609.53	69.201	5.4197	634.6	584.45	7.354	0.977
4	680.89	73.8265	1.5585	705.97	669.32	4.6492	0.2385
5	748.41	72.2581	6.8418	788.91	717.54	8.6637	1.4747
6	842.92	76.361	1.7528	862.21	788.91	8.0422	0.5344
7	898.86	75.6682	2.3444	945.15	862.21	9.4436	0.551
8	1037.74	68.595	4.9262	1087.89	945.15	20.2982	2.2034
9	1188.19	49.6711	13.0037	1230.63	1101.39	30.1503	5.4675
10	1267.27	40.3461	21.4351	1309.71	1230.63	23.2443	6.7883
11	1373.36	57.8172	0.3144	1375.29	1352.14	5.3449	0.0727
12	1450.52	43.1256	3.6181	1456.3	1400.37	16.3815	0.5722
13	1573.97	19.9733	1.0523	1606.76	1570.11	22.877	0.1183
14	1645.33	26.1959	0.8434	1654.98	1639.55	8.8584	0.105
15	2357.09	50.7986	16.2055	2409.17	2054.26	60.5106	7.4416
16	2899.11	32.9878	6.965	2947.33	2409.17	164.1604	-0.3947
17	2987.84	36.05	1.1501	3024.48	2947.33	33.6841	0.5597
18	3074.63	34.134	3.1541	3163.36	3024.48	61.6478	2.343
19	3306.1	30.0745	6.6269	3350.46	3213.51	63.9629	4.3376
20	3402.54	26.6576	11.059	3460.41	3350.46	53.7353	7.2286
21	3759.39	46.2365	0.4449	3761.32	3749.74	3.8122	0.0581

Comment;
NAME: DR. B.E. EZEMA, SAMPLE: 16 (KBr)

User; Administrator

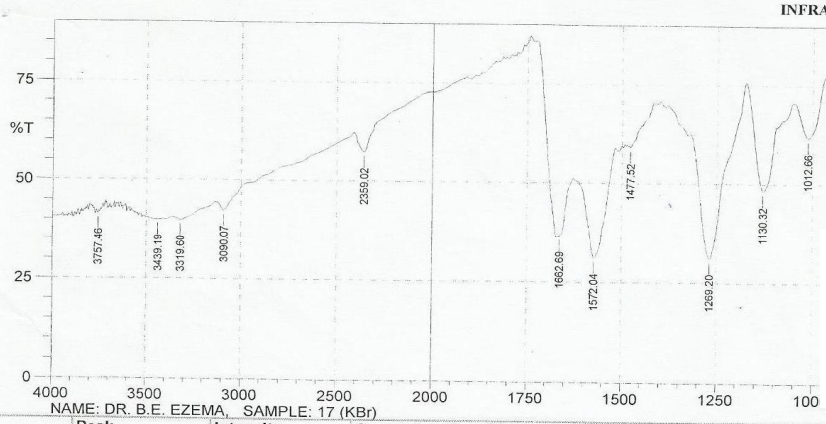
No. of Scans;
Date/Time; 11/11/2013 12:31:29 PM
Resolution;

FIG 8: IR-spectral chart of 11-amino-6-chloro-9-thiol-8,10-diazabenzo[a]phenoxazin-5-one

SHIMADZU

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



NAME: DR. B.E. EZEMA, SAMPLE: 17 (KBr)

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	468.72	69.4336	0.3377	518.87	466.79	7.2102	0.1173
2	592.17	71.4519	1.4087	599.88	549.73	6.8413	0.2381
3	624.96	68.7698	3.8839	667.39	599.88	10.3289	0.9953
4	698.25	62.7707	13.0618	744.55	667.39	12.3118	3.3128
5	823.63	58.1251	1.1861	864.14	819.77	8.7485	0.1871
6	937.44	76.3898	1.6864	956.72	920.08	4.1157	0.1739
7	1012.66	61.7084	11.7429	1049.31	956.72	15.4677	3.4229
8	1130.32	48.2281	25.6211	1176.62	1049.31	27.2855	9.9669
9	1269.2	31.125	36.2961	1321.28	1176.62	44.1295	20.7531
10	1477.52	59.0573	0.8385	1481.38	1465.95	3.4395	0.0422
11	1572.04	31.1444	22.9469	1614.47	1516.1	37.0929	11.1692
12	1662.69	36.5697	0.4519	1666.55	1658.84	3.347	0.0173
13	2359.02	57.2865	6.3386	2411.1	2034.97	67.8061	2.787
14	3090.07	42.3751	3.2713	3138.29	2411.1	202.5829	-0.3664
15	3319.6	39.8453	0.0935	3356.25	3315.74	16.0367	0.0163
16	3439.19	39.9361	0.0427	3441.12	3435.34	2.3056	0.0019
17	3757.46	41.3804	0.614	3761.32	3755.53	2.2058	0.0214

Comment;
NAME: DR. B.E. EZEMA, SAMPLE: 17 (KBr)

User; Administrator

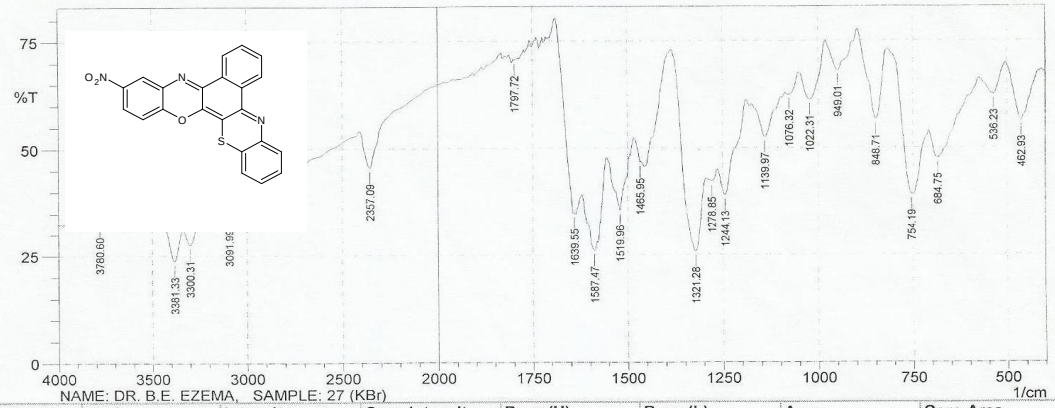
No. of Scans;
Date/Time; 11/11/2013 11:14:46 AM
Resolution;

FIG 9 : IR-spectral chart of 6,8-dichlorobenzo[a]phenoxazin-5-one

SHIMADZU

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM
INFRARED SPECTROPHOTOMETER



NAME: DR. B.E. EZEMA, SAMPLE: 27 (KBr)

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	462.93	56.2137	12.7808	503.44	408.92	19.1081	3.8057
2	536.23	62.4081	5.6168	574.81	503.44	13.3147	1.2964
3	684.75	47.6324	7.4923	707.9	630.74	22.3938	2.871
4	754.19	39.1571	2.1062	815.92	750.33	16.2914	-1.5038
5	848.71	56.595	18.2736	896.93	815.92	14.6408	4.6501
6	949.01	67.9598	6.9272	979.87	910.43	10.3748	1.6481
7	1022.31	61.1872	9.331	1051.24	979.87	13.1092	2.5508
8	1076.32	62.4303	0.3786	1078.24	1051.24	5.191	0.1226
9	1139.97	52.4906	8.408	1174.69	1097.53	18.7915	2.2131
10	1244.13	39.0159	10.1284	1263.42	1188.19	24.3553	3.2785
11	1278.85	42.2876	1.683	1294.28	1263.42	11.2997	0.2832
12	1321.28	25.7786	26.2758	1383.01	1294.28	35.2317	12.8512
13	1465.95	46.7126	0.8859	1483.31	1464.02	5.8369	-0.0276
14	1519.96	35.6186	14.4239	1554.68	1483.31	26.224	4.7723
15	1587.47	25.9771	3.5656	1604.83	1579.75	13.7694	0.7592
16	1639.55	34.7483	1.7612	1687.77	1637.62	13.8019	-0.1045
17	1797.72	70.5241	0.1835	1801.57	1795.79	0.8751	0.0038
18	2357.09	45.5674	10.0201	2411.1	2056.19	87.8489	5.9391
19	3091.99	34.8708	4.3933	3142.15	2976.26	70.2033	3.9881
20	3300.31	27.6106	4.3866	3336.96	3217.37	60.9823	2.7739
21	3381.33	23.7998	7.5873	3439.19	3336.96	57.0525	5.6898
22	3780.6	33.1156	1.8914	3784.46	3772.89	5.4325	0.1384

Comment;
NAME: DR. B.E. EZEMA, SAMPLE: 27 (KBr)

User; Administrator

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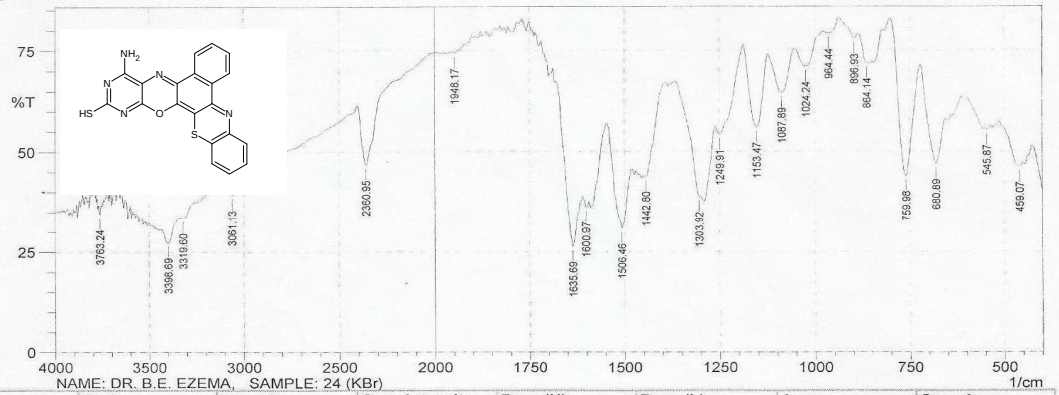
Date/Time; 11/11/2013 11:37:51 AM

FIG 10: IR-spectral chart of 14-nitrobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine

SHIMADZU

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	459.07	46.1018	2.4028	501.51	451.36	15.1552	0.6381
2	545.87	55.2752	0.4904	549.73	534.3	3.9304	0.0379
3	680.89	46.8721	16.2433	721.4	655.82	16.9953	4.4351
4	759.98	43.9285	32.9236	802.41	721.4	18.0643	8.8277
5	864.14	71.8476	0.5635	887.28	862.21	3.0916	0.0196
6	896.93	77.86	2.0505	937.44	887.28	4.7881	0.2177
7	964.44	79.141	1.7326	979.87	937.44	4.0982	0.2863
8	1024.24	71.0425	6.0979	1055.1	979.87	9.6169	1.2865
9	1087.89	64.6596	10.1069	1124.54	1055.1	11.1458	2.3599
10	1153.47	55.9918	19.1227	1188.19	1124.54	12.3748	4.4997
11	1249.91	54.2104	5.0788	1261.49	1188.19	15.4197	1.9258
12	1303.92	38.7818	1.7018	1367.58	1300.07	18.0288	-1.6046
13	1442.8	43.9675	0.8936	1444.73	1392.65	13.4045	-0.3842
14	1506.46	30.8945	19.4797	1545.03	1479.45	26.0448	6.9018
15	1600.97	36.2572	2.0047	1610.61	1593.25	7.4276	0.1966
16	1635.69	26.2995	22.9229	1680.05	1610.61	27.2619	7.095
17	1948.17	74.327	0.6386	1952.03	1928.88	2.9021	0.0565
18	2360.95	46.7175	16.1094	2405.31	2218.21	44.068	8.2429
19	3061.13	39.2946	3.5759	3105.5	2972.4	50.1892	1.8833
20	3319.6	33.4855	0.4696	3325.39	3261.74	28.8096	0.1591
21	3398.69	27.3246	4.4133	3431.48	3338.89	48.2636	2.6295
22	3763.24	34.4925	2.3624	3770.96	3755.53	6.8803	0.1909

Comment;
NAME: DR. B.E. EZEMA, SAMPLE: 24 (KBr)

User; Administrator

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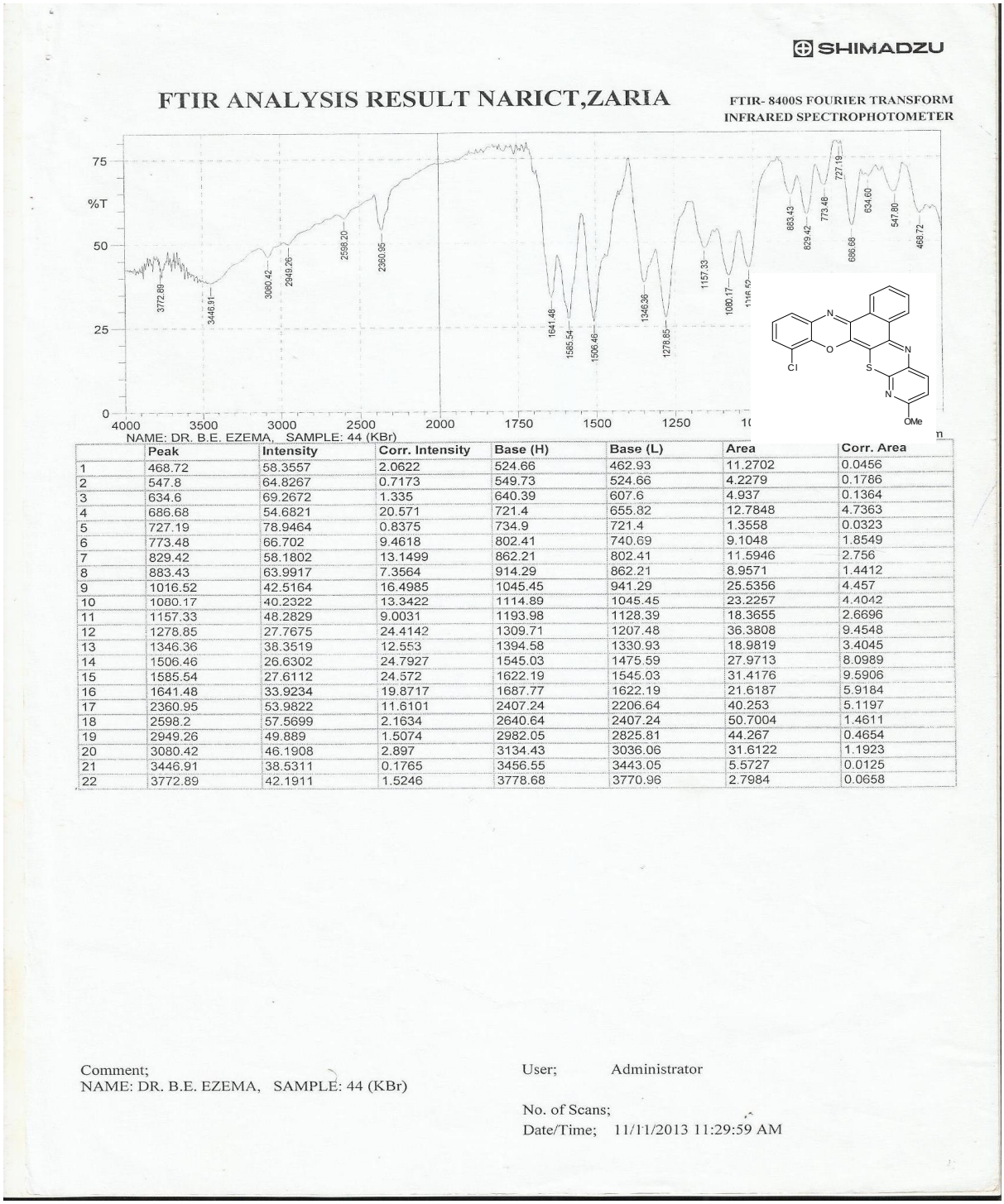
Date/Time; 11/11/2013 12:13:06 PM

FIG 11 : IR-spectral chart of 15-amino-13-thiol-12,14-diazabenzo[a]benzoxazino[1,4]

[3,2-c]phenothizine



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	468.72	58.3557	2.0622	524.66	462.93	11.2702	0.0456
2	547.8	64.8267	0.7173	549.73	524.66	4.2279	0.1786
3	634.6	69.2672	1.335	640.39	607.6	4.937	0.1364
4	686.68	54.6821	20.571	721.4	655.82	12.7848	4.7363
5	727.19	78.9464	0.8375	734.9	721.4	1.3558	0.0323
6	773.48	66.702	9.4618	802.41	740.69	9.1048	1.8549
7	829.42	58.1802	13.1499	862.21	802.41	11.5946	2.756
8	883.43	63.9917	7.3564	914.29	862.21	8.9571	1.4412
9	1016.52	42.5164	16.4985	1045.45	941.29	25.5356	4.457
10	1080.17	40.2322	13.3422	1114.89	1045.45	23.2257	4.4042
11	1157.33	48.2829	9.0031	1193.98	1128.39	18.3655	2.6696
12	1278.85	27.7675	24.4142	1309.71	1207.48	36.3808	9.4548
13	1346.36	38.3519	12.553	1394.58	1330.93	18.9819	3.4045
14	1506.46	26.6302	24.7927	1545.03	1475.59	27.9713	8.0989
15	1585.54	27.6112	24.572	1622.19	1545.03	31.4176	9.5906
16	1641.48	33.9234	19.8717	1687.77	1622.19	21.6187	5.9184
17	2360.95	53.9822	11.6101	2407.24	2206.64	40.253	5.1197
18	2598.2	57.5699	2.1634	2640.64	2407.24	50.7004	1.4611
19	2949.26	49.889	1.5074	2982.05	2825.81	44.267	0.4654
20	3080.42	46.1908	2.897	3134.43	3036.06	31.6122	1.1923
21	3446.91	38.5311	0.1765	3456.55	3443.05	5.5727	0.0125
22	3772.89	42.1911	1.5246	3778.68	3770.96	2.7984	0.0658

Comment;
NAME: DR. B.E. EZEMA, SAMPLE: 44 (KBr)

User; Administrator

No. of Scans;
Date/Time; 11/11/2013 11:29:59 AM

FIG 12 : IR-spectral chart of 12-chloro-9-methoxy-8-azabenzo[a][1,4]benzoxazino [3,2-c]phenothiazine

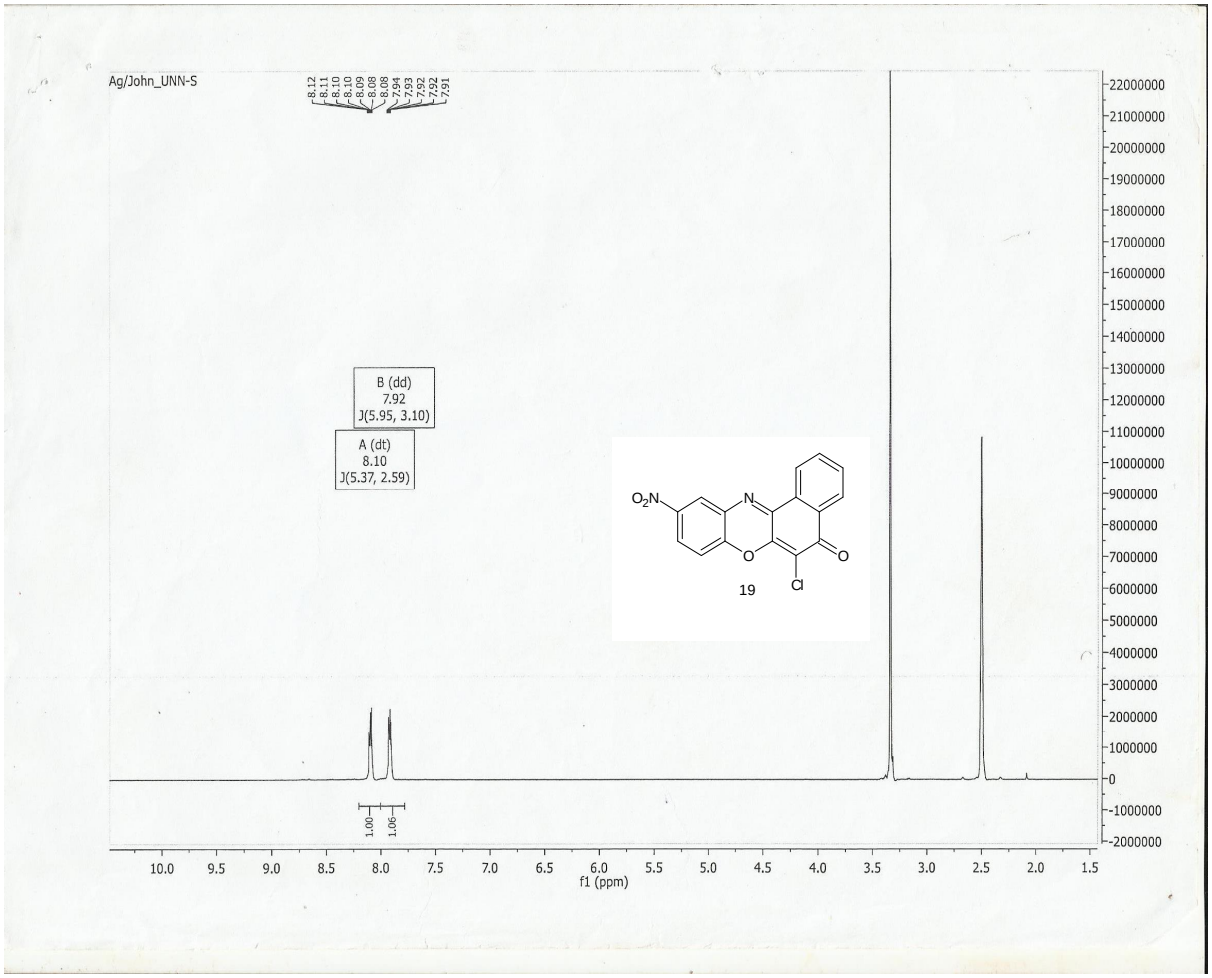


FIG 13 : ^{1}H-NMR-spectral chart of 6-chloro-10-nitro-5Hbenzo[a]phenoxazin-5-one

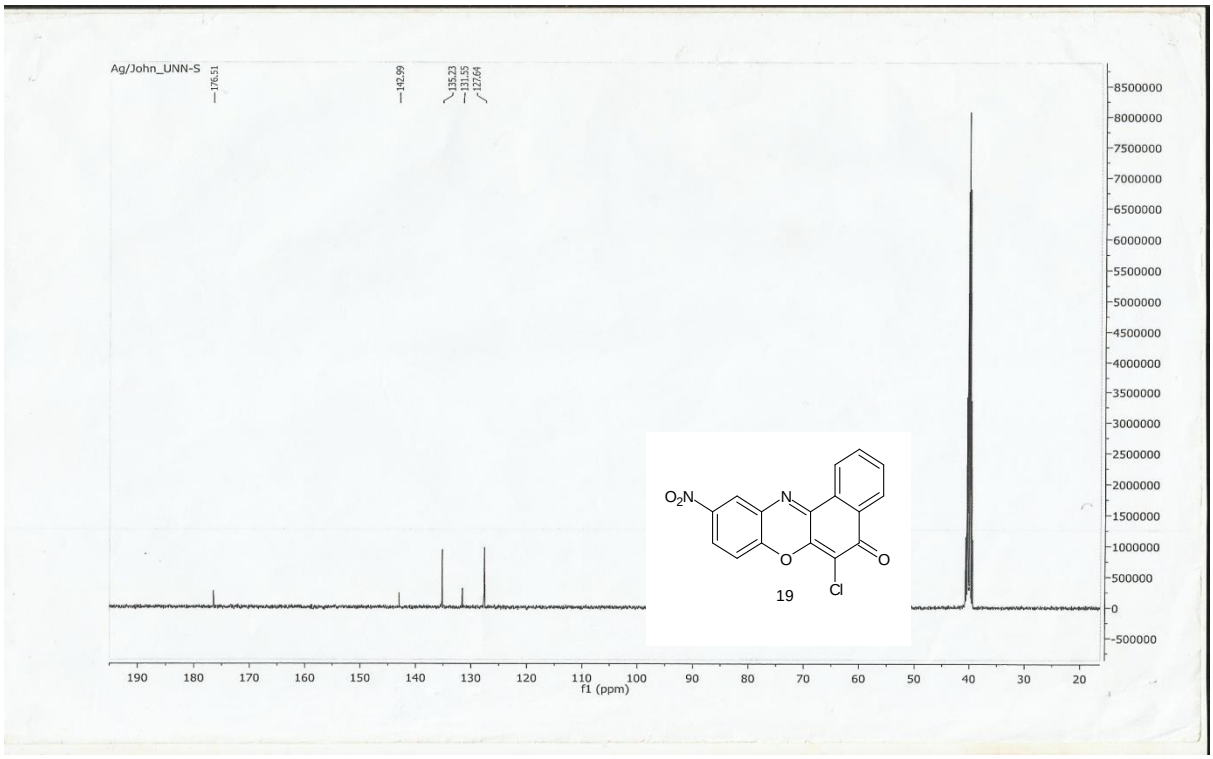


FIG 14 : ^{13}C-NMR-spectral chart of 6-chloro-10-nitro-5H-benzo[a]phenoxazin-5-one

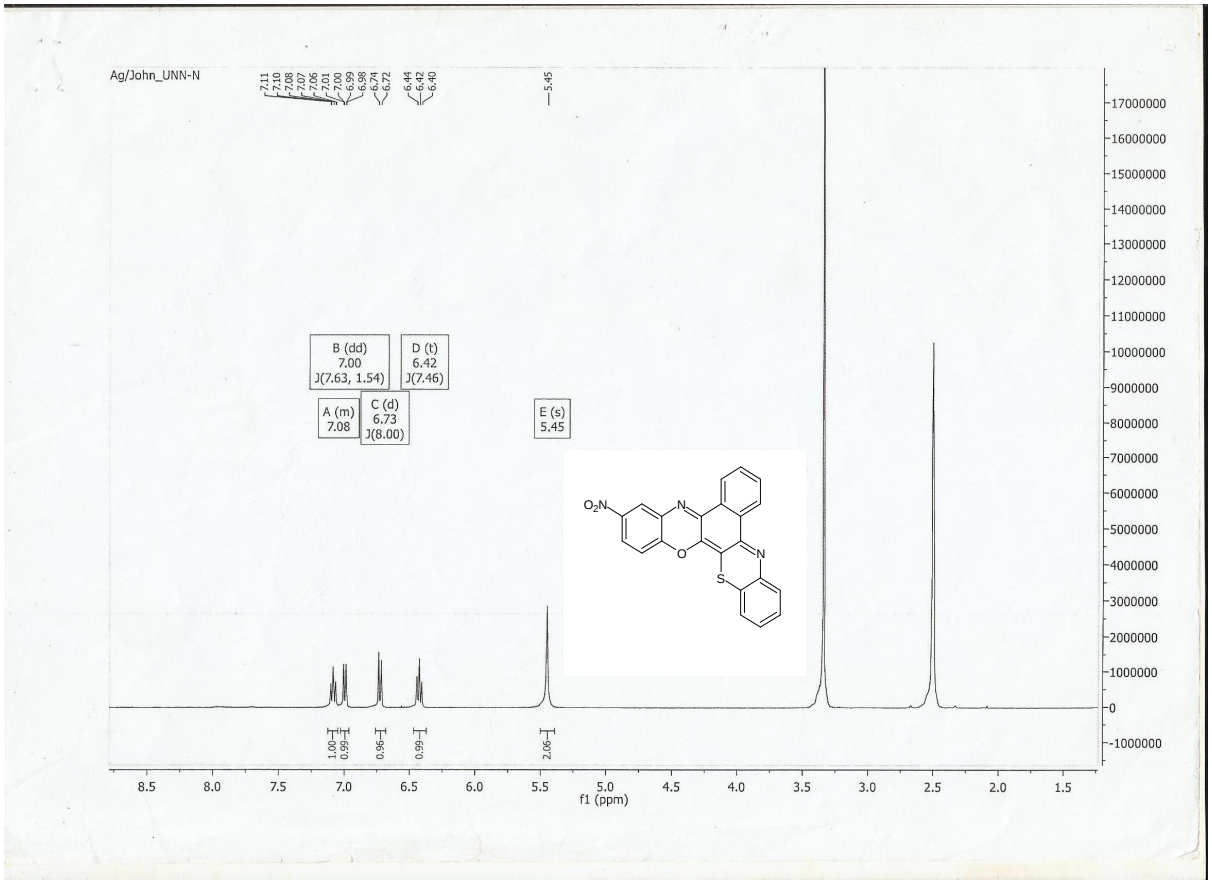


FIG 15 : ^{1}H-NMR-spectral chart of 14-nitrobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine

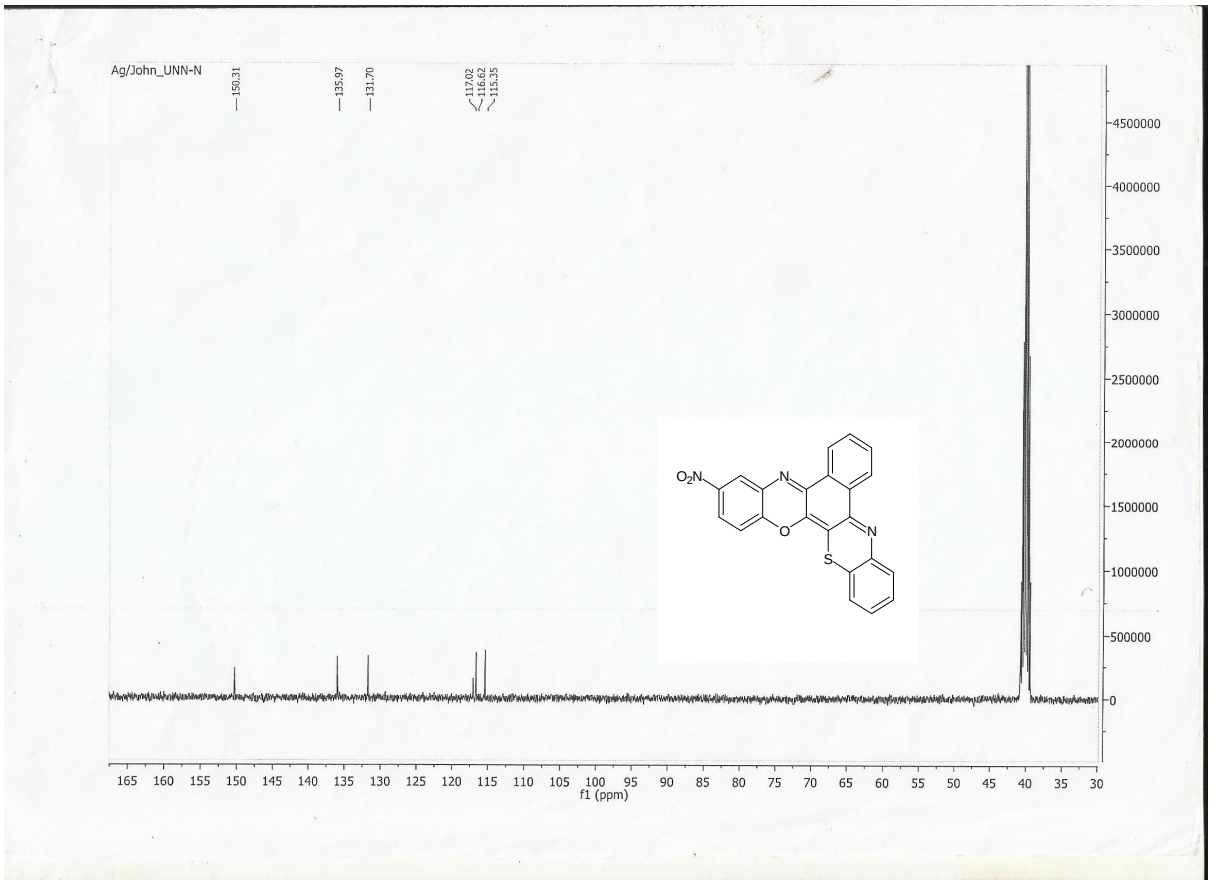


FIG 16 : ^{13}C-NMR-spectral chart of 14-nitrobenzo[a][1,4]benzoxazino[3,2-c]phenothiazine

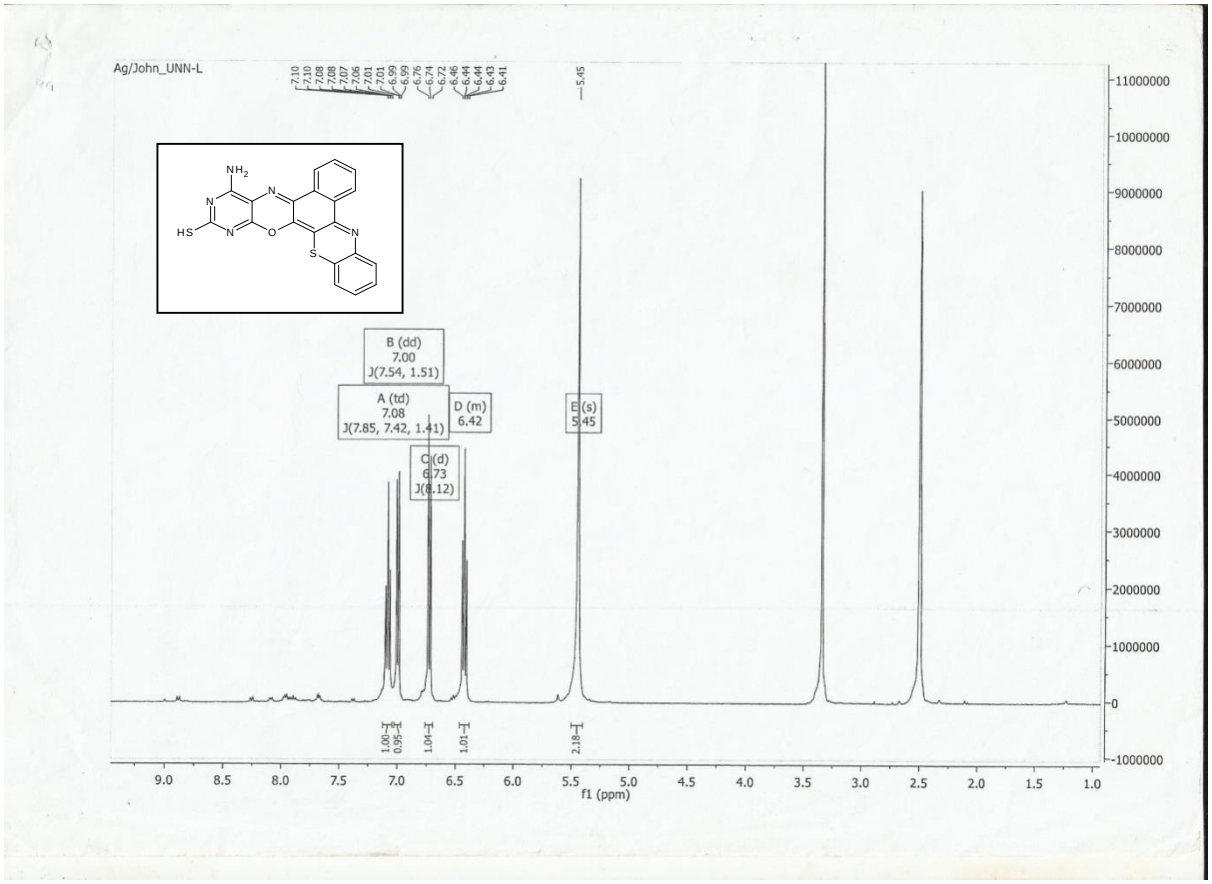


FIG 17 : ^{1}H-NMR-spectral chart of 15-amino-13-thiol-12,14-diazabenzo[a][1,4]benzoxazino [3,2-c]phenothiazine

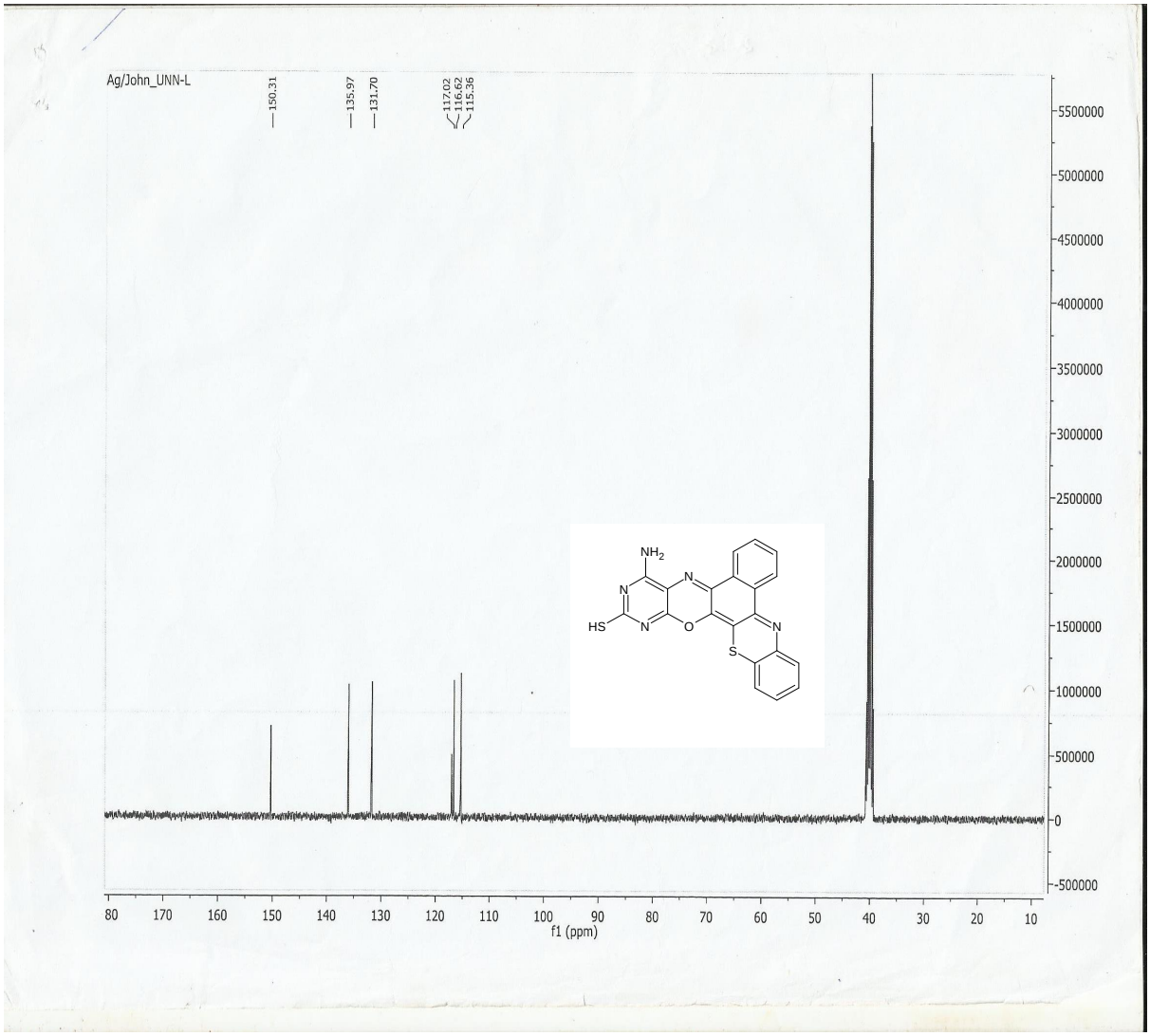


FIG 18 : ^{13}C-NMR-spectral of 15-amino-13-thiol-12,14-diazabenzo[a][1,4]benzoxazino [3,2-c]phenothiazine

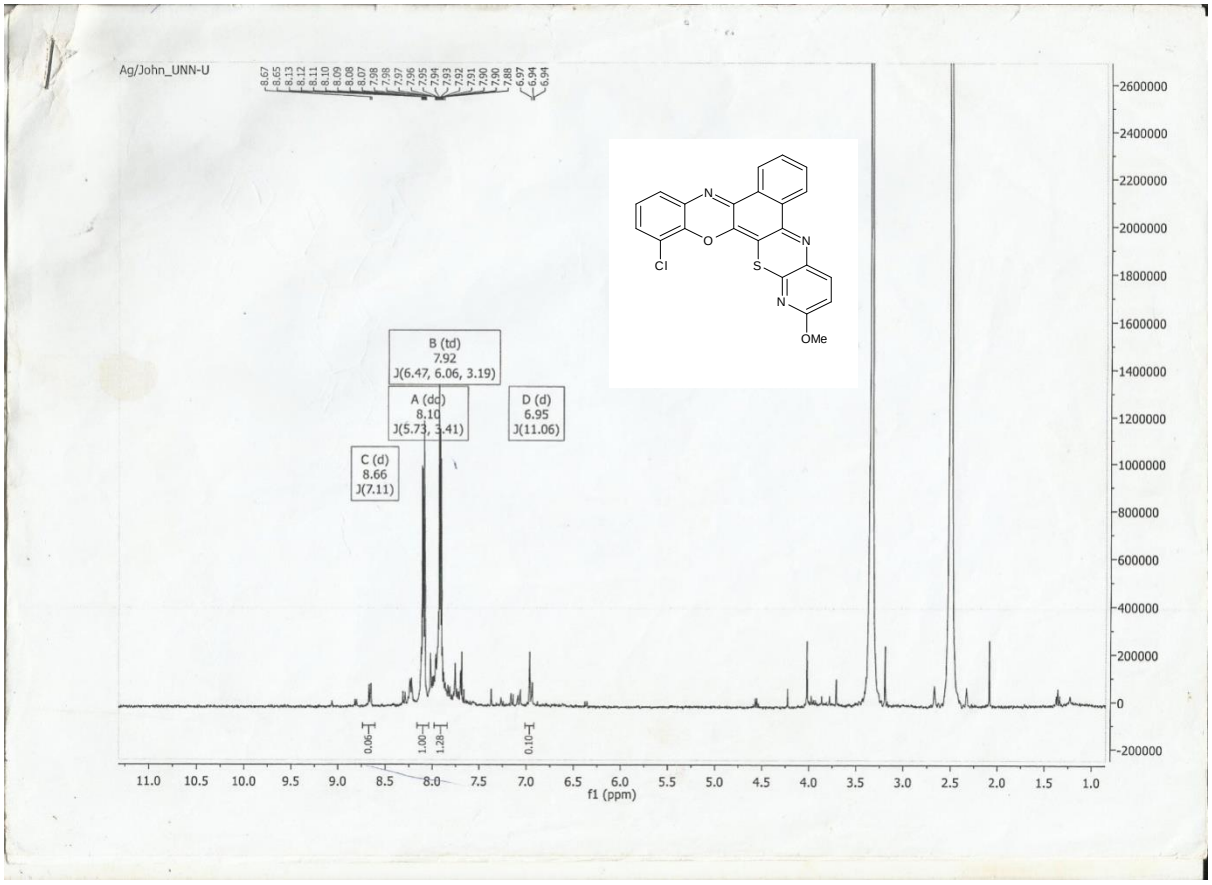


FIG 19 : ^{1}H-NMR-spectral chart of 12-chloro-9-methoxy-8-azabenzo[a][1,4]benzoxazino [3,2-c]phenothiazine

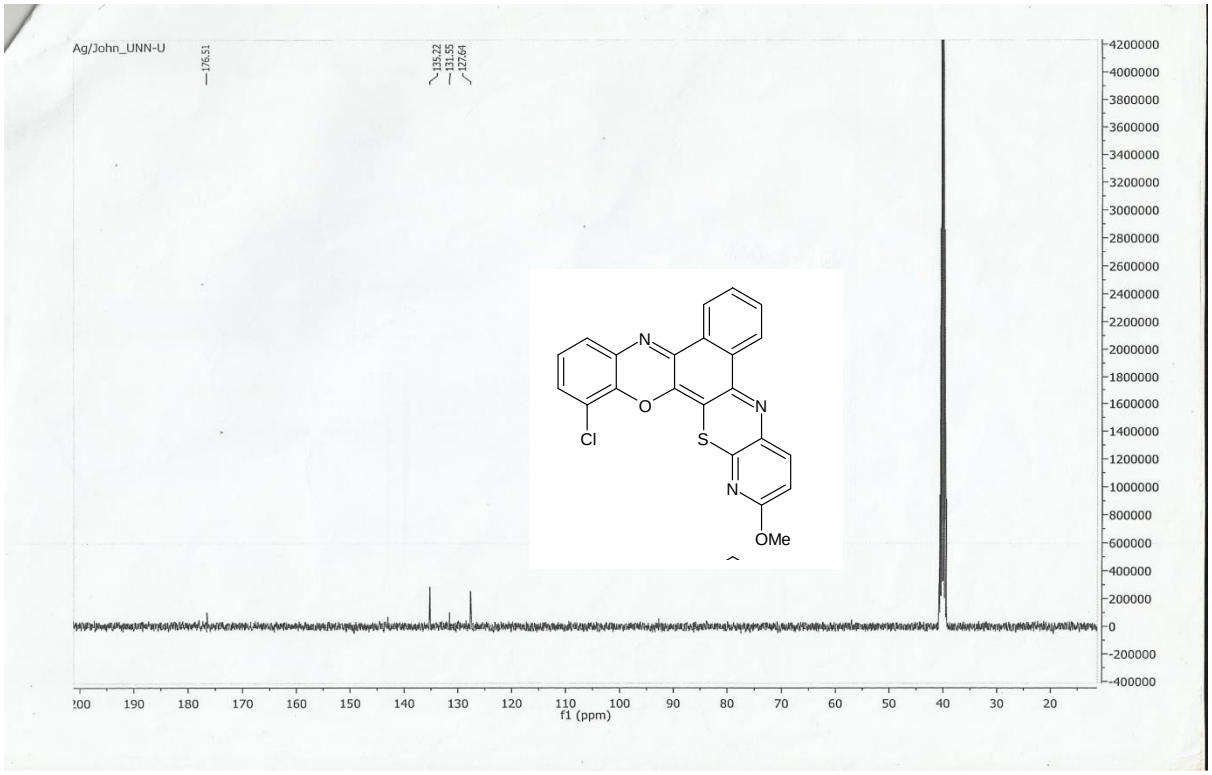


FIG 20 : ^{13}C-NMR-spectral chat of 12-chloro-9-methoxy-8-azabenzo[a][1,4]benzoxazino [3,2-c]phenothiazine