Research Paper

Volume : 3 | Issue : 12 | December 2014 • ISSN No 2277 - 8179

The Synthesis, Characterisation and Antimicrobial Screening of 8-Amino-6-Chloro-1,9,11-Triazabenzo[A]Phenothiazin-5-One

Science

KEYWORDS : synthesis, phenothiazine, antimicrobial activity

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ABSTRACT

The synthesis of 8-amino-6-chloro-1,9,11-triazabenzo[a]phenothiazin-5-one and its regio isomer, 8-amino-6-chloro-4,9,11-triazabenzo[a]phenothiazin-5-one from 8-hydroxyquinoline is reported. 8-Hydroxyquinoline was converted to 6,7-dichloro-5,8-quinolinequinone, a key intermediate, by stepwise nitrosation, nitration, reduction and chloroxidation. 4,6-Diaminopyrimidine-5-thiol another key intermediate was obtained by thiocyanation, followed by alkaline hydrolysis of 4,6-diaminopyrimidine. These two intermediates were coupled under anhydrous basic condition to furnish isomeric mixture of the two isomers which were then separated by column chromatography. The new compounds were characterized using spectral and analytical data .The antimicrobial tests were carried out on multi-resistant bacterial strains isolated under clinical conditions using agar- well diffusion method .Both the Inhibition Zone Diameter(IZD) and the Minimum Inhibito ry Concentration (MIC) were determined. These new compounds were found to show significant activities against the tested microbes

INTRODUCTION

Phenothiazines are important group of compounds which have for long been used as antimalarial, antipsychotic and antischizophrenic agents (Kalkanidis et al, 2002),(Sharma, 2012), (Rayhuvanshi,2012). They are also of interest as carcinostatic agents and in treatment of prion disease (korth, 2001), (okafor, 1988). Phenothiazines have application as antioxidant in petroleum products and as vat dyes in textile industries (Zhang et al, 2007), (Okafor & Okoro, 1988). It is the importance of phenothiazines in medicine and industry that prompted our synthesis of these two new phenothiazines. The phenothiazines were tested for antimicrobial activities against five microorganisms which are *Staphylococcus aureus* (G101 and G102), *Escherichia coli* (Eco3and Eco 12) and *Pseudomonas aerugenosa* which are known for their increased resistance to existing antibiotics (Bae & Freeman, 2007). Antimicrobial resistance is a major cause of significant morbidity and motility globally (David & Heyman, 2007), hence efforts are continuously being made to identify compounds with antimicrobial properties and so can be potential new antibiotics.

EXPERIMENTAL SECTION

Melting points of the synthesized compounds were determined by the use of Fischer John's electro-thermal melting point apparatus in open capillaries and were uncorrected. Ultraviolet visible spectra were done on scan Buffer 16 CEUL CE 9050 spectrophotometer using matched 1cm quartz cells in Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka. The absorption maxima were given in nanometer (nm). Infrared spectra were recorded with FTIR-8400s Fourier Transform Infrared Spectrophotometer in NARICT, Zaria, Nigeria using KBr discs, and the absorption values were given in per centimeter (cm^{-1}). Nuclear Magnetic Resonance (NMR) was determined with Varian NMR – Mercury 200BB spectrometer in Central Science Laboratory Obafemi Awolowo University, Ile –Ife. Chemical shift δ is reported in ppm. MS spectra were obtained from GCMS – QP2010 PLUS SHIMADZU, in NARICT, Zaria, Nigeria. Elemental analyses were done on a CE 440 Elemental Analyser at the Central Science Laboratory University of Cairo, Cairo, Egypt. Most of the chemicals were purchased from Aldrich chemical company and were used without further purification. Column chromatography was done using silica gel (mesh 60-80).

Synthesis of 8-Hydroxy-5-nitrosoquinoline 2

8-hydroxyquinoline (29.0g, 0.2mol) was placed in a 500ml conical flask immersed in an ice bath and water (100ml) mixed with concentrated hydrochloric acid (38ml) was added and vigorously stirred. Aqueous solution of sodium nitrite made of sodium nitrite (15.0g, 0.21mmol) and water (50ml) was added in portions to the mixture maintained at 0°C for over 1h. The slurry was allowed to stand overnight at 0°C before it was filtered and washed with cold water. The yellow product was air dried. The bright yellow the compound obtained (40g, 91%) melted with decomposition at 183°

Synthesis of 8-Hydorxy-5-nitroquinoline 3

The conversion of 8-hydroxy-5-nitroso-quinoline to 8-hydroxy-5-nitroquinoline was carried out according to procedure reported by Petrow and Sturgeon (1954). Finely divided powder of 8-hydroxy-5-nitrosoquinoline hydrochloride (15.0g, 0.07 mol) was placed in a 500ml conical flask in a water-ice bath. A mixture of concentrated nitric acid (45 ml) and water (30ml) at 17°C was poured into) flask and stirred for 1¼ h at 17°C.after which the reaction mixture was worked –up.the crude yellow product obtained was recrystallised from ethanol. The compound 8-hydroxy-5-nitroquinoline (10.9g, 80%) obtained melted with decomposition at 180°C (Lit. 179.5-181.5°C) (Pratt & Drake, 1960).

Synthesis of 5-Amino-8-hydroxyquinoline 4

Finely divided powder 8-hydroxy-5-nitroquinoline (9.5 g, 0.05 mol) was mixed with zinc dust (9.8 g, 0.15mol) in 250 ml round bottom flask containing a magnetic stirring bar and 40 ml of acetic acid was gradually added while stirring for 45 min. The reaction mixture was thereafter refluxed for 3h at 50°C and cooled. It was neutralized with cold dilute sodium hydroxide solution and the precipitate filtered. The crude product was washed with water and dried in an oven maintained at 40°C. The yellowish brown compound (5.5 g, 68%) obtained melted at 172°C.

Synthesis of 6, 7-Dichloro-5, 8-quinolinequinone 5 .

5-amino-8-hydroxyquinoline (8.0 g, 0.05mol) was dissolved in concentrated hydrochloric acid (50 ml) with vigorous stirring at room temperature. An aqueous solution of potassium chlorate

(7.5 g, 0.06 mol) in of water (50 ml) was gradually added to it. It was stirred at 50°C for 2 h and then at room temperature for 3 h. Cold water was added and the precipitate was filtered out and subsequently worked-up to obtain a bright yellow powder (6.8 g, 60%) which melted at 217-219°C (Lit 220 – 222°C) (Johnson et al,1986), UV λ_{max} 344, IR (KBr)ν_{max}(cm^{-1}) 1640 ,1579 , 1505 , 1458 and 793.

Synthesis of 4, 6,-Diamino-5-thiocyanatopyrimidine 7

This compound was prepared using a procedure reported by Okafor (1975). 4,6-diamino-pyrimidine (5.5 g, 0.05 mol) was placed in 500 ml three necked flask equipped with a mechanical stirrer, dropping funnel and thermometer. Glacial acetic acid pre-cooled to 17°C was then added to the mixture and cooled to 0 – 5°C in a freezing mixture of salt and ice. Potassium thiocyanate (40.0 g, 0.41 mol) was added and the mixture mechanically stirred for 30 min. Bromine (8 ml) in glacial acetic acid (30 ml) was added. The reaction mixture was stirred for 2 h at 0°C and at room temperature for another 10 h. It was allowed to stand overnight and then worked up to obtain 4,6-diamino-5-thiacyanatopyrimidine as glistering light yellow crystals (5.8 g, 70%) which melts above 300 °C.

Synthesis of 4,6-Diaminopyrimindine-5-thiol

The conversion of 4,6-diamino-5-thionyanatopyrimidine to its thiol derivative was done using alkaline hydrolysis. 4,6-Diamino-5-thioyanatopyrimidine (3.3 g, 0.02 mol) was refluxed in 50 ml of 20% potassium hydroxide for 8 h, there was obtained 4,6-diaminopyrimidine-5-thiol (1.9 g, 63%) melting above 300°C IR (KBr) ν_{max} cm^{-1} 3398, 3295, 3179 , 1629, 1561, 1510, 1468.

8-Amino-6-chloro-1,9,11-triazabenzo[a]phenothiazin-5-one 9

4,6-Diaminophyrimidine-5-thiol (0. 71 g, 5 mmol) was suspended in chloroform(40ml) mixed DMF(4 ml) in a 100 ml two-necked flask bearing a reflux condenser, magnetic stirring bar and, thermometer and sodium carbonate (0.53 g, 5 mmol) added. The mixture was heated with stirring at 75 – 80°C for 45 min and 6,7-dichloro-5,8-quinolinequinone (1.5 g, 5mmol) was added and refluxed for 6 h. At the end of the 6 h the reaction mixture was filtered hot. Removal of the solvent gave a deep red residue of two isomers which were separated on a silica gel column eluting with chloroform-acetone. The first fraction intense red-colour 8-amino-6-chloro-1,9,11-triazabenzo[a]phenothiazin-5-one (0.96 g, 60%) mp = 242°C ,UV (EtOH) λ_{max} (nm) 278, 236, and 506, IR (KBr) ν_{max} (cm^{-1}) 3240, 1672, 1614 and 1543 . ^{1}H-NMR ($_d$DMSO) δ(ppm) 7.9-7.5 aromatic protons. MS; mz, (rel.int.%), 315 (M^+ , 25%), 317 (M+2, 10%), 280(-Cl,100%), 252(-CO,25%). Calculated for $C_{13}H_6N_5OSCl$, C = 49.52, H = 1.90, N = 22.22, O = 5.07, S = 10.15, Cl = 11.11. Found : C = 49.50; H = 1.92; N = 22.23; S = 10.20; Cl = 11.32.

Synthesis of 8-Amino-6-chloro-4,9,11-triazabenzo[a]phenothiazin-5-one 10.

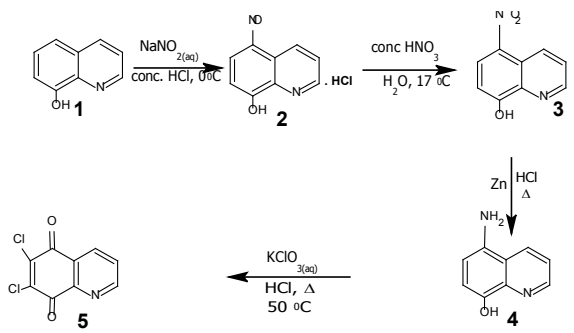
The second isomer 8-amino-6-chloro-4, 9, 11-triazabenzo[a] phenothiazin-5-one **10** (0.32, 20%) which is brown in colour decomposed at 270°C. UV (EtOH) λ_{max} (nm) 239, 265, and 501. IR (KBr) ν_{max} (cm^{-1}) 3369, 1566, 1541and 1453. ^{1}H-NMR ($_d$DMSO) δ(ppm)7.9(s,1H), 7.8(m,1H), 7.6(m,1H),7.5(m,1H), 7.4(s,2H). ^{13}C-NMR ($_d$DMSO) δ 188(C=O),167(C=N),142,134,125,(C=C). MS: m/z(rel.int.%), 315 (M^+, 80%) ,317(M^++2,30%),299(-NH_2,60%),254(-Me,20%,). Calculated for $C_{13}H_6N_5OSCl$. C = 49.52, H = 1.90, N = 22.22, O = 5.07, S = 10.15, Cl = 11.11 Found: C = 49.60; H = 2.01; N = 22.21; S = 10.09; Cl = 11.31.

Evaluation of antimicrobial activities

The assay was conducted using agar-well diffusion method[14]. 20 mg of each compound was dissolved in 1 ml of DMSO. A single colony of each test isolate was suspended in 2 ml sterile nutrient broth. The suspension of each isolate was adjusted to 0.5 McFarland turbidity standards (corresponding to approximately 10^8Cfu/mL) and used to inoculate the surface of the iso-sensitive agar and the excess fluid was drained into discarded pot containing disinfectant. The inoculated agar surface was allowed to dry and the plates appropriately labeled. Using a cork borer of 6 mm in diameter wells were bored in the inoculated iso-sensitive agar. With a micro pipette, 50 µl of the test compound was delivered into each well. Plates were left on the bench for 30 min to allow the compound to diffuse into the agar. Thereafter the plates were incubated at 37°C for 24 h. After incubation the plates were observed for inhibition zones around the wells. The diameters of the zones were measured with meter rule to the nearest whole millimeter

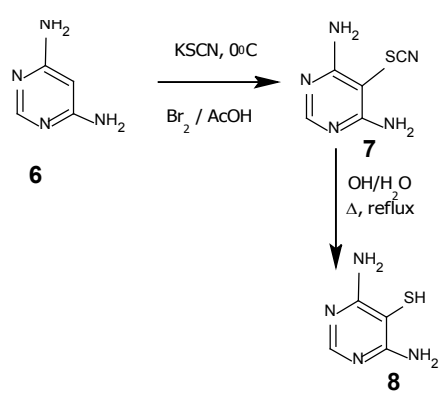
RESULT AND DISCUSSION

6,7-dichloro-5,8-quinolinequinone **5** required for the synthesis was prepared from 8-hydroxyquinoline by the multistep procedure depicted by Scheme 1 below



\sScheme 1: Steps in conversion of 8-hydroxyquinoline to 6,7-dicholoroquinolinequinone. 5

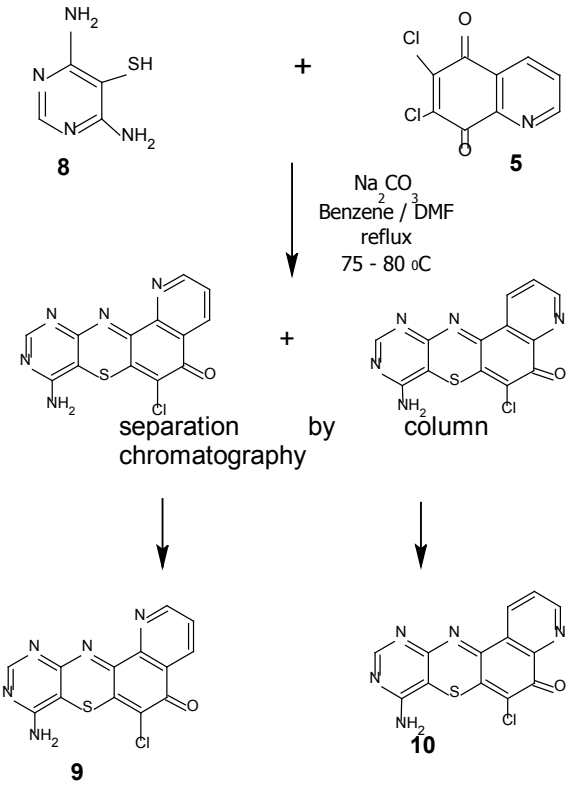
On the other hand 4,6-diaminopyrimidine-5-thiol **8** was obtained from 4,6-diaminopyrimidine **6** by the procedure shown in scheme 2



SCHEME 2: Preparation of 4-6-Diaminopyrimidine-5-Thiol

An equimolar mixture of properly dried 4,6-diaminopyrimidine-5-thiol and 6,7-dichloro-5,8-quinolinequinone in benzene and DMF (ratio 10:1) was treated with anhydrous sodium carbonate and refluxed for 6 hours at 70°C – 75°C to furnish a mixture of the two isomers which were separated by column chromatography using benzene-acetone as eluent. Microanalysis, NMR, IR and mass spectroscopy results obtained supported the structure of the compounds. The steps in the formation of these isomers are represented by the scheme below.

Volume : 3 | Issue : 12 | December 2014 • ISSN No 2277 - 8179



Scheme 3: Synthesis Of Compounds 9 and 10

Antimicrobial Susceptablity Test

One of the methods of measuring the effectiveness of a chemical agent as is to determine its zone of inhibition using the agar well diffusion method (Perez et al, 1990). Zone of inhibition is a qualitative means to measure the ability of an antimicrobial agent to inhibit the growth of micro organism. Zone of inhibition is the area on an agar plate where the growth of a control organism is prevented by a chemical agent usually placed on the surface of agar. An effective chemical agent will inhibit the growth of the microbe and measurement of the diameter of the zone of inhibition can be done. The relative effectiveness of the test compound is determined by comparing its inhibition zone diameter(IZD) value with values in a standard table or IZD value of a standard drug. The larger the value, the more effective the compound is likely to be as antimicrobial agent. For instance, test compound with IZD of less than 10mm is said to have no effect on the test microbe, those with IZD of 11-15mm are said to exhibit moderate activity while IZD of 16mm and above are said to be effective (Delahaye et al, 2009). The values of IZD obtained for the synthesized compounds are given in Table 1 together with the values for standard drugs Ampicillin and Gemtamin at 20mg/ml

The synthesized compound showed higher IZD than both Ampicillin and Gentamicin for G101, G102 and *bacillius*. Of the six micro organisms tested *pseudomonas aeauginosa* showed highest resistance to the synthesized compounds.

Table 1: Inhibition Zone Diameter.

Compound	Escherichia coli		Staphylococcus aureus		Bacillus spp	Pseudomonas aerugenosa
	Eco3	Eco 12	G101	G102		
8-amino-6-chloro-1,9,11-triazabenzo[a] phenothiazin-5-one	10	0	11	12	14	0
8-amino-6-chloro-4,9,11-triazabenzo[a] phenothiazin-5-one	27	28	28	35	27	13
Ampicilin	100	50	0.313	70.313	1.25	20
Gentamicin	6.25	10	0.393	0.393	0.156	10

Figures are in millimeter. (Inhibition zone diameter) at 20mg/ml concentration level.

Minimum Inhibitory Concentration (MIC).

The synthesized compounds gave encouraging results for inhibition zone diameter at 20 mg/ml for *Eco* 3and *Bacillus spp*. and were further investigated for concentrations between 10 mg/ml and 0.3125 mg/ml to determine their Minimum Inhibitory Concentration (MIC). The lowest concentration of test compounds that produced no zone of inhibition is regarded as the MIC and this is an index of minimal concentration of drugs that inhibits visible growth of microorganisms after overnight incubation.

Table 2: Minimum Inhibition Concentration.

Eco 3							Bacillus spp								G 102							
cpd	a	b	c	d	e	f	a	b	c	d	e	f	g		a	b	c	d	e	f	G	H
10	25	22	20	17	11	0	24	22	20	19	18	17	15	0	35	32	30	24	18	13	0	
9	0						14	11	0						0							

a= 10mg/ml, b= 5mg/ml, c= 2.5mg/ml, d=1.25mg/ml, e= 0.845mg/ml, f= 0.625,0.3125mg/ml

Compound **10** showed highest activity amongst the synthesized compounds since it showed activity against all the three microorganisms tested even at concentration of 0.625 mg/ml. From the values obtained from the inhibition zone diameter and minimum inhibition concentration, the compounds are potential antimicrobial agents.

REFERENCE

Andrzej Z.B., Zenon P.C And Wojciech K.L.(2007). In vitro antimicrobial activity of novel azaphenothiazine derivatives. Acta Poloniae Pharmaceutica ñ Drug Research, 69 No. 6 pp. 1149ñ1152 | Bae S., Freeman, H.S. (2007). Aquatic Toxicity Evaluation of copper-Complexed direct dyes to the Daphnia Magna. Dye and Pigments73; 126-132. | David L, Heyman MM,(2013) Antimicrobial Resistance : an emerging public health problem across sectors. World Health Summit , Singapore. | Johnson F., Grollman A.P. and Shaikh I.A. (1986). Streplonigrin.1. Structure-Activity Relationships among Simple Bicyclic Analogues. Rate Dependence of DNA degradation on Quinone Reduction Potential. Jour.Med.Chem.29(8); 1320-1340. | Kalkanidis, M., Klonis, N., Tilley, L. and Deady, L. (2002).Novel Phenothiazine Antimalarials: Synthesis, Antimalarial activities and Inhibition of beta-Haematin. Biochem Pharmacol.63(5), 833-842 . | Korth Carsten, May C. H., Cohen E.F. and Prusiner B.S. (2001). Acridine and Phenothiazine Derivatives as Pharmacotherapeutics from prion diseases. National Academy of science USA98(17); 9836-9841. | Okafor C.O. and Okoro U.C. (1991). Dyes and Pigments. 16(2) pg 149-163 | Okafor CO,(1975¬).Studies in Heterocyclic Series X ..J.Org Chem 40;2753-2755 | Perez. C., Pauli M. and Bazerque, P. (1990). Antibiotic assay by agar well diffusion method. Acta-Biological et Medicine Experimentalis. 15; 113-115. | Petrow V, and Sturgeon B. (1954) Some Quinoline-5,8-quinone. J.Chem Soc.570. | Pratt Y.T, and Drake N.L. (1960). Qinolinequinones V. 6-Chloro and 7-Chloro-5,8-quinolinequinones. J.Am. Chem.Soc. 82; 1155-1160. | Raghvanshi Dharaj; Sharma, Pramod Kumar Manocha, Nimita Agrawal, Shikha; Chaturved, Prerna (2012). Design, Synthesis and Characteristics of some Novel Amino and Aldehyde Substituted Azaphenothiazines. Asian Journal of Research in Chemistry 5(4): 532 | Zhang X-H, Kim, S.H., Lee S.I., Gai, C.J., Yang S.I., Ahn K-H.(2007). Synthesis, Photophysical and Electrical Properties of Novel Conjugated Donor-Acceptor Molecules Based on Phenothiazines and Benzimidiazole. Bull.Korean.Chem.Soc.28(8); 1389. |