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Synthesis and Antibacterial Studies of Some Alkynylated Benzo[a]phenoxazin-5-one and 1,4-Naphthoquinone Derivatives

M.A. EZEOKONKWO[1,*], F.O. UGWUONA[1] and I.C. UGWU[2]

[1]Department of Pure & Industrial Chemistry, University of Nigeria, Nsukka, Enugu State, Nigeria
[2]Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria

*Corresponding author: Tel: +234 8035486024; E-mail: mercy.ezeokonkwo@unn.edu.ng; mercy.ezeokonkwo@yahoo.com

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Various 6-alkynyl-5H-benzo[a]phenoxazin-5-ones (**5a-e**) and 2-chloro-3-alkynyl-1,4-naphthoquinones (**6a-e**) were synthesized using modified Sonogashira protocol. The chemical structures of the synthesized compounds were established by spectroscopic data. *In vitro* antibacterial screening of these compounds was carried out on multi-resistant bacterial strains using gentamycin and ampicillin as a reference standard. The bacteria strains used were Gram-positive bacterium (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* I, *Escherichia coli* 12 and *Klebsiella pneumonia*). All the tested compounds showed moderate to excellent activity against one or more tested micro-organisms. Compounds **6d** (MIC 0.30 mg/mL) and **6b** (MIC 1.05 mg/mL) showed highest activity against Gram-negative (*Klebsiella pneumoniae*) and Gram-positive (*Staphylococcus aureus*) respectively.

Keywords: Sonogashira cross-coupling reaction, Benzo[a]phenoxazin-5-ones, 1,4-Naphthoquinones, Terminal alkynes.

INTRODUCTION

Organic synthesis has been greatly enhanced by the use of reactions catalyzed by transition metal complexes especially palladium and this has led to the development of new methods of constructing carbon-carbon bonds and carbon heteroatom bonds[1]. One of the most general and widely used palladium-catalyzed cross-coupling reactions is the alkynylation of aryl halides using terminal alkynes, generally known as the Sonogashira cross-coupling reaction[2]. Other palladium catalyzed coupling reactions that have tremendously changed the face of organic synthesis include Heck-Mizoroki coupling reaction, Buchwald-Hartwig coupling reaction, Suzuki-Miyaura coupling reaction and Negishi coupling reaction. The Sonogashira cross-coupling reaction has the advantage of occurring at or slightly above room temperature unlike the harsh conditions required for the alternative Castro-Stephens coupling. It tolerates a wide range of functional groups, making the process a useful tool in total synthesis. However, some drawbacks[3-5] are experienced when copper co-catalyst is used in the reaction. Researchers have attempted to overcome this by modifying the reaction conditions. Some of the modifications that have proved successful include the use of pyrrolidine as a base with a platinum catalyst in the absence of copper salt[6], the addition of tetra-*n*-butylammonium fluoride (TBAF) as a base under a nitrogen atmosphere without using copper or amine[7] and the use of hydrogen to degas the reaction[5]. The Sonogashira reaction is used in the synthesis of various organic compounds and in the production of pharmaceuticals, agricultural chemicals and natural products. In the present study, the modified Sonogashira cross-coupling reaction is used to synthesize alkynylated derivatives of 6-chloro-5H-benzo[a]phenothiazin-5-one and 2,3-dichloro-1,4-naphthoquinone.

Interest in the synthesis of phenoxazine and its derivatives has continued to grow due to the various biological properties as well as industrial applications. Since the discovery of the parent ring phenoxazine by Bernthsen[8], researchers have continued to engage in structural modifications to improve the biological properties, reduce side effects and open new areas of applications. Such modifications have led to an array of derivatives of pharmacological and industrial interest. Some of the pharmacological applications of phenoxazine and its derivatives include as anti-epileptic[9], antitumour[10,11], anticancer[12], antituberculosis[13], antibacterial[14,15], anthelminthic[16], spasmolytic, C.N.S. depressants[17,18], herbicides, tranquillizers, sedatives[19] and parasiticidal agents[20]. Phenoxazine derivatives have also been used as antioxidants[21], biological stains[22,23], acid-base indicators[24] and bromometric and stannometric redox indicators[25-28].

Naphthoquinone and its derivatives have attracted much interest also due to their biological activities. The naphthoquinone fragments are often encountered in natural biologically active compounds. Natural naphthoquinone derivatives found in

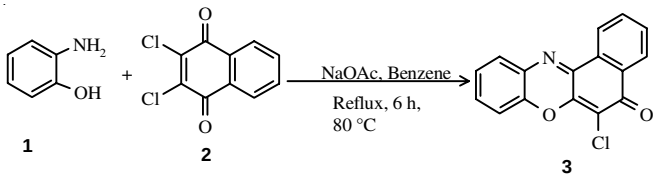
plants, such as Juglone Lawsone, Plumbagine and Lapachol, demonstrate antibacterial effect on several species of aerobic and anaerobic organism[29,30]. The natural naphthoquinone products alkannin and shikonin and their derivatives are also active against Gram-positive bacterial such as *Staphylococcus aureus*, *Enterococcus faecium* and *Bascillus substilis*, but they are inactive against Gram-negative bacteria[31]. Reports have also shown that 2,3-disubstituted-1,4-naphthoquinones have potent antibacterial[32], antifungal[33], anticoagulant[34,35], anti-malarial[36], cytotoxic and anticoplastics activities[37,38]. It has been observed that the extensive use of antibiotics has resulted in increased prevalence of antibiotic resistant bacteria. This may render the current antimicrobial agents insufficient to control some bacterial infectious with time[39]. There is therefore the need to synthesize new derivatives of antimicrobial phenoxazines and naphthoquinones to improve their pharmacological properties; peradventure they could form part of the solution to the growing problem of the rising new infectious diseases and ever-increasing multi-drug resistance of microbial pathogen[40].

Despite the synthesis and various biological activities of phenoxazine derivatives, which abound in the literatures, the antimicrobial study is still under studied. Moreover, synthesis and biological activities of alkynylated phenoxazine and 1,4-naphthoquinone derivatives is scarcely and poorly investigated. Herein is reported the successful synthesis of biologically active alkynyl derivatives of 6-chloro-5*H*-benzo[a]phenoxazin-5-one and 2,3-dichloro-1,4-naphthoquinone *via* modified Sonogashira cross-coupling reaction.

EXPERIMENTAL

Some of the reactions were carried out under an atmosphere of nitrogen. The intermediate 6-chloro-5*H*-benzo[a]phenoxazin-5-one (**3**) was prepared following the literature procedure[41]. Melting points were determined with Fischer John's melting point apparatus and are uncorrected. UV and visible spectra were recorded in ethanol on a unicon UV-2500PC spectrophotometer using matched 1 cm quartz cells; absorptions are measured in nanometer (nm), the figure in parenthesis are the molar absorptivity coefficient (ε) value. IR spectra were recorded on 8400s Fourier transform infrared (FTIR) spectrophotometer using KBr disc and in some cases NaCl disc and are reported in wave numbers (cm^{-1}). Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) were determined using Joel 400 MHz at Strathclyde University, Scothland. Chemical shifts are reported in delta (δ) scale. The antimicrobial screening was done at the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. All reagents were of analytical grade and were used as supplied. 2-Aminophenol, *bis*(triphenylphosphine)palladium(II)chloride [$PdCl_2(PPh_3)_2$], tetrabutylammonium fluoride trihydrate (TBAF·$3H_2O$), 2,3-dichloro-1,4-naphthoquinone and all the terminal alkynes were purchased from Sigma Aldrich Chemical Company in sure-seal bottles.

Synthesis of 6-chloro-5*H*-benzo[a]phenoxazin-5-one (3): This compound was prepared according to the procedure by Okafor[41]. 2-Aminophenol (**1**) (4 g, 37 mmol) and anhydrous sodium acetate (8.2 g, 37 mmol) were dissolved in stirring benzene (100 mL) and the mixture warmed to boil in a three necked flask. 2,3-Dichloro-1,4-naphthquinone (**2**) (8.2 g, 37 mmol) was gradually added and the mixture refluxed on a water bath with stirring for 6 h at 80 °C. The reaction mixture was allowed to cool and poured into a beaker containing crushed ice. The precipitate formed was air dried and recrystallized from ethanol-water (2:1) to give yellow solid of 6-chloro-5*H*-benzo[a]phenoxazin-5-one **3** (9.4 g, 97 %) with a melting point of 200-201 °C (dec) (lit. 201-202 °C)[41].



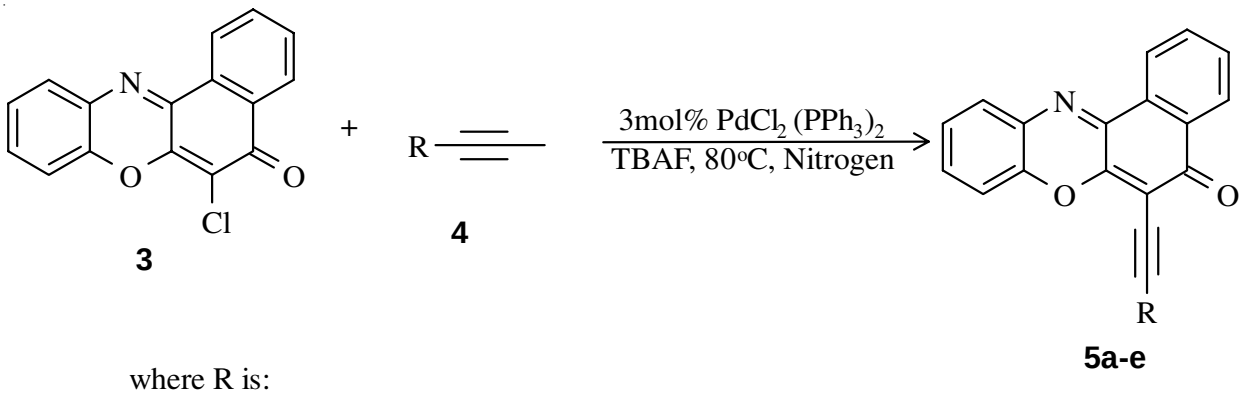
Scheme-I: Synthesis of 6-chloro-5*H*-benzo[a]phenoxazin-5-one (**3**)

General procedure for synthesis of 6-alkynyl-5*H*-benzo[a]phenoxazin-5-ones (5a-e) and 2-chloro-3-alkynyl-1,4-naphthoquinones (6a-e): The alkynylated compounds were prepared according to the procedure developed by Liang *et al.*[7] with some modifications. A mixture of 6-chloro-5*H*-benzo[a]phenoxazin-5-one (**3**) (0.14 g, 0.5 mmol) or 2,3-dichloro-1,4-naphthoquinone (**2**) (0.114 g, 0.5 mmol), terminal alkyne (**4**) (0.07 mL, 0.6 mmol), $PdCl_2(PPh_3)_2$ (3 mol %) and TBAF·$3H_2O$ (3 equivalent) was stirred under nitrogen at 80 °C for 10-45 min. The mixture was then washed with water, extracted with diethyl ether and evaporated. The resulting crude product was recrystallized from ethanol to afford 6-alkynyl-5*H*-benzo[a]phenoxazin-5-ones (**5a-e**) and 2-chloro-3-alkynyl-1,4-naphthoquinones (**6a-e**) respectively in good to excellent yield.

6-Phenylethynyl-5*H*-benzo[a]phenoxazin-5-one (5a): Dark brown solid, yield 0.153 g (85 %), m.p.: 140-142 °C (dec). UV-visible λ_{max}: 356 (2.03), 368.50 (2.04) and 738 (1.23) nm. IR (KBr, ν_{max}, cm^{-1}): 1667 (C=O and C=N), 2220 (C≡C), 1450 (C=C aromatic), 1040, (C-O-C and C-N). ^{1}H NMR (DMSO-d_6) δ: 8.66 (d, J = 7.69 Hz, 1H, Ar-H), 8.21 (d, J = 7.58 Hz, 1H, Ar-H), 8.08 (dd, J_1 = 5.65 Hz, J_2 = 3.21 Hz, 1H, Ar-H), 8.01 (dd, J_1 = 14.70 Hz, J_2 = 7.67 Hz, 1H, Ar-H), 7.90 (m, 4H, Ar-H), 7.76 (t, J = 7.47 Hz, 2H, Ar-H), 7.62 (t, J = 7.62 Hz, 1H, Ar-H), 7.51 (dd, J_1 = 8.33 Hz, J_2 = 3.75 Hz, 2H, Ar-H), 7.19 (dd, J_1 = 8.93 Hz, J_2 = 3.96 Hz, 2H, Ar-H), 7.07(m, 5H, Ar-H). ^{13}C NMR (DMSO-d_6) δ: 177 (C=O), 143.99-124.16 (C=C aromatic), 89.38 (C≡C).

6-(3-Hydroxy-3-methylbut-1-yn-1-yl)-5*H*-phenoxazin-5-one (5b): Brown solid, yield 0.18 g (88 %), m.p.: 148-150 °C (dec). UV-visible λ_{max}: 355 (2.10), 431.50 (2.0), 738.00 (1.18) nm. IR (NaCl, ν_{max}, cm^{-1}): 2354 (C≡C), 1652 (C=O and C=N), 1454 (C=C aromatic), 3414 (O-H), 2930 (C-H aliphatic), 1035 (C-O-C). ^{1}H NMR (DMSO-d_6) δ: 8.67 (d, J = 7.48 Hz, 2H, Ar-H), 8.22 (d, J = 7.38 Hz, 2H, Ar-H), 8.09 (dd, J = 5.99 Hz, 3.07 Hz, 2H, Ar-H), 7.91 (m, 4H, Ar-H), 7.66 (m, 4H, Ar-H), 2.5 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6): δ 176 (C=O), 146.63-124.17 (C=C aromatic), 89.38 (C≡C), 23.64-19.78 (aliphatic carbon).

6-(Hex-1-yn-1-yl)-5*H*-benzo[a]phenoxazin-5-one (5c): Reddish-brown solid, yield 0.175 g (92 %), m.p.: 144-146 °C (dec). UV-visible λ_{max}: 370.50 (2.19), 738.50 (1.17) nm. IR



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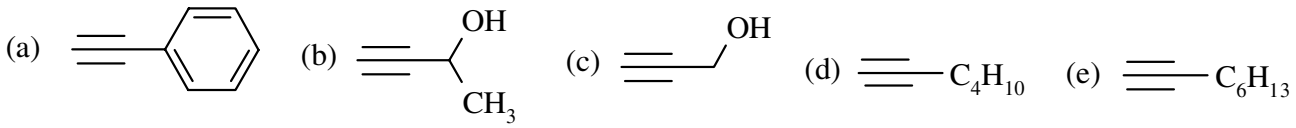
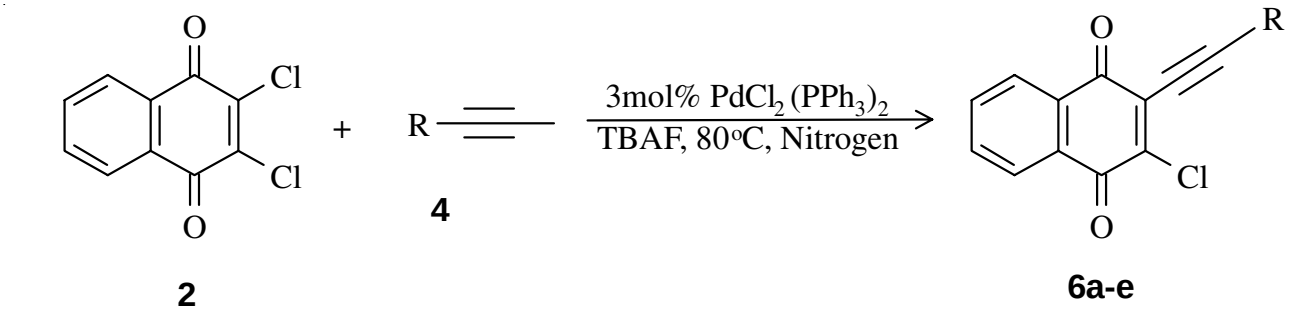


Scheme-II: Synthesis of 6-alkynylated benzo[a]phenoxazin-5-ones (**5a-e**)



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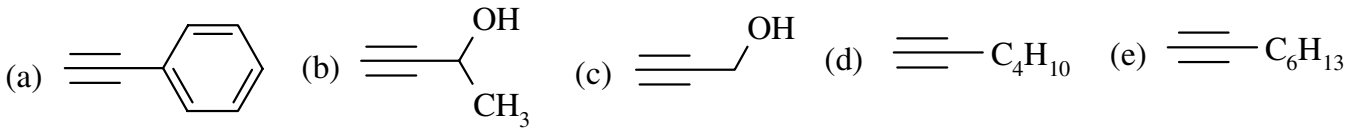


Scheme-III: Synthesis of 2-alkynylated-3-chloronaphthoquinones (**6a-e**)

(NaCl, ν_{max}, cm^{-1}): 2297 (C≡C), 1473 (C=C aromatic), 1635 (C=O and C=N), 2953 (C-H aliphatic), 1120 (C-O and C-N). ^{1}H NMR (DMSO-d_6) δ: 8.68(dd, J = 7.77 Hz,1.51 Hz, 2H, Ar-H), 8.23(m, 4H, Ar-H), 8.01 (m, 4H, Ar-H), 7.92 (m, 4H, Ar-H), 3.16 (m, 2H, CH_2), 1.56 (m, 2H, CH_2), 1.31 (t, J = 7.28 Hz, 2H, CH_2), 0.93 (t, J = 7.27 Hz, 3H, CH_3). ^{13}C NMR (DMSO): δ 177.23 (C=O), 146.63-123.49 (C=C aromatic), 89.37 (C≡C), 23.63-14.07 (aliphatic carbon).

6-(3-Hydroxyprop-1-yn-1-yl)-5*H*-benzo[a]phenoxazin-5-one (5d): Greyish-brown solid, yield 0.13 g (86.7 %), m.p.: 138-140 °C (dec). UV-visible λ_{max}: 351.50 (2.72), 363 (2.70), 420 (1.53), 738 (1.15) nm. IR (KBr, ν_{max}, cm^{-1}): 2365 (C≡C), 1650 (C=O and C=N), 1576 (C=C aromatic), 1283 (C-O-C), 3385 (O-H), 2955 (C-H aliphatic). ^{1}H NMR (DMSO-d_6): δ 8.67 (d, J = 7.89 Hz, 1H, Ar-H), 8.22 (d, J = 7.52 Hz, 1H, Ar-H), 8.01 (dd, J = 12.38 Hz, 7.64 Hz, 2H, Ar-H), 7.64 (m, 8H, Ar-H), 7.50 (dt, J = 9.56Hz, 7.17 Hz, 4H, Ar-H), 1.56 (s, 2H) for aliphatic proton). ^{13}C NMR (DMSO-d_6): δ 177.21 (C=O), 148.06-123.49 (C=C aromatic), 116.72 (C≡C), 23.63-14.07 (aliphatic carbon).

6-(Oct-1-yn-1-yl)-5*H*-benzo[a]phenoxazin-5-one (5e): Brown solid, yield 0.15 g (79.8 %), m.p.: 158-160 °C (dec). UV-visible λ_{max}: 351.50 (2.24), 431.50 (2.14), 738 (1.16), 767.50 (1.15) nm. IR (NaCl, ν_{max}, cm^{-1}): 2300 (C≡C), 1639 (C=O and C=N), 1464 (C=C aromatic), 2949 (C-H aliphatic), 1040 (C-N and C-O). ^{1}H NMR (DMSO-d_6) δ: 8.63 (d, J = 7.73 Hz, 2H, Ar-H), 8.19 (d, J = 7.67 Hz, 2H, Ar-H),7.87 (m, 4H, Ar-H), 7.57 (m, 4H, Ar-H), 3.15 (t, 2H, CH_2), 1.55 (t, J = 9.13 Hz, CH_2), 0.93 (t, J = 7.28 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ: 177.29 (C=O), 146.31-125.19 (C=C aromatic), 112.99 (C≡C), 23.61-14.04 (aliphatic carbon).

2-Chloro-3-(phenylethynyl)-1,4-naphthoquinone (6a): Reddish-brown solid, yield 0.16 g (90 %), m.p.: 139-140 °C (dec). UV-visible λ_{max}: 326.50 (23), 350.50 (2.21), 737 (1.09) nm. IR (NaCl, ν_{max}, cm^{-1}): 2365 (C≡C), 1669 (C=O), 1486 (C=C aromatic), 693 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.09 (dd, J = 5.71 Hz, 3.24 Hz, 2H, Ar-H), 7.91 (dd, J = 5.80 Hz, 3.34 Hz, 2H, Ar-H), 7.8 (m, 5H, Ar-H). ^{13}C NMR (DMSO-d_6): δ 176 (C = O), 142-125 (C=C aromatic), 113(C≡C), 58.09 (C-Cl).

2-Chloro-3-(3-hydroxy-3-methylbut-1-yn-1-yl)-1,4-naphthoquinone (6b): Red solid, yield 0.159 g (89.8 %), m.p.: 158-160 °C (dec). UV-visible λ_{max}: 351 (1.70), 738.50 (1.03) nm. IR (KBr, ν_{max}, cm^{-1}): 2300 (C≡C), 1654 (C=O), 1455 (C=C aromatic), 3394 (O-H), 691 (C-Cl), 2968 (C-H aliphatic). ^{1}H NMR (DMSO-d_6) δ: 8.08 (dd, *J* = 5.78 Hz, 3.32 Hz, 2H, Ar-H), 7.91 (dt, *J* = 6.71 Hz, 3.29 Hz, 4H, Ar-H), 1.56 (m, 6H, CH_3). ^{13}C NMR (DMSO-d_6): δ 176.46 (C=O), 142.99-127.64 (C=C aromatic), 58.09 (C-Cl), 89 (C≡C), 23.66-19.79 (aliphatic carbon).

2-Chloro-3-(hex-1-yn-yl)-1,4-naphthoquinone (6c): Red solid, yield 0.15 g (93.8 %), m.p.: 138-140 °C (dec). UV-visible λ_{max}: 350.50 (2.0), 739.00 (1.03) nm. IR (KBr, ν_{max}, cm^{-1}): 2356 (C≡C), 1470 (C=C aromatic), 755 (C-Cl), 2954 (C-H aliphatic). ^{1}H NMR (DMSO-d_6): δ 8.09 (dd, *J* = 5.71 Hz, 3.07 Hz, 2H, Ar-H), 7.91 and 7.51 (m, 4H, Ar-H), 3.17 (m, 2H), 1.57 (tt, 2H), 1.31 (h, 2H), 0.93 (t, 3H) *i.e.* 3.17-0.93 for aliphatic protons in hexyne. ^{13}C NMR (DMSO-d_6): δ 176.49 (C=O), 142.99-127.64 (C=C aromatic), 58.11 (C-Cl), 23.65-19.79 (aliphatic carbon), 89 (C≡C).

2-Chloro-3-(3-hydroxyprop-1-yn-1-yl)-1,4-naphthoquinone (6d): Dark-brown solid, yield 0.1 g (83.3 %), m.p.: 152-153 °C (dec). IR (KBr, ν_{max}, cm^{-1}): 2351 (C≡C), 1670 (C=O), 3335 (O-H), 1458 (C=C aromatic), 2955 (C-H aliphatic), 635 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 8.03 (m, 4H, Ar-H), 7.86 (m, 4H, Ar-H), 1.52 (s, 2H, CH_2 aliphatic proton). ^{13}C NMR (DMSO): δ 176.49 (C=O), 143.01-127.69 (C=C aromatic), 58.09 (C-Cl), 23.53 (aliphatic carbon).

2-Chloro-3-(oct-1-yn-1-yl)-1,4-naphthoquinone (6e): Purple solid, yield 0.14 g (87.5 %), m.p.: 138-140 °C (dec). UV-visible λ_{max}: 350.50 (2.26), 738 (1.13) nm. IR (KBr, ν_{max}, cm^{-1}): 2230 (C≡C), 1458 (C=C aromatic), 1678 (C=O), 629 (C-Cl), 2952 (C-H aliphatic). ^{1}H NMR (DMSO-d_6) δ: 8.06 (dd, *J* = 5.73 Hz, 2H, Ar-H), 3.16 (m, 2H, CH_2), 1.56 (dq, *J* = 12.09 Hz, 7.55 Hz, 2H), 1.30 (h, *J* = 7.31 Hz, 2H,), 0.92 (t, *J* = 7.28 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 176.47 (C=O), 142.99-126.20 (C=C aromatic), 58.10 (C-Cl), 23.62-14.05 (aliphatic carbon), 89 (C≡C).

Evaluation of antimicrobial activity: The bacterial strains used in this study were one Gram-positive bacterium (*Staphylococcus aureus*) and four Gram-negative bacteria (*Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli* 1 and *Esccherichia coli* 12). These were all multi-resistant bacterial strains freshly cultured under clinical conditions. The antimicrobial properties of the alkynylated compounds were investigated in form of the general sensitivity test and minimum inhibitory concentration (MIC) with respect to these bacterial strains using agar-well diffusion method[42,43]. Gentamycin and ampicillin were used as a reference standard.

Sensitivity testing: The antibacterial activity of the alkynylated compounds were determined using agar diffusion well method described by Perez *et al.*[42]. A single colony of each test isolate was suspended in 2 mL sterile Mueller-Hinton agar. The suspension of each isolate was standardized by adjusting to 0.5 McFarland turbidity standards equivalent to approximately 10^8 cfu/mL and used to inoculate the surface of the nutrient agar. The inoculated agar surface was allowed to dry and 6 mm diameter cork borer was used to bore wells (7 mm in diameter and 2.5 mm deep) into the agar. A 20 mg/mL concentration of each compound was prepared by dissolving 0.04 g of each in 2 mL dimethyl sulfoxide (DMSO) and 0.05 mL of each solution was delivered into each agar well. The plates were incubated at 37 °C for 24 h. The diameters of the inhibition zones around the wells were measured with metre rule to the nearest whole millimetre. The test was done in triplicate and the mean inhibition zone calculated.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) of the alkynylated compounds and the reference standard was determined using agar well diffusion method described by Ojo *et al.*[43]. Serial dilutions of the synthesized products were prepared from 2 mg/mL solution of the products synthesized to give final concentrations ranging from 10 mg/mL to 0.625 mg/mL. 1 mL of each serial dilution was mixed with 19mL of sterile Mueller-Hinton agar maintained at 45 °C, poured into a sterile plate and allowed to set. The amended culture media was spot-inoculated with 0.025 mL of an overnight broth culture of the test bacterial strains and incubated at 37 °C for 24 h. The plates were examined for presence of visible growth. The minimum concentration that completely inhibited growth of the organisms was taken as the minimum inhibition concentration of the respective alkynylated compounds. The procedure was repeated for the reference standard (gentamycin and ampicillin).

RESULTS AND DISCUSSION

6-Chloro-5*H*-benzo[a]phenoxazin-5-one derivatives: The intermediate 6-chloro-5*H*-benzo[a]phenoxazin-5-one (**3**) was prepared according to a reported procedure by base catalyzed reaction of 2-aminophenol (**1**) with 2,3-dichloro-1,4-naphthoquinone **2** at 80 °C giving the product of interest **3** as a yellow solid in 97 % yield (**Scheme-I**). The first step in the reaction is the abstraction of proton from the hydroxyl group of the phenol **1** by the base. The phenoxide ion **1a** formed mounts a nucleophilic attack on the 2,3-dichloro-1,4-napthoquinone **2** by displacing one of the halogen atoms to form a diaryl intermediate **7**. By doing so, cyclization took place by a second nucleophilic attack from the amino group on the carbon atom of the carbonyl group to form a second intermediate **8**, which on elimination of water molecule yields 6-chloro-5*H*-benzo[a]phenoxazin-5-one **3** (Scheme 4). Direct nucleophilic alkynylation of compound **3** with various terminal alkynes **4** was done in the absence of copper(I) salt and amine. The reaction was performed in the presence of *bis*(triphenylphosphine) palladium(II) chloride as a catalyst and tetrabutyl ammonium fluoride trihydrate as a base under nitrogen atmosphere to yield the corresponding 6-alkynyl-5*H*-benzo[a]phenoxazin-5-ones (**5a-e**) (**Scheme-II**). The proposed mechanism follows the modified Sonogashira coupling reaction which begins with the oxidative addition of Pd(0) with 6-chloro-5*H*-benzo[a]-phenoxazin-5-one **3** to form ArPdX complex **9** (Ar = phenoxazine molecule, X = Cl). The application of electron-rich amino phosphine ligand, *bis*(triphenylphosphine), makes this step easier. This first step is followed by the activation of the terminal alkyne. Because no copper salt was employed and the base is not strong enough to abstract a proton from the alkyne, a transmetallation step could be excluded[10]. The terminal alkyne

C-H bond activation is accomplished by the coordination of the alkyne to the ArPdX complex. Upon coordination, the C-H bond is weakened and HX is removed in the presence of the base to form complex **10**, which subsequently undergoes reductive elimination to afford the product of interest **5** and regenerates the catalyst (**Scheme-V**). The electron-rich and bulky aminophosphine ligand is likely to play key roles in facilitating the reductive elimination step. It is also believed that the tetra-*n*-butylammonium fluoride might have played some beneficial roles in the reaction, which includes the activation of the active Pd(0) species with the formation of anionic Pd species and deprotonation of the acidic hydrogen in the alkyne[7].

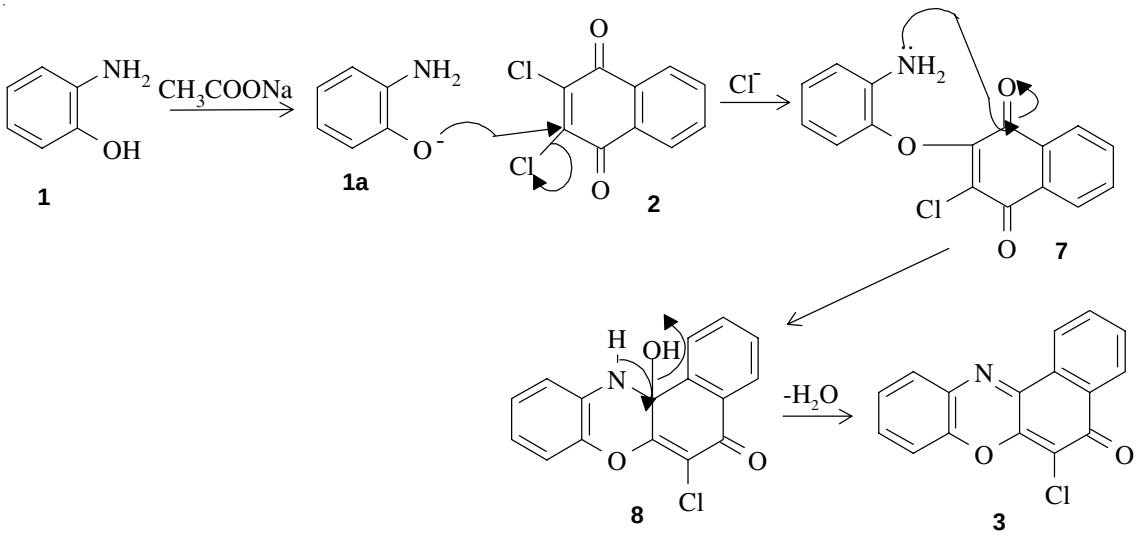
The structures of the 6-alkynyl-5H-benzo[a]phenoxazin-5-ones (**5a-e**) were established on the bases of FT-IR and NMR spectroscopy. The IR spectra of the compounds **5a-e** showed, in each case stretching band of C=O and C=N, C≡C and C=C aromatics in the region of 1667-1635 cm^{-1}, 2365-2220 cm^{-1} and 1576-1450 cm^{-1} respectively. The peaks around 1283-1040 cm^{-1} that are characteristic of phenoxazine ring C-O-C and C-N stretching frequency were so assigned. The ^{1}H NMR spectra in each case, showed signals at δ 7.50-8.68 ppm assigned to Ar-H. The ^{13}C NMR spectra further support the assigned structures. The peaks at 176-177.29 ppm and 123.49-148.06 ppm were attributed to C=O and C=C aromatics respec-tively. Other peaks are in agreement with the rest of the carbons in the structure of the compounds.

2,3-Dichloro-1,4-naphthoquinone derivatives: These derivatives were prepared by the reaction of 2,3-dichloro-1,4-naphthoquinone (**2**) with terminal alkynes (**4a-e**) under similar modified Sonogashira reaction conditions. The products of interest 2-chloro-3-substituted alkynylated-1,4-naphthoquinones (**6a-e**) were obtained in good to excellent yield. The mechanism of nucleophilic alkynylation of **2** similarly follows the modified Sonogashira reaction wherein the terminal alkyne C-H bond is activated by the coordination of the alkyne to the ArPdX complex **11** (where Ar = 2-chloro1,4-naphthoquinone and X = Cl). Hydogen chloride HX is removed in the presence of the base to furnish the product **6** while regenerating the catalyst. The terminal alkynes yielded 3-(phenylethynyl)-, 3-(3-hydroxy-3-methylbut-1-yn-1-yl)-, 3-(hex-1-yn-1-yl)-, 3-(3-hydroxy prop-1-yn-1-yl), 3-(oct-1-yn-1-yl)- derivatives (**6a-e**, 83.3-93.8 %).

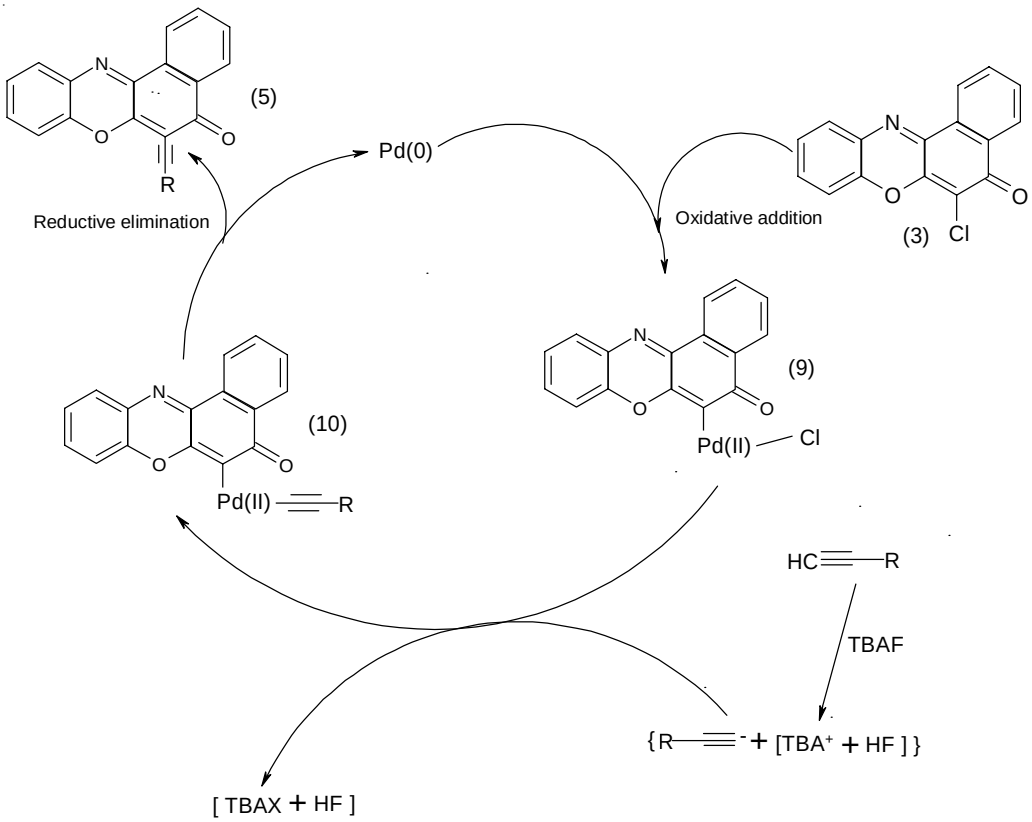
Compounds **6a-e** were characterized based on their FT-IR, ^{1}H NMR and ^{13}C NMR spectra. In each case, the IR of the compounds showed prominent absorption signals due to the stretching vibrations of C≡C, C=O, C=C aromatics and Cl at 2365-2230 cm^{-1}, 1678-1654 cm^{-1}, 1486-1455 cm^{-1} and 755-629 cm^{-1} respectively. From the ^{1}H NMR spectra the signals at δ 7.10-8.09 ppm were assigned to aromatic protons while δ 0.92-3.17 ppm were attributed to aliphatic protons. The ^{13}C NMR spectra showed the absorption bands due to C=O, C=C aromatics, C≡C and Cl at 176-176.49 ppm, 125-143.03 ppm, 89 ppm and 58.09-58.11 ppm respectively. Every other peak is consistent with the assigned structures.

Antimicrobial activities: Due to the considerable biological and pharmaceutical activities of phenoxazines and naphthoquinones, it was necessary to evaluate the antibacterial activity of the alkynylated products (**5a-e** and **6a-e**). The compounds were screened *in vitro* for antibacterial activities against Gram-positive bacterium (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* I, *Escherichia coli* 12 and *Klebsiella pneumonia*) using the disc-agar well diffusion method[42].

The results of the antimicrobial activity of the compounds are expressed as inhibition zone diameter (IZD, mm) and minimum inhibition concentration (MIC, mg/mL) and shown in Tables 1 and 2 respectively. All the tested compounds showed at least some activity against one or more tested microorganisms. Table-1 shows the IZD produced by each compound against the bacterial organism at 10 mg/mL concentration. The IZD ranged between 9 and 17 mm in diameter; the higher the IZD, the higher the sensitivity. All the synthesized compounds were very active against *K. pneumonia*. However, compounds **6c** and **5d** showed activity only to *Klebsiella pneumonia* with IZD of 15 mm; other bacteria strains were resistant to them. Compound **6d** has the highest activity against *Klebsiella pneumoniae* with IZD of 17 mm. All the tested compounds were active to *Staphylococcus aureus* except compounds **6c** and **5d**. *Pseudomonas aeruginosa* was resistant to compounds

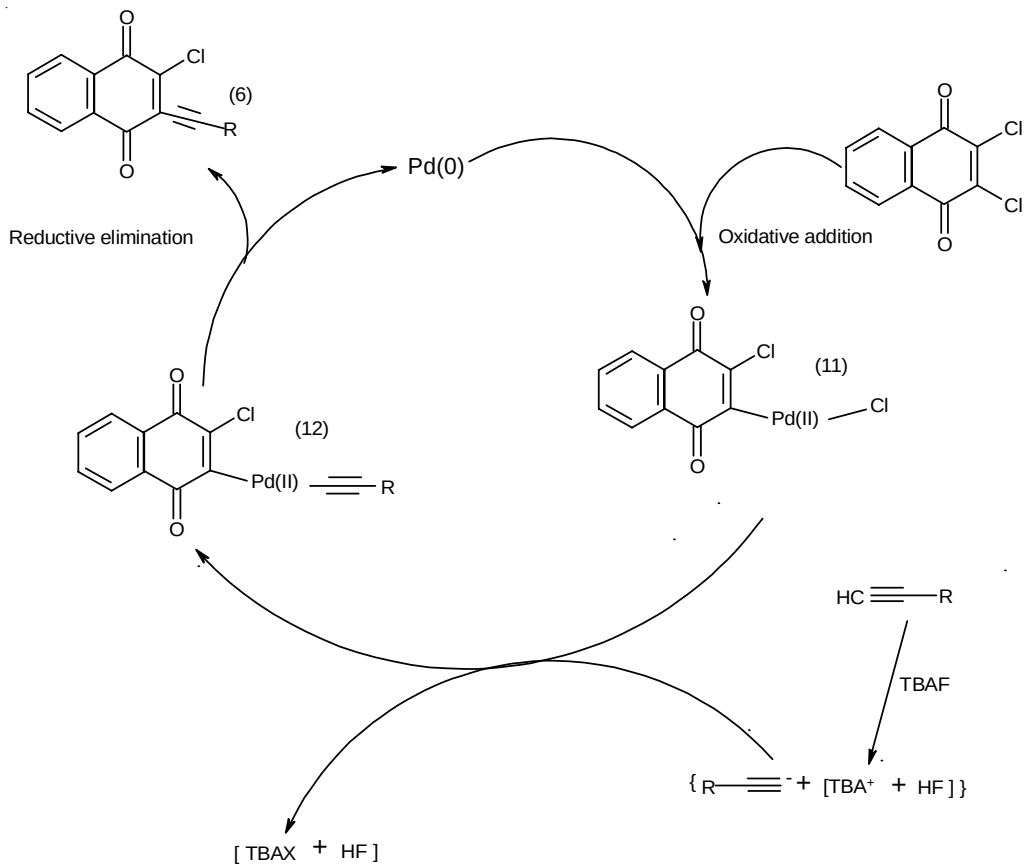


Scheme-IV: Proposed mechanism of synthesis of 6-chloro-5H-benzo[a]phenoxazin-5-one (**3**)



Ligand was omitted for simplicity.

Scheme-V: Proposed mechanism of the preparation of 6-alkynylated-5*H*-benzo[a]phenoxazin-5ones



Ligand was omitted for simplicity.

Scheme-VI: Proposed mechanism of the preparation of 2-chloro-3-alkynyl-1,4-naphthoquinones

5a, **6a**, **5c**, **6c**, **5d** and **5e** but was sensitive to other compounds with IZD ranging between 9 and 11. *E. coli* 1 was resistant to compounds **5b**, **6b**, **6c** and **5d**. *E. coli* 12 was resistant to compounds **5c**, **6c** and **5d** but sensitive to others. The Gram-positive bacterium *Staphylococcus aureus* was sensitive to all the compounds (IZD 11-13 mm) except **6c** and **5d**.

Minimum inhibitory concentration (MIC) of the compounds was also determined by agar-well diffusion method already described[43] with various concentrations ranging between 10 mg/mL to 0.625 mg/mL. The lowest concentration of each compound that produced no zone was regarded as MIC. Hence, essence of MIC is to determine the least concentration of the compounds that can inhibit the growth of the micro-organism. Table-2 also compares the MICs of the synthesized compounds with those of some standard drugs (ampicillin and gentamycin) that are known to have biological activity. Compared to standard drugs, compounds **5a-e** and **6a-e** showed significant antibacterial activity against both Gram-positive and negative bacteria. Compound **6d** (MIC 0.3 mg/mL) with electron donating group (3-hydroxyprop-1-yne) at position 3 of the quinone ring exhibited highest and excellent activity against Gram-negative bacterium (*K. pneumonia*). While compound **6b** (MIC 1.05 mg/mL) with also an alkynol group at position 3 showed very good and highest activity against the Gram-positive bacterium (*S. aureus*). All the synthesized products are very active against *K. pneumonia*. The MIC for gentamycin and ampicillin is 5 mg/mL, which is very high when compared to MIC values of the synthesized compounds, which ranges from 0.30 to 1.26 mg/mL. The MIC value of gentamycin and ampicillin against *E. coli* I and *E. coli* 12 is 100 mg/mL, which is still higher than the MIC values for most of the synthesized compounds. The same explanation goes for *P. aeruginosa* and *S. aureus* showing that the synthesized phenoxazines and naphthoquinones are highly biologically active, hence they are of pharmaceutical interest.

From structure activity relationship, it was observed that in some cases the antimicrobial activity of compounds **5** and **6** were the same despite the difference in structure. For instance, compounds **5a** and **6a** (MIC 1.78 mg/mL) and **5e** and **6e** (MIC 1.99, 1.26 mg/mL) have the same antagonistic effect both on *E. coli* 1 and *E. coli* 12 respectively (Table-2). Similarly compounds **5b** and **6b** (MIC 0.79 mg/mL), have the same antibacterial activity against *K. pneumonia*. Compounds **5a-e** are structurally related with compounds **6a-e**, but the only difference is that compounds **5a-e** were obtained by first coupling 2,3-dichloro-1,4-naphthoquinone (**2**) with 2-aminophenol (**1**) before alkynylating with terminal alkynes whereas compounds **6a-e** were obtained by direct alkynylation of 2,3-dichloro-1,4-naphthoquinone (**2**). Considering the fact that highly resistant bacterial strains were used in this study, the potency of the test compounds can be exploited to serve as a chemotherapeutic agent.

TABLE-1
SENSITIVITY TEST OF THE SYNTHESIZED COMPOUNDS SHOWING INHIBITION ZONE DIAMETER (IZD)

Compd. No.	Gram-negative bacteria				Gram-positive bacteria
	Pseudomonas aeruginosa	*Escherichia coli* I	*Escherichia coli* 12	*Klebsiella pneumonia*	*Staphylococcus aureus*
5a	-	11	11	14	12
6a	-	11	11	15	11
5b	9	-	11	14	12
6b	10	-	10	14	13
5c	-	11	-	14	12
6c	-	-	-	15	-
5d	-	-	-	15	-
6d	11	12	10	17	12
5e	9	12	10	14	12
6e	-	12	10	12	12

All activity data are given in mm; - = resistant

TABLE-2
RESULTS OF MINIMUM INHIBITION CONCENTRATION (MIC)

Compd. No.	Gram-negative bacteria				Gram-positive bacteria
	Pseudomonas aeruginosa	*Escherichia coli* I	*Escherichia coli* 12	*Klebsiella pneumoniae*	*Staphylococcus auerus*
5a	-	1.78	1.78	0.79	1.26
6a	-	1.78	1.78	0.50	1.78
5b	2.50	-	1.78	0.79	1.26
6b	1.99	-	1.99	0.79	1.05
5c	-	1.78	-	0.79	1.26
6c	-	-	-	0.50	-
5d	-	-	-	0.50	-
6d	1.78	1.26	1.99	0.30	1.26
5e	2.50	1.26	1.99	0.79	1.26
6e	-	1.26	1.99	1.26	1.26
Gentamycin	10	100	100	5	2.5
Ampicillin	20	100	100	5	2.5

All activity data are given in mg/mL; - = resistant

Conclusion

The study has shown that palladium catalyzed Sonogashira coupling reaction offers excellent routes to the synthesis of alkynylated angular phenoxazinones and alkynylated napthoquinones. These coupling reactions proceeded excellently under copper-, amine- and solvent free conditions. High yields of products at short time were also recorded. The new compounds were characterized based on FT-IR, ^{1}H NMR and ^{13}C NMR spectra. Antimicrobial screening of the synthesized compounds revealed that they have high potency against the test micro-organisms. Therefore, these compounds could be of pharmaceutical interest if properly harnessed.

REFERENCES

1. Z. Xu and E. Negishi, *Org. Lett.*, **10**, 4311 (2008).
2. K. Sonogashira, Y. Tohda and N. Hagihara, *Tetrahedron Lett.*, **16**, 4467 (1975).
3. V.P.W. Bohm and W.A. Herrmann, *Eur. J. Org. Chem.*, 3679 (2000).
4. D. Mery, K. Heuze and D. Astruc, *Chem. Commun.*, **15**, 1934 (2003).
5. A. Elangovan, Y. Wang and T.-I. Ho, *Org. Lett.*, **5**, 1841 (2003).
6. B. Liang, M. Dai, J. Chen and Z. Yang, *J. Org. Chem.*, **70**, 391 (2005).
7. Y. Liang, Y. Xie and J. Li, *J. Org. Chem.*, **71**, 379 (2006).
8. A. Bernthsen, *Ber. Deut. Chem. Ges.*, **20**, 942 (1887).
9. H. Brockmann, The Chemistry of Natural Product, Butterworths Publishers and Co. Ltd, London p. 405 (1961).
10. T. Shimamoto, A. Tomoda, R. Ishida and K. Ohyashiki, *Am. Assoc. Cancer Res.*, **7**, 704 (2001).
11. M.R. Lewis, P.P. Goland and H.A. Sloviter, *Cancer Res.*, **9**, 736 (1949).
12. J.K. Horton, K.N. Thimmaiah, F.C. Harwood, J.F. Kuttesch and P.J. Houghton, *Mol. Pharmocol. Abstr.*, **44**, 552 (1993).
13. B. Boothroyd and E.R. Clark, *J. Chem. Soc.*, 1499 (1953).
14. T.U.S. Chu Daniel, *Chem. Abstr.*, **104**, 109663k (1986).
15. T. Yuasa, *Ann. Res. Inst. Tuberc.*, **11**, 265 (1953).
16. W.P. Rogers, J. Cymerman-Craig and G.P. Warwick, *Br. J. Pharmacol.*, **10**, 340 (1955).
17. S. Gao, T. Takano, H.J. Sadak, C. Noda, N. Hori-Tamura, A. Tomoda and H. Yamamura, *Br. J. Pharmacol*., **137**, 749 (2002).
18. A. Ribbentrop and N. Schauwann, *Arch. Int. Pharmacodyn. Ther.*, **149**, 374 (1964).
19. P.N. Craig, *Chem. Abstr*., **55**, 582 (1960); US Patent 2,947,747 (1961).
20. H.B. Rickert and W.M. Geiger, US Patent 2,945, 856 (1960).
21. C.M. Murphy, H. Ravner and N.L. Smith, *Ind. Eng. Chem.*, **42**, 2479 (1950).
22. W.C. Holmes and A.R. Peterson, *Stain Technol.*, **6**, 79 (1931).
23. W.C. Holmes, *Amer. Dye Stuff Rept*, **17**, 626 (1929).
24. G. Mannelli and E. Martiini, *Ann. Chim. (Rome)*, **41**, 68 (1951).
25. S. Musha and T. Kitagawa, *Chem. Abstr.*, **51**, 127737 (1957).
26. E. Ruzioka, *Chem. Commun.*, **24**, 930 (1964).
27. E. Ruzioka, *Abstract*, **51**, 1193 (1957).
28. E. Ruzioka, *Chem. Listy*, **51**, 969 (1959).
29. N. Didry, M. Pinkas, and L. Dubreil, *Ann Pharm.*, **44**, 73 (1986).
30. V.D. Kerkar, R.B. Mhaske, P.L. Kulkarni and A.R. Kulkarni, *J. Pharm. Soc.*, **49**, 184 (1987).
31. V.P. Papageorgiou, A.N. Assimopoulou, E.A. Couladouros, D. Hepworth and K.C. Nicolaou, *Angew. Chem. Int.*, **38**, 270 (1999).
32. R.F. Silver and H.L. Holmes, *J. Chem.*, **46**, 1859 (1968).
33. H. Tomozane, Y. Takeuchi, T. Choshi, S. Kishida and M. Yamato, *Chem. Pharm. Bull. (Tokyo)*, **80**, 925 (1990).
34. C.K. Ryc, J.C. Ryu, C.Y. Chung and D.H. Kim, Proceeding Internal Congress of New Drug Development of the Pharmaceutical Society in Commemoration of the Society 40th Anniversary (1990).
35. E.M. Hodnett, C. Wongwiechintana, W.J. Dunn and P. Marrs, *J. Med. Chem.*, **26**, 570 (1983).
36. T.S. Lin, L.Y. Zhu, S.P. Xu, A. Divo and A. Sartorelli, *J. Med. Chem.*, **34**, 1643 (1991).
37. C.Y. Chung, C.K. Ryu, J.C. Ryu and D.H. Kim, Proceedings of the Spring Convention of the Pharmaceutical Society, **34**, 125 (1990).
38. Y. Take, M. Sawada, H. Kunai, Y. Inouye and S. Nakamura, *J. Antibiotics*, **39**, 557 (1986).
39. T. Ngoc-Chau and N. Minh–Trile, *Sciforum Electronic Conference Series*, **13**, c012 (2009).
40. T.G. Emori and R.P. Gaynes, *Clin. Microbiol. Rev.*, **6**, 428 (1993).
41. C.O. Okafor, *Int. J. Rev. Commun. Heterocycl. Chem.*, **7**, 392 (1977).
42. C. Perez, M. Pauli and P. Bazerque, *Acta Biol. Med. Exp.*, **15**, 113 (1990).
43. O.O. Ojo, A.O. Ajayi and I.I. Anibijuwon, *J. Zhejiang Univ. Sci.*, **8**, 189 (2007).