Research Article

Synthesis and Antimicrobial Activity of Novel α- Aminophosphonates Bearing Pyrazoloquinoxaline Moiety

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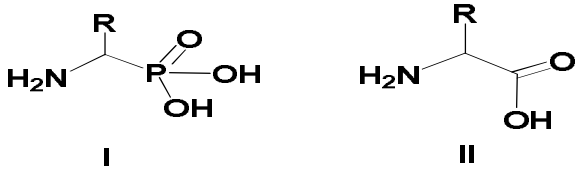
ABSTRACT

Syntheses of novel N-protected α-aminophosphonates **6** were achieved with high yields through lithium per chlorate catalyzed one-pot three component reaction process. It involves the reaction of aryl substituted Quinoxalinealdehydes, benzyl carbamate, aniline, p-methoxy aniline, p-chloro aniline, p-methyl aniline, propyl amine, pentyl amine, p-amino benzoic acid, 1,4-phenylenediamine, amino uracil, N-glycosyl amine and triphenylphosphite using lithium perchlorate as Lewis acid catalyst in dry dichloromethane at room temperature. A mechanism for this condensation reaction is proposed. Cleavage of the N-phenyloxycarbonyl group under acid hydrolysis afforded the free α-aminophosphonates 8 in quantitative yields. The structures of all new compounds were established by IR, ^{1}HNMR, ^{13}CNMR and mass spectral data. All the synthesized compounds were screened for in vitro antibacterial activity and most of them showed potency against both gram positive and gram negative bacteria.

Keywords: Pyrazoloquinoxaline, amines, triphenylphosphite, Lewis Acid, α-aminophosphonates, Antimicrobial Activity.

INTRODUCTION

Organophosphorus compounds have found a wide range of applications in the areas of industrial, agricultural, and medicinal chemistry owing to their biological and physical properties as well as their utility as synthetic intermediates[1]. α-Functionalized phosphonic acids are valuable intermediates for the preparation of medicinal compounds and synthetic intermediates[2–4]. Among α-functional phosphonic acids, α-aminophosphonic acids are an important class of compounds that exhibit a variety of interesting and useful properties. α-Aminophosphonic acids **I**, as structural mimics of α-amino acids **II (Fig.1)**, exhibit a broad spectrum of biological activities[5-12].



I II

Figure 1

These compounds have already been found to act as antibacterial agents, neuroactive compounds, anticancer drugs, and pesticides, with some of them already commercialized[13-18]. In this context, the therapeutic potential for modified α-aminophosphonates with improved pharmacokinetic properties, potency or spectrum, and lower side effects, prompted us to start a synthetic program to explore new Quinoxaline α - aminophosphonate conjugates. We focused on Quinoxaline and its derivatives because it is an important class of compounds and attracted widespread attention due to their pharmacological properties, being reported to have a large spectrum of biological effects, especially analgesic, antibacterial, antifungal, anticancer and anti-inflammatory properties. In this paper we would like to present the synthesis of novel Quinoxaline modified α-aminophosphonates conjugates and biological evaluate as antibacterial activity.

MATERIALS AND METHODS

All ^{1}HNMR and ^{13}CNMR experiments (solvent DMSO) were carried out with a 300 MHz at the University of Ulm, Germany, Okayama university, Japan. Chemical shifts are reported in part per million (ppm) relative to the respective solvent or tetramethylsilane (TMS). The mass spectroscopy experiments and IR spectroscopy were performed at Cairo University, Egypt. Melting points were recorded on Stuart scientific melting point apparatus and are uncorrected. The microanalysis was performed in department of Bacteriology, Mycology and Immunology Faculty of Veterinary Medicine University of Sadat City, Menoufia, Egypt. All reactions were followed by thin layer chromatography (TLC) on kiesel gel F254 precoated plates (Merck). Starting materials, MeOH, DMF, acetonitrile, CH_2Cl_2, hexane and diethyl ether were either commercially available as reported in literature.

Synthesis of (1S, 2S)-1-(1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) propane-1, 2, 3-triol: (.i)

A solution of L-glucose (1.8 gm., 0.01 mol.) in water (800 ml.) was heated with O-phenylenediamine (1.08 gm., 0.01 mol.), phenyl hydrazine hydrochloride (7.2 gm., 0.01 mol.), glacial acetic acid (11 ml.) and 0.2 gm. Sodium acetate. The reaction mixture was treated as previously mentioned method; Recrystallization from ethanol gave

yellow needles and Show the following data; MP. =235 °C Yield= 80%, Infra-red spectra of compound **(.i)** show: 3461.6, 2932.2, 1598.7, 1500.35 and 757.88 cm^{-1}, ^{1}H NMR (DMSO-400 MHz): δ ppm = 3.43-3.54(m, 2H , 3-H), 4.34-4.45 (m, 1H , 2-H), 4.64 (t, 1H , J= 5.49 Hz, OH), 4.88 (d.1H ,J= 5.56 Hz, OH),5.34 (t.1H ,J= 5.58 Hz, OH),5.56 (d.1H ,J= 6.18 Hz, 1H),7.35-8.46 (m, 9H , H aroma.), ^{13}CNMR (DMSO-d): δ ppm = 60.77-95.89 (Sugar-C), 119.52-144.90 (aroma.-C).

Synthesis of 1-phenyl-1H-pyrazolo [3, 4-b] quinoxaline-3-carbaldehyde: (.ii)

A solution of periodic acid (2.28 gm. 1mol.) in water (100 ml.) was added drop wise during 3 h. at room temperature to a stirred suspension of **(.i)** (0.336 gm. 0.001 mol.) in water (50 ml.).

The mixture is then stirred for 20 h. the yellow precipitate was filtered. Off and successively washed with water and 50 % propanol, dried, recrystallization from 80% propanol gave yellow crystals and Show the following data Mp. 150 °C; Yield: (85%), Infra-red spectra of compound **(.ii)** show: 1701.27, 1598.7, 1500.13, and 761.35 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ = 7.26– 8.49 (m, 9H, H aroma), 10.49 (s, 1H, formyl 1-H), ^{13}CNMR (DMSO-d): δ ppm = 119.52-144.90 (aroma.-C), 185.19 (1 CHO).

The mass spectra show the molecular ion peak at m/e = 274 $[M]^+$ (84.47 % is base peak), The ion peak at m/e = 245 (- CHO) $[M]^+$.

Reaction of 1-phenyl-1H-pyrazolo [3, 4-b] quinoxaline-3-carbaldehyde with Amines and Triphenylphosphite; General Procedure:

1-phenyl-1H-pyrazolo [3, 4-b] quinoxaline-3-carbaldehyde (1mlmol.), amines (1mlmol.) and triphenylphosphite (1mlmol.) Were dissolved in (5 ml) of dry dichloromethane. The Lewis acid* (10 mol. %) is added in one portion.

The mixture was stirred at room temperature, until TLC analysis showed the complete consumption of 1-phenyl-1H-pyrazolo $^{3,4-b}$ quinoxaline-3-carbaldehyde after 72 h.

The dichloromethane is then evaporated and the residue dissolved in diethyl ether (10 ml) the product was precipitated from this solution by storing at – 20 °C for 3 – 6 h. followed by the collection of the precipitate by filtration afford the protected aminophosphonates **5** in good to excellent yields.

* $LiClO_4$ was added as 1 mol. Solution in dry dichloromethane.

Di phenyl ((1-phenyl -1H- pyrazolo [3, 4-b] qunaxolin-3-yl) (p-tolylamino) methyl) phosphonate: (.iii)

Show the following data MP. = 98-102 °C, Yield = 85 %, Infra-red spectra of compound **(.iii)** show: 3439.42, 2863.52, 1643.87, 1511.07, 1151.16, 1118.46 and 761.35 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ =5.48-5.62 (s, 1H, NH), 3.1 (s, 3H, CH_3), 4.5-4.7 (m, 1H, CHP), 7.36-7.65(m, 10H, H aroma.), 7.88-8.41(m, 5H, H aroma.), The mass spectra show the molecular ion peak at m/e = (597$[M]^+$, 5.54%), the base ion peak at m/e = 215$[M]^+$ - ($C_{23}H_{18}N_5$3.91%).

Di phenyl ((((4-methoxy phenyl) amino (1-phnyl – 1H-pyrazolo [3, 4, b] quinaxolin-3-yl) methyl) phosphonate: (.iv)

Show the following data MP. = 100 °C, Yield = 87%, Infra-red spectra of compound **(.iv)** show: 3442.89, 2851.26, 1643.57, 1506.71, 1142.99, 1116.52 and 758.83 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ =2.5-2.8 (s,3H,CH_3O),5.12-5.15 (m, 1H,CHP), 5.7-6.0 (s,1H,NH) ,7.36-7.65(m, 10H,H aroma.),7.88-8.44(m, 5H,H aroma.), The mass spectra show the molecular ion peak at m/e = (614$[M]^+$, 77.59%), the base ion peak at m/e = 370$[M]^+$-($C_{15}H_9N_4$65.52%).

Diphenyl (((4-chlorophenyl) amino) (1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) methyl) phosphonate: (.v)

Show the following data MP. = 98-100 °C, Yield = 80 %, Infra-red spectra of compound **(.v)** show: 3438.82, 1642.41, 1503.9, 1147.42, 1114.31 and 758.46 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ =5.07-5.14 (m, 1H, CHP), 6.25-6.32 (s, 1H, NH), 7.37-7.64(m, 10H, H aroma.), 7.89-8.42(m, 5H, H aroma.), ^{13}CNMR (DMSO-d): δ ppm = 119.62-148.68 (C- aroma.), 153.26-159 (1 NH), The mass spectra show the molecular ion peak at m/e = 618$[M]^+$, 84.07%), the base ion peak at m/e = 373$[M]^+$ - ($C_{15}H_9N_4$69.03%).

Diphenyl ((1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) (phenylamino) methyl) phosphonate: (.vi)

Show the following data MP. = 95-98 Yield = 80%, Infra-red spectra of compound **(.vi)** show: 3442.89, 1643.57, 1510.79, 1151.16, 1090.46 and 767 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ =5.06-5.92 (m, 1H, CHP), 6.69-6.71 (s, 1H, NH), 7.38-7.65(m, 10H, H aroma.), 7.90-8.43(m, 5H, H aroma.), The mass spectra show the molecular ion peak at m/e = 583.58$[M]^+$, 45.08%), the base ion peak at m/e = 338$[M]^+$ - ($C_{15}H_8N_4$73.77%).

Diphenyl ((1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) (propylamino) methyl) phosphonate: (.vii)

Show the following data MP. = 198 – 201 °C, Yield = 75%, Infra-red spectra of compound **(.vii)** show: 3438.21, 2984.51, 1638.28, 1503.45, 1152.52, 1006.55 and 763 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ =1.98-2.0 (m,4H,CH_2), 3.70-3.80 (m,3H,CH_3), 5.40-5.50 (m, 1H,CHP), 5.80-6.0 (s,1H,NH) ,7.13-7.66(m, 10H,H aroma.),7.66-8.44(m, 5H,H aroma.), The mass spectra show the molecular ion peak at m/e = 549.56$[M]^+$, 15.61%), the base ion peak at m/e = 215$[M]^+$–($C_{19}H_{18}N_5$10.87 %).

Diphenyl ((pentylamino) (1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) methyl) phosphonate: (.viii)

Show the following data MP. = 230-233°C, Yield = 75 %, Infra-red spectra of compound **(.viii)** show: 3437, 1636.93, 1502.62, 1147.07, 1009.28 and 762.92 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ =1.98-2.0 (m,8H,CH_2), 3.50-

3.60 (m,3H,CH_3), 5.06-5.08 (m, 1H,CHP), 5.80-6.0 (s,1H,NH) ,7.23-7.91(m, 10H,H aroma.),7.91-8.44(m, 5H,H aroma.), The mass spectra show the molecular ion peak at m/e = 577.61[M]$^+$, 71.82%), the base ion peak at m/e = 332[M]$^+$–($C_{15}H_8N_4$51.82 %).

Diphenyl((1-phenyl-1H-pyrazolo[3,4-b]quinoxalin-3-yl)(((2R,3R,4R,5R,6R)-2,4,5-trihy droxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)amino) methyl) phosphonate: (.ix)

Show the following data MP = 155 °C, Yield = 65 %, Infra-red Spectra of compound **(.ix)** show: 3422, 1627.63, 1498.42, 1145.51, 1110.8 and 756.923 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ =4.47 (d,2H,4-OH), 5.07-5.09 (m, 1H,CHP), 6.90-7.0 (s,1H,NH) ,7.37-7.64(m, 10H,H aroma.),7.99-8.42(m, 5H,H aroma.).

The mass spectra show the molecular ion peak at m/e = 669.62[M] $^+$, 25.49%), the base ion peak at m/e = 178[M] $^+$–($C_{28}H_{19}N_4O_3P$ 23.20 %).

4-(((diphenoxyphosphoryl) (1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) methyl) amino) benzoic acid: (.x)

Show the following data MP. = 130°C, Yield = 85 %, Infra-red spectra of compound **(.x)** show: 3427, 1602.56, 1498.42, 1144.55, 1089.58 and 756.923 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ = 5.06-5.08 (m, 1H,CHP), 5.84 (s, 1H,H-acid),6.52-6.55 (s,1H,NH) ,7.38-7.66(m, 10H,H aroma.),7.67-8.44(m, 5H,H aroma.), ^{13}CNMR (DMSO-d): δ ppm = 112.61-146.31 (C- aroma.), 167.73-178.40 (1-COO).

The mass spectra show the molecular ion peak at m/e = 627.58[M] $^+$, 17.60%), the base ion peak at m/e = 385[M+2] $^+$–($C_{15}H_8N_4$23.46 %).

Diphenyl(((2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)amino)(1-phenyl-1H-pyrazolo[3,4-b]quinoxalin-3-yl)methyl)phosphonate: (.xi)

Show the following data MP. = 167-170°C Yield = 60%, Infra-red spectra of compound **(.xi)** show: 3415, 1710.55, 1627.63, 1501.31, 1238.08, 896.74 and 759.82 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ = 5.08-5.10 (m, 1H,CHP), 6.27 (s,1H,NH) ,7.37-7.66(m, 10H,H aroma.),7.66-8.43(m, 5H,H aroma.),10.21(s, 1H,OH),11.12(s, 1H,NH), ^{13}CNMR (DMSO-d): δ ppm = 119.52-148.85 (aroma. - C), 155.43-167.73 (2 C=O).

Tetraphenyl((1,4-phenylenebis(azanediyl))bis((1-phenyl-1H-(pyrazolo[3,4-b]quinoxalin-3-yl) methylene))bis(phosphonate): (.xii)

Show the following data MP. = 145-148°C. Yield = 80 %, Infra-red spectra of compound **(.xii)** show: 3428.81, 1630.52, 1499.38, 1230.36, 902.523 cm^{-1} and 757.888 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ = (m, 1H,CHP) 4.47-4.49,5.07-5.10(m, 1H,CHP), 5.87(s,1H,NH) ,7.37-7.66(m, 20H,H aroma.),7.88-8.44(m, 8H,H aroma.).

The mass spectra show the molecular ion peak at m/e = 1089.04[M] $^+$, 66.23%), the base ion peak at m/e = 843[M+2] $^+$–($C_{15}H_8N_4$29.80%).

Diphenyl (((4-aminophenyl) amino) (1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) methyl) phosphonate: (.xiii)

Show the following data MP. = 200-203°C. Yield = 90%, Infra-red spectra of compound **(.xiii)** show: 3457, 1624.93, 1501.31, 1235.18, 900.594 and 756.923 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ = 5.07-5.09 (s, 2H, NH_2), 5.87 (m, 1H, CHP), 6.70 (s, 1H, NH), 7.38-7.92(m, 10H, H aroma.), 8.00-8.44(m, 5H, H aroma.), ^{13}CNMR (DMSO-d): δ ppm = 119.40-148.82 (C- aroma.), The mass spectra show the molecular ion peak at m/e = 577.61[M] $^+$, 71.82%), the base ion peak at **m/e** = 354[M] +–(C15H8N4 41.95 %).

Benzyl((diphenoxyphosphoryl)(1-phenyl-1H-pyrazolo[3,4-b]quinoxalin-3-yl)methyl) carbamate: (.xiv)

Show the following data MP. = 125°C Yield = 65%, Infra-red spectra of compound **(.xiv)** show: 3074.44, 1702.66, 1644.06 , 1500.74, 1194.86, 1037.27 and 760.89 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ = 4.98 (m, 1H, CHP), 5.14-5.22 (m, 2H, phCH_2O), 6.12 (s, 1H, NH), 7.17-7.65(m, 10H, H aroma.), ^{13}CNMR (DMSO-d): δ ppm = 119.47-149.73 (aroma. - C), 155.98-180.00 (1 C=O).

Diphenyl (amino(1-phenyl-1H-pyrazolo[3,4-b]quinoxalin-3-yl)methyl)phosphonate: (.xv)

A solution of ((diphenoxyphosphoryl)(1-phenyl-1H-pyrazolo[3,4-b]quinoxalin-3-yl)methyl) carbamate (0.128 g – 0.199mlmol.), 3-4 drop of tri ethyl amine and 2-4 drop of (HBr/Acetic) in (5 ml) of dry dichloromethane .The mixture was stirred at room temperature, until TLC analysis showed the complete consumption after 1-2 hr. the dichloromethane is then evaporated. Show the following data MP. = 145°C yield 65%, Infra-red spectra of compound **(.xv)** show: 3413, 1594.84, 1495.53, 1122.37, 1031.73 and 757.888 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ = 5.01-5.14 (m, 1H, CHP), 5.17-5.21 (m, 2H, NH_2), 7.33-7.66 (m, 10H, H aroma.), 7.95-8.43(m, 5H, H aroma.).

Synthesis of diphenyl ((1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) ((4-(3-phenylthioureido) phenyl) amino) methyl) phosphonate: (.xvi)

A solution of Diphenyl (((4-aminophenyl) amino) (1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) methyl) phosphonate (0.334mlmol) and isothiocyanatobenzene (0.334mlmol) were dissolved in (5 ml) of dry dichloromethane. The mixture was stirred at room temperature, until TLC analysis showed the complete consumption after 2-4 hr. the dichloromethane is then evaporated and the residue dissolved in di ethyl ether (10 ml) the product was precipitated from this solution by storing at – 20 °C for 3 – 6hr. Show the following data MP. = 187 – 190 °C Yield = 80 %, Infra-red spectra of compound **(.xvi)** show: 3460, 3228.25, 1709.59, 1597.73, 1502.28, 1237.11, 906.379 and 756.923 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ = 3.68-3.88 (d,2H,NH) ,4.47-4.64 (m, 1H,CHP), 5.07-5.08 (s,1H,NH) ,5.85 (s,1H,SH) ,7.33-7.68(m, 10H,H aroma.),7.89-8.45(m, 8H,H aroma.), The

mass spectra show the molecular ion peak at m/e = 733.78[M] $^{+}$, 20.44%), the base ion peak at m/e = 489[M+1] $^{+}$–($C_{15}H_8N_4$18.87 %).

2-(1, 3-dioxoisoindolin-2-yl) propanoic acid: (.xvii)

A mixture of phthalic anhydride (10mlmol.) and L-alanine (10mlmol.) mixed to completely fusion in sand passes. Then leave to cold and boiling the product with ethanol. Filtered as a hot, take the filtrate and cold it Show the following data MP. =153 °C. Yield = 90 %, Infra-red spectra of compound **(.xvii)** show: 3208 cm^{-1}, 1699.94, 1531.2 cm^{-1} and 726.07 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ = 1.03-1.06 (m, 2H, CH_2), 3.41-3.46 (m, 3H, CH_3), 7.79-7.88(m, 5H, H aroma.), 12.38(s, 1H, H acid), ^{13}CNMR (DMSO-d): δ ppm = 123.06-167.66 (aroma. - C), 172.19 (1 C=O).

Diphenyl((2-(1,3-dioxoisoindolin-2-yl) propanamido)(1-phenyl-1H-pyrazolo[3,4-b] quinoxalin-3-yl) methyl)phosphonate: (.xviii)

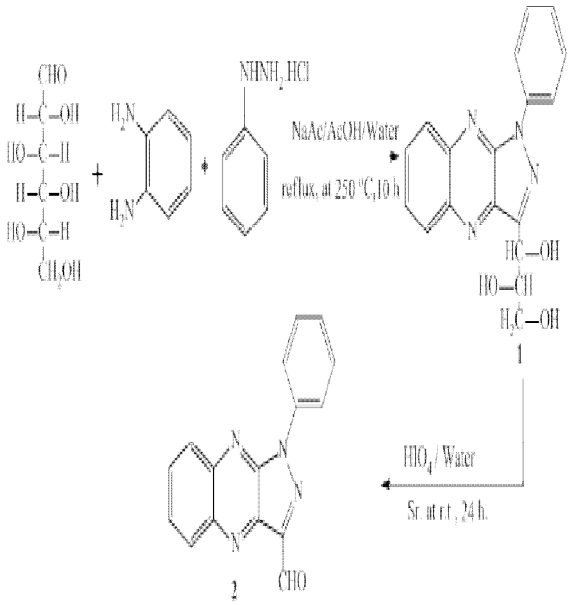
A solution of diphenyl (amino(1-phenyl-1H-benzo[g]pyrazolo[3,4-b]quinoxalin-3-yl)methyl) phosphonate (0.179 mlmol.), 2-(1,3-dioxoisoindolin-2-yl) propanoic acid (0.456mlmol.) and 2 drop of triethylamine in (5 ml) of dry dichloromethanein presence of little amount of TBTU as a catalyst . The mixture was stirred at room temperature, until TLC analysis showed the complete consumption. The dry dichloromethane is then evaporated and the residue dissolved in (10 ml) of di ethyl ether the product was precipitated from this solution by storing at – 20 °C for 3 – 6h. Show the following data MP. = 140 °C Yield = 65 %, Infra-red spectra of compound **(.xviii)** show: 2955.3, 1733.29, 1599.02, 1502.62, 1186.76, 1003.02 cm^{-1} and 760.38 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ = 2.49-2.60(q, 1H,CH),3.09-3.10(d, 3H,CH_3), 5.10 (m, 1H,CHP), 6.60 (s,1H,NH) ,7.10-7.20(m, 10H,H aroma.),7.25-7.84(m, 8H,H aroma.).

Diphenyl ((2-aminopropanamido) (1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) methyl) phosphonate: (.xix)

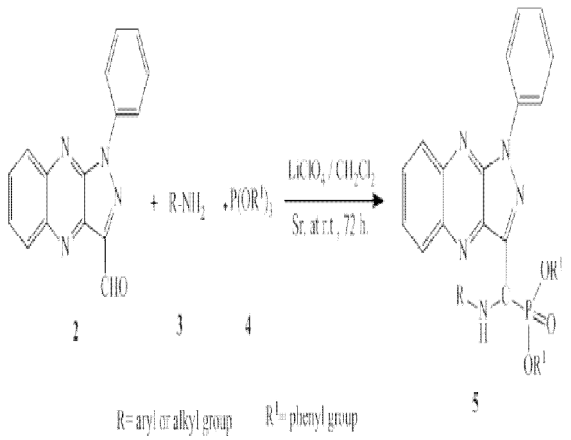
To a solution of biphenyl ((2-(1, 3-dioxoisoindolin-2-yl) propanamido) (1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) methyl) phosphonate (0.299mlmol.) and hydrazine hydrochloride (0.8mlmol.) add mixture of tri ethyl amine (0.7 ml) and dry methanol (5ml) drop by drop to the previous solution. At reflux in water bass at 40 °C with stirring at 4h. Leave it to cold. Add (30ml) of water and (30ml) of ethyl acetate. The lower layer formed with (water, tri ethyl amine and methanol). The upper layer formed with (ethyl acetate and the product). Using separating funnel to separation of the tow layer. By using rotary vapor to the upper layer to the dispersion of ethyl acetate, and by using di ethyl ether formed the product. Show the following data MP. = 135 °C Yield = 65 %, Infra-red spectra of compound **(.xix)** show: 3437, 1636.93, 1502.62, 1147.07, 1114.38 and 762.92 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ =1.28(d, 3H,CH_3),3.74(q, 1H,CH), 4.90 (m, 1H,CHP), 5.11 (d,2H,NH_2) ,7.21-8.24(m, 19H,H aroma.),8.03(d, 1H,NH).

Diphenyl ((2-benzamidopropanamido) (1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) methyl) phosphonate: (.xx)

To a solution of diphenyl ((2-aminopropanamido) (1-phenyl-1H-pyrazolo [3, 4-b] quinoxalin-3-yl) methyl) phosphonate (0.099-mmol), benzoyl chloride (0.099mlmol.) And 2 drop of tri ethyl amine in (5 ml) of dry dichloromethane the mixture was stirred at room temperature, until TLC analysis showed the complete consumption after 3-4 hr. Then CH_2Cl_2 was evaporated and the residue dissolved in di ethyl ether (10 ml) the product was precipitated from this solution by storing at – 20 °C for 3 – 6hr. Show the following data MP. = 186-170 °C Yield = 65 %, Infra-red spectra of compound **(.xx)** show: 3437, 1636.93, 1502.62, 1147.07, 1114.38 and 762.92 cm^{-1}, ^{1}HNMR (DMSO, 400 MHz) δ = 1.48(d, 3H,CH_3),4.71 (q, 1H,CH), 4.90 (m, 1H,CHP), 5.11 (d,2H,NH_2) ,7.21-8.03(m, 24H,H aroma),8.03,8.45(d, 2H,NH), The mass spectra show the molecular ion peak at m/e = 682.66[M]$^{+}$, 34.84%), the base ion peak at m/e = 437[M]$^{+}$–($C_{15}H_8N_4$ 17.74 %).



Scheme 1



Scheme 2

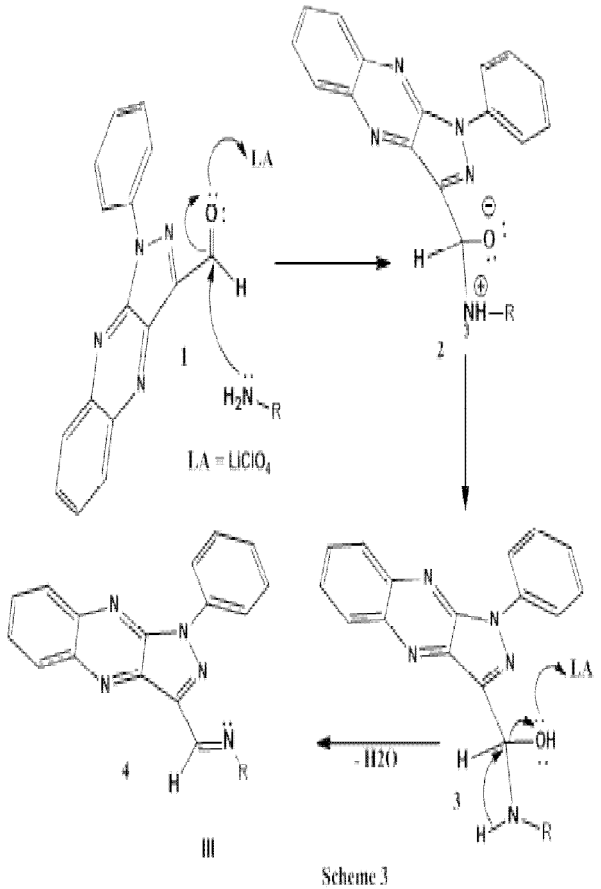
RESULTS AND DISCUSSION

The synthesis of mono- and disubstituted diphenyl α-aminophosphonates **5** were accomplished in good yield using 1-phenyl-1H-pyrazolo[3,4-b] quinoxaline-3-carbaldehyde, a different and triphenylphosphite in the presence of a Lewis acid such as lithium per chlorate according to **scheme 2**. The required aldehydes needed for this study were synthesized according to published method[19] using Vilsmeier reagent as shown in **scheme 1**.

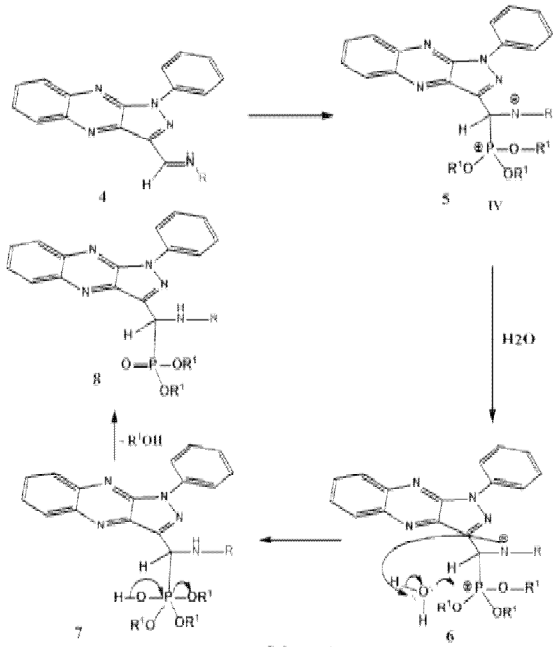
Having a diverse series of quinoxalinealdehyde derivatives affording the opportunity to obtain a various structures diversity of α-amino- phosphonates **5** by a fast and convenient one-pot three component reaction route according to **scheme 2**.

Optimal conditions for the Lewis acid were found to be 10 mol% in dichloromethane. At 5 mol%, the reaction afforded the same yield but required longer reaction times. The reactions are clean and complete within hours. The reaction conditions are very mild and α-aminophosphonates are exclusively formed without the formation of any undesired side products another important feature of this reaction is the survival of a variety of functional groups such as ester under the reaction conditions.

Moreover, the mechanism of this reaction has not been investigated in detail. We suppose that after reaction of the carbonyl compound with the amines in presence of Lewis acid catalyst,

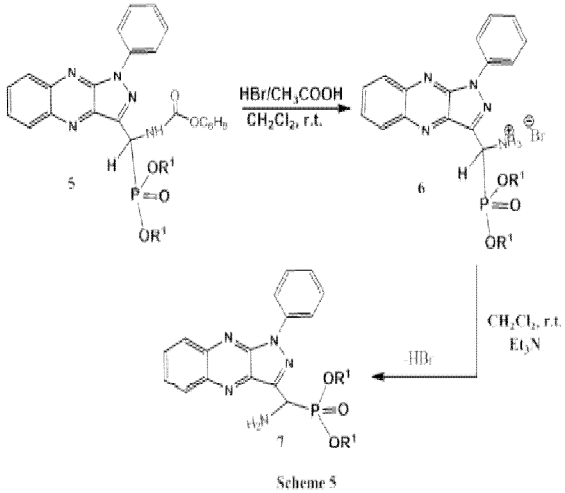


Scheme 3



Scheme 4

The acylimine intermediate **III** is attacked by nucleophilicphosphite with the formation of a phosphonium intermediate **IV** and that both reactions are catalyzed by the Lewis acid. Reaction of phosphonium intermediate **IV** with water affords the target compound **5** after elimination of phenol as shown in **scheme 3,4**. Finally, in especially reaction with benzyl carbamated protection or cleavage of the phenyloxycarbonly group by acidic hydrolysis using HBr/acetic cleanly affords the free α-aminophosphonates **7** in high yields as shown in **scheme 5**. In all cases, the reaction proceeded smoothly at ambient temperatures with high selectivity.



Scheme 5

In summary, we found that a Lewis acid such as $LiClO_4$ effectively promoted the condensation of heterocyclic aldehydes bearing quinoxaline moiety with benzyl carbamate and triphenylphosphite or at room temperature. In addition to we have demonstrated a novel and efficient protocol for the synthesis of α-aminophosphonates which can serve as peptide mimetic. The method is effective for heterocyclic aldehydes such as quinoxalinealdehyde and provides excellent yields of

the products, which makes it useful and attractive process for the synthesis of α-aminophosphonates. It is believed that this method presents a better and more practical alternative to the existing methodologies[20] for the synthesis of α-aminophosphonates.

Antimicrobial Screening

Antibacterial agents are crucial in reducing the widely spreaded global burden of infectious bacterial diseases. As consequence to development of resistant pathogens and spread, the efficacy of many antibiotics is diminished. This type of bacterial resistance to the antimicrobial agents possesses critical threat to public health, and for all kinds of antibiotics, including the major last-resort drugs, the frequencies of resistance are increasing worldwide (Mandal and Mandal, 2011) and Mandal (2009). Pseudomonas aeruginosa is widely spreaded an opportunistic Gram negative pathogen Implicated in multiple infections as respiratory, urinary, gastrointestinal infections, keratitis, otitis media, and bacteremia in patients with compromised host defenses [e.g., burn, cancer, cystic fibrosis (CF) and HIV]. Significant morbidity and mortality are the main coincidence of such notorious infectious agent Morita (2014).

Staphylococcus aureus affects humans is a major cause of morbidity, and economic loss in production in companion and food animals causing community acquired and nosocomial infections Rubin (2011).

Wide spectrum of diseases in man and animals are caused by **Aeromonas** species Ghenghesh. Recently, some motile Aeromonas species are becoming food and waterborne pathogens of great importance Ansari et al., (2011). They have been associated with several food-borne outbreaks and are progressively being isolated from patients with traveler's diarrhea Von Graevenitz et al., (2007).

Pasteurella multocida It is azoonotic Gram negative bacterium responsible for arrange of infections in domestic animals, fowl cholera in domestic and wild birds, bronchopneumonia and hemorrhagic septicemia in bovids, atrophic rhinitis in porcines and snuffles in rabbits causing substantial economic losses (Steen) and (Hunt).

Vibrio cholerae O1 and enterotoxigenic **Escherichia coli** (ETEC) considered two major bacterial pathogens responsible for a high proportion of diarrhoeal disease and death in adults and children in many countries in Africa and Asia (Svennerholm, 2011) also, Shiga toxin–producing Escherichia coli (STEC) are a leading cause of bacterial enteric infections Brooks.

Salmonella enterica species are widely dispersed in nature and are common inhabitants and highly adapted to the intestinal tract of domesticated and wild mammals, reptiles, birds, and even insects. S. enterica Typhi causes typhoid fever only in humans, whereas other serotypes, namely nontyphoid Salmonella serotypes, can cause a wide spectrum of diseases in humans and animals, such as acute gastroenteritis, bacteremia, and extraintestinally localized infections involving many organs Su et al., (2004).

Antimicrobial resistance has emerged in the past few years as a major problem and many programs have been set up for its surveillance in human and veterinary medicine. These programs are aimed mainly at human pathogens, agents of zoonoses, and indicator bacteria of the normal intestinal flora from animals (LANZ). The potential transfer of antibiotic resistance from animals to humans through the use of antibiotics in animal production has been implicated as a cause of treatment failure, prolongation of illness and death, and increased costs of treatment (Kelly) and (Kolar).

Hence, there is an urgent need for alternative antibacterial strategies, and thus this condition has led to a re-evaluation of the therapeutic use of new chemical modifications and derivatives against most notorious bacterial agents those responsible for serious drastic infections.

Materials used

1- Tryptic Soy Broth for culturing and refreshment of identified bacterial isolates in order to detect the effect of modified antibacterial agents upon.

2-Muller Hinton agar that's used to test the effect of modified antibacterial agents.

3- Identified bacterial isolates (**Pseudomonasaeruginosa, Staphylococcus aurous, Aeromonashydrophila, Pasteurellamultocida, Ornithobacteriumrhinotracheale, Escherichia coli, Salomnellatyphimurium and Salmonella enterritidis**).

4- Antibiotic reference like **neomycin, doxycycline, chloramphenicol, Cefixitin, streptomycin, Ampicillin, penicillin, ofloxacin, erythromycin, amoxicillin and tetracycline**.

Methods:

1-detection of the bacterial count after 24hrs growth according to Wiegand the isolated and identified bacterial isolates under test was cultured in Tryptic Soy Broth (TSB) for 24 hrs., then the concentration of bacterial cells in 1ml medium was measured using spectrophotometer at 660 nm so as to adjust the concentration to $1x10^8$colony forming unit (CFU) per 1ml. So as to reach this bacterial concentration sterile TSB used for dilution of the concentrated bacterial isolates. from the adjusted 1 10^8CFU $/l^{-1}$1ml was taken and separated on the surface of Muller Hinton agar plates and the excess decanted away then the plates are left to dry at 40 ^{0}C in the incubator for 20 minutes. Wells made in agar plates by using the wide end of a blunted sterile Pasteur pipette, inserting it and twisting it slightly to remove the plug of agar. Alternatively, cork borers sterilized with alcohol may be used. A mounted needle or a pair of forceps, sterilized by flaming in alcohol, may be required to remove the agar

then, these wells were numbered with relevant numbers of the utilized chemical substances furthermore, these wells were filled with 120µl of the utilized modified chemicals originated from antibiotics and then incubated at 37^0C for 24-48hrs

The zone of inhibition traced on to an acetate grid with felt pen and measured against squared or graph paper or measured ruler to determine its size BSAC (2003). Wiegand, I.; Hilpert, K.and Hancock, R. E. (2008): Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances.

Journal of Nature Protocols| 3(2):163-175. BSAC (2003) Disc Diffusion Method for Antimicrobial Susceptibility Testing Version 2.1.5 pages 1-41.

The results *In Vitro* Screening of samples for antimicrobial activity after solubility in DMSO solvent on cold show:

The efficacy of the novel derivatives of quinoxaline ring from (**.i**) to (**.xx**) with concentration of 100% against **Pseudomonas aeruginosa as gram negative bacteria** was eminent as follows: No. (**.i**) (15mm), No. (**.ii**) (15mm), No. (**.iv**) (13mm), No. (**.v**) (11mm), No. (**.vi**) (13mm), No. (**.vii**) (10mm), No. (**.viii**) (12mm), No. (**.ix**) (12mm), No. (**.x**) (15mm), No. (**.xi**) (15mm), No. (**.xii**) (18mm), No. (**.xiii**) (8mm), No. (**.xiv**) (13mm), No. (**.xv**) (10mm), No. (**.xvi**) (10mm), No. (**.xviii**) (12mm) and No. (**.xx**) (13mm) .As it is shown in (**figure 2**).

Was noticeable when being compared with the diminished efficacy of the original **sulfaquinoxaline which contains quinoxaline ring** as well as resistance to most antibiotics as **Amoxicillin, Ampicillin, Penicillin, Tetracycline, Neomycin, Chloramphenicol, Ofloxacin, Doxycycline, and Erythromycin**. As it is shown in (**figure 3**).

Furthermore, there located illustrious efficacy against **staphylococcus aureus as gram positive bacteria** was eminent as follows with derivatives No. (**.i**) (11mm), No. (**.ii**) (17mm), No. (**.iii**) (10 mm), No. (**.iv**) (12mm), No. (**.vi**) (10mm), No. (**.vii**) (15mm), No. (**.viii**) (10mm), No. (**.ix**) (10mm), No. (**.x**) (18mm), No. (**.xi**) (7mm), No. (**.xiii**) (8mm), No. (**.xv**) (7mm), No. (**.xvi**) (11mm), No. (**.xviii**) (8mm) and No. (**.xx**) (8mm) .As it is shown in (**figure 2**). This compared with the absence of efficacy of the original **sulfaquinoxaline which contains quinoxaline ring** and resistance to **Amoxicillin, Tetracycline, Ofloxacin, Doxycycline and Penicillin**. As it is shown in (**figure 3**).

While, the efficacy of these derivatives was prominent against **Aeromonas hydrophila as gram negative bacteria** was eminent as follows: No. (**.i**) (13 mm), No. (**.ii**) (15mm), No. (**.iii**) (20 mm), No. (**.iv**) (22mm), No. (**.v**) (23mm), No. (**.vi**) (18mm), No. (.vii) (20mm), No. (.viii) (22mm), No. (**.ix**) (15mm), No. (**.x**) (13mm), No. (**.xi**) (14mm), No. (**.xii**) (17mm), No. (**.xiii**) (18mm), No. (**.xiv**) (12mm), No. (**.xv**) (10mm), No. (**.xvi**) (15mm) and No. (**.xiii**) (10mm) furthermore, it was apparent that derivatives (**.iii**), (**.v**), (**.vii**) and (**.viii**) expressed synergistic effect. As it is shown in (**figure 2**).

The effect of these products are promising with regard to resistance to **sulfaquinoxaline and most antibiotics as Amoxicillin, ampicillin, Tetracycline, Ofloxacin, Doxycycline, Penicillin, Chloramphenicol and Erythromycin** that have no effect against pseudomonas aeruginosa. As it is shown in (**figure 3**).

Concerning efficacy against **pasteurella multocida as gram negative bacteria** was eminent as follows on the contrary to the wider range of efficacy of most derivatives on previous bacterial species only No. (**.vi**) (7mm), No. (**.vii**) (10mm) and No. (**.viii**) (10mm).

As it is shown in (**figure 2**).gave efficacy but still conspicuous when compared with the resistance to **sulfaquinoxaline, Amoxicillin, Ampicillin, Penicillin, Tetracycline, Neomycin, Streptomycin, Ofloxacin, Doxycycline and Erythromycin**. As it is shown in (**figure 3**).

Focusing upon the efficacy upon **Ornithobacterium rhinotracheale as gram negative bacteria** was eminent as follows only derivative's. No. (**.i**) (15mm), No. (**.ii**) (18mm), No. (**.iii**) (10mm), No. (**.iv**) (12mm), No. (**.vi**) (10mm), No. (**.vii**) (10mm), No. (**.viii**) (7mm), No. (**.ix**) (10mm), No. (**.x**) (10mm), No. (**.xii**) (12mm), No. (**.xiii**) (10mm), No. (**.xv**) (10mm) and No. (**.xvi**) (7mm). As it is shown in (**figure 2**).

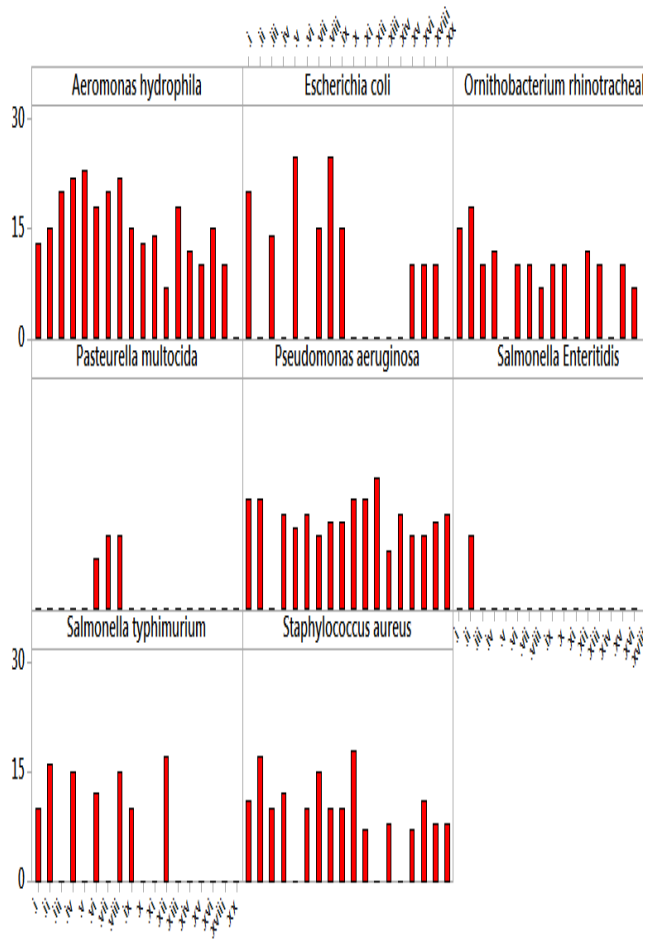
When compared with the no effect of **quinoxaline ring** and most antibiotics as **Amoxicillin, Ampicillin, Chloramphenicol, Tetracycline, Neomycin, Streptomycin, Ofloxacin, Doxycycline and Erythromycin**. As it is shown in (**figure 3**).

Interpreting the effect against **Escherichia coli as gram negative bacteria** was eminent as follows only derivative's .No. (**.i**) (20mm), No. (**.iii**) (14mm), No. (**.v**) (25mm), No. (**.vii**) (15mm), No. (**.viii**) (25mm), No. (**.ix**) (15mm), No. (**.xv**) (10mm), No. (**.xvi**) (10mm) and No. (**.xviii**) (10mm).

As it is shown in (**figure 2**). with regard to **sulfaquinoxaline** which expressed less effect against E.coli as well as **Ampicillin, Penicillin, Tetracycline, Neomycin, Streptomycin, Ofloxacin, Doxycycline, Erythromycin and Chloramphenicol**. As it is shown in (**figure 3**).

Concerning the susceptibility of **Salmonella typhimurium as gram negative bacteria** was eminent as follows to most derivatives' only products .No. (**.i**) (10mm), No. (**.ii**) (16mm), No. (**.iv**) (15mm), No. (**.vi**) (12mm), No. (**.viii**) (15mm), No. (**.ix**) (10mm) and No. (**.xii**) (17mm). As it is shown in (**figure 2**). when compared with **sulfaquinoxaline** which gave no efficacy against **Salomnella typhimurium as well as Ampicillin, Amoxicillin, Penicillin, Neomycin, quinolones as**

Ofloxacin, Doxycycline, Erythromycin and Chloramphenicol. As it is shown in (**figure 3**).



(Figure 2)

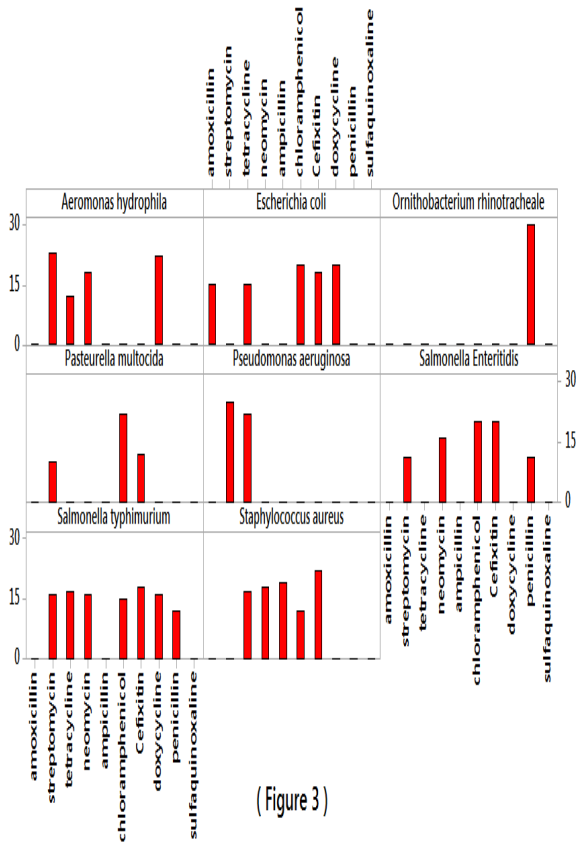
On the contrary to most tested bacterial species **Salmonella enteritidis as gram negative bacteria** was eminent as follows the lowest susceptible one only derivative. No. (**.ii**) (10 mm)

As it is shown in (**figure 2**). Gave efficacy against it but it still encouraging when compared with resistance to **sulfaquinoxaline, Ampicillin, Amoxicillin, Penicillin, Neomycin, quinolones as Ofloxacin, Doxycycline, Erythromycin, Chloramphenicol, Tetracycline and Streptomycin**. As it is shown in (**figure 3**).

CONCLUSION

The synthesized derivatives from the quinoxaline ring are promising due to the salient effect against the tested antibacterial species.

This mainly opens the door to evade the concurrent problem of bacterial resistance but further investigations are required to test and detect the pharmacokinetics (absorption, distribution, metabolism, and excretion), Toxic kinetics in animal's effective dosage, over dosage of these valuable products.



(Figure 3)

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REFERENCES

1. I. El Sayed, S. M. El Kosy, M. F. Abdel Magied, M. A. Hamed, A. Abdel A. ElGokha and M. M. Abd El-Sattar, One-pot Synthesis of Novel α-Aminophosphonate Derivatives Containing a Pyrazole Moiety ; Journal of American Science, 7, 2011, 604-608 and references cited therein.
2. I. El Sayed, S. El Kosy , M., Hawata M. A., El Gokha , A. A., Tolan A. Abd El-Sattar M. M. Journal of American Science, 7, 2011, 357.
3. Naydenova E. D., Todorov P.T., Trove K. D., Amino Acids, 38, 2010, 23. Lejczak try Department, Faculty of Science, El Menoufeia University, Shebin El Koom, Egypt.
4. B., Kafarski P., Topics in Heterocyclic Chemistry, 20, 2009, 31.
5. M. Casteleijn, M. Alahuhta, K. Groebel, I. El-Sayed, K. Augustyns, A.Lambeir, P. Neubauer, R. Wierenga; Functional Role of the Conserved Active Site Proline of TriosephosphateIsomerase, Biochemistry, 45(51), 2006, 15483–15494.
6. P. Van der Veken, I. El Sayed, J. Joossens, C. V. Stevens, K. Augustyns and A. Haemers; The Lewis acid catalyzed synthesis of N-protected diphenyl 1-aminoalkylphosphonates, Synthesis, 2005, 634-638.
7. Pudovik A. N., DokladyAkad. NaukSSSR. 83, 1952, 865; Chem.Abstr. 47, 1953, 4300.
8. (a) Kabachinik M. I., Medved T. Y, DokladyAkad. Nauk SSSR. 83, 1952, 689; Chem. Abstr. 47, 1953, 2724. (b) Kabachinik

M. I., Medved T. Y, Izv. Akad., Nauk SSSR, Ser. Chim. 1953, 1126. (c) Fields E., Journal of American Chemical Society, 74, 1952, 1528.

9. Cherkasov R. A., Galkin V. I., Russian Chemistry Review, 67, 1998, 857; Chem. Abstr. 47, 1953, 2724b.
10. (a) Galkina I., Zvereva E., Galkin V., Sobanov A., Cherkasov R., Zhur. Obshch. Khim. (Russian Journal of General Chemistry), 68, 1998, 1453. (b) Galkina I., Sobanov A., Galkin V., Cherkasov R., Zhur . Obshch. Khim., (Russian Journal of General Chemistry), 68, 1998, 1465.
11. Galkina I., Galkin V., Cherkasov R., Zhur .Obshch. Khim. (Russian Journal of General Chemistry), 68, 1998, 1469.
12. Mandal M., Mandal S. (2011): Honey: its medicinal property and antibacterial activity. Asian Pac J Trop Biomed., 1(2), 2011, 154-160.
13. Mandal S, Pal NK, Chowdhury IH, Deb Mandal M. Antibacterial activity of ciprofloxacin and trimethoprim, alone and in combination, against Vibrio cholerae O1 biotype El Tor serotype Ogawa isolates. Polish J Microbiol, 58, 2009, 57-60.
14. J. E. Rubin, K.R. Ball, M. Chirino-Trejo (2011): Antimicrobial susceptibility of Staphylococcus aureus and Staphylococcus pseudintermedius isolated from various animals. Can Vet J., 52, 2011, 153–157.
15. K. S. Ghenghesh, S. F. Ahmed, R. A. El-Khalek, A. Al-Gendy, and J. Klena, "Aeromonas infections in developing countries," Journal of Infection in Developing Countries, 2(2), 2008, 81–98.
16. M. Ansari, E. Rahimi, and M. Raissy, "Antibiotic susceptibility and resistance of Aeromonas spp. isolated from fish," African Journal of Microbiology Research, 5(31), 2011, 5772–5775.
17. A. von Graevenitz, "The role of Aeromonas in diarrhea: a review," Infection, 35(2), 2007, 59–64.
18. Harrison P., Seemann T., Wilkie I., Harper M., (2010). Fis is essential for capsule production in Pasteurella multocida and regulates expression of other important virulence factors. PLOS Pathog. 6:e1000750. doi: 10.1371/journal.ppat.1000750.
19. Hunt M.L., Adler B. and Townsend K. M. (2000). The molecular biology of Pasteurella multocida. Vet. Microbiol. 91, 2000, 73-84.
20. Svennerholm A. (2011): From cholera to enterotoxigenic Escherichia coli (ETEC) vaccine development. Indian J Med Res., 133, 2011, 188-194.

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