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Synthesis, characterization, and antimicrobial activity of some new phosphorus macrocyclic compounds containing pyrazole rings

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Abstract: A simple synthetic method is reported for the preparation of some new phosphorus macrocycles in which the pyrazole rings are appended to a phosphorus atom. The methodology is based on the cyclocondensation reaction of *bis*(4-formylpyrazolyl) phosphine oxides (**1a**, **1b**) with nitrogen nucleophiles that contain active terminal amino groups. The antimicrobial activities of the synthesized compounds were assayed.

Key words: Phosphorus macrocycles, pyrazole, cyclocondensation, nitrogen nucleophiles

1. Introduction

The discovery of a family of macrocyclic compounds (cyclophanes) was a milestone in chemistry and opened new frontiers in the synthesis of supramolecular host molecules. This group of compounds was recognized as a source of receptors for many chemical species, such as metal cations and inorganic and organic anions, as well as structurally variable organic molecules.[1] Therefore, they are widely used for construction of metal sequestering agents, selective sensors, mimetics of enzymes, and carriers for transport through membranes.[2–10] Phosphorus-containing macrocycles are interesting molecules with potential application in supramolecular and synthetic organic chemistry.[11] They were synthesized as phosphine oxides, phosphines, phosphonium salts, phosphates, phosphonates, and phosphoranes.[12] The importance of these molecules, as phosphorous analogs of crown ethers, is their potential catalytic activity and ion-carrier properties. The synthesis of host molecules that are capable of binding neutral organic molecules as guests is an area of rapidly expanding interest.[13] Tucker et al.,[14] Fages et al.,[15] Wallon et al.,[16] Seward et al.,[17] and Brelow et al.[18] have made significant advances in the field of host–guest complexation. Some of the past and present research has led to the construction of large reorganized macrocyclic cavities bearing concave functionalities.[19] They are also expected to function as good 'hosts' in the 'host–guest chemistry'. This particular property enables them to carry the drug molecule to the required site in the living system, thus suggesting a great future for them in the pharmaceutical industry. On the other hand, the introduction of a pyrazole ring into molecular structures is one of the important aims of the present work, which promises an approach leading to new biological properties.[20,21] In view of these and several other possible applications, the present work deals with the synthesis and characterization of some new phosphorus macrocycles with nitrogen, oxygen, sulfur, and phosphorus as rich electronic centers. In addition, their antimicrobial activities were also evaluated.

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2. Experimental

The melting point was determined in an open capillary tube on a digital Stuart SMP-3 apparatus. Infrared spectra were measured on a PerkinElmer 293 spectrophotometer (cm^{-1}), using KBr disks. ^{1}H NMR spectra were measured on a Gemini-200 spectrometer (200 MHz), using DMSO-d_6 as a solvent and TMS (δ) as the internal standard. ^{31}P NMR spectra were registered on a Varian Inova 202 MHz spectrometer at room temperature using DMSO-d_6 as a solvent, TMS as the internal standard, and 85% H_3PO_4 as the external reference. Mass spectra were recorded on a gas chromatographic GCMSqp 1000-EX Shimadzu instrument at 70 eV. Elemental microanalyses were performed at the microanalysis center of the National Research Center, Giza. The results of elemental microanalyses were satisfactory and did not exceed 0.4% for carbon and hydrogen and 0.42% for nitrogen. The purity of the synthesized compounds was checked by thin layer chromatography (TLC). Bis(4-formylpyrazolyl)phosphine oxides (**1a**, **1b**),[22] carbohydrazide (**2a**),[23] thiocarbohydrazide (**2b**),[23] phosphonic dihydrazide (**3a**),[24] and P-methoxyphosphonic dihydrazide (**3b**)[24] were prepared according to the reported methods in the literature.

2.1. General procedure for macrocycles 4a, 4b and 5a, 5b

A hot ethanolic solution (15 mL) of *bis*(4-formyl-3-phenyl-1*H*-pyrazol-1-yl) phosphine oxide (**1a**) (0.78 g, 2 mmol) was mixed with a hot aqueous solution of appropriate dihydrazide (2 mmol in 5 mL of water), namely carbohydrazide (**2a**), thiocarbohydrazide (**2b**), phosphonic dihydrazide (**3a**), and P-methoxyphosphonic dihydrazide (**3b**) in the presence of 2 drops of glacial acetic acid**.** The solution mixture was heated under reflux for 4 h. The resulting solids were filtered off and crystallized from dimethylformamide to give the corresponding macrocycles **4a**, **4b** and **5a**, **5b**, respectively, as yellow crystals with yields of 58%–71%.

13,33-Diphenyl-6,8-dihydro-5,6,8,9-tetraaza-2-phosphoryl-7-oxo-1,3-(1,4)-dipyrazola-cyclodcaphane-4,9-diene (4a): Mp 211–212 °C. IR (KBr, cm^{-1}): 3300 (NH), 3029 (C–H_{arom}), 2500 (P–H), 1690 (C=O), 1601 (C=$N_{exocyclic}$), 1539 (C=C), 1219 (P=O). ^{1}H NMR (DMSO-d_6, δ): 6.66 (d, 2H, J = 7.0 Hz, Ph–H), 7.18 (d, 1H, J = 8.0 Hz, Ph–H), 7.37–7.62 (m, 4H, Ph–H), 7.68 (d, 1H, J_{PH} = 584 Hz, P–H), 8.00 (d, 3H, J = 8.6 Hz, Ph–H), 8.42 (brs, 2H, C_5–$H_{pyrazole}$), 9.86 (s, 2H, CH=$N_{exocyclic}$), 11.81 (br, 2H, NH exchangeable with D_2O). m/z (relative intensity %): 444 (M^+, 25), 408 (50), 301 (50), 186 (50), 156 (50), 65 (100). Anal. Calcd. for $C_{21}H_{17}N_8O_2P$ (444.38): C, 56.76; H, 3.86; N, 25.22. Found: C, 56.37; H, 3.53; N, 25.35%.

13,33-Diphenyl-6,8-dihydro-5,6,8,9-tetraaza-2-phosphoryl-7-thioxo-1,3-(1,4)-dipyrazo-lacyclodecaphane-4,9-diene (4b): Mp 214–216 °C. IR (KBr, cm^{-1}): 3135 (NH), 3060 (C–H_{arom}), 2500 (P–H), 1597 (C=$N_{exocyclic}$), 1540 (C=$N_{endocyclic}$), 1500 (C=C), 1218 (P=O), 1129 (C=S). ^{1}H NMR (DMSO-d_6, δ): 7.64 (d, 1H, J_{PH} = 612 Hz, P–H), 6.51–7.96 (m, 10H, Ph–H), 8.49 (s, 2H, C_5–$H_{pyrazole}$), 9.80 (s, 2H, CH=$N_{exocyclic}$), 11.72 (br, 2H, NH exchangeable with D_2O). m/z (relative intensity %): 462 (M+2, 0.02), 461 (M+1, 0.13), 460 (M^+, 0.45), 369 (2), 321 (1.4), 246 (7), 221 (18), 161 (12), 104 (47), 77 (100), 51 (16). Anal. Calcd. for $C_{21}H_{17}N_8OP$ (460.45): C, 54.78; H, 3.72; N, 24.34. Found: C, 54.42; H, 3.39; N, 24.47%.

13,33-Diphenyl-6,8-dihydro-5,6,8,9-tetraaza-2,7-diphosphoryl-1,3-(1,4)-dipyrazolacyc-lodecaphane-4,9-diene (5a): Mp 242–244 °C. IR (KBr, cm^{-1}): 3427 (NH), 3056 (C–H_{arom}), 2500 (P–H), 1615 (C=$N_{exocyclic}$), 1595 (C=$N_{endocyclic}$), 1533 (C=C), 1294, 1202 (2P=O). ^{1}H NMR (DMSO-d_6, δ): 6.52 (d, 1H, J = 6.0 Hz, Ph–H), 7.16 (d, 1H, J =8.2 Hz, Ph–H), 7.45–7.98 (m, 8H, Ph–H), 7.39 (d, 1H, J_{PH} = 512 Hz,

P–H), 8.06 (d, 1H, J_{PH} = 406 Hz, P–H), 8.47 (s, 2H, C_5–$H_{pyrazole}$), 9.80 (s, 2H, CH=$N_{exocyclic}$). ^{31}P NMR (DMSO-d_6, δ): 8.29 (pyrazole–P–pyrazole), 17.70 (NH–P–NH). m/z (relative intensity %): 462 (M–2, 0.2), 324 (100), 231 (18), 104 (16), 77 (70), 65 (24), 51 (24). Anal. Calcd. for $C_{20}H_{18}N_8O_2P_2$ (464.36): C, 51.73; H, 3.91; N, 24.13. Found: C, 51.45; H, 3.59; N, 23.72%.

13,33-Diphenyl-6,8-dihydro-5,6,8,9-tetraaza-2-phosphoryl-7-methoxyphosphoryl-1,3-(1,4)-dipyrazolacyclodecaphan-4,9-diene (5b): Mp 232–234 °C. IR (KBr, cm^{-1}): 3429 (br, NH), 3055 (C–H_{arom}), 2992 (C–H_{aliph}), 2353 (P–H), 1602 (C=$N_{exocyclic}$), 1534 (C=C), 1294, 1209 (2 P=O), 1053 (P–O–C). ^{1}H NMR (DMSO-d_6, δ): 3.35 (s, 3H, OCH_3), 5.24 (brs, 2H, NH exchangeable with D_2O), 6.52 (d, 1H, J = 8.4 Hz, Ph–H), 7.02–7.20 (m, 3H, Ph–H), 7.40 (d, 1H, J_{PH} = 513 Hz, P–H), 7.32–7.54 (m, 4H, Ph–H), 7.76 (d, 1H, J = 7.2 Hz, Ph–H), 8.02 (d, 1H, J = 7.2 Hz, Ph–H), 8.49 (s, 2H, C_5–$H_{pyrazole}$), 9.81 (s, 2H, CH=$N_{exocyclic}$). m/z (relative intensity %): 494 (M^+, 3.3), 463 (3), 246 (41), 231 (15), 116 (12), 77 (100), 51 (32). Anal. Calcd. for $C_{21}H_{20}N_8O_3P_2$ (494.38): C, 51.02; H, 4.08; N, 22.67. Found: C, 50.79; H, 3.69; N, 22.25%.

2.2. General procedure for macrocycles 8a, 8b and 9a, 9b

A hot ethanolic solution (10 mL) of *bis*(4-formyl-3-(4'-biphenyl)-1*H*-pyrazol-1-yl) phosphine oxides (**1b**) (1.08 g, 2 mmol) was mixed with a hot ethanolic solution of appropriate diamine (2 mmol in 10 mL), namely hydrazine hydrate (**6a**), 1,2-ethylenediamine (**6b**), 1,2-phenylenediamine (**7a**), and 1,4-phenylenediamine (**7b**) in the presence of 2 drops of glacial acetic acid. The solution mixture was heated under reflux for 4 h. The resulting solids were filtered off and crystallized from dimethylformamide to give the corresponding macrocycles **8a**, **8b** and **9a**, **9b**, respectively, as yellow crystals with yields of 48%–85%.

13,33,83,103-Tetris(4'-biphenyl)-5,6,12,13-tetraaza-2,9-diphosphoryl-1,3,8,10(1,4)-tetrapyrazolacyclotetradecaphane-4,6,11,13-tetraene (8a): Mp 232–235 °C. IR (KBr, cm^{-1}): 3057 (C–H_{arom}), 2363 (P–H), 1614 (C=$N_{exocyclic}$), 1597 (C=$N_{endocyclic}$), 1533 (C=C), 1209 (P=O). ^{1}H NMR (DMSO-d_6, δ): 7.56 (d, 1H, J_{PH} = 590 Hz, P–H), 7.67 (d, 1H, J_{PH} = 612 Hz, P–H), 6.50–7.99 (m, 36H, Ar–H), 8.46 (s, 2H, C_5–$H_{pyrazole}$), 8.65 (s, 2H, C_5–$H_{pyrazole}$), 9.78 (s, 4H, CH=$N_{exocyclic}$). m/z (relative intensity %): 1073 (M–4H, 0.1), 882 (0.2), 734 (0.3), 452 (0.5), 307 (3), 293 (11), 167 (24), 149 (100), 127 (13), 96 (11), 57 (35). Anal. Calcd. for $C_{64}H_{46}N_{12}O_2P_2$ (1077.07): C, 71.37; H, 4.30; N, 15.61. Found: C, 71.63; H, 4.44; N, 15.23%.

13,33,103,123-Tetris(4'-biphenyl)-5,8,14,17-tetraaza-2,11-diphosphoryl-1,3,10,12(1,4)-tetrapyrazolacyclooctadecaphane-4,8,13,17-tetraene (8b): Mp 218–220 °C. IR (KBr, cm^{-1}): 3053 (C–H_{arom}), 2880, 2844 (C–H_{aliph}), 2362 (P–H), 1633 (C=$N_{exocyclic}$), 1595 (C=$N_{endocyclic}$), 1539 (C=C), 1214 (P=O). ^{1}H NMR (DMSO-d_6, δ): 3.79 (t, 8H, 2 CH_2CH_2), 7.59 (d, 1H, J_{PH} = 619 Hz, P–H), 7.69 (d, 1H, J_{PH} = 633 Hz, P–H), 6.51–8.28 (m, 36H, Ar–H), 8.49 (s, 2H, C_5–$H_{pyrazole}$), 8.87 (s, 2H, C_5–$H_{pyrazole}$), 9.80 (s, 2H, CH=$N_{exocyclic}$), 9.99 (s, 2H, CH=$N_{exocyclic}$). Anal. Calcd. for $C_{68}H_{54}N_{12}O_2P_2$ (1133.18): C, 72.07; H, 4.80; N, 14.83. Found: C, 71.72; H, 4.59; N, 14.42%.

13,33,93,113-Tetris(4'-biphenyl)-6,14(1,2)-dibenzene-5,7,13,15-tetraaza-2,10-diphosph-oryl-1,3,9,11(1,4)-tetrapyrazolacyclohexadecaphane-4,7,12,15-tetraene (9a): Mp 299–300 °C. IR (KBr, cm^{-1}): 3057 (C–H_{arom}), 2361 (br, P–H), 1615 (C=$N_{exocyclic}$), 1595 (C=$N_{endocyclic}$), 1533 (C=C), 1209 (P=O). ^{1}H NMR (DMSO-d_6, δ): 7.70 (d, 1H, J_{PH} = 610 Hz, P–H), 7.79 (d, 1H, J_{PH} = 631 Hz, P–H), 6.64–

8.01 (m, 44H, Ar–H), 8.63 (s, 4H, C_5–$H_{pyrazole}$), 9.87 (s, 2H, CH=$N_{exocyclic}$), 10.05 (s, 2H, CH=$N_{exocyclic}$). Anal. Calcd. for $C_{76}H_{54}N_{12}O_2P_2$ (1229.268): C, 74.26; H, 4.43; N, 13.67. Found: C, 73.86; H, 4.12; N, 13.25%.

13,33,93,113-Tetris(4'-biphenyl)-6,14(1,4)-dibenzene-5,7,13,15-tetraaza-2,10-diphosph-oryl-1,3,9,11(1,4)-tetrapyrazolacyclohexadecaphane-4,7,12,15-tetraene (9b): Mp 313–315 °C. IR (KBr, cm^{-1}): 3023 (C–H_{arom}), 2363 (P–H), 1607 (C=$N_{exocyclic}$), 1537 (C=C), 1213 (P=O). ^{1}H NMR (DMSO-d_6, δ): 7.70 (d, 1H, J_{PH} = 610 Hz, P–H), 7.79 (d, 1H, J_{PH} = 630 Hz, P–H), 6.59–8.07 (m, 44H, Ar–H), 8.56 (s, 2H, C_5–$H_{pyrazole}$), 8.60 (s, 2H, C_5–$H_{pyrazole}$), 9.86 (s, 2H, CH=$N_{exocyclic}$), 10.05 (s, 2H, CH=$N_{exocyclic}$). ^{31}P NMR (DMSO-d_6, δ): 8.38. m/z (relative intensity %): 1214 (M–16, 0.2), 643 (0.2), 501(0.4), 353 (30), 309 (100), 177 (10), 104 (16), 69 (53), 57 (48). Anal. Calcd. for $C_{76}H_{54}N_{12}O_2P_2$ (1229.268): C, 74.26; H, 4.66; N, 13.64. Found: C, 73.87; H, 4.27; N, 13.29%.

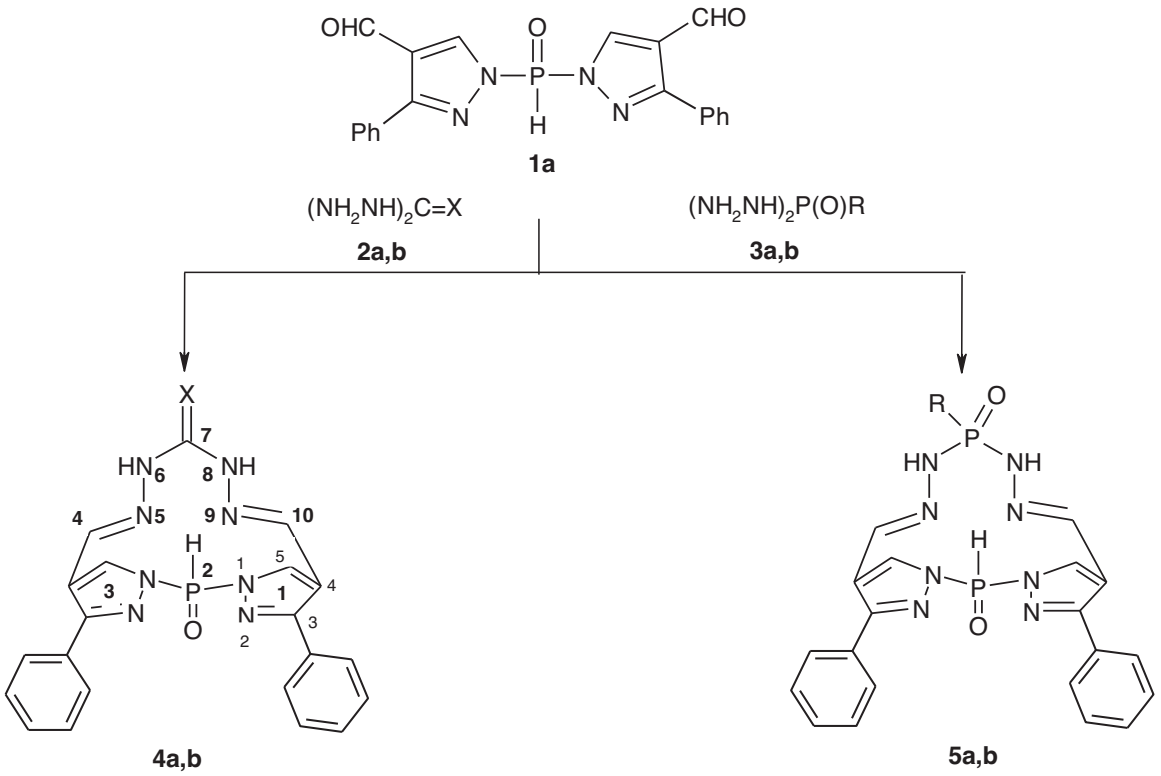
3. Results and discussion

A series of some new phosphorus macrocycles containing pyrazole rings were achieved via reaction of *bis*(4-formylpyrazolyl)phosphine oxides (**1a**, **1b**) with some dihydrazides containing oxygen, sulfur, and/or phosphorus atoms and also with aliphatic/aromatic diamines. The reactions of dicarbonyl compounds with diamines are much more complicated and produce a wide spectrum of products that can be identified by mass spectrometry.[25] The type [1+1] macrocycle is usually formed as the major product when any flexible diamine reacts with a dicarbonyl compound.[26] Thus, equimolar amounts (approximately $<10^{-2}$ mol) of *bis*-{4-formyl-3-phenyl-1*H*-pyrazol-1-yl}phosphine oxide (**1a**) and carbohydrazide (**2a**), thiocarbohydrazide (**2b**), phosphonic dihydrazide (**3a**), and P-methoxyphosphonic dihydrazide (**3b**) were condensed in aqueous ethanol under reflux, leading to the formation of [1+1] macrocycles **4a**, **4b** and **5a**, **5b**, respectively (Scheme 1).

When 2 amino groups are located in close proximity to each other (e.g., α,β- and α,γ-diamines), the respective diamines can react with dicarbonyl compounds to produce [2+2] macrocycles.[27,28] Thus, macrocycles **8a**, **8b** and **9a**, **9b** were obtained via reaction of *bis*-{4-formyl-3-(4'-biphenyl)-1*H*-pyrazol-1-yl}phosphine oxide (**1b**) with hydrazine hydrate (**6a**), 1,2-ethylenediamine (**6b**), 1,2-phenylenediamine (**7a**), and 1,4-phenylenediamine (**7b**), respectively, under the same previous conditions (Scheme 2).

3.1. The IR spectral study

The absorption bands corresponding to free aldehyde (–CHO) and primary amine ($-NH_2$) groups were not observed for the prepared macrocycles. This confirmed that there is a complete condensation of the terminal amino groups of nitrogen nucleophiles with *bis*(4-formyl-pyrazolyl)phosphine oxides (**1a**, **1b**). The recorded absorption bands in the region of 3135–3429 cm^{-1} in the IR spectra of **4a**, **4b** and **5a**, **5b** were assignable to NH stretching vibrations. In addition, the azomethine bonds were observed from the presence of strong bands in the frequency region of 1597–1615 cm^{-1} for all the newly formed macrocycles. The pure characteristic ν (C–H) modes of aromatic rings were also observed in the region of 3023–3060 cm^{-1} in all macrocycles, while ν (C–H) modes of aliphatic groups in macrocycles **5b** and **8b** were displayed in the region of 2880–2992 cm^{-1}. The vibrational frequencies of C=O and C=S functional groups in macrocycles **4a** and **4b** appeared at 1690 and 1129 cm^{-1}, respectively. Moreover, the P=O groups in all macrocycles were also recorded in the wave region of 1209–1294 cm^{-1}.



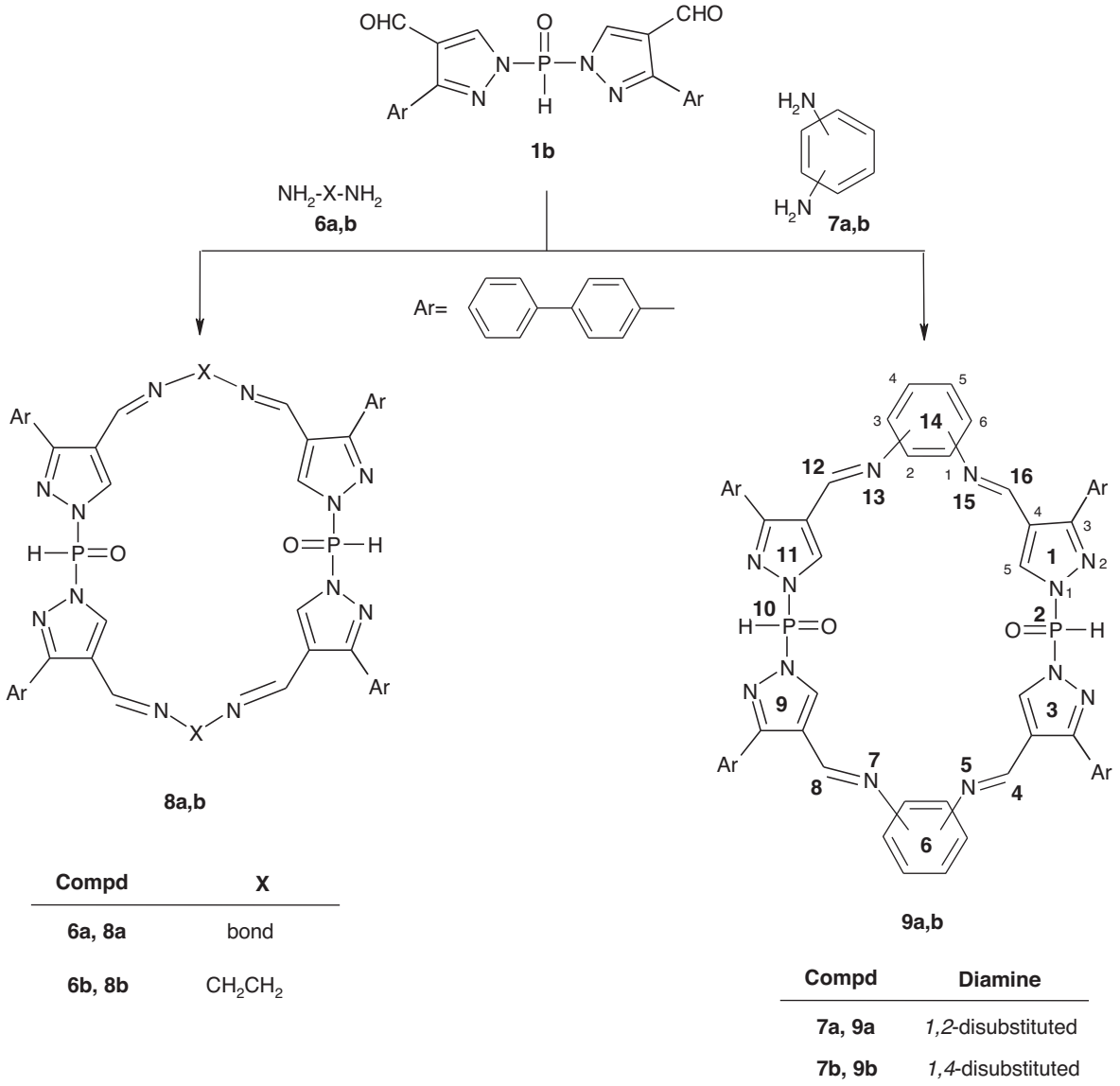
Compd	X
2a, 4a	O
2b, 4b	S

Compd	R
3a, 5a	H
3b, 5b	OCH_3

Scheme 1. Synthetic route for the preparation of the macrocycles **4a**, **4b** and **5a**, **5b**.

3.2. The ^{1}H NMR spectral study

The ^{1}H NMR spectra of the formed macrocycles in DMSO-d_6 did not give any signal corresponding to free aldehyde (–CHO) or primary amine ($-NH_2$) protons; instead, they showed broad signals in the region of δ 11.72, 11.81, and 5.24 ppm corresponding to NH protons in macrocycles **4a**, **4b**, and **5b**, respectively. The signals of NH–P(O)H–NH moiety in the ^{1}H NMR spectrum of macrocycle **5a** were displaced, presumably as a result of rapid proton exchange in 2 types of tautomeric equilibrium. First, there is the tautomeric amide–imide equilibrium (I, II, III) (Scheme 3). Second, the hydrophosphoryl unit in solutions easily undergoes the tautomeric transition, providing it a unique combination of properties of pentavalent and trivalent phosphorus atoms (phosphite–phosphonate I and IV) (Scheme 3).[29–32] The protons of hydrophosphoryl units that attached to pyrazole rings in all macrocycles appeared as doublets in the region of δ 7.39–8.06 ppm with coupling constants in the range of 406–634 Hz. The high field protons for exocyclic CH=N and C_5–H pyrazole rings were attributed to δ 9.78–10.05 and 8.46–8.87 ppm, respectively. It was interesting that the signals of protons of exocyclic CH=N and C_5–H pyrazole rings were duplicated at different values of chemical shifts in macrocycles **8a**, **8b** and **9a**, **9b** (see Experimental). This can be explained as the result of the presence of each 2 pyrazole rings separated with the hydrophosphoryl unit at different planes. We failed to obtain suitable crystals to



Compd	X
6a, 8a	bond
6b, 8b	CH_2CH_2

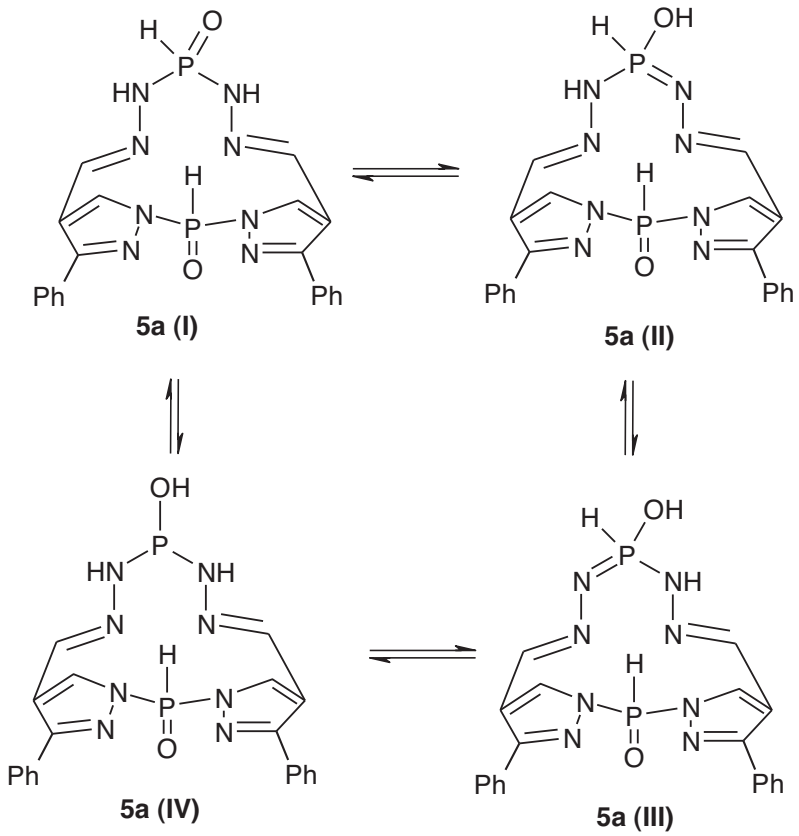
Compd	Diamine
7a, 9a	*1,2*-disubstituted
7b, 9b	*1,4*-disubstituted

Scheme 2. Synthetic route for the preparation of the macrocycles **8a**, **8b** and **9a**, **9b**.

conduct X-ray crystallography to confirm this suggestion. The chemical shifts of aromatic protons for all the macrocycles appeared in the region of δ 6.50–8.28 ppm. In addition, the methoxy protons in macrocycle **5b** were observed as singlet at δ 3.35 ppm, while protons of the $-CH_2CH_2-$ group in macrocycle **8b** were displayed as a triplet at δ 3.79 ppm.

3.3. The ^{31}P NMR spectral study

The ^{31}P NMR spectra of the selected macrocycles in DMSO-d_6 were measured. Macrocycle **5a** showed 2 doublets signals at δ 8.29 and 17.70 ppm with coupling constants 612 and 572 Hz, respectively, because of the presence of 2 hydrophosphoryl units. The hydrophosphoryl unit of macrocycle **9b** was also observed at δ 8.38 ppm with coupling constant 605 Hz.



Scheme 3. The tautomeric structures of compound **5a**.

3.4. The mass spectral study

The mass spectral study of macrocycles **4a**, **4b** and **5a**, **5b** confirmed that the cyclocondensation process occurred at a 1:1 ratio. The mass spectral data of **4a** and **4b** showed the molecular ion peaks at m/z 444 (M^+, 25 %) and 460 (M^+, 0.45 %) with their base peaks at m/z 65 and 77, respectively. The mass spectral data of **5a** and **5b** designated the molecular ion peaks at m/z 462 (M–2H, 0.2%) and 494 (M^+, 3.3%) with their base peaks at m/z 324 and 77, respectively. On the other hand, the mass spectra of macrocycles **8a** and **9b** supported their formation at a 2:2 ratio, although they did not show the molecular ion peaks, indicating the fragile nature of these macrocycles. Thus, the mass spectrum of **8a** showed the highest value peak at 1073 (M–4H) with a base peak at m/z 149, whereas macrocycle **9b** recorded the highest peak at m/z 1214 corresponding to its fragment ion peak after losing an oxygen atom, while its base peak was recorded at m/z 309.

3.5. Antimicrobial activity

All the newly synthesized compounds were evaluated in vitro for their antibacterial activities against *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615) as representatives of gram-positive bacteria and *Pseudomonas fluorescens* (S 97) and *Pseudomonas phaseolicola* (GSPB 2828) as examples of gram-negative bacteria. They were also examined in vitro for their antifungal activities against *Fusarium oxysporum* and *Aspergillus fumigatus* fungal strains. The agar-diffusion technique was used for the determination of the

preliminary antibacterial and antifungal activities.[33] The test was performed on medium potato dextrose agar that contained an infusion of 200 g potatoes, 6 g dextrose, and 15 g agar.[34] Uniformly sized filter paper disks (3 disks per compound) were impregnated by an equal volume (10 μL) of compounds dissolved in dimethylformamide at concentrations of 1000 and 2000 μg/mL and were carefully placed on inoculated agar surface. After incubation for 36 h at 27 °C in the case of bacteria and 48 h at 24 °C in the case of fungi, the antimicrobial activities were determined by measuring the inhibition zones (Table 1). Cephalothin, chloramphenicol, and cycloheximide were used as reference drugs (30 μg/mL) for gram-positive bacteria, gram-negative bacteria, and fungi, respectively. The minimum inhibitory concentration (MIC, μg/mL) for some selected compounds against all species of microbes was also determined (Table 2). The tube dilution technique[35] was applied for the determination of the MIC of the tested compounds against microbes. Dilution series were set up with 500, 250, 125, 62.5.....6.25 μg/mL of nutrient broth medium to each tube, 100 mL of standardized suspension of the test microbes (107 cell/mL) was added, and tubes were incubated at 37 °C for 24 h (Table 2).

Table 1. In vitro antimicrobial activities of the synthesized macrocycles **1a**, **1b**, **4a**, **4b**, **5a**, **b**, **8a**, **b** and **9a**, **b** at 1000 and 2000 μg/mL by disk diffusion assay.

Compd.* no.	Concentration (μg/mL)	Zone of inhibition (mm)**					
		Gram-positive bacteria		Gram-negative bacteria		Fungi	
		S. aureus	*S. pyogenes*	*P. phaseolicola*	*P. fluorescens*	*F. oxysporum*	*A. fumigatus*
1a	1000	8	–	8	6	–	–
	2000	10	7	10	8	–	–
1b	1000	8	5	–	–	–	–
	2000	10	8	–	6	–	–
4a	1000	7	6	–	–	–	–
	2000	10	10	5	9	–	–
4b	1000	13	14	–	5	19	14
	2000	16	19	8	10	22	17
5a	1000	13	14	–	–	9	11
	2000	16	19	8	7	13	13
5b	1000	5	5	–	–	5	–
	2000	9	9	8	6	8	6
8a	1000	5	–	–	–	–	–
	2000	8	5	5	6	–	–
8b	1000	7	11	6	5	–	–
	2000	12	14	6	8	–	–
9a	1000	12	12	–	–	11	12
	2000	17	16	–	–	15	15
9b	1000	5	–	–	–	–	–
	2000	8	6	–	5	8	6
R1	30	28	30	NT	NT	NT	NT
R2	30	NT	NT	25	30	NT	NT
R3	30	NT	NT	NT	NT	28	31

*R1 = cephalothin, R2 = chloramphenicol, R3 = cycloheximide; used as reference drugs at concentration of 30 μg/mL.
**Lowly active: 6–12 mm; moderately active: 13–19 mm; highly active: 20–30 mm; –: no inhibition or inhibition less than 5 mm; NT: not tested.

The obtained results were recorded for each tested compound as the average diameter of inhibition zones of both the bacteria and fungi around the disks in millimeters at the concentrations of 1000 and 2000 μg/mL (Table 1) and as MIC values (Table 2). The following points were revealed:

1) The results recorded in Table 1 revealed that most of the synthesized macrocycles possessed various antimicrobial activities towards all the microorganisms.

Table 2. Minimum inhibitory concentration (MIC) of compounds **4b**, **5a**, and **9a**.

Compd.	MIC*, μg/mL					
no.	*S. aureus*	*S. pyogenes*	*P. phaseolicola*	*P. fluorescens*	*F. oxysporum*	*A. fumigatus*
4b	500	250	>500	>500	250	>500
5a	500	250	>500	>500	500	>500
9a	250	500	>500	>500	500	>500
R1	6.25	6.25	–	–	–	–
R2	–	–	6.25	6.25	–	–
R3	–	–	–	–	12.50	6.25

*R1 = cephalothin, R2 = chloramphenicol, and R3 = cycloheximide; used as reference drugs.

2) Although compounds **4b**, **5a**, and **9a** had the highest inhibition zones for antibacterial and antifungal activities, they did not have strong MIC values.

3) All the synthesized macrocycles revealed better effects against gram-positive bacteria strains in comparison with the starting *bis*-(4-formylpyrazolyl)phosphine oxides (**1a**, **1b**).

4) Most of the small macrocycles, **4a**, **4b** and **5a**, **5b**, revealed better effects than the large macrocycles, **8a**, **8b** and **9a**, **9b**.

5) Small macrocycles **4a** and **5a** showed more antibacterial activities than antifungal activities, while macrocycles **4b** and **5b** revealed more antifungal activities than antibacterial activities.

6) Large macrocycles **8a**, **8b** and **9a**, **9b** displayed more antibacterial activities than antifungal activities.

7) The macrocycles that contained aromatic units, **9a** and **9b**, had more effects than the macrocycles that contained aliphatic units, **8a** and **8b**.

8) Generally, the macrocycles showed lower to moderate activities. However, none of the tested compounds were nearly equal to or more active than the reference drugs.

4. Conclusion

We achieved an efficient route for the preparation of previously unreported phosphorus macrocycles containing pyrazole rings. The characterization of these macrocycles was discussed. The preliminary antimicrobial activities of the tested compounds showed that they had lower to moderate activities. Although compounds **4b**, **5a**, and **9a** had the highest recorded inhibition zones for antibacterial and antifungal activities, they did not have strong MIC values. However, none of these tested compounds was equal to or better than the reference drugs in terms of activity.

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