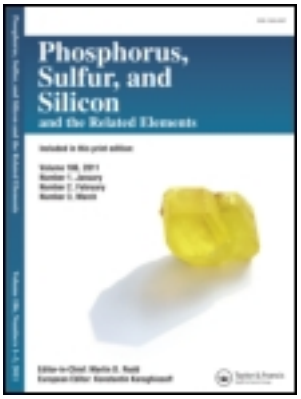

Phosphorus, Sulfur, and Silicon and the Related Elements

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Antimicrobial Activities of a Series of Diphenyl (4′-(Aryldiazenyl)Biphenyl-4-Ylamino) (Pyridin-3-YL)Methylphosphonates

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ANTIMICROBIAL ACTIVITIES OF A SERIES OF DIPHENYL (4′-(ARYLDIAZENYL)BIPHENYL-4-YLAMINO)(PYRIDIN-3-YL)METHYLPHOSPHONATES

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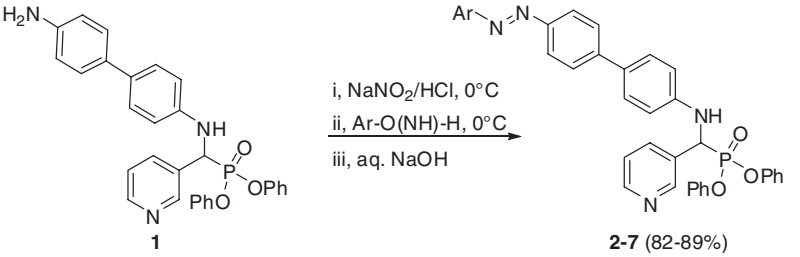
GRAPHICAL ABSTRACT



Ar in **2-7** = 4-hydroxynaphthalen-1-yl (**2**), 2-hydroxynaphthalen-1-yl (**3**), 2,4-dihydroxyphen-1-yl (**4**), 5-amino-2-hydroxyphen-1-yl (**5**), 2-amino-5-hydroxy-7-sulphonylnaphthalen-1-yl (**6**), 2-amino-5-methylphen-1-yl (**7**)

Abstract *A series of diphenyl (4′-(aryldiazenyl)biphenyl-4-ylamino)(pyridin-3-yl)methylphosphonates 2–7 was synthesized in high yields and their antimicrobial activities were investigated. Some compounds showed high antimicrobial activities against* Escherichia coli *as a gram-negative bacterium,* Bacillus subtilis *and* Staphylococcus aureus *as gram-positive bacteria, and* Candida albicans *and* Schccaromycies cerevisiae *as fungi and at low concentrations (10–1000 μg/mL). Supplemental materials are available for this article. Go to the publisher's online edition of* Phosphorus, Sulfur, and Silicon and the Related Elements *to view the free supplemental file.*

Keywords α-Aminophosphonates; antimicrobial; gram-negative and gram-positive bacteria; fungi; minimum inhibitory concentrations

INTRODUCTION

α-Aminophosphates constitute an important class of biologically active compounds as they are structurally related to α-amino acids.[1–3] Therefore, they continue to attract

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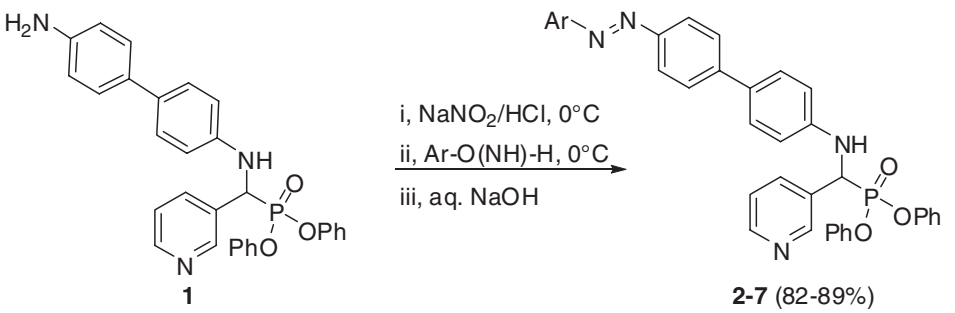
researchers because of their important pharmaceutical applications. For example, α-aminophosphonates act as peptide mimics,[4] enzyme inhibitors,[5,6] antibiotics,[7] crop protection agents,[8] and catalytic antibodies.[9] As a result, the synthesis of such compounds were developed over the years. The synthetic procedures of α-aminophosphonates involve use of Lewis acids, such as lanthanide triflates/$MgSO_4$,[10] $InCl_3$,[11] scandium (*tris*-dodecyl sulfate),[12] $TaCl_5$-SiO_2,[13] $Ln(OTf)_3$,[14] lanthanide triflates/ionic liquids,[15] and $AlCl_3$.[16]

As part of our continuing interest in heterocyclic chemistry,[17–31] we have reported a simple and convenient approach for the synthesis of a series of 3-arylazo-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-ones as azodisperse dyes.[32] The work reported in this paper describes the synthesis of diphenyl (4′-(aryldiazenyl)biphenyl-4-ylamino)(pyridin-3-yl)methylphosphonates. The synthesized compounds were evaluated for their antimicrobial activities against gram-positive and gram-negative bacteria and fungi.

RESULTS AND DISCUSSION

Diazotization of diphenyl (4′-aminobiphenyl-4-ylamino)(pyridin-3-yl)methylphosphonate (**1**) was performed by using a mixture of sodium nitrite and hydrochloric acid at low temperature (0 °C) to produce the corresponding diazonium salt. Coupling of diazonium salt of compound **1** with 1-naphthol, 2-naphthol, resorcinol, 2-aminophenol, 7-amino-4-hydroxynaphthalene-2-sulfonic acid, and 4-toludine, in aqueous sodium hydroxide solution (10%), gave the corresponding azo dyes **2**–**7** (Scheme 1) in high yields (82%–89%; Table 1).



Ar in **2-7** = 4-hydroxynaphthalen-1-yl (**2**), 2-hydroxynaphthalen-1-yl (**3**), 2,4-dihydroxyphen-1-yl (**4**), 5-amino-2-hydroxyphen-1-yl (**5**), 2-amino-5-hydroxy-7-sulphonylnaphthalen-1-yl (**6**), 2-amino-5-methylphen-1-yl (**7**)

Scheme 1

The structures of diazo dyes **2**–**7** were confirmed by their elemental analyses and various spectroscopic techniques including IR, ^{1}H-NMR, and mass spectral data (see experimental section for details).

Antimicrobial Activities

The synthesized α-aminophosphonates **2**–**7** were screened for their in vitro antibacterial and antifungal activities against *Escherichia coli* NCIM 2065 as a gram-negative

Table 1 Synthesis of azo dyes **2–7** according to Scheme 1

Product	Ar	Color	mp (°C)	Yield (%)[a]
2	4-Hydroxynaphthalen-1-yl	Rubine	179–181	88
3	2-Hydroxynaphthalen-1-yl	Dark red	190–193	84
4	2,4-Dihydroxyphen-1-yl	Red violet	171–173	89
5	5-Amino-2-hydroxyphen-1-yl	Reddish burgundy	149–152	84
6	2-Amino-5-hydroxy-7-sulphonylnaphthalen-1-yl	Burgundy	161–163	83
7	2-Amino-5-methylphen-1-yl	Buff	201–203	82

[a]Yield of pure products after crystallization from ethanol.

bacterium, *Bacillus subtilis* PC 1219 and *Staphylococcus aureus* ATCC 25292 as gram-positive bacteria, and *Candida albicans* and *Schccaromycies cerevisiae* as fungi. The inhibition zones were measured in triplicates and the results of antimicrobial testing are reported in Table S1 (Supplemental Materials).

The results recorded in Table S1 showed that all compounds showed high antimicrobial activities against *S. aureus* and *S. cerevisiae*. Compounds **2–6** showed moderate to high antimicrobial activities against *E. coli*. Compounds **2**, **5**, and **6** showed moderate to high antimicrobial activities, while compounds **3**, **4**, and **7** showed no antimicrobial activities against *B. subtilis*. It was found that compound **7** was the only active compound against *C. albicans*. Compound **7** contains an NH_2 group but does not have a hydroxyl group.

Minimum Inhibitory Concentrations (MICs)

The minimum inhibitory concentrations (MICs) of compounds **2–7** were determined for each antimicrobial agent by using agar diffusion method. The inhibition zone was measured in triplicates in four different concentrations (10–1000 μg/mL) and the mean value $\pm$ standard deviation (SD) is recorded in Table S2 (Supplemental Materials).

Table S2 showed that compound **6** was the most active compound against all organisms except for *C. albicans* and showed the lowest MIC value (10 μg/mL). On the other hand, compound **3** was found to be the least effective compound and showed the highest MIC value (1000 μg/mL). The activities of other compounds can be arranged in the following order: **2 > 5 > 7 > 4**.

CONCLUSIONS

A series of diphenyl (4′-(aryldiazenyl)biphenyl-4-ylamino)(pyridin-3-yl)methylphosphonates were synthesized and their antimicrobial activities were investigated. Some of the synthesized compounds exhibit a remarkable inhibition of the growth of gram-positive bacteria and gram-negative bacteria and fungi at low concentrations.

Experimental

Melting point determinations were performed by the open capillary method using an Electrothermal MEL–TEMP II apparatus and are reported uncorrected. IR spectra were recorded on a Perkin–Elmer 1430 Spectrophotometer using KBr disc technique. ^{1}H NMR spectra were recorded on a Bruker AC400 spectrometer operating at 400 MHz. The spectra

were recorded in DMSO–d_6. Chemical shifts δ are reported in parts per million (ppm) relative to TMS and coupling constants J are in Hz and have been rounded to the nearest whole number. EI mass spectra were recorded at energy 70 eV with a 7070 EQ mass spectrometer. Microanalysis was performed by analytical service at Cairo and Tanta Universities, Egypt. Reagents and solvents were used from commercial sources without purification.

Chemistry

Synthesis of Diphenyl (4′-Aminobiphenyl-4-Ylamino)(Pyridin-3-yl-) Methylphosphonate (1). A mixture of pyridine-3-carboxaldehyde (1.07 g, 10 mmol), benzidine (1.84 g, 10 mmol), triphenylphosphite (3.72 g, 12 mmol), and titanium tetrachloride (0.19 g, 1 mmol) in dichloromethane (10 mL) was stirred at room temperature for 26 h. The solvent was removed under reduced pressure and the residue obtained was treated with methanol (20 mL) and then filtered to remove the solid materials. A mixture of water (10 mL) and dichloromethane (10 mL) was added to the filtrate. The organic layer was separated and washed with water (10 mL), dried over anhydrous Na_2SO_4 and removed under reduced pressure to give the crude product, which was recrystallized from ethanol to give pure **1**.

Yield 88%; colorless; mp 120–122 °C; IR (KBr): Selected IR data (KBr) ν_{max}: 3329 (NH), 1597 (C═N), 1322 (P═O) and 823 (P—O—C) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.17 (br s, exch., 1H, NH), 9.15–6.76 (m, 22H, aromatic), 5.16 (d, $J = 16$ Hz, 1H, CH) and 3.97 (s, exch., 2H, NH_2). MS, *m/z* (%): 507 (M^+, 5), 449 (12), 390 (22), 314 (35), 265 (44), 396 (5), 225 (100), 214 (12), and 168 (11). Anal calcd for $C_{30}H_{26}N_3O_3P$ (507.52): C, 71.00; H, 5.16; N, 8.28; P, 6.10; Found: C, 71.12; H, 4.94; N, 8.01; P, 6.15%.

General Procedure for the Synthesis of Diphenyl (4′-(Aryldiazenyl)Biphenyl-4-Ylamino)(Pyridin-3-yl)Methylphosphonates 2–7. A cold (0 °C) solution of sodium nitrate (0.76 g, 11 mmol) in H_2O (10 mL) was added gradually to a cold (0 °C) suspension of **1** (5.07 g, 10 mmol) in conc. HCl (5 mL). The diazonium salt thus obtained was added with continuous stirring to a cold (0 °C) solution of coupler (10 mmol) in aqueous NaOH solution (10%; 15 mL). The mixture was stirred at 0 °C for 30 min and the dye obtained was filtered, washed with H_2O, dried and crystallized from ethanol to give pure **2–7**. The physical properties of products **2–7** are recorded in Table 1.

Diphenyl (4′-((4-hydroxynaphthalen-1-yl)diazenyl)biphenyl-4-ylamino)(pyridin-3-yl)methylphosphonate (2): Selected IR data (KBr) ν_{max}: 3494 (OH/NH), 1613 (C═N), 1581 (N═N), 1323 (P═O) and 893 (P—O—C) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.10 (s, exch., 1H, OH), 8.73 (br s, exch., 1H, NH), 8.50–7.21 (m, 28H, H-arom) and 6.95 (d, $J = 12$ Hz, 1H, CH). MS, *m/z* (%): 662 (M^+, 3), 613 (2), 531 (3), 439 (59), 410 (4), 396 (5), 362 (29), 273 (12), and 98 (100). Anal. Calcd. for $C_{40}H_{31}N_4O_4P$ (662.67): C, 72.50; H, 4.72; N, 8.45; P, 4.67; Found: C, 72.62; H, 4.74; N, 8.41; P, 4.65%.

Diphenyl (4′-((2-hydroxynaphthalen-1-yl)diazenyl)biphenyl-4-ylamino)(pyridin-3-yl)methylphosphonate (3): Selected IR data (KBr) ν_{max}: 3337(OH/NH), 1613 (C═N), 1580 (N═N), 1325 (P═O), and 890 (P—O—C) cm^{-1}. ^1H–NMR (400 MHz, DMSO–d_6) δ (ppm): 9.09 (s, exch., 1H, OH), 8.81 (br s, exch., 1H, NH), 8.72–7.43 (m, 28H, H-arom) and 6.95 (d, $J = 12$ Hz, 1H, CH). MS, *m/z* (%): 662 (M^+, 4), 613 (4), 531 (6), 439 (66), 410 (6), 396 (4), 362 (32), 273 (15) and 98 (100). Anal. Calcd. for $C_{40}H_{31}N_4O_4P$ (662.67): C, 72.50; H, 4.72; N, 8.45; P, 4.67; Found: C, 72.60; H, 4.77; N, 8.39; P, 4.69%.

Diphenyl (4′-((2,4-dihydroxyphenyl)diazenyl)biphenyl-4-ylamino)(pyridin-3-yl)methylphosphonate (4): Selected IR data (KBr) ν_{max}: 3332 (OH/NH), 1610 (C=N), 1490 (N=N), 1320 (P=O), and 896 (P−O−C) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.11 (s, exch., 1H, OH), 8.81 (br s, exch., 1H, NH), 8.73 (s, exch., 1H, OH), 8.50–7.08 (m, 25H, H-arom) and 6.92 (d, $J = 13$ Hz, 1H, CH). MS, *m/z* (%): 628 (M^+, 3), 564 (4), 514 (3), 436 (25), 362 (100), 282 (28) and 273 (12). Anal. Calcd. for $C_{36}H_{29}N_4O_5P$ (628.61): C, 68.78; H, 4.65; N, 8.91; P, 4.93; Found: C, 68.62; H, 4.84; N, 8.86; P, 4.95%.

Diphenyl (4′-((5-amino-2-hydroxyphenyl)diazenyl)biphenyl-4-ylamino)(pyridin-3-yl)methylphosphonate (5): Selected IR data (KBr) ν_{max}: 3399 (OH/NH), 1613 (C=N), 1582 (N=N), 1327 (P=O), and 884 (P−O−C) cm^{-1}. ^1H–NMR (400 MHz, DMSO–d_6) δ (ppm): 9.09 (s, exch., 1H, OH), 8.81 (br s, exch., 1H, NH), 8.72–6.93 (m, 25H, H-arom), 6.63 (d, $J = 13$ Hz, 1H, CH) and 6.37 (s, exch., 2H, NH_2). MS, *m/z* (%): 627 (M^+, 3), 413 (2), 362 (100), 282 (38), 273 (13) and 152 (38). Anal. Calcd. for $C_{36}H_{30}N_5O_4P$ (627.63): C, 68.89; H, 4.82; N, 11.16; P, 4.94; Found: C, 68.62; H, 4.94; N, 11.01; P, 4.85%.

7-Amino-3-((4′-((diphenoxyphosphoryl)(pyridin-3-yl)methylamino)biphenyl-4-yl)diazenyl)-4-hydroxynaphthalene-2-sulfonic acid (6): Selected IR data (KBr) ν_{max}: 3424 (OH/NH), 1615 (C=N), 1519 (N=N), 1299 (P=O), and 892 (P−O−C) cm^{-1}. ^1H–NMR (400 MHz, DMSO–d_6) δ (ppm): 9.09 (s, exch., 1H, OH), 8.90 (s, exch., 1H, SO_3H), 8.80 (br s, 1H, NH), 8.72–6.93 (m, 26H, H-arom), 6.75 (d, $J = 12$ Hz, 1H, CH) and 6.83 (s, exch., 2H, NH_2); MS, *m/z* (%): 757 (M^+, 6), 515 (39), 439 (15), 362 (72), 282 (100) and 273 (11). Anal. Calcd. for $C_{40}H_{32}N_5O_7PS$ (757.75): C, 63.40; H, 4.26; N, 9.24; P, 4.09; Found: C, 63.62; H, 4.24; N, 9.21; P, 4.15%.

Diphenyl (4′-((2-amino-5-methylphenyl)diazenyl)biphenyl-4-ylamino)(pyridin-3-yl)methylphosphonate (7): Selected IR data (KBr) ν_{max}: 3408 (OH/NH), 1612 (C=N), 1582 (N=N), 1323 (P=O), and 890 (P−O−C) cm^{-1}. ^1H–NMR (400 MHz, DMSO–d_6) δ (ppm): 9.09 (br s, exch., 1H, NH), 8.80–6.49 (m, 25H, H-arom), 5.65 (d, $J = 12$ Hz, 1H, CH), 5.26 (s, exch., 2H, NH_2) and 2.30 (s, 3H, CH_3). MS, *m/z* (%): 625 (M^+, 9), 442 (15), 362 (100), 282 (97), 273 (40) and 152 (95). Anal. Calcd. for $C_{37}H_{32}N_5O_3P$ (625.65): C, 71.03; H, 5.16; N, 11.19; P, 4.95; Found: C, 71.02; H, 5.04; N, 11.01; P, 4.85%.

Biological Assay

Bacteria. After gram-staining procedure, gram-negative cells appear pink. The gram-negative bacterium used in this study was *E. coli*, which is known as the backbone example for gram-negative bacteria and cause urinary infection, wound infection, and gastroenteritis. The thick cell wall of a gram-positive organism retains the crystal violet dye used in the gram-staining procedure, so the stained cells appear purple under magnification. Gram-positive bacteria used in this study were *B. subtilis* and *S. aureus*. *B. subtilis* is mostly involved in Urinary infection, wound, ulceration, and septicemia. *S. aureus* is the mild stone of gram-positive bacteria and it is a causative agent of pneumonia, meningitis, and food poisoning.

Fungi. Pathogenic fungi spatially yeasts are responsible for a number of diseases in human and animals. A number of pathogenic strains of fungi are represented in *C. albicans* and *S. cerevisiae*. The tested organisms were obtained from the culture collection of Bacteriology Unit, Department of Botany, Faculty of Science, Tanta University, Egypt.

Media Used and Antimicrobial Assay. Compounds that produced the highest inhibition zones were selected and assayed further at different concentrations in suspensions

to quantify their inhibitory effects. Nutrient and Sabouraud's broths were used in activation of organisms.[33]

Determination of Minimum Inhibitory Concentrations (MICs). The antimicrobial activities of the tested samples were determined by measuring the diameter of zone of inhibition expressed in millimeter. The inhibition zones were measured in triplicates and expressed as mean ± SD.[34]

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