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ORIGINAL PAPER

Efficient synthesis routes for various phthalimido phosphor esters as antimicrobial agents in terms of structure–activity relationship

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Abstract A series of phthalimido phosphor derivatives—α-amino-phosphonates, amidophosphonates, phosphonic acid diamides, and oxadiazaphospholes—were prepared by applying different types of phosphorus(III) reagents to 2-methoxy-1*H*-isoindole-1,3(2*H*)-dione and 2-anilino-1*H*-isoindole-1,3(2*H*)-dione. On the basis of bioassay results, some of the new phosphoryl imides could be considered as lead molecules to be modified in order to improve their antibiotic activity.

Keywords Phosphorylation · 1*H*-Isoindole-1,3(2*H*)-dione-2-derivatives · Structure–activity relationship · Amidophosphates · Antibiotics

Introduction

Trivalent phosphorous acid derivatives are mainly used for preparation of organophosphorus compounds suitable as flame retardants, corrosion inhibitors, detergents, and lubricants [1], and additives to these, as well as agricultural chemicals and pharmaceuticals [2]. Some work has been done on the reactions of tri- and pentavalent phosphorus reagents with *N*-phthalimides in order to synthesize the relevant phosphorylated derivatives for applications [3–9]. On the whole, the reactions followed the predicted routes, although surprising outcomes were observed in a few cases [9]. In our present work, a new and efficient procedure for phosphorylating two phthalimide derivatives is described. The strategy depends on applying trialkyl phosphites **2a**–**2c**, dialkyl hydrogenphosphonates **11a**–**11c**, and tris-(dialkylamino)phosphines **9a** and **9b** to 2-methoxy-1*H*-isoindole-1,3(2*H*)-dione (**1**, *N*-methoxyphthalimide) and 2-anilino-1*H*-isoindole-1,3(2*H*)-dione (**15**).

Consecutively, a series of phosphorylated isoindole derivatives were synthesized and screened for their pharmacological activities. Even though many phosphorylated phthalimides are well known as pesticides [2], several derivatives have recently been reported to exhibit anesthetic activity superior to that of procaine [10, 11], and in addition they represent the core unit found in a number of alkaloid families [12, 13].

Results and discussion

In the presence of excess trialkyl phosphites **2a**–**2c**, the reaction with *N*-methoxyphthalimide (**1**) led to the formation of the corresponding α-aminophosphonates **6a**–**6c** in ~77% yield (Scheme 1). The identities of **6a** and **6b** were established by comparing their melting points and infrared (IR) spectra with the appropriate authentic samples [14–18]. Diisopropyl [(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)methyl]phosphonate (**6c**), prepared in this work for the first time, gave comparable analytical and spectroscopic data.

Remarkably, the mass spectra of α-aminophosphonates **6** did not display the corresponding ion peak; nevertheless, they showed a base ion peak at $m/z = 160$ that was assigned to the *N*-methylphthalimide ion, and another prominent peak for the corresponding phosphonic acid anion $[(RO)_2PO^{\ominus}]$. The major pathways of the fragmentation of **6** (e.g., **6c**) to positive ions are outlined in Scheme 2. In favor of this result, upon heating **6c** as a representable example above its melting point (120 °C)

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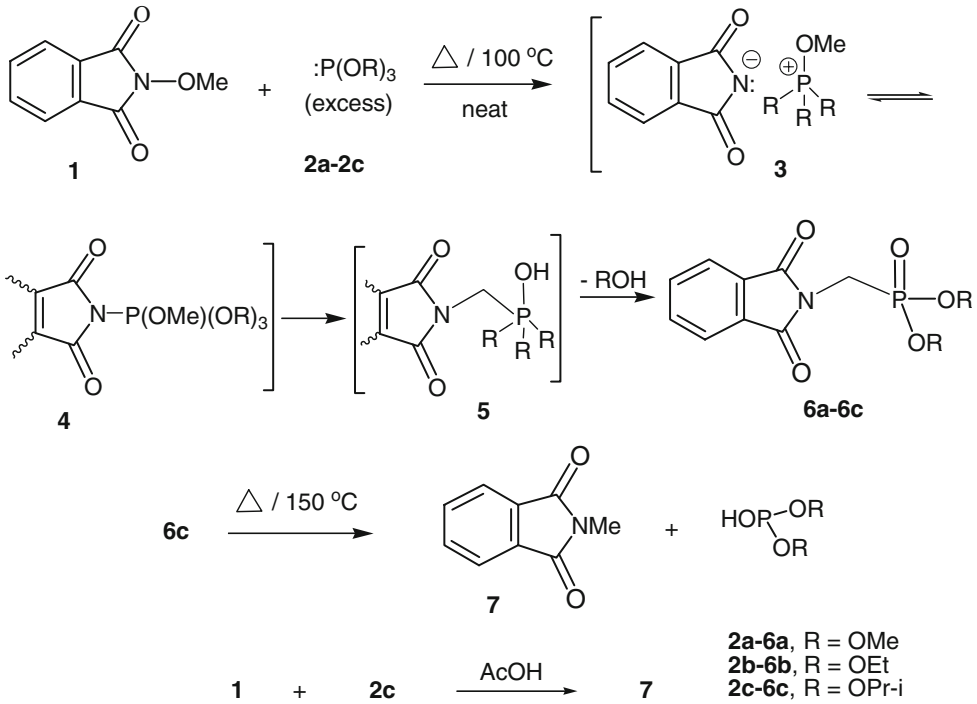
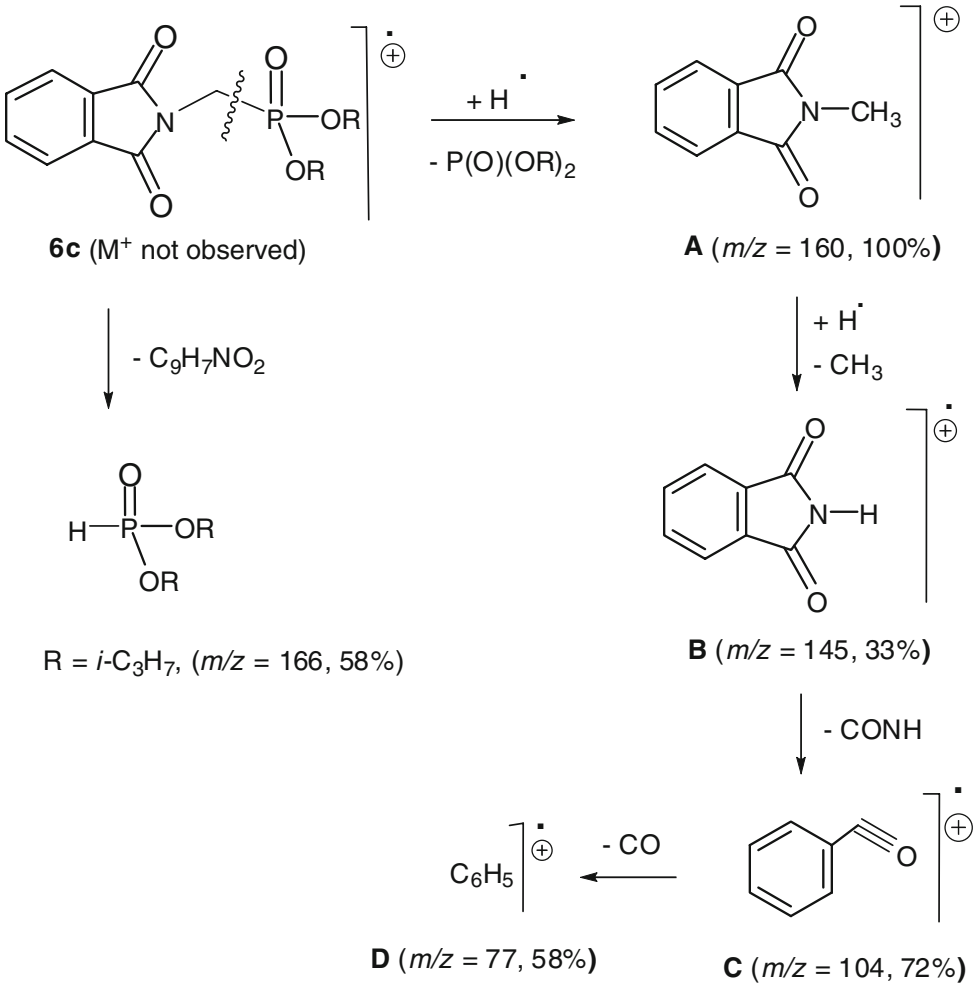
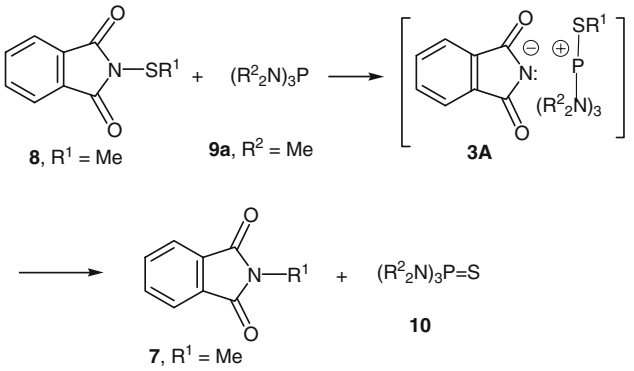
Scheme 1

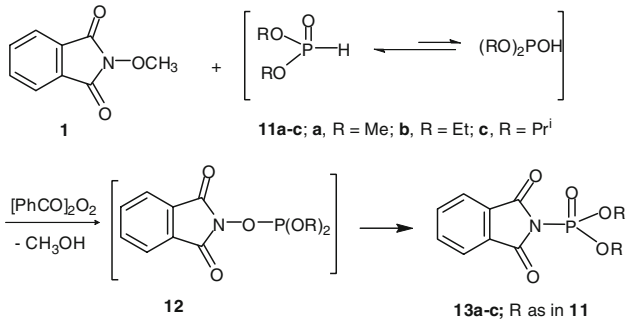


Scheme 2





Scheme 3



Scheme 4

under reduced pressure, *N*-methylphthalimide (**7**) was isolated (Scheme 1). Furthermore, when the same reaction (**1** + **2c**) was carried out in the presence of a protonating agent (AcOH), compound **7** was again obtained (Scheme 1).

A possible mechanism of this reaction involves the initial formation of the phosphonium salt **3**, which is present in equilibrium with the salt **4**, leading to the intermediate **5**. Further extrusion of an alcohol molecule from **5** generates α-aminophosphonates **6**. The latter step is thought to involve a deoxygenation process, as the methoxyl species serves as a source of oxygen necessary for the formation of the phosphorus–oxygen bond in **6**.

Similar formation of the salts **3** and **4** has been found previously [8, 9] to proceed in reactions between sulfenamides **8** and tris(dimethylamino)phosphine (**9a**), whereby *N*-alkylphthalimide **7** and tris(dimethylamino)thiophosphinate (**10**) were the reaction products (Scheme 3).

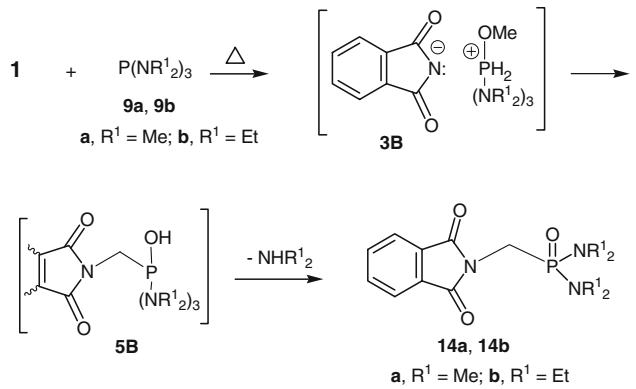
It is worth mentioning here that the methods previously described for the preparation of isoindolylmethyl phosphonates **6a** and **6b** are lengthy and indirect. For example, compound **6b** was synthesized in fair yield by treating diethyl bromomethylphosphonate with *N*-(*tert*-butyldimethylsilyl)-phthalimide in the presence of tetrabutylammonium fluoride [16], or by applying diethyl 1-hydroxyalkyl phosphonates to phthalimide in the presence of a complex formed from diethyl diazenedicarboxylate and triphenylphosphine [17]. Furthermore, the preparation of *N*-alkyl-phthalimide in the present study provides an alternative to Gabriel synthesis [19] from the halogen precursor for some branched alkylamines such as isopropyl, for which a direct synthesis of *N*-isopropylphthalimide was unsuccessful [9, 19].

The reaction of dialkyl hydrogenphosphonates **11a–11c** with *N*-methoxyphthalimide (**1**) was studied next. However, the reaction between **1** and **11a–11c** proceeded only when a catalytic amount of triethylamine or benzoylperoxide was present in the medium (best yield was obtained with the peroxide), to yield the respective amidophosphonates **13a–13c** as the sole reaction products in ~66% yield. This has been rationalized as proceeding via rearrangement of the intermediate **12**, which in turn can be envisaged as arising by the condensation of **1** and **11** accompanied with elimination of a methanol molecule (Scheme 4).

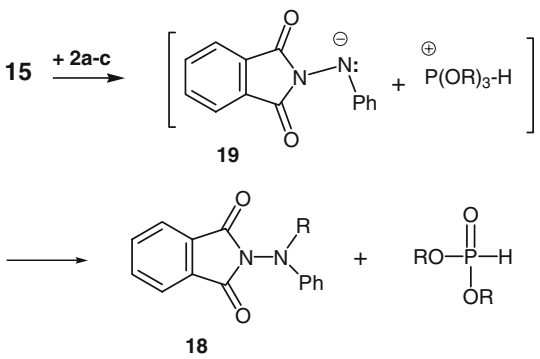
The recorded infrared and mass spectra and the analytical data of the products **13a** and **13b** are consistent with their assignments [20]. Diisopropyl (1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)phosphonate (**13c**) displayed a ^{31}P nuclear magnetic resonance (NMR) chemical shift of 14.6 ppm, which is in agreement with the presence of a P–N linkage in the molecule [21]. Its IR spectrum showed absorption bands at 1,780 and 1,722 cm^{-1}, assigned to the coupled C=O vibration of cyclic imides. The phosphonate moiety of **13c** in the ^{1}H NMR spectrum appeared at δ_H = 1.12, 1.13 (2dd, J_{H-H} = 6.6, J_{P-H} = 5.7 Hz, 12H, $(Me)_2COP$), 3.94 (sept, J_{P-H} = 12.3 Hz, 2H, HCOP) ppm and at δ_C = 15.9 (d, J_{P-C} = 8.4 Hz, CH_3COP) and 71.4 (d, J_{P-C} = 17 Hz, CHOP) ppm in its ^{13}C NMR spectrum.

Furthermore, we have established that tris(dialkylamino)phosphines **9a** and **9b** react with **1** by a pathway analogous to that previously discussed for trialkyl phosphites in Scheme 1. However, the phosphines **9a** and **9b** were found to be more reactive towards the imide derivative **1**, leading to *P*-[(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)methyl]-*N*,*N*,*N*′,*N*′-tetramethylphosphonic diamide (**14a**) or the known tetraethyl derivative **14b** [22] in dramatically shorter time and higher yields, according to the mechanism outlined in Scheme 5 [8, 9]. Thus, the formation of the products **6** or **14** (Schemes 1 and 5) was attributed to the efficiency of the phosphorus moiety for the abstraction of a proton from the methyl group in **1**.

Turning now to the scope of the above three reactions of **1** with phosphorus reagents **2**, **9**, and **11**, the reactivity observed in all these reagents seems to be attributed to the formation of a P–O bond between the phosphorus reagent and the methoxy oxygen of *N*-methoxyphthalimide.



Scheme 5



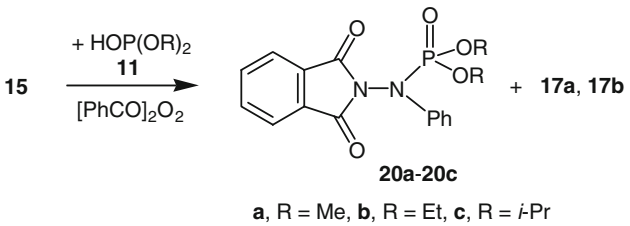
Scheme 7

Next, since potential biological activities are recorded for several substrates incorporating a N–P linkage, trialkyl phosphites **2a**–**2c**, dialkyl hydrogenphosphonates **11a**–**11c**, and tris(dialkylamino)phosphines **9a** and **9b** were applied in a systematic study to 2-anilino-1*H*-isoindole-1,3(2*H*)-dione (**15**, 2-anilinophthalimide). On heating **15** at 100 °C with **2a**–**2c** for ~20 h, dialkyl (3-alkoxy-1-hydroxy-2*H*-isoindol-2-yl)phenylphosphoramidoates **17a**–**17c** were obtained in ~74% yield. Furthermore, *N*-alkylated hydrazines **18a** [23] and **18b** [24] were isolated in ~13% yield in the case of the reactions with **2a** and **2b** (Scheme 6). Conceivably, the nucleophilic addition of the phosphite phosphorus to the terminal nitrogen of the aza-group takes place to yield the dipolar species **16**. The intermediate **16** then undergoes intramolecular group translocation to yield **17a** and **17b**.

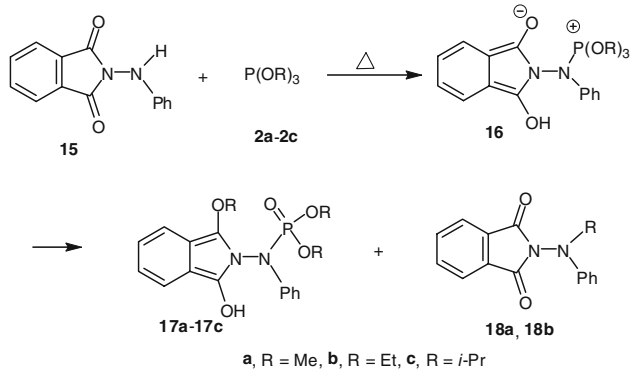
The mechanism for the *N*-alkylation process is depicted in Scheme 7. This is based on direct attack by the anionic center in **19** on the alkoxy-alkyl group of the reagent **2** to give *N*-alkylated products **18a** and **18b**.

Similarly, when a mixture of the hydrazine **15**, dialkyl hydrogen-phosphonates **11a**–**11c**, and benzoylperoxide was heated at 100 °C, phosphoramidoates **20a**–**20c** were formed through the loss of a molecule of H_2O according to Scheme 8. The parallel *N*-alkyl derivatives **18a** (10%) or **18b** (11%) were also formed in the reactions of **15** with **11a** and **11b**. *N*-alkylation of **15** by trialkyl phosphites and dialkyl hydrogenphosphonates contributes, however, to their potentialities as alkylating agents for acids, phenols, thiols, and amino groups [25, 26]. Activity of the phosphorus compounds as alkylating agents increases in the order: $(RO)_2P(O)R < (RO)_2P(O)H < (RO)_3P(O)$; ($R=CH_3$) [27].

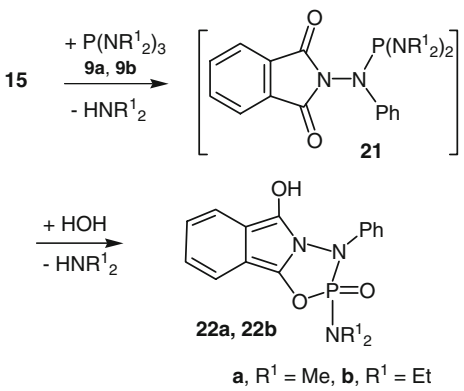
Conversely, when tris(dialkylamino)phosphines **9a** and **9b** were applied to the hydrazine **15**, oxadiazaphospholes **22a** and **22b** were isolated in ~72% yield (Scheme 9). The structure elucidation of **22** was based on elemental analysis, molecular weight determination (MS), and spectroscopic data [27, 28]. The IR spectra of **22a** and **22b**



Scheme 8



Scheme 6



Scheme 9

showed the presence of an intense band at 1,258 cm^{-1} for free P=O absorption, two bands due to absorption of the PNR^1 moiety (1,320, 838 cm^{-1}), and a broad band at 3,430 cm^{-1} assigned to the OH group. The ^{31}P NMR spectrum of **22a** recorded a positive shift at $\delta_P =$ 14.4 ppm, which is within the range expected for the assigned structure [29, 30]. ^1H and ^{13}C NMR spectra also supported this conclusion. In the mass spectrum, a prominent peak at $m/z = 91$ arises from $OP^{\oplus}NMe_2$, at 182 from $C_6H_5NP^{\oplus}$ $(O)NMe_2$, and the base peak $m/z = 237$ from $[M^{\oplus}–(O–PNMe_2)]$. Obviously, compounds **22** were formed through an initial condensation of the aminophosphines **9**–**15** to give the intermediate **21** accompanied by elimination of a dialkyl amine molecule. Stabilization of **21** was attained by reacting with fortuitous water leading to the formation of oxadiazaphospholes **22a** and **22b**, accompanied by extrusion of dialkyl amine.

Pharmacological evaluation

The prepared phthalimide derivatives bearing a phosphorus moiety were screened for their antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, and *Klebsiella pneumoniae* (recultured) bacterial strains by the disc diffusion method [31, 32]. The data were compared with amoxicillin (**A**) as a standard drug. The diameter of the zone of inhibition and minimum inhibitory concentration values are given in Table 1.

Furthermore, the prepared compounds were tested for their antifungal activity against *Saccharomyces cerevisiae*, *Candida albicans*, and *Aspergillus niger* (recultured) by the agar diffusion method [33]. The data were compared with fluconazole (**F**) as a standard drug. The diameter of the zone of inhibition and minimum inhibitory concentration values are given in Table 1.

Inspection of the data obtained in the antibacterial and antifungal screening of different phosphorylated phthalimides revealed that all tested compounds have moderate to good antimicrobial properties when compared with available drugs **A** and **F**. In general, these data indicate only marginal differences in potency of the tested phosphorus derivatives due to the phosphorus ester substituent, i.e., R or R^1 in phosphorus moiety $P(O)(OR)_2$ or $P(O)(NR^1_2)$. Nevertheless, it has been pointed out that an important role could be played by the nature of the type of the phosphorus moiety. Thus, according to our results, phosphoramidoates **14a**, **14b**, **20a**–**20c**, **22a**, and **22b**, which include a P–N bond, seem to be the most active antibiotic agents for both tested bacteria and fungi strains. Finally, pharmacological activity of different types of organophosphorus compounds tested here increases in the order: $N–P(O)(OR)_2 < CH_2$-$P(O)(NR^1_2)_2 < N–P(O)(NR^1_2)_2$.

These preliminary results lead us to conclude that this type of compounds should be studied in detail for their applications in diverse areas, although their toxicity should also be tested. Further study is in progress.

Table 1 Antibiotic activity of synthesized phthalimido phosphor derivatives

Sample	Bacterial strains			Fungal strains		
	K. pneumoniae	*E. coli*	*B. subtilis*	*S. cerevisiae*	*C. albicans*	*A. niger*
6a	11[a] (8.32)[b]	19 (8.32)	14 (30.6)	15 (30.6)	9 (30.6)	25 (8.32)
6b	13 (8.32)	20 (8.32)	12 (30.6)	13 (30.6)	11 (30.6)	22 (8.32)
6c	18 (8.32)	28 (8.32)	15 (30.6)	10 (30.6)	30 (8.32)	21 (8.32)
13a	20 (8.32)	27 (8.32)	20 (8.32)	23 (8.32)	32 (8.32)	23 (8.32)
13b	28 (8.32)	25 (8.32)	22 (8.32)	18 (8.32)	34 (8.32)	24 (8.32)
13c	25 (8.32)	28 (8.32)	24 (8.32)	25 (8.23)	25 (14.5)	16 (14.5)
14a	18 (8.32)	27 (8.32)	20 (8.32)	23 (8.23)	21 (8.32)	22 (8.32)
14b	18 (8.32)	25 (8.32)	28 (8.32)	26 (8.32)	24 (8.32)	20 (8.32)
17a	15 (30.6)	12 (30.6)	10 (30.6)	22 (8.32)	17 (14.5)	25 (8.32)
17b	11 (30.6)	13 (30.6)	14 (30.6)	21 (8.32)	18 (14.5)	22 (8.32)
17c	9 (14.5)	16 (30.6)	11 (30.6)	25 (8.32)	29 (8.23)	20 (8.32)
20a	16 (14.5)	21 (8.32)	23 (8.32)	28 (8.32)	18 (8.23)	27 (8.32
20b	22 (8.32)	22 (8.32)	14.6 (14.5)	30 (8.32)	25 (8.23)	21 (8.32)
20c	28 (14.52)	28 (14.52)	55 (2.2)	27 (8.32)	32 (8.23)	32 (8.32)
22a	21 (8.23)	19 (8.23)	20 (8.32)	18 (14.5)	19 (14.5)	25 (14.5)
22b	28 (8.23)	20 (8.23)	20 (8.32)	25 (14.5)	18 (14.5)	26 (8.32)
A	24 (8.23)	25 (8.23)	21 (8.23)	–	–	–
F	–	–	–	28 (8.23)	30 (8.23)	25 (8.23)

[a] Diameter (mm) of zone of inhibition

[b] Minimum inhibitory concentration values (MIC/ μg cm^{-3})

A: Amoxicillin is used as an antibacterial standard

F: Fluconazole is used as an antifungal standard

Conclusion

We have been successful in utilizing 2-methoxy- and 2-anilinophthalimide for one-pot synthesis of novel and rather inaccessible phosphonates and amidoates with potential biological activity. Compounds **6c**, **13c**, **14a**, **17a**–**17c**, **20a**–**20c**, **22a**, and **22b** are new phthalimido-phosphorylated derivatives and have been characterized by spectral and analytical data. Finally, on the basis of bioassay results, products **20a**–**20c** could be considered as lead molecules to be modified in order to improve their antibiotic activity.

Experimental

Melting points were measured on an Electrothermal melting point apparatus. IR spectra were recorded on a Perkin Elmer 317 grating IR spectrophotometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were measured on a Jeol ECA 500 MHz instrument using $SiMe_4$ as an internal reference. ^{31}P NMR spectra were recorded by using the same instrument, relative to external H_3PO_4 (85%). Mass spectra were recorded on a Jeol JMS-A X 500 spectrometer. Elemental analyses were carried out at the Microanalysis Laboratory, Cairo University, Cairo, Egypt. The appropriate precautions in handling moisture-sensitive compounds were considered. Solvents were dried by standard techniques. TLC: Merck 0.2 mm silica gel 60 F154 aluminum plates. Column chromatography (CC): silica gel (Kieselgel 60 mesh, particle size 0.2–0.5 mm; E. Merck, Darmstadt). All yields are based on the starting substrate **1** or **15**.

*Reaction of N-methoxyphthalimide (**1**) with trialkyl phosphites **2a**–**2c***

(A) In absence of solvent. Synthesis of compounds **6a**–**6c**
A mixture of 0.8 g **1** (0.5 mmol) and 4 cm^3 trialkyl phosphite (trimethyl **2a**, triethyl **2b**, or triisopropyl phosphite **2c**) was heated at 100 °C in absence of solvent for ~20 h (TLC). Excess of the phosphite was removed under vacuum. The residue was washed several times with light petroleum and crystallized from the appropriate solvent to give the corresponding phosphonate **6a**–**6c**.

*Dimethyl [(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl) methyl]phosphonate (**6a**)*
Yield 77%; m.p.: 115–117 °C (pentane; Refs. [13, 15] m.p.: 113–117 °C).

*Diethyl [(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)methyl] phosphonate (**6b**)*
Yield 78%; m.p.: 66–68 °C (pentane; Ref. [16–18] m.p.: 67 °C).

Diisopropyl [(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl) methyl]phosphonate (**6c**, $C_{15}H_{20}NO_5P$)
Yield 77%; m.p.: 58–60 °C (pentane); IR (KBr): $\overline{\nu}$ = 1,781, 1,724 (C(1,3)=O), 1268 (P=O), 1,048 (P–O–C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.13, 1.24 (2dd, J_{H-H} = 6.6, $^{4}J_{P-H}$ = 5.7 Hz, 12H, iso-Me_2COP), 3.28, 3.42 (2d, $^{2}J_{P-H}$ = 22.5 Hz, 2H, H_2C), 4.31 (septet, $^{3}J_{P-H}$ = 13.2 Hz, 2H, HCOP), 7.76, 7.88 (2d, J_{H-H} = 4.4 Hz, 4H, H–Ar) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 23.19 (d, $^{3}J_{P-C}$ = 7.6 Hz, Me_2COP), 34.76 (d, $^{1}J_{P-C}$ = 136.4 Hz, N–C–P), 71.2 (d, $^{2}J_{P-C}$ = 17.4 Hz, CHOP), 127.8, 132.5, 133.6 (C–Ar), 170.4, 170.6 (C(1,3)=O) ppm; ^{31}P NMR (202 MHz, $CDCl_3$): δ = 27.8 ppm; MS (EI, 70 eV): *m/z* (%) = 160 (100) [M^+–$OP(OC_3H_7)_2$], 166 (58) [$OP(OC_3H_7)_2$], 145 (33), 104 (72), 77 (8).

(B) Reaction of **1** with **2c** in the presence of glacial acetic acid. Synthesis of compound **7**
A mixture of 0.8 g **1** (0.5 mmol) and 4 cm^3 triisopropyl phosphite (**2c**, as a representative example) was heated at 100 °C in the presence of 0.5 cm^3 AcOH for ~15 h (TLC). After evaporation of the volatile materials in vacuo, the residue was triturated with 5 cm^3 hexane to give *N*-phenylphthalimide (**7**). Yield 90%; m.p.: 133–135 °C (cyclohexane; Ref. [19] m.p.: 133–135 °C).

(C) Pyrolysis of **6c**
In a cold finger sublimator 0.3 g **6c** was heated at 120 °C (bath temperature) under reduced pressure (5 torr) for 30 min. The material that sublimed was collected, recrystallized from cyclohexane, and proved to be *N*-methylphthalimide (**7**) by mixed melting points (100 mg, 63%). Diethyl hydrogenphosphonate was also detected in the receiver by the development of violet color on addition of 3,5-dinitrobenzoic acid in the presence of alkali [32].

*Reaction of **1** with dialkyl hydrogenphosphonates **11a**–**11c**. Synthesis of compounds **13a**–**13c***

A mixture of 0.8 g *N*-methoxyphthalimide (**1**, 0.5 mmol) and 4 cm^3 dialkyl hydrogenphosphonate (dimethyl **11a**, diethyl **11b**, or diisopropyl phosphonate **11c**) was heated at 100 °C in the presence of a catalytic amount of benzoyl-peroxide for ~15 h (TLC). Excess of the volatile materials was removed under vacuum, and the residue was washed several times with light petroleum and crystallized from the appropriate solvent to give the corresponding phosphonate **13a**–**13c**.

*Dimethyl (1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl) phosphonate (**13a**)*
Yield 77%; m.p.: 112–113 °C (pentane; Ref. [20] m.p.: 112 °C).

*Diethyl (1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)phosphonate (**13b**)*
Yield 68%; m.p.: 74–76 °C (cyclohexane; Ref. [20] m.p.: 73 °C).

Diisopropyl (1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)phosphonate (**13c**, $C_{14}H_{18}NO_5P$)
Yield 66%; m.p.: 132–134 °C (CH_2Cl_2); IR (KBr): $\bar{\nu}$ = 1,780, 1,722 (C(1,3)=O), 1262 (P=O), 1080 (P–O–C) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.12, 1.13 (2dd, J_{H-H} = 6.6, $^4J_{P-H}$ = 5.7 Hz, 12H, Me_2COP), 3.94 (sept, $^3J_{P-H}$ = 12.3 Hz, 2H, HCOP), 7.78, 7.88 (2d, J_{H-H} = 5.8 Hz, 4H, H–Ar) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 15.9 (d, $^3J_{P-C}$ = 8.4 Hz, CH_3COP), 71.4 (d, $^2J_{P-C}$ = 17 Hz, CHOP), 126.7, 135.2, 133.8, 135.8 (C–Ar), 175.7, 169.4 (C(1,3)=O) ppm; ^{31}P NMR (202 MHz, $CDCl_3$): δ = 8.6 ppm; MS (EI, 70 eV): *m/z* (%) = 145 (100) [M^+–$OP(OC_3H_7)_2$], 166 (68) [$OP(OC_3H_7)_2$], 104 (72).

*Reaction of **1** with tris(dialkylamino)phosphines **9a** and **9b**. Synthesis of compounds **14a** and **14b***

Phosphine **9a** (R^1 = Me) or **9b** (R^1 = Et) in 4 cm^3 dry THF was added dropwise to 0.8 g **1** (0.5 mmol) in 15 cm^3 of the same solvent, and the reaction mixture was stirred at room temperature for ~10 h (TLC). Working up the product mixture in the usual manner afforded **14a** or **14b**.

P-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)methyl]-N,N,N′,N′-tetramethylphosphonic diamide
(**14a**, $C_{13}H_{18}N_3O_3P$)
Yield 80%; m.p.: 82–84 °C (pentane); IR (KBr): $\bar{\nu}$ = 1,772, 1,720 (C(1,3)=O), 1256 (P=O), 860 [($N(Me_2)$] cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 2.77, 2.84 (2d, $^3J_{P-H}$ = 10.8 Hz, 12H, (Me_2N–P), 3.28, 3.42 (2d, $^2J_{P-H}$ = 22.5 Hz, 2H, H_2C), 7.77, 7.92 (2d, J_{HH} = 5.8 Hz, 4H, H–Ar) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 35.5, 35.9 (2d, $^2J_{P-C}$ = 14.2 Hz, Me_2N–P), 36.76 (d, $^1J_{P-C}$ = 141.2 Hz, N–C–P), 126.6, 132.5 (C- Ar), 175.8 (C(1,3)=O) ppm; ^{31}P NMR (202 MHz, $CDCl_3$): δ = 26.4 ppm; MS (EI, 70 eV): *m/z* (%) = 160 (100) [M^+–$OP(NMe_2)_2$], 135 (48) [$OP(NMe_2)_2$], 145 (38), 104 (66).

*P-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)ethyl]-N,N,N′,N′-tetraethylphosphonic diamide (**14b**)*
Yield 82%; m.p.: 102–104 °C (cyclohexane; Ref. [22] m.p.: 101–102 °C).

*Reaction of 2-anilino-1H-isoindole-1,3(2H)-dione (**15**) with **2a–2c**. Synthesis of compounds **17a–17c***

A mixture of 0.5 g **15** (2.1 mmol) and 4 cm^3 **2a–2c** was heated at 100 °C in absence of solvent for ~20 h (TLC). Working up the product mixture in the usual manner afforded **17a–17c**. *N*-alkylated hydrazines **18a** and **18b** were also isolated from the reactions of **2a** and **2b** by fractional crystallization.

Dimethyl N-(1-hydroxy-3-methoxy-2H-isoindol-2-yl)-N-phenylphosphoramidoate (**17a**, $C_{17}H_{19}N_2O_5P$)
Yield 72%; m.p.: 126–128 °C (benzene); IR (KBr): $\bar{\nu}$ = 3,340 (OH), 1,778, 1,726 (C(1,3)=O), 1,256 (P=O), 1,050 (P–O–C) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 3.31 (s, 3H, MeO), 3.55 (d, $^3J_{P-H}$ = 11.8 Hz, 6H, MeOP), 5.85 (br, 1H, HO, exchanges with D_2O), 7.25–8.02 (m, 9H, H–Ar, H–Ph) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 53.6 (d, $^3J_{P-C}$ = 7.7 Hz, MeOP), 58.3 (COMe), 98.3, 103.4, 103.5, 103.8, 119.7, 120.6, 121.3, 125.6, 133.2, 138.7 (C–Ar, C–Ph), 140.7 (d, $^3J_{P-C}$ = 6.4 Hz, COMe), 152.6 (d, $^3J_{P-C}$ = 8.4 Hz, COH) ppm; ^{31}P NMR (202 MHz, $CDCl_3$): δ = 14.2 ppm; MS (EI, 70 eV): *m/z* (%) = 361 (<5) [M^+-1], 346 (9), 236 (100), 221 (52), 144 (62), 110 (52), 104 (28), 77 (54).

Diethyl N-(3-ethoxy-1-hydroxy-2H-isoindol-2-yl)-N-phenylphosphoramidoate (**17b**, $C_{20}H_{25}N_2O_5P$)
Yield 76%; m.p.: 116–118 °C (CH_2Cl_2); IR (KBr): $\bar{\nu}$ = 3,345 (OH), 1,782, 1720 (C(1,3)=O), 1264 (P=O), 1082 (P–O–C) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.21 (t, J_{H-H} = 6.8 Hz, 3H, MeCO), 1.36 (dt, J_{H-H} = 6.4, $^4J_{P-H}$ = 4.8 Hz, 6H, MeCOP), 3.45 (q, J_{H-H} = 6.8 Hz, 2H, H_2CO), 3.82 (dq, $^3J_{P-H}$ = 12.5 Hz, 4H, H_2COP), 6.35 (s, 1H, OH, exchanges with D_2O), 7.25–8.02 (m, 9H, H–Ph, H–Ar) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.3 (MeCO), 16.5 (MeCOP), 62.7 (d, $^3J_{P-C}$ = 6 Hz, CH_2OP), 64.2 (CH_2OC), 97.8, 102.3, 103.5, 103.8, 114.9, 120.6, 123.1, 125.4, 133.2, 140.3 (C–Ar, C–Ph), 141.7 (d, $^3J_{P-C}$ = 6 Hz, $COCH_2$), 140.8 (d, $^2J_{P-C}$ = 8 Hz, C–N), 152.6 (d, $^3J_{P-C}$ = 5.5.4 Hz, COH) ppm; ^{31}P NMR (202 MHz, $CDCl_3$): δ = 14.8 ppm; MS (EI, 70 eV): *m/z* (%) = 403 (11) [M^+-1], 374 (17), 358 (28), 236 (100), 221 (35), 144 (62), 110 (52), 104 (28), 77 (54).

Diisopropyl N-(1-hydroxy-3-isopropoxy-2H-isoindol-2-yl)-N-phenylphosphoramidoate (**17c**, $C_{23}H_{31}N_2O_5P$)
Yield 74%; m.p.: 121–122 °C (cyclohexane); IR (KBr): $\bar{\nu}$ = 3,355 (OH), 1,774, 1,724 (C(1,3)=O), 1,262 (P=O), 1088 (P–O–C) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.12–1.36 (m, 18H, iso-Me_2CO), 3.41 (sept, J_{H-H} = 6.4 Hz, 1H, HCO), 3.82 (dsept, 2H, HCOP), 6.35 (br, 1H, OH, exchanges with D_2O), 7.25–8.02 (m, 9H,

H–Ph, H–Ar) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.3 (MeCOP), 22.7 (MeCO), 72.7 (d, $^2J_{P–C}$ = 22.6 Hz, CHOP), 74.2 (OCHC), 92.8, 97.5, 102.5, 103.5, 114.6, 120.6, 121.3, 125.6, 133.2, 140.8 (C–Ar, C–Ph), 137.6 (d, $^3J_{P–C}$ = 8.6 Hz, $COCH_2$), 152.4 (d, $^3J_{P–C}$ = 5.5.4 Hz, COH) ppm; ^{31}P NMR (202 MHz, $CDCl_3$): δ = 15.3 ppm; MS (EI, 70 eV): *m/z* (%) = 445 (11) [M^+-1], 402 (17), 386 (29), 237 (100), 221 (35), 144 (62), 110 (52), 104 (28), 77 (54).

2-(N-methyl-N-phenylamino)-1H-isoindole-1,3(2H)-dione ***(18a)***
Yellow prisms; yield 14%; m.p.: 124–125 °C (hexane; Ref. [23] m.p.: 124 °C).

2-(N-ethyl-N-phenylamino)-1H-isoindole-1,3(2H)-dione ***(18b)***
Yellow prisms; yield 16%; m.p.: 134–136 °C (hexane; Ref. [24] m.p.: 134 °C).

Reaction of ***14*** *with* ***11a–11c****. Synthesis of compounds* ***20a–20c***

A mixture of 0.5 g **15** (2.1 mmol) and 4 cm^3 **11a–11c** was heated at 100 °C in the presence of a catalytic amount of benzoylperoxide for ~20 h (TLC). Working up the product mixture in the usual manner afforded the phosphoramidoates **20a–20c**. *N*-alkylated hydrazides **18a** (10% yield) and **18b** (11% yield) were also isolated from the reactions of **11a** and **11b** by fractional crystallization.

Dimethyl N-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-N-phenylphosphoramidoate (**20a**, $C_{16}H_{15}N_2O_5P$)
Yield 68%; m.p.: 84–86 °C (pentane); IR (KBr): $\overline{\nu}$ = 1,780, 1,723 (C(1,3)=O), 1,266 (P=O), 1,058 (P–O-C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.57 (d, $J_{P–H}$ = 14.3 Hz, 6H, 2OMe), 7.19–7.98 (m, 9H, H–Ph, H–Ar) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 53.8 (d, $^2J_{P–C}$ = 11.7 Hz, MeOP), 118.6, 126.2, 127.3, 127.6, 127.8, 131.2, 132.7, 140.5 (C–Ar, C–Ph), 167.8, 172.3 (2d, $^3J_{P–C}$ = 6.4 Hz, C=O) ppm; ^{31}P NMR (202 MHz, $CDCl_3$): δ = 11.3 ppm; MS (EI, 70 eV): *m/z* (%) = 345 (15) [M^+-1], 236 (100), 144 (72), 110 (60), 104 (28), 77 (58).

Diethyl N-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-N-phenylphosphoramidoate (**20b**, $C_{18}H_{19}N_2O_5P$)
Yield 70%; m.p.: 90–92 °C (pentane); IR (KBr): $\overline{\nu}$ = 1,780, 1,724 (C(1,3)=O), 1,260 (P=O), 1,048 (P–O–C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.14, 1.25 (2dt, $J_{H–H}$ = 6.6, $^4J_{P–H}$ = 4.8 Hz, 6H, Me_2COP), 3.72–3.82 (2dq, 4H, H_2COP), 6.85–8.13 (m, 9H, H–Ph, H–Ar) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.4 (MeCOP), 62.7 (d, $^2J_{P–C}$ = 12.6 Hz, CH_2OP), 119.3, 120.3, 126.8, 127.4, 127.6, 131.6, 132.5, 133.2, 138.9 (C–Ar, C–Ph), 169.2, 170.3 (2d, $^3J_{P–C}$ = 9.4 Hz, C=O) ppm; ^{31}P NMR (202 MHz, $CDCl_3$): δ = 10.4 ppm; MS (EI, 70 eV): *m/z* (%) = 373 (9) [M^+-1], 358 (28), 236 (100), 221 (13), 144 (52), 110 (44), 104 (18), 77 (54).

Diisopropyl N-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-N-phenylphosphoramidoate (**20c**, $C_{20}H_{23}N_2O_5P$)
Yield 74%; m.p.: 96–98 °C (pentane); IR (KBr): $\overline{\nu}$ = 1,776, 1,726 (C(1,3)=O), 1,260 (P=O), 1,100 (P–O-C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.12, 1.36 (2dd, $J_{H–H}$ = 6.6, $^4J_{P–H}$ = 4.7 Hz, 12H, Me_2COP), 3.72–3.82 (sept, $J_{P–H}$ = 10.4 Hz, 2H, HCOP), 6.82–8.22 (m, 9H, H–Ph, H–Ar) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (Me_2COP), 72.2 (d, $^2J_{P–C}$ = 6 Hz, CHOP), 119.2, 125.9, 126.3, 127.3, 127.6, 131.6, 132.1, 141.6 (C–Ar, C–Ph), 167.8, 169.8 (2d, $^3J_{P–C}$ = 6.4 Hz, C=O) ppm; ^{31}P NMR (202 MHz, $CDCl_3$): δ = 10.8 ppm; MS (EI, 70 eV): *m/z* (%) = 401 (12) [M^+-1], 236 (100), 144 (72), 110 (60), 104 (28), 77 (58).

Reaction of ***15*** *with* ***9a*** *and* ***9b****. Synthesis of compounds* ***22a*** *and* ***22b***

Phosphine **9a** (R^1 = Me) or **9b** (R^1 = Et) in 4 cm^3 dry THF was added dropwise to 0.5 g **15** (2.1 mmol) in 15 cm^3 of the same solvent, and the reaction mixture was stirred at room temperature for ~6 h (TLC). Working up the product mixture in the usual manner afforded **22a** and **22b**.

2-(Dimethylamino)-2,3-dihydro-3-phenyl[1–4]oxadiazaphospholo[5,4-a]isoindol-5-ol 2-oxide
(**22a**, $C_{16}H_{16}N_3O_3P$)
Yield 69%; m.p.: 102–104 °C (MeCN); IR (KBr): $\overline{\nu}$ = 3,425 (OH), 1,258 (P=O), 1,320, 838 (PNMe) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.47, 2.49 (2d, $^3J_{P–H}$ = 10.8 Hz, 6H, Me_2N–P), 6.91–8.12 (m, 9H, H–Ph, H–Ar), 9.49 (s, 1H, OH, exchanges with D_2O) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 35.5 (d, $^3J_{P–C}$ = 6 Hz, Me_2NP), 96.7, 100.9, 103.9, 122.5, 123.4, 126.3, 131.6, 132.3, 133.6, 141.4 (C–Ar, C–Ph), 147.7 (d, $^2J_{P–C}$ = 21 Hz, CO–P), 150.7 (d, $^3J_{P–C}$ = 4.8 Hz, COH) ppm; ^{31}P NMR (202 MHz, $CDCl_3$): δ = 14.6 ppm; MS (EI, 70 eV): *m/z* (%) = 329 (14) [M^+-1], 237 (100), 221 (71), 146 (53), 104 (29), 77 (11).

2-(Diethylamino)-2,3-dihydro-3-phenyl[1–4]oxadiazaphospholo[5,4-a]isoindol-5-ol 2-oxide
(**22b**, $C_{18}H_{20}N_3O_3P$)
Yield 75%; m.p.: 114–116 °C (MeCN); IR (KBr): $\overline{\nu}$ = 3,430 (OH), 1,260 (P=O), 1,320, 838 (NMe_2) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.77, 2.84 (2d, $^3J_{P–H}$ = 10.8 Hz, 12H, $(H_3C)_2N$–P), 3.28, 3.42 (2d, $^2J_{P–H}$ = 22.5 Hz, 2H, H_2C), 7.77, 7.92 (2d, $J_{H–H}$ = 5.8 Hz, 4H, H–Ar) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 35.5,

35.9 (2d, $^{2}J_{P-C} = 14.2$ Hz, CH_3N–P), 36.76 (d, $^{1}J_{P-C} = 141.2$ Hz, N–C–P), 126.6, 132.5 (C–Ar), 175.8 (C(1,3)=O) ppm; ^{31}P NMR (202 MHz, $CDCl_3$): $\delta = 13.6$ ppm; MS (EI, 70 eV): m/z (%) = 356 (13) [M^+-1], 237 (100), 221 (44), 146 (71), 104 (25), 77 (36).

Antibacterial assay

The prepared compounds were screened for antibacterial activity against three bacterial strains by the disc diffusion method. A standard inoculum (1–2 × 10^7 c.f.u. cm^3 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 8.32 cm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37 °C. The inhibition zones were measured and compared with the controls. Minimum inhibitory concentration (MIC) was determined by broth dilution technique. The nutrient broth, which contained logarithmic serially twofold-diluted amounts of test compound and controls, were incubated with approximately 5 × 10^5 c.f.u. cm^3 of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as MIC. Amoxicillin (**A**) was used as a standard drug; all data are presented in Table 1.

Antifungal assay

The prepared compounds were screened for their antifungal activity against three fungal strains by the agar diffusion method. Sabouraud agar media was prepared by dissolving 1 g peptone, 4 g D-glucose, and 2 g agar in 100 cm^3 distilled water, and adjusting pH to 5.7 using buffer. Normal saline was used to make a suspension of spores of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 cm^3 saline to get a suspension of corresponding species. Into each Petri dish was poured 20 cm^3 of agar media. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C. Using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. Inhibition zone diameter was measured and compared with controls. The nutrient broth, which contained logarithmic serially twofold-diluted amount of the tested compound and control, were inoculated with approximately 1.6 × 10^4 to 6 × 10^4 c.f.u. cm^3. The cultures were incubated for 48 h at 35 °C, and growth was monitored visually. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as MIC. Fluconazole (**F**) was used as a standard drug; all data are presented in Table 1.

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