Synthesis of Antimicrobial N-Phthaloyl-alanyl-derived Amidophosphates and Triazoles

Wafaa M. Abdou, Neven A. Ganoub, and Eman Sabry

Chemical Industries Division, National Research Centre, Elbohouth St. D-12622, Dokki, Cairo, Egypt

Reprint requests to Professor Wafaa M. Abdou. E-mail: wabdou@intouch.com

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Dedicated to Professor Richard Neidlein on the occasion of his 77th birthday

N-Phthaloyl-alanylazide reacts smoothly with trialkyl phosphites producing the corresponding α-aminophosphates. With dialkyl hydrogenphosphonates in the presence of benzoyl peroxide, amidophosphates were the isolated products whereas the oxoaziridin-1-yl-phosphonic diamide was preferentially provided from the reaction of the azide with tris(dimethylamino)phosphine. The azide was also allowed to react with α-keto-, α-ethoxycarbonyl- and α-cyanomethylenetriphenylphosphorane to give the corresponding linear disubstituted 1,2,3-triazoles. Screening results of antibiotic potency for the products were discussed in terms of structure-activity relationship (SAR), and an attempt was made to define the structural features for lead compounds.

Key words: α-Aminophosphates, Amidophosphates, Disubstituted 1,2,3-Triazoles, N-Phthaloyl-alanylazide

Introduction

Phthalimides constitute a class of compounds which has attracted considerable attention in heterocyclic chemistry [1, 2]. In effect, phthalimide derivatives have demonstrated significant and potential biological activities in agricultural [3, 4] and in medicinal chemistry [5, 6]. With the aim to develop expanded applications of phthalimides, very recently [7] we elaborated an efficient one-pot procedure for the synthesis of α- and β-phosphono-substituted phthalimides with antibiotic activity. The method was based on the reaction of phosphorus(III) reagents with 2-methoxy- and 2-anilino-isoindole-1,3 (2H)-dione. In our continuing development of phosphono-substituted phthalimides for application purposes, we now apply trivalent and pentavalent phosphorus reagents to 2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)propionylazide (1, also known as N-phenylalanylalanylazide). Trialkyl phosphites 2a–c, dialkyl hydrogenphosphonates 12a–c and tris(dimethylamino)phosphine (16) were the applied phosphorus(III) reagents whereas triphenylalkylidenephosphoranes 18 and 20a, b were the phosphorus(V) reagents. The reactions studied and the products obtained are depicted in Schemes 1 – 6.

Results and Discussion

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

In the presence of an excess of trimethyl phosphite (2a), the reaction with azide 1 led to the formation of the substituted imidoylphosphate 6a in 72% yield. Obviously, the mechanism of condensation of the azide 1 with 2a involved the formation of the Staudinger phosphoranimine intermediate 4a that arose from the denitrogenation of the initially formed triazenyldiene phosphorane 3a [8, 9]. Subsequent ring closure followed by ring opening [10], and rearrangement (tautomeric conversion) [8] through alkyl group shift led to the phosphate product 6a (Scheme 1). The composition and the structure of 6a–c are based on the recorded elemental analyses, molecular weight determinations (MS), and spectroscopic data [11, 12]. Compound 6a showed a 31P NMR chemical shift around δ = 3.8 ppm, which indicates the presence of a P–O linkage in the molecule, and readily eliminates a structure like 7 for which a signal at δ = 11 – 16 ppm for P=N (or P–N) would be expected. The IR spectrum of 6a revealed the absence of the stretching vibration bands at 1708 and 2200 cm⁻¹ related to the carbonyl
and the azido group, respectively, present in the IR spectrum of the azide 1, and showed strong absorption bands at 1580 assigned to the imide (C=\(\text{N}\)) stretching, and at 1778 and 1728 cm\(^{-1}\) assigned to a coupled C=O vibration of cyclic imides. In the \(^1\)H NMR spectrum (CDCl\(_3\)) of 6a the exocyclic methine proton, present in the spectrum of 1 at \(\delta=4.78\) ppm (q), was displayed at \(\delta=5.17\) (dq) ppm. The deshielding is clearly due to the phosphorus entity. Although the physical and spectroscopic data indicated that 6 existed in the imino form, the stable amino structure 6A clearly could not be overlooked. Treatment of 6a with methyl iodide in acetone, containing potassium carbonate, gave the expected \(N,\text{N}\)-dialkylated product 8 in 80% yield.

In a similar way, the reaction product of 1 with triethyl phosphite (2b) was assigned the analogous structure 6b (74% yield). Conversely, treatment of 1 with triisopropyl phosphite (2c) afforded a mixture of the expected analog 6c together with the vinyl phosphate 11 (Scheme 2). This behavior is not unexpected since the bulky isopropyl group would impede the Arbusov reaction. It appears that a partial hydrolysis at the stage of the dipolar intermediate 9 has occurred to give the final product 6c along with 11 via the intermediate 10. It is known that lengthening or branching the alkyl rad-
Reactions of 1 with dialkyl hydrogenphosphonates 12a – c

The behavior of 1 toward 12a – c was studied next, and the products obtained were those depicted in Scheme 3. However, the reaction between 1 and 12 proceeded only when a catalytic amount of benzoyl peroxide (13) was present in the medium [15, 16] to yield the respective amido-phosphates 14a – c along with the known [14] phthaloyl-DL-α-alanylamide (15) (Scheme 3). The reaction products 14a – c were obtained as colorless crystals in ∼ 62 % yield. Satisfactory elemental analyses and molecular weight determinations (MS) confirmed structure 14. 31P NMR signals of 14a – c were found at δ = ∼ 14 ppm [17]. The 1H NMR spectrum (CDCl 3 ) of 14b showed among others the NH proton at δ = 6.32 ppm.

Reaction of 1 with Tris(dimethylamino)phosphine (16)

Addition of an excess of tris(dimethylamino)phosphine (16) in dry tetrahydrofuran to the carbonyl azide 1 in dry THF at 20 °C led to the precipitation of the triazenyldiene-phosphorane 17 (88 % yield). A 31P NMR signal was observed at δ = 40.3 ppm. Correct elemental analysis and expected 1H, 13C NMR data also confirmed the assigned structure (Scheme 4).

Reactions of N-phthaloyl-alanylazide (1) with alkylidenephosphoranes

The present study was extended to investigate the application of P(V) reagents namely, alkylidenephosphoranes, to the azide 1, in order to prepare as yet unknown 1-(N-phthaloyl-alanyl)-1,2,3-triazoles. Thus, the reaction of 1 with benzoylmethylenetriphenylphosphorane (18) was carried out in boiling chloroform for 36 h to furnish the 1,5-disubstituted 1,2,3-triazole 19 in moderate 42 % yield via 1,3-cycloaddition [18 – 20]. Triphenylphosphine oxide was also isolated from the reaction medium. However, when the reaction was carried out in dimethylformamide (DMF) solution, and the mixture was heated in a microwave oven for 20 min, product 19 was obtained in 90 % yield (Scheme 5). The triazole structure 19 was deduced from analytical and spectroscopic data.

The reaction of α-ethoxycarbonyl- (20a) and α-cyanomethylenetriphenylphosphorane (20b), however, did not proceed in this way, but gave the triazoles 22a or 22b in quantitative yields (Scheme 6). Conceivably an addition of the ylides 20a, 20b to the terminal nitrogen of the azide takes place (Staudinger reaction), followed by an aza-Wittig reaction to give the
Table 1. Antibacterial activity data of 6a–c, 14a, b, 19 and 22a, b.

<table>
<thead>
<tr>
<th>Compound</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>K. pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>22* (5.85)</td>
<td>27 (5.85)</td>
<td>20 (5.85)</td>
<td>23 (5.85)</td>
</tr>
<tr>
<td>6b</td>
<td>18 (5.85)</td>
<td>27 (5.85)</td>
<td>23 (5.85)</td>
<td>21 (5.85)</td>
</tr>
<tr>
<td>6c</td>
<td>–</td>
<td>15 (12.5)</td>
<td>20 (12.5)</td>
<td>–</td>
</tr>
<tr>
<td>14a</td>
<td>21 (5.85)</td>
<td>25 (5.85)</td>
<td>20 (5.85)</td>
<td>25 (5.85)</td>
</tr>
<tr>
<td>14b</td>
<td>22 (5.85)</td>
<td>26 (5.85)</td>
<td>24 (5.85)</td>
<td>22 (5.85)</td>
</tr>
<tr>
<td>22a</td>
<td>12 (12.5)</td>
<td>15 (12.5)</td>
<td>12 (25)</td>
<td>19 (8.5)</td>
</tr>
<tr>
<td>22b</td>
<td>14 (12.5)</td>
<td>14 (12.5)</td>
<td>12 (12.5)</td>
<td>13 (12)</td>
</tr>
<tr>
<td>Standardd</td>
<td>22 (5.85)</td>
<td>28 (5.85)</td>
<td>24 (5.85)</td>
<td>25 (5.85)</td>
</tr>
</tbody>
</table>

* Indicates the diameter of zone of inhibition; b minimum inhibitory concentration values (MIC, µg mL⁻¹); c Amoxicillin is used as standard.

Table 2. Antifungal activity data of compounds 6a–c, 14a, b, 19 and 22a, b.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>A. niger</th>
<th>C. albicans</th>
<th>A. fumigatus</th>
<th>P. marneffei</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>25* (3.85)</td>
<td>23 (5.85)</td>
<td>22 (5.85)</td>
<td>22 (5.85)</td>
</tr>
<tr>
<td>6b</td>
<td>22 (8.4)</td>
<td>21 (6.25)</td>
<td>25 (6.25)</td>
<td>25</td>
</tr>
<tr>
<td>6c</td>
<td>20 (5.85)</td>
<td>21 (6.25)</td>
<td>23 (8.8)</td>
<td>19 (8.8)</td>
</tr>
<tr>
<td>14a</td>
<td>27 (5.85)</td>
<td>23 (5.85)</td>
<td>25 (5.85)</td>
<td>22 (5.85)</td>
</tr>
<tr>
<td>14b</td>
<td>28 (5.85)</td>
<td>25 (5.85)</td>
<td>26 (5.85)</td>
<td>28 (5.85)</td>
</tr>
<tr>
<td>19</td>
<td>18 (12.5)</td>
<td>12 (25)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>22a</td>
<td>9 (22)</td>
<td>14 (13)</td>
<td>–</td>
<td>–</td>
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<tr>
<td>22b</td>
<td>10 (22)</td>
<td>18 (8.8)</td>
<td>20 (5.85)</td>
<td>10 (12.5)</td>
</tr>
<tr>
<td>Standardd</td>
<td>25 (5.85)</td>
<td>21 (5.85)</td>
<td>23 (5.85)</td>
<td>22 (5.85)</td>
</tr>
</tbody>
</table>

* Indicates the diameter of zone of inhibition; c minimum inhibitory concentration values (MIC, µg mL⁻¹); c Fluconazole is used as standard.

4,5-disubstituted triazoles 22a, b via the intermediates 21a, b, and extrusion of TPPO.

Formulas 22a and 22b are consistent with analytical and spectroscopic data. It is noteworthy that the position of the N-H hydrogen atom in the N-unsubstituted triazoles such as structure 22 has been the subject of a contradictory discussion [21] (Scheme 6).

Pharmacological screening

Antibacterial activity

A selection of the newly prepared compounds were screened for their antibacterial activity against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae (recultured) bacterial strains by the disc diffusion method [22, 23]. The antibacterial screening data shown in Table 1 indicate that compounds 6a, 6b, 14a, and 14b exhibit good antibacterial activity against all tested bacterial strains almost equivalent to that of the standard drug Amoxicillin.

Antifungal activity

Selected compounds were screened for their antifungal activity against Aspergillus niger, Candida albicans, Aspergillus fumigatus, and Pencillium marneffei (recultured) by the agar diffusion method [23]. The results revealed that compounds 6a, 14a and 14b show significant antifungal activity. Other tested compounds, however, showed moderate activity as compared to that of the standard drug Fluconazole.

Summary and Conclusion

To summarize, we have reported an efficient access to a series of phthalimidoyl-phosphorus derivatives and linear disubstituted vic-triazoles that may complement those existing in the literature. This sequence included a number of α-amino-phosphate, amidophosphate and triazenylphosphine derivatives from the reactions of the carboxyl azide 1 with different types of P(III) reagents. In the second part, disubstituted triazole derivatives were obtained from the reactions of 1 with phosporus ylides. The latter reactions fit into the general class of concerted 1,3-dipolar reactions of azides to dipolarophiles [18–20]. Furthermore, the antimicrobial screening studies revealed that compounds with a phosphorus moiety (phosphate, phosphazane, or phosphoryl diamide) showed excellent activity. Finally, the results of the present investigation reflect the versatility of the phosphorus reagents and of the azido group as well.

Experimental Section

General remarks

Melting points were measured on an Electrothermal melting point apparatus. The IR spectra were recorded on a Perkin Elmer 317 Grating IR spectrophotometer, using KBr pellets. The 1H and 13C NMR spectra were measured on a Jeol E.C.A. 500 MHz instrument using SiMe₄ as internal standard. The 31P NMR spectra were recorded with the same instrument, relative to external H₃PO₄ (85 %). The mass spectra were performed on a Jeol JMS-A X 500 spectrometer. Elemental analyses were carried out at the Microanalytical Laboratory, Cairo University, Cairo, Egypt. All reactions were performed under argon. 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 (silica gel 60 mesh, particle size 0.2–0.5 mm; E. Merck, Darmstadt). The substrate 2-(1,3-dioxo-1,3-dihydro-2H-isoinol-2-yl)propionyl-azide (1) was prepared according to the reported method [24].
General procedure for the reaction of 1 with trialkyl phosphites 2a–c

A mixture of N-phthaloyl-alanylazide (1) (0.7 g, 2.87 mmol) and a trialkyl phosphate 2a–c (4 mL) was heated at 100 °C, in absence of a solvent for ∼15 h (TLC). The excess of the phosphate was removed under vacuum, and then the residue was washed several times with light petroleum (40–60 °C), and crystallized from the proper solvent to give compounds 6a, 6b or 6c and 11, respectively.

(1Z)-2-(1,3-Dioxo-1,3-dihydro-2H-isooindol-2-yl)-N-methyl-propanamidinio dimethyl phosphate (6a)

Straw-yellow prisms. Yield: 700 mg (72%). M. p. 153–155 °C (from acetone/ether). – IR (film): v = 1778, 1728 (C(1,3)=O), 1580 (C=O), 1256 (P=O), 1044 (P-O-C) cm⁻¹. – ¹H NMR (500 MHz, CDCl₃, 25 °C, TMS): δ = 1.55 (d, J = 6.8 Hz, 3 H, C-Me), 3.18 (s, 3 H, N-Me), 3.79 (d, J = 6.6 Hz, 3 H, H₃C-CN), 1.22 (dt, J = 10.5 Hz, 4 H, H-Ar). – ¹³C NMR (500 MHz, CDCl₃, TMS): δ = 14.6 (Me-C), 41.6 (d, J = 10.5 Hz, CHMe), 42.4 (NMe), 72.7 (d, J = 12.9 Hz, 2 H, MeOP), 24.3 (NMe₂), 54.3 (d, J = 14.8 Hz, MeOP), 124.3, 134.6, 135.3 (all C-Ph), 165.5 (C(1,3)=O), 174.6 (d, J = 18.4 Hz, C=O), – ³¹P NMR (500 MHz, CDCl₃, H₂PO₄): δ = 3.8 ppm. – MS (EI, 70 eV): m/z (%) = 340 (22) [M⁺], 325 (9), 310 (29), 280 (100), 185 (30), 171 (83), 146 (80), 110 (54), 104 (41), 77 (56). – C₁₇H₂₃N₂O₆P (382.4): calcd. C 53.4, H 6.0, N 7.3, P 8.1; found C 53.3, H 6.1, N 7.2, P 8.2.

(1Z)-2-(1,3-Dioxo-1,3-dihydro-2H-isooindol-2-yl)-N-isopropyl-propanamidinio diisopropyl phosphate (6b)

Straw-yellow needles. Yield: 810 mg, 74%. M. p. 135–137 °C (from CH₂Cl₂). – IR (film): v = 1782, 1720 (C(1,3)=O), 1587 (C=O), 1265 (P=O), 1000 (P-O-C) cm⁻¹. – ¹H NMR (500 MHz, CDCl₃, 25 °C, TMS): δ = 0.88 (t, J = 6.6 Hz, 3 H, H₃C-C(3)), 1.22 (dt, J = 6.6 Hz, 4 J = 4.8 Hz, 6 H, H₃C-C(3)), 1.57 (d, J = 6.8 Hz, 3 H, H₃C-CN), 3.65 (q, J = 6.6 Hz, H₃C-CN), 4.08 (dq, J = 6.6 Hz, J' = 5.2 Hz, 4 H, H₂COP), 5.17 (dq, J = 6.8 Hz, J' = 4.2 Hz, 1 H, H-CMe), 7.68, 7.78 (2 × d, J = 7.4 Hz, 4 H, H-Ar). – ¹³C NMR (500 MHz, CDCl₃, TMS): δ = 15.5 (CH₃CN), 15.8 (CH₃CH), 16.3 (CH₂COP), 38.4 (CH₂N), 42.6 (d, J = 8.4 Hz, CH-Me), 64.7 (d, J = 18.6 Hz, CH₂OP), 124.2, 133.4, 135.7 (all C-Ar), 166.5 (C(1,3)=O), 173.4 (d, J = 18.5 Hz, C=O). – ³¹P NMR (500 MHz, CDCl₃, H₂PO₄): δ = 7.2 ppm. – MS (EI, 70 eV): m/z (%) = 381 (17) [M–1]+, 338 (18), 309 (31), 280 (100), 185 (16), 174 (96), 146 (90), 110 (52), 104 (28), 77 (54). – C₁₇H₂₃N₂O₆P (382.4): calcd. C 53.4, H 6.0, N 7.3, P 8.1; found C 53.3, H 6.1, N 7.2, P 8.2.

Methylation of the phosphate 6a

A mixture of the phosphate 6a (0.5 g, 1.47 mmol), methyl iodide (3.0 mL), and anhyd. K₂CO₃ (2 g) in dry acetone (50 mL) was refluxed for 2 h and then filtered while hot. After evaporation of the volatile materials in vacuum, the residue was triturated with light petroleum ether, and then allowed to cool in an ice-chest. The solid obtained was recrystallized from aqueous ethanol to give 8.
(1E)-1-(Dimethylamino)-2-(1,3-dioxo-1,3-dihydro-2H-isindol-2-yl)prop-1-en-1-yl dimethyl phosphate (8)

Colorless needles. Yield: 416 mg (80 %). M. p. 118–120 °C. – IR (film): v = 1778, 1728 (C(1,3)=O), 1622 (C=C, exocyclic), 1268 (P=O), 1050 (P-O-C) cm⁻¹. – ¹H NMR (500 MHz, CDCl₃, 25 °C, TMS): δ = 1.57 (s, 3 H, H₃CC), 2.98, 3.01 (2 × s, 2 × 3 H, (H₂C₂N)), 3.85 (d, 3J = 12.5 Hz, 6 H, H₂COP), 7.77, 7.90 (2 × d, J = 4.4 Hz, 4 H, H- Ar). – ¹³C NMR (500 MHz, CDCl₃, TMS): δ = 12.3 (CH₃C), 41.6, 41.8 (N(CH₃)₂), 54.3 (d, 2J = 8.3 Hz, CH₂OP), 93.8 (d, 3J = 10.3 Hz, CH=C), 124.3, 131.6, 133.3 (all C- Ar), 162.5 (C(1,3)=O), 166.2 (d, 2J = 28.3 Hz, =COP). – ³¹P NMR (500 MHz, CDCl₃, TMS): δ = 4.8 ppm. – MS (EI, 70 eV): m/z (%) = 354 (15) [M⁺], 325 (21), 310 (23), 295 (46), 280 (100), 185 (29), 171 (81), 146 (82), 116 (54), 104 (40), 77 (52), – C₁₅H₁₄N₂O₆P (354.3); calcld. C 50.8, H 5.4, N 7.9, P 8.7; found C 50.7, H 5.5, N 7.8, P 8.8.

General procedure for the reaction of 1 with dialkyl hydrogengaphosphonates 12a–c

A mixture of 1 (0.8 g, 3.28 mmol) and 6 mmol of dimethyl (12a), diethyl (12b) or diisopropyl phosphonate (12c) was refluxed in benzene (20 mL) containing a catalytic amount of benzoyl peroxide (13) for ≈ 4 h (TLC). After cooling, the colorless material that precipitated was collected, crystallized, and identified as phthaloyl-DMα-alanilamidine (15, 12 %). M. p. 214–216 °C (from ethanol) (lit. [14]: 214–215.5 °C). The filtrate was evaporated under reduced pressure, and then the residue was crystallized from the proper solvent to give the corresponding amidophosphates 14a, 14b or 14c.

Diethyl [2-(1,3-dioxo-1,3-dihydro-2H-isindol-2-yl)propanoyl]amidophosphate (14a)

Colorless crystals. Yield: 620 mg (58 %). M. p. 164–167 °C (from acetone). – IR (film): v = 3320 (NH), 1777, 1732 (C(1,3)=O), 1708 (C=O, amide), 1232 (P=O, bonded), 1048 (POC), 978 (P-O-C) cm⁻¹. – ¹H NMR (500 MHz, CDCl₃, 25 °C, TMS): δ = 1.55 (d, J = 6.8 Hz, 3 H, H₃CC), 3.79 (d, 3J = 12.4 Hz, 6 H, H₂COP), 5.17 (q, J = 6.8 Hz, 1 H, HCMε), 6.28 (br, 1 H, HN), 7.76, 7.88 (2 × d, J = 4.4 Hz, 4 H, H- Ar). – ¹³C NMR (500 MHz, CDCl₃, TMS): δ = 17.6 (CH₃C), 52.3 (d, 2J = 21.0 Hz, CH₂OP), 54.3 (d, 3J = 12.3 Hz, CH=C), 123.7, 134.5, 135.3 (all C- Ar), 164.5 (d, 2J = 10.8 Hz, C=O, amide), 168.4 (C(1,3)=O). – ³¹P NMR (500 MHz, CDCl₃, TMS): δ = 14.3 ppm. – MS (EI, 70 eV): m/z (%) = 325 (18) [M⁻I]⁺, 311 (31), 281 (44), 202 (68), 174 (100), 146 (80), 110 (54), 104 (49) 77 (33), – C₁₅H₁₄N₂O₆P (325.2); calcld. C 50.8, H 4.6, N 8.6, P 9.5; found C 47.7, H 4.7, N 8.7, P 9.4.

Diisopropyl [2-(1,3-dioxo-1,3-dihydro-2H-isindol-2-yl)propanoyl]amidophosphate (14c)

Colorless crystals. Yield: 800 mg (64 %). M. p. 176–178 °C (from EtOH). – IR (film): v = 3342 (NH), 1782, 1728 (C(1,3)=O), 1705 (C=O, amide), 1235 (P=O, bonded), 1105 (POC), 974 (P-O-C) cm⁻¹. – ¹H NMR (500 MHz, CDCl₃, 25 °C, TMS): δ = 0.98, 1.14 (2 × d, J = 6.6 Hz, 2J = 5.4 Hz, 12 H, iso-(H₃C)₂C=O), 1.57 (d, J = 6.8 Hz, 3 H, H₂C=C), 4.12 (d-sept, J = 6.6 Hz, 2J = 8.9 Hz, 2 H, HCOPO), 4.94 (q, J = 6.6 Hz, 1 H, HN), 6.24 (br, 1 H, HN), 7.67, 7.78 (2 × d, J = 4.4 Hz, 4 H, H- Ar). – ¹³C NMR (500 MHz, CDCl₃, TMS): δ = 12.8 (CH₂OP), 17.8 (CH₃C), 54.7 (CH-Me), 69.8 (d, 2J = 11.8 Hz, CHOP), 123.2, 133.4, 135.7 (all C- Ar), 164.5 (d, 2J = 22.2, C=O, amide), 166.4 (C(1,3)=O). – ³¹P NMR (500 MHz, CDCl₃, TMS): δ = 14.3 ppm. – MS (EI, 70 eV): m/z (%) = 381 (22) [M–I]⁺, 367 (18), 334 (36), 306 (18), 263 (14), 202 (48), 174 (100), 146 (55), 110 (23), 104 (44), 77 (43), – C₁₅H₁₄N₂O₆P (382.4); calcld. C 53.4, H 6.0, N 7.3, P 8.1; found C 53.3, H 6.1, N 7.2, P 8.2.

Reaction of 1 with tris(dimethylamino)phosphine (16)

Trisaminophosphine 16 (6 mL, 4.5 mmol) in super-dry tetrahydrofuran (THF) (5 mL) was added dropwise to the carbonyl azide 1 (0.5 g, 2.1 mmol) in THF (5 mL), and the reaction mixture was stirred at r. t. for ≈ 10 h (TLC). After cooling, the precipitated material was collected and dried to give compound 17.

3-[2-(1,3-Dioxo-1,3-dihydro-2H-isindol-2-yl)propanoyl]-2-triazenylidenetris(dimethylamino)phosphorane (17)

Pale yellow material (stable for ca. 2 weeks in a refrigerator). Yield: (88 %). M. p. 208–211 °C. – IR (film): v =
1774, 1730 (C(1,3)=O), 1696 (C≡O), 1355 (P=N), 1335, 860 [P(Me=2)]cm⁻¹, –¹H NMR (500 MHz, CDCl₃, 25 °C, TMS): δ = 1.55 (d, J = 4.8 Hz, 3 H, H₂-C(3)), 2.45, 2.63 (2d, J = 10.8 Hz, 18 H, (CH₃)₂-N=P), 5.98 (q, J = 4.8 Hz, 1 H, H-C(7)), 7.77, 7.85 (2d, J = 4.8 Hz, 4 H, H-Ar). –¹³C NMR (500 MHz, CDCl₃, TMS): δ = 16.8 (CH₃-C(3)), 37.4 (d, J = 30.6 Hz, (CH₃)₂-N=P), 44.4 (C-Me), 124.3, 130.4, 134.7 (all C-Ar), 150.4, 166.4 (C(1,3)=O), 179.8 (C≡O). –³¹P NMR (500 MHz, CDCl₃, H₂PO₄): δ = 40.3 ppm. – MS (EI, 70 eV): m/z (%) = 407 (15) [M⁺], 392 (25) [M–Me]⁺, 379 (56) [M–N₂ or –CO]⁺, 191 (36) [C₆H₈(N₃)⁺], 173 (100) [M–C₆H₅N₅OP]⁺, 146 (82) [M–C₆H₅N₅OP]⁺, 116 (35), 104 (23), 77 (56). – C₁₇H₁₆N₂O₄P (407.4): calcld. C 50.1, H 6.4, N 24.1, P 7.5; found C 50.2, H 6.3, N 24.2, P 7.5.

Reaction of I with alkylidenephosphoranes

Azide 1 (0.8 g, 3.28 mmol) and benzoylmethylenetriphenylphosphorane (18) (1.3 g, 3.5 mol) in dry chloroform (20 mL) were boiled under reflux for 36 h. After removing the solvent, the residue was chromatographed on silica gel to afford the triazole 19 (42 %). Next, the azide 1 (0.8 g, 3.28 mmol) and 3.5 mmol of benzoyl- (18), ethoxycarbonyl- (20a) or cyanoalkylidenetriphenylphosphorane (20b) in dry dimethylformamide (DMF) (8 mL) in a Pyrex glass beaker were heated under microwave irradiation for 20–25 min. After the completion of the reaction (TLC analysis), the volatile materials were evaporated under reduced pressure to dryness. The resulting residue was chromatographed on silica gel (n-hexane/AcOEt) to give the triazoles 19, 22a and 22b, respectively. Triphenylphosphine oxide was also isolated from the above four reactions, eluent: n-hexane/AcOEt (2:3, v/v).

5-[1-(1,3-Dioxy-1,3-dihydro-2H-isooindol-2-yl)ethyl]-2H-1,2,3-triazole-4-carbonitrile (22b)

Eluent: n-hexane/AcOEt (1:1, v/v). Yield: 740 mg (85 %). M. p. 193–195 °C (from MeCN). – IR (film): ν = 3452 (NH), 2218 (CN), 1756, 1732 (C(1,3)=O), 1163 (triazole) cm⁻¹. –¹H NMR (500 MHz, CDCl₃, 25 °C, TMS): δ = 1.82 (d, J = 6.6 Hz, 3 H, H₂-C(3)), 5.24 (q, J = 6.6 Hz, 1 H, HMe), 7.98, 8.08 (2 d, J = 4.8 Hz, 4 H, H-Ar), 8.67 (s, 1 H, HN). –¹³C NMR (500 MHz, CDCl₃, TMS): δ = 14.7 (CH₃), 46.8 (CH-Me), 108.7 (C-CN), 124.3 (C), 131.4, 133.7, 134.6 (all C-Ar), 137.2 (C(5)), triazole, 166.2 (C(1,3)=O). – MS (EI, 70 eV): m/z (%) = 267 (36) [M⁺], 266 (32), 251 (29), 241 (31), 225 (45), 147 (100), 146 (88), 116 (68), 104 (36), 77 (25). – C₁₉H₁₄N₅O₂ (267.2): calcld. C 58.4, H 3.4, N 26.2; found C 58.3, H 3.5, N 26.1.

Antibacterial assay

The prepared compounds were screened for their activity against four bacterial strains by the disc diffusion method. A standard inoculum (1 × 10⁵ c.f.u. mL of acetone) 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 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile disc 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. The minimum inhibitory concentration (MIC) was determined by the broth dilution technique. The nutrient broth, which contained a logarithmic subculture of the test microorganism, was inoculated with approximately 5 × 10⁵ c.f.u. mL of actively dividing bacteria cells. The plates were incubated for 24 h at 37 °C, and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was re-
garded as minimum inhibitory concentration (MIC). Amoxicillin was used as the standard drug.

**Antifungal assay**

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


