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Demonic axe-like conjugated alkynes in combating microbes

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ABSTRACT

A new series of disubstituted alkynes was obtained by microwave induced internal splitting of the corresponding β-oxo-alkylidenetriphenylphosphoranes. The antimicrobial potential of these conjugated alkynes and phosphoranes was assayed *in vitro* against three *Gram-positive* bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis*), three *Gram-negative* bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) and five fungal strains (*Aspergillus niger*, *Candida albicans*, *Aspergillus flavus*, *Candida rugosa*, *Saccharomyces cerevisiae*). The 3-pyridylalkyne derivatives *viz*., 3-(6-chloropyridin-3-yl)propynenitrile (**6a**), 3-(2-chloropyridin-3-yl)propynenitrile (**6b**), ethyl 3-(6-chloropyridin-3-yl)propiolate (**6c**), *iso*-propyl 3-(6-chloropyridin-3-yl)propiolate (**6d**) and 3-(2,6-dichloro-5-fluoropyridin-3-yl) propynenitrile (**6e**) were found to be highly potent towards all tested microorganisms except *E. coli*.

1. Introduction

Development of new antimicrobial drugs would remain a continuous activity to address the problems caused by rapid development of microbial resistance to most of the known antibiotics [1]. If suitable new drugs were not made available, the drug resistance caused by mutations in the microbial genomes would subject the immunocompromised patients who have undergone bone-marrow transplantation, or those suffering from AIDS or diabetes and receiving chemotherapy to greater risk [2]. The resistance of *candidiasis*, a common fungal infection found in both adults and infants caused by *Candida albicans*, to treatment with azole antifungal drugs is currently a burning problem [3,4]. Most of the available antifungal agents could be classified into polyenes, nucleoside analogs, echinocandins and azoles [5] and their longer term use in treatment was fraught with severe side effects. For example, the use of amphotericin B (a polyene antifungal drug) has been restricted due to its adverse side effects like renal toxicity [5,6]. Such a scenario demands a renewed global effort seeking continuous development of new antimicrobial agents effective against pathogenic microorganisms resistant to currently available treatments. Our continued interest in the synthesis and conversion of disubstituted acetylenes into various heterocyclic compounds [7,8] has prompted us to prepare a new series of propynenitriles and alkyl propynoic acid esters having a bulky substituent attached directly to the acetylenic carbon atom for evaluating their antimicrobial efficacy. Acetylenic carboxylic acid esters and alkynenitriles were not properly investigated for their antimicrobial potential. Conjugated alkynenitriles and acetylenic carboxylic acid esters with their ability to undergo nucleophilic addition reactions are good synthons for preparing heterocyclic compounds [9]. Several pyridine and furan ring containing heterocyclic compounds have found use in pharmaceutical and agrochemical applications [10]. For example, a chloronicotinyl insecticide like imidacloprid is used worldwide for controlling pests due to its broad spectrum of activity and low mammalian toxicity [11]. The present investigation was aimed at preparing a new series of conjugated alkynes with a bulky group attached to the *sp*-carbon atom, by an elegant process involving microwave induced intramolecular Wittig reaction of the corresponding β-oxo-alkylidenetriphenylphosphoranes, for assaying their antibacterial and antifungal potential. Conjugated alkynenitriles are rod like compounds, having all the atoms forming the two adjacent and rigid triple bonds along with

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the carbon directly attached to the *sp*-carbon atom in a straight line. With a planar and bulky substituent like 2-chloropyridin-3-yl- or 6-chloropyridin-3-yl- moiety attached to the *sp*-carbon atom, these conjugated alkynenitriles and the corresponding acetylenic carboxylic acid esters resemble a demonic axe with the chloropyridinyl substituent mimicking the axe-head and the linear part of the molecule forming the handle. The chloropyridinyl moiety attached to the stick-like alkynes could possibly interact with microbial DNA and inhibit the growth as reported earlier in the case of pyrimidones [12] and pyridinium compounds [13].

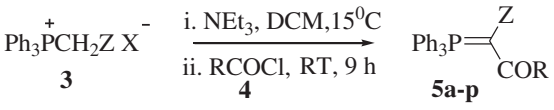
2. Results and discussion

2.1. Chemistry

Triphenylphosphine (**1**) was reacted with chloroacetonitrile (**2a**) in refluxing toluene for 7 h to obtain cyanomethyltriphenyl phosphonium chloride (**3a**) in quantitative yield [7,8]. The reaction of alkyl chloro/bromoacetate (**2b**) with **1** in dry dichloromethane has resulted in the corresponding alkyl bromo/chloro-triphenyl phosphoranylacetic acid ester (**3b**). Treatment of cyanomethyltriphenylphosphonium chloride salt (**3a**) or alkyl bromo/chloro-triphenylphosphoranylacetic acid ester (**3b**) with triethylamine has resulted in quantitative formation of the respective α-(cyano/alkoxycarbonyl)methylenetriphenylphosphorane [7,8]. These reactive phosphorous ylides, without isolation, were acylated in the same reaction flask with the corresponding carboxylic acid chloride (**4a**–**p**) under transylidation conditions to afford the desired β-oxo-ylides (**5a**–**p**) in high yield. The reaction sequence was depicted in Scheme 1. Thus, a series of α-cyanomethylenetriphenylphosphoranes and α-(ethoxy/*iso*-propyloxycarbonyl)methylenetriphenylphosphoranes (**5a**–**p**) having 6-chloronicotinoyl-, 2-chloronicotinoyl-, 2,6-dichloro-5-fluoronicotinoyl-, *o*-toluoyl-, 2-furoyl- or *t*-butyroyl-substituent on the ylide carbon were synthesized in high yield (Table 1). The chloronicotinoyl-substituted phosphorus ylides (**5a**–**e**, **5i**–**k**) along with the *o*-toluoyl- (**5f**), furoyl- (**5m**) and pivaloyl- (**5o**–**p**) derivatives were prepared for the first time. The α-(ethoxycarbonyl)methylenetriphenylphosphoranes containing *o*-toluoyl- (**5g**), *t*-butyroyl- (**5h**), 2-furoyl- (**5l**) substituent, and the α-(2-furoyl)-α-cyanomethylenetriphenylphosphorane (**5n**) were previously reported [14–16]. However, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data was not available for the stabilized ylide **5n**.

The oxo-ylides **5a**–**h** were converted into disubstituted conjugated alkynes (**6a**–**h**) *via* intramolecular Wittig reaction under microwave irradiation conditions. All these alkynes containing a bulky substituent were screened against six bacterial strains *viz.*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and five fungal species *viz.*, *Aspergillus niger*, *Aspergillus flavus*, *C. albicans*, *Candida rugosa*, *Saccharomyces cerevisiae* to obtain their MIC values and zone of inhibition, respectively.

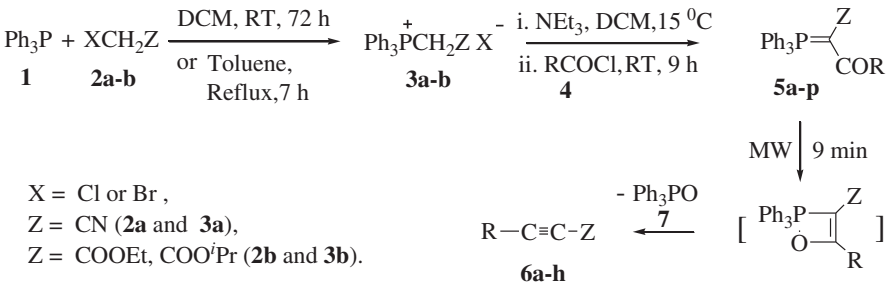
Table 1
List of β-oxo-ylides synthesized.



S. No.	R	Z	Isolated yield, %
5a	6-Chloropyridin-3-yl	CN	95
5b	2-Chloropyridin-3-yl	CN	89
5c	6-Chloropyridin-3-yl	CO_2Et	92
5d	6-Chloropyridin-3-yl	$CO_2{}^{i}Pr$	91
5e	2,6-Dichloro-5-fluoropyridin-3-yl	CN	88
5f	*o*-Tolyl	CN	94
5g	*o*-Tolyl	CO_2Et	93
5h	*t*-Butyl	CO_2Et	84
5i	2-Chloropyridin-3-yl	CO_2Et	90
5j	2,6-Dichloro-5-fluoropyridin-3-yl	CO_2Et	90
5k	2,6-Dichloro-5-fluoropyridin-3-yl	$CO_2{}^{i}Pr$	91
5l	2-Furyl	CO_2Et	87
5m	2-Furyl	$CO_2{}^{i}Pr$	87
5n	2-Furyl	CN	89
5o	*t*-Butyl	CN	85
5p	*t*-Butyl	$CO_2{}^{i}Pr$	82

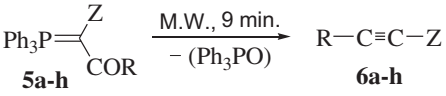
The phosphoranes **5a**–**p** were characterized with the help of IR, NMR (^{1}H & ^{13}C) and mass spectroscopy. The IR spectra of the nitrile group containing oxo-ylides (**5a**–**b**, **5e**–**f**, **5n** and **5o**) showed sharp nitrile stretching absorption band in the region of 2164–2186 cm^{-1}. The alkyl ester containing oxo-ylides (**5c**–**d**, **5i**, **5j**–**k**, **5g**–**h**, **5l**–**m** and **5p**) showed strong ester carbonyl stretching IR absorption band in the region of 1656–1692 cm^{-1}. The β-keto stretching absorption band of the oxo-ylides **5a**–**p** was shifted to 1532–1577 cm^{-1} region due to delocalization of electrons involving the ylide carbon. Also present was a sharp absorption band each in 1435–1440 and 995–1095 cm^{-1} regions, a common feature observed in compounds containing phosphorus linked to aryl group [17–19]. The ^{13}C NMR signal for the ylide carbon of phosphoranes **5a**–**p** appeared as a doublet ($^{1}J_{(C-P)}$ = 122–127 Hz) between δ 50–70 ppm, indicating greater localization of negative charge on this carbon and imparting partial ionic character to P=C bond. The signals due to the eighteen carbon atoms present in three phenyl rings attached to P-atom were observed as four distinct doublets in the range δ 123–133 ppm with $^{1}J_{(C-P)}$, $^{2}J_{(C-P)}$, $^{3}J_{(C-P)}$, $^{4}J_{(C-P)}$ values of 93–94 Hz, 9–10 Hz, 11–13 Hz and 3–4 Hz, respectively. The spectral data was presented in Experimental section.

Thermal decomposition of keto-ylides at elevated temperatures to obtain alkynes was prior art [20]. The heat sensitive alkynes formed in this reaction have to be distilled simultaneously under high vacuum, resulting in their lower isolated yield. We have overcome this drawback by applying microwave energy under



Scheme 1. Reaction sequence leading to alkynes (**6**).

Table 2
List of conjugated alkynes synthesized.



5a-h **6a-h**

S. No.	R	Z	Isolated yield (%)	
			MW	Thermal
6a	6-Cloropyridin-3-yl	CN	76	52
6b	2-Cloropyridin-3-yl	CN	73	55
6c	6-Cloropyridin-3-yl	CO_2Et	79	56
6d	6-Cloropyridin-3-yl	CO_2^iPr	78	49
6e	2,6-Dichloro-5-fluoropyridin-3-yl	CN	71	52
6f	*o*-Tolyl	CN	81	59
6g	*o*-Tolyl	CO_2Et	83	61
6h	*t*-Butyl	CO_2Et	59	31

controlled conditions over a shorter duration to facilitate intramolecular Wittig reaction of the oxo-ylides at relatively lower temperature and obtained the conjugated alkynes (**6a**–**h**) in good yield (Table 2). The 3-(6-chloropyridin-3-yl)prop-2-ynenitrile (**6a**), 3-(2-chloropyridin-3-yl) prop-2-ynenitrile (**6b**), ethyl 3-(6-chloro pyridin-3-yl)prop-2-ynoic acid ester (**6c**), *iso*-propyl 3-(6-chloro pyridin-3-yl)prop-2-ynoic acid ester (**6d**) and 3-(2,6-dichloro-5-fluoropyridin-3-yl)prop-2-ynenitrile (**6e**) were new compounds, while 3-(2-methylphenyl)prop-2-ynenitrile (**6f**) was prepared by this method for the first time. Compound **6f** was obtained earlier by a different route involving cyanation of alkyne [21]. The preparation of ethyl 3-(2-methylphenyl)prop-2-ynoic acid ester (**6g**) and ethyl 4,4-dimethylpent-2-ynoate (**6h**) was also reported earlier [14,22]. The 2-chloronicotinoyl containing α-(alkoxycarbonyl)methylenetriphenylphoshoranes **5i**–**k** did not undergo triphenylphosphine oxide (**7**) extrusion under the controlled microwave irradiation conditions and failed to give the corresponding alkynes. The disubstituted acetylenes (**6a**–**h**) were thoroughly characterized with the help of IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic data. The IR spectra of the conjugated alkynenitriles (**6a**–**b**, **6e**–**f**) contained sharp nitrile stretching absorption band at 2265–2271 cm^{-1} and the alkyne triple bond absorption band at 2140–2148 cm^{-1} region. The IR absorption band due to acetylene function of the alkyl acetylenic carboxylic acid esters has appeared in the region of 2206–2227 cm^{-1}. The characteristic ester carbonyl stretching absorption band of the acetylenic carboxylic acid esters (**6c**, **6d**, **6g** and **6h**) was seen in 1701–1708 cm^{-1} region. The ^{13}C NMR spectra contained signals due to acetylenic carbons in the δ 70–85 ppm region. The nitrile carbon signal in alkynenitriles (**6a**–**b**, **6e**–**f**) appeared at δ 105 ppm, while the signal due to ester carbonyl of the acetylenic carboxylic acid esters (**6c**–**d**, **6g**–**h**) was seen around δ 153 ppm.

2.2. Biological activity

An *in vitro* antimicrobial assay was carried out in respect of all the synthesized phosphorous ylides **5a**–**p** and conjugated alkynes **6a**–**h** against six bacterial strains (*B. subtilis*, *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*) and five fungal strains (*A. niger*, *A. flavus*, *C. albicans*, *C. rugosa*, *S. cerevisiae*). Stabilized phosphorus ylides containing substituted benzoyl moiety on the ylide carbon were reported [23] to exhibit high antibacterial and moderate antifungal activity *in vitro*. In contrast to this report, no effectiveness was exhibited by the oxo-ylides **5a**–**p** against all the tested bacterial strains, with their MIC values higher than 150 μg/mL. They did not show any noticeable antifungal activity either, with a near zero mm zone of inhibition even at 100 μg/mL concentration against all the five fungal species studied. However, the 3-pyridinyl-substituted acetylenic compounds (**6a**–**e**) were more effective against all the tested microorganisms except *E. coli*, suggesting a crucial role for the 3-pyridyl moiety in enhancing the antimicrobial properties of this class of compounds.

2.2.1. Antibacterial activity of conjugated alkynes (*6a*–*h*)

3-(6-Chloropyridin-3-yl)propynenitrile (**6a**), 3-(2-chloropyridin-3-yl)propynenitrile (**6b**), ethyl 3-(6-chloropyridin-3-yl)propiolate (**6c**), *iso*-propyl 3-(6-chloropyridin-3-yl)propiolate (**6d**) and 3-(2,6-dichloro-5-fluoropyridin-3-yl)propynenitrile (**6e**) were very potent *in vitro* towards all tested *Gram-positive* microorganisms (*B. subtilis*, *S. aureus* & *S. epidermidis*) and two *Gram-negative K. pneumoniae* & *P. aeruginosa* bacterial strains. Their effectiveness was comparable to the standards used *viz.*, streptomycin and penicillin. However, they were not so effective against *E. coli*. The aryl- and *t*-butyl-substituted acetylenes (**6f**–**h**) were ineffective against all the tested microorganisms. Compounds **6a**–**d**, with minimum inhibitory concentration (MIC) value ranging from 0.78 to 1.56 μg/mL against *P. aeruginosa*, were comparable in their antibacterial activity to streptomycin and considerably more potent than penicillin. The antibacterial activity of conjugated alkynes (**6a**–**h**) and the standards was shown in Table 3.

2.2.2. Antifungal activity of conjugated alkynes (*6a*–*h*)

The 3-(halopyridin-3-yl)alkynes (**6a**–**e**) have displayed very good zone of inhibition values of 10–44 mm at 50 μg/mL concentration against *A. niger*, *A. flavus*, *C. albicans*, *C. rugosa* and *S. cerevisiae*. The *in vitro* antifungal activity of 3-(2/6-chloropyridin-3-yl)alkynenitriles (**6a**–**b**) against *A. niger*, *A. flavus*, *C. albicans* and

Table 3
In vitro antibacterial activity assay of acetylenic compounds **6a**–**h**.

Compound	MIC (μg/mL)					
	Gram-positive			*Gram-negative*		
	B. subtilis	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
6a	1.56	1.56	6.25	100	0.78	3.125
6b	3.125	6.25	50	100	0.78	12.5
6c	6.25	1.56	50	100	0.78	6.25
6d	50	1.56	1.56	100	1.56	12.5
6e	6.25	12.5	25	100	100	12.5
6f	>150	>150	>150	>150	>150	˃150
6g	>150	>150	>150	>150	>150	˃150
6h	>150	>150	>150	>150	>150	˃150
Penicillin	1.562	1.562	3.125	12.5	12.5	6.25
Streptomycin	6.25	6.25	3.125	6.25	1.562	3.125

C. rugosa was slightly better than that of amphotericin B (standard). However, they were ineffective against *S. cerevisiae*. Ethyl 3-(6-chloropyridin-3-yl)propynoate (**6c**) was very potent towards *S. cerevisiae* and *A. niger* and its activity was comparable to amphotericin B. The aryl- and *t*-butyl-substituted acetylenic compounds **6f**–**h** were ineffective against all tested fungal strains. The antifungal assay of conjugated alkynes **6a**–**h** was displayed in Table 4.

3. Conclusions

Conjugated alkynes containing a bulky β-substituent were synthesized in high yield from β-oxo-alkylidenetriphenyl-phosphoranes *via* microwave induced intramolecular Wittig reaction and tested *in vitro* for their antimicrobial activity against three *Gram-positive* and three *Gram-negative* bacteria, along with five fungal species. Halopyridin-3-yl substituted acetylenes have displayed very good broad spectrum antimicrobial activity, a first observation for disubstituted acetylenes. The antibacterial efficacy of 3-(6-chloropyridin-3-yl)propynenitrile, 3-(2-chloropyridin-3-yl) propynenitrile and ethyl 3-(6-chloropyridin-3-yl)propynoic acid ester against *B. subtilis*, *S. aureus*, *P. aeruginosa* and *K. pneumoniae* was comparable to penicillin and streptomycin. The same compounds have also exhibited good antifungal activity comparable to amphotericin B against *A. niger*, *C. albicans*, *A. flavus*, *C. rugosa*, *S. cerevisiae*. However, they were less affective against *E. coli*. The results indicated that conjugated alkynenitriles and acetylenic carboxylic acid esters armed with a suitable bioactive scaffold have the potential to become a new class of antimicrobial agents.

4. Experimental protocols

4.1. Chemistry

All the reagents and solvents were purchased from commercial sources. The solvents were redistilled and dried before use. Melting points were determined on the veego (VMP-MP) melting point apparatus and were uncorrected. Thin layer chromatography was carried out on Merck 60 F_{254} silica gel coated glass sheets. The acetylenic compounds were purified by column chromatography on silica gel (60–120 mesh). Infrared spectra were recorded on Perkin–Elmer FT-IR 1600 spectrometer. ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded on Bruker Avance 300 MHz and Inova 500 MHz spectrometer with TMS as internal standard. Chemical shifts (δ) were given in parts per million and coupling constants were given as absolute values expressed in Hertz. Mass spectral analysis using electrospray ionization (ESI) and high resolution mass spectrometry (HRMS) experiments were performed using a quadrupole time-of-flight mass spectrometer (QSTAR XL).

4.1.1. General procedure for the synthesis of β-oxo-alkylidenetriphenylphosphoranes (**5a**–**p**)

The general protocol followed for preparing β-oxo-ylides was described here taking α-(6-chloronicotinoyl)-α-(cyano)methyl-enetriphenylphosphorane (**5a**) as an example. The remaining β-oxo-ylides (**5b**–**p**) were prepared in a similar manner.

Triethylamine (1.06 g, 0.01 mol) was added drop wise to a stirred suspension of cyanomethyltriphenylphosphonium chloride (**3a**, 1.68 g, 0.005 mol) in 10 mL dry CH_2Cl_2 at 10–15 °C under N_2 atmosphere. The reaction mixture was stirred at room temperature for 30 min and a solution of 6-chloronicotinoyl chloride (**4a**, 0.88 g, 0.005 mol) in 5 mL CH_2Cl_2 was added drop wise in about 15 min. After vigorous stirring for 12 h at room temperature, the reaction mixture was diluted with 10 mL CH_2Cl_2 and washed with 3 × 10 mL water. The organic layer was separated, dried over anhydrous sodium sulfate and the solvent was removed on rotavapor. The crude product was recrystallized from a 1:4 mixture of CH_2Cl_2 and hexane to obtain α-(6-chloronicotinoyl)-α-(cyano)methylenetriphenylpho sphorane (**5a**). Yield: 2.09 g (95%).

*4.1.1.1. α-(6-Chloronicotinoyl)-α-(cyano)methylene-triphenylphosphorane (**5a**).* Colorless solid; mp 175–177 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 500 MHz): 7.34 (1H, d, J = 7.8 Hz, Py–H), 7.54–7.60 (6H, m, Ar–Hs), 7.64–7.71 (9H, m, Ar–Hs), 8.30 (1H, dd, J = 1.9, 7.8 Hz, Py–H), 8.93 (1H, d, J = 1.9 Hz, Py–H); ^{13}C NMR (δ ppm in $CDCl_3$, 75 MHz): 50.4 (d, $^1J_{C-P}$ = 125.1 Hz, P=C), 121.5 (d, $^2J_{C-P}$ = 15.3 Hz, –CN), 122.5 (d, $^1J_{C-P}$ = 94.4 Hz, P–Ph), 129.3 (d, $^3J_{C-P}$ = 10.9 Hz, P–Ph), 133.4 (d, $^4J_{C-P}$ = 3.4 Hz, P–Ph), 133.6 (d, $^2J_{C-P}$ = 10.9 Hz, P–Ph), 128.7, 132.0, 138.3, 149.6, 153.0, 186.3 (d, $^2J_{C-P}$ = 4.3 Hz, –C=O); IR (KBr) υ cm^{-1}: 3055, 2176, 1573, 1482, 1437, 1356, 1128, 1105, 1014, 938; ESI-MS m/z (M + H)$^+$: 441; HRMS m/z calcd for $C_{26}H_{19}ON_2ClP$ [M + H]$^+$: 441.0918; found: 441.0903.

*4.1.1.2. α-(2-Chloronicotinoyl)-α-(cyano)methylene-triphenylphosphorane (**5b**).* Colorless solid; mp 135–137 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 300 MHz): 7.25 (1H, dd, J = 4.5, 7.5 Hz, Pyridine–H), 7.41–7.49 (9H, m, Ar–Hs), 7.62–7.70 (6H, m, Ar–Hs), 7.80 (1H, dd, J = 2.2, 7.5 Hz, Pyridine–H), 8.39 (1H, dd, J = 2.2, 4.5 Hz, Pyridine–H); IR (KBr) υ cm^{-1}: 3054, 2186, 1568, 1481, 1437, 1338, 1188, 1114, 1064, 997; ESI-MS m/z (M + H)$^+$: 441; HRMS m/z calcd for $C_{26}H_{19}ON_2ClP$ [M + H]$^+$: 441.0918; found: 441.0905.

*4.1.1.3. α-(6-Chloronicotinoyl)-α-(ethoxycarbonyl)methylene-triphenylphosphorane (**5c**).* Colorless solid; mp 165–167 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 300 MHz): 0.68 (3H, t, J = 6.7 Hz, $-CH_3$),

Table 4
Invitro antifungal activity data of acetylenic compounds **6a h**.

Compound	*C.albicans*		*C.rugosa*		*S.cerevisiae*		*A.niger*		*A.flavus*	
	Zone of inhibition (mm)									
	50[a]	100[a]	50[a]	100[a]	50[a]	100[a]	50[a]	100[a]	50[a]	100[a]
6a	35	40	33	28	0	10	39	43	44	47
6b	23	28	23	29	0	11	26	30	35	39
6c	15	20	10	15	25	29	25	29	20	25
6d	13	19	0	11	11	17	17	23	12	19
6e	11	16	0	11	0	10	20	24	20	25
6f	0	0	0	0	0	0	0	0	0	0
6g	0	0	0	0	0	0	0	0	0	0
6h	0	0	0	0	0	0	0	0	0	0
Amphote-ricin-B	23.5		21		22		25		25	

[a] Concentration of compound in g/mL.

3.69 (2H, q, J = 6.7 Hz, $-CH_2$), 7.26 (1H, d, J = 8.3 Hz, Py–H), 7.44–7.60 (9H, m, Ar–Hs), 7.68–7.77 (6H, m, Ar–Hs), 7.85 (1H, dd, J = 2.2, 8.3 Hz, Py–H), 8.64 (1H, d, J = 2.2 Hz, Py–H); ^{13}C NMR (δ ppm in $CDCl_3$, 125 MHz): 13.6, 54.1 (d, $^1J_{C-P}$ = 127.3 Hz, P=C), 58.6, 125.4 (d, $^1J_{C-P}$ = 92.2 Hz, P–Ph), 128.9 (d, $^3J_{C-P}$ = 10.9 Hz, P–Ph), 132.1 (d, $^4J_{C-P}$ = 3.4 Hz, P–Ph), 133.3 (d, $^2J_{C-P}$ = 10.9 Hz, P–Ph) 128.4, 132.0, 138.7, 149.6, 151.5, 164.9 (d, $^2J_{C-P}$ = 14.3 Hz, ester carbonyl), 183.7 (d, $^2J_{C-P}$ = 4.3 Hz, –C=O); IR (KBr) υ cm^{-1}: 3053, 2975, 1655, 1575, 1482, 1441, 1326, 1256, 1083, 1023, 895; ESI-MS m/z $(M + Na)^+$: 510; HRMS m/z calcd for $C_{28}H_{23}O_3NClNaP$ $[M + Na]^+$: 510.0996; found: 510.1009.

*4.1.1.4. α-(6-Chloronicotinoyl)-α-(isopropyloxycarbonyl)methylenetriphenylphosphorane (**5d**).* Colorless solid; mp120–122 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 500 MHz): 0.65 (6H, d, J = 5.5 Hz, two CH_3 groups), 4.56–4.62 (1H, m, –CH), 7.26 (1H, d, J = 2.7 Hz, Py–H), 7.39–7.51 (9H, m, Ar–Hs), 7.64–7.70 (6H, m, Ar–Hs), 7.79 (1H, dd, J = 2.7, 1.8 Hz, Py–H), 8.58 (1H, d, J = 1.8 Hz, Py–H); ^{13}C NMR (δ ppm in $CDCl_3$, 125 MHz): 21.4, 50.1 (d, $^1J_{C-P}$ = 127.3 Hz, P=C), 66.0, 125.5 (d, $^1J_{C-P}$ = 92.2 Hz, P–Ph), 128.9 (d, $^3J_{C-P}$ = 10.9 Hz, P–Ph), 131.7 (d, $^4J_{C-P}$ = 3.2 Hz, P–Ph), 133.2 (d, $^2J_{C-P}$ = 10.9 Hz, P–Ph), 128.4, 137.5, 138.7, 149.7, 151.5, 166.7 (d, $^2J_{C-P}$ = 13.1 Hz, ester carbonyl), 179.1 (d, $^2J_{C-P}$ = 4.3 Hz, –C=O); IR (KBr) υ cm^{-1}: 3055, 2976, 1656, 1577, 1444, 1358, 1324, 1256, 1095, 924; ESI-MS m/z $(M + H)^+$: 502; HRMS m/z calcd for $C_{29}H_{26}O_3NClP$ $[M + H]^+$: 502.1333; found: 502.1328.

*4.1.1.5. α-(2,6-Dichloro-5-fluoro-nicotinoyl)-α-(cyano)methylenetriphenylphosphorane (**5e**).* Colorless solid; mp 205–207 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 500 MHz): 7.57 (1H, m, Py–H), 7.58–7.62 (6H, m, Ar–Hs), 7.67–7.74 (9H, m, Ar–Hs); ^{13}C NMR (δ ppm in $CDCl_3$, 75 MHz): 52.6 (d, $^1J_{C-P}$ = 122.0 Hz, P=C), 120.7 (d, $^2J_{C-P}$ = 15.2 Hz, –CN), 122.1 (d, $^1J_{C-P}$ = 93.0 Hz, P–Ph), 129.6 (d, $^3J_{C-P}$ = 13.7 Hz, P–Ph), 133.5 (d, $^4J_{C-P}$ = 3.7 Hz, P–Ph), 133.8 (d, $^2J_{C-P}$ = 10.6 Hz, P–Ph), 126.1, 137.9, 139.2, 141.2, 154.5, 185.3 (d, $^2J_{C-P}$ = 6.1 Hz, –C=O); IR (KBr) υ cm^{-1}: 3062, 2180, 1578, 1481, 1438, 1391, 1108; ESI-MS m/z $(M + H)^+$: 493; HRMS m/z calcd for $C_{26}H_{17}ON_2Cl_2FP$ ($[M + H]^+$): 493.0434; found: 493.0441.

*4.1.1.6. α-(o-Toluoyl)-α-(cyano)methylenetriphenylphosphorane (**5f**).* Colorless solid; mp 125–127 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 300 MHz): 2.40 (3H, s, Ar–CH_3), 7.15 (2H, t, J = 6.7 Hz, Ar–Hs), 7.19 (1H, d, J = 2.2 Hz, Ar–H), 7.21 (1H, d, J = 2.2 Hz, Ar–H), 7.50–7.60 (9H, m, Ar–Hs), 7.60–7.71 (6H, m, Ar–H); ESI-MS m/z $(M + H)^+$: 420; HRMS m/z calcd for $C_{28}H_{23}ONP$ $[M + H]^+$: 420.1511; found: 420.1499.

*4.1.1.7. α-(Pivaloyl)-α-(ethoxycarbonyl)methylenetriphenylphosphorane (**5h**).* Colorless solid; mp 149–150 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 300 MHz): 0.68 (3H, t, J = 6.7 Hz, $-CH_3$), 1.27 (9H, s, three $-CH_3$ groups), 3.58 (2H, q, J = 6.7 Hz, $-CH_2$), 7.35–7.50 (9H, m, Ar–Hs), 7.55–7.65 (6H, m, Ar–Hs); ^{13}C NMR (δ ppm in $CDCl_3$, 75 MHz): 13.6, 27.2, 42.5 (d, $^3J_{C-P}$ = 6.5 Hz, ^{t}Bu–quaternary carbon), 50.5 (d, $^1J_{C-P}$ = 125.3 Hz, P=C), 58.5, 128.0 (d, $^1J_{C-P}$ = 94.4 Hz, P–Ph), 128.5 (d, $^3J_{C-P}$ = 13.1 Hz, P–Ph), 131.1 (d, $^4J_{C-P}$ = 2.9 Hz, P–Ph), 132.7 (d, $^2J_{C-P}$ = 10.9 Hz, P–Ph), 202.3 (d, $^2J_{C-P}$ = 4.3 Hz, –C=O); IR (KBr) υ cm^{-1}: 3048, 2940, 1662, 1537, 1483, 1438, 1329, 1251, 1075; ESI-MS m/z $(M + H)^+$: 433; HRMS m/z calcd for $C_{27}H_{30}O_3P$ $[M + H]^+$: 433.1927; found: 433.1919.

*4.1.1.8. α-(2-Chloronicotinoyl)-α-(ethoxycarbonyl)methylenetriphenylphosphorane (**5i**).* Colorless solid; mp 123–125 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 300 MHz): 0.65 (3H, t, J = 6.7 Hz, $-CH_3$), 3.69 (2H, q, J = 6.7 Hz, $-CH_2$), 7.20 (1H, dd, J = 4.5, 7.5 HZ, Py–H), 7.44–7.60 (9H, m, Ar–Hs), 7.68–7.77 (6H, m, Ar–Hs), 7.80 (1H, dd, J = 1.5, 7.5 Hz, Py–H), 8.28 (1H, dd, J = 1.5, 4.5 Hz, Py–H); IR (KBr) υ cm^{-1}: 3053, 2975, 1655, 1532, 1482, 1436, 1187, 995; ESI-MS m/z $(M + Na)^+$: 510; HRMS m/z calcd for $C_{28}H_{23}O_3NClNaP$ $[M + Na]^+$: 510.0994; found: 510.1007.

*4.1.1.9. α-(2,6-Dichloro-5-fluoronicotinoyl)-α-(ethoxycarbonyl)methylenetriphenylphosphorane (**5j**).* Colorless solid; mp 133–135 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 300 MHz): 0.65 (3H, t, J = 7.1 Hz, $-CH_3$), 3.69 (2H, q, J = 7.1 Hz, $-CH_2$), 7.35 (1H, d, J = 7.1 Hz, Py–H), 7.46–7.60 (9H, m, Ar–Hs), 7.69–7.79 (6H, m, Ar–Hs); ^{13}C NMR (δ ppm in $CDCl_3$, 75 MHz): 13.3, 50.0 (d, $^1J_{C-P}$ = 121.8 Hz, P=C), 58.8, 124.4 (d, $^1J_{C-P}$ = 93.3 Hz, P–Ph), 128.6 (d, $^3J_{C-P}$ = 12.6 Hz, P–Ph), 131.9 (d, $^4J_{C-P}$ = 4.3 Hz, P–Ph), 133.2 (d, $^2J_{C-P}$ = 9.8 Hz, P–Ph), 125.0, 129.9, 133.7, 135.0, 154.1, 166.7 (d, $^2J_{C-P}$ = 13.1 Hz, ester –C=O), 185.1 (d, $^2J_{C-P}$ = 6.5 Hz, –C=O); IR (KBr) υ cm^{-1}: 3055, 2985, 1664, 1547, 1479, 1439, 1385, 1327, 1256, 1104, 900; ESI-MS m/z $(M + H)^+$: 540; HRMS m/z calcd for $C_{28}H_{22}O_3NCl_2FP$ $[M + H]^+$: 540.0692; found: 540.0708.

*4.1.1.10. α-(2-Furoyl)-α-(ethoxycarbonyl)methylenetriphenylphosphorane (**5l**).* Red color solid; mp 100–102 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 300 MHz): 0.65 (3H, t, J = 6.9 Hz, $-CH_3$), 3.64 (2H, q, J = 6.9 Hz, $-CH_2$), 6.40 (1H, dd, J = 2.2, 3.0 Hz, Furan–H), 7.12 (1H, d, J = 3.0 Hz, Furan–H), 7.40 (1H, d, J = 2.2 Hz, Furan–H), 7.41–7.55 (9H, m, Ar–Hs), 7.67–7.76 (6H, m, Ar–Hs); ^{13}C NMR (δ ppm in $CDCl_3$, 125 MHz): 13.8, 49.6 (d, $^1J_{C-P}$ = 125.1 Hz, P=C), 58.8, 126.1 (d, $^1J_{C-P}$ = 94.4 Hz, P–Ph), 128.6 (d, $^3J_{C-P}$ = 13.1 Hz, P–Ph), 131.7 (d, $^4J_{C-P}$ = 3.7 Hz, P–Ph), 133.3 (d, $^2J_{C-P}$ = 8.7 Hz, P–Ph), 110.9, 114.4, 131.8, 143.0, 167.2 (d, $^2J_{C-P}$ = 13.1 Hz, ester carbonyl), 179.2 (d, $^2J_{C-P}$ = 6.5 Hz, –C=O); IR (KBr) υ cm^{-1}: 3058, 2981, 1671, 1575, 1473, 1438, 1326, 1260, 1077, 1012, 933; ESI-MS m/z $(M + Na)^+$: 465; HRMS m/z calcd for $C_{27}H_{23}O_4NaP$ $[M + Na]^+$: 465.1226; found: 465.1252.

*4.1.1.11. α-(2-Furoyl)-α-(isopropyloxycarbonyl)methylenetriphenylphosphorane (**5m**).* Colorless solid; mp 145–147 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 300 MHz): 0.82 (6H, d, J = 6.4 Hz, two $-CH_3$ groups), 4.69–4.78 (1H, m, –CH), 6.42 (1H, dd, J = 1.7, 3.3 Hz, Furan–H), 7.13 (1H, dd, J = 0.9, 3.3 Hz, Furan–H), 7.42 (1H, dd, J = 0.9, 1.7 Hz, Furan–H), 7.44–7.54 (9H, m, Ar–Hs), 7.64–7.81 (6H, m, Ar–Hs); IR (KBr) υ cm^{-1}: 3067, 2976, 1692, 1668, 1512, 1475, 1435, 1363, 1254, 1106, 1071, 1002, 932; ESI-MS m/z $(M + H)^+$: 457; HRMS m/z calcd for $C_{28}H_{26}O_4P$ $[M + H]^+$: 457.1563; found: 457.1555.

*4.1.1.12. α-(Pivaloyl)-α-(cyano)methylenetriphenylphosphorane (**5o**).* Colorless solid; mp 167–169 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 300 MHz): 1.35 (9H, s, three $-CH_3$ groups), 7.45–7.54 (9H, m, Ar–Hs), 7.55–7.62 (6H, m, Ar–Hs); ^{13}C NMR (δ ppm in $CDCl_3$, 75 MHz): 26.9, 42.5 (d, $^3J_{C-P}$ = 6.5 Hz, ^{t}Bu–quaternary carbon), 51.2 (d, $^1J_{C-P}$ = 127.3 Hz, P=C), 122.5 (d, $^2J_{C-P}$ = 17.5 Hz, –CN), 123.9 (d, $^1J_{C-P}$ = 94.4 Hz, P–Ph), 128.7 (d, $^3J_{C-P}$ = 13.1 Hz, P–Ph), 132.5 (d, $^4J_{C-P}$ = 4.3 Hz, P–Ph), 133.1 (d, $^2J_{C-P}$ = 8.7 Hz, P–Ph), 202.5 (d, $^2J_{C-P}$ = 4.3 Hz, –C=O); IR (KBr) υ cm^{-1}: 3052, 2164, 1565, 1479, 1437, 1327, 1160, 1105, 1027, 996; ESI-MS m/z $(M + H)^+$: 386; HRMS m/z calcd for $C_{25}H_{25}ONP$ $[M + H]^+$: 386.1668; found: 386.1665.

*4.1.1.13. α-(Pivaloyl)-α-(isopropyloxycarbonyl)methylenetriphenylphosphorane (**5p**).* Colorless solid; mp 145–147 °C; ^{1}H NMR (δ ppm in $CDCl_3$, 300 MHz): 0.72 (6H, d, J = 6.2 Hz, two $-CH_3$ groups), 1.27 (9H, s, three CH_3 groups), 4.58–4.69 (1H, m, –CH), 7.35–7.47 (9H, m, Ar–Hs), 7.56–7.66 (6H, m, Ar–Hs); ^{13}C NMR (δ ppm in $CDCl_3$, 75 MHz): 21.4, 27.2, 42.6 (d, $^3J_{C-P}$ = 6.5 Hz, ^{t}Bu–quaternary carbon), 51.5 (d, $^1J_{C-P}$ = 125.3 Hz, P=C), 65.6, 128.0 (d, $^1J_{C-P}$ = 92.2 Hz, P–Ph), 128.4 (d, $^3J_{C-P}$ = 13.1 Hz, P–Ph), 131.1 (d,

$^4J_{C-P}$ = 2.3 Hz, P–Ph), 132.7 (d, $^2J_{C-P}$ = 8.7 Hz, P–Ph), 202.4 (d, $^2J_{C-P}$ = 6.5 Hz, –C=O); IR (KBr) υ cm^{-1}: 3050, 2974, 1655, 1526, 1483, 1437, 1354, 1254, 1083, 1075, 998; ESI-MS *m/z* $(M + H)^+$: 447; HRMS *m/z* calcd for $C_{28}H_{32}O_3P$ $[M + H]^+$: 447.2083; found: 447.2076.

*4.1.2. General procedure for the synthesis of conjugated acetylenes (**6a**–**h**)*

4.1.2.1. Microwave irradiation method. The β-oxo-ylide (**5**, 0.045 mol) was taken in a sealed tube and subjected to microwave irradiation (CEM discover, 250 W, 120 °C, 25 psi) for 6–10 min. The dark brown reaction mixture was dissolved in 10 mL CH_2Cl_2 and purified by column chromatography using silica gel (60–120 mesh). Initial fractions, eluted with pet ether, on solvent removal have afforded the corresponding conjugated alkynes. The fractions eluted with ethyl acetate and pet ether (1:9) contained the unreacted phosphorane (**5**) followed by triphenylphosphine oxide (**7**).

4.1.2.2. Thermal decomposition method. The β-oxo-ylide (0.045 mol) was taken in a short path distillation flask and heated at 250–260 °C under 1 mm vacuum for 25 min and the distillate was collected in a cold trap. The distillate and the dark brown reaction mass consisting of disubstituted acetylene (**6**) and triphenylphosphine oxide (**7**) was dissolved in CH_2Cl_2 (20 mL) and subjected to column chromatography as described above to obtain the pure alkyne.

*4.1.2.3. 3-(6-Chloropyridin-3-yl)prop-2-ynenitrile (**6a**).* Colorless solid; mp 118–120 °C; ^1H NMR (δ ppm in $CDCl_3$, 300 MHz): 7.40 (1H, d, *J* = 8.3 Hz, Py–H), 7.84 (1H, dd, *J* = 2.2, 8.3 Hz, Py–H), 8.64 (1H, d, *J* = 2.2 Hz, Py–H); ^{13}C NMR (δ ppm in $CDCl_3$): 72.6, 78.3, 104.7, 115.9, 124.5, 142.4, 151.2, 153.9; IR (KBr) υ cm^{-1}: 3421, 3090, 3038, 2925, 2855, 2364, 2270, 2148, 1974, 1874, 1710, 1578, 1534, 1456, 1114, 1024, 848; ESI-MS *m/z* $(M + H)^+$: 163; HRMS *m/z* calcd for $C_8H_4N_2Cl$ $[M + H]^+$: 163.0036; found: 163.0040; Anal. ($C_8H_3N_2Cl$): C 59.10%, H 1.86%, N 17.23%; found: C 59.03%, H 1.98%, N 17.15%.

*4.1.2.4. 3-(2-Chloropyridin-3-yl)prop-2-ynenitrile (**6b**).* Colorless solid; mp 96–98 °C; ^1H NMR (δ ppm in $CDCl_3$, 300 MHz): 7.31 (1H, dd, *J* = 2.2, 7.5 Hz, Pyridine–H), 7.92 (1H, dd, *J* = 1.5, 7.5 Hz, Pyridine–H), 8.51 (1H, dd, *J* = 2.2, 1.5 Hz, Pyridine-H); ^{13}C NMR (δ ppm in $CDCl_3$): 69.2, 77.1, 104.7, 115.7, 122.0, 143.4, 151.3, 153.9; IR (KBr) υ cm^{-1}: 3422, 3079, 2923, 2853, 2271, 2145, 1735, 1571, 1447, 1397, 1089, 808, 735; ESI-MS *m/z* $(M + H)^+$: 163; HRMS *m/z* calcd for $C_8H_4N_2Cl$ $[M + H]^+$: 163.0057; found: 163.0061; Anal. ($C_8H_3N_2Cl$): C 59.10%, H 1.86%, N 17.23%; found: C 59.05%, H 1.92%, N 17.18%.

*4.1.2.5. Ethyl 3-(6-chloropyridin-3-yl) prop-2-ynoate (**6c**).* Colorless solid; mp 72–74 °C; ^1H NMR (δ ppm in $CDCl_3$, 300 MHz): 1.37 (3H, t, *J* = 7.1 Hz, $-CH_3$), 4.29 (2H, q, *J* = 7.1 Hz, $-CH_2$), 7.35 (1H, d, *J* = 8.3 Hz, Pyridine–H), 7.80 (1H, dd, *J* = 2.2, 8.3 Hz, Pyridine–H), 8.59 (1H, d, *J* = 2.2 Hz, Pyridine–H); ^{13}C NMR (δ ppm in $CDCl_3$): 14.0, 62.3, 80.8, 84.4, 115.8, 124.2, 142.0, 151.2, 152.9, 153.2; IR (KBr) υ cm^{-1}: 3395, 3092, 3039, 2983, 2928, 2855, 2235, 2206, 1985, 1707, 1577, 1544, 1458, 1366, 1291, 1188, 1101; ESI-MS *m/z* $(M + H)^+$: 210; HRMS *m/z* calcd for $C_{10}H_9O_2NCl$ $[M + H]^+$: 210.0316; found: 210.0319; Anal. ($C_{10}H_8O_2NCl$): C 57.29%, H 3.85%, N 6.68%; found: C 56.98%, H 3.89%, N 6.48%.

*4.1.2.6. Propan-2-yl 3-(6-chloropyridin-3-yl)prop-2-ynoate (**6d**).* Colorless solid; mp 58–60 °C; ^1H NMR (δ ppm in $CDCl_3$, 300 MHz): 1.34 (6H, d, *J* = 6.2 Hz, two $-CH_3$ groups), 5.08–5.18 (1H, m, –CH), 7.36 (1H, d, *J* = 8.3 Hz, Pyridine–H), 7.80 (1H, dd, *J* = 2.2, 8.3 Hz, Pyridine–H), 8.59 (1H, d, *J* = 2.2 Hz, Pyridine–H); ^{13}C NMR (δ ppm in $CDCl_3$): 21.1, 70.1, 80.0, 84.3, 115.5, 123.7, 141.5, 151.2, 152.3, 152.7; IR (KBr) υ cm^{-1}: 3377, 3035, 2985, 2928, 2223, 1701, 1578, 1545, 1455, 1291, 1204, 1099; ESI-MS *m/z* $(M + H)^+$: 224; HRMS *m/z* calcd for $C_{11}H_{11}O_2NCl$ $[M + H]^+$: 224.0300; found: 224.0317; Anal. ($C_{11}H_{10}O_2NCl$): C 59.07%, H 4.51%, N 6.26%; found: C 59.02%, H 4.72%, N 6.15%.

*4.1.2.7. 3-(2,6-Dichloro-5-fluoropyridin-3-yl)prop-2-ynenitrile (**6e**).* Colorless solid; mp 87–89 °C; ^1H NMR (δ ppm in $CDCl_3$): 7.65 (1H, d, *J* = 7.1 Hz, Pyridine–H); IR (KBr) υ cm^{-1}: 3057, 2924, 2854, 2229, 1727, 1617, 1551, 1404, 1241, 1125, 1061, 824, 721; ESI-MS *m/z* $(M + H)^+$: 215; HRMS *m/z* calcd for $C_8H_2N_2Cl_2F$ $[M + H]^+$: 214.9579; found: 214.9577; Anal. ($C_8H_1N_2Cl_2F$): C 44.69%, H 0.47%, N 13.03%; found: C 44.62%, H 0.53%, N 12.98%.

*4.1.2.8. 3-(2-Methylphenyl)prop-2-ynenitrile (**6f**).* Light yellow color liquid; ^1H NMR (δ ppm in $CDCl_3$, 300 MHz): 2.48 (3H, S, $-CH_3$), 7.20 (1H, d, *J* = 7.5 Hz, Ar–H), 7.27 (1H, d, *J* = 6.7 Hz, Ar–H), 7.41 (1H, t, *J* = 6.7, 7.5 Hz, Ar–H), 7.56(1H, d, *J* = 6.7 Hz, Ar–H); ^{13}C NMR (δ ppm in $CDCl_3$): 20.4, 82.3, 85.0, 105.5, 117.4, 126.0, 130.0, 131.7, 134.0, 143.9; IR (KBr) υ cm^{-1}: 3065, 2924, 2265, 2140, 760; ESI-MS *m/z* $(M + H)^+$:142; HRMS *m/z* calcd for $C_{10}H_8N$ ($[M + H]^+$): 142.0657; found: 142.0649; Anal. ($C_{10}H_7N$): C 85.08%, H 5.00%, N 9.92%; found: C 85.02%, H 5.08%, N 9.79%.

*4.1.2.9. Ethyl 3-(2-methylphenyl)prop-2-ynoate (**6g**).* Light yellow color liquid; ^1H NMR (δ ppm in $CDCl_3$, 300 MHz): 1.35 (3H, t, *J* = 7.1 Hz, $-CH_3$), 2.48 (3H, s, $-CH_3$), 4.30 (2H, q, *J* = 7.1 Hz, $-CH_2$), 7.14–7.35 (3H, m, Ar–Hs), 7.53 (1H, d, *J* = 6.9 Hz, Ar–H); ^{13}C NMR (δ ppm in $CDCl_3$): 13.6, 20.0, 61.5, 83.9, 84.6, 119.0, 125.2, 129.2, 130.0, 132.9, 141.7, 153.7; IR (KBr) υ cm^{-1}: 3399, 2983, 2206, 1708, 1187, 765; ESI-MS *m/z* $(M + H)^+$: 189; HRMS *m/z* calcd for $C_{12}H_{13}O_2$ ($[M + H]^+$): 189.0916; found: 189.0912; Anal. ($C_{12}H_{12}O_2$): C 76.57%, H 6.43%; found: C 76.65%, H 6.50%.

*4.1.2.10. Ethyl 4,4-dimethylpent-2-ynoate (**6h**).* Red color liquid; ^1H NMR (δ ppm in $CDCl_3$, 300 MHz): 0.71 (3H, t, *J* = 7.1 Hz, $-CH_3$), 1.27 (9H, S, three $-CH_3$ groups), 3.59 (2H, q, *J* = 7.1 Hz, $-CH_2$); IR (KBr) υ cm^{-1}: 2926, 2852, 2227, 1725; ESI-MS *m/z* $(M + H)^+$:155; HRMS *m/z* calcd for $C_9H_{15}O_2$ $[M + H]^+$: 155.0982; found: 155.0979; Anal. ($C_9H_{14}O_2$): C 70.10%, H 9.15%; found: C 70.08%, H 9.21%.

4.2. Biology

All the test organisms used for antimicrobial activity study were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh, India.

4.2.1. Antibacterial activity

The minimum inhibitory concentration (MIC) of various synthetic compounds (**5a**–**p**, **6a**–**h**) were obtained against three representative *Gram-positive* microorganisms *viz*., *B. subtilis* (MTCC 441), *S. aureus* (MTCC 96), *S. epidermidis* (MTCC 435) and *Gram-negative* microorganisms *viz*., *E. coli* (MTCC 443), *P. aeruginosa* (MTCC 741), and *K. pneumoniae* (MTCC 618) using broth dilution method recommended by National Committee for Clinical Laboratory (NCCL) standards [24,25].

Standard antibacterial agents like penicillin and streptomycin were also screened under identical conditions for comparison.

4.2.2. Antifungal activity

The *in vitro* antifungal efficacy of all the synthesized phosphoranes (**5a**–**p**) and alkynes (**6a**–**h**) was determined against five fungal strains *viz*., *C. albicans* (MTCC 227), *C. rugosa* (NCIM 3462), *S. cerevisiae* (MTCC 36), *A. niger* (MTCC 344), *A. flavus* (MTCC 277) by agar well diffusion method. The fungal strains were obtained from

the Institute of Microbial Technology, Chandigarh. The protocol used for the antibacterial activity was followed using potato dextrose agar as media instead of nutrient agar and amphotericin B as positive control. The treated specimens and controls with DMSO were kept at 27 °C for 48 h. Inhibition zones were measured and the diameter was calculated in millimeters. Three to four replicates were maintained for each treatment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.07.013.

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