Monatsh Chem (2012) 143:283–288
DOI 10.1007/s00706-011-0652-x

ORIGINAL PAPER

Synthesis, characterization, and antifungal activity of biaryl-based bis(1,2,3-triazoles) using click chemistry

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Received: 9 February 2010 / Accepted: 4 September 2011 / Published online: 22 October 2011

Abstract Click chemistry was used to synthesize a series of biaryl-based bis(1,2,3-triazoles). Their antifungal activity was evaluated against three soil-borne plant pathogenic fungi, viz. *Rhizoctonia bataticola*, *Sclerotium rolfsii*, and *Fusarium oxysporum*, using the food poison technique at concentrations of 62.5–500 μg/cm^3.

Keywords Biaryls · Antifungal · Click chemistry · 1,2,3-Triazoles

Introduction

Huisgen's 1,3-dipolar cycloaddition reaction of azides with substituted acetylenes is a well-established route to 1,2,3-triazoles [1], but the reaction requires high temperature, long reaction times, and is not regiospecific. Copper(I)-catalyzed click chemistry discovered by Sharpless and co-workers [2, 3] has put this reaction center stage and since then it has become a reliable method to synthesize 1,2,3-triazoles. The presence of Cu(I) dramatically accelerates the rate and makes the reaction highly regioselective, leading to only 1,4-disubstituted isomers [4]. Moreover, the reaction can be performed at room temperature and under a variety of conditions [5].

1,2,3-Triazoles have attracted the interest of organic chemists for development of new biologically active molecules owing to their stability, resistance to hydrolysis and metabolic degradation, and capability to form hydrogen bonds. 1,2,3-Triazoles have been shown to possess diverse biological activities, e.g., they are antifungal and antibacterial agents, enzyme inhibitors [6–8], and are useful for the treatment of various diseases [9, 10]. In spite of much progress in diverse areas of research [11–13], the agrochemical potential of 1,2,3-triazoles has not been explored fully so far. Plant pathogenic fungi are of concern as they cause a substantial loss of crop. Among various pathogenic fungi, which alone cause nearly 20% reduction in the yield of major food and cash crops, *Sclerotium rolfsii*, *Rhizoctonia bataticola*, and *Fusarium oxysporum* are soil-borne fungi which devastate a wide range of hosts. These fungi infect seeds, seedlings, and mature plants in the field causing collar rot, wilt, damping off, dry root rot, and spoilage [14–16]. To the best of our knowledge, reports of antifungal activity of 1,2,3-triazoles against plant pathogenic strains are scarce. Here, we report synthesis and antifungal activity of a series of highly substituted biaryl-based bis(1,2,3-triazoles) against three soil-borne pathogenic strains.

Electronic supplementary material The online version of this article (doi:10.1007/s00706-011-0652-x) contains supplementary material, which is available to authorized users.

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Results and discussion

The triazoles were synthesized in good yields (85–98%) from three new bisazides obtained from their respective amines. The position of the azide group was confirmed by 2D NMR spectroscopy of the corresponding nitro compound. The reaction conditions were optimized by examining the reaction of bisazide **1** with phenyl acetylene in different solvents (Table 1), the best results being obtained using *t*-butanol/water (3:1) and ethanol. However, product isolation was found to be easier in the former case. In the presence of acetonitrile several products were formed, whereas the reaction in tetrahydrofuran (THF) was not complete even after 3 days.

A series of compounds were synthesized bearing a variety of substituents such as alkyl, aromatic, and heterocyclic groups (Table 2).

Studies on antifungal activity

The compounds were tested for their potential antifungal activity using the food poison technique. The results obtained in terms of percentage inhibition (mean of three replicates) and ED_{50} values are shown in Table 3.

The newly synthesized triazoles showed moderate to good antifungal activity. They were found to be more effective against *R. bataticola* (55–100% inhibition) than *S. rolfsii* and *F. oxysporum* at a concentration of 500 μg/cm^3. ED_{50} values of most of these compounds were in the range of 200–500 μg/cm^3 against all three fungi. The ED_{50} values of these compounds against *R. bataticola* and *S. rolfsii* were in the range of ~55–800 and ~350–875 μg/cm^3, respectively. Except for compounds **1a** and **2d**, the ED_{50} values were 110–450 μg/cm^3 against *F. oxysporum*. Carboxin was used as a standard fungicide and its ED_{50} values were 8.73, 11.78, and 10.34 μg/cm^3 against *R. bataticola*, *S. rolfsii*, and *F. oxysporum*, respectively. Further work on the synthesis and structure–activity relationship is currently under progress.

Table 1 Effect of solvent on reaction of bisazide **1** and phenyl acetylene

Solvent	Ratio	Yield/%	Time/h
Water	Neat	64	16
Acetonitrile	Neat	Mixture	–
THF	Neat	Incomplete	>72
Ethanol	Neat	99	14
Ethanol/water	3:1	98.9	14
t-Butanol	Neat	91.9	12
t-Butanol/water	3:1	99.9	12
t-Butanol/water	1:1	98.0	14

The reaction was performed using the bisazide (0.05 mmol), alkyne (0.1 mmol), sodium ascorbate (10^{-2} mmol), and $CuSO_4$ (10^{-3} mmol)

Experimental

Chemicals and solvents were purchased from Sigma-Aldrich and E. Merck. Culture media (PDA) were purchased from HiMedia. Reactions were monitored by thin-layer chromatography (TLC) on fluorescent coated plates purchased from E. Merck. Melting points were measured on an electrothermal melting point apparatus. IR spectra were recorded on a Spectrum BX series spectrophotometer using KBr disks. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz spectrometer. TMS was used as the internal standard in all cases. Mass spectra were recorded on a Qstar XL instrument and processed using Analyst QS software.

5,5′-Diazido-2,2′,3,3′-tetramethoxy-1,1′-biphenyl (**1**, $C_{16}H_{16}N_6O_4$)

The diamine (260 mg, 0.73 mmol) was dissolved in 5 cm^3 diluted HCl (1:1) and diazotized with 210 mg sodium nitrite (0.03 mmol) below 5 °C. The solution was filtered and added slowly to a freshly prepared solution of 150 mg NaN_3 (2.3 mmol) containing excess sodium acetate. The reaction mixture was stirred for 2 h and the resulting solid was filtered, washed with water, and dried. It was recrystallized from petroleum ether to yield 120 mg (40%) **1**. M.p.: 143–144 °C; IR: $\bar{\nu}$ = 2,939, 2,828, 2,111 (N_3), 1,583, 1,488, 1,314, 1,246, 1,112, 1,038, 1,002, 954 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 3.6 (s, 6H, –OCH_3), 3.8 (s, 6H, –OCH_3), 6.5 (s, 4H, Ar-H, J = 3 Hz) ppm; ^{13}C NMR ($CDCl_3$): δ = 55.9, 60.8, 103.4, 112.6, 132.8, 135.2, 144.0, 153.7 ppm.

5,5′-Diazido-2,2′,3,3′,6,6′-hexamethoxy-1,1′-biphenyl (**2**, $C_{18}H_{20}N_6O_6$)

The diamino compound (500 mg, 1.3 mmol) was dissolved in 2 cm^3 conc. hydrochloric acid and cooled in ice bath. It was diazotized by adding sodium nitrite solution (200 mg in 1 cm^3 water) slowly. The diazotized solution was filtered and subsequently added to a stirred solution of 190 mg sodium azide and 10 cm^3 sodium acetate in water. The reaction mixture was stirred for 1 h, and the resulting light yellow solid was filtered. Yield 478 mg (83.7%); m.p.: 83–84 °C; IR: $\bar{\nu}$ = 2,942, 2,834, 2,104 (N_3), 1,590, 1,482, 1,246, 1,046, 969, 816 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 3.6 (6H, s, –OCH_3), 3.7 (6H, s, –OCH_3), 3.9 (6H, s, –OCH_3), 6.6 (2H, s, Ar-H) ppm; ^{13}C NMR ($CDCl_3$): δ = 56.1, 60.7, 61.5, 104.1, 124.0, 127.9, 149.5 ppm.

Table 2 Synthesis of highly substituted biaryl-based bis(1,2,3-triazoles)

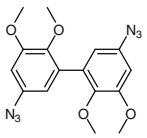
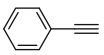
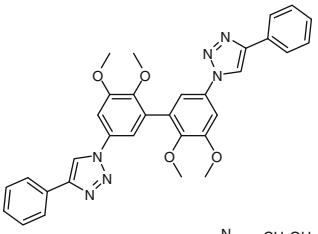
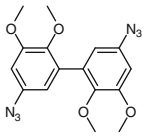
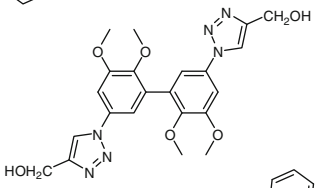
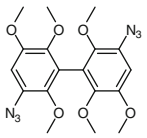
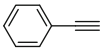
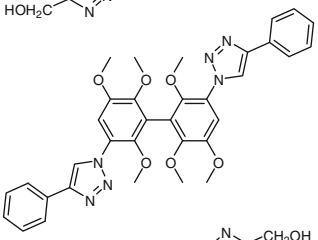
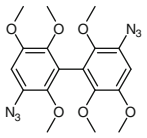
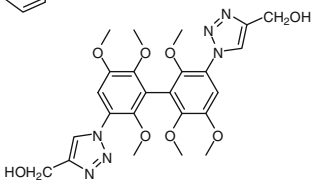
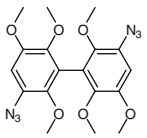
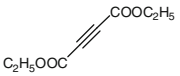
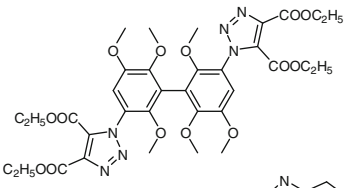
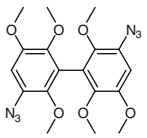
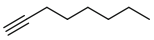
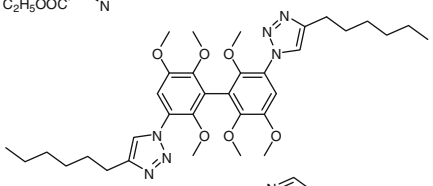
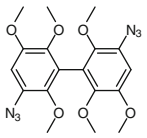
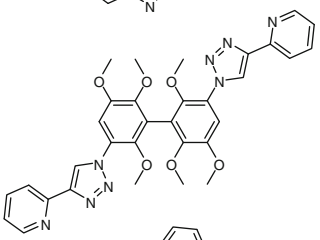
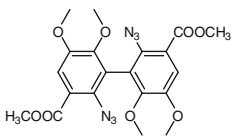
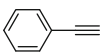
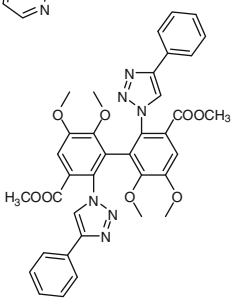
Bisazide	Alkyne	Product	Yield/%
		**1a** 	97.7
		**1b** 	88.1
		**2a** 	95.1
		**2b** 	87.0
		**2c** 	85.0
		**2d** 	89.3
		**2e** 	93.1
		**3a** 	88.3

Table 3 Antifungal activity of newly synthesized triazoles

Compound	Fungi	Inhibition (%) at different concentrations/μg cm^{-3}				ED_{50}/μg cm^{-3}
		500	250	125	62.5	
1a	Rb	100	87.7	78.8	54.4	56.6
	Sr	25.0	11.1	0	0	828.9
	Fo	34.4	25.5	21.1	17.7	2,829.8
1b	Rb	56.6	45.5	35.0	28.8	331.9
	Sr	50.0	38.8	33.0	22.2	508.2
	Fo	65.5	57.7	35.5	21.2	224.0
2a	Rb	100	78.4	55.0	23.3	114.7
	Sr	32.2	16.6	11.1	0	871.3
	Fo	73.8	62.7	55.0	38.3	111.9
2b	Rb	54.4	38.3	32.2	20.2	416.1
	Sr	36.6	7.7	1.1	0	634.1
	Fo	55.0	45.5	36.6	26.1	344.0
2c	Rb	65.5	50.5	16.4	8.3	298.0
	Sr	66.1	43.2	12.2	0	325.9
	Fo	51.0	37.7	23.3	8.8	442.7
2d	Rb	55.0	41.6	38.3	13.8	353.4
	Sr	54.4	38.3	12.2	0	395.9
	Fo	34.4	21.1	18.8	13.3	2,047.8
2e	Rb	70.5	53.8	31.6	21.1	225.8
	Sr	54.4	14.4	0	0	463.5
	Fo	52.7	38.8	30.5	21.6	452.0
3a	Rb	74.2	48.3	28.3	13.6	244.6
	Sr	34.4	21.6	7.3	0	709.9
	Fo	67.7	45.5	38.8	24.4	240.1

Rb, *Rhizoctonia bataticola*; Sr, *Sclerotium rolfsii*; Fo, *Fusarium oxysporum*; no inhibition was observed in dimethyl sulfoxide (DMSO) used as control

Dimethyl 2,2′-diazido-5,5′,6,6′-tetramethoxy-1,1′-biphenyl-3,3′-dicarboxylate (**3**, $C_{20}H_{20}N_6O_8$)

The diamine (140 mg, 3 mmol) was dissolved in 2.5 cm^3 conc. HCl and diazotized with 414 mg sodium nitrite (6 mmol) below 5 °C. The solution was filtered and added slowly to a freshly prepared solution of NaN_3 (260 mg in 1 cm^3 water) containing excess sodium acetate. The reaction mixture was stirred for 2 h and left overnight in the cold. The next day the white solid was filtered, dried, and recrystallized from petroleum ether. Yield 103 mg (92.2%); m.p.: 94–95 °C; IR: $\bar{\nu}$ = 3,099, 3,003, 2,947, 2,843, 2,125 (N_3), 1,719, 1,588, 1,481, 1,427, 1,295, 1,211, 1,161, 1,025, 1,005, 794 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 3.7 (s, 6H, –$COOCH_3$), 3.9 (s, 6H, –OCH_3), 4.0 (s, 6H, –OCH_3), 7.5 (s, 2H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$): δ = 52.4, 56.1, 60.7, 115.1, 119.3, 124.8, 128.8, 133.2, 149.8, 150.9, 165.5 ppm.

General procedure for the synthesis of triazoles [2]

The bisazide (0.4 mmol) and the alkyne (0.4 mmol) were added to a mixture of *t*-butanol/water (3:1). To the stirred reaction mixture sodium ascorbate (0.04 mmol) and $CuSO_4$ (0.004 mmol) were added. Completion of the reaction was monitored by TLC. After completion of the reaction (14–48 h) the reaction mixture was diluted with water and the resulting solid filtered. TLC showed that all compounds were pure.

1,1′-(5,5′,6,6′-Tetramethoxy-1,1′-biphenyl-3,3′-diyl)bis(4-phenyl-1H-1,2,3-triazole) (**1a**, $C_{32}H_{28}N_6O_4$)

Yield 97.7%; m.p.: 132–134 °C; R_f = 0.45 ($CHCl_3$); IR: $\bar{\nu}$ = 3,435, 3,132, 2,941, 1,595, 1,498, 1,466, 1,420, 1,276, 1,233, 1,154, 1,054, 844 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 3.8 (s, 6H, –OCH_3), 4.0 (s, 6H, –OCH_3), 7.4, 7.5, 7.9 (6H, Ar-H), 8.2 (triazole-H) ppm; ^{13}C NMR ($CDCl_3$): δ = 56.1, 60.9, 105.0, 113.9, 117.8, 125.6, 128.3, 128.8, 129.9, 131.9, 132.5, 146.8, 148.3, 153.6 ppm.

1,1′-(5,5′,6,6′-Tetramethoxy-1,1′-biphenyl-3,3′-diyl)-bis(1H-1,2,3-triazole-4-methanol) (**1b**, $C_{22}H_{24}N_6O_6$)

Yield 88.1%; m.p.: 152–154 °C; R_f = 0.3 (ethyl acetate); IR: $\bar{\nu}$ = 3,368, 2,939, 1,592, 1,490, 1,465, 1,417, 1,267, 1,238, 1,150, 1,066, 1,035, 872 cm^{-1}; ^{1}H NMR (acetone-d_6): δ = 3.7 (s, 6H, –OCH_3), 4.1 (s, 6H, –OCH_3), 4.8 (s, 4H, –CH_2–), 7.4 (d, Ar-H, J = 2.1 Hz), 7.6 (d, Ar-H, J = 2.1 Hz), 8.5 (triazole-H) ppm; ^{13}C NMR (acetone-d_6): δ = 56.6, 60.9, 105.7, 114.7, 121.3, 133.5, 133.7, 147.6, 154.5, 206.2, 206.5 ppm.

1,1′-(2,2′,5,5′,6,6′-Hexamethoxy-1,1′-biphenyl-3,3′-diyl)-bis(4-phenyl-1H-1,2,3-triazole) (**2a**, $C_{34}H_{32}N_6O_6$)
Yield 95.1%; m.p.: 120–122 °C; $R_f = 0.40$ ($CHCl_3$); IR: $\bar{\nu} = 3{,}435, 3{,}144, 2{,}935, 1{,}599, 1{,}478, 1{,}420, 1{,}226, 1{,}147, 1{,}053, 831\ cm^{-1}$; ^1H NMR ($CDCl_3$): $\delta = 3.3$ (s, 6H, $–OCH_3$), 3.8 (s, 6H, $–OCH_3$), 4.0 (s, 6H, $–OCH_3$), 7.3, 7.4, 7.5, 7.9 (6X Ar-H), 8.4 (triazole-H) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 56.3, 60.8, 61.2, 109.1, 121.3, 123.4, 125.8, 126.0, 128.3, 128.9, 130.3, 143.8, 147.9, 148.0, 149.5$ ppm.

1,1′-(2,2′,5,5′,6,6′-Hexamethoxy-1,1′-biphenyl-3,3′-diyl)-bis(1H-1,2,3-triazole-4-methanol) (**2b**, $C_{24}H_{28}N_6O_8$)
Yield 87%; m.p.: 192–194 °C; $R_f = 0.3$ (ethyl acetate); IR: $\bar{\nu} = 3{,}411, 3{,}158, 2{,}941, 1{,}601, 1{,}492, 1{,}471, 1{,}418, 1{,}220, 1{,}054, 1{,}036, 840\ cm^{-1}$; ^1H NMR (DMSO-d_6): $\delta = 3.3$ (s, 6H, $–OCH_3$), 3.8 (s, 6H, $–OCH_3$), 3.9 (s, 6H, $–OCH_3$), 4.9 (s, 4H, $–CH_2–$), 7.4 (s, 2H, Ar-H), 8.1 (triazole-H) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 54.8, 56.2, 59.7, 60.2, 60.9, 110.3, 122.8, 124.4, 125.6, 144.5, 147.4, 148.3, 148.7, 170.3$ ppm.

Tetraethyl 1,1′-(2,2′,5,5′,6,6′-hexamethoxy-1,1′-biphenyl-3,3′-diyl)bis(1H-1,2,3-triazole-4,5-dicarboxylate) (**2c**, $C_{34}H_{40}N_6O_{14}$)
Yield 85%; m.p.: 140–142 °C; $R_f = 0.62$ ($CHCl_3$/ethyl acetate 9:1); IR: $\bar{\nu} = 3{,}104, 2{,}981, 2{,}943, 1{,}747, 1{,}602, 1{,}560, 1{,}489, 1{,}421, 1{,}376, 1{,}350, 1{,}284, 1{,}225, 1{,}085, 1{,}021, 841\ cm^{-1}$; ^1H NMR ($CDCl_3$): $\delta = 1.2$ (t, 3H, CH_3), 1.4 (t, 3H, CH_3), 4.2 (q, 4H, $–CH_2$), 4.5 (q, 4H, $–CH_2$), 3.2 (s, 6H, $–OCH_3$), 3.8 (s, 6H, $–OCH_3$), 3.9 (s, 6H, $–OCH_3$), 7.2 (s, 2H, Ar-H) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 13.7, 14.2, 56.2, 60.8, 61.3, 61.5, 61.8, 62.6, 110.3, 123.0, 124.5, 133.3, 138.7, 145.1, 149.1, 149.5, 158.4, 159.7$ ppm.

1,1′-(2,2′,5,5′,6,6′-Hexamethoxy-1,1′-biphenyl-3,3′-diyl)-bis(4-hexyl-1H-1,2,3-triazole) (**2d**, $C_{34}H_{48}N_6O_6$)
Yield 89.3%; m.p.: 54–56 °C; $R_f = 0.65$ ($CHCl_3$/ethyl acetate 9:1); IR: $\bar{\nu} = 2{,}929, 2{,}855, 1{,}601, 1{,}496, 1{,}421, 1{,}221, 1{,}143, 1{,}059, 839\ cm^{-1}$; ^1H NMR ($CDCl_3$): $\delta = 0.8$ (s, 6H, CH_3), 1.3 (s, 12H, CH_2), 1.7 (s, 4H, CH_2), 2.8 (s, 4H, CH_2), 3.2 (s, 6H, $–OCH_3$), 3.7 (s, 6H, $–OCH_3$), 3.9 (s, 6H, $–OCH_3$), 7.4 (s, 2H, Ar-H), 7.8 (triazole-H) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 13.9, 22.5, 25.5, 28.7, 29.3, 29.6, 31.4, 56.1, 60.7, 60.8, 109.1, 122.4, 123.3, 126.2, 143.7, 147.5, 148.6, 149.4$ ppm.

1,1′-(2,2′,5,5′,6,6′-Hexamethoxy-1,1′-biphenyl-3,3′-diyl)-bis[4-(2-pyridinyl)-1H-1,2,3-triazole] (**2e**, $C_{32}H_{30}N_8O_6$)
Yield 93.1%; m.p.: 98–100 °C; $R_f = 0.22$ ($CHCl_3$); IR: $\bar{\nu} = 3{,}424, 2{,}924, 2{,}837, 1{,}606, 1{,}492, 1{,}469, 1{,}425, 1{,}221, 1{,}149, 1{,}058, 1{,}028, 930\ cm^{-1}$; ^1H NMR ($CDCl_3$): $\delta = 3.3$ (s, 6H, $–OCH_3$), 3.8 (s, 6H, $–OCH_3$), 3.9 (s, 6H, $–OCH_3$), 7.4, 7.8, 8.2, 8.6 (Ar-H and pyridine-H), 8.7 (triazole-H) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 56.2, 60.8, 61.2, 109.0, 115.0, 121.0, 122.8, 123.7, 125.0, 137.0, 144.0, 148.0, 148.1, 149.5, 149.9$ ppm.

Dimethyl 5,5′,6,6′-tetramethoxy-2,2′-bis(4-phenyl-1H-1,2,3-triazol-1-yl)-1,1′-biphenyl-3,3′-dicarboxylate (**3a**, $C_{36}H_{32}N_6O_8$)
Yield 88.3%; m.p.: 128–130 °C; $R_f = 0.55$ ($CHCl_3$/ethyl acetate 9:1); IR: $\bar{\nu} = 3{,}444, 2{,}947, 1{,}718, 1{,}589, 1{,}479, 1{,}352, 1{,}216, 1{,}049, 997, 772\ cm^{-1}$; ^1H NMR ($CDCl_3$): $\delta = 3.5$ (s, 6H, $–OCH_3$), 3.8 (s, 6H, $–OCH_3$), 3.9 (s, 6H, $–OCH_3$), 7.3, 7.4, 7.8 (6X Ar-H), 8.3 (triazole-H) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 52.54, 56.07, 61.50, 114.95, 123.44, 124.00, 125.78, 125.94, 127.97, 128.75, 129.15, 130.49, 146.63, 149.57, 152.75, 164.77$ ppm.

Antifungal assay

Microorganisms and media used

The newly synthesized triazoles were tested for antifungal activity against three soil-borne pathogenic fungi, namely *Sclerotium rolfsii* (ITCC 5226), *Rhizoctonia bataticola* (ITCC 0842), and *Fusarium oxysporum* (ITCC 2042), by the food poison technique. These fungi were collected from the Indian Type Culture, Division of Plant Pathology, Indian Agricultural Research Institute (New Delhi, India) and were maintained on potato dextrose agar (PDA) at 25 °C and were subcultured on PDA petri dishes for 5–6 days at 28 °C prior to use as inoculums.

Food poison technique

The ready-made PDA medium (39 g) was suspended in distilled water (1,000 cm^3) and heated to boiling until completely dissolved. The medium and petri dishes were autoclaved at 120 °C for 30 min. The compounds were tested at concentrations of 500, 250, 125, and 62.5 μg/cm^3. A stock solution of 1,000 μg/cm^3 was prepared, which was further diluted with DMSO to give the required concentrations. DMSO (1 cm^3) was used as the control. These solutions were added to the media (65 cm^3) contained in conical flasks to obtain the desired concentrations of the test compounds in the media. The medium was poured into a two petri dishes (90 cm in diameter) under aseptic conditions in a laminar flow hood. The plates were kept under UV light in the laminar flow chamber for solidification of the media. After solidification, a 5-mm mycelial plug cut from the actively growing front of a 2-week-old colony of the desired pathogenic fungus was then placed with the inoculum side down in the center of each treatment plate, aseptically. Treated petri dishes were then incubated at 28 °C until the fungal growth was almost complete in the control plates.

Calculation of ED_{50} values

The mycelial growth of fungus (cm) in both treated and control petri dishes was measured diametrically. The mean and standard errors were calculated from the three replicates of each treatment, and the percentage inhibition of growth (I) was calculated using the following equation:

$$I(\%) = \frac{C - T}{C} \times 100 \quad (1)$$

where C is the diameter of fungal growth in the control and T is the diameter fungal of growth in the treated plates.

For calculation of ED_{50} values (effective dose required for 50% inhibition of growth), the percentage inhibition was converted to corrected inhibition by using Abbott's formula:

$$\text{Corrected inhibition } (\%) = \frac{\%I - \text{CF}}{100 - \text{CF}} \times 100 \quad (2)$$

where CF is the correction factor $[(9 - C)/C]$. ED_{50} values were calculated (effective dose for 50% inhibition, μg/cm^3) for inhibition of growth using the Basic LD_{50} program, version 1.1 [17].

Acknowledgments We thank the Principal of St. Stephen's College, Delhi for providing facilities. We thank the Defence Research Development Organisation (DRDO) and University Grants Commission (UGC), Govt. of India, New Delhi for the grant for research projects. We are grateful to Dr. Vairmani, Head, Chemical & Instrumental Analysis, IICT, Hyderabad for mass spectrometric studies. We thank Prof. Amit Basu for introducing click chemistry to one of us (SVE) during a visit to Prof. David Cane's laboratory in the Department of Chemistry, Brown University, Providence, RI, USA under a St. Stephen's College–Brown University exchange program. One of the authors (LS) thanks UGC, New Delhi for the award of junior research fellowship.

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