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ORIGINAL PAPER

Efficient synthesis of bis-isoxazole ethers via 1,3-dipolar cycloaddition catalysed by Zn/Zn^{2+} and their antifungal activities

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An efficient method was developed for synthesising isoxazoles. A series of novel bis-isoxazole ether compounds *VI*, *VII* and *VIII* were synthesised starting from different substituted aldehydes (*I*) via a 1,3-dispolar cycloaddition using Zn/Zn^{2+} as a catalyst; these were characterised by FT-IR, HRMS, ^{1}H NMR and ^{13}C NMR spectroscopy. In addition, the antimicrobial properties of the synthesised products were investigated. The synthesised compounds exhibited significant antifungal activities in comparison with the standard drugs, fluconazole and itraconazole. It was found that *Candida albicans* was sensitive to 2-substituted phenyl bis-isoxazole ethers bearing pyridyl.

Keywords: bis-isoxazole ether, 1,3-dispolar cycloaddition, Zn/Zn^{2+}, anti-fungal activity, nitrile oxide

Introduction

Isoxazole and pyridine compounds are important members of heterocycles in pharmaceuticals. In particular, isoxazole (Tanaka et al., 2010; Barbachyn et al., 2003; Pirrung, et al., 2002) and pyridine derivatives (Renard et al., 2009; Liu et al., 1996; Li et al., 2006) are, respectively, one of many important pharmacophores of medicinal interest, possessing potent biological activity. Hence, the development of simple and practical methods for synthesising their derivatives is an important target in organic and medicinal chemistry. Recently, many syntheses of isoxazoles have been reported, including 1,3-dipolar cycloaddition reaction starting from nitrile oxides and alkynyl or alkenyl (Shen et al., 2011; Daliboyena & Nefzi, 2012; Bhuniya et al., 2009; Gothelf & Jørgensen, 1998; Stanley & Sibi, 2008; Kanemasa & Tsuge, 1990), the Claisen reaction of hydroxylamine with the β-unsaturated ketone and ester or 1,3-dicarbonyl compound (Cuadrado et al., 2002; Kurangi et al., 2007; Heravi et al., 2008), the addition reaction of 2-alkyne-1-one *O*-methyl oximes with the unsaturated compound (Waldo & Larock, 2005) and the reaction between halogenated cyclopropane and nitrosyl cation (Lin et al., 1997), etc. Among them, 1,3-dipolar cycloaddition is recognised as one of the most effective methods in medicinal chemistry; because the raw materials with different functional groups are facile, the desired products might be synthesised with high yields by combining varieties of alkynes and nitrile oxides (Stevens, 1976; van Mersbergen et al., 1998; Katritzky et al., 2000). Furthermore, the 1,3-dipolar cycloaddition of alkynes and nitrile oxides was also identified as the most direct approach to obtaining heterocycle compounds (Katritzky et al., 2000; Minakata et al., 2011; Yoshimura et al., 2013). In the previous work, copper(I), $ZnCl_2$, ruthenium(II) and other Lewis acids as catalysts have been revealed to react regioselectively with nitrile oxides to generate 3,5-disubstituted isoxazoles and/or 3,4- and 3,4,5-substituted isoxazoles (Grecian & Fokin, 2008; Tanaka et al., 2010; Himo et al., 2005; Hansen et al., 2005). However, the synthesis of bis-isoxazole ethers starting from 3-phenyl-5-((prop-2-yn-1-yloxy)methyl)isoxazole has not previously been reported as using the above

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catalysts for the reaction without the catalyst or the unsuitable catalyst added was puzzled by low yields or trace. Accordingly, it is important to develop a novel catalyst or catalytic process for the 1,3-dipolar cycloaddition reaction to construct synthesise fused isoxazole derivatives with high yields. In the present study, Zn/Zn^{2+} was chosen to catalyse the 1,3-dipolar cycloaddition reaction by employing 3-substituted phenyl-5-prop-2-ynyloxymethyl-isoxazoles and *N*-hydroxynicotinimidoyl chloride. In pursuit of new potent compounds, a series of new 3-(substituted-phenyl)-5-(((3-aryl-isoxazol-5-yl)methoxy)methyl) isoxazole were prepared, and their activities were studied, with the results of those studies reported here.

Experimental

Various aromatic aldehydes were of analytical-reagent grade from Aladdin reagent (China) and used without further purification. The other solvents and reagents used were supplied by Tianjin Tiantai Chemical (China) and Beijing Chemical Plant (China). (3-Substituted phenylisoxazol-5-yl)methanols were prepared according to the reported procedure (Shen et al., 2011). 3-Substituted phenyl-5-prop-2-ynyloxymethyl-isoxazoles were synthesised from (3-phenylisoxazol-5-yl)methanols using the reported method (Ma et al., 2009; Grischenko et al., 2013). Fluconazole and itraconazole were of analytical-reagent grade from Aladdin reagent, with purity of 99 %. DMSO was further purified prior to use. All melting points were determined on an XT–4 melting point apparatus (China) and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured using a Varian Mercury-300 NMR (USA) spectrometer or a Bruker AVANCE-500 NMR (Germany) spectrometer and with TMS as an internal standard. The chemical shift is given in δ relative to TMS. MS was collected using an Agilent HP1100/ 6890 LC/ MS (USA) spectrometer and an Agilent 1290-micrOTOF Q II spectrometer, respectively. X-ray photoelectron spectroscopy (XPS) was collected using an ESCALAB MK II (UK). FT-IR spectra were obtained by the KBr technique using an IRAffinity-1 instrument (Shimadzu, Japan) in the range of 500–3500 cm^{-1}

General procedure for synthesis of (3-phenylisoxazol-5-yl)methanols

To a solution of aldoximes (20 mmol) in *N*,*N*-dimethylformamide (DMF; 15 mL), *N*-chlorosuccin imide (NCS; 20.2 mmol, 2.7 g) was added under glacial bath conditions. Triethylamine (1.5 mmol, 4.17 mL) was added to the reaction solution followed by propargyl alcohol (60 mmol, 3.5 mL). The mixture was stirred at ambient temperature until the reaction was complete as monitored by TLC monitoring (ethyl acetate/petroleum ether $\varphi_r = 1 : 2$). Following the reaction, the slurry was extracted with ethyl acetate. The organic layers were dried over anhydrous sodium sulphate, filtered and then evaporated under vacuum to provide the crude product which was purified by column chromatography (silica gel, 200–300 mesh; Merck, Germany) to furnish the product with a 44–62 % yield.

General procedure for synthesis of 3-substituted phenyl-5-prop-2-ynyloxymethyl-isoxazoles

(3-Phenylisoxazol-5-yl)methanols (10 mmol) was poured into a stirred mixture of sodium hydride (60 mmol, 1.44 g) and anhydrous THF (20 mL) previously cooled in a glacial bath. Propargyl bromide (18 mmol, 1.4 mL) was added, and the mixture was stirred at ambient temperature until the reaction was complete as monitored by TLC (ethyl acetate/petroleum ether $\varphi_r = 1 : 4$). The slurry was filtered using a Showalter funnel. The filtrate was evaporated under vacuum to provide the crude product which was purified by column chromatography to furnish the desired product with a 68–96 % yield.

General procedure for synthesis of bis-isoxazole ethers

3-Substituted phenyl-5-prop-2-ynyloxymethyl-isoxazoles (1.0 mmol), and *N*-hydroxynicotinimidoyl chloride (1.4 mmol, 0.22 g) were dissolved in anhydrous THF (5 mL). After 10 min of stirring, Zn powder (1.5 mmol, 0.98 g) was added, then triethylamine (2.5 mmol, 0.35 mL) was poured into the mixture. The reaction process was monitored at ambient temperature by TLC (ethyl acetate/petroleum ether $\varphi_r = 1 : 1$). Once the reaction was complete, the slurry was extracted with ethyl acetate. The organic layers were dried over anhydrous sodium sulphate, filtered, then evaporated under vacuum to provide the crude product which was purified by column chromatography to furnish the product. The yields of bis-isoxazole ethers (*VIa–VIi*, *VIIa–VIIg* and *VIIIa–VIIIe*) thus obtained were 50–77 %, 63–83 % and 76–81 %, respectively.

Antimicrobial activity test

The in-vitro antimicrobial activities of all the synthesised compounds *VIa–VIi*, *VIIa–VIIg and VIIIa–VIIIe* were screened against the standard strains: *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923), *Pseudomonas aeruginosa* (ATCC9027), *Candida albicans* (ATCC10231).

A microdilution assay entitled 96-well plate is a standard used to assess antimicrobial activities. Starters of the bacterial strains were grown overnight under shaking at 37℃. The inoculum was diluted 1000 times. The compounds were dissolved in DMSO

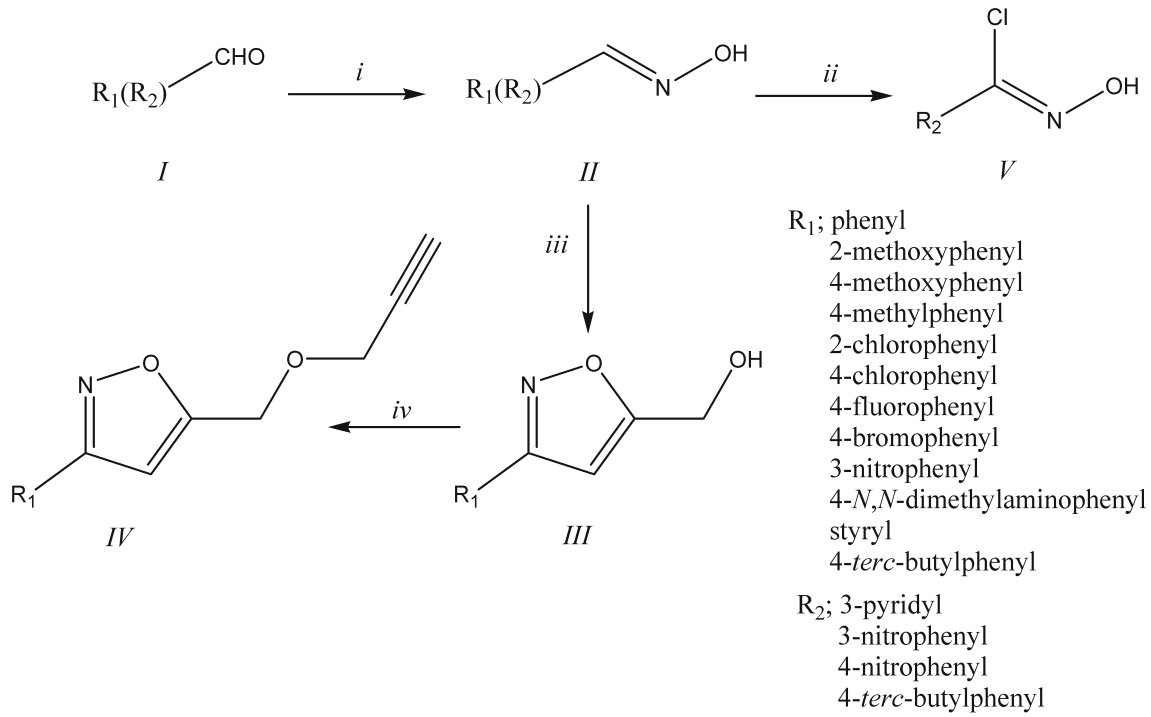


Fig. 1. Route for synthesising isoxazole alkyne and chloro-oxime derivatives from aromatic aldehydes: *i*) NH_2OHHCl, 6 M aq. NaOH, EtOH, reflux, 3–6 h, 95–98 %; *ii*) NCS, DMF, 0–20 °C, 8–14 h, 78–90 %; *iii*) NCS, DMF, 0–20 °C, 8–14 h, then propargyl alcohol, Et_3N, utrasonication, 0–20 °C, 30–50 min, 44–62 %; *iv*) propargyl bromide, NaH, THF, 0–20 °C, 3–6 h, 68–96 %.

and the antimicrobial activity was determined using the broth microdilution technique at concentrations of 64 μg mL^{-1}, 32 μg mL^{-1}, 16 μg mL^{-1}, 8 μg mL^{-1}, 4 μg mL^{-1}, 2 μg mL^{-1}, 1 μg mL^{-1}, 0.5 μg mL^{-1} and 0.25 μg mL^{-1}, respectively. Minimal inhibitory concentrations (MICs) were determined by incubating the organisms in 96-well microplates at 37 °C for 24 h, with the exception of fungi, which were cultured at 28 °C. In the case of filamentous fungi, spores were cultured for 48–72 h. DMSO and untreated inoculum were used as negative controls, while fluconazole was used as a positive control for bacteria.

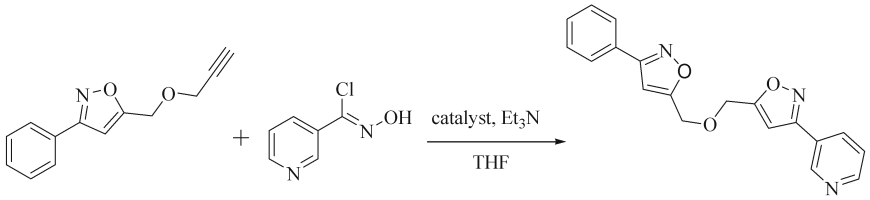
Results and discussion

3-Substituted phenyl-5-prop-2-ynyloxymethyl-isoxazoles (*IV*) were synthesised in three steps starting from substituted benzaldehyde (Fig. 1). Aldoximes were readily synthesised from substituted benzaldehyde and hydroxylamine, which was an important intermediate for the whole synthetic process and prepared with a yield in excess of 95 % in accordance with the literature (Cuadrado et al., 2002). The nucleophilic substitution reaction between aldoximes and NCS afforded *N*-hydroxynicotinimidoyl chloride and *N*-hydroxy-3(4)-nitrobenzimidoyl chloride with 88 % and 90 % yields, respectively. (3-Substituted phenylisoxazol-5-yl)methanols (*III*) were synthesised using the one-pot method following the reported procedures via the 1,3-dipolar cycloaddition reaction where the aldoximes are subjected to successive reactions in just one reactor using the ultrasound technology as in the literature (Shen et al., 2011). A series of intermediates *IV* were prepared by employing intermediates *III* and propargyl bromide under NaH with a 68–96 % yields at ambient temperature (Fig. 1).

In addition, the synthesis of 3-phenyl-5-(((3-(pyridin-3-yl)isoxazol-5-yl)methoxy)methyl)isoxazole (*VIa*) was explored by treating 3-phenyl-5-((prop-2-yn-1-yloxy)methyl)isoxazole (*IVa*) with *N*-hydroxynicotinimidoyl chloride (*Va*) employing $ZnCl_2$ as a catalyst and anhydrous THF as a solvent with reference to the literature (Tanaka et al., 2010). However, the desired compound *VIa* was not obtained (Table 1, entry 1). This might be because: *i*) nitrile oxides bearing electron-rich substituents (3-pyridyl, etc.) are not very stable and dimerise readily (Günanger et al., 1991); *ii*) the pyridine-ring promoted the formation of a complex between nitrile oxide and $ZnCl_2$, according to the literature (Wagner et al., 2007; Kanemasa et al., 1991; Tanaka et al., 2010). The present study sought a more effective catalyst to synthesise the designed compounds. The different catalysts were introduced into the reaction between *IVa* and *Va* (Table.1). The effects of the different catalysts (Lewis acid, metal and the corresponding salt) and the different loading on the yield of the *VIa* so obtained are summarised in Table 1.

Table 1 shows that the desired product *VIa* was successfully synthesised in the presence of the differ-

Table 1. Synthesis of *VIa* under different reaction conditions



IVa *Va* *VIa*

Entry	Catalyst	Amount/eq.	Et_3N/eq.	Yield[a] (%)
1	$ZnCl_2$	2.0	2.5	0
2	–	–	2.5	46
3	$ZnCl_2$	2.0	none	0
4	$ZnCl_2$	1.0	2.5	15
5	$ZnCl_2$	0.2	2.5	34
6	$FeCl_3$	0.2	2.5	21
7	CuI	0.2	2.5	10
8	$SnCl_2$	0.2	2.5	38
9	Zn	2.0	2.5	62
10	Zn	1.5	2.5	62
11	Zn	1.0	2.5	51
12	Zn	0.2	2.5	48
13	Fe	1.5	2.5	48
14	$Cu/CuSO_4$	1.5	2.5	51

a) Isolated product yields.

ent catalysts (Table 1, entries 1 and 3–14) and under catalyst-free conditions (Table 1, entry 2), except that *VIa* was not obtained in the absence of Et_3N (Table 1, entry 3) or 2.0 eq. of $ZnCl_2$ (Table 1, entry 1) as the catalyst, respectively, since Et_3N may promote aldoxime to transform the corresponding nitrile oxide (Hansen et al., 2005). The yield clearly decreased as the amount of $ZnCl_2$ increased from 0.2 eq. (catalyst amount) to 2.0 eq. (Tanaka et al., 2010) under the otherwise same conditions (Table 1, entries 1 and 4–5). A possible reason is that $ZnCl_2$ readily formed the complex with heterocyclic compounds containing nitrogen (Su et al., 2014; Lane et al., 1962). However, the desired compound *VIa* was obtained when Zn powder was optionally added into the reaction. With an increase in Zn powder up to a Zn powder loading of 1.5 eq. (62.0 %), the yield increased rapidly. Beyond this value, the yield decreased slightly (61.5 %) (Table 1, entries 9–12). In comparison with the situation when catalyst was absent (Table 1, entry 2), the other metal and the corresponding salt or Lewis acids, such as Fe, $FeCl_3$, CuI, $Cu/CuSO_4$, $SnCl_2$ (Table 1, entries 6–8 and 13–14), Zn/Zn^{2+} was found to be the best catalyst (Table 1, entry 10). The reason may lie in the fact that the Zn powder reacted with HCl generated by aldoxime chloride forming the corresponding nitrile oxide to afford a certain amount of $ZnCl_2$. Also, Zn prevented the generation of red complex. It is deduced that the mutual action of Zn and Zn^{2+} catalysed the reaction. The result was confirmed by XPS (Fig. 2).

Following the reaction, a grey Zn solid was separated and dried under vacuum; its chemical states were studied by XPS, as presented in Fig. 2a. The XPS spectra indicated that the grey Zn solid was largely composed of Zn, O, N, C, Cl, which is consonant with the species of elements in the reaction. Fig. 2b shows that there are two strong peaks centred at 1044.8 eV and 1021.7 eV, which arose from the binding energies (BE) of Zn $2p^{1/2}$ and Zn $2p^{3/2}$, respectively. This confirms that Zn is present as Zn^{2+} and Zn^0 in accordance with what is reported in the literature (Chen et al., 2000; Biesinger et al., 2010; Lu et al., 2011, 2012), since the BE at 1021.7 eV is much closer to the Zn $2p^{3/2}$ than BE of Zn^0 of 1021.65 eV. Fig. 2c shows the XPS spectra in the Zn 2p region. The BE component at 499.3 eV is usually attributed to the BE of Zn^{2+} which may be associated with O 1s or Cl $2p^{1/2}$. The BE at approximately 494.3 eV can be attributed to Zn^0. Hence, the reaction was catalysed by Zn/ Zn^{2+}. Also, the optimal catalyst loading was 1.5 eq.

Subsequently, the other 3-substituted phenyl-5-(((3-(pyridin-3-yl)isoxazol-5-yl)methoxy)methyl)isoxazoles (*VI*) were also successfully synthesised with 50–77 % yields from intermediates *IV* and *N*-hydroxynicotinimidoyl chloride (*Va*) by the same method with 1.5 eq. of Zn/Zn^{2+} added as a catalyst (Table 2). The structures of bis-isoxazole ethers were confirmed by FT-IR, ^1H NMR and ^{13}C NMR and HRMS analyses. The $–CH_2—O—CH_2–$ protons of compounds exhibited resonances at δ 4.77–4.82 using $CDCl_3$ as solvent, while the resonances for the corresponding

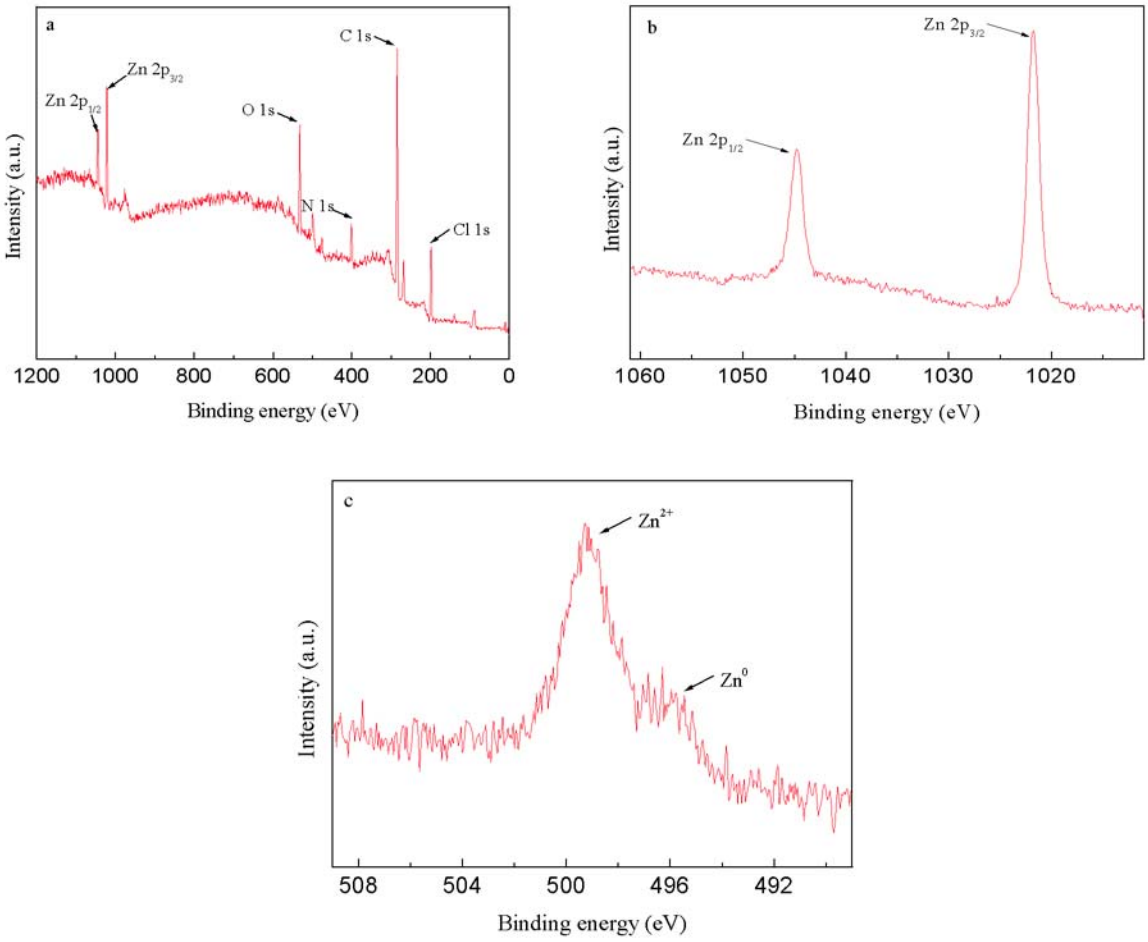
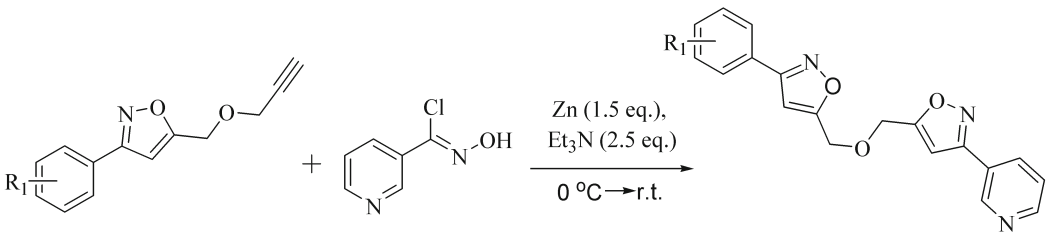
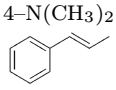


Fig. 2. XPS spectra of product show Zn 2p (a) and Zn^{2+} (b), Zn^0 (c) XPS spectra, respectively.

Table 2. Bis-isoxazole (5-(3-pyridyl)) ether *VIa–VIi* yield using Zn/Zn^{2+} as catalyst

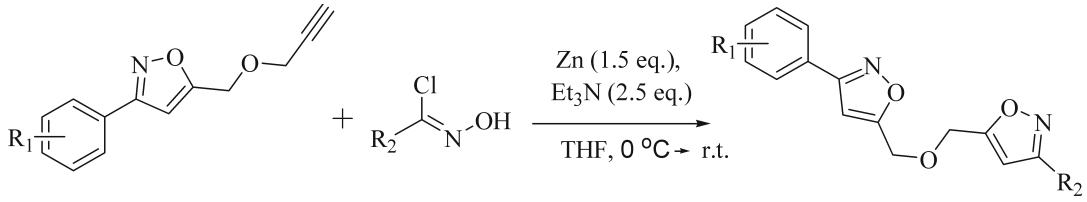


IV *Va* *VI*

Entry	Alkyne	R_1	Product	Yield[a] (%)	Reaction time (h)	M.p. (℃)
1	*IVa*	H	*VIa*	62	14	76–77
2	*IVb*	2–OCH_3	*VIb*	77	11	56–58
3	*IVc*	4–OCH_3	*VIc*	74	12	58–59
4	*IVd*	4–CH_3	*VId*	73	11	72–73
5	*IVe*	2–Cl	*VIe*	66	12	76–78
6	*IVf*	4–F	*VIf*	70	12	76–78
7	*IVg*	4–Br	*VIg*	63	14	98–100
8	*IVh*	4–$N(CH_3)_2$	*VIh*	69	14	99–100
9	*IVi*		*VIi*	50	16	89–90

a) Isolated product yields.

Table 3. Synthesis and yield of bis-isoxazole ethers *VIIa–VIIh* and *VIIIa–VIIIg*



IV *Vb* or *Vc* *VII* or *VIII*

Entry	Alkyne	R_1	R_2	Product	Yield[a] (%)	Reaction time (h)	M.p. (°C)
1	*IVa*	2–OCH_3	3–NO_2–C_6H_4	*VIIa*	81	7	84–86
2	*IVb*	4–OCH_3	3–NO_2–C_6H_4	*VIIb*	80	8	102–104
3	*IVc*	4–CH_3	3–NO_2–C_6H_4	*VIIc*	83	8	86–88
4	*IVd*	2–Cl	3–NO_2–C_6H_4	*VIId*	78	9	102–104
5	*IVe*	4–Cl	3–NO_2–C_6H_4	*VIIe*	77	9	108–110
6	*IVf*	4–F	3–NO_2–C_6H_4	*VIIf*	82	9	112–114
7	*IVg*	3–NO_2–C_6H_4	3–NO_2–C_6H_4	*VIIg*	63	14	150–152
8	*IVa*	2–OCH_3	4–NO_2–C_6H_4	*VIIIa*	80	8	144–146
9	*IVb*	4–OCH_3	4–NO_2–C_6H_4	*VIIIb*	78	8	132–134
10	*IVc*	4–CH_3	4–NO_2–C_6H_4	*VIIIc*	81	8	128–130
11	*IVd*	2–Cl	4–NO_2–C_6H_4	*VIIId*	76	9	122–124
12	*IVe*	4–Cl	4–NO_2–C_6H_4	*VIIIe*	76	8	125–126
13	*IVh*	4–$(CH_3)_3$	3–NO_2–C_6H_4	*VIIh*	65	15	–
14	*IVi*	4–$(CH_3)_3$	4–$(CH_3)_3$–C_6H_4	*VIIIf*	30	20	–
15	*IVe*	4–Cl	4–$(CH_3)_3$–C_6H_4	*VIIIg*	44	20	–

a) Isolated product yields.

Table 4. Antifungal activities of bis-isoxazole ethers *VI*, *VII* and *VIII*

Entry	Compound	R_1	R_2	MIC[a] (μg mL^{-1})
1	*VIb*	2–OCH_3	3–C_5H_4N	1
2	*VIc*	4–OCH_3	3–C_5H_4N	> 64
3	*VId*	4–CH_3	3–C_5H_4N	> 64
4	*VIe*	2–Cl	3–C_5H_4N	4
5	*VIf*	4–F	3–C_5H_4N	> 64
6	*VIIIe*	4–Cl	4–NO_2–C_6H_4	> 64
7	*A*	fluconazole	–	8
8	*B*	itraconazole	–	1

a) Minimum inhibitory concentrations (MIC) were defined as the lowest concentration at which the growth of one hundred percent of the tested organism (*C. albicans*, ATCC10231 strain) were inhibited by the microbroth dilution method.

–CH_2—O—CH_2– carbon atom were observed as two peaks at both δ 63.3–63.5.

Table 2 shows that nine novel bis-isoxazoles bearing 3-pyridyl were synthesised with a 50–77 % yield by using intermediates *IV* and *Va* using 1.5 eq. of Zn/Zn^{2+} as the catalyst. In order to investigate its applicability, the Zn/Zn^{2+} catalyst was used to synthesise other substituted bis-isoxazole ethers (Table 3). Two different series of compounds *VII* and *VIII* were synthesised with 63–83 % and 76–81 % yields starting from *N*-hydroxy-3-nitrobenzimidoyl chloride (*Vb*) or *N*-hydroxy-4-nitrobenzimidoyl chloride (*Vc*) and intermediates *IV* in the presence of 1.5 eq. of Zn/Zn^{2+} catalyst and anhydrous THF. It is clear that this approach could be applied to a broad range.

Table 3 shows that the synthesis was relatively sensitive to terminal alkynes bearing a wide variety of functional groups. When the benzene ring of R_1 on terminal alkynes was substituted with the ortho- and para-position directing group (Table 3, entries 1–6 and 8–12), the yields of the synthesised compounds were higher than that of the benzene ring of R_1 substituted with the meta-position directing group (Table 3, entry 7). In addition, the nitrile oxides-bearing 3(4)-nitrophenyl groups were insensitive to the electronic and steric properties of the nitrile oxide (Table 3), which were consistent with the results reported in the literature (Grecian & Fokin, 2008). Nonetheless, the object product 5,5'-(oxybis(methylene))bis(3-(4-(*tert*-butyl)phenyl)isoxazole) or 5,5'-(oxybis(methylene)) bis(3-(4-chlorophenyl)isoxazole) was prepared with a lower yield (30 % or 44 %) under the same condi-

Table 5. Spectral data of newly prepared compounds

Compound	Spectral data
VIa	IR (KBr), $\tilde{\nu}$/cm^{-1}: 3106, 3047, 2924, 2895, 1617, 1596, 1582, 1569, 1479, 1444, 1411, 1352, 1295, 1187, 1103, 985, 952, 843, 692 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 9.02 (d, J = 1.9 Hz, 1H, pyridyl H2), 8.70 (dd, J = 4.9 Hz, 1.5 Hz, 1H, pyridyl H6), 8.13 (dt, J = 7.9 Hz, 1.9 Hz, 1H, pyridyl H4), 7.86–7.74 (m, 2H, phenyl H2, H6), 7.57–7.34 (m, 4H, phenyl H3, H4, H5, pyridyl H5), 6.69 (s, 1H, isoxazolyl H4), 6.64 (s, 1H, isoxazolyl H4), 4.80 (d, J = 2.9 Hz, 4H, CH_2) ^{13}C NMR ($CDCl_3$, 125 MHz), δ: 169.4, 168.5, 160.1 (isoxazolyl C1, C3), 151.2, 147.9, 134.1, 130.2 (pyridyl C1, C2, C3, C5), 129.0, 128.7, 126.8, 125.0 (phenyl C), 123.8 (pyridyl C4), 101.8, 101.4 (isoxazolyl C2), 63.5, 63.4 (CH_2) HRMS, m/z: 334.1238 (calc. 334.1193) $[M + H]^+$
VIb	IR (KBr), $\tilde{\nu}$/cm^{-1}: 3125, 3065, 2924, 2887, 1606, 1581, 1512, 1471, 1441, 1419, 1346, 1244, 1090, 1024, 910, 819, 754, 702, 620 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 9.02 (s, 1H, pyridyl H2), 8.69 (d, J = 3.5 Hz, 1H, pyridyl H6), 8.14 (dt, J = 7.9 Hz, 1.7 Hz, 1H, pyridyl H4), 7.88 (dd, J = 7.7 Hz, 1.7 Hz, 1H, pyridyl H6), 7.42 (td, J = 8.0 Hz, 3.2 Hz, 2H, pyridyl H5, phenyl H4), 7.03 (dd, J = 14.5 Hz, 7.9 Hz, 2H, phenyl H3, H5), 6.84 (s, 1H, isoxazolyl H4), 6.68 (s, 1H, isoxazolyl H4), 4.79 (d, J = 2.6 Hz, 4H, CH_2), 3.90 (s, 3H, OCH_3) ^{13}C NMR ($CDCl_3$, 125 MHz), δ: 169.6, 167.0, 160.2, 160.0 (isoxazolyl C1, C3), 157.2 (phenyl C2), 151.1, 147.9, 134.1, 129.4 (pyridyl C1, C2, C3, C5), 123.8, 121.0 (phenyl C4, C6), 117.6 (pyridyl C4), 111.4 (phenyl C1, C3, C5), 105.5, 101.3 (isoxazolyl C2), 63.5, 63.3 (CH_2), 55.6 (OCH_3) HRMS, m/z: 364.1298 (calc. 364.1299) $[M + H]^+$
VIc	IR (KBr), $\tilde{\nu}$/cm^{-1}: 3118, 3040, 2925, 2898, 1616, 1582, 1531, 1467, 1441, 1415, 1346, 1253, 1180, 1112, 1028, 912, 822, 704, 620 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 9.02 (s, 1H, pyridyl H2), 8.69 (d, J = 3.5 Hz, 1H, pyridyl H6), 8.14 (dt, J = 7.9 Hz, 1.7 Hz, 1H, pyridyl H4), 7.88 (dd, J = 7.7 Hz, 1.7 Hz, 1H, pyridyl H5), 7.42 (td, J = 8.0 Hz, 3.2 Hz, 2H, phenyl H2, H6), 7.03 (dd, J = 14.5 Hz, 7.9 Hz, 2H, phenyl H3, H5), 6.84 (s, 1H, isoxazolyl H4), 6.68 (s, 1H, isoxazolyl H4), 4.79 (d, J = 2.6 Hz, 4H, CH_2), 3.90 (s, 3H, OCH_3) ^{13}C NMR ((CD_3)$_2$SO, 125 MHz), δ: 169.4, 168.2 (isoxazolyl C1), 162.1 (phenyl C4), 161.1, 160.0 (isoxazolyl C1), 151.1, 147.9, 134.1, 128.2 (pyridyl C1, C2, C3, C5), 125.0, 123.8 (phenyl C2, C6), 121.1 (pyridyl C4), 114.4, (phenyl C1, C3, C5), 101.6, 101.4 (isoxazolyl C2), 63.5, 63.4 (CH_2), 55.4 (OCH_3) HRMS, m/z: 364.1286 (Calc. 364.1299) $[M + H]^+$
VId	IR (KBr), $\tilde{\nu}$/cm^{-1}: 3108, 3033, 2925, 2896, 1617, 1594, 1575, 1464, 1446, 1416, 1352, 1295, 1185, 1101, 984, 953, 822, 708, 617 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 9.02 (s, 1H, pyridyl H2), 8.70 (d, J = 4.8 Hz, 1H, pyridyl H6), 8.22–8.09 (m, 1H, pyridyl H4), 7.69 (d, J = 8.1 Hz, 2H, phenyl H2, H6), 7.41 (dd, J = 8.1 Hz, 4.9 Hz, 2H, phenyl H3, H5), 7.28 (d, J = 7.9 Hz, 1H, pyridyl H5), 6.69 (s, 1H, isoxazolyl H4), 6.62 (s, 1H, isoxazolyl H4), 4.80 (d, J = 4.8 Hz, 4H, CH_2), 2.41 (s, 3H, CH_3) ^{13}C NMR ((CD_3)$_2$SO, 125 MHz), δ: 169.4, 168.3, 162.5, 160.0 (isoxazolyl C1, C3), 151.1, 147.9, 140.4, 134.1 (pyridyl C1, C2, C3, C5), 129.7, 126.7, 125.8, 125.0 (phenyl C), 123.8, (pyridyl C4), 101.7, 101.4 (isoxazolyl C2), 63.5, 63.4 (CH_2), 21.4 (CH_3) HRMS, m/z: 348.1347 (calc. 348.1349) $[M + H]^+$
VIe	IR (KBr), $\tilde{\nu}$/cm^{-1}: 3128, 3058, 2924, 2861, 1608, 1563, 1505, 1466, 1448, 1419, 1364, 1236, 1095, 1049, 948, 774, 703, 652 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 9.03 (d, J = 2.1 Hz, 1H, pyridyl H2), 8.70 (dd, J = 4.8 Hz, 1.6 Hz, 1H, pyridyl H6), 8.23–8.10 (m, 1H, pyridyl H4), 7.73 (dd, J = 7.4 Hz, 2.0 Hz, 1H, phenyl H6), 7.61–7.32 (m, 4H, phenyl H3, H4, H5, pyridyl H5), 6.81 (s, 1H, isoxazolyl H4), 6.70 (s, 1H, isoxazolyl H4), 4.82 (s, 4H, CH_2) ^{13}C NMR ((CD_3)$_2$SO, 125 MHz), δ: 169.4, 167.8, 161.1, 160.0 (isoxazolyl C1, C3), 151.1, 147.9, 134.1, 132.9 (pyridyl C1, C2, C3, C5), 131.0, 130.4, 128.0, 127.2, 125.0 (phenyl C), 123.8 (pyridyl C4), 105.1, 101.4 (isoxazolyl C2), 63.5 (CH_2) HRMS, m/z: 368.0806 (calc. 368.0803) $[M + H]^+$
VIf	IR (KBr), $\tilde{\nu}$/cm^{-1}: 3110, 3046, 2924, 2896, 1617, 1588, 1575, 1530, 1473, 1437, 1397, 1352, 1295, 1246, 1187, 1161, 1103, 983, 953, 835, 708, 639, 618 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 9.01 (d, J = 2.2 Hz, 1H, pyridyl H2), 8.70 (dd, J = 4.8 Hz, 1.7 Hz, 1H, pyridyl H6), 8.19–8.08 (m, 1H, pyridyl H4), 7.84–7.73 (m, 2H phenyl H2, H6), 7.41 (ddd, J = 8.0 Hz, 4.8 Hz, 0.8 Hz, 1H, pyridyl H5), 7.21–7.09 (m, 2H phenyl H3, H5), 6.68 (s, 1H, isoxazolyl H4), 6.60 (s, 1H, isoxazolyl H4), 4.80 (d, J = 4.2 Hz, 4H, CH_2) ^{13}C NMR ($CDCl_3$, 125 MHz), δ: 169.4, 168.8, 164.9, 162.9 (isoxazolyl C1, C3), 161.6, 160.0, 151.2 (pyridyl C1, C3, C5), 147.9 (phenyl C4), 134.1 (pyridyl C2), 128.7, 123.8, 116.2 (phenyl C1, C2, C3, C5, C6), 116.0 (pyridyl C4), 101.6, 101.4 (isoxazolyl C2), 63.5 (CH_2), 63.4 (CH_2) HRMS, m/z: 352.1074 (calc. 352.1099) $[M + H]^+$

Table 5. (continued)

Compound	Spectral data
VIg	IR (KBr), $\tilde{\nu}$/cm^{-1}: 3106, 3046, 2925, 2896, 1617, 1594, 1563, 1464, 1426, 1403, 1352, 1295, 1186, 1101, 1015, 989, 952, 900, 830, 706, 687 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 9.02 (s, 1H, pyridyl H2), 8.71 (d, J = 4.8 Hz, 1H, pyridyl H6), 8.15 (dt, J = 7.9 Hz, 1.9 Hz, 1H, pyridyl H4), 7.72–7.54 (m, 4H, phenyl H2, H3, H5, H6), 7.43 (dd, J = 8.0 Hz, 4.9 Hz, 1H, pyridyl H4), 6.69 (s, 1H, isoxazolyl H4), 6.62 (s, 1H, isoxazolyl H4), 4.80 (d, J = 3.4 Hz, 4H, CH_2) ^{13}C NMR ($(CD_3)_2SO$, 125 MHz), δ: 169.3, 168.9, 161.6, 160.1 (isoxazolyl C1, C3), 151.2, 147.9, 134.0, 132.2 (pyridyl C1, C2, C3, C5), 127.6, 124.9 (phenyl C2, C3, C4, C5), 124.5 (pyridyl C4), 123.8 (phenyl C4), 101.6, 101.4 (isoxazolyl C2), 63.5 (CH_2) HRMS, m/z: 412.0260 (calc. 412.0298) $[M + H]^+$
VIh	IR (KBr), $\tilde{\nu}$/cm^{-1}: 3122, 3065, 2924, 2917, 1614, 1562, 1537, 1467, 1437, 1415, 1359, 1289, 1202, 1174, 1092, 996, 957, 914, 812, 705, 619 ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.02 (s, 1H, pyridyl H2), 8.69 (d, J = 5.8 Hz, 1H, pyridyl H6), 8.13 (d, J = 8.0 Hz, 1H, pyridyl H4), 7.65 (dd, J = 15.8 Hz, 5.5 Hz, 2H, phenyl H2, H6), 7.40 (dd, J = 8.0 Hz, 4.9 Hz, 1H, pyridyl H5), 6.74 (d, J = 8.8 Hz, 2H, phenyl H3, H5), 6.68 (s, 1H, isoxazolyl H4), 6.56 (s, 1H, isoxazolyl H4), 4.78 (d, J = 9.2 Hz, 4H, CH_2), 3.02 (d, J = 41.8 Hz, 6H, $N(CH_3)_2$).^{13}C NMR (DMSO, 125 MHz), δ: 169.6, 167.7, 162.5, 160.0 (isoxazolyl C1, C3), 151.6 (phenyl C4), 151.1, 148.0, 134.1, 125.9 (pyridyl C1, C2, C3, C5), 123.8 (phenyl C2, C6), 119.9 (pyridyl C4), 116.1, 112.0 (phenyl C1, C3, C5), 101.4 (isoxazolyl C2), 63.6, 63.3 (CH_2), 43.4, 40.2 ($N(CH_3)_2$) HRMS, m/z: 377.1603 (calc. 377.1615) $[M + H]^+$
VIi	IR (KBr), $\tilde{\nu}$/cm^{-1}: 3110, 3059, 2925, 1614, 1594, 1575, 1493, 1464, 1436, 1346, 1289, 1104, 1001, 961, 913, 827, 808, 754, 692, 615 ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.02 (s, 1H, pyridyl H2), 8.69 (d, J = 4.8 Hz, 1H, pyridyl H6), 8.21–8.10 (m, 1H, pyridyl H4), 7.56–7.28 (m, 6H, phenyl C, pyridyl H5), 7.14 (d, J = 6.1 Hz, 2H, CH═CH), 6.68 (s, 1H, isoxazolyl H4), 6.57 (s, 1H, isoxazolyl H4), 4.77 (d, J = 9.7 Hz, 4H, CH_2) ^{13}C NMR ($CDCl_3$, 125 MHz), δ: 169.4, 168.5, 162.6, 160.1 (isoxazolyl C1, C3), 151.2, 147.9 (pyridyl C1, C5), 134.1, 130.2 (Ph–CH=, phenyl C2, C3, C5, C6), 129.0, 128.7 (pyridyl C2, C3), 126.8 (phenyl C4), 125.0 (pyridyl C4), 123.8 (=CH-isoxazolyl), 101.8, 101.4 (isoxazolyl C2), 63.5, 63.4 (CH_2) HRMS, m/z: 360.1340 (calc. 360.1350) $[M + H]^+$
VIIa	IR (KBr), $\tilde{\nu}$/cm^{-1}: 3110, 3079, 2920, 1720, 1620, 1597, 1540, 1500, 1467, 1430, 1350, 1270, 1090, 1000, 914, 876, 817, 756, 700, 674, 582 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.67–8.55 (m, 1H, 3-nitrophenyl H2), 8.31 (dd, J = 6.6 Hz, 1.6 Hz, 1H, 3-nitrophenyl H4), 8.22–8.09 (m, 1H, 3-nitrophenyl H6), 7.87 (dd, J = 7.6 Hz, 1.7 Hz, 1H, 2-methoxyphenyl H6), 7.66 (t, J = 8.0 Hz, 1H, 3-nitrophenyl H5), 7.42 (dd, J = 11.2 Hz, 4.6 Hz, 1H, 2-methoxyphenyl H4), 7.02 (dd, J = 12.1 Hz, 7.9 Hz, 2H, 2-methoxyphenyl H3, H5), 6.85 (s, 1H, isoxazolyl H4), 6.72 (s, 1H, isoxazolyl H4), 4.80 (d, J = 3.5 Hz, 4H, CH_2), 3.90 (s, 3H, OCH_3) ^{13}C NMR ($(CD_3)_2SO$, 125 MHz), δ: 170.0, 167.1, 160.8, 160.2 (isoxazolyl C1, C3), 157.2 (2-methoxyphenyl C2), 148.6 (3-nitrophenyl C3), 132.5, 131.4, 130.6 (3-nitrophenyl C1, C2, C6), 130.1, 129.4 (2-methoxyphenyl C4, C6), 124.7, 121.8 (3-nitrophenyl C4, C5), 121.0, 117.5, 111.5 (2-methoxyphenyl C1, C3, C5), 105.5, 101.5 (isoxazolyl C2), 63.5, 63.3 (CH_2), 55.6 (OCH_3) HRMS, m/z: 408.1185 (calc. 408.1197) $[M + H]^+$
VIIb	IR (KBr), $\tilde{\nu}$/cm^{-1}: 3120, 3086, 2950, 1730, 1620, 1574, 1540, 1500, 1460, 1430, 1350, 1250, 1080, 1030, 937, 870, 818, 748, 694, 528 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.62 (t, J = 2.0 Hz, 1H, 3-nitrophenyl H2), 8.31 (d, J = 8.3 Hz, 1H, 3-nitrophenyl H4), 8.16 (d, J = 7.7 Hz, 1H, 3-nitrophenyl H6), 7.78–7.60 (m, 3H, 3-nitrophenyl H5, 4-methoxyphenyl H2, H6), 6.97 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H3, H5), 6.71 (s, 1H, isoxazolyl H4), 6.58 (s, 1H, isoxazolyl H4), 4.80 (d, J = 7.3 Hz, 4H, CH_2), 3.86 (s, 3H, OCH_3) ^{13}C NMR ($(CD_3)_2SO$, 125 MHz), δ: 169.9, 168.2, 162.1, 161.1 (isoxazolyl C1, C3), 160.7 (4-methoxyphenyl C4), 148.6, 132.5, 130.5, 130.1 (3-nitrophenyl C1, C2, C3, C6), 128.2 (4-methoxyphenyl C2, C6), 124.7, 121.8 (3-nitrophenyl C4, C5), 121.1, 114.4 (4-methoxyphenyl C1, C3, C5), 101.6, 101.5 (isoxazolyl C2), 63.6, 63.4 (CH_2), 55.4 (OCH_3) HRMS, m/z: 408.1193 (calc. 408.1197) $[M + H]^+$
VIIc	IR (KBr), $\tilde{\nu}$/cm^{-1}: 3120, 3092, 2920, 1920, 1730, 1620, 1577, 1540, 1500, 1460, 1430, 1350, 1278, 1100, 1010, 903, 876, 818, 739, 702, 513 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.61 (t, J = 1.9 Hz, 1H, 3-nitrophenyl H2), 8.30 (dd, J = 9.4 Hz, 1.1 Hz, 1H, 3-nitrophenyl H4), 8.15 (dd, J = 6.4 Hz, 1.3 Hz, 1H, 3-nitrophenyl H6), 7.72–7.53 (m, 4H, 4-methylphenyl H2, H3, H5, H6), 7.25 (d, J = 7.9 Hz, 1H, 3-nitrophenyl H5), 6.71 (s, 1H, isoxazolyl H4), 6.61 (s, 1H, isoxazolyl H4), 4.80 (d, J = 5.9 Hz, 4H, CH_2), 2.39 (s, 3H, CH_3) ^{13}C NMR ($CDCl_3$, 125 MHz), δ: 169.8, 168.5, 162.5, 160.7 (isoxazolyl C1, C3), 148.6 (3-nitrophenyl C3), 132.5, 130.5, 130.2 (3-nitrophenyl C1, C2, C6), 130.1, 129.0, 128.6, 126.8 (4-methylphenyl C), 124.7, 121.8 (3-nitrophenyl C4, C5), 101.8, 101.5 (isoxazolyl C2), 63.6, 63.4 (CH_2), 21.4 (CH_3) HRMS, m/z: 392.1240 (calc. 392.1248) $[M + H]^+$

Table 5. (continued)

Compound	Spectral data
VIId	IR (KBr), $\tilde{\nu}/cm^{-1}$: 3130, 3073, 2920, 1730, 1620, 1577, 1540, 1500, 1447, 1408, 1350, 1110, 1051, 926, 866, 756, 694, 674, 555 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.64 (t, $J = 1.8$ Hz, 1H, 3-nitrophenyl H2), 8.43 (d, $J = 1.4$ Hz, 1H, 3-nitrophenyl H4), 8.37–8.27 (m, 1H, 3-nitrophenyl H6), 8.23–8.14 (m, 1H, 3-nitrophenyl H5), 7.77–7.61 (m, 2H, 2-chlorophenyl H3, H6), 7.54–7.45 (m, 1H, 2-chlorophenyl H5), 7.38 (dd, $J = 11.7$ Hz, 3.8 Hz, 1H, 2-chlorophenyl H4), 6.82 (s, 1H, isoxazolyl H4), 6.74 (s, 1H, isoxazolyl H4), 4.83 (d, $J = 0.9$ Hz, 4H, CH_2) ^{13}C NMR ($(CD_3)_2SO$, 125 MHz), δ: 169.8, 167.7, 161.2, 160.7 (isoxazolyl C1, C3), 148.6, 132.9, 132.5 (3-nitrophenyl C1, C3, C6), 131.1, 131.0 (2-chlorophenyl C2, C3), 130.6 (3-nitrophenyl C2), 130.5, 130.1, 127.9, 127.2 (2-chlorophenyl C1, C4, C5, C6), 124.7, 121.8 (3-nitrophenyl C4, C5), 105.1, 101.5 (isoxazolyl C2), 63.6, 63.5 (CH_2) HRMS, m/z: 412.0686 (calc. 412.0701) $[M + H]^+$
VIIe	IR (KBr), $\tilde{\nu}/cm^{-1}$: 3130, 3092, 2920, 1730, 1620, 1577, 1540, 1500, 1460, 1430, 1350, 1090, 999, 906, 869, 810, 739, 694, 501 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.61 (s, 1H, 3-nitrophenyl H2), 8.30 (s, 1H, 3-nitrophenyl H4), 8.15 (s, 1H, 3-nitrophenyl H6), 7.75 (d, $J = 2.1$ Hz, 1H, 3-nitrophenyl H5), 7.72 (s, 1H, 4-chlorophenyl H2), 7.66 (s, 1H, 4-chlorophenyl H6), 7.44 (s, 2H, 4-chlorophenyl H3, H5), 6.73 (s, 1H, isoxazolyl H4), 6.62 (s, 1H, isoxazolyl H4), 4.82 (d, $J = 4.4$ Hz, 4H, CH_2) ^{13}C NMR ($(CD_3)_2SO$, 125 MHz), δ: 169.8, 168.9, 161.6, 160.7 (isoxazolyl C1, C3), 148.6, 136.3, 132.5, 130.5 (3-nitrophenyl C1, C2, C3, C6), 130.1, 129.3, 128.1, 127.1 (4-chlorophenyl C), 124.8, 121.8 (3-nitrophenyl C4, C5), 101.7, 101.6 (isoxazolyl C2), 63.6, 63.5 (CH_2) HRMS, m/z: 411.9960 (calc. 412.0701) $[M + H]^+$
VIIf	IR (KBr), $\tilde{\nu}/cm^{-1}$: 3132, 2866, 1869, 1734, 1531, 1352, 1229, 1090, 1003, 822, 696 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.64–8.60 (m, 1H, 3-nitrophenyl H2), 8.32 (ddd, $J = 8.2$ Hz, 2.1 Hz, 0.9 Hz, 1H, 3-nitrophenyl H4), 8.20–8.15 (m, 1H, 3-nitrophenyl H6), 7.82–7.76 (m, 2H, 4-fluorophenyl H2, H6), 7.67 (t, $J = 8.0$ Hz, 1H, 3-nitrophenyl H5), 7.19–7.11 (m, 2H, 4-fluorophenyl H3, H5), 6.73 (s, 1H, isoxazolyl H4), 6.61 (s, 1H, isoxazolyl H4), 4.81 (d, $J = 8.6$ Hz, 4H, CH_2) ^{13}C NMR ($(CD_3)_2SO$, 125 MHz), δ: 169.8, 168.7 (isoxazolyl C1), 164.9 (4-fluorophenyl C4), 162.9, 161.6 (isoxazolyl C3), 160.7, 132.5, 130.5, 130.1 (3-nitrophenyl C1, C2, C3, C6), 128.8, 128.7, 124.9, 124.8 (4-fluorophenyl C1, C2, C4, C6), 121.8, 116.2 (3-nitrophenyl C4, C5), 116.0 (4-fluorophenyl C3, C5), 101.6, 101.5 (isoxazolyl C2), 63.6, 63.5 (CH_2) HRMS, m/z: 396.0991 (calc. 396.0996) $[M + H]^+$
VIIg	IR (KBr), $\tilde{\nu}/cm^{-1}$: 3111, 2926, 1699, 1533, 1348, 1097, 910, 694 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.62 (s, 2H, 3-nitrophenyl H2), 8.32 (d, $J = 7.3$ Hz, 2H, 3-nitrophenyl H4), 8.18 (d, $J = 7.7$ Hz, 2H, 3-nitrophenyl H6), 7.67 (t, $J = 8.0$ Hz, 2H, 3-nitrophenyl H5), 6.74 (s, 2H, isoxazolyl H4), 4.84 (s, 4H, CH_2) ^{13}C NMR ($(CD_3)_2SO$, 125 MHz), δ: 169.9, 160.7 (isoxazolyl C1, C3), 148.7, 132.5, 130.5, 130.2, 124.8, 121.8 (3-nitrophenyl C), 101.6 (isoxazolyl C2), 63.6 (CH_2) HRMS, m/z: 423.0945 (calc. 423.0941) $[M + H]^+$
VIIIa	IR (KBr), $\tilde{\nu}/cm^{-1}$: 3120, 3092, 2960, 2040, 1720, 1600, 1520, 1440, 1350, 1260, 1090, 953, 852, 768, 698, 579, 486 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.36–8.26 (m, 2H, 4-nitrophenyl H3, H5), 7.97 (d, $J = 9.0$ Hz, 2H, 4-nitrophenyl H2, H6), 7.86 (dd, $J = 7.7$ Hz, 1.7 Hz, 1H, 2-methoxyphenyl H6), 7.48–7.36 (m, 1H, 2-methoxyphenyl H4), 7.09–6.95 (m, 2H, 2-methoxyphenyl H3, H5), 6.84 (s, 1H, isoxazolyl H4), 6.70 (s, 1H, isoxazolyl H4), 4.80 (d, $J = 4.2$ Hz, 4H, CH_2), 3.89 (s, 3H, OCH_3) ^{13}C NMR ($(CD_3)_2SO$, 125 MHz), δ: 170.1, 167.0, 160.7, 160.2 (isoxazolyl C1, C3), 157.2 (2-methoxyphenyl C2), 148.8, 134.9 (4-nitrophenyl C1, C4), 131.5, 130.9 (2-methoxyphenyl C4, C6), 129.4, 128.9, 127.7, 124.2 (4-nitrophenyl C2, C3, C5 C6), 121.0, 117.5, 111.5 (2-methoxyphenyl C1, C3, C5), 105.5, 101.6 (isoxazolyl C2), 63.5, 63.3 (CH_2), 55.6 (OCH_3) HRMS, m/z: 408.1192 (calc. 408.1197) $[M + H]^+$
VIIIb	IR (KBr), $\tilde{\nu}/cm^{-1}$: 3130, 3092, 2940, 1720, 1600, 1520, 1469, 1430, 1340, 1260, 1180, 1090, 1030, 914, 856, 698, 482 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.32 (d, $J = 8.9$ Hz, 2H, 4-nitrophenyl H3, H5), 7.97 (d, $J = 8.9$ Hz, 2H, 4-nitrophenyl H2, H6), 7.73 (d, $J = 8.9$ Hz, 2H, 4-methoxyphenyl H2, H6), 6.97 (d, $J = 8.8$ Hz, 2H, 4-methoxyphenyl H3, H5), 6.70 (s, 1H, isoxazolyl H4), 6.58 (s, 1H, isoxazolyl H4), 4.79 (d, $J = 8.0$ Hz, 4H, CH_2), 3.86 (s, 3H, OCH_3) ^{13}C NMR ($(CD_3)_2SO$, 125 MHz), δ: 170.6, 169.2 (isoxazolyl C1), 162.0 (4-methoxyphenyl C4), 161.2, 161.0 (isoxazolyl C3), 148.9, 134.9 (4-nitrophenyl C1, C4), 128.6 (4-methoxyphenyl C2, C6), 128.4, 124.8 (4-nitrophenyl C2, C3, C5, C6), 121.2, 115.0 (4-methoxyphenyl C1, 3C, 5C), 103.1, 102.4 (isoxazolyl C4), 63.2, 63.1 (CH_2), 55.8 (OCH_3) HRMS, m/z: 408.1173 (calc. 408.1197) $[M + H]^+$
VIIIc	IR (KBr), $\tilde{\nu}/cm^{-1}$: 3130, 3087, 2920, 1730, 1620, 1520, 1430, 1340, 1100, 1000, 852, 810, 698, 513 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.36–8.26 (m, 2H, 4-nitrophenyl H3, H5), 8.02–7.92 (m, 2H, 4-nitrophenyl H2, H6), 7.72–7.54 (m, 2H, 4-methylphenyl H2, H6), 7.26 (d, $J = 7.9$ Hz, 2H, 4-methylphenyl H3, H5), 6.70 (s, 1H, isoxazolyl H4), 6.60 (s, 1H, isoxazolyl H4), 4.80 (d, $J = 6.6$ Hz, 4H, CH_2), 2.40 (s, 3H, CH_3) ^{13}C NMR ($(CD_3)_2SO$, 125 MHz), δ: 169.4, 162.3, 161.0 (isoxazolyl C1, C3), 148.9, 140.5 (4-nitrophenyl C1, C4), 134.9, 132.6, 130.1 (4-methylphenyl C3, C4, C5), 129.1, 128.4 (4-nitrophenyl C2, C6), 127.0, 126.1 (4-methylphenyl C1, C2, C6), 124.8 (4-nitrophenyl C3 C5), 103.1, 102.6 (isoxazolyl C2), 63.2, 63.1 (CH_2), 21.4 (CH_3) HRMS, m/z: 392.1233 (calc. 392.1248) $[M + H]^+$

Table 5. (continued)

Compound	Spectral data
VIIId	IR (KBr), $\tilde{\nu}/cm^{-1}$: 3140, 3068, 2930, 1700, 1600, 1520, 1458, 1430, 1350, 1180, 1090, 937, 862, 837, 760, 704, 653, 501 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.38–8.28 (m, 2H, 4-nitrophenyl H3, H5), 8.04–7.95 (m, 2H, 4-nitrophenyl H2, H6), 7.72 (dd, $J = 7.1$ Hz, 2.3 Hz, 1H, 2-chlorophenyl H6), 7.55–7.30 (m, 3H, 2-chlorophenyl H3, H4, H5), 6.81 (s, 1H, isoxazolyl H4), 6.72 (s, 1H, isoxazolyl H4), 4.82 (d, $J = 2.2$ Hz, 4H, CH_2) ^{13}C NMR ($(CD_3)_2SO$, 125 MHz), δ: 169.9, 167.6, 161.2, 160.7 (isoxazolyl C1, C3), 148.8, 134.8 (4-nitrophenyl C1, C3), 132.9 (2-chlorophenyl 3C), 131.1, 131.0, 130.5, 127.9, 127.7 (2-chlorophenyl C), 127.2, 124.3 (4-nitrophenyl C2, C3, C5, C6), 105.2, 101.7 (isoxazolyl C2), 63.6, 63.5 (CH_2) HRMS, m/z: 412.0683 (calc. 412.0701) $[M + H]^+$
VIIIe	IR (KBr), $\tilde{\nu}/cm^{-1}$: 3120, 3087, 2920, 1700, 1600, 1520, 1458, 1430, 1350, 1170, 1060, 918, 859, 818, 760, 698, 517 ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.32 (d, $J = 9.0$ Hz, 2H, 4-nitrophenyl H3, H5), 8.02–7.93 (m, 2H, 4-nitrophenyl H2, H6), 7.78–7.67 (m, 2H, 4-chlorophenyl H2, H6), 7.48–7.38 (m, 2H, 4-chlorophenyl H3, H5), 6.71 (s, 1H, isoxazolyl H4), 6.61 (s, 1H, isoxazolyl H4), 4.80 (d, $J = 4.9$ Hz, 4H, CH_2) ^{13}C NMR ($(CD_3)_2SO$, 125 MHz), δ: 169.8, 168.8, 161.6, 160.7 (isoxazolyl C1, C3), 148.8, 136.3 (4-nitrophenyl C1, C4), 134.8, 129.3, 128.1, 127.7 (4-chlorophenyl C), 127.1, 124.2 4-nitrophenyl C2, C3, C5, C6), 101.7, 101.6 (isoxazolyl C2), 63.6 (CH_2), 63.5 (CH_2) HRMS, m/z: 412.0691 (calc. 412.0701) $[M + H]^+$

tions when R_2 was replaced by 4-(*tert*-butyl)phenyl (Table 3, entry 14) or 4-chlorophenyl (Table 3, entry 15).

The isoxazoles-ring and pyridine-ring were important and effective drug cores (Gagneux, 1965; Lunn et al., 2003; Murugesan et al., 2000, 2005; Pirrung et al., 2002; Renard et al., 2009; Liu et al., 1996; Li et al., 2006). Fluconazole and itraconazole were drugs for treating different subtypes of fungal infections, and had a therapeutic effect on humans and animals in clinical therapy. Herein, the in-vitro antifungal and antibacterial activities of bis-isoxazole ether derivatives were evaluated by preparing solutions with different concentrations (Table 4). The bacteria thus evaluated includes *E. coli* (ATCC25922), *S. aureus* (ATCC25923), *P. aeruginosa* (ATCC9027) and *C. albicans* (ATCC10231). The results showed that these compounds had no significant effect on the bacteria; the most potent compounds *VIb* and *VIe* exhibited MIC of 1 μg mL^{-1} and 4 μg mL^{-1} to *C. albicans* (ATCC10231), respectively (Table 4, entries 1 and 4). This suggested a more potent antifungal activity against *Candida albicans* than fluconazole, commonly used as antifungal agent in clinical practice. *VIb* exhibited the same predominant antifungal activity against *Candida albicans* as itraconazole (Table 4, entries 1 and 8). In addition to Table 4, the bacteriostatic test of other compounds was also carried out but did not exhibit antibacterial activity. In addition, it was found that substitution at the 2-position of the benzene ring (compounds *VIb* and *VIc*) and replacement of the benzene ring by the pyridine-ring (compounds *VIIIe* and *VIe*) were preferred.

Conclusions

A convenient, efficient and novel process using Zn/Zn^{2+} as the catalyst to synthesise bis-isoxazole ethers was developed by monitoring the XPS spectra of the product. Twenty-one novel bis-isoxazole ethers were successfully synthesised starting from 3-substituted phenyl-5-prop-2-ynyloxymethyl-isoxazoles *IV* and different substituted chloro oximes. The intermediates *III* were first obtained in one-pot by the 1,3-dipolar cycloaddition reaction. Subsequently, Zn/Zn^{2+} was found to be the best catalyst for synthesising compounds *VI* and the better catalyst for synthesising compounds *VII* and *VIII*. The antifungal and antibacterial activities of the synthesised compounds were studied in comparison with the standard drugs fluconazole, itraconazole. The MIC of compounds *VIb* and *VIe* were, respectively, 1 μg mL^{-1} and 4 μg mL^{-1} which were close to itraconazole and was superior to the drug fluconazole. It was found that 2-substituted phenyl bis-isoxazole ethers with pyridyl exhibited excellent antifungal activities. Such a functional compound might possess potential applications in clinical practice and medicine. The side-effects of compounds *VIb* and *VIe* on the human body require further investigation. In addition, the synthesis of other compounds with biological and pharmacological activity is in progress.

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