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Discovery of 1,2,4-Triazole-1,3-Disulfonamides as Dual Inhibitors of Mitochondrial Complex II and Complex III

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ABSTRACT The respiratory chain succinate-ubiquinone oxidoreductase (SQR or complex II) and ubihydroquinone-cytochrome (cyt) c oxidoreductase (cyt bc_1 or complex III) have been demonstrated as the promising targets of numerous antibiotics and fungicides. As a continuation of our research work on the development of new fungicides, a series of 1,2,4-triazole-1,3-disulfonamide derivatives with dual function targeting both SQR and cyt bc_1 were designed and synthesized by coupling diverse diphenyl ether moiety with triazolesulfonamide unit. These newly synthesized compounds were characterized by elemental analyses, ^{1}H NMR and ESI-MS spectrometry. The *in vitro* assay indicated that most of the synthesized compounds displayed good inhibition against porcine succinate-cytochrome reductase (SCR) with IC_{50} values ranging from 3.2 to 81.8 μM, revealing much higher activity than that of the commercial control amisulbrom whose IC_{50} value is 93.0 μM. Further evaluation against respective SQR and cyt bc_1 indicated that most compounds exhibited SQR-inhibiting activity as well as cyt bc_1-inhibiting activity, but the inhibition potency against SQR is much higher than that towards cyt bc_1, showing the SCR inhibition might be contributed greatly from the SQR inhibition. The further antibacterial evaluation against *Xanthomonasoryzae* pv. *oryzae* revealed that four compounds showed excellent potency at the concentration of 20 μg/mL. In particular, compounds **6h** and **6j** exhibited much better antibacterial activity than the commercial control bismerthiazol in terms of their EC_{50}. Impressively, **6j** has an EC_{90} of 33.62 μg/mL, more than 10-fold higher than that of bismerthiazol.

Key words: Cytochrome bc_1, 1,2,4-Triazole-1,3-Disulfonamide, Fungicides, Molecular docking

1. Introduction

Oxidative phosphorylation and tricarboxylic acid (or Krebs) cycle are two bioprocesses which coupled with electron transport and proton pump flow constituted the highly effective mitochondrial respiration. In animals and bacteria, the oxidative phosphorylation system comprises five multiprotein complexes (complexes I to V) and two mobile electron carriers (ubiquinone and cytochrome c) embedded in the lipid bilayer of the mitochondrial inner membrane. Among them, succinate-ubiquinone oxidoreductase (SQR or complex II) and ubihydroquinone-cytochrome (cyt) c oxidoreductase (cyt bc_1 or complex III) are two essential and central components of the cellular respiratory chain and of the photosynthetic apparatus in photosynthetic bacteria. SQR specifically catalyzes the oxidation of succinate to fumarate with concomitant reduction of ubiquinone to ubiquinol. This enzyme is a four subunit membrane-bound dehydrogenase that comprises a FAD-containing flavoprotein, a subunit containing multiple iron-sulfur clusters and two smaller hydrophobic cytochrome b containing subunits that anchor the catalytic portion to the mitochondrial membrane. Whereas, the function of bc_1 complex is to catalyze the electron transfer (ET) from ubiquinol (hydroxyquinones, QH_2) to a water-soluble cytochrome c (cyt c) and couples this electron transfer to the translocation of protons across the membrane to generate a proton gradient and membrane potential for ATP synthesis.[1–5] Though their subunit composition varied among different organisms from three or more subunits in bacterial to ten or eleven subunits in mitochondrial forms, the catalytic central of bc_1 complexes usually contain three redox-active subunits: a cyt. *b* possessing two *b*-type hemes, *b*H and *b*L; a cyt. c_1 bearing one *c*-type heme; and an ISP containing a 2Fe-2S cluster.[6] In case of the electron transport process of complex II and/or cyt bc_1 was disturbed or disrupted, the cellular respiration will be blocked and resulted in cell death, thus complex II and III have been identified as the promising action of target for numerous antiparastic agents, antibiotics as well as agricultural fungicides.

So far, approximately 30 crystal structures of SQR, including native structures and inhibitor-bound complexes, have been reported since the first crystal structure of *E. coli* SQR was solved at 2.6 resolution.[7] The small molecules involved in these structures include ubiquinone (UQ) and some ubiquinone binding site (*Q*-site) inhibitors. X-ray crystallographic studies of *Q*-site inhibitors bound to SQR have indicated that the essential bonding residues were absolutely conserved and the amino acid residues of the IP and of the transmembrane subunits constitute the putative *Q*-site[8,9]. Under these circumstances, 18 specific SQR inhibitors have been developed and commercialized as agricultural fungicides. These structurally diverse inhibitors are categorized into carboxamide fungicides binding to *Q*-site.[10,11] Though the first generation of carboxamide fungicides such as carboxin and benodanil have manifested a narrow fungicidal spectrum, continuous efforts have led to a range of chemical structures such as boscalid and bixafen, which exhibit broadened biological spectrum and improved potency to match the requirement for modern agricultural protection as well.

Unlike SQR system, in which the binding site is formed by a pocket near the cytoplasmic interface with residues from chains B, C and D, the catalytic core of cyt bc_1 comprises of two discrete binding sites according to the *Q*-cycle reaction mechanism[12], termed the quinone reduction site near the negative side of the membrane (Q_i) and the quinol oxidation site close to the positive side of the membrane (Q_o).[13,14] Consequently, the inhibitors targeted at bc_1 complex are divided into two types according to the point of action:[15,16] type I for Q_o site inhibitors and type II for the Q_i site inhibitors. The strobilurin-type fungicides like azoxystrobin and kresoxim-methyl are typical representatives of Q_o site agrochemical class.[17,18] Over 15 strobilurin fungicides were commercially available since the first strobilurin fungicide azoxystrobin was developed and launched in 1996. In comparison, only two Q_i inhibitors namely cyazofamid and amisulbrom[19–20] have been launched into the agricultural fungicide market. Though strobilurin type fungicides have achieved great success, the significant resistance issues were observed in a wide range of important

plant pathogens after a short period of field applications. Thus, discovery of novel fungicides targeting Q_i site of cyt bc_1 represents an attractive approach to fight against the explosive development of resistance that the Q_o site inhibitors are facing.

Antimycin A,[21] a nature product isolated from *streptomyces sp.* showing a dissociation constant with bovine heart mitochondrial particles of 32 pM,[22] was shown to bind to the Q_i site of bc_1 to block the mitochondrial electron transfer between cytochrome b and c. It is a valuable starting point for the development of agricultural fungicides with the action of Q_i site of mitochondrial.[23–25] Bolgunas and Tokutake have successfully simplified antimycin scaffold by replacing the dilactone portion of the molecule with biphenyl and biphenyl ether group (Figure 1).[26] These analogues possess comparable *in vitro* activity as potent Q_i site inhibitors. On the other hand, cyazofamid and amisulbrom, the two commercialized Q_i site inhibitors are featured with a sulfonamide azolyl pharmacophore.[27-28] Initially, we envisioned that the integration of the biphenyl ether, which has been demonstrated their inherent importance in agrochemistry, with the sulfonamide azolyl pharmacophore may result in a novel class of structure as depicted in Figure 1 with efficient Q_i site inhibition. Unexpectedly, the bioassay results indicated that these inhibitors showed not only significant bc_1-inhibiting activity but also remarkable SQR-inhibiting activity. To the best of our knowledge, this is the first observation of the inhibitors possessing dual target inhibition toward mitochondrial complex II and complex III. These inhibitors may serve as novel lead for further fungicide discovery in terms of the dual-targeting inhibitors have the advantageous to improve the potency by synergistic effect and provide a new approach to overcome the resistance issues that affect marketing fungicides.

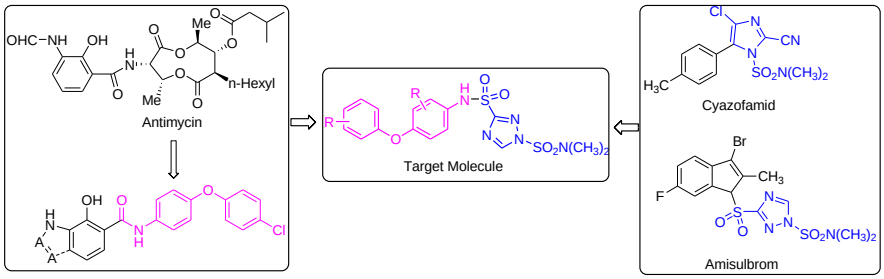


Figure 1. Antimycin, cyazofamid, amisulbrom and the designed compounds.

2. Experimental

2.1 General Techniques

All chemical reagents were commercially available and treated with standard methods before use. Solvents were dried and redistilled before use. Silica gel column chromatography (silica gel 200-300 mesh, Qingdao Makall Group Co., Ltd, Qingdao, China).^{1}H NMR spectra were recorded on a VARIAN Mercury-Plus 600 or 400 spectrometer in $CDCl_3$ or DMSO-d_6 with TMS as the internal reference, ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a VARIAN Mercury-Plus 600 (151 MHz) or 400 (101 MHz) spectrometer, and chemical shifts (δ) are given in ppm relative to the center line of a triplet at 77.0 ppm of $CDCl_3$. The following abbreviations are used to designate multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Elementary analysis was taken with a Vario EL III elementary analysis instrument. MS spectra were determined using a Trace MS 2000 organic mass spectrometry, and the signals were given in m/z. Melting points were taken on a Buchi B-545 melting point apparatus and are uncorrected.

2.2 Preparation of the Designed Compounds

***Preparation of 1,2-di(1H-1,2,4-triazol-3-yl)disulfane (1)*:** To a mechanically stirred slurry of 1.01 g (10 mmol) 3-mercapto-1,2,4-triazole in 5 mL dichloromethane was added 0.79 g (10 mmol) dry pyridine. The resulting mixture was cooled in an ice bath and 0.88 g (5 mmol) benzenesulfonyl chloride was added dropwise over a period of 1 h. The ice bath was removed and the mixture was stirred for 16 h at room temperature. Dichloromethane was then evaporated and the resulting residue was mechanically

stirred with a mixture of 5 mL water and 3 mL ethyl acetate for 1 h. The mixture was filtered to isolate the resulting precipitate, which was then washed sequentially with 20 mL water and 20 mL ethyl acetate. Drying the precipitate under vacuum at 60-70 ℃ gave 0.92 g (92%) of the desired 3,3'-(dithiobis)-1,2,4-triazole as a white solid.[29]

***Preparation of 3,3'-disulfanediylbis(N,N-dimethyl-1H-1,2,4-triazole-1- sulfonamide) (2)*:** To a mixture of 0.2 g (1 mmol) of bis[1,2,4-triazole-3-yl]disulfide and 5 mL of DMF was added 0.276 g (2 mmol) of potassium carbonate. The temperature was raised to 30 ℃, and 0.317 g (2.2 mmol) of N, N-dimethylsulfamoyl chloride was added dropwise at a temperature between 28 to 32 ℃ over 2 hours. After the completion of the reaction (monitored by TLC), 30 mL of 1,2-dichloroethane was added, and the resulting solution was added in a mixture of 10 mL of 35% hydrochloric acid and 40 mL of water at a temperature ranging from 20 to 25 ℃. The organic phase was collected to obtain a 1,2-dichloroethane solution containing 0.373 g bis[1-(N,N-dimethylsulfamoyl) -1,2,4-triazole -3-yl]disulfide, yield 90%.[30]

***Preparation of 1-(N,N-dimethylsulfamoyl)-1H-1,2,4-triazole-3-sulfonyl chloride (3)*:** To 10 mL of a 1,2-dichloroethane solution containing 0.829 g (2 mmol) of bis[1-(N,N-dimethyl-sulfamoyl)-1,2,4-triazole-3-yl]disulfide was added 20 mL of water, and the mixture was cooled to 0 ℃. 10 mL of formic acid was added and then chlorine gas was bubbled at a temperature ranging from 15 to 20 ℃ over 3 hours. Thereafter, the resulting mixture was stirred at 15 to 20 ℃ for 0.5 hour. After the completion of the reaction, the solution was subjected to phase seperation, washed with water (30 mL x 3) to obtain a 1,2-dichloroethane solution containing 0.986 g of 3-chlorosulfonyl-1-(N,N-dimethyl-sulfamoyl)-1,2,4-triazole, yield 91%.[30]

***General procedure for the preparation of compounds (5)*:** Under N_2 atmosphere, a three-neck round bottom flask was charged with nitroarenes (1.1 mmol), phenols (1.0 mmol), and K_2CO_3 (1.5 mmol) in DMF (5 mL) at room temperature, the mixture was stirred constantly at 60 ℃ (oil bath temperature) for 8 hours. After the completion of

the reaction, as monitored by TLC, the reaction mixture was cooled to room temperature, diluted with ethyl acetate, and filtrated. The filtrate was concentrated under vacuum, and the resulting residue was purified by silica gel column chromatography to afford compounds **4**. Then to a solution of **4** (5 mmol) in dichloromethane (25 mL) was added 10% Pd-C (15% by weight based on **4**) at room temperature. When the reaction was complete (monitored by TLC), the reaction mixture was filtered and the solvent was evaporated under reduced pressure to give the residue, which was then purified through flash chromatography to give compound **5**.

Data for **5a**[31]: White solid (92%), mp: 239-240 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.45 (d, *J*=8.4 Hz, 2H), 6.81 (d, *J*=8.4 Hz, 2H), 6.77 (d, *J*=8.4 Hz, 2H), 6.59 (d, *J*=8.4 Hz, 2H), 5.10 (s, 2H). EI-MS; m/z = 265.20 (M^+).

Data for **5b**: White solid (42%); mp: 55-56 ℃; ^{1}H NMR (600 MHz, $CDCl_3$): δ=7.69 (s, 1H), 7.36 (d, *J*=9.0 Hz, 1H), 6.88 (d, *J*=8.4 Hz, 2H), 6.82 (d, *J*=9.0 Hz, 1H), 6.71 (d, *J*=8.4 Hz, 2H), 3.68 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.64, 146.45, 143.95, 127.40, 125.67, 124.87, 123.27, 122.94, 122.61, 122.43, 122.17, 121.10, 116.44, 115.07; EI-MS: m/z = 287.21 (M^+).

Data for **5c**: White solid (90%); mp: 184-185 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.67 (d, *J*=2.3 Hz, 1H), 7.32 (dd, *J*=8.9, 2.5 Hz, 1H), 6.78 (m, 3H), 6.61 (d, *J*=8.7 Hz, 2H), 5.11 (s, 2H).; ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.24, 145.46, 145.31, 129.61, 128.15, 126.19, 123.44, 120.34, 118.46, 115.43; EI-MS: m/z = 255.16(M^+).

Data for **5d**[32]: White solid (95%); mp: 88-89 ℃; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.09 – 6.99 (m, 1H), 6.96 (d, *J*=7.8 Hz, 1H), 6.88 – 6.77 (m, 4H), 6.64 (d, *J*=8.4 Hz, 2H), 3.87 (s, 3H), 3.47 (s, 2H). EI-MS: m/z = 215.19 (M^+).

Data for **5e**[33]: White solid (65%); mp: 63-64℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.36 – 7.26 (m, 1H), 7.10 (t, *J*=7.8 Hz, 1H), 7.06 (s, 1H), 6.88 (t, *J*=8.4 Hz, 1H), 6.76 (d, *J*=8.4 Hz, 2H), 6.59 (d, *J*=8.0 Hz, 2H), 5.02 (s, 2H); EI-MS: m/z = 203.19 (M^+).

Data for **5f**[32]: White solid (93%), mp: 77-78 ℃;^{1}H NMR (600 MHz, $CDCl_3$) δ =7.17 (t, *J*=7.8 Hz, 1H), 6.88 (d, *J*=8.4 Hz, 2H), 6.85 (d, *J*=7.8 Hz, 1H), 6.83 – 6.71 (m, 4H), 4.38 (s, 2H), 2.31 (s, 3H); EI-MS: m/z = 199.25 (M^+).

Data for **5g**[34]: White solid (98%); Mp: 116-117℃; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.62 (d, *J*=7.2 Hz, 1H), 7.41 (t, *J*=7.6 Hz, 1H), 7.05 (t, *J*=7.2 Hz, 1H), 6.91 (d, *J*=7.2 Hz, 2H), 6.78 (d, *J*=8.4 Hz, 1H), 6.72 (d, *J*=7.2 Hz, 2H), 3.78 (s, 2H); EI-MS: m/z = 210.20 (M^+).

Data for **5h**[35]: White solid (94%); Mp: 111-112℃; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (d, *J*=8.4 Hz, 2H), 6.94 (d, *J*=8.4 Hz, 2H), 6.88 (d, *J*=8.4 Hz, 2H), 6.72 (d, *J*=8.4 Hz, 2H), 3.80 (s, 2H); EI-MS: m/z = 210.24(M^+).

Data for **5i**[36]: White solid (77%); Mp: 130-131℃; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51 – 7.35 (m, 4H), 7.33 (d, *J*=7.2 Hz, 1H), 6.89 (s, 4H), 6.82 (d, *J*=8.8 Hz, 2H), 6.65 (d, *J*=8.4 Hz, 2H), 5.02 (s, 2H), 3.44 (s, 2H); EI-MS: m/z = 291.32(M^+).

Data for **5j**[37]: Yield: 70%; Mp: 138-139℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.51-7.28 (m, 5H), 7.19 (d, *J*=8.2 Hz, 1H), 6.75 (d, *J*=5.8 Hz, 2H), .6.65 (d, *J*=6.7 Hz, 1H), 6.58 (d, *J*=5.6 Hz, 2H), 6.48 (s, 1H), 6.40 (d, *J*=5.6 Hz, 1H), 5.04 (s, 2H), 5.01 (s, 2H); EI-MS: m/z = 291.28(M^+).

Data for **5k**[38]: White solid (90%); Mp: 66 - 68 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ=8.01 (d, *J*=8.4 Hz, 1H), 7.54 (d, *J*=7.8 Hz, 2H), 7.15 (d, *J*=8.4 Hz, 2H), 6.54 (d, *J*=7.8 Hz,1H), 6.48 (d, *J*=7.2 Hz, 2H), 5.57 ppm (s, 2H); EI-MS: m/z = 186.08 (M^+).

Data for **5l**: White solid (73%); Mp: 127-129 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.08 (s, 1H), 7.94 (s, 1H), 7.15 (d, =8.4 Hz, 1H), 6.74 (s, 1H), 6.58 (d, *J*=7.2 Hz, 1H), 5.77 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.66, 150.91, 139.21, 136.98, 130.39, 129.46, 125.54, 124.95, 113.22, 112.63, 109.44; EI-MS: m/z = 288.15 (M^+).

Data for **5m**: White solid (68%); Mp: 131-132 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.09-7.97 (m, 2H), 7.12-7.09 (m, 1H), .6.46-6.42 (m, 2H), 5.78 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.35, 156.54, 155.89, 151.61, 139.12, 137.06, 128.99, 125.30, 114.96, 109.54, 99.97, 99.75; EI-MS: m/z = 271.11 (M^+).

Data for **5n**: White solid (57%); Mp: 208-210 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.13 (d, *J*=2.4 Hz, 1H), 8.04 (d, *J*=2.4 Hz, 1H), 6.73 (s, 2H), 6.08 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.19, 151.22, 139.81, 136.75, 132.34, 125.79, 121.81, 112.39, 110.20; EI-MS: m/z = 324.08 (M^+).

Data for **5o**: White solid (73%); Mp: 95-96 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.69 (d, *J*=2.5 Hz, 1H), 7.31 (dd, *J*=9.0, 2.4 Hz, 1H), 6.95 (d, *J*=8.4 Hz, 1H), 6.74 (d, *J*=2.4 Hz, 1H), 6.61 (d, *J*=8.9 Hz, 1H), 6.57 (d, *J*=8.4 Hz, 1H), 5.44 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.76, 147.71, 139.19, 129.68, 128.13, 126.14, 125.50, 123.22, 122.51, 116.72, 114.54, 113.74; EI-MS: m/z = 288.15(M^+).

Data for **5p**: White solid (59%); Mp: 99-100 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.06 (s, 1H), 7.72 (d, *J*=7.8 Hz, 1H), 7.24 (s, 2H), 7.17 (d, *J*=8.4 Hz, 1H), 5.34 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.76, 151.54, 127.79, 125.92, 125.05, 124.69, 124.10, 121.98, 121.18, 119.01; EI-MS: m/z = 324.13(M^+).

Data for **5q**: White solid (86%); Mp: 96-97 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.80 (s, 1H), 7.35 (s, 1H), 6.95 (s, 1H), 6.73 (s, 1H), 6.57 (s, 2H), 5.44 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.77, 147.61, 139.38, 132.38, 128.62, 126.42, 125.53, 123.20, 116.46, 114.59, 113.73, 111.59; EI-MS: m/z = 333.11(M^+).

Data for **5r**: White solid (75%); Mp: 58-60 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.97 (s, 1H), 7.63 (d, *J*=8.4 Hz, 1H), 7.05 (d, *J*=8.4 Hz, 1H), 6.84 – 6.69 (m, 2H), 6.61 (d, *J*=8.4 Hz, 1H), 5.52 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.73, 148.23, 138.45, 127.51, 125.70, 124.81, 124.01, 123.66, 123.34, 122.11, 122.01, 115.41, 114.52, 113.84; EI-MS: m/z = 319.18 (M^+).

Data for **5s**: White solid (55%); Mp: 90-92 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.01 (s, 1H), 7.63 (d, *J*=7.8 Hz, 1H), 6.76-6.70 (m, 3H), 5.81 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.42, 148.57, 133.92, 127.91, 127.74, 125.89, 124.74, 124.14, 123.82, 122.04, 121.77, 114.61, 113.35; EI-MS: m/z = 355.37 (M^+).

Data for **5t**: White solid (82%); Mp: 46-47 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.70 (s, 1H), 7.33 (d, *J*=9.0 Hz, 1H), 6.96 (t, *J*=9.0 Hz, 1H), 6.73 (d, *J*=9.0 Hz, 1H), 6.51 (d, *J*=12.0 Hz, 1H), 6.41 (d, *J*=9.0 Hz, 1H), 5.46 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.33, 153.09, 152.91, 148.21, 148.11, 130.33, 130.19, 129.68, 128.26, 126.18, 123.40, 122.34, 116.58, 110.05, 101.68, 101.47; EI-MS: m/z = 271.16 (M^+).

Data for **5u**: White solid (55%); Mp: 102-104 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.74 (d, *J*=2.4 Hz, 1H), 7.35 (d, *J*=9.0 Hz, 1H), 6.81 (d, *J*=9.0 Hz, 1H), 6.39 (d, *J*=10.8 Hz, 2H), 5.84 (s, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 156.40, 154.77, 152.65, 148.18, 148.08, 129.84, 128.38, 126.68, 121.97, 118.36, 118.25. 118.14, 115.47, 97.18, 97.03; EI-MS: m/z = 289.16 (M^+).

Data for **5v**[39]: White solid (46%); Mp: 168-169 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.72 (s, 2H), 6.62 (s, 2H), 5.59 (s, 2H); EI-MS: m/z = 357.08 (M^+).

Data for **5w**: White solid (37%); Mp: 104-106 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.76 (s, 2H), 6.26 (d, *J*=11.4 Hz, 2H), 5.60 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.28, 155.21, 152.87, 152.80, 148.19, 146.26, 146.13, 129.21, 129.08, 127.48, 122.02, 97.01, 96.77; EI-MS: m/z = 323.35 (M^+).

Data for **5x**[39]: White solid (54%); Mp: 142-143 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.85 (s, 2H), 6.71 (d, *J*=2.4 Hz, 1H), 6.38 (dd, *J*=8.4, 2.4 Hz, 1H), 6.29 (d, *J*=8.4 Hz, 1H), 5.14 (s, 2H); EI-MS: m/z = 323.08 (M^+).

Data for **5y**: White solid (83%); Mp: 114-115 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.29 (s, 1H), 7.94 (d, *J*=9.0 Hz, 1H), 7.58 (m, 3H), 7.35 (s, 1H), 6.99 (d, *J*=8.4 Hz, 1H), 6.77 (d, *J*=2.4 Hz, 1H), 6.63 – 6.58 (m, 1H), 6.50 (d, *J*=7.8 Hz, 1H), 5.39 (s, 2H);

^{13}C NMR (100 MHz, DMSO-d_6): δ 157.01, 156.84, 154.13, 151.47, 139.70, 139.41, 134.75, 132.52, 132.38, 126.21, 124.42, 124.18, 121.58, 112.42, 111.19, 108.50; EI-MS: m/z = 269.57(M^+).

Data for **5z**: White solid (64%); Mp: 105-106 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.35 (m, 1H), 7.95 (t, *J*=8.4 Hz, 1H), 7.60 (m ,3H), 7.34 (t, *J*=7.8 Hz, 1H), 6.79 (s, 2H), 6.43 (d, *J*=7.8 Hz, 1H), 5.73 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ=152.76, 147.89, 135.19, 134.31, 128.46, 127.56, 126.73, 125.84, 124.06, 121.66, 121.37, 113.46, 106.35; EI-MS: m/z = 303.15 (M^+).

Data for 5I: White solid (56%); Mp: 90-92 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.38 – 8.28 (m, 1H), 8.02 – 7.90 (m, 1H), 7.67 – 7.54 (m, 3H), 7.37 (t, *J*=8.4 Hz, 1H), 6.64 (d, *J*=7.8 Hz, 1H), 6.41 (d, *J*=10.8 Hz, 2H), 5.77 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ=157.24, 154.80, 153.86, 147.90, 147.75, 134.25, 127.62, 126.87, 125.97, 125.89, 124.06, 121.92, 121.26, 106.41, 97.28, 97.07; EI-MS: m/z = 271.20 (M^+).

General procedure for the preparation of compound (6):A solution of triazole-3-sulfonyl chloride **3** (1.1 mmol) in dry THF (5 mL) was added dropwise to a solution of aniline (1.0 mmol) in dry THF (5 mL) and NEt_3 (2 mmol) at room temperature under N_2 atmosphere. After stirring overnight, the reaction mixture was poured into water, and extracted with CH_2Cl_2 (30 mL). The organic layer was washed with 2 M HCl (10 mL × 2), brine (10 mL × 2), saturated aqueous $NaHCO_3$ (10 mL), brine (10 mL × 2), and dried over Na_2SO_4. The solvent was evaporated and the crude product was purified through flash chromatography to give the pure product **6**.[40]

Data for **6a**: White solid (86%); Mp: 209-210 ℃; ^{1}H NMR (400 MHz, DMSO-d_6): δ=11.03 (s, 1H), 9.40 (s, 1H), 7.57 – 7.50 (m, 2H), 7.20 (d, *J*=8.8 Hz, 2H), 7.01 (d, *J*=8.8 Hz, 2H), 6.90 (d, *J*=8.8 Hz, 2H), 2.84 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ=162.62, 156.69, 153.65, 148.78, 133.19, 132.80, 123.97, 120.66, 122.21, 115.45,

38.68; EI-MS: m/z = 503.14 (M^+). Anal. Calcd for $C_{16}H_{16}BrN_5O_5S_2$ (502.98): C, 38.25; H, 3.21; N, 13.94; S, 12.77; Found: C, 38.43; H, 3.337; N, 14.01; S, 12.93.

Data for **6b**: White solid (63%); Mp: 160-161 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.14 (s, 1H), 9.42 (s, 1H), 8.03 (s, 1H). 7.86 (d, *J*=10.8 Hz, 1H), 7.49 (d, *J*=8.4 Hz, 2H), 7.10(d, *J*=8.4 Hz, 2H), 7.03 (d, *J*=8.4 Hz, 1H), 2.86 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.61, 156.03, 152.37, 148.78, 133.75, 128.25, 126.35, 125.41, 125.20, 124.58, 123.76, 122.85, 120.38, 119.45, 38.65; EI-MS: m/z = 525.27 (M^+). Anal. Calcd for $C_{17}H_{15}ClF_3N_5O_5S_2$ (525.02): C, 38.82; H, 2.87; N, 13.32; S, 12.19; Found: C, 38.99; H, 2.755; N, 13.59; S, 12.17.

Data for **6c**: White solid (83%); Mp: 140-142 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.02 (s, 1H), 9.40 (s, 1H), 7.76 (s, 1H), 7.42 (d, *J*=8.7 Hz, 1H), 7.19 (d, *J*=8.4 Hz, 2H), 7.03 (d, *J*=8.4 Hz, 1H), 6.96 (d, *J*=8.4 Hz, 2H), 2.84 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.58, 153.76, 151.23, 148.81, 132.66, 130.58, 129.26, 128.92, 125.92, 124.05, 122.24, 118.90, 38.68; EI-MS: m/z = 491.22 (M^+). Anal. Calcd for $C_{16}H_{15}Cl_2N_5O_5S_2$ (490.99): C, 39.03; H, 3.07; Cl, 14.22; S, 13.03; Found: C, 39.19; H, 3.255; N, 14.49; S, 12.93.

Data for **6d**: White solid (88%); Mp: 132-134 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.85 (s, 1H), 9.39 (s, 1H), 7.19 (d, *J*=7.8 Hz, 1H), 7.16 (d, *J*=7.8Hz, 1H), 7.09 (d, *J*=8.4 Hz, 2H), 7.00 – 6.93 (m, 2H), 6.77 (d, *J*=8.4 Hz, 2H), 3.72 (s, 3H), 2.82 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.61, 155.94, 151.74, 148.79, 143.81, 130.79, 126.16, 124.51, 122.01, 121.56, 117.01, 113.84, 56.02, 38.63; EI-MS: m/z = 453.27(M^+). Anal. Calcd for $C_{17}H_{19}N_5O_6S_2$ (453.08): C, 45.02; H, 4.22; N, 15.44; S, 14.14; Found: C, 45.25; H, 4.326; N, 15.53; S, 13.99.

Data for **6e**: White solid (72%); Mp: 168-169 ℃; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 9.40 (s, 1H), 7.35 (t, *J*=7.6 Hz, 2H), 7.24 – 7.13 (m, 2H), 7.11 (d, *J*=7.6 Hz, 1H), 7.04 (s, 1H), 6.90 (d, *J*=8.4 Hz, 2H), 2.86 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.18, 154.34, 142.81, 142.73, 131.66, 125.68, 125.55, 123.81,

122.00, 117.60, 117.31, 117.21, 38.24; EI-MS: m/z = 441.29 (M^+). Anal. Calcd for $C_{16}H_{16}FN_5O_5S_2$ (441.06): C, 43.53; H, 3.65; F, 4.30; N, 15.86; S, 14.53; Found: C, 43.79; H, 3.605; N, 16.07; S, 14.68.

Data for **6f**: White solid (83%); Mp: 117-118 ℃; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.97 (s, 1H), 9.39 (s, 1H), 7.23 (d, *J*=7.6 Hz, 1H), 7.17 (d, *J*=8.8 Hz, 2H), 6.95 (m, 3H), 6.77 (s, 1H), 6.73 (d, *J*=7.6 Hz, 1H), 2.84 (s, 6H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.62, 157.10, 154.42, 148.81, 140.21, 132.16, 130.17, 124.61, 124.09, 119.82, 119.31, 115.81, 38.66, 21.34; EI-MS: m/z = 437.31(M^+). Anal. Calcd for $C_{17}H_{19}N_5O_5S_2$ (437.08): C, 46.67; H, 4.38; N, 16.01; S, 14.66; Found: C, 46.56; H, 4.410; N, 16.18; S, 14.41.

Data for **6g**: White solid (86%); Mp: 128-130 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.15 (s, 1H), 9.39 (s, 1H), 7.89 (d, *J*=7.6 Hz, 1H), 7.65 (t, *J*=7.6 Hz, 1H), 7.27 (dd, *J*=18.4, 8.2 Hz, 3H), 7.13 (d, *J*=8.4 Hz, 2H), 6.88 (d, *J*=8.4 Hz, 1H), 2.85 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.16, 158.80, 151.93, 148.45, 135.32, 134.19, 133.45, 123.79, 123.31, 120.43, 117.29, 115.93, 102.74, 38.31; EI-MS: m/z = 448.22(M^+). Anal. Calcd for $C_{17}H_{16}N_6O_5S_2$ (448.06): C, 45.53; H, 3.60; N, 18.74; S, 14.30; Found: C, 45.59; H, 3.468; N,18.69; S, 14.37.

Data for **6h**: White solid (81%); Mp: 170-172 ℃; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.16 (s, 1H), 9.42 (s, 1H), 7.83 (d, *J*=8.4 Hz, 2H), 7.26 (d, *J*=8.4 Hz, 2H), 7.13 (d, *J*=8.4 Hz, 2H), 7.03 (d, *J*=8.4 Hz, 2H), 2.85 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.19, 161.18, 151.35, 148.48, 134.65, 133.60, 123.25, 121.22, 118.74, 117.76, 105.12, 38.29; EI-MS: m/z = 448.32(M^+). Anal. Calcd for $C_{17}H_{16}N_6O_5S_2$ (448.06): C, 45.53; H, 3.60; N, 18.74; S, 14.30; Found: C, 45.67; H, 3.736; N, 18.50; S, 14.49.

Data for **6i**: White solid (78%); Mp: 151-153 ℃; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.90 (s, 1H), 9.39 (s, 1H), 7.45 (d, *J*=7.2 Hz, 2H), 7.40 (t, *J*=7.2 Hz, 2H), 7.34 (d, *J*=6.8 Hz, 1H), 7.17 – 7.10 (m, 2H), 7.03 (d, *J*=9.2 Hz, 2H), 6.97 – 6.91 (m, 2H), 6.87 (d, *J*=8.8Hz, 2H), 5.08 (s, 2H), 2.83 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ

162.25, 155.41, 154.75, 149.66, 148.39, 137.04, 131.06, 128.45, 127.87, 127.73, 123.94, 120.47, 118.11, 116.03, 69.64, 38.24; EI-MS: m/z = 529.27(M^+). Anal. Calcd for $C_{23}H_{23}N_5O_6S_2$ (529.11): C, 52.16; H, 4.38; N, 13.22; S, 12.11; Found: C, 52.51; H, 4.178; N, 13.46; S, 12.26.

Data for **6j**: White solid (60%); Mp: 97-98 ℃; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.99 (s, 1H), 9.38 (s, 1H), 7.36 (m, 6H), 7.25 (t, *J*=8.4 Hz, 1H), 7.17 (d, *J*=8.8 Hz, 2H), 6.96 (d, *J*=8.9 Hz, 2H), 6.77 (d, *J*=8.0 Hz, 1H), 6.57 (s, 1H), 6.47 (d, *J*=8.0 Hz, 1H), 5.06 (s, 2H), 2.81 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.65, 160.16, 158.39, 153.90, 148.81, 137.22, 132.51, 130.96, 128.86, 128.20, 123.94, 120.15, 110.80, 110.21, 105.57, 69.82, 38.65; EI-MS: m/z = 529.38(M^+). Anal. Calcd for $C_{23}H_{23}N_5O_6S_2$ (529.11): C, 52.16; H, 4.38; N, 13.22; S, 12.11; Found: C, 52.18; H, 4.431; N, 13.40; S, 11.98

Data for **6k**: White solid (41%); Mp: 183-185 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.08 (s, 1H), 9.43 (s, 1H), 8.12 (s, 1H), 7.84 (t, *J*=7.6 Hz, 1H), 7.20 (d, *J*=8.8 Hz, 2H), 7.12 (s, 1H), 7.08 (d, *J*=8.8 Hz, 2H), 7.01 (d, *J*=8.4 Hz, 1H), 2.83 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 163.34, 162.61, 151.35, 148.93, 147.75, 140.62, 133.25, 123.25, 122.50, 119.48, 111.87, 38.67; EI-MS: m/z = 424.85 (M^+). Anal. Calcd for $C_{15}H_{16}N_6O_5S_2$ (424.06): C, 42.45; H, 3.80; N, 19.80; S, 15.11; Found: C, 42.36; H, 3.919; N, 19.66; S, 15.23.

Data for **6l**: White solid (56%); Mp: 169-170 ℃; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.75 (s, 1H), 9.45 (s, 1H), 8.13 (s, 1H), 8.02 (s, 1H), 7.58 (d, *J*=8.0 Hz, 1H), 7.45 (s, 1H), 7.29 (s, 1H), 2.88 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.33, 156.60, 149.16, 140.07, 139.43, 136.41, 133.58, 131.53, 130.87, 126.06, 120.38, 119.43, 110.54, 38.77; EI-MS: m/z = 528.01 (M^+). Anal. Calcd for $C_{15}H_{13}Cl_3N_6O_5S_2$ (527.94): C, 34.13; H, 2.48; N, 15.92; S, 12.15; Found: C, 34.16; H, 2.254; N, 15.97; S, 11.02.

Data for **6m**: White solid (47%); Mp: 214-216 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.75 (s, 1H), 9.45 (s, 1H), 8.11 (s, 1H), 8.05 (s, 1H), 7.54 (d, *J*=8.4 Hz, 1H), 7.21

(d, *J*=11.4 Hz, 1H), 7.14 (d, *J*=8.4 Hz, 1H), 2.88 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.30, 156.54, 156.02, 140.04, 139.87, 139.81, 136.56, 130.44, 125.84, 123.54, 123.45, 116.03, 110.65, 38.75; EI-MS: m/z = 510.02 (M^+). Anal. Calcd for $C_{15}H_{13}Cl_2FN_6O_5S_2$ (509.97): C, 35.23; H, 2.56; N, 16.44; S, 12.54; Found: C, 35.49; H, 2.659; N, 16.71; S, 12.38.

Data for **6n**: White solid (38%); Mp: 126-128 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 12.05 (s, 1H), 9.48 (s, 1H), 8.21 (s, 1H), 8.13 (s, 1H), 7.46 (s, 2H), 2.92 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.10, 156.00, 140.78, 140.05, 133.66, 133.52, 130.81, 126.30, 119.17, 118.33, 111.43, 38.78; EI-MS: m/z = 562.09 (M^+). Anal. Calcd for $C_{15}H_{12}Cl_4N_6O_5S_2$ (561.90): C, 32.04; H, 2.15; N, 14.95; S, 11.41; Found: C, 32.28; H, 2.316; N, 14.83; S, 11.44.

Data for **6o**: White solid (72%); Mp: 145-146 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.38 (s, 1H), 9.43 (s, 1H), 7.79 (s, 1H), 7.40 (s, 2H), 7.16 (s, 1H), 7.07(d, *J*=7.8 Hz, 1H), 6.88 (d, *J*=7.2 Hz, 1H), 2.98 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.38, 151.09, 148.24, 134.44, 130.64, 129.18, 128.77, 124.81, 123.14, 121.85, 121.33, 120.40,107.41, 38.68; EI-MS: m/z = 527.26 (M^+). Anal. Calcd for $C_{16}H_{14}Cl_3N_5O_5S_2$ (526.95): C, 36.48; H, 2.68; N, 13.29; S, 12.17; Found: C, 37.19; H, 2.256; N, 14.43; S, 12.93.

Data for **6p**: White solid (63%); Mp: 136-137 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.79 (s, 1H), 9.48 (s, 1H), 7.79 (s, 1H), 7.44 (s, 2H), 7.32 – 7.28 (m, 1H), 6.58 (d, *J*=9.0Hz, 1H), 2.93 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.10, 151.08, 150.23, 142.42, 136.62, 130.61, 129.03, 128.82, 127.64, 122.65, 120.87, 115.92, 38.69; EI-MS: m/z = 561.08 (M^+). Anal. Calcd for $C_{16}H_{13}Cl_4N_5O_5S_2$ (560.91): C, 34.24; H, 2.33; N, 12.48; S, 11.43; Found: C, 34.70; H, 2.256; N, 12.23; S, 11.93.

Data for **6q**: White solid (66%); Mp: 140-141 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.35 (s, 1H), 9.43 (s, 1H), 7.89 (s, 1H), 7.41 (m, 2H), 7.16 (s, 1H), 7.05 (d, *J*=8.4 Hz, 1H), 6.85 (d, *J*=9.0 Hz, 1H), 2.87 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ

162.39, 152.24, 148.95, 148.35, 134.43, 133.40, 129.72, 129.00, 124.68, 123.20, 121.87, 121.35, 120.20, 114.08 38.70; EI-MS: m/z = 571.00 (M^+). Anal. Calcd for $C_{16}H_{14}BrCl_2N_5O_5S_2$ (570.90): C, 33.64; H, 2.47; N, 12.26; S, 11.23; Found: C, 33.62; H, 2.454; N, 12.41; S, 11.50.

Data for **6r**: White solid (57%); Mp: 136-137 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.47 (s, 1H), 9.44 (s, 1H), 8.05 (s, 1H), 7.66 (d, *J*=8.4 Hz, 1H), 7.44 (s, 1H), 7.30 (d, *J*=8.4 Hz, 1H), 7.24 (s, 1H), 6.88 (d, *J*=8.4 Hz, 1H), 2.89 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.39, 155.57, 148.96, 146.92, 135.59, 128.40, 126.44, 125.66, 125.40, 125.17, 124.65, 123.62, 123.23, 122.81, 121.64, 117.76, 38.68; EI-MS: m/z = 558.96 (M^+). Anal. Calcd for $C_{17}H_{14}Cl_2F_3N_5O_5S_2$ (558.98): C, 36.44; H, 2.52; N, 12.50; S, 11.44; Found: C, 36.35; H, 2.712; N, 12.75; S, 11.30.

Data for **6s**: White solid (52%); Mp: 141-142 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.48 (s, 1H), 9.47 (s, 1H), 8.07 (s, 1H), 7.66 (d, *J*=8.4 Hz, 1H), 7.45 (s, 1H), 6.76 (d, *J*=9.0 Hz, 2H), 2.89 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.13, 154.73, 146.56, 142.00, 141.42, 136.90, 133.84, 128.92, 128.82, 126.35, 125.14, 125.00, 124.54, 122.76, 122.44, 120.90, 120.06, 115.23, 38.68; EI-MS: m/z = 595.21 (M^+). Anal. Calcd for $C_{17}H_{13}Cl_3F_3N_5O_5S_2$ (594.93): C, 34.33; H, 2.20; N, 11.77; S, 10.78; Found: C, 35.09; H, 2.275; N, 11.49; S, 10.93.

Data for **6t**: White solid (55%); Mp: 144-146 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.39 (s, 1H), 9.43 (s, 1H), 7.78 (s, 1H), 7.39 (d, *J*=7.8 Hz, 1H), 7.22 (d, *J*=12.0 Hz, 1H), 7.14 (d, *J*=9.0 Hz, 1H), 7.02 (d, *J*=8.4 Hz, 1H), 6.93 (d, *J*=8.4Hz, 1H), 2.89 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.41, 153.84, 152.18, 151.49, 148.78, 139.52, 134.64, 130.46, 128.97, 128.57, 124.49, 122.07, 119.66, 117.98, 110.34, 110.20, 38.35; EI-MS: m/z = 509.09 (M^+). Anal. Calcd for $C_{16}H_{14}Cl_2FN_5O_5S_2$ (508.98): C, 37.65; H, 2.77; N, 13.72; S, 12.57; Found: C, 37.73; H, 2.387; N, 13.99; S, 12.31.

Data for **6u**: White solid (57%); Mp: 161-163 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.80 (s, 1H), 9.46 (s, 1H), 7.78 (s, 1H), 7.33 (d, *J*=9.0 Hz, 1H), 7.10 (t, *J*=4.8 Hz, 2H), 6.86 (d, *J*=8.0 Hz, 1H), 2.91 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.12, 156.05, 154.39, 152.00, 148.95, 135.96, 130.41, 128.80, 128.02, 126.53, 122.79, 116.53, 104.91, 104.76, 38.62; EI-MS: m/z = 527.27 (M^+). Anal. Calcd for $C_{16}H_{13}Cl_2F_2N_5O_5S_2$ (526.97): C, 36.37; H, 2.48; N, 13.26; S, 12.14; Found: C, 36.36; H, 2.549; N, 13.37; S, 12.32.

Data for **6v**: White solid (36%); Mp: 193-195 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.62 (s, 1H), 9.42 (s, 1H), 7.79 (s, 2H), 7.30 (s, 2H), 2.90 ppm (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.13, 148.98, 147.05, 144.49, 134.78, 129.71, 129.61, 126.72, 126.16, 121.26, 38.63 ppm; EI-MS: m/z = 595.05 (M^+). Anal. Calcd for $C_{16}H_{12}Cl_5N_5O_5S_2$ (594.87): C, 32.26; H, 2.03; N, 11.76; S, 10.77; Found: C, 32.50; H, 2.160; N, 11.56; S, 10.94.

Data for **6w**: White solid (35%); Mp: 191-193 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.61 (s, 1H), 9.43 (s, 1H), 7.84 (s, 2H), 7.01 (d, *J*=10.1 Hz, 2H), 2.88 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.10, 154.07, 152.44, 149.01, 147.64, 133.72, 130.70, 129.64, 129.38, 127.91, 105.33, 107.17, 38.62; EI-MS: m/z = 563.13 (M^+). Anal. Calcd for $C_{16}H_{12}Cl_5F_2N_5O_5S_2$ (562.93): C, 34.15; H, 2.15; N, 12.44; S, 11.40; Found: C, 34.02; H, 2.326; N, 12.71; S, 11.43.

Data for **6x**: White solid (43%); Mp: 212-213 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.21 (s, 1H), 9.42 (s, 1H), 7.92 (s, 2H), 7.37 (s, 1H), 7.04 (d, *J*=9.0 Hz, 1H), 6.64 (d, *J*=8.4 Hz, 1H), 2.85 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.44, 149.19, 148.89, 145.50, 132.76, 131.67, 130.00, 129.67, 123.94, 122.18, 121.73, 115.43. 38.66; EI-MS: m/z = 561.14 (M^+). Anal. Calcd for $C_{16}H_{13}Cl_3N_5O_5S_2$ (560.91): C, 34.24; H, 2.33; N, 12.48; S, 11.43; Found: C, 34.07; H, 2.348; N, 12.58; S, 11.71.

Data for **6y**: White solid (75%); Mp: 141-143 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.35 (s, 1H), 9.44 (s, 1H), 8.11 (d, *J*=7.8 Hz, 1H), 7.99 (d, *J*=7.8 Hz, 1H), 7.72 (d,

J=8.0 Hz, 1H), 7.60 (d, *J*=7.8 Hz, 2H), 7.43-7.40 (m, 2H), 7.17 (s, 1H), 7.10 (s, 1H), 6.72 (d, *J*=7.8 Hz, 1H), 2.88 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.49, 152.52, 149.32, 148.98, 134.95, 134.12, 128.29, 127.36, 126.81, 126.38, 125.50, 125.18, 123.79, 123.37, 122.12, 121.56, 111.69, 38.68; EI-MS: m/z = 507.26.(M^+). Anal. Calcd for $C_{20}H_{18}ClN_5O_5S_2$ (507.04): C, 47.29; H, 3.57; N, 13.79; S, 12.62; Found: C, 47.23; H, 3.769; N, 14.04; S, 12.89.

Data for **6z**: White solid (75%); Mp: 172-175 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.82 (s, 1H), 9.47 (s, 1H), 8.34-8.32 (m, 1H), 7.97(t, *J*=8.0 Hz, 1H), 7.65-7.62 (m, 3H), 7.62 (d, *J*=9.6 Hz, 2H), 7.32 (d, *J*=6.6 Hz, 1H), 6.37 (d, *J*=7.8 Hz, 1H), 2.86 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.19, 152.23, 149.13, 143.47, 136.11, 134.83, 129.51, 128.11, 127.42, 126.62, 126.18, 124.35,122.92, 121.70, 121.16, 107.03, 38.63; EI-MS: m/z = 542.70.(M^+). Anal. Calcd for $C_{20}H_{17}Cl_2N_5O_5S_2$ (541.00): C, 44.29; H, 3.16; N, 12.91; S, 11.82; Found: C, 44.22; H, 3.010; N, 12.97; S, 12.05.

Data for **6I**: White solid (78%); Mp: 158-160 ℃, ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.76 (s, 1H), 9.47 (s, 1H), 8.29 (s, 1H), 7.99 (s, 1H), 7.67 (d, *J*=7.8 Hz, 1H), 7.62 (s, 2H), 7.36 (s, 1H), 7.14 (d, *J*=9.6 Hz, 2H), 6.63 (d, *J*=7.8 Hz, 1H), 2.92 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.19, 156.61, 154.97, 153.38, 149.10, 135.55, 134.70, 128.11, 127.47, 126.73, 126.16, 124.35, 123.19, 121.50, 107.40, 105.19, 38.65; EI-MS: m/z = 509.23.(M^+). Anal. Calcd for $C_{20}H_{17}F_2N_5O_5S_2$ (509.06): C, 47.15; H, 3.36; N, 13.75; S, 12.59; Found: C, 47.16; H, 3.592; N, 13.76; S, 12.60.

2.3 *Enzyme assay*

The preparation of succinate-cytochrome c reductase (SCR, mixture of respiratory complex II and bc1 complex) from porcine heart was essentially as reported.[41] The activity of SCR was measured by monitoring the increase of cytochrome c at 550 nm, by using the extinction coefficient of 18.5 mM^{-1} cm^{-1}. The succinate–ubiquinone reductase (complex II) activity was measured by monitoring the decrease of 2,6-dichlorophenolindophenol (DCIP) at 600 nm, by using the extinction coefficient of 21 mM^{-1} cm^{-1}. The reaction mixture may be scaled down to 1.8 mL with final

concentrations of PBS (pH 7.4), 100 mM; EDTA, 0.3 mM; succinate, 20 mM; oxidized cytochrome c, 60 μM (or DCIP, 53 μM); and appropriate amounts of enzyme to start the reaction.[42]

2.4 *Determination of median effective concentration (EC_{50})*

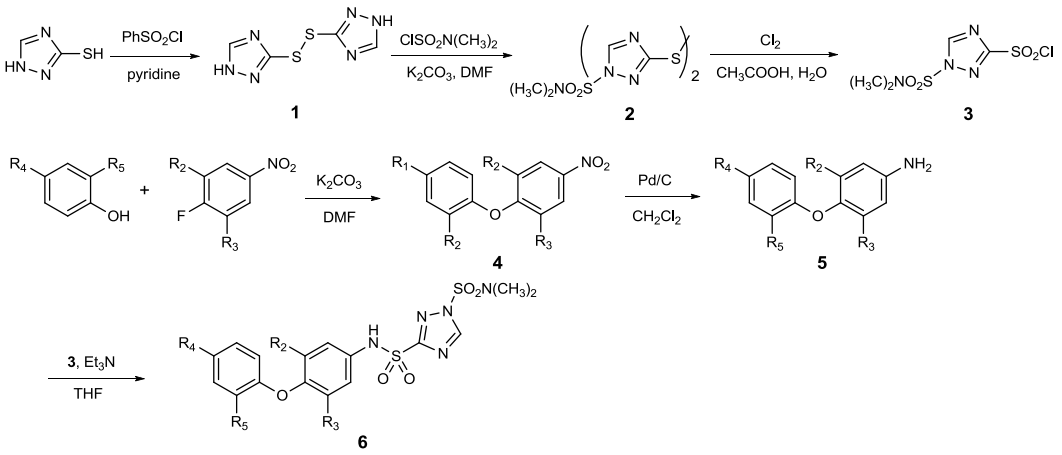
EC_{50} was determined by bacterial growth inhibition according to literature method with slight modification.[43] The bacteria were grown in nutrient broth (NB) at 28 ℃ to late logarithmic growth phase, and the suspension was diluted to a density of approximately 10^7 CFU / mL. Aliquots (100 μL) of the suspension were added to 25 mL of NB culture in 50-ml Erlenmeyer flasks containing various concentrations of the test compounds, and flasks were placed on an orbital shaker (28 ℃, 170 rpm). When the concentration of bacterial suspension in the control flask increased to approximately 10^8 CFU / mL, the values of optical density of bacterial suspension in all flasks were measured with a nephelometer (WCY-WOG; Baoli, Beijing, China). The toxicity regression equation was deduced with the values of optical density, and the EC_{50} was determined.

3. Results and Discussion

3.1 Chemistry

The designed compounds 1,2,4-triazole-1,3-disulfonamide derivatives were synthesized as shown in Scheme 1. The target molecules consist of two parts: the triazole-disulfonamides unit and the diphenyl ether moiety. Firstly, triazolesulfonyl chloride was prepared in three steps *via* 3-mercapto-1,2,4-triazole as the starting material. The reaction of 3-mercapto-1,2,4-triazole with benzenesulfonylchloride affords the intermediate triazolesulfonothioate, which react with another molecular thiol to generate the symmetric disulfide ether in the presence of the pyridine as a base. The disulfide ether was then treated with N, N-dimethylsulfamoyl chloride in the presence of potassium carbonate to give bis[1-(N,N-dimethylsulfamoyl)-1,2,4-triazole-3-yl]disulfide. Thereafter, the oxidation of the disulfide was performed by bubbling chlorine gas in the acetic acid solvent. It conveniently gives access to the triazolesulfochloride **3**.

The diphenyl ether moiety was prepared by nucleophilic reaction of appropriate substituted phenol with *para*-nitro fluorobenzene to generate the intermediate **4**, which was reduced with palladium catalytic hydrogenation to produce the corresponding aromatic amine **5**. The triazolesulfochloride and varied diphenyl ether was assembled to furnish the designed product **6** in good to excellent yield.



Scheme 1. Synthetic route of the designed compounds.

3.2 Inhibition activities of compounds against porcine succinate-cytochrome c reductase (SCR), succinate-ubiquinone oxidoreductase (SQR) and ubihydroquinone-cytochrome (cyt) c oxidoreductase (cyt bc_1)

The *in vitro* activities of the prepared compounds were assayed against porcine succinate-cyctochrome reductase (SCR), which compose of respiratory complex II （SQR） and complex III (bc_1 complex), and they also deemed to form complex II-complex III supercomplexes. Complex II (SQR) firstly passes electrons from succinate to ubiquinone, and then the cytochrome bc_1 complex passes electrons from reduced ubiquinone to cytochrome c. The activity of complex II in SCR was selectively determined using succinate and dichlorophenolindophenol (DCIP) as substrates, and the activity of only the cytochrome bc_1 complex in SCR was determined using decylubiquinol (DBH_2) and cytochrome c as substrates, whereas the overall activity of SCR (both complex II and bc_1 complex) was determined using succinate and cytochrome c as substrates.

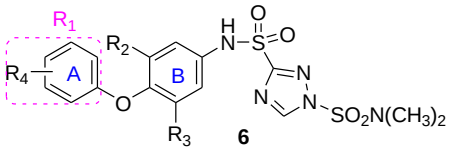
The inhibition results against SCR derived from porcine heart mitochondria were listed in Table 1. For clarity, the two benzene rings of the biphenyl ether component were marked as A and B, respectively. The activities of these prepared compounds were varied significantly depending on the substituted pattern of the biphenyl ether moiety. Although some analogues were inactive, most of them showed good to excellent inhibitory capability toward bc_1 complex compared to the commercial control amisulbrom, which exhibited an IC_{50} value of 93.0 μM. Some conclusion about the structure activity relationship can be drawn based on the biological results. It was clear that the feature of phenyl ring B affect the inhibition activity dramatically. When there is no further substituent on phenyl ring B other than phenoxyl and triazolesulfonamidyl, the introduction of electron-withdrawing substituent such as halogen (**6a** and **6c**) or trifluromethyl (**6b**) but not cyano group (**6h**) on the para position of the phenoxyl ring seems favorable to maintain bc_1 complex inhibition since compounds with substitution on other position (*ortho* or *meta*) all showed much less activity regardless of electron-donating or electron-withdrawing groups were introduced. Interestingly, substituting a halogen atom to the *ortho* position of phenoxyl group on phenyl ring B leads to considerable improvement in the bc_1 inhibition. For example, the activity of compound **6o** (IC_{50} = 9.6 μM) with chlorine tied at the *ortho* position of phenoxyl group displayed 3-fold enhancement over that of the unsubstituted compound **6c** (IC_{50} = 28.8 μM). Similarly, compound **6r** also showed a little higher inhibition activity (IC_{50} = 15.5 μM) as compared to compound **6b** (IC_{50} = 27.4 μM) without chlorine substitution on the *ortho* position of phenyl ring B. It was worthy to note that the activity was further improved by introducing another halogen substituent on ring B. For instance, the activity of compound **6p** and **6s** bearing dichloro-substituent of phenyl ring B increased a further 2-fold and 3-fold over compound **6o** and **6r** with monochloro-substitution, respectively. When the substituted pattern of ring B was fixed with two chlorine substituents, 2,4,6-trichloro substituted phenoxyl was selected as ring A, the most potent compound **6v** with IC_{50} value of 3.2 μM was discovered. In comparison, chlorine substitution is superior to fluorine substitution on ring B. when the substituent was changed from chlorine (**6o**)

to fluorine (**6t**), the bc_1-inhibiting activity was reduced sharply with IC_{50} value decreased from 9.6 μM to 37.6 μM, nearly 4-fold reduction. Similarly, difluoro substituted compound (**6u**) also displayed higher activity than monofluorine substituted counterpart (**6t**). These observations demonstrated that the substituted pattern of phenyl ring B played a crucial role for their interaction with the target enzyme. Further investigation indicated that phenyl ring A can be replaced with more bulky group such as naphthyl without affecting their activity when compared the activity of compounds **6z** and **6I** with that of compounds **6p** and **6u**. However, when heterocycle such as substituted pyridyl was selected instead of phenyl ring A, the bc_1 inhibition activity was decreased significantly and some of them even lost activity.

Because SCR is composed of respiratory complex II (SQR) and complex III (bc_1 complex), it is very interesting to further determine which one, SQR or bc_1, is responsible for the inhibition activity against SCR system. Then, those inhibitors with IC_{50} values less than 100 μM were assayed against SQR and bc_1 alone. Interestingly, apart from several compounds such as **6a**, **6b**, **6c**, **6n** and **6w**, which did not show significant inhibition effect toward bc_1, most compounds showed characteristics of dual inhibitors of SQR and bc_1. Additionally, the inhibition potency against SQR is usually over one order of magnitude higher than that to complex bc_1. Their IC_{50} values against porcine SQR ranged from 0.2 to 43.1 μM, whereas the IC_{50} values against complex bc_1 varied from 35.5 to 105.0 μM. These results might indicate that the inhibition against succinate-cyctochrome reductase (SCR) is mainly attributed to the interruption of the electrons transfer from succinate to ubiquinone in SQR. Considering the inhibition activity against SQR system, it can be concluded that the IC_{50} values are generally smaller than those against SCR system. Nevertheless, the interaction regularity between the inhibitors and SQR and the tendency of their inhibitory potency are consistent with that of SCR system. For instance, introduction of halogen atom such as chlorine or fluorine to the *ortho* position of phenoxyl group on phenyl ring B is favorable to the activity against SQR. This is evidenced by the chlorinated inhibitor **6o** (IC_{50} = 4.3 μM) and **6r** (IC_{50} = 2.7 μM) whose IC_{50} are 5-fold

and 4-fold higher as compared to inhibitor **6c** (IC_{50} = 23.0 μM) and **6b** (IC_{50} = 9.9 μM). Similarly, the activity of dihalogenated inhibitor such as **6p**, **6s** and **6u** are superior to the counterpart monohalogenated inhibitors **6o**, **6r** and **6t**.

Table 1 Inhibitory activity of the synthesized compounds against porcine SCR, SQR and cyt bc_1 [a]



6

Entry	R_1	R_2	R_3	IC_{50} (μM)		
				SCR	SQR	cyt bc_1
6a		H	H	81.8±2.1	43.1±2.1	1%[a]
6b		H	H	27.4±1.0	9.9 ±1.1	23%[a]
6c		H	H	28.8±1.1	23.0±1.3	3%[a]
6d		H	H	>100	-	-
6e		H	H	>100	-	-
6f		H	H	>100	-	-
6g		H	H	>100	-	-
6h		H	H	>100	-	-
6i		H	H	>100	-	-
6j		H	H	>100	-	-
6k		H	H	>100	-	-
6l		H	Cl	>100	-	-
6m		H	F	>100	-	-
6n		Cl	Cl	37.8±3.8	10.7±1.1	5%[a]
6o		H	Cl	9.6±1.2	4.3 ±1.2	40%[a]
6p		Cl	Cl	5.0±0.8	0.2 ±0.1	52.6±1.5
6q		H	Cl	17.6±2.6	3.1 ±1.5	105.0±1.0
6r		H	Cl	15.5±2.0	2.7 ±1.9	39%[a]
6s		Cl	Cl	5.3±1.1	0.3 ±0.1	51.8 ±1.0
6t		H	F	37.6±1.8	9.5 ±1.1	37%[a]
6u		F	F	12.5±1.2	2.5 ±1.2	64.9 ±1.2
6v		Cl	Cl	3.2±0.1	0.5±0.0	35.5±1.0
6w		F	F	15.8±1.2	0.9 ±0.1	0
6x		H	Cl	14.7±1.1	3.8 ±1.2	55.1 ±1.1
6y		H	Cl	16.6±1.1	3.3 ±1.1	45.7 ±1.1

6z		Cl	Cl	5.9±1.2	0.7±0.1	49.2 ±1.1
6l		F	F	9.5±1.1	1.8±1.1	58.2±1.1
amisulbrm				93.0±1.3	0	29%[a]
Antimycin				0.033×10^{-3}±0.00027	-	0.26×10^{-3}±0.046

[a] the inhibition ratio was determined at the concentration of 100 μM.

3.3 Inhibition activities of selected compounds against plant-pathogenic bacterium *Xanthomonasoryzae* pv. *oryzae*

Bacterial blight of rice, caused by *Xanthomonasoryzae* pv. *oryzae* is one of the bacterial diseases of rice in many rice-growing regions of the world including southern China.[44] The inhibitory potency of some selected compounds against *Xanthomonasoryzae* pv. *oryzae* were evaluated at a concentration of 20 μg/mL and, the results listed in Table **2** indicated that these compounds displayed varied antibacterial activity with the inhibition ratios ranging from 1.5% to 95.6% depending on the substitution pattern of the diphenyl ether component of the inhibitors. It is difficult to draw some reliable conclusions about the structure-activity relationship according to these results. Evidently, substituting a cyano group on aromatic ring A is unambiguously facilitate the antibacterial activity since compound **6g** with a *ortho*-cyano group and compound **6h** with a *para*-cyano substituent are showed 94.7% and 94.3% inhibition potency, respectively. Furthermore, benzyloxyl substituent also demonstrated its importance for conserve inhibition activity against *Xanthomonasoryzae* pv. *oryzae* because compound **6j** with benzyloxyl group on the *meta*-position of phenyl-ring A exhibited 95.6% inhibition ratio. However, when the benzyloxyl substituent was moved from the *meta*-position to *para*-position in phenyl-ring A, the resulting compound **6i** produced much lower activity. The analog **6u** bearing a 2,4-dichlorinated phenyl-ring A is also favorable to antibacterial activity. Other halogenated samples such as **6v**, **6s** and **6e** showed moderate inhibition rate, namely 73.0%, 58.5% and 56.5% respectively.

The preliminary antibacterial assay against *Xanthomonasoryzae* pv. *oryzae* at the concentration of 20μg/mL revealed that compounds **6g**, **6h**, **6j** and **6u** showed excellent potency. Therefore, these four compounds were selected for further

evaluation. Bismerthiazol, the most frequently used effective bactericide which has both protective and curative activity against *Xanthomonasoryzae* pv. *oryzae*, was selected as positive control. The EC_{50} values were determined based on *in vitro* inhibition of bacterial growth according to a previous study.[44]

As listed in Table 2, compound **6h** (EC_{50} = 6.62 μg/mL) and **6j** (EC_{50} = 6.76 μg/mL) exhibited better antibacterial activity against *Xanthomonasoryzae* pv. *oryzae* than that of bismerthiazol (EC_{50} = 12.46 μg/mL). Whereas compounds **6g** and **6u** are much less active, their EC_{50} values are 45.07 μg/mL and 78.72 μg/mL, respectively. Impressively, compound **6j** showed more than 10-fold higher antibacterial activity than bismerthiazol when compared their EC_{90} values. However, compound **6h**, which has the highest EC_{50} value, displayed more than 4-fold lower EC_{90} values than the positive control bismerthiazol. The other two compounds, **6g** and **6u** showed much less active with regard to the EC_{90} values. It is worthy to note that three of the four compounds namely **6**g, **6**h and **6**j, which showed good antibacterial activity against *Xanthomonasoryzae* pv. *oryzae*, exhibited much lower inhibition potency toward SCR in terms of their respective IC_{50}. At the same time, some compounds such as 6p, 6s and 6v, displaying excellent SCR inhibitory activity, are inactive against *Xanthomonasoryzae* pv. *oryzae*. One possible reason may due to the inhibitors must suffer from complicated biological process such as absorption, distribution, metabolism and excretion property, resulting some inhibitors with high enzyme inhibition inactive during *in vivo* evaluation, alternatively, enzymes/protein other than SCR that interact with the inhibitors may account for the antibacterial activity.

Table 2 Inhibition effect of selected compounds against *x. oryzae* pv. *oryzae*

Compd.	Inhibition ratio (%, 20 μg/mL)	toxic regression equation	EC_{50} (μg/mL)	EC_{90} (μg/mL)
6a	11.2			
6b	1.5			
6e	56.5			
6f	74.5			
6g	94.7	$y = 0.4401x + 4.6404$	45.07	36382.71
6h	94.3	$y = 0.5543x + 4.5449$	6.62	1357.89
6j	95.6	$y = 1.8401x + 3.4724$	6.76	33.63

6n	8.7			
6r	34.3			
6s	58.5			
6u	93.1	$y = 0.6081x + 4.2194$	78.72	14274.24
6v	73.0			
Bismerthiazol		$y = 0.8855x + 4.0297$	12.46	349.169

4. Conclusion

In conclusion, a new series of 1,2,4-triazole-1,3-disulfonamide derivatives were designed and synthesized by coupling diverse diphenyl ether moiety with triazolesulfonamide unit. The *in vitro* bioassay results indicated that these newly prepared compounds exhibited varied inhibition toward porcine succinate-cyctochrome reductase (SCR) dependent on the substituted pattern of the diphenyl ether moiety. Further evaluation against respective SQR and cyt bc_1 indicated that most of the title compounds are dual inhibitors of SQR and bc_1 complex activity. In general, the potency of these dual inhibitors against SQR is much higher than that of cyt bc_1, showing the SCR inhibition might be contributed greatly from the SQR inhibition. Notablely, placing the halogen substituent adjacent to the phenoxyl group on the phenyl ring B is crucial in determining the inhibitory activity. Compounds **6p**, **6s**, **6v** and **6z**, in which dichloro-substitutent was introduced in the middle phenyl ring B displayed good succinate-cyctochrome reductase inhibition with the IC_{50} values of 5.0, 5.3, 3.2 and 5.9 μM, respectively. In comparison, the commercial control showed much lower inhibition activity with the IC_{50} value of 93.0 μM. Further antibacterial assay against *Xanthomonasoryzae* pv. *oryzae* indicated that four compounds **6g**, **6h**, **6j** and **6u** showed excellent potency at the concentration of 20 μg/mL. In particular, **6h** and **6j** exhibited much better antibacterial activity than the commercial control bismerthiazol in terms of their EC_{50}. Impressively, **6j** has an EC_{90} of 33.62 μg/mL, more than 10-fold higher than that of bismerthiazol, may recognize as one potential fungicide for combat bacterial blight of rice, the bacterial diseases of rice caused by *Xanthomonasoryzae* pv. *oryzae*.

ACKNOWLEDGMENT

The research was supported by the National Key Technologies R&D Program (2011BAE06B05) and the National Natural Science Foundation of China (No. 21332004 and 21372094).

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