JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Inhibition of Tobacco Bacterial Wilt with Sulfone Derivatives Containing an 1,3,4-Oxadiazole Moiety

Wei-Ming Xu,[†] Fei-Fei Han,[†] Ming He, De-Yu Hu, Jiang He, Song Yang,*[,‡] and Bao-An Song*[,‡]

State Key Laboratory Breeding Base of Green Pesticide and Agricultural Bioengineering, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Huaxi District, Guiyang 550025, China

Supporting Information

ABSTRACT: A series of new sulfone compounds containing the 1,3,4-oxadiazole moiety were designed and synthesized. Their structures were identified by ^{1}H and ^{13}C nuclear magnetic resonance and elemental analyses. Antibacterial bioassays indicated that most compounds exhibited promising in vitro antibacterial bioactivities against tobacco bacterial wilt at 200 μg/mL. The relationship between structure and antibacterial activity was also discussed. Among the title compounds, **5′c**, **5′h**, **5′i**, and **5′j** could inhibit mycelia growth of *Ralstonia solanacearum* in vitro by approximately 50% (EC_{50}) at 39.8, 60.3, 47.9, and 32.1 μg/mL, respectively. Among them, compound **5′j** was identified as the most promising candidate due to its stronger effect than that of Kocide 3000 [$Cu(OH)_2$] within the same concentration range. Field trials demonstrated that the control effect of compound **5′j** against tobacco bacterial wilt was better than that of the commercial bactericide Saisentong. For the first time, the present work demonstrated that sulfone derivatives containing 1,3,4-oxadiazole can be used to develop potential bactericides for plants.

KEYWORDS: *antibacterial activity, sulfone derivatives, 1, 3, 4-oxadiazole moiety, tobacco bacterial wilt*

INTRODUCTION

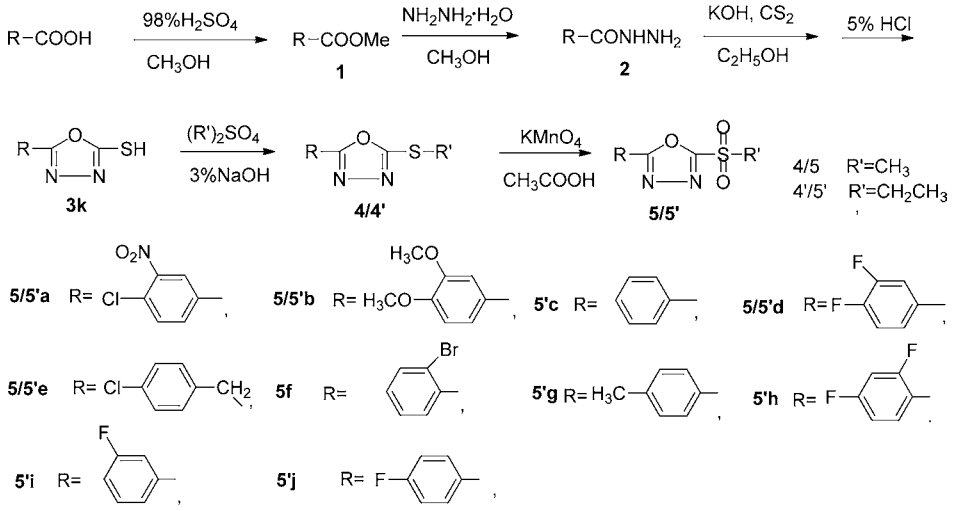
Sulfone derivatives are an important class of bioactive compounds that have a wide spectrum of activities. These compounds have varied activities as antibacterial,[1] insecticidal,[2] antifungal,[3] herbicidal,[4] antihepatitis,[5] antitumor,[6] anti-inflammatory,[7] anticancer,[8] anti-HIV-1,[9] and antitubercular agents.[10] There is evidence that the key feature of these compounds is a five- or six-membered heterocycle attached to the sulfone.[11] Modifications on these heterocycles have been performed. For example, 2-((4-chlorobenzyl)sulfonyl)-5-(methylsulfonyl)-1,3,4-thiadiazole has been prepared by Joachim et al.[12] This compound exhibits good inhibitory activity against *Plasmopara viticola* at 10 mg/kg concentration. On the other hand, 2,4-dibromo-5-methyl-1-((2-methyl-5-nitrophenyl)sulfonyl)-1*H*-imidazole has been reported by Assmann et al.[13] This compound exhibits strong activity against *Phytophthora infestans* and *P. viticola* at 50 g/ha dosage. Moreover, 2-(5-ethyl-1-methyl-1*H*-pyrazol-3-yl)-5-(methylsulfonyl)-1,3,4-oxadiazole has been prepared by Yuan et al.[14] This compound exhibits medium inhibitory activity against *Phoma asparagi*. Tthe first commercialized fungicide of this structure type was Oxycarboxin, developed by the U.S. Uniroyal Co. in 1966. In the past few decades, a large number of other fungicides containing sulfone (e.g., Tolylfluanid, Dichlofluanid, Cyazofamid, Amisulbrom, and Oryzaemate) with potent bioactivities have been introduced in the market.[15] With growing applications mentioned above, the research on the synthesis and bioactivity of sulfone derivatives have attracted extensive attentions from chemists and biologists in recent years. In our previous work we had demonstrated antifungal activities of a series of novel sulfone derivatives of 2-sulfonyl-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (**I**)[16] and 2-sulfinyl-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (**II**).[17] Further in vitro bioassays have disclosed that **Ia** [2-(methylsulfonyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole] and **IIa** [2-(benzylsulfinyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole] possess high antifungal activities against 10 kinds of fungi [effective dose for 50% inhibition (EC_{50}) = 19.9−93.3 μg/mL]. Structure−activity relationship (SAR) analyses have suggested that 2-(methylsulfonyl)-1,3,4-oxadiazole moiety is crucial for the potent antifungal activities.

Controlling of plant bacterial diseases has long been a challenging mission in the agricultural sector. The constantly increasing threats caused by tobacco bacterial wilt (*Ralstonia solanacearum*), tomato bacterial wilt (*Pseudomonas solanacearum*), and *Xanthomonas oryzae* have become a matter of great concern throughout the world. The application of traditional pesticides has not proved very effective, and at the same time high residue level or negative impact on the environment was caused. Copper formulation, a commercial bactericide, is a type of inorganic pesticide which can enhance resistance in host tobacco and tomato plant. Despite being useful in the treatment of plants affected by tobacco bacterial wilt, the use of copper formulation for field trial is largely limited due to its phytotoxicity, strong alkali, and low mobility. Therefore, the search for new antibacterial agents still remains a daunting task in pesticide science.[18]

To aid the development of high activity, proper alkali, high mobility, and readily available plant bacteria inhibitors, we developed herein a series of new 2-(methyl/ethylsulfonyl)-1,3,4-oxadiazole sulfone derivatives and evaluated them for their antibacterial activities. The title compounds displayed different antibacterial activities at 200 μg/mL; **5′c**, **5′h**, **5′i**, and **5′j** could inhibit mycelia growth of *R. solanacearum* in vitro by

Received: September 16, 2011
Revised: December 28, 2011
Accepted: December 29, 2011
Published: December 29, 2011

Scheme 1. Synthetic Route of 5a−5′j



approximately 50% (EC_{50}) at 39.8, 60.3, 47.9, and 32.1 μg/mL. The field trials demonstrated that the control effect of compound **5′j** was better than that of the commercial bactericide Saisentong, a kind of organocopper formulation. The present work demonstrates that sulfone derivatives containing 1,3,4-oxadiazole can be used to develop potential agrochemicals. To the best of our knowledge, this is the first report on the antibacterial activity of 2-(methyl/ethyl sulfonyl)-1,3,4-oxadiazole derivatives.

MATERIALS AND METHODS

Instruments. ^{1}H and ^{13}C NMR (solvent $CDCl_3$ or acetone-d_6 or DMSO-d_6) spectral analyses were performed on a JEOL-ECX 500 NMR spectrometer at room temperature using TMS as an internal standard. The IR spectra were obtained from a KBr pellet using a SHIMADZU-IR Prestige-21 spectrometer. The melting points were determined on an XT-4 digital microscope (Beijing Tech Instrument Co.) Analytical thin-layer chromatography was performed on silica gel GF254 (400 mesh). Column chromatographic operations were performed on silica gel (100−200 or 200−300 mesh, Qingdao Haiyang Chemical Co.). A Sephadex LH-20 column chromatographic instrument (Beijing Huideyi Tech Instrument Co.) and an XAD-7HP macroporous resin (Rohm and Haas Co.) were employed for the extraction and purification of chemical composition. Tobacco seeds (Yun 85) were kindly provided by Guizhou Institute of Tobacco. Fertilized soil (Baltisches substrate, HAWITA BALTIC) was bought from Hawita Gruppe GmbH for plant cultivation.

General Synthetic Procedures for Title Compounds. Compounds **5a−5′j** were synthesized according to known methods,[19] as shown in Scheme 1. The starting materials were substituted benzoic acid, fatty acids, and heterocyclic acid. 2-Thiol-5-substituted-1,3,4-oxadiazole was synthesized in three steps via esterification, hydrazidation, and cyclization. The oxadiazole analogs were then converted into their corresponding thioether derivatives **4** by thioetherification. Finally, the target sulfones **5** were obtained via oxidation of the thioether. The physical characteristics, IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis data for all the synthesized compounds are reported in the experimental protocols. The data for **5′j** is shown below, while data for the others can be found in the Supporting Information.

2-(Ethylsulfonyl)-5-(4-fluorophenyl)-1,3,4-oxadiazole (5′j). Yield 80.3% of a white solid; mp 127−129 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15−7.75 (m, 4H, ArH), 3.53(q, J = 7.45, 2H, CH_2), 1.47 (t, J = 7.45, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.4, 163.3, 135.3, 130.6, 131.3, 129.6, 50.3, 6.7; IR (KBr, cm^{-1}) ν 3038, 2913, 1605, 1557, 1357, 1153. Anal. Calcd for $C_{10}H_9FN_2O_3S$: C, 46.87; H, 3.54; N, 10.93. Found: C, 46.62; H, 3.81; N, 10.59.

In Vitro Antibacterial Bioassay. The antibacterial activities of some of the synthesized compounds were tested against tobacco bacterial wilt by the turbidimeter test.[20] The compounds were tested at concentrations of 500 and 200 μg/mL. Acetone in sterile distilled water served as the blank control, whereas Kocide 3000 was the positive control. About 40 μL of solvent NB (3 g of beef extract, 5 g of peptone, 1 g of yeast powder, 10 g of glucose, 18 g of agar, 1 L of distilled water, pH 7.0−7.2) containing tobacco bacterial wilt was added to 5 mL of solvent NA (3 g of beef extract, 5 g of peptone, 1 g of yeast powder, 10 g of glucose, 1 L of distilled water, pH 7.0−7.2) containing the test compounds and Kocide 3000. The inoculated test tubes were incubated at 30 ± 1 °C and continuously shaken at 180 rpm for 2 d. The growth of the cultures was monitored on a spectrophotometer by measuring the optical density at 600 nm (OD_{600}) given by $\text{turbidity}_{\text{corrected values}} = OD_{\text{bacterial wilt}} - OD_{\text{no bacterial wilt}}$, I (%) = $(C_{tur} - T_{tur})/C_{tur} \times 100$. C_{tur} represents the corrected turbidity values of bacterial growth on untreated NA (blank control), and T_{tur} means the corrected turbidity values of bacterial growth on treated NA. I denotes the inhibition rate.

Some of the title compounds were tested against tobacco bacterial wilt under different concentrations (200, 100, 50, 25, 12.5, and 0 μg/mL). EC_{50} values were statistically estimated by probit analysis with the probit package of the SPSS 17.0 software using a personal computer. The average EC_{50} was computed from at least three separate analyses for growth inhibition using the basic EC_{50} program version SPSS 17.0.

In Vivo Antibacterial Activities Tests Under Greenhouse Conditions. Seedling plates were covered with thus prepared soil. Three to four uniform tobacco seeds were sown in each spot of the seedling plate. The seedling plates were then placed in sterilized water for floating cultivation at 25 °C. After 30 d, the tobacco plants (with about three leaves) were carefully transplanted to pots containing 500 g of soil, one plant for each pot. After 10 d, the uniformly grown tobacco plants will have four to six leaves and are ready to test. Four compounds were used in this study, compounds **5′c**, **5′j**, commercially available $Cu(OH)_2$ (Kocide 3000, Dupont, 46.1% WDG), and Saisentong. All compounds were dissolved in DMSO at 50 mg/mL and then diluted with deionized water containing 0.1% Tween-20 to the final concentration of 500 μg/mL. The $Cu(OH)_2$ WDG was diluted directly with deionized water to 500 μg/mL.

TTC media was inoculated with tobacco bacterial wilt and cultured for 24−48 h. Single colonies with strong virulence were then picked out and transferred into 100 mL of NB culture medium and were then incubated for 24 h, at 30 °C, 180 rpm to give the bacterial stock suspension. Then 10 mL of stock suspension was added into 1 L of NB culture media and was cultured for a further 24 h to give the

dx.doi.org/10.1021/jf203772d | J. Agric.Food Chem. 2012, 60, 1036−1041

Table 1. Inhibition Effect and Toxicity of the Testing Compounds (500, 200 μg/mL) against Tobacco Bacterial Wilt[a]

	inhibition (%)				
compd	500 μg/mL	200 μg/mL	toxic regression equation	r	EC_{50}(μg/mL)
5a	20.1 ± 0.8c	0.0 ± 0.3 g			
5′a	46.0 ± 0.4a	17.0 ± 0.7 cd			
5b	10.1 ± 1.0de	6.0 ± 2.4efg			
5′b	9.3 ± 0.4e	2.8 ± 0.3f			
5′c	100.0 ± 2. 9a	100.0 ± 2.7a	$y = 3.1363x - 0.0223$	0.8883	39.8 ± 4.9a
5d	12.0 ± 1.1de	11.1 ± 1.3def			
5′d	52.0 ± 1.5a	46.7 ± 0.8b			
5e	31.0 ± 1.5b	19.2 ± 0.5c			
5′e	31.4 ± 0.3ab	18.7 ± 2.1cde			
5f	14.2 ± 0d	9.9 ± 0.4e			
5′g	39.0 ± 0.4ab	15.0 ± 0.3d			
5′h	100.0 ± 2.6a	92.3 ± 4.4a	$y = 3.8065x - 1.7789$	0.9912	60.3 ± 2.8c
5′i	100.0 ± 3.1a	100.0 ± 1.9ag	$y = 1.4616x + 2.5443$	0.9583	47.9 ± 3.3b
5′j	100.0 ± 12.2abcd	100.0 ± 4.9a	$y = 1.8065x + 2.2789$	0.8843	32.1 ± 2.3c
Saisentong	100.0 ± 10.0a	100.0 ± 8.7ab			
Kocide 3000		100.0 ± 5.7a	$y = 4.8739x - 3.1000$	0.9792	
$Cu(OH)_2$					45.9 ± 6.6bc

[a]The statistic analysis was conducted by ANOVA method at the condition of equal variances assumed ($p > 0.05$) and equal variances not assumed ($p < 0.05$). The different lowercase letters indicate the values of inhibition and EC_{50} with significantly difference among different treatment groups at $p < 0.05$.

bacteria working suspension ready for infection.[21,22] Testing compound solution was poured into the tobacco root soil, 40 mL for each root. After 24 h, the tobacco roots were intentionally damaged by cutting two or three times with knives. Then the prepared bacteria working suspension was poured into the root soil, 20 mL for each root. Deionized water without compound or bacteria was set as blank control, while bacteria treatment without compound treatment was set as negative control.[23,24] Three replicates were carried out for each treatment. The disease infection rates of the tobacco plants were investigated at 7, 14, and 21 days and then the plants were treated with testing compounds solutions. The plant disease infection rate, infection index, etc. were recorded according to the "Protocols of Disease Investigation and Classification".[25]

Field Trials of 5′j against Tobacco Bacterial Wilt. Field trials of **5′j** against tobacco bacterial wilt were conducted in Guizhou Province, China, in May 2011. The effect of the natural infection of *R. solanacearum* was studied in a field with tobacco having suffered tobacco bacterial wilt for 1 year. Sterile distilled water served as the control, whereas the commercial bactericide Saisentong ($C_5H_4N_6S_4Cu$, 5,5′-(methylenediimino)bis[1,3,4-thiadiazole-2(3*H*)-thione] copper salt; China Zhejiang Dongfeng Chem. Ind. Co., Ltd.) was the positive control. The experimental design area of the plot was 30 m^2. The field was flooded twice, and then compounds were sprayed on the foliage of the tobaccos. For each treatment, three replicates were conducted. The tobacco bacterial wilt disease index system was previously introduced as follows: 0 = healthy, 1 = one leaf wilting, 2 = two or three leaves wilting, 3 = two or three healthy leaves and all others wilting, and 4 = all leaves wilting. The tobacco bacterial wilt index values were determined as follow: disease index = $(4W + 3X + 2Y + Y)/4A$, where W, X, and Y are equal to the corresponding sum of the disease classification of plants with symptoms after 7, 14, or 21 d. A is equal to the sum of the total investigated plants. The inhibiting effects of the field trials were calculated by the formula I (%) = $(CK - PT_1)/CK \times 100$, where CK represents the disease index of fungi growth on an untreated field, PT_1 represents the disease index of fungi on a treated field after treatment. I represents the inhibition rate.[26] SPSS 17.0 was used for the statistical analyses of the data of inhibition. ANOVA (least significant difference method) was performed to analyze the differences among the treatment groups.

RESULTS AND DISCUSSION

The syntheses of **5a**–**5′j** are shown in Scheme 1, the electron-rich (methyl/ethyl)thio moiety **4** can be oxidized to sulfone by a variety of agents, such as *m*-CPBA[27] and H_2O_2.[28] However, the C–S bond between methyl/ethyl and sulfonyl can be easily broken. In the present experiments, the methyl/ethylthio moiety **4** was oxidized with potassium permanganate to yield the corresponding methyl/ethylsulfonyl **5**. The oxidation reaction was very fast and completed in 10 min at room temperature. The C–S bond between methyl/ethyl and sulfonyl could break if the reaction time was prolonged. As indicated by ^{1}H NMR, the methyl absportion peaks shown at 3.5–2.8 ppm disappeared, in favor of the broad peaks between 9.5 and 11 ppm.

In Vitro Antibacterial Activities of Oxadiazole Sulfones. *R. solanacearum* infects over 50 families of plants, including vegetables, ornamentals, crops, fruits, and woody perennial plants. Host plants of global importance include tomato, eggplant, pepper, tobacco, ginger, and groundnut. The inhibition effects of oxadiazole methyl or ethyl sulfone compounds on tobacco bacterial wilt were studied. The results of the preliminary bioassays were compared with those of DuPont Kocide 3000 fungicide/bactericide; as indicated in Table 1, the compounds had low to satisfactory antibacterial activities against the tested bacteria. It was found that compounds **5′c**, **5′h**, **5′i**, and **5′j** exhibited significant inhibition effects against tobacco bacterial wilt, showing a control efficacy of almost 100% at 500 or 200 μg/mL, which was equal to that of the most active fungicide Kocide 3000, with control efficacy = 100% at 200 μg/mL. The variances among the different substitutes on phenyl greatly affected the antibacterial activities of the compounds against tobacco bacterial wilt. For example, when R = 3-fluorophenyl or 4-fluorophenyl, there was an apparent increase in antibacterial activity. On the other hand, when R was introduced to the other substituted phenyls, the corresponding compounds inhibited tobacco bacterial wilt by 0%–46.7% at 200 μg/mL. Further toxicity bioassays disclosed that **5′c**, **5′h**, **5′i**, and **5′j** remarkably inhibited tobacco bacterial

Table 2. Inhibition Effect of Testing Compounds against Tobacco Bacterial Wilt under Greenhouse Conditions at 500 μg/mL[a]

	7 days after spraying			14 days after spraying			21 days after spraying		
compd	morbidity (%)	disease index (%)	control efficiency (%)	morbidity (%)	disease index (%)	control efficiency (%)	morbidity (%)	disease index (%)	control efficiency (%)
5′c	60.0	60.0	34.6 ± 2.2c	100.0	86.7	12.0 ± 1.0c	100.0	91.7	8.3 ± 1.5c
5′j	13.3	13.3	85.5 ± 0.7b	60.0	40.0	59.3 ± 2.0b	100.0	61.7	38.3 ± 0.7b
Saisentong	6.7	6.7	92.7 ± 1.6a	73.3	40.0	59.3 ± 2.7b	100.0	78.3	21.7 ± 0.5b
$Cu(OH)_2$	50.3	53.3	41.9 ± 1.4c	100.0	95.0	3.4 ± 0.5d	100.0	100.0	0.0 ± 0.0d
CK1[b]	0.0	0.0	100.0 ± 0.0a	0.0	0.0	100.0 ± 0.0a	0.0	0.0	100.0 ± 0.0ad
CK2[b]	100.0	91.7	–	100.0	98.3	–	100.0	100.0	–

[a]The statistical analysis was conducted by ANOVA method at the condition of equal variances assumed ($p > 0.05$) and equal variances not assumed ($p < 0.05$). The different lowercase letters indicate the values of inhibition and EC_{50} with significantly difference among different treatment groups at $p < 0.05$. [b]CK1: blank control; CK2: negative control.

Table 3. Results of Field Trials of 5′j against Tobacco Bacterial Wilt

		7 d after spraying		14 d after spraying		21 d after spraying	
compd	dosage (g ai/ha)[a]	index values	inhibition (%)[c]	index values	inhibition (%)	index values	inhibition (%)
Saisentong	540	3.8	61.2 ± 12.7a	3.9	71.1 ± 6.4a	6.4	67.3 ± 10.5a
5′j	405	3.4	65.3 ± 8.0a	4.2	68.8 ± 8.7a	5.5	71.9 ± 11.2a
CK[b]		9.8		13.5		19.6	

[a]ai means active ingredient. [b]CK means untreated blank control. [c]The statistical analysis was conducted by ANOVA method at the condition of equal variances assumed ($p > 0.05$) and equal variances not assumed ($p < 0.05$). Different lowercase letters indicate the values of inhibition and EC_{50} with significantly difference among different treatment group at $p < 0.05$.

wilt, exhibiting EC_{50} values of 39.8, 60.3, 47.9, and 32.1 μg/mL, respectively. These compounds possessed good activities against the tested bacteria and were even superior to the commercial agent Kocide 3000 (EC_{50} = 45.9 μg/mL). This finding suggested that **5′c**, **5′h**, **5′i**, and **5′j** may be promising lead structures for the further discovery of new antibacterial agents.

In Vivo Antibacterial Activities of Some Compounds under Greenhouse Conditions. All CK2 (positive control) were seriously infected in 1 week after the bacteria suspension treatment, indicating that this *R. solanacearum* strain has strong virulence. At the same time, CK1 (negative control) was not infected throughout the whole month, indicating that tobacco plants were healthy in the whole growth period and were not infected by other pathogens.

It can be seen that the controlling effect of compounds **5′c** was 34.6%, which was lower than that of the positive control $Cu(OH)_2$, 41.9%. The other compounds **5′j** and Saisentong were much more potent against tobacco bacterial wilt with efficacies of 85.5% and 92.7%, respectively, which were much higher than that of $Cu(OH)_2$. The controlling effects at 14 d after the second treatment of compounds **5′c**, **5′j**, and Saisentong were all prominent, while $Cu(OH)_2$ was almost inactive. It can be seen from Table 2 that at 21 d after the third treatment, both tested compounds and Saisentong still exhibited certain controlling effect, while $Cu(OH)_2$ was totally inactive. In general, among the tested compounds, the effects of compounds **5′j** and Saisentong were much better than the others, and compound **5′j** was persistently and stably effective against tobacco bacterial wilt.

Field Trials of 5′j against Tobacco Bacterial Wilt. Field trials of **5′j** against tobacco bacterial wilt were carried out, and the results are summarized in Table 3. The inhibition rates of **5′j** at 405 g of active ingredient/ha WP dosage were 65.3%, 68.8%, and 71.9% after 7, 14, and 21 d, respectively. The effect of **5′j** was better than that of the commercial bactericide Saisentong; furthermore, compound **5′j** was safe for the tobacco, similar to the commercial fungicide, and no visible injury was observed on the aerial parts of the tobacco. The data was statistically analyzed by ANOVA (least significant difference), and the results showed no significant differences ($p > 0.05$) between Saisentong and **5′j** at 7 d, 14 d, and 21 d after spraying. Comparing the same treatment at different time, the results showed no differences between any 7 d, 14 d, and 21 d after spraying ($p > 0.05$) for **5′j** and Saisentong.

SAR Analysis of the Antibacterial Activities against Tobacco Bacterial Wilt. As summarized in Table 1, some of the compounds showed promising potency against tobacco bacterial wilt. To examine the SAR, different substituent groups were introduced into the 1,3,4-oxadiazole ring. When R was fixed and R′ = methyl or ethyl, no significant activity changes were observed. When R was phenyl or 3- or 4-fluoro substituted phenyl, the corresponding target compounds had excellent activities, and some title compounds showed good activities comparable with that of Saisentong. On the basis of the activity values indicated in Table 1, the relationships of the antibacterial activities with the different phenyls (type, position, and number of substituents) were deduced. Three main conclusions were drawn. First, at the same substituted position of the phenyl, the electron-withdrawing group was superior to the electron-donating group in terms of antibacterial activity. For example, inhibition of **5′d** (R = 3,4-difluorophenyl) on tobacco bacterial wilt was 52.0% and 46.7% at 500 μg/mL, whereas **5′b** (R = 3,4-dimethoxyphenyl) was only 9.3% and 2.8% under the same condition. EC_{50} of **5′j** (R = 4-fluorophenyl) on tobacco bacterial wilt was 32.1 μg/mL, and compound **5′j** showed 100% inhibition against tobacco bacterial wilt at 500 μg/mL, changing the fluoro group into a H or CH_3 atom, EC_{50} of **5′c** (R = phenyl) was 39.9 μg/mL and inhibition of **5′g** (R = 4-methylphenyl) was 39.0% at 500 μg/mL. Second, compared with the same substituents on phenyl, the monosubstituents had higher activities than the polysubstituents. For example, inhibiton of **5′j** (R = 4-fluorophenyl) on tobacco bacterial wilt was 100% against tobacco bacterial wilt at

dx.doi.org/10.1021/jf203772d | J. Agric.Food Chem. 2012, 60, 1036–1041

500 μg/mL, whereas EC_{50} of **5′h** (R = 2,4-difluorophenyl) was 60.3 μg/mL and **5e** (R = 3,4-difluorophenyl) had 31.0% efficacy at 500 μg/mL. Third and last, comparing compounds with the same substituents on the phenyl, those with substituents at the 3- or 4-position always had higher inhibition rates for tobacco bacterial wilt. For example, EC_{50} of **5′i** (R = 3-fluorophenyl) was 47.9 μg/mL and EC_{50} of **5′j** (4-fluorophenyl) on tobacco bacterial wilt was 32.1 μg/mL.

In summary, a series of new sulfone compounds containing the 1,3,4-oxadiazole moiety were designed and synthesized on the basis of the lead compound **Ia**. The title compounds **5′c**, **5′h**, **5′i**, and **5′j** exhibited favorable activity against tobacco bacterial wilts in vitro as well as in vivo compared to the commercial fungicides/bactericides Kocide 3000 and Saisentong. The antibacterial tests showed that when the electron withdrawing group at the 3- or 4-position of phenyl was attached to the 5-position of oxadiazole, the corresponding compounds presented good antibacterial activities. The field trials demonstrated that the control effect of compound **5′j** was better than that of the commercial bactericide Saisentong. To our knowledge, this is the first report of sulfone compounds containing 1,3,4-oxadiazole moieties with potent controlling effect against tobacco bacterial wilt on tobacco plants. Further field studies on the biological efficacies, crop safety, and toxicities of these compounds as bactericide candidates need to be conducted.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The characterizations of **5a−5′j**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +86(851)362-0521. Fax: +86(851)362-2211. E-mail: songbaoan22@yahoo.com; fcc.syang@gzu.edu.cn.

Present Address

‡State Key Laboratory Breeding Base of Green Pesticide and Agricultural Bioengineering, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Huaxi District, Guiyang 550025, China.

Author Contributions

†These authors have contributed equally to the manuscript.

Funding

We gratefully acknowledge the financial support of the National Key Program for Basic Research (No. 2010CB 126105), Key Technologies R&D Program (Nos. 2011BAE06B05), and Special Fund for Agro-scientific Research in the Public Interest (No.201203022)

■ ABRREVIATIONS USED

R. solanacearum, *Ralstonia solanacearum*; EC_{50}, 50% effective concentration; ^{1}H NMR, ^{1}H nuclear magnetic resonance; ^{13}C NMR, ^{13}C nuclear magnetic resonance

■ REFERENCES

(1) Richter, H. G. F.; Angehrn, P.; Hubschwerlen, C.; Kania, M.; Page, M. G. P.; Specklin, J.-L.; Winkler, F. K. Design, synthesis, and evaluation of 2β-alkenyl penam sulfone acids as inhibitors of β-lactamases. *J. Med. Chem.* **1996**, *39* (19), 3712−3722.

(2) Fitzjohn, S.; Robinson, M. P. Benzoxazole and benzothiazole derivatives. WO 9406783, 1994 [*Chem. Abstr.* **1994**, *121*, 9394*f*].

(3) Hiromichi, I.; Masakazu, T.; Ten, U.; Seiichi, K. Preparation of disulfonylthiadiazoles and their use as agrochemical microbicides. JP 94116252, 1994 [*Chem. Abstr.* **1994**, *121*, 127847*d*].

(4) Andrew, P.; Jutta, E. B.; Janice, B.; Timothy, D. S. Isoxazoline derivatives and their preparation, herbicidal composition, and use as herbicides to control weeds or plant growth inhibition. WO 2006024820, 2006 [*Chem. Abstr.* **2006**, *144*, 274262*v*].

(5) Gong, P.; Chai, H. F.; Zhao, Y. F.; Zhao, C. S. Synthesis and *in vitro* anti-hepatitis B virus activities of some ethyl 5-hydroxy-1*H*-indole-3-carboxylates. *Bioorg. Med. Chem.* **2006**, *14*, 2552−2558.

(6) Tai, X. S.; Yin, X. H.; Tan, M. Y. Crystal structure and antitumor activity of tri[2-[*N*-(4′-methyl-benzylsulfonyl)amino]ethyl]-amine. *Chin. J. Struc. Chem.* **2003**, *22*, 411−414.

(7) Fang, S. H.; Padmavathi, V.; Rao, Y. K.; Subbaiah, D. R. C.; Thriveni, P.; Geethangili, M.; Padaja, A.; Tzeng, Y. M. Biological evaluation of sulfone derivatives as anti-inflammatory and tumor cells growth inhibitory agents. *Int. Immunopharmacol.* **2006**, *6*, 1699−1705.

(8) Vedula, M. S.; Pulipaka, A. B.; Venna, C.; Chintakunta, V. K.; Jinnapally, S.; Kattuboina, V. A.; Vallakati, R. K.; Basetti, V.; Akella, V.; Rajgopai, S.; Reka, A. K.; Teepireddy, S. K.; Mamnoor, P. K.; Rajagopalan, R.; Bulusu, G.; Khandelwal, A.; Upreti, V. V.; Mamidi, S. R. New styryl sulfones as anticancer agents. *Eur. J. Med. Chem.* **2003**, *38*, 811−824.

(9) Silvestri, R.; Artico, M.; Regina, G. L. Anti-HIV-1 activity of pyrryl aryl sulfone (PAS) derivatives: Synthesis and SAR studies of novel esters and amides at the position 2 of the pyrrole nucleus. *Farmaco* **2004**, *59*, 201−210.

(10) Talath, S.; Gadad, A. K. Synthesis, antibacterial and antitubercular activities of some 7-[4-(5-amino-[1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl] fluoroquinolonic derivatives. *Eur. J. Med. Chem.* **2006**, *41*, 918−924.

(11) Ohshima, T.; Komyojia, T.; Mitani, S. Development of a novel fungicide, eyazofamid. *J. Pestic. Sci.* **2004**, *29*, 136−138.

(12) Joachim, D. H.; Albrecht, M.; Wilhelm, B.; Gerd, H. Preparation of 2,5-bis(alkylsulfonyl)-1,3,4-thiadiazoles as agrochemical fungicides. DE 3838432, 1990 [*Chem. Abstr.* **1990**, *113*, 191364*f*].

(13) Assmann, L.; Stenzel, K.; Erdelen, C.; Kugler, M.; Wachtler, P. Nitrophenyl sulfonyl imidazoles and use thereof for controlling vegetable and animal pests. US 20020094936 A1, 2002 [*Chem. Abstr.* **2002**, *130*, 125074].

(14) Yuan, D. K.; Li, Z. M.; Zhao, W. G.; Chen, H. S. Synthesis and bioactivity of 2-substituted amino-5-pyrazolyl-1,3,4-oxadiazoles. *Chin. J. Appl. Chem.* **2003**, *20*, 624−628.

(15) Komyji, N.; Terumasa, K.; Kazumi, S.; Keiichiro, I. Imidazole compounds and biocidal compositions comprising the same. EP 0298196A1, 1989 [*Chem. Abstr.* **1989**, *110*, 192824].

(16) Chen, C. J.; Song, B. A.; Yang, S.; Xu, G. F.; Bhadury, P. S.; Jin, L. H.; Hu, D. Y.; Li, Q. Z.; Liu, F.; Xue, W.; Chen, Z. Synthesis and antifungal activities of 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-thiadiazole and 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-oxadiazole derivatives. *Bioorg. Med. Chem.* **2007**, *15*, 3981−3989.

(17) Liu, F.; Luo, X. Q.; Song, B. A.; Bhadury, P. S.; Yang, S.; Jin, L. H.; Xue, W.; Hu, D. Y. Synthesis and antifungal activity of novel sulfoxide derivatives containing trimethoxyphenyl substituted 1,3,4-thiadiazole and 1,3,4-oxadiazole moiety. *Bioorg. Med. Chem.* **2008**, *16*, 3632−3640.

(18) Hayward, A. C. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annu. Rev. Phytopathol.* **1991**, *29*, 65−87.

(19) Song, B. A.; Chen, C. J.; Yang, S.; Jin, L. H.; Xue, W.; Zhang, S. M.; Zou, Z. H.; Hu, D. Y.; Liu, G. Synthesis, structure and antitumor activity of 2-alkylthio-5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole compounds. *Acta. Chim. Sin.* **2005**, *63*, 1720−1726.

(20) Paw, D.; Thomas, R.; Laura, K.; Karina, N.; Thomas, A. M. Estimation of bacterial growth rates from turbidimetric and viable count data. *Int. J. Food Microbiol.* **1994**, *23*, 391−404.

(21) Nonomura, T.; Matsuda, Y.; Bingo, M.; Onishi, M.; Matsuda, K.; Harada, S.; Toyoda, H. Algicidal effect of 3-(3-indolyl) butanoic

acid, a control agent of the bacterial wilt pathogen *Ralstonia solanacearum*. *Crop Prot.* **2001**, *20*, 935−939.
(22) Geraats, B. P. J; Bakker, P. A. H. M.; Lawrence, C. B.; Achuo, E. A.; Höfte, M.; van Loon., L. C. Ethylene-insensitive tobacco shows differentially altered susceptibility to different pathogens. *Phytopathology* **2003**, *93*, 813−821.
(23) Fang, Z. D. *The Study Methods of Plant Pathogenesis*; Agricultural Press: Bejing, 1998; pp 388−390.
(24) Krause, M. S.; De Ceuster, T. J. J.; Tiquia, S. M.; Michel, F. C. Jr; Madden, L. V.; Hoitink, H. A. J. Isolation and characterization of rhizobacteria from composts that suppress the severity of bacterial leaf spot of radish. *Phytopathology* **2003**, *93*, 1292−1300.
(25) China state tobacco monopoly administration. *YC/T39−1996, Grade and Investigating Method of Tobacco Disease*; China Standard Press: Beijing, 1996.
(26) Liu, G. S.; Sun, P.; Ren, H. Y.; Lu, D. P. Control experiment of Kangdileide against tobacco bacterial wilt. *Mod. Agric. Sci. Technol.* **2009**, *20*, 165−168.
(27) Pees, B.; Paul, J. M.; Oget, N.; Sindt, M.; Mieloszynski, J. L. Synthesis of fluoro-substituted monomers bearing a functionalised lateral chain: Part 2. Preparation of sulfoxides and sulfones containing monomers. *J. Fluorine Chem.* **2003**, *124*, 139−146.
(28) Yamazaki, S. Selective synthesis of sulfones and sulfoxides by methytrioxorhenium catalyzed oxidation of sulfides with hydrogen peroxide. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2955−2959.