Design, synthesis and antifungal activity of a fenfuram-diarylamine hybrid

Hongyu Wang, Xuheng Gao, Xiaoxiao Zhang, Hong Jin, Ke Tao, Taiping Hou

PII: S0960-894X(16)31169-6
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.11.026
Reference: BMCL 24421

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 5 May 2016
Revised Date: 21 October 2016
Accepted Date: 11 November 2016

Please cite this article as: Wang, H., Gao, X., Zhang, X., Jin, H., Tao, K., Hou, T., Design, synthesis and antifungal activity of a fenfuram-diarylamine hybrid, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.11.026

Design, synthesis and antifungal activity of a fenfuram-diarylamine hybrid

Hongyu Wang, Xuheng Gao, Xiaoxiao Zhang, Hong Jin *, Ke Tao, Taiping Hou *

Key Laboratory of Bio-Resource and Eco-environment of Ministry of Education, College of Life Sciences, Sichuan University, Chengdu, 610064, China

*Corresponding authors:

E-mail: jinhong@scu.edu.cn; houtplab@scu.edu.cn

Phone No: 86-28-85415611

ABSTRACT

Ten novel fenfuram-diarylamine hybrids were designed and synthesized. And their antifungal activities against four phytopathogenic fungi have been evaluated *in vitro* and most of the compounds demonstrated a significant antifungal activity against *Rhizoctonia solani and Sclerotinia sclerotiorum*. Compound **5e** exhibited the most potent antifungal activity against *R. solani* with an EC_{50} value of 0.037 mg/L, far superior to the commercially available fungicide boscalid (EC_{50} = 1.71 mg/L) and lead fungicide fenfuram (EC_{50} = 6.18 mg/L). Furthermore, scanning electron microscopy images show that the mycelia on treated media grew abnormally compared to the negative control with tenuous, wizened and overlapping colonies. Molecular docking studies revealed that compound **5e** features a higher affinity for succinate dehydrogenase (SDH) than fenfuram. Furthermore, it was shown that the 3-chlorophenyl group in compound **5e** forms a CH-π interaction with B/Trp-206 and a Cl-π interaction with D/Tyr-128, rendering compound **5e** more active than fenfuram against SDH.

Keywords: Fenfuram-diarylamine hybrid; Synthesis; Antifungal activity; Molecular docking

Plant diseases have been recognized as a worldwide threat to crop production.[1] The uses of fungicides are, and will remain, critical for the effective controls of most plant diseases in agriculture and have contributed greatly to higher crop yields and quality benefits in China and other countries worldwide.[2,3] Fungicides based on carboxylic amides, as one class of the most important classes of agrochemical fungicides, have been intensively studied across the globe to fight highly destructive plant pathogens, including *Rhizoctonia spp.*, *Sclerotinia spp.* and others.[4-7] The first carboxylic amide fungicide used for crop protection was carboxin (Uniroyal). Newly discovered compounds include fenfuram (Bayer), mepronil (Kumiai), boscalid (BASF), penflufen (Bayer) and fluxapyroxad (BASF) (Figure 1).[8]The initial narrow biological spectrums of these compounds were broadened with progressive modification of the chemical structures. By comparison of all chemical structures of these commercial carboxylic amides, Dehne found that they indeed shared common chemical features, essential for fungicidal activity.[9] This finding is most notably due to the fact that these compounds bind to their biological target in a similar fashion. The common target receptor for carboxylic amide fungicides is succinate dehydrogenase (SDH).[10] Most of these compounds act as SDH inhibitors (SDHIs) and disrupt the mitochondrial tricarboxylic acid cycle and respiration chain of various fungi species.[11-13]

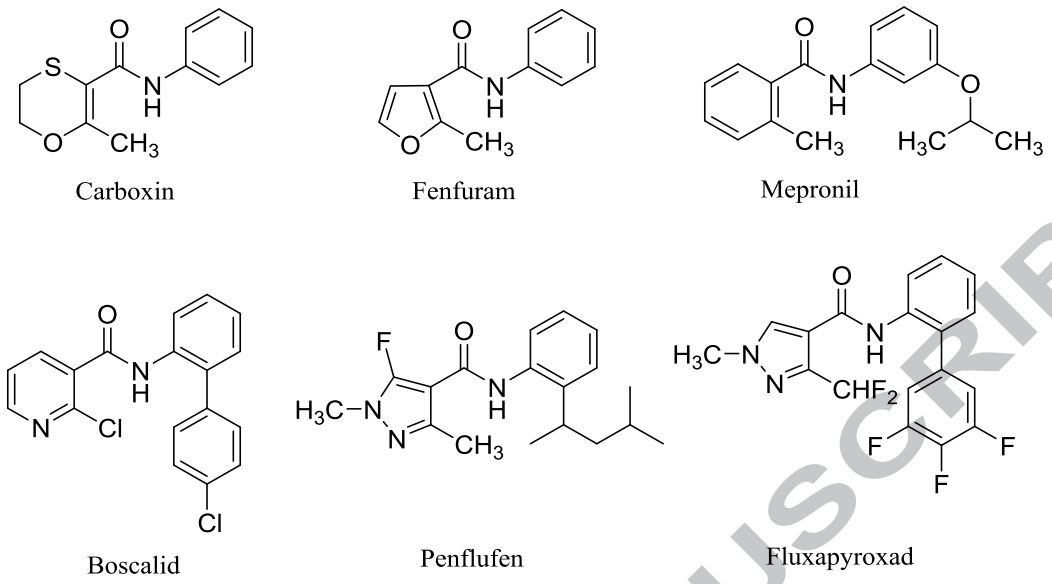


Figure 1. The carboxylic amides fungicides.

Diarylamines represent an important structural motif for many bioactive compounds used in the agrochemical field over the years.[14]Their derivatives feature significant biological activities, including fungicidal, insecticidal, acaricidal, rodenticidal and herbicidal activities.[15-19] Therefore, diarylamines may represent a promising bioactive motif to integrate with other pharmacophores. To extend the research on the development of novel carboxylic amide derivatives as fungicides, fenfuram was applied as a lead molecule and diarylamines were introduced in order to replace the phenyl group in fenfuram based on the principle of "splicing-up" bioactive substructures.[20] A series of novel fenfuram-diarylamine hybrids were designed and synthesized (Figure 2). Bioassay studies have been carried out and demonstrated that some target molecules exhibited good antifungal activities and may be useful as potential lead compounds. To the best of our knowledge, this is the first time the antifungal activities of all synthetic compounds shown in this paper have been studied.

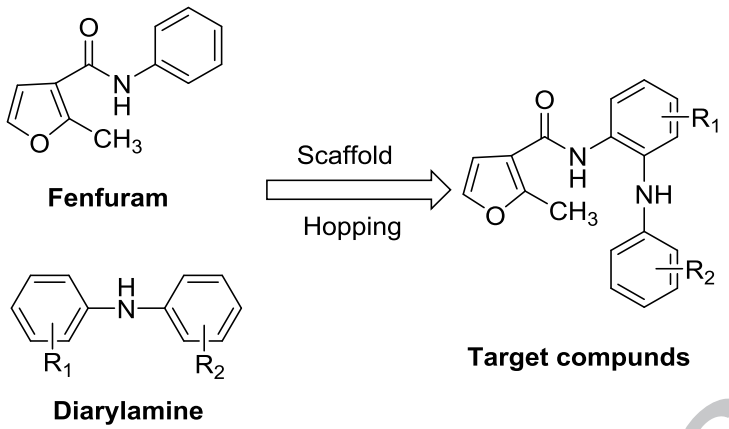
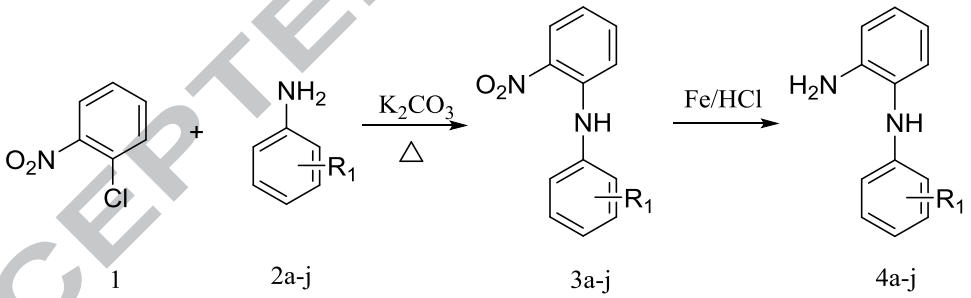
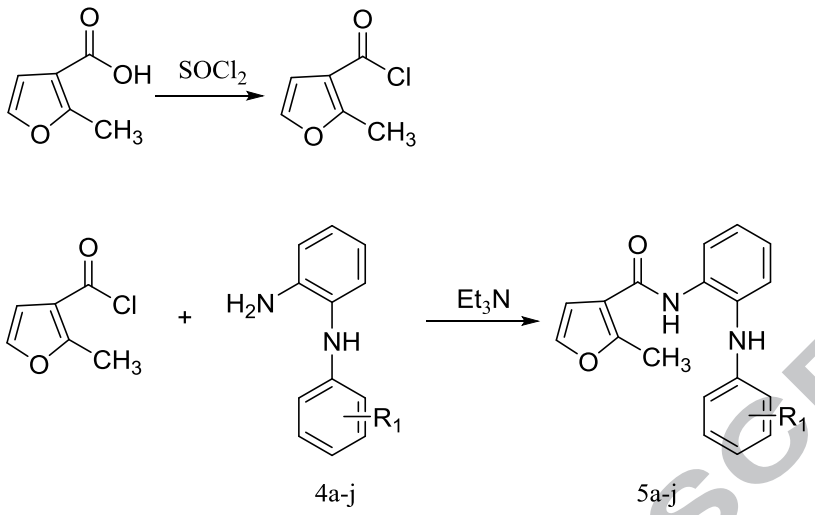


Figure 2. Design strategy of the target compounds.

Scheme 1, Scheme 2 and Table 1 summarize the synthesis and chemical structures of the fenfuram-diarylamine hybrids. The key intermediates **4a-j** shown in Scheme 1 were obtained by classic condensation reactions and reduction with Fe/HCl. And the intermediate 2-methylfuran-3-carboxylic acid shown in Scheme 2 was synthesized in good yield following reported procedures and was characterized by ^{1}H NMR.



Scheme 1. Synthesis of 2-amine-diarylamines **4a-j**.



Scheme 2. Synthesis of target compounds **5a-j**.

The results of the *in vitro* antifungal activity of compounds **5a-j**, boscalid and fenfuram at a dosage of 20 mg/L against *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Botrytis cinerea* and *Fusarium oxysporum* are listed in Table 1. Here, the antifungal activities are expressed as the inhibition percentage. Although it seems impossible to devise an obvious structure activity relationship from the data shown in Table 1, it was found that the target compounds exhibited fungicidal activity to varying extent against four fungi. Most of the target compounds showed very strong activity against *R. solani* and *S. sclerotiorum*, however, poor activity against *B. cinerea* and *F. oxysporum* could be observed. For example, compound **5a** displayed a much higher fungicidal activity against *R. solani* and *S. sclerotiorum* (90.34 and 88.55 %) than *B. cinerea* and *F. oxysporum* (59.34 and 27.43 %).

Table 1

Antifungal activities of fenfuram-diarylamine hybrid at 20 mg L^{-1}

Compound	R_1	Inhibition rate[a] (%)			
		R.solani	*S.sclerotiorum*	*B. cinerea*	*F. axysporum*

5a	H	90.34	88.55	59.34	27.43
5b	4-F	81.12	79.45	53.96	29.86
5c	2,4-F_2	89.82	82.88	52.53	18.77
5d	3,4-F_2	86.43	79.98	43.87	7.62
5e	3-Cl	90.93	78.64	41.06	26.07
5f	4-Cl	83.18	76.37	53.70	44.21
5g	2,4-Cl_2	74.95	61.26	15.21	13.25
5h	4-Br	83.72	86.58	47.07	35.38
5i	4-CH_3	55.60	87.90	53.12	24.17
5j	4-OCH_3	57.12	80.96	47.94	19.75
Fenfuram	-	75.6	65.61	21.44	9.34
Boscalid	-	70.5	54.85	34.43	44,58

[a]Average of three replicates.

By comparing the different substitute groups of compounds **5a-j** it was found that compound **5i** or **5j** (R_1 = 4-CH_3 or 4-OCH_3, electron-donating group; 55.60 or 57.12 %) showed a poorer fungicidal activity against *R. solani* than the other compound **5b**, **5f**, or **5h** (R_1 = 4-F, 4-Cl or 4-Br, electron-donating group; 81.12, 83.18, or 83.72 %). And the position of substitute groups of comparing compounds **5b**, **5e**, **5f** and **5h**, it also was seen that compound with substitute group in third position was better than it in fourth position, such as compound **5e** (R_1 = 3-Cl, 90.93 %) had better antifungal activity against against *R. solani* than compounds **5b** (R_1 = 4-F, 81.12 %), **5f** (R_1 = 4-Cl, 83.18 %) and **5h** (R_1 = 4-Br, 83.72 %).

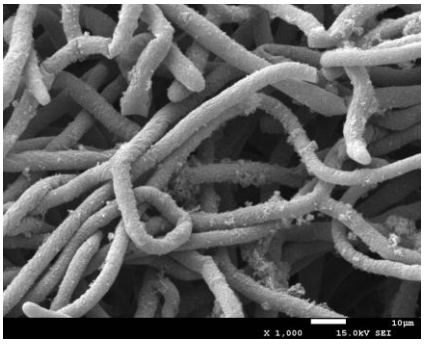
To analyze the antifungal activities of the compounds, compounds **5a** and **5e** exhibiting stronger antifungal activities against *R. solani* were selected for further studies. The corresponding EC_{50} values were listed in Table 2. Compounds **5a** and **5e** exhibited a higher antifungal activity against *R. solani* than boscalid or fenfuram, and

their EC_{50} values were found to be much smaller than boscalid or fenfuram. For example, compound **5e** (R_1 = 3-Cl) exhibited the greatest antifungal activity in the group.

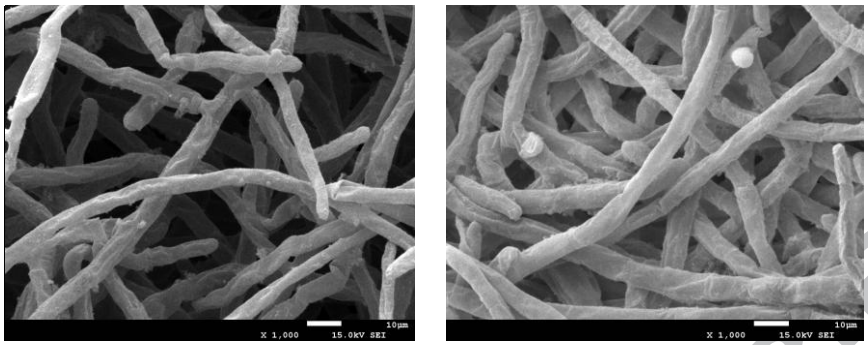
Table 2
EC_{50} values of some compounds with excellent effect against *R.solani*

Compound	Regression equation	Value of regression	EC_{50} (mg/L)
5a	y=5.6455+0.9906x	0.95	0.223
5e	y=6.045+0.7278x	0.96	0.037
fenfuram	y=4.3706+0.7957x	0.97	6.18
boscalid	y=4.7886+0.9048x	0.98	1.71

And the SEM images (Figure 3) clearly showed *R.solani* cultivated on media without addition of any drugs feature dense, sturdy, and smooth mycelia with a fine morphology. In contrast, the morphology of the mycelia of *R. solani* changed when cultured on media with addition of 0.037 mg/L of compound **5e** or boscalid. The mycelia grew abnormally with a comparatively tenuous, wizened, and overlapping colony, with the surface being rough and less ramified.



A



B C

Figure 3. Scanning electron micrographs of negative control (A) and treated colony with boscalid (B) and compound **5e** (C) of 20 mg/L.

Meanwhile, the theoretical binding mode of compound **5e** or fenfuram to SDH is shown in Figures 4, 5 and 6. Compound **5e** fitted in the cavity composed of subunits B, C and D (Figure 4). The phenyl group in compound **5e** bound to the hydrophobic pocket was surrounded by residues B/Pro-202, B/Ile-251, C/Ile-77 and C/Trp-73, while the 2-methylfuryl moiety in compound **5e,** located at another hydrophobic pocket, was surrounded by residues B/Trp-205, B/Trp-206, C/Phe-64 and C/Trp-73. A detailed analysis showed that a π-π interaction was observed between the phenyl group of compound **5e** and side chain of residue C/Trp-73. Fenfuram also fitted in the cavity composed of subunits B, C and D (Figure 5) and shared a similar binding mode with compound **5e** (Figure 6). The main difference between **5e** and fenfuram was that the 3-chlorophenyl group in compound **5e** formed a CH-π interaction with B/Trp-206 and a Cl-π interaction with D/Tyr-128, which rendered compound **5e** more active against SDH than fenfuram.

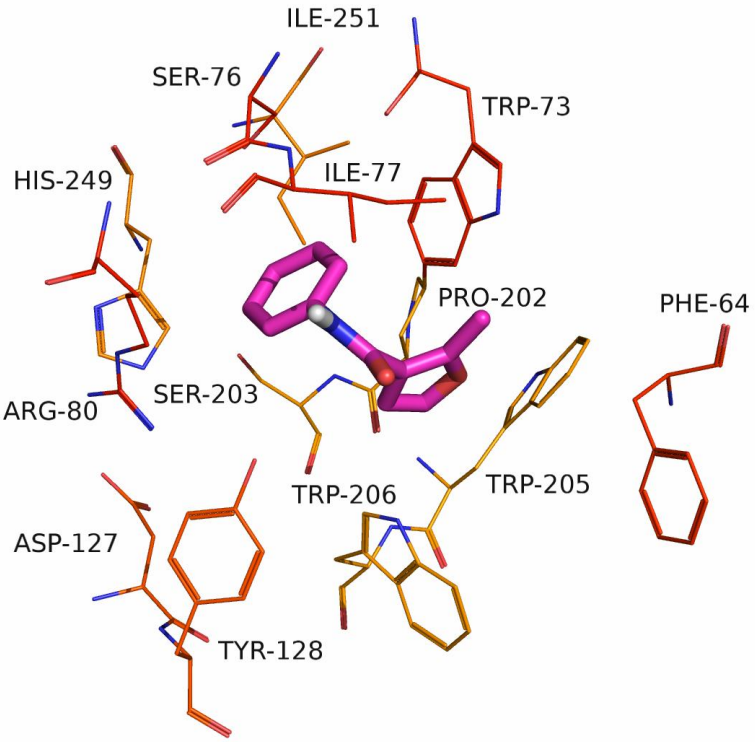


Figure 4. The theoretical binding mode between compound **5e** and SDH, and the result was shown by PyMoL 1.7.6.

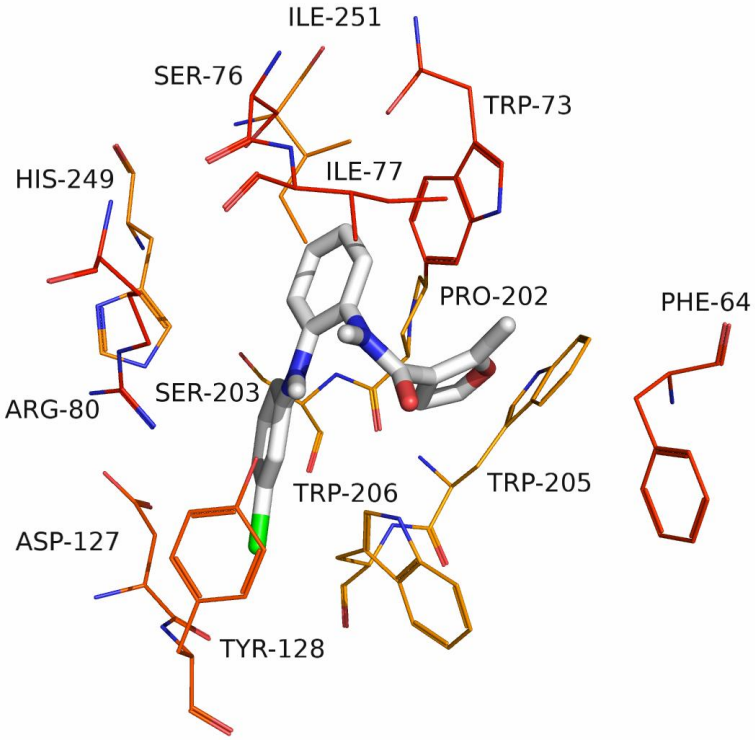


Figure 5. The theoretical binding mode of between **Fenfuram** and SDH, and the result was shown by PyMoL 1.7.6.

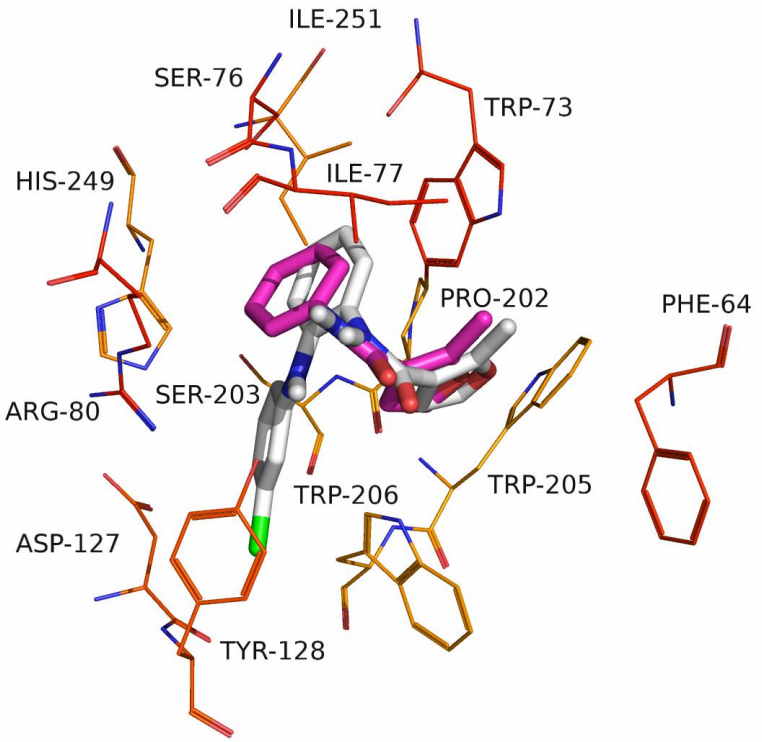


Figure 6. The theoretical binding mode of compound **5e** and **Fenfuram** to SDH, and the result was shown by PyMoL 1.7.6 (overlap).

In addition, via energy calculations of **5e** or fenfuram and SDH's interaction, the estimated binding energies were determined to be -7.9 kcal/mol for compound **5e**, and -6.2 kcal/mol for fenfuram, respectively. Docking results revealed that the two compounds from the screen might be potential SDH inhibitors. Furthermore, the different free binding energies of compound **5e** and fenfuram suggested that compound **5e** featured a higher affinity for SDH than fenfuram, consistent with the results of the *in vitro* antifungal activity assay (Table 2). The molecular simulations described above provided a rational explanation of the interactions between the compound **5e** or fenfuram with SDH and therefore offered valuable insights for the future development of SDH inhibitors.

In order to investigate whether the SDH is a potential target enzyme of title compounds or not, the fungal SDH inhibition assay was performed. Compound **5e** and fenfuram were selected and tested against SDH enzyme *in vitro* from mitochondria of *R. solani.* As demonstrated in Table 3, the selected compound **5e** (IC_{50} = 0.13 μg/mL) showed higher inhibition abilities against SDH enzyme than fenfuram (IC_{50} = 0.81 μg/mL). And this indicated that the inhibition ability of compound **5e** was 6-fold higher than fenfuram. It proved that the SDH enzyme is one of the important action targets of title compounds.

Table 3
IC_{50} (μg/mL) values of fungal SDH inhibition activity (*in vitro*).

compound	*R. solani*
5e	0.13±0.0174
fenfuram	0.81±0.3918

In summary, a series of fenfuram-diarylamine hybrids were designed, synthesized, and screened for their antifungal activity against four phytopathogenic fungi. Compounds **5a** and **5e** exhibited EC_{50} values of 0.223 and 0.037 mg/L against *R. solani*, respectively, superior to the positive controls. The marked changes in the SEM images when comparing the negative control with the treated media in combination with molecular docking studies provided further insights into the interactions between the ligand and the receptor proteins. The fenfuram-diarylamine hybrids exhibited broad antifungal activities and the selective potent inhibition against *R. solani* may offer a promising lead compound for the potential development of fungicides.

Acknowledgments

This study was supported by National Natural Science Foundation of China (31272068), National Key Research and Development Program of China (Grant No. 2016YFC0502004-2), Hi-Tech Research and Development of China (863 program, 2011AA10A202-3), National Key Technology R&D Program of China (2011BAE06B01-23) and Applied Basic Research Program of Sichuan Province (2014JY0063) for financial support.

Supplementary data

Supplementary data associated with this article can be found in the online version.

References and notes

1. Fisher, M. C.; Henk, D. A.; Briggs, C. J.; Brownstein, J. S.; Madoff, L. C.; McCraw, S. L.; Gianessi, L.; Reigner, N. **2006**, *Outlook Pest Manag.* 17, 209.
2. Gurr, S. J. **2012**, *Nature* 484, 186.
3. Qiu, S. S.; Bai, Y. L. **2014**, *Mod. Agrochem.* 13, 1.
4. Schmeling, B. V.; Kulka, M. **1996**, *Science* 3722, 659.
5. Yang, J. C.; Zhang, J. B.; Chai, B. S.; Liu, C. L. **2008**, *Agrochem.* 1, 6.
6. Xiao, Y. S.; Yan, X. J.; Xu, Y. J.; Huang, J. X.; Yuan, H. Z.; Liang, X. M.; Zhang, J. J.; Wang, D. Q. **2013**, *Pest Manag. Sci.* 69, 814.
7. Zhou, S. F.; Li, F. B.; Zhang, P. Z.; Jiang, L. **2013**, *Res. Chem. Intermediat.* 39, 1735.
8. Wu, Z. B.; Hu, D. Y.; Kuang, J. Q.; Cai, H.; Wu, S. X.; Xue, W. **2012**, *Molecules* 17, 14205.

9. Dehne, H. W.; Deising, D. H.; Gisi, U.; Kuck, K. H.; Russell, P. E.; Lyr, H. **2010**, *In Proceedings of the 16th International Reinhardsbrunn Symposium*, Friedrichroda, Germany, 25-29 April, 161.
10. Krämer, W.; Schirmer, U.; Jeschke, P.; Witschel, M. **2012**, *Modern Crop Protection Compounds.* 627.
11. Scalliet, G.; Bowler, J.; Luksch, T.; Kirchhofer-Allan, L.; Steinhauer, D.; Ward, K.; Niklaus, M.; Verras, A.; Csukai, M.; Daina, A. **2012**, *PLoS One* 7, 35429.
12. Ralph, S. J.; Moreno-Sanchez, R.; Neuzil, J.; Rodriguez-Enriquez, S. **2011**, *Pharmaceut. Res.* 28, 2695.
13. Huang, Q. C. **2004**, *World Pest.* 26, 23.
14. MacBean, C. **2012**, *The Pesticide Manual; British Crop Protection Council*: Alton, Hants,UK, 389.
15. Dreikorn, B. A. **1978**, *US Patent* 4,152,460.
16. Dreikorn, B. A.; Kramer, K. E.; **1983**, *US Patent* 4,407,820.
17. Dreikorn, B. A.; Kramer, K. E.; Berard, D. F.; Harper, R. W.; Tao, E.; Thompson, L. G.; Mollet, J. A. **1992**, *ACS Symp. Ser.* 504, 336.
18. Allen, D. B. **1977**, *DE Patent* 2,642,148.
19. Collins, D. J.; Slater, J. W.; Hunt, J. D.; Freeman, P. F. H.; **1979**, *GB Patent* 1,544,078.
20. Chen, M. J.; Jin, H.; Tao, K.; Hou, T. P. **2014**, *J. Pestic. Sci.* 39, 187.

Graphical abstract