DOI 10.1007/s10600-018-2281-7
Chemistry of Natural Compounds, Vol. 54, No. 1, January, 2018

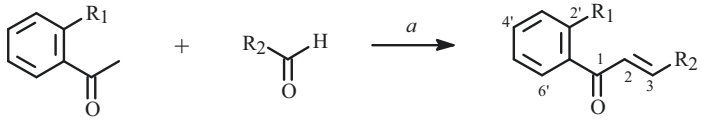
SYNTHESIS OF SUBSTITUTED CHALCONES AND ASSESSMENT OF THEIR ANTIFUNGAL ACTIVITY AGAINST *Trichophyton rubrum*

Kai-Xia Zhang,[1] Wei Wang,[2*] Fei Zhao,[1] Qing-Guo Meng,[1*] and Yong-Sheng Jin[1,3*]

Fungal infections can include superficial and systemic ones. The anthropophilic species *Trichophyton rubrum* is an important etiologic agent of dermatophytosis and accounts for 69.5% of all dermatophytic infections [1, 2]. Infections caused by this species are difficult to treat, especially in immunocompromised patients. Although many antifungal drugs are available, their side effects and interactions with other drugs, as well as the emergence of resistant strains, have limited their clinical use [3]. Therefore, novel drugs with more specific and effective mechanisms of action against dermatophytes are urgently needed.

Some studies have reported that chalcones can exert antitumor, anti-inflammatory, and cardiovascular effects [4–6]. In light of their extremely high values, chalcones have become a popular focus of research and development. Several studies [7–10] have reported that certain natural and synthetic chalcone derivatives could exert antifungal effects. Bitencourt et al. reported that non-substituted chalcones were effective against *T. rubrum* [11] by down-regulating fatty acid synthase gene expression and reducing ergosterol content. We therefore designed and synthesized chalcones and screened them for antifungal activity against *T. rubrum*. Previous studies identified structure–activity relationships (SARs) between chalcones and antifungal activity, but to our knowledge the actions of 2′-substituted chalcones have not been characterized. In this work, we synthesized 2′-substituted chalcones and screened them for antifungal activity against *T. rubrum*. As expected, most compounds exhibited good activity (Table 1). Herein we report the results and discuss the SARs.

In total, 25 compounds were synthesized (Scheme 1) and evaluated for *in vitro* antifungal activity against *T. rubrum*; Table 1 summarizes the findings.



1 - 25

1-13: R_1 = OH, **14-24:** R_1 = NH_2
1: R_2 = 3-NO_2-Ph; **2:** R_2 =2-F-Ph; **3:** R_2 = 3-F-Ph; **4:** R_2 = 4-F-Ph
5: R_2 = 3-Cl-Ph; **6:** R_2 = 3,4-diCl-Ph; **7:** R_2 = 2,4-diCl-Ph
8: R_2 = 2-Br-Ph; **9:** R_2 = 4-Br-Ph; **10:** R_2 =2-Furyl
11: R_2 = 5-CH_2OH-2-Furyl; **12:** R_2 = 2-Naphthyl
13: R_2 = 4-OH-3-OCH_3-Ph; **14:** R_2 = 3,4-$(OCH_3)_2$-Ph
15: R_2 = 3-NO_2-Ph; **16:** R_2 = 4-NO_2-Ph; **17:** R_2 = 2-F-Ph
18: R_2 = 3-F-Ph; **19:** R_2 = 4-F-Ph; **20:** R_2 = 2,4-diCl-Ph
21: R_2 = 3,4-diCl-Ph; **22:** R_2 = 2-Br-Ph; **23:** R_2 = 2-Naphthyl
24: R_2 = 2-Furyl; **25:** R_1 = NO_2, R_2 = 3,4-$(OCH_3)_2$-Ph

a. 10% NaOH, EtOH, r.t.

Scheme 1

1) School of Pharmacy, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Yantai University, 264005, Yantai, P. R. China, fax: +86 21 81871227, e-mail: ysjinsmmu@163.com, ysjin@smmu.edu.cn; 2) No. 455 Hospital of PLA, 200052, Shanghai, P. R. China; 3) School of Pharmacy, Second Military Medical University, 200433, Shanghai, P. R. China. Published in *Khimiya Prirodnykh Soedinenii*, No. 1, January–February, 2018, pp. 133–135. Original article submitted March 9, 2016.

TABLE 1. Antifungal Activities of the Title Compounds *in vitro* (MIC_{80}, μg/mL)

Compound	MIC_{80}, μg/mL	Compound	MIC_{80}, μg/mL
1	16	**2**	8
3	16	**4**	16
5	4	**6**	16
7	> 64	**8**	> 64
9	> 64	**10**	4
11	4	**12**	> 64
13	1	**14**	> 64
15	0.5	**16**	0.5
17	1	**18**	0.5
19	1	**20**	> 64
21	> 64	**22**	1
23	> 64	**24**	> 64
25	16	Fluconazole	0.25

Sixteen compounds (**1**–**6**, **10**, **11**, **13**, **15**–**19**, **22**, and **25**) exhibited antifungal activity. Values of the minimum inhibitory concentration required to inhibit 80% of cells (MIC_{80}) ranged from 0.5 to 16 μg/mL; compounds **15**, **16**, and **18** were the most potent (MIC_{80} = 0.5 μg/mL). These findings suggest that 2′-amino substituents are more effective than 2′-hydroxy substituents. In addition, almost all compounds exhibited potent activity, with the exception of compounds **14**, **20**, **21**, **23**, and **24**. Substituents of the B-ring appeared to exert notable effects on antifungal activity. In general, nitro substituents (**1**, **15**, and **16**), fluorosubstituents (**2**–**4**, **17**–**19**), and a hydroxy substituent (**11**) exhibited better antifungal activity than other compounds. A benzene ring appeared to be superior to a naphthalene nucleus (**12** and **23**), and a single substituent appeared to be better than disubstituents (**6**, **7**, **14**, **20**, **21**, and **25**). The natural compound **13** also exhibited good antifungal activity (MIC_{80} = 1 μg/mL) [12], suggesting that some natural chalcones could act as antifungal agents. The 2′-nitrochalcone (**25**) exerted an antifungal effect against *T. rubrum*, but replacing the 2′-nitro group with a 2′-amino group (**14**) resulted in no antifungal activity. The 2′-nitro substituent could therefore be a factor in antifungal activity, but this hypothesis requires further study.

In conclusion, 25 chalcones were synthesized and screened for antifungal activity against *T. rubrum*. Seven compounds (**13**, **15**–**19**, and **22**) displayed promising antifungal activities ($MIC_{80} \leq 1$ μg/mL). Assessment of the SARs indicated that the introduction of $–NH_2$ on ring A at the 2 position may improve antifungal activity. The present findings may guide the design of chalcone analogs with high antifungal activity, although the underlying mechanism has not been elucidated yet. Further work is already in progress.

General Comments. ^{1}H NMR spectra were obtained on a Bruker Avance II 300 or 600 spectrometer (Bruker, Billerica, MA, USA), and chemical shifts are reported in parts per million relative to $CDCl_3$ (7.27 ppm for ^{1}H) and DMSO-d_6 (2.53 ppm for ^{1}H). Unless otherwise noted, the materials were obtained from commercially available sources and were used without further purification.

Synthesis of Chalcones (general method). A solution of acetophenone (1 mmol) in ethanol (10 mL) was treated with 10% NaOH aqueous solution (4 mmol) and benzaldehyde (1 mmol) in ethanol with stirring at room temperature for 12–24 h. The reaction was monitored by TLC. The mixture was then cooled on ice, and AcOH was added; pH was adjusted to 3–4. The mixture was then filtered, dried, and recrystallized from ethanol to yield the title compounds.

Identification of all title compounds was performed by NMR and MS [13].

(*E*)-1-(2′-Hydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one (1), mp 162–164°C.

(*E*)-3-(2-Fluorophenyl)-1-(2′-hydroxyphenyl)prop-2-en-1-one (2), mp 80–82°C.

(*E*)-3-(3-Fluorophenyl)-1-(2′-hydroxyphenyl)prop-2-en-1-one (3), mp 104–106°C.

(*E*)-3-(4-Fluorophenyl)-1-(2′-hydroxyphenyl)prop-2-en-1-one (4), mp 114–115°C.

(*E*)-3-(3-Chlorophenyl)-1-(2′-hydroxyphenyl)prop-2-en-1-one (5), mp 94–96°C.

(*E*)-3-(3,4-Dichlorophenyl)-1-(2′-hydroxyphenyl)prop-2-en-1-one (6), mp 153–155°C.

(*E*)-3-(2,4-Dichlorophenyl)-1-(2′-hydroxyphenyl)prop-2-en-1-one (7), mp 168–170°C.

(*E*)-3-(2-Bromophenyl)-1-(2′-hydroxyphenyl)prop-2-en-1-one (8), mp 92–94°C.

(*E*)-3-(4-Bromophenyl)-1-(2′-hydroxyphenyl)prop-2-en-1-one (9), mp 146–148°C.

(*E*)-3-(Furan-2-yl)-1-(2′-hydroxyphenyl)prop-2-en-1-one (10), mp 100–102°C.

(*E*)-3-(5-(Hydroxymethyl)furan-2-yl)-1-(2′-hydroxyphenyl)prop-2-en-1-one (11), mp 92–94°C.
(*E*)-1-(2′-Hydroxyphenyl)-3-(naphthalen-2-yl)prop-2-en-1-one (12), mp 152–154°C.
(*E*)-3-(4-Hydroxy-3-methoxyphenyl)-1-(2′-hydroxyphenyl)prop-2-en-1-one (13), mp 123–125°C.
(*E*)-1-(2′-Aminophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (14), mp 84–86°C.
(*E*)-1-(2′-Aminophenyl)-3-(3-nitrophenyl)prop-2-en-1-one (15), mp 152–153°C.
(*E*)-1-(2′-Aminophenyl)-3-(4-nitrophenyl)prop-2-en-1-one (16), mp 144–147°C.
(*E*)-1-(2′-Aminophenyl)-3-(2-fluorophenyl)prop-2-en-1-one (17), mp 91–93°C.
(*E*)-1-(2′-Aminophenyl)-3-(3-fluorophenyl)prop-2-en-1-one (18), mp 102–104°C.
(*E*)-1-(2′-Aminophenyl)-3-(4-fluorophenyl)prop-2-en-1-one (19), mp 87–90°C.
(*E*)-1-(2′-Aminophenyl)-3-(2,4-dichlorophenyl)prop-2-en-1-one (20), mp 132–134°C.
(*E*)-1-(2′-Aminophenyl)-3-(3,4-dichlorophenyl)prop-2-en-1-one (21), mp 118–120°C.
(*E*)-1-(2′-Aminophenyl)-3-(2-bromophenyl)prop-2-en-1-one (22), mp 76–78°C.
(*E*)-1-(2′-Aminophenyl)-3-(naphthalen-2-yl)prop-2-en-1-one (23), mp 118–121°C.
(*E*)-1-(2′-Aminophenyl)-3-(furan-2-yl)prop-2-en-1-one (24), mp 63–66°C.
(*E*)-3-(3,4-Dimethoxyphenyl)-1-(2′-nitrophenyl)prop-2-en-1-one (25), mp 120–122°C.

Antifungal Activity Assay. The *in vitro* antifungal activities of the synthesized compounds were evaluated against *T. rubrum*, and fluconazole served as the positive control. The *in vitro* MIC values of the compounds were determined by the broth microdilution method in 96-well microtest plates using the protocols of the Clinical and Laboratory Standards Institute, USA [14]. The MIC_{80} was determined as the first well to exhibit an approximately 80% reduction in growth compared with growth in drug-free wells. The compounds were dissolved in DMSO, serially diluted in growth medium, inoculated, and incubated at 35°C. The growth MIC was determined at 24 h. The data points in Table 1 represent the mean of the replicates. All tests were conducted in triplicate for each compound.

REFERENCES

1. M. Ameen and O. Welsh, *Clin. Dermatol.*, **28** (2), 197 (2010).
2. B. K. Chen and S. F. Friedlander, *Curr. Opin. Pediatr.*, **13** (4), 331 (2001).
3. T. C. White, S. Holleman, F. Dy, L. F. Mirels, and D. A. Stevens, *Antimicrob. Agents Chemother.*, **46** (6), 1704 (2002).
4. M. Leao, J. Soares, S. Gomes, L. Raimundo, H. Ramos, C. Bessa, G. Queiroz, S. Domingos, M. Pinto, A. Inga, H. Cidade, and L. Saraiva, *Life Sci.*, **142**, 60 (2015).
5. F. A. Pinho-Ribeiro, M. S. Hohmann, S. M. Borghi, A. C. Zarpelon, C. F. Guazelli, M. F. Manchope, R. Casagrande, and W. A. Verri, Jr., *Chem. Biol. Interact.*, **228**, 88 (2015).
6. D. K. Mahapatra and S. K. Bharti, *Life Sci.*, **148**, 154 (2016).
7. P. K. Mishra, B. K. Sarma, P. K. Singhai, and U. P. Singh, *Mycobiology*, **35** (2), 72 (2007).
8. D. Gupta and D. K. Jain, *J. Adv. Pharm. Technol. Res.*, **6** (3), 114 (2015).
9. R. R. Kulkarni, S. G. Tupe, S. P. Gample, M. G. Chandgude, D. Sarkar, M. V. Deshpande, and S. P. Joshi, *Nat. Prod. Res.*, **28** (4), 245 (2014).
10. S. N. Lopez, M. V. Castelli, S. A. Zacchino, J. N. Dominguez, G. Lobo, J. Charris-Charris, J. C. Cortes, J. C. Ribas, C. Devia, A. M. Rodriguez, and R. D. Enriz, *Bioorg. Med. Chem.*, **9** (8), 1999 (2001).
11. T. A. Bitencourt, T. T. Komoto, B. G. Massaroto, C. E. Miranda, R. O. Beleboni, M. Marins, and A. L. Fachin, *BMC Complement Altern. Med.*, **13**, 229 (2013).
12. Q. Wang and D. Chen, *Helv. Chim. Acta*, **90** (12), 2432 (2007).
13. F. Zhao, Q. J. Zhao, J. X. Zhao, D. Z. Zhang, Q. Y. Wu, and Y. S. Jin, *Chem. Nat. Compd.*, **49**, 206 (2013).
14. W. P, Clinical and Laboratory Standards Institute (CLSI): USA, Approved standard M27-A3, 2008.