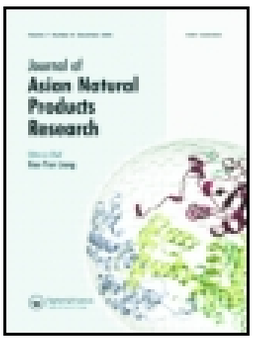


Journal of Asian Natural Products Research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ganp20

Synthesis and biological profiling of half-calycanthaceous alkaloid analogues

Rui Zhu , Yang Wei , Ke Han , Xin-Wei Shi , Yong-Dong Gu , Hong-Jin Bai & Shao-Jun Zheng

To cite this article: Rui Zhu , Yang Wei , Ke Han , Xin-Wei Shi , Yong-Dong Gu , Hong-Jin Bai & Shao-Jun Zheng (2020): Synthesis and biological profiling of half-calycanthaceous alkaloid analogues, Journal of Asian Natural Products Research, DOI: 10.1080/10286020.2020.1791100

To link to this article: https://doi.org/10.1080/10286020.2020.1791100

JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH
https://doi.org/10.1080/10286020.2020.1791100

Synthesis and biological profiling of half-calycanthaceous alkaloid analogues

Rui Zhu[a], Yang Wei[a], Ke Han[a], Xin-Wei Shi[b], Yong-Dong Gu[a], Hong-Jin Bai[c] and Shao-Jun Zheng[a]

[a]School of Environmental and Chemical Engineering, Jiangsu University of Science and Technology, Zhenjiang 212003, China; [b]Engineering Center of Qinling Mountains Natural Products, Shaanxi Academy of Sciences, Institute of Botany of Shaanxi Province, Xi'an 710061, China; [c]Key Laboratory of Protection & Utilization of Biological Resources in Tarim Basin of Xinjiang Production and Construction Corps/College of Life Sciences, Tarim University, Alar 843300, China

ABSTRACT

During our continuous efforts to pursue antifungal agents, some calycanthaceous alkaloid analogs showed diverse and promising bioactivities. Therefore, 34 new calycanthaceous alkaloid derivatives were further prepared and screened for bioactivities. As a result of the evaluation against a great deal of plant pathogen fungi, bacteria and human pathogenic fungi, a majority of them displayed potent bioactivity. In particular, compound **b6** displayed remarkably activity and might be novel potential leading compound for further development of antifungal agent. The relationship between structure and biological activity was also discussed.

ARTICLE HISTORY
Received 8 March 2020
Accepted 27 June 2020

KEYWORDS
Calycanthaceous alkaloids; synthesis; plant pathogen fungi; biological activity

1. Introduction

Plant diseases are caused mainly via fungal pathogens, threating to the global food security. The majority of the currently antifungal agents possess serious drawbacks, such as serious drug resistance, and severe drug interactions. Therefore, novel antifungal agents with improved fungicidal potency are the key to control those plant diseases.

As a well-established lead optimization method, structure modification is continually used to discover compounds with structural novelty, and increased potency. With regard to pesticide design, structure optimization is also extensively recommended to

CONTACT Shao-Jun Zheng sz281cam@just.edu.cn; Hong-Jin Bai bhj67@163.com
Supplemental data for this article is available online at https://doi.org/10.1080/10286020.2020.1791100.

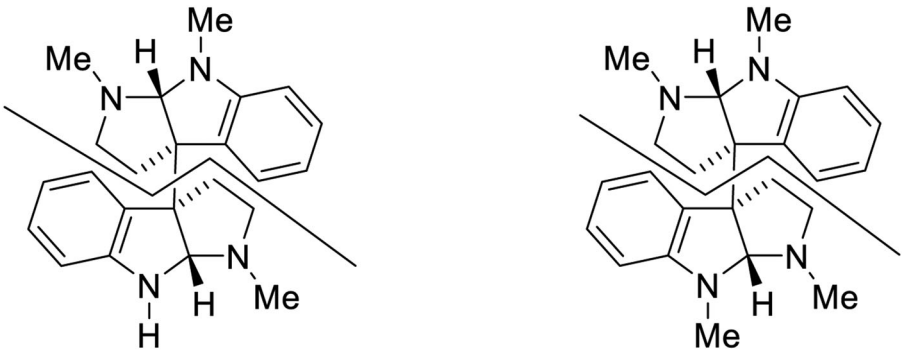


Figure 1. Structures of calycanthaceous alkaloids.

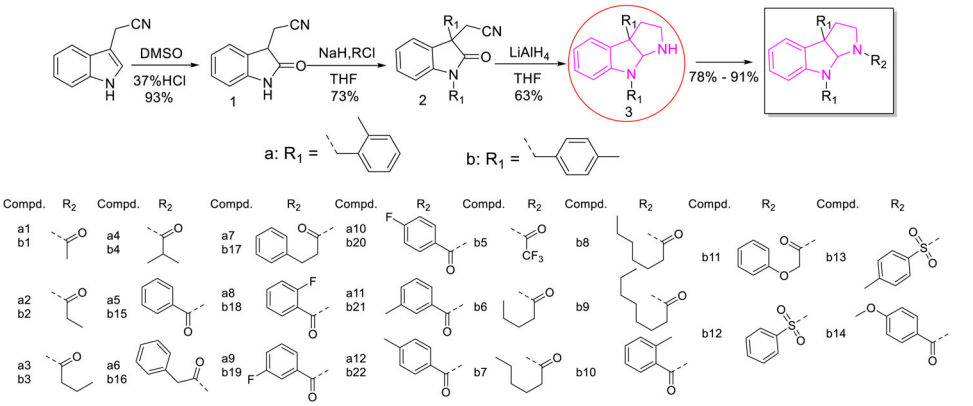
change the lead compounds. Calycanthaceous alkaloids [1–4] (Figure 1), bearing hexahydropyrroloindole skeletons, are an important class of alkaloid which have been utilised as traditional Chinese medicines for the treatment of funga [5], tumor, inflammatory, and melanogenesis [6, 7]. Due to the wide spectrum of biological activities, a great deal of studies towards the synthesis and antimicrobial activity of calycanthaceous alkaloids have been reported [8–17]. In our previous work, calycanthaceous alkaloids derivatives were designed and synthesized. Among them, some derivatives showed diverse and promising bioactivities, such as, compound **c4** showed remarkably high activity against *Ralstonia solanacearum,* with a MIC value of 1.96 μg ml^{-1}, compound **b9** showed better against *Verticillium dahlia* compared with chlorothalonil, with a MIC value of 7.81 μg ml^{-1}, compound **a1** displayed a significant activities against *B. cereus*, *Escherichia sp* and *R. solanacearum*, even better than the positive control streptomycin and penicillin, with the same MIC value of 15.63 mg ml^{-1}, and compounds **c2** and **c11** revealed potent activity against *acetylcholinesterase*, with MIC values of 0.01 and 0.1 ng ml^{-1}, respectively [18–24]. As a part of our continuous work in pursuit of antifungal agents with novel structure and high efficiency, we initiated a structural modification campaign focusing on introducing functional group at the *N*-position to compound **3**. Taking the synthetic accessibility into consideration, three novel series of *N*-substituted calycanthaceous alkaloid derivatives were identified as the target compounds for synthesis.

To the best of our knowledge, the biological activities of the synthetic derivatives are reported here for the first time.

2. Results and discussion

2.1. Rational design and synthesis of calycanthaceous alkaloid analogues

According to our previous study, the introduction of functional group at the *N*-position to compound **3** could affect the bioactivities. To explore novel antifungal agents with novel structure and high efficiency, the calycanthaceous alkaloid analogs were prepared from indole-3-acetonitrile via acylation at the *N*-position based on our reported methods and confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS according to a previously reported procedure in our group (Scheme 1) [19, 20].



Scheme 1. Synthetic route to the title compounds **a1**–**a12** and **b1**–**b22** [19].

2.2. Antimicrobial activity

Chlorothalonil, fluconazole, carbendazim, gentamicin, penicillin, streptomycin, or amphotericin B were used as the positive control. The fungicidal activities of all the target compounds were evaluated against *V. dahliae, Fusarium oxysperium sp. vasinfectum, Cytospora juglandis, Aspergillus flavus, Penicillium citrinum, Fusarium oxysporum, Colletotrichum orbiculare, Aspergillus niger, B. cinerea Pers, Curvularia lunaia, Escherichia sp, Pseudomonas aeruginosa, R. solanacearum, B. cereus, Staphylococcus aureus, Candida krusei, Crytococcus neoformans* and *Candida tropicalis*, and the results are provided in Table 1.

The antibacterial results are displayed in Table 1. The biological testing showed that a majority of the compounds showed potent antifungal activities. Compounds **b1**, **b6**, **b7**, **b8**, **b9**, **b9**, and **b13** revealed excellent activity against *V. dahlia*, with MIC values of 62.5, 15.6, 62.5, 31.3, 31.3, 62.5, and 31.3 μg ml^{-1}, respectively. Among them, Compound **b6** showed more effective activity against *V. dahlia* than that of chlorothalonil (MIC = 31.3 μg ml^{-1}), with a MIC value of 15.6 μg ml^{-1}. All of the synthesied compounds displayed activity against *C. juglandis*, *A. flavus*, *F. oxysporum*, and *A. niger*, but only compound **a3** is similar to that of chlorothalonil against *C. juglandis*, all with the same MIC value of 62.5 μg ml^{-1}. Compound **b1** manifested comparable control efficacy against *P. citrinum* to that of amphotericin (MIC = 31.3 μg ml^{-1}), with a MIC value of 31.3 μg ml^{-1}. Compound **b1** displayed moderate activity against *Escherichia sp*, with a MIC value of 31.3 μg ml^{-1}. The activity of compound **b1** is more potent than that of streptomycin against *Escherichia sp*, with a MIC value of 31.3 μg ml^{-1}. Compounds **a10** and **b9** indicated more activity against *Escherichia sp* than that of Streptomycin, with a MIC value of 62.5 μg ml^{-1}. Compounds **a2**, **b1**, and **b2**, displayed moderate activity against *B. cereus*, with MIC values of 15.6, 31.3 and 62.5 μg ml^{-1}, respectively. Compounds **b1** and **b2** displayed moderate activity against *S. aureus*, with MIC values of 31.3 and 62.5 μg ml^{-1}, respectively.

Although it is difficult to extract apparent structure-activity relationships from the bioassay results, some conclusions can still be obtained. Firstly, compounds (**a2**, **a3**, **b1**, **b2**, and **b6-b9**) with alkyl carbonyl substituted groups at *N*-position indicated

Table 1. MICs of compounds against plant pathogenic fungi, gram-negative bacteria, gram-positive bacteria and human pathogenic fungi.

	Plant pathogen fungi										Gram-negative bacteria			Gram-positive bacteria		Human pathogenic fungi		
Comp.	*V.d.*	*F.v.*	*C.j.*	*A.s.*	*P.c.*	*F.o.*	*C.o.*	*A.n.*	*B.p.*	*C.l.*	*E.s.*	*P.a.*	*R.s.*	*B.c.*	*S.a.*	*C.k.*	*C.N.*	*C.t.*
											MIC (μg/ml)							
a1	250	—	250	250	250	125	250	125	—	125	—	—	—	—	—	250	—	125
a2	250	—	125	250	—	125	250	250	—	250	—	—	—	15.6	—	250	—	250
a3	250	—	62.5	250	—	125	125	125	—	—	—	—	—	—	—	250	—	—
a4	125	—	250	250	250	125	250	250	—	250	—	—	—	—	—	250	—	—
a5	125	—	125	250	250	250	250	250	—	—	—	—	—	250	—	—	125	—
a6	250	250	250	250	—	250	—	250	—	—	—	—	—	—	—	—	—	—
a7	—	250	250	250	—	250	—	—	—	—	—	—	—	—	—	—	—	—
a8	—	—	125	250	—	250	—	250	—	—	—	—	—	—	—	250	—	—
a9	—	—	250	250	—	250	—	250	—	—	—	—	—	—	—	250	—	—
a10	—	—	250	250	250	125	—	250	250	—	—	—	62.5	—	—	—	—	250
a11	—	—	125	250	—	250	—	250	—	—	—	—	—	—	—	—	—	—
a12	—	—	125	250	250	250	—	250	250	250	—	—	—	—	—	—	—	—
b1	62.5	—	125	125	31.3	125	250	250	250	125	31.3	—	—	31.3	31.3	—	—	—
b2	250	—	62.5	250	—	125	250	125	—	—	—	—	—	62.5	62.5	250	—	—
b3	125	—	250	250	250	125	125	125	—	—	—	250	250	250	—	250	—	250
b4	250	—	125	250	250	125	250	125	—	—	—	—	—	250	—	250	—	250
b5	250	—	250	250	—	125	250	250	—	—	—	—	—	—	—	250	—	125
b6	15.6	250	250	250	125	125	250	250	—	—	125	125	125	125	125	250	—	125
b7	62.5	250	250	250	250	125	250	250	—	—	—	125	250	250	—	250	—	—
b8	31.3	—	62.5	250	250	250	250	250	—	—	—	250	250	250	—	125	—	—
b9	31.3	250	125	250	250	125	250	125	—	125	250	125	62.5	250	250	250	250	250
b10	62.5	—	250	250	250	250	—	125	—	—	—	—	—	—	—	250	—	—
b11	—	—	250	250	250	250	—	250	—	—	—	—	—	—	—	—	—	—
b12	62.5	125	250	250	—	250	250	250	—	—	—	—	—	125	—	250	—	—
b13	31.3	125	250	250	—	250	250	250	—	—	250	—	250	125	—	250	—	250
b14	125	—	250	250	250	250	—	250	—	—	—	—	—	—	—	250	—	125
b15	250	—	250	250	250	250	—	250	—	—	—	—	—	—	—	—	—	—
b16	250	125	250	250	—	250	250	250	—	—	—	—	—	—	—	—	—	—
b17	250	—	250	250	—	250	250	250	—	—	—	—	—	—	—	—	—	—
b18	—	—	250	250	—	250	—	250	—	—	—	—	—	—	—	—	—	—
b19	—	—	250	250	250	250	—	250	—	—	—	—	—	—	—	—	—	—
b20	125	—	250	250	—	250	—	250	—	—	—	—	—	—	—	250	—	—
b21	—	—	250	250	250	250	—	250	—	—	—	—		—	—	—	—	—
b22	—	—	125	250	—	250	—	250	—	—	—	—	—	—	—	—	—	—
Ca	7.8	62.5	31.3	7.8	1.9	125	125	—	1.9	250						250	—	250
A	1.9	—	250	31.3	31.3	62.5	250	3.9	—	7.8						250	1.9	—
Ch	31.3	250	62.5	7.8	15.6	62.5	250	15.6	31.3	125						7.8	—	1.9
G											1.9	1.9	62.5					
S											31.3	—	250					
P														7.8	15.6			
F																62.5	—	250

Note: Carbendazim and chlorothalonil were used as the positive controls; "—" means no inhibition effect. MIC: Minimal Inhibitory Concentration; *V.d.: V. dahliae; F.v.: F. oxysperium sp. Vasinfectum; C.j.: C. juglandis; A.s.: A. flavus; P.c.: P. citrinum; F.o.*: *F. oxysporum*; *C.o.*: *C. orbiculare*; *A.n.*: *A. niger*; *B.c.: B. cinerea; C.l.*: *C. lunaia; E.s.*: *Escherichia sp*; *P.a.*: *P. aeruginosa*; *R.s.*: *R. solanacearum; B.c.*: *B. cereus*; *S.a.*: *S. aureus*; *C.k: C. krolimus; C.N.: C. neoformans; C.t.: C. tropicalis; Ca: carbendazim; A: amphotericin B; Ch: chlorothalonil; G: gentamicin; S: streptomycin; P: penicillin: F: fluconazole.*

more activities and a broad antifungal spectrum than the compounds (**a5-a12** and **b11-b14**) with aryl carbonyl substituted groups, even better than the positive control carbendazim, chlorothalonil, and streptomycin. Secondly, compounds (**a2**, **b1**, and **b2**) with shorter alkyl carbonyl substituted groups at *N*-position showed a greater activities than the compounds (**a3**, and **b6-b9**) with longer alkyl carbonyl substituted groups. Thirdly, almost all synthesised compounds showed better activity against a broad range of plant pathogen fungi than bacteria and human pathogenic fungi. Fourth, compounds of series b displayed distinctive activity against *V. dahlia*. Fifth, the biological activity shows that the electron donor substituents are beneficial to the improvement of biological activity.

In conclusion, a total of 34 novel tetrahydropyrroloindole-based calycanthaceous alkaloid analogs were prepared via acylation at the *N*-position, and the activity against a great deal of plant pathogen fungi, bacteria and human pathogenic fungi were evaluated. The biological testing showed that a majority of the compounds indicated potent antifungal activities, in which compound **b6** was the optimal one and identified as the most potential candidate for further study. Further structure optimization of calycanthaceous alkaloids and the QSAR studies are well underway in our group.

3. Experimental

3.1. General experimental procedures

All chemicals were purchased from market and purified based on standard routes before use. The ^{1}H NMR (500 MHz), and ^{13}C NMR (125 MHz) spectra were obtained on an AM − 500 FT − NMR spectrometer (Bruker Corporation, Karlsruhe, Switzerland) with $CDCl_3$ as the solvent and TMS as the internal standard. Melting points were taken on an Electrothermal digital apparatus (Beijing, China) and are uncorrected. MS were recorded under ESI conditions using a LCQ Fleet instrument (Thermo Fisher, Waltham, MA, USA). Analytical thin-layer chromatography (TLC) was performed with silica gel plates using silica gel 60 GF_{254} (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). The title compounds were synthesized under a nitrogen atmosphere. Yields were not optimized.

3.2. Synthesis

3.2.1. Synthesis of intermediates 1-3

Intermediates **1-3** were synthesized according to our previously reported method [18–24].

3.2.2. Synthesis of compounds a1–a12 and b1–b22

Intermediate **3** was added in pyridine (15 ml) in a round flask. Then, the corresponding desired reagent was added portionwise at 0 °C. After refluxing for 2 h, the mixture was warmed to room temperature. Then, the resulting mixture was reacted for 1 h. When TLC monitoring showed that the intermediate **3** had disappeared, the resulting mixture was quenched. The solvent was evaporated under reduced pressure and then washed with water, extracted with ethyl acetate and the extracts were dried over

Na_2SO_4. At last, the solvent was concentrated and purified to afford the compounds **a1–a12** and **b1–b22** in yields from 78% to 91%.

3.2.2.1. Synthesis of intermediate a1. The acetyl chloride was added portionwise to a stirred solution of intermediate **3** in pyridine (15 ml) in a round flask at 0 °C. After refluxing for 1.5 h, the mixture was warmed to room temperature. Then, the resulting mixture was reacted for 1 h. When TLC monitoring showed that the intermediate **3** had disappeared, the resulting mixture was quenched. The solvent was evaporated under reduced pressure and then washed with water, extracted with ethyl acetate and the extracts were dried over Na_2SO_4. At last, the solvent was concentrated and purified to afford compounds **a1.**

1-(3a,8-bis(2-methylbenzyl)-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indol-1(2H)-yl)ethan-1-one(a1) Yellow oil, ^{1}H- NMR (500 MHz, $CDCl_3$) δ 7.19 − 6.88 (m, 9H), 6.77 − 6.56 (m, 2H), 6.19 (d, J = 7.9 Hz, 1H), 5.54 (s, 1H), 4.87 − 4.15 (m, 2H), 3.60 − 3.51 (m, 1H), 3.31 − 3.14 (m, 2H), 2.95 (d, J = 13.7 Hz, 1H), 2.35 (s, 2H), 2.30 − 2.15 (m, 3H), 2.03 − 1.72 (m, 6H). ^{13}C- NMR (125 MHz, $CDCl_3$) δ 170.02(C), 150.34(C), 137.41(C), 137.03(C), 135.72(C), 135.54(C), 131.30(C), 130.52(CH), 130.4(CH), 130.1(CH), 128.6 (CH), 126.7 (CH), 126.4 (CH), 125.9 (CH), 125.7 (CH), 125.7 (CH), 123.2 (CH), 117.1 (CH), 106.3 (CH), 83.3 (CH), 57.0 (C), 47.6 (CH_2), 44.0 (CH_2), 39.9 (CH_2), 36.8 (CH_2), 22.8 (CH_3), 19.9 (CH_3), 19.4 (CH_3). HRESIMS: m/z 411.2434 $[M+H]^+$ (calcd for $C_{28}H_{31}N_2O$, 411.2436).

The data of the other compounds and the NMR spectral details can be found in the Supplementary data.

3.3. Biological activity

The bioactivities of calycanthaceous alkaloid derivatives were tested based on the reported procedure [25]. The concentrations were 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9 and 2 μg ml^{-1}, respectively. The antibacterial test cells were incubated at 37 °C for 24 h. All tests were manipulated in triplicate and repeated if the results differed.

Disclosure statement

The authors declare no conflict of interest.

Funding

This work was financially supported by the National Natural Science Foundation of China (21502073), the Natural Science Foundation of Jiangsu Province (Grants No BK 20150465 and BK20180978), and the Key Research and Development Program (Modern Agriculture) of Zhenjiang City (NY2018002), Xinjiang Production Construction Corps Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin (BRYB1703), and Science and Technology Research Project of Shaanxi Province Academy of Sciences (2018nk-01), and Zhoushan Public Welfare Science and Technology Project (2019C31066).

References

[1] J.W. Zhang, Z. Hu, S.K. Li, and W.J. Wu, *Int. J. Mol. Sci.* **12**, 9596 (2011).
[2] L. Xiang, K. Zhao, and L. Chen, *Plant Physiol. Biochem.* **48**, 845 (2010).
[3] J.W. Zhang, S.K. Li, Z.Q. Ji, Z.N. Hu, and W.J. Wu, *Chem. Nat. Compd.* **45**, 507 (2009).
[4] Z.M. Yang, Z.Q. Ji, Q.F. Ye, and W.J. Wu, *J. Label. Compd. Radiopharm.* **51**, 109 (2008).
[5] W.R. Bowman, M.O. Cloonan, A.J. Fletcher, and T. Stein, *Org. Biomol. Chem.* **3**, 1460 (2005).
[6] Z.Z. Ma, Y. Hano, T. Nomura, and Y.J. Chen, *Heterocycles* **46**, 541 (1997).
[7] N. Haider and S. Nuß, *Molecules* **17**, 11363 (2012).
[8] P. Ruiz-Sanchis, S.A. Savina, F. Albericio, and M. Álvarez, *Chemistry* **17**, 1388 (2011).
[9] T. Hino and S. Yamada, *Tetrahedron Lett.* **4**, 1757 (1963).
[10] E.S. Hall, F. McCapra, and A.I. Scott, *Tetrahedron* **23**, 4131 (1967).
[11] C.L. Fang, S. Horne, N. Taylor, and R. Rodrigo, *J. Am. Chem. Soc.* **116**, 9480 (1994).
[12] Y.X. Li, H.X. Wang, S. Ali, X.F. Xia, and Y.M. Liang, *Chem. Commun. (Camb.)* **48**, 2343 (2012).
[13] T. Araki, Y. Manabe, K. Fujioka, H. Yokoe, M. Kanematsu, M. Yoshida, and K. Shishido, *Tetrahedron Lett.* **54**, 1012 (2013).
[14] Y. Peng, L. Luo, C.S. Yan, J.J. Zhang, and Y.W. Wang, *J. Org. Chem.* **78**, 10960 (2013).
[15] J. Kim and M. Movassaghi, *Acc. Chem. Res.* **48**, 1159 (2015).
[16] J.B. Xu and K.J. Cheng, *Molecules* **20**, 6715 (2015).
[17] M. Movassaghi and M.A. Schmidt, *Angew. Chem. Int. Ed. Engl.* **46**, 3725 (2007).
[18] S. Zheng, Y. Gu, L. Li, R. Zhu, X. Cai, H. Bai, and J. Zhang, *Nat. Prod. Res.* **31**, 1142 (2017).
[19] S. Zheng, X. Zhou, S. Xu, R. Zhu, H. Bai, and J. Zhang, *Molecules* **21**, 1207 (2016).
[20] S. Zheng, L. Li, Y. Wang, R. Zhu, H. Bai, and J. Zhang, *Nat. Prod. Commun.* **11**, 1429 (2016).
[21] S. Zheng, Y. Gu, R. Zhu, L. Li, H. Bai, and J. Zhang, *Chem. Nat. Compd.* **54**, 127 (2018).
[22] S. Zheng, D. Yang, R. Zhu, and D.R. Spring, *Chem. Nat. Compd.* **54**, 289 (2018).
[23] S. Zheng, R. Zhu, X. Zhou, L. Chen, H. Bai, and J. Zhang, *Bioorg. Med. Chem.* **27**, 115088 (2019).
[24] S. Zheng, R. Zhu, B. Tang, L. Chen, H. Bai, and J. Zhang, *Nat. Prod. Res*, 1 (2019).
[25] W.J. Zhang, S.P. Wei, J.W. Zhang, and W.J. Wu, *Molecules* **18**, 2763 (2013).