Received: 14 May 2021 | Revised: 21 July 2021 | Accepted: 8 August 2021

DOI: 10.1002/jccs.202100194

COMMUNICATION

JOURNAL OF THE CHINESE CHEMICAL SOCIETY

Synthesis and structure confirmation of 7-ester-8-aminomethylene-substituted baicalein derivatives

Jiajia Mou[1] | **Qiang Wang**[2] | **Yanru Deng**[1] | **Danghui Chen**[1] | **Shuang Qiu**[1]

[1]Department of Medicinal Chemistry, School of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, Tianjin, China

[2]College of Pharmacy, South-central University for Nationalities, Wuhan, China

Correspondence
Jiajia Mou, Department of Medicinal Chemistry, School of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, Health Industry Park, Jinghai District, Tianjin 301617, China.
Email: moujiajia66@163.com

Funding information
National Natural Science Foundation of China, Grant/Award Number: 21502142

Abstract

Twelve baicalein derivatives, derivatized with 7-ester and 8-aminomethylene groups, were synthesized as cyclin-dependent kinase 1 (CDK1) inhibitors, by the addition of one of four secondary amines to C-8, using the Mannich reaction, followed by mono-esterification with one of three carboxylic acids. Analysis by ^{1}H-, ^{13}C-NMR, and HMBC spectra, as well as a chemical test, confirmed that the esterification occurred at the 7-phenolic hydroxyl position of baicalein but not at the 5- or 6-positions.

KEYWORDS

baicalein derivatives, esterification reaction, Mannich reaction, strontium chloride identification reaction

1 | INTRODUCTION

Flavonoids are polyphenolic compounds, which have diverse pharmacological effects, such as antioxidant, antiinflammatory, antibacterial,[1–3] liver protection,[4,5] antiviral,[6,7] and antitumor,[8,9] so they are frequently exploited as lead compounds for drug development.[10]

Baicalein (Figure 1), one of the flavone class of compounds, has often been used as a lead compound for structural modification and screening for pharmacological activity. Modifications were mainly of the phenolic hydroxyl groups and the active hydrogen at C-8 on the A ring. Etherification and esterification are common modifications of phenolic hydroxyl groups[11–16]; benzyl ether derivatives have higher antitumor and antiinflammatory activities than baicalein itself.[11,12] Common modifications of the active hydrogen are aminomethylation and sulphonation[16–19]; aminomethylation improves the antitumor activity of baicalein,[16,17] and sulphonation improves its antioxidant capacity.[18] The replacement of the B ring by a substituted thiophene ring produced derivatives with anti-hepatitis effects.[20] Modifying both the phenolic hydroxyl groups and the active hydrogen,[13] or the phenolic hydroxyl groups and the B ring,[21] increased antitumor activity. Baicalein can induce apoptosis and cell-cycle arrest by downregulating cyclin-dependent kinase 1 (CDK1), CDK2, cyclin D2, and cyclin A, as well as upregulating endogenous cyclin-dependent kinase inhibitors (CDKIs) in the G1 and G2 cell-cycle phases. It can also attenuate the expression of the CDK4/cyclin B- and D-complexes.[22] Baicalein appears to be a promising lead compound for anticancer drug development, based on its cell-cycle arrest-inducing effects. We previously reported a new series of derivatives (**1**, Figure 1), which were got by the esterification of the 7-phenolic hydroxyl group and aminomethylation at position-8 of baicalein. The newly synthesized compounds enhanced their CDK1 inhibition activities (IC_{50} = 2.36–30.17 μM) compared with baicalein (IC_{50} = 62.67 μM).[23] Here, we report the synthesis and structural characterization of the various derivatives.

2 | RESULTS AND DISCUSSION

The synthesis of the target compounds can, in principle, be carried out by either esterification followed by aminomethylation (Scheme 1), or vice versa (Scheme 2).

There are three phenolic hydroxyl groups in baicalein, at positions-5, -6, and -7 of the A ring. The 7-hydroxyl is in the *para*-position, relative to the 4-carbonyl of the C ring, so it is susceptible to electrophilic attack, because of the p-π conjugation effect. The 5-hydroxyl forms an intramolecular hydrogen bond with the 4-carbonyl group, resulting in relatively low reactivity. Therefore, the order of reactivity with electrophiles is 7- > 6- > 5-. The 7-ester **2** can be obtained by esterification, catalyzed by *N*, *N*′-dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBT, Scheme 1).[24–26] However, **2** was unreactive with formaldehyde and secondary amines.[17] The relatively high reactivity of the 8-hydrogen is attributable to the keto-enol tautomerism with the 7-hydroxyl group, which is inhibited by esterification of the latter. This, in turn, prevents nucleophilic attack by C-8 on the methylene ammonium cation formed by the amine and formaldehyde (Mannich reaction), so this step must be performed before esterification (Scheme 2).

The Mannich reaction of baicalein, however, proceeds smoothly to yield the 8-aminomethyl derivatives **3** (Table 1),[17] which were then esterified successfully to yield the target compounds **1** (Table 2). (Scheme 2) Compounds **1** need to be purified by silica gel column chromatography. Sometimes, further recrystallization is required. So, some compounds **1** were obtained with low yield, for example, **1b–d** and **1l**.

Although the original reactivity order of the three phenolic hydroxyl groups in baicalein is 7- > 6- > 5-, the introduction of steric hinderance from the bulky aminomethylene groups at position-8 (**3**) would be expected to inhibit reaction at position-7, making the prediction of the esterification site difficult (Figure 2). It is also difficult to confirm the esterification position by ^{1}H, or ^{13}C-NMR spectra, because of the small differences in chemical shift. Because there is no hydrogen atom on the ester bond of the target compound, ^{1}H-^{13}C heteronuclear multiple bond correlation spectroscopy (HMBC) cannot determine the esterification position (Figure 3).

A comparison of the ^{1}H, ^{13}C-NMR, and HMBC spectra of target compound **1a** with those of **3a**, however, was successful in determining the position of esterification.

The ^{1}H and ^{13}C-NMR spectra, and HMBC (Figure 3) of **3a** determined the chemical shifts of each carbon atom (Figure 4). In the ^{1}H-NMR spectrum of compound **1a**, the signal at δ 13.14 was consistent with the existence of a free 5-OH, which indicated that the benzoylation of **3a**

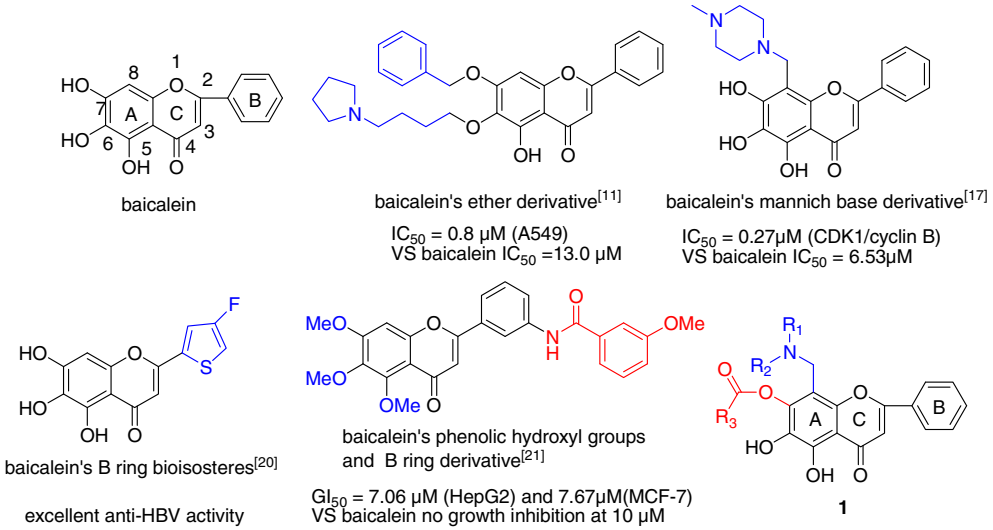
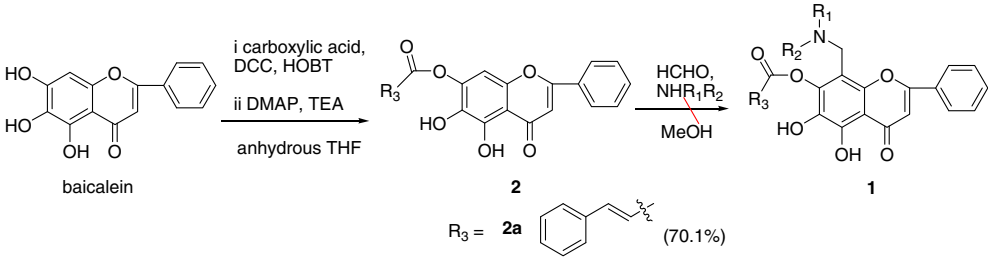


FIGURE 1 The structures of baicalein and the target derivatives



SCHEME 1 Attempted synthesis of the target compounds

SCHEME 2 Synthesis of the target compounds

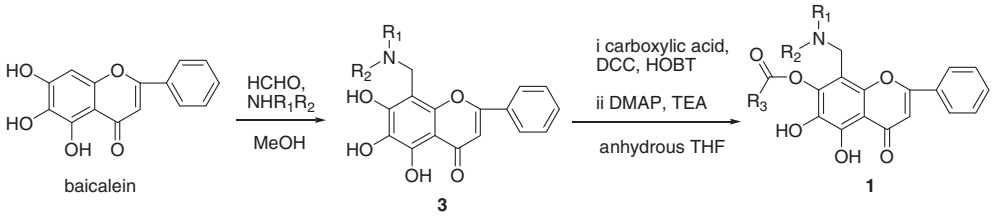
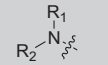
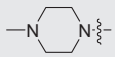


TABLE 1 Synthesis of compounds **3**[a]

Comp.		Temperature (°C)	Reaction time (hr)	Yield (%)[b]
3a		50	4.0	88.7
3b		50	4.0	89.6
3c		45	3.5	92.4
3d		55	4.5	93.5

[a]Mannich reaction of baicalein (4 mmol) with formaldehyde solution (37%, 6 mmol) and one of four secondary amines (4.8 mmol) in methanol (30 ml) until a large amount of yellow precipitate appeared.
[b]Isolated yield.

took place at the 6- or 7-OH. Comparing the ^{13}C-NMR spectra of **1a** and **3a**, the chemical shifts of C-6 and C-8 in **3a** (129.7 and 100.7) move upfield by 7.2 and 1.9 ppm in **1a** (122.5 and 98.8), respectively, whereas those of C-7 moved downfield by 5.6 ppm (155.0–160.6). These shift changes are consistent with the reported chemical shift changes of the carbon atom bonded to the esterified hydroxyl group (down-field) and those of carbon atoms *ortho* to it (upfield).[27] Therefore, the benzoylation of **3a** took place at the 7-OH position (Figure 4).

To verify the above result, a chemical test was also carried out. Flavonoids with *O*-diphenol groups can form a green, brown, or black precipitate with strontium chloride ($SrCl_2$) in methanolic ammonia solution.[28] The position of esterification can be determined, because a 7-ester (Scheme 3) would form a precipitate, whereas a 6-ester, lacking the *O*-diphenol groups, would not.

Baicalein, 5,6,7-trihydroxy-2-phenyl-8-(thiomorpholinomethyl)-4*H*-chromen-4-one (**3a**, Figure 5) and 7-(benzyloxy)-5, 6-dihydroxy-2-phenyl-4*H*-chromen-4-one (**4**, Figure 5) that have *O*-diphenol groups in their structures were selected as positive controls, and chrysin (Figure 5), with no *O*-diphenol hydroxyl groups, was selected as a negative control.

The colored precipitates resulting from the reaction of the test compounds with $SrCl_2$ were observed (Figure 6). Compound **1a** and the positive controls yielded dark precipitates, indicating the presence of an *O*-diphenol groups. Only chrysin, the negative control, produced no precipitate. This result is consistent with the spectral data analysis, and similar results were obtained with compounds **1b-l**. Even with bulky groups at the 7- and 8-positions, compounds **1** still reacted with $SrCl_2$.

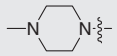
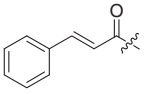
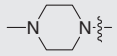
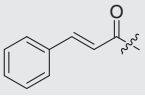
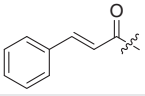
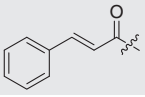
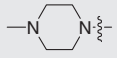
Combining the ^{1}H, ^{13}C-NMR, and HMBC data with the results from the strontium chloride test of compounds **1** and **3**, it can be concluded that the conjugation of the 7-hydroxyl with the 4-carbonyl group had a greater influence on the position of esterification than steric hindrance from an 8-aminomethyl group in **3**, resulting in esterification at position-7 and confirming the structure shown in Scheme 2.

3 | EXPERIMENTAL SECTION

3.1 | General

All solvents were of analytical grade, from local suppliers, and purified according to standard procedures. Thin-layer chromatography (TLC) with silica gel-precoated glass plates, with a fluorescent indicator, was used to monitor the reactions. Melting points (uncorrected) were determined on a micromelting point apparatus. ^{1}H, ^{13}C-NMR, and HMBC spectra were recorded on a Bruker AV-III-600 instrument. High-resolution mass spectral (HRMS) data were determined on an Agilent 6,540 UHD accurate mass Q-TOF MS instrument in a low resonance electrospray mode (ESI) (Data S1).

TABLE 2 Synthesis of compounds **1**[a]

Comp.	R_1, R_2	R_3	Reaction time (hr)[b]	Yield (%)[c]
1a			8	70.1
1b			12	18.9
1c			10	29.8
1d			10	21.2
1e			8	69.5
1f			8	52.9
1g			8	62.3
1h			8	50.2
1i			8	59.8
1j			10	40.3
1k			8	52.5
1l			10	19.5

[a]Mono-esterification of one of three carboxylic acids (2.2 mmol) with one of four compounds **3** (2.2 mmol) under the catalyst of HOBT (2.6 mmol), DCC (2.6 mmol), DMAP (0.43 mmol), and TEA (5.7 mmol) in anhydrous THF (100 ml).
[b]The time used to activate various of carboxylic acids is the same 12 hr. But the final esterification reaction time varies.
[c]Total yield.

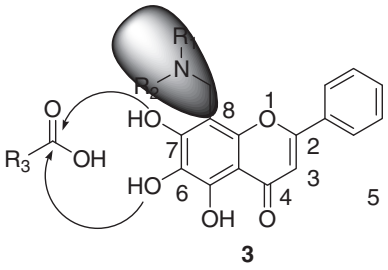
3.2 | General synthetic procedure for compounds 1a-1f

3.2.1 | Aminomethylation

Formaldehyde solution (37%, 0.17 ml, 6 mmol) was added to baicalein (1.08 g, 4 mmol) in methanol (30 ml), followed by one of the secondary amines (4.8 mmol, Scheme 2). The mixture was stirred at 30–70°C until a large amount of yellow precipitate appeared. The precipitate was filtered under vacuum, washed with a small amount of methanol, and dried in a vacuum oven to obtain one of the 5,6,7-trihydroxy-2-phenyl-8-aminomethylene-4*H*-chromen-4-ones (**3**).[17]

3.2.2 | Activation of the carboxylic acid

HOBT (0.35 g, 2.6 mmol) was added to one of the carboxylic acids (2.2 mmol, Scheme 2) in anhydrous tetrahydrofuran (THF) (30 ml), maintained at −5°C. DCC (0.54 g,



3

FIGURE 2 The possible modes of esterification in compounds **3**

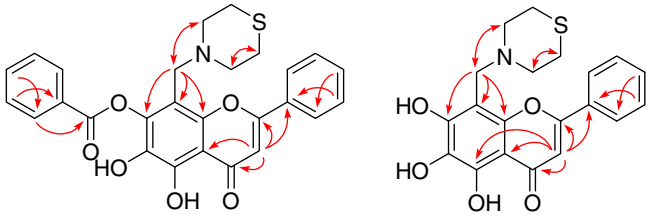
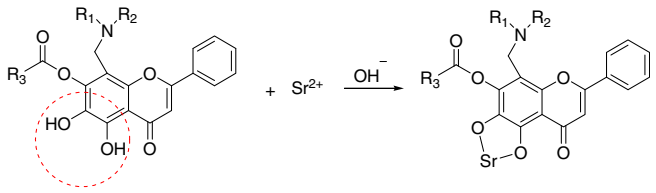


FIGURE 3 Main HMBC correlations (red arrow, from ^{1}H to ^{13}C) of compounds **1a** and **3a**

2.6 mmol) in anhydrous THF (20 ml) was then added dropwise. The mixture was stirred at −5°C for 12 hr, and then, the resulting precipitate was filtered under vacuum, and the filtrate containing activated carboxylic acid was retained.

3.2.3 | Esterification reaction

Dimethylaminopyridine (DMAP, 0.05 g, 0.43 mmol) and triethylamine (0.8 ml, 5.7 mmol) were added with stirring, to one of the aminomethylene derivatives (**3**, 2.2 mmol) in anhydrous THF (50 ml). Then, the filtrate containing one of the activated esters was added



SCHEME 3 The reaction of *O*-diphenol groups in flavonoids with strontium chloride

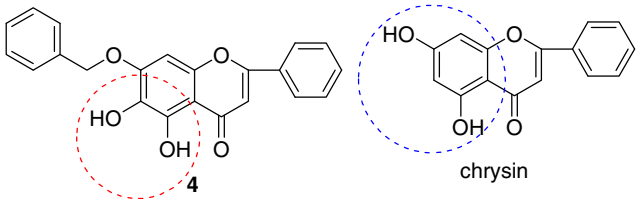


FIGURE 5 The structures of compound **4** and chrysin

dropwise, with continued stirring. After the completion of the reaction, the organic solvent was removed, and then, the residue was purified by silica gel column chromatography, as described previously, to isolate the desired aminomethyl ester derivatives **1**; sometimes, further recrystallization was needed.[24]

3.2.4 | Analytical data

5,6-Dihydroxy-4-oxo-2-phenyl-8-(thiomorpholinomethyl)-4*H*-chromen-7-yl benzoate (**1a**) yellow crystals. 70.1% yield. mp 176.9–177.1°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 13.14 (s, 1H, 5-OH), 8.12–8.14 (m, 4H, H-2″,6″,2′,6′), 7.75–7.77 (m, 1H, H-4″), 7.60–7.64 (m, 5H, H-3′, 4′,5′,3″,5″), 7.01 (s, 1H, H-3), 4.20 (s, 2H, H-9), 3.06 (brs, 4H, H-11,14), 2.75 (brs, 4H, H-12,13). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 181.6 (C-4), 163.5 (C-10), 162.6 (C-2),

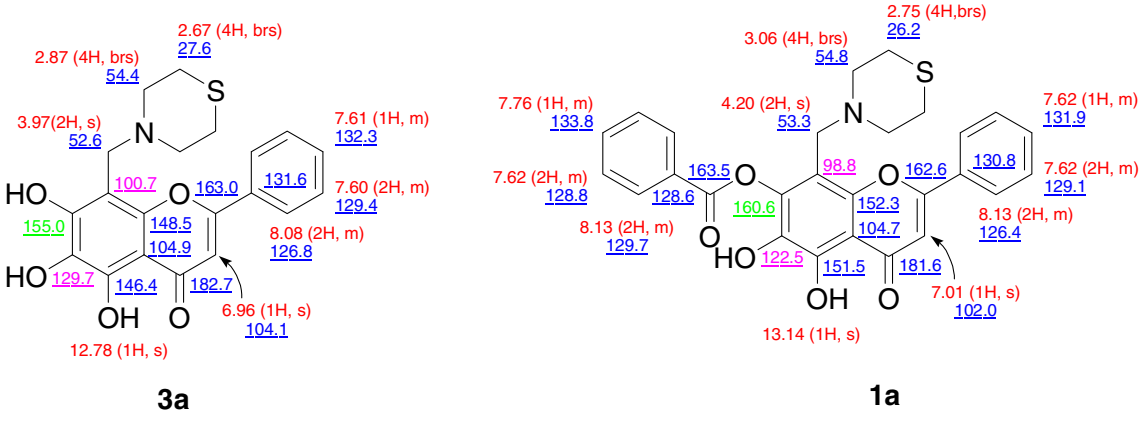


FIGURE 4 Carbon and hydrogen atom signal attributions of compounds **3a** and **1a**

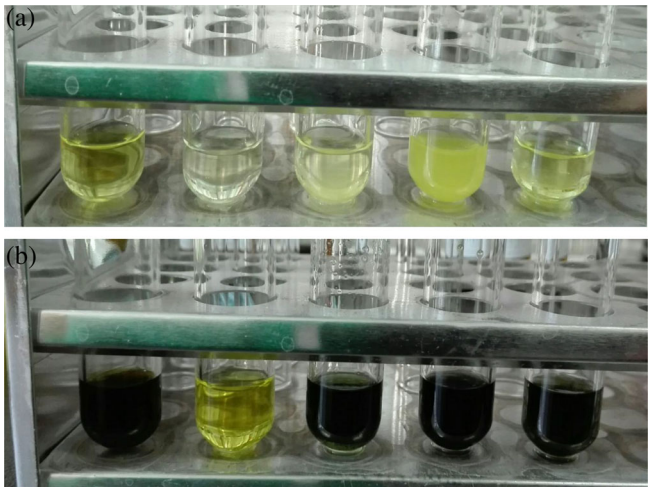


FIGURE 6 The appearance of the test compounds in methanolic ammonia solution (a) before and (b) after the addition of strontium chloride. From left to right: baicalein, chrysin, the 7-ether (**4**), 8-aminomethyl derivative (**3a**), and the 7-ester-8-aminomethyl derivative (**1a**)

160.6 (C-7), 152.3 (C-8a), 151.5 (C-5), 133.8 (C-4″), 131.9 (C-4′), 130.8 (C-1′), 129.7 (C-2″,6″), 129.1 (C-3′,5′), 128.8 (C-3″,5″), 128.6 (C-1″), 126.4 (C-2′,6′), 122.5 (C-6), 104.7 (C-4a), 102.0 (C-3), 98.8 (C-8), 54.8 (C-11,14), 53.3 (C-9), 26.2 (C-12,13). HRMS (ESI) *m/z* $[M + H]^+$ Calcd for $C_{27}H_{24}NO_6S$: 490.1324, found : 490.1346.

4 | CONCLUSIONS

In summary, 7-ester-8-aminomethylene-substituted baicalein derivatives (**1**) were successfully synthesized by a Mannich reaction, followed by esterification. Comparison of the ^1H, ^{13}C-NMR, HMBC data, and strontium chloride reactivity of **1** with those of the 7-ester derivatives **3** confirmed the proposed structure of **1**.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support from the National Natural Science Foundation of China (Grant No. 21502142). Prof. Yi Zhang is acknowledged for the structural analysis of compound **1**.

REFERENCES

[1] S. Chen, *J. Chin. Chem. Soc.* **2020**, *67*, 1661.
[2] C. Tian, H. Wang, Y. Guo, P. Qiu, C. Cui, M. Liu, *Ind. Crop. Prod.* **2019**, *134*, 100.
[3] C. Tian, Y. Chang, Z. Zhang, H. Wang, S. Xiao, C. Cui, M. Liu, *Heliyon* **2019**, *5*, e02234.
[4] J. Xie, W. Wang, C. Dong, L. Huang, H. Wang, C. Li, S. Nie, M. Xie, *Food Chem. Toxicol.* **2018**, *119*, 392.
[5] X. Zhu, W. Ouyang, Y. Lan, H. Xiao, L. Tang, G. Liu, K. Feng, L. Zhang, M. Song, Y. Cao, *Food Biosci.* **2020**, *35*, 100574.
[6] L. Ling, Y. Lu, Y. Zhang, H. Zhu, P. Tu, H. Li, D. Chen, *Phytomedicine* **2020**, *67*, 153150.
[7] M. Zou, H. Liu, J. Li, X. Yao, Y. Chen, C. Ke, S. Liu, *Biochem. Pharmacol.* **2020**, *177*, 113962.
[8] R. Liu, X. Bao, X. Sun, Y. Cai, T. Zhang, X. Ye, X. Li, *Tetrahedron Lett.* **2020**, *61*, 151803.
[9] C. Liu, R. Li, J. Peng, D. Qu, M. Huang, Y. Chen, *Process Biochem.* **2019**, *86*, 80.
[10] S. Yan, M. Xie, Y. Wang, Q. Xiao, N. Ding, Y. Li, *Tetrahedron* **2020**, *76*, 130950.
[11] R. Luo, J. Wang, L. Zhao, N. Lu, Q. You, Q. Guo, Z. Li, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1334.
[12] X. Jiang, J. Zhou, Q. Lin, G. Gong, H. Sun, W. Liu, Q. Guo, F. Feng, W. Qu, *Bioorg. Med. Chem.* **2018**, *26*, 4481.
[13] M. P. Neves, H. Cidade, M. Pinto, A. M. S. Silva, L. Gales, A. M. Damas, R. T. Lima, M. H. Vasconcelos, M. de Sao Jose Nascimento, *Eur. J. Med. Chem.* **2011**, *46*, 2562.
[14] Y. Lee, H. Yeo, S. Liu, Z. Jiang, R. M. Savizky, D. J. Austin, Y. Cheng, *J. Med. Chem.* **2004**, *47*, 5555.
[15] J. Wang, N. Ding, W. Zhang, P. Wang, Y. Li, *Helv. Chim. Acta* **2011**, *94*, 2221.
[16] L. Li, W. Liu, F. Feng, C. Wu, N. Xie, *Chin. J. Nat. Med.* **2013**, *11*, 284.
[17] S. Zhang, J. Ma, Y. Bao, P. Yang, L. Zou, K. Li, X. Sun, *Bioorg. Med. Chem.* **2008**, *16*, 7127.
[18] N. Wang, D. Ren, S. Deng, X. Yang, *Chem. Biol. Interact.* **2014**, *221*, 99.
[19] X. Lv, H. Liu, Z. Ren, W. Wang, F. Tang, H. Cao, *Mol. Divers.* **2019**, *23*, 299.
[20] J. Ma, T. Li, X. Han, H. Yuan, H. Liang, Y. Wang, X. Wang, Y. Duan, A. Li, H. Song, D. Yang, *Virology* **2017**, *507*, 199.
[21] B. Yun, Y. Lee, K. Park, S. Jung, Y. Lee, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 4170.
[22] S. Hsu, Y. Hsieh, W. Hsieh, C. Chou, *Eur. J. Pharmacol.* **2001**, *425*, 165.
[23] D. Chen, Y. Deng, J. Mou, Y. Jia, *Nat. Prod. Res. Dev.* **2019**, *31*, 1258.
[24] J. Mou, H. Fang, F. Jing, Q. Wang, Y. Liu, H. Zhu, W. Xu, *Bioorg. Med. Chem.* **2009**, *17*, 4666.
[25] G. Xu, L. Zhang, H. Shen, C. Hu, J. Shanghai, *Med. Univ.* **1993**, *20*, 187.
[26] Y. Zhao, H. Li, X. Zhou, S. Wei, J. Li, K. Li, CH patent CN 108794441 a, Nov. 13, **2018**.
[27] Z. Zhu, W. Wang, Z. Wang, L. Chen, J. Zhang, X. Liu, S. Wu, Y. Zhang, *Eur. J. Med. Chem.* **2014**, *75*, 297.
[28] L. Wu, H. Lou, J. Zhou, *Medicinal Chemistry of Natural Products*, Vol. 6, Peoples Medical Publishing House Co., LTD, Beijing **2012**, p. 197.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: J. Mou, Q. Wang, Y. Deng, D. Chen, S. Qiu, *J. Chin. Chem. Soc.* **2021**, 1. https://doi.org/10.1002/jccs.202100194