


 Cite this: *RSC Adv.*, 2023, 13, 4859

Collective synthesis of aspulvinone and its analogues by vinylogous aldol condensation of substituted tetronic acids with aldehydes†

 Xiaotan Yu,[‡] Xiaoxia Gu,[‡] Yunpeng Zhao, Fengqing Wang, Weiguang Sun, Changxing Qi,^{*} Lianghu Gu^{*} and Yonghui Zhang[‡]

 Received 21st December 2022
 Accepted 30th January 2023

DOI: 10.1039/d2ra08133d

rsc.li/rsc-advances

A mild, modular and efficient synthetic method with broad substrate scope was developed for aspulvinones. Nine natural aspulvinones were synthesized, six of which were for the first time. The aldol condensation delivered Z-configuration products predominantly in MeCN. Meanwhile, alkoxy exchange occurred in MeOH and EtOH. Aspulvinone O and E, and substrate 49, 50, and 51 exhibited modest anti-SARS-CoV-2 activity in a high-throughput screening and enzyme kinetics assay.

Introduction

Aspulvinones, mainly isolated from fungus metabolites, were first reported in the 19th century.¹ Up to now, more than 40 aspulvinones have been reported. The core structure of aspulvinone is characterized by a tetronic acid ring, a 5-membered heterocycle connecting two substituted aromatic rings. Aspulvinones exhibit a wide range of bioactivities, such as antibacterial,² anti-inflammatory,³ anti-DPPH radical,⁴ anti-fungal,⁵ and anti- α -glucosidase effects.⁶ Recently, a study showed that some aspulvinones could inhibit SARS-CoV-2 infection and reduce inflammatory reactions caused by SARS-CoV-2.⁷

As part of our work, aspulvinone O and H were isolated from the rice culture of *Aspergillus terreus* and identified as potent bioactive inhibitors of GOT1 and novel anti-tumor agents for PDAC therapy.^{8,9} However, the poor isolated yield and limited structure variation restricted our further study of their pharmaceutical properties. The diverse activities of aspulvinone have attracted extensive attention from synthetic chemists. The synthetic approaches can be divided into three categories: noble metal catalysis,^{10–13} harsh conditions,^{14–16} and other conditions.^{2,17,18} Some of the methods reported after 2010 are listed in Scheme 1. However, noble metal catalysts often face the problem of high cost and scarce availability. The use of organolithium reagents requires low temperature (–78 °C) and the

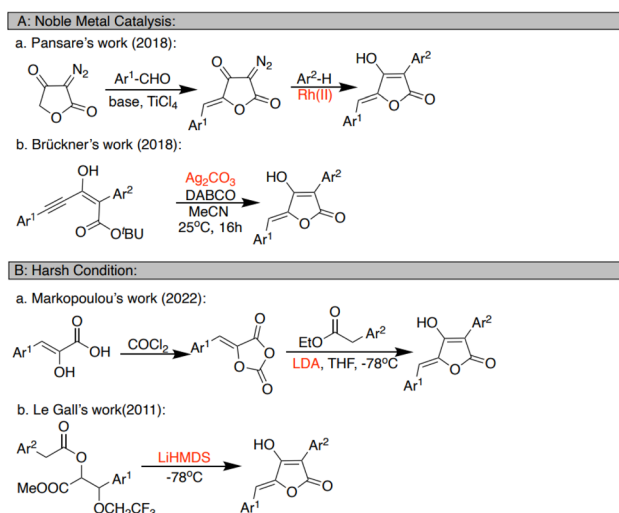
Schlenk technique, which is relatively hard to operate in labs and factories.

Our group aims to develop a new method which is economical and easy to operate in a short route. In Liu's work, a two-step method was developed to synthesize the precursor of aspulvinone in a mild condition (Scheme 2A).² Besides, in synthesizing aspulvinone H, Brückner and co-workers obtained unexpected aspulvinone A after deprotection. They assumed that deprotection of methyl ether using BBr₃ resulted in the cyclization of the prenyl chain with the neighboring phenol group (Scheme 2B).¹³ Based on their work, we modified the starting material to 4-(benzyloxy)-3-phenyl-5-furan-2(5H)-one, hoping to find a one-step method for the aldol condensation, which can also be used in a broad substrate scope (Scheme 2C).

Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China. E-mail: zhangyh@mails.tjmu.edu.cn; gulianghu@hust.edu.cn; qichangxing@hust.edu.cn

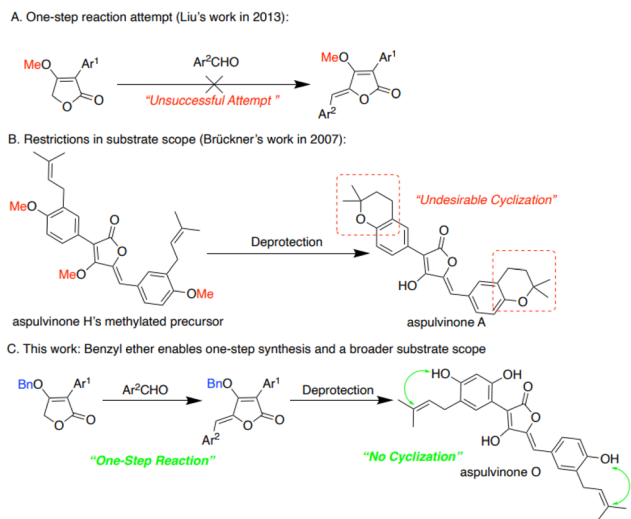
† Electronic supplementary information (ESI) available. CCDC 2183460 and 2204675–2204677. For ESI and crystallographic data in CIF or other electronic format see DOI: <https://doi.org/10.1039/d2ra08133d>

‡ These authors contributed equally to this work.



Scheme 1 Previous synthetic approaches for the aspulvinone.



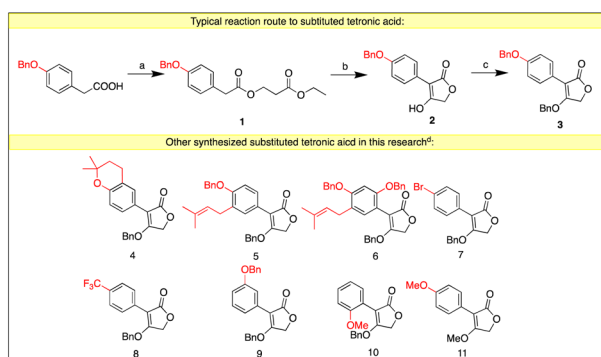


Scheme 2 Synthetic Strategies of the aspulvinone.

Results and discussion

Our work started from the synthesis of the substituted tetronic acid, 4-(benzyloxy)-3-(4-(benzyloxy) phenyl)furan-2(5*H*)-one (**3**). The commercially available *p*-benzyloxy phenylacetic acid was treated with ethyl chloroacetate and triethylamine to afford the acyclic ester (**1**), which then underwent an intramolecular cyclization by *t*-BuOK to afford the 4-hydroxy-3-(2-methoxyphenyl)furan-2(5*H*)-one (**2**). It was protected by the use of BnBr or BnOH to afford compound **3**. Other substituted tetronic acids were synthesized using the same route from the different starting materials (Scheme 3).

Next, optimization of the aldol condensation between tetronic acid **3** and 4-(benzyloxy)benzaldehyde was performed. In Chopin's work, they proposed a method for aldol condensation using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the base in acetonitrile (MeCN) at 65 °C.¹⁹ Initially, we used this method for aspulvinone's synthesis and successfully gained the desired product (Table 1, Entry 1). Then, the reactions were



Scheme 3 The synthesis of substituted tetronic acid. Reagents and conditions: ^aethyl chloroacetate, Et₃N, THF, reflux, 12 h, 90%. ^b*t*-BuOK, DMF, 0 °C to rt, 2 h, 82%. ^cK₂CO₃, BnBr, DMF, rt, 12 h, 35% or BnOH, Ph₃P, DEAD, rt, 12 h, 65%. ^dDetailed procedures and yields are in ESI.†

conducted at 65 °C employing DBU (2 eq.) as the base in various solvents, including tetrahydrofuran (THF), dichloromethane (DCM), methanol (MeOH) and isopropanol (*i*-PrOH) (Table 1, entry 2–5). With these tested solvents, MeOH afforded a methylated product, and *i*-PrOH afforded a mixture of *Z/E* products. MeCN showed the best yield in all solvents. To be noted, these results were quite different from Chopin's work, probably due to the difference in substrates. Next, several bases, including triethylamine (Et₃N), 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), 4-dimethylaminopyridine (DMAP), potassium *tert*-butoxide (*t*-BuOK), sodium hydroxide (NaOH), and potassium carbonate (K₂CO₃), were tested for this reaction (Table 1, entry 6–11), among which DBN showed a slight increase in yield. Then, different temperatures were tested (Table 1, entry 12–16), and the reaction at 30 °C gave the best yield. High and low temperatures lead to low yield, which is an interesting phenomenon that deserves more study. Finally, different times and equivalents of reactants and reagents were checked (Table 1, entry 17–20). It was found that extending reaction time, decreasing DBN, and increasing the aldehyde could increase the reaction yield (Table 1, entry 20). The reaction of 1.0 g scale was also performed in the same condition and gained the same yield.

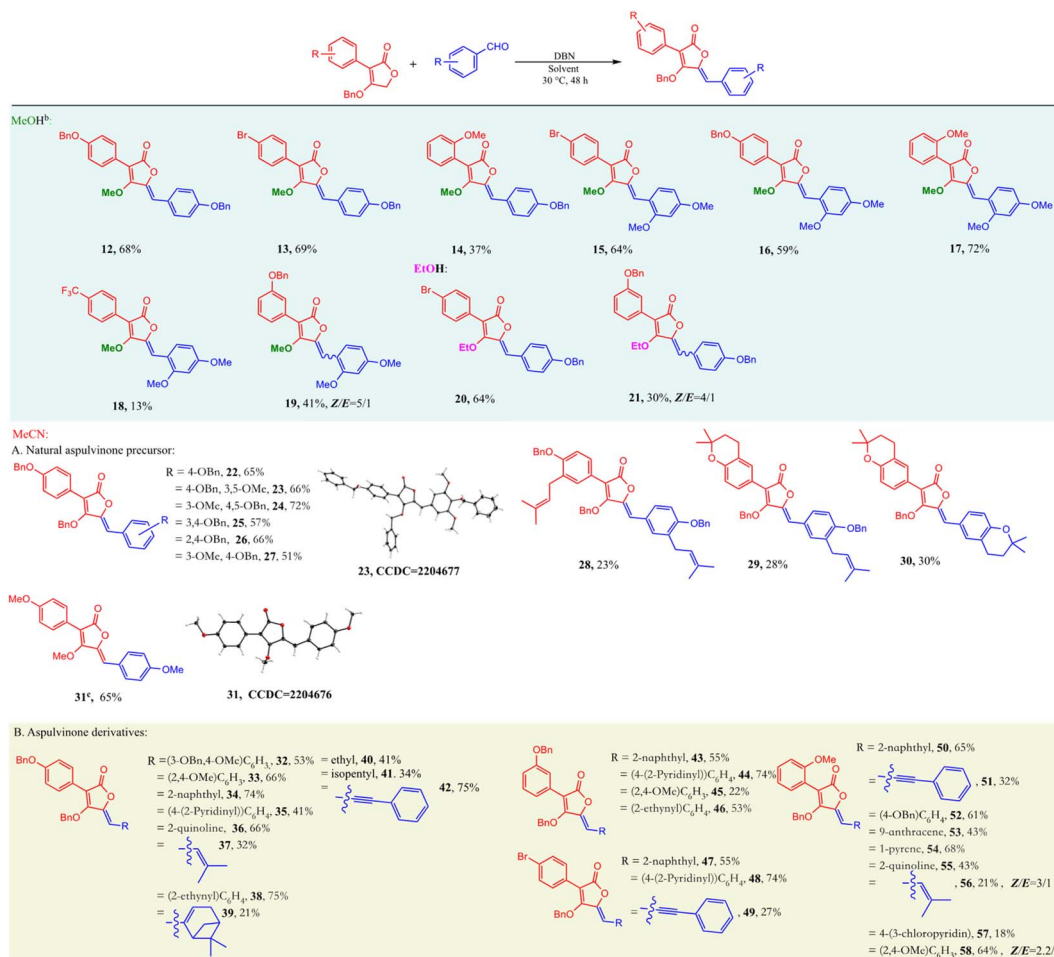
After optimization, the modular condensation between different substituted tetronic acids and aldehydes to afford various aspulvinone-skeleton products was investigated.

Table 1 Screening of the Reaction Solvent and Base^a

Entry	Temperature (°C)	Time (h)	Solvent	Base (eq.)	Yield (%) ^b
1	65	12	MeCN	DBU (2)	34
2	65	12	THF	DBU (2)	21
3	40	12	DCM	DBU (2)	6
4	65	12	MeOH	DBU (2)	36 ^c
5	65	12	<i>i</i> -PrOH	DBU (2)	28 ^d
6	65	12	MeCN	Et ₃ N (2)	Trace
7	65	12	MeCN	DBN (2)	38
8	65	12	MeCN	DMAP (2)	Trace
9	65	12	MeCN	<i>t</i> -BuOK (2)	16
10	65	12	MeCN	NaOH (2)	25
11	65	12	MeCN	K ₂ CO ₃ (2)	12
12	80	12	MeCN	DBN (2)	10
13	45	12	MeCN	DBN (2)	42
14	30	12	MeCN	DBN (2)	46
15	15	12	MeCN	DBN (2)	24
16	0	12	MeCN	DBN (2)	5
17	30	24	MeCN	DBN (2)	49
18	30	48	MeCN	DBN (2)	55
19	30	48	MeCN	DBN (1)	55
20 ^e	30	48	MeCN	DBN (1)	65

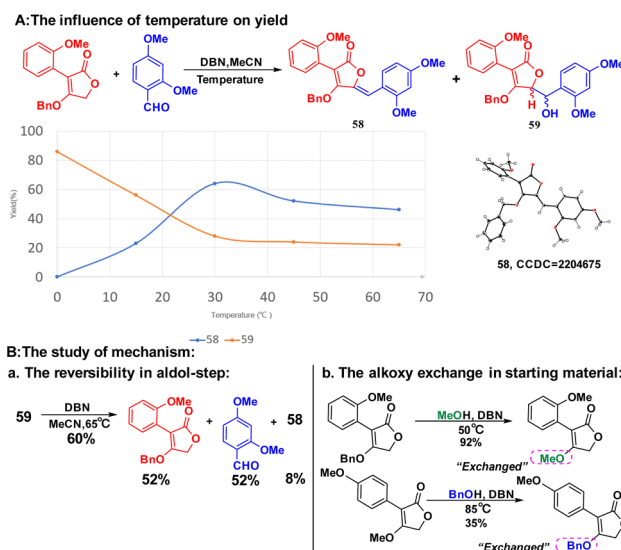
^a Standard condition: tetronic acid (50 mg), aldehyde (57 mg, 2 eq.), solvent (1 ml), base (2 eq.). ^b Isolated yields. ^c Methylated product. ^d *Z/E* = 1.7 : 1. ^e The equivalent of aldehyde is 3.



Scheme 4 Substrate scope. ^aIsolated yields. ^b60 °C. ^cCompound **31** is a natural product.²⁰

Firstly, a series of fully methylated products (**12–19**) and partly ethylated ones (**20–21**) were synthesized using MeOH or EtOH as solvent. Then, some natural products protected by benzyl groups (**22–30**) were synthesized with similar conditions using MeCN as solvent. Some of them can be purified by filtration since precipitation of the products formed in the solvent. Next, more aspluvone derivatives were synthesized from heterocyclic aldehyde and alkyl ones (**32–58**). Most compounds are pure *Z* isomers except some derivatives from compound **10** (Scheme 4). Next, we conducted an investigation to gain insights into the factors controlling the reaction.

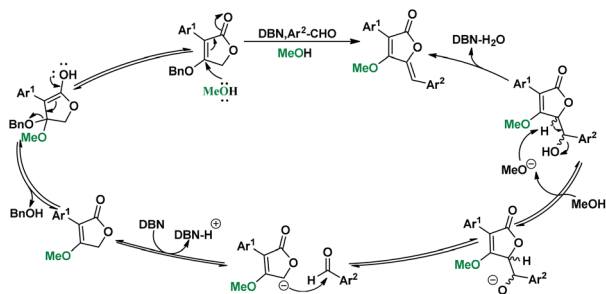
In the substrate exploration, the reaction for synthesizing compound **58** is formed with by-product **59**, which is the only reaction where an addition intermediate can be obtained. Then, the influence of temperatures was studied on the yield of **58** and **59** (Scheme 5, A). It was found that the yield of **58** was gradually increased from 0 to 30 °C and then decreased from 30 to 65 °C. While the best yield of **59** was at 0 °C, its yield decreased as the temperature increased. Additional controlled experiments using **59** further illustrated this (Scheme 5, Ba). However, no elimination product can be found in low temperatures, indicating the necessity of high-temperature for elimination. Interestingly, a unique equilibrium occurred at



Scheme 5 The study of mechanism.

30 °C, where both the aldol step and the elimination proceeded smoothly and afforded the best yield. Then, the alkoxy exchange mechanism was studied by using the starting

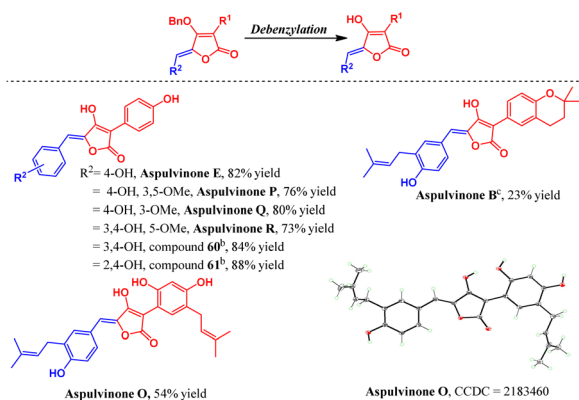




Scheme 6 Plausible Mechanism.

material in MeOH condition (Scheme 5, Bb). We found that the exchange only took place in the starting material stage, thus proposing a mechanism containing both alkoxy exchange and aldol condensation (Scheme 6).

Next, the natural aspulvinone precursors were deprotected, and eight new natural aspulvinones were obtained. Six of them, including aspulvinone O, P, Q, R, compound **60** and **61**, were synthesized for the first time. Pd/C and H₂ were used for most compounds to cleave the *O*-benzyl bond. However, the deprotection of aspulvinone B precursor using this method invariably resulted in partial hydrogenation of the prenyl



Scheme 7 The deprotection and synthesis of natural aspulvinones in this study. ^a isolated yields. ^bUnnamed natural aspulvinones. ^cAspulvinone B use C₂NMe and BCl₃ for deprotection.¹³

double bond. Finally, the Lewis acid BCl₃ was used for deprotection and successfully delivered aspulvinone B (Scheme 7).¹³

In the optimization process, we found that the mixture of *Z/E* products can be formed in aspulvinone E precursor using *i*-PrOH in reflux condition. Through screening, two natural product precursors were formed with *Z/E* mixed configurations and transformed to natural aspulvinones of different configurations by deprotecting them. Isoaspulvinone have been observed in some researches.^{18,21–23} Our NMR data for isoaspulvinone E are consistent with those reported in Gao's work.²¹ The *E* configuration of aspulvinone E and P showed similar NMR changes compared to the *Z* configuration, which is consistent with Campbell's work.¹⁸

M^{Pro} and PL^{Pro} are two important proteases for SARS-CoV-2 and other coronaviruses and essential for viral replication and transcription.^{24,25} These aspulvinone analogues exhibited modest antiviral activity in the high-throughput screening assay. Aspulvinone E and **49** inhibited SARS-CoV-2 M^{Pro} activity with IC₅₀ of 39.93 ± 2.42 μM and 28.25 ± 2.37 μM, respectively (Fig. S1B, Table S1†). **51** and **50** inhibited SARS-CoV-2 PL^{Pro} activity with IC₅₀ of 23.05 ± 0.07 μM and 17.43 ± 2.60 μM, respectively (Fig. S1A, Table S2†). Notably, aspulvinone O exhibited dual-antiviral activity towards SARS-CoV-2 M^{Pro} and PL^{Pro}, with IC₅₀ of 12.41 ± 2.40 μM and 21.34 ± 0.94 μM, respectively (Fig. S1A and S1B, Table S1 and S2†).

Further enzyme kinetics assays revealed that aspulvinone E (Fig. S1C†) and **49** (Fig. 1A) showed uncompetitive inhibition against SARS-CoV-2 M^{Pro}, with reduced *V*_{max} and *K*_m values. In contrast, aspulvinone O showed competitive inhibition against SARS-CoV-2 M^{Pro}, with almost unchanged *V*_{max} values and gradually increased *K*_m values (Fig. 1A). The same assays were also performed against SARS-CoV-2 PL^{Pro}, which revealed that aspulvinone O, **51**, and **50** all behaved as competitive inhibitors, consistent with GRL0617 (Fig. 1B, S1D†). These results gave substantial evidence for the docking analysis in Hawary's work.²⁷

Conclusions

In conclusion, we have developed a method with high yield, mild conditions, and wide substrate tolerance to synthesize

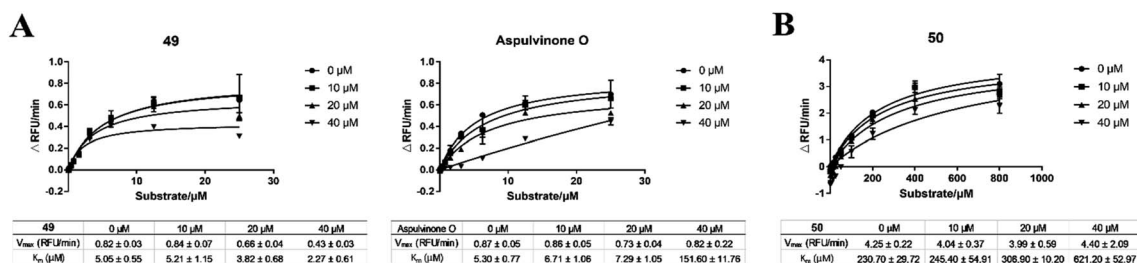


Fig. 1 Antiviral activity of aspulvinone analogues. (A) Mechanisms of action of **49** and aspulvinone O against SARS-CoV-2 M^{Pro}. **49** inhibited M^{Pro} in an uncompetitive way, while aspulvinone O in a competitive way. (B) The mechanism of action of **50** against SARS-CoV-2 PL^{Pro}, which inhibited PL^{Pro} in a competitive way.



aspulvinones and their derivatives. This is a useful protocol for researchers who are interested in aspulvinones to quickly obtain their desired products. We found an interesting alkoxy exchange mechanism during the synthesis, further broadening the substrate scope. By using *i*-PrOH, we successfully synthesized aspulvinones with *E* configuration after separation by HPLC. In addition, our synthetic aspulvinone O, E, and derivatives **49**, **50**, and **51** exhibited modest anti-SARS-CoV-2 activity, which may provide new ideas for the design of lead compounds to treat COVID-19 in the future.

Author contributions

Xiaotan Yu and Xiaoxia Gu contributed equally to this work. Xiaotan Yu completed the research of the organic synthesis part, and Xiaoxia Gu completed the research of the pharmacological part.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was financially supported by the Program for Changjiang Scholars of Ministry of Education of the People's Republic of China (No. T2016088), the National Natural Science Foundation for Distinguished Young Scholars (81725021), the National Natural Science Foundation of China (81903461 and 82003633), and the Fundamental Research Funds for the Central Universities (2172019kfyXJJS166 and 2019kfyXJJS168). We thank the Medical Subcenter of HUST Analytical & Testing Center for data acquisition. We thank Prof. Chaomei Xiong at the School of Pharmacy, Huazhong University of Science and Technology, for NMR spectrum acquisition, and we thank Prof. Zimin Lv at the School of Pharmacy, Huazhong University of Science and Technology, for mass spectrum acquisition.

References

- 1 N. Ojima, S. Takenaka and S. Seto, New butenolides from *Aspergillus terreus*, *Phytochemistry*, 1973, **12**, 2527–2529.
- 2 H.-W. Xu, C. Xu, Z.-Q. Fan, L.-J. Zhao and H.-M. Liu, A facile synthesis, antibacterial activity of pulvinone and its derivatives, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 737–739.
- 3 G.-R. Hsiao, W.-C. Chi, C.-H. Chang, Y.-R. Chiang, Y.-J. Fu and T.-H. Lee, Bioactive pulvinones from a marine algicolous fungus *Aspergillus terreus* NTU243, *Phytochemistry*, 2022, **200**, 113229.
- 4 P. Zhang, X.-M. Li, J.-N. Wang, X. Li and B.-G. Wang, New butenolide derivatives from the marine-derived fungus *Paecilomyces variotii* with DPPH radical scavenging activity, *Phytochem. Lett.*, 2015, **11**, 85–88.
- 5 N. Bunbamrung, C. Intaraudom, A. Dramaee, S. Komwijit, T. Laorob, S. Khamsaeng and P. Pittayakhajonwut, Antimicrobial, antimalarial and anticholinesterase substances from the marine-derived fungus *Aspergillus terreus* BCC51799, *Tetrahedron*, 2020, **76**, 131496.
- 6 C.-J. Wu, X. Cui, L.-Z. Sun, J.-J. Lu, F. Li, M.-H. Song, Y.-X. Zhang, X.-Q. Hao, C.-K. Tian, M.-P. Song and X.-M. Liu, Aspulvinones Suppress Postprandial Hyperglycemia as Potent alpha-Glucosidase Inhibitors From *Aspergillus terreus* ASM-1, *Front. Chem.*, 2021, **9**, 736070.
- 7 X.-X. Liang, X.-J. Zhang, Y.-X. Zhao, J. Feng, J.-C. Zeng, Q.-Q. Shi, J. S. Kaunda, X.-L. Li, W.-G. Wang, W.-L. Xiao and A.-H. Aspulvins, Aspulvinone Analogues with SARS-CoV-2 M-pro Inhibitory and Anti-inflammatory Activities from an Endophytic *Cladosporium* sp, *J. Nat. Prod.*, 2022, **85**, 878–887.
- 8 W.-G. Sun, S.-S. Luan, C.-X. Qi, Q.-Y. Tong, S. Yan, H. Li and Y.-H. Zhang, Aspulvinone O, a natural inhibitor of GOT1 suppresses pancreatic ductal adenocarcinoma cells growth by interfering glutamine metabolism, *Cell Commun. Signal.*, 2019, **17**, 111.
- 9 S. Yan, C.-X. Qi, W. Song, Q.-Q. Xu, L.-H. Gu, W.-G. Sun and Y.-H. Zhang, Discovery of GOT1 Inhibitors from a Marine-Derived *Aspergillus terreus* That Act against Pancreatic Ductal Adenocarcinoma, *Mar. Drugs*, 2021, **19**, 588.
- 10 A. Manchoju, R. A. Annadate, L. Desquien and S. V. Pansare, Functionalization of diazotetronic acid and application in a stereoselective modular synthesis of pulvinone, aspulvinones A-E, G, Q and their analogues, *Org. Biomol. Chem.*, 2018, **16**, 6224–6238.
- 11 D. Hermann and R. Brückner, Silver-Catalyzed *tert*-Butyl 3-Oxopent-4-ynoate π -Cyclizations: Controlling the Ring Size-Hydroxypyrrone or Pulvinone Formation-by Counterion and Additive Optimization, *Org. Lett.*, 2018, **20**, 7455–7460.
- 12 Y. Sadamitsu, K. Komatsuki, K. Saito and T. Yamada, Access to Tetronic Acids via Silver-Catalyzed CO₂ Incorporation into Conjugated Ynones, *Org. Lett.*, 2017, **19**, 3191–3194.
- 13 D. Bernier and R. Brückner, Novel synthesis of naturally occurring pulvinones: A heck coupling, transesterification, and Dieckmann condensation strategy, *Synthesis*, 2007, **15**, 2249–2272.
- 14 K. C. Prousis, S. Katsamakas, J. Markopoulos and O. Igglessi-Markopoulou, A novel synthetic protocol for the synthesis of pulvinones, and naturally occurring Aspulvinone E, molecules of medicinal interest, *Synth. Commun.*, 2022, **52**, 117–128.
- 15 B. Nadal, J. Rouleau, H. Besnard, P. Thuery and T. Le Gall, Synthesis of pulvinones via tandem Dieckmann condensation-alkoxide beta-elimination, *Tetrahedron*, 2011, **67**, 2605–2611.
- 16 N. Kaczybura and R. Brückner, Tandem claisen condensation/transesterification between arylacetate enolates and arylmethylene-substituted 2,2-dimethyl-1,3-dioxolan-4-ones: An improved synthesis of *Z*-configured pulvinones, *Synthesis*, 2007, **1**, 118–130.
- 17 D. Bernier, F. Moser and R. Brückner, Synthesis and Cyclization of 3-aryl-2-(arylacetoxy)acrylates: A three-step access to pulvinones, *Synthesis*, 2007, **15**, 2240–2248.



- 18 A. C. Campbell, M. S. Maidment, J. H. Pick and D. F. M. Stevenson, Synthesis of (*E*)- and (*Z*)-pulvinones, *J. Chem. Soc., Perkin trans. 1*, 1985, 1567–1576.
- 19 N. Chopin, H. Yanai, S. Iikawa, G. Pilet, J. P. Bouillon and M. Medebielle, A Rapid Entry to Diverse gamma-Ylidenetetronate Derivatives through Regioselective Bromination of Tetronic Acid Derived gamma-Lactones and Metal-Catalyzed Postfunctionalization, *Eur. J. Org. Chem.*, 2015, **2015**, 6259–6269.
- 20 S. Chokpaiboon, P. Unagul, S. Kongthong, K. Danwisetkanjana, A. Pilantanapak, S. Suetrong and T. Bunyapaiboonsri, A pyrone, naphthoquinone, and cyclic urea from the marine-derived fungus *Astrosphaeriella nypae* BCC 5335, *Tetrahedron Lett.*, 2016, **57**, 1171–1173.
- 21 H.-Q. Gao, W.-Q. Guo, Q. Wang, L.-Q. Zhang, M.-L. Zhu, T.-J. Zhu, Q.-Q. Gu, W. Wang and D.-H. Li, Aspulvinones from a mangrove rhizosphere soil-derived fungus *Aspergillus terreus* Gwq-48 with anti-influenza A viral (H1N1) activity, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 1776–1778.
- 22 E. Geib, M. Gressler, I. Viediernikova, F. Hillmann, I. Jacobsen, S. Nietzsche, C. Hertweck and M. Brock, A Non-canonical Melanin Biosynthesis Pathway Protects *Aspergillus terreus* Conidia from Environmental Stress, *Cell Chem. Biol.*, 2016, **23**, 587–597.
- 23 R. T. Dewi, S. Tachibana, S. Fajriah and M. Hanaf, α -Glucosidase inhibitor compounds from *Aspergillus terreus* RCC₁ and their antioxidant activity, *Med. Chem. Res.*, 2015, **24**, 737–743.
- 24 Z.-M. Jin, X.-Y. Du, Y.-C. Xu, Y.-Q. Deng, M.-Q. Liu, Y. Zhao, B. Zhang, X.-F. Li, L.-K. Zhang, C. Peng, Y.-K. Duan, J. Yu, L. Wang, K.-L. Yang, F.-J. Liu, R.-D. Jiang, X.-L. Yang, T. You, X.-C. Liu, X.-N. Yang, F. Bai, H. Liu, X. Liu, L. Guddat, W.-Q. Xu, G.-F. Xiao, C.-F. Qin, Z.-L. Shi, H.-L. Jiang, Z.-H. Rao and H.-T. Yang, Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors, *Nature*, 2020, **582**, 289–293.
- 25 D. Shin, R. Mukherjee, D. Grewe, D. Bojkova, K. Baek, A. Bhattacharya, L. Schulz, M. Widera, A. R. Mehdipour, G. Tascher, P. P. Geurink, A. Wilhelm, G. J. van der Heden van Noort, H. Ovaa, S. Müller, K.-P. Knobeloch, K. Rajalingam, B. A. Schulman, J. Cinatl, G. Hummer, S. Ciesek and I. Dikic, Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity, *Nature*, 2020, **587**, 657–662.
- 26 N. Ojima, S. Takenaka and S. Seto, Structures of pulvinone derivatives from *Aspergillus terreus*, *Phytochemistry*, 1975, **14**, 573–576.
- 27 S. S. El-Hawary, R. Mohammed, H. S. Bahr, E. Z. Attia, M. H. El-Katatny, N. Abelyan, M. M. Al-Sanea, A. S. Moawad and U. R. Abdelmohsen, Soybean-associated endophytic fungi as potential source for anti-COVID-19 metabolites supported by docking analysis, *J. Appl. Microbiol.*, 2021, **131**, 1193–1211.

