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

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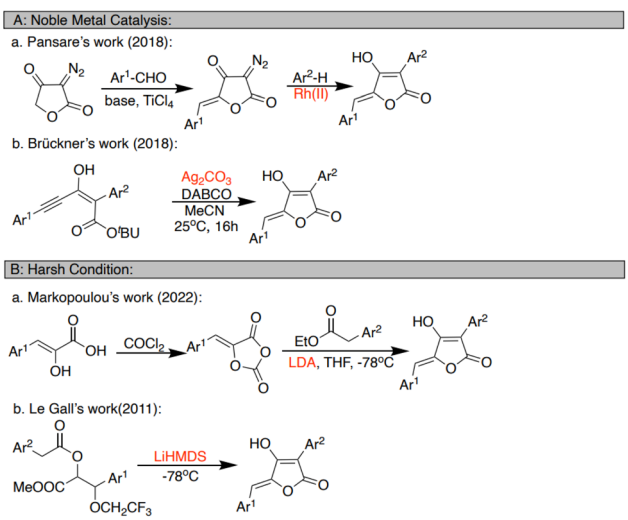
A mild, modular and efficient synthetic method with broad substrate scope was developed for aspulvinones. Nine natural aspulvinones were synthesized, six of which were for the first time. The aldol condensation delivered *Z*-configuration products predominantly in MeCN. Meanwhile, alkoxy exchange occurred 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 BBr_3 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(5*H*)-one, hoping to find a one-step method for the aldol condensation, which can also be used in a broad substrate scope (Scheme 2C).



Scheme 1 Previous synthetic approaches for the aspulvinone.

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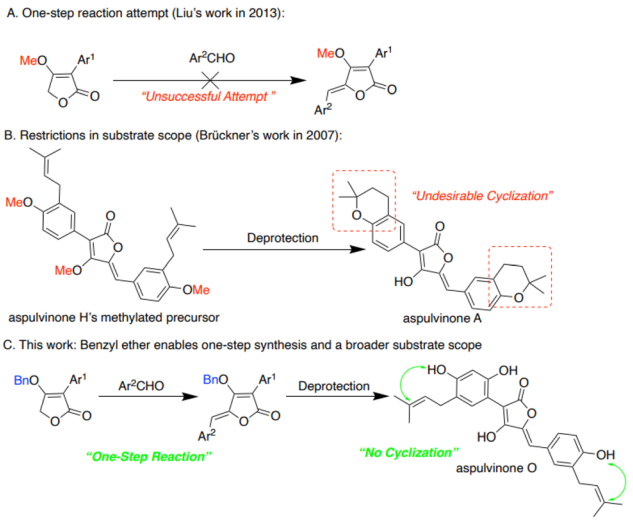


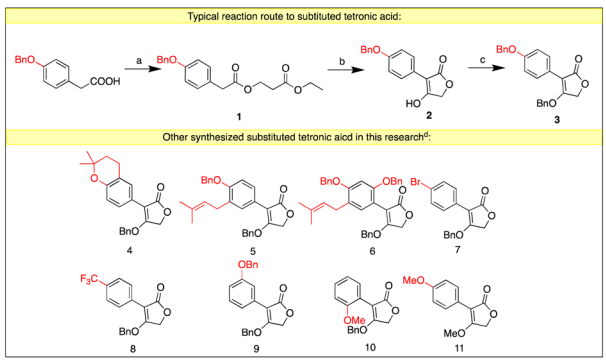
Scheme 2 Synthetic Strategies of the aspulvinone.

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).

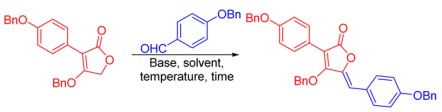
Next, optimization of the aldol condensation between tetronic acid **3** and 4-(benzyloxy)benzaldehyde was performed. In Chopin's work, they proposed a method for aldol condensation using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the base in acetonitrile (MeCN) at 65 °C.[19] Initially, we used this method for aspulvinone's synthesis and successfully gained the desired product (Table 1, Entry 1). Then, the reactions were 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 (Et_3N), 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), 4-dimethylaminopyridine (DMAP), potassium *tert*-butoxide (*t*-BuOK), sodium hydroxide (NaOH), and potassium carbonate (K_2CO_3), 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.



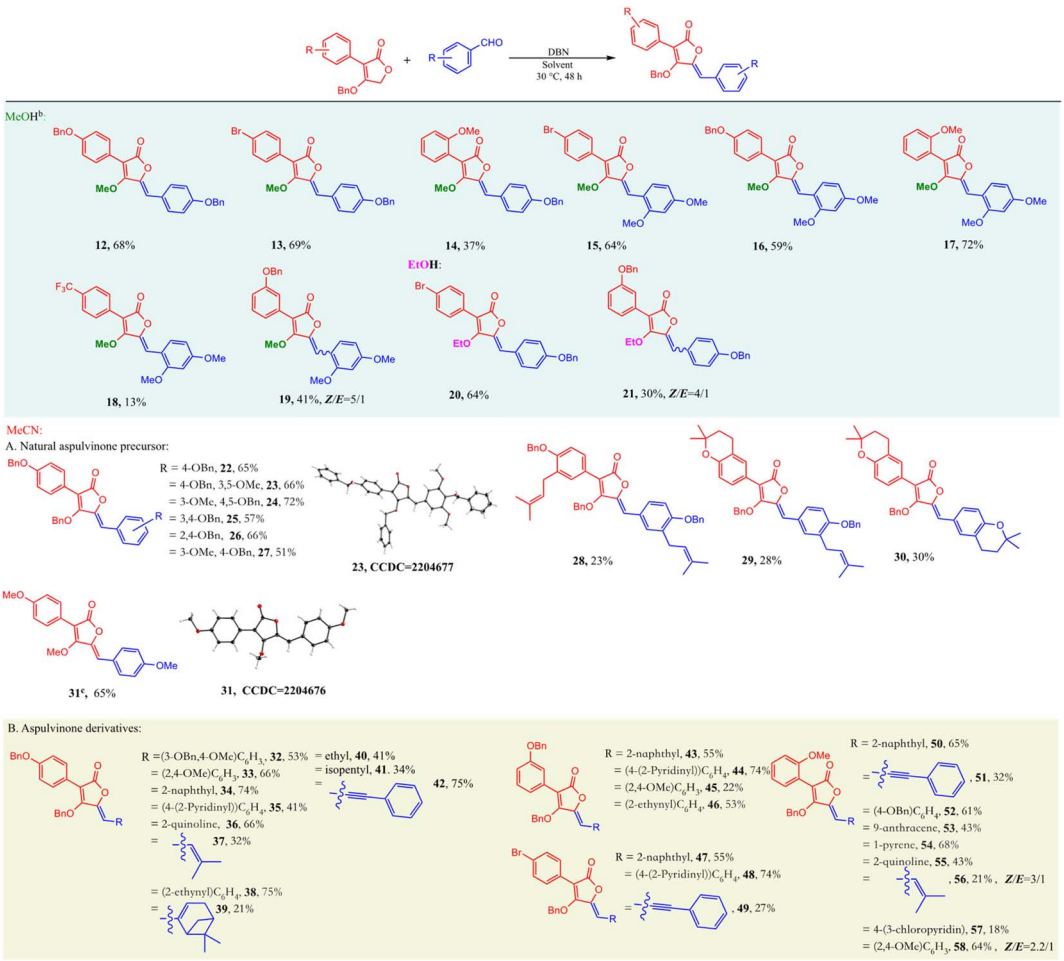
Scheme 3 The synthesis of substituted tetronic acid. Reagents and conditions: [a]ethyl chloroacetate, Et_3N, THF, reflux, 12 h, 90%. [b]*t*-BuOK, DMF, 0 °C to rt, 2 h, 82%. [c]K_2CO_3, BnBr, DMF, rt, 12 h, 35% or BnOH, Ph_3P, DEAD, rt, 12 h, 65%. [d]Detailed procedures and yields are in ESI.†

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*-PrOH	DBU (2)	28[d]
6	65	12	MeCN	Et_3N (2)	Trace
7	65	12	MeCN	DBN (2)	38
8	65	12	MeCN	DMAP (2)	Trace
9	65	12	MeCN	*t*-BuOK (2)	16
10	65	12	MeCN	NaOH (2)	25
11	65	12	MeCN	K_2CO_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

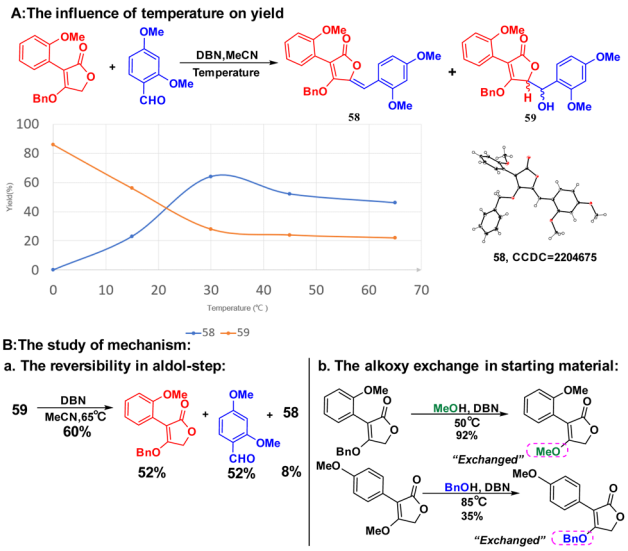
[a] Standard condition: tetronic acid (50 mg), aldehyde (57 mg, 2 eq.), solvent (1 ml), base (2 eq.). [b] Isolated yields. [c] Methylated product. [d] $Z/E = 1.7:1$. [e] The equivalent of aldehyde is 3.



Scheme 4 Substrate scope. [a]Isolated yields. [b]60 °C. [c]Compound **31** is a natural product.[20]

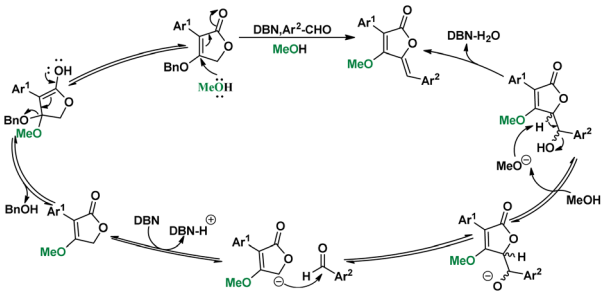
Firstly, a series of fully methylated products (**12–19**) and partly ethylated ones (**20–21**) were synthesized using MeOH or EtOH as solvent. Then, some natural products protected by benzyl groups (**22–30**) were synthesized with similar conditions using MeCN as solvent. Some of them can be purified by filtration since precipitation of the products formed in the solvent. Next, more aspulvinone derivatives were synthesized from hetero cyclic 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.

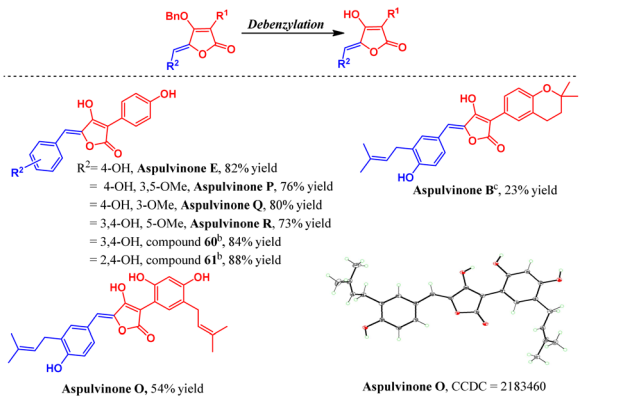
30 °C, where both the aldol step and the elimination proceeded smoothly and afforded the best yield. Then, the alkoxy exchange mechanism was studied by using the starting



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 H_2 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 double bond. Finally, the Lewis acid BCl_3 was used for deprotection and successfully delivered aspulvinone B (Scheme 7).[13]



Scheme 7 The deprotection and synthesis of natural aspulvinones in this study. [a] isolated yields. [b]Unnamed natural aspulvinones.[26] [c]Aspulvinone B use Cy_2NMe and BCl_3 for deprotection.[13]

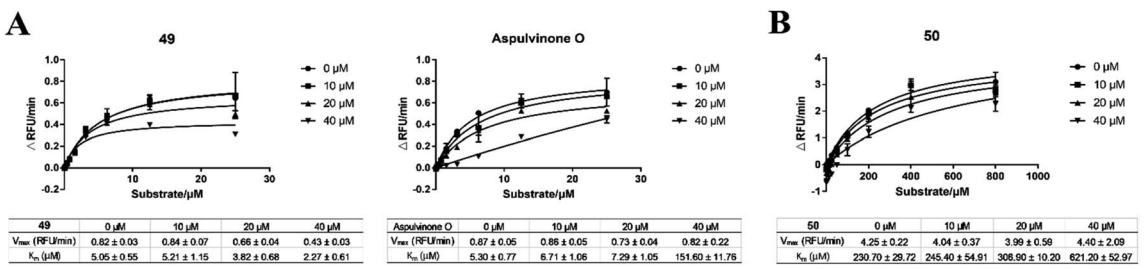
In the optimization process, we found that the mixture of *Z*/*E* products can be formed in aspulvinone E precursor using *i*-PrOH in reflux condition. Through screening, two natural product precursors were formed with *Z*/*E* mixed configurations and transformed to natural aspulvinones of different 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 IC_{50} 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 IC_{50} 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 IC_{50} 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



49	0 μM	10 μM	20 μM	40 μM
V_{max} (RFU/min)	0.82 ± 0.03	0.84 ± 0.07	0.66 ± 0.04	0.43 ± 0.03
K_m (μM)	5.05 ± 0.55	5.21 ± 1.15	3.82 ± 0.68	2.27 ± 0.61

Aspulvinone O	0 μM	10 μM	20 μM	40 μM
V_{max} (RFU/min)	0.67 ± 0.05	0.86 ± 0.05	0.73 ± 0.04	0.82 ± 0.22
K_m (μM)	5.30 ± 0.77	6.71 ± 1.06	7.29 ± 1.05	151.60 ± 11.76

50	0 μM	10 μM	20 μM	40 μM
V_{max} (RFU/min)	4.25 ± 0.22	4.04 ± 0.37	3.99 ± 0.59	4.40 ± 2.09
K_m (μM)	230.70 ± 29.72	245.40 ± 54.91	306.90 ± 10.20	621.20 ± 52.97

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

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