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Synthesis of Bioactive Fluoropyrrolidines *via* Copper(I)-Catalyzed Asymmetric 1,3-Dipolar Cycloaddition of Azomethine Ylides

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Chiral pyrrolidinyl units are important building blocks in biologically active natural products and drugs, thereupon, searching approaches for the synthesis of diverse structured pyrrolidine derivatives is of great importance. Meanwhile, owing to the special physicochemical properties of fluorine atoms, the introduction of fluorine containing groups into small molecules often change their activities to a great extent. Herein, we reported an efficient route to chiral 3,3-difluoro- and 3,3,4-trifluoropyrrolidinyl derivatives by Cu(I)-catalyzed enantioselective 1,3-dipolar cycloaddition of azomethine ylides with less active 1,1-difluoro- and 1,1,2-trifluoroalkenes. A series of new fluorinated pyrrolidines have been prepared in high yields (up to 96%) and excellent stereoselectivities (up to > 20:1 dr and 97% ee), and these unique structural blocks could be readily introduced into some natural compounds and pharmaceuticals. Additionally, antifungal activity investigation against four common plant funguses showed that some products possess general and high biological activities (up to 93% inhibition rate), the comparison with the antifungal activities of corresponding non-fluorinated compounds (low to 13% inhibition rate) revealed that the fluorine atoms at pyrrolidinyl rings play a crucial role in the antifungal activity, which prove the special properties of fluorine atoms in organic molecules.

Fluoro-organic chemistry is one of the most fiery-hot areas in modern chemistry, and has currently become crucial in the evolution of many transdisciplinary research fields.¹⁻⁴ The determinant impact of fluorine is undoubtedly associated with pharmaceutical and agrochemical industry. Due to the special properties, introduction of fluorine containing groups into compounds often affect their biological activity a lot (Figure 1) and thus has always been regarded as a powerful tactics in agrochemicals and pharmaceuticals creation.⁵⁻¹⁴ Accounting to recent survey, about half of newly developed pesticides and 35% of new drug candidates in phase II-III clinical trials contain distinct kinds of fluorine-substituted moieties.¹⁵⁻¹⁷ During the past decades, the remarkable success and huge requirement of fluorine compounds in various fields have provided infinite vitality for organic chemists to explore methods for the construction of fluorinated molecules.¹⁸⁻²²

In the other hand, enantioenriched pyrrolidinyl groups, which are important building blocks in many biologically active natural alkaloids and pharmaceuticals, has attracted much attention recent years.²³⁻²⁸ The transition-metal-catalyzed asymmetric 1,3-dipolar cycloaddition of azomethine ylides with electron deficient alkenes represent one of the most powerful routes for the stereo-controlled synthesis of these molecules.²⁹⁻³⁴ Taking the crucial property of fluorine atoms in consideration, engrafting fluorine atoms into heterocycle rings in chiral pyrrolidine derivatives would change their bioactivities to a great extent. In principle, the asymmetric 1,3-dipolar cycloaddition of azomethine ylides with fluoroalkenes could lead to multiple fluorinated pyrrolidines, however, rare research work focused on the construction and bioactivity evaluation of such molecules have been reported to date.

Most recently, *gem*-difluoroalkenes, which could be easily prepared by witting reaction of difluoro-phosphorus ylide with various aldehydes and ketones,³⁵ gain a lot of interest. Owing to their multiple reactivities, these reactants could participated in various organic transformations to access prosperous substituted fluorocompounds. The transition metal or photocatalytic addition-

elimination of nucleophiles or free radicals to *gem*-difluoroalkenes could lead to monofluoro-alkenes (Scheme 1a).³⁶⁻⁴⁴ While nucleophiles or free radicals could also add directly to *gem*-difluoroalkenes without fluorine elimination, giving difluoro-motifs (Scheme 1b).⁴⁵⁻⁴⁷ Moreover, novel strategies have been reported by taking advantage of *gem*-difluoroalkenes as reliable trifluoromethyl (CF₃) precursors through F⁺/F⁻ addition initialized cascade reactions (Scheme 1c).⁴⁷⁻⁵² In 2020, Zhang and coworkers realized an asymmetric fluoroarylation of *gem*-difluoroalkenes by using their newly developed TY-Phos, which is heretofore the only asymmetric transformation of *gem*-difluoroalkenes.⁵³ Despite of the comprehensive application of *gem*-difluoroalkenes in the synthesis of structurally diverse fluoro-compounds, asymmetric cycloaddition of these substrates still remain blank.

In this work, an enantioselective 1,3-dipolar cycloaddition of azomethine ylides with *gem*-1,1-difluoroalkenes and 1,1,2-trifluoroalkenes was achieved by a Cu(I)/DTBM-Segphos catalyst. To achieve this transformation, two challenges shown in Scheme 1d must be overcome. First, the high activation barrier is a big

Figure 1 | Fluorine factors on biological activity.

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Previous work

(a) Synthesis of monofluoro-alkenes by nucleophilic addition-elimination reactions

(b) Synthesis of difluoro-compounds via nucleophilic addition reactions

(c) Synthesis of trifluoro-compounds through F-addition induced cascade reactions

This work:

(d) Synthesis of bioactive fluoropyrrolidines by asymmetric [3+2] cycloaddition

- Regioselective & Stereoselective
- Broad substrate scope (55 cases)
- High antifluor activities

Enantioenriched
3,3-Difluoropyrrolidines &
3,3,4-Trifluoropyrrolidines

Challenge I

Less reactive olefins
High activation barrier

Reactive olefins
Low activation barrier

Challenge II

F-elimination

Scheme 1 | Synthesis of fluorochemicals from difluoroalkenes.

difficulty, as known, 1,3-dipolar cycloaddition of azomethine ylide is generally limited to highly activated dipolarophiles bearing strong electron withdrawing substituents,²⁹⁻³⁴ only a few cases are associated with less reactive olefins. Martín and coworkers reported a Ag(I)-catalyzed asymmetric 1,3-dipolar cycloaddition of azomethine ylide with **C60**,⁵⁴ while the reaction with less activated alkenyl arenes was achieved by Carretero et. al.⁵⁵⁻⁵⁷ In spite of these rare examples, the reaction of inactive alkenes remains a great challenge in some sense. Besides, as shown scheme 1a and 1d, the elimination of fluorine atoms in intermediates and products is another big problem for these reactions.⁵⁸

Results and discussion

Reaction condition optimizations. In the initial studies, imino ester **1a** was chose as azomethine ylide precursor and 4-Cl-*gem*-difluoroalkene **2a** as dipolarophile. In the presence of Cu(CH₃CN)₄PF₆/(*S*)-BINAP complex (5 mol%) and KO^tBu (20 mol%) in toluene, the reaction did not happen at room temperature at all (Table 1, entry 1), when the reaction temperature raised to 60 °C, the desired product **3a** was obtained in only 18% yield after 48 h (Table 1, entry 2), these results revealed that the energy barrier of this transformation is extremely high. As known, high temperature often cause side reactions and poor stereoselectivity, however, when the reaction was carried out at 80 °C for 48 h, **3a** was obtained in 60% yield with > 20:1 dr and 60% ee (Table 1, entry 3). Encouraged by this result, we next tested other chiral diphosphine ligands, (*S*)-Segphos **L2** gave similar catalytic reactivity with (*S*)-BINAP (61% yield) but with poorer enantioselectivity (28% ee) (Table 1, entry 4). To our delight, (*S*)-DTBM-segphos **L3** performed very well in this reaction, giving desired product **3a** in 91% yield together with > 20:1 dr and 95% ee (Table 1, entry 5). As shown in Table 1, Lewis acids play an important role in this reaction, when anion was changed to BF₄⁻, yield decreased to 57% although stereoselectivities maintained at the same level (> 20:1 dr

Table 1 | Investigation of optimal reaction conditions.^a

Entry	[M] ^c	Ligand	Solvent	Base	Yield (%) ^d	Ee (%) ^e
1	CuPF ₆	L1	Toluene	KO ^t Bu	trace	- ^f
2	CuPF ₆	L1	Toluene	KO ^t Bu	18	- ^g
3	CuPF ₆	L1	Toluene	KO ^t Bu	60	60
4	CuPF ₆	L2	Toluene	KO ^t Bu	61	28
5	CuPF₆	L3	Toluene	KO^tBu	91	95
6	CuBF ₄	L3	Toluene	KO ^t Bu	57	92
7	CuOAc	L3	Toluene	KO ^t Bu	trace	-
8	Cu(OAc) ₂	L3	Toluene	KO ^t Bu	trace	-
9	AgOAc	L3	Toluene	KO ^t Bu	85	77
10	CuPF ₆	L3	THF	KO ^t Bu	81	95
11	CuPF ₆	L3	AcOEt	KO ^t Bu	61	94
12	CuPF ₆	L3	1,4-Dioxane	KO ^t Bu	48	91
13	CuPF ₆	L3	Toluene	K ₂ CO ₃	42	95
14	CuPF ₆	L3	Toluene	DIPEA	90	95

^a All reactions were carried out with 0.40 mmol of **1a** and 0.20 mmol of **2a** in 2 mL solvent. ^b Dr was determined by crude ¹H NMR. ^c CuPF₆ = Cu(CH₃CN)₄PF₆, CuBF₄ = Cu(CH₃CN)₄BF₄. ^d Isolated yield. ^e Ee was determined by chiral HPLC analysis. ^f The reaction was carried out at 25 °C for 48 h. ^g The reaction was carried out at 60 °C for 48 h.

and 92% ee) (Table 1, entry 6). Neither CuOAc nor Cu(OAc)₂ could make this reaction happen (Table 1, entries 7 and 8). Other metal salt such as AgOAc also worked in this transformation, however, providing the corresponding product **3a** with moderate enantioselectivity (Table 1, entry 9). With the best catalytic combination in hand, we next examined various common solvents in lab like tetrahydrofuran, ethyl acetate, and 1,4-dioxane, in which, both reactivities and stereoselectivities decreased of varying degrees (Table 1, entries 10-12). It seems that bases did not affect stereoselectivity control at all, but compared with KO^tBu or DIPEA (Table 1, entry 14), inorganic base K₂CO₃ lead to poor yield (42%) which may due to the poor solubility of K₂CO₃ in toluene (Table 1, entry 13). Through the experiments above, we finally confirmed the optimal reaction conditions: 5 mol% of Cu(CH₃CN)₄PF₆, 6 mol% of (*S*)-DTBM-segphos, 20 mol% of KO^tBu, 2 mL of toluene, under 80 °C.

Substrate scope of azomethine ylides and *gem*-difluoroalkenes. Then we investigated the reaction of various substituted imino esters **1** with **2a** under optimal conditions, and results are summa-

Table 2 | Substrate scope of imino esters **1 and gem-difluoroalkene **2**^a**

Reaction scheme showing the synthesis of 3-4 from 1 and 2:

1: $\text{MeO}_2\text{C}-\text{CH}=\text{N}-\text{Ar}^1$

2: $\text{Ar}^2-\text{CF}=\text{CF}_2$

Reaction conditions:

- $\text{Cu}(\text{CH}_3\text{CN})_4\text{PF}_6$ (5 mol%)
- (S)-DTBM-Segphos (6 mol%)
- KO^tBu (20 mol%)
- Toluene, 80 °C, 48 h

Product 3-4: Ar^1 -substituted 2,3-difluoro-4-methoxycarbonylpyrrolidine derivative.

Substrate scope of imino esters

Substrate scope of imino esters (3a-3r):

3a, 91% > 20:1 dr, 95% ee

3b, 66% > 20:1 dr, 97% ee

3c, 95% > 20:1 dr, 94% ee

3d, 88% > 20:1 dr, 97% ee

3e, 60% > 20:1 dr, 90% ee

3f, 71% > 20:1 dr, 94% ee

3g, 81% > 20:1 dr, 96% ee

3h, 79% > 20:1 dr, 97% ee

3i, 74% > 20:1 dr, 86% ee

3j, 96% > 20:1 dr, 97% ee

3k, 78% > 20:1 dr, 85% ee

3l, 81% > 20:1 dr, 94% ee

3m, 80% > 20:1 dr, 94% ee

3n, 83% > 20:1 dr, 89% ee

3o, 97% > 20:1 dr, 87% ee

3p, 54% > 20:1 dr, 71% ee

3q, 85% > 20:1 dr, 83% ee

3r, 56% > 20:1 dr, 90% ee

3s, 87% > 20:1 dr, 97% ee

3t, 68% > 20:1 dr, 79% ee

3u, 79% > 20:1 dr, 91% ee

X-ray of (2R, 4S, 5R)-3u

Substrate scope of gem-difluoroalkenes

Substrate scope of gem-difluoroalkenes (4a-4l):

4a, 85% > 20:1 dr, 90% ee

4b, 94% > 20:1 dr, 95% ee

4c, 96% > 20:1 dr, 97% ee

4d, 76% > 20:1 dr, 86% ee

4e, 81% > 20:1 dr, 95% ee

4f, 72% > 20:1 dr, 94% ee

4g, 59% > 20:1 dr, 94% ee

4h, 74% > 20:1 dr, 94% ee

4i, 57% > 20:1 dr, 92% ee

4j, 75% > 20:1 dr, 94% ee

4k, 91% > 20:1 dr, 81% ee

4l, 79% > 20:1 dr, 92% ee

^a All reactions were carried out with 0.40 mmol of **1** and 0.20 mmol of **2** in 2 mL toluene. Isolated yield, dr was determined by crude ¹H NMR and ee was determined by chiral HPLC.

rized in Table 2. Besides product **3a**, various substituted chiral difluoropyrrolidines can be prepared as one single diastereomer by this method. Steric effect of substituents did not influence the stereoselectivities, *ortho*- and *para*-Me substituted products **3b**-**3c** were obtained with excellent enantioselectivities (97%, 94% ee respectively), while reactivities were affected a lot, *ortho*-Me imino ester gave product **3b** only in 66% yield, while *para*-Me substituted substrate gave 95% yield. Fluoro-substituted products **3d**-**3f** were obtained with similar results, except *meta*-fluoro-product **3e** (60%).

Other halogen substituents like chloride or bromide also tolerant this reaction condition, providing corresponding products **3g** and **3h** in good yields (81%, 79%) and high enantioselectivities (96%, 97% ee). Biphenyl group containing derivative **3i** was created in 74% yield and with high enantioselectivity (86% ee). As shown in Table 2, substrates bearing strong electron withdrawing substituents like -CF₃, -CN, and -CO₂Me performed very well in this reaction, leading to the desired products **3j**-**3l** with good reactivities (78-96% yields) and stereoselectivities (> 20:1 dr, 85-

97% ee). While strong electron donating group -OMe showed no influence on this reaction (**3m**). Both 1-Naphthyl and 2-Naphthyl substituted difluoropyrrolidines **3n** and **3o** were readily prepared with satisfied results. Considering the important role of heterocycle units in biologically active compounds, we investigated four substrates containing furyl, theinyl, indolyl and triazolyl in this transformation to our delight, all of the corresponding products **3p**, **3q**, **3r** and **3s** were obtained in moderate to good yields (54-87%) and enantioselectivities (71-97% ee). We also tried an imino ester bearing ferrocene group (**3t**). The reaction worked although with moderate yield (68%) and enantioselectivity (79% ee). Finally, the absolute configuration of **3u** was determined to be (2*R*, 4*S*, 5*R*) by x-ray analysis. Encouraged by the cheering results of imino esters,

we next examined a series of substituted *gem*-difluoroalkenes. As mentioned before, normal aromatic alkenes could not be used in 1,3-dipolar cycloaddition of azomethine ylide, except that activated by strong electron withdrawing substituents on phenyl rings, however, due to the electron withdrawing inductive effect of fluorine atom, all substrates with electron rich, neutral and deficient substituents can be used in this reaction. Results are summarized in Table 2, product **4a** with bromide substituent was prepared with similar result to **3a** (85% yield, > 20:1 dr, 90% ee).

As expected, substrates with electron withdrawing groups such as -CF₃ and -CO₂Me performed very well in this reaction and provided corresponding products **4b** and **4c** in high yields (94%, 96%) and excellent enantioselectivities (95%, 97% ee). Diphenyl alkene can be used in this transformation although the enantioselectivity is somewhat lower (86% ee). It seems that steric effect does not affect stereoselective control in this reaction, for example, 1-Naphthyl and 2-Naphthyl substituted *gem*-difluoroalkenes showed similar results (81%, 95% ee and 72%, 94% ee respectively). Meanwhile, ortho-, meta- and para-methyl substituted products **4g-4i** could be created by this method with same enantioselectivities (94%, 94%, 92% ee) while in different yields (59%, 74%, 57%). Substrate with strong electron donating group -OMe went smoothly and gave product **4j** with > 20:1 dr and 94% ee despite of moderate yield (75%). Meanwhile, we synthesized two *gem*-difluoroalkenes bearing 1-triazolyl and 6-indolyl substituents and tested their applicability in this

Table 3 | Modification of natural compounds and pharmaceuticals.^a

^a All reactions were carried out with 0.40 mmol of **1** and 0.20 mmol of **2** in 2 mL toluene. ^b The reactions were carried out at 60 °C for 96 h.

Table 4 | Synthesis of trifluoropyrrolidine derivatives from trifluoroalkenes.^a

^a All reactions were carried out with 0.40 mmol of **1** and 0.20 mmol of **6** in 2 mL toluene. ^b The reactions were carried out at 60 °C for 96 h.

transformation, to our delight, the desired products **4k** and **4l** were prepared with satisfied results.

Gem-difluorodiene **2m** derived from cinnamaldehyde also participated in this reaction, giving product **4m** bearing styryl group at 4-position with > 20:1 dr and 88% ee although in moderate yield (53%). Similar result also obtained with *gem*-difluoroalkyne **2n** as fluorinating precursor to access the corresponding product **4n** with an alkynyl group. (Scheme 2)

Modification of natural compounds and drugs. Additionally, since the modification of biologically active compounds is an important method for the development of agrochemicals and pharmaceuticals, the introduction of certain units into biologically active molecules is of great significance. Therefore, we further demonstrate the broad applicability of the asymmetric transformation protocol by examination of imino esters derived from natural compounds and currently existed synthetic drugs. As shown in Table 3, the desired product **5a** bearing cholesterol unit could be readily prepared in 72% yield and with > 20:1 dr under the optimized reaction conditions, similarly, androsterone derived imino ester could participate in this reaction to access adduct **5b**, notably, their diastereomers **5a'** and **5b'** could also be prepared in high yield and stereoselectivity by using Cu(I)/(*R*)-DTBM-segphos as catalyst. Pitavastatin, which is an important building skeleton in drugs, could also be employed in this reaction to give **5c** in good yield (78%) and with excellent stereoselectivity (> 20:1 dr and 91% ee). Meanwhile, the well-known commercial analgesic indomethacin has been modified by this method, leading to the corresponding derivative **5d** in moderate yield (47%) together with excellent stereoselectivity (> 20:1 dr and 87% ee). Encouraged by these results, we next tested saccharide derivatives which are important components in medicines while contain multiple sensitive functional groups. The asymmetric cycloaddition of imino esters derived from fructose and glucose were readily achieved, leading to the corresponding products **5e-5g** respectively in high yields (69-78%) and stereoselectivities (18:1 - >20:1 dr). Finally, L-menthol is no doubt one of the most important chiral precursors for the synthesis of agrochemicals and pharmaceuticals, in this work, we combined this unit with difluoro-pyrrolidinyl motif easily to give product **5h** in 79% yield and > 20:1 dr.

1,3-Dipolar cycloaddition of 1,1,2-trifluoroalkenes. Besides 3,3-difluoropyrrolidines, chiral 3,3,4-trifluoropyrrolidinyl derivatives were also prepared by using 1,1,2-trifluoroalkenes as substrates in this transformation. As shown in table 4, various substituted 1,1,2-trifluorostyrenes could readily participate in this reaction to access corresponding chiral 3,3,4-trifluoro pyrrolidine derivatives. All simply structured imino esters and trifluoroalkenes can be applied as compatible reactants, leading to desired 3,3,4-trifluoropyrrolidines **7a-7f** in moderate to good yields (59-82%) and with excellent stereoselectivities (> 20:1 dr and 72-96% ee). Meanwhile, similar to the synthesis of 3,3-difluoropyrrolidines, we introduced these unique structural motifs into some kinds of biologically active natural compounds such as glucose (**7g**), fructose (**7h**), L-menthol (**7i**) and androsterone (**7j**), it needs to be mentioned that these reactions should be conducted at 60 °C to prevent decomposition of the obtained products.

Scale-up reactions. To test the practicability of this methodology, gram-scale synthesis of **3u** and **7b** were examined under the optical conditions (Scheme 3). The reaction of 7.5 mmol of imino ester **1g** with 5 mmol of *gem*-difluoroalkene **2b** performed very well and gave 1.66 g of product **3u** in 77% yield, and with high stereoselectivity (> 20:1 dr and 91% ee), while 1.45 g of 3,3,4-trifluoro pyrrolidine **7b** can be prepared by the reaction of 7.5 mmol of imino ester **1g** with 5 mmol of 1,1,2-trifluoroalkene **6a**.

Scheme 3 | Gram-scale reactions.

Table 5 | Investigation of “fluorine” influence on antifungal activities.

Antifungal activities evaluation. With these unique fluorinated compounds in hands, we next tested their antifungal activities. In this part, four common fungi *Sclerotinia sclerotiorum*, *Pestalotiopsis*, *Brown-rot fungi* and *Rhizoctonia solani* as experimentally inhibitory subjects. Meanwhile, two popular and commercial antifungal insecticides hymexazol, azoxystrobin as contraposition. As shown in Table S1, most 3,3-difluorinated products showed general antifungal activities against four fungi, however, in contrast, most 3,3,4-trifluoro-compounds gave poorer results. Among all biologically active difluoropyrrolidines, products **3f**, **3k**, **3q**, **4b**, **4k** provided similar and even better antifungal inhibition effects in compare with hymexazol and azoxystrobin (Table 5). It seems that additional fluorine groups contribute a lot to the antifungal inhibition. Product **3f** with an additional fluorine atom at the *para*-position of phenyl group gave 84.60%, 80.37%, 74.49%, 74.65% inhibition rate against *Sclerotinia sclerotiorum*, *Pestalotiopsis*, *Brown-rot fungi* and *Rhizoctonia solani* respectively, and similarly, compound **4b** which has a trifluoromethyl (CF₃) substituent also showed great results (85.66%, 85.03%, 70.62%, 79.86%). Meanwhile, product **3k** bearing a cyano-group (CN) also performed very well towards all fungi (90.26%, 69.09%, 83.64%, 73.78%). Heterocycles, which are important building blocks in agricultural compounds, often provided key contribution to the bioactivity, for example, thienyl substituted product **3q** can inhibit these fungi effectively (82.83%, 86.39%, 68.46%, 79.32%), and the same results were obtained by compound **4k** with 1,2,4-triazolyl unit (92.65%, 70.59%, 85.05%, 78.38%). These experimental results reveal that

the combination of certain functional groups is necessary to enhance the biological activities.

As mentioned in the beginning, fluorine factors plays an crucial role in biological activity, therefore, in order to confirm the contribution of fluorine atoms in these structures on the antifungal inhibitory reactivity, a series of corresponding chiral pyrrolidinyl derivatives **3f'**, **3k'**, **3q'**, **4b'**, **4k'** without fluorine atoms at the heterocyclic rings were readily prepared according to J. C. Carretero's methods.⁵⁵ Antifungal inhibition results of these contrapositive compounds were summarized in Table 5 and Table S2, the antifungal activities between fluorinated compounds and their contrasts are quite different, as excepted, all the contrapositive samples gave poor results in antifungal experiments. **3f'** showed only about half of the inhibition rate of **3f** against *Sclerotinia sclerotiorum*, *Pestalotiopsis* and *Brown-rot fungi*, *Rhizoctonia solani* (48.38%, 49.34%, 38.44%, 41.62% respectively). **3k'** and **3q'** lead to even lower biological reactivities (32.06%, 43.38%, 38.44%, 28.24% and 38.09%, 54.83%, 37.81%, 55.44%). Compound **4b'** bearing a trifluoromethyl (CF₃) group also provided moderate inhibition rate towards the four fungi (51.18%, 46.36%, 34.06%, 33.53%). Furthermore, the none-fluorinated triazolyl-substituted contrast **4k'** showed almost no effect on the antifungal inhibition (13.24%, 22.68%, 14.02%, 27.65%). Through these control experiments, we can see that the two fluorine atoms at the pyrrolidine ring play a crucial role in their biological activities.

Meanwhile, antifungal experiments of racemic samples were carried out under same conditions, as shown in Table S2, (±)-**3f**,

Table 6 | Inhibitory effect on *Sclerotinia sclerotiorum* plant pathogenic fungi under different concentration.

C/mg·L ⁻¹	6.25	12.50	25.00	50.00	100.00
3f	40.00	44.56	64.41	83.24	84.60
3k	38.53	50.15	69.26	78.82	90.26
3q	0.00	28.68	62.35	71.62	82.83
4b	56.00	63.97	81.47	82.50	85.66
4k	0.00	13.18	47.95	70.62	92.65
Hymexazol	59.33	59.18	65.14	83.61	100.00
Azoxystrobin	45.32	55.30	92.55	95.38	92.56

(±)-**3q** and (±)-**4b** gave slight poor results compare with chiral samples, but (±)-**3k** and (±)-**4k** showed very poor antifungal inhibition. This revealed that stereoselectivity is also a key factor on biologically activity.

Considering the requirement for low pesticide dosage in modern practical application, we finally examined the antifungal inhibition effects of these active compounds under different concentrations (6.25, 12.50, 25.00, 50.00, 100.00 mg·L⁻¹) with *Sclerotinia sclerotiorum* as research subject. As shown in Scheme 4, all inhibition rates decreased as the concentration reduced from 100.00 to 6.25 mg·L⁻¹, but their rates of decline are different. The inhibition effects of **3q** and **4k** drop sharply and no effects was observed with 6.25 mg·L⁻¹ concentration, while that of **3f** and **3k** declined slower and still possess similar inhibition rates with commercial pesticides Hymexazol and Azoxystrobin at 6.25 mg·L⁻¹. The best result was obtained by **4b**, whose antifungal effect maintained at the same level from 100.00 to 25.00 mg·L⁻¹, and gave 56% inhibition rate even with 6.25 mg·L⁻¹ concentration. This result illustrate that this structure has some kind of potential application in agricultural compound creation.

Conclusion

In conclusion, we achieved an asymmetric 1,3-dipolar cycloaddition of azomethine ylides with less active 1,1-*gem*-difluoroalkenes and 1,1,2-trifluoroalkenes, leading to a series of novel chiral 3,3-difluoro- and 3,3,4-trifluoropyrrolidinyl derivatives. This methodology features with high yields (up to 96% yield), stereoselectivity (up to >20:1 dr and 97% ee) and broad substrate scope (55 cases). Biological activity of these newly prepared compounds has been evaluated *via* antifungal experiments, some of these fluorinated pyrrolidines performed very well in antifungal experiments against *Sclerotinia sclerotiorum*, *Pestalotiopsis*, *Brown-rot fungi* and *Rhizoctonia solani*. Control experiments proved that the fluorine atoms on heterocyclic rings are crucial to the biological activity. This work not only developed a facile route for the synthesis of novel biologically active fluorinated pyrrolidinyl derivatives, but also provide a direct evidence for the impact of fluorine atom on the antifungal activity. We believe this research work will give some elicitation for bioactive molecules creation.

Experimental section

General procedure for the synthesis of 3,3-difluoro- and 3,3,4-trifluoropyrrolidines. Under N₂ atmosphere, Cu(CH₃CN)₄PF₆ (3.7 mg, 0.01 mmol) and (*S*)-DTBM-segphos (14.2 mg, 0.012 mmol) were dissolved in 2.0 mL of toluene and stirred at room temperature for 1 h. Then, KO^tBu (0.04 mmol, 0.2 eq.), imino esters **1** (0.4 mmol, 2.0 eq.), 1,1-*gem*-difluoroalkenes **2** or 1,1,2-trifluoroalkenes **6** (0.2 mmol, 1.0 eq.) were added successively, the reaction was kept at 80 °C for 48 h. After completion determined by TLC, solvent was removed under vacuum and diastereoselectivity was determined by crude ¹H NMR, then the reaction mixture was purified by flash chromatography on silica gel to give the corresponding fluorinated pyrrolidines, whose enantiomeric excess was analyzed directly by chiral HPLC.

Methods for antifungal activity investigation. All tested compounds (10 mg) were dissolved in 1.0 mL acetone, then the solution was added to 100 mL of Potato Dextrose Agar medium to prepare a drug-loaded PDA medium plate having a concentration of 100 mg·L⁻¹, and 1.0 mL acetone was added to the PDA as a blank sample. Commercial pesticides azoxystrobin and hymexazol were chose as compared positive fungicides samples. *Sclerotinia sclerotiorum*, *Pestalotiopsis*, *Brown-rot fungi* and *Rhizoctonia solani* were activated beforehand (prepared by a punch with Φ=0.50 cm), and then they were cut from the edge of the colony and inoculated into the above PDA plate, each strain and blank control experiment were repeated twice. After sealed with a sealing film to prevent contamination of other bacteria, the poisonous PDA culture dishes were placed in a constant temperature incubator at the temperature condition of 24±1 °C. After the blank control group culture was grown to about two-thirds of the culture dish, the colony diameter was measured by the cross method in millimeters (mm) with a cross and a vertical method, and the average value was taken, and the inhibition rate of each compound against the fungus was calculated.

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Supplementary Information

Available in the online version of the paper, experimental procedures, antifungal experimental results, characterization data, ¹H NMR, ¹³C NMR, and HPLC spectra (PDF)..

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X. X. developed the synthetic methods. L. B. performed the antifungal activity investigations. L. R and H. T. participated in the synthetic work. D. Y. took charge in the characterization of compounds. C.-J. W. and H. T. wrote the manuscript with revisions provided by the other authors.

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