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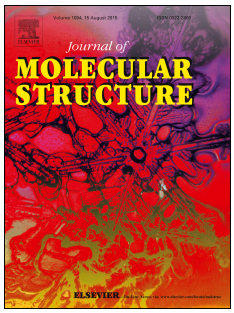

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PII: S0022-2860(18)30751-8

DOI: 10.1016/j.molstruc.2018.06.050

Reference: MOLSTR 25337

To appear in: *Journal of Molecular Structure*

Received Date: 9 April 2018

Revised Date: 11 June 2018

Accepted Date: 11 June 2018

Please cite this article as: H. Wang, Z.-W. Zhai, Y.-X. Shi, C.-X. Tan, J.-Q. Weng, L. Han, B.-J. Li, X.-H. Liu, Novel trifluoromethylpyrazole acyl urea derivatives: Synthesis, crystal structure, fungicidal activity and docking study, *Journal of Molecular Structure* (2018), doi: 10.1016/j.molstruc.2018.06.050.

Novel Trifluoromethylpyrazole Acyl Urea Derivatives: Synthesis, Crystal Structure, Fungicidal Activity and Docking Study

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Abstract Thirteen novel trifluoromethylpyrazole acyl urea derivatives were designed and synthesized. *In vivo* fungicidal activities of these compounds were tested against *Fusarium oxysporum*, *Corynespora mazei*, *Botrytis cinerea* and *Pseudomonas syringae* respectively, particularly compounds exhibited significant control effective at 100 mg/L. More importantly, some compounds showed the good control effective at 10 mg/L. Furthermore, docking was established to study the structure-activity relationship of the title compounds. It is possible that trifluoromethylpyrazole acyl urea derivatives, which possess good control effective against *Fusarium oxysporum*, *Corynespora mazei* and *Botrytis cinerea*, may become novel lead compounds for the development of fungicides with further structure modification.

Keywords: trifluoromethylpyrazole • urea • fungicidal activity • docking • synthesis

Introduction

Pesticide is an effective way to protect crops and increase yields in agriculture[1], within which fungicides play a very important role in modern agriculture. But the fungicide-resistance problem has appeared in recent years[2]. It is urgent to develop novel active compounds with lower doses, environmental friendly and high effective in order to overcome the resistance of fungal[3]. In the past half century, since the found of the first fungicide carboxin target succinate dehydrogenase (SDH), many SDHI fungicides had been introduced into the market for effective treatment of fruit and vegetable crops[4,5], such as furametpyr, penthiopyrad, isopyrazam, sedaxane, bixafen, fluxapyroxad, pydiflumetofen, benzovindiflupyr and so on (Figure 1). Among them, many of these fungicides contain pyrazole ring with F, CHF_2 or CF_3 moiety. Also carboxamide is a main key group for SDHI fungicides[6,7]. Recent years, the carbon chain was introduced between the carboxamide and aromatic ring in most of the commercial SDHI fungicides.

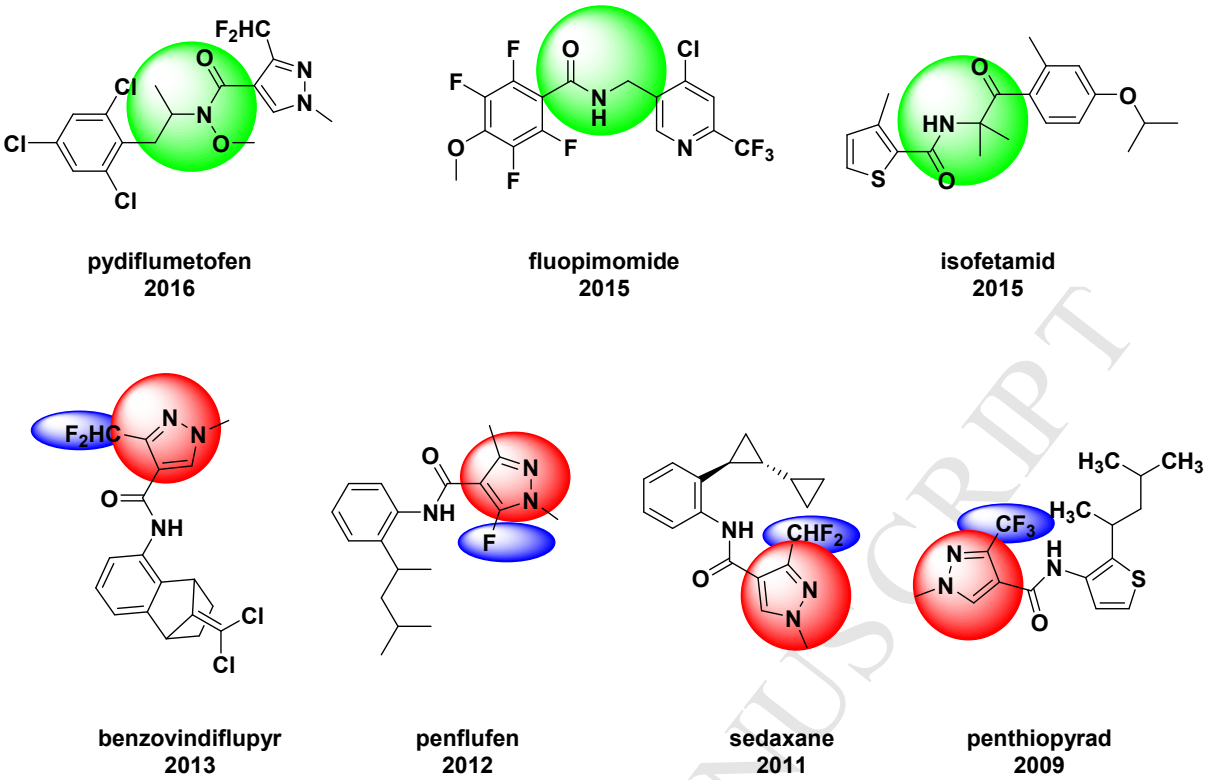


Figure 1 The representative SDH inhibitors

In our previous work, to find high active fungicides with efficacy, lower toxicity, broad spectrum and environmental friendly, we have been devoted to synthesizing heterocyclic derivatives and researching their pesticidal activity[8-29], especially pyrazole ring with antifungal[6] and nematocidal activity[30-37]. The title trifluoromethylpyrazole derivatives had been designed by introducing urea group between the trifluoromethylpyrazole ring and benzene ring in order to find high active fungicidal compounds (Figure 2). Surprisingly, all these compounds possessed good fungicidal activity, especially against *Botrytis cinerea*.

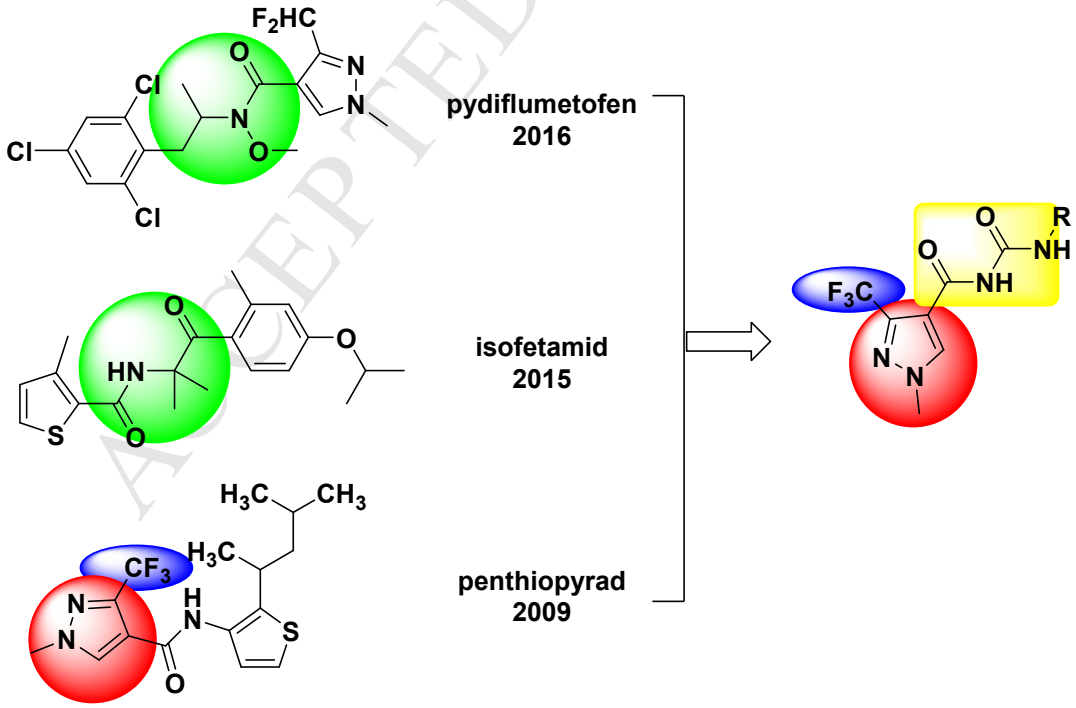


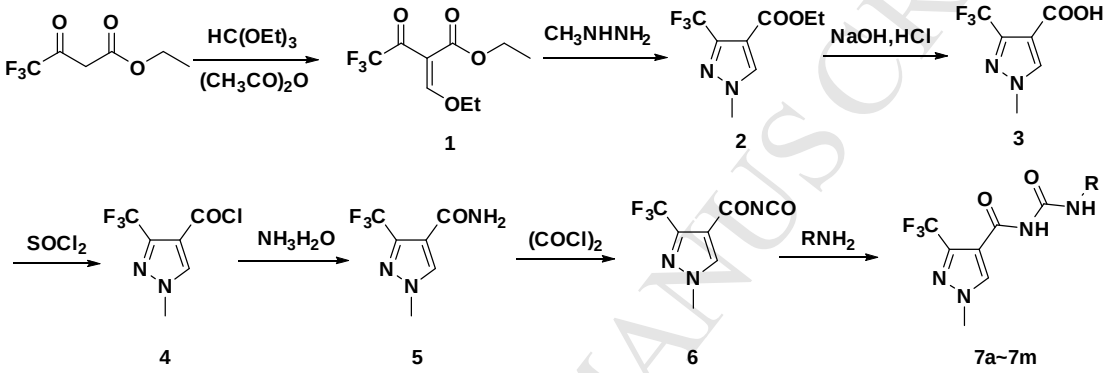
Figure 2 Design strategy of the title compounds.

In this study, we reported for the firstly trifluoromethylpyrazole acyl urea derivatives and their *in vivo* fungicidal activities. We also discussed the relationship between structure and fungicidal activity preliminarily.

Results and Discussion

Synthesis and Spectrum

The synthetic route of key intermediate 1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carbonyl chloride is outlined in Scheme **1**. The synthetic route of title compounds is outlined in Scheme **2** and Scheme **3**. Tifluoromethyl-1*H*-pyrazole is an important skeleton in many pesticides. Many reference reported the synthetic methods of 3-(tifluoromethyl)-1-methyl-1*H*-pyrazole- 4-carboxylic acid, such as α,β-unsaturated ketones, 1,3-diketones, β-keto esters, perfluoro acetylenes. Among them, the α,β-unsaturated ketone method was applied, which cyclized from the starting material 2,2,2-tifluoroethyl 2-(ethoxymethyl)-4,4-difluoro-3-oxobutanoate and methylhydrazine under base condition. In this step, the reaction temperature is below 60 ^{o}C. While the temperature increase, the yield decrease. Then the key intermediate 1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carbonyl chloride was obtained by hydrolysis and acyl chlorination. Finally, the acyl isocyanate reacted with substituted aniline at room temperature. The target compounds were given easily with less by-products under this mild condition.



Scheme 1 The synthetic route of title compounds

All the compounds were identified and characterized by FTIR, ^{1}H NMR and HRMS. The infrared spectrum of acyl urea derivatives **7** showed absorption bands around at 3200 and 3100 cm^{-1} for N-H stretching. The characteristic stretching vibrations ν (C=O) appears at 1700 and 1690 cm^{-1} respectively. In the ^{1}H NMR spectra of target compounds, all the -NH proton signals of the title compounds can be found around 9~13 ppm. The appearance of signals at ~8.0 ppm are assigned to CH of pyrazole ring. The signal of methyl group of pyrazole is assigned at about 4.0 ppm. Meanwhile, most of the title compounds exhibited the M+H^{+} peak in the ESI-MS results.

Crystal

The structure of compound **7d** was further confirmed by single crystal X-ray diffraction analysis (Figure **3**). The crystallographic data of compound 7d and selected bond lengths (Å) and bond angles (°) for compound 7d are given in Table 1 and Table 2 respectively. In the molecular structure of title compound, the pyrazole ring nearly paralleled with the benzene ring with the angle of 4.2 º. The title compound has an extensive two dimension polymer of hydrogen bonding involving the atom O. Also it exist intermolecular hydrogen bond in the molecule (N4-H4—O1)(see Supplementary material). The average bond lengths and bond angles of the phenyl ring[38], the pyrazole ring[39], acyl urea group[40] are normal.

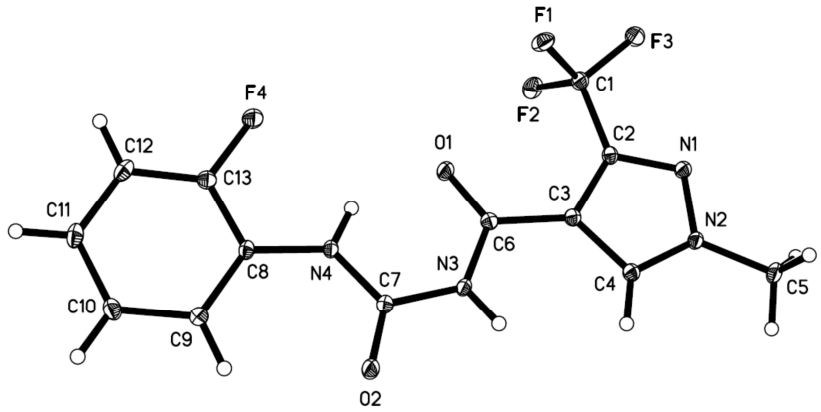


Figure 3 The crystal structure of high active compound 7d

Table 1 Crystal Structure and Data Refinement Parameters

Compound	7d
Empirical Formula	$C_{13}H_{10}F_4N_4O_2$
Color/shape	colorless / rectangle
Formula Weight	330.25
Crystal System / Space Group	Monoclinic, P2(1)/c
a / Å	4.6181(11)
b / Å	18.857(4)
c / Å	15.180(4)
α / °	90
β / °	97.672(8)
γ / °	90
V / $Å^3$	1310.1(5)
Z	4
D_{calc} (g/cm^3)	1.674
μ (mm^{-1})	0.154
Crystal size (mm)	0.2 × 0.18 × 0.12
F(000)	672
Temp (K)	113(2)
Theta range for collection	3.46-27.53°
Reflections collected	16507
Independent reflections	2977
Data/restraints/parameters	2977 / 2 / 215
Goodness of fit on F^2	1.020
Final R indices [I > 2σ(I)]	R1 = 0.0313, wR2 = 0.0782
R indices (all data)	R1 = 0.0424, wR2 = 0.0834
Largest difference peak/hole	0.309 and -0.273

Table 2. Selected Bond lengths (Å), Selected Bond angles (°) for the Compound 7d.

Bond	Dist.	Angle	(°)
F(1)-C(1)	1.3406(16)	C(2)-N(1)-N(2)	104.70(10)
F(2)-C(1)	1.3432(15)	C(4)-N(2)-N(1)	112.61(10)
F(3)-C(1)	1.3363(15)	C(4)-N(2)-C(5)	128.30(11)
O(1)-C(6)	1.2247(15)	N(1)-N(2)-C(5)	118.95(10)
O(2)-C(7)	1.2333(15)	C(6)-N(3)-C(7)	127.50(11)
N(2)-C(4)	1.3448(16)	O(1)-C(6)-C(3)	121.16(11)
N(3)-C(7)	1.4044(16)	O(2)-C(7)-N(4)	125.56(12)
N(4)-C(7)	1.3412(16)	C(9)-C(8)-C(13)	117.57(11)
N(4)-C(8)	1.4106(15)	F(4)-C(13)-C(12)	119.51(11)
C(1)-C(2)	1.4976(18)	C(12)-C(13)-C(8)	123.45(12)
C(2)-C(3)	1.4162(17)	N(4)-C(7)-N(3)	115.69(11)

Greenhouse in vivo fungicidal activity and SAR

The *in vivo* fungicidal activity of compounds **7a~7m** against *Fusarium oxysporum*, *Corynespora mazei* and *Botrytis cinerea* in greenhouse at different concentration are listed in Table **3**. Fluxapyroxad was used as control. Meanwhile, all of these tested compounds were found safe for the cucumber plants. As an initial evaluation, all of the title compounds were tested at a concentration of 100 μg/mL against *Fusarium oxysporum*, *Corynespora mazei* and *Botrytis cinerea*. As shown in Table **3**, most of the title compounds were found to exhibit good control effect against *Botrytis cinerea*. For example, acyl urea compound **7a** (88.55±2.26), **7c** (82.42±2.32), **7d** (96.13±3.16), **7i** (94.37±3.11), **7k** (86.18±2.85) displayed >80% control effect against *Botrytis cinerea* respectively, which is better than that of control. All of the title compounds possessed good control effect (>50%) against *Botrytis cinerea* except compound **7f, 7g, 7h**. It can be seen that substitution on the benzene ring can greatly influence the fungicidal activity against *Botrytis cinerea*. When the substitution is 2,6-2F, 2,5-2F or 2,3,4-3F in urea

7, there is an apparent decrease of fungicidal activity. For *Corynespora mazei*, acyl urea compound **7a**, **7d**, **7l**, **7m** possessed efficacy rates 70.62±3.05, 71.10±2.85, 70.01±2.30, 77.97±2.04, 83.07±2.63, and 74.29±2.33% against *Corynespora mazei*, respectively. All of the three compounds were more effective than that of control. For this fungi, when compounds with an electron-donating group in the benzene ring will affect higher fungicidal activities than those of others. However, the large electron-donating group in the benzene ring of title compounds is favorable. Compounds **7a** and **7m** displayed moderate efficacy rates against *Corynespora mazei*, while all of them were less effective than all of the contrasts. It was found that compounds with multi substituted in the benzene ring have a higher level of fungicidal activity than others. Unfortunately, most of the compounds had low fungicidal activities against *Fusarium oxysporum*.

CK(Water) against *B. cinerea* The control efficacy of **7d** against *B. cinerea* at 100 mg/L

CK(Water) against *C. mazei* The control efficacy of **7m** against *C. mazei* at 100 mg/L

Figure 4 The control efficacy against *B. cinerea* and *C. mazei* of compound **7d** and **7m**

On the basis of the preliminary *in vivo* fungicidal results, the title compounds were selected for further bioassay(>50% inhibitory) at lower dose (50 μg/mL and 10 μg/mL) for fungicidal activity. The subsequent results in Table **4** and Table **5** showed that the most of tested compounds had good fungicidal activity against *Botrytis cinerea* with around 80% control efficacy at 10 μg/mL. For example, acyl urea compound **7d** (87.35±2.35, shown in Figure **4**), **7j** (83.35±2.36), **7k** (93.91±3.11), **7l** (86.12±3.01) displayed >80% control efficacy against *Botrytis cinerea* at 10 mg/L respectively, which is same as that of control. As the concentration decreased, but the fungicidal activity of these compounds (**7d, 7k** and **7l**) was slightly affected. For the fungal *Fusarium oxysporum*, however, it was found that the inhibitory activities decreased very quickly with the decreasing of the treatment concentration. All of them held no inhibitory at these two concentrations, even some of them can increase the fungal growth.

Table 3 The fungicidal activity of title compounds against three fungus at 100 mg/L

No	R	*Fusarium oxysporum*	*Botrytis cinerea*	*Corynespora mazei*
7a	3,5-2Me	5.19±0.29	88.55±2.26	70.62±3.05
7b	2-MeO	12.99±1.02	60.97±2.77	45.22±0.88
7c	2,4,6-3M e	7.79±0.85	82.42±2.32	12.78±0.46
7d	2-F	16.88±0.13	96.13±3.16	71.10±2.85
7e	3,4-2F	0	60.30±2.47	40.02±1.24
7f	2,6-2F	10.39±0.91	46.76±2.62	48.08±2.24
7g	2,5-2F	10.39±0.22	35.32±2.11	36.65±2.09
7h	2,3,4-3F	18.18±0.71	39.88±2.62	17.99±1.47
7i	3-F	16.88±0.62	94.37±3.11	54.16±2.31
7j	2-CF_3	11.69±0.47	75.83±2.85	54.40±2.15
7k	2-Cl	-1.30±0.02	86.18±2.85	11.32±0.22
7l	4-Et	7.79±0.62	62.61±2.22	70.01±2.30

7m	2,6-2Et	27.27±1.12	52.43±2.06	77.97±2.04
Control	Fluxapyroxad	38.33±0.15	83.87±0.23	88.33±1.05
CK	water	0	0	0

Table 4 The fungicidal activity of some compounds against *Botrytis cinerea* and *Corynespora mazei* at 50 mg/L

No	R	*Botrytis cinerea*	*Corynespora mazei*
7a	3,5-2Me	52.50±2.85	43.69±1.02
7b	2-MeO	84.35±3.44	-
7c	2,4,6-3M e	81.73±2.55	-
7d	2-F	62.05±2.24	65.49±2.65
7e	3,4-2F	25.22±1.95	-
7i	3-F	76.98±2.05	38.52±2.04
7j	2-CF_3	87.80±3.01	41.86±2.06
7k	2-Cl	87.44±2.65	-
7l	4-Et	69.88±2.85	72.46±2.00
7m	2,6-2Et	43.29±1.74	64.81±1.85
Control	Fluxapyroxad	83.87±0.23	74.06±1.01
CK	water	0	0

Table 5 The fungicidal activity of some compounds against *Botrytis cinerea* and *Corynespora mazei* at 10 mg/L

No	R	*Botrytis cinerea*	*Corynespora mazei*
7a	3,5-2Me	65.61±3.01	64.81±1.63
7b	2-MeO	70.06±3.01	-
7c	2,4,6-3M e	50.31±2.06	-
7d	2-F	87.35±2.35	79.28±3.03
7i	3-F	68.25±3.11	-
7j	2-CF_3	83.35±2.36	-
7k	2-Cl	93.91±3.11	-
7l	4-Et	86.12±3.01	62.05±1.14
7m	2,6-2Et	-	81.64±2.39
Control	Fluxapyroxad	64.16±0.25	64.56±1.02
CK	water	0	0

Docking Study

In order to study the action mode of high active compound and the target, the binding modes between SDH (PDB:2FBW) and the active compound **7d** were selected as exemplified in the case of representative compound by using the Discovery studio. The compound **7d** can tightly occupy binding site of SDH, the docking results is shown in Figure **5**. From the docking results, the compound **7d** held two hydrogen bonds. The O atom and F atom of NHCO group in pyrazole ring of **7d** can form tightly interaction with Tyr58 and Trp173 in hinge domain of SDH via two hydrogen bonds, respectively. The results indicated that the key active group is trifluoromethyl group and acyl amide group.

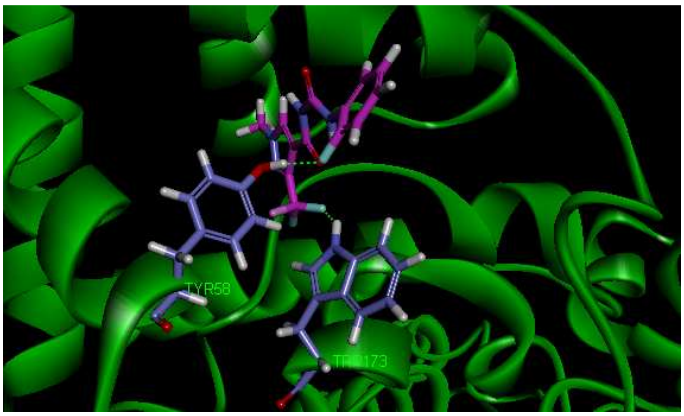


Figure **5** The docking mode of compound **7d** and the SDH

Conclusions

In summary, a series of novel pyrazole acyl urea derivatives were designed and synthesized, and their fungicidal activity against four species of fungals was evaluated. The title compound **7d** possessed high in vivo activity against *B. cinerea* with > 80 % control efficacy at 10 mg/L. Furthermore, the combination of DOCK provided meaningful clues as to the structural features of these new family herbicides that will be helpful in the design of more potent compounds in the future.

Experimental Section

General

Melting points were determined by an X-4 apparatus and uncorrected. ^{1}H NMR spectra were measured on a Bruker AV-400 or 500 MHz instrument using TMS as an internal standard and $CDCl_3$ or DMSO-d_6 as the solvent. FT IR spectra were measured on a NICOLET-670 instrument using KBr. HRMS was determined on a JOEL AccuTOF (JMS-T 100LC) instrument equipped with electrospray ionization (ESI) ion source.

The raw materials of trifluoroacetoacetate ethyl-(4,4,4-trifluoro)-3-oxobutyrate, triethyl orthoformate and methyl hydrazine were purchased from Nanjing Duodian Co. Ltd., acetic anhydride, ethyl acetate, potassium hydroxide, $SOCl_2$, aqueous ammonia, 1,2-dichloroethane, oxalyl chloride and other routine solvents were purchased by Shanghai Chemical Reagent Co. Ltd. and used without any purification. The course of the reactions was monitored by TLC; analytical TLC was performed on silica gel GF_{254}.

Experimental

Ethyl 2-(ethoxymethylene)-4,4,4-trifluoro-3-oxobutanoate (**1**).

In a 500 mL four-necked flask, trifluoroacetoacetate ethyl-(4,4,4-trifluoro)-3-oxobutyrate (18400 mg, 100 mmol), triethyl orthoformate (44400 mg, 300 mmol) and acetic anhydride (61200 mg, 600 mmol) were added and heated at 120 °C for 6 hours. Then, the solvent was removed and the product was then distilled under vacuum over a column to give a colorless liquid, yield 90%.

ethyl 1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxylate (**2**).

To a solution of methyl hydrazine (1900 mg, 41 mmol) in ethyl acetate, was added 2-ethoxy methylene 4,4,4-trifluoroacetoethylacetate (9700 mg, 41 mmol) dropwisely. The mixture was stirred under 5 °C for 1h, then further stirred at reflux temperature for 3 hours. After the reaction is completed, the mixture was cooled and concentrated. The residue was recrystallized with yield 69%, m. p. 55~56 °C. ^{1}H NMR ($CDCl_3$, 500 MHz), δ: 1.35 (t, 3H, *J*=7.32 Hz, CH_3), 3.97 (s, 3H, CH_3), 4.31(m, 2H, CH_2), 7.92 (s, 1H, CH).

1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid (**3**)

ethyl 1-Methyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxylate **2** (5600 mg, 25 mmol) and potassium hydroxide (4740 mg, 30 mmol) were added to water (50 mL). The mixture was stirred for overnight, then acidified to pH = 1 using HCl, to give 3 as a white solid that was filtered off and dried with yield 59%, m.p. 195~196 °C, ^{1}H NMR (500 MHz, DMSO-d_6), δ: 12.80 (s, 1H, COOH), 8.44 (s, 1H, CH), 3.93 (s, 1H, CH_3).

1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carbonyl chloride (**4**)

To 1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid **3** (1500 mg, 7.5 mmol) and $SOCl_2$ (3500 mg, 30 mmol) was refluxed for 4 h. After the reaction is completed, the excess of $SOCl_2$ was evaporated to give **4** as a yellow liquid that was used without further purification.

1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (**5**)

The resulting 1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carbonyl chloride was dissolved in 50 mL of dry THF and added to a mixture of aqueous ammonia (25%, 2720 mg, 40 mmol) at 0 °C. After stirring overnight, a large amount of solid formed and was filtered, which was further purified by a silica gel column eluted with petroleum ether and ethyl acetate to give the 1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide, white solid; yield 91%; m.p. 148~149 °C. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 3.99 (s, 1H, CH_3), 8.41 (s, 1H, CH), 7.65 (s, 2H, NH).

1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carbonyl isocyanate (6)

1-Methyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (1930 mg, 10 mmol) and anhydrous 1,2-dichloroethane(8 mL) were added into a three-neck flask and cooled to 0-5 °C with ice water. Oxalyl chloride(2520 mg, 20 mmol) was added slowly to this mixture. The reaction mixture was stirred for 1 h at room temperature, then kept at 50-55 °C for about 3 h, further heated to 75 °C and refluxed for 6 h. Unreacted oxalyl chloride was evaporated under reduced pressure to leave, the 1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carbonyl isocyanate as an oily solution, which was reserved for subsequent reaction.

General Synthetic Procedure for urea 7a~7n

A 10 mL round bottom was charged with 1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carbonyl isocyanate 6 (219 mg, 1 mmol) with various substituted aniline (1 mmol) in CH_2Cl_2 (4 mL). The mixture was stirred at room temperature and the correspondence product was given. The target compounds were filtered and crude solids were recrystallized from ethanol to give the title compounds **7a~7n**.

N-((3,5-dimethylphenyl)carbamoyl)-1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide**(7a)**, White solid, yield 59.7%, m.p. 209~210°C; FTIR(ν, cm^{-1}): 3242 (N-H), 3131 (N-H), 1701 (C=O), 1692 (C=O), 1614 (Ar), 1569 (Ph,C=C), 1541 (C-N), 1509 (Ph, C=C), 1475 (Ph, C=C), 1298 (C-N), 1229 (C-N), 1000-1200 (C-F), 771 (Ar-H); ^{1}HNMR ($CDCl_3$, 400MHz), δ: 2.26(s, 6H, $2CH_3$), 3.98(s, 3H, CH_3), 6.75(s, 1H, Ph), 7.20(s, 2H, Ph), 8.74(s, 1H, CH), 10.53(s, 1H, NH), 10.94(s, 1H, NH). ESI-HRMS for $C_{15}H_{15}F_3N_4O_2$ m/z: Calculated, 341.1220, Found, 341.1218 [M+1]$^+$.

N-((2-methoxyphenyl)carbamoyl)-1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide(7b), White solid, yield 72.6% , m.p. 246~247°C; FTIR(ν, cm^{-1}): 3243 (N-H), 3134 (N-H), 1705 (C=O), 1688 (C=O), 1604 (Ar), 1565 (Ph, C=C), 1541 (C-N), 1492 (Ph, C=C), 1466 (Ph, C=C), 1301 (C-N), 1247 (C-N), 1000-1200 (C-F), 761 (Ar-H), 745 (Ph, C-H). ^{1}HNMR ($CDCl_3$, 400MHz), δ: 3.89(s, 3H, O-CH_3), 3.98(s, 3H, CH_3), 6.94-6.97(m, 1H, Ph), 7.08-7.10(m, 2H, Ph), 8.17(d, *J*=7.5Hz, 1H, Ph), 8.73(s, 1H, CH), 10.88(s, 1H, NH), 10.97(s, 1H, NH). ESI-HRMS for $C_{14}H_{13}F_3N_4O_3$ m/z: Calculated, 343.1013, Found, 343.1016 [M+1]$^+$.

N-(mesitylcarbamoyl)-1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide**(7c)**, White solid, yield 54.8%, m.p. 222~223°C; FTIR(ν, cm^{-1}): 3273 (N-H), 3127 (N-H), 1697 (C=O), 1625 (Ar), 1607 (Ph, C=C), 1540 (C-N), 1503 (Ph, C=C), 1478 (Ph, C=C), 1299 (C-N), 1249 (C-N), 1000-1200 (C-F), 777 (Ar-H), 759, 679 (Ph, C-H). ^{1}HNMR ($CDCl_3$, 400MHz), δ: 3.35(s, 3H, CH_3), 3.98(s, 3H, CH_3), 6.91(s, 2H, Ph), 8,74(s, 1H, CH), 9.72(s, 1H, NH), 10.88(s, 1H, NH). ESI-HRMS for $C_{16}H_{17}F_3N_4O_2$ m/z: Calculated, 355.1376 , Found, 355.1377 [M+1]$^+$.

N-((2-fluorophenyl)carbamoyl)-1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide**(7d)**, White solid, yield 76.2%, m.p. 227~228°C; FTIR(ν, cm^{-1}): 3243 (N-H), 3160 (N-H), 1701 (C=O), 1684 (C=O), 1621 (Ar), 1599 (Ph, C=C), 1543 (C-N), 1515 (Ph, C=C), 1458 (Ph, C=C), 1301 (C-N), 1247 (C-N), 1000-1200 (C-F), 772 (Ar-H), 759 (Ph, C-H). ^{1}HNMR ($CDCl_3$, 400MHz), δ: 3.99(s, 3H, CH_3), 7.113-7.17(m, 1H, Ph), 7.22(t, *J*=7.5Hz, 1H, Ph), 7.31-7.34(m, 1H, Ph), 8.14-8.18(m, 1H, Ph), 8.75(s, 1H, CH), 10.86(s, 1H, NH), 11.15(s, 1H, NH). ESI-HRMS for $C_{13}H_{10}F_4N_4O_2$ m/z: Calculated, 331.0813, Found, 331.0812[M+1]$^+$.

N-((3,4-difluorophenyl)carbamoyl)-1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide**(7e)**, White solid, yield 69.2%, m.p. 192~193°C; FTIR(ν, cm^{-1}): 3207 (N-H), 3142 (N-H), 1704 (C=O), 1681 (C=O), 1615 (Ar), 1582 (Ph, C=C), 1542 (C-N), 1521 (Ph, C=C), 1481 (Ph, C=C), 1300 (C-N), 1269 (C-N), 1000-1200 (C-F), 771 (Ar-H), 839, 762, (Ph,C-H). ^{1}HNMR ($CDCl_3$, 400MHz), δ: 3.98(s, 3H, CH_3), 7,39-7,44(m, 2H, Ph), 7.78-7.83(m, 1H, Ph), 8.74(s, 1H, CH), 10.59(s, 1H, NH), 11.04(s, 1H, NH). ESI-HRMS for $C_{13}H_9F_5N_4O_2$ m/z: Calculated, 349.0718, Found, 349.0715[M+1]$^+$.

N-((2,6-difluorophenyl)carbamoyl)-1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide**(7f)**, White solid, yield 91.2%, m.p. 211~212°C; FTIR(ν, cm^{-1}): 3245 (N-H), 3127 (N-H), 1704(C=O), 1687 (C=O), 1606 (Ar), 1587 (Ph, C=C), 1568 (Ph, C=C), 1540 (C-N), 1472 (Ph, C=C), 1298 (C-N), 1246 (C-N), 1000-1200 (C-F), 763 (Ar-H), 786, 713 (Ph,C-H). ^{1}HNMR ($CDCl_3$, 400MHz), δ: 3.99(s, 3H, CH_3), 7.17-7.21(m, 2H, Ph), 7.36-7.42(m, 1H, Ph), 8.75(s, 1H, CH), 9.93(s, 1H, NH), 11.14(s, 1H, NH). ESI-HRMS for $C_{13}H_9F_5N_4O_2$ m/z: Calculated, 349.0718 , Found,349.0721 [M+1]$^+$.

N-((2,5-difluorophenyl)carbamoyl)-1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide**(7g)**, White solid, yield 97.5%, m.p. 230~231°C; FTIR(ν, cm^{-1}): 3243 (N-H), 3158 (N-H), 1704(C=O), 1697 (C=O), 1629 (Ar), 1587 (Ph, C=C), 1568 (Ph, C=C), 1541 (C-N), 1473 (Ph, C=C), 1300 (C-N), 1242 (C-N), 1000-1200 (C-F), 772 (Ar-H), 761, 731, (Ph, C-H). ^{1}HNMR ($CDCl_3$, 400MHz), δ: 3.99(s, 3H, CH_3), 6.97-7.01(m, 1H, Ph), 7.37-7.42(m, 1H, Ph), 8.01-8.05(m, 1H, Ph), 8.76(s, 1H, CH), 11.02(s, 1H, NH), 11.26(s, 1H, NH). ESI-HRMS for $C_{13}H_9F_5N_4O_2$ m/z: Calculated, 349.0718 , Found, 349.0717 [M+1]$^+$.

1-methyl-3-(trifluoromethyl)-N-((2,3,4-trifluorophenyl)carbamoyl)-1*H*-pyrazole-4-carboxamide**(7h)**, White solid, yield 47.8%, m.p. 240~241°C; FTIR(ν, cm^{-1}): 3209 (N-H), 3156 (N-H), 1707(C=O), 1683 (C=O), 1625 (Ar), 1581 (Ph, C=C), 1543 (C-N), 1517 (Ph, C=C), 1483 (Ph, C=C), 1301 (C-N), 1254 (C-N), 1000-1200 (C-F), 773 (Ar-H), 821, 761 (Ph, C-H). ^{1}HNMR ($CDCl_3$, 400MHz), δ: 4.00(s, 3H, CH_3), 7.34(s, 1H, Ph), 7.86(s, 1H, Ph), 8.76(s, 1H, CH), 10.77(s, 1H, NH), 11.23(s, 1H, NH). ESI-HRMS for $C_{13}H_8F_6N_4O_2$ m/z: Calculated, 367.0624 , Found, 367.0620 $[M+1]^+$.

N-((3-fluorophenyl)carbamoyl)-1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide**(7i)**, White solid, yield 70.0%, m.p. 205~206°C, FTIR(ν, cm^{-1}): 3237 (N-H), 3146 (N-H), 1710 (C=O), 1684 (C=O), 1606 (Ar), 1541 (C-N), 1513 (Ph, C=C), 1481 (Ph, C=C), 1448 (Ph, C=C), 1300 (C-N), 1279 (C-N), 1000-1200 (C-F), 771 (Ar-H), 884, 847, 711 (Ph, C-H). ^{1}HNMR ($CDCl_3$, 400MHz), δ: 3.99(s, 3H, CH_3), 6.93-6.96(m, 1H, Ph), 7.33-7.38(m, 2H, Ph), 7.60-7.62(m, 1H, Ph), 8.74(s, 1H, CH), 10.65(s, 1H, NH), 11.03(s, 1H, NH). ESI-HRMS for $C_{13}H_{10}F_4N_4O_2$ m/z: Calculated, 331.0813, Found, 331.0809 $[M+1]^+$.

1-methyl-3-(trifluoromethyl)-N-((2-(trifluoromethyl)phenyl)carbamoyl)-1*H*-pyrazole-4-carboxamide(**7j**), White solid, yield 90.8%, m.p. 192~193°C; FTIR(ν, cm^{-1}): 3241 (N-H), 3137 (N-H), 1705 (C=O), 1695(C=O), 1616 (Ar), 1559 (Ph, C=C), 1541 (C-N), 1477 (Ph, C=C), 1456 (Ph, C=C), 1322 (C-N), 1301 (C-N), 1000-1200 (C-F), 767 (Ar-H); ^{1}HNMR ($CDCl_3$, 400MHz), δ: 3.99(s, 3H, CH_3), 7.38(t, *J*=7.5Hz, 1H, Ph), 7.71(t, *J*=7.5Hz, 1H, Ph), 7.75(d, *J*=8.0Hz, 1H, Ph), 8.76(s, 1H, CH), 10.95(s, 1H, NH), 11.20(s, 1H, NH). ESI-HRMS for $C_{14}H_{10}F_6N_4O_2$ m/z: Calculated, 381.0781, Found, 381.0780 $[M+1]^+$.

N-((2-chlorophenyl)carbamoyl)-1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide(**7k**), White solid, yield 96.4%, m.p. 218~219°C; FTIR(ν, cm^{-1}): 3259 (N-H), 3127 (N-H), 1701 (C=O), 1682 (C=O), 1596 (Ph, C=C), 1540 (C-N), 1510 (Ph, C=C), 1291 (C-N), 1236 (C-N), 1000-1200 (C-F), 757 (Ar-H), 734 (Ph, C-H). ^{1}HNMR ($CDCl_3$, 400MHz), δ: 3.99(s, 3H, CH_3), 7.15-7.18(m, 1H, Ph), 7.37-7.39(m, 1H, Ph), 7.55-7.56(m, 1H, Ph), 8.76(s, 1H, CH), 11.09(s, 1H, NH), 11.17(s, 1H, NH). ESI-HRMS for $C_{13}H_{10}ClF_3N_4O_2$ m/z: Calculated, 347.0517, Found, 347.0512 $[M+1]^+$.

N-((4-ethylphenyl)carbamoyl)-1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide(**7l**), White solid, yield 72.3% , m.p. 246°C; FTIR(ν, cm^{-1}): 3244 (N-H), 3127 (N-H), 1701(C=O), 1697 (C=O), 1598 (Ph, C=C), 1541 (C-N), 1514 (Ph, C=C), 1471 (Ph, C=C), 1296 (C-N), 1235 (C-N), 1000-1200 (C-F), 826 (Ph, C-H), 765 (Ar-H). ^{1}HNMR ($CDCl_3$, 400MHz), δ: 1.17(t, *J*=7.5Hz, 3H, CH_3), 2.58(m, 2H, CH_2), 3.98(s, 3H, CH_3), 7.18(d, *J*=8.5Hz, 2H, Ph), 7.48(d, *J*=8.5Hz, 2H, Ph), 8,74(s, 1H, CH), 10.49(s, 1H, NH), 10.93(s, 1H, NH). ESI-HRMS for $C_{15}H_{15}F_3N_4O_2$ m/z: Calculated, 341.1220, Found, 341.1222 $[M+1]^+$.

N-((2,6-diethylphenyl)carbamoyl)-1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide(**7m**) , White solid, yield 46.3% , m.p. 219~220°C; FTIR(ν, cm^{-1}): 3234 (N-H), 3124 (N-H), 1705(C=O), 1697 (C=O), 1591 (Ph, C=C), 1521 (C-N), 1299 (C-N), 1230 (C-N), 1000-1200 (C-F), 777 (Ar-H), 760 (Ph, C-H). ^{1}HNMR ($CDCl_3$, 400MHz), δ: 1.13(t, *J*=7.5Hz, 6H, $2CH_3$), 2.56(m, 4H, $2CH_2$), 3.98(s, 3H, CH_3), 7.14(d, *J*=7.5Hz, 2H, Ph), 7.21-7.24(m, 1H, Ph), 8,75(s, 1H, CH), 9.81(s, 1H, NH), 10.92(s, 1H, NH). ESI-HRMS for $C_{17}H_{19}F_3N_4O_2$ m/z: Calculated, 369.1533, Found, 369.1533 $[M+1]^+$.

Greenhouse in vivo fungicidal evaluation

Antifungal activities of compound **7a~7m** against *Fusarium oxysporum*, *Corynespora mazei* and *Botrytis cinerea* were determined according to our previous work[41]. The potted plants cucumbers were used. The determine concentration of control fluxapyroxad and the title compounds is 100 μg/mL, 50μg/mL, 10μg/mL respectively. The three fungals *Fusarium oxysporum*, *Corynespora mazei* and *Botrytis cinerea* were inoculated when the cucumber is at the stage of two seed leaves. The relative control efficacy of compounds compared to the blank assay was calculated via the following equation:

relative control efficacy (%)=*(CK-PT)/CK* × 100%

where CK is the average disease index during the blank assay and PT is the average disease index after treatment during testing. All experiments were replicated three times.

Crystal Structure Determination

The crystal of compound **7d** with dimensions of 0.20mm × 0.18mm × 0.12 mm was mounted on a Rigaku saturn diffractometer with a graphite-monochromated MoKα radiation (λ = 0.71073Å) by using a phi and scan modes at 113(2) K in the range of $3.46°\leq\theta\leq27.53°$. The crystal belongs to monoclinic system with space group P2(1)/c and crystal parameters of a= 4.6181(11) Å, b = 18.857(4) Å, c = 15.180(4) Å, α = 90°, β = 97.672(8)°, γ = 90°, V= 1310.1(5) A^3, Dc= 1.674 g/cm^3. The absorption coefficient μ= 0.154 mm^{-1}, and Z = 4. The structure was solved by direct methods with SHELXS-97[42] and refined by the full-matrix least squares method on F^2 data using SHELXL-97. The empirical absorption corrections were applied to all intensity data. H atom of N-H was initially located in a difference Fourier map and were refined with the restraint Uiso(H) = 1.2Ueq(N). Other H atoms were positioned geometrically and refined using a riding model, with d(C---H) = 0.93-0.97 Å and Uiso(H) = 1.2Ueq(C) or 1.5Ueq(Cmethyl). The final full-matrix least squares refinement gave R = 0.0313 and *wR* = 0.0782 ($w = 1/[\sigma^2(F_o^2) + (0.0426P)^2]$ *where* $P = (F_o^2 + 2F_c^2)/3$] *S* = 1.02.

Supplementary material

CCDC 1848186 contain the supplementary crystallographic data for 7d. Copies of this information can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html/datarequest/cif, or from The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, Fax :(+44)-1223-336033/ Email: deposit@ccdc.cam.ac.uk. Also the NMR, FTIR and HRMS data can be found in supplementary material.

Acknowledgements

This work was supported financially by the Opening Foundation of the Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, grant No. 2018GDGP0104 and Zhejiang Provincial Natural Science Foundation of China (No. LY16C140007).

Author Contribution Statement

It is no conflict.

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Highlights

►The new trifluoromethylpyrazole acyl urea derivatives were designed and synthesized.

► All the structures were determined by NMR, FTIR and HRMS. The highest active compound were determined by X-ray diffraction.

► All newly synthesized urea derivatives are potent antifungal agents compared to commercial drug Fluxapyroxad.

► Among all the synthesized urea derivatives, compound **7d** possessed high in vivo activity against *B. cinerea* with > 80 % control efficacy at 10 mg/L.

► The mode of action was done using Docking method.