



Synthesis and Bioactivity of Novel Sulfonate Scaffold-Containing Pyrazolecarbamide Derivatives as Antifungal and Antiviral Agents

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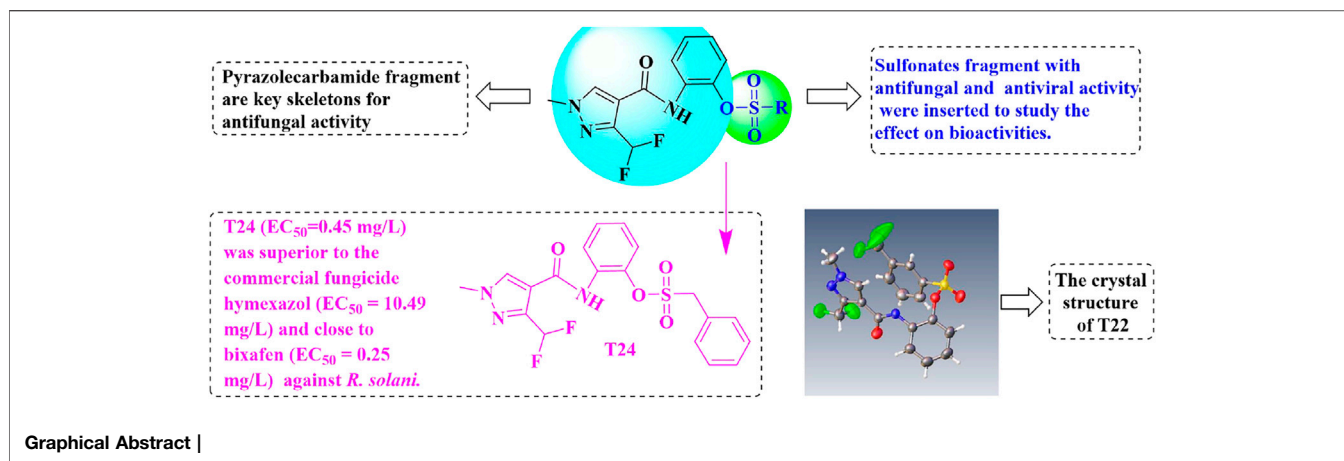
Novel pyrazolecarbamide derivatives bearing a sulfonate fragment were synthesized to identify potential antifungal and antiviral agents. All the structures of the key intermediates and target compounds were confirmed by nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS). The single-crystal X-ray diffraction of the compound **T22** showed that pyrazole carbamide is a sulfonate. The *in vitro* antifungal activities of the target compounds against *Colletotrichum camelliae*, *Pestalotiopsis theae*, *Gibberella zeae*, and *Rhizoctonia solani* were evaluated at 50 µg/ml. Among the four pathogens, the target compounds exhibited the highest antifungal activity against *Rhizoctonia solani*. The compound **T24** (EC₅₀ = 0.45 mg/L) had higher antifungal activity than the commercial fungicide hymexazol (EC₅₀ = 10.49 mg/L) against *R. solani*, almost similar to bixafen (EC₅₀ = 0.25 mg/L). Additionally, the target compounds exhibited protective effects *in vivo* against TMV. Thus, this study reveals that pyrazolecarbamide derivatives bearing a sulfonate fragment exhibit potential antifungal and antiviral activities.

Keywords: pyrazolecarbamide, sulfonate, antifungal activity, antiviral activity, synthesis

INTRODUCTION

Phytopathogenic microorganisms, such as *Rhizoctonia solani*, *Gibberella zeae*, *Pestalotiopsis theae*, *Colletotrichum camelliae*, and tobacco mosaic virus (TMV) reduce the yield and quality of food and cash crops (Fisher et al., 2012). Chemical pesticides are still the most commonly used control measure for these diseases; however, the associated pesticide resistance and environmental hazards (Wei et al., 2020) impede their usage. Therefore, there is an urgent need to develop novel eco-friendly antifungal and antiviral agents agent with low toxicity and high efficiency.

Pyrazole and its derivatives have received considerable attention because of their diverse agrochemical and pharmaceutical applications. Most pyrazole derivatives exhibit a broad spectrum of biological activities, including antifungal (Kanungo and Joshi, 2014; Mu et al., 2016; Yan et al., 2018), insecticidal (Wu et al., 2012; Jiang et al., 2020), antibacterial (El Shehry et al., 2018; Wang et al., 2021), and other antimicrobial activities (Kasiotis et al., 2014; Saleh et al., 2020). Especially, pyrazole carboxamide derivatives, such as penthiopyrad, furametpyr, penflufen,



isopyrazam, and bixafen, which could inhibit the succinate dehydrogenase, have been developed and commercialized as fungicides (Si et al., 2019).

Sulfonates are also widely applied in agrochemical and medical industries because of their insecticidal (Sun et al., 2013; Wang et al., 2015), antifungal (Kang et al., 2019; Zhou et al., 2022), and antibacteria (Su et al., 2021). Moreover, the heterocyclic compounds containing aryl sulfonate moiety exhibit excellent antiviral activities (Zeng et al., 2010; Huang et al., 2015; Hadházi et al., 2017).

Therefore, we designed and synthesized a series of novel pyrazolecarbamide derivatives bearing a sulfonate moiety based on the active splicing principle and used the mycelial growth rate and half-leaf blight spot methods to evaluate their antifungal and antiviral activities.

MATERIALS AND METHODS

Chemistry

The ^1H and ^{13}C NMR spectra were recorded in CDCl_3 using 400 and 101 MHz spectrophotometers (Bruker BioSpin GmbH, Rheinstetten, Germany), respectively, while high-resolution mass spectrometry (HRMS) was performed using Thermo Scientific Q Exactive (Thermo Fisher Scientific, Massachusetts, America). The X-ray crystallographic data were collected and processed on a D8 Quest X-ray diffractometer (Bruker BioSpin GmbH, Rheinstetten, German). All solvents were dried using the standard methods and distilled before use.

3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxylic Acid (4)

As shown in Scheme 1, the key intermediate 4 was synthesized using a previously published three-step procedure (Wang et al., 2020). White powder, yield 46%. m.p. 201.1–201.9°C. ^1H NMR (400 MHz, CDCl_3) δ 7.98 (s, 1H), 7.12 (t, $J = 54.3$ Hz, 1H), 4.02 (s, 3H). HRMS (ESI): calculated for $\text{C}_6\text{H}_6\text{F}_2\text{N}_2\text{O}_2$ $[\text{M} + \text{Na}]^+$: 199.02950, found: 199.02896.

2-(Difluoromethyl)-N-(2-Hydroxyphenyl)-1-Methyl-1H-Pyrazole-4-Carboxamide (6)

A mixture of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI, 120 mmol), Intermediate 4 (100 mmol) and *o*-aminophenol (100 mmol), and dimethylaminopyridine (DMAP, 10 mmol) were dissolved in CH_2Cl_2 (500 ml) at -10°C for 1 h. Thereafter, the mixture was stirred at room temperature for 8 h, and the key intermediate 6 was purified using column chromatography. Light yellow solid, yield 62%. m.p. 181.1–182.3°C. ^1H NMR (400 MHz, CDCl_3) δ 8.99 (s, 1H), 8.37 (s, 1H), 8.08 (s, 1H), 7.17 (td, $J = 7.7, 1.6$ Hz, 1H), 7.05 (ddd, $J = 7.8, 6.0, 1.5$ Hz, 2H), 6.91 (dd, $J = 7.4, 1.5$ Hz, 1H), 6.88 (t, $J = 54.1$ Hz, 1H), 3.97 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 160.74, 149.12 (t, $J = 26.5$ Hz), 136.77, 127.62, 125.42, 122.63, 120.51, 120.10, 115.5, 112.18, 110.40 (t, $J = 235.3$ Hz), 39.71. HRMS (ESI): calculated for $\text{C}_{12}\text{H}_{11}\text{F}_2\text{N}_3\text{O}_2$ $[\text{M} + \text{Na}]^+$: 290.07170, found: 290.07126.

General Procedure for the Preparation of the Target Compounds (T1–27)

Catalytic DMAP, arylsulfonyl chloride (1.1 mmol), and Et_3N (2 mmol) were added to a stirred CH_3CN (20 ml) solution of the key intermediate 6 (1 mmol), and the reaction was monitored at room temperature using TLC. Thereafter, the solvent was removed by rotary evaporation, and 10 ml of water was added to the residue, followed by extraction of the aqueous layer three times (30 ml \times 3) using ethyl acetate. The organic layers were then combined and dried using anhydrous Na_2SO_4 and later concentrated under reduced pressure to form a crude product, purified using flash chromatography to obtain the target product.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl Benzenesulfonate (T1)

Gray powder, yield 72%. m.p. 138.3–139.6°C. ^1H NMR (400 MHz, CDCl_3) δ 8.33 (s, 1H), 8.27 (dd, $J = 8.3, 1.6$ Hz,

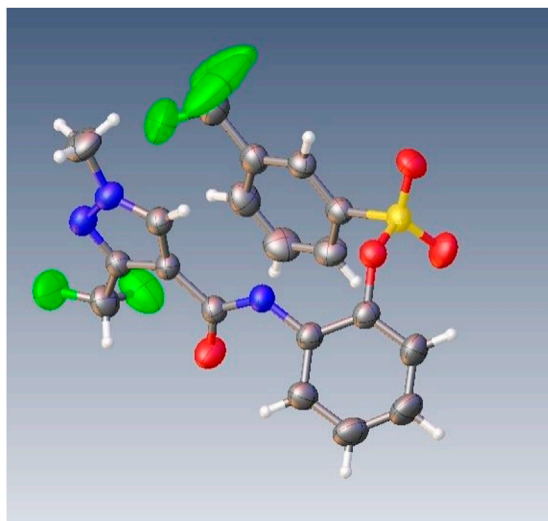
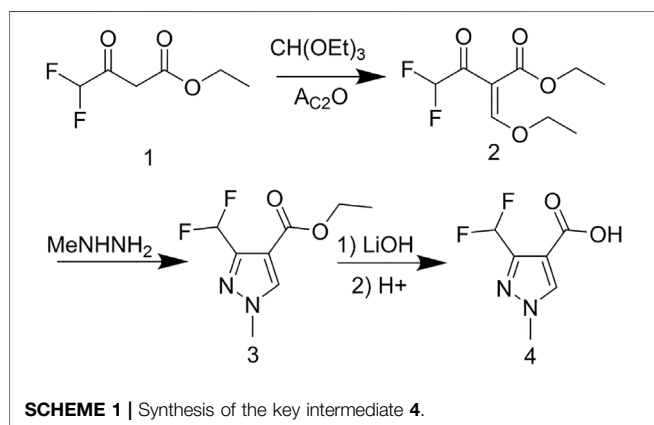


FIGURE 1 | The single-crystal X-ray diffraction of compound T22.

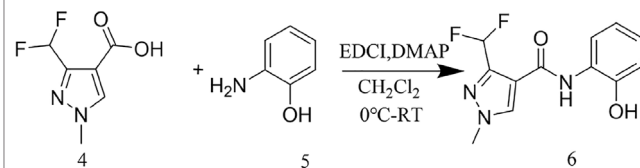


SCHEME 1 | Synthesis of the key intermediate 4.

1H), 7.92 (s, 1H), 7.87–7.80 (m, 2H), 7.70–7.61 (m, 1H), 7.52–7.45 (m, 2H), 7.26 (dd, $J = 15.7, 1.5$ Hz, 1H), 7.08 (t, $J = 54.1$ Hz, 1H), 7.04–6.97 (m, 1H), 6.90 (dd, $J = 8.2, 1.5$ Hz, 1H), 4.00 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 159.33, 144.86(t, $J = 26.5$ Hz), 139.41, 134.99, 134.50, 133.41, 131.01, 129.44($\times 2$), 128.65($\times 2$), 128.01, 124.78, 123.27, 122.71, 116.68, 110.50 (t, $J = 235.3$ Hz), 39.92. HRMS (ESI): calculated for $\text{C}_{18}\text{H}_{15}\text{F}_2\text{N}_3\text{O}_4\text{S}[\text{M} + \text{Na}]^+$: 430.06490, found: 430.06531.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 4-Methylbenzenesulfonate (T2)

Light yellow powder, yield 79%. m.p. 126.2–126.9°C. ^1H NMR (400 MHz, CDCl_3) δ 8.35 (s, 1H), 8.28 (dd, $J = 8.3, 1.6$ Hz, 1H), 7.92 (s, 1H), 7.76–7.64 (m, 2H), 7.31–7.21 (m, 4H), 7.09 (t, $J = 54.1$ Hz, 1H), 7.00 (td, $J = 7.9, 1.6$ Hz, 1H), 6.88 (dd, $J = 8.2,$



SCHEME 2 | Synthesis of the key intermediate 6.

1.5 Hz, 1H), 4.00 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 159.35, 146.34, 145.05(t, $J = 29.3$ Hz), 139.50, 133.19, 131.54, 131.13, 130.07($\times 2$), 128.72($\times 2$), 127.94, 124.73, 123.20, 122.78, 116.82, 110.44(t, $J = 235.8$ Hz), 39.92, 21.87. HRMS (ESI): calculated for $\text{C}_{19}\text{H}_{17}\text{F}_2\text{N}_3\text{O}_4\text{S}[\text{M} + \text{Na}]^+$: 444.08055, found: 444.08109.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 2-Fluorobenzenesulfonate (T3)

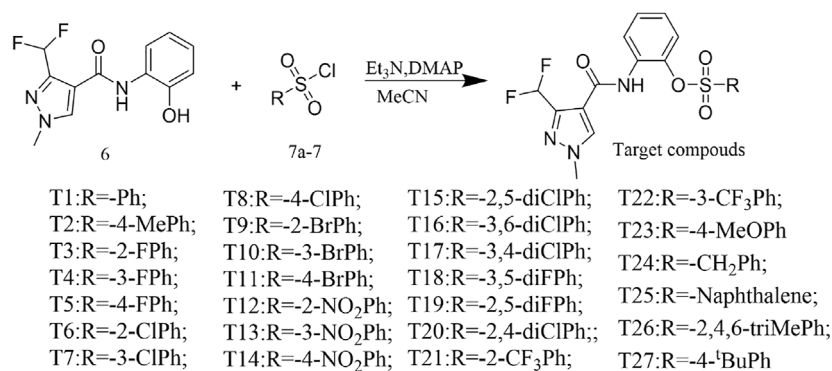
White powder, yield 78%. m.p. 123.9–124.5°C. ^1H NMR (400 MHz, CDCl_3) δ 8.40 (s, 1H), 8.33 (dd, $J = 8.3, 1.6$ Hz, 1H), 7.96 (s, 1H), 7.89 (ddd, $J = 8.3, 6.9, 1.8$ Hz, 1H), 7.76–7.66 (m, 1H), 7.30 (qd, $J = 7.7, 1.3$ Hz, 3H), 7.26–7.19 (m, 2H), 7.12 (t, $J = 54.0$ Hz, 1H), 7.15 (dd, $J = 8.2, 1.5$ Hz, 1H), 7.10–7.02 (m, 1H), 4.02 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 160.92, 159.53, 158.34, 138.73, 137.67, 137.58, 132.71, 131.57, 131.09, 128.31, 125.00, 124.96, 124.88, 123.24, 122.66, 117.81, 117.60, 116.77, 112.53, 110.19, 39.97. HRMS (ESI): calculated for $\text{C}_{18}\text{H}_{14}\text{F}_3\text{N}_3\text{O}_4\text{S}[\text{M} + \text{Na}]^+$: 448.05548, found: 448.05454.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 3-Fluorobenzenesulfonate (T4)

Gray powder, yield 76%. m.p. 119.3–120.9°C. ^1H NMR (400 MHz, CDCl_3) δ 8.31–8.19 (m, 2H), 7.92 (s, 1H), 7.63–7.53 (m, 2H), 7.47 (td, $J = 8.1, 5.2$ Hz, 1H), 7.33 (tdd, $J = 8.3, 2.5, 1.0$ Hz, 1H), 7.30–7.27 (m, 1H), 7.05 (ddd, $J = 8.7, 7.2, 1.5$ Hz, 1H), 7.02 (t, $J = 54.1$ Hz, 1H), 7.00 (dd, $J = 8.2, 1.7$ Hz, 1H), 3.99 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 163.61, 161.09, 159.26, 144.67, 144.40, 144.13, 139.44, 136.61, 136.53, 133.98, 131.37, 131.30, 130.85, 128.18, 124.96, 124.58, 124.54, 123.55, 122.56, 122.35, 122.14, 116.55, 116.08, 115.83, 113.16, 110.82, 108.49, 39.86. HRMS (ESI): calculated for $\text{C}_{18}\text{H}_{14}\text{F}_3\text{N}_3\text{O}_4\text{S}[\text{M} + \text{Na}]^+$: 448.05548, found: 448.05454.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 4-Fluorobenzenesulfonate (T5)

Light yellow powder, yield 69%. m.p. 165.2–165.9°C. ^1H NMR (400 MHz, CDCl_3) δ 8.32–8.21 (m, 2H), 7.93 (s, 1H), 7.88–7.80



SCHEME 3 | Synthesis of the target compounds.

TABLE 1 | Inhibition rate *in vitro* of target compounds **T1-27** at 50 µg/ml.

Compounds	Inhibition Rate (%)			
	R. Solani (36 h)	C.camelliae (120 h)	P. Theae (120 h)	G.Zeae (120 h)
T1	29.37 ± 1.02 k	20.30 ± 1.22 kl	30.30 ± 0.42 ij	30.20 ± 1.33 j
T2	51.59 ± 1.31 e	41.09 ± 1.10 de	31.60 ± 1.69 hi	42.50 ± 1.23 d
T3	30.95 ± 1.19 k	26.90 ± 1.09 i	33.75 ± 0.19 fg	26.88 ± 2.09 k
T4	10.14 ± 0.24 q	11.74 ± 0.26o	12.04 ± 1.04 r	18.21 ± 1.04 no
T5	13.23 ± 0.97 op	16.20 ± 0.27 n	18.23 ± 1.08 p	19.69 ± 0.97 n
T6	44.97 ± 0.92 g	34.17 ± 0.12 f	40.02 ± 0.42 d	30.67 ± 0.62 j
T7	62.96 ± 1.27 d	12.90 ± 1.16o	32.16 ± 0.17 gh	36.16 ± 1.36 h
T8	61.38 ± 1.39 d	30.18 ± 1.09 h	29.08 ± 0.19 jk	41.38 ± 2.49 de
T9	23.02 ± 1.06 m	21.02 ± 0.76 k	20.19 ± 0.46o	25.02 ± 1.16 kl
T10	30.56 ± 1.42 k	20.66 ± 1.02 kl	28.51 ± 0.32 k	25.69 ± 1.02 kl
T11	36.77 ± 1.21 i	30.07 ± 0.41 h	33.71 ± 0.42 fg	38.27 ± 1.41 fg
T12	12.43 ± 1.01 p	19.73 ± 0.70 kl	17.40 ± 0.80 p	16.43 ± 1.21o
T13	13.46 ± 1.09 op	19.40 ± 1.17 lm	12.66 ± 0.19 r	10.26 ± 1.49 p
T14	20.45 ± 0.91 n	23.25 ± 0.78 j	22.05 ± 0.88 mn	22.45 ± 0.71 m
T15	24.34 ± 1.08 m	21.06 ± 0.98 k	23.04 ± 0.13 lm	26.64 ± 1.00 k
T16	48.15 ± 1.26 f	28.05 ± 0.16 i	33.18 ± 0.19 fgh	43.19 ± 0.26 d
T17	34.13 ± 1.10 j	24.03 ± 1.01 j	29.03 ± 1.00 jk	33.03 ± 0.16 i
T18	81.48 ± 1.06 c	40.40 ± 1.78 de	35.98 ± 0.76 e	40.08 ± 0.96 ef
T19	45.74 ± 1.02 g	35.04 ± 1.12 f	34.74 ± 0.92 ef	38.87 ± 0.46 f
T20	44.18 ± 1.00 g	40.01 ± 0.90 e	24.18 ± 0.10 l	36.58 ± 0.90 gh
T21	14.81 ± 0.98o	17.80 ± 0.68 mn	14.81 ± 0.78 q	24.73 ± 0.88 kl
T22	27.25 ± 0.93 l	23.15 ± 0.63 j	17.25 ± 0.13 p	26.35 ± 0.73 kl
T23	20.11 ± 0.95 n	20.71 ± 0.36 kl	13.05 ± 0.65 r	24.41 ± 0.65 l
T24	100.00 ± 0.00 a	45.31 ± 0.47 c	62.40 ± 0.51 c	48.00 ± 1.10 c
T25	29.37 ± 0.40 k	31.07 ± 0.69 gh	20.30 ± 0.16o	39.07 ± 0.64 f
T26	30.69 ± 0.73 k	32.19 ± 0.33 g	21.30 ± 0.44 no	32.64 ± 0.91 i
T27	40.21 ± 0.98 h	42.12 ± 1.84 d	20.20 ± 0.61°	26.26 ± 0.68 kl
hymexazol	84.28 ± 0.96 b	54.91 ± 1.80 b	66.11 ± 3.20 b	67.33 ± 2.19 b
bixafen	100.00 ± 0.00 a	79.49 ± 1.36 a	93.40 ± 1.77 a	100.00 ± 0.00 a

Note: Data in the table are mean ± SD. Different lowercase letters in the same column indicate significant difference at $p < 0.05$ level by Duncan's new multiple range test. The meaning of bold is only to emphasize the good activity of the two compounds.

(m, 2H), 7.33–7.26 (m, 1H), 7.18–7.07 (m, 2H), 7.05 (ddd, $J = 8.9, 7.4, 1.6$ Hz, 1H), 7.00 (t, $J = 54.0$ Hz, 1H), 6.98 (dd, $J = 8.3, 1.6$ Hz, 1H), 4.00 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 166.37(d, $J = 259.6$ Hz), 159.22, 144.15(t, $J = 26.2$ Hz), 144.18, 139.45, 134.24, 131.69, 131.59, 130.89, 130.69, 128.13, 124.92, 123.48, 122.78, 116.96, 116.73, 116.65, 110.90(t, $J = 235.8$ Hz), 105.41, 39.90. HRMS (ESI): calculated for C₁₈H₁₄F₃N₃O₄S [M + Na]⁺:448.05548, found: 448.05454.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 2-Chlorobenzenesulfonate (T6)

Gray powder, yield 70%. m.p. 116.3–117.2°C. ¹H NMR (400 MHz, CDCl₃) δ 8.48 (s, 1H), 8.39–8.30 (m, 1H), 8.03 (dd, $J = 8.0, 1.5$ Hz, 1H), 7.97 (s, 1H), 7.67–7.57 (m, 2H), 7.44 (ddd, $J = 8.0, 7.1, 1.6$ Hz, 1H), 7.29 (ddd, $J = 8.6, 5.6, 3.4$ Hz, 1H), 7.11 (d, $J = 54.1$ Hz, 1H), 7.06–6.99 (m, 2H), 4.01 (s, 3H). ¹³C NMR

TABLE 2 | EC₅₀ values of T24 against R. solani.

Compound	Regression Equation	EC ₅₀ (mg/L)	R ²	95% confidence Interval (mg/L)
T24	y = 5.7941 + 1.3307x	0.45	0.9588	0.32-0.61
hymexazol	y = 3.9940 + 0.9853x	10.49	0.9949	6.35-17.33
bixafen	y = 5.7941 + 1.3307x	0.25	0.9976	0.13-0.47

TABLE 3 | Antiviral activity of the target compounds against TMV *in vivo* (500 mg/L).

Compound	Curative effect(%)	Protective effect(%)	Inactivation effect(%)
T1	30.9 ± 2.4 fg	40.1 ± 2.2 ghi	54.6 ± 3.2 jkl
T2	35.2 ± 1.6 f	43.1 ± 1.4 defghi	53.2 ± 1.3 jkl
T3	32.8 ± 3.2 fg	49.8 ± 2.3 b	63.3 ± 2.3 efgh
T4	40.8 ± 2.9 e	43.8 ± 2.6 cdefgh	62.6 ± 4.2 efghi
T5	42.4 ± 4.5 de	50.4 ± 1.5 b	59.5 ± 1.7 ghijk
T6	42.5 ± 2.0 de	42.4 ± 2.4 efghi	57.6 ± 2.5 hijk
T7	43.8 ± 1.7 de	43.5 ± 1.4 defghi	56.5 ± 3.0 ijk
T8	45.9 ± 2.5 cde	47.2 ± 3.0 bcde	62.8 ± 2.2 efghi
T9	35.2 ± 2.7 f	41.1 ± 3.2 ghi	50.5 ± 3.9 lmn
T10	32.2 ± 2.3 fg	42.5 ± 2.4 defghi	55.1 ± 3.4 hijk
T11	33.8 ± 4.0 f	49.8 ± 1.9 b	57.3 ± 3.5 jkl
T12	41.9 ± 2.0 de	50.2 ± 3.6 b	60.6 ± 2.4 fghij
T13	43.0 ± 3.7 de	45.4 ± 3.5 bcdefg	58.5 ± 4.7 hijk
T14	44.5 ± 3.1 de	43.0 ± 3.9 defghi	49.6 ± 4.5 lmn
T15	40.8 ± 0.7 e	49.5 ± 4.4 b	46.5 ± 3.7 no
T16	42.9 ± 3.1 de	48.0 ± 3.0 bcd	72.8 ± 4.9 bc
T17	41.5 ± 3.7 e	41.1 ± 4.2 fghi	67.6 ± 4.3 de
T18	54.2 ± 3.6 ab	49.1 ± 4.4 bc	70.2 ± 4.6 bcd
T19	46.9 ± 3.4 cd	40.1 ± 3.2 ghi	74.6 ± 4.2 b
T20	49.8 ± 3.9 bc	45.8 ± 4.6 bcdef	68.6 ± 3.9 cde
T21	28.4 ± 2.9 g	40.3 ± 1.5 fghi	65.3 ± 2.1 defg
T22	41.2 ± 2.0 e	32.4 ± 1.8 j	58.7 ± 3.8 hijk
T23	33.8 ± 1.7 f	38.6 ± 2.6 hi	66.3 ± 3.9 def
T24	35.9 ± 2.5 f	37.9 ± 3.1 i	42.8 ± 3.7o
T25	33.8 ± 1.7 f	45.6 ± 1.7 bcdefg	57.5 ± 1.9 hijk
T26	45.9 ± 2.5 cde	43.2 ± 2.8 defghi	49.8 ± 2.9 lmn
T27	30.9 ± 1.7 fg	40.2 ± 2.9 fghi	57.8 ± 2.1 hijk
Chitosan oligosaccharides	54.6 ± 2.7 a	57.6 ± 2.2 a	47.9 ± 1.5 mno
Ningnanmycin	55.3 ± 1.2 a	50.7 ± 1.1 b	98.1 ± 1.0 a

Note: Data in the table are mean ± SD., Different lowercase letters in the same column indicate significant difference at p < 0.05 level by Duncan's new multiple range test.

(101 MHz, CDCl₃) δ 159.59, 145.46(t, J = 26.5 Hz), 139.00, 135.92, 133.48, 133.15, 132.92, 132.54, 132.50, 131.21, 128.26, 127.52, 124.89, 123.48, 122.61, 116.70, 110.23 (t, J = 236.3 Hz), 39.96. HRMS (ESI): calculated for C₁₈H₁₄ClF₂N₃O₄S [M + Na]⁺: 464.02593, found: 464.02521.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 3-Chlorobenzenesulfonate (T7)

Light yellow powder, yield 73%. m.p. 110.0-111.9°C. ¹H NMR (400 MHz, CDCl₃) δ 8.24 (dd, J = 8.2, 1.4 Hz, 1H), 8.19 (s, 1H), 7.91 (s, 1H), 7.86 (t, J = 1.9 Hz, 1H), 7.63 (dt, J = 7.9, 1.4 Hz, 1H), 7.58 (ddd, J = 8.1, 2.1, 1.0 Hz, 1H), 7.41 (t, J = 8.0 Hz, 1H), 7.29 (ddd, J = 8.5, 6.6, 2.4 Hz, 1H), 7.11-7.02 (m, 2H), 7.01 (d, J = 54.1

Hz, 1H), 3.99 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.16, 144.30(t, J = 27.3 Hz), 139.40, 136.43, 135.79, 134.99, 134.11, 130.81, 130.70, 128.47, 128.22, 126.77, 124.98, 123.52, 122.72, 116.53, 110.90(t, J = 235.3 Hz), 39.88. HRMS (ESI): calculated for C₁₈H₁₄ClF₂N₃O₄S [M + Na]⁺: 464.02593, found: 464.02521.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 4-Chlorobenzenesulfonate (T8)

Light yellow powder, yield 79%. m.p. 185.6-185.9°C. ¹H NMR (400 MHz, CDCl₃) δ 8.24 (dd, J = 8.2, 1.5 Hz, 2H), 8.22 (s, 1H), 7.93 (s, 1H), 7.77-7.69 (m, 2H), 7.44-7.36 (m, 2H), 7.29 (ddd, J = 8.6, 7.2, 1.8 Hz, 1H), 7.07 (td, J = 7.7, 1.5 Hz, 2H), 7.02 (dd, J = 8.2, 1.8 Hz, 1H), 6.98 (t, J = 54.1 Hz, 1H), 3.99 (s, 3H). ¹³C NMR

(101 MHz, CDCl₃) δ 159.15, 143.97(t, J = 26.7 Hz), 141.62, 139.54, 134.48, 133.37, 130.80, 130.04(\times 2), 129.75(\times 2), 128.15, 125.00, 123.59, 122.89, 116.64, 111.03(t, J = 234.7 Hz), 39.88. HRMS (ESI): calculated for C₁₈H₁₄ClF₂N₃O₄S [M + Na]⁺: 464.02593, found: 464.02521.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 2-Bromobenzenesulfonate (T9)

Gray powder, yield 69%. m.p. 133.9-134.2°C. ¹H NMR (400 MHz, CDCl₃) δ 8.48 (s, 1H), 8.36-8.28 (m, 1H), 8.04 (dd, J = 7.8, 1.9 Hz, 1H), 7.98 (s, 1H), 7.81 (dd, J = 7.8, 1.4 Hz, 1H), 7.53 (td, J = 7.6, 1.9 Hz, 1H), 7.48 (td, J = 7.7, 1.4 Hz, 1H), 7.32-7.25 (m, 1H), 7.00 (t, J = 54.0 Hz, 1H), 7.04-6.98 (m, 2H), 4.00 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.59, 145.42(t, J = 25.8 Hz), 139.06, 136.05, 135.79, 134.98, 132.99, 132.79, 131.24, 128.23, 128.07, 124.89, 123.52, 122.66, 121.38, 116.82, 110.22(t, J = 235.6 Hz), 39.94. HRMS (ESI): calculated for C₁₈H₁₄BrF₂N₃O₄S [M + Na]⁺: 507.97542, found: 507.97227.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 3-Bromobenzenesulfonate (T10)

Light yellow powder, yield 80%. m.p. 128.4-128.5°C. ¹H NMR (400 MHz, CDCl₃) δ 8.30-8.21 (m, 1H), 8.18 (s, 1H), 8.01 (t, J = 1.9 Hz, 1H), 7.91 (s, 1H), 7.73 (ddd, J = 8.1, 1.9, 1.0 Hz, 1H), 7.66 (ddd, J = 7.9, 1.8, 1.0 Hz, 1H), 7.33 (t, J = 8.0 Hz, 1H), 7.29 (td, J = 6.1, 3.3 Hz, 1H), 7.11-7.03 (m, 2H), 7.01 (t, J = 54.1 Hz, 1H), 3.99 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.13, 144.26 (t, J = 27.3 Hz), 139.35, 137.90, 136.47, 134.16, 131.24, 130.87, 130.75, 128.23, 127.18, 125.00, 123.50, 123.38, 122.78, 116.47, 110.91(t, J = 235.3 Hz), 39.91. HRMS (ESI): calculated for C₁₈H₁₄BrF₂N₃O₄S [M + Na]⁺: 507.97542, found: 507.97227.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 4-Bromobenzenesulfonate (T11)

Light yellow powder, yield 79%. m.p. 175.8-176.4°C. ¹H NMR (400 MHz, CDCl₃) δ 8.23 (dd, J = 8.2, 1.5 Hz, 1H), 8.20 (s, 1H), 7.94 (s, 1H), 7.68-7.61 (m, 2H), 7.60-7.50 (m, 2H), 7.29 (ddd, J = 8.5, 7.0, 1.9 Hz, 1H), 7.07 (ddd, J = 8.6, 7.1, 1.5 Hz, 1H), 7.03 (dd, J = 8.2, 1.9 Hz, 1H), 6.97 (t, J = 54.1 Hz, 1H), 4.00 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.12, 143.91(t, J = 28.8 Hz), 139.54, 134.57, 133.94, 132.73(\times 2), 130.77, 130.26, 130.02(\times 2), 128.15, 125.02, 123.60, 122.92, 116.60, 111.06(t, J = 235.02 Hz), 39.89. HRMS (ESI): calculated for C₁₈H₁₄BrF₂N₃O₄S [M + Na]⁺: 507.97542, found: 507.97227.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 2-Nitrobenzenesulfonate (T12)

Light yellow powder, yield 83%. m.p. 146.0-147.8°C. ¹H NMR (400 MHz, CDCl₃) δ 8.31 (s, 1H), 8.26 (dd, J = 8.3, 1.6 Hz, 1H),

7.92 (dd, J = 7.9, 1.4 Hz, 1H), 7.90 (d, J = 1.2 Hz, 1H), 7.81 (td, J = 7.8, 1.4 Hz, 1H), 7.69 (td, J = 7.8, 1.3 Hz, 1H), 7.66 (dd, J = 7.9, 1.3 Hz, 1H), 7.38 (dd, J = 8.3, 1.5 Hz, 1H), 7.34-7.28 (m, 1H), 7.13 (ddd, J = 8.3, 7.4, 1.6 Hz, 1H), 7.11 (t, J = 54.1 Hz, 1H), 4.00 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.55, 148.37, 146.02(t, J = 25.3 Hz), 138.50, 136.28, 132.83, 132.35(\times 2), 130.76, 128.57, 128.06, 125.10, 124.90, 123.43, 123.06, 116.02(t, J = 2.7 Hz), 109.75(t, J = 236.8 Hz), 39.91. HRMS (ESI): calculated for C₁₈H₁₄F₂N₄O₆S [M + Na]⁺: 475.04998, found: 475.04948.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-3-Carboxamido)Phenyl 4-Nitrobenzenesulfonate (T13)

Gray powder, yield 79%. m.p. 160.4-160.9°C. ¹H NMR (400 MHz, CDCl₃) δ 8.70 (t, J = 2.0 Hz, 1H), 8.42 (ddd, J = 8.2, 2.2, 1.1 Hz, 1H), 8.14 (dd, J = 8.2, 1.6 Hz, 1H), 8.11 (d, J = 4.6 Hz, 1H), 7.99 (dt, J = 8.0, 1.3 Hz, 1H), 7.88 (s, 1H), 7.66 (t, J = 8.1 Hz, 1H), 7.32 (ddd, J = 8.3, 7.5, 1.5 Hz, 1H), 7.26 (dd, J = 8.3, 1.5 Hz, 1H), 7.14 (ddd, J = 8.5, 7.4, 1.6 Hz, 1H), 6.92 (t, J = 54.1 Hz, 1H), 3.98 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 158.87, 148.24, 143.29(t, J = 27.7 Hz), 139.43, 136.92, 135.14, 134.01, 130.78, 130.38, 129.07, 128.44, 125.34, 123.96, 123.77, 122.79, 116.11, 111.46(t, J = 234.2 Hz), 39.83. HRMS (ESI): calculated for C₁₈H₁₄F₂N₄O₆S [M + Na]⁺: 475.04998, found: 475.04948.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 4-Nitrobenzenesulfonate (T14)

Light yellow powder, yield 80%. m.p. 198.9-199.6°C. ¹H NMR (400 MHz, CDCl₃) δ 8.24-8.17 (m, 2H), 8.15 (dd, J = 8.3, 1.6 Hz, 1H), 8.07 (s, 1H), 7.99-7.94 (m, 2H), 7.86 (s, 1H), 7.33 (td, J = 7.8, 1.6 Hz, 1H), 7.21 (dd, J = 8.3, 1.6 Hz, 1H), 7.14 (ddd, J = 8.5, 7.3, 1.6 Hz, 1H), 6.88 (t, J = 54.1 Hz, 1H), 3.98 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 158.82, 151.19, 144.20(t, J = 26.5 Hz), 140.75, 139.69, 135.60, 130.30, 130.00(\times 2), 128.42, 125.40, 124.41(\times 2), 124.16, 123.00, 116.31, 111.61(t, J = 234.7 Hz), 39.81. HRMS (ESI): calculated for C₁₈H₁₄F₂N₄O₆S [M + Na]⁺: 475.04998, found: 475.04948.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 2,5-Dichlorobenzenesulfonate (T15)

Light yellow powder, yield 82%. m.p. 155.6-157.3°C. ¹H NMR (400 MHz, CDCl₃) δ 8.40 (s, 1H), 8.35-8.27 (m, 1H), 8.01 (d, J = 2.4 Hz, 1H), 7.95 (s, 1H), 7.57 (dd, J = 8.6, 2.4 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.31 (ddd, J = 8.5, 5.4, 3.5 Hz, 1H), 7.10-7.03 (m, 2H), 7.06 (t, J = 54.1 Hz, 1H), 3.99 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.52, 145.01(t, J = 26.2 Hz), 138.96, 135.74, 134.44, 133.74, 133.54, 133.36, 132.06, 131.70, 131.01, 128.43, 125.11, 123.77, 122.47, 116.62, 110.43(t, J = 235.3 Hz), 39.93. HRMS (ESI): calculated for C₁₈H₁₃Cl₂F₂N₃O₄S [M + Na]⁺: 497.98696, found: 497.98602.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 3,5-Dichlorobenzenesulfonate (T16)

Gray powder, yield 78%. m.p. 128.9–129.5°C. ¹H NMR (400 MHz, CDCl₃) δ 8.27–8.22 (m, 1H), 8.15 (s, 1H), 7.94 (s, 1H), 7.68 (d, J = 1.9 Hz, 2H), 7.56 (t, J = 1.9 Hz, 1H), 7.33 (ddd, J = 8.5, 5.7, 3.3 Hz, 1H), 7.15–7.12 (m, 2H), 7.06 (t, J = 54.0 Hz, 1H), 3.99 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.03, 143.78 (t, J = 25.6 Hz), 139.33, 137.56, 136.50, 134.78, 134.76, 130.61, 128.44, 126.84 (×2), 125.19, 123.82, 122.63, 116.40, 111.22 (t, J = 234.9 Hz), 76.84, 39.89. HRMS (ESI): calculated for C₁₈H₁₃C₂F₂N₃O₄S [M + Na]⁺: 497.98696, found: 497.98602.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 3,4-Dichlorobenzenesulfonate (T17)

Gray powder, yield 79%. m.p. 173.4–174.4°C. ¹H NMR (400 MHz, CDCl₃) δ 8.18 (dd, J = 8.2, 1.5 Hz, 1H), 8.11 (d, J = 4.1 Hz, 1H), 7.92 (d, J = 2.4 Hz, 2H), 7.56–7.43 (m, 2H), 7.32 (ddd, J = 8.5, 7.0, 1.9 Hz, 1H), 7.20–7.09 (m, 2H), 6.93 (t, J = 54.1 Hz, 1H), 3.99 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 158.92, 143.41 (t, J = 28.3 Hz), 139.81, 139.59, 135.04, 134.65, 134.32, 131.42, 130.50, 130.29, 128.31, 127.52, 125.24, 123.89, 123.05, 116.33, 111.39 (t, J = 234.8 Hz), 39.84. HRMS (ESI): calculated for C₁₈H₁₃C₂F₂N₃O₄S [M + Na]⁺: 497.98696, found: 497.98602.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 3,5-Difluorobenzenesulfonate (T18)

Gray powder, yield 86%. m.p. 143.1–144.0°C. ¹H NMR (400 MHz, CDCl₃) δ 8.33–8.12 (m, 2H), 7.94 (s, 1H), 7.45–7.22 (m, 3H), 7.11–7.03 (m, 3H), 7.00 (t, J = 54.0 Hz, 1H), 3.98 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 164.11, 164.00, 161.57, 161.45, 159.20, 144.23, 143.95, 143.68, 139.45, 137.91, 137.82, 137.73, 134.57, 130.68, 128.36, 125.15, 123.86, 122.38, 116.42, 113.44, 112.50, 112.41, 112.30, 112.21, 111.11, 110.83, 110.58, 110.33 (t, J = 235.4 Hz), 39.84. HRMS (ESI): calculated for C₁₈H₁₃F₄N₃O₄S [M + Na]⁺: 466.04606, found: 466.04663.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 2,5-Difluorobenzenesulfonate (T19)

Light yellow powder, yield 80%. m.p. 141.4–141.6°C. ¹H NMR (400 MHz, CDCl₃) δ 8.33 (s, 1H), 8.29 (dd, J = 8.2, 1.6 Hz, 1H), 7.95 (s, 1H), 7.58 (ddd, J = 7.0, 5.2, 3.2 Hz, 1H), 7.43–7.32 (m, 1H), 7.33–7.27 (m, 1H), 7.25–7.20 (m, 1H), 7.18 (dd, J = 8.1, 1.7 Hz, 2H), 7.09 (t, J = 54.1 Hz, 1H), 7.08 (dd, J = 15.6, 1.6 Hz, 2H), 3.99 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.50, 159.06, 156.94, 156.55, 154.39, 145.44, 145.18, 144.92, 138.78, 133.15, 130.87, 128.43, 125.06, 124.42, 124.34, 124.27, 124.19, 124.10, 124.04, 123.55, 122.51, 119.42, 119.34, 119.18, 119.10, 118.33, 118.06, 116.57, 112.73, 110.39, 108.05, 39.91. HRMS (ESI): calculated for C₁₈H₁₃F₄N₃O₄S [M + Na]⁺: 466.04606, found: 466.04663.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 2,4-Difluorobenzenesulfonate (T20)

light yellow powder, yield 79%. m.p. 148.7–149.6°C. ¹H NMR (400 MHz, CDCl₃) δ 8.36 (s, 1H), 8.30 (dd, J = 8.3, 1.6 Hz, 1H), 7.95 (s, 1H), 7.90 (ddd, J = 8.9, 7.8, 5.9 Hz, 1H), 7.30 (ddd, J = 8.5, 7.4, 1.6 Hz, 1H), 7.15 (dd, J = 8.3, 1.6 Hz, 1H), 7.10–7.05 (m, 1H), 7.08 (t, J = 54.1 Hz, 1H), 7.04–6.95 (m, 2H), 4.00 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.46, 145.53, 138.87, 135.14, 133.40, 133.01, 132.81, 132.69, 130.97, 129.09, 129.00, 128.94, 128.76, 128.36, 124.93, 123.41, 123.01, 121.07, 116.49, 112.41, 110.07, 107.73, 39.90. HRMS (ESI): calculated for C₁₈H₁₃F₄N₃O₄S [M + Na]⁺: 466.04606, found: 466.04663.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 2-(Trifluoromethyl)Benzenesulfonate (T21)

Gray powder, yield 69%. m.p. 120.6–121.2°C. ¹H NMR (400 MHz, CDCl₃) δ 8.36 (s, 1H), 8.30 (dd, J = 8.3, 1.6 Hz, 1H), 7.95 (s, 1H), 7.90 (ddd, J = 8.9, 7.8, 5.9 Hz, 1H), 7.30 (ddd, J = 8.5, 7.4, 1.6 Hz, 1H), 7.15 (dd, J = 8.3, 1.6 Hz, 1H), 7.10–7.05 (m, 2H), 7.09 (t, J = 54.1 Hz, 1H), 7.04–6.95 (m, 2H), 4.00 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 168.62, 166.13, 166.02, 162.04, 161.91, 159.48, 159.31, 145.37, 145.11, 144.85, 138.85, 133.57, 133.46, 133.26, 130.97, 128.36, 125.00, 123.51, 122.57, 119.62, 119.49, 116.67, 112.78, 112.75, 112.56, 112.53, 110.44, 108.10, 106.65, 106.41, 106.39, 106.15, 39.92. HRMS (ESI): calculated for C₁₉H₁₄F₅N₃O₄S [M + Na]⁺: 498.05229, found: 498.05078.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-3-Carboxamido)Phenyl 3-(Trifluoromethyl)Benzenesulfonate (T22)

Light yellow powder, yield 73%. m.p. 147.2–148.3°C. ¹H NMR (400 MHz, CDCl₃) δ 8.27–8.15 (m, 2H), 8.12 (s, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.91 (s, 1H), 7.87 (d, J = 7.9 Hz, 1H), 7.62 (t, J = 7.9 Hz, 1H), 7.29 (ddd, J = 8.6, 5.8, 3.1 Hz, 1H), 7.13–7.03 (m, 2H), 6.96 (t, J = 54.1 Hz, 1H), 3.97 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.09, 144.17, 143.89, 143.62, 139.32, 136.04, 134.52, 132.64, 132.30, 131.96, 131.82, 131.63, 131.46, 131.43, 131.39, 131.36, 130.75, 130.30, 128.28, 125.69, 125.65, 125.62, 125.58, 125.02, 124.22, 123.59, 122.64, 121.50, 116.36, 113.43, 111.11, 108.78, 39.79. HRMS (ESI): calculated for C₁₉H₁₄F₅N₃O₄S [M + Na]⁺: 498.05229, found: 498.05078.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 4-Methoxybenzenesulfonate (T23)

Gray powder, yield 83%. m.p. 130.0–131.1°C. ¹H NMR (400 MHz, CDCl₃) δ 8.35 (s, 1H), 8.28 (dd, J = 8.2, 1.6 Hz, 1H), 7.91 (s, 1H), 7.78–7.70 (m, 2H), 7.30–7.23 (m, 1H), 7.09 (t, J = 54.0 Hz, 1H), 7.01 (td, J = 7.8, 1.6 Hz, 1H), 6.93–6.86 (m, 3H), 4.00 (s, 3H), 3.86 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 164.70, 159.36, 144.97 (t, J = 25.8 Hz), 139.56, 133.27, 131.15, 131.02 (×2), 127.90, 125.68, 124.72, 123.18, 122.93, 116.83, 114.64 (×2), 110.48 (t, J = 235.4 Hz), 55.93, 39.90. HRMS (ESI): calculated for C₁₉H₁₇F₂N₃O₅S [M + Na]⁺: 460.07547, found: 460.07503.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl Phenylmethanesulfonate (T24)

Light yellow powder, yield 83%. m.p. 123.4–124.2°C. ¹H NMR (400 MHz, CDCl₃) δ 8.35 (dd, J = 8.3, 1.6 Hz, 1H), 8.27 (s, 1H), 7.68 (s, 1H), 7.51–7.44 (m, 2H), 7.40 (dd, J = 5.0, 2.0 Hz, 3H), 7.31 (td, J = 7.9, 1.5 Hz, 1H), 7.20 (m, 1H), 7.15 (t, J = 54.0 Hz, 1H), 7.09 (td, J = 7.8, 1.6 Hz, 1H), 7.02–6.97 (m, 1H), 4.65 (s, 2H), 3.97 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.54, 145.26(t, J = 25.8 Hz), 138.25–132.95, 131.24, 131.09(×2), 129.67, 129.24, 128.23(×2), 126.88, 125.03, 123.19, 122.90, 115.83, 110.39 (t, J = 235.4 Hz), 57.29, 39.92. HRMS (ESI): calculated for C₁₉H₁₇F₂N₃O₄S [M + Na]⁺: 444.08055, found: 448.07975.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl Naphthalene-2-Sulfonate (T25)

Light yellow powder, yield 80%. m.p. 158.7–159.5°C. ¹H NMR (400 MHz, CDCl₃) δ 8.43 (d, J = 1.9 Hz, 1H), 8.30–8.17 (m, 2H), 7.94–7.81 (m, 3H), 7.71 (ddd, J = 13.8, 8.5, 1.6 Hz, 2H), 7.65–7.58 (m, 2H), 7.31–7.21 (m, 1H), 7.04–6.99 (m, 2H), 6.98 (t, J = 54.1 Hz, 1H), 3.90 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.11, 144.39(t, J = 25.7 Hz), 139.66, 135.70, 133.52, 131.91, 131.81, 130.94, 130.72, 129.89, 129.82, 129.55, 128.15, 128.01, 128.00, 124.87, 123.32, 123.04, 122.76, 116.48, 110.66 (t, J = 234.8 Hz, 1H), 39.76. HRMS (ESI): calculated for C₂₂H₁₇F₂N₃O₄S [M + Na]⁺: 480.08055, found: 448.08005.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 2,4,6-Trimethylbenzenesulfonate (T26)

White powder, yield 81%. m.p. 155.0–155.4°C. ¹H NMR (400 MHz, CDCl₃) δ 8.56 (s, 1H), 8.35 (dd, J = 8.3, 1.6 Hz, 1H), 7.95 (s, 1H), 7.28–7.23 (m, 1H), 7.15 (t, J = 54.1 Hz, 1H), 7.01 (s, 2H), 6.91 (td, J = 7.8, 1.6 Hz, 1H), 6.52 (dd, J = 8.2, 1.5 Hz, 1H), 4.01 (s, 3H), 2.55 (s, 6H), 2.35 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.50, 145.40 (t, J = 25.3 Hz), 144.77, 140.77(×2), 139.25, 132.64, 132.05(×2), 131.52, 129.59, 127.78, 124.57, 123.25, 121.97, 116.80, 110.09 (t, J = 234.8 Hz, 1H), 39.85, 22.85(×2), 21.23. HRMS (ESI): calculated for C₂₁H₂₁F₂N₃O₄S [M + Na]⁺: 472.11185, found: 472.11150.

2-(3-(Difluoromethyl)-1-Methyl-1H-Pyrazole-4-Carboxamido)Phenyl 4-(Tert-butyl)Benzenesulfonate (T27)

Light yellow powder, yield 82%. m.p. 149.7–150.5°C. ¹H NMR (400 MHz, CDCl₃) δ 8.41 (s, 1H), 8.31 (dd, J = 8.3, 1.6 Hz, 1H), 7.94 (s, 1H), 7.81–7.73 (m, 2H), 7.56–7.46 (m, 2H), 7.26 (td, J = 7.8, 1.5 Hz, 1H), 7.11 (t, J = 54.1 Hz, 1H), 7.00 (td, J = 7.9, 1.6 Hz, 1H), 6.89 (dd, J = 8.2, 1.5 Hz, 1H), 4.00 (s, 3H), 1.32 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 159.33, 159.29, 145.04(t, J = 25.2 Hz), 139.43, 133.31, 131.48, 131.22, 128.57(×2), 127.93,

126.46(×2), 124.66, 123.12, 122.75, 116.81, 110.45 (t, J = 236.3 Hz), 39.91, 35.54, 31.05(×3). HRMS (ESI): calculated for C₂₂H₂₃F₂N₃O₄S [M + Na]⁺: 486.12750, found: 486.12686.

In Vitro Biological Evaluation

In Vitro Antifungal Assay

The test strains were *Colletotrichum camelliae* (C. camelliae), *Pestalotiopsis theae* (P. theae) provided by Guizhou Tea Research Institute, and *Gibberella zeae* (G. zeae), *Rhizoctonia solani* (R. solani) provided by Guizhou Institute of Plant Protection. In this study, the *in vitro* antifungal activity of the target compounds **T1–27** against four plant pathogens was screened by the mycelial growth rate method (Zhang et al., 2019). The tested compounds were dissolved in DMSO to prepare a 10 mg/ml stock solution before mixing with PDA. The PDA containing compounds at a concentration of 50 mg/L were then poured into sterilized Petri dishes for primary screening. Data Processing System (DPS, V9.50) was used for statistical analysis of test data, and Duncan's new multiple range method was used to test the significance of differences. The EC₅₀ values and 95% confidence limits were calculated after testing the inhibition rates, based on the above method. The inhibition rate of the potent compounds was further tested and the corresponding EC₅₀ values were calculated by using DPS. This test method is provided in the Supporting information.

In Vivo Antiviral Activities Assay

The *in vivo* antiviral activities of target compounds **T1–27** against TMV were tested by the half leaf blight spot method previously reported in the literature (Chen et al., 2021; Xie et al., 2018). TMV was propagated in *Nicotiana tabacum* cv. K326 by the Gooding method. Antiviral activities of the target compounds against TMV *in vivo* were at 500 mg/L. The commercial antiviral agents Ningnanmycin and Chitosan oligosaccharides were served as the positive controls. Data is processed in the same way as that of antifungal activity.

RESULTS AND DISCUSSION

Chemistry

The reaction between the starting material, ethyl 4,4-difluoro-3-oxobutanoate (**1**) and triethyl orthoformate in acetic anhydride at 140°C, yielded ethyl 2-(ethoxymethylene)-4,4-difluoro-3-oxobutanoate (compound **2**) (Sun and Zhou, 2015). Compound **2** was then treated with methylhydrazine to yield compound **3**, which was successively hydrolyzed with lithium hydroxide and hydrochloric acid to obtain a white solid of the key intermediate 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid (compound **4**) (Scheme 1). Thereafter, compound **6**, a light yellow solid, was formed by conjugating compound **4** with 2-aminophenol in CH₂Cl₂ using EDCI and DMAP (Scheme 2). Finally, different substituted moieties of arylsulfonyl chloride were reacted with compound **5** to yield

the target compounds (Scheme 3). The structures of all key intermediates and target compounds were confirmed *via* ^1H and ^{13}C NMR and HRMS, and their spectra data are shown in the **Supplementary Material**. The single-crystal X-ray diffraction of compound **T22** showed that the compound is a sulfonate and not a sulfonamide. **Figure 1** shows the crystal structure of **T22**, whose deposition number is CCDC 2168151.

In Vitro Biological Evaluation

In Vitro Antifungal Assay

The preliminary *in vitro* antifungal activities of the 27 target compounds are presented in **Tables 1, 2**. Most of the target compounds exhibited some degree of antifungal activities against the four plant pathogens at 50 $\mu\text{g}/\text{ml}$ (**Table 1**). Among the four plant pathogens, the target compounds, particularly **T24**, exhibited remarkable antifungal activity against *R. solani*. When R group was nitro group, the antifungal activity against *R. solani* was no more than 20%. It can be known from these data that the substituent on the benzene ring was a strong electron-withdrawing group, the antifungal activity was adversely affected. We also found that the activity of **T24** against *R. solani* was much higher than that of **T1** (**Table 1**). The only structural difference between these two compounds is the presence of an extra methylene group in **T24**, which is thought to enhance its antifungal activity. The compound **T24** ($\text{EC}_{50} = 0.45 \text{ mg}/\text{L}$) was superior to the commercial fungicide hymexazol ($\text{EC}_{50} = 10.49 \text{ mg}/\text{L}$), but closer to bixafen ($\text{EC}_{50} = 0.25 \text{ mg}/\text{L}$) in its activity against *R. solani* (**Table 2**).

In Vivo Antiviral Activities of Compounds T1-27

The phenylsulfonyl fragment has been reported to increase the antiviral activity (Hadházi et al., 2017), we synthesised novel sulfonate scaffold-containing pyrazolecarbamide and evaluated their antiviral activities. The curative, protective, and inactivation effects of the 27 target compounds against TMV were evaluated using the half leaf blight spot method (Liu et al., 2021; Zhang et al., 2021), and the commercial agents, Ningnanmycin and Chitosan oligosaccharide, served as positive controls. Compound **T18** (54.2%) exhibited a close curative activity to ningnanmycin (55.3%) at 500 mg/ml . Additionally, most of the target compounds exhibited protective effects *in vivo*, and the protective effects of compounds **T5** (50.4%) and **T12** (50.2%) were similar to that of Ningnanmycin (50.7%). Although the target compounds had lower inactivation effects than ningnanmycin, most of them exhibited better inactivation activities than Chitosan oligosaccharides (**Table 3**).

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CONCLUSION

In summary, 27 novel pyrazolecarbamide derivatives bearing a sulfonate fragment were synthesized and screened for their *in vitro* antifungal and *in vivo* antiviral activities against four plant pathogens (*C. camelliae*, *P. theae*, *G. zeae*, and *R. solani*). The structures of these compounds were identified using the single-crystal X-ray diffraction and spectral data obtained *via* ^1H and ^{13}C NMR and HRMS spectroscopy. The preliminary bioassay results showed that the target compounds exhibited certain inhibitory activities against the test fungi and TMV. Compound **T24** exhibited excellent antifungal activities against *R. solani* compared to the commercial fungicide hymexazol, almost similar to bixafen. Moreover, the target compounds displayed protective effects *in vivo* against TMV. Thus, our research group is conducting further structural optimization of the target compounds for wide-scale field application.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Z-WL and WY conceived and designed the paper. Z-WL and HL contributed to the synthesis, purification, characterization of all compounds. JY and CM performed the biological activity research. Z-WL wrote the manuscript. All authors have read and reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fchem.2022.928842/full#supplementary-material>

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