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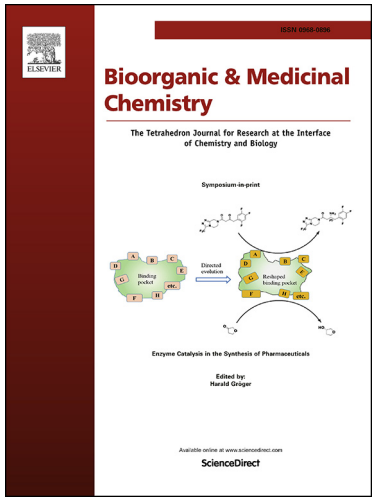

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Graphical Abstract

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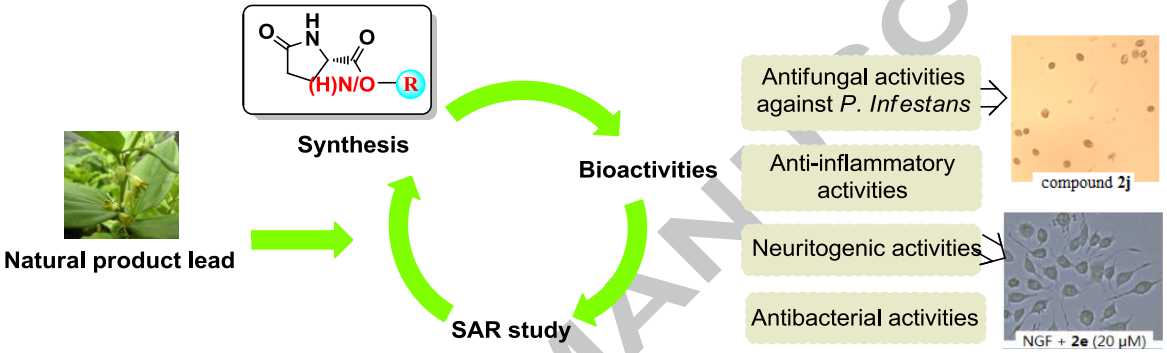

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ABSTRACT

A series of L-pyroglutamic acid analogues from natural product lead were designed and synthesized, as well as their antifungal activities against *Phytophthora infestans*, neuritogenic activities, antibacterial activities and anti-inflammatory activities are described. The bioassays and SAR study showed that the majority of L-pyroglutamic acid esters have a significant antifungal activity against *P. infestans*, especially **2d** and **2j** demonstrated the best activities with EC_{50} values of 1.44 and 1.21 $\mu g \cdot mL^{-1}$, which were about seven times that of commercial azoxystrobin (7.85 $\mu g \cdot mL^{-1}$). Moreover, compounds **2e**, **2g** and **4d** displayed anti-inflammatory activity against LPS-induced NO production in BV-2 microglial cells; neuritogenic activity in NGF-induced PC-12 cells is the same activity. This study demonstrates that compounds **2d** and **2j** are potential drugs to control *P. infestans*.

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1. Introduction

Plant is a natural molecular manufacturing factory and a superb organic chemist. There is no doubt that natural products are a treasure trove of drug development, giving a constant stream of inspiration to human drug design. Many plant-derived biologically active natural compounds origin possess insecticidal,[1-2] antibacterial,[3] anti-microbial,[3-4] anti-tumor,[5] anti-malarial,[6] anti-tuberculosis[7] or nematocidal[8] activities, and can be considered as ideal lead in the development of agricultural or medical chemicals.[9-14] Moreover, some of the secondary metabolites in plants are formed in order to resist stress damage during the long-term survival competition. Typically, they are unique new structures; tend to have very good biological activity; better compatibility with the environment. All these advantages make pesticide scientists, medical experts and toxicologists pay more attention on the traditional herbal.

L-pyroglutamic acid (**1**), according to our previous study, is a biologically active substance that has been isolated from *Disporopsis aspersa* (H_{UA}) E_{NGL}. ex D_{IELS} against *Pseudoperonospora cubensis* and *Phytophthora infestans*.[15] Significantly, L-pyroglutamic acid showed excellent antifungal activity against *P. infestans* and *P. cubensis* with EC_{50} values of 9.48, 10.82 μg/mL, respectively. In addition, **1** also exhibited dual effect of protection and treatment against *P. cubensis in vivo*.

In recent years, complex compounds contained L-pyroglutamic acid analogues, which exhibited human farnesyltransferase inhibitory activity[16], anti-HIV-1 and anti-HCV activity[17], antioxidant activity[18], Gram-negative antibacterial activity[19-21], anti-colitic activity[22], anti-inflammatory activity on RAW 264.7 macrophages[23], antidepressant-like activity[24] and antiplatelet efficacy (*in vitro*) [25], have received great attention from chemists and biologists.

Motivated by the above findings and to continue our efforts in studying the structure−activity relationships of L-pyroglutamic acid (**1**) toward exploring lead compounds for botanical medicines,[26] we herein designed and synthesized 42 L-pyroglutamic acid analogues, and their biological activity was systematically evaluated. To the best of our knowledge, this is the first report on antifungal activity against *P. infestans*, neuritogenic activity and anti-inflammatory activity of this series of pyroglutamic acid derivatives.

2. Results and discussion

2.1. Chemistry

Synthesis of the target compounds **1a-h**, **2a-k**, **3a-j**, **4a-k**, and **5a-5b** was performed as illustrated in **Scheme 1**. Briefly, various precursor L-pyroglutamic acid derivatives were obtained from L-pyroglutamic acid and the different substituted alcohols/phenols/amines as the starting materials in one-pot with satisfactory

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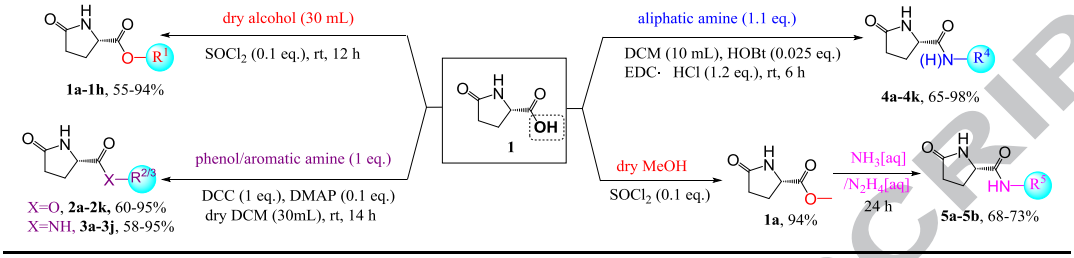
yields (55% to 95%). Of these, 32 compounds were synthesized for the first time. In addition, during the reaction, the stereochemistry of these products did not change, and still maintained the same configuration as L-pyroglutamic acid.

The structures of synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, MS. The physical characteristics, ^{1}H NMR, ^{13}C NMR, MS data for target compounds are reported in the *Supporting Information*, and the data of representative compound **1g** are shown below.

(S)-Prop-2-yn-1-yl 5-oxopyrrolidine-2-carboxylate: (**1g**)

Yield: 87%, yellowish oil. $[\alpha]_D^{24}$ –8.2° (*c* 1.0, DMSO). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.02 (s, 1H), 4.78 (d, *J* = 1.4 Hz, 2H), 4.26 (dd, *J* = 9.1, 4.1 Hz, 1H), 3.51 (t, *J* = 2.4 Hz, 1H), 2.49 – 2.33 (m, 1H), 2.29 – 2.07 (m, 2H), 2.05 – 1.99 (m, 1H).**^{13}C NMR** (125 MHz, DMSO-d_6) δ 177.7, 172.7, 78.3, 75.0, 55.1, 52.9, 29.3, 24.9. **MS** (ESI, MeOH) m/z calcd for $C_8H_9NO_3$ (M + H) $^+$: 168.06; found 168.06.



1a: R^1 = CH_3
1b: R^1 = C_2H_5
1c: R^1 = *n*-C_3H_7
1d: R^1 = *n*-C_4H_9
1e: R^1 = *i*-C_3H_7
1f: R^1 = *i*-C_4H_9
1g: R^1 = C_3H_3
1h: R^1 = C_3H_5

2a: R^2 = Ph
2b: R^2 = 2-NO_2-Ph
2c: R^2 = 2-Cl-Ph
2d: R^2 = 4-Cl-Ph
2e: R^2 = 4-OH-Ph
2f: R^2 = 2-CH_3-Ph
2g: R^2 = 2-CH_3-4-Cl-Ph
2h: R^2 = 2-C_2H_5COO-Ph
2i: R^2 = 2-*i*-propyl-5-CH_3-Ph
2j: R^2 = naphthyl
2k: R^2 = 2-CN-Ph

3a: R^3 = Ph
3b: R^3 = 4-CH_3-Ph
3c: R^3 = 4-C_2H_5O-Ph
3d: R^3 = 4-NO_2-Ph
3e: R^3 = 4-F-Ph
3f: R^3 = 2-F-Ph
3g: R^3 = 2-NH_2-Ph
3h: R^3 = 4-CF_3-Ph
3i: R^3 = 2-CH_3COO-Ph
3j: R^3 = 2-OH-Ph

4a: R^4 = CH_3
4b: R^4 = *n*-C_3H_7
4c: R^4 = *n*-C_4H_9
4d: R^4 = C_3H_5
4e: R^4 = C_3H_3
4f: R^4 = $(CH_3)_2$
4g: R^4 = $(CH_2CH_3)_2$
4h: R^4= *i*-C_3H_7
4i: R^4 = *t*-C_4H_9
4j: R^4 = tryptamino
4k: R^4 = 1-CH_3-piperazino

5a: R^5 = H
5b: R^5 = NH_2

Scheme 1. Synthesis of L-pyroglutamic acid analogues.

2.2. *Antifungal activity against P. infestans*

In this study, the target 43 compounds were evaluated for their antifungal activity against *P. infestans* via inhibition of zoospore release assay[27-28] at concentrations of 100 μg/mL (**Figure 1**). The results of the preliminary bioassays exhibited that compounds **1**, **1b**, **1c**, **1d**, **2a**, **2b**, **2c**, **2d**, **2j**, **2h** and **2k** have excellent antifungal activities against *P. infestans*, and similar to the positive control with inhibition rate as 99.23%. As can be seen in **Table 1**, most of L-pyroglutamic acid esters (**1a-1h**, **2a-2k**) showed good activities except **1g** and **2e**, which were superior to the activities of L-pyroglutamines (**3a-3j**, **4a-4k**, **and 5a-5b**). Moreover, the antifungal activities of L-pyroglutamic acid esters containing fatty chains (**1a-f**) are slightly lower than that of phenols (**2a-k** except **2e**).

Table 1 Inhibitory effect of L-pyroglutamic acid derivatives at a concentration of 100 μg/mL on zoospore release of *P. infestans*

compound	Inhibition rate ± SD (%)[b]	compound	Inhibition rate ± SD（%）	compound	Inhibition rate ± SD (%)[b]
azoxystrobin [a]	99.23 ± 0.88	**2f**	48.56 ± 1.49	**3j**	33.68 ± 2.75
1	98.85 ± 2.17	**2g**	39.75 ± 3.06	**4a**	1.17 ± 0.97
1a	26.10 ± 0.95	**2h**	98.22 ± 2.12	**4b**	-
1b	95.81 ± 2.01	**2i**	13.16 ± 1.56	**4c**	17.76 ± 2.64
1c	95.12 ± 1.15	**2j**	98.47 ± 0.90	**4d**	49.32 ± 3.11
1d	98.95 ± 1.68	**2k**	98.32 ± 2.51	**4e**	-
1e	36.46 ± 3.10	**3a**	-	**4f**	44.26 ± 2.91
1f	64.90 ± 0.66	**3b**	-	**4g**	35.78 ± 1.79
1g	-	**3c**	-	**4h**	-
1h	84.19 ± 4.10	**3d**	47.52 ± 3.44	**4i**	-
2a	98.27 ± 2.04	**3e**	-	**4j**	21.52 ± 2.60
2b	96.34 ± 1.45	**3f**	-	**4k**	-
2c	98.39 ± 1.67	**3g**	-	**5a**	13.58 ± 2.14
2d	98.09 ± 1.33	**3h**	-	**5b**	-
2e	-	**3i**	2.37 ± 1.33		

[a]Positive control.
[b]Values represent the means ± SD based on three independent experiments.

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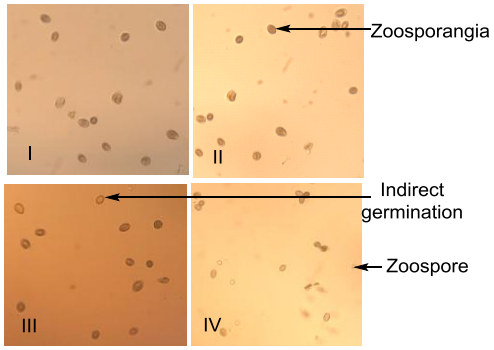

Figure 1. Effect on indirect germination (zoospore release from zoosporangia), zoospore motility, and cystospore germination: I compound **2j**; II compound **2d**; III L-pyroglutamic acid; IV blank control

To further determine the activity and structure-activity relationship of the target compounds, the EC_{50} values of compounds with activities greater than 90% were determined in this study. As shown in **Table 2**, compounds **2d** and **2j** revealed better activity against *P. infestans* with EC_{50} values of 1.44 and 1.21 $\mu g \cdot mL^{-1}$; these values are even better than pyroglutamic acid (7.85 $\mu g \cdot mL^{-1}$) and the positive control azoxystrobin (1.98 $\mu g \cdot mL^{-1}$). Moreover, compounds **2b** and **2k** exhibited relatively good antifungal activity, comparable to the positive control at the same magnitude as L-pyroglutamic acid. In addition, the introduction of straight-chain alcohols (**1b**, **1c** and **1d**) and phenols (**2a-2d, 2h, 2j** and **2k**) on the pyroglutamate ring led to a significant increase in antifungal activity against *P. infestans*. For the effect of substituents on phenols, available data show that the electron-withdrawing groups were useful for fungicidal activity increasing.

Table 2 EC_{50} values of **L**-pyroglutamic acid derivatives against *P. infestans*

Compound	Toxic regression equation	R^2 (Chi-square value)	EC_{50} ($\mu g \cdot mL^{-1}$)[b]
Azoxystrobin [a]	y= 3.23x + 4.04	0.995	1.98 ± 0.50
1	y= 2.58x + 2.69	0.988	7.85 ± 0.58
1b	y= 3.12x + 1.31	0.991	15.25 ± 0.74
1c	y= 1.67x + 3.17	0.975	12.43 ± 1.22
1d	y= 3.05x + 1.80	0.969	11.20 ± 0.65
2a	y= 2.14x + 2.24	0.990	19.51 ± 0.25
2b	y= 3.71x + 1.54	0.989	8.58 ± 0.88
2c	y= 2.89x + 1.64	0.996	14.56 ± 1.02
2d	y= 1.09x + 4.83	0.992	1.44 ± 0.33
2h	y= 2.28x + 2.23	0.993	16.42 ± 0.81
2j	y= 1.12x + 4.91	0.984	1.21 ± 0.67
2k	y= 1.55x + 4.02	0.984	9.62 ± 0.36

[a]Positive control.
[b]Values represent the means ± SD based on three independent experiments.

2.3. Anti-inflammatory activity

The title compounds were evaluated for their inhibitory activities against NO production induced by lipopolysaccharide (LPS) in BV-2 microglial cells using the Griess assays[29-31]. Comparison of the inhibitory effects of L-pyroglutamines, pyroglutamic acid esters have better activities, and with the increase of chain growth activity (**Figure 2**). It is worth noting that allyl and propargyl are very useful groups for inhibitory activity against NO production, and the propargyl activity is higher than the allyl group.

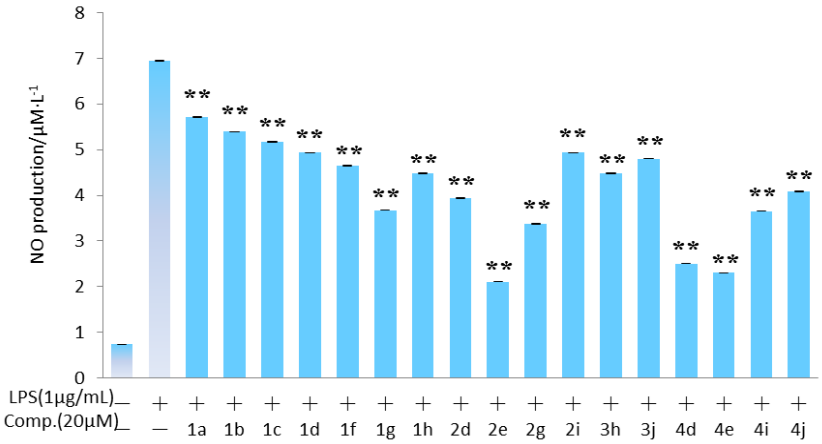

Figure 2. Inhibitory effects of L-pyroglutamic acid derivatives against LPS-induced NO production in BV-2 cells; Data represent the mean ± SD of three dependent experiments; ** P <0.01 represent differences compared with LPS-induced NO production in BV-2 cells

As illustrated in **Table 3**, compounds **2e**, **2g**, **4d** and **4e** showed anti-inflammatory activities with IC_{50} values in the range of 4.5-19.4 μM. Among them, the value of **2e** is basically the same as the positive control quercetin (IC_{50} = 4.3 μM), a well-known NO inhibitor[29-30]. These 4 compounds did not show significant cytotoxicity with LPS treatment for 24 hours using SRP assay, which indicates that inhibition activity of the active compounds and their cytotoxicity independent.

Table 3 IC_{50} values of the active compounds against LPS-induced NO production in BV-2 cells

compound	IC_{50} (μM) [a]	cell viability (%) [b]
2e	4.5 ± 0.9	92.6
2g	19.4 ± 1.2	91.1
4d	9.1 ± 0.6	95.4
4e	6.0 ± 0.8	89.5
quercetin [c]	4.3 ± 0.3	99.5

[a]Values represent the means ± SD based on three independent experiments.
[b]Cell viability was expressed as a percentage (%) of control group.
[c]Positive control.

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2.4. Neuritogenic activity

In this study, the neuritogenic activities of target compounds were measured using rat pheochromocytoma (PC-12) cells as a neuronal differentiation model[32-33] and are given in **Figure 3**. Among the target compounds, **1g**, **2e**, **2g**, **2h**, **2i**, **4d**, **4i**, **4j** and **5b** in combination with NGF (20 ng/mL) showed different degrees of increase in neurite bearing cells compared to positive control. Significantly, the percentage of neurite-bearing NGF-induced PC-12 cells treated with 20 μM **2e** and **4d** reached 15.443 ± 0.49% and 14.962 ± 0.36%, respectively. These were compatible with positive control (11.90 ± 0.25%) and far higher than negative control (0.77 ± 0.13%). In addition, the vast majority of test pyroglutamic acid derivatives contained fatty alcohols or aromatic amines had no effect on neurite outgrowth in NGF-induced PC-12 cells.

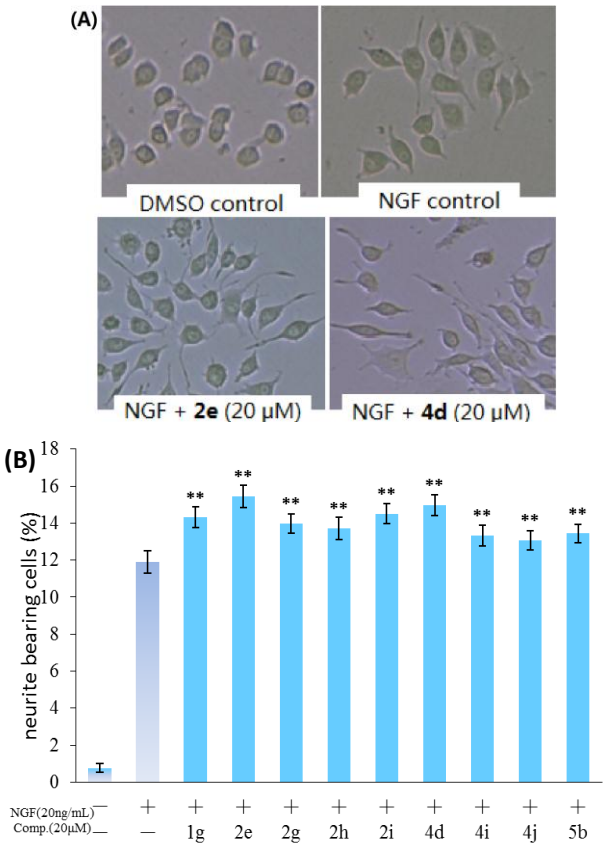


Figure 3. Effects of compounds **1g**, **2e**, **2g**, **2h**, **2i**, **4d**, **4i**, **4j** and **5b** on the neurite outgrowth in NGF-induced PC-12 cells; (A) Cell morphology (B) Neurite bearing cells analyze. Data represent the mean ± SD of three dependent experiments. ** P <0.01 represent differences compared with NGF-treated PC-12 cells.

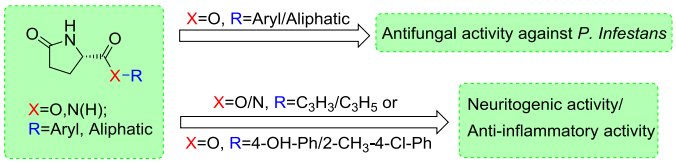
2.5. Antibacterial activity

To evaluate antibacterial activities of our compounds against 5 bacteria (*B. thuringiensis*, *S. aureus*, *B. cereus*, *R. solanacearum* and *E. carotovora*), we adapted a commonly-used way namely filter paper dispersion method[34-36], and then quantifying the size and transparency of the inhibitory ring as shown in *Supporting Information*, **Table S.1.** In general, L-pyroglutamines have higher activity than L-pyroglutamic acid esters, and derivatives containing benzene rings higher than fatty chains. Moreover, in the bacterial bioassay experiments, the title compound showed relatively high activities against *B. thuringiensis*, while showing lower activities against *S. aureus*, *B. cereus*, *R. solanacearum* and *E. carotovora*.

2.6. Structure – activity relationship analysis (SAR)

Based on the above bioassay experiments, we further discuss its structure-activity relationship (**Scheme 2**). Concerning the presence of L-pyroglutamic acid derivatives involved alcohols or phenols, the corresponding compounds exhibited good antifungal activity against *P. infestans* (**Table 1-2**), wherein L-pyroglutamate containing alcohol has a lower antifungal activity, and significant increase in activities with the growth of the carbon chain (**1d**>**1c**>**1b**>**1a**), branched reduced (**1d**>**1f, 1c** > **1e**). In particular, for aryl pyroglutamate, the activity of naphthyl is higher than the phenyl, and electron-withdrawing group on the aromatic ring has a significant effect on the activity enhancement (**2b**, **2c**, **2d**, **2h** and **2k**). In addition, alkynyl group introduced to title compounds (**1g** and **4e**) is unfavorable for antibacterial activity against *P. infestans*, which is contrary to cellular activities (**Table 3** and **Figure 2-3**). Compounds **1g**, **2e**, **2g** and **4d** displayed anti-inflammatory against LPS-induced NO production in BV-2 microglial cells; same as neuritogenic activity in NGF-induced PC-12 cells. L-pyroglutamic acid derivatives have no significant activity against 5 bacteria (*B. thuringiensis*, *S. aureus*, *B. cereus*, *R. solanacearum* and *E. carotovora*).

In a word, the SAR analysis demonstrated pyroglutamates derivatives contained naphthol, long-chain alcohol or electron-withdrawing group have a significant meaning for the design of drugs against *P. infestans*, and C_3H_3/C_3H_5 or 4–OH/2-CH_3-4-Cl on the phenyl ring of phenols is beneficial for the increase of anti-inflammatory activity/neuritogenic activity.



Scheme 2. SAR study of title compounds.

3. Conclusion

In summary, a series of L-pyroglutamic acid derivatives were synthesized and their antifungal activity against *P. infestans*, neuritogenic activity, anti-inflammatory activity and antibacterial activity were systematically evaluated. From the bioassay results and the structure – activity relationship (SAR) analysis, most of L-pyroglutamic acid esters have good antifungal activity against *P. infestans*. Notably, compounds **2d** and **2j** demonstrated the best activities with EC_{50} values of 1.44 and 1.21 $\mu g \cdot mL^{-1}$, respectively, which were superior to commercial azoxystrobin. Moreover, compounds **2e**, **2g** and **4d** displayed anti-inflammatory against LPS-induced NO production in BV-2 microglial cells; same as neuritogenic activity in NGF-induced PC-12 cells.

To the best of our knowledge, this is the first report on the antifungal, neuritogenic and anti-inflammatoryof L-pyroglutamic acid derivatives. In particular, compounds **2d** and **2j** can be used as a potential drug for controlling *P. infestans*. Further detailed mechanism and field experiments are ongoing.

4. Experimental section

4.1. General

All reagents were of analytical grade or chemically pure. Melting points were determined on a WRS-1B melting point

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apparatus (Shanghai YiCe Apparatus and Equipment Co., Ltd, Shanghai, China) and are uncorrected; A Bruker AMX-500 spectrometer was used to record ^{1}H NMR and ^{13}C NMR, spectra (solvent $CDCl_3$, DMSO-d_6) with tetramethylsilane (TMS) as an internal standard at room temperature; thin-layer chromatography (TLC) analysis was carried out on GF254 silica gel plates, and the spots were coloured by an ultraviolet lamp or 0.5% ninhydrin in ethanol or 0.1 g/mL phosphomolybdic acid in ethanol; All compounds were purified by using Silica gel (200–300 mesh.

4.2. Chemical synthesis

4.2.1 General procedure for the synthesis of L-pyroglutamic acid esters (**1a-1h**):

To a solution of L-pyroglutamic acid (10 mmol) in anhydrous alcohol (30 mL) was added catalytic amount of $SOCl_2$ (1 mmol). The reaction mixture was stirred at room temperature for 12 h until no change was observed by TLC. After evaporating the solvent under reduced pressure, the residue was dissolved in EtOAc (100 mL), stirred over K_2CO_3, and dried with $MgSO_4$ to afford the crude product. Then the residue passed through a flash column chromatography (MeOH/ CH_2Cl_2=1/10) on silica gel and removal of all volatiles were in vacuo to give the pure products.

4.2.2 General procedure for the synthesis of L-pyroglutamic acid ester (**2a-2k**) and L-pyroglutamines (**3a-3j**):

To a round bottom flask with a stirring bar, L-pyroglutamic acid (10 mmol), phenol/aromatic amine (11 mmol) and DMAP (1 mmol) in dry CH_2Cl_2 (30 mL) was stirred for 10 min, followed by addition of DCC (11 mmol). The mixture was allowed to stir at room temperature overnight. The reaction was filtered through a coarse frit and the filtrate diluted with CH_2Cl_2 (50 mL), washed with brine (3 × 20 mL), dried over $MgSO_4$, and concentrated to dryness. The crude mixture was purified using flash column chromatography (MeOH/ CH_2Cl_2=1/30) to give the pure products.

4.2.3 General procedure for the synthesis of L-pyroglutamines (**4a-4k**):

The reaction was conducted with fatty amine (11 mmol), L-pyroglutamic acid (10 mmol), HOBt (0.25 mmol) and EDC·HCl (12 mmol) in dry CH_2Cl_2 (30 mL) was stirred for 6 h at room temperature. After the completion of the reaction, as monitored by TLC, the reaction was diluted with 10% NaCl, extracted with CH_2Cl_2 (3 × 30 mL), dried over $MgSO_4$, organic layer was collected and concentrated to dryness. The reaction residues were purified by flash column chromatography on silica (MeOH/ CH_2Cl_2=1/2) to provide pure products.

4.2.4 General procedure for the synthesis of L-pyroglutamines (**5a-5b**):

Stirred L-pyroglutamic acid methyl ester (10 mmol) in NH_3 [aq] / N_2H_4 [aq] (50 mL) for 12 h at room temperature. Then the mixture was allowed to stand for one day at -10 °C. Next the crystallization washed with ethanol, ether, dried over $MgSO_4$, The crude mixture was purified by flash column chromatography on silica (MeOH/ CH_2Cl_2=1/6) to provide pure product as white solid.

4.3. Antifungal activity against P. infestans

A zoosporangia suspension (5×10^4 zoosporangia/mL) was used to evaluate the effects on title compounds against *P. infestans*. Sporulation has taken gently from *P. infestans* to prepare spore suspension with a concentration of each field 60-70 best. Next, spore suspension (0.1 mL) and compounds solution (0.1 mL, 0.2 mg/mL) were added to 0.5 mL EP tube separately. After mixing, they were placed in a 4 °C environment and cultured in darkness for 4 h. Solvent as negative control and azoxystrobin as positive control. The zoospore release was recorded and the inhibition rate was calculated when the control spore release rate reached 90%. Toxicity bioassay was then performed the concentration for 50% of maximal effect (EC_{50}) of compounds with inhibition rate > 50%. Inhibition rate (%) [36] was calculated as equation (1):

$$\text{Inhibition rate (\%)} = \frac{P_0 - P_1}{P_0} \times 100 \quad (1)$$

Where P_0 is control germination rate; P_1 is treated-groups germination rate

4.4. Anti-inflammatory activity and cell viability

LPS-induced BV-2 microglial cells as an inflammation model were plated into a 96-well plate at a density of 1×10^5 cells/mL. After 24 h incubation, cells were pretreated with target compounds at the specified concentration and stimulated with 1 μg/mL LPS for another 24 h. Quercetin was used as positive control. The cell-free supernatant (50 μL) was mixed with 100 μL Griess reagent, and the absorbance was measured at 540 nm using a microplate reader. Cell viability was evaluated using an SRB assay.

4.5. Neuritogenic activity

All target compounds were evaluated for their neuritogenic activity by rat pheochromocytoma (PC-12) cells as a neuronal differentiation model. The evaluation was carried out using morphological analysis and quantification of neurite-bearing cells. PC-12 cells were seeded in poly-L-lysine-coated 24-well plates in high serum medium (10% HS and 5% FBS) for 48 h with a density of 1×10^4 cells/mL, and then starved for 14 h with low serum media (1% HS and 0.5% FBS). The hungry cells were treated with tested compounds (20 μM) in the presence of NGF (20 ng/mL) in three parallels. NGF-treated cells were used as positive control; solvent-only cells were used as negative control. After 48 h incubation, neurite outgrowth of PC-12 cells was observed under an inverted microscope. Ten images were selected randomly under the microscope in each well, with at least 100 cells in each field. A cell that contains one or more neuritis greater than the length of its body was positive for neurite outgrowth, and neurite bearing cells (%) was calculated as:

$$\text{neurite bearing cells (\%)} = \frac{N_a}{N_t} \times 100 \quad (2)$$

Where N_a is the number of neurite bearing cells; N_t is the number of total cell number

4.6. Antibacterial activity

The antibacterial activities of the title compounds against 5 bacteria were evaluated using filter paper dispersion method. The activated-bacteria were brushed with sterile water to form a bacterial suspension, which is then mixed well with Beef extract peptone medium (BAP), and took 3 mL to a 9 cm petri dish. The test compounds (5 μL, 2 mg/mL) were applied to a 6 mm filter paper in three parallels. Fosfomycin was used as positive control; solvent-only was used as negative control. The air-dried filter paper is affixed to the culture medium. After 4-10 h incubation at 37°C, recorded the size and transparency of the inhibitory ring using the crisscross method.

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Supplementary Material

Supporting information associated with this article (antibacterial activity, characterization data of products and NMR spectra of all products) can be found at http://dx.doi.org/10.1002/MS-number.

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