Bioorganic & Medicinal Chemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

C ring may be dispensable for β-carboline: Design, synthesis, and bioactivities evaluation of tryptophan analog derivatives based on the biosynthesis of β-carboline alkaloids

Yuanqiong Huang, Yongxian Liu, Yuxiu Liu, Hongjian Song *, Qingmin Wang *

State Key Laboratory of Elemento-Organic Chemistry, Research Institute of Elemento-Organic Chemistry, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin 300071, People's Republic of China

ARTICLE INFO

Article history:
Received 25 June 2015
Revised 3 August 2015
Accepted 13 August 2015
Available online xxxx

Keywords:
β-Carboline
Tryptophan
Biosynthesis
Anti-TMV
Fungicide

ABSTRACT

According to our previous work and the latest research on the biosynthesis of β-carboline, and using the reverse thinking strategy, tryptophan, the biosynthesis precursor of β-carboline alkaloids, and their derivatives were synthesized, and their biological activities and structure–activity relationships were studied. This bioassay showed that these compounds exhibited good inhibitory activities against tobacco mosaic virus (TMV); especially (*S*)-2-amino-3-(1*H*-indol-3-yl)-*N*-octylpropanamide (**4**) (63.3 ± 2.1%, 67.1 ± 1.9%, 68.7 ± 1.3%, and 64.5 ± 3.1%, 500 μg/mL) exhibited the best antiviral activity both in vitro and in vivo. Compound **4** was chosen for the field trials and the acute oral toxicity test, the results showed that the compound exhibited good anti-TMV activity in the field and low acute oral toxicity. We also found that these compounds showed antifungal activities and insecticidal activities.

1. Introduction

In our previous work, series of tetrahydro-β-carboline derivatives were found to exhibit excellent bioactivities.[1–3] As we all know, β-carbolines are synthesized in organisms using tryptophan as precursor via the catalysis of enzymes. Recently, Ju and co-workers[4] successfully parsed the biosynthetic machinery of β-carboline alkaloids arrived from the microorganisms in deep-sea. They found these marinacarbolines are biosynthesized using tryptophan and acetate as precursors via enzyme-catalyzed Pictet–Spengler cyclization (Fig. 1); tryptophan can also be used as precursor of biosynthesis for other indole alkaloids.[5,6]

In addition to being important synthetic precursor in biosynthesis, L-tryptophan is one of the eight kinds of essential amino acids in the human body, which participates in the synthesis of various proteins and in the regulation of metabolic network. For the past decades, L-tryptophan is used as not only intravenous nutrition, but also eutherapeutic drug for tristimania, insomnia, hypertension, dermatitis and schizophrenia, etc.[7,8] D-Tryptophan, as one of non-protein amino acids, also has a certain value, especially in the pharmaceutical industry; it is an important synthetic precursor of anticancer agent and immune inhibitor.[9–11] Furthermore, some tryptophan derivatives also have great value in the fields of biology and medicine.[12–15]

Although there are a lot of research on the biology activities of the important biosynthetic precursor, there is little study focus on its application in the field of pesticides. As we have found tetrahydro-β-carboline derivatives exhibited excellent pesticidal activity, we wonder whether their biosynthetic precursor (tryptophan) exhibited similar activities. Based on the above points, firstly, tryptophan and their ester, amides derivatives were synthesized (**1**–**22**); then, the ureido moiety was introduced into the tryptophan derivatives (**23**–**27R**) as we have found that it was beneficial to increase the anti-TMV activity of β-carboline derivatives when increasing hydrogen bond donor;[16] lastly, in order to investigate the influence of the amino acid part on the activities, the ureido and ester moieties were transformed to cyclothioureas structure via the cyclization reaction (**28**–**32**). The bioactivities and structure–activity relationships (SAR) of these derivatives were studied for the first time (Fig. 2).

2. Results and discussion

2.1. Synthesis

Using tryptophan as starting material, compound **1** and **2** could be synthesized via a classical esterification by reacting with thionyl chloride and ethanol (Scheme 1a). Compound **3** could be obtained

* Corresponding authors. Tel./fax: +86 (0)222 350 3952 (Q.W.).
E-mail address: wangqm@nankai.edu.cn (Q. Wang).

http://dx.doi.org/10.1016/j.bmc.2015.08.016
0968-0896/

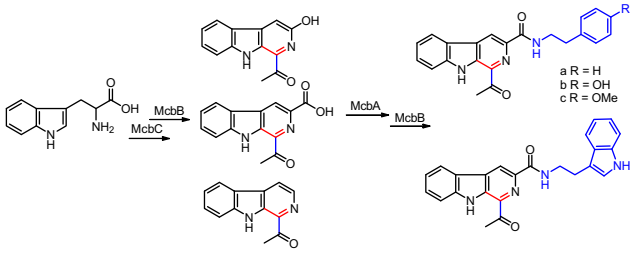


Figure 1. Biosynthesis of marinacarbolines using tryptophan as a precursor.

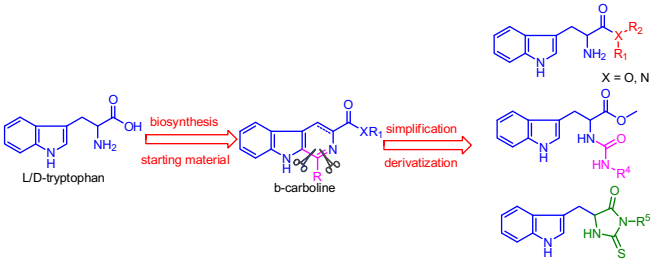


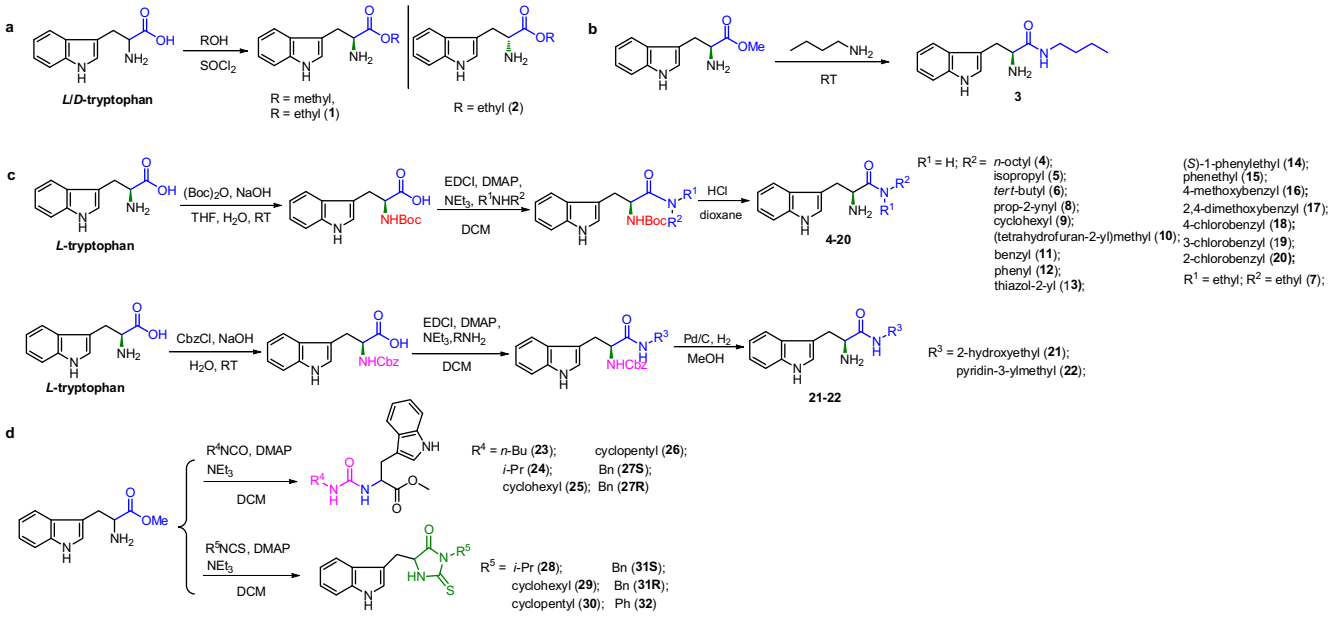
Figure 2. Design of target compounds.

from (*S*)-methyl 2-amino-3-(1*H*-indol-3-yl)propanoate by reaction with *n*-butylamine which also acted as solvent (Scheme 1b). However, other amines does not apply to this reaction conditions. Instead, compounds **5**–**22** were synthesized (Scheme 1c) via carbodiimide (such as EDCI) mediated condensation and the subsequent deprotection. Compounds **23**–**27R** were obtained via the reaction of methyl 2-amino-3-(1*H*-indol-3-yl)propanoate with isocyanates. When methyl 2-amino-3-(1*H*-indol-3-yl)propanoate reacted with isothiocyanates, the cyclization compounds **28**–**32** could be obtained.

2.2. Antiviral activities

The results of antiviral activities, in vitro and in vivo (inactivation, curative, and protection), of these compounds are listed in Table 1. To our delight, most of them exhibited good antiviral activity. For the in vitro activity, as we can see, tryptophan itself showed moderate antiviral activity and the chirality (L/D) affected slightly: the in vitro activity of L-tryptophan (33.3 ± 2.3%, 500 μg/mL) was slightly lower than that of D-tryptophan (37.6 ± 3.0%, 500 μg/mL). Compounds **1** and **2** showed similar antiviral activities (35.9 ± 2.3% and 38.4 ± 2.0%, respectively, at 500 μg/mL) as L- or D-tryptophan. However, when we changed the ester group to amide group, most of these compounds exhibited desirable anti-TMV activity in vitro. Compounds **4**, **9**, **11**, **14**, **15**, **19**, **20**, **23**, **31S**, and **31R** showed obviously higher inhibition than that of ribavirin at 500 and 100 μg/mL; especially, the activity of compound **4** reached 31.5 ± 2.4% at 100 μg/mL, it means increasing liposolubility is beneficial to the anti-TMV activity of these compounds. All these results above proved that the introduction of the amide group to the tryptophan skeleton was advantageous to the antiviral activities.

Additionally, the substituents of the amide moiety had a significant impact on anti-TMV activity. Firstly, when R^1 was hydrogen and R^2 was an aliphatic substituent, the longer and less branched the aliphatic chain was, the higher the anti-TMV activity was, compound **4** (63.3 ± 2.1%, 500 μg/mL) displayed obviously higher anti-TMV activity than compound **3** (46.6 ± 1.2%, 500 μg/mL). It was noteworthy that the existence of N—H bond of the amide moiety is of crucial importance: the activity of compound **7** (26.1 ± 1.7%, 500 μg/mL) was reduced sharply. Compound **8** (41.4 ± 3.4%, 500 μg/mL) containing a propargyl exhibited the same anti-TMV activity as ribavirin; compound **9** (47.7 ± 2.7%, 500 μg/mL) containing a saturated carbocycle exhibited obviously higher antiviral activities than ribavirin; However, when the substituent was changed to a saturated heterocycle, the activity of compound **10** (38.8 ± 1.5%, 500 μg/mL) was reduced. Furthermore, it was noteworthy that compound **21** (23.7 ± 2.1%, 500 μg/mL) showed much lower inhibitory effect compared with those of compounds **3**–**9**. These results above meant that the introduction of heteroatom was adverse to the anti-TMV activity. On the other hand, when R^1 was hydrogen and R^2 was a benzyl, compound **11** (26.3 ± 2.0% and 48.6 ± 2.5% at 100 μg/mL and 500 μg/mL, respectively) exhibited good inhibitory effect; In contrast, compound**12** (0 and 38.7 ± 1.7% at 100 μg/mL and 500 μg/mL, respectively)



Scheme 1. Synthesis of compounds **1**–**32**.

Table 1
In vitro and in vivo antiviral activity of compounds L/D-tryptophans, **1–32** against TMV

Compd	Concn (μg/mL)	In vitro inhibition rate (%)	In vivo		
			Inactivation effect (%)	Curative effect (%)	Protection effect (%)
Ribavirin	500	40.0 ± 1.3	37.4 ± 2.1	36.2 ± 1.9	38.5 ± 2.5
	100	12.9 ± 1.1	13.7 ± 2.3	16.9 ± 1.8	16.2 ± 1.4
L-Tryptophan	500	33.3 ± 2.3	34.1 ± 1.2	32.5 ± 2.5	33.0 ± 1.6
	100	0	10.0 ± 1.1	0	8.2 ± 1.4
D-Tryptophan	500	37.6 ± 3.0	39.1 ± 2.1	36.2 ± 1.5	37.3 ± 2.7
	100	0	13.1 ± 1.3	10.3 ± 1.5	11.7 ± 2.3
1	500	35.9 ± 2.3	42.5 ± 3.2	37.0 ± 1.7	36.3 ± 1.2
	100	8.6 ± 2.0	17.3 ± 1.5	12.0 ± 1.2	15.1 ± 2.3
2	500	34.8 ± 1.2	43.0 ± 1.3	39.2 ± 3.0	37.3 ± 2.1
	100	0	15.1 ± 1.3	15.9 ± 2.1	18.2 ± 3.1
3	500	46.6 ± 1.2	56.7 ± 1.5	50.3 ± 3.1	48.2 ± 2.7
	100	0	23.6 ± 1.5	17.5 ± 1.2	17.0 ± 2.0
4	500	**63.3 ± 2.1**	**67.1 ± 1.9**	**68.7 ± 1.3**	**64.5 ± 3.1**
	100	**31.5 ± 2.4**	**26.3 ± 1.3**	**29.8 ± 3.0**	**24.0 ± 1.2**
5	500	45.8 ± 1.4	49.5 ± 2.1	53.4 ± 2.3	47.1 ± 1.8
	100	8.4 ± 1.2	11.4 ± 1.1	18.5 ± 2.1	6.9 ± 2.3
6	500	40.3 ± 1.5	42.0 ± 2.2	45.4 ± 2.9	43.8 ± 1.8
	100	18.2 ± 1.1	16.8 ± 1.4	19.7 ± 1.0	12.1 ± 1.5
7	500	26.1 ± 1.7	29.7 ± 2.3	28.6 ± 2.7	32.3 ± 1.9
	100	0	0	0	0
8	500	41.4 ± 3.4	44.2 ± 2.1	45.1 ± 1.9	38.7 ± 2.5
	100	0	0	15.9 ± 1.0	7.6 ± 1.4
9	500	47.7 ± 2.7	45.4 ± 2.0	42.6 ± 1.5	48.2 ± 1.7
	100	17.8 ± 1.0	10.8 ± 1.3	12.6 ± 1.7	19.0 ± 1.1
10	500	38.8 ± 1.5	40.0 ± 3.0	35.9 ± 1.2	38.1 ± 2.1
	100	7.9 ± 1.1	8.7 ± 1.4	14.4 ± 2.0	11.6 ± 1.2
11	500	**48.6 ± 2.5**	**52.3 ± 3.2**	**54.6 ± 3.7**	**46.1 ± 2.7**
	100	**26.3 ± 2.0**	**17.4 ± 1.2**	**12.8 ± 1.4**	**13.2 ± 1.9**
12	500	38.7 ± 1.7	46.5 ± 1.2	44.2 ± 1.4	48.9 ± 2.3
	100	0	16.6 ± 2.0	13.0 ± 1.1	6.4 ± 1.2
13	500	38.2 ± 1.1	46.7 ± 1.9	45.9 ± 2.4	40.4 ± 1.2
	100	0	9.8 ± 1.0	11.7 ± 1.2	16.7 ± 1.5
14	500	**51.1 ± 3.2**	**50.0 ± 2.1**	**44.6 ± 1.7**	**46.1 ± 3.3**
	100	**19.6 ± 1.4**	**18.9 ± 2.0**	**12.4 ± 2.4**	**23.9 ± 1.3**
15	500	**50.2 ± 2.3**	**48.4 ± 2.0**	**51.8 ± 4.0**	**51.3 ± 2.8**
	100	**23.5 ± 1.4**	**18.2 ± 1.0**	**22.9 ± 2.1**	**17.6 ± 1.9**
16	500	30.5 ± 1.3	33.7 ± 1.5	34.0 ± 1.3	28.9 ± 2.1
	100	0	0	0	0
17	500	40.0 ± 1.7	29.8 ± 2.3	37.7 ± 1.2	39.4 ± 1.9
	100	0	0	0	7.6 ± 1.0
18	500	44.0 ± 1.5	49.5 ± 1.4	48.0 ± 2.1	43.5 ± 2.5
	100	13.7 ± 1.0	15.3 ± 1.3	20.4 ± 2.0	9.9 ± 1.1
19	500	**53.3 ± 3.8**	**48.6 ± 2.1**	**51.1 ± 1.6**	**50.0 ± 2.8**
	100	**26.9 ± 1.3**	**20.1 ± 1.6**	**18.3 ± 1.3**	**23.2 ± 2.0**
20	500	48.6 ± 1.1	46.2 ± 1.9	45.3 ± 3.0	48.3 ± 1.3
	100	17.3 ± 1.2	21.3 ± 1.3	18.8 ± 1.8	13.2 ± 1.0
21	500	23.7 ± 2.1	31.5 ± 1.9	29.0 ± 1.5	27.7 ± 2.4
	100	0	0	0	0
22	500	31.4 ± 2.1	32.9 ± 1.3	34.6 ± 1.9	38.3 ± 2.3
	100	0	0	0	0
23	500	**58.1 ± 2.5**	**51.3 ± 1.9**	**53.4 ± 3.1**	**47.1 ± 2.1**
	100	**14.9 ± 1.4**	**17.2 ± 1.7**	**23.3 ± 2.0**	**21.6 ± 1.1**
24	500	32.5 ± 1.5	40.0 ± 1.6	36.2 ± 2.3	34.7 ± 1.3
	100	0	15.4 ± 1.2	7.3 ± 1.0	10.0 ± 1.7
25	500	47.2 ± 2.6	46.1 ± 2.1	43.5 ± 2.4	52.8 ± 1.6
	100	8.8 ± 2.1	19.2 ± 1.4	14.8 ± 1.4	23.9 ± 1.3
26	500	43.3 ± 1.9	50.5 ± 2.0	46.2 ± 1.4	55.8 ± 4.0
	100	9.8 ± 2.0	21.4 ± 1.5	16.3 ± 1.1	27.1 ± 1.9
27S	500	49.2 ± 1.9	51.3 ± 2.3	43.8 ± 2.7	46.2 ± 1.3
	100	14.7 ± 1.0	10.8 ± 1.4	15.4 ± 1.2	7.6 ± 1.9
27R	500	48.6 ± 2.3	39.8 ± 2.5	41.6 ± 1.7	46.9 ± 1.0
	100	0	10.1 ± 1.1	15.0 ± 1.5	11.7 ± 1.7
28	500	35.7 ± 2.3	43.1 ± 1.9	40.6 ± 1.3	48.0 ± 2.1
	100	0	14.5 ± 1.2	8.3 ± 1.5	17.2 ± 1.0
29	500	45.0 ± 1.4	40.1 ± 2.1	46.2 ± 2.6	42.8 ± 1.7
	100	6.9 ± 1.2	16.6 ± 1.4	10.4 ± 1.2	11.5 ± 1.2
30	500	38.7 ± 1.3	47.5 ± 1.5	42.3 ± 3.0	50.9 ± 2.1
	100	21.5 ± 1.5	12.2 ± 1.2	18.0 ± 1.0	21.4 ± 2.1
31S	500	**56.8 ± 3.1**	**50.0 ± 2.3**	**53.8 ± 2.1**	**45.5 ± 1.5**
	100	**25.4 ± 1.4**	**19.6 ± 2.1**	**21.7 ± 1.4**	**12.2 ± 1.0**
31R	500	**53.3 ± 2.3**	**57.6 ± 3.8**	**49.8 ± 1.9**	**51.4 ± 1.5**
	100	**15.2 ± 1.2**	**28.1 ± 1.7**	**25.4 ± 2.3**	**20.0 ± 1.1**
32	500	38.9 ± 1.8	43.7 ± 2.3	41.7 ± 2.1	48.0 ± 1.8
	100	0	11.3 ± 1.2	20.0 ± 1.7	22.3 ± 1.3

The bold values were used to highlight that these compounds showed good activities.

Please cite this article in press as: Huang, Y.; et al. *Bioorg. Med. Chem.* (2015), http://dx.doi.org/10.1016/j.bmc.2015.08.016

Table 2
Fungicidal activities of L/D-tryptophans, compounds **1**, **3**, **4**, and **6**–**32** against fourteen kinds of phytopathogens

Compd	Fungicidal activity (%)/50 mg/kg													
	F.C.[a]	*C.H.*	*P.P.*	*A.S.*	*F.G.*	*F.M.*	*S.S.*	*P.C.*	*R.C.*	*B.M.*	*W.A.*	*P.I.*	*R.S.*	*B.C.*
L-Tryptophan	4.5	18.2	42.4	9.5	46.2	35.3	7.7	6.1	40.5	28.0	24.0	8.7	1.4	41.7
D-Tryptophan	4.5	18.2	45.5	9.5	**69.2**	23.5	15.4	15.2	43.2	28.0	20.0	17.4	0.0	66.7
1	18.2	18.2	30.3	14.3	46.2	29.4	17.3	9.1	62.2	28.0	16.0	21.7	27.8	52.8
3	38.5	58.8	**83.3**	45.0	62.1	21.4	**82.4**	**78.9**	**88.2**	32.0	33.3	36.0	32.9	**72.7**
4	13.6	36.4	54.5	52.4	53.8	47.1	**75.0**	**78.8**	54.1	48.0	48.0	34.8	36.1	61.1
6	13.6	9.1	45.5	19.0	38.5	29.4	11.5	15.2	40.5	32.0	36.0	26.1	1.4	50.0
7	13.6	**72.7**	**81.8**	33.3	53.8	35.3	48.1	12.1	56.8	32.0	32.0	26.1	29.2	**69.4**
8	15.4	29.4	55.6	15.0	44.8	21.4	58.8	18.4	60.8	16.0	22.2	12.0	13.2	4.5
9	4.5	27.3	57.6	19.0	53.8	29.4	11.5	36.4	54.1	24.0	28.0	21.7	9.7	47.2
10	26.9	23.5	**69.4**	0.0	65.5	21.4	**76.5**	7.9	47.1	16.0	18.5	28.0	26.3	4.5
11	30.8	35.3	55.6	0.0	27.6	21.4	35.3	23.7	52.9	20.0	22.2	24.0	13.2	9.1
12	30.8	35.3	50.0	30.0	20.7	14.3	**82.4**	36.8	62.7	24.0	22.2	28.0	22.4	13.6
13	23.1	35.3	**69.4**	5.0	27.6	14.3	64.7	18.4	52.9	20.0	18.5	16.0	13.2	0.0
14	4.5	27.3	54.5	33.3	38.5	35.3	28.8	36.4	56.8	32.0	32.0	17.4	1.4	66.7
15	4.5	36.4	51.5	33.3	69.2	29.4	30.8	48.5	51.4	36.0	32.0	17.4	0.0	**69.4**
16	4.5	27.3	48.5	23.8	46.2	41.2	9.6	15.2	43.2	36.0	36.0	21.7	11.1	61.1
17	9.1	36.4	36.4	33.3	53.8	35.3	1.9	21.2	54.1	32.0	32.0	17.4	1.4	61.1
18	27.3	54.5	36.4	57.1	46.2	41.2	46.2	**81.8**	62.2	52.0	52.0	43.5	33.3	75.0
19	0.0	0.0	21.2	23.8	53.8	35.3	26.9	21.2	43.2	24.0	28.0	13.0	1.4	52.8
20	9.1	27.3	48.5	38.1	46.2	35.3	44.2	**81.8**	59.5	36.0	32.0	30.4	8.3	**69.4**
21	30.8	41.2	**97.2**	20.0	34.5	21.4	**94.1**	23.7	52.9	24.0	25.9	32.0	13.2	9.1
22	9.1	9.1	48.5	14.3	46.2	29.4	3.8	3.0	29.7	32.0	24.0	8.7	8.3	55.6
23	10.5	10.3	33.3	18.5	17.9	7.1	14.8	19.6	34.3	8.1	12.9	23.5	10.5	10.3
24	10.5	24.1	-2.0	25.9	26.8	7.1	11.1	19.6	27.1	2.7	16.1	23.5	7.1	35.2
25	7.9	13.8	0.0	22.2	17.9	7.1	11.1	25.5	25.7	16.2	16.1	8.8	7.1	35.2
26	18.4	6.9	0.0	33.3	53.6	7.1	**77.8**	29.4	31.4	16.2	9.7	35.3	7.1	42.3
27S	26.3	10.3	0.0	29.6	26.8	7.1	12.3	23.5	27.1	16.2	25.8	8.8	7.1	28.2
27R	57.9	17.2	39.2	22.2	35.7	10.7	16.0	29.4	37.1	10.8	16.1	35.3	10.7	39.4
28	13.2	20.7	29.4	48.1	21.4	3.6	24.7	43.1	34.3	16.2	25.8	17.6	3.6	32.4
29	**71.1**	41.4	**92.2**	63.0	75.0	35.7	**85.2**	68.6	**84.3**	35.1	38.7	67.6	35.7	**88.7**
30	31.6	58.6	**78.4**	44.4	35.7	28.6	17.3	52.9	**74.3**	45.9	38.7	29.4	28.6	39.4
31S	15.8	10.3	39.2	37.0	14.3	14.3	12.3	23.5	24.3	13.5	12.9	5.9	14.3	35.2
31R	10.5	10.3	33.3	14.8	26.8	10.7	12.3	27.5	18.6	13.5	16.1	8.8	10.7	31.0
32	13.2	17.2	39.2	18.5	25.0	10.7	21.0	49.0	34.3	21.6	19.4	23.5	10.7	35.2
Carbendazim	<50	<50	<50	<50	100	<50	100	<50	100	100	100	100	100	<50
Chlorothalonil	100	73.3	100	73.3	<50	100	<50	100	100	91.3	91.3	86.4	100	100

The bold values were used to highlight that these compounds showed good activities.

[a] *F.C.*: *Fusarium oxysporiumf.* sp. *cucumeris*; *C.H.*: *Cercospora arachidicola Hori*; *P.P.*: *Physalospora piricola*; *A.S.*: *Alternaria solani*; *F.G.*: *Fusarium graminearum*; *F.M.*: *Fusarium moniliforme*; *S.S.*: *Sclerotinia sclerotiorum*; *P.C.*: *Phytophthora capsici*; *R.C.*: *Rhizoctonia cerealis*; *B.M.*: *Bipolaris maydis*; *W.A.*: *Watermelon-anthracnose*; *P.I.*: *Phytophthora infestans*; *R.S.*: *Rhizoctonia solani*; *B.C.*: *Botrytis cinerea*.

containing a phenyl was far less effective, the activity was also reduced when the benzyl was changed to an aryl heterocycle (**13**). Compounds **14**(51.1 ± 3.2%, 500 μg/mL) and **15**(50.2 ± 2.3%, 500 μg/mL) showed the same inhibition as compound **13**. In order to investigate SAR further, a representative set of compounds **16**–**20** containing substituted benzyl structure were synthesized. The result demonstrated that the electronic effect of substituents on the benzene ring did have effect on anti-TMV activity. For instance, it was detrimental to anti-TMV activity when the electron-donating group (methoxyl-, **16** (30.5 ± 1.3%, 500 μg/mL), **17** (40 ± 1.7%, 500 μg/mL)) was introduced to the benzene ring comparing with the activity of compounds **18**–**20**(44 ± 1.5%, 53.3 ± 3.8%, and 48.6 ± 1.1% at 500 μg/mL, respectively) containing an electron-withdrawing group (chloro-). However, when the benzyl was changed to pyridin-3-ylmethyl (**22**), the activity (31.4 ± 2.1%, 500 μg/mL) was greatly reduced. The introduction of the ureido moiety was beneficial to the anti-TMV activity; most of the compounds (**23**–**27R**) exhibited higher activities than compounds **1** and **2**. Especially, the compounds **23** exhibited 58.1 ± 2.5% at 500 μg/mL. For these compounds which containing cyclothioureas structure, the substituents of the imide moiety had a significant impact on anti-TMV activity. Compounds **31S** (56.8 ± 3.1% at 500 μg/mL) and **31R** (53.3 ± 2.3% at 500 μg/mL) which have a benzyl on the imide moiety exhibited the best activities.

Further bioassay was conducted to investigate their inactivation, curative, and protection effect in vivo, which showed similar SAR as the antiviral activities in vitro. Most of the compounds exhibited excellent antiviral activity, especially the activities of compounds **3** (56.7 ± 1.5%, 50.3 ± 3.1%, and 48.2 ± 2.7% at 500 μg/mL), **4** (67.1 ± 1.9%, 68.7 ± 1.3%, and 64.5 ± 3.1% at 500 μg/mL), **11** (52.3 ± 3.2%, 54.6 ± 3.7%, and 46.1 ± 2.7% at 500 μg/mL), **14** (50.0 ± 2.1%, 44.6 ± 1.7%, and 46.1 ± 3.3% at 500 μg/mL), **15** (48.4 ± 2.0%, 51.8 ± 4.0%, and 51.3 ± 2.8% at 500 μg/mL), **19** (48.6 ± 2.1%, 51.1 ± 1.6%, and 50.0 ± 2.8% at 500 μg/mL), **23** (51.3 ± 1.9%, 53.4 ± 3.1%, and 47.1 ± 2.1% at 500 μg/mL), **31S** (50.0 ± 2.3%, 53.8 ± 2.1%, and 45.5 ± 1.5% at 500 μg/mL), and **31R** (57.6 ± 3.8%, 49.8 ± 1.9%, and 51.4 ± 1.5% at 500 μg/mL) were higher than ribavirin (37.4 ± 2.1%, 36.2 ± 1.9%, and 38.5 ± 2.5% at 500 μg/mL). Compound**4**, containing a long-chain, exhibited the best antiviral activity both in vitro and in vivo. Considering its good solubility, stability and simple chemical structure, it is worthy to conduct further research and exploitation.

2.3. Fungicidal activities

Overall, most of these derivatives showed fungicidal activities against 14 kinds of phytopathogens (Table 2). Especially compound **3** and **29** exhibited more than 70% inhibition to several fungi. Some of these compounds exhibited fungicidal activities to certain fungi, for instance, the activities of compound **12** (against *Sclerotinia sclerotiorum*) and compound **20** (against *Physalospora piricola* and

Table 3
Insecticidal activity of compounds **1**, **3**, **4**, **6**–**20**, and **22**–**32** against four kinds of insects

Compd	Larvicidal activity (%) at concn (mg/kg)						
	M. separata	*H. armigera*	*O. nubilalis*	*C. pipiens pallens*			
	600/200 mg/kg	600 mg/kg	600 mg/kg	10 mg/kg	5 mg/kg	2 mg/kg	1 mg/kg
L-Tryptophan	65	45	55	100	70	—	
D-Tryptophan	50	45	35	100	10	—	
1	55	50	55	100	30	—	
3	30	20	35	75	—		
4	20	25	15	35	—		
6	70	50	50	100	80	—	
7	30	20	30	65	—		
8	65	65	25	100	70	—	
9	5	30	0	10	—		
10	45	35	40	100	30	—	
11	15	10	20	35	—		
12	30	25	25	50	—		
13	30	30	35	55	—		
14	5	30	5	15	—		
15	20	30	15	45	—		
16	20	25	30	35	—		
17	25	20	20	55	—		
18	20	25	10	40	—		
19	10	25	20	20	—		
20	65	50	50	100	50	—	
22	5	5	5	15	—		
23	20	70	50	100	0		
24	100/20	30	20	65	—		
25	65	65	65	100	10	—	
26	50	70	50	65	—		
27S	60	45	55	100	40	—	
27R	50	45	35	**100**	**100**	**100**	**60**
28	70	50	55	60	—		
29	60	20	35	100	20	—	
30	60	65	45	45	—		
31S	50	50	45	**100**	**100**	**100**	**50**
31R	50	55	50	15	—		
32	60	65	45	100	75	—	

The bold values were used to highlight that these compounds showed good activities.
'—' not test.

Table 4
Results of anti-TMV activities of field trials in 2014 after 10 days of application of **4**amino oligosaccharins, and moroxydine hydrochloride-cupric acetate

Compd[a]	Concn (gai/ha)	I'_{eq}[b]	I_{eq}[c]	E[d] (%)
4 (1% ME)	100	3.82	5.56	66.81
	50	5.80	10.55	58.51
	10	2.67	8.02	31.49
Blank control	—	2.81	12.32	—
Amino oligosaccharins (5% aqueous solution)	100	3.33	5.01	65.70
Moroxydine hydrochloride-cupric acetate (20% WP)	600	1.23	1.80	66.57

[a] ME: microemulsion, WP: wettable powder.
[b] I'_{eq}: The average disease index of the spraying area before spraying.
[c] I_{eq}: The average disease index of the spraying area 10 days after the third time spraying.
[d] E: The average control effect 10 days after the third time spraying.

Sclerotinia sclerotiorum) were >80% at 50 mg/kg, which was much higher than that against other fungi.

2.4. Insecticidal activities

These selected derivatives also showed insecticidal activities (Table 3). Of which, compounds **27R** and **31S** exhibited more than 70% activities against *culex pipiens pallens* at 1 mg/kg. It was interesting that their enantiomers (**27S** and **31R**) exhibited low or no activity at 5 mg/kg.

2.5. Field trials

As compound **4** exhibited excellent anti-TMV activity both in vitro and in vivo in laboratory, 1% EC of compound **4** was employed to evaluate its anti-TMV activity in field trials in Tengchong County (Yunnan Province, China) using amino oligosaccharins, moroxydine hydrochloride-cupric acetate, and water as controls and blank control respectively. The result exhibited that compound **4** showed the same efficacy as the controls. Table 4 showed part of the results of the field trials.

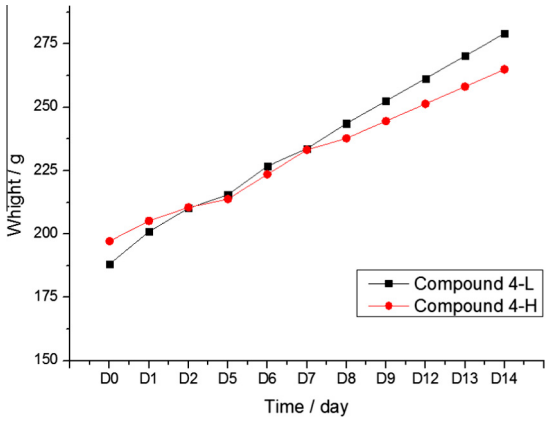


Figure 3. Average weight of rats. 4-L means the dosage of compound **4** is 50 mg/kg, 4-H means dosage of compound **4** is 500 mg/kg.

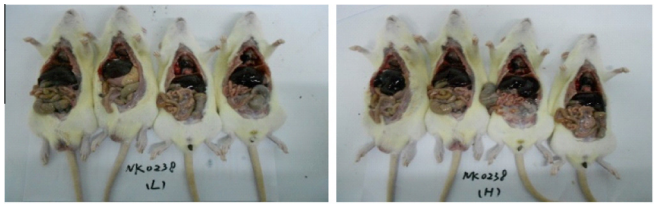


Figure 4. Autopsy results of the rats.

2.6. Rat acute oral toxicity of compound 4

To balance the toxicity versus efficacy of compound **4**, we next tested its tolerated dose (50 mg/kg and 500 mg/kg) using a group of rats (female rats and male rats). The test was conducted using our previously reported methods,[17] and monitoring morbidity (body weight loss) and mortality in the meantime. There was no obvious weight difference between the two doses (50 mg/kg and 500 mg/kg) (Fig. 3). After dissected, all rats showed no abnormal state under the macroscopic observation (Fig. 4). The results above proved that compound **4** display low toxicity to rats.

3. Conclusions

In conclusion, according to our previous study on the activities of β-carboline and their derivatives and the latest research work on their biosynthesis, and using the reverse thinking strategy, tryptophan and its derivatives containing amide or ester moiety were designed and synthesized via simplifying the β-carboline alkaloid. Most of the target compounds showed good anti-TMV activity both in vitro and in vivo (inactivation, curative, and protection) in the laboratory, activities of compounds **3**, **4**, **11**, **14**, **15**, and **19** were much higher than that of ribavirin and the relevant SAR was summarized. Meanwhile, the field trials of compound **4** further demonstrated its antiviral efficacy against TMV, and the rat acute oral toxicity test showed low toxicity. Because of the outstanding features, compound **4** is a promising candidate to develop into an inhibitor of plant virus. In addition, these compounds also showed fungicidal activity and insecticidal activity. For example, compound **3** showed more than 70% inhibition against five kinds of phytopathogens at 50 mg/kg, and **27R** and **31S** exhibited more than 70% activities against *culex pipiens pallens* at 1 mg/kg. The substituents on nitrogen of these compounds containing cyclothioureas structure played important role in the anti-TMV activity. These compounds containing benzyl group (**31S** and **31R**) exhibited the best activities.

4. Experimental

4.1. Chemistry

^{1}H NMR spectra were obtained at 400 MHz using a Bruker AV400 spectrometer in $CDCl_3$ or DMSO-d_6 solution with tetramethylsilane as the internal standard. HRMS data were obtained on an FTICR-MS instrument (Ionspec 7.0 T). The melting points were determined on an X-4 binocular microscope melting point apparatus and are uncorrected. All anhydrous solvents were dried and purified by standard techniques. The synthetic routes were given in Scheme 1.

4.1.1. Synthesis of (*S*)-2-(*tert*-butoxycarbonylamino)-3-(1*H*-indol-3-yl)propanoic acid[18]

To a mixture of L-tryptophan (10.00 g, 49.02 mmol) and NaOH (4.30 g, 107.5 mmol) in a mixed solvent of THF (450 mL) and H_2O (490 mL) was added di-*tert*-butyl dicarbonate (23.50 g, 107.7 mmol) at room temperature, and the resulting solution was stirred at room temperature for 36 h. THF was removed under reduced pressure, and the aqueous layer was acidified with citric acid to pH = 4. The mixture was extracted with ethylacetate, and concentrated to give the product as a white solid (14.90 g) in quantitative yield: ^{1}H NMR (400 MHz, DMSO-d_6):δ 11.15 (s, 1H, COOH), 8.17 and 8.14 (s, 1H, NH), 7.59 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.36–7.29 (m, 1H, Ar-H), 7.19 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 7.11 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 6.95 and 6.81 (s, 1H, Ar-H), 6.26 and 5.10 (d, $^3J_{HH}$ = 7.6 Hz, 1H, O═C—NH), 4.71–4.61 and 4.45–4.40 (m, 1H, C**H**), 3.39–3.22 and 3.12–3.01 (m,2H, C$\mathbf{H}_2$), 1.42 and 1.27 (s, 9H, C$\mathbf{H}_3$).

4.1.2. Synthesis of (*S*)-2-(benzyloxycarbonylamino)-3-(1*H*-indol-3-yl)propanoic acid[19]

To a stirred mixture of L-tryptophan (20.07 g, 98.04 mmol) and NaOH (9.41 g, 235.25 mmol) in water (250 mL) was added dropwise benzyl chloroformate (20.18 g, 117.65 mmol) in an ice bath. The mixture was allowed to stir overnight at room temperature, then acidified to pH = 1 with hydrochloric acid, and filtered. The cake was washed with cold water and dried to give the product as a white solid (21.02 g) in 64% yield: ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.85 (s, 1H,COOH), 7.54 (d, $^3J_{HH}$ = 7.8 Hz, 1H, Ar-H), 7.40–7.20 (m, 6H, Ar-H), 7.14 (s, 1H, Ar-H), 7.06 (t, $^3J_{HH}$ = 7.5 Hz, 1H, Ar-H), 6.96 (t, $^3J_{HH}$ = 7.5 Hz, 1H, Ar-H), 4.97 (s, 2H, C$\mathbf{H}_2$), 4.25–4.10 (m, 1H, C**H**), 3.21 (dd, $^3J_{HH}$ = 4.2 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H}_2$), 2.99 (dd, $^3J_{HH}$ = 9.0 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H}_2$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.0, 173.9, 155.8, 137.1, 136.0, 128.3, 127.6, 127.5, 123.6, 120.7, 118.2, 111.3, 110.5, 65.1, 55.4, 27.1.

4.1.3. Synthesis of (*S*)-methyl 2-amino-3-(1*H*-indol-3-yl)-propanoate

To a stirred solution of L-tryptophan(10 g, 49.02 mmol) in methanol (150 mL) was added dropwise sulfur dichloride(10 mL) in an ice bath, the reaction solution was heated at reflux for 4 h. After cooling and evaporation of methanol, the residue was partitioned between ethyl acetate (80 mL) and a saturated solution of $NaHCO_3$ (50 mL). The organic layer was washed with saturated brine, dried over Na_2SO_4 and concentrated to give a brown solid (9.16 g) in 86% yield: mp = 90–91 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.47 (s, 1H, Ar-NH), 7.60 (d, $^3J_{HH}$ = 7.8 Hz, 1H, Ar-H), 7.32 (d, $^3J_{HH}$ = 8.1 Hz, 1H, Ar-H), 7.18 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 7.11 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 7.00 (s, 1H, Ar-H), 3.82 (dd, $^3J_{HH}$ = 4.8 Hz, 7.5 Hz, 1H, C**H**), 3.71 (s, 3H, C$\mathbf{H}_3$), 3.28 (dd, $^3J_{HH}$ = 4.5 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H}_2$), 3.04 (dd, $^3J_{HH}$ = 7.5 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H}_2$), 1.67 (s, 2H, NH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.7, 136.3, 127.3, 123.2, 121.9, 119.3, 118.6, 111.3, 110.5, 54.9, 52.0, 30.7.

Compounds **1** and **2** were synthesized using similar procedure as (*S*)-methyl 2-amino-3-(1*H*-indol-3-yl)propanoate.

4.1.4. Data for (*S*)-ethyl 2-amino-3-(1*H*-indol-3-yl)propanoate (1)

This compound was obtained as a yellow solid in 87% yield: mp = 58–60 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (s, 1H, Ar-NH), 7.64 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.37 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.20 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 7.13 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.08 (s, 1H, Ar-H), 4.16 (q, $^3J_{HH}$ = 7.2 Hz, 2H, OC$\mathbf{H}_2$CH$_3$), 3.82 (dd, $^3J_{HH}$ = 5.2 Hz, $^2J_{HH}$ = 7.6 Hz, 1H, C**H**), 3.29 (dd, $^3J_{HH}$ = 4.5 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H}_2$), 3.05 (dd, $^3J_{HH}$ = 8.0 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H}_2$), 1.24 (t, $^3J_{HH}$ = 7.2 Hz, 3H, OCH$_2$C$\mathbf{H}_3$).^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.2, 136.3, 127.4, 123.1, 122.0, 119.3, 118.7, 111.2, 110.8, 61.0, 54.9, 30.6, 14.1; HRMS (ESI) calcd for $C_{13}H_{17}N_2O_2$ [M+H]$^+$ 233.1285, found 233.1289.

4.1.5. Data for (*R*)-ethyl 2-amino-3-(1*H*-indol-3-yl)propanoate (2)

This compound was obtained as a yellow solid in 84% yield: mp = 58–60 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (s, 1H, Ar-NH), 7.64 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.37 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.20 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 7.13 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.08 (s, 1H, Ar-H), 4.16 (q, $^3J_{HH}$ = 7.2 Hz, 2H, O**C**$\mathbf{H_2}$$CH_3$), 3.82 (dd, $^3J_{HH}$ = 5.2 Hz, $^2J_{HH}$ = 7.6 Hz, 1H, C**H**), 3.29 (dd, $^3J_{HH}$ = 4.5 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H_2}$), 3.05 (dd, $^3J_{HH}$ = 8.0 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H_2}$), 1.24 (t, $^3J_{HH}$ = 7.2 Hz, 3H, OCH_2**C**$\mathbf{H_3}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.2, 136.3, 127.4, 123.1, 122.0, 119.3, 118.7, 111.2, 110.8, 61.0, 54.9, 30.6, 14.1; HRMS (ESI) calcd for $C_{13}H_{17}N_2O_2$ [M+H]$^+$ 233.1285, found 233.1289.

4.1.6. Synthesis of ((*S*)-2-amino-*N*-butyl-3-(1*H*-indol-3-yl)-propanamide) (3)

(*S*)-Methyl 2-amino-3-(1*H*-indol-3-yl)propanoate (0.40 g, 1.83 mmol) was added to butan-1-amine (10 mL) and this reaction solution was stirred for 2 h at room temperature. The solvent was removed, the residue was partitioned between dichloromethane (20 mL) and H_2O (15 mL). The combined organic layer was dried over Na_2SO_4 and concentrated to give yellow oil (0.33 g) in 70% yield: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (s, 1H, Ar-NH), 7.69 (d, $^3J_{HH}$ = 7.8 Hz, 1H, Ar-H), 7.38 (d, $^3J_{HH}$ = 7.8 Hz, 1H, Ar-H), 7.21 (t, $^3J_{HH}$ = 7.5 Hz, 1H, Ar-H), 7.13 (t, $^3J_{HH}$ = 7.5 Hz, 1H, Ar-H), 7.08 (s, 1H, Ar-H), 3.71 (dd, $^3J_{HH}$ = 9.0 Hz, 3.9 Hz, 1H, C**H**), 3.40 (dd, $^3J_{HH}$ = 3.9 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H_2}$), 3.25 (q, $^3J_{HH}$ = 6.6 Hz, 1H, C$\mathbf{H_2}$), 2.91 (dd, $^3J_{HH}$ = 9.0 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H_2}$), 1.57–1.20 (m, 4H, C$\mathbf{H_2}$C$\mathbf{H_2}$), 0.91 (t, $^3J_{HH}$ = 7.5 Hz, 3H, C$\mathbf{H_3}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.7, 136.4, 127.5, 123.1, 122.2, 119.5, 119.0, 111.8, 111.3, 55.7, 38.8, 31.6, 30.9, 20.1, 13.8. HRMS (ESI) calcd for $C_{15}H_{22}N_3O$ [M+H]$^+$ 260.1757, found 260.1760.

4.1.7. Synthesis of (*S*)-2-amino-3-(1*H*-indol-3-yl)-*N*-octylpropanamide (4)[20]

To a stirred solution of (*S*)-2-(*tert*-butoxycarbonylamino)-3-(1*H*-indol-3-yl)propanoic acid(0.50 g, 1.64 mmol), 4-dimethylaminopyridine (DMAP, 0.40 g, 3.28 mmol) and triethylamine (0.33 g, 3.28 mmol) in dichloromethane (40 mL) was added 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDCI, 0.63 g, 3.28 mmol) in one portion. After the mixture was stirred at 0 °C for 0.5 h, *n*-octylamine (0.32 g, 2.46 mmol) was added to the above mixture and the mixture was then kept at room temperature for another 8 h until the reaction was complete detected by TLC. A saturated solution of ammonium chloride (30 mL) was added to the reaction, and the layers were separated. The organic phase was washed with saturated brine, dried over Na_2SO_4 and concentrated. The crude product was chromatographed on silica gel (petroleum ether/ethyl acetate 2:1) to give a yellow solid. Then a mixture of HCl (4 M, 4 mL)/dioxane (4 mL) was added, and the mixture was stirred for 3 h at room temperature. After the solvent was removed under reduced pressure, the residue was resolved in H_2O (20 mL) and the pH was adjusted to around 10 by progressively adding solid Na_2CO_3. The aqueous solution was extracted with ethyl acetate (25 mL × 4), and the combined organic layer was washed with saturated brine, dried over Na_2SO_4, and concentrated to give the expected compound **4** (0.39 g) as yellow oil in 75% yield: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.49 (s, 1H, Ar-NH), 7.64 (d, $^3J_{HH}$ = 7.8 Hz, 1H, Ar-H), 7.35 (d, $^3J_{HH}$ = 8.1 Hz, 1H, Ar-H), 7.32–7.24 (m, 1H, O=C—NH), 7.17 (t, $^3J_{HH}$ = 7.5 Hz, 1H, Ar-H), 7.13–7.05 (m, 2H, Ar-H), 3.79 (dd, $^3J_{HH}$ = 7.5 Hz, 4.5 Hz, 1H, C**H**), 3.36 (dd, $^3J_{HH}$ = 3.9 Hz, $^2J_{HH}$ = 14.7 Hz, 1H, C$\mathbf{H_2}$), 3.24–3.08 (m, 2H, N-C$\mathbf{H_2}$), 2.97 (dd, $^3J_{HH}$ = 8.4 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H_2}$), 2.76 (br, 2H, NH_2), 1.45–1.34 (m, 2H, C$\mathbf{H_2}$), 1.34–1.13 (m, 10H, C$\mathbf{H_2}$), 0.87 (t, $^3J_{HH}$ = 6.6 Hz, 3H, C$\mathbf{H_3}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 136.4, 127.5, 123.5, 122.2, 119.5, 118.9, 111.4, 111.0, 55.4, 39.3, 31.8, 30.3, 29.4, 29.3, 29.2, 26.9, 22.7, 14.1. HRMS (ESI) calcd for $C_{19}H_{30}N_3O$ [M+H]$^+$ 316.2383, found 316.2384.

Compounds **5**–**20** were synthesized using similar procedure as compound **4**.

4.1.8. Data for (*S*)-2-amino-3-(1*H*-indol-3-yl)-*N*-isopropylpropanamide (5)

This compound was obtained as a yellow solid in 76% yield: mp = 113–115 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.71 (s, 1H, Ar-NH), 7.65 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.37 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.18 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.14–7.05 (m, 2H, Ar-H and O=C—NH), 7.04 (s, 1H, Ar-H), 4.17–4.04 (m, 1H, C**H**), 3.67 (dd, $^3J_{HH}$ = 8.8 Hz, 4.4 Hz, 1H, C**H**), 3.37 (dd, $^3J_{HH}$ = 4.0 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H_2}$), 2.90 (dd, $^3J_{HH}$ = 8.8 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H_2}$), 1.56 (s, 2H, NH_2), 1.10 (t, $^3J_{HH}$ = 6.4 Hz, 6H, C$\mathbf{H_3}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.9, 136.5, 127.5, 123.2, 122.1, 119.4, 118.9, 111.5, 111.3, 55.6, 40.8, 30.8, 22.7, 22.7. HRMS (ESI) calcd for $C_{14}H_{20}N_3O$ [M+H]$^+$ 246.1601, found 246.1605.

4.1.9. Data for (*S*)-2-amino-*N*-*tert*-butyl-3-(1*H*-indol-3-yl)-propanamide (6)

This compound was obtained as a yellow solid in 71% yield: mp = 53–55 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (s, 1H, Ar-NH), 7.64 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.36 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.17 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.11 (s, 1H, O=C—NH), 7.08 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.04 (s, 1H, Ar-H), 3.63 (dd, $^3J_{HH}$ = 8.4 Hz, 4.4 Hz, 1H, C**H**), 3.34 (dd, $^3J_{HH}$ = 4.4 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C$\mathbf{H_2}$), 2.20 (br, 2H, NH_2), 1.31 (s, 9H, C$\mathbf{H_3}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 136.5, 127.5, 123.4, 122.1, 119.5, 118.9, 111.4, 55.4, 50.6, 30.6, 28.7. HRMS (ESI) calcd for $C_{15}H_{22}N_3O$ [M+H]$^+$ 250.1757, found 250.1756.

4.1.10. Data for (*S*)-2-amino-*N*,*N*-diethyl-3-(1*H*-indol-3-yl)-propanamide (7)

This compound was obtained as yellow oil in 62% yield: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 1H,NH), 7.57 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.36 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.18 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 7.11 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 7.05 (s, 1H, Ar-H), 3.96 (t, $^3J_{HH}$ = 6.8 Hz, C**H**), 3.45 (dd, $^3J_{HH}$ = 6.8 Hz, $^2J_{HH}$ = 13.6 Hz, 1H, C$\mathbf{H_2}$), 3.31–3.01 (m, 4H, C$\mathbf{H_2}CH_3$), 2.94 (dd, $^3J_{HH}$ = 7.6 Hz, $^2J_{HH}$ = 14.0 Hz, 1H, C$\mathbf{H_2}$), 2.46 (br, 1H, NH_2), 1.13–0.94 (m, 6H, CH_2C$\mathbf{H_3}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.1, 136.4, 127.5, 123.3, 122.0, 119.4, 118.4, 111.4, 51.7, 41.6, 40.5, 32.4, 14.6, 13.0. HRMS (ESI) calcd for $C_{15}H_{22}N_3O$ [M+H]$^+$ 260.1758, found 260.1756.

4.1.11. Data for (*S*)-2-amino-3-(1*H*-indol-3-yl)-*N*-(prop-2-ynyl)-propanamide (8)

This compound was obtained as yellow oil in 71% yield: ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.85 (s, 1H, NH), 8.29 (s, 1H, O=C—NH), 7.56 (d, $^3J_{HH}$ = 7.5 Hz, 1H, Ar-H), 7.33 (d, $^3J_{HH}$ = 8.1 Hz, 1H, Ar-H), 7.15 (s, 1H, Ar-H), 7.06 (t, $^3J_{HH}$ = 7.5 Hz, 1H, Ar-H), 6.97 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 3.86 (s, 2H, N-C$\mathbf{H_2}$), 3.46 (dd, $^3J_{HH}$ = 7.8 Hz, 4.8 Hz, 1H, CH), 3.17–2.97 (m, 2H, CC**H** and C$\mathbf{H_2}$), 2.74 (dd, $^3J_{HH}$ = 8.1 Hz, $^2J_{HH}$ = 14.1 Hz, 1H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.5, 136.2, 127.4, 123.8, 120.9, 118.5, 118.2, 111.3, 110.5, 81.3, 72.8, 55.3, 31.0, 27.9. HRMS (ESI) calcd for $C_{14}H_{16}N_3O$ [M+H]$^+$ 242.1288, found 242.1291.

4.1.12. Data for (*S*)-2-amino-*N*-cyclohexyl-3-(1*H*-indol-3-yl)-propanamide (9)

This compound was obtained as a yellow solid in 78% yield: mp = 58–60 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (s, 1H, Ar-NH), 7.63 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.35 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.22–7.11 (m, 2H, O=C—NH and Ar-H), 7.07 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.03 (s, 1H, Ar-H), 3.83–3.63 (m, 2H, C**H**), 3.35 (dd,

$^3J_{HH}$ = 3.6 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C**H**$_2$), 2.91 (dd, $^3J_{HH}$ = 8.8 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C**H**$_2$), 2.20 (s, 2H, NH$_2$), 1.89–1.73 (m, 2H, C**H**$_2$), 1.73–1.49 (m, 3H, C**H**$_2$), 1.40–1.22 (m, 2H, C**H**$_2$), 1.21–1.00 (m, 3H, C**H**$_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 136.5, 127.5, 123.4, 122.1, 119.4, 118.9, 111.4, 111.3, 55.4, 47.8, 33.0, 32.9, 30.7, 25.5, 24.8. HRMS (ESI) calcd for $C_{17}H_{24}N_3O$ $[M+H]^+$ 286.1914, found 286.1914.

4.1.13. Data for (2*S*)-2-amino-3-(1*H*-indol-3-yl)-*N*-((tetrahydrofuran-2-yl)methyl)propanamide (10)

This compound was obtained as yellow oil in 80% yield: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (s, 1H, Ar-NH), 7.67 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.63–7.50 (m, 1H, O=C—NH), 7.37 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.19 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.15–7.08 (m, 1H, Ar-H), 7.05 (t, $^4J_{HH}$ = 2.8 Hz, 1H, Ar-H), 3.98–3.88 (m, 1H, O-C**H**), 3.86–3.78 (m, 1H, O-C**H**$_2$), 3.76–3.69 (m, 2H, O-C**H**$_2$ and C**H**), 3.59–3.49 (m, 1H, N-C**H**$_2$), 3.39 (dd, $^3J_{HH}$ = 4.0 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C**H**$_2$), 3.23–3.12 (m, 1H, N-C**H**$_2$), 2.90 and 2.89 (dd, $^3J_{HH}$ = 9.2 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C**H**$_2$), 1.99–1.90 (m, 1H, C**H**$_2$), 1.90–1.80 (m, 2H, C**H**$_2$), 1.78 (br, 2H, N**H**$_2$), 1.55–1.44 (m, 1H, C**H**$_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.1 and 175.0, 136.5, 127.5 and 127.5, 123.2, 122.2, 119.5, 119.0, 111.7, 111.3, 77.8 and 77.8, 68.1 and 68.1, 55.7 and 55.6, 43.0 and 43.0, 30.9 and 30.8, 28.7 and 28.7, 25.9. HRMS (ESI) calcd for $C_{16}H_{22}N_3O_2$ $[M+H]^+$ 288.1707, found 288.1708.

4.1.14. Data for (*S*)-2-amino-*N*-benzyl-3-(1*H*-indol-3-yl)-propanamide (11)

This compound was obtained as a yellow solid in 73% yield: mp = 41–43 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.90 (s, 1H,NH), 8.45 (s, 1H, O=C—NH), 7.59 (d, $^3J_{HH}$ = 6.8 Hz, 1H, Ar-H), 7.35 (d, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 7.30–7.10 (m, 6H, Ph-H and Ar-H), 7.07 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 6.98 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 4.35–4.19 (m, 1H, C**H**$_2$), 3.89 (br, 1H, NH$_2$), 3.65–3.56 (m, 1H, C**H**), 3.20–3.07 (m, 2H, C**H**$_2$), 2.85 (dd, $^3J_{HH}$ = 7.2 Hz, $^2J_{HH}$ = 14.0 Hz, 1H, C**H**$_2$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.6, 139.3, 136.3, 128.2, 127.4, 127.2, 126.7, 124.0, 120.9, 118.6, 118.3, 111.4, 110.0, 55.1, 42.0, 30.6. HRMS (ESI) calcd for $C_{18}H_{20}N_3O$ $[M+H]^+$ 294.1601, found 294.1606.

4.1.15. Data for (*S*)-2-amino-3-(1*H*-indol-3-yl)-*N*-phenylpropanamide (12)

This compound was obtained as a yellow solid in 77% yield: mp = 48–49 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.87 (s, 1H,NH), 7.66–7.56 (m, 3H, Ar-H and Ph-H), 7.36–7.26 (m, 3H, Ar-H and Ph-H), 7.17 (d, $^4J_{HH}$ = 2.0 Hz, 1H, Ar-H), 7.09–7.01 (m, 2H, Ar-H and Ph-H), 6.96 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 3.66 (dd, $^3J_{HH}$ = 7.2 Hz, 5.6 Hz, 1H, C**H**), 3.16 (dd, $^3J_{HH}$ = 5.2 Hz, $^2J_{HH}$ = 14.0 Hz, 1H, C**H**$_2$), 2.89 (dd, $^3J_{HH}$ = 7.6 Hz, $^2J_{HH}$ = 14.0 Hz, 1H, C**H**$_2$), 1.56 (br, 2H, NH$_2$), 1.10 (t, $^3J_{HH}$ = 6.4 Hz, 3H, C**H**$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.5, 138.8, 136.2, 128.7, 127.4, 123.8, 123.2, 120.9, 119.3, 118.5, 118.2, 111.3, 110.3, 56.1, 30.7. HRMS (ESI) calcd for $C_{17}H_{18}N_3O$ $[M+H]^+$ 280.1444, found 280.1446.

4.1.16. Data for (*S*)-2-amino-3-(1*H*-indol-3-yl)-*N*-(thiazol-2-yl)-propanamide (13)

This compound was obtained as a yellow solid in 76% yield: mp = 74–76 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.86 (s, 1H,NH), 7.58 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.46 (s, 1H, thiazole-H), 7.32 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.20 (s, 1H, thiazole-H), 7.13 (s, 1H, Ar-H), 7.05 (t, $^3J_{HH}$ = 6.8 Hz, 1H, Ar-H), 6.94 (t, $^3J_{HH}$ = 6.8 Hz, 1H, Ar-H), 5.33 (br, 1H, NH$_2$), 3.85–3.74 (m, 1H, C**H**), 3.14 (dd, $^3J_{HH}$ = 5.2 Hz, $^2J_{HH}$ = 13.6 Hz, 1H, C**H**$_2$), 2.91 (dd, $^3J_{HH}$ = 7.2 Hz, $^2J_{HH}$ = 13.6 Hz, 1H, C**H**$_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.9, 158.7, 138.3, 137.1, 128.1, 124.0, 123.1, 120.5, 119.4, 114.2, 112.1, 111.5, 56.0, 31.1. HRMS (ESI) calcd for $C_{14}H_{15}N_4OS$ $[M+H]^+$ 287.0961, found 287.0964.

4.1.17. Data for (2*S*)-2-amino-3-(1*H*-indol-3-yl)-*N*-((*S*)-1-phenylethyl)propanamide (14)

This compound was obtained as a yellow solid in 75% yield: mp = 91–92 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (s, 1H,NH), 7.69 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.53 (d, $^3J_{HH}$ = 8.0 Hz, 1H, O=C—NH), 7.37 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.34–7.24 (m, 5H, Ph-H), 7.21 (t, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.13 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.04 (d, $^4J_{HH}$ = 1.6 Hz, 1H, Ar-H), 5.17–5.07 (m, 1H, C**H**), 3.71 (dd, $^3J_{HH}$ = 8.8 Hz,4.0 Hz, 1H, C**H**), 3.40 (dd, $^3J_{HH}$ = 4.0 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C**H**$_2$), 2.96 (dd, $^3J_{HH}$ = 8.8 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C**H**$_2$), 1.60 (s, 1H, NH$_2$), 1.42 (d, $^3J_{HH}$ = 6.8 Hz, 3H, C**H**$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 143.5, 136.4, 128.6, 127.5, 127.2, 126.2, 123.1, 122.3, 119.7, 119.0, 111.8, 110.3, 55.5, 48.3, 30.8, 22.0. HRMS (ESI) calcd for $C_{19}H_{22}N_3O$ $[M+H]^+$ 308.1758, found 308.1764.

4.1.18. Data for (*S*)-2-amino-3-(1*H*-indol-3-yl)-*N*-phenethylpropanamide (15)

This compound was obtained as yellow oil in 75% yield: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (s, 1H, NH), 7.64 (d, $^3J_{HH}$ = 6.8 Hz, 1H, O=C—NH), 7.35 (d, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 7.33–7.05 (m, 8H, Ar-H and Ph-H), 7.02 (s, 1H, Ar-H), 3.74–3.63 (m, 1H, C**H**), 3.56–3.40 (m, 2H, C**H**$_2$), 3.39–3.26 (m,1H, C**H**$_2$), 2.98–2.85 (m, 1H, C**H**$_2$), 2.06 (br, 1H, NH$_2$), 1.79–1.65 (m, 2H, C**H**$_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.6, 139.0, 136.4, 128.8, 128.5, 127.5, 126.4, 123.2, 122.2, 119.6, 119.0, 111.5, 111.3, 55.6, 40.3, 35.7, 30.7. HRMS (ESI) calcd for $C_{19}H_{22}N_3O$ $[M+H]^+$ 308.1758, found 308.1764.

4.1.19. Data for (*S*)-2-amino-3-(1*H*-indol-3-yl)-*N*-(4-methoxybenzyl)propanamide (16)

This compound was obtained as yellow oil in 80% yield: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (s, 1H,NH), 7.63 (d, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 7.55 (s, 1H, O=C—NH), 7.34 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.16 (t, $^3J_{HH}$ = 6.8 Hz, 1H, Ar-H), 7.13–7.03 (m, 3H, Ph-H and Ar-H), 6.97 (s, 1H, Ar-H), 6.80 (d, $^3J_{HH}$ = 7.2 Hz, 2H, Ph-H), 4.34 (d, $^4J_{HH}$ = 2.8 Hz, 1H, C**H**$_2$), 3.85–3.63 (m, 4H, C**H**$_3$ and C**H**), 3.36 (d, $^3J_{HH}$ = 13.2 Hz, 1H, C**H**$_2$), 3.01–2.86 (m, 1H, C**H**$_2$), 1.77 (br, 1H, NH$_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.8, 158.9, 136.5, 130.5, 129.1, 123.4, 122.1, 119.5, 118.9, 114.0, 111.4, 111.4, 55.6, 55.3, 42.7, 30.9. HRMS (ESI) calcd for $C_{19}H_{22}N_3O_2$ $[M+H]^+$ 324.1707, found 324.1697.

4.1.20. Data for (*S*)-2-amino-*N*-(2,4-dimethoxybenzyl)-3-(1*H*-indol-3-yl)propanamide (17)

This compound was obtained as yellow oil in 69% yield: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (s, 1H,NH), 7.66 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.52 (t, $^3J_{HH}$ = 5.2 Hz, 1H, O=C—NH), 7.35 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.19 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.13 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ph-H), 7.11 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 6.98 (d, $^4J_{HH}$ = 1.2 Hz, 1H, Ar-H), 6.47–6.38 (m, 2H, Ph-H), 4.37 (dd, $^4J_{HH}$ = 4.0 Hz, $^3J_{HH}$ = 5.6 Hz, 2H, C**H**$_2$), 3.80 (s, 3H, C**H**$_3$), 3.76 (s, 3H, C**H**$_3$), 3.70 (dd, $^3J_{HH}$ = 8.8 Hz, 4.4 Hz, 1H, C**H**$_2$), 3.38 (dd, $^3J_{HH}$ = 4.4 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C**H**$_2$), 2.90 (dd, $^3J_{HH}$ = 8.8 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C**H**$_2$), 1.58 (br, 1H, NH$_2$).^{13}C NMR (100 MHz, $CDCl_3$): δ 174.4, 160.4, 158.6, 136.4, 130.3, 127.5, 123.1, 122.2, 119.6, 119.1, 119.0, 111.9, 111.2, 103.9, 98.6, 55.8, 55.4, 55.3, 38.4, 30.9. HRMS (ESI) calcd for $C_{20}H_{24}N_3O_3$ $[M+H]^+$ 354.1812, found 354.1806.

4.1.21. Data for (*S*)-2-amino-*N*-(4-chlorobenzyl)-3-(1*H*-indol-3-yl)propanamide (18)

This compound was obtained as yellow oil in 83% yield: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (s, 1H,NH), 7.70–7.59 (m, 2H, Ar-H and O=C—NH), 7.36 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.24–7.16 (m, 3H, Ar-H and Ph-H), 7.14–7.03 (m, 3H, Ph-H and Ar-H), 7.00 (s, 1H, Ar-H), 4.37 (t, $^4J_{HH}$ = 5.2 Hz, $^3J_{HH}$ = 5.2 Hz, 1H, C**H**$_2$), 3.75 (dd, $^3J_{HH}$ = 8.4 Hz, 4.4 Hz, 1H, C**H**$_2$), 3.37 (dd, $^3J_{HH}$ = 4.4 Hz, $^2J_{HH}$ = 14.4 Hz, 1H,

C**H**$_2$), 2.99 (dd, $^3J_{HH}$ = 8.4 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C**H**$_2$), 1.60 (br, 1H, NH$_2$). ^{13}C NMR (100 MHz, CDCl$_3$): δ 174.9, 137.0, 136.5, 133.1, 129.0, 128.7, 127.5, 123.2, 122.3, 119.7, 119.0, 111.5, 111.3, 55.5, 42.4, 30.8. HRMS (ESI) calcd for $C_{18}H_{19}N_3OCl$ $[M+H]^+$ 328.1211, found 328.1210.

4.1.22. Data for (*S*)-2-amino-*N*-(3-chlorobenzyl)-3-(1*H*-indol-3-yl)propanamide (19)

This compound was obtained as yellow oil in 79% yield: ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.39 (s, 1H,NH), 7.72–7.60 (m, 2H, Ar-H and O=C—NH), 7.37 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.23–7.14 (m, 4H, Ar-H and Ph-H), 7.11 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.05 (d, $^3J_{HH}$ = 5.6 Hz, 1H, Ph-H), 7.01 (d, $^3J_{HH}$ = 0.8 Hz, 1H, Ar-H), 4.42–4.36 (m, 1H, C**H**$_2$), 3.76 (dd, $^3J_{HH}$ = 8.0 Hz, 4.0 Hz, 1H, C**H**$_2$), 3.38 (dd, $^3J_{HH}$ = 4.4 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C**H**$_2$), 3.01 (dd, $^3J_{HH}$ = 8.4 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C**H**$_2$), 1.52 (br, 1H, NH$_2$). ^{13}C NMR (100 MHz, CDCl$_3$): δ 175.0, 140.6, 136.4, 134.4, 129.9, 127.7, 127.5, 127.5, 125.9, 123.3, 122.3, 119.7, 118.9, 111.5, 111.4, 55.6, 42.6, 30.8. HRMS (ESI) calcd for $C_{18}H_{19}N_3OCl$ $[M+H]^+$ 328.1211, found 328.1210.

4.1.23. Data for (*S*)-2-amino-*N*-(2-chlorobenzyl)-3-(1*H*-indol-3-yl)propanamide (20)

This compound was obtained as yellow oil in 77% yield: ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.35 (s, 1H,NH), 7.90–7.56 (m, 2H, Ar-H and O=C—NH), 7.52–6.86 (m, 8H, Ar-H and Ph-H), 4.53 (s, 1H, C**H**$_2$), 3.86–3.62 (m, 1H, C**H**), 3.54–3.25 (m, 1H, C**H**$_2$), 3.11–2.82 (m, 1H, C**H**$_2$), 1.56 (br, 1H, NH$_2$). ^{13}C NMR (100 MHz, CDCl$_3$): δ 174.9, 136.4, 135.8, 133.7, 129.9, 129.5, 128.8, 127.5, 127.0, 123.2, 122.3, 119.6, 118.9, 111.6, 111.3, 55.7, 41.1, 30.8. HRMS (ESI) calcd for $C_{18}H_{19}N_3OCl$ $[M+H]^+$ 328.1211, found 328.1208.

4.1.24. Synthesis of (*S*)-2-amino-*N*-(2-hydroxyethyl)-3-(1*H*-indol-3-yl)propanamide (21)[21]

To a stirred solution of (*S*)-2-(benzyloxycarbonylamino)-3-(1*H*-indol-3-yl)propanoic acid(0.50 g, 1.48 mmol), DMAP (0.36 g, 1.48 mmol) and triethylamine (0.30 g, 2.96 mmol) in dichloromethane (40 mL) was added EDCI (0.57 g, 2.96 mmol) in one portion. The mixture was stirred at 0 °C for 0.5 h, ethanolamine (0.13 g, 2.13 mmol) was added to the above mixture and then kept at room temperature for another 8 h, the reaction was complete detected by TLC. A saturated solution of ammonium chloride (30 mL) was added to the reaction, and the layers were separated. The organic phase was washed with saturated brine, dried over Na_2SO_4 and concentrated. The crude product was chromatographed on silica gel (dichloromethane/methanol 30:1) to give a white solid. Then methanol (30 mL) and Pd/C (0.02 g, 10%) was added, and the solution was purged with H_2 overnight at room temperature. The mixture was filtered through a Celite pad, and the filtrate was concentrated to give compound **21** (0.15 g) as brown oil in 41% yield: ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.97 (s, 1H,NH), 8.33 (t, $^3J_{HH}$ = 5.2 Hz, 1H, O=C—NH), 7.63 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.35 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.19 (d, $^4J_{HH}$ = 2.0 Hz, 1H, Ar-H), 7.08 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 6.99 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 5.91 (br, 2H,NH$_2$), 4.77 (s, 1H, OH), 3.72 (dd, $^3J_{HH}$ = 8.0 - Hz, 5.6 Hz, 1H, C**H**), 3.45–3.35 (m, 2H, C**H**$_2$), 3.25–3.05 (m, 3H, C**H**$_2$), 2.94 (dd, $^3J_{HH}$ = 8.0 Hz, $^2J_{HH}$ = 14.4 Hz, 1H, C**H**$_2$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.2, 136.2, 127.2, 124.3, 121.0, 118.4, 118.3, 111.3, 108.6, 59.6, 53.7, 41.5, 28.9. HRMS (ESI) calcd for $C_{13}H_{18}N_3O_2$ $[M+H]^+$ 248.1394, found 248.1397.

Compounds **22** were synthesized using the similar procedure as compound **21**.

4.1.25. Data for (*S*)-2-amino-3-(1*H*-indol-3-yl)-*N*-(pyridin-3-ylmethyl)propanamide (22)

This compound was obtained as a yellow solid in 49% yield: ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.89 (s, 1H, Ar-NH), 8.50–8.40 (m, 3H, Py-H and O=C—NH), 7.56 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.48–7.43 (m, 1H, Py-H), 7.35 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.26 (dd, $^3J_{HH}$ = 4.8 Hz, 7.6 Hz, 1H, Py-H), 7.14 (d, $^4J_{HH}$ = 2.0 Hz), 7.06 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 6.97 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 4.28 (t, $^4J_{HH}$ = 2.4 Hz, 2H, Py-C**H**$_2$), 3.52 (dd, $^3J_{HH}$ = 5.2 Hz, 7.6 Hz, 1H, C**H**), 3.09 (dd, $^3J_{HH}$ = 5.2 Hz, $^2J_{HH}$ = 14.0 Hz, 1H, C**H**$_2$), 2.80 (dd, $^3J_{HH}$ = 7.6 Hz, $^2J_{HH}$ = 14.0 Hz, 1H, CH$_2$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.9, 148.8, 148.0, 136.4, 135.1, 135.1, 127.5, 124.0, 123.5, 121.0, 118.6, 118.4, 111.5, 110.5, 55.6, 40.4, 31.1. HRMS (ESI) calcd for $C_{17}H_{19}N_4O$ $[M+H]^+$ 295.1553, found 295.1558.

4.1.26. Synthesis of (*S*)-methyl 2-(3-butylureido)-3-(1*H*-indol-3-yl)propanoate (23)

A mixture of (*S*)-methyl 2-amino-3-(1*H*-indol-3-yl)propanoate (0.60 g, 2.75 mmol), NEt$_3$ (0.3 mL), *n*-butyl isocyanate (0.33 g, 3.3 mmol) in dichloromethane (40 mL) were stirred at rt overnight. The mixture was concentrated in vacuum when the reaction was complete detected by TLC, and the crude product was chromatographed on silica gel (petroleum oil/ethyl acetate 30:1) to give compound **23** (0.65 g) as oil in 85% yield: ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.68 (s, 1H, Ar-NH), 7.50 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.30 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.14 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.07 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 6.90 (d, $^3J_{HH}$ = 2.0 Hz, 1H, Ar-H), 5.27 (br, 1H, C=ONH), 4.94 (br, 1H, C=ONH), 4.79 (dd, $^3J_{HH}$ = 13.2 Hz, $^3J_{HH}$ = 5.6 Hz, 1H, CHC=O), 3.62 (s, 3H, OCH$_3$), 3.22 (d, $^3J_{HH}$ = 5.2 Hz, 2H, C**H**$_2$CH), 2.97 (br, 1H, C**H**$_2$NH), 1.37–1.15 (m, 4H, CH$_2$), 0.93–0.77 (m, 3H, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 174.0, 157.9, 136.2, 127.6, 123.2, 122.0, 119.4, 118.5, 111.4, 109.8, 53.7, 52.2, 40.2, 32.0, 28.1, 19.9, 13.7; HRMS (ESI) calcd for $C_{17}H_{23}N_3O_3$ $[M+H]^+$ 318.1812, found 318.1816.

Compounds **24**–**27R** were synthesized using the similar procedure as compound **23**.

4.1.27. Data for (*S*)-methyl 3-(1*H*-indol-3-yl)-2-(3-isopropylureido)propanoate (24)

This compound was obtained as a yellow solid in 80% yield: mp = 58–61 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 1H, Ar-NH), 7.44 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.34 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.15–7.02 (m, 2H, Ar-H), 6.98 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 6.01 (d, $^3J_{HH}$ = 7.6 Hz, 1H, CONH), 5.96 (d, $^3J_{HH}$ = 8.0 Hz, 1H, CONH), 4.45 (dd, $^3J_{HH}$ = 13.6 Hz,$^3J_{HH}$ = 6.4 Hz, 1H, C=OCH), 3.68–3.59 (m, 1H, CH), 3.57 (s, 3H, OCH$_3$), 3.14–2.99 (m, 2H, CH$_2$), 1.00 (d, $^3J_{HH}$ = 6.8 Hz, 6H, CH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.9, 157.2, 136.5, 127.7, 124.2, 121.4, 118.8, 118.6, 111.8, 109.6, 53.8, 52.1, 41.3, 28.3, 23.7, 23.6; HRMS (ESI) calcd for $C_{16}H_{21}N_3O_3$ $[M+H]^+$ 304.1656, found 304.1662.

4.1.28. Data for (*S*)-methyl 2-(3-cyclohexylureido)-3-(1*H*-indol-3-yl)propanoate (25)

This compound was obtained as a yellow solid in 81% yield: mp = 84–87 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (s, 1H, Ar-NH), 7.44 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.33 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.12–7.02 (m, 2H, Ar-H), 6.97 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 6.08 (d, $^3J_{HH}$ = 8.0 Hz, 1H, NH), 5.98 (d, $^3J_{HH}$ = 8.0 Hz, 1H, NH), 4.45 (dd, $^3J_{HH}$ = 13.6 Hz, $^3J_{HH}$ = 6.4 Hz, 1H, C=OCH), 3.57 (s, 3H, OCH$_3$), 3.33–3.27 (br, 1H, CH), 3.14–2.98 (m, 2H, CH$_2$), 1.80–0.94 (m, 10H, cyclohexyl–CH$_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.4, 156.6, 136.1, 127.3, 123.6, 120.9, 118.3, 118.1, 111.3, 109.1, 53.3, 51.6, 47.6, 33.2, 33.1, 27.8, 25.2, 24.3; HRMS (ESI) calcd for $C_{19}H_{26}N_3O_3$ $[M+H]^+$ 344.1969, found 344.1975.

4.1.29. Data for (*S*)-methyl 2-(3-cyclopentylureido)-3-(1*H*-indol-3-yl)propanoate (26)

This compound was obtained as a yellow solid in 82% yield: mp = 60–62 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 1H, Ar-NH), 7.44 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.34 (d, $^3J_{HH}$ = 8.0 Hz,

1H, Ar-H), 7.13–7.03 (m, 2H, Ar-H), 6.97 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 6.17 (d, $^3J_{HH}$ = 7.2 Hz, 1H, NH), 5.93 (d, $^3J_{HH}$ = 8.0 Hz, 1H, NH), 4.46 (dd, $^3J_{HH}$ = 14.0 Hz, $^3J_{HH}$ = 6.4 Hz, 1H, C═OCH), 3.89–3.75 (m, 1H, CH), 3.57 (s, 3H, OCH_3), 3.15–2.99 (m, 2H, CH_2), 1.81–1.40 (m, 8H, cyclopentyl-CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.4, 156.9, 136.1, 127.3, 123.7, 120.9, 118.3, 118.1, 111.3, 109.1, 53.3, 51.6, 50.8, 50.8, 33.0, 32.8, 27.8, 23.1; HRMS (ESI) calcd for $C_{18}H_{23}N_3O_3$ $[M+H]^+$ 330.1812, found 330.1815.

4.1.30. Data for (*S*)-methyl 2-(3-benzylureido)-3-(1*H*-indol-3-yl)-2-(3-isopropylureido)propanoate (27S)

This compound was obtained as a white solid in 74% yield: mp = 151–153 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H, Ar-NH), 7.46 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.38–7.17 (m, 6H, Ar-H), 7.11 (d, $^3J_{HH}$ = 2.4 Hz, 1H, Ar-H), 7.09–7.04 (m, 1H, ArH), 7.01–6.95 (m, 1H, Ar-H), 6.62 (t, $^3J_{HH}$ = 6.0 Hz, 1H, N**H**CH_2), 6.26 (d, $^3J_{HH}$ = 8.0 Hz, 1H, C═ONH), 4.54–4.46 (m, 1H, C**H**NH), 4.19 (d, $^3J_{HH}$ = 6.0 Hz, 2H, CH_2CH), 3.58 (s, 3H, OCH_3), 3.17–3.00 (m, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.8, 157.9, 141.1, 136.5, 128.7, 127.7, 127.4, 127.7, 124.2, 121.4, 118.9, 118.6, 111.8, 109.6, 54.0, 52.2, 43.2, 28.3; HRMS (ESI) calcd for $C_{20}H_{21}N_3O_3$ $[M+H]^+$ 352.1656, found 352.1657.

4.1.31. Data for (*R*)-methyl 2-(3-benzylureido)-3-(1*H*-indol-3-yl)-2-(3-isopropylureido)propanoate (27R)

This compound was obtained as a yellow solid in 75% yield: mp = 190–193 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.95 (s, 1H, Ar–NH), 10.56 (s, 1H, N**H**), 7.58 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.37 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.15–6.99 (m, 5H, Ar-H), 6.96 (t, $^3J_{HH}$ = 6.4 Hz, 1H, Ar-H), 6.56(d, $^3J_{HH}$ = 6.8 Hz, 1H, Ar-H), 6.26 (d, $^3J_{HH}$ = 8.0 Hz, 1H, C═ONH),4.67 (m, 3H, C**H**NH+C**H**$_2$Ph), 3.58 (s, 3H, OCH_3),3.28 (d, $^2J_{HH}$ = 15.2 Hz, 1H, C**H**$_2$CH), 3.18 (d, $^2J_{HH}$ = 14.8 Hz, 1H, C**H**$_2$CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.6, 175.5, 137.0, 129.1, 128.5, 127.7, 127.3, 125.5, 122.1, 119.8, 119.6, 112.4, 108.1, 61.0, 44.1, 26.7; HRMS (ESI) calcd for $C_{20}H_{21}N_3O_3$ $[M+H]^+$ 352.1656, found 352.1657.

4.1.32. Synthesis of (*S*)-5-((1*H*-indol-3-yl)methyl)-3-isopropyl-2-thioxoimidazolidin-2,4-dione (28)

A mixture of (*S*)-methyl 2-amino-3-(1*H*-indol-3-yl)propanoate (0.60 g, 2.75 mmol), NEt_3 (0.3 mL), isopropyl isothiocyanates (0.34 g, 3.3 mmol) in dichloromethane (40 mL) were stirred at 40 °C overnight. The mixture was concentrated in vacuum when the reaction was complete detected by TLC, and the crude product was chromatographed on silica gel (petroleum oil/ethyl acetate 30:1) to give compound **23** (0.63 g) as yellow solid in 85% yield: mp = 166–169 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 1H, Ar–NH), 10.31 (s, 1H, N**H**), 7.52 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.28 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.09–6.99 (m, 2H, Ar-H), 6.95 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 4.42 (m, 2H, C**H**NH+C**H**$(CH_3)_2$), 3.19 (dd, $^2J_{HH}$ = 14.8 Hz, $^3J_{HH}$ = 4.0 Hz, 1H, C**H**$_2$), 3.07 (dd, $^2J_{HH}$ = 14.8 Hz, $^3J_{HH}$ = 4.4 Hz, 1H, C**H**$_2$), 1.17 (d, $^3J_{HH}$ = 6.8 Hz, 3H, C**H**$_3$), 0.68 (d, $^3J_{HH}$ = 6.8 Hz, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.2, 175.1, 136.2, 127.7, 124.7, 121.2, 119.1, 118.7, 111.5, 107.1, 59.0, 45.7, 26.4, 19.1, 18.3; HRMS (ESI) calcd for $C_{15}H_{17}N_3OS$ $[M+H]^+$ 288.1165, found 288.1164.

Compounds **29**–**32** were synthesized using the similar procedure as compound **28**.

4.1.33. Data for (*S*)-5-((1*H*-indol-3-yl)methyl)-3-cyclohexyl-2-thioxoimidazolidin-4-one (29)

This compound was obtained as a yellow solid in 85% yield: mp = 69–72 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H, Ar-NH), 10.30 (s, 1H, N**H**), 7.52 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.28 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.06–6.99 (m, 2H, Ar-H), 6.95 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 4.42 (t, $^3J_{HH}$ = 4.0 Hz, 1H, C**H**NH), 4.08 (m, 1H, C**H**$(CH_2)_2$), 3.18 (dd, $^2J_{HH}$ = 15.2 Hz, $^3J_{HH}$ = 4.4 Hz, 1H, CH_2), 3.07 (dd, $^2J_{HH}$ = 14.8 Hz, $^3J_{HH}$ = 4.4 Hz, 1H, CH_2), 1.53 (m, 4H), 1.08–0.55 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.3, 175.1, 136.2, 127.7, 124.6, 121.2, 119.1, 118.7, 111.5, 107.1, 59.0, 53.6, 28.5, 27.6, 26.4, 25.8, 25.7, 25.2; HRMS (ESI) calcd for $C_{18}H_{21}N_3OS$ $[M+H]^+$ 328.1478, found 328.1694.

4.1.34. Data for (*S*)-5-((1*H*-indol-3-yl)methyl)-3-cyclopentyl-2-thioxoimidazolidin-4-one (30)

This compound was obtained as a yellow solid in 83% yield: mp = 155–158 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H, Ar-NH), 10.33 (s, 1H, N**H**), 7.52 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.29 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.07–7.00 (m, 2H, Ar-H), 6.95 (t, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 4.66–4.52 (m, 1H, CH$(CH_2)_2$), 4.44 (t, $^3J_{HH}$ = 4.0 Hz, 1H, C**H**NH), 3.19 (dd, $^2J_{HH}$ = 14.8 Hz, $^3J_{HH}$ = 4.4 1H, CH_2), 3.09 (dd, $^2J_{HH}$ = 15.2 Hz,$^3J_{HH}$ = 4.4, 1H,CH_2),1.92–1.27 (m, 8H), 1.21–1.11 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.1, 174.3, 135.7, 127.2, 124.1, 120.8, 118.5, 118.2, 111.0, 106.8, 58.6, 53.2, 27.7, 27.2, 25.9, 24.7, 24.6; HRMS (ESI) calcd for $C_{17}H_{19}N_3OS$ $[M+H]^+$ 314.1322, found 314.1613.

4.1.35. Data for (*S*)-5-((1*H*-indol-3-yl)methyl)-3-benyl-2-thioxoimidazolidin-4-one (31S)

This compound was obtained as a yellow solid in 86% yield: mp = 185–188 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (s, 1H, Ar-NH), 10.55 (s, 1H, N**H**), 7.58 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.37 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.06 (m, 5H, Ar-H), 6.96 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 6.56 (d, $^3J_{HH}$ = 7.6 Hz, 2H, Ar-H), 4.68 (d, $^3J_{HH}$ = 3.6 Hz, 1H, C**H**NH), 4.66 (s, 2H, C**H**$_2$), 3.27 (dd, $^2J_{HH}$ = 14.8 Hz, $^3J_{HH}$ = 4.0 Hz, 1H, C**H**$_2$CH), 3.17 (dd, $^2J_{HH}$ = 14.8 Hz, $^3J_{HH}$ = 4.0 Hz, 1H, C**H**$_2$CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.9, 174.7, 136.3, 136.3, 128.4, 127.8, 127.0, 126.6, 124.8, 121.4, 119.0, 118.9, 111.7, 107.4, 60.2, 43.4, 26.0; HRMS (ESI) calcd for $C_{19}H_{17}N_3OS$ $[M+H]^+$ 336.1165, found 336.1167.

4.1.36. Data for (*R*)-5-((1*H*-indol-3-yl)methyl)-3-benyl-2-thioxoimidazolidin-4-one (31R)

This compound was obtained as a yellow solid in 75% yield: mp = 190–193 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.95 (s, 1H, Ar-NH), 10.56 (s, 1H, N**H**), 7.58 (d, $^3J_{HH}$ = 7.6 Hz, 1H, Ar-H), 7.37 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.15–6.99 (m, 5H, Ar-H), 6.96 (t, $^3J_{HH}$ = 6.4 Hz, 1H, Ar-H), 6.56 (d, $^3J_{HH}$ = 6.8 Hz, 2H, Ar-H), 4.67 (m, 3H, C**H**NH+C**H**$_2$Ph), 3.28 (d, $^2J_{HH}$ = 15.2 Hz, 1H, C**H**$_2$CH), 3.18 (d, $^2J_{HH}$ = 14.8 Hz, 1H, C**H**$_2$CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.6, 175.5, 137.0, 129.1, 128.5, 127.7, 127.3, 125.5, 122.1, 119.8, 119.6, 112.4, 108.1, 61.0, 44.1, 26.7; HRMS (ESI) calcd for $C_{19}H_{17}N_3OS$ $[M+H]^+$ 336.1165, found 336.1165.

4.1.37. Data for (*S*)-5-((1*H*-indol-3-yl)methyl)-3-phenyl-2-thioxoimidazolidin-4-one (32)

This compound was obtained as a yellow solid in 83% yield: mp = 176–179 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.98 (s, 1H, Ar-NH), 10.57 (s, 1H, N**H**), 7.56 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.36 (d, $^3J_{HH}$ = 8.0 Hz, 1H, Ar-H), 7.33–7.27 (m, 3H, Ar-H), 7.15 (d, $^3J_{HH}$ = 2.0 Hz, 1H, Ar-H), 7.08 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 6.97 (t, $^3J_{HH}$ = 7.2 Hz, 1H, Ar-H), 6.68–6.62 (m, 2H, Ar-H), 4.72 (t, $^3J_{HH}$ = 4.0 Hz, 1H, C**H**NH), 3.31 (d, $^2J_{HH}$ = 14.8 Hz, $^3J_{HH}$ = 4.0 Hz, 1H, C**H**$_2$), 3.22 (dd, $^2J_{HH}$ = 14.8 Hz, $^3J_{HH}$ = 4.8 Hz, 1H, C**H**$_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.8, 174.6, 136.3, 133.6, 128.9, 128.8, 127.6, 124.9, 121.4, 119.1, 118.9, 111.7, 107.3, 60.5, 26.8; HRMS (ESI) calcd for $C_{18}H_{15}N_3OS$ $[M+H]^+$ 322.1009, found 328.1010.

4.2. Biological assay

The bioassay was repeated in triplicate at 25 ± 1 °C. The error of the experiments was 5%. Assessments were made on a dead/alive

basis, and mortality rates were corrected using Abbott's formula. Evaluations were based on a percentage scale of 0–100, where 0 equals no activity and 100 equals total kill.

4.2.1. Antiviral activity of compounds against TMV in vitro

Fresh leaf of the 5–6 growth stage of tobacco inoculated by the juice-leaf rubbing method (concentration of TMV is 5.88×10^{-2} μg/mL) was cut into halves along the main vein. The halves were immersed into the solution of 500 μg/mL of the compounds and double-distilled water for 20 min, respectively, and then cultured at 25 °C for 72 h. Each compound was replicated at least 3 times.

4.2.2. Protective effect of compounds against TMV in vivo

The compound solution was smeared on the left side, and the solvent served as a control on the right side of growing *Nicotiana tabacum L.* leaves of the same ages. The leaves were then inoculated with the virus after 12 h. A brush was dipped in TMV of 6×10^{-3} mg/mL to inoculate the leaves, which were previously scattered with silicon carbide. The leaves were then washed with water and rubbed softly along the nervature once or twice. The local lesion numbers appearing 3–4 days after inoculation were counted. There are three replicates for each compound.

4.2.3. Inactivation effect of compounds against TMV in vivo

The virus was inhibited by mixing with the compound solution at the same volume for 30 min. The mixture was then inoculated on the left side of the leaves of *N. tabacum L.*, whereas the right side of the leaves was inoculated with the mixture of solvent and the virus for control. The local lesion numbers were recorded 3–4 days after inoculation. There are three replicates for each compound.

4.2.4. Curative effect of compounds against TMV in vivo

Growing leaves of *N. tabacum L.* of the same ages were selected. TMV (concentration of 6.0×10^{-3} mg/mL) was dipped and inoculated on the whole leaves. Then, the leaves were washed with water and dried. The compound solution was smeared on the left side, and the solvent was smeared on the right side for control. The local lesion numbers were then counted and recorded 3–4 days after inoculation. There are three replicates for each compound.

The in vitro and in vivo inhibition rates of the compound were then calculated according to the following formula ('av' means average, and controls were not treated with compound): inhibition rate (%) = [(av local lesion number of control − av local lesion number of drug-treated)/av local lesion number of control] × 100%.

4.2.5. Fungicide activity

A stock solution of each compound was prepared at 500 mg/kg using *N*,*N*-dimethylformamide (DMF) as a solvent. A working solution (50 mg/kg) was then prepared by diluting the stock solution (0.1 mL) with sterilized water (0.9 mL) in a 10 cm diameter Petri dish. Potato dextrose agar (PDA, 9 mL) was then added to prepare the plate. Before the plate solidification, the PDA was thoroughly mixed by turning around the Petri dish in the sterilized operation desk 5 times to scatter the compounds in PDA evenly. Then,4 mm of diameter of fungi cake was inoculated on the plate and cultured in the culture tank at 24–26 °C. The diameter of fungi spread was measured 2 days later. Growth inhibition was then calculated using the corresponding control. Fungi used in this study included *Fusarium oxysporiumf.* sp. *cucumeris* (*F.C.*); *Cercospora arachidicola Hori* (*C.H.*); *Physalospora piricola* (*P.P.*); *Alternaria solani* (*A.S.*); *Fusarium graminearum* (*F.G.*); *Fusarium moniliforme* (*F.M.*); *Sclerotinia sclerotiorum* (*S.S.*); *Phytophthora capsici* (*P.C.*); *Rhizoctonia cerealis* (*R.C.*); *Bipolaris maydis* (*B.M.*); *Watermelon-anthracnose* (*W.A.*); *Phytophthora infestans* (*P.I.*); *Rhizoctonia solani* (*R.S.*); *Botrytis cinerea* (*B.C.*).

4.2.6. Stomach toxicity against oriental armyworm (*Mythimna separate*), cotton bollworm (*Helicoverpa armigera*), pyrausta nubilalis (*Ostrinia nubilalis*)

The stomach toxicities of the title compounds **1**, **3**, **4**, **6**–**20**, and **22**–**32** against oriental armyworm, cotton bollworm, pyrausta nubilalis were evaluated by foliar application using the reported procedure. For the foliar tests, individual corn leaves were placed on moistened pieces of filter paper in Petri dishes. The leaves were then sprayed with the test solution and allowed to dry. The dishes were infested with 10 fourth-instar larvae. Percentage mortalities were evaluated 4 days after treatment. Each treatment was performed three times.

4.2.7. Larvicidal activity against mosquito

The larvicidal activity of the title compound **1**, **3**, **4**, **6**–**20**, and **22**–**32**against mosquito were tested. These compounds were prepared to a terminal concentration of 10 and 5 mg/kg by dissolving them in acetone and adding distilled water. Ten fourth-instar mosquitolarvae were put into the 10 mL of the test solution and raised for 8 days; the results were expressed by death percentage.

4.2.8. The field trials

The severity of the virus disease was divided into 0–4 grade. The disease index and control effect can be calculated by using the following formulae:

$$\text{Disease index } (I)\% = [\Sigma(N_n \times n)/(N \times 4)] \times 100$$

$$\text{Control effect } (\%) = [1 - (I'_{bc} \times I)/(I_{bc} \times I')] \times 100$$

where N_n is the number of diseased plants of each relative level values, n is the relative level values (n = 0–4), N is the total number of diseased plants, I'_{bc} is the disease index of the blank control area before spraying, I_{bc} is the disease index of the blank control area 10 days after the third time spraying, I' is the disease index of the spraying area before spraying; And I is the disease index of the spraying area 10 days after the third time spraying.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (21132003, 21421062, 21372131), and the Specialized Research Fund for the Doctoral Program of Higher Education (20130031110017) and the '111' Project of Ministry of Education of China (B06005).

References and notes

1. Song, H. J.; Liu, Y. X.; Liu, Y. X.; Wang, L. Z.; Wang, Q. M. *J. Agric. Food Chem.* **2014**, *62*, 1010.
2. Song, H. J.; Liu, Y. X.; Liu, Y. X.; Huang, Y. Q.; Li, Y. Q.; Wang, Q. M. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5228.
3. Liu, Y. X.; Song, H. J.; Huang, Y. Q.; Li, J. R.; Zhao, S.; Song, Y. C.; Yang, P. W.; Xiao, Z. X.; Liu, Y. X.; Li, Y. Q.; Shang, H.; Wang, Q. M. *J. Agric. Food Chem.* **2014**, *62*, 9987.
4. Chen, Q.; Ji, C. T.; Song, Y. X.; Huang, H. B.; Ma, J. Y.; Tian, X. P.; Ju, J. H. *Angew. Chem., Int. Ed.* **2013**, *52*, 9980.
5. Yan, W.; Ge, H. M.; Wang, G.; Jiang, N.; Mei, Y. N.; Jiang, R.; Li, S. J.; Chen, C. J.; Jiao, R. H.; Xu, Q.; Ng, S. W.; Tan, R. X. PNAS2014, 111, 18138.
6. Lin, H. C.; Chiou, G.; Chooi, Y. H.; McMahon, T. C.; Xu, W.; Garg, N. K.; Tang, Y. *Angew. Chem., Int. Ed.* **2015**, *54*, 3004.
7. Song, W. X.; Wang, R. M. *Acad. Period. Farm Prod. Process.* **2005**, *3*, 18.
8. Zuo, Z. Z.; Huang, Q. G.; Wu, W. B.; Cai, A. J.; Weng, X. Q.; Zhao, Y. Y.; Shi, Q. Q. *Anhui. Agric. Sci. Bull.* **2010**, *16*, 38.
9. Kang, G. Y.; Li, Z. P.; Liu, X. L.; Wu, X. R.; Shen, H. Q.; Wang, Q. I. *Carcinog. Teratogenesis. Mutagen.* **2015**, *25*, 272.
10. Hipskind, P. A.; Lobb, K. L. US 005792760 A, 1998.
11. Wang, D. H.; Wei, P.; Ouyang, P. K. *Chem. Ind. Eng. Prog.* **2002**, *21*, 103.
12. Kohki, T.; Keisuke, S.; Yutaka, S. EP 0032708 A1, 1981.
13. Appelt, C.; Schrey, A. K.; Arvid Söderhäll, J.; Schmieder, P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2334.

14. Li, W. M.; Tan, H. Z.; Wang, J. G.; Li, Y. H.; Li, Z. M. *Chem. J. Chin. Univ.* **2009**, *30*, 728.
15. Nongonierma, A. B.; FitzGerald, R. J. *Food Funct.* **1843**, *2013*, 4.
16. Lu, A. D.; Wang, Z. W.; Zhou, Z. H.; Chen, J. X.; Wang, Q. M. *J. Agric. Food Chem.* **2015**, *63*, 1378.
17. Li, L.; Li, Z.; Wang, K. L.; Zhao, S.; Feng, J. M.; Li, J. R.; Yang, P. W.; Liu, Y. X.; Wang, L. Z.; Li, Y. Q.; Shang, H.; Wang, Q. M. *J. Agric. Food Chem.* **2014**, *62*, 11080.
18. Xiao, J. C.; Xu, J. L.; Cui, S.; Liu, H. B.; Wang, S.; Li, Y. L. *Org. Lett.* **2008**, *10*, 645.
19. Kleijn, L. H. J.; Müskens, F. M.; Oppedijk, S. F.; Bruin, G. D.; Martin, N. I. *Tetrahedron Lett.* **2012**, *53*, 6430.
20. Zhu, J.; Chen, T.; Chen, L. L.; Lu, W. Q.; Che, P.; Huang, J.; Li, H. L.; Li, J.; Jiang, H. L. *Molecules* **2009**, *14*, 494.
21. Yaeko, K. Y.; Chiharu, O.; Kiminari, Y.; Yasuyuki, U.; Shiho, A.; Noriko, S.; Toshitsugu, K.; Hiroaki, T.; Yoshihiro, H. *Tetrahedron* **2002**, *58*, 7851.

Please cite this article in press as: Huang, Y.; et al. *Bioorg. Med. Chem.* (2015), http://dx.doi.org/10.1016/j.bmc.2015.08.016