有机化学 DOI: 10.6023/cjoc202302012 研究论文
Chinese Journal of Organic Chemistry ARTICLE

5-硝基亚氨基[1,4-2*H*]-1,2,4-三唑啉烯式吡虫啉类似物的合成及生物活性研究

刘 敏[†,a] 杨冬燕[†,b] 肖玉梅*[,a] 苏旺苍[c] 赵峰海[a] 覃兆海*[,a]

([a]中国农业大学理学院 北京 100193)
([b]仲恺农业工程学院化学化工学院 广州 510225)
([c]河南省农业科学院植物保护研究所 郑州 450002)

摘要 烯式吡虫啉是吡虫啉的高活性代谢产物. 为解决烯式吡虫啉的高蜂毒问题, 根据生物电子等排原理, 合成了一系列烯式吡虫啉的类似物 1,3-二取代-5-硝基亚氨基三唑啉化合物, 研究了该系列化合物对蚜虫、褐飞虱和西花蓟马的杀虫活性和离体杀菌活性. 结果表明, 这些化合物在 400 μg/mL 浓度下表现出一定的杀虫活性, 其中化合物**II-7** 在 100 μg/mL 浓度对西花蓟马的致死率达 100%. 用 admetSAR 预测了部分化合物对蜜蜂的毒性, 结果均表现为低毒. 目标化合物在 50 μg/mL 浓度下对 9 种植物病原真菌均表现出不同程度的抑菌活性, 其中化合物 **I-15** 对 5 种病原菌的抑菌率高于或等同于对照药恶霉灵. 构效关系分析发现, 3-位脂肪烃基取代的化合物的杀虫活性高于芳香烃取代, 提高 3 号位取代基的亲脂性也可明显提高化合物的抑菌活性, 1-位吡啶环和噻唑环的取代对化合物抑菌活性没有影响. 与乙酰胆碱结合蛋白(AchBP)的分子对接结果表明, 化合物**II-7** 与吡虫啉(IMI)的结合模式相似, 说明该类化合物也具有与 AChBP 结合的潜力, 为进一步分子优化提供了新的借鉴.

关键词 生物电子等排; 5-硝基亚氨基-1,2,4-三唑; 杀虫活性; 抑菌活性; 分子对接

Synthesis and Bioactivities of 5-Nitroimino-[1,4-2*H*]-1,2,4-triazolines as Olefin-Imidacloprid Mimics

Liu, Min[†,a] Yang, Dongyan[†,b] Xiao, Yumei*[,a] Su, Wangcang[c]
Zhao, Fenghai[a] Qin, Zhaohai*[,a]

([a] *College of Science*, *China Agricultural University*, *Beijing*, *100193*)
([b] *College of Chemistry and Chemical Engineering*, *Zhongkai University of Agriculture and Engineering*, *Guangzhou 510225*)
([c] *Institute of Plant Protection*, *Henan Academy of Agricultural Sciences*, *Zhengzhou 450002*)

Abstract Olefin-imidacloprid is a highly active metabolite of imidacloprid. To solve the problem of high bee toxicity of olefin-imidacloprid, according to the bioisosterism, a series of novel olefin-imidacloprid mimics containing 1,3-disubstituted-1,2,4-triazoline were designed and synthesized. The insecticidal activity of these compounds against *A. craccivora*, *Nilaparvata lugens* and *F. occidentalis* and *in vitro* antifungal activity were determined. The results showed that these compounds showed certain insecticidal activity at 400 μg/mL and 100% lethal rate was obtained from compound **II-7** at 100 μg/mL against *F. occidentalis*. The toxicity of some compounds to bees was predicted by admetSAR, and they showed low level of toxicity. On the other hand, the target compounds showed different degrees of antifungal activity against 9 plant pathogenic fungi at the concentration of 50 μg/mL. Compound **I-15** exhibited the highest activity with the inhibition rate higher or equal to hymexazol against five pathogens. The structure-activity relationship analysis showed that the compounds bearing alkyl substituent at site 3 in triazoline unit displayed higher insecticidal activity than that of an aryl substitution, meanwhile, the more lipophilic groups, the higher fungicidal activity. The substitution of pyridine ring or thiazole ring at site 1 in triazoline unit had little effect on their antifungal activity. The molecular docking results showed that the binding pattern of compound **II-7** to acetylcholine binding protein (AChBP) was similar to that of imidacloprid (IMI), indicating that these compounds also had the potential

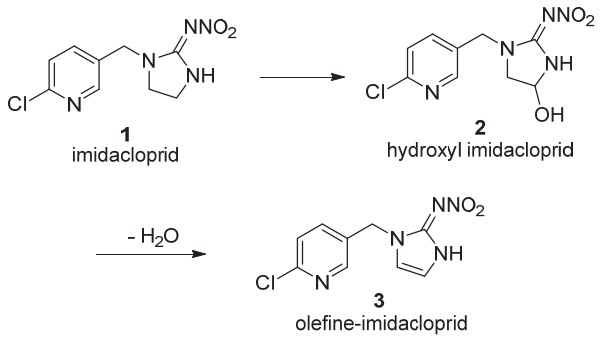
* Corresponding authors. E-mail: qinzhaohai@263.net; xiaoyumei@cau.edu.cn
Received February 12, 2023; revised April 4, 2023; published online June 26, 2023.
Project supported by the National Key Research and Development Plan of China (No. 2016YFD0300709) and the Guangzhou Science and Technology Plan (No. 202102020214).
国家重点研发计划(No. 2016YFD0300709)和广州市科技计划(No.202102020214)资助项目.
† 共同第一作者(These authors contributed equally to this work).

to bind to AChBP, which provided a new reference for further molecular optimization.
Keywords bioisosterism; 5-nitroimine-1,2,4 triazoline; insecticidal activity; fungicidal activity; molecular docking

1 Introduction

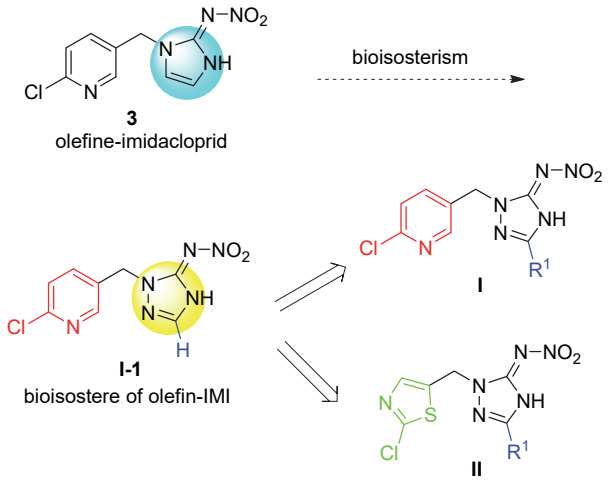
Since imidacloprid (IMI, **1**) was introduced to the market in 1991,[1] neonicotinoid insecticides have grown rapidly and become one of the main types of insecticides.[2-10] However, the safety of bees has become a global concern and led to the restriction or prohibition of some neonicotinoid insecticides in certain regions.[11-14] So it calls for the discovering of novel insecticides with different modes of action to solve this problem.[15-25]

As shown in Scheme 1, imidacloprid can be oxidized to hydroxyl imidacloprid (**2**) and then dehydrated to olefin-imidacloprid (olefin-IMI, **3**) *in vivo*.[26] Nauen *et al.*[27] reported the bioactivity of metabolites of IMI against aphid. Studies have shown that the olefin-IMI is approximately ten times more active than IMI.[28] These results indicated that olefin-IMI can be used as a potential parent compound with high level of activity. However, due to the structural similarity between olefin-IMI and IMI, its toxicity to bees is still high.[29-30]



Scheme 1 Catabolic pathway of imidacloprid

Bioisosterism is a unique approach for lead compounds reasonable modification, which have produced much more effective, economical, and therapeutically attractive drugs.[31] In the field of agrochemicals, bioisosterism has also been widely and successfully applied for development of pesticides.[32-33] 1,2,4-Triazole nucleus and its derivatives have received considerable attention due to its wide biological activities, such as antifungal,[34] antibacterial,[35] and antiviral.[36] 1,2,4-Triazole derivatives have been used in the plant protection as pesticides.[37-38] In previous research, we developed an efficient and practical method for the synthesis of 1,2,4-triazoles from nitroguanidine, and extensively investigated the cyclization mechanism.[39] Herein, 4-C atom in the imidazole ring of olefin-IMI was replaced by N atom according to bioisosterism, a series of novel 1,2,4-triazole derivatives **I** and **II** were designed as shown in Scheme 2. We anticipated that the compounds processed high level of activity and low level of toxicity to honeybees.



Scheme 2 Design strategy for target compounds

2 Results and discussion

2.1 Chemistry

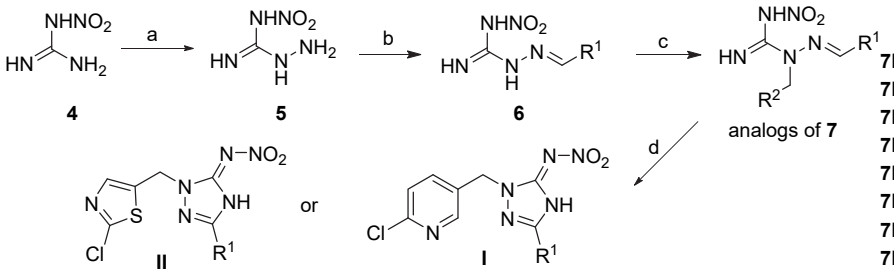
As illustrated in Scheme 3, the target compounds were prepared through a four-step reaction from nitroguanidine as our reported procedure.[39] The intermediate **5** was obtained by nitroguanidine (**4**) with hydrazine hydrate. Then compound **5** with different aldehydes in the presence of a catalytic amount of acetic acid formed intermediate **6**. Compound **6** was reacted with 2-chloro-5-(chloromethyl)-pyridine or 2-chloro-5-chloromethylthiazole in the presence of sodium hydride to produce precursors **7**. Under alkaline conditions, precursors **7** was cyclized to 5-nitroimino-1,2,4-triazoles. Among these, compounds **I-3**～**I-6** have been reported in our previous work.[39]

2.2 Honey bee toxicity prediction of the synthesized compounds

An important reason for the failure of drug candidates is unfavorable bioavailability.[40-41] Presently, the toxicity of the compounds can be predicted by computational methods such as ProTox-II,[42] admetSAR,[43] CompTox Chemicals Dashboard,[44] and so on.[45] Han *et al.*[43,46] successfully obtained a series of novel piperonyl-tethered rhodanine derivatives with low toxicity to honey bees by admetSAR. We also test some of our designed compounds on the toxicity to bees by admetSAR (http://lmmd.ecust.edu.cn/admetsar2/).[43] Delightfully, most of the compounds were less toxic to honey bees (Table 1).

2.3 Insecticidal activity

The initial insecticidal activities at the concentration of 400, 100 and 50 μg/mL were determined and the results are summarized in Table 2.



II-1 R^1 = H
II-2 R^1 = CH_3
II-3 R^1 = C_2H_5
II-4 R^1 = *n*-Pr
II-5 R^1 = *i*-Pr
II-6 R^1 = *n*-Bu
II-7 R^1 = *sec*-Bu
II-8 R^1 = *t*-Bu
II-9 R^1 = *n*-hexyl
II-10 R^1 = *n*-octyl
II-11 R^1 = $CH(OCH_3)_2$
II-12 R^1 = 4-ClC_6H_4

I-1 R^1 = H
I-2 R^1 = CH_3
I-3 R^1 = C_2H_5
I-4 R^1 = *n*-Pr
I-5 R^1 = *i*-Pr
I-6 R^1 = *n*-Bu
I-7 R^1 = *sec*-Bu
I-8 R^1 = *t*-Bu
I-9 R^1 = *n*-hexyl
I-10 R^1 = *n*-octyl
I-11 R^1 = $CH(OCH_3)_2$
I-12 R^1 = 4-ClC_6H_4
I-13 R^1 = 4-$CH_3C_6H_4$
I-14 R^1 = 4-$CH_3OC_6H_4$
I-15 R^1 = 4-$(CH_3)_3CC_6H_4$
I-16 R^1 = C_6H_5
I-17 R^1 = 3-$O_2NC_6H_4$
I-18 R^1 = 4-CNC_6H_4

7I-1 R^1 = H
7I-2 R^1 = CH_3
7I-3 R^1 = C_2H_5
7I-4 R^1 = *n*-Pr
7I-5 R^1 = *i*-Pr
7I-6 R^1 = *n*-Bu
7I-7 R^1 = sec-Bu
7I-8 R^1 = *t*-Bu
7I-9 R^1 = *n*-hexyl
7I-10 R^1 = *n*-octyl
7I-11 R^1 = $CH(OCH_3)_2$
7I-12 R^1 = 4-ClC_6H_4
7I-13 R^1 = 4-$CH_3C_6H_4$
7I-14 R^1 = 4-$CH_3OC_6H_4$
7I-15 R^1 = 4-$(CH_3)_3CC_6H_4$
7I-16 R^1 = C_6H_5
7I-17 R^1 = 3-$O_2NC_6H_4$
7I-18 R^1 = 4-CNC_6H_4
7I-19 R^1 = CF_3
7I-20 R^1 = $COOCH_3$

7II-1 R^1 = H
7II-2 R^1 = CH_3
7II-3 R^1 = C_2H_5
7II-4 R^1 = *n*-Pr
7II-5 R^1 = *i*-Pr
7II-6 R^1 = *n*-Bu
7II-7 R^1 = *sec*-Bu
7II-8 R^1 = *t*-Bu
7II-9 R^1 = *n*-hexyl
7II-10 R^1 = *n*-octyl
7II-11 R^1 = $CH(OCH_3)_2$
7II-12 R^1 = 4-ClC_6H_4

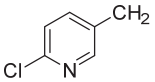
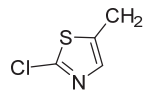
Reaction conditions: [a] N_2H_4•H_2O/H_2O, 50 ℃; [b] R^1CHO/CH_3COOH/MeOH, 50 ℃; [c] 2-chloro-5-chloromethylthiazole or 2-chloro-5-chloromethylpyridine/NaH/DMF, 0 ℃; [d] K_2CO_3/MeCN, reflux.

Scheme 3 Synthetic route for the title compounds

Table 1 Honey bee toxicity prediction of the synthesized compounds

Compd.	Honey bee toxicity	Compound	Honey bee toxicity	Compd.	Honey bee toxicity
I-3	Low	**II-2**	Low	**7I-19**	Low
I-7	Low	**II-5**	Low	**7I-20**	Low
I-9	Low	**II-7**	Low	**3**	High[29-30]
I-13	Low	**II-10**	Low		
I-18	Low	**II-12**	Low		

Table 2 Insecticidal activity of 5-nitroimine-[1,4-2*H*]-1,2,4-triazoline analogues (lethality, %)

Compd.	R^2	R^1	*A.craccivora*/(μg•mL^{-1})		*N. lugens*/(μg•mL^{-1})		*F. occidentalis*/(μg•mL^{-1})		
			400	100	400	100	400	100	50
I-1		H	0	—	0	—	6.67	—	—
I-2		CH_3	44	—	4.44	—	0	—	—
I-3		C_2H_5	52.87	—	2.13	—	39.33	—	—
I-4		*n*-Pr	41.77	—	4.35	—	8.33	—	—
I-5		*i*-Pr	42.35	—	0	—	3.96	—	—
I-6		*n*-Bu	26.25	—	0	—	91.09	0	—
I-7		*sec*-Bu	22.62	—	0	—	2.27	—	—
I-8		*t*-Bu	44.71	—	0	—	43.37	—	—
I-9		*n*-Hexyl	62.9	—	6.25	—	3.88	—	—
I-10		*n*-Octyl	0	—	2.13	—	9.09	—	—
I-11			2.47	—	4.55	—	22.33	—	—
I-12		4-ClC_6H_4	3.8	—	0	—	4.3	—	—
I-13		4-$CH_3C_6H_4$	5.43	—	13.64	—	54.26	—	—
I-14		4-$CH_3OC_6H_4$	0	—	0	—	64	—	—
I-15		4-$(CH_3)_3CC_6H_4$	0	—	0	—	4.3	—	—
I-16		C_6H_5	2.86	—	0	—	94.51	0	—
I-17		3-$NO_2C_6H_4$	9.86	—	8.89	—	2.13	—	—
I-18		4-CNC_6H_4	3.03	—	0	—	4.4	—	—
II-1		H	0	—	0	—	0	—	—
II-2		CH_3	86.25	0	0	—	10.42	—	—
II-3		C_2H_5	58.02	—	2.17	—	45.26	—	—
II-4		*n*-Pr	52.78	—	0	—	3.49	—	—

Continued

Compd.	R^2	R^1	*A.craccivora*/(μg•mL^{-1})		*N. lugens*/(μg•mL^{-1})		*F. occidentalis*/(μg•mL^{-1})		
			400	100	400	100	400	100	50
II-5		*i*-Pr	4.11	—	0	—	25.53	—	—
II-6		*n*-Bu	24.29	—	0	—	3.26	—	—
II-7		*sec*-Bu	100	0	6.38	—	100	100	0
II-8		*t*-Bu	28.79	—	0	—	80.23	0	—
II-9		*n*-Hexyl	3.49	—	0	—	28.05	—	—
II-10		*n*-Octyl	42.68	—	2.22	—	2.97	—	—
II-11			4.84	—	13.64	—	4.3	—	—
II-12		4-ClC_6H_4	2.44	—	2.17	—	0	—	—
7I-19	—	—	48.48	—	0	—	4.44	—	—
7I-20	—	—	2.41	—	46.51	—	0	—	—
3	—	—	100	100	97.78	41.3	100	100	100
Nitenpyram	—	—	100	—	—	—	—	—	—
Dinotefuran	—	—	—	100	100	100	—	—	97.56
Spinosad	—	—	—	—	—	—	100	100	—
DMF	—	—	4.76	1.06	0	0	0	4.9	2.35

[a] "—" not detected.

Most of the compounds exhibited less insecticidal activity against the *Nilaparvata lugens*. However, most of the compounds showed certain insecticidal activities against *Aphis craccivora*, and **II-2** and **II-7** displayed lethality rates of 86.25% and 100% at 400 μg/mL, respectively. Compounds **I-2** to **I-9** and **I-12** to **I-18** (R^2 is 6-chloro-3-pyridylmethyl) showed moderate activities when R^1 is alkyl. As R^1 changed to aryl, the lethality decreased. For substituent R^1, in addition to *t*-Bu, *n*-hexyl and *n*-Bu, the replacement of R^2 by 2-chloro-5-thiazolylmethyl had higher insecticidal activities than 6-chloro-3-pyridylmethyl. For the substituent R^2, 2-chloro-5-thiazolylmethyl and 6-chloro-3-pyridylmethyl didn't show obvious activity pattern.

Also, most of the compounds exhibited certain insecticidal activities against *Frankliniella occidentalis* at 400 μg/mL, and compounds **I-6**, **I-16**, **II-7** and **II-8** displayed a lethality rate of over 80% at 400 μg/mL. It is worthwhile to note that compound **II-7** led to a 100% mortality rate for *Frankliniella occidentalis* at 100 μg/mL, which is equivalent to the insecticidal activity of the olefin-IMI and Spinosad. The structure-activity relationship of the compounds against *Frankliniella occidentalis* is summarized as follows: except that R^1 is *n*-Bu, 2-chloro-5-thiazolylmethyl derived compounds showed higher or similar activities than that of 6-chloro-3-pyridylmethyl. It was worth noting that for 6-chloro-3-pyridylmethyl derived compounds, as R^1 changed to an aryl with electron withdrawing group or a big group like *t*-Bu, there was an obvious reduction in insecticidal activities.

Compared to olefin-IMI, compound **I-1** showed lower activity to the pests, which may be caused by lone pairs on nitrogen atoms forming hydrogen bonds, influencing the binding ability of compound **I-1** to receptors. Meanwhile, nitrogen atom may increase the water solubility of the compound, so it is hard for the compound to move to the action site and bond to the receptor. The replacement of R^1 by an alkyl substituent or an aryl substituent will enhance the lipid solubility of the compounds, resulting in increased activity. For R^1, an aryl with an electron-withdrawing group or a big group has no benefit to enhance activity. Perhaps steric hindrance prevents the binding of the compound with the receptor.

2.4 Antifungal activity

The activities of synthesized compounds against 9 plant pathogenic fungi at 50 μg/mL are shown in Table 3. The fungicidal activities of all the target compounds were below 50% against *Colletotrichum gloeosporioides*, *Fusarium graminearum Schwabe*, *Magnaporthe grisea* and *Fusarium oxysporumf. sp. Vasinfectum*, also exhibited low inhibition against *Botrytis cinerea*. But fortunately, compound **I-15** displayed 61.15% antifungal activities against *Botrytis cinerea*, which was better than hymexazol. Compounds **I-15**, **II-10** and **7I-20** showed no less than 50% inhibitory rates against *Rhizoctonia solani Kuhn*. Compounds **I-9** and **II-10** showed over 50% inhibition rate against *Phytophthora capsici*, which is better than hymexazol. Compounds **I-10** and **I-15** exhibited $>$ 50% inhibitions against *Fusarium moniliforme*. 8 compounds showed over 60% inhibition rate against *Pythium aphanidermatum*.

To sum up, keeping the substituent R^2 unchanged, the replacement of R^1 by a long-chain alkyl substituent like *n*-hexyl and *n*-octyl would led to a higher inhibition rate. Good lipophilicity makes the compound molecule have good penetration ability, so it might bind to the receptor better and show better activity (Table 4). For most of compounds, the substituent R^2 with 2-chloro-5-thiazolylmethyl and 6-chloro-3-pyridylmethyl make no difference in activity. But for those compounds with more than 20% activity difference, the activity of the substituent R^2 with 2-chloro-5-thiazolylmethyl was higher than 6-chloro-3-pyridylmethyl, except compounds **I-1** and **II-1** against *Rhizoctonia solani*. It was worth noting that compound **I-15** exhibited the best activity, with the inhibition rate

Table 3 Antifungal activity of synthesized compounds against 9 pathogenic fungi

Compd.	Inhibition rate/% (50 μg/mL)								
	PA	BC	RS	PC	CG	FG	MG	FO	FM
I-1	0.0±0.8	14.5±3.7	30.0±2.7	6.6±0.8	8.0±4.6	1.0±2.0	11.1±1.0	0.1±2.4	5.1±0.2
I-2	0.0±1.8	11.2±1.6	20.5±2.0	0.6±0.8	5.9±3.1	12.1±4.1	6.8±1.4	1.0±0.5	2.4±1.4
I-3	3.6±0.4	11.3±3.2	16.9±1.6	3.4±1.1	0.0±0.4	14.9±0.7	1.6±0.7	0.3±0.9	0.2±3.9
I-4	0.0±0.9	13.3±1.1	11.6±3.7	0.0±1.4	11.8±0.8	13.6±8.8	4.1±0.3	2.3±4.2	1.3±3.3
I-5	3.2±1.8	6.5±1.3	7.6±4.3	8.3±0.2	5.8±1.1	0.7±3.6	5.6±0.4	3.7±3.5	4.2±1.4
I-6	4.8±0.8	10.7±2.8	14.0±1.6	15.5±1.1	13.3±0.6	0.0±1.2	5.4±1.2	0.1±0.3	2.4±1.6
I-7	5.4±0.4	13.8±1.4	13.3±2.4	20.8±0.7	10.9±0.3	0.9±0.4	4.1±0.7	0.2±0.7	4.2±1.7
I-8	12.3±2.7	14.8±1.0	10.4±4.6	11.5±1.6	6.1±3.1	0.0±0.2	6.2±1.2	5.4±1.4	6.8±4.9
I-9	68.2±1.0	35.5±0.9	34.1±4.7	50.8±2.1	26.4±0.9	12.1±1.2	7.0±2.2	21.0±1.4	13.5±1.5
I-10	89.4±0.1	25.2±3.0	33.1±2.2	48.7±2.4	18.2±3.8	9.2±1.3	20.2±0.5	4.9±2.7	54.9±1.9
I-11	0.0±3.5	8.4±4.7	15.9±4.2	1.7±1.4	7.1±2.3	0.0±2.5	5.4±0.7	4.6±1.4	7.437±23
I-12	69.7±0.9	20.4±1.3	31.8±3.5	10.5±3.4	0.0±1.4	9.2±3.9	6.1±3.4	13.3±1.9	42.1±1.0
I-13	75.8±1.1	26.5±1.5	23.0±1.6	25.3±0.9	11.5±2.2	19.0±2.5	7.5±2.8	15.3±1.8	47.9±3.3
I-14	45.7±3.9	16.7±2.9	19.3±1.7	14.8±2.2	0.0±1.4	17.7±1.5	5.8±0.7	8.7±2.7	32.2±2.5
I-15	87.7±0.5	61.2±3.4	59.3±2.3	44.0±1.2	21.2±4.2	17.2±0.5	10.7±3.1	22.7±2.3	59.0±0.7
I-16	0.0±0.8	15.5±3.5	21.0±1.9	6.3±0.7	3.7±0.9	8.8±2.1	10.4±1.2	5.4±0.4	37.6±1.6
I-17	0.0±2.7	11.6±0.9	21.2±1.1	5.3±0.6	10.1±2.5	1.5±1.2	2.5±1.0	10.3±2.6	6.3±0.3
I-18	0.0±2.2	10.8±2.6	23.9±3.7	0.0±0.7	4.7±4.6	9.5±2.1	4.9±1.8	1.5±1.7	36.1±4.5
II-1	0.0±1.5	10.4±1.7	5.1±1.9	2.4±3.2	15.5±0.8	0.4±0.7	5.8±0.4	10.6±1.4	3.8±1.8
II-2	2.0±2.0	6.1±4.7	12.4±3.4	0.9±0.7	16.8±0.4	4.1±2.4	3.3±0.7	4.5±3.8	1.5±2.2
II-3	0.0±2.0	8.6±2.9	21.2±4.8	0.0±1.6	0.0±2.2	5.6±4.4	3.0±1.8	0.0±1.2	0.0±2.6
II-4	3.6±0.6	13.2±4.0	14.9±3.4	10.9±2.6	7.2±1.4	9.1±1.5	6.5±0.9	0.0±0.4	4.2±0.7
II-5	13.8±1.2	9.7±3.9	6.1±4.0	13.0±4.1	9.4±3.2	21.9±2.7	4.9±0.4	3.6±1.7	13.7±0.7
II-6	23.1±1.5	20.1±3.8	6.4±1.6	27.9±1.7	9.5±2.5	6.4±0.8	36.3±4.3	8.4±1.2	44.9±3.5
II-7	12.4±1.7	13.6±0.2	18.9±4.3	11.7±4.6	10.9±3.2	0.0±2.0	3.1±0.5	1.1±1.4	7.0±0.3
II-8	9.4±0.8	13.7±3.7	10.4±1.6	20.1±2.0	0.0±0.3	0.0±3.7	6.4±0.5	3.2±1.4	8.9±2.2
II-9	64.8±0.2	48.9±1.6	45.5±2.0	34.4±3.2	12.8±2.4	6.6±1.4	11.8±0.7	22.8±1.8	12.1±4.0
II-10	90.0±0.7	41.7±4.1	52.4±2.8	54.1±1.4	17.5±2.0	13.4±2.4	24.0±1.1	6.8±4.7	46.5±8.7
II-11	2.3±2.8	5.6±0.6	19.2±3.7	2.3±1.7	5.8±0.3	0.0±0.3	9.6±4.9	3.3±1.1	1.1±1.6
II-12	86.9±1.3	34.7±1.8	46.6±1.6	16.2±1.1	0.0±0.6	4.8±2.1	10.3±1.0	0.0±2.6	45.6±5.0
7I-19	10.2±1..0	17.3±3.8	25.3±3.7	27.5±1.1	10.9±3.8	2.8±1.0	10.9±0.5	9.7±2.8	9.7±1.1
7I-20	8.7±1.5	0.4±1.8	52.4±2.8	6.1±2.6	11.4±4.7	0.8±1.6	4.7±0.0	5.5±0.9	2.0±2.1
3	0.0±3.8	9.4±4.6	2.3±3.9	0.0±1.2	15.7±1.1	0.0±0.5	5.5±1.1	0.6±1.6	4.1±0.3
Hymexazol	100.0±0.0	50.4±1.9	25.8±2.0	1.5±1.2	37.5±0.4	41.4±0.7	43.0±1.3	51.6±0.3	36.4±2.0

[a] PA=*Pythium aphanidermatum*; BC=*Botrytis cinerea*; RS=*Rhizoctonia solani*; PC=*Phytophthora capsici*; CG=*Colletotrichum gloeosporioides*; FG=*Fusarium graminearum*; MG=*Magnaporthe grisea*; FO=*Fusarium oxysporum*; FM=Fusarium moniliforme.

Table 4 Log *P* values of the compounds predicted by ChemDraw 19.0.0.22

Compd.	Log *P*	Comd.	Log *P*	Compd.	Log *P*
Ⅰ-1	2.56	**Ⅰ-9**	5.64	**Ⅰ-13**	5.06
Ⅰ-3	3.81	**Ⅰ-10**	6.55	**Ⅰ-14**	4.78
Ⅰ-6	4.72	**Ⅰ-12**	5.26	**Ⅰ-15**	6.52

higher than or equivalent to that of boscalid against five pathogens.

2.5 Molecular docking

The imidazole ring in 1,3-disubstituted-1,2,4-triazoline 5-nitroimines is different with traditional neonicotinoids, and we speculate whether these compounds also target at nicotine acetylcholine receptor (nAChR) and form hydrogen bonds at the pocket. Molecular docking of **II-7** and IMI was carried out for evaluation of its potent mode of action of target compounds. The crystal structure of the soluble homologous acetylcholine-binding protein (AChBP) extracted from the sea snail (Aplysia californica) is highly similar to the structure of nAChR. Therefore, AChBP from Aplysia is often used instead of nAChR in many studies on the structure, function and ligand binding of nAChR. The complex crystal of AChBP with imidacloprid bound (PDB ID: 3C79) was chosen for the molecular docking study. To illustrate the binding mode between the ligand and receptor, **II-7** was selected as an example. As shown in Figure 1A, **II-7** located in the pocket which was similar to imidacloprid in the three-dimensional schematic diagrams. It can be seen from the binding mode that the nitro group also acts as a hydrogen bond acceptor, forming a hydrogen bond with the hydroxyl group in the glutamine residue Gln57. The nitro group formed the other hydrogen bonds with the amino acid residues Ser 189. Besides these, IMI could form more hydro-

gen bonds (Figure 1B). The nitrogen atom in pyridine acts as a hydrogen bond acceptor, forming a hydrogen bond with water. The binding mode of **II-7** was similar to that of imidacloprid which indicated that the target compounds also possessed a strong interaction with AChBP.

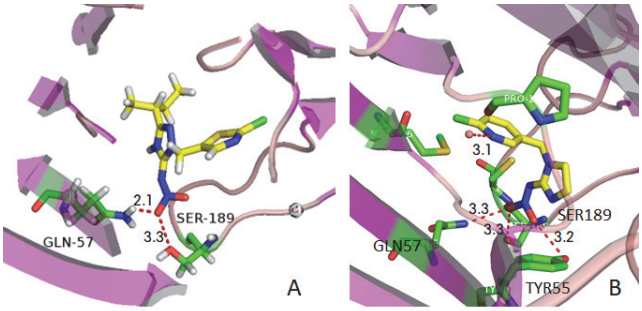


Figure 1 Binding modes of **II-7** (A) and imidacloprid (B) with AChBP

3 Conclusions

In summary, according to bioisosterism, a novel series of 1,3-disubstituted-1,2,4-triazoline 5-nitroimines analogues were designed and synthesized. The bioassays showed that some compounds exhibited different degrees of insecticidal activity against *Aphis craccivoras* and *Frankliniella occidentalis*. The bioisosteric compound **I-1** did not keep the high level of activity as olefin-IMI. But substituting R^1 group with an alkyl or an aryl group to enhance its lipid solubility can improve insecticidal activity. Meanwhile, a big group for R^1 had no benefit to enhance insecticidal activity. It is found that 2-chloro-5-thiazolylmethyl derived compounds did not necessarily lead to higher insecticidal activity than 6-chloro-3-pyridylmethyl. Compound **II-7** displayed the best activity with a 100% mortality rate against *Frankliniella occidentalis* at 100 μg/mL. Delightfully, our structural modification reduced the toxicity of the compounds to honeybees by the predicting result. The molecular docking results showed that the binding pattern of compound **II-7** to AChBP was similar to that of IMI, indicating that it also had the potential to bind to AChBP. The replacement of R^1 by lipophilic groups showed better antifungal activity. For those compounds with more than 20% activity difference, the substituent R^2 with 2-chloro-5-thiazolylmethyl showed higher activity than 6-chloro-3-pyridylmethyl, except compounds **I-1** and **II-1** against *Rhizoctonia solani*. Compound **I-15** exhibited the best activity with the inhibition rate higher or equal to hymexazol against five pathogens. Our results provided a new clue for the molecular design of the novel highly active nAChR agonists with low toxicity to honeybees.

4 Experimental section

4.1 Chemistry

Chemicals and reagents used were obtained from commercial sources without further purification. Melting points were determined by an uncorrected Cole-Parmer microscope melting point apparatus. The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained on a Bruker AVANCE NEO 500 MHz spectrometer using tetramethylsilane as the internal standard. High-resolution mass spectra (HRMS) were obtained on an Shimadzu AB SCIEX X500 QTOF spectrometer.

4.2 General procedures for the *N*-nitrohydrazinecarboximidamide (**5**)

Compound **4** (1.00 equiv.) was added into H_2O and heated to 50 ℃ in an oil bath. The hydrazine hydrate (1.20 equiv.) was added with the reaction temperature at 55～58 ℃. The reaction system was clarified and ice bath was used after 15 min reaction. Concentrated hydrochloric acid was added to adjust pH to 5～6 and a lot of yellow precipitation was appeared. The product was collected by suction. Compound **5** was used directly without any characterization.

4.3 General procedures for the compound **6**

These compounds were synthesized according to the method we reported before.[39] Compound **5** (0.05 equiv.) and catalytic amount of CH_3COOH were added to 50 mL of MeOH. When the mixture was heated to 50 ℃, R^1CHO wad added. The resulting mixture was stirred at reflow temperature for about 2 h and monitored by thin-layer chromatography (TLC). The compounds **6** were recrystallized from ethyl acetate/petroleum ether (V∶V=1∶2) in 92% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.44 (s, 1H), 8.67 (s, 1H), 7.98 (s, 1H), 7.44 (d, J=5.8 Hz, 1H), 2.37～2.26 (m, 1H), 1.56～1.46 (m 1H), 1.44～1.34 (m, 1H), 1.04 (d, J=6.9 Hz, 3H), 0.86 (t, J=7.4 Hz, 3H).

4.4 General procedures for the compound **7**

These compounds were synthesized according to the method we reported before.[39] The mixture of compound **6** (1.00 equiv.) and catalytic amount of KI in *N*,*N*-dimethylformamide (DMF) (40 mL) were stirred to 0 ℃, and then NaH (1.50 equiv.) was added slowly. After 1 h, 2-chloro-5-chloromethylthiazole or 2-chloro-5-chloromethylpyridine (1.10 equiv.) was added dropwise and the mixture was monitored by TLC. About 12 h later, 200 mL of water was added to the reaction system, and a lot of yellow precipitation was appeared, which was collect by suction to get the crude product. Then the crude product was recrystallized from ethyl acetate/petroleum ether (V∶V=1∶5) to get compound **7** in 78% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.01 (s, 2H), 8.28 (d, J=2.5 Hz, 1H), 7.62 (dd, J=8.3, 2.6 Hz, 1H), 7.51 (d, J=8.2 Hz, 1H), 7.37 (t, J=5.4 Hz, 1H), 5.24 (s, 2H), 2.30～2.23 (m, 2H), 1.50～1.39 (m, 2H), 0.79 (t, J=7.4 Hz, 3H).

(*E*)-1-((6-Chloropyridin-3-yl)methyl)-*N*-nitro-2-(2,2,2-trifluoroethylidene)hydrazine-1-carboximidamide (**7I-19**): White crystal, yield 62%, m.p. 152～153 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.00 (s, 2H), 8.34 (d, J=2.5 Hz, 1H), 7.74 (q, J=3.8 Hz, 1H), 7.68 (dd, J=8.3, 2.6 Hz, 1H), 7.53 (d, J=8.3 Hz, 1H), 5.29 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 158.3, 150.0, 149.1, 138.7, 130.8 (q, J=38.3

Hz), 129.4, 124.8, 120.8 (q, J=271.8 Hz), 44.6. ESI-HRMS calcld for $C_9H_8ClF_3N_6O_2$ $[M+H]^+$ 325.0422, found 325.0420.

Methyl (*E*)-2-(2-((6-chloropyridin-3-yl)methyl)-2-(*N*-nitrocarbamimidoyl)hydrazineylidene)acetate (**7I-20**): White solid, yield 71%, m.p. 164～165 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.96 (s, 1H), 8.72 (s, 1H), 8.33 (d, J=2.6 Hz, 1H), 7.68 (dd, J=8.3, 2.6 Hz, 1H), 7.52 (d, J=8.3 Hz, 1H), 7.41 (s, 1H), 5.34 (s, 2H), 3.76 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 162.9, 158.4, 150.0, 149.0, 138.7, 133.6, 129.7, 124.9, 52.7, 44.4; ESI-HRMS calcld for $C_{10}H_{11}ClN_6O_4$ $[M+H]^+$ 315.0603, found 315.0602.

4.5 General procedures for the compounds I and II

These compounds were synthesized according to the method we reported before.[39] Compound **7** (1.00 equiv.) was dissolved in 40 mL of acetonitrile, and then K_2CO_3 (1.50 equiv.) was added. The mixture was stirred and then heated to reflux for 12 h. When reacted with different analogs of **7**, it needed make a little change in reaction condition. After evaporating the solvent *in vacuo*, 50 mL of H_2O was added and then 1 mol/L HCl (aq.) was added to adjust pH to 5～6. The solid was filtered out and washed three times with water. The solid was dried and purified by column chromatography to give compounds **I** and **II**.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-1**): White solid, yield 71%, m.p. 186～187 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 14.02 (s, 1H), 8.53 (s, 1H), 8.41 (d, J=2.5 Hz, 1H), 7.80 (dd, J=8.3, 2.5 Hz, 1H), 7.53 (d, J=8.2 Hz, 1H), 5.27 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 150.5, 150.4, 150.0, 140.1, 139.3, 130.8, 124.8, 47.8; ESI-HRMS calcld for $C_8H_8ClN_6O_2$ $[M+H]^+$ 255.0392, found 255.0393.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-2**): White crystal, yield 76%, m.p. 173～174 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.84 (s, 1H), 8.40 (d, J=2.5 Hz, 1H), 7.79 (dd, J=8.3, 2.5 Hz, 1H), 7.52 (d, J=8.3 Hz, 1H), 5.21 (s, 2H), 2.32 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 150.8, 150.4, 149.9, 148.2, 140.0, 131.0, 124.8, 47.5, 11.8; ESI-HRMS calcld for $C_9H_{10}ClN_6O_2$ $[M+H]^+$ 269.0548, found 269.0548.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-5-ethyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-3**): White crystal, yield 81%, m.p. 161～162 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.84 (s, 1H), 8.40 (d, J=2.6 Hz, 1H), 7.79 (dd, J=8.3, 2.6 Hz, 1H), 7.53 (d, J=8.3 Hz, 1H), 5.23 (s, 2H), 2.70 (q, J=7.5 Hz, 2H), 1.19 (t, J=7.6 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 152.6, 150.9, 150.4, 149.8, 139.9, 131.0, 124.8, 47.6, 19.4, 11.4; ESI-HRMS calcld for $C_{10}H_{12}ClN_6O_2$ $[M+H]^+$ 283.0705, found 283.0704.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-5-propyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-4**): White crystal, yield 85%, m.p. 134～135 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.84 (s, 1H), 8.39 (d, J=2.5 Hz, 1H), 7.78 (dd, J=8.3, 2.5 Hz, 1H), 7.52 (d, J=8.3 Hz, 1H), 5.23 (s, 2H), 2.65 (t, J=7.5 Hz, 2H), 1.71～1.60 (m, 2H), 0.89 (t, J=7.4 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 151.5, 150.9, 150.4, 149.8, 139.9, 131.0, 124.8, 47.6, 27.4, 20.4, 13.7; ESI-HRMS calcld for $C_{11}H_{14}ClN_6O_2$ $[M+H]^+$ 297.0861, found 297.0861.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-5-isopropyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-5**): White crystal, yield 76%, m.p. 165～168 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.83 (s, 1H), 8.39 (d, J=2.5 Hz, 1H), 7.78 (dd, J=8.3, 2.6 Hz, 1H), 7.53 (d, J=8.2 Hz, 1H), 5.24 (s, 2H), 3.15～3.03 (m, 1H), 1.24 (d, J=6.9 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 156.1, 151.0, 150.3, 149.8, 139.9, 131.0, 124.8, 47.7, 26.4, 20.6; ESI-HRMS calcld for $C_{11}H_{14}ClN_6O_2$ $[M+H]^+$ 297.0861, found 297.0861.

(*E*)-*N*-(5-Butyl-2-((6-chloropyridin-3-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-6**): White crystal, yield 79%, m.p. 120～122 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.84 (s, 1H), 8.40 (d, J=2.5 Hz, 1H), 7.79 (dd, J=8.2, 2.5 Hz, 1H), 7.53 (d, J=8.2 Hz, 1H), 5.23 (s, 2H), 2.69 (t, J=7.6 Hz, 2H), 1.67～1.57 (m, 2H), 1.37～1.26 (m, 2H), 0.88 (t, J=7.4 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 151.6, 150.8, 150.4, 149.8, 139.9, 131.0, 124.8, 47.6, 28.9, 25.3, 21.9, 13.9; ESI-HRMS calcld for $C_{12}H_{16}ClN_6O_2$ $[M+H]^+$ 311.1018, found 311.1017.

(*E*)-*N*-(5-(*sec*-Butyl)-2-((6-chloropyridin-3-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-7**): White crystal, yield 64%, m.p. 139～142 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.83 (s, 1H), 8.39 (d, J=2.5 Hz, 1H), 7.78 (dd, J=8.3, 2.6 Hz, 1H), 7.54 (d, J=8.2 Hz, 1H), 5.25 (s, 2H), 2.94 (h, J=6.9 Hz, 1H), 1.78～1.51 (m, 2H), 1.23 (d, J=7.0 Hz, 3H), 0.83 (t, J=7.4 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 155.2, 150.9, 150.3, 149.7, 139.8, 131.0, 124.8, 47.7, 32.8, 27.7, 17.9, 11.6; ESI-HRMS calcld for $C_{12}H_{16}ClN_6O_2$ $[M+H]^+$ 311.1018, found 311.1017.

(*E*)-*N*-(5-(*tert*-Butyl)-2-((6-chloropyridin-3-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-8**): White solid, yield 72%, m.p. 176～179 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.66 (s, 1H), 8.39 (d, J=2.5 Hz, 1H), 7.79 (dd, J=8.3, 2.5 Hz, 1H), 7.53 (d, J=8.2 Hz, 1H), 5.24 (s, 2H), 1.34 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 158.3, 151.6, 150.4, 149.8, 139.9, 130.9, 124.8, 47.7, 32.7, 28.0; ESI-HRMS calcld for $C_{12}H_{16}ClN_6O_2$ $[M+H]^+$ 311.1018, found 311.1017.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-5-hexyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-9**): White crystal, yield 85%, m.p. 93～95 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.83 (s, 1H), 8.39 (d, J=2.5 Hz, 1H), 7.78 (dd, J=8.3, 2.5 Hz, 1H), 7.52 (d, J=8.2 Hz, 1H), 5.22 (s, 2H), 2.66 (t, J=7.6 Hz, 2H), 1.62～1.57 (m, 2H), 1.32～1.20 (m, 6H), 0.87～0.80 (m, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 151.7, 150.9, 150.4, 149.8, 139.9, 131.0, 124.8, 47.6, 31.2, 28.3, 26.8, 25.6, 22.4, 14.3; ESI-HRMS calcld for $C_{14}H_{20}ClN_6O_2$ $[M+H]^+$ 339.1331, found 339.1330.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-5-octyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-10**): White crystal, yield 73%, m.p. 101～102 ℃; ^{1}H NMR (500 MHz,

DMSO-d_6) δ: 13.85 (s, 1H), 8.40 (d, J=2.4 Hz, 1H), 7.79 (dd, J=8.3, 2.6 Hz, 1H), 7.53 (d, J=8.3 Hz, 1H), 5.23 (s, 2H), 2.67 (t, J=7.6 Hz, 2H), 1.63 (p, J=7.3 Hz, 2H), 1.31～1.20 (m, 10H), 0.85 (t, J=6.9 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 151.6, 150.8, 150.4, 149.8, 139.9, 131.0, 124.8, 47.6, 31.6, 29.0, 28.9, 28.7, 26.8, 25.6, 22.5, 14.4; ESI-HRMS calcld for $C_{12}H_{24}ClN_6O_2$ $[M+H]^+$ 367.1644, found 367.1643.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-5-(dimethoxymethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene) nitramide (**I-11**): White solid, yield 74%, m.p. 92～95 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 14.07 (s, 1H), 8.42 (d, J=2.5 Hz, 1H), 7.81 (dd, J=8.3, 2.6 Hz, 1H), 7.54 (d, J=8.3 Hz, 1H), 5.55 (s, 1H), 5.28 (s, 2H), 3.36 (s, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 151.1, 150.5, 150.0, 147.8, 140.1, 130.7, 124.8, 96.2, 54.2, 48.0; ESI-HRMS calcld for $C_{11}H_{14}ClN_6O_4$ $[M+H]^+$ 329.0760, found 329.0759.

(*E*)-*N*-(5-(4-Chlorophenyl)-2-((6-chloropyridin-3-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene) nitramide (**I-12**): White solid, yield 64%, m.p. 168～169 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.47 (d, J=2.5 Hz, 1H), 8.08～8.01 (m, 2H), 7.86 (dd, J=8.3, 2.6 Hz, 1H), 7.64～7.57 (m, 2H), 7.54 (d, J=8.3 Hz, 1H), 5.35 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 151.7, 150.5, 150.0, 149.1, 140.1, 136.3, 130.7, 129.4, 129.4, 124.8, 124.5, 48.1; ESI-HRMS calcld for $C_{14}H_{11}Cl_2N_6O_2$ $[M+H]^+$ 365.0315, found 365.0315.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-5-(*p*-tolyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-13**): White solid, yield 75%, m.p. 177～178 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 14.02 (s, 1H), 8.48 (d, J=2.5 Hz, 1H), 7.95～7.90 (m, 2H), 7.86 (dd, J=8.3, 2.5 Hz, 1H), 7.54 (d, J=8.3 Hz, 1H), 7.34 (d, J=8.0 Hz, 2H), 5.34 (s, 2H), 2.37 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 151.6, 150.4, 150.0, 149.6, 141.7, 140.1, 130.8, 129.8, 127.5, 124.8, 122.6, 48.0, 21.5; ESI-HRMS calcld for $C_{15}H_{14}ClN_6O_2$ $[M+H]^+$ 345.0861, found 345.0861.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-5-(4-methoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene) nitramide (**I-14**): White solid, yield 72%, m.p. 191～192 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 14.03 (s, 1H), 8.47 (d, J=2.5 Hz, 1H), 8.02～7.95 (m, 2H), 7.86 (dd, J=8.3, 2.5 Hz, 1H), 7.54 (d, J=8.3 Hz, 1H), 7.11～7.04 (m, 2H), 5.32 (s, 2H), 3.83 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 161.9, 151.6, 150.4, 150.0, 149.5, 140.1, 130.9, 129.3, 124.8, 117.7, 114.7, 55.9, 48.0; ESI-HRMS calcld for $C_{15}H_{14}ClN_6O_3$ $[M+H]^+$ 361.0810, found 361.0810.

(*E*)-*N*-(5-(4-(*tert*-butyl)phenyl)-2-((6-chloropyridin-3-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene) nitramide (**I-15**): White solid, yield 77%, m.p. 177～178 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 14.04 (s, 1H), 8.47 (d, J=2.5 Hz, 1H), 8.01～7.94 (m, 2H), 7.86 (dd, J=8.3, 2.5 Hz, 1H), 7.61～7.51 (m, 3H), 5.35 (s, 2H), 1.30 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 154.6, 151.6, 150.4, 150.0, 149.5, 140.1, 130.8, 127.4, 126.1, 124.8, 122.6, 48.1, 35.2, 31.3; ESI-HRMS calcld for $C_{18}H_{20}ClN_6O_2$ $[M+H]^+$ 387.1331, found 387.1330.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-5-phenyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-16**): White solid, yield 61%, m.p. 169～170 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.92 (s, 1H), 8.48 (d, J=2.5 Hz, 1H), 8.06～8.00 (m, 2H), 7.87 (dd, J=8.3, 2.5 Hz, 1H), 7.60～7.50 (m, 4H), 5.36 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 151.7, 150.5, 150.0, 149.7, 140.1, 131.7, 130.8, 129.3, 127.6, 125.4, 124.8, 48.1; ESI-HRMS calcld for $C_{14}H_{12}ClN_6O_2$ $[M+H]^+$ 331.0705, found 331.0704.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-5-(3-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-17**): Yellow solid, yield 66%, m.p. 263～265 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.67 (t, J=2.0 Hz, 1H), 8.38 (d, J=2.5 Hz, 1H), 8.36～8.30 (m, 1H), 8.26～8.20 (m, 1H), 7.78～7.70 (m, 2H), 7.51 (d, J=8.2 Hz, 1H), 5.24 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 157.6, 156.7, 149.9, 149.7, 148.6, 139.8, 133.5, 132.5, 132.0, 130.9, 124.7, 123.7, 120.2, 47.7; ESI-HRMS calcld for $C_{14}H_{11}ClN_7O_4$ $[M+H]^+$ 376.0556, found 376.0556.

(*E*)-*N*-(2-((6-Chloropyridin-3-yl)methyl)-5-(4-cyanophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**I-18**): Yellowish solid, yield 64%, m.p. 157～159 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.47 (d, J=2.5 Hz, 1H), 8.23～8.18 (m, 2H), 8.03～7.97 (m, 2H), 7.87 (dd, J=8.3, 2.5 Hz, 1H), 7.54 (d, J=8.3 Hz, 1H), 5.37 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 151.9, 150.5, 150.0, 149.4, 140.2, 133.2, 130.7, 130.3, 128.2, 124.8, 118.7, 113.6, 48.3; ESI-HRMS calcld for $C_{15}H_{11}ClN_7O_2$ $[M+H]^+$ 356.0657, found 356.0657.

(*E*)-*N*-(2-((2-Chlorothiazol-5-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**II-1**): Yellowish solid, yield 68%, m.p. 151～152 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.99 (s, 1H), 8.53 (s, 1H), 7.76 (s, 1H), 5.44 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 152.1, 150.1, 142.2, 139.3, 134.3, 43.6; ESI-HRMS calcld for $C_6H_6ClN_6O_2S$ $[M+H]^+$ 260.9956, found 260.9955.

(*E*)-*N*-(2-((2-Chlorothiazol-5-yl)methyl)-5-methyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**II-2**): White crystal, yield 71%, m.p. 166～167 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.86 (s, 1H), 7.75 (s, 1H), 5.38 (s, 2H), 2.32 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 152.0, 150.3, 148.3, 142.1, 134.5, 43.3, 40.5, 40.3, 11.8; ESI-HRMS calcld for $C_7H_8ClN_6O_2S$ $[M+H]^+$, 275.0112, found 275.0112.

(*E*)-*N*-(2-((2-Chlorothiazol-5-yl)methyl)-5-ethyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**II-3**): White crystal, yield 84%, m.p. 148～150 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.86 (s, 1H), 7.75 (s, 4H), 5.39 (s, 9H), 2.70 (q, J=7.6 Hz, 9H), 1.20 (t, J=7.5 Hz, 14H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 152.7, 152.0, 150.4, 142.0, 134.5, 19.4, 11.3; ESI-HRMS calcld for $C_8H_{10}ClN_6O_2S$ $[M+H]^+$ 289.0269, found 289.0268.

(*E*)-*N*-(2-((2-Chlorothiazol-5-yl)methyl)-5-propyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**II-4**): White crystal, yield 80%, m.p. 122～123 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.85 (s, 1H), 7.75 (s, 1H), 5.39 (s, 2H), 2.66 (t, J=7.4 Hz, 2H), 1.66 (h, J=7.4 Hz, 2H), 0.90 (t, J=7.4

Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 152.0, 151.5, 150.4, 142.0, 134.6, 43.5, 40.5, 27.4, 20.3, 13.7; ESI-HRMS calcld for $C_9H_{12}ClN_6O_2S$ $[M+H]^+$ 303.0425, found 303.0425.

(*E*)-*N*-(2-((2-Chlorothiazol-5-yl)methyl)-5-isopropyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**II-5**): White crystal, yield 74%, m.p. 147～149 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.86 (s, 1H), 7.76 (s, 1H), 5.40 (s, 2H), 3.09 (hept, J=7.0 Hz, 1H), 1.25 (d, J=6.9 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 156.1, 152.0, 150.5, 142.0, 134.5, 43.6, 26.4, 20.5; ESI-HRMS calcld for $C_9H_{12}ClN_6O_2S$ $[M+H]^+$ 303.0425, found 303.0425.

(E)-N-(5-Butyl-2-((2-chlorothiazol-5-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**II-6**): White crystal, yield 81%, m.p. 95～96 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.83 (s, 1H), 7.74 (s, 1H), 5.39 (s, 2H), 2.68 (t, J=7.6 Hz, 2H), 1.67～1.57 (m, 2H), 1.36～1.27 (m, 2H), 0.88 (t, J=7.4 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 152.0, 151.7, 150.4, 142.0, 134.6, 43.4, 28.9, 25.3, 21.9, 13.9; ESI-HRMS calcld for $C_{10}H_{14}ClN_6O_2S$ $[M+H]^+$ 317.0582, found 317.0581.

(*E*)-*N*-(5-(*sec*-Butyl)-2-((2-chlorothiazol-5-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**II-7**): Yellow solid, yield 74%, m.p. 107～109 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.86 (s, 1H), 7.76 (s, 1H), 5.42 (s, 2H), 2.99～2.88 (m, 1H), 1.80～1.69 (m, 1H), 1.63～1.52 (m, 1H), 1.24 (d, J=7.0 Hz, 3H), 0.84 (t, J=7.4 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 155.3, 152.0, 150.4, 141.9, 134.6, 43.6, 32.8, 27.7, 17.9, 11.6; ESI-HRMS calcld for $C_{10}H_{14}ClN_6O_2S$ $[M+H]^+$ 317.0582, found 317.0581.

(*E*)-*N*-(5-(*tert*-Butyl)-2-((2-chlorothiazol-5-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**II-8**): White solid, yield 79%, m.p. 153～155 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.69 (s, 1H), 7.76 (s, 1H), 5.40 (s, 2H), 1.34 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 158.3, 152.1, 151.0, 142.0, 134.4, 43.7, 32.7, 28.0; ESI-HRMS calcld for $C_{10}H_{14}ClN_6O_2S$ $[M+H]^+$ 317.0582, found 317.0581.

(*E*)-*N*-(2-((2-Chlorothiazol-5-yl)methyl)-5-hexyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**II-9**): White crystal, yield 81%, m.p. 98～100 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.87 (s, 1H), 7.76 (s, 1H), 5.40 (s, 2H), 2.68 (t, J=7.5 Hz, 2H), 1.68～1.59 (m, 2H), 1.35～1.19 (m, 6H), 0.88～0.82 (m, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 152.0, 151.7, 150.3, 142.0, 134.6, 43.4, 31.2, 28.3, 26.7, 25.5, 22.4, 14.3; ESI-HRMS calcld for $C_{12}H_{18}ClN_6O_2S$ $[M+H]^+$, 345.0895, found 345.0894.

(*E*)-*N*-(2-((2-Chlorothiazol-5-yl)methyl)-5-octyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**II-10**): White crystal, yield 79%, m.p. 95～96 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 13.87 (s, 1H), 7.76 (s, 1H), 5.40 (s, 2H), 2.68 (t, J=7.5 Hz, 2H), 1.69～1.59 (m, 2H), 1.31～1.18 (m, 10H), 0.85 (t, J=6.9 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 151.9, 151.6, 150.3, 142.0, 134.6, 43.4, 31.7, 29.0, 28.9, 28.7, 26.8, 25.5, 22.5, 14.4; ESI-HRMS calcld for $C_{14}H_{22}ClN_6O_2S$ $[M+H]^+$ 373.1208, found 373.1207.

(*E*)-*N*-(2-((2-Chlorothiazol-5-yl)methyl)-5-(dimethoxymethyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**II-11**): White solid, yield 69%, m.p. 162～163 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.64 (s, 1H), 5.25 (s, 1H), 5.24 (s, 2H), 3.27 (s, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 158.0, 156.3, 151.4, 141.0, 136.8, 99.0, 53.3, 43.2; ESI-HRMS calcld for $C_9H_{12}ClN_6O_4S$ $[M+H]^+$ 335.0324, found 335.0323.

(*E*)-*N*-(5-(4-Chlorophenyl)-2-((2-chlorothiazol-5-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ylidene)nitramide (**II-12**): White solid, yield 63%, m.p. 164～165 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.08～8.01 (m, 2H), 7.81 (s, 1H), 7.65～7.58 (m, 2H), 5.51 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 152.2, 151.2, 149.2, 142.3, 136.4, 134.2, 129.4, 129.4, 124.5, 44.0; ESI-HRMS calcld for $C_{12}H_9Cl_2N_6O_2S$ $[M+H]^+$ 370.9879, found 370.9882.

4.6 Larvicidal activity against *Aphis craccivora* and *Nilaparvata lugens*

The activities against *A. craccivora* were tested using a spraying method.[47-48] About 30 live adults of *A. craccivora* were transferred to a bean leaf and sprayed with Potter spray tower with 2.5 mL of the test solution. Nitenpyram, compound **3** and Tween-80 solution served as the positive controls and blank control, respectively. After 72 h, the number of pest deaths was counted to determine mortality. A similar method was used to test the insecticidal activities against *Nilaparvata lugens*.

4.7 Larvicidal activity against *Frankliniella occidentalis*

The activities against *F. occidentalis* were tested by leaf impregnation method. Centrifuge tubes of 2 mL were filled with each concentration of test solution and placed for 4 h. Then, the test solution was poured out and the centrifuge tubes on the experimental table were dried. The cabbage leaves were cut into long strips of 0.7 cm×2 cm and immersed in each concentration solution for 10 s. After treatment, the cabbage leaves were dried naturally at room temperature, and put into the centrifuge tube with the corresponding concentration of liquid medicine with small tweezers, then put the pests into the tubes. Three replicates were set for each measurement, and spinosad, compound **3** and Tween-80 solution served as the positive controls and blank control, respectively. After 48 h, the number of pest deaths was counted to determine mortality.

4.8 *In vitro* antifungal activity assay

The *in vitro* antifungal activity was determined by the mycelium growth rate method.[49] When the mycelium in the blank group was fully grown in the whole Petri dish, the diameter of mycelium formation was measured with the criss-cross method. The mycelial inhibition rate was calculated according to the method reported before.[49] Preliminary results of in vitro antifungal activity at a concentration of 50 μg/mL were shown in Table 3.

Supporting Information The ^{1}H NMR, ^{13}C NMR, HRMS spectra of compounds **I**, **II**, **7I-19**, **7I-20**, and the ^{1}H

NMR of intermediates that were not reported before compounds **6-7**～**6-20**, **7I-4**, **7I-7**, **7I-9**～**7I-18** and **7II-7**～**7II-12**. The physical data of compounds **I** and **II** in detail. The Supporting Information is available free of charge via the Internet at http://sioc-journal.cn.

References

[1] Elbert, A.; Becker, B.; Hartwig, J.; Erdelen, C. *Pflanzenschutz-Nachr. Bayer* **1991**, *44*, 113.
[2] Casida, J. E.; Quistad, G. B. *Annu. Rev. Entomol.* **1998**, *43*, 1.
[3] Jeschke, P.; Nauen, R. *Pest Manage. Sci.* **2008**, *64*, 1084.
[4] Tomizawa, M.; Casida, J. E. *Acc. Chem. Res.* **2009**, *42*, 260.
[5] Casida, J. E. *J. Agric. Food Chem.* **2011**, *59*, 2923.
[6] Kagabu, S. *J. Agric. Food Chem.* **2011**, *59*, 2887.
[7] Tomizawa, M.; Casida, J. E. *J. Agric. Food Chem.* **2011**, *59*, 2883.
[8] Jeschke, P.; Nauen, R.; Beck, M. E. *Angew. Chem., Int. Ed.* **2013**, *52*, 9464.
[9] Leite, S. A.; Guedes, R. N. C.; da Costa, D. R.; Colmenarez, Y. C.; Matsumoto, S. N.; dos Santos, M. P.; Coelho, B. S.; Moreira, A. A.; Castellani, M. A. *Pest Manage. Sci.* **2022**, *78*, 2581.
[10] Alsafran, M.; Rizwan, M.; Usman, K.; Saleem, M. H.; Jabri, H. A. *J. Environ. Chem. Eng.* **2022**, *10*, 108485.
[11] Britt, E. E. *Chem. Eng. News* **2023**, *101*, 14.
[12] Lourencetti, A. P. S.; Azevedo, P.; Miotelo, L.; Malaspina, O.; Nocelli, R. C. F. *Environ. Pollut.* **2023**, *318*, 120842.
[13] European Food Safety, A. *EFSA J.* **2018**, *16*, e05178.
[14] Demortain, D. *Curr. Opin. Insect. Sci.* **2021**, *46*, 78-82.
[15] Onozaki, Y.; Horikoshi, R.; Ohno, I.; Kitsuda, S.; Durkin, K. A.; Suzuki, T.; Asahara, C.; Hiroki, N.; Komabashiri, R.; Shimizu, R.; Furutani, S.; Ihara, M.; Matsuda, K.; Mitomi, M.; Kagabu, S.; Uomoto, K.; Tomizawa, M. *J. Agric. Food Chem.* **2017**, *65*, 7865.
[16] Xu, Y.; Yang, D. Y.; Zou, X. G.; Rui, C. H.; Zhou, Z. Y.; Ma, Y. Q.; Qin, Z. H. *Pest Manage. Sci.* **2017**, *73*, 1927.
[17] Yang, S.; Lai, Q.; Lai, F.; Jiang, X.; Zhao, C.; Xu, H. *Pest Manage. Sci.* **2021**, *77*, 1013.
[18] Nauen, R.; Jeschke, P.; Velten, R.; Beck, M. E.; Ebbinghaus-Kintscher, U.; Thielert, W.; Wolfel, K.; Haas, M.; Kunz, K.; Raupach, G. *Pest Manage. Sci.* **2015**, *71*, 850.
[19] Wernecke, A.; Eckert, J. H.; Forster, R.; Kurlemann, N.; Odemer, R. *J. Plant Dis. Protect.* **2021**, *129*, 93.
[20] Tamburini, G.; Wintermantel, D.; Allan, M. J.; Dean, R. R.; Knauer, A.; Albrecht, M.; Klein, A. M. *Sci. Total Environ.* **2021**, *778*, 146084.
[21] Jeschke, P.; Nauen, R.; Gutbrod, O.; Beck, M. E.; Matthiesen, S.; Haas, M.; Velten, R. *Pest. Biochem. Physiol.* **2015**, *121*, 31.
[22] Zhu, C.; Li, G.; Xiao, K.; Shao, X.; Cheng, J.; Li, Z. *Chin. Chem. Lett.* **2019**, *30*, 255.
[23] Markussen, M. D. K.; Kristensen, M. *Pest. Biochem. Physiol.* **2010**, *98*, 50.
[24] Kim, J.; Chon, K.; Kim, B.-S.; Oh, J.-A.; Yoon, C.-Y.; Park, H.-H. *Pest Manage. Sci.* **2022**, *78*, 5402.
[25] Zhang, D.; Zhang, J.; Liu, T.; Wu, S.; Wu, Z.; Wu, S.; Song, R.; Song, B. *J. Agric. Food Chem.* **2022**, *70*, 8598.
[26] Sur, R. *B. Insectol.* **2003**, *56*, 35.
[27] Nauen, R.; Tietjen, K.; Wagner, K.; Elbert, A. *Pestic. Sci.* **1998**, *52*, 53.
[28] Nauen, R.; Reckmann, U.; Armborst, S.; Stupp, H.-P.; Elbert, A. *Pestic. Sci.* **1999**, *55*, 265.
[29] Liu, J.; Zhang, Y.; Dong, F.; Wu, X.; Pan, X.; Xu, J.; Zheng, Y. *J. Sep. Sci.* **2022**, *45*, 3567.
[30] Suchail, S.; Guez, D.; Belzunces, L. P. *Environ. Toxicol. Chem.* **2001**, *20*, 2482.
[31] Jayashree, B. S.; Nikhil, P. S.; Paul, S. *Med. Chem.* **2022**, *18*, 915-925.
[32] Kang, O.-Y.; Kim, E.; Lee, W. H.; Ryu, D. H.; Lim, H. J.; Park, S. J. *RSC Adv.* **2023**, *13*, 2004.
[33] Wu, H.; Maimaitijiang, A.; Tang, D.; Xie, B.; Niu, C.; Aisa, H. A. *Heterocycles* **2023**, *106*, 94.
[34] Guzel, E.; Acar Cevik, U.; Evren, A. E.; Bostanc, H. E.; Gul, U. D.; Kays, U.; Ozkay, Y.; Kaplanckl, Z. A. *ACS Omega* **2023**, *8*, 4369.
[35] Li, J.; Zhang, J. *Curr. Top. Med. Chem.* **2022**, *22*, 41.
[36] Gupta, O.; Pradhan, T.; Chawla, G. *J. Mol. Struct.* **2023**, *1274*, 134487.
[37] Xie, R.; Mei, X.; Ning, J. *Chem. Pharm. Bull.* **2019**, *67*, 345.
[38] Li, B.; Yu, Y.; Duan, W.; Lin, G.; Wen, R.; Zhang, Z. *Chem. Biodivers.* **2022**, *19*, e202200726.
[39] Zhao, F.; Singh, T.; Xiao, Y.; Su, W.; Yang, D.; Jia, C.; Li, J.-Q.; Qin, Z. *Synthesis* **2021**, *53*, 1901.
[40] Ouyang, Y.; Huang, J.-j.; Wang, Y.-L.; Zhong, H.; Song, B.-A.; Hao, G.-f. *J. Agric. Food Chem.* **2021**, *69*, 10761.
[41] Chen, D.; Hao, G.; Song, B. *J. Agric. Food Chem.* **2022**, *70*, 10090.
[42] Banerjee, P.; Eckert, A. O.; Schrey, A. K.; Preissner, R. *Nucleic Acids Res.* **2018**, *46*, W257.
[43] Cheng, F.; Li, W.; Zhou, Y.; Shen, J.; Wu, Z.; Liu, G.; Lee, P. W.; Tang, Y. *J. Chem. Inf. Model.* **2012**, *52*, 3099.
[44] Williams, A. J.; Grulke, C. M.; Edwards, J.; McEachran, A. D.; Mansouri, K.; Baker, N. C.; Patlewicz, G.; Shah, I.; Wambaugh, J. F.; Judson, R. S.; Richard, A. M. *J. Cheminf.* **2017**, *9*, 61/61-61/27.
[45] Perez Santin, E.; Rodriguez Solana, R.; Gonzalez Garcia, M.; Garcia Suarez, M. D. M.; Blanco Diaz, G. D.; Cima Cabal, M. D.; Moreno Rojas, J. M.; Lopez Sanchez, J. I. *Wiley Interdiscip. Rev.: Comput. Mol. Sci.* **2021**, *11*, e1516.
[46] Han, Q.; Wu, N.; Liu, Y.-Y.; Zhang, J.-Y.; Zhang, R.-L.; Li, H.-L.; Jiang, Z.-Y.; Huang, J.-X.; Duan, H.-X.; Yang, Q. *J. Agric. Food Chem.* **2022**, *70*, 7387.
[47] Zhao, P.-L.; Wang, F.; Zhang, M.-Z.; Liu, Z.-M.; Huang, W.; Yang, G.-F. *J. Agric. Food Chem.* **2008**, *56*, 10767.
[48] Su, W.; Zhou, Y.; Ma, Y.; Wang, L.; Zhang, Z.; Rui, C.; Duan, H.; Qin, Z. *J. Agric. Food Chem.* **2012**, *60*, 5028.
[49] Wang, J.; Liu, X.; Zhang, X.; Du, S.; Han, X.; Li, J. Q.; Xiao, Y.; Xu, Z.; Wu, Q.; Xu, L.; Qin, Z. *J. Agric. Food Chem.* **2022**, *70*, 111.

(Lu, Y.)

