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Chiral 1,5-disubstituted 1,3,5-hexahydrotriazine-2-*N*-nitroimine analogues as novel potent neonicotinoids: Synthesis, insecticidal evaluation and molecular docking studies

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ABSTRACT

A new series of 1,5-disubstituted 1,3,5-hexahydrotriazine-2-*N*-nitroimines (**4a**–**4x**) were designed and synthesized as novel chiral neonicotinoid analogues. The single-crystal structure of **4n** was further determined by X-ray diffraction, and its *S* configuration was confirmed. Preliminary bioassay showed that compound **4e**, **4k**, **4u**, **4v** exhibited excellent insecticidal activities at 100 mg/L, while **4k** had >90% mortality at 10 mg/L, which suggested it could be used as a lead for future development. Modeling the inhibitor-nAChR complexes by molecular docking studies explained the structure–activity relationships observed in vitro, and revealed an intriguing molecular binding mode at the active site of nAChR, which raised the possibility that these analogues may arbitrate their insecticidal activity through a mechanism other than imidacloprid.

1. Introduction

Neonicotinoid insecticides [1] (NNs) have recently received considerable interests from both agricultural chemistry and medicinal fields, due to their broad spectrum of biological activities and high selectivity for crop protection and public health. Since imidacloprid [2] (IMI) was introduced to the market, many new generation NNs, such as thiacloprid [3] (THIA), thiamethoxam [4] and dinotefuran [5] are now on the market with their own prominence (Fig. 1) and they account for one-fifth of the global insecticide market. These NNs have agonistic effects on the nicotinic acetylcholine receptor (nAChR) [6] and confer high selectivity [7–9] and much lower toxicity [10,11] against mammals, birds, aquatic life than insects, due to the differential binding affinities with the nAChR of their neurosystem [12].

However, a well-recognized potential problem facing all insecticides is the insects' acquisition of resistance [13,14]. Although the neonicotinoids have proven relatively resilient to the development of resistance [15–17], significant increases in resistance and cross-resistance to NNs have been observed in a range of species after frequent field applications during the past decades [18–20]. Especially, recently collected strains of the whitefly exhibited much stronger resistance to imidacloprid and thiamethoxam [21,22]. Hence, research in the design and screening for novel insecticidal lead compounds with less resistance is a high priority.

Since structure modification of the existing NNS is one of the most effective resistance-management tactics, we developed a new design strategy to innovate a novel serious of neonicotinoid analogues. As well known, electron-withdrawing groups such as nitro play important roles in the major classes of NNs insecticides, and the corresponding NNs showed excellent insecticidal activities [22,23]. In addition, the hydroheterocyles and amino acid esters have also been proved to be useful sections in the scaffold of active insecticides, which may be involved in the recognition and interactions with the insect target(s) [24–26]. Based on these reports, a new series of chiral neonicotinoid analogues (**4a**–**4x**) were designed and synthesized by introducing the 1,3,5-hexahydrotriazine pharmacophore with various ethoxycarbonyl-alkyl substituents at 5-position into the scaffolds of imidacloprid and thiamethoxam, and their insecticidal activities against *Aphis medicagini* were evaluated. To obtain the precise three-dimensional structural information, compound **4n** was further crystallized and its single-crystal structure was determined by X-ray diffraction. The

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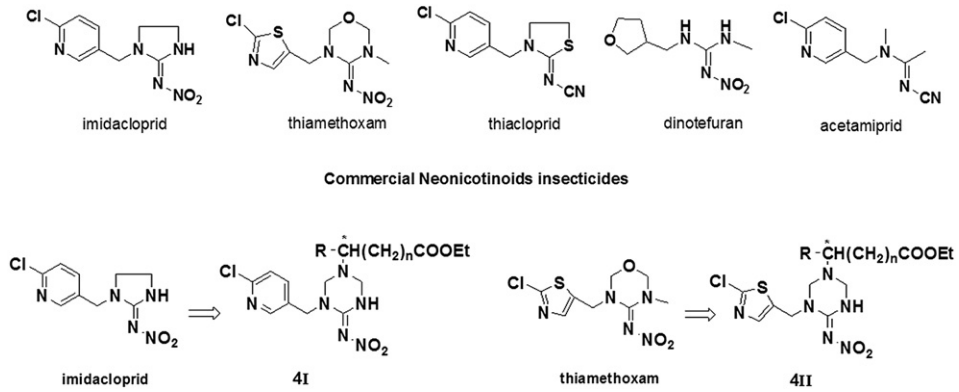


Fig. 1. Structures of commercial available neonicotinoid insecticides, **4I** and **4II** are the target compounds based on imidacloprid and thiamethoxam, respectively.

binding activities of these analogues into the nAChR model were also studied by molecular docking investigations, which prompted some useful information for future design of new NNs pesticides.

2. Chemistry

The route adapted for the synthesis of the target compounds (**4a**–**4x**) is summarized in Scheme 1. Amino acid ethyl ester hydrochlorides **2a**–**2l** were prepared via acylation of various amino acids **1** with dichloro sulfoxide [27], followed by alcoholysis at 70 °C with >80% yield. These amino acids included Gly, γ-aminobutanoic acid, β-aminopropanoic acid, L-α-(Ala, Leu, Ile, Val, Met, Phe, Asp, Glu), and D-4-Chlorophenylglycine. Correspondingly, 5-substituted 1,3,5-hexahydrotriazine-2-*N*-nitroimines **3a**–**3l** were synthesized by Manich reaction with 50–60% yield [28], in the presence of Et_3N. The three components of this Manich reaction were nitroguanidine, 37% formaldehyde aqueous solution, and an excess of amino acid ethyl ester hydrochlorides **2a**–**2l** in the ratio of 1: 3: 1.5. The target compounds **4a**–**4x** were then prepared by reaction of **3a**–**3l**, 2-chloro-5-chloromethly thiazole (Cl-Thy-CH_2Cl) or 6-chloro-3-chloromethly pyridine (Cl-Pyr-CH_2Cl) and K_2CO_3 with catalysis amount of CsCl at 60 °C [29,30], which promoted the conversion and selectivity. Aprotic solvent must be selected in this reaction, since the chloro atom on the thiazolemethyl and pyridinemethyl group may be apt to react with proton solvent. The best choice of aprotic solvent herein is acetonitrile, because of its high polarity and low boiling point.

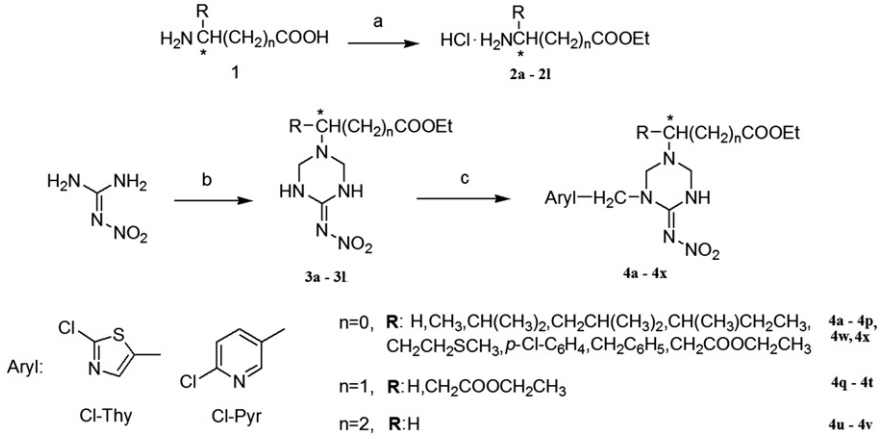
3. Result and discussion

3.1. Single-crystal structure of compound *4n*

To further obtain precise three-dimensional structural information, the single-crystal structure of chiral compound **4n** was investigated by X-ray diffraction analysis (CCDC Number: 751235), with its *S* configuration confirmed. As indicated in Fig. 2, the molecules with chiral carbon atom (C10) were symmetrical to the center of unit cell and adopted the single *S* configuration, which was also supported by the value of abs_structure_Flack (−0.01). Due to the electron-withdrawing force of nitro, the distance of N (3)-C(7) (1.337 Å), N(2)-C(2) (1.341 Å) were remarkably shorter than standard C–N single bond (1.47 Å) but close to C=N (1.33 Å). The crystal data also showed that the dihedral angles made by the triazine ring with the pyridine ring are approximate 65.99° and the hexahydrotriazine ring of **4n** was in a chair form conformation, which makes this molecule more stable.

3.2. Evaluation of insecticidal activities

As depicted in Table 1, most of our designed compounds exhibited good insecticidal activities against *A. medicagini* and had >60% mortality at 100 mg/L. Among all these analogues, **4k** afforded the best in vitro activity, and had >90% mortality at 10 mg/L, which is comparable to that of imidacloprid and thiamethoxam. When different substituents were introduced to the 5-position of



Scheme 1. Synthesis of 1,5-disubstituted 1,3,5-hexahydrotriazine-2-*N*-nitroimines (**4a**–**4x**). Reagents and conditions: (a) (1) $SOCl_2$, 0 °C; (2) EtOH, 70 °C; (b) **2a**–**2l**, HCHO, Et_3N, EtOH, 60 °C; (c) 2-chloro-5-chloromethyl-thiazole and 2-chloro-5-chloromethyl-pyridine, K_2CO_3, CsCl, CH_3CN, 60 °C.

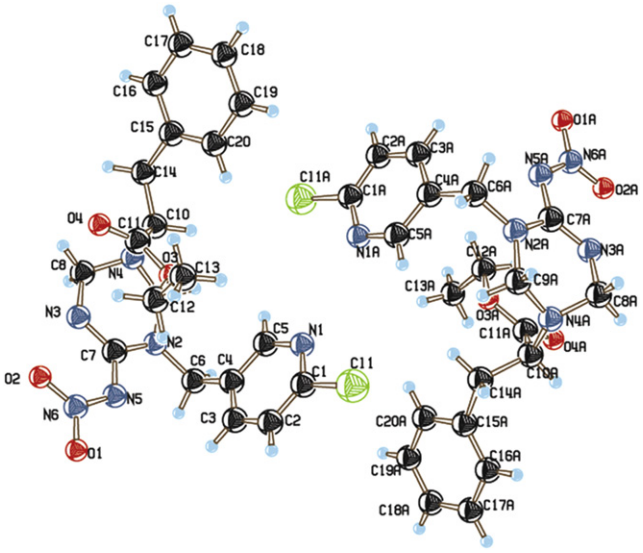
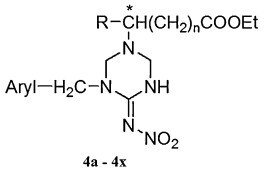


Fig. 2. Molecular structure of compound **4n** (Number CCDC 751235), with its single *S* configuration confirmed by X-ray diffraction.

Table 1
Insecticidal activities of 1,5-disubstituted 1,3,5-hexahydrotriazine-2-*N*-nitroimines (**4a**–**4x**) against *Aphis medicagini*



4a - 4x

Compd	n	R	Aryl	Concn (mg/L)[a]		
				500	100	10
4a	0	CH_3	Thy-Cl	++++	++	++
4b	0	CH_3	Pyr-Cl	+++	++	+
4c	0	$(CH_3)_2CH$	Thy-Cl	++++	++	+
4d	0	$(CH_3)_2CH$	Pyr-Cl	++++	+++	++
4e	0	$(CH_3)_2CHCH_2$	Thy-Cl	++++++	++++++	++++
4f	0	$(CH_3)_2CHCH_2$	Pyr-Cl	++++++	++++	+++
4g	0	$CH_3CH_2(CH_3)CH$	Thy-Cl	++++	++	+
4h	0	$CH_3CH_2(CH_3)CH$	Pyr-Cl	++++	++++	++
4i	0	$CH_3SCH_2CH_2$	Thy-Cl	+++++	++	+
4j	0	$CH_3SCH_2CH_2$	Pyr-Cl	+++	++	nt[b]
4k	0	4-Cl–C_6H_4	Thy-Cl	++++++	++++++	+++++
4l	0	4-Cl–C_6H_4	Pyr-Cl	+++	++	+
4m	0	$C_6H_5CH_2$	Thy-Cl	+++	++	nt
4n	0	$C_6H_5CH_2$	Pyr-Cl	+++	++	+
4o	0	$CH_2COOCH_2CH_3$	Thy-Cl	+++++	++++	+++
4p	0	$CH_2COOCH_2CH_3$	Pyr-Cl	+++++	+++	++
4q	1	H	Thy-Cl	++++++	+++	++
4r	1	H	Pyr-Cl	++++++	++++	+++
4s	1	$CH_2COOCH_2CH_3$	Thy-Cl	+++++	+++	++
4t	1	$CH_2COOCH_2CH_3$	Pyr-Cl	++++++	+++	++
4u	2	H	Thy-Cl	++++++	++++++	++++
4v	2	H	Pyr-Cl	++++++	++++++	++++
4w	0	H	Thy-Cl	++++	+++	++
4x	0	H	Pyr-Cl	++++	+++	++
–	–	Imidacloprid	Thy-Cl	++++++	++++++	++++++
–	–	Thiamethoxam	Pyr-Cl	++++++	++++++	++++++

[a] Rating system for the mortality percentage: ++++++, 100%; +++++, ≥90%; ++++, ≥80%; +++, ≥70%; ++, ≥60%; +, ≥50%; –, <50%.
[b] nt = not tested.

the hexahydrotriazine ring, their insecticidal activities decreased in the order **4u**, **4v** ($n = 2$) > **4q**, **4r** ($n = 1$) >**4a**, **4x** ($n = 0$), respectively, which indicated that the parameter **n** was strongly related to their insecticidal potency. However, in most cases, the substitution of the 6-chloro-3-pyridylmethyl (Cl-Pyr-CH_2) or 2-chloro-5-thiazolylmethyl (Cl-Thy-CH_2) had no distinct influence on their insecticide activities.

As for the substituent **R**, compounds (**4e**, **4f**) with a long and flexible alkyl group at α-position exhibited excellent inhibitory activities at 10 mg/L, whereas the corresponding compounds substituted with benzyl (**4m**, **4n**) showed much lower inhibitory efficiency, which is possibly due, in part, to the relatively rigid structure of this substituent. In addition, the introduction of electron-withdrawing ester groups (**4o**) as **R** resulted in higher insecticidal potency than the corresponding analogues with electron-donating groups (**4a**). Compared with **4b**, compound **4p** also displayed increasing tendency in biological activity. Hence, the observations herein suggest that compound **4k** could be used as a lead for further developing new neonicotinoid type products, and the insecticidal potency of our designed analogues depends significantly on the length and flexibility of the substituted ethyl ester groups on the 5-position of the hexahydrotrizine ring.

3.3. Molecular docking study

To validate observed structure–activity relationships, molecular docking studies of selected analogues were performed. Since the amino acids forming the active pockets are both structurally and functionally consistent in the diverse nAChRs and AchBPs, the homomeric *ls*-AChBP (PDB ID: 2zju) was used as a structural surrogate of the insect nAChR. Among all the modeled molecules, compound **4k** attained the highest score (data not shown) and fitted the best in the interfacial binding pocket between the two adjacent subunits. As illustrated in Fig. 3, seven hydrogen bonds were found between compound **4k** and the active site residues, versus five for compound **4e**, **4u** and six for **4v**, which was in good agreement with their high insecticidal efficiency observed in vitro. The binding conformation of **4k** exhibited two hydrogen bonds via its nitryl O21 and O22 with the side chain H–N of Gln55 and phenolic hydroxy of Tyr164 (Fig. 3b), respectively. Its ethoxycarbonyl interacts with the phenolic hydroxy O of Tyr192 and its thiazole N hydrogen bonds the Tyr185 side chain. Moreover, **4k** also showed the important additional H bonding interactions with Met114 at the interface of two adjacent subunits. These distinctions likely account for the high inhibitory potency of inhibitor **4k**.

As indicated in the ligand superimposition in Fig. 4, most other active compounds also exhibited significant hydrogen bonding interactions with the receptor, and shared a quite similar binding mode with **4k** (colored in magenta). However, the backbones of **4j** and **4m** were not nicely nestled in the subunit interfacial binding pocket of this receptor (data not shown). Consistent with this simulation, they had little biological activity in vitro. In summary, the structural similarity to interactions of neonicotinoids with other nAChRs, calculated binding affinities, and similar rank order of complementarity scores suggest that our predicted binding modes accurately capture the ligand–receptor interactions. Meanwhile, the different binding mode observed herein has raised the possibility that some analogues may arbitrate their insecticidal activity through a mechanism other than imidacloprid.

4. Conclusion

This paper describes the design and synthesis of a new series of chiral 1,5-disubstituted 1,3,5-hexahydrotriazine-2-*N*-nitroimines (**4a**–**4x**) analogues as potent NNs. Most of these compounds

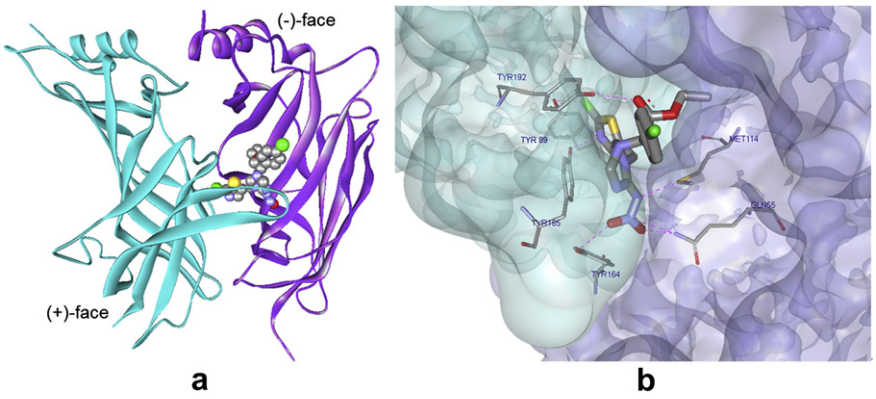


Fig. 3. View of the binding mode of compound **4k** in the binding site of *ls*-AChBP structural surrogate of the insect nAChR, suggested by molecular docking studies. (a) **4k** is bound into the interfacial binding pocket between the (+)-face and (−)-face of two adjacent subunits. (b) The binding interactions between **4k** and the active site residues. For clarity, only two subunits (A and B) of the five identical subunits are extracted and shown from the homomeric AChBP pentamer, and they are colored cyan and purple, respectively. Key H-bonds are indicated by magenta dotted lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

exhibited good insecticidal activity against *A. medicagini* at 100 mg/L, while analogue **4k** afforded the best in vitro activity, and had >90% mortality at 10 mg/L. The single-crystal structure of **4n** was further determined by X-ray diffraction, with its single *S* configuration confirmed. In addition, to further explore the structural features important for better activity, molecular docking studies were also performed to model the ligand-nAChR complexes and analyze their binding interactions. The simulation demonstrated new binding modes of **4k** and other active analogues at the active site of nAChR, which is in good agreement with their high insecticidal activities observed in vitro. Further pharmacological studies on these compounds are ongoing. Hence, this study has gained some insight into the molecular mechanism of this new series of neonicotinoids, which may provide some useful information, and facilitate future receptor structure-guided design of novel insecticides with less resistance.

5. Experimental section

5.1. Chemistry reagents and instruments

All reagents obtained from commercial sources were of analytical reagent grade and used without further purification, except for acetonitrile, which was dried by refluxing in the presence of CaH_2 and distilled prior to use. All α-amino acids are L types, except for D-*p*-Chlorophenylglycine. Melting points were measured using a XT4A

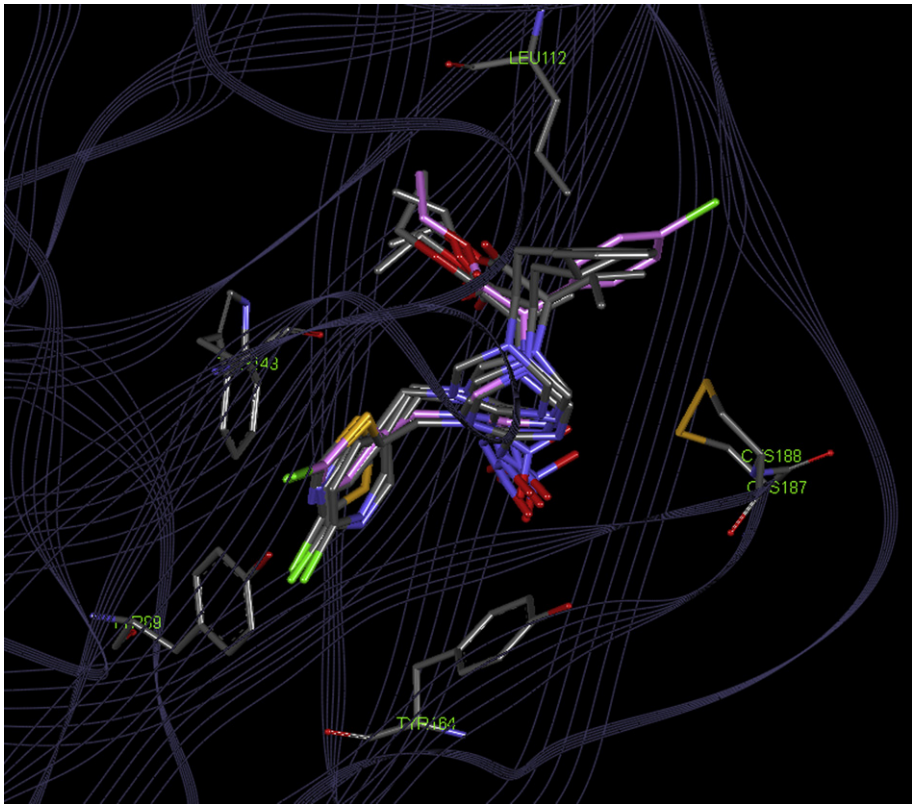


Fig. 4. Superimposition of active compounds docked into nAChR. The backbone of the most potent 4k is shown in magenta, while the protein is represented as line. The atoms of other molecules are colored as follows: O, red; N, blue; C, grey; S, yellow; Cl, light green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

microscopic melting point apparatus. IR spectra were obtained on a Nicolet5DX FT-IR spectrophotometer in the region 4000–400 cm^{-1} using KBr discs. ^1H NMR spectrum ($CDCl_3$) was recorded on a Bruker AVANCE-400 MHz with TMS as an internal standard. Coupling constants (*J* values) are in Hertz. Elemental analysis was performed with a Perkin–Elmer 2400 instrument. Mass spectra (MS-ESI) were recorded on a TOF-LCT mass spectrometer. The single-crystal structure of compound **4n** was determined on a Bruker SMART 1000 CCD diffractometer.

5.2. General procedure for the synthesis of compounds **2a**–**2l** (exemplified by **2a**)

A mixture of alanine (3.56 g, 0.04 mol) in ethanol (30 mL), with thionyl chloride (3.6 mL) dropwise added at −5 °C, was refluxed in ethanol at 78 °C under stirring within 1.5 h. Subsequently, the solution was distilled in vacuum to afford Alanine ethyl ester hydrochloride **2a** in >80% yield. The physical constants of **2a**–**2l** are identical with the corresponding data in reference [24].

5.3. General method for preparation of compounds **3a**–**3l** (exemplified by **3a**)

A mixture of 37% formaldehyde aqueous (3.65 g, 0.045 mol), triethylamine (3.15 g, 0.03 mol) and Alanine ethyl ester hydrochloride **2a** (3.44 g, 0.0225 mol) in ethanol were added into to a solution of nitroguanidine (1.58 g, 0.015 mol) in 20 mL ethanol dropwise, with stirring at 60 °C for 6 h. When the mixture was cooled to room temperature, the crude product was deposited from the solution and removed by filtration. Compound **3a** was recrystallized using the solvent mixture of EtOH–H_2O to afford the pure product.

5.3.1. 5-(1-ethoxycarbonylethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**3a**)

White crystals, yield 55.3%, m.p.129–131 °C ^1H NMR(δ, ppm, $CDCl_3$): 9.04 (2H, s, 2NH), 4.65–4.62 (2H, m, J = 1.6 Hz, triazine-2H), 4.53–4.49 (2H, m, J = 6.4 Hz, triazine-2H), 4.26–4.19 (2H, m, OCH_2), 3.75–3.70(1H, m, CH), 1.48 (3H, d, J = 7.2 Hz, CH_3CH), 1.28–1.32 (3H, t, J = 7.2 Hz, CH_2CH_3). IR (potassium bromide, cm^{-1}) ν: 3351 (N–H), 2973, 2936 (CH_3), 1739 (C=O), 1586 (C=N), 1376 (NO_2), 1187 (C–O–C), 1110 (C–N). Anal. calcd for $C_8H_{15}N_5O_4$:C 39.18,H 6.17, N 28.56; found C 39.26, H 6.11,N 28.52. ESI-MS (M + H) *m/z*: 245.4, $[\alpha]_D^{20} = -12.08$(*c* = 0.1 g/L in acetone).

5.3.2. 5-(2-Methyl-1-ethoxycarbonylpropyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**3b**)

White crystals, yield 58.3%, m.p.169–171 °C ^1H NMR(δ, ppm, $CDCl_3$): 9.06 (2H, s, 2NH), 4.61–4.58 (2H, m, J = 1.2 Hz, triazine-2H), 4.50–4.46 (2H, m, J = 1.6 Hz, triazine-2H), 4.10–4.18 (2H, m, OCH_2), 3.12 (1H, J = 7.8 Hz d, CHC=O), 2.15–2.06 (1H, m, CH), 1.25–1.28 (3H, t, J = 6.8 Hz, CH_3CH_2), 1.01–1.02 (3H, d, J = 6.4 Hz, $CHCH_3$), 0.93–0.95 (3H, d, J = 6.4 Hz, $CHCH_3$). IR(potassium bromide, cm^{-1}) ν: 3443 (N–H), 2926 (CH_3), 1742 (C=O) 1642 (C=N), 1366(NO_2), 1189(C–O–C), 1101 (C–N). Anal. calcd for $C_{10}H_{19}N_5O_4$: C43.95, H 7.01 N 25.63; found C 43.89, H 7.10, N 25.57. ESI-MS (M + H) *m/z*: 274.3, $[\alpha]_D^{20} = +10.47$ (*c* = 0.1 g/L in acetone).

5.3.3. 5-(3-Methyl-1-ethoxycarbonylbutyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**3c**)

White crystals, yield 63.6%, m.p.153–155 °C. ^1H NMR(δ, ppm, $CDCl_3$): 9.01 (2H, s, 2NH), 4.61–4.58 (4H, s, triazine-4H), 4.20–4.11 (2H, m, OCH_2), 3.61–3.57 (1H, dd, J = 6 Hz, J = 6.8 Hz, CHC=O), 1.73–1.65 (2H, m, CH_2), 1.45–1.41 (1H,t, J = 7.2 Hz CH), 1.29–1.26 (3H, t, J = 7.2 Hz, OCH_2CH_3), 0.96–0.93 (6H, t, J = 6 Hz, $2CH_3$). IR (potassium bromide, cm^{-1}) ν: 3454 (N–H), 2961 (CH_3), 1742 (C=O) 1642 (C=N), 1367 (NO_2), 1189 (C–O–C), 1101 (C–N). Anal. calcd for $C_{11}H_{21}N_5O_4$: C45.98, H 7.37, N 24.38; found C 46.07, H 7.43, N 24.31. ESI-MS (M + H) *m/z*: 288.3, $[\alpha]_D^{20} = -16.70$(*c* = 0.1 g/L in acetone).

5.3.4. 5-(2-Methyl-1-ethoxycarbonylbutyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**3d**)

White crystals, yield 60.8%, m.p.167–169 °C. ^1H NMR(δ, ppm, $CDCl_3$): 9.00 (2H, s, 2NH), 4.60–4.57 (2H, d, J = 13.2 Hz, triazine-2H), 4.50–4.47 (2H, d, J = 13.2 Hz, triazine-2H), 4.19–4.10 (2H, m, OCH_2), 3.27–3.25 (1H, d, J = 6.8 Hz, CHC=O), 1.44–1.41 (2H, t, J = 7.2 Hz CH_2CH_3), 1.28–1.25 (3H, t, J = 7.2 Hz, OCH_2CH_3), 1.20–1.17 (1H,m, CH), 0.94–0.91 (6H, t, J = 6.8 Hz, $2CH_3$). IR(potassium bromide, cm^{-1}) ν: 3424 (N–H), 2969 (CH_3), 1721 (C=O) 1618 (C=N), 1377 (NO_2), 1181 (C–O–C), 1110 (C–N). Anal. calcd for $C_{11}H_{21}N_5O_4$: C45.98, H 7.37, N 24.38; found C 46.04, H 7.33, N 24.42. ESI-MS (M + H) *m/z*: 288.5, $[\alpha]_D^{20} = +8.95$(*c* = 0.1 g/L in acetone).

5.3.5. 5-(3-Methylthioyl-1-ethoxycarbonylpropyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**3e**)

White crystals, yield 52.4%, m.p. 83–85 °C. ^1H NMR(δ, ppm, $CDCl_3$): 9.17 (2H, s, 2NH), 4.59–4.48(4H, m, triazine-4H), 4.21–4.11 (2H, m, OCH_2), 3.76–3.73(1H, t, J = 6.8 Hz, CHC=O), 2.56–2.52 (2H, m, SCH_2), 2.10(3H,d, J = 6.8 Hz, SCH_3), 2.07–2.05(2H, m, J = 6.4 Hz, CH_2), 1.29–1.25(3H,ddd, J = 1.2 Hz, 1.2 Hz, 0.8 Hz, CH_3). IR (potassium bromide, cm^{-1}) ν: 3423(N–H), 2976(CH_3), 1740(C=O), 1644(C=N), 1371(NO_2), 1190(C–O–C), 1087 (C–N), 712(C–S). Anal. calcd for $C_{10}H_{19}N_5O_4S$: C39.33, H 6.27, N 22.94; found C 39.37, H 6.35, N 22.89. ESI-MS (M + H) *m/z*: 306.3, $[\alpha]_D^{20} = -21.93$(*c* = 0.1 g/L in acetone).

5.3.6. 5-(1-(4-chlorophenyl)-1-ethyoxycarbonylmethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**3f**)

White crystals, yield 58.5%, m.p.211–213 °C. ^1H NMR(δ, ppm, $CDCl_3$): 8.85 (2H, s, 2NH), 7.46–7.38 (4H, m, phenyl), 4.64 (1H, s, CHC=O), 4.55–4.52 (2H, d, J = 13.6 Hz, triazine-2H), 4.40–4.37 (2H, d, J = 13.6 Hz, triazine-2H), 4.25–4.14 (2H, m, OCH_2), 1.27–1.31 (3H, t, J = 7.2 Hz, CH_3); IR(potassium bromide, cm^{-1}) ν: 3438 (N–H), 3082(phenyl), 2973 (CH_3), 1727(C=O) 1647 (C=N), 1342 (NO_2), 1215 (C–O–C), 1112 (C–N). Anal. calcd for $C_{13}H_{16}N_5O_4Cl$: C 47.40, H 4.83, N 19.74; found C 47.46, H 4.91, N 19.67. ESI-MS (M + H) *m/z*: 355.4, $[\alpha]_D^{20} = +24.33$(*c* = 0.1 g/L in acetone).

5.3.7. 5-(2-Phenyl-1-ethyoxycarbonylethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**3g**)

White crystals, yield 62.6%, m.p.140–142 °C. ^1H NMR(δ, ppm, $CDCl_3$): 9.13 (2H, s, 2NH), 7.31–7.18 (5H, m, phenyl), 4.61 (4H, m, triazine-4H),4.06–3.97 (2H, m, OCH_2), 3.85–3.81 (1H, t, J = 7.2 Hz, CHC=O), 3.15–3.10 (2H, m, ph-CH_2), 1.09–1.06 (3H, t, J = 7.2 Hz, CH_3); IR(potassium bromide, cm^{-1}) ν: 3438 (N–H), 3082 (phenyl), 2973 (CH_3), 1727 (C=O) 1647 (C=N), 1342 (NO_2), 1215 (C–O–C), 1112 (C–N). Anal. calcd for $C_{14}H_{19}N_5O_4$: C 52.33, H 5.96, N 21.79; found C 52.40, H 5.91, N 21.85. ESI-MS (M + H) *m/z*: 322.3, $[\alpha]_D^{20} = -10.52$(*c* = 0.1 g/L in acetone).

5.3.8. 5-(1,2-diethoxycarbonylethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**3h**)

White crystals, yield 53.8%, m.p.119–121 °C ^1H NMR(δ, ppm, $CDCl_3$): 9.02 (2H, s, 2NH), 4.58 (4H, d, J = 2 Hz, triazine-4H), 4.26–4.08 (4H, m, $2OCH_2$), 4.06–4.02 (1H, t, J = 7.2 Hz, CHC=O), 2.95–2.89 (1H, m, CH_2C=O), 2.83–2.77 (1H, m, CH_2C=O), 1.30–1.28(3H, t, J = 6.4 Hz, CH_3), 1.27–1.25 (3H, t, J = 6.4 Hz, CH_3). IR(potassium bromide, cm^{-1}) ν: 3350 (N–H), 2983,2936 (CH_3), 1721 (C=O) 1603 (C=N), 1372 (NO_2), 1240 (C–O–C), 1109 (C–N). Anal. calcd for $C_{11}H_{19}N_5O_6$: C 41.64, H 6.04, N 22.07; found C 41.72, H 5.98, N 22.16. ESI-MS (M + H) *m/z*: 318.3, $[\alpha]_D^{20} = -15.12$(*c* = 0.1 g/L in acetone).

*5.3.9. 5-(2-ethoxycarbonylethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**3i**)*

White crystals, yield 64.7%, m.p.164–166 °C ^{1}H NMR(δ, ppm, $CDCl_3$): 9.08 (2H, s, 2NH), 4.45–4.44 (4H, d, J = 2 Hz, triazine-4H), 4.20–4.15 (2H, m, OCH_2), 3.11–3.08 (2H, t, J = 6.4 Hz, NCH_2), 2.56–2.59 (2H, t, J = 6.8 Hz, CH_2C=O), 1.27–1.31 (3H,t,J = 7.2 Hz, CH_3). IR(potassium bromide, cm^{-1}) ν: 3398 (N–H), 2975 (CH_3), 1730 (C=O) 1650 (C=N), 1381 (NO_2), 1210 (C–O–C), 1089 (C–N). Anal. calcd for $C_8H_{15}N_5O_4$: C39.18, H 6.17, N 28.56; found C 39.14, H 6.24, N 28.60. ESI-MS (M + H) m/z: 246.5.

*5.3.10. 5-(1,3-Diethoxycarbonyl-2-propyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**3j**)*

White crystals, yield 46.7%, m.p. 101–103 °C ^{1}H NMR(δ, ppm, $CDCl_3$): 8.99 (2H, s, 2NH), 4.59–4.50 (4H, m, triazine-4H), 4.21–4.11 (4H, m, $2OCH_2$), 3.64–3.61 (1H, t, J = 7.2 Hz, CHC=O), 2.48–2.33 (2H, m, CH_2C=O), 2.19–2.07 (2H, m, $CHCH_2$), 1.30–1.26 (6H, ddd, J = 1.2 Hz, 1.2 Hz, 1.2 Hz, CH_3). IR (potassium bromide, cm^{-1}) ν: 3352 (N–H), 2984 (CH_3), 1722 (C=O) 1612 (C=N), 1375 (NO_2), 1231 (C–O–C), 1108 (C–N). Anal. calcd for $C_{12}H_{21}N_5O_6$: C 43.50, H 6.39, N 21.14; found C 43.43, H 6.42, N 21.18. ESI-MS (M + H) m/z: 332.4, $[\alpha]_D^{20}$ = −2.82 (c = 0.1 g/L in acetone).

*5.3.11. 5-(3-ethoxycarbonylpropyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**3k**)*

White crystals, yield 70.6%, m.p.131–133 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.06 (2H, s, 2NH), 4.42–4.41 (4H, d, J = 2 Hz, triazine-4H), 4.17–4.12 (2H, m, OCH_2), 2.82–2.78 (2H, t, J = 7.2 Hz, NCH_2), 2.43–2.39 (3H, t, J = 7.2 Hz, CH_2C=O), 1.92–1.85 (2H,m, CH_2), 1.29–1.26 (3H, t, J = 7.2 Hz, CH_2CH_3). IR (potassium bromide, cm^{-1}) ν: 3398 (N–H), 2975(CH_3), 1730(C=O) 1650(C=N), 1381(NO_2), 1210 (C–O–C), 1089 (C–N). Anal. calcd for $C_9H_{17}N_5O_4$: C 41.69, H 6.61, N 27.01; found C 41.75, H 6.68, N 27.14, ESI-MS (M + H) m/z: 260.5.

*5.3.12. 5-(1-ethoxycarbonylmethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**3l**)*

Yellow crystals, yield 60.6%, m.p.185–187 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 9.15 (2H, s, 2NH), 4.74 (4H, s, $2CH_2$), 4.29–4.22 (2H, m, CH_2CH_3), 3.61 (2H, s, NCH2C=O), 1.33–1.30 (3H, t, J = 7.2 Hz, CH_2CH_3). IR (potassium bromide, cm^{-1}) ν: 3462 (N–H), 2977(CH_3), 1743 (C=O) 1604 (C=N), 1371 (NO_2), 1223 (C–O–C), 1108 (C–N) cm^{-1}. Anal. calcd for $C_7H_{13}N_5O_4$: C36.36, H 5.67, N 30.29; found C 36.30, H 5.72, N 30.31. ESI-MS m/z: 232.1.

*5.4. General procedure for the synthesis of analogues **4a**–**4x** (exemplified by **4a**)*

The solution of compound **3a** (1.22 g, 0.005 mol) in dry acetonitrile (20 mL) was mixed with K_2CO_3 (0.7 g, 0.005 mol) and a spot of CsCl, and was heated to 55 °C. The solution of 2-chloro-5-chloromethyl thiazole (2.86 g, 0.017 mol) in 15 mL dry acetonitrile was slowly added to this mixture. After stirring under 55 °C for 6 h, the mixture was filtrated and poured into water (10 mL), and extracted with ethyl acetate (3 × 20 mL). Then it was washed with saturation salt water and dried with anhydrous $MgSO_4$. After removing the solvent under vacuum, the residue was purified by silica gel chromatography using ethyl acetate and petroleum ether in the ratio of 1:1 as the flush to afford **4a**.

*5.4.1. 1-(2-Chloro-5-thiazolmethyl)-5-(1-ethoxycarbonylethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4a**)*

White crystals, yield 40.2%, m.p.129–131 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.47 (1H,s, NH), 7.45 (1H, s, thiazole-H), 4.73–4.70 (2H, d, J = 15.2 Hz, CH_2-thiazole), 4.61–4.46 (4H, m, triazine-4H), 4.21–4.03 (2H, m, OCH_2), 3.49–3.44 (2H, s, CHC=O), 1.32–1.29 (3H, d, J = 11.2 Hz, CH_3), 1.26–1.23 (3H, t, J = 7.2 Hz, CH_3). IR (potassium bromide, cm^{-1}) ν: 3282 (N–H), 2995 (thiazole), 1732 (C=O) 1584 (C=N), 1395 (NO_2), 1242 (C–O–C), 1100 (C–N). Anal. calcd for $C_{12}H_{17}ClN_6O_4S$: C 38.25, H 4.55, N 22.30; found C 38.21, H 4.62, N 22.36. ESI-MS (M + H) m/z: 377.1, $[\alpha]_D^{20}$ = +28.08(c = 0.1 g/L in acetone).

*5.4.2. 1-(6-Chloro-3-pyridinemethyl)-5-(1-ethoxycarbonylethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4b**)*

White crystals, yield 38.2%, m.p.126–128 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.64 (1H,s, NH), 8.33–8.32 (1H, d, J = 2.4 Hz, pyridine-H), 7.85–7.82 (1H,dd, J = 2.4 Hz, 2.4 Hz, pyridine-H), 7.38–7.36 (1H, d, J = 4.4 Hz, pyridine-H), 4.70–4.66 (1H, d, J = 15.6 Hz, CH_2-pyridine), 4.56–4.46 (4H, m, triazine-4H), 4.38–4.35 (1H, d, J = 12.8 Hz, CH_2-pyridine), 4.22–4.04 (2H, m, OCH_2), 3.55–3.50 (2H, s, CHC=O), 1.33–1.31 (3H, d, J = 6.8 Hz, CH_3), 1.28–1.24 (3H, t, J = 7.2 Hz, CH_3). IR (potassium bromide, cm^{-1}) ν: 3132 (N–H), 2986 (pyridine), 1739 (C=O) 1584 (C=N), 1395 (NO_2), 1248 (C–O–C), 1111 (C–N),. Anal. calcd for $C_{14}H_{19}ClN_6O_4$: C 45.35, H 5.16, N 22.67; found C 45.29, H 5.20, N 22.74. ESI-MS (M + H) m/z: 371.2, $[\alpha]_D^{20}$ = +22.33 (c = 0.1 g/L in acetone).

*5.4.3. 1-(2-Chloro-5-thiazomethyl)-5-(2-methyl-1-ethoxycarbonylpropyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4c**)*

White crystals, yield 35.2%, m.p.96–98 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.52 (1H, s, NH), 7.46 (1H, s, thiazole-H), 4.76–4.72 (1H, d, J = 16 Hz, CH_2-thiazole), 4.65 (1H, d, J = 2 Hz, CH_2-thiazole), 4.18–4.03 (2H, m, OCH_2), 3.00–2.97 (1H, d, J = 10 Hz, CHC=O), 2.02–1.96 (1H, m, CH), 1.27–1.23 (3H, t, J = 7.2 Hz, CH_3), 0.99–0.97 (3H, d, J = 6.8 Hz, CH_3), 0.92–0.91 (3H, d, J = 6.4 Hz, CH_3). IR (potassium bromide, cm^{-1}) ν: 3128 (N–H), 2926 (thiazole), 1736 (C=O), 1642 (C=N), 1395 (NO_2), 1194 (C–O–C), 1106 (C–N). Anal. calcd for $C_{14}H_{21}ClN_6O_4S$: C 41.53, H 5.23 N 20.76; found C 41.60, H 5.14, N 20.81. ESI-MS (M + H) m/z: 405.3 $[\alpha]_D^{20}$ = +27.83(c = 0.1 g/L in acetone).

*5.4.4. 1-(6-Chloro-3-pyridinemethyl)-5-(2-methyl-1-ethoxycarbonylpropyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4d**)*

White crystals, yield 37.5%, m.p.126–128 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 9.64 (1H, s, NH), 8.33 (1H, d, J = 8 Hz, pyridine-H), 7.81–7.79 (1H, d, J = 8 Hz, pyridine-H), 7.36–7.34 (1H, d, J = 8 Hz, pyridine-H), 4.80–4.77 (1H, d, J = 15.2 Hz, CH_2-pyridine), 4.66–4.63 (1H, d, J = 12.4 Hz, CH_2-pyridine), 4.47–4.30 (4H, m, triazine-4H), 4.18–4.04 (2H, m, OCH_2), 2.98–2.96 (1H, d, J = 10 Hz, CHC=O), 1.98–1.94 (1H, m, CH), 1.28–1.24 (3H, m, CH_3), 0.97–0.95 (3H, d, J = 6.8 Hz, $CHCH_3$), 0.91–0.90 (3H, d, J = 6.4 Hz, $CHCH_3$). IR (potassium bromide, cm^{-1}) ν: 3307 (N–H), 2963 (pyridine), 1714 (C=O), 1587 (C=N), 1397 (NO_2), 1186 (C–O–C), 1118 (C–N). Anal. calcd for $C_{16}H_{23}ClN_6O_4$: C 48.18, H 5.81, N 21.07; found C 48.25, H 5.90, N 21.14. ESI-MS (M + H) m/z: 399.7, $[\alpha]_D^{20}$ = +6.69 (c = 0.1 g/L in acetone).

*5.4.5. 1-(2-Chloro-5-thiazomethyl)-5-(3-methyl-1-ethoxycarbonylbutyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4e**)*

White crystals, yield 46.2%, m.p.130–132 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 9.54 (1H, s, NH), 7.47 (1H, s, thiazole-H), 4.73–4.69 (1H, d, J = 15.6 Hz, CH_2-thiazole), 4.61–4.57 (1H, m, CH_2-thiazole), 4.55–4.47 (4H, m, triazine-4H), 4.21–4.03 (2H, m, OCH_2), 3.47–3.43 (1H, m, CHC=O), 1.60–1.52 (2H, m, CH_2), 1.37–1.35 (1H, m, CH),1.28–1.24 (3H, t, J = 7.2 Hz, OCH_2CH_3), 0.91–0.90 (3H, d, J = 2 Hz, $CHCH_3$), 0.89–0.88 (3H, d, J = 1.6 Hz, $CHCH_3$). IR (potassium bromide, cm^{-1}) ν: 3307 (N–H), 2963 (thiazole), 1714 (C=O), 1587 (C=N), 1397 (NO_2), 1186 (C–O–C), 1118 (C–N). Anal. calcd for $C_{15}H_{23}ClN_6O_4S$: C 43.01, H 5.53, N 20.06; found C 42.97, H 5.59, N 20.10. ESI-MS (M + H) m/z: 419.2, $[\alpha]_D^{20}$ = −4.68(c = 0.1 g/L in acetone).

*5.4.6. 1-(6-Chloro-3-pyridinemethyl)-5-(3-methyl-1-ethoxycarbonylbutyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4f**)*

White crystals, yield 50.2%, m.p.104–106 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 9.67 (1H, s, NH), 8.33 (1H, d, J = 2 Hz, pyridine-H), 7.83–7.80 (1H, dd, J = 2.4 Hz, 2.4 Hz, pyridine-H), 7.37–7.35 (1H, d, J = 8 Hz, pyridine-H), 4.77–4.73 (1H, d, J = 15.6 Hz, CH_2-pyridine), 4.64–4.60 (1H, dd, J = 2.4 Hz, 2.4 Hz, CH_2-pyridine), 4.47–4.29 (4H, m, triazine-4H), 4.16–4.07 (2H, m, OCH_2), 3.48–3.45 (1H,m, CHC═O), 1.64–1.51 (2H, m, CH_2), 1.49–1.42 (1H, m, CH), 1.28–1.24 (3H,t, J = 7.2 Hz, OCH_2CH_3), 0.90–0.89 (3H, d, J = 2.4 Hz, $CHCH_3$), 0.89–0.88 (3H, d, J = 2 Hz, $CHCH_3$). IR (potassium bromide, cm^{-1}) ν: 3259 (N–H), 2961 (pyridine), 1742 (C═O), 1587 (C═N), 1397 (NO_2), 1186 (C–O–C), 1105 (C–N). Anal. calcd for $C_{17}H_{25}ClN_6O_4$: C 49.45, H 6.10, N 20.36; found C 49.52, H 6.14, N 27.35. ESI-MS (M + H) m/z: 413.4, $[\alpha]_D^{20}$ = −5.97 (c = 0.1 g/L in acetone).

*5.4.7. 1-(2-Chloro-5-thiazomethyl)-5-(2-methyl-1-ethoxycarbonylbutyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4g**)*

White crystals, yield 45.3%, m.p.135–137 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 9.52 (1H, d, J = 0.8 Hz, NH), 7.46 (1H, s, thiazole-H), 4.74–4.70 (1H, d, J = 15.6 Hz, CH_2-thiazole), 4.63–4.59 (1H, dd, J = 2 Hz, 2.4 Hz, CH_2-thiazole), 4.53–4.49 (2H, m, triazine-2H), 4.43–4.39 (2H, m, triazine-2H), 4.19–4.04 (2H, m, OCH_2), 3.13–3.11 (1H, d, J = 9.6 Hz, CHC═O), 1.61–1.55 (2H, m, CH_2), 1.27–1.24 (3H, t, J = 6.8 Hz, OCH_2CH_3), 1.17–1.13 (1H, t, J = 7.2 Hz, CH), 0.91–0.87 (6H, m, $2CH_3$). IR (potassium bromide, cm^{-1}) ν: 3291 (N–H), 2972 (thiazole), 1733 (C═O), 1585 (C═N), 1395 (NO_2), 1195 (C–O–C), 1046 (C–N). Anal. calcd for $C_{15}H_{23}ClN_6O_4S$: C 43.01, H 5.53, N 20.06; found C 42.95, H 5.58, N 20.17. ESI-MS (M + H) m/z: 419.1 $[\alpha]_D^{20}$ = +11.53 (c = 0.1 g/L in acetone).

*5.4.8. 1-(6-Chloro-3-pyridinemethyl)-5-(2-methyl-1-ethoxycarbonylbutyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4h**)*

White crystals, yield 43.9%, m.p.141–143 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 9.66 (1H, s, NH), 8.33–8.32 (1H, d, J = 6.4 Hz, pyridine-H), 7.82–7.79 (1H, dd, J = 2.4 Hz, 2.4 Hz, pyridine-H), 7.37–7.35 (1H, d, J = 8 Hz, pyridine-H), 4.81–4.77 (1H, d, J = 15.6 Hz, CH_2-pyridine), 4.64–4.60 (1H, dd, J = 2.4 Hz, 2.4 Hz, CH_2-pyridine), 4.47–4.29 (4H, m, triazine-4H), 4.19–4.06 (2H, m, OCH_2), 3.12–3.09 (1H, d, J = 9.6 Hz, CHC═O), 1.59 (3H, s, $CHCH_2$), 1.28–1.25 (3H, t, J = 7.2 Hz, OCH_2CH_3), 0.89–0.85 (6H, m, $2CH_3$). IR (potassium bromide, cm^{-1}) ν: 3288 (N–H), 2965 (pyridine), 1732 (C═O), 1585 (C═N), 1396 (NO_2), 1189 (C–O–C), 1106 (C–N). Anal. calcd for $C_{17}H_{25}ClN_6O_4$: C 49.45, H 6.10 1, N 20.36; found C 49.40, H 6.18, N 20.40 ESI-MS (M + H) m/z: 413.5, $[\alpha]_D^{20}$ = −6.48 (c = 0.1 g/L in acetone).

*5.4.9. 1-(2-Chloro-5-thiazomethyl)-5-(3-meththioyl-1-ethoxycarbonylpropyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4i**)*

White crystals, yield 35.8%, m.p.89–91 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 9.55 (1H, d, J = 0.8 Hz, NH), 7.47 (1H, s, thiazole-H), 4.72–4.68 (1H, d, J = 16 Hz, CH_2-thiazole), 4.62–4.57 (1H, m, CH_2-thiazole), 4.53–4.45 (4H, m, triazine-4H), 4.21–4.04 (2H, m, OCH_2), 3.65–3.62 (1H, t, J = 6.8 Hz, CHC═O), 2.57–2.45 (2H, m, SCH_2), 2.09 (3H, s, CH_3), 2.02–1.95 (2H, m, CH_2), 1.28–1.25 (3H, t, J = 7.2 Hz, OCH_2CH_3). IR (potassium bromide, cm^{-1}) ν: 3395 (N–H), 3008 (thiazole), 1744 (C═O), 1589 (C═N), 1396 (NO_2), 1197 (C–O–C), 1108 (C–N). Anal. calcd for $C_{14}H_{21}ClN_6O_4S_2$: C 38.48, H 4.84, N 19.23; found C 38.39, H 4.89, N 19.31. ESI-MS (M + H) m/z: 437.6, $[\alpha]_D^{20}$ = +8.48 (c = 0.1 g/L in acetone).

*5.4.10. 1-(6-Chloro-3-pyridinemethyl)-5-(2-methanthioyl-1-ethoxycarbonylpropyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4j**)*

White crystals, yield 33.6%, m.p.68–70 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 9.68 (1H, s, NH), 8.43–8.33 (1H, d, J = 2.4 Hz, pyridine-H), 7.82–7.79 (1H, dd, J = 2.4 Hz, 2.4 Hz, pyridine-H), 7.37–7.35 (1H, d, J = 8 Hz, pyridine-H), 4.77–4.73 (1H, d, J = 15.2 Hz, CH_2-pyridine), 4.63–4.60 (1H, m, CH_2-pyridine), 4.51–4.36 (4H, m, triazine-4H), 4.22–4.03 (2H, m, OCH_2), 3.66–3.63 (1H, t, J = 7.2 Hz, CHC═O), 2.53–2.46 (2H, m, SCH_2), 2.08 (3H, s, SCH_3), 2.02–1.91 (2H, m, CH_2), 1.28–1.25 (3H, t, J = 7.2 Hz, OCH_2CH_3). IR (potassium bromide, cm^{-1}) ν: 3394 (N–H),2980 (pyridine),1734 (C═O),1544 (C═N),1398 (NO_2), 1193 (C–O–C), 1094 (C–N). Anal. calcd for $C_{16}H_{23}ClN_6O_4S$: C 44.60, H 5.38, N 19.50; found C 44.53, H 5.43, N 19.55. ESI-MS (M + H) m/z: 431.2, $[\alpha]_D^{20}$ = −3.73 (c = 0.1 g/L in acetone).

*5.4.11. 1-(2-Chloro-5-thiazomethyl)-5-(1-(4-chlorophenyl)-1-ethyoxycarbonylmethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4k**)*

White crystals, yield 44.7%, m.p.226–228 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.41 (1H, d, J = 0.8 sHz, NH), 7.37 (1H, s, thiazole-H), 7.35 (1H, s, phenyl-H), 7.30 (1H, s, phenyl-H), 7.24–7.22 (2H, d, J = 8.8 Hz, phenyl-H), 4.64–4.60 (1H, d, J = 15.6 Hz, CH_2-thiazole), 4.48–4.46 (1H, d, J = 16 Hz, CH_2-thiazole), 4.41–4.27 (4H, m, triazine-4H), 4.09–4.05 (2H, m, OCH_2), 1.59 (1H, t, J = 6.8 Hz, CHC═O), 1.15–1.11 (3H, t, J = 7.2 Hz, OCH_3). IR (potassium bromide, cm^{-1}) ν: 3254 (N–H), 2965 (thiazole), 1740 (C═O), 1590 (C═N), 1399 (NO_2), 1190 (C–O–C), 1053 (C–N). Anal. calcd for $C_{18}H_{19}Cl_2N_6O_4S$: C 43.14, H 3.83, N 17.75; found C 43.09, H 3.89, N 17.81. ESI-MS (M + H) m/z: 473.3 $[\alpha]_D^{20}$ = −12.75 (c = 0.1 g/L in acetone).

*5.4.12. 1-(6-Chloro-3-pyridinemethyl)-5-(1-(4-chlorophenyl)-1-ethyoxycarbonylmethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4l**)*

White crystals, yield 41.5%, m.p.160–162 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 9.56 (1H, s, NH), 8.23 (1H, d, J = 2.4 Hz, pyridine-H), 7.81–7.78 (1H, dd, J = 2 sHz, 2.4 Hz, pyridine-H), 7.37–7.35 (1H, d, J = 7.6 sHz, pyridine-H), 7.33 (1H, s, phenyl-H), 7.28 (1H, s, phenyl-H), 7.24–7.22 (2H, d, J = 8.4 Hz, phenyl-H), 4.60–4.56 (1H, d, J = 15.2 Hz, CH_2-pyridine), 4.51–4.48 (1H, dd, J = 0.4 Hz, 0.4 Hz, CH_2-pyridine), 4.45–4.23 (4H, m, triazine-4H), 4.12–4.01 (2H, m, OCH_2), 1.27 (1H, s, CHC═O), 1.14–1.11 (3H, t, J = 7.2 Hz, CH_3). IR (potassium bromide, cm^{-1}) ν: 3342 (N–H), 2985 (pyridine), 1727 (C═O), 1545 (C═N), 1397 (NO_2), 1193 (C–O–C), 1112 (C–N). Anal. calcd for $C_{20}H_{21}Cl_2N_6O_4$: C 48.83, H 4.31, N 17.98; found C 48.78, H 4.38, N 18.02. ESI-MS (M + H) m/z: 467.3 $[\alpha]_D^{20}$ = −22.50 (c = 0.1 g/L in acetone).

*5.4.13. 1-(2-Chloro-5-thiazomethyl)-5-(2-pheny-1-ethyoxycarbonylethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4m**)*

White crystals, yield 52.8%, m.p.106–108 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.50 (1H, d, J = 0.8 Hz, NH), 7.46 (1H, s, thiazole-H), 7.30–7.25 (3H, m, phenyl-H), 7.11–7.09 (2H, m, phenyl-H), 4.63–4.59 (2H, m, CH_2-thiazole), 4.56–4.51 (4H, m, triazine-4H), 4.07–3.90 (2H, m, OCH_2), 3.69–3.65 (1H, m, CHC═O), 3.06–2.94 (2H, m, CH_2Ph), 1.09–1.05 (3H, t, J = 7.2 Hz, OCH_3). IR (potassium bromide, cm^{-1}) ν: 3289 (N–H), 2962 (thiazole), 1729 (C═O), 1585 (C═N), 1396 (NO_2), 1189 (C–O–C), 1049 (C–N). Anal. calcd for $C_{18}H_{21}ClN_6O_4S$: C 47.73, H 4.67, N 18.56; found C 47.80, H 4.72, N 18.52. ESI-MS (M + H) m/z: 453.6, $[\alpha]_D^{20}$ = +13.67 (c = 0.1 g/L in acetone).

*5.4.14. 1-(6-Chloro-3-pyridinemethyl)-5-(2-pheny-1-ethyoxycarbonylethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4n**)*

White crystals, yield 50.5%, m.p.131–133 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 9.64 (1H, s, NH), 8.32 (1H, d, J = 2 Hz, pyridine-H), 7.82–7.79 (1H, dd, J = 2.4 Hz, 2.4 Hz, pyridine-H), 7.36–7.34 (1H, d, J = 7.6 Hz, pyridine-H), 7.30–7.25 (3H, m, phenyl-H), 7.12–7.10 (2H, t, J = 2 Hz, phenyl-H), 4.65–4.60 (2H, m, CH_2-pyridine), 4.54–4.38 (4H, m, triazine-4H), 4.09–3.92 (2H, m, OCH_2), 3.71–3.67 (1H, m, CHC = O), 3.06–2.92 (2H, m, CH_2Ph), 1.11–1.08 (3H, t, J = 7.2 Hz, CH_3). IR

(potassium bromide, cm^{-1}) ν: 3300 (N–H), 2982 (pyridine), 1715 (C=O), 1545 (C=N), 1392 (NO_2), 1185 (C–O–C), 1122 (C–N),. Anal. calcd for $C_{20}H_{23}ClN_6O_4$: C 53.75, H 5.19, N 18.81; found C 53.81, H 5.23, N 18.78. ESI-MS (M + H) *m/z*:447.4, $[\alpha]_D^{20} = +16.58$ ($c = 0.1$ g/L in acetone).

5.4.15. 1-(2-Chloro-5-thiazomethyl)-5-(1,2-diethoxycarbonylethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4o**)

White crystals, yield 34.8%, m.p.100–102 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 9.53 (1H, s, NH), 7.48 (1H, s, thiazole-H), 4.77–4.74 (1H, d, $J = 15.6$ Hz, CH_2-thiazole), 4.60–4.56 (1H, dd, $J = 2$ Hz, 3 Hz, CH_2-thiazole), 4.54–4.46 (4H, m, triazine-4H), 4.30–4.01 (4H, m, $2OCH_2$), 3.97–3.93 (1H, t, $J = 7.2$ Hz, CHC = O), 2.83–2.67 (2H, m, CH_2C=O), 1.28–1.27 (3H, m, CH_3), 1.26–1.24 (3H, m, CH_3). IR (potassium bromide, cm^{-1}) ν: 3423 (N–H), 2986 (thiazole), 1735 (C=O), 1584 (C=N), 1373 (NO_2), 1155 (C–O–C), 1056 (C–N). Anal. calcd for $C_{15}H_{21}ClN_6O_6S$: C 40.14, H 4.72, N 18.72; found C 40.20, H 4.65, N 18.75. ESI-MS (M + H) *m/z*: 449.5, $[\alpha]_D^{20} = +11.92$ ($c = 0.1$ g/L in acetone).

5.4.16. 1-(6-Chloro-3-pyridinemethyl)-5-(1,2-diethoxycarbonylethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4p**)

White crystals, yield 38.6%, m.p.109–111 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.67 (1H, s, NH), 8.34 (1H, d, $J = 2.4$ Hz, pyridine-H), 7.81–7.79 (1H, dd, $J = 2.4$ Hz, 2.4 Hz, pyridine-H), 7.37–7.35 (1H, d, $J = 8$ Hz, pyridine-H), 4.83–4.79 (1H, d, $J = 11.6$ Hz, CH_2-pyridine), 4.61 (1H, d, $J = 2$ Hz, CH_2-pyridine), 4.58–4.38 (4H, d, $J = 0.8$ Hz, triazine-4H), 4.25–4.03 (4H, m, $2OCH_2$), 3.97–3.93 (1H, t, $J = 7.2$ Hz, NCH), 2.83–2.66 (2H, m, CH_2C=O), 1.29–1.27 (3H, m, CH_3), 1.26–1.25 (3H, m, CH_3). IR (potassium bromide, cm^{-1}) ν: 3424 (N–H), 2989 (pyridine), 1734 (C=O), 1584 (C=N), 1386 (NO_2), 1178 (C–O–C), 1113 (C–N). Anal. calcd for $C_{17}H_{23}ClN_6O_6$: C 46.11, H 5.23, N 18.98; found C 46.13, H 5.22, N 19.02. ESI-MS (M + H) *m/z*: 443.6 $[\alpha]_D^{20} = +13.37$ ($c = 0.1$ g/L in acetone)

5.4.17. 1-(2-Chloro-5-thiazomethyl)-5-(2-ethoxycarbonylethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4q**)

White crystals, yield 60.8%, m.p.134–136 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 9.54 (1H, d, $J = 0.8$ Hz, NH), 7.48 (1H, s, thiazole-H), 4.64 (2H, m, CH_2-thiazole), 4.39 (4H, d, $J = 2$ Hz, triazine-4H), 4.19–4.13 (2H, m, OCH_2), 2.88–2.85 (2H, t, $J = 6.4$ Hz, NCH_2), 2.46–2.43 (2H, t, CH_2C=O), 1.03–1.26 (3H, t, $J = 7.2$ Hz, OCH_2CH_3). IR (potassium bromide, cm^{-1}) ν: 3270 (N–H), 2988 (thiazole), 1716 (C=O), 1585 (C=N), 1396 (NO_2), 1197 (C–O–C), 1047 (C–N). Anal. calcd for $C_{12}H_{17}ClN_6O_4S$: C 38.25, H 4.55, N 22.30; found C 38.28, H 4.70, N 22.26. ESI-MS (M + H) *m/z*: 377.1, $[\alpha]_D^{20} = +3.29$ ($c = 0.1$ g/L in acetone).

5.4.18. 1-(6-Chloro-3-pyridinemethyl)-5-(2-ethoxycarbonylethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4r**)

White crystals, yield 62.7%, m.p.157–159 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.59 (1H, s, NH), 8.34 (1H, d, $J = 2$ Hz, pyridine-H), 7.83–7.81 (1H, d, $J = 8$ Hz, pyridine-H), 4.64 (2H, s, CH_2-pyridine), 4.41 (2H, s, triazine-2H), 4.31 (2H, s, triazine-2H), 4.18–4.12 (2H, m, OCH_2), 2.92–2.89 (2H, t, $J = 6.4$ Hz, NCH_2), 2.43–2.40 (2H, t, $J = 6.4$ Hz, CH_2C=O), 1.28–1.25 (3H, t, $J = 6.8$ Hz, CH_3). IR (potassium bromide, cm^{-1}) ν: 3300 (N–H), 2978 (pyridine), 1730 (C=O), 1542 (C=N), 1396 (NO_2), 1189 (C–O–C), 1126 (C–N). Anal. calcd for $C_{14}H_{19}ClN_6O_4$: C 45.35, H 5.16, N 22.67; found C 45.33, H 5.20, N 22.72. ESI-MS (M + H) *m/z*: 371.2, $[\alpha]_D^{20} = -5.08$ ($c = 0.1$ g/L in acetone).

5.4.19. 1-(2-Chloro-5-thiazomethyl)-5-(1,3-diethoxycarbonyl-2-propyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4s**)

White crystals, yield 42.5%, m.p.103–105 °C. ^{1}H NMR(δ, ppm,$CDCl_3$): 9.53 (1H, s, NH), 7.47 (1H, s, thiazole-H), 4.68–4.60 (2H, t, $J = 16$ Hz, thiazole-CH_2), 4.56–4.47 (4H, m, triazine-4H), 4.20–4.03 (4H, m, $2OCH_2$), 3.51–4.48 (1H, m, NCH), 2.45–2.23 (2H, m, O=CCH_2), 2.11–1.97 (2H, m, CH_2), 1.29–1.24 (6H, m, $2CH_3$). IR (potassium bromide, cm^{-1}) ν: 3450 (N–H), 2930 (thiazole), 1725 (C=O), 1586 (C=N), 1390 (NO_2), 1185 (C–O–C), 1095 (C–N). Anal. calcd for $C_{16}H_{23}ClN_6O_6S$: C 41.51, H 5.01, N 18.15; found C 41.48, H 5.06, N 18.12. ESI-MS (M + H) *m/z*:463.2, $[\alpha]_D^{20} = -57.08$($c = 0.1$ g/L in acetone).

5.4.20. 1-(6-Chloro-3-pyridinemethyl)-5-(1,3-diethoxycarbonyl-2-propyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4t**)

White crystals, yield 38.6%, m.p.101–103 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.67 (1H, s, NH), 8.34 (1H, d, $J = 2$ Hz, pyridine-H), 7.82–7.79 (1H, dd, $J = 2.4$ Hz, 2.4 Hz, pyridine-H), 7.37–7.35 (1H, d, $J = 8$ Hz, pyridine-H), 4.75–4.71 (1H, d, $J = 11.6$ Hz, CH_2-pyridine), 4.61–4.57 (1H, dd, $J = 2$ Hz, 2.4 Hz, CH_2-pyridine), 4.54–4.35 (4H, m, triazine-4H), 4.22–4.03 (4H, m, $2OCH_2$), 3.53–3.50 (1H, t, $J = 7.2$ Hz, NCH), 2.41–2.28 (2H, m, CH_2C=O), 2.07–1.98 (2H, m, CH_2), 1.28–1.25 (6H, m, $2CH_3$). IR (potassium bromide, cm^{-1}) ν: 3450 (N–H), 2922 (pyridine), 1738 (C=O), 1586 (C=N), 1388 (NO_2), 1182 (C–O–C), 1113 (C–N). Anal. calcd for $C_{18}H_{25}ClN_6O_6$: C 47.32, H 5.52, N 18.39; found C 47.29, H 5.55, N 18.35. ESI-MS (M + H) *m/z*: $[\alpha]_D^{20} = +4.22$ ($c = 0.1$ g/L in acetone).

5.4.21. 1-(2-Chloro-5-thiazomethyl)-5-(3-ethoxycarbonylpropyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4u**)

White crystals, yield 56.4%, m.p. 127–129 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.49 (1H, s, NH), 7.47 (1H, s, thiazole-H), 4.63 (2H, s, CH_2-thiazole), 4.38–4.35 (4H, m, triazine-4H), 4.16–4.10 (2H, m, OCH_2), 2.60–2.56 (2H, t, $J = 7.2$ Hz, NCH_2), 2.35–2.31 (2H, t, $J = 7.2$ Hz, CH_2C=O), 1.77–1.73 (2H, m, CH_2), 1.28–1.24 (3H, t, $J = 7.2$ Hz, OCH_2CH_3). IR (potassium bromide, cm^{-1}) ν: 3423 (N–H), 2969 (thiazole), 1720 (C=O), 1588 (C=N), 1375 (NO_2), 1189 (C–O–C), 1055 (C–N). Anal. calcd for $C_{13}H_{19}ClN_6O_4S$: C 39.95, H 4.90, N 21.50; found C 40.00, H 4.86, N 21.52. ESI-MS (M + H) *m/z*:391.2.

5.4.22. 1-(6-Chloro-3-pyridinemethyl)-5-(3-ethoxycarbonylpropyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4v**)

White crystals, yield 58.7%, m.p.70–72 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.61 (1H, s, NH), 8.33 (1H, d, $J = 2.4$ Hz, pyridine-H), 7.81–7.79 (1H, m, 1.2 Hz, pyridine-H), 7.36–7.34 (1H, d, $J = 8$ Hz, pyridine-H), 4.62 (2H, s, CH_2-pyridine), 4.39 (2H, d, $J = 0.8$ Hz, triazine-2H), 4.28 (2H, s, triazine-2H), 4.14–4.09 (2H, m, OCH_2), 2.64–2.60 (2H, t, $J = 7.2$ Hz, NCH_2), 2.33–2.29 (2H, t, $J = 7.2$ Hz, CH_2C=O), 1.75–1.68 (2H, m, CH_2), 1.27–1.23 (3H, m, CH_3). IR (potassium bromide, cm^{-1}) ν: 3473 (N–H), 3083 (pyridine), 1720 (C=O), 1544 (C=N), 1383 (NO_2), 1189 (C–O–C),1121 (C–N). Anal. calcd for $C_{15}H_{21}ClN_6O_4$: C 46.82, H 5.50, N 21.84; found C 46.83, H 5.53, N 21.88. ESI-MS (M + H) *m/z*: 385.4.

5.4.23. 1-(6-Chloro-3-thiazolmethyl)-5-(1-ethoxycarbonylmethyl)-1,3,5-hexahydrotriazine-2-N-nitroimine (**4w**)

White crystals, yield 46.7%, m.p.175–177 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.51 (1H,s, NH), 7.44 (1H,s, thiazole-H), 4.61 (2H,s, CH_2-thiazole), 4.48–4.49 (4H, d, $J = 5.2$ Hz, triazine-4H), 4.21–4.15 (2H, m, OCH_2), 3.32 (2H, s, CHC = O) 1.29–1.26 (3H, t, $J = 7.2$ Hz, CH_3). IR (potassium bromide, cm^{-1}) ν: 3288 (N–H), 3000 (thiazole), 1730 (C=O) 1587 (C=N), 1398 (NO_2), 1224 (C–O–C), 1105 (C–N). Anal. calcd for $C_{11}H_{15}ClN_6O_4S$: C 36.42, H 4.17, N 23.16; found C 36.40, H 4.23, N 23.19. ESI-MS *m/z*: 363.8.

5.4.24. 1-(6-Chloro-3-pyridinemethyl)-5-(1-ethoxycarbonylmethyl)- 1,3,5-hexahydrotriazine-2-N-nitroimine (**4x**)

White crystals, yield 51.7%, m.p.170–172 °C. ^{1}H NMR(δ, ppm, $CDCl_3$): 9.66 (1H,s, N–H), 8.33–8.32 (1H, d, $J = 2.4$ Hz, pyridine-H),

7.83–7.81 (1H, dd, J = 2 Hz, 2.4 Hz, pyridine-H), 7.38–7.37 (1H, d, J = 4.4 Hz, pyridine-H), 4.62 (2H,s, pyridine-CH_2), 4.52 (2H, s, triazine-2H), 4.43 (2H, s, triazine-2H), 4.20–4.14 (2H, m, OCH_2), 3.38 (2H, s, $CH_2C{=}O$), 1.29–1.25 (3H, t, J = 7.2 Hz, CH_3). IR(potassium bromide, cm^{-1}) ν: 3278 (N–H), 2994 (thiazole), 1731 (C=O), 1586 (C=N), 1391 (NO_2), 1215 (C–O–C), 1111 (C–N). Anal. calcd for $C_{13}H_{17}ClN_6O_4$: C 43.77, H 4.80, N 23.56; found C 43.75, H 4.84, N 23.52. ESI-MS m/z: 357.8.

5.5. Crystal growth and X-ray data for crystal structure of **4n**

Colorless crystal of compound **4n** with approximate dimensions of 0.16 mm × 0.12 mm × 0.10 mm, which is suitable for single-crystal X-ray diffraction, was obtained by slowly evaporating a solution of **4n** in mixed with acetone and petroleum at 25 °C. The crystal was mounted on a glass fiber for data collection on a Bruker Smart Apex CCD diffractometer equipped with a graphite-monochromatic Mo $K\alpha$ radiation (λ = 0.71073 Å). The φ-ω scan mode in the range of $1.54 \le \theta \le 26.00^\circ$ and $2.29 \le \theta \le 25.50^\circ$ at 298 K was used. A total of 6826 reflections were collected with 5544 unique ones (R_{int} = 0.0546), of which 4717 with $I > 2\sigma(I)$ were considered as observed and used in the subsequent refinements. The structure was refined by full-matrix least-squares method on F^2 with anisotropic thermal parameters for all nonhydrogen atoms.

Crystal data for **4n**: space group $P2_1/c$ with a = 8.877(2) Å, b = 9.176(2) Å, c = 13.661(2) Å, V = 1068.2(4)Å^3, Dc = 1.386 g/cm^3, F(000) = 466, μ = 0.219 mm^{-1}, Z = 24,.The final refinement gave S = 1.017, R = 0.0504 and $w = 1/[\sigma^2(F_o^2) + (0.0996P)^2] + 0.000P$, where $P = (F_o^2 + 2F_c^2)/3$. The crystal was analyzed with SHELXTL-97[27] software package and the structural plots were drawn with Otrep. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 751235. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

5.6. Insecticidal activity assay

The insecticidal activities of compounds **4a**–**4x** were measured against *A. medicagini* according to the standard test [31] with a slight modification. The test analogues were dissolved in DMF and serially diluted with water containing Triton X-80 (0.1 mg/L) to get the required concentrations. The insects were reared at 25° (±1) °C, and groups of 10 were transferred to glass Petridishes and sprayed with the aforementioned solutions using a Potter sprayer. Assessments were made after 72 h by the number and size of live insects relative to that in the negative control, and evaluations are based on a percentage scale of 0–100, in which 100 total kill and 0 no activity. The mortality rates were subjected to probit analysis. The reference compounds were imidacloprid and thiamethoxam, while water containing Triton X-80 (0.1 mg/L) was used as a negative control. All experiments were carried out in three replicates according to statistical requirements, and the results were shown in Table 1.

5.7. Experimental protocol of docking study

The *Lymnaea stagnalis* AchBP (*ls*-AchBP, PDB ID: 2zju) [32] was used as the template to construct the docking model. The molecular docking studies were performed using AutoDock version 4.0 [33].

5.7.1. Preparation of the receptor and ligands

The crystal structure of *ls*-AchBP complexed with imidacloprid was retrieved from the RCSB Protein Data Bank. Since it's a pentameric macromolecules composed of five identical α subunits, the subunits A and B, which are adjacent, were extracted from the homopentamer for docking, and the interfacial binding pocket between the (+)-face and (−)-face of these two subunits was used for modeling. The protein was prepared for docking by addition of polar hydrogens, and subjected to a side chain conformational search. The putative active binding site was characterized by selecting all residues within a 12 Å radius of the original binding substrate in the X-ray structure. All co-crystallized molecules were deleted, and each ligand was iteratively minimized and assigned the Gasteiger–Hückel charges.

5.7.2. Molecular modeling and results analysing

All selected compounds were flexibly docked and investigated for their binding activities into protein nAChR. The AMBER force field was used to calculate a three-dimensional grid of interaction energies for the target nAChR by the AutoGrid component of the program, and these grids were precomputed to store the electrostatic and van der Waals values. Default values were used for all docking parameters with 15 independent docking runs for each ligand. Intermolecular energy, torsional free energy and intermolecular hydrogen bonds were included to evaluate their binding free energy. The docking conformations were subjected to cluster analysis using a root mean square deviation (RMSD) tolerance of 0.5 Å. The top hits of each cluster were examined and the conformations with the lowest binding energy were chosen for further comparison and analysis. Accelrys DS visualizer 2.5[Accelrys Inc., San Diego, CA (2009)] was used for molecular modeling to determine the binding orientations of these analogues and their interactions with the active site.

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References

[1] S. Kagabu, R. Ishiara, Y. Hieda, K. Nishimura, Y. Naruse, J. Agric. Food Chem. 55 (2007) 812–818.
[2] M. Tomizawa, J.E. Casida, Toxicol. Appl. Pharmacol. 169 (2000) 114–120.
[3] S. Kagabu, K. Nishimura, Y. Naruse, I. Ohno, J. Pest Sci. 33 (2008) 58–66.
[4] a) R. Nauen, U.E. Kintscher, V.L. Salgado, M. Kaussmannb, Pestic Biochem. Physiol. 76 (2003) 559;
b) P. Maienfisch, Z. Naturforsch. B Chem. Sci. 61 (2006) 353.
[5] T. Wakita, K. Kinoshita, E. Yamada, N. Yasui, N. Kawahara, A. Naoi, M. Nakaya, K. Ebihara, H. Matsuno, K. Kodaka, Pest Manag. Sci. 59 (2003) 1016–1022.
[6] K. Matsuda, S.D. Buckingham, D. Kleier, J.J. Rauh, M. Grauso, D.B. Sattelle, Trends Pharmacol. Sci. 22 (2001) 573–580.
[7] K. Matsuda, M. Shimomura, M. Ihara, M. Akamatsu, D.B. Sattelle, Biosci. Biotechnol. Biochem. 69 (2005) 1442–1452.
[8] Z.Z. Tian, X.S. Shao, Z. Li, X.H. Qian, Q.C. Huang, J. Agric. Food Chem. 55 (2007) 2288–2292.
[9] M. Tomizawa, D.L. Lee, J.E. Casida, J. Agric. Food Chem. 48 (2000) 6016–6024.
[10] S.J. Lee, M. Tomizawa, J.E. Caida, J. Agric. Food Chem. 51 (2003) 2646–2652.
[11] M. Tomizawa, J.E. Casida, Annu. Rev. Entomol. 48 (2003) 339–364.
[12] M. Tomizawa, I. Yamamoto, Pestic. Sci. 18 (1993) 91–98.
[13] R. Nauen, I. Denholm, Arch. Insect Biochem. Physiol. 58 (2005) 200–215.
[14] R. Nauen, A. Elbert, Pest Manag. Sci. 56 (2000) 60–64.
[15] M. Reyes, P. Franck, P.J. Charmillot, C. Ioriatti, J. Olivares, E. Pasqualini, B. Sauphanor, Pest Manag. Sci. 63 (2000) 890–902.
[16] Z.W. Liu, Z.J. Han, Y.C. Wang, L.C. Zhang, H.W. Zhang, C.J. Liu, Pest Manag. Sci. 59 (2003) 1355–1359.
[17] K.D. Ninsin, Pest Manag. Sci. 60 (2004) 839–841.
[18] D. Mota-Sanchez, R.M. Hollingworth, E.J. Grafius, Pest Manag. Sci. 62 (2006) 30–37.
[19] K. Gorman, G. Devine, J. Bennison, P. Coussons, N. Punchard, I. Denholm, Pest Manag. Sci. 63 (2007) 555–558.

[20] M. Kristensen, J.B. Jespersen, Pest Manag. Sci. 64 (2008) 126–132.
[21] K. Ishimitsu, J. Suzuki, H. Ohishi, T. Yamada, R. Hatano, N. Takakusa, J. Mitsui, WO Patent 9104965, 1991.
[22] I. Ohno, M. Tomizawa, K.A. Durkin, J.E. Casida, S. Kagabu, J. Agric. Food Chem. 57 (2009) 2436–2440.
[23] P. Maienfisch, H. Huerlimann, A. Rindlisbacher, L. Gsell, H. Dettwiler, J. Haettenschwiler, E. Sieger, M. Walti, Pest Manag. Sci. 57 (2001) 165–176.
[24] P. Lewer, P.R. Graupner, D.R. Hahn, L.L. Karr, J. Nat. Prod. 66 (2006) 1506–1510.
[25] I. Yamamoto, M. Tomizawa, T. Saito, T. Miyamoto, E.C. Walcott, K. Sumikawa, Arch. Insect Biochem. Physiol. 37 (1998) 24–32.
[26] E. Luttmann, J. Ludwig, A. Höffle-Maas, M. Samochocki, A. Maelicke, G. Fels, ChemMedChem. 4 (2009) 1874–1882.
[27] a) X.Q. Lu, G.Q. Zhao, X. Song, J.M. Xie, Chem. World 48 (2007) 357–359;
b) W.J. Chen, D.H. Liao, Chem. World 47 (2006) 285–286.
[28] J. Hernndez-Toribio, R.G. Arrays, J.C. Carretero, J. Am. Chem. Soc. 130 (2008) 16150–16151.
[29] H. Kayser, C. Lee, A. Decock, M. Baur, J. Haettenschwiler, P. Maienfisch, Pest Manag. Sci. 60 (2004) 945–958.
[30] P. Dapporto, A. Guerri, P. Paoli, P. Rossi, M. Altamura, J. Mol. Struct. (Theochem) 617 (2002) 189–199.
[31] P.L. Zhao, F. Wang, Z.M. Zhang, J. Agric. Food Chem. 56 (2008) 10767–10773.
[32] M. Ihara, M. Okajima, A. Yamashita, T. Oda, K. Hirata, H. Nishiwaki, T. Morimoto, M. Akamatsu, Y. Ashikawa, S. Kuroda, R. Mega, S. Kuramitsu, D.B. Sattelle, K. Matsuda, Invert. Neurosci. 8 (2008) 71–81.
[33] R. Huey, G.M. Morris, A.J. Olson, J. Comput. Chem. 28 (2007) 1145–1152.