# Synthesis, Insecticidal Activities, and Molecular Docking Studies of 1,5-Disubstituted-1,3,5-hexahydrotriazine-2-(*N*-nitro)imines



Chuan-Wen Sun,[a]* Hai-Feng Wang,[a] Jun Zhu,[a] Ding-Rong Yang,[a] Jia Jin,[a] and Jia-Hua Xing[b]

[a]College of Life and Environment Sciences, Shanghai Normal University, Shanghai 200234, China
[b]Bioassay Department, Branch of National Pesticide R&D South Center, Hangzhou 310023, China
*E-mail: willin112@163.com

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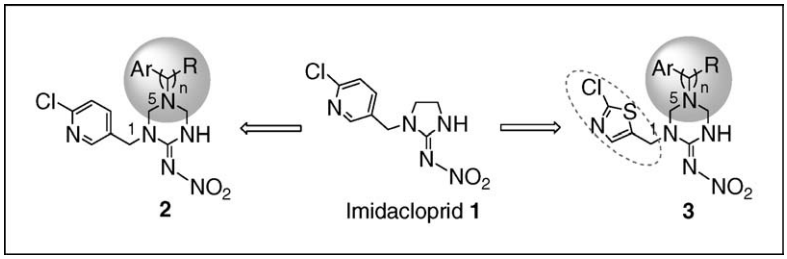


A series of novel neonicotinoids analogs were designed by modifying the pharmacophore of imidacloprid to 1,3,5-hexahydrotriazine conjugated to nitroimine (=$NNO_2$) and introducing the phenyl or arylmethyl at the 5-position, and their insecticidal activities were evaluated. Introducing a heterocyclic methyl at 5-position increased the insecticidal activities, whereas other phenyl, phenylmethyl or phenylethyl substituents were unfavorable to activities. Molecular docking study was also performed to clarify the interactions of the most potent analog 1-((6-chloropyridin-3-yl)methyl)-5-(3-pyridylmethyl)-1,3,5-hexahydrotriazine-2-(*N*-nitro) imine (**7s**) with the target nicotinic acetylcholine receptor, which explained the structure-activity relationships observed *in vitro*, and revealed further possibilities for insecticide development.

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## INTRODUCTION

The nicotinic acetylcholine receptor (nAChR) is the target for a major group of the neonicotinoid insecticides, which have been increasingly used to control various insects during recent years and account for one-fifth of the global insecticide market. As the first commercially available insecticide from this class, the supreme biological profile of Imidacloprid (IMI) [1] (**1**, Fig. 1) has driven the development of new products by modifying the structural features. Six other neonicotinoids insecticides (**NNs**): acetamiprid [2], nitenpyram [3], thiamethoxam [4,5], thiacloprid [6], clothianidin [7], and dinotefuran [8] were successively commercialized, which are all nAChR agonists that act on the ligand-gated ion channels responsible for rapid neurotransmission of insects [9,10]. These insecticides combine high potency with low mammalian toxicity and are used extensively for crop protection [11–13]. However, during the past decade, significant increases in resistance and cross resistance were observed in a range of species after frequent field applications [14–21]. Especially, some resistant species increased in potency, with more recently collected strains exhibiting more than 100-fold resistance to IMI and comparable levels of resistance to thiamethoxam and acetamiprid [22]. One of the effective tactics to handle resistance is the design and screening for more insecticides with novel structures or mode of actions, the development of new neonicotinoids with better activities against resistance strains is therefore a high priority.

The common molecular structural features of neonicotinoids consist of four sections: (1) aromatic heterocycle, (2) flexible linkage, (3) hydroheterocyles or guanidine/amidine, and (4) electron-withdrawing segment [23–25]. Similarly, as for IMI, the electron-withdrawing moiety nitro group and the chlorpyridinylmethyl, which joined to a second heterocyclic ring, may play important roles in its activities [26–28]. Encouraged by these reports, we took manifold modifications of IMI by changing imidazolidine to 1,3,5-hexahydrotriazine and introducing the phenyl or aryl methyl at the 5-position. To explore the influence of appending 6-chloro-3-pyridylmethyl ($ClPyrCH_2$) or 2-chloro-5-thiazolylmethyl ($ClThyCH_2$) at the 1-position and different substituents at the 5-position on the bioactivities, a series of neonicotinoids analogs **2** and **3** (Fig. 1) were designed, synthesized, and evaluated for their insecticidal activity against Pseudaletia separate Walker. The interactions of the most potent analog with its target (nAChR) were also studied by

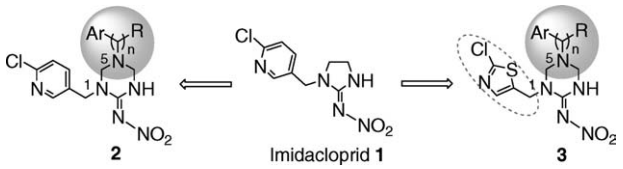


**Figure 1.** Regions of IMI chosen for modification.

molecular docking simulations, which explained the structure-activity relationships observed *in vitro* and may gain insight into their mechanism of action.

## RESULTS AND DISCUSSION

**Synthesis of compounds.** Synthesis procedures for the novel neonicotinoid derivatives are summarized in Scheme 1. A three-step synthetic strategy was adopted to prepare 1,5-disubstituted-1,3,5-hexahydrotriazine-2-(*N*-nitro)imines derivatives **7a–f**. Firstly, fusing of the substituted aniline hydrochloride and formaldehyde at 25°C for 1 h provided the various substituted imine hydrochlorides **5a–f** in 77–82% yields, which were condensed with nitroguanidine in catalytic amount of hydrochloric acid to give 5-substituted-1,3,5-hexahydrotriazine-2-(*N*-nitro)imines **6a–f** in 56–67% yields. 1,5-substituted-1,3,5-hexahydrotriazine-2-(*N*-nitro)imines **7a–f** were obtained by reacting **6a–f** with 2-chloro-5-chloromethyl pyridine or 2-chloro-5-chloromethyl thiazole in the presence of catalytic amount of cesium chloride and potassium carbonate in the mixture of absolute acetonitrile and *N,N*-dimethylformamide. Synthesis of 5-substituted-1,3,5-hexahydrotriazine- 2-(*N*-nitro)imines **6g–z** was accomplished by using a well-known ''3-component'' method as described in literature [29]. The three components(substituted amine, nitroguanidine, and 37% formaldehyde in 1:1:2 ratio) were refluxed in the presence of catalytic amount of concentrated hydrochloric acid in alcohol to give **6g–z** in 76–90% yields. Subsequent treatment of **6g–z** with 2-chloro-5-chloromethyl pyridine or 2-chloro-5-chloromethyl thiazole gave 1,5-disubstituted-1,3,5-hexahydrotriazine-2-(*N*-nitro)imines **7g–z** in 51–68% yields. All compounds synthesized herein are freely soluble in both polar aprotic solvent and polar protic solvents at 25°C, and their structures were well characterized by IR, $^1$H-NMR, and mass spectroscopy.

**Insecticidal activity.** The insecticidal activities of compounds **7a–z** and IMI were screened against Pseudaletia separate Walker using the standard testing method [30]. Results of the *in vitro* insecticidal activities of compounds **7a–z** were summarized in Table 1. As indicated in **Table** 1, most of our designed compounds exhibited significant insecticidal activities against Pseudaletia separate Walker and had > 90% mortality at 500 mg/L. Among all the analogs, **7s** afforded the best *in vitro* activity, which is comparable to that of IMI. Other compounds' potential varied drastically, depending upon the size, types, and characters of the 5-position substitution on 1,3,5-hexahydrotriazine. The introduction of 3-pyridyl methyl, 2-furanyl methyl, 2-chloro-5-thiazolyl methyl and 2-tetrahydrofuranyl methyl at the 5-position of 1,3,5-hexahydrotriazine (**7s–7z**) showed excellent inhibitory activities, even at the rate of 20 mg/L, whereas the appending 6-chloro-3-pyridylmethyl ($ClPyrCH_2$) and 2-chloro-5-thiazolylmethyl ($ClThyCH_2$) groups had no conspicuous influence on their insecticide activities. In addition, the insecticidal activities of the corresponding analogs decreased in the order heterocyclic methyl (**7s–7z**) > phenyl (**7a–7f**) > phenylmethyl (**7g–7l**) > phenylethyl (**7q–7r**), and compounds **7m**, **7n**, and **7o**, **7p** with a methyl group at the methenyl demonstrated higher activities than **7g–7h**. However, the introductions of other phenyl (**7a–7f**), phenylmehtyl (**7g–7l**), and phenylethyl (**7q–7r**) seemed to be unfavorable to activities. Interestingly, due to the existence of fluorine, compounds **7c**, **7d** also showed higher activities than **7a, 7b** and **7e, 7f**. The observations herein corroborate our point of view that a heterocyclic methyl or phenyl with electron-withdrawing group introduced at 5-position will increase the insecticidal activity of neonicotinoid analogs.

**Molecular docking study.** To understand the binding mode of action of our newly synthesized compounds, we performed a docking study using AutoDock 4.0 [31]. Consistent with the above *in vitro* insecticide activities, the scoring function of AutoDock ranked the analogs in

**Scheme 1.** Synthesis of novel neonicotinoid derivatives **7a–z**. Reagents and conditions: (a) concd HCl, abs ethanol, r.t; (b) nitroguanidine, concd HCl, abs ethanol, refluxing; (c) concd HCl, abs ethanol, refluxing; and (d) 2-chloro-5-chloromethyl pyridine or 2-chloro-5-chloromethyl thiazole, $CH_3CN$+DMF, CsCl, $K_2CO_3$, 60–65°C.

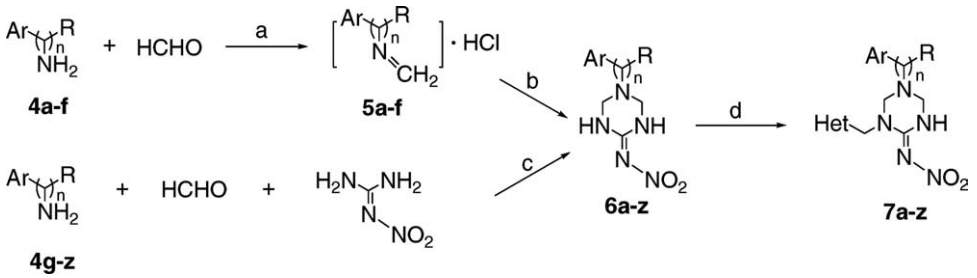

**Table 1**

Insecticidal activities of compounds **7a–z** and imidacloprid against *Pseudaletia separate Walker*.

| Compd | Ar | R | n | Het | Concn (mg/L)[a] 500 | 100 | 20 |
|---|---|---|---|---|---|---|---|
| 7a | $C_6H_5$ | | 0 | ClPyr | +++++ | +++ | ++ |
| 7b | $C_6H_5$ | | 0 | ClThy | ++++++ | ++++ | ++ |
| 7c | 4-$FC_6H_4$ | | 0 | ClPyr | ++++++ | +++++ | +++ |
| 7d | 4-$FC_6H_4$ | | 0 | ClThy | ++++++ | +++++ | ++ |
| 7e | 2,4-$(CH_3)_2C_6H_3$ | | 0 | ClPyr | ++++ | +++ | + |
| 7f | 2,4-$(CH_3)_2C_6H_3$ | | 0 | ClThy | ++++ | ++++ | ++ |
| 7g | $C_6H_5$ | H | 1 | ClPyr | ++++ | ++ | − |
| 7h | $C_6H_5$ | H | 1 | ClThy | +++ | +++ | − |
| 7i | 4-$CH_3C_6H_4$ | H | 1 | ClPyr | +++ | +++ | + |
| 7j | 4-$CH_3C_6H_4$ | H | 1 | ClThy | ++++ | ++ | − |
| 7k | 4-$CH_3OC_6H_4$ | H | 1 | ClPyr | +++ | − | n.t |
| 7l | 4-$CH_3OC_6H_4$ | H | 1 | ClThy | ++ | − | n.t |
| 7m[b] | $C_6H_5$ | $CH_3$ | 1 | ClPyr | ++++ | ++ | + |
| 7n[b] | $C_6H_5$ | $CH_3$ | 1 | ClThy | +++++ | ++ | − |
| 7o[c] | $C_6H_5$ | $CH_3$ | 1 | ClPyr | ++++ | +++ | − |
| 7p[c] | $C_6H_5$ | $CH_3$ | 1 | ClThy | ++++ | ++ | + |
| 7q | $C_6H_5CH_2$ | H | 1 | ClPyr | ++ | − | n.t |
| 7r | $C_6H_5CH_2$ | H | 1 | ClThy | ++ | − | n.t |
| 7s | 3-pyridyl | H | 1 | ClPyr | ++++++ | ++++++ | ++++++ |
| 7t | 3-pyridyl | H | 1 | ClThy | ++++++ | ++++ | ++++ |
| 7u | 2-chloro-5-thiazolyl | H | 1 | ClPyr | ++++++ | +++ | +++ |
| 7v | 2-chloro-5-thiazolyl | H | 1 | ClThy | ++++++ | ++++ | ++++ |
| 7w | 2-furanyl | H | 1 | ClPyr | ++++++ | +++++ | ++++ |
| 7x | 2-furanyl | H | 1 | ClThy | ++++++ | ++++ | ++ |
| 7y | 2-tetrahydrofuranyl | H | 1 | ClPyr | ++++++ | ++++++ | ++ |
| 7z | 2-tetrahydrofuranyl | H | 1 | ClThy | ++++++ | ++++++ | +++ |
| 1 | Imidacloprid | | | | ++++++ | ++++++ | ++++++ |

[a] Rating system for the mortality percentage: ++++++, 100%; +++++, ≥ 90%; ++++, ≥ 80%; +++, ≥ 70%; ++, ≥ 60%; +, ≥ 50%; −, <50%.
[b] (±)-.
[c] (*R*)-.
[d] n.t means not tested.

the same general order as observed experimentally, and all the modeled potent compounds exhibited significant hydrogen bonding interactions with nAChR(data not shown). As expected, the most active compound **7s** attained the highest score amongst all molecules and fitted the best in the active site of nAChR (Fig. 2a), which located at the interface between its two adjacent subunits. The chloropyridine N of **7s** substantially interacted with Tyr185 OH, and its nitryl O10 hydrogen bonds the side chain of Cys187 as illustrated [Fig. 2(b,c)]. Besides,

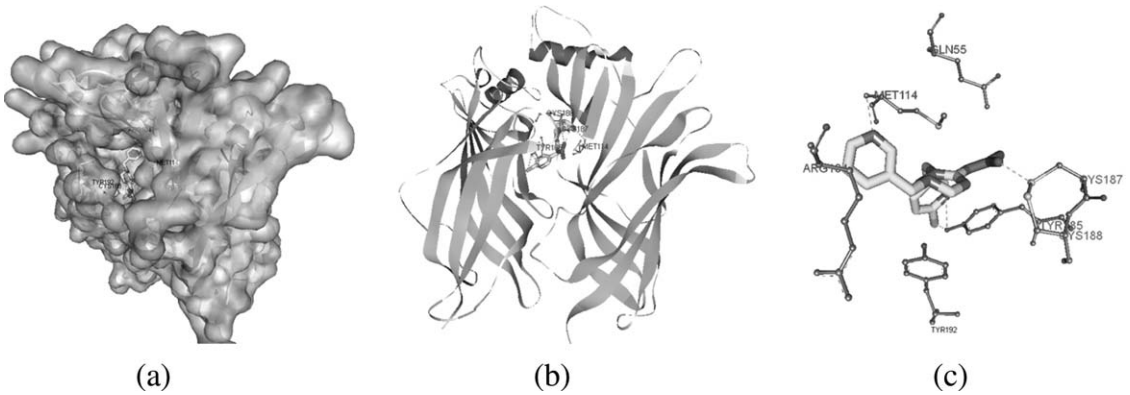


**Figure 2.** Model of the active compound **7s** docked in the active site of nAChR (PDB ID: 2zju). (a) The protein surface of the extracellular domain of nAChR. For clarity, only two of its five subunits are displayed; (b) **7s** is bound into the subunit interfacial binding pocket between two faces of adjacent subunits; (c) The binding site interactions of **7s** with the active site residues. The hydrogen bonds between the inhibitor and residues are shown in green lines.

analog **7s** also showed the important additional H bonding interactions between its N22 and H–N of Met114 (N–H. . .N22: 2.21 Å, 126.1). This is due to the newly introduced 3-pyridylmethyl group of **7s**, which is present at the fifth position of its hexahydrotriazine ring. Therefore, the substituent group at the 5-position on 1,3,5-hexahydrotriazine ring definitely plays an important role in the binding interactions with nAChR.

In summary, a series of new 1,5-disubstituted-1,3,5-hexahydrotriazine-2-(*N*-nitro) imines analogs were prepared and tested for the insecticidal activity against Pseudaletia separate Walker, and their binding interactions with nAChR were investigated by molecular docking study. Among all synthesized compounds, **7s** exhibited the best inhibitory activity, displaying 90% mortality at 20 mg/L. In addition, the docking results were in good agreement with their high insecticidal potential, and modeling the inhibitor-nAChR complexes explained the structure-activity relationships observed *in vitro*. Further studies are underway to assess their inhibitory activities against insect species resistant to IMI, as well as searching for new specific insect target(s). The preliminary structure activity relationship (SAR) and molecular modeling study herein have shed a light on the further insight into their mechanism of action and novel insecticide design.

## EXPERIMENTAL

Thin-layer chromatography (TLC) was carried out on silica gel 60 $F_{254}$ plates Merck KGaA). $^1$H-NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer, using dimethylsulfoxide (DMSO) as solvents and tetramethylsilane as internal standard. The chemical shift (δ) values are expressed in parts per million (ppm) relative to tetramethylsilane as an internal standard: s = singlet, d = doublet, dd = double doublet, and m = multiplet. Melting points were determined by an RK1 microscopic melting apparatus uncorrected. Elemental analysis was performed with a Perkin-Elmer 2400 instrument. IR spectra were obtained on a Nicolet 5DX FT-IR spectrophotometer in the region 4000–400 $cm^{-1}$ using KBr discs. MS spectra were recorded on a Trace DSQ mass spectrograph. All reactions were performed in oven-dried glassware with magnetic stirring. All the chemical reagents purchased were of analytical grade and used without further purification, except for the toluene, which was dried by refluxing in the presence of sodium and distilled prior to use.

**General synthetic procedure for synthesis of 7a–f.** The title compounds **6a–6m** were synthesized by the method outlined in Scheme 1. Commercial (substituted) anilines were converted to their hydrochloride with hydrogen chloride in ether in 91–96% yields. Then the mixture of the aniline hydrochloride and 37% formaldehyde was gently stirred at room temperature for 1 h provided imine hydrochloride. Then, the various substituted imine hydrochlorides were condensed with nitroguanidine and 37% formaldehyde in catalytic amount of hydrochloric acid to give 5-substituted-1,3,5-hexahydroriazine **6a–6f**. Progress of the reaction was monitored by TLC. The absence of nitroguanidine in the reaction mixture was ascertained by carrying out the 5-substituted-1,3,5-hexahydro triazine test. For all of the compounds, precipitation of the product occurred during the reaction process. Upon completion of the reaction, the reaction mixture was vacuum filtered and washed with cold ethanol. The product obtained was then oven dried, and purification was achieved by recrystallization using admixtures of ethanol and water. The filtrate was kept in the refrigerator for a few days, and the crystalline products harvested by vacuum filtration. The final product was dried in a vacuum oven at 50°C for at least 24 h before further use.

Drying 5-substituted 1,3,5-hexahydrotriazine (0.015 mol) **6a–6f** was dissolved in the the mixture of 35 mL absolute acetonitrile and 5 mL *N,N*-dimethylformamide 35 mL, followed by addition of $K_2CO_3$ (0.017 mol), a spot of CsCl. Heated to 55°C, the mixture was slowly added dropwise a solution of 2-chloro-5-chloromethylthiazole or 2-chloro-5-chloromethylpyridine (0.017 mol) in 15 mL dry acetonitrile. After stirring under 55°C for 6 h, cooled to room temperature, and then concentrated under reduced pressure. The residue was purified by flash chromatography eluting with dichloromethane/acetone (v/v 2:1) to afford desired products **7a–7f**.

***1-((6-Chloropyridin-3-yl)methyl)-5-phenyl-1,3,5-hexahydrotriazine-2-(N-nitro)imine(7a).*** Yield: 42.8%; m.p.: 132–133°C; IR (KBr disc): 3288, 1593, 1338, 1175, 1103 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 4.31 (s, 2H, triazine), 4.46 (s, 2H, triazine), 4.69 (s, 2H, -Pyri), 6.83 (t, $J$ = 7.2 Hz, 1H, Ph), 6.94 (d, $J$ = 8.0 Hz, 2H, Ph), 7.24 (t, $J$ = 8.0 Hz, 2H, Ph), 7.49 (d, $J$ = 8.0 Hz, 1H, Pyri-H), 7.78–7.81 (m, 1H, Pyri-H), 8.36 (d, $J$ = 2.4 Hz, 1H, Pyri-H), 9.78 (s, 1H, N—H) ppm; $[M+H]^+$: 347.5. Anal. Calcd. for $C_{15}H_{15}ClN_6O_2$: C, 51.95; H, 4.36; N, 24.24.; Found C, 51.86; H, 4.43; N, 24.11.

***1-((2-Chlorothiazol-5-yl)methyl)-5-phenyl-1,3,5-hexahydrotriazine-2-(N-nitro)imine(7b).*** Yield: 38.5%; m.p.: 121–122°C; IR (KBr disc): 3302, 1585, 1538, 1171, 1134 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 4.36 (s, 2H, triazine), 4.39 (s, 2H, triazine $CH_2$), 4.63 (s, 2H, $CH_2$-thiazole), 6.86 (t, $J$ = 7.2 Hz, 1H, Ph), 6.98 (d, $J$ = 8.0 Hz, 2H, Ph), 7.27 (t, $J$ = 8.0 Hz, 2H, Ph), 7.47 (s,1H, thiazole-H), 9.61 (s, 1H, N—H) ppm; $[M+H]^+$: 353.5; Anal. Calcd. for $C_{13}H_{13}ClN_6O_2S$: C, 44.26; H, 3.71; N, 23.82.; Found C, 44.41; H, 3.63; N, 23.75.

***1-((6-Chloropyridin-3-yl)methyl)-5-(4-fluorophenyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7c).*** Yield: 32.6%; m.p.: 143–145°C; IR (KBr disc): 3266, 1585, 1544, 1140, 1103 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 3.74 (d, $J$ = 9.6 Hz, 2H, $CH_2$—Ph), 4.25 (s, 2H, triazine), 4.43 (s, 2H, triazine), 4.58 (s, 2H, $CH_2$-Pyri), 7.09 (m, 2H, Ph—H), 7.10–7.32 (m, 2H, Ph—H), 7.36 (d, $J$ = 8.0 Hz, 1H, Pyri-H), 7.81–7.84 (m, 1H, Pyri-H), 8.25 (d, $J$ = 2.4 Hz, 1H, Pyri-H), 9.71 (s,1H, N—H) ppm; $[M+H]^+$: 365.8; Anal. Calcd. for $C_{15}H_{14}ClFN_6O_2$: C, 49.23; H, 3.92; N, 23.18.; Found C, 49.39; H, 3.87; N, 23.04.

***1-((2-Chlorothiazol-5-yl)methyl)-5-(4-fluorophenyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7d).*** Yield: 34.8%; m.p.: 127–128°C; IR (KBr disc): 3302, 1585, 1538, 1171, 1134 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 4.36 (s, 2H, triazine), 4.39 (s, 2H, triazine), 4.63 (s, 2H, $CH_2$-thiazole), 7.12 (d, $J$ = 8.0 Hz, 2H, Ph—H), 28 (d, $J$ = 8.0 Hz, 2H, Ph—H), 7.47 (s,1H, thiazole-H), 9.58 (s,1H, N—H) ppm; $[M+H]^+$: 371.5; Anal. Calcd. for $C_{13}H_{12}ClFN_6O_2S$: C, 42.11; H, 3.26; N, 22.67.; Found C, 42.29; H, 3.41; N, 22.48.

***1-((6-Chloropyridin-3-yl)methyl)-5-(2,4-dimethylphenyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7e).*** Yield: 48.0%; m.p.: 106–108°C; IR (KBr disc): 3286, 1587, 1343, 1183, 1107 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 2.14 (s, 3H, $CH_3$), 2.31 (s, 3H, $CH_3$), 4.56 (s, 2H, triazine), 4.60 (s, 2H, triazine), 4.77 (d, $J$ = 5.6 Hz, 2H, $CH_2$-Pyri), 6.79 (d, $J$ = 8.4 Hz, 1H, Ph—H), 6.88 (d, $J$ = 8.4 Hz, 1H, Ph—H), 7.09 (d, $J$ = 8.4 Hz, 1H, Ph—H), 7.49 (d, $J$ = 8.0 Hz, 1H, Pyri-H), 8.13–8.15 (m, 1H, Pyri-H), 8.69 (d, $J$ = 2.4 Hz, 1H, Pyri-H), 9.87 (s,1H, N—H) ppm; $[M+H]^+$: 375.7; Anal. Calcd. for $C_{17}H_{19}ClN_6O_2$: C, 54.47; H, 5.11; N, 22.42.; Found C, 54.32; H, 5.23; N, 22.38.

***1-((2-Chlorothiazol-5-yl)methyl)-5-(2,4-dimethylphenyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7f).*** Yield: 39.4%; m.p.: 116–117°C; IR (KBr disc): 3298, 1583, 1363, 1167, 1101 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 2.16 (s, 3H, $CH_3$), 2.32 (s, 3H, $CH_3$), 4.47 (s, 2H, triazine-H), 4.50–4.63 (t, 2H, $J$ = 9.6 Hz, triazine-H), 4.63 (s, 2H, $CH_2$-thiazole), 6.81 (d, $J$ = 8.4 Hz, 1H, Ph—H), 6.87 (d, $J$ = 8.0 Hz, 1H, Ph—H), 7.13 (d, $J$ = 8.0 Hz, 1H, Ph—H), 7.47 (s, 1H, thiazole-H), 9.54 (s, 1H, N—H) ppm; $[M+H]^+$: 381.9; Anal. Calcd. for $C_{15}H_{17}ClN_6O_2$: C, 47.30; H, 4.50; N, 22.07.; Found C, 47.23; H, 4.58; N, 22.19.

***General synthetic procedure for 7g–z.*** A mixture of the substituted amine (0.05 mol) **4g–z**, nitroguanidine (0.05 mol), 37% formaldehyde (0.01 mol), concentrated hydrochloric acid (0.05 mol) and absolute ethanol (15 mL) was refluxed with stirring for 6–10 h, depending on the type of substituted amine used. The reaction mixture became clear within 30–40 min, followed by the precipitation of the product during reflux. Progress of the reaction was monitored by TLC. The absence of nitroguanidine in the reaction mixture was ascertained by carrying out the 5-substituted-1,3,5-hexahydrotriazine test. For all of the compounds, precipitation of the product occurred during the reaction process. Upon completion of the reaction, the reaction mixture was vacuum filtered and washed with cold ethanol. The product obtained was then oven dried and purification was achieved by recrystallization using admixtures of ethanol and water. The filtrate was kept in the refrigerator for a few days and the crystalline products harvested by vacuum filtration. The final product **6g–6z** was dried in a vacuum oven at 50°C for at least 24 h before further use [29]. The further reactions of 5-substituted-1,3,5-hexahydrotriazine **6g–6z** with 2-chloro-5-chloromethyl pridine or 2-chloro-5-chloromethyl thiazole were adopted for the synthesis of **7a–7f** to give **7g–7z** in 50–70% yields.

***1-((6-Chloropyridin-3-yl)methyl)-5-benzyl-1,3,5-hexahydrotriazine-2-(N-nitro)imine(7g).*** Yield: 59.4%; m.p.: 110–112°C; IR (KBr disc): 3297, 1590, 1553, 1125, 1104 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 3.74 (d, $J$ = 9.6 Hz, 2H, $CH_2$—Ph), 4.25 (s, 2H, triazine), 4.43 (s, 2H, triazine), 4.58 (s, 2H, $CH_2$-Pyri), 7.09 (m, 2H, Ph—H), 7.10–7.32 (m, 3H, Ph—H), 7.36 (d, $J$ = 8.0 Hz, 1H, Pyri-H), 7.81–7.84 (dd, $J_1$ = $J_2$ = 2.4 Hz, 1H, Pyri-H), 8.25 (d, $J$ = 2.4 Hz, 1H, Pyri-H), 9.66 (s,1H, N—H) ppm; $[M+H]^+$: 361.1; Anal. Calcd. for $C_{16}H_{17}ClN_6O_2$: C, 53.26; H, 4.75; N, 23.29.; Found C, 53.23; H, 4.83; N, 23.34.

***1-((2-Chlorothiazol-5-yl)methyl)-5-benzyl-1,3,5-hexahydrotriazine-2-(N-nitro)imine(7h).*** Yield: 62.7%; m.p.: 165–166°C; IR (KBr disc): 3311, 1590, 1553, 1125, 1104 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 3.90 (s, 2H, $CH_2$Ph), 4.32 (s, 2H, triazine), 4.44 (s, 2H, triazine), 4.58 (s, 2H, $CH_2$-thiazole), 7.10 (q, $J$ = 2.8 Hz, 2H, Ph—H), 7.28 (t, $J$ = 5.6 Hz, 3H, Ph—H), 7.59 (s, 1H, thiazole-H), 9.45 (s, 1H, N—H) ppm; $[M+H]^+$: 367.2; Anal. Calcd. for $C_{14}H_{15}ClN_6O_2S$: C, 45.84; H, 4.12; N, 22.91.; Found C, 45.90; H, 4.01; N, 22.94.

***1-((6-Chloropyridin-3-yl)methyl)-5-(4-methylbenzyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7i).*** Yield: 61.8%; m.p.: 115–117°C; IR (KBr disc): 3286, 1586, 1249, 1189, 1110 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 2.35 (s, 3H, $CH_3$), 3.70 (s, 2H, $CH_2$—Ph), 4.24 (s, 2H, triazine), 4.43 (s, 2H, triazine), 4.58 (s, 2H, $CH_2$-Pyri), 6.97 (d, $J$ = 8.0 Hz, 2H, Ph—H), 7.12 (d, $J$ = 8.0 Hz, 2H, Ph—H), 7.36 (d, $J$ = 8.0 Hz, 1H, Pyri-H), 7.82–7.85 (dd, $J_1$ = 2.4 Hz, $J_2$ = 2.8, 1H, Pyri-H), 8.26 (d, $J$ = 2.4 Hz, 1H, Pyri-H), 9.65 (s, 1H, N—H) ppm; $[M+H]^+$: 375.5; Anal. Calcd. for $C_{17}H_{19}ClN_6O_2$: C, 54.47; H, 5.11; N, 22.42.; Found C, 54.39; H, 5.23; N, 22.48.

***1-((2-Chlorothiazol-5-yl)methyl)-5-(4-methylbenzyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine(7j).*** Yield: 61.5%; m.p.: 154–155°C; IR (KBr disc): 3289, 1587, 1543, 1165, 1103 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 2.36 (s, 3H, $CH_3$—Ph), 3.68 (s, 2H, $CH_2$—Ph), 4.30 (s, 2H, triazine),4.43 (s, 2H, triazine), 4.57 (s, 2H, $CH_2$-thiazole), 6.99 (d, $J$ = 8.0 Hz, 2H, Ph—H), 7.14 (d, $J$ = 8.0 Hz, 3H, Ph—H), 7.35 (s, 1H, thiazole-H), 9.52 (s, 1H, N—H) ppm; $[M+H]^+$: 381.2; Anal. Calcd. for $C_{15}H_{17}ClN_6O_2S$: C, 47.30; H, 4.50; N, 22.07.; Found C, 47.17; H, 4.45; N, 22.16.

***1-((6-Chloropyridin-3-yl)methyl)-5-(4-methoxybenzyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7k).*** Yield: 60.3%; m.p.: 113–115°C; IR (KBr disc): 3297, 15911534, 1152, 1100 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 3.82 (s, 3H, $CH_3$), 3.68 (s, 2H, $CH_2$—Ph), 4.23 (s, 2H, triazine), 4.43 (s, 2H, triazine), 4.57 (s, 2H, $CH_2$-Pyri), 6.84 (d, $J$ = 8.4 Hz, 1H, Ph—H), 6.90 (d, $J$ = 8.4 Hz, 1H, Ph—H), 7.36 (d, $J$ = 8.0 Hz, 1H, Pyri-H), 7.81–7.83 (dd, $J_1$ = $J_2$ = 2.4 Hz, Pyri-H), 8.26 (d, $J$ = 2.4 Hz, 1H, Pyri-H), 9.63 (s, 1H, N—H) ppm; $[M+H]^+$: 391.1; Anal. Calcd. for $C_{17}H_{19}ClN_6O_3$: C, 52.24; H, 4.90; N, 21.50.; Found C, 52.15; H, 4.99; N, 21.37.

***1-((2-Chlorothiazol-5-yl)methyl)-5-(4-methoxybenzyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7l).*** Yield: 63.6%; m.p.: 168–170°C; IR (KBr disc): 3278, 1634, 1590, 1152, 1102 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 3.66 (s, 2H, $CH_2$—Ph), 3.83 (s, 3H, $CH_3$-O), 4.30 (s, 2H, triazine),4.42 (t, $J$ = 0.8 Hz 2H, triazine), 4.57 (s, 2H, $CH_2$-thiazole), 6.86 (d, $J$ = 6.0 Hz 2H, Ph—H), 7.02 (d, $J$ = 4.0 Hz, 3H, Ph—H), 7.36 (s, 1H, thiazole-H),9.52 (s,1H, N—H)ppm; $[M+H]^+$: 397.1; Anal. Calcd. for $C_{15}H_{17}ClN_6O_3S$: C, 45.40; H, 4.32; N, 21.18.; Found C, 45.38; H, 4.30; N, 21.22.

***(±)-1-((6-Chloropyridin-3-yl)methyl)-5-(1-phenylethyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine(7m).*** Yield: 55.3%; m.p.: 165∼166°C; IR (KBr disc): 3278, 1586, 1531, 1152, 1107 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 2.11–2.13 (m, 3H, $CH_3$), 3.64 (t, $J$ = 4 Hz, $CH_2$—Ph), 4.31 (s, 2H, triazine), 4.42 (s, 2H, triazine), 4.57 (s, 2H, $CH_2$-thiazole), 7.22–7.35 (m, 5H, Ph—H), 7.36 (d, $J$ = 8.0 Hz, 1H, Pyri-H), 7.84 (dd, $J_1$ = $J_2$ = 2.4 Hz, 1H, Pyri-H), 8.25 (d, $J$ = 2.4 Hz, 1H, Pyri-H),, 9.52 (s, 1H, N—H) ppm; $[M+H]^+$: 375.7; Anal. Calcd. for $C_{17}H_19ClN_6O_2$: C, 54.47; H, 5.11; N, 22.42; Found C, 54.28; H, 5.17; N, 22.38.

***(±)-1-((2-Chlorothiazol-5-yl)methyl)-5-(1-phenylethyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7n).*** Yield: 56.7%; m.p.: 157∼158°C; IR (KBr disc): 3301, 1590, 1553, 1125, 1104 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 2.13–2.16 (m, 3H, $CH_3$), 3.68 (d, $J$ = 4.2 Hz, CH—Ph), 4.37 (s, 2H, triazine), 4.45 (s, 2H, triazine), 4.57 (s, 2H, $CH_2$-thiazole), 7.21–7.34 (m, 5H, Ph—H), 7.37 (s,1H, thiazole-H), 9.56 (s, 1H, N—H) ppm; $[M+H]^+$:

381.1; Anal. Calcd. for $C_{15}H_{17}ClN_6O_2S$: C, 47.30; H, 4.50; N, 22.07.; Found C, 47.47; H, 4.43; N, 22.10.

***(R)-1-((6-Chloropyridin-3-yl)methyl)-5-(1-phenylethyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7o).*** Yield: 57.6%; m.p.: 165∼166°C; IR (KBr disc): 3289, 1587, 1534, 1152, 1100 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 1.39–1.41 (m, 3H, $CH_3$), 3.64 (dd, J1 = J2 = 6.4 Hz, $CH_2$—Ph), 4.31 (s, 2H, triazine), 4.42 (s, 2H, triazine), 4.57 (s, 2H, $CH_2$-thiazole), 7.24–7.35 (m, 5H, Ph—H), 7.37 (d, J = 8.0 Hz, 1H, Pyri-H), 7.82–7.85 (m, 1H, Pyri-H), 8.33 (d, J = 2.4 Hz, 1H, Pyri-H), 9.61 (s, 1H, N—H) ppm; $[M+H]^+$: 375.7; Anal. Calcd. for $C_{17}H_{19}ClN_6O_2$: C, 54.47; H, 5.11; N, 22.42; Found C, 54.33; H, 5.18; N, 22.48; $[\acute{A}]_D^{25}$ = +23.38° (C = 0.01 g/mL, $CH_3CH_2OH$).

***(R)-1-((2-Chlorothiazol-5-yl)methyl)-5-(1-phenylethyl)-1,3,5-hexahydro-triazine-2-(N-nitro) imine(7p).*** Yield: 62.3%; m.p.: 145∼146°C; IR (KBr disc): 3291, 1584, 1541, 1148, 1105 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 1.42–1.44 (m, 3H, $CH_3$), 3.63 (dd, $J_1 = J_2$ = 6.4 Hz, $CH_2$—Ph), 4.34 (s, 2H, triazine), 4.42 (s, 2H, triazine), 4.56 (s, 2H, $CH_2$-thiazole), 7.28–7.36 (m, 5H, Ph—H), 7.38 (d, J = 8.0 Hz, 1H, Pyri-H), 7.81–7.83 (m, 1H, Pyri-H), 8.34 (d, J = 2.4 Hz, 1H, Pyri-H), 9.63 (s, 1H, N—H) ppm; $[M+H]^+$: 381.1; Anal. Calcd. for $C_{15}H_{17}ClN_6O_2S$: C, 47.30; H, 4.50; N, 22.07.; Found C, 47.37; H, 4.38; N, 22.11; $[\acute{A}]_D^{25}$ = +27.44° (C = 0.01 g/mL, $CH_3CH_2OH$).

***1-((6-Chloropyridin-3-yl)methyl)-5-phenethyl-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7q).*** Yield: 54.3%; m.p.: 147–149°C; IR (KBr disc): 3311, 1590, 1553, 1125, 1104 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 2.66–2.71 (m, 2H, $CH_2$—Ph), 2.73–2.90 (m, N-2H-Bz, 4.24 (s, 2H, triazine), 4.44 (s, 2H, triazine), 4.57 (s, 2H, $CH_2$-Pyri), 7.05 (d, J = 7.2 Hz, 1H, Ph—H), 7.22–7.36 (m, 1H, Ph—H), 7.83 (d, J = 8.0 Hz, 1H, Pyri-H), 8.27–8.31 (dd, $J_1 = J_2$ = 2.4 Hz, Pyri-H), 8.74 (d, J = 2.4 Hz, 1H, Pyri-H), 9.65 (s, 1H, N—H) ppm; $[M+H]^+$: 375.8; Anal. Calcd. for $C_{17}H_{19}ClN_6O_2$: C, 54.47; H, 5.11; N, 22.42.; Found C, 54.32; H, 5.29; N, 22.37.

***1-((2-Chlorothiazol-5-yl)methyl)-5-phenethyl-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7r).*** Yield: 57.1%; m.p.: 159–160°C; IR (KBr disc): 3260, 1586, 1531, 1152, 1107 cm-1; $^1$H-NMR (DMSO-$d_6$), 2.74–2.75 (m,2H, $CH_2$—Ph), 2.75–2.76 (m, N-2H-Bz), 4.58 (m, 2H, $CH_2$-thiazole), 4.38 (s, 2H, triazine), 4.41 (s, 2H, triazine),7.06–7.11 (d, 1H, Ph—H,), 7.20–7.35 (m, 4H, Ph—H), 7.43 (s,1H, thiazole-H), 9.52 (s, 1H, N—H) ppm; $[M+H]^+$: 381.1; Anal. Calcd. for $C_{15}H_{17}ClN_6O_2S$: C, 47.30; H, 4.50; N, 22.07.; Found C, 47.17; H, 4.58; N, 22.04.

***1-((6-Chloropyridin-3-yl)methyl)-5-(3-pyridylmethyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7s).*** Yield: 51.7%; m.p.: 132–134°C; IR (KBr disc): 3288, 1593, 1338, 1175, 1103 $cm^{-1}$; $^1$H NMR (DMSO-$d_6$), 3.89 (s, 2H, triazine), 4.35 (s, 2H, triazine), 4.50 (d, J = 1.2 Hz, 2H, $CH_2$-Pyri), 4.63 (d, 2H, $CH_2$-Pyri), 7.12 (d, J = 7.6 Hz, 1H, Pyri-H), 7.23–7.27 (m, 1H, Pyri-H), 7.36 (d, J = 8.0 Hz, 1H, Pyri-H), 7.65–7.67 (m, 1H, Pyri-H), 7.49 (d, J = 8.0 Hz, 1H, Pyri-H), 8.13–8.15 (m, 1H, Pyri-H), 8.69 (d, J = 2.4 Hz, 1H, Pyri-H), 9.87 (s, 1H, N—H) ppm; $[M+H]^+$: 362.1; Anal. Calcd. for $C_{15}H_{16}ClN_7O_2$: C, 49.80; H, 4.46; N, 27.10.; Found C, 49.93; H, 4.51; N, 27.22.

***1-((2-Chlorothiazol-5-yl)methyl)-5-(3-pyridylmethyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7t).*** Yield: 58.3%; m.p.: 188–189°C; IR (KBr disc): 3445, 1537, 1399, 1107, 1047 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 3.89 (s, 2H, pyridine-$CH_2$), 4.42 (s, 2H, triazine-2H), 4.50 (s, 2H, triazine-H), 4.63 (s, 2H, $CH_2$-thiazole), 7.11–7.13 (d, J = 8 Hz, 1H, pyri-H), 7.36 (s, 1H, thiazole-H), 7.69–7.72 (d, 1H, J = 8 Hz, pyri-H), 7.61–7.60 (d, 1H, J = 4.4 Hz, pyri-H), 9.59 (s, 1H, N—H) ppm; $[M+H]^+$: 368.1; Anal. Calcd. for $C_{13}H_{14}ClN_7O_2S$: C, 42.45; H, 3.84; N, 26.66.; Found C, 42.49; H, 3.87; N, 24.61.

***1-((6-Chloropyridin-3-yl)methyl)-5-((5-chlorothiazol-3-yl)methyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine(7u).*** Yield: 63.6%; m.p.: 168–170°C; IR (KBr disc): 3278, 1634, 1590, 1152, 1102 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 4.30 (s, 2H, triazine),4.42 (t, J = 0.8 Hz 2H, triazine), 4.57 (s, 2H, $CH_2$-Pyri), 4.61 (s, 2H, $CH_2$-thiazole), 7.36 (s,1H, thiazole-H), 7.45 (d, J = 3.6 Hz, 1H, Pyri-H), 7.75–7.77 (dd, $J_1$ = 2.4 Hz, $J_2$ = 2.8, 1H, Pyri-H), 8.30 (d, J = 2.4 Hz, 1H, Pyri-H), 9.52 (s, 1H, N—H) ppm; $[M+H]^+$: 403.1; Anal. Calcd. for $C_{13}H_{13}Cl_2N_7O_2S$: C, 38.82; H, 3.26; N, 24.37.; Found C, 38.71; H, 3.35; N, 24.22.

***1-((2-Chlorothiazol-5-yl)methyl)-5-((2-chlorothiazol-5-yl)methyl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7v).*** Yield: 57.9%; m.p.: 153–155°C; IR (KBr disc): 3283, 1655, 1593, 1158, 1107 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 4.22 (d, J = 3.2 Hz 2H, triazine), 4.60 (d, J = 1.2 Hz 2H, triazine), 4.55 (s, 2H, $CH_2$-thiazole), 4.62 (s, 2H, $CH_2$-thiazole), 7.38 (s, 1H, thiazole-H), 7.42 (s, 1H, thiazole-H), 9.45 (s, 1H, N—H) ppm; $[M+H]^+$: 409.3; Anal. Calcd. for $C_{11}H_{11}Cl_2N_7O_2S_2$: C, 32.36; H, 2.72; N, 24.01.; Found C, 32.28; H, 2.83; N, 24.17.

***1-((6-Chloropyridin-3-yl)methyl)-5-(2-furfuryl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7w).*** Yield: 54.3%; m.p.: 147–149°C; IR (KBr disc): 3289, 1587, 1543, 1165, 1103 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 3.61 (s, 2H, $CH_2$-furan), 4.30 (s, 2H, triazine),4.43 (s, 2H, triazine), 4.63 (s, 2H, $CH_2$-Pyri), 6.22 (d, J = 3.2 Hz, 1H, furan-H), 6.33–6.35 (m, 1H, furan-H), 7.40–7.41 (dd, $J_1 = J_2$ = 0.8 Hz, 1H, furan-H), 7.36 (d, J = 8.0 Hz, 1H, Pyri-H), 7.81–7.84 (m, 1H, Pyri-H), 8.25 (d, J = 2.4 Hz, 1H, Pyri-H), 9.68 (s, 1H, N—H) ppm; $[M+H]^+$: 351.9; Anal. Calcd. for $C_{14}H_{15}ClN_6O_3$: C, 47.94; H, 4.31; N, 23.96.; Found C, 47.82; H, 4.37; N, 23.84.

***1-((2-Chlorothiazol-5-yl)methyl)-5-(2-furfuryl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7x).*** Yield: 62.5%; m.p.: 154–155°C; IR (KBr disc): 3289, 1592, 1548, 1165, 1101 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 3.74 (s, 2H, $CH_2$-furan), 4.36 (s, 2H, triazine),4.45 (d, J = 0.8 Hz, 2H, triazine), 4.62 (s, 2H, thiazole-$CH_2$), 6.07 (d, J = 2.8 Hz, 1H, furan-H), 6.34–6.35 (m, 1H, furan-H), 7.40–7.41 (dd, $J_1 = J_2$ = 1.2 Hz, 1H, furan-H), 7.43 (1H,s,thiazole-H), 9.55 (s, 1H, N—H) ppm; $[M+H]^+$: 357.1; Anal. Calcd. for $C_{12}H_{13}ClN_6O_3S$: C 40.40, H 3.67, N 23.55; found C 40.39, H 3.72, N 23.52.

***(±)-1-((6-Chloropyridin-3-yl)methyl)-5-(2-tetrahydrofurfuryl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine (7y).*** Yield: 59.4%; m.p.: 134–135°C; IR (KBr disc): 3260, 1586, 1531, 1152, 1107 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 1.40–1.44 (m, 1H, H-THF), 1.80–1.88 (m, 3H, H-THF), 2.53–2.56 (m,2H, H-THF), 3.85–3.92 (m, 1H, H-THF), 4.36–4.40 (t, J = 12.4 Hz, 2H, triazine), 4.48–4.54 (t, J = 12.4 Hz, 2H, triazine), 4.61 (s, 2H, $CH_2$-Pyri), 7.83 (d, J = 8.0 Hz, 1H, Pyri-H), 8.27–8.31 (dd, $J_1 = J_2$ = 2.4 Hz, Pyri-H), 8.74 (d, J = 2.4 Hz, 1H, Pyri-H), 9.53 (s, 1H, N—H) ppm; $[M+H]^+$: 356.1; Anal. Calcd. for $C_{14}H_{19}ClN_6O_3$: C 47.39, H 5.40, N 23.69; found C 47.28, H 5.37, N 23.52.

***(±)-1-((2-Chlorothiazol-5-yl)methyl)-5-(2-tetrahydrofurfuryl)-1,3,5-hexahydrotriazine-2-(N-nitro) imine(7z).*** Yield: 63.3%; m.p.: 127–128°C; IR (KBr disc): 3260, 1586, 1531, 1152, 1107 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$), 1.38–1.48 (m, 1H, H-THF), 1.81–1.99 (m, 3H, H-THF), 2.55–2.66 (m,2H, H-THF), 3.94–3.99 (m, 1H, H-THF), 4.38–4.43 (t, J = 12.4 Hz, 2H, triazine), 4.53–4.61 (t, J = 11.2 Hz, 2H, triazine), 4.63 (s, 2H, thiazole-

$CH_2$), 7.46 (s, 1H, thiazole-H), 9.50 (s, 1H, N—H) ppm; $[M+H]^+$: 361.8; Anal. Calcd. for $C_{12}H_{17}ClN_6O_3S$: C 39.94, H 4.75, N 23.29; found C 39.81; H 4.88, N 23.32.

***Biology assay.*** The insecticidal activities of compounds **7a–7z** and IMI were screened against Pseudaletia separate Walker using the standard testing method [30]. Test solutions of each compound were prepared in DMF and serially diluted with water containing Triton X-80 (0.1 mg $L^{-1}$) to get the required concentrations. The insects were reared at 25 (±2)°C, 60 (±5)% relative humidity, and groups of 12 were transferred to glass Petri dishes and sprayed with the aforementioned solutions using a Potter sprayer. The control experiment was carried out under the same conditions, with 1 mL of DMF at a concentration of 1.0 g $L^{-1}$ applying on each insect. Assessments were made after 72 h by the number and size of live insects relative to that in the negative control, and evaluations of mortality rate are based on a percentage scale of 0∼100, in which 0 no activity and 100 total kill.

***Experimental protocol of docking study.*** To understand the binding mode of action of our newly synthesized compounds, we performed a docking study using AutoDock 4.0 [31]. All potent inhibitors were chosen to get insight into the binding preferences in detail. As the amino acids forming the active pockets are both structurally and functionally consistent both in the diverse nAChRs and AchBPs, the *lymnaea stagnalis* AchBP was used to construct the docking model, and the simulations were carried out based on its X-ray structure cocrystallized with bound IMI (protein data bank code: 2zju) (Fig. 2) [32]. As it is a pentameric macromolecule composed of five identical subunits, the subunits A and B of the homopentameric *ls*-AChBP, which are adjacent, were used as the template for modeling. The macromolecule was prepared for docking by addition of hydrogen atoms and removal of cocrystallized molecules, and iteratively minimized and subjected to a side chain conformational search. The active sites were assigned at a radius of 10 Å around the original binding substrate. All ligands were flexibly docked and subjected to cluster analysis using a root mean square deviation 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 analysis.

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