# AChR Inhibiting Properties of 1,2,3,4-tetrahydropyrimidines: Synthesis and In-silico Studies

**AS Devi[1], RR Baruah[3], MA Laskar[3], JN Vishwakarma*[1, 2]**

[1]Organic Research Lab, Department of Chemistry, St. Anthony's College, Shillong 793 001.
[2]Department of Chemical Sciences, Don Bosco University, Azara, Guwahati 781 017.
[3]Department of Biotechnology, St. Anthony's College, Shillong 793 001, India.

*Correspondence to: jnvishwakarma@rediffmail.com

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**Abstract**

Novel tetrahydropyrimidines (THP-1 to THP-7) have been synthesized by the reaction of enaminones 1a-d, primary amine and formaldehyde in very good yields and their structures have been established with the help of spectral and analytical data. These tetrahydropyrimidines along with THP-8 to THP-15 previously reported by us were subjected to docking by using fast and flexible method to study their Acetylcholine Receptor (AChR) inhibiting properties. A knowledge-based approach has been followed for assigning proper parameters into the binding site of the protein. Our studies on these THPs reveal that THP-4 possesses best binding conformation within the binding site of the target protein with the energy value of -23.66 KJ/mol.

**Keywords:** Tetrahydropyrimidine; enaminones; acetylcholinesterase receptor; docking; knowledge-based approach.

## 1. Introduction

Keeping in view the pesticidal properties of 5-nitro-1,2,3,4-tetrahydropyrimidine derivatives [1-8], we recently reported the synthesis of novel 5-aroyl-6-methylthio-1,2,3,4-tetrahydropyrimidine analogues [9], the biological properties of which remain unexplored. In a recent report by Chuanwen Sun *et al.* insecticidal properties and molecular docking of some of the analogues of 5-nitrotetrahydropyrimidines have been studied [10] and the analogue (Figure 1) has been found to be most active candidate.

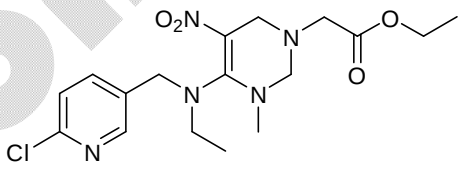



**Figure 1:** Ethyl 2-(4-(((6-chloropyridin-3-yl)methyl)(ethyl)amino)-3-methyl-5-nitro-2,3-dihydropyrimidin-1(6H)-yl)acetate.

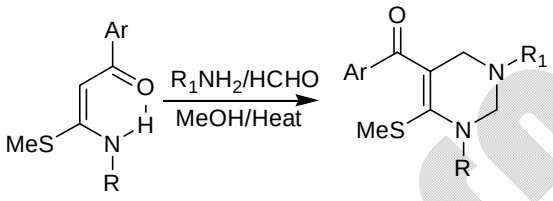
Prompted by these reports, we have now decided to extend our synthetic strategy [9], to prepare a library of novel tetrahydropyrimidines (THPs) in order to see their efficiency for pesticide activities. Recently, docking studies have proved to be very useful in sorting out the most potent derivatives from a library of compounds [11]. Thus, we intend to subject the library of molecules presented herein to docking and identify the most potent derivative. Nicotinic acetylcholine receptors (nAChRs) are most widely used targeted proteins for insecticidal activities by different classes of THPs because of their cholinergic neurotransmitter function in vertebrates and invertebrates [12]. The binding affinity in vertebrates has been found to be much lower [13] because of which further synthetic studies on this class of compounds deserves attention. The binding site of the pentameric protein shows structural and functional similarity with Acetylcholine Binding Protein (AChBP) within different classes of insects [10, 12]. In AChBP, the active site is located at the interface between two subunits. The ligand binding domain of the protein is found to be mostly β-structured with a series of loops from the adjacent subunit [14].

Because of the similarity in the ligand binding site, AChBP from *Lymnaea stagnalis* (PDB ID: 2ZJU) [10] was selected as the target protein to perform our in-silico studies. A knowledge-based approach has been followed for generating the proper binding mode into the active site of the protein with ligand-flexible docking as the scoring function, the results of which are reported herein.

## 2. Methods

### 2.1. Synthesis

Synthesis of the tetrahydropyrimidines (**THP1-7**) was achieved by the reaction of **1a-d** with primary amine and formaldehyde as shown in **Scheme-1** following our previously reported procedure. The structures of the products were established with help of spectral and analytical data. Thus, IR spectra of the THPs showed carbonyl band close to 1600 $cm^{-1}$ due to intensive delocalization of the enaminone skeleton. The absence of N-H and vinylic proton signals of the starting materials and presence of singlets for N-$CH_2$-N and N-$CH_2$-C in $^1$H NMR spectra of the compounds confirmed the formation of the cyclic product. The aromatic protons resonated in the usual range of 6.80-7.95 ppm. The mass spectra of the products provided additional support for the confirmation of the proposed structures.




**1a-g** **THP1-7**

**Scheme 1:** Synthesis of 1,3-disubstituted-5-aroyl-6-methylthio-1,2,3,4-tetrahydropyrimidines.

| 1 | Ar | R |
|---|---|---|
| **a** | $C_6H_5$ | $C_6H_5CH_2$ |
| **b** | $C_6H_5$ | $C_6H_5$ |
| **c** | $C_6H_5$ | Me |
| **d** | $C_6H_5$ | Et |

| THP | Ar | R | $R_1$ |
|---|---|---|---|
| **THP-1** | $C_6H_5$ | $C_6H_5CH_2$ | $C_6H_5CH_2$ |
| **THP-2** | $C_6H_5$ | $C_6H_5CH_2$ | $C_6H_5$ |
| **THP-3** | $C_6H_5$ | $C_6H_5CH_2$ | 4-$ClC_6H_4$ |
| **THP-4** | $C_6H_5$ | $C_6H_5$ | $C_6H_5CH_2$ |
| **THP-5** | $C_6H_5$ | $C_6H_5$ | $C_6H_5$ |
| **THP-6** | $C_6H_5$ | Me | $C_6H_5CH_2$ |
| **THP-7** | $C_6H_5$ | Et | $C_6H_5CH_2$ |
| **THP-8** | $C_6H_5$ | $C_6H_5CH_2$ | 4-$MeOC_6H_4$ |
| **THP-9** | 4-$MeC_6H_4$ | $C_6H_5CH_2$ | $C_6H_5CH_2$ |
| **THP-10** | 4-$MeOC_6H_4$ | $C_6H_5$ | $C_6H_5CH_2$ |
| **THP-11** | 4-$ClC_6H_4$ | $C_6H_5$ | 4-$ClC_6H_4$ |
| **THP-12** | 4-$ClC_6H_4$ | $C_6H_5$ | 4-$MeC_6H_4$ |
| **THP-13** | 4-$ClC_6H_4$ | $C_6H_5$ | 4-$MeOC_6H_4$ |
| **THP-14** | 4-$ClC_6H_4$ | $C_6H_5$ | Et |
| **THP-15** | 4-$ClC_6H_4$ | $C_6H_5$ | Me |

#### 2.1.1. Experimental

Melting points were determined on Thomas Hoover apparatus and are uncorrected. The reaction progress was monitored by TLC on silica gel. The infrared spectra of the products were recorded on Perkin Elmer 983 spectrophotometer and $^1$H NMR spectra on Varian EM-390 spectrometer using TMS as internal standard. Mass spectra were recorded on JEOL D-300 mass spectrometer. The starting materials **1a-d** were prepared by our previously reported procedures [15].

**1-alkyl/aralkyl/aryl-3-alalky/aryl-5-benzoyl-6-methylthio-1,2,3,4-tetrahydro-pyrimidines (THP-1 to THP-7). General Procedure.**

A mixture of primary amine (1 mmol) and formaldehyde (2 mmol, 40% solution) in 2 ml methanol was stirred at room temperature for 5 minutes. To this was added N,S-acetal **1** (1 mmol) in 4-5 ml methanol and the

resulting mixture was stirred at room temperature. A precipitate was observed after 5-10 minutes. After the completion of the reaction (3-6 hours, monitored by TLC), the reaction mixture was cooled in ice-water and the precipitate was collected by filtration, washed with cold methanol and dried to give analytically pure **THP-1** to **THP-7** in 72-87% overall yields. These products were further purified by recrystallization from methanol.

**1,3-Dibenzyl-5-benzoyl-6-methylthio-1,2,3,4-tetrhydropyrimidine (THP-1)** was obtained as colourless crystalline solid; m.p.105-106$^0$ C; reaction time 3 hrs; yield, 87%; IR (KBr): 1630, 1580 cm$^{-1}$; PMR ($CDCl_3$): 2.00 (s, 3H), 3.48 (s, 2H), 3.55 (s, 2H), 3.60 (s, 2H), 4.40 (s, 2H), 6.80-7.49 (m, 13H), 7.56-7.90 (m, 2H); MS: m/z 399 ($M^+$-15, 17%), 248 (11%).

*Anal.* Calcd. for $C_{26}H_{26}N_2OS$: C, 75.33; H, 6.32; N, 6.75; found: C, 75.49; H, 6.17; N, 6.49%..

**1-Benzyl-3-phenyl-5-benzoyl-6-methylthio-1,2,3,4-tetrhydropyrimidine (THP-2)** was obtained as colourless crystalline solid; m.p.96-97$^0$ C; reaction time 5 hrs; yield, 80%; IR (KBr): 1582, 1550; PMR ($CDCl_3$): 2.35 (s, 3H), 4.45 (s, 2H), 4.52 (s, 2H), 5.51 (s, 2H), 7.10-7.42 (m, 13H), 7.50-7.82 (m, 2H).

*Anal.* Calcd. for $C_{25}H_{24}N_2OS$: C, 74.96; H, 6.04; N, 6.99; found: C, 75.49; H, 6.17; N, 6.49%..

**1-Benzyl-3-(4-chlorophenyl)-5-benzoyl-6-methylthio-1,2,3,4-tetrahydropyrimidine (THP-3)** was obtained as colourless crystalline solid; m.p.129-130$^0$ C; reaction time 6 hrs; yield, 86%; IR (KBr): 1580, 1540 cm$^{-1}$; PMR ($CDCl_3$): 2.40 (s, 3H), 4.45 (s, 2H), 4.50 (s, 2H), 5.52 (s, 2H), 7.08-7.33 (m, 12H), 7.65-7.80 (m, 2H).

*Anal.* Calcd. for $C_{25}H_{23}ClN_2OS$: C, 69.03; H, 5.32; N, 6.44; found: C, 69.29; H, 5.07; N, 6.65%.

**1-Phenyl-3-benzyl-5-benzoyl-6-methylthio-1,2,3,4-tetrahydropyrimidine (THP-4)** was obtained as colourless crystalline solid; m.p.155-156$^0$ C; reaction time 4 hrs; yield, 85%; IR (KBr): 1620, 1550 cm$^{-1}$; PMR ($CDCl_3$): 2.65 (s, 3H), 3.65 (s, 2H), 3.71 (s, 2H), 4.31 (s, 2H), 6.85-7.58 (m, 13H), 7.60-7.95 (m, 2H). MS: m/z 385 ($M^+$-15, 17%), 281 (1%), 234 (98%).

*Anal.* Calcd. for $C_{25}H_{24}N_2OS$: C, 74.96; H, 6.03; N, 6.99; found: C, 74.72; H, 6.29; N, 7.27%.

**1,3-Diphenyl-5-benzoyl-6-methylthio-1,2,3,4-tetrahydropyrimidine (THP-5)** was obtained as colourless crystalline solid; m.p. 166-167$^0$ C; reaction time 3 hrs; yield, 72%; IR (KBr): 1620, 1550 cm$^{-1}$; PMR ($CDCl_3$): 1.70 (s, 3H), 4.14 (s, 2H), 4.55 (s, 2H), 6.34-7.46 (m, 13H), 7.50-7.90 (m, 2H). MS: m/z 371 ($M^+$-15, 37%), 234 (98%).

*Anal.* Calcd. for $C_{24}H_{22}N_2OS$: C, 74.50; H, 5.73; N, 7.24; found: C, 74.27; H, 5.49; N, 7.51%.

**1-Methyl-3-benzyl-5-benzoyl-6-methylthio-1,2,3,4-tetrahydropyrimidine (THP-6)** was obtained as colourless crystalline solid; m.p.129-130$^0$ C; reaction time 5 hrs; yield, 79%; IR (KBr): 1605, 1590, 1550 cm$^{-1}$; PMR ($CDCl_3$): 1.91 (s, 3H), 2.89 (s, 3H), 3.45 (s, 2H), 3.68 (two s, 4H), 7.05-7.48 (m, 8H), 7.50-7.85 (m, 2H). MS: m/z 338 ($M^+$, 2%), 323 ($M^+$-15, 79%), 172 (100%).

*Anal.* Calcd. for $C_{20}H_{22}N_2OS$: C, 70.98; H, 6.55; N, 8.28; found: C, 71.23; H, 6.41; N, 8.01%.

**1-Ethyl-3-benzyl-5-benzoyl-6-methylthio-1,2,3,4-tetrahydropyrimidine (THP-7)** was obtained as colourless crystalline solid; m.p.135-136$^0$ C; reaction time 4 hrs; yield, 75%; IR (KBr): 1600, 1592, 1530 cm$^{-1}$; PMR ($CDCl_3$): 1.04 (3H, J=7 Hz), 1.91 (s, 3H), 3.31 (q, 2H, J=7 Hz), 3.42 (s, 2H), 3.61 (s, 2H), 3.70 (s, 2H), 6.92-7.41 (m, 8H), 7.45-7.75 (m, 2H). MS: m/z 337 ($M^+$-15, 34%), 186 (52%).

*Anal.* Calcd. for $C_{21}H_{24}N_2OS$: C, 71.56; H, 6.86; N, 7.59; found: C, 71.72; H, 7.11; N, 7.68%.

**2.2. In-silico evaluation**

Correctly assigned atom and bond type information is an absolute prerequisite for proper protein-ligand interaction. FlexX provides all those facilities to predict probable interaction patterns in a natural conformation. Fast and flexible method used by FlexX mainly relies on an incremental construction algorithm and program MIMUMBA used in the software generates conformation based on empirical observation [16].

Target protein AChR (PDB ID 2ZJU) was loaded from Protein Data Bank (http://www.rcsb.org/) and receptor site was defined using co-crystallized reference ligand information (Imidacloprid). Structural comparison between the reference and test ligands was done manually to ensure accuracy of receptor definition.

**2.2.1. Pharmacophore definition**

A knowledge-based approach has been followed for providing proper interaction type into the active site of the protein. PDB Ligand Explorer used during the study, provides useful information for generating hydrogen bonds and hydrophobic interactions between the best docked conformational pose of the ligand and the amino acid residues of the target protein.

**2.2.2. Ligand preparation**

The cdx files generated by ChemDraw Ultra 8.0 were converted to mol-MDL MOL/SDF file and energy minimization was done using PRODRG2 server [17]. The output file was converted to mol2 format by the cheminformatics tool OpenBabel ver. 2.2.3 (http://openbabel.sourceforge.net/) and explicit hydrogens were added. These mol2 files were loaded onto FlexX and docking was performed. The efficiency of binding conformations was judged based on the FlexX score (in KJ/mol) and the binding geometries generated by the module PoseView.

**3. Results and Discussion**

Out of the fifteen THPs under investigation, THP-4 (ΔG= -23.66 KJ/mol) has been found to be accommodated most comfortably into the binding pocket of the protein (Figure 2) through hydrophobic interactions involving amino acid residues Trp-143, Tyr-185, Tyr-164, Trp-53, Leu-112, Arg-104 and tyr-192 showing almost all interactions to hold the molecule tightly into the binding pocket as reported for 5-nitrotetrahydropyrimidine analogues [10]. The carbonyl group of **THP-4** is found strongly hydrogen bonded with Tyr-192 (1.51Å) whereas this hydrogen bonding was absent in **THP-12** probably because of the changed orientation of the ligand arising due to para substituted Cl present in it. However, nitrogen at position 3 of **THP-12** ring is hydrogen bonded with Tyr-192 with a bond length of 1.93 Å. The longer and hence weaker bonding is reflected by its higher binding energy (ΔG= -23.39 KJ/mol). Our analyses of the docking results on the remaining THPs reveal that these were not as comfortably accommodated in the bonding pocket of AChR as were **THP-4** and **THP-12** and binding energies were found to be higher accordingly. This could probably be due to their unsuitable molecular orientations.

It is interesting to mention here that Chuanwen Sun *et al.* (2010) have reported hydrogen bond length of oxygen atom of nitro group with tyr192 as 2.85Å in case of the most active nitro-THP (Figure 1), whereas in the present study it has been found to be 1.51 Å in **THP-4** (Figure 3) when the nitro group was replaced by benzoyl group in the most comfortably bound ligand. Moreover, the newly introduced benzoyl group was found to be involved in hydrophobic interaction with Met114, Leu112, Thr144 and Arg104 and methylthio group of the ligand with Tyr185 and Tyr192. This difference in the interactions with previously reported THPs could be probably due the presence of newly introduced groups in the present study.

**4. Conclusion**

In the light of the above observations, it can be concluded that for best binding of the ligand, presence of unsubstituted benzoyl group at position 5 of the newly designed tetrahydropyrimidine derivatives appears to be necessary. Thus a number of new molecules with different substituents in position 1, 3 and 6 of the tetrahydropyrimidine ring could be synthesized keeping benzoyl group in position 5 intact with a view to arrive at a molecule better than **THP-4**. It has been reported [10] that Try 192 plays an important role in holding the compound comfortably into the binding pocket of the protein thereby contributing to the activity of nitro-THPs. Presence of similar interactions in 5-benzoyl-THPs, particularly in **THP-4** and **THP-12** is encouraging and *in vitro* studies of these tetrahydropyrimidines is worth pursuing. In addition, when nitro-THP (Figure 1) was docked under identical conditions, the results found were inferior to those obtained in our cases.

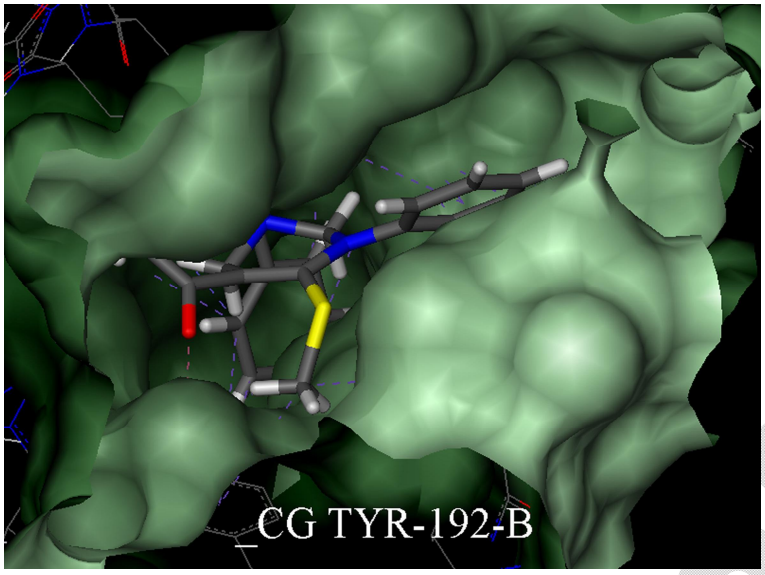



**Figure 2:** THP-4 inside the binding pocket of AChBP (PDB ID: 2ZJU).

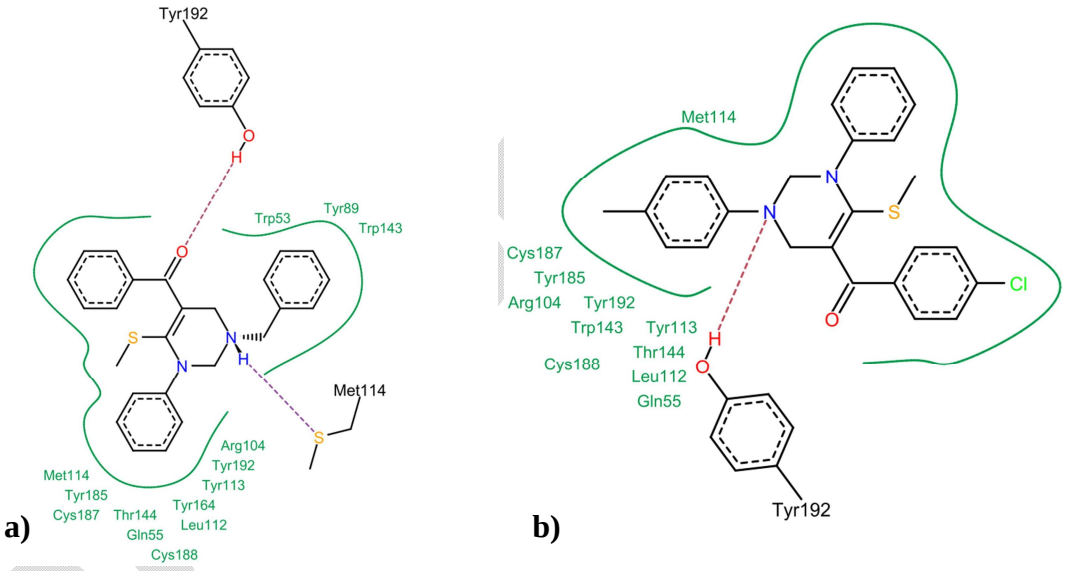



**Figure 3:** The most comfortable binding geometries generated by PoseView after docking of a) THP-4 (ΔG= -23.66 KJ/mol) and b) THP-12 (ΔG= -23.39 KJ/mol) with AChR.

## Competing Interests

Authors do not have any competing interests.

## Authors' Contributions

ASD carried out the synthesis of the THPs and RRB carried out docking studies of the molecules presented in this paper. MAL took part in the analysis of the docking results. JNV designed the synthetic strategy and analyzed spectral and analytical data.

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