Design, Molecular Docking and Synthesis of Pyrazole-Oxadiazole in search of potent insecticidal agents

Amar Patil,[1] Rahul Jadhav,[1] Nachiket More,[1,2] Hemant Raundal,[1] Ashok Desai,[3] Sonali Deore,[4] Vivek Bobade[1*]

[1] Organic Chemistry Research Centre, P.G Department of Chemistry, H.P.T Arts and R.Y.K Science College, Nashik, 422 005, MS, India

[2] School of Chemistry, University of St. Andrews, North Haugh, St. Andrews, KY169ST, United Kingdom

[3] Department of Zoology, K.T.H.M College, Nashik 422 002, MS, India

[4] Department of Zoology, KAANMS Arts, Commerce AND Science College, Satana, MS, India

[*]Corresponding author

Tel.:+ +91 253 2574684; Fax: +91 253 2574684

E-mail: v_bobade31@rediffmail.com, vdbobade@hptrykcollege.com

Abstract

A series of sixteen substituted pyrazole oxadiazole derivatives are designed, synthesised and characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometry. The ligands have been docked with Acetylcholine receptor to understand the binding efficiency and amino acids interactions, indicative of all of the sixteen molecules bind efficiently better than Fipronil and Pyrafluprole. These compounds will provide a lead for designing new compounds with improved insecticidal activity.

Key Words: Pyrazole oxadiazole, Acetylcholine receptor, insecticidal activity, Bruchus chinensis

Introduction

Quantitative as well as qualitative loss[1] of grains due to insects during storage by various pests is a major concern for the agricultural industry. A wide range of insects attack stored products, the commonest among them being beetles and moths. Pulse beetle, Bruchus chinensis and moths are serious threat to the stored food grains as they causes damage to the grains by eating out the entire content of the grain, leaving behind the empty shell or the seed coat. Insect pest damage of stored grains causes economic losses to farmers throughout the world which is estimated at 20-30% (Dick 1988). The ecological problem associated with conventional insecticides warrants a need to discover alternative as well as effective insecticides as many pests become resistant to routine insecticides.[2,3] These insecticides should have eco-friendly properties such as easy degradability to nontoxic residues and should be harmless to human being and animals.

Pyrazole has an outstanding performance in controlling the pest and thus has attracted considerable attention over the last few decades[7]. Many of the marketed pesticides such as fripronil[4], tebunpyrad[5], chlorantraniliporle[6] have pyrazole nucleus in their structure. Fipronil is the first phenyl pyrazole insecticide with good selectivity between insects and mammals that disrupts the insect central nervous system (CNS) by blocking the passage of chloride ions through the GABA receptor and glutamate-gated chloride (GluCl) channels, components of the CNS. However, a survey of the literature revealed that linked biheterocyclic compounds that affect two targets simultaneously always have a better bioactivity and are less prone to resistance than the single target heterocyclic compounds[8]. Pyrazole nucleus could play an important role in these types of biheterocyclic compounds. 1, 3, 4- Oxadiazole derivatives being fungicidal[9, 10] and insecticidal[11] could be a good candidate in these bi heterocyclic compounds. Organic fluorides have good and extensive biological activities allowing their possible applications in pharmaceuticals and pesticides.[12] Literature also revealed that trifluoromethyl group is responsible for the biological activity and therefore is the subject of considerable growing interest[13.] The increased activity is attributed to the presence of fluorine atoms (highly electronegative) in the molecules which increases the lipophilicity and affects the partitioning of molecules into membranes and facilitates hydrophobic interactions of the molecules with specific binding sites on either receptor or enzymes[14].

Considering the above facts, in this paper we report synthesis and insecticidal activity of novel pyrazole oxadiazole derivatives. A total number of sixteen new pyrazole oxadiazole derivatives were synthesized and these compound showed good to moderate insecticidal activity even at very minute (ppm) concentration. The bioassay tests showed that some compounds exhibited acute toxic activity.

Material and Methods

1. Instruments and reagents

All anhydrous solvent were dried and purified by standard techniques prior to their use. The progress of the reaction was monitored by thin layer chromatography (TLC) using pre-coated Merck silica gel 60 F_{254} TLC plate. The spots were visualized by UV or by iodine vapour. Melting points (m.p. values) were determined on melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker at 400 MHz spectrometer (Germany) using tetramethylsilane (TMS) as an internal standard. The chemical shift values are recorded on δ scale and the coupling constants (J) are in Hertz. Mass spectrometry was recorded on waters, Q-TOF MICROMASS (LC-MS).

2. Chemical synthesis

All pyrazole oxadiazole (ethyl 3-(3, 5-bistrifluoromethyl) phenyl)-1-(4-fluorophenyl)-1H-pyrazole-5-carbohydrazide) derivatives were synthesized by the known literature reported procedure[15] (Fig. 1)

2.1 General Synthesis

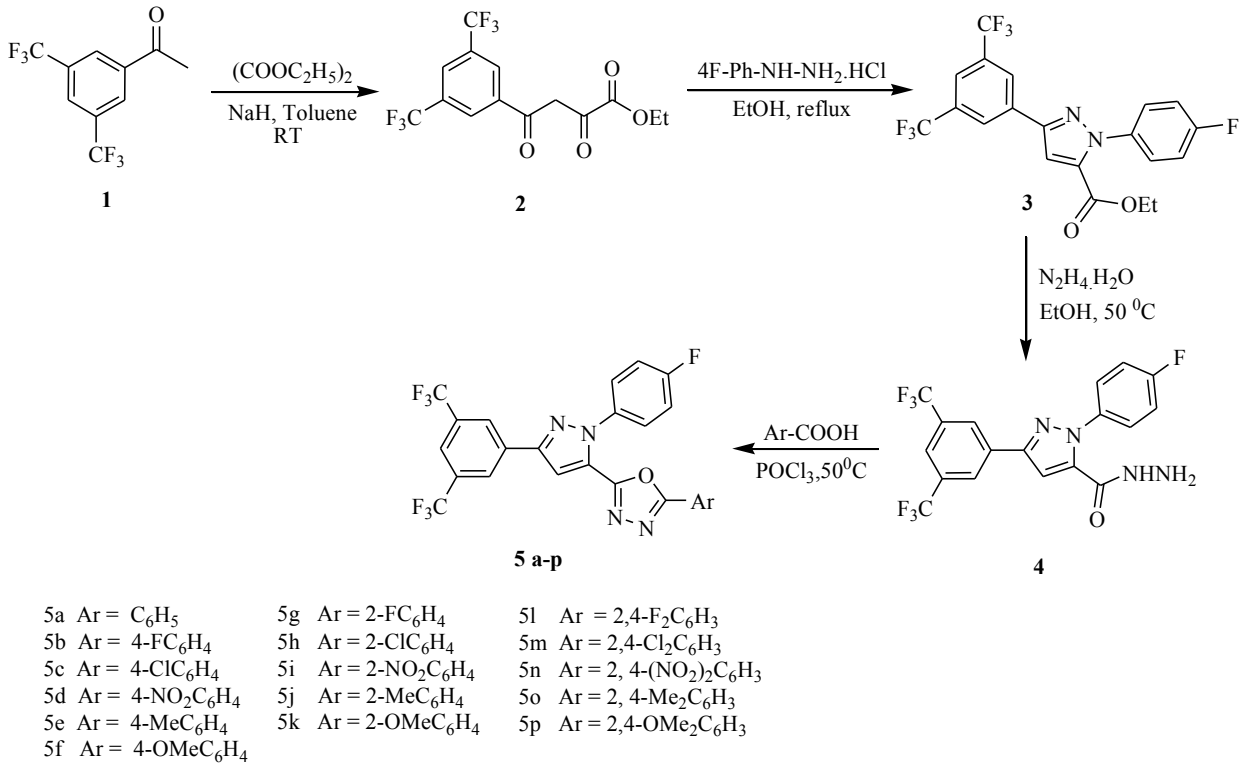
Synthesis of ethyl 4-(3, 5-bistrifluoromethyl) phenyl)-2, 4-dixobutanoate (2): 3, 5 bis-trifluoro acetophenone (1 mol) was reacted with diethyl oxalate (1.5 mol) and sodium hydride (2 mol) in toluene. The reaction mixture was stirred overnight and progress of the reaction was monitored on TLC (hexane: ethyl acetate, 7: 3 v/v). After completion of the reaction, the solvent was evaporated and the mixture was then poured on to crushed ice and acidified with dil. HCl to obtain the product.

Synthesis of ethyl3-(3, 5-bistrifluoromethyl) phenyl)-1-(4-fluorophenyl)-1H-pyrazole-5-carboxylate (3): The mixture of ethyl ester **2** (1.0 mol) and 4-fluorophenyl hydrazine hydrochloride (1.1 mol) in ethanol: acetic acid (2:1) was refluxed

at 100°C. After completion of the reaction, the solvent was evaporated and the mixture was poured on to crushed ice to obtain the product.

Synthesis of ethyl3-(3, 5-bistrifluoromethyl) phenyl)-1-(4-fluorophenyl)-1H-pyrazole-5-carbohydrazide (4): The mixture of ethyl ester **3** and hydrazine hydrate in ethanol was refluxed for 6 h on water bath. After the reaction was complete, the reaction mixture was cooled. The solid obtained was filtered and washed with water.

Synthesis of 2-(3(3, 5 bis trifluoromethyl) phenyl) 1H- pyrazol-5-yl)-5-phenyl-1, 3, 4-oxadiazole derivatives (5): The mixture of ethyl-3-(3, 5-bistrifluoromethyl) phenyl)-1-(4-fluorophenyl)-1*H*-pyrazole-5-carbohydrazide **4** (1.0 mol) and different substituted benzoic acid (1.0 mol) in $POCl_3$ was refluxed for 12 h. The reaction mixture was then cooled and poured onto the crushed ice. The solid separated was filtered, washed with water. The crude products (**5a-p**) were purified by column chromatography using hexane: ethyl acetate as eluent.



5a Ar = C_6H_5 5g Ar = 2-FC_6H_4 5l Ar = 2,4-$F_2C_6H_3$
5b Ar = 4-FC_6H_4 5h Ar = 2-ClC_6H_4 5m Ar = 2,4-$Cl_2C_6H_3$
5c Ar = 4-ClC_6H_4 5i Ar = 2-$NO_2C_6H_4$ 5n Ar = 2, 4-$(NO_2)_2C_6H_3$
5d Ar = 4-$NO_2C_6H_4$ 5j Ar = 2-MeC_6H_4 5o Ar = 2, 4-$Me_2C_6H_3$
5e Ar = 4-MeC_6H_4 5k Ar = 2-$OMeC_6H_4$ 5p Ar = 2,4-$OMe_2C_6H_3$
5f Ar = 4-$OMeC_6H_4$

Fig. 1: Synthesis of 2-(3(3, 5 bis trifluoromethyl) phenyl) 1-(4-fluorophenyl) - 1H- pyrazol-5-yl)-5-phenyl-1, 3, 4-oxadiazole derivatives

2.2 Chemical data

The molecular formula, molecular weight (MW), melting point, % yield, mass analysis, ^{1}H NMR, ^{13}C NMR of all the synthesized compounds are given below:

2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-phenyl-1, 3, 4-oxadiazole (**5a**):
yield, 72%, pale yellow solid, m.p 218-220°C. ^{1}H NMR, 7.38-7.42(2H, t, Ar-H), 7.56-7.58(2H, m, Ar-H), 7.63-7.69(3H, m, Ar-H), 7.84(1H, s, -CH-pyrazole), 8.00(2H, s, Ar-H), 8.11-8.14(2H, m, Ar-H), 8.18(1H, s, Ar-H).

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(4-fluorophenyl)-1, 3, 4-oxadiazole (**5b**)*:
yield, 64%, white solid, m.p 152-154°C. ^{1}H NMR, δ ($CDCl_3$): 7.15-7.20 (2H, t, Ar-H), 7.35-7.39 (2H, m, Ar-H), 7.40(1H, s, -CH-pyrazole), 7.50-7.53(2H, t, Ar-H), 7.70 (2H, s, Ar-H), 7.89(1H, s, Ar-H), 8.12-8.15(2H, m, Ar-H).

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(4-Chlorophenyl)-1, 3, 4-oxadiazole (**5c**)*:
yield, 68%, White solid, m.p 158-160°C. ^{1}H NMR, δ ($CDCl_3$): ^{1}H NMR, δ (CDCl3): 7.15-7.18(2H, t, Ar-H), 7.35-7.38(2H, m, Ar-H), 7.39(1H, S, -CH- pyrazole), 7.50-7.53(2H, t, Ar-H), 7.70(2H, s,Ar-H), 7.89(1H, s, Ar-H), 8.12-8.15(2H, m, Ar-H).

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(4-nitrophenyl)-1, 3, 4-oxadiazole (**5d**)*: yield, 70%, White solid, m.p 202-204°C. ^{1}H NMR, δ ($CDCl_3$): 7.17-7.21 (2H, t, Ar-H), 7.37-7.40(2H, m, Ar-H), 7.44(1H, s, -CH-Pyrazole), 7.72(2H, s, Ar-H),7.90(1H, s, Ar-H), 8.40(4H, m, Ar-H).

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-p-tolyl-1, 3, 4-oxadiazole (**5e**)*: yield, 72%, White solid, m.p 206-208°C. ^{1}H NMR, δ ($CDCl_3$): 2.44(3H, s, Ar-H)7.14-7.19(2H, t, Ar-H), 7.32 -7.34(2H, m, Ar-H), 7.35-7.39 (2H, m, Ar-H), 7.38(1H, s, -CH-Pyrazole), 7.70(2H, s, Ar-H), 7.88(1H, s, Ar-H), 8.07-8.09(2H, t, Ar-H). ^{13}C NMR : 21.61, 108.71, 116.52, 116.75, 118.65, 120.77, 121.36, 122.60, 122.64, 120.07, 126.79, 127.64, 127.72, 128.66, 129.76, 131.07, 131.89, 132.23, 134.66, 134.70, 138.93, 141.99, 142.57, 159.18, 161.42, 163.92, 164.98. Lc-MS (M+1): 533.1252.

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(4-methoxyphenyl)-1, 3, 4-oxadiazole (**5f**)*: yield, 62-64%, White solid, m.p 195-197°C. ^{1}H NMR, δ ($CDCl_3$): 3.89(3H, s, Ar-H), 7.01-7.03(2H, t, Ar-H), 7.14-7.19(2H, t, Ar-H), 7.35-7.39(2H, m, Ar-H), 7.38(1H, s, Ar-H), 7.70(2H, s, Ar-H), 7.88(1H, s, Ar-H), 8.12-8.14(2H, m, Ar-H). ^{13}C NMR: 55.45, 114.49, 116.03, 116.52, 116.75, 118.63, 121.36, 122.56, 122.60, 122.63, 124.07, 126.79, 127.63, 127.72, 128.62, 129.01, 131.09, 131.89, 132.22, 132.56, 132.89, 134.67, 134.70, 138.99, 141.96, 158.97, 161.41, 162.56, 163.91, 164.79. Lc-MS (M+1): 549.1169.

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(2-fluorophenyl)-1, 3, 4-oxadiazole (**5g**)*: yield, 66%, White solid, m.p 222-224°C. ^{1}H NMR, δ ($CDCl_3$): 7.14-7.19 (2H, t, Ar-H), 7.26-7.32(2H, m, Ar-H), 7.35-7.39(2H, m, Ar-H), 7.40(1H,s,-CH-pyrazole)7.54-7.60(1H, m, Ar-H), 7.70(2H, s, Ar-H), 7.88(1H, s, Ar-H), 8.16-8.20(1H,m,Ar-H).

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(2-chlorophenyl)-1, 3, 4-oxadiazole (**5h**)*: yield, 74 %, pale yellow solid, m.p 160-162°C. ^{1}H NMR, δ ($CDCl_3$): 7.13-7.18 (2H, t, Ar-H), 7.34-7.39(2H, m, Ar-H), 7.40(1H, s, -CH-pyrazole), 7.41-7.45(1H, m, Ar-H), 7.46-7.52(1H, m, Ar-H), 7.57-7.60(1H, m, Ar-H), 7.70(2H, s, Ar-H), 7.97(1H, s, Ar-H), 8.05-8.11(1H, m, Ar-H).

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(2-nitrophenyl)-1, 3, 4-oxadiazole (**5h**)*: yield, 67 %, pale yellow solid, m.p 160-162°C. ^{1}H NMR, δ ($CDCl_3$): 7.14-7.16 (2H, t, Ar-H), 7.30-7.34(2H, m, Ar-H), 7.40(1H, s,-CH-pyrazole), 7.70(2H, s, Ar-H), 7.90(1H, s, Ar-H), 8.09-8.10(1H, m, Ar-H), 8.52-8.55(1H, m, Ar-H), 8.49-8.47(1H, m, Ar-H), 8.44-8.46(1H, m.Ar-H).

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-o-tolyl-1, 3, 4-oxadiazole (**5j**)*: yield, 76 %, White solid, m.p 206-208°C. ^{1}H NMR, δ ($CDCl_3$): 2.79(3H, s, Ar-H), 7.14-7.18(2H, t, Ar-H), 7.32-7.37(4H, m, Ar-H), 7.38(1H, s,-CH-pyrazole), 7.42-7.46(1H, m, Ar-H), 7.71(2H, s, Ar-H), 7.88(1H, s, Ar-H),8.08-8.10(1H, m, Ar-H). ^{13}C NMR, δ ($CDCl_3$):13.83, 21.59, 22.07, 28.69, 28.99, 31.28, 108.93, 116.13, 118.63, 121.34, 122.23, 124.06, 126.26, 128.15, 129.20, 130.86, 130.96, 131.19,131.37,134.69, 134.72, 137.57, 137.74, 137.81, 141.90, 158.72, 160.73, 163.19, 164.07.

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(2-methoxyphenyl)-1, 3, 4-oxadiazole (**5h**)*: yield, 72%, White solid, m.p 170-172°C. ^{1}H NMR, δ ($CDCl_3$): 4.01(3H, s, Ar-H), 7.07-7.11(2H, m, Ar-H), 7.13-7.18(2H, t, Ar-H), 7.35-7.39(2H, m, Ar-H), 7.39(1H, s, -CH-pyrazole), 7.51-7.55(1H, m, Ar-H), 7.705(2H, s, Ar-H), 7.87(1H, s, Ar-H), 8.03-8.06(1H, s, Ar-H)

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(2, 4-difluorophenyl)-1, 3, 4-oxadiazole (**5l**)*: yield, 70%, White solid, m.p 180-182°C. ^{1}H NMR, δ ($CDCl_3$): 7.02-7.09(2H, m, Ar-H), 7.14-7.19(2H, t, Ar-H), 7.34-7.38(2H, m, Ar-H), 7.39(1H, s,-CH-pyrazole), 7.70(2H, s, Ar-H), 7.88(1H,s, Ar-H),8.16-8.22(1H, m, Ar-H).LC-MS (M+1): (555.0881)

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazole (**5l**)*: yield, 77%, White solid, m.p 180-182°C. ^{1}H NMR, δ ($CDCl_3$): 7.14-7.18(2H, t, Ar-H), 7.34-7.38(2H, m, Ar-H),7.39 (1H, s,-CH-pyrazole), 7.41-7.44(1H, m, Ar-H), 7.61(1H, m, Ar-H), 7.70(2H, s, Ar-H), 7.89(1H, s, Ar-H), 8.05-8.07(1H, m, Ar-H).

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(2, 4-dinitrophenyl)-1, 3, 4-oxadiazole (**5n**)*: yield, 64%, yellow solid, m.p 135-137°C. ^{1}H NMR, δ ($CDCl_3$): 7.14-7.19(2H, t, Ar-H), 7.32-7.37(2H, m, Ar-H), 7.38(1H, s,-CH-pyrazole), 7.70(2H, s, Ar-H), 7.90(1H, s, Ar-H), 8.33-8.36(1H, m, Ar-H), 8.62-8.65(1H, m, Ar-H), 8.86-8.89(1H, m, Ar-H).

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(2, 4-dimethyl phenyl)-1, 3, 4-oxadiazole (**5o**)*: yield, 73%, White solid, m.p 163-165°C. ^{1}H NMR, δ ($CDCl_3$): 2.39(3H, s, Ar-H), 2.75(3H, s, Ar-H), 7.14-7.18(4H, m, Ar-H), 7.35-7.38(2H, m, Ar-H), 7.38(1H, s, -CH-pyrazole), 7.70(2H, s, Ar-H), 7.88(1H,s, Ar-H), 7.97-7.99(1H, m, Ar-H).^{13}C NMR, δ ($CDCl_3$): 21.37, 22.10, 108.73, 116.49, 116.72 ,118.66, 119.86, 121.37, 122.59, 124.08, 126.80, 126.91, 127.62, 127.70, 128.68, 129.25, 131.14, 131.89, 132.22, 132.53, 132.56, 132.89, 134.69, 134.72, 138.61, 139.00, 141.86, 141.94, 158.73, 161.40, 163.89, 165.16, LC-MS (M+1):547.1351

*2-(3(3, 5 bis trifluoromethyl) phenyl) -1-(4-fluorophenyl)-1H- pyrazol-5-yl)-5-(2, 4-dimethoxy phenyl)-1, 3, 4-oxadiazole (**5p**)*: yield, 71%, White solid, m.p 170-172°C. ^{1}H NMR, δ ($CDCl_3$): 3.83(3H, s, Ar-H), 3.98(3H, s, Ar-H), 6.58-6.62(2H, m, Ar-H), 7.13-7.17(2H, t, Ar-H), 7.35-7.38(2H, m, Ar-H), 7.38(1H, s,-CH- pyrazole), 7.70 (2H, s, Ar-H), 7.81(1H, s, Ar-H),8.98-8.01(1H, m, Ar-H)

3. Molecular Docking

The target, Acetylcholine receptor was chosen to understand the binding efficiency of ligands and in order to screen the ligands virtually. The target, PDB ID 2BG9 (Refined Structure of the Nicotinic Acetylcholine Receptor) was downloaded from RCSB PDB[15] in pdb format, later uploaded in UCSF Chimera[16] to remove the ligand and the file was again saved in pdb format. The ligands were drawn in ChemDraw Ultra 8.0 and were saved in mol format and later was uploaded in the Avogadro[17] to generate the 3-D co-ordinates, optimize the energy and then saved in pdb format. The protein-ligand was docked using AutoDockTools 1.5.7.[18] Initially the protein and ligand were prepared using defined protocols and files were saved in pdbqt format and later from Computed Atlas of Surface Topography of proteins (CASTp)[19] webserver and the amino acids in the catalytic pocket of active site were feeded in the selection from string tab and active sites were defined. The grid box of 60 X 60 X 60 dimension was designed encompassing 226981 grid points and spacing angstrom was kept 0.375. The search grid of Acetylcholine receptor was identified as centre: 65.452, centre: 80.011 and centre: 160.022 and the results were saved in grid point file and later autogrid was run followed by using Lamarckian Genetic Algorithm, autodock was run. After completion of autodock, dlg file was checked and docking was confirmed. The results were analyzed using analysing conformations of top 10 conformers and the complex formed was initially saved in pdbqt format and followed by saving it in the pdb format for understanding the docking pose, visualization of 2D interaction of target with ligands and to study the participation and contribution of amino acids in the catalytic pocket of target was carried out using BIOVIA Discovery Studio Visualizer Cilent.[20] The binding energy and amino acid interactions were compared with 2 standard drugs, Fipronil and Pyrafluprole along with the binding energies of the best conformer with high binding energy and amino acids interactions is mentioned in the Table 1.

Ligands	Binding Energy (kcal/mol)	H-Bond with Amino Acids	Other Interactions with Amino Acids
5a	- 7.74	**TRP67**	LEU65, ASN14, ARG64, LEU11, LEU80, TYR15, LEU110, LEU12, ALA9, LYS17, VAL8,
5b	- 7.85	**PRO81**	ARG79, VAL85, ASN10, ASN14, ILE78, ARG56, ASP62, ILE116, TRP60, ARG64, LEU11, ASP84, TYR15, LEU11, TRP67, LEU65
5c	- 8.07	**TRP67**	ASN14, LEU11, ARG64, LEU65, THR5, LEU80, TYR15, LYS17, LEU12, ALA9, LEU110, VAL8
5d	- 6.86	**TYR15**	ASP84, LEU11, ARG64, TRP67, ASN14, LEU110, THR5, LEU65, LEU80, ALA9, LEU12, VAL8

5e	- 7.36	**LYS17**	LEU12, TYR15, GLU13, ASP84, LEU7, ASN16, ASP71, ALA70, TRP67, LEU65, VAL8, LEU11
5f	- 7.17	**LYS17, ASP71**	TYR15, LEU12, GLU13, ARG64, ASP84, ASN16, LEU65, TRP67, ILE78, ALA70, VAL7, LEU7
5g	- 6.89	-	VAL85, ALA9, LEU12, LYS17, LEU65, LEU80, VAL8, ASP84, TYR15
5h	- 7.22	-	ASP84, VAL8, ALA9, LEU12, LEU80, TYR15, LYS17, VAL85
5i	- 6.18	-	LEU65, LEU80, VAL8, LEU12, LEU11, ASP84, TYR15, GLU13, TRP67, ILE78, ALA9, ASP71
5j	- 7.53	**LYS17**	VAL8, ILE78, LEU11, TRP67, LEU65, ASN16, ASP71, ASP84, LEU12, TYR15, GLU13, ARG64
5k	- 7.30	**LYS17, ASN16**	TYR15, GLU13, LEU12, ILE78, TRP67, VAL8, LEU80, LEU110, LEU65, ASP71, ASP84, LEU11
5l	- 7.10	-	ASN14, ARG64, ASP84, THR5, LEU110, TRP67, ALA9, LEU12, LEU65, LEU80, LEU11, TYR15, VAL8
5m	- 7.94	**LYS17**	LEU12, GLU13, TYR15, ASP84, ALA70, ARG64, LEU7, ILE78, LEU65, TRP67, ASP83, ASN16, ASP71, VAL8, LEU11
5n	- 7.10	**ARG79**	ASP84, LEU11, ALA70, VAL85, LEU110, ILE116, ILE75, VAL8, LEU7, ASP71, TYR15, TRP67, LEU80, ILE78, LEU65
5o	- 7.40	**TYR15**	ASP84, ARG64, LEU110, THR5, ALA9, TRP67, LEU12, LEU11, LEU65, LEU80, VAL8,
5p	- 6.68	-	ASP71, ILE78, LEU12, VAL85, LEU80, LEU65, TRP67, ALA9, ASP84, VAL8, LEU11,
Fipronil	- 5.15	**ASP84**	VAL8, LEU65, TRP67, LEU80, LEU110, ALA9, TYR15, LEU12, LEU11
Pyrafluprole	- 5.86	**ASP84**	ARG64, VAL8, LEU12, ASN14, VAL85, LEU11, LEU65, LEU80, PRO81, TRP67,TYR15

Table 1: Docking Analysis of Designed Ligands With Refined Structure of The Nicotinic Acetylcholine Receptor
H-Bonding, Vander waal Interactions, Halogen Interactions,
Alkyl & Pi-Alkyl, Pi-Pi Stacking, Pi-Sigma, Pi-Anion, Pi-Lone Pair

Results and discussion

1. Synthesis

Pyrazole oxadiazole (**5a-p**) were synthesized by reacting 3, 5-bistrifluoromethyl acetophenone **1** with diethyl oxalate in presence of NaH to obtain ester **2**. The ester **2** on reaction with 4-Fluoro phenyl hydrazine hydrochloride afforded pyrazole ester **3**. The pyrazole ester derivative **3** was further converted to the corresponding carbohydrazide derivative **4** using hydrazine hydrate. This carbohydrazide was then refluxed in $POCl_3$ with different substituted benzoic acids to afford the target compounds **5a-p**.

2. Molecular Docking

The amino acids interactions, binding energies of the protein-ligand complexes are clearly evident that all the heterocyclic scaffolds designed are binding with the acetylcholine receptor and have fairly good energy. As per our hypothesis we have observed a high number of halogen interactions in the conformers due to presence of CF_3 groups which are contributing to increase in binding. These interactions have been highlighted in blue in table and 2-D diagrams. All the derivatives have shown better docking score(-6.18 kcal/mol to 8.07 kcal/mol) than the standard drugs, Fipronil (-5.15 kcal/mol) & Pyrafluprole (-5.86 kcal/mol). In the all sixteen derivatives we could observe that greater binding energies were for para/four - substituted, highest for 4-Cl (5c) and lowest for 4-NO_2 (5d) , suggestive of that electron withdrawing substituents are responsible in decrease in binding efficiency and electron donating are helping in binding to catalytic pocket of the protein. This can also be explained on basis of scores obtained for other derivatives, we could observe that for 4-F, 4-Me, 4-OMe has binding energy less than -7.0 kcal/mol, which assures that they are binding better than electron withdrawing substituent. The significance of para substitution could also be understood from the binding energies observed for ortho substitution, in case of all the derivatives there was decreasein binding energies. In case of the 2,4 substitution the binding energies were in between of para and ortho substitution, which indicates that the para position is contributing to binding but the ortho position is blocking due to crowding.

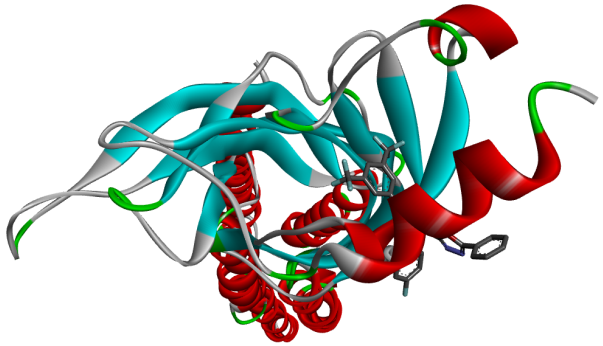
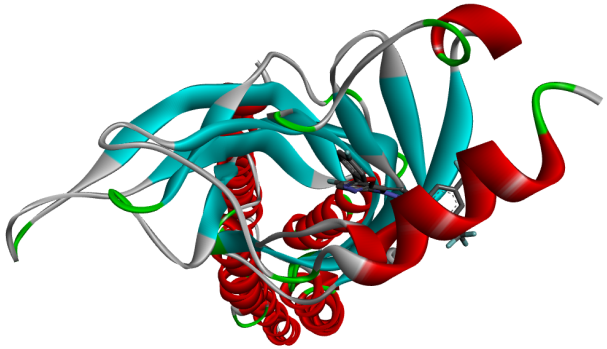
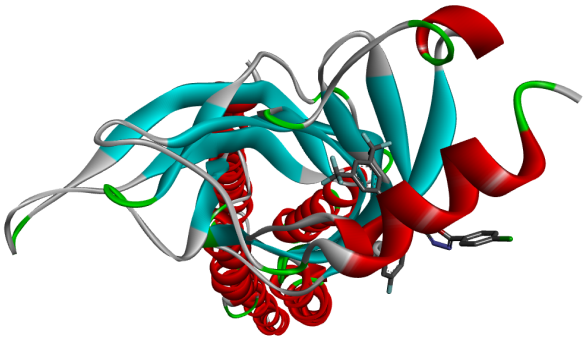
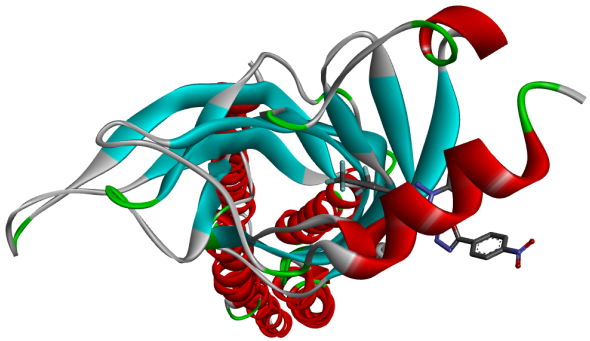
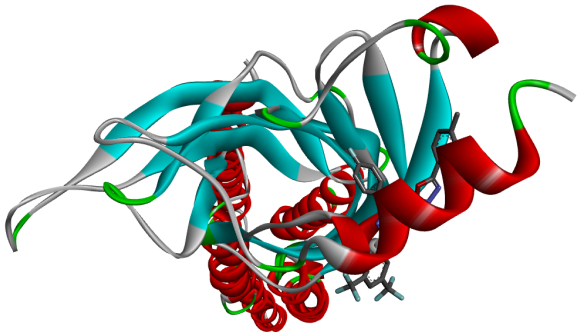
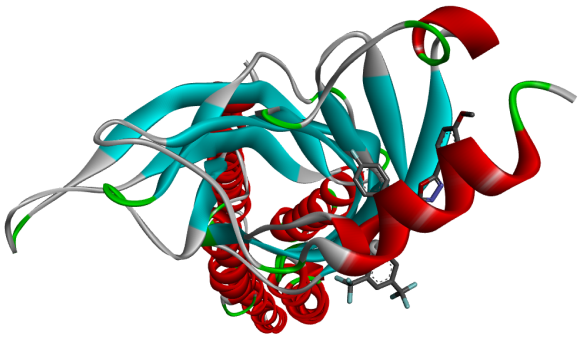
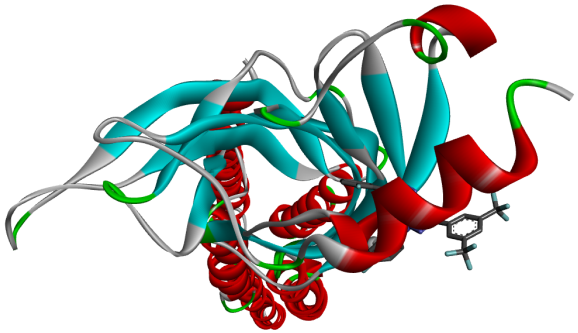
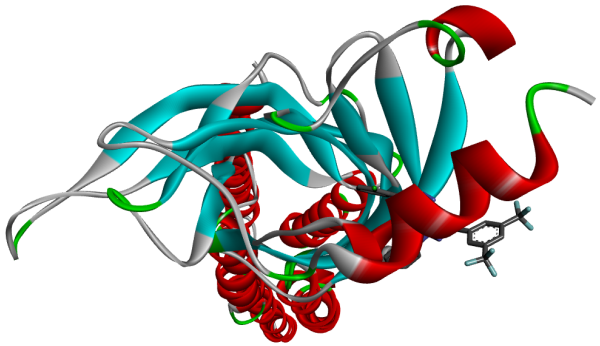
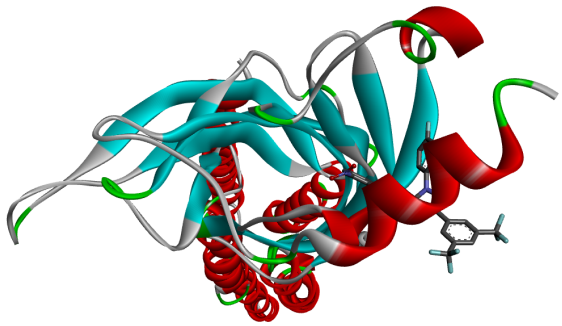
5a 	5b 	5c 
5d 	5e 	5f 
5g 	5h 	5i 

Table 2: Docking poses of 5a to 5i

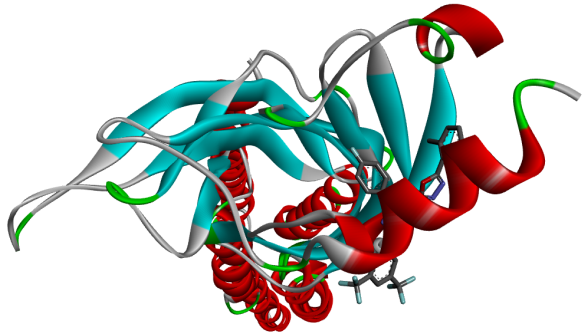
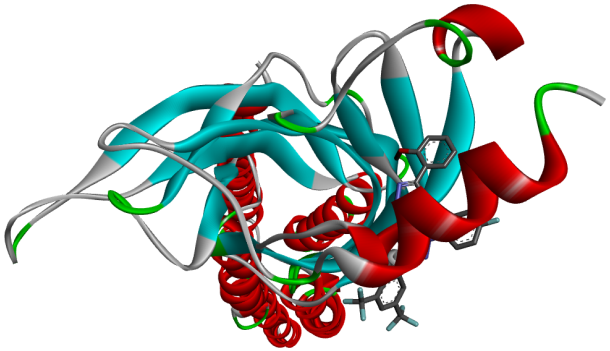
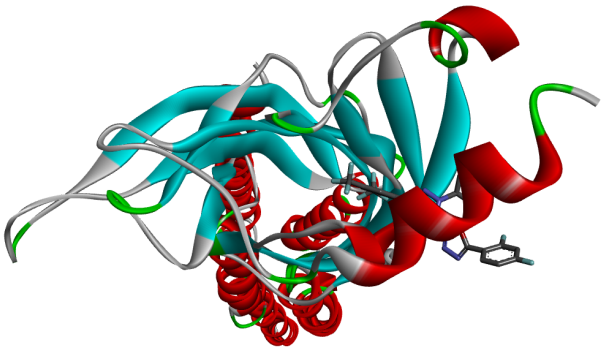
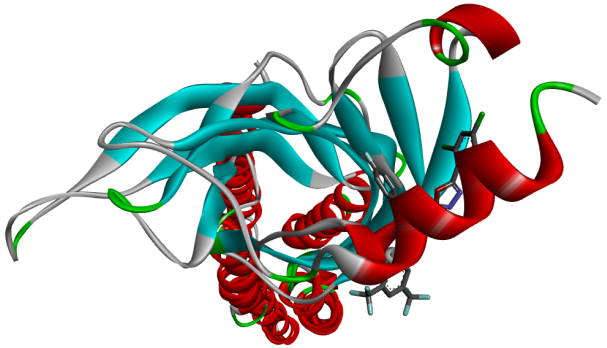
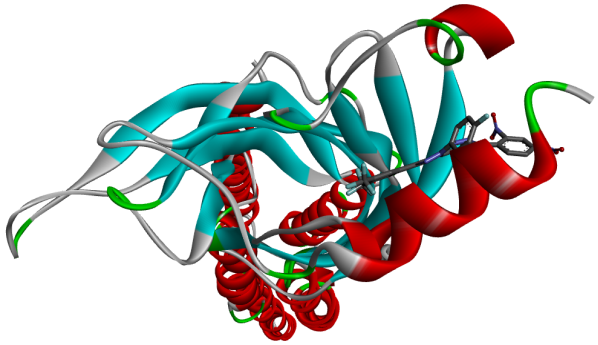
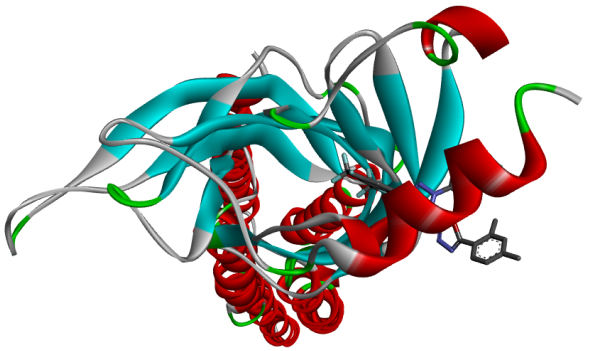
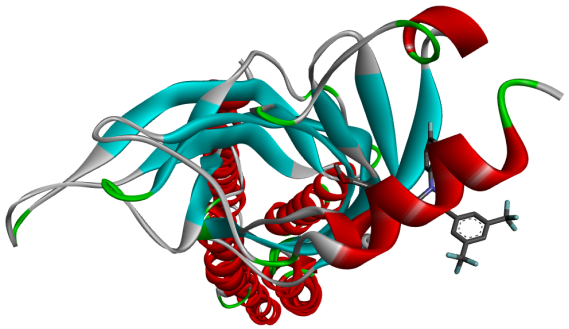
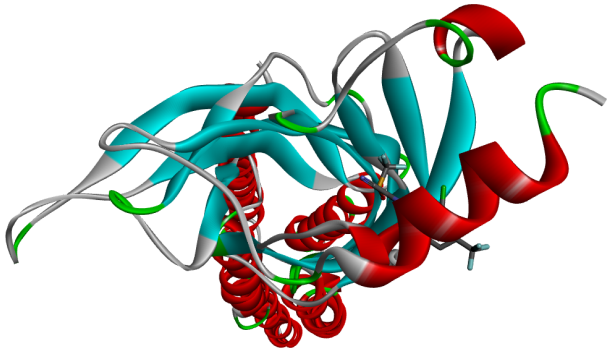
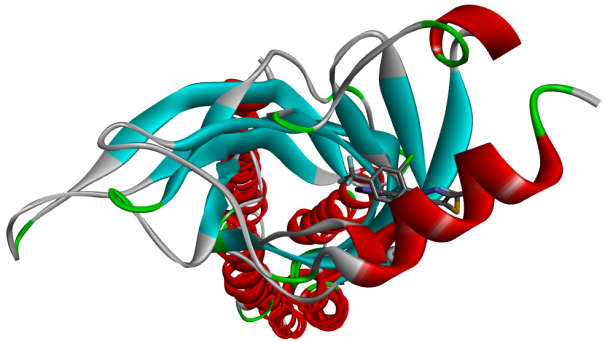
5j	5k	5l
		
5m	5n	5o
		
5p	Fipronil	Pyrafluprole
		

Table 2 (continued) Docking Poses of 5j to 5p and Standard/Reference Molecules

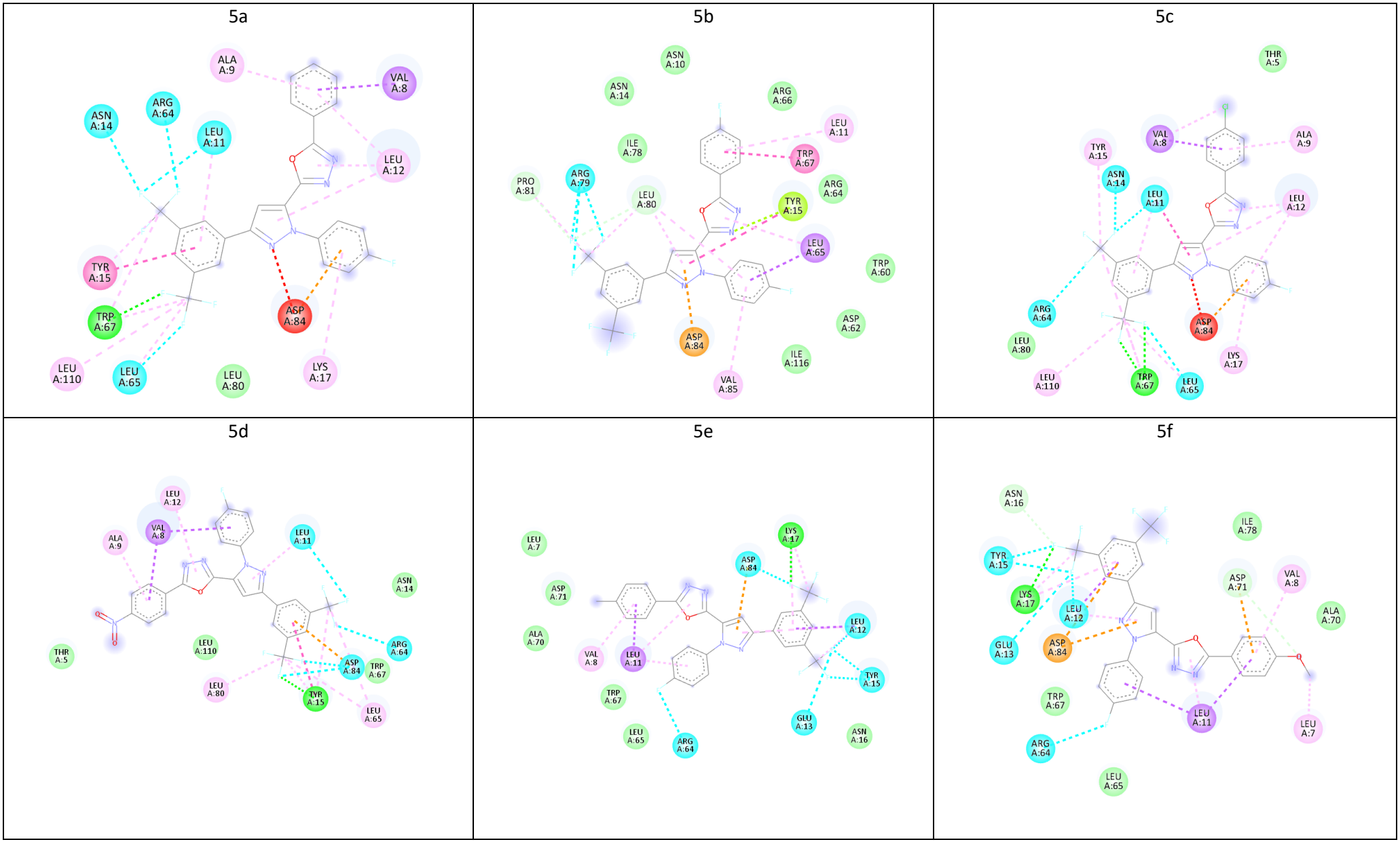


Table 3: 2D- Interactions of Ligands with Amino Acids present in catalytic pocket of active site of target

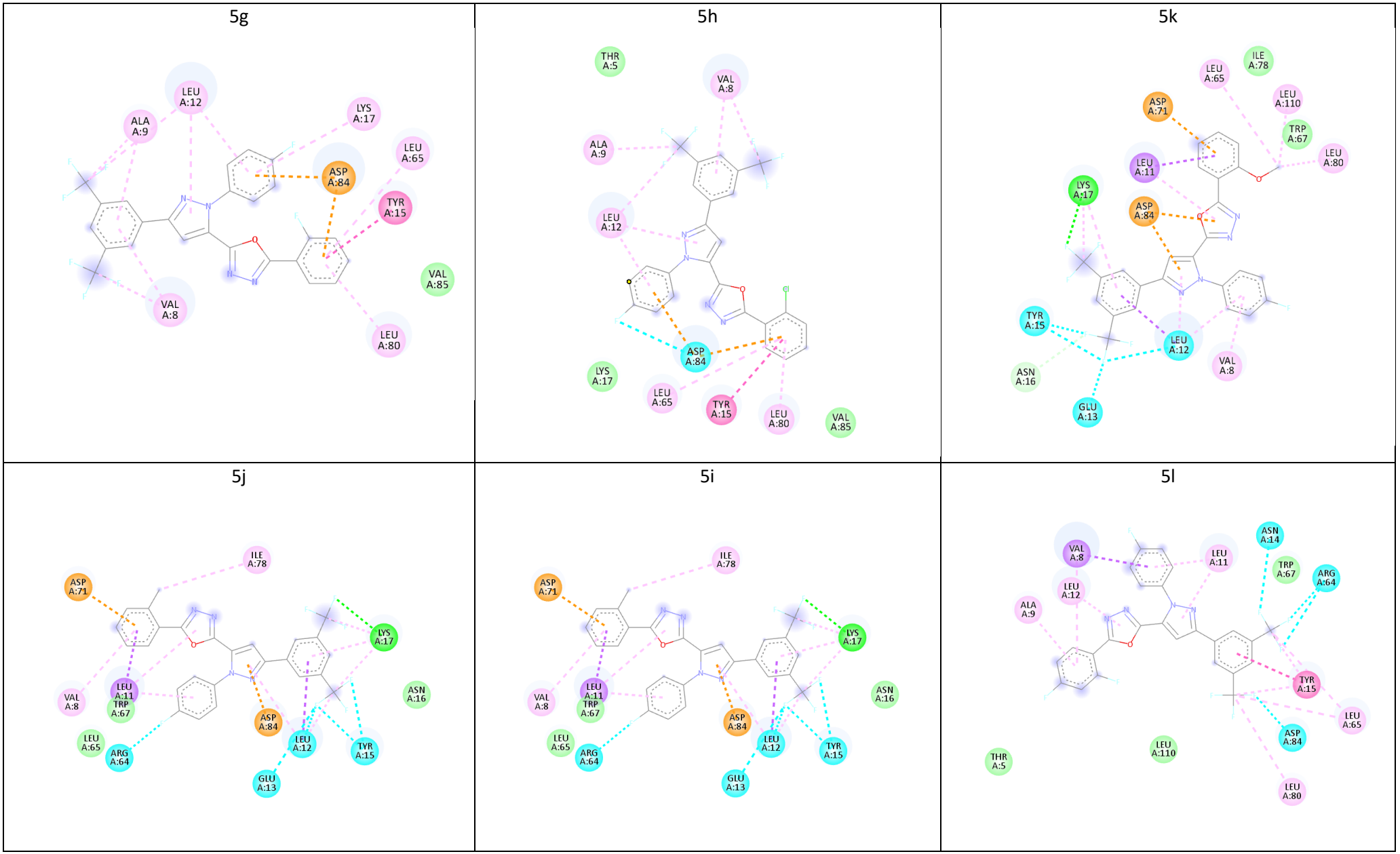


Table 3 (continued): 2D- Interactions of Ligands (5g to 5i) with Amino Acids present in catalytic pocket of active site of target

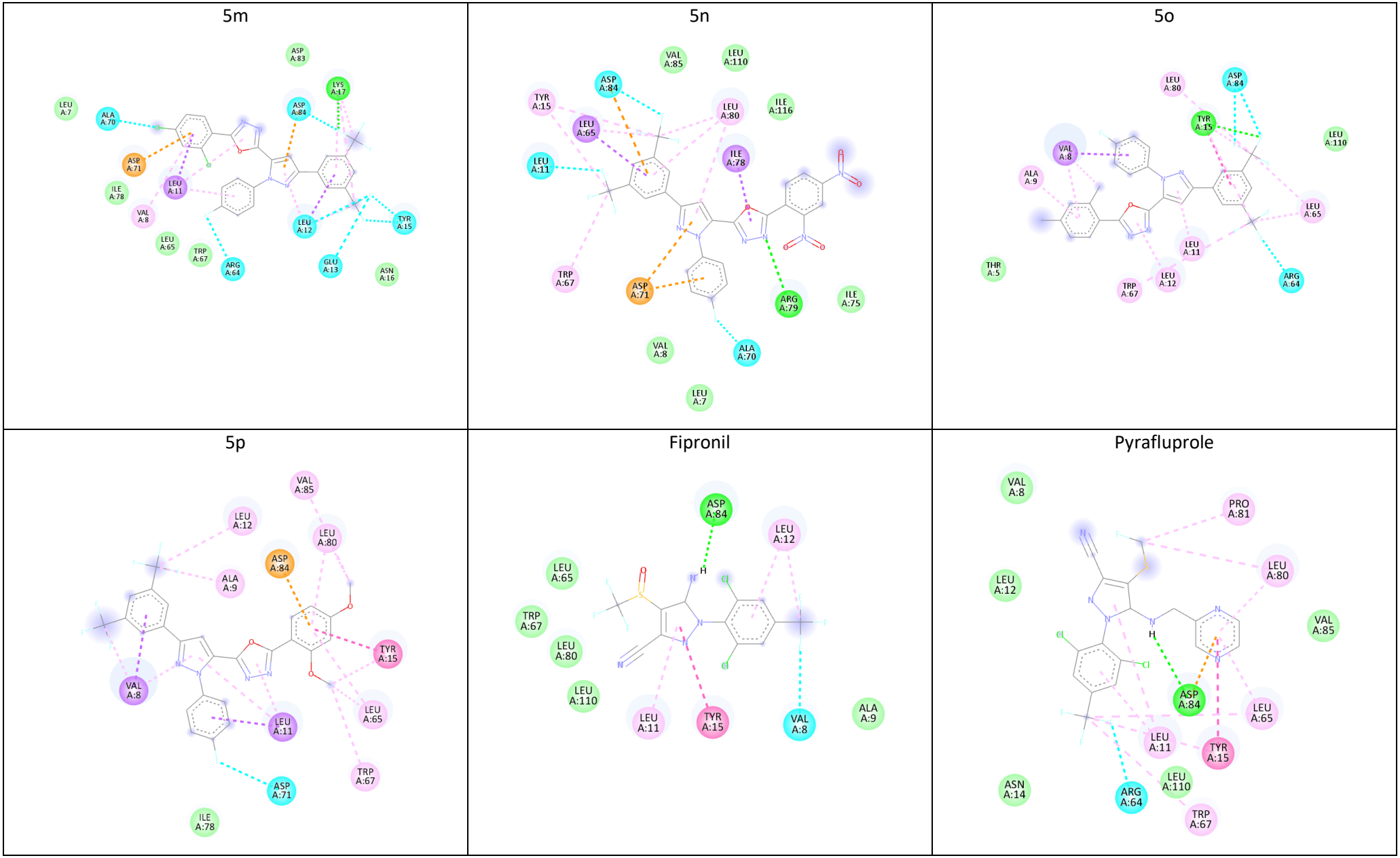


Table 3 (continued): 2D- Interactions of Ligands (5m to 5p, Fipronil & Pyrafluprole) with Amino Acids present in catalytic pocket of active site of target

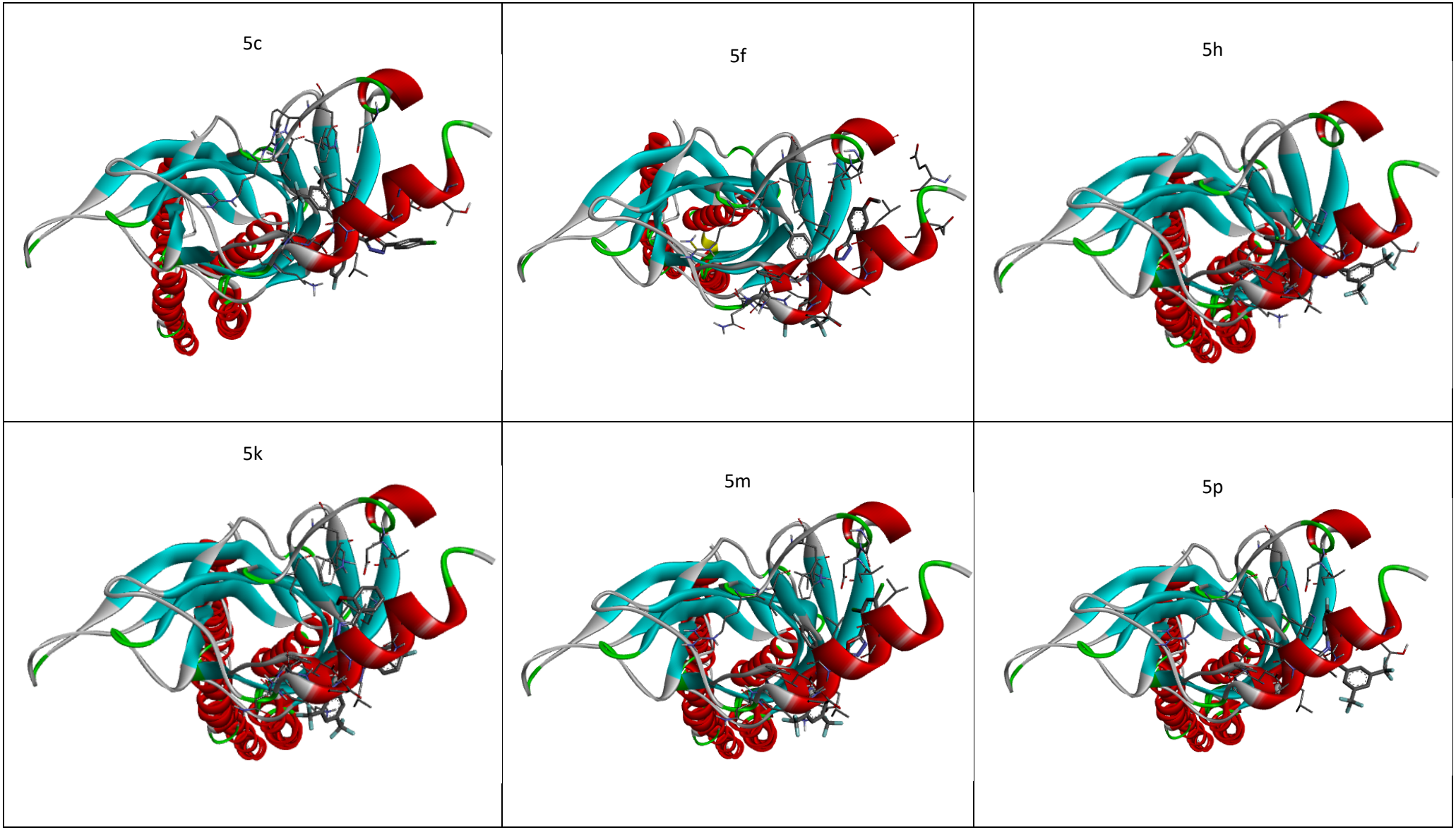


Table 4: Interactions of Ligands (5c, 5f, 5h, 5k, 5m, 5p) with pocket atoms present in target

The other amino acid interactions contributing to the efficient binding of the derivatives are H-bonds, in most of complexes except in case of 5g, 5h, 5i & 5l and in these cases the binding efficiencies were less in comparison with other derivatives, suggestive of that H-bonding is playing role in increasing efficiency of protein-ligand complex. The other interactions were π-π stacking, π-cation, π-anion & π-sigma which were observed in case of most of complexes. The plain derivative also binded to the receptor with a binding energy of -7.74 kcal/mol, indicative of that substitution is not playing much in increasing the binding efficiency derivatives.

Acknowlgements

Authors are thankful to CSIR (Council of Scientific and Industrial Research), New Delhi for the financial support. Authors would like to acknowledge SAIF, Punjab University, Chandigarh for the spectral analysis.

References

1. Srivastava K P and Butani D K 2009, "Pest Management in Vegetable," 2nd Ed, Vol. I, Studium Press (India) Pvt., New Delhi, India, pp. 192–193
2. Champ B R and Dyte C E 1976 "Report of the FAO Global Survey of Pesticide Susceptibility of Stored Grain Pests. FAO Plant Production and Protection Series No. 5, Food and Agricultural Organisation of the United Nations, Rome 297
3. Srivastava C and Sinha S N *Ann. Pl. Prot. Sci.* (2008) **16**, 337–340.
4. Moffat A S (1993) New chemical seek to outwit insect pests. Science 261, 550-551.
5. Okada I.; Okusi S.; Tsakahasi Y. *et al*1996 E. P. Patent 289, 879,.
6. Lahm G P,. Selby T P, Frendenberger J H, Stevenson T M, Myers B J, Seburyamo G, Smith B K, Flexner L, Clark C E, Cordova D (2005) Insecticidal anthranilic diamides: A new class of potent ryanodine receptor activators Bioorg. Med. Chem. Lett.15 4898-4906,
7. (a)Okada I.; Suzuki S.; Okusi S.; Tsakahasi Y,; Fukuchi T,; Nakajima T. E. P. Patent 329020. (b) Okada I.; Okusi S.; Tsakahasi Y. J. P. Patent 02264760, 1990 (c) Okada I.; Suzuki S.; Okusi S.; Tsakahasi Y. J. Pestic Sci 22(3), 230-232, 1997. (d) Okada I.; Okusi S.; Wada M.; Tsakahasi Y. J. Pestic Sci 21(3),305-301, 1996.
8. Guo, Y.S.; Guo, Z. R. Acta Pharma. Sinica 44, 276-281, 2009.
9. AshourF.A.; El-Hawash S. A. M.; Mahran, M.A.; et al. Bull.Pharm.Sci.Assiut univ 17(1), 17, 1994.
10. Funatsukuri, G.; Ueda, M. Japanese Patent 20, 944, 1996.
11. Xiaoyoung Xu, Xuhong Qian, Zhong Li, Qingchun Huang, Gang Chen J. Fluorine Chem.121, 51-54, (2003).
12. X.Qian, J.Tang, W.Chen, R.Zhang, Chem. J. Chin. Uni.15, 224-227,(1994).
13. N. Plant, Drug Discov. Today 9, 328,(2004).
14. Pushpan Puthiyapurayil, Boja Poojary, Chandrashekhar Chikkanna, Sunil Kumar Buridipad E. J. Med. Chem 53, 203-210,(2012).
15. Nigel Unwin, J Mol Biol., 346(4), 967-89. (2005)
16. UCSF Chimera--a visualization system for exploratory research and analysis. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. J Comput Chem. 2004 Oct;25(13):1605-12.
17. Marcus D Hanwell, Donald E Curtis, David C Lonie, Tim Vandermeersch, Eva Zurek and Geoffrey R Hutchison; "Avogadro: An advanced semantic chemical editor, visualization, and analysis platform" Journal of Cheminformatics 2012, 4:17.
18. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J. (2009) Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. J. Computational Chemistry 2009, 16: 2785-91.
19. Wei Tian, Chang Chen, Xue Lei, Jieling Zhao, Jie Liang, Nucleic Acids Res. 46(W1):W363-W367 (2018)
20. BIOVIA, Dassault Systèmes, [BIOVIA Discovery Studio Visualizer], [v21.1.0.20298], San Diego: Dassault Systèmes, (2021)