Section A-Research paper
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SYNTHESIS AND EVALUATION OF THE SELECTIVITY AND POTENCY OF TRIPHENYL IMIDAZOLES AS POTENTIAL ANTI TUBERCULAR AGENTS

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Abstract

Tuberculosis is an infectious disease with a high rate of mortality; it requires a prolonged period of treatment with many multi drug resistant strains of the bacterium highly prevalent. This problem needs all hands on deck to ensure a varied arsenal of molecules to ensure proper treatment of this scrouge preying on the world. This paper explores the susceptibility of *Mycobaterium tuberculosis* H37RV strain to triphenyl imidazoles synthesized from benzils. Two of the synthesized compounds exhibited anti tubercular activity comparable to that of the standard drug.

Key words: Imidazoles, anti tubercular activity, MABA assay, anti microbial activity, FtsZ protein.

Introduction

The Global Alliance for TB drug development got approval in 2019; for an imidazole containing new molecular entity - Pretomanid for the treatment of multi drug resistant tuberculosis.[1] Delamanid is another nitroimidazole drug which is on the list of WHO approved list of essential medicines for the treatment of drug resistant tuberculosis.[2] Compounds containing imidazole ring system play an important role in many biochemical processes. The

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potency and wide range of applicability of imidazole pharmacophore is due to its hydrogen bond donor-acceptor capability as well as its high affinity for metals (Zn, Fe, Mg) which are present in many protein active sites.[3]

Imidazoles are the core structures present in histidine, histamine and biotin involved in several biological systems.[4] The imidazole pharmacophore is present in drug molecules like Losartan, Olmesartan and Eprosartan. Some other drugs with imidazole moiety include Omeprazole which acts as proton pump inhibitor, Flumazenil (benzodiazepine antagonist), Trifenagrel (a platelet coagulation drug in animal and human beings), Cimetidine (antiulcerative agent), Temozolomide (lymphoma malignant melanoma agent), Misonidazole (inhibitor of *de novo* purine synthesis), Clotrimazole, Metronidazole (antiprotozoal *drugs* in treatment of *Trichomonas vaginalis, Entamoeba histolytica and Giardia lamblia,* Capravirine(Anti-HIV). This literature prompted our research for newer imidazoles with potential anti tubercular activity.

Materials and methods:

Reagents and all the chemicals were purchased from Aldrich Chemical Company (USA) and were used without further purification. The purity of the compounds was checked by TLC using methanol and chloroform as elutant and visualized in UV-chamber or by using Iodine vapour. Also the compounds can be visualized using the reagents 2,4 Dinitrophenylhydrazine and Ninhydrin. The melting points were taken in open capillary tube of SRS-EZMelt automated melting point instrument and are uncorrected. The IR spectra of the compounds were recorded on Bruker FT-IR spectrometer (software-OPUS 6.4) using KBr disc method and the values are expressed in cm^{-1}. The 1H-NMRspectra of the compounds were recorded using DMSO-d6 or $CDCl_3$ on BRUKER AVANCE 400MHz NMR spectrometer (software-Topspin) and chemical

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shifts were expressed in δ (ppm).The mass spectra were recorded on AGILENT QQQ LC-MS (ESI-MS) spectrometer (software- Mass Hunter B.03.01).

Method of Synthesis

Synthesis of substituted benzyl [5]:

Anisole (5 g) was taken as starting material and to that 2.3 ml of oxalyl chloride was added under ice-cold conditions. After sometime aluminum chloride was added in equal proportions to the reaction mixture under stirring. The reaction was monitored using TLC with 20% ethylacetate in hexane as mobile phase. It was considered as completed by observing that the starting material spot has completely disappeared and a new spot was formed under UV light. Then the reaction mixture was quenched with dil. HCl until fumes disappear. Later it was extracted with ethylacetate and the organic layer was distilled in order to attain the concentrate. The concentrate was kept in freezer overnight in order to obtain crystals which were filtered and washed with cold hexane to obtain pure product.

Synthesis of trisubstitutedimidazoles[6]:

Substituted benzil (1 m.mol), aldehyde (1 m.mol) and ammonium acetate (2.5 m.mol) were taken in a tube and mixed thoroughly. Then 5 ml of glacial acetic acid was added and the mixture was irradiated in microwave at 680 W for 10 min with a successive shaking for a time gap of every 30 s (Figure 1). Reaction was monitored using TLC with 10% methanol in chloroform as elutant. The reaction was considered to be complete when starting material spot has disappeared and new spot was found under UV light. Also the formed spot was confirmed by using visualizing reagents ninhydrin and 2,4-dinitrophenylhydazine. Once it is confirmed that the

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reaction has completed, it is allowed to attain room temperature and the solid obtained was filtered. The filtrate obtained was neutralized with ammonium hydroxide in ice cold conditions to give solid and it was filtered. The solid mass obtained in first and second crop was dried in vacuum and recrystallized from absolute ethanol.

Spectral data of the synthesized compounds

4-(4,5-bis(4-methoxyphenyl)-1H-imidazol-2-yl)-N,N-dimethylaniline (TM-1);

Yield 42%; m.pt 126-128 ºC; m/z 400 [M+H$^+$]; IR(KBr, *v* max cm^{-1}) Ar C-Hstr(3031), Ar C=C str (1509,1609), C-O str (1171), aliphatic C-H str(2896), C-N(1244), N-H(3616); ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.13 (s, 1H, NH); 7.86 (d, *J*=8.Hz, 2H) ; 7.40 (m, 4H); 6.97 (d, *J*=8Hz, 2H); 6.85 (d, *J* =8Hz, 2H); 6.77 (d, *J*= 8Hz, 2H); 3.75 (s,6H); 2.96 (6H, N-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 42.7, 55.4, 112.7, 115.1, 116.2, 154.2, 161.5, 177.3.

4-(4,5-bis(4-methoxyphenyl)-1H-imidazol-2-yl)phenol (TM-2);

Yield 38%; m.pt 156-158 ºC; m/z 400 [M+H$^+$]; IR (KBr, υ max cm^{-1}) Ar C-H str(3009), OH str(3544), Ar C=C str (1506), C-O str(1110), N-H (3640), aliphatic C-H str(2932), C-N(1247); ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.23 (s, 1H, NH); 7.85 (d, *J*=8.Hz, 2H); 7.41 (m, 4H); 6.91 (d, *J*=8Hz, ,2H); 6.82 (d, *J*=8Hz, 2H);6.77 (d, *J*=8Hz, 2H); 3.76 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.8, 114.2, 114.9, 116.5, 128.5, 129.2, 131.1, 159.5, 160.6, 176.9.

2,4,5-tris(4-methoxyphenyl)-1H-imidazole (TM-3);

Yield 47%; m.pt 160-161 ºC; m/z 400 [M+H$^+$]; IR(KBr, υ max cm^{-1}) Ar C-H str(3001), aliphatic C-H str(2952), Ar C=C(1508) , Ar C=N(1613), C-N str(1248), C-Ostr(1029); ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.33 (s, 1H, NH); 7.97 (d, *J*=8.4Hz, 2H); 7.41 (d, *J*= 6.4 Hz, 2H); 7.03

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(d, *J*= 7.6Hz, 2H); 6.90 (m, 4H); 6.60 (d, *J*=8Hz, 2H); 3.812 (s, 6H); 3.766 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.6, 56.8, 113.5, 114.6, 114.8, 129.2, 129.9, 130.5, 159.9, 160.8, 176.8.

2-(2,4-dichlorophenyl)-4,5-bis(4-methoxyphenyl)-1H-imidazole (TM-4);

Yield 42%; m.pt 78-81 ºC; m/z 400 [M+H$^+$]; IR (KBr, *v* max cm^{-1}) N-H(3674), Ar C-H str(3000),Ar C=C str(1585), C-N (1247),C-O str(1105), C=N(1700); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.953 (s, 1H); 7.76 (d, *J*=2Hz, 1H); 7.61 (d, *J*=2Hz, 1H); 7.39 (m, 4H); 7.24 (m, 4H); 3.76 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9, 114.5, 126.9, 128.5, 129.5, 129.9, 130.4, 130.8, 133.6, 135.7, 136.9, 147.5, 161.1.

2-(3,4,5-trimethoxyphenyl)-4,5-bis(4-methoxyphenyl)-1H-imidazole (TM-5);

Yield 49%; m.pt 217-218 ºC; m/z 400 [M+H$^+$]; IR (KBr, *v* max cm^{-1}) N-H(3546), aliphatic C-H str(2938), Ar C=C str(1588) , Ar C-N (1245), C-O str(1125); ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.44 (s, 1H, NH); 7.47 (d, *J*=8.4Hz, 2H) ; 7.37 (m, 4H); 7.01 (d, *J*=8.4Hz, 2H); 3.86 (s, 9H); 3.55 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.5, 58.9, 60.1, 104.5, 114.9, 124.8, 139.1, 129.5, 130.6, 139.9, 154.5, 160.2, 177.2.

4-(4,5-bis(4-methoxyphenyl)-1H-imidazol-2-yl)-2-ethoxyphenol (TM-6);

Yield 53%; m.pt 185-188 ºC; m/z 400 [M+H$^+$]; IR (KBr, *υ* max cm^{-1}) OH str(3392), aliphatic C-H str(2979), N-H (3738), Ar C=C str(1510), C-O str(1123), C-N(1250); ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.23 (s, 1H, NH); 7.59 (s, 1H); 7.48 (d, *J*=8Hz, 1H); 7.41 (m, 5H); 6.84 (m, 4H); 4.07 (q, 2H); 3.76 (s, 6H); 1.27 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.5, 55.5, 63.9, 113.1, 114.9, 115.4, 122.2, 124.1, 128.3, 129.5, 130.5, 147.9, 148.3, 160.1, 176.9.

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4,5-bis(4-methoxyphenyl)-2-(4-nitrophenyl)-1H-imidazole (TM-7);

Yield 44%; m.pt 178-179 ºC; m/z 400 [M+H$^+$]; IR(KBr, υ max cm^{-1}) N-H(3362), Ar C=C(1515,1597), aliphatic C-H str(2836), N-O (1335), C-O str(1108), C-N(1248), C=N(1656); ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.72(s,1H,NH); 8.411 (m, 4H); 7.27 (m, 8H); 3.784 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.7, 114.6, 124.4, 127.1, 128.5, 129.2, 130.4, 135.1, 147.8, 160.4, 172.5.

2-(2,4-dimethoxyphenyl)-4,5-bis(4-methoxyphenyl)-1H-imidazole (TM-8);

Yield 40%; m.pt 126-127 ºC; m/z 400 [M+H$^+$]; IR (KBr, υ max cm^{-1}) N-H(3617), aliphatic C-H str(2929), C=N(1612), Ar C=C str(1507) C-N(1250), C-O str(1120); ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.51 (s, 1H, NH); 7.92 (d, *J*=8.4Hz, 1H); 7.42 (d, *J*=7.2Hz, 2H); 7.36 (d, *J*=7.6Hz, 2H); 6.97 (d, *J*=7.2Hz, 2H); 6.85 (d, *J*=6.8Hz, 2H); 6.64 (m, 2H); 3.90 (s, 3H); 3.83 (s, 3H); 3.79 (s, 3H); 3.75(s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.6, 55.8, 56.2, 99.5, 107.2, 110.3, 114.8, 128.4, 129.2, 129.6, 130.2, 148.7, 156.9, 160.5, 160.8.

4,5-bis(4-methoxyphenyl)-2-p-tolyl-1H-imidazole (TM-9);

Yield 35%; m.pt 116-118 ºC; m/z 400 [M+H$^+$]; IR(KBr, υ max cm^{-1}) N-H(3425), Ar C-H str(3001), aliphatic C-H str(2925), Ar C=C str(1503), C=N(1615), C-N(1246), C-O str(1108); ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.41 (s, 1H, NH); 7.93 (s, 2H); 7.43 (m, 4H); 7.25 (m, 2H); 6.85 (m, 4H); 3.76 (s, 6H); 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.2, 16.3, 114.9, 128.5, 128.8, 129.2, 129.5, 129.9, 130.6, 131.9, 161.2, 177.2.

2-(3,4-dimethoxyphenyl)-4,5bis(4-methoxyphenyl)-1H-imidazole (TM-10);

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Yield 41%; m.pt 106-108 ºC; m/z 400 [$M+H^+$]; IR (KBr, υ max cm^{-1}) N-H(3323), Ar C-H str(3073), aliphatic C-H str(2935), Ar C=C str(1509), C=N(1677), C-N(1249), C-O str(1137); ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.34 (s, 1H, NH); 7.63 (s, 2H); 7.40 (m,3H); 7.01 (m, 3H); 6.87 (m, 2H); 6.57 (m, 1H); 3.78 (s, 6H);3.75(s,3H);3.69(s,3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.3, 55.8, 56.6, 110.5, 112.3, 115.1, 122.8, 123.2, 128.9, 129.6, 130.6, 150.1, 150.8, 160.6, 177.2.

In vitro screening

The synthesized compounds were screened for their anti microbial[7] activity by cup plate method and anti tubercular activity by micro plate alamar blue assay[8,9,10] using standard protocols from literature. The zone of inhibition from the anti microbial assay and the minimum inhibitory concentration from the MABA assay were recorded.

Docking analysis

FtsZ is a protein involved in bacterial cell division. The binding affinity of substituted benzimidazoles to the FtsZ protein has previously been investigated by others.[11] Docking studies for the triphenyl imidazoles with Mycobacterium FtsZ protein have been conducted to explore a probable mechanism of action for the synthesized compounds using Molegro Virtual Docker, version 6.0. The protein data bank ID of the selected protein is 1RQ2. The structure of the protein was validated by generating a Ramachandran plot using Mol Probity server (Figure 2). Five binding pockets were identified in the protein. Citric acid was the co-crystallized ligand and it was found to bind in the fifth cavity. The docking poses were analyzed using PyMOL viewer.

Results

None of the synthesized compounds showed anti microbial activity comparable to that of the standard drug Rifampicin. Compound TM6 exhibited the highest anti tubercular activity in the series and its activity is comparable to that of the standard drugs Pyrazinamide and Ciprofloxacin (Table 1). Compound TM9 showed anti tubercular activity comparable to that of Streptomycin. The results of docking analysis correlated with the results from MABA assay. Compound TM6 showed the highest Mol Dock score of -147.569 KJ/Mol (Table 2).

Discussion

Compound TM6 with an ethoxy group at the third position and a hydroxyl group at the fourth position showed the highest anti tubercular activity, compounds with a methoxy group have not shown similar activity. This shows that the presence of the electron releasing ethoxy group at position 3 and hydroxyl group at the fourth position is essential for activity. Replacement of the ethoxy group with a methoxy group or the imidazole obtained from p-anisaldehyde failed to show activity comparable to that of TM6. The compound TM6 showed hydrogen bonding interactions with the following residues; Asn 22(B), Asn 25(B), Gln 30(B) and Asp 51(B) in the receptor pocket (Figure 3). The total energy due to hydrogen bond interactions between the ligand and protein is -6.232. The hydroxyl group is responsible for two hydrogen bond interactions of the order 2.6 Å and 1.9 Å. The hydrogen atom on the imidazole ring is responsible for a hydrogen bond interaction of the order 2.9 Å (Figure 4, 5).

Compounds with methoxy groups at various positions have not shown anti tubercular activity comparable to that of the standard; this shows that replacement of the ethoxy group with a methoxy group leads to a drastic decrease in anti tubercular activity. Out of these four compounds, TM3 with a single methoxy group (at the para position) showed the best activity.

This shows that increasing the number of methoxy groups leads to a decrease in activity. Compounds with strongly electron releasing groups like the hydroxyl group or the dimethylamino group at the para position; compounds TM2 and TM1 respectively showed anti tubercular activity lesser that of the standard. Compound TM9, with a methyl group at the para position showed good anti tubercular activity. Compounds with other electron releasing groups at other positions have not shown activity comparable to that of the standard.

The docking results indicate that the probable mechanism of action of the triphenyl imidazoles as inhibitors of the FtsZ protein. Further *in vitro* binding studies need to be carried out to assess the reliability of the docking results.

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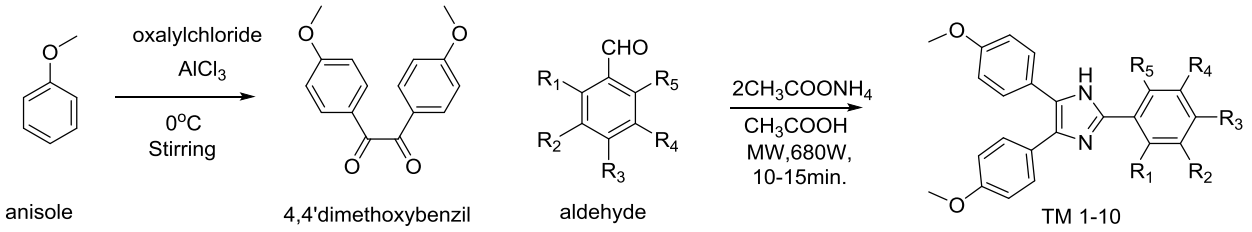
2	Ramachandran Plot analysis of protein using Mol Probity server.
3	Docking pose of TM6 into the receptor pocket of 1RQ2
4	View of TM6 in the receptor pocket of 1RQ2 showing hydrogen bond interactions.
5	View of TM6 in the receptor pocket of 1RQ2 showing hydrogen bond interactions.



Code	R_1	R_2	R_3	R_4	R_5
TM1	-H	-H	$-N(CH_3)_2$	-H	-H
TM2	-H	-H	-OH	-H	-H
TM3	-H	-H	$-OCH_3$	-H	-H
TM4	-H	-H	-Cl	-H	-H
TM5	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	-H
TM6	-H	$-OC_2H_5$	-OH	-H	-H
TM7	-H	-H	$-NO_2$	-H	-H
TM8	$-OCH_3$	-H	$-OCH_3$	-H	-H
TM9	-H	-H	$-CH_3$	-H	-H
TM10	-H	$-OCH_3$	$-OCH_3$	-H	-H

Figure I: Scheme for the synthesis of triphenylimidazoles

S.No.	Compound code	Anti microbial activity (mm)						Anti TB
		S.aureus		*E.coli*		*A.oryzae*		*M.tuberculosis*
		100	150	100	150	100	150	MIC value (µg/ml)
		Zone Of Inhibition						
1.	TM-1	-	-	-	-	-	-	25
2.	TM-2	8	11	6	9	4	6	12.5
3.	TM-3	-	-	-	-	4	6	12.5
4.	TM-4	-	-	-	-	5	7	25
5.	TM-5	-	-	-	-	-	-	50
6.	TM-6	-	-	-	-	6	10	3.12
7.	TM-7	5	5	6	7	-	-	50
8.	TM-8	-	4	-	6	-	-	50
9.	TM-9	-	-	5	6	5	9	6.25
10.	TM-10	-	5	-	7	8	10	50

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Standard drugs used and their activity				
Rifampicin	At 10µg, 21mm	At 10µg, 21mm	-	-
Fluconazole	-		At 10µg, 18mm	-
Pyrazinamide	-		-	3.125
Streptomycin	-		-	6.25
Ciprofloxacin	-		-	3.125

Table 1: Results of anti microbial and anti tubercular activity screening of the synthesized.

Table 2: Docking results on FtsZ protein (PDB ID: 1RQ2).

Compound Code	Mol Dock Score
TM1	-137.609
TM 2	-137.583
TM 3	-132.816
TM 4	-119.832
TM 5	-132.784
TM 6	-147.569
TM 7	-124.664
TM 8	-144.575
TM 9	-106.449
TM 10	-126.218

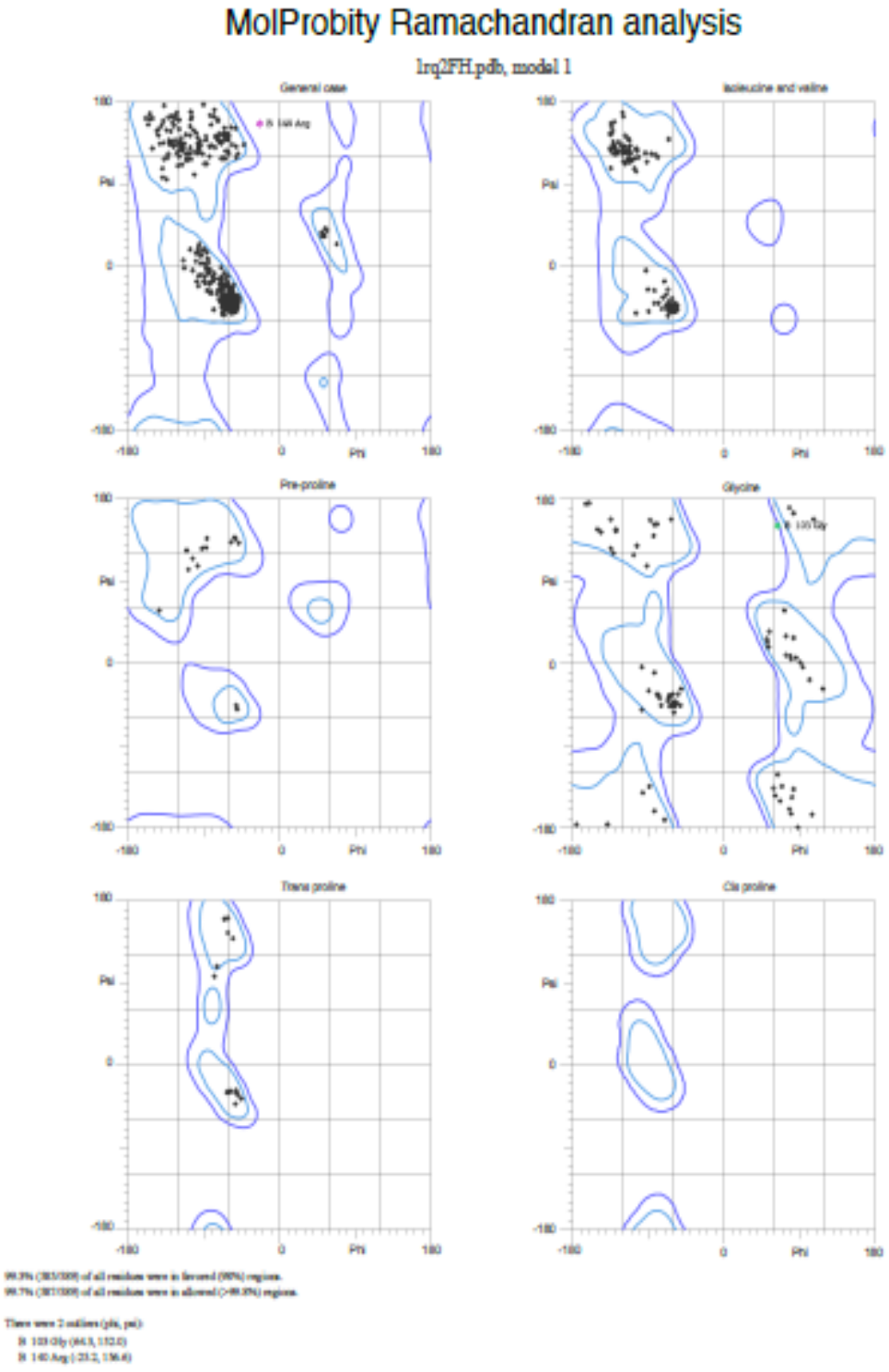

Figure 2: Ramachandran Plot analysis of protein using Mol Probity server.

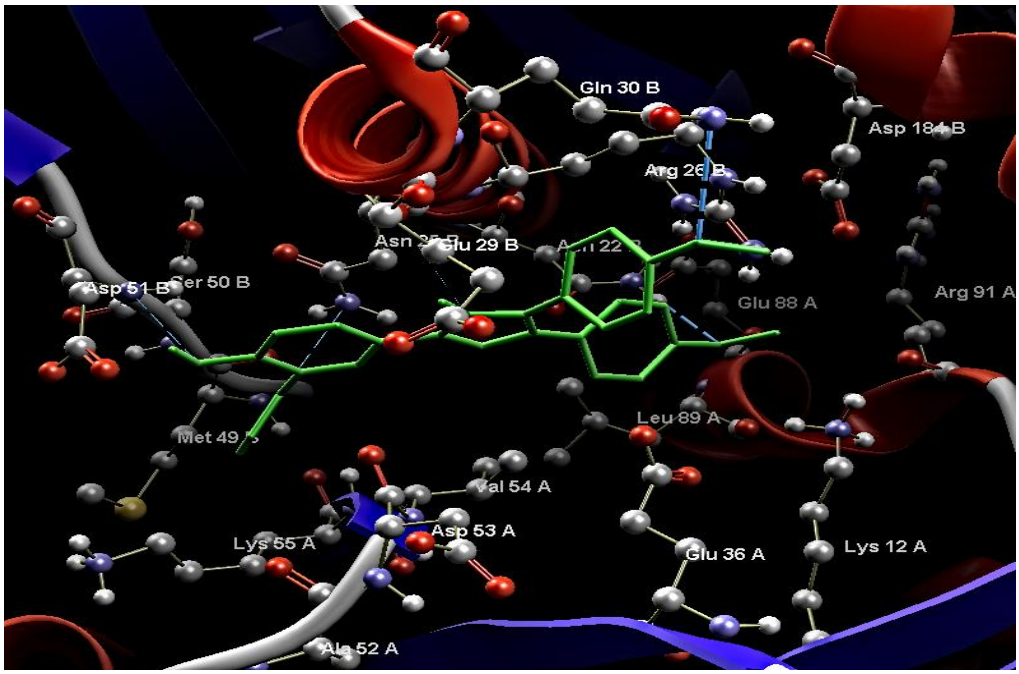

Figure 3: Docking pose of TM6 into the receptor pocket of 1RQ2 (Protein resolution: 1.86 Å, Method: X-ray diffraction, R Value Free: 0.222).

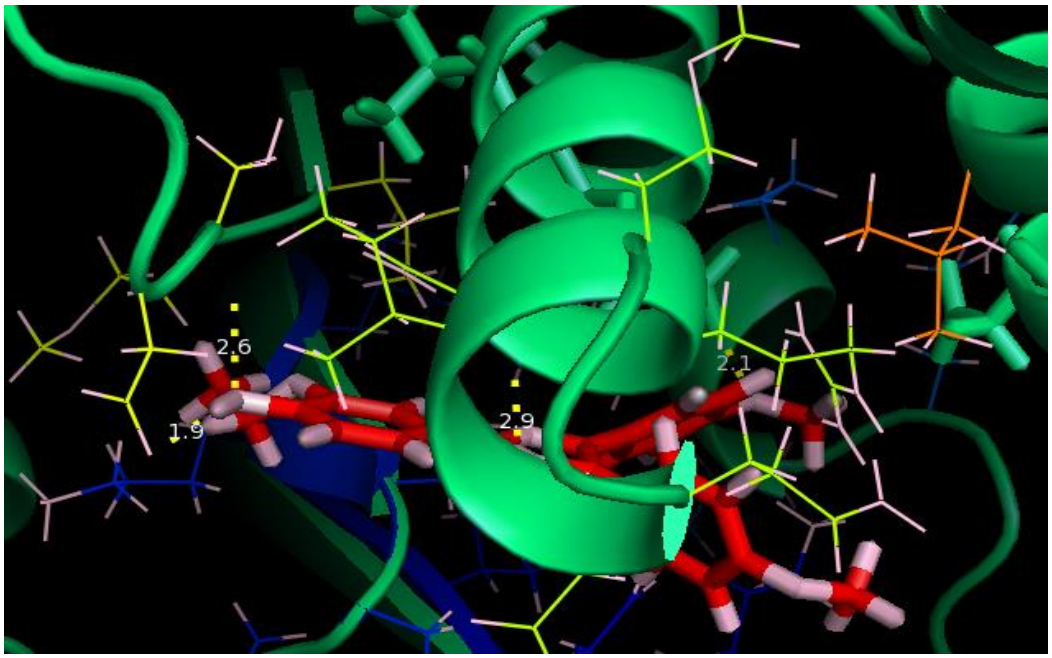

Figure 4: View of TM6 in the receptor pocket of 1RQ2 showing hydrogen bond interactions.

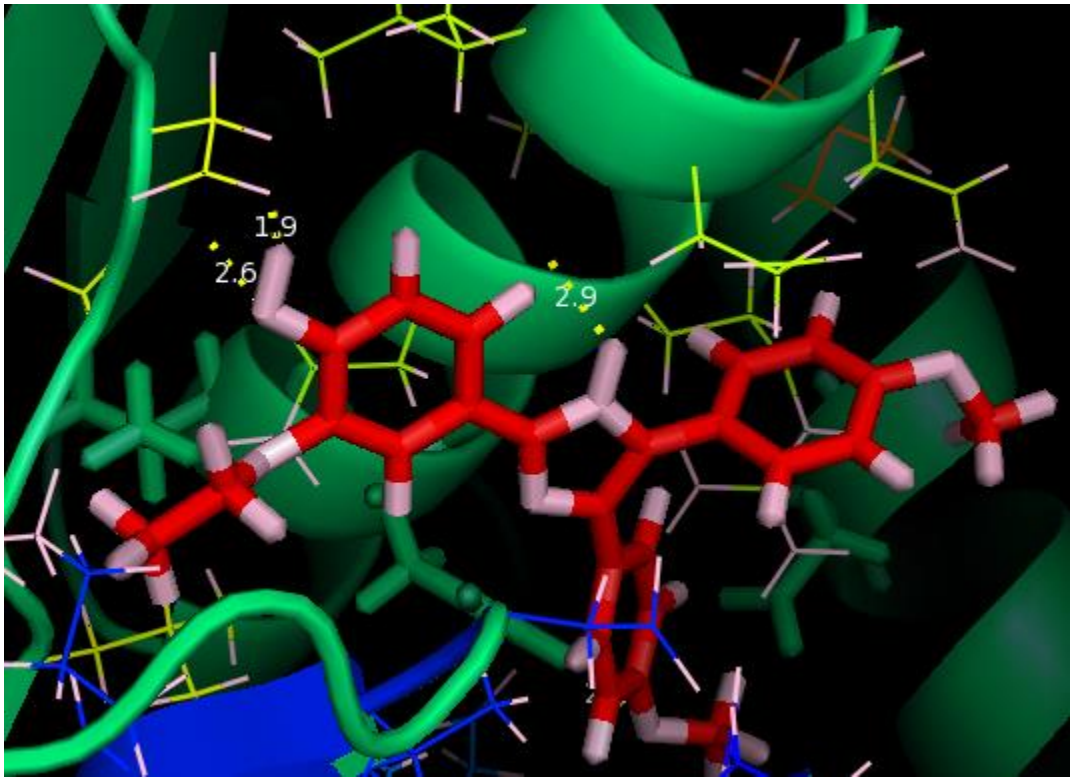

Figure 5: View of TM6 in the receptor pocket of 1RQ2 showing hydrogen bond interactions.