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Design of small chain peptides as inhibitors of *Mycobacterium tuberculosis* Protein Tyrosine Phosphatase

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Abstract

Tuberculosis is one of the leading causes of death caused by *Mycobacterium tuberculosis*. To prevent multi-drug resistant and extensively drug resistant TB, there is a need to develop safer smaller chain peptide. Protein tyrosine phosphatase B (mPTPB) is chosen as a target for our study which is crucial for Mtb intracellular survival resulting in decreased macrophage apoptotic activity and secretion of inflammatory cytokines IL-6. Our aim is to study proline-based dipeptide as potent inhibitors of mPTPB. With proline as vital amino acid, different smaller chain dipeptides were selected. Swiss DOCK online tool was used for docking (PDB code: 1c83) along with Chimera and Discovery studio visualizer for detailed investigation of 3D interactions. Pro-Arg was found to be the most promising lead as potent mPTPB inhibitor with ΔG value of -10.11 Kcal/mol in comparison to cefsulodin (ΔG = -11.54 Kcal/mol) followed by Pro-Cys (ΔG = -9.37 Kcal/mol) and Pro-Val (ΔG = -9.06 Kcal/mol) respectively. Additionally, Pro-Arg dipeptide showed good conventional hydrogen bonding with the bond distance as 2.72 A°, 1.90 A° and 2.13 A° (Arg 254: HH 21; Asp 29: H5; Gly 259: HN-), in comparison to cefsulodin with best bond distances of 2.62 A° and 2.01 A° respectively (Arg 254: HH11; Gly 259: HN-). The results of cefsulodin's conventional hydrogen bond with respective bond distance as 2.69 A° and 2.94 A° (Arg 24: HH21; Gln 262: HE 22), clearly indicated that the interaction with arginine and glutamine site of mPTPB is attributed to best therapeutic antitubercular candidate.

Key words: *Dipeptides, Docking, Protein Tyrosine Phosphatase B (mPTPB)*

Introduction

Tuberculosis is an infectious disease caused by intracellular pathogen *Mycobacterium tuberculosis* (Mtb). According to Tuberculosis report given by WHO in 2018, an estimated population of 10 million developed TB and over 1.2 million individuals succumbed to it. According to WHO, there are around 50 antibiotics currently available in the clinical pipeline [1]. However, the pharmaceutical industry is not sufficient to rectify the problem of increasing antibiotic resistance. This is mainly due to factors such as inadequate diagnosis, lack of patient's compliance, poor tabledrugs and mutation transfer [2].

Protein tyrosine phosphatases (PTPs) catalyzes the removal of the phosphoryl groups from substrate proteins. In conjunction with protein tyrosine kinases, protein tyrosine phosphatases carry out regulation of cellular functions such as cell growth, proliferation, differentiation, metabolism and immune response. To evade the host environment of macrophages, bacterial pathogens have developed diverse strategies to change the host signaling processes [3]. Mtb encodes for two PTPs, Mycobacterium protein tyrosine phosphatase A (mPTPA; RV2234) and Mycobacterium protein tyrosine phosphatase B (mPTPB; RV0153c), which are secreted by bacterium into the cytoplasm of host macrophages [4]. Both the phosphatases directly alter the host signaling to evade the antimicrobial functions of the host, thereby promoting Mtb survival within the macrophages [5,6]. Hence mPTPA and mPTPB represents striking targets for drug development of anti-TB drugs. Upon Mtb infection, macrophages decrease secretion of inflammatory cytokines and suppresses macrophages apoptosis [7], which indicates that both mPTPA and mPTPB are inevitable for Mtb intracellular survival. mPTPB also promotes macrophage survival by augmenting Akt phosphorylation and suppressing caspase-3 activity [6]. All the above research findings suggest that mPTPB acts to interfere with host defense mechanisms by blocking the bactericidal immune responses and increasing macrophage survival.

Charlotte *et al.*, in 2001 have reported the activity of antitubercular peptide containing proline arginine rich amino acid for potent in vitro activity [8]. Chen *et al.*, in 2010 have found that mPTPB is an essential virulence factor needed for Mtb survival in host macrophages with screening of numerous compounds against mPTPB and identifies several 2-oxo-1,2-dihydrobenzo[cd]indole-6-sulfonamide and piperazinyl-thiophenyl-ethyl-oxalamide derivatives as novel mPTPB inhibitors [9].

He *et al.*, in 2013 have investigated a benzofuran salicylic acid scaffold as highly potent and selective mPTPB inhibitor with IC50 value of 38 nM [10]. He *et al.*, in 2014 have successfully developed a small molecule salicylic acid based and drug like mPTPB inhibitor with an IC50 of 2 µM for targeting tuberculosis [11]. In 2015, He et al. have identified α-sulfophenyl acetic amide (SPAA) from the third generation β-lactam cephalosporin antibiotic cefsulodin as most potent and selective mPTPB inhibitor with ki value of 7.9 nM and extremely high ligand efficiency of 0.46 [12].

Chen *et al.*, in 2016 have reported that bostrycin and one of its analogues exhibited inhibitory activity against mPTPB as potent antitubercular agent (IC50 = 327.6 µM and 64.6 µM) [13]. In 2021, Chen et al. have isolated fusarielins M, fusarielins N and fusarielins G from marine-derived fungus Fusarium graminearum and identified fusarielins M as the most potent compound against mPTPB with its IC50 of 1.05±0.08 µM and an inhibition constant (Ki) of 1.03±0.39 µM [14].

Erik *et al.*, in 2018 have reported the activity of plectasin as an antifungal peptide against Mycobacterium species [15]. Zhang *et al.*, in 2019 have identified thiobarbiturate-based drug like mPTPB non-competitive inhibitor with a Ki of 2.47 µM and IC50 of 22.4 µM through structure-based virtual screening

strategy [16]. Shamima *et al.*, in 2019 have reported the use of amino acids to form peptides showing activity in prediction, identification and treatment of TB [17]. Our objective is to design a smaller chain peptide lead, preferably a dipeptide as potent inhibitors of *Mycobacterium tuberculosis* protein tyrosine phosphatase B through a detailed docking investigation approach.

Materials and Methods

Materials:

The Configuration of the system in which docking study was performed along with other online tools of drug design was running on 1.80 GHZ Hewlett Packard Lap top Intel ® Core ™ is processor, 8 GB RAM and 64-Bit window operating system.

Methodology:

Based on thorough literature studies [18,19], seventeen smaller chain dipeptide molecules Pro- Ala, Pro-Arg, Pro-Asp, Pro-Cys, Pro-Glu, Pro-Gln, Pro-Gly, Pro-His, Pro-Ile, Pro-Leu, Pro-Met, Pro-Phe, Pro-Ser, Pro-Lys, Pro-Trp, Pro-Tyr, Pro-Val were taken for the study and subjected to SWISS ADME online to check the drug likeliness property of Lipinski rule of five along with pharmacokinetics [20]. Bioavailability of all compounds were tested against permeability glycoprotein (PGP) substrate along with metabolic profile of drug against various cytochrome P450 inhibition (CYP1 A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4).

Molinspiration cheminformatics online tool was used for all the seventeen smaller chain dipeptides to predict the various physicochemical parameters along with bioactivity score on enzyme inhibition as a general way of identifying the best suitable potent molecule [21,22]. The same set of compounds were subjected to way 2 drug online PASS software [23] for identifying the activity against a specific target with possible adverse and toxic effects. Online free Swiss Dock tool [24] was used for docking the selective dipeptides based on preliminary results obtained with a target protein, protein tyrosine phosphatase B (mPTPB), which are consecutively obtained from zinc database I. D and PDB code (1c83). The standard drug used for comparison of docking with the tested dipeptides is cefsulodin. Visualization and detailed analysis of bonding interaction in 3D and 2D was further done with UCSF Chimera tool and Biovia Discovery studio visualizer.

Results and Discussion

Swiss ADME tool was used for all the selected seventeen smaller chain dipeptides with the physicochemical properties (molecular weight, number of heavy atoms, number of aromatic heavy atoms, number of rotatable bonds, number of hydrogen bond donors, number of hydrogen bond acceptors, molar refractivity, total polar surface area), lipophilicity (consensus log Po/w), water solubility (log S), all ADME properties (GI absorption, blood brain barrier permeation, p-gp substrate, cytochrome P450 inhibitors-CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, skin permeation (log kp), Lipinski rule of drug likeness characteristics, bioavailability score, lead likeness and synthetic accessibility as multiple parameters and the results of all seventeen dipeptides are expressed in Table 1a and 1b.

Further, the same set of compounds were subjected to Molinspiration cheminformatics tools and the results are expressed in Table 2. This tool has clearly indicated that most of the dipeptides were filtered off with relative bioactivity score as general enzyme inhibitors and found that only 7 dipeptides were selected for further study, viz., Pro-Val, Pro-Cys, Pro-Arg, Pro-Trp, Pro-Tyr, Pro-His and Pro-Phe.

In addition, to access the further bioactivity of smaller chain dipeptides against a specific target for tuberculosis with possible adverse and toxic effects prediction, PASS online way 2 drug software tool was used for all the seventeen dipeptide molecules with their isomeric SMILES notation. The results of PASS activity value are expressed in Table 3.

By comparing the Molinspiration data and PASS data, only 4 dipeptides were selected for further docking investigation, namely pro-Arg, Pro-Cys, Pro-Val and Pro-Trp against the

specific target mPTPB (PDB Code: 1c83) with cefsulodin as the standard drug. The results of docking study with ΔG value (Kcal/mol) are expressed in Table 4.

The detailed docking investigation of all tested compounds, Pro-Arg, Pro-Trp, Pro-Cys, Pro-Val, Pro-Tyr, Pro-His, Pro-Phe and cefsulodin standard are tabulated in Table 5, and their best molecules docking interactions are clearly visualized in Figures 1-8.

Among the seven dipeptide templates (Pro-Val, Pro-Cys, Pro-Arg, Pro-Trp, Pro-Tyr, Pro-His, Pro-Phe) obtained, Pro-Arg was found to be the most promising compound as potent mPTPB inhibitor with ΔG value of -10.11 Kcal/mol (full energy: 1983.90 Kcal/mol) in comparison to the standard drug cefsulodin having ΔG value of -11.54 Kcal/mol (full energy: 1773.45 Kcal/mol) followed by Pro-Cys (ΔG value = -9.37 K. Cal/mol) and Pro-Val (ΔG value = -9.06. Cal/mol) respectively. In addition, the dipeptide Pro-Arg showed good conventional hydrogen bond interaction with the bond distance as 2.72 A°, 1.90 A° and 2.13 A° (Arg 254: HH 21; Asp 29: H5; Gly 259: HN-), as compared to cefsulodin with best bond distances as 2.62 A° and 2.01 A° respectively (Arg 254: HH 11; Gly 259: HN-). The results of cefsulodin's conventional hydrogen bond have extra features with respective bond distance as 2.69 A° and 2.94 A° (Arg 24: HH 21; Gln 262: HE 22), clearly indicated that the interaction with arginine and glutamine site of mPTPB is attributed to best therapeutic potency of antitubercular drug. As the hydrogen bond interaction indicates a more stable complex between the chosen dipeptide & protein target, all the observed interactions of dipeptide Pro-Arg is less than the ideal bond distance (3 A°).

Detailed docking investigation identifies the most potent drug as Pro-Arg with ΔG value as -10.11 Kcal/mol as compared with standard cefsulodin (containing 4 H-bond interactions Arg 254, Arg 24, Gln 262 and Gly 259), our potent drug Pro-Arg has shown 3 hydrogen bond interactions (Arg 254, Asp 29 and Gly 259) in which, instead of Arg 24 and Gln 259 in standard, our drug shows only Asp 29 active site as H-bond interaction.

The binding mode of Pro-Arg was further explored via molecular docking (Fig. 1c) which suggested that Pro-Arg binds to the active site of mPTPB, forming a hydrogen bond with the side chain of Asp 29; this is unique in the p-loop of mPTPB and the contact between Pro-Arg and Asp 29 in the catalytic loop provides a potential basis for inhibitor. Selectivity (bond distance: 1.90 A°), which may not be seen in the standard drug cefsulodin's molecular docking. Therefore, Pro-Arg shows great potential as an anti-TB drug candidate with respect to detailed docking investigation alone.

As from SWISS ADME pharmacokinetic profile data of Table 1, the most potent drug Pro-Arg through docking score seems to be identified with permeability glycoprotein as suitable substrate and hence the drug Pro-Arg may not be chosen further as lead molecule for synthesis, since their efflux is more with reduced bioavailability and probably may fail in clinical phase II stage if chosen as lead.

The second most potent drug through docking study was identified as Pro-Tyr with ΔG values as -9.68 Kcal/mol followed by Pro-Trp (ΔG value = -9.55 Kcal/mol), Pro-His (ΔG value = -9.52 Kcal/mol) and Pro-Phe (ΔG value = -9.51 Kcal/mol) respectively in comparison with standard cefsulodin (ΔG value = -11.54 Kcal/mol). As compared with H-bond interaction with cefsulodin, Pro-Tyr has 4H-bond interactions in which 3 of them are almost similar to standard Cefsulodin with active site residues of Arg-254 (2.96 A° versus standard 2.62 A°), Arg-24 (3.08 A° versus standard 2.69 A°) and Gln-262 (2.83 A° versus standard 2.94 A°), Whereas the 4th active site residue of H-bond interaction of Gly 259 (2.01 A°) of Cefsulodin is replaced by Ala 27 (1.96 A°) residue in Pro-Tyr lead. Similarly, Pro-Trp has 4H-bond interactions which are slightly different without having glycine active site for H-bond interaction. Arg 254 active site shows 4 conventional H-bond interactions in which at HH 11 itself shown 2 interactions with ligand 1:

O18 and O3 with 2.70 A° and 2.61 A° respectively.

Targeting mPTPB for the treatment of TB offers an alternative strategy to the traditional antibiotic approaches and could provide therapeutic agents that overcome the antibiotic resistance. In practice, the success of a small molecule- based drug discovery project depends on the availability of potent and specific inhibitors with drug-like properties.

Many synthetic PTP inhibitors are based on pTyr mimetic as the central building block [25]. This approach has the advantage that it is relatively easy to find starting points for inhibitors. However, the phosphotyrosine mimetic poses a challenge to introduce sufficient selectivity, because the phosphotyrosine-binding pockets of different PTP enzymes are similar. This problem is reinforced by the observation that isolated PTP catalytic domains often exhibit modest substrate specificity, with cellular localization and the activities of appended substrate-binding domains enhancing selectivity in vivo [26]. This may be the case observed with our smaller chain peptide Pro-Tyr with similar probable mode of action specificity.

Significant advances were made in the mPTPB inhibitor development by Ghattas and co-workers, conducted a druggability assessment on 17 PTPs [27]. Their assessment revealed that only two of 17 PTPs, namely mPTPB and GLEP-1 are likely druggable due to their large hydrophobic active sites.

Due to the presence of aromatic amino acid tyrosine in Pro-Tyr and tryptophan in Pro-Trp, we may predict that the hydrophobic interaction is possible with dipeptide (Tyr and Trp) and the active site of mPTPB (Tyr). Mycobacterium phosphatases have emerged as key players, especially mPTPB in interfering with the MAPK and AKT signal transduction pathways. The potent dipeptide Pro-Tyr and Pro-Trp discussed above are not only useful as starting points for the development of therapeutic agents for TB but can also serve as molecular probes in understanding the structure and function of these enzymes in TB pathogenesis. Hence PTPB inhibition by small molecules could impact Mtb survival in the host and open the way for the development of innovative therapeutic strategies. Particularly, the localization outside of the mycobacterium cell wall, which is difficult to penetrate, renders these enzymes attractive targets with utmost safety, against any resistance type, MDR and XDR tuberculosis. Further in vitro investigation of tuberculosis with automated microbial detection system is necessary for subjecting the potent lead Pro-Tyr and Pro-Trp after synthesizing the same.

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Table 1a: Swiss ADME Physicochemical characteristic features of smaller chain dipeptides

S. No.	Dipeptides	Physicochemical Properties								Lipophilicity Consensus	
		Mol. Weight	No. of Heavy Atoms	No. of Aromatic Heavy Atoms	No. of Rotatable bonds	No. of H-Bond acceptors	No. of H-Bond donors	Molar Refractivity	TPSA A^2	Log $P_{o/w}$	H_2O Solubility Log S (ESOL)
1	Pro-Ala	186.21	13	0	4	4	3	49.95	78.43	-0.89	1.62
2	Pro-Arg	271.32	19	0	8	5	5	73.47	142.83	-1.58	2.11
3	Pro-Asp	230.22	16	0	6	6	4	56.52	115.73	-1.63	2.03
4	Pro-Cys	218.27	14	0	5	4	3	57.88	117.23	-0.93	1.54
5	Pro-Glu	244.24	17	0	7	6	4	61.33	115.73	-1.24	1.78
6	Pro-Gln	243.26	17	0	7	5	4	62.47	121.52	-1.62	2.19
7	Pro-Gly	172.18	12	0	4	4	3	45.14	78.43	-1.17	1.76
8	Pro-His	252.27	18	5	6	5	4	66.58	107.11	-1.10	1.24
9	Pro-Ile	228.29	16	0	6	4	3	64.37	78.43	0.04	0.75
10	Pro-Leu	228.29	16	0	6	4	3	64.37	78.43	0.04	0.66
11	Pro-Met	246.33	16	0	7	4	3	67.15	103.73	-0.38	1.04
12	Pro-Phe	262.30	19	6	6	4	3	74.43	78.43	0.46	0.00
13	Pro-Ser	202.21	14	0	5	5	4	51.11	98.66	-1.69	2.25
14	Pro-Lys	243.30	17	0	8	5	4	67.07	104.45	-0.88	1.68
15	Pro-Trp	301.34	22	9	6	4	4	86.29	94.22	0.50	-0.62
16	Pro-Tyr	278.30	20	6	6	5	4	76.46	98.56	0.02	0.14
17	Pro-Val	214.26	15	0	5	4	3	59.56	78.43	-0.29	1.00

Table 1b: Swiss ADME pharmacokinetic profile and drug likeness characteristics of smaller chain dipeptides

S. No.	Dipeptides	Pharmacokinetic Properties									Drug Likeness			
		GI Absorption	BBB Permeant	PGP Substrate	CYP 1A2 Inhibitor	CYP 2C19 Inhibitor	CYP 2C9 Inhibitor	CYP 2D6 Inhibitor	CYP 3A4 Inhibitor	Log K_p (Skin Permeation) CM/S	Lipinski	Bioavailability Score	Lead Likeness	Synthetic Accessibility
1	Pro-Ala	High	No	No	No	No	No	No	No	-10.08	Yes	0.55	No	2.26
2	Pro-Arg	Low	No	Yes	No	No	No	No	No	-11.46	Yes	0.55	No	3.14
3	Pro-Asp	High	No	No	No	No	No	No	No	-10.97	Yes	0.56	No	2.74
4	Pro-Cys	High	No	No	No	No	No	No	No	-10.34	Yes	0.55	No	2.67
5	Pro-Glu	High	No	No	No	No	No	No	No	-10.80	Yes	0.56	No	2.72
6	Pro-Gln	Low	No	No	No	No	No	No	No	-11.26	Yes	0.55	No	2.71
7	Pro-Gly	High	No	No	No	Yes	No	No	No	-10.06	Yes	0.55	No	1.91
8	Pro-His	High	No	No	No	No	No	No	No	-10.60	Yes	0.55	Yes	2.98
9	Pro-Ile	High	No	Yes	No	No	No	No	No	-9.51	Yes	0.55	No	2.78
10	Pro-Leu	High	No	Yes	No	No	No	No	No	-9.40	Yes	0.55	No	2.59
11	Pro-Met	High	No	Yes	No	No	No	No	No	-10.00	Yes	0.55	No	2.99
12	Pro-Phe	High	No	No	No	No	No	No	No	-9.37	Yes	0.55	Yes	2.58
13	Pro-Ser	High	No	No	No	No	No	No	No	-10.93	Yes	0.55	No	2.42
14	Pro-Lys	High	No	Yes	No	No	No	No	No	-10.60	Yes	0.55	No	2.74
15	Pro-Trp	High	No	No	No	No	No	No	No	-9.26	Yes	0.55	Yes	2.95
16	Pro-Tyr	High	No	No	No	No	No	No	No	-9.72	Yes	0.55	Yes	2.62
17	Pro-Val	High	No	No	No	No	No	No	No	-9.68	Yes	0.55	No	2.48

Table 2: Molinspiration features with bioactivity score of smaller chain dipeptides

S. No.	Dipeptides	Mi Log P	TPSA	n Atoms	MW	nON	nOHNH	n violations	nRot	Volume	Enzyme Inhibitors Bioactivity Score
1	Pro-Ala	-2.29	78.42	13	186.21	5	3	0	3	173.21	0.11
2	Pro-Arg	-3.36	142.83	19	271.32	8	7	1	7	252.9	0.63
3	Pro-Asp	-3.11	115.72	16	230.22	7	4	0	5	200.5	0.42
4	Pro-Cys	-2.31	78.42	14	218.28	5	3	0	4	191.1	0.62
5	Pro-Glu	-2.84	115.72	17	244.25	7	4	0	6	217.3	0.47
6	Pro-Gln	-3.36	121.52	17	243.26	7	5	0	6	220.5	0.44
7	Pro-Gly	-2.07	78.42	12	172.18	5	3	0	3	156.6	0.08
8	Pro-His	-2.60	107.11	18	252.27	7	4	0	5	225.7	0.75
9	Pro-Ile	-1.00	78.42	16	228.29	5	3	0	5	223.4	0.30
10	Pro-Leu	-0.98	78.42	16	228.29	5	3	0	5	223.4	0.36
11	Pro-Met	-1.83	78.42	16	246.33	5	3	0	6	224.9	0.46
12	Pro-Phe	-0.83	78.42	19	262.31	5	3	0	5	244.9	0.39
13	Pro-Ser	-3.26	98.65	14	202.21	6	4	0	4	181.46	0.39
14	Pro-Lys	-2.78	104.45	17	243.31	6	5	0	7	235.14	0.59
15	Pro-Trp	-0.68	94.22	22	301.35	6	4	0	5	273.8	0.47
16	Pro-Tyr	-1.30	98.65	20	278.31	6	4	0	5	252.9	0.43
17	Pro-Val	-1.51	78.42	15	214.26	5	3	0	4	206.6	0.23

Table 3: Pass online bioactivity score & toxicity prediction of smaller chain dipeptides

S. No.	Dipeptides	Target for TB	Activity		Possible Adverse and Toxic Effects
			P_A	P_I	
1	Pro-Ala	Proteasome ATPase Inhibitor	0.724	0.011	Twitching, Shivering (may be)
2	Pro-Arg	Glutamate-5-Semialdehyde dehydrogenase Inhibitor	0.859	0.009	Nil
3	Pro-Asp	Membrane Integrity antagonist	0.749	0.010	Hyperglycemia (may be)
4	Pro-Cys	Proteasome ATPase Inhibitor	0.856	0.003	Stomatitis (may be)
5	Pro-Glu	Methylene THF reductase NADPH Inhibitor	0.910	0.006	Nil
6	Pro-Gln	Methylene THF reductase NADPH Inhibitor	0.817	0.016	Nil
7	Pro-Gly	Proteasome ATPase Inhibitor	0.844	0.004	Nil
8	Pro-His	No Specific Activity against TB	NA	NA	Nil
9	Pro-Ile	Proteasome ATPase Inhibitor	0.718	0.012	Nil
10	Pro-Leu	Proteasome ATPase Inhibitor	0.730	0.010	Nil
11	Pro-Met	Methylene THF reductase NADPH Inhibitor	0.841	0.013	Nil
12	Pro-Phe	NADPH-Cytochrome-C_2 reductase Inhibitors	0.722	0.014	Nil
13	Pro-Ser	Membrane Integrity antagonist	0.816	0.006	Nil
14	Pro-Lys	Membrane Integrity antagonist	0.840	0.005	Nil
15	Pro-Trp	Glutamate-5-Semialdehyde dehydrogenase Inhibitor	0.823	0.014	Nil
16	Pro-Tyr	Glutamate-5-Semialdehyde dehydrogenase Inhibitor	0.853	0.010	Nil
17	Pro-Val	Glutamate-5-Semialdehyde dehydrogenase Inhibitor	0.874	0.008	Nil
		Proteasome ATPase Inhibitor	0.753	0.008	

Table 4: Docking Score of dipeptides with ΔG value

S. No.	Dipeptide	Estimated ΔG (Kcal/mol)	Simple fitness	Full energy (Kcal/mol)	ΔG (Vdw)
1	Pro-Arg	-10.11	-50.03	-1983.90	-123.78
2	Pro-Val	-9.057	-14.41	-1802.86	-107.8
3	Pro-Cys	-9.37	-12.35	-1816.43	-97.93
4	Pro-Trp	-9.55	-3.01	-1809.13	-106.07
5	Pro-Phe	-9.51	-5.92	-1804.70	-117.65
6	Pro-His	-9.52	-23.3	-1833.64	-110.62
7	Pro-Tyr	-9.68	-7.32	-1816.73	-115.3
8	Cefsulodin	-11.54	93.09	-1773.45	-156.60

Table 5: Conventional Hydrogen bond interactions residues of various dipeptides & standard drug

S. No.	Dipeptide	Hydrogen bond interactions
1	Pro-Arg	Arg 254: HH 21 (2.72 A°) Asp 29: H5 (1.90 A°) Gly 259: HN (2.13 A°)
2	Pro-Val	Arg 254: HH 11 (2.79 A°) Arg 24: HH 21 (2.99 A°) Arg 24: HE (1.81 A°) Arg 254: HH 21 (1.84 A°)
3	Pro-Cys	Gln 262: HE 22 (1.95 A°) Gly 259: HN (2.37 A°)
4	Pro-Trp	Arg 254: HH 12; LIG1: O_{18} (2.91 A°) Arg 254: HH 11: LIG1: O_{18} (2.70 A°) Arg 254: HH 11: LIG1: O_3 (2.70 A°) Arg 254: HH 21: LIG1: O_3 (2.61 A°) Gln 262: HE 22: LIG1: O_1 (2.53 A°)
5	Pro-Phe	Arg 24: HH 21: LIG1: O_1 (2.35 A°) Gln 262: HE 22: LIG1: O_1 (2.43 A°) Gly 259: HN: LIG1: O_{15} (2.49 A°)
6	Pro-His	LIG1: H 26: Ser 28: O (2.77 A°) Arg 254: HH 11: LIG1: O_3 (2.01 A°) Gln 262: HE 22: LIG1: O_1 (2.76 A°) Arg 254: HH 21: LIG1: O_3 (2.23 A°) Arg 254: HH 12: LIG1: N_{11} (2.61 A°) Gly 259: HN: LIG1: N_{11} (2.46 A°) LIG1: H21: Asp 48: O (1.87 A°)
7	Pro-Tyr	LIG1: H 29: Gln 262: OE_1 (2.83 A°) Arg 24: HE: LIG1: O_1 (3.08 A°) Arg 254: HH 12: LIG1: O_{16} (2.96 A°) LIG1: H 23: Ala 27: O (1.96 A°)
8	Cefsulodin	Arg 254: HH 11 (2.62 A°) Arg 24: HH 21 (2.69 A°) Gln 262: HE 22 (2.94 A°) Gly 259: HN-(2.01 A°)

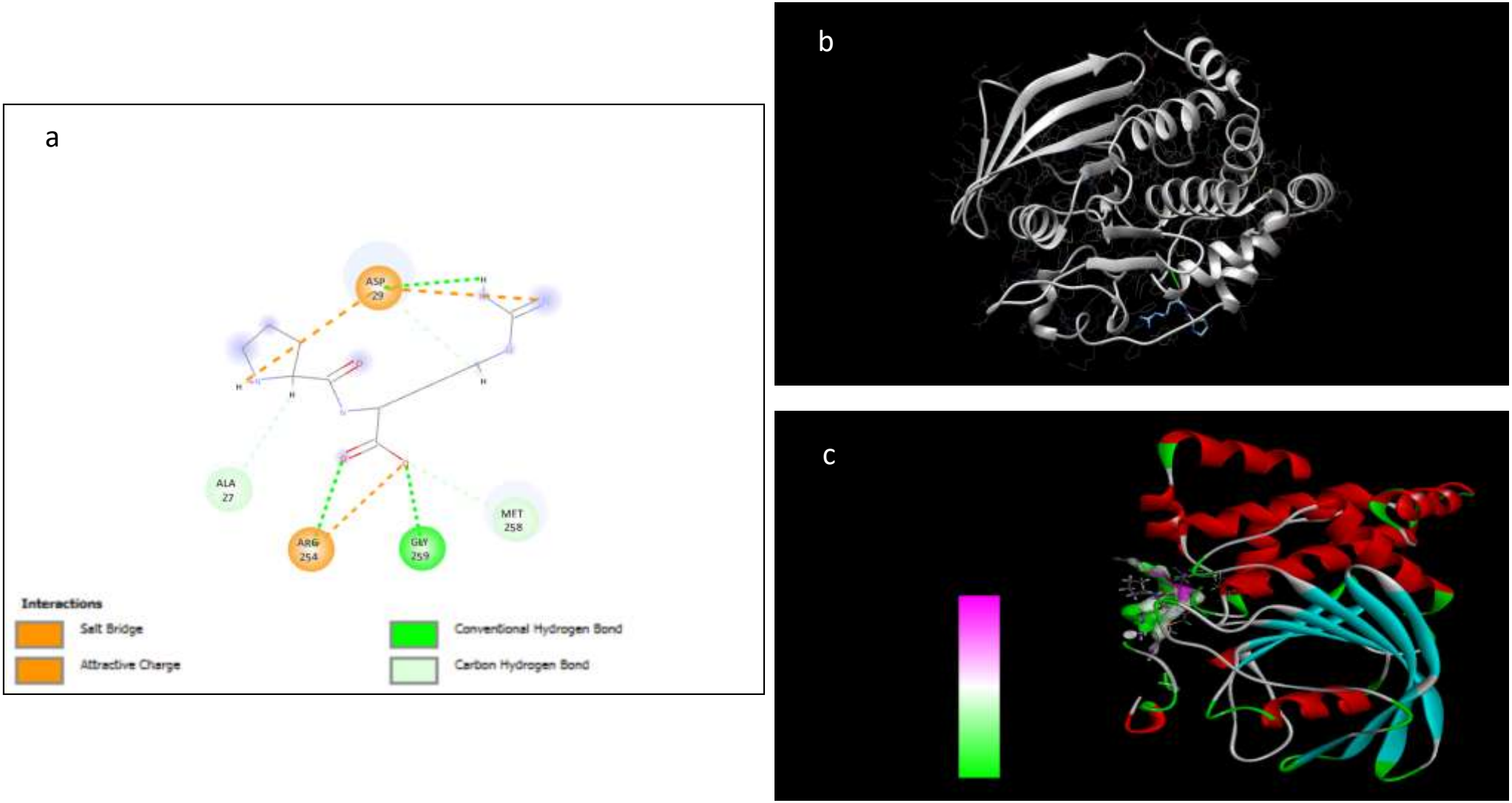

Figure 1: Docking Investigation of dipeptide Pro-Arg against the target Protein tyrosine phosphatase
a) 2D Map of ligand's non-bonded interactions b) Computed secondary structure of assignments of Pro-Arg against tyrosine phosphatase target using UCSF Chimera model c) 3D Docking of Pro-Arg with target in ribbon model

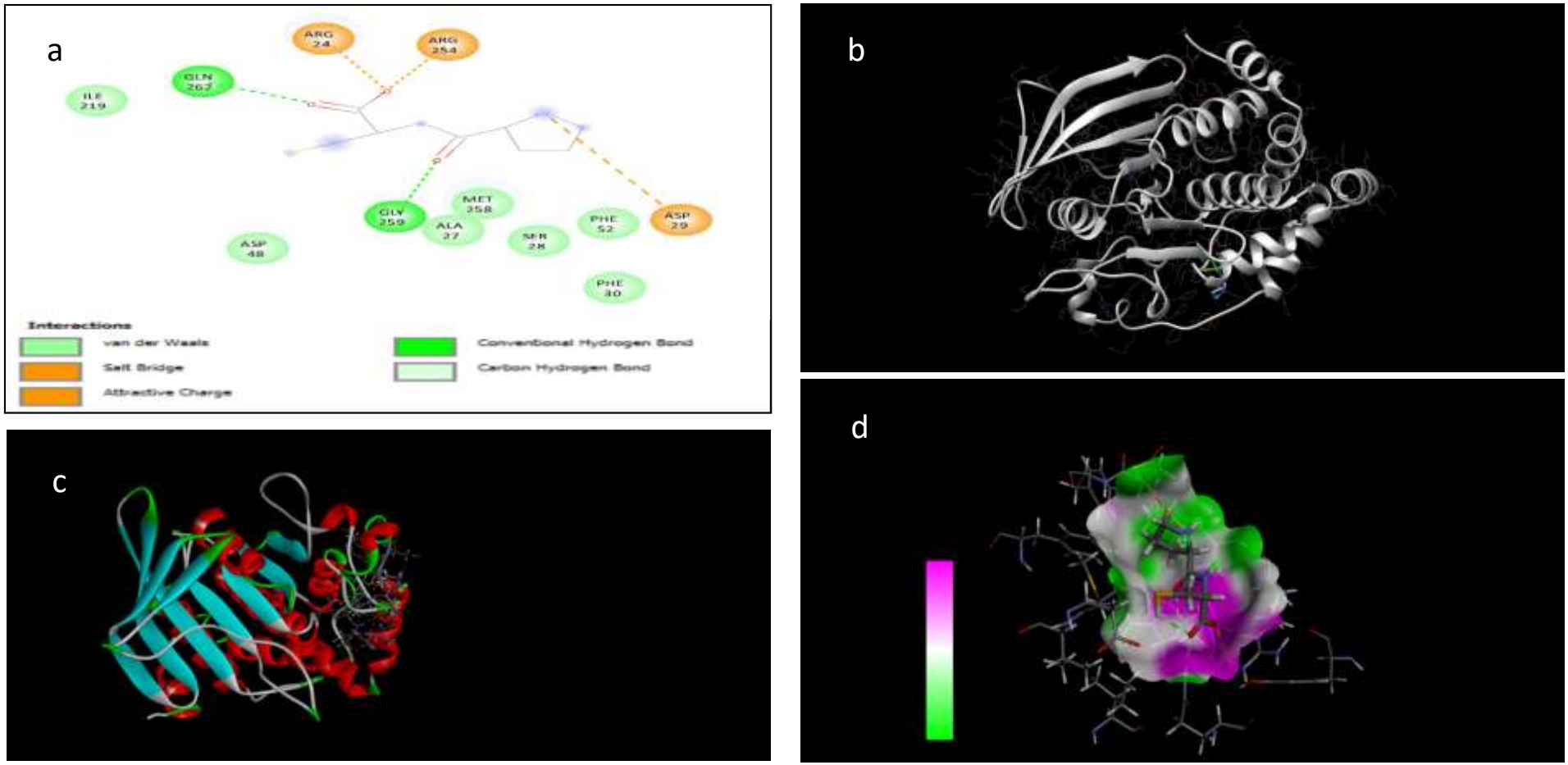

Figure 2: Docking Investigation of dipeptide Pro-Cys against the target Protein tyrosine phosphatase
a) 2D Map of ligand's non-bonded interactions b) Computed secondary structure of assignments of Pro-Cys against tyrosine phosphatase target using UCSF Chimera model c) 3D Docking of Pro-Cys with target in ribbon model d) 3D Pocket view of ligand interaction with enzyme

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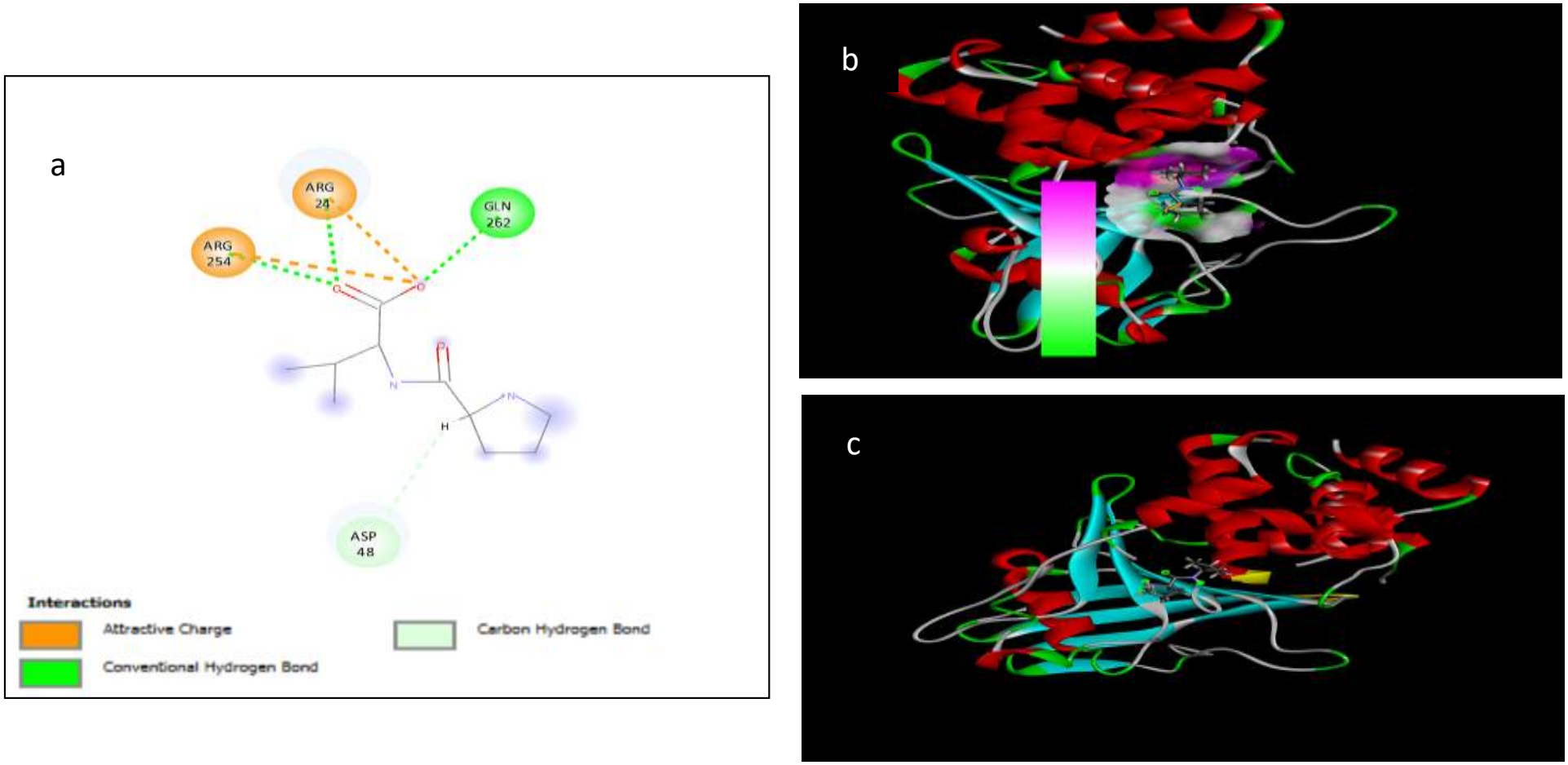


Figure 3: Docking Investigation of dipeptide Pro-Val against the target Protein tyrosine phosphatase
a) 2D Map of ligand's non-bonded interactions b) 3D Pocket view of ligand interaction with enzyme c) 3D Docking of Pro-Val with target in ribbon model

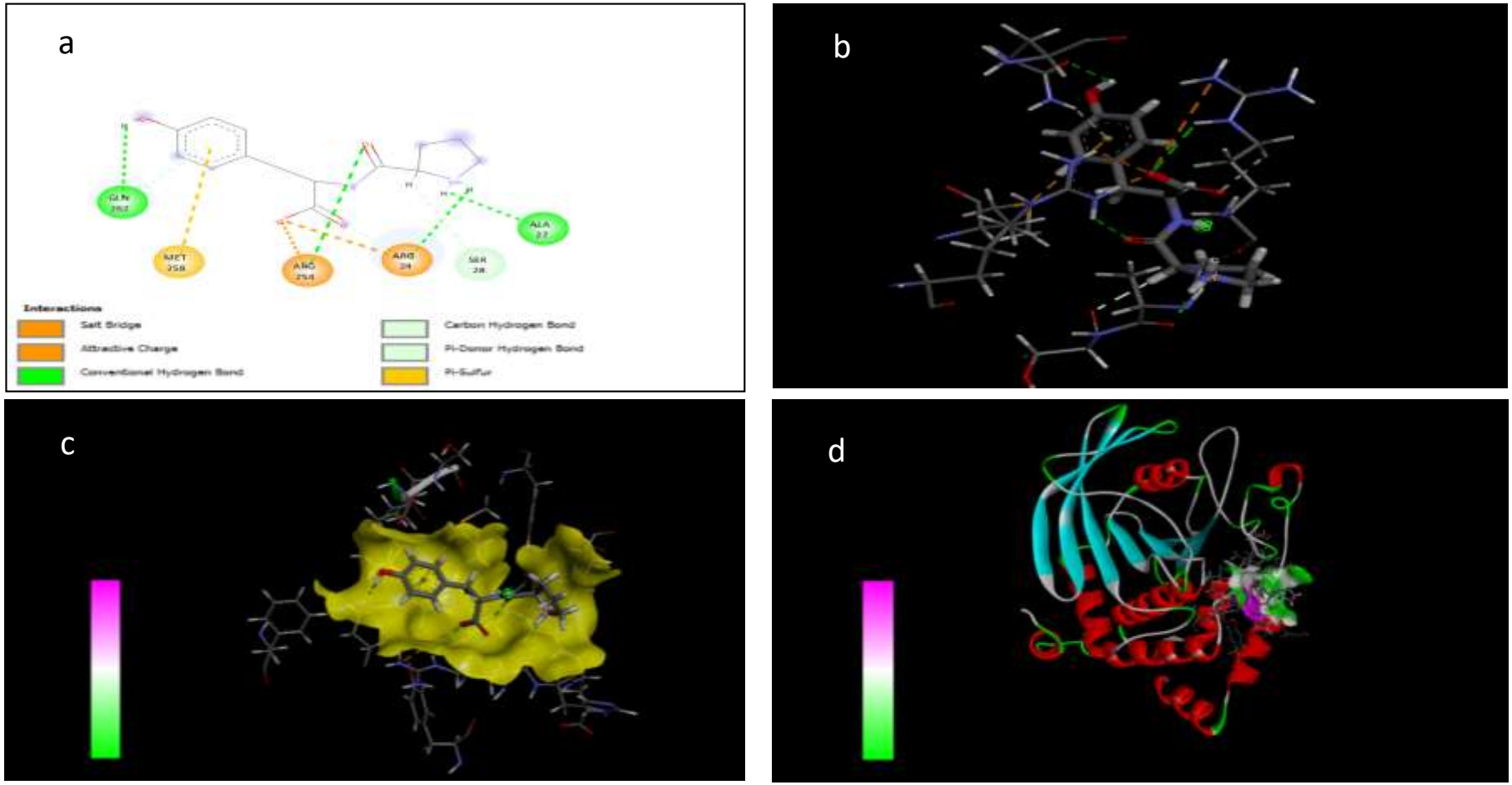


Figure 4: Docking Investigation of dipeptide Pro-Tyr against the target Protein tyrosine phosphatase
a) 2D Map of ligand's non-bonded interactions b) Conventional pharmacophoric distance measurement of H Bonds between drug and targe using Biovia Studio visualizer c) 3D Pocket view of ligand interaction with enzyme d) 3D Docking of Pro-Tyr with target in ribbon model

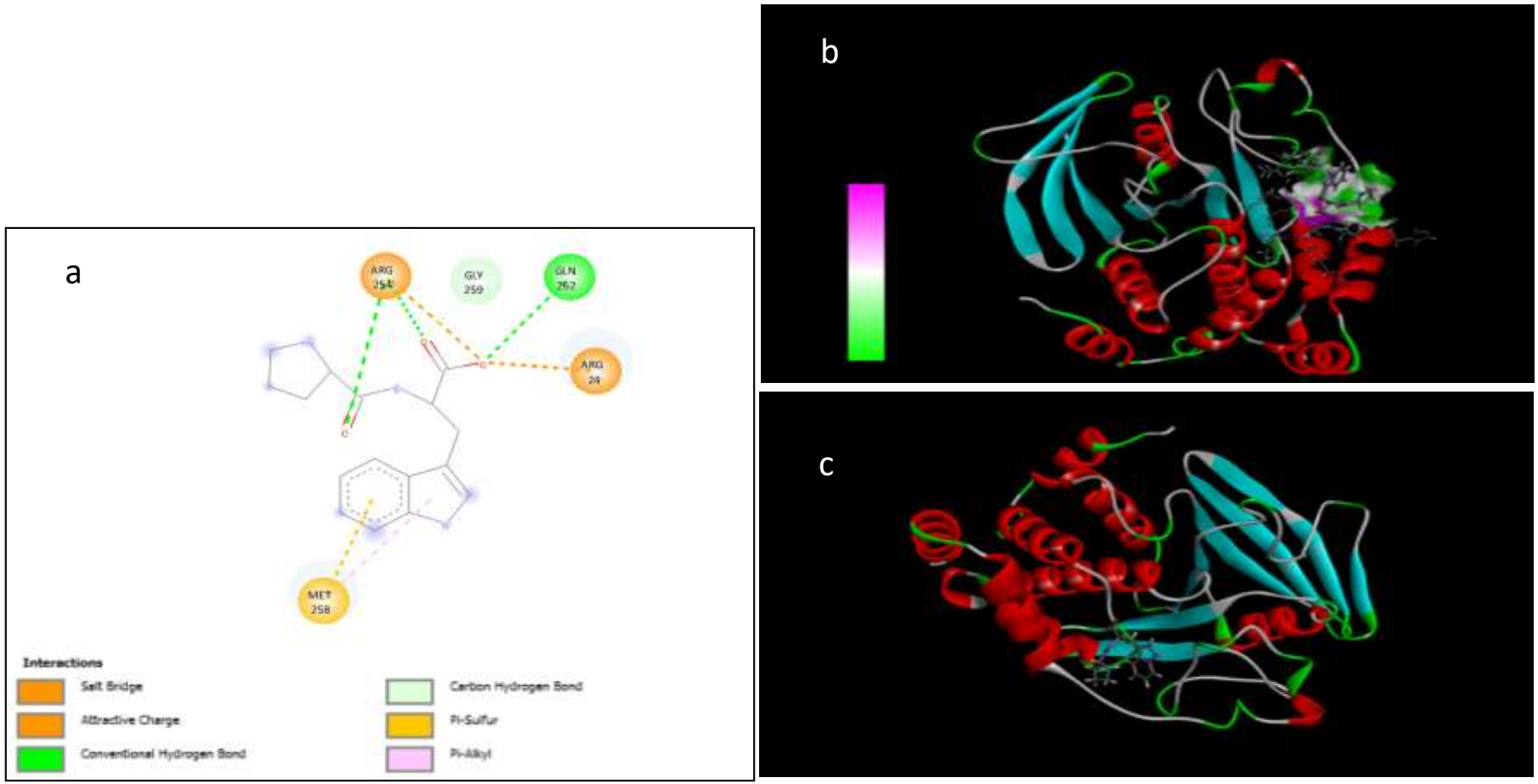
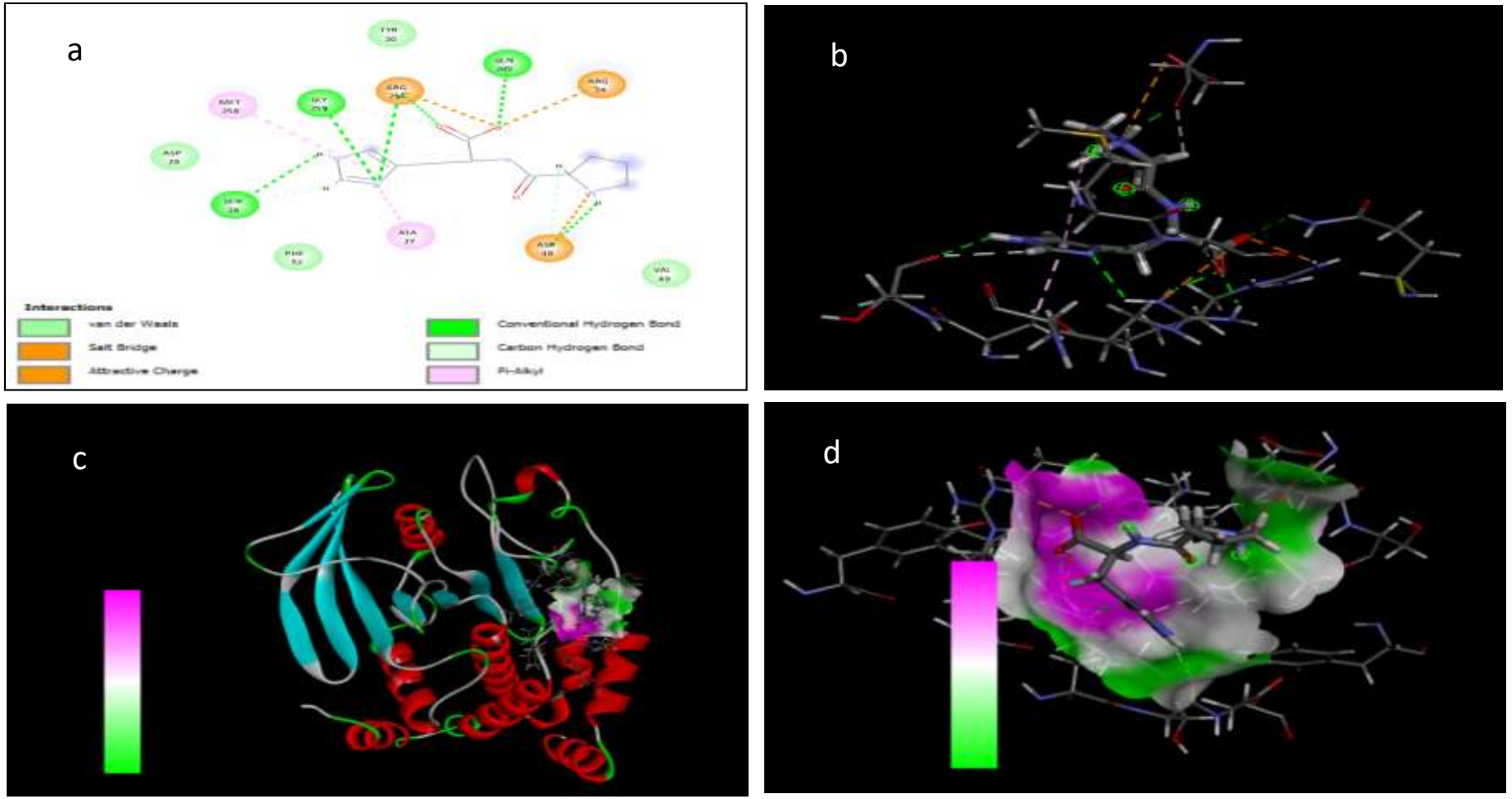


Figure 5: Docking Investigation of dipeptide Pro-Trp against the target Protein tyrosine phosphatase
a) 2D Map of ligand's non-bonded interactions b) 3D Pocket view of ligand interaction with enzyme c) 3D Docking of Pro-Trp with target in ribbon model

Figure 6: Docking



Investigation of dipeptide Pro-His against the target Protein tyrosine phosphatase
a) 2D Map of ligand's non-bonded interactions b) Conventional pharmacophoric distance measurement of H Bonds between drug and targe using Biovia Studio visualizer c) 3D Docking of Pro-His with target in ribbon model d) 3D Pocket view of ligand interaction with enzyme

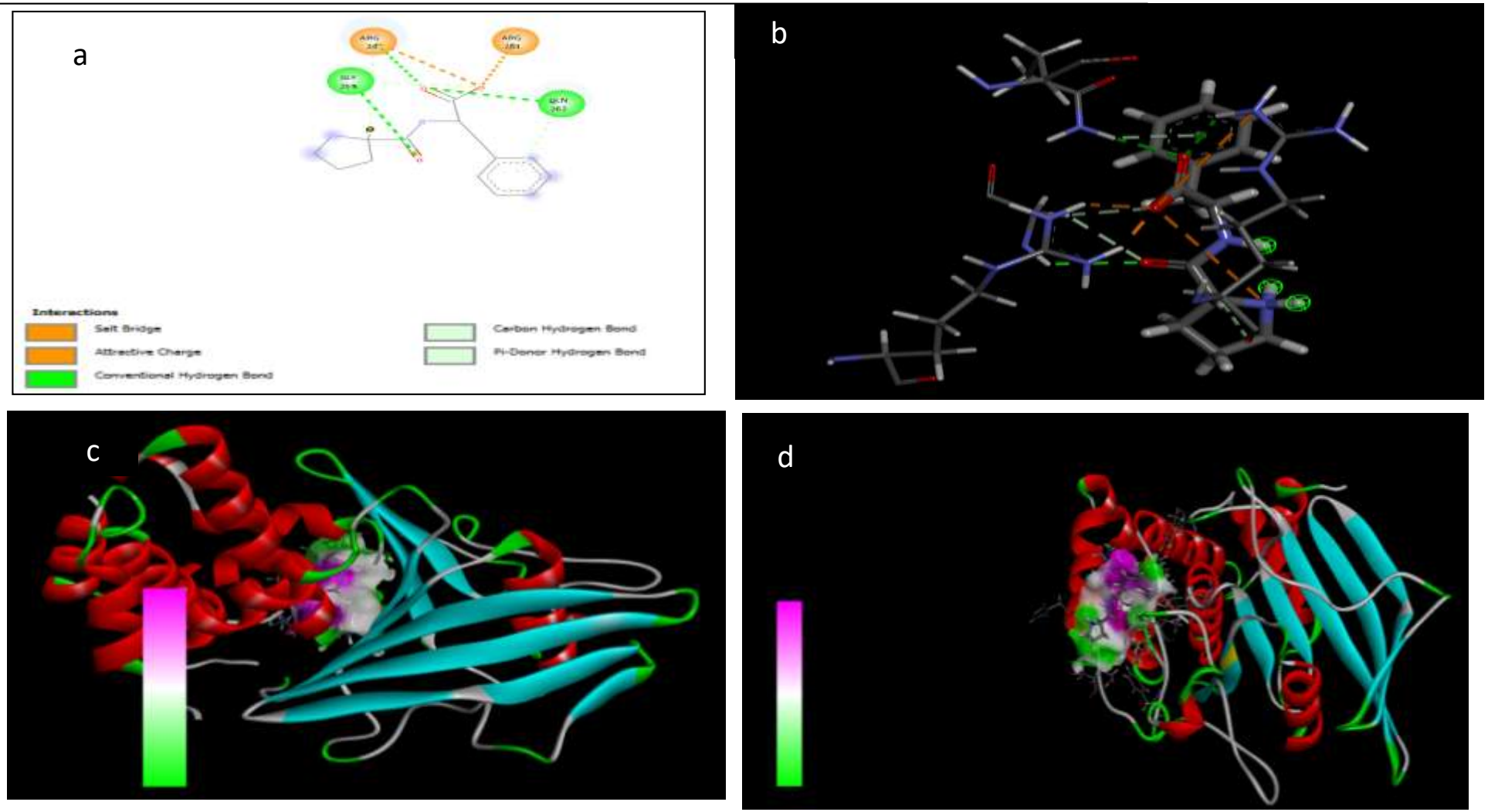


Figure 7: Docking Investigation of dipeptide Pro-Phe against the target Protein tyrosine phosphatase
a) 2D Map of ligand's non-bonded interactions b) Conventional pharmacophoric distance measurement of H Bonds between drug and targe using Biovia Studio visualizer c) 3D Pocket view of ligand interaction with enzyme d) 3D Docking of Pro-Phe with target in ribbon model

visualizer c) 3D pocket view of ligand interaction with enzyme d) 3D docking of cefsulodin with target in ribbon model

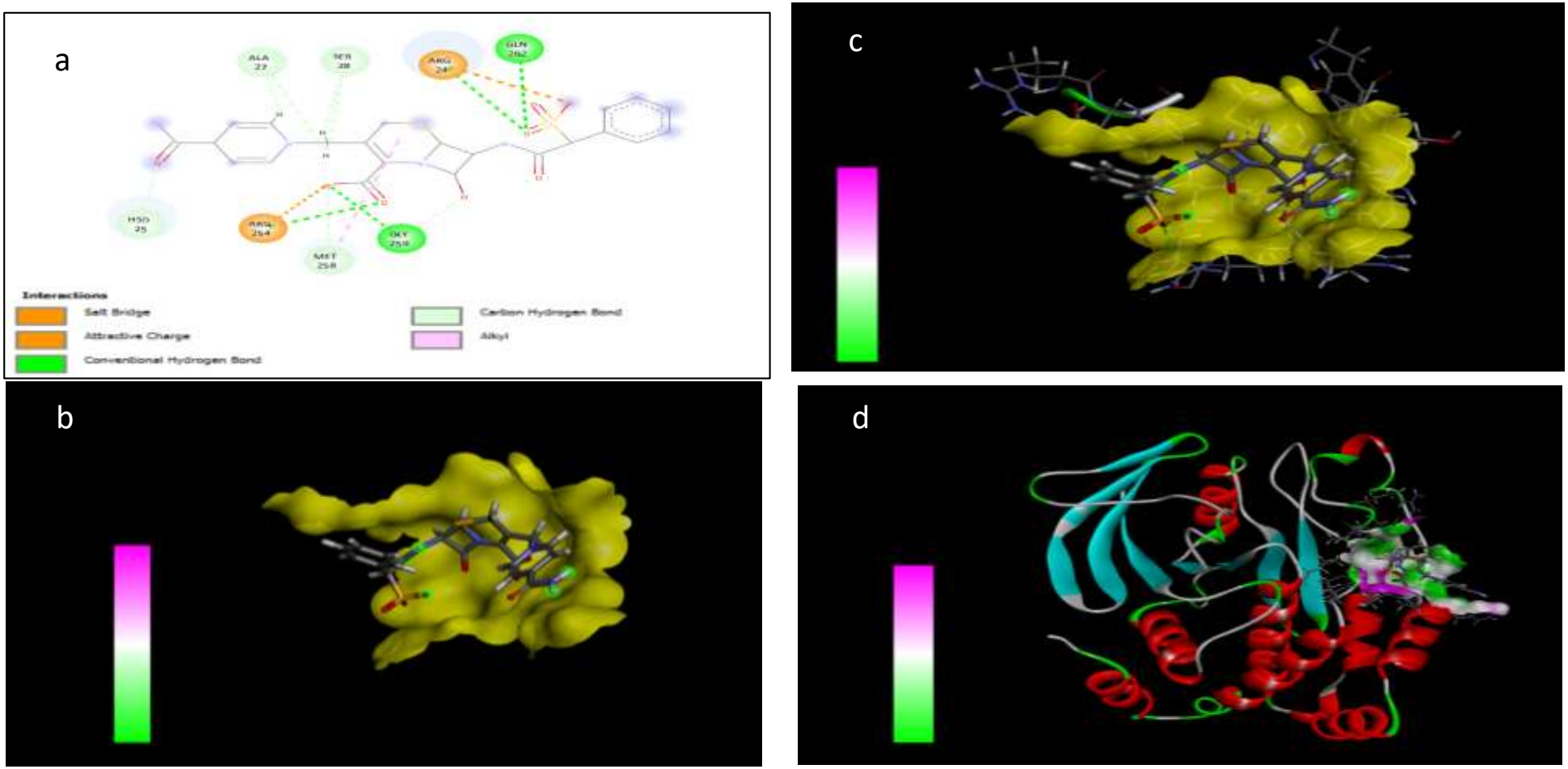


Figure 8: Docking Investigation of Cefsulodin standard against the target Protein tyrosine phosphatase
a) 2D Map of ligand's non bonded interactions b) conventional pharmacophoric distance measurement of H-bonds between drug and target using Biovia studio