Clinical Evaluation of [^{99m}Tc]Tc-PSMA-P1: A Promising SPECT Radiotracer for Prostate Cancer Imaging

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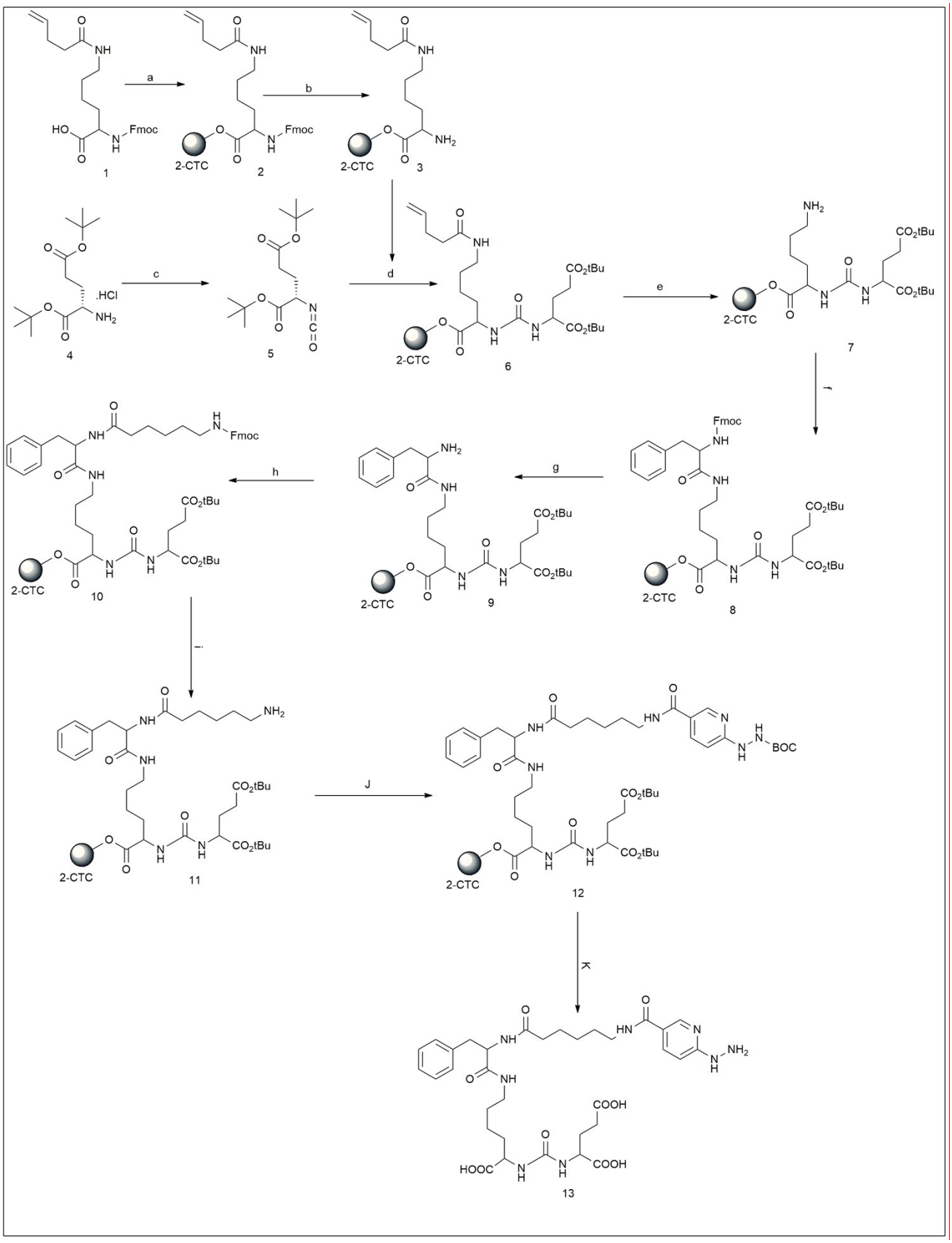

Scheme S1: Reagents and conditions; (a) 2-CTC, DCM (b) 20% piperidine in DMF (c) BTC, DIPEA, 0 °C (d) H-Lys(Alloc)-2CTC-Resin, DCM (e) $Pd[P(C_6H_5)_3]_4$, Morpholine, DCM (f) Fmoc-Phe-OH, DIPEA, HOBT, TBTU, DMF (g) 20% piperidine in DMF (h) Fmoc-Ahx-OH, DIPEA, HOBT, TBTU, DMF (i) 20% piperidine in DMF (j) HYNIC-BOC (k) TFA, DCM.

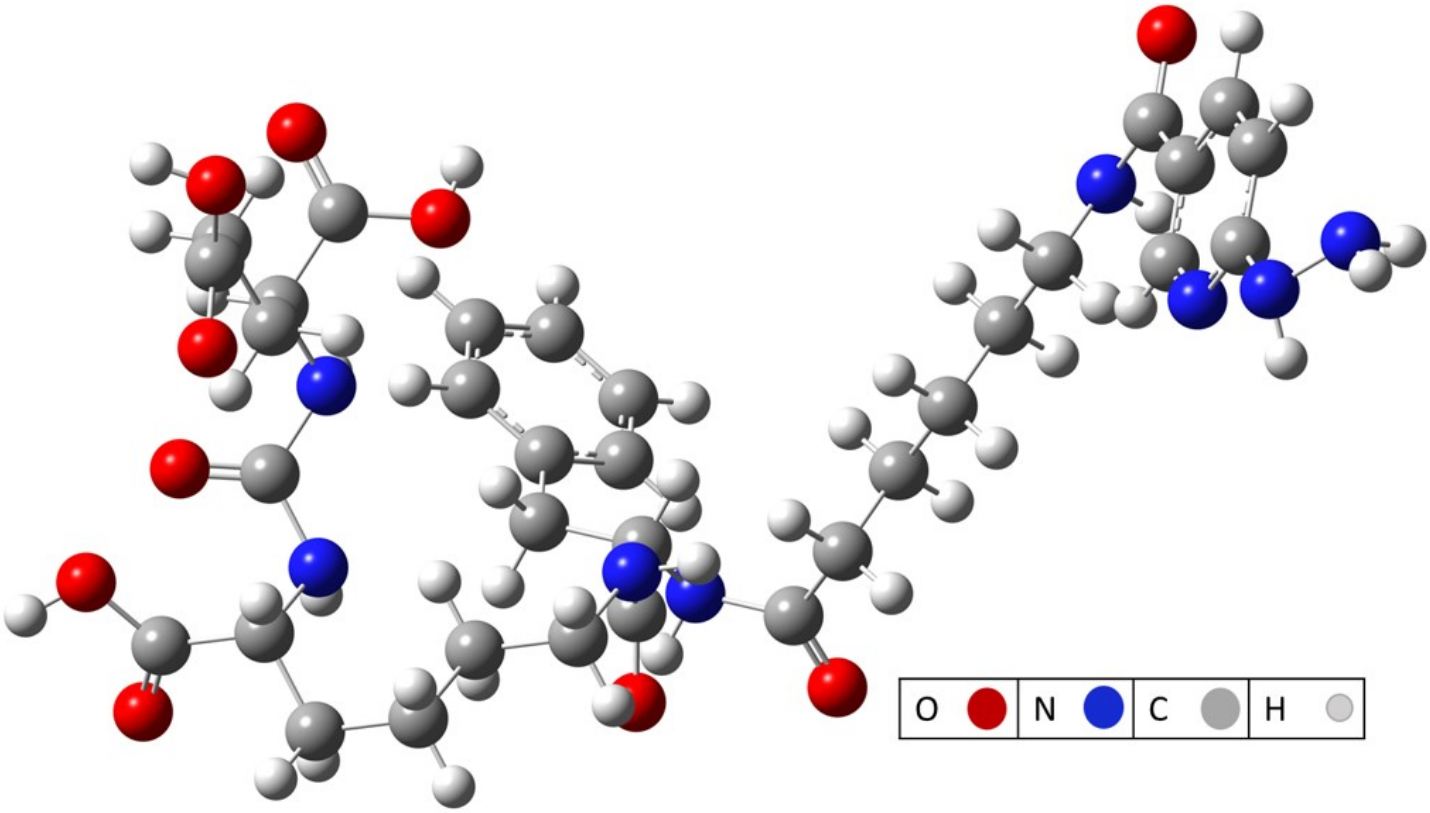

Figure S1 3D optimized structures of PSMA-P1 ligand

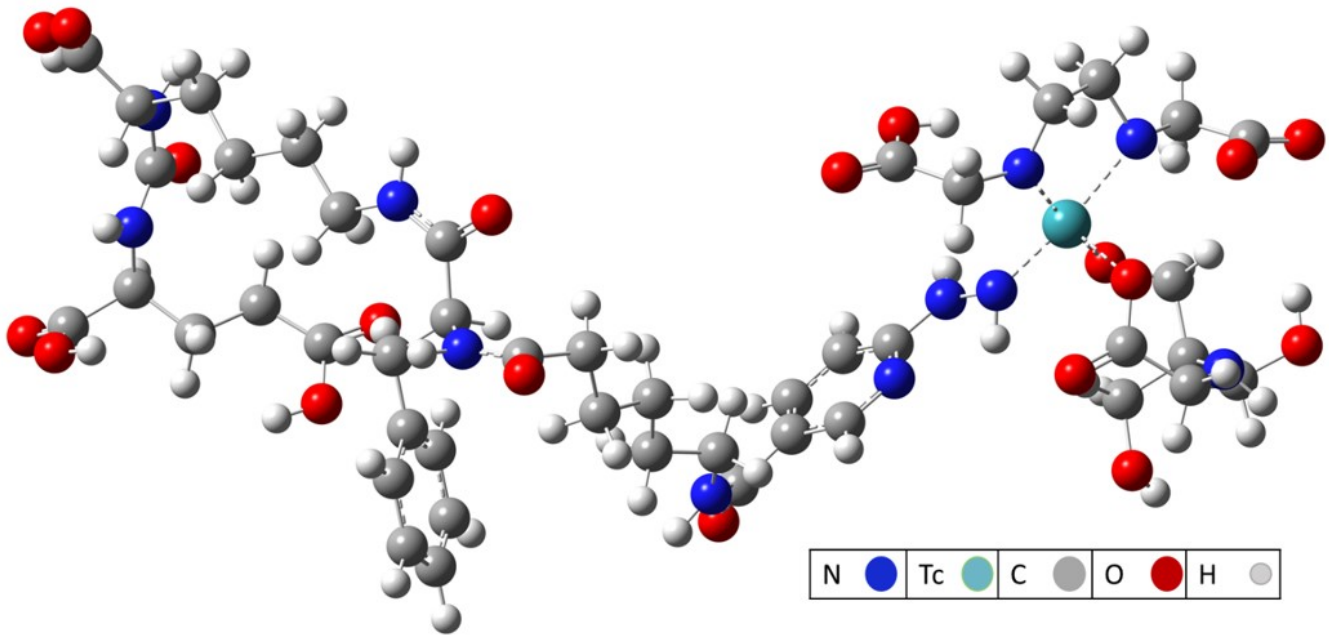

Figure S2 3D optimized structures of [^{99m}Tc]Tc-PSMA-P1 complex

Table S1. Scoring results and binding sites to target protein using AutoDock

Protein inhibitor	Receptor PDB ID	Interactions with receptor residues	Kolmann charges	Binding Energy (Kcal/mol)	kI (inhibition constant)	Intermolecular Energy	Internal Energy	Torsional Energy	Unbound Extended Energy	Ref RMS
HYNIC-PSMA-P1	**2ZCH**	SER192,PHE95H,HIS57,SER214,TYR228,TRP215,SER195,SER226,LEU95I,GLY216,CYS42,THR190,THR213,LEU227,GLY193,LEU95D,CYS220,ARG60,ARG95G	11	-0.41	500.07mM	8.76	4.54	8.35	4.54	50.8
[^{99m}Tc]Tc-HYNIC-PSMA-P1	**2ZCH**	SER35, ASN61, LYS62, ARG60, ARG36, LEU95D, ARG38, PHE149	11	-0.55	393.28mM	-12.19	-10.02	11.63	10.02	47.46
HYNIC-PSMA-P1	**2XW1**	GLU425,LEU463,LYS432,SER193,GLN459,ASP108,TYR452,HIS146,LYS195,LYS190,ASN429,TYR148,ALA191,ALA194	-6.672	-1.55	72.8mM	9.9	0.09	8.35	0.09	9.07
[^{99m}Tc]Tc-HYNIC-PSMA-P1	**2XW1**	GLU354, THR478, LEU347, LYS351, LYS323, ARG209, LYS205, PHE206, LEU481, GLU479, VAL482	-6.672	0.85	0.74mM	-10.78	-9.33	11.63	9.33	17.94
HYNIC-PSMA-P1	**2XV7**	CYS142,ALA117,ARG141,THR116,HIS186,VAL118,ASN138,GLU115,GLU123	-1.459	-0.73	291.71mM	-9.08	-0.58	8.35	-0.58	37.66
[^{99m}Tc]Tc-HYNIC-PSMA-P1	**2XV7**	GLU123, TYR160, LYS182, ALA184, LEU124	-1.459	-2.79	2.38mM	-7.18	-12.89	11.63	12.89	33.17
HYNIC-PSMA-P1	**2OOT**	GLY548,ALA701,ARG210,GLY206,ARG534,ASN698,VAL208,ASP465,LYS207,TYR700,PHE209,ARG463,ASN519,GLY702	1.731	-4.46	222.81uM	-13.33	-2.78	8.35	-2.78	71.21
[^{99m}Tc]Tc-HYNIC-PSMA-P1	**2OOT**	PRO237,VAL239,GLY238,PHE235,TRP246,LYS240,PHE565	1.731	-4.98	69.14 uM	-14.12	-9.65	11.34	9.65	86.56

Table S2. Scoring results and binding sites of reference compound

Protein inhibitor	Receptor PDB ID	Interactions with receptor residues	Kolmann charges	Binding Energy (Kcal/mol)	kI (inhibition constant)	Intermolecular Energy	Internal Energy	Torsional Energy	Unbound Extended Energy	Ref RMS
HYNIC-ALUG	2OOT	LYS207,ARG463,GLY548,TYR234,LYS539,PRO237,ALA236,ASP233,PHE235,PRO231,GLY206,ALA232	1.731	-2.19	253.45uM	-12.07	-3.94	7.16	-3.94	65.18
[^{99m}Tc]Tc-HYNIC-ALUG	2OOT	TYR234, LYS539, GLY548, GLY702, LYS610, ARG511, GLY206, LYS207, ALA701		-2.86	80.2µM	-13.0	-5.38	10.14	-5.38	73.52

Table S3 Physicochemical properties of HYNIC PSMA-P1

Molinspiration property engine		Molinspiration Bioactivity	Data Warrior Calculations				
miLogP	-3.14	Bioactivity	Score/value	Property	Value	Toxicity & Molecular Structure	Prediction
TPSA	291.26	GPCR ligand	-0.58	cLogP	0.9846	Mutagenic	None
Natoms	51	Ion channel modulator	-1.69	cLogS	-4.349	Tumorigenic	High
M.W	714	Kinase inhibitor	-1.24	H-Acceptors	18	Reproductive effective	None
nON	18	Nuclear receptor ligand	-1.54	H-Donors	10	Irritant	None
nOHNH	11	Protease inhibitor	- 0.16	Total surface area	554.98	Nasty functions	Hydrazine
nviolations	3	Enzyme inhibitor	- 0.93	Relative PSA	0.40647	Shape Index	0.60784
Nrotb	24	-	-	Polar surface area	291.27	Molecular Flexibility	0.59581
Volume	646.04	-	-	Drug likeness	-19.354	Molecular complexity	0.78235

Table S4 Physicochemical properties of [^{99m}Tc]Tc-HYNIC PSMA-P1

Molinspiration property engine		**Molinspiration Bioactivity**	**Data Warrior Calculations**				
miLogP	-5.72	Bioactivity	Score/value	Property	Value	Toxicity & Molecular Structure	Prediction
TPSA	446.36	GPCR ligand	-3.70	cLogP	-4.5177	Mutagenic	High
Natoms	76	Ion channel modulator	-3.81	cLogS	-1.007	Tumorigenic	None
M.W	1163	Kinase inhibitor	-3.82	H-Acceptors	30	Reproductive effective	None
nON	30	Nuclear receptor ligand	-3.86	H-Donors	15	Irritant	None
nOHNH	15	Protease inhibitor	- 3.59	Total surface area	802.37	Nasty functions	
nviolations	3	Enzyme inhibitor	- 3.74	Relative PSA	0.43851	Shape Index	0.48684
nrotb	32	-	-	Polar surface area	446.38	Momolecular Flexibility	0.55703
volume	963.31	-	-	Drug likeness	-28.54	Momolecular complexity	0.90142

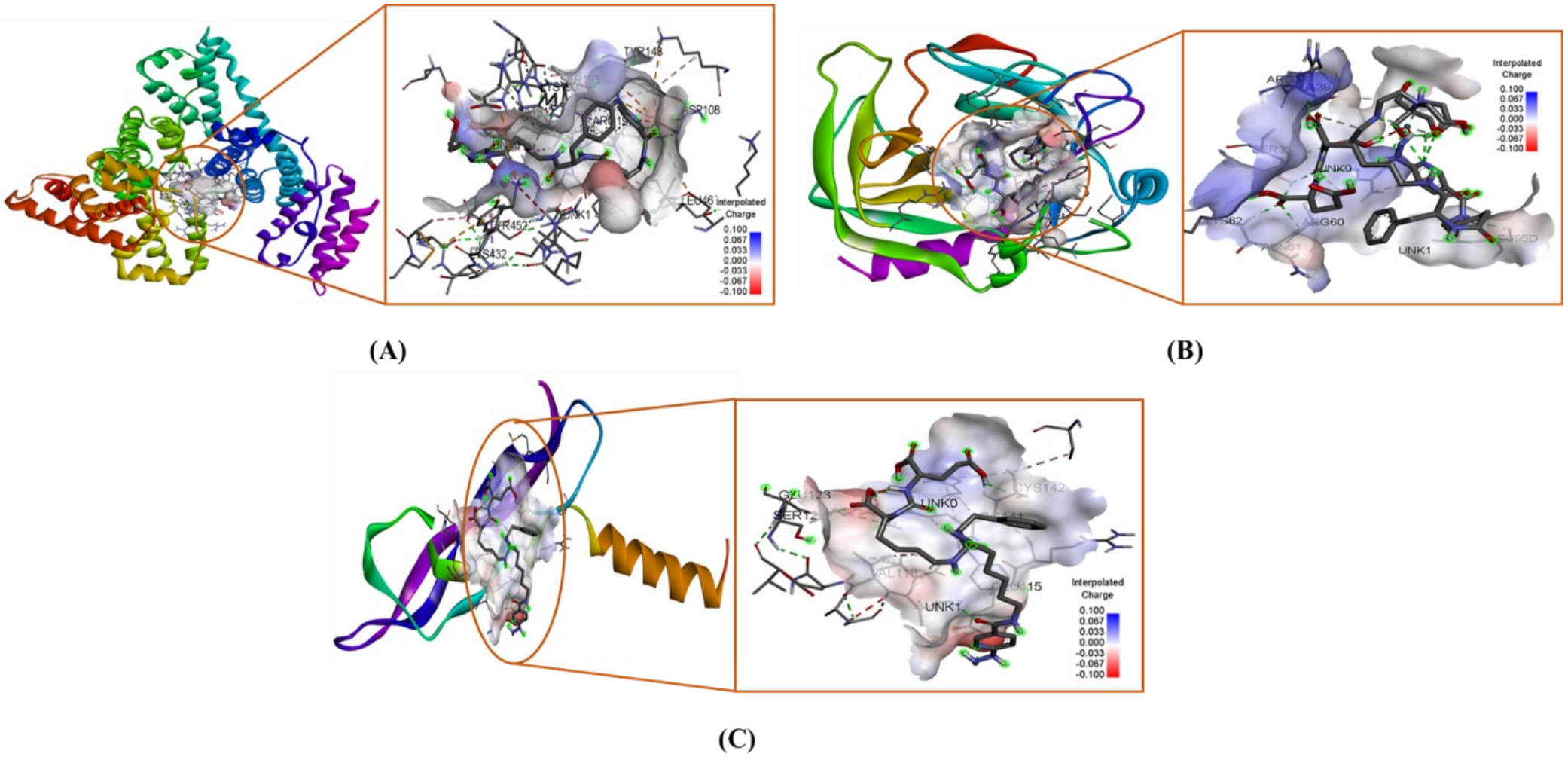

Figure S3: PSMA-P1 interactions with receptor **PDB ID's: 2XW1**, **2ZCH** and **2XV7** represented in ribbon form and magnified inside the active site with electrostatic potential on molecule surface **A**), (**B**) and (**C**) respectively

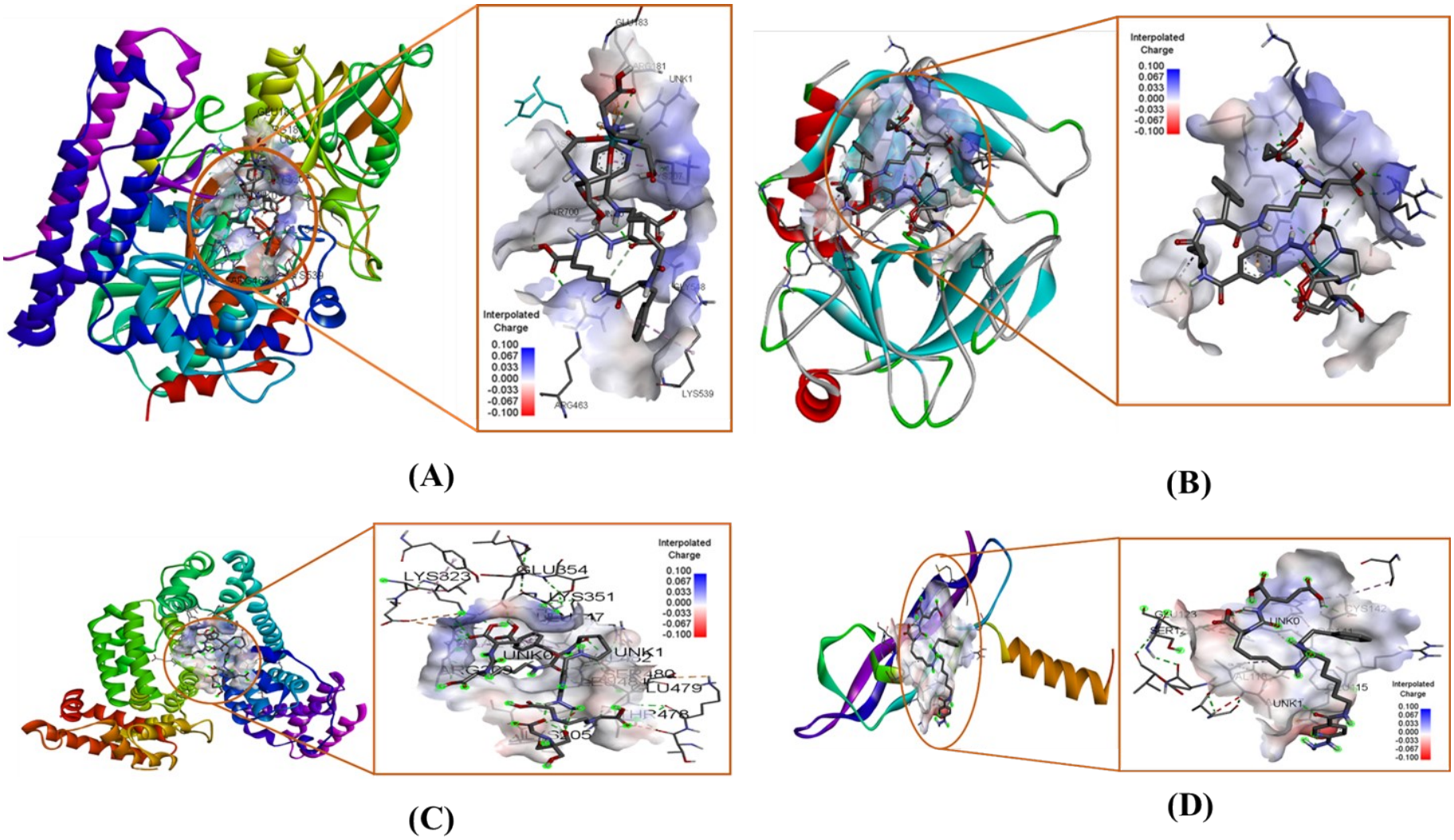

Figure S4: [^{99m}Tc]Tc-PSMA-P1 interactions with receptor **PDB ID's: 2OOT 2XW1**, **2ZCH** and **2XV7** represented in ribbon form and magnified inside the active site with electrostatic potential on molecule surface in (**A**), (**B**), (**C**) and (**D**) respectively.

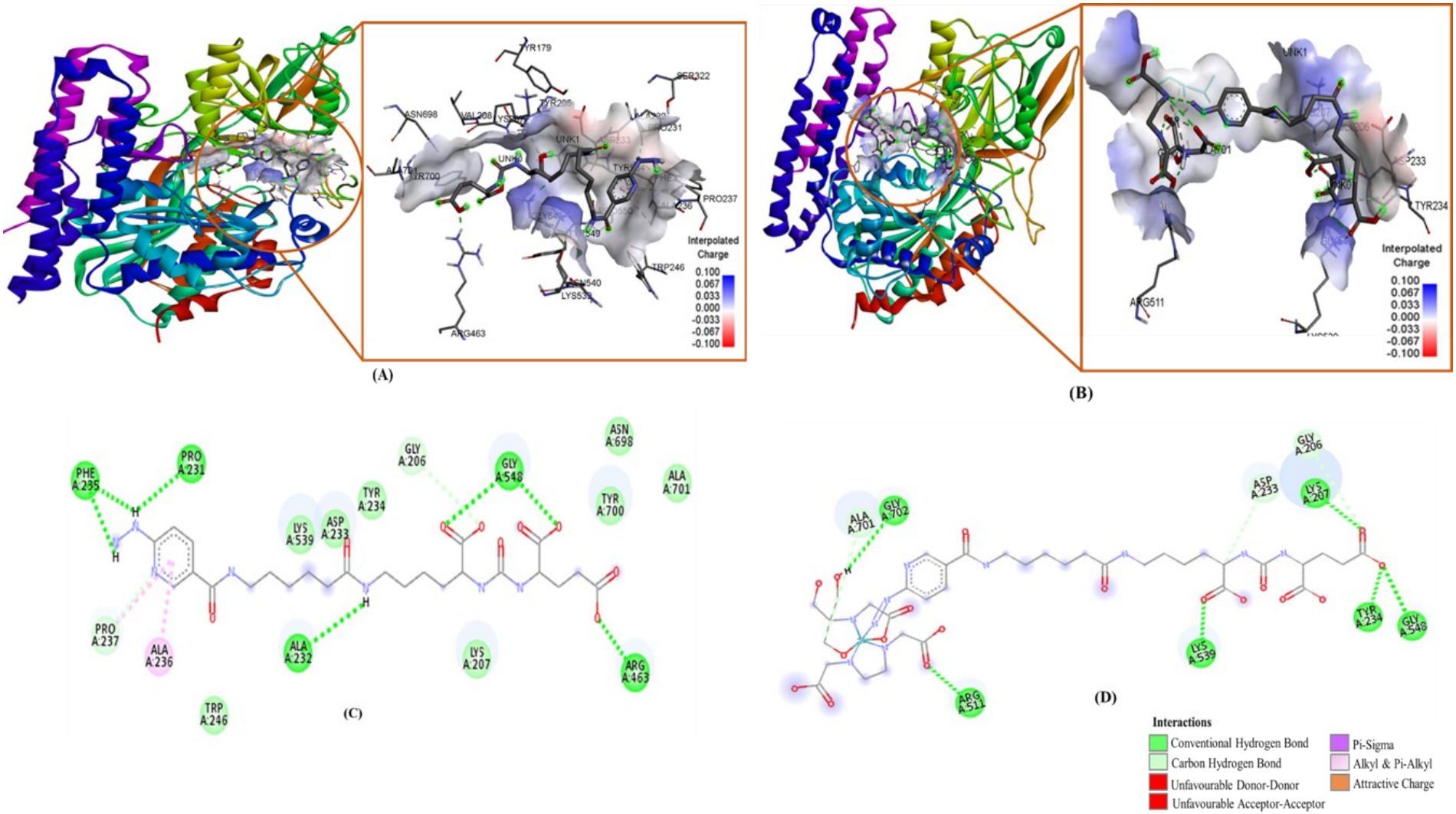

Figure S5: HYNIC-ALUG and [^{99m}Tc]Tc-HYNIC-ALUG interactions with receptor **PDB ID's: 2OOT** represented in ribbon form and magnified inside the active site with electrostatic potential on molecule surface in (**A**) and (**B**), while interaction types are presented through 2D diagrams (**C**) and (**D**) respectively.

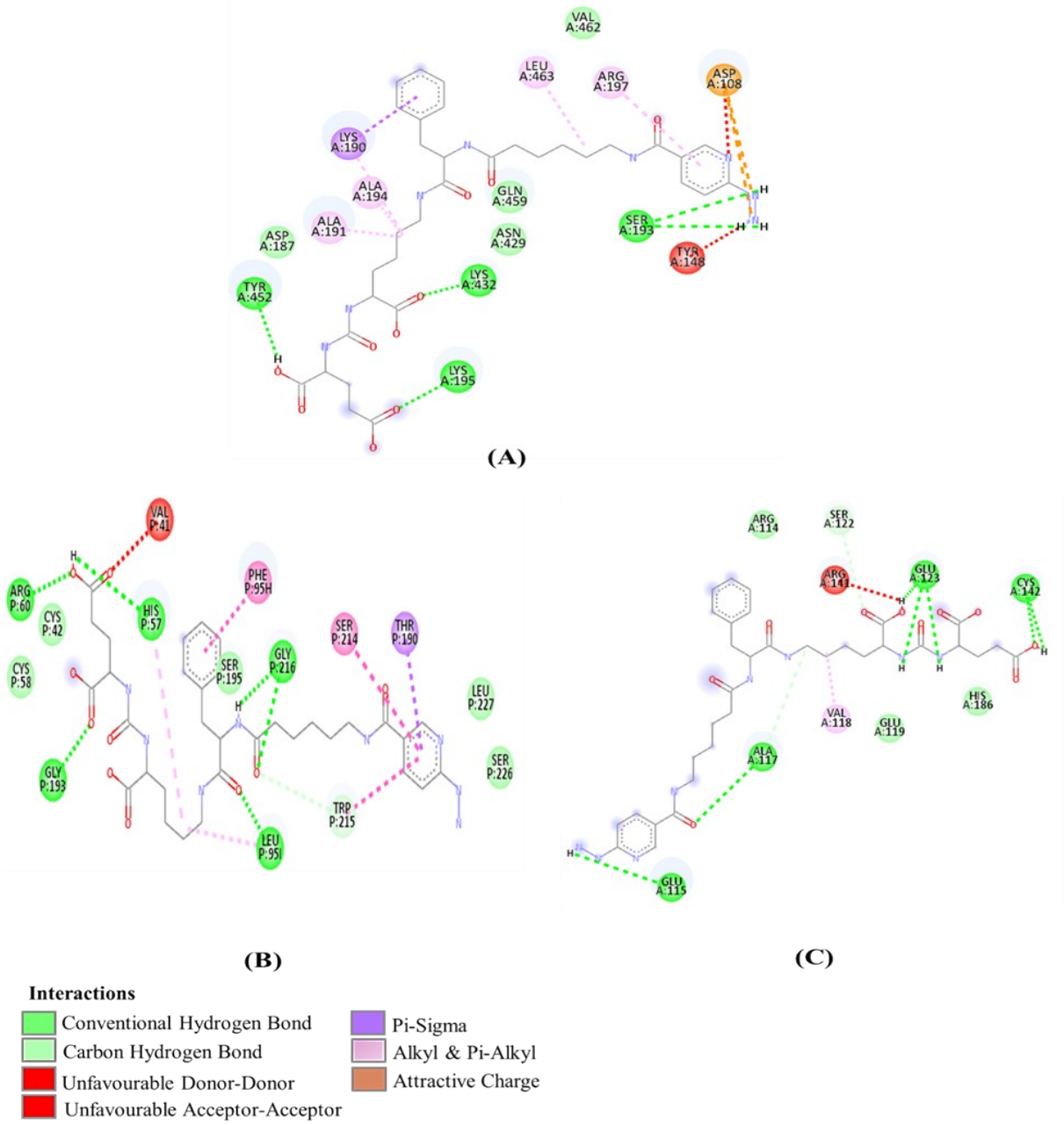

Figure S6: PSMA-P1 interaction types with receptor **PDB ID's: 2XW1**, **2ZCH** and **2XV7** residues represented in 2D diagram (**A**), (**B**) and (**C**) respectively.

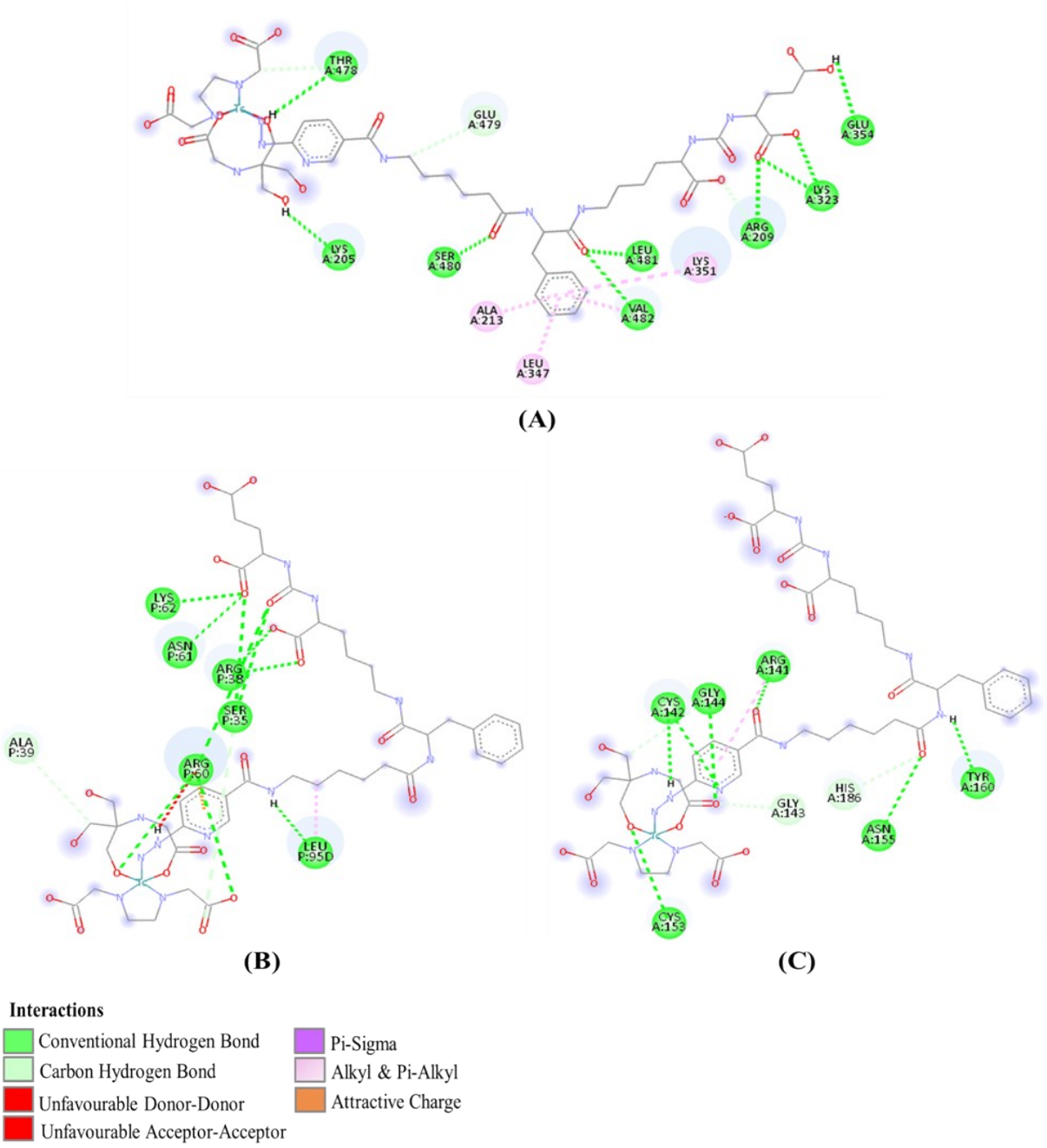

Figure S7: [^{99m}Tc]Tc-PSMA-P1 interaction types with receptor **PDB ID's: 2XW1**, **2ZCH** and **2XV7** residues represented in 2D diagram (**A**), (**B**) and (**C**) respectively.

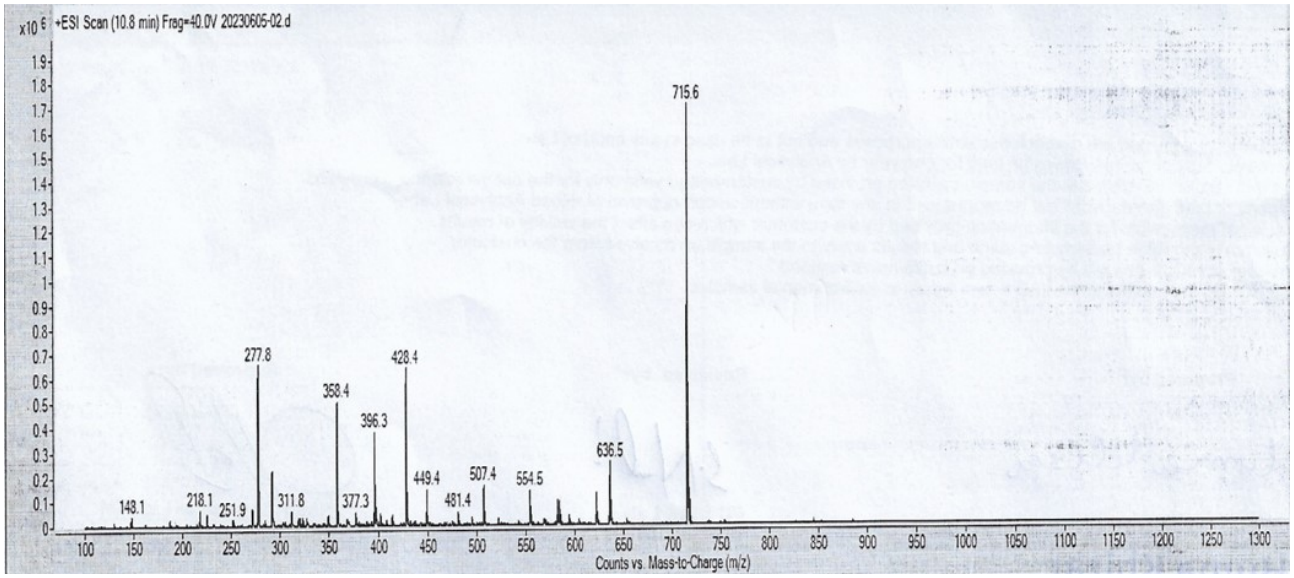

Figure S8 : LC-MS of PSMA-P1

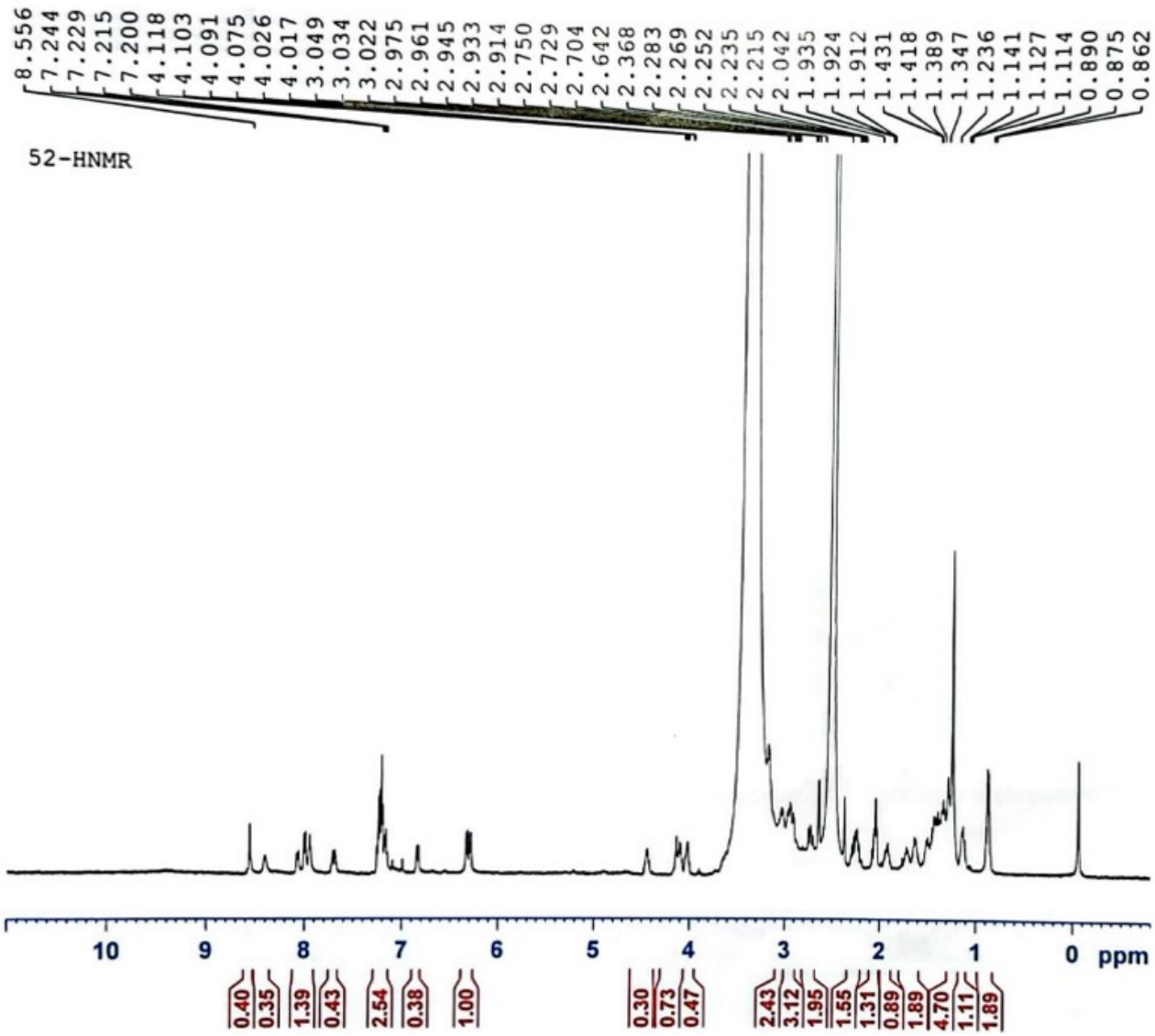

Figure S9 : ^{1}H-NMR spectrum PSMA-P1

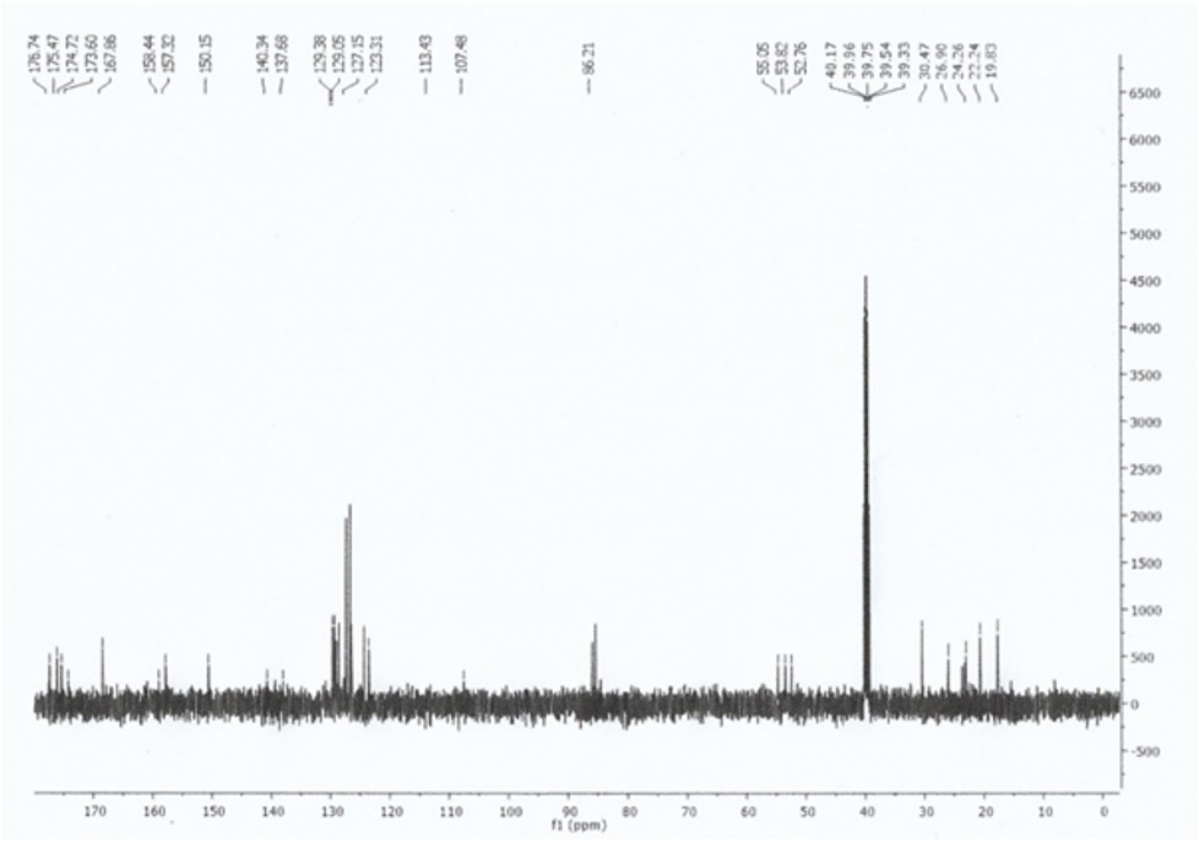

Figure S 10: ^{13}C-NMR spectrum PSMA-P1

Abbreviations list

Abbreviation's	Meanings
2-CTC	2-Chloro-trityl
DIPEA	N-N diisopropylethylamine
Alloc	ε-allyloxycarbonyl
Fmoc	Fluorenyl methoxycarbonyl
Fmoc Lys(Alloc)-OH	Fmoc-protected L-lysine
K	Lysine
DMF	N,N-dimethylformamide
DCM	Dichloromethane
t-Bu-Glu.HCl	Bis(t-Bu)-L-glutamate hydrochloride
E	Glutamate
BTC	Triphosgene
TTPP	Tetrakis(triphenyl)palladium
U	Urea
$(C_2H_5)_2NCSSNa.3H_2O$	Sodium Diethyldithiocarbamate trihydrate
GUL/KUE	Glutamate urea lysine
Fmoc-Phe-OH/F	Fmoc-L-phenylalanine
HOBT	1H-1,2,3-Benzotriazol-1-ol
HBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
Fmoc-Ahx-OH	6-(Fmoc-amino)hexanoic acid
BOC-HYNIC	6-[2-(tert-Butoxycarbonyl)hydrazino]nicotinic acid
TFA	Trifluoroacetic acid
ACN	acetonitrile
PET	Positron emission tomography
SPECT	Single photon emission computed tomography
PCa	Prostate cancer
PSMA	Prostate-specific membrane antigen