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Neat synthesis and *in silico* screening of pyrazole α- amino phosphonates as potent inhibitors of cancer targets

C.Vinodhini*, K .Chitra
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai-116, India.
***Corresponding author: E.Mail: vin_pharma@yahoo.com**

ABSTRACT

Thymidylate synthase has been unique in selectivity and preferred target for designing and developing novel inhibitors which could be used as anticancer drugs. Research is widely exposed to find new chemical entities that lodge in the active site of the glycoprotein, blocking its action from inside. Pyrazoles are widely used in medicinal, pesticidal chemistry and recently reported to display non-nucleoside HIV- I reverse transcriptase inhibitor activity. Because of their structural similarity to the natural α- amino carboxylic acids and as enzyme inhibitors, α- amino phosphonates are quite interesting compounds in the development of potential drugs against several metabolic disorders. Ten different analogues of pyrazole α- amino phosphonates were synthesised using pyrazole aldehyde, wide variety of amines and diethyl phosphite in the presence of indium chloride as catalyst under neat condition at ambient temperature to exert product in good yield. The physical characteristics like appearance, melting point, R_f value, % yield were determined after purification of the products by TLC and colum chromatography. Characterisation of the products were done by spectral studies like IR, NMR, and Mass Spectroscopy. The docking was performed using autodock, version 4.0 with genetic algorithm as the optimization protocol at both thymidylate synthase and P- glycoprotein specific cancer targets for ten analogues of pyrazole α- amino phosphonates. The clusters with largest number of conformations were chosen for binding site analysis. Binding energy and affinity were used for the prioritisation of lead molecules to promote for *in vitro* studies.

KEY WORDS: Pyrazole α--aminophosphonate, *in silico, in vitro,* neat, Thymidylate synthase, P-Glycoprotein.

1. INTRODUCTION

In recent years considerable attention has been focused on α- amino phosphonates in particular to pyrazole α- amino phosphonates (PV), due to their interesting biological activities, medicinal importance such as enzyme inhibitors, HIV protease, antibiotics, herbicides, fungicides, insecticides, plant growth regulators, antithrombotic agents, peptidases and proteases, antimicrobial, antitumor, antibiotic, anticonvulsant, potential antileukaemic, local anaesthetic and antiviral activities. But the literature review reveals that number of synthetic methodologies with respect to pyrazole α- amino phosphonates involved the usage of less effective catalyst and toxic solvents were reported earlier and few reports were available with respect to synthetic strategies of pyrazole α- amino phosphonates.

Colorectal and breast cancer are the third and second most common malignancy leading cause for death across the world. Drugs like Cisplatin, Gemcitabine and 5-Fluorouracil are supportive and the best way to mix with radiation targeted therapy and immunotherapy for advanced cancers. New chemo tamoxifen causes side effects such as blood clots, strokes, uterine cancer and cataracts. The side effect of these drugs make the need for the new improved drugs. Hence in this research study, a suitable green synthesis of pyrazole α- amino phosphonates analogues with high binding affinity were tried for its efficacy as better anti-cancer profile by *in silico* model[7], which could generate a possible lead molecule to treat colororectal and breast cancer by any of these mechanism like retarding the cell cycle and /or activating the cellular apoptotic response in the cancerous cells.

2. MATERIALS AND METHODS

2.1. Materials: All chemicals were purchased from sigma Aldrich USA, Analytical TLC was performed on precoated aluminium sheets of silica gel G/UV-254 of 0.2 mm thickness (Merck, Germany).Column chromatography was performed on silica gel (100-200 mesh, Merck, India).Melting point were determined in capillary tubes using melting point apparatus (Technico, India). IR spectra were recorded as KBr pellets on a perkin elmer spectrum RXI FT-IR.^{1}HNMR (500 MHz) and ^{13}CNMR (125 MHz) spectra were recorded in $CDCl_3$ with TMS as internal standard on a JEOL Spectrometer. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage MAX 6000 ESI spectrometer. Chemical shift with reference to H_3PO_4 was record in ^{31}PNMR.

2.2. General Procedure For Synthesis Of Pyrazole α- - Amino Phosphonates: In the initial endeavour a mixture of pyrazole aldehyde, 1 mmol (1), aniline,1 mmol (2e) and diethyl phosphate,1.5 m.mol (3) in the presence of indium chloride (20 mol %) as catalyst under neat condition was stirred at room temperature.After completion of the reaction as indicated by TLC, it was poured into water and extracted with ethyl acetate. The organic layer was dried over

sodium sulphate and concentrated under vacuum and purified by column chromatography. Further the reaction was amenable to a wide variety of arylamines bearing various substituents like methyl, methoxy, nitro, bromo, chloro (2a-2j) and aliphatic amine (2i).The reaction was carried out for about 8 hours at ambient temperature.

2.3. Anti-Oxidany Activity By DPPH Assay Method: The antioxidant activity of the test compounds and standard were assessed by the technique based on the DPPH scavenging ability using 96 well microtitre plate. To 190 µl of DPPH solution, 10 µl of each of the test samples (PV1-PV10) and standard in the concentration range of 1.97- 1000 µg/ml was added separately in well of the microtitre plate. The plates were incubated at 37° C for 20 minutes and the decrease in absorbance of test compounds and standard (due to quenching of DPPH free radicals) were measured at 517 nm in ELISA Reader. Absorbance of control blank, sample blank and control were measured as well. The experiment was performed in triplicates and the % scavenging activity was calculated using the below formula.

$$\%\ Scavenging\ activity = \frac{C - T}{C} \times 100$$

Control(C) = C - CB, Test (T) = S – SB, where,

Control (C) = 10 µl methanol*+ 190 µl DPPH

Control Blank (CB) = 10 µl diluents (1 % DMSO in ethanol) + 190 µl DPPH

Sample blank (SB) = 10 µl test solution+190 µl diluents

Sample(S) = 10 µl sample solution + 190 µl DPPH

*Solvent used to dissolve DPPH.

The IC_{50} values for each drug compounds as well as standard preparation were calculated. The effective concentration of sample required to scavenge DPPH radical by 50 % (IC_{50} value) was obtained by linear regression analysis of dose – response curve plotting between % scavenging activity on y-axis and concentration on x-axis.

2.4. Molecular Docking using Autodock Software

2.4.1. Generation of 3D coordinates for the Ligand: The molecular structure of PV(1-10) were drawn using 'jchempaint' and saved as Simplified Molecular Input Line Entry Specification format (SMILES). The 2D SMILES files were converted into 3D coordinates in two steps using the modules available in the program smi23D. Using the first module 'smi2sdf', 2D SMILES were converted to an intermediate 3D structure data format (SDF). For energy minimization, forcefield and Gasteiger protocol based charge addition was used. Prior to energy minimization, all hydrogen atoms were added with proper consideration for steric interactions.

2.4.2. Generation of 3D coordinates for enzyme/protein target: The 3D coordinates for Human thymidylate synthase (Pdb: 1HVY) and P-Glycoprotien (3G61) were downloaded from the RSCB website (http://www.rcsb.org). Out of the 4 homomeric subunits (A-D) of 1HVY, subunit A was chosen for the study. Similarly, in the case of 3G61, chain A was chosen for docking analysis. The co-ordinates of water, ligands and other co-factors already present in the structure were removed for both the proteins. The resulted pdb's were imported into chimera and energy was minimized using 1000 and 5000 steps of steepest descent and conjugate gradient methods, respectively.

2.4.3. Preparation of ligand structure for docking: The energy minimized structures of the ligand were imported into ADT tools for addition of all hydrogen atoms. Gasteiger protocol based charges were added and residual non-integral charges were evenly distributed over the whole molecule, so as to ensure that the total charge of the molecule remains an integral value. The root atom and the rotatable bonds for the ligand was defined and the aromatic criteria was adjusted so that all the bonds present in an aromatic ring remains non-rotatable. The structure was finally saved in 'pdbqt' format.

2.4.4. Preparation of enzyme/protein structure for docking: Similar to ligand, the protein was imported into ADT tools and the missing hydrogen atoms were added. Kollman protocol based charges were added to the protein molecule. The grid box for blind docking was defined by centering the grid box with respect to the center of the protein and adjusting the dimensions of the box so that it encloses all the atoms of the protein well within the box. The grid parameters were saved as 'gpf' file and the coordinates of the protein were saved in pdbqt format.

2.4.5. Automated blind docking of the ligand to the enzyme/protein: Prior to docking, grid maps were generated for each ligand (total: 10) through the program autogrid4 using the grid parameter file (gpf) and the pdbqt of the ligand, target protein as its input. The docking was performed using autodock4 with genetic algorithm as the optimization protocol. The population size was set to 200 and the number of generation was set to 27000.100 runs were performed and the elitist from each run was selected for the final cluster analysis. The mutation and the cross-

over rates were kept at 2 % and 80 % respectively. The final 100 conformations were clustered with threshold RMSD criteria of 2A unit. The clusters with largest number of conformations were chosen for binding site analysis.

3. RESULTS AND DISCUSSION

Scheme.1.Neat Synthesis of Pyrazole α-- Amino Phosphonate analogues (PV1-PV10)

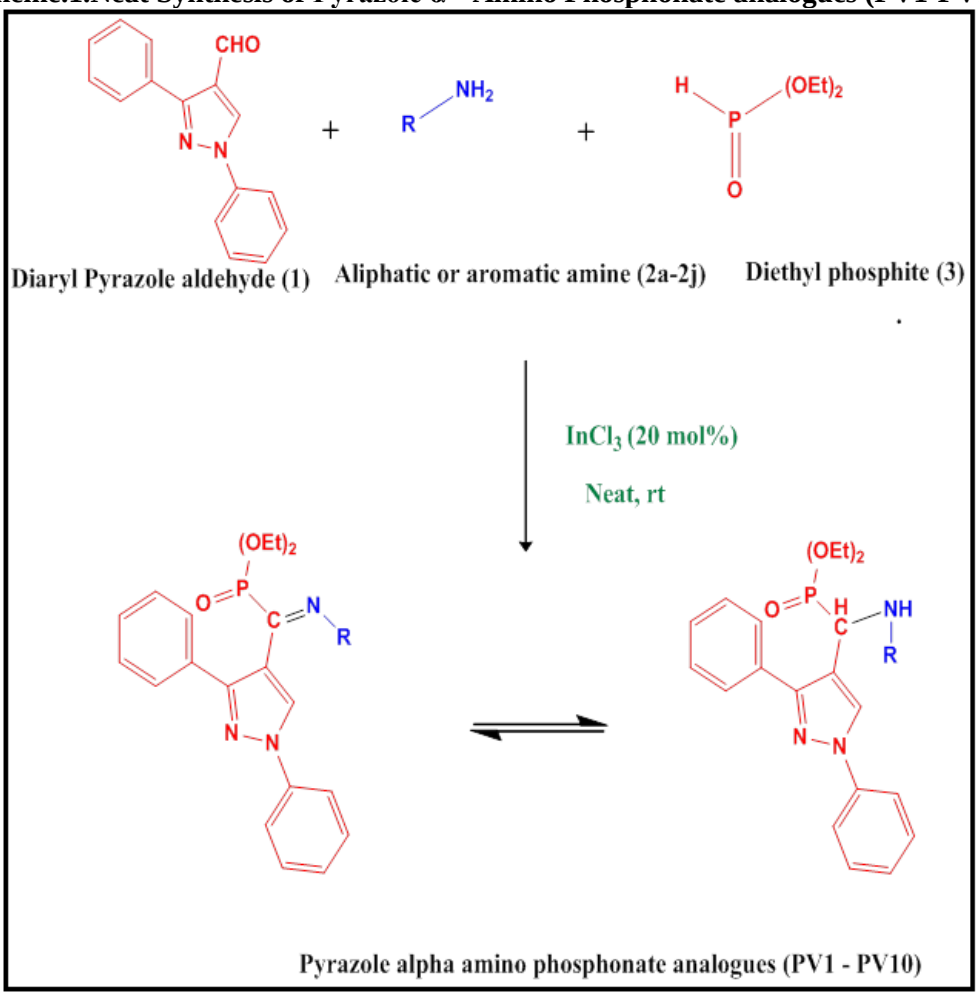


Table.1.Synthetic protocol and Physical characteristics of Pyrazole α-Amino Phosphonate analogues (PV1-PV10)

Compound Id	Pyrazole aldehyde	Substituted aniline	Diethyl phosphate	Product	Appearance	Molecular weight	Melting point (°C)	Rf Value	% yield
PV1	1	2a	3		white solid	495	170-172	0.35	87
PV2	1	2b	3		white solid	495	156-158	0.53	80
PV3					white solid	540	194-196	0.53	79

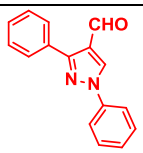
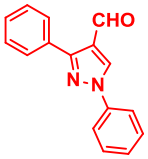
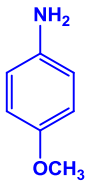
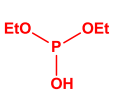
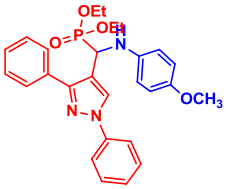
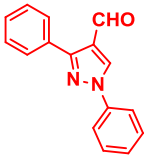
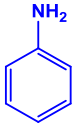
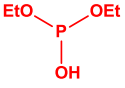
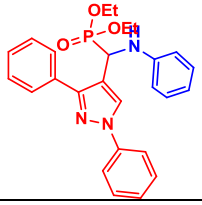
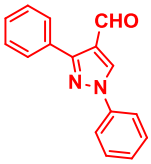
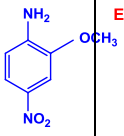
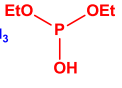
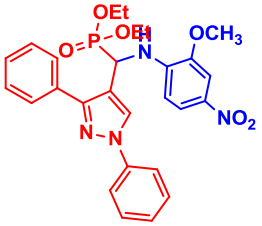
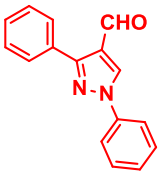
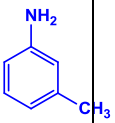
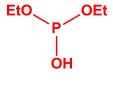
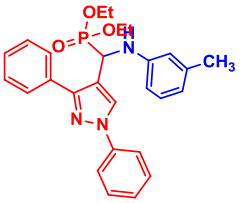
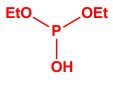
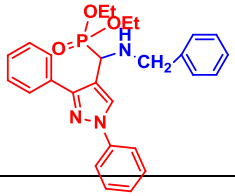
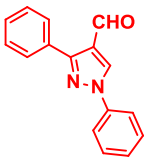
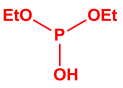
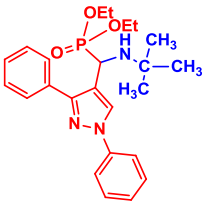
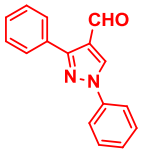
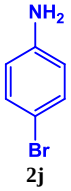
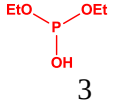
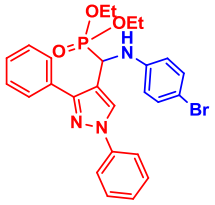
	 1	2c	3						
PV4	 1	 2d	 3		Pale brown solid	491	190-192	0.30	88
PV5	 1	 2e	 3		Off white solid	461	166-168	0.43	86
PV6	 1	 2f	 3		Off white solid	536	178-180	0.43	79
PV7	 1	 2g	 3		Off white solid	475	176-178	0.66	85
PV8	 1	 2h	 3		Off white solid	475	184-186	0.29	80
PV9	 1	 2i	 3		Pale brown solid	441	180-182	0.40	78
PV10	 1	 2j	 3		Off white solid	540	172-174	0.38	87

Table.2.DPPH Free radical scavenging activity of Pyrazole α-- amino phosphonate analogues

COMPOUND ID	CONC(µg/ml)										IC_{50} (µg/ml)
	1000	500	250	125	62.5	31.25	15.63	7.81	3.95	1.97	
PV1	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
PV2	2.05± 0.34	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
PV3	5.67± 1.28	1.38± 0.56	NI	NI	NI	NI	NI	NI	NI	NI	NI
PV4	**72.4± 0.56**	**67.9± 0.46**	**58.6± 0.56**	**30.6± 0.56**	**12.6± 0.00**	**5.9± 0.92**	**NI**	**NI**	**NI**	**NI**	**143.90**
PV5	1.25± 0.76	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
PV6	4.32± 1.36	2.56± 0.58	NI	NI	NI	NI	NI	NI	NI	NI	NI
PV7	**43.4± 0.16**	**40.6± 0.96**	**38.6± 0.96**	**34.6± 1.26**	**15.2± 0.24**	**9.6± 0.24**	**2.4±0 .04**	**NI**	**NI**	**NI**	**NI**
PV8	**39.2± 0.06**	**18.6± 0.14**	**7.4±1 .26**	**5.8±1 .38**	**2.6±0 .98**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**
PV9	11.6± 1.34	4.7±0 .16	NI	NI	NI	NI	NI	NI	NI	NI	NI
PV10	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Ascorbic acid	**94.3± 0.06**	**90.12 ±0.76**	**89.41 ±0.98**	**87.65 ±0.98**	**84.53 ±0.96**	**56.88±0. 26**	**28.33 ±0.67**	**15.48± 0.12**	**13.28 ±1.02**	**4.34± 1.24**	**25.20**

Figure.1.DPPH free radical scavenging activities of pyrazole alpha amino phosphonate analogues and the standard, Ascorbic acid. The data represents the percentage inhibition on DPPH radicals with respect to concentration. All data expressed as mean ± SE (n=3)

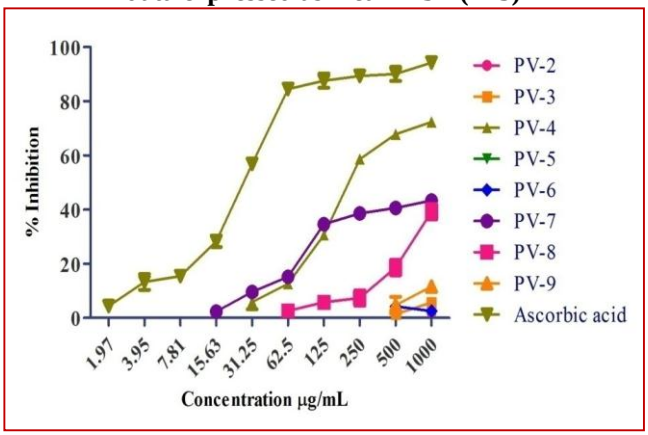


Figure.2.IC_{50} values of Pyrazole α-- amino phosphonate on anti-oxidant activity by DPPH scavenging assay

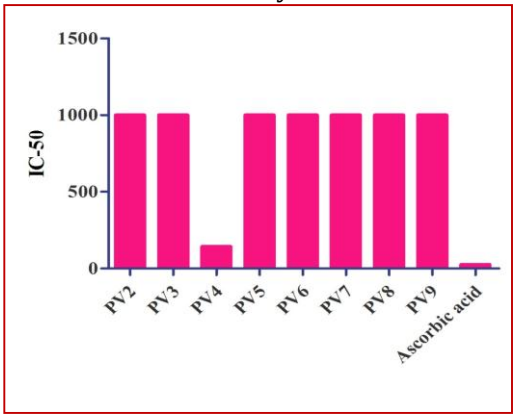


Table.3.Binding energy and docking score of Pyrazole α--amino phosphonates analogues at colon cancer targets

THYMIDYLATE SYNTHASE(PBD: ID: IHVY) COLON CANCER TARGET						
Compound ID	Structure	Cluster	Poses	RMSD Distance A G	Energy (Kcal/mol)	Ki (uM)
PV_1HVY/1HVY_3.dlg	R= HN-C_6H_4-Br(o)	3	8	24.451	-5.09	184.29
PV_1HVY/1HVY_10.dlg	R= HN-C_6H_4-Br(p)	1	10	15.328	-5.08	189.43
PV_1HVY/1HVY_1.dlg	R= HN-C_6H_4-Cl (p)	6	7	16.706	-4.79	305.93
PV_1HVY/1HVY_7.dlg	R= HN-C_6H_4-Me(m)	2	4	21.375	-4.66	386.30
PV_1HVY/1HVY_4.dlg	R= HN-C_6H_4-OMe(p)	5	4	20.397	-4.60	422.37
PV_1HVY/1HVY_6.dlg	R= HN-C_6H_3-OMe (o) NO_2(p)	14	4	25.648	-4.53	474.26
PV_1HVY/1HVY_5.dlg	R= HN-C_6H_5	5	9	25.272	-4.47	528.96
PV_1HVY/1HVY_2.dlg	R= HN-C_6H_4-Cl (o)	5	8	24.981	-3.98	1210.00
PV_1HVY/1HVY_8.dlg	R= HN-CH_2-C_6H_5	1	5	18.185	-3.67	2030.00
PV_1HVY/1HVY_9.dlg	R=HN-C-$(CH_3)_3$	4	6	13.393	-2.93	7170.00

Table.4.Binding energy and docking score of Pyrazole α--amino phosphonates analogues at Breast cancer targets

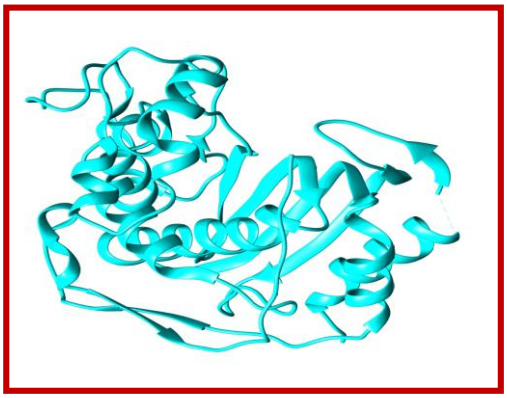
P-GLYCOPROTEIN(PDB ID : 3G61) BREAST CANCER TARGET						
Compound ID	Structure	Cluster	Poses	RMSD Distance A G	Energy (Kcal/mol)	Ki (uM)
PV_3G61/3G61_3.dlg	R= HN-C_6H_4-Br(o)	8	3	31.476	-4.78	315.97
PV_3G61/3G61_1.dlg	R= HN-C_6H_4-Cl (p)	11	3	40.565	-4.76	324.92
PV_3G61/3G61_6.dlg	R= HN-C_6H_3-OMe(o)-NO_2(p)	1	2	30.812	-4.68	371.62
PV_3G61/3G61_4.dlg	R= HN-C_6H_4-OMe(p)	8	3	28.067	-4.51	492.36
PV_3G61/3G61_10.dlg	R= HN-C_6H_4-Br(p)	6	2	40.196	-4.27	740.95
PV_3G61/3G61_7.dlg	R= HN-C_6H_4-Me(m)	8	5	35.953	-3.94	1290.00
PV_3G61/3G61_2.dlg	R= HN-C_6H_4-Cl (o)	3	4	30.182	-3.77	1730.00
PV_3G61/3G61_5.dlg	R= HN-C_6H_5	19	4	31.055	-3.74	1810.00
PV_3G61/3G61_8.dlg	R= HN-CH_2-C_6H_5	7	3	37.579	-3.29	3890.00
PV_3G61/3G61_9.dlg	R=HN-C-$(CH_3)_3$	13	5	36.963	-2.38	17930.00

Table.5.Set of 3 potent leads displaying various H-bond interacting residues in the active binding site of colon cancer target Thymidylate synthase enzyme -1HVYand breast cancer target P-Glycoprotein - 3G61.

	Thymidylate synthase enzyme -1HVY			P-Glycoprotein - 3G61		
Designed compound	**Key Interacting residues**	**Distance Å**	**No of H-Bond**	**Key Interacting residues**	**Distance Å**	**No of H-Bond**
PV4	Ph-CH_3-O---------HN- M311 CH_3-CH_2-O-----HN- K77 Hydrophobic interactions -N-Ph--------F80 -C-Ph-------------F225, I108	3.02 3.73	2	Hydrophobic interactions Pyrazole -N-Ph--------F71, F953, F332 CH_3O-Ph------F332, I336 Pyrazole-phe----------A981,V978	-	-
PV7	No hydrophilic interaction Hydrophobic interactions CH_3-Ph----------F80, I108 -N-Ph(CH_3)------F225,W109	-	-	No hydrophilic interaction Hydrophobic interactions Pyrazole -N-Ph--------F974, F953, F71 Pyrazole –Ph----------V978, A981 -N-Ph------L64, M67, I336	-	-
PV8	P=O--------HN-M309 Hydrophobic interactions -N-Ph-----F80, K107	3.07	1	No hydrophilic interaction Pyrazole -N-Ph--------F332, F71, F953, S-CH3-M309 Pyrazole –Ph----------Y949 -N-CH_2-Ph------I977, V978, F974	-	-

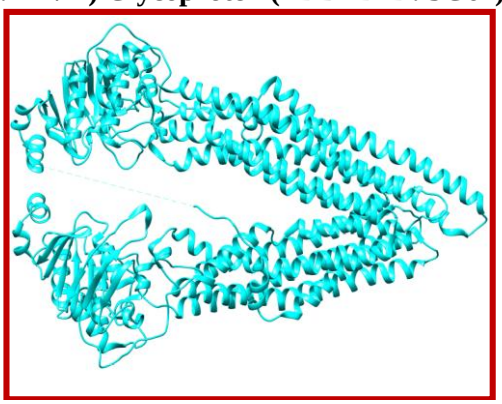
F = PHENYL ALANINE, K = LYSINE, M = METHIONINE, I = ISOLEUCINE, W =TRYPTOPHAN, V = VALINE, L = LEUCINE, Y=TYROSINE, A=ARGININE

Figure.3.3D Structure of Thymidylate synthase



A Chain: Cys104, Cys292, Cys593, Cys169, Cys284, Cys287, Cys99 Arg140, Arg147, Arg163, Asp148, Asp152, Asp159, Asp166, Asp186, Asp289, Asp290, Glu156, Glu100, Glu145, Glu150,Gly155, Gly157, Gly94, His141, Ile92, Ser151, Ser154, Ser95, Tyr153.

Figure.4.3D Structure of P- enzyme(PDB ID:1HVY) Glycoprotein(PDB IDID: 3G61)



A Chain: Arg460, Arg 543, Asn 383, Asn 544, Asp 382, Gln 385, Glu 464, Ile 338, Ile 465, Cys 380, Cys 546, Pro 381, Pro 545, Tyr 461.
B Chain: Asp 382, Ile 384, Pro 351, Tyr 461.

Figure.5.Docked pose of leads PV4, 7& 8 (A-C) of Pyrazole α--amino phosphonate scaffold in the active site of Thymidylate synthase enzyme (PDB ID:1HVY)

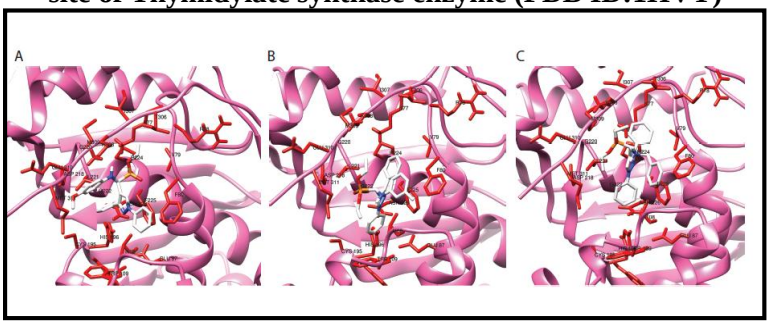
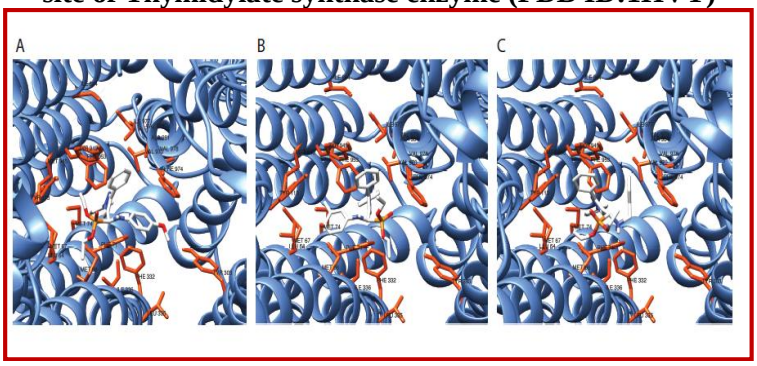


Figure.6.Docked pose of leads PV4, 7& 8 (A-C) of Pyrazole α--amino phosphonate scaffold in the active site of Thymidylate synthase enzyme (PDB ID:1HVY)



DISCUSSION

Characterization of synthesized compounds: The structures of the compounds (PV1-PV10) were investigated with spectral studies and demonstrated for compound PV2. In IR spectrum, the – NH stretching frequency appeared at 3298 cm^{-1}. In the ^{1}H NMR spectra there are two doublets in the region of δ 4.95 (J=8.4 and 20.6Hz) and 5.07 (J = 6.1 and 8.4 Hz) ppm with the integral value of one assigned as –CH and –NH protons respectively. Based on the above, the coupling constants J = 20.6 (on –CH proton) and 6.1Hz (on –NH proton) were due to the presence of phosphorus atom (one bond and three bonds respectively). In ^{13}C NMR spectrum, the peak at δ 46.8 ppm doublet ascertained the presence of methane group (Ar-CH-P). A distinguishable singlet at δ23.3 ppm in the ^{31}P NMR showed the presence of one phosphorus atom. The mass spectrum displayed the molecular ion [M]+ peak at *m/z*: 495.13.

***In vitro* DPPH Assay:** The free radical scavenging activity of pyrazole α- amino phosphonates analogues PV (1-10) was conducted and the effect of the different functional groups attached to amino (N-H) group such as p-chlorophenyl (PV1), o-chlorophenyl (PV2), o-bromophenyl (PV3), p-methoxyphenyl (PV4), o-methoxy p-nitrophenyl (PV6), o- tolyl (PV7) , benzyl (PV8), t-butyl (PV9), o-bromophenyl (PV10) in the phenyl parent scaffold (PV5) were studied. Among the ten compounds tested, PV4, PV7, PV8 have shown moderate scavenging activity and the rest of the seven analogues were deficient in activity by DPPH method. From the result (Table-2 Fig - 1 & 2) it was inferred that PV4, the compound with p-methoxy phenyl substitution possessed moderate activity with 143.90 µg/ml, comparable with that of ascorbic acid at IC_{50} 25.02 µg/ml.The compound bearing o- tolyl (PV7) and benzyl (PV8) substitutions shown temperate percentage inhibition of 43.4 ± 0.16 and 39.2 ± 0.06 respectively at the concentration of 1000 µg/ml.

The effect of substituents at para and meta positions of the phenyl ring was studied. Interestingly the radical scavenging ability of this scaffold was supported by the presence of moderately active methoxy group at the para position on the phenyl ring. However the presence of electron withdrawing group such as chloro, bromo at the ortho and para position did not favour the activity. But the compounds having chloro (PV2) and bromo (PV3) at ortho position exhibited action when compared to that of parent compound (PV5) and with that of compound having chloro (PV1) and bromo (PV10) at para position having no inhibition.

***In silico* Autodocking:** The docking results for PV ligands 1 -10 against 1HVY and 3G61 were summarized in Table 3 and 4 respectively. When docked against 1HVY the binding affinity for PV ligands (1-10) ranged within 184.29 to 7170.0 µM with an average value of 1290 µM. The binding affinity of PV7, 4 was 386.3 and 422.3 µM respectively. Unlike PV7 and 4, PV 8 showed a weak affinity towards 1HVY with Ki of 2030 µM. The docking results of PV1-10 against 3G61 followed a similar binding affinity profile ranging within 315.9 to 17930 µM with an average of 2889.5 µM. As seen with 1HVY, PV 4 and 7 showed better inhibition against 3G61 with ki value of 492.4 and 1290 µM compared to 3890 µM of PV8.

The binding site analysis was carried out by overlaying the docked ligands individually and fortunately found that they were binding to the same pocket of thymidylate synthase enzyme, P-Glycoprotein (Fig :3 & 4) and the key interacting residues were highlighted for each best fit within a zone of 5A (Table:5).

Binding site of PV ligands in 1HVY: According to the docking result there are three compounds with high affinity namely PV7, PV4 & PV8 with binding affinities Ki=386.30, 422.37 & 2030.00 µM respectively were identified as best fit. The shared functional groups Ph-O-CH_3 in PV4 has hydrogen bonding interaction with NH- M311 (3.02À) and CH_3-CH_2-O- with NH-K77 (3.73 À).The compound tolyl side chain did not show any hydrophilic interaction but PV8 having benzyl side chain has considerable hydrogen bonding in P=O with NH of M309 (3.07À). In all the three compounds hydrophobic interaction due to N-Ph, CH_3 –Ph and N-Ph with F80 for PV4,7,8 were observed respectively (Fig:5).

Binding site of PV ligands in 3G61: According to the docking result there are three compounds with high affinity namely PV4, PV7 & PV8 with binding affinities Ki= 492.36,1290,3890 µM respectively were identified as best fit. It was found that there were no hydrophilic interactions seen in any of these inhibitors. But the hydrophobic interaction due to Pyrazol –Phe with R981,V978, Pyrazole-N-Ph with F71, F953, F332, F974, M309 were the characteristics features in all of these three inhibitors (Fig:6).

4. CONCLUSION

In this present study, we have demonstrated a simple and environmental friendly protocol for the synthesis of pyrazole α- amino phosphonates analogues under neat condition in good yield. It was inferred that the 3 synthetic inhibitors have proven to be effective free radical scavengers by DPPH assay. But it is noteworthy that all the compounds proven to be effective in *in-silico* molecular docking studies at both the specific cancer targets. Therefore based on these findings, the study explores the primary leads PV4, PV7, PV8 for cytotoxicity assay in specific cancer lines like HT-29(colon), MCF-7 (breast) and anti-cancer mechanism in future with a greater hope that the results may provide a novel candidate for pre-clinical toxicological and efficacy studies in carcinogenesis induced xenograft models..

5. ACKNOWLEDGMENTS

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