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Theoretical Bio-Estimation of 1,2,3-triazolo[4,5-d]pyrimidine Hybrids using DFT, Multiple Linear Regression (MLR) and Docking Approaches

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ABSTRACT

26 sets of molecular compounds were studied for anti-esophageal cancer activity. B3LYP/6-31+G basis set was used for Quantum chemical calculation and the obtained molecular descriptors were used for QSAR studies via Gretl software. Thus, the developed model predicted efficiently and it was used to predict the cytotoxicity of the proposed compounds. Furthermore, molecular docking study was carried out on esophageal cancer cell line (2leo) and it was observed that A13 inhibited more than all the studied compounds. Also, all the studied compounds were more effective than the standard (5-FU).*

Keywords: 1,2,3-triazolo[4, 5-d]pyrimidine hybrids, DFT, QSAR, Molecular docking.

INTRODUCTION

Recently, the broad uses of antibiotics against microorganism have brought about a new search for more effective drug-like molecules and modification of the existing antibiotics. Cancer, a frenzied cell growth, is positioned high amidst every disease attacking humans and have been reported to be the cause of death of several human beings [1]. Despite the continuous effort of scientist over the world to overcome cancer, the more the number of people with cancer upturns [2]. Globally, its position as an agent of death still remains the second. According to Fridlender et al., and Gali-Muhtasib et alcancer could be caused by malfunction of basic cellular processes in human body [3,4].

Also, esophageal cancer is a deadly tumor. It is ranked to be one of the top five most dreaded tumor ever recorded [5]. It is majorly sub-divided into two, esophageal squamous-cell carcinoma (ESCC) and esophageal adenocarcinoma (EA). ESCC is a common malevolence that is rated to be fourth leading cause of deaths in Asia region [6]. It diagnosis is poor and the cure for this malignant disease is still a serious threat to medicinal world [7,8].

In the designing and development of drug-like molecules, the role play by heterocyclic compounds is very vital. 1, 2, 3-triazolo[4,5-d]pyrimidine analogues as heterocyclic molecules have two major active compounds i.e. triazole and pyrimidine. Both triazole and pyrimidine are important class of molecular compounds with reputable bio-activities and this has drawn the attention of researchers over the world. Triazole and Pyrimidine derivatives possess many biological activities such as antimicrobial [9,10], anticancer [11], antioxidant [12], anti-inflammatory

[13,14], diuretics [15] and analgesics [16,17].

As shown in Figure 1, twenty-six selected molecular compounds used in this work were optimized using density functional theory. The compounds used were randomly selected from two experimental research work and were subjected to further studies. Compounds A1-A15 as shown in Figure 1 were taken from Li et al., [18] and compound A16-A26 were chosen from Li et al., [19]. The optimized molecular compounds are 3-benzyl-7-(2-(2-chlorobenzylidene)hydrazinyl)-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (A1), 3-benzyl-7-(2-(4-nitrobenzylidene)hydrazinyl)-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (A2), 4-((2-(3-benzyl-5-(propylthio)-3H-[1,2,3]triazole-[4,5-d]pyrimidin-7-yl)hydrazono)methyl)-N,N-dimethylaniline (A3), 7-(2-((1H-indol-3-yl)methylene)hydrazinyl)-3-benzyl-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (A4), 1-((2-(3-benzyl-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)hydrazono)methyl)naphthalen-2-ol (A5), 2-(1-(2-(3-benzyl-5-(propylthio)-3H-[1,2,3]-triazolo[4,5-d]-pyrimidin-7-yl)-hydrazono)-ethyl)phenol (A6), 3-benzyl-7-(2-(1-(4-bromophenyl)ethylidene)hydrazinyl)-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (A7), 2-(1-(2-(3-(3-chlorobenzyl)-5-(propylthio)-3H-[1,2,3]-triazolo[4,5-d]pyrimidin-7-yl)-hydrazono)-ethyl)phenol (A8), 2-(1-(2-(3-(4-chlorobenzyl)-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)hydrazono)ethyl)-phenol (A9), 2-(1-(2-(3-(furan-2-ylmethyl)-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]-pyrimidin-7-yl)-hydrazono)ethyl)phenol (A10), 2-(1-(2-(3-(4-hydroxybenzyl)-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)hydrazono)ethyl)-phenol (A11), 3-benzyl-5-(prop-2-yn-1-ylthio)-7-(2-(3,4,5-trimethoxy-benzylidene)hydrazinyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (A12), 1-((2-(3-benzyl-5-(prop-2-yn-1-ylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)hydrazono)methyl)naphthalen-2-ol (A13), 2-(1-(2-(3-benzyl-5-(prop-2-yn-1-ylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)hydrazono)-ethyl)phenol (A14), 3-benzyl-5-(benzylthio)-7-(2-(3,4,5-trimethoxybenzylidene)hydrazinyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (A15), 1-(2-((3-Phenethyl-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)ami-no) ethyl)-3-(3-(trifluoromethyl)phenyl)thiourea (A16), 1-(3-Chlorophenyl)-3-(2-((3-phenethyl-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d] pyrimidin-7-yl)amino)ethyl)thiourea (A17), 1-(4-Chlorophenyl)-3-(2-((3-phenethyl-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d] pyrimidin-7-yl)amino)ethyl)thiourea (A18), 1-(4-Butylphenyl)-3-(2-((3-phenethyl-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d] pyrimidin-7-yl)amino)ethyl)thiourea (A19), 1-(2-((3-Phenethyl-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)amino) ethyl)-3-(pyridin-2-yl)thiourea (A20), 1-(2-((3-Phenethyl-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)amino) ethyl)-3-(pyridin-3-yl)thiourea (A21), 1-Benzyl-3-(2-((3-phenethyl-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)amino)ethyl)thiourea (A22), 1-(2-((3-Cyclopentyl-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)amino)ethyl)-3-(pyridin-3-yl)thiourea (A23), 1-(2-((3-(2-hydroxyethyl)-5-(propylthio)-3H-[1,2,3]-triazolo[4,5-d]-pyrimidin-7-yl) amino)ethyl)-3-(pyridin-3-yl)thiourea (A24), 1-(2-((3-benzyl-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)amino) ethyl)-3-(pyridin-3-yl)thiourea (A25), 1-(2-((3-(2-(1H-indol-3-yl)ethyl)-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d] pyrimidin-7-yl)amino)ethyl)-3-(pyridin-3-yl)thiourea (A26).

Thus, this work is aimed at developing effective QSAR model via multiple linear regression (MLR) method using the calculated molecular descriptors of 1,2,3-triazolo[4,5-d]pyrimidine Hybrids as well as examining the non-bonding interactions between 1,2,3-triazolo[4,5-d]pyrimidine Hybrids and Esophageal cancer cell line (PDB ID: 2leo) [20] via molecular docking.

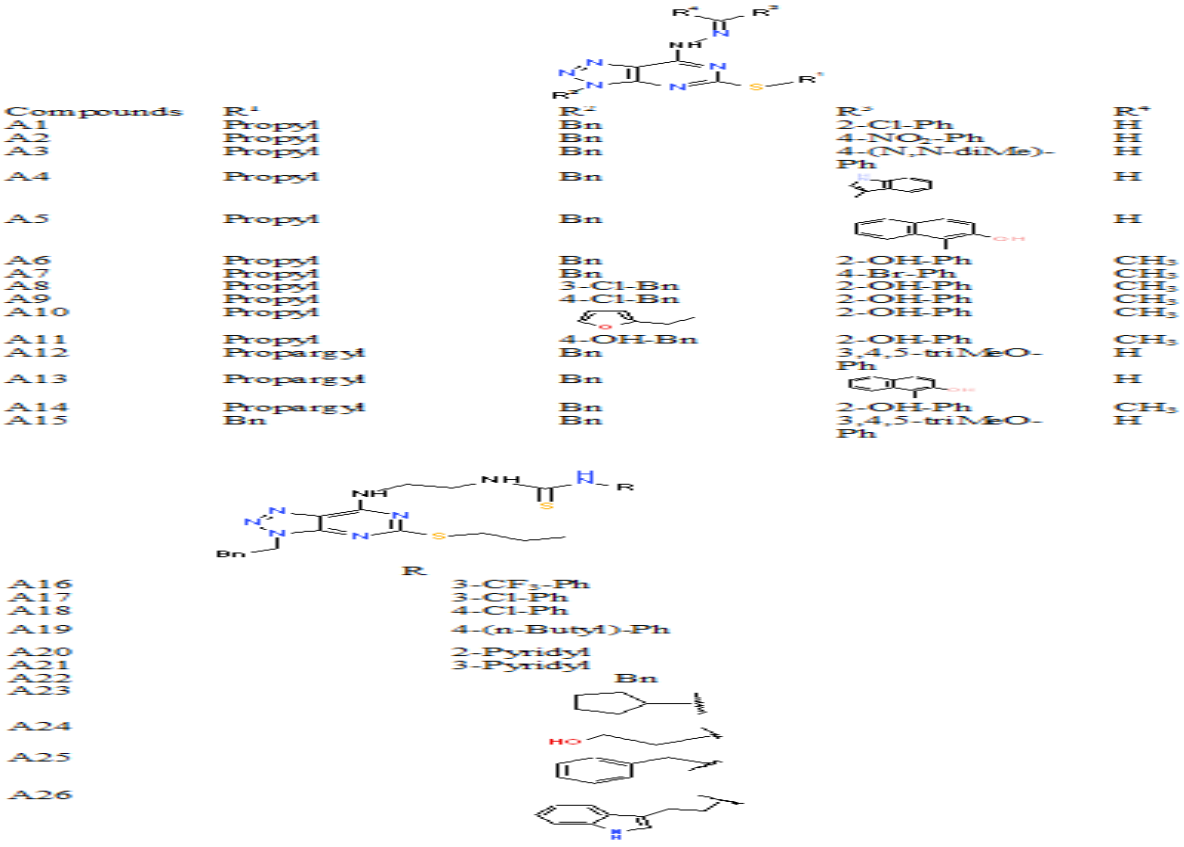


Figure 1: The schematic structures of 1,2,3-triazolo[4, 5-d]pyrimidine analogues

METHODS AND MATERIALS

Methodology

Quantum chemical method via Density functional theory method (6-31+G* basis set) was used for optimization of the studied compounds (1, 2, 3-triazolo[4, 5-d]pyrimidine derivatives) in gas phase and the energy calculation was executed in water using Spartan 14 [21]. As shown in Table 1, several parameters were obtained from quantum chemical study and were used further study in this work. Moreover, the obtained calculated descriptors were used to develop QSAR model in order to predict inhibition concentration (IC_{50}). The calculated molecular descriptors were highest occupied molecular orbital energy (E_{HOMO}), the lowest unoccupied molecular orbital energy (E_{LUMO}), band gap (eV), Ovality, dipole moment (Debye), log P, and molecular weight (amp). The developed QSAR model for multiple linear regression was achieved by using Gretl [22].

Moreover, several statistical parameters such as squared correlation coefficient (R^2), adjusted squared correlation coefficient R^2_{adj}, cross validation ($CV.R^2$) (equations 1 and 2) and the significance level (p-value) [23] were used for QSAR model validation. Also, non-bonding interactions between the studied ligand-receptor complex as well as the binding energy were observed using docking approach. In this study, the grid dimension (grid size) used for the studied receptor (2leo) is 28 × 24 × 28 Å and the grid centre used for X, Y and Z coordinate are -3.266 Å, 0.702 Å and 0.427 Å respectively. Inhibition constant (Ki) was calculated using equation 3.

$$CV.R^2 = 1 - \frac{\sum (Y_{obs} - Y_{cal})^2}{\sum (Y_{obs} - \bar{Y}_{obs})^2} \qquad (1)$$

The R^2 adjusted could be calculated using equation (2)

$$R_a^2 = \frac{(N-1) \times R^2 - P}{N - 1 - P} \qquad (2)$$

$$K_i = e^{\frac{-\Delta G}{RT}} \qquad (3)$$

RESULTS AND DISCUSSION

DFT and QSAR Studies

In this work, the entire studied compounds were divided into twenty (20) and six (6) molecular compounds as training set and test set respectively. The calculated descriptors from 1,2,3-triazolo-4,5-d-pyrimidine hybrids via density functional theory at 6-31+G* described anti-*Esophageal* cancer activity. Seven selected descriptors (HOMO, Area, and polarizability, N4N5, N3, N5 and NOH) and inhibition concentration (IC_{50}) were employed in developing QSAR model via multiple linear regression method. Inhibition concentration (IC_{50}) served as dependent variable and the selected molecular descriptor served as independent variable. The developed QSAR model predicted the bioactivity of 1,2,3-triazolo-4,5-d-pyrimidine derivatives and this was confirmed via the calculated squared correlation coefficient (R^2), adjusted squared correlation coefficient (R^2_{adj}), cross validation (CVR^2), P value, MSE. The set of compounds used as test set (which was denoted by *) were observed to be closer to their observed IC_{50} as shown in Table 1. According to values obtained for the test set using equation 4, it was discovered that the observed IC_{50} for A6* is greater than the predicted IC_{50}, for A18*, the predicted IC_{50} was the same with the observed IC_{50}. Mores so, the predicted value for A1*, A12*, A23* and A26* were greater than the observed IC_{50}. This mean that the developed model (Equation 4) used was effective and efficient (Table 1).

Moreover, the contribution of each molecular descriptors used in the development of the QSAR model were displayed in equation 4. It was observed in equation 4 that, polarizability, bond length between N4 and N5 and Nitrogen on position 3 (N3) contributed positively to the inhibition concentration and E_{HOMO}, Area, N5 as well as NOH negatively contributed to the predicted IC_{50}. Thus, as shown in Table 1, A3 proved to be more potent than other studied compounds and this could be attributed to the derivative attached to it (Table 1).

IC_{50} = -107.202-0.792953(HOMO)-0.0102402(AREA)+0.152360(POL)+71.7702(N4N5)+1.31188(N3) -2.08087 (N5)-0.144364(NOH)----(4)

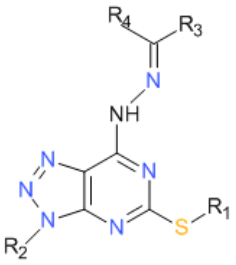
N= 20, F = 16.28, P < 0.0001, R^2 = 0.905, R^2_{adj} = 0.849, $C.VR^2$ = 0.994, MSE = 0.004

Table 1: Observed IC50 and predicted IC_{50}

	Observed IC_{50}	Fitted IC_{50}
A1*	-0.79	-0.94
A2	-0.82	-0.88
A3	-1.30	-1.32
A4	-0.93	-0.94
A5	-0.96	-0.89
A6*	-1.04	-1.00
A7	-0.66	-0.79
A8	-1.11	-0.99
A9	-1.05	-1.13
A10	-0.76	-0.66
A11	-1.20	-1.26
A12*	-0.81	-0.89
A13	-0.81	-0.84
A14	-0.72	-0.66
A15	-0.64	-0.66
A16	-1.24	-1.24
A17	-1.07	-0.98
A18*	-1.02	-1.02
A19	-1.27	-1.24
A20	-0.73	-0.80
A21	-0.69	-0.78
A22	-1.13	-1.08
A23*	-1.09	-1.25
A24	-1.26	-1.22
A25	-1.03	-1.01
A26*	-0.90	-1.46
*denote test set		

Proposed Novel Molecular Compounds

Eight molecular compounds were proposed and their inhibition concentration were predicted using the developed QSAR model (equation 4). The calculated inhibition concentration for the proposed compounds are -0.65,-0.64, 0.39, -0.83, -0.52, -1.04, -1.69, -0.84 for AN1 –AN8 respectively. It was discovered that all the proposed molecules were more potent than the standard ligand (5FU) used in the model paper (Figure 2). More so, the derivatives attached to 1,2,3-triazolo[4, 5-d]pyrimidine derivatives increase the potency of the proposed compounds thereby leading to increase IC_{50} value (in term of negativity). The replacement of Propyl (R_1), Bn (R_2), 4-(N,N-diMe)-Ph (R_3) and H (R_4) in A3 with Ethyl (R_1), C_6H_5Br(R_2), CF_3 (R_3), and Br (R_4) in AN7 respectively resulted in to more effective and potent drug-like compounds (AN7).



	R_1	R_2	R_3	R_4
AN1	Propyl	F	3,4,5-triMeO-Ph	H
AN2	Propyl	F	3,4,5-triMeO-Ph	F
AN3	Propyl	-OMe	3,4,5-triMeO-Ph	F
AN4	H	Cl	3,4,5-triMeO-Ph	F
AN5	Propyl	F	No_2	F
AN6	Butyl	S	CN	Cl
AN7	Ethyl	C_6H_5Br	CF_3	Br
AN8	Ethyl	NO_2	CH_3	H

Figure 2: The schematic structures of the proposed compounds

Molecular Docking

The result obtained for docking studies revealed the compound with ability to inhibit *Esophageal* cancer cell line via the calculated binding affinity. It was observed that all the studied compounds in this research inhibited more effectively than the standard used. More so, out of the entire studied compounds, A13 inhibited more effectively than other studied compounds (Table 2), since compounds with lowest binding affinity value prove to inhibit the most. Also, the residues involve in 1-((2-(3-benzyl-5-(prop-2-yn-1-ylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)hydrazono)methyl)naphthalen-2-ol (A13)-2leo complex were displayed in Figure 3.

Table 2: Interactions between 1,2,3-triazolo[4,5-d]pyrimidine Derivatives and receptor (2leo)

	Scoring **(kcal/mol)**	K_i **(μM)**	Amino Acid Residue
A1	-6.6	6.93×10^4	ASP-82, SER-20, ALA-22, LYS-28, LEU-50
A2	-7	1.36×10^5	GLU-21, LYS-28, VAL-52, LEU-50, PRO-51, SER-20, ASP-82, ALA-22
A3	-7.1	1.61×10^5	GLU-21, VAL-52, LEU-50, PRO-51, ASP-82, SER-20, ALA-22, PHE-79, LYS-28
A4	-7.1	1.61×10^5	VAL-52, LEU-50, ALA-22, ASP-82, GLU-21, LYS-28, GLU-63
A5	-7	1.36×10^5	GLU-63, LEU-50, VAL-52, LYS-29, VAL-30, PRO-46
A6	-7.1	1.61×10^5	LYS-28, GLU-21, LEU-50, PRO-51, SER-20, ASP-82, PHE-79, ALA-22
A7	-6.8	9.71×10^4	LEU-50, LYS-28, ASP-31, ILE-34, GLU-63, VAL-30, ASP-82
A8	-6.8	9.71×10^4	LYS-28, GLU-21, ASP-82, ALA-22, PHE-79, GLU-63
A9	-6.7	8.20×10^4	SER-20, GLU-21, LYS-28, ASP-82, ALA-22, PRO-51, LEU-50, GLU-63
A10	-7.2	1.90×10^5	GLU-21, LYS-28, LEU-50, PHE-79, ASP-82, SER-20, ALA-22
A11	-6.4	4.94×10^4	ILE-34, TYR-38, PRO-44, GLU-63, PRO-46, LYS-28
A12	-6.6	6.93×10^4	ASP-82, ALA-22, VAL-52, LEU-50, GLU-63, ASP-31, LYS-29, LYS-28, GLU-21
A13	-7.7	4.44×10^5	LYS-28, GLU-21, ASP-82, ALA-22, PRO-51, LEU-50, GLU-63
A14	-7.5	3.16×10^5	PHE-79, GLU-21, LEU-50, VAL-52, PRO-51, SER-20, ASP-82, LYS-28, SER-26, ALA-26, ALA-22
A15	-7	1.36×10^5	GLU-21, SER-20, ASP-82, ALA-22, PRO-51, VAL-30, ASP-31, LYS-29, GLU-63, LYS-28
A16	-6.8	9.71×10^4	PHE-79, GLU-21, ASP-82, ALA-22, PRO-51, LEU-50, VAL-30, LYS-29, PHE-79
A17	-6.9	1.15×10^5	PHE-79, GLU-63, LYS-28, PRO-51, LEU-50, SER-20, ASP-82, ALA-22
A18	-6.8	9.71×10^4	ALA-22, LYS-28, GLU-63, LEU-50, PRO-51, PHE-79, SER-20, ASP-82
A19	-5.8	1.79×10^4	PRO-51, SER-20, LEU-50, GLU-21, LYS-28, PHE-79, ALA-22,
A20	-6.2	3.52×10^4	LEU-50, GLU-63, VAL-52, LYS-28, GLU-21, ASP-82, ALA-22
A21	-5.9	2.12×10^4	LEU-50, SER-20, LYS-28, ASP-82, ALA-22
A22	-6.3	4.17×10^4	LEU-50, GLU-63, VAL-52, LYS-28, SER-20, GLU-21, ALA-22, ASP-82, PRO-51
A23	-5.6	1.28×10^4	LYS-28, SER-20, ALA-22, PRO-51, LEU-50
A24	-5.8	1.79×10^4	GLU-21, SER-20, ASP-82, PRO-51, PHE-79, LYS-28
A26	-6.7	8.20×10^4	GLU-21, ASP-82, SER-20, PRO-51, LEU-50, LYS-28
A27	-6.9	1.15×10^5	LEU-50, LYS-29, LYS-28, TYR-35, PRO-46, GLU-63, ASP-31
5-FU	-4	0.85×10^3	SER-33, CYS-32, VAL-30, GLN-78

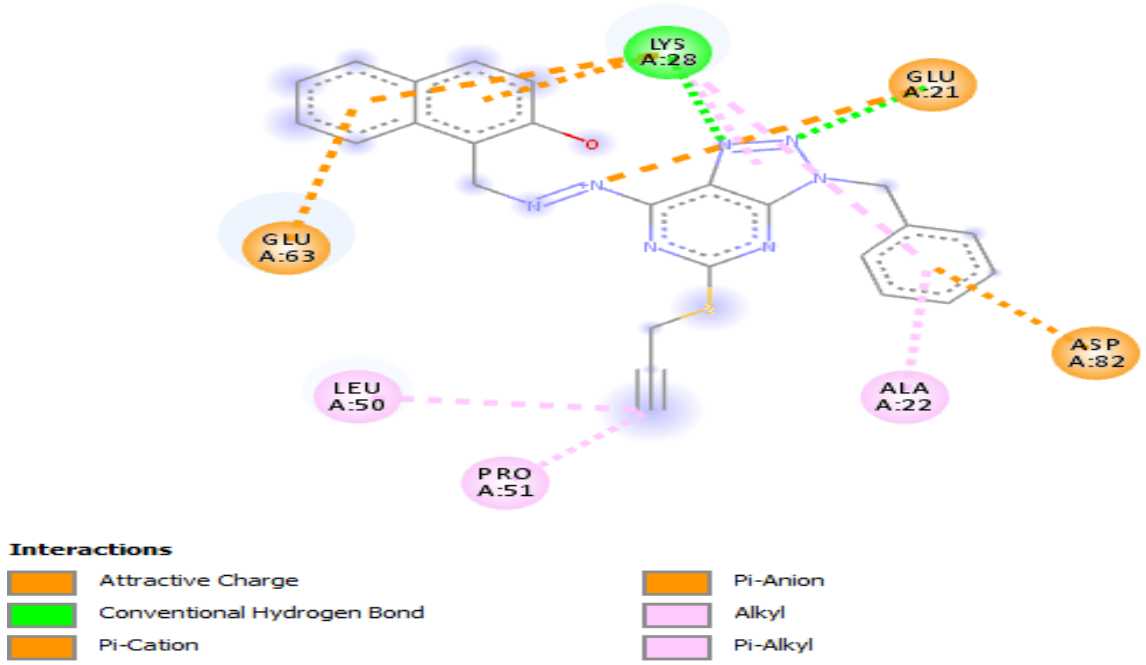


Figure 3: Interactions of A13 with the residue in the active gouge of Esophageal cancer cell line (2leo)

CONCLUSION

Bioactivity of 26 sets of 1, 2, 3-triazolo[4, 5-d]pyrimidine derivatives were studied by observing the calculated electronic descriptors using density functional theory method via 6-31+G(d,p) basis set. The selected calculated molecular descriptors which described anti-Esophageal cancer activity of 1, 2, 3-triazolo[4, 5-d]pyrimidine analogues were used in QSAR study. The QSAR method used was predictive and the docking study was also observed to show the non-bonding interaction as well as the binding affinity between the studied compounds and the receptor. Thus, in the docking study, 1-((2-(3-benzyl-5-(prop-2-yn-1-ylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)hydrazono)methyl)naphthalen-2-ol (A13) inhibited more effectively than other compounds.

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