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Theoretical Studies on Triazoles of 3-Acetylbetulin and Betulone as Anticancer Agents

[1]Erazua Ehimen A., [2]Adepoju Adewusi John, [3]Ajayi Ayomide Peter, [3]Josiah Olubunmi Modupe, [3]Adelowo Joy M, [3]Odoemene Simon N., [4]Akintelu Sunday A and [3]Oyebamiji Abel Kolawole

[1]Department of Chemistry, University of Ibadan, Ibadan, Nigeria.
[2]Department of Pure and Applied Chemistry, LadokeAkintola University of Technology, PMB 4000 Ogbomoso, Nigeria.
[3]Department of Basic Sciences, Adeleke University, P.M.B. 250, Ede, Osun State, Nigeria
[4]School of Chemistry and Chemical Engineering, Beijing Institute of Technology, Beijing, China.
erazuaann@gmail.com

Abstract: Several types of cancer cell lines are still resistant to various substances (drug-like molecules) obtained from plant. Globally, the threat posed by cancer to human beings i.e. men and women; young and old still remain challenge to medical world. Thus, the need to discover effective and efficient drug-like molecule such as Triazoles of 3-Acetylbetulin and Betulone to combat/ suppress cancer in human being remain a continuously effort among the researchers. Molecular docking approach is recently used to improve the understanding of the interaction between drug and receptor, hence, leading to the development of novel drugs with better properties. Derivatives of 3-acetylbetulin and betulone bearing 1,2,3-triazole moiety were evaluated against amelanotic melanoma C-32 cancer cell lines using Density Functional Theory (DFT), and Docking approaches. Docking of the triazoles of 3-acetylbetulin and betulone compounds with target proteins of PDB code 5vau were performed using Discovery Studio 4.1 visualizer, Autodock tool, AutoDockVina. Biovia Discovery Studio 2017 was used for the post docking analysis. The correlation between calculated molecular descriptors and calculated binding affinity were studied. Therefore, 28-[1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl]carbonylbetulone (H) was observed to have the highest inhibition efficiency while 28-Propynoylbetulone (B) has the least inhibition efficiency. Also, six molecular descriptors i.e. molecular weight, area, volume, polar surface area, polarizibility, and hydrogen bond acceptor played significant role in the binding affinity of 28-Propynoylbetulone (B) in the active site of amelanotic melanoma C-32 cancer cell lines (5vau). Four descriptors (band gap, E_{LUMO} electrophilicity index and Chemical potential) also played a significant role in the binding affinity of compound H to the studied protein. Thus, 28-[1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl]carbonylbetulone (H) proved to have highest ability to inhibit **5vau** than other studied compounds. Also, correlation between the calculated binding affinity and the calculated molecular descriptors were observed.

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1. Introduction

There is an increase in the cancer incidence and mortality. Melanomas are malignant tumors derived from melanocytes. The most common site of involvement is the skin, although occasionally primary melanoma develops in other organs (eye, oral and nasal mucosa, vulval and anorectal mucosa, other gastrointestinal mucosa and the central nervous system (CNS)) [1]. Amelanotic melanoma (AM) refers to any melanoma lacking melanin pigmentation. AM accounts for 2-8% of all melanomas [2]. Studies show that survival after diagnosis of amelanotic melanoma is poorer than after pigmented melanoma, probably because the diagnosis is difficult and is made in more advanced stage [3]. Despite the significant development of new surgical techniques, radio-, chemo-, and targeted therapy, failures in tumour treatment are still the most important challenges to oncology [4]. Additionally, the acquired drug resistance by tumour cells is considered to be responsible for the failure of conventional types of oncological therapy, including cytostatic drugs and radiation [5]. A novel approach to the cancer treatment has appreciated the key components of specifically altered signalling pathways in neoplastic cells or targeting of the tumour microenvironment

without affecting non cancerous cells. There are a lot of plant-derived substances that are effective antitumour and chemopreventive agents, but there are also a lot of tumour types that do not respond, or become resistant to them. This lead to d continues search for of new active compounds [6].

A promising group of anticancer or chemopreventive agents has been derived from natural product and this has lead to the development of drugs or supplements for the treatment of several human cancers [7, 8]. Among the most abundant natural products identified are the triterpenes. Triterpenesare one of terpenes classes, formed from six isoprene units and occur as complex cyclic structures called triterpenoids [9]. Pentacyclictriterpenes and its derivatives, are being marketed as therapeutic agents or dietary supplements around the world. Betulin (3-lup-20(29)-ene-3β,28-diol) is a pentacycliclupane-type triterpenoid isolated from a bark of many species of birch [10, 11]. It has being a good material for the preparation of new derivatives with a broad spectrum of biological activities, such as anticancer, antiviral, antimalarial, antibacterial, anti-inflammatory, and hepatoprotective [10] activities. Among all the pharmacological properties of betulin, the anticancer and chemopreventive activity is attracting a lot of attention. The natural and synthetic derivative of betulin act specifically on cancer cells with low cytotoxicity towards normal cells [6].

On the other hand Triazole are nitrogen heterocyclic compounds known to exhibit interesting biological activities. The 1,2,3-triazole ring is an attractive connecting unit, which exhibits a high stability under acid/base hydrolysis conditions. They are capable of forming hydrogen bonds, which can be important for their bioavailability and solubility [12]. The triazoles can be used as a biological linker, it is known that the triazole linkage is stable inside cells, and can interact with viral proteins [13]. Triazole compounds in biological system display versatile biological activities and many of them have been identified as clinical drugs or candidates for the treatment of various types of diseases [14]. Most of the triazole analogs of natural compounds have been investigated for their anticancer activity [15-17]. This expanded the interest to synthesize new triazoles of pentacyclic triterpenes and study their anticancer activity [18].

Great advancements in computational methods have made it possibile for the development of novel drugs with robust properties. There is an increasing interest on potential targets of bioactive small molecules. A computational approach known as molecular docking is frequently used to predict the binding modes of small molecules to their targeted proteins. This gives a better understanding on the drug–enzyme interaction hence, it plays an important role in rational drug design [19, 20]. Density functional theory (DFT) with its different levels of calculations has been found effective and reliable in successfully predicting the properties of various compounds within limits of the experimental values. It has been used to obtain some fundamental properties of compounds which could not be easily derived from laboratory procedures [21].

Bebenek*et al*., (2018) [18] synthesized a series of new derivatives of 3-acetylbetulin and betulone bearing 1,2,3-triazole moiety and evaluated them for their anticancer activity in vitro against amelanotic melanoma C-32, ductal carcinoma T47D and glioblastoma SNB-19 cell lines [18]

In this work, the objectives of this study are (i) to use quantum chemical method via density functional theory for the calculation of descriptors which describe the cytotoxicity of the studied compounds (ii) to discover the non-bonding interactions that is present between Triazoles of 3-Acetylbetulin and Betulone and the studied receptor.

2. Material and Methods

2.1 Quantum Chemical Methods

Twelve triazoles of 3-acetylbetulin and betulone(Table 1) were obtained from literature reported by Bebenek *et al*., (2018) [18]. Geometry optimized structures was generated for the selected compounds (A-L) using Spartan'14 (Wavefunction, Inc). DFT calculations was carried out using the Becke's gradient exchange correction [22] with the Lee–Yang–Parr correlation functional (B3LYP) [23], together with the 6-31G*(d,p) basic set.

2.2 Molecular Docking Study

All compounds were docked to catalytic binding sites of amelanotic melanoma cancer cell lines (5PDB:5vau) downloaded from protein data bank [24] to predict their binding modes and approximate binding free energies. The receptor protein was prepared using Discovery Studio 4.1 visualizer. Autodock tool was used to convert the optimized ligands and the receptor to pdbqt format. Computational docking was executed with the AutoDockVina software. Biovia Discovery Studio 2017 was used to analyze the output of docking process. The lowest energy conformation was identified and binding energies were evaluated.

3. Results

3.1 Molecular Descriptors and Docking Studies

Calculated molecular descriptors namely; molecular weight (MW), partition coefficient (Log P), volume (V), Area, polar surface area (PSA), E_{HOMO} (highest occupied molecular orbital energy), E_{LUMO} (lowest unoccupied molecular orbital energy),dipole

moment (DM), Band gap (BG), electrophilicity index (ω),Chemical potential(μ) hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA). The binding affinity/ binding energy for each complex formed by compound A-L with the receptor protein 5vau is also shown in Table 2. From the study the values obtained for the binding affinity are -5.5 Kcal/mol, -4.9Kcal/mol, -6.0Kcal/mol, -6.1Kcal/mol, -5.8Kcal/mol, -5.3Kcal/mol, -6.3Kcal/mol, -6.6Kcal/mol, -5.0Kcal/mol, -5.5Kcal/mol, -6.2Kcal/moland -6.0Kcal/mol,for compounds **A-L** respectively.

This revealed that compound H had the greatest tendency to inhibit the active site of amelanotic melanoma cell line with a binding energy of -6.60kcal/mol, while compound B has the least tendency to inhibit the active site of amelanotic melanoma cell line with a binding energy of -4.9 kcal/mol, Hence compound H formed the most stable complex with the protein. This may be attributed to the presence cyano group in the compound.

3.2 Correlation between Calculated Descriptors and Binding Affinities.

The correlation of some calculated molecular descriptors with binding affinity is discussed here. The energy difference between the HOMO and the LUMO (band gap) establishes correlation in various chemical and bio-chemical systems [25] and is very relevance to drug-receptor interactions. Molecules with narrower energy gap have higher chemical reactivity, lower stability and better ability to react with the neighboring molecule [26]. As shown in Table 2, Compound H has the lowest value of energy gap, this correspond to the lowest binding affinity (-6.60kcal/mol). This greatly influenced the behaviour of the compound H in the gouge of the amelanotic melanoma cancer cell line (5vau) and thereby increasing the ability of H to inhibit well more than other studied compounds. Therefore, it could be suggested that band gap play a crucial role in enhancing the ability of compound H to inhibit amelanotic melanoma cancer cell line than other studied compounds.

Moreover, the E_{LUMO} gives qualitative facts about the excitation features of molecules and it represents the ability of a molecule to obtain an electron from the compounds that have the ability to release it [27]. Compound H with a greater ability to inhibit 5vau than other compounds has the lowest value of E_{LUMO}. Thus, the ability of compound H to inhibit 5vau is attributed to its ability to receive electron from amelanotic melanoma cancer cell line (5vau) that have the ability to release it which resulted to it better inhibiting strength. In this work, it could be suggested that LUMO energy contributed to increased ability of compound H to inhibit than other compounds.

Chemical potential, μ indicates reactivity. It is a measure of the ability of a molecule to cause chemical reaction due to internal chemical energy or external energy. It was observed that Compound H has the lowest value of μ (-4.04) enhancing its ability to inhibit 5vau more than other studied compounds. Another important descriptor that shows the correlation with the binding affinty is the electrophilicity index, .It is the electrophilic power and describes the biological activity of the molecule. [28]. It is also observed that compound H has the highest electrophilicity index, ω. This further emphasized the reason why it has the lowest binding affinity and hence greater ability to inhibit 5vau than other studied compound.

Polarizability of a molecule plays an important role in modeling molecular properties and biological activities [29]. Larger molecules in which electrons are far from the positively charged nucleus are more polarizable than smaller molecules and are expected to have strong attractions with other molecules. From the result compound B has lowest value of polarizability (do not have a strong attraction with other molecule), molecular weight, volume area andcorrespond to the highest binding affinity (-4.9 kcal/mol).This greatly influenced the behaviour of the compound B in the gouge of the amelanotic melanoma cancer cell lines (5vau), reducing the inhibiting strength of the ligand which translated into higher binding affinity and hence least tendency to inhibit the active site of protein.

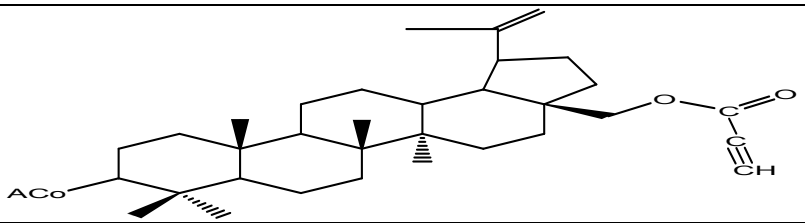
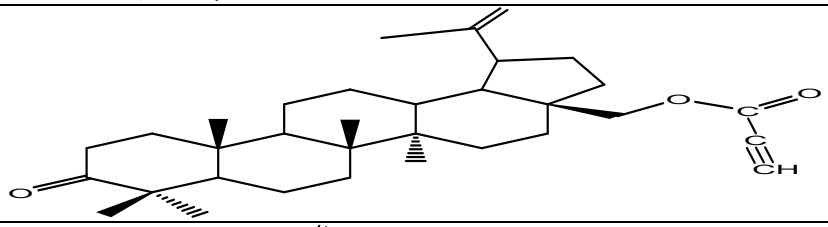
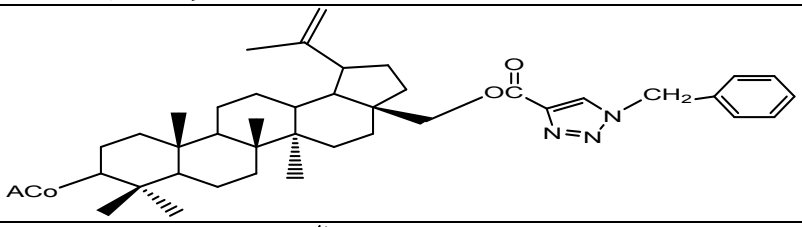
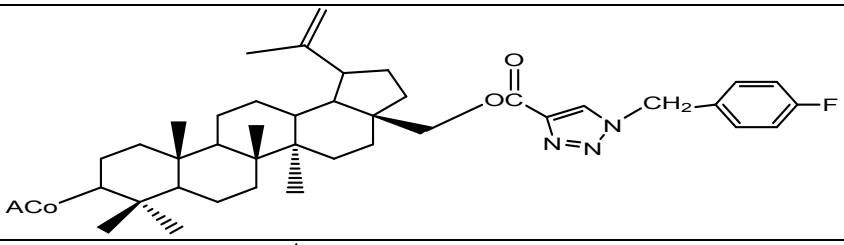
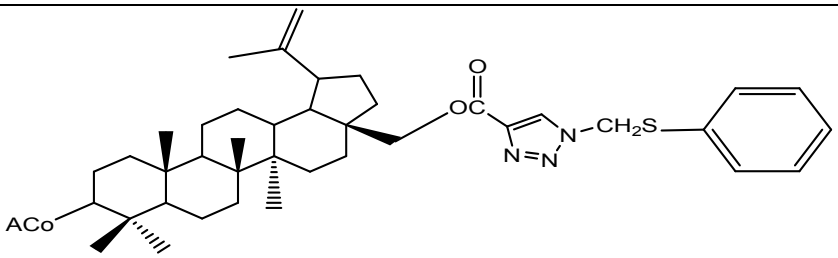
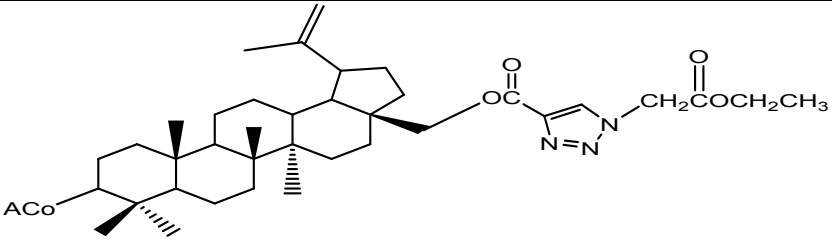
Also Polar surface area (PSA) is a sum of surfaces of polar atoms in a molecule. PSA is an indicator of the ligand hydrophilicity. It strongly reflects hydrogen bonding capacity and polarity and measure the ability of a drug to permeate/penetrate cells. It plays an important role in shaping the protein-ligand interaction by affecting the non-bonded contribution to the binding energy. The allowed PSA value is $\leq 90 Å^2$ [30], Compound B has the lowest PSA value(34.18 $Å^2$), while compound H has a PSA value (71.99 $Å^2$) closer to the standard PSA value, hence this may also suggest why it has a greater inhibiting ability and a lower binding affinity.

3.3 Interaction between Ligands and 5vau Receptor

The interaction between the ligand and the receptor are shown in Table 3and Figure 1. Alot of interactions were observed in the complexes. Vanderwaal forces of interaction were observed in all the complexes. Conventional hydrogen bonding was observed in all the complexes except for the complex formed by compound I. Pi-Anion coordination was observed between Glu B:89 and the ligands in

complexes formed by compounds C, D, E, and H. Pi-Donor Hydrogen Bond coordination was also observed in complexes formed by compound D,F H,K and L. for complexes formed by compounds F, G, K and L Carbon Hydrogen Bond interaction was also observed. Amide-Pi Stacked coordination was only observed between GLY B:193 and the ligands in the complex formed by compound G.

Table 1: IUPAC Name and Chemical Structure and of the Studied Compounds.

	IUPAC NAME	CHEMICAL STRUCTURE
A	3-Acetyl-28-propynoyl betulin	
B	28-Propynoylbetulone	
C	3-Acetyl-28-(1-benzyl-1H-1, 2,3-triazol-4yl)carbonyl betulin	
D	3-Acetyl-28-[1-(4-fluoro ben zyl) -1H-1,2,3-triazol-4-yl] carbonylbetulin	
E	3-Acetyl-28-(1-phenylthio methyl-1H-1,2,3-triazol-4-yl) carbonylbetulin	
F	3-Acetyl-28-(1-ethylacetyl-1H-1,2,3-triazol-4-yl)carbony l betulin	

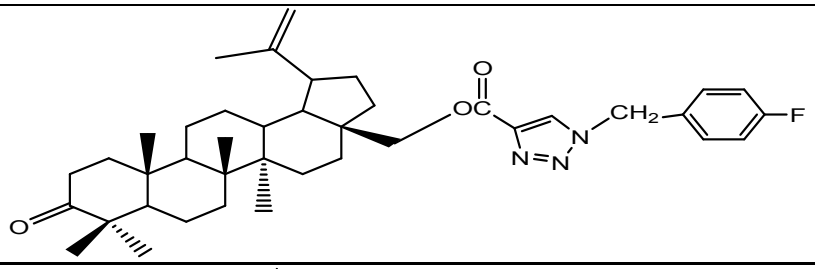
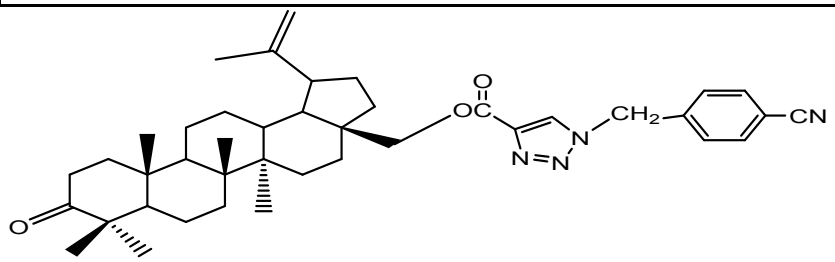
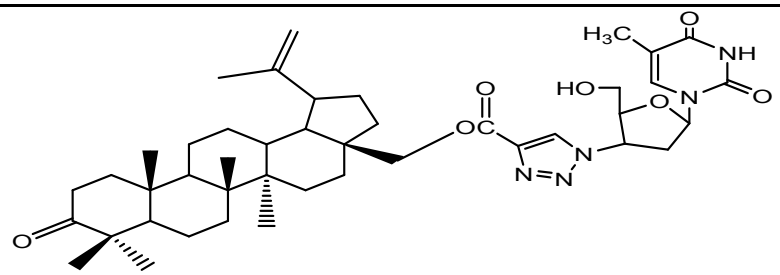
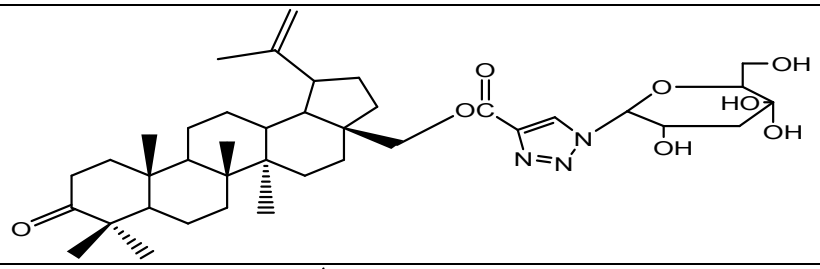
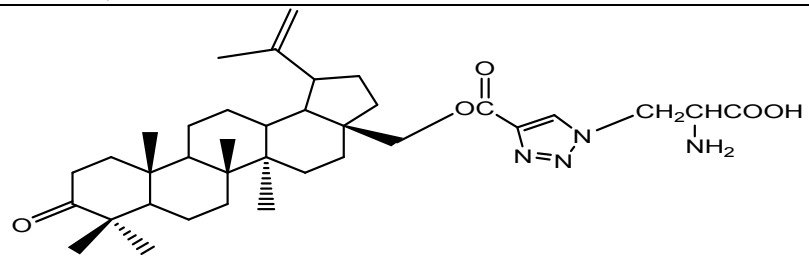
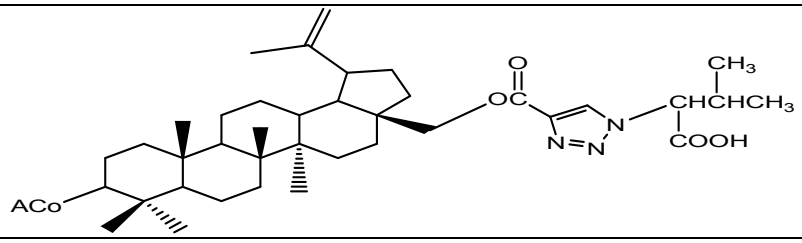
G	28-[1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl]carbonyl betulone	
H	28-[1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl]carbonyl betulone	
I	28-[1-(3'-Deoxythymidine-5'-yl)-1H-1,2,3-triazol-4-yl] carbonylbetulone	
J	28-[1-(1-Deoxy-β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl]carbonylbetulone.	
K	2-Amino-3-[4-(3-acetyl-28-betulonylcarbonyl)-1H-1,2,3-triazol-1-yl]propanoic acid.	
L	3-Methyl-3-[4-(28-betulonyl carbonyl) -1H-1,2,3-triazol-1-yl]butyric acid.	

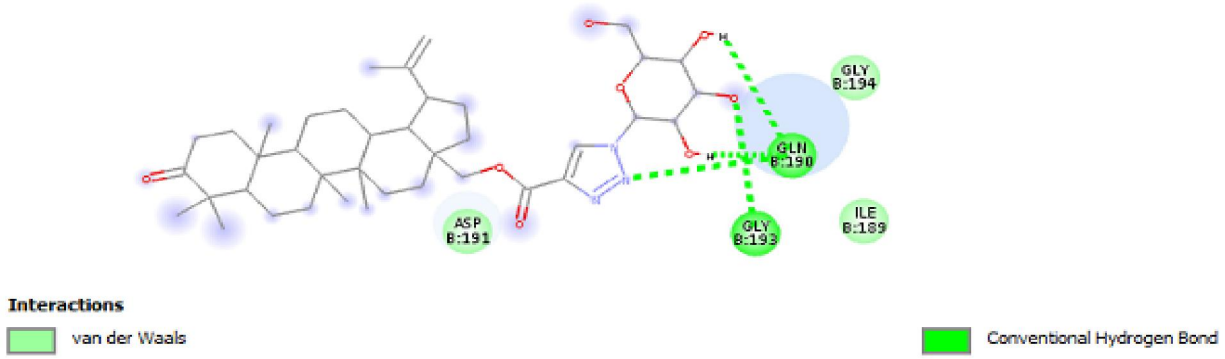
Table 2: The Calculated Molecular Descriptors for the Studied Compounds

Mol	E_{Homo}(eV)	E_{Lumo} (eV)	BG (eV)	DM (Debye)	μ	ω	Ƞ	MW (amu)	Log P	Area (A^2)	Vol (A^3)	PSA ($Å^2$)	HBD	HBA	Pol	AffinityKcal/Mol)
A	-6.44	-1.16	5.28	1.77	-3.8	2.74	2.64	536.797	8.09	572.54	596.91	39.75	0	2	88.55	-5.5
B	-6.25	-1.20	5.05	2.83	-3.73	2.75	2.53	492.744	8.35	522.16	551.08	34.178	0	2	84.88	-4.9
C	-6.42	-0.78	5.64	7.58	-3.6	2.30	2.82	669.951	9.60	695.47	722.91	61.222	0	5	98.68	-6.0
D	-6.49	-0.86	5.63	6.79	-3.68	2.40	2.82	687.941	9.76	701.14	727.45	61.237	0	5	99.06	-6.1
E	-6.36	-1.07	5.29	2.63	-3.72	2.62	2.65	702.017	10.38	718.64	742.77	63.703	0	6	100.38	-5.8
F	-6.34	-0.71	5.63	5.04	-3.53	2.21	2.82	665.916	7.74	688.08	705.61	83.172	0	6	97.28	-5.3
G	-6.16	-0.95	5.21	7.63	-3.56	2.43	2.61	643.888	10.02	652.52	681.79	57.421	0	5	95.45	-6.3
H	-6.24	-1.84	4.4	4.01	-4.04	3.71	2.2	650.908	9.90	671.45	697.25	71.991	0	6	96.90	-6.6
I	-6.26	-1.24	5.02	5.26	-3.75	2.80	2.51	759.989	7.12	750.74	776.70	119.538	2	11	103.19	-5.0
J	-6.24	-1.27	4.97	1.90	-3.76	2.84	2.49	697.914	6.03	680.98	708.88	130.205	4	10	97.70	-5.5
K	-6.28	-1.07	5.21	1.43	-3.68	2.59	2.61	622.851	6.65	628.74	650.04	110.272	1	7	92.87	-6.2
L	-6.13	-0.98	5.15	7.23	-3.56	2.45	2.58	635.890	8.78	647.32	675.63	88.572	1	6	94.96	-6.0

Table 3: Binding Energy, Interactions between Ligands and 5vau receptor.

Mol	Interaction between Ligands and 5vau receptor
A	(i) ASP-191 (ii) GLY-193, LIG:O (iii) GLN-190, LIG:N, H, H (iv) GLY -194 (v) ILE-189
B	(i) ASP-196 (ii) GLY-194 (iii) GLN-190, LIG:O (iv) GLY -193 (v) ASP-191
C	(i) ASP-10 (ii) GLU-89 (iii) HIS-186, (iv) TRP-195 (v) GLY-194 (vi)GLY-193 (vii)ASP-191 (viii) GLN-190, LIG:N
D	(i) ASP-191 (ii) GLY-193 (iii) HIS-186, (iv) ASP-10, LIG:H (v) GLU-89 (vi)GLY-194 (vii)GLN-190 (viii) TRP-195
E	(i) ASP-10 (ii) GLU-89 (iii) HIS-186 (iv) ASP-196 (v) TRP-195 (vi) GLY-194 (vii)GLN-190, LIG:N (viii) GLY-193 (ix) ASP-191
F	(i) ASP-191 (ii) GLU-190, LIG:N (iii) GLY-193(iv) GLU-89 (v) ASP-10 (vi) HIS-186 (vii) GLY-194 (viii) ILE-189 (ix) TRP-195
G	(i) HIS-186 (ii) TRP-195 (iii) GLU-89 (iv) ASP-196 (v) THR-187 (vi) ASP-191 (vii)GLN-190 (viii) GLY-194, LIG:N (ix) GLY-193, LIG:N,O
H	(i) ASP-191 (ii) ASP-10, LIG:N (iii) GLU-89 (iv) HIS-186 (v) TRP-195 (vi) GLN-190, LIG:N (vii) GLY-194 (viii) GLY-193
I	(i) ASN-192 (ii) ASP-191(iii) GLY-193 (iv) GLN-190
J	(i) GLY-194 (ii) GLY-193, LIG:O (iii) GLN-190, LIG:N, N, O (iv) ASP-191
K	(i) ASP-191 (ii) GLY-193 (iii) GLN-190, LIG:H (iv) GLU-89 (v) ASP-10 (vi)ASN-11 (vii) TRP-195 (viii) ILE-14(ix) HIS-186 LIG:N (x) GLY-194 (xi) ILE-189
L	(i) ASP-191 (ii) GLY-193, (iii) ASP-196 (iv) GLY-194, (v) ASP-196 (vi) GLU-89 (vii) HIS-186, LIG:O,O (viii) TRP-195, LIG:O (ix)

A.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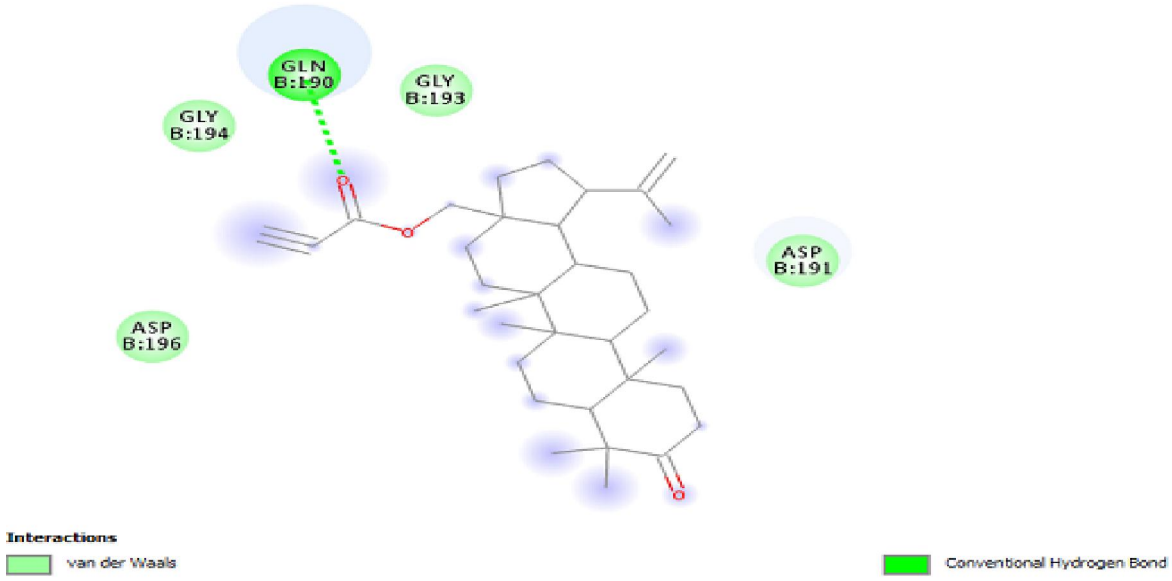
GLN B:190
ASP B:191
GLY B:193
ILE B:189
Interactions
van der Waals
Conventional Hydrogen Bond

B.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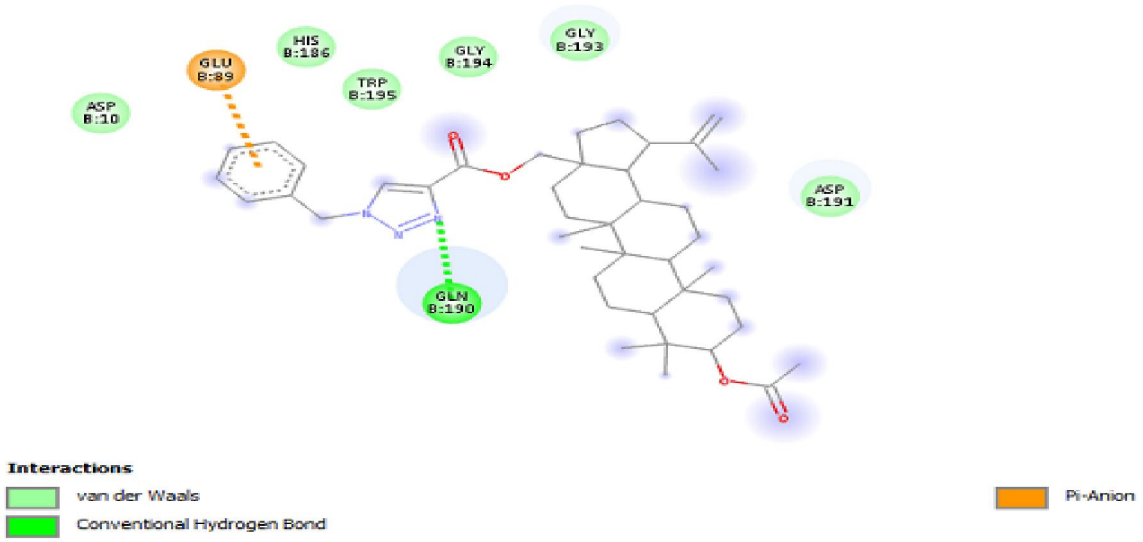
GLY B:193
GLY B:194
ASP B:191
ASP B:196
Interactions
van der Waals
Conventional Hydrogen Bond

C.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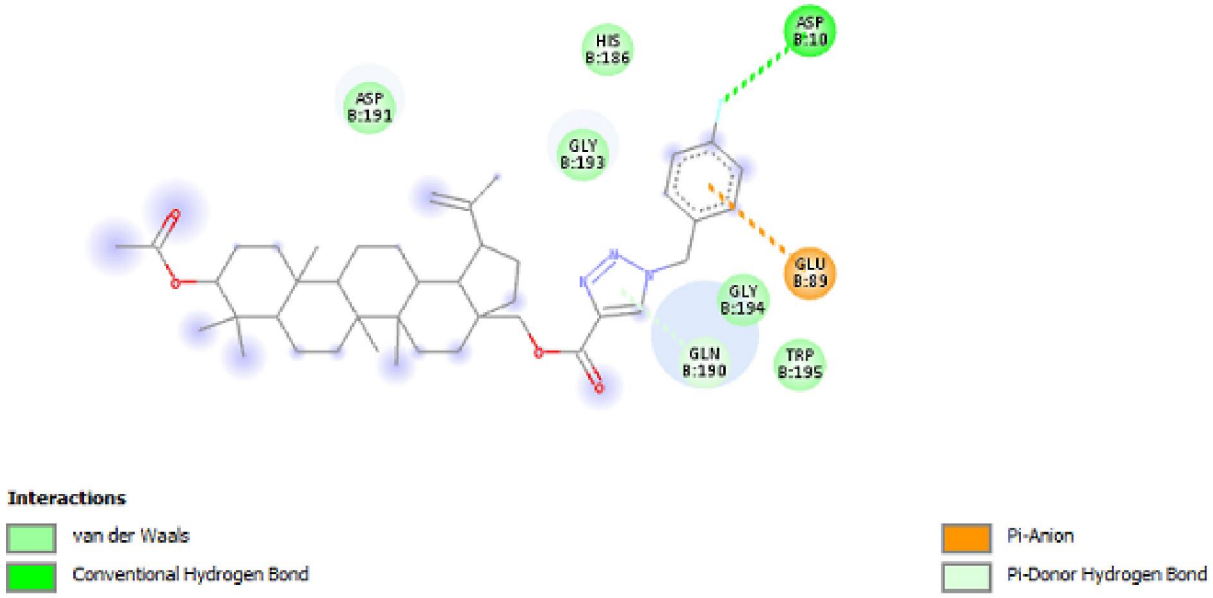
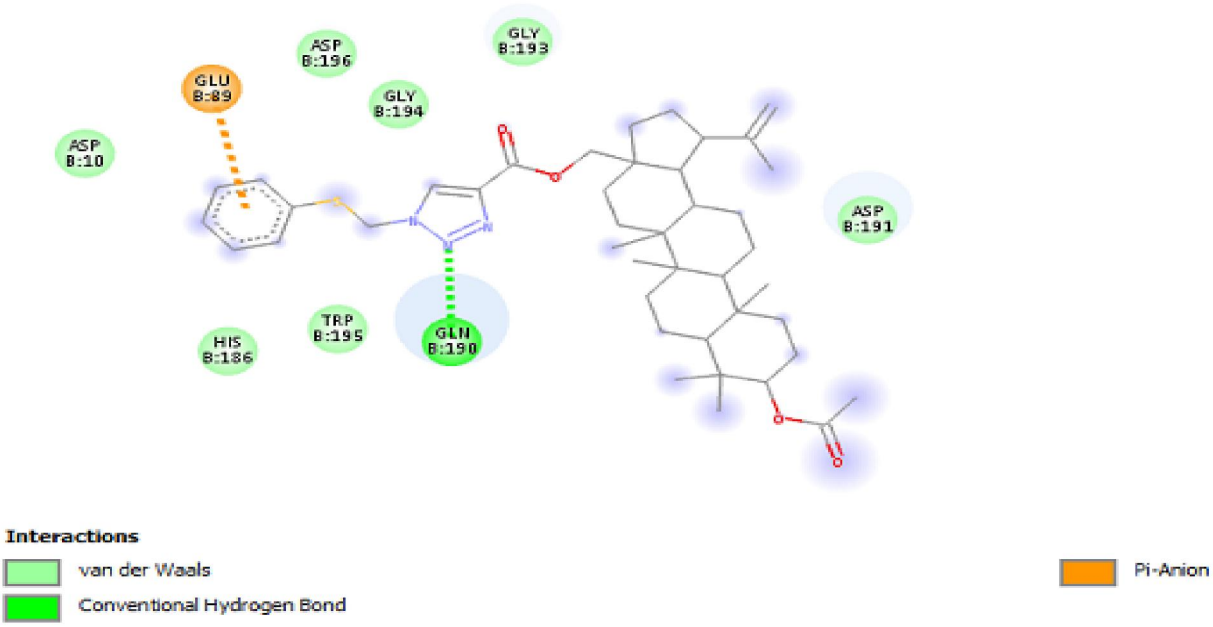
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Anion

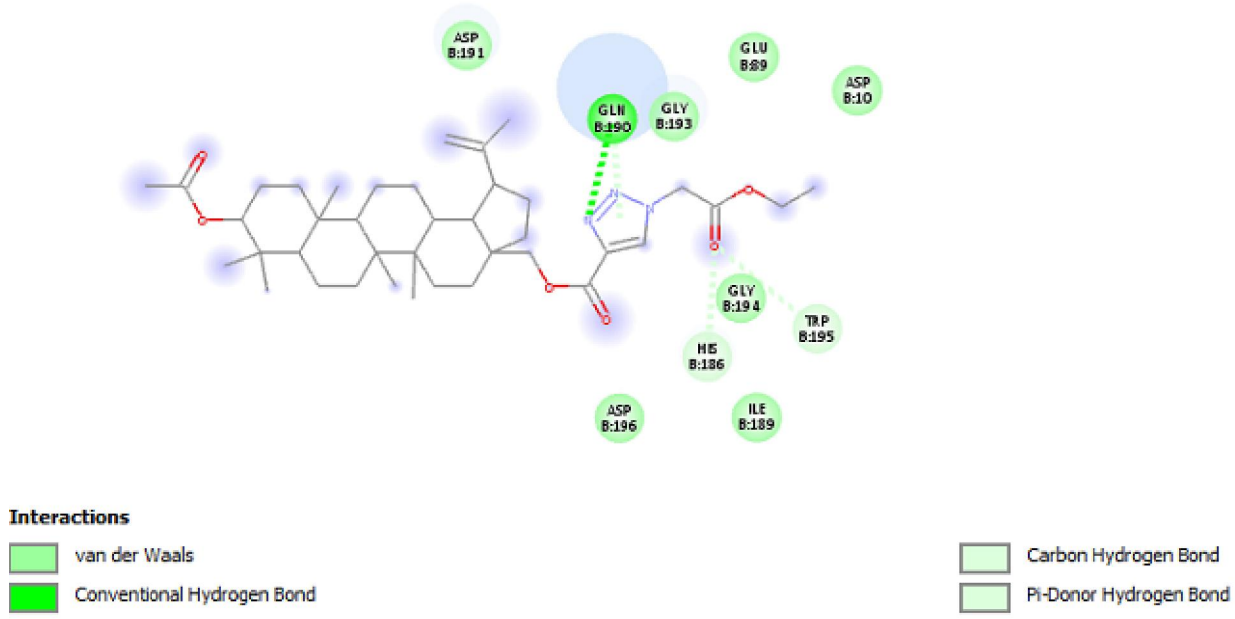
D.


ASP B:10
HIS B:186
ASP B:191
GLY B:193
GLU B:89
GLY B:194
GLN B:190
TRP B:195
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Anion
Pi-Donor Hydrogen Bond

E.


GLY B:193
ASP B:196
GLU B:89
GLY B:194
ASP B:10
ASP B:191
TRP B:195
HIS B:186
GLN B:190
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Anion

F.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ASP B:10
GLN B:190
GLY B:193
GLY B:194
TRP B:195
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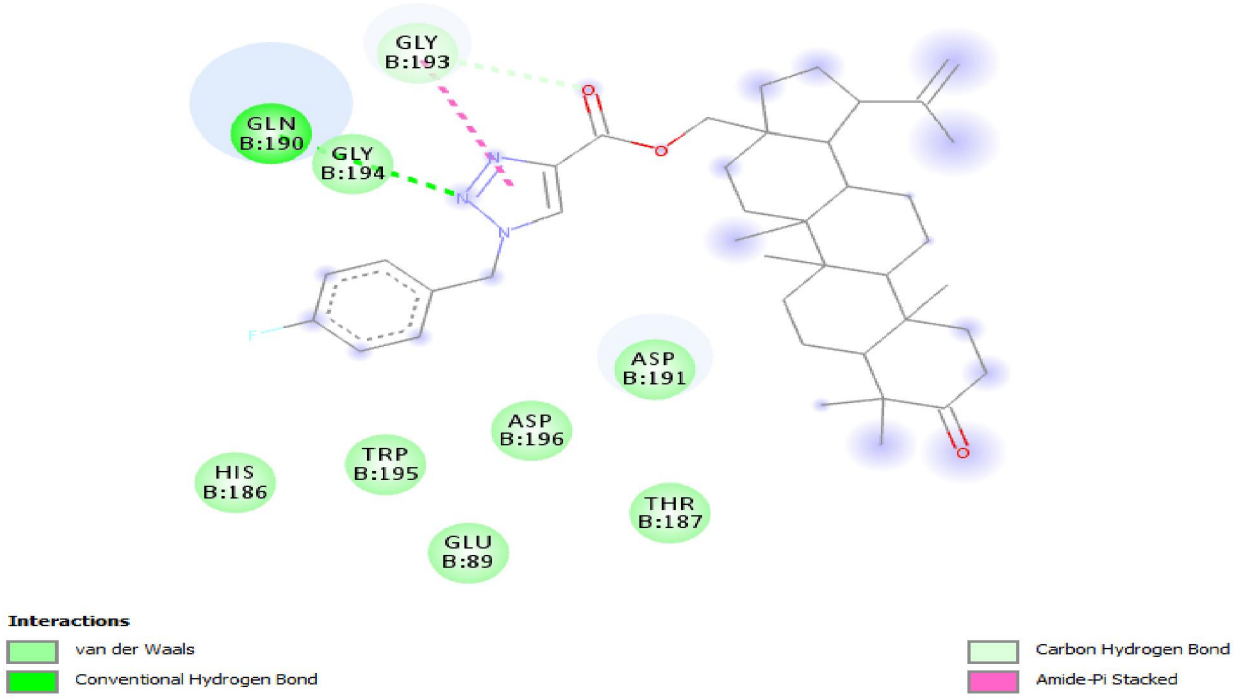
ILE B:189
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Donor Hydrogen Bond

G.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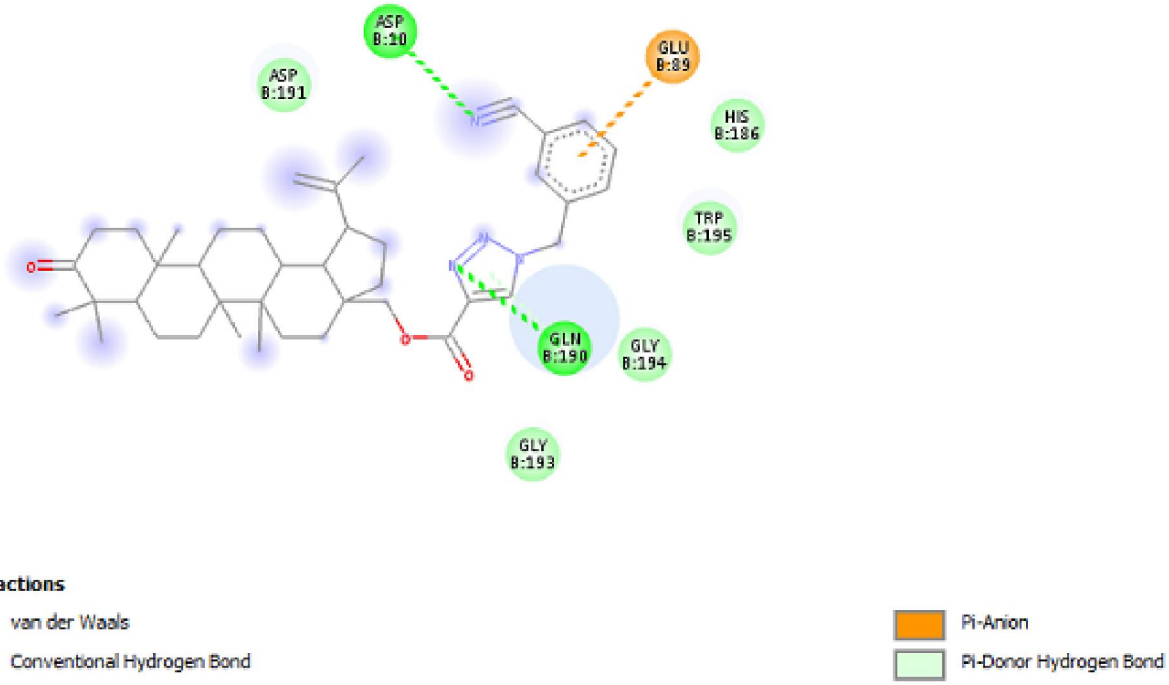
GLN B:190
GLY B:194
ASP B:191
ASP B:196
TRP B:195
HIS B:186
THR B:187
GLU B:89
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Amide-Pi Stacked

H.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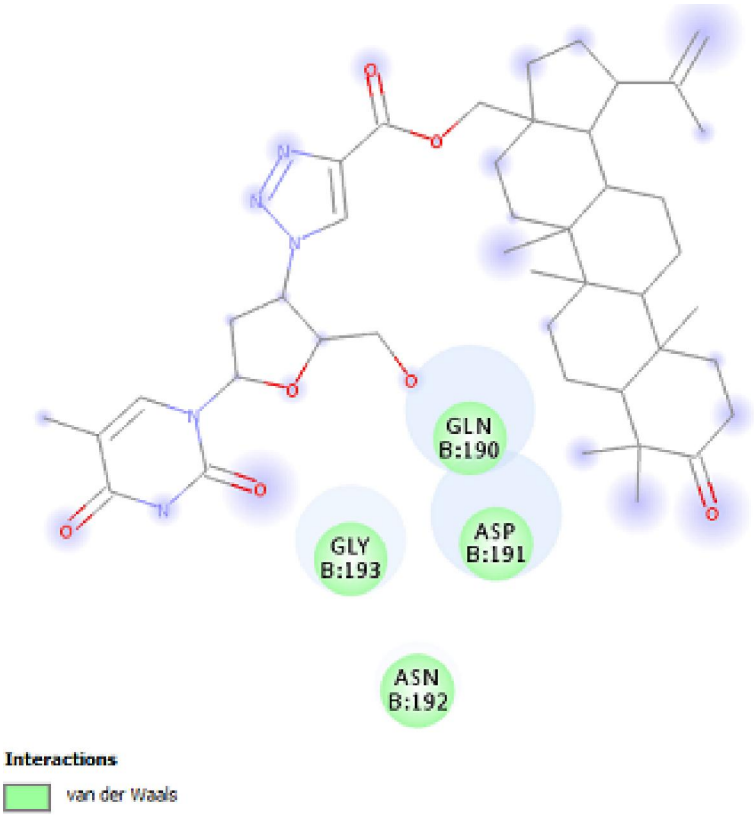
ASP B:191
HIS B:186
TRP B:195
GLN B:190
GLY B:194
GLY B:193
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Anion
Pi-Donor Hydrogen Bond

I.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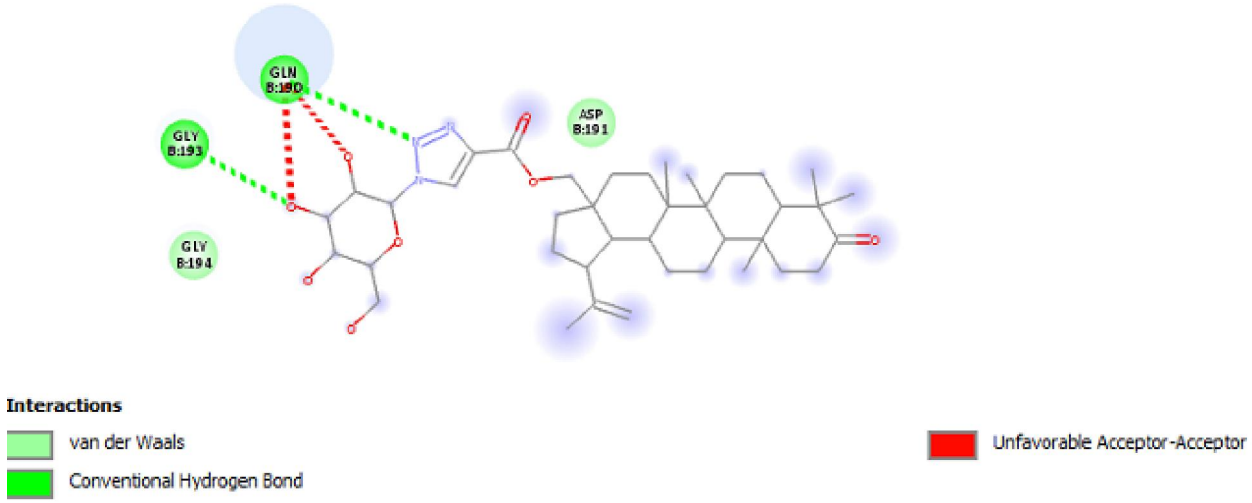
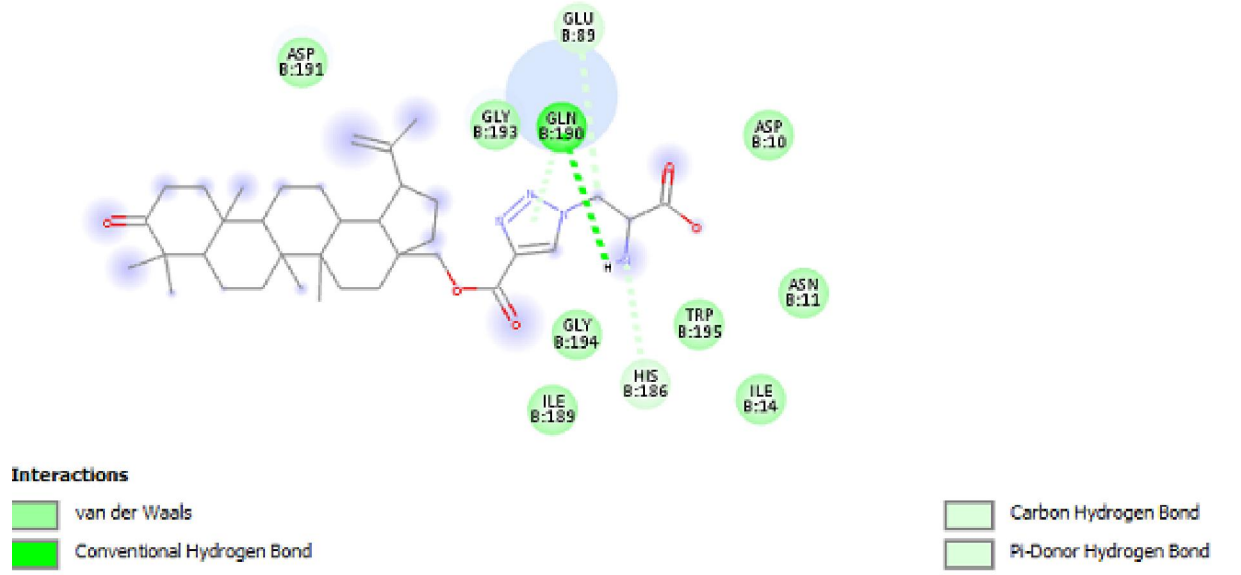
GLY B:193
ASP B:191
ASN B:192
Interactions
van der Waals

J.


GLN B:190
ASP B:191
GLY B:193
GLY B:194
Interactions
van der Waals
Conventional Hydrogen Bond
Unfavorable Acceptor-Acceptor

K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ASP B:191
GLY B:193
GLN B:190
ASP B:10
ASN B:11
TRP B:195
GLY B:194
HIS B:186
ILE B:189
ILE B:14
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Donor Hydrogen Bond

L.

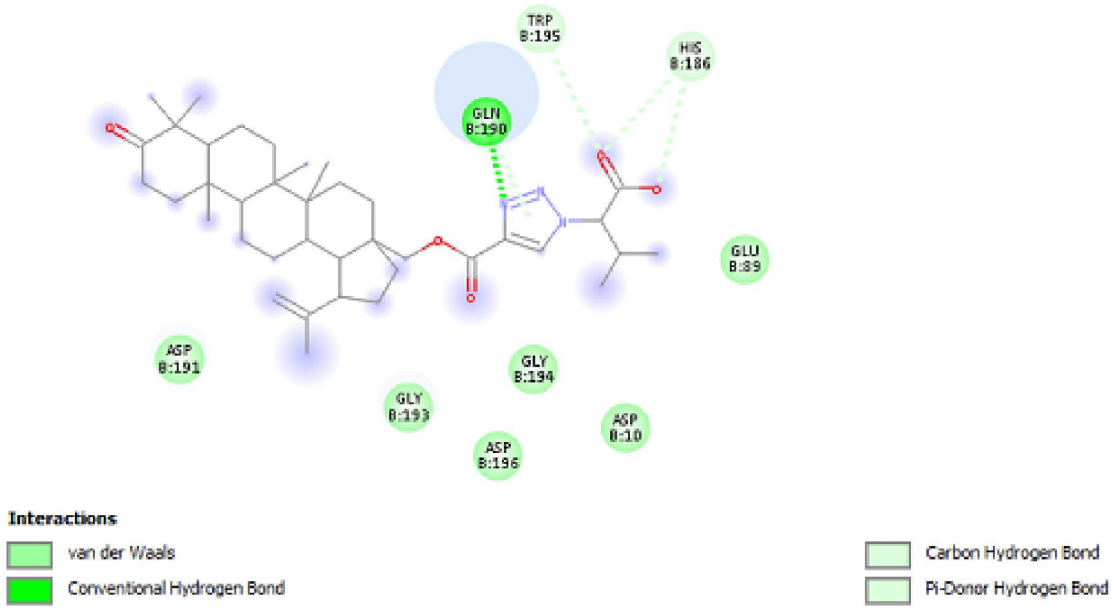

Figure 1: Binding interactions between the studied compounds and 5vau receptor

4. CONCLUSIONS

Twelve triazoles of 3-acetylbetulin and betulone compounds were optimized and there molecular parameters were obtained by Density functional theory calculation. The compounds were docked to catalytic binding sites of 5vau (C-32 receptor protein) and binding affinity of the studied compounds were obtained. It was observed that 28-[1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl]carbonylbetulone (H) inhibited more than any other studied compound. Also the correlation between calculated descriptors and calculated binding affinity were studied. This shows that lower band gap value, lower value of E_{LUMO}, lower chemical reactivity and higher value of electrophilicity index enhanced the inhibiting strength of the ligands thereby resulting to lower binding affinity. On the other hand 28-Propynoylbetulone (B) has the lowest value of molecular weight, area, volume, PSA, polarizibility, and HBA. This shows why it has the least inhibiting ability among the studied compound. Hence an increase in molecular weight, area, volume, PSA, polarizibility, and HBA enhanced the inhibiting strength of the ligand.

CONFLICT OF INTERESTS

Authors have declared that there is no conflict of interest.

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