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Triazole analogues as novel antioxidants: *In vitro* bioassay and molecular modeling studies

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ABSTRACT

The free radical scavenging activity of triazole analogues was estimated by the novel spectrophotometric method. The antioxidant property of triazole is influenced to a great extent by the increase in the strength of electron donating group attached on five member triazole ring. The semi empirical quantum chemical methods, AM1 and PM3 were used to estimate different physicochemical parameters. The electron affinity and softness were found to be responsible for high antioxidant activity. Docking studies were also performed with the active site of cyclo-oxygenase-2 to identify hydrogen bonding, hydrophobic and ionic interactions. The suitable chemical environment of triazole analogues for high efficacy is proposed.

KEYWORDS: Triazoles, Spectrophotometer, Antioxidant, QSAR, Cyclo-oxygenase-2 inhibitor

1. INTRODUCTION

Much attention has been paid to the synthesis of nitrogen containing heterocyclic compounds like isoxazole and triazole mainly due to their broad spectrum of biological and pharmacological activities [1]. Analogues of these [2-4] have played a crucial role in the history of heterocyclic chemistry and being used extensively important pharmacophores and synthons in the field of organic chemistry. Owing to their versatile chemotherapeutic importance, a significant amount of research effort has been focused on these nuclei. These analogues exhibit various biological activities such as, antibacterial, anticonvulsant, anticholestermic, anticancer, anthelmintics, anti-inflammatory, adenosine antagonist, fungicidal, herbicidal, hypoglycemic, muscle relaxant, nematocidal, insecticidal, antiviral and antimicrobial [5].

Triazole analogues also provide an antioxidant protective effect that may contribute to their pharmacological activities [6]. The antioxidant properties of triazoles are known to be influenced to a greater extent by the aryl structures of the substitutions on aryl rings (Fig.1). Especially, the free radical donor substituents were one of the key groups to enhance greatly the antioxidant activity of triazole mainly due to its easy conversion to free radicals through the hydrogen atom transfer mechanism [7].

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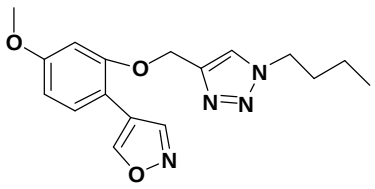
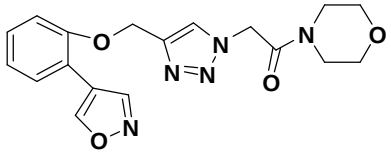
(I).



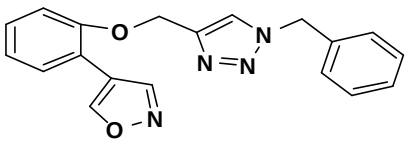
4-((2-(isoxazol-4-yl)-5-methoxyphenoxy)methyl)
-1-butyl-1H-1,2,3-triazole

(II).



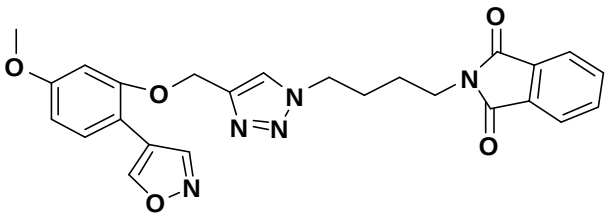
2-(4-((2-(isoxazol-4-yl)-5-methoxyphenoxy)methyl)
-1H-1,2,3-triazol-1-yl)-1-morpholinoethanone

(III).



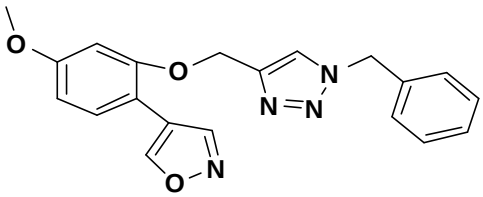
4-((2-(isoxazol-4-yl)phenoxy)methyl)
-1-benzyl-1H-1,2,3-triazole

(IV).



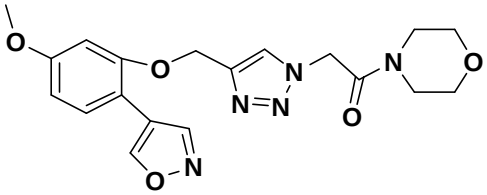
2-(4-(4-((2-(isoxazol-4-yl)-5-methoxyphenoxy)methyl) -1H-1,2,3-triazol-1-yl)butyl)isoindoline-1,3-dione

(V).



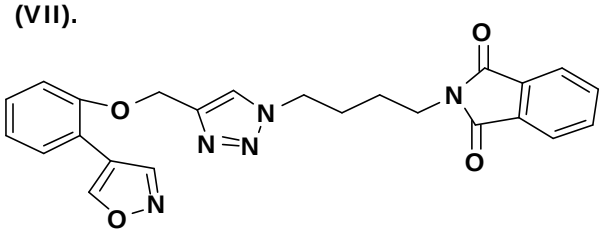
4-((2-(isoxazol-4-yl)-5-methoxyphenoxy)methyl) -1-benzyl-1H-1,2,3-triazole

(VI).



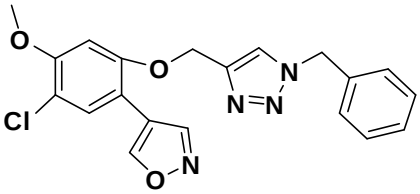
2-(4-((2-(isoxazol-4-yl)-5-methoxyphenoxy)methyl) -1H-1,2,3-triazol-1-yl)-1-morpholinoethanone

(VII).



2-(4-(4-((2-(isoxazol-4-yl)phenoxy)methyl) -1H-1,2,3-triazol-1-yl)butyl)isoindoline-1,3-dione

(VIII).



4-((4-chloro-2-(isoxazol-4-yl)-5-methoxyphenoxy)methyl) -1-benzyl-1H-1,2,3-triazole

Fig.1. Structures of triazole analogues

Compounds that displayed promising antioxidant profile was further evaluated for their inhibitory activity against cyclooxygenase enzyme (COX-2), by *in vitro* spectrophotometric method. The results revealed that the compounds **I, V**, and **VII** exhibited effective inhibition against COX-2. In an attempt to understand the ligand–protein interactions in terms of the binding affinity, docking studies were performed using GOLD2.0 and Autodock4.0 for those compounds, which showed good antioxidant activity. It was observed that the binding affinities calculated were in agreement with the IC_{50} values. Hence, it prompted us to develop triazoles as antioxidants. The QSAR studies help us to identify the pharmacophore and suitable chemical environment for high efficacy. Docking studies further help in understanding the various interactions between the ligands and enzyme active site in detail and thereby helps to design and synthesize novel potent inhibitors.

1. MATERIALS AND METHODS

1.1. Antioxidant Bioassay

All the chemicals were used of analytical grade. A Systronics UV-Visible PC based double beam spectrophotometer-2202 equipped with 1.0 cm quartz cells with a fixed slit width (2*nm*) was used to record the absorption spectra.

The antioxidant activity of triazoles was measured by using spectrophotometer. This method is based on the CTC which formed between triethyl amine and DDQ. This method is followed by measuring the maximum absorbance at 470*nm* under the optimized conditions. To the 10mL of 3×10^{-4}M CT complex, 10mL of 10^{-4}M substituted triazole was added. The mixture was allowed to stand 5 min at room temperature and then the absorbance of coloured solution was measured at 440*nm*. The capacity of free radical scavenging activity of triazole was calculated using the following equation:

RSA (radical scavenging activity) of triazole, $\mathbf{A_i}$ initial absorbance of the CTC, $\mathbf{A_f}$ is the absorbance of the test/ standard compound. From this the obtained %RSA values is calculated for the 1mM concentration of title compounds. Then the concentration needed to inhibit half of the maximum biological response of the against is called IC_{50}. It is calculated by

$$\textbf{Activity} = \log \frac{1}{IC_{50}}$$

The optical density was recorded as decrease in intensity of purple red colour of CTC. The antioxidant activity is expressed as IC_{50}.The Lower IC_{50} value represents higher antioxidant activity **(Table 1) [8]**. The antioxidant activity was compared with ascorbic acid, used as a

standard. DMSO (dimethyl sulfoxide) is the polar aprotic solvent used for dissolving the title compounds.

Table 1. Antioxidant activity of triazole analogues

Compound	(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)	(VIII)	Std
IC_{50} (mM)	0.803	1.024	1.094	1.914	0.855	1.500	0.976	1.136	0.603
Activity	3.09	2.99	2.96	2.72	3.07	2.82	3.01	2.94	3.213

mM = milli molar; Std= Standard

2.2. Computational Methodology

2.2.1.Construction of molecular structures

A series of triazole compounds tested for inhibitory activity was selected for the present study and the program of window Hyperchem software Inc (http://www.warezdestiny.com/free-hyp) was used in modeling studies. The molecules were generated and the energy was minimized using molecular modeling pro. The window version software SPSS10 (SPSS Software; Consult http://www.spss.com) was used in the regression analysis.

2.2.2. Calculation of quantum chemical descriptors

All of the molecular structures of the compounds were initially optimized geometrically using the semi-empirical method AM1 (Austin Model 1) and PM3 (Parameterization Model 3) [9]. The quantum chemical descriptors (variables) [10-13] obtained for model building in this work include: energy of cation (E_{cation}), energy of anion (E_{anion}), the electron affinity (EA)(calculated from $E_{neutral}$-E_{anion}), ionization potential (IP) (calculated from E_{cation}-$E_{neutral}$), electro negativity(?), hardness(?), softness(S), electrophilic index (?), partition coefficient (LogP), hydration energy (HE),chemical potential (μ) and polarisability (Pol)were obtained for triazole analogues.

2.2.3. Molecular modeling studies

The computational technique was applied to the triazole analogues that were varied on aryl ring position. The appropriate descriptors or parameters for the compounds were used as independent variables for deciding in cyclo-oxygenase-2 (4COX) inhibitory activity.

Molecular docking methodologies ultimately seek to predict the best mode by which a compound which fit into a binding site of a macro molecular target. In addition to the synthetic work which was made by our collaborative group, an attempt to explore docking studies on triazole analogues was made to explain observed variance in biological activity. This predicts the best candidate providing an insight on substitution and configuration for optimum receptor pit which leads to the development of best pharmacophore activity.

2.2.3.1. GOLD 2.0.

The GOLD 2.0 (Genetic Optimization for Ligand Docking) program uses a genetic algorithm (GA) to explore the full range of ligand flexibility and the rotational flexibility of selected receptor hydrogen's [14,15].The mechanism for ligand placement is based on fitting points. The program adds fitting points to hydrogen-bonding groups on the protein and ligand and maps acceptor points in the ligand, on donor points in the protein and vice versa. The docking poses are ranked based on a molecular mechanics-like scoring function. There are two different built in scoring functions in the GOLD program -Gold Score and Chem Score. The interaction of the ligands with the receptor in the modeled complexes was investigated and observed for the fitness function ability on protein of cyclo-oxygenase-2 by using synthesized moieties.

The 3D structure of selected Protein cyclo-oxygenase-2 (4COX) was selected from PDB(Protein Data Bank) Bank RCSB with an X-ray resolution in the range of 2.90A^0 [16].The fitness function that was implemented in GOLD consisted basically of H-bonding, complexing energy, and ligand internal energy terms. The GOLD Score was calculated by defining the site using the list of atom numbers and retaining all the other default parameters. The docking studies are frequently used to predict the binding orientations of small molecules of drug candidates to their protein targets in order to predict the affinity of the small molecules *viz;* **(I)-(VIII)**. A population of possible docked orientations of the ligand is set up at random. Each member of the population is encoded as a chromosome, which contains information about the mapping of ligand H-bond atoms onto protein H-bond atoms, mapping of hydrophobic points all the conformation around flexible ligand bonds and protein OH groups. All docking runs were carried out using standard default settings with a population size of 100, a selection pressure of 1.1, a maximum of 100000 operations, number of islands as 5, a niche size of 2, and a mutation and cross over rate of 95. Docking poses were obtained by applying both Chemscore and Gold score. In the present study of the GOLD Program, the performance of both Gold Score, Chemscore are good. These protein-ligand complexes were prepared for docking studies by adding hydrogen atoms, removing water molecules and co-crystallized inhibitors and refined by using the SPDBV3.7 [17]. Enzyme-inhibitor interactions within a radius equal to 15Å centered on reported bound inhibitors were taken into account.

2.2.3.2. Argus Lab 4.0.1

Argus Lab 4.0.1[18] was used for molecular modeling studies, which is very flexible and can reproduce crystallographic binding orientation. Argus lab provides a user friendly graphical interface and uses shape dock algorithm, to carry out docking studies. This helps to

visualize the binding conformations of these analogues, within the active site region of cyclo-oxygenase-2 protein.

2.2.3.3Auto dock 4.0

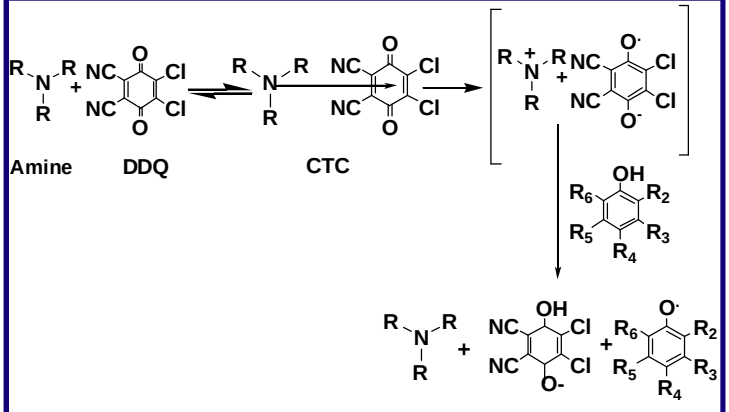
Autodock 4.0 [19] was used to estimate binding free energy and inhibition constant (K_i).

3. RESULTS AND DISCUSSIONS

3.1. Free radical scavenging activity

The photometric methods based on molecular interactions are simple and suitable since they result in the rapid formation of the complexes. It is a general phenomenon in organic chemistry and Mullikan considered bond between the components of the complex being postulated to arise from the base to the empty orbital of the acceptor. The charge transfer complex (CTC) is formed between triethyl amine as ***n***-donor (D) and DDQ as *p*-acceptor. The intermolecular CTCs are formed when electron donors and electron acceptors interact. The CTC have unique absorption bands in ultra violet-visible region. 2, 3-Dichloro-5, 6-dicyano 1, 4- Benzoquinone (DDQ) is a ***p***-electron acceptor often it forms highly colored electron donor-acceptor complex or CTC with the triethyl amine [20]. The molecular interactions between electron donors and acceptors are generally associated with the formation of intensity coloured CTC, which absorb radiation in the visible region. Triethyl amine is good ***n***-electron donor which forms CTC with ***p***-acceptors. The CTC becomes a free radical source on its decomposition [21].

The Beer's law is obeyed over the concentration ranges. The described method is successfully applied to the determination of antioxidant activity. To accommodate the observed results, the following reaction mechanism is proposed in **Scheme-1.**



Scheme-1:

The CTC decomposes to give DDQ free radical which in turn forms R· radical of abstraction of hydrogen from triazole (RH). R· radical will then undergo further reactions which control the overall stoichiometry *i.e*., the number of molecules DDQ reduced by RH. Mixing of the DDQ solution to donor resulted in a decrease in intensity of color *i.e.* shifted to shorter wavelength.The biological activity values calculated for the compounds with respect to standard Ascorbic acid by other methods like DPPH assay, TRAP, ORAC, TEAC, NO *etc*, showed somewhat lower activity values with respect to standard Ascorbic acid. In this method the used chemicals are cheaper and accuracy is more than other methods like DPPH assay etc. This method involves in lesser time. According to these activity results the compounds **I**, **V**, **VII** were showed best *in vitro* biological activity compare standard ascorbic acid. The compounds **III, VIII** and **II** showed smaller greater value of biological activity evaluated standard ascorbic acid.

3.2. Linear regression model analysis

The biological activity data and the physicochemical properties IP, EA, ? , EN, ?, S, LogP, HE, μ and Pol of the triazole analogues are given in **Table 2** and **Table 3.** The data from these tables were subjected to regression analysis. The correlation matrices were generated with eight triazole analogues**.** The term close to **1** indicates high co-linearity, while the value below 0.5 indicates that no co-linearity exist between more than the two parameters.

Table 2. Antioxidant activities and molecular descriptor values of triazole analogues in AM1 method

Compound	Obs. Act	Eq-1 Pre. Act	Residual	Eq-2 Predicted	Residual
(I)	3.09	2.93	.16	2.96	.14
(II)	2.99	3.09	-.10	3.12	-.13
(III)	2.96	3.01	-.05	3.04	-.08
(IV)	2.72	2.77	-.05	2.76	-.05
(V)	3.07	2.93	.14	2.96	.11
(VI)	2.82	2.96	-.13	-	-
(VII)	3.01	2.95	.06	2.95	.06
(VIII)	2.94	2.97	-.02	2.99	-.05

Compound	Molecular descriptors IP(eV)	EA(eV)	EN(eV)	h (eV)	S(eV^{-1})
(I)	8.76	.33	4.54	4.22	2.11
(II)	9.26	.44	4.85	4.41	2.21
(III)	8.99	.27	4.63	4.36	2.18
(IV)	8.53	1.28	4.90	3.63	1.81
(V)	8.77	.35	4.56	4.21	2.10
(VI)	8.84	.36	4.60	4.24	2.12
(VII)	9.06	1.24	5.15	3.91	1.95
(VIII)	8.92	.51	4.72	4.20	2.10

Compound	Molecular descriptors w	HE(K.cal/mole)	LogP	Pol(A^0)	μ(eV)
(I)	43.53	-15.99	-.70	34.94	-4.54
(II)	51.84	-16.7.	-2.08	37.44	-4.85
(III)	46.73	-16.74	.10	36.63	-4.63
(IV)	43.60	-17.80	-1.66	49.02	-4.90
(V)	43.78	-18.06	-.89	39.10	-4.56
(VI)	44.85	-17.27	-3.08	39.91	-4.60
(VII)	51.86	-16.38	-.67	46.55	-5.15
(VIII)	46.75	-17.38	-1.11	41.03	-4.72

Obs. Act = observed Activity; Pre. Act = Predicted Activity

Table 3. Antioxidant activities and molecular descriptor values of triazole analogues in PM3 method

Compound	Obs. Act	Eq-1 Pre. Act	Residual	Eq-2 Predicted	Residual
(I)	3.09	2.94	.16	2.97	.12
(II)	2.99	3.08	-.09	3.12	-.13
(III)	2.96	2.98	-.01	3.01	-.05
(IV)	2.72	2.77	-.05	2.77	-.05
(V)	3.07	2.97	.10	3.00	.06
(VI)	2.82	2.99	-.16	-	-
(VII)	3.01	2.93	.08	2.93	.08
(VIII)	2.94	2.95	-.01	2.98	-.03

Compound	Molecular descriptors IP(eV)	EA(eV)	EN(eV)	h (eV)	S(eV^{-1})
(I)	8.89	.35	4.62	4.27	2.14
(II)	9.36	.49	4.92	4.44	2.22
(III)	9.03	.46	4.75	4.29	2.14
(IV)	8.71	1.34	5.03	3.68	1.84
(V)	9.00	.40	4.70	4.30	2.15
(VI)	9.07	.47	4.77	4.30	2.15
(VII)	9.17	1.31	5.24	3.93	1.96
(VIII)	8.98	.53	4.75	4.23	2.11

Compound	Molecular descriptors w	HE(K.cal/mole)	LogP	Pol(A^0)	μ(eV)
(I)	45.57	-15.99	-.70	34.94	-4.62
(II)	53.81	-16.7.	-2.08	37.44	-4.92
(III)	48.30	-16.74	.10	36.63	-4.75
(IV)	46.53	-17.80	-1.66	49.02	-5.03
(V)	47.49	-18.06	-.89	39.10	-4.70
(VI)	48.88	-17.27	-3.08	39.91	-4.77
(VII)	53.93	-16.38	-.67	46.55	-5.24
(VIII)	47.73	-17.38	-1.11	41.03	-4.75

Obs. Act = observed Activity; Pre. Act = Predicted Activity

The perusal of correlation matrix indicates that EA and S are the predicted parameters of AM1 method. The enter, backward, forward, removed and stepwise regression methods are used. The EA and S were found to be explainable varied. The regression technique was applied through the origin using these explainable parameters.

Activity = 0.246(0.098)*EA+1.352(0.034)* S..... (1)

N = 8; R = 0.999; R^2 = 0.999; R^2adj=0.998; %EV = 99.9; SEE = 0.1169; F= 2549.313; Q =8.54577;

In addition, the plot of observed activity versus predicted activity was not found to be satisfactory. Hence, the predictive ability of the model is not good. **Eq.1** shows that the values of %EV are less and to improve its value, outlier (**VI**) were sought and eliminated. After the elimination of the outlier (**VI**), a second model was developed. Overall, there is an increase in R (0.999-1.000) and %EV values, and a decrease in SEE (**0.1169- 0.1108**).

Activity = 0.221(0.095)*EA+1.369(0.034)* S.....(2)

N =7; R= 1.000; R^2= 0.999; R^2adj =0.999; %EV = 99.9; SEE = 0.1108; F =2516.890; Q = 9.02527;

Eq.2 is an improved model since it explains the biological activity to the extent of (99.9%).

From the correlation matrix table, it reveals EA and S are found to be explainable variables. In both AM1 and PM3 a di-parametric EA and S QSAR equations are generated.

Activity = 0.225(0.097)*EA+1.340(0.036)* S ..... (3)

N = 8; R = 0.999; R^2 = 0.999; R^2adj=0.999; %EV = 99.9; SEE = 0.1144; F= 2664.65; Q =8.7325;

Eq.3 shows that the values of % EV is less and to improve its value, outlier(**VI**) were sought and eliminated, In addition, the plot of observed activity versus predicted activity was not found to be satisfactory. Hence, the predictive ability of the model is not good. After the elimination of the outlier (**VI**), a second model was developed.

Activity = 0.197(0.083)*EA+1.361(0.032)* S ..... (4)

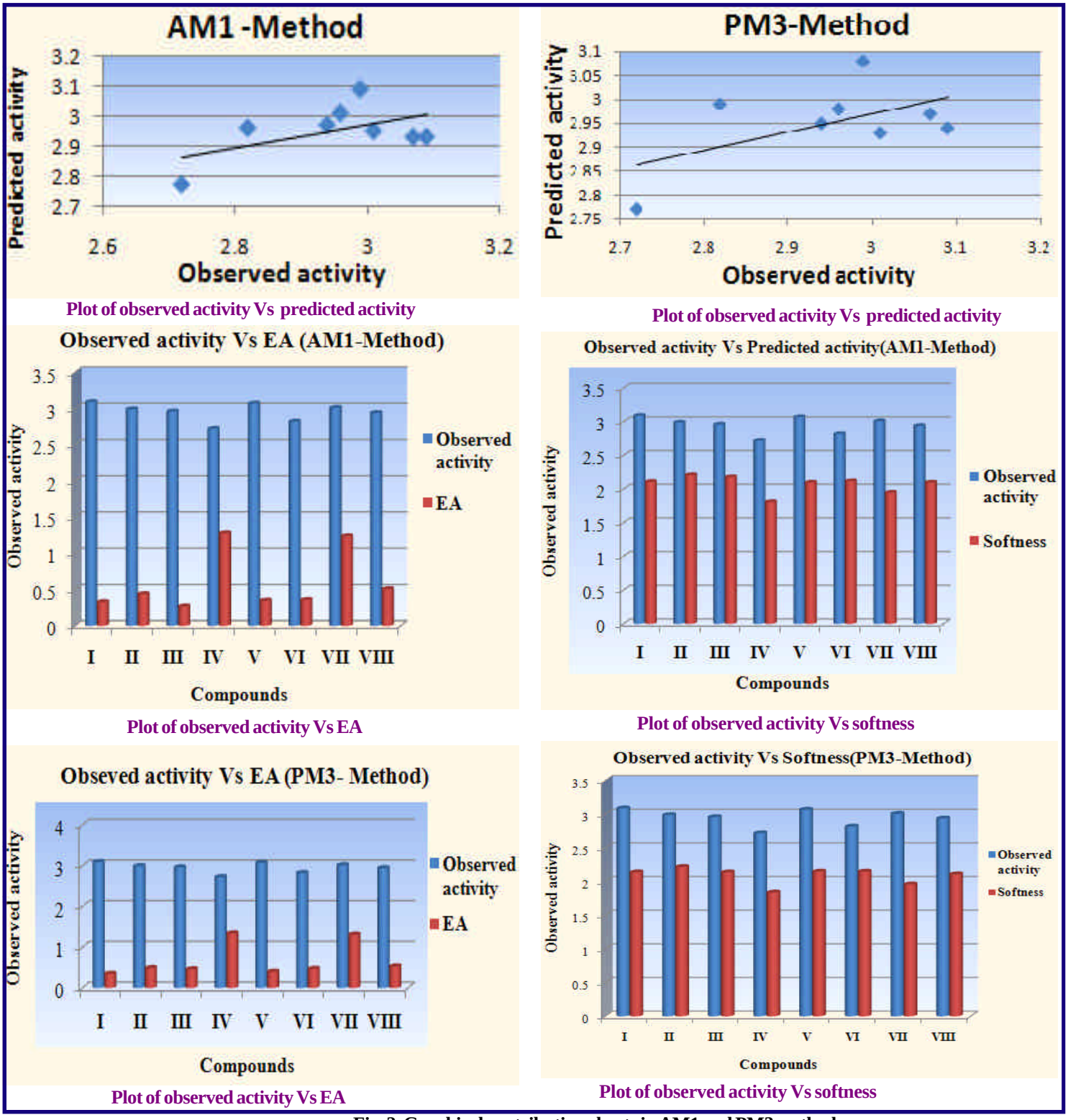
N = 7; R = 1.000; R^2 = 0.999; R^2adj=0.999; %EV = 99.9; SEE = 0.09676; F= 3298.426; Q =10.334;

In an attempt to investigate the predictive potential of the proposed models, the cross-validation parameters (q^2_{cv} and PRESS) were calculated and used. The predictive power of the equations was confirmed by leave-one-out (LOO) cross-validation method (**Table-2** and **3**). **Eq.3** and **4** of AM1 and PM3 methods respectively give a good q^2_{cv} value, which should be always smaller than %EV. A model is considered to be significant when q^2_{cv}= (>0.82). Another cross-validation parameter, PRESS which is the sum of the squared differences between the actual and that predicted when the compound is omitted from the fitting process, also supports the predictive ability of **Eqs.2** and **4**. Its value decreases from **Eq.1** to **Eq.3**.

The quality factor Q [22], is defined as the ratio of regression constants (R) to the standard error estimation (SEE), that is, Q = R/SEE. This indicates that the higher the value of R, and the lower the value of SEE, the higher is the magnitude of Q and the better will be the correlation. In present case, Q increases from 8.545 to 9.025 and 8.7325 to 10.334 **(Eq. 1** to **4)**.

In the final AM1 and PM3 modelled **Eq-2** and **Eq-4** respectively**,** the contribution of the physicochemical parameters shown graphically in contribution charts (Fig.2). Soft acids and bases can be explained on the HSAB principle. Softness of chemical species linked with large atomic/ionic radius, low or zero oxidation state, high polarisability, low electro negativity. Soft bases have HOMO of higher energy than hard bases, and soft acids have LUMO of lower energy than hard acids. The soft molecules are more reactive than hard molecules if electron transfer or rearrangement is necessary for the reaction. The

softness is important in understanding the chemistry of large, delocalized molecules or ions [23]. Therefore, the high efficacy of antioxidant nature is due to high softness. The electron affinity is characterized by the susceptibility of the compound in relation to attacks by nucleophiles. Therefore, one can conclude that electronic effects have a very important role when one is trying to understand the activity of triazole analogues with antioxidant activity.The correlation between the actual and predicted activity of the compounds are shown in Table 2, 3 and Fig. 2.



Plot of observed activity Vs predicted activity

Plot of observed activity Vs predicted activity

Plot of observed activity Vs EA

Plot of observed activity Vs softness

Plot of observed activity Vs EA

Plot of observed activity Vs softness

Fig. 2. Graphical contribution charts in AM1 and PM3 methods

3.3. Docking Analysis

Among all the triazole compounds tested for docking studies, showed good inhibitory activity values against cyclo-oxygenase-2 (Table 4 and Table 5). The compound-**(I)**, **(V)** and **(VII)** showed high affinities with low energy of with employed protein. The binding between 4COX and compound-**(I)**, **(V)** and **(VII)** indicates very good inhibition. The compound **((I)-(VIII))** showed good inhibition with their affinity ranges. In the active site of **4COX**,Thr 212,Asn 68,Glu 67,His 388,Ser 530,Tyr 355,Tyr 402,Asn 382,Thr 70,Glu1 40,Asn 144 amino acids play important role and they are shown in Fig.3.

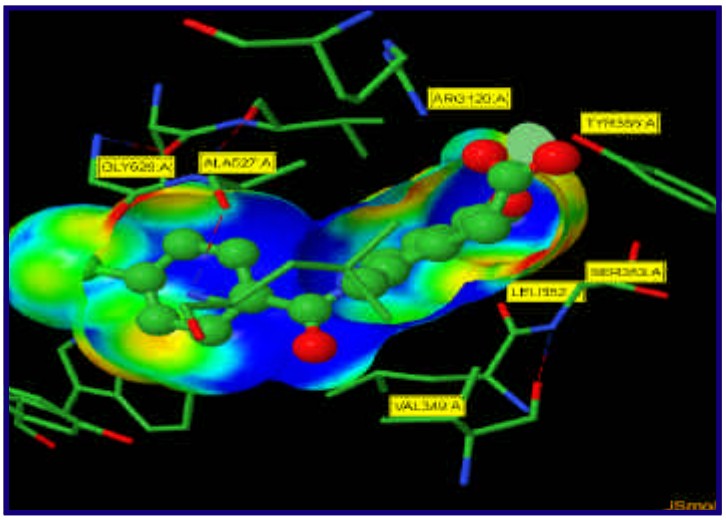


Fig. 3. Active site amino acid residues of crystallographic protein 4COX

Table 4. Docking values obtained from GOLD in fitness score with cyclo-oxygenase-2

Compound	Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(vdw_int)
(I)	46.37	6.71	35.38	0.00	-8.99
(II)	46.94	1.89	39.10	0.00	-8.70
(III)	51.79	7.43	37.10	0.00	-6.65
(IV)	65.04	9.22	48.06	0.00	-10.27
(V)	49.54	1.15	41.37	0.00	-8.50
(VI)	47.69	12.39	34.27	0.00	-11.82
(VII)	60.78	7.95	45.45	0.00	-9.67
(VIII)	49.28	1.44	39.82	0.00	-6.91

Table 5. Docking values obtained from GOLD in Chemscore function with cyclo-oxygenase-2

Compound	Score	DG	S(h-bond)	S(metal)	S(lipo)	DE(clash)	DE(int)
(I)	19.54	-22.07	2.71	0.00	104.38	0.03	2.50
(II)	20.31	-21.97	2.74	0.00	103.01	0.08	1.58
(III)	21.20	-23.22	2.53	0.00	112.65	0.02	2.00
(IV)	21.41	-24.10	2.09	0.00	158.17	0.02	2.66
(V)	21.37	-24.13	2.64	0.00	121.70	0.23	2.54
(VI)	20.02	-21.87	2.73	0.00	107.83	0.06	1.80
(VII)	19.58	-21.52	1.94	0.00	135.07	0.03	1.91
(VIII)	20.01	-22.04	1.62	0.00	132.81	0.11	1.92

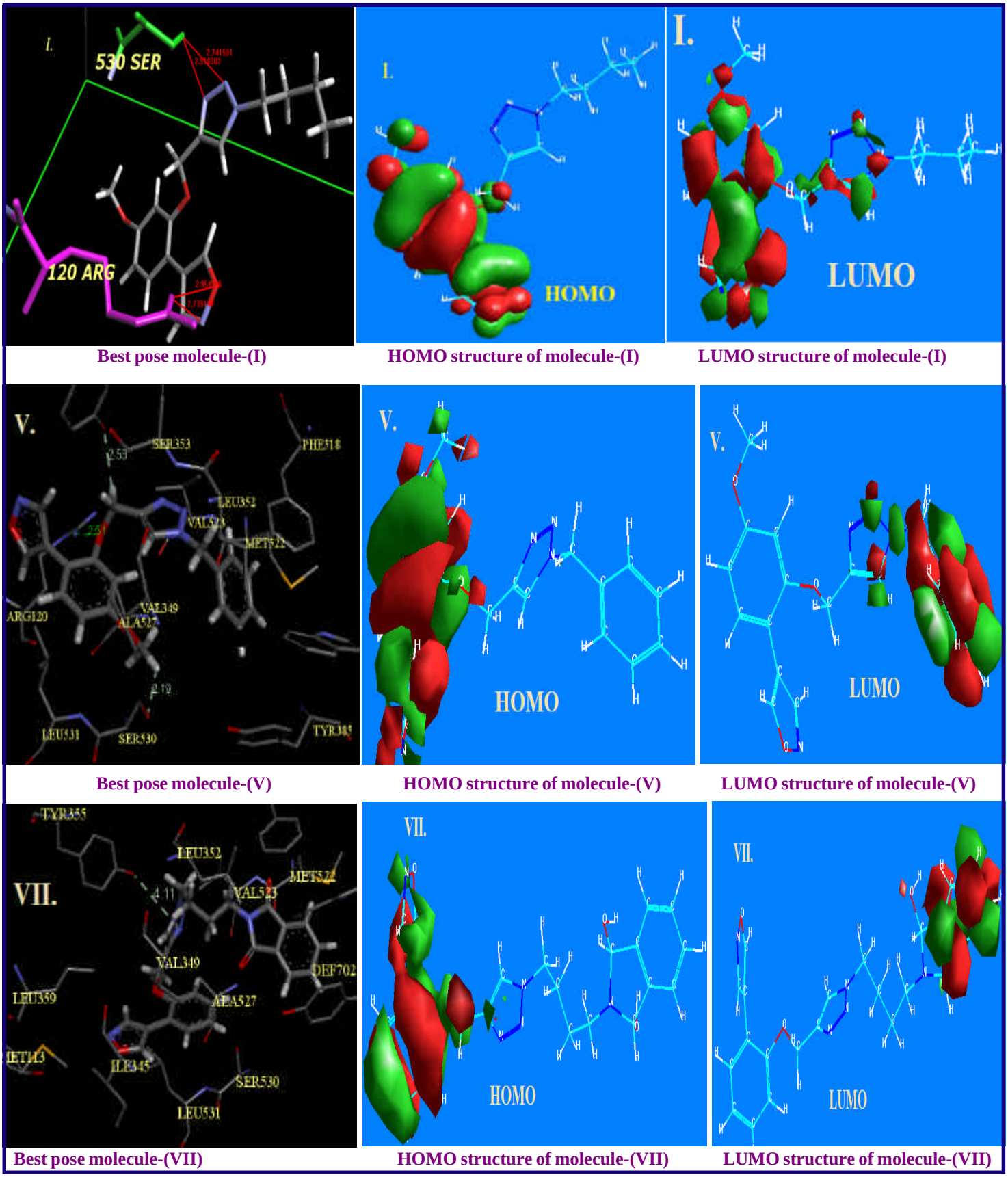
The docking results from the crystal structure of cyclooxegenase-2 (4COX) agreed well with the observed *in vitro* data. .The docked score of compounds-**(I), (V)** and **(VII)** (46.37, 49.54 and 60.78) indicates tight binding to the active site cyclooxegenase-2 and it agreed with biological activity. The high score of these compounds is due to the best fitting of ligand containing electron releasing groups on the substituted aromatic ring of triazole analogues with the cyclooxegenase-2 protein. Therefore these compounds are expected to be potent inhibitors of cyclooxegenase-2.The remaining compounds have next good fitness score due to presence of electron releasing groups on aromatic ring and highest score due to *inter* molecular hydrogen bondings with the electron releasing groups of triazole analogues.

Highest Occupied Molecular Orbital (HOMO) energy and Lowest Unoccupied Molecular Orbital (LUMO) energy were constructed from of HQSAR (Hologram QSAR) [24]. The theoretical calculations of molecular properties such as the maps of molecular orbital's (HOMO, LUMO), Autodock and Argus lab binding energies showed a good antioxidant activity of the title compounds (Table 6 and Fig.4.).

Table 6. HOMO, LUMO (AM1 and PM3), Auto Dock and Argus Lab energies of triazoles

Compound	AM1		PM3	
	$-e_{HOMO(eV)}$	$-e_{LUMO(eV)}$	$-e_{HOMO(eV)}$	$-e_{LUMO(eV)}$
(I)	-8.76	-.33	-8.89	-.35
(II)	-9.26	-.44	-9.36	-.49
(III)	-8.99	-.27	-9.03	-.46
(IV)	-8.53	-1.28	-8.71	-1.34
(V)	-8.77	-.35	-9.00	-.40
(VI)	-8.84	-.36	-9.07	-.47
(VII)	-9.06	-1.24	-9.17	-1.31
(VIII)	-8.92	-.51	-8.98	-.53

Compound	Auto dock		Argus binding energies
	B.E in K. cal/mol	K_i in uM or mM	K.cal/mol (elapsed time in seconds)
(I)	-7.59	2.74(uM)	-9.62(8)
(II)	-0.36	548.38(mM)	-9.87(7)
(III)	-8.79	358.04(uM)	-12.78(8)
(IV)	-5.49	95.19(uM)	-11.46(16)
(V)	-1.97	35.81(mM)	-11.03(9)
(VI)	-3.92	1.33(mM)	-8.51(6)
(VII)	-5.50	93.52(uM)	-13.29(12)
(VIII)	-3.95	1.26(mM)	-12.32(12)



Best pose molecule-(I) | **HOMO structure of molecule-(I)** | **LUMO structure of molecule-(I)**

Best pose molecule-(V) | **HOMO structure of molecule-(V)** | **LUMO structure of molecule-(V)**

Best pose molecule-(VII) | **HOMO structure of molecule-(VII)** | **LUMO structure of molecule-(VII)**

Fig.4. Best docking poses of molecule (I), (V) and (VII). HOMO, LUMO energy maps of molecule (I), (V) and (VII) and green color indicate positive regions, while red color indicate negative region for the activity.

The HQSAR maps show positive (green) and negative (red) contributions. The positive contributions of the most potent compounds- **(I)**, **(V)** and **(VII)** indicate the importance of polar contacts for biological activity. The higher energy of the HOMO and lower energy of the LUMO indicate the greater electron-donating ability and smaller resistant to accept electrons respectively. Therefore the HOMO and LUMO energies support the QSAR and docking results.

4. CONCLUSIONS

The antioxidant activity of triazole analogues was determined by using CTC of DDQ. The determined contents of triazoles could be corrected for the ascorbic acid content to eliminate or minimize the misinterpretations of the ratio of real values when comparing the content of triazoles. In the present study, it is established the predictive QSAR models that are quite reliable to the experimental antioxidant activity of triazoles. The best predictive AM1 model resulted in cross-validated R^2, R^2adj and standard error of estimate (AM1), comprising EA and S. Similarly the best predictive PM3 model was derived with R^2, R^2adj and standard error of estimate, comprising EA and S. The linear dependence of inhibitory nature on EA and S are evident from **Fig. 2** in both AM1 and PM3 methods.

The main contribution of the high score compounds to the cyclo-oxygenase-2 enzyme is due to hydrophobic interactions. These findings demonstrated that these high score compounds could be identified as the best antioxidants. The QSAR equations show good predictive performance and have ability to provide an insight into the softness and electron affinity of triazole analogues. QSAR shows good predictive performance and has ability to provide some insight into the relative importance of the individual compounds involved in determining the biological activity or binding with receptor. Based on the activity data, the compounds **(I)**, **(V)** and **(VII)** serve as an important pharmacophore for the design and develop as antioxidant agents. The QSAR study reveals that the relationship between physiochemical parameters with structures and antioxidant activity. The antioxidant property of triazole is influenced to a great extent by the increase in the strength of electron donating groups attached on five member triazole ring. In the presence of electron releasing groups on aromatic ring and highest score due to *inter*-molecular hydrogen bonding with the electron releasing groups. The docking also gives insight into the pharmacophore and residues of cyclo-oxygenase-2 active site. Finally, it is concluded that the work presented here will play an important role in understanding the relationship of physiochemical parameters with structure and biological activity.

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