ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Novel *In vitro* Antioxidant Bioassay, QSAR and Docking Studies of β-Aminoketone analogues

Ramesh Macha*, Ravindrachary Kanchanapally, and Parthasarathy Tigulla*.

Department of chemistry, Osmania University Hyderabad- 500007, India.

ABSTRACT

The antioxidant properties of β-Aminoketone analogues were evaluated by spectrophotometric method. This *in vitro* novel bioassay was monitored at maximum wave length. The free radical scavenging activities of the analogous are expressed as IC_{50}values. AM1 and PM3 semi empirical methods were used to estimate different physicochemical parameters. The antioxidant activity was correlated with these physicochemical parameters and different QSAR models were generated. The QSAR results reveal electron affinity (EA), softness(S), eletrophilic index (**ω**) and LogP are responsible for high antioxidant activity. Docking studies were also performed with in the active site of cyclo-oxygenase-2 to identify hydrogen bonding, hydrophobic and ionic interactions. GOLD, Auto dock and Argus lab docking results explain the active site residues. All most all compounds showed very good inhibitory activity values against cyclo-oxygenase-2 with the formation of strong hydrogen bond interactions with the residues of active site. This type of chemical environment may serve as a starting point for synthesis of cyclo-oxygenase-2 inhibitors with improved efficacy.

Keywords: β-Aminoketone analogues, antioxidant activity, semi empirical methods, QSAR, docking

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Nitrogen containing molecules are significant synthetic targets owing to their wide range of applications as pharmaceutical and bioactive compounds. Mannich reaction has been one of the classical methods for the construction of nitrogenous compounds especially β-amino carbonyl compounds. These are versatile intermediates for the synthesis of β-amino alcohols or acids, which have great deal of biological significance. In the β-Aminoketones ((1-Propanones (1,3-diphenyl-3-(phenyl amino) propan-1-ones)) the formation of carbon-carbon bonds is crucial to the development of organic molecules such as medicines, biodegradable plastics and natural products. These were used as photo initiators in printing applications. They were used as intermediates for the preparation of known keto-methylene pseudo peptides which were used as antibiotics, antibiotic enhancers or inhibitors, antioxidants etc **[1].**

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 **5, 7, 11** and **12** were showed best in vitro biological activity compare standard ascorbic acid.

The β-Aminoketones are expected to act as free radical scavengers because of their structure features (**Fig.1).**The free radicals source **[2]** is charge transfer complex (CTC). The CTC is formed between **n**-electron donor and the sigma-acceptor, iodine **[3]**.

The aim of the present work is to develop *in vitro* antioxidant property of β-Aminoketones by spectrophotometric method. The molecular modeling studies also help us to understand the various interactions between the ligand and enzyme active site in detail and there by facilitating in design of novel antioxidants. The suitable chemical environment may serve as a starting point for synthesis of cyclo-oxygenase-2 (PDB ID: 4COX) inhibitors with improved antioxidant efficacy.

EXPERIMENTAL METHODOLOGY

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 (2nm) was used to record the absorption spectra.

Antioxidant activity of β-Aminoketones was measured by using spectrophotometer methods. This method is based on the charge transfer complex (CTC) formation of the donor with Iodine. This method is followed by measuring the maximum absorbance at 370*nm* under the optimized conditions.

To the 10mL of 3×10^{-3}M CTC, 10mL of 10^{-3}M substituted β-Aminoketone was added. The mixture was allowed to stand for 5 min at room temperature and then the absorbance of the purple-red coloured solution was measured at 370*nm* (Systronics UV–Vis. double beam spectrophotometer Model 2202). Ascorbic acid was used as standard. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capacity of free radical scavenging activity of β-Aminoketone was calculated using the following equation:

$$\%Rs = \frac{Ai - Af}{Ai} x\ 100$$

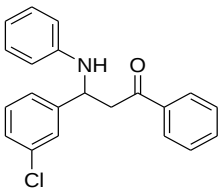
R_s radical scavenging activity of β-Aminoketones, A_i initial absorbance of the CTC, A_f is the absorbance of the test/standard compound.

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**) **[4]**.The antioxidant activity was compared with ascorbic acid, used as a 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}. 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). 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.

Figure 1: Structures of β-Aminoketone derivatives

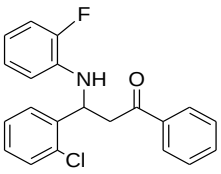
1.



3-(3-chlorophenyl)-1-phenyl-3-(phenylamino)propan-1-one

M.wt = 335.83

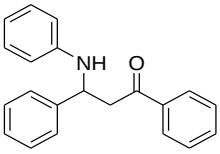
2.



3-(2-fluorophenylamino)-3-(2-chlorophenyl)-1-phenylpropan-1-one

M.wt = 353.82

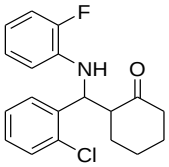
3.



1,3-diphenyl-3-(phenylamino)propan-1-one

M.wt = 301.38

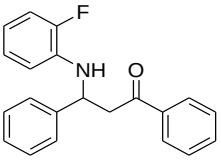
4.



2-((2-fluorophenylamino)(2-chlorophenyl)methyl)cyclohexanone

M.wt = 331.81

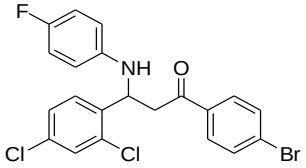
5.



3-(2-fluorophenylamino)-1,3-diphenylpropan-1-one

M.wt. = 319.37

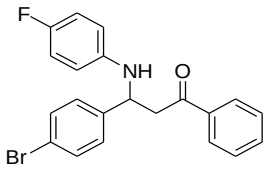
6.



3-(4-fluorophenylamino)-1-(4-bromophenyl)-3-(2,4-dichlorophenyl)propan-1-one

M.wt = 467.16

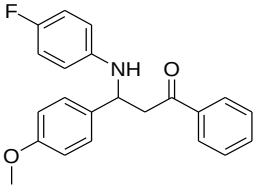
7.



3-(4-fluorophenylamino)-3-(4-bromophenyl)-1-phenylpropan-1-one

M.wt = 398.27

8.

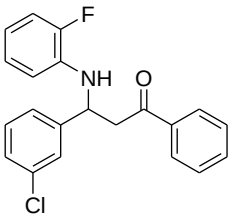


3-(4-fluorophenylamino)-3-(4-methoxyphenyl)-1-phenylpropan-1-one

M.wt = 349.40

ISSN: 0975-8585

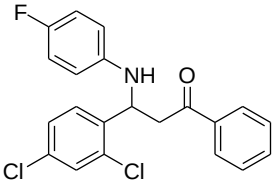
9.



3-(2-fluorophenylamino)-3-(3-chlorophenyl)-1-phenylpropan-1-one

M.wt = 352.82

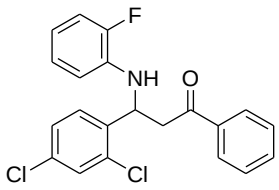
10.



3-(4-fluorophenylamino)-3-(2,4-dichlorophenyl)-1-phenylpropan-1-one

M.wt = 388.26

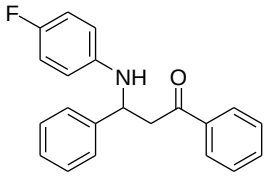
11.



3-(2-fluorophenylamino)-3-(2,4-dichlorophenyl)-1-phenylpropan-1-one

M.wt = 388.26

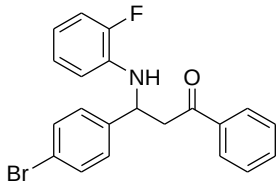
12.



3-(4-fluorophenylamino)-1,3-diphenylpropan-1-one

M.wt = 319.37

13.



3-(2-fluorophenylamino)-3-(4-bromophenyl)-1-phenylpropan-1-one

M.wt. = 398.27

Table 1: Antioxidant activity of β-Aminoketone analogues.

comp	1	2	3	4	5	6	7	8	9	10	11	12	13	Ascorbic Acid(std
$IC_{50(mM)}$	0.06	0.90	0.04	0.11	0.03	0.24	0.03	0.04	0.10	-0.19	0.03	0.02	-0.05	0.018
Act	4.20	3.04	4.36	3.95	4.46	3.62	4.48	4.38	3.97	2.30	4.46	4.68	4.30	5.20

Comp = compound, %RSA = %Radical Scavenging Activity, Act = Activity. Std = standard

Computational Methodology

Construction of molecular structures

A series of β-Aminoketone compounds tested for inhibitory activity was selected for the present study and the program of window Hyperchem software in (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. Consulthttp://www.spss.com) was used in the regression analysis.

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) **[5]** The quantum chemical descriptors (variables) **[6-9]** 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(χ), hardness(η), softness(S), electrophilic index (ω), partition coefficient (Log P), hydration energy (HE),chemical potential(μ) and polarisability (Pol)were obtained for β-Aminoketone analogues.

Molecular modeling studies

QSAR technique was applied to the analogs of β-Aminoketone analogues that were varied on aryl ring position. The appropriate descriptors or parameters for the compounds were used as independent variables.

In addition to the synthetic work, an attempt to explore docking studies on β-Aminoketone 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.

GOLD Software

The GOLD2.0 (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 **[10,11]**. 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 **[12](**http://www.rcsb.org/pdb**).**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*; **1-13**. 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. 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 **[13]** Enzyme-inhibitor interactions within a radius equal to 15Å centered on reported bound inhibitors were taken into account.

Argus Lab

Argus Lab 4.0.1 **[14]** 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.

Auto dock 4.0

Autodock 4.0 **[15]** was used to estimate binding free energy and inhibition constant (K_i).

ISSN: 0975-8585

RESULTS AND DISCUSSIONS

Free radical scavenging activity

Iodine itself acts as a sigma acceptor. The bonding type involved in iodine is ***n-σ***. In the present case the resulted CTC is evidenced by hypsochromic shift. Formation of CTC is due to excitation of electrons from orbital of donor to orbital of acceptor **[16-17].** Therefore the method is based on the reaction of ***n***-electron donors with the sigma-acceptor iodine.

To accommodate the observed results, the following reaction mechanism is proposed:

$$\underset{\text{Donar}}{D} + \underset{\text{Iodine}}{I_2} \rightleftharpoons \underset{\text{CTC}}{[D\text{---}I^{+}\text{---}I^{\bullet}]} \xrightarrow{RH} R^{\bullet} + D + HI$$

The CTC is formed between triethyl amine as a donor (D) and iodine as sigma(***σ***)-acceptor(I_2). The CTC decomposes to give iodine free radical which in terns forms $R^{\bullet}$ radical on abstraction of hydrogen from RH. $R^{\bullet}$radical will then undergo further reactions which control the overall stoichiometry *i.e*., the number of molecules Iodine reduced by RH. Mixing of iodine solution to donors resulted in decrease in intensity of color *i.e.* shifted to shorter wave length (hypsochromic shift). Formation of CTC is due to excitation of electrons from orbital of donor to orbital of acceptor.

Simple linear regression model analysis

The biological activity data and the physicochemical properties IP, EA, ω, EN, η, S, LogP, HE, μ and Pol of the β-Aminoketone 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 **thirteen** β-Aminoketone 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 descriptors values of β-Aminoketone analogues in AM1 method

Comp	Obs. Act.	Eq-1		Eq-2		Molecular descriptors									
		Predicted	residual	Predicted	residual	$IP_{(eV)}$	$EA_{(eV)}$	$EN_{(eV)}$	$\eta_{(eV)}$	$S_{(eV)}$	ω	HE(K.cal/mol)	LogP	Pol (A^0)	μ
1	4.20	4.13	.07	4.08	.12	7.89	0.60	4.25	3.65	.14	2.48	-5.33	3.08	38.75	-4.25

ISSN: 0975-8585

2	3.04	3.54	-.50	3.51	-.46	7.94	0.59	4.26	3.67	.14	2.48	-5.01	1.97	38.53	-4.26
3	4.36	4.41	-.05	4.37	-.01	8.09	0.51	4.30	3.79	.13	2.44	-5.48	2.79	36.70	-4.30
4	3.95	4.27	-.31	4.24	-.29	8.22	0.26	4.24	3.98	.13	2.26	-2.69	1.90	35.44	-4.24
5	4.46	4.60	-.15	4.53	-.08	7.93	-0.47	3.73	4.20	.12	1.66	-5.23	2.19	36.61	-3.73
6	3.62	3.61	.00	3.61	.01	8.02	1.00	4.51	3.51	.14	2.90	-4.22	1.80	43.09	-4.51
7	4.48	4.40	.08	4.39	.09	8.26	0.62	4.44	3.82	.13	2.58	-4.86	2.24	39.23	-4.44
8	4.38	3.92	.45	3.93	.45	8.32	0.50	4.41	3.91	.13	2.49	-6.59	1.20	39.08	-4.41
9	3.97	3.24	.73	-	-	7.76	0.40	4.08	3.68	.12	2.27	-3.82	1.97	38.53	-4.08
10	2.30	2.78	-.49	-	-	7.64	0.50	4.07	3.57	.12	2.32	-4.97	1.74	40.46	-4.07
11	4.46	4.77	-.31	4.79	-.33	8.58	0.57	4.58	4.00	.12	2.62	-4.30	1.74	40.46	-4.58
12	4.68	4.29	.39	4.26	.42	8.14	0.23	4.19	3.95	.13	2.22	-5.05	2.19	36.61	-4.19
13	4.30	4.22	.08	4.20	.10	8.17	0.61	4.39	3.78	.13	2.55	-4.81	2.24	39.23	-4.39

Table 3: Antioxidant activities and molecular descriptors values of β-Aminoketone analogues in PM3 method

Comp		Eq-3		Eq-4		Molecular descriptors									
	Obs. Act.	Predicted	residual	Predicted	residual	$IP_{(eV)}$	$EA_{(eV)}$	$EN_{(eV)}$	$\eta_{(eV)}$	$S_{(eV)}$	ω	HE(K.cal/mol)	LogP	Pol(A^0)	μ
1	4.20	4.17	.03	3.95	.25	8.08	.63	4.35	3.72	.13	2.55	-5.34	3.08	38.75	-4.35

2	**3.04**	**3.55**	**-.51**	**4.08**	**-.04**	**8.15**	**.38**	**4.26**	**3.88**	**.13**	**2.34**	**-5.04**	**1.97**	**38.53**	**-4.26**
3	**4.36**	**4.37**	**-.02**	**4.19**	**.16**	**8.27**	**.29**	**4.28**	**3.99**	**.13**	**2.30**	**-5.35**	**2.79**	**36.70**	**-4.28**
4	**3.95**	**4.15**	**-.19**	**4.28**	**-.33**	**8.47**	**.32**	**4.40**	**4.08**	**.12**	**2.37**	**-2.64**	**1.90**	**35.44**	**-4.40**
5	**4.46**	**3.70**	**.76**	**3.98**	**.48**	**8.14**	**.65**	**4.40**	**3.74**	**.13**	**2.58**	**-5.17**	**2.19**	**36.61**	**-4.40**
6	**3.62**	**3.86**	**-.25**	**3.99**	**-.37**	**8.28**	**1.01**	**4.64**	**3.64**	**.14**	**2.96**	**-4.36**	**1.80**	**43.09**	**-4.64**
7	**4.48**	**4.44**	**.04**	**4.23**	**.24**	**8.50**	**.46**	**4.48**	**4.02**	**.12**	**2.50**	**-4.77**	**2.24**	**39.23**	**-4.48**
8	**4.38**	**3.83**	**.54**	**4.30**	**.08**	**8.56**	**.38**	**4.47**	**4.09**	**.12**	**2.44**	**-6.54**	**1.20**	**39.08**	**-4.47**
9	**3.97**	**3.43**	**.54**	**-**	**-**	**8.09**	**.58**	**4.34**	**3.75**	**.13**	**2.51**	**-3.82**	**1.97**	**38.53**	**-4.34**
10	**2.30**	**3.18**	**-.88**	**-**	**-**	**8.04**	**.59**	**4.31**	**3.73**	**.13**	**2.50**	**-5.03**	**1.74**	**40.46**	**-4.31**
11	**4.46**	**4.46**	**.00**	**4.34**	**.12**	**8.69**	**.44**	**4.57**	**4.12**	**.12**	**2.53**	**-4.40**	**1.74**	**40.46**	**-4.57**
12	**4.68**	**4.20**	**.48**	**4.21**	**.47**	**8.40**	**.40**	**4.40**	**4.00**	**.13**	**2.42**	**-5.10**	**2.19**	**36.61**	**-4.40**
13	**4.30**	**4.84**	**-.54**	**4.37**	**-.07**	**8.72**	**.40**	**4.56**	**4.16**	**.12**	**2.50**	**-4.79**	**2.24**	**39.23**	**-4.56**

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

Activity = -3.713(0.692)*EA-54.516(16.239)* **S** + 4.825(0.942)* ω +0.618(0.263)*LogP------- (1)

N = 13; R = 0.996; R^2 = 0.993; R^2adj=0.989; %EV = 99.3; SEE = 0.4228; F= 298.30; Q =2.355;

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, outliers (**9 & 10**) were sought and eliminated.

After the elimination of the outlier (**9&10**), a second model was developed. Overall, there is an increase in R (0.996-0.998) and %EV (99.3– 99.8) values, and a decrease in SEE (**0.4228- 0.3429**).

Activity = -3.758(0.778)*EA-57.17(23.379)* **S** + 4.978(1.259)* ω +0.605(0.261)*LogP-------- (2)

N =11; R= 0.998; R^2= 0.996; R^2adj =0.993; %EV = 99.8; SEE = 0.3429; F =410.568; Q =2.910;

Eq.2 is an improved model since it explains the biological activity to the extent of (99.5%).

From the correlation matrix table, it reveals EA, ω, S and LogP are found to be explainable variables. A tetra-parametric QSAR equation with EA, ω, S and LogP and tetra-parametric in AM1 Method & di-parametric QSAR equation with EA and ω were generated in PM3 method also.

Activity = -2.971(1.083)*EA-54.54(27.663)* S + 4.424(1.411)* ω +0.685(0.363)*LogP-------- (3)

ISSN: 0975-8585

N =13; R = 0.993; R^2= 0.987; R^2adj =0.981; %EV = 98.70; SEE = 0.5601;
F = 169.019; Q = 1.7729;

Eq.3 shows that the values of % EV is less and to improve its value, outliers(**9&10**) 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 outliers (**9&10**), a second model was developed.

Activity = -2.254(0.853)*EA+2.111(0.179)* ω ---------- (4)

N = 11; R = 0.995; R^2 = 0.990; R^2adj=0.988; %EV = 99.0; SEE = 0.4660; F = 441.942; Q =2.1351;
In an attempt to investigate the predictive potential of 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 Table 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}= (>0.68). 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 **[18]**, 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 2.355 to 2.910 and 1.7729 to 2.1351 **(Eq. 1 to 4).**

In the final AM1 and PM3 modelled **Eq-2** and **Eq-4,** contribution of the physicochemical parameters shown graphically in contribution charts (**Fig.2. & Fig.3**). In AM1 method the indicative parameters are EA, S, ω and logP. The variation of S and logP are found to be negligible (**Table 2**). Hence, EA and ω are the main contributory factors for deciding antioxidant property. The electron affinity is characterized by the susceptibility of the compound in relation to attacks by nucleophiles. Electrophilicity is a property of atoms which signifies the energy lowering process on soaking electrons from donors. The electrophilicity index measures the stabilization in energy when the system acquires an additional electronic charge from the environment **[19]**. The correlation between actual and predicted activity for the compounds are shown in **Tables 2, 3** and **Figs. 4-7**. Therefore, one can conclude that electronic effects have a very important role when one is trying to understand the activity of β-Aminoketone analogues with anti-oxidant activity.

Figure 2: Plot of Observed activity verses Predicted activity (AM1- Method).

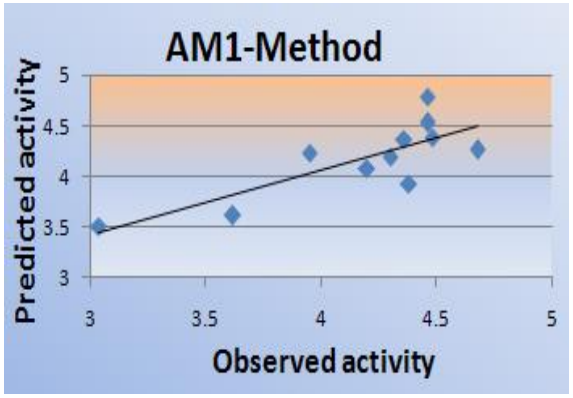


Figure 3: Plot of Observed activity verses Predicted activity (PM3- Method).

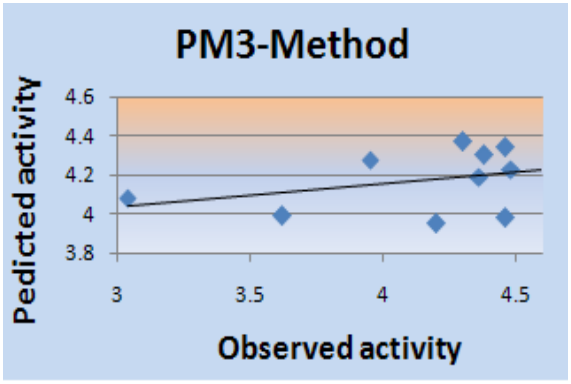


Figure 4: Plot of Observed activity Verses EA (AM1-Method).

Figure 5 Plot of Observed activity Verses Softness (AM1- Method).

ISSN: 0975-8585

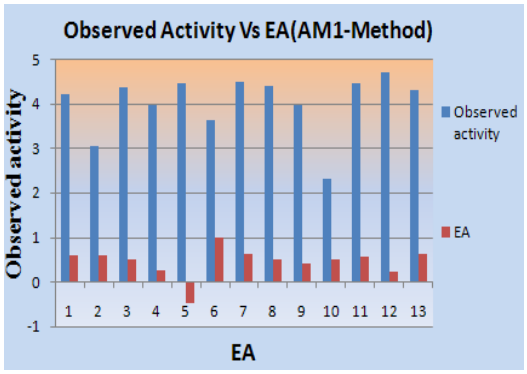


Figure 6: Plot of Observed activity verses ω (AM1-Method)

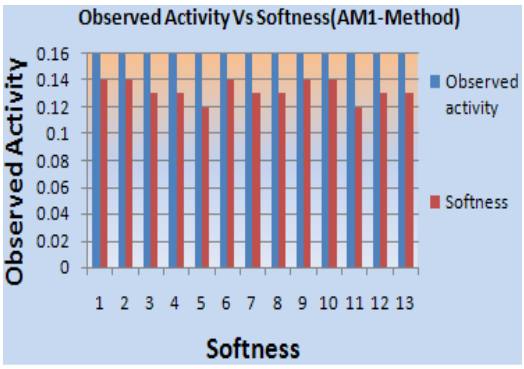


Figure 7: Plot of Observed activity verses LogP (AM1-Method)

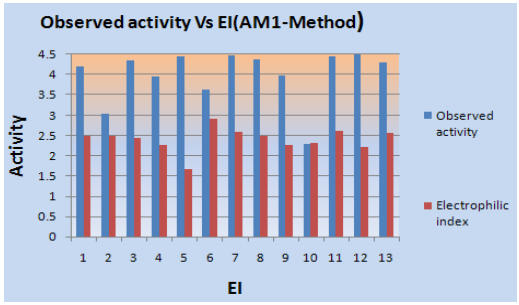


Figure 8: Plot of Observed activity verses EA (PM3-Method).

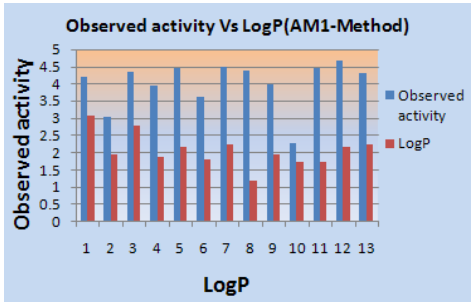
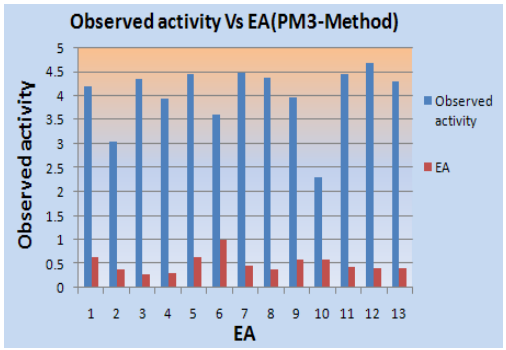
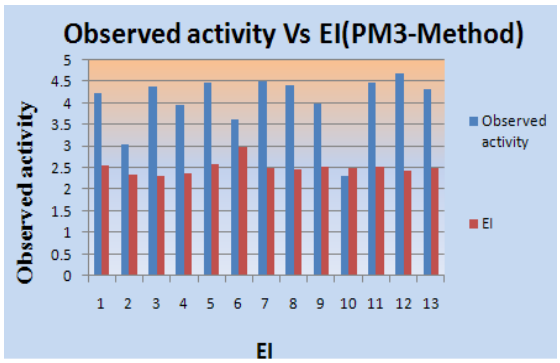


Figure 9: Plot of Observed activity verses ω (PM3-Method).



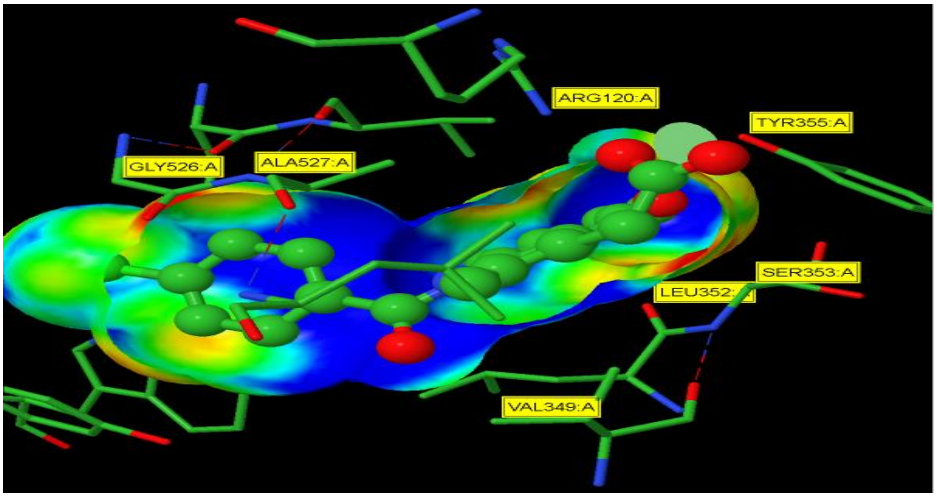


Docking Analysis

Among all the β-Aminoketone compounds tested for docking study, showed good inhibitory activity values against cyclo-oxygenase-2 (**Table 4** and **Table 5**).The compound **7 & 12** showed high affinities with low energy of with employed protein. It indicates the binding between 4COX and compound-1**2** indicates very good inhibition. The compounds **(1-13)** showed good inhibition with affinity ranges. In the active site of **4COX**,Thr212,Asn68,Glu67,His388,Ser 530,Tyr355,Tyr 402,Asn 382,Thr70,Glu140,Asn144 amino acids play important role and they are shown in **Fig.10**.

ISSN: 0975-8585

Figure 10: Active site amino acids of crystallographic protein 4COX



The docking results from the crystal structure of cyclooxegenase-2(4COX) in the modeling study agreed well with the observed *in vitro* data, which indicated that compound-1**2** **(IC_{50}=0.020)** expected to be a potent inhibitor of cyclooxegenase-2.The docked score of compound-1**2** (54.19) indicates tight binding to the active site cyclooxegenase-2 and it agreed with biological activity. The high score of compound-1**2** is due to the best fitting of ligand containing electron releasing group (F) in the *para* position of aromatic ring of β-Aminoketone analogues with the cyclooxegenase-2 protein. The second highest score for the compound-**7** is due to electron releasing groups (F & Br) on aromatic rings of compound-**7**. The compounds **11, 5** and **3** have next highest score due to presence of electron releasing groups on aromatic ring and highest score due to *inter* molecular hydrogen bindings' with the electron releasing groups. The remaining compounds have medium gold docking score due to presence of less capacity of electron donating groups present on the aromatic ring of β-Aminoketone analogues.

Table 4: Docking values obtained from GOLD in fitness score with cyclo-oxygenase-2 (PDB ID = 4COX)

Comp	Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(vdw_int)
1	57.09	0.00	46.65	0.00	-7.06
2	54.87	0.00	46.59	0.00	-9.18
3	57.57	0.00	46.05	0.00	-5.75
4	50.22	0.00	41.00	0.00	-6.16
5	**55.92**	**0.00**	**46.12**	**0.00**	**-7.49**
6	59.64	0.00	47.20	0.00	-5.27
7	**57.12**	**0.00**	**45.71**	**0.00**	**-5.73**
8	56.56	0.00	47.52	0.00	-8.78
9	58.79	0.00	47.74	0.00	-6.85
10	55.57	0.00	46.04	0.00	-7.73
11	54.31	0.00	45.35	0.00	-8.04
12	**54.19**	**0.00**	**44.56**	**0.00**	**-7.08**
13	55.88	0.00	45.95	0.00	-7.30

Table 5: Docking values obtained from GOLD in Chemscore function with cyclo-oxygenase-2 (PDB ID = 4COX)

Comp	Score	DG	S(h-bond)	S(metal)	S(lipo)	DE(clash)	DE(int)
1	35.02	-38.69	0.00	0.00	312.12	1.89	1.78
2	33.50	-37.24	0.00	0.00	301.18	1.84	1.90
3	35.71	-38.48	0.00	0.00	310.29	2.09	0.68
4	28.28	-34.29	0.00	0.00	273.27	3.85	2.17

ISSN: 0975-8585

5	**34.66**	**-36.17**	**0.00**	**0.00**	**292.04**	**0.86**	**0.65**
6	34.34	-43.15	0.00	0.00	351.74	4.48	4.33
7	**34.18**	**-36.90**	**0.00**	**0.00**	**298.28**	**1.14**	**1.58**
8	33.96	-37.41	0.00	0.00	307.11	1.78	1.67
9	36.44	-41.42	0.00	0.00	336.92	2.15	2.83
10	34.23	-40.63	0.00	0.00	330.17	5.61	0.79
11	33.00	-42.18	0.00	0.00	343.42	6.23	2.95
12	**35.80**	**-37.44**	**0.00**	**0.00**	**302.89**	**1.15**	**0.49**
13	35.51	-40.36	0.00	0.00	327.84	2.87	1.98

The construction of HQSAR (Hologram QSAR) **[20] (**Vinícius G et al.,2012**)** and molecular docking studies were performed. The theoretical calculations of some molecular properties, such as the maps of molecular orbitals (HOMO, LUMO), Auto dock and Argus lab binding energies showed a good correlation between the title compounds of antioxidant activity and are confirmed in **Table 6** and **Fig.9**. HQSAR maps show positive (green) and negative (blue) contributions. The HQSAR map of compounds **5, 7 & 12**(the most potent), showed a positive contributions, indicating the importance of polar contacts to biological activity.

The energy of Highest Occupied Molecular Orbital Energy (HOMO) and Lowest Unoccupied Molecular Orbital Energy (LUMO) are the quantum-chemical descriptors, which play an important role in chemical reactions**.** The delimited region for the HOMO orbital which measures the electron-donor character of β-Aminoketones, and the LUMO which measures the electron-acceptor character in Figure 9. The higher the energy of the HOMO greater electron-donating ability and the lower the energy of the LUMO will be lowest resistance to accept electrons. The HOMO and LUMO energies also support the QSAR and docking studies in Table 6.

Table 6: HOMO, LUMO (AM1 & PM3), Auto Dock and Argus Lab energies of β-Aminoketone analogues.

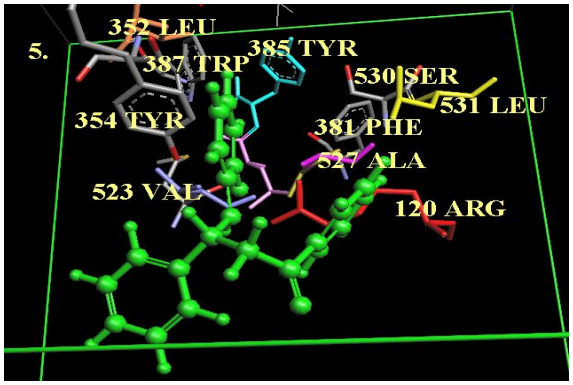
Compound	AM1		PM3		Auto dock B.E in K. cal/mol	Argus B.E in K cal/mol(elapsed time in seconds)
	$-\varepsilon_{HOMO\ (eV)}$	$-\varepsilon_{LUMO(eV)}$	$-\varepsilon_{HOMO(eV)}$	$-\varepsilon_{LUMO(eV)}$		
1	**-8.69**	**-.42**	**-8.79**	**-.33**	**+13.04**	**-14.79(6)**
2	**-8.43**	**-.48**	**-8.59**	**-.54**	**+23.47**	**-13.76(7)**
3	**-8.59**	**-.34**	**-8.70**	**-.28**	**+5.15**	**-14.11(8)**
4	**-8.64**	**.02**	**-8.72**	**-.12**	**+3.64**	**-12.75(5)**
5	**-8.42**	**-.35**	**-8.64**	**-.32**	**-1.25**	**-14.05(7)**
6	**-8.48**	**-.84**	**-8.67**	**-.88**	**+19.62**	**-15.14(6)**
7	**-8.73**	**-.50**	**-8.91**	**-.46**	**+20.10**	**-14.17(7)**
8	**-8.60**	**-.39**	**-8.80**	**-.37**	**+20.76**	**-13.53(7)**
9	**-8.62**	**-.44**	**-8.55**	**-.41**	**+25.51**	**-14.67(6)**
10	**-8.72**	**-.37**	**-8.52**	**-.42**	**+43.15**	**-14.30(6)**
11	**-8.87**	**-.43**	**-8.92**	**-.32**	**+25.07**	**-15.04(7)**
12	**-8.45**	**-.23**	**-8.81**	**-.40**	**+23.35**	**-13.65(6)**

ISSN: 0975-8585

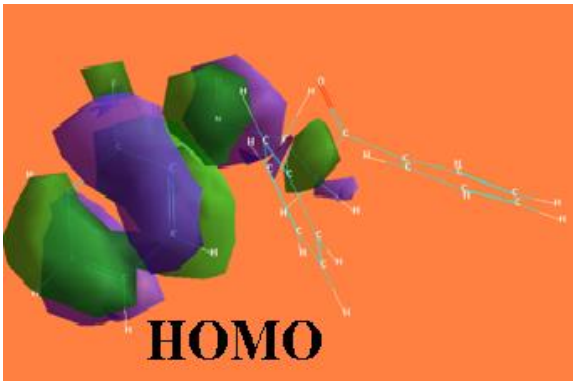
13	-8.65	-.45	-9.02	-.38	+22.48	-14.91(7)

Figure 9 Best docking poses of molecule 5,7 &12.HOMO,LUMO energy maps of molecule (5,7&12) and green colour indicate favorable regions, while blue colour indicate unfavorable region for the activity.

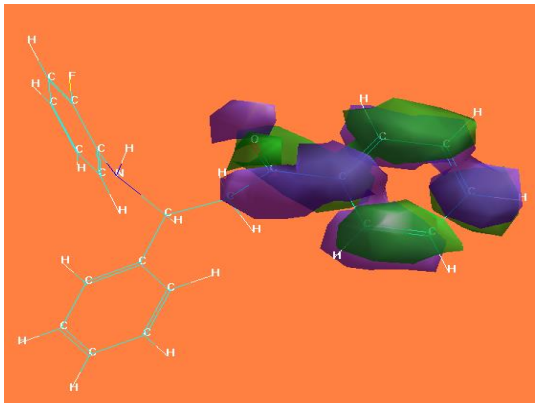
Best pose molecule-5



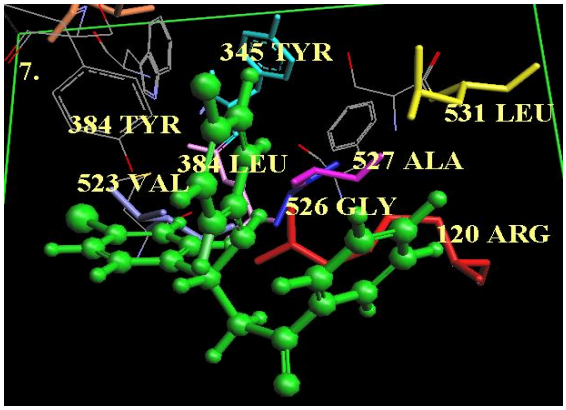
HOMO structure of molecule-5



LUMO structure of molecule-5

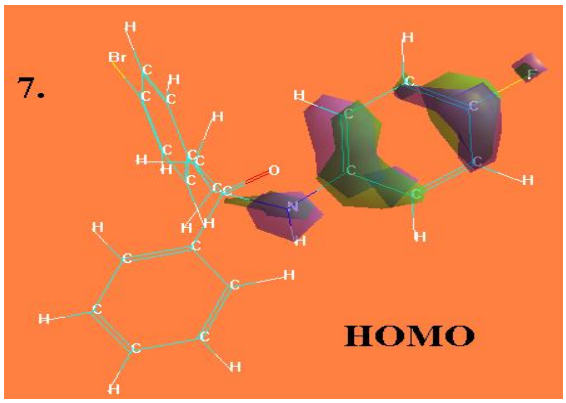


Best pose molecule-7

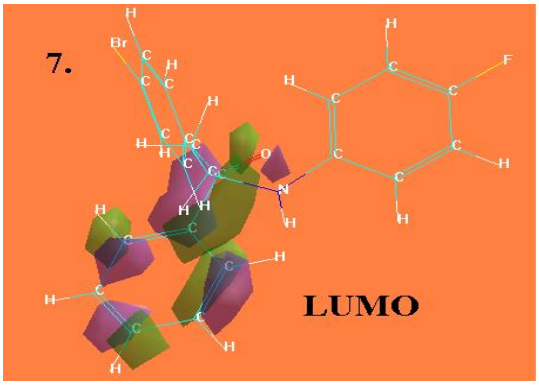


HOMO structure of molecule-7

LUMO structure of molecule-7

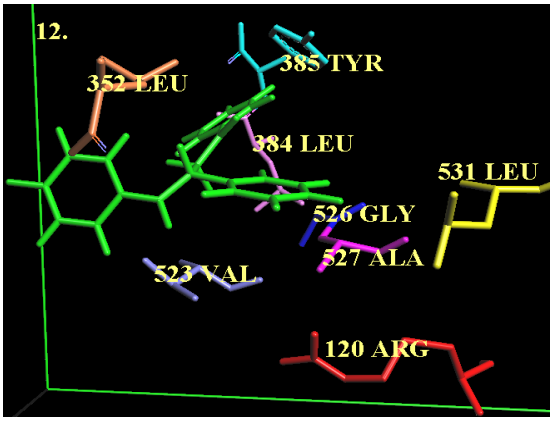


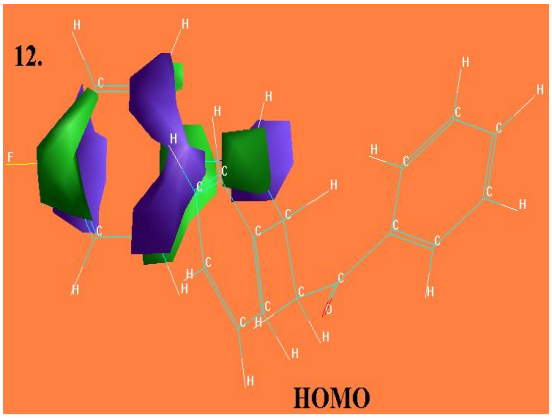
Best pose molecule-12



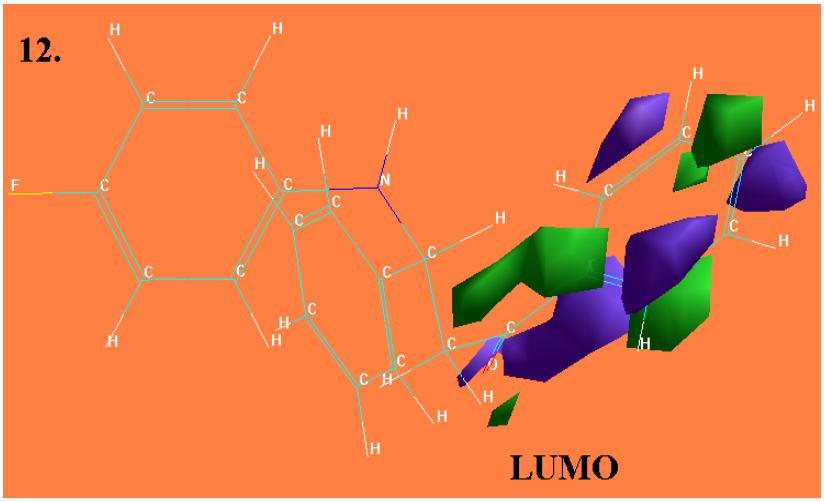
HOMO structure of molecule-12

ISSN: 0975-8585





LUMO structure of molecule-12



CONCLUSIONS

The antioxidant activity of β-Aminoketone analogues was determined using CTC of Iodine and β-Aminoketone analogues. The determined contents of β-Aminoketones 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 β-Aminoketone. In our present study, it was established the predictive QSAR models that are quite reliable to the experimental antioxidant activity of β-Aminoketone. The QSAR studies and molecular docking were performed on **thirteen** β-Aminoketone analogues. The best predictive AM1 model resulted in cross-validated R^2 value of 0.996, R^2adj value of 0.993 and standard error of estimate 0.2910(AM1), comprising EA, S, ω and LogP. Similarly the best predictive PM3 model was derived with R^2 of 0.990, R^2adj of 0.988 and standard error of estimate of 0.2135, comprising EA and ω. The linear dependence of inhibitory nature on EA and ω were evident from (**Figure 2** and **3)** in both AM1 and PM3 method**.**

In the presence of electron releasing groups on aromatic ring and highest score due to *inter*-molecular hydrogen bonding with the electron releasing groups. These findings demonstrated that these compounds could be developed into novel lead molecules for treating antioxidant. Further experiments would be required for investigating the detailed interaction and *in vitro* testing their inhibitory activity against cyclo-oxygenase-2. QSAR shows good predictive performance and has ability to provide some insight into the relative importance of the individual compounds involved in determining the biologic activity or binding with receptor. Based on the activity data, from the series **5, 7, 11** and **12** serve as an important pharmacophore for the design and development of new lead as antioxidant agent. 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.

ACKNOWLEDGMENTS

The author **RM** wishes to thank Head, Department of Chemistry, and University College of science, Campus, Hyderabad, Osmania University, for providing the facilities for the work.

Conflict of interest: We declare that none of the authors have a conflict of interest.

REFERENCES

[1] Borovic, S., Tirzitis, G., Tirzite, D., Cipak, A., Khoschsorur, G. A., Waeg, G., Tatzber, F., Scukanec-Spoljar, M., Zarkovic, N., 2006. Bioactive 1,4-dihydroisonicotinic acid Derivatives. prevent oxidative damage of liver cells. Eur. J. Pharmacol. 12,537-540.

[2] Contreras, R., Domingo, L.R., Andrés, J., Pérez, P. and Tapia, O., 1999. Nonlocal (Pair Site) Reactivity from Second- Order Static Density Response Function: Gas- and Solution- Phase Reactivity of the Acetaldehyde Enolate as a Test Case. The Journal of Physical Chemistry A,103, 1291-1436.

[3] Hesham, Salem., 2002. Spectrophotometric determination of β-adrenergic blocking agents in pharmaceutical formulations. Journal of Pharmaceutical and Biomedical Analysi. 29(3),527- 538.

[4] Manuela Silva1 M, Marta R. Santos1, Gonçalo Caroço1, Rui Rocha1, Gonçalo Justino1 and Lurdes Mira, "Structure-antioxidant Activity Relationships of Flavonoids: A Re-examination." *Free Radical Research*, vol. 36(11), pp.1219-1227, 2002.

[5] Mauro, Reis, Benedito, Lobato, Jeronimo, Lameira, Alberdan S, Santos and Cla´udio N. Alves,"A theoretical study of phenolic compounds with antioxidant properties." *European Journal of Medicinal Chemistry,* vol. 42, pp. 440-446, 2007.

[6] M.Kawase, A.Shah, H.Gaveriya, N.Motohashi, H.Sakagami and A.V. Molnar, "3,5-Dibenzoyl-1,4-dihydropyridines: synthesis and MDR reversal in tumor cells." *Bioorganic and Medicinal Chemistry*, vol.10, pp.1051–1055, 2002.

[7] Heravi M. M, Behbahani F. K, Oskooie H. A and Shoar R. H, "Catalytic aromatization of Hantzsch 1,4-dihydropyridines by ferric perchlorate in acetic acid."*Tetrahedron Lett*, vol.46, pp. 2775-2777, 2005.

[8] S.Borovic G.Tirzitis D.Tirzite, A.Cipak, G. A.Khoschsorur, G.Waeg, F.Tatzber, M. Scukanec-Spoljar and N. Zarkovic, *European Journal of pharmacology*, vol.12, pp. 537, 2006.

[9] M.Ramesh, Ganesh Arram and T.Parthasarathy, "Novel Antioxidant Bioassay, QSAR and Docking studies of 1-Phenyl, 3-(Nitro Furan) prop-2-ene-1-one derivatives." *World Journal of Pharmacy & Pharmaceutical Sciences,* Vol. 3(9), pp.442-459, 2014.

[10] (a) P.Jones G,Willett and R. C. Glen, "Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation." *Journal of Molecular Biology*, 245, 43. 1995. (b) G. Jones, P.Willett, R. C.Glen, A. R.Leach and R.Taylor, "Development and validation of a genetic algorithm for flexible docking."*, Journal of Molecular Biology,* vol. 267, pp. 727-748, 1997.

[11] (a) Vijay Kumar Chityalaa, K. Sathish Kumar, Ramesh, Macha, Parthasarathy Tigulla and Shivaraj, "DNA-Cleavage, Cytotoxic Activities and Antimicrobial Studies of Ternary Copper (II) Complexes of Isoxazole Schiff base and Heterocyclic Compounds." *Bioinorganic chemistry and applications,* Vol. **2014**, Article ID 691260, 11 pages. (b) V.Srimai, M,Ramesh, K.Satyaparmeshwar and T.Parthasarathy, "Computer aided design of selective cytochromeP450 inhibitors and docking studies of alkyl resorcinol derivatives." *Medicinal Chemistry Research*, vol. 22(11), pp.314-323, 2013.

[12] http://www.rcsb.org/pdb.

[13] M. L. Verdonk, J. C.Cole, M. J. Hartshorn, C.W. Murray and R. D Taylor, "Improved protein-ligand docking using GOLD." *Proteins:Structure Function Bioinformatics*, vol. 52(4), pp. 609-623, 2003.

[14] M.A. Thompson, "Molecular docking using ArgusLab, an efficient shape-based search algorithm and the AScore scoring function." ACS meeting, *Philadelphia 172 CINF 42 PA,*2004.

[15] O .Trott and A.J. Olson, "Auto dock vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading." *Journal of Computational Chemistry*, vol. 31, pp. 455-461, 2010.

[16] Hesham and Salem, "Spectrophotometric determination of β-adrenergic blocking agents in pharmaceutical formulations." *Journal of Pharmaceutical and Biomedical Analysis*. Vol. 29(3), pp. 527-538, 2002.

[17] T. Parthasarathy, K. NageshwarRao, B.Sethuram and T.NavaneethRao, "Polymerization of acrylonitrile by N-bromosuccinimide-Fe(ii)redox system." *Acta Ciencia Indica*, vol. 23(1), pp. 15, 1997.

[18] N.Rameshwar, K.Krishna, B.Ashok Kumar and T, Parthasarathy, "QSAR studies of N1-(5- chloro-2-pyridyl)-2-{[4-(alkyl methyl)] amino}-5-chlorobenzamide analoges." *Bioorganic and Medicinal Chemistry*, vol.14, pp.319-325, 2006.

[19] W. Yang and R.G. Parr, "Hardness, softness, and the fukui function in the electronic theory of metals and catalysis." *Proc. Natl. Acad. Sci*. USA 82: 6723-6726,(**1985**).

[20] G.Vinícius, Maltarollo, C.Danielle, M.Silvaand Káthia and Honório, "Advanced QSAR Studies on PPARd Ligands Related to Metabolic Diseases." *Journal of Bazillion. Chemical. Society*, vol. 23(1), pp. 85-95, 2012.