IJPSR (2018), Volume 9, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 29 April, 2017; received in revised form, 08 July, 2017; accepted, 25 July, 2017; published 01 January, 2018

SPECTROPHOTOMETRIC ANTIOXIDANT BIOASSAY AND MOLECULAR MODELING STUDIES OF ETHYL 4-SUBSTITUTED-1, 4, 5, 6, 7, 8-HEXAHYDRO-2, 7, 7-TRIMETHYL-5-OXOQUINOLINE-3-CARBOXYLATE DERIVATIVES

Ramesh Macha[*1], K. Ravindrachary [2], G. P. L. Jayasree [3] and Parthasarathy Tigulla [1]

Department of Chemistry [1], Osmania University, Hyderabad - 500007, Telangana, India.
Department of Chemistry [2], Sri Venkateshwara College, Suryapet, Nalgonda - 508213, Telangana, India.
Department of Biotechnology [3], Sreenidhi Institute of Science and Technology (SNIST), Hyderabad - 501301, Telangana, India.

Keywords:

4-Aryl-hexahydroquinolines, Spectrophotometer, Antioxidant, QSAR, Docking

Correspondence to Author:
Dr. Ramesh Macha

Department of Chemistry, UCSS, Osmania University, Hyderabad - 500007, Telangana, India.

Email: machachem2006@gmail.com

ABSTRACT: 4-Aryl-hexahydroquinolines *in vitro* antioxidant properties were evaluated by spectrophotometric method. This method is based on the charge transfer complex which is formed between 4-aryl-hexahydroquinolines and chloranil. 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. Docking studies were also performed in the active site of prenyl transferase to identify the structural features of the antioxidants. Docking scores with appreciable binding energy values also exactly correlated with the experimental activity. The chemscore estimated by Gold Software was found to have a good correlation with the experimental activity. The Docking and QSAR results reveal that the electron donating substituents on benzene ring were responsible to improve the antioxidant activity of the molecules.

INTRODUCTION: Free radicals play a vital role in a number of biological process, some of which are necessary for life, such as the intracellular killing of microorganisms by phagocytic cells [1] but unnecessary amounts of free radicals can lead to damage of bio-molecules such as lipids, proteins, enzymes, DNA in cells and tissues [2] damage in diseases such as atherosclerosis, heart failure, neurodegenerative disorders, cancer, diabetes mellitus, hypertension, aging and several other diseases are becoming increasingly recognized [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(1).67-79

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(1).67-79

Generation of free radicals from metabolism and other activities like environmental pollutants, deep fried and spicy foods, radiation, chemicals, toxins gives rise to oxidative stress [4], physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins and arthritis, ischemia and reperfusion damaged to many tissues, central nervous system injury, gastritis, cancer and AIDS [5, 6].

The free radicals are atoms or molecules which comprise a non-paired electron at the level of their external orbit (NO', 'OH, ' O2, ROO', RO') called reactive oxygen radicals (ROR) or reactive oxygen species (ROS). These compounds are extremely uneven and can react with their electron. They are formed permanently in the organism and their enormous reactivity; can cause serious injuries in the cell.

Reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are yield of normal cellular metabolism and they are well recognized for playing a dual role as both toxic and beneficial species, since they can be either harmful or beneficial to living systems [7]. The oxidation process is one of the most significant routs for producing free radicals in food, drugs and even living systems.

The evidence show that oxidative damage to cellular components such as cell membrane by free radicals is believed to be associated with the increase of degenerative diseases including cardiovascular disorders, cancer, inflammation, immune system decline, arthritis, brain, dysfunction, and cataract [8, 9], age related degenerative diseases, and that dietary antioxidants oppose this and lower risk of disease [10]. Due to depletion of immune system, natural antioxidants in different maladies consuming antioxidants as free radical scavengers might be necessary [11-14]. Antioxidants are the substances which are present in low concentrations compared to those of an oxidisable substrate significantly delays or prevent oxidation of that substance [15].

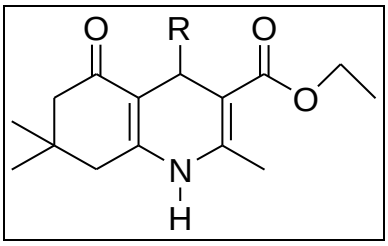
Quinolines are an important group of pharmaceutical compounds, which occur predominately in nature among the various heterocyclic compounds [16, 17] and exhibit a broad spectrum of biological activities such as antioxidant [18], anti-proliferation [19], anti-inflammation [20], and anticancer [21] in the treatment of malaria [22] and more recently of tumours [23]. The poly-hydroquinolines possessing a bioactive 1, 4-dihydropyridine moiety also have an important biological activities such as vasodilator, bronchodilator, anti-atherosclerotic, antitumor, gero-protective, hepatoprotective and anti-diabetic activity [24]. Although the antioxidant activities of quinolines and 1, 4-dihydropyridines [25, 26] have been extensively studied, the poly-hydroquinolines are not well explored. 4-Aryl-1, 4-dihydropyridines are analogues of NADH coenzymes, which have been explored for their calcium channel activity [27].

Some compounds were screened for their *in vitro* biological activities showing good DPPH and $ABST^+$ radical scavenging activities. These quinolines showed the best antioxidant activities against DPPH and $ABST^+$ which suggest further studies to discover the potential chemotherapeutic treatment again tumors or other free radical induced diseases [28].

The quinolines are expected to act as free radical scavengers because of their structural features (**Fig. 1**). In addition, recent studies have suggested that 1, 4-dihydropyridine derivatives also provide an antioxidant protective effect that may contribute to their pharmacological activities [18]. These derivatives were synthesized by our collaborative research group.

The aim of the present work is to estimate antioxidant property of quinolines quantitatively by spectro-photometric method. The molecular modelling studies 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 prenyl transferase inhibitors with improved antioxidant efficacy.

Basic skeleton of ethyl 4 substituted-1,4,5,6,7,8-hexa hydro- 2, 7, 7-trimethyl-5-oxoquinoline-3-carboxylate derivatives

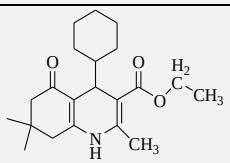
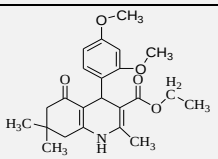
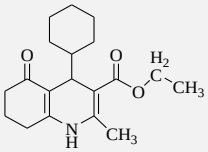
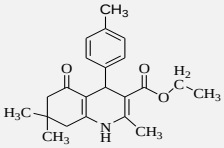
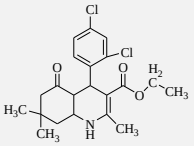
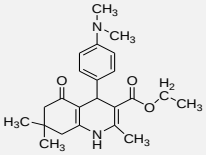
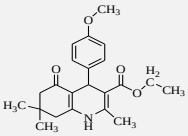
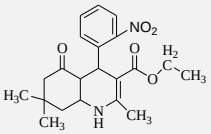
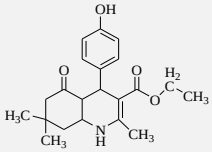
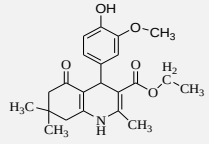
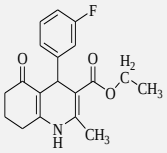
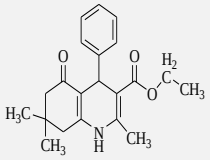
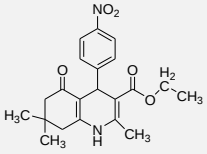
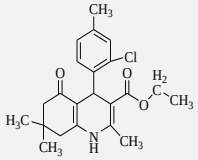
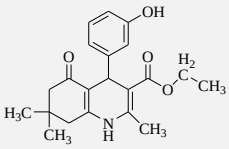
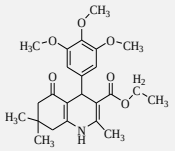
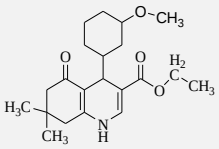
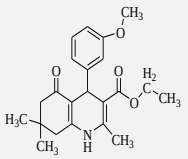
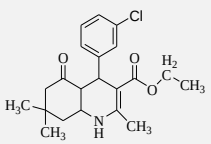


Experimental Methodology:

General Analytical Procedure: Chloranil (2, 3, 5, 6- Tetrachloro- 1, 4-benzoquinone) was procure from the AVRA synthesis laboratory. L-Ascorbic acid, 1, 4-dioxane and acetone solvents were purchased from the SD FINE-Systronics version1. 1PC based Double Beam Spectrophotometer 2202 with matched, 1cm quartz cuvettes was used. Into a 100mL calibrated flask, 25mg of 2, 3, 5, 6-tetrachloro- 1, 4- benzoquinone (chloranil) was weighed exactly and dissolved in 2mL of 1, 4-dioxane, and the volume made up to the mark with the same solvent then add 1mL of the dimethyl amine solution, within 5 minutes complex was formed. It was then diluted quantitatively to obtain the accurate concentration.

Into a 10mL calibrated flask, 10mM concentration of ascorbic acid was prepared by weighing 17.6mg of ascorbic acid absolutely and dissolved in 2mL of distilled water. The volume was made up to the mark with the same solvent. It was used as a standard and by maintaining the concentration each time 10mL of polyhydroquinoline derivatives solution was prepared.

TABLE 1: ANTIOXIDANT ACTIVITY BY CHLORANIL FREE RADICAL SCAVENGING METHOD OF OXO-QUINOLINE DERIVATIVES

Compounds	Structure	IC_{50}	Activity	Compounds	Structure	IC_{50}	Activity
1		2.82	1.54	11		4.9	1.30
2		3.21	1.49	12		2.82	1.54
3		3.58	1.44	13		0.90	2.04
4		1.22	2.02	14		0.67	2.11
5		1.06	1.97	15		0.77	2.17
6		1.73	1.76	16		0.77	2.11
7		1.92	1.71	17		0.90	2.04
8		1.92	1.71	18		0.86	2.06
9		14.53	0.83	19		0.78	2.1
10		2.70	1.56				

In 10mL calibrated flasks, 4mL of the charge transfer complex (CTC) solution was placed and then 1mL of the sample solution was added. The absorbance of the solution was considered at the wavelength of maximum charge transfer bands *i.e.* at 412nm after the suitable time interval at room temperature against blank. Absorbance was recorded and percentage of radical scavenging activity was measured, the lower IC_{50} value represents higher antioxidant activity [29, 30] (**Table 1**).

$$\% \text{ RSA} = (Ai - Af) / Ai \times 100$$

RSA (radical scavenging activity) of poly-hydroquinoline derivatives, Ai initial absorbance of the CTC, Af is the absorbance of the test / standard compound.

Computational Methodology:

Construction of Molecular Structures: The compounds tested for inhibitory activity was selected for the present study and the program of window Hyperchem software Inc [31] was used in modelling studies. The molecules were generated and the energy was optimized using molecular modelling pro.

Calculation of Quantum Chemical Descriptors: The molecular structure of the compounds were initially optimized geometrically using the semi-empirical method AM1 (Austin Model 1) and PM3 (parameterization method 3) [32]. The quantum chemical descriptors (variables) [33-36] obtained for model building in this work consist of 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), electrophilic index (ω), hardness(η), softness(S), partition coefficient (LogP), hydration energy (HE), chemical potential (μ) and polarisability (Pol) were obtained for oxo-quinoline derivatives.

Regression Analysis of Antioxidant Derivatives: The windows version software SPSS10 (SPSS Software; Consult http://www.spss.com) [37] was used in the regression analysis study. A relation between biological activity (expressed as Log ($1/IC_{50}$) and the physicochemical parameters was analyzed statistically by fitting the data to correlation equations consisting of various combinations of these parameters. The statistical optimization was used to put forward the best correlation model.

The regression coefficient assumes a value between -1 and +1. If one variable tends to increase the other decreases, the correlation coefficient is negative. On the other hand, if the two variables tend to increase together the correlation coefficient is positive. We achieved the correlation matrix between inhibitory activities and respective calculated properties for nineteen ethyl 4 substituted-1, 4, 5, 6, 7, 8-hexa hydro-2, 7, 7-tri-methyl-5- oxoquinoline- 3- carboxylate derivatives. The more relevant regression models were selected from following criteria: The correlation coefficient (R), the Fisher ratio values (F) and the standard deviations (SD), percentage of effective variable (% EV), standard error estimate (SEE) and R^2 adjusted (R^2 adj).

The best equation was also tested for their analytical command using a cross-validation procedure. The cross-validation is a practical and dependable method for testing this significance. In principle, the so-called "leave-one-out" approach consists in developing a number of models with one sample omitted at the time.

After developing each model, the omitted data is predicted and the differences between actual and predicted potential values are calculated. The sum of squares of these differences is computed and to conclude the performance of the model (its predictive ability) is given by PRESS (Predictive Sum of Squares) and S_{PRESS} (Standard deviation of cross validation). The predictive ability of the model was also computed in terms of the Q^2_{cv} [38].

Molecular Docking Studies: Molecular docking methodologies eventually look 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 research group, an attempt to explore docking studies on quinoline derivatives was made to explain observed variance in biological activity. This expects the best candidate providing an insight on substitution and configuration for optimum receptor pit which leads to the perfection of best pharmacophore activity.

The 3D structure of selected protein Prenyl transferase with PDB ID-1ZDW was selected from PDB (Protein Data Bank) RCSB with an X-ray resolution in the range of 2.0 Å [39]. This protein contains an antioxidant naphterpin, a natural product that isoprene chains, which possesses anti-microbial, anti-oxidant, anti-inflammatory, anti-viral and anticancer activities. The X-ray crystallographic studies of this protein contain FLV (Flaviolin) and geranyl S-thiolodiphosphate (GST) which is external ligands present in the enzyme. The docking studies are carried out by creating a binding site at the FLV ligand site. The FLV and the Quinoline compounds have common structure, the naphthalene ring, in both of these compounds and the amino acids like tyrosine (288) serine (213, 214) is linked to the FLV (**Fig. 1**) ligand. Hence, FLV site in this enzyme can be used as a targeted site for our compounds.

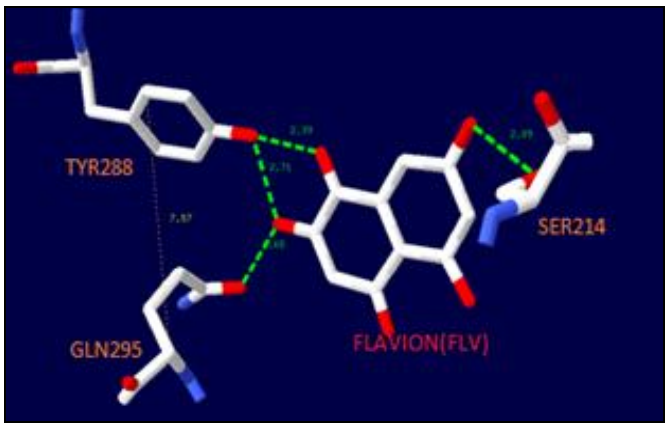


FIG. 1: FLAVIOLIN AND HYDROGEN BOND LINKAGE TO SERINE 214, TYROSINE 288 AND GLUTAMINE 295 IN PRENYL TRANSFERASE WITH PDB ID-1ZDW

GOLD 2.0: The GOLD2.0[40] (Genetic Optimization for Ligand Docking) program uses a genetic algorithm (GA) to explore the full range of rotational flexibility and the ligand flexibility of selected receptor hydrogen's. The mechanism for ligand position is based on fitting points. The program adds fitting points to hydrogen-bonding groups on the protein, 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 procedure-like scoring function. There are two different built in scoring functions in the GOLD program -Gold Score and Chemscore. In GOLD software the ligands in *.mol* format and the protein in *.Pdb* format are given as input.

The interaction of the ligands with the receptor in the modelled complexes was investigated and observed for the fitness function potential on protein of cyclo-oxygenase-2 by using synthesized moieties.

SPDBV3.7 software [41] was used for preparation of protein-ligand complexes by adding hydrogen atoms, removing water molecules, co-crystallization of inhibitors. The minimized protein was used for docking and all compounds were docked one after the other in GOLD. The atom numbers with respect to the amino acids as a receptor site are given in a box in gold parameter. The GOLD is run several times at all the atom numbers and in different scoring functions. All the parameters and settings used are in standard settings configured in GOLD 2.0. The compounds are evaluated in both scoring functions *i.e.* Fitness function and the Chemscore. All the gold fitness scores and the Chemscore are evaluated and tabulated.

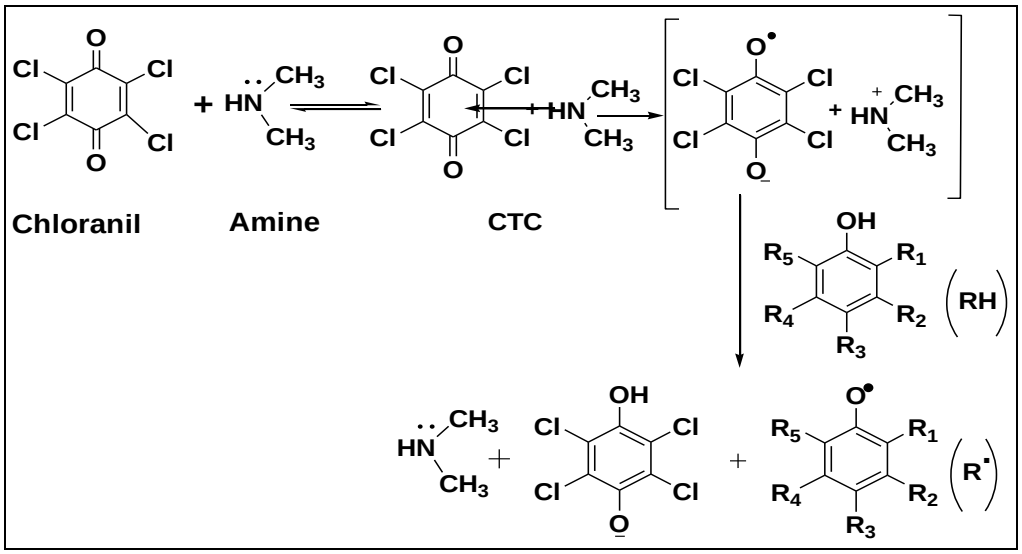
Autodock 4.0: Autodock4.0 [42] was used to calculate in the region of binding free energy and inhibition constant (Ki).

Argus Lab 4.0.1: Argus Lab 4.0.1 [43] was used for molecular modelling studies, which is very flexible and can reproduce crystallographic binding orientation. Argus lab gives a user friendly graphical interface and uses shape dock algorithm, to perform docking studies. This helps for the representation of the binding conformations of these derivatives, within the active site region of prenyl transferase protein.

RESULTS AND DISCUSSIONS:

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. The CTC is formed between dimethyl amine as *n*-donor (D) and chloranil as electron acceptor [44] (**Scheme 1**). 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. Therefore, the method is based on the reaction of *n*- donors with the electron acceptor. The Beer's law is obeyed over the concentration ranges.

The described method was successfully applied to the determination of antioxidant activity. To accommodate the observed results, the following reaction mechanism is proposed in **Scheme 1**.



SCHEME: 1

On studying the CTC maximum peak (**Fig. 2**) was exhibited at 412nm and the formation of CTC followed by chloranil radical. A simple method that has been developed to determine the antioxidant activity of oxo-quinoline compounds which utilize the stable chloranil radical. These compounds act as a radical scavenger by reducing chloranil free radical.

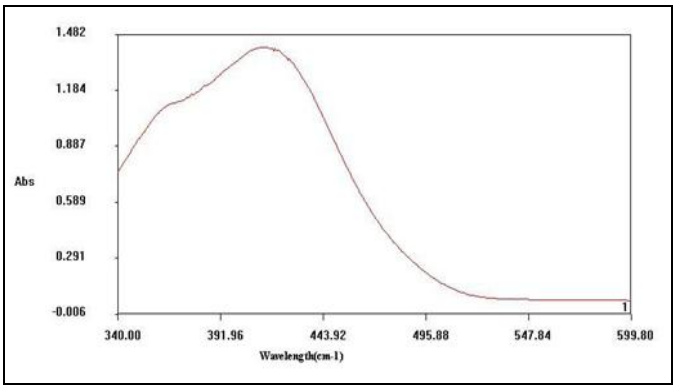


FIG. 2: ABSORPTION SPECTRUM OF CTC WITH CHLORANIL IN 1, 4-DIOXANE

The resulting decolourization with respect to number of electrons captured. The CTC decomposes to give chloranil free radical which in turn forms R· radical on abstraction of hydrogen from oxo-quinoline (RH). R· radical will then undergo further reactions which control the overall stoichiometry *i.e*. the number of molecules chloranil reduced by Rh. Mixing of chloranil solution to donor resulted in decrease in intensity of color *i.e.* shifted to shorter wavelength [29, 30].

Linear Regression Model: The biological activity data and the physicochemical properties IP, EA, EN, ω, η, S, LogP, HE and Pol of the quinolines derivatives are given in **Table 2** and **Table 3**. The data from these tables were subjected to regression analysis. The correlation matrices were generated with quinoline derivatives. 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 ETHYL 4-SUBSTITUTED-1, 4, 5, 6, 7, 8-HEXA HYDRO-2, 7, 7-TRIMETHYL-5-OXOQUINOLINE-3-CARBOXYLATE DERIVATIVES IN AM1 METHOD

		Eq-1		Eq-2		Molecular descriptors								
Comp.	Obs. Activity	Predicted	residual	Predicted	residual	IP (eV)	EA (eV)	EN (eV)	H (eV)	S (eV^{-1})	Ω	HE (K.cal/ mol)	LogP	Pol (Å)
1	1.55	1.17	0.38	1.2	0.35	8.98	-0.35	4.32	4.67	0.11	2	0.33	2.44	38.76
2	1.49	1.64	-0.15	1.6	-0.11	7.47	1.19	4.33	3.14	0.16	2.99	-0.31	1.68	35.09
3	1.45	1.73	-0.28	1.84	-0.39	7.45	-1.03	3.21	4.24	0.12	1.22	-1.11	1.71	42.24
4	2.03	1.73	0.3	1.73	0.3	7.97	1.14	4.56	3.42	0.15	3.04	-3.39	0.49	40.66
5	1.97	1.67	0.3	1.65	0.32	7.73	1.13	4.43	3.3	0.15	2.97	-8.68	1.13	39.02
6	1.76	1.97	-0.21	1.97	-0.21	7.57	1.37	4.47	3.1	0.16	3.22	-2.19	0.12	34.43

7	1.72	1.82	-0.1	1.79	-0.07	7.81	1.68	4.74	3.06	0.16	3.67	-8.21	0.46	38.82
8	1.72	1.72	0	1.74	-0.02	7.88	0.86	4.37	3.51	0.14	2.72	-7.44	0.67	40.03
9	0.84	1.66	-0.82	-	-	7.45	1.19	4.32	3.13	0.16	2.98	-1.28	1.63	39.4
10	1.57	1.56	0.01	1.54	0.03	7.48	0.95	4.21	3.27	0.15	2.72	-1.43	1.93	40.31
11	1.31	1.95	-0.64	-	-	7.34	1.04	4.19	3.15	0.16	2.79	-3.12	0.5	43.13
12	1.55	1.67	-0.12	1.64	-0.09	7.42	1.27	4.34	3.07	0.16	3.07	-0.8	1.64	40.02
13	2.05	2.22	-0.17	2.23	-0.18	6.77	1.27	4.02	2.75	0.18	2.94	-0.7	0.53	43.21
14	2.11	2.17	-0.06	2.21	-0.1	7.48	1.14	4.31	3.17	0.16	2.93	-9.91	-0.64	41.3
15	2.17	1.61	0.56	-	-	7.85	1.78	4.81	3.03	0.16	3.82	-3.54	1.34	40.32
16	2.11	1.7	0.41	-	-	7.45	1.28	4.36	3.09	0.16	3.08	-1.68	1.48	38.19
17	2.05	1.7	0.35	-	-	7.48	1.3	4.39	3.09	0.16	3.12	-0.28	1.41	41.95
18	2.07	1.66	0.41	2.32	0.25	13.27	-0.7	6.28	6.98	0.07	2.83	-4.3	-1.5	45.6
19	2.11	1.94	0.17	1.84	0.17	7.41	1.28	4.34	3.07	0.16	3.07	-3.13	0.49	40.66

TABLE 3: ANTIOXIDANT ACTIVITIES AND MOLECULAR DESCRIPTOR VALUES OF ETHYL 4-SUBSTITUTED - 1, 4, 5, 6, 7, 8-HEXA HYDRO-2, 7, 7-TRIMETHYL-5-OXOQUINOLINE-3-CARBOXYLATE DERIVATIVES IN PM3 METHOD

		Eq-3		Eq-4		Molecular descriptors								
Comp.	**Obs. Activity**	**Predicted**	**residual**	**Predicted**	**residual**	**IP (eV)**	**EA (eV)**	**EN (eV)**	**H (eV)**	**S (eV^{-1})**	**ω**	**HE(K.cal/mol)**	**LogP**	**Pol (A^0)**
1	1.55	1.89	-0.34	1.84	-0.29	6.29	1.28	3.79	2.51	0.2	2.86	-0.77	2.44	38.76
2	1.49	1.51	-0.02	1.47	0.02	7.58	1.21	4.39	3.19	0.16	3.03	-0.53	1.68	35.09
3	1.45	1.55	-0.1	1.5	-0.05	7.84	2.09	4.97	2.88	0.17	4.29	-2.24	1.71	42.24
4	2.03	1.91	0.12	1.89	0.14	7.44	1.17	4.3	3.14	0.16	2.95	-3.66	0.49	40.66
5	1.97	1.68	0.29	1.65	0.32	7.48	0.97	4.23	3.26	0.15	2.75	-8.81	1.13	39.02
6	1.76	1.97	-0.21	1.96	-0.2	7.59	1.26	4.43	3.16	0.16	3.1	-2.29	0.12	34.43
7	1.72	1.84	-0.12	1.82	-0.1	7.81	1.6	4.71	3.1	0.16	3.57	-7.81	0.46	38.82
8	1.72	1.93	-0.21	1.9	-0.18	7.45	1.61	4.53	2.92	0.17	3.51	-7.8	0.67	40.03
9	0.84	1.58	-0.74	-	-	7.43	1.2	4.31	3.11	0.16	2.99	-1.29	1.63	39.4
10	1.57	1.43	0.14	1.39	0.18	7.54	1.04	4.29	3.25	0.15	2.83	-1.7	1.93	40.31
11	1.31	1.73	-0.42	-	-	7.74	0.15	3.94	3.79	0.13	2.05	-3.12	0.5	43.13
12	1.55	1.57	-0.02	1.53	0.02	7.43	1.13	4.28	3.15	0.16	2.91	-0.79	1.64	40.02
13	2.05	2.03	0.02	2.01	0.04	7.09	1.15	4.12	2.97	0.17	2.86	-0.97	0.53	43.21
14	2.11	2.06	0.05	2.06	0.05	7.98	1.13	4.55	3.42	0.15	3.03	-8.48	-0.64	41.3
15	2.17	1.66	0.51	-	-	7.41	1.12	4.26	3.15	0.16	2.89	-3.42	1.34	40.32
16	2.11	1.61	0.5	-	-	7.46	1.16	4.31	3.15	0.16	2.95	-1.9	1.48	38.19
17	2.05	1.62	0.43	-	-	7.48	1.17	4.33	3.16	0.16	2.97	-0.54	1.41	41.95
18	2.07	2.04	0.03	2.13	-0.06	12.04	-2.74	4.65	7.39	0.07	1.46	-4.9	-1.5	45.6
19	2.11	1.91	0.2	1.89	0.22	7.43	1.16	4.29	3.14	0.16	2.94	-3.28	0.49	40.66

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

Activity = -0.231*****LogP (0.100) - 0.325 *****EA (0.149) + 15.158*****S (1.394) ---------- (1)

R= 0.980; R^2 = 0.961; R^2_{adj} = 0.954; SEE = 0.3871; F= 132.096; PRESS = 0.1156; S.D = 1015.06; Q^2 = 0.9998; Q = 2.531.

In addition, the plot of observed activity *vs* predicted activity was not found to be acceptable. Hence, the predictive potential value of the model is not good. Eq. 1 shows that the values of % EV are less and to improve its value, outlier (9, 11, 15, 16 and 17) were sought and eliminated. After the elimination of the outlier (9, 11, 15, 16 and 17), a second model was build. Overall, there is an increase in R (0.980-0.993) and % EV values, and a decrease in SEE (0.325- 0.247).

Activity= -0.264*****LogP (0.068)-0.406*****EA (0.101) + 15.898*****S (0.915) ---------- (2)

R= 0.993; R^2= 0.985; R^2_{adj} = 0.981; SEE= 0.2470; F=247.69; PRESS=0.0625; S.D=545.3893; Q^2= 0.9999; Q = 4.020.

Eq. 2 is an improved model since it gives explanation of the biological activity to the extent

of (98.5%). From the correlation matrix table, it reveals LogP, EA and S are found to be explainable variables. In both AM1 and PM3 a tri-parametric LogP, EA and S QSAR equations are generated.

Activity = -0.295*LogP (0.108) - 0.226 *EA (0.111) + 14.531*S (0.897) ---------- (3)

R= 0.985; R^2 = 0.971; R^2_{adj} = 0.965; SEE = 0.3371; F= 175.96; PRESS = 0.0121; S. D. = 1015.06; Q^2=0.9999; Q = 2.921.

Eq. 3 showed that the values of % EV are less and to improve its value, outlier (9, 11, 15, 16 and 17) were sought and reduced. In addition, the plot of observed activity versus predicted activity was not found to be satisfactory. Hence, the projected ability of the model is not good. After the elimination of the outlier (9, 11, 15, 16 and 17), a second model was developed.

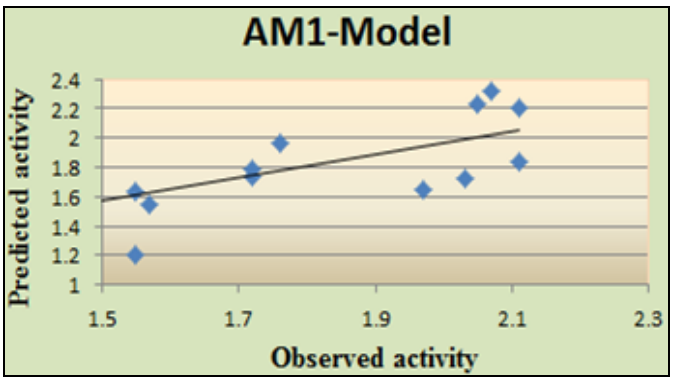
Activity = -0.311*LogP (0.063) - 0.245 *EA (0.063) + 14.619*S (0.521) ---------- (4)

R = 0.996; R^2 = 0.992; R^2_{adj} = 0.990; SEE= 0.1848; F= 445.26; PRESS = 0.0121; S.D = 545.3893; Q^2 = 0.9999; Q = 5.389.

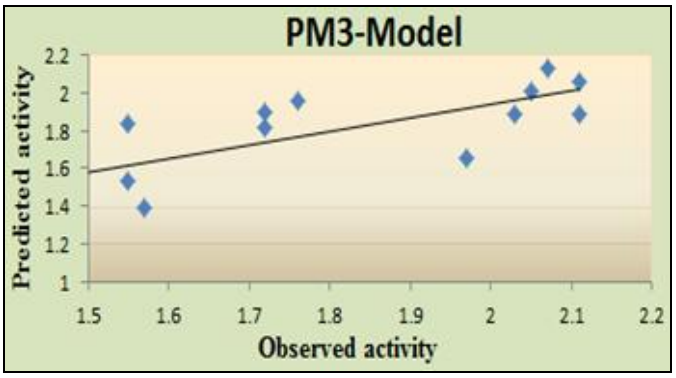
In an effort to investigate the predictive potential of proposed models, the cross-validation parameters (q^2_{cv} and PRESS) were calculated and used. The predictive power of the equations was confirmed by leave-one-out (LOO) cross-validation method (**Table 2** and **3**).

Eq. 3 and 4 of AM1 and PM3 method respectively give a very good q^2_{cv} value, which should be always smaller than % EV. A model is considered to be significant when q^2_{cv} = (> 0.78). Another cross-validation parameter, PRESS which is the sum of the squared differences between the actual and that of predicted when the compound is omitted from the fitting process, also maintains the predictive ability of Eqs. 2 and 4. Its value decreases from Eq. 1 to Eq. 3. The quality factor Q, is defined as the ratio of regression constants (R) to the standard error estimation (SEE), that is, Q = R/SEE. This values point out that the higher the value of R, 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 2.531 to 4.020 and 2.921 to 5.389 (Eq. 1 to 4).

In the final AM1 and PM3 modelled Eq- 2 and Eq-4 respectively, the contribution of the physico-chemical parameters shown graphically in contribution charts (**Graph 1** and **Graph 2**). The activity decrease with increase LogP and EA. Lipophilicity means the attraction of the compound to partition between organic phase (immiscible with water) and polar aqueous phase. The value of lipophilicity most commonly refers to logarithm of partition coefficient P (LogP) between these two phases. Up to specific limit, the compounds with higher lipophilicity have higher permeation across biological membranes (but lower aqueous solubility). Relationship between LogP and permeation is however non-linear, permeation decreasing in both low and high end of LogP values. The electron affinity is characterized by the susceptibility of the compound in relation to attacks by nucleophiles.



GRAPH 1: PLOT OF OBSERVED *vs* PREDICTED ACTIVITY



GRAPH 2: PLOT OF OBSERVED *vs* PREDICTED ACTIVITY

The electron affinity of an atom or molecule is defined as the amount of energy released when an electron is added to a neutral atom or molecule to form a negative ion. As softness of ligand increases the activity also increases. Soft acids and bases can be explained on the HSAB principal. Softness of

chemical species linked with large atomic/ionic radius, high polarisability, low or zero oxidation state, low electro negativity. Soft bases have HOMO of higher energy than hard bases, and soft acids have LUMO of lower power than hard acids. The soft molecules are more reactive than hard molecules if rearrangement or electron transfer is necessary for the reaction. The softness is important in understanding the chemistry of large, delocalized molecules or ions.

Docking Analysis: The successful docking has been performed for all newly synthesized target compounds using genetic optimization for ligand docking (GOLD) algorithm version 2.0. The GOLD program uses a genetic algorithm (GA) to explore the full range of the rotational flexibility of selected receptor hydrogens and ligand flexibility. The 3D crystallographic structure of 1ZDW protein was used template selected from RCSB protein data bank (PDB). Swiss PDB-viewer, GOLD 2.0, Autodock and Argus lab are molecular modelling and docking softwares, which help in virtual screening for finding a lead compound. All the compounds were designed in Hyper Chem and the protein from the protein data bank. Docking procedure is carried out with all the standard parameters. As a conclusive part of docking, we expect that the generated results should yield RMSD values below1.5Å. The successful docking has been performed for the selected oxo-quinoline derivatives of protein prenyl transferase inhibitors and their corresponding fitness, Chemscore, Autodock and Argus energy values with the respective RMSD (**Table 4 - 6**). Discovery studio visualizer has been utilized to visualize the binding conformations of these analogues in the active site of 1ZDW protein and good binding orientation poses were shown in **Fig. 3**.

TABLE 4: DOCKING VALUES OBTAINED FROM GOLD FITNESS SCORE WITH PRENYL TRANSFERASE (PDBID = 1ZDW)

Compound	Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(vdw_int)
1	51.61	6.26	36.20	0.00	-4.42
2	42.63	0.31	34.24	0.00	-4.77
3	50.68	3.54	37.86	0.00	-4.91
4	57.77	7.17	39.41	0.00	-3.58
5	40.18	6.11	37.52	0.00	-17.52
6	47.30	1.77	35.06	0.00	-2.67
7	52.20	9.62	36.00	0.00	-6.92
8	44.23	9.97	32.76	0.00	-10.78
9	45.45	6.02	35.99	0.00	-10.06
10	37.30	6.00	34.18	0.00	-15.70
11	40.37	0.91	39.66	0.00	-15.06
12	51.59	8.38	34.34	0.00	-4.00
13	55.72	6.04	40.52	0.00	-6.03
14	59.49	12.88	38.46	0.00	-6.26
15	47.63	8.58	33.07	0.00	-6.43
16	42.75	9.99	31.49	0.00	-10.54
17	53.33	2.54	40.16	0.00	-4.42
18	59.70	6.25	46.09	0.00	-9.93
19	50.79	1.31	38.56	0.00	-3.54

TABLE 5: DOCKING VALUES OBTAINED FROM GOLD IN CHEMSCORE FUNCTION WITH PRENYLTRANSFERASE (PDBID = 1ZDW)

Compound	Score	DG	S(hbond)	S(metal)	S(lipo)	DE(clash)	DE(int)
1	36.53	-39.56	2.97	0.00	232.59	0.23	2.80
2	31.69	-35.23	2.47	0.00	210.27	0.29	3.24
3	32.85	-37.85	0.98	0.00	274.98	1.58	3.42
4	39.12	-43.31	3.08	0.00	265.92	2.40	1.79
5	31.89	-36.58	1.68	0.00	256.00	3.14	1.55
6	32.75	-34.26	2.19	0.00	211.49	0.51	0.99
7	32.89	-36.77	1.85	0.00	253.00	2.99	0.89
8	35.02	-38.94	2.15	0.00	251.80	2.36	1.56
9	33.38	-39.16	2.68	0.00	241.36	1.52	4.26
10	33.08	-40.22	1.96	0.00	267.27	5.22	1.92

11	35.65	-44.31	2.73	0.00	288.50	5.87	2.79
12	35.79	-39.26	1.73	0.00	265.47	1.77	1.70
13	35.42	-40.82	1.67	0.00	283.59	3.67	1.73
14	34.64	-40.21	2.80	0.00	261.41	3.09	2.47
15	31.33	-34.94	0.84	0.00	254.89	2.23	1.38
16	35.64	-38.45	2.22	0.00	244.90	1.15	1.66
17	35.44	-42.79	2.15	0.00	283.83	4.71	2.64
18	32.63	-40.48	1.69	0.00	289.81	4.09	3.75
19	36.30	-39.48	2.43	0.00	251.62	1.77	1.41

TABLE 6: ARGUS BINDING ENERGY AND AUTODOCK ENERGY (IN k.cal/mol) OBTAINED FROM DOCKING RESULTS OF PROTEIN PRENYL TRANSFERASE (PDBID =1ZDW)

Compound	Argus B.E K.cal/mol	Autodock B.E K.cal/mol
1	-11.46	-8.94
2	-10.44	-8.93
3	-13.96	-8.54
4	-9.50	-8.96
5	-10.08	-8.51
6	-12.35	-8.37
7	-12.25	-9.53
8	-9.23	-8.34
9	-8.66	-8.74
10	-11.87	-8.85
11	-9.68	-8.25
12	-12.61	-8.97
13	-11.11	-8.93
14	-9.76	-8.28
15	-10.57	-8.47
16	-11.75	-8.54
17	-12.75	-9.31
18	-8.56	-7.84
19	-11.34	-7.85

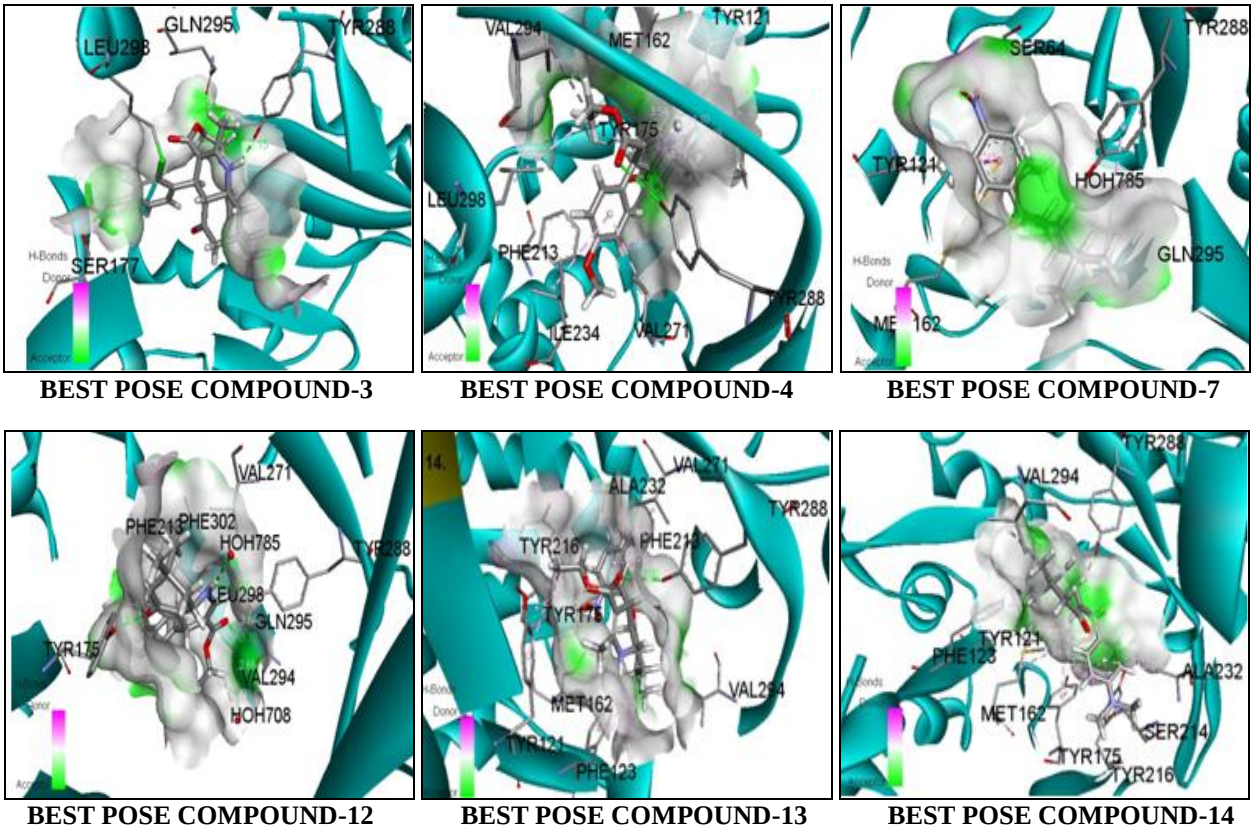
The binding energies obtained in Argus lab ranged from 8.56-13.96 kcal/mol and the Chemscore values are ranged from 31.33 to 39.12, to the antioxidant activity of quinolone aryl compounds, and fitness values range from 37.30 to 59.70 respectively. The docking simulation of the most active quinoline derivatives showed that enzyme inhibits or complex was mostly stabilized by hydrophobic interactions occurring between the aromatic moieties of the ligand and lipophilic residues of the binding site. The protein prenyl-transferase has interaction through serine 214, tyrosine 288, glutamine 295 amino acids with the quinoline derivatives show hydrogen bonding interactions and correlation with docking binding energy values. The protein ligand best interactions were found with amino acid serine 214 at atom number 2025. The GOLD fitness and chemscore values showing very good scores are the best fitting values. The compounds 1, 3, 4, 7, 12, 13, 14 and 18 showed a good fitness and chemscore values (**Table 4 - 6**).

Among the compound- 4, 13, 14 and 18 are having the highest fitness values due to the interactions of protein ligand complex at the binding site obtained from parameters like external protein ligand hydrogen bond energy, external Vander Waals energy *etc*. Especially, compound 14 is having the highest fitness value 59.49 with a good, S(hb_ext) =12.88, S(vdw_ext) = 38.46. It was supported with the experimental biological data also. From the Chemscore compounds 1, 4, 8, 12, 13 and 14 are having good docking scores. However the compound-1, 3, 4, 7, 12, 13, 14 and 18 are having good scores when chemscore and fitness scores were compared.

As these compounds are having good docking scores, they are expected to show good inhibiting activity. The substituents like cyclohexane, 4-methoxyphenyl, tolylquinoline, dimethyl amino phenyl, 4-methyl-3-chlorophenyl and 3-methoxy-phenyl are responsible at 4^{th} position on aryl group for high binding scores (**Table 4 - 6**).

From all the docking scores obtained from GOLD 2.0, Autodock 4.0 and Argus lab 4.0.1, the compounds 1, 3, 4, 7,12,13,14 and 18 showed good protein ligand interactions. In this investigation, we discovered that the oxoquinolines which showed good Chem score and Gold score functions also exhibited good binding energy values. Finally, molecular docking studies showed good correlation between the *in vitro* anti-oxidant activity of final compounds and their binding interactions with 1ZDW as well as their Gold fitness scores.

CONCLUSION: The antioxidant activity of oxo-quinoline derivatives was determined by using CTC of chloranil. In our present study, it is established that the predictive QSAR models are quite reliable to efficiently guide further modification in the molecules for obtaining better antioxidants. AM1 and PM3 are the semi empirical methods employed in QSAR studies. Both of them provided good statistical results in terms of R^2, R^2_{adj} and standard error of estimate (SEE), suggesting the significant correlations of molecular structures with its biological activities. Both methods reveal LogP, EA and S are indicative variables for antioxidant nature of oxo-quinolines. For high efficacy of these compounds low LogP and EA and high S are desirable. The molecular docking studies were performed on nineteen oxo-quinoline compounds for the interpretation of their biological activity (**Tables 2** and **3**). Introducing groups like cyclohexane, 4-methoxyphenyl, tolylquinoline, dimethyl amino phenyl, 4-methyl-3-chlorophenyl and 3-methoxyphenyl (**Table 1** and **Fig. 3**) increase the antioxidant activity.



BEST POSE COMPOUND-3 **BEST POSE COMPOUND-4** **BEST POSE COMPOUND-7**

BEST POSE COMPOUND-12 **BEST POSE COMPOUND-13** **BEST POSE COMPOUND-14**

FIG. 3: BINDING ORIENTATIONS OF DATABASE BALL CYLINDER LOW MODEL COMPOUNDS (3, 4, 7, 12, 13, AND 14) WITH CRYSTALLOGRAPHIC CONFORMATION OF ACTIVE SITE (PDB ID: 1ZDW). HYDROGEN BONDS ARE SHOWN IN DOTTED LINES

These Oxo-quinoline molecules interact mainly *via* electrostatic forces, including hydrogen bonds or hydrogen bonding networks, often formed through water molecules. The best protein ligand interactive poses of molecule 1, 3, 4, 7, 12, 13, 14 and 18 (**Fig. 3**) show good hydrogen bonding energies. The protein prenyl-transferase has interaction through serine 214, tyrosine 288 and glutamine 295 amino acids with the quinoline derivatives show hydrogen bonding interactions and correlation with

docking binding energy values. The inhibitory activity of the most potent compounds is explained by hydrophobic interactions in docking studies. So, the compound - 1, 3, 4, 7, 12, 13, 14 and 18 were found to possess a promising antioxidant activity and significant inhibitory activity on prenyl transferase. Therefore one can conclude that electronic effects have a very important role for antioxidant activity and this chemical environment may serve as a starting point for synthesis of cyclo-oxygenase-2 inhibitors with improved efficacy. It also gives insight into the pharmacophore and residues of prenyl transferase active site.

ACKNOWLEDGEMENT: One of the author's RM (Ramesh Macha) is thankful to the Head, Department of Chemistry, University College of Science, Osmania University, for providing the laboratory facilities for the research work.

CONFLICT OF INTEREST: None Declared.

REFERENCES:

1. Pacher P, Beckman JS and Liaudet L: Nitric oxide and peroxynitrite in health and disease. Physiological reviews 2007; 87(1): 315-424. (b) Marjan K and Mehdi H: The Role of Nitric Oxide in Health and Diseases. Scimetr 2015; 3(1): e20987.
2. Karal N, Güzel Ö, Özsoy N, Özbey S and Salman A: Synthesis of new spiroindolinones incorporating a benzothiazole moiety as antioxidant agents. Europian. Journal of Medicinal. Chemistry. 2010; 45(3): 1068-1077.
3. Flora SJ: Role of free radicals and antioxidants in health and disease. Cell Mol Biol. 2007; 53(1): 1-2.
4. Chowdhury KAA, Hosen SMZ, Mohammad NI, Huq I, Md. A, Md. NUC, Md. Imtiazul K, Reedwan BJA, Md. RU, Mohammed S and Mohammad AG: Cytotoxic and thrombolytic activity of roots of *Musa paradisiaca* (Linn). The Pharma Innovation Journal 2016; 5(8): 97-100.
5. Kumpulainen JT and Salonen JT: (Natural Antioxidants and Anti carcinogens 1999) in Nutrition, Health and Disease, The Royal Society of Chemistry, UK 1999; 178-187.
6. Cook NC and Samman S: Flavonoids- chemistry, metabolism, cardio protective effects, and dietary sources. Nutritional Biochemistry 1996; 7: 66- 76.
7. Valko M, Izakovic M, Mazur M, Rhodes C.J and Telser J: Role of oxygen radicals in DNA damage and cancerincidence. Mol Cell Biochem 2004; 266: 37-56.
8. Aruoma OI: Free Radicals, Oxidative Stress and Antioxidants in Human Health and Disease. Journal of American Oil Chemists Society 1998; 75: 199-212.
9. Lee JN, Koo and Min DB: Reactive Oxygen Species, Aging, and Antioxidative Nutraceuticals. Comprehensive Reviews in Food Science and Food Safety 2004; 21-33.
10. Atoui AK, Mansouri A, Boskou G and Kefalas P: Tea and herbal infusions: their antioxidant activity and phenolic profile. Food Chemistry 2005; 89: 27-36.
11. Halliwell B and Gutteridge JMC: Free radicals in biology and medicine (2nd ed.). Oxford: Clarendon press 1989.
12. Kuhnan J: The flavonoids. A class of semi-essential food components; their role in human nutrition. World Review of Nutrition and Dietetics 1976; 24: 117- 191.
13. Kumpulainen JT and Salonen JT: Natural Antioxidants and Anti carcinogens in Nutrition, Health and Disease. The Royal Society of Chemistry UK 1999; 178- 187.
14. Younes M: Inhibitory action of some flavonoids on enhanced spontaneous lipidperoxidation following glutathione depletion. Planta Medica 1981; 43: 240- 245.
15. Halliwell B and Gutteridge JMC: Free radicals in biology and medicine (2nd ed.). Oxford: Clarendon press 1989.
16. Nordell P and Lincoln PJ: Mechanism of DNA threading intercalation of binuclear Ru complexes: uni- or bimolecular pathways depending on ligand structure and binding density. Am. Chem. Soc. 2005; 127(27): 9670-9671.
17. Barraja P, Diana P, Montalbano A, Dattolo G, Cirrincione G, Viola G, Vedaldi D and Dall'Acqua F: Pyrrolo[2, 3-h]quinolinones: a new ring system with potent photo-antiproliferative activity. Bioorg Med Chem 2006; 14: 8712–28.
18. Chung HS and Woo WSJ: A quinolone alkaloid with antioxidant activity from the aleurone layer of anthocyanin-pigmented rice. Nat. Prod. 2001; 64(12): 1579.
19. Sedic M, Poznic M, Gehrig P and Scott M: Differential antiproliferative mechanisms of novel derivative of benzimidazo[1,2-alpha]quinoline in colon cancer cells depending on their p53 status. Mol. Cancer Ther. 2008; 7(7): 2121-32.
20. El-Sayed OA, Al-Turki TM, Al-Daffiri HM, Al-Bassam BA, Hussein ME, Boll Gakhar G, Ohira T, Shi A, Hua DH and Nguyen TA: Drug Dev. Res. 2009; 69: 526.
21. AI-khalil SA, Atkofahi D, EI-Eisawi and AI-shibib A: Transtorine, a new Quinoline alkaloid from *Ephedra fransitoria*. J. Nat. Prod. Feb. 1998; 61(2): 262-283.
22. Bergh JCA, Lazovios G, Somogyi L, Lengyel and Feher J: The first clinical pilotot Roquinimax (Linomide) cancer patient swîthspesial focus onimmunological effects. Canoer Invest 2006; 15(3): 204-211.
23. Kawase M, Shah A, Gaveriya H, Motohashi N, Sakagami H and Molnar AV: 3, 5-dibenzoyl-1, 4-dihydropyridines: synthesis and MDR reversal in tumor cells. J. Bioorg .Med. Chem 2002; 10(4): 1051-1055. b) Mannhold R, Jablonka B, Voigdt W, Schoenafinger K and Schravan K: Eur. J. Med. Chem 2002; 27: 229.
24. Heravi MM, Behbahani FK, Oskooie HA and Shoar RH: Tetrahedron Lett. 2005; 46: 2775.
25. Borovic S, Tirzitis G, Tirzite D, Cipak A, Khoschsorur GA, Waeg G, Tatzber F, Scukanec-Spoljar M and Zarkovic N: Bioactive 1, 4-dihydroisonicotinic acid derivatives prevent oxidative damage of liver cells. Eur. J. Pharmacol. 2006; 53: 12.
26. Visentin S, Rolando B, Stilo DA, Fruttero R, Novara M, Carbone E, Roussel C, Vanthuyne N and Gasco A: J. Chem 2004; 475: 2688.
27. Toniolo R, Narda DF, Bontempelli G and Ursini F: Bioelectro Chem 2000; 51: 193.
28. Xiao-Hui Y, Ping-Hu Z, Yong-Hong Z, Cheng-Guo L, Xiao-Yu L and Jing-Fang C: Synthesis and antioxidant evaluation of novel 4-arylhexahydroquinolines from lignin. ARKIVOC 2011; 335-345.
29. Braca A, Sortino C and Politi M *et al*.: Antioxidant activity of flavonoids from licanialicniaeflora. J Ethnopharmacol 2002; 79: 379-381.
30. Ramesh M, Ravindrachary K and Parthasarathy T: Novel *in vitro* Antioxidant Bioassay, QSAR and Docking Studies

of β-Aminoketone analogues. RJPBCS 2017; 8(3): 2458-2473.
31. Hypercube Inc. HyperChem 8.0 package. Gainesville, USA. http://www.hyper.com 2007.
32. Mauro R, Benedito L, Jeronimo L, Alberdanm S, Santos, Cla´udio N and Alves: A theoretical study of phenolic compounds with antioxidant properties. European Journal of Medicinal Chemistry 2007; 42: 440.
33. Parr RG, Szentpaly LV and Liu S: Electrophilicity index. American Chemical Society 1999; 121: 1922-1924.
34. Parr RG, and Pearson RG: Absolute hardness: companion parameter to absolute electronegativity. American Chemical Society 1983; 105(26): 7512–7516.
35. Parr RG, Donnelly RA, Levy M and Palke WE: Electro-negativity: the density functional view point. The Journal of Chemical Physics 1977; 68(8): 3801–3807.
36. Shanthi V, Ramesh M, Srimai V, Srinivas P and Parthasarathy T: QSAR, Docking and *in vitro* antioxidant study of novelchromone derivatives. Modern Chemistry 2013; 1(1): 8-17.
37. SPSS Software. Consult http://www.spss.com. (a) Abul KLM, Salma U and A: Chandra Bhowmick: Cytotoxicity and 2D-QSAR study of some heterocyclic compounds" Arabian Journal of Chemistry. 2014; 7(5): 639–646. (b) Ramesh M, Ganesh Arram and Parthasarathy T: Novel Antioxidant Bioassay, QSAR and Docking studies of 1-Phenyl, 3-(Nitro Furan) prop-2-ene-1-one derivatives. World Journal of Pharmacy and Pharmaceutical Sciences. 2014; 3(9): 442-459.
38. Macha R, Ravindrachary K, Himabindu M, Shyam P, Rajesh R, Jayasree GPL, Tigulla P: Triazole analogues as novel antioxidants: *In vitro* bioassay and molecular modeling studies. Journal of Pharmacy Research 2016; 10(3): 136-145.
39. Tomohisa K, Joseph PN and Stéphane BR: Structural basis for the promiscuous biosynthetic prenylation of aromatic natural products" Nature 2005; 435: 983-987.
40. (a) Jones G, Willett P and Glen RC: Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. J Mol Biol. 1995; 245: 43. (b) Jones G, Willett P, Glen RC, Leach AR and Taylor R: Development and validation of a genetic algorithm for flexible docking, J Mol Biol. 1997; 267: 727-748. (c) Nissink JWM, Murray C, Hartshorn M, Verdonk ML, Cole JC and Taylor RA: New test set for validating predictions of protein-ligand interaction. Proteins: Structure Function Bioinformatics 2002; 49(1): 457-471.
41. Verdonk ML, Cole JC, Hartshorn MJ, Murray CW and Taylor RD: Improved protein-ligand docking using GOLD. Proteins: Structure Function Bioinformatics. 2003; 52(4): 609-623.
42. Trott O and Olson AJ: Auto dock vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comp Chem 2010; 31: 455-461.
43. Thompson MA: Molecular docking using ArgusLab, an efficient shape-based search algorithm and the A Score scoring function. ACS meeting, Philadelphia 172 CINF 42 P, 2004.
44. Hesham and Salem: Spectrophotometric determination of β-adrenergic blocking agents in pharmaceutical formulations, Journal of Pharmaceutical and Biomedical Analysis 2002; 29(3): 527-538.

How to cite this article:
Macha R, Ravindrachary K, Jayasree GPL and Tigulla P: Spectrophotometric antioxidant bioassay and molecular modelling studies of ethyl 4-substituted-1, 4, 5, 6, 7, 8-hexahydro-2, 7, 7-trimethyl-5-oxoquinoline-3-carboxylate derivatives. Int J Pharm Sci Res 2018; 9(1): 67-79. doi: 10.13040/IJPSR.0975-8232.9(1).67-79.