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Experimental and Computational Study of studies of Thienopyridine Derivatives

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Abstract: Thienopyridine derivatives provide an antioxidant protective effect that may contribute to their pharmacological activities. The antioxidant properties of thienopyridine are known to be influenced to a greater extent by the aryl structures of the substitutions on aryl rings. Especially, the free radical donor substituents were one of the key groups to enhance greatly the antioxidant activity of thienopyridine mainly due to its easy conversion to phenoxy radicals through the hydrogen atom transfer mechanism .

Keywords: Thienopyridine Derivatives, Antioxidant Bioassay, Computational study

1.1 Introduction

Thienopyridine (4, 5, 6, 7-tetrahydrothieno [3, 2-c] pyridines) and their derivatives are important heterocyclic compounds (figure1) that are widely distributed in nature. Many of these compounds contain thienopyridine skeletons which were reported to have antibacterial[**1]**, non-peptide GPIIb/IIIa antagonists[**2]**, platelet aggregators and antithrombotic agents[**3]**.The incorporation of benzylic or substituted benzylic groups on the nitrogen of the thienopyridine ring can bring an extensive modification in the biological activities of parent compound. Among the substitutions occurred at nitrogen of the thienopyridine moiety, increased the biological activity of the parent moiety, showed good antithrombotic activity in Ticlopidine and with more increased activity in Clopidogrel. Later on, the studies proved that the Prasugrel to be more efficient drug candidate than the existing Clopidogrel by making the structural modifications to the parent thienopyridine moiety **[4]**. Hence,

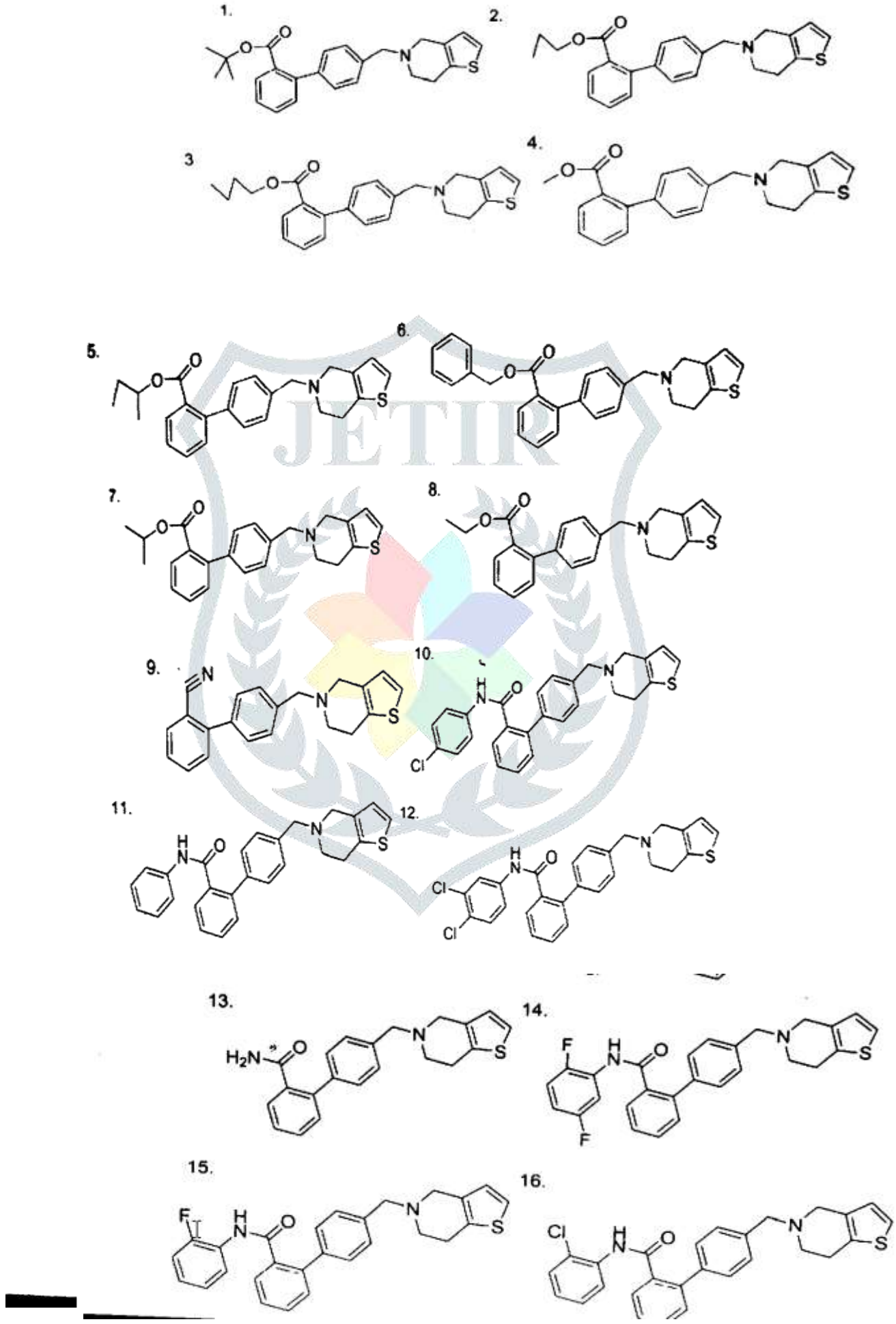
different substitutions at nitrogen of the thienopyridine moiety may bring extensive improvement in the biological activity of the new chemical entities (NCEs). Biphenyl system is the key structural unit in most of the sartans, non-peptide antagonists of angiotensin II receptors. Along with their well-known antihypertensive activity **[5, 6],** biphenyl substituent's have also been demonstrated as stimulators of growth hormone release [**7]** metallo protease inhibitors **[8,9]** antibiotics [**10]** and chloride channel blockers [**11]**. In this context, biphenyls and its derivatives had received substantial attention as good therapeutics. Thienopyridine derivatives also provide an antioxidant protective effect that may contribute to their pharmacological activities **[12]**. The antioxidant properties of thienopyridine are known to be influenced to a greater extent by the aryl structures of the substitutions on aryl rings. Especially, the free radical donor substituents were one of the key groups to enhance greatly the antioxidant activity of thienopyridine mainly due to its easy conversion to phenoxy radicals through the hydrogen atom transfer mechanism **[13]**.

The intermolecular charge transfer complex (CTC) is formed between electron donor and electron acceptor. It is a general phenomenon in organic chemistry and Mullikan considered bond between the components of the complex being postulated to arise from the base to the empty orbital of the acceptor. The CTC have unique absorption bands in ultra violet-visible region. 2, 3-Dichloro-5, 6-dicyano 1,4- Benzoquinone (DDQ) is a ***π***-electron acceptor often it forms highly colored electron donor-acceptor or CTC with the triethyl amine. The molecular interactions between electron donors and acceptors are generally associated with the formation of intensity colored CTC. The photometric methods based on molecular interactions are simple and suitable since they result in the rapid formation of the complexes. Triethyl amine is a good ***n***-electron donor and forms CTC with π-acceptor. DDQ forms charge transfer complex and radical anions with a variety of electron donors **[14,15]**. Thienopyridine derivatives may act as free radical scavengers due to their structural features.

Several methods are used for the estimation of antioxidant activity of synthetic or natural source [**16-19]**. In order to develop simple and sensitive spectrophotometric method charge transfer concept is used. A CTC is formed between DDQ and ***n***-donor which was used to investigate their antioxidant activity.

The aim of the present work is to develop *in vitro* antioxidant property of thienopyridines by spectrometric method. The *in vitro* free radical scavenging activities of thienopyridine derivatives may be quantitatively estimated. Molecular modeling studies further help in understanding the various interactions between the ligands and enzyme active sites, physicochemical parameters and type of substituents are responsible for high antioxidant efficacy of thienopyridine derivatives.

Figure 1 Structures of thienopyridine derivatives



1.2. Experimental Methods

Antioxidant Bioassay

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

Antioxidant activity of thienopyridines was measured by using spectrophotometer. This method is based on the charge transfer complex (CTC) which is formed between triethyl amine and DDQ. To the 10ml of 3×10^{-4}M CT complex, 10ml of 10^{-4}M substituted thienopyridine was added. The mixture was allowed to stand 5 min at room temperature and then the absorbance of colored solution was measured at 440*nm.*The capacity of freeradical scavenging activity of thienopyridine was calculated using the following equation:

$$\%RSA = \frac{Ai - Af}{Ai} x100$$

RSA (radical scavenging activity) of thienopyridine, $\mathbf{A_i}$ initial absorbance of the CTC, $\mathbf{A_f}$is the absorbance of the test/ standard compound.

The optical density was recorded as decrease in intensity of purple red color of CTC. The antioxidant activity is expressed as IC_{50}. **(Table 1) [20]**. The antioxidant activity was compared with ascorbic acid, used as a standard.

Table 1. Antioxidant activity of thienopyridine derivatives

comp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
%RSA	41.73	42.60	44.7	50.8	50.8	43.9	53.04	48.26	35.65	55.21	68.69	76.95	64.78	71.73	67.39	75.65
IC_{50}	1.19	1.17	1.11	0.98	0.98	1.138	0.942	1.036	1.40	0.90	0.73	0.65	0.77	0.697	0.74	0.66
Act	2.93	2.93	3.90	3.01	3.01	.86	3.03	2.98	2.85	3.04	3.14	3.19	3.11	3.16	3.13	3.18

Comp = compound, %RSA = %Radical Scavenging Activity, Act = Activity

1.3. Computational Methodology

1.3.1Construction of molecular structures

A series of thienopyridine compounds tested for inhibitory activity was selected for the present study and the program of window Hyperchem software Inc (http://www.warezdestiny.com/free-hyp)**[21]** was used in modeling studies. The molecules were generated and the energy was minimized using molecular modeling pro. The window version software SPSS10 (SPSS Software;Consult http://www.spss.com)**[22]** was used in the regression analysis.

1.3.2 ***Calculation of quantum chemical descriptors***

All of the molecular structures of the compounds were initially optimized geometrically using the semi-empirical method AM1 (Austin Model 1) and PM3 (Parameterization Model 3) [**23]**. The quantum chemical descriptors (variables)[**24-27]** 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 (LogP), hydration energy (HE),chemical potential (μ) and polarisability (Pol)were obtained for thienopyridine derivatives.

1.3.3 ***Molecular Modeling Studies***

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

Molecular docking methodologies ultimately seek to predict the best mode by which a compound fit into a binding site of a macro molecular target. This predicts the best candidate providing an insight on substitution and configuration for optimum receptor pit which leads to the development of best pharmacophore activity.

2.2.2.1 . GOLD2.0 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 hydrogens **[28,29]**. 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. The 3D structure of 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 [**30]**. Cyclooxygenases are enzymes that take part in a complex biosynthetic cascade that results in the conversion of polyunsaturated fatty acids to prostaglandins and thromboxane(s)

. Their main role is to catalyze the transformation of arachidonic acid into the intermediate prostaglandin H2, which is the precursor of a variety of prostanoids with diverse and potent biological actions.COX-2 plays a major role in prostaglandin biosynthesis in inflammatory cells and in the central nervous system. Prostaglandin synthesis in these sites is a key factor in the development of inflammation and hyperalgesia. COX-2 inhibitors have analgesic and anti-inflammatory activity by blocking the transformation of arachidonic acid into prostaglandin H2 selectively. The three-step mechanism explains behind the inhibitory effects of selective COX-2 inhibitors. The first step accounts for the contact of the inhibitor with the gate

of the hydrophobic channel (called the lobby region). The second step could account for the movement of the inhibitor from the lobby region to the active site of the COX enzyme. The last step probably represents repositioning of the inhibitor at the active site, which leads to strong interactions of the phenylsulfonamide or phenylsulfone group of the inhibitor and the amino acids of the side pocket. It is directly inhibition to postaglanding.

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 active 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;***1-16**. A population of possible docked orientations of the ligand is set up at random. Each member of the population is encoded as a chromosome, which contains information about the mapping of ligand H-bond atoms onto protein H-bond atoms, mapping of hydrophobic points, all the conformation around flexible ligand bonds and protein OH groups. All docking runs were carried out using standard default settings with a population size of 100, a selection pressure of 1.1, a maximum of 100000 operations, number of islands as 5, a niche size of 2, and a mutation and cross over rate of 95. Docking poses were obtained by applying both Chemscore and Gold score. In the present study of the GOLD Program, the performance of both Gold Score, Chemscore are found to be good. SPDBV3.7 software **[31]** was used for preparation of protein-ligand complexes by adding hydrogen atoms, removing water molecules, co-crystallization of inhibitors. Enzyme-inhibitor interactions within a radius equal to 15Å centered on reported bound inhibitors were taken into account.

2.2.2.2 Argus Lab

Argus Lab 4.0.1[**32]** was used for molecular modeling studies, which is very flexible and can reproduce crystallographic binding orientation. Argus lab provides a user-friendly graphical interface and uses shape dock algorithm, to carry out docking studies. This helps to visualize the binding conformations of these analogues, within the active site region of cyclo-oxygenase-2 protein.

2.2.2.3 Auto dock

Autodock4.0 [**33]** was used to estimate binding free energy and inhibition constant

(K_i).

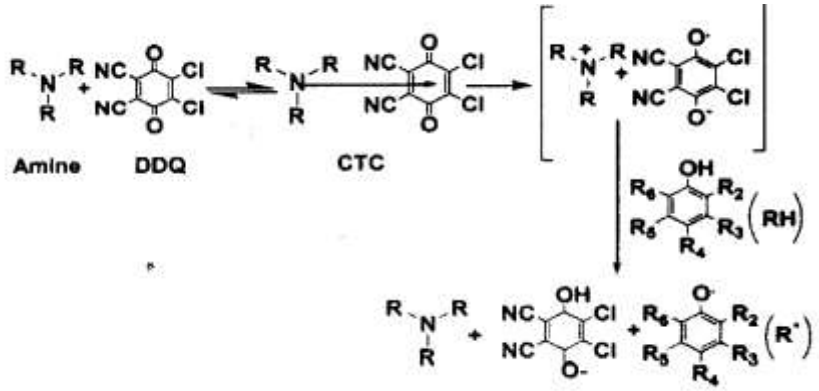
2.3 Results and Discussions

2.3.2 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 triethyl amine as ***n***-donor (D) and DDQ as π-acceptor. The Beer's law is obeyed over the concentration ranges. The described method was successfully applied to the determination of antioxidant activity. Lower absorbance of the reaction mixture indicates higher free radical

scavenging activity. The Lower IC_{50} value represents higher antioxidant activity. To accommodate the observed results, the following reaction mechanism is proposed in **Scheme-1.**

Scheme-1:



The CTC decomposes to give DDQ free radical which in turn forms R˙radical on abstraction of hydrogen from thienopyridine (RH). The R˙ radical then undergoes further reactions which control the overall stoichiometry *i.e*., the number of molecules DDQ reduced by RH. Mixing of DDQ solution to donor resulted in decrease in intensity of color

i.e. shifted to shorter wave length (hypsochromic shift).

2.3.3 Linear regression model analysis

The biological activity data and the physicochemical properties IP, EA, ω, EN, η, *S*, LogP, HE and Pol of the thienopyridine 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 sixteenth Thienopyridine derivatives**.** The term close to **1** indicates high co-linearity, while the value below 0.5 indicates that no co-linearity exists between more than the two parameters.

Table 2. Antioxidant activities and molecular descriptors values of thienopyridine derivatives in **AM1** method

Compd	Obs. Act.	Eq-1		Eq-2		Molecular descriptors								
		Predicted	residual	Predicted	Residual	IP (eV)	EA (eV)	EN (eV)	η (eV)	S (eV^{-1})	ω	HE (K.cal/mol)	LogP	Pol (A^o)
1	2.93	2.96	-.03	3.08	.16	8.87	.70	4.78	4.09	.12	2.80	-5.51	-.32	53.98
2	2.93	2.90	.03	3.06	.13	8.87	.54	4.71	4.16	.12	2.66	-5.92	-.09	52.05
3	3.90	3.12	.78	3.22	-.68	8.65	.91	4.78	3.87	.13	2.96	-6.21	-.37	49.74
4	3.01	2.98	.03	3.11	.11	8.79	.68	4.74	4.05	.12	2.77	-5.64	1.28	50.22
5	3.01	3.01	.00	3.15	.15	8.66	.63	4.65	4.02	.12	2.69	-2.49	1.49	47.09
6	.86	3.03	-2.16	-	-	8.72	.73	4.72	3.99	.13	2.80	-5.66	1.51	43.42
7	3.03	3.05	-.02	3.17	.15	8.69	.76	4.72	3.96	.13	2.81	-3.29	.63	47.09

8	2.98	2.95	.03	3.07	.08	8.93	.75	4.84	4.09	.12	2.86	-6.30	.59	45.26
9	2.85	2.89	-.04	3.05	.20	8.87	.52	4.69	4.18	.12	2.64	-5.92	-.09	41.59
10	3.04	2.89	.16	3.05	.01	8.87	.51	4.69	4.18	.12	2.63	-5.92	.59	39.05
11	3.14	2.97	.16	3.11	-.03	8.79	.67	4.73	4.06	.12	2.76	-6.37	-.09	52.05
12	3.19	2.96	.23	3.07	-.11	8.91	.74	4.83	4.09	.13	2.85	-6.30	-.61	40.47
13	3.11	2.58	.53	2.98	-.13	8.64	-.72	3.96	4.68	.11	1.67	-6.30	-.61	45.26
14	3.16	2.82	.34	2.94	-.21	9.21	.64	4.93	4.29	.12	2.83	-5.34	-1.07	47.09
15	3.13	3.46	-.33	3.60	.47	7.86	.88	4.37	3.49	.14	2.74	-6.21	-.47	50.03
16	3.18	2.69	.49	2.79	-.39	9.69	.70	5.20	4.50	.11	3.00	-6.15	-.09	49.94

Table 3. Antioxidant activities and molecular descriptors values of thienopyridine derivatives in **PM3** method

Com pd	**Obs. Act.**	**Eq-3**		**Eq-4**		**Molecular descriptors**								
		Predicted	**residual**	**Predicted**	**Residual**	**IP (eV)**	**EA (eV)**	**EN (eV)**	**η (eV)**	**S (eV^{-1})**	**ω**	HE(K.cal/mol)	**Log P**	**Pol (Aº)**
1	2.93	2.96	-.03	3.12	.20	9.09	.33	4.71	4.38	.11	2.53	-5.78	-.32	53.98
2	2.93	2.95	-.02	3.13	.20	9.08	.30	4.69	4.39	.11	2.50	-6.18	-.09	52.05
3	3.90	3.06	.84	3.14	-.76	8.98	.52	4.75	4.23	.12	2.67	-6.38	-.37	49.74
4	3.01	2.98	.02	3.04	.03	9.21	.54	4.88	4.34	.12	2.74	-5.78	1.28	50.22
5	3.01	3.22	-.22	3.26	.25	8.66	.63	4.65	4.02	.12	2.69	-2.49	1.49	47.09
6	.86	2.95	-2.09	-	-	9.33	.55	4.94	4.39	.11	2.78	-5.34	1.51	43.42
7	3.03	2.30	.72	2.99	.12	10.47	-.77	4.85	5.62	.09	2.10	-2.78	.63	47.09
8	2.98	2.97	.02	3.11	.20	9.11	.38	4.75	4.37	.11	2.58	-3.57	.59	45.26
9	2.85	3.01	-.16	3.05	.07	9.18	.58	4.88	4.30	.12	2.76	-2.42	-.09	41.59
10	3.04	2.95	.09	3.11	.06	9.11	.34	4.73	4.38	.11	2.55	-2.97	.59	39.05
11	3.14	3.19	-.05	3.20	-.16	8.79	.67	4.73	4.06	.12	2.76	-6.35	-.09	52.05
12	3.19	3.00	.19	3.03	-.03	9.23	.60	4.91	4.32	.15	2.80	-6.33	-.61	40.47
13	3.11	2.93	.18	3.08	-.03	9.19	.36	4.78	4.42	.11	2.58	-5.65	-.61	45.26
14	3.16	2.91	.25	3.12	-.02	9.13	.23	4.68	4.45	.12	2.46	-7.20	- 1.07	47.09

15	3.13	2.95	.17	3.10	-.09	9.12	.36	4.74	4.38	.13	2.57	-3.16	-.47	50.03
16	3.18	2.96	.22	3.09	.12	9.15	.40	4.77	4.38	.11	2.60	-2.47	-.09	49.94

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

Activity = 24.154 (1.307) * *S* ..... (1)

N = 16; R = 0.979; R^2 = 0.958; R^2adj=0.955; %EV = 95.80; SEE = 0.64; F= 341.566; Q =1.53;

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 were sought and eliminated.

After the elimination of the outlier (**6**), a second model was developed. Overall, there is an increase in R (0.979-0.997) and %EV (95.80– 99.5) values, and a decrease in SEE (0.64 - 0.24).

Activity = 26.458(1.216) * *S* – 0.220 (0.0213) * EA ---------- (2)

N =15; R= 0.996; R^2= 0.993; R^2adj =0.992; %EV = 99.6; SEE = 0.2841; F =894.019; Q =3.5058;

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 ***S*** and **EA** are found to be explainable variables. A mono-parametric QSAR equation with Soft and di-parametric QSAR equation with S and **EA** were generated in PM3 method also.

Activity = 25.889(1.367) * *S* ..... (3) N =16; R = 0.980; R^2= 0.960; R^2adj =0.957; %EV = 96.0; SEE = 0.62; F = 358.871; Q = 1.56;

Eq.3 shows that the values of %EV is less and to improve its value, outliers were sought and eliminated, In addition, the plot of observed activity versus predicted activity was not found to be satisfactory. Hence, the predictive ability of the model is not good. After the elimination of the outlier (**6**), a second model was developed.

Activity = 28.910*(0.910) * *S* – 0.540 (0.212) * EA ---------- (4)

N = 15; R = 0.997; R^2 = 0.994; R^2adj=0.994; %EV = 99.4; SEE = 0.2509; F = 1148.341; Q =3.9736;

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} = (>0.82). Another cross-validation parameter, PRESS which is the sum of the squared differences between the actual and that predicted when the compound is omitted from the fitting process, also supports the predictive ability of **Eqs.2** and **4**. Its value decreases from **Eq.1** to **Eq.3.**

The quality factor Q,**[34]** 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 1.53to 3.505 and 1.56 to 3.973**(Eq. 1** to **4)**.

As softness of ligand increases the activity also increases. The activity decrease with increase in electron affinity. Soft acids and bases can be explained on the HSAB principal. Softness of chemical species linked with large atomic/ionic radius, low or zero oxidation state, high polarisability, low electro negativity. Soft bases have HOMO of higher energy than hard bases, and soft acids have LUMO of lower energy than hard acids. The soft molecules are more reactive than hard molecules if electron transfer or rearrangement is necessary for the reaction. The softness is important in understanding the chemistry of large, delocalized molecules or ions **[35]**. The electron affinity is characterized by the susceptibility of the compound in relation to attacks by nucleophiles. 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 an negative ion. In principle, any molecule can act as an electron donor to all molecules with superior values to it. In the final AM1 and PM3 modelled **Eq-2** and **Eq-4,** contribution of the physicochemical parameters shown graphically in contribution charts (**figure 2** and **figure 3**). The correlation between actual and predicted activity for the compounds are shown in **table 2, table 3** and **figure 4-7**.

Figure 2 Plot of observed activity Vs activity

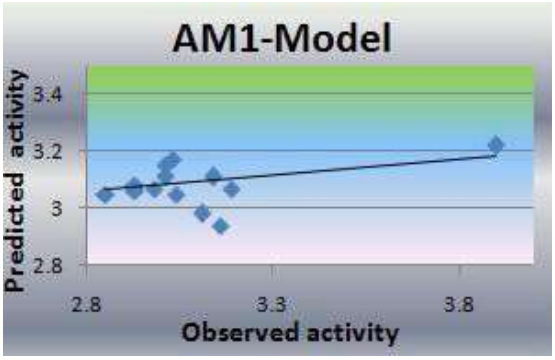


Figure 3 Plot of observed activity Vs predicted predicted activity

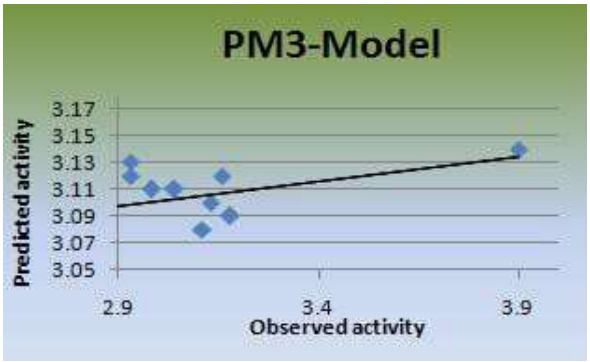


Figure 4 Plot of observed activity Vs **EA**

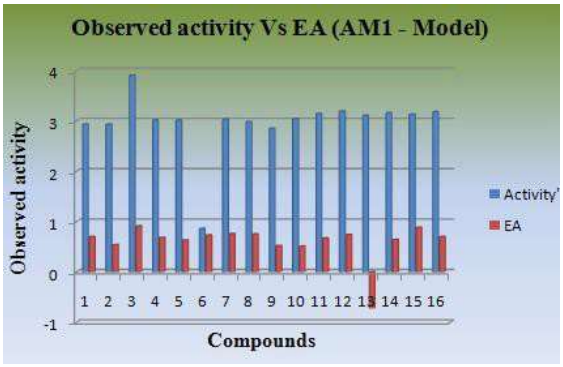


Figure 5 Plot of observed activity Vs ***S***

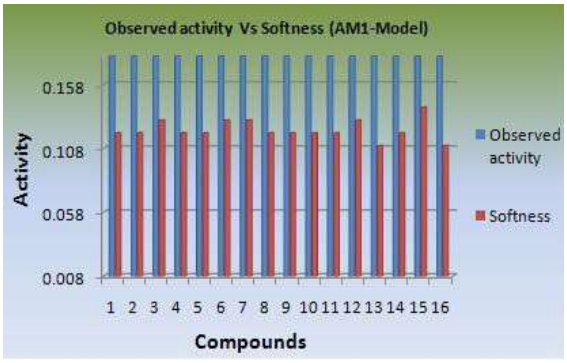


Figure 6 Plot of observed activity Vs **EA**

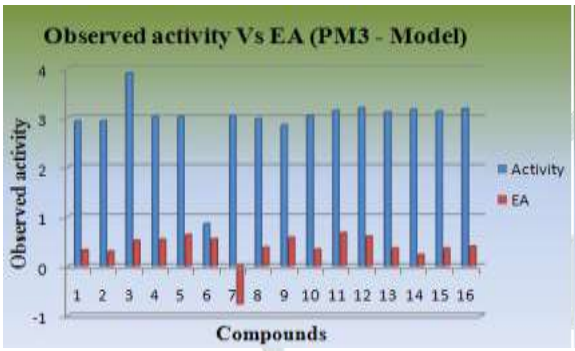
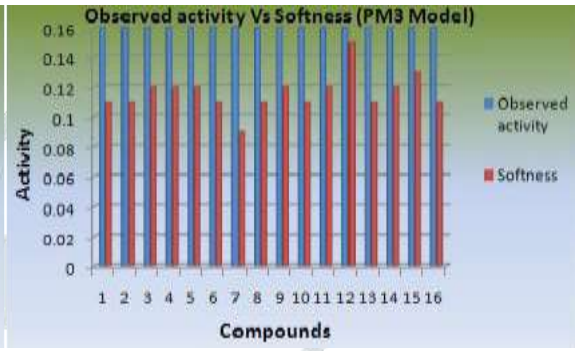


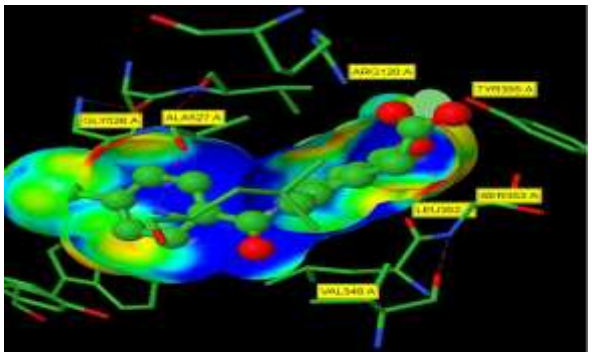
Figure 7 Plot of observed activity Vs ***S***



5.3.3. Docking Analysis

Among all the compounds tested for docking study, showed good inhibitory activity values against cyclo-oxygenase-2 (**table 4** and **table 5**). The compound 1**2** and **15** showed high affinities with low energy of with employed protein. It indicates the binding between 4COX and compound-**12** indicates very good inhibition. The compounds **(1-16)** 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 **figure 8**.

Figure 8 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-**12 (IC_{50}=0.649)** expected to be a potent inhibitor

of cyclooxegenase-2.The docked score of compound-**12** (55.19) 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 groups (-Cl) in the aromatic ring of thienopyridine derivatives with the cyclooxegenase-2 protein. The second highest score for the compound-**15** is due to electron releasing group (–F) on aromatic ring of compound-**15**.The compounds 1**4, 11,10** and **2** have next highest score due to presence of electron releasing groups on aromatic ring and highest score due to *intra* and *inter* molecular hydrogen bondings 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 thienopyridine derivatives.

Table 4 Docking Values Obtained from Gold in Fitness Score with cyclo-oxygenase-2 (PDB ID = 4COX)

Compound	Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(vdw_int)
1	46.39	0.00	37.80	0.00	-5.58
2	249.20	0.25	39.11	0.00	-4.83
3	45.71	0.16	38.43	0.00	-7.28
4	49.13	0.12	36.38	0.00	-1.01
5	545.52	0.21	36.57	0.00	-4.97
6	52.90	0.00	43.52	0.00	-6.94
7	46.76	0.18	38.31	0.00	-6.09
8	47.31	0.21	37.22	0.00	-4.08
9	44.57	0.00	33.26	0.00	-1.16
10	51.27	3.39	40.95	0.00	-8.43
11	52.00	4.48	41.78	0.00	-9.92
12	55.19	2.61	43.59	0.00	-7.35
13	46.23	0.30	37.52	0.00	-5.66
14	52.67	2.23	42.48	0.00	-7.97
15	53.73	0.78	43.71	0.00	-7.15
16	-20.33	0.24	40.03	0.00	-75.62

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

Comp	**Score**	**DG**	**S(hbond)**	**S(metal)**	**S(lipo)**	**DE(clash)**	**DE(int)**
1	20.17	-22.81	0.99	0.00	145.86	0.282.36	
2	21.01	-24.32	0.99	0.00	160.01	0.203.10	
3	21.23	-24.71	0.94	0.00	165.41	0.163.32	

4	21.09	-23.50	0.97	0.00	152.37	0.112.29
5	20.51	-23.24	0.99	0.00	150.57	0.042.69
6	24.66	-27.85	1.00	0.00	191.04	0.712.48
7	20.44	-23.30	0.98	0.00	151.18	0.232.62
8	20.59	-24.62	0.93	0.00	163.39	1.632.40
9	21.34	-22.94	0.83	0.00	148.81	0.401.20
10	19.93	-25.06	0.92	0.00	164.72	1.723.41
11	21.26	-25.80	0.91	0.00	171.32	1.493.05
12	22.02	-29.07	1.19	0.00	191.23	2.704.35
13	21.06	-24.02	0.96	0.00	155.55	0.062.90
14	21.97	-27.92	0.96	0.00	188.76	0.305.65
15	21.63	-27.55	0.99	0.00	184.23	1.993.93
16	20.61	-24.53	0.95	0.00	159.31	0.263.66

Highest Occupied Molecular Orbital (HOMO) energy and Lowest Unoccupied Molecular Orbital (LUMO) energy were constructed from of HQSAR (Hologram QSAR)**[36]**.The theoretical calculations of molecular properties such as the maps of Molecular Orbitals (HOMO, LUMO), Autodock and Argus lab binding energies showed a good antioxidant activity of the title compounds (**Table 6**and**Fig.9**.). The HQSAR maps show positive (green) and negative (blue) contributions. The positive contributions of the most potent compounds-**12, 14** and **15** indicate the importance of polar contacts for biological activity. The higher energy of the HOMO and lower energy of the LUMO indicate the greater electron-donating ability and smaller resistant to accept electrons respectively. Therefore, the HOMO and LUMO energies also support the QSAR and docking results.

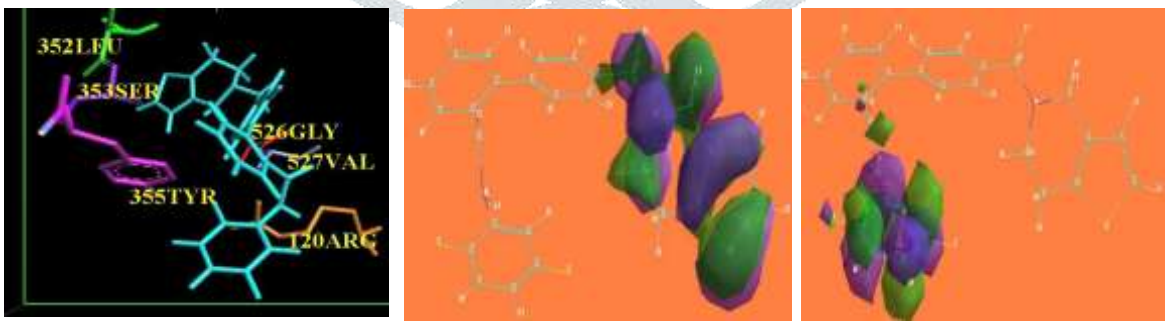
Table 6 HOMO, LUMO, AUTODOCK and Argus Lab energies of thienopyridine derivatives

Compound	$-\varepsilon_{HOMO}$	$-\varepsilon_{LUMO}$	Auto dock K_i in uM	Auto dock B.E in K cal/mol	Argus B.E in K cal/mol(elapsed time in seconds)
1	-9.03	-0.45	68.56	-5.68	-15.99(7)
2	-9.02	-0.44	-	+12.77	-14.19(8)
3	-9.03	-0.45	+40.59	-	-15.91(6)
4	-9.03	-0.35	50.40	-5.86	-13.47(6)
5	-9.03	-0.41	+41.19	-	-15.66(8)
6	-3.59	-0.22	+62.01	-	-16.69(8)

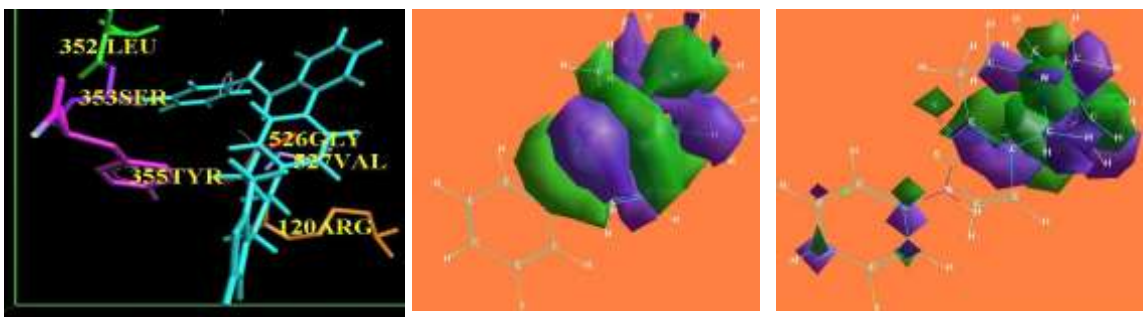
7	-9.02	-0.41	65.61	-5.71	-14.35(7)
8	-9.03	-0.42	99.25	-5.46	-14.34(6)
9	-9.26	-0.65	20.51	-6.40	-15.10(8)
10	-8.98	-0.40	124.59	-5.33	-16.09(8)
11	-9.14	-0.35	21.35	-6.37	-15.11(7)
12	-9.03	-0.43	152.95	-5.21	-14.82(6)
13	-9.12	-0.38	34.13	-6.09	-13.88(5)
14	-9.14	-0.63	82.14	-5.57	-14.46(8)
15	-9.01	-0.49	8.33	-6.93	-16.77(7)
16	-8.79	-0.61	-	+227.44	-12.99(7)

Figure 9 Best docking poses of molecule 12,14 and 15. HOMO, LUMO energy maps of molecule (12,14 and 15) and green color indicate favorable regions, while blue color indicate unfavorable region for the activity.

Best pose molecule-12HOMO structure of molecule-12LUMO structure of molecule-12



Best pose molecule-14 HOMO structure of molecule-14 LUMO structure of molecule-14



Best pose molecule-15 HOMO structure of molecule-15 LUMO structure of molecule-15

2.4 CONCLUSIONS

The antioxidant activity of thienopyridine derivatives was determined using CTC of DDQ. In our present study, it was established the predictive QSAR models that are quite reliable to the experimental antioxidant activity of thienopyridines. The main contribution of the high score compounds to the cyclo-oxygenase-2 enzyme is due to hydrophobic interactions. These findings demonstrated that these compounds could be developed into novel antioxidant pharmacophore. 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. Based on the activity data, from the series **12, 15, 14, 11, 10** and **2** serve as an important pharmacophore for the design and development of new lead as antioxidant agent. Therefore, it gives insight into the pharmacophore and residues of cyclo-oxygenase-2active site. The docking studies helped in understanding the various interactions between the ligands and enzyme active sites. The QSAR studies revealed the indicative physicochemical parameters and type of substituents are responsible for high antioxidant efficacy of thienopyridine derivatives.

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