Original Research Article

Antioxidant activity of thiazolidinedione analogues: Results of descriptor-basedquantitativestructure-activity relationship studies

ABSTRACT

Background: Diabetes mellitus, a chronic metabolic disorder is characterized by defective insulin secretion(β-cell dysfunction), insulin action (insulin resistance) and reduced bio-antioxidant potential.Antioxidants play key role in diabetes by reducing the oxidative stress and alleviating diabeticcomplications. Thiazolidinediones (TZDs) attenuate insulin resistance and produce antioxidanteffect.

Study Design: In continuation of our goal to develop thiazolidine-2,4-dione analogues that can addressboth oxidative stress and Type 2 diabetes, the *in vitro* antioxidant potential of a fewsynthesized thiazolidinediones (**1-61**) were evaluated for their DPPH and nitric oxide free radical scavengingassays. Descriptor-based QSAR analysis was utilized to study the structural contributionto the radical scavenging potential.

Results: Among all test compounds, the DPPH radical scavenging activity of compound **12** wasfound to be significant (IC_{50} 22.7±0.43 µM). The compound **11** (IC_{50}: 13.8±0.5 µM) showedsuperiornitricoxideradicalscavengingpotential,whencomparedtoascorbicacid(IC_{50}:14.8±0.7 µM). Among various developed QSAR models, 16 and 29 models were foundto be best for DPPH and nitric oxide radical scavenging activities, respectively. The R^2 value0.745 and 0.890 in the above models are indicative of good correlation between *in vitro* and *insilico* antioxidant activity.

Conclusion: The QSAR studies revealed the potential contribution of the partition-coefficient,hydrogen bond acceptor and donors andmolecular weight towards the antioxidant activity inboth theassaymodels.

Keywords:Diabetesmellitus;Freeradical;Oxidativestress;Knoevenagelcondensation;Thiazolidine-2,4-dione;moleculardescriptor.

1. INTRODUCTION

Diabetes mellitus, a metabolic disease characterized by hyperglycemia associated with longterm damage, results in dysfunction and failure of various organs (eyes, kidneys, nerves, heartand blood vessels) [1, 2]. The role of oxidative stress (OS) in the pathogenesis of micro andmacro vascular complications (retinopathy, nephropathy,atherosclerosis and coronary arterydisease) of diabetes is well characterized [3-6]. OS reduces antioxidant enzyme activities byincreasing lipid peroxidation and altering glutathione (GSH) redox state. Generation of reactiveoxygen species (ROS) such as superoxide anion radicals ($O_2^{\bullet-}$), hydroxyl radicals ($^{\bullet}OH$), etc. ishigh during diabetes and is involved in the lipid peroxidation. The depleted GSH in OS isresponsible for the reduction of the hydrogen peroxide detoxification [6].Several natural andsynthetic antioxidants neutralize ROS by donating their hydrogen and prevent cell damage [7].The antioxidants reduce oxidative stress and thereby alleviate diabetic complications [8, 9]. Oralhypoglycemic agents such as glibenclamide, glipizide and metformin scavenge free radicals anddecreasesintracellularROSlevel[10,11].Hence,thesearchformoleculeshavingbothhypoglycemic as well as antioxidant potential offers a novel gateway in the diabetes therapy. TheQuantitativeStructuralActivityRelationship(QSAR)studiessuggesttheimportanceofquantum-chemical descriptors in producingtheantioxidant potential [12,13].

The thiazolidinedione (TZD) class of drugs (pioglitazone and rosiglitasone) attenuatetheinsulin resistance [14, 15]. The antioxidant potential of various 5-substituted-1,3-thiazolidine-2,4-diones and N,5-

disubstituted-1,3-thiazolidine-2,4-diones were reported in the literature [16,17].Promptedbytheabovementionedfacts,the*invitro*DPPHandniricoxideradicalscavenging potential of TZDs, **1-61** were evaluated. The descriptor-based QSAR analysis wasutilized to identifythe molecularproperties contributingto theantioxidantactivity[18].

2. METHODOLOGY

2.1 Tools

Double-beamShimadzuUV-Visible spectrophotometer1800wasused formeasuring theabsorbance of chromophore formed in both the *in vitro* assay models. The PIC_{50} values werecalculated using free Web Sanjeev's lab software. QSAR studies was performed using Strike 1.9module, (Schrodinger, USA) on Dell Precision T-1500 workstation Intel(R) Core(TM) i7 CPU860 @ 2.80 GHz; 12.0 GB Ram, 1 TB Hard disk. The structures of the ligands were drawn usingMarvinsketch and the geometrywas optimized usingLigprep 2.4(Schrodinger *Inc*).

2.2 Chemistry

The structures of TZDs (**1-61**) utilized in the present investigation were shown in Fig. 1 and 2along with their synthetic scheme. The 5-substituted aryl/heteroaryl-1,3-thiazolidine-2,4-diones(**1-13**)werepreparedbyKnoevenagelcondensationofaryl/heteroarylaldehydeswith1,3-thiazolidine-2,4-dione.ThebasecatalysedN-alkylationofthiazolidine-2,4-dionewithalkylhalidesyieldedN-substituted-1,3-thiazolidine-2,4-dioneanalogues(**14-17**).Knoevenagelcondensation of compounds **14-17** with various arylaldehydes produced N,5-disubstituted-1,3-thiazolidine-2,4-diones(**18-49**).Reactionof(2,4-dioxo-1,3-thiazolidin-5methylidene)benzenesulphonylchloride(**1a**)withvariousaminesandaminoacidsaffordedTZDs**50-61**.Thesyntheticdetails ofcompounds**1-61**weredescribedin ourpreviouscommunications[19-22].

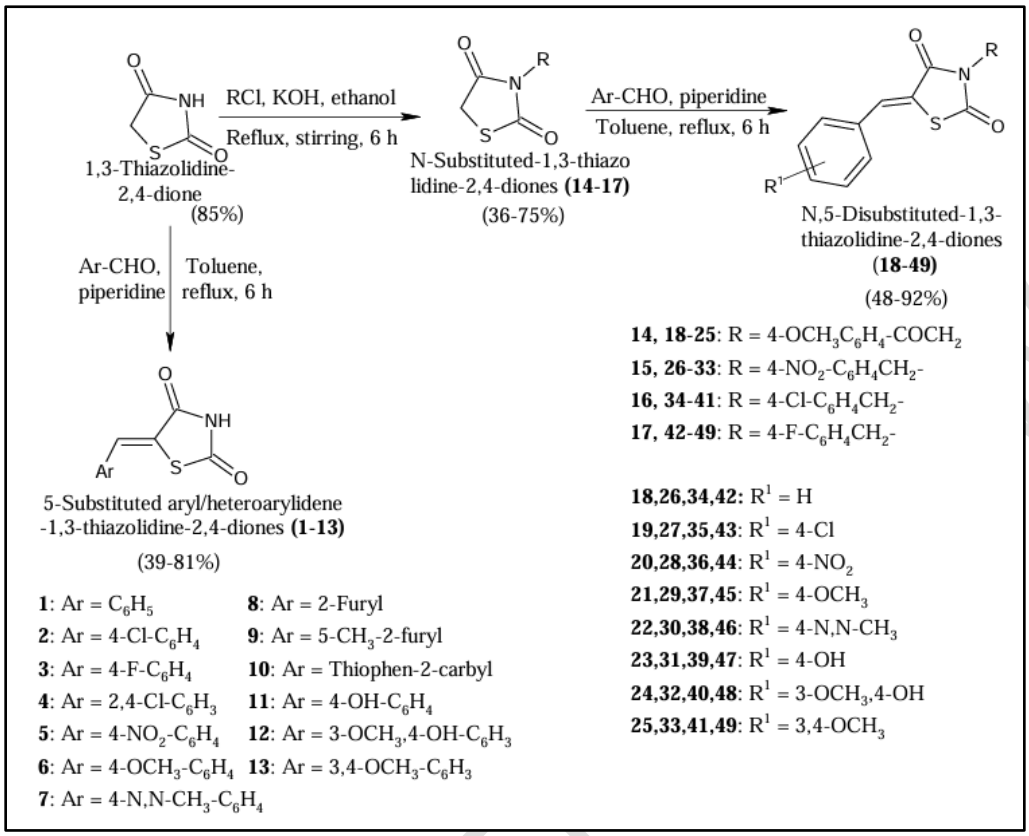

Fig.1. Schemeshowingthesynthesisofthiazolidinedioneanalogues, **1-49**.

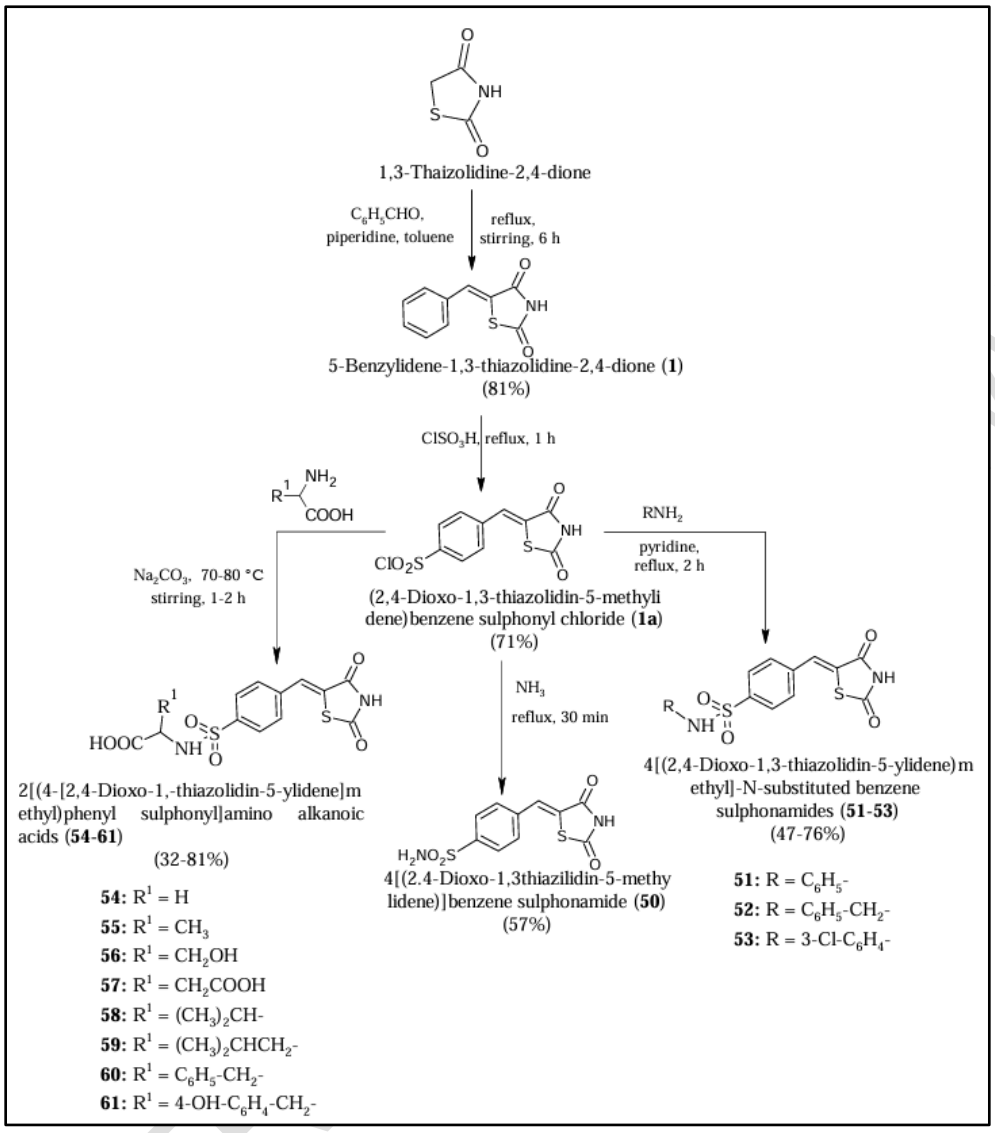

Fig.2. Schemeshowingsynthesisofthiazolidinedioneanalogues,**50-61**.

2.3 Evaluationof *invitro*antioxidantactivity

2.3.1 DPPHfreeradicalscavengingactivity

TheDPPHradicalscavengingactivityoftheTZDs,**1-61**wasdeterminedbyfollowingmethod given in literature [23]. The test substances of different concentrations (10-100 μM) indimethyl formamide (2 mL) were added to test tubes containing methanolic DPPH solution (0.3mM, 1 mL). The mixture was incubated in the dark for 30 min and the absorbance was measuredat 517 nm. Reaction mixture without DPPH solution was taken as control. The %DPPH radicalscavenging activity of the TZDs was calculated using following formula and the activity wasrepresentedin terms ofconcentration at 50% inhibition (IC_{50}).

2.3.2 Nitricoxidefreeradicalscavengingactivity

The nitric oxide scavenging activity of the synthesized compounds (**1-61**) was determined byfollowing the literature method with modification [24]. The test/reference compounds (10-100µM) were incubated with sodium nitroprusside (10 µM) in phosphate buffer pH 7.4 at 25 ^{o}C.After 2½ h, the incubation solution (1 mL) was removed and diluted with Griess reagent (1 mL)and solvent (1 mL). The mixture was kept aside for 20 min. The absorbance of the chromophoreformedduringdiazotizationofnitritewithsulphanilamideandsubsequentN-napthylethylenediaminewasmeasuredat546nm.Thepercentageofnitricoxideinhibitionwascalculated usingtheformula:

2.4 Quantitativestructure-antioxidantactivityrelationshipanalysis

AllTZDsstructureswheresketchedwithMarvinSketch[25] andgeometryoptimizationwasperformed with LigPrep 2.4 program [26] using MMFF force field at pH 7±2.0. Molecular properties,such as partition coefficient (Log P), hydrogen bond donors and acceptors (HBD, HBA), energyof highest occupied molecular orbital (HOMO), energy of lowest unoccupied molecular orbital(LUMO),dipolemoment(DM),solvent-accessiblesurfacearea(SASA),molecularweight(MW), polar surface area (PSA) and molecular volume (MV) were calculated for optimizedstructures.HOMOandLUMOweredeterminedfromMOPACusingthePM3method.Remainingproperties werecalculated usingquickprop 3.3 [27].

Two data sets containing 33 and 38 TZDs were utilized for quantitative structure-DPPH radicalscavengingactivityandquantitativestructure-nitricoxideradicalscavengingactivityrelationshipanalysis, respectively.Theabovementionedmoleculardescriptorswereconsideredastheindependent variables and the DPPH and nitric oxide radical scavenging activities (PIC_{50}) wereconsideredasthedependentvariables.MultipleLinearRegression(MLR)analysiswasperformed using Strike 1.9 (Schrodinger, 2010). Various QSAR models were generated and werevalidated by internal validation measures. The DPPH and nitric oxide radical scavenging activity(IC_{50}) of the molecules was converted to corresponding PIC_{50} values using the formula,PIC_{50} =-Log(IC_{50}) [28] andthe samevalues were considered asdependent variable.

3. RESULTS AND DISCUSSION

3.1 Evaluationofantioxidantpotential

3.1.1 DPPHradical scavengingactivity

TheTZDs**1-61**wereevaluatedfortheirDPPHradicalscavengingactivityandtheresultswere compared with ascorbic acid, butylated hydroxy anisole andbutylated hydroxy toluene.The DPPH radical is reduced by an electron transfer from the antioxidant molecule and, followedby protonation and therefore the colour intensity is decreased. A decrease in the absorbance at517 nm indicates the DPPH radical scavenging activity of the test compound. The activity wasexpressed as mean IC_{50} (µM)±SD of triplicate measurements and were given in Table 1. Thecompound**12**witha*p*-hydroxylgrouponthebenzylideneringshownhighestDPPHradicalscavenging activity (22.7±0.43 µM) among the test compounds. It was indicated from this studythat the compounds with electron-donating groups showed significant DPPH radical scavengingpotential.Compoundswithelectron-withdrawing(-$NO_2$and–Cl)groupsandthosewithunsubstituted benzylidene group on thiazolidinedione ring have shown poor activity (IC_{50}> 100µM).

Table 1.*In vitro* radical scavenging activity of compounds

Compound Code	Radical scavenging activity (IC_{50} (μM)±SD, n=3)	
	DPPH	Nitric oxide
5	48.7±0.73	93.6±4.9
6	74.6±0.67	72.3±3.8
7	32.1±0.44	65.1±3.8
9	86.4±0.52	97±3.4
11	24.3±0.12	13.8±0.5
12	22.7±0.43	14.3±0.4
13	33.2±0.70	66.2±3.3
14	78.8±0.58	76.9±2.9
15	> 100	86.2±3.9
18	> 100	69.2±3.8
19	> 100	89.1±3.2
20	> 100	73.8±3.6
21	> 100	59.4±2.3
22	33.4±0.24	19±0.4
23	28.1±0.52	14.2±0.7
24	26.3±0.48	14.1±0.5
25	35.5±0.72	56.2±2.5
29	> 100	68.3±3.4
30	68.2±0.66	21.4±1.8
31	29.1±0.57	17.3±0.6
32	29.0±0.52	18.8±1.4
37	> 100	76.2±3.7
38	72.2±0.43	23.8±0.7
39	31.0±0.58	18.7±0.6
40	38.7±0.77	19.2±0.5
41	89.1±0.63	68.1±3.6
45	> 100	81.8±4.9
46	76.4±0.46	25.1±1.6
47	37.8±0.82	18.4±0.6
48	44.3±0.73	19.4±0.8
49	96.4±0.27	68±3.9
50	35.2±1.4	24.5±1.9
52	39.6±2.0	29.3±0.9
54	45.4±1.3	21.8±0.9
55	48.1±1.7	25.3±1.0
56	65.7±2.1	42.4±1.9
57	61.3±2.2	> 100
58	53.6±1.8	34±2.0
Ascorbic acid	13.0±0.48	14.8±0.7
Butylated hydoxy anisole	61[a]	NA
Butylated hydroxyl toluene	220[a]	NA

[a]Ref29; NA: Not available

3.1.2 Nitricoxideradicalscavengingactivity

Nitric oxide radical scavenging activity of theTZDs, **1-61** was determined and the resultswere compared with ascorbic acid (Table 1). Nitric oxide, generated as a result of decompositionof sodium nitroprusside in the aqueous medium, $^{-}$ interacts with dissolved oxygen at physiologicalpHandproducenitriteions(NO_2^-).Diazotizationofsulphanilamidewithnitriteinacidicconditions gives a transient diazonium compound which on subsequent coupling with naphthylethylenediamine(NED)formsastablepurpleazocompound.Thischromophorehasanabsorption maximum at 546 nm. The antioxidant, as it competes with Griess reagent for thenitrite, reduction in the absorbance was observed. The blank without the test substance showshigher absorbance.The TZDs **11, 12, 23** and **24** with strong electron-donating hydroxyl group (IC_{50}: 13.8±0.45,14.3±0.4, 14.2±0.7 and 14.1±0.5 µM, respectively) showed high nitric oxide inhibitory activityamong the test compounds and they were found to be superior to ascorbic acid (IC_{50}: 14.8±0.7µM).Theresultsindicatedtheimportanceofelectron-donatingfunctionalities,suchashydroxy (-OH),methoxy(-OCH_3)andN,N-dimethylamino(-N,N-CH_3)groups,astheirpresenceincreasedthe*in vitro* antioxidant activity.

3.2 Developmentofdescriptor-basedQSARmodel

QSARanalysiswasperformedtoidentifythemoleculardescriptorsnecessaryfortheantioxidant activity. It was performed for two different data sets, one utilizing DPPH radicalscavengingactivity(n=33)andanotherusingnitricoxidescavengingactivity(n=38)asdependent variable. Stepwise multiple linear regression analysis method was utilized to performQSARanalysis. Among the developed models, the best model is selected from various statistically significant equations on the basis of squared correlationcoefficient (R^2), standard deviation (SD), sequential Fischer test (*F*) and pearson-r (*P*). TheQSAR equation with the lowest SD, and P and the R^2 value reaching to unity is considered asbest.A high *F*value explains thestrongrelation betweenthe variables understudy.

3.2.1 Qunatitativestructure-DPPHradicalscavengingactivityrelationshipanalysis

AnumberofQSARmodelsweregeneratedby consideringthemoleculardescriptors in different combinations and few of them are given in Table 2. The model 16 with lowest SD (0.146), higher R^2 value (0.743), higher *F* (15.6) and smaller *P*($3.03x10^{-7}$) values was considered as the best QSAR model. QSAR equation for model16in DPPH radical scavengingactivityis as follows:

PIC_{50}=-$2.8472e^{-002}$(±$5.0088e^{-002}$)*LogP+$2.9133e^{-002}$(±$4.0857e^{-002}$)*HBA+ $1.8701e^{-001}$(±$4.6319e^{-002}$)*HBD-$1.1159e^{-002}$(±$2.029e^{-002}$)*HOMO+ $1.5423e^{-003}$(±$1.0842e^{-003}$)*MW+2.6244(±$2.7583e^{-001}$) ---------- (1)

n =33;SD=0.146;R^2= 0.745.

Table2.ThestatisticalrelevanceofQSARmodelsinDPPHradicalscavengingactivity(n=33).

Model No.	Descriptors	SD	R^2	F	P
1	LogP, HBD,MW	0.205	0.451	7.9	$5.115X10^{-4}$
6	LogP, HOMO,MW	0.179	0.584	13.6	$1.02X10^{-5}$
11	LogP, HOMO, MW, HBD	0.144	0.738	19.8	$7.993X10^{-8}$
12	LogP, HOMO, MW, HBA	0.181	0.588	10.0	$3.733X10^{-5}$
16*	LogP,HOMO, MW,HBD, HBA	0.146	0.745	15.6	$3.03X10^{-7}$

*Bestmodel;LogP:Partitioncoefficient;HBD:Hydrogenbonddonor;HBA:Hydrogenbondacceptor;HOMO: Highestoccupied molecular orbital;MW: Molecularweight.

A perusal to Table 2 indicated that model 11 is equally a good fit, because it contained fourparameters,yet the R^2 value (0.738) was appreciable. Since for calculation of each descriptor5to 6 molecules are required, as the present study involves a total of 33 molecules, it would beappropriate to consider the model 16 with five descriptors. The experimental antioxidant activityresultswere wellcorrelated withthepredictedactivity(Table3) withR^2valueof0.745(**Fig. 3**).

The QSAR equation (1) reveals that molecular descriptos like, HBA, HBD and MW werepositively contributed, while Log P and HOMO were negatively related to the DPPH radicalscavengingactivity.The QSARmodel was validated internally for its robustness and predictive ability based on the value ofleave-one-outcross-validatedsquaredcorrelationcoefficient(LOO-Q^2).Themodel16hasshownLOO-Q^2valueof 0.58 (greater than 0.5)and considered to beagood model.

Table 3. Experimental and Strike 1.9 predicted DPPH scavenging activity of compounds (n=33)

Compound Code	PIC_{50}[a]		Residual[c]
	Experimental	Predicted[b]	
5	4.31	4.40	-0.09
6	4.13	4.25	-0.12
7	4.49	4.21	0.28
9	4.07	4.20	-0.13
11	4.61	4.44	0.17
12	4.66	4.49	0.17
13	4.48	4.33	0.15
14	4.11	4.23	-0.12
22	4.48	4.28	0.2
23	4.55	4.50	0.05
24	4.59	4.59	0
25	4.46	4.38	0.08
30	4.17	4.3	-0.06
31	4.53	4.46	0.07
32	4.56	4.52	0.04
38	4.14	4.13	0.01
39	4.51	4.36	0.15
40	4.42	4.43	-0.01
41	4.05	4.23	-0.18
46	4.12	4.12	0
47	4.43	4.35	0.08
48	4.36	4.41	-0.05
49	4.02	4.22	-0.2
50	4.68	4.74	-0.06
52	4.86	4.93	-0.07
54	4.42	4.45	-0.03
55	4.41	4.43	-0.02
56	4.44	4.48	-0.04
57	4.33	4.32	0.01
58	4.74	4.76	-0.02
Ascorbic acid	4.89	4.99	-0.1
Butylated hydoxy anisole	4.21	4.01	0.2
Butylated hydroxyl toluene	3.66	4.02	-0.36

[a]PIC_{50}=-(LogIC_{50});[b]Predictedactivityvaluesaspermodel16inTable 2.[c]Residual =Experimentalactivity-Predicted activity.

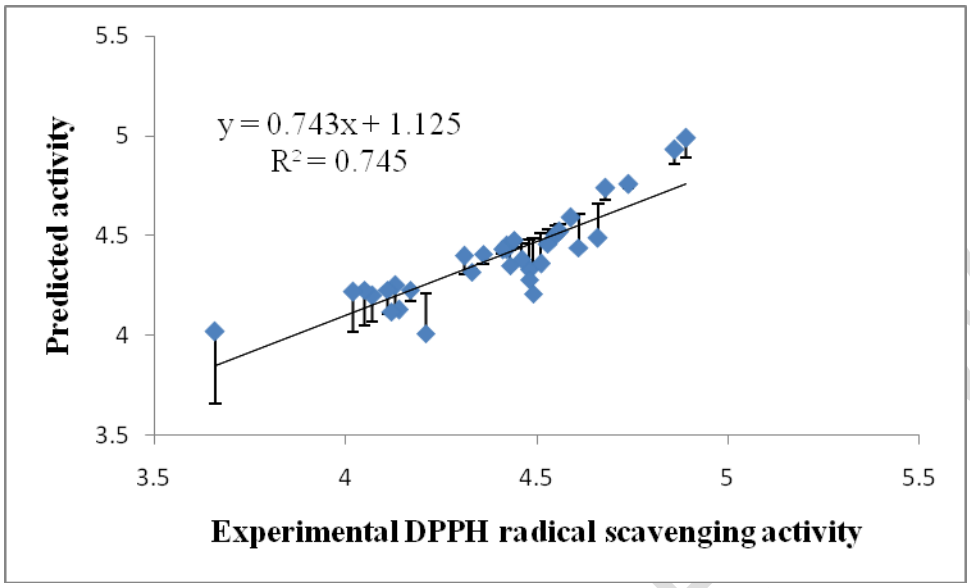

Fig. 3. A plot showing experimental versus predicted DPPH radical scavenging activity of TZDs(n=33)with residual representation usingQSARmodel

3.2.2 Qunatitativestructure-nitricoxideradical scavengingactivityrelationshipanalysis

A number of QSAR models were generated by considering the molecular descriptors asindependentvariablesandnitric oxide radicalscavenging activity (PIC_{50}) of compoundsasdependent variable. Some of the models were given in Table 4. The model 29 with lowest SD(0.0881),higherR^2value(0.890),higher*F*(42.1)andsmaller*P*($1.454x10^{-13}$)valueswasconsidered as the best QSAR model. The nitric oxide radical scavenging activity of the TZDs (asrepresented by PIC_{50}) is best predicted by regression equation (2).It evidences the positivecontribution of Log P, HBD, DM and MV and negative contribution of HBA and MW towardsthenitricoxide radical scavengingactivity.

PIC_{50} $=1.5849e^{-002}(\pm4.9755e^{-002})$*Log P-$8.6896e^{-002}(\pm3.5278e^{-002})$*HBA+$3.9092e^{-001}(\pm2.5836e^{-002})$ *HBD+$4.9026e^{-003}(\pm9.5644e^{-003})$*DM-$4.4276e^{-003}(\pm2.0299e^{-003})$*MW+$2.620e^{-003}(\pm9.8161e^{-004})$*MV+ $3.4047e^{+000}(\pm1.2392e^{-001}$ ---------- (2)

n=38;R^2=0.890;SD=0.0881.

Table4.ThestatisticalrelevanceofQSARmodelsinnitric oxide radicalscavengingactivity(n=38).

Model No.	Descriptors	SD	R^2	F	P
2	LogP, HBD,MV	0.102	0.839	59.0	$1.458X10^{-13}$
9	LogP,HBD,MW,HBA	0.103	0.84	43.4	$1.087X10^{-12}$
14	LogP,HBD, MV,DM	0.0946	0.866	53.2	$6.214X10^{-14}$
23	LogP,HBD,DM, HBA, MV	0.0931	0.874	44.4	$1.810x10^{-13}$

29*	LogP,HBD, DM,HBA,MV,MW	0.0881	0.890	42.1	$1.454x10^{-13}$

*Bestmodel;LogP:Partitioncoefficient;HBD:Hydrogenbonddonor;HBA:Hydrogenbondacceptor;DM: Dipole moment; MV:Molecularvolume; MW: Molecularweight.

Theexperimentalantioxidantactivityresultswerewellcorrelatedwiththepredictedactivity(Table 5) with R^2 value of 0.890 (Fig. 4). The model29has shown LOO-Q^2 value of 0.8385 (greater than 0.5) and thus the model was predicted to begood.

Table 5. Experimental and Strike 1.9 predicted nitric oxide radical scavenging activity of compounds (n=38)

Compound Code	PIC_{50}[a]		Residual[c]
	Experimental	Predicted[b]	
5	4.18	4.21	-0.03
6	4.28	4.32	-0.04
7	4.27	4.46	-019
9	4.15	4.27	-0.12
11	4.71	4.62	0.09
12	4.68	4.61	0.07
13	4.23	4.31	-0.08
14	4.11	3.92	0.19
15	4.06	3.95	0.11
18	4.16	4.28	-0.12
19	4.21	4.24	-0.03
20	4.13	4.18	-0.05
21	4.23	4.28	-0.05
22	4.48	4.41	0.07
23	4.68	4.58	0.1
24	4.61	4.56	0.05
25	4.25	4.23	0.02
29	4.17	4.29	-0.12
30	4.41	4.43	-0.02
31	4.63	4.60	0.03
32	4,61	4.59	0.02
37	4.32	4.36	-0.04
38	4.55	4.50	0.05
39	4.73	4.66	0.07
40	4.72	4.65	0.07
41	4.36	4.37	-0.01
45	4.25	4.35	-0.1
46	4.6	4.49	0.11
47	4.74	4.66	0.08
48	4.71	4.65	0.06
49	4.29	4.37	-0.08
50	4.62	4.63	-0.01
52	4.54	4.52	0.02
54	4.45	4.42	0.03
55	4.55	4.54	0.01
56	4.38	4.38	0
58	5.03	5.14	-0.11
Ascorbic acid	4.87	4.89	-0.02

[a]PIC_{50}=-($LogIC_{50}$);[b]Predictedactivityvaluesaspermodel29inTable 4.[c]Residual =Experimentalactivity-Predicted activity.

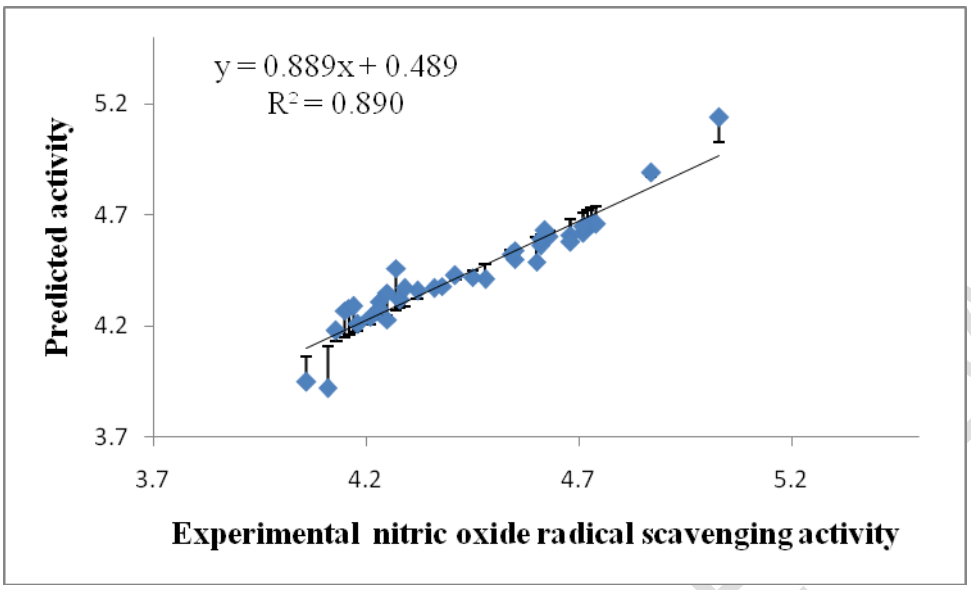

Fig. 4. A plot showing experimental versus predicted nitric oxide radical scavenging activity ofTZDs(n=38)with residual representation using QSARmodel.

4. CONCLUSION

The antioxidant potential of few TZDs (**1-61**) was assessed by *in vitro* DPPH and nitric oxideradicalscavengingactivities.Thestatisticalrelevancebetweenmoleculardescriptorsandantioxidant activity was established through descriptor-based QSAR analysis. The compoundswithelectron-donatinggroupsatthebenzylideneportionoftheanalogueshaveshownpredominant antioxidant activity in comparison of molecules with electron-withdrawing groups.The QSAR studies revealed the contribution of hydrophobic (Log P), electronic (HBD, HBA,HOMO andDM)and steric(MW) descriptorstowards theantioxidant activity.in both the*invitro* antioxidant assays, compounds **11**, **12**, **23** and **24** showed significant radical scavengingactivities in comparison with reference compounds. The difference between experimental andpredicted activity data (residual) of the above compounds was also small. From these findings,compounds **11**, **12**, **23** and **24** were identified as potential leads for the further synthesis of analogues and *in vivo*investigations.

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