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Rosiglitazone Metabolism: A Molecular modeling study using PM6 Model

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ABSTRACT

Molecular Modelling studies based on molecular mechanics and semi-empirical methods have been carried out for RGT and its metabolites in order to gain insight into excretion, reactivity and toxic effects of RGT. All metabolites are kinetically labile as indicated by LUMO-HOMO energy difference. The molecular modelling analysis shows that M4 and M10 metabolites are readily excreted in urine as their calculated solvation energy is highest.Further, it is found that M1 metabolite might be responsible for edema as it contains acidic group which can form salt bridge with Arg 120 of COX-1.All the metabolites are more reactive than RGT towards nucleophilic attack as indicated by lowest electron affinity of RGT. M13 has the highest HOMO (lowest ionization potential) energy so it will be most susceptible towards electrophilic attack. M1 possesses highest ionization potential in both gaseous as well as aqueous phase.

Keywords : Rosiglitazone, Molecular Modelling ,PM6,Toxicity , Metabolites

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INTRODUCTION

Rosiglitazone(±)-5-[[4-[2-methyl-2-(pyridinylamino)ethoxy]phenyl]methyl]-2,4- thiazolidinedione is a potent antihyperglycemic agent that reduces insulin resistance in patients with type 2 diabetes.Rosiglitazone belongs to the thiazolidinedione class of oral antidiabetic agents. The mechanism of action of RGT involves its binding to peroxisome proliferator-activated receptors gamma (PPARγ), which binds to DNA as heterodimers and activate transcription of a large number of metabolic regulators which are involved in the differentiation of stem cells into adipocytes. This also increases the expression of a number of genes involved in the regulation of glucose and lipid metabolism.The metabolism of rosiglitazone includes mainly N-

demethylaon,hydroxylation and subsequent conjugation. Out of these, about 44% of the metabolites of rosiglitazone are N-demethylated and around 76 % are hydroxylated on pyridine ring. The ortho- and para- hydroxylation metabolites ratio is estimated to be 1 to 9 and that of sulfation to glucuronidation is 6 to 1 .The metabolite N-desmethyl-rosiglitazone (M12) is 20-fold less potent than and unconjugated para-hydroxyrosiglitazone (M13) is equipotent to rosiglitazone respectively (1).The CYP enzymes cause the scission of thiazolidinediones during metabolism of rosiglitazone and the reactive metabolites are trapped by glutathiones. (2). The CYP2C8 is the major P450 involved in the metabolism of Rosiglitazone (3). But CYP2C9 plays a minor role in the metabolism of Rosiglitazone(4) . Molecular modelling of metabolism of many drugs e.g. Zileuton (5). Troglitazone (6) etc. has been described in literature. The side effect profile of RGT includes weight gain, edema, (7) hypersensitivity (urticaria and angioedema), myocardial infarction (8), macular edema,dyspnoea,athralgia and fractures of the upper arm, hand and foot in women etc (9).

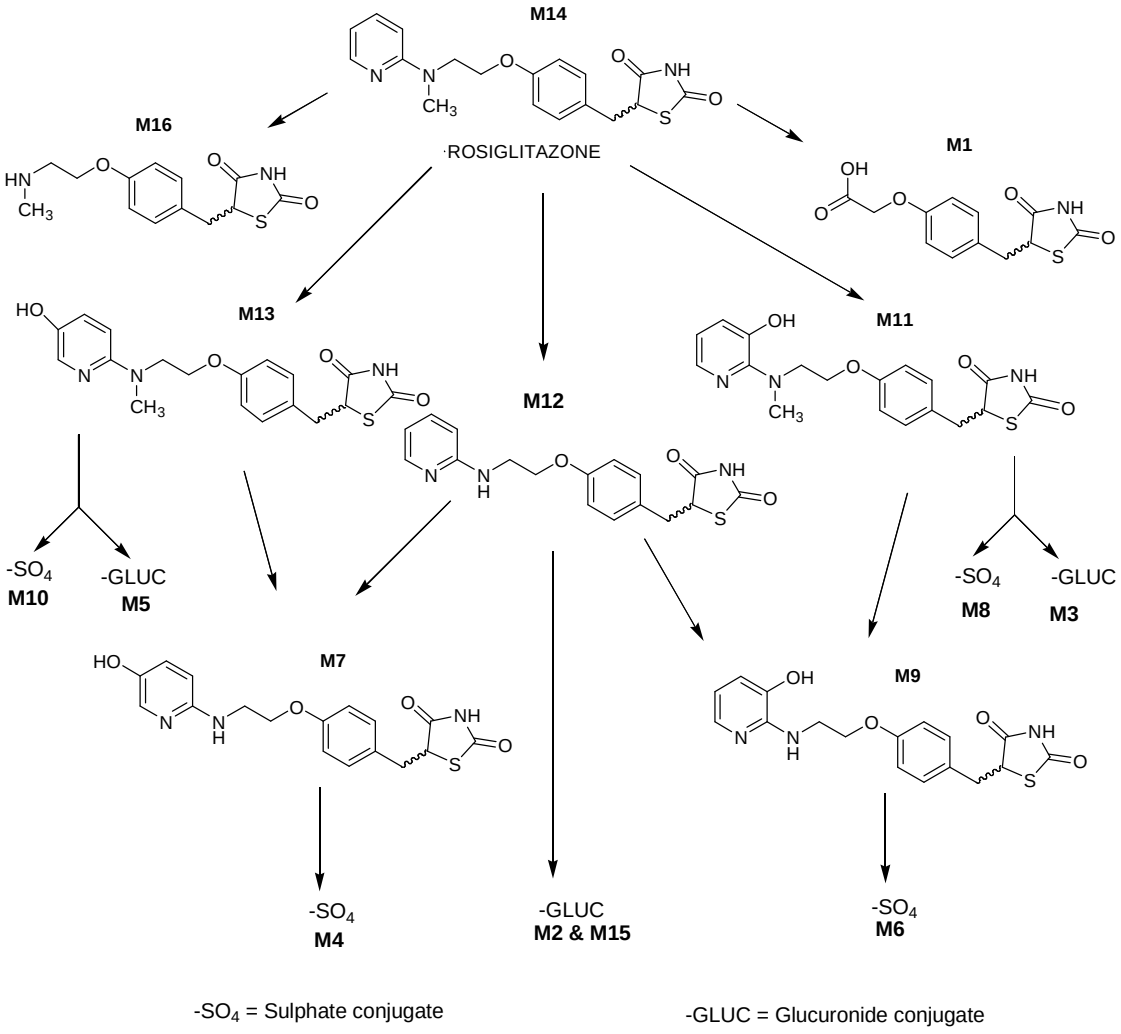


Figure 1: Metabolic Scheme of Rosiglitazone in Human Body (1)

MATERIALS AND METHODS

Molecular modeling calculations were performed in this study.

Computational Methods

The geometries of Rosiglitazone and its metabolites were fully optimized/minimized using molecular mechanics MM2 and semi-empirical methods (PM3 and PM6) using the Baker's Eigenvector Following routine (10). The geometry was considered to be fully optimized when the gradient norm was less than 0.1. The single point calculations were carried out for the geometry minimized structures to obtain heat of formation, total energy, dipole moment, cosmo area, cosmo volume, HOMO and LUMO energies, electronic energy, ionization potential. Further for studying the effect of aqueous environment, all the optimizations and calculations were performed using the Conductor –like Screening Model (COSMO), a continuum approach to solvent effec (11) . The new model PM6 i.e. Parameterized Model 6 (12) was used because it is more accurate than older semi-empirical methods and also almost equally efficient as DFT method. The advantage over DFT method is its speed. It is much faster than DFT methods and results are comparable. (13) .All the calculations were performed using MOPAC 2009 (14).The generated structure files were visualized using Jmol. (Version : 11.8.24).

RESULTS AND DISCUSSION

Thermodynamic and other parameters like (Heat of Formation, Total Energy, Electronic Energy, Ionization Potential, Dipole Moment, HOMO & LUMO energies, Cosmo Area and Cosmo Volume etc.) of RGT and its metabolites were calculated and are given in Table 1. Further, to see the effect of solvent on the structure and reactivity of compounds under study, the calculations were also performed using COSMO model with dielectric constant 78.4. The electrostatic potential on molecular surface, (where red indicates negative, blue indicates positive and green indicates neutral) ,HOMOs and LUMOs are presented in Figures 2-13.

The solvation energies calculated as per heat of formation based data by using PM6 method for RGT and its metabolites M1, M4, M6, M7, M8, M9, M10, M11, M12, M13 and M16 are : -86.52219, -93.65967, -146.74344, -130.73145, -104.95904, 0, -90.81945, -140.71742, -84.63463, 0, -97.28011 and -73.45784 respectively and dipole moment in gaseous phase is : 2.84281, 3.24931, 1.82242, 4.20242, 2.49971, 3.94423, 2.25672, 4.63530, 3.14773, 2.24623, 1.47202 and 4.26321 respectively. Similarly dipole moment in aqueous phase is: 4.42403, 6.10025, 6.99828, 6.85217, 6.11388, 3.94423, 2.57437, 6.58638, 3.63256, 2.24623, 1.68822 and 4.56289 respectively. This data clearly indicates that RGT and its metabolites differ in their solubility in water.

Table 1: Calculated Thermodynamic and other Parameters of Rosiglitazone and its metabolites

Molecule	Heat of Formation (kJ)	Total Energy (eV)	Electronic energy (eV)	Ionization Potential (eV)	Dipole momer (debye)	HOMO (eV)	LUMO (eV)	Cosmo Area (square angstorms)	Cosmo Volume (cubic angstorms)	LUMO HOMO (eV)
In Gas Phase										
RGT	-292.86348	-4049.95490	-29783.4487	8.723445	2.84281	-8.723	-0.866	367.12	417.87	7.857
M1	-769.76970	-3430.89457	-20335.53249	9.590639	3.24931	-9.591	-1.165	283.43	303.87	8.426
M4	-941.65225	-5240.51717	-37576.85369	9.094417	1.82242	-9.094	-1.277	420.47	462.00	7.817
M6	-939.59628	-5240.49521	-39659.60267	8.948134	4.20242	-8.948	-1.494	402.82	461.52	7.454
M7	-461.56354	-4191.79099	-29303.97736	8.759672	2.49971	-8.760	-0.994	371.46	406.16	7.766
M8	-930.83733	-5390.02926	-42513.15504	8.742476	3.94423	-8.742	-1.455	413.75	482.96	7.287
M9	-455.82557	-4191.73203	-29621.43523	9.108137	2.25672	-9.108	-0.993	366.39	404.94	8.115
M10	-937.58368	-5390.09940	-40538.02343	8.904871	4.63530	-8.905	-1.243	434.54	482.76	7.662
M11	-456.60972	-4341.36491	-31446.36785	8.976530	3.14773	-8.977	-0.957	386.58	429.59	8.020
M12	-289.63208	-3900.29576	-27131.47507	8.902151	2.24623	-8.902	-0.992	360.79	393.49	7.910
M13	-451.89383	-4341.31495	-31297.8787	8.520081	1.47202	-8.520	-0.989	387.83	428.30	7.531
M16	-432.63531	-3217.97564	-20486.2798	8.879612	4.26321	-8.880	-0.919	305.68	327.55	7.961
In Water										
RGT	-379.38567	-4050.85260	-6154.76753	8.906831	4.42403	-8.907	-0.920	370.47	418.85	7.987
M1	-863.42937	-3431.86256	-4640.47119	9.407040	6.10025	-9.407	-1.042	279.01	306.38	8.365
M4	-1088.39569	-5242.03871	-7232.53677	9.095430	6.99828	-9.095	-1.151	418.05	467.24	7.944
M6	-1070.32773	-5241.85137	-7603.89129	9.068445	6.85217	-9.068	-1.244	403.47	465.39	7.824
M7	-566.52258	-4192.87886	-6040.24497	8.844823	6.11338	-8.845	-1.020	369.78	410.68	7.825
M8	-930.83733	-5390.02926	-42513.1550	8.742476	3.94423	-8.742	-1.455	413.75	482.96	7.287
M9	-546.64502	-4192.67321	-6074.41140	9.167554	2.57437	-9.168	-1.029	367.05	407.64	8.139
M10	-1078.30110	-5391.55737	-7774.97140	8.839795	6.58638	-8.840	-1.100	425.74	490.75	7.740
M11	-541.24435	-4342.24200	-6893.47708	9.061661	3.63256	-9.062	-1.024	370.65	434.37	8.038
M12	-289.63208	-3900.29576	-27131.4750	8.902151	2.24623	-8.902	-0.992	360.79	393.49	7.910
M13	-549.17394	-4342.32300	-6312.01334	8.651942	1.68822	-8.652	-1.024	389.30	431.80	7.628
M16	-506.09315	-3218.73702	-4513.96445	9.104240	4.56289	-9.104	-1.024	305.78	330.40	8.080

The calculated LUMO-HOMO energy difference for RGT and its metabolites shows that they differ in their kinetic lability. It has been found that metabolites M6 and M8 possess the lowest LUMO-HOMO energy difference in gaseous phase and thus are the most labile in gaseous phase. Similarly, in aqueous phase, metabolites M8 and M13 are found to be with the lowest LUMO-HOMO energy difference and are most labile in aqueous phase. The metabolite M1 has been found to possess the highest LUMO-HOMO energy difference in both gas and water and hence expected to be the least reactive. The highest solvation energy is possessed by the metabolite M4 and M10 and hence are most soluble in water and least soluble in lipid and readily excreted in urine .The experimental findings also support this fact (1).On the other hand, metabolites M8 and M12 possess least solvation energies and hence these are least soluble in water and not readily excreted in urine.

The metabolites M6 and M8 show dominating electron-deficient regions on their molecular surfaces and react readily with reduced glutathione and bases in DNA and cause decrease in the level of glutathione and damage to DNA respectively .The decreased level of glutathione causes cellular toxicity which leads to oxidative stress .

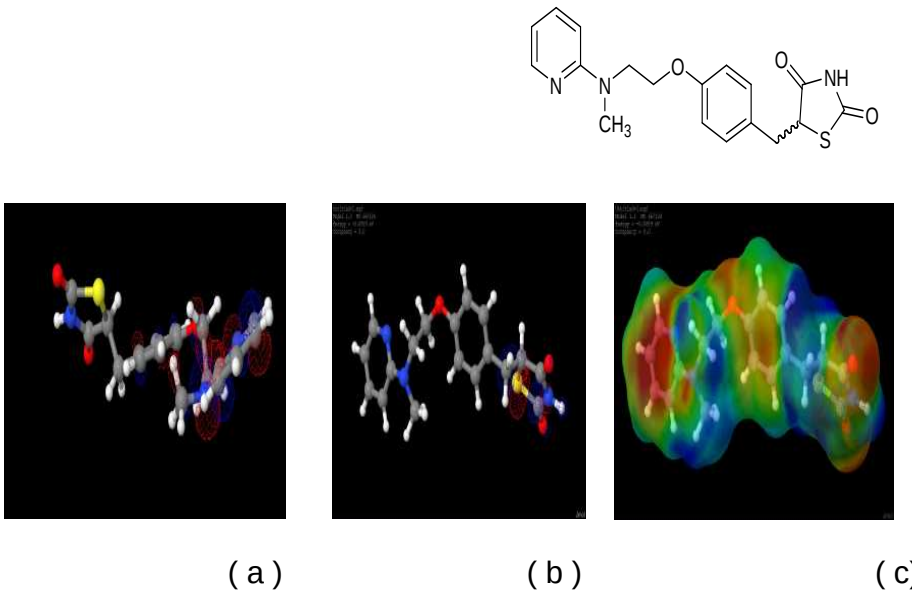
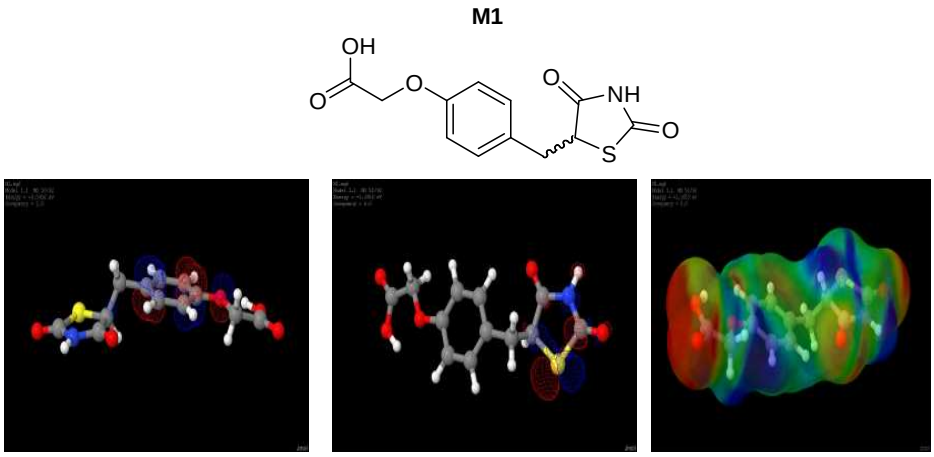
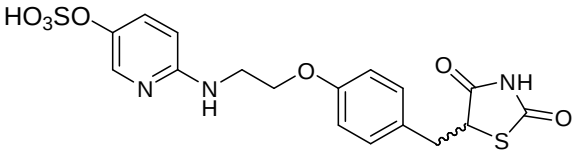


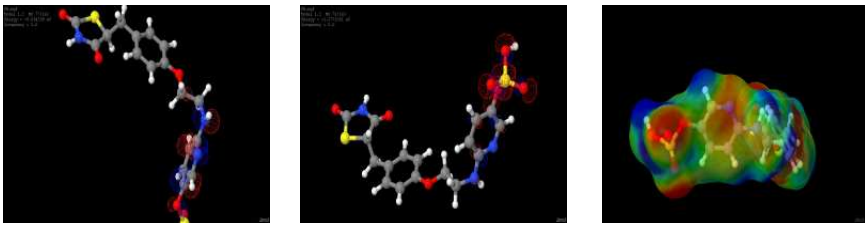
Figure 2: Structure of RGT : (a) HOMOs,(where red indicates HOMOs with high electron density) (b)LUMOs (where blue indicates LUMOs) and (c) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral).



(a) (b) (c)

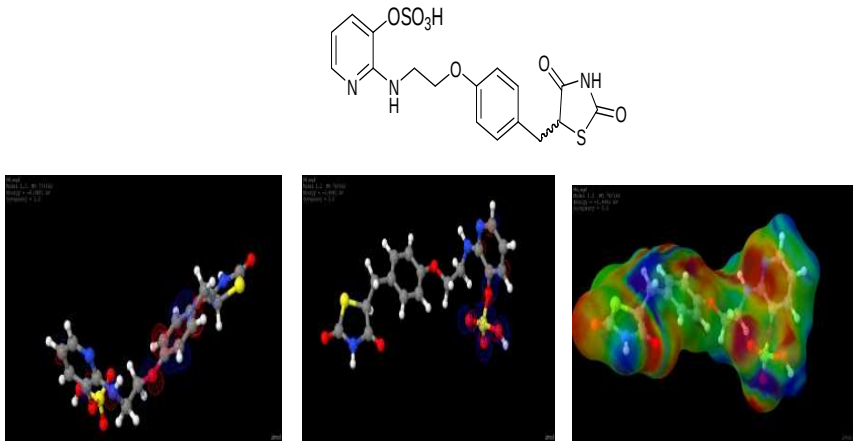
Figure 3: Structure of M1 : (a) HOMOs,(where red indicates HOMOs with high electron density) (b) LUMOs (where blue indicates LUMOs) and (c) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral).





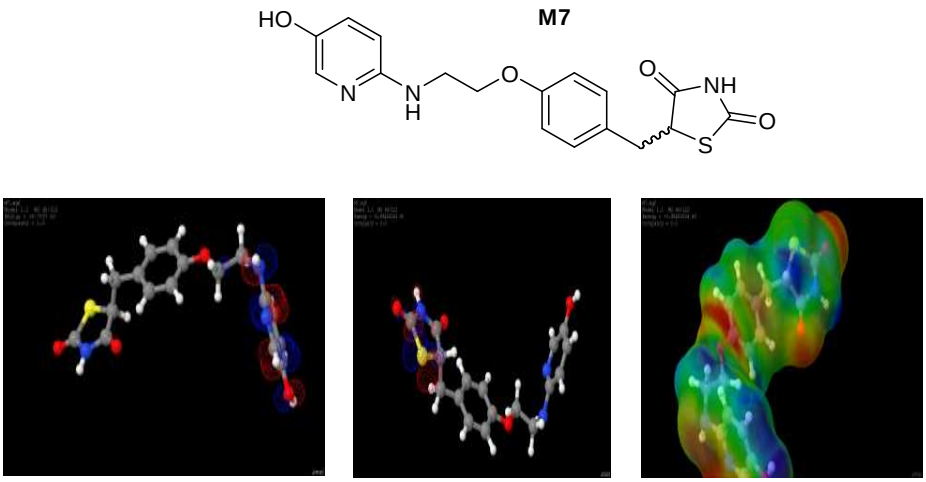
(a) (b) (c)

Figure 4: Structure of M4: (a) HOMOs,(where red indicates HOMOs with high electron density) (b) LUMOs (where blue indicates LUMOs) and (c) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral).



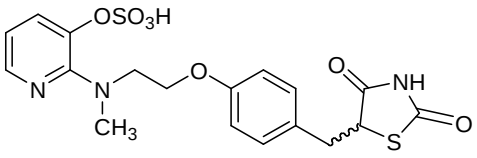
(a) (b) (c)

Figure 5: Structure of M6: (a) HOMOs,(where red indicates HOMOs with high electron density) (b) LUMOs (where blue indicates LUMOs) and (c) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral).



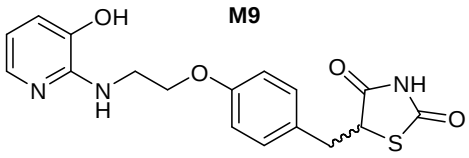
(a) (b) (c)

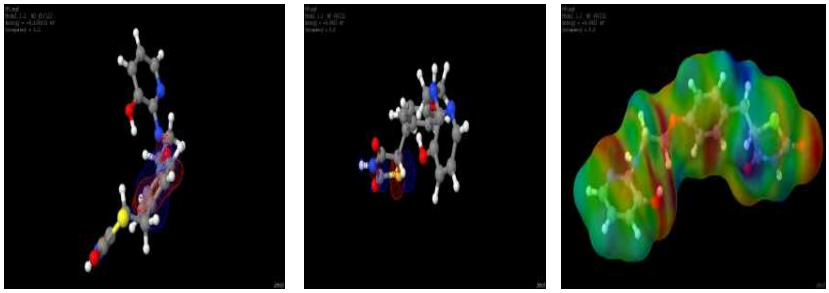
Figure 6: Structure of M7: (a) HOMOs,(where red indicates HOMOs with high electron density) (b) LUMOs (where blue indicates LUMOs) and (c) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral).



(a) (b) (c)

Figure 7: Structure of M8: (a) HOMOs,(where red indicates HOMOs with high electron density) (b) LUMOs (where blue indicates LUMOs) and (c) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral).





(a) (b) (c)

Figure 8: Structure of M9: (a) HOMOs,(where red indicates HOMOs with high electron density) (b) LUMOs (where blue indicates LUMOs) and (c) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral).

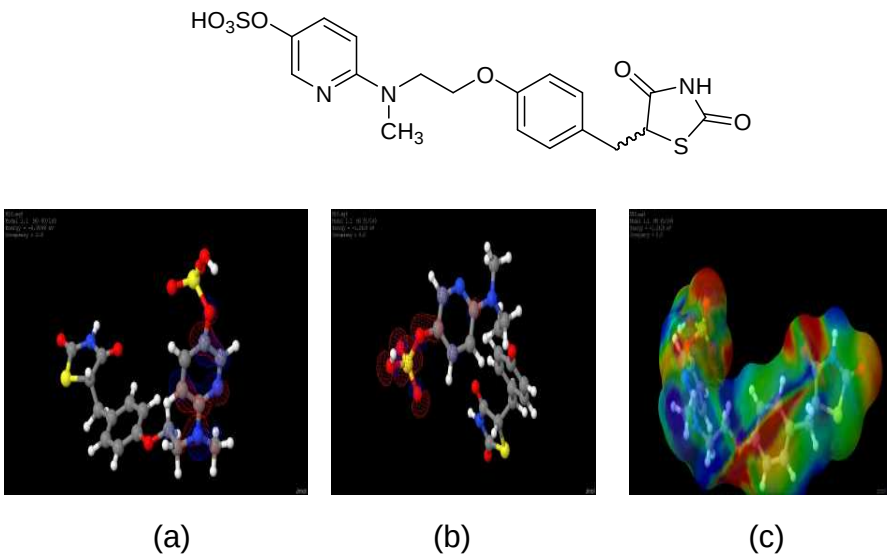


Figure 9: Structure of M10: (a) HOMOs,(where red indicates HOMOs with high electron density) (b) LUMOs (where blue indicates LUMOs) and (c) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral).

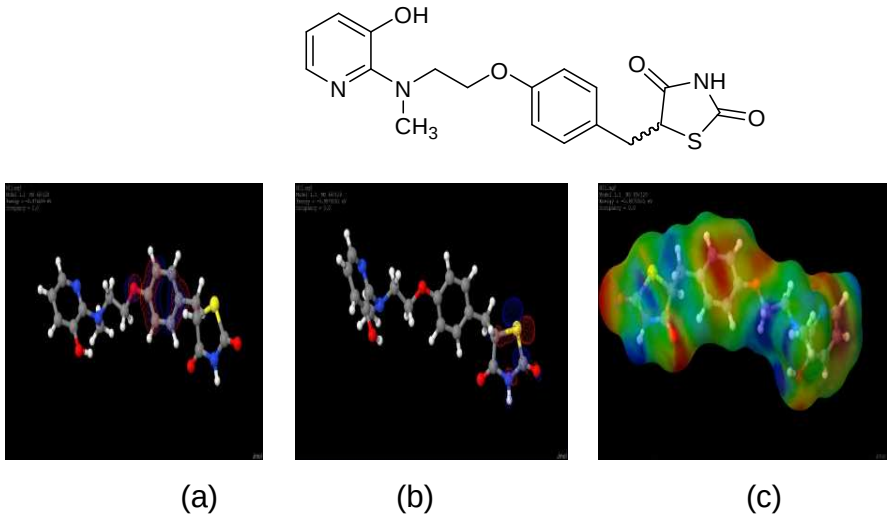
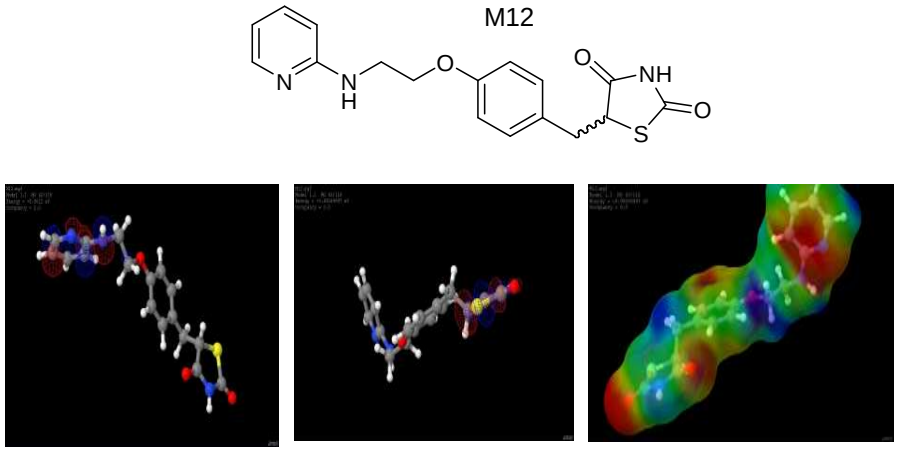
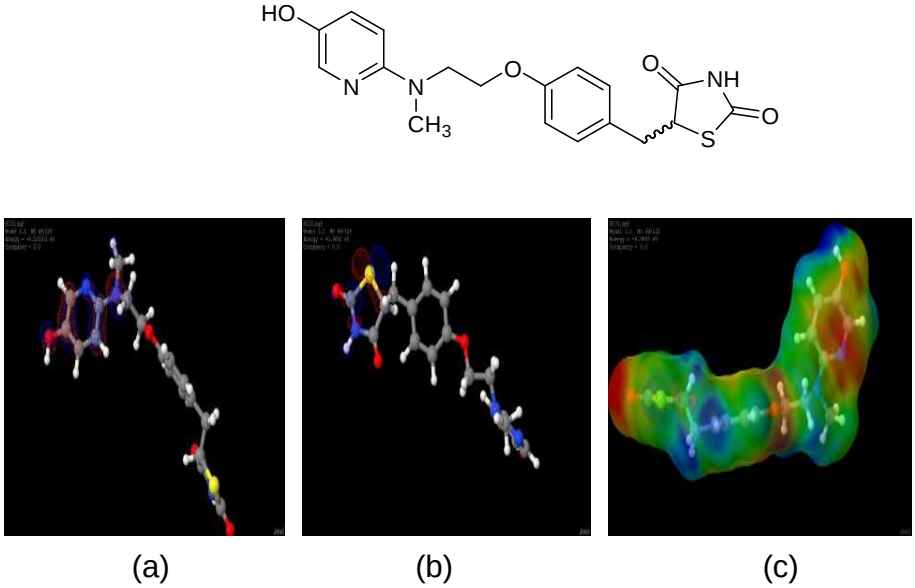


Figure 10: Structure of M11: (a) HOMOs,(where red indicates HOMOs with high electron density) (b) LUMOs (where blue indicates LUMOs) and (c) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral).



(a) (b) (c)

Figure11: Structure of M12: (a) HOMOs,(where red indicates HOMOs with high electron density) (b) LUMOs (where blue indicates LUMOs) and (c) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral).



(a) (b) (c)

Figure12: Structure of M13: (a) HOMOs,(where red indicates HOMOs with high electron density) (b) LUMOs (where blue indicates LUMOs) and (c) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral).

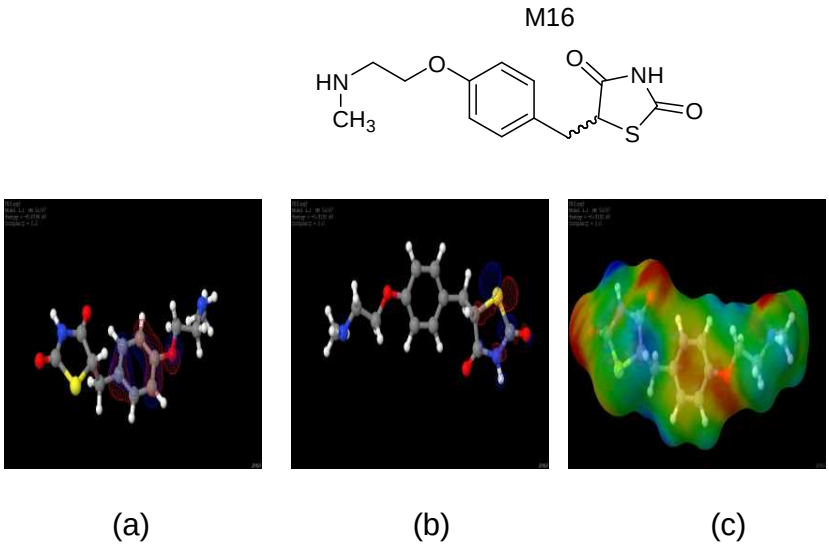


Figure13: Structure of M16: (a) HOMOs,(where red indicates HOMOs with high electron density) (b) LUMOs (where blue indicates LUMOs) and (c) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral).

In most of the metabolites of RGT, the HOMOs with high electron density are found mainly near pyridine ring region whereas LUMOs are centred mainly on thiazolidinedione ring suggesting that the pyridine ring and thiazolidinedione rings are susceptible to electrophilic and nucleophilic attack respectively. All the gaseous as well as aqueous phase metabolites are more reactive than RGT towards nucleophilic attack as indicated by lowest electron affinity (-LUMO) of RGT. M1 has the highest ionization potential energy. So, it will be most susceptible towards electrophilic attack.

The comparison of cosmo volume of RGT with its metabolites shows that M7 and M13 closely match with RGT cosmo volume. Therefore, M7 and M13 can be a substrate for the binding site of PPAR-γ receptor. The potency of M13 has also been proved experimentally and it is found equipotent with RGT. (1)

Flurbiprofen and Indomethacin cause a slow, time dependent inhibition of COX-1 and COX-2, apparently via formation of salt bridge between a carboxylate on the drug and Arg -120 (15). Similarly, metabolite M1 contains phenoxyacetic acid group which can also form salt bridge with Arg-120. Also, both compounds have comparable cosmo volume (303.87 CUBIC ANGSTROMS for M1 and 292.78 CUBIC ANGSTROMS for flurbiprofen). In figure-14, overlay of both compounds is shown which indicates that metabolite M1 can fit into the site of COX.

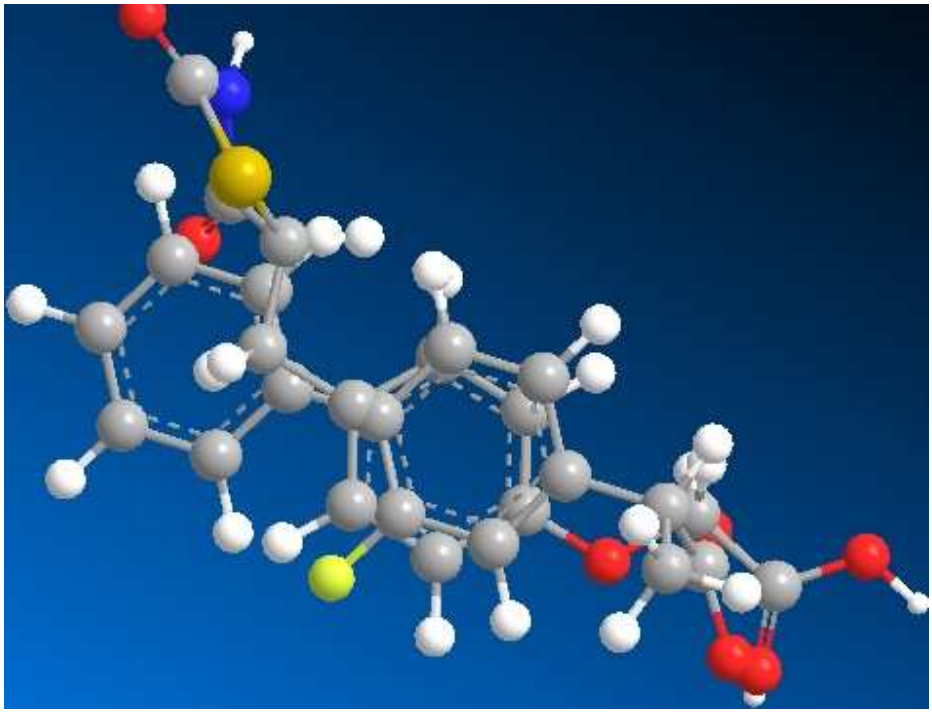


Figure 14: Overlay of metabolite M1 and flurbiprofen

CONCLUSION

Rosiglitazone is an antihyperglycaemic drug that acts through peroxisome proliferator –activated receptor gamma and decreases hyperglycemia by reducing insulin resistance in patients with type 2 diabetes mellitus. The major routes of metabolism of Rosiglitazone are N-demethylation and hydroxylation with subsequent conjugation .Molecular modeling study has been performed by using PM6 method which has not been used earlier in any study of this type .The study shows that metabolites M4 and M10 are readily excreted in urine because of their highest solvation energy. This fact has been proved experimentally also. The metabolite M13 is the most susceptible to electrophilic attack because of highest HOMO energy .Further, the metabolite M1 might be responsible for edema as it contains acidic group which can form salt bridge with Arg-120 of COX 1. Overlay of these two compounds shows that M1 can bind with COX. Further insight into this matter is needed.

Abbreviations:

RGT	: Rosiglitazone
LUMO	: Lowest unoccupied molecular orbital
HOMO	: Highest occupied molecular orbital
COX	: Cycloxygenase
Arg	: Arginine
CYP450	: Cytochrome P450
COSMO	: Conductor –like Screening Model

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