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Original Article

In-silico study, synthesis and biological evaluation of some substituted xanthone derivatives as alpha-glucosidase inhibitor

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ABSTRACT

Xanthone possess varieties of pharmacological activities associated with tricyclic scaffold depending on the position of different substituent(s). The main aim of this paper is to present a series of substituted hydroxy and alkoxy xanthone derivatives as novel α-glucosidase inhibitors. 27 xanthone derivatives were screened to identify α-glucosidase inhibitor with the help of molecular docking by using Discovery Studio software version 2.5. In the *in-silico* study designed compound showed binding energy ranging from **-31.02207** to **-121.31928 kcal/mol**. Compound P16 showed highest binding energy followed by P7, P13, P8, P15, and P1. Five best scoring ligands were randomly selected and synthesized. The structure of the synthesized compounds were established by means of FT-IR, NMR (^{1}H and ^{13}C) and Mass spectrum data. All the synthesized compounds were tested for their anti-diabetic activity. 3,6-bis(hydroxyethoxy)9H-xanthene-9-one; 3,6-bis(benzyloxy)9H-xanthene-9-one and 3,6-bis(heptyloxy) 9H-xanthene-9-one showed significant anti-diabetic activity as compared to the standard drug Miglitol. 3,6-bis(oxiran-2-ylmethoxy)9H-xanthene-9-one showed moderate and 1-hydroxy-3-(phenethoxy)9H-xanthene-9-one showed less anti-diabetic activity as compared to the standard drug. From the above result it may be concluded that substituted hydroxy and alkoxy xanthone derivatives are potential key approach to design of new α-glucosidase inhibitors.

Keywords: alpha-glucosidase inhibitors, anti-diabetic activity, molecular docking study, xanthone derivatives

INTRODUCTION

Xanthones are a class of heterocyclic compounds containing simple three-membered rings and they have diversed physicochemical and pharmacological activities. They are active against a wide variety of pathogens and have exhibited interesting pharmacological properties such anticancer [1], antioxidant [2], antifungal [3], anti-viral [4], anti-inflammatory [5], anti-mycobacterial [6], anti-diabetic [7] etc.

Various synthetic xanthone derivatives have been reported as potent anti-diabetic agents. In this project, our main target to enhance the anti-diabetic activity by modifying the phenolic hydroxyl group of xanthone moiety with other alkoxy substituent(s). In this project, we have done molecular docking study which help us to identify new molecule come towards the market. Molecular docking studies are performed to generate the bio-active binding poses of α-glucosidase inhibitors in the active site of the protein by using the LibDock program from Discovery Studio 2.5 (Accelrys, San Diego, CA, USA).

MATERIALS AND METHOD

Retrieval of 3D Structure: The 3D structure of the protein was downloaded from RCSB (Research Collaboratory for Structural Bioinformatics), Protein Data Bank (PDB, http://www.pdb.org). The PDB ID of the selected protein was found to be 3L4W [8] and downloaded as PDB text file format. All the bound water molecules and co-crystallized ligand attached to the protein have been removed.

Analogue design: Several polyhydroxy derivatives of xanthone such as 3-hydroxy; 1,3-dihydroxy; 3,6-dihydroxy; 1,3,6-trihydroxy, 1,3,7- trihydroxy; 1,3,6,7,8-penta hydroxy xanthones were proven as potential anti anti-diabetic agents as published in numerous literatures. In this study, the one hydroxyl groups at 3rd position of 1,3- dihydroxy xanthone series; two hydroxyl group at 3,6 position of 3,6-dihydroxyxanthone series and one hydroxyl

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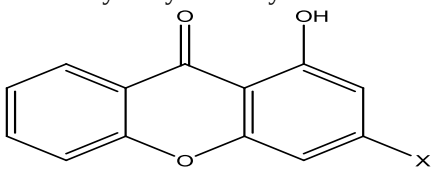
group at 3rd position of 3-hydroxy xanthone series was substituted with other alkoxy substituents to obtain the targeted compounds. The three series of xanthone derivatives along with their different substituent(s) are given in the Table 1, Table 2, Table 3 .

Series I: 1-hydroxy-3-alkoxy substituted xanthone

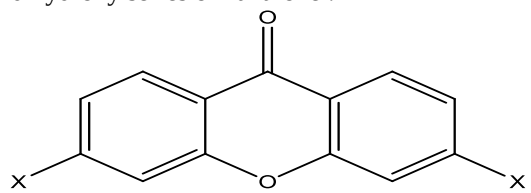


X=alkoxy substituent(s)

Table 1: 1-hydroxy-3alkoxy series of xanthone derivatives along with their different substituent(s) :

Ligand code	Substituent's (X)	Name of the compound
P1	OC_5H_{11}	1-hydroxy-3-(pentyloxy) xanthone
P2	OC_6H_{13}	1-hydroxy-3-(hexyloxy) xanthone
P3	OC_7H_{15}	1-hydroxy-3-(heptyloxy) xanthone
P4	OC_8H_{17}	1-hydroxy-3-(octyloxy) xanthone
P5	$OCH_2C_6H_5$	3-(benzyloxy) -1-hydroxy xanthone
P6	$OCH_2CH_2C_6H_5$	1-hydroxy-3-(phenethoxy) xanthone
P7	$OCH_2C_3H_2O$	1-hydroxy-3-(oxiran 2ylmethoxy)xanthone
P8	OCH_2CH_2OH	1-hydroxy-3-(2-hydroxyethoxy) xanthone

Series II : 3,6-dihydroxy series of xanthone :

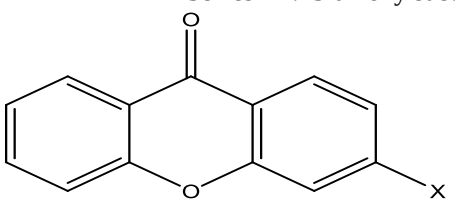


X=alkoxy substituent(s)

Table 2 : 3,6-dihydroxy series of xanthone derivatives along with their different substituent(s)

Ligand code	Substituent's (X)	Name of the compound
P9	OC_5H_{11}	3,6-bis(pentyloxy) xanthone
P10	OC_6H_{13}	3,6-bis(hexyloxy) xanthone
P11	OC_7H_{15}	3,6-bis(heptyloxy) xanthone
P12	OC_8H_{17}	3,6-bis(octyloxy) xanthone
P13	$OCH_2C_6H_5$	3,6-bis(benzyloxy) xanthone
P14	$OCH_2CH_2C_6H_5$	3,6-bis(phenethoxy) xanthone
P15	$OCH_2C_3H_2O$	3,6-bis(oxiran 2ylmethoxy)xanthone
P16	OCH_2CH_2OH	3,6-bis(2-hydroxyethoxy) xanthone

Series III: 3-alkoxy substituted xanthone



X=alkoxy substituent(s)

Table 3 : 3-alkoxy substituted xanthone derivatives along with their different substituent(s)

Ligand code	Substituent's (X)	Name of the compound
P17	OC_5H_{11}	3-(pentyloxy) xanthone
P18	OC_6H_{13}	3-(hexyloxy) xanthone
P19	OC_7H_{15}	3-(heptyloxy) xanthone
P20	OC_8H_{17}	3-(octyloxy) xanthone
P21	$OCH_2C_6H_5$	3-(benzyloxy) xanthone
P22	$OCH_2CH_2C_6H_5$	3-(phenethoxy) xanthone
P23	$OCH_2C_3H_2O$	3-(oxiran- 2-ylmethoxy)xanthone
P24	OCH_2CH_2OH	3-(2-hydroxyethoxy) xanthone

Ligand Preparation, toxicity and drug likeness: Chem Draw Ultra 11.0, freeware was used for drawing and converting 2D chemical structure of the compound to 3D structure (.mol format) before submission for docking studies. The energies of ligand structures were previously minimized using the semi-empirical AM1 method with Discovery Studio 2.5 software.OSIRIS property explorer [9] was used to screen the drug likeness. Toxicity risks were also evaluated by calculating mutagenic, tumorigenic, irritant, reproductive effective, solubility, drug likeliness and drug score which are given in the Table 4.*In silico* prediction, the molecular properties of the compounds were calculated such as molecular weight, LogP, MiLogP, (nRB) number of rotatable bonds and Total Polar Surface Area (TPSA), nON, nOHNH, nViolations(number of violations)which are given in the Table 5. After calculating OSIRIS property 10 best score ligands were selected for docking studies and their Molecular properties were calculated out using **Molinspiration Property calculator** and **Chemdraw ultra 11.0 software.**

Table 4: Solubility, drug score and drug likeness accounted by OSIRIS Property of the substituted xanthone derivatives are given in the following table

Compound Code	Solubility	Drug likeness	Drug Score
P16	-4.55	-6.74	0.13
P8	-4.44	-0.07	0.34
P13	-7.61	-11.72	0.05
P7	-7.00	-0.06	0.04
P15	-7.92	-1.41	0.15
P1	-5.76	-8.02	0.15
P24	-4.74	-1.13	0.27
P6	-6.08	-1.34	0.17
P21	-6.27	-6.15	0.14
P9	-7.18	-14.76	0.05

Table 5: Molecular properties were calculated for the following novel substituted xanthone derivatives

Compound Code	MilogP	nRB	TPSA	nON	nOHNH	nViolations
P16	**3.067**	**0**	**89.135**	**6**	**2**	**0**
P8	**2.95**	**4**	**79.901**	**5**	**2**	**0**
P13	**6.825**	**6**	**48.679**	**4**	**0**	**1**
P7	**3.145**	**3**	**72.201**	**5**	**1**	**0**
P15	**3.302**	**6**	**73.735**	**6**	**0**	**0**
P1	**5.255**	**5**	**59.673**	**4**	**1**	**0**
P24	**2.971**	**3**	**59.673**	**4**	**1**	**1**
P6	**5.116**	**4**	**59.673**	**4**	**1**	**1**
P21	**5.198**	**3**	**39.445**	**3**	**0**	**1**
P9	**7.522**	**10**	**48.679**	**4**	**0**	**1**

(TPSA=Total Polar Surface Area, nRB = number of rotatable bond, nOHNH= number of OH & NH bonds, nRB = number of rotatable bond)

Protein-Ligand Docking Studies: Docking studies were carried out using Discovery Studio version 2.5 software to understand the alpha glucosidase inhibitory behaviour of the designed compound. The scoring function and a number of hydrogen bondings formed with the surrounding amino acids are used to predict their binding modes, binding affinities in the active sites of the protein. Lamarckian genetic algorithm (LGA) which is a hybrid of a genetic algorithm and a local search algorithm, were used for ligand conformational searching. The active site of the enzyme was found to include residues within a 10.0Å radius to any of the inhibitor atoms and the scoring functions for docked compounds were calculated from minimized ligand-protein complexes.

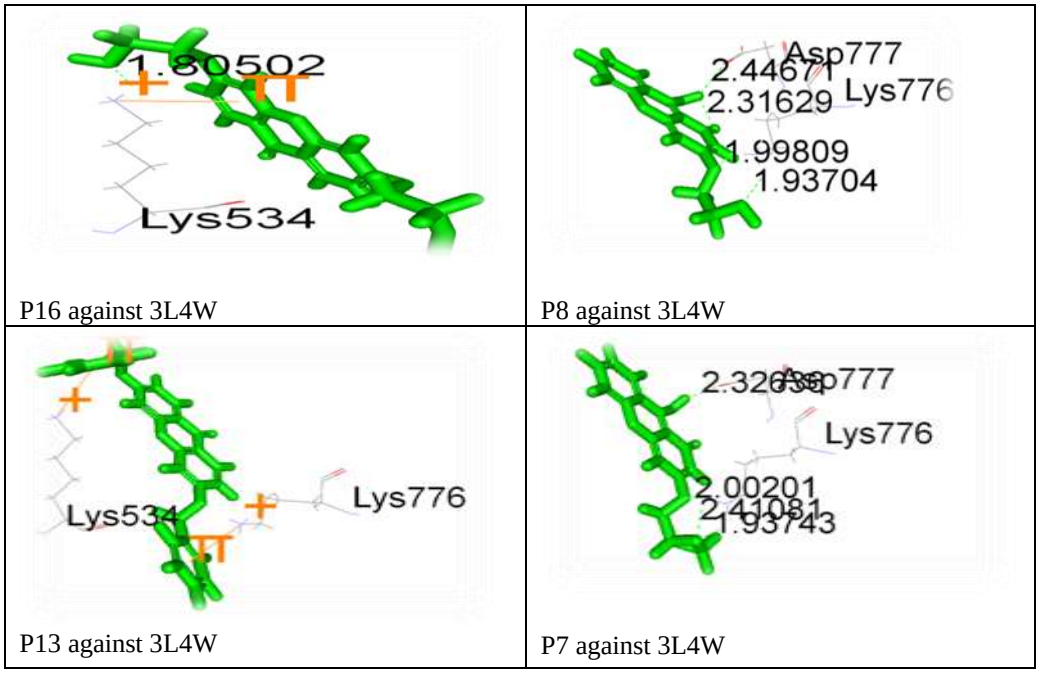


Fig 1: The interactions of some ligands against 3L4W
'.....' indicates hydrogen bonding interaction, '+'indicates cationic interaction and 'π' indicates Pi interaction

Binding Site Analysis: Binding Site analysis is a fast detection program for 'the identification and visualization of possible binding sites' and 'the distribution of surrounding residues in the active sites'. Scoring function which estimates the free binding energy of the ligand from a given pose was used to rank the final poses. For the improvement of the docking results, force field evaluation, torsional and rigid body movements of ligand is quantified. Ligands with their mode of interactions with different residues present on the binding sites of the protein (**PDB ID: 3L4W)** during docking simulations are given in the Table 6. The ligand-enzyme complex with lowest score was selected.

SYNTHETIC METHOD

After docking studies,five best scoring ligands were randomly selected and synthesized to check its anti-diabetic activity.Those were MM1[3,6-bis(hydroxyethoxy)9H-xanthene-9-one]; MM2[3,6-bis(benzyloxy)9H-xanthene-9-one]; MM3[3,6-bis(oxiran-2-ylmethoxy)9H-xanthene-9-one];MM4[1-hydroxy-3-phenethoxy-9H-xanthen-9-one] and MM5 [3,6-bis(heptyloxy) 9H-xanthene-9-one]. Their binding energy (docking score) with substituent's are represented in Table 7.

Table 6: Ligands with their mode of interactions with different residues present on the binding sites of the protein (PDB ID: 3L4W) during docking simulations.

Compc ode	Residues involves in hydrogen bonding interactions	Residues involed in hydrophobic interactions	Binding energy (Kcal/mol)
P16	Lys534	----	-121.31928
P8	Asp777	Lys776	-111.52668
P13	----	Lys534,Lys776	-96.79375
P7	Asp777,Lys776	----	-93.62844
P15	Lys776	----	-88.42792
P1	Lys776	Asp777	-86.11852
P24	Lys776	Arg520	-85.47606
P6	----	Lys776	-80.93656
P21	----	Lys776,Lys534,Arg283	-79.54650
P9	Lys776	Lys534	-78.65802

Table 7 : Ligands Selected For Synthesis After Docking Studies

Ligand code	Compound code	Substitutent's	Binding energy (Kcal/mol)
P18	MM1	3,6-OCH_2CH_2OH	-121.31928
P15	MM2	3,6-$OCH_2C_6H_5$	-96.79375
P17	MM3	3,6-$OCH_2C_2H_3O$	-88.42792
P7	MM4	1-OH, 3-$OCH_2CH_2C_6H_5$	-80.93656
P13	MM5	3,6-OC_7H_{15}	-61.53474

Materials

Table 8 : List of chemicals used for synthetic work

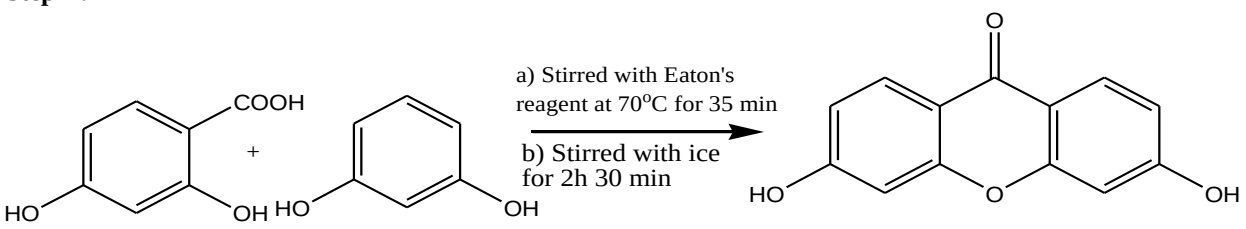
Name of the Chemicals	Specification	Manufacturer
Methanesulphonic acid	LR Grade	Spectrochem Pvt. Ltd Mumbai
Acetone AR	LR Grade	
Streptozotocin	LR Grade	
Pentylchloride or 1-chloropentane	LR Grade	
1-chlorohexane	LR Grade	
1-bromo heptanes	LR Grade	
1-bromo octane	LR Grade	
Benzyl chloride	LR Grade	
Epichlorohydrin	LR Grade	
2-chloroethanol	LR Grade	
Phloroglucinol	LR Grade	HiMedia Lab. Pvt. Ltd Mumbai
Salicylic acid	AR Grade	

2,4- dihydroxy benzoic acid	LR Grade	
Resorcinol	LR Grade	
Phosphorus pentoxide	LR Grade	
(2-bromo ethyl)benzene	LR Grade	
(2-bromo ethyl)benzene	LR Grade	
Tween 80	LR Grade	
Silica Gel-G for TLC	LR Grade	CDH Pvt. Ltd. New Delhi-02
Petroleum ether	LR Grade	Rankem, Ranbaxy fine chemicals Ltd.(RFCL), New Delhi
Potassium carbonate	LR Grade	

METHODS

Synthesis of MM1,MM2, MM3 and MM5

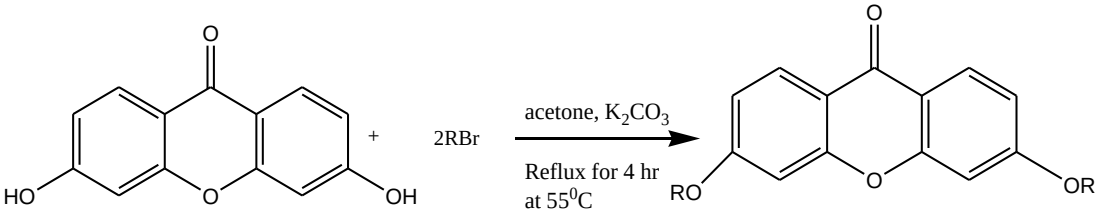
Step I :



2,4-dihydroxy benzoic acid — Resorcinol — 3,6-dihydroxyxanthone int.1

Procedure: Eaton's reagent was prepared by dissolving phosphorus pentoxide in methane sulphonic acid in 1:10 ratio i.e.10gm phosphorus pentoxide was dissolved in 100 ml methane sulphonic acid. 100 ml of Eaton's reagent was added slowly to a mixture of 2, 4-dihydroxy benzoic acid (60 mmol) and Resorcinol (60 mmol). The mixture was warmed up to 70˚C for 35 min under stirring. Then cooled to room temperature and poured the reaction mixture into an ice, maintain temperature 0-4˚C and stirred for 2hr 30 min. The resulting solid collected by filtration, washed with water until ph 6 and dried at 60˚C [10].

Step II:



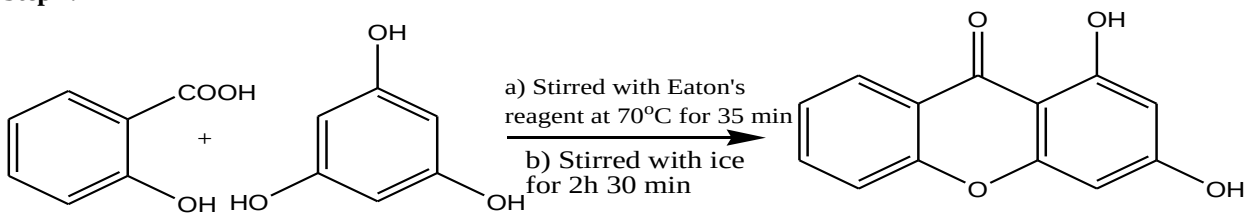
3,6-dihydroxy xanthone — Alkyl bromide — 3,6-bis (alkoxy substituted)xanthone

MM1: R=CH_2CH_2OH
MM2: R=$CH_2C_6H_5$
MM3: R=$CH_2C_2H_3O$
MM5: R=C_7H_{15}

Procedure: To a solution of 3, 6-dihydroxy xanthone (2 mmol) and 2-chloroethanol (for MM1), Benzyl chloride (for MM2), Epichlorohydrin (for MM3), 1-bromo heptanes (for MM5) (3 mmol) in acetone (50-60 mL) was added K_2CO_3 (2.5 mmol). The mixture was refluxed at 55˚C under stirring for 4 hrs. After cooling, the mixture was filtered and the organic filtrate was concentrated as yellow solid [7].

Synthesis of MM4

Step I:

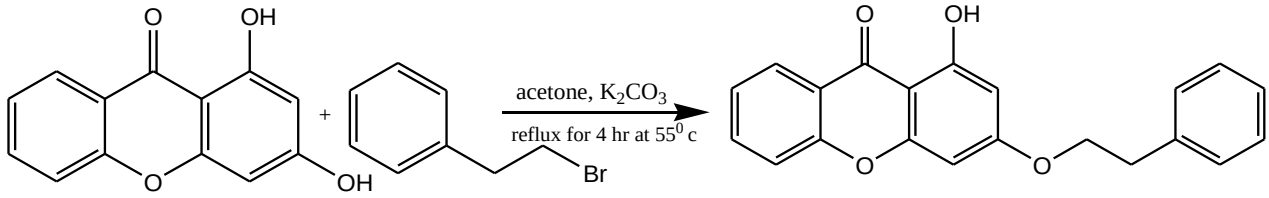


Salicylic acid Phloroglucinol 1,3-dihydroxyxanthone Int-2

Procedure:

Eaton's reagent was prepared by dissolving phosphorus pentoxide in methane sulphonic acid in 1:10 ratio i.e.10gm phosphorus pentoxide was dissolved in 100 ml methane sulphonic acid. 100 ml of Eaton's reagent was added slowly to a mixture of salicylic acid (60 mmol) and Phloroglucinol (60 mmol). The mixture was warmed up to 70˚C for 35 min under stirring. Then cooled to room temperature and poured the reaction mixture into an ice, maintain temperature 0-4˚C and stirred for 2hr 30 min. The resulting solid collected by filtration, washed with water until pH 6 and dried at 60˚C [10].

Step II:



1,3-dihydroxy xanthone 2-bromo ethyl benzene 1-hydroxy-3-(phenethoxy)9*H*-xanthen-9-one

Procedure: To a solution of 1,3-dihydroxy xanthone (2 mmol) and 1-bromo heptane (3 mmol) in acetone (50-60 mL) was added K_2CO_3 (2.5 mmol). The mixture was refluxed at 55˚C under stirring for 4 hrs. After cooling, the mixture was filtered and the organic filtrate was concentrated as off yellow solid [7].

CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS

Physicochemical characterization: All the synthesized compounds were characterized by various analytical methods. Physiochemical properties like physical state, colour, melting point, Rf value [11] of the synthesized compounds are given in Table 9.

Table 9 : Physicochemical characterisation of synthetic compound

Comp.code	State	Colour	Melting point(˚c)	Mol. wt(g)	Rf value*
MM1	Solid	Yellow	237-238˚C	316.30	0.65
MM2	Solid	Brownish yellow	247-249˚C	408.14	0.58
MM3	Solid	Yellow	232-234˚C	340.09	0.80
MM4	Solid	Greyish yellow	240-243˚C	332.10	0.67
MM5	Solid	Deep yellow	239-241˚C	424.26	0.63

* Solvent system for TLC- Acetone: Ethyl acetate: Methanol:: 3:1:1

SPECTRAL ANALYSIS

All the synthesized compounds were characterized with UV-VIS spectrophotometry, FTIR, ^{1}H [12] and ^{13}C NMR spectrum and MASS spectrum.

Compound MM1: 3,6-bis(hydroxyethoxy)-9H-xanthene-9-one: UVλmax (Acetone) : 542 nm; FTIR (cm^{-1}) : 3503.15 (O-H stretch, phenolic),2855.50 (C-H Str. aromatic), 1514.94 (C=C Aromatic, Str.in ring), 1679.25 (C=O str., keto group), 1238.44 (C-O str.,6 membered cyclic ether); ^{1}H NMR(400MHz) $DMSOd_{6,}$ δ(ppm) : 3.6541 (OH, alcohol); 3.6817, 4.4888 ($CH_{2,}$methylene); 6.8309, 6.8498, 7.9002 (CH, 1-benzene); MASS (m/z %) : 317.1106(M^{+})

Compound MM2: 3, 6-bis(benzyloxy)-9H-xanthene-9-one: UV λmax (Acetone) : 544 nm; FTIR (cm^{-1}) : 2900.07 (C-H Str. aromatic), 1612.18 (C=C Aromatic,Str. in ring), 1693.34 (C=O str., keto group),1101.11 (C-O str., 6 membered cyclic ether); ^{1}H NMR(400MHz) $DMSOd_{6,}$δ(ppm) : 5.1205 ($CH_{2,}$ methylene); 6.6888, 7.3637, 6.7493, 7.7887, 7.3848 (CH, 1-benzene); MASS (m/z %) : 409.26(M^{+})

Compound MM3: 3,6-bis(oxiran-2-ylmethoxy)-9H-xanthene-9-one: UV λmax (Acetone) : 544 nm; FTIR (cm^{-1}) : 2888.22 (C-H Str. aromatic), 1513.76 (C=C Aromatic, Str. in ring), 1693.77 (C=O str., keto group),1097.56 (C-O str., 6 membered cyclic ether); ^{1}H NMR(400MHz) $DMSOd_{6,}$ δ(ppm) : 2.4951,2.5276 ($CH_{2,}$ oxiran); 3.6731(CH, oxiran);6.5220,7.1372,7.5273 (CH, 1-benzene);3.9978,4.2070 ($CH_{2,}$ methylene); MASS (m/z %): 340.25 (M^{+})

Compound MM4: 1-hydroxy-3-(phenethoxy)-9H-xanthene-9-one: UV λmax (Acetone): 556 nm; FTIR (cm^{-1}): 3215.91 (O-H str. phenolic), 2900.07 (C-H Str. aromatic), 1519.66 (C=C Aromatic, Str.in ring), 1741.47 (C=O str., keto group),1051.90 (C-O str., 6 membered cyclic ether); ^{1}H NMR(400MHz) $DMSOd_{6,}$ δ(ppm): 2.5124, 3.4178 ($CH_{2,}$methylene); 5.3984 (OH, aromatic, C-OH); 5.5303,5.5268, 7.2438, 7.5704,7.9305(CH,1-benzene); ^{13}CNMR (400MHz) $DMSOd_{6,}$δ(ppm):38.82, 79.17 ($CH_{2,}$ aliphatic), 102.12,122.70, 154.67,157.86, 162.45 (C,1-benzene),180.37(C,1-carbonyl), 116.55, 120.82, 124.46, 132.90 (CH, 1-benzene); MASS (m/z %): 333.12 (M^{+})

Compound MM5: 3,6-bis(heptyloxy)-9H-xanthene-9-one: UV λmax (Acetone): 542 nm; FTIR (cm^{-1}): 2920.70,2855.04 (C-H Str. aromatic), 1503.15 (C=C Aromatic, Str.in ring), 1698.36 (C=O str., keto group),1162.45 (C-O str., 6 membered cyclic ether); ^{1}HNMR(400MHz) $DMSOd_{6,}$δ(ppm):6.4579,7.4971(CH,1-benzene); 1.3515, 1.3714, 1.3907, 1.7162,3.9665 ($CH_{2,}$ methylene); ^{13}C NMR (400MHz) $DMSOd_{6,}$ δ(ppm):158.78,163.92,113.52 (C,1-benzene);172.15(C,1-carbonyl); 101.61,108.50, 131.01(CH,1-benzene); 67.51, 28.54,25.42, 28.39,31.20,22.02 ($CH_{2,}$ aliphatic); 13.92 ($CH_{3,}$ aliphatic); MASS (m/z %) : 425.25 (M^{+})

ANTI-DIABETIC ACTIVITY EVALUATION

The activity of synthesized compounds were evaluated by –

Acute Toxicity Study: The toxicity of the proposed synthesized drugs were determined according to the OECD-423 guidelines (acute toxic class method). The animals were kept fasting overnight and provided water *ad-libitum*. The synthesized drugs were administrated orally at 5mg/kg body weight. If mortality was not observed, the procedure was repeated for higher doses such as 50,100, 500, 1000 and 1500mg/kg body weight. Animals were observed individually after dosing for first 30 min, 1hr, 2hr and daily thereafter, till 15 days. No serious toxicity signs or death was observed during the whole acute toxicity studies for each compounds upto dose level 1500mg/kg body weight.

Induction of Diabetes: Streptozotocin (65mg/kg; *i.p.*) was prepared in cold citrate buffer (0.1M, pH 4.5) and used for induction of Type 2 diabetes [13] and injected in overnight fasting experimental rats. Diabetes was confirmed by measuring blood glucose level after 72 hrs of injecting STZ. Every time blood from tail vein of rats is collected and Blood glucose was measured in AccuSure Glucometer™ (TaiDoc Technology Corporation) during whole study. Animals were kept in laboratory condition for one week to stabilize Diabetes and animals showing blood glucose >220mg/dl taken for activity assessment of synthesized drugs.

Experimental Design: Animals were divided into 13 groups and each group containing five animals. The 1st group that was the control group which received vehicle (1%Tween 80) and the 2nd group was considered as diabetic control and no drug administered throughout the study period. The 3rd group received standard drug Miglitol (25mg/kg body weight). Other treatment groups received synthesized drugs of two dose level such as 50mg/kg and 100mg/kg body weight. For determination of blood glucose level, blood samples were collected from the vein of tail on 0, 5th, 10th and 15th day [14]. Glucometer™ (Accusure) was used to measure the blood glucose level throughout the two weeks of treatment.

Blood glucose level: Before starting the treatments, blood glucose level of all the animals was within normal range. After 72 hours of STZ treatment the blood glucose level was significantly changed more

than 240 mg/dl. Statistical analysis by one way ANOVA revealed that there was significant decrease (**p< 0.01) in blood glucose level of the animals after treatment with drugs on 5th day, 10th day and 15th day. The observed blood glucose level of animals are represented in Table X showing the effect of synthesized drug on blood glucose level of normal and diabetic rats after drug treatment.

Table 10 : Effect of synthesized drugs on blood glucose level of normal and diabetic rats

Group	Dose level(mg /kg, p.o)	0day	5th day	10th day	15th day
Normal Control	----	97 ± 0.433	115 ± 0.4933	115.6 ± .6977	94 ± 0.678
Diabetic control	----	244.6 ± 1.022	254.8 ± 1.363	272 ± 2.563	273 ± 0.535
Standard (Miglitol)	25	276.64 ± 6.4	258.36 ± 6.18$^{\#\#}$	194.04 ± 4.732$^{\#\#}$	155.64 ± 6.561$^{\#\#}$
MM1	50	263.44 ± 12.11	238.76 ± 9.07$^{\#\#}$	178.94 ± 12.664**$^{\#\#}$	142.26 ± 6.181**$^{\#\#}$
	100	285.54 ± 5.793	228.4 ± 6.712$^{\#\#}$	163.02 ± 11.786**$^{\#\#}$	136.36 ± 8.125**$^{\#\#}$
MM2	50	283.84 ± 13.271	261.9 ± 11.667$^{\#\#}$	155.8 ± 9.842**$^{\#\#}$	135.56 ± 5.183**$^{\#\#}$
	100	273.44 ± 6.719	244.72 ± 5.826*$^{\#\#}$	139.96 ± 5.99**$^{\#\#}$	129.3 ± 3.637**$^{\#\#}$
MM3	50	285.5 ± 7.499	273.02 ± 8.146$^{\#\#}$	183.56 ± 7.962**$^{\#\#}$	162.76 ± 9.116**$^{\#}$
	100	278.44± 6.246	254.28 ± 6.752$^{\#\#}$	160.94 ± 5.496**$^{\#\#}$	139.72 ± 7.987**$^{\#}$
MM4	50	259.88 ± 7.841	238± 5.412*$^{\#\#}$	250.78 ± 7.664**$^{\#\#}$	220.2 ±5.34**$^{\#}$
	100	262.72± 7.136	229.67± 7.295$^{\#\#}$	179.72 ± 7.551**$^{\#\#}$	168.96 ± 6.254**$^{\#}$
MM5	50	279.74± 8.641	267.18 ± 6.189*$^{\#\#}$	159.8 ± 5.945**$^{\#\#}$	139.26 ± 3.72**
	100	271.3± 6.51	249.9 ± 6.145**$^{\#\#}$	192.8 ± 5.32**$^{\#\#}$	147.92 ± 6.821**

All the values are given as Mean ± SEM (n=5), Standard vs all group (** p<0.01, * p< 0.05), Normal control vs all group ($^{\#\#}$ p<0.01, $^{\#}$p<0.05).

Body weight: Statistical analysis revealed that there is significant (p<0.01) difference among the group at 5th day, 10th day and 15th day when compared with normal control group. But there are no significant changes in body weight among the groups when compared with standard drug. Effects of drugs over body weight are given in the Table 11 showing the changes in the body weight after two weeks of drug treatment.

Biochemical Estimation: Serum marker such as serum glutamic oxaloacetic transaminase (SGOT), serum alkaline phosphatase (ALP) and serum glutamic pyruvic transaminase (SGPT) were measured during biochemical estimation [15]. Lipid profile like triglyceride (TG) and total cholesterol were estimated with the help of testing kits in colorimeter.

Effect on serum transaminase level: SGOT, SGPT and ALP values were measured from collected blood samples after 14 days of treatment. After completion of treatment it was pointed out that there is a mark increase in the serum transaminase level among the untreated diabetic control group and the level decreases to a significant mark among drug treatment groups. The serum transaminase values and ALP values are given in the Table 12.

Effect on lipid profile: After 14 days of drug treatment, total cholesterol and triglyceride were reduced significantly in the test group as compared to diabetic control group. The effect of synthesized drugs on blood lipid profile of normal rats, diabetic rats as well as test sample injecting rats are given here in Table 13.

Table 11 : Effect Of Synthesized Drugs On Body Weight Of Normal And Diabetic Rats

Group	Dose level(mg/kg,p.o) BW	0th day	5th day	10th day	15th day
Normal control	--	110.5± 2.804	110.6 ±2.804	110.6 ± 2.804	109.9± 2.404
Diabetic control	--	100.4 ± 1.470	100± 1.594	80.6 ± 2.337	82.4 ± 2.205
Standard (Miglitol)	25	120.12± 8.396	115.10± 8.339##	120.00± 8.135##	120.42± 9.29##
MM1	50	110.68± 8.666	110.00± 8.772##	90.22± 8.224##	109.66± 8.074##
	100	110.38± 7.441	90.58± 7.494##	100.32± 7.405##	100.84± 7.158##
MM2	50	120.6± 8.685	100.64± 8.306##	115.12± 8.035##	115.68± 8.014##
	100	120.54± 6.687	120.28± 6.828##	115.38± 6.42##	119.00± 6.351##
MM3	50	100.32± 4.425	85.06± 4.512##	78.64± 4.603##	84.16± 4.591##
	100	90.65± 4.385	112.22± 4.722##	100.7± 4.918##	115.96± 5.033##
MM4	50	125.54± 4.33	120.68± 4.215##	120.1± 4.263##	128.22± 3.996##
	100	125.46± 9.004	125.58± 8.745##	120.5± 8.356##	125.38± 8.113##
MM5	50	120.18± 6.729	115.54± 6.646##	115.78± 6.371##	119.32± 6.394##
	100	100.26± 6.511##	90.22± 7.136##	90.98± 7.224##	97.84± 7.387##

Table 12 : Effect Of Synthesized Drugs On The Serum Markers Of Normal And Diabetic Rats

Group	Dose level(mg/kg,p.o)	SGOT(mg/dl)	SGPT(mg/dl)	ALP(mg/dl)
Normal control	--	71.2± 2.14	74.37 ± 2.23	95.6 ± 2.31
Diabetic control	--	128.87 ± 2.43	123.3 ± 1.16	187.12 ± 2.34
Miglitol	25	79.83 ± 2.417	96.12 ± 2.824	163.15 ± 3.897
MM1	50	97.46 ± 4.497	110.81 ± 6.597	157.87 ± 4.515
	100	120.56 ± 3.741	137.67 ± 7.052	192.2 ± 3.518
MM2	50	106.45 ± 4.174	121.35 ± 6.135	178.13 ± 5.357

	100	129.94 ± 2.605	143.6 ± 10.657	186.13 ± 12.826
MM3	50	99.68 ± 2.667	110.23 ± 3.156	194.24 ± 5.147
	100	121.787 ± 2.508	137.16 ± 2.705	192.3± 3.251
MM4	50	74.187 ± 2.325	89.23 ± 1.048	130.87 ± 5.302
	100	110.8 ± 2.926	115.403 ± 3.545	178.267 ± 2.19
MM5	50	135.31 ± 3.564	143.57 ± 2.677	189.57 ± 4.824
	100	91.85 ± 0.854	122.23 ± 6.29	190.63 ± 6.21

Table 13 : Effect of synthesized drugs on blood lipid profile of normal and diabetic rats

Group	Dose level(mg/kg,p.o)	Total cholesterol(mg/dl)	Triglyceride(mg/dl)
Normal control	--	129.46 ± 5.3	110.2 ± 3.56
Diabetic control	--	255.2 ± 1.16	219.1 ± 0.85
Standard (Miglitol)	25	178.12 ± 2.41	144.43 ± 3.4
MM1	50	177.51 ± 3.54	144.69± 1.23
	100	243.9 ± 5.87	168.79 ± 3.45
MM2	50	187.6 ± 2.21	165.3 ± 3.12
	100	191.5 ± 2.43	166.2 ± 5.36
MM3	50	211.0 ± 2.37	149.12 ± 3.66
	100	199.3 ± 4.02	153.21 ±7.97
MM4	50	177.45 ± 2.2	139.87 ± 4.21
	100	179.7 ± 4.87	169.25 ± 2.23
MM5	50	183.34 ± 8.76	155.24 ± 2.14
	100	210.5 ± 6.71	159.2± 2.42

All the values are given as Mean ± SEM (n=5), Standard vs all group (** $p<0.01$, * $p< 0.05$), Normal control vs all group ($^{\#\#}$ $p<0.01$, $^{\#}p<0.05$).

RESULTS AND DISCUSSION

The molecular docking study clearly indicates that the compounds (MM1, MM2, MM3, MM4, MM5) shows better interaction with the protein 3L4W than the standard drug (Miglitol). Among these compounds, xanthone with 3,6-bis(hydroxyethoxy) substitution (MM1) showed best binding efficacy than other compound as well as the standard drug (Miglitol). Binding energy of the compound MM1 was found to be -121.41928. Because of the presence of hydroxyl group in 3,6 position of the xanthone moieties, the compound 3,6-bis (hydroxyethoxy)-9H-xanthene-9-one showed better binding interaction. In case of 3,6-bis (benzyloxy)-9H-xanthene-9-one [MM2] and 3,6-bis (phenethoxy)-9H-xanthene-9-one[MM4] because of the presence of aromatic ring in the side chain at

3,6 positions, they also showed better binding interaction than the standard drug (Miglitol). For compound MM3 because of having epoxy substituents at 3,6 positions, it interacts better with the binding pocket of the protein 3L4W and showed significant binding energy. Compound having aliphatic side chain showed less binding interaction as compared to the other compound having aromatic ring as well as epoxy substituents in the side chain. That is why 3,6-bis(heptyloxy)-9H-xanthene-9-one [MM5] showed less binding interaction as compared to the other compound but have significant binding energy than the standard drug (Miglitol).

From the evaluation of anti-diabetic activity we have found the compound MM1, MM2 and MM5 showed significant anti-diabetic activity as compared to the standard drug (Miglitol) with $^{\#}p<0.05$. They decrease blood glucose level by 46 % especially at dose level 50mg/kg body weightand 52 % at dose level 100mg/kg body weight as compared to the standard drug (Miglitol).Similarly compound MM3 showed moderate anti-diabetic activity and compound MM4 showed less anti-diabetic activity as compared to the standard drug (Miglitol) at dose level 50mg/kg body weight. After the drug treatment, body weight increases significantly. Similarly lipid profile like total cholesterol, triglycerides increases significantly after the drug treatment to the Wister albino rats of either sex. The above results indicate that the synthesized xanthone compounds in the laboratory possess significant, moderate and in case of compound MM4 less anti-diabetic activity.

CONCLUSION

Molecular docking study shows that most of the designed ligand revealed better binding potential than standard drug (Miglitol) and thus they acts as attractive inhibitors of α-glucosidase. These compounds showed excellent correlation between docking results, synthetic data and *in-vivo* anti-diabetic activity. From this research effort, it can be concluded that the compound having hydroxyl groups in 1,3 and 6 position of the xanthone moieties as well as the presence of aromatic and epoxy substituents at 3,6 position showed better binding interaction and biologically significant. Further modification can be done by increasing side chain and incorporated aromatic substituents in 3,6 position so that to increase the binding affinity as well as to minimize toxicity profile.

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