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Discovery of synthetic bioactive flavonoid derivatives as potential antidiabetic agents

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ABSTRACT

Diabetes mellitus (DM) is a growing problem that threatens human health and life in both developed and third world countries. According to reports from the WHO, around 250 million people are currently living with diabetes and this number is expected to be more than 366 million by 2030. Bioactive flavonoid chalcones are naturally occurring phenolic compounds that are widely distributed in plants and some of them have been described as potential antidiabetic agents with multiple mechanisms which earlier reported as PPARγ agonist, α-glucosidase inhibitors, insulin mimetics, insulin-stimulated glucose uptake regulators, and aldose reductase inhibitors respectively. Bioactive flavoinoids chalcones reaction sequence intended for the preparation of title compounds (MVC1-MVC5) were synthesized using the Claisen-Schmidt base incremental method. The investigation of in vitro glucose uptake by yeast cells activity screening data revealed that the compounds MCV4 and MCV5 demonstrated comparatively the most potent in-vitro antidiabetic activity, with significant percentages of glucose uptake by yeast cells.

Keywords: Diabetes mellitus (DM), bioactive flavonoids, chalcones

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INTRODUCTION

Medicinal chemistry is the science that deals with the discovery and design of new therapeutic chemicals or biochemical and biochemical and their development into useful medicines. There has been increasing interest in the research on flavonoids from synthetic or plant sources because of their versatile health benefits reported in various epidemiological studies [1-3]. Since flavonoids are directly associated with human dietary ingredients and health, there is need to evaluate structure and function relationship. Flavonoids are polyphenolic molecules containing 15 carbon atoms and are soluble in water [4-10]. They consist of two benzene rings connected by a short three carbon chain. One of the carbons in this chain is connected to a carbon in one of the benzene rings, either through an oxygen bridge or directly, which gives a third middle ring. [11-17] Diabetes mellitus is a complex metabolic disorder in the endocrine system characterized by abnormalities in insulin secretion and/or insulin action that leads to progressive deterioration of glucose tolerance and causes hyperglycemia [18-22]. This disease is a major public health problem worldwide and is rapidly becoming more common. As an ultimate goal on improving the quality of human being life, there are many new drug discoveries as potential anti-diabetic agents[23-29]. Chalcone is a class of open-chain flavonoids that is not only biosynthesized by plants but also can be prepared synthetically. Chalcones, considered to be the precursor of flavonoids and isoflavonoids, are abundant in edible plants. They consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α, β-unsaturated carbonyl system.

Introduction of various substituents into the two aryl rings is also a subject of interest because it leads to useful structure-activity relationship (SAR). Several studies have demonstrated that chalcones either from natural or synthetic sources can influence carbohydrate pathways, especially glucose metabolism. These studies verified the effectiveness of chalcones as antihyperglycemic and/or hypoglycemic agents through in vitro and in vivo experimental responses.

MATERIALS AND METHODS

Materials

Instrumentation

Melting points were taken in open capillary tubes. Purity of the compounds was checked on silica gel G TLC plates of 2 mm thickness using n-hexane and ethyl acetate as solvent system. The visualization of spot was carried out in an UV-chamber.

Reagents and chemicals

Substituted aldehydes and ketones, sodium hydroxide, potassium hydroxide, pyridine, triethylamine, methanol, ethanol, iodine, H2SO4 spraying reagent, silica gel (column & TLC) and other regular laboratory chemical of AR grade.

Methods

General procedure for the synthesis of chalcone derivatives

The reaction sequence intended for the preparation of title compounds (MVC1-MVC5) is shown in Scheme 1; we have followed the pre-existing methods for the proposed synthesis.

Scheme 1: Synthesis of Chalcone

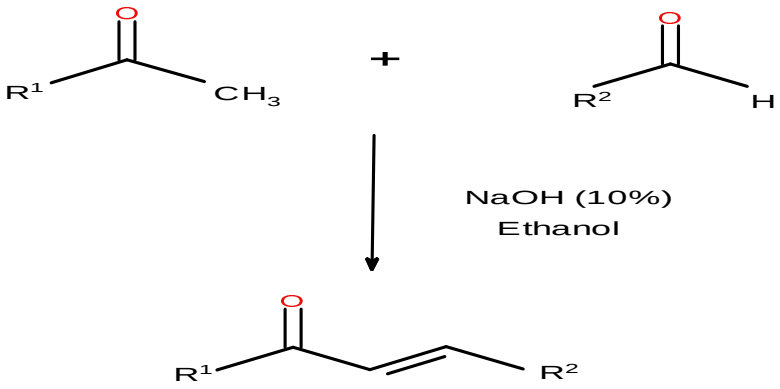
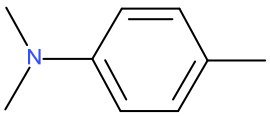


Table 5.1 Chemical structure of molecules

Compound	R^1	R^2
1) MVC1	C_6H_5	C_6H_5
2) MVC2		C_6H_5

Part A

The reaction as shown in scheme 1 part-A was started by adding 1:1 mole concentration of substituted ketone and respective substituted aromatic aldehydes in q.s. amount of ethanol. To this reaction mixture1.5mL of 20% NaOH solution was added in drop-wise until the reaction mixture turns to yellow. Further the reaction mixture kept aside overnight and examined the TLC profile. After completion of the reaction, the reaction mixture was poured in

crushed ice, acidified if necessary with 1:1 dilute hydrochloric acid, and the yellow color solid which separated out was isolated by filtration using Buchner funnel under vacuum filtration setup, dried and purified by recrystallization with ethanol [5-7]. The crystals which separated out from the solvent were collected and used for doing physical characterization and bioassay.[1-3,29-33] The physical properties are depicted in separate table as given under chapter 5 (Tables 5.1-5.2) for an every individual compound.

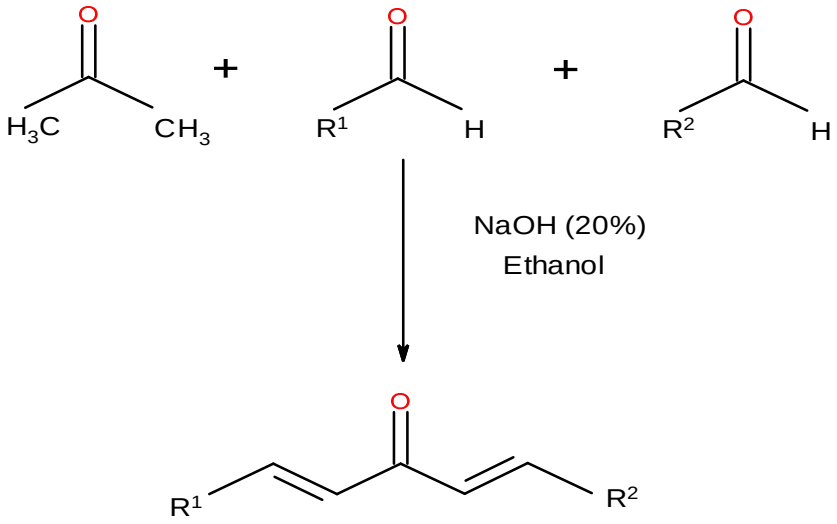
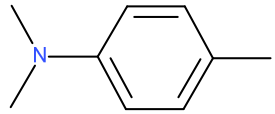


Table 5.2 Chemical structure of molecules

Compound	R^1	R^2
1) MVC3	C_6H_5	C_6H_5
2) MVC4	C_6H_5	
3) MVC5	C_6H_5	C_6H_5

The reaction as shown in scheme 1 part-B was started by adding substituted benzaldehydes to acetone in 1:2 mole concentrations in q.s. amount of ethanol. To this reaction mixture1.5mL of 20% NaOH solution was added in drop-wise until the reaction mixture turns to yellow. Further the reaction mixture kept aside overnight and examined the TLC profile. After completion of the reaction, the reaction mixture was poured in crushed ice, acidified if necessary with 1:1 dilute hydrochloric acid, and the yellow color solid which separated out was isolated by filtration using Buchner funnel under vacuum filtration setup, dried and purified by recrystallization with ethanol. The crystals which separated out from the solvent were collected and used for doing physical characterization and bioassay. The physical properties are depicted in separate table as given under chapter 5 (Tables 5.3-5.5) for an every individual compound.

Identification of chalcones

The identification of individual compound, analysed by Co-TLC technique with the comparison with the starting materials.

Characterization of chalcones

The chemical structures of the synthetic chalcones were established on the basis of their Physical, chemical and spectral analytical data.

Biological evaluation of chalcones

***In-vitro* glucose uptake by yeast cells**

Yeast cells were prepared according to the method of Cirillo [13].Commercial baker's yeast was washed by repeated centrifugation (4200 r/min, 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of the flavonoid chalcones MVC1-MVC5 (5-15 mg/mL) were added to 1 mL of glucose solution (10-25 mmol/L) and incubated together for 10 min at 37 °C. The reaction was started by adding 100 µL of yeast suspension, vortexed and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (3 800 r/min, 5 min) and glucose was estimated in the supernatant. The percent increase in glucose uptake by yeast cells was calculated using UV method based on the absorbance of blank reaction (containing all reagents except the test sample) and test sample [48-50].

UV method for estimation of glucose

Glucose estimation was done according to the sulfuric acid-UV method of Ammar [2]. A 1 mL of aliquot of glucose concentrations 5, 7.5, 10, 12.5 and 15 mM respectively was rapidly mixed with 2 mL of concentrated sulfuric acid in a test tube and vortexed for 30 s. The temperature of the reaction mixture was raised rapidly within 10 s after addition of sulfuric acid. Then the solution was cooled in ice for 2 min and brought it back to the room temperature. Finally, UV light absorption at 315 nm was recorded using UV-double beam spectrophotometer (Jasco V-630). The glucose content was estimated from a standard curve prepared with standard solutions. Standard glucose solutions (5-15 mM) were prepared using serial dilution method. The calibration curve for glucose estimations was plotted with a correlation coefficient (r2) of 0.99, indicating acceptable precision and accuracy of the method

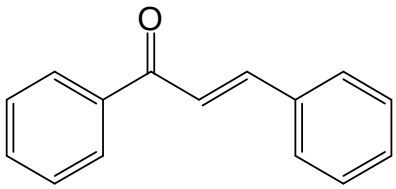
Statistical analysis

The SPSS 20 software was used in data analysis. Data was expressed as mean and SEM.

RESULTS

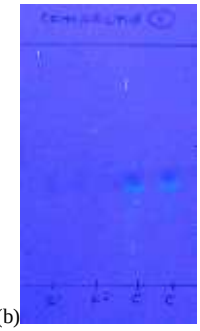
Table 2.1.Physical characterization data of flavonoid chalcone MVC1

Flavonoid Chalcone (MVC1)



MVC1

Physical state	Solid crystals
Color	Yellow
Nomenclature	*(E)*-Chalcone
Molecular weight	208
Molecular formula	$C_{15}H_{12}O$
Melting point (°C)	55-60
Yield (%)	85
Recrystallization solvent	Ethanol
Thin Layer Chromatography (TLC)	Thin Layer Chromatography (TLC)
• **Mobile phase concentration**	• 25% Ethylacetate/Hexane
• **R_f value**	• 0.56 cm
• **Image of TLC plate**	

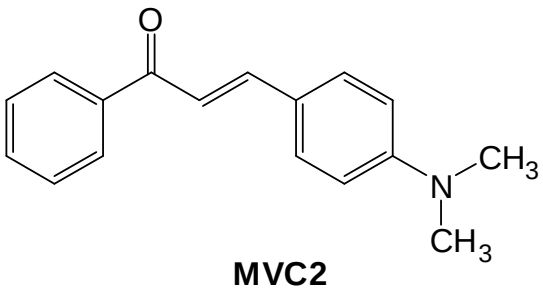
TLC plate **(a) Developed in Iodine Chamber** **(b) Developed under UV lamp**	 (a)  (b)
IR spectrum data (cm^{-1})	Not determined
^{1}H NMR spectrum data (δ ppm)	Not determined
^{13}C NMR spectrum data (δ ppm)	Not determined
Mass spectrum data (m/z)	Not determined
Elemental Analysis (Calculated)	C, 86.51%; H, 5.81%; O, 7.68%
Elemental Analysis (Found)	Not determined
H_2SO_4 spray test	Pink color spot
3D Model structure	

Literature Report

Rocchi, D., González, J. F., & Menendez, J. C. (2014). Montmorillonite Clay-Promoted, Solvent-Free Cross-Aldol Condensations under Focused Microwave Irradiation. Molecules, 19(6), 7317-7326.

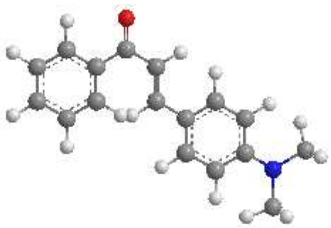
Table 2.2.Physical characterization data of flavonoid chalcone MVC2

Flavonoid Chalcone (MVC2)



MVC2

Physical state	Solid crystals
Color	Yellow
Nomenclature	*(E)*-3-(4-(dimethylamino)phenyl)-1-phenylprop-2-en-1-one
Molecular weight	251
Molecular formula	$C_{17}H_{17}NO$
Melting point (°C)	90-95
Yield (%)	70
Recrystallization solvent	Ethanol
Thin Layer Chromatography (TLC)	Thin Layer Chromatography (TLC)
• **Mobile phase concentration**	• 25% Ethylacetate/Hexane
• **R_f value**	• 0.30 cm
• **Image of TLC plate**	

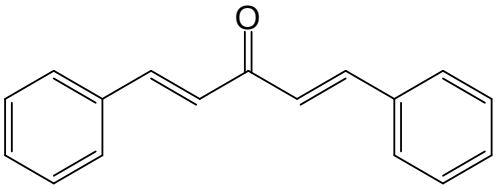
TLC plate **(a)Developed in Iodine Chamber** **(b) Developed under UV lamp**	 (a)  (b)
IR spectrum data (cm^{-1})	Not determined
^{1}H NMR spectrum data (δ ppm)	Not determined
^{13}C NMR spectrum data (δ ppm)	Not determined
Mass spectrum data (m/z)	Not determined
Elemental Analysis (Calculated)	C, 81.24%; H, 6.82%; N, 5.57%; O, 6.37%
Elemental Analysis (Found)	Not determined
H_2SO_4 spray test	Pink color spot
3D Model structure	

Literature Report

Li, J. T., Yang, W. Z., Wang, S. X., Li, S. H., & Li, T. S. (2002). Improved synthesis of chalcones under ultrasound irradiation. UltrasonicsSonochemistry, 9(5), 237-239.

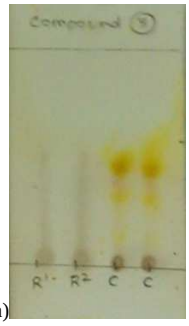
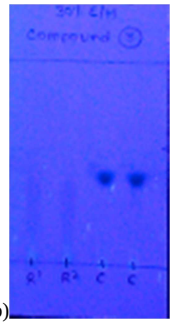
Table 2.3.Physical characterization data of flavonoid chalcone MVC3

Flavonoid Chalcone (MVC3)



MVC3

Physical state	Solid crystals
Color	Yellow
Nomenclature	*(1E,4E)*-1,5-diphenylpenta-1,4-dien-3-one
Molecular weight	234
Molecular formula	$C_{17}H_{14}O$
Melting point (°C)	110-111
Yield (%)	92
Recrystallization solvent	Ethanol
Thin Layer Chromatography (TLC)	Thin Layer Chromatography (TLC)
• **Mobile phase concentration**	• 25% Ethylacetate/Hexane
• **R_f value**	• 0.50 cm

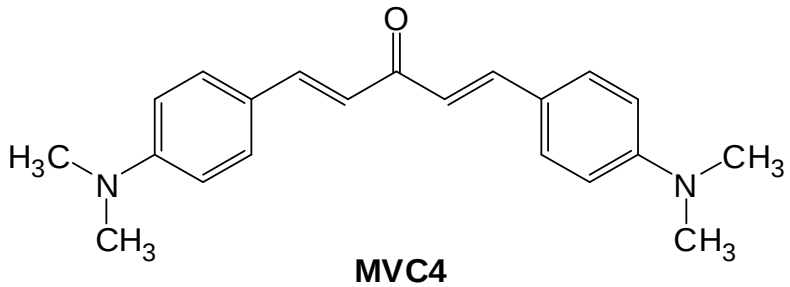
TLC plate **(a) Developed in Iodine Chamber** **(b) Developed under UV lamp**	 (a)  (b)
IR spectrum data (cm^{-1})	Not determined
^{1}H NMR spectrum data (δ ppm)	Not determined
^{13}C NMR spectrum data (δ ppm)	Not determined
Mass spectrum data (m/z)	Not determined
Elemental Analysis (Calculated)	C, 87.15%; H, 6.02%; O, 6.83%
Elemental Analysis (Found)	Not determined
H_2SO_4 spray test	Pink color spot
3D Model structure	

Literature Report

Sudha, S., Sundaraganesan, N., Vanchinathan, K., Muthu, K., &Meenakshisundaram, S. P. (2012). Spectroscopic (FTIR, FT-Raman, NMR and UV) and molecular structure investigations of 1, 5-diphenylpenta-1, 4-dien-3-one: A combined experimental and theoretical study. Journal of Molecular Structure, 1030, 191-203.

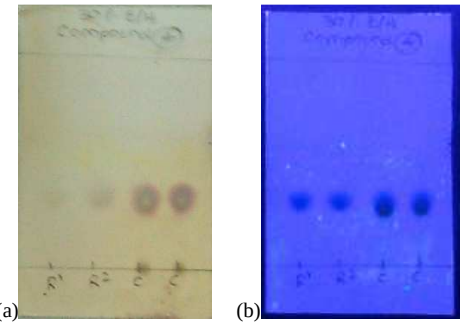
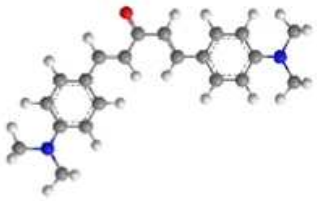
Table 2.4.Physical characterization data of flavonoid chalcone MVC4

Flavonoid Chalcone (MVC4)



MVC4

Physical state	Solid crystals
Color	Yellow
Nomenclature	*(1E,4E)*-1,5-bis(4-(dimethylamino)phenyl)penta-1,4-dien-3-one
Molecular weight	320
Molecular formula	$C_{21}H_{24}N_2O$
Melting point (°C)	70-75
Yield (%)	81
Recrystallization solvent	Ethanol
Thin Layer Chromatography (TLC)	Thin Layer Chromatography (TLC)
• **Mobile phase concentration**	• 25% Ethylacetate/Hexane
• **R_f value**	• 0.37 cm

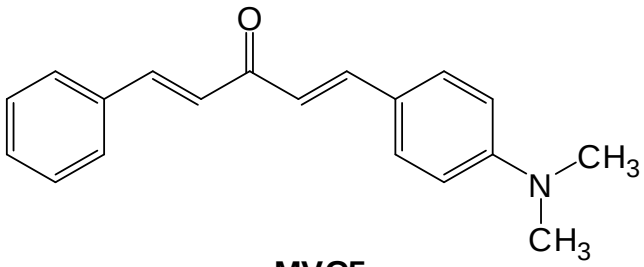
TLC plate **(a) Developed in Iodine Chamber** **(b) Developed under UV lamp**	 (a) (b)
IR spectrum data (cm^{-1})	Not determined
^{1}H NMR spectrum data (δ ppm)	Not determined
^{13}C NMR spectrum data (δ ppm)	Not determined
Mass spectrum data (m/z)	Not determined
Elemental Analysis (Calculated)	C, 78.71%; H, 7.55%; N, 8.74%; O, 4.99%
Elemental Analysis (Found)	Not determined
H_2SO_4 spray test	Pink color spot
3D Model structure	

Literature Report

Lin, H., Hu, G. X., Guo, J., Ge, Y., Liang, G., Lian, Q. Q., &Ge, R. S. (2013). Mono-carbonyl curcumin analogues as 11β-hydroxysteroid dehydrogenase 1 inhibitors. Bioorganic & medicinal chemistry letters, 23(15), 4362-4366.

Table 2.5.Physical characterization data of flavonoid chalcone MVC5

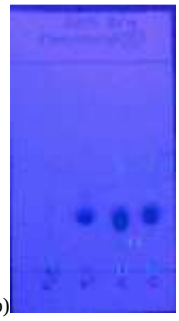
Flavonoid Chalcone (MVC5)



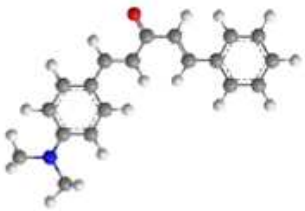
MVC5

Physical state	Solid crystals
Color	Yellowish orange
Nomenclature	*(1E,4E)*-1-(4-(dimethylamino)phenyl)-5-phenylpenta-1,4-dien-3-one
Molecular weight	277
Molecular formula	$C_{19}H_{19}NO$
Melting point (°C)	65-70
Yield (%)	65
Recrystallization solvent	Ethanol
Thin Layer Chromatography (TLC)	Thin Layer Chromatography (TLC)
• **Mobile phase concentration**	• 25% Ethylacetate/Hexane
• **R_f value**	• 0.33 cm

TLC plate
(a) Developed in Iodine Chamber
(b) Developed under UV lamp


(a)

(b)

IR spectrum data (cm^{-1})	Not determined
^{1}H NMR spectrum data (δ ppm)	Not determined
^{13}C NMR spectrum data (δ ppm)	Not determined
Mass spectrum data (m/z)	Not determined
Elemental Analysis (Calculated)	C, 82.28%; H, 6.90%; N, 5.05%; O, 5.77%
Elemental Analysis (Found)	Not determined
H_2SO_4 spray test	Pink color spot
3D Model structure	



Literature Report
Bento, A. P., Gaulton, A., Hersey, A., Bellis, L. J., Chambers, J., Davies, M.,…&Overington, J. P. (2014). The ChEMBL bioactivity database: an update. Nucleic acids research, 42(D1), D1083-D1090.

Biological Evaluation

Table 2.6.Standard plots for estimation of glucose using sulfuric acid based UV method at 315 nm

S.No	Concentration of glucose solution (mM)	Absorbance response (λmax) at 315 nm
1	5	1.0012
2	7.5	1.2621
3	10	1.6551
4	12.5	2.0516
5	15	2.5007

The results in Fig. 5.1 and in Table 5.6 clearly show that there is strong linear correlation with coefficient of determination R^2 = 0.992, Intercept = 0.178, Slope = 0.151 between the glucose solutions (mM) and UV-light absorbance at 315 nm, measured using sulfuric acid method.

Table 2.7.UV absorbance of test samples for different glucose concentrations

S.No	Test Sample (1 mg/mL)	Absorbance response to different glucose concentrations using Sulfuric Acid-UV method at 315 nm		
		5 mM±SEM	10 mM±SEM	15 mM±SEM
1	**MVC1**	0.7048±0.06	0.9015±0.07	1.5923±0.08
2	**MVC2**	0.5735±0.06	0.6832±0.07	1.4732±0.08
3	**MVC3**	0.836±0.06	0.9793±0.07	1.6542±0.08
4	**MVC4**	0.3954±0.06	0.4569±0.07	1.1173±0.08
5	**MVC5**	0.5872±0.06	0.6528±0.07	1.3349±0.08
6	**Standard***	0.4562±0.06	0.5983±0.07	1.236±0.08

**Metformin HCl*
The mean difference is significant at P value < 0.05.

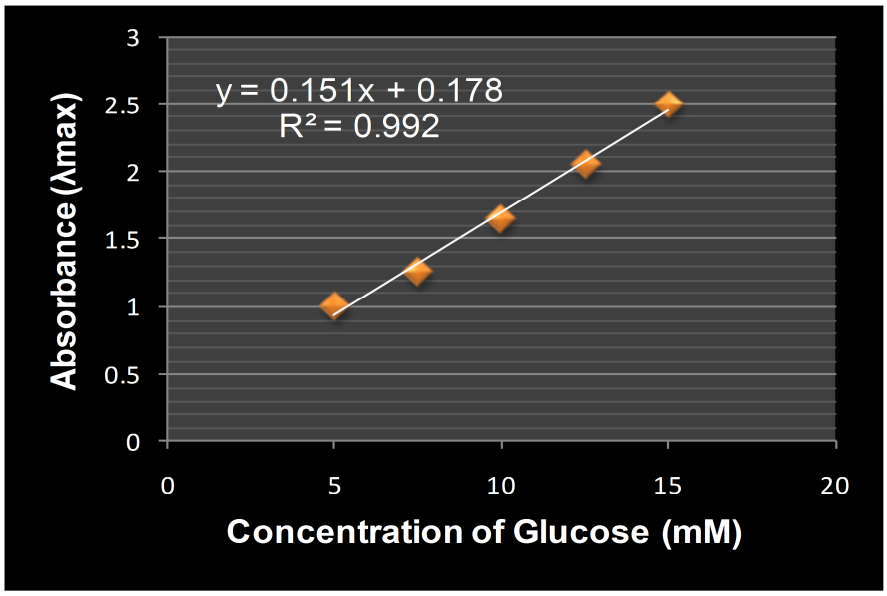


Figure 2.1. Standard graph for estimation of glucose using sulfuric acid based UV method at 315 nm

Concentration (mM) = (Absorbance) - 0.178
0.151

Table 2.8.Anova Single Test (1mg/ml)

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
5	6	3.5531	0.592183	0.025944		
10	6	4.272	0.712	0.037939		
15	6	8.4079	1.401317	0.0436		
ANOVA						
ce of Varic	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between	2.288421	2	1.14421	31.93659	0.000004	3.68232
Within Gr	0.537414	15	0.035828			
Total	2.825834	17				

At the degree of freedom (2, 15), the "F" value for the ANOVA shows = 31.93 and "P"≤ 0.05 is 0.000 which shows there is statically significant different. Thereby, the alternative hypothesis is acceptable.

Table 2.9.Relative percentages of glucose uptake by yeast cells

S.No	Test Sample (1 mg/mL)	5 mM Glucose solution		10 mM Glucose solution		15 mM Glucose solution	
		Glucose Conc. (mM)	Glucose Uptake (%)	Glucose Conc. (mM)	Glucose Uptake (%)	Glucose Conc. (mM)	Glucose Uptake (%)
1	**MVC1**	4.2	84	9.1	91	13.4	89
2	**MVC2**	4.4	88	9.2	92	13.5	90
3	**MVC3**	4.2	90	9.0	90	13.3	88
4	**MVC4**	4.7	94	9.5	95	13.9	93
5	**MVC5**	4.5	90	9.3	93	13.7	91
6	**Standard***	4.6	92	9.4	94	13.8	92

**Metformin HCl*

Figure 2.2.In-vitro Percentages of Glucose (5mM) Uptake by Yeast Cells

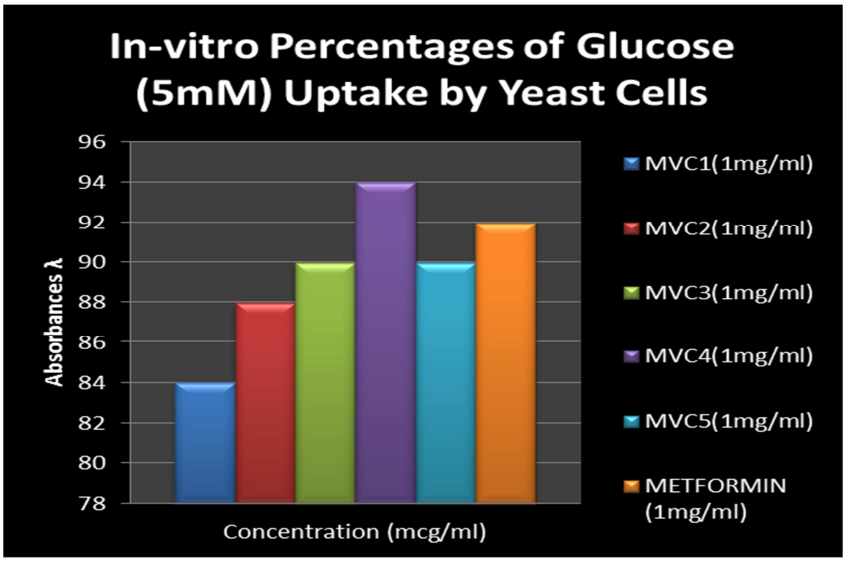


Figure 2.3.In-vitro Percentages of Glucose (10mM) Uptake by Yeast Cells

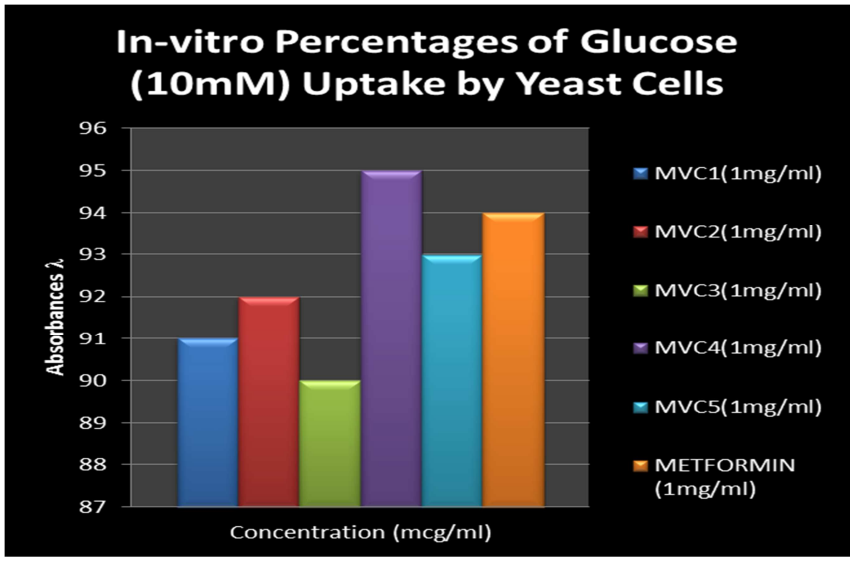


Figure 2.4.In-vitro Percentages of Glucose (15mM) Uptake by Yeast Cells

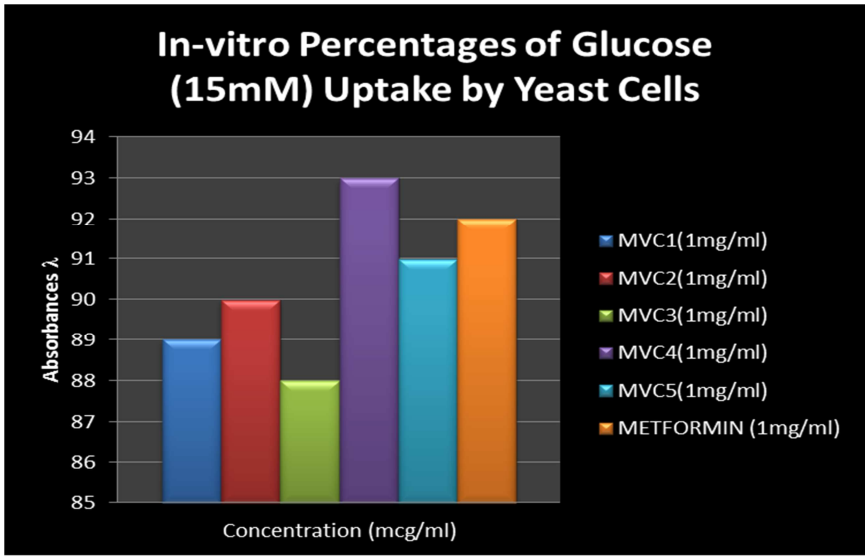


Table 2.10.UV absorbance of test samples for different glucose concentrations

S.No	Test Sample (5 mg/mL)	Absorbance response to different glucose concentrations using Sulfuric Acid-UV method at 315 nm		
		5 mM±SEM	10 mM±SEM	15 mM±SEM
1	**MVC1**	0.6916±0.08	0.8865±0.08	1.6723±0.09
2	**MVC2**	0.5682±0.08	0.6701±0.08	1.4388±0.09
3	**MVC3**	0.8765±0.08	0.8994±0.08	1.6512±0.09
4	**MVC4**	0.3658±0.08	0.4184±0.08	1.1033±0.09
5	**MVC5**	0.4523±0.08	0.5722±0.08	1.3294±0.09
6	**Standard***	0.3987±0.08	0.4983±0.08	1.1949±0.09

**Metformin HCl*

The mean difference is significant at P value < 0.05.

Table 2.11. Anova Single Test (5mg/ml)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
5	6	3.3531	0.55885	0.038576
10	6	3.9449	0.657483	0.040181
15	6	8.3899	1.398317	0.054764

ANOVA

ce of Varic	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between (	2.526534	2	1.263267	28.38359	0.000008	3.682320344
Within Gr	0.667604	15	0.044507			
Total	3.194138	17				

At the degree of freedom (2, 15), the "F" value for the ANOVA shows = 31.93 and "P"≤ 0.05 is 0.000 which shows there is statically significant different. Thereby, the alternative hypothesis is acceptable.

Table 2.12.Relative percentages of glucose uptake by yeast cells

S.No	Test Sample (5 mg/mL)	5 mM Glucose solution		10 mM Glucose solution		15 mM Glucose solution	
		Glucose Conc. (mM)	Glucose Uptake (%)	Glucose Conc. (mM)	Glucose Uptake (%)	Glucose Conc. (mM)	Glucose Uptake (%)
1	**MVC1**	4.3	86	9.1	91	13.3	89
2	**MVC2**	4.4	88	9.3	93	13.6	90
3	**MVC3**	4.1	82	9.1	91	13.3	89
4	**MVC4**	4.6	92	9.6	96	13.9	93
5	**MVC5**	4.5	90	9.4	94	13.7	91
6	**Standard***	4.6	92	9.5	95	13.8	92

**Metformin HCl*

Figure 2.5.In-vitro Percentages of Glucose (5mM) Uptake by Yeast Cells

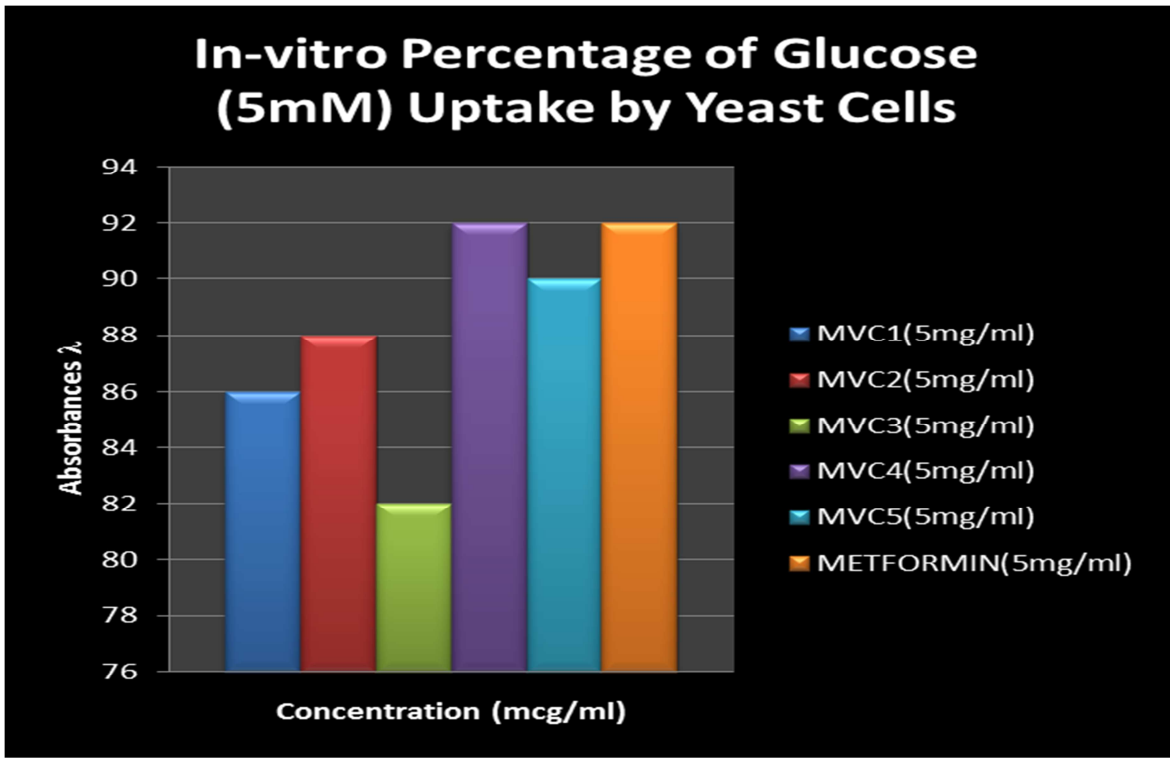


Figure 2.6.In-vitro Percentages of Glucose (10mM) Uptake by Yeast Cells

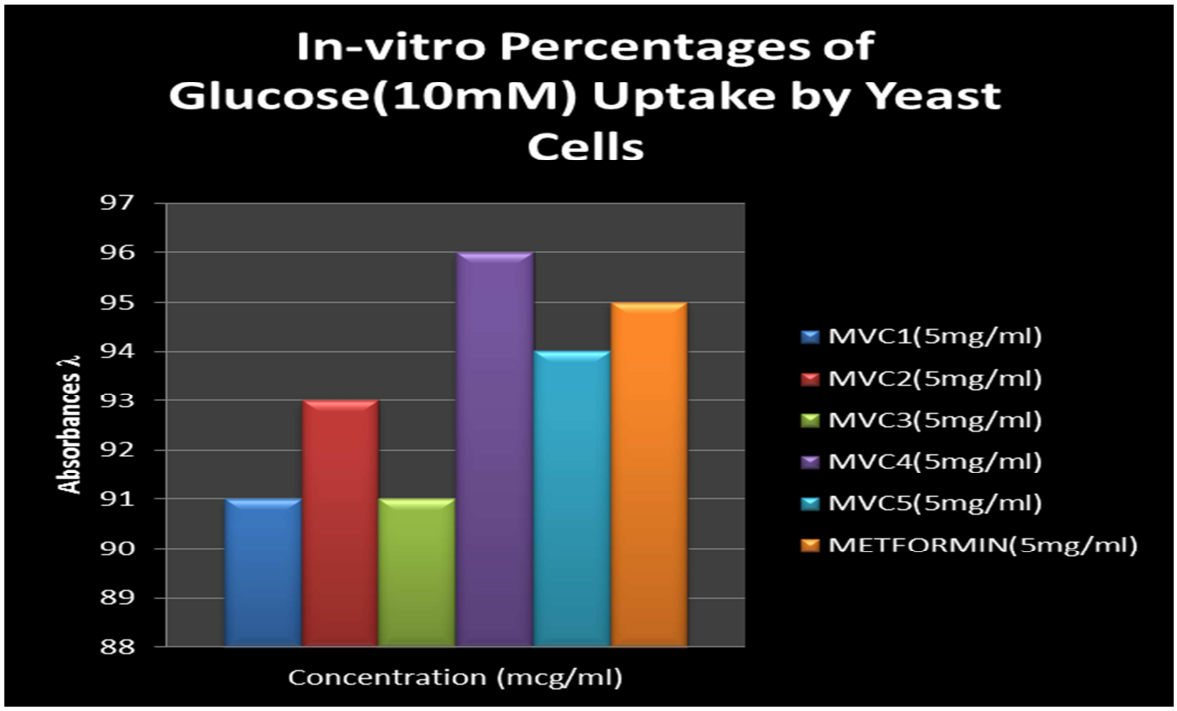
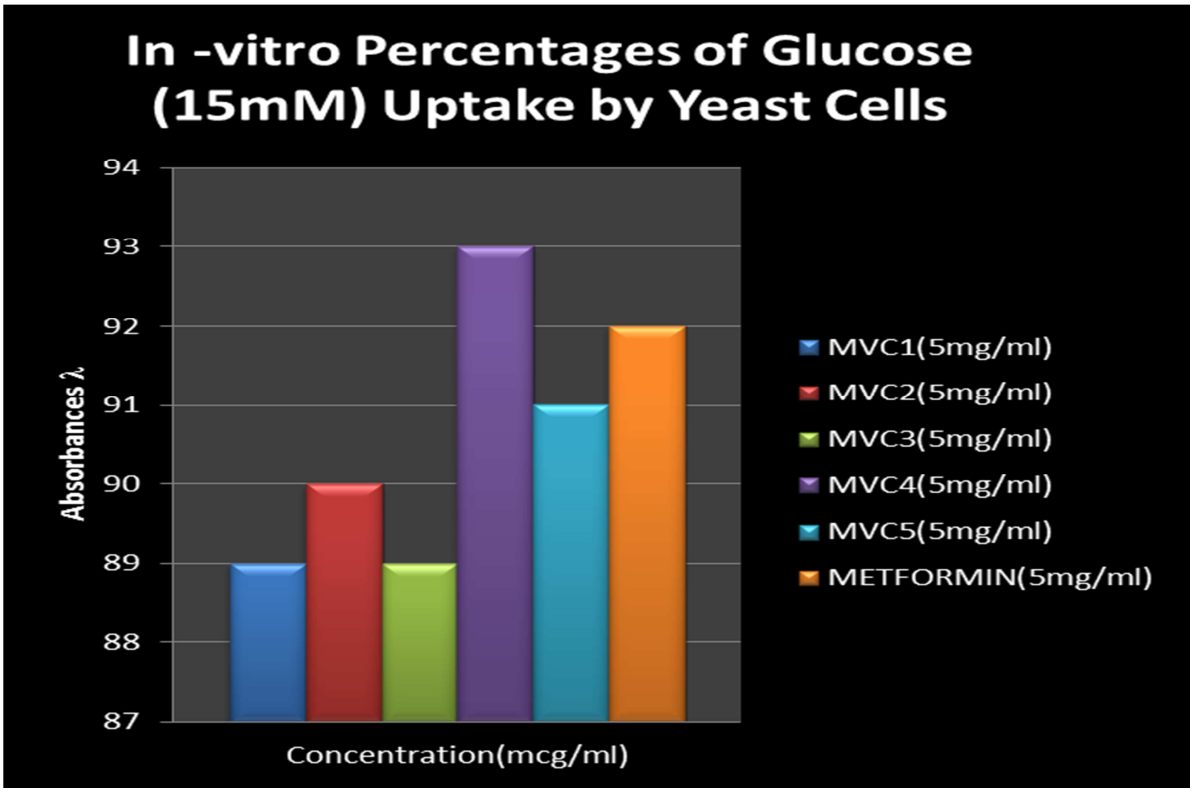


Figure 2.7.In-vitro Percentages of Glucose (15mM) Uptake by Yeast Cells



DISCUSSION

We started our study using the Claisen-Schmidt condensation classical method. As shown in Table 1 and Scheme 1 (Part A and B), the synthesis of chalcones **(MVC1 – MVC5)** was carried out using acetophenone with various aromatic aldehydes in the presence of sodium hydroxide (NaOH) to obtain good yield. The choice of catalyst was based on literature precedent for the Claisen-Schmidt condensation method of acetophenone and benzaldehydes. Melting points were measured in open capillary tubes and Silica gel-G plates (Merck) were used for TLC analysis. As for the mobile phase concentration, 25% of Ethylacetate/Hexane was optimized for each compound synthesized namely **MVC1 – MVC5.** Elemental analyses (EA) were measured by the Chemdraw Ultra sketch. Eventually all the compounds **MVC1-MVC5** were confirmed based on literature reports.

The reaction as shown in scheme 1 part-A was started by adding 1:1 mole concentration of substituted ketone and respective substituted aromatic aldehydes in q.s. amount of ethanol. To this reaction mixture1.5mL of 20% NaOH solution was added in drop-wise until the reaction mixture turns to yellow. Further the reaction mixture kept aside overnight and examined the TLC profile. After completion of the reaction, the reaction mixture was poured in crushed ice, acidified if necessary with 1:1 dilute hydrochloric acid, and the yellow color solid which separated out was isolated by filtration using Buchner funnel under vacuum filtration setup, dried and purified by recrystallization with ethanol. The crystals which separated out from the solvent were collected and used for doing physical characterization and bioassay [1-3,29-33] The physical properties are depicted in separate table as given under chapter 5 (Tables 5.1-5.2) for an every individual compound.

The reaction as shown in scheme 1 part-B was started by adding substituted benzaldehydes to acetone in 1:2 mole concentrations in q.s. amount of ethanol. To this reaction mixture1.5mL of 20% NaOH solution was added in drop-wise until the reaction mixture turns to yellow. Further the reaction mixture kept aside overnight and examined the TLC profile. After completion of the reaction, the reaction mixture was poured in crushed ice, acidified if necessary with 1:1 dilute hydrochloric acid, and the yellow color solid which separated out was isolated by filtration using Buchner funnel under vacuum filtration setup, dried and purified by recrystallization with ethanol [5-10]. The crystals which separated out from the solvent were collected and used for doing physical characterization and bioassay. The physical properties are depicted in separate table as given under chapter 5 (Tables 5.3-5.5) for an every individual compound.

For Part B, the physical properties are depicted in separate Tables for an every individual compound. The substituted benzaldehydes were dissolved in minimum amount of alcohol. Then 1:2 mole ratio quantities of substituted benzaldehydes and acetone were mixed together. Over a period of 15 minutes, 20% NaOH solution was added to the mixture while swirling thoroughly. After completion of the reaction, the mixture was poured on to crushed ice, acidified if necessary with dilute hydrochloric acid, and the solid that separated was isolated by filtration using Erlenmeyer flask , dried and purified by recrystallization with ethanol. Completion of the reaction was identified by TLC using silica gel-G.

For each compound **(MVC1-MVC5)** IR, ^{1}HNMR and ^{13}CNMR spectral data were not determined as all compounds were earlier reported. Besides, a pink color spot was observed during the sulphuric acid spraying test. Characterization data for previously reported compounds **(MVC1-MVC5)** are given below. For full characterization data, see the Supporting Information [34-39].

MVC1: (E)-Chalcone, Solid crystals, Yellow, MW: 208, MF: C15H12O, M.p: 55-60°C(consistency), % yield:85, Rf:0.56 cm, E.A: C, 86.51%; H, 5.81%; O, 7.68% , IR & NMR: were adapted from Gonzalez, J.F et al., 2014.

MVC2: (E)-3-(4-(dimethylamino)phenyl)-1-phenylprop-2-en-1-one, Solid crystals, Yellow, MW:215, MF: C17H17NO, M.p: 90-95°C(consistency), % yield:70, Rf:0.30 cm, E.A: C, 81.24%; H, 6.82%; N, 5.57%; O, 6.37%, IR & NMR: were adapted from Yang, W.Z et al., 2002.

MVC3: 1E,4E)-1,5-diphenylpenta-1,4-dien-3-one, Solid crystals, Yellow, MW:234, MF: C17H11O, M.p: 110-111°C(consistency), % yield:92, Rf:0.50 cm, E.A: C, 87.15%; H, 6.02%; O, 6.83%, IR & NMR: were adapted from Sundaraganesan, N et al., 2012.

MVC4: (1E,4E)-1,5-bis(4-(dimethylamino)phenyl)penta-1,4-dien-3-one), Solid crystals, Yellow, MW:340, MF: C21H24N2O, M.p: 70-75°C(consistency), % yield:81, Rf:0.37 cm, E.A: C, 78.71%; H, 7.55%; N, 8.74%; O, 4.99%, IR & NMR: were adapted from Hu, G.X et al., 2013.

MVC5: (1E,4E)-1-(4-(dimethylamino)phenyl)-5-phenylpenta-1,4-dien-3-one, Solid crystals, Yellowish orange, MW:277, MF: C19H19NO, M.p: 65-70°C(consistency), % yield: 65, Rf:0.33 cm, E.A: C, 82.28%; H, 6.90%; N, 5.05%; O, 5.77%, IR & NMR: were adapted from Gaulton, A et al., 2014.

The investigation of in vitro glucose uptake by yeast cells activity screening data (Table 5.1 to 5.5) revealed that the compounds **MVC4** and **MVC5** demonstrated comparatively the most potent in-vitro antidiabetic activity, with significant percentages of glucose uptake by yeast cells [35-41]. Various concentrations (5mM, 10mM, and 15mM) of glucose solution were prepared. As seen in case of compound **MVC4**, percentages of glucose uptake by yeast cells were 94%, 95% and 93% respectively at dose concentration of 1mg/mL. Likewise, compound **MVC5** has exhibited 90%, 93%, and 91% of glucose uptake by yeast cells. It is interesting to note that the compound **MVC2**has also showed appreciable activity with moderate percentage of 88%, 92% and 90% for its glucose uptake by yeast cells. Correspondingly, for the compounds **MVC1** and **MVC3**the percentages of glucose uptake activity showed (84%, 91%, 89%) and (90%, 90%, 88%) respectively.

From the results, at the dose concentration of 5mg/mL, it was found that the percentages in glucose uptake by yeast cells at 5mM, 10mM and 15mM glucose concentration for compound **MVC4** were 92%, 96% and 93% respectively. Similarly, as depicted in case of compound **MVC5**, the percentages of glucose uptake by yeast cells were 90%, 94% and 91% respectively. It is remarkable to note that the compound **MVC2** showed reasonably activity of glucose uptake at 5mM (88%), 10mM (93%), and 15mM (90%). The other compounds such as **MVC1** and **MVC3** denoted less glucose uptake activity by yeast cells at concentrations of 5mM, 10mM and 15mM, where for **MVC1** and **MVC3** the glucose uptake percentages were (86%,91%,89%) and (82%,91%,89%) respectively.

Although, compounds of present study exhibited in-vitro potential as glucose uptake inducer, still there were some limitations which were figured out from our observations and obtained results. Moreover, the standard drug comparison is significant as seen in case of compound **MVC4** and **MVC5**. Since the quantity of yeast suspension added to the reaction tubes for initiating the reaction was retained same hence the possible uptake of glucose by yeast cells in various concentrations of glucose will also remain same because the concentration of yeast cells was same in each case.

A close insight into chemical structure of the molecules synthesized in the present study clearly displayed the significance of chalcone moiety where they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α, β-unsaturated carbonyl system as glucose uptake enhancer by yeast cells [23-29,42-46]. This observation was maintained constant only with the standard drug i.e. Metformin, there was no other compound maintained it's potential to enhance the glucose uptake by yeast cells as constant in varied concentrations [47] Consequently, more specific bioassay that has to be performed to study the exact mechanism of action of the compounds synthesized in the present investigation.

CONCLUSION

In summary we could synthesis and characterize some substituted flavonoid chalcones **MVC1-MVC5**. These compounds were screened for in-vitro antidiabetic bioassay i.e. in-vitro glucose uptake by yeast cells and the results revealed the positive and significant contribution of **MVC4** consisting of bis-dimethylamino substitution at position 4 of ring A and B on dibenzalacetone towards the observed glucose uptake by yeast cells at 10mM glucose solution concentration. The compound **MVC4** has exhibited dose dependent activity at 1 and 5 mg/mL with 95% and 96% of glucose uptake by yeast cells, which could be the remarkable comparison with that of the standard drug Metformin at similar dose concentrations 1 and 5 mg/mL with 94% and 95% of glucose uptake by yeast cells. The observed activity of **MVC4** may be due to the bis-α,β-unsaturation with central core ketone moiety forming part of the basic structure of this molecule.

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